Organic Chemistry

With a Biological Emphasis

Volume I: Chapters 1-8

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Notes to the reader:

This textbook is intended for a sophomore-level, two-semester course in Organic Chemistry targeted at Biology, Biochemistry, and Health Science majors. It is assumed that readers have taken a year of General Chemistry and college level Introductory Biology, and are concurrently enrolled in the typical Biology curriculum for sophomore Biology/Health Sciences majors.

This textbook is meant to be a constantly evolving work in progress, and as such, feedback from students, instructors, and all other readers is greatly appreciated. Please send any comments, suggestions, or notification of errors to the author at soderbt@morris.umn.edu.

If you are looking at a black and white printed version of this textbook, please be aware that most of the figures throughout are meant to contain color, which is used to help the reader to understand the concepts being illustrated. It will often be very helpful to refer to the full-color figures in a digital version of the book, either at the Chemwiki site (see below) or in a PDF version which is available for free download at:

http://facultypages.morris.umn.edu/~soderbt/textbook_website.htm

An online version is accessible as part of the **Chemwiki** project at the University of California, Davis:

http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_Biological_Emphasis.

This online version contains some additional hyperlinks to animations, interactive 3D figures, and online lectures that you may find useful. *Note*: The online (Chemwiki) version currently corresponds to the older (2012) edition of this textbook. It is scheduled to be updated to this 2016 edition during the spring and summer of 2016.

Where is the index? There is no printed index. However, an electronic index is available simply by opening the digital (pdf) version of the text (see above) and using the 'find' or 'search' function of your pdf viewer.

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Chapter 1

Introduction to organic structure and bonding, part I

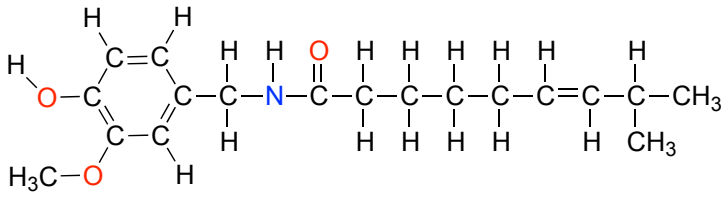
Habanero peppers

(credit: https://www.flickr.com/photos/jeffreyww/)

It's a hot August evening at a park in the middle of North Hudson, Wisconsin, a village of just under 4000 people on the St. Croix river in the western edge of the state. A line of people are seated at tables set up inside a canvas tent. In front of a cheering crowd of friends, family, and neighbors, these brave souls are about to do battle . . .with a fruit plate.

Unfortunately for the contestants, the fruit in question is the habanero, one of the hotter varieties of chili pepper commonly found in markets in North America. In this particular event, teams of five people will race to be the first to eat a full pound of peppers. As the eating begins, all seems well at first. Within thirty seconds, though, what begins to happen is completely predictable and understandable to anyone who has ever mistakenly poured a little to much hot sauce on the dinner plate. Faces turn red, sweat and tears begin to flow, and a copious amount of cold water is gulped down.

Although technically the contestants are competing against each other, the real opponent in this contest - the cause of all the pain and suffering - is the chemical compound 'capsaicin', the source of the heat in hot chili peppers.

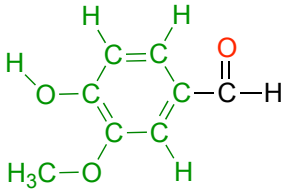


capsaicin

Composed of the four elements carbon, hydrogen, oxygen and nitrogen, capsaicin is produced by the pepper plant for the purpose of warding off hungry mammals. The molecule binds to and activates a mammalian receptor protein called TrpV1, which in normal circumstances has the job of detecting high temperatures and sending a signal to the brain - 'it's hot, stay away!' This strategy works quite well on all mammalian species except one: we humans (some of us, at least) appear to be alone in our tendency to actually seek out the burn of the hot pepper in our food.

Interestingly, birds also have a heat receptor protein which is very similar to the TrpV1 receptor in mammals, but birds are not at all sensitive to capsaicin. There is an evolutionary logic to this: it is to the pepper's advantage to be eaten by a bird rather than a mammal, because a bird can spread the pepper seeds over a much wider area. The region of the receptor which is responsible for capsaicin sensitivity appears to be quite specific - in 2002, scientists were able to insert a small segment of the (capsaicin-sensitive) rat TrpV1 receptor gene into the non-sensitive chicken version of the gene, and the resulting chimeric (mixed species) receptor was sensitive to capsaicin (*Cell* **2002**, *108*, 421).

Back at the North Hudson Pepperfest, those with a little more common sense are foregoing the painful effects of capsaicin overload and are instead indulging in more pleasant chemical phenomena. A little girl enjoying an ice cream cone is responding in part to the chemical action of another organic compound called vanillin.



vanillin

What is it about capsaicin and vanillin that causes these two compounds to have such dramatically different effects on our sensory perceptions? Both are produced by plants, and both are composed of the elements carbon, hydrogen, oxygen, and (in the case of

capsaicin) nitrogen. Since the birth of chemistry as a science, chemists have been fascinated - and for much of that history, mystified - by the myriad properties of compounds that come from living things. The term 'organic', from the Greek *organikos*, was applied to these compounds, and it was thought that they contained some kind of 'vital force' which set them apart from 'inorganic' compounds such as minerals, salts, and metals, and which allowed them to operate by a completely different set of chemical principles. How else but through the action of a 'vital force' could such a small subgroup of the elements combine to form compounds with so many different properties?

Today, as you are probably already aware, the term 'organic,' - when applied to chemistry - refers not just to molecules from living things, but to all compounds containing the element carbon, regardless of origin. Beginning early in the 19th century, as chemists learned through careful experimentation about the composition and properties of 'organic' compounds such as fatty acids, acetic acid and urea, and even figured out how to synthesize some of them starting with exclusively 'inorganic' components, they began to realize that the 'vital force' concept was not valid, and that the properties of both organic and inorganic molecules could in fact be understood using the same fundamental chemical principles.

They also began to more fully appreciate the unique features of the element carbon which makes it so central to the chemistry of living things, to the extent that it warrants its own subfield of chemistry. Carbon forms four stable bonds, either to other carbon atoms or to hydrogen, oxygen, nitrogen, sulfur, phosphorus, or a halogen. The characteristic bonding modes of carbon allow it to serve as a skeleton, or framework, for building large, complex molecules that incorporate chains, branches and ring structures.

Although 'organic chemistry' no longer means exclusively the study of compounds from living things, it is nonetheless the desire to understand and influence the chemistry of life that drives much of the work of organic chemists, whether the goal is to learn something fundamentally new about the reactivity of a carbon-oxygen bond, to discover a new laboratory method that could be used to synthesize a life-saving drug, or to better understand the intricate chemical dance that goes on in the active site of an enzyme or receptor protein. Although humans have been eating hot peppers and vanilla-flavored foods for centuries, we are just now, in the past few decades, beginning to understand how and why one causes searing pain, and the other pure gustatory pleasure. We understand that the precise geometric arrangement of the four elements in capsaicin allows it to fit inside the binding pocket of the TrpV1 heat receptor - but, as of today, we do not yet have a detailed three dimensional picture of the TrpVI protein bound to capsaicin. We also know that the different arrangement of carbon, hydrogen and oxygen atoms in vanillin allows it to bind to specific olfactory receptors, but again, there is much yet to be discovered about exactly how this happens.

In this chapter, you will be introduced to some of the most fundamental principles of organic chemistry. With the concepts we learn about, we can begin to understand how carbon and a very small number of other elements in the periodic table can combine in predictable ways to produce a virtually limitless chemical repertoire.

As you read through, you will recognize that the chapter contains a lot of review of topics you have probably learned already in an introductory chemistry course, but there will likely also be a few concepts that are new to you, as well as some topics which are already familiar to you but covered at a greater depth and with more of an emphasis on biologically relevant organic compounds.

We will begin with a reminder of how chemists depict bonding in organic molecules with the 'Lewis structure' drawing convention, focusing on the concept of 'formal charge'. We will review the common bonding patterns of the six elements necessary for all forms of life on earth - carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus - plus the halogens (fluorine, chlorine, bromine, and iodine). We'll then continue on with some of the basic skills involved in drawing and talking about organic molecules: understanding the 'line structure' drawing convention and other useful ways to abbreviate and simplify structural drawings, learning about functional groups and isomers, and looking at how to systematically name simple organic molecules. Finally, we'll bring it all together with a review of the structures of the most important classes of biological molecules - lipids, carbohydrates, proteins, and nucleic acids - which we will be referring to constantly throughout the rest of the book.

Before you continue any further in your reading, you should do some review of your own, because it will be assumed that you already understand some basic chemistry concepts. It would be a very good idea to go back to your introductory chemistry textbook and remind yourself about the following topics:

Topics to review from introductory chemistry

atomic structure: protons, neutrons, and electrons; atomic number, atomic mass and radius, isotopes
s and *p* orbitals, nodes
electron configuration of atoms, valence electrons
covalent vs ionic bonds
drawing Lewis structures, lone-pair (nonbonding) electrons, the octet rule

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Atomic structure, electron configuration, and Lewis structure review exercises

Exercise 1.1: How many neutrons do the following isotopes have?

a) ^{31}P, the most common isotope of phosphorus
b) ^{32}P, a radioactive isotope of phosphorus used often in the study of DNA and RNA.
c) ^{37}Cl, one of the two common isotopes of chlorine.
d) tritium (^{3}H), a radioactive isotope of hydrogen, used often by biochemists as a 'tracer' atom.
e) ^{14}C, a radioactive isotope of carbon, also used as a tracer in biochemistry.

Exercise 1.2: The electron configuration of a carbon atom is $1s^2 2s^2 2p^2$, and that of a sodium cation (Na^+) is $1s^2 2s^2 2p^6$. Show the electron configuration for

a) a nitrogen atom
b) an oxygen atom
c) a fluorine atom
d) a magnesium atom
e) a magnesium cation (Mg^{2+})
f) a potassium atom
g) a potassium ion (K^+)
h) a chloride anion (Cl^-)
i) a sulfur atom
j) a lithium cation (Li^+)
k) a calcium cation (Ca^{2+})

Exercise 1.3: Draw Lewis structures for the following species (use lines to denote bonds, dots for lone-pair electrons). All atoms should have a complete valence shell of electrons.

a) ammonia, NH_3
b) ammonium ion, NH_4^+
c) amide ion, NH_2^-
d) formaldehyde, HCOH
e) acetate ion, CH_3COO^-
f) methyl amine, CH_3NH_2
g) ethanol, CH_3CH_2OH
h) diethylether, $CH_3CH_2OCH_2CH_3$
i) cyclohexanol (molecular formula C_6H_6O, with six carbons bonded in a ring and an OH group)
j) propene, CH_2CHCH_3
k) pyruvate, CH_3COCO_2H

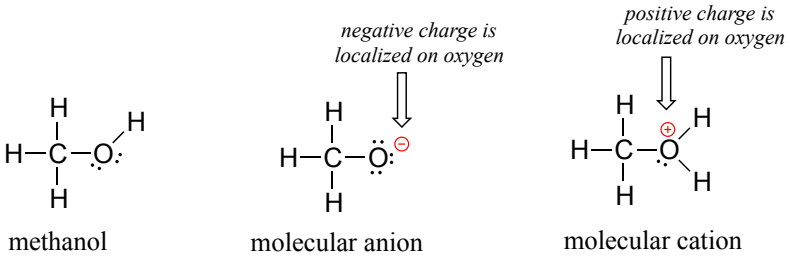
Section 1.1: Drawing organic structures

1.1A: Formal charges

Now that you have had a chance to go back to your introductory chemistry textbook to review some basic information about atoms, orbitals, bonds, and molecules, let's direct our attention a little more closely to the idea of charged species. You know that an **ion** is a molecule or atom that has an associated positive or negative charge. Copper, for example, can be found in both its neutral state (Cu^0, which is the metal), or in its Cu^{+2} state, as a component of an ionic compound like copper carbonate ($CuCO_3$), the green substance called 'patina' that forms on the surface of copper objects.

Organic molecules can also have positive or negative charges associated with them. Consider the Lewis structure of methanol, CH_3OH (methanol is the so-called 'wood

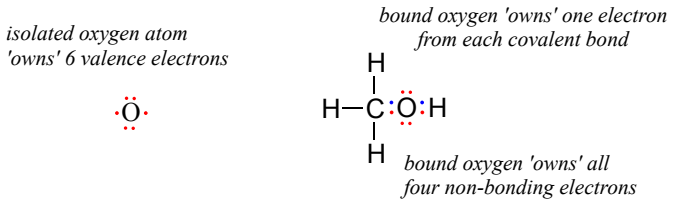
alcohol' that unscrupulous bootleggers sometimes sold during the prohibition days in the 1920's, often causing the people who drank it to go blind). Methanol itself is a neutral molecule, but can lose a proton to become a molecular anion (CH_3O^-), or gain a proton to become a molecular cation ($CH_3OH_2^{+)}$.



The molecular anion and cation have overall charges of -1 and +1, respectively. But we can be more specific than that - we can also state for each molecular ion that a **formal charge** is located specifically on the oxygen atom, rather than on the carbon or any of the hydrogen atoms.

Figuring out the formal charge on different atoms of a molecule is a straightforward process - it's simply a matter of adding up valence electrons.

A unbound oxygen atom has 6 valence electrons. When it is bound as part of a methanol molecule, however, an oxygen atom is surrounded by 8 valence electrons: 4 nonbonding electrons (two 'lone pairs') and 2 electrons in each of its two covalent bonds (one to carbon, one to hydrogen). In the formal charge convention, we say that the oxygen 'owns' all 4 nonbonding electrons. However, it only 'owns' one electron from each of the two covalent bonds, because covalent bonds involve the sharing of electrons between atoms. Therefore, the oxygen atom in methanol owns 2 + 2 + (½ x 4) = 6 valence electrons.



The formal charge on an atom is calculated as the number of valence electrons owned by the isolated atom minus the number of valence electrons owned by the bound atom in the molecule:

Determining the formal charge on an atom in a molecule:

formal charge =

(number of valence electrons owned by the isolated atom)
- (number of valence electrons owned by the bound atom)

or . . .

formal charge =

(number of valence electrons owned by the isolated atom)
- (number of non-bonding electrons on the bound atom)
- (½ the number of bonding electrons on the bound atom)

Using this formula for the oxygen atom of methanol, we have:

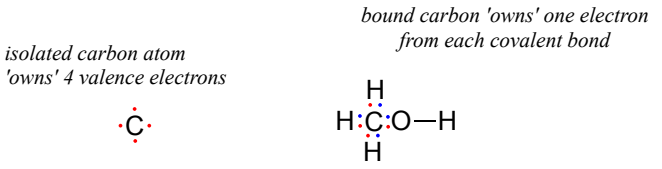
formal charge on oxygen =

(6 valence electrons on isolated atom)
- (4 non-bonding electrons)
- (½ x 4 bonding electrons)

= 6 - 4 - 2 = 0

Thus, oxygen in methanol has a formal charge of zero (in other words, it has *no formal charge*).

How about the carbon atom in methanol? An isolated carbon owns 4 valence electrons. The bound carbon in methanol owns (½ x 8) = 4 valence electrons:



formal charge on carbon =

(4 valence electron on isolated atom)
- (0 nonbonding electrons)
- (½ x 8 bonding electrons)

= 4 - 0 - 4 = 0

. . . so the formal charge on carbon is zero.

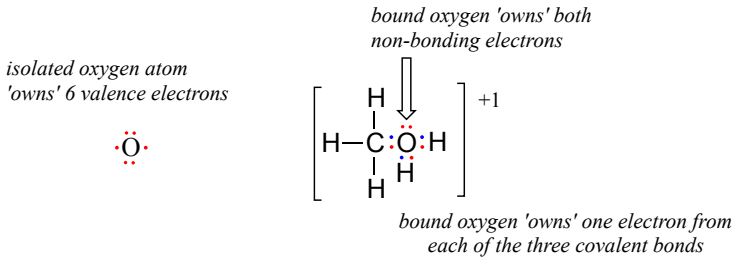
For each of the hydrogens in methanol, we also get a formal charge of zero:

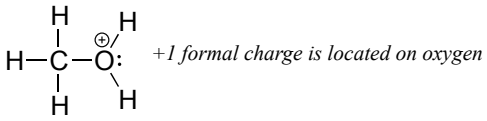
formal charge on hydrogen =

(1 valence electron on isolated atom)
- (0 nonbonding electrons)
- (½ x 2 bonding electrons)

= 1 - 0 - 1 = 0

Now, let's look at the cationic form of methanol, $CH_3OH_2^+$. The bonding picture has not changed for carbon or for any of the hydrogen atoms, so we will focus on the oxygen atom.





The oxygen owns 2 non-bonding electrons and 3 bonding elections, so the formal charge calculations becomes:

formal charge on oxygen =

(6 valence electrons in isolated atom)
- (2 non-bonding electrons)
- (½ x 6 bonding electrons)

= 6 - 2 - 3 = 1.

A formal charge of +1 is located on the oxygen atom.

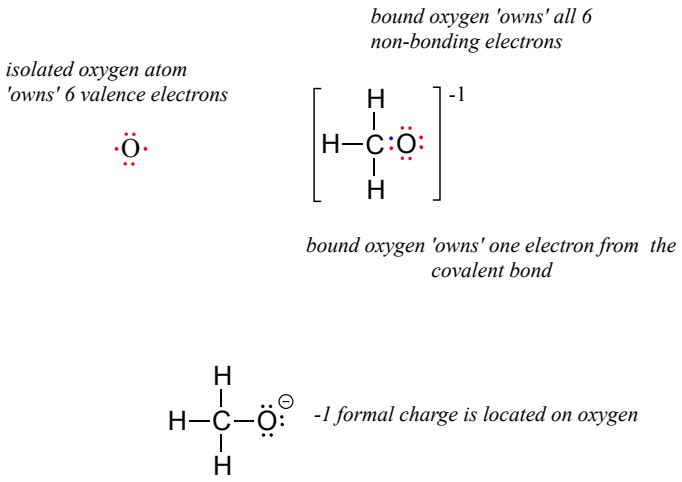
For methoxide, the anionic form of methanol, the calculation for the oxygen atom is:

formal charge on oxygen =

(6 valence electrons in isolated atom)
- (6 non-bonding electrons)
- (½ x 2 bonding electrons)

= 6 - 6 - 1 = -1

. . . so a formal charge of -1 is located on the oxygen atom.

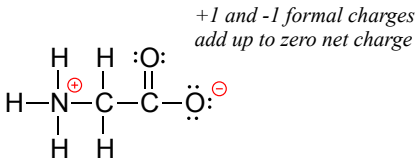


A very important rule to keep in mind is that *the sum of the formal charges on all atoms of a molecule must equal the net charge on the whole molecule.*

When drawing the structures of organic molecules, it is very important to show all non-zero formal charges, being clear about where the charges are located. *A structure that is missing non-zero formal charges is not correctly drawn, and will probably be marked as such on an exam!*

At this point, thinking back to what you learned in general chemistry, you are probably asking "What about dipoles? Doesn't an oxygen atom in an O-H bond 'own' more of the electron density than the hydrogen, because of its greater electronegativity?" This is absolutely correct, and we will be reviewing the concept of bond dipoles later on. For the purpose of calculating formal charges, however, bond dipoles don't matter - we always consider the two electrons in a bond to be shared equally, even if that is not an accurate reflection of chemical reality. Formal charges are just that - a formality, a method of electron book-keeping that is tied into the Lewis system for drawing the structures of organic compounds and ions. Later, we will see how the concept of formal charge can help us to visualize how organic molecules react.

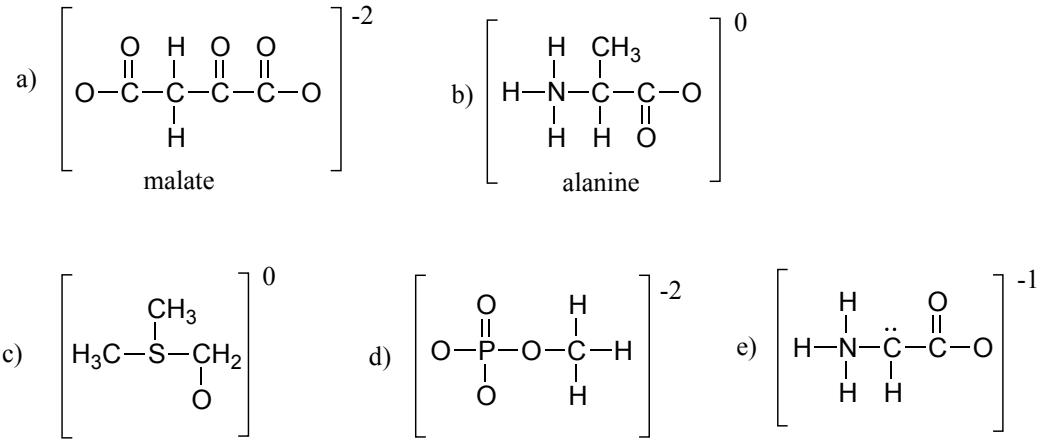
Finally, don't be lured into thinking that just because the net charge on a structure is zero there are no atoms with formal charges: one atom could have a positive formal charge and another a negative formal charge, and the net charge would still be zero.
Zwitterions, such as amino acids, have both positive and negative formal charges on different atoms:



glycine
(an amino acid)

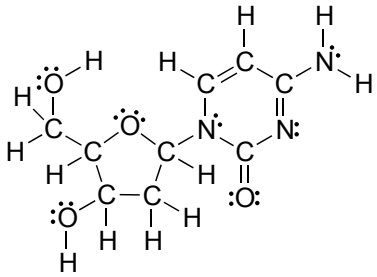
Even though the net charge on glycine is zero, it is still mandatory to show the location of the positive and negative formal charges.

Exercise 1.4: Fill in all missing lone pair electrons and formal charges in the structures below. Assume that all atoms have a complete valence shell of electrons. Net charges are shown outside the brackets.



1.1B: Common bonding patterns in organic structures

The electron-counting methods for drawing Lewis structures and determining formal charges on atoms are an essential starting point for a novice organic chemist, and work quite well when dealing with small, simple structures. But as you can imagine, these methods become unreasonably tedious and time-consuming when you start dealing with larger structures. It would be unrealistic, for example, to ask you to draw the Lewis structure below (of one of the four nucleoside building blocks that make up DNA) and determine all formal charges by adding up, on an atom-by-atom basis, all of the valence electrons.

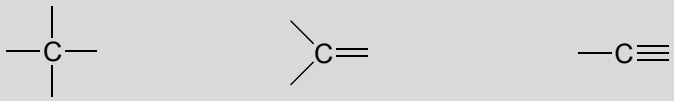


2'-deoxycytidine

And yet, as organic chemists, and especially as organic chemists dealing with biological molecules, you will be expected soon to draw the structures of large molecules on a regular basis. Clearly, you need to develop the ability to quickly and efficiently draw large structures and determine formal charges. Fortunately, this ability is not terribly hard to come by - all it takes is learning a few shortcuts and getting some practice at recognizing **common bonding patterns**.

Let's start with carbon, the most important element for organic chemists. *Carbon is **tetravalent**, meaning that it tends to form four bonds.* If you look again carefully at the structure of the DNA nucleoside 2'-deoxycytidine above, you should recognize that each carbon atom has four bonds, no lone pairs, and a formal charge of zero. Some of the carbon atoms have four single bonds, and some have one double bond and two single bonds. These are the two most common bonding patterns for carbon, along with a third option where carbon has one triple bond and one single bond.

Common bonding patterns for carbon



four single bonds
no lone pairs

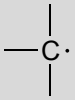
one double bond
two single bonds
no lone pairs

one triple bond
one single bond
no lone pairs

These three bonding patterns apply to most organic molecules, but there are exceptions. Here is a very important such exception: in carbon dioxide, the four bonds to the carbon atom take the form of two double bonds. (O=C=O).

Carbon is also sometimes seen with a formal charge of +1 (a **carbocation**) or -1 (a **carbanion**). Notice that a carbocation does not have a full octet of valence electrons.

Additional bonding patterns for carbon:
(high energy intermediate species)

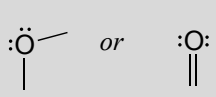
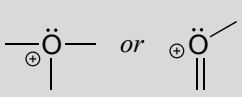
carbocation:	carbanion:	carbon radical:
		
three bonds no lone pairs positive charge	three bonds one lone pair negative charge	three bonds one lone pair neutral

Carbocations, carbanions, and carbon radicals are very high-energy (unstable) species and thus we do not expect to see them in the structure of a stable compound. However, they are important in organic chemistry because they often form as transient (short-lived) **intermediates** in reactions - they form, then very quickly change into something else. We will have much more to say about carbocation, carbanion, and radical intermediates in later chapters.

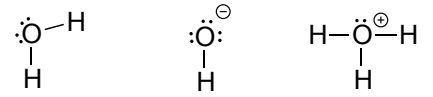
The bonding pattern for hydrogen atoms is easy: only one bond, no nonbonding electrons, and no formal charge. The exceptions to this rule are the proton, (H^+, just a single proton and no electrons) and the hydride ion, H^-, which is a proton plus two electrons. Because we are concentrating in this book on organic chemistry as applied to living things, however, we will not be seeing 'naked' protons and hydrides as such: they are far too reactive to be present in that form in aqueous solution. Nonetheless, the *idea* of a proton will be very important when we discuss acid-base chemistry, and the *idea* of a hydride ion will become very important much later in the book when we discuss organic oxidation and reduction reactions.

Let us next turn to oxygen atoms. Typically, you will see an oxygen bonding in one of three ways.

Common bonding patterns for oxygen:

zero formal charge:	-1 formal charge:	+1 formal charge:
		
two bonds two lone pairs	one bond three lone pairs	three bonds one lone pair

If oxygen has two bonds and two lone pairs, as in water, it will have a formal charge of zero. If it has one bond and three lone pairs, as in hydroxide ion, it will have a formal charge of -1. If it has three bonds and one lone pair, as in hydronium ion, it will have a formal charge of +1.

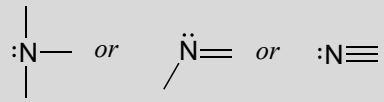


water hydroxide hydronium

Nitrogen has two major bonding patterns: three bonds and one lone pair, or four bonds and a positive formal charge.

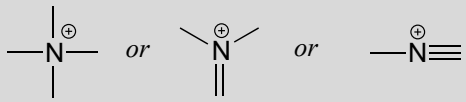
Common bonding patterns for nitrogen:

zero formal charge:



three bonds
one lone pair

+1 formal charge:



four bonds
no lone pairs

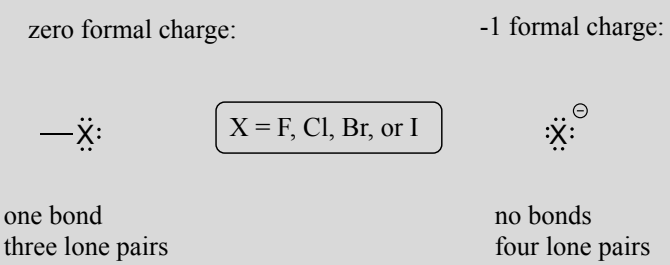
Nitrogen is occasionally also seen with two bonds, two lone pairs, and a negative formal charge - however, these species are extremely reactive and not very relevant to biological chemistry.

Two third row elements are commonly found in biological organic molecules: sulfur and phosphorus. Although both of these elements have other bonding patterns that are relevant in laboratory chemistry, in a biological context sulfur most commonly follows the same bonding/formal charge pattern as oxygen, while phosphorus is seen in a form in which it has five bonds (almost always to oxygen), no nonbonding electrons, and a formal charge of zero. Remember that elements in the third row of the periodic table have *d* orbitals in their valence shell as well as *s* and *p* orbitals, and thus the octet rule does not apply.

Common bonding pattern for phosphorus (phosphate)

Finally, the halogens (fluorine, chlorine, bromine, and iodine) are very important in laboratory and medicinal organic chemistry, but less common in naturally occurring organic molecules. Halogens in organic chemistry usually are seen with one bond, three lone pairs, and a formal charge of zero, or as ions in solution with no bonds, four lone pairs, and a negative formal charge.

Common bonding pattern for halogens:



These patterns, if learned and internalized so that you don't even need to think about them, will allow you to draw large organic structures, complete with formal charges, quite quickly.

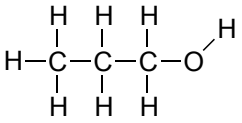
Once you have gotten the hang of drawing Lewis structures in this way, it is not always necessary to draw lone pairs on heteroatoms, as you can assume that the proper number of electrons are present around each atom to match the indicated formal charge (or lack thereof). Often, though, lone pairs are drawn, particularly on nitrogen, if doing so helps to make an explanation more clear.

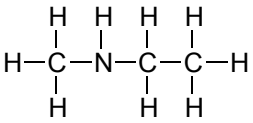
Exercise 1.5: Draw one structure that corresponds to each of the following molecular formulas, using the common bonding patters covered above. Be sure to include all lone pairs and formal charges where applicable, and assume that all atoms have a full valence shell of electrons. More than one correct answer is possible for each, so you will want to check your answers with your instructor or tutor.

a) $C_5H_{10}O$ b) C_5H_8O c) $C_6H_8NO^+$ d) $C_4H_3O_2^-$

1.3C: Using condensed structures and line structures

If you look ahead in this and other books at the way organic compounds are drawn, you will see that the figures are somewhat different from the Lewis structures you are used to seeing in your general chemistry book. In some sources, you will see **condensed structures** for smaller molecules instead of full Lewis structures:

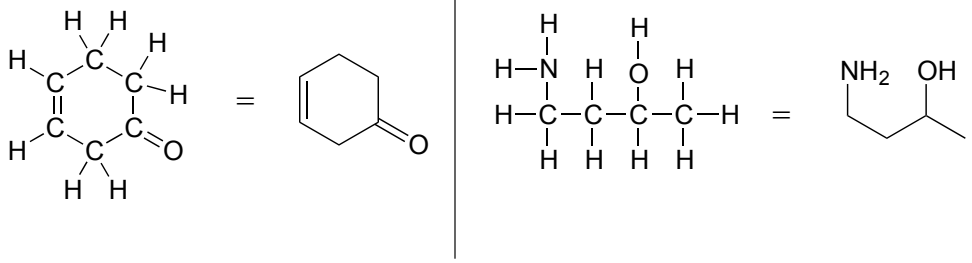




Lewis structures

$CH_3CH_2CH_2OH$ $CH_3NHCH_2CH_3$ Condensed structures

More commonly, organic and biological chemists use an abbreviated drawing convention called **line structures**. The convention is quite simple and makes it easier to draw molecules, but line structures do take a little bit of getting used to. Carbon atoms are depicted not by a capital C, but by a 'corner' between two bonds, or a free end of a bond. Open-chain molecules are usually drawn out in a 'zig-zig' shape. Hydrogens attached to carbons are generally not shown: rather, like lone pairs, they are simply implied (unless a positive formal charge is shown, all carbons are assumed to have a full octet of valence electrons). Hydrogens bonded to nitrogen, oxygen, sulfur, or anything other than carbon *are* shown, but are usually drawn without showing the bond. The following examples illustrate the convention.

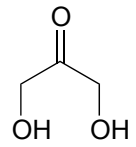


As you can see, the 'pared down' line structure makes it much easier to see the basic structure of the molecule and the locations where there is something other than C-C and C-H single bonds. For larger, more complex biological molecules, it becomes impractical to use full Lewis structures. Conversely, very small molecules such as ethane should be drawn with their full Lewis or condensed structures.

Sometimes, one or more carbon atoms in a line structure will be depicted with a capital C, if doing so makes an explanation easier to follow. *If you label a carbon with a C, you also must draw in the hydrogens for that carbon*.

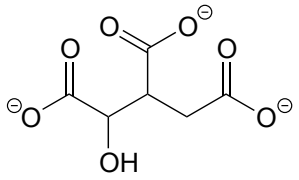
Exercise 1.6: A good way to test your understanding of the line structure convention is to determine the molecular formula of a molecule from its line structure. Give the molecular formula, (including overall charge, if any) of the molecules/ions below.

a)



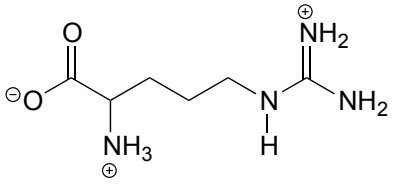
dihydroxyacetone

b)



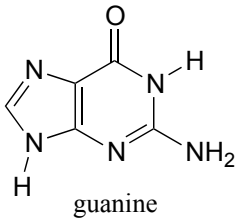
isocitrate

c)



arginine

d)

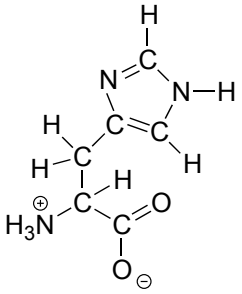


guanine

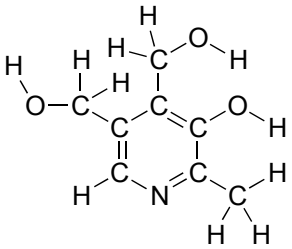
Exercise 1.7:

a) Draw a line structure for the DNA base 2-deoxycytidine (the full structure was shown earlier)

b) Draw line structures for histidine (an amino acid) and pyridoxine (Vitamin B_6).

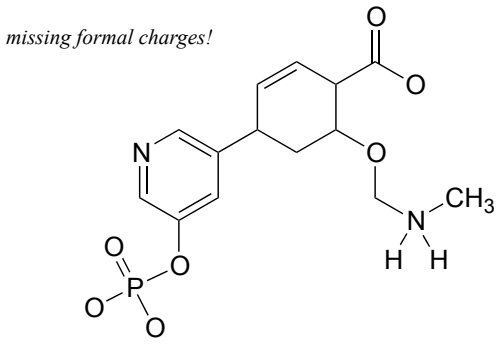


histidine



pyridoxine
(Vitamin B_6)

Exercise 1.8: Add lone pair electrons and non-zero formal charges to the structural drawing below:



Exercise 1.9: Find, anywhere in chapters 2-17 of this textbook, one example of each of the common bonding patterns specified below. Check your answers with your instructor or tutor.

a) carbon with one double bond, two single bonds, no lone pairs, and zero formal charge
b) oxygen with two single bonds, two lone pairs, and zero formal charge
c) oxygen with one double bond, two lone pairs, and zero formal charge
d) nitrogen with one double bond, two single bonds, and a +1 formal charge
e) oxygen with one single bond, three lone pairs, and a negative formal charge

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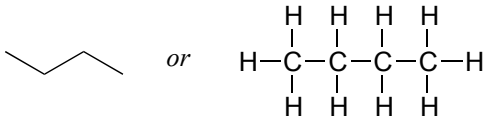
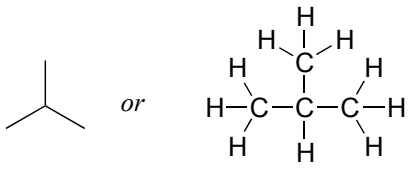
1.3D: Constitutional isomers

Imagine that you were asked to draw a structure for a compound with the molecular formula C_4H_{10}. This would not be difficult - you could simply draw:



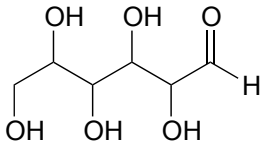
But when you compared your answer with that of a classmate, she may have drawn this structure:



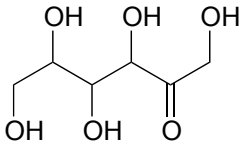
Who is correct? The answer, of course, is that both of you are. A molecular formula only tells you *how many* atoms of each element are present in the compound, not what the actual atom-to-atom connectivity is. There are often many different possible structures for one molecular formula. Compounds that have the same molecular formula but

different connectivity are called **constitutional isomers** (sometimes the term '**structural isomer**' is also used). The Greek term '*iso'* means 'same'.

Fructose and glucose are constitutional isomers with the molecular formula $C_6H_{12}O_6$.



glucose



fructose

Exercise 1.10: Draw a constitutional isomer of ethanol, CH_3CH_2OH.

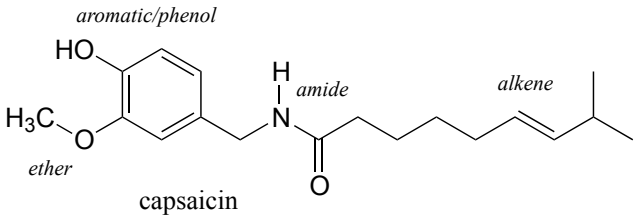
Exercise 1.11: Draw all of the possible constitutional isomers with the given molecular formula.

a) C_5H_{12}
b) C_4H_{10}
c) C_3H_9N

Section 1.2: Functional groups and organic nomenclature

1.4A: Functional groups in organic compounds

Functional groups are structural units within organic compounds that are defined by specific bonding arrangements between specific atoms. The structure of capsaicin, the compound discussed in the beginning of this chapter, incorporates several functional groups, labeled in the figure below and explained throughout this section.

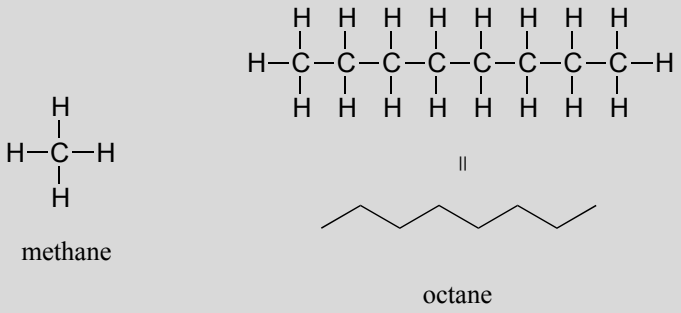


capsaicin

As we progress in our study of organic chemistry, it will become extremely important to be able to quickly recognize the most common functional groups, because *they are the key structural elements that define how organic molecules react*. For now, we will only worry about drawing and recognizing each functional group, as depicted by Lewis and line structures. Much of the remainder of your study of organic chemistry will be taken up with learning about how the different functional groups behave in organic reactions.

The 'default' in organic chemistry (essentially, the *lack* of any functional groups) is given the term **alkane**, characterized by single bonds between carbon and carbon, or between carbon and hydrogen. Methane, CH_4, is the natural gas you may burn in your furnace. Octane, C_8H_{18}, is a component of gasoline.

Alkanes

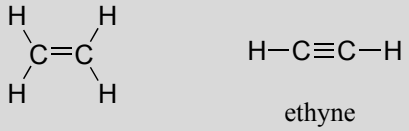


methane

octane

Alkenes (sometimes called **olefins**) have carbon-carbon double bonds, and **alkynes** have carbon-carbon triple bonds. Ethene, the simplest alkene example, is a gas that serves as a cellular signal in fruits to stimulate ripening. (If you want bananas to ripen quickly, put them in a paper bag along with an apple - the apple emits ethene gas, setting off the ripening process in the bananas). Ethyne, commonly called acetylene, is used as a fuel in welding blow torches.

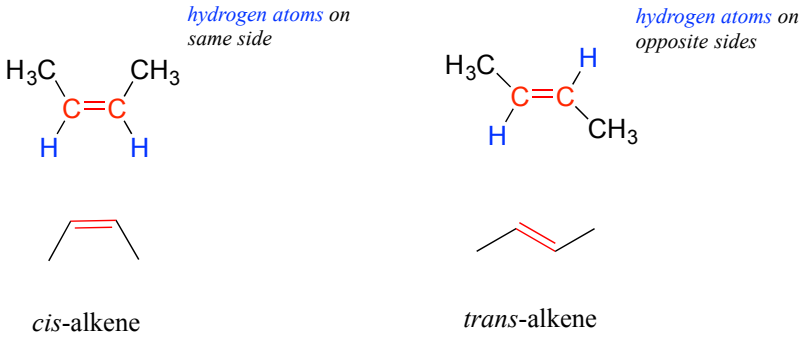
Alkenes and alkynes



ethene
(an alkene)

ethyne
(an alkyne)

In chapter 2, we will study the nature of the bonding on alkenes and alkynes, and learn that that the bonding in alkenes is trigonal planar in in alkynes is linear. Furthermore, many alkenes can take two geometric forms: *cis* or *trans*. The *cis* and *trans* forms of a given alkene are different molecules with different physical properties because, as we will learn in chapter 2, there is a very high energy barrier to rotation about a double bond. In the example below, the difference between *cis* and *trans* alkenes is readily apparent.



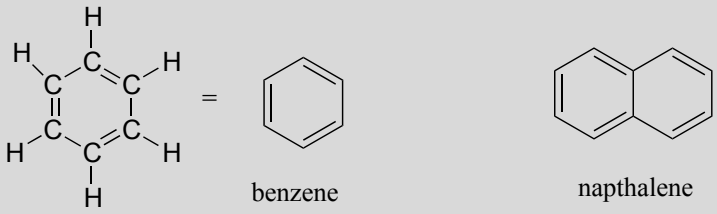
cis-alkene *trans*-alkene

We will have more to say about the subject of *cis* and *trans* alkenes in chapter 3, and we will learn much more about the reactivity of alkenes in chapter 14.

Alkanes, alkenes, and alkynes are all classified as **hydrocarbons**, because they are composed solely of carbon and hydrogen atoms. Alkanes are said to be **saturated hydrocarbons**, because the carbons are bonded to the maximum possible number of hydrogens - in other words, they are *saturated* with hydrogen atoms. The double and triple-bonded carbons in alkenes and alkynes have fewer hydrogen atoms bonded to them - they are thus referred to as **unsaturated hydrocarbons**. As we will see in chapter 15, hydrogen can be added to double and triple bonds, in a type of reaction called 'hydrogenation'.

The **aromatic** group is exemplified by benzene (which used to be a commonly used solvent on the organic lab, but which was shown to be carcinogenic), and naphthalene, a compound with a distinctive 'mothball' smell. Aromatic groups are planar (flat) ring structures, and are widespread in nature. We will learn more about the structure and reactions of aromatic groups in chapters 2 and 14.

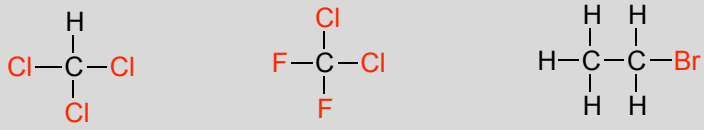
aromatics



benzene napthalene

When the carbon of an alkane is bonded to one or more halogens, the group is referred to as a **alkyl halide** or **haloalkane**. Chloroform is a useful solvent in the laboratory, and was one of the earlier anesthetic drugs used in surgery. Chlorodifluoromethane was used as a refrigerant and in aerosol sprays until the late twentieth century, but its use was discontinued after it was found to have harmful effects on the ozone layer. Bromoethane is a simple alkyl halide often used in organic synthesis. Alkyl halides groups are quite rare in biomolecules.

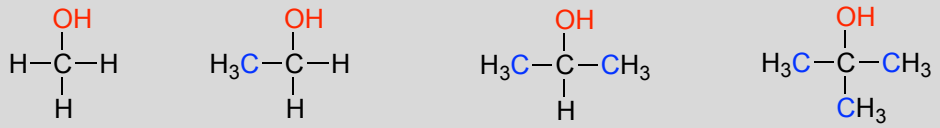
alkyl halides



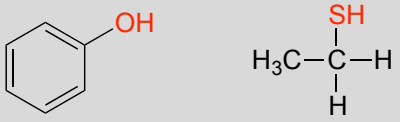
trichloromethane (chloroform) | dichlorodifluoromethane (Freon-12) | bromoethane

In the **alcohol** functional group, a carbon is single-bonded to an OH group (the OH group, by itself, is referred to as a **hydroxyl**). Except for methanol, all alcohols can be classified as primary, secondary, or tertiary. In a **primary alcohol**, the carbon bonded to the OH group is also bonded to only one other carbon. In a **secondary alcohol** and **tertiary alcohol,** the carbon is bonded to two or three other carbons, respectively. When the hydroxyl group is *directly* attached to an aromatic ring, the resulting group is called a **phenol**. The sulfur analog of an alcohol is called a **thiol** (from the Greek *thio*, for sulfur).

alcohols, phenols, and thiols



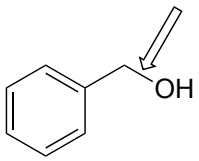
methanol | primary alcohol | secondary alcohol | tertiary alcohol



phenol | thiol

Note that the definition of a phenol states that the hydroxyl oxygen must be *directly* attached to one of the carbons of the aromatic ring. The compound below, therefore, is *not* a phenol - it is a primary alcohol.

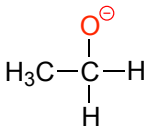
hydroxyl is not attached to carbon in aromatic ring



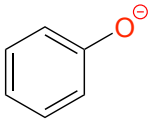
primary alcohol
(*not* a phenol)

The distinction is important, because as we will see later, there is a significant difference in the reactivity of alcohols and phenols.

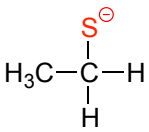
The deprotonated forms of alcohols, phenols, and thiols are called **alkoxides**, **phenolates**, and **thiolates**, respectively. A protonated alcohol is an **oxonium** ion.



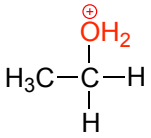
alkoxide



phenolate



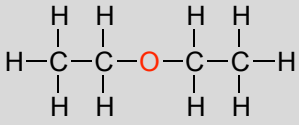
thiolate

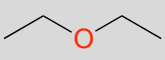


an oxonium

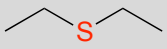
In an **ether** functional group, a central oxygen is bonded to two carbons. Below is the structure of diethyl ether, a common laboratory solvent and also one of the first compounds to be used as an anesthetic during operations. The sulfur analog of an ether is called a **thioether** or **sulfide**.

ethers and sulfides





an ether

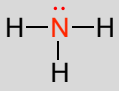


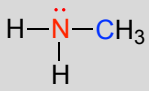
a sulfide

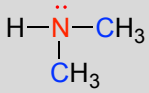
Amines are characterized by nitrogen atoms with single bonds to hydrogen and carbon. Just as there are primary, secondary, and tertiary alcohols, there are primary, secondary, and tertiary amines. Ammonia is a special case with no carbon atoms.

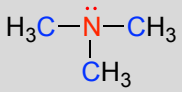
One of the most important properties of amines is that they are basic, and are readily protonated to form **ammonium** cations. In the case where a nitrogen has four bonds to carbon (which is somewhat unusual in biomolecules), it is called a quaternary ammonium ion.

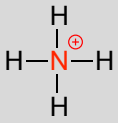
amines

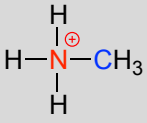

ammonia

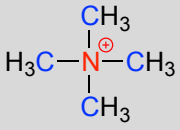

a primary amine


a secondary amine


a tertiary amine

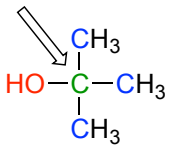

ammonium ion


a primary ammonium ion

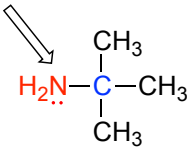

a quaternary ammonium ion

Note: Do not be confused by how the terms 'primary', 'secondary', and 'tertiary' are applied to alcohols and amines - the definitions are different. In alcohols, what matters is how many other carbons the alcohol *carbon* is bonded to, while in amines, what matters is how many carbons the *nitrogen* is bonded to.

***carbon** is bonded to **three** carbons*

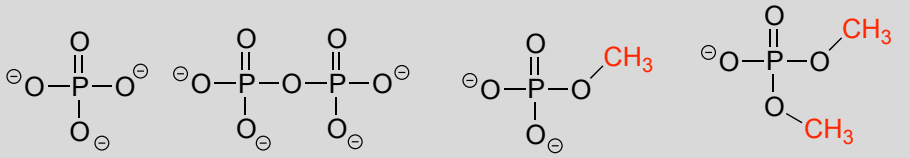

a *tertiary* alcohol

***nitrogen** is bonded to **one** carbon*


a *primary* amine

Phosphate and its derivative functional groups are ubiquitous in biomolecules. Phosphate linked to a single organic group is called a **phosphate ester**; when it has two links to organic groups it is called a **phosphate diester**. A linkage between two phosphates creates a **phosphate anhydride**.

phosphate functional groups



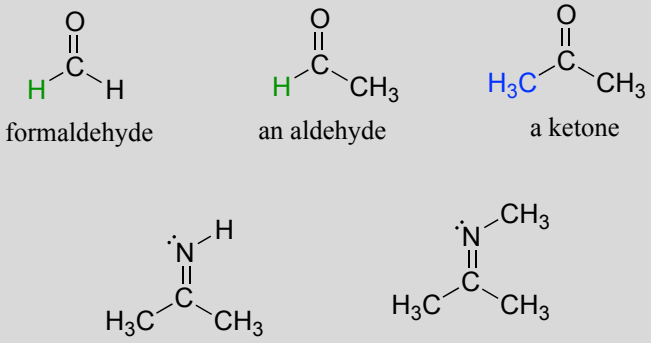
phosphate | a phosphate anhydride | a phosphate ester | a phosphate diester

Chapter 9 of this book is devoted to the structure and reactivity of the phosphate group.

There are a number of functional groups that contain a carbon-oxygen double bond, which is commonly referred to as a **carbonyl**. **Ketones** and **aldehydes** are two closely related carbonyl-based functional groups that react in very similar ways. In a ketone, the carbon atom of a carbonyl is bonded to two other carbons. In an aldehyde, the carbonyl carbon is bonded on one side to a hydrogen, and on the other side to a carbon. The exception to this definition is formaldehyde, in which the carbonyl carbon has bonds to two hydrogens.

A group with a carbon-nitrogen double bond is called an **imine**, or sometimes a **Schiff base** (in this book we will use the term 'imine'). The chemistry of aldehydes, ketones, and imines will be covered in chapter 10.

aldehydes, ketones, and imines



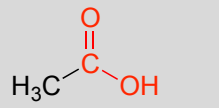
formaldehyde | an aldehyde | a ketone

imines

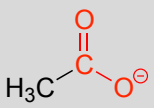
When a carbonyl carbon is bonded on one side to a carbon (or hydrogen) and on the other side to an oxygen, nitrogen, or sulfur, the functional group is considered to be one of the '**carboxylic acid derivatives**', a designation that describes a set of related functional groups. The eponymous member of this family is the **carboxylic acid** functional group, in which the carbonyl is bonded to a hydroxyl group. The conjugate base of a carboxylic

acid is a **carboxylate**. Other derivatives are carboxylic esters (usually just called **'esters'**), **thioesters**, **amides**, **acyl phosphates**, **acid chlorides**, and **acid anhydrides**. With the exception of acid chlorides and acid anhydrides, the carboxylic acid derivatives are very common in biological molecules and/or metabolic pathways, and their structure and reactivity will be discussed in detail in chapter 11.

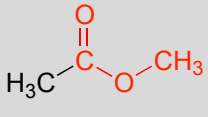
Carboxylic acid derivatives



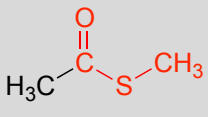
a carboxylic acid



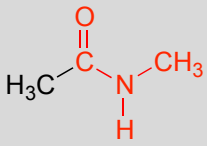
a carboxylate ion



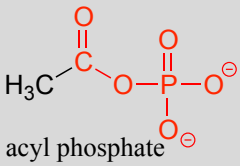
a carboxylic ester



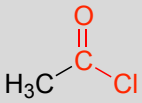
a thioester



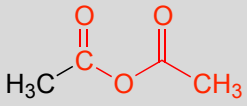
an amide



an acyl phosphate

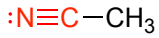


an acid chloride



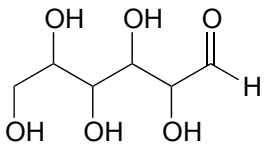
an acid anhydride

Finally, a **nitrile** group is characterized by a carbon triple-bonded to a nitrogen.

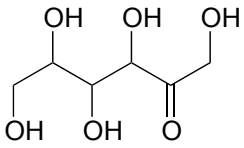


a nitrile

A single compound often contains several functional groups, particularly in biological organic chemistry. The six-carbon sugar molecules glucose and fructose, for example, contain aldehyde and ketone groups, respectively, and both contain five alcohol groups (a compound with several alcohol groups is often referred to as a '**polyol**').

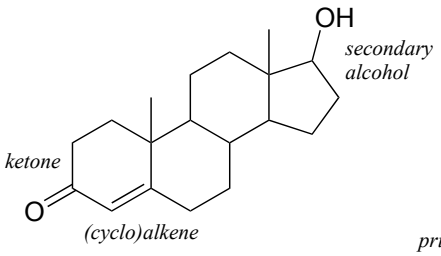


glucose

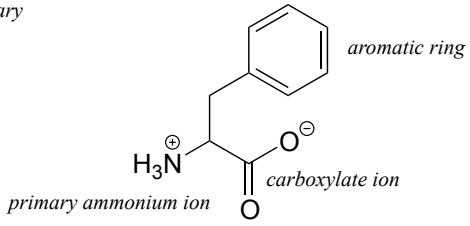


fructose

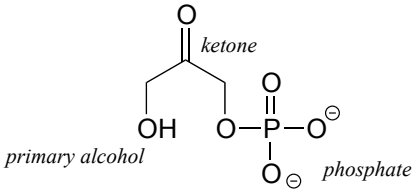
The hormone testosterone, the amino acid phenylalanine, and the glycolysis metabolite dihydroxyacetone phosphate all contain multiple functional groups, as labeled below.



testosterone



phenylalanine

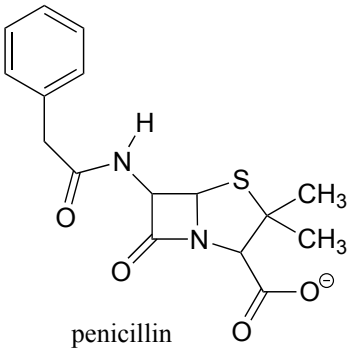


dihydroxyacetone phosphate
(DHAP)

While not in any way a complete list, this section has covered most of the important functional groups that we will encounter in biological organic chemistry. Table 9 in the tables section at the back of this book provides a summary of all of the groups listed in this section, plus a few more that will be introduced later in the text.

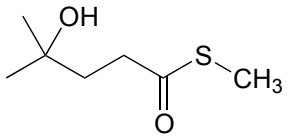
Exercise 1.12: Identify the functional groups (other than alkanes) in the following organic compounds. State whether alcohols and amines are primary, secondary, or tertiary.

a)

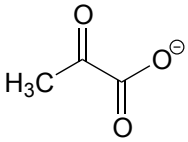


penicillin

b)

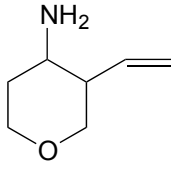


c)



pyruvate

d)



Exercise 1.13: Draw one example each of compounds fitting the descriptions below, using line structures. Be sure to designate the location of all non-zero formal charges. All atoms should have complete octets (phosphorus may exceed the octet rule). There are many possible correct answers for these, so be sure to check your structures with your instructor or tutor.

a) a compound with molecular formula $C_6H_{11}NO$ that includes alkene, secondary amine, and primary alcohol functional groups

b) an ion with molecular formula $C_3H_5O_6P^{2-}$ that includes aldehyde, secondary alcohol, and phosphate functional groups.

c) A compound with molecular formula C_6H_9NO that has an amide functional group, and does *not* have an alkene group.

1.2B: Naming organic compounds

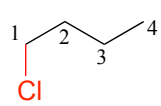
A system has been devised by the International Union of Pure and Applied Chemistry (IUPAC, usually pronounced ***eye-you-pack***) for naming organic compounds. While the IUPAC system is convenient for naming relatively small, simple organic compounds, it is not generally used in the naming of biomolecules, which tend to be quite large and complex. It is, however, a good idea (even for biologists) to become familiar with the basic structure of the IUPAC system, and be able to draw simple structures based on their IUPAC names.

Naming an organic compound usually begins with identify what is referred to as the **'parent chain'**, which is the longest straight chain of carbon atoms. We'll start with the simplest straight chain alkane structures. CH_4 is called **methane**, and C_2H_6 **ethane**. The table below continues with the names of longer straight-chain alkanes: be sure to commit these to memory, as they are the basis for the rest of the IUPAC nomenclature system (and are widely used in naming biomolecules as well).

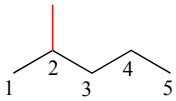
Names for straight-chain alkanes:

Number of carbons	Name
1	methane
2	ethane
3	propane
4	butane
5	pentane
6	hexane
7	heptane
8	octane
9	nonane
10	decane

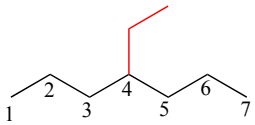
Substituents branching from the main parent chain are located by a carbon number, with *the lowest possible numbers* being used (for example, notice in the example below that the compound on the left is named 1-chlorobutane, *not* 4-chlorobutane). When the substituents are small alkyl groups, the terms **methyl**, **ethyl**, and **propyl** are used.



1-chlorobutane

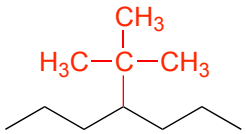


2-methylpentane

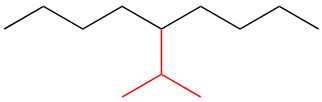


4-ethylheptane

Other common names for hydrocarbon substituent groups **isopropyl,** ***tert*-butyl** and **phenyl**.

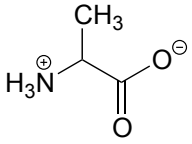


4-*tert*-butylheptane

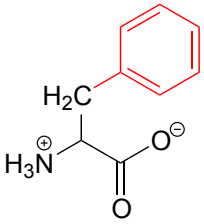


5-isopropylnonane

(non-IUPAC example):

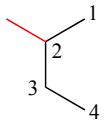


alanine
(an amino acid)

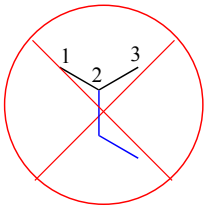


phenylalanine
(an amino acid)

Notice in the example below, an 'ethyl group' (in blue) is not treated as a substituent, rather it is included as part of the parent chain, and the methyl group is treated as a substituent. The IUPAC name for straight-chain hydrocarbons is always based on the *longest possible parent chain*, which in this case is four carbons, not three.



2-methylbutane



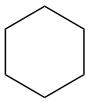
not 2-ethylpropane

Cyclic alkanes are called cyclopropane, cyclobutane, cyclopentane, cyclohexane, and so on.

cyclopropane

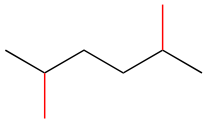
cyclobutane

cyclopentane

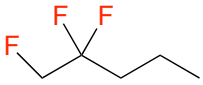


cyclohexane

In the case of multiple substituents, the prefixes *di*, *tri*, and *tetra* are used.

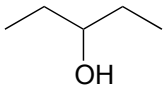


2,5-dimethylhexane

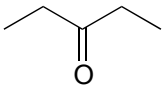


1,2,2,-trifluoropentane

Functional groups have characteristic suffixes. Alcohols, for example, have ‘ol’ appended to the parent chain name, along with a number designating the location of the hydroxyl group. Ketones are designated by ‘one’.

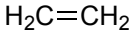


3-pentanol

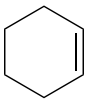


3-pentanone

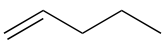
Alkenes are designated with an 'ene' ending, and when necessary the location and geometry of the double bond are indicated. Compounds with multiple double bonds are called dienes, trienes, etc.



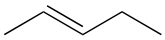
ethene



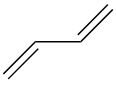
cyclohexene



1-pentene

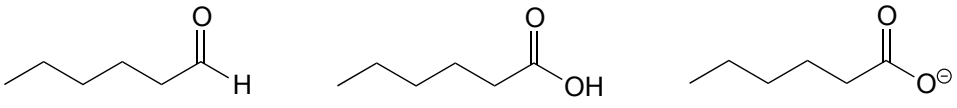


trans-2-pentene



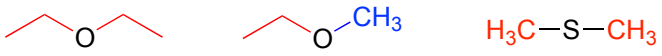
1,3-butadiene

Some groups can only be present on a terminal carbon, and thus a locating number is not necessary: aldehydes end in ‘al’, carboxylic acids in ‘oic acid’, and carboxylates in ‘oate’.



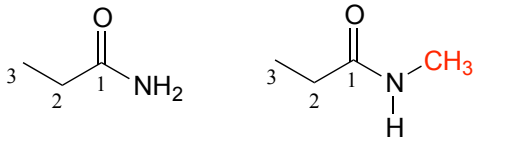
hexanal hexanoic acid hexanoate

Ethers and sulfides are designated by naming the two groups on either side of the oxygen or sulfur.



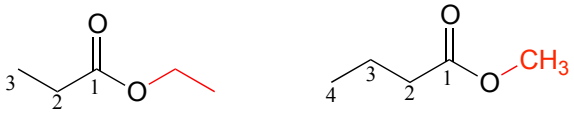
diethyl ether ethyl methyl ether dimethyl sulfide

If an amide has an unsubstituted $–NH_2$ group, the suffix is simply 'amide'. In the case of a substituted amide, the group attached to the amide nitrogen is named first, along with the letter '*N*' to clarify where this group is located. Note that the structures below are both based on a three-carbon (propan) parent chain.



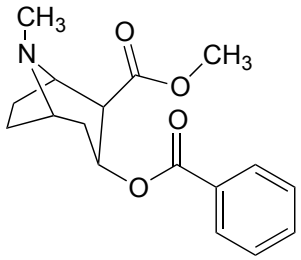
propanamide *N*-methylpropanamide

For esters, the suffix is 'oate'. The group attached to the oxygen is named first.



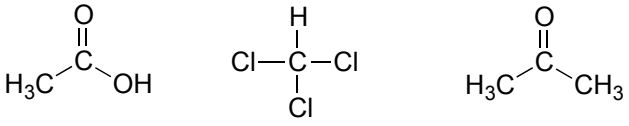
ethyl propanoate methyl butanoate

All of the examples we have seen so far have been simple in the sense that only one functional group was present on each molecule. There are of course many more rules in the IUPAC system, and as you can imagine, the IUPAC naming of larger molecules with multiple functional groups, ring structures, and substituents can get very unwieldy very quickly. The illicit drug cocaine, for example, has the IUPAC name 'methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate' (this name includes designations for stereochemistry, which is a structural issue that we will not tackle until chapter 3).



cocaine
(methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate)

You can see why the IUPAC system is not used very much in biological organic chemistry - the molecules are just too big and complex. A further complication is that, even outside of a biological context, many simple organic molecules are known almost universally by their 'common', rather than IUPAC names. The compounds acetic acid, chloroform, and acetone are only a few examples.



acetic acid chloroform acetone

In biochemistry, nonsystematic names (like 'cocaine', 'capsaicin', 'pyruvate' or 'ascorbic acid') are usually used, and when systematic nomenclature is employed it is often specific to the class of molecule in question: different systems have evolved, for example, for fats and for carbohydrates. We will not focus very intensively in this text on IUPAC nomenclature or any other nomenclature system, but if you undertake a more advanced study in organic or biological chemistry you may be expected to learn one or more naming systems in some detail.

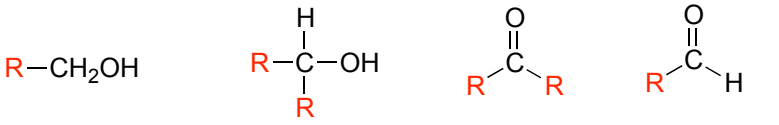
Exercise 1.14: Give IUPAC names for acetic acid, chloroform, and acetone.

Exercise 1.15: Draw line structures of the following compounds, based on what you have learned about the IUPAC nomenclature system:

a) methylcyclohexane
b) 5-methyl-1-hexanol
c) 2-methyl-2-butene
d) 5-chloropentanal
e) 2,2-dimethylcyclohexanone
f) 4-penteneoic acid
g) *N*-ethyl-*N*-cyclopentylbutanamide

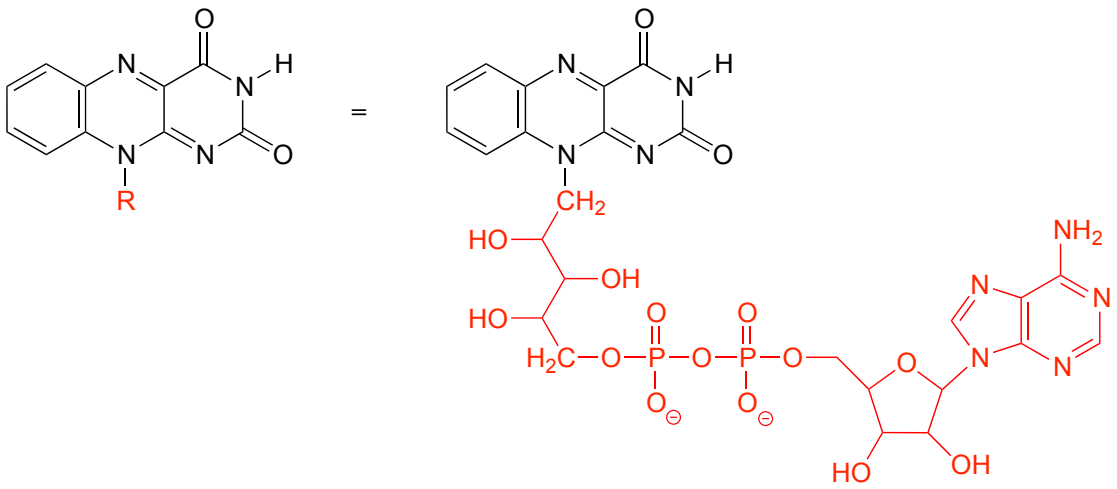
1.2C: Abbreviated organic structures

Often when drawing organic structures, chemists find it convenient to use the letter 'R' to designate part of a molecule outside of the region of interest. If we just want to refer in general to a functional group without drawing a specific molecule, for example, we can use 'R groups' to focus attention on the group of interest:



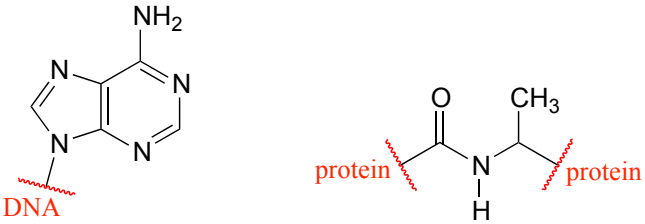
a primary alcohol a secondary alcohol a ketone an aldehyde

The R group is a convenient way to abbreviate the structures of large biological molecules, especially when we are interested in something that is occurring specifically at one location on the molecule. For example, in chapter 15 when we look at biochemical oxidation-reduction reactions involving the flavin molecule, we will abbreviate a large part of the flavin structure which does not change at all in the reactions of interest:



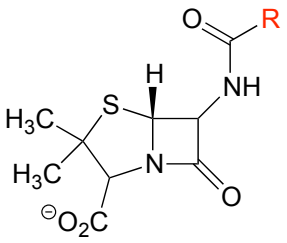
flavin adenine dinucleotide (FAD)

As an alternative, we can use a 'break' symbol to indicate that we are looking at a small piece or section of a larger molecule. This is used commonly in the context of drawing groups on large polymers such as proteins or DNA.

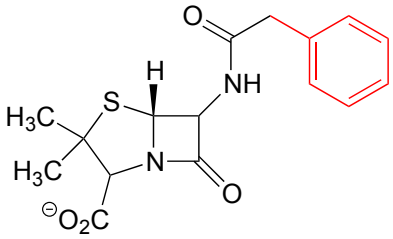


an adenine base in DNA a small section of a protein

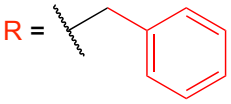
Finally, R groups can be used to concisely illustrate a series of related compounds, such as the family of penicillin-based antibiotics.



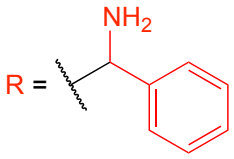
penicillin framework



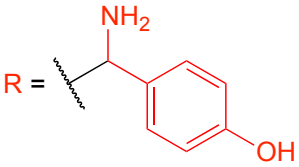
benzyl penicillin



benzyl penicillin

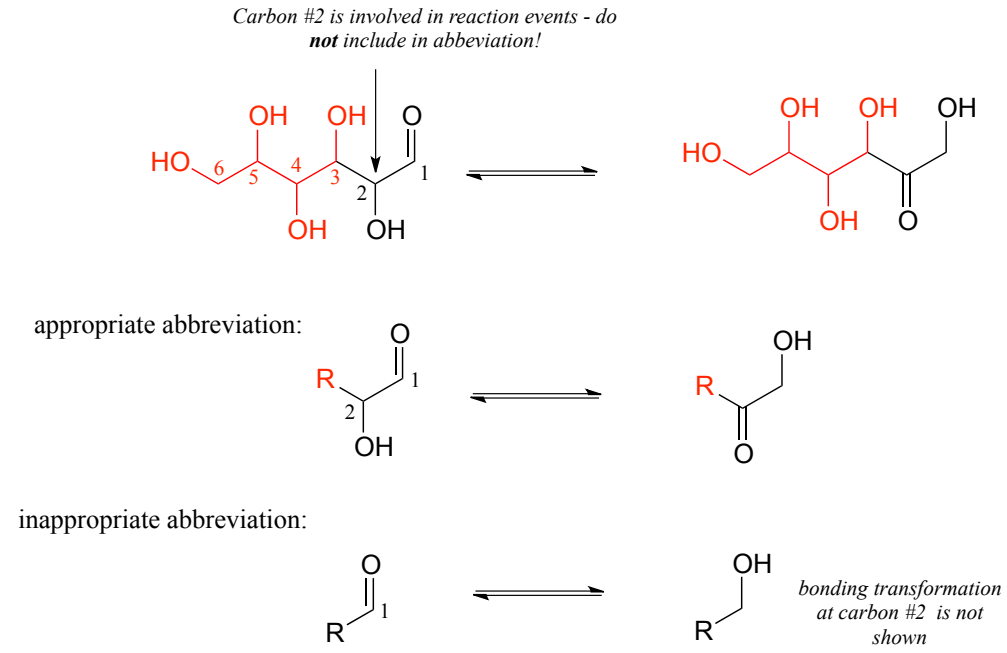


ampicillin



amoxicillin

Using abbreviations appropriately is a very important skill to develop when studying organic chemistry in a biological context, because although many biomolecules are very large and complex (and take forever to draw!), usually we are focusing on just one small part of the molecule where a change is taking place. As a rule, *you should never abbreviate any atom involved in a bond-breaking or bond-forming event that is being illustrated:* only abbreviate that part of the molecule which is not involved in the reaction of interest. For example, carbon #2 in the reactant/product below most definitely is involved in bonding changes, and therefore should not be included in the 'R' group.

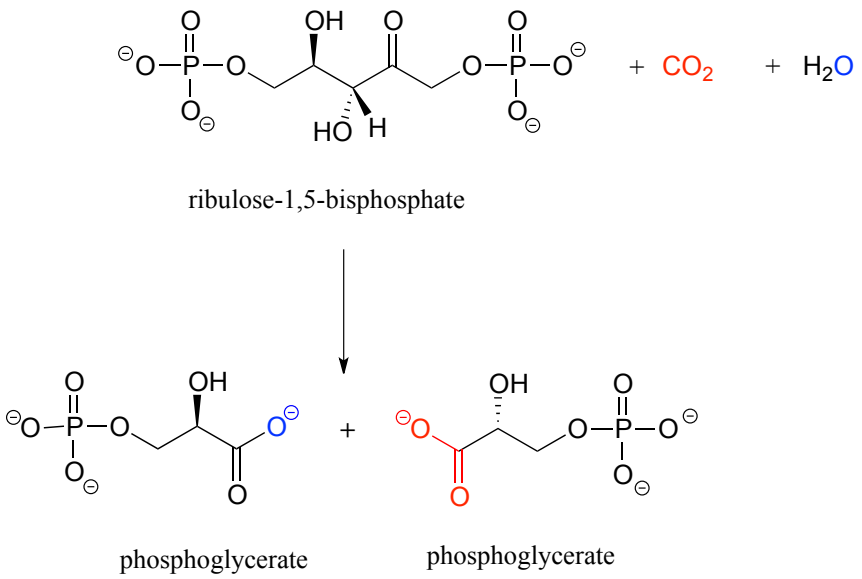


If you are unsure whether to draw out part of a structure or abbreviate it, the safest thing to do is to draw it out.

Exercise 1.16:

a) If you intend to draw out the chemical details of a reaction in which the methyl ester functional group of cocaine (see earlier figure) was converted to a carboxylate plus methanol, what would be an appropriate abbreviation to use for the cocaine structure (assuming that you *only* wanted to discuss the chemistry specifically occurring at the ester group)?

b) Below is the (somewhat complicated) reaction catalyzed by an enzyme known as 'Rubisco', by which plants 'fix' carbon dioxide. Carbon dioxide and the oxygen of water are colored red and blue respectively to help you see where those atoms are incorporated into the products. Propose an appropriate abbreviation for the starting compound (ribulose 1,5-bisphosphate), using two different 'R' groups, R_1 and R_2.



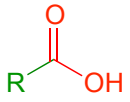
Section 1.3: Structures of important classes of biological molecules

Because we are focusing in this textbook on biologically relevant organic chemistry, we will frequently be alluding to important classes of biological molecules such as lipids, carbohydrates, proteins, and nucleic acids (DNA and RNA). Now is a good time to go through a quick overview of what these molecules look like. These are large, complex molecules and there is a *lot* of information here: you are not expected to memorize these structures or even, at this point, to fully understand everything presented in this section. For now, just read through and get what you can out of it, and work on recognizing the fundamental things you have just learned: common bonding patterns, formal charges, functional groups, and so forth. Later, you can come back to this section for review when these biomolecules are referred to in different contexts throughout the remainder of the book.

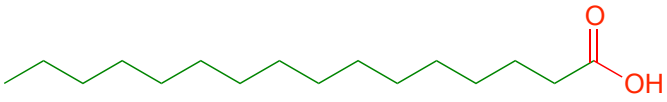
1.3A: Lipids

We'll start a large subgroup of a class of biomolecules called **lipids**, which includes fats, oils, waxes, and isoprenoid compounds such as cholesterol.

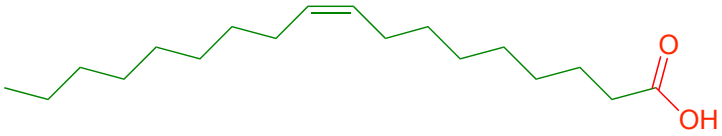
Fatty acids are composed of hydrocarbon chains terminating in a carboxylic acid/carboxylate group (we will learn in Chapter 7 that carboxylic acids are predominantly in their anionic, carboxylate form in biological environments). Saturated fatty acids contain only alkane carbons (single bonds only), mononsaturated fatty acids contain a single double bond, and polyunsaturated fatty acids contain two or more double bonds. The double bonds in naturally occurring fatty acids are predominantly in the *cis* configuration.



fatty acid

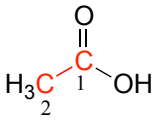


palmitic acid
(a 16-carbon saturated fatty acid)

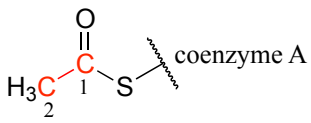


oleic acid
(an 18-carbon monounsaturated fatty acid)

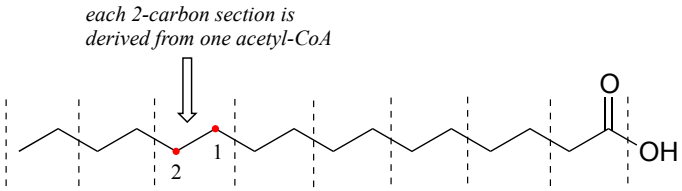
Fatty acids are synthesized in the body by a process in which the hydrocarbon chain is elongated two carbons at a time. Each two-carbon unit is derived from a metabolic intermediate called **acetyl-coA**, which is essentially an acetic acid (vinegar) molecule linked to a large 'carrier' molecule, called coenzyme A, by a thioester functional group. We will see much more of coenzyme A when we study the chemistry of thioesters in chapter 11.



acetic acid

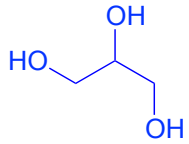


acetyl-coA

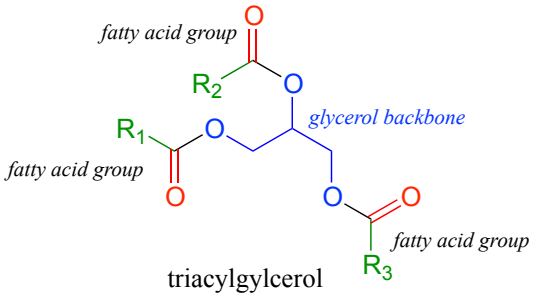


The breakdown of fatty acids in the body also occurs two carbons at a time, and the endpoint is again acetyl-coenzyme A. We will learn about the details of all of the reactions in these metabolic pathways at various places in this book. If you go on to take a biochemistry course, you will learn more about the big picture of fatty acid metabolism - how it is regulated, and how is fits together with other pathways of central metabolism.

Fats and oils are forms of **triacylglycerol**, a molecule composed of a glycerol backbone with three fatty acids linked by ester functional groups.



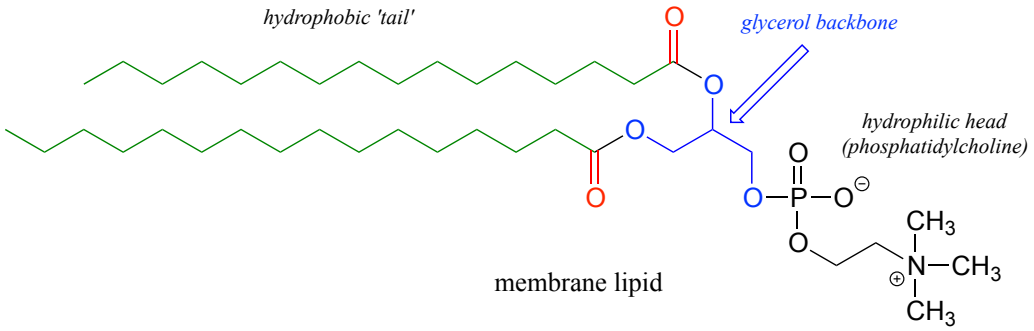
glycerol



triacylgylcerol

Solid fats (predominant in animals) are triacylglycerols with long (16-18 carbon) saturated fatty acids. Liquid oils (predominant in plants) have unsaturated fatty acids, sometimes with shorter hydrocarbon chains. In chapter 2 we will learn about how chain length and degree of unsaturation influences the physical properties of fats and oils.

Cell membranes are composed of **membrane lipids**, which are **diacylglycerols** linked to a hydrophilic **'head group'** on the third carbon of the glycerol backbone. The fatty acid chains can be of various lengths and degrees of saturation, and the two chains combined make up the hydrophobic 'tail' of each membrane lipid molecule.

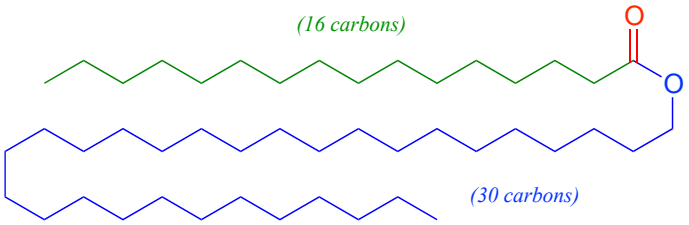


membrane lipid

In chapter 2 we will see how these molecules come together to form a cell membrane.

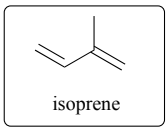
Exercise 1.17: What functional group links the phosphatidylcholine 'head' group to glycerol in the membrane lipid structure shown above?

Waxes are composed of fatty acids linked to long chain alcohols through an ester group. Tricontanyl palmitate is a major component of beeswax, and is constituted of a 16-carbon fatty acid linked to a 30-carbon alcohol.

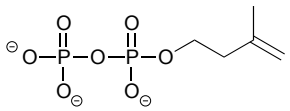


tricontanyl palmitate

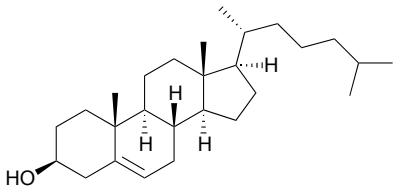
Isoprenoids, a broad class of lipids present in all forms of life, are based on a five-carbon, branched-chain building block called isoprene. In humans, cholesterol and hormones such as testosterone are examples of isoprenoid biomolecules. In plants, isoprenoids include the deeply colored compounds such as lycopene (the red in tomatoes) and carotenoids (the yellows and oranges in autumn leaves).



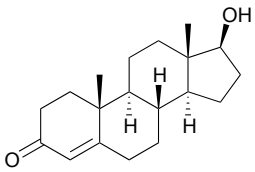
isoprene



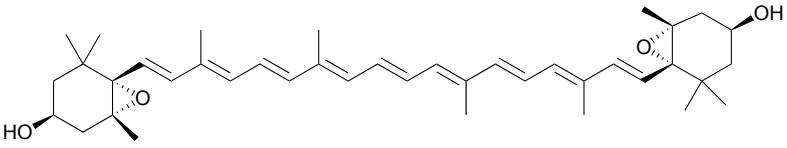
isopentenyl diphosphate



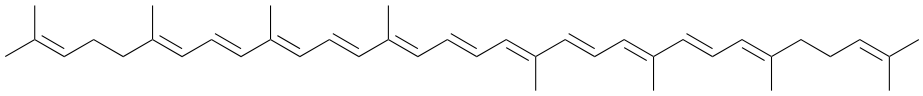
cholesterol



testosterone

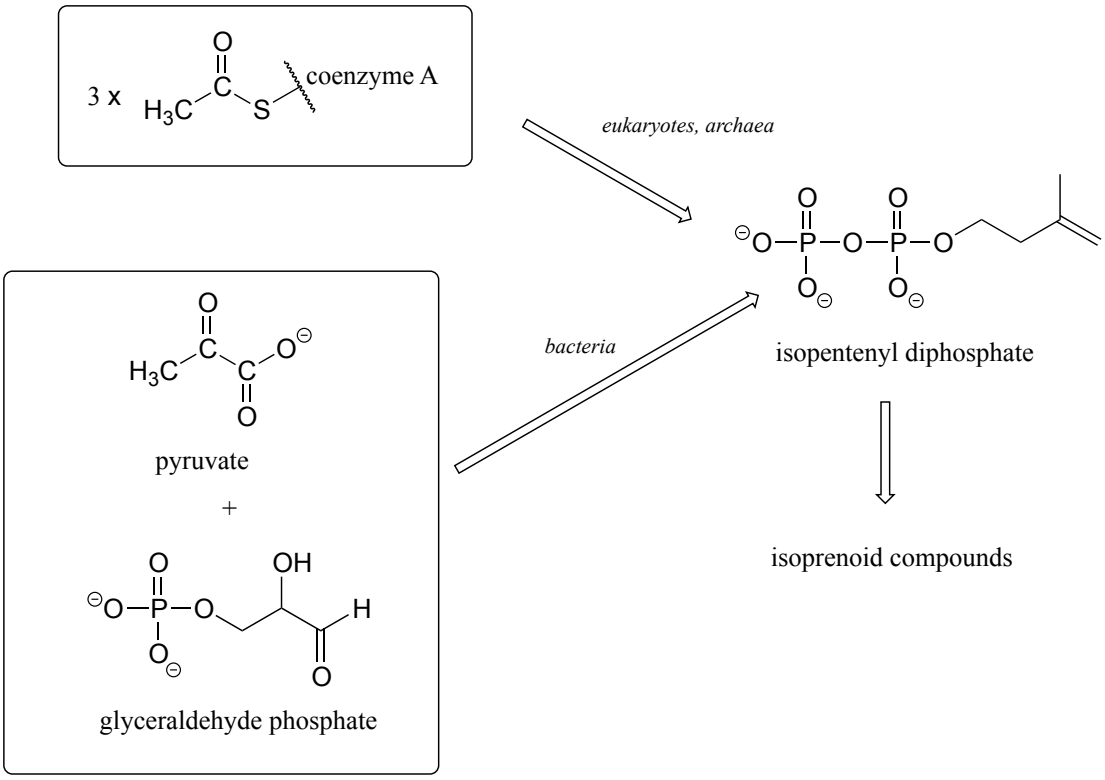


violaxanthin
(a carotenoid)



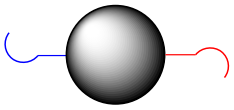
lycopene

In almost all eukaryotes, isopentenyl diphosphate (the building block molecule for all isoprenoid compounds) is synthesized from three acetyl-Coenzyme A molecules. Bacteria and the plastid organelles in plants have a different biosynthetic pathway to isopentenyl diphosphate, starting with pyruvate and glyceraldehyde phosphate.

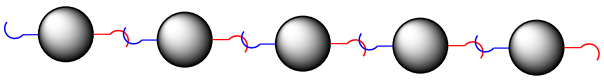


1.3B: Biopolymer basics

Carbohydrates, proteins, and nucleic acids are the most important **polymers** in the living world. To understand what a polymer is, simply picture a long chain made by connecting lots of individual beads, each of which is equipped with two hooks. In chemical terminology, each bead is a **monomer**, the hooks are **linking groups**, and the whole chain is a polymer.



monomer



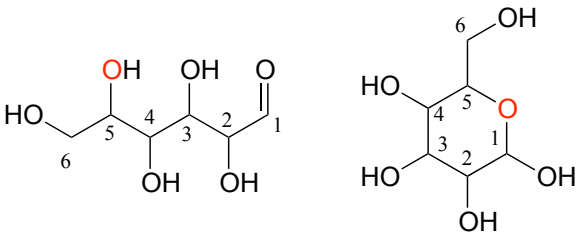
polymer

1.3C: Carbohydrates

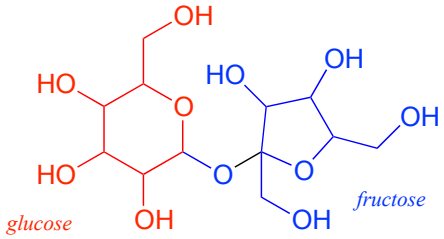
The term 'carbohydrate', which literally means 'hydrated carbons', broadly refers to monosaccharides, disaccharides, oligosaccharides (shorter polymers) and polysaccharides

(longer polymers). We will cover the chemistry of carbohydrates more completely in chapter 10, but the following is a quick overview.

Monosaccharides (commonly called 'sugars') are four- to six-carbon molecules with multiple alcohol groups and a single aldehyde or ketone group. Many monosaccharides exist in aqueous solution as a rapid equilibrium between an open chain and one or more cyclic forms. Two forms of a six-carbon monosaccharide are shown below.

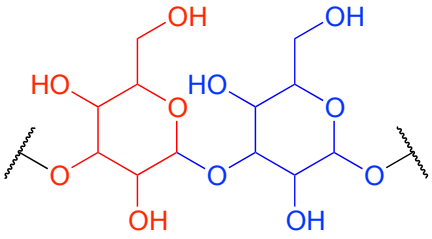


Disaccharides are two monosaccharides linked together: for example, sucrose, or table sugar, is a disaccharide of glucose and fructose.



sucrose

Oligosaccharides and **polysaccharides** are longer polymers of monosaccharides. **Cellulose** is a polysaccharide of repeating glucose monomers. As a major component of the cell walls of plants, cellulose is the most abundant organic molecule on the planet! A two-glucose stretch of a cellulose polymer is shown below.



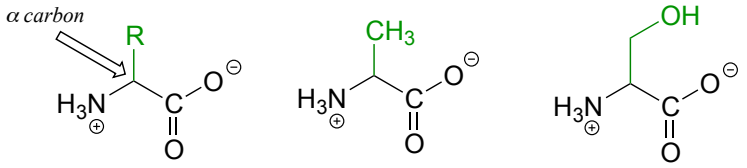
cellulose

The linking group in carbohydrates is not one that we have covered in this chapter - in organic chemistry this group is called an acetal, while biochemists usually use the term **glycosidic bond** when talking about carbohydrates (again, the chemistry of these groups will be covered in detail in chapter 10).

The possibilities for carbohydrate structures are vast, depending on which monomers are used (there are many monosaccharides in addition to glucose and fructose), which carbons are linked, and other geometric factors which we will learn about later. Multiple linking (branching) is also common, so many carbohydrates are not simply linear chains. In addition, carbohydrate chains are often attached to proteins and/or lipids, especially on the surface of cells. All in all, carbohydrates are an immensely rich and diverse subfield of biological chemistry.

1.3D Amino acids and proteins

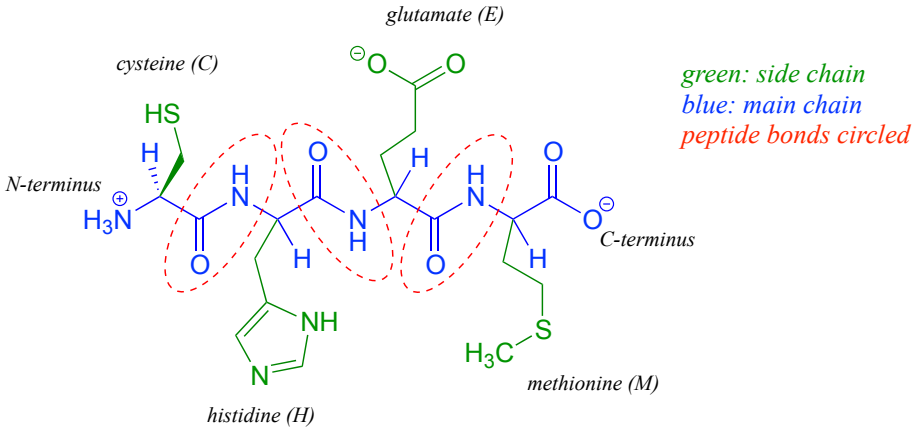
Proteins are polymers of amino acids, linked by amide groups known as peptide bonds. An amino acid can be thought of as having two components: a 'backbone', or 'main chain', composed of an ammonium group, an 'α-carbon', and a carboxylate, and a variable 'side chain' (in green below) bonded to the α-carbon.



amino acid alanine serine

There are twenty different side chains in naturally occurring amino acids (see Table 5 in the tables section at the back of this book), and it is the identity of the side chain that determines the identity of the amino acid: for example, if the side chain is a $-CH_3$ group, the amino acid is alanine, and if the side chain is a $-CH_2OH$ group, the amino acid is serine. Many amino acid side chains contain a functional group (the side chain of serine, for example, contains a primary alcohol), while others, like alanine, lack a functional group, and contain only a simple alkane.

The two 'hooks' on an amino acid monomer are the amine and carboxylate groups. Proteins (polymers of ~50 amino acids or more) and peptides (shorter polymers) are formed when the amino group of one amino acid monomer reacts with the carboxylate carbon of another amino acid to form an amide linkage, which in protein terminology is a **peptide bond**. Which amino acids are linked, and in what order - the protein **sequence** - is what distinguishes one protein from another, and is coded for by an organism's DNA. Protein sequences are written in the amino terminal (N-terminal) to carboxylate terminal (C-terminal) direction, with either three-letter or single-letter abbreviations for the amino acids (see table 5). Below is a four amino acid peptide with the sequence "cysteine - histidine - glutamate - methionine". Using the single-letter code, the sequence is abbreviated CHEM.



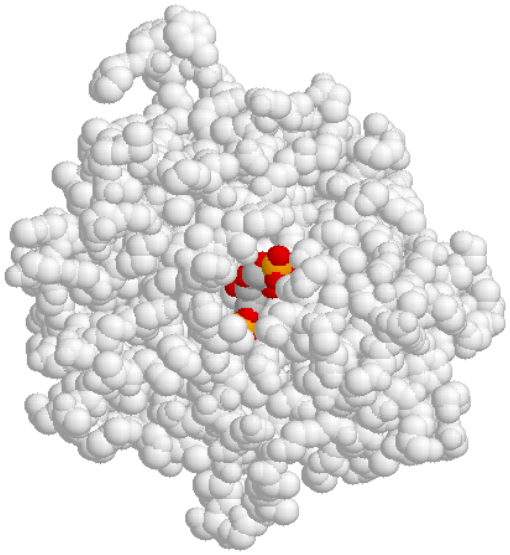
CHEM peptide

When an amino acid is incorporated into a protein it loses a molecule of water and what remains is called a **residue** of the original amino acid. Thus we might refer to the 'glutamate residue' at position 3 of the CHEM peptide above.

Once a protein polymer is constructed, it in many cases folds up very specifically into a three-dimensional structure, which often includes one or more 'binding pockets' in which other molecules can be bound. It is this shape of this folded structure, and the precise arrangement of the functional groups within the structure (especially in the area of the binding pocket) that determines the function of the protein.

Enzymes are proteins which catalyze biochemical reactions. One or more reacting molecules - often called **substrates** - become bound in the **active site** pocket of an enzyme, where the actual reaction takes place. **Receptors** are proteins that bind specifically to one or more molecules - referred to as **ligands** - to initiate a biochemical process. For example, we saw in the introduction to this chapter that the TrpVI receptor in mammalian tissues binds capsaicin (from hot chili peppers) in its binding pocket and initiates a heat/pain signal which is sent to the brain.

Shown below is an image of the glycolytic enzyme fructose-1,6-bisphosphate aldolase (in grey), with the substrate molecule bound inside the active site pocket.

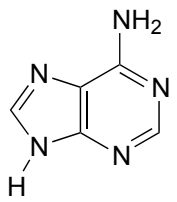


x-ray crystallographic data are from *Protein Science* **1999**, 8, *291*; pdb code 4ALD. Image produced with JMol First Glance

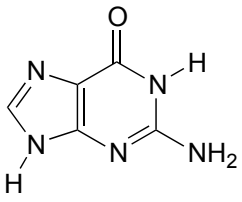
1.3E: Nucleic Acids (DNA and RNA)

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are polymers composed of monomers called **nucleotides**. An RNA nucleotide consists of a five-carbon sugar phosphate linked to one of four **nucleic acid bases**: guanine (G), cytosine (C), adenine (A) and uracil (U).

purine bases

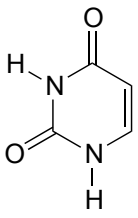


adenine (A)

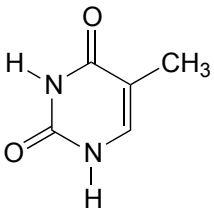


guanine (G)

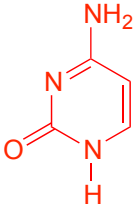
pyrimidine bases



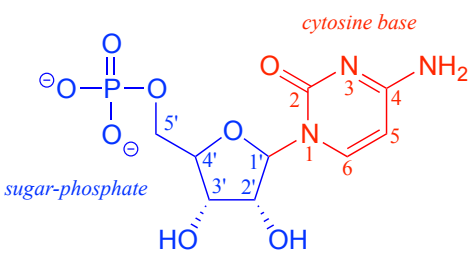
uracil (U)
(RNA)



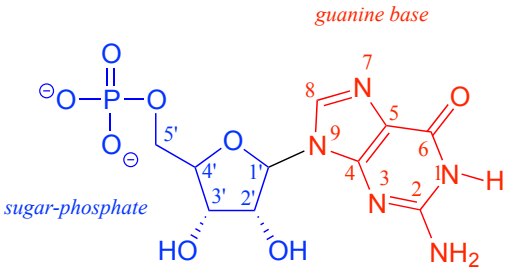
thymine (T)
(DNA)



cytosine (C)



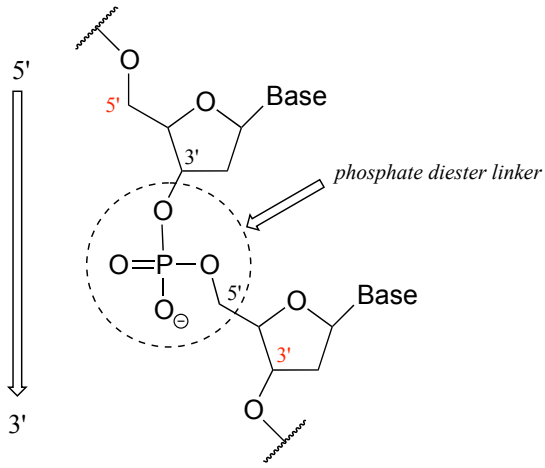
cytidine nucleotide



guanosine nucleotide

In a DNA nucleototide, the sugar is missing the hydroxyl group at the 2' position, and the thymine base (T) is used instead of uracil. (The conventional numbering system used for DNA and RNA is shown here for reference - the prime (') symbol is used to distinguish the sugar carbon numbers from the base carbon numbers.)

The two 'hooks' on the RNA or DNA monomer are the 5' phosphate and the 3' hydroxyl on the sugar, which in DNA polymer synthesis are linked by a 'phosphate diester' group.



In the polymerization process, individual nucleotides are successively added to the 3' hydroxyl group of a growing polymer: in other words, DNA and RNA synthesis occurs in the 5' to 3' direction, and conventionally this is the order in which sequences are written.

A DNA polynucleotide (often referred to as **single-stranded** DNA) combines with a complementary strand to form the famous Watson-Crick **double-helix** structure of **double-stranded DNA**. RNA can also form a double helix within a variety of single- and double-stranded structures depending on its function. Further discussion of higher-order polysaccharide, protein, and DNA/RNA structure is beyond the scope of this textbook, but you will learn many more details in biochemistry and molecular biology

courses. All references to these biomolecules in this textbook will focus on organic chemistry details at the monomer and linking group level.

Summary of Key Concepts

Before you move on to the next chapter, you should:

Thoroughly review (from your General Chemistry course) the fundamental principles of atomic structure and electron configuration, and review the rules for drawing Lewis structures.

Be very familiar with the **common bonding patterns in organic molecules** - you should be able to quickly recognize where lone pairs exist, even when they are not drawn explicitly, and you should be able to readily recognize incorrectly drawn structures -for example, when carbon is drawn with five bonds.

Be able to determine the **formal charge** on all atoms of a compound - with practice, you should be able to look at an organic structure and very rapidly recognize when there is a formal charge on a carbon, oxygen, or nitrogen.

Become adept at interpreting and drawing **line structures** for organic molecules (line structures will be used almost exclusively for the remainder of this textbook). A good test is to determine the molecular formula of a molecule from a line structure.

Understand the meaning of **constitutional isomer**, and be able to recognize and/or draw constitutional isomers of a given compound.

Be able to recognize and come up with your own examples of the most important **functional groups** in organic chemistry, introduced in this chapter and summarized in table 9 in the tables section at the back of this book.

Be familiar with the basic rules of the **IUPAC nomenclature system**, at the level presented in this textbook, and be able to draw a structure based on its IUPAC name.

Understand how and when to use **abbreviated organic structures** appropriately.

You need not memorize in detail the **structures of common classes of biological molecules** illustrated in this chapter (fats, isoprenoids, carbohydrates, proteins, and nucleic acids), but you should be able to recognize examples when you see them. Also, you should be prepared to refer to section 1.3 when references are made to these structures throughout the rest of this textbook and you need a review.

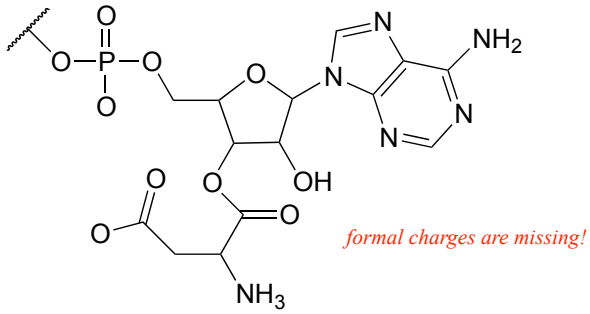
As always, you should be familiar with the meaning of all of the **terms** written in bold in this chapter.

Problems

P1.1: The figure below illustrates a section of an intermediate compound that forms during the protein synthesis process in the cell. Lone pairs are not shown, as is typical in drawings of organic compounds.

a) Draw in lone pair electrons on all nitrogen and oxygen atoms.
b) The structure as drawn is incomplete, because it is missing formal charges - fill them in.
c) Fill in all of the hydrogen atoms that are not already shown.

d) Identify the two important biomolecule classes (covered in section 1.3) in the structure.



P1.2: Find, in Table 6 ('Structures of common coenzymes', in the tables section at the back of this book), examples of the following:

a) a thiol b) an amide c) a secondary alcohol d) an aldehyde

e) a methyl substituent on a ring f) a primary ammonium ion

g) a phosphate anhydride h) a phosphate ester

P1.3: Draw line structures corresponding to the following compounds. Show all lone pair electrons (and don't forget that non-zero formal charges are part of a correctly drawn structure!)

a) 2,2,4-trimethylpentane b) 3-phenyl-2-propenal

c) 6-methyl-2,5-cyclohexadienone d) 3-methylbutanenitrile

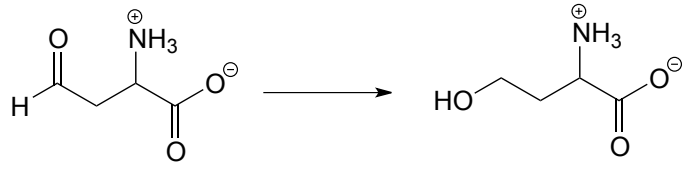
e) 2,6-dimethyldecane f) 2,2,5,5-tetramethyl-3-hexanol

g) methyl butanoate h) *N*-ethylhexanamide

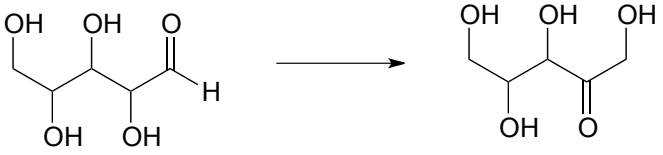
i) 7-fluoroheptanoate j)) 1-ethyl-3,3-dimethylcyclohexene

P1.4: Reaction A below is part of the biosynthetic pathway for the amino acid methionine, and reaction B is part of the pentose phosphate pathway of sugar metabolism.

A

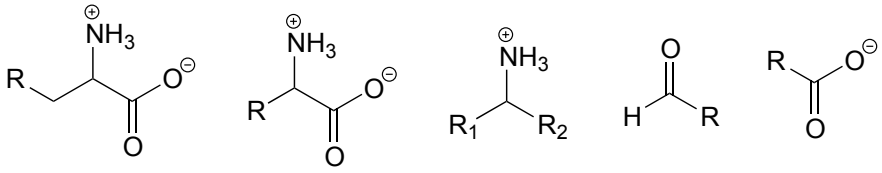


B



a) What is the functional group transformation that is taking place in each reaction?

b) Keeping in mind that the 'R' abbreviation is often used to denote parts of a larger molecule which are *not* the focus of a particular process, which of the following abbreviated structures could be appropriate to use for aspartate semialdehyde when drawing out details of reaction A?



c) Again using the 'R' convention, suggest an appropriate abbreviation for the reactant in reaction B.

P1.5: Find, in the table of amino acid structures (Table 5), examples of the following:

a) a secondary alcohol b) an amide c) a thiol

d) a sulfide e) a phenol f) a side chain primary ammonium

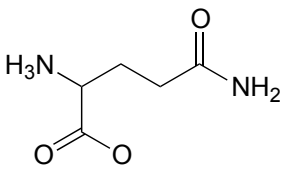
g) a side chain carboxylate h) a secondary amine

P1.6: Draw correct Lewis structures for ozone (O_3), azide, (N_3^-), and bicarbonate, HCO_3^-. Include lone pair electrons, formal charges, and correct bond geometry.

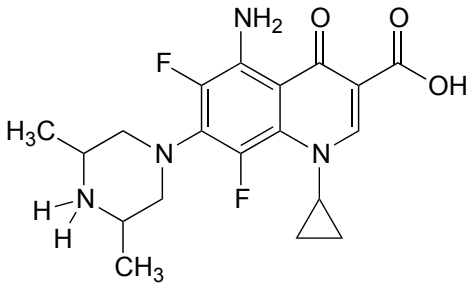
P1.7: Draw one example each of compounds fitting the descriptions below, using line structures. Be sure to include all non-zero formal charges. All atoms should fit one of the common bonding patters discussed in this chapter. There are many possible correct answers - be sure to check your drawings with your instructor or tutor.

a) an 8-carbon molecule with secondary alcohol, primary amine, amide, and *cis*-alkene groups
b) a 12-carbon molecule with carboxylate, diphosphate, and lactone (cyclic ester) groups.
c) a 9-carbon molecule with cyclopentane, alkene, ether, and aldehyde groups

P1.8: Three of the four structures below are missing formal charges.

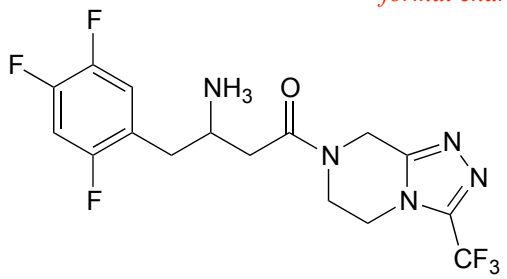


glutamine
(amino acid)

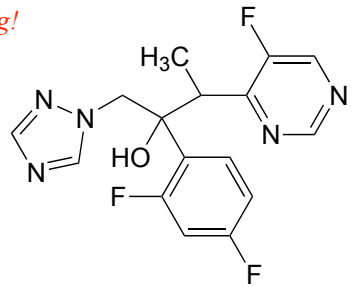


Sparfloxacin
(antibiotic)

formal charges are missing!



Sitagliptin
(diabetes drug)



Voriconazol
(antifungal agent)

a) Fill in all missing formal charges (assume all atoms have a complete octet of valence electrons).

b) Identify the following functional groups or structural elements (there may be more than one of each): carboxylate, carboxylic acid, cyclopropyl, amide, tertiary amine, ketone, secondary ammonium ion, tertiary alcohol.

c) Determine the number of hydrogen atoms in each compound.

P1.9:

a) Draw four constitutional isomers with the molecular formula C_4H_8.
b) Draw two open-chain (non-cyclic) constitutional isomers of cyclohexanol (there are more than two possible answers).

P1.10: Draw structures of four different amides with molecular formula C_3H_7NO.

Chapter 2

Introduction to organic structure and bonding, part II

(Credit: https://www.flickr.com/photos/biodivlibrary/)

"Towards thee I roll, thou all-destroying but unconquering whale; to the last I grapple with thee; from hell's heart I stab at thee; for hate's sake I spit my last breath at thee. Sink all coffins and all hearses to one common pool! and since neither can be mine, let me then tow to pieces, while still chasing thee, though tied to thee, thou damned whale! THUS, I give up the spear!" The harpoon was darted; the stricken whale flew forward; with igniting velocity the line ran through the grooves;--ran foul. Ahab stooped to clear it; he did clear it; but the flying turn caught him round the neck, and voicelessly as Turkish mutes bowstring their victim, he was shot out of the boat, ere the crew knew he was gone. Next instant, the heavy eye-splice in the rope's final end flew out of the stark-empty tub, knocked down an oarsman, and smiting the sea, disappeared in its depths.

(Herman Melville, *Moby Dick*)

In the classic 19th century novel 'Moby Dick', Herman Melville's Captain Ahab obsessively hunts down the enormous albino sperm whale which years before had taken one of his legs, the monomaniacal quest ending with Ahab being dragged by the neck to the bottom of the sea by his enormous white nemesis. It is fitting, somehow, that one of the most memorable fictional characters in modern literature should be a real-life 50 ton monster – sperm whales are such fantastic creatures that if they didn't in fact exist, it would stretch the imagination to make them up. They are the largest predator on the planet, diving to depths of up to three kilometers and staying down as long as 90 minutes to hunt the giant squid and other deep-dwelling species that make up the bulk of their diet.

It would be hard for anyone to mistake a sperm whale for any other creature in the ocean, due to their enormous, squared-off foreheads. It is what is inside this distinctive physical feature, though, that brought them to the edge of extinction in the middle of the 20th century. For over 200 years, sperm whales had been prized by whalers for the oil that fills the 'spermaceti' and 'melon' compartments which make up the bulk of the front part of their bodies. Whalers in the 17th and 18th centuries would lower one of their crew into a hole cut into a captured whale, and he would literally ladle out the 'sperm oil ' by the bucketful, often filling eight barrels from the head of one animal. The different processed components obtained from raw sperm oil had properties that were ideal for a multitude of applications: as a lubricant for everything from sewing machines to train engines, as a fuel for lamps, and as a prized ingredient in cosmetics and skin products.

'Sperm oil' is not really an oil – it is mostly liquid wax (refer back to section 1.3A for a reminder of how the structure of oils differs from that of waxes). The composition of waxes in sperm oil is complex and variable throughout the life of the animal, but in general contains waxes with saturated and unsaturated hydrocarbon chains ranging from 16 to 24 carbons.

Remarkably, scientists are still not sure about the function of the enormous wax-filled reservoirs in the sperm whale's forehead. The most prevalent hypothesis holds that they play a role in echolocation. In the pitch-black void of the deep ocean a whale's eyes are useless, but it is able to navigate and locate prey in the same way that a bat does, using the reflection of sound waves. In fact, the sonic clicks generated by the sperm whale are the loudest sounds generated by any animal on earth. The wax reservoirs may be used somehow for the directional focusing of these sound waves.

Another intriguing but unproven hypothesis is that the reservoir serves as a buoyancy control device. The wax is normally liquid and buoyant at the whale's normal body temperature, but solidifies and becomes denser than water at lower temperatures. If the diving whale could cool the wax by directing cold seawater around the reservoir and restricting blood flow to the region, it could achieve negative buoyancy and thus conserve energy that otherwise would be expended in swimming down. When it needs to return to the surface, blood could be redirected to the wax, which would melt and become positively buoyant again, thus conserving energy on the upward trip.

Whatever its natural function, it is inarguable that the physical and chemical properties of sperm oil make it valuable, both to the whale and to humans. Fortunately for the world's population of whales, both economic forces and conservation efforts have made virtually all trade in sperm oil a thing of the past. Beginning in the late 19th century, the discovery of new oil fields and advances in petroleum processing led to the use of cheaper mineral oil alternatives for many of the major applications of sperm oil, one of the most notable substitutions being the use of kerosene for lamps. More recently, the 'oil' from the Jojoba plant, a native of the American southwest, has been found to be an excellent substitute for sperm oil in cosmetics and skin products, exhibiting many of the same desirable characteristics. Jojoba oil, like sperm oil, is composed primarily of liquid waxes rather than actual oils, and a major selling point of both is that the oily substance produced by human skin, called sebum, is also composed of about 25% wax.

While organic and biological chemistry is a very diverse field of study, one fundamental question that interests all organic chemists is how the structure of an organic molecule determines its physical properties. To understand why sperm oil has properties that made it both a useful industrial lubricant for humans and an effective buoyancy control and/or sonic lens for a hunting sperm whale, we first have to understand the nature of both the forces holding each wax molecule together – the covalent single and double bonds between atoms – and also the forces governing the noncovalent interactions between one wax molecule and all the others around it – the so-called 'intermolecular forces' which determine physical properties such as viscosity, melting point, and density.

That is what we will learn about in this chapter. First, we will look more closely at the nature of single and double covalent bonds, using the concepts of 'hybrid orbitals' and 'resonance' to attempt to explain how orbital overlap results in characteristic geometries and rotational behavior for single and double bonds, as well as bonds that have characteristics of somewhere in between single and double. Then we will move on to a review of the noncovalent interactions between molecules - Van der Waals, ion-ion, dipole-dipole and ion-dipole interactions, and hydrogen bonds - and how they are manifested in the observable physical properties of all organic substances.

Before reading any further on this chapter, you will probably need to go back and review some topics from your Introductory Chemistry course. Be sure that you understand the concepts of atomic orbitals, atomic electron configuration, and that you are able to describe *s* and *p* orbitals and orbital lobes and nodes. Now would also be a very good time to review *VSEPR* theory.

Section 2.1: Covalent bonding in organic molecules: valence bond theory

As we have been discussing how to use Lewis structures to depict the bonding in organic compounds, we have been very vague so far in our language about the actual nature of

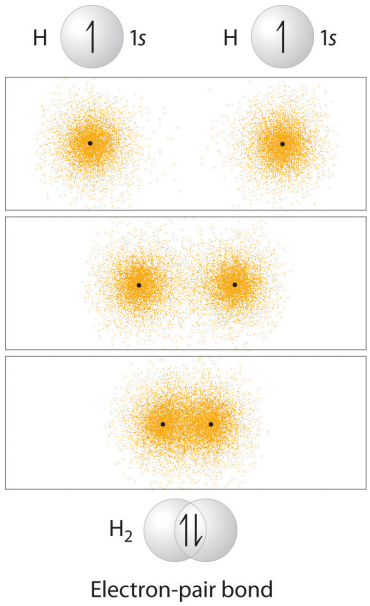
the chemical bonds themselves. We know that a covalent bond involves the 'sharing' of a pair of electrons between two atoms - but how does this happen, and how does it lead to the formation of a bond holding the two atoms together?

Valence bond theory is most often used to describe bonding in organic molecules. In this model, bonds are considered to form from the overlap of two atomic orbitals on different atoms, each orbital containing a single electron. In looking at simple inorganic molecules such as molecular hydrogen (H_2) or hydrogen fluoride (HF), our present understanding of *s* and *p* atomic orbitals will suffice. In order to explain the bonding in organic molecules, however, we will need to introduce the concept of **hybrid orbitals**.

Many of the concepts in this section involve three-dimensional visualization, and are quite challenging for many students to understand when presented solely in a static, textbook-style format. You may find it very helpful to refer as well to lecture videos produced by Kahn Academy, which can be found at: https://www.khanacademy.org/science/organic-chemistry/gen-chem-review/hybrid-orbitals-jay.

2.1A: The σ bond in the H_2 molecule

The simplest case to consider is the hydrogen molecule, H_2. When we say that the two hydrogen nuclei share their electrons to form a covalent bond, what we mean in valence bond theory terms is that the two spherical 1*s* orbitals (the grey spheres in the figure below) overlap, and contain two electrons with opposite spin.



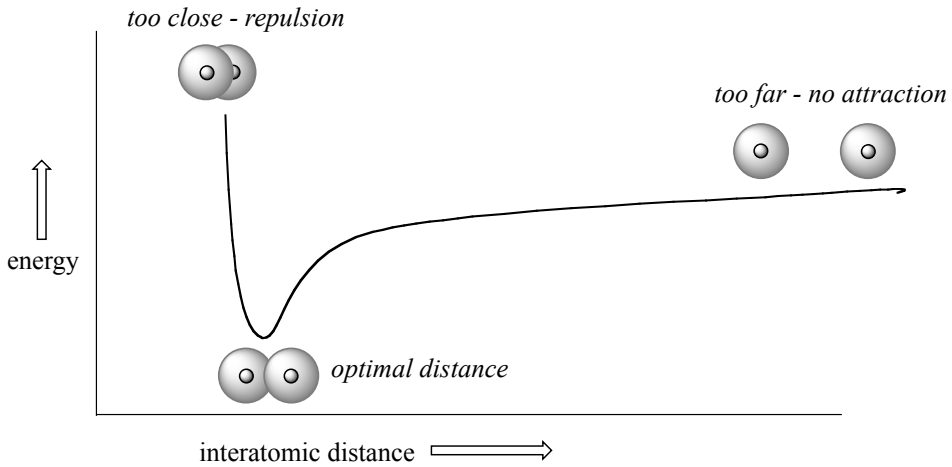
Electron-pair bond

chemwiki.ucdavis.edu

These two electrons are now attracted to the positive charge of *both* of the hydrogen nuclei, with the result that they serve as a sort of 'chemical glue' holding the two nuclei together.

How far apart are the two nuclei? If they are too far apart, their respective 1*s* orbitals cannot overlap, and thus no covalent bond can form - they are still just two separate hydrogen atoms. As they move closer and closer together, orbital overlap begins to occur, and a bond begins to form. This lowers the potential energy of the system, as new, *attractive* positive-negative electrostatic interactions become possible between the nucleus of one atom and the electron of the second.

But something else is happening at the same time: as the atoms get closer, the *repulsive* positive-positive interaction between the two nuclei also begins to increase.



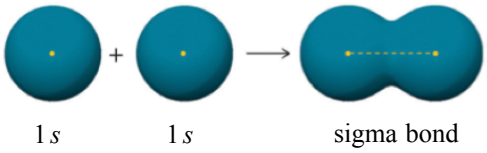
At first this repulsion is more than offset by the attraction between nuclei and electrons, but at a certain point, as the nuclei get even closer, the repulsive forces begin to overcome the attractive forces, and the potential energy of the system rises quickly. When the two nuclei are 'too close', we have an unstable, high-energy situation. There is a defined optimal distance between the nuclei in which the potential energy is at a minimum, meaning that the combined attractive and repulsive forces add up to the greatest overall attractive force. This optimal internuclear distance is the **bond length**. For the H_2 molecule, the distance is 74 pm (picometers, 10^{-12} meters). Likewise, the difference in potential energy between the lowest energy state (at the optimal internuclear distance) and the state where the two atoms are completely separated is called the **bond dissociation energy,** or, more simply**, bond strength**. For the hydrogen molecule, the H-H bond strength is equal to about 435 kJ/mol.

Every covalent bond in a given molecule has a characteristic length and strength. In general, the length of a typical carbon-carbon single bond in an organic molecule is about 150 pm, while carbon-carbon double bonds are about 130 pm, carbon-oxygen double bonds are about 120 pm, and carbon-hydrogen bonds are in the range of 100 to 110 pm. The strength of covalent bonds in organic molecules ranges from about 234 kJ/mol for a

carbon-iodine bond (in thyroid hormone, for example), about 410 kJ/mole for a typical carbon-hydrogen bond, and up to over 800 kJ/mole for a carbon-carbon triple bond. You can refer to tables in reference books such as the CRC Handbook of Chemistry and Physics for extensive lists of bond lengths and energies, and many other data for specific organic compounds.

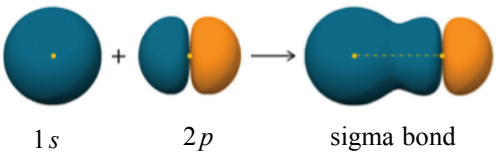
It is not accurate, however, to picture covalent bonds as rigid sticks of unchanging length - rather, it is better to picture them as *springs* which have a defined length when relaxed, but which can be compressed, extended, and bent. This 'springy' picture of covalent bonds will become very important in chapter 4, when we study the analytical technique known as infrared (IR) spectroscopy.

One more characteristic of the covalent bond in H_2 is important to consider at this point. The two overlapping 1*s* orbitals can be visualized as two spherical balloons being pressed together. This means that the bond has **cylindrical symmetry**: if we were to take a cross-sectional plane of the bond at any point, it would form a circle. This type of bond is referred to as a **σ (sigma) bond**.

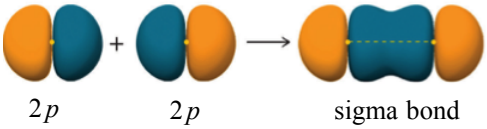


(chemwiki.ucdavis.edu)

A σ bond can be formed by overlap of an *s* atomic orbital with a *p* atomic orbital. Hydrogen fluoride (HF) is an example:

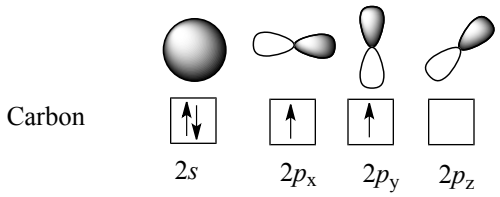


A σ bond can be formed by the overlap of two *p* orbitals. The covalent bond in molecular fluorine, F_2, is a σ bond formed by the overlap of two half-filled 2*p* orbitals, one from each fluorine atom.

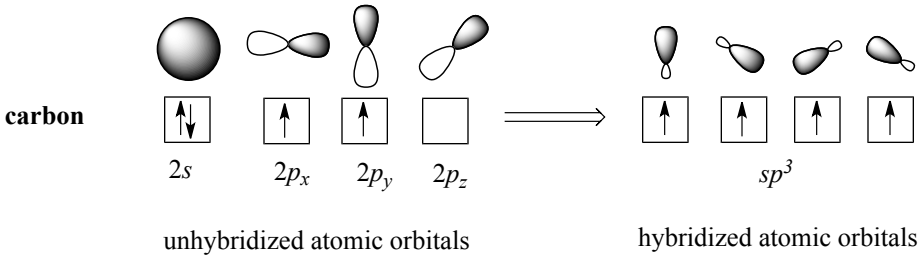


2.1B: sp^3 hybrid orbitals and tetrahedral bonding

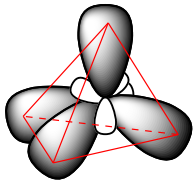
Now let's look more carefully at bonding in organic molecules, starting with methane, CH_4. Recall the valence electron configuration of a carbon atom:



This picture, however, is problematic in terms of bonding. How does the carbon form four bonds if it has only two half-filled *p* orbitals available for bonding? A hint comes from the experimental observation that the four C-H bonds in methane are arranged with **tetrahedral** geometry about the central carbon, and that each bond has the same length and strength. In order to explain this observation, valence bond theory relies on a concept called **orbital hybridization**. In this picture, the four valence orbitals of the carbon (one 2*s* and three 2*p* orbitals) combine mathematically (remember: orbitals are described by wave equations) to form four equivalent **hybrid orbitals**, which are named ***sp*^{3} orbitals** because they are formed from mixing one *s* and three *p* orbitals. In the new electron configuration, each of the four valence electrons on the carbon occupies a single sp^3 orbital.



The sp^3 hybrid orbitals, like the *p* orbitals of which they are partially composed, are oblong in shape, and have two lobes of opposite sign. Unlike the *p* orbitals, however, the two lobes are of very different size. While the unhybridized *p* orbitals are oriented perpendicular (90°) to each other, the sp^3 hybrids are directed towards the four corners of a tetrahedron, meaning that the angle between any two orbitals is 109.5°.

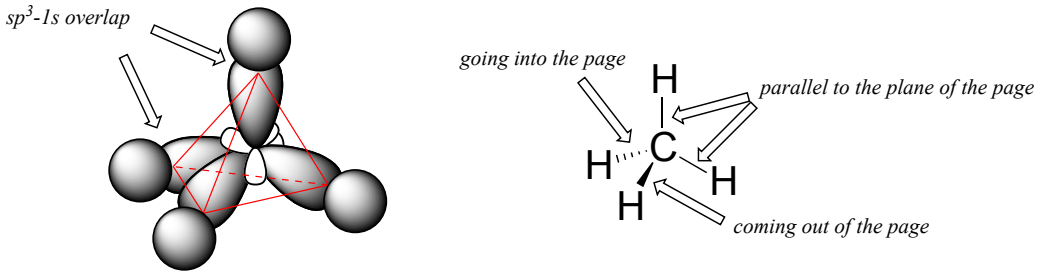


four *sp3* orbitals in tetrahedral orientation

This geometric arrangement makes perfect sense if you consider that it is precisely this angle that allows the four orbitals (and the electrons in them) to be as far apart from each

other as possible. This is simply a restatement of the Valence Shell Electron Pair Repulsion (VSEPR) theory that you learned in General Chemistry: electron pairs (in orbitals) will arrange themselves in such a way as to remain as far apart as possible, due to negative-negative electrostatic repulsion.

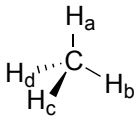
Each C-H bond in methane, then, can be described as an overlap between a half-filled 1*s* orbital in a hydrogen atom and the larger lobe of one of the four half-filled sp^3 hybrid orbitals in the central carbon. The length of the carbon-hydrogen bonds in methane is 109 pm.

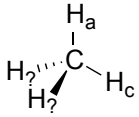


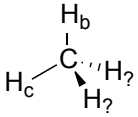
tetrahedral sp^3 bonding in methane

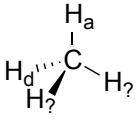
While previously we drew a Lewis structure of methane in two dimensions using lines to denote each covalent bond, we can now draw a more accurate structure in three dimensions, showing the tetrahedral bonding geometry. To do this on a two-dimensional page, though, we need to introduce a new drawing convention: the solid / dashed wedge system. In this convention, a solid wedge simply represents a bond that is meant to be pictured emerging from the plane of the page. A dashed wedge represents a bond that is meant to be pictured pointing into, or behind, the plane of the page. Normal lines imply bonds that lie in the plane of the page. This system takes a little bit of getting used to, but with practice your eye will learn to immediately 'see' the third dimension being depicted.

Exercise 2.1: Imagine that you could distinguish between the four hydrogen atoms in a methane molecule, and labeled them H_a through H_d. In the images below, the *exact same* methane molecule is rotated and flipped in various positions. Draw the missing hydrogen atom labels. (It will be much easier to do this if you make a model.)



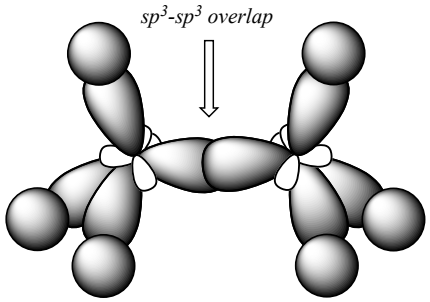
a) 

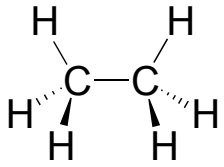
b) 

c) 

Exercise 2.2: What kind of orbitals overlap to form the C-Cl bonds in chloroform, $CHCl_3$?

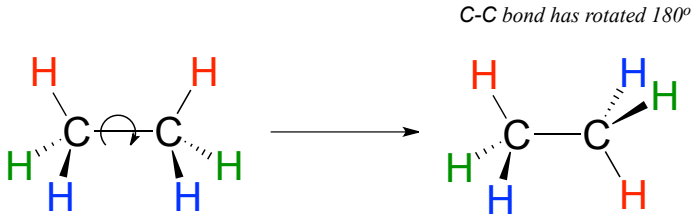
How does this bonding picture extend to compounds containing carbon-carbon bonds? In ethane (CH_3CH_3), both carbons are sp^3-hybridized, meaning that both have four bonds with tetrahedral geometry. The carbon-carbon bond, with a bond length of 154 pm, is formed by overlap of one sp^3 orbital from each of the carbons, while the six carbon-hydrogen bonds are formed from overlaps between the remaining sp^3 orbitals on the two carbons and the 1*s* orbitals of hydrogen atoms. All of these are σ bonds.





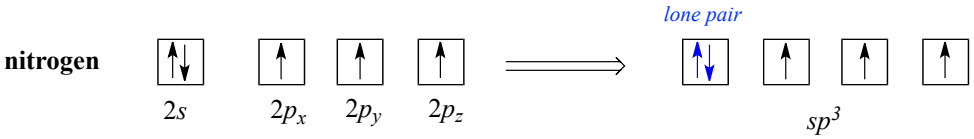
ethane

Because they are formed from the end-on-end overlap of two orbitals, *σ bonds are free to rotate*. This means, in the case of ethane molecule, that the two methyl (CH_3) groups can be pictured as two wheels on an axle, each one able to rotate with respect to the other.

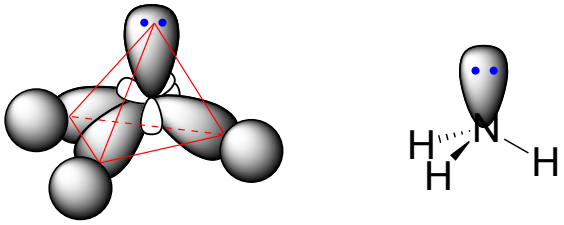


In chapter 3 we will learn more about the implications of rotational freedom in σ bonds, when we discuss the 'conformation' of organic molecules.

The sp^3 bonding picture is also used to described the bonding in amines, including ammonia, the simplest amine. Just like the carbon atom in methane, the central nitrogen in ammonia is sp^3-hybridized. With nitrogen, however, there are five rather than four valence electrons to account for, meaning that three of the four hybrid orbitals are half-filled and available for bonding, while the fourth is fully occupied by a nonbonding pair (lone pair) of electrons.



unhybridized atomic orbitals hybridized atomic orbitals

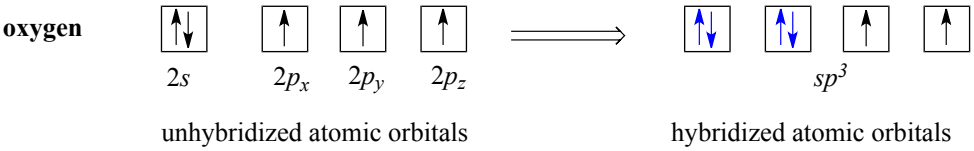


ammonia

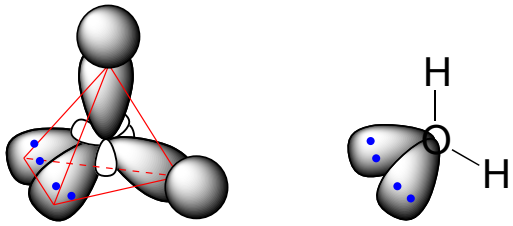
The bonding arrangement here is also tetrahedral: the three N-H bonds of ammonia can be pictured as forming the base of a trigonal pyramid, with the fourth orbital, containing the lone pair, forming the top of the pyramid.

Recall from your study of VSEPR theory in General Chemistry that the lone pair, with its slightly greater repulsive effect, 'pushes' the three N-H σ bonds away from the top of the pyramid, meaning that the H-N-H bond angles are slightly less than tetrahedral, at 107.3˚ rather than 109.5˚.

VSEPR theory also predicts, accurately, that a water molecule is 'bent' at an angle of approximately 104.5˚. The bonding in water results from overlap of two of the four sp^3 hybrid orbitals on oxygen with 1*s* orbitals on the two hydrogen atoms. The two nonbonding electron pairs on oxygen are located in the two remaining sp^3 orbitals.



unhybridized atomic orbitals hybridized atomic orbitals



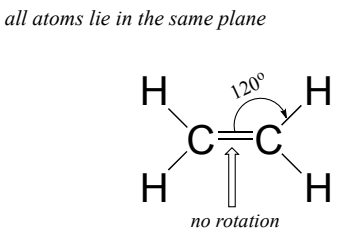
water

Exercise 2.3: Draw, in the same style as the figures above, orbital pictures for the bonding in a) methylamine, and b) ethanol.

2.1C: sp^2 and sp hybrid orbitals and π bonds

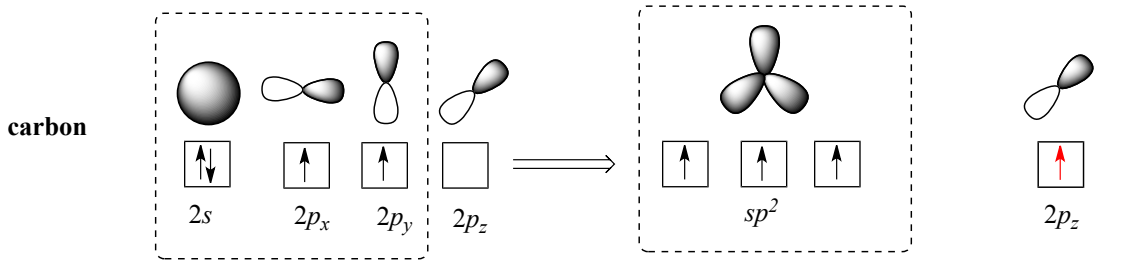
The valence bond theory, along with the hybrid orbital concept, does a very good job of describing double-bonded compounds such as ethene. Three experimentally observable characteristics of the ethene molecule need to be accounted for by a bonding model:

1) Ethene is a planar (flat) molecule.
2) Bond angles in ethene are approximately 120°, and the carbon-carbon bond length is 1.34 Å, significantly shorter than the 1.54 Å single carbon-carbon bond in ethane.
3) There is a significant barrier to rotation about the carbon-carbon double bond.

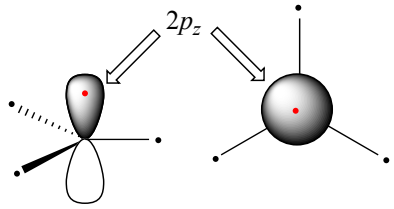


ethene

Clearly, these characteristics are not consistent with an sp^3 hybrid bonding picture for the two carbon atoms. Instead, the bonding in ethene is described by a model involving the participation of a different kind of hybrid orbital. Three atomic orbitals on each carbon – the 2*s*, $2p_x$ and $2p_y$ orbitals – combine to form three ***sp*^{2} hybrids**, leaving the $2p_z$ orbital unhybridized.

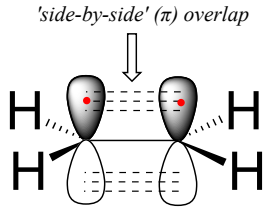


The three sp^2 hybrids are arranged with trigonal planar geometry, pointing to the three corners of an equilateral triangle, with angles of 120° between them. The unhybridized $2p_z$ orbital is *perpendicular* to this plane (in the next several figures, sp^2 orbitals and the σ bonds to which they contribute are represented by lines and wedges; only the $2p_z$ orbitals are shown in the 'space-filling' mode).



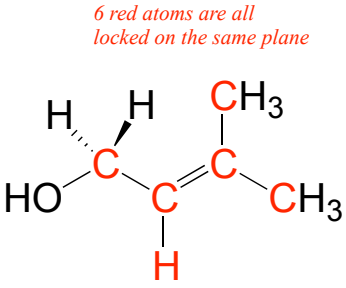
side view top view

The carbon-carbon double bond in ethene consists of one σ bond, formed by the overlap of two sp^2 orbitals, and a second bond, called a **π (pi) bond**, which is formed by the *side-by-side* overlap of the two unhybridized $2p_z$ orbitals from each carbon.

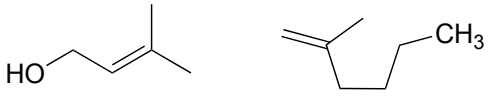


(see also https://www.youtube.com/watch?v=C2W-yDPcpl4 for a 3D animated view of the bonding in ethene)

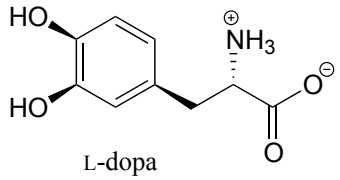
Unlike a σ bond, a π bond does *not* have cylindrical symmetry. If rotation about this bond were to occur, it would involve disrupting the side-by-side overlap between the two $2p_z$ orbitals that make up the π bond. The presence of the π bond thus 'locks' the six atoms of ethene into the same plane. This argument extends to larger alkene groups: in each case, six atoms lie in the same plane.



Exercise 2.4: Redraw the structures below, indicating the six atoms that lie in the same plane due to the carbon-carbon double bond.

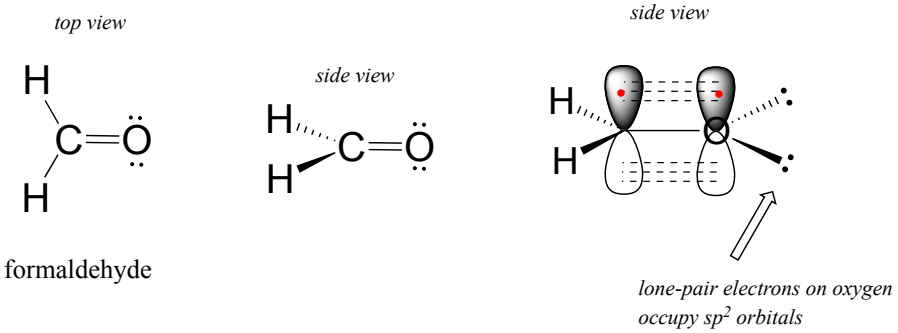


Exercise 2.5: What is wrong with the way the following structure is drawn?



L-dopa

A similar picture can be drawn for the bonding in carbonyl groups, such as formaldehyde. In this molecule, the carbon is sp^2-hybridized, and we will assume that the oxygen atom is also sp^2 hybridized. The carbon has three σ bonds: two are formed by overlap between sp^2 orbitals with 1*s* orbitals from hydrogen atoms, and the third σ bond is formed by overlap between the remaining carbon sp^2 orbital and an sp^2 orbital on the oxygen. The two lone pairs on oxygen occupy its other two sp^2 orbitals.



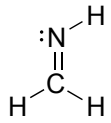
formaldehyde

The π bond is formed by side-by-side overlap of the unhybridized $2p_z$ orbitals on the carbon and the oxygen. Just like in alkenes, the $2p_z$ orbitals that form the π bond are perpendicular to the plane formed by the σ bonds.

Exercise 2.6:

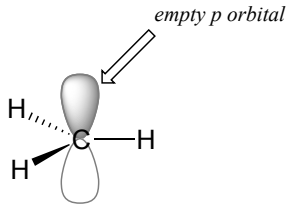
a) Draw a diagram of hybrid orbitals in an sp^2-hybridized nitrogen.

b) Draw a figure showing the bonding picture for the imine below.



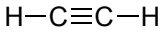
c) In your drawing for part b, what kind of orbital holds the nitrogen lone pair?

Recall from section 1.2 that carbocations are transient, high-energy species in which a carbon only has three bonds (rather than the usual four) and a positive formal charge. We will have much more to say about carbocations in this and later chapters. For now, though, the important thing to understand is that a carbocation can be described as an sp^2-hybridized carbon with an ***empty*** *p* orbital perpendicular to the plane of the σ bonds.



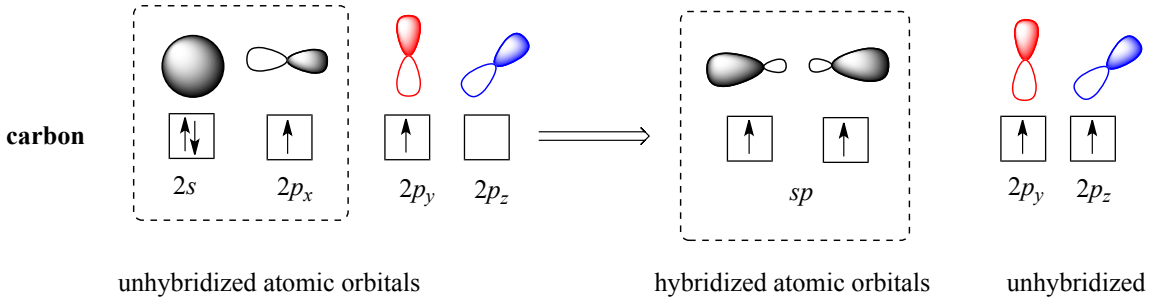
methyl carbocation, CH_3^+

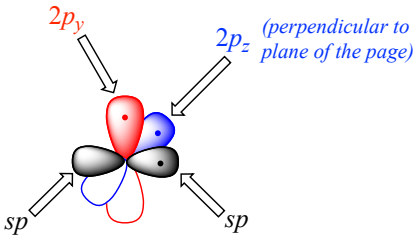
Finally, the hybrid orbital concept applies as well to triple-bonded groups, such as alkynes and nitriles. Consider, for example, the structure of ethyne (common name acetylene), the simplest alkyne.



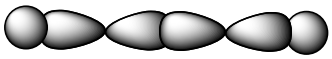
ethyne
(common name acetylene)

Both the VSEPR theory and experimental evidence tells us that the molecule is linear: all four atoms lie in a straight line. The carbon-carbon triple bond is only 120 pm long, shorter than the double bond in ethene, and is very strong, about 837 kJ/mol. In the hybrid orbital picture of acetylene, both carbons are ***sp*-hybridized**. In an *sp*-hybridized carbon, the 2*s* orbital combines with the $2p_x$ orbital to form two *sp* hybrid orbitals that are oriented at an angle of 180° with respect to each other (eg. along the x axis). The $2p_y$ and $2p_z$ orbitals remain unhybridized, and are oriented perpendicularly along the y and z axes, respectively.

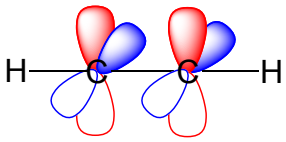




The carbon-carbon σ bond, then, is formed by the overlap of one *sp* orbital from each of the carbons, while the two carbon-hydrogen σ bonds are formed by the overlap of the second *sp* orbital on each carbon with a 1*s* orbital on a hydrogen. Each carbon atom still has two half-filled $2p_y$ and $2p_z$ orbitals, which are perpendicular both to each other and to the line formed by the σ bonds. These two perpendicular pairs of *p* orbitals form two π bonds between the carbons, resulting in a triple bond overall (one σ bond plus two π bonds).



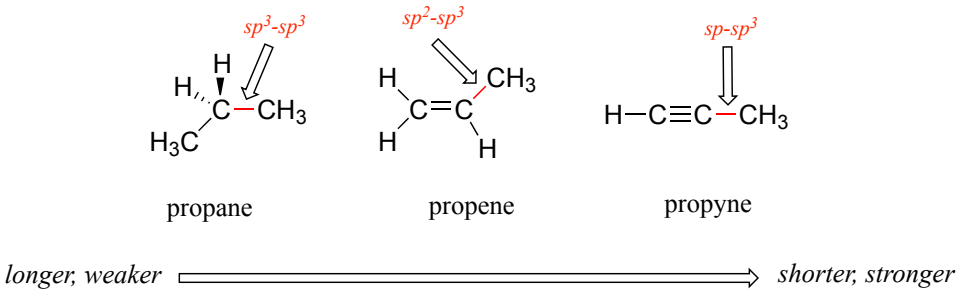
σ bonding framework in ethyne



π overlap in ethyne

Exercise 2.7: Look at the structure of thiamine diphosphate in Table 6. Identify the hybridization of all carbon atoms in the molecule.

The hybrid orbital concept nicely explains another experimental observation: single bonds adjacent to double and triple bonds are progressively shorter and stronger than single bonds adjacent to other single bonds. Consider for example, the carbon-carbon single bonds in propane, propene, and propyne.

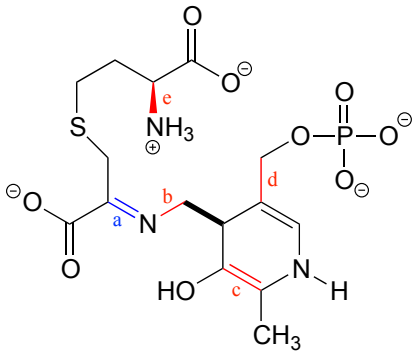


All three are single, σ bonds; the bond in propyne is shortest and strongest, while the bond in propane is longest and weakest. The explanation is relatively straightforward. An *sp* orbital is composed of one *s* orbital and one *p* orbital, and thus it has 50% *s* character and 50% *p* character. sp^2 orbitals, by comparison, have 33% *s* character and 67% *p* character, while sp^3 orbitals have 25% *s* character and 75% *p* character. Because of their spherical shape, 2*s* orbitals are smaller, and hold electrons closer and 'tighter' to the nucleus, compared to 2*p* orbitals. It follows that electrons in an *sp* orbital, with its greater *s* character, are closer to the nucleus than electrons in an sp^2 or sp^3 orbital. Consequently, bonds involving *sp*-sp^3 overlap (as in propyne) are shorter and stronger than bonds involving sp^2-sp^3 overlap (as in propene). Bonds involving sp^3-sp^3 overlap (as in propane) are the longest and weakest of the three.

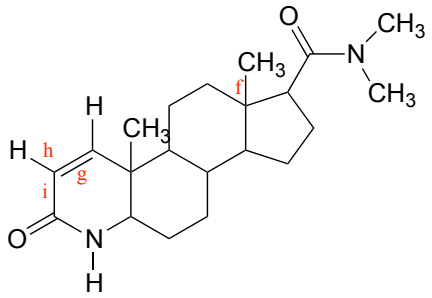
Exercise 2.8:

a) What kinds of orbitals are overlapping in bonds b-i indicated below? Be sure to distinguish between σ and π bonds. An example is provided for bond 'a'.

b) In what kind of orbital is the lone pair of electrons located on the nitrogen atom of bond a? Of bond e?



intermediate compound in amino acid metabolism



Finasteride
(trade name Propecia, a hair-loss drug for men from Merck Pharmacueticals)

Bond a:
σ bond: overlap of sp^2 orbital on N and sp^2 orbital on C
π bond: overlap of 2*p* orbital on N and 2*p* orbital on C

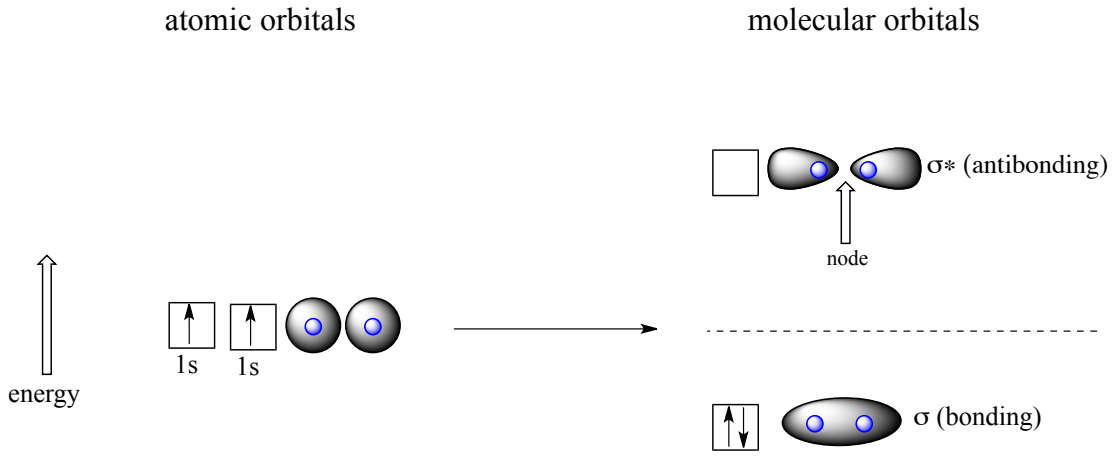
Section 2.2: Molecular orbital theory, conjugation, and aromaticity

Valence bond theory does a remarkably good job at explaining the bonding geometry of many of the functional groups in organic compounds. There are some areas, however, where the valence bond theory falls short. It fails to adequately account, for example, for some interesting properties of compounds that contain alternating double and single bonds. In order to understand these properties, we need to think about chemical bonding in a new way, using the ideas of molecular orbital (MO) theory.

2.2A: Another look at the H_2 molecule using molecular orbital theory

Let's go back and consider again the simplest possible covalent bond: the one in molecular hydrogen (H_2). When we described the hydrogen molecule using valence bond theory, we said that the two 1*s* orbitals from each atom overlap, allowing the two electrons to be shared and thus forming a covalent bond. In molecular orbital theory, we make a further statement: we say that the two atomic 1*s* orbitals mathematically combine to form two new orbitals. Recall that an atomic orbital (such as the 1s orbital of a hydrogen atom) describes a region of space around a single atom inside which electrons are likely to be found. *A molecular orbital describes a region of space around two or more atoms inside which electrons are likely to be found.*

Mathematical principles tell us that when orbitals combine, the number of orbitals before the combination takes place must equal the number of new orbitals that result from the combination – orbitals don't just disappear! We saw this previously when we discussed hybrid orbitals: one *s* and three *p* orbitals make four sp^3 hybrids. When two atomic 1*s* orbitals combine in the formation of H_2, the result is *two* **sigma (σ) molecular orbitals**.



According to MO theory, one σ orbital is lower in energy than either of the two isolated atomic 1*s* orbitals –this lower σ orbital is referred to as a **bonding molecular orbital**. The second, **σ* orbital** is higher in energy than the two atomic 1*s* orbitals, and is referred to as an **antibonding molecular orbital**.

The bonding σ orbital, which holds both electrons in the ground state of the molecule, is egg-shaped, encompassing the two nuclei, and with the highest likelihood of electrons being in the area between the two nuclei. The high-energy, antibonding σ* orbital can be visualized as a pair of droplets, with areas of higher electron density near each nucleus and a 'node', (area of zero electron density) midway between the two nuclei.

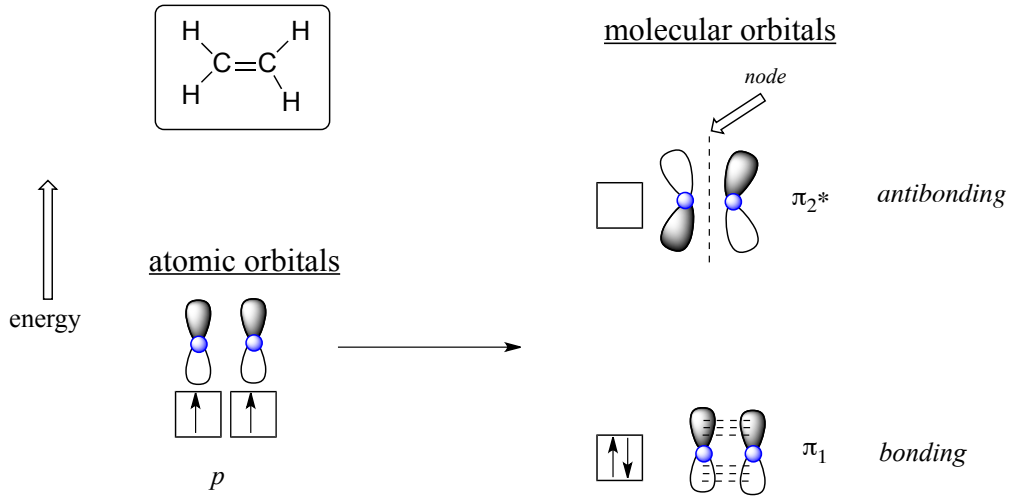
Remember that we are thinking here about electron behavior as *wave behavior*. When two separate waves combine, they can do so with **constructive interference**, where the two amplitudes build up and reinforce one another, or **destructive interference**, where the two amplitudes cancel one another out. Bonding MOs are the consequence of constructive interference between two atomic orbitals, which results in an attractive interaction and an increase in electron density between the nuclei. Antibonding MO's are the consequence of destructive interference which results in a repulsive interaction and a region of zero electron density between the nuclei (in other words, a node).

Following the same *aufbau* ('building up') principle you learned in General Chemistry for writing out electron configurations, we place the two electrons in the H_2 molecule in the lowest energy molecular orbital, which is the (bonding) σ orbital. The bonding (attracting) MO is full, and the antibonding (repulsing) MO is empty.

2.2B: MO theory and conjugated π bonds

The advantage of using MO theory to understand bonding in organic molecules becomes more apparent when we think about π bonds. Let's first consider the π bond in ethene

from an MO theory standpoint (in this example we will be disregarding the σ bonds in the molecule, and thinking *only* about the π bond). We start with two atomic orbitals: one unhybridized 2p orbital from each carbon. Each contains a single electron. In MO theory, the two atomic combine mathematically to form two **π molecular orbitals**, one a low-energy π bonding orbital and one a high-energy π* antibonding orbital.
(need better artwork)

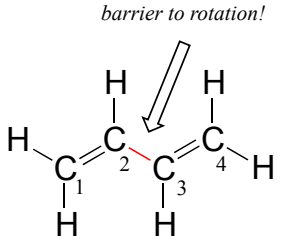


In the bonding π orbital, the two shaded lobes of the *p* orbitals interact *constructively* with each other, as do the two unshaded lobes (remember, the arbitrary shading choice represents mathematical (+) and (-) signs for the mathematical wavefunction describing the orbital). There is increased electron density between the two carbon nuclei in the molecular orbital - it is a bonding interaction.

In the higher-energy antibonding π* orbital, the shaded lobe of one *p* orbital interacts *destructively* with the unshaded lobe of the second *p* orbital, leading to a node between the two nuclei and overall repulsion between the carbon nuclei.

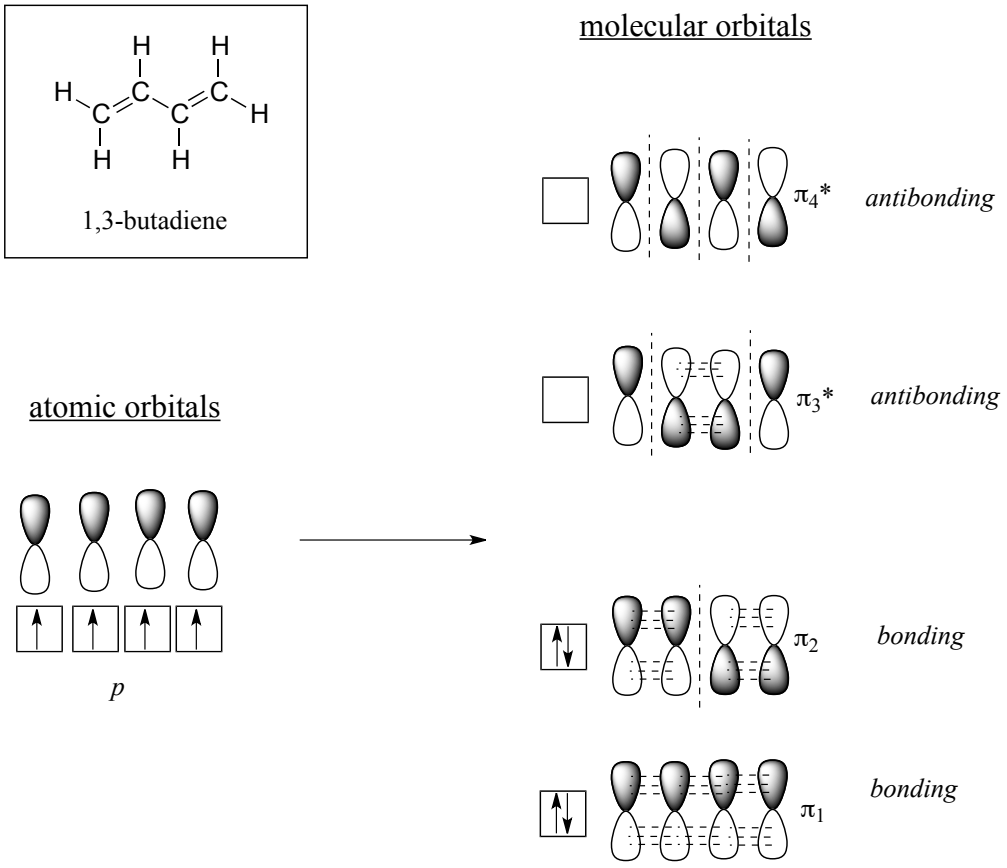
Again using the 'building up' principle, we place the two electrons in the lower-energy, bonding π molecular orbital. The antibonding π* orbital remains empty.

Next, we'll consider the 1,3-butadiene molecule. From valence orbital theory alone we might expect that the C_2-C_3 bond in this molecule, because it is a σ bond, would be able to rotate freely.



Experimentally, however, it is observed that there is a significant barrier to rotation about the C_2-C_3 bond, and that the entire molecule prefers to be planar. In addition, the C_2-C_3 bond is 148 pm long, shorter than a typical carbon-carbon single bond (about 154 pm), though longer than a typical double bond (about 134 pm).

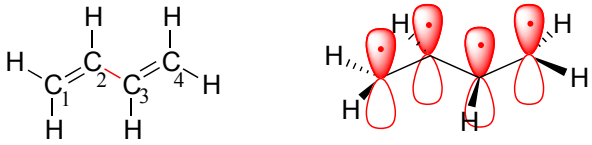
Molecular orbital theory accounts for these observations with the concept of **delocalized π bonds**. In this picture, the four *p* atomic orbitals combine mathematically to form four π molecular orbitals of increasing energy. Two of these - the bonding π orbitals - are lower in energy than the *p* atomic orbitals from which they are formed, while two - the antibonding π orbitals - are higher in energy.



The lowest energy molecular orbital, π_1, has only contructive interaction and zero nodes. Higher in energy, but still lower than the isolated *p* orbitals, the π_2 orbital has one node but two constructive interactions - thus it is still a bonding orbital overall. Looking at the two antibonding orbitals, π_3* has two nodes and one constructive interaction, while π_4* has three nodes and zero constructive interactions.

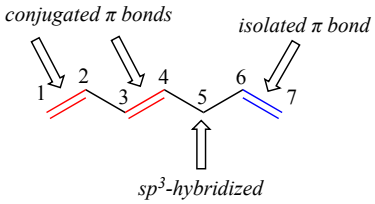
By the *aufbau* principle, the four electrons from the isolated $2p_z$ atomic orbitals are placed in the bonding π_1 and π_2 MO's. Because π_1 includes constructive interaction between C_2 and C_3, there is a degree, in the 1,3-butadiene molecule, of π-bonding interaction between these two carbons, which accounts for its shorter length and the

barrier to rotation. The valence bond picture of 1,3-butadiene shows the two π bonds as being isolated from one another, with each pair of π electrons 'stuck' in its own π bond. However, molecular orbital theory predicts (accurately) that the four π electrons are to some extent delocalized, or 'spread out', over the whole π system.

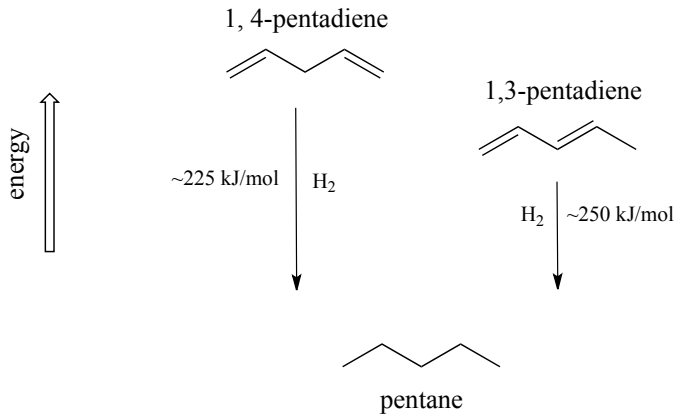


1,3-butadiene

1,3-butadiene is the simplest example of a system of **conjugated π bonds**. To be considered conjugated, two or more π bonds must be separated by only one single bond – in other words, there cannot be an intervening sp^3-hybridized carbon, because this would break up the overlapping system of parallel *p* orbitals. In the compound below, for example, the C_1-C_2 and C_3-C_4 double bonds are conjugated, while the C_6-C_7 double bond is **isolated** from the other two π bonds by sp^3-hybridized C_5.

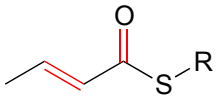


A very important concept to keep in mind is that *there is an inherent thermodynamic stability associated with conjugation.* This stability can be measured experimentally by comparing the **heat of hydrogenation** of two different dienes. (Hydrogenation is a reaction type that we will learn much more about in chapter 15: essentially, it is the process of adding a hydrogen molecule - two protons and two electrons - to a π bond). When the two *conjugated* double bonds of 1,3-pentadiene are 'hydrogenated' to produce pentane, about 225 kJ is released per mole of pentane formed. Compare that to the approximately 250 kJ/mol released when the two *isolated* double bonds in 1,4-pentadiene are hydrogenated, also forming pentane.

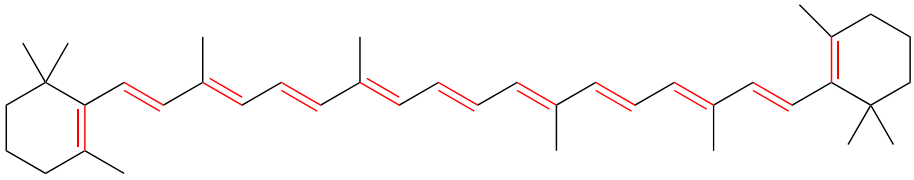


The conjugated diene is lower in energy: in other words, it is more stable. In general, conjugated π bonds are more stable than isolated π bonds.

Conjugated π systems can involve oxygen and nitrogen atoms as well as carbon. In the metabolism of fat molecules, some of the key reactions involve alkenes that are conjugated to carbonyl groups.

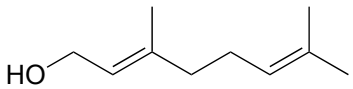


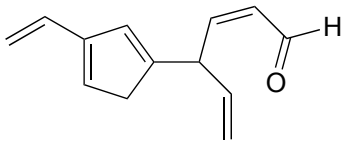
In chapter 4, we will see that MO theory is very useful in explaining why organic molecules that contain extended systems of conjugated π bonds often have distinctive colors. β-carotene, the compound responsible for the orange color of carrots, has an extended system of 11 conjugated π bonds.



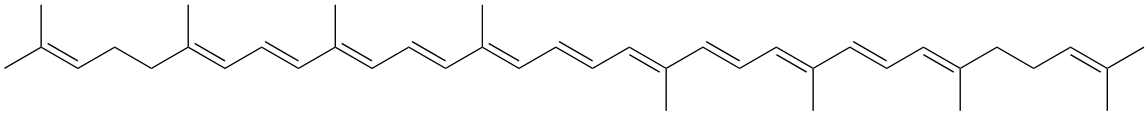
β-carotene

Exercise 2.9: Identify all conjugated and isolated double bonds in the structures below. For each conjugated π system, specify the number of overlapping *p* orbitals, and how many π electrons are shared among them.





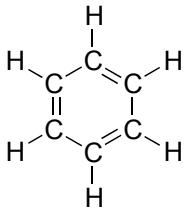
Exercise 2.10: Identify all isolated and conjugated π bonds in lycopene, the red-colored compound in tomatoes. How many π electrons are contained in the conjugated p system?



lycopene

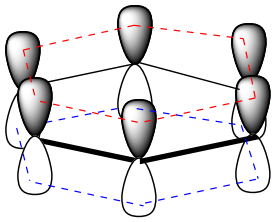
2.2C: Aromaticity

Molecular orbital theory is especially helpful in explaining the unique properties of **aromatic** compounds such as benzene.



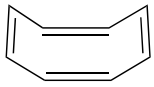
Although it is most often drawn with three double bonds and three single bonds, in fact all of the carbon-carbon bonds in benzene are exactly the same length (138 pm). In addition, the π bonds in benzene are significantly less reactive than 'normal' π bonds, either isolated or conjugated. Something about the structure of benzene makes its π bonding arrangement especially stable. This 'something' has a name: it is called 'aromaticity'.

What exactly is this 'aromatic' property that makes the π bonds in benzene so stable? In a large part, the answer to this question lies in the fact that benzene is a *cyclic* molecule in which all of the ring atoms are sp^2-hybridized. This allows the π electrons to be delocalized in molecular orbitals that extend all the way around the ring, above and below the plane. For this to happen, of course, the ring must be planar – otherwise the *p* orbitals couldn't overlap properly. Benzene is indeed known to be a flat molecule.



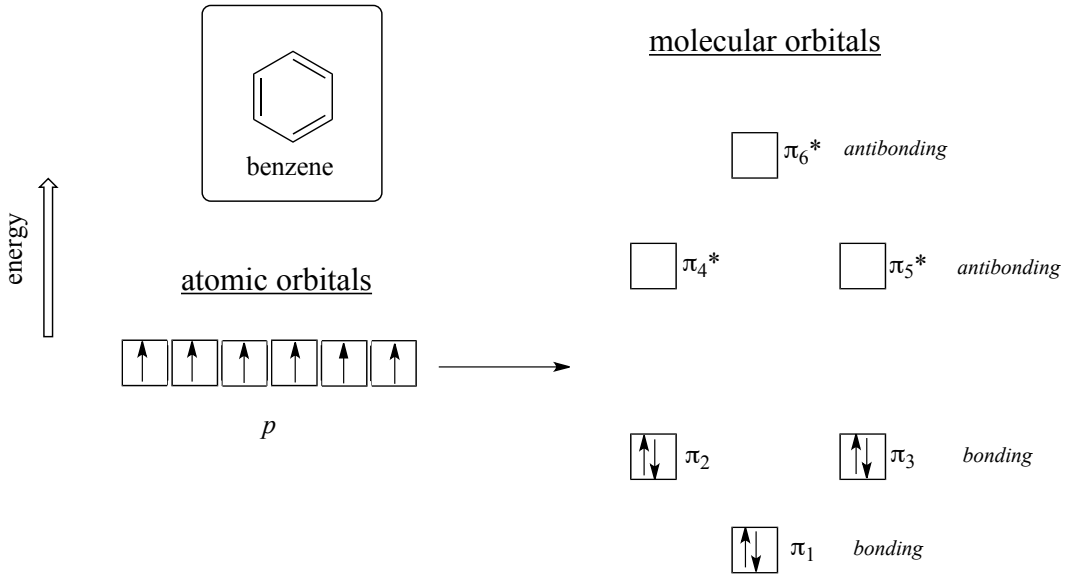
π electrons delocalized around the ring, above and below the plane

Do all cyclic molecules with alternating single and double bonds have this same aromatic stability? The answer, in fact, is 'no'. The eight-membered cyclooctatetraene ring shown below is *not* flat, and its π bonds react like 'normal' alkenes.



cyclooctatetraene
(not aromatic)

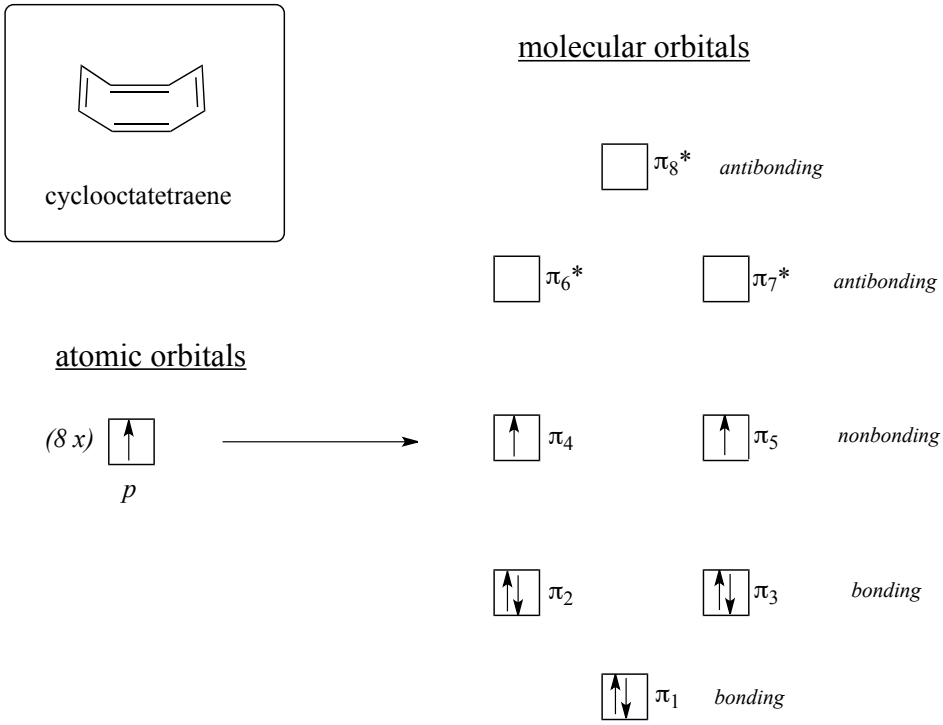
Clearly it takes something more to be aromatic, and this can best be explained with molecular orbital theory. Let's look at an energy diagram of the π molecular orbitals in benzene.



Quantum mechanical calculations tell us that the six π molecular orbitals in benzene, formed from six atomic *p* orbitals, occupy four separate energy levels. π_1 and π_6* have unique energy levels, while the π_2 - π_3 and π_4*- π_5* pairs are **degenerate**, meaning they are at the same energy level. When we use the *aufbau* principle to fill up these orbitals with the six π electrons in benzene, we see that the bonding orbitals are completely filled, and the antibonding orbitals are empty. This gives us a good clue to the source of the special stability of benzene: a full set of bonding MO's is similar in many ways to the

'full shell' of electrons in the atomic orbitals of the stable noble gases helium, neon, and argon.

Now, let's do the same thing for cyclooctatetraene, which we have already learned is *not* aromatic.



The result of molecular orbital calculations tells us that the lowest and highest energy MOs (π_1 and π_8*) have unique energy levels, while the other six form degenerate pairs. Notice that π_4 and π_5 are at the same energy level as the isolated $2p_z$ atomic orbitals: these are therefore neither bonding nor antibonding, rather they are referred to as **nonbonding MOs**. Filling up the MOs with the eight π electrons in the molecule, we find that the last two electrons are unpaired and fall into the two degenerate nonbonding orbitals. Because we don't have a perfect filled shell of bonding MOs, our molecule is not aromatic. As a consequence, each of the double bonds in cyclooctatetraene acts more like an *isolated* double bond.

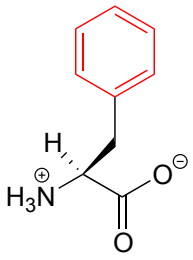
Here, then, are the conditions that must be satisfied for a molecule or group to be considered aromatic:

Criteria for aromaticity:

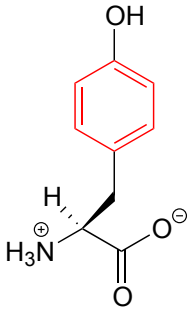
1) The molecule or group must be cyclic.
2) The ring must be planar.
3) Each atom in the ring must be sp^2-hybridized.
4) The number of π electrons in the ring must equal $4n+2$, where n is any positive integer including zero.

Rule #4 is known as the **Hückel rule**, named after Erich Hückel, a German scientist who studied aromatic compounds in the 1930's. If $n = 0$, the Hückel number is **2**. If $n = 1$, the Hückel number is **6** (the Hückel number for benzene). The series continues with **10, 14, 18, 22,** and so on. Cyclooctatetraene has eight π electrons, which is *not* a Hückel number. Because six is such a common Hückel number, chemists often use the term **'aromatic sextet'**.

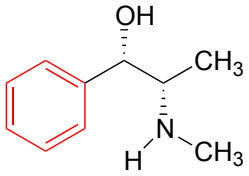
Benzene rings are ubiquitous in biomolecules and drugs - below are just a few examples.



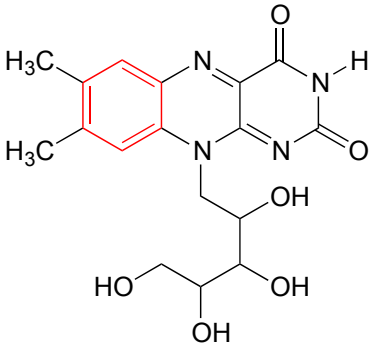
phenylalanine
(an amino acid)



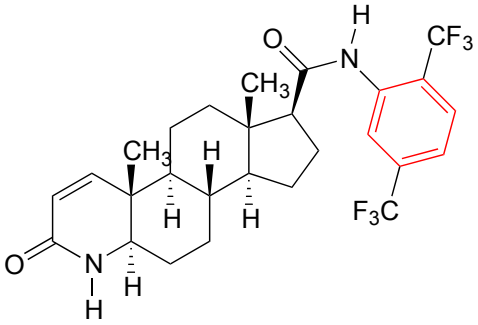
tyrosine
(an amino acid)



pseudoephidrine
(decongestant)



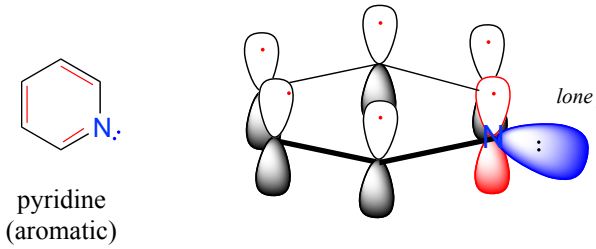
riboflavin
(a vitamin)



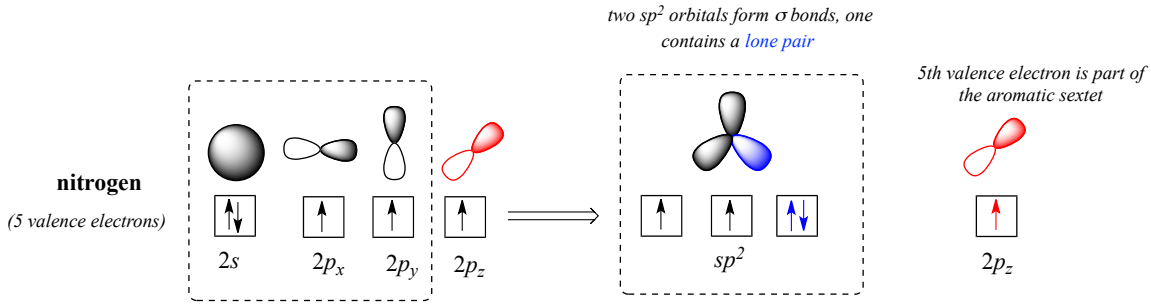
dutasteride
(GlaxoSmithKline drug for urinary condition in men)

Recall that a benzene ring with a hydoxyl substituent -such as seen in the tyrosine structure above - is called a phenol.

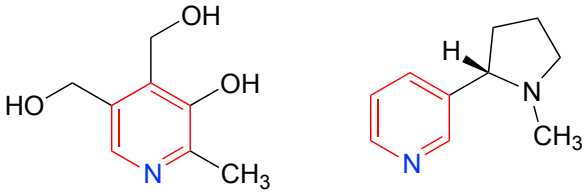
Heterocycles - cyclic structures in which the ring atoms may include oxygen or nitrogen - can also be aromatic. Pyridine, for example, is an aromatic heterocycle. In the bonding picture for pyridine, the nitrogen is sp^2-hybridized, with two of the three sp^2 orbitals forming σ overlaps with the sp^2 orbitals of neighboring carbon atoms, and the third nitrogen sp^2 orbital containing the lone pair. The unhybridized *p* orbital contains a single electron, which is part of the 6 π-electron system delocalized around the ring.



pyridine
(aromatic)



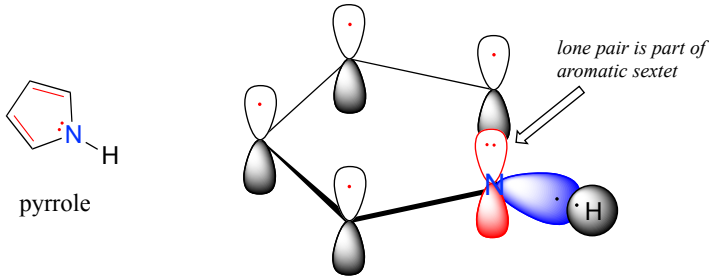
Pyridoxine, commonly known as vitamin B_6, and nicotine are both substituted pyridines.

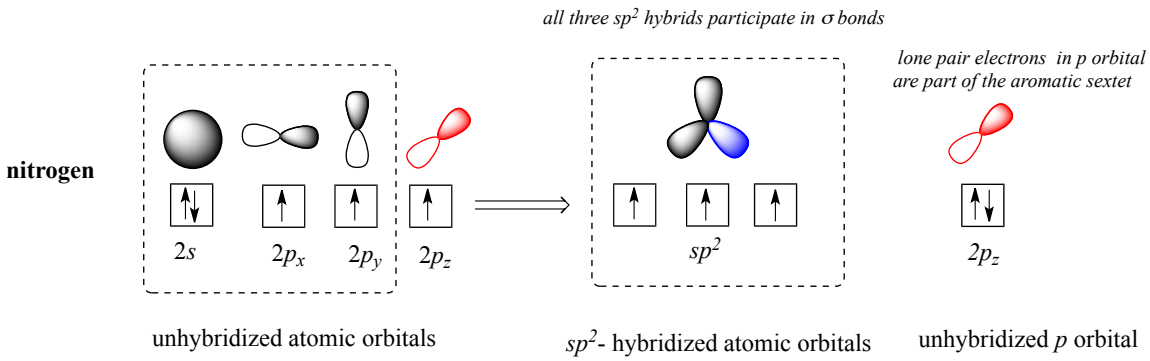


pyridoxine
(vitamin B_6)

nicotine

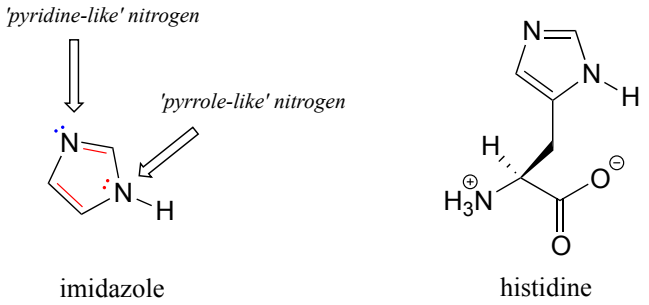
Pyrrole is a five-membered aromatic heterocycle. In pyrrole, the lone pair electrons on the sp^2-hybridized nitrogen are part of the aromatic sextet (contrast this to pyridine, where the lone pair occupies one of the sp^2 hybrid orbitals).





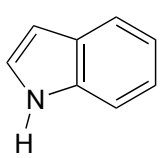
Why do we not assume that the nitrogen in pyrrole is sp^3-hybridized, like a normal secondary amine? The answer is simple: if it were, then pyrrole could not be aromatic, and thus it would not have the stability associated with aromaticity. In general, *if a molecule or group can be aromatic, it will be,* just as water will always flow downhill if there is a downhill pathway available.

Imidazole is another important example of an aromatic heterocycle found in biomolecules - the side chain of the amino acid histidine contains an imidazole ring.

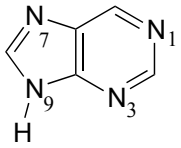


In imidazole, one nitrogen is 'pyrrole-like' (the lone pair contributes to the aromatic sextet) and one is 'pyridine-like' (the lone pair is located in an sp^2 orbital, and is *not* part of the aromatic sextet).

Fused-ring structures can also fulfill the Hückel criteria, and often have many of the same properties as monocyclic aromatic compounds, including a planar structure. Indole (a functional group on the side chain of the amino acid tryptophan) and purine (found in guanine and adenine nucleotide bases) both have a total of ten π electrons delocalized around two rings.



indole

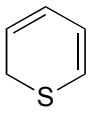


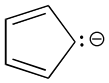
purine

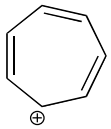
Exercise 2.11: Classify the nitrogen atoms of indole and purine as either 'pyrrole-like' or 'pyridine-like', in terms of where the lone pair electrons are located.

Exercise 2.12: Are the following molecules/ions aromatic? Explain, using criteria you learned in this section.

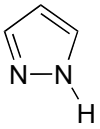
a) 

b) 

c) 

d) 

e) 

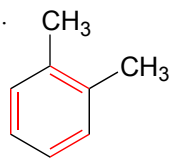
f) 

Section 2.3: Resonance

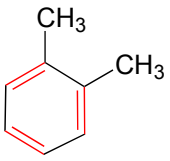
2.3A: What is resonance?

If we were to draw the structure of 1,2-dimethylbenzene, there are two ways that we could draw the double bonds:

is this correct. . .



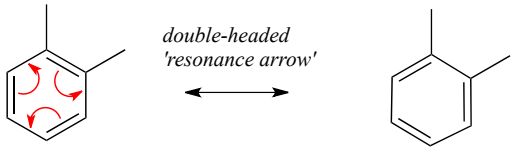
or is this?



Which way is correct? There are two simple answers to this question: 'both' and 'neither one'. Both ways of drawing the molecule are equally acceptable approximations of the bonding picture for the molecule, but neither one, by itself, is an accurate picture of the

delocalized π bonds. The two alternative drawings, however, *when considered together*, give a much more accurate picture than either one on its own. The two drawings imply, when considered together, that the carbon-carbon bonds are not double bonds, not single bonds, but halfway in between.

These two drawings are an example of what is referred to in organic chemistry as **resonance contributors**: two or more different Lewis structures depicting the same molecule or ion that, when considered together, do a better job of approximating delocalized π bonding than any single structure. By convention, resonance contributors are linked by a double-headed arrow:



resonance contributor A resonance contributor B

*A and B are different drawings of the **same molecule!***

In order to make it easier to visualize the difference between two resonance contributors, small **curved arrows** are often used. Each of these arrows (colored red for emphasis in the figure above) depicts the 'movement' of two π electrons. A few chapters from now when we begin to study organic reactions - a process in which electron density shifts and covalent bonds between atoms break and form - this 'curved arrow notation' will become extremely important in depicting electron movement. In the drawing of resonance contributors, however, this electron 'movement' occurs only in our minds, as we try to visualize delocalized π bonds. Nevertheless, use of the curved arrow notation is an essential skill that you will need to develop in drawing resonance contributors.

The depiction of benzene using the two resonance contributors A and B in the figure above does *not* imply that the molecule at one moment looks like structure A, then at the next moment shifts to look like structure B. Rather, at all moments, the molecule is a combination, or **resonance hybrid** of both A and B.

Caution! It is very important to be clear that in drawing two (or more) resonance contributors, we are not drawing two different molecules: they are simply *different depictions of the exact same molecule*. Furthermore, the double-headed resonance arrow does NOT mean that a chemical reaction has taken place.

Usually, derivatives of benzene (and phenyl groups, when the benzene ring is incorporated into a larger organic structure) are depicted with only one resonance contributor, and it is assumed that the reader understands that resonance hybridization is implied. This is the convention that will be used for the most part in this book. In other books or articles, you may sometimes see benzene or a phenyl group drawn with a circle inside the hexagon, either solid or dashed, as a way of drawing a resonance hybrid.

resonance contributor drawings resonance hybrid drawings

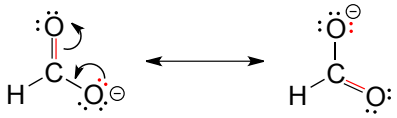
2.3B: Resonance contributors for the carboxylate group

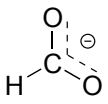
The convention of drawing two or more resonance contributors to approximate a single structure may seem a bit clumsy to you at this point, but as you gain experience you will see that the practice is actually very useful when discussing the manner in which many functional groups react. Let's next consider the carboxylate ion (the conjugate base of a carboxylic acid). As our example, we will use formate, the simplest possible carboxylate-containing molecule. The conjugate acid of formate is formic acid, which causes the painful sting you felt if you have ever been bitten by an ant.

formate formic acid

Usually, you will see carboxylate groups drawn with one carbon-oxygen double bond and one carbon-oxygen single bond, with a negative formal charge located on the single-bonded oxygen. In actuality, however, the two carbon-oxygen bonds are the same length, and although there is indeed an overall negative formal charge on the group, it is shared equally (delocalized) between the two oxygens. Therefore, the carboxylate can be more accurately depicted by a *pair* of resonance contributors. Alternatively, a single structure can be used, with a dashed line depicting the resonance-delocalized π bond and the negative charge located in between the two oxygens.

negative charge is delocalized to both oxygens

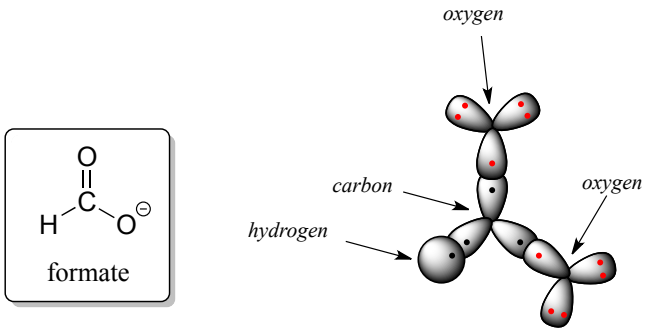




resonance hybrid drawing

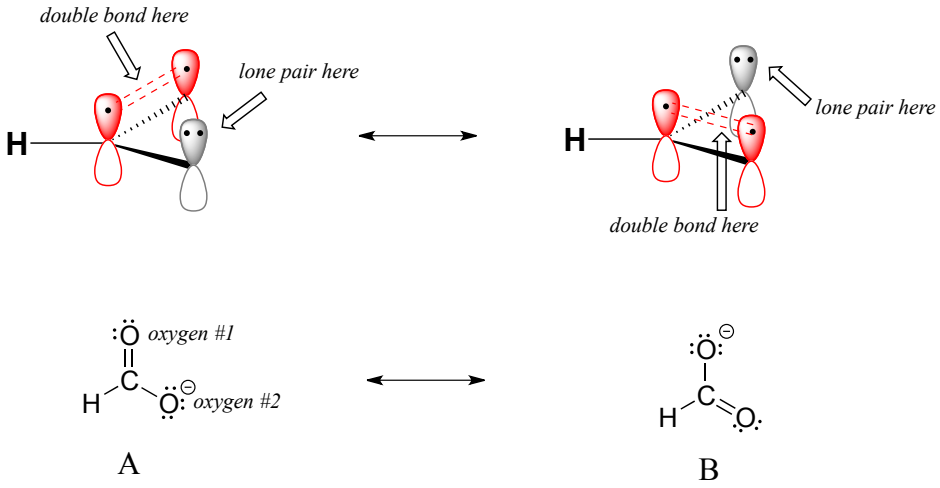
Let's see if we can correlate these drawing conventions to valence bond theory. The carbon is sp^2-hybridized: the bond angles are close to 120˚, and the molecule is planar.

Both carbon-oxygen σ bonds, then, are formed from the overlap of carbon sp^2 orbitals and oxygen sp^2 orbitals.



the σ-bonding framework of formate

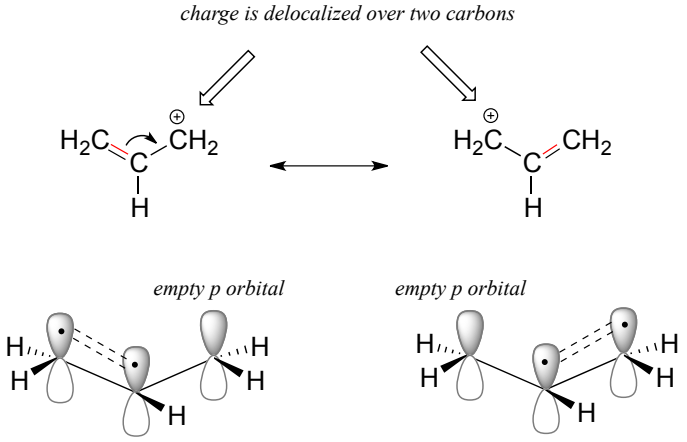
In addition, the carbon and both oxygens each have an unhybridized $2p_z$ orbital situated perpendicular to the plane of the σ bonds. These three $2p_z$ orbitals are parallel to each other, and can overlap in a side-by-side fashion to form a delocalized π bond.



Resonance contributor A shows oxygen #1 sharing a pair of electrons with carbon in a π bond, and oxygen #2 holding a lone pair of electrons in its *p* orbital. Resonance contributor B, on the other hand, shows oxygen #2 participating in the π bond with carbon, and oxygen #1 holding a lone pair in its *p* orbital. Overall, the situation is one of ***three*** *parallel, overlapping p orbitals sharing* ***four*** *delocalized π electrons*. Because there is one more electron than there are *p* orbitals, the system has an overall charge of –1. This is the kind of 3D picture that resonance contributors are used to approximate, and once you get some practice you should be able to quickly visualize overlapping *p* orbitals and delocalized π electrons whenever you see resonance structures being used. In this text, carboxylate groups will usually be drawn showing only one resonance contributor for the sake of simplicity, but you should always keep in mind that the two C-O bonds are equal, and that the negative charge is delocalized to both oxygens.

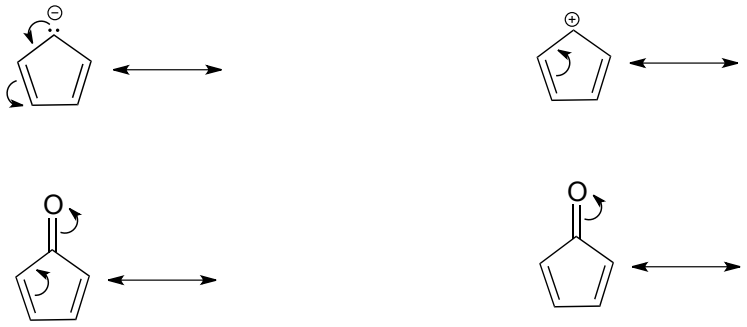
Exercise 2.13: A third resonance contributor for formate (which we will soon learn is considered a 'minor' contributor), has a positive formal charge on the carbon and negative formal charges on both oxygens. Draw this resonance contributor, and draw an orbital picture showing the location of the π electrons in this contributor.

Here's another example, this time with a carbocation. Recall from section 2.1C that carbcations are sp^2-hybridized, with an empty *p* orbital oriented perpendicular to the plane formed by three σ bonds. If a carbocation is adjacent to a double bond, then three *p* orbitals can overlap and share the two π electrons - another kind of conjugated π system in which the positive charge is shared over two carbons.

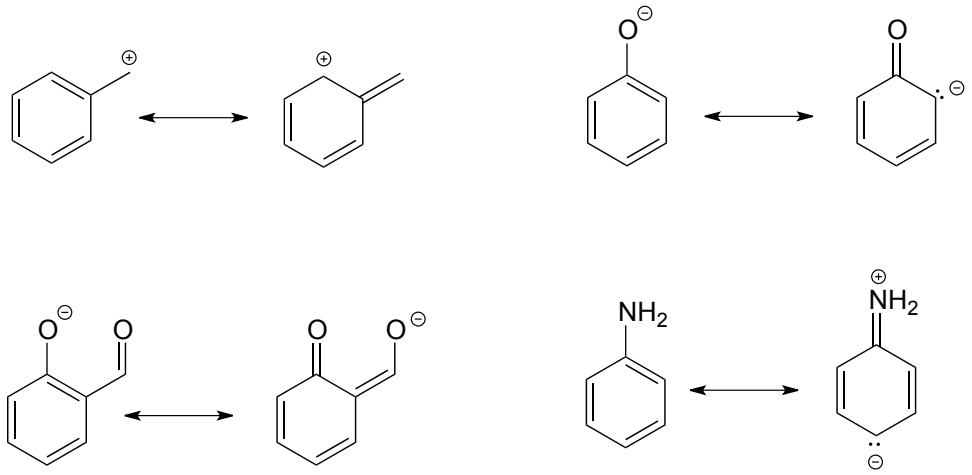


three *p* orbitals sharing two π electrons

Exercise 2.14: Draw the resonance contributors that correspond to the curved, two-electron movement arrows in the resonance expressions below.



Exercise 2.15: In each resonance expression, draw curved two-electron movement arrows on the left-side contributor that shows how we get to the right-side contributor. Be sure to include formal charges.



2.3C: Rules for drawing resonance structures

As you work on learning how to draw and interpret resonance structures, there are a few basic rules that you should keep in mind in order to avoid drawing nonsensical structures. All of these rules make perfect sense as long as you keep in mind that resonance contributors are merely a human-invented convention for depicting the delocalization of π electrons in conjugated systems.

Rules for drawing resonance structures:

1) When you see two different resonance contributors, you are *not* seeing a chemical reaction! Rather, you are seeing the exact same molecule or ion depicted in two different ways.

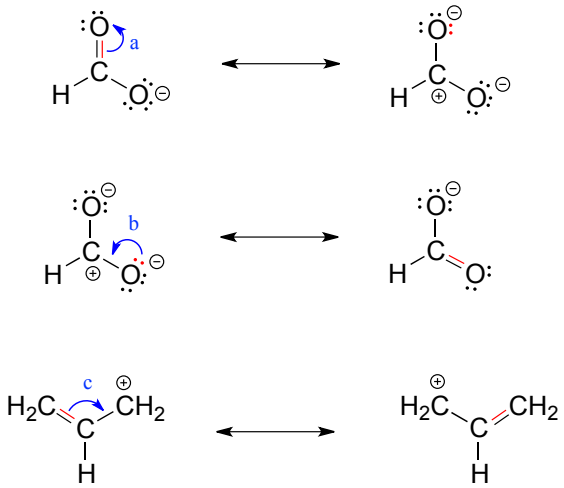
2) Resonance contributors involve the 'imaginary movement' of π-bonded electrons or of lone-pair electrons that are adjacent to (*i.e*. conjugated to) π bonds. You can *never* shift the location of electrons in σ bonds – if you show a σ bond forming or breaking, you are showing a chemical reaction taking place (see rule #1). Likewise, the positions of *atoms* in the molecule cannot change between two resonance contributors.

3) All resonance contributors for a molecule or ion must have the same *net* charge.

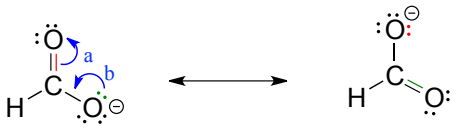
4) All resonance contributors must be drawn as proper Lewis structures, with correct formal charges. Never show curved 'electron movement' arrows that would lead to a situation where a second-row element (ie. carbon, nitrogen, or oxygen) has more than eight electrons: this would break the 'octet rule'. Sometimes, however, we will draw resonance contributors in which a carbon

atom has only six electrons (ie. a carbocation). In general, all oxygen and nitrogen atoms should have a complete octet of valence electrons.

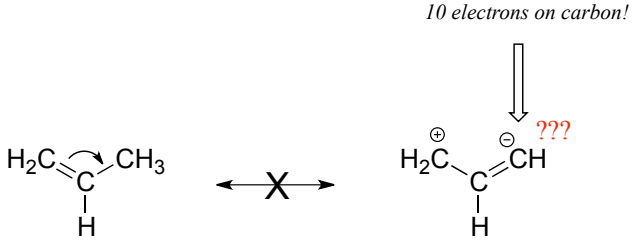
To expand a bit on rule #4, there are really only three things we can do with curved arrows when drawing resonance structures. First, we can take the two electrons in a π bond and shift them to become a lone pair on an adjacent atom (arrow 'a' below). Second, we can take a lone pair on an atom and put those two electrons into a π bond on the same atom (arrow 'b'). Third, we can shift a π bond one position over (arrow c).



Resonance arrows can also be combined - below, we show arrows a and b together:

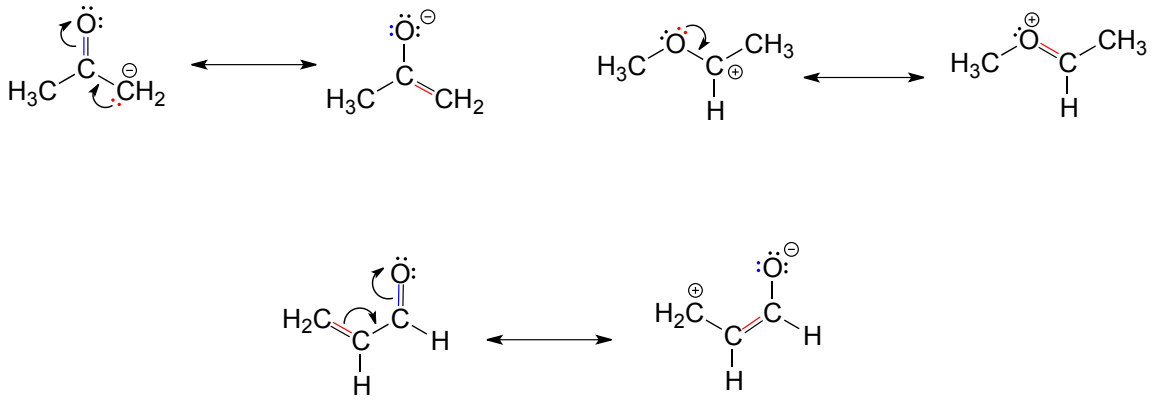


Notice that we do not exceed the octet rule on any atoms when we move electrons with arrows a, b and c. The resonance picture below shows an 'illegal' movement of electrons, because it would result in a carbon with five bonds, or 10 valence electrons (this would break the octet rule):

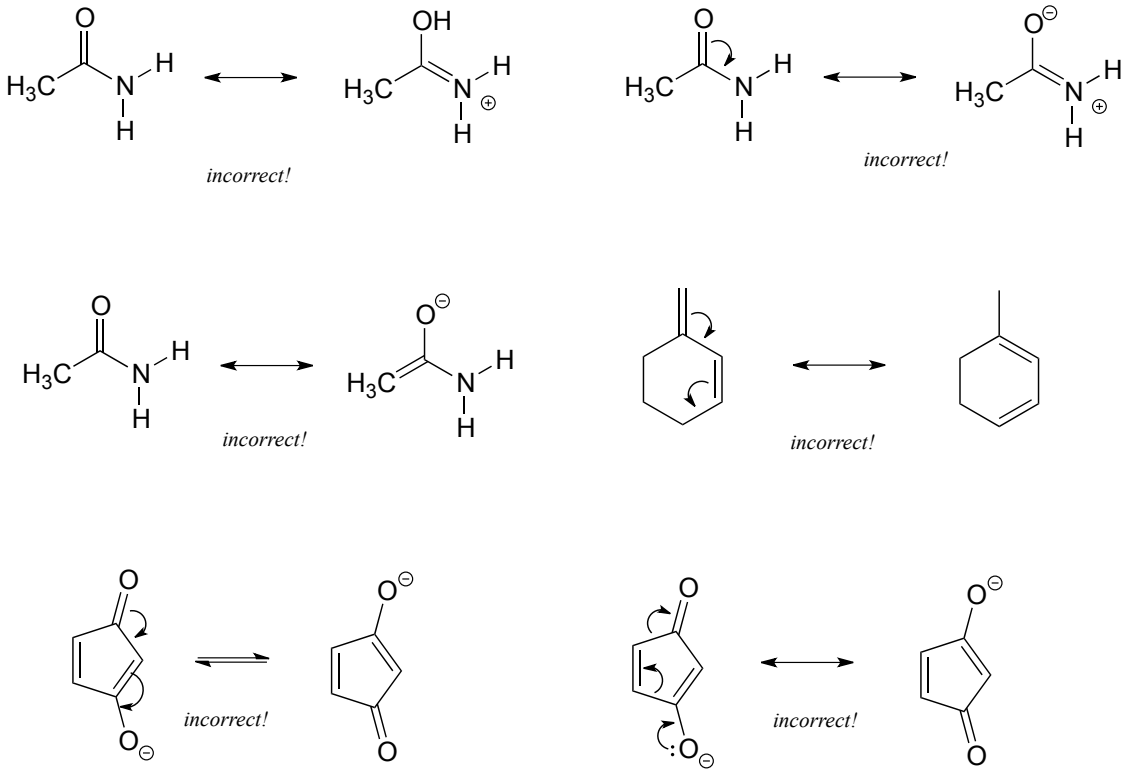


Always be very careful when drawing resonance structures that your arrows do ***only*** the three types of electron movement described above, and that you ***never*** exceed the octet rule on a second-row element. It is often helpful (but optional), to include all lone-pair electrons on oxygen and nitrogen in the drawing in order to keep track of valence electrons, avoid breaking the octet rule, and recognize when atoms have a negative or positive formal charge. Getting the 'electron accounting' correct is a big part of working with resonance contributors.

Below are a few more examples of 'legal' resonance expressions. Confirm for yourself that the octet rule is not exceeded for any atoms, and that formal charges are correct.

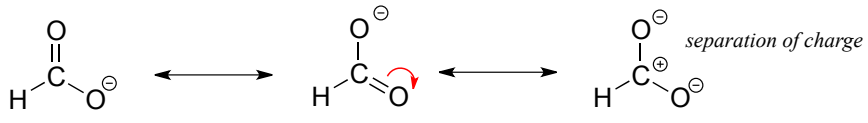


Exercise 2.16: Each of the 'illegal' resonance expressions below contains one or more mistakes. Explain what is incorrect in each.



2.3D: Major vs minor resonance contributors

Different resonance contributors do not always make the same contribution to the overall structure of the hybrid - rather, in many cases one contributor comes closer to depicting the actual bonding picture than another. In the case of carboxylates, contributors A and B below are equivalent in terms of their relative contribution to the hybrid structure. However, there is also a third resonance contributor 'C, in which the carbon bears a positive formal charge and both oxygens are single-bonded and bear negative charges.



A B C
(minor contributor)

Structure C makes a less important contribution to the overall bonding picture of the group relative to A and B. How do we know that structure C is the 'minor' contributor? There are four basic rules which you need to learn in order to evaluate the relative importance of different resonance contributors. We will number them 5-8 so that they may be added to in the 'rules for resonance' list from section 2.2C.

Rules for determining major and minor resonance contributors:

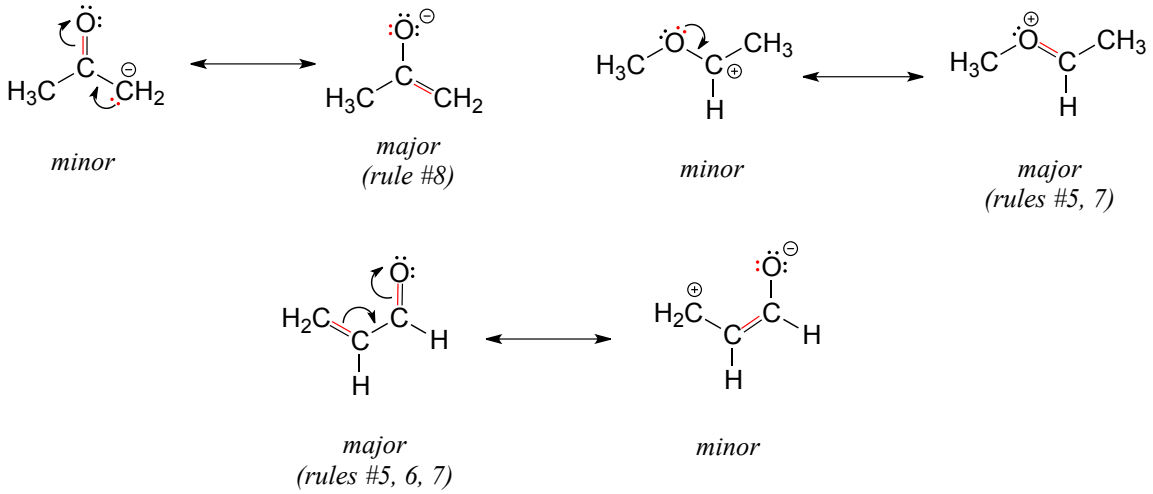
5) The carbon in contributor C does not have an octet – in general, resonance contributors in which a carbon does not fulfill the octet rule are relatively less important.

6) In structure C, a separation of charge has been introduced that is not present in A or B. In general, resonance contributors in which there is a greater separation of charge are relatively less important.

7) In structure C, there are only three bonds, compared to four in A and B. In general, a resonance structure with a lower number of total bonds is relatively less important.

8) The resonance contributor in which a negative formal charge is located on a more electronegative atom, usually oxygen or nitrogen, is more stable than one in which the negative charge is located on a less electronegative atom such as carbon. *An example is in the upper left expression in the next figure.*

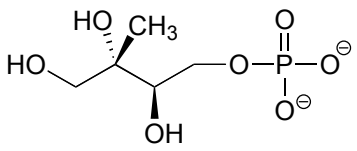
Below are some additional examples of major and minor resonance contributors:



Why do we worry about a resonance contributor if it is the minor one? We will see later that very often a minor contributor can still be extremely important to our understanding of how a molecule reacts.

Exercise 2.17: a) Draw a minor resonance structure for acetone (IUPAC name 2-propanone). Explain why it is a minor contributor. b) Are acetone and 2-propanol resonance contributors of each other? Explain.

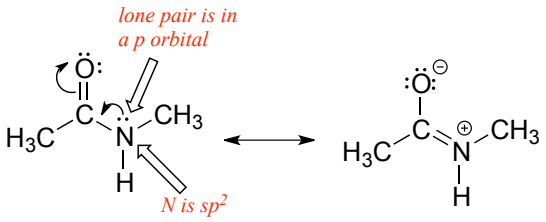
Exercise 2.18: Draw four additional resonance contributors for the molecule below. Label each one as major or minor (the structure below is of a major contributor).



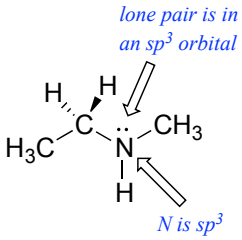
Exercise 2.19: Draw three resonance contributors of methyl acetate (IUPAC name methyl methanoate), and order them according to their relative importance to the bonding picture of the molecule. Explain your reasoning, and include curved arrows showing how you get from contributor #1 (most important) to #2, and from #2 to #3 (least important).

2.3E: Resonance applied to peptide bonds

What is the hybridization state of the nitrogen atom in an amide? At first glance, it would seem logical to say that it is sp^3-hybridized, because, like the nitrogen in an amine, the Lewis structure shows three single bonds and a lone pair. The picture looks quite different, though, if we consider another resonance contributor in which the nitrogen has a double bond to the carbonyl carbon: in this case, we would have to say that applicable hybridization is sp^2, and the bonding geometry trigonal planar rather than tetrahedral.



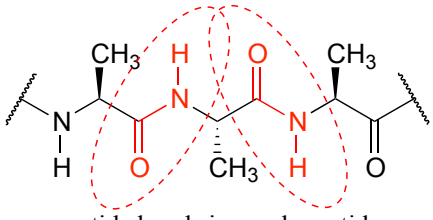
amide



amine

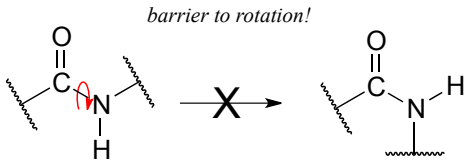
In fact, the latter picture is more accurate: the lone pair of electrons on an amide nitrogen are not localized in an sp^3 orbital, rather, they are delocalized as part of a conjugated π system, and the bonding geometry around the nitrogen is trigonal planar as expected for sp^2 hybridization. This is a good illustration of an important point: conjugation and the corresponding delocalization of electron density is stabilizing, thus if conjugation can occur, it probably will.

One of the most important examples of amide groups in nature is the 'peptide bond' that links amino acids to form polypeptides and proteins.



peptide bonds in a polypeptide

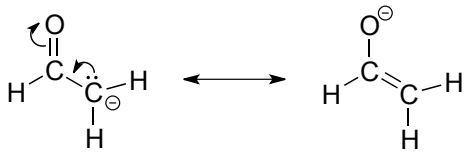
Critical to the structure of proteins is the fact that, although it is conventionally drawn as a single bond, *the C-N bond in a peptide linkage has a significant barrier to rotation*, indicating that to some degree, C-N π overlap is present - in other words, there is some double bond character, and the nitrogen is sp^2 hybridized with trigonal planar geometry.



The barrier to rotation in peptide bonds is an integral part of protein structure, introducing more rigidity to the protein's backbone. If there were no barrier to rotation in a peptide bond, proteins would be much more 'floppy' and three dimensional folding would be very different.

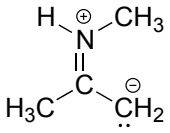
Exercise 2.20: Draw two pictures showing the unhybridized *p* orbitals and the location of π electrons in methyl amide. One picture should represent the major resonance contributor, the other the minor contributor. How many overlapping *p* orbitals are sharing how many π-bonded electrons?

Exercise 2.21: Draw two pictures showing the unhybridized *p* orbitals and the location of π electrons in the 'enolate' anion shown below. One picture should represent the major resonance contributor, the other the minor contributor. How many overlapping *p* orbitals are sharing how many π-bonded electrons?



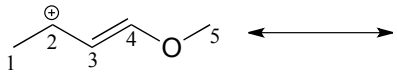
Exercise 2.22: Below is a minor resonance contributor of a species known as an 'enamine', which we will study more in chapter x. Draw the major resonance contributor

for the enamine, and explain why your contributor is the major one (refer to resonance rules #5-8 from this section).

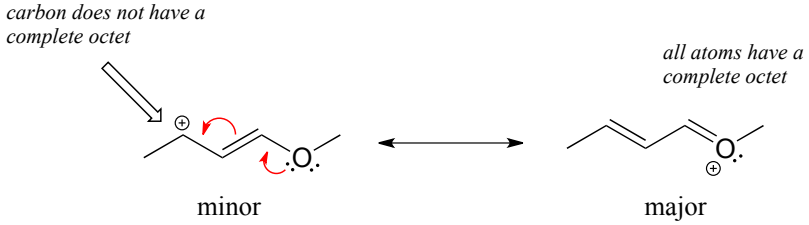


Solved example:

Draw the major resonance contributor of the structure below. Include in your figure the appropriate curved arrows showing how you got from the given structure to your structure. Explain why your contributor is the major one. In what kind of orbitals are the two lone pairs on the oxygen?

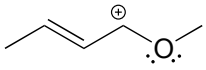


Solution: In the structure above, the carbon with the positive formal charge does not have a complete octet of valence electrons. Using the curved arrow convention, a lone pair on the oxygen can be moved to the adjacent bond to the left, and the electrons in the double bond shifted over to the left (see the rules for drawing resonance contributors to convince yourself that these are 'legal' moves).



The resulting resonance contributor, in which the oxygen bears the formal charge, is the major one because all atoms have a complete octet, and there is one additional bond drawn (resonance rules #5 and #7 both apply). This system can be thought of as four parallel *p* orbitals (one each on C_2, C_3, and C_4, plus one on oxygen) sharing four π electrons. One lone pair on the oxygen is in an unhybridized *p* orbital and is part of the conjugated π system, and the other is located in an sp^2 orbital.

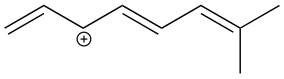
Also note that one additional contributor can be drawn, but it is also minor because it has a carbon with an incomplete octet:



minor

Exercise 2.23:

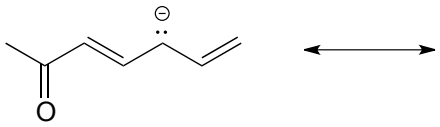
a) Draw three additional resonance contributors for the carbocation below. Include in your figure the appropriate curved arrows showing how one contributor is converted to the next.

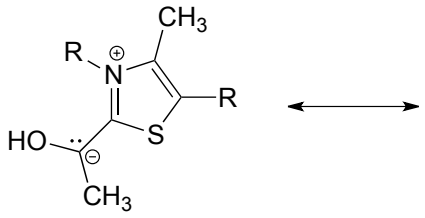


b) Fill in the blanks: the conjugated π system in this carbocation is composed of _____ *p* orbitals sharing _____ delocalized π electrons.

Exercise 2.24:

Draw the major resonance contributor for each of the anions below.

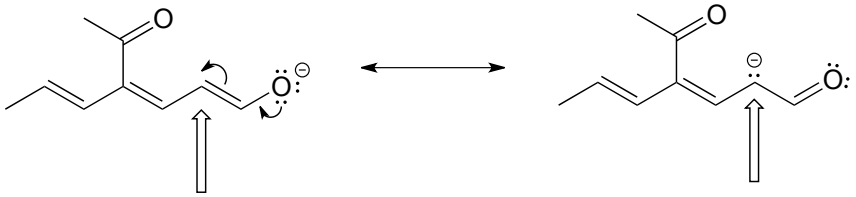




c) Fill in the blanks: the conjugated π system in part (a) is composed of _____ *p* orbitals containing _____ delocalized π electrons.

Exercise 2.25: The figure below shows how the negative formal charge on the oxygen can be delocalized to the carbon indicated by an arrow. More resonance contributors can be drawn in which negative charge is delocalized to three other atoms on the molecule.

a) Circle these atoms.
b) Draw the two most important resonance contributors for the molecule.



A word of advice

Becoming adept at drawing resonance contributors, using the curved arrow notation to show how one contributor can be converted to another, and understanding the concepts of conjugation and resonance delocalization are some of the most challenging but also most important jobs that you will have as a beginning student of organic chemistry. If you work hard now to gain a firm grasp of these ideas, you will have come a long way toward understanding much of what follows in your organic chemistry course. Conversely, if you fail to come to grips with these concepts now, a lot of what you see later in the course will seem like a bunch of mysterious and incomprehensible lines, dots, and arrows, and you will be in for a rough ride, to say the least. More so than many other topics in organic chemistry, *understanding bonding, conjugation, and resonance is something that most students really need to work on 'in person' with an instructor or tutor, preferably using a molecular modeling kit*. Keep working problems, keep asking questions, and keep at it until it all makes sense!

Section 2.4: Non-covalent interactions

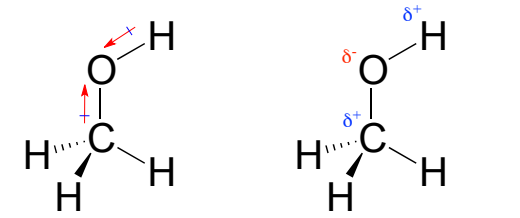
Until now we have been focusing on understanding the covalent bonds that hold individual molecules together. We turn next to a review on the subject of non-covalent interactions *between* molecules, or between different functional groups within a single molecule. You have probably learned all of these concepts already in your general chemistry course, but this review will focus on applications to organic and biological chemistry, and specifically will allow us to explain differences in physical properties - such boiling points, melting points, and solubility - between different organic compounds. An understanding of noncovalent interactions is also critical for looking at the environment inside the active site of an enzyme, where much of the chemistry that we will study in this book takes place.

2.4A: Dipoles

To understand the nature of noncovalent interactions, we first must return to covalent bonds and delve into the subject of dipoles. Many of the covalent bonds that we have seen – between two carbons, for example, or between a carbon and a hydrogen –involve the approximately equal sharing of electrons between the two atoms in the bond. In these

examples, the two atoms have approximately the same **electronegativity**. Recall from your general chemistry course that electronegativity refers to " the power of an atom in a molecule to attract electrons to itself" (this is the definition offered by Linus Pauling, the eminent 20th-century American chemist who was primarily responsible for developing many of the bonding concepts that we have been learning).

However, quite often in organic chemistry we deal with covalent bonds between two atoms with different electronegativities, and in these cases the sharing of electrons is not equal: the more electronegative nucleus pulls the two electrons closer. In the carbon-oxygen bond of an alcohol, for example, the two electrons in the σ bond are held more closely to the oxygen than they are to the carbon, because oxygen is significantly more electronegative than carbon. The same is true for the oxygen-hydrogen bond, as hydrogen is slightly less electronegative than carbon, and much less electronegative than oxygen.

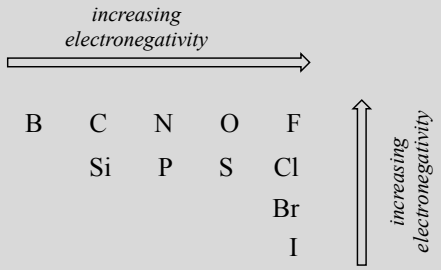


bond dipole arrows partial charge notation

The result of this unequal sharing is what we call a **bond dipole**, which exists in a **polar covalent bond**. A bond dipole has both negative and positive ends, or poles, where electron density is lower (the positive pole) and higher (the negative pole). The difference in electron density can be expressed using the Greek letter δ (delta) to denote 'partial positive' and 'partial negative' charge on the atoms. 'Dipole arrows', with a positive sign on the tail, are also used to indicated the negative (higher electron density) direction of the dipole.

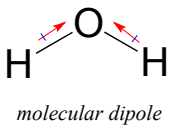
The degree of polarity in a covalent bond depends on the difference in electronegativity between the two atoms. Electronegativity is a periodic trend: it increases going from left to right across a row of the periodic table of the elements, and also increases as we move up a column. Therefore, oxygen is more electronegative than nitrogen, which is in turn more electronegative than carbon. Oxygen is also more electronegative than sulfur. Fluorine, in the top right corner of the periodic table, is the most electronegative of the elements. Hydrogen is slightly less electronegative than carbon.

Periodic trends in electronegativity

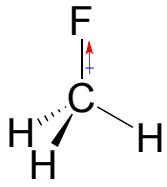


Exercise 2.26: Using what you about atomic orbitals, rationalize the periodic trends in electronegativity. Why does it increase from left to right, and decrease from top to bottom? This is a good question to talk through with classmates and an instructor or tutor.

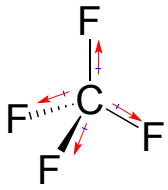
Most molecules contain both polar and nonpolar covalent bonds. Depending on the location of polar bonds and bonding geometry, molecules may posses a net polarity, called a **molecular dipole moment**. Water, as you probably recall, has a dipole moment that results from the combined dipoles of its two oxygen-hydrogen bonds. Fluoromethane also has a dipole moment.



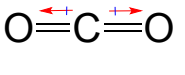
molecular dipole



molecular dipole



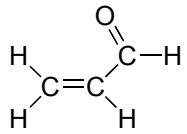
no *molecular dipole*

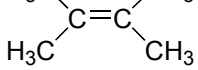


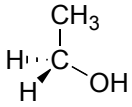
no *molecular dipole*

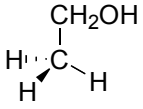
Tetrafluoromethane, however, has four polar bonds that pull equally in to the four corners of a tetahedron, meaning that although there are four bond dipoles there is no overall *molecular* dipole moment. Carbon dioxide also lacks a molecular dipole moment.

Exercise 2.27: Which of the molecules below have molecular dipole moments?

a) 

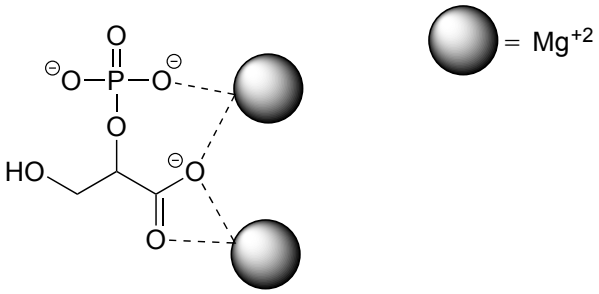
b) 

c) 

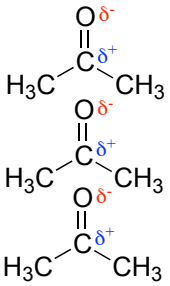
d) 

2.3B: Ion-ion, dipole-dipole and ion-dipole interactions

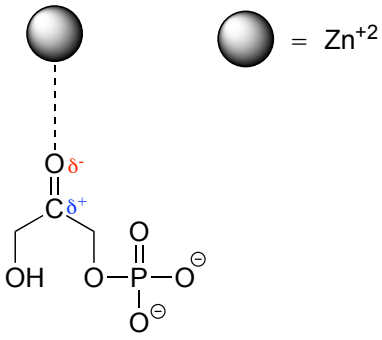
The strongest type of non-covalent interaction is between two ionic groups of opposite charge (an **ion-ion** or **charge-charge interaction**). You probably saw lots of examples of ionic bonds in inorganic compounds in your general chemistry course: for example, table salt is composed of sodium cations and chloride anions, held in a crystal lattice by ion-ion interactions. One of the most common examples in biological organic chemistry is the interaction between a magnesium cation (Mg^{+2}) and an anionic carboxylate or phosphate group. The figure below shows 2-phosphoglycerate, an intermediate in the glycolysis pathway, interacting with two Mg^{+2} ions in the active site of a glycolytic enzyme called enolase.



Polar molecules – those with an overall dipole moment, such as acetone – can align themselves in such a way as to allow their respective positive and negative poles to interact with each other. This is called a **dipole-dipole interaction**.



When a charged species (an ion) interacts favorably with a polar molecule or functional group, the result is called an **ion-dipole interaction**. A common example of ion-dipole interaction in biological organic chemistry is that between a metal cation, most often Mg^{+2} or Zn^{+2}, and the partially negative oxygen of a carbonyl.



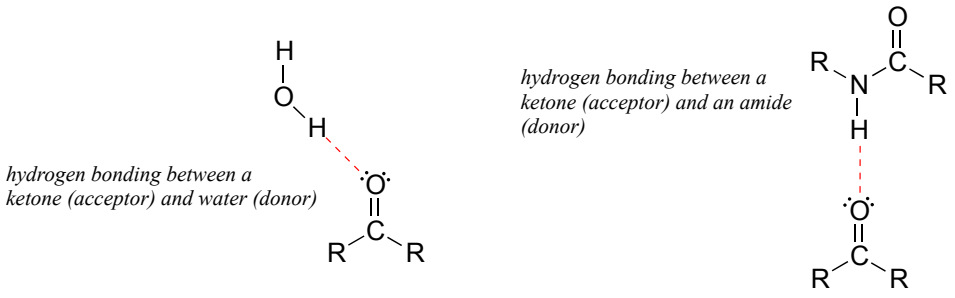
As we shall in later chapters, these types of ion-dipole interactions have important implications in terms of the reactivity of carbonyl groups in biochemical reactions.

2.4C: Van der Waals forces

Nonpolar molecules such as hydrocarbons also are subject to relatively weak but still significant attractive noncovalent forces. **Van der Waals forces** (also called **London dispersion forces** or **nonpolar interactions**) result from the constantly shifting electron density in any molecule. Even a nonpolar molecule will, at any given moment, have a weak, short-lived dipole. This transient dipole will induce a neighboring nonpolar molecule to develop a corresponding transient dipole of its own, with the end result that a transient dipole-dipole interaction is formed. These van der Waals forces are relatively weak, but are constantly forming and dissipating among closely-packed nonpolar molecules, and when added up the cumulative effect can become significant.

2.4D: Hydrogen bonds

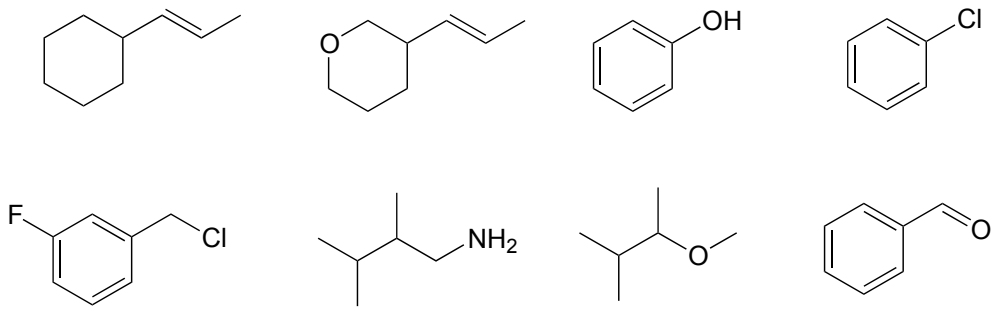
Hydrogen bonds result from the interaction between a hydrogen bonded to an electronegative heteroatom – specifically a nitrogen, oxygen, or fluorine – and lone-pair electrons on a nitrogen, oxygen, or fluorine a neighboring molecule or functional group. Because a hydrogen atom is just a single proton and a single electron, when it loses electron density in a polar bond it essentially becomes an approximation of a 'naked' proton, capable of forming a strong interaction with a lone pair on a neighboring electronegative atom.



Hydrogen bonds are usually depicted with dotted lines in chemical structures. A group that provides a proton to a hydrogen bond is said to be acting as a **hydrogen bond donor**.

A group that provides an oxygen or nitrogen lone pair is said to be acting as a **hydrogen bond acceptor**. Many common organic functional groups can participate in the formation of hydrogen bonds, either as donors, acceptors, or both. Water and alcohols, for example, can be both hydrogen bond donors and acceptors. A carbonyl, as it lacks a hydrogen bound to an oxygen or nitrogen, can only act as a hydrogen bond acceptor.

Exercise 2.28: Classify the structures below as: A) capable of being both a hydrogen bond donor and acceptor, B) capable of being a hydrogen bond acceptor, but not a donor, or C) not capable of participating in hydrogen bonding.

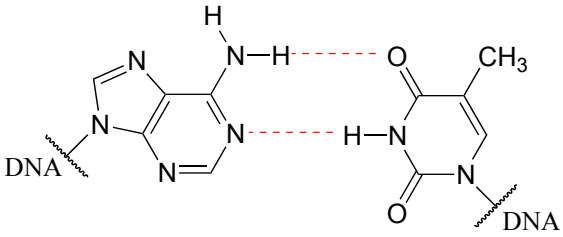


Exercise 2.29: Draw figures that show the hydrogen bonds described below.

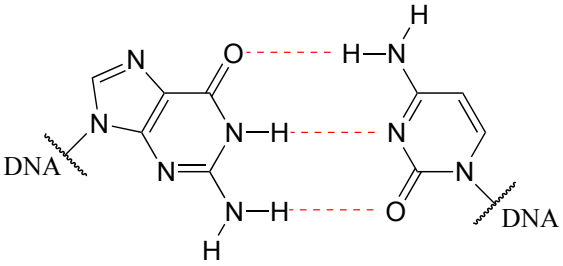
a) A hydrogen bond between methanol (donor) and water (acceptor).
b) A hydrogen bond between methanol (acceptor) and water (donor).
c) Two possible hydrogen bonds between methyl acetate and methylamine.

In general, hydrogen bonds are stronger than dipole-dipole interactions, but also much weaker than covalent bonds. The strength of hydrogen bonds has enormous implications in biology. Copying of DNA in the cell, for example, is based on very specific hydrogen bonding arrangements between DNA bases on complimentary strands: adenine pairs with thymine, while guanine pairs with cytidine:

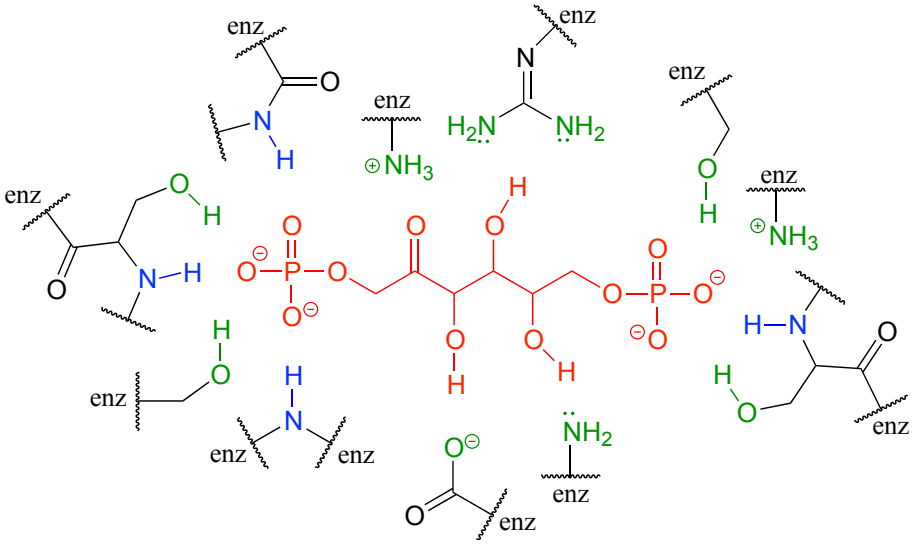
A-T base pair:



G-C base pair:



Hydrogen bonds, as well as the other types of noncovalent interactions, are very important in terms of the binding of a ligand to a protein. In section 1.3D, we saw a 'space-filling' picture of an enzyme with its substrate bound in its active site. Here, in a two-dimensional approximation, is an image of the same substrate-enzyme pair showing how amino acid side chain (green) and parent chain (blue) groups surround and interact with functional groups on the substrate (red).



Section 2.5: Physical properties of organic compounds

An understanding of the various types of noncovalent forces allows us to explain, on a molecular level, many observable physical properties of organic compounds. In this section, we will concentrate on solubility (especially solubility in water), melting point, and boiling point.

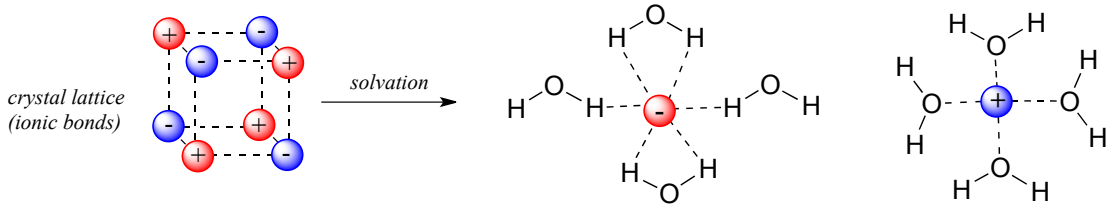
2.5A: Solubility

Virtually all of the organic chemistry that you will see in this course takes place in the solution phase. In the organic laboratory, reactions are often run in nonpolar or slightly polar solvents such as toluene (methylbenzene), dichloromethane, or diethylether. In recent years, much effort has been made to adapt reaction conditions to allow for the use of 'greener' (in other words, more environmentally friendly) solvents such as water or ethanol, which are polar and capable of hydrogen bonding. In biochemical reactions the solvent is of course water, but the 'microenvironment' inside an enzyme's active site - where the actual chemistry is going on - can range from very polar to very non-polar, depending on which amino acid residues are present.

You probably remember the 'like dissolves like' rule you learned in general chemistry, and even before you took any chemistry at all, you probably observed at some point in your life that oil does not mix with water. Let's revisit this rule, and put our knowledge of covalent and noncovalent bonding to work.

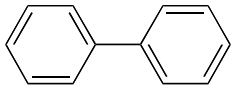
When considering the solubility of an organic compound in a given solvent, the most important question to ask ourselves is: how strong are the noncovalent interactions between the compound and the solvent molecules? If the solvent is polar, like water, then a smaller hydrocarbon component and/or more charged, hydrogen bonding, and other polar groups will tend to increase the solubility. If the solvent is non-polar, like hexane, then the exact opposite is true.

Imagine that you have a flask filled with water, and a selection of substances that you will test to see how well they dissolve in the water. The first substance is table salt, or sodium chloride. As you would almost certainly predict, especially if you've ever inadvertently taken a mouthful of water while swimming in the ocean, this ionic compound dissolves readily in water. Why? Because water, as a very polar molecule, is able to form many ion-dipole interactions with both the sodium cation and the chloride anion, the energy from which is more than enough to make up for energy required to break up the ion-ion interactions in the salt crystal.



The end result, then, is that in place of sodium chloride crystals, we have individual sodium cations and chloride anions surrounded by water molecules – the salt is now *in solution*. Charged species as a rule dissolve readily in water: in other words, they are very **hydrophilic** (water-loving).

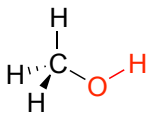
Now, we'll try a compound called biphenyl, which, like sodium chloride, is a colorless crystalline substance.



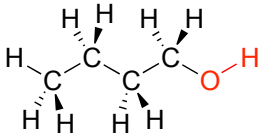
biphenyl

Biphenyl does not dissolve at all in water. Why is this? Because it is a very non-polar molecule, with only carbon-carbon and carbon-hydrogen bonds. It is able to bond to itself very well through nonpolar van der Waals interactions, but it is not able to form significant attractive interactions with very polar solvent molecules like water. Thus, the energetic cost of breaking up the biphenyl-to-biphenyl interactions in the solid is high, and very little is gained in terms of new biphenyl-water interactions. Water is a terrible solvent for nonpolar hydrocarbon molecules: they are very **hydrophobic** (water-fearing).

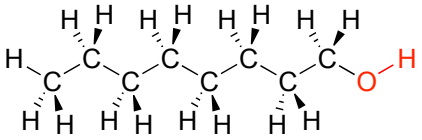
Next, you try a series of increasingly large alcohol compounds, starting with methanol (1 carbon) and ending with octanol (8 carbons).



methanol
very soluble in water



butanol
slightly soluble in water



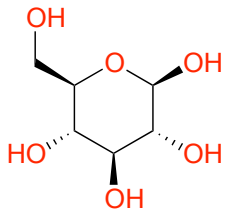
octanol
very insoluble in water

You find that the smaller alcohols - methanol, ethanol, and propanol - dissolve easily in water, at any water/alcohol ratio that you try. This is because the water is able to form hydrogen bonds with the hydroxyl group in these molecules, and the combined energy of formation of these water-alcohol hydrogen bonds is more than enough to make up for the energy that is lost when the alcohol-alcohol (and water-water) hydrogen bonds are broken up. When you try butanol, however, you begin to notice that, as you add more and more to the water, it starts to form a layer on top of the water. Butanol is only sparingly soluble in water.

The longer-chain alcohols - pentanol, hexanol, heptanol, and octanol - are increasingly non-soluble in water. What is happening here? Clearly, the same favorable water-alcohol hydrogen bonds are still possible with these larger alcohols. The difference, of course, is that the larger alcohols have larger nonpolar, hydrophobic regions in addition to their hydrophilic hydroxyl group. At about four or five carbons, the influence of the

hydrophobic part of the molecule begins to overcome that of the hydrophilic part, and water solubility is lost.

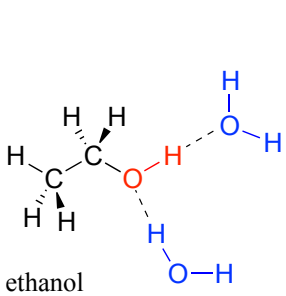
Now, try dissolving glucose in the water – even though it has six carbons just like hexanol, it also has five hydrogen-bonding, hydrophilic hydroxyl groups in addition to a sixth oxygen that is capable of being a hydrogen bond acceptor.



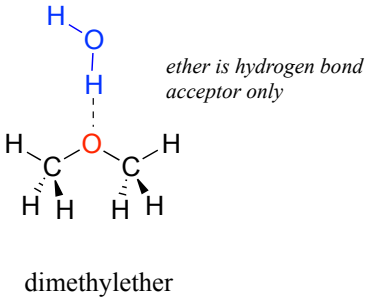
glucose

We have tipped the scales to the hydrophilic side, and we find that glucose is quite soluble in water.

We saw that ethanol was very water-soluble (if it were not, drinking beer or vodka would be rather inconvenient!) How about dimethyl ether, which is a constitutional isomer of ethanol but with an ether rather than an alcohol functional group? We find that diethyl ether is much less soluble in water. Is it capable of forming hydrogen bonds with water? Yes, in fact, it is –the ether oxygen can act as a hydrogen-bond acceptor. The difference between the ether group and the alcohol group, however, is that the alcohol group is both a hydrogen bond donor *and* acceptor.



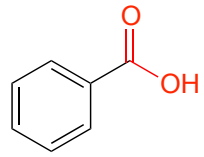
ethanol



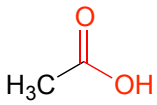
dimethylether

The result is that the alcohol is able to form more energetically favorable interactions with the solvent compared to the ether, and the alcohol is therefore much more soluble.

Here is another easy experiment that can be done (with proper supervision) in an organic laboratory. Try dissolving benzoic acid crystals in room temperature water – you'll find that it is not soluble. As we will learn when we study acid-base chemistry in a later chapter, carboxylic acids such as benzoic acid are relatively weak acids, and thus exist mostly in the acidic (protonated) form when added to pure water.



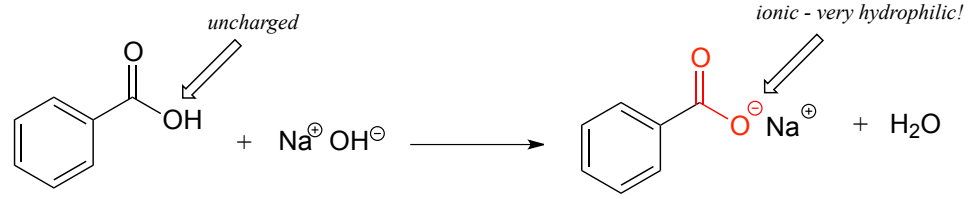
benzoic acid
insoluble in water



acetic acid
soluble in water

Acetic acid (vinegar) is quite soluble. This is easy to explain using the small alcohol vs large alcohol argument: the hydrogen-bonding, hydrophilic effect of the carboxylic acid group is powerful enough to overcome the hydrophobic effect of a single hydrophobic methyl group on acetic acid, but not the larger hydrophobic effect of the 6-carbon benzene group on benzoic acid.

Now, try slowly adding some aqueous sodium hydroxide to the flask containing undissolved benzoic acid. As the solvent becomes more and more basic, the benzoic acid begins to dissolve, until it is completely in solution.



benzoic acid
insoluble in water

sodium benzoate
soluble in water

What is happening here is that the benzoic acid is being converted to its conjugate base, benzoate. The *neutral* carboxylic acid group was not hydrophilic enough to make up for the hydrophobic benzene ring, but the carboxylate group, with its *full negative charge*, is much more hydrophilic. Now, the balance is tipped in favor of water solubility, as the powerfully hydrophilic anion part of the molecule drags the hydrophobic part into solution. Remember, charged species usually dissolve readily in water. If you want to precipitate the benzoic acid back out of solution, you can simply add enough hydrochloric acid to neutralize the solution and reprotonate the carboxylate.

If you are taking a lab component of your organic chemistry course, you will probably do at least one experiment in which you will use this phenomenon to physically separate an organic acid like benzoic acid from a hydrocarbon compound like biphenyl.

Similar arguments can be made to rationalize the solubility of different organic compounds in nonpolar or slightly polar solvents. In general, the greater the content of charged and polar groups in a molecule, the less soluble it tends to be in solvents such as hexane. The ionic and very hydrophilic sodium chloride, for example, is not at all soluble in hexane solvent, while the hydrophobic biphenyl is very soluble in hexane.

Because we are concentrating on the biologically relevant chemistry, let's take a minute to review how to evaluate a compound's solubility in water, the biological solvent:

Summary of factors contributing to water solubility:

A: How many carbons? All else being equal, more carbons means more of a non-polar/hydrophobic character, and thus lower solubility in water.

B: How many, and what kind of hydrophilic groups? The more, the greater the water solubility. In order of importance:

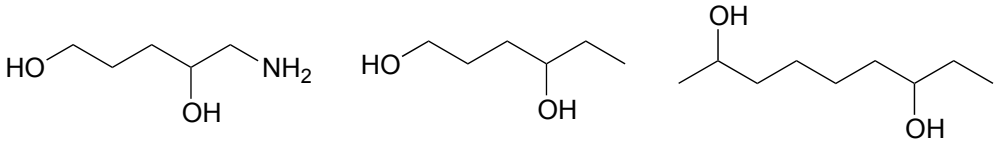
#1) Anything with a **charged** group (eg. ammonium, carboxylate, phosphate) is almost certainly water soluble, unless has a vary large nonpolar group, in which case it will most likely be soluble in the form of micelles, like a soap or detergent (see next section).

#2) Any functional group that can **donate a hydrogen bond** to water (eg. alcohols, amines) will significantly contribute to water solubility.
#3) Any functional group that can only **accept a hydrogen bond** from water (eg. ketones, aldehydes, ethers) will have a somewhat smaller but still significant effect on water solubility.

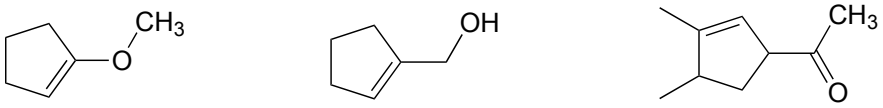
#4) Other groups that contribute to polarity (eg. alkyl halides, thiols sulfides) will make a small contribution to water solubility.

Exercise 2.30: Rank each set of three compounds below according to their solubility in water (most soluble to least):

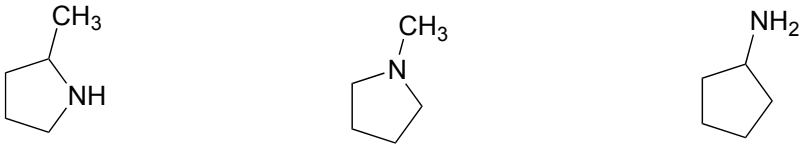
a)



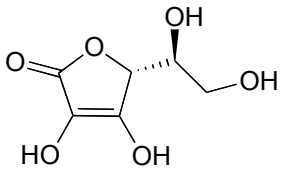
b)



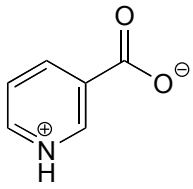
c)



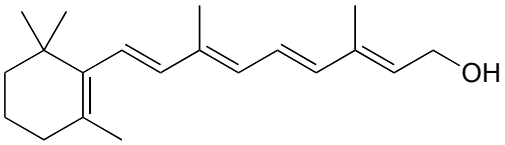
Exercise 2.31: Vitamins can be classified as water-soluble or fat-soluble (consider fat to be a very non-polar 'solvent'. Decide on a classification for each of the vitamins shown below.



Vitamin C
(ascorbic acid)

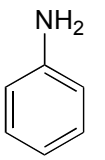


Vitamin B_3
(niacin)

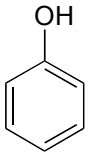


Vitamin A
(retinol)

Exercise 2.32: Both aniline and phenol are mostly insoluble in pure water. Predict the solubility of these two compounds in 10% aqueous hydrochloric acid, and explain your reasoning. *Hint* –aniline is much more basic than phenol!



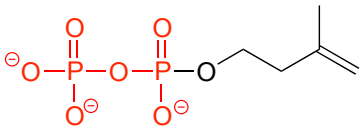
aniline



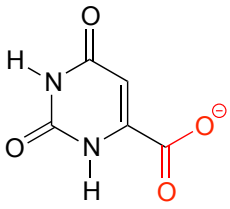
phenol

Exercise 2.33: Would you predict methanol or 2-propanol (rubbing alcohol) to be a better solvent for cyclohexanone? Why?

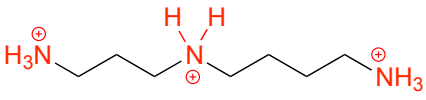
Because water is the biological solvent, most biological organic molecules, in order to maintain water-solubility, contain one or more charged functional groups: most often phosphate, ammonium or carboxylate.



ispentenyl diphosphate
an early precuror to cholesterol



orotate
a precursor to the RNA base uridine



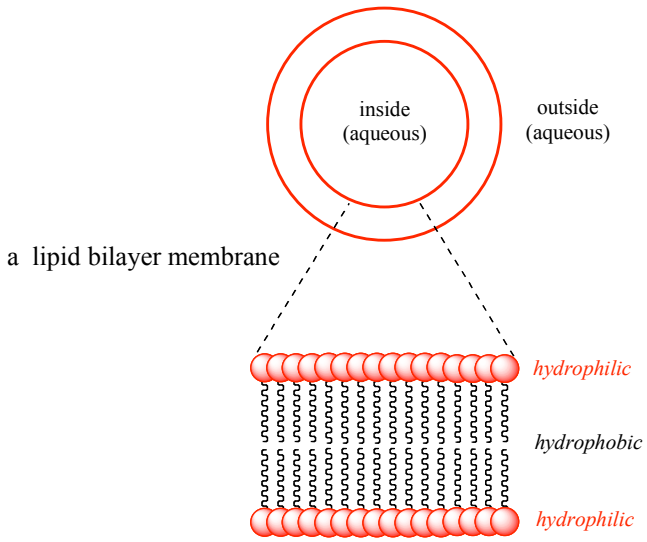
spermidine
binds tightly to DNA in rapidly dividing cells

Note that the charge on these functional groups depends on their protonation state: spermidine, for example, could be drawn with three (uncharged) amine groups rather than the charged ammonium groups as shown, and orotate could be drawn in the uncharged

carboxylic acid form. It turns out, however, that these three functional groups are all charged when in a buffer at the physiological pH of approximately 7.3. We will have much more to say about the acid-base aspects of these groups in chapter x.

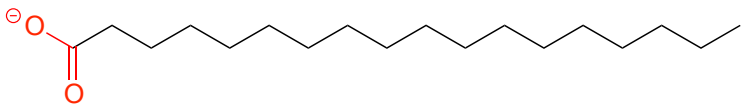
Carbohydrates often lack charged groups, but as we discussed in our 'thought experiment' with glucose, they are quite water-soluble due to the presence of multiple hydroxyl groups, which can hydrogen bond with water.

Some biomolecules, in contrast, contain distinctly hydrophobic components. Membrane lipids are **amphipathic**, meaning that they contain both hydrophobic and hydrophilic components. Cell membranes are composed of membrane lipids arranged in a 'bilayer', with the hydrophobic 'tails' pointing inward and the hydrophilic 'heads' forming the inner and outer surfaces, both of which are in contact with water.



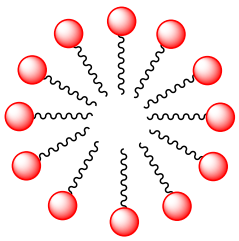
The nonpolar interior of the lipid bilayer is able to 'dissolve' hydrophobic biomolecules such as cholesterol. Polar and charged biomolecules, on the other hand, are not able to cross the membrane, because they are repelled by the hydrophobic environment of the bilayer's interior. The transport of water-soluble molecules across a membrane can be accomplished in a controlled and specific manner by special transmembrane transport proteins, a fascinating topic that you will learn more about if you take a class in biochemistry.

A similar principle is the basis for the action of soaps and detergents. Soaps are composed of fatty acids such as stearate obtained through basic hydrolysis of triacylglycerols (section 1.3A) in fats and oils.



stearate
a common fatty acid in animal fats

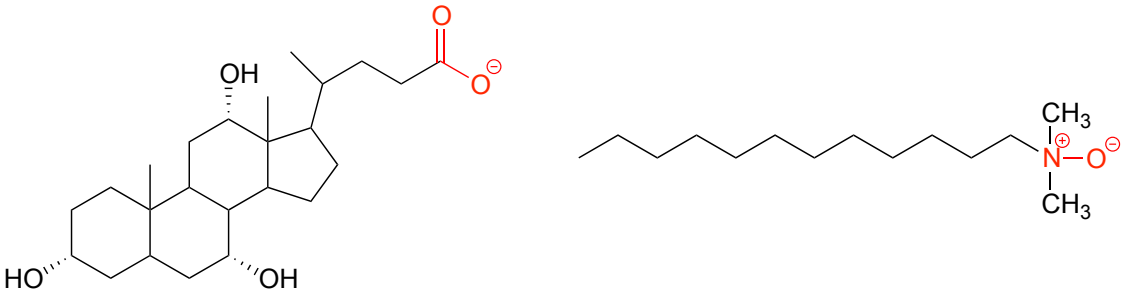
Like membrane lipids, fatty acids are amphipathic. In aqueous solution, the fatty acid molecules in soaps will spontaneously form **micelles**, a spherical structure that allows the hydrophobic tails to avoid contact with water and simultaneously form favorable van der Waals contacts with each other.



micelle

Because the outside of the micelle is charged, the structure as a whole is soluble in water. Micelles will form spontaneously around small particles of oil that normally would not dissolve in water, and will carry the particle away with it into solution. We will learn more about the chemistry of soap-making in chapter 11.

Synthetic detergents are non-natural amphipathic molecules that work by the same principle as that described for soaps.



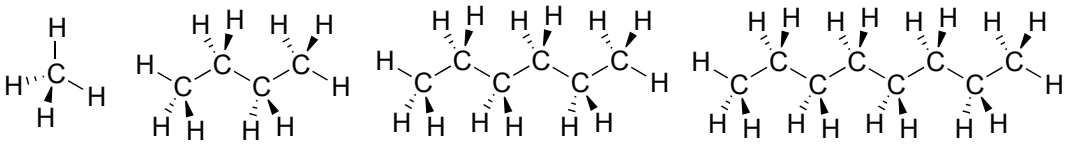
synthetic detergents

2.5B: Boiling point and melting point

The observable melting and boiling points of different organic molecules provides an additional illustration of the effects of noncovalent interactions. The overarching principle involved is simple: *how well can a compound bind to itself*? Melting and boiling are processes in which noncovalent interactions between identical molecules in a pure sample are disrupted. The stronger the noncovalent interactions, the more energy that is required, in the form of heat, to break them apart.

As a rule, larger molecules have higher boiling (and melting) points. Consider the boiling points of increasingly larger hydrocarbons. More carbons and hydrogens means a greater surface area possible for van der Waals interaction, and thus higher boiling points.

Below zero degrees centigrade (and at atmospheric pressure) butane is a liquid, because the butane molecules are held together by Van der Waals forces. Above zero degrees, however, the molecules gain enough thermal energy to break apart and enter the gas phase. Octane, in contrast, remains in the liquid phase all the way up to 128°C, due to the increased van der Waals interactions made possible by the larger surface area of the individual molecules.



methane
bp = -164°C

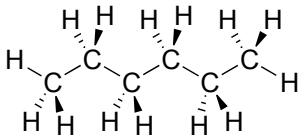
butane
bp = 0°C

hexane
bp = 68°C

octane
bp = 128°C

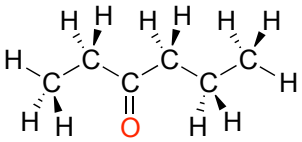
The strength of intermolecular hydrogen bonding and dipole-dipole interactions is reflected in higher boiling points. Look at the trend for hexane (van der Waals interactions only), 3-hexanone (dipole-dipole interactions), and 3-hexanol (hydrogen bonding). In all three molecules, van der Waals interactions are significant. The polar ketone group allows 3-hexanone to form intermolecular dipole-dipole interactions, in addition to the weaker van der Waals interactions. 3-hexanol, because of its hydroxyl group, is able to form intermolecular hydrogen bonds, which are stronger yet.

van der Waals only



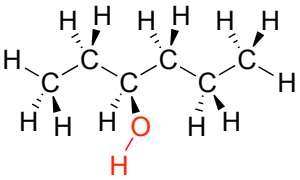
hexane
bp = 69°C

vdW plus dipole-dipole



3-hexanone
bp = 123 °C

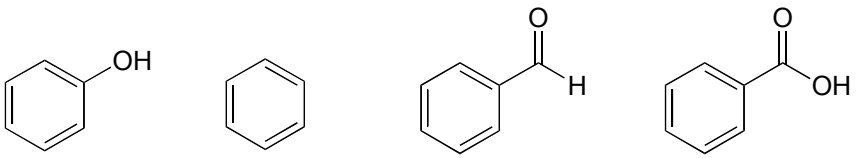
vdW plus hydrogen bonding



3-hexanol
bp =135°C

Of particular interest to biologists (and pretty much anything else that is alive on the planet) is the effect of hydrogen bonding in water. Because it is able to form tight networks of intermolecular hydrogen bonds, water remains in the liquid phase at temperatures up to 100 °C despite its small size. The world would obviously be a very different place if water boiled at 30 °C.

Exercise 2.34: Based on their structures, rank phenol, benzene, benzaldehyde, and benzoic acid in terms of lowest to highest boiling point. Explain your reasoning.



By thinking about noncovalent intermolecular interactions, we can also predict relative melting points. All of the same principles apply: stronger intermolecular interactions result in a higher melting point. Ionic compounds, as expected, usually have very high melting points due to the strength of ion-ion interactions. Just like with boiling points, the presence of polar and hydrogen-bonding groups on organic compounds generally leads to higher melting points. The size of a molecule influences its melting point as well as its boiling point, again due to increased van der Waals interactions between molecules.

What is different about melting point trends, that we don't see with boiling point or solubility trends, is the importance of a molecule's shape and its ability of pack tightly together. Picture yourself trying to make a stable pile of baseballs in the floor. It just doesn't work, because spheres don't pack together well - there is very little area of contact between each ball. It is very easy, though, to make a stack of flat objects like books.

The same concept applies to how well molecules pack together in a solid. The flat shape of aromatic compounds allows them to pack efficiently, and thus aromatics tend to have higher melting points compared to non-planar hydrocarbons with similar molecular weights. Comparing the melting points of benzene and toluene, you can see that the extra methyl group on toluene disrupts the molecule's ability to pack tightly, thus decreasing the cumulative strength of intermolecular van der Waals forces and lowering the melting point.

benzene
mp = 5.5 °C
bp = 80 °C

toluene
mp = -93 °C
bp = 111 °C

lower mp, higher bp!

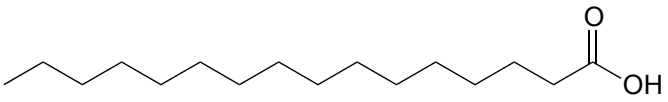
Note also that the boiling point for toluene is significantly above the boiling point of benzene! The key factor for the boiling point trend in this case is *size* (toluene has one more carbon), whereas for the melting point trend, *shape* plays a much more important role. This makes sense when you consider that melting involves 'unpacking' the molecules from their ordered array, whereas boiling involves simply separating them from their already loose (liquid) association with each other.

Exercise 2.35: Which would you expect to have the higher melting point, 2,3-dimethylbutane or hexane? Explain.

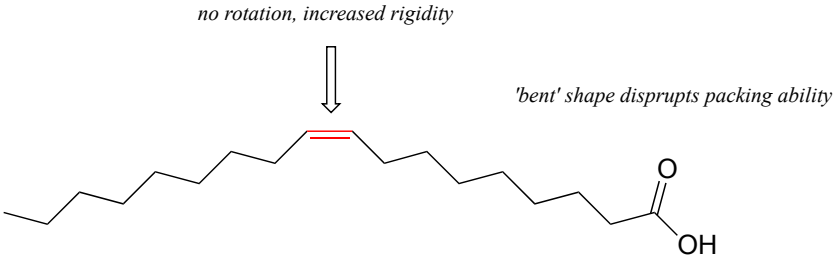
2.5C: Physical properties of lipids and proteins

Lipids

An interesting biological example of the relationship between molecular structure and melting point is provided by the observable physical difference between animal fats like butter or lard, which are solid at room temperature, and vegetable oils, which are liquid. Recall (section 1.3A) that fats and oils are triacylglycerols: fatty acids linked to a glycerol backbone. In vegetable oils, the fatty acid components are unsaturated, meaning that they contain one or more double bonds. Solid animal fat, in contrast, contains mainly saturated hydrocarbon chains, with no double bonds.



palmatic acid
(a saturated fatty acid)



oleic acid
(an unsaturated fatty acid)

The double bond(s) in vegetable oils cause those hydrocarbon chains to be more rigid, and ‘bent’ at an angle (remember that rotation is restricted around double bonds), with the result that they don’t pack together as closely, and thus can be broken apart (melted) more readily.

In a related context, the fluidity of a cell membrane (essentially, the melting point) is determined to a large extent by the length and degree of unsaturation of the fatty acid 'tails' on the membrane lipids. Longer and more saturated fatty acids make the membrane less fluid (they are able maximize van der Waals interactions), while shorter and more unsaturated fatty acids cause the membrane to be more fluid.

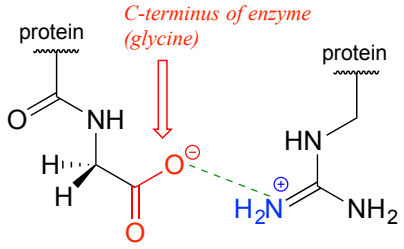
Proteins

The very same noncovalent forces we have just learned about are also integral to protein structure: when a protein folds up, it does so in such a way that very specific non-covalent interactions form between amino acid residues on different regions of the chain, each one becoming part of the 'molecular glue' that holds the chain together in its

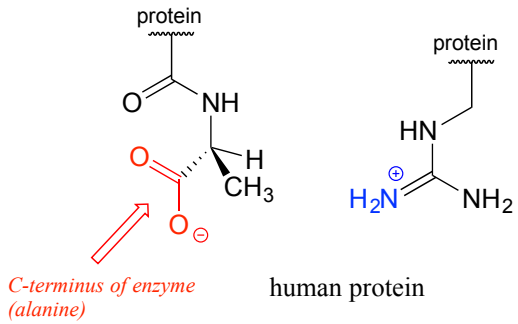
correctly folded shape. Hydrogen bonds and charge-charge interactions are particularly important in this respect. In general, the interior of a folded protein is relatively hydrophobic, while the outside surface, which of course is in constant contact with water, is very hydrophilic - many charged side chains such as aspartate, glutamate, lysine, and arginine point out of the surface of a protein structure.

Most of the proteins of 'mesophilic' organisms (those who thrive in intermediate temperatures, including humans) will denature - come unfolded - at high temperatures, as the heat disrupts the specific noncovalent interactions holding the protein chain together. Unfolded proteins usually are not water soluble because the more hydrophobic interior regions are no longer hidden from the solvent, so denaturing is accompanied by precipitation. Obviously, an unfolded protein also loses its functionality.

In the last few decades, we have become aware that a wide variety of microbes naturally inhabit extremely hot environments such as the boiling water of hot springs in Yellowstone National Park, or the base of a deep-sea thermal vent. How do the proteins of these 'thermophiles' hold up to the heat? There is nothing extraordinary about these proteins that makes them so resistant to heat, other than the fact that they have evolved so that they simply have *more* molecular 'glue' holding them together - in particular, more ionic interactions between oppositely charged residues. In just one of many examples, the three-dimensional structure of an enzyme from *Pyrococcus horikoshii*, a microbe isolated from a thermal vent deep in the Pacific Ocean, was compared to a very similar enzyme in humans. The thermophilic protein has a stabilizing charge-charge interaction between the terminal carboxylate group on the last amino acid in the chain and an arginine residue near the beginning of the chain.



thermostable protein



human protein

This interaction is not present in the human version of the protein because the terminal carboxylate group is angled away from the positively-charged group on the arginine. The single charge-charge interaction is not by itself responsible for the thermostability of *the P. horikoshii* protein - other similar interactions throughout the protein structure also contribute. (*PLOS Biology* **2011**, *9*, e1001027),

Conversely, proteins from 'psychrophilic' organisms - those which live in extremely cold temperatures, such as in arctic soils or in small water pockets in polar ice - have fewer stabilizing charge-charge interactions. This gives them the flexibility to function at temperatures in which mesophilic human or *E. coli* proteins would be frozen and

inactive. On the other hand, a typical psychrophilic protein will rapidly unfold, precipitate, and lose its functionality at room temperature.

Scientists are extremely interested in thermostable proteins, because the ability to function at high temperatures can be a very desirable trait for a protein used in industrial processes. In fact, thermostable DNA polymerase from *Thermus aquaticus* (the enzyme is known to molecular biologists as *'Taq* polymerase') is the enzyme that makes the PCR (polymerase chain reaction) process possible, and has earned billions of dollars in royalties for drug company Hoffman La Roche, the patent owner. Many research groups are searching for useful enzymes in thermophilic species, and others are working on ways to engineer heat stability into existing mesophilic enzymes by tinkering with their amino acid sequences to introduce new stabilizing charge-charge interactions.

Summary of Key Concepts

Understand **valence bond theory** and the **hybrid orbital** concept. Be able to identify the hybridization of all carbons in any organic molecule. Be able to accurately draw the π-bonding picture for a double bond (side-by-side overlapping *p* orbitals). Be able to describe what types of orbitals are overlapping to form the single and double bonds in an organic molecule.

Be able to distinguish **conjugated double bonds** from **isolated double bond**s. Be able to accurately draw the π-bonding picture for a conjugated π system.

Understand the concept of **resonance**, and understand that **resonance contributors** are *not* different molecules, just different ways of drawing the same molecule.

Be able to draw resonance contributors correctly. Be able to draw **curved arrows** between any two contributors.

Major and minor resonance contributors: be able to evaluate the relative importance of contributors.

Understand the concept of resonance **delocalization** - what it means when we say that a charge is delocalized, or that π electrons are delocalized.

Recognize and understand **noncovalent interactions**: Van der Waals, ion-ion, ion-dipole, dipole-dipole, and hydrogen bonding. Be able to recognize whether a group is acting as a hydrogen bond donor or acceptor.

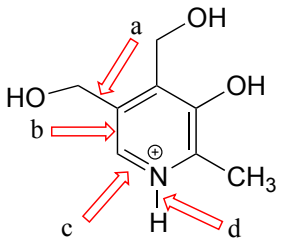
Be able to use your knowledge of noncovalent intermolecular interactions to predict trends in solubility, melting point, and boiling point.

Problems

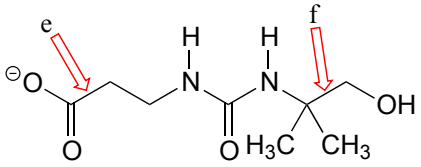
In questions involving drawing resonance contributors, assume that all second-row atoms should have a complete octet in all structures with the exception of positively-charged carbons.

P2.1: For each of the bonds indicated by arrows b-f in the figures below, describe the bonding picture. An example is given for bond 'a'. Note that for a double bond (bond 'c'), you will need to describe *two* bonds.

bond a: "this is a σ bond formed by the overlap of an sp^3 orbital on one carbon and an sp^2 orbital on another carbon."



pyridoxine
(vitamin B_6)

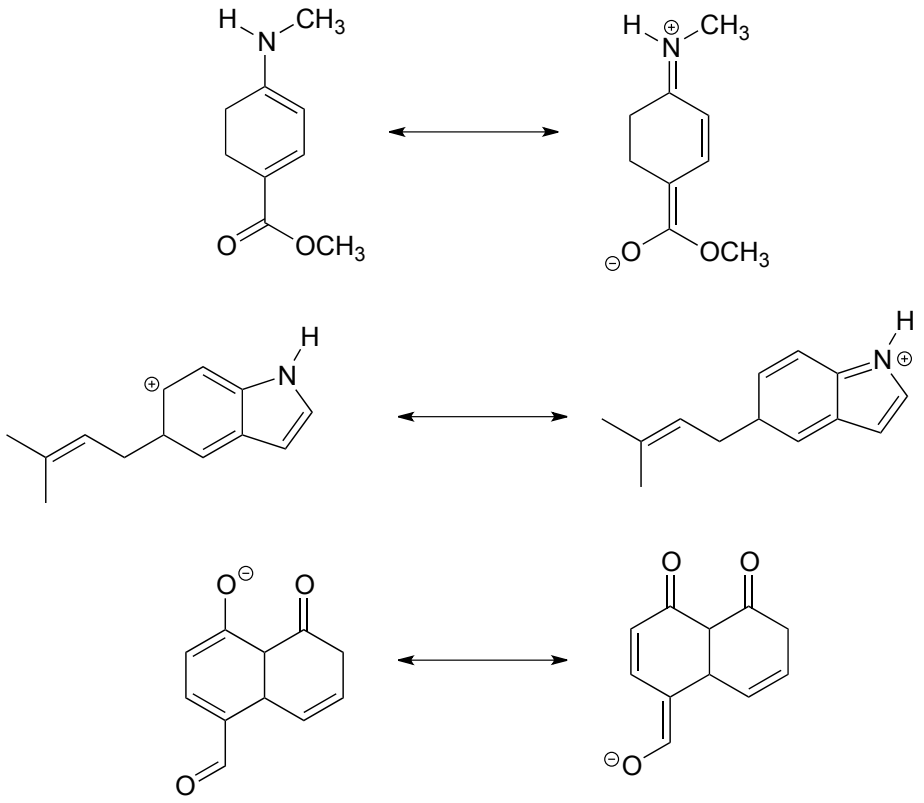


pantothenate
(vitamin B_5)

P2.2:

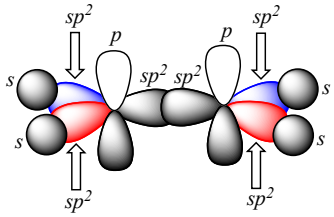
a) Draw curved arrows showing how each of the resonance contributors on the left could be converted to the one on the right.

b) Label contributors as major, minor, or approximately equivalent to each other.



P2.3: Draw a 3D-accurate picture showing the orbitals involved in bonding in the molecules below. Draw *all* bonds, both σ and π, as overlapping orbitals. Indicate whether each orbital is *s*, *p*, *sp*, sp^2, or sp^3, and indicate (with words or a color scheme) orbitals that are pointed into or out of the plane of the page. Locate all lone pairs in their appropriate orbitals. It is highly recommended to check your drawing with your instructor or tutor.

An example is provided for ethene, CH_2CH_2:



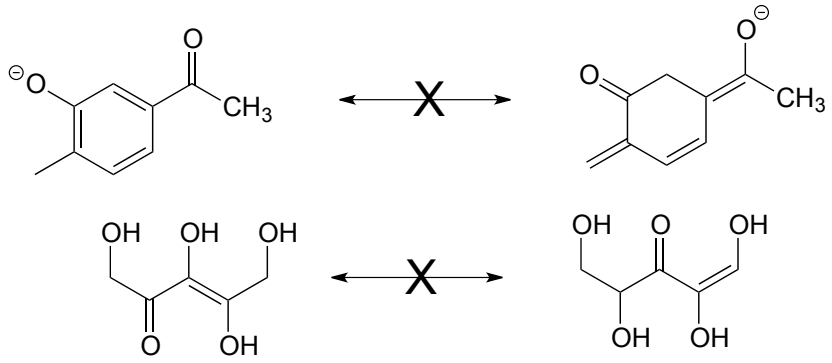
blue orbitals pointing into page
red orbitals pointing out of page

orbital overlap in ethene

a) dimethyl ether (CH_3OCH_3)
b) ethanol (CH_3CH_2OH)
c) acetaldehyde (CH_3COH)
d) hydrogen cyanide (HCN)

P2.4: Neither of the pairs of structures below are pairs of resonance contributors.

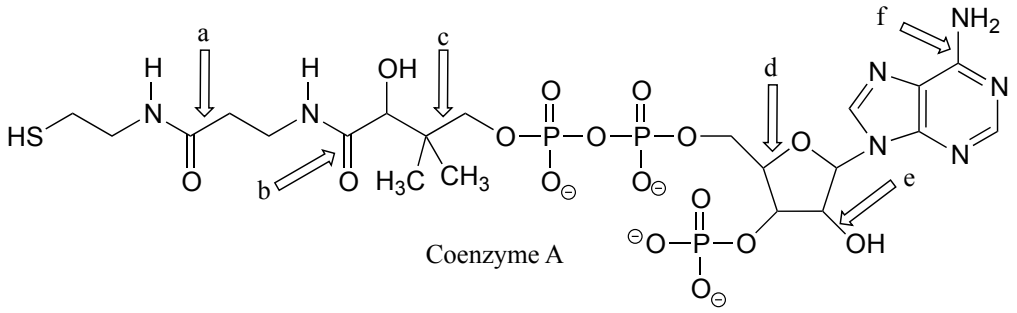
a) Explain why not.
b) What in fact is the relationship between them?

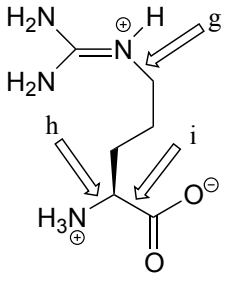


P2.5:

a-i) Describe the orbitals involved in the bonds indicated by the arrows, as in problem 2.1.

j) Give the molecular formula for arginine





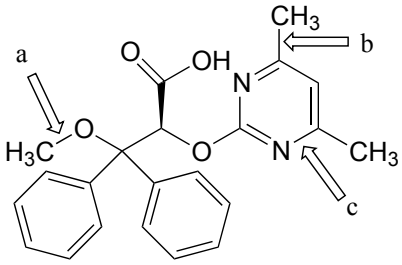
arginine

P2.6: The four compounds below appeared in the October 9 and October 25, 2006 issues of Chemical and Engineering News.

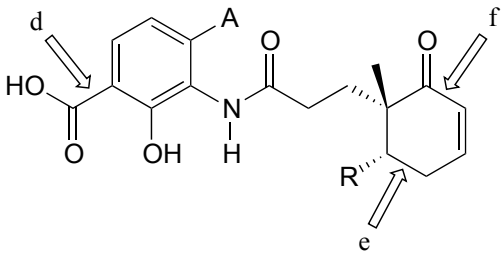
a-k) For each bond indicated by an arrow, specify the types of orbitals that are overlapping.

l) (functional group review) Which compound contains two aldehydes? Which contains an ether? Which contains an amide? Which contains a terminal alkene? Which contains an amine (and is this amine primary, secondary, tertiary, or quaternary?)

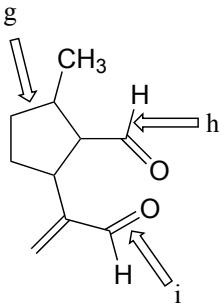
m) Give the molecular formula for the walking-stick compound



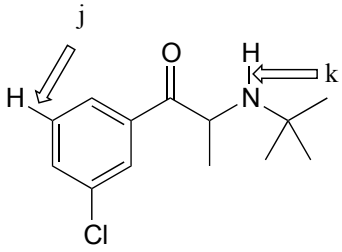
a hypertension drug



a newly-discovered antibiotic compound

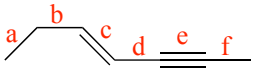


a defensive chemical extracted from a walking stick insect

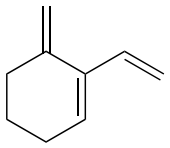


a drug for smoking cessation

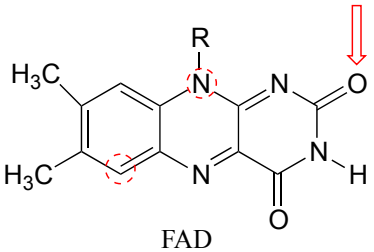
P2.7: Rank the bonds a-f below according to increasing bond length.



P2.8: Redraw the structure below, showing the $2p_z$ orbitals that make up the conjugated π bond system.

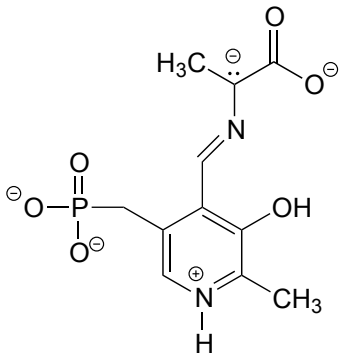


P2.9: Draw two different (minor) resonance contributors of the structure below (the flavin group of flavin adenine dinucleotide (FAD), a biochemical oxidation/reduction molecule) in which the oxygen indicated by an arrow bears a negative formal charge and one of the circled atoms bears a positive formal charge. Include curved arrows to account for the changing positions of electrons and π bonds.



FAD

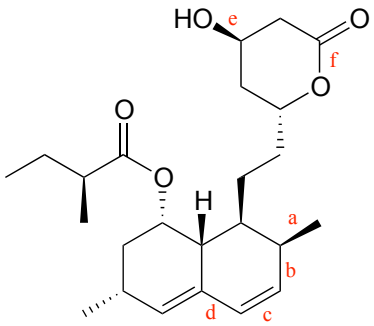
P2.10: The structure below shows an intermediate species in a reaction involving the amino acid alanine, attached to pyridoxal phosphate (vitamin B6). Draw a resonance contributor in which the only formal charges are on the oxygens.



P2.11: Below is the structure of the cholesterol-lowering drug Lovastatin. Predict the trend in bond length for

a) bonds a, b, c, and d

b) bonds e and f



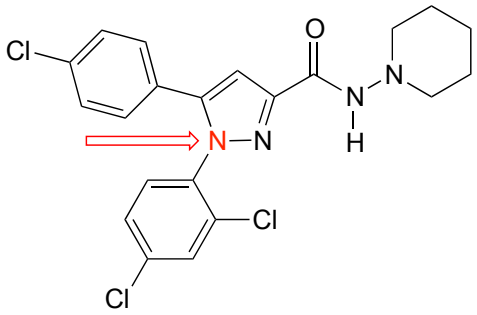
Lovastatin

P2.12: In problem P1.12, you were asked to draw four different amides with molecular formula C_3H_7NO. One of these constitutional isomers is significantly less soluble in water than the other three. Which one, and why?

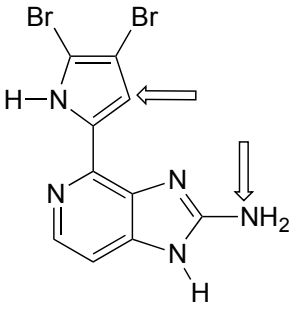
P2.13: Below is the structure of Rimonabant, a drug candidate which is being tested as a possible treatment for alcohol/tobacco dependence and obesity (see *Chemical and Engineering News*, October 15, 2006, p. 24). Draw minor resonance contributors in which

a) there is a separation of charge between the nitrogen indicated by the arrow and the oxygen.

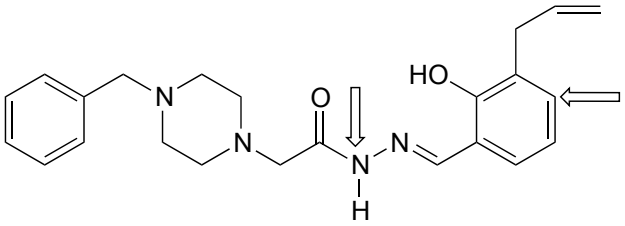
b) there is a separation of charge between a chlorine (positive) and one of the three nitrogens.



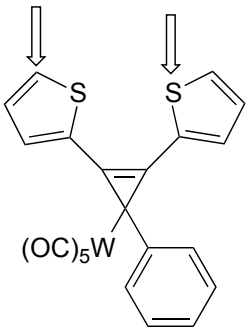
P2.14: For the molecules below, draw minor resonance contributors in which formal charges are placed on the atoms indicated by arrows. Use curved arrows to show how you are rearranging electrons between resonance contributors.



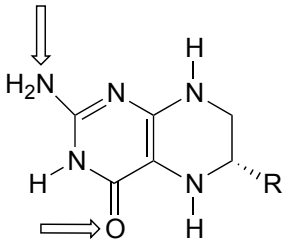
ageladine A, a potential cancer drug
Org. Lett. 2006, 8, 1443



PAC-1, a molecule that trigers cell death
Nature Chemical Biology 2006, 2, 543

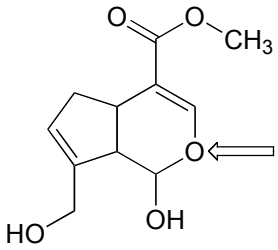


a new building block molecule for optoelectronic materials
Organometallics 2006, 25, 5176



tetrahydrofolate, a coenzyme

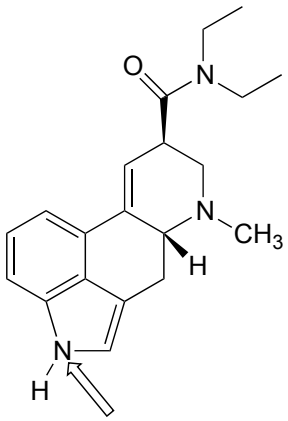
P2.15: Genipin was recently identified as the active compound in gardenia fruit extract, a traditional Chinese medicine for the treatment of diabetes (*Chemical and Engineering News* June 12, 2006, p. 34; *Cell Metab*. 2006, *3*, 417). Resonance contributors can be drawn in which the oxygen atom indicated by an arrow bears a positive formal charge. Indicate where the corresponding negative formal charge would be located in the most important of these contributors.



genipin

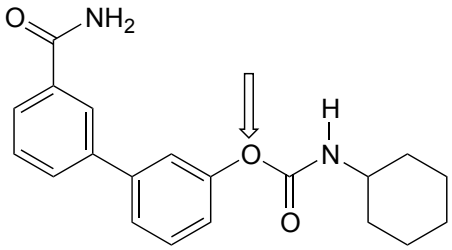
P2.16: Identify any *isolated* alkene groups in the PAC-1 structure in problem P2.14, and in the genipin structure in problem P2.15.

P2.17: The February 27, 2006 issue of *Chemical and Engineering News* contains an interesting article on the 100th birthday of Albert Hofmann, the inventor of the hallucinogen LSD. The structure of LSD is shown below. Several minor resonance contributors can be drawn in which the nitrogen atom indicated by an arrow bears a positive formal charge. Indicate atoms where a corresponding negative formal charge could be located in these contributors.



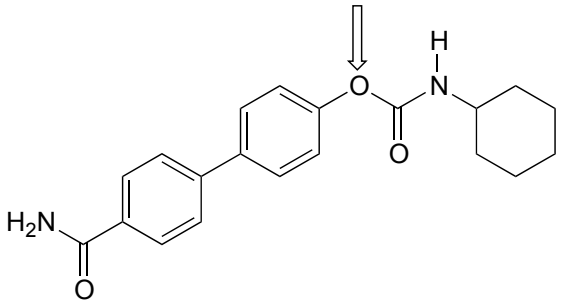
D-lysergic acid diethylamine (LSD)

P2.18: The human brain contains naturally occurring cannabinoid compounds which are related in structure to Δ^9-tetrahydrocannabinol, the active compound in cannabis. Cannabinoids are thought to exert an antidepressant effect. Because cannabis itself is not a realistic candidate for the treatment of depression due to its use as a recreational drug, researchers at the University of California, Irvine are studying synthetic compounds, such as the one shown below, which inhibit the degradation of natural cannabinoids in the brain. This compound has been shown to have antidepressant-like effects in rats and mice. (*Chemical and Engineering News*, December 19, 2005, p. 47; *Proc. Natl. Acad. Sci. USA* 2005, *102*, 18620.)



a) Several minor resonance contributors can be drawn in which the oxygen atom indicated by an arrow bears a positive formal charge. Indicate atoms where a corresponding negative formal charge could be located in these contributors.

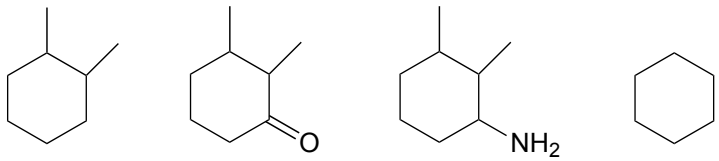
b) Answer the same question again, this time with the structural isomer shown below.



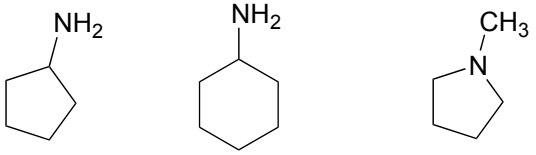
P2.19: Give the expected trend (lowest to highest) in boiling points for the following series of compounds.

a) CH_3CHF_2 CH_2F_2 HF CH_3F

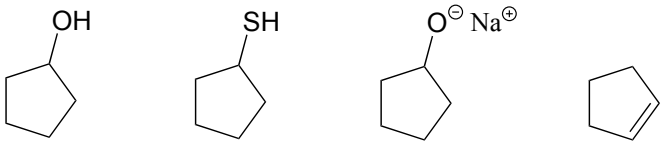
b)



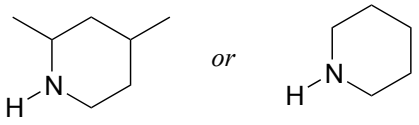
c)

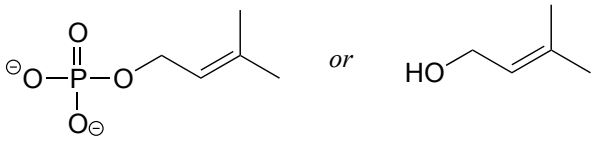


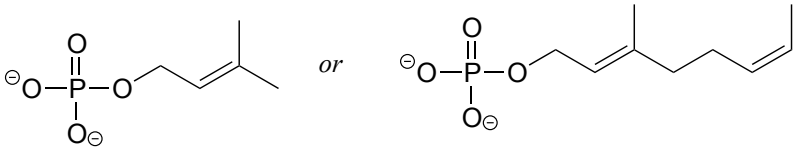
d)

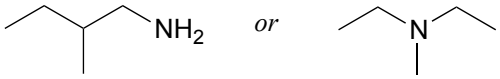


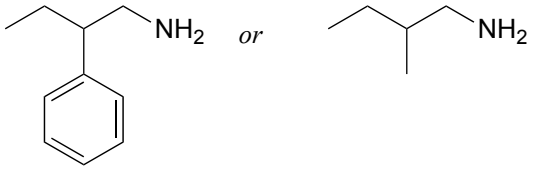
P2.20: For each pair of molecules below, choose the one that is more water-soluble, and explain your choice.

a)  *or*

b)  *or*

c)  *or*

d)  *or*

e)  *or*

P2.21: Intermolecular forces: For *a-c* below, you may want to review amino acid/protein structure basics in section 1.3D and Table 5 at the end of the book. Use abbreviations as appropriate to focus the viewer's attention on the interaction in question.

a) Which of the 20 natural amino acids have *side chains* capable of forming hydrogen bonds with water?

b) Draw a picture of a hydrogen bond in a protein between an alanine main chain nitrogen and a glutamate side chain.

c) Draw a picture of a hydrogen bond in a protein between a tyrosine main chain (acting as donor) and a threonine side chain (acting as acceptor).

d) Draw a picture of an charge-charge (ionic) interaction in a protein between an aspartate and a lysine.

P2.22: In properly folded protein structures, main chain nitrogens often participate in hydrogen bonding interactions in the role of donor, but rarely as acceptor. Speculate as to why this might be so, using what you have learned in this chapter.

P2.23: Ozone, O_3, is an uncharged, non-cyclic molecule. Draw a Lewis structure for ozone. Are the two oxygen-oxygen bonds the likely to be the same length? What is the bond order? Explain.

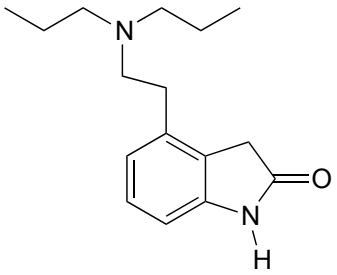
P1.24: Imagine that you hear a description of the bonding in water as being derived directly from the atomic orbital theory, without use of the hybrid orbital concept. In other words, the two bonds would be formed by the overlap of the half-filled $2p_y$ and $2p_z$ orbitals of oxygen with the 1s orbitals of hydrogen, while the two lone pairs on oxygen would occupy the 2s and $2p_x$ orbitals. What is wrong with this picture? How would the bonding geometry differ from what is actually observed for water?

P2.25:

a) Draw a picture showing the geometry of the overlapping orbitals that form the bonding network in allene, H_2CCCH_2. Then, draw a Lewis structure for the molecule, using the solid/dash wedge bond convention as necessary to indicate the correct geometry of the σ bonds.

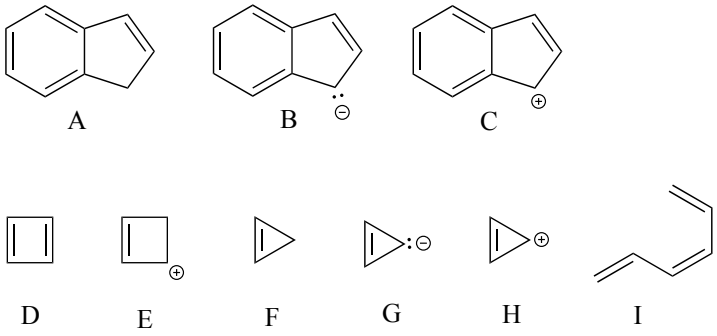
b) Draw a picture showing the geometry of the overlapping orbitals that form the bonding network in carbon dioxide.

P2.26 Below is the structure of ropinerol, a drug made by GlaxoSmithKline for the treatment of Parkinson's disease. Is the five-membered ring part of the aromatic system? Explain your answer.



roprinol

P2.27: Classify each of the molecules/ions below as aromatic or not aromatic. Explain your reasoning.


A
B
C
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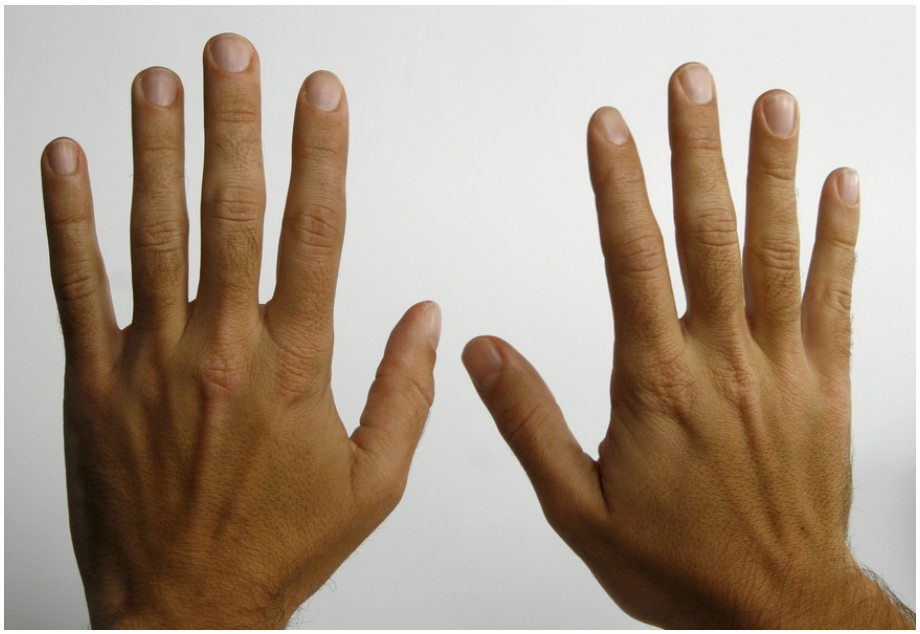
I

Chapter 3

Conformation and stereochemistry



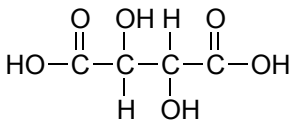
(Credit: https://www.flickr.com/photos/nate/)

In 1848, a 25 year old chemist named Louis Pasteur made a startling - and some thought brash - claim to the scientific community. Pasteur was inexperienced, to say the least: he had only earned his doctorate the previous year, and had just started his first job as an assistant to a professor at the *Ecole normale superieure*, a university in Paris. Jean-Baptiste Biot, a highly respected physicist who had already made major contributions to scientific fields as diverse as meteorites, magnetism, and optics, was intrigued but unconvinced by Pasteur's claim. He invited the young man to come to his laboratory and reproduce his experiments.

Decades earlier, Biot had discovered that aqueous solutions of some biologically-derived substances, such as tartaric acid, quinine, morphine, and various sugars, were optically active: that is, plane polarized light would rotate in either a positive (clockwise, or right-

handed) or negative (counter-clockwise, or left-handed) direction when passed through the solutions. Nobody understood the source of this optical property.

One of the biological substances known to be optically active was a salt of tartaric acid, a compound found in abundance in grapes and a major by-product of the wine-making industry.



tartaric acid

The compound was dextrorotatory in solution – in other words, it rotated plane-polarized light in the positive (right-handed, or clockwise) direction. Curiously, though, chemists had also found that another form of processed tartaric acid was optically *in*active, despite that fact that it appeared to be identical to the optically active acid in every other respect. The optically inactive compound was called '*acide racemique*', from the Latin *racemus*, meaning 'bunch of grapes'.

Louis Pasteur's claims had to do with experiments he said he had done with the 'racemic' acid. Jean-Babtise Biot summoned Pasteur to his laboratory, and presented him with a sample of racemic acid which he himself had already confirmed was optically inactive. With Biot watching over his shoulder, and using Biot's reagents, Pasteur prepared the salt form of the acid, dissolved it in water, and left the aqueous solution in an uncovered flask to allow crystals to slowly form as the water evaporated.

Biot again summoned Pasteur to the lab a few days later when the crystallization was complete. Pasteur placed the crystals under a microscope, and began to painstakingly examine their shape, just as he had done in his original experiments. He had recognized that the crystals, which had a regular shape, were asymmetric: in other words, they could not be superimposed on their mirror image. Scientists referred to asymmetric crystals and other asymmetric objects as being 'chiral', from the Greek word for 'hand'. Your hands are chiral objects, because although your right hand and your left hand are mirror images of one another, they cannot be superimposed. That is why you cannot fit your right hand in a left-handed glove.

More importantly, Pasteur had claimed that the chiral crystals he was seeing under the lens of his microscope were of two different types, and the two types were mirror images of each other: about half were what he termed 'right handed' and half were 'left-handed'. He carefully separated the right and left-handed crystals from each other, and presented the two samples to Biot. The eminent scientist then took what Pasteur told him were the left-handed crystals, dissolved them in water, and put the aqueous solution in a polarimeter, an instrument that measures optical rotation. Biot knew that the processed tartaric acid he had provided Pasteur had been optically inactive. He also knew that unprocessed tartaric acid

from grapes had right-handed optical activity, whereas left-handed tartaric acid was unheard of. Before his eyes, however, he now saw that the solution was rotating light to the left. He turned to his young colleague and exclaimed, " *Mon cher enfant, j'ai tant aime´ les sciences dans ma vie que cela me fait battre le coeur*!' (My dear child, I have loved science so much during my life that this makes my heart pound!)

Biot had good reason to be so profoundly excited. Pasteur had just conclusively demonstrated, for the first time, the concept of **molecular chirality**: molecules themselves - not just macroscopic objects like crystals - could exhibit chirality, and could be separated into distinct right-handed and left-handed 'stereoisomers'. Tying together ideas from physics, chemistry, and biology, he had shown that nature could be chiral *at the molecular level*, and in doing do he had introduced to the world a new subfield which came to be known as 'stereochemistry'.

About ten years after his demonstration of molecular chirality, Pasteur went on to make another observation with profound implications for biological chemistry. It was already well known that 'natural' tartaric acid (the right-handed kind from grapes) could be fermented by bacteria. Pasteur discovered that the bacteria were selective with regard to the chirality of tartaric acid: no fermentation occurred when the bacteria were provided with pure left-handed acid, and when provided with racemic acid they specifically fermented the right-handed component, leaving the left-handed acid behind.

Pasteur was not aware, at the time of the discoveries described here, the details of the structural features of tartaric acid at the molecular level that made the acid chiral, although he made some predictions concerning the bonding patterns of carbon which turned out to be remarkably accurate. In the more than 150 years since Pasteur's initial tartaric acid work, we have greatly expanded our understanding of molecular chirality, and it is this knowledge that makes up the core of this chapter. Put simply, stereochemistry is the study of how bonds are oriented in three-dimensional space. It is difficult to overstate the importance of stereochemistry in nature, and in the fields of biology and medicine in particular. As Pasteur so convincingly demonstrated, life itself is chiral: living things recognize different stereoisomers of organic compounds and process them accordingly.

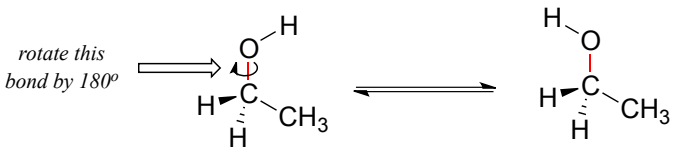
Molecular models are your friend!

Because this chapter deals extensively with concepts that are inherently three-dimensional in nature, it will be very important for you to use a molecular modeling kit that is specifically intended for organic chemistry. Many of the ideas we will be exploring can be extremely confusing if you are limited to the two dimensions of this page. Be prepared to follow along with these discussions in ***three*** *dimensions, with a molecular model in your hands!*

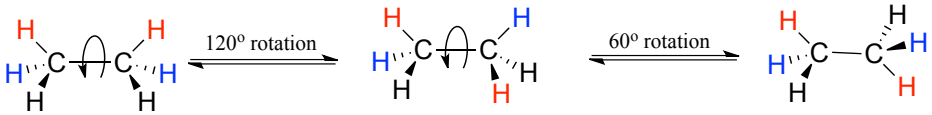
Section 3.1: Conformations of open-chain organic molecules

Before we begin our exploration of stereochemistry and chirality, we first need to consider the subject of **conformational isomerism**, which has to do with rotation about single bonds.

We learned in section 2.1B that single bonds in organic molecules are free to rotate, due to the 'end-to-end' (σ) nature of their orbital overlap. Consider the carbon-oxygen bond in ethanol, for example: with a 180° rotation about this bond, the shape of the molecule would look quite different:

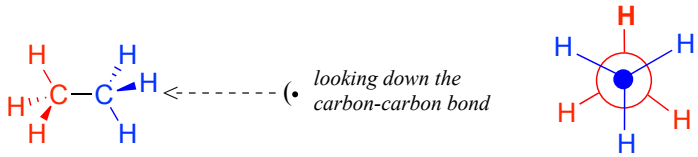


Or ethane: rotation about the carbon-carbon σ bond results in many different possible three-dimensional arrangements of the atoms.



These different arrangements, resulting from σ bond rotation, are referred to in organic chemistry as **conformations**. Any one specific conformation is called a **conformational isomer**, or **conformer**.

In order to better visualize different conformations of a molecule, it is convenient to use a drawing convention called the **Newman projection**. In a Newman projection, we look lengthwise down a specific bond of interest – in this case, the carbon-carbon bond in ethane. We depict the 'front' atom as a dot, and the 'back' atom as a larger circle.



'staggered' conformation
(Newman projection)

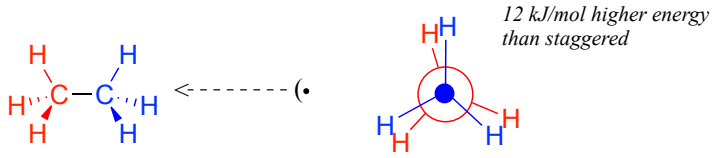
The six carbon-hydrogen bonds are shown as solid lines protruding from the two carbons. Note that we do *not* draw bonds as solid or dashed wedges in a Newman projection.

Looking down the C-C bond in this way, the angle formed between a C-H bond on the front carbon and a C-H bond on the back carbon is referred to as a **dihedral angle**. (The

dihedral angle between the hour hand and the minute hand on a clock is 0° at noon, 90° at 3:00, and so forth).

The lowest energy conformation of ethane, shown in the figure above, is called the '**staggered'** conformation: all of the dihedral angles are 60°, and the distance between the front and back C-H bonds is maximized.

If we now rotate the front CH_3 group 60° clockwise, the molecule is in the highest energy '**eclipsed'** conformation, where the dihedral angles are all 0° (we stagger the bonds slightly in our Newman projection drawing so that we can see them all).

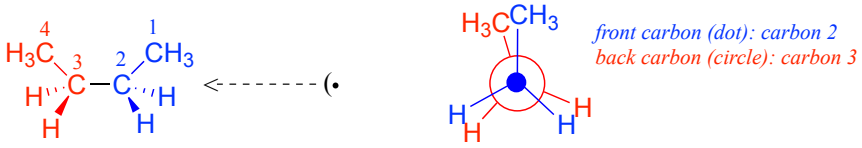


'eclipsed' conformation

The energy of the eclipsed conformation, where the electrons in the front and back C-H bonds are closer together, is approximately 12 kJ/mol higher than that of the staggered conformation.

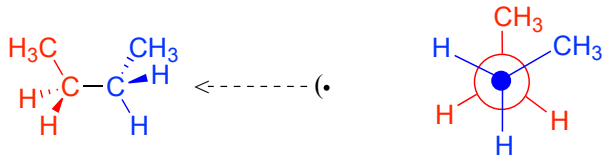
Another 60° rotation returns the molecule to a second staggered conformation. This process can be continued all around the 360° circle, with three possible eclipsed conformations and three staggered conformations, in addition to an infinite number of conformations in between these two extremes.

Now let's consider butane, with its four-carbon chain. There are now three rotating carbon-carbon bonds to consider, but we will focus on the middle bond between C_2 and C_3. Below are two representations of butane in a conformation which puts the two CH_3 groups (C_1 and C_4) in the eclipsed position, with the two C-C bonds at a 0° dihedral angle.



eclipsed (A)

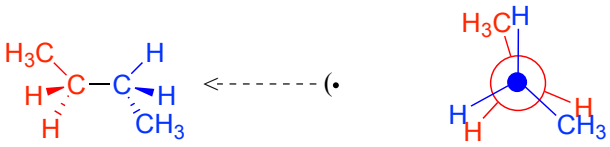
If we rotate the front, (blue) carbon by 60° clockwise, the butane molecule is now in a staggered conformation.



gauche

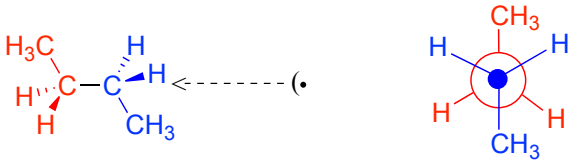
This is more specifically referred to as the **gauche** conformation of butane. Notice that although they are staggered, the two methyl groups are not as far apart as they could possibly be.

A further rotation of 60° gives us a second eclipsed conformation (B) in which both methyl groups are lined up with hydrogen atoms.



eclipsed (B)

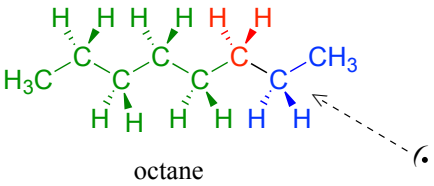
One more 60 rotation produces another staggered conformation called the **anti** conformation, where the two methyl groups are positioned opposite each other (a dihedral angle of 180°).



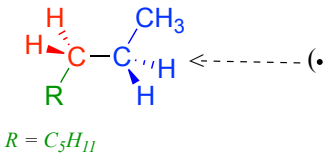
anti

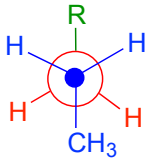
As with ethane, the staggered conformations of butane are energy 'valleys', and the eclipsed conformations are energy 'peaks'. However, in the case of butane there are two different valleys, and two different peaks. The gauche conformation is a higher energy valley than the anti conformation due to **steric strain**, which is the repulsive interaction caused by the two bulky methyl groups being forced too close together. Clearly, steric strain is lower in the anti conformation. In the same way, steric strain causes the eclipsed A conformation - where the two methyl groups are as close together as they can possibly be - to be higher in energy than the two eclipsed B conformations.

Because the anti conformation is lowest in energy (and also simply for ease of drawing), it is conventional to draw open-chain alkanes in a 'zigzag' form, which implies anti conformation at all carbon-carbon bonds. The figure below shows, as an example, a Newman projection looking down the C_2-C_3 bond of octane.



octane

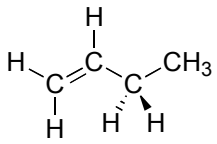




anti

Exercise 3.1: Draw Newman projections of the lowest and highest energy conformations of propane.

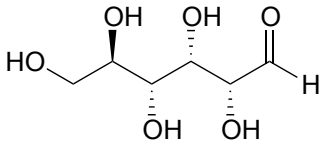
Exercise 3.2: Draw a Newman projection, looking down the C_2-C_3 bond, of 1-butene in the conformation shown below (C_2 should be your *front* carbon).



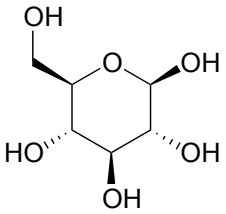
Section 3.2: Conformations of cyclic organic molecules

Browse through a biochemistry textbook and you will see any number of molecules with cyclic structures. Many of these cyclic structures are aromatic, and therefore planar. Many others, though, are composed of sp^3-hybridized atoms, and it is these cyclic structures that are the topic of discussion in this section.

When discussing cyclic organic molecules, we will often use sugars as examples, because they are such important molecules in biological chemistry. It is important to recall (section 1.3C) that many sugars exist in aqueous solution as both open-chain and cyclic forms. You need not worry at this point about understanding how the cyclic form is named, or the reaction by which the cyclization occurs - this will be covered in chapter x.

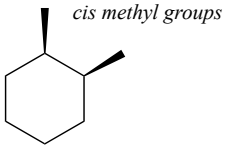


open-chain glucose

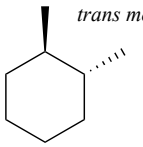


cyclic glucose (β-glucopyranose)

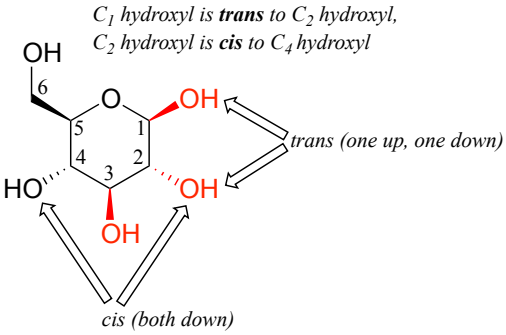
One thing that you should notice in the cyclic structure shown above is that atoms or groups bonded to tetrahedral ring carbons are either pointing up (out of the plane of the page) or down (into the plane of the page), as indicated by the use of dashed or solid wedge bonds. When two substituents on the same ring are both pointing toward the same side of the ring, they are said to be ***cis*** to each other. When they are pointed to opposite sides, they are said to be ***trans*** to each other.



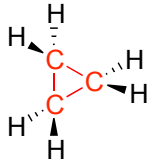
cis methyl groups



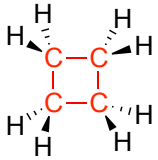
trans methyl groups



Ring structures in organic molecules are usually five-membered or six-membered. Three- and four-membered rings are occasionally found in nature, but are significantly higher in energy. The relative instability of these smaller ring structures can be explained by a concept called **angle strain**, in which the four bonds around the sp^3-hybridized carbons are forced out of their preferred tetrahedral angles.

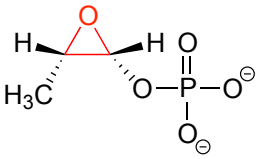


cyclopropane

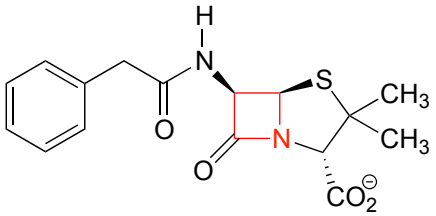


cyclobutane

If one of the carbon-carbon bonds is broken, the ring will 'spring' open, releasing energy as the bonds reassume their preferred tetrahedral geometry. The effectiveness of two antibiotic drugs, fosfomycin and penicillin, is due in large part to the high reactivity of the three- and four-membered rings in their structures.

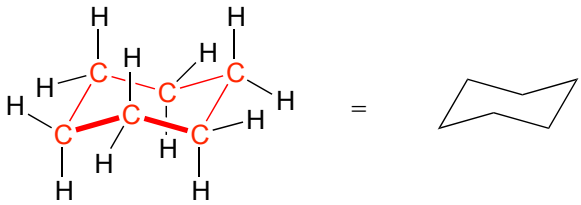


fosfomycin



penicillin G

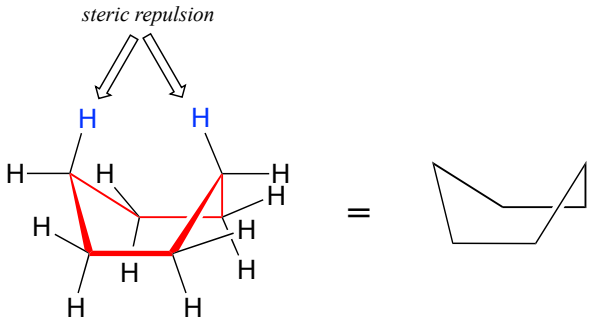
In six-membered cycloalkane structures, bonding angles are close to tetrahedral, and thus ring strain is not a factor – these rings are in fact very stable. However, the 'flat' drawings we have been using up to now do not accurately show the actual three-dimensional shape of a five- or six-membered ring. If cyclohexane were indeed flat, the bond angles would have to be distorted from 109.5° to 120°. If you build a model, though, you will find that when you rotate the carbon-carbon bonds so as to put the ring into a shape that resembles a reclining beach chair, all of the carbon-carbon bonds are able to assume tetrahedral bonding angles.



'chair' conformation of cyclohexane

This **chair conformation** is the lowest energy conformation for cyclohexane and other six-membered rings.

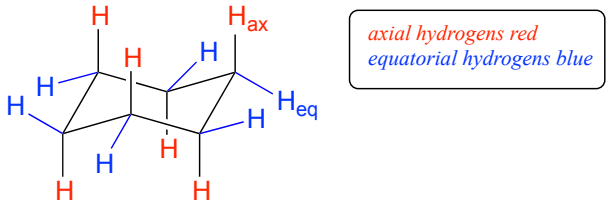
An alternate conformation for a six-membered ring is called the **'boat'**:



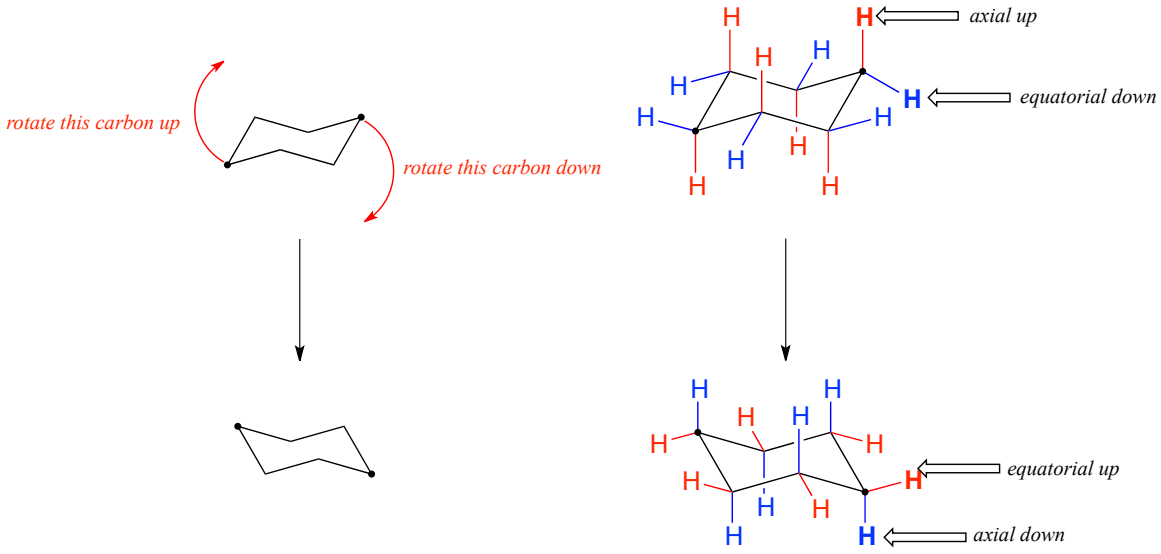
'boat' conformation of cyclohexane

In the boat conformation, two of the substituents – those on the 'bow' and the 'stern' if you will – are brought close enough to each other to cause steric strain. An additional cause of the higher energy of the boat conformation is that adjacent hydrogen atoms on the 'bottom of the boat' are forced into eclipsed positions. For these reasons, the boat conformation is a high energy conformation of cyclohexane, about 30 kJ/mol less stable than the chair conformation.

If you look carefully at your model of cyclohexane in the chair conformation, you will see that all twelve hydrogens are not equivalent in terms of their three-dimensional arrangement in space. Six hydrogens are **axial** – that is, they are pointing either straight up or straight down relative to the ring. The other six hydrogens are **equatorial**, meaning that they are pointing away from the perimeter of the ring, either slightly up or slightly down. *(The equatorial vs axial distinction is often hard to see at first - it would be a very good idea at this point to sit down with your instructor or tutor and work with a modeling kit).*



This is not the only possible chair conformation for cyclohexane. On your model, rotate one of the 'up' carbons down, and one of the 'down' carbons up. You now have a new, alternate chair conformation – this process is called **ring inversion**.

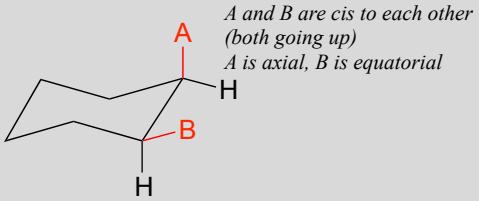


What you should recognize here is that, as a result of the ring inversion process, all of the axial and equatorial hydrogens have traded positions – axial hydrogens have become

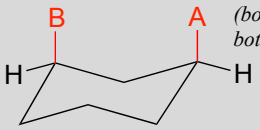
equatorial, and vice-versa. Notice, however, that the 'down' hydrogens are still pointing down, and the 'up' hydrogens are still pointing up regardless of whether they are axial or equatorial. At room temperature, cyclohexane is constantly inverting between two chair forms of equal energy – it is a rapid equilibrium situation. Thus, except at very low temperatures, we are not able to distinguish between axial and equatorial hydrogens, as they are constantly switching back and forth.

axial/equatorial *vs cis/trans*

A very common error made by organic chemistry students as they begin to learn about chair conformations is to confuse the terms axial and equatorial with the terms *cis* and *trans*. **These are completely different things!** For example, when two substituents on a ring are *cis* in relation to one another, it means that they are pointed to the same side of the ring (both up or both down). Depending on their positions on the ring, they might both be axial, both be equatorial, or one of each.



A and B are cis to each other (both going up) A is axial, B is equatorial

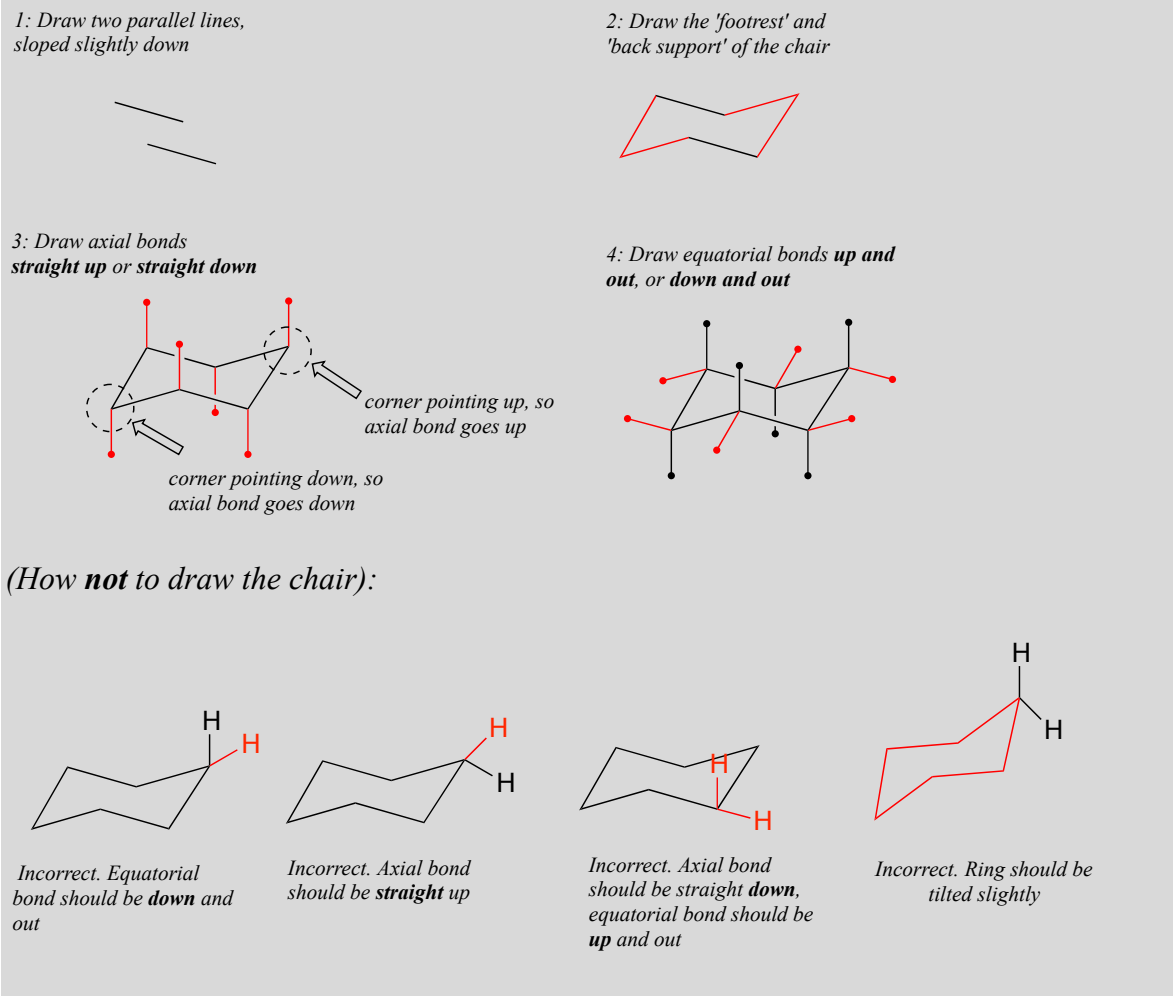


A and B are cis to each other (both going up) both are axial

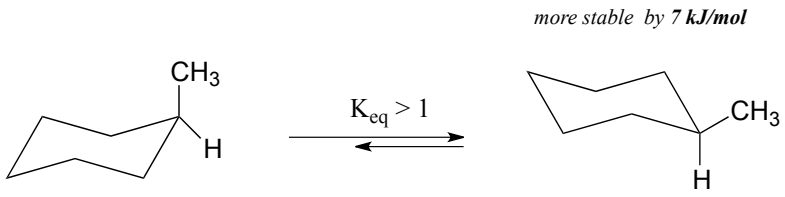
Do not make the mistake of calling two substituents *trans* to each other merely because one is equatorial and one is axial, or *cis* because the are both axial or both equatorial.

How to draw the cyclohexane chair conformation:

As an organic chemistry student, you will be expected to be able to draw an accurate representation of the chair conformations of six-membered cycloalkanes, which includes being able to draw axial and equatorial substituents with their correct orientations. Here, then, are some guidelines to follow:



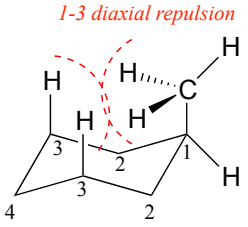
The picture is different when we replace one of the hydrogen substituents with something bigger, such as a methyl group. Now, the two chair conformations are quite different: in one, the methyl group is equatorial and in the other it is axial.



methyl group axial

methyl group equatorial

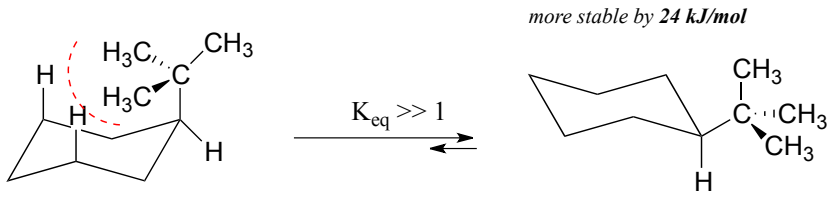
When the methyl group is in the axial position, it is brought close enough to the axial hydrogens on carbons two bonds away to cause destabilizing steric repulsion: this is referred to as **1,3-diaxial repulsion**.



When in the equatorial position, the methyl group is pointing up and *away* from the rest of the ring, eliminating the unfavorable 1,3-diaxial interaction. As a consequence, the conformation in which the methyl group is in the equatorial position is more stable, by approximately 7 kJ/mol. At room temperature, methylcyclohexane exists as a rapid equilibrium between the two chair forms (and many other intermediate conformations), but the equilibrium constant (K_{eq}) favors the conformation where the methyl group is equatorial.

Exercise 3.3: Here's some General Chemistry review: what is the value of K_{eq} at 25 ^{o}C for the chair-chair interconversion of methylcyclohexane as shown in the previous figure?

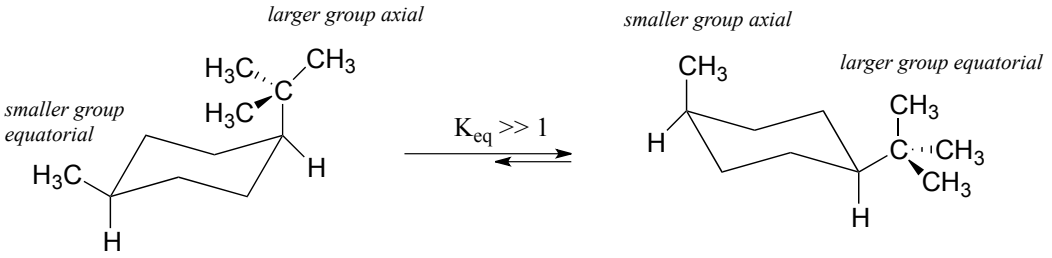
The importance of the steric strain factor increases with the increasing size of a substituent. For example, the difference in energy between the two chair conformations of *tert*-butyl cyclohexane (24 kJ/mol) is much larger than for methylcyclohexane (7 kJ/mol), because a *tert*-butyl group is larger than a methyl group and results in more energetically unfavorable 1,3-diaxial interactions.



tert-butyl group axial

tert-butyl group equatorial

In the case of a disubstituted cyclohexane ring in which both substituents cannot be equatorial, the lower energy conformation generally places the bulkier substituent in the equatorial position.



more stable conformation

As a general rule, the most stable chair conformation of a six-membered ring will be that in which the bulkiest groups are in the equatorial position.

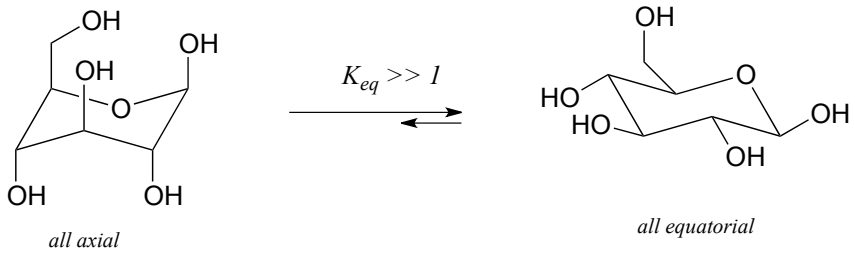
Exercise 3.4: Draw the lower energy chair conformations of a) *trans*-1,2-dimethylcyclohexane, and b) *trans*-1-isopropyl-3-methylcyclohexane. Draw all substituents on all carbons (including hydrogens), being sure that the axial or equatorial orientation is clear. Be sure to check your drawing with your instructor or tutor.

Exercise 3.5: Predict which of the following disubstituted hexanes has a greater energy difference between its two chair conformations, and state your reasons for your choices.

a) *cis*-1,3-dimethylcyclohexane or *cis*-1,4-dimethylcyclohexane
b) *cis*-1,2-dimethylcyclohexane or *trans*-1,2-dimethylcyclohexane
c) *trans*-1,2-dimethylcyclohexane or *trans*-1-isopropyl-2-methylcyclohexane

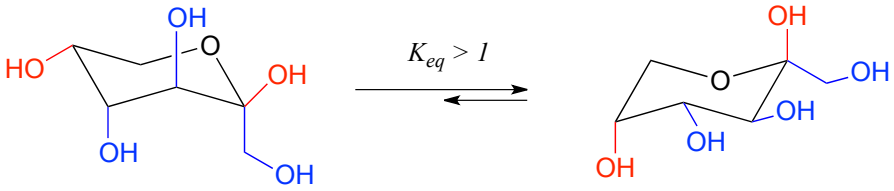
Exercise 3.6: Can a 'ring flip' change a *cis*-disubstituted cyclohexane to *trans*? Explain.

Recall that five- and six-carbon sugars such as glucose and fructose exist in solution in open chain and cyclic forms. Glucose, in its most abundant form in solution, is a six-membered ring adopting a chain conformation with all substituents equatorial.



glucose
(β-glucopyranose form)

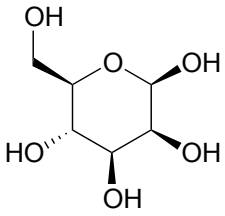
The most abundant form of fructose in aqueous solution is also a six-membered ring.



fructose
(β-fructopyranose form)

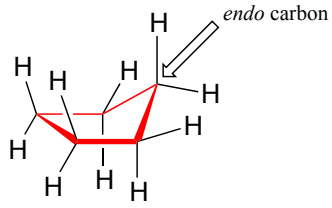
The lower energy chair conformation is the one with three of the five substituents (including the bulky $–CH_2OH$ group) in the equatorial position.

Exercise 3.7: Draw the two chair conformations of the six-carbon sugar mannose, being sure to clearly show each non-hydrogen substituent as axial or equatorial. Predict which conformation is likely to be more stable, and explain why.



mannose

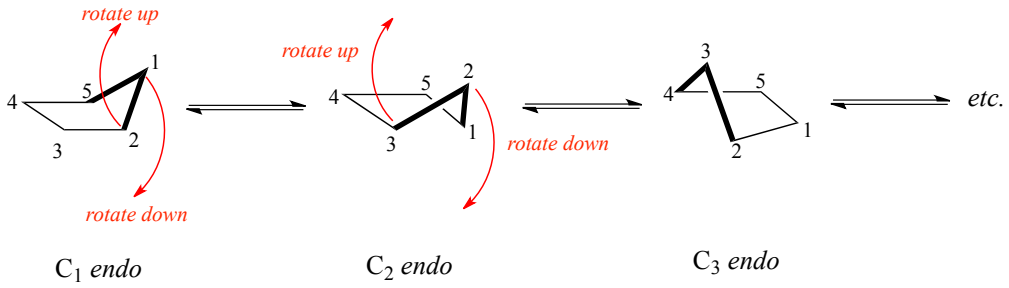
The lowest energy conformation of cyclopentane and other five-membered rings is known as the 'envelope', with four of the ring atoms in the same plane and one out of plane (notice that this shape resembles an envelope with the flap open). The out-of-plane carbon is said to be in the ***endo*** position ('*endo*' means 'inside').



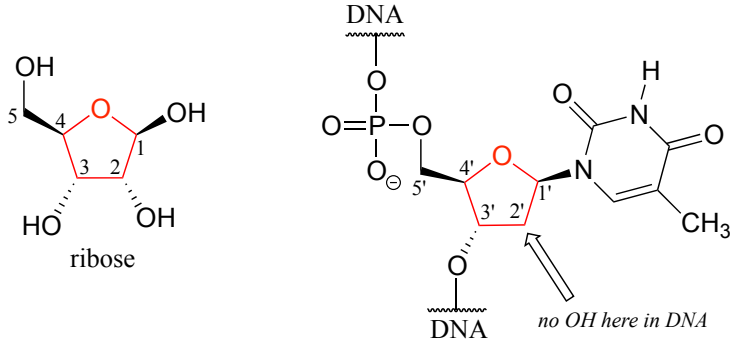
'envelope' conformation of cyclopentane

The 'equatorial' vs 'axial' distinction discussed in the context of 6-membered rings does not apply to five-membered rings.

At room temperature, cyclopentane undergoes a rapid **pseudorotation** process in which each of the five carbons takes turns being in the *endo* position.



One of the most important five-membered rings in nature is a sugar called ribose – recall from section 1.3E that DNA and RNA are both constructed upon 'backbones' derived from ribose. Pictured below is one thymidine (T) deoxy-nucleotide from a stretch of DNA:



The lowest-energy conformations for ribose are envelope forms in which either the 3' or 2' carbons are *endo*. This has very important implications for oligonucleotide structure – in a DNA double helix, it is C_2 that is in the *endo* position, while in RNA it is C_3.

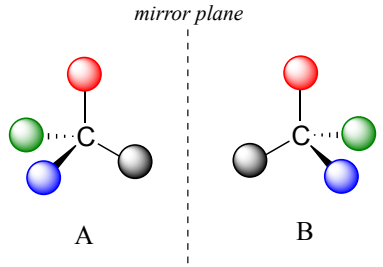
Section 3.3: Chirality and stereoisomers

We turn now to concept of chirality that formed the basis of the story about Louis Pasteur in the beginning of this chapter. Recall that the term **chiral**, from the Greek work for 'hand', refers to anything which cannot be superimposed on its own mirror image. Your hands, of course, are chiral - you cannot superimpose your left hand on your right, and you cannot fit your left hand into a right-handed glove (which is also a chiral object). Another way of saying this is that your hands do not have a **mirror plane of symmetry**: you cannot find any plane which bisects your hand in such a way that one side of the plane is a mirror image of the other side. *Chiral objects do not have a plane of symmetry*.

Your face, on the other hand is **achiral** - lacking chirality - because, some small deviations notwithstanding, you could superimpose your face onto its mirror image. If someone were to show you a mirror image photograph of your face, you could line the image up, point-

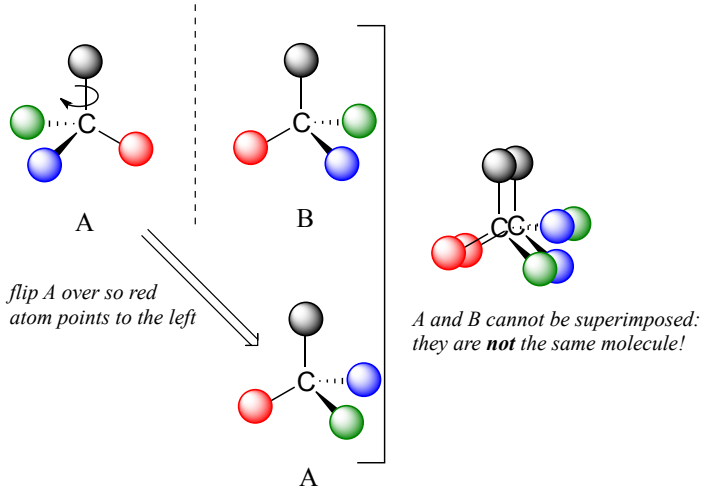
for-point, with your actual face. Your face has a plane of symmetry, because the left side is the mirror image of the right side.

What Pasteur, Biot, and their contemporaries did not yet fully understand when Pasteur made his discovery of molecular chirality was the *source* of chirality at the molecular level. It stood to reason that a chiral molecule is one that does not contain a plane of symmetry, and thus cannot be superimposed on its mirror image. We now know that chiral molecules contain one or more **chiral centers**, which are almost always tetrahedral (sp^3-hybridized) carbons with four different substituents. Consider the cartoon molecule A below: a tetrahedral carbon, with four different substituents denoted by balls of four different colors (for the time being, don't worry about exactly what these substituents could be - we will see real examples very soon).



The mirror image of A, which we will call B, is drawn on the right side of the figure, and an imaginary mirror is in the middle. Notice that every point on A lines up through the mirror with the same point on B: in other words, if A looked in the mirror, it would see B looking back.

Now, if we flip compound A over and try to superimpose it point for point on compound B, we find that we cannot do it: if we superimpose any two colored balls, then the other two are misaligned.

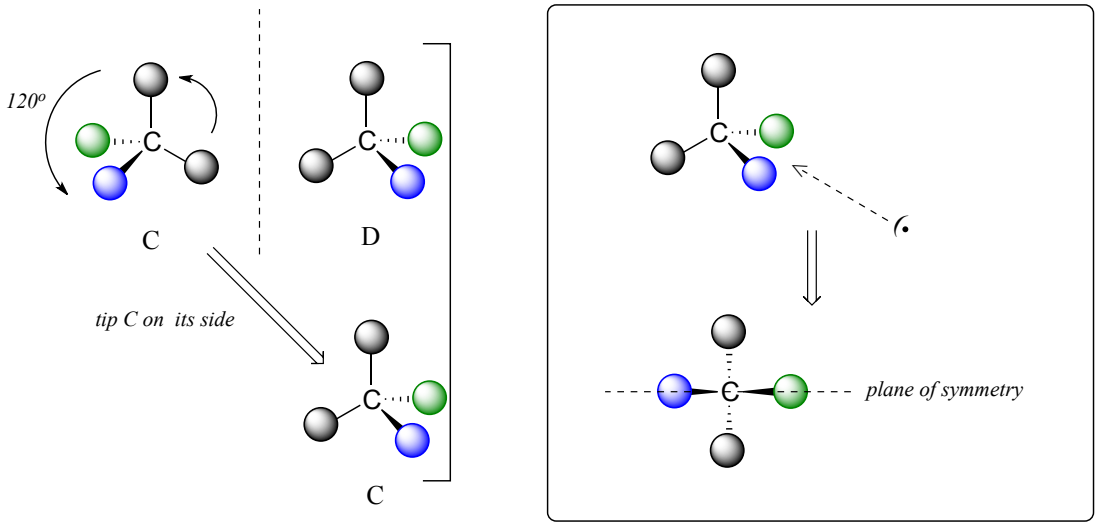


A is not superimposable on its mirror image (B), thus by definition A is a chiral molecule. It follows that B also is not superimposable on *its* mirror image (A), and thus it is also a chiral molecule. Also notice in the figure below (and convince yourself with models) that neither A nor B has an internal plane of symmetry.

A and B are **stereoisomers**: molecules with the same molecular formula and the same bonding arrangement, but *a different arrangement of atoms in space*. There are two types of stereoisomers: enantiomers and diastereomers. **Enantiomers** are pairs of stereoisomers which are mirror images of each other: thus, A and B are enantiomers. It should be self-evident that a chiral molecule will always have one (and *only* one) enantiomer: enantiomers come in pairs. Enantiomers have identical physical properties (melting point, boiling point, density, and so on). However, enantiomers do differ in how they interact with polarized light (we will learn more about this soon) and they may also interact in very different ways with other chiral molecules - proteins, for example. We will begin to explore this last idea in later in this chapter, and see many examples throughout the remainder of our study of biological organic chemistry.

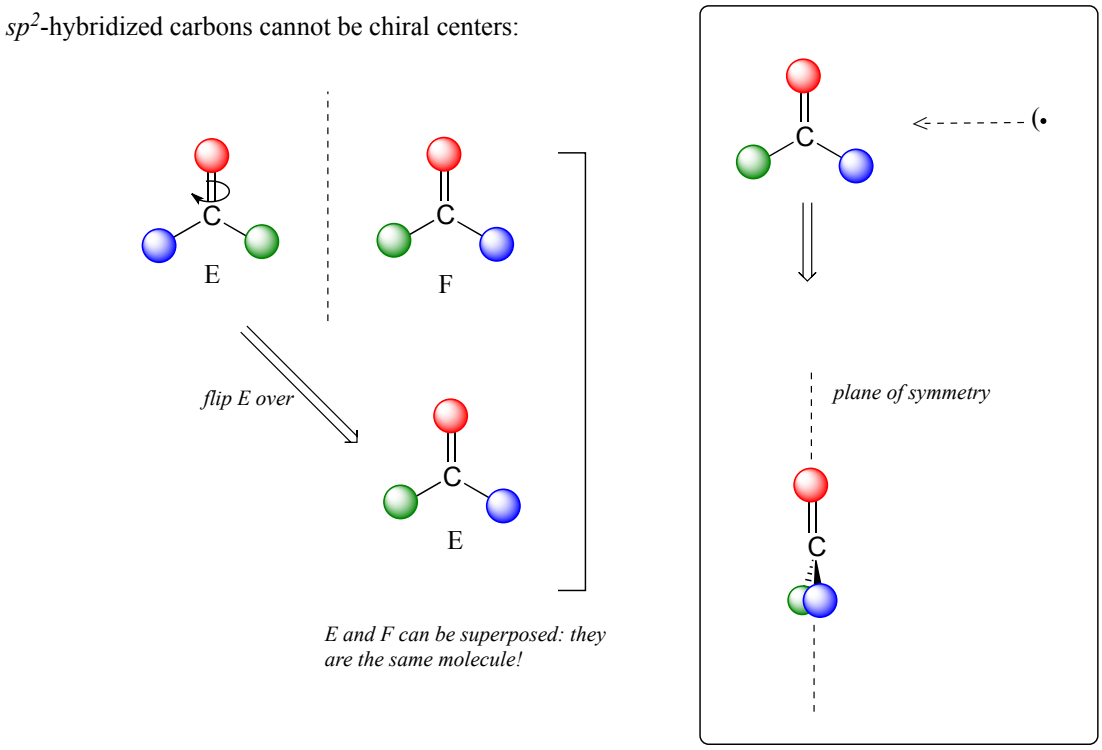
Diastereomers are stereoisomers which are *not* mirror images of each other. For now, we will concentrate on understanding enantiomers, and come back to diastereomers later.

We defined a chiral center as a tetrahedral carbon with four different substituents. If, instead, a tetrahedral carbon has two identical substituents (two black atoms in the cartoon figure below), then of course it still has a mirror image (*everything* has a mirror image, unless we are talking about a vampire!) However, it is superimposable on its mirror image, and has a plane of symmetry.



C and D can be superposed: they are the same molecule!

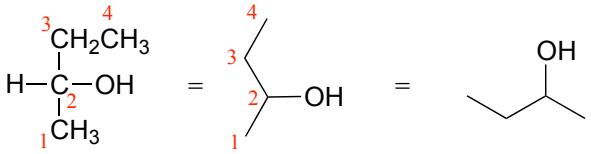
This molecule is **achiral** (lacking chirality). Using the same reasoning, we can see that a trigonal planar (sp^2-hybridized) carbon is also not a chiral center.



Notice that structure E can be superimposed on F, its mirror image - all you have to do is pick E up, flip it over, and it is the same as F. This molecule has a plane of symmetry, and is achiral.

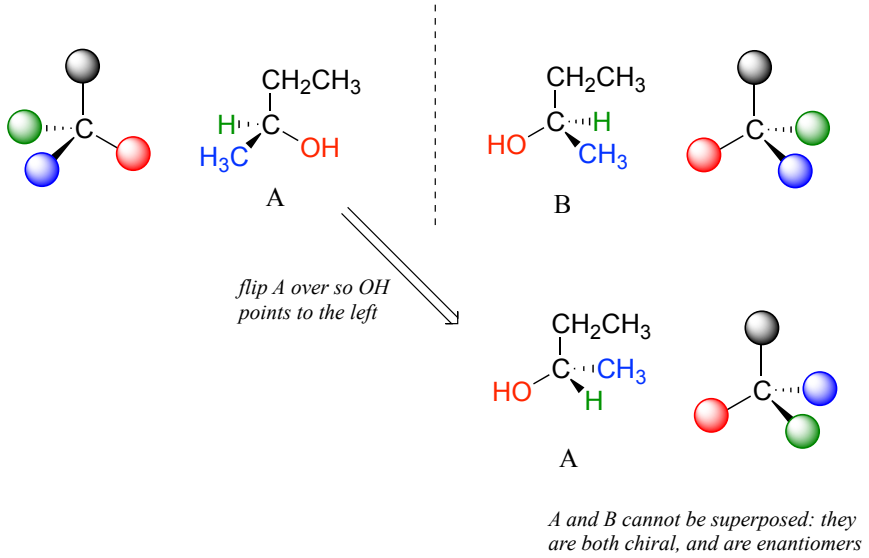
Let's apply our general discussion to real molecules. For now, we will limit our discussion to molecules with a single chiral center. It turns out that tartaric acid, the subject of our chapter introduction, has *two* chiral centers, so we will come back to it later.

Consider 2-butanol, drawn in two dimensions below.



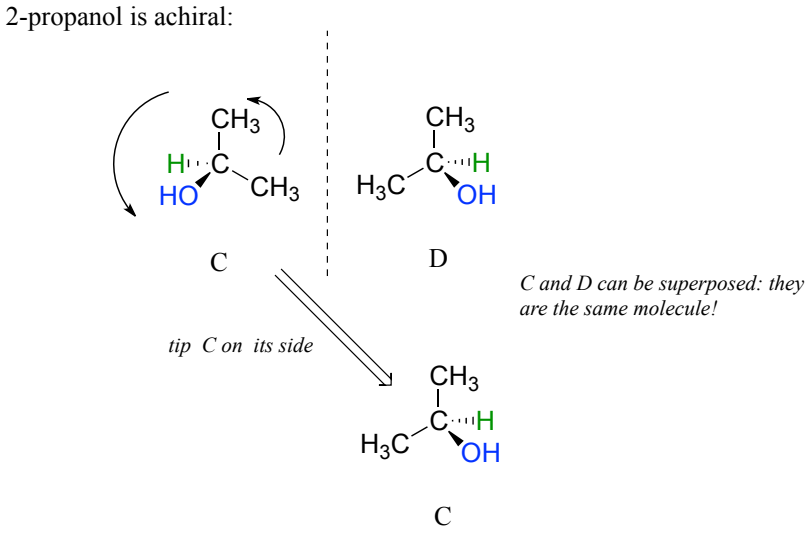
2-butanol
(drawn in 2D)

Carbon #2 is a chiral center: it is sp^3-hybridized and tetrahedral (even though it is not drawn that way above), and the four things attached to is are different: a hydrogen, a methyl ($-CH_3$) group, an ethyl ($-CH_2CH_3$) group, and a hydroxyl (OH) group. Let's draw the bonding at C_2 in three dimensions, and call this structure A. We will also draw the mirror image of A, and call this structure B.



When we try to superimpose A onto B, we find that we cannot do it. A and B are both chiral molecules, and they are enantiomers of each other.

2-propanol, unlike 2-butanol, is *not* a chiral molecule. Carbon #2 is bonded to two identical substituents (methyl groups), and so it is not a chiral center.

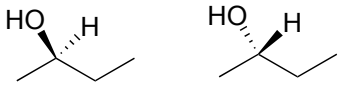


Notice that 2-propanol is superimposable on its own mirror image.

When we look at very simple molecules like 2-butanol, it is not difficult to draw out the mirror image and recognize that it is not superimposable. However, with larger, more complex molecules, this can be a daunting challenge in terms of drawing and three-dimensional visualization. The easy way to determine if a molecule is chiral is simply to look for the presence of one or more chiral centers: molecules with chiral centers will (almost always) be chiral. We insert the 'almost always' caveat here because it is possible

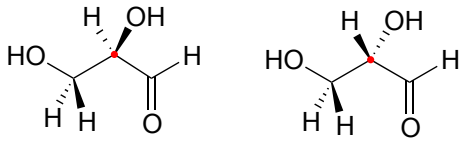
to come up with the exception to this rule - we will have more to say on this later, but don't worry about it for now.

Here's another trick to make your stereochemical life easier: if you want to draw the enantiomer of a chiral molecule, it is not necessary to go to the trouble of drawing the point-for-point mirror image, as we have done up to now for purposes of illustration. Instead, keep the carbon skeleton the same, and simply reverse the solid and dashed wedge bonds on the chiral carbon: that accomplishes the same thing. You should use models to convince yourself that this is true, and also to convince yourself that swapping *any two substituents* about the chiral carbon will result in the formation of the enantiomer.

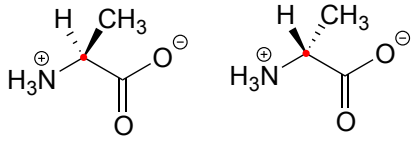


two enantiomers of 2-butanol

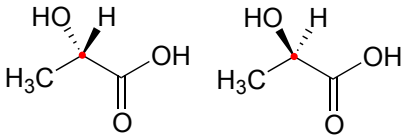
Here are four more examples of chiral biomolecules, each one shown as a pair of enantiomers, with chiral centers marked by red dots.



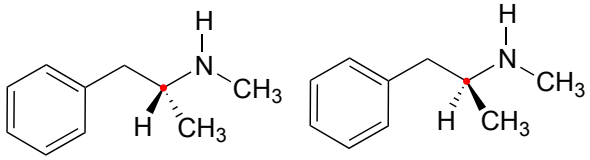
glyceraldehyde



alanine

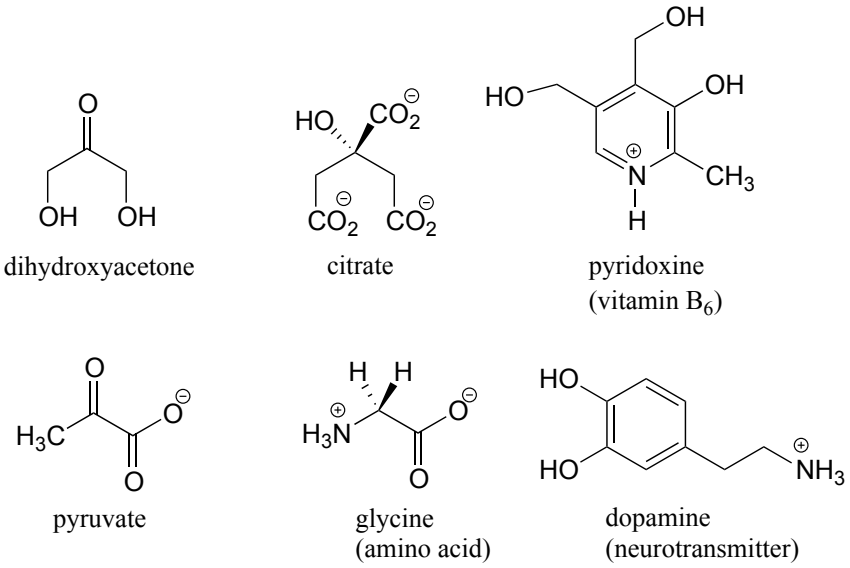


lactic acid



methamphetamine

Here are some examples of achiral biomolecules - convince yourself that none of them contains a chiral center:

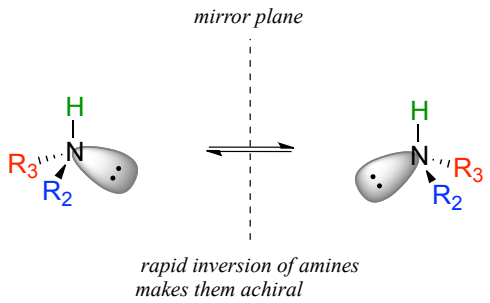


When looking for chiral centers, it is important to recognize that the question of whether or not the dashed/solid wedge drawing convention is used is irrelevant. Chiral molecules are sometimes drawn without using wedges (although obviously this means that stereochemical information is being omitted). Conversely, wedges may be used on carbons that are *not* chiral centers – look, for example, at the drawings of glycine and citrate in the figure above.

Can a chiral center be something other than a tetrahedral carbon with four different substituents? The answer to this question is 'yes' - however, these alternative chiral centers are very rare in the context of biological organic chemistry, and outside the scope of our discussion here.

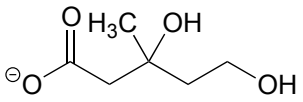
You may also have wondered about amines: shouldn't we consider a secondary or tertiary amine to be a chiral center, as they are tetrahedral and attached to four different substituents, if the lone-pair electrons are counted as a 'substituent'? Put another way, isn't an amine non-superimposable on its mirror image?

The answer: yes it is, in the static picture, but in reality, the nitrogen of an amine is rapidly and reversibly inverting, or turning inside out, at room temperature.

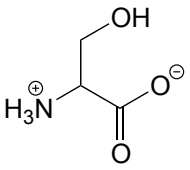


If you have trouble picturing this, take an old tennis ball and cut it in half. Then, take one of the concave halves and flip it inside out, then back again: this is what the amine is doing. The end result is that the two 'enantiomers' if the amine are actually two rapidly interconverting forms of the same molecule, and thus the amine itself is not a chiral center. This inversion process does *not* take place on a tetrahedral carbon, which of course has no lone-pair electrons.

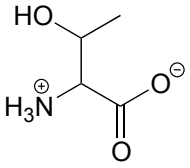
Exercise 3.8: Locate all of the chiral centers (there may be more than one in a molecule). Remember, hydrogen atoms bonded to carbon usually are not drawn in the line structure convention - but they are still there!



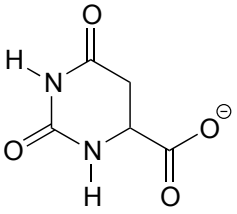
mevalonate



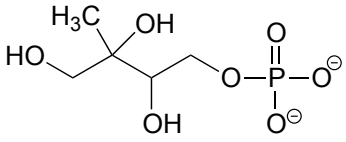
serine



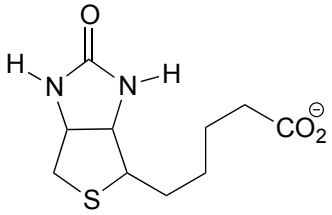
threonine



dihydroorotate



2-methylerythritol-4-phosphate

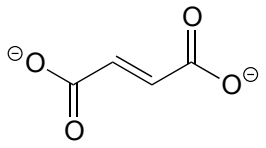


biotin

Exercise 3.9: Draw two enantiomers of a) mevalonate and b) serine.

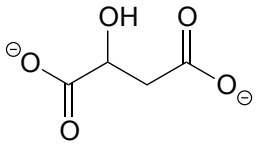
Exercise 3.10: Label the molecules below as chiral or achiral, and locate all chiral centers.

a)



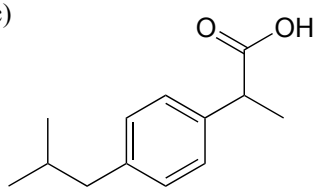
fumarate
(a citric acid cycle intermediate)

b)



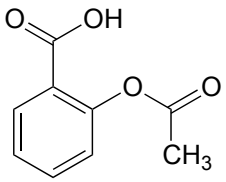
malate
(a citric acid cycle intermediate)

c)



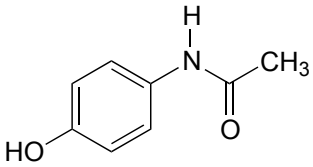
ibuprofen

d)



acetylsalicylic acid
(aspirin)

e)



acetaminophen
(active ingredient in Tylenol)

Section 3.4: Labeling chiral centers

Chemists need a convenient way to distinguish one stereoisomer from another. The **Cahn-Ingold-Prelog system** is a set of rules that allows us to unambiguously define the stereochemical configuration of any stereocenter, using the designations '***R***' (from the Latin *rectus*, meaning right-handed) or '***S***' (from the Latin *sinister*, meaning left-handed).

The rules for this system of stereochemical nomenclature are, on the surface, fairly simple.

Rules for assigning an R/S designation to a chiral center:

1: Assign priorities to the four substituents, with #1 being the highest priority and #4 the lowest. Priorities are based on the atomic number.

2: Trace a circle from #1 to #2 to #3.

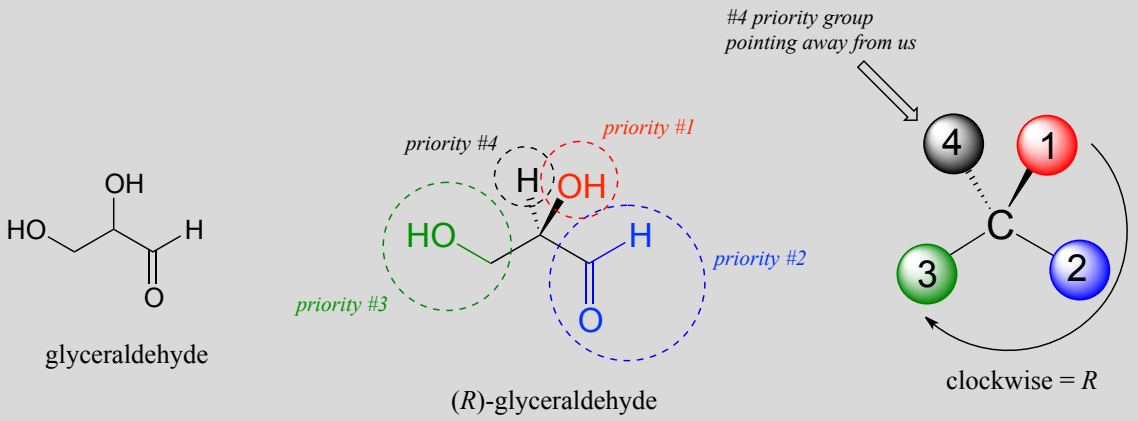
3: Determine the orientation of the #4 priority group. If it is oriented into the plane of the page (away from you), go to step 4a. If it is oriented out of the plane of the page (toward you) go to step 4b.

4a: *(#4 group pointing away from you)*: a clockwise circle in part 2 corresponds to the *R* configuration, while a counterclockwise circle corresponds to the *S* configuration.

4b: *(#4 group pointing toward you)*: a clockwise circle in part 2 corresponds to the *S* configuration, while a counterclockwise circle corresponds to the *R* configuration.

We'll use the 3-carbon sugar glyceraldehyde as our first example. The first thing that we must do is to assign a **priority** to each of the four substituents bound to the chiral center. We first look at the atoms that are directly bonded to the chiral center: these are H, O (in the hydroxyl), C (in the aldehyde), and C (in the CH_2OH group).

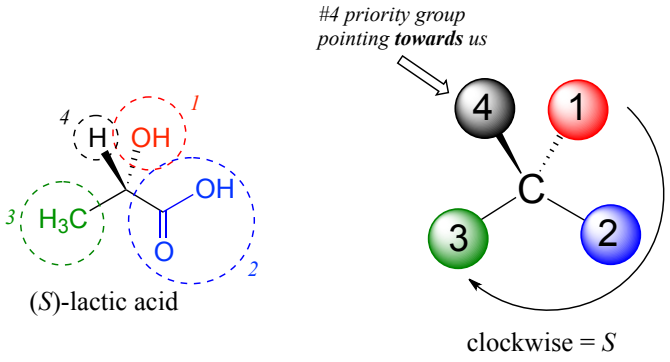
Assigning R/S configuration to glyceraldehyde



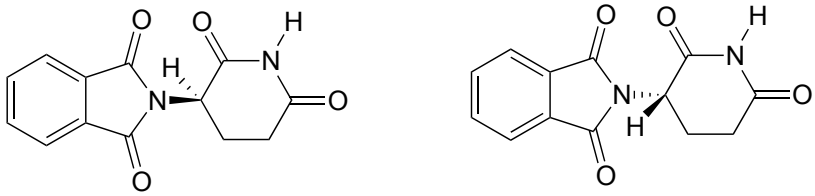
Two priorities are easy: hydrogen, with an atomic number of 1, is the lowest (#4) priority, and the hydroxyl oxygen, with atomic number 8, is priority #1. Carbon has an atomic number of 6. Which of the two 'C' groups is priority #2, the aldehyde or the CH_2OH? To determine this, we move one more bond away from the chiral center: for the aldehyde we have a *double* bond to an oxygen, while on the CH_2OH group we have a *single* bond to an oxygen. If the atom is the same, double bonds have a higher priority than single bonds. Therefore, the aldehyde group is assigned #2 priority and the CH_2OH group the #3 priority.

With our priorities assigned, we look next at the #4 priority group (the hydrogen) and see that it is pointed back away from us, into the plane of the page - thus step 4a from the procedure above applies. Then, we trace a circle defined by the #1, #2, and #3 priority groups, in increasing order. The circle is clockwise, which by step 4a tells us that this carbon has the '*R*' configuration, and that this molecule is (*R*)-glyceraldehyde. Its enantiomer, by definition, must be (*S*)-glyceraldehyde.

Next, let's look at one of the enantiomers of lactic acid and determine the configuration of the chiral center. Clearly, H is the #4 substituent and OH is #1. Owing to its three bonds to oxygen, the carbon on the acid group takes priority #2, and the methyl group takes #3. The #4 group, hydrogen, happens to be drawn pointing *toward* us (out of the plane of the page) in this figure, so we use step 4b: The circle traced from #1 to #2 to #3 is clockwise, which means that the chiral center has the *S* configuration.

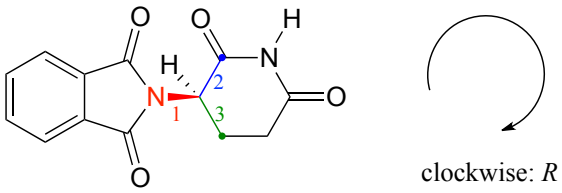


The drug thalidomide is an interesting - but tragic - case study in the importance of stereochemistry in drug design. First manufactured by a German drug company and prescribed widely in Europe and Australia in the late 1950's as a sedative and remedy for morning sickness in pregnant women, thalidomide was soon implicated as the cause of devastating birth defects in babies born to women who had taken it. Thalidomide contains a chiral center, and thus exists in two enantiomeric forms. It was marketed as a **racemic mixture**: in other words, a 50:50 mixture of both enantiomers.



two enantiomers of thalidomide

Let's try to determine the stereochemical configuration of the enantiomer on the left. Of the four bonds to the chiral center, the #4 priority is hydrogen. The nitrogen group is #1, the carbonyl side of the ring is #2, and the $-CH_2$ side of the ring is #3.



(R)-thalidomide

The hydrogen is shown pointing away from us, and the prioritized substituents trace a clockwise circle: this is the *R* enantiomer of thalidomide. The other enantiomer, of course, must have the S configuration.

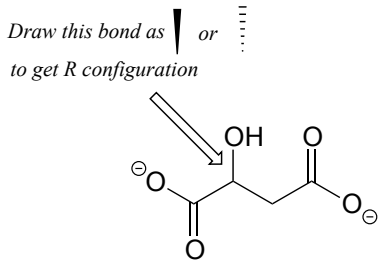
Although scientists are still unsure today how thalidomide works, experimental evidence suggests that it was actually the *R* enantiomer that had the desired medical effects, while the *S* enantiomer caused the birth defects. Even with this knowledge, however, pure (*R*)-thalidomide is not safe, because enzymes in the body rapidly convert between the two enantiomers - we will see how that happens in chapter 12.

As a historical note, thalidomide was never approved for use in the United States. This was thanks in large part to the efforts of Dr. Frances Kelsey, a Food and Drug officer who, at peril to her career, blocked its approval due to her concerns about the lack of adequate safety studies, particularly with regard to the drug's ability to enter the bloodstream of a developing fetus. Unfortunately, though, at that time clinical trials for new drugs involved widespread and unregulated distribution to doctors and their patients across the country, so families in the U.S. were not spared from the damage caused.

Very recently a close derivative of thalidomide has become legal to prescribe again in the United States, with strict safety measures enforced, for the treatment of a form of blood cancer called multiple myeloma. In Brazil, thalidomide is used in the treatment of leprosy - but despite safety measures, children are still being born with thalidomide-related defects.

Exercise 3.11: Determine the stereochemical configurations of the chiral centers in the alanine, lactic acid and amphetamine structures.

Exercise 3.12: Draw the (*R*) enantiomer of malate (just copy the structure below, and use a solid or dash wedge for the C-O bond at the chiral center.

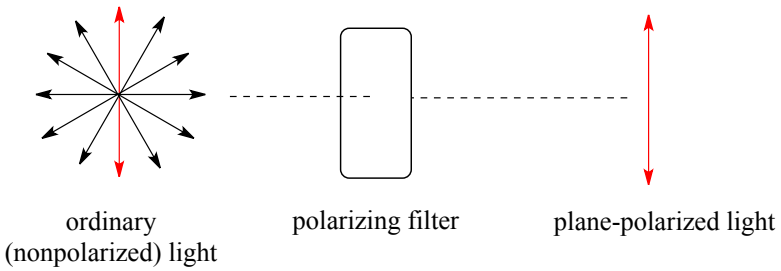


(*R*)-malate

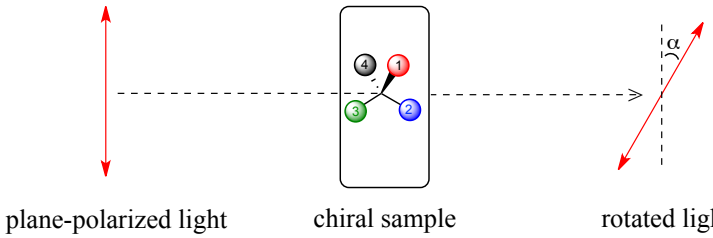
Exercise 3.13: Using solid or dashed wedges to show stereochemistry, draw the (*R*) enantiomer of ibuprofen (structure in exercise 3.10), and the (*S*) enantiomer of mevalonate, and dihydroorotate (structures are shown earlier in this chapter).

Section 3.5: Optical activity

Chiral molecules, as we learned in the introduction to this chapter, have an interesting optical property. You may know from studying physics that light waves are oscillating electric and magnetic fields. In ordinary light, the oscillation is randomly oriented in an infinite number of planes. When ordinary light is passed through a polarizer, all planes of oscillation are filtered out except one, resulting in **plane-polarized light**.



A beam of plane-polarized light, when passed through a sample of a chiral compound, interacts with the compound in such a way that the angle of oscillation will rotate. This property is called **optical activity**.



If a compound rotates plane polarized light in the clockwise (+) direction, it is said to be **dextrorotatory**, while if it rotates light in the counterclockwise (-) direction it is **levorotatory**. (We mentioned L- and D-amino acids in the previous section: the L-amino acids are levorotatory). The magnitude of the observed optical activity is dependent on temperature, the wavelength of light used, solvent, concentration of the chiral sample, and the path length of the sample tube (path length is the length that the plane-polarized light travels through the chiral sample). Typically, optical activity measurements are made in a 1 decimeter (10 cm) path-length sample tube at 25 °C, using as a light source the so-called "D-line" from a sodium lamp, which has a wavelength of 589 nm. The **specific rotation** [α] of a compound at 25° is expressed by the expression:

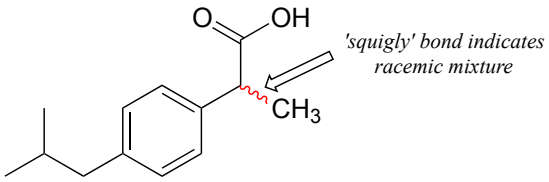
specific rotation:

$$[\alpha]_{D}^{25} = \frac{\alpha_{obs}}{lc}$$

. . . where α_{obs} is the observed rotation, *l* is path length in dm, and *c* is the concentration of the sample in grams per 100 mL. Every chiral molecule has a characteristic specific rotation, which is recorded in the chemical literature as a physical property just like melting point or density.

Different enantiomers of a compound will always rotate plane-polarized light with an equal but opposite magnitude. (*S*)-ibuprofen, for example, has a specific rotation of +54.5° (dextrorotatory) in methanol, while (*R*)-ibuprofen has a specific rotation of -54.5°. *There is no relationship between chiral compound's R/S designation and the direction of its specific rotation.* For example, the *S* enantiomer of ibuprofen is dextrorotatory, but the *S* enantiomer of glyceraldehyde is levorotatory.

A 50:50 mixture of two enantiomers (a racemic mixture) will have no observable optical activity, because the two optical activities cancel each other out. In a structural drawing, a 'squigly' bond from a chiral center indicates a mixture of both *R* and *S* configurations.



(+/-) ibuprofen
(racemic)

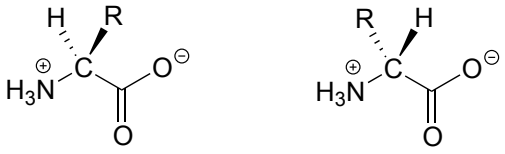
Chiral molecules are often labeled according to whether they are dextrorotatory or levorotatory as well as by their *R/S* designation. For example, the pure enantiomers of ibuprofen are labeled (*S*)-(+)-ibuprofen and (*R*)-(-)-ibuprofen, while (±)-ibuprofen refers to the racemic mixture, which is the form in which the drug is sold to consumers.

Exercise 3.14: The specific rotation of (*R*)-limonene is +11.5° in ethanol. What is the expected observed rotation of a sample of 6 g (*S*)-limonene dissolved in ethanol to a total volume of 80 mL?

Exercise 3.15: The specific rotation of (*S*)-carvone is +61°, measured 'neat' (pure liquid sample, no solvent). The optical rotation of a mixture of *R* and *S* carvone is measured at -23°. Which enantiomer is in excess in the mixture?

All of the twenty natural amino acids except glycine have a chiral center at their α-carbon (recall that basic amino acid structure and terminology was introduced in section 1.3D). Virtually all of the amino acids found in nature, both in the form of free amino acids or incorporated into peptides and proteins, have what is referred to in the biochemical literature as the 'L' configuration:

R = *amino acid side chain*



L-amino acids
(common in nature)

D-amino acids
(rare in nature)

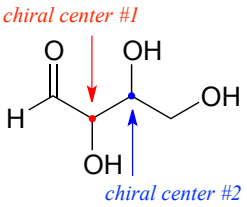
The 'L' indicates that these amino acid stereoisomers are levorotatory. All but one of the 19 L-amino acids have *S* stereochemistry at the α–carbon, using the rules of the *R/S* naming system.

Exercise 3.16: Which L-amino acid has the *R* configuration?

D-amino acids (the D stands for dextrorotatory) are very rare in nature, but we will learn about an interesting example of a peptide containing one D-amino acid residue later in chapter 12.

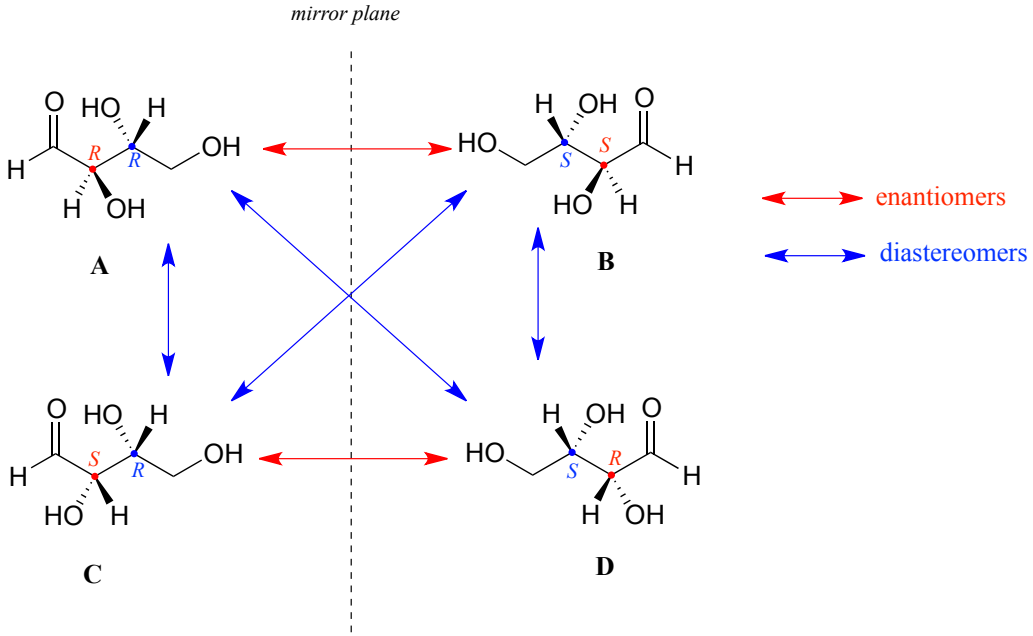
Section 3.6: Compounds with multiple chiral centers

So far, we have been analyzing compounds with a single chiral center. Next, we turn our attention to those which have multiple chiral centers. We'll start with some stereoisomeric four-carbon sugars with two chiral centers.



To avoid confusion, we will simply refer to the different stereoisomers by capital letters.

Look first at compound A, below. Both chiral centers in have the *R* configuration (you should confirm this for yourself!). The mirror image of Compound A is compound B, which has the *S* configuration at both chiral centers. If we were to pick up compound A, flip it over and put it next to compound B, we would see that they are *not* superimposable (again, confirm this for yourself with your models!). A and B are nonsuperimposable mirror images: in other words, enantiomers.



Now, look at compound C, in which the configuration is *S* at chiral center 1 and *R* at chiral center 2. Compounds A and C are stereoisomers: they have the same molecular formula and the same bond connectivity, but a different arrangement of atoms in space (recall that this is the definition of the term 'stereoisomer). However, they are *not* mirror images of each other (confirm this with your models!), and so they are *not* enantiomers. By definition, they are **diastereomers** of each other.

Notice that compounds C and B also have a diastereomeric relationship, by the same definition.

So, compounds A and B are a pair of enantiomers, and compound C is a diastereomer of both of them. Does compound C have its own enantiomer? Compound D is the mirror image of compound C, and the two are not superimposable. Therefore, C and D are a pair of enantiomers. Compound D is also a diastereomer of compounds A and B.

This can also seem very confusing at first, but there some simple shortcuts to analyzing stereoisomers:

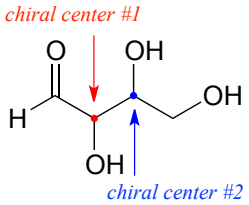
Stereoisomer shortcuts

If ***all*** of the chiral centers are of opposite R/S configuration between two stereoisomers, they are enantiomers.

If ***at least one, but not all*** of the chiral centers are opposite between two stereoisomers, they are diastereomers.

(*Note: these shortcuts to not take into account the possibility of additional stereoisomers due to alkene groups: we will come to that later)*

Here's another way of looking at the four stereoisomers, where one chiral center is associated with red and the other blue. Pairs of enantiomers are stacked together.

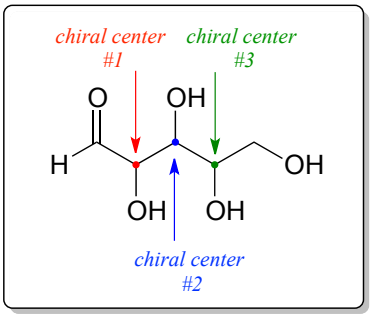


The four possible configurations:

RR	*RS*
SS	*SR*

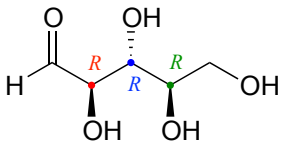
We know, using the shortcut above, that the enantiomer of RR must be SS - both chiral centers are different. We also know that RS and SR are diastereomers of RR, because in each case one - but not both - chiral centers are different.

Now, let's extend our analysis to a sugar molecule with three chiral centers. Going through all the possible combinations, we come up with eight total stereoisomers - four pairs of enantiomers.



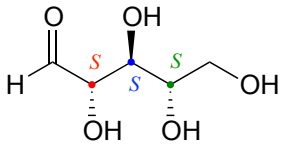
RRR	RRS	RSR	RSS
SSS	SSR	SRS	SSR

Let's draw the *RRR* stereoisomer. Being careful to draw the wedge bonds correctly so that they match the *RRR* configurations, we get:



RRR stereoisomer

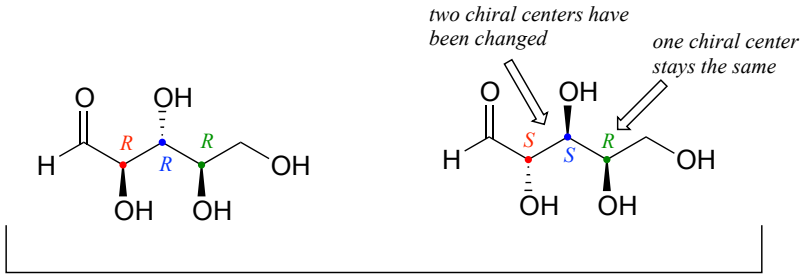
Now, using the above drawing as our model, drawing any other stereoisomer is easy. If we want to draw the enantiomer of *RRR*, we don't need to try to visualize the mirror image, we just start with the *RRR* structure and invert the configuration at *every* chiral center to get *SSS*.



SSS stereoisomer

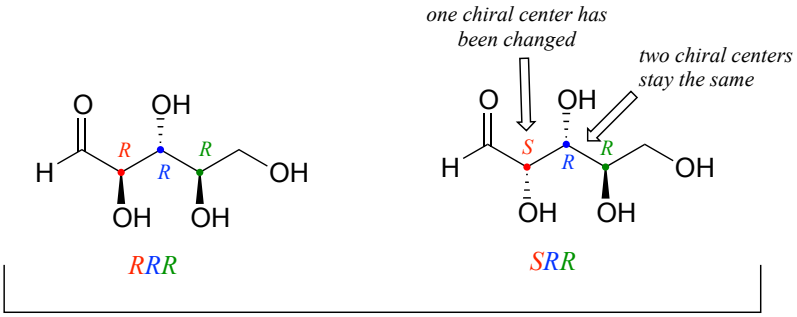
Try making models of *RRR* and *SSS* and confirm that they are in fact nonsuperimposable mirror images of each other.

There are six diastereomers of *RRR*. To draw one of them, we just invert the configuration of at least one, but not all three, of the chiral centers. Let's invert the configuration at chiral center 1 and 2, but leave chiral center 3 unchanged. This gives us the *SSR* configuration.



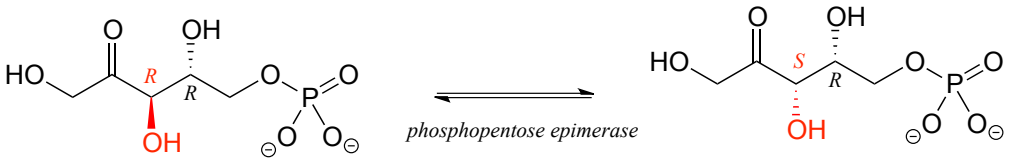
diastereomers

One more definition at this point: diastereomers which differ at only a single chiral center are called **epimers**. For example, *RRR* and *SRR* are epimers:



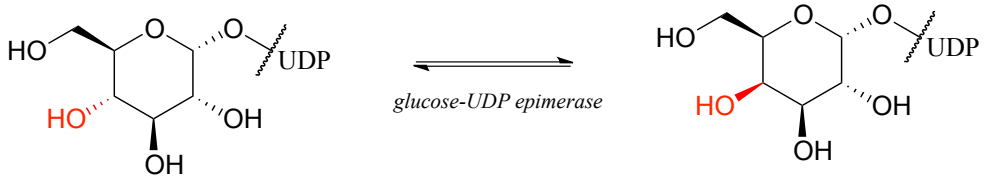
The *RRR* and *SSR* stereoisomers shown above are diastereomers but *not* epimers because they differ at *two* of the three chiral centers.

The epimer term is useful because in biochemical pathways, compounds with multiple chiral centers are isomerized at one specific center by enzymes known as **epimerases**. Two examples of epimerase-catalyzed reactions are below.



ribulose-5-phosphate

xylulose-5-phosphate



glucose-UDP

galactose-UDP

We know that enantiomers have identical physical properties and equal but opposite magnitude specific rotation. Diastereomers, in theory at least, have *different* physical properties – we stipulate 'in theory' because sometimes the physical properties of two or more diastereomers are so similar that it is very difficult to distinguish between them. In addition, the specific rotation values of diastereomers are unrelated – they could be the same sign or opposite signs, similar in magnitude or very dissimilar.

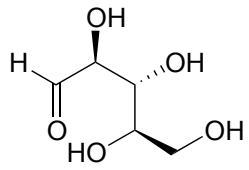
Exercise 3.17:

a) Draw the structure of the *enantiomer* of the *SRS* stereoisomer of the sugar used in the previous example.

b) List (using the *XXX* format, not drawing the structures) all of the epimers of *SRS*.

c) List all of the stereoisomers that are diastereomers, but not epimers, of *SRS*.

Exercise 3.18: The sugar below is one of the stereoisomers that we have been discussing.



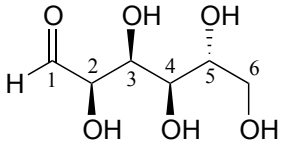
?

The only problem is, it is drawn with the carbon backbone in a different orientation from what we have seen. Determine the configuration at each chiral center to determine which stereoisomer it is.

Exercise 3.19: Draw the enantiomer of xylulose-5-phosphate.

Exercise 3.20: Draw all of the stereoisomers of the amino acid threonine, and identify pairs of enantiomers.

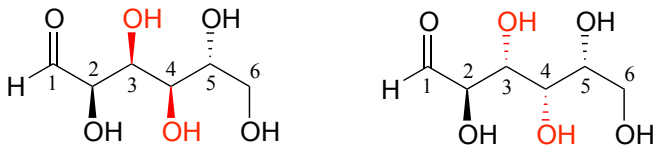
Here is some more practice in identifying isomeric relationships. D-glucose is the monosaccharide that serves as the entrance point for the glycolysis pathway and as a building block for the carbohydrate biopolymers starch and cellulose. The 'D' in D-glucose stands for *dextrarotatory* and is part of the specialized nomenclature system for sugars, which we will not concern ourselves with here. The open-chain structure of the sugar is shown below.



D-glucose

Because D-glucose has four chiral centers, it can exist in a total of $2^4 = 16$ different stereoisomeric forms: it has one enantiomer and 14 diastereomers.

Now, let's compare the structures of the two sugars D-glucose and D-gulose, and try to determine their relationship.

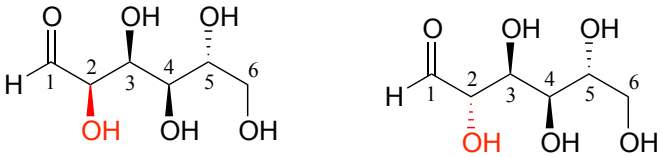


D-glucose D-gulose

(diastereomers)

The two structures have the same molecular formula and the same connectivity, therefore they must be stereoisomers. They each have four chiral centers, and the configuration is different at two of these centers (at carbons #3 and #4). They are diastereomers.

Now, look at the structures of D-glucose and D-mannose.

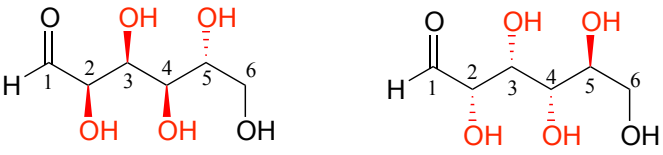


D-glucose D-mannose

(epimers)

Here, everything is the same except for the configuration of the chiral center at carbon #2. The two sugars differ at only one of the four chiral centers, so again they are diastereomers, and more specifically they are epimers.

D-glucose and L-glucose are enantiomers, because they differ at all four chiral centers.

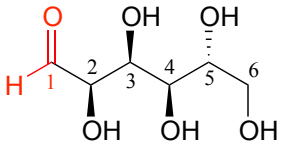


D-glucose L-glucose

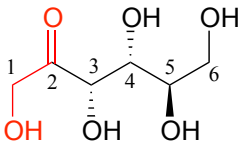
(enantiomers)

D-glucose is the enantiomer commonly found in nature.

D-glucose and D-fructose are not stereoisomers, because they have different bonding connectivity: glucose has an aldehyde group, while fructose has a ketone. The two sugars do, however, have the same molecular formula, so by definition they are constitutional isomers.



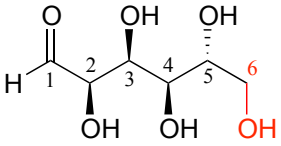
D-glucose



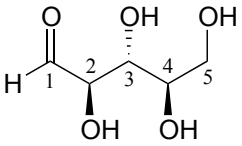
D-fructose

(constitutional isomers)

D-glucose and D-ribose are not isomers of any kind, because they have different molecular formulas.



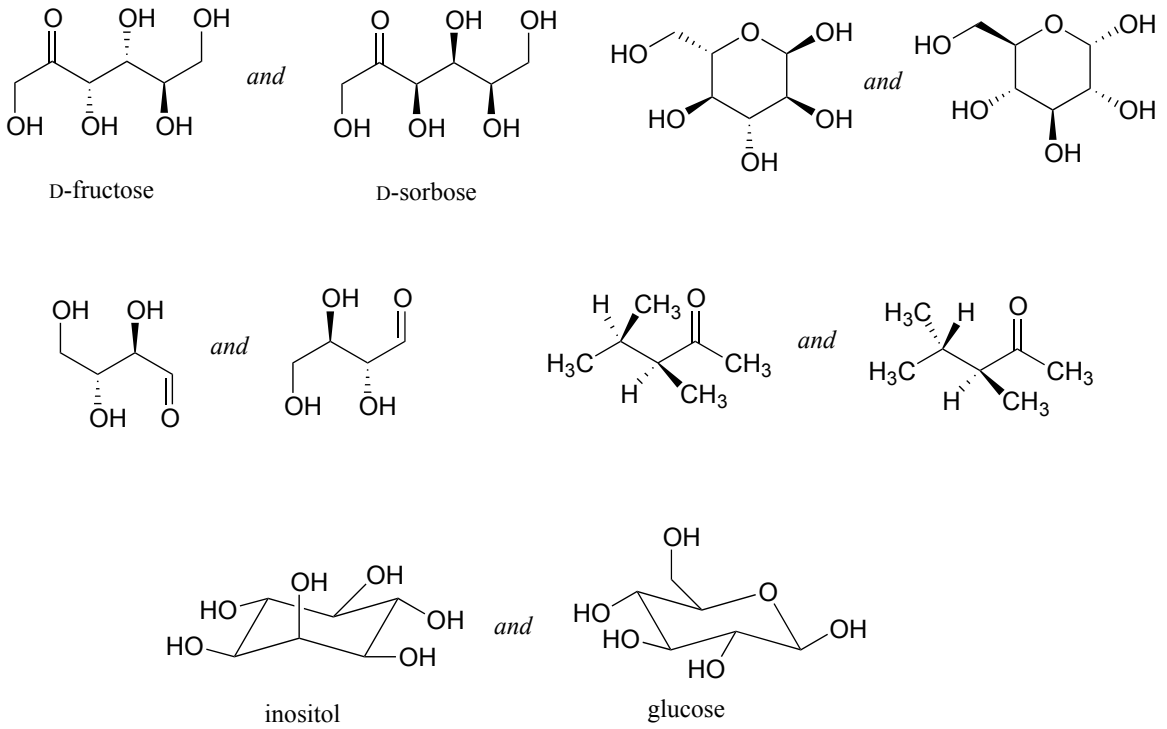
D-glucose



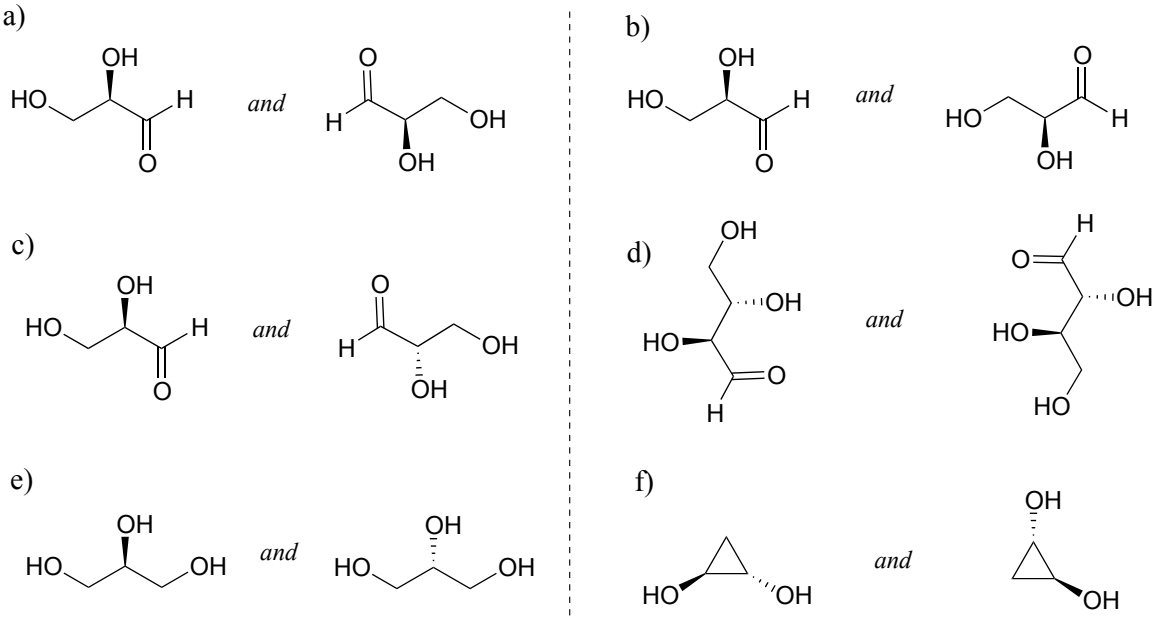
D-ribose

(not isomers)

Exercise 3.21: Identify the relationship between each pair of structures. Your choices are: not isomers, constitutional isomers, diastereomers but not epimers, epimers, enantiomers, or same molecule.

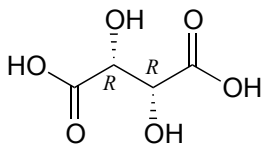


Exercise 3.22: Identify the relationship between each pair of structures.

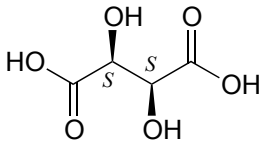


Section 3.7: *Meso* compounds

The levorotatory and dextrorotatory forms of tartaric acid studied by Louis Pasteur were, as we now know, the (*S,S*) and (*R,R*) enantiomers, respectively.



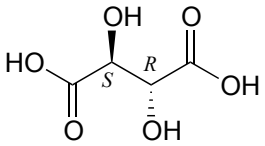
(*R,R*)-(+)-tartaric acid
('natural' isomer from grapes)



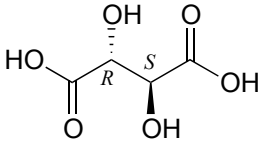
(*S,S*)-(-)-tartaric acid

What the 19th century chemists referred to as *'acide racemique'* was just that: a racemic mixture of the *R,R* and *S,S* enantiomers, the racemization a result of how the natural *R,R* isomer had been processed.

But tartaric acid has two chiral centers: shouldn't there be another pair of enantiomers?

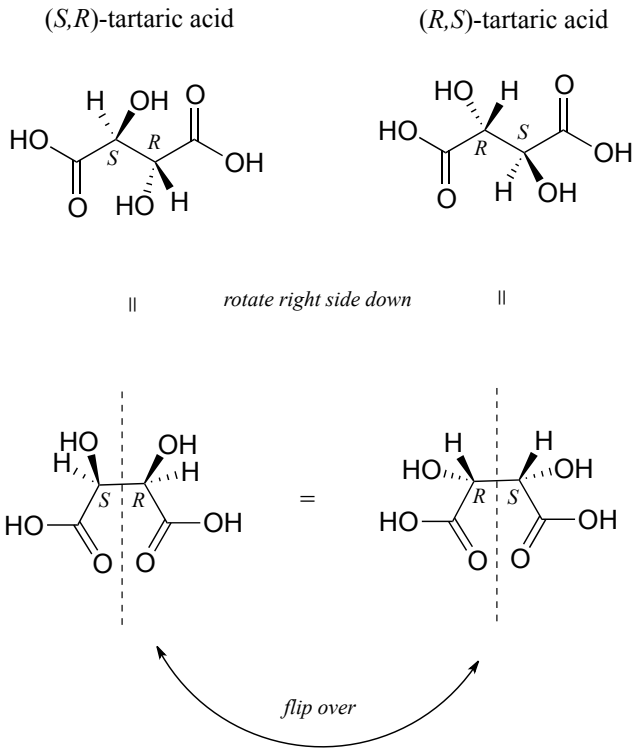


(*S,R*)-tartaric acid



(*R,S*)-tartaric acid

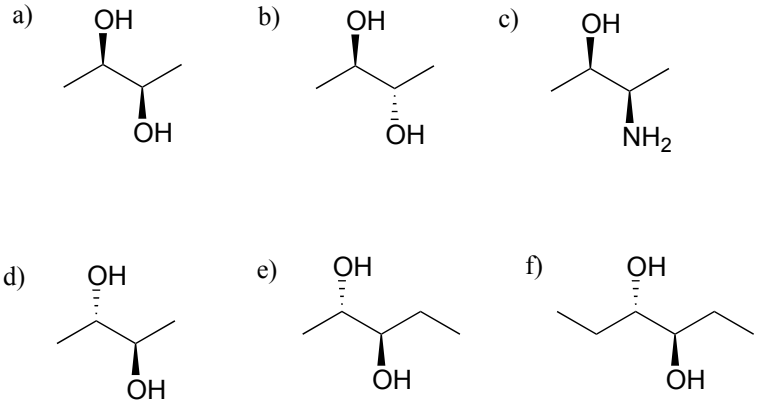
There in fact is another stereoisomer of tartaric acid: *but only one*. The two structures above are actually superimposable on one another: they are the exact same molecule. The figure below illustrates this, and also that the structure has a plane of symmetry. However, you should be sure to build models and confirm these assertions for yourself.



This tartaric acid isomer is an *achiral* diastereomer of the both the levorotatory and dextrorotatory isomers. It is a special case, called a ***meso*** compound: it has two apparent chiral centers but due to its internal symmetry it is not in fact chiral, and does not exhibit optical activity. Note that the *meso* form of tartaric acid did not play a part in Pasteur's experiments.

There are many more possible examples of *meso* compounds, but they really can be considered 'exceptions to the rule' and quite rare in biologically relevant chemistry.

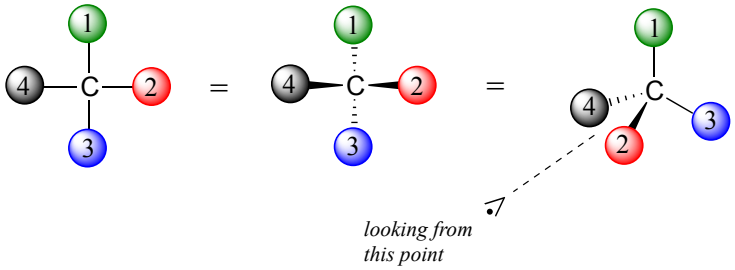
Exercise 3.23: Which of the following compounds are *meso*? Hint: build models, and then try to find a conformation in which you can see a plane of symmetry.



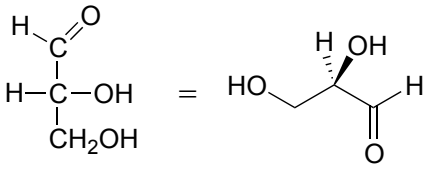
Section 3.8: Fischer and Haworth projections

When reading the chemical and biochemical literature, you are likely to encounter several different conventions for drawing molecules in three dimensions, depending on the context of the discussion. While organic chemists prefer to use the dashed/solid wedge convention to show stereochemistry, biochemists often use drawings called **Fischer projections** and **Haworth projections** to discuss and compare the structure of sugar molecules.

Fisher projections show sugars in their open chain form. In a Fischer projection, the carbon atoms of a sugar molecule are connected vertically by solid lines, while carbon-oxygen and carbon-hydrogen bonds are shown horizontally. Stereochemical information is conveyed by a simple rule: vertical bonds point into the plane of the page, while horizontal bonds point out of the page.

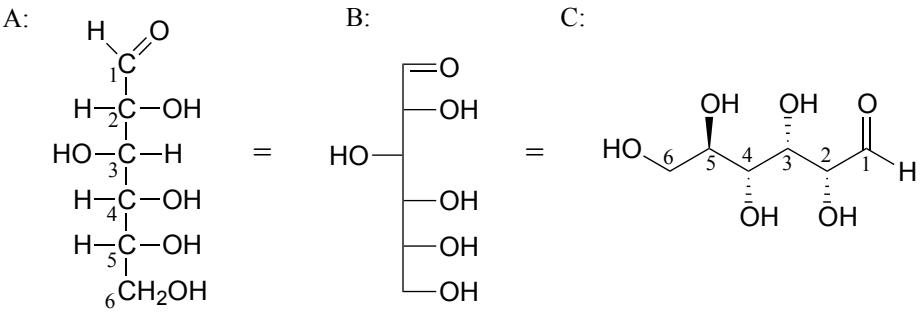


Below are two different representations of (R)-glyceraldehyde, the smallest sugar molecule (also called D-glyceraldehyde in the stereochemical nomenclature used for sugars)

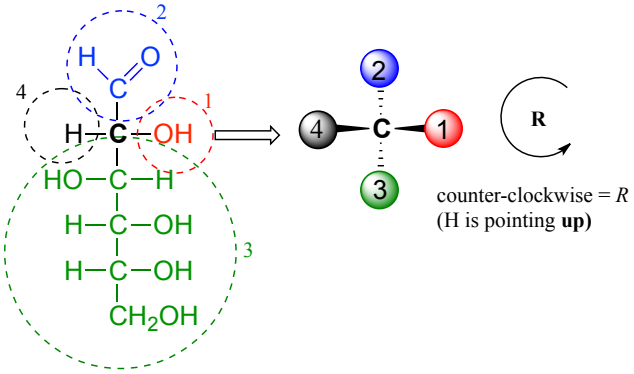


(R)-glyceraldehyde
(D-glyceraldehyde)

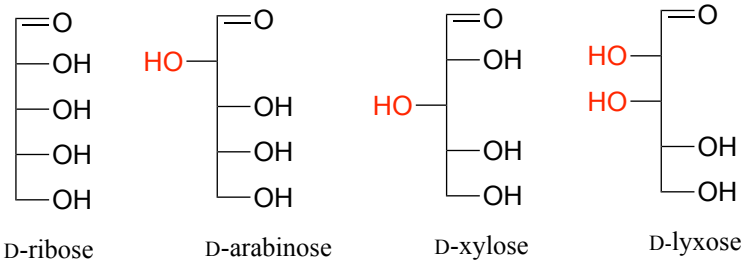
Below are three representations of the open chain form of D-glucose: in the conventional Fischer projection (A), in the “line structure” variation of the Fischer projection in which carbons and hydrogens are not shown (B), and finally in the 'zigzag' style (C) that is preferred by organic chemists.



Care must be taken when 'translating' Fischer projection structures into' zigzag' format – it is easy to get the stereochemistry wrong. Probably the best way to make a translation is to simply assign *R*/*S* configurations to each stereocenter, and proceed from there. When deciding whether a stereocenter in a Fischer projection is *R* or *S*, realize that the hydrogen, in a horizontal bond, is pointing *towards* you – therefore, a counterclockwise circle means *R*, and a clockwise circle means *S* (the opposite of when the hydrogen is pointing away from you).

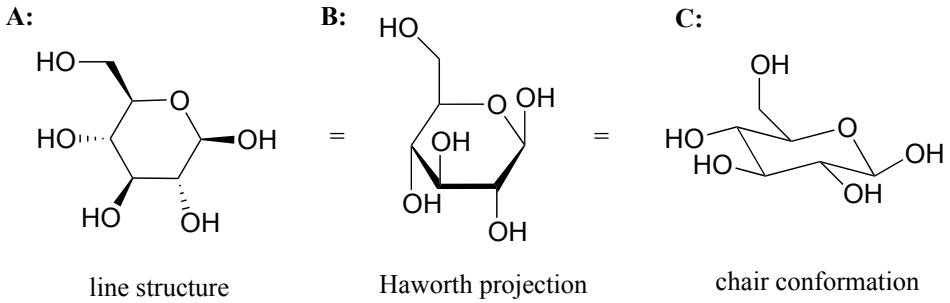


Fischer projections are useful when looking at many different diastereomeric sugar structures, because the eye can quickly pick out stereochemical differences according to whether a hydroxyl group is on the left or right side of the structure.



Exercise 3.24: Draw 'zigzag' structures (using the solid/dash wedge convention to show stereochemistry) for the four sugars in the figure above. Label all stereocenters *R* or *S*.

While Fischer projections are used for sugars in their open-chain form, Haworth projections are often used to depict sugars in their cyclic forms. The β diastereomer of the cyclic form of glucose is shown below in three different depictions, with the Haworth projection in the middle.

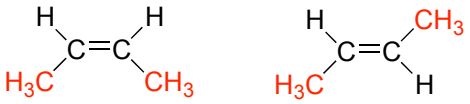


line structure Haworth projection chair conformation

Notice that although a Haworth projection is a convenient way to show stereochemistry, it does not provide a realistic depiction of conformation. To show *both* conformation and stereochemistry, you must draw the ring in the chair form, as in structure C above.

Section 3.9: Stereochemistry of alkenes

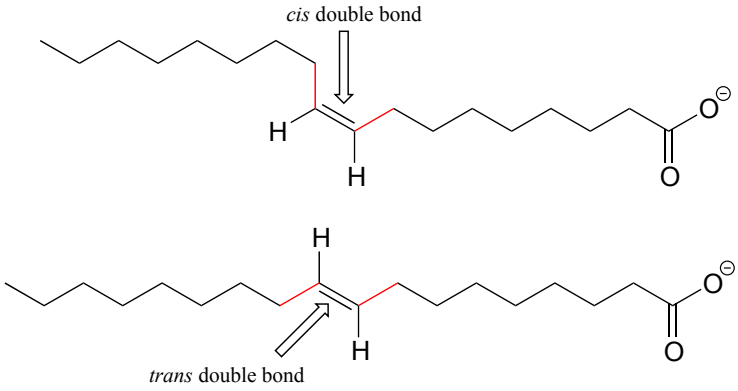
When we talk about stereochemistry, we are not always talking about chiral compounds and chiral centers. Consider *cis*- and *trans*-2-butene:



cis-2-butene *trans*-2-butene

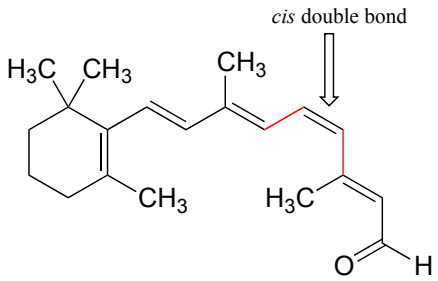
Each can be superimposed on its own mirror image, and neither is chiral (also, note the lack of a chiral center!) However, they both have the same molecular formula and the same bonding connectivity, so by definition they are stereoisomers of each other. Because they are not mirror images, they must be diastereomers. An alkene group which can exist in two stereoisomeric forms is referred to as **stereogenic**.

Alkene groups in natural unsaturated fatty acids are normally *cis*, but *trans*-fatty acids (which are thought to be associated with heart disease and other health problems) are found in some food products.

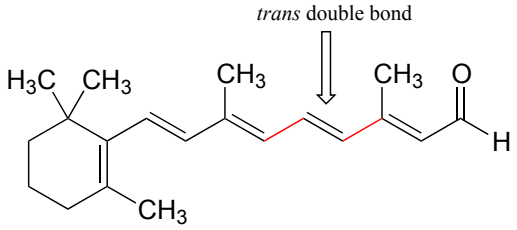


cis and *trans* fatty acids

Retinal is a light-sensitive molecule, derived from vitamin A, that is found in the rod cells of the eye. When light enters the eye through the retina, one form of retinal is converted to a diastereomer when a *cis* double bond is converted to *trans* (we"ll learn how this happens in chapter x). This changes the shape of the molecule and the way that it binds to the vision protein rhodopsin, which in turn initiates a chain of events that leads to a signal being sent to the vision center of the brain.



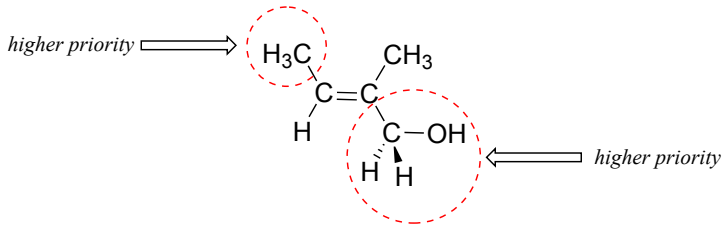
11-*cis*-retinal



all-t*rans*-retinal

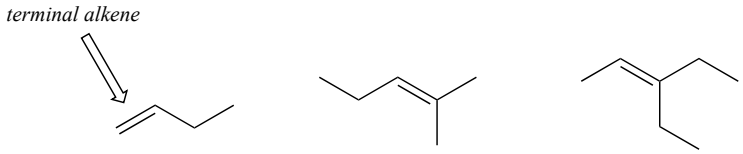
While the terms *cis* and *trans* are quite clear in the examples above, in some cases they can be ambiguous, and a more rigorous stereochemical designation is required. To unambiguously designate alkene stereochemistry, it is best to use the designators '*E*' and 'Z' rather than *trans* and *cis*. To use this naming system, we first decide which is the higher priority group on each carbon of the double bond, using the same priority rules that we learned for the *R/S* system. If the higher-priority groups are one the same side of the double bond, it is a *Z*-alkene, and if they are on the opposite side it is an *E*-alkene. A memory device that many students find helpful is the phrase *'Z = zame zide'*.

Shown below is an example of an *E*-alkene: notice that, although the two methyl groups are on the same side relative to one another, the alkene has *E* stereochemistry according to the rules of the *E/Z* system because one of the methyl groups takes a higher priority (relative to a hydrogen) and the other takes lower priority (relative to a primary alcohol). The *cis/trans* terms would be ambiguous for this compound.



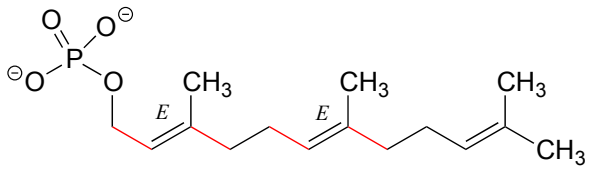
an *E*-alkene

Not all alkenes can be labeled *E* or *Z:* if one of the double-bonded carbons has identical substituents, the alkene is not stereogenic, and thus cannot be assigned an E or Z configuration. Terminal alkenes, in which one of the alkene carbons is bonded to two hydrogen atoms, are the most commonly seen type of nonstereogenic alkene.

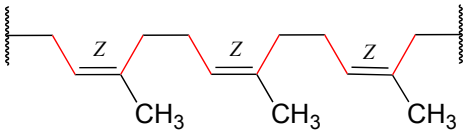


nonstereogenic alkenes (cannot be labelled *E* or *Z)*

Natural rubber is a polymer composed of five-carbon isoprenoid building blocks (see section 1.3A) linked with *Z* stereochemistry. The same isoprenpoid building blocks can also be connected with *E* stereochemistry, leading to a polymer that is a precursor to cholesterol and many other natural isoprenoid compounds found in all forms of life.



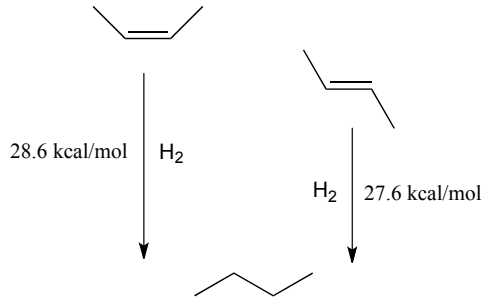
E-isoprenoids
(eg. a precursor to cholesterol)



Z-isoprenoids
(eg. rubber)

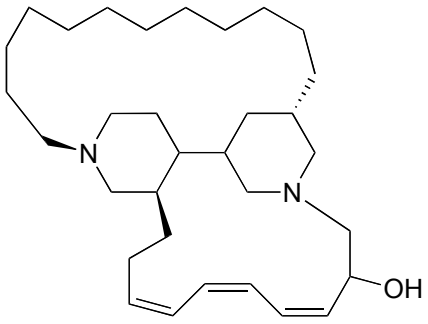
Alkenes located inside a five- or six-membered ring, such as in cyclohexene, are not generally labeled *E* or *Z*, simply because the closed geometry of the ring allows for only one stereochemical possibility. (*E*)-cyclohexene is not physically possible, so it is not necessary to include the (*Z*) designator for cyclohexene. Larger rings, however, can hypothetically have *E* or *Z* alkene groups: two actual examples are included in exercise 3.26 below.

As a general rule, alkenes with the bulkiest groups on opposite sides of the double bond are more stable, due to reduced steric strain. The *trans* (*E)* diastereomer of 2-butene, for example, is slightly lower in energy than the *cis (Z)* diastereomer, as seen by their relative heats of hydrogenation to butane (see section 2.2C for a reminder of the meaning of 'heat of hydrogenation'.)

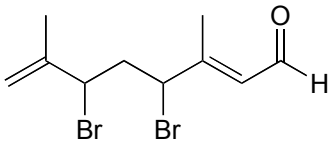


Exercise 3.25: Label the alkene groups below as *E*, *Z*, or *N* (for a nonstereogenic alkene).

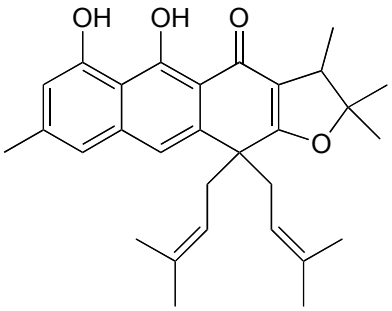
Exercise 3.26: The compounds shown below were all isolated from natural sources and their structures reported in a 2007 issue of the *Journal of Natural Products*, an American Chemical Society publication. Label all alkene groups that are not inside 5- or 6-membered rings as *E*, *Z*, or *N* (for a nonstereogenic alkene).



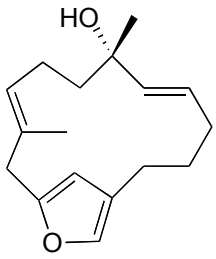
from marine sponge
J. Nat. Prod. **2007**, *70*, 538



from algea
J. Nat. Prod. **2007**, *70*, 596



from Nketto plant in Cameroon
J. Nat. Prod. **2007**, *70*, 600



from tree bark in Thailand
J. Nat. Prod. **2007**, *70*, 659

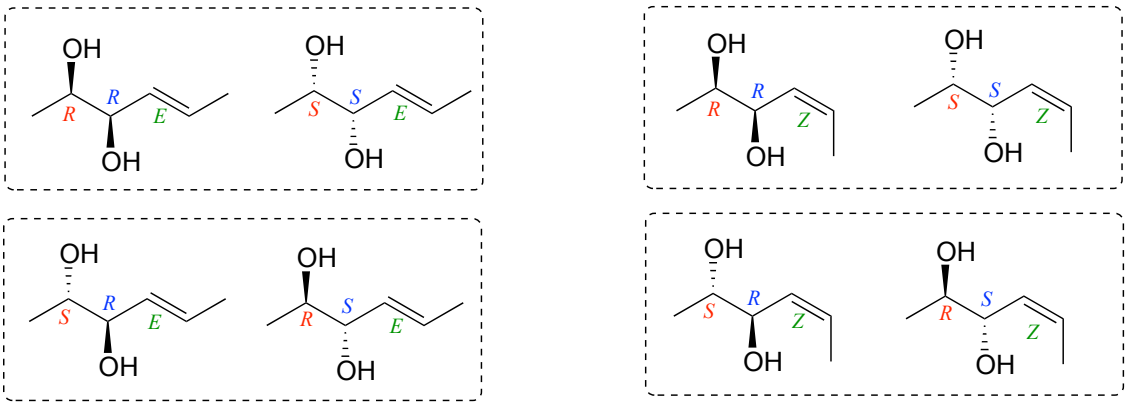
How do we know how many stereoisomers are possible for a given structure? There is actually a straightforward way to figure this out. All we need to do is count the number of chiral centers and stereogenic alkene groups, the use this following rule:

number of stereoisomeric forms = 2^n

. . . where *n* = the number of chiral centers ***plus*** the number of stereogenic alkene groups

(the rare exception to this rule is when a meso *form is possible - in this case, the rule becomes* 2^n*-1)*

Consider for example a molecule with two chiral centers and one stereogenic alkene. By the rule stated above, we know right away that there must be eight possible stereoisomers. Drawing out all the possibilities, we see:

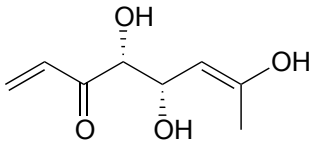


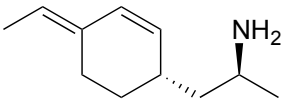
8 stereoisomers of a compound with two chiral centers plus one stereogenic alkene

We see that, for example, *RRE* has one enantiomer, the *SSE* compound. The six other stereoisomers are all diastereomers of *RRE*.

It needs to be stressed that the enantiomer of the *RRE* compound is the *SSE* compound, ***not*** the *SSZ* compound. Remember, the *E/Z* relationship is diastereomeric, not enantiomeric. Use models to convince yourself that the *RRE* and the *SSE* isomers are mirror images of each other, while *RRE* and *SSZ* compounds are not. In general, to get the enantiomer of a compound, we invert all chiral centers *but leave all stereogenic alkenes the same.*

Exercise 3.27: Draw the enantiomer of each the compounds below, and assign configurations to all chiral centers and stereogenic alkenes. How many diastereomers are possible for each of the structures you drew?

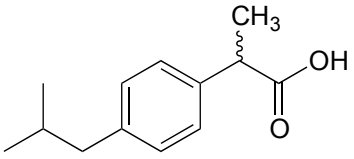




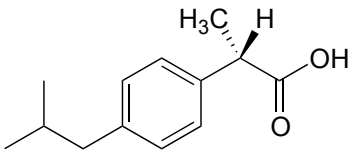
Section 3.10: Stereochemistry in biology and medicine

While challenging to understand and visualize, the stereochemistry concepts we have explored in this chapter are integral to the study of living things. The vast majority of biological molecules contain chiral centers and/or stereogenic alkene groups. Most importantly, proteins are chiral, which of course includes all of the the enzymes which catalyze the chemical reactions of a cell, the receptors which transmit information within or between cells, and the antibodies which bind specifically to potentially harmful invaders. You know from your biology classes that proteins, because they fold up into a specific three dimensional shape, are able to very specifically recognize and bind to other organic molecules. The ligand or substrate bound by a particular protein could be a small organic molecule such as pyruvate all the way up to a large biopolymer such as a specific region of DNA, RNA, or another protein. Because they are chiral molecules, *proteins are very sensitive to the stereochemistry of their ligands*: a protein may bind specifically to (*R*)-glyceraldehyde, for example, but not bind to (*S*)-glyceraldehyde, just as your right hand will not fit into a left-handed baseball glove.

The over-the-counter painkiller ibuprofen is currently sold as a racemic mixture, but only the *S* enantiomer is effective, due to the specific way it is able to bind to and inhibit the action of prostaglandin H_2 synthase, an enzyme in the body's inflammation response process.



racemic ibuprofen

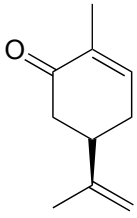


(*S*)-ibuprofen
(active enantiomer)

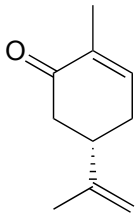
The *R* enantiomer of ibuprofen does not bind to prostaglandin H_2 synthase in the same way as the *S* enantiomer, and as a consequence does not exert the same inhibitory effect on the enzyme's action (*Nature Chemical Biology* **2011**, *7*, 803). Fortunately, (*R*)-ibuprofen apparently does not cause any harmful side effects, and is in fact isomerized gradually by an enzyme in the body to (*S*)-ibuprofen.

Earlier in this chapter we discussed the tragic case of thalidomide, and mentioned that it appears that it is specifically the *S* enantiomer which caused birth defects. Many different proposals have been made over the past decades to try to explain the teratogenic (birth defect-causing) effect of the drug, but a clear understanding still evades the scientific community. In 2010, however, a team in Japan reported evidence that thalidomide binds specifically to a protein called 'thereblon'. Furthermore, when production of thereblon is blocked in female zebra fish, developmental defects occur in her offspring which are very similar to the defects caused by the administration of thalidomide, pointing to the likelihood that thalidomide binding somehow inactivates the protein, thus initiating the teratogenic effect. (http://news.sciencemag.org/2010/03/thalidomides-partner-crime)

You can, with a quick trip to the grocery store, directly experience the biological importance of stereoisomerism. Carvone is a chiral, plant-derived molecule that contributes to the smell of spearmint in the *R* form and caraway (a spice) in the *S* form.



(*R*)-carvone
(spearmint odor)

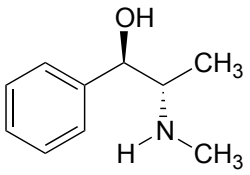


(*S*)-carvone
(caraway odor)

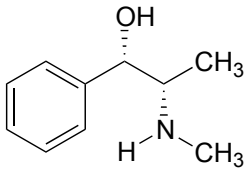
Although details are not known, the two enantiomers presumably interact differently with one or more smell receptor proteins in your nose, generating the transmission of different chemical signals to the olfactory center of your brain.

Exercise 3.28: Ephedrine, found in the Chinese traditional medicine *ma huang*, is a stimulant and appetite suppressant. Both pseudoephedrine and levomethamphetamine are

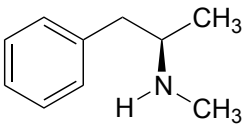
active ingredients in over-the-counter nasal decongestants. Methamphetamine is a highly addictive and illegal stimulant, and is usually prepared in illicit 'meth labs' using pseudoephedrine as a starting point.



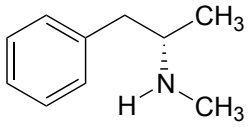
ephedrine



pseudoephedrine



methamphetamine

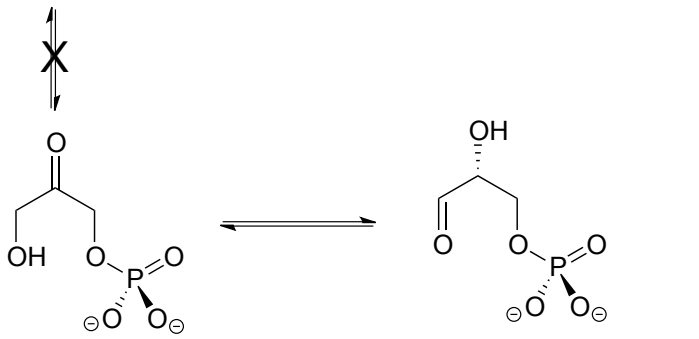


levomethamphetamine

What is the relationship between ephedrine and pseudoephedrine? Between methamphetamine and levomethamphetamine? Between pseudoephedrine and methamphetamine? Your choices are: not isomers, constitutional isomers, diastereomers, enantiomers, or same molecule.

Enzymes are very specific with regard to the stereochemistry of the reactions they catalyze. *When the product of a biochemical reaction contains a chiral center or a stereogenic alkene, with very few exceptions only one stereoisomer of the product is formed*. In the glycolysis pathway, for example, the enzyme triose-phosphate isomerase catalyzes the reversible interconversion between dihydroxyacetone (which is achiral) and (*R*)-glyceraldehyde phosphate. The (*S*)-glyceraldehyde enantiomer is not formed by this enzyme in the left-to-right reaction, and is not used as a starting compound in the right-to-left reaction - it does not 'fit' in the active site of the enzyme.

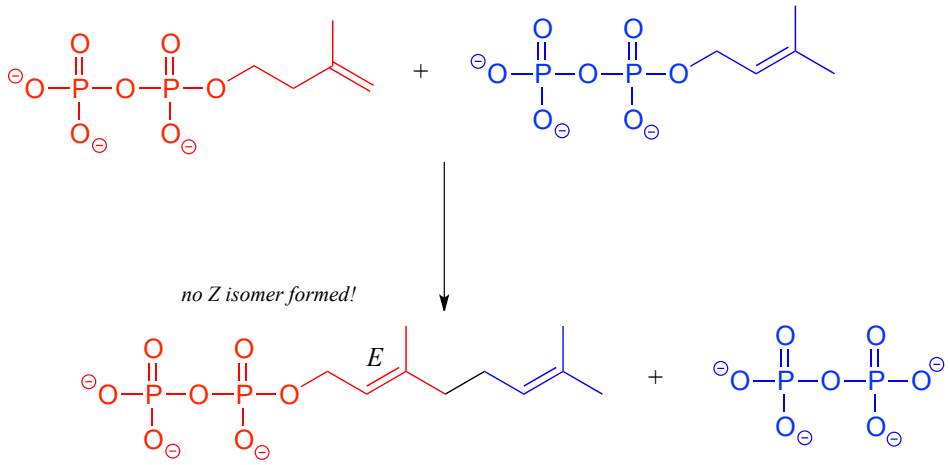
(*S*)-glyceraldehyde phosphate



dihydroxyacetone phosphate
(achiral)

(*R*)-glyceraldehyde phosphate

In the isoprenoid biosynthesis pathway, two five-carbon building-block molecules combine to form a ten-carbon chain containing an *E*-alkene group. The enzyme does *not* catalyze formation of the *Z* diastereomer.

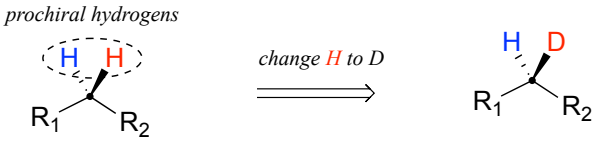


In chapters 9-17 of this book, and continuing on into your study of biological and organic chemistry, you will be learning about how enzymes are able to achieve these feats of stereochemical specificity. If you take a more advanced class in organic synthesis, you will also learn how laboratory chemists are figuring out ingenious ways to exert control over the stereochemical outcomes of nonenzymatic reactions, an area of chemistry that is particularly important in the pharmaceutical industry.

Section 3.11: Prochirality

3.11A: *pro-R* and *pro-S* groups on prochiral carbons

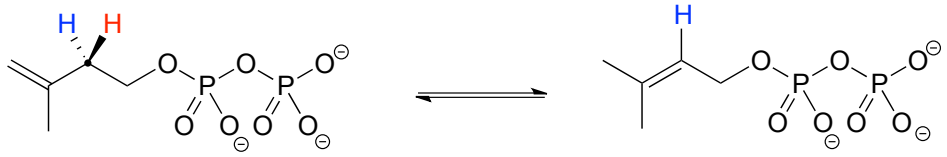
When a tetrahedral carbon can be converted to a chiral center by changing only one of the attached groups, it is referred to as a '**prochiral'** carbon. The two hydrogens on the prochiral carbon can be described as 'prochiral hydrogens'.



prochiral carbon chiral carbon

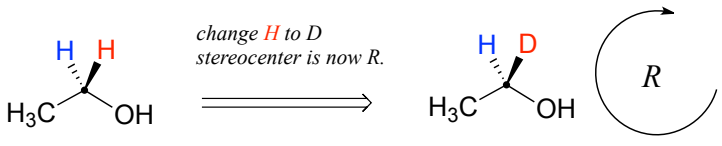
Note that if, in a 'thought experiment', we were to change either one of the prochiral hydrogens on a prochiral carbon center to a deuterium (the ^{2}H isotope of hydrogen), the carbon would now have four different substituents and thus would be a chiral center.

Prochirality is an important concept in biological chemistry, because enzymes can distinguish between the two 'identical' groups bound to a prochiral carbon center due to the fact that *they occupy different regions in three-dimensional space*. Consider the isomerization reaction below, which is part of the biosynthesis of isoprenoid compounds. We do not need to understand the reaction itself (it will be covered in chapter 14); all we need to recognize at this point is that the isomerase enzyme is able to distinguish between the prochiral 'red' and the 'blue' hydrogens on the isopentenyl diphosphate (IPP) substrate. In the course of the left to right reaction, IPP specifically loses the 'red' hydrogen and keeps the 'blue' one.

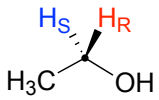


isopentenyl diphosphate dimethylallyl diphosphate

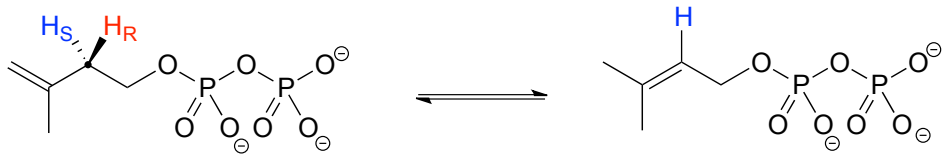
Prochiral hydrogens can be unambiguously designated using a variation on the *R/S* system for labeling chiral centers. For the sake of clarity, we'll look at a very simple molecule, ethanol, to explain this system. To name the 'red' and 'blue' prochiral hydrogens on ethanol, we need to engage in a thought experiment. If we, in our imagination, were to arbitrarily change red H to a deuterium, the molecule would now be chiral and the chiral carbon would have the *R* configuration (D has a higher priority than H).



For this reason, we can refer to the red H as the *pro-R* hydrogen of ethanol, and label it H_R. Conversely, if we change the blue H to D and leave red H as a hydrogen, the configuration of the molecule would be *S*, so we can refer to blue H as the *pro-S* hydrogen of ethanol, and label it H_S.

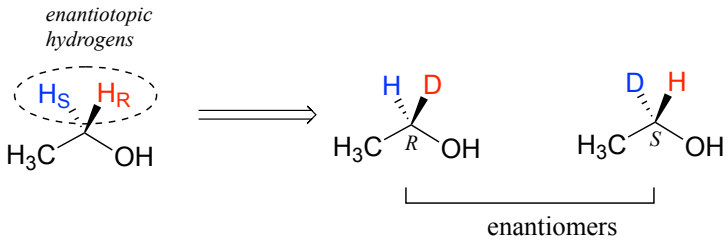


Looking back at our isoprenoid biosynthesis example, we see that it is specifically the *pro-R* hydrogen that the isopentenyl diphosphate substrate loses in the reaction.



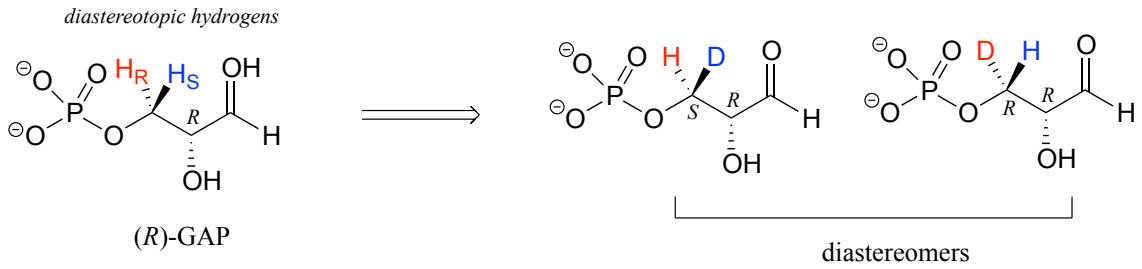
isopentenyl diphosphate dimethylallyl diphosphate

Prochiral hydrogens can be designated either enantiotopic or diastereotopic. If either H_R or H_S on ethanol were replaced by a deuterium, the two resulting isomers would be enantiomers (because there are no other stereocenters anywhere on the molecule)



Thus, these two hydrogens are referred to as **enantiotopic**.

In (*R*)-glyceraldehyde-3-phosphate ((*R*)-GAP), however, we see something different:

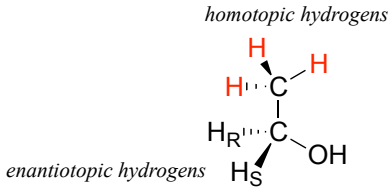


(*R*)-GAP diastereomers

(*R*)-GAP already has one chiral center. If either of the prochiral hydrogens H_R or H_S is replaced by a deuterium, a second chiral center is created, and the two resulting molecules will be diastereomers (one is *S,R,* one is *R,R*). Thus, in this molecule, H_R and H_S are referred to as diastereotopic hydrogens.

Finally, hydrogens that can be designated neither enantiotopic nor diastereotopic are called **homotopic**. If a homotopic hydrogen is replaced by deuterium, a chiral center is

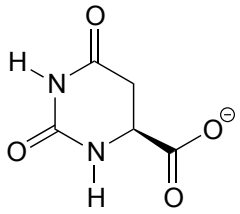
not created. The three hydrogen atoms on the methyl (CH_3) group of ethanol (and on *any* methyl group) are homotopic.



An enzyme cannot distinguish among homotopic hydrogens.

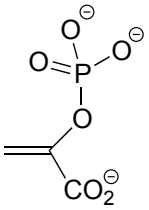
Exercise 3.29: Identify in the molecules below all pairs/groups of hydrogens that are homotopic, enantiotopic, or diastereotopic. When appropriate, label prochiral hydrogens as H_R or H_S.

a)



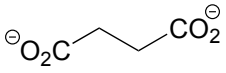
dihydroorotate
(a nucleotide biosynthesis intermediate)

b)



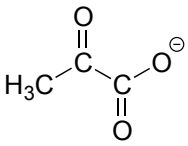
phosphoenolpyruvate
(a glycolysis intermediate)

c)



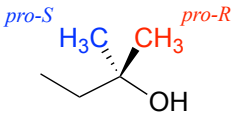
succinate
(a citric acid cycle intermediate)

d)

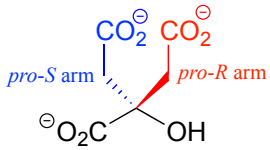


pyruvate
(endpoint of glycolysis)

Groups other than hydrogens can be considered prochiral. The alcohol below has two prochiral methyl groups - the red one is *pro-R*, the blue is *pro-S*. How do we make these designations? Simple - just arbitrarily assign the red methyl a higher priority than the blue, and the compound now has the *R* configuration - therefore red methyl is *pro-R*.

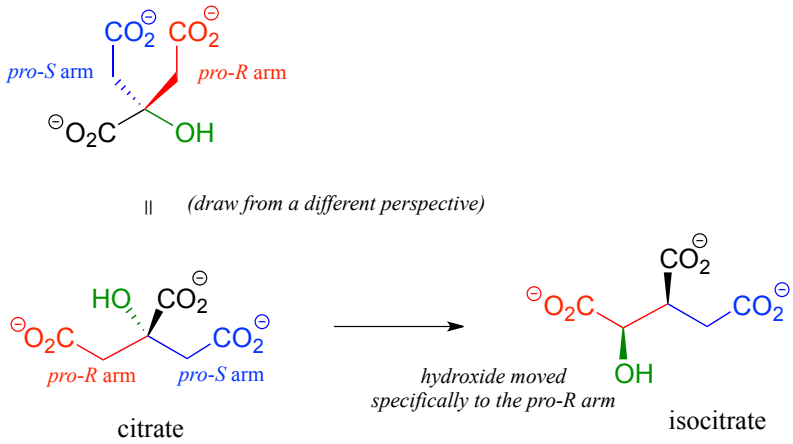


Citrate is another example. The central carbon is a prochiral center with two 'arms' that are identical except that one can be designated *pro-R* and the other *pro-S*.



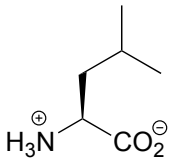
citrate

In an isomerization reaction of the citric acid (Krebs) cycle, a hydroxide is shifted specifically to the *pro-R* arm of citrate to form isocitrate: again, the enzyme catalyzing the reaction distinguishes between the two prochiral arms of the substrate (we will study this reaction in chapter 13).



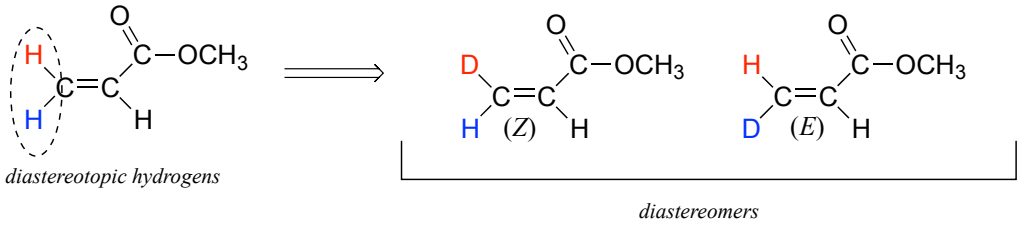
citrate isocitrate

Exercise 3.30: Assign *pro-R* and *pro-S* designations to all prochiral groups in the amino acid leucine. (*Hint*: there are two pairs of prochiral groups!). Are these prochiral groups diastereotopic or enantiotopic?



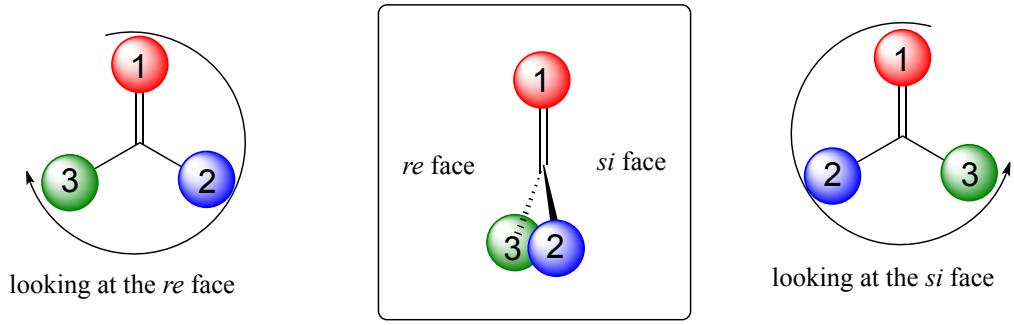
leucine

Although an alkene carbon bonded to two identical groups is *not* considered a prochiral center, these two groups *can* be diastereotopic. H_a and H_b on the alkene below, for example, are diastereotopic: if we change one, and then the other, of these hydrogens to deuterium, the resulting compounds are *E* and *Z* diastereomers.

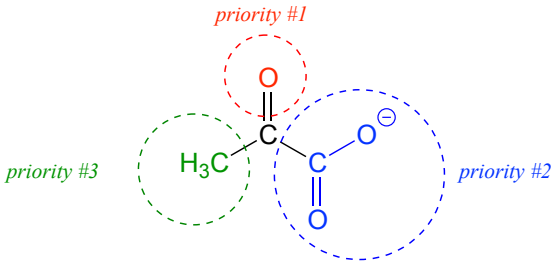


3.11B: The *re* and *si* faces of carbonyl and imine groups

Trigonal planar, sp^2-hybridized carbons are not, as we well know, chiral centers– but they can be prochiral centers if they are bonded to three different substitutuents. We (and the enzymes that catalyze reactions for which they are substrates) can distinguish between the two planar 'faces' of a prochiral sp^2 - hybridized group. These faces are designated by the terms ***re*** and ***si***. To determine which is the *re* and which is the *si* face of a planar organic group, we simply use the same priority rankings that we are familiar with from the R/S system, and trace a circle: *re* is clockwise and *si* is counterclockwise.

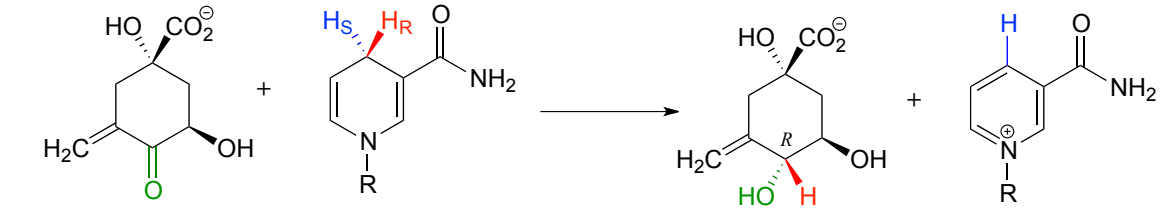


Below, for example, we are looking down on the *re* face of the ketone group in pyruvate:



If we flipped the molecule over, we would be looking at the *si* face of the ketone group. Note that the carboxylate group does not have *re* and *si* faces, because two of the three substituents on that carbon are identical (when the two resonance forms of carboxylate are taken into account).

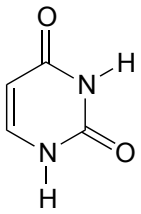
As we will see in chapter 10, enzymes which catalyze reactions at carbonyl carbons act specifically from one side or the other.



we are looking at the si face of the ketone

We need not worry about understanding the details of the reaction pictured above at this point, other than to notice the stereochemistry involved. The *pro-R* hydrogen (along with the two electrons in the C-H bond) is transferred to the *si* face of the ketone (in green), forming, in this particular example, an alcohol with the *R* configuration. If the transfer had taken place at the *re* face of the ketone, the result would have been an alcohol with the *S* configuration.

Exercise 3.31: For each of the carbonyl groups in uracil, state whether we are looking at the *re* or the *si* face in the structural drawing below.



uracil

Summary of Key Concepts

Before you move on to the next chapter, you should be confortable with the following concepts:

Conformations of open-chain compounds:

Be able to distinguish between **eclipsed, staggered, gauche, and anti conformations**, and the rational for trends in stability.

Be able to draw and interpret **Newman projections**.

Conformations of cyclic compounds:

Understand the concept of **angle strain** in 3- and 4-membered rings.

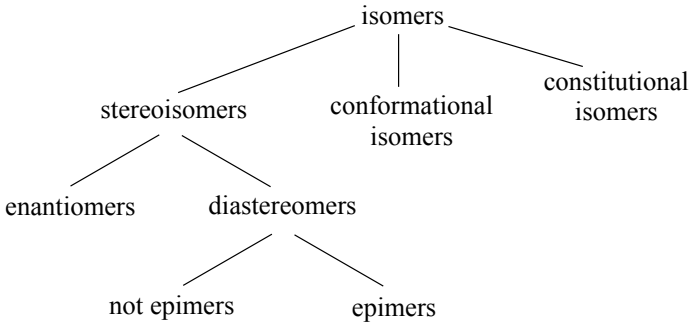
Be able to draw the **envelope conformation** of five-membered rings

Be able to draw the **chair and boat conformations** of six-membered rings.

In the chair conformation, be able to draw **equatorial** and **axial** substituents. Understand that large groups in the axial position experience considerable 1**,3-diaxial repulsion**, and thus are more stable in the equatorial position.

Stereochemistry:

Hierarchy of isomeric relationships:



You should understand the relevant terms and concepts:

A **chiral** object or molecule is cannot be superimposed on its mirror image.
A **chiral center** is an sp^3-hybridized (tetrahedral) carbon bonded to four different groups. A chiral center can be labeled *R* or *S*.
A **stereogenic alkene** is an alkene is one in which both sides of the alkene are asymmetric, and which can therefore be labeled *E* or *Z*.

Stereoisomers have the same molecular formula and same connectivity, but a different orientation of atoms in space.

- **Enantiomers** are stereoisomers which are mirror images.
  - In practice, the enantiomer of a compound is the one in which ***all*** chiral centers are in the opposite configuration.
  - Every chiral molecule has one and only one enantiomer.
  - Achiral molecules are superimposable on their mirror image, and thus cannot have an enantiomer.
  - Enantiomers have equal but opposite specific rotations, but identical physical properties otherwise.
- **Diastereomers** are stereoisomers which are *not* mirror images. They have different physical properties.
  - In practice, a diastereomer of a chiral molecule with have ***at least one, but not all*** chiral centers in the opposite configuration.
  - Alternatively, two diastereomers may contain a stereogenic alkene with the opposite *E/Z* configuration.
  - A molecule has 2^n-2 diastereomers, where *n* is the number of chiral centers plus stereogenic alkene groups. *Meso* compounds are an exception to this rule.
- **Epimers** are diastereomers which differ at only one chiral center.
- A **racemic mixture** is a 50:50 mixture of two enantiomers.
- A ***meso* compound** has multiple chiral centers but, because it has a plane of symmetry, is achiral.

You should know how to assign **R/S and E/Z configuration** to chiral centers and stereogenic alkenes, respectively.

You should understand the concept of **optical rotation** and the definition of **specific rotation.**

You should recognize that in general, a protein can distinguish between its natural ligand and a stereoisomer of that ligand.

You should also recognize that enzymes are highly specific with respect to stereochemistry, catalyzing the formation of only one stereoisomer of their products.

You should be able to recognize and label *pro-R* and *pro-S* groups on prochiral tetrahetral carbons.

You should be able to recognize *re* and *si* faces of carbonyl and imine groups

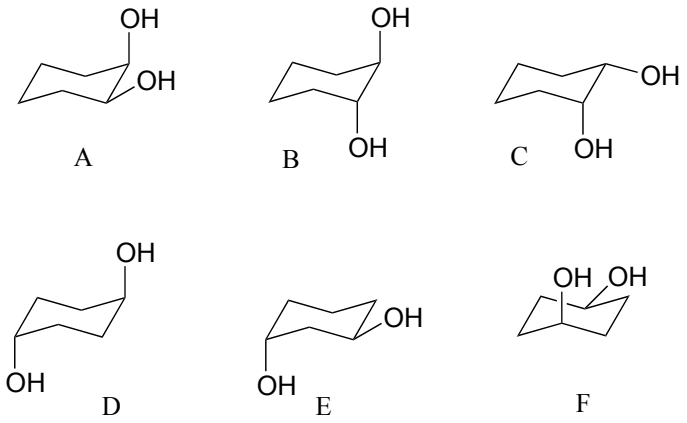
Problems

P3.1: Draw an energy vs dihedral angle graph for rotations about the C_2-C_3 bond of 2-methylbutane. Start with the highest-energy conformation as the 0^o point. For each energy peak and valley, draw a corresponding Newman projection.

P3.2:

a) Which has the highest energy diaxial chair conformation: *trans*-1,2-dimethylcyclohexane, *cis*-1,3-dimethylcyclohexane, or *trans*-1,4-dimethylcyclohexane? Explain.

b) Which of the following are *trans* disubstituted cyclohexanes?



c) Draw A-F above in two dimensions (rings in the plane of the page, substituents drawn as solid or dashed wedges).

d) Structure D does not have any chiral centers. Explain.

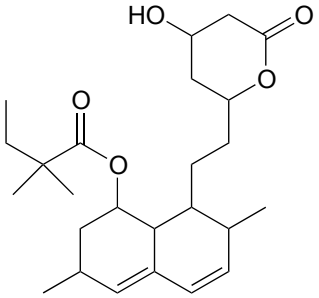
e) Draw a diastereomer of structure D (in two dimensions, as in part c).

f) Are structure D and its diastereomer chiral?

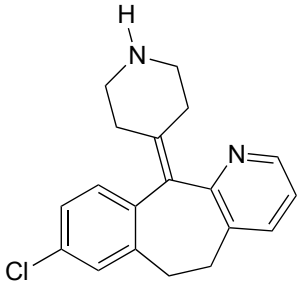
g) Assign R/S designations to the two chiral centers in structure B (hint: making a model will be *very* helpful!)

P 3.3: The following are structures, drawn in two dimensions, of drugs listed on the products web page of Merck Pharmaceutical. One of the compounds is achiral.

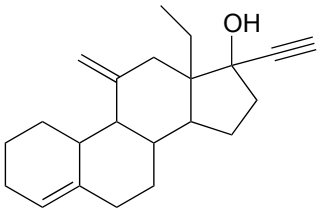
a) Circle all chiral centers. (Hint: *Don't panic*! Remember - you are looking for sp^3-hybridized carbons with four different substituents.)



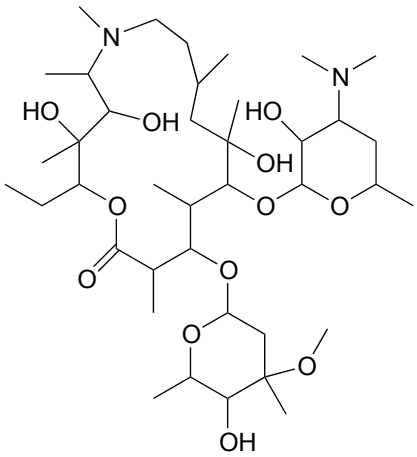
simvastatin
(active ingredient in Zocor, for high cholesterol



desloratadine
(Clarinex, for seasonal allergies)



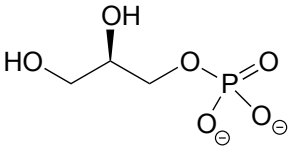
desogestrel
(an active ingredient in Cyclessa, an oral contraceptive)



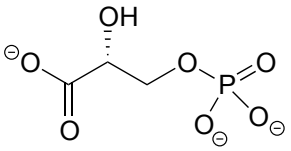
azithromycin
(an antibiotic in AzaSite, for conjunctivitis)

b) How many diastereomers are possible for desogestrel?
c) Draw two epimeric forms of simvastatin

P3.4: Three of the four structures below are chiral. Assign R/S designations to all chiral centers, and identify the achiral molecule.

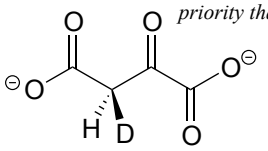


glycerol phosphate

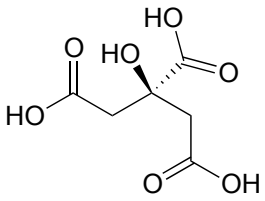


phosphoglycerate

D = deuterium, the ^2H isotope of hydrogen. D has a higher priority than H, lower priority than C.

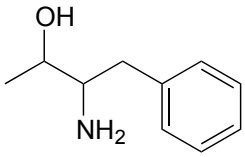


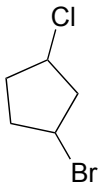
(^{2}H)-oxaloacetate

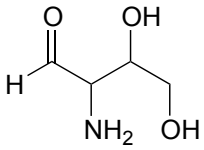


citric acid

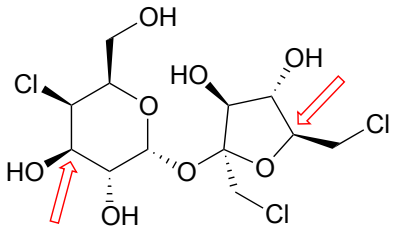
P3.5: Draw the *R,R* stereoisomers of the structures below.



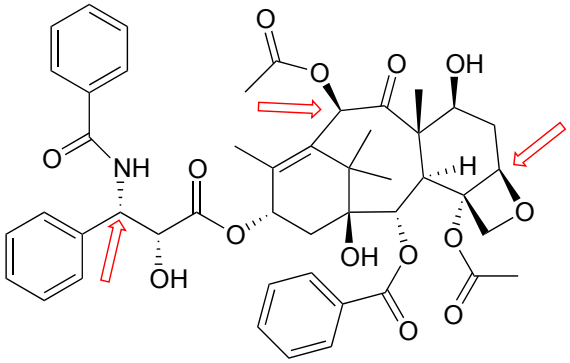




P3.6: Below are the structures of sucralose, the artificial sweetener with the brand name Splenda (TM), and the cancer drug Paclitaxel. Give an *R* or *S* designation to chiral centers indicated with an arrow.



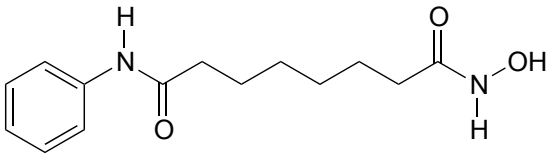
sucralose



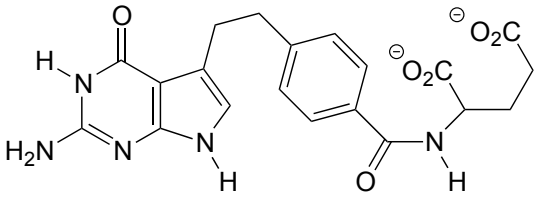
Paclitaxel

P3.7: The four drugs below were featured in a *Chemical & Engineering News* article (April 16, 2007, p. 42) on new drugs that had been developed in university labs.

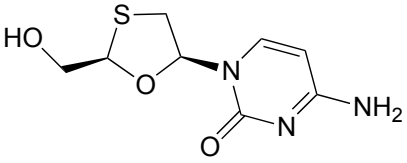
a) Identify each as chiral or achiral, and identify all chiral centers (in most cases, specific stereochemistry is not shown in the structures below). Also, state how many possible stereoisomers exist for each structure.



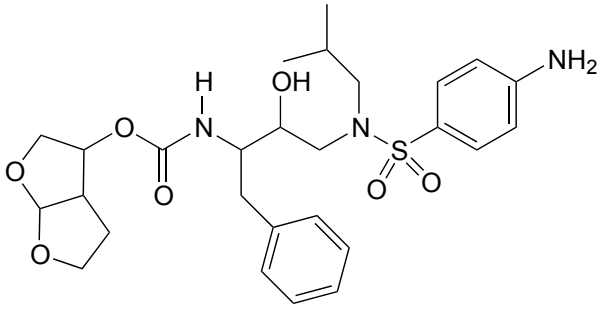
SAHA (suberoylanilide hydroxamic acid)



Alimta

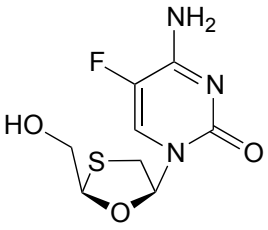


Epivir

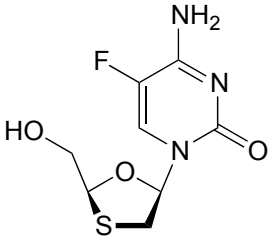


Darunavir

b) Visualization challenge: two fluorinated derivatives of Epivir were also mentioned in the article. The structures are below, drawn in what is referred to as a 'Haworth projection'. What is the relationship between them? (Your choices: not isomers, constitutional isomers, diastereomers but not epimers, epimers, enantiomers, identical)

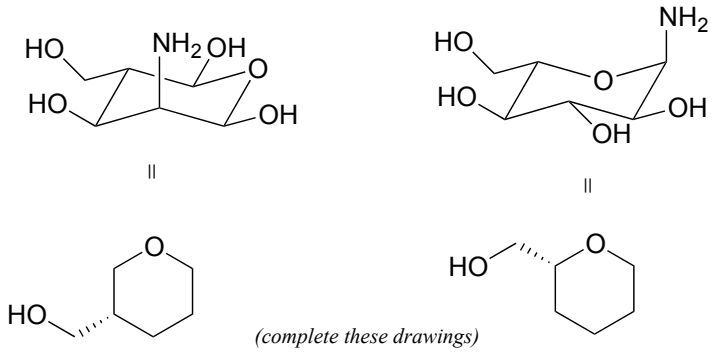


A



B

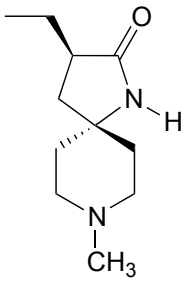
P3.8: Redraw the following structures in the flat ring, solid/dash wedge convention (the drawings have been started for you).



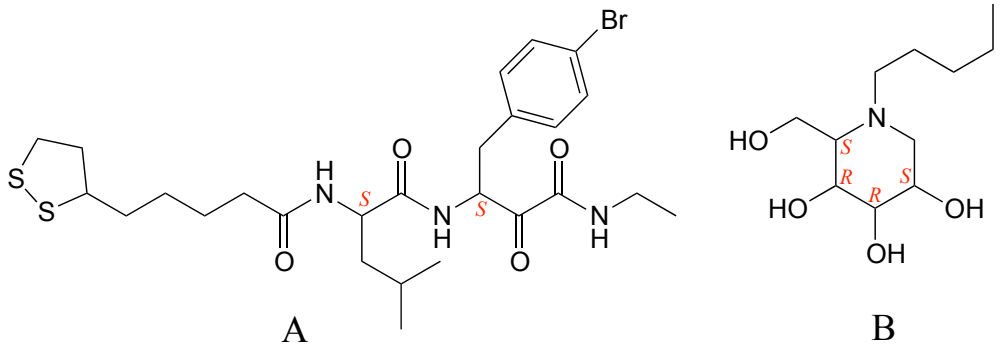
P3.9: Below is an experimental drug for Alzheimer's disease that was mentioned in the March 13, 2007 issue of *Chemical and Engineering News*.

a) Label the chiral center(s) *R* or *S*.

b) Draw the enantiomer of the molecule shown.



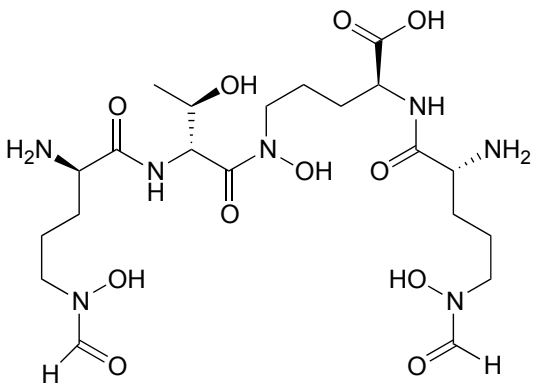
P3.10: The molecules below are potential new drugs for the treatment of Duchenne muscular dystrophy (molecule A) and skin cancer (molecule B) (*Chemical and Engineering News* Sept 26, 2005, p. 39). Given the *R*/*S* designations, redraw the structure showing the correct stereochemistry.



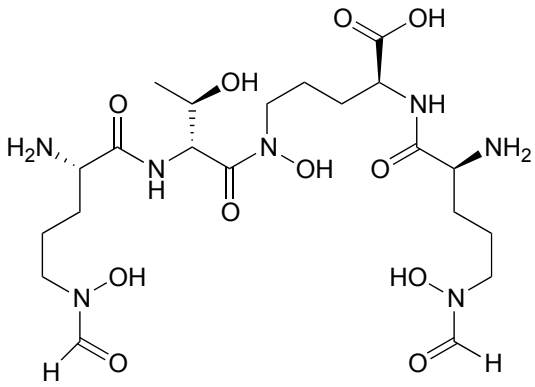
P3.11: Draw the structure of the following molecules:

a) (*R*)-3-methyl-3-hexanol
b) (*R*)-1-chloro-1-phenylethane
c) (2*R*, 3*R*)-2,3-dihydroxybutanedioic acid (tartaric acid)
d) (*S*)-(*E*)-4-chloro-3-ethyl-2-pentenoic acid
e) (1*S*, 3*R*)-1-chloro-3-ethylcyclohexane

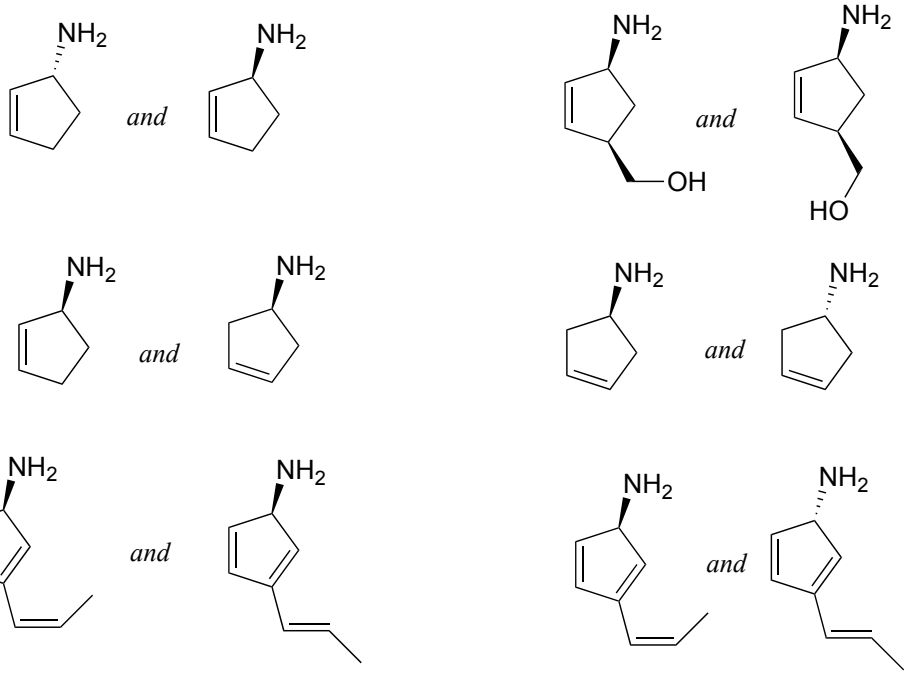
P3.12: Coelichelin (the structure below to the left) is a natural product from soil bacteria that was identified using a technique known as 'genome mining' (*Chemical and Engineering News* Sept. 19, 2005, p. 11). What is the relationship between coelichelin and the compound shown to the right? (Note: the two structures are drawn with the same conformation of the carbon backbone - just trace them through from end to end and identify where they are different!)



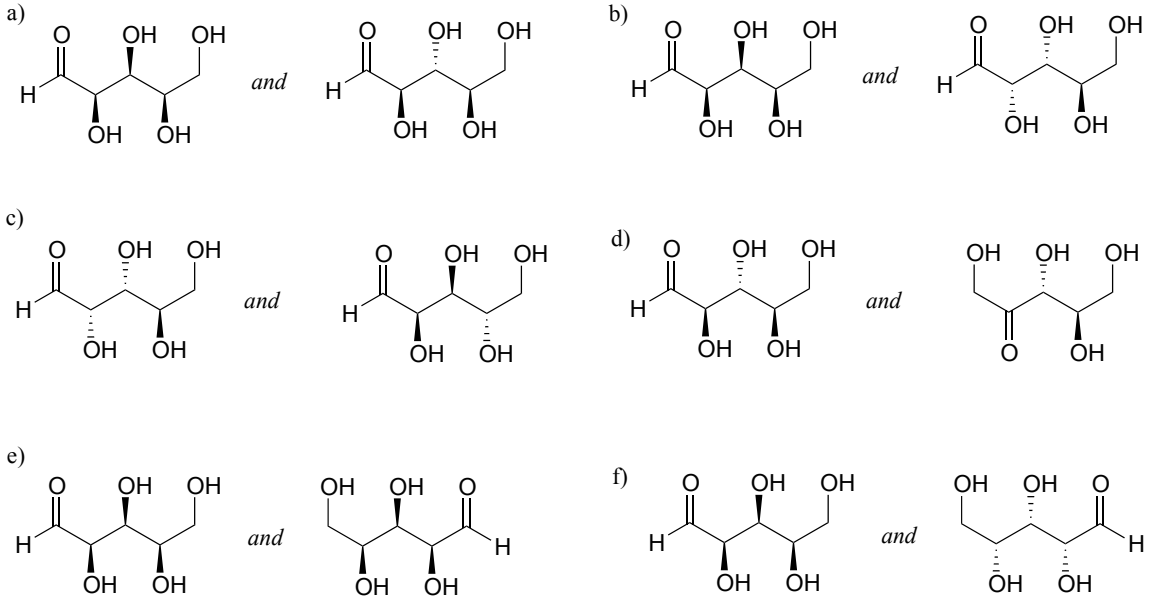
ceolichelin



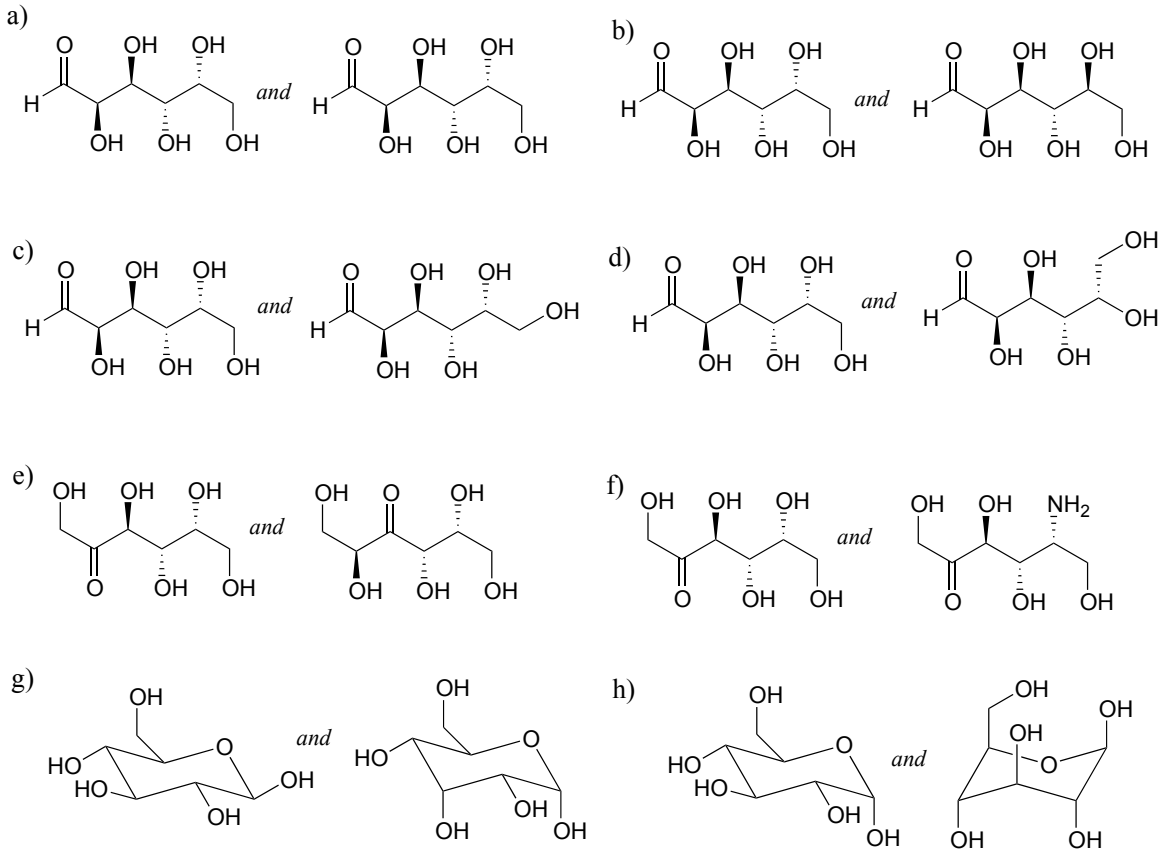
P3.13: Identify the relationships between the following pairs of structures (Not isomers, constitutional isomers, diastereomers but *not* epimers, epimers, enantiomers, identical)



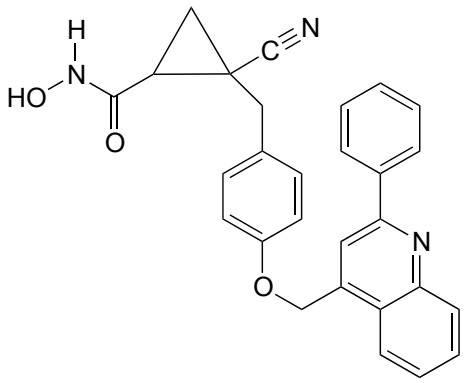
P3.14: Identify the relationships between each of the following pairs of pentose sugars (not isomers, constitutional isomers, diastereomers but *not* epimers, epimers, enantiomers, identical).



P3.15: Identify the relationships between each of the following pairs of hexose sugars (not isomers, constitutional isomers, diastereomers but *not* epimers, epimers, enantiomers, identical).



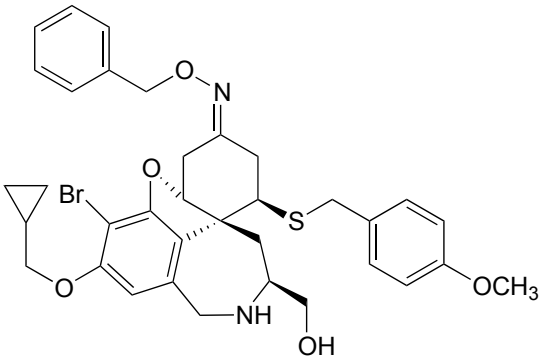
P3.16: The compound drawn below (not showing stereochemistry) has been identified as a potential anti-inflammatory agent by scientists at Schering-Plough a pharmaceutical company (see *Chemical and Engineering News* Nov. 28, 2005 p. 29). How many stereoisomers are possible for the compound?



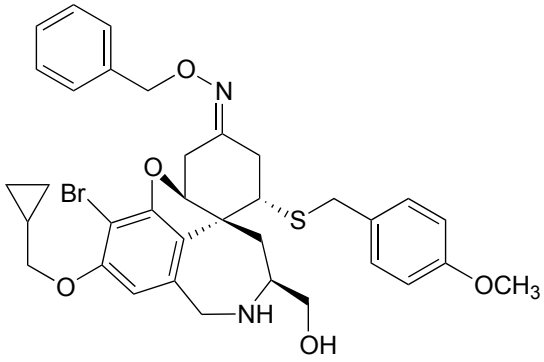
P3.17: Secramine is a synthetic compound that has been shown to interfere with the transport of newly synthesized proteins in the cell (see *Chemical and Engineering News* Nov. 28, 2005, p. 27). Also drawn below is a (hypothetical) isomer of secramine.

a) Identify the relationship between the two isomers: are they consitutional isomers, confomational isomers, enantiomers, or diastereomers?

b) Locate a five-membered ring in the secramine structure.

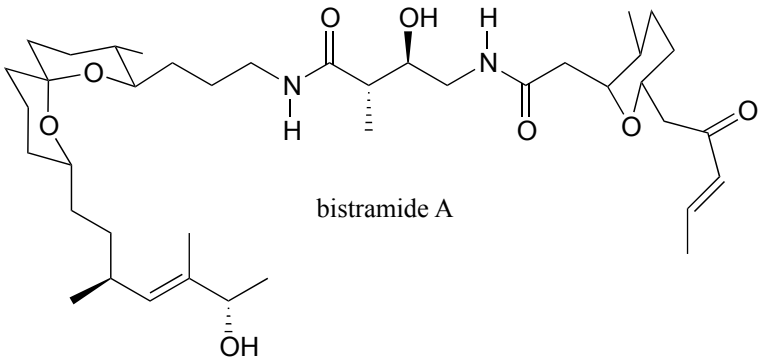


secramine



isomer of secramine

P3.18: The natural product bistramide A has been shown to bind to actin, an important structural protein in the cell, and suppress cell proliferation (see *Chemical and Engineering News* Nov. 21, 2005, p. 10).



bistramide A

a) Label the alkene functional groups as *E*, *Z*, or *N* (no *E*/*Z* designation possible)

b) Theoretically, how many diastereomers are possible for bistramide A?

P3.19:

a) Draw Newman projections of the *gauche* and the *anti* conformations of 1,2-ethanediol.

b) Why might the *gauche* conformation be expected to be the more stable of the two?

c) Do you think that *gauche* is also the most stable conformation of 1,2-dimethoxyethane? Explain.

P3.20: Draw the chair conformation of *cis*-1,2-dimethylcyclohexane.

a) Label the stereochemical configuration at C_1 and C_2 for the structure you drew.

b) Build a model of your molecule, and try out different possible boat conformations. Can you find one in which there is a plane of symmetry?

c) Is *cis*-1,2-dimethylcyclohexane a chiral molecule?

d) is *cis* -1,4-dimethylcyclohexane chiral? How about *trans*-1,4-dimethylcyclohexane? How about *trans*-1-chloro-4-fluorocyclohexane?

P3.21: In some special cases, a 'chiral center' can be composed of several atoms instead of just one, and molecules which contain such multi-atom chiral centers are indeed chiral. What is the relationship between the two two difluorallene compounds below? It will be very helpful to make models, and review the fundamental definitions in this chapter.

Chapter 4

Structure determination, part I:

Mass spectrometry, infrared and UV-visible spectroscopy

(Credit: https://www.flickr.com/photos/vamapaull/)

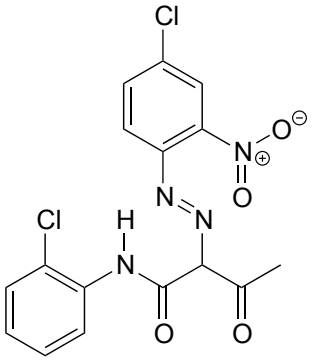
In the end, it was a 'funky yellow color' that led to the demise of Charles Heller's not-so-illustrious career in the world of collectable art.

William Aiken Walker was a 19th-century 'genre' painter, known for his small scenes of sharecroppers working the fields in the post-Civil War south. For much of his career, he traveled extensively, throughout the southern states but also to New York City and even as far as Cuba. He earned a decent living wherever he went by setting up shop on the

sidewalk and selling his paintings to tourists, usually for a few dollars each. While he never became a household name in the art world, he was prolific and popular, and his paintings are today considered collectible, often selling for upwards of ten thousand dollars.

In August, 1994, Robert Hicklin, an art gallery owner in Charleston, South Carolina, was appraising a Walker painting brought to him by another South Carolina art dealer named Rick Simons. Hicklin's years of experience with Walker paintings told him that something just wasn't right with this one - he was particularly bothered by one of the pigments used, which he later described in a story in the *Maine Antique Digest* as a 'funky yellow color'. Reluctantly, he told Simons that it almost certainly was a fake.

Hoping that Hicklin was wrong, Simons decided to submit his painting to other experts for analysis, and eventually it ended up in the laboratory of James Martin, whose company *Orion Analytical* specializes in forensic materials analysis. Using a technique called infrared spectroscopy, Martin was able to positively identify the suspicious yellow pigment as an organic compound called 'pigment yellow 3'.



pigment yellow 3

As it turns out, Pigment Yellow 3 had not become available in the United States until many years after William Aiken Walker died.

Simons had purchased his painting from a man named Robert Heller for $9,500. When Heller approached him again to offer several more Walker paintings for sale, Simons contacted the FBI. A few days later, with FBI agents listening in, Simons agreed to buy two more Walker paintings. When he received them, they were promptly analyzed and found to be fake. Heller, who turned out to be a convicted felon, was arrested and eventually imprisoned.

In the first three chapters of this text, we have focused our efforts on learning about the structure of organic compounds. Now that we know what organic molecules look like, we can begin to address, in the next two chapters, the question of *how* we get this knowledge

in the first place. How are chemists able to draw with confidence the bonding arrangements in organic molecules, even simple ones such as acetone or ethanol? How was James Martin at Orion Analytical able to identify the chemical structure of the pigment compound responsible for the 'funky yellow color' in the forged William Aiken Walker painting?

This chapter is devoted to three very important techniques used by chemists to learn about the structures of organic molecules. First, we will learn how **mass spectrometry** can provide us with information about the mass of a molecule as well as the mass of fragments into which the molecule has been broken. Then, we will begin our investigation of **molecular spectroscopy**, which is the study of how electromagnetic radiation at different wavelengths interacts in different ways with molecules - and how these interactions can be quantified, analyzed, and interpreted to gain information about molecular structure. After a brief overview of the properties of light and the elements of a molecular spectroscopy experiment, we will move to a discussion of **infrared (IR) spectroscopy**, the key technique used in the detection of the Walker forgery, and a way to learn about functional groups present in an organic compound. Then, we will consider **ultraviolet-visible (UV-vis) spectroscopy**, with which chemists gain information about conjugated π-bonding systems in organic molecules. Among other applications, we will see how information from UV-vis spectroscopy can be used to measure the concentration of biomolecules compounds in solution.

Looking ahead, Chapter 5 will be devoted to **nuclear magnetic resonance (NMR) spectroscopy**, where we use ultra-strong magnets and radio frequency radiation to learn about the electronic environment of individual atoms in a molecule and use this information to determine the atom-to-atom bonding arrangement. For most organic chemists, NMR is one of the most powerful analytical tools available in terms of the wealth of detailed information it can provide about the structure of a molecule.

In summary, the structure determination techniques we will be studying in this chapter and the next primarily attempt to address the following questions about an organic molecule:

<u>Chapter 4:</u>

Mass spectrometry (MS): *What is the atomic weight of the molecule and its common fragments?*
Infrared (IR) spectroscopy: *what functional groups does the molecule contain?*
Ultraviolet-visible (UV-Vis) spectroscopy: *What is the nature of conjugated π-bonding systems in the molecule?*

<u>Chapter 5:</u>

Nuclear magnetic resonance spectroscopy (NMR): *What is the overall bonding framework of the molecule?*

Section 4.1: Mass Spectrometry

Mass spectrometry (MS) is a powerful analytical technique widely used by chemists, biologists, medical researchers, and environmental and forensic scientists, among others. With MS, we are looking at the mass of a molecule, or of different fragments of that molecule.

4.1A: Overview of mass spectrometry

There are many different types of MS instruments, but they all have the same three essential components:

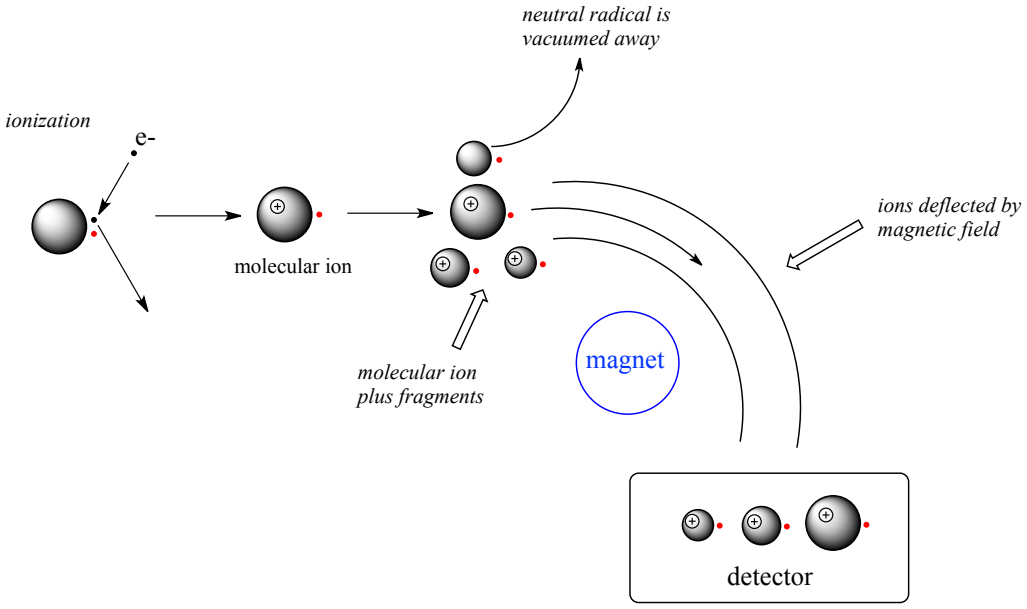
1) First, there is an ionization source, where the molecule is given a positive electrical charge, either by removing an electron or by adding a proton.

2) Depending on the ionization method used, the ionized molecule may or may not break apart into a population of smaller fragments

3) Next in line there is a mass analyzer, where the positively-charged fragments are separated according to mass.

4) Finally, there is a detector, which detects and quantifies the separated ions.

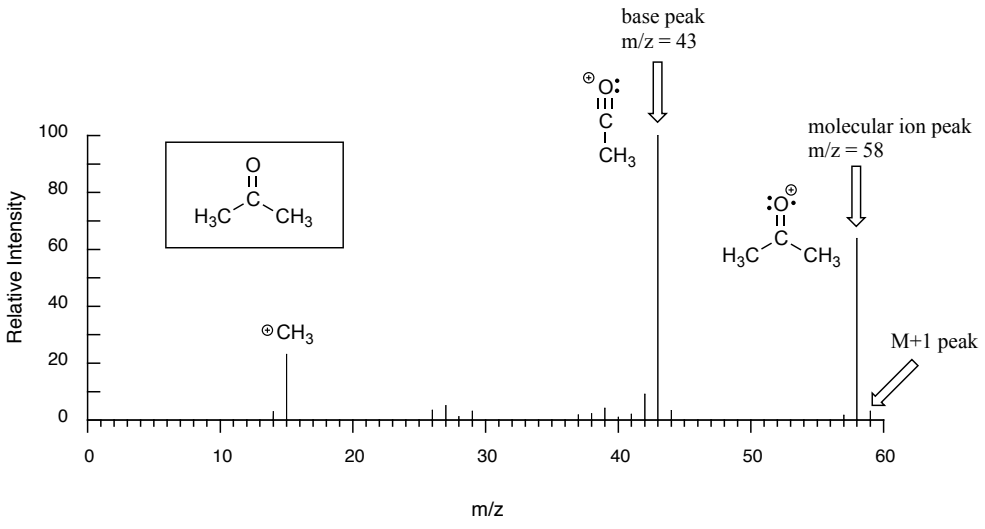
One of the more common types of MS techniques used in the organic laboratory is **electron ionization** (EIMS). Although biomolecules are usually too polar for this technique, it is useful for the analysis of intermediates and products in laboratory synthesis, and is a good starting place to learn about MS in general. In a typical EIMS experiment, the sample molecule is bombarded by a high-energy electron beam, which has the effect of knocking a valence electron off of the molecule to form a **radical cation**. Because a great deal of energy is transferred by this bombardment process, the radical cation quickly begins to break up into smaller **fragments**, some of which are cations (both radical and non-radical) and some of which are neutral. A subset of the initially formed radical cations do not fragment at all - these are referred to as **molecular ions**. The neutral fragments are either adsorbed onto the walls of the chamber or are removed by a vacuum source. The molecular ion and all other cationic fragments are accelerated down a curved tube by an electric field.



As they travel down the curved tube, the ions are deflected by a strong magnetic field. Cations of different **mass to charge (*m*/*z*) ratios** are deflected to a different extent, resulting in a sorting of ions by mass (virtually all ions have charges of $z = +1$, so sorting by the mass to charge ratio is essentially the same thing as sorting by mass). A detector at the end of the curved flight tube records and quantifies the sorted ions.

4.1B: Looking at mass spectra

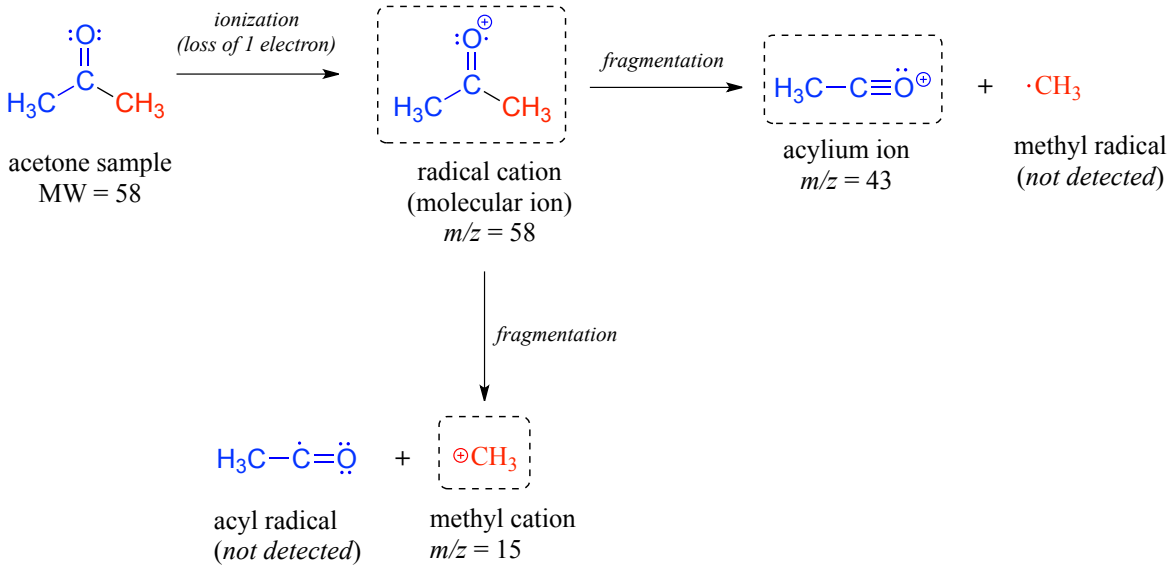
Below is typical output for an electron-ionization MS experiment (MS data in the section is derived from the Spectral Database for Organic Compounds, a free, web-based service provided by AIST in Japan: http://riodb01.ibase.aist.go.jp/sdbs/).



The sample is acetone. On the horizontal axis is the value for *m/z* (as we stated above, the charge *z* is almost always +1, so in practice this is the same as mass). On the vertical axis is the relative abundance of each ion detected. On this scale, the most abundant ion, called the **base peak**, is set to 100%, and all other peaks are recorded relative to this value. In the acetone spectrum above, the base peak is at *m/z* = 43, representing a fragment of acetone with a mass of 43 amu. The molecular weight of acetone is 58, so we can identify the peak at *m/z* = 58 as that corresponding to the **molecular ion peak**, or **parent peak**. In some mass spectra, the molecular ion peak is the most abundant peak, and so it is also the base peak by definition.

The data collected in a mass spectrum - a series of m/z values, each associated with a relative abundance value - is a unique 'fingerprint' of the compound being analyzed, so the spectrum of an unknown sample can be used to search a computer database of MS spectra to come up with a 'hit', just as fingerprint information can be used by the police to identify a suspect from a fingerprint database. In fact, mass spectrometry is a powerful tool in criminal forensics.

An experienced chemist can gain a wealth of useful structural information from a mass spectrum, even without access to a reference database. The parent peak provides the molecular weight of the compound being analyzed, which of course is a very useful piece of information. However, much of the utility in electron-ionization MS comes from the fact that the radical cations generated in the electron-bombardment process tend to fragment in predictable ways. The base peak at *m/z* = 43 in the acetone spectrum, for example, is the result of the molecule breaking apart into what is termed an acylium cation and a methyl radical. The methyl radical fragment has a mass of 15 amu, but is not detected because it is neutral, not a cation.



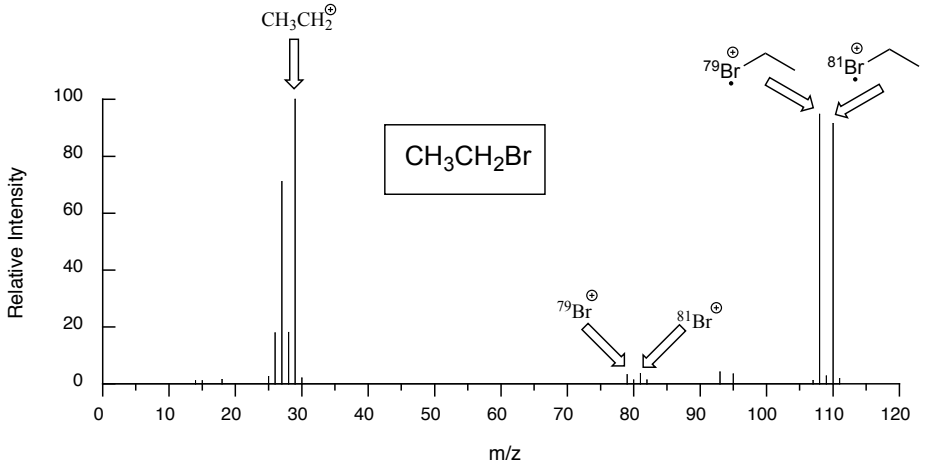
Notice that we do in fact see a peak in the spectrum at m/z = 15, which corresponds to a methyl cation, formed in a different fragmentation event in which the second product is a neutral (and thus undetected) radical fragment.

Exercise 1: Using the fragmentation patterns for acetone above as a guide, predict the signals that you would find in the mass spectra of:
a) 2-butanone; b) 3-hexanone; c) cyclopentanone.

Many other organic functional groups and bonding arrangements have predictable fragmentation patterns. As a rule, the cationic fragments that form in greatest abundance are those which are, relatively speaking, the most stable (we will learn about carbocation stability in chapter 8). A discussion of the mechanisms for fragmentation in mass spectrometry is outside the scope of this book, but will be covered if you take more advanced courses in organic or analytical chemistry.

Notice in the mass spectrum of acetone that there is a small peak at $m/z = 59$: this is referred to as the **M+1 peak**. How can there be an ion that has a greater mass than the molecular ion? A small fraction - about 1.1% - of all carbon atoms in nature are actually the ^{13}C rather than the ^{12}C isotope. The ^{13}C isotope has an extra neutron in its nucleus, and thus is heavier than ^{12}C by 1 mass unit. The M+1 peak corresponds to those few acetone molecules in the sample which contained a ^{13}C.

Molecules with several oxygen atoms sometimes show a small **M+2 peak** (2 *m/z* units greater than the parent peak) in their mass spectra, due to the presence of a small amount of ^{18}O (the most abundant isotope of oxygen is ^{16}O). Because there are two abundant isotopes of both chlorine (about 75% ^{35}Cl and 25% ^{37}Cl) and bromine (about 50% ^{79}Br and 50% ^{81}Br), chlorinated and brominated compounds have very large and recognizable M+2 peaks. Fragments containing both isotopes of bromine can be seen in the mass spectrum of bromoethane: notice that the equal abundance of the two isotopes is reflected in the equal height of their corresponding peaks.



The spectrum for bromoethane shows a parent peak at m/z = 64, and an M+2 peak at 1/3 the height of the parent peak. These characteristic M+2 patterns are useful for identifying the presence of bromine or chlorine in a sample.

Exercise 4.2: Predict some signals that you would expect to see in a mass spectrum of 2-chloropropane.

Exercise 4.3: The mass spectrum of an aldehyde shows a parent peak at m/z = 58 and a base peak at m/z = 29. Propose a structure, and identify the two species whose m/z values were listed.

4.1C: Gas Chromatography - Mass Spectrometry

Quite often, mass spectrometry is used in conjunction with a separation technique called gas chromatography (GC). The combined GC-MS procedure is very useful when dealing with a sample that is a mixture of two or more different compounds, because the various compounds are separated from one another before being subjected individually to MS analysis. We will not go into the details of gas chromatography here, although if you are taking an organic laboratory course you might well get a chance to try your hand at GC, and you will almost certainly be exposed to the conceptually analogous techniques of thin layer and column chromatography. Suffice it to say that in GC, a very small amount of a liquid sample is vaporized, injected into a long, coiled column, and pushed though the column by a gas such as helium. Along the way, different compounds in the sample stick to the walls of the column to different extents, and thus travel at different speeds and emerge separately from the end of the column. In GC-MS, each separated compound is sent directly from the end of the GC column into the MS instrument, so in the end we get a separate mass spectrum for each of the components in the original mixed sample. Ideally, this data can be used to search an MS database and identify each compound in the sample.

The extremely high sensitivity of modern GC-MS instrumentation makes it possible to detect and identify very small trace amounts of organic compounds. GC-MS is being used increasingly by environmental chemists to detect the presence of harmful organic contaminants in food and water samples.

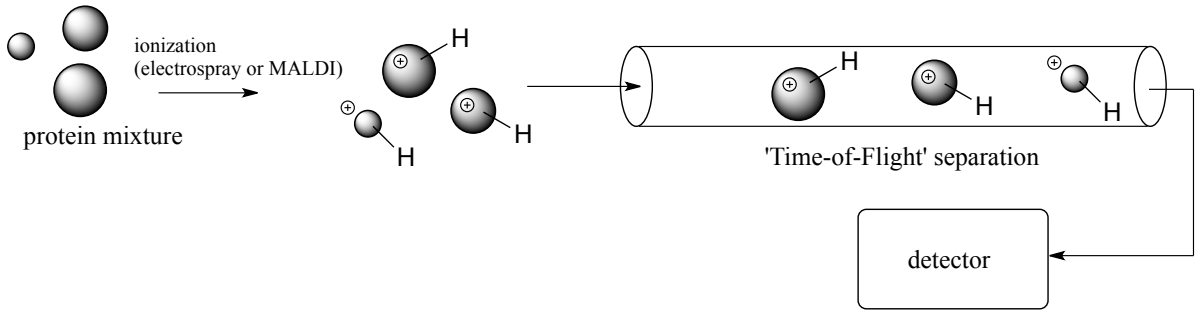
4.1D: Mass spectrometry of biomolecules

As was mentioned earlier, biomolecules, electron ionization mass spectrometry is generally not very useful for analyzing biomolecules: their high polarity makes it difficult to get them into the vapor phase, the first step in EIMS. Mass spectrometry of biomolecules has undergone a revolution over the past few decades, with many new ionization and separation techniques being developed. Generally, the strategy for biomolecule analysis involves **soft ionization**, in which much less energy (compared to techniques such as EIMS) is imparted to the molecule being analyzed during the

ionization process. Usually, soft ionization involves adding protons rather than removing electrons: the cations formed in this way are significantly less energetic than the radical cations formed by removal of an electron. The result of soft ionization is that little or no fragmentation occurs, so the mass being measured is that of an intact molecule. Typically, large biomolecules are digested into smaller pieces using chemical or enzymatic methods, then their masses determined by 'soft' MS.

New developments in soft ionization MS technology have made it easier to detect and identify proteins that are present in very small quantities in biological samples. In **electrospray ionization** (ESI), the protein sample, in solution, is sprayed into a tube and the molecules are induced by an electric field to pick up extra protons from the solvent. Another common 'soft ionization' method is 'matrix-assisted laser desorption ionization' (**MALDI**). Here, the protein sample is adsorbed onto a solid matrix, and protonation is achieved with a laser.

Typically, both electrospray ionization and MALDI are used in conjunction with a time-of-flight (TOF) mass analyzer component.



The proteins are accelerated by an electrode through a column, and separation is achieved because lighter ions travel at greater velocity than heavier ions with the same overall charge. In this way, the many proteins in a complex biological sample (such as blood plasma, urine, etc.) can be separated and their individual masses determined very accurately. Modern protein MS is extremely sensitive – recently, scientists were even able to detect the presence of *Tyrannosaurus rex* protein in a fossilized skeleton! (*Science* **2007**, *316*, 277).

Soft ionization mass spectrometry has become in recent years an increasingly important tool in the field of **proteomics**. Traditionally, protein biochemists tend to study the structure and function of individual proteins. Proteomics researchers, in contrast, want to learn more about how large numbers of proteins in a living system interact with each other, and how they respond to changes in the state of the organism. One important subfield of proteomics is the search for protein 'biomarkers' for human disease: in other words, proteins which are present in greater quantities in the tissues of a sick person than in a healthy person. Detection in a healthy person of a known biomarker for a disease such as diabetes or cancer could provide doctors with an early warning that the patient may be especially susceptible to the disease, so that preventive measures could be taken to prevent or delay onset.

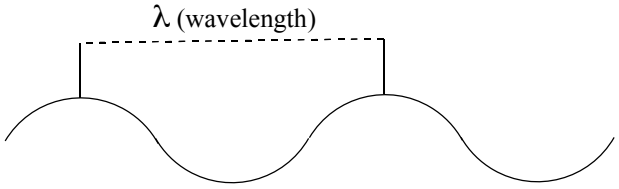
In a 2005 study, MALDI-TOF mass spectrometry was used to compare fluid samples from lung transplant recipients who had suffered from tissue rejection to samples from recipients who had not suffered rejection. Three peptides (short proteins) were found to be present at elevated levels specifically in the tissue rejection samples. It is hoped that these peptides might serve as biomarkers to identify patients who are at increased risk of rejecting their transplanted lungs. (*Proteomics* **2005**, *5*, 1705).

Section 4.2: Introduction to molecular spectroscopy

4.2A: The electromagnetic spectrum

Electromagnetic radiation, as you may recall from a previous chemistry or physics class, is composed of oscillating electrical and magnetic fields. Visible light is electromagnetic radiation. So are the gamma rays that are emitted by spent nuclear fuel, the x-rays that a doctor uses to visualize your bones, the ultraviolet light that causes a painful sunburn when you forget to apply sun block, the infrared light that the army uses in night-vision goggles, the microwaves you use to heat up your frozen burritos, and the radio-frequency waves of your cell phone signal.

While the speed of a wave in the ocean can vary, the speed of electromagnetic waves – commonly referred to as the speed of light – is a constant, approximately 300 million meters per second, whether we are talking about gamma radiation, visible light, or FM radio waves. Electromagnetic radiation is defined by its **wavelength**, which is the distance between one wave crest to the next.



Because electromagnetic radiation travels at a constant speed, each wavelength corresponds to a given **frequency**, which is the number of times per second that a crest passes a given point. Longer waves have lower frequencies, and shorter waves have higher frequencies. Frequency is commonly reported in hertz (Hz), meaning 'cycles per second', or 'waves per second'. The equivalent standard unit for frequency is s^{-1}.

When talking about electromagnetic waves, we can refer either to wavelength or to frequency - the two values are inversely proportional:

equation 4.1 $$\lambda \upsilon = c$$

where λ (Greek '*lambda*') is wavelength, υ (Greek '*nu*') is frequency in s^{-1} (or Hz), and *c* is the speed of light, a constant value of 3.0 x 10^8 m·s^{-1}.

Electromagnetic radiation transmits energy in discreet quantum 'packages' called **photons**. Shorter wavelengths (and higher frequencies) correspond to higher energy.
High energy radiation, such as gamma radiation and x-rays, is composed of very short waves – as short as 10^{-16} m. Longer wavelengths are far less energetic, and thus are less dangerous to living things. Visible light waves are in the range of 400 – 700 nm (nanometers, or 10^{-9} m), while radio waves can be several hundred meters in length.

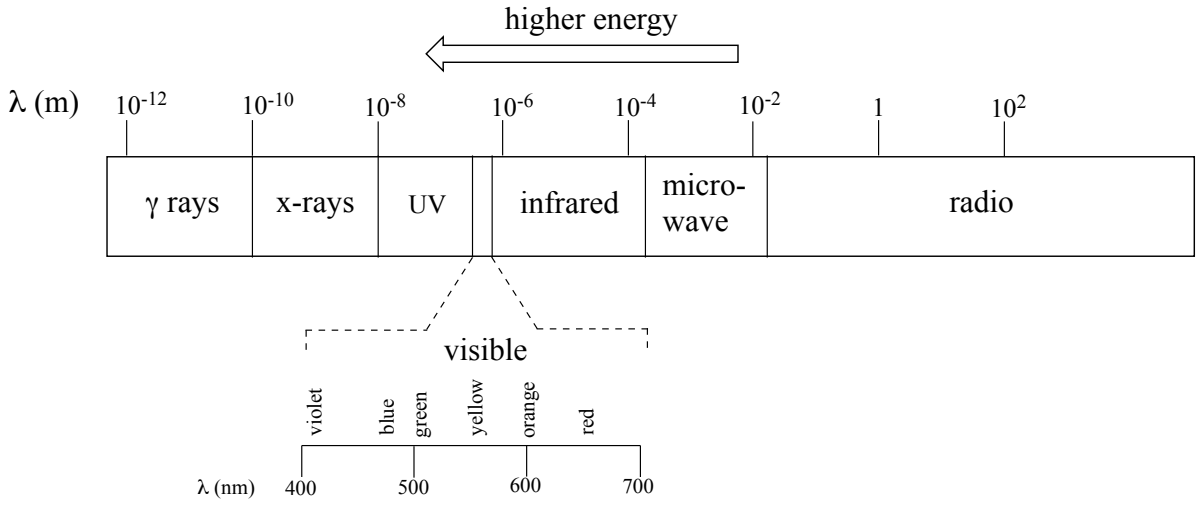
The energy of a particular wavelength of electromagnetic radiation can be expressed as:

equation 4.2 $$E = hc/\lambda = h\nu$$

where E is energy in kJ/mol of photons and *h* is 6.63 x 10^{-34} J·s, a number known as **Planck's constant**.

Using equations 4.1 and 4.2, we can calculate, for example, that visible red light with a wavelength of 700 nm has a frequency of 4.29 x 10^{14} Hz, and an energy of 171 kJ per mole of photons.

The full range of electromagnetic radiation wavelengths is referred to as the **electromagnetic spectrum**.



Notice in the figure above that visible light takes up just a narrow band of the full spectrum. White light from the sun or a light bulb is a mixture of all of the visible wavelengths plus some UV. You see the visible region of the electromagnetic spectrum divided into its different wavelengths every time you see a rainbow: violet light has the shortest wavelength, and red light has the longest.

Exercise 4.4: Visible light has a wavelength range of about 400-700 nm. What is the corresponding frequency range? What is the corresponding energy range, in kJ/mol of photons?

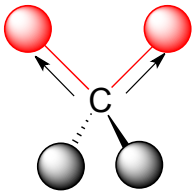
4.2B: Overview of the molecular spectroscopy experiment

In a molecular spectroscopy experiment, electromagnetic radiation of a specified range of wavelengths is allowed to pass through a sample containing a compound of interest. The sample molecules absorb energy from some of the wavelengths, and as a result jump from a low energy 'ground state' to some higher energy 'excited state'. Other wavelengths are *not* absorbed by the sample molecule, so they pass on through. A detector records which wavelengths were absorbed, and to what extent they were absorbed.

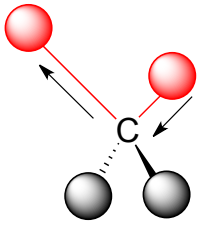
As we will see in this chapter, we can learn a lot about the structure of an organic molecule by quantifying how it absorbs (or does not absorb) different wavelengths in the electromagnetic spectrum. Three of the most useful types of molecular spectroscopy for organic chemists involve absorption of radiation in the infrared, ultraviolet/visible, and radio regions of the electromagnetic spectrum. We will focus first on infrared spectroscopy.

Section 4.3: Infrared spectroscopy

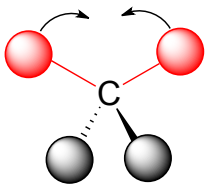
Covalent bonds in organic molecules are not rigid sticks – rather, they behave more like springs. At room temperature, organic molecules are always in motion, as their bonds stretch, bend, and twist. These complex vibrations can be broken down mathematically into individual **vibrational modes**, a few of which are illustrated below.



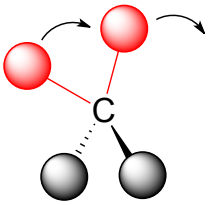
symmetric stretching



asymmetric stretching



scissoring



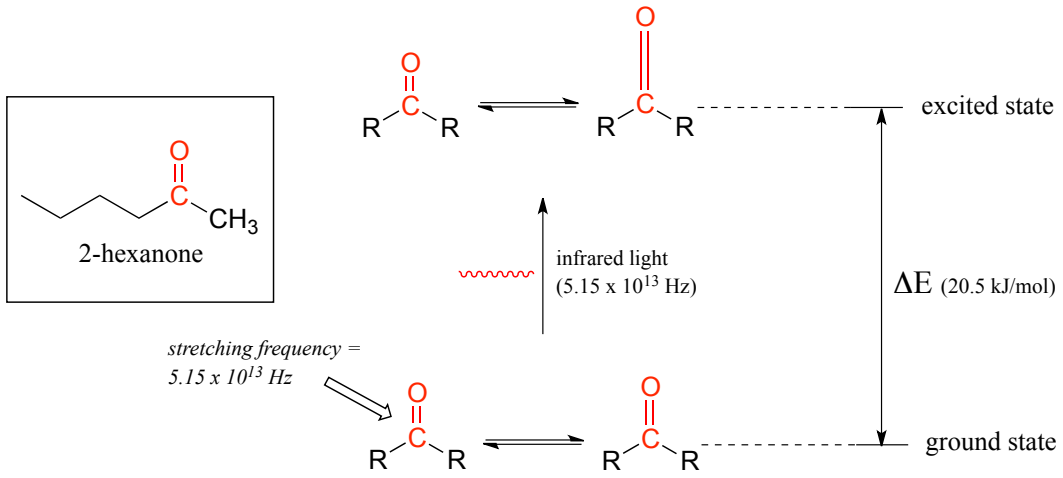
rocking

Each vibrational mode for any bond in an organic molecule has a characteristic ground state frequency of vibration, which will be somewhere between 10^{13} and 10^{14} vibrations per second. This range of frequencies corresponds to the infrared region of the electromagnetic spectrum.

If a molecule is exposed to electromagnetic radiation that matches the frequency of one of its vibrational modes, it will absorb energy from the radiation and jump to a higher vibrational energy state - the *amplitude* of the vibration will increase, but the vibrational *frequency* will remain the same. The difference in energy between the two vibrational states is equal to the energy associated with the wavelength of radiation that was absorbed.

Let's take 2-hexanone as an example. Picture the carbonyl bond of the ketone group as a spring. This spring is constantly bouncing back and forth, stretching and compressing, pushing the carbon and oxygen atoms further apart and then pulling them together. This is the **stretching mode** of the carbonyl bond. In the space of one second, the spring 'bounces' back and forth 5.15 x 10^{13} times - in other words, its stretching frequency is 5.15 x 10^{13} Hz (remember that Hz means 'per second').

If our ketone sample is irradiated with infrared light, the carbonyl bond will absorb light of a frequency matching its stretching frequency of 5.15 x 10^{13} Hz, which by equations 4.1 and 4.2 corresponds to a wavelength of λ = 5.83 x 10^{-6} m and an energy of 20.5 kJ/mol. When the carbonyl bond absorbs this energy, it jumps up to its excited vibrational state.



The molecule does not remain in the excited vibrational state for very long, but quickly releases energy to the surrounding environment in form of heat, and returns to the ground state.

In an **infrared spectrophotometer**, light in the infrared region (frequencies ranging from about 10^{13} to 10^{14} Hz) is passed though our sample of 2-hexanone. Most frequencies pass right through the sample. The 5.15 x 10^{13} Hz carbonyl stretching frequency, however, is absorbed by the 2-hexanone sample, and a detector in the instrument records that the

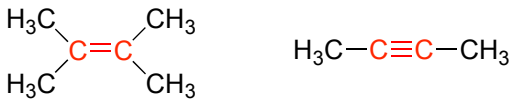
intensity of this frequency, after having passed through the sample, is something less than 100% of its initial value.

The vibrations of a 2-hexanone molecule are not, of course, limited to the simple stretching of the carbonyl bond. The various carbon-carbon bonds also stretch and bend, as do the carbon-hydrogen bonds, and all of these vibrational modes also absorb different frequencies of infrared light.

The power of infrared spectroscopy arises from the observation that *the bonds characterizing different functional groups have different characteristic absorption frequencies*. The carbonyl bond in a ketone, as we saw with our 2-hexanone example, typically absorbs in the range of 5.11 - 5.18 x 10^{13} Hz, depending on the molecule. The carbon-carbon triple bond of an alkyne, on the other hand, absorbs in the range of 6.30 - 6.80 x 10^{13} Hz. The technique is therefore very useful as a means of identifying which functional groups are present in a molecule of interest. If we pass infrared light through an unknown sample and find that it absorbs in the carbonyl frequency range but not in the alkyne range, we can infer that the molecule contains a carbonyl group but not an alkyne.

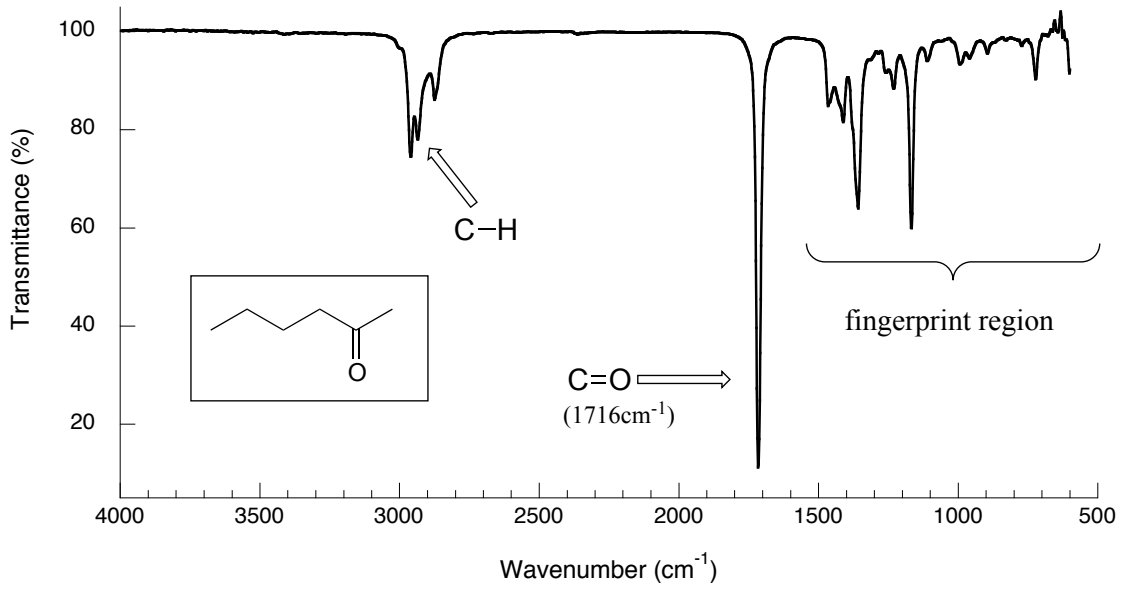
Some bonds absorb infrared light more strongly than others, and some bonds do not absorb at all. *In order for a vibrational mode to absorb infrared light, it must result in a periodic (repeating) change in the dipole moment of the molecule*. Such vibrations are said to be **infrared active**. In general, the greater the polarity of the bond, the stronger its IR absorption. The carbonyl bond is very polar, and therefore absorbs very strongly. The carbon-carbon triple bond in most alkynes, in contrast, is much less polar, and thus a stretching vibration does not result in a large change in the overall dipole moment of the molecule. We will see below that IR signals for carbonyl bonds are very strong, but the signals for carbon-carbon double and triple bonds are relatively weak.

Some kinds of vibrations are **infrared inactive**. The stretching vibrations of completely symmetrical double and triple bonds, for example, do not result in a change in dipole moment, and therefore do not result in any absorption of light (but other bonds and vibrational modes in these molecules *do* absorb IR light).



infrared-inactive double and triple bonds

Now, let's look at some actual output from IR spectroscopy experiments. Below is the IR spectrum for 2-hexanone.



There are a number of things that need to be explained in order for you to understand what it is that we are looking at. On the horizontal axis we see IR wavelengths expressed in terms of a unit called **wavenumber** (cm^{-1}), which tells us how many waves fit into one centimeter.

equation 4.3: wavenumber (in cm^{-1}) = $1/100\lambda = \nu/100c$

where λ is the wavelength in meters, v is frequency in s^{-1}, and *c* is the speed of light, 3.0×10^8 m/s. Wavenumber units are typically used in IR spectra, but you may also occasionally see micrometer (μm) units) on the horizontal axis.

On the vertical axis we see '% transmittance', which tells us how strongly light was absorbed at each frequency (100% transmittance, at the high end of the axis, means no absorption occurred at that frequency). The solid line traces the values of % transmittance for every wavelength passed through the sample – the downward-pointing **absorbance bands** show regions of strong absorption.

Exercise 5.5: Express the wavenumber value of 3000 cm^{-1} in terms of wavelength (in meter units) frequency (in Hz), and associated energy (in kJ/mol).

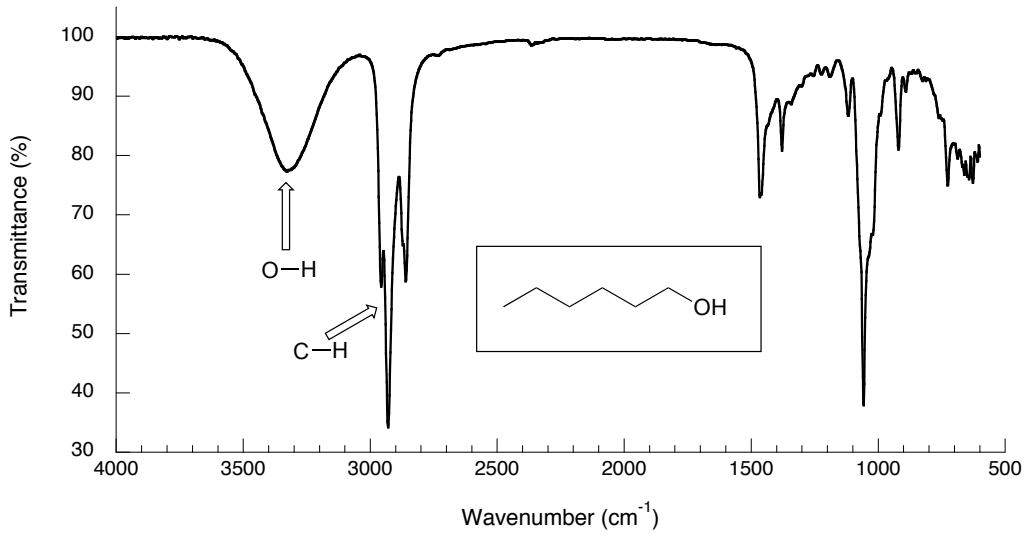
The key absorption band in this spectrum is that from the carbonyl double bond, at 1716 cm^{-1}, corresponding to a wavelength of 5.86 μm, a frequency of 5.15×10^{13} Hz, and a ΔE value of 20.5 kJ/mol. Notice how strong this band is, relative to the others on the spectrum: *a strong absorbance band in the 1650-1750 cm^{-1} region tells us that a carbonyl*

group is present. Within that range, carboxylic acids, esters, ketones, and aldehydes tend to absorb in the shorter wavelength end (1700-1750 cm-1), while conjugated unsaturated ketones and amides tend to absorb on the longer wavelength end (1650-1700 cm^{-1}).

The jagged band at approximately 2900-3000 cm^{-1} is characteristic of the stretching modes of sp^3-hybridized carbon-hydrogen bonds. This band is not terribly useful, as just about every organic molecule that you will have occasion to analyze has these bonds. Nevertheless, it can serve as a familiar reference point to orient yourself in a spectrum.

You will notice that there are many additional band in this spectrum in the longer-wavelength 400 -1400 cm^{-1} region. This part of the spectrum is called the **fingerprint region**. While it is usually very difficult to pick out anything specific to a functional group from this region, the fingerprint region nevertheless contains valuable information. Just like a human fingerprint, the pattern of absorbance bands in the fingerprint region is unique to every molecule, meaning that the data from an unknown sample can be compared to a database of IR spectra of known standards in order to make a positive identification. In our introductory story about the forged paintings, it was the IR fingerprint region of the suspicious yellow paint that allowed for its identification as a pigment that could not possibly have been used by the purported artist, William Aiken Walker. (Recall from earlier in this chapter that a mass spectrum of a molecule can also be thought of as a kind of 'fingerprint' by which the molecule can be identified.)

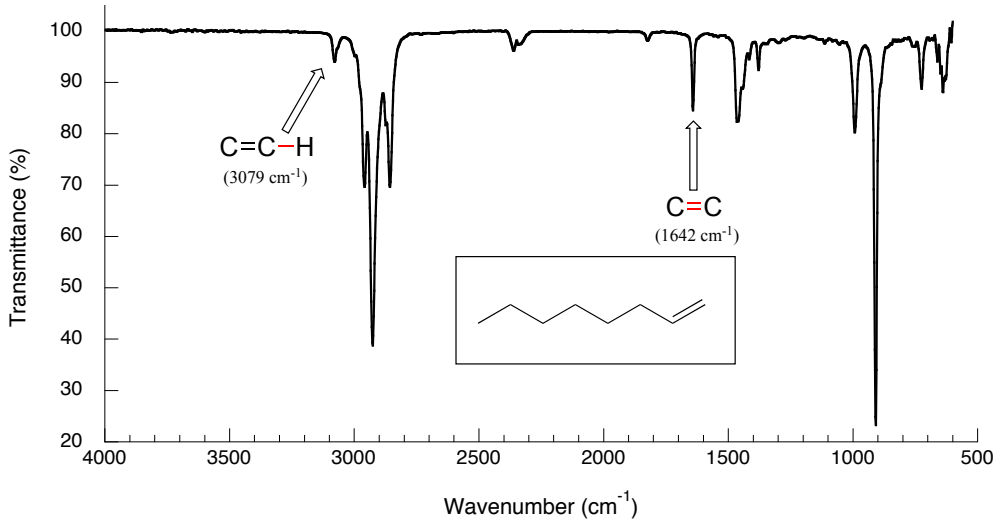
Now, let's take a look at the IR spectrum for 1-hexanol.



As expected, there is no carbonyl band at around 1700 cm^{-1}, but we can see a very broad 'mountain' centered at about 3400 cm^{-1}. This signal is characteristic of the O-H stretching

mode of alcohols, and tells us that our sample has an alcohol group. The breadth of this signal is a consequence of hydrogen bonding between molecules.

The spectrum for 1-octene shows two bands that are characteristic of alkenes: the one at 1642 cm^{-1} is due to stretching of the carbon-carbon double bond, and the one at 3079 cm-1 is due to stretching of the σ bond between the sp^2-hybridized alkene carbons and their attached hydrogens.



Alkynes have characteristic weak but sharp IR absorbance bands in the range of 2100-2250 cm^{-1} due to stretching of the carbon-carbon triple bond, and terminal alkynes can be identified by their absorbance at about 3300 cm^{-1}, due to stretching of the bond between the *sp*-hybridized carbon and the terminal hydrogen.*)*

Exercise 4.6: Explain how you could use the C-H stretching frequencies in IR spectra to distinguish between four constitutional isomers: 1,2-dimethylcyclohexene, 1,3-octadiene, 3-octyne, and 1-octyne.

Other functional groups can be identified IR, but we will confine our discussion here to a small set of the most easily identifiable groups. These are summarized below.

Characteristic IR absorbances

Functional group	Characteristic IR absorbance(s) (cm^{-1})	Source of signal
carbonyl	1650-1750 (strong)	C=O stretching
alcohol	3200 - 3600 (broad)	O-H stretching
carboxylic acid	1700-1725 (strong)	C=O stretching
	2500-3000 (broad)	O-H stretching
alkene	1620 - 1680 (weak)	C=C stretching
	3020 - 3080	vinylic C-H stretching
alkyne	1620 - 1680 (weak)	triple bond stretching
	3250-3350	terminal C-H stretching

A somewhat more detailed summary is provided in Table 1 at the back of the book.

An IR spectrum usually does not provide enough information for us to figure out the complete structure of a molecule. In conjunction with other analytical methods, however, IR spectroscopy can prove to be a very valuable tool, given the information it provides about the presence or absence of key functional groups. IR can also be a quick and convenient way for a chemist to check to see if a reaction has proceeded as planned. If we were to run a reaction in which we wished to convert cyclohexanone to cyclohexanol, for example, a quick comparison of the IR spectra of starting compound and product would tell us if we had successfully converted the ketone group to an alcohol.

Exercise 4.7: Using the online Spectral Database for Organic Compounds, look up IR spectra for the following compounds, and identify absorbance bands corresponding to those listed in the table above.

a) 1-methylcyclohexanol
b) 4-methylcyclohexene
c) 1-hexyne
d) 2-hexyne
e) 3-hexyne-2,5-diol
f) *ortho*-acetoxybenzoic acid (acetylsalicylic acid, better known as aspirin)

Exercise 4.8: A carbon-carbon single bond absorbs in the fingerprint region, and we have already seen the characteristic absorption wavelengths of carbon-carbon double and triple bonds. Rationalize the trend in wavelengths. (*Hint* - remember, we are thinking of bonds as springs, and looking at the frequency at which they 'bounce').

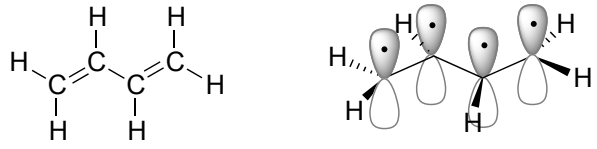
Section 4.4: Ultraviolet and visible spectroscopy

Ultraviolet and visible (UV-Vis) spectroscopy provides us with information about aromatic groups and other conjugated π systems in organic compounds. In looking at IR spectroscopy, we saw how interaction with infrared light causes molecules to undergo vibrational transitions. The shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes organic molecules with conjugated π bonds to undergo **electronic transitions**.

4.4A: The electronic transition

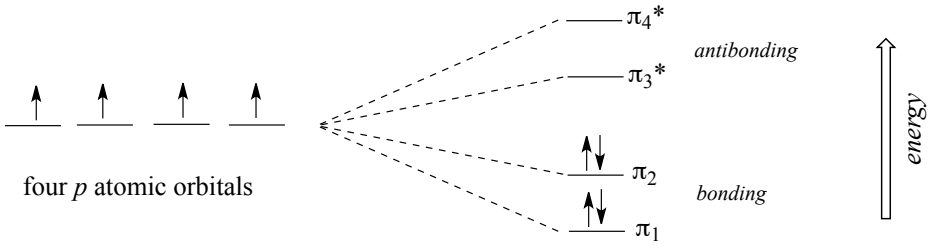
Recall from chapter 2 that molecular orbital (MO) theory can be a useful way to think about covalent bonding in molecules containing conjugated double bonds and aromatic rings. Keep in mind that molecular orbitals result from the mathematical combination of two or more atomic orbitals, and describe a region in space around a *group of atoms* (rather than a single atom) in which electrons are likely to be found. We'll see here that MO theory is indispensible to a discussion of ultraviolet and visible spectroscopy.

A **chromophore** is any molecule or part of a molecule that has the property of absorbing light in the ultraviolet or visible region of the spectrum. 1,3-butadiene is a chromophore. Recall from section 2.2B that the conjugated π bonds in1,3-butadiene can be pictured as four parallel, overlapping *p* orbitals sharing four electrons:



1,3-butadiene

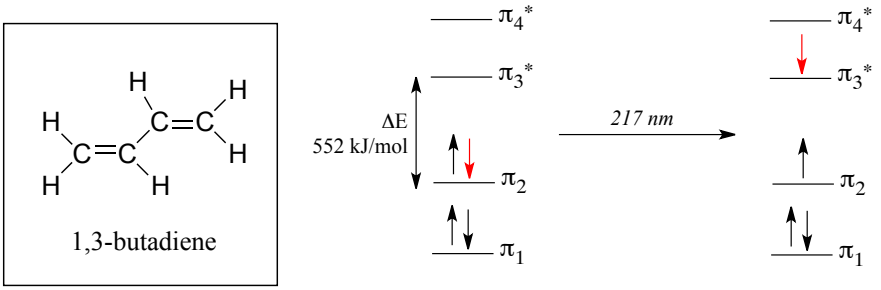
Using the language of MO theory, we can say that the four *p* atomic orbitals combine to form four π molecular orbitals. The two lower energy orbitals π_1 and π_2 are bonding orbitals, while the two higher energy orbitals π_3* and π_4* are antibonding orbitals.



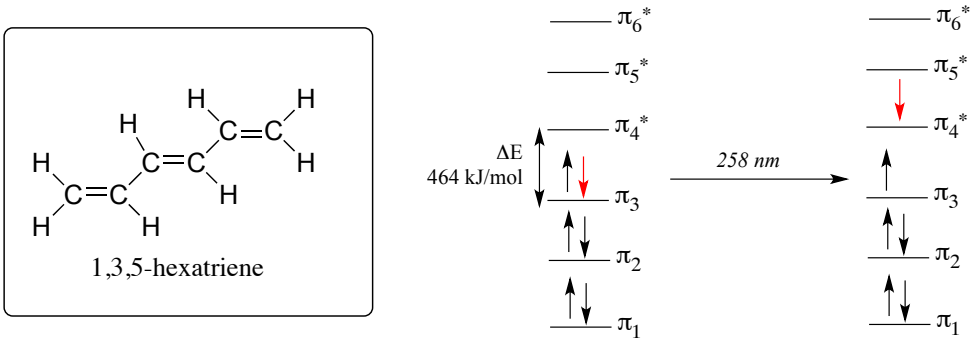
four π molecular orbitals

In the electronic ground state, all four π electrons in the conjugated system reside in the two bonding orbitals. A **π→ π* transition** occurs when an electron in π_2, the **highest occupied molecular orbital (HOMO)** jumps up to π_3*, the **lowest unoccupied molecular orbital (LUMO)**. The gap between these two molecule orbitals is often called the **HOMO-LUMO energy gap**.

For 1,3-butadiene, the energy difference in the HOMO-LUMO gap is 552 kJ/mol. This energy corresponds to light with a wavelength of 217 nm, which is in the ultraviolet region of the spectrum. When a sample of 1,3-butadiene is irradiated with UV radiation, the molecule absorbs the light at 217 nm as an electron undergoes a π-π* transition.



The absorbance due to the π → π* transition in 1,3,5-hexatriene occurs at 258 nm, corresponding to a HOMO-LUMO gap of 464 kJ/mol.



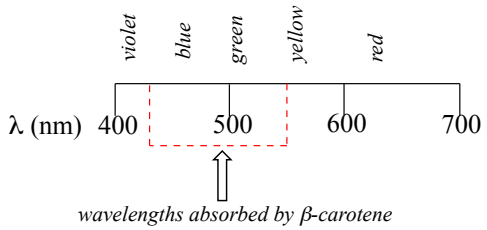
Notice what happened here: when the conjugated π system was extended by two carbons, the HOMO-LUMO energy gap shrunk from 552 to 464 kJ/mol. A lower energy transition corresponds to longer wavelength absorbance (look again at equation 4.2 in the previous section). In general:

As conjugated π systems become more extended,

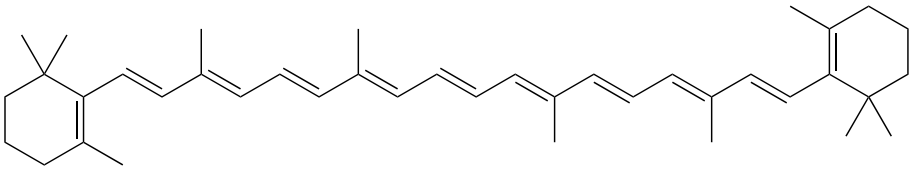
a) the HOMO-LUMO gap shrinks, and
b) the wavelength of absorbed light becomes longer.

In molecules with very large, extended conjugated π systems, the π → π* energy gap becomes so small that absorption occurs in the visible rather than the UV region of the electromagnetic spectrum.

The visible region of the electromagnetic spectrum:



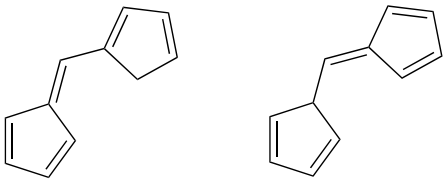
β-carotene, with its system of 11 conjugated double bonds, absorbs light in a broad band between approximately 420-550 nm, with a λ_{max} at 470, spanning the blue and green regions of the visible spectrum. Because blue/green wavelengths are absorbed while those in the red and yellow regions are able to pass through the sample, β-carotene appears to our eyes as orange. β-carotene is abundant in carrots, and is the compound mainly responsible for their orange color.



β-carotene

Exercise 4.9: what is value of ΔE (in kJ/mol) for the π → π* transition in 4-methyl-3-penten-2-one?

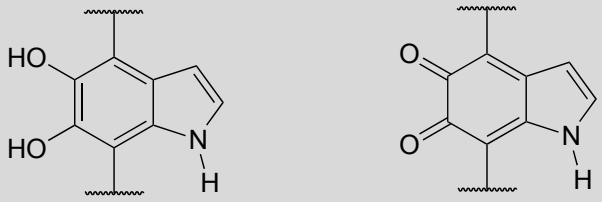
Exercise 4.10: Which of the following molecules would you expect absorb at a longer wavelength in the UV region of the electromagnetic spectrum? Explain your answer.



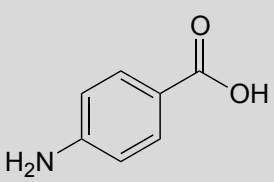
A B

Protecting yourself from sunburn

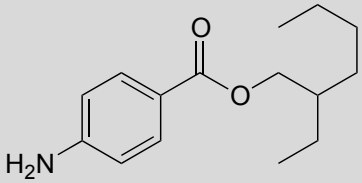
Human skin can be damaged by exposure to ultraviolet light from the sun. We naturally produce a pigment, called melanin, which protects the skin by absorbing much of the ultraviolet radiation. Melanin is a complex polymer, two of the most common monomers units of which are shown below.



common monomer units in melanin



PABA

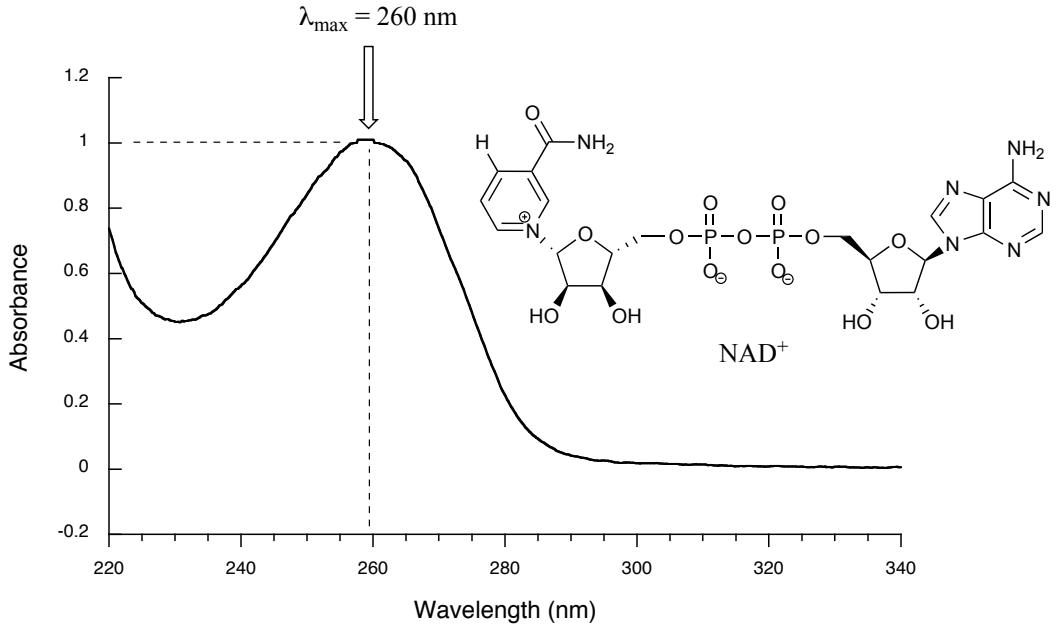


Padimate-O

Overexposure to the sun is still dangerous, because there is a limit to how much radiation our melanin can absorb. Most commercial sunscreens claim to offer additional protection from both UV-A and UV-B radiation: UV-A refers to wavelengths between 315-400 nm, UV-B to shorter, more harmful wavelengths between 280-315 nm. PABA (*para*-aminobenzoic acid) was used in sunscreens in the past, but its relatively high polarity meant that it was not very soluble in oily lotions, and it tended to rinse away when swimming. Many sunscreens today contain, among other active ingredients, a more hydrophobic derivative of PABA called Padimate O.

4.4B: Looking at UV-vis spectra

We have been talking in general terms about how molecules absorb UV and visible light – now let's look at some actual examples of data from a UV-vis absorbance spectrophotometer. The setup is conceptually the same as for IR spectroscopy: radiation with a range of wavelengths is directed through a sample of interest, and a detector records which wavelengths were absorbed and to what extent the absorption occurred. Below is the absorbance spectrum of an important biological molecule called nicotinamide adenine dinucleotide, abbreviated NAD^+. The adenine nucleotide base in the compound absorbs light in the neighborhood of 260 nm, which is within the UV range of the electromagnetic spectrum.



The convention in UV-vis spectroscopy is to show the baseline at the bottom of the graph with the absorbance bands pointing up (opposite to the way IR spectra are normally plotted). Wavelength values on the x-axis are generally displayed in nanometer (nm) units. On the y-axis is a measure of how much light was absorbed at each wavelength, expressed as **absorbance (A)**. The absorbance value contains the same information as the 'percent transmittance' number usually used in IR spectroscopy, just expressed in different terms. To calculate absorbance at a given wavelength, the computer in the spectrophotometer simply takes the intensity of light at that wavelength *before* it passes through the sample (I_0), divides this value by the intensity of the same wavelength *after* it passes through the sample (I), then computes the $\log_{10}$ of that number.

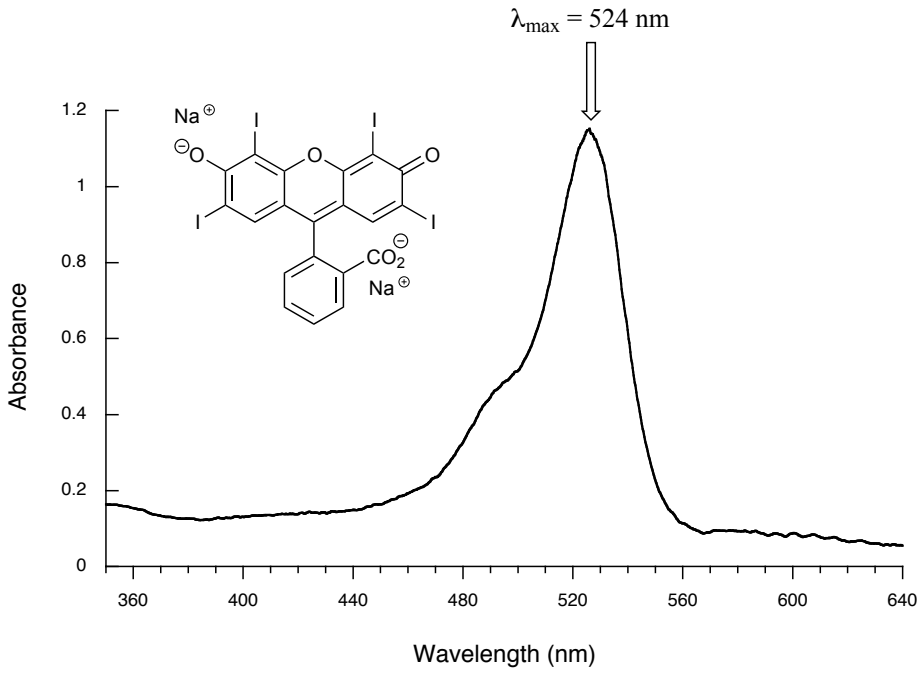
equation 4.4 $$A = \log (I_0/I)$$

Note that in this expression the units cancel, so A is a unitless value.

Typically, there are two things that we look for and record from a UV-Vis spectrum. The first is λ_{max}, the wavelength at maximal absorbance, which visually is the very top of the broad absorbance band. We also record the value of A at λ_{max}, which depends on the concentration of the sample. The NAD^+ spectrum above shows a maximal absorbance of 1.0 at 260 nm. In the next section, we will see how we can use this data to calculate the concentration of NAD^+ in the sample.

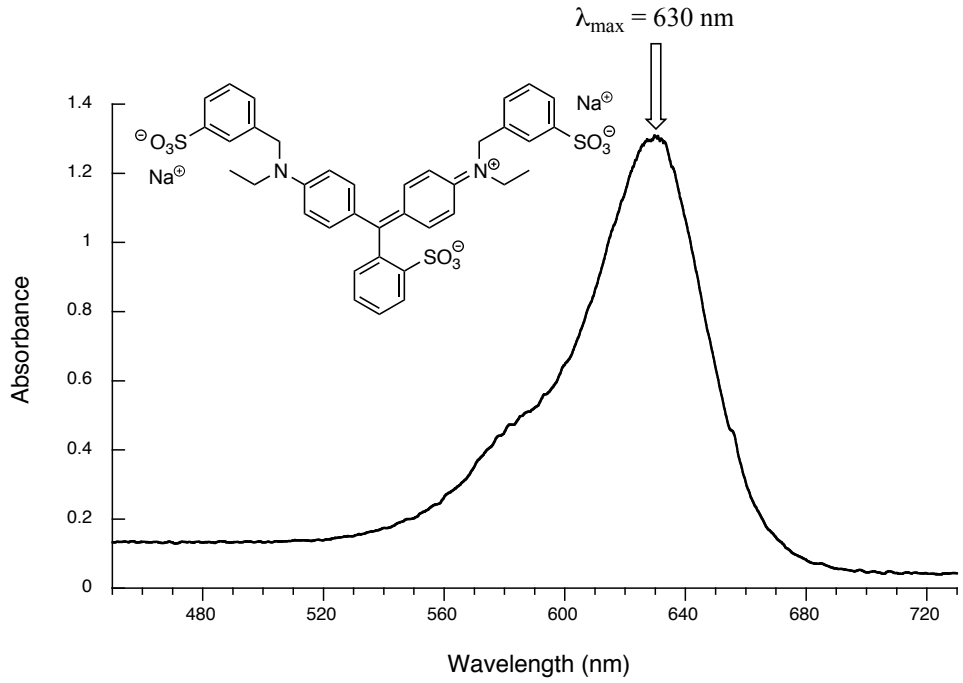
Exercise 4.10: Express A = 1.0 in terms of percent transmittance (%T, the unit usually used in IR spectroscopy (and sometimes in UV-vis as well).

Here is the absorbance spectrum of the common food coloring Red #3:



Here, we see that the extended system of conjugated π bonds causes the molecule to absorb light in the visible range - specifically, with a λ_{max} of 524 nm, which is in the blue/green range. Red wavelengths pass through without being absorbed, so the solution appears red to our eyes.

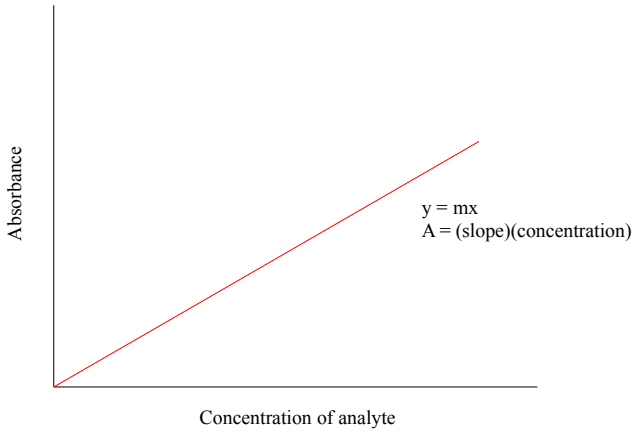
Now, take a look at the spectrum of another food coloring, Blue #1:



Maximum absorbance is at 630 nm, in the orange range of the visible spectrum, and the compound appears blue.

4.3D: Applications of UV spectroscopy in organic and biological chemistry

UV-Visible spectroscopy has many applications in organic and biological chemistry. The **Beer - Lambert Law** is often used to determine the concentration of a UV-active sample. You most likely have performed a Beer – Lambert experiment in a General Chemistry lab. The law is simply an application of the observation that, within certain ranges, the absorbance of UV-active compound at a given wavelength varies in a linear fashion with its concentration: the higher the concentration, the greater the absorbance.



If, for a particular sample, we divide the observed value of A at λ_{max} by the concentration of the sample (c, in mol/L), we obtain the **molar absorptivity**, or **extinction coefficient** (ε), which is a characteristic value for a given compound that we can look up.

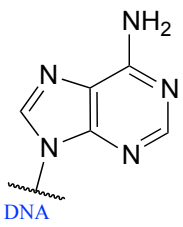
equation 4.4 $\varepsilon = A/cl$

In the above equation, 'l' is **path length** - the distance that the beam of light travels though the sample - expressed in cm. Most sample holders are designed with a path length of1.0 cm, so the conventional unit for molar absorptivity is $L \cdot mol^{-1} \cdot cm^{-1}$. In all UV-Vis discussion or problems in this book, you should assume that the relevant path length is 1.0 cm.

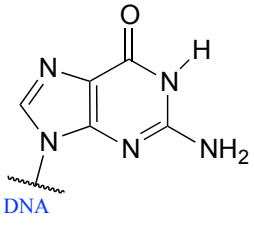
For example: In the spectrum of NAD^+ (section 4.3C), we see that A = 1.0 at the λ_{max} of 260 nm. We look up the molar absorptivity of NAD^+ at 260 nm and find that it is listed as $\varepsilon(260) = 18{,}000\ L \cdot mol^{-1} \cdot cm^{-1}$. Using equation 4.4, it is easy to calculate that the concentration of the sample is 5.6×10^{-5} M.

Exercise 4.11: The literature value of ε for 1,3-pentadiene in hexane is 26,000 $L \cdot mol^{-1} \cdot cm^{-1}$ at its λ_{max} of 224 nm. You prepare a sample and take a UV spectrum, finding that $A_{224} = 0.850$. What is the concentration of your sample?

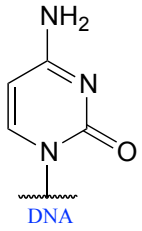
The bases of DNA and RNA are good chromophores:



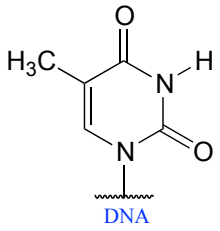
Adenine



Guanine



Cytosine

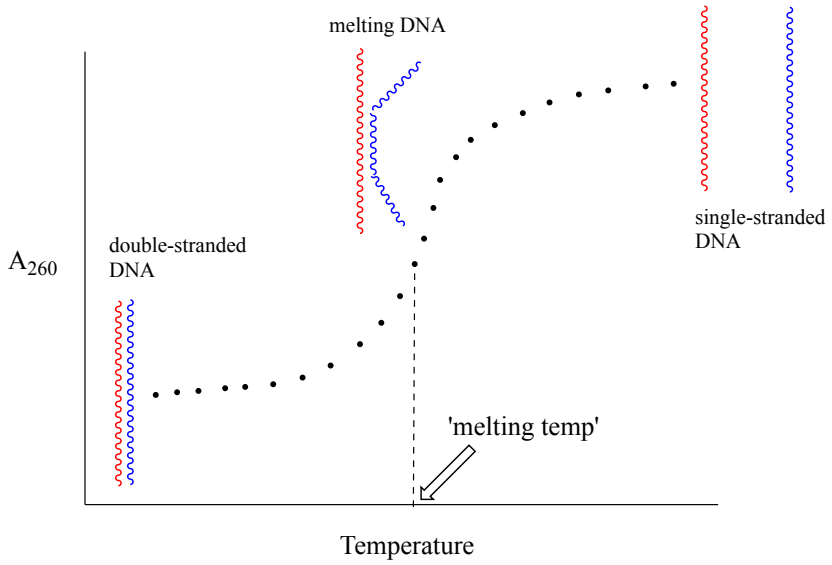


Thymine

Biochemists and molecular biologists often determine the concentration of a DNA or oligonucleotide sample by assuming an average value of $\varepsilon_{260} = 0.020\ ng \cdot \mu L^{-1} \cdot cm^{-1}$ for double-stranded DNA at its λ_{max} of 260 nm (notice that concentration here is expressed in terms of mass rather than moles: nanograms per microliter is a convenient unit for DNA concentration when doing molecular biology).

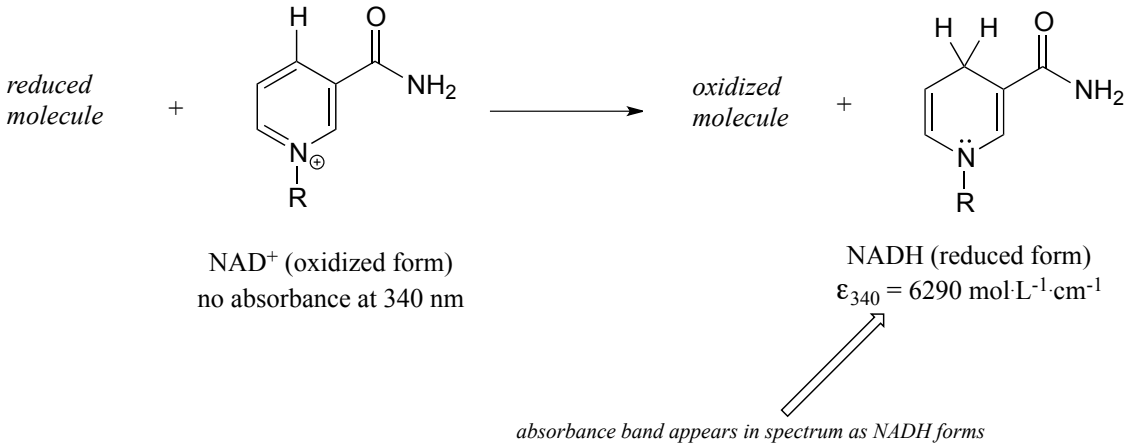
Exercise 4.12: 50 µL of an aqueous sample of double stranded DNA is dissolved in 950 µL of water. This diluted solution has a maximal absorbance of 0.326 at 260 nm. What is the concentration of the original (more concentrated) DNA sample, expressed in µg/µL?

Because the extinction coefficient of double stranded DNA is slightly lower than that of single stranded DNA, we can use UV spectroscopy to monitor a process known as DNA melting, an important factor in molecular cloning experiments. If a short stretch of double stranded DNA is gradually heated up, it will begin to 'melt', or break apart, as the temperature increases (recall that two strands of DNA are held together by a specific pattern of hydrogen bonds formed by 'base-pairing').

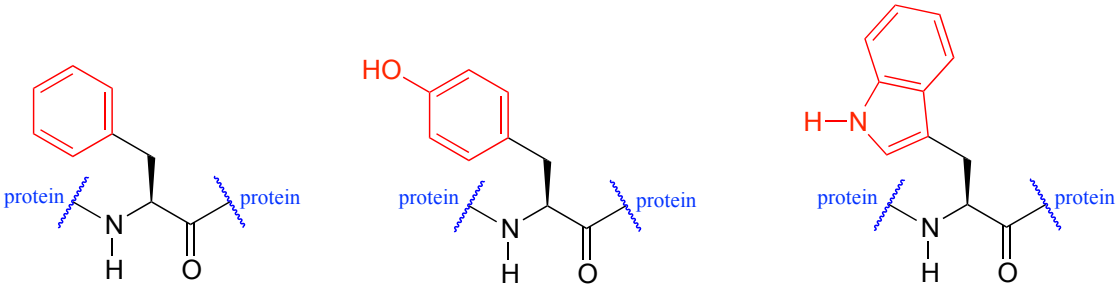


As melting proceeds, the absorbance value for the sample increases, eventually reaching a high plateau as all of the double-stranded DNA has melted into two strands. The mid-point of this process, called the 'melting temperature', provides a good indication of how tightly the two complementary strands of DNA are able to bind to each other.

In chapter 15 we will see how the Beer - Lambert Law and UV spectroscopy provides us with a convenient way to follow the progress of many different enzymatic redox (oxidation-reduction) reactions. In biochemistry, oxidation of an organic molecule often occurs concurrently with reduction of nicotinamide adenine dinucleotide (NAD^+, the compound whose spectrum we saw earlier in this section) to NADH:



Proteins absorb light in the UV range due to the presence of the aromatic amino acids tryptophan, phenylalanine, and tyrosine, all of which are chromophores. Many coenzymes are also UV-active.



phenylalanine residue tyrosine residue tryptophan residue

Biochemists have used UV spectroscopy to study conformational changes in proteins - how they change shape in response to different conditions. When a protein undergoes a conformational shift (due to binding of a ligand, for example), the resulting change in the environment around an aromatic amino acid or coenzyme chromophore can cause the UV spectrum of the protein to be altered.

Key concepts for review

Mass spectrometry:

Understand the fundamentals of an MS experiment.
In a given mass spectrum, recognize the molecular ion peak along with M+1 and M+2 peaks and be able to explain what these are and why they are there.
Recognize characteristic mass spectral data for chloro- and bromo-alkanes (M+2 peaks in 1:1 or 3:1 ratios, respectively).

Spectrometry basics:

Be able to put the following regions of the electromagnetic spectrum in order from shortest to longest wavelength: gamma radiation, x-rays, ultraviolet, blue visible light, red visible light, infrared, microwave, radio wave.

Be able to mathematically convert among the following: wavelength expressed in meters, wavelength expressed in wavenumbers (cm-1), frequency, and energy in kJ/mol of photons. Non-quantitatively, you should be able to quickly recognize relationships between the above: in other words, you should have internalized the idea that radiation with shorter wavelengths corresponds to higher wavenumbers, higher frequency, and higher energy.

Be able to describe, in 'generic' terms, a molecular spectroscopy experiment, and understand its elements: a spectrum of wavelengths passing into a sample, a quantum transition from a ground to an excited state in the sample molecules with absorbance of corresponding wavelength(s) of radiation, and detection of wavelengths absorbed along with the intensity of each absorbance.

IR spectroscopy:

Understand the basic idea of a vibrational transition
Be able to identify IR-active and inactive functional groups, and predict which functional groups will lead to more intense absorbance (ie., those with greater dipole moments)
Based on an IR spectrum, be confident in predicting the presence (or absence) of the functional groups listed in table x.

UV-visible spectroscopy:

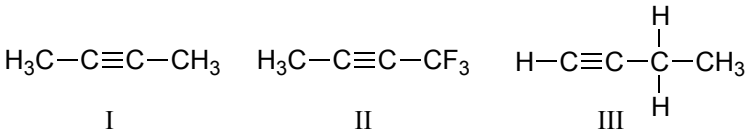
Understand the basic idea of an electronic (π-π^*, n-π^*) transition.
Be able to recognize a chromophore.
Be able to predict trends in absorbance maxima based on degree of conjugation. Be able to use the Beer-Lambert Law to do simple calculations (eg. figuring out concentration of a compound based on observed A and literature value of molar absorptivity).

Problems

P4.1: Which represents a higher energy frequency of electromagnetic radiation, 1690 cm^{-1} or 3400 cm^{-1}? Express each of these in terms of wavelength (meters) and frequency (Hz).

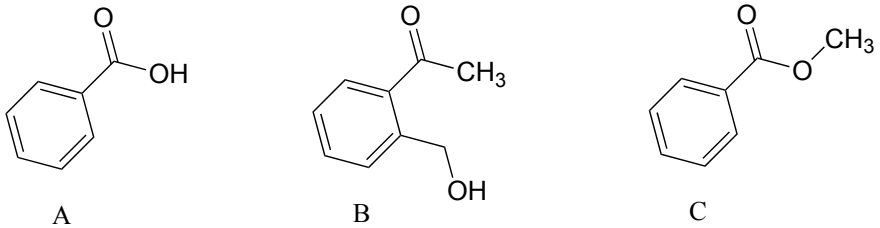
P4.2: Calculate the value, in kJ/mol, of the ΔE associated with a typical alkyne carbon-carbon stretching frequency at 2100 cm^{-1}.

P4.3: Explain how you could use IR spectroscopy to distinguish between compounds I, II, and III.



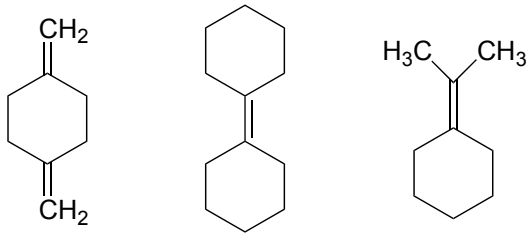
I II III

P4.4: Explain how you could use IR spectroscopy to distinguish between compounds A, B, and C.



A B C

P4.5: Explain how you could use IR spectroscopy to distinguish between compounds X, Y, and Z.

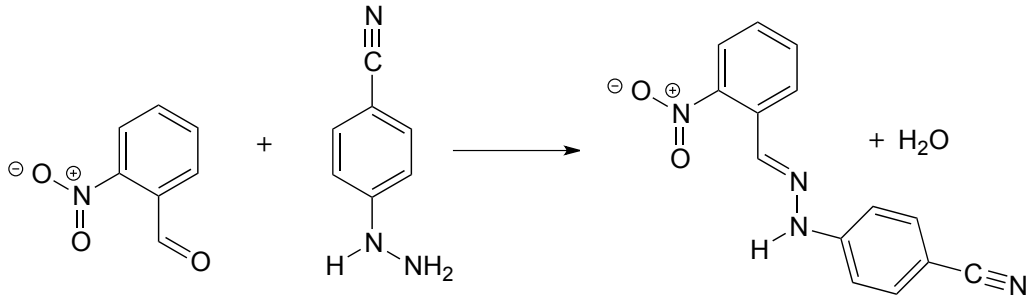


X Y Z

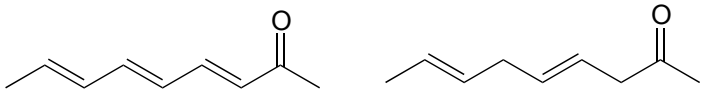
P4.6: A 0.725 mL aqueous solution of NADH shows an absorbance at 340 nm of A = 0.257. Express (in nanomole (nm) units) how much NADH is contained in the sample.

P4.7: A 1 mL enzymatic reaction mixture contains NADH as one of the reactants, and has a starting $A_{340} = 0.345$. After the reaction is allowed to run for five minutes, the researcher records a value of $A_{340} = 0.125$. How many nm of NADH are used up per minute, on average, in this reaction?

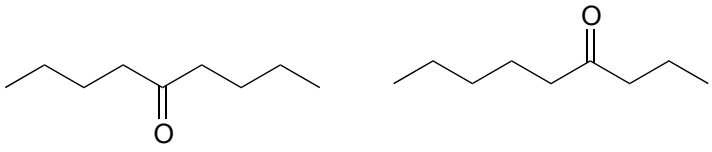
P4.8: The reaction below is of a type that we will study in Chapter 11. While the two starting materials are only slightly colored, the product is an intense orange-red. Account for this observation.



P4.9: Which would be more useful in distinguishing the two compounds shown below: IR or UV spectroscopy? Explain.



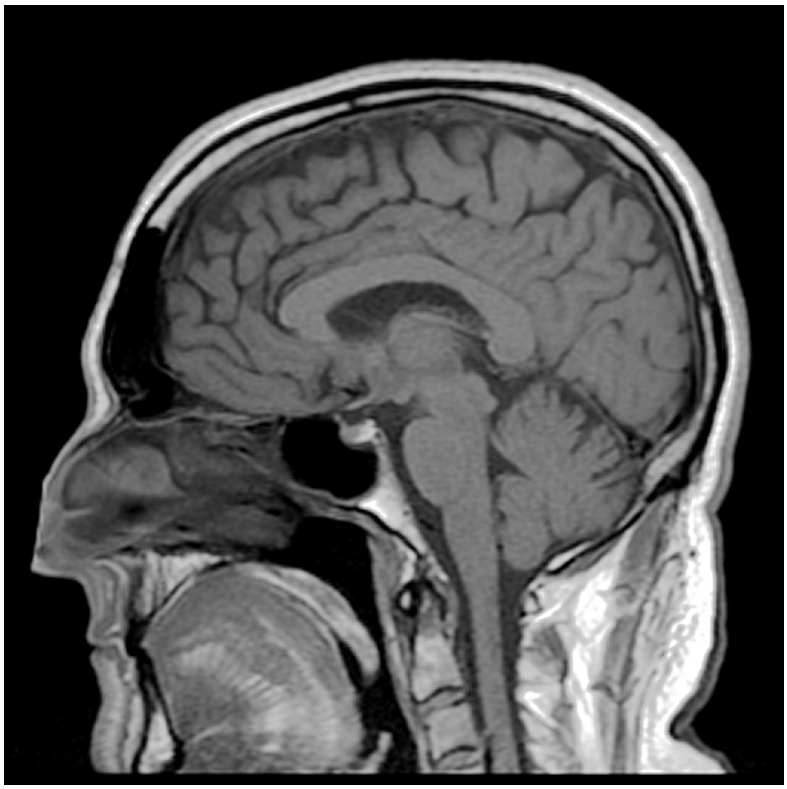
P4.10: Which analytical technique – IR, UV, or MS - could best be used to distinguish between the two compounds below? Explain.



Chapter 5

Structure determination, part II:

Nuclear magnetic resonance spectroscopy



(credit: https://www.flickr.com/photos/reighleblanc/)

One morning in a suburb of Edinburgh, Scotland, an active, athletic teenager named Charli found that she did not have her usual appetite for breakfast. She figured she was just feeling a little under the weather, and was not to worried. But as the days passed, her appetite did not return. Before long, she stopped eating lunch as well, and eventually she was hardly eating anything at all. She had to withdraw from her soccer team because she didn't have enough energy to make it through the practices. When her weight began to

drop alarmingly and she began to suffer from crippling headaches, her parents took her to her doctor, who diagnosed a glandular disorder.

To make things worse, Charli started getting teased at her school, enduring constant comments from other kids about her weight loss and gossip about an eating disorder. Almost two years went by, filled with doctors visits and various diagnoses and treatments, none of which were effective.

Finally, on a September day when Charli was fifteen, things came to a head. She was rushed to the hospital after suffering a massive stroke. Once she was stabilized, her doctors ordered an MRI scan of her brain. The images showed that she had a large tumor in her brain – it was benign, but its sheer size and the pressure it exerted had been enough to cause the devastating symptoms that Charli had been suffering for the past year and a half. Her doctors told her that if the tumor had not been detected, it could eventually have been fatal. After enduring an 8-hour brain surgery, Charli finally was able to start down her road to recovery. Speaking later to a journalist, Charli said of her stroke, “it was the best thing that ever happened to me”.

***

In Austin, Texas, a 28 year-old man named Alex was fed up with the back pain he had been suffering, the result, he assumed, of the damage from some old sports injuries catching up to him. His friend John, who was a radiological technician, convinced him to come in for an MRI scan on the chance that doctors might be able to spot something that could lead to a treatment. Alex agreed, and took a day off work to come in to his friend's clinic. With John at the controls, Alex tried to relax as he was slowly rolled into the claustrophobic MRI chamber. After finishing the scan of his friend's back and saving the images, John decided to ask a little favor. He had just installed some new software for head scans and needed to test it out on an actual subject, so he asked Alex if he would mind lying still for just a few minutes more so that he could take a test scan of his head. Unlike x-rays and CAT scans, the MRI procedure does not subject patients to potentially harmful radiation - just strong but harmless magnetic fields combined with radio waves – so there was no risk to undergoing an unnecessary scan. Alex agreed, and John proceeded with the test scan.

When the first image appeared, John was alarmed by what he saw. The new software was working just fine, but there was an ominous-looking lump behind Alex's right eye that should not have been there. Not wanted to scare his friend unduly, he merely mentioned that he thought he might have seen something that should be checked out by a neurologist. Alex was feeling fine other than the back pain – no headaches, blurred vision, or dizziness, so it was probably nothing at all to worry about.

It turned out that Alex had a golf ball-sized brain tumor. His neurologist told him that because it happened to be located in an area of the brain that was not responsible for any critical functions, he was not yet experiencing any symptoms. But if the tumor had remained undetected for a few more years, it would have continued to grow and begun to

press on other areas of Alex's brain - and at that point, it probably would have been very difficult to remove safely.

Alex underwent a successful surgery to remove the tumor and was able to go on with his life, thanks to having an observant friend in the right place at the right time, with access to a powerful diagnostic technology.

***

The common denominator in these two stories – and in countless others from around the world – is the power of MRI to detect hidden but deadly medical problems, without causing any harm or pain to the patient. In this chapter, we are going to learn about an analytical tool used by organic chemists called nuclear magnetic resonance (NMR) spectroscopy, which works by the same principles as an MRI scanner in a hospital. While doctors use MRI peer inside the human body, we will see how NMR allows chemists to piece together, atom by atom and bond by bond, the structure of an organic molecule.

Section 5.1: The source of the NMR signal

5.1A: The magnetic moment

Nuclear magnetic resonance spectroscopy is an incredibly powerful tool for organic chemists because it allows us to analyze the connectivity of carbon and hydrogen atoms in molecules. The basis for NMR is the observation that many atomic nuclei generate their own magnetic field, or **magnetic moment**, as they spin about their axes. Not all nuclei have a magnetic moment. Fortunately for organic chemists, though, hydrogen (^{1}H), the ^{13}C isotope of carbon, the ^{19}F isotope of fluorine, and the ^{31}P isotope of phosphorus all have magnetic moments and therefore can be observed by NMR – they are, in other words, NMR-active. Other nuclei - such as the common ^{12}C and ^{16}O isotopes of carbon and oxygen - do not have magnetic moments, and cannot be directly observed by NMR. Still other nuclei such as the hydrogen isotope deuterium (^{2}H) and nitrogen (^{14}N) have magnetic moments and are NMR-active, but the nature of their magnetic moments is such that analysis of these nuclei by NMR is more complex.

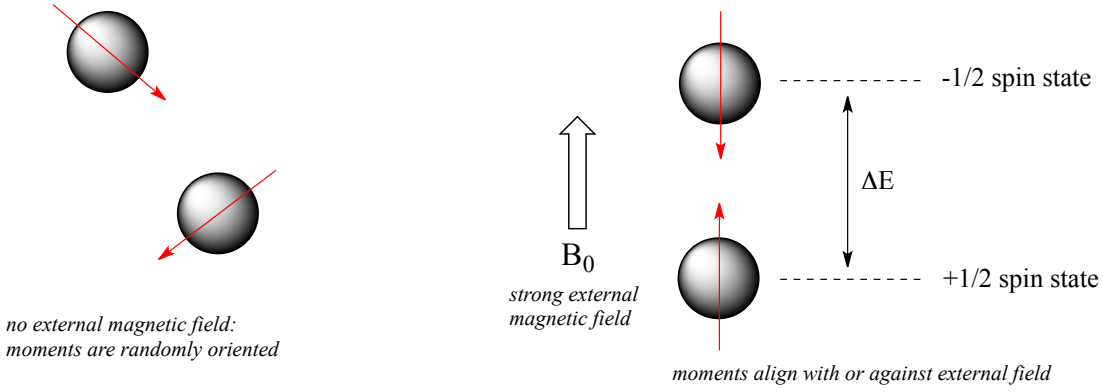
In practice it is ^{1}H and ^{13}C nuclei that are most commonly observed by NMR spectroscopy, and it is on those techniques that we will focus in this chapter, beginning with ^{1}H-NMR. The terms ‘proton’ and ‘hydrogen’ are used interchangeably when discussing because the ^{1}H nucleus is just a single proton.

Common nuclei in organic compounds

Magnetic nuclei	Nonmagnetic nuclei
^{1}H	^{12}C
^{2}H	^{16}O
^{13}C	^{32}S
^{14}N	
^{19}F	
^{31}P	

5.1B: Spin states and the magnetic transition

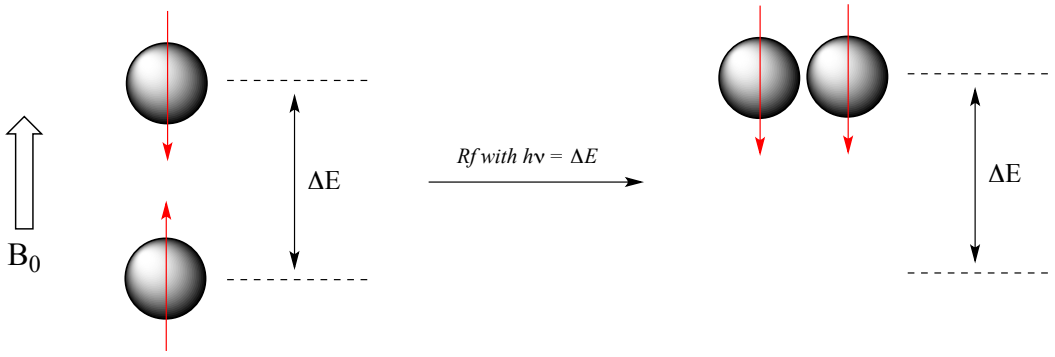
When a sample of an organic compound is sitting in a flask on a laboratory bench, the magnetic moments of all of its protons are randomly oriented. However, when the same sample is placed within the field of a very strong superconducting magnet (this field is referred to by NMR spectroscopists as the **applied field**, abbreviated **B_0**) each proton will assume one of two possible **quantum spin states**. In the +½ spin state, the proton's magnetic moment is aligned *with* the direction of B_0, while in the -½ spin state it is aligned *opposed to* the direction of B_0.



The +½ spin state is slightly lower in energy than the -½ state, and the energy gap between them, which we will call ΔE, depends upon the strength of B_0: a stronger applied field results in a larger ΔE. For a large population of organic molecules in an applied field, slightly *more* than half of the protons will occupy the lower energy +½ spin state, while slightly *less* than half will occupy the higher energy -½ spin state. It is this population difference (between the two spin states) that is exploited by NMR, and the difference increases with the strength of the applied magnetic field.

Think back for a second to the other two spectroscopic techniques we have learned about: IR and UV-Vis spectroscopy. Recall from section 4.2 that photons of electromagnetic

radiation of a given frequency correspond to energy (E) given by E = *h*ν, where *h* is Plank's constant and ν is the frequency in waves per second, or Hz. Recall also from section 4.3 that the energy gap between *vibrational* states corresponds to the energy associated with infrared radiation, and from section 4.4 that the energy gap between *electronic* states in conjugated π-bonding systems corresponds to the energy associated with light in the ultraviolet and visible regions of the electromagnetic spectrum. It turns out that the energy gap ΔE between the +½ and -½ *spin* states of an atomic nucleus corresponds to the energy associated with radiation in the radio frequency (Rf) region of the spectrum. ***If a population of protons in a strong applied magnetic field is subjected to radio waves of a frequency that corresponds to ΔE, those in the +½ spin state will absorb the radiation and use the energy to 'spin flip' up to the higher-energy -½ state.***

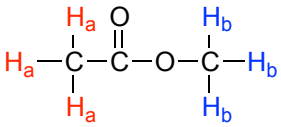


By detecting the frequency of Rf radiation that is absorbed, we can gain information about the chemical environment of protons in an organic molecule.

Exercise 5.1: In a general sense, how big is the energy gap for the nuclear spin transition observed in NMR compared to the energy gap for the vibrational transition observed in IR spectroscopy? Much bigger? Much smaller? Slightly bigger or smaller? About the same? How can you tell from the information presented in this section?

Section 5.2: Chemical equivalence

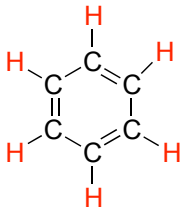
The frequency of radiation absorbed by a proton (or any other nucleus) during a spin transition in an NMR experiment is called its '**resonance frequency**'. If all protons in all organic molecules had the same resonance frequency, NMR spectroscopy but would not be terribly useful for chemists. Fortunately for us, however, resonance frequencies are not uniform for different protons in a molecule - rather, the resonance frequency varies according to the electronic environment that a given proton inhabits. In methyl acetate, for example, there are two distinct 'sets' of protons.



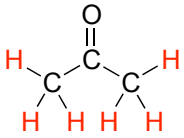
methyl acetate

The three methyl acetate protons labeled H_a above have a different resonance frequency compared to the three H_b protons, because the two sets of protons are in non-identical electronic environments: the H_a protons are on a carbon next to a carbonyl carbon, while the H_b protons or on a carbon next to the an oxygen. In the terminology of NMR, all three H_a protons are **chemically equivalent** to each other, as are all three H_b protons. The H_a protons are, however, **chemically nonequivalent** to the H_b protons. As a consequence, the resonance frequency of the H_a protons is different from that of the H_b protons. For now, do not worry about *why* the different electronic environment gives rise to different resonance frequencies - we will get to that soon.

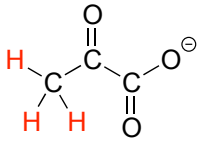
The ability to recognize chemical equivalancy and nonequivalency among atoms in a molecule will be central to understanding NMR. Each of the molecules below contains only one set of chemically equivalent protons: all six protons on benzene, for example, are equivalent to each other and have the same resonance frequency in an NMR experiment. Notice that any description of the bonding and position of one proton in benzene applies to all five other protons as well.



benzene

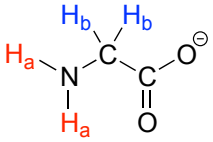


acetone

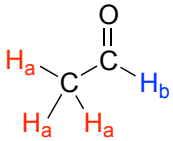


pyruvate

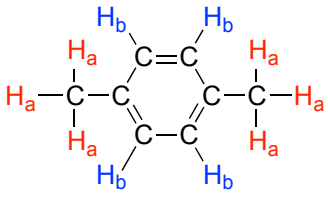
Each of the molecules in the next figure contains *two* sets of chemically equivalent protons, just like our previous example of methyl acetate, and again in each case the resonance frequency of the H_a protons will be different from that of the H_b protons.



glycine



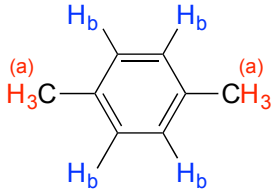
acetaldehyde



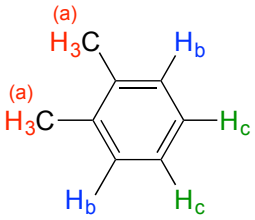
1,4-dimethylbenzene

Take acetaldehyde as an example: a description of the bonding and position of the H_b proton does *not* apply to the three H_a protons: H_b is bonded to an sp^2-hybridized carbonyl carbon while the H_a protons are bonded to an sp^3-hybridized methyl carbon.

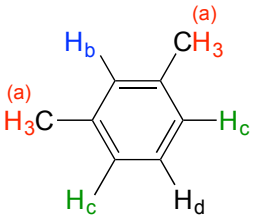
Note that while all four aromatic protons in 1,4-dimethylbenzene are chemically equivalent, its constitutional isomer 1,2 dimethylbenzene has *two* sets of aromatic protons in addition to the six methyl (H_a) protons. The 1,3-substituted isomer, on the other hand, has *three* sets of aromatic protons.



1,4-dimethylbenzene



1,2-dimethylbenzene

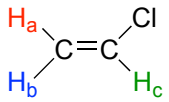


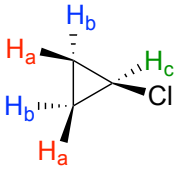
1,3-dimethylbenzene

In 1,2-dimethylbenzene, both H_b protons are adjacent to a methyl substituent, while both H_c protons are two carbons away. In 1,3-dimethylbenzene, H_b is situated between two methyl groups, the two H_c protons are one carbon away from a methyl group, and H_d is two carbons away from a methyl group.

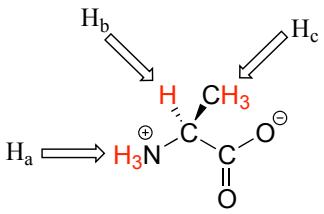
As you have probably already realized, chemical equivalence or non-equivalence in NMR is closely related to *symmetry*. Different planes of symmetry in the three isomers of dimethylbenzene lead to different patterns of equivalence.

Stereochemistry can play a part in determining equivalence or nonequivalence of nuclei in NMR. In the chloroethene (commonly known as vinyl chloride, the compound used to make polyvinyl chloride or PVC), H_a and H_b are in nonequivalent electronic environments, because H_a is *cis* to the chlorine atom while H_b is *trans*. Likewise, H_a and H_b in chlorocyclopropane are nonequivalent.

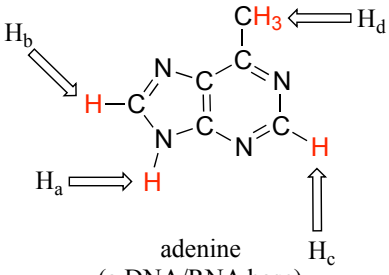




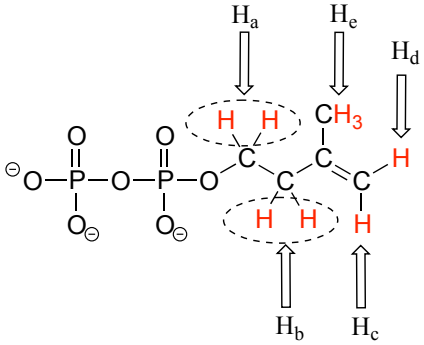
Most organic molecules have several sets of protons in different chemical environments, and each set will have a different resonance frequency in ^{1}H-NMR spectroscopy. Below we see some examples of multiple sets of protons in biological molecules.



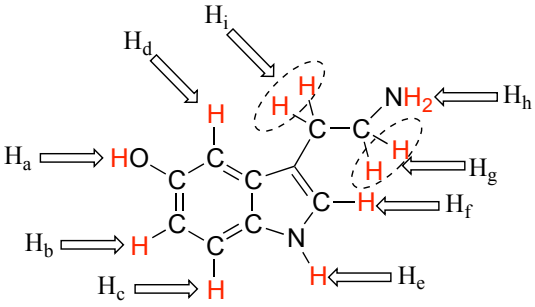
alanine
(an amino acid)



adenine
(a DNA/RNA base)

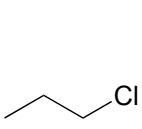


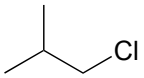
isopentenyl diphosphate
(a isoprenoid building block)

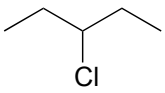


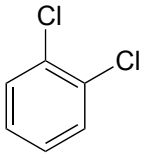
serotonin
(a neurotransmitter)

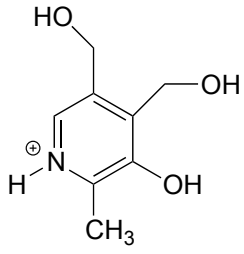
Exercise 5.2: How many sets of equivalent protons do the following molecules contain?



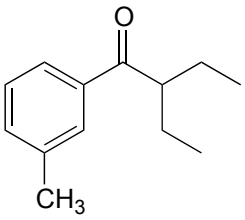




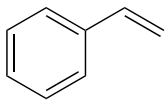




pyridoxine
(vitamin B_6)



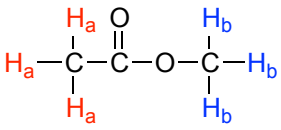
DEET
(an insect repellent)



styrene
(used to make polystyrene
in styrofoam)

Section 5.3: The ^{1}H-NMR experiment

In an NMR experiment, a sample compound (we'll again use methyl acetate as our example) is placed inside a very strong applied magnetic field (B_0) generated by a superconducting magnet in the instrument. (The magnetic fields generated by modern NMR instruments are strong enough that users must take care to avoid carrying any magnetics objects anywhere near them. They are also notorious for erasing the magnetic strips on credit cards!)



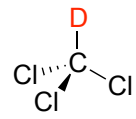
methyl acetate

At first, the magnetic moments of (slightly more than) half of the protons in the sample are aligned with B_0, and half are aligned against B_0. Then, the sample is exposed to a range of radio frequencies. Out of all of the frequencies which hit the sample, only two - the resonance frequencies for H_a and H_b - are absorbed, causing those protons which are aligned *with* B_0 to 'spin flip' so that they align themselves *against* B_0. When the 'flipped' protons flip back down to their ground state, they emit energy, again in the form of radio-frequency radiation. The NMR instrument detects and records the frequency and intensity of this radiation, making using of a mathematical technique known as a 'Fourier Transform'.

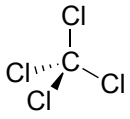
Note: the above description of an NMR experiment is an oversimplification, but is adequate for our purpose here. If you take a more advanced course in molecular spectroscopy you will learn about the process is much greater detail.

In most cases, a sample being analyzed by NMR is in solution. If we use a common laboratory solvent (diethyl ether, acetone, dichloromethane, ethanol, water, etc.) to dissolve our NMR sample, however, we run into a problem – there many more solvent protons in solution than there are sample protons, so the signals from the sample protons will be overwhelmed. To get around this problem, we use special NMR solvents in which all protons have been replaced by deuterium. Deuterium is NMR-active, but its resonance frequency is far outside of the range in which protons absorb, so it is `invisible` in ^{1}H-NMR. Some common NMR solvents are shown below.

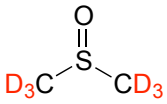
Common NMR solvents



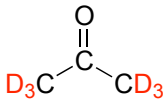
deuterated chloroform



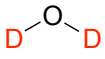
carbon tetrachloride



d_6-dimethylsulfoxide

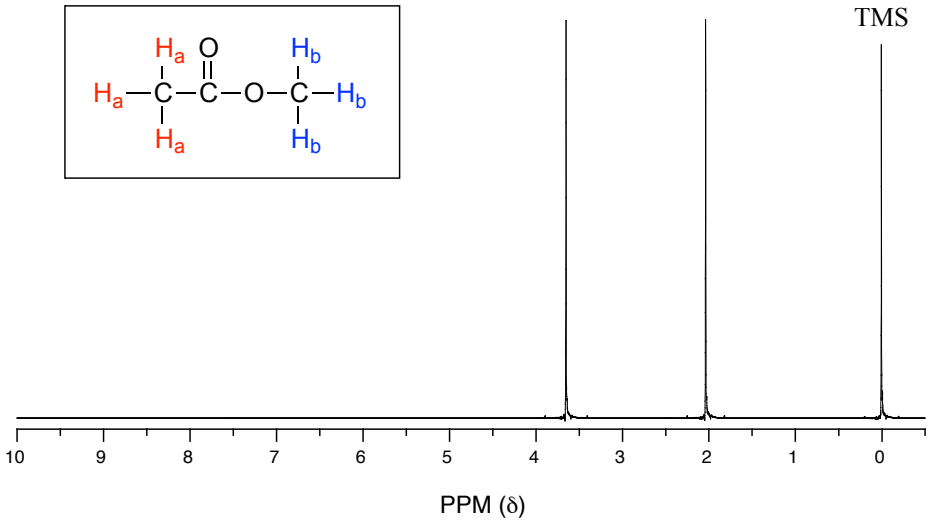


d_6-acetone



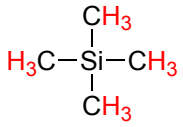
D_2O

Let's look at an actual ^{1}H-NMR spectrum for methyl acetate. Just as in IR and UV-vis spectroscopy, the vertical axis corresponds to intensity of absorbance, the horizontal axis to frequency. However, you will notice right away that a) there is no *y*-axis line or units drawn in the figure, and b) the *x*-axis units are not Hz, which you would expect for a frequency scale. Both of these mysteries will become clear very soon.



The ^{1}H-NMR spectrum of methyl acetate

We see three absorbance signals: two of these correspond to H_a and H_b (don't worry yet which is which), while the peak at the far right of the spectrum corresponds to the 12 chemically equivalent protons in tetramethylsilane (**TMS**), a standard reference compound that was added to our sample.



tetramethylsilane (TMS)

First, let's talk about the *x*-axis. The **'ppm'** label stands for 'parts per million', and simply tells us that the two sets of equivalent protons in our methyl acetate sample have resonance frequencies about 2.0 and 3.6 parts per million higher than the resonance frequency of the TMS protons, which we are using as our reference standard. This is referred to as their **chemical shift**.

The reason for using a relative value (chemical shift expressed in ppm) rather than the actual resonance frequency (expressed in Hz) is that every NMR instrument will have a different magnetic field strength, so the actual value of resonance frequencies expressed in Hz will be different on different instruments - remember that ΔE for the magnetic transition of a nucleus depends upon the strength of the externally applied magnetic field. However, the resonance frequency values *relative to the TMS standard* will always be the same, regardless of the strength of the applied field. For example, if the resonance frequency for the TMS protons in a given NMR instrument is exactly 300 MHz (300 million Hz), then a chemical shift of 2.0 ppm corresponds to an actual resonance frequency of 300,000,600 Hz (2 parts per million of 300 million is 600). In another instrument (with a stronger magnet) where the resonance frequency for TMS protons is 400 MHz, a chemical shift of 2.0 ppm corresponds to a resonance frequency of 400,000,800 Hz.

A frequently used symbolic designation for chemical shift in ppm is the lower-case greek letter δ (*delta*). Most protons in organic compounds have chemical shift values between 0 and 10 ppm relative to TMS, although values below 0 ppm and up to 12 ppm and above are occasionally observed. By convention, the left-hand side of an NMR spectrum (higher chemical shift) is called **downfield**, and the right-hand direction is called **upfield**.

In modern research-grade NMR instruments, it is no longer necessary to actually add TMS to the sample: the computer simply calculates where the TMS signal *should* be, based on resonance frequencies of the solvent. So, from now on you will not see a TMS peak on NMR spectra - but the 0 ppm point on the *x*-axis will always be defined as the resonance frequency of TMS protons.

A Chemical Shift Analogy

If you are having trouble understanding the concept of chemical shift and why it is used in NMR, try this analogy: imagine that you have a job where you travel frequently to various planets, each of which has a different gravitational field strength. Although your body mass remains constant, your measured weight is variable - the same scale may show that you weigh 60 kg on one planet, and 75 kg on another. You want to be able to keep track your body mass in a meaningful, reproducible way, so you choose an object to use as a standard: a heavy iron bar, for example. You record the weight of the iron bar and yourself on your home planet, and find that the iron bar weighs 50 kg and you weigh 60 kg. You

are 20 percent (or pph, parts per hundred) heavier than the bar. The next day you travel (with the iron bar in your suitcase) to another planet and find that the bar weighs 62.5 kg, and you weigh 75 kg. Although your measured weight is different, you are still 20% heavier than the bar: you have a 'weight shift' of 20 pph relative to the iron bar, no matter what planet you are on.

Exercise 5.3:

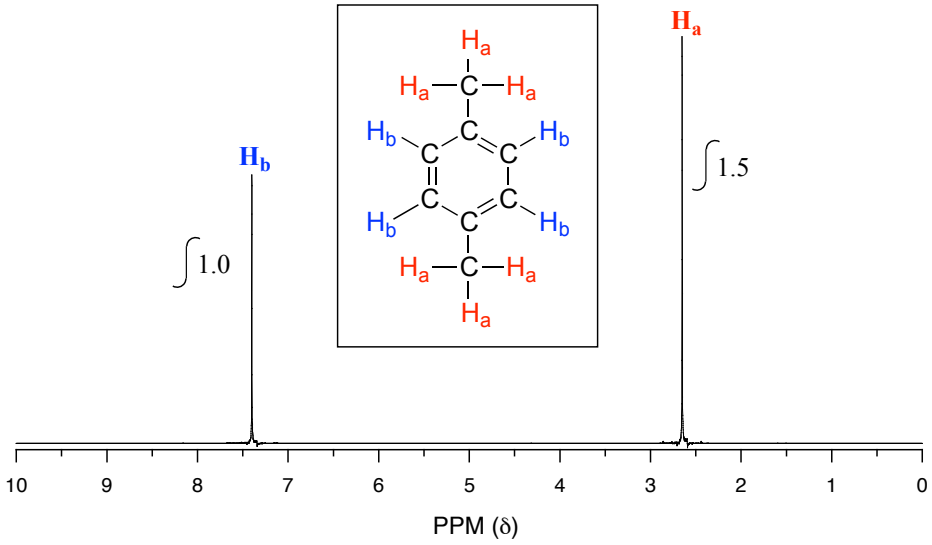
a) What is the *actual resonance frequency* (in Hz) of proton signals at 2.0 ppm and 3.6 ppm for an NMR instrument in which the TMS protons have a resonance frequency of 500 MHz?

b) What is the *chemical shift* of these two protons in that same instrument, expressed in Hz (*not* in ppm)?

We have already pointed out that, on our spectrum of methyl acetate, there is there is no *y*-axis scale indicated. With *y*-axis data it is *relative* values, rather than absolute values, that are important in NMR. The computer in an NMR instrument can be instructed to mathematically integrate the area under a signal or group of signals. The **signal integration** process is very useful, because *in ^{1}H-NMR spectroscopy the area under a signal is proportional to the number of protons to which the signal corresponds*. When we instruct the computer to integrate the areas under the H_a and H_b signals in our methyl acetate spectrum, we find that they have approximately the same area. This makes sense, because each signal corresponds to a set of *three* equivalent protons.

Be careful not to assume that you can correlate apparent peak *height* to number of protons - depending on the spectrum, relative peak heights will not always be the same as relative peak *areas*, and it is the relative areas that are meaningful. Because it is difficult to compare relative peak area by eye, we rely on the instrument's computer to do the calculations.

Take a look next at the spectrum of 1,4-dimethylbenzene:



As we discussed earlier, this molecule has two sets of equivalent protons: the six methyl (H_a) protons and the four aromatic (H_b) protons. When we instruct the instrument to integrate the areas under the two signals, we find that the area under the peak at 2.6 ppm is 1.5 times greater than the area under the peak at 7.4 ppm. The ratio 1.5 to 1 is of course the same as the ratio 6 to 4. This integration information (along with the actual chemical shift values, which we'll discuss soon) tells us that the peak at 7.4 ppm must correspond to H_b, and the peak at 2.6 ppm to H_a.

The integration function can also be used to determine the relative amounts of two or more compounds in a *mixed* sample. If we take a ^{1}H-NMR spectrum of a sample that is a equimolar mixture of benzene and acetone, for example, we will see two signals, one for the six equivalent acetone protons and one for the six equivalent benzene protons. The integrated area under the acetone signal will be the same as the area under the benzene sample, because both signals represent six protons. If we have an equimolar mixture of acetone and cyclopentane, on the other hand, the ratio of the acetone peak area to the cylopentane peak area will be 3:5 (or 6:10), because the cyclopentane signal represents ten protons.

Exercise 5.4: You take a ^{1}H-NMR spectrum of a mixed sample of acetone and dichloromethane. The integral ratio of the two signals (acetone : dichloromethane) is 2.3 to 1. What is the molar ratio of the two compounds in the sample?

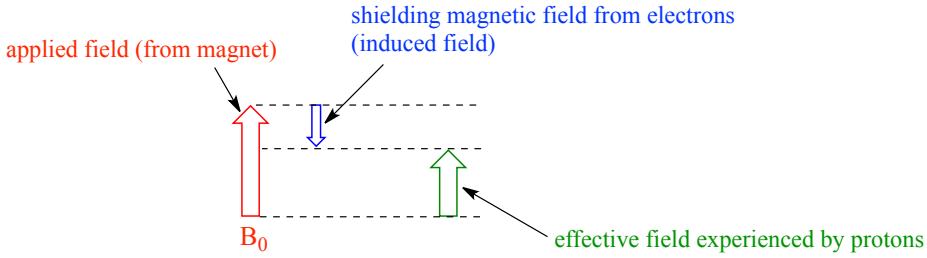
Exercise 5.5: You take the ^{1}H-NMR spectrum of a mixed sample of 36% 1,4-dimethylbenzene and 64% acetone (these are mole percentages). What is the expected integration ratio of the signals that you would observe? Order the ratio from highest to lowest numbers.

Section 5.4: The basis for differences in chemical shift

5.4A: Diamagnetic shielding and deshielding

We come now to the question of *why* nonequivalent protons have different resonance frequencies and thus different chemical shifts. The chemical shift of a given proton is determined primarily by interactions with the nearby electrons. The most important thing to understand is that *when electrons are subjected to an external magnetic field, they form their own small* ***induced magnetic fields*** *in opposition to the external field.*

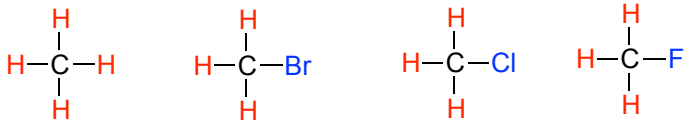
Consider the methane molecule (CH_4) in which the four equivalent protons have a chemical shift of 0.23 ppm (this is a value we can look up in any chemistry reference source). The valence electrons around the methyl carbon, when subjected to B_0, generate their own very small induced magnetic field that opposes B_0. This induced field, to a small but significant degree, *shields* the nearby protons from experiencing the full force of B_0, an effect known as **local diamagnetic shielding**. In other words, the methane protons do not quite experience the full force of B_0 - what they experience is called $\mathbf{B_{eff}}$, or the **effective field**, which is slightly *weaker* than B_0 due to the influence of the nearby electrons.



Because B_{eff} is slightly weaker than B_0, the resonance frequency (and thus the chemical shift) of the methane proton is slightly lower than what it would be if it did not have electrons nearby and was feeling the full force of B_0. (You should note that the figure above is not to scale: the applied field is generated by a superconducting magnet and is *extremely* strong, while the opposing induced field from the electrons is comparatively very small.)

Now consider methyl fluoride, CH_3F, in which the protons have a chemical shift of 4.26 ppm, significantly higher than that of methane. This is caused by something called the **deshielding effect**. Recall that fluorine is very electronegative: it pulls electrons towards itself, effectively *decreasing* the electron density around each of the protons. For the protons, being in a lower electron density environment means less diamagnetic shielding, which in turn means a greater overall exposure to B_0, a stronger B_{eff}, and a higher resonance frequency. Put another way, the fluorine, by pulling electron density away from the protons, is *deshielding* them, leaving them more exposed to B_0. As the electronegativity of the substituent increases, so does the extent of deshielding, and so does the chemical shift. This is evident when we look at the chemical shifts of methane

and three halomethane compounds (remember that electronegativity increases as we move up a column in the periodic table, so flourine is the most electronegative and bromine the least).

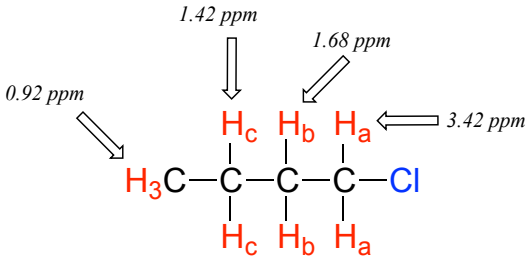


0.23 ppm 2.68 ppm 3.05 ppm 4.26 ppm

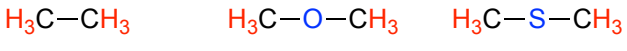
To a large extent, then, we can predict trends in chemical shift by considering how much deshielding is taking place near a proton. The chemical shift of trichloromethane is, as expected, higher than that of dichloromethane, which is in turn higher than that of chloromethane.

0.23 ppm 3.05 ppm 5.30 ppm 7.27 ppm

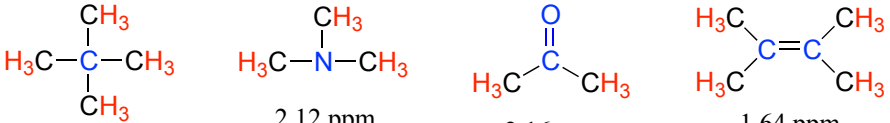
The deshielding effect of an electronegative substituent diminishes sharply with increasing distance:



The presence of an electronegative oxygen, nitrogen, sulfur, or sp^2-hybridized carbon also tends to shift the NMR signals of nearby protons slightly downfield:

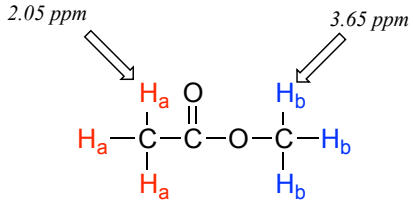


0.87 ppm 3.42 ppm 2.12 ppm



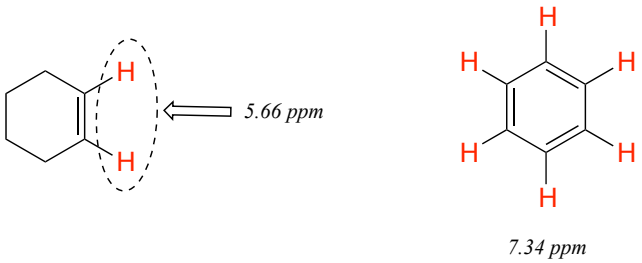
0.90 ppm 2.12 ppm 2.16 ppm 1.64 ppm

Now we can finally assign the two peaks in the the ^{1}H-NMR spectrum of methyl acetate. We can predict that the methyl ester protons (H_b), which are deshielded by the adjacent oxygen atom, will have a higher chemical shift than the acetate protons (H_a), which are deshielded to a lesser extent by the carbonyl group. Therefore, the signal at 3.7 must correspond to H_b, and the signal at 2.0 to H_a.



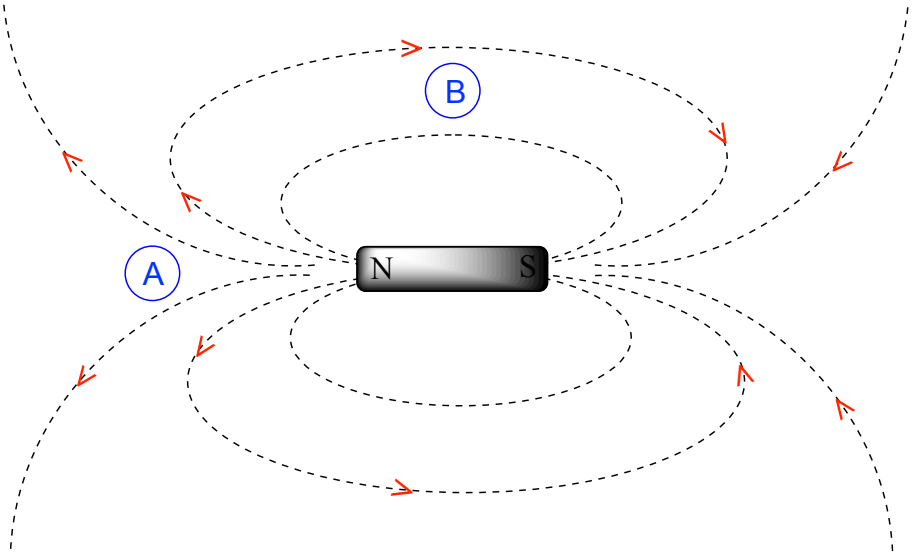
5.4B: Diamagnetic anisotropy

Vinylic protons (those directly bonded to an alkene carbon) and aromatic protons resonate much further downfield than can be accounted for simply by the deshielding effect of nearby electronegative atoms. Note the chemical shifts of the vinylic and aromatic protons in cyclohexene and benzene:



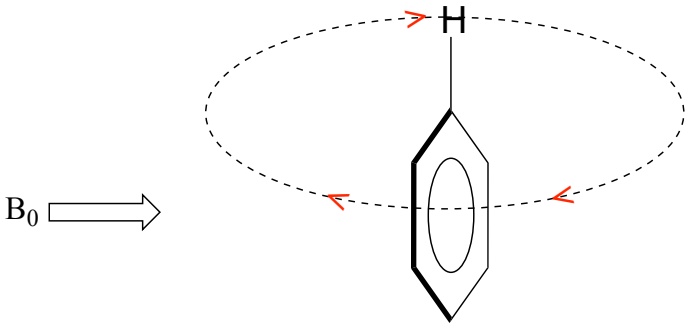
We'll consider the aromatic proton first. Recall that in benzene and many other aromatic structures, a sextet of π electrons is delocalized around the ring. When the molecule is exposed to B_0, these π electrons begin to circulate in a **ring current**, generating their own induced magnetic field that opposes B_0. In this case, however, the induced field of the π electrons does not shield the aromatic protons from B_0 as you might expect– rather, it causes the protons to experience a *stronger* magnetic field in the direction of B_0 – in other words, it *adds* to B_0 rather than subtracting from it.

To understand how this happens, we need to understand the concept of **diamagnetic anisotropy** (anisotropy means `non-uniformity`). So far, we have been picturing magnetic fields as being oriented in a uniform direction. This is only true over a small area. If we step back and take a wider view, however, we see that the lines of force in a magnetic field are actually anisotropic. They start in the 'north' direction, then loop around like a snake biting its own tail.



If we are at point A in the figure above, we feel a magnetic field pointing in a northerly direction. If we are at point B, however, we feel a field pointing to the south.

In the induced field generated by the aromatic ring current, the aromatic protons are at the equivalent of 'point B' – this means that the induced current in this region of space is oriented in the *same* direction as B_0, so it *adds* to B_0 rather than subtracting from it.



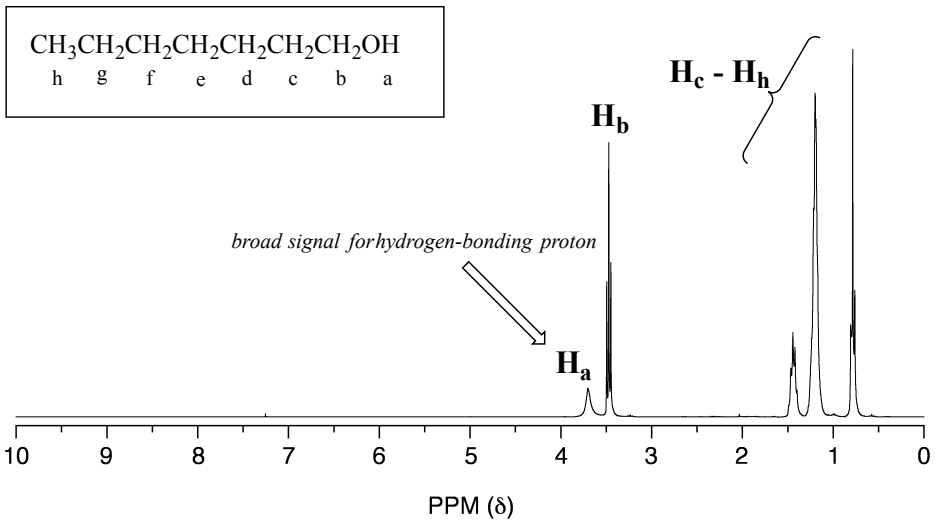
The end result is that aromatic protons, due to the anisotropy of the induced field generated by the π ring current, appear to be highly deshielded. Their chemical shift is far downfield, in the 6.5-8 ppm region.

Diamagnetic anisotropy is also responsible for the downfield chemical shifts of vinylic protons (4.5-6.5 ppm) and aldehyde protons (9-10 ppm). These groups are not aromatic and thus do not generate ring currents does benzene, but the π electrons circulate in such a way as to generate a magnetic field that *adds* to B_0 in the regions of space occupied by the protons. Carboxylic acid protons are very far downfield (10-12 ppm) due to the combined influence of the electronegative oxygen atom and the nearby π bond.

5.4C: Hydrogen bonded protons

Protons that are directly bonded to oxygen or nitrogen have chemical shifts that can vary widely depending on solvent and concentration. These protons can participate to varying degrees in hydrogen bonding interactions, and the degree hydrogen bonding greatly influences the electron density around the proton - and thus the chemical shift. Signals for hydrogen bonding protons also tend to be *broader* than those of hydrogens bonded to carbon, a phenomenon that is also due to hydrogen bonding.

Alcohol protons, for example, will usually show broad signals anywhere between 1-5 ppm. The signal for H_a in the spectrum of 1-heptanol is a typical example of a broadened NMR signal for an alcohol proton.

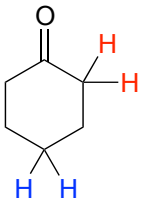


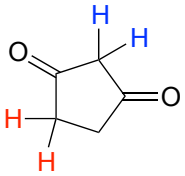
The table below provides a summary of approximate chemical shift ranges for protons in different bonding arrangements. A more detailed table can be found in the appendix.

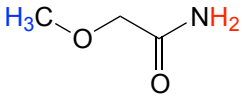
Typical chemical shift ranges in ^{1}H-NMR

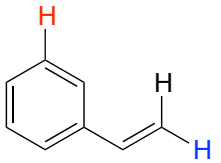
type of proton	**chemical shift range (ppm)**
bonded to sp^3 carbon	0.5 - 4
bonded to N (amine)	1 - 3
bonded to O (alcohol)	1 - 5
alkene/ vinylic	3.5 - 6.5
terminal alkyne	2 - 3
bonded to N (amide)	5 - 9
aromatic	6 - 9
aldehyde	9.5 - 10
carboxylic acid	10 - 13

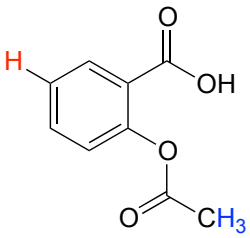
Exercise 5.6: For each pair of protons colored red and blue in the structures below, state which is expected to have the higher chemical shift in ^{1}H-NMR. For some of these it will be helpful to consult Table X in the appendix.



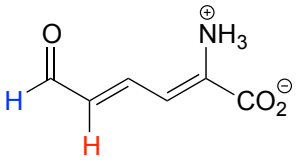




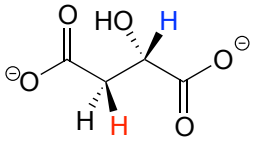




acetylsalicyclic acid
(aspirin)

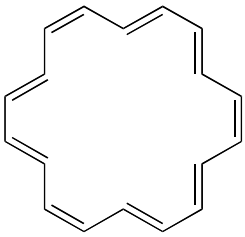


intermediate in tryptophan degradation



malate
(citric acid cycle intermediate)

Exercise 5.7: The ^{1}H-NMR spectrum of the aromatic compound [18] annulene has two peaks, at 8.9 ppm and -1.8 ppm (a *negative* chemical shift, upfield of TMS!) with an integration ratio of 2:1. Explain the unusual chemical shift of the latter peak.

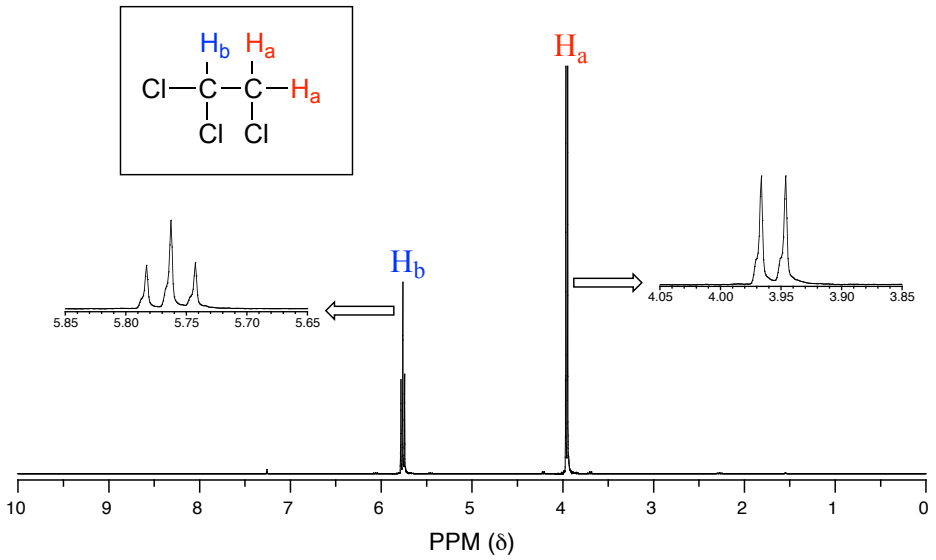


[18] annulene

Section 5.5: Spin-spin coupling

The ^{1}H-NMR spectra that we have seen so far (of methyl acetate and 1,4-dimethylbenzene) are somewhat unusual in the sense that in both of these molecules, each set of protons generates a single NMR signal. In fact, the ^{1}H-NMR spectra of most organic molecules contain proton signals that are 'split' into two or more sub-peaks. Rather than being a complication, however, this splitting behavior is actually very useful because it provides us with more information about our sample molecule.

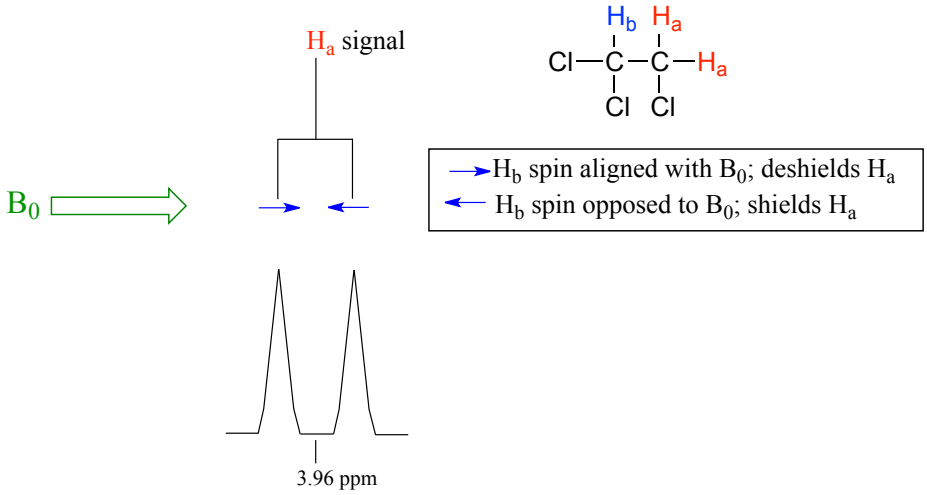
Consider the spectrum for 1,1,2-trichloroethane. In this and in many spectra to follow, we show enlargements of individual signals so that the signal splitting patterns are recognizable.



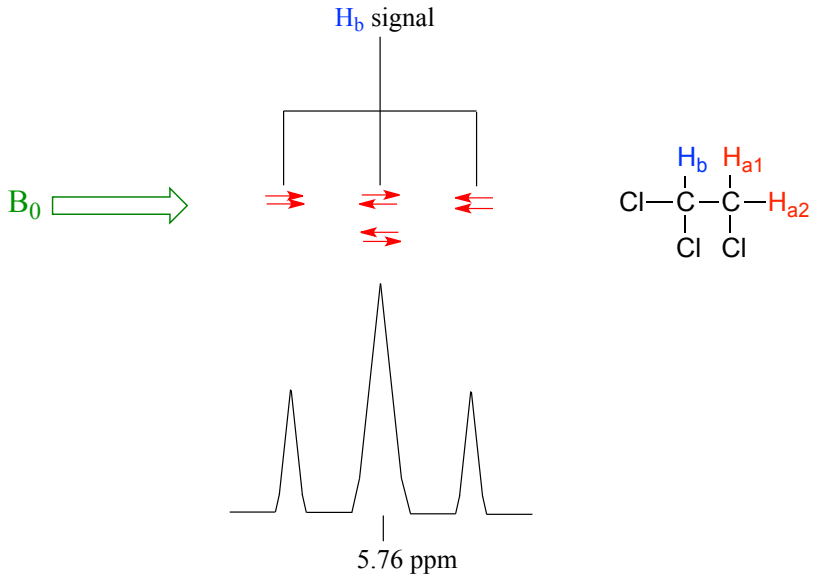
The signal at 3.96 ppm, corresponding to the two H_a protons, is split into two subpeaks of equal height (and area) – this is referred to as a **doublet**. The H_b signal at 5.76 ppm, on the other hand, is split into three sub-peaks, with the middle peak higher than the two

outside peaks - if we were to integrate each subpeak, we would see that the area under the middle peak is twice that of each of the outside peaks. This is called a **triplet**.

The source of signal splitting is a phenomenon called **spin-spin coupling**, a term that describes the magnetic interactions between neighboring, non-equivalent NMR-active nuclei. (The terms 'splitting' and 'coupling' are often used interchangeably when discussing NMR.) In our 1,1,2 trichloromethane example, the H_a and H_b protons are spin-coupled to each other. Here's how it works, looking first at the H_a signal: in addition to being shielded by nearby valence electrons, each of the H_a protons is also influenced by the small magnetic field generated by H_b next door (remember, each spinning proton is like a tiny magnet). The magnetic moment of H_b will be aligned *with* B_0 in slightly more than half of the molecules in the sample, while in the remaining molecules it will be opposed to B_0. The B_{eff} 'felt' by H_a is a slightly weaker if H_b is aligned against B_0, or slightly stronger if H_b is aligned with B_0. In other words, in half of the molecules H_a is *shielded* by H_b (thus the NMR signal is shifted slightly upfield) and in the other half H_a is *deshielded* by H_b (and the NMR signal shifted slightly downfield). What would otherwise be a single H_a peak has been split into two sub-peaks (a doublet), one upfield and one downfield of the original signal. These ideas an be illustrated by a **splitting diagram**, as shown below.

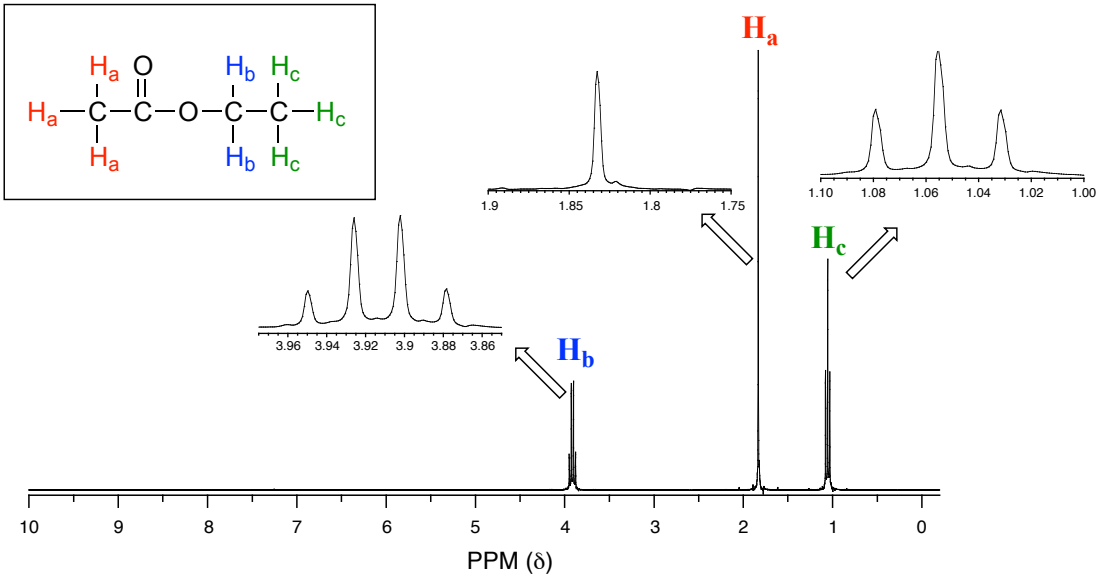


Now, let's think about the H_b signal. The magnetic environment experienced by H_b is influenced by the fields of both neighboring H_a protons, which we will call H_{a1} and H_{a2}. There are four possibilities here, each of which is equally probable. First, the magnetic fields of both H_{a1} and H_{a2} could be aligned with B_0, which would deshield H_b, shifting its NMR signal slightly downfield. Second, both the H_{a1} and H_{a2} magnetic fields could be aligned opposed to B_0, which would shield H_b, shifting its resonance signal slightly upfield. Third and fourth, H_{a1} could be with B_0 and H_{a2} opposed, or H_{a1} opposed to B_0 and H_{a2} with B_0. In each of the last two cases, the shielding effect of one H_a proton would cancel the deshielding effect of the other, and the chemical shift of H_b would be unchanged.



So in the end, the signal for H_b is a **triplet**, with the middle peak twice as large as the two outer peaks because there are *two* ways that H_{a1} and H_{a2} can cancel each other out.

Consider the spectrum for ethyl acetate:



We see an unsplit **'singlet'** peak at 1.83 ppm that corresponds to the acetyl (H_a) protons – this is similar to the signal for the acetate protons in methyl acetate that we considered earlier. This signal is unsplit because there are no adjacent protons on the molecule. The signal at 1.06 ppm for the H_c protons is split into a triplet by the two H_b protons next door. The explanation here is the same as the explanation for the triplet peak we saw previously for 1,1,2-trichloroethane.

The H_b protons give rise to a **quartet** signal at 3.92 ppm – notice that the two middle peaks are taller then the two outside peaks. This splitting pattern results from the spin-coupling effect of the *three* adjacent H_c protons, and can be explained by an analysis similar to that which we used to explain the doublet and triplet patterns.

Exercise 5.8:

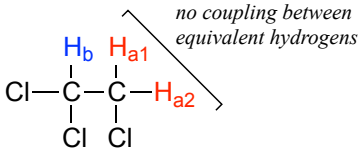
a) Explain, using a diagram similar to those in figures 21 and 22, the possible combinations of nuclear spin states for the H_c protons in ethyl acatate, and why the H_b signal is split into a quartet.

b) The integration ratio of the two 'sub-peaks' in a doublet is 1:1, and in triplets it is 1:2:1. What is the integration ratio of the H_b quartet in ethyl acetate? (Hint – use the illustration that you drew in part a to answer this question.)

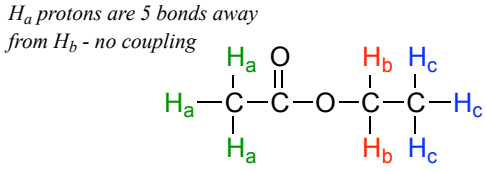
By now, you probably have recognized the pattern which is usually referred to as the ***n* + 1 rule**: if a set of protons has *n* neighboring, non-equivalent protons, it will be split into *n* + 1 subpeaks. Thus the two H_b protons in ethyl acetate split the H_c signal into a triplet, and the three H_c protons split the H_b signal into a quartet. H_a, with zero neighboring protons, is a singlet. This is very useful information if we are trying to determine the structure of an unknown molecule: if we see a triplet signal, we know that the corresponding proton or set of protons has two `neighbors`. When we begin to determine structures of unknown compounds using ^{1}H-NMR spectral data, it will become more apparent how this kind of information can be used.

Four important points need to be emphasized at this point.

First, signal splitting only occurs between non-equivalent protons – in other words, H_{a1} in 1,1,2-trichloroethane is *not* split by H_{a2}, and vice-versa.



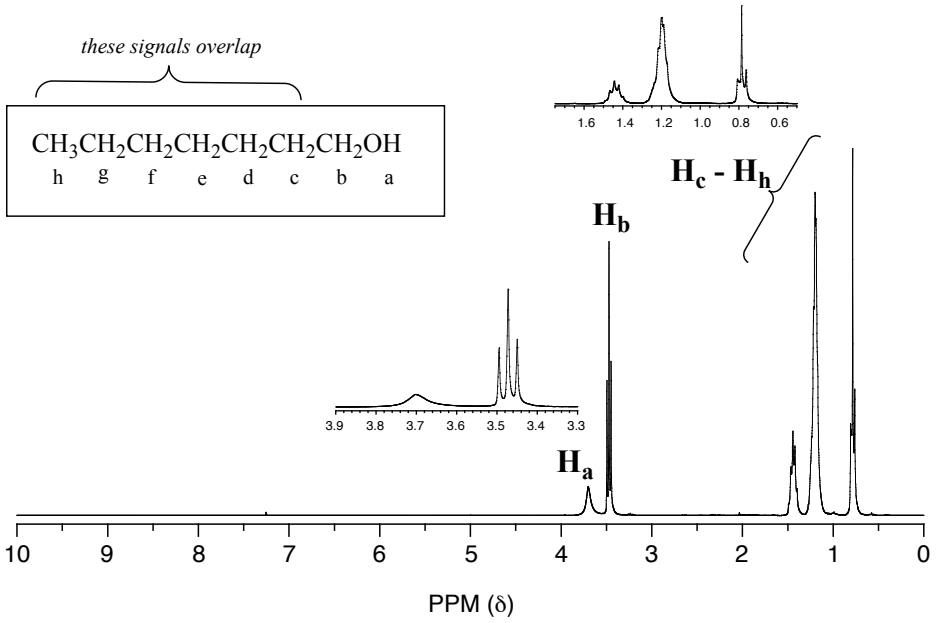
Second, splitting occurs primarily between protons that are separated by three or fewer bonds. This is why the H_a protons in ethyl acetate form a singlet– the nearest proton neighbors are five bonds away, too far for coupling to occur.



With more sensitive instruments we will sometimes see 4-bond and even 5-bond splitting, but in our treatment of NMR, for the sake of simplicity we will always assume that only three-bond splitting is seen.

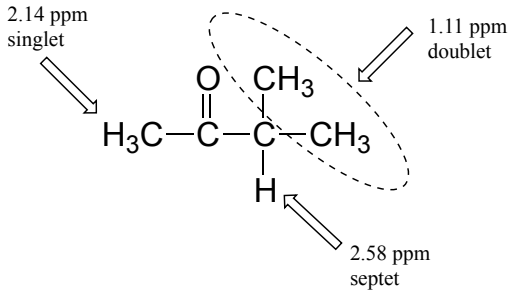
Third, protons that are bonded to oxygen or nitrogen generally do not split - and are not split by - adjacent protons. OH and NH protons are acidic enough to rapidly exchange between different molecules, so the neighboring protons never actually 'feels' their influence.

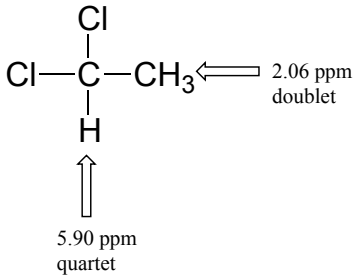
The spectrum of 1-heptanol has a characteristically broad alcohol proton signal at 3.7 ppm (labeled H_a below).

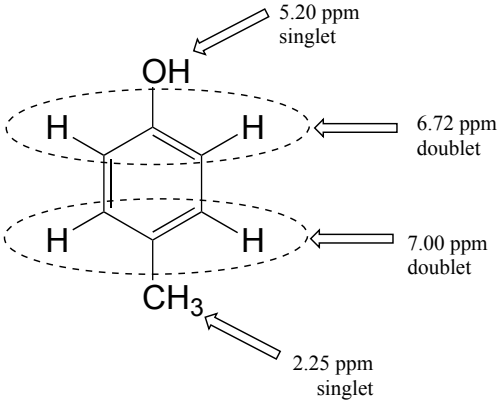


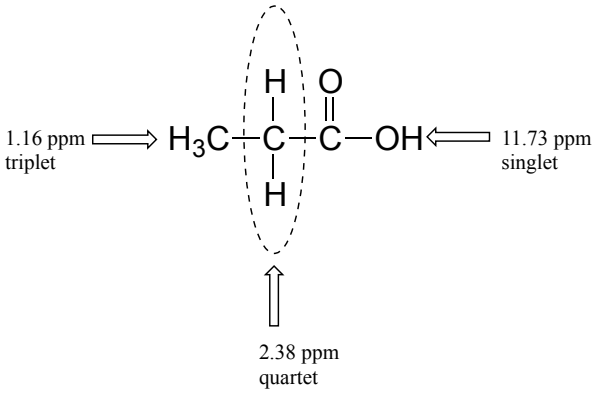
Notice in this spectrum that H_b is a triplet, coupled to the two H_c protons but *not* coupled to H_a. The signals corresponding to H_c through H_h are complex due to overlapping - when this happens (as it often does!), detailed analysis becomes more challenging.

Below are a few more examples of chemical shift and splitting pattern information for some relatively simple organic molecules.

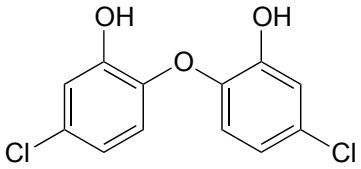






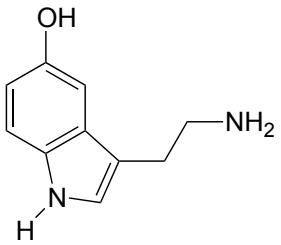


Exercise 5.9: How many proton signals would you expect to see in the ^{1}H-NMR spectrum of triclosan (a common antimicrobial agent in soap)? For each of the proton signals, predict the splitting pattern, assuming that you can see only 3-bond splitting.



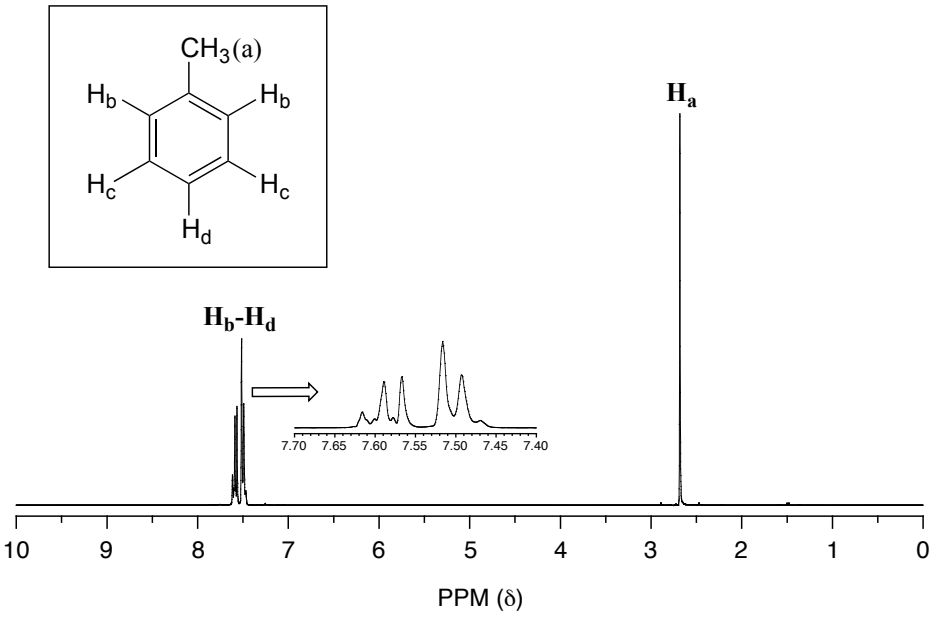
Triclosan

Exercise 5.10: How many proton signals would you expect to see in the ^{1}H-NMR spectrum of the neurotransmitter serotonin? For each of the proton signals, predict the splitting pattern, again assuming only 3-bond splitting.



serotonin

In an ideal world, all NMR spectra would be as easy to interpret as those we have seen so far: every peak would be separated from the others, the peak integration would be obvious, and the multiplicity (singlet, doublet, etc.) would be easy to recognize. The real world, unfortunately, is not always so pretty: peaks with similar chemical shifts overlap, making interpretation much more difficult. We have already seen this is the spectrum of 1-heptanol above. In the spectrum of methylbenzene, we would expect the signal for H_a to be a singlet, H_b to be a doublet, and H_c and H_d to be triplets. Looking at relative integration values for the four peaks, we would expect to see a 3:2:2:1 ratio.

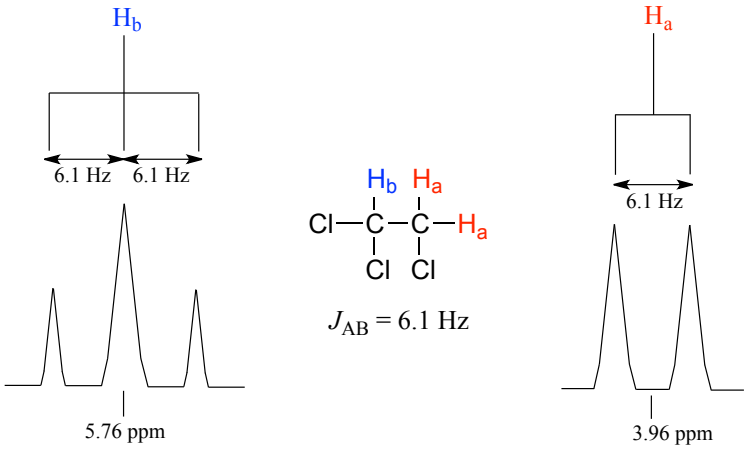


In practice, however, the three aromatic proton sets H_b, H_c and H_d have very similar chemical shifts so their signals overlap substantially, and we cannot recognize doublet or triplet splitting patterns. In this case, we would refer to the aromatic part of the spectrum as a **multiplet**. We can report the integration ratio of the H_a peak compared to the combined aromatic peaks as 3 to 5, or the equivalent 1 to 1.67.

The magnitude of spin-spin coupling can be expressed using the **coupling constant**, abbreviated with the capital letter J. The coupling constant is simply the difference,

expressed in Hz, between two adjacent sub-peaks in a split signal, and is a measure of the extent to which one nucleus 'feels' the magnetic dipole of its neighbor.

For our doublet in the 1,1,2-trichloroethane spectrum, for example, the two subpeaks are separated by 6.1 Hz, and thus we write $^{3}J_{a\text{-}b} = 6.1$ Hz.

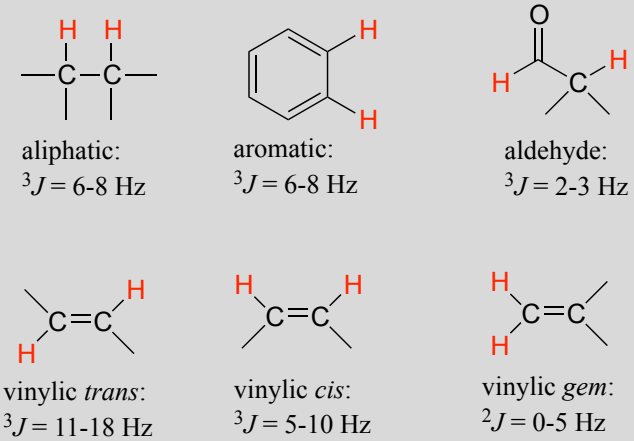


The superscript '3' tells us that this is a three-bond coupling interaction, and the 'a-b' subscript tells us that we are talking about coupling between H_a and H_b. Unlike the chemical shift value, ***the coupling constant, expressed in Hz, is the same regardless of the applied field strength of the NMR magnet***. The strength of the magnetic moment of a neighboring proton, which is the source of the spin-spin coupling phenomenon, does *not* depend on the applied field strength. For this reason, coupling constants are normally given in Hz, *not* ppm.

When we look closely at the triplet signal in 1,1,2-trichloroethane, we see that the coupling constant - the 'gap' between subpeaks - is 6.1 Hz, the same as for the doublet. The coupling constant $^{3}J_{a\text{-}b}$ quantifies the magnetic interaction between the H_a and H_b hydrogen sets, and *this interaction is of the same magnitude in either direction*. In other words, spin-spin coupling is *reciprocal*: H_a influences H_b to the same extent that H_b influences H_a.

Coupling constants between proton sets on neighboring sp^3-hybridized carbons is typically in the region of 6-8 Hz. Coupling constants for neighboring vinylic protons can range from 0 Hz (no coupling at all) to 18 Hz, depending on the bonding arrangement.

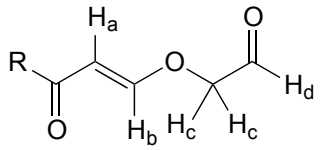
Typical proton-proton coupling constants



For vinylic protons in a *trans* configuration, we see coupling constants in the range of ^3J = 11-18 Hz, while *cis* protons couple in the ^3J = 5-10 Hz range. The 2-bond coupling between protons on the same alkene carbon (referred to as *geminal* protons) is very fine, generally 5 Hz or lower.

Fine coupling (2-3 Hz) is often seen between an aldehyde proton and a three-bond neighbor.

Exercise 5.11: Give the expected splitting patterns and approximate coupling constants for the labeled protons in the compound below.



Presenting NMR data in table format

Information from NMR can be recorded conveniently in a condensed form without having to reproduce the actual spectrum. For example, the information from the ^{1}H-NMR specta of ethyl acetate and methylbenzene (see earlier figures) can be presented in tabular format, listing the chemical shift, the peak splitting pattern, and the relative area under peaks (usually, the smallest peak is set to 1). Coupling constant information is not shown in the example tables below.

^{1}H-NMR spectrum of ethyl acetate:

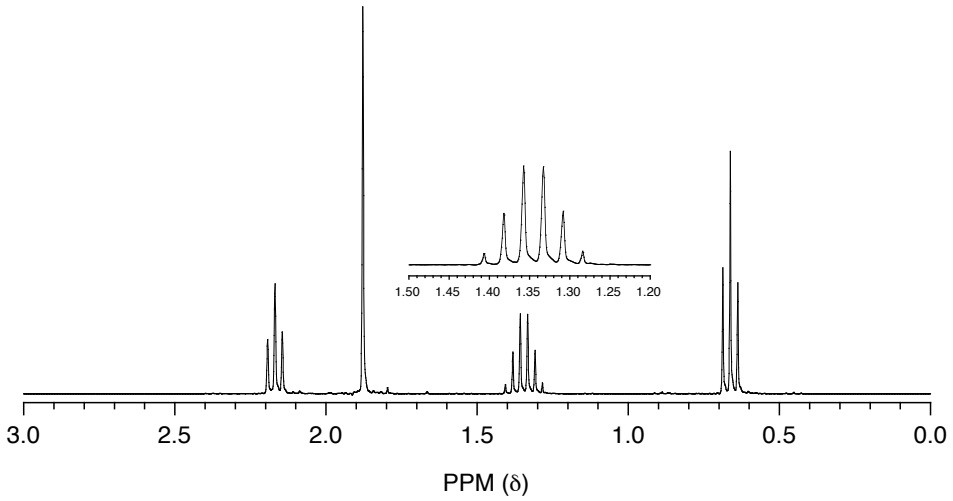
δ (ppm)	splitting	integration
3.92	q	1
1.83	s	1.5
1.06	t	1.5

^{1}H-NMR spectrum of methylbenzene:

δ (ppm)	splitting	integration
7.45-7.63	m	1.67
2.64	s	1

(abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet)

Exercise 5.12: Match the ^{1}H-NMR spectrum below to its corresponding compound, and assign all of the signals.

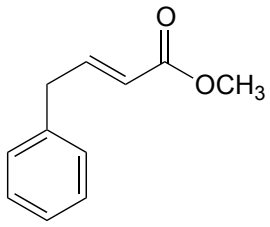


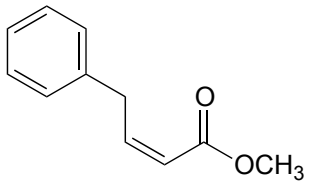
a) cyclopentanone b) 3-pentanone c) butaldehyde
d) 2-pentanone e) 4-heptanone f) 1-butene

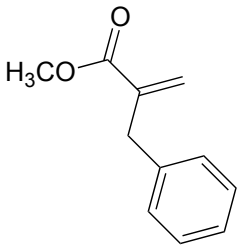
Exercise 5.13:

a) Which of the compounds in the previous exercise is expected to have an ^{1}H-NMR spectrum consisting of two triplets and a sextet?
b) Which would have a spectrum consisting of a triplet and a pentet?
c) Which would have a spectrum that includes a signal above 8 ppm?

Exercise 5.14: Explain how you could use coupling constants in ^{1}H-NMR spectra to distinguish between the three isomers below: (hint - you will need to consider stereochemistry).







Section 5.6: ^{13}C-NMR spectroscopy

The ^{12}C isotope of carbon - which accounts for up about 99% of the carbons in organic molecules - does not have a nuclear magnetic moment, and thus is NMR-inactive. Fortunately for organic chemists, however, the ^{13}C isotope, which accounts for most of the remaining 1% of carbon atoms in nature, has a magnetic dipole moment just like protons. Most of what we have learned about ^{1}H-NMR spectroscopy also applies to ^{13}C-NMR, although there are several important differences.

The magnetic moment of a ^{13}C nucleus is much weaker than that of a proton, meaning that NMR signals from ^{13}C nuclei are inherently much weaker than proton signals. This, combined with the low natural abundance of ^{13}C, means that it is much more difficult to observe carbon signals: more sample is required, and often the data from hundreds of scans must be averaged in order to bring the signal-to-noise ratio down to acceptable levels. Unlike ^{1}H-NMR signals, the area under a ^{13}C-NMR signal cannot easily be used to determine the number of carbons to which it corresponds. The signals for some types of carbons are inherently weaker than for other types – peaks corresponding to carbonyl carbons, for example, are much smaller than those for methyl or methylene (CH_2) peaks. For this reason, peak integration is generally not useful in ^{13}C-NMR spectroscopy.

The resonance frequencies of ^{13}C nuclei are lower than those of protons in the same applied field - in an instrument with a 7.05 Tesla magnet, protons resonate at about 300 MHz, while carbons resonate at about 75 MHz. This is fortunate, as it allows us to look at ^{13}C signals using a completely separate 'window' of radio frequencies. Just like in ^{1}H-

NMR, the standard used in ^{13}C-NMR experiments to define the 0 ppm point is tetramethylsilane (TMS), although of course in ^{13}C-NMR it is the signal from the four equivalent *carbons* in TMS that serves as the standard. Chemical shifts for ^{13}C nuclei in organic molecules are spread out over a much wider range than for protons – up to 200 ppm for ^{13}C compared to 10-12 ppm for protons (see Table 3 for a list of typical ^{13}C-NMR chemical shifts).

The chemical shift of a ^{13}C nucleus is influenced by essentially the same factors that influence a proton's chemical shift: bonds to electronegative atoms and diamagnetic anisotropy effects tend to shift signals downfield (higher resonance frequency). In addition, sp^2 hybridization results in a large downfield shift. The ^{13}C-NMR signals for carbonyl carbons are generally the furthest downfield (170-220 ppm), due to both sp^2 hybridization and to the double bond to oxygen.

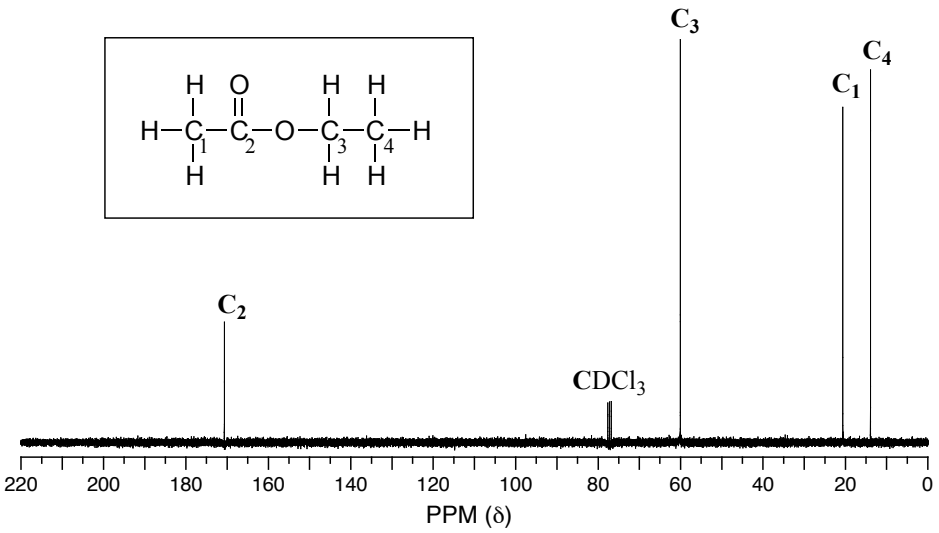
Exercise 5.15: How many sets of non-equivalent carbons are there in each of the molecules shown in exercise 5.2?

Exercise 5.16: How many sets of non-equivalent carbons are there in:

a) methylbenzene
b) 2-pentanone
c) 1,4-dimethylbenzene
d) triclosan

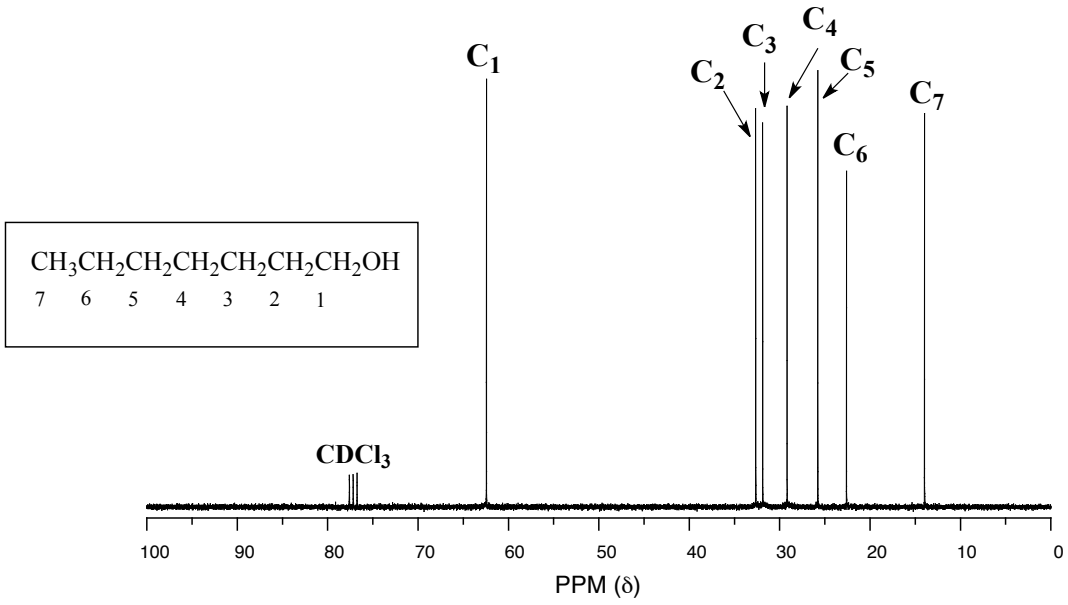
(all structures are shown earlier in this chapter)

Because of the low natural abundance of ^{13}C nuclei, it is very unlikely to find two ^{13}C atoms near each other in the same molecule, and thus *we do not see spin-spin coupling between neighboring carbons in a ^{13}C-NMR spectrum*. ^{13}C nuclei are coupled to nearby protons, however, which results in complicated spectra. For clarity, chemists generally use a technique called **broadband decoupling**, which essentially 'turns off' C-H coupling, resulting in a spectrum in which all carbon signals are singlets. Below is the proton-decoupled ^{13}C-NMR sectrum of ethyl acetate, showing the expected four signals, one for each of the carbons. We can also see a signal for the carbon atom in the deuterated chloroform ($CDCl_3$) solvent (although a detailed discussion is beyond our scope here, it is interesting to note that this signal is split into a triplet by deuterium, which is NMR active and has *three* possible spin states rather than two). We can ignore the solvent signal when interpreting ^{13}C-NMR spectra.



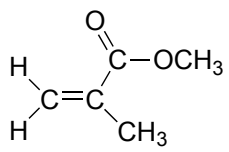
While broadband decoupling results in a much simpler spectrum, useful information about the presence of neighboring protons is lost. However, another NMR technique called DEPT (Distortionless Enhancement by Polarization Transfer) allows us to determine how many hydrogens are bound to each carbon. This information is usually provided in problems in which you are asked to interpret the ^{13}C-NMR spectrum of an unknown compound. (Details of how the DEPT technique works is beyond the scope of this book, but will be covered if you take a more advanced course in spectroscopy.)

One of the greatest advantages of ^{13}C-NMR compared to ^{1}H-NMR is the breadth of the spectrum - recall that carbons resonate from 0-220 ppm relative to the TMS standard, as opposed to only 0-12 ppm for protons. Because of this, ^{13}C signals rarely overlap, and we can almost always distinguish separate peaks for each carbon, even in a relatively large compound containing carbons in very similar environments. In the proton spectrum of 1-heptanol we saw earlier only the broad singlet of the alcohol proton (H_a) and the triplet for (H_b) are easily analyzed. The other proton signals overlap, making analysis difficult. In the ^{13}C spectrum of the same molecule, however, we can easily distinguish each carbon signal, and we know from this data that our sample has seven nonequivalent carbons. (Notice also that, as we would expect, the chemical shifts of the carbons get progressively lower as they get farther away from the deshielding oxygen.)

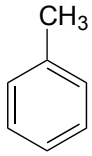


This property of ^{13}C-NMR makes it very helpful in the elucidation of larger, more complex structures.

Exercise 5.17 : Below are ^{13}C-NMR spectra for methylbenzene (common name toluene) and methyl methacrylate. Match the spectra to the correct structure, and make peak assignments.

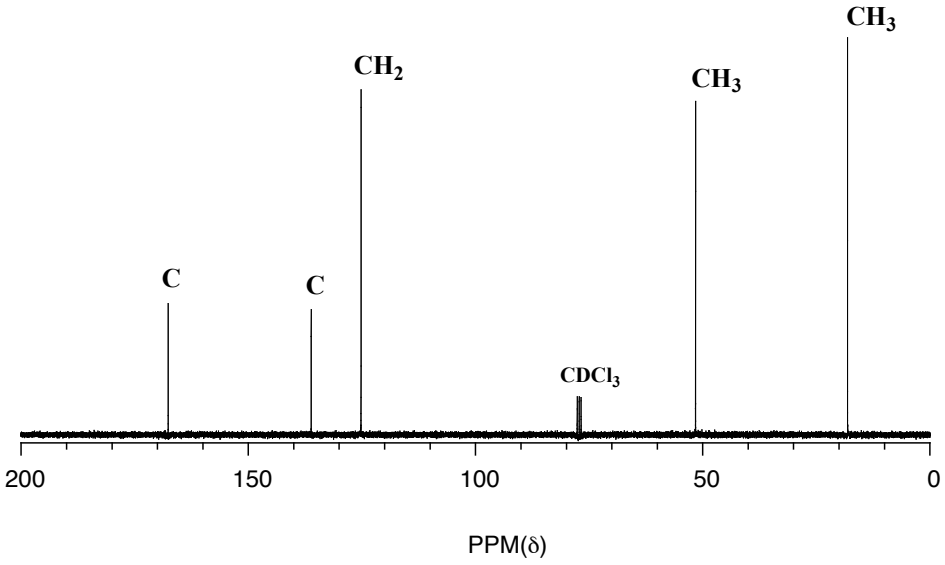


methyl methacrylate

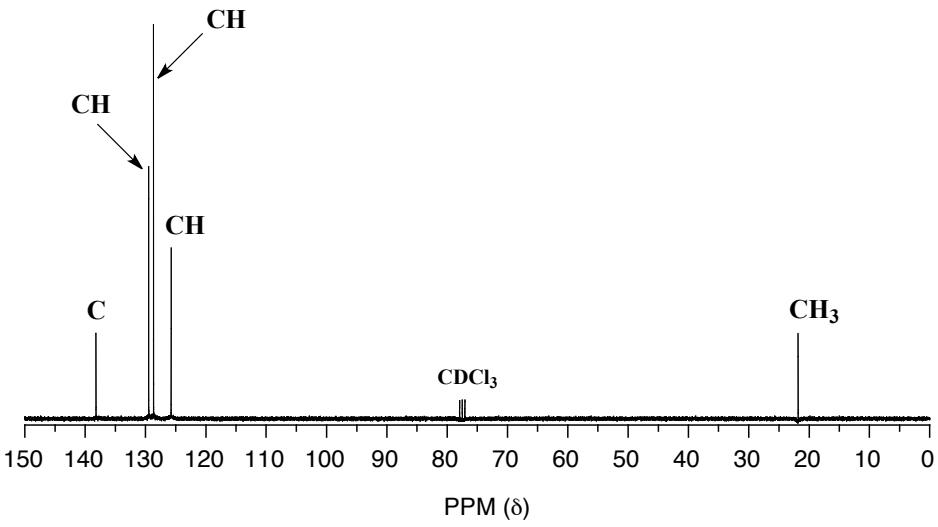


toluene

Structure A:

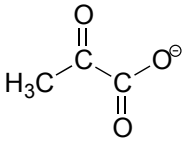


Structure B:

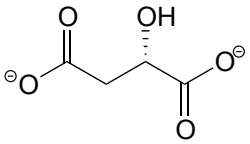


Exercise 5.18: ^{13}C-NMR data for some common biomolecules are shown below (data is from the Aldrich Library of ^{1}H and ^{13}C NMR). Match the NMR data to the correct structure, and make complete peak assignments.

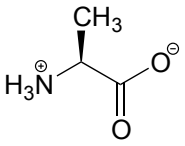
spectrum a: 168.10 ppm (C), 159.91 ppm (C), 144.05 ppm (CH), 95.79 ppm (CH)
spectrum b: 207.85 ppm (C), 172.69 ppm (C), 29.29 ppm (CH_3)
spectrum c: 178.54 ppm (C), 53.25 ppm (CH), 18.95 ppm (CH_3)
spectrum d: 183.81 ppm (C), 182. 63 ppm (C), 73.06 ppm (CH), 45.35 ppm (CH_2)



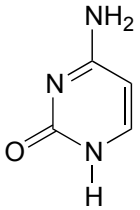
pyruvate



(S)-malate



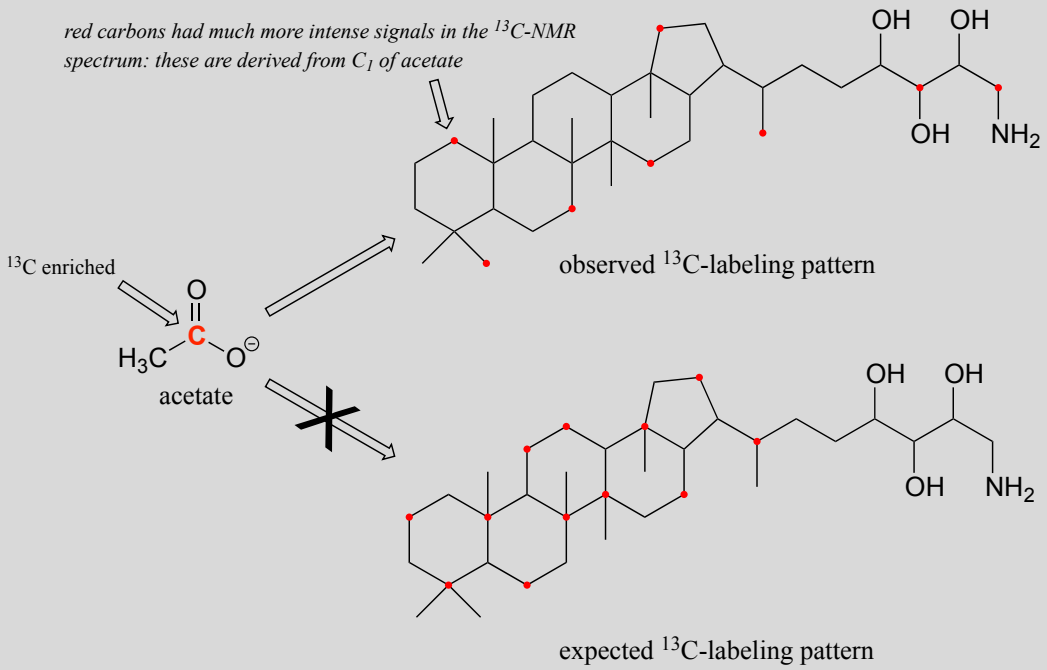
alanine



cytosine

^{13}C-NMR in isotopic labeling studies

Although only about 1 out of 100 carbon atoms in a naturally occurring organic molecule is a ^{13}C isotope, chemists are often able to synthesize molecules that are artificially enriched in ^{13}C at specific carbon positions. This can be very useful in biochemical studies, because it allows us to 'label' one or more carbons in a small precursor molecule and then trace the presence of the ^{13}C label through a biosynthetic pathway all the way to the final product, providing insight into how the biosynthesis occurs. For example, scientists were able to grow bacteria in a medium in which the only source of carbon was acetate enriched in ^{13}C at the C_1 (carbonyl) position. When they isolated an isoprenoid compound called amino-bacterio-hopanetriol synthesized by the bacteria and looked at its ^{13}C-NMR spectrum, they observed that the ^{13}C label from acetate had been incorporated at eight specific positions. They knew this because the ^{13}C-NMR signals for these carbons were much stronger compared to the same signals in a control (unlabeled) compound.



This result was very surprising - the scientists had expected a completely different pattern of ^{13}C incorporation based on what they believed to be the isoprenoid biosynthesis pathway involved. This unexpected result led eventually to the discovery that bacteria synthesize isoprenoid compounds by a completely different pathway then yeasts, plants, and animals. The newly discovered bacterial metabolic pathway is currently a key target for the development of new antibiotic and antimalaria drugs. (*Eur. J. Biochem*. **1988**, *175*, 405).

Section 5.7: Solving unknown structures

Now it is finally time to put together all that we have studied about structure determination techniques and learn how to actually solve the structure of an organic molecule 'from scratch' - starting, in other words, with nothing but the raw experimental data. For this exercise, we will imagine that we have been given a vial containing a pure sample of an unknown organic compound, and that this compound to our knowledge has never before been synthesized, isolated, or characterized - we are the first to get our hands on it. Can we figure out its structure? While of course the exact method of determining an unknown structure will depend on the compound in question and, in the real world of research, will probably involve some techniques that are beyond the scope of this book, here is an overview of an approach that could be taken to analyze a pure sample of a relatively simple organic compound, using the techniques we have learned about.

Step 1: Use MS and combustion analysis to determine the molecular formula

Before we start analyzing spectroscopic data, we need one very important piece of information about our compound - its molecular formula. This can be determined through the combined use of mass spectrometry and **combustion analysis**. We will not go into the details of combustion analysis - for now, it is enough to know that this technique tells us the mass percent of each element in the compound. Because molecular oxygen is involved in the combustion reaction, oxygen in the sample is not measured directly - but we assume that if the mass percentages do not add up to 100%, the remainder is accounted for by oxygen.

When we obtain our unknown compound, one of the first things we will do is to send away a small quantity to an analytical company specializing in combustion analysis. They send us back a report stating that our compound is composed, by mass, of 52.0% carbon, 38.3% chlorine, and 9.7% hydrogen. This adds up to 100%, so our compound does not contain any oxygen atoms.

In order to determined the molecular formula of our compound from this data, we first need to know its molar mass. This piece of information, as you recall from chapter 4, we determine by looking at the 'molecular ion peak' in the mass spectrum of our compound. In this example, we find that our MS data shows a molecular ion peak at $m/z = 92$, giving us a molar mass of 92 g/mole (remember that in the MS experiment, charge (z) is almost always equal to 1, because we are looking at +1 cations).

So, one mole of our compound is 92g. How many moles of carbon atoms are in one mole of the compound? Simple: 52% of 92g is 47.8g. So in one mole of our compound, there is about 48 g of carbon, which means four moles of carbon. With similar calculations, we find that one mole of our compound contains nine hydrogens and one chlorine. Therefore our molecular formula is C_4H_9Cl.

Step 2: Calculate the Index of Hydrogen Deficiency

The next step is to calculate a number called the Index of Hydrogen Deficiency (IHD) from the molecular formula. The IHD will tell us how many multiple bonds and/or ring structures our molecule has - very useful information. The idea behind the IHD is very simple: the presence of a double bond or a ring structure means that two fewer hydrogen atoms can be part of the compound. The formula for calculating IHD from a molecular formula is:

Calculating Index of Hydrogen Deficiency:

$$\text{IHD} = \frac{(2n+2) - \text{A}}{2}$$

fig 36

where:

n = number of carbon atoms
A = (number of hydrogen atoms) + (number of halogen atoms) - (number of nitrogen atoms) - (net charge)

For example, a molecule with the molecular formula C_6H_{14} would have $n = 6$ and A = 14, so we can calculate that IHD = 0 and thereby know that a compound with this formula has no double bonds or ring structures. Hexane and 2-methyl pentane are two examples of compounds that apply.

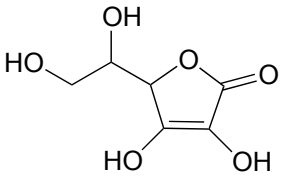
A molecular formula of C_6H_{12}, on the other hand, corresponds to IHD = 1, so a compound with this formula should have one double bond *or* one ring structure. Cyclohexane (one ring structure) and 2-hexene (one double bond) are two possibilities. Benzene (C_6H_6) , and methyl benzene (C_7H_8) both have IHD = 4, corresponding in both cases to three π bonds and one ring. An IHD value of 4 or greater is often an indicator that an aromatic ring is present.

Exercise 5.19:

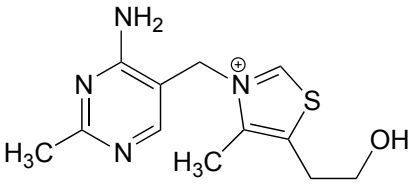
a) What is the IHD that corresponds to a molecular formula $C_6H_{12}O$? Draw the structures of three possible compounds that fit.

b) The amino acid alanine has molecular formula $C_2H_8NO_2^+$ in aqueous buffer of pH = 2. Calculate the IHD. Then, draw the relevant structure to confirm that this IHD makes sense.

c) What is the IHD of the compounds below? (*Hint*: you don't need to figure out molecular formulas!)



ascorbic acid



thiamine

The formula for our structure determination sample, C_4H_9Cl, corresponds to IHD = 0, meaning that our compound contains no multiple bonds or rings.

Step 3: Use available spectroscopy data to identify discrete parts of the structure.

In this problem, we have proton and carbon NMR data to work with (other problems may include IR and/or UV/Vis data).

^{1}H-NMR:

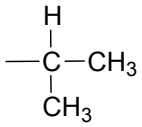
δ (ppm)	splitting	integration
3.38	d	2
1.95	m	1
1.01	d	6

^{13}C-NMR:

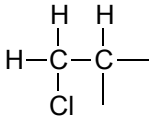
52.49 (CH_2)
31.06 (CH)
20.08 (CH_3)

The process of piecing together an organic structure is very much like putting together a puzzle. In every case we start the same way, determining the molecular formula and the IHD value. After that, there is no set formula for success- what we need to do is figure out as much as we can about individual pieces of the molecule from the NMR (and often IR, MS, or UV-Vis) data, and write these down. Eventually, hopefully, we will be able to put these pieces together in a way that agrees with all of our empirical data. Let's give it a go.

We see that there are only three signals in each NMR spectrum, but four carbons in the molecule, which tells us that two of the carbons are chemically equivalent. The fact that the signal at 1.01 ppm in the proton spectrum corresponds to *six* protons strongly suggests that the molecule has two equivalent methyl (CH_3) groups. Because this signal is a doublet, there must be a CH carbon bound to each of these two methyl groups. Taken together, this suggests:

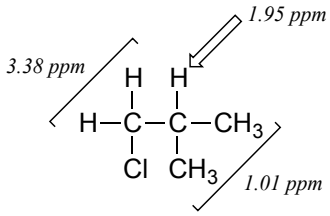


The ^{1}H-NMR signal at 3.38 ppm must be for protons bound to the carbon which is in turn bound to the chlorine (we infer this because this signal is the furthest downfield in the spectrum, due to the deshielding effect of the electronegative chlorine). This signal is for two protons and is a doublet, meaning that there is a single nonequivalent proton on an adjacent carbon.

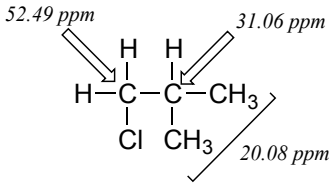


Step 4: Try to put the pieces of the puzzle together, and see if everything fits the available data.

At this point, we have accounted for all of the atoms in the structure, and we have enough information to put together a structure that corresponds to 1-chloro-2-methylpropane.



^{1}H-NMR assignments



^{13}C-NMR assignments

To confirm, we make assignment all NMR signals to their corresponding atoms and make sure that our structure fits all of the NMR data. Notice that the proton peak at 1.95 ppm might be expected to be a '9-tet' because of its eight 3-bond neighbors: however, two of the neighbors are different from the other six, and may not couple to exactly the same extent. The signal at 1.95 will not, then, be a 'clean' 9-tet, and we would call it a multiplet.

Exercise 5.20:

Three constitutional isomers of 1-chloro-2-methylpropane produce the following NMR data. Assign structures to the three compounds, and make all peak assignents.

Compound A:

^{1}H-NMR:
1.62 ppm, 9H, s

^{13}C-NMR:
67.14 ppm (C)
34. 47 ppm (CH_3)

Compound B:

^{1}H-NMR:

3.42 ppm, 2H, t
1.68 ppm, 2H, p
1.41 ppm, 2H, sextet
0.92 ppm, 3H, t

^{13}C-NMR:

44.74 ppm (CH_2)
34.84 ppm (CH_2)
20.18 ppm (CH_2)
13.34 ppm (CH_3)

Compound C:

^{1}H-NMR:

3.97 ppm, 1H, sextet
1.71 ppm, 2H, p
1.50 ppm, 3H, d
1.02 ppm, 3H, t

^{13}C-NMR:

60.34 ppm (CH)
33.45 ppm (CH_2)
24.94 ppm (CH_3)
11.08 ppm (CH_3)

Let's try another problem, this time incorporating IR information. The following data was obtained for a pure sample of an unknown organic compound:

Combustion analysis:

C: 85.7%
H: 6.67%

MS: Molecular ion at *m/z* = 210

^{1}H-NMR:

7.5-7.0, 10H, m
5.10, 1H, s
2.22, 3H, s

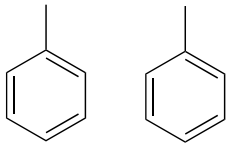
^{13}C-NMR:

206.2 (C) 128.7 (CH) 30.0 (CH_3)
138.4 (C) 127.2 (CH)
129.0 (CH) 65.0 (CH)

IR: 1720 cm^{-1}, strong *(there are of course many other peaks in the IR spectrum, but this is the most characteristic one)*

The molecular weight is 210, and we can determine from combustion analysis that the molecular formula is $C_{15}H_{14}O$ (the mass percent of oxygen in the compound is assumed to be 100 - 85.7 - 6.67 = 7.6 %). This gives us IHD = 9.

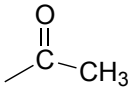
Because we have ten protons with signals in the aromatic region (7.5-7.0 ppm), we are probably dealing with two phenyl groups, each with one substituted carbon. The ^{13}C-NMR spectrum shows only four signals in the range for aromatic carbons, which tells us that the two phenyl groups must be in equivalent electronic environments (if they are in different environments, they would give rise to eight signals).



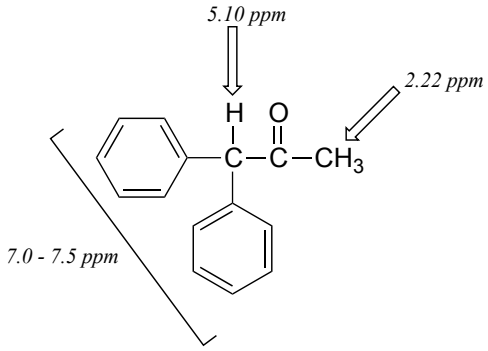
This accounts for 12 carbons, 10 hydrogens, and 8 IHD units. Notice that the carbon spectrum has only six peaks - and only four peaks in the aromatic region! This again indicates that the two phenyl groups are in chemically equivalent positions

The IR spectrum has a characteristic carbonyl absorption band, so that accounts for the oxygen atom in the molecular formula, the one remaining IHD unit, and the ^{13}C-NMR signal at 206.2 ppm.

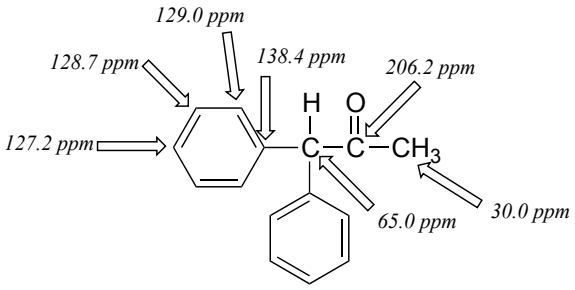
Now we only have two carbons and four hydrogens left to account for. The proton spectrum tells us we have a methyl group (the 2.22 ppm singlet) that is not split by neighboring protons. Looking at the table of typical chemical shifts, we see that this chemical shift value is in the range of a carbon next to a carbonyl.



Finally, there is one last proton at 5.10 ppm, also a singlet. Putting the puzzle together, the only possibility that fits is 1,1-diphenyl-2-propanone:



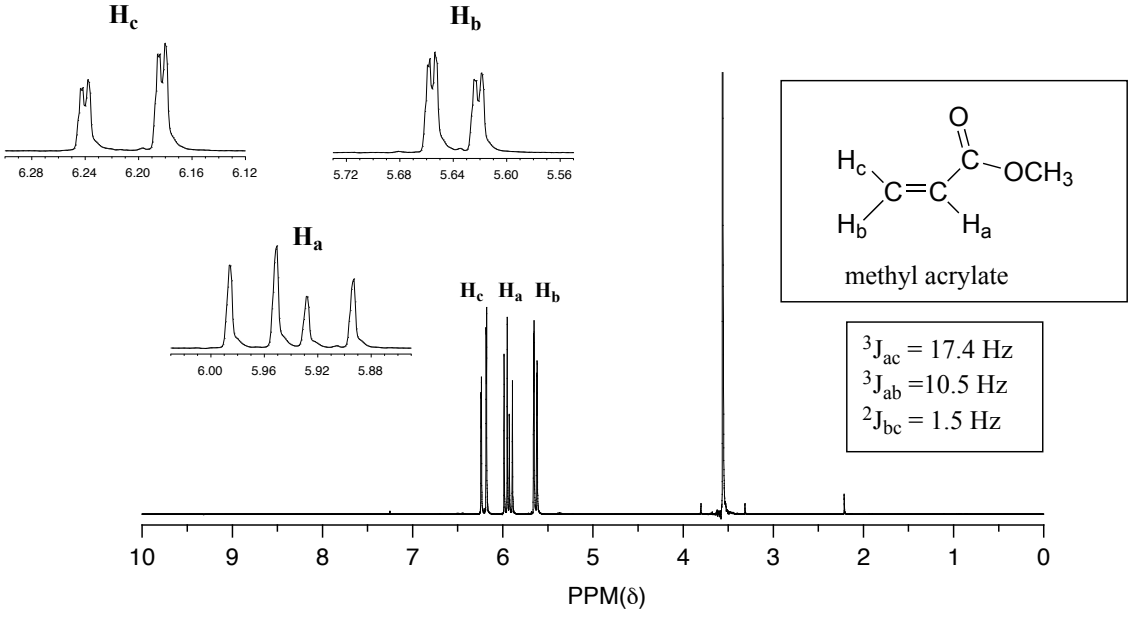
^{1}H-NMR assignments



^{13}C-NMR assignments

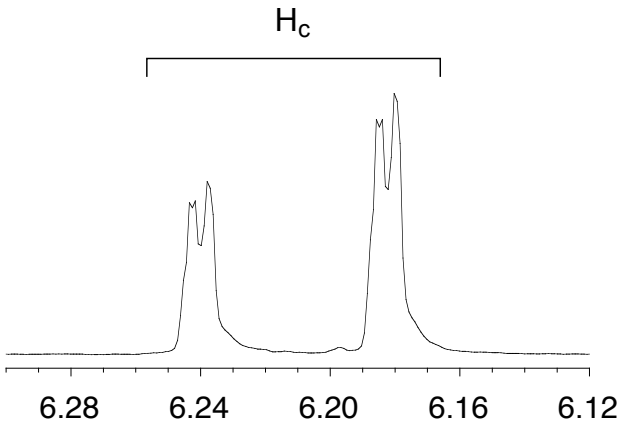
Section 5.8: Complex coupling in ^{1}H-NMR spectra

In all of the examples of spin-spin coupling we saw in our discussion of proton NMR, the observed splitting resulted from the coupling of one set of protons to *just one* neighboring set of protons. When a set of protons is coupled to *two* sets of nonequivalent neighbors, with significantly different coupling constants, the result is a phenomenon called **complex coupling**. A good illustration is provided by the ^{1}H-NMR spectrum of methyl acrylate:

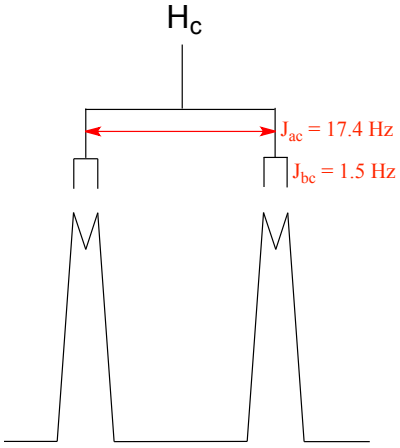


Note that all three vinylic protons in methyl acrylate (designated above as H_a, H_b and H_c) are separated from each other by three bonds or less, and thus are all spin-coupled. For example, H_c is *gem*-coupled to H_b (J = 1.5 Hz), and H_c is also *trans*-coupled to H_a (J = 17.4 Hz). You might think that the n+1 rule would tell us that because H_c has two nonequivalent neighbors - H_a and H_b - its NMR signal should be a triplet. This would be

correct *if* J_{ac} and J_{bc} were the same, or very close. However, because the two coupling constants are in fact very different from each other, the signal for H_c is clearly *not* a triplet. Here is a further expansion of the Hc signal:



You can see that the H_c signal is actually composed of four sub-peaks. Why is this? A splitting diagram can help us to understand what we are seeing. H_a is *trans* to H_c across the double bond, and splits the H_c signal into a doublet with a coupling constant of $^3J_{ac}$ = 17.4 Hz. In addition, each of these H_c doublet sub-peaks is split again by H_b (*geminal* coupling) into two more doublets, each with a much smaller coupling constant of $^2J_{bc}$ = 1.5 Hz.

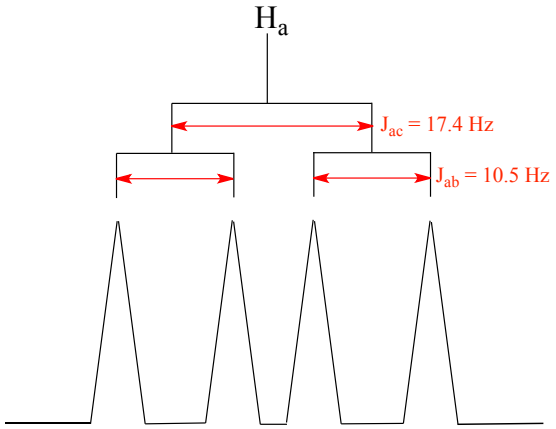


The result of this `double splitting` is a pattern referred to as a **doublet of doublets**, abbreviated `**dd**`.

The reported chemical shift of H_c is 6.210 ppm, the average of the four sub-peaks.

Exercise 5.21: Assume that on a 300 MHz spectrometer, the chemical shift of H_c, expressed in Hz, is 1863 Hz. What is the chemical shift, in Hz, of the furthest downfield subpeak in the H_c signal?

The signal for H_a at 5.950 ppm is also a doublet of doublets, with coupling constants $^3J_{ac}$ = 17.4 Hz and $^3J_{ab}$ = 10.5 Hz.



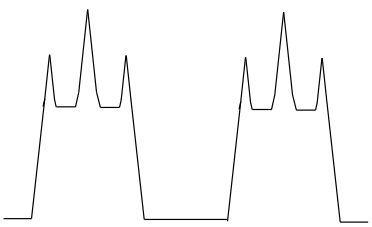
The signal for H_b at 5.64 ppm is split into a doublet by H_a, a *cis* coupling with $^3J_{ab}$ = 10.4 Hz. Each of the resulting sub-peaks is split again by H_c, with the same *geminal* coupling constant $^2J_{bc}$ = 1.5 Hz that we saw previously when we looked at the H_c signal. The overall result is again a doublet of doublets, this time with the two `sub-doublets` spaced slightly closer due to the smaller coupling constant for the *cis* interaction.

Exercise 5.22 : Construct a splitting diagram for the H_b signal in the ^{1}H-NMR spectrum of methyl acrylate. The chemical shift of H_b, in Hz, is 1691 Hz - label the chemical shifts (in Hz) of each of the four sub-peaks.

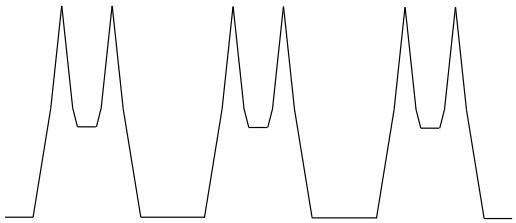
Exercise 5.23: Explain how the signals for H_b and H_c of methyl acrylate can be unambiguously assigned.

When constructing a splitting diagram to analyze complex coupling patterns, it is conventional (and simpler) to show the broader splitting first, followed by the finer splitting: thus we show the broad H_a-H_c splitting first, then the fine H_b-H_c splitting.

In the methyl acrylate spectrum, the signals for each of the three vinylic protons was a doublet of doublets (abbreviated 'dd'). Other complex splitting patterns are possible: triplet of doublets (td), doublet of triplets (dt), doublet of quartets (dq), and so on. Remember that the broader splitting is listed first, thus a triplet of doublets is different from a doublet of triplets.

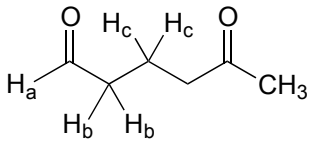


a doublet of triplets



a triplet of doublets

Exercise 5.24: Draw a predicted splitting diagram for the signal corresponding to H_b in the structure below, using approximate coupling constants. What would you call the splitting pattern for the H_b signal in this example?



Exercise 5.25: A signal in a proton NMR spectrum has multiple sub-peaks with the following chemical shifts values, expressed in Hz: 1586, 1583, 1580, 1572, 1569, 1566. Sketch the signal, identify the splitting pattern, and give the coupling constant(s) and the overall chemical shift value (in Hz).

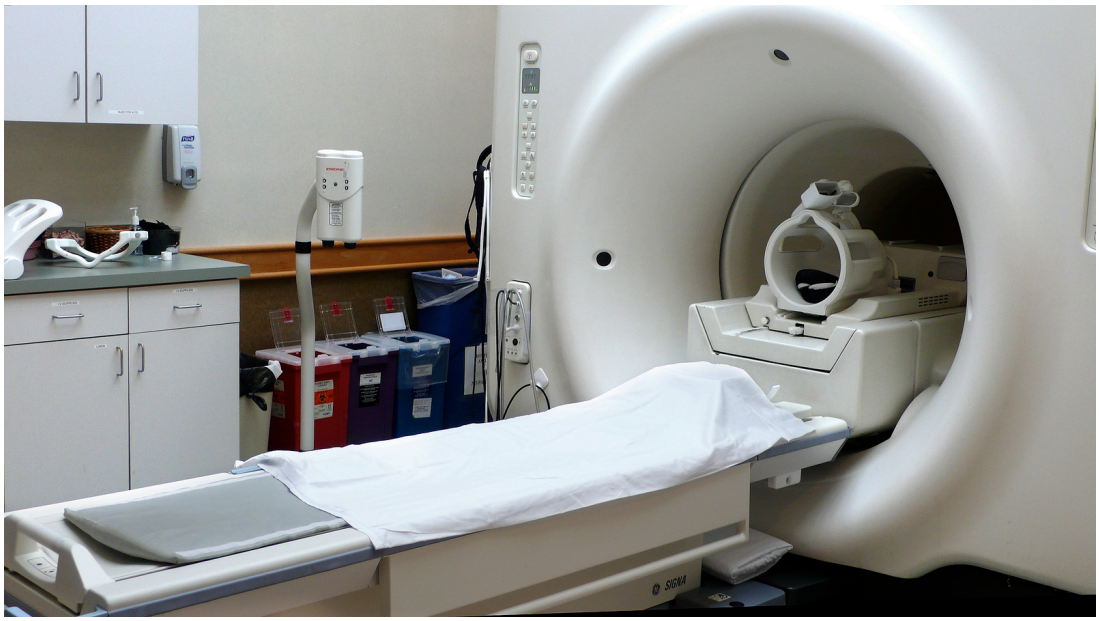
When we start trying to analyze complex splitting patterns in larger molecules, we gain an appreciation for why scientists are willing to pay large sums of money (hundreds of thousands of dollars) for higher-field NMR instruments. Quite simply, the stronger our magnet is, the more resolution we get in our spectrum. In a 100 MHz instrument (with a magnet of approximately 2.4 Tesla field strength), the 12 ppm frequency 'window' in which we can observe proton signals is 1200 Hz wide. In a 500 MHz (~12 Tesla) instrument, however, the window is 6000 Hz - five times wider. In this sense, NMR instruments are like digital cameras and HDTVs: better resolution means more information and clearer pictures (and higher price tags!)

Section 5.9: Other applications of NMR

A: Magnetic resonance imaging

In the introduction to this chapter, we heard two stories about people whose lives were potentially saved when brain tumors were discovered during a magnetic resonance imaging (MRI) scan. MRI is a powerful diagnostic technique because it allows doctors to visualize internal body tissues while sparing the patient from surgery and potentially

harmful, high energy x-rays. The basis for MRI is essentially the same as for NMR: an MRI scanner has a very strong superconducting magnet large enough to completely surround a whole person, much the same way in which a small glass sample tube in an NMR experiment is surrounded by the instrument's magnet. Once exposed to the strong magnetic field, water protons in the body resonate at different radio frequencies - the variation in resonance frequencies is due to water binding in different ways to different tissue types, creating slightly different electronic environments for the protons. The software in the MRI scanner then translates variations in resonance frequencies to a color scheme, which creates a visual image of the body tissues in the scanned area.



A typical MRI scanner (credit Liz West: https://www.flickr.com/photos/calliope/)

B: NMR of proteins and peptides

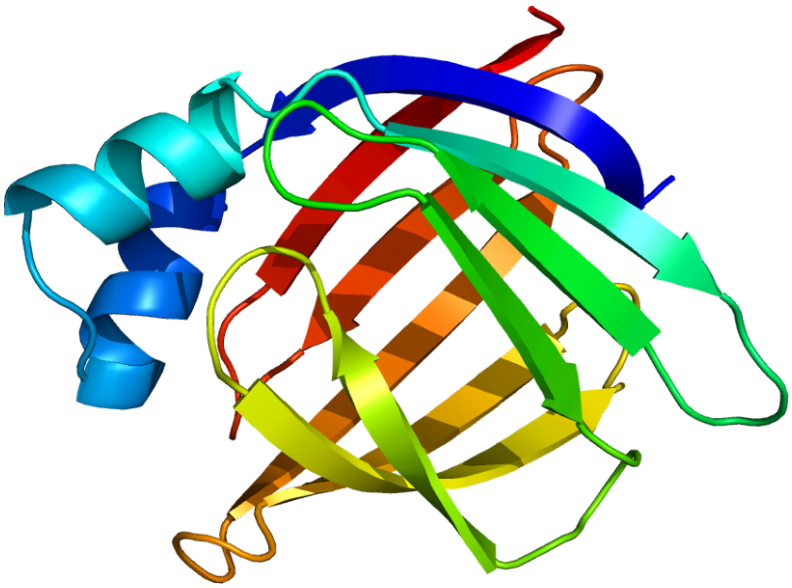
In this chapter you have learned enough about NMR to be able to understand how it is used to solve the structures of relatively small organic molecules. But what about really big organic molecules, like proteins?

X-ray crystallography, not NMR, is the most common way to determine the precise three-dimensional structure of a protein, and in a biochemistry class you will look at many images of protein structures derived from x-ray crystallography. While it is an immensely powerful tool for analyzing protein structure, crystallography has two major drawbacks. First, it relies on a researcher being able to get a protein to form regular, ordered crystals, which can be very challenging. Most proteins are globular, meaning they are (very roughly) spherical in shape. For a molecule to form crystals, it must pack together tightly in an ordered, repeating way: think of a neat stack of cube-shaped objects. Spheres,

however, are inherently difficult to pack this way. Imagine trying to make a pile of tennis balls - they just roll apart, because so little of each ball's surface area comes into contact with its neighbor, thus there is very little friction (ie. noncovalent interactions!) holding them together. A large percentage of known proteins simply will not crystallize under any conditions that have been tried - therefore, we cannot determine their structure using x-ray crystallography.

Secondly, a lot of what is most interesting about proteins is how they move: flaps open and close when a substrate binds, or one part of the protein moves over to connect with another part. Protein action is dynamic. A crystal, on the other had, is static, or frozen. A protein structure determined by x-ray crystallography is like a still photograph of leaping dancer: we can infer from the picture what kind of movement might be taking place, but we can't get a full appreciation of the motion.

This leads to NMR, which of course is done in solution. It is easy to get most proteins into aqueous solution, so there are no worries about trying to make crystals. Also, a protein in solution is free to move, so NMR can potentially capture elements of protein dynamics. So why don't scientists always use NMR to look at proteins?



Structure of an intestinal fatty acid binding protein determined by NMR

(credit: Wikipedia commons: https://commons.wikimedia.org/wiki/User:Emw; *Biochemistry* **2003**, *42*, 7339)

After working through a few NMR structure determination problems in this chapter, you have an appreciation for the brainwork required to figure out the structure of a small organic molecule based on its NMR structure: now imagine doing this with a protein, with its thousands of carbon and hydrogen atoms! Nevertheless, spectroscopists are gradually

getting better and better at using NMR and computer-power to do just this. The advanced NMR techniques and methods of analysis are far beyond the scope of our discussion here, but you can see how useful it could be to protein scientists to be able to 'see' what a protein looks like using NMR, and if you are interested in this area of research you can learn about it in more advanced courses.

Summary of Key Concepts

Before moving on to the next chapter, you should:

Be able to identify groups of chemically equivalent protons and carbon atoms in a structure.

Be able to explain the basis of an NMR experiment in terms of the spin state of a nucleus, the ground state to excited state transition involved, and the frequency of radiation absorbed.

Understand the differences between proton and carbon NMR experiments, and explain why carbon spectra generally have more noise, do not show coupling, and do not suffer from the disadvantage of overlapping peaks. You should be able to explain why ^{13}C-NMR peaks are not usually integrated.

Understand how to look at an NMR spectrum, including the meaning of the ppm label on the x-axis, the meaning of 'chemical shift', and the definition of zero ppm on the chemical shift scale.

Be able to predict trends in chemical shifts for protons and carbon atoms in different bonding positions, and provide a rationale for the trend. You should also be able to roughly estimate the chemical shift of a given proton or carbon using Table X or a similar table from another source.

Understand how to use proton peak integration values to determine how many protons a particular peak is 'worth'.

Be able to explain the physical basis for spin-spin coupling in ^{1}H-NMR spectra, and be able to use the 'n+1 rule'.

Be able to interpret, and draw splitting diagrams for, ^{1}H-NMR spectra with complex coupling.

Be able to use a ^{13}C-NMR spectrum to identify the number of magnetically nonequivalent types of carbon in an unknown compound."

Be confident at working problems in which you are asked to match structures to ^{1}H- and/or ^{13}C-NMR spectra.

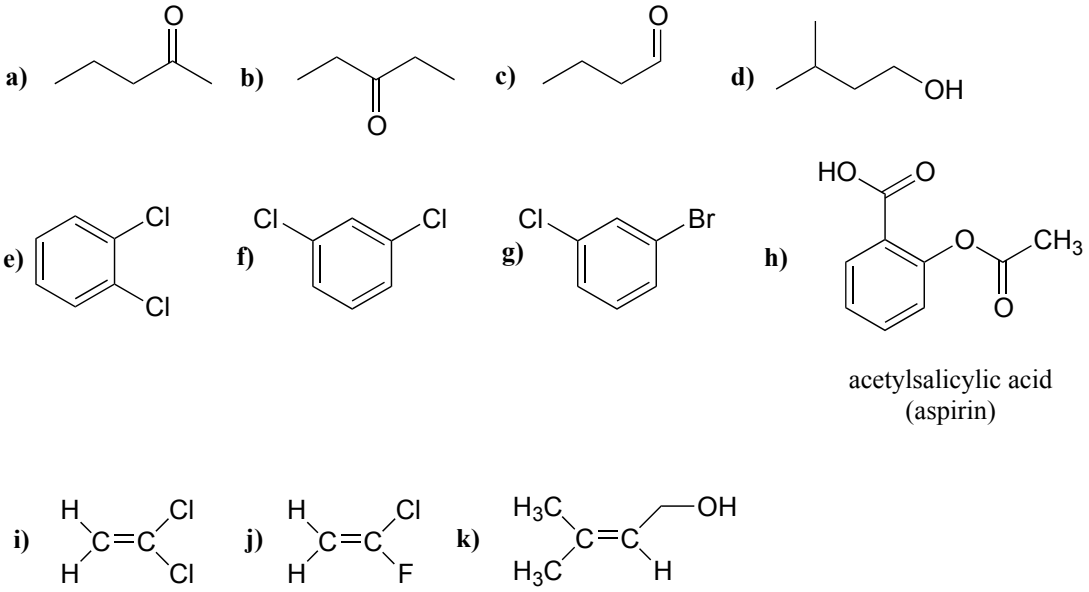
Given a molecular formula (or a combination of combustion and MS data), you should be confident in your ability to solve an unknown structure based on a ^{1}H- spectrum, possibly in combination with data from ^{13}C-NMR, IR, or UV-Vis spectroscopy.

Problems

P5.1:

a) For each molecule below, draw in all hydrogen atoms, and label them H_a, H_b, etc., with chemically equivalent hydrogens having the same label.

b) Predict splitting patterns for all proton signals.

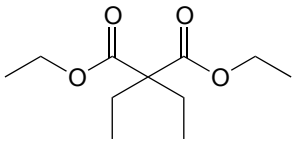


P5.2: For each of the 20 common amino acids, predict the number of signals in the proton-decoupled ^{13}C-NMR spectrum.

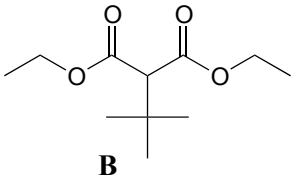
P5.3: Match spectra below to their corresponding structures A-F. Make complete peak assignments for all structures.

(in all ^{1}H-NMR data tables in the following problems, peak relative integration values are listed in which the smallest area peak is equal to 1)

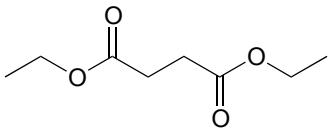
Structures:



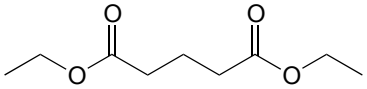
A



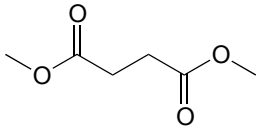
B



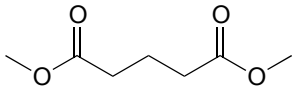
C



D



E



F

Spectrum 1:

δ (ppm)	splitting	integration
4.13	q	2
2.45	t	2
1.94	p	1
1.27	t	3

Spectrum 2:

δ (ppm)	splitting	integration
3.68	s	3
2.99	t	2
1.95	p	1

Spectrum 3:

δ (ppm)	splitting	integration
4.14	q	1
2.62	s	1
1.26	t	1.5

Spectrum 4:

δ (ppm)	splitting	integration
4.14	q	4
3.22	s	1
1.27	t	6
1.13	s	9

Spectrum 5

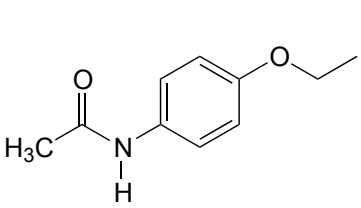
d (ppm)	splitting	integration
4.18	q	1
1.92	q	1
1.23	t	1.5
0.81	t	1.5

Spectrum 6:

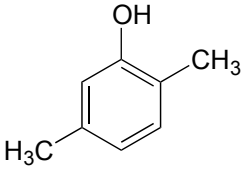
d (ppm)	splitting	integration
3.69	s	1.5
2.63	s	1

P5.4: Match the ^{1}H-NMR spectra 7-12 to their corresponding structures G-L. Make complete peak assignments for all structures.

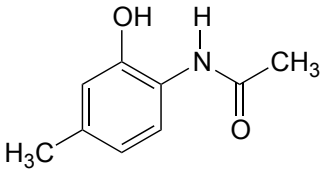
Structures:



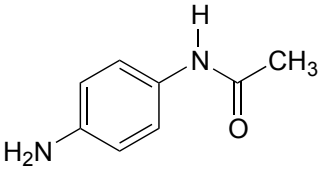
G



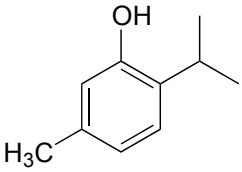
H



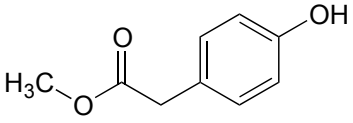
I



J



K



L

Spectrum 7:

δ (ppm)	splitting	integration
6.98	d	1
6.64	d	1
6.54	s	1
4.95	s	1
2.23	s	3
2.17	s	3

Spectrum 8:

δ (ppm)	splitting	integration
7.08	d	1
6.72	d	1
6.53	s	1
4.81	s	1
3.15	septet	1
2.24	s	3
1.22	d	6

Spectrum 9:

δ (ppm)	splitting	integration
7.08	d	2
6.71	d	2
6.54	s	1
3.69	s	3
3.54	s	2

Spectrum 10:

δ (ppm)	splitting	integration
9.63	s	1
7.45	d	2
6.77	d	2
3.95	q	2
2.05	s	3
1.33	t	3

Spectrum 11:

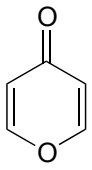
δ (ppm)	splitting	integration
9.49	s	1
7.20	d	2
6.49	d	2
4.82	s	2
1.963	s	3

Spectrum 12:

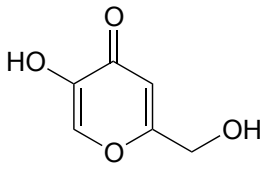
δ (ppm)	splitting	integration
9.58	s(b)	1
9.31	s	1
7.36	d	1
6.67	s	1
6.55	d	1
2.21	s	3
2.11	s	3

P5.5: Match the ^{1}H-NMR spectra 13-18 to their corresponding structures M-R. Make complete peak assignments for all structures.

Structures:



M



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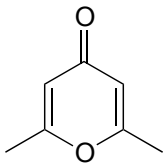
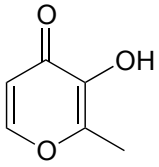
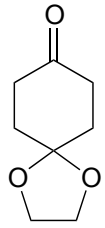


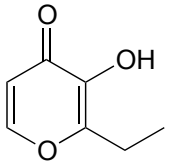
O



P



Q



R

Spectrum 13:

δ (ppm)	splitting	integration
8.15	d	1
6.33	d	1

Spectrum 14:

δ (ppm)	splitting	integration
6.05	s	1
2.24	s	3

Spectrum 15:

δ (ppm)	splitting	integration
8.57	s (b)	1
7.89	d	1
6.30	d	1
2.28	s	3

Spectrum 16:

δ (ppm)	splitting	integration
9.05	s (b)	1
8.03	s	1
6.34	s	1
5.68	s (b)	1
4.31	s	2

Spectrum 17:

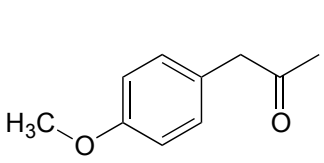
δ (ppm)	splitting	integration
7.76	d	1
7.57	s (b)	1
6.44	d	1
2.78	q	2
1.25	t	3

Spectrum 18:

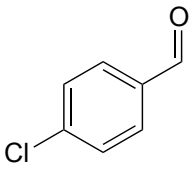
δ (ppm)	splitting	integration
4.03	s	1
2.51	t	1
2.02	t	1

P5.6: Match the ^{1}H-NMR spectra 19-24 below to their corresponding structures S-X. Make complete peak assignments for all structures.

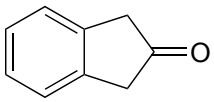
Structures:



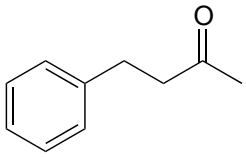
S



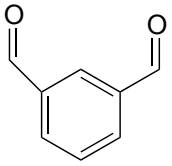
T



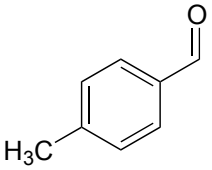
U



V



W



X

Spectrum 19:

δ (ppm)	splitting	integration
9.94	s	1
7.77	d	2
7.31	d	2
2.43	s	3

Spectrum 20:

δ (ppm)	splitting	integration
10.14	s	2
8.38	s	1
8.17	d	2
7.75	t	1

Spectrum 21:

δ (ppm)	splitting	integration
9.98	s	1
7.81	d	2
7.50	d	2

Spectrum 22:

δ (ppm)	splitting	integration
7.15-7.29	m	2.5
2.86	t	1
2.73	t	1
2.12	s	1.5

Spectrum 23:

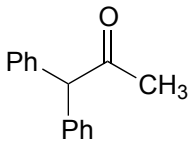
δ (ppm)	splitting	integration
7.10	d	1
6.86	d	1
3.78	s	1.5
3.61	s	1
2.12	s	1.5

Spectrum 24:

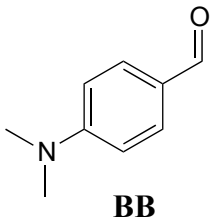
δ (ppm)	splitting	integration
7.23-7.30	m	1
3.53	s	1

P5.7: Match the ^{1}H-NMR spectra 25-30 below to their corresponding structures AA-FF.

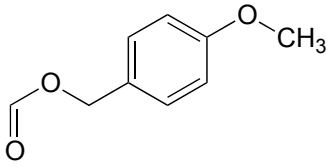
Structures:



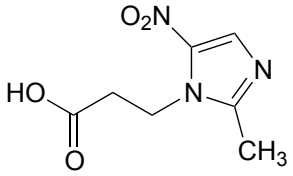
AA



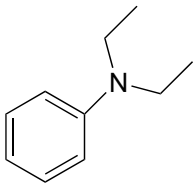
BB



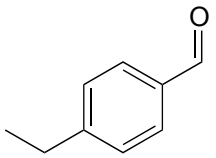
CC



DD



EE



FF

Spectrum 25:

δ (ppm)	splitting	integration
9.96	s	1
7.79	d	2
7.33	d	2
2.72	q	2
1.24	t	3

Spectrum 26:

δ (ppm)	splitting	integration
9.73	s	1
7.71	d	2
6.68	d	2
3.06	s	6

Spectrum 27:

δ (ppm)	splitting	integration
11.2 (b)	s	1
7.20-7.35	m	10
5.12	s	1
2.22	s	3

Spectrum 28:

δ (ppm)	splitting	integration
8.08	s	1
7.29	d	2
6.87	d	2
5.11	s	2
3.78	s	3

Spectrum 29:

δ (ppm)	splitting	integration
7.18	d	1
6.65	m	1.5
3.2	q	2
1.13	t	3

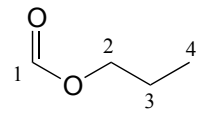
Spectrum 30:

δ (ppm)	splitting	integration
8.32	s	1
4.19	t	2
2.83	t	2
2.40	s	3

P5.8:

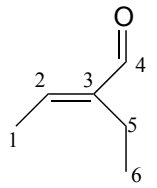
^{13}C-NMR data is given for the molecules shown below. Complete the peak assignment column of each NMR data table.

a)



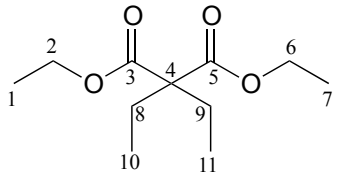
δ (ppm)		carbon #(s)
161.12	CH	
65.54	CH_2	
21.98	CH_2	
10.31	CH_3	

b)



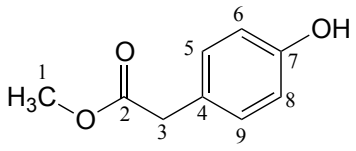
δ (ppm)		carbon #(s)
194.72	C	
149.10	C	
146.33	CH	
16.93	CH_2	
14.47	CH_3	
12.93	CH_3	

c)



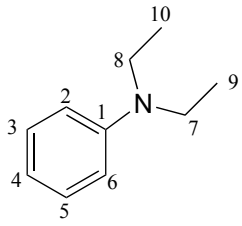
δ (ppm)		carbon #(s)
171.76	C	
60.87	CH_2	
58.36	C	
24.66	CH_2	
14.14	CH_3	
8.35	CH_3	

d)



δ (ppm)		carbon #(s)
173.45	C	
155.01	C	
130.34	CH	
125.34	C	
115.56	CH	
52.27	CH_3	
40.27	CH_2	

e)



δ (ppm)		carbon #(s)
147.79	C	
129.18	CH	
115.36	CH	
111.89	CH	
44.29	CH_2	
12.57	CH_3	

P5.9: Use the NMR data given to deduce structures.

a) Molecular formula: C_5H_8O

^{1}H-NMR:

δ (ppm)	**splitting**	**integration**
9.56	s	1
6.25	d	1
5.99	d	1
2.27	q	2
1.18	t	3

^{13}C-NMR

δ (ppm)	
194.60	CH
151.77	C
132.99	CH_2
20.91	CH_2
11.92	CH_3

b) Molecular formula: $C_7H_{14}O_2$

^{1}H-NMR:

δ (ppm)	**splitting**	**integration**
3.85	d	2
2.32	q	2
1.93	m	1
1.14	t	3
0.94	d	6

^{13}C-NMR

δ (ppm)	
174.47	C
70.41	CH_2
27.77	CH
27.64	CH_2
19.09	CH_3
9.21	CH_3

c) Molecular weight: 88

Combustion analysis:
C: 68.2%
H: 13.6%

IR: ~3349 cm^{-1} (broad)

^{1}H-NMR:

δ (ppm)	**splitting**	**integration**
3.38	s	2H
2.17	s	1H
0.91	s	9H

^{13}C-NMR

δ (ppm)	
73.35	CH_2
32.61	C
26.04	CH_3

d) Molecular weight: 148

Combustion analysis:

C: 81.1%
H: 8.1%

IR: 1713 cm^{-1} (strong)

^{1}H-NMR:

δ (ppm)	splitting	integration
7.18-7.35	m	2.5
3.66	s	1
2.44	q	1
1.01	t	1.5

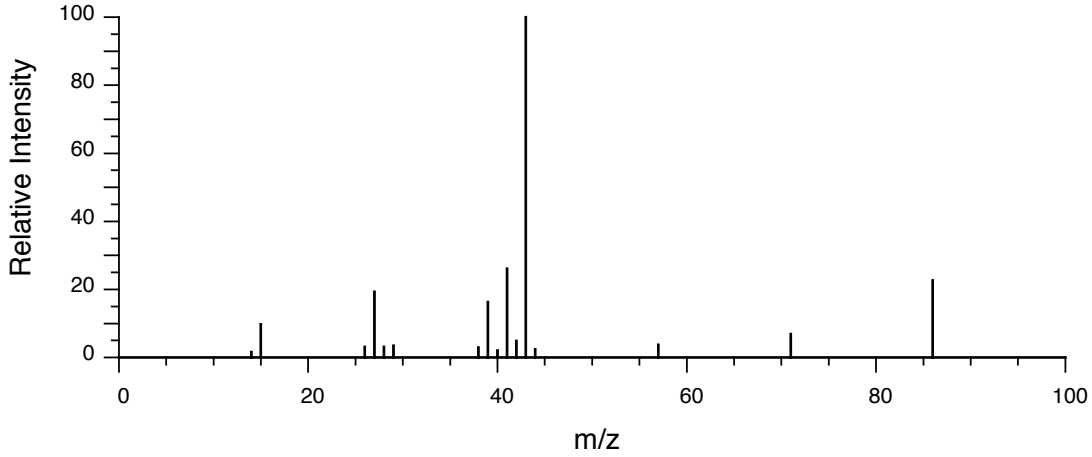
^{13}C-NMR

δ (ppm)	
208.79	C
134.43	C
129.31	CH
128.61	CH
126.86	CH
49.77	CH_2
35.16	CH_2
7.75	CH_3

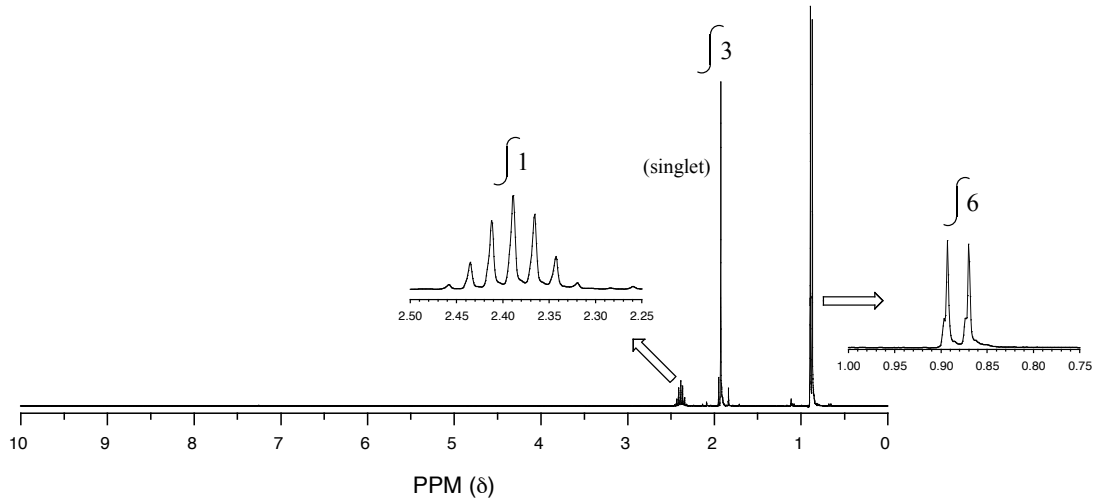
P5.10: You obtain the following data for an unknown sample. Deduce its structure.

Combustion analysis: C (69.7%); H (11.7%)

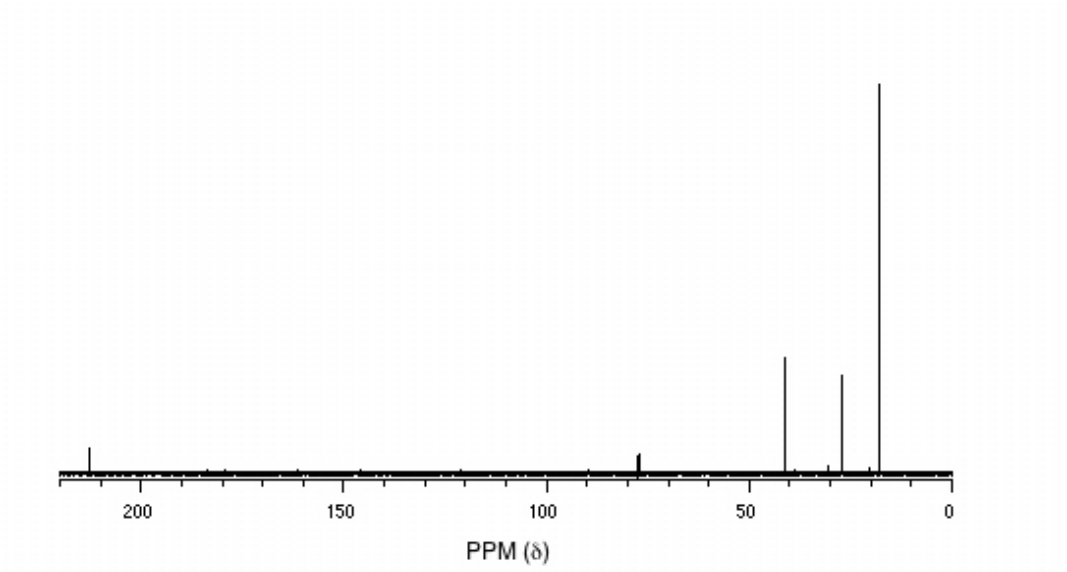
Mass Spectrometry:



^{1}H-NMR:



^{13}C-NMR:



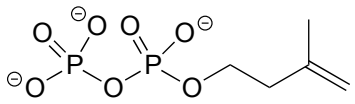
P5.11: You take a ^{1}H-NMR spectrum of a sample that comes from a bottle of 1-bromopropane. However, you suspect that the bottle might be contaminated with 2-bromopropane. The NMR spectrum shows the following peaks:

δ (ppm)	splitting	Integration
4.3	septet	0.0735
3.4	t	0.661
1.9	sextet	0.665
1.7	d	0.441
1.0	t	1.00

How badly is the bottle contaminated? Specifically, what percent of the molecules in the bottle are 2-bromopropane?

P5.12:

a) The proton-decoupled ^{13}C-NMR spectrum of isopentenyl diphosphate, the building block compound for isoprenoids (see section 1.3) is composed of five signals, two of which are doublets. Explain.



isopentenyl diphosphate

b) Recall that other magnetically active nuclei besides ^{1}H and ^{13}C can be observed by NMR. Without trying to predict chemical shift values, describe what the ^{31}P-NMR spectrum for isopentenyl diphosphate might look like.

P4.11: One would expect the mass spectrum of cyclohexanone to show a molecular ion peak at m/z = 98. However, the m/z = 98 peak in the cyclohexanone spectrum is unusually abundant, compared to the molecular ion peaks in the mass spectra of other ketones such as 2-hexanone or 3-hexanone. Explain.

Chapter 6

Overview of organic reactivity

A hot spring in Yellowstone National Park

(Credit: https://www.flickr.com/photos/pavdw)

September 5, 1966, turned out to be a very good day for Hudson Freeze. An undergraduate microbiology major at Indiana University, he was a few weeks away from the first day of classes in his junior year, but on this September day he was far away from the oppressive heat and humidity of late summer in the midwest. Instead, he was working at the edge of Mushroom Spring in Yellowstone National Park, one of one of the many geothermal hot springs for which the park is so famous.

At the end of his sophomore year, Freeze had approached Dr. Thomas Brock, one of his microbiology professors, to ask about the possibility of working as a research assistant

over the summer. Brock took him up on the offer, inviting him to come out to Yellowstone for a few weeks in late summer to help with some fieldwork.

For the past few years, Brock had been studying microbes that inhabited the hot springs: these 'extremophile' organisms were fascinating to him because they appeared to thrive in conditions that until quite recently had been thought to be too hot to support life. The currently accepted upper limit at which life was believed to be possible was 73 ^{o}C, but during his work in Yellowstone the previous summer Brock was convinced that he had observed microbial life – a pink colored, filamentous bacteria - in water as hot as 88 ^{o}C. Unfortunately, all of his attempts to culture these life forms in the lab had been unsuccessful. He had decided to focus his efforts this summer on Mushroom Spring, where the water was 73 ^{o}C, right at the supposed limit for life, and assigned Freeze the task of collecting microbial samples from the waters of the spring. On September 5, Freeze collected a promising-looking sample, which he took back to a makeshift lab in Brock's cabin to prepare for transport back to Indiana.

A few weeks later, working in his professor's lab in between classes and homework, Freeze was engaged in the challenge of figuring out how to get his microbes to grow outside their natural environment, so that he could isolated and eventually characterize them. The work was frustrating at first – attempting to get the bacterial to grow in a liquid medium, he never observed the characteristic turbidity that usually signals success. In some samples, however, he did observe the appearance of salt crystals on the bottom of the test tubes. He allowed these samples to incubate for a few more days, and noticed that more crystals had formed. Just to be thorough, he decided to look at some of the crystals under a microscope – and hit the jackpot. Clinging to the crystals themselves were the recognizable shapes of microbial cells.

In subsequent work with Thomas Brock, Freeze was able to improve his culturing techniques and characterize the new species of bacterium, which was later named *Thermus aquaticus,* or *'Taq'* for short. He also was able to demonstrate that enzymes isolated from the bacterium remained intact and active even in boiling water.

Even though Yellowstone is a beautiful place to spend a few weeks in the summer doing field work, it turned out that making the trip to Wyoming was not really necessary – Thomas Brock later was able to isolate cultures of *Taq* from samples taken from the hot water system right there on the Indiana University campus, as well as from many other hot-water environments around the world. Brock and Freeze went on to publish a paper in the *Journal of Bacteriology (***1969**, *98*, 289) describing their newly discovered species, and donated live cultures of *Taq* to the American Type Culture Collection, a biological repository in Washington D.C.

Years later, a scientist named Kary Mullis working at Cetus, a biotechnology firm in the San Francisco Bay area, purchased a culture of *Taq* - a direct descendent of the very culture that Hudson Freeze had taken from Mushroom Spring on September 5, 1966 - from the ATCC repository. Cetus paid $35 for the sample. It turned out to be a pretty good investment.

Mullis and his colleagues at Cetus were intrigued by Freeze's report years earlier that enzymes isolated from *Taq* were stable at high temperatures, unlike enzymes isolated from *E. coli* and other common model organisms. They cultured their *Taq* sample, purified the DNA-copying enzyme DNA polymerase from the *Taq* cells, and using the heat-stable polymerase were able to come up with a remarkably efficient method for copying short stretches of DNA. Their 'polymerase chain reaction', or PCR, went on to revolutionize the fields of molecular/cellular biology and biochemistry – read through the experimental section of any recent research paper in one of these fields and chances are you will see that the researchers used PCR. If you take a lab course in molecular biology, you will probably perform at least one PCR procedure. When your professor purchases the purified *Taq* polymerase enzyme and other reagents for your lab, part of the price will go towards paying royalty fees to the pharmaceutical giant Hoffmann-LaRoche: Kary Mullis and Cetus obtained a patent for their PCR process, and in 1992 sold patent rights to Hoffmann-LaRoche for $300 million. Mullis was awarded the 1993 Nobel Prize in Chemistry for his work on PCR.

What makes the PCR technique so powerful is that it harnesses a biological catalyst - the DNA polymerase enzyme naturally produced by the *Taq* microbe - to vastly increase the rate of a very specific and very useful chemical reaction, under environmental conditions (high temperature) that until then had been fatal for other enzymes. *Taq* polymerase, the $300 million molecule, is the most visible example (for now!) of how scientists might harness the power of biological catalysis to great advantage, but many researchers are hard at work, in Yellowstone and many other locations around the world, writing more chapters in the story that was begun by Hudson Freeze and Thomas Brock on a September day in 1966.

Up to this point, we have been focusing on the *structure* of organic molecules: essentially, how these molecule are put together. Now our focus shifts to the study of *reactivity*: what happens, in other words, when covalent bonds within a molecule break and new covalent bonds form, as molecule A is transformed into molecule B. The story of *Taq* and PCR is centered around a biochemical reaction - the polymerization of DNA starting with an existing DNA 'template'. We are about to begin our exploration of chemical reactivity: how a reaction is depicted on paper, whether it absorbs or releases energy, how fast it goes, and how a catalyst might be able to make it go much faster.

In your previous college general chemistry and high school chemistry courses (and perhaps in biology courses as well), you have no doubt seen many different examples of chemical reactions. Most likely, these reactions were depicted by chemical *equations*, showing the starting materials (reactants) and the finished products connected by a 'reaction arrow'. In most cases, the structures of reactants and products were not considered - they were defined only by molecular formula and perhaps by physical state (solid, liquid, gas, or solution). The reaction below, showing the decomposition of dinitrogen pentoxide (N_2O_5) to nitrogen dioxide and oxygen, is a typical example of the

'equation' treatment of chemical reactivity which you might have seen in your General Chemistry textbook.

$N_2O_{5\,(s)} \rightarrow 2NO_{2\,(g)} + \frac{1}{2}\, O_{2\,(g)}$

This way of talking about chemical reactions is perfectly adequate in introductory chemistry classes, when fundamental chemical concepts like stoichiometry, thermodynamics, and kinetics are being explained for the first time. In organic chemistry, beginning with this chapter, we will go much further. We will certainly review the important fundamental concepts of thermodynamics and kinetics that you learned previously. But in our discussion of organic reactivity, we will bring our understanding of organic *structure* into the picture, and think about *how* reactions take place: which bonds break, which bonds form, *why* a particular bond breaks or forms, the order in which bond-breaking and bond-forming takes place, and the nature of any intermediate species that might form during the course of the reaction. We also will think about how catalysts - enzymes in particular - are able to increase the rate of a particular reaction. Taken together, a description of a chemical reaction at this level is called a **reaction mechanism**. Beginning here, and continuing throughout the rest of the text, our main job will be to understand the mechanisms of the most important types of reactions undergone by organic molecules in living organisms.

Section 6.1: A first look at some reaction mechanisms

6.1A: An acid-base (proton transfer) reaction

We'll begin with a relatively simple type of reaction that you are no doubt familiar with from previous chemistry classes: an acid-base reaction. Note that in chapter 7, we will come back to consider acid-base reactivity in much greater detail.

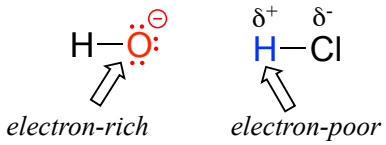
Here is the acid-base reaction between hydroxide ion and hydrochloric acid:

> Note: A reaction such as the one above would of course include a spectator cation, such as sodium (Na^+) or potassium (K^+). In most of the reaction figures that we'll see throughout this book, the spectator ion is not shown in the interest of simplicity.

A proton is transferred from HCl, the acid, to hydroxide ion, the base. The product is water (the conjugate acid of hydroxide ion) and chloride ion (the conjugate base of HCl). Despite its simplicity (and despite the fact that the reactants and products are *inorganic* rather than organic), this reaction allows us to consider for the first time many of the

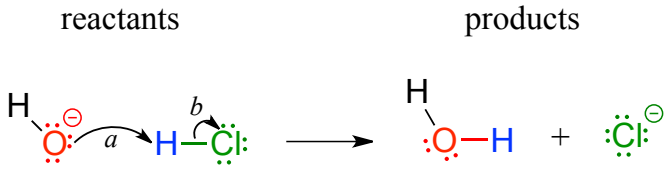
fundamental ideas of organic chemistry that we will be exploring in various contexts throughout this text.

One very important key to understanding just about any reaction mechanism is the concept of **electron density**, and how it is connected to the **electron movement** (bond-breaking and bond-forming) that occurs in a reaction. The hydroxide ion – specifically, the oxygen atom bearing the negative formal charge – has high electron density: it is **electron-rich**.



The hydrogen atom in HCl, on the other hand, has low electron density: it is **electron-poor**, because chlorine is more electronegative than hydrogen. As you might expect, an atom that is electron-rich is likely to be attracted to an atom that is electron-poor. As hydroxide and HCl move closer to each other, a new bond forms between oxygen and hydrogen, and the hydrogen-chlorine bond breaks. The end result is a water molecule and a chloride anion.

In organic chemistry terms, a **reaction mechanism** is a formalized description of how a reaction takes place - how we get, in other words, from reactants to products. Previously (section 2.3) , we saw how **curved arrows** were used to depict the 'imaginary' movement of two electrons when illustrating the conversion between two resonance contributors of the same molecule or ion (remember from that discussion that the conversion between two resonance contributors is *not* a reaction - it is merely an illustration of two different ways to draw the same molecule). The same curved arrow convention is used in **mechanism drawings** to show the electron movement that takes place in chemical reactions, where bonds are actually broken and formed. The mechanism for the HCl + OH^- reaction, for example, can be depicted by drawing two curved arrows.

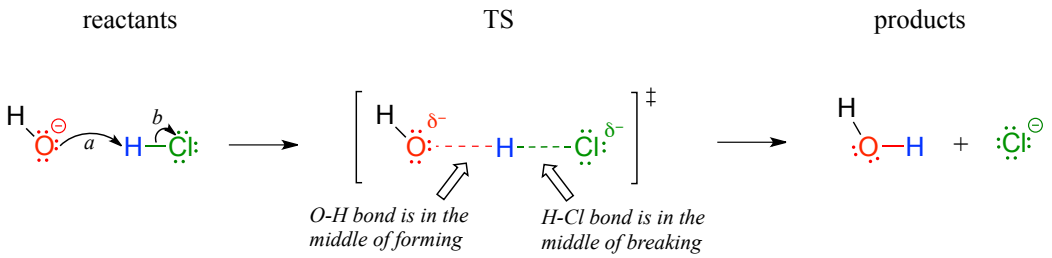


Arrow (a) in the mechanistic drawing originates at one of the lone pairs on the hydroxide oxygen and points to the 'H' symbol in hydrochloric acid, illustrating the 'attack' of the oxygen lone pair and subsequent formation of a new hydrogen-oxygen bond. Arrow (b) originates at the middle of hydrogen-chlorine bond and points to the 'Cl' symbol, indicating that this bond is breaking: the two electrons that make the bond are 'leaving' and becoming a lone pair on chloride ion. Always keep in mind that these curved arrows

by definition depict the movement of *two* electrons. (When we study radical reactions in chapter x, we will see how to depict the movement of a single electron.)

When two (or more) curved arrows are drawn in the same figure of a mechanism, the intended meaning is that the electron movements being shown are occurring *simultaneously*. For example, in the figure above, the electron movement illustrated by arrow (a) (O-H bond formation) is occurring at the same time as the H-Cl bond breaking illustrated by arrow (b).

The **transition state (TS)** of a chemical step is a point at which bonds are in the process of breaking and/or forming. (More specifically, we will see below when discussing energy diagrams that the transition state is the point of *highest energy* in the chemical step). Transition states are illustrated by drawing the forming/breaking bonds as dotted lines, and are enclosed by brackets with the 'double-dagger' symbol. For example, the transition state in the acid-base reaction between hydroxide and HCl can be illustrated as:



Notice in the drawing above that both the oxygen and the chlorine bear *partial* negative charges at the transition state: the formal charge on oxygen changes from -1 to 0 during the course of the reaction step, while the formal charge on chlorine changes from 0 to -1.

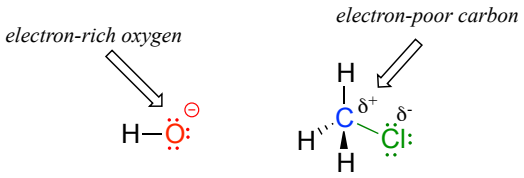
While it can sometimes be instructive to include a transition state drawing in an organic mechanism diagram, they are not 'obligatory' elements of such a diagram. When asked to draw a reaction mechanism in the exercises and problems in this book, you need not include TS drawings in your answer unless specifically directed to do so.

Exercise 6.1: Draw electron movement arrows to illustrate the acid-base reaction between acetic acid, CH_3CO_2H, and ammonia, NH_3. Draw out the full Lewis structures of reactants and products, including all lone pairs and, of course, formal charges. Include a transition state drawing in your mechanism.

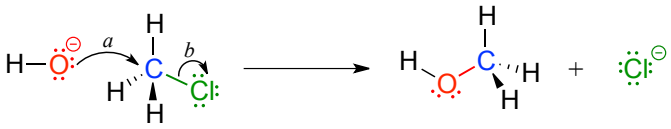
Exercise 6.2: Draw electron movement arrows to illustrate the acid-base reaction between acetate ion ($CH_3CO_2^-$, acting as a base) and methylamine ($CH_3NH_3^+$, acting as an acid). Draw out the full Lewis structures of reactants and products, including all lone pairs and formal charges.

6.1B: A one-step nucleophilic substitution reaction

The reaction between hydroxide and HCl is a simple example of a Brønsted acid-base (proton transfer) reaction. We now continue our introduction to the essential ideas of organic reactivity with a different type of reaction in which bonds to a *carbon* atom are rearranged. Consider what might happen if a hydroxide ion encountered a chloromethane molecule. The hydroxide is an electron-rich species, and thus might be expected to act as a base and 'attack' a hydrogen as it did in the previous example with hydrochloric acid. In this case, though, the three hydrogens on chloromethane are not electron-poor, as they are bound not to chlorine but to carbon, which is not very electronegative. However, there *is* a relatively electron-poor atom in chloromethane: the carbon itself.



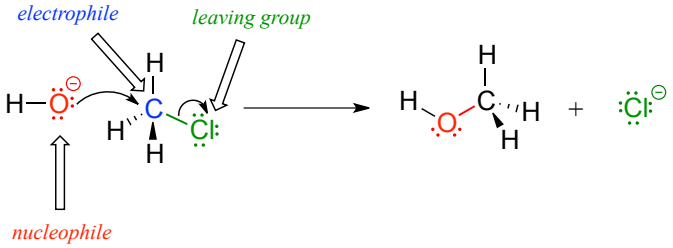
Due to the relative electronegativity of chlorine, the carbon-chlorine bond is polar. It stands to reason that a lone pair of electrons on the electron-rich hydroxide oxygen will be attracted to the electron-poor carbon nucleus.



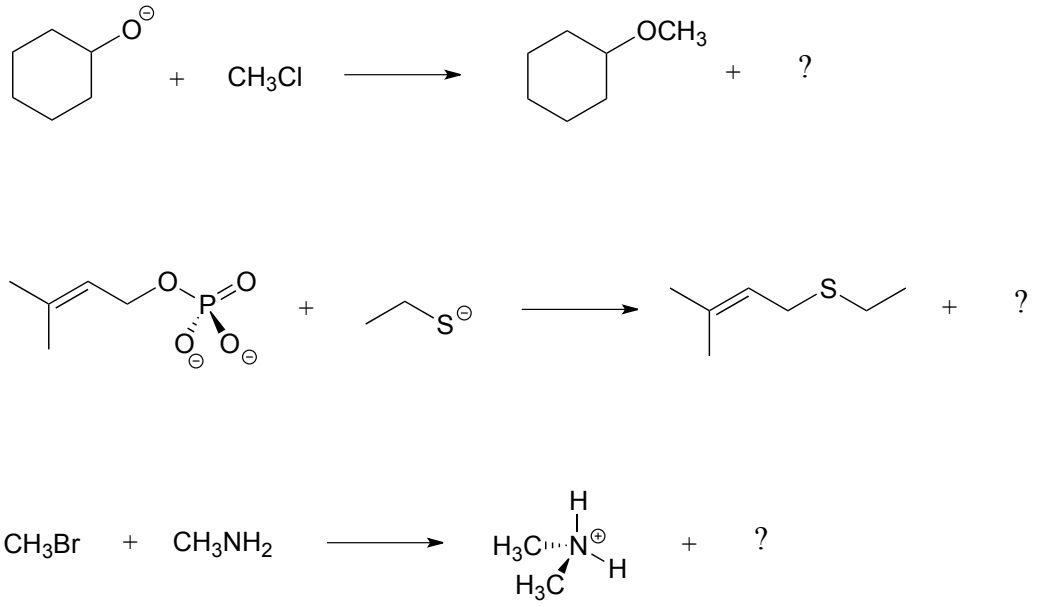
In the mechanism drawing above, curved arrow (a) shows the lone pair electrons on the hydroxide oxygen moving to fill up an sp^3 orbital on chloromethane, forming a new carbon-oxygen σ bond. However, in order for this new bond to form, one of the bonds already on the carbon must simultaneously break - otherwise, there will be five bonds to carbon and the octet rule will be violated (remember that the 'octet rule' tells us that elements in the second row of the periodic table can have a maximum of eight valence electrons). Curved arrow (b) illustrates how the two electrons in the carbon-chloride bond break out of their σ bond and become a lone pair on the chloride ion product. In other words, arrow (b) illustrates the breaking of the carbon-chlorine bond. (We will see a transition state drawing for this reaction in chapter 8, when we study this type of reaction mechanism in much greater detail).

The reaction mechanism illustrated above called a **nucleophilic substitution**. The 'substitution' term is easy to understand: just recognize how hydroxide *substitutes* for chlorine as the fourth bond to the central carbon. The term 'nucleophilic' means 'nucleus-loving' and refers to the electron-rich species, the hydroxide oxygen. This oxygen is a **nucleophile**: it is electron-rich and attracted to the electron-poor nucleus of the carbon atom, and 'attacks' with a lone pair to form a new covalent bond.

There are two more terms that come into play here, both of which you will see again and again as you continue to study organic reactions. Because the carbon atom in methyl chloride is electron-poor, it is attracted to anything that is electron rich - anything nucleophilic, in other words. Thus, the carbon is referred to in this context as an **electrophile**. The chlorine, because it leaves with two electrons to become a chloride ion, is termed a **leaving group.**



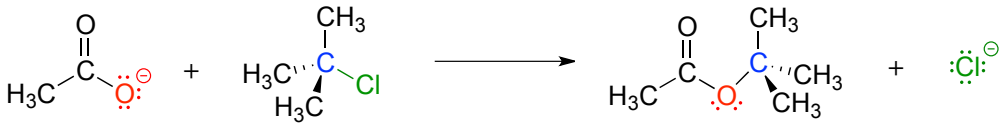
Exercise 6.3: In each of the nucleophilic substitution reactions below, identify the nucleophile, electrophile, and leaving group, and fill in the missing product.



6.1C: A two-step nucleophilic substitution mechanism

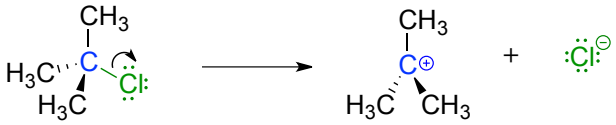
Reaction mechanisms describe not only the electron movement that occurs in a chemical reaction, but also the *order* in which bond-breaking and bond-forming events occur. Some nucleophilic substitution reactions, for example, can occur by a two-step mechanism that is different from the one-step mechanism we just saw between hydroxide

ion and chloromethane. Look, for example, at the substitution reaction between acetate ion and 2-chloro-2-methyl propane (common name *tert*-butyl chloride).



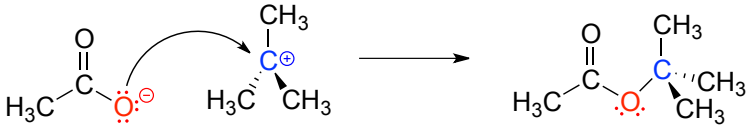
Unlike the chloromethane plus hydroxide reaction, in which the substitution process took place in a single, concerted step, it turns out that this mechanism involves two separate steps. The leaving group, chloride anion, leaves first, *before* the acetate nucleophile attacks.

Step 1: loss of leaving group (slow)

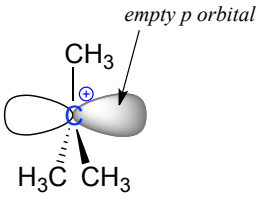


carbocation intermediate

Step 2: nucleophilic attack (fast)



Because the central carbon (colored blue in the figure above) has lost its share of the two electrons in what was the carbon-chlorine bond, it is now positively charged. Recall from section 2.1C that we can picture a carbocation as a planar, sp^2-hybridized carbon center with three bonds, an empty *p* orbital, and a full positive charge.



The carbocation is highly reactive, and does not exist for very long before participating in a subsequent bond-forming event. In the language of organic mechanisms, it is referred to as a **reaction intermediate**.

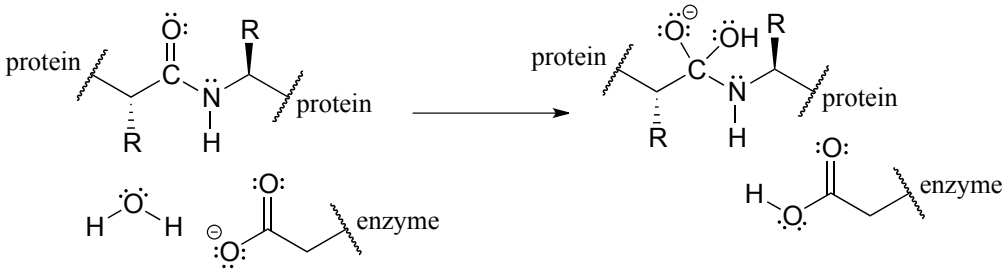
With its empty *p* orbital, the carbocation intermediate is clearly electron-poor, and thus is a powerful electrophile. The negatively charged acetate ion is electron dense and a nucleophile, and as such is strongly attracted to the carbocation electrophile. Attack by

the nucleophile results in a new carbon-oxygen σ bond and formation of the substitution product.

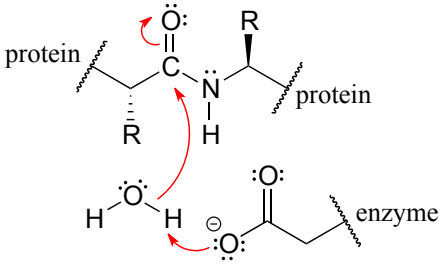
We will have much more to say about nucleophilic substitutions, nucleophiles, electrophiles, and leaving groups in chapter 8. The take home message at this point, however, is simply that two reactions that look quite similar in terms of the reactants and products can occur by different mechanisms.

You can probably appreciate by now how essential it is to understand and be able to work with curved arrows - it is something that will use constantly during the remainder of your study of organic reactivity.

For example, although you are not yet familiar with the relevant reaction mechanism (it is the HIV protease reaction, covered in chapter 11), given reactant and intermediate structures:



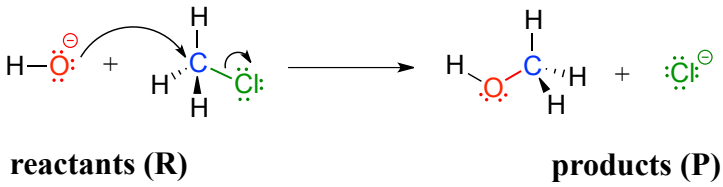
with practice you should at this point be able to recognize the bond-forming and bond-breaking electron movement that is taking place, and draw the appropriate curved arrows:



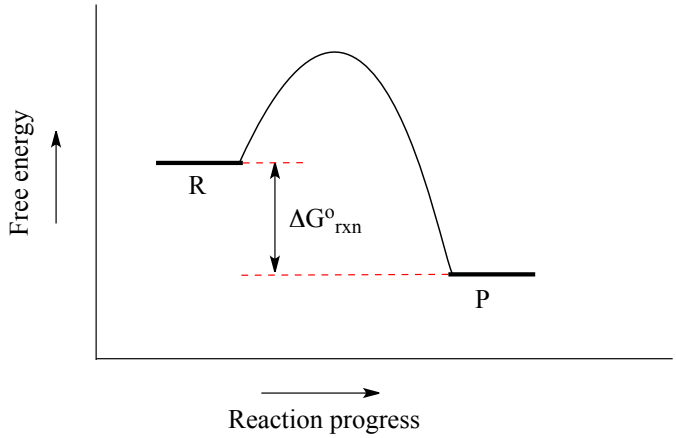
An additional word of caution: many beginning organic students make the mistake of using curved arrows to depict the motion of *atoms*. This is incorrect! The curved arrows in an organic mechanism ***always*** refer to the motion of *electrons*.

Section 6.2: A brief review of thermodynamics and kinetics

You may recall from general chemistry that it is often convenient to illustrate the energetics of a chemical reaction with a **reaction coordinate diagram**. In a reaction coordinate diagram, the vertical axis represents the overall potential energy of the reactants, while the horizontal axis is the 'reaction coordinate', tracing from left to right the progress of the reaction from starting reactants R to final products P. (Many students find it helpful to envision the horizontal axis in an reaction coordinate diagram as being analogous to the progress bar at the bottom of a youtube vieo). The diagram for a typical one-step nucleophilic substitution reaction such as that between hydroxide and methyl chloride might look like this:



reactants (R) **products (P)**



Despite its apparent simplicity, this diagram conveys some very important ideas about the thermodynamics and kinetics of the reaction. Recall that when we talk about the **thermodynamics** of a reaction, we are concerned primarily with the difference in energy between reactants (R) and products (P): whether the reaction as a whole is uphill or downhill. When we talk about **kinetics**, on the other hand, we are concerned with the *rate* of the reaction: how fast it goes from reactants to products, regardless of whether that transformation is energetically uphill or downhill.

6.2A: Thermodynamics

First, a quick review of some key thermodynamics terms (you may also want to go back to your General Chemistry text for a more complete review). Recall that the **standard Gibbs free-energy change of a reaction (ΔG°)** is the difference in energy between

reactants and products at standard conditions. Gibbs free-energy change is a combination of enthalpy change (ΔH^o) and entropy change (ΔS^o):

$\Delta G = \Delta H^o - T\Delta S^o$

. . .where T is the temperature in Kelvin (recall that the Kelvin temperature is simply the Celsius temperature plus 273.15).

Enthalpy change (ΔH^o) is the heat released or absorbed by the reaction.

Entropy change (ΔS^o) is the change in disorder from reactants to products. In a reaction in which one molecule cleaves into two smaller molecules, for example, disorder increases, so (ΔS^o) is positive.

The **equilibrium constant (K_{eq})** for a reaction is an expression of the relative concentrations of reactants and products *after the reaction has reached equilibrium*. The equilibrium constant is defined as:

this means that w molecules of compound A react with x molecules of compound B)

$$wA + xB \rightleftharpoons yC + zD$$

$$Keq = \frac{[products]}{[reactants]} = \frac{[C]^y\,[D]^z}{[A]^w[B]^x}$$

Now, let's review what the above energy diagram tells us about the thermodynamics of the reaction. Note that the energy level of the products is *lower* than that of the reactants. This tells us that the Gibbs free-energy change for the reaction is negative, and the step is **exergonic**, or energy releasing. We can also say the reaction is 'thermodynamically favorable', or, more informally, 'downhill'.

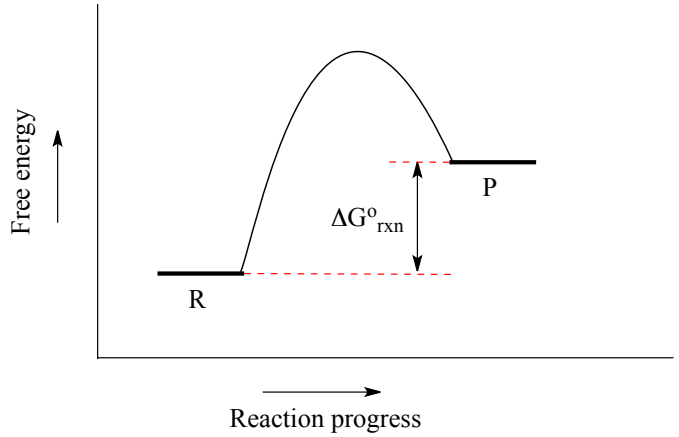
Recall from General Chemistry that the standard Gibbs free energy change for a reaction can be related to the reaction's equilibrium constant (K_{eq}) by the equation:

$\Delta G^\circ = -RT \ln K_{eq}$

. . . where R is the gas constant (8.314 J/mol·K) and T is the temperature in Kelvin (K).

If you do the math, you see that a negative value for ΔG°_{rnx} (an exergonic reaction) corresponds to K_{eq} being greater than 1, an equilibrium constant which favors product formation.

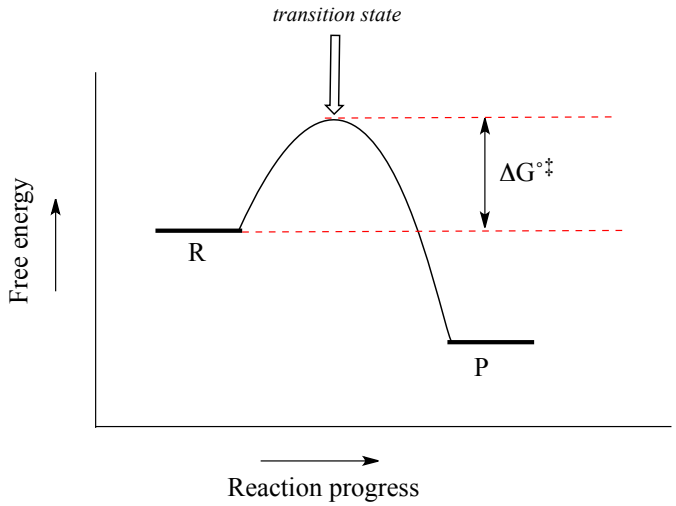
Conversely, an **endergonic** reaction is one in which the products are higher in energy than the reactants, and energy is absorbed. An endergonic reaction has a positive value of ΔG°_{rnx}, and a K_{eq} between 0 and 1.



Acid-base reactions provide convenient examples of thermodynamically favorable and unfavorable reactions. The reaction of a strong acid like HCl with a strong base like hydroxide ion, for example, is highly favorable, and has an equilibrium constant much greater than one. The reaction of a weak acid such as acetic acid with a weak base such as water, on the other hand, is unfavorable and has an equilibrium constant that is a very small (much less than 1) positive number: we can visualize this in a reaction coordinate diagram as an 'uphill' reaction, in which ΔG°_{rnx} is positive.

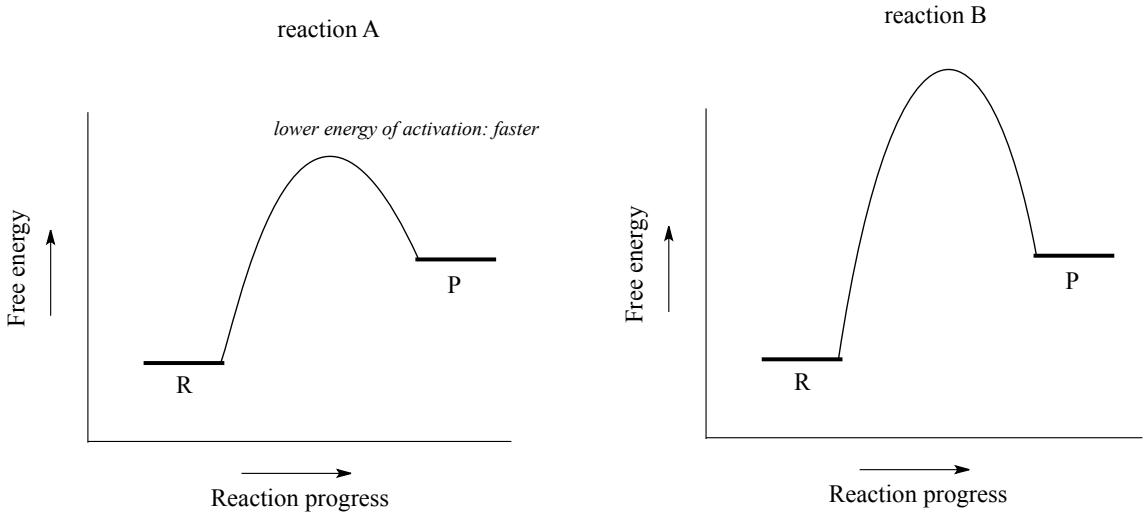
When talking about exergonic, or 'downhill' reactions, chemists sometimes use the term **'driving force'** to describe the chemical factor or factors that drive the reaction from higher energy reactant to lower energy product. Using the 'downhill' analogy again, when water flows downhill, the driving force is gravity. In an exergonic chemical reaction, the driving force generally is based on a combination of two factors: a) the stability of positive and negative charges in the product relative to those in the reactant, and b) the total bond energy in the product relative to the reactant. That may not make a lot of sense right now, but keep it in the back of your mind and we will come back to the idea of driving force when we study different reaction types in greater detail.

Now, let's move to kinetics. Look again at the diagram for an exergonic reaction: although it is ‘downhill’ overall, it isn’t a straight downhill run.



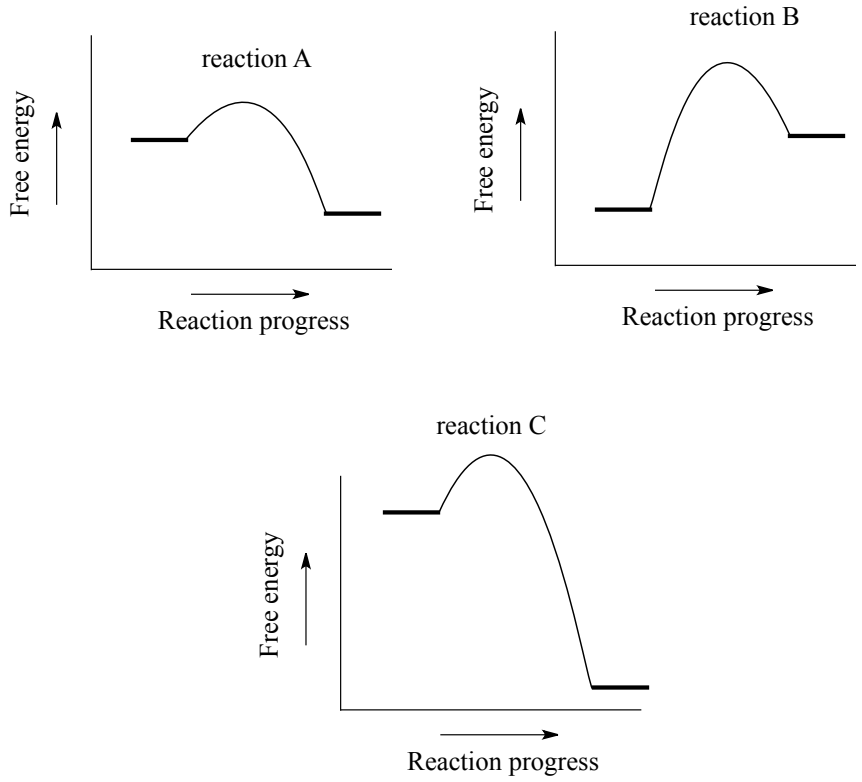
First, an 'energy barrier' must be overcome to get to the product side. The height of this energy barrier is called the **standard free energy of activation** ($\Delta G^{\circ\ddagger}$). The activation energy, in combination with the temperature at which the reaction is being run, determines the rate of a reaction: the higher the activation energy, the slower the reaction. At the very top of the energy barrier, the reaction is at its **transition state** (TS), which you should recall is defined as the highest energy structure in the transition from reactant to product.

Consider the hypothetical reaction reaction coordinate diagrams below.



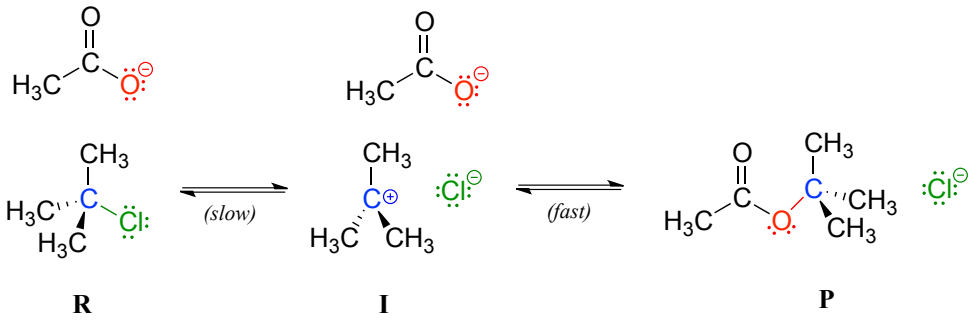
Both reaction A and reaction B are slightly endergonic, or uphill: ΔG°_{rxn} for both is positive, meaning that K_{eq} for both is between 0 and 1. However, the energy of activation is higher for reaction B. From this observation, we know that reaction A will proceed faster than reaction B in both forward and reverse directions (temperature and other conditions being equal), so reaction A will reach equilibrium in less time.

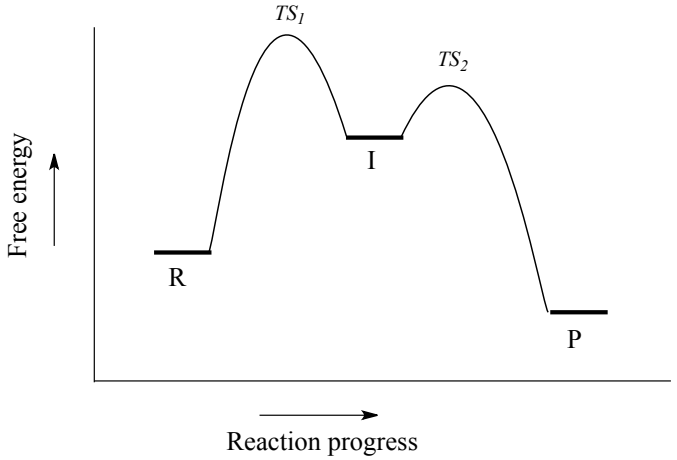
Exercise 6.4: Consider the hypothetical reaction coordinate diagrams below, and assume that they are on the same scale.



a)Which of the diagrams describe(s) a reaction with $K_{eq} < 1$?
b) Which of the diagrams describes the fastest reaction?
c) Which of the diagrams describes the reaction with the highest value of K_{eq}?
d) Which of the diagrams describes the reaction with the largest $\Delta G^{\circ\ddagger}$ for the *reverse* reaction?
d) Copy the diagram for your answer to part (d), and add a label which graphically illustrates the value of $\Delta G^{\circ\ddagger}$ for the reaction in the *reverse* direction.

We turn our attention next to a two-step reaction mechanism, such as the nucleophilic substitution reaction between acetate and *tert*-butyl chloride. The reaction coordinate diagram for this reaction looks somewhat different from what we have seen until now:



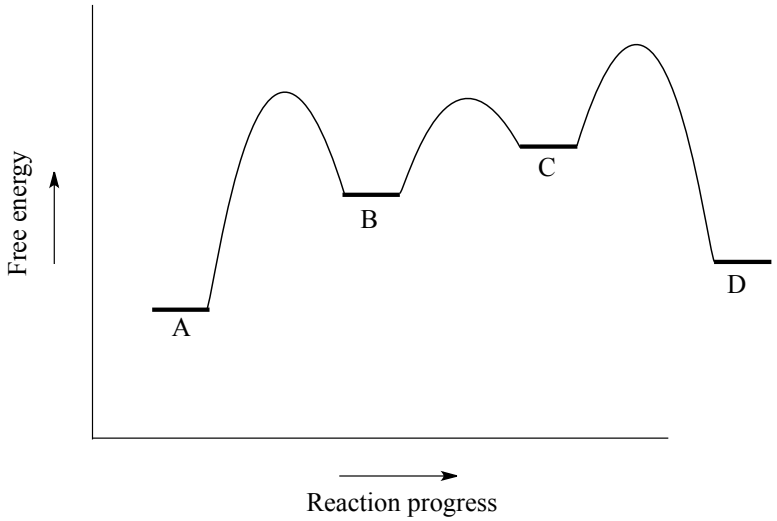


Because there are two steps involved, there are also two transition states and two activation energies to consider, as well as a carbocation intermediate (denoted by the letter I). The first, bond-breaking step from R to I, passing over transition state TS_1, can be depicted as a highly endergonic (uphill) reaction, because the carbocation-chloride ion pair is significantly higher in energy than the reactants. The second step, attack on the carbocation electrophile by the acetate nucleophile and formation of the new carbon-oxygen bond, is a highly exergonic step that passes over a second, lower energy transition state TS_2. The intermediate (I) is thus depicted as an energy 'valley' (a local energy minimum) situated between the two energy peaks TS_1 and TS_2.

Notice that the activation energy for the first step is higher than the activation energy for the second, meaning that the first step is slower. This should make intuitive sense, because the first step involves bond-breaking, separation of charge, and formation of a carbocation, which is high in energy due to lacking a complete octet. Conversely, the second step involves bond-forming and neutralization of charge. In a multi-step reaction, the slowest step - the step with the highest energy of activation - is referred to as the **rate-determining step** (rds). The rds can be thought of as the 'bottleneck' of the reaction: a factor which affects the rds will affect the overall rate of the reaction. Conversely, a factor which affects a much faster step will not significantly affect the rate of the overall reaction.

Exercise 6.5: Imagine that you are trying to extinguish a burning campfire using buckets of water filled from a faucet some distance from the fire. It takes 20 seconds to fill a bucket at the faucet, and two seconds to carry the bucket to the fire and dump it on the flames. a) If you double the speed at which you carry the buckets by running instead of walking, by what percent will you speed up the entire process? b) If, instead, you realize you can double the speed at which you fill up the buckets by opening the faucet a couple of turns, by what percent will you speed up the entire process? c) What is the rate-determining step for the process?

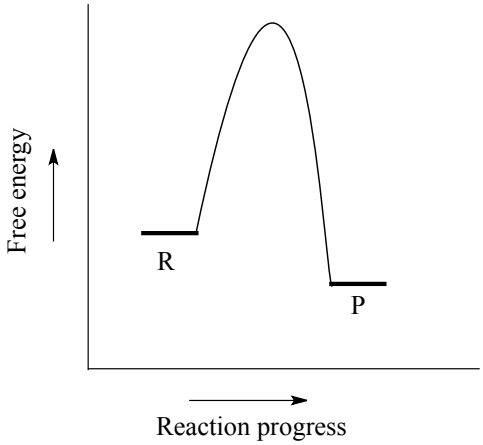
Exercise 6.6: Use the reaction coordinate diagram below to answer the questions.



a) Is the overall reaction endergonic or exergonic in the forward (A to D) direction?
b) How many steps does the reaction mechanism have?
c) How many intermediates does the reaction mechanism have?
d) Redraw the diagram and add a label showing the activation energy for the rate-determining step of the forward reaction.
e) Add a label showing ΔG^{o}_{rxn} for the *reverse* reaction (D to A).
f) What is the fastest reaction step, considering *both* the forward and reverse directions?

Section 6.3: Catalysis

Consider a hypothetical reaction R → P described by the diagram below.



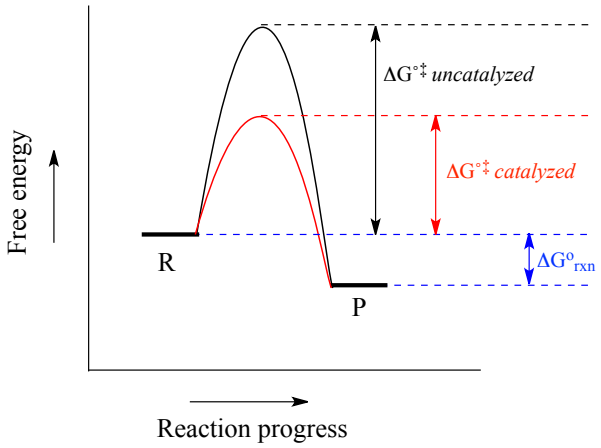
We notice two things about this reaction: it is exergonic, and it has a high activation energy. What this means is that although it is thermodynamically favorable, it is also slow: in other words, equilibrium favors product over reactants, but it will take a long time to reach equilibrium.

There are three ways that we could increase the rate of the reaction. First, we could add energy to the system by increasing the temperature, which gives the reacting molecules more energy to pass over the transition state. Increasing the temperature will increase the value of the rate constant *k* in the rate expression:

rate = *k* [R]

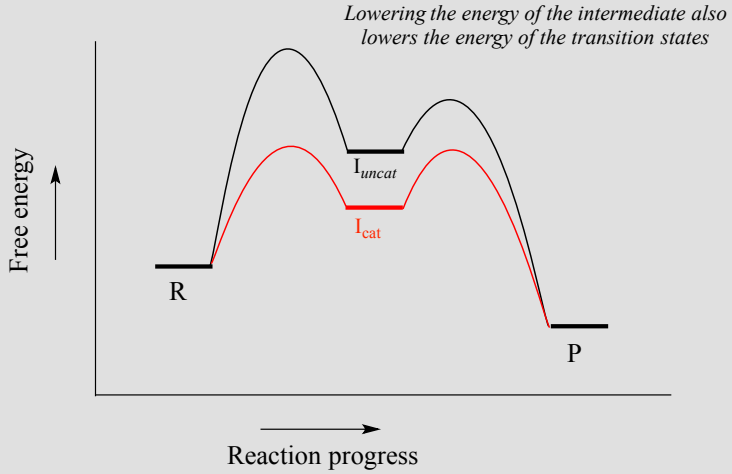
In the laboratory, many organic reactions are run at high temperatures for this very purpose. We could also increase the concentration of the reaction R, which would increase the rate of the reaction without increasing the value of *k*.

When talking about the biochemical reactions happening in a living cell, however, increasing the reaction temperature or reactant concentration is not a reasonable option. As an alternative, we could provide a *new route* from point R to point P in which the activation energy is lower. The role of a **catalyst** is to accelerate a reaction by stabilizing the transition state, and thus lowering the activation energy.



Catalysts, while they participate in the mechanism, are not consumed, so one catalyst molecule can catalyze multiple reaction cycles. Notice also that while the catalyst lowers the energy of the transition state (and thus the activation energy), it has no effect on ΔG_{rxn}. ***A catalyst increases the rate of a reaction, but does not get consumed in the reaction and does not alter the equilibrium constant***. In other words, a catalyst affects the *kinetics* of a reaction, but not the *thermodynamics*. Catalysts play a hugely important role in biochemical reactions.

Most organic reactions involve more than a single mechanistic step. Below is a reaction coordinate diagram illustrating rate acceleration of a two-step reaction by a catalyst:



Notice that the catalyst lowers the energy of the intermediate species. A concept known as the **Hammond Postulate** (the details of which are beyond our scope here) tells us that ***when a catalyst lowers the energy of an intermediate, it also lowers the energy of the adjacent transition states.*** Note in this diagram above that the energy barrier for the rate-determining first step is much lower in the catalyzed reaction - thus, the overall reaction is faster. When studying how an enzyme catalyzes a biochemical reaction, chemists often are actually looking at how the enzyme interacts with - and stabilizes - an intermediate species following a rate-determining step. The Hammond postulate tells us that an

understanding of enzyme-*intermediate* interactions will also apply to enzyme-*transition state* interactions.

Acids and bases as are commonly used as catalysts in organic chemistry, and chemists have come up with a huge arsenal of catalysts, many of them metals, to speed up the rates of useful laboratory reactions. Almost all biochemical reactions are catalyzed by **enzymes**, which are protein catalysts. In the introduction to this chapter, we heard the story of the discovery of a heat-stable DNA polymerizing enzyme which turned out to be very useful to the scientific world.

How do enzymes accomplish their role as biochemical catalysts? Recall from section 1.3D that enzymes have an active site pocket in which substrates (reactant molecules) are bound. It is inside these active site pockets that most biochemical reactions take place. Enzymes achieve catalysis in the active site by some combination of the following:

I: By positioning two reacting molecules close to each other in the active site, in precisely the orientation necessary for them to react. Compare this to an uncatalyzed reaction in which completion depends on the two reactant molecules happening to collide, by chance, in the correct orientation.

II: By binding substrates in such a way that they assume the proper conformation necessary for a reaction to occur.

III: By increasing the reactivity of the substrates: making acidic protons more acidic, nucleophiles more nucleophilic, electrophiles more electrophilic, and leaving groups better at leaving. Very often, these feats are accomplished with acidic and/or basic amino acid side chains lining the active site pocket. As we go on to study many different types of biochemical reactions, we get a better of how this works.

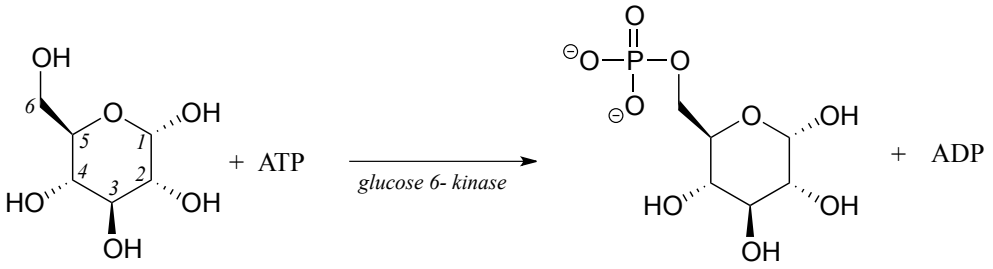
IV: By stabilizing the transition states of the slower, rate-determining steps of the reaction. If a transition state has a negative charge, for example, the enzyme might provide a positively charged amino acid side chain, or a bound metal cation such as Zn^{+2}, as a stabilizing factor. A lower-energy transition state, of course, means a lower activation energy and a faster reaction step.

Enzymes are capable of truly amazing rate acceleration. Typical enzymes will speed up a reaction by anywhere from a million to a billion times, and the most efficient enzyme currently known to scientists is believed to accelerate its reaction by a factor of about 10^{17} over the uncatalyzed reaction (see *Chemical and Engineering News*, March 13, 2000, p. 42 for an interesting discussion about this nucleotide biosynthesis enzyme called 'orotidine monophosphate decarboxylase').

At this point, it is not necessary for you to fully understand the four ideas listed above: just keep them in mind as we go on to study a variety of common biological organic reactions and see in greater detail how enzymes have evolved to catalyze them.

Exercise 6.7: Table sugar, or sucrose, is a high-energy dietary compound, as are the fats in vegetable oil. Conversion of these compounds, along with oxygen gas (O_2), to water and carbon dioxide releases a lot of energy - fat contains a little over twice as much energy as sugar, per gram. If they are both so high in energy (in other words, thermodyamically *unstable*), how can they sit for years on your kitchen shelf without reacting?

Another very important point to keep in mind about enzymes is the *specificity* with which they catalyze reactions. We have already discussed, in chapter 3, the idea that enzymes exert a very high level of control over the stereochemistry of a reaction: if two or more stereoisomeric products could potentially form in a reaction, an enzyme will likely only catalyze the formation of one stereoisomer, with negligible formation of other side products. Likewise, enzymes demonstrate remarkable control of **regiochemistry** in their reactions. The glycolysis pathway enzyme glucose-6-kinase, for example, transfers a phosphate group specifically to the hydroxyl group on carbon #6 of glucose, and not to any of the other four hydroxyl groups. We'll look more closely at this reaction and others like it in chapter 9.



glucose glucose-6-phosphate

Finding ways to maintain control over stereochemistry and regiochemistry is a constant challenge for synthetic organic chemists working with non-enzymatic reactions, and the techniques that have been developed in this arena are a big part of what you will study if you go on to take a more advanced course focusing on organic synthesis.

Finally, we will encounter many biochemical reactions in this book in which the enzyme catalyzing the reaction does so with the assistance of a **coenzyme**. A coenzyme is a small (relative to a protein) molecule that binds in the active site of a partner enzyme and participates in some manner with the reaction being catalyzed. Table 6 at the back of the book shows the structures of several coenzymes commonly seen in biochemical reactions.

Section 6.4: Comparing biological reactions to laboratory reactions

The focus of this book is on organic chemistry in a biological context. At various points in our investigation of organic reactivity, however, we will also be considering some non-biological, laboratory counterparts of reactions that occur in living cells. The reason for this is two-fold: first of all, it is often instructive to compare and contrast similar reactions taking place in very different environments, and sometimes the similarities are quite striking. Secondly, even those who intend to pursue a career in the life or health sciences can benefit from some exposure to the kind of challenges that professional organic or medicinal chemists work on: if you are working as a biologist for a pharmaceutical company for example, you will be better able to appreciate the contributions of your chemist colleagues if you are able to make the intellectual connection between the reactions they are running in flasks and the those that are taking place in the cells you are studying.

Below we briefly outline the differences between laboratory and biological reactions:

Catalysts: The vast majority of biological organic reactions are catalyzed by enzymes. While chemists synthesizing molecules in the laboratory sometimes make use of enzyme-catalyzed reactions, it is much more common to use non-biological catalysts (often containing transition metals), acids or bases as catalysts, or no catalyst at all.

Solvent: Biological organic reactions occur in the aqueous environment of the cell. In the laboratory, organic reactions can be run in a wide variety of solvents, ranging from the very nonpolar (such as hexane) to the very polar, such as methanol,water, or even ionic liquids. Most commonly, though, laboratory reactions are run in relatively non-polar solvents such as diethyl ether or dichloromethane.

Reactant mixture: The aqueous environment of a cell is an extremely complex mixture of thousands of different biomolecules in solution at low concentrations (usually nanomolar to millimolar), whereas the components of a laboratory reaction have usually been purified, and are present in much higher concentrations.

Temperature: Biological reactions take place within a narrow temperature range specific to the organism: a little too cold and the enzymes catalyzing the reactions are 'frozen', a little too hot and the enzymes will come unfolded, or 'denature'. Laboratory reactions can be run at a variety of temperatures, sometimes at room temperature, sometimes at the boiling point of the solvent, and sometimes at very low temperatures (such as when a reaction flask is immersed in a dry ice-acetone bath).

pH: Biological reactions take place in aqueous solution buffered to a specific pH: about pH 7 for most living things. Accordingly, highly acidic or basic species are unlikely to be reactants or intermediates in a biological reaction mechanism. Laboratory reactions are often carried out in the presence of strong acids or bases.

Key concepts for review

You should be confident in interpreting and using the **curved arrow** drawing convention for showing two-electron movement. Given a set of curved arrows describing a reaction step, you should be able to draw the product indicated by the arrows. Alternatively, given the starting structure and a product for a reaction step, you should be able to draw the curved arrows showing how bonds were broken and formed. You need not understand (yet) the chemistry behind these steps, you just need to be able to use the drawing formality.

You should be able to recognize three reaction mechanism types: an **acid-base reaction,** a **one-step nucleophilic substitution**, and a **two-step nucleophilic substitution**.

Given an example reaction, you should be able to identify a **nucleophile**, **electrophile**, and in many cases a **leaving group**.

Given an example reaction mechanism, you should be able to recognize one or more **reaction intermediates**.

Given a reaction coordinate diagram for a hypothetical reaction, you should be able to recognize whether the reaction is endergonic or exergonic, and whether the equilibrium constant is greater than or less than 1. You should be able to identify the point(s) on the diagram corresponding to **transition state**(s) and reaction intermediate(s). In a multi-step reaction diagram, you should be able to identify the **rate determining step**.

Given a detailed reaction process showing starting reactant(s), intermediate(s), and product(s) with associated curved arrows, you should be able to sketch a reaction coordinate diagram that that is consistent with the details of the reaction mechanism.

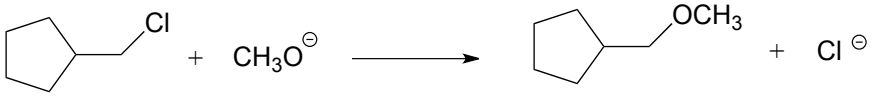
You should be able to explain the role of a **catalyst** in a reaction.

You should be able to list the major differences between a typical biological reaction and a typical laboratory reaction.

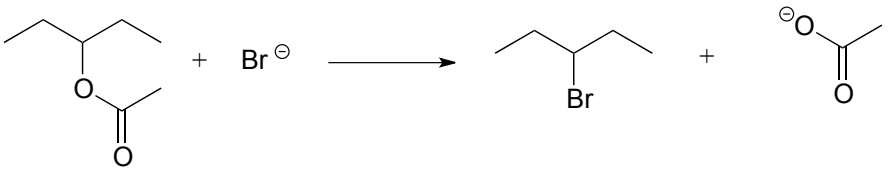
Problems

P6.1: For each of the nucleophilic substitution reactions below, identify the atoms which are acting as nucleophile, electrophile, and leaving group, and draw a curved-arrow diagram showing a *one-step* mechanism.

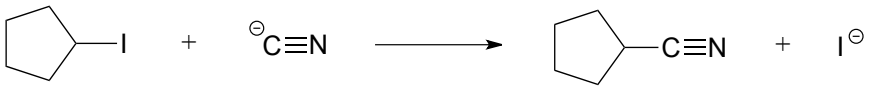
a)



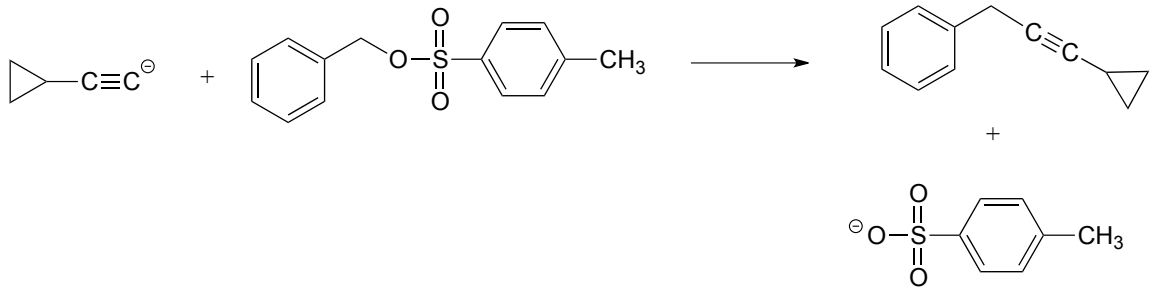
b)



c)

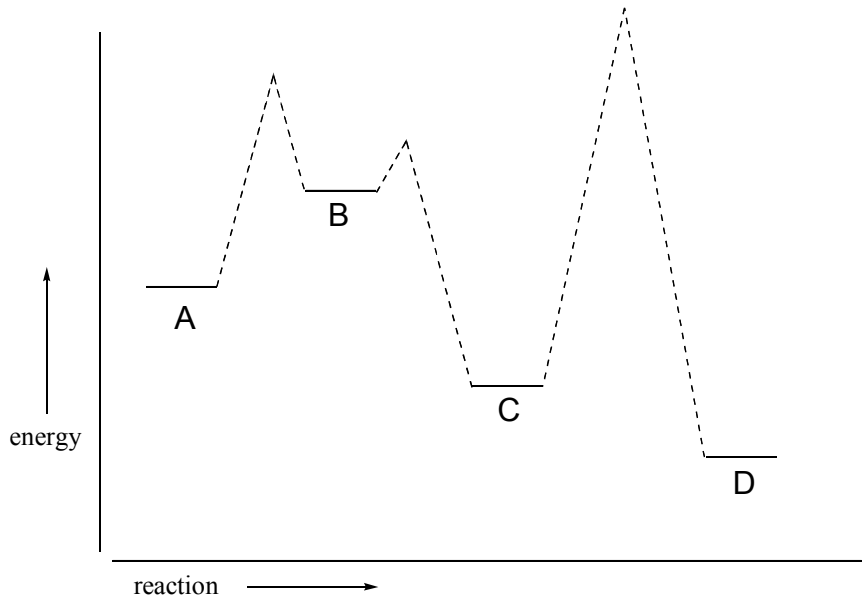


d)

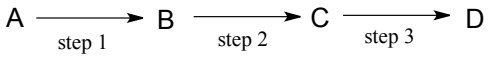


P6.2: Below is a reaction coordinate diagram for a hypothetical reaction.

a) What can you say about the value of K_{eq} for the overall A to D transformation?
b) What is the rate determining step for the overall A to D transformation?
c) Which step is faster, A to B or B to C?
d) Which transformation is more thermodynamically favorable, A to B or C to D?

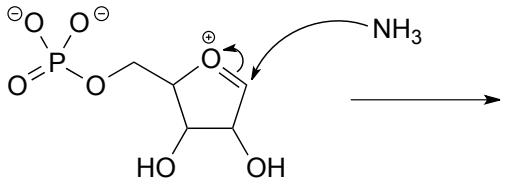


e) Below is a diagram of a hypothetical reaction. Step 2 is the rate-determining step, C is the least stable species, B is higher energy than D, and the overall reaction has an equilibrium constant $K_{eq} = 0.33$. Draw a diagram that corresponds to all of this information.

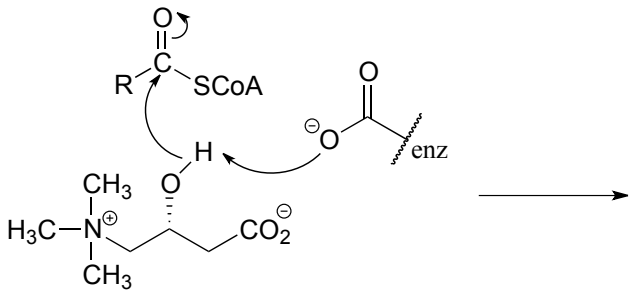


P6.3: Illustrated below are individual steps in some biochemical reaction mechanisms that we will be studying later. For each step, draw the products or intermediate species that would form according to the electron-movement arrows given. Be sure to include all formal charges. You do not need to show stereochemistry.

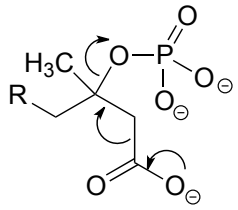
a)



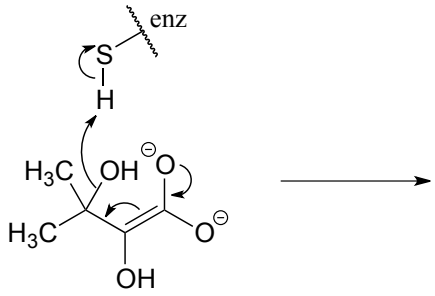
b)



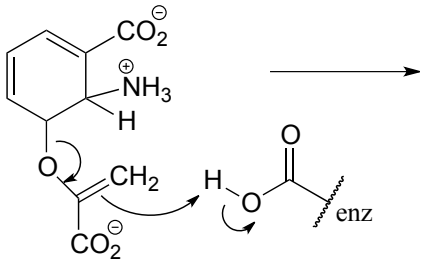
c)



d)

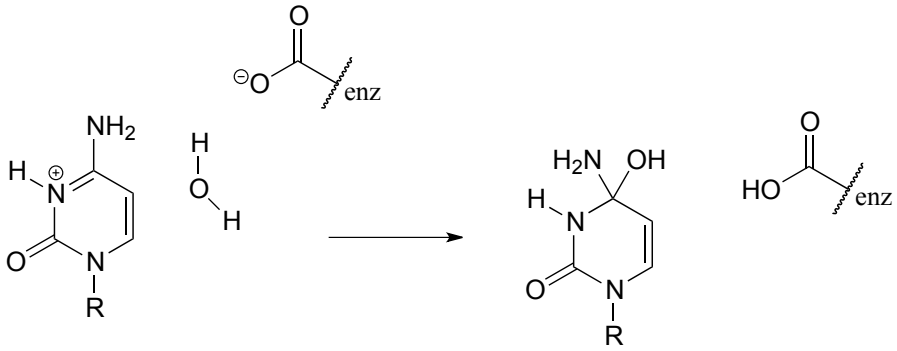


e)

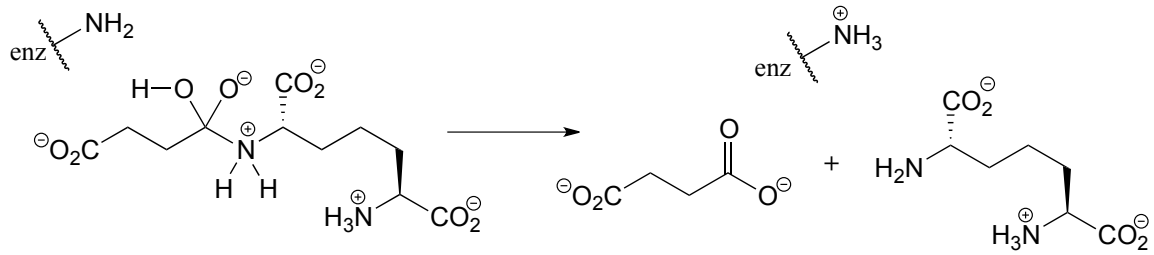


P6.4: Shown below are individual steps in some biochemical reaction mechanisms that we will be studying later. For each, draw curved arrows showing the electron movement taking place.

a)



b)



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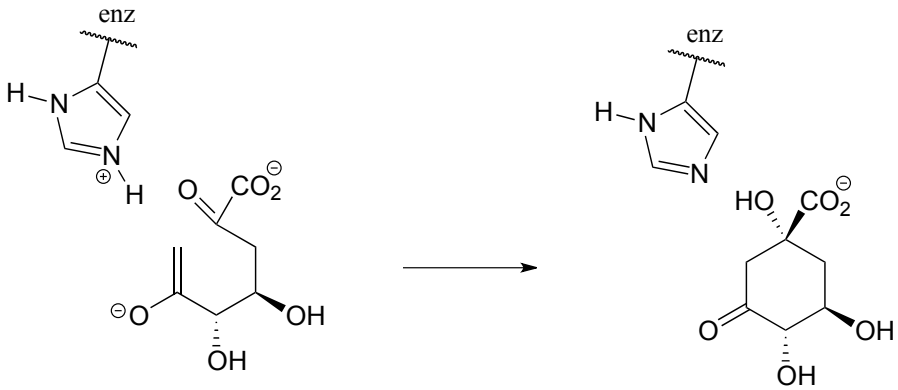
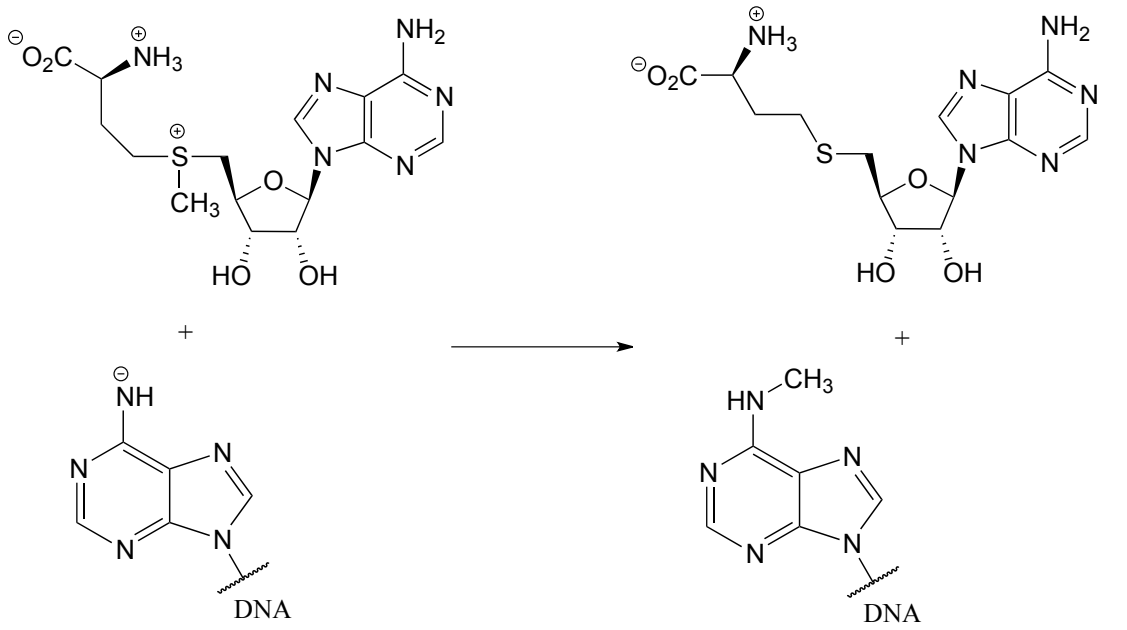


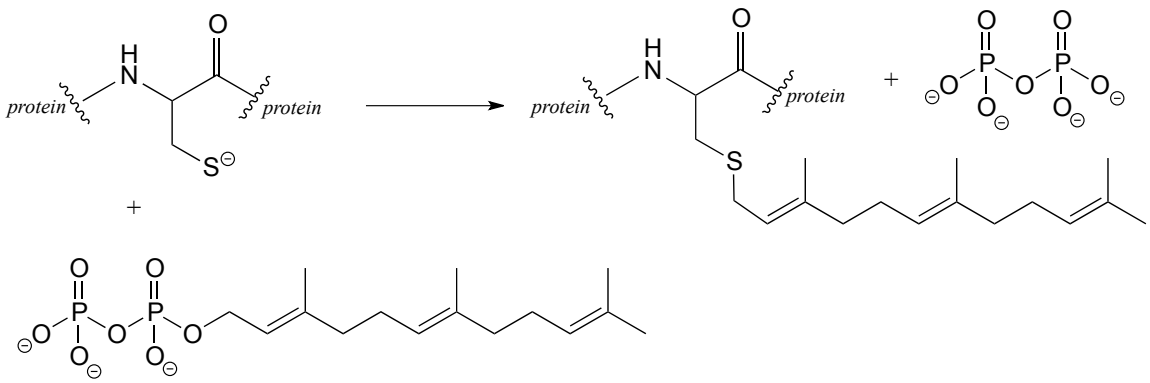
P6.5:

a) In the biochemical nucleophilic substitution reactions illustrated below, identify the atoms which are acting as nucleophile, electrophile, and leaving group.

Reaction 1:



Reaction 2:



b) Using appropriate 'R' abbreviations for regions of the molecules that are not directly involved in bond-breaking or bond-forming events above, draw curved arrows showing the electron movement that takes place in each step. Assume that both are one-step mechanisms.

Chapter 7

Acid-base reactions

Helicobacter pylori

Credit: https://www.flickr.com/photos/ajc1/

The glass flask sitting on a bench in Dr. Barry Marshall's lab in Perth, Western Australia, contained about thirty milliliters of a distinctly unappetizing murky, stinking yellowish liquid.

A few days earlier, Dr. Marshall, in consultation with his mentor and research partner Dr. J. Robin Warren, had poured a nutrient broth into the flask, then dropped in a small piece of tissue sample taken from the stomach of a patient suffering from chronic gastritis. Gastritis is an inflammation of the stomach lining characterized by lingering pain, nausea, and vomiting, and is often is the precursor to a peptic ulcer. Now, after several days of incubating in a warm water bath, the flask contained a living liquid culture, swarming with billions of bacterial cells. According to the medical textbooks lining Dr. Marshall's bookshelves, the bacteria should not have been there – nothing should have grown in the broth, because the highly acidic environment inside the

stomach was supposed to be sterile. Also according to the textbooks, the cause of his patient's stomach ailment was stress, or perhaps a poor diet – but most definitely not a bacterial infection.

Dr. Marshall took a good, long, look at the contents of the flask. Then he gave it a final swirl, and drank it down.

Five days later, his stomach started to hurt.

This is the story of how two doctors dared to think what no one else had thought, and turned an established medical doctrine upside-down. Dr. J. Robin Warren was a pathologist at the Royal Perth Hospital in Perth, Western Australia. One day, when examining an image taken from a stomach biopsy from a patient with severe gastritis, he noticed what appeared to be spiral-shaped bacteria in the tissue, a surprising observation given the medical consensus that bacteria could not live in the stomach.

The microbe-like shapes were very hard to see, but when he tried treating the sample with a silver stain they became much more apparent. He decided to start looking at silver-stained sections of every stomach biopsy he examined, and before long he noticed a pattern: the presence of the spiral bacteria coincided with gastritis in the patient, and the tissues which were more severely inflamed seemed also to have more bacteria present. Could there be a causal link between the bacteria and the illness?

When he discussed his observations with colleagues, they dismissed the results as coincidental contamination – nothing important.

Warren wasn't willing to just let it go. He was able to interest Barry Marshall, a young internal medicine resident training at the same hospital, in taking on a research project to try and solve the mystery of the bacteria that were not supposed to exist. Marshall started by doing a thorough search of the literature, and found that his mentor was not the first to report seeing spiral-shaped bacteria in stomach tissue – there were in fact several such observations in the literature, the oldest going back to the middle of the 19^{th} century. All of them had been dismissed as unimportant artifacts.

Warren and Marshall started sending stomach biopsies from gastritis patients to the microbiology lab in the hospital, to see if bacteria from the samples could be cultured in a petri dish. For many months, they got nothing. Then one Tuesday morning, right after the four-day Easter holiday, Marshall got a call from an excited microbiology technician: he had neglected to dispose of the latest round of test cultures before going home for the holiday, and instead had left them in the incubator the whole time. After growing for a full five days, the dishes had colonies of bacteria growing in them. All this time, the technicians had been throwing the cultures away after two days when no bacterial growth was evident – standard procedure when working with other bacteria – but apparently these bacteria were especially slow-growing.

Now that he could culture the bacteria in the lab, he was able to isolate and study them in more detail, and gave them the name *Helicobacter pylori*. Marshall confirmed Warren's earlier observation of a correlation between stomach bacteria and gastritis: only those patients suffering from gastritis or ulcers seemed to have the bacteria in their stomachs. There seemed to be strong evidence that *H. pylori* infection led to gastritis, which in turn lead eventually to stomach ulcers.

Despite the new data, Warren and Marshall's colleagues in the gastroenterology field were still unconvinced of the link between bacteria and gastritis or ulcers. Scientists are inherently skeptical people, and it was difficult to overcome the long-entrenched theory that the stomach was sterile, and that ulcers were caused by stress. Interviewed much later by Discovery Magazine, Marshall recalled: "To gastroenterologists, the concept of a germ causing ulcers was like saying that the Earth is flat".

Poring once more over the available literature, Marshall learned that acid-reducing drugs – an enormously profitable product– were able to relieve ulcer symptoms, but only temporarily. One interesting piece of information stood out to him, though: an over-the-counter antacid containing the element bismuth (similar the brand-name medicine Pepto-Bismol) provided much longer-lasting relief compared to the other drugs – and in some cases seemed to effect a permanent cure. Marshall soaked a small circle of filter paper in the bismuth medicine, and placed it on a petri dish that he had inoculated with *H. pylori*. After five days in the incubator, there was a clear circle of non-growth around the filter paper. The medicine had killed the bacteria.

Everything seemed to fit together: almost all patients with gastritis or ulcers had *H. pylori* infections, and a drug which was able to kill the bacteria was also effective against the stomach ailment. But to convince the medical community that the root cause of ulcers was *H. pylori* infection, Warren and Marshall needed more direct evidence: they had to show that a healthy stomach – free from gastritis and uninfected by *H. pylori* – would develop gastritis as a result of intentional infection, and that clearing up the infection would also cure the gastritis. They tried experiments with pigs first, then rats, and then mice, but to no avail – they were not able to induce an *H. pylori* infection in the animal models. Marshall was getting desperate: all around him he saw patients suffering terribly, getting only temporary relief from acid blockers and eventually needing to have parts of their stomachs removed, and he was convinced that simple antibiotic therapy would cure them if only their doctors could be convinced to try it. Because animal models had failed, he decided to move to a model system that he knew would work: humans. Ethical and regulatory considerations prevented him from intentionally infecting human volunteers - so his only option was to use himself as a guinea pig.

We are now at the point in the story where Barry Marshall, in the name of science and medicine, took his disgusting but undeniably courageous gulp of bacteria-laden broth. He had already undergone an endoscopy to ascertain that his stomach was free from both inflammation and *H. pylori*. As we already know, he started to develop symptoms of gastritis about five days after drinking the bacteria – the same amount of time that it

took for *H. pylori* colonies to appear in the petri dish cultures. After a few more days, he underwent another endoscopy, and was overjoyed to be told that his stomach was indeed inflamed, and was infested with spiral-shaped bacteria. He initially wanted to carry on the experiment for a few more days of further tests, but his wife had a different opinion on the matter and convinced him to begin antibiotic treatment, which quickly cleared up both his infection and his stomach inflammation.

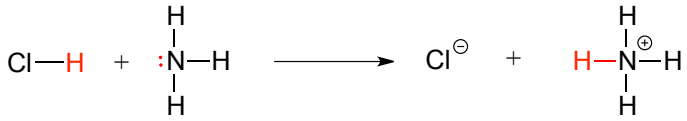
Marshall and Warren now had clear, direct evidence that the stomach inflammation which leads to ulcers was caused by bacteria, and could be cured with antibiotics. They submitted a summary of their results for presentation at a meeting of the Gastroenterological Society of Australia, but were rejected. Apparently, 67 submissions had come in and there was only time for 56 presentations; unfortunately their results were not considered important enough to make the cut. They persevered, and eventually published their findings in the June, 1984 issue of the British medical journal *The Lancet*.

The paper gained some notice, especially from microbiologists, but did not have an immediate impact in clinical practice. Around the world, gastroenterologists continued to treat ulcers with acid blockers. Outside of the mainstream medical community, however, word was getting out that two Australian doctors had a cure for ulcers, and more people started coming to them for treatment. Stories about Warren and Marshall appeared in places like Reader's Digest and The National Enquirer, and eventually in the United States the National Institutes of Health and the Food and Drug Administration responded by fast-tracking the clinical testing and approval process, and publicizing the new treatment option.

Stomach ulcers, which have been tormenting human beings since the beginning of recorded history, are today considered an easily curable condition, and the idea that they are caused by *H. pylori* infection is fully accepted by the medical community. Drs. J. Robin Warren and Barry Marshall shared the 2005 Nobel Prize in Medicine.

The now-discredited idea that the stomach is a sterile place made perfectly good biochemical sense at the time: the stomach is like a bathtub full of hydrochloric acid, which you probably recall from previous chemistry classes and labs is a very strong, dangerously corrosive acid. It is very difficult to imagine how a microbe could survive in such an environment. We now know that *H. pylori* can thrive there in part because its spiral shape allows it to burrow deep into the protective layer of mucus that coats the stomach wall. In addition, *H. pylori* cells produce large amounts of an enzyme called urease, which catalyzes a reaction between urea and water to form carbon dioxide and ammonia.

Ammonia - the main component of window-washing liquid - is a fairly strong base, and reacts rapidly and completely with hydrochloric acid to neutralize it.



Although scientists are still unsure of all the details, it is likely that these two protective strategies used by *H. pylori* somehow play a role in causing the inflammation that can lead to peptic ulcers, where the stomach lining becomes exposed to the harsh action of hydrochloric acid.

The idea of acidity is at the heart of our story about the discovery of *H. pylori*, and is the subject of this chapter. From here on in our study of organic chemistry, we will be learning about how organic molecules react, and how their structure determines their reactivity. The reaction between an acid and a base - where a proton is donated from the former and accepted by the latter - is the first kind of organic reaction that we will explore. After reviewing some basic ideas about acid-base equilibria with which you are probably already familiar from General Chemistry, we will dive into some very challenging new waters, as we attempt to use our understanding of organic structure to predict how different organic functional groups will react in an acid-base context. Many of the ideas that are introduced in this chapter, though perhaps difficult to grasp at first, will be crucial to understanding not only acid base chemistry but all of the other organic reaction types that we will see throughout the remainder of the book.

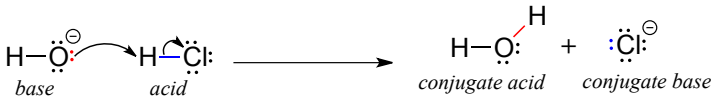
Additional reading:

Discover Magazine, March 7, 2010. *The Dr. Who Drank Infectious Broth, Gave Himself an Ulcer, and Solved a Medical Mystery*

Section 7.1: Overview of acid-base reactions

7.1A: The Brønsted-Lowry definition of acidity and basicity

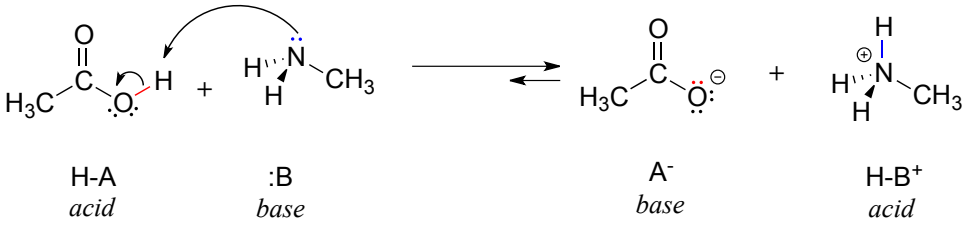
We'll begin our discussion of acid-base chemistry with a couple of essential definitions. The first of these was proposed in 1923 by the Danish chemist Johannes Brønsted and the English chemist Thomas Lowry, and has come to be known as the **Brønsted-Lowry definition of acidity and basicity**. An acid, by the Brønsted-Lowry definition, is a species which acts as a proton donor, while a base is a proton acceptor. We have already discussed in the previous chapter one of the most familiar examples of a Brønsted-Lowry acid-base reaction, between hydrochloric acid and hydroxide ion:



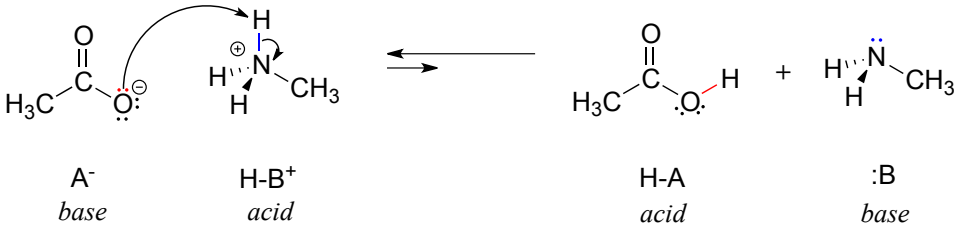
In this reaction, a proton is transferred from HCl (the acid, or proton *donor*) to hydroxide ion (the base, or proton *acceptor*). As we learned in the previous chapter, curved arrows depict the movement of electrons in this bond-breaking and bond-forming process.

After a Brønsted-Lowry acid donates a proton, what remains is called the **conjugate base**. Chloride ion is thus the conjugate base of hydrochloric acid. Conversely, when a Brønsted-Lowry base accepts a proton it is converted into its **conjugate acid** form: water is thus the conjugate acid of hydroxide ion.

Here is an organic acid-base reaction, between acetic acid and methylamine:



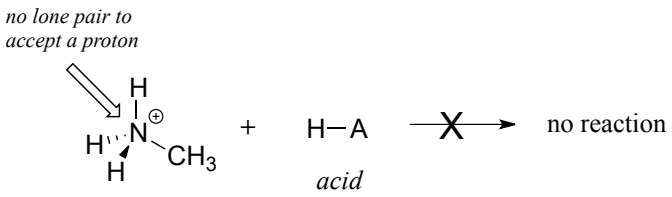
In the reverse of this reaction, acetate ion is the base and methylammonium ion (protonated methylamine) is the acid.



What makes a compound acidic (likely to donate a proton) or basic (likely to accept a proton)? Answering that question is one of our main jobs in this chapter, and will require us to put to use much of what we learned about organic structure in the first two chapters, as well as the ideas about thermodynamics that we reviewed in chapter 6.

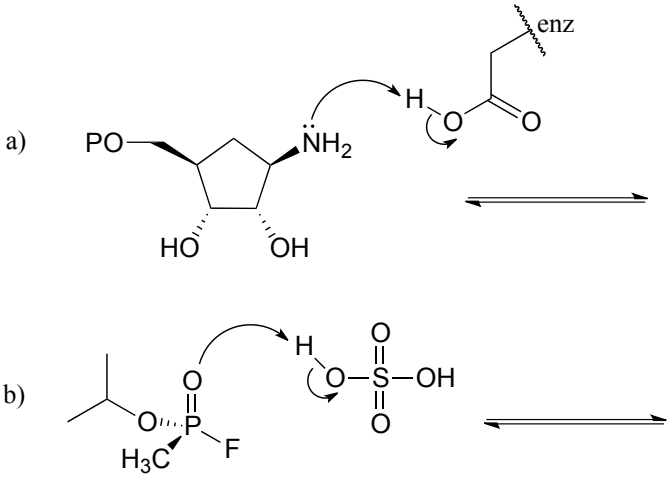
For now, let's just consider one common property of bases: *in order to act as a base, a molecule must have a reactive pair of electrons*. In all of the acid-base reactions we'll see in this chapter, the basic species has an atom with a lone pair of electrons. When acetate ion acts as a base in the lower reaction shown in figure 2 above, for example, one of its oxygen lone pairs is used to form a new bond to a proton. The same can be said for an amine acting as a base in upper part of figure 2.

Clearly, methylammonium ion cannot act as a base – it does not have a reactive pair of electrons with which to accept a proton.



Later, in chapter 14, we will study reactions in which a pair of electrons in a π bond of an alkene or aromatic ring act in a basic fashion - but for now, will concentrate on the basicity of non-bonding (lone pair) electrons.

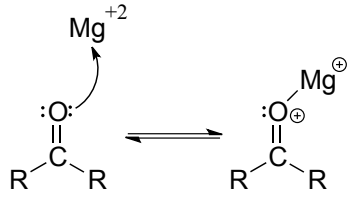
Exercise 7.1: Complete the reactions below - in other words, draw structures for the missing conjugate acids and conjugate bases that result from the curved arrows provided.



7.1B: The Lewis definition of acidity and basicity

The Brønsted-Lowry picture of acids and bases as proton donors and acceptors is not the only definition in common use. A broader definition is provided by the **Lewis definition of acidity and basicity**, in which a **Lewis acid** is an electron-pair acceptor and a **Lewis base** is an electron-pair donor. This definition covers Brønsted-Lowry proton transfer reactions, but also includes reactions in which no proton transfer is involved. The interaction between a magnesium cation (Mg^{+2}) and a carbonyl oxygen is a common example of a Lewis acid-base reaction in enzyme-catalyzed biological

reactions. The carbonyl oxygen (the Lewis base) donates a pair of electrons to the magnesium cation (the Lewis acid).



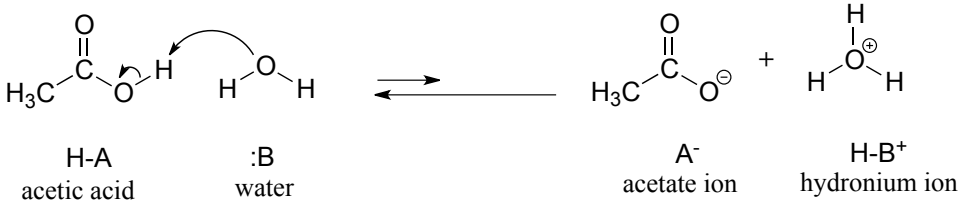
While it is important to be familiar with the Lewis definition of acidity, the focus throughout the remainder of this chapter will be on acid-base reactions of the (proton-transferring) Brønsted-Lowry type.

Section 7.2: The acidity constant

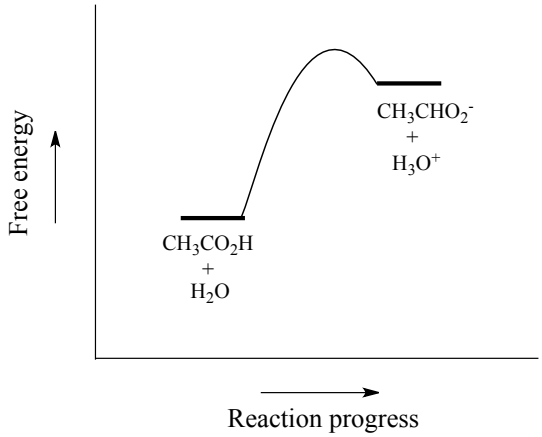
7.2A: Defining the acidity constant

You are no doubt aware that some acids are stronger than others. The relative acidity of different compounds or functional groups – in other words, their relative capacity to donate a proton to a common base under identical conditions – is quantified by a number called the **acidity constant**, abbreviated $\mathbf{K_a}$. The common base chosen for comparison is water.

We will consider acetic acid as our first example. If we make a dilute solution of acetic acid in water, an acid-base reaction occurs between the acid (proton donor) and water (proton acceptor).



Acetic acid is a weak acid, so the equilibrium favors reactants over products - it is thermodynamically 'uphill'. This is indicated in the figure above by the relative length of the forward and reverse reaction arrows.



The equilibrium constant K_{eq} is defined as:

$$K_{eq} = \frac{[\text{products}]}{[\text{reactants}]} = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]\,[H_2O]}$$

Remember that this is a dilute aqueous solution: we added a small amount of acetic acid to a large amount of water. Therefore, in the course of the reaction, the concentration of water (approximately 55.6 mol/L) changes very little, and can be treated as a constant.

If we move the constant term for the concentration of water to the left side of the equilibrium constant expression, we get the expression for K_a, the acid constant for acetic acid:

$$K_a = K_{eq}[H_2O] = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]}$$

In more general terms, the dissociation constant for a given acid HA or HB^+ is expressed as:

$$HA + H_2O \rightleftharpoons :A^- + H_3O^+ \qquad HB^+ + H_2O \rightleftharpoons :B + H_3O^+$$

$$K_a = \frac{[:A^-]\,[H_3O^+]}{[HA]} \qquad \textit{or} \qquad K_a = \frac{[:B]\,[H_3O^+]}{[HB^+]}$$

The value of K_a for acetic acid is 1.75×10^{-5} - much less than 1, indicating there is much more acetic acid in solution at equilibrium than acetate and hydronium ions.

Conversely, sulfuric acid, with a K_a of approximately 10^9, or hydrochloric acid, with a K_a of approximately 10^7, both undergo essentially complete dissociation in water: they are very strong acids.

A number like 1.75×10^{-5} is not very easy either to say, remember, or visualize, so chemists usually use a more convenient term to express relative acidity. The **pKa** value of an acid is simply the log (base 10) of its K_a value.

$pKa = -\log Ka$ $\qquad$ $Ka = 10^{-pKa}$

Doing the math, we find that the pKa of acetic acid is 4.8. The pKa of sulfuric acid is -10, and of hydrochloric acid is -7. The use of pKa values allows us to express the relative acidity of common compounds and functional groups on a numerical scale of about –10 (for a very strong acid) to 50 (for a compound that is not acidic at all). ***The lower the pKa value, the stronger the acid.***

The ionizable (proton donating or accepting) functional groups relevant to biological organic chemistry generally have pKa values ranging from about 5 to about 20. The most important of these are summarized below, with *very rough* pKa values for the conjugate acid forms. More acidic groups with pKa values near zero are also included for reference.

Typical pKa values

group	**approximate pKa**
hydronium ion (H_3O^+)	0
protonated alcohol	0
protonated carbonyl	0
carboxylic acids	5
protonated imines	7
protonated amines	10
phenols	10
thiols	10
alcohols, water	15
α-carbon acids*	20

**α-carbons acids will be explained in section 7.6A*

It is highly recommended to commit these rough values to memory now - then if you need a more precise value, you can always look it up in a more complete pKa table. The appendix to this book contains a more detailed table of typical pKa values, and much more complete tables are available in resources such as the *Handbook of Chemistry and Physics*.

pKa vs. pH

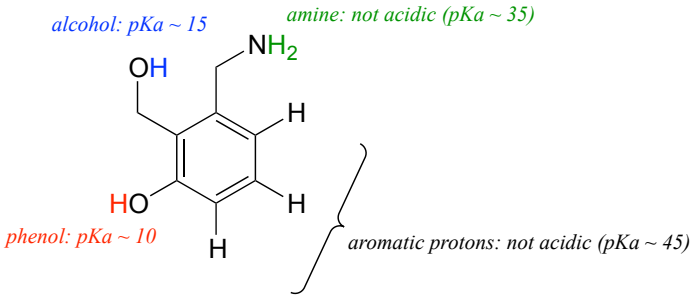
It is important to realize that pKa is *not* the same thing as pH: the former is an inherent property of a compound or functional group, while the latter is a measure of hydronium ion concentration in a given aqueous solution:

$pH = -\log [H_3O^+]$

Knowing pKa values not only allows us to compare acid strength, it also allows us to compare base strength. The key idea to remember is this: ***the stronger the conjugate acid, the weaker the conjugate base.*** We can determine that hydroxide ion is a stronger base than ammonia (NH_3), because ammonium ion (NH_4^+, pKa = 9.2) is a stronger acid than water (pKa = 15.7).

Exercise 7.2: Which is the stronger base, CH_3O^- or CH_3S^-? Acetate ion or ammonia? Hydroxide ion or acetate ion?

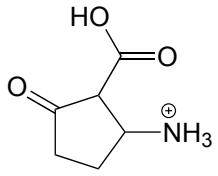
Let's put our understanding of the pKa concept to use in the context of a more complex molecule. For example, what is the pKa of the compound below?



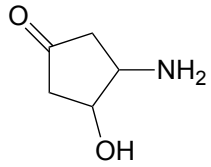
We need to evaluate the potential acidity of *four* different types of protons on the molecule, and find the most acidic one. The aromatic protons are not all acidic - their pKa is about 45. The amine group is also not acidic, its pKa is about 35. (Remember, uncharged amines are basic: it is positively-charged *protonated* amines, with pKa values around 10, that are weakly acidic.) The alcohol proton has a pKa of about 15, and the phenol proton has a pKa of about 10: thus, the most acidic group on the molecule above is the phenol. (Be sure that you can recognize the difference between a phenol and an alcohol - remember, in a phenol the OH group is bound *directly* to the aromatic ring). If this molecule were to react with one molar equivalent of a strong base such as sodium hydroxide, it is the phenol proton which would be donated to form a phenolate anion.

Exercise 7.3: Identify the most acidic functional group on each of the molecules below, and give its approximate pKa.

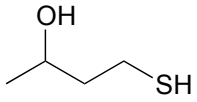
a)



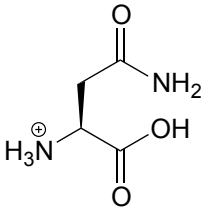
b)



c)

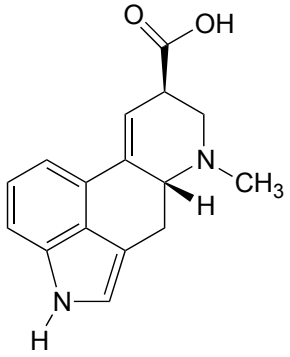


d)



asparagine

e)



lysergic acid

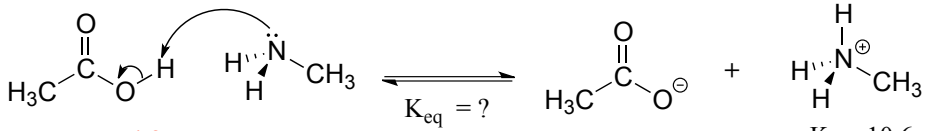
7.2B: Using pKa values to predict reaction equilibria

By definition, the pKa value tells us the extent to which an acid will react with water as the base, but by extension we can also calculate the equilibrium constant for a reaction between any acid-base pair. Mathematically, it can be shown that:

$$K_{eq} = 10^{\Delta pKa}$$

where ΔpKa = (pKa of product acid minus pKa of reactant acid)

Consider a reaction between methylamine and acetic acid:



$pK_a = 4.8$ *(stronger acid)*

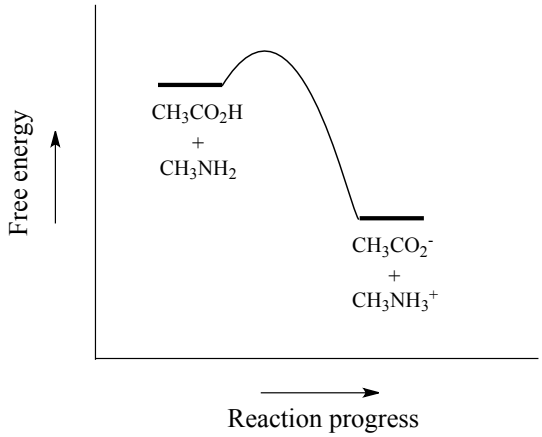
$pK_a = 10.6$

The first step is to identify the acid species on either side of the equation, and look up or estimate their pKa values. On the left side, the acid is of course acetic acid while on the right side the acid is methyl ammonium ion (in other words, methyl ammonium ion is the acid in the reaction going from right to left). We can look up the precise pKa values in table 7 (at the back of the book), but we already know (because we have this information memorized, right?!) that the pKa of acetic acids is about 5, and methyl ammonium is about 10. More precise values are 4.8 and 10.6, respectively.

Without performing any calculations at all, you should be able to see that this equilibrium lies far to the right-hand side: acetic acid has a lower pKa, meaning it is a stronger acid than methyl ammonium, and thus it wants to give up its proton more than methyl ammonium does. Doing the math, we see that

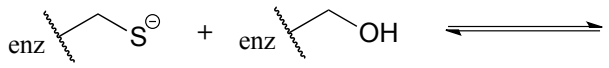
$K_{eq} = 10^{\Delta pKa} = 10^{(10.6 - 4.8)} = 10^{5.8} = 6.3 \times 10^5$

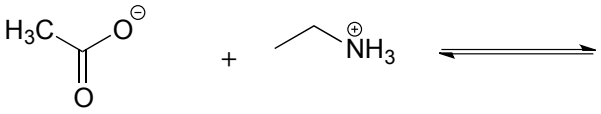
So K_{eq} is a very large number (much greater than 1) and the equilibrium for the reaction between acetic acid and methylamine lies far to the right-hand side of the equation, just as we had predicted. This also tells us that the reaction has a negative Gibbs free energy change, and is thermodynamically favorable.

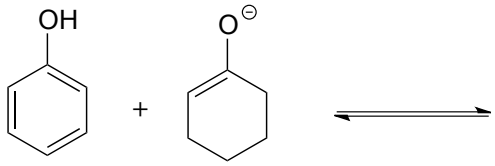


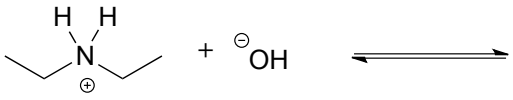
If you had just wanted to quickly approximate the value of K_{eq} without benefit of precise pKa information or a calculator, you could have approximated pKa ~ 5 (for the carboxylic acid) and pKa ~10 (for the ammonium ion) and calculated in your head that the equilibrium constant should be somewhere in the order of 10^5.

Exercise 7.4 Show the products of the following acid-base reactions, and roughly estimate the value of K_{eq}.

a) 

b) 

c) 

d) 

7.2C: Organic molecules in buffered solution: the Henderson-Hasselbalch equation

The environment inside a living cell, where most biochemical reactions take place, is an aqueous buffer with pH ~ 7. Recall from your General Chemistry course that a buffer is a solution of a weak acid and its conjugate base. The key equation for working with buffers is the Henderson-Hasselbalch equation:

The Henderson-Hasselbalch equation

$$pH = pK_a + \log\left(\frac{\textit{concentration of conjugate base}}{\textit{concentration of weak acid}}\right)$$

The equation tells us that if our buffer is an equimolar solution of a weak acid and its conjugate base, the pH of the buffer will equal the pKa of the acid (because the log of 1 is equal to zero). If there is more of the acid form than the base, then of course the pH of the buffer is lower than the pKa of the acid.

Exercise 7.5: What is the pH of an aqueous buffer solution that is 30 mM in acetic acid and 40 mM in sodium acetate? The pKa of acetic acid is 4.8.

The Henderson-Hasselbalch equation is particularly useful when we want to think about the protonation state of different biomolecule functional groups in a pH 7 buffer. When we do this, we are always assuming that the concentration of the biomolecule is small compared to the concentration of the buffer components. (The actual composition of physiological buffer is complex, but it is primarily based on phosphoric and carbonic acids).

Imagine an aspartic acid residue located on the surface of a protein in a human cell. Being on the surface, the side chain is in full contact with the pH 7 buffer surrounding the protein. In what state is the side chain functional group: the protonated state (a carboxylic acid) or the deprotonated state (a carboxylate ion)? Using the Henderson-Hasselbalch equation, we fill in our values for the pH of the buffer and a rough pKa approximation of pKa = 5 for the carboxylic acid functional group. Doing the math, we find that the ratio of carboxylate to carboxylic acid is about 100 to 1: the carboxylic acid is almost completely ionized (in the deprotonated state) inside the cell. This result extends to all other carboxylic acid groups you might find on natural biomolecules or drug molecules: in the physiological environment, carboxylic acids are almost completely deprotonated.

Now, let's use the equation again, this time for an amine functional group, such as the side chain of a lysine residue: inside a cell, are we likely to see a neutral amine (R-NH_2) or an ammonium cation (R-NH_3^+?) Using the equation with pH = 7 (for the biological buffer) and pKa = 10 (for the ammonium group), we find that the ratio of neutral amine to ammonium cation is about 1 to 100: the group is close to completely protonated inside the cell, so we will see R-NH_3^+, not R-NH_2.

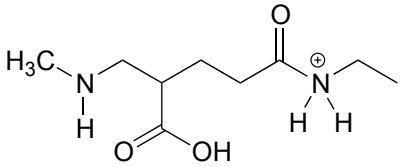
We can do the same rough calculation for other common functional groups found in biomolecules.

At physiological pH:

Carboxylic acids are deprotonated (in the carboxylate anion form)
Amines are protonated (in the ammonium cation form)
Thiols, phenols, alcohols, and amides are uncharged
Imines are a mixture of the protonated (cationic) and deprotonated (neutral) states.

We will talk about the physiological protonation state of phosphate groups in chapter 9.

Exercise 7.6: The molecule below is not drawn in the protonation state that we would expect to see it at physiological pH. Redraw it in the physiologically relevant protonation state.



While we are most interested in the state of molecules at pH 7, the Henderson-Hasselbalch equation can of course be used to determine the protonation state of functional groups in solutions buffered to other pH levels. The exercises below provide some practice in this type of calculation.

Exercise 7.7: What is the ratio of acetate ion to neutral acetic acid when a small amount of acetic acid (pKa = 4.8) is dissolved in a buffer of pH 2.8? pH 3.8? pH 4.8? pH 5.8? pH 6.8?

Exercise 7.8: Would you expect phenol to be soluble in an aqueous solution buffered to pH 2? pH 7? pH 12? Explain your answer.

Exercise 7.9: Methylamine is dissolved in a water buffered to pH 9.0 What percent of the solute molecules are charged? What is the average charge on solute molecules?

Exercise 7.10: What is the approximate net charge on a tetrapeptide Cys-Asp-Lys-Glu in pH 7 buffer?

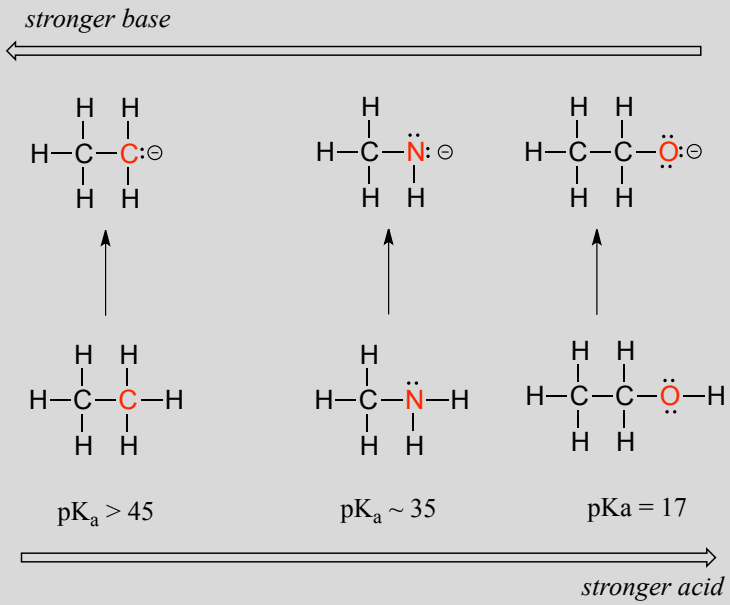
Section 7.3: Structural effects on acidity and basicity

Now that we know how to quantify the strength of an acid or base, our next job is to gain an understanding of the fundamental reasons behind *why* one compound is more acidic or more basic than another. This is a big step: we are, for the first time, taking our knowledge of organic *structure* and applying it to a question of organic *reactivity*. Many of the ideas that we'll see for the first here will continue to apply throughout the book as we tackle many other organic reaction types.

7.3A: Periodic trends

First, we will focus on individual atoms, and think about trends associated with the position of an element on the periodic table. We'll use as our first models the simple organic compounds ethane, methylamine, and ethanol, but the concepts apply equally to more complex biomolecules with the same functionalities, for example the side chains of the amino acids alanine (alkane), lysine (amine), and serine (alcohol).

Horizontal periodic trend in acidity and basicity



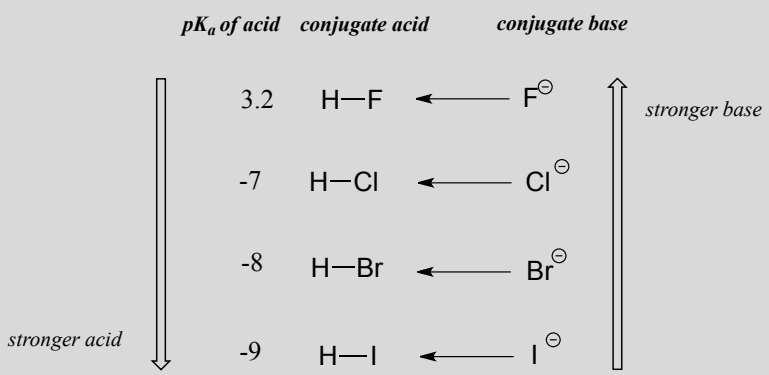
We can see a clear trend in acidity as we move from left to right along the second row of the periodic table from carbon to nitrogen to oxygen. The key to understanding this trend is to consider the hypothetical conjugate base in each case*: the more stable (weaker) the conjugate base, the stronger the acid*. Look at where the negative charge ends up in each conjugate base. In the conjugate base of ethane, the negative charge is borne by a carbon atom, while on the conjugate base of methylamine and ethanol the negative charge is located on a nitrogen and an oxygen, respectively. Remember from section 2.4A that electronegativity also increases as we move from left to right along a row of the periodic table, meaning that oxygen is the most electronegative of the three atoms, and carbon the least.

The more electronegative an atom, the better it is able to bear a negative charge. Weaker bases have negative charges on more electronegative atoms; stronger bases have negative charges on less electronegative atoms.

Thus, the methoxide anion is the most stable (lowest energy, least basic) of the three conjugate bases, and the ethyl carbanion anion is the least stable (highest energy, most basic). Conversely, ethanol is the strongest acid, and ethane the weakest acid.

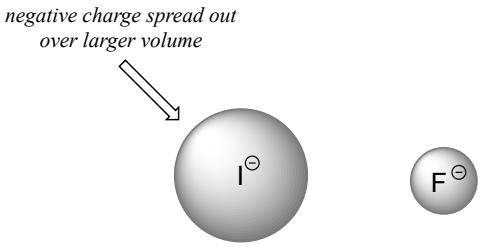
When moving vertically within a given column of the periodic table, we again observe a clear periodic trend in acidity. This is best illustrated with the halogens: basicity, like electronegativity, increases as we move up the column.

Vertical periodic trend in acidity and basicity



Conversely, acidity in the haloacids increases as we move *down* the column.

In order to make sense of this trend, we will once again consider the stability of the conjugate bases. Because fluorine is the most electronegative halogen element, we might expect fluoride to also be the least basic halogen ion. But in fact, it is the *least* stable, and the most basic! It turns out that when moving vertically in the periodic table, the *size* of the atom trumps its electronegativity with regard to basicity. The atomic radius of iodine is approximately twice that of fluorine, so in an iodide ion, the negative charge is spread out over a significantly larger volume:



This illustrates a fundamental concept in organic chemistry:

Electrostatic charges, whether positive or negative, are more stable when they are 'spread out' over a larger area.

We will see this idea expressed again and again throughout our study of organic reactivity, in many different contexts. For now, we are applying the concept only to the influence of atomic radius on base strength. Because fluoride is the least stable (most basic) of the halide conjugate bases, HF is the least acidic of the haloacids, only slightly stronger than a carboxylic acid. HI, with a pKa of about -9, is almost as strong as sulfuric acid.

More importantly to the study of biological organic chemistry, this trend tells us that ***thiols are more acidic than alcohols***. The pK_a of the thiol group on the cysteine side chain, for example, is approximately 8.3, while the pKa for the alcohol group on the serine side chain is on the order of 17.

Remember the concept of 'driving force' that was introduced in section 6.2A? Recall that the driving force for a reaction is usually based on two factors: relative charge stability, and relative total bond energy. Let's see how this applies to a simple acid-base reaction between hydrochloric acid and fluoride ion:

$HCl + F^- \rightarrow HF + Cl^-$

We know that HCl (pKa -7) is a stronger acid than HF (pKa 3.2), so the equilibrium for the reaction lies on the product side: the reaction is exergonic, and a 'driving force' pushes reactant to product.

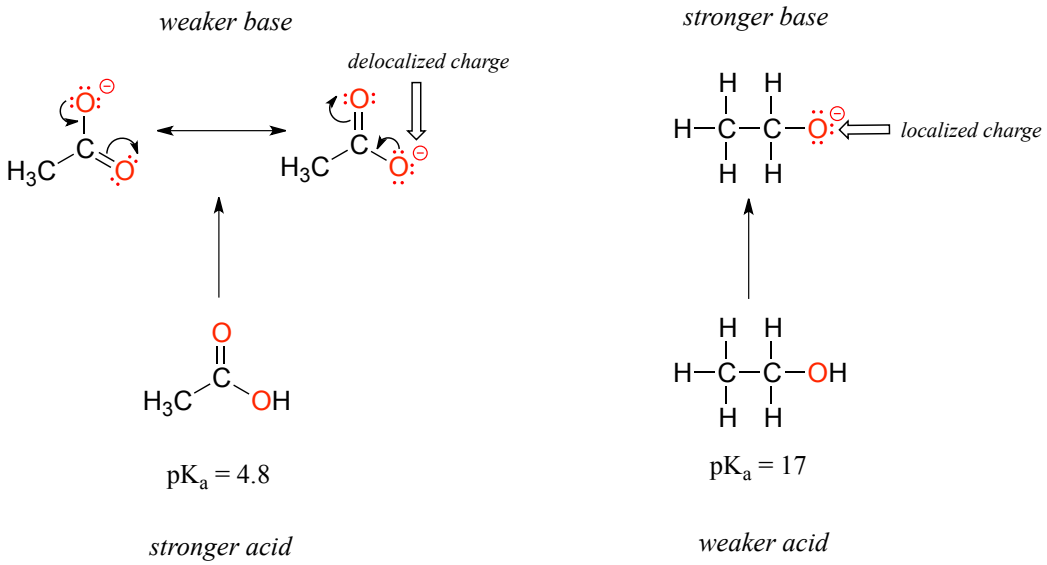
What explains this driving force? Consider first the charge factor: as we just learned, chloride ion (on the product side) is more stable than fluoride ion (on the reactant side). This partially accounts for the driving force going from reactant to product in this reaction: we are going from less stable ion to a more stable ion.

What about total bond energy, the other factor in driving force? If you consult a table of bond energies, you will see that the H-F bond on the product side is more energetic (stronger) than the H-Cl bond on the reactant side: 570 kJ/mol vs 432 kJ/mol, respectively). This also contributes to the driving force: we are moving from a weaker (less stable) bond to a stronger (more stable) bond.

7.3B: Resonance effects

In the previous section we focused our attention on periodic trends - the differences in acidity and basicity between groups where the exchangeable proton was bound to different elements. Now, it is time to think about how the structure of different organic groups contributes to their relative acidity or basicity, even when we are talking about *the same element acting as the proton donor/acceptor*. The first model pair we will consider is ethanol and acetic acid, but the conclusions we reach will be equally valid for all alcohol and carboxylic acid groups.

Despite the fact that they are both oxygen acids, the pKa values of ethanol and acetic acid are strikingly different. What makes a carboxylic acid so much more acidic than an alcohol? As before, we begin by considering the stability of the conjugate bases, remembering that a more stable (weaker) conjugate base corresponds to a stronger acid.



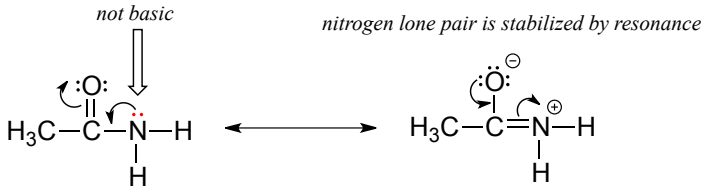
In both species, the negative charge on the conjugate base is located on oxygen, so periodic trends cannot be invoked. For acetic acid, however, there is a key difference: two resonance contributors can be drawn for the conjugate base, and the negative charge can be delocalized (shared) over two oxygen atoms. In the ethoxide ion, by contrast, the negative charge is localized, or 'locked' on the single oxygen – it has nowhere else to go. This makes the ethoxide ion much less stable.

Recall the important general statement that we made a little earlier: 'Electrostatic charges, whether positive or negative, are more stable when they are 'spread out' than when they are confined to one location.' Now, we are seeing this concept in another context, where a charge is being 'spread out' (in other words, delocalized) *by resonance*, rather than simply by the size of the atom involved.

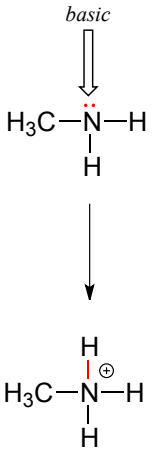
The delocalization of charge by resonance has a very powerful effect on the reactivity of organic molecules, enough to account for the difference of over 12 pKa units between ethanol and acetic acid (and remember, pKa is a log expression, so we are talking about a factor of 10^{12} between the Ka values for the two molecules!)

The resonance effect also nicely explains why a nitrogen atom is basic when it is in an amine, but *not* basic when it is part of an amide group. Recall that in an amide, there is significant double-bond character to the carbon-nitrogen bond, due to a minor but still important resonance contributor in which the nitrogen lone pair is part of a π bond.

amide



amine



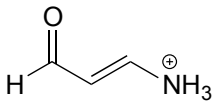
$pK_a = 10.6$

Whereas the lone pair of an amine nitrogen is 'stuck' in one place, the lone pair on an amide nitrogen is delocalized by resonance. Notice that in this case, we are extending our central statement to say that *electron density* – in the form of a lone pair – is stabilized by resonance delocalization, even though there is not a negative charge involved. Here's another way to think about it: the lone pair on an amide nitrogen is not available for bonding with a proton – these two electrons are too 'comfortable' being part of the delocalized π-bonding system. The lone pair on an amine nitrogen, by contrast, is not so comfortable - it is *not* part of a delocalized π system, and is available to form a bond with any acidic proton that might be nearby.

If an amide group is protonated, it will be at the oxygen rather than the nitrogen.

Exercise 7.11:
a) Draw the Lewis structure of nitric acid, HNO_3.
b) Nitric acid is a strong acid - it has a pKa of -1.4. Make a structural argument to account for its strength. Your answer should involve the structure of the conjugate base of nitric acid.

Exercise 7.12: Rank the compounds below from most acidic to least acidic, and explain your reasoning.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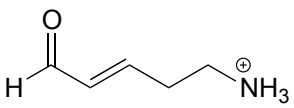
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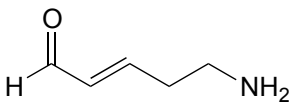
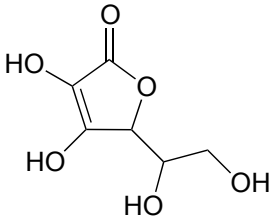

C

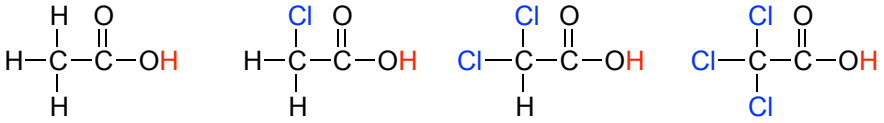
Exercise 7.13 (challenging): Often it requires some careful thought to predict the most acidic proton on a molecule. Ascorbic acid, also known as Vitamin C, has a pKa of 4.1 - the fact that this is in the range of carboxylic acids suggest to us that the negative charge on the conjugate base can be delocalized by resonance to *two* oxygen atoms. Which if the four OH protons on the molecule is most acidic? Draw the structure of ascorbate, the conjugate base of ascorbic acid, then draw a second resonance contributor showing how the negative charge is delocalized to a second oxygen atom. Hint - try deprotonating each OH group in turn, then use your resonance drawing skills to figure out whether or not delocalization of charge can occur.



ascorbic acid
(Vitamin C)

7.3C: Inductive effects

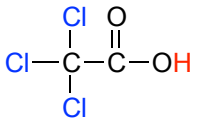
Compare the pKa values of acetic acid and its mono-, di-, and tri-chlorinated derivatives:



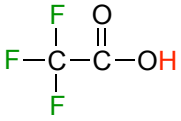
$pK_a = 4.8$ $pK_a = 2.8$ $pK_a = 1.3$ $pK_a = 0.64$

The presence of the chlorine atoms clearly increases the acidity of the carboxylic acid group, but the argument here does not have to do with resonance delocalization, because no additional resonance contributors can be drawn for the chlorinated molecules. Rather, the explanation for this phenomenon involves something called the **inductive effect**. A chlorine atom is more electronegative than a hydrogen, and thus is able to 'induce', or 'pull' electron density towards itself, away from the carboxylate group. In effect, the chlorine atoms are helping to further spread out the electron density of the conjugate base, which as we know has a stabilizing effect. In this context, the chlorine substituent can be referred to as an **electron-withdrawing group**. Notice that the pKa-lowering effect of each chlorine atom, while significant, is not as dramatic as the delocalizing resonance effect illustrated by the difference in pKa values between an alcohol and a carboxylic acid. In general, *resonance effects are more powerful than inductive effects*.

Because the inductive effect depends on electronegativity, fluorine substituents have a more pronounced pKa-lowered effect than chlorine substitutents.



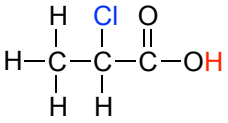
trichloroacetic acid
$pK_a = 0.64$

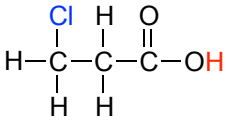


trifluoroacetic acid
$pK_a = -0.25$

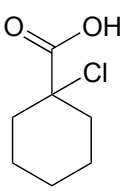
In addition, the inductive takes place through covalent bonds, and its influence decreases markedly with distance – thus a chlorine two carbons away from a carboxylic acid group has a decreased effect compared to a chlorine just one carbon away.

stronger acid





Exercise 7.14: Rank the compounds below from most acidic to least acidic, and explain your reasoning.



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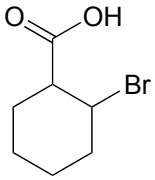

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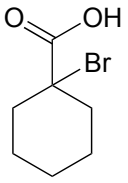
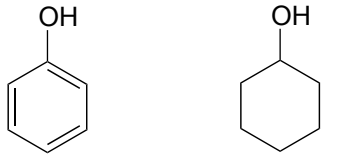


C

Section 7.4: Acid-base properties of phenols

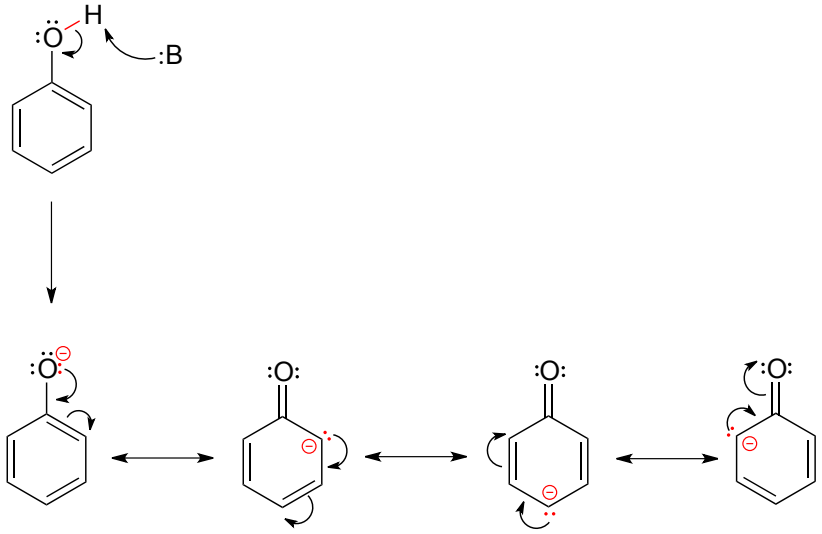
Resonance effects involving aromatic structures can have a dramatic influence on acidity and basicity. Notice, for example, the difference in acidity between phenol and cyclohexanol.



phenol
$pK_a = 10$

cyclohexanol
$pK_a = 16$

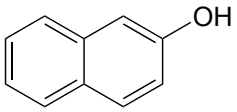
Looking at the conjugate base of phenol, we see that the negative charge can be delocalized by resonance to three different carbons on the aromatic ring.



Although these are all minor resonance contributors (negative charge is placed on a carbon rather than the more electronegative oxygen), they nonetheless have a significant effect on the acidity of the phenolic proton. Essentially, the benzene ring is acting as an electron-withdrawing group by resonance.

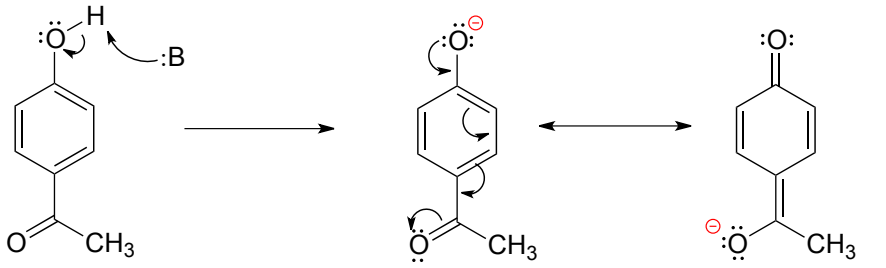
As we begin to study in detail the mechanisms of biological organic reactions, we'll see that the phenol side chain of the amino acid tyrosine (see table 5 at the back of the book), with its relatively acidic pKa of 9-10, often acts as a catalytic proton donor/acceptor in enzyme active sites.

Exercise 7.15: Draw the conjugate base of 2-napthol (the major resonance contributor), and on your drawing indicate with arrows all of the atoms to which the negative charge can be delocalized by resonance.



2-napthol

The base-stabilizing effect of an aromatic ring can be accentuated by the presence of an additional electron-withdrawing substituent, such as a carbonyl. For the conjugate base of the phenol derivative below, an additional resonance contributor can be drawn in which the negative formal charge is placed on the carbonyl oxygen.

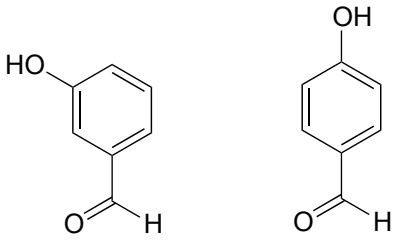


negative charge can be delocalized to a second oxygen

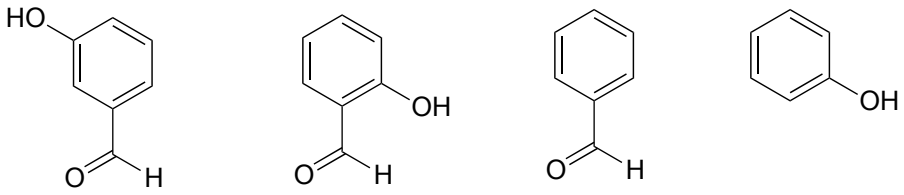
para-hydroxyacetophenone
(pKa = 4.2)

Now the negative charge on the conjugate base can be spread out over *two* oxygens (in addition to three aromatic carbons). The phenol acid therefore has a pKa similar to that of a carboxylic acid, where the negative charge on the conjugate base is also delocalized to two oxygen atoms. The ketone group is acting as an electron withdrawing group - it is 'pulling' electron density towards itself, through both inductive and resonance effects.

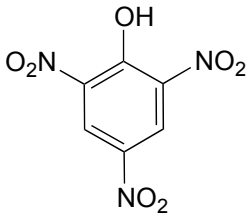
Exercise 7.16: The position of the electron-withdrawing substituent relative to the phenol hydroxyl is very important in terms of its effect on acidity. Which of the two phenols below is more acidic? Use resonance drawings to explain your answer.



Exercise 7.17: Rank the four compounds below from most acidic to least.



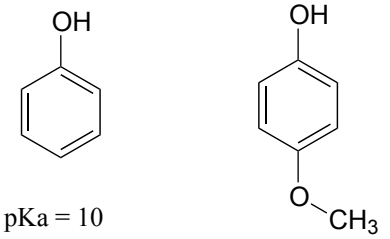
Exercise 7.18: Nitro groups are very powerful electron-withdrawing groups. The phenol derivative picric acid has a pKa of 0.25, lower than that of trifluoroacetic acid.



picric acid
pKa = 0.25

Use a resonance argument to explain why picric acid has such a low pKa.

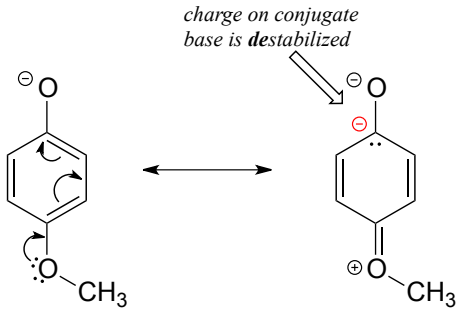
Consider the acidity of 4-methoxyphenol, compared to phenol.



pKa = 10

pKa = 10.6

Notice that the methoxy group increases the pKa of the phenol group - it makes it *less* acidic. Why is this? At first inspection, you might assume that the methoxy substituent, with its electronegative oxygen, would be an electron-withdrawing group by induction. That is correct, but only to a point. The oxygen atom does indeed exert an electron-withdrawing inductive effect, but the lone pairs on the oxygen cause the exact opposite effect – *the methoxy group is an* ***electron-donating group by resonance***. A resonance contributor can be drawn in which a formal negative charge is placed on the carbon adjacent to the negatively-charged phenolate oxygen.

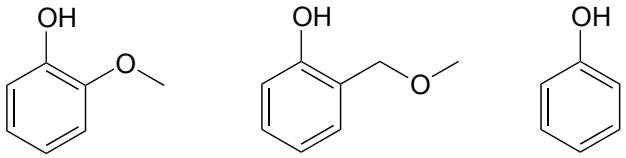


Because of like-charge repulsion, this *destabilizes* the negative charge on the phenolate oxygen, making it more basic. It may help to visualize the methoxy group 'pushing' electrons towards the lone pair electrons of the phenolate oxygen, causing them to be less 'comfortable' and more reactive.

When resonance and induction compete, resonance usually wins!

The example above is a somewhat confusing but quite common situation in organic chemistry - a functional group, in this case a methoxy group, is exerting both an inductive effect and a resonance effect, *but in opposite directions* (the inductive effect is electron-withdrawing, the resonance effect is electron-donating). As stated earlier, as a general rule a resonance effect is more powerful than an inductive effect - so overall, the methoxy group is acting as an electron donating group.

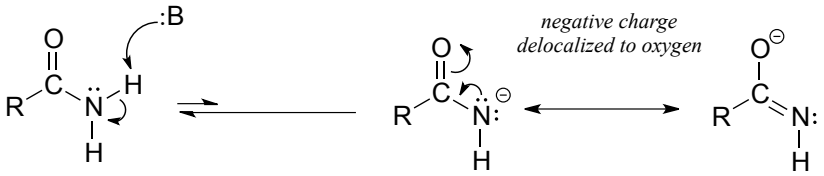
Exercise 7.19: Rank the three compounds below from lowest pKa to highest, and explain your reasoning. *Hint* - think about both resonance and inductive effects!



Section 7.5: Acid-base properties of nitrogen-containing functional groups

Many of the acid-base reactions we will see throughout our study of biological organic chemistry involve functional groups which contain nitrogen. In general, a nitrogen atom with three bonds and a lone pair of electrons can potentially act as a proton-acceptor (a base) - but basicity is reduced if the lone pair electrons are stabilized somehow. We already know that amines are basic, and that the pKa for a protonated amine is in the neighborhood of 10. We also know that, due to resonance with the carbonyl bond, amide nitrogens are not basic (in fact they are very slightly acidic, with a pKa around 20).

amide can act as a weak acid



$pK_a \sim 20$

Next, let's consider the basicity of some other nitrogen-containing functional groups.

7.5A: Anilines

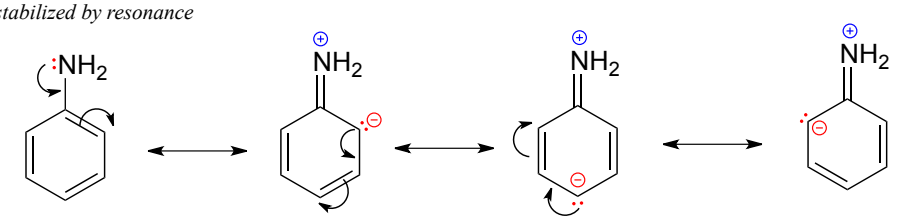
Aniline, the amine analog of phenol, is substantially less basic than an amine.

protonated amine
$pK_a \sim 10$

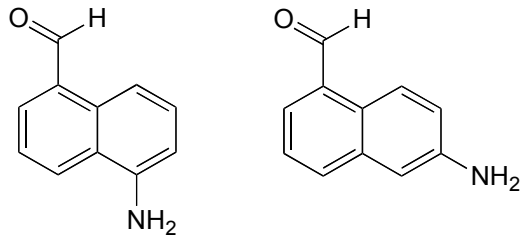
anilinium
(protonated aniline)
$pK_a \sim 5$

We can use the same reasoning that we used when comparing the acidity of a phenol to that of an alcohol. In aniline, the lone pair on the nitrogen atom is stabilized by resonance with the aromatic π system, making it less available for bonding and thus less basic.

lone pair is stabilized by resonance



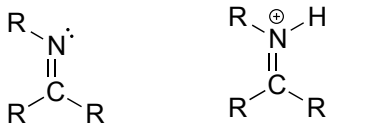
Exercise 7.20: With anilines just as with phenols, the resonance effect of the aromatic ring can be accentuated by the addition of an electron-withdrawing group, and diminished by the addition of an electron-donating group. Which of the two compounds below is expected to be more basic? Use resonance drawings to explain your reasoning.



A B

7.5B: Imines

Imines are somewhat less basic than amines: pKa for a protonated imine is in the neighborhood of 5-7, compared to ~10 for protonated amines. Recall that an imine functional group is characterized by an sp^2-hybridized nitrogen double-bonded to a carbon.



imine

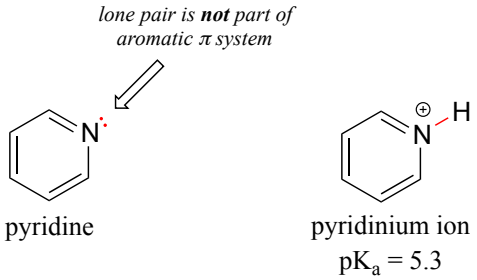
imminium ion
(protonated imine)
pK_a ~ 5 - 7

The lower basicity of imines compared to amines can be explained in the following way:

- The lone pair electrons on an imine nitrogen occupy an sp^2 hybrid orbital, while the lone pair electrons on an amine nitrogen occupy an sp^3 hybrid orbital.
- sp^2 orbitals are composed of one part *s* and two parts *p* atomic orbitals, meaning that they have about 33% *s* character. sp^3 orbitals, conversely, are only 25% *s* character (one part *s*, three parts *p*).
- An *s* atomic orbital holds electrons closer to the nucleus than a *p* orbital, thus *s* orbitals are more electronegative than *p* orbitals.
- Therefore, sp^2 hybrid orbitals, with their higher *s*-character, are more electronegative than sp^3 hybrid orbitals.
- Lone pair electrons in the more electronegative sp^2 hybrid orbitals of an imine are held more tightly to the nitrogen nucleus, and are therefore less 'free' to break away and form a bond to a proton - in other words, they are less basic.

The aromatic compound pyridine, with an imine-like nitrogen, has a pKa of 5.3. Recall from section 2.2C that the lone pair electrons on the nitrogen atom of pyridine occupy

an sp^2-hybrid orbital, and are *not* part of the aromatic sextet - thus, they are available for bonding with a proton.

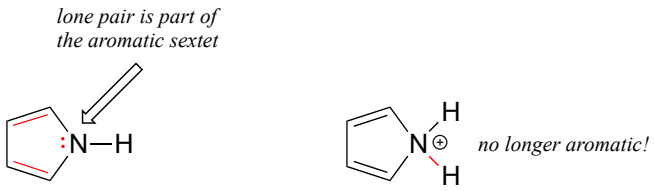


pyridine

pyridinium ion
$pK_a = 5.3$

7.5C: Pyrrole

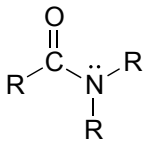
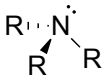
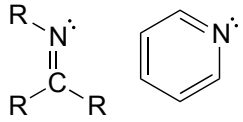
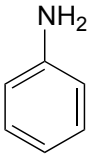
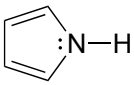
In the aromatic ring of pyrrole, the nitrogen lone pair electrons *are* part of the aromatic sextet, and are therefore much less available for forming a new bonding to a proton. Pyrrole is a very weak base: the conjugate acid is a strong acid with a pKa of 0.4.



pyrrole

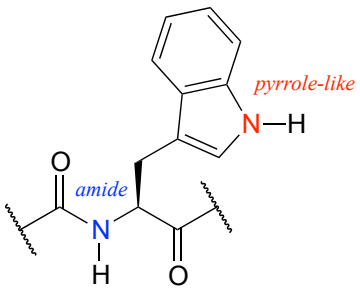
pyrrolium ion
$pK_a = 0.4$

Below is a summary of the five common bonding arrangements for nitrogen and their relative basicity:

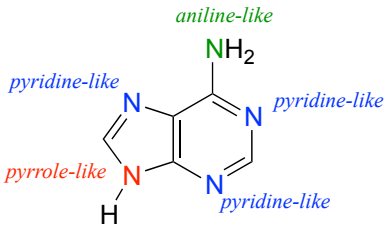
nitrogen group	structure	pKa of conjugate acid
amide		NA (amide nitrogens are not basic)
amine		~ 10
imine/pyridine		~ 5 - 7
aniline		~ 5
pyrrole		~ 0

Learning and being able to recognize these five different 'types' of nitrogen can be very helpful in making predictions about the reactivity of a great variety of nitrogen-containing biomolecules. The side chain of the amino acid tryptophan, for example, contains a non-basic 'pyrrole-like' nitrogen (the lone pair electrons are part of the 10-electron aromatic system), and the peptide chain nitrogen, of course, is an amide. The nucleotide base adenine contains three types of nitrogen.

.

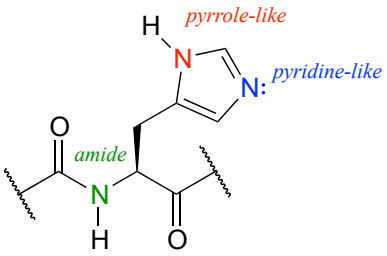


tryptophan

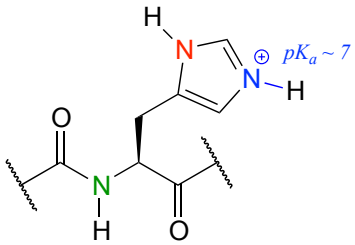


adenine

The side chain on a histidine amino acid has both a 'pyrrole-like' nitrogen and a 'pyridine-like' imine nitrogen. The pKa of a protonated histidine residue is approximately 7, meaning that histidine will be present in both protonated and deprotonated forms in physiological buffer. Histidine residues in the active site of enzymes are common proton donor-acceptor groups in biochemical reactions.

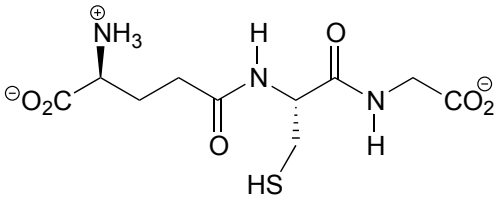


histidine
(deprotonated)

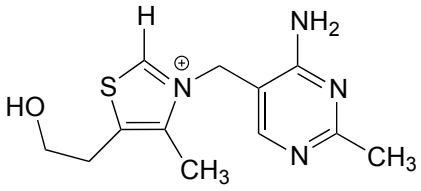


histidinium
(protonated)

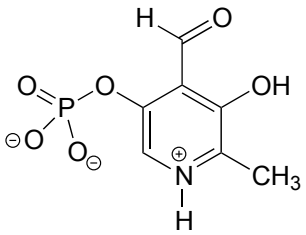
Exercise 7.21: Below are the structures of four 'coenzyme' molecules necessary for human metabolism (we will study the function of all of these in chapter 17).



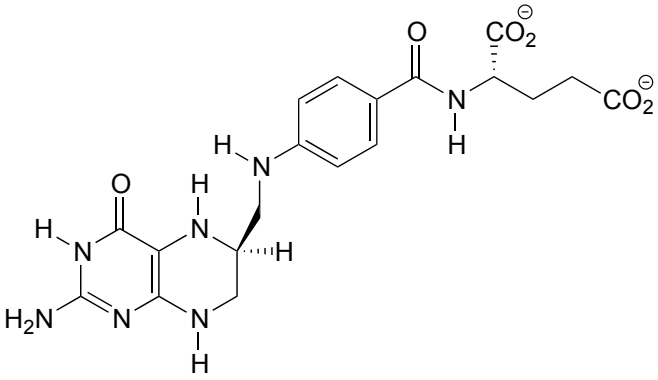
glutathione



thiamine
(vitamin B1)



pyridoxal phosphate
(derived from vitamin B6)



tetrahydrofolate
(derived from folic acid)

a) When appropriate, assign a label to each nitrogen atom using the basicity classifications defined in this section ('pyrrole-like', etc.).

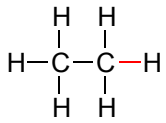
b) There is one nitrogen that does not fall into any of these types - is it basic? Why or why not? What would be a good two-word term to describe the group containing this nitrogen?

Section 7.6: Carbon acids

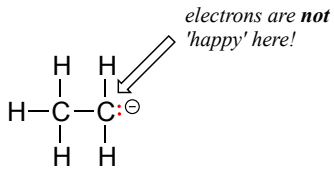
So far, we have limited our discussion of acidity and basicity to heteroatom acids, where the acidic proton is bound to an oxygen, nitrogen, sulfur, or halogen. However, **carbon acids** - in which the proton to be donated is bonded to a carbon atom - play an integral role in biochemistry.

7.6A: The acidity of α-protons

A hydrogen on an alkane is not at all acidic – its pKa is somewhere on the order of 50, about as non-acidic as it gets in the organic chemistry world. The reason for this is that if the hydrogen were to be abstracted, the electrons from the broken bond would be localized on a single carbon atom.



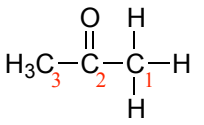
ethane
$pK_a > 45$



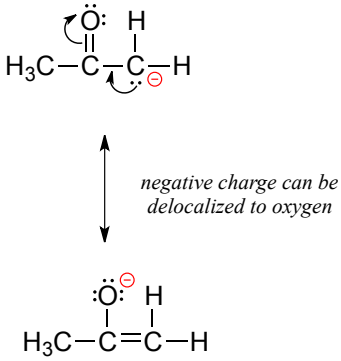
hypothetical conjugate base of ethane

Because carbon is not electronegative and is terrible at holding a negative charge, such carbanion species are extremely unstable.

How, then, can a proton bonded to a carbon be acidic? Remember that an acid becomes stronger if the conjugate base is stabilized, an in particular if the negative charge on the conjugate base can be delocalized to an electronegative atom such as an oxygen. This is possible when a carbon is located adjacent to a carbonyl group. Consider, for example, the conjugate base of acetone.

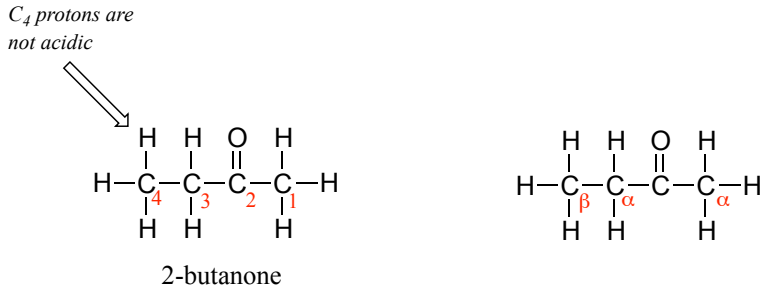


acetone
$pK_a = 19$



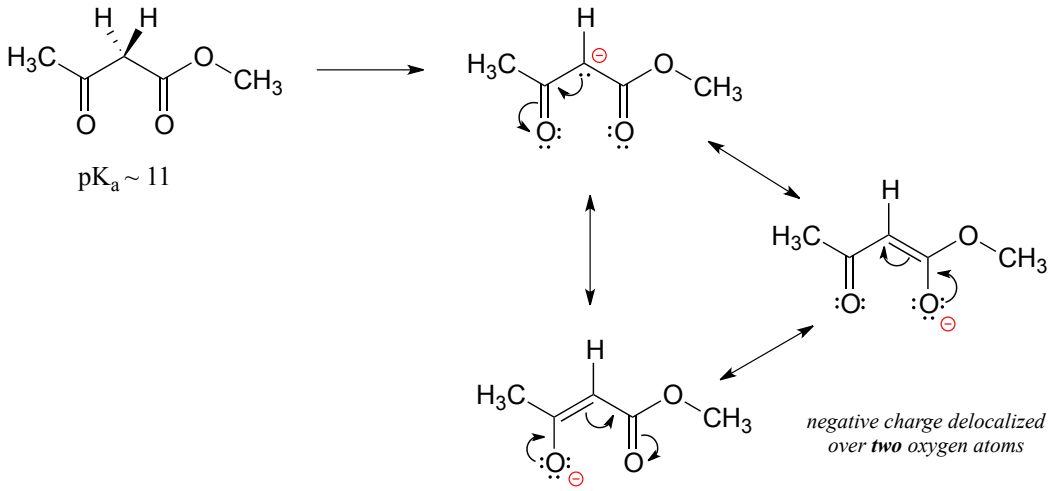
conjugate base of acetone
(enolate)

One resonance contributor puts the negative charge on the carbon #1. Due to the presence of the adjacent carbonyl group, however, a second resonance contributor can be drawn in which the negative charge is located on the carbonyl oxygen, where it is much more stable. This type of stabilized carbanion species is specifically referred to as an **enolate**. Acetone is in fact weakly acidic, with a pKa of about 19. The importance of the position of the carbonyl group is evident when we consider 2-butanone: here, the protons on carbons #1 and #3 are somewhat acidic (in the neighborhood of pKa = 20), but the protons on carbon #4 are not acidic at all, because carbon #4 is not adjacent to the carbonyl.

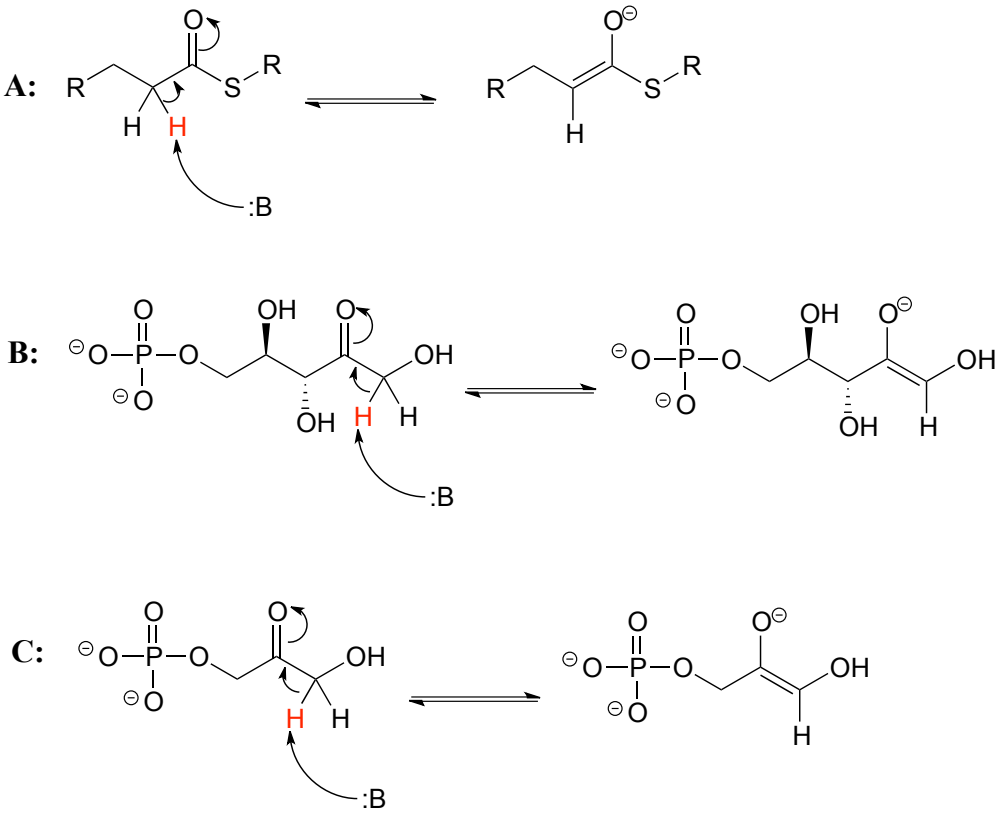


A carbon that is located next to a carbonyl group is referred to as an **α-carbon**, and any proton bound to it is an **α-proton**. In 2-butanone, carbons #1 and #3 are α-carbons, and their five protons are α-protons. Carbon #4 is a **β-carbon**, as it is two positions removed from the carbonyl carbon

An **active methylene** is a carbon in the α position relative to *two* carbonyl groups rather than just one. Protons on active methylene carbons are more acidic than other α-protons, because the charge on the conjugate base can be localized to *two* different oxygen atoms, not just one. This keto-ester compound, for example, has a pKa of approximately 11, close to that of phenol.

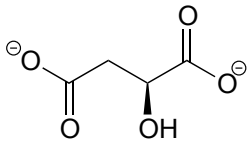


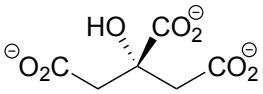
As we alluded to above, the acidity of α-protons is an extremely important concept in biological organic chemistry. Look through a biochemistry textbook, and you will see reaction after reaction in which the first mechanistic step is the abstraction of an α-proton to form an enolate intermediate. Two chapters in this book (chapters x and y) are devoted to such reactions, and the initial proton-extraction step of three example reactions are previewed below. Reaction A is from fatty acid oxidation, while reactions B and C are both part of carbohydrate metabolism.

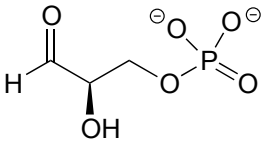


Exercise 7.22: For each molecule shown below:

a) Show the location of all α-protons.
b) Draw the structure(s) of all possible enolate conjugate bases.

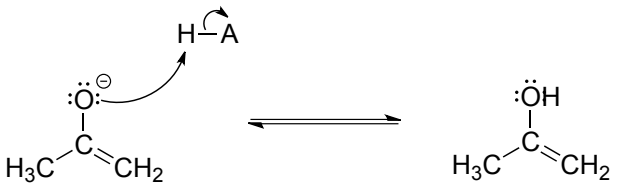

malate


citrate


glyceraldehyde-3-phosphate

7.6B: Keto-enol tautomers

An enolate ion can, of course, be reprotonated at the α-carbon to return the molecule to the ketone or aldehyde form. An alternate possibility is that the oxygen atom, rather than the α-carbon, could be protonated. The species that results from this step is referred to as an **enol** (this term reflects the fact that an enol contains structural elements of both an akl**en**e and an alcoh**ol**).

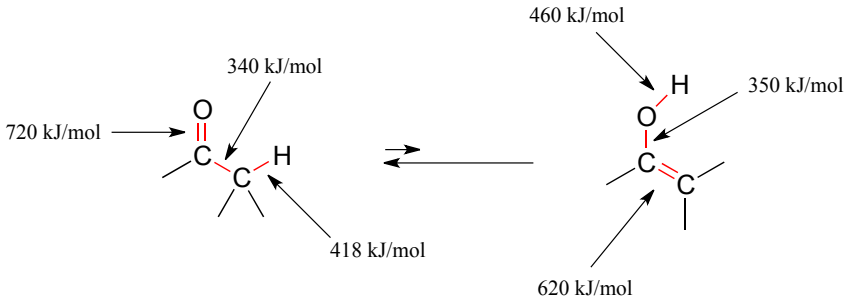

enolate enol

In fact, most ketones and aldehydes exist in rapid equilibrium with their enol form. A ketone/aldehyde and its corresponding enol are **tautomers**: a pair of constitutional isomers which can be rapidly and reversibly interconverted, and which vary in terms of the site of protonation and location of a double bond. As we will see going forward, **tautomerization** - the interconversion of two tautomers - is a ubiquitous step in biological organic chemistry. Often, when discussing tautomerization, the ketone (or aldehyde) isomer is referred as the **keto** form.

As a general rule, the keto form is lower in energy than the corresponding enol form, and thus the keto form predominates at equilibrium. Acetone, for example, is present at >99% keto form at equilibrium, and the enol form at less than 1%.


keto form of acetone (>99% at equilibrium) enol form of acetone (<1% at equilibrium)

The 'driving force' for the enol to keto conversion can be understood in terms of the energies of the three bonds involved in the process: the sum of the three bond energies is about 48 kJ/mol greater in the keto form than in the enol.



keto
total 1478 kJ/mol

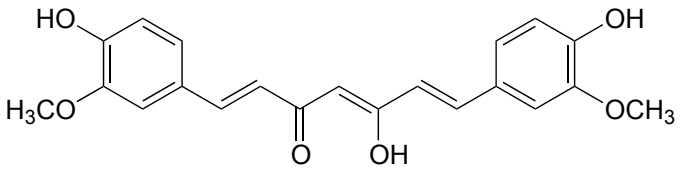
enol
total 1430 kJ/mol

Exercise7.23: Draw all of the possible enol forms of the following aldehydes/ketones. There may be more than one possible enol form (consider stereochemistry as well as regiochemistry!)

a) 3-pentanone
b) acetaldehyde (IUPAC name ethaldehyde)
c) cyclohexanone
d) 2-pentanone

Exercise 7.24: Draw three examples of aldehyde or ketone compounds for which there is no possible enol form.

Exercise 7.25: In some special cases, the enol form of a compound is more stable than the keto form and thus predominates at equilibrium. Curcumin is the compound mainly responsible for the characteristic yellowish color of tumeric, a ubiquitous spice in indian cuisine. The extended system of π bonds present in the enol form causes it to be lower in energy than the keto form. Draw the keto form of curcumin, and explain how the conjugated π system is disrupted in this form.

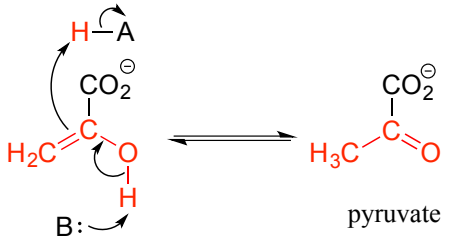


curcumin
(enol form)

Exercise 7.26: The phenol functional group can also be thought of a kind of enol.

a) Draw the 'keto' form of phenol.
b) The 'keto' form of phenol is highly disfavored compared to the 'enol' form - why?

There is an enol to keto tautomerization step in the glycolysis reaction catalyzed by pyruvate kinase (EC 2.7.1.40). Below is just the tautomerization part of this reaction; we will see the complete reaction in chapter 9.

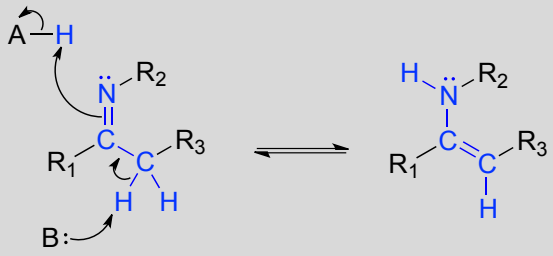


enolpyruvate pyruvate

7.6C: Imine-enamine tautomerization

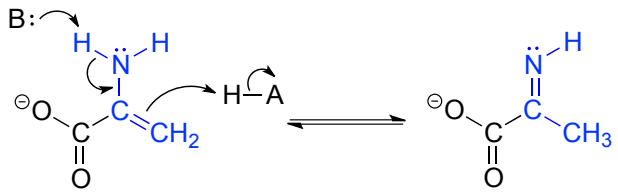
Another common tautomeric relationship in biological organic chemistry is the equilibrium between imines (also known as Schiff bases) and **enamines**, which are essentially the nitrogen equivalents of enols.

Mechanism for imine - enamine tautomerization



imine enamine

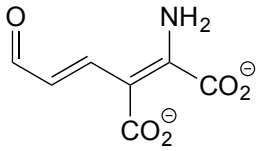
The degradation of serine, for example, involves an enamine to imine tautomerization step: (EC 4.2.1.13)



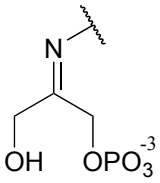
enamine imine

Exercise 7.27: The structures below all contain either an imine or an enamine group. For each, draw the structure of an alternate tautomer.

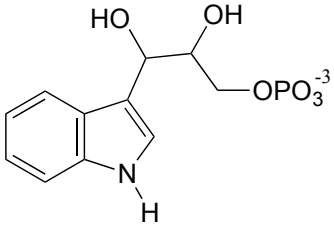
a)



b)



c)



7.6D: Acidity of terminal alkynes

Terminal alkynes are another kind of carbon acid which are relevant more to laboratory organic chemistry than to biological chemistry.

a terminal alkyne
$pK_a \sim 26$

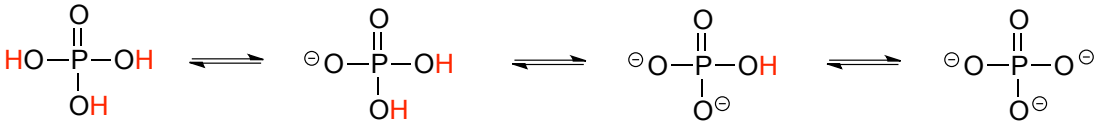
conjugate base

Terminal alkynes are more acidic than alkenes or alkanes for the same reason that protonated imines are more acidic than protonated amines: the alkyne carbon is *sp*-hybridized, meaning that it has 50% *s*-orbital character and is therefore more electronegative. With a pKa of approximately 26, alkynes are only weakly acidic, but nonetheless can be fully deprotonated through the use of a strong base such as sodium amide ($NaNH_2$).

Exercise 7.28: Hydrogen cyanide, HCN, is another example of a relatively strong carbon acid, with a pKa of 9.2. Suggest a rationale for the acidity of this proton.

Section 7.7: Polyprotic acids

Polyprotic acids are capable of donating more than one proton. The most important polyprotic acid group from a biological standpoint is triprotic phosphoric acid. Because phosphoric acid has three acidic protons, it also has three pKa values.



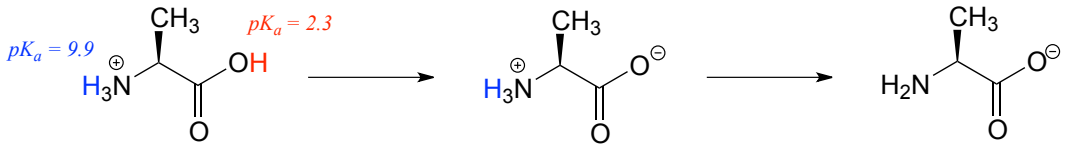
$pK_{a1} = 2.1$ $pK_{a2} = 7.2$ $pK_{a3} = 12.3$

The pKa values for any polyprotic acid always get progressively higher, because it becomes increasingly difficult to stabilize the additional electron density that results from each successive proton donation. H_3PO_4 is a strong acid because the (single) negative charge on its conjugate base $H_2PO_4^-$ can be delocalized over two oxygen atoms.

$H_2PO_4^-$ is substantially less acidic, because proton donation now results in the formation of an additional negative charge: a –2 charge is inherently higher in energy than a –1 charge because of negative-negative electrostatic repulsion. The third deprotonation, resulting in formation of a third negative charge, has an even higher pKa. We will have more to say about the acidity of phosphate groups in chapter xx, when we study the reactions of phosphate groups on biomolecules.

Exercise 7.29: In a pH 7 buffer, what form(s) of phosphoric acid predominate? What is the average net charge?

Free amino acids are polyprotic, with pKa values of approximately 2 for the carboxylic acid group and 9-10 for the ammonium group. Alanine, for example, has the acid constants $pK_{a_1} = 2.3$ and $pK_{a_2} = 9.9$.

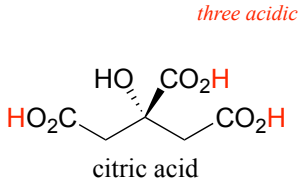


cation at low pH zwitterion at pH 7 anion at high pH

The Henderson-Hasselbalch equation tells us that alanine is almost fully protonated and positively charged when dissolved in a solution that is buffered to pH 0.5. At pH 7, alanine has lost one proton from the carboxylic acid group, and thus is a **zwitterion** (it has both a negative and a positive formal charge). At pH levels above 12, the ammonium group is fully deprotonated, and alanine has a negative overall charge.

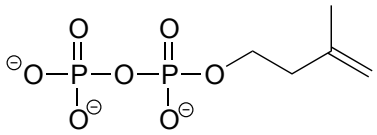
Some amino acids (arginine, lysine, aspartate, glutamate, tyrosine, and histidine) are triprotic, with a third pKa value associated with an ionizable functional group on the side chain.

Many biological organic molecules have several potentially ionizable functional groups and thus can be considered polyprotic acids. Citric acid, found in abundance in oranges, lemons, and other citrus fruits, has three carboxylic acid groups and pKa values of 3.1, 4.8, and 6.4.



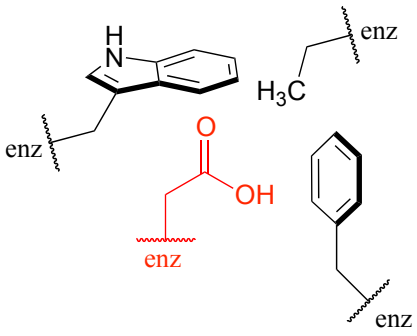
citric acid

Exercise 7.30: Predict the structure of the organic diphosphate compound below in the protonation state where it has a -2 charge.



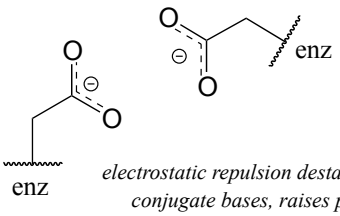
Section 7.8: Effects of enzyme microenvironment on acidity and basicity

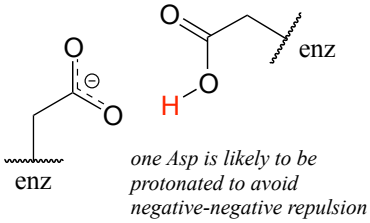
Virtually all biochemical reactions take place inside the active site pocket of an enzyme, rather than free in aqueous solution. The **microenvironment** inside an enzyme's active site can often be very different from the environment outside in the aqueous solvent. Consider, for example, the side chain carboxylate on an aspartate residue in an enzyme. The literature pKa of this carboxylic acid group is listed as 3.9, but this estimate assumes that the group is positioned on the surface of the protein, exposed to water. In the physiological buffer of pH ~ 7, a carboxylic acid group with pKa = 3.9 will be fully deprotonated and negatively charged. If, however, an aspartate side chain happens to be buried deep inside the interior of the protein's active site, and is surrounded primarily by nonpolar side chains such as alanine, phenylalanine, tryptophan, etc., the situation is very different.



Cut off from the environment of the bulk solvent, the carboxylate group (red in the above figure) is now in a very nonpolar microenvironment, a situation in which the protonated, uncharged state is stabilized relative to the deprotonated, negatively charged state (this is simply another application of the 'like dissolves like' principle you learned in General Chemistry - a charged group is highly destabilized by a nonpolar environment). The overall effect is that the pKa for this aspartate residue is actually higher than 3.9 – it is less acidic, and more likely to be in its protonated form inside the protein.

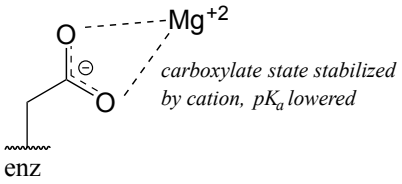
A similar effect would be observed in a situation where the side chain carboxylate groups of two aspartate residues are located in close proximity to one another in an enzyme active site. Two negatively charged groups close to each other represents a very high energy, repulsive situation, and this can be relieved if one of the two side chains is protonated.





In this microenvironment, the proximity of one amino acid group directly effects the pKa of its neighbor.

Now consider a situation where a metal ion such as magnesium (Mg^{+2}) or zinc (Zn^{+2}) is bound in the interior of the enzyme, in close contact with an aspartate side chain. With a cation to interact with, the anionic, deprotonated state of the amino acid is stabilized, so the pKa of this Asp residue is likely to be substantially *lower* than 3.9.

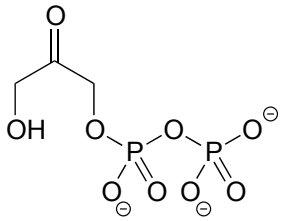


The metal ion in this situation is considered to be acting as a Lewis acid, accepting electron density from the carboxylate group.

The pK_a-lowering effect of a metal cation can be dramatic – it has been estimated that a water molecule coordinated to a Cu^{+2} or Zn^{+2} ion can have a pK_a as low as 7 (compare this to the 'normal' water pK_a of 15.7!)

Exercise 7.31: A lysine residue located deep in the interior of a protein is surrounded by nonpolar residues. In what direction will this alter the 'normal' pK_a of the lysine side chain, and why?

Exercise 7.32: In many biochemical reactions which involve the formation of an enolate intermediate, the carbonyl oxygen of the substrate is coordinated to a divalent metal ion (usually zinc or magnesium) in the active site. Explain, with structural drawings, how this ion-dipole interaction effects the acidity of the α-protons of dihydroxyacetone phosphate (DHAP), an intermediate compound in the glycolysis pathway.



DHAP

Summary of key concepts

Before you move on to the next chapter, you should:

Know the **Bronsted-Lowry definition of acidity and** basicity: a Bronsted acid is a proton donor, a Bronsted base is a proton acceptor.

Know the **Lewis definition of acidity and basicity**: a Lewis acid is an electron acceptor, a Lewis base is an electron donor.

Understand that the Lewis definition is broader: all Bronsted acids are also Lewis acids, but not all Lewis acids are also Bronsted acids.

Be able to draw a curved arrow mechanism for both Bronsted and Lewis acid-base reactions.

Know the expressions for **K_a** and **pK_a**.

Commit to memory the approximate pK_a values for the following functional groups:

H_3O^+, protonated alcohol, protonated carbonyl (~ 0)

carboxylic acids (~ 5)

imines (~ 7)

protonated amines, phenols, thiols (~ 10)

water, alcohols (~ 15)

α-carbon acids (~ 20)

Be able to use pK_a values to compare acidity: a lower pK_a corresponds to a stronger acid.

Know that:

For a given pair of acids, the stronger acid will have the weaker conjugate base.

For a given pair of basic compounds, the stronger base will have the weaker conjugate acid.

Be able to identify the most acidic/basic groups on a polyfunctional molecule.

Be able to calculate the equilibrium constant of an acid base equation from the pKa values of the acids on either side of the equation.

Be able to use the **Henderson-Hasselbalch equation** to determine the protonation state/charge of an organic compound in an aqueous buffer of a given pH.

Understand the idea that the best way to compare the strength of two acids is to compare the stability of their conjugate bases: the more stable (weaker) the conjugate base, the stronger the acid.

Be able to compare the acidity or basicity of compounds based on **periodic trends**:

acidity increases left to right on the table, so alcohols are more acidic than amines

acidity increases top to bottom on the table, so a thiol is more acidic than an alcohol.

Be able to compare the acidity or basicity of compounds based on protonation state: H_3O^+ is more acidic than H_2O, NH_4^+ is more acidic than NH_3.

Understand how the **inductive effect** exerted by electronegative groups influences acidity.

Understand how **resonance delocalization** of electron density influences acidity.

Be able to explain/predict how orbital hybridization affects the relative acidity of terminal alkynes, alkenes, and alkanes.

Be able to explain why phenols are more acidic than alcohols, and how electron-withdrawing or donating groups influence the acidity of phenols.

Be able to identify the relative basicity of a nitrogen-containg group in a compound, based on whether it is an amine, amide, imine, aniline, or 'pyrrole-like'.

Be able to identify **α-carbon**(s) on a carbonyl compound, and explain why α–protons are weakly acidic. You should be able to draw the enolate conjugate base of a carbonyl compound.

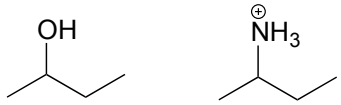
Be able to identify **tautomeric** relationships, specifically keto-enol and imine-enamine tautomers.

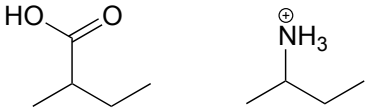
Understand what a **polyprotic acid** is, what is meant by multiple pK_a values, and why these values get progressively higher.

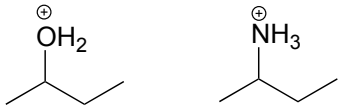
Problems

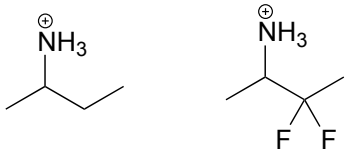
P7.1: For each pair of molecules below, choose the stronger acid, and explain your choice.

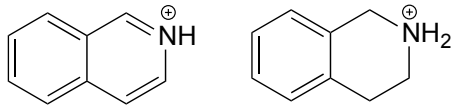
a) 

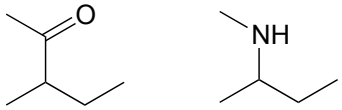
b) 

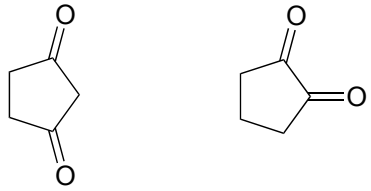
c) 

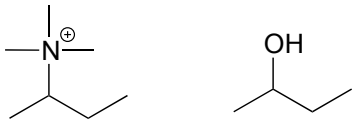
d) 

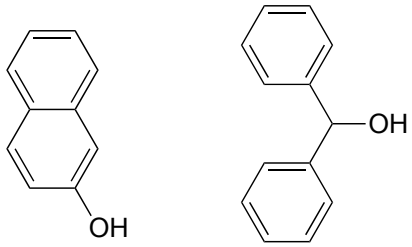
e) 

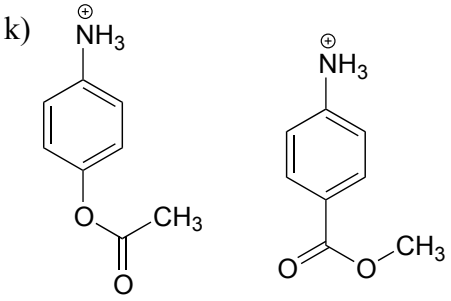
f) 

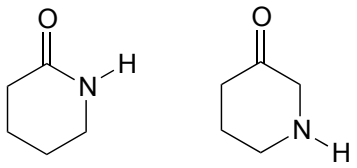


h) 

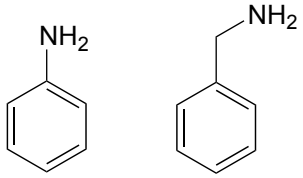
i) 

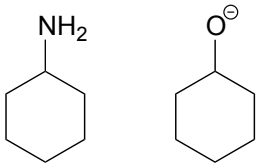
j) 

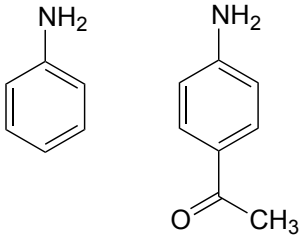
k) 

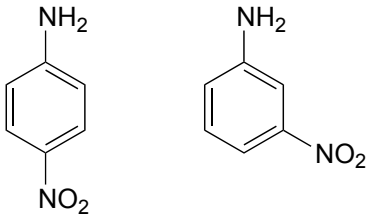
l) 

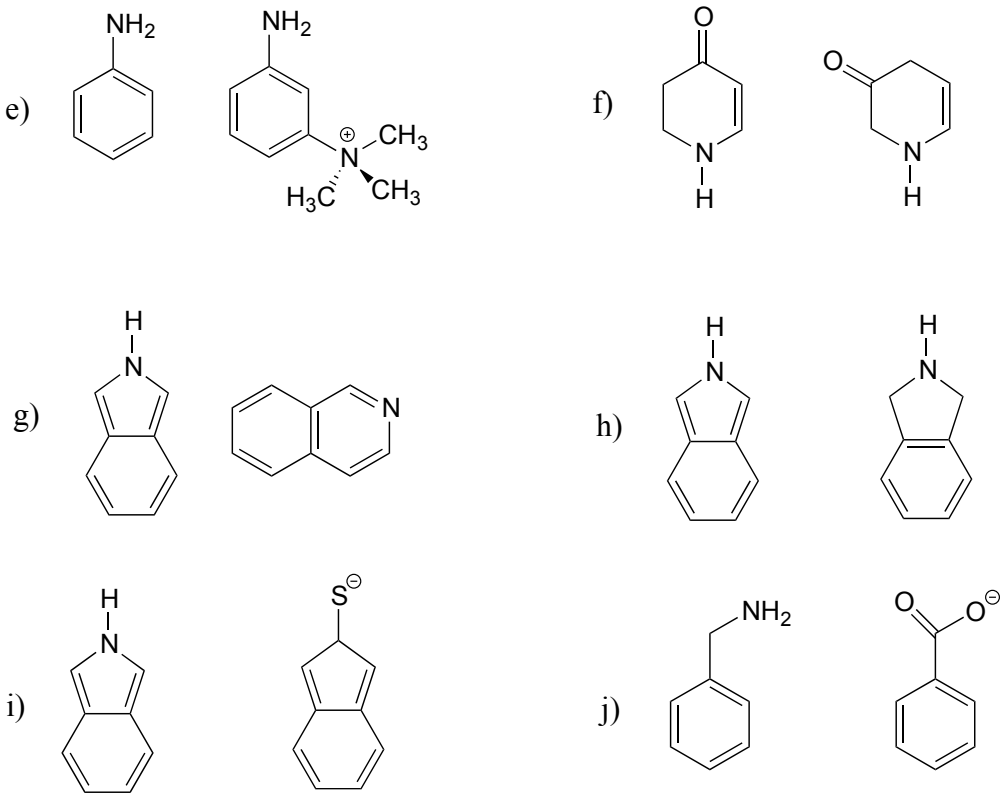
P7.2: For each pair of molecules below, choose the stronger base, and explain your choice.

a) 

b) 

c) 

d) 



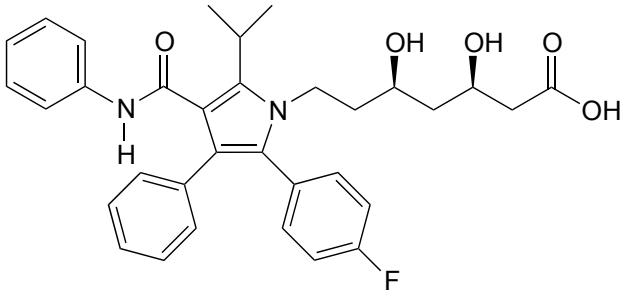
P7.3: Below are the structures of some well-known drugs.

a) What is the most acidic proton on Lipitor? What is its approximate pK$_a$ value? (Lipitor is a brand name for atorvastatin, a cholesterol-lowering drug.)

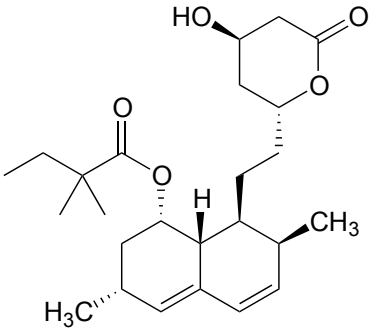
b) What is the most acidic proton on Zocor? What is its approximate pK$_a$ value? (Zocor is a brand name for simvastatin, a cholesterol-lowering drug.)

c) Where is the most basic site on Plavix? What is the approximate pK$_a$ value of the conjugate acid? (Plavix is a brand name for clopidogrel, a drug to prevent blood clots after a stroke.)

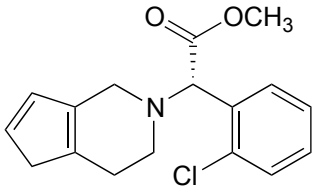
d) Identify the most acidic proton on methadone, and draw the conjugate base that would form if this proton were abstracted by a base. (Methadone is an opiate used in the treatment of heroin addiction.)



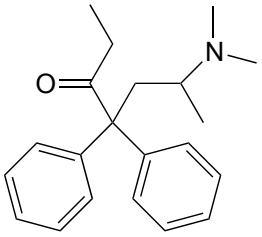
Lipitor



Zocor

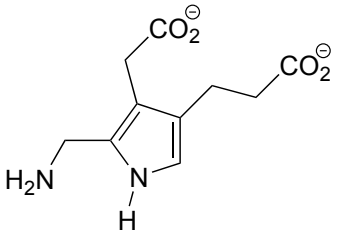


Plavix



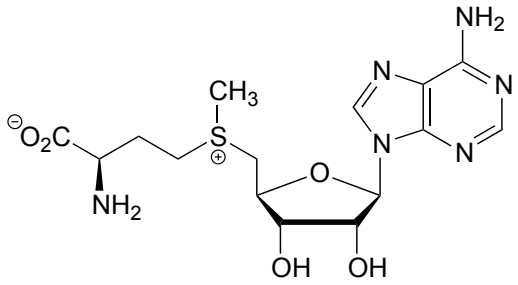
methadone

P7.4: Porphobilinogen is a precuror to the biosynthesis of chlorophyll and many other biological molecules. Draw the form of the molecule with a +1 net charge. What would the net charge of the molecule be at physiological pH?



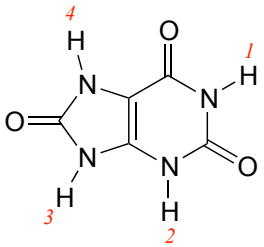
porphobilinogen

P7.5: Classify each of the nitrogen atoms in the coenzyme S-adenosylmethionine as an amine, amide, 'aniline-like', 'pyridine-like', or 'pyrrole-like'. Which nitrogen is most basic? Which is least basic?



S-adenosylmethionine

P7.6: Uric acid, an intermediate in the catabolism (breakdown) of the nucleotide adenosine, has four protons. Which would you expect to be the least acidic? Use resonance structures to explain your reasoning. *Hint*: consider protons 1-4 in turn, and what the conjugate base would look like if each proton were donated to a base: how well could the resulting negative charge be stabilized by resonance?



uric acid

P7.7: Estimate the net charge on a peptide with the sequence P-E-P-T-I-D-E (single-letter amino acid code), when it is dissolved in a buffer with pH = 7 (don't forget to consider the terminal amino and carboxylate groups).

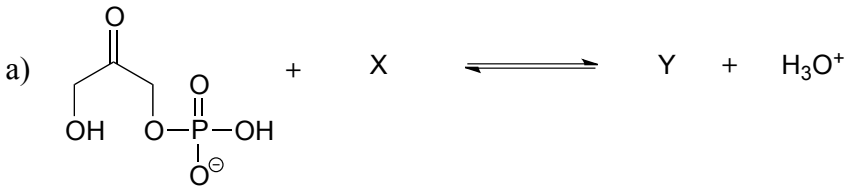
P7.8: Estimate the net charge on a dipeptide of sequence D-I.

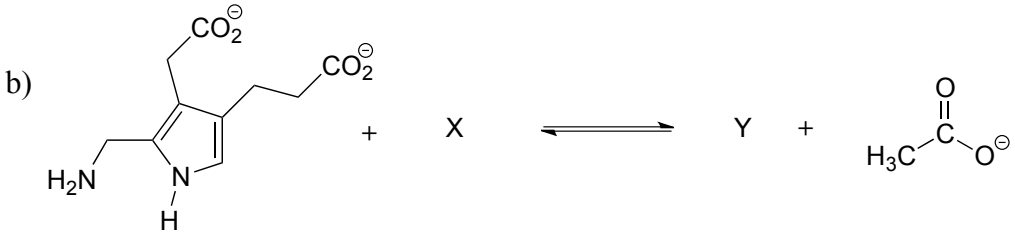
a) in a buffer with pH = 4.0

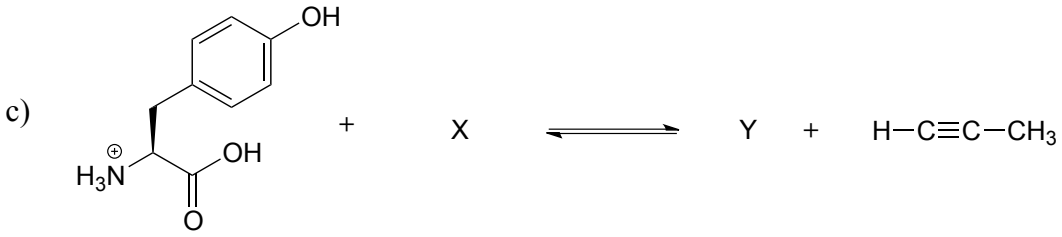
b) in a buffer with pH = 7.3

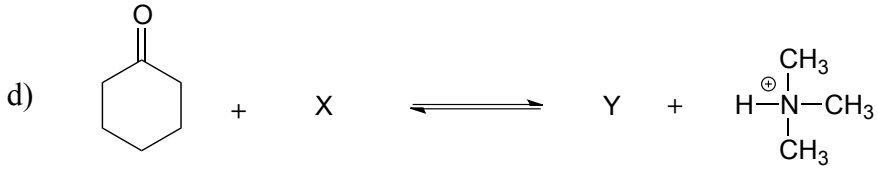
c) in a buffer with pH = 9.6

P7.9: Show the structures of species X and Y in the following acid-base reactions, and estimate the value of K_{eq} using the pK_a table. Assume that reactions involve equimolar amounts of acid and base.

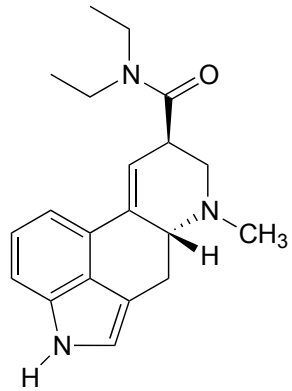
a) 

b) 

c) 

d) 

P7.10: Locate the most basic site on the structure of the hallucinogenic drug known as LSD.

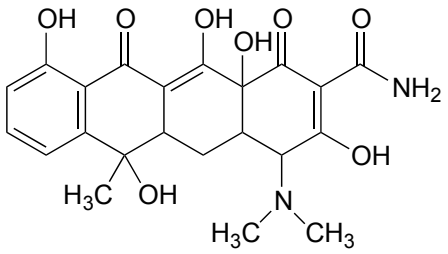


lysergic acid diethylamide
(LSD)

P7.11:

a) Identify the most acidic proton on the antibiotic tetracycline, and explain your choice.

b) Identify two additional protons which would be expected to have pKa values close to 5.



tetracycline

P7.12: In an enzyme active site, a lysine side chain is surrounded by phenylalanine, alanine, tryptophan, and leucine residues. Another lysine side chain is located on the surface of the protein, pointing out into the surrounding water. Which residue has the higher pKa, and why?

P7.13: (a-d) How would the immediate proximity of a magnesium ion affect the pKa of the side chains of the following amino acids (relative to the 'typical' pKa values given in the text)? Assume that all residues are located in the interior of the protein structure, not in direct contact with the outside buffer solution.

a) a glutamate residue?

b) a lysine residue?

c) a histidine residue?

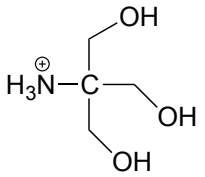
d) a tyrosine residue?

e) How would contact with a magnesium ion effect the pKa of a bound water molecule in the interior of a protein?

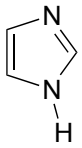
P7.14: The side chain of lysine has a pKa of approximately 10.5, while the pKa of the arginine side chain is approximately 12.5. Use resonance structures to rationalize this difference.

P7.15: The α-protons of ketones are, in general, significantly more acidic than those of esters. Account for this observation using structural arguments.

P7.16: 2-amino-2-hydroxymethylpropane-1,3-diol, (commonly known as 'Tris') and imidazole are very commonly used as buffers in biochemistry and molecular biology laboratories. You make two buffer solutions: One is 50 mM Tris at pH 7.0, the other 50 mM imidazole at pH 6.7. For each solution, calculate the concentration of buffer molecules in the cationic protonation state.



'Tris' buffer
$pK_a = 8.1$



imidazole
$pK_a = 7.0$

P7.17: The compound pictured below is an unusual carbon acid (pKa ~ 16) that does not contain any heteroatoms. Explain why it is so much more acidic than other hydrocarbons.

$pK_a \sim 16$

Chapter 8

Nucleophilic substitution reactions

(Credit: https://www.flickr.com/photos/12567713@N00/)

Introduction

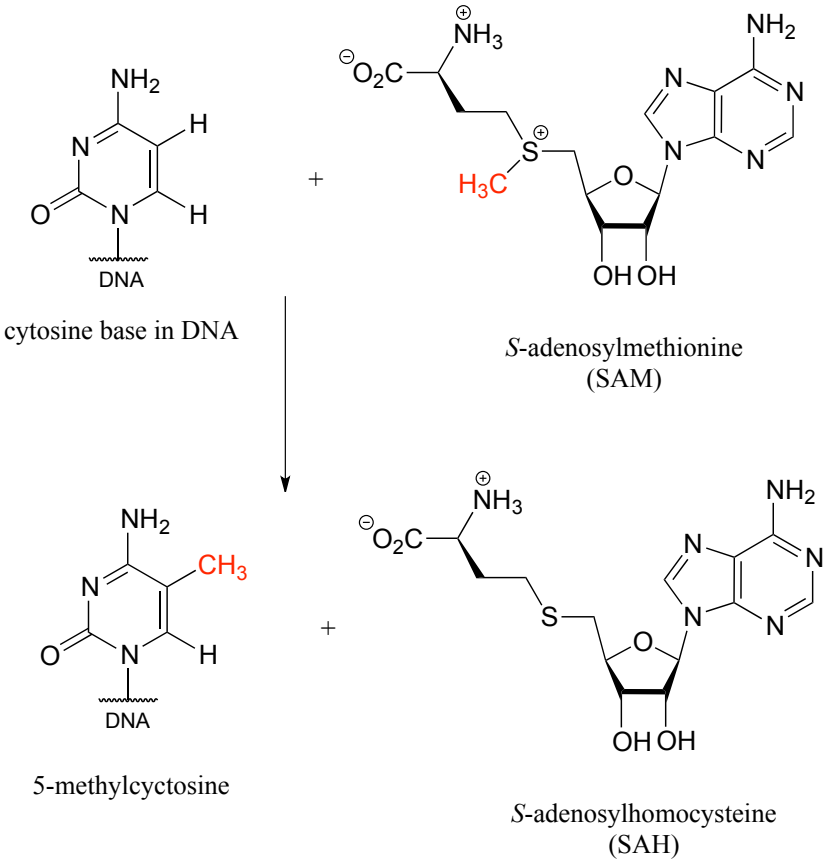
Dr. Tim Spector, Professor of Genetic Epidemiology at Kings College in London, knows a thing or two about twins. He should: as head of the Department of Twin Research at Kings College, Spector works with about 3500 pairs of identical twins, researching the influence of a person's genetic blueprint on everything from how likely they are to be obese, to whether or not they hold religious beliefs, to what kind of person they fall in love with. Anyone who is a twin, or has ever known a pair of identical twins, can attest to how remarkably similar they are to each other, even in the rare cases of adopted twins raised in separate homes. Dr. Spector, however, has over the course of his research become much more interested in how they are different.

A recent article about Spector in the British newspaper *The Guardian* (June 1, 2013) begins with an introduction to two middle-aged twin sisters named Barbara and Christine, one of the pairs of twins in the Kings College study group. Although they were treated almost as a single person when growing up, with identical haircuts and clothes, the twins began to diverge in their teenage years as they gained the freedom to make their own choices. They began to dress quite differently, with Christine choosing much more conservative styles than Barbara. Christine describes herself as being self-conscious, while Barbara has always been more confident. Christine suffers from depression, but Barbara does not.

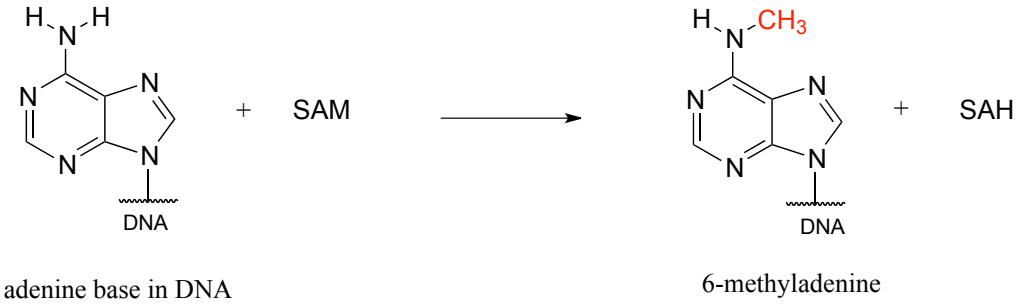
Given that they were born with the exact same DNA and were raised in the same home, where do these differences come from? In public debates about why people are the way they are, a catch phrase that often comes up is 'nature vs. nurture': people argue, in other words, about the relative influence of a person's genes vs. the influence of their environment. In Barbara and Christine's case, one would assume that the 'nature' is identical, and given that they grew up in the same house, the 'nurture' side of the equation should also be quite similar.

As it turns out, the 'nature' component may not be so identical after all. Based on his work with twins, Spector now thinks that subtle changes to Barbara's and Christine's DNA *after* conception - and indeed, throughout their lifetimes - may be a much more important determinant of their physical and psychological characteristics than was previously believed. As we age from infants to adulthood, some of our DNA bases are modified by methylation: in other words, a methyl (CH_3) group replaces a hydrogen. In humans and other mammals, this mainly happens to cytosine (C) bases, while in bacteria it is mainly adenosine (A) bases which are methylated. The biomolecule that serves as the methyl group donor in both cases is called *S*-adenosyl methionine, or 'SAM' for short.

Methylation of cytosine:



Methylation of adenine:



In mammals, gene methylation seems to occur in different patterns in different people - even in identical twins - in response to environmental factors. Methylation also seems to have the effect of amplifying or muting a gene's function, by altering how it interacts with regulatory proteins. The combined effect of many gene methylation events can be profound, as groups of interrelated genes are 'turned up' or 'turned down' in concert. Professor Spector thinks that the many differences between Barbara and Christine probably stem, at least in part, from differences in how their genes have been methylated over the course of their lives so far.

In this chapter, we delve for the first time into 'real' organic reactions, beyond the simple proton transfer events of Bronsted acid-base reactions that we looked at in chapter 7. The methylation of DNA is an excellent example of a type of organic reaction called *nucleophilic substitution*, to which we were introduced briefly in chapter 6 as a model for learning about some of the fundamental concepts of organic reactivity. Now we will delve more deeply into three crucial players in this bond-forming and bond-breaking process: the nucleophile, the electrophile, and the leaving group. In doing so, we will get a chance to practice and refine our skills in drawing organic reaction mechanisms using the curved arrow formality, and we will think about what a transition state and a reactive intermediate of a reaction might look like, and how the structure of these species determines the regiochemical and stereochemical outcome of a nucleophilic substitution reaction. Perhaps, in the time spent working on this chapter, some of the cytosines in your DNA will undergo nucleophilic substitution reactions to become methylated - and who knows how this will influence who you go on to become?

Additional Reading:

Spector, Tim, 2013. *Identically Different: Why We Can Change Our Genes*. Overlook Hardcover. ISBN 978-1468306606

Section 8.1: Two mechanistic models for nucleophilic substitution

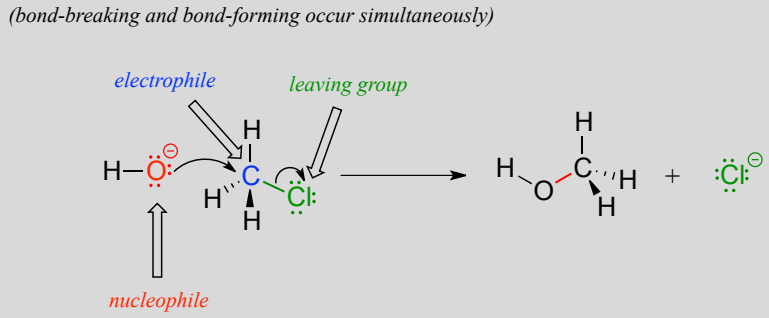
As we begin our study of nucleophilic substitution reactions, we will focus at first on simple alkyl halide compounds. While the specific reactions we'll initially consider do not occur in living things, it is nonetheless useful to start with alkyl halides as a model to illustrate some fundamental ideas that we must cover. Later, we will move on to apply what we have earned about alkyl halides to the larger and more complex biomolecules that are undergoing nucleophilic substitution right now in your own cells.

8.1A: The S_N2 mechanism

You may recall from our brief introduction to the topic in chapter 6 that there are two mechanistic models for how a nucleophilic substitution reaction can proceed. In one mechanism, the reaction is **concerted**: it takes place in a single step, and bond-forming

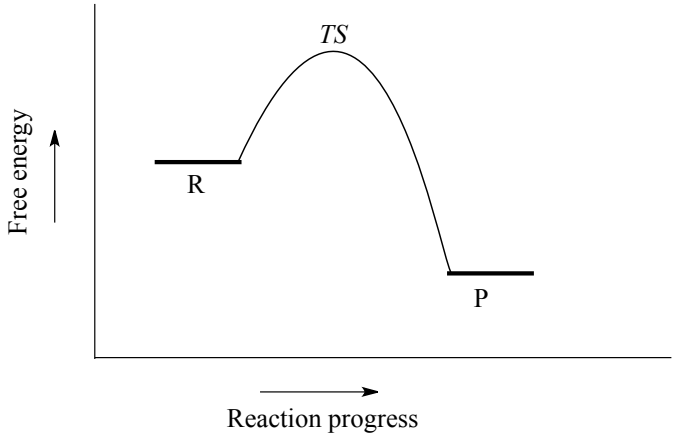
and bond-breaking occur simultaneously. This is illustrated by the reaction between chloromethane and hydroxide ion:

An S_N2 mechanism:

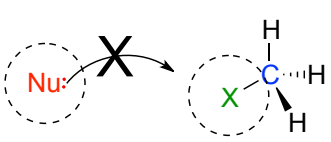


Recall that the hydroxide ion in this reaction is acting as a **nucleophile** (an electron-rich, nucleus-loving species), the carbon atom of chloromethane is acting as an **electrophile** (an electron-poor species which is attracted to electrons), and the chloride ion is the **leaving group** (where the name is self-evident).

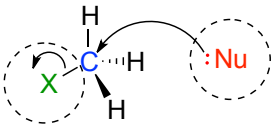
Organic chemists refer to this mechanism by the term '**S_N2',** where *S* stands for 'substitution', the subscript *N* stands for 'nucleophilic', and the number *2* refers to the fact that this is a **bimolecular reaction**: the overall rate depends on a step in which two separate species collide. A potential energy diagram for this reaction shows the transition state (TS) as the highest point on the pathway from reactants to products.



The geometry of an S_N2 reaction is specific: the reaction can only occur when the nucleophile collides with the electrophilic carbon from the *opposite* side relative to the leaving group. This is referred to as **backside attack**. Approach from the front side simply doesn't work: the leaving group - which, like the nucleophile is an electron-rich group - blocks the way.

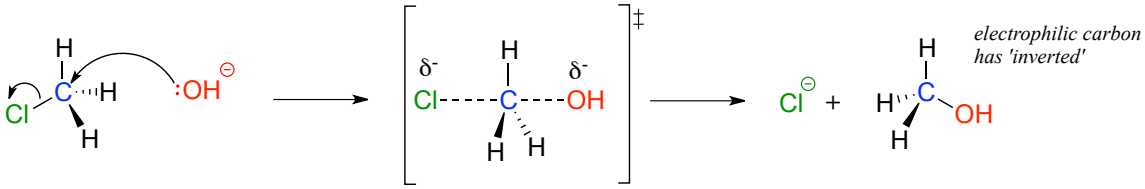


nucleophilic attack is blocked from the front side . . .



. . .so attack occurs from the back side

The result of backside attack is that the bonding geometry at the electrophilic carbon *inverts* (turns inside-out) as the reaction proceeds.



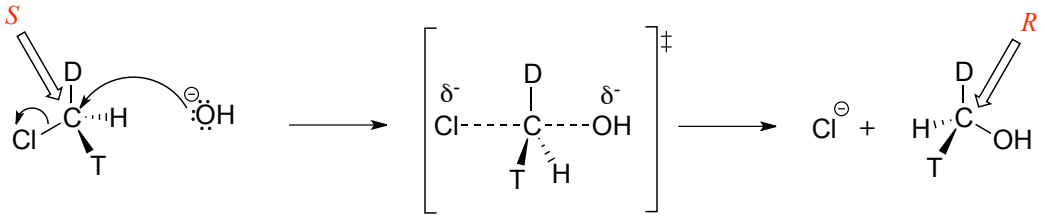
transition state

The transition state of the reaction is illustrated by drawing dotted lines to represent the covalent bonds that are in the process of breaking or forming. Because the formal charge on the oxygen nucleophile changes from negative one to zero as the reaction proceeds, and conversely the charge on the chlorine leaving group changes from zero to negative one, at the transition state both atoms are shown bearing a *partial* negative charge (the symbol δ^-). One other drawing convention for transition states is to use brackets, with the double-dagger symbol in subscript.

Notice that the transition state for an S_N2 reaction has **trigonal bipyramidal geometry**: the nucleophile, electrophile, and leaving group form a straight line, and the three substituents on carbon (all hydrogen atoms in this case) are arranged in the same plane at 120° angles.

Exercise 8.1: What is the value of the H-C-O angle in the S_N^2 transition state illustrated above?

Consider what would happen if we were to replace one of the hydrogen atoms in chloromethane with deuterium (the ^2H isotope), and one with tritium (the radioactive ^3H isotope). Now, because it has four different substituents, our carbon electrophile is a chiral center. We'll arbitrarily assume that we start with the *S* enantiomer.



As the hydroxide nucleophile attacks from the backside and the bonding geometry at carbon inverts, we see that the stereochemistry of the product reflects this inversion: we end up with the *R* enantiomer of the chiral product.

S_N2 reactions proceed with inversion of stereochemical configuration at the electrophilic carbon.

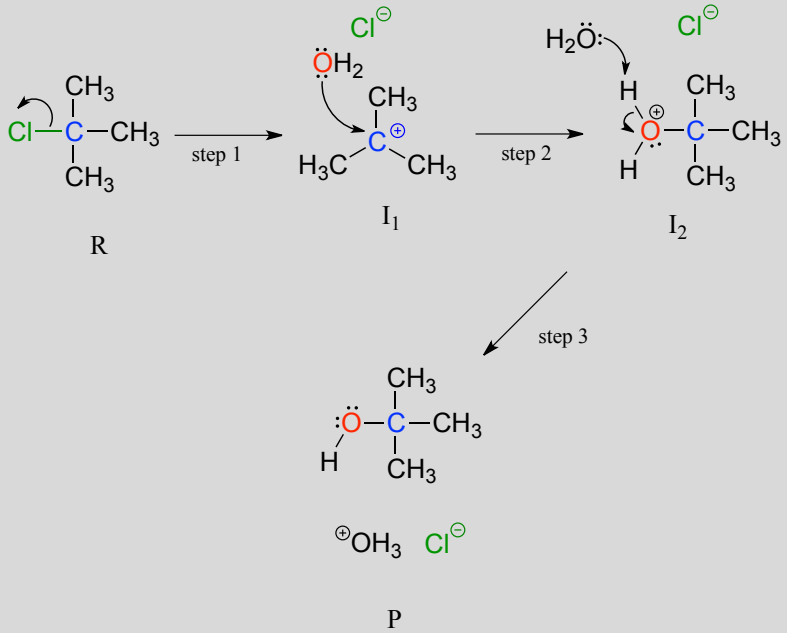
8.1B: The S_N1 mechanism

A second model for the nucleophilic substitution reaction is called the **S_N1 mechanism**. The '1' in S_N1 indicates that the rate-determining step of the reaction is *unimolecular*: in other words, the rate-determining step involves a single molecule breaking apart (rather than two molecules colliding as was the case in the S_N2 mechanism.)

In an S_N1 mechanism the carbon-leaving group bond breaks *first*, before the nucleophile approaches, resulting in formation of a carbocation intermediate (step 1):

An S_N1 mechanism:

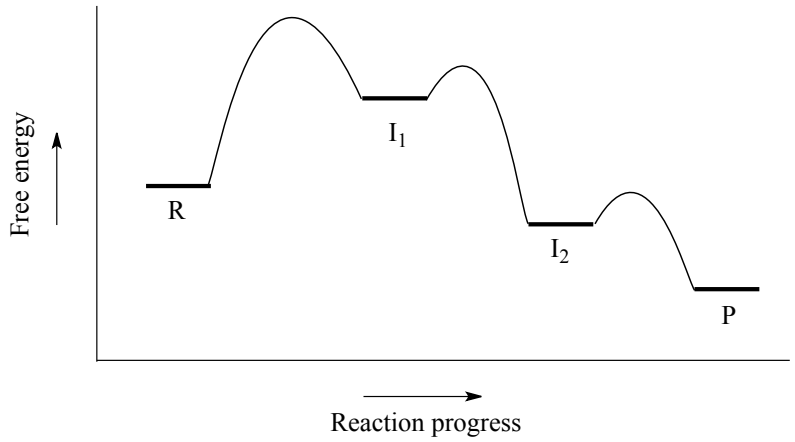
*(bond-breaking occurs **before** bond-forming)*



A carbocation is a powerful electrophile: because the carbon lacks a complete octet of valence electrons, it is 'electron-hungry'. In step 2, a lone pair of electrons on the water nucleophile fills the empty *p* orbital of the carbocation to form a new bond.

Notice that this is actually a three-step mechanism, with a final, rapid acid-base step leading to the alcohol product.

A potential energy diagram for this S_N1 reaction shows that each of the two positively-charged intermediate stages (I_1 and I_2 in the diagram) can be visualized as a valley in the path of the reaction, higher in energy than both the reactant and product but lower in energy than the transition states.



The first, bond-breaking step is the slowest, rate-determining step - notice it has the highest activation energy and leads to the highest-energy species (I_1, the carbocation intermediate). Step 2 is rapid: a new covalent bond forms between a carbocation and and a water nucleophile, and no covalent bonds are broken. Recall from chapter 7 that Bronsted-Lowry proton transfer steps like step 3 are rapid, with low activation energies.

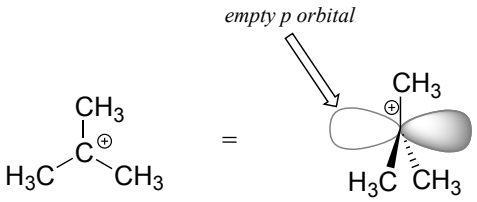
Hydrolysis

The nucleophilic substitution reactions we have seen so far are examples of **hydrolysis**. This term is one that you will encounter frequently in organic and biological chemistry. Hydrolysis means '*breaking with water*': in a hydrolysis reaction, a water molecule (or hydroxide ion) participates in the breaking of a covalent bond. There are many reaction types other than nucleophilic substitution that can accurately be described as hydrolysis, and we will see several examples throughout the remaining chapters of this book.

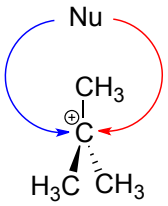
Solvolysis is a more general term, used when a bond in a reagent is broken by a solvent molecule: usually, the solvent in question is water or an alcohol such as methanol or ethanol.

Exercise 8.2: Draw a mechanism for the S_N1 solvolysis of *tert*-butyl chloride in methanol. What new functional group has been formed?

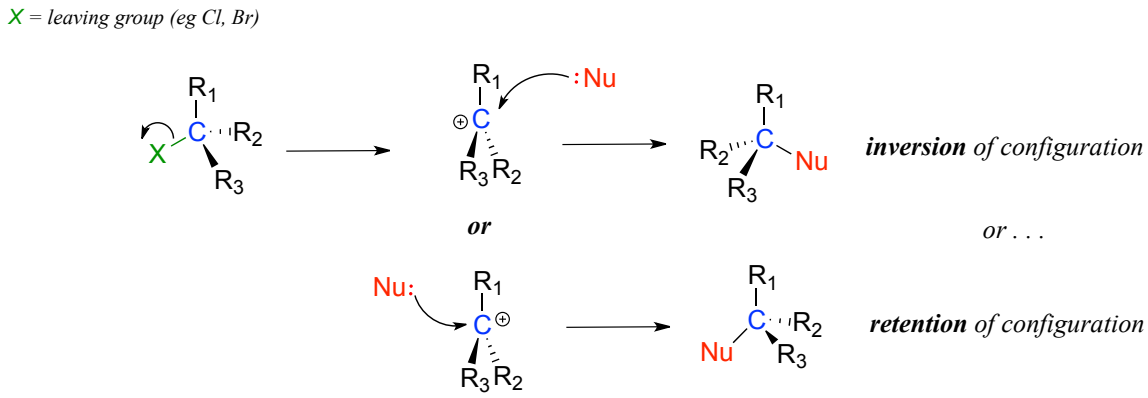
We saw that S_N2 reactions result in inversion of stereochemical configuration at the carbon center. What about the stereochemical outcome of S_N1 reactions? Recall from section 2.1C that a carbocation is sp^2-hybridized, with an empty *p* orbital perpendicular to the plane formed by the three sigma bonds:



In the second step of an S_N1 reaction, the nucleophile can attack from *either side* of the carbocation (the leaving group is already gone, and thus cannot block attack from one side like in an S_N2 reaction).



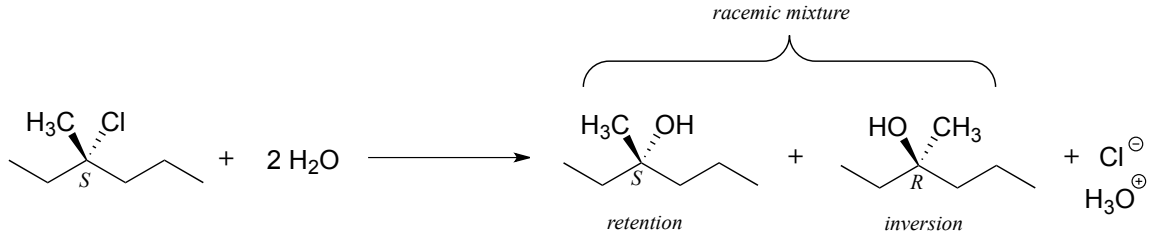
Consider an S_N1 reaction with a chiral tertiary alkyl chloride:



Because the nucleophile is free to attack from either side of the carbocation electrophile, the reaction leads to a 50:50 mixture of two stereoisomeric products. In other words:

In general *nonenzymatic* S_N1 reaction can occur with either **retention** or **inversion** of configuration at the electrophilic carbon, leading to **racemization** if the carbon is chiral.

For an example, consider the hydrolysis of (*S*)-3-chloro-3-methylhexane.



The result of this (nonenzymatic) reaction is a racemic mixture of chiral alcohols.

It is important to remember, however, that *enzymatic* reactions are in almost all cases very specific with regard to stereochemical outcome. A biochemical S_N1 reaction, as we shall see later, can result in either inversion or retention of configuration at the electrophilic carbon, but generally *not* a mixture of both: the two reactants are bound with specific geometry in the enzyme's active site, so that the nucleophile can approach from one side only.

(The following exercises refer to nonbiological reactions)

Exercise 8.3:

a) Draw a complete mechanism for the hydrolysis reaction in the previous figure, showing all bond-breaking and bond-forming steps, and all intermediate species.

b) Draw structures representing TS_1 and TS_2 in the reaction. Use the solid/dash wedge convention to show three dimensions.

c) What is the expected optical rotation of the product mixture?

d) Could the two organic products be separated on a silica column chromatography?

Exercise 8.4:

a) Draw the product(s) of the hydrolysis of (R)-3-chloro-3-methyl heptane.

b) What can you predict, if anything, about the optical rotation of the product(s)?

c) Draw the product(s) of the hydrolysis of (3R,5R)-3-chloro-3,5-dimethyl heptane.

d) What can you predict, if anything, about the optical rotation of the product(s)?

Exercise 8.5: Predict the product(s) of the reaction between methanol and *tert*-butyl chloride (IUPAC name 2-chloro-2-methylpropane).

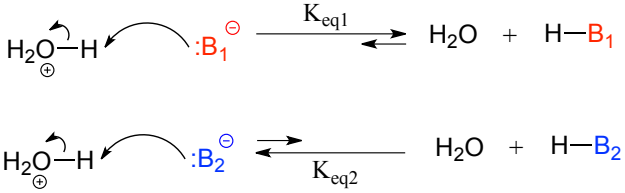
Before we go on to look at some actual biochemical nucleophilic substitution reactions, we first need to lay the intellectual groundwork by focusing more closely on the characteristics of the three principal partners in the nucleophilic substitution reaction: the nucleophile, the electrophile, and the leaving group. In addition, we need to consider the carbocation intermediate that plays such a key role in the S_N1 mechanism. For the sake of simplicity, we will continue to use simple, non-biological organic molecules and reaction examples as we work through the basic concepts.

Section 8.2: Nucleophiles

8.2A: What is a nucleophile?

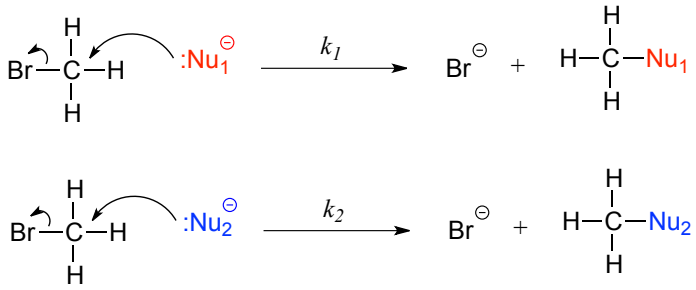
A nucleophile is an atom or functional group with a pair of electrons (usually a non-bonding, or lone pair) that can be shared. The same, however, can be said about a base: in fact, bases can act as nucleophiles, and nucleophiles can act as bases. What, then, is the difference between a base and a nucleophile?

A Bronsted-Lowry base, as you will recall from chapter 7, uses a lone pair of electrons to form a new bond with an acidic proton. We spent much of chapter 7 discussing how to evaluate how basic a species is. Remember that when we evaluate basicity - the strength of a base - we speak in terms of *thermodynamics*: where does equilibrium lie in a reference acid-base reaction?



$Base_1$ is stronger than $Base_2$: equilibrium lies further to the right in reaction with a common acid.
$Keq_1 > Keq_2$

We will spend much of this section discussing how to evaluate how nucleophilic a species is - in other words, its **nucleophilicity**. A nucleophile shares its lone pair of electrons with an electrophile - an electron-poor atom other than a hydrogen, usually a carbon. When we evaluate nucleophilicity, we are thinking in terms of *kinetics* - how fast does the nucleophile react with a reference electrophile?

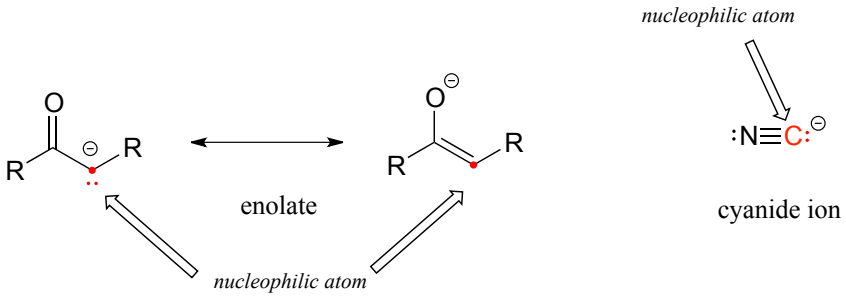


*Nucleophile$_1$ is more powerful than Nucleophile$_2$: reacts **faster** with a common electrophile. rate constant $k_1 > k_2$*

In both laboratory and biological organic chemistry, the most common nucleophilic atoms are oxygen, nitrogen, and sulfur, and the most common nucleophilic compounds and functional groups are water/hydroxide ion, alcohols, phenols, amines, thiols, and sometimes carboxylates.

In laboratory (non-biological) reactions, halide (I^-, Br^-, Cl^-, F^-) and azide (N_3^-) anions are also commonly seen acting as nucleophiles in addition to the groups mentioned above.

Carbon atoms can also be nucleophiles - enolate ions (section 7.6) are common carbon nucleophiles in biochemical reactions, while the cyanide ion (CN^-) is just one example of a carbon nucleophile commonly used in the laboratory.



Understanding carbon nucleophiles will be critical when we study, in chapters 12 and 13, the enzyme-catalyzed reactions in which new carbon-carbon bonds are formed in the synthesis of biomolecules such as DNA and fatty acids. In the present chapter, however, we will focus on heteroatom (non-carbon) nucleophiles.

Now, let's consider a number of factors that influence how nucleophilic an atom or functional group is. We'll start with protonation state.

8.2B: Protonation state

The protonation state of a group has a very large effect on its nucleophilicity. A negatively-charged hydroxide ion is much more nucleophilic (and basic) than a water molecule. In practical terms, this means that a hydroxide nucleophile will react in an S_N2 reaction with chloromethane several orders of magnitude faster than will a water nucleophile.

Likewise, a thiolate anion is more nucleophilic than a neutral thiol, and a neutral amine is nucleophilic, whereas an ammonium cation is not.

In a non-biological context, S_N2 reactions tend to occur with more powerful, anionic nucleophiles, where the nucleophile can be thought of as actively displacing ('pushing') the leaving group off the carbon. S_N1 reactions, in contrast, tend to be solvolysis reactions, with a weak, neutral nucleophile such as water or an alcohol.

8.2C: Periodic trends in nucleophilicity

Just as with basicity, there are predictable periodic trends associated with nucleophilicity. Moving horizontally across the second row of the periodic table, the trend in nucleophilicity parallels the trend in basicity:

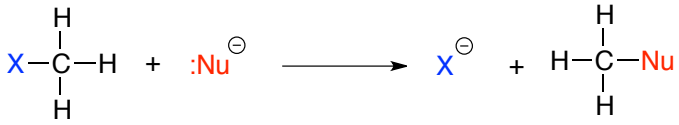
The horizontal periodic trend in nucleophilicity

more nucleophilic $NH_2^- > OH^- > F^-$ *less nucleophilic*

more nucleophilic $R\text{-}NH_2 > R\text{-}OH$ *less nucleophilic*

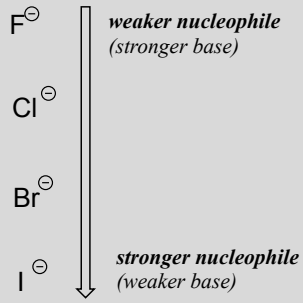
Recall from section 7.3A that the basicity of atoms decreases as we move vertically down a column on the periodic table: thiolate ions are less basic than alkoxide ions, for example, and bromide ion is less basic than chloride ion, which in turn is less basic than fluoride ion. Recall also that this trend can be explained by considering the increasing size of the 'electron cloud' around the larger ions: the electron density inherent in the negative charge is spread around a larger volume, which tends to increase stability (and thus reduce basicity).

The vertical periodic trend for nucleophilicity is somewhat more complicated that that for basicity, and depends on the solvent in which the reaction is taking place. Take the general example of the S_N2 reaction below:



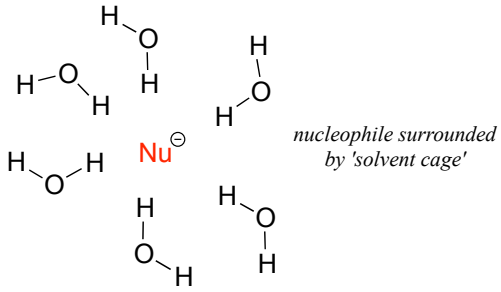
. . .where Nu^- is one of the halide ions: fluoride, chloride, bromide, or iodide, and X is a common leaving group. If this reaction is occurring in a **protic solvent** (that is, a solvent that has a hydrogen atom bonded to an oxygen or nitrogen - water, methanol and ethanol are protic solvents), then *the reaction will go fastest when iodide is the nucleophile, and slowest when fluoride is the nucleophile*, reflecting the relative strength of the nucleophile.

The vertical periodic trend in nucleophilicity in water and other protic solvents
(opposite of the trend in basicity!)



This is the *opposite* of the vertical periodic trend in basicity (section 7.3A), where iodide is the *least* basic. What is going on here? Shouldn't the stronger base, with its more reactive unbonded valence electrons, also be the stronger nucleophile?

As mentioned above, it all has to do with the solvent. Remember, we are talking now about the reaction running in a *protic* solvent like water. Protic solvent molecules form strong noncovalent interactions with the electron-rich nucleophile, essentially creating a 'solvent cage' of hydrogen bonds:
artwork needed

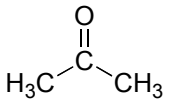


For the nucleophile to attack in an S_N2 reaction, the nucleophile-solvent hydrogen bonds must be disrupted - in other words, the nucleophilic electrons must 'escape through the bars' of the solvent cage. A weak base like iodide ion interacts weakly with the protons of the solvent, so these interactions are more readily disrupted. Furthermore, because the valence electrons on iodide ion are far from the nucleus, the electron cloud is **polarizable** - electron density can readily be pulled away from the nucleus, through the solvent cage and toward the electrophile.

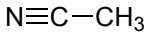
A smaller, more basic anion such as fluoride is more highly shielded by stronger interactions with the solvent molecules. The electron cloud of the fluoride ion is smaller and much less polarizable than that of an iodide ion: in water solvent, the larger iodide ion is a more powerful nucleophile than the smaller fluoride ion.

The above discussion applies to biochemical reactions, because of course the biological solvent is water. The picture changes for laboratory reactions if we switch to a **polar aprotic solvent**, such as acetone, which is polar enough to solvate the polar and ionic compounds in the reaction but *is not a hydrogen bond donor*, and does not form a strong 'solvent cage' like water does. In acetone and other polar aprotic solvents, the trend in nucleophilicity is the same as the trend in basicity: fluoride is the strongest base *and* the strongest nucleophile.

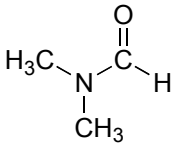
Structures of some of the most common polar aprotic solvents are shown below. These solvents are commonly used in laboratory nucleophilic substitution reactions.



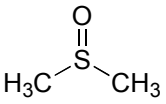
acetone



acetonitrile

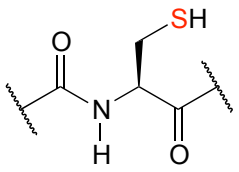


dimethylformamide (DMF)

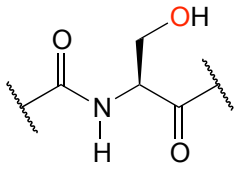


dimethylsulfoxide (DMSO)

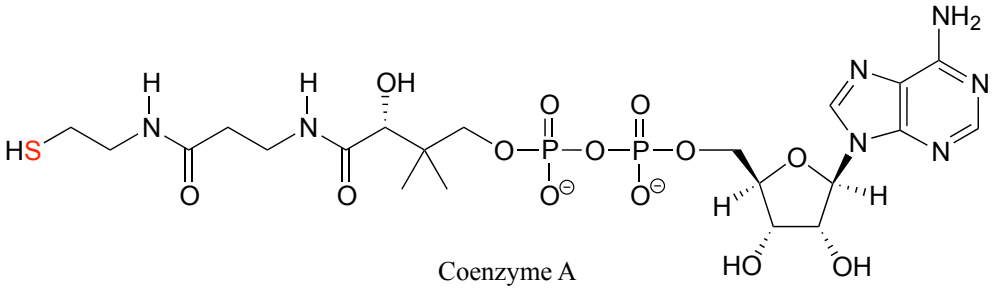
In biological chemistry, the most important implication of the vertical periodic trend in nucleophilicity is that *thiols are more nucleophilic than alcohols*. The thiol group in a cysteine amino acid residue, for example, is more nucleophilic than the alcohol group on a serine, and cysteine often acts as a nucleophile in enzymatic reactions. The thiol group on coenzyme A is another example of a nucleophile we will see often in enzymatic reactions later on. Of course, reactions with oxygen and nitrogen nucleophiles are widespread in biochemistry as well.



cysteine residue



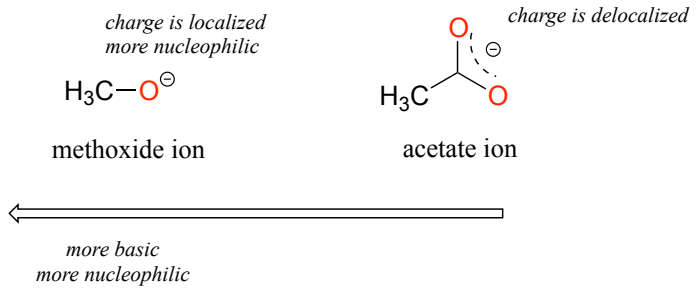
serine residue



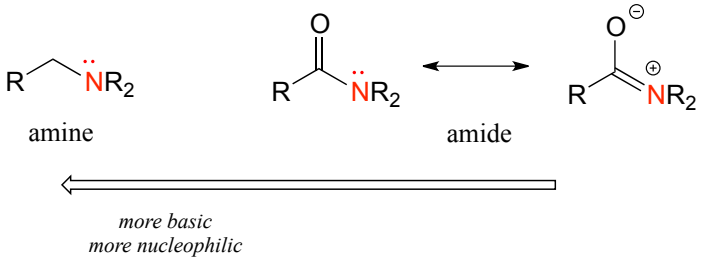
Coenzyme A

8.2D: Resonance effects on nucleophilicity

Resonance effects also come into play when comparing the inherent nucleophilicity of different molecules. The reasoning involved is the same as that which we used to understand resonance effects on basicity (see section 7.3B). If the electron lone pair on a heteroatom is delocalized by resonance, it is inherently less reactive - meaning less nucleophilic, and also less basic. An alkoxide ion, for example, is more nucleophilic and more basic than a carboxylate group, even though in both cases the nucleophilic atom is a negatively charged oxygen. In an alkoxide, the negative charge is localized on a single oxygen, while in the carboxylate the charge is delocalized over two oxygen atoms by resonance.



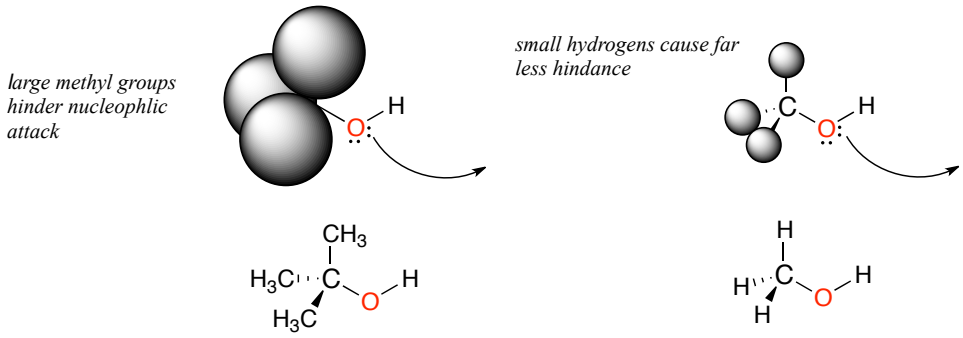
The nitrogen atom on an amide is less nucleophilic than the nitrogen of an amine, due to the resonance stabilization of the nitrogen lone pair provided by the amide carbonyl group.



Exercsice 8.5: Which amino acid has the more nucleophilic side chain - serine or tyrosine? Explain.

8.2E: Steric effects on nucleophilicity

Steric hindrance is an important consideration when evaluating nucleophility. For example, tert-butanol is less potent as a nucleophile than methanol. The comparatively bulky methyl groups on the tertiary alcohol effectively block the route of attack by the nucleophilic oxygen, slowing the reaction down considerably (imagine trying to walk through a narrow doorway while carrying three large suitcases!).



A final note: when it comes to comparing the rate of nucleophilic substitution reactions, the strength of the nucleophile only matters for S_N2 reactions. It is irrelevant for S_N1 reactions, because the rate-determining step (when the leaving group departs and a carbocation intermediate forms) does *not* involve the nucleophile.

Exercise 8.6: Which is the better nucleophile - a cysteine side chain or a methionine side chain? A serine or a threonine? Explain.

Exercise 8.7: In each of the following pairs of molecules/ions, which is expected to react more rapidly with CH_3Cl in acetone solvent? Explain your choice.

a) phenolate (deprotonated phenol) or benzoate (deprotonated benzoic acid)?
b) water or hydronium ion?
c) trimethylamine or triethylamine?
d) chloride anion or iodide anion?
e) CH_3NH^- or $CH_3CH_2NH_2$?
f) acetate or trichloroacetate?
g) aniline or 4-methoxyaniline?
h) phenolate or 2,6-dimethylphenolate?

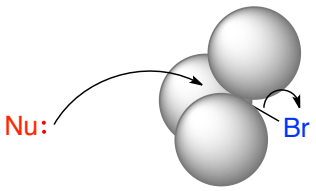
Section 8.3: Electrophiles: steric hindrance and carbocation stability

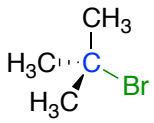
Next, we turn to electrophiles. In the vast majority of the nucleophilic substitution reactions you will see in this and other organic chemistry texts, the electrophilic atom is a carbon bonded to an electronegative atom, usually oxygen, nitrogen, sulfur, or a halogen. The concept of electrophilicity is relatively simple: an electron-poor atom is an attractive target for something that is electron-rich, *i.e.* a nucleophile. However, we must also consider the effect of steric hindrance on electrophilicity.

Steric hindrance at the electrophile

One of the most important factors to consider when looking at the electrophile in a nucleophilic substitution reaction is *steric hindrance*. Consider two hypothetical S_N2 reactions: one in which the electrophile is a methyl carbon and another in which it is tertiary carbon.

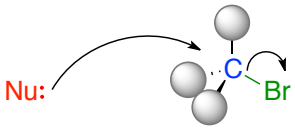
tertiary alkyl halide

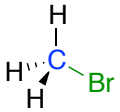




electrophile is hindered: no S_N2 possible (S_N1 only)

methyl halide



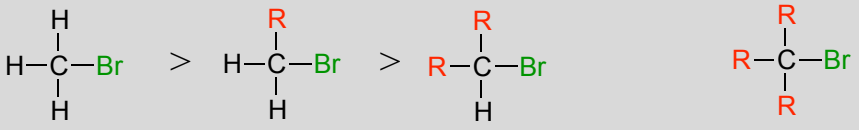


electrophile is unhindered: S_N2 is fast

Because the three substituents on the methyl electrophile are hydrogen atoms, the nucleophile has a relatively clear path for backside attack, and the S_N2 reaction will take place readily. However, backside attack on the *tertiary* carbon electrophile is blocked by the bulky methyl groups, preventing access to the site of electrophilicity.

S_N2 reactions occur at methyl, primary, and secondary carbon electrophiles. The degree of steric hindrance determines relative rates of reaction: unhindered methyl electrophiles react fastest, and more hindered secondary carbon electrophiles react slowest, assuming all other reactions conditions are identical. S_N2 reactions do *not* occur to an appreciable extent at tertiary carbon electrophiles.

relative rate of S_N2 reaction:



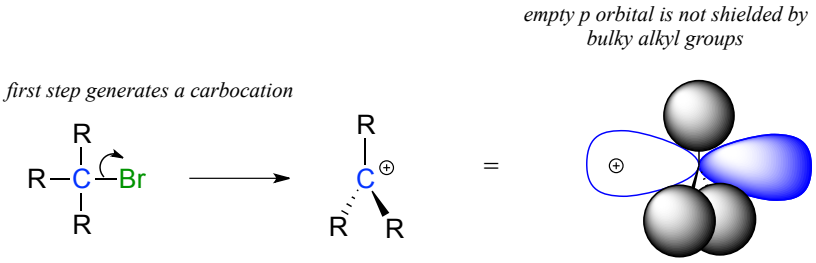
methyl | 1° | 2°

3°

(S_N2 not observed)

Exercise 8.8: Which would be expected to react more rapidly with an azide ion (N_3^-) nucleophile in acetone solvent: 1-bromo-2,2-dimethylbutane or 1-bromo-3-methylbutane?

What about the S_N1 pathway? Steric hindrance around the electrophilic carbon is *not* a significant factor in slowing down an S_N1 reaction. This makes perfect sense from a geometric point of view: the limitations imposed by sterics are significant in an S_N2 displacement because the electrophile being attacked is an sp^3-hybridized tetrahedral carbon with relatively 'tight' angles of 109.5°. Remember that in an S_N1 mechanism, the leaving group leaves first, and then the nucleophile attacks an sp^2-hybridized carbocation intermediate, which has trigonal planar geometry with 'open' 120° angles.
artwork needed



With this open geometry, the empty *p* orbital of the carbocation is no longer significantly shielded from the approaching nucleophile by the bulky alkyl groups, and is an 'easy target' for a nucleophile: this step is fast, and is *not* the rate-determining step for an S_N1 reaction.

Carbocation stability

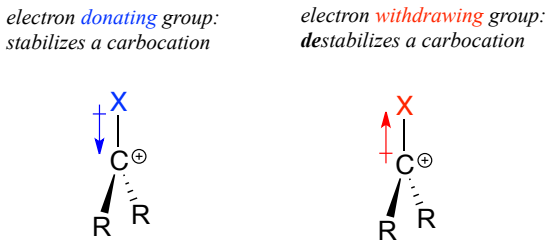
What, then, are the characteristics of an electrophile that favor an S_N1 reaction pathway as opposed to an S_N2 pathway? We know that the rate-limiting step of an S_N1 reaction is the first step: loss of the leaving group and formation of the carbocation intermediate. Accordingly, ***the rate of an S_N1 reaction depends to a large extent on the stability of the carbocation intermediate.***

The critical question now becomes:

What stabilizes a carbocation?

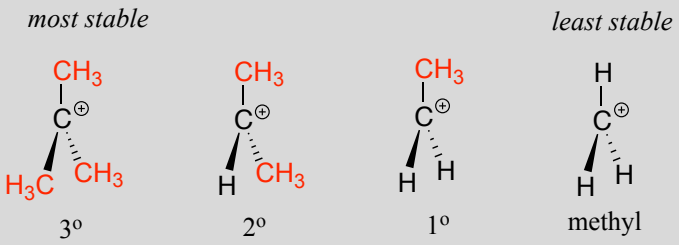
Think back to Chapter 7, when we were learning how to evaluate the strength of an acid. The critical question there was: "how stable is the conjugate base that results when this acid donates its proton"? In many cases, this conjugate base was an anion – a center of excess electron density. Anything that can draw some of this electron density away– in other words, any electron withdrawing group – will stabilize the anion.

Conversely, a carbocation is stabilized by an electron *donating* group, and **de**stabilized by an electron withdrawing group.

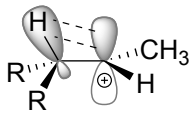


A positively charged species such as a carbocation is electron-poor, and thus anything which donates electron density to the center of electron poverty will help to stabilize it. Alkyl groups, because of the electrons in their carbon-carbon and carbon-hydrogen bonds, are weak electron-donating groups, and will stabilize nearby carbocations. What this means is that, in general, ***more substituted carbocations are more stable***: a *tert*-butyl carbocation, for example, is more stable than an isopropyl carbocation. Primary carbocations are highly unstable and not often observed as reaction intermediates; methyl cations are even less stable.

More substituted carbocations are more stable:



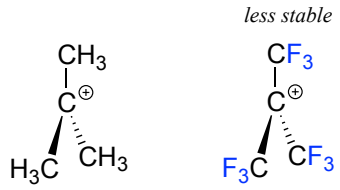
Another way to explain this trend in carbocation stability involves the phenomenon of **hyperconjugation**, in which the empty *p* orbital of a carbocation is stabilized by overlap with a σ bond on an adjacent carbon. This overlap effectively spreads the positive charge over a larger area. The figure below shows the empty π orbital of a secondary carbocation being stabilized by hyperconjugation with an adjacent C-H σ bond.



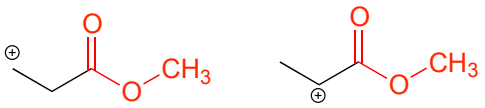
hyperconjugation

Hyperconjugation is not possible with a methyl cation as there is no adjacent s bond available to overlap the empty p orbital. As the degree of substitution on a carbocation increases, so does the capacity for stabilizing hyperconjugation interactions.

The presence of an electron-withdrawing group - such as a fluorine atom - will significantly *destabilize* a carbocation through the inductive effect.



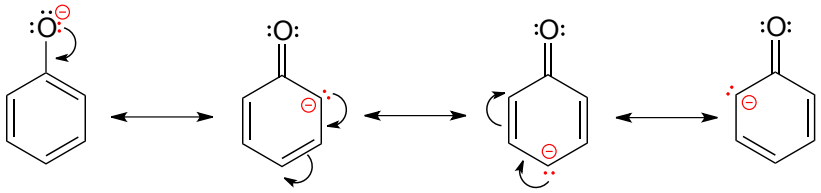
Carbonyl groups are electron-withdrawing by inductive effects, due to the polarity of the C=O double bond. It is possible to demonstrate in the laboratory (we'll see how in problem 14.x) that carbocation A below is more stable than carbocation B, even though A is a primary carbocation and B is secondary.



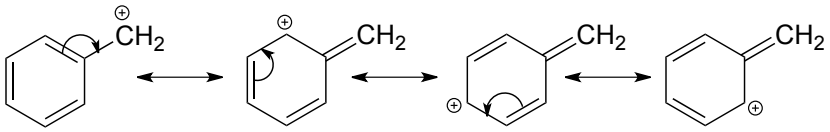
A: more stable **B**: less stable

The positive charge in cation B is closer to the electron withdrawing carbonyl substitution, and as we learned in section 7.3C, the inductive effect of an electron withdrawing group decreases with distance.

Stabilization of a carbocation can also occur through resonance effects. Recall from section 7.4 that the negative charge on a phenolate ion is stabilized by resonance, because the charge can be delocalized to three of the carbons on the aromatic ring.

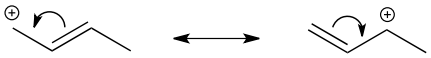


A positive charge is also stabilized when it can be delocalized over more than one atom. Consider a **benzylic carbocation**, where the positively-charged carbon is bonded directly to an aromatic ring. A benzylic carbocation is stabilized by the resonance electron-donating effect of the aromatic ring. Three additional resonance structures can be drawn for the carbocation in which the positive charge is located on one of three aromatic carbons:



Exercise 8.9: Fill in the missing numbers in this statement: The conjugated *p* system in the benzylic carbocation above is composed of _____ *p* orbitals overlapping to share _____ π electrons.

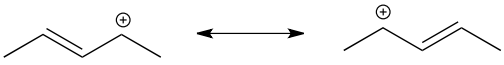
Allylic carbocations, where the positively charged carbon is adjacent to a double bond, are stabilized by resonance delocalization of the posive charge.



an allylic carbocation

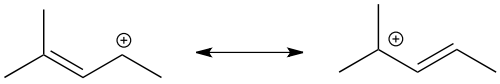
Often, we must consider more than one factor when predicting carbocation stability. For example, the carbocation on the right in the figure below is more stable than the carbocation on the left. Both are allylic with the charge delocalized over two carbons, but on the more stable carbocation, one of the carbons is tertiary.

charged delocalized over two 2º carbons



less stable

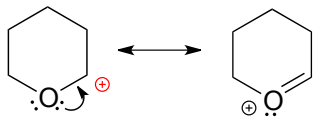
charged delocalized over 2º carbon and 3º carbon



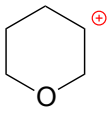
more stable

Because heteroatoms such as oxygen and nitrogen are more electronegative than carbon, you might expect that they would be carbocation-destabilizing electron withdrawing groups. In fact, the opposite is often true: if the oxygen or nitrogen atom is in the right position, the overall effect can be carbocation *stabilization*. Although these heteroatoms are indeed electron withdrawing groups by induction, they can be electron *donating* groups by resonance, and, as we learned earlier (section 7.3) in the context of acid-base chemistry, resonance effects are in general more powerful than inductive effects when the two operate in opposite directions.

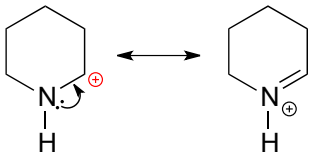
Consider the two pairs of carbocation species below:



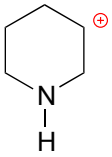
more stable



less stable
(no resonance delocalization)



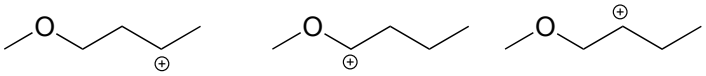
more stable



less stable
(no resonance delocalization)

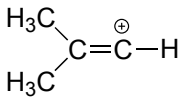
In the more stable carbocations, the heteroatom acts as an electron *donating* group by resonance: in effect, the lone pair on the heteroatom is available to delocalize the positive charge. Note also that every atom in the major resonance contributor has a complete octet of valence electrons.

Exercise 8.10: rank the following carbocations from most to least stable:



A B C

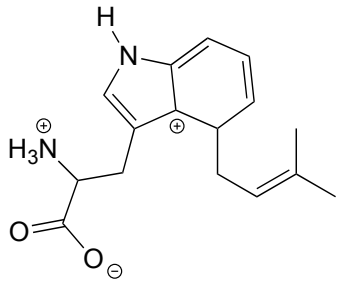
Finally, **vinylic** carbocations, in which the positive charge resides on a double-bonded carbon, are highly unstable.



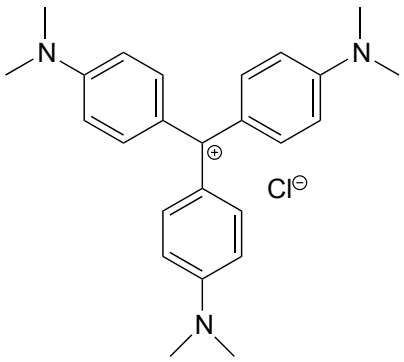
a vinylic carbocation
(very unstable)

Exercise 8.11: Explain why vinylic carbocations are unstable. (Hint: think about hybridization and electronegativity)

Exercise 8.12: The carbocation below is an intermediate species in a reaction that is part of the biosynthesis of a hallucinogenic compound in a fungus. Draw a resonance contributor that shows how it is stabilized by resonance with the nitrogen atom.



For the most part, carbocations - even 'relatively stable' carbocations such as those that are tertiary and/or benzylic - are still highly reactive, transient intermediate species in organic reactions, which briefly form and then react again right away. However, there are some unusual examples of carbocation species that are so stable that they can be put in a jar and stored on the shelf as a salt. Crystal violet is the common name for the chloride salt of the carbocation whose structure is shown below. Notice the structural possibilities for extensive resonance delocalization of the positive charge, and the presence of three electron-donating amine groups.



crystal violet

Exercise 8.13:

a) Draw a resonance structure of the crystal violet cation in which the positive charge is delocalized to one of the nitrogen atoms.

b) Notice that crystal violet is deeply colored. Explain why you could have predicted this from looking at its chemical structure.

c) The conjugated system of crystal violet consists of how many overlapping *p* orbitals sharing how many π electrons?

Summary of factors influencing carbocation stability:

I: More substituted carbocations are more stable than less substituted carbocation (eg. tertiary carbocations are more stable than secondary carbocations).

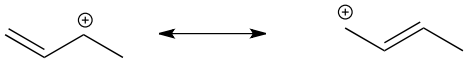
II: Nearby electronegative atoms can decrease carbocation stability by the inductive effect.

III: Allylic and benzylic carbocations are stabilized by resonance delocalization of the positive charge .

IV: Delocalization of the positive charge by resonance with the lone pair electrons on a heteroatom contributes to carbocation stability.

Below are three examples illustrating how we can make predictions about relative carbocation stability:

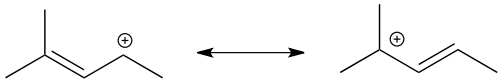
charged delocalized over 1º and 2º carbons



less stable

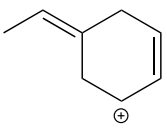
vs

charged delocalized over 2º carbon and 3º carbon



more stable

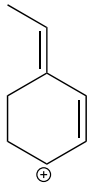
*charge delocalized over **two** 2º carbons*



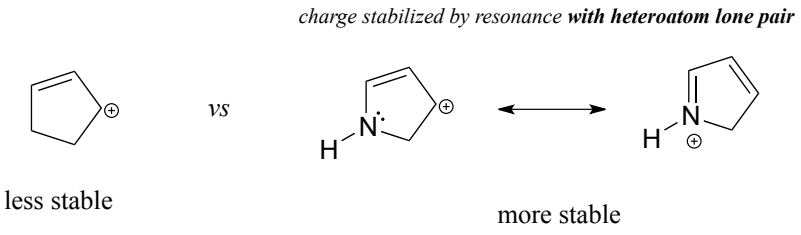
less stable

vs

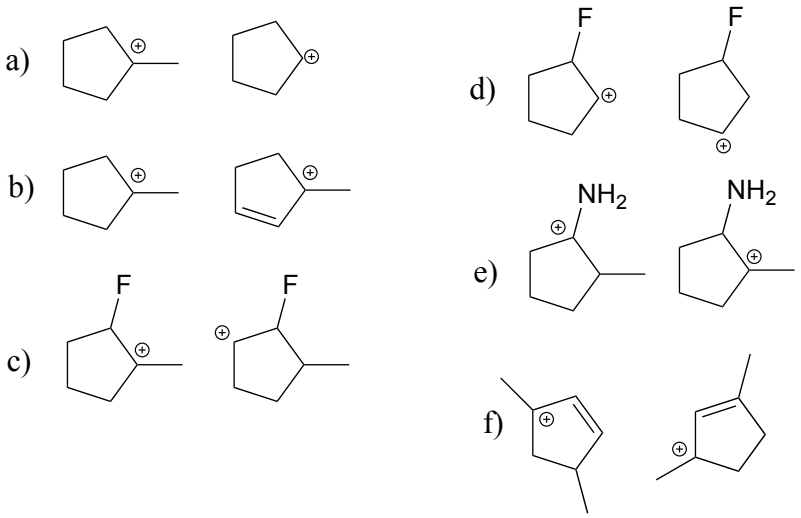
*charge delocalized over **three** 2º carbons*



more stable



Exercise 8.14: State which carbocation in each pair below is more stable, or if they are expected to be approximately equal. Explain your reasoning.



Now, back to our discussion of the electrophile in an S_N1 reaction:

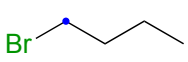
An S_N1 reaction requires a stabilized carbocation intermediate. The more stable the relevant carbocation intermediate, the more favored the S_N1 reaction pathway.

S_N1 reactions in general *do not* occur at methyl or primary carbon electrophiles: the carbocation intermediates involved would be too unstable and the rate-determining (carbocation-generating) step would have a very high energy barrier. Substitution on these electrophiles will occur through the S_N2 pathway.

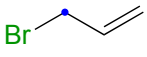
The S_N1 reaction pathway *is* possible, however, with secondary and tertiary carbon electrophiles, or with any other carbon electrophile in which departure of the leaving group generates a carbocation which is stabilized by resonance.

For example: a primary alkyl bromide would *not* be expected to undergo nucleophilic substitution by the S_N1 pathway. An allylic primary alkyl bromide, on the other hand,

would generate a relatively stable allylic carbocation and thus the S_N1 pathway is possible.

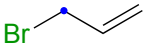


1°: no S_N1

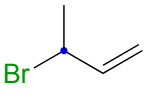


allylic 1°
S_N1 possible

An allylic secondary alkyl bromide would undergo S_N1 substitution more rapidly than the allylic primary alkyl bromide, because the relevant carbocation is more substituted and thus more stable.



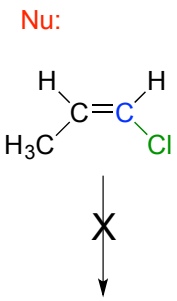
allylic 1°
slower S_N1



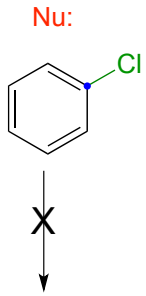
allylic 2°
faster S_N1

<u>*sp2*-hybridized carbons</u>

Nucleophilic substitution generally does *not* occur at *sp*^{2}-hybridized carbons, either by the S_N2 or S_N1 pathway.



no reaction



no reaction

Bonds on *sp*^{2}-hybridized carbons are inherently shorter and stronger than bonds on *sp*^{3}-hybridized carbons, meaning that it is harder to break the bond between an *sp*^{2} carbon and a potential leaving group (such as the chlorine atom in the figure above). In addition, steric considerations play a part here: in order to attack from behind the leaving group in an S_N2-like fashion, the nucleophile would need to approach *in the plane* of the carbon-carbon double bond.

Substitution by an S_N1 pathway is equally unlikely because of the inherent instability of a vinylic (double-bonded) carbocation.

Section 8.4: Leaving groups

Next, we investigate what makes a good leaving group. It's really quite straightforward: everything that we learned in chapter 7 about evaluating base strength will apply to leaving groups:

Weaker bases are better leaving groups.

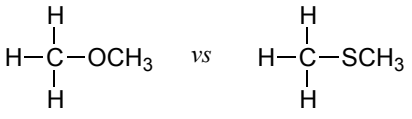
In our general discussion of nucleophilic substitution reactions, we have until now been using chloride ion as our common leaving group. Alkyl chlorides are indeed common reactants in laboratory nucleophilic substitution reactions, as are alkyl bromides and alkyl iodides. Iodide, which is the *least* basic of the four common halides (F, Cl, Br, and I), is the *best* leaving group among them. Fluoride is the least effective leaving group among the halides, because fluoride anion is the most basic. This rule applies to both S_N2 and S_N1 reactions, because in both cases the rate-determining step involves loss of the leaving group.

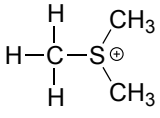
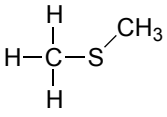
best leaving group $I^- > Br^- > Cl^- > F^-$ *worst leaving group*

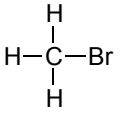
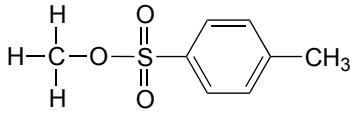
This trend is evident when you compare the relative rates of S_N2 reactions of four halomethanes with a common nucleophile and solvent: iodomethane reacts fastest, fluoromethane the slowest.

fastest S_N2 reaction $CH_3I > CH_3Br > CH_3Cl > CH_3F$ *slowest S_N2 reaction*

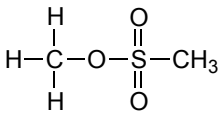
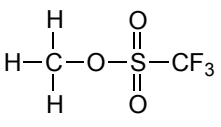
Exercise 8.15: In each pair (A and B) below, which electrophile would be expected to react more rapidly with cyanide ion nucleophile in acetone solvent? Explain your reasoning.

a)  *vs*

b)  *vs* 

c)  *vs* 

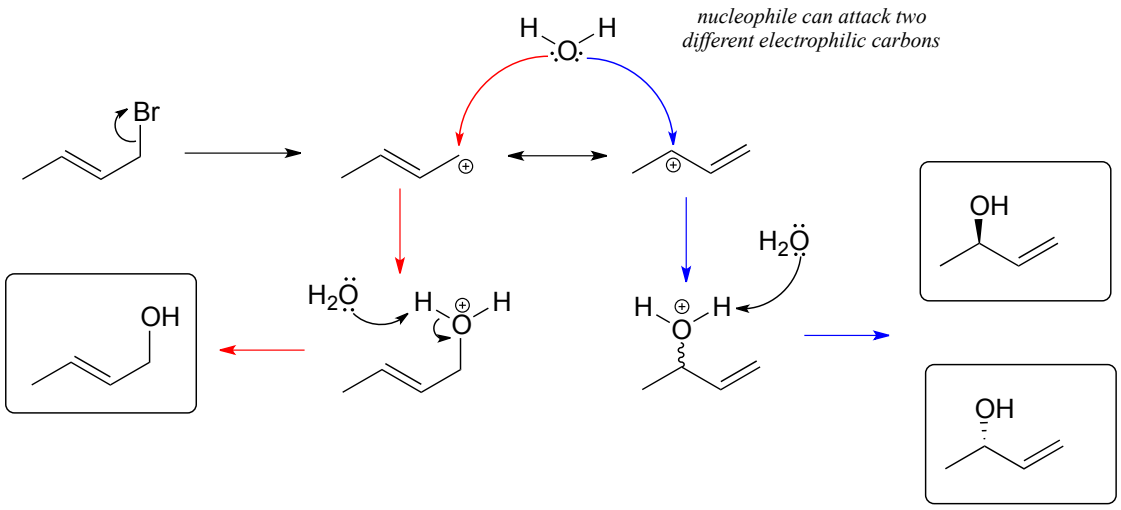
(hint - use a pKa table)

d)  *vs* 

Beginning later in this chapter and throughout the rest of our study of organic reactivity, we will see examples of leaving group 'activation': in other words, conversion of a strong base/poor leaving group into a weak base/good leaving group. In some cases this is as simple as protonation: an acidic group may be positioned in the active site in order to protonate a poor leaving group (eg. hydroxide ion in the case of an alcohol) as it leaves, thus converting it into a weak base and good leaving group. In many other enzymatic reactions, leaving groups are phosphate or pyrophosphate ions, species which we will learn much more about in chapter 9.

Section 8.5: Regiochemistry of S_N1 reactions with allylic electrophiles

S_N1 reactions with allylic electrophiles can often lead to more than one possible regiochemical outcome - resonance delocalization of the carbocation intermediate means that more than one carbon is electrophilic. For example, hydrolysis of this allylic alkyl bromide leads to a mixture of primary and secondary allylic alcohols.



In an enzyme-catalyzed reaction of this kind, however, generally only one product will form, because enzymes maintain strict control over the regiochemistry and stereochemistry of the reactions they catalyze. The nucleophilic and electrophilic substrates are bound specifically in the active site so that nucleophilic attack is directed at one - and only one - electrophilic carbon. Problem 15, 17, and 19 at the end of this chapter provide some examples of regio- and stereospecific biochemical substitution reactions at allylic carbon electrophiles.

Section 8.6: S_N1 or S_N2? Predicting the mechanism

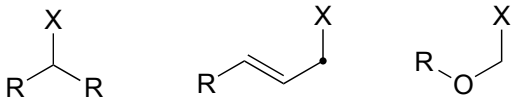
First of all, it is important to understand that the S_N1 and S_N2 mechanism models are just that: models. While many nucleophilic substitution reactions can be described as

proceeding through 'pure' S_N1 or S_N2 pathways, other reactions - in particular some important biochemical reactions we'll see later - lie somewhere in the continuum between the S_N1 and the S_N2 model (more on this later). With that being said, here are some guidelines to help you predict whether a reaction is likely to have more of an SN1 or SN2 character.

First, look at the electrophile: as stated above, an S_N1 reaction requires that a relatively **stable carbocation intermediate** be able to form. An S_N2 reaction requires a relatively **unhindered electrophilic center**. Therefore, methyl and primary carbon electrophiles will react by the S_N2 pathway, and tertiary carbon electrophiles will react by the S_N1 pathway.

Secondary carbon electrophiles, or primary carbon electrophiles adjacent to a potential carbocation-stabilizing group (double bond or heteroatom) can react by either or both pathways. The reasoning here is that these electrophiles are unhindered (favoring S_N2), but can also form stabilized carbocation intermediates (favoring S_N1)

Can react by S_N1 *or* S_N2



Next, look at the nucleophile. More powerful nucleophiles, particularly anionic nucleophiles such as hydroxides, alkoxides or thiolates, favor an S_N2 pathway: picture the powerful nucleophile 'pushing' the leaving group off the electrophile. Weaker, uncharged nucleophiles like water, alcohols, and amines, favor the S_N1 pathway: they are not nucleophilic enough to displace the leaving group, but will readily attack a carbocation intermediate.

Finally look at the solvent in the reaction. As a general rule, water and other protic solvents (for example methanol or ethanol) favor S_N1 pathways, due to the ability of the solvents to stabilize carbocation intermediates, combined with their tendency to weaken the nucleophile by enclosing it in a 'solvent cage'. In laboratory reactions, the presence of a polar aprotic sovent such as acetone or dimethylformamide points to the probability of an S_N2 reaction.

Factors favoring the S_N1 pathway:

hindered electrophile
potential for a tertiary, secondary, or resonance-stabilized carbocation intermediate
uncharged nucleophile
protic solvent such as water

Factors favoring the S_N2 pathway:

Unhindered (methyl or primary) electrophile
powerful, anionic nucleophile
polar aprotic solvent

Section 8.7: Biological nucleophilic substitution reactions

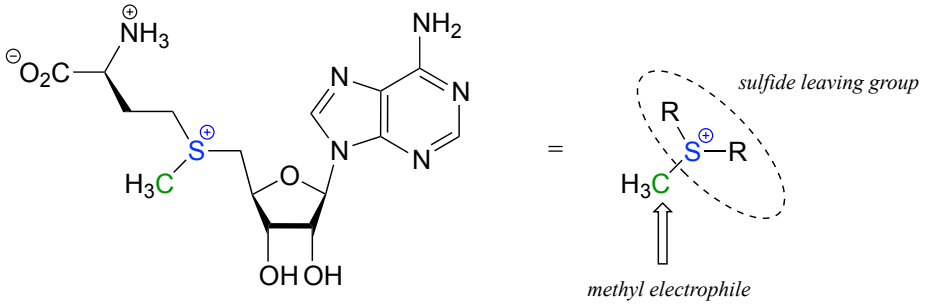
The nucleophilic substitution reactions we have seen so far have all been laboratory reactions, rather than biochemical ones. Now, finally, let's take a look at a few examples of nucleophilic substitutions in a biological context. All of the principles we have learned so far still apply to these biochemical reactions, but in addition we need to consider the roles of the enzyme catalysts.

A word of encouragement:

This is the first time that we will be seeing 'real' biological organic reaction mechanisms. ***Do not be intimidated by the size and complexity of the reacting biomolecules*** - they are just organic molecules, with the same bonding patterns and functional groups that you are already familiar with. Focus on the *reacting* parts of the molecule: what is the nucleophile? The electrophile? The leaving group? In most biological organic reactions, the main bulk of the biomolecule is just 'going along for the ride', and can often be abbreviated with an 'R group' (section 1.2C) to simplify the picture.

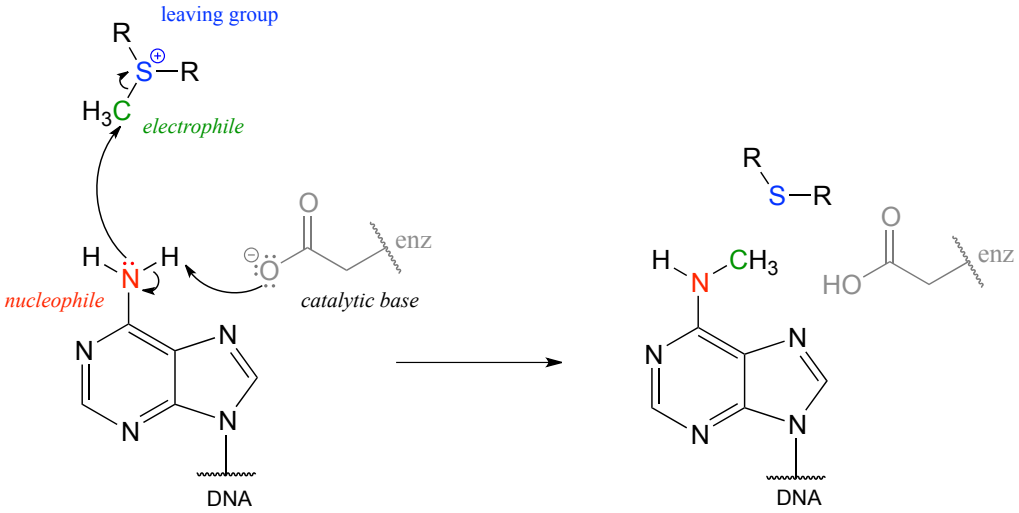
8.8A: A biochemical S_N2 reaction

One very important class of nucleophilic substitution reactions in biochemistry are the S_N2 reactions catalyzed by ***S*-adenosyl methionine** (SAM) – dependent **methyltransferase** enzymes. SAM is a coenzyme (section 6.3) that plays the role of methyl group donor: you can think of SAM in this context as being simply a methyl carbon electrophile attached to a sulfide leaving group.

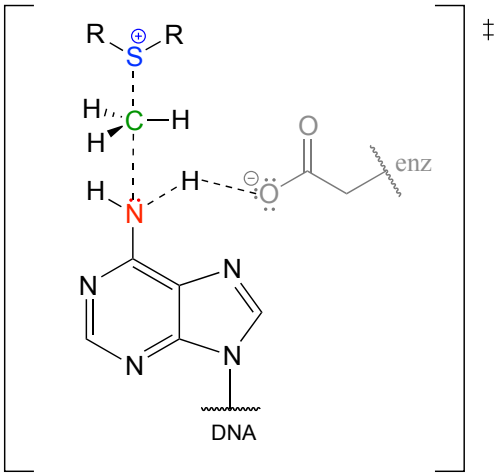


S-adenosylmethionine
(SAM)

There are many variations of SAM-dependent methylation reactions in nature. In the introduction to this chapter, we were introduced to a reaction occurring in bacterial DNA in which a methyl carbon is transferred from SAM to a nitrogen atom on adenine (this type of reaction is often referred to as *N*-methylation).



In the figure above, we are showing how an aspartate residue in the active site of the enzyme acts as a catalytic base: transfer of a proton from substrate to the aspartate side chain begins to enhance the nucleophilicity of the amine nitrogen as it approaches the electrophilic methyl carbon of SAM, and formation of the new N-C bond and cleavage of the C-S bond begins. These four bond-rearranging events probably take place in concerted fashion. A likely transition state is approximated below:



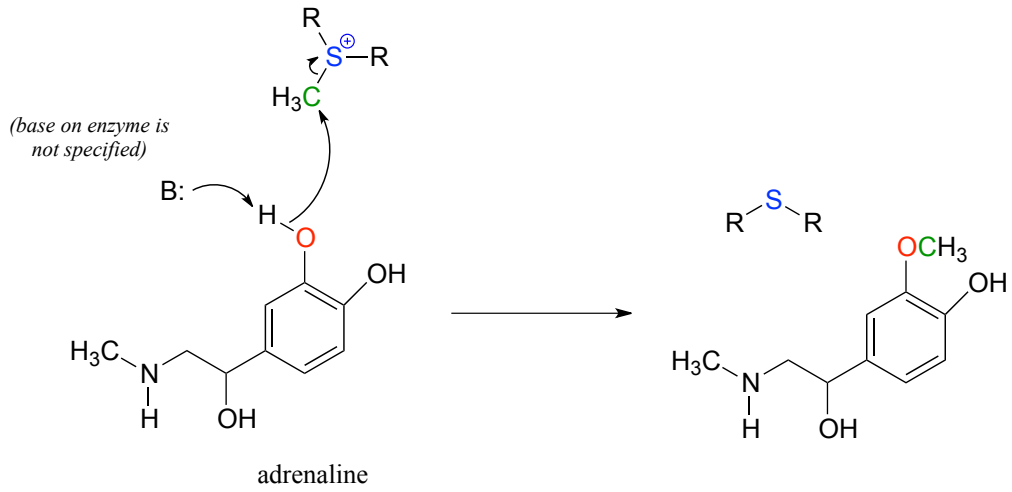
Of course, there are many other noncovalent interactions between active site enzyme residues and the substrate (the adenine base) and cofactor (SAM), but in the interest of clarity these are not shown. These interactions, many of which are hydrogen-bonds, help to position the adenine base and SAM in just the right relative orientation for the nucleophilic attack to take place. (If you have access to American Chemical Society journals, a paper about an enzyme catalyzing a similar *N*-methylation reaction contains some detailed figures showing hydrogen-bond and charge-dipole interactions between the enzyme and the two substrates: *Biochemistry* **2003**, *42*, 8394).

The electrophile is a methyl carbon, so there is little steric hindrance to slow down the nucleophilic attack. The carbon is electrophilic (electron-poor) because it is bonded to a positively-charged sulfur, which is a powerful electron withdrawing group. The positive charge on the sulfur also makes it an excellent leaving group, because as it leaves, it becomes a neutral and very stable sulfide. All in all, we have a good nucleophile (enhanced by the catalytic base), an unhindered electrophile, and an excellent leaving group. We can confidently predict that this reaction is S_N2. An S_N1 mechanism is extremely unlikely: a methyl cation is very unstable and thus is not a reasonable intermediate to propose.

Notice something else about the SAM methylation mechanism illustrated in the previous figure. It is ***ter****molecular*: there are *three* players acting in concert: the catalytic base, the nucleophile, and the electrophile. This is possible because the all three players are bound in a very specific geometry in the active site of the enzyme. In a reaction that takes place free in solution, rather than in an active site, the likelihood of three separate molecules colliding all at once, with just the right geometry for a reaction to take place, is very, very low. You should notice going forward that when we illustrate the mechanism of a reaction that takes place free in solution, we will only see *bimolecular* steps - *two* molecules colliding. Almost all of the biochemical reactions we see in this book will be enzyme-catalyzed - and termolecular steps will be common - while almost all of the laboratory reactions we see will take place free in solution, so we will only see unimolecular and bimolecular steps. (Synthetic chemists often employ non-biological catalysts that mimic enzyme active sites, but these examples are well beyond the scope of our discussion).

Exercise 8.16: Think back to the acid-base chapter: the pKa of a protonated ether is approximately zero, indicating that an ether is a very weak base. Considering periodic trends in acidity and basicity, what can you say about the relative basicity of a sulfide?

Another SAM-dependent methylation reaction is catalyzed by an enzyme called catechol-*O*-methyltransferase. The substrate here is epinephrine, also known as adrenaline, and the reaction is part of the pathway by which adrenaline is degraded in the body.



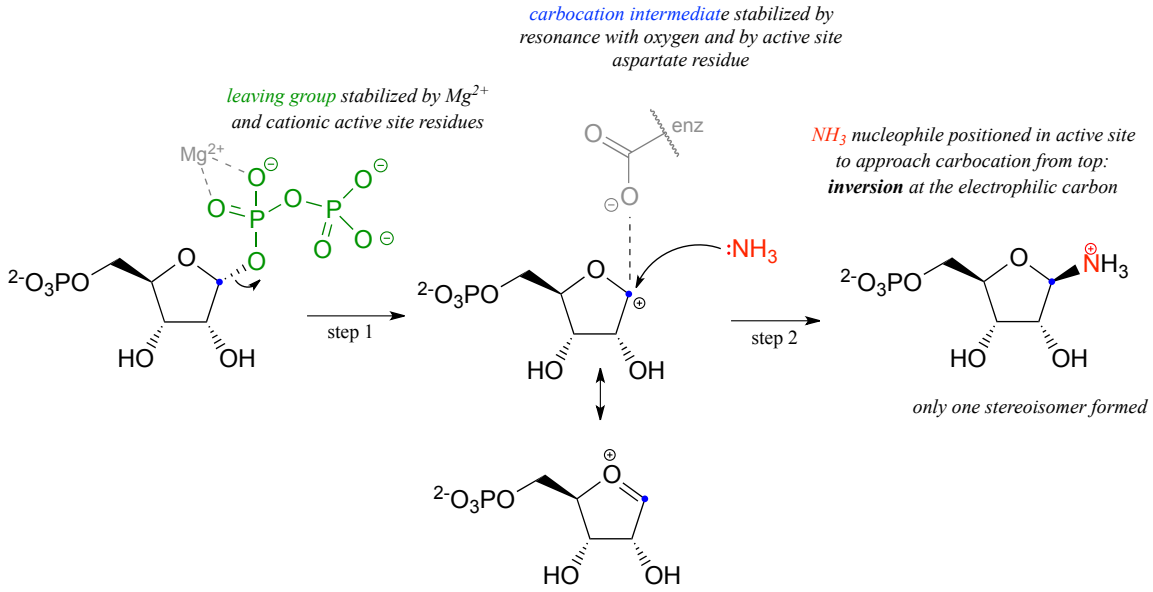
adrenaline

Notice that in this example, the attacking nucleophile is a phenol oxygen rather than a nitrogen (that's why the enzyme is called an *O*-methyltransferase). In many cases when drawing biochemical reaction mechanisms, we use the abbreviations B: for a catalytic base and H-A for a catalytic acid, in order to keep the drawings from getting too 'busy' (it's also possible that the identity of the acidic or basic group may not be known).

Exercise 8.17: SAM is formed by a nucleophilic substitution reaction between methionine and adenosine triphosphate (ATP). Draw a mechanism for this reaction, and explain why you chose either an S_N1 or and S_N2 pathway.

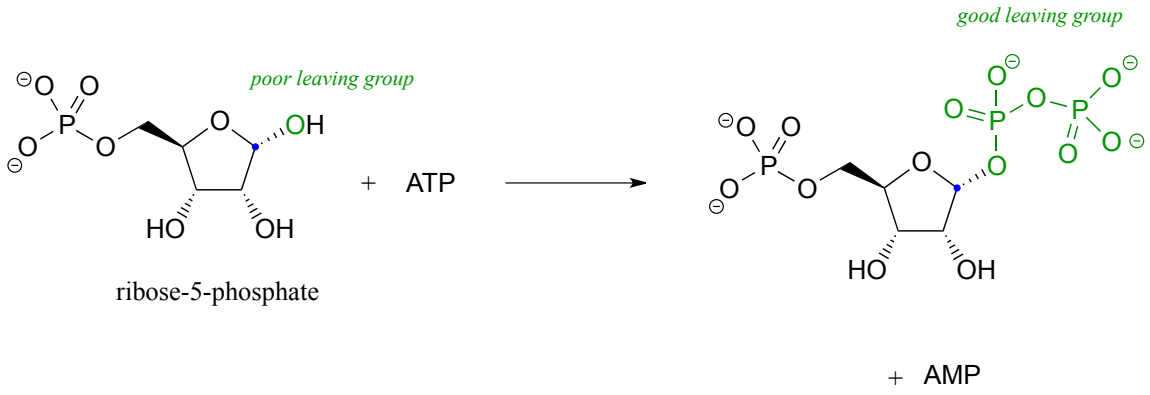
8.8B: A biochemical S_N1 reaction

As we will see in chapter 10, enzyme-catalyzed S_N1 reactions play a critical role in carbohydrate and DNA/RNA nucleotide metabolism. The reaction below is part of nucleotide biosynthesis:



Notice a few things here: first, the diphosphate leaving group is stabilized by interactions with Mg^{+2} ion bound in the active site and also by hydrogen-bonding with active site amino acid residues (not shown). The carbocation intermediate is stabilized by resonance with the lone pairs on the oxygen (see section 8.5), and also by an active site aspartate side chain. The ammonia nucleophile is positioned in the active site so that it approaches from the 'top' side of the planar carbocation intermediate, and the substitution results in inversion of configuration. Remember: S_N1 reactions which occur free in solution tend to result in a mixture of stereoisomers, but enzyme-catalyzed reactions - including enzymatic SN1 reactions such as this one - are generally stereo- and regio-specific, meaning that they almost always result in a *single* isomeric product, not a mixture of products.

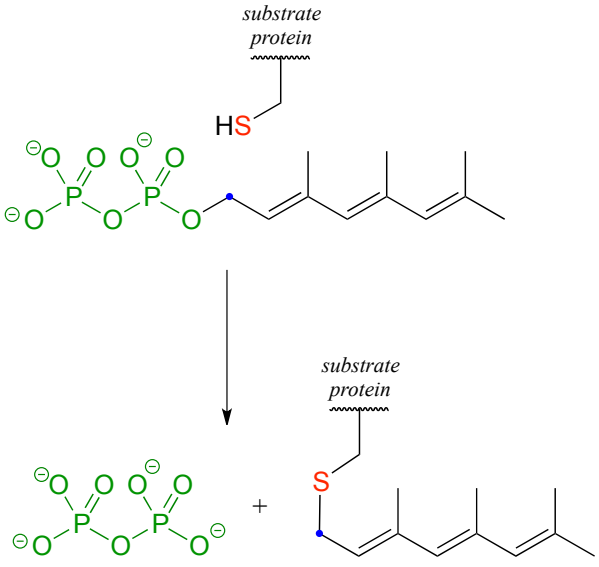
Recall the statement from section 8.4 that poor leaving groups often need to be converted into good leaving groups. Backing up one metabolic step from the reaction depicted above, we see that a poor (hydroxide) leaving group on ribose-5-phosphate is first converted to a good (diphosphate) leaving group, which can stabilized through interactions with the active site of the enzyme catalyzing the S_N1 reaction.



This preliminary phosphorylation step, which requires ATP (adenosine triphosphate) as the donor of the diphosphate group, is a reaction that we will study in much more detail in chapter 9.

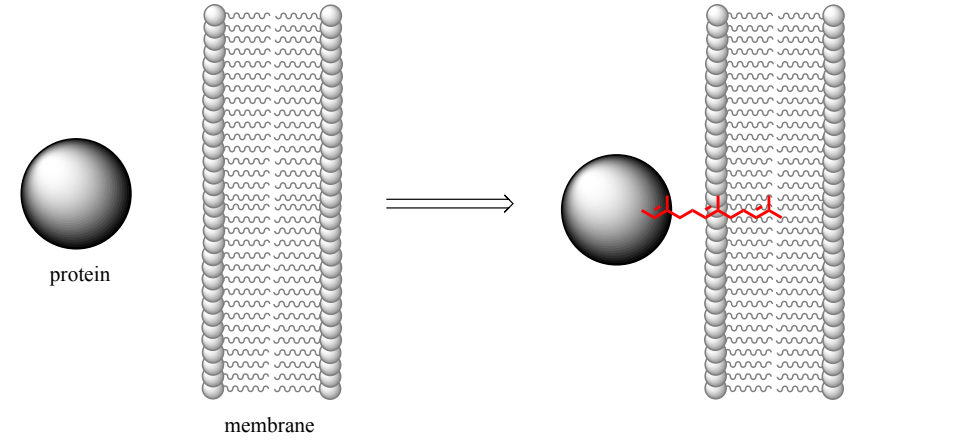
8.8C: A biochemical S_N1/S_N2 hybrid reaction

The cysteine residues of certain proteins are modified by addition of a 15-carbon isoprene chain (section 1.3A) to the side chain thiol group.



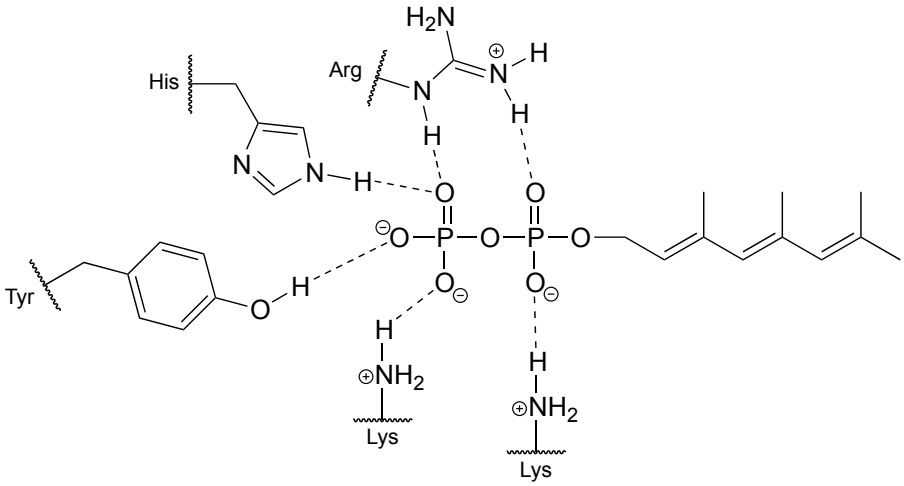
protein with modified cysteine side chain

The mechanistic details of this reaction are of particular interest to biomedical scientists. The proteins that are substrates for this type of modification are involved in cell signaling processes, and they are not able to carry out their biological functions unless they are anchored to a cell's lipid membrane. The hydrocarbon group that becomes attached to a cysteine residue in this reaction serves as the anchor.



Some of these proteins have been implicated in tumor formation. Scientists hope that if they can find a way to shut down the cysteine modification reaction, the tumor-causing proteins will not be able to anchor to cell membranes and thus will remain inactive. The search is on for an effective inhibitor of this enzyme to serve as a potential anti-tumor drug.

How does the enzyme lower the energy barrier for this reaction? Experimental evidence indicates that when a substrate protein is bound to the active site of the enzyme, the cysteine thiol associates with a zinc ion bound in the active site. As we learned in section 7.8, this association will lower the pK_a of the thiol to the point where it loses a proton and exists as a thiolate anion in the active site - a thiolate is a *very* potent nucleophile! Studies also show that the diphosphate group forms stabilizing interactions with several amino acid residues (two lysines, an arginine, a histidine, and a tyrosine) in the enzyme's active site, making it a weaker base and thus a better leaving group.



Biochemistry **1998**, *37*, 16601

Is protein prenylation an S_N1 or S_N2 reaction? In other words, to what extent does the nucleophile displace, or 'push' the leaving group off, or to what extent does the leaving group leave on its own, without a 'push' from the nucleophile? Along the same lines, to what extent does a positive charge develop on the carbon center (development of a full positive charge implies an S_N1 mechanism). First, consider the electrophile: it is a primary allylic carbon, so either pathway is possible (it is relatively unhindered for S_N2 attack, but could also form a resonance-stabilized carbocation intermediate in an S_N1 pathway). The nucleophile is a very powerful thiolate ion, suggestive of an S_N2 mechanism where a strong nucleophile actively displaces the leaving group.

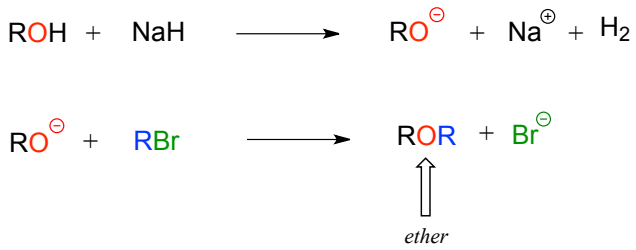
In fact, experiments designed to address this very question (see problem P8.19) have provided evidence that the reaction is a mechanistic hybrid: essentially S_N2, but with *elements* of S_N1. In other words, at the transition state the electrophilic carbon takes on some degree of positive charge, but a true carbocation intermediate does not form. The take-home message here is that the S_N1 and S_N2 mechanistic pictures we have studied in this chapter are models, and while they are useful for learning about chemical principles and accurate for describing many substitution reactions, other reactions are not necessarily 'pure' S_N1 or S_N2, but actually lie somewhere in between.

Section 8.8: SN In the lab - the Williamson ether synthesis and organic tosylates

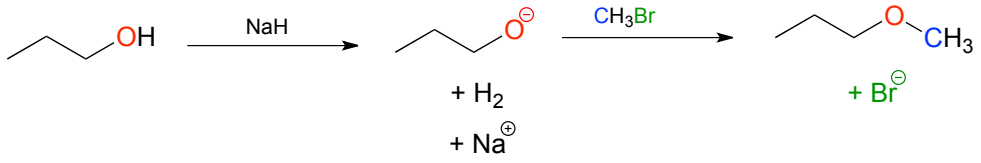
8.9A: The Williamson ether synthesis

Synthetic organic chemists often make use of a reaction that is conceptually very similar to the SAM-dependent methylation reactions we saw earlier. The 'Williamson ether synthesis' is named for Alexander William Williamson, who developed the reaction in 1850.

In the Williamson ether synthesis, an alcohol is first deprotonated by a strong base, typically sodium hydride. An alkyl halide is then added to the reaction mixture, and the alkoxide ion, a powerful nucleophile, displaces the halide leaving group in an S_N2 reaction.

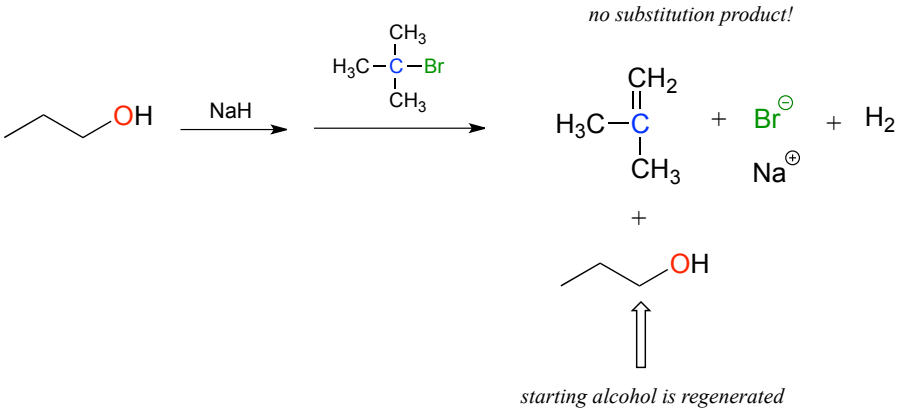


For example, below we see methyl bromide performing the role of methyl group donor, analogous to the role played by SAM in biochemical methylation reactions:



Notice the difference between this non-biological laboratory reaction and the biological, enzyme-catalyzed SAM methylation reaction we saw earlier. Deprotonation of the nucleophile occurs as a separate step, before the nucleophile attacks. Contrast this solution reaction (with two bimolecular steps) to the enzyme-catalyzed S_N2 reaction (SAM methylation) we saw earlier, which involves a single, concerted trimolecular step. Also notice that this non-biological reaction involves a highly basic reagent (sodium hydride) and intermediate (propanoate anion), which would be unreasonable to propose for a reaction taking place under physiological conditions.

The Williamson ether synthesis will only work with methyl or primary alkyl halides. If a secondary or tertiary alkyl halide is used, the result will be formation of an *alkene* in what is called an 'elimination' reaction:



We will study elimination reactions in chapter 14.

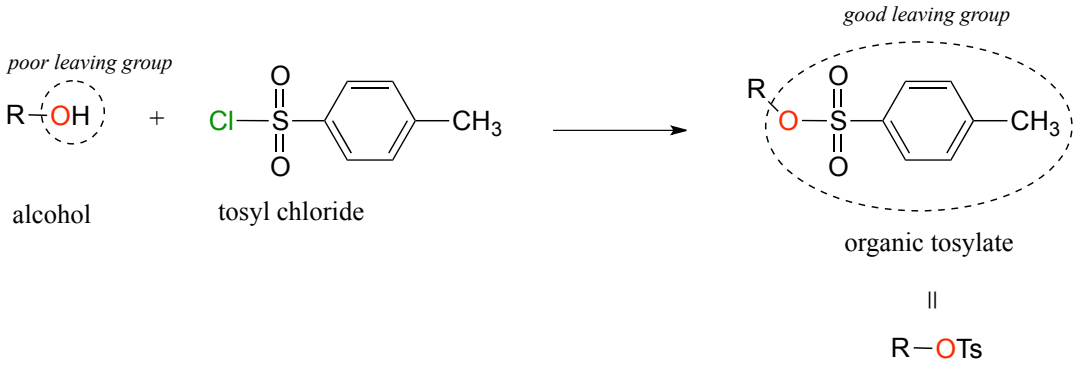
Exercise 8.18: A rookie organic chemist ran the reaction shown above, hoping to synthesize an ether. Instead, he got the alkene shown. What alkyl halide/alcohol combination should he have used instead to get the ether product he was trying for?

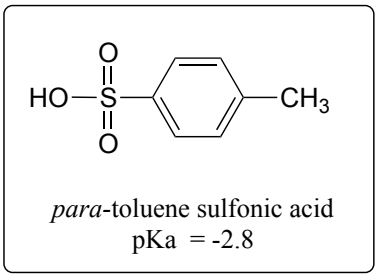
8.9B: Turning a poor leaving group into a good one - tosylates

In section 8.4 it was mentioned how, in metabolic pathways, the relatively poor OH leaving group of an alcohol can be converted into a phosphate or diphosphate, which

when stabilized by noncovalent interactions inside an enzyme active site can be a very good leaving group.

In laboratory synthesis, a similar goal can be accomplished by converting an alcohol (a poor leaving group)to an organic **tosylate** (a good leaving group) using tosyl chloride (the terms 'tosylate' and 'OTs', are abbreviations for *para*-toluene sulfonate). The alcohol to tosylate reaction is not something we are equipped yet to understand, but if we consider that the pKa of *para*-toluene sulfonic acid is -2.8, we realize that the *para*-toluene sulfonate anion is a very weak base and thus an excellent leaving group. Conversion of alcohols to organic tosylates is a very common step in organic synthesis schemes.





Key Concepts to Review

Before you move on to the next chapter, you should be comfortable with the following concepts and skills:

Nucleophilic substitution basics:

Draw a complete mechanism for an S_N2 reaction

Illustrate the transition state for an S_N2 reaction

Understand how S_N2 reactions result in inversion of configuration at the electrophilic carbon.

Draw a complete mechanism for an S_N1 reaction, in particular a hydrolysis or other solvolysis S_N1 reaction.

Draw an energy diagram illustrating the energy profile of a typical S_N1 solvolysis reaction.

Illustrate all transition states that are part of an S_N1 reaction.

Understand that *non-enzymatic* S_N1 reactions result in both inversion and retention of configuration (racemization) at the electrophilic carbon. *Enzymatic* S_N1 reactions are stereospecific, usually resulting in inversion at the electrophilic carbon.

Nucleophiles:

Understand the factors that influence nucleophilicity. Be able to evaluate the relative nucleophilicity of two or more compounds, and predict relative rates of S_N2 reactions with different nucleophiles and a common electrophile.

Be able to recognize the nucleophile, electrophile, and leaving group in an S_N1 or S_N2 reaction.

Understand that – with the exception of the vertical periodic trend in protic solvents – *in most cases anything that makes something a stronger base also makes it a more powerful nucleophile:*

The horizontal periodic trend in nucleophilicity: for example, NH_3 is a better nucleophile than H_2O.

The vertical periodic trend in nucleophilicity *for reactions in polar aprotic solvents*: chloride ion is a better nucleophile than bromide ion in acetone solvent.

Protonation state: for example, hydroxide ion is a better nucleophile than water.

Inductive effect: electron-withdrawing groups decrease nucleophilicity

Resonance effects:

Delocalization of negative charge/electron density decreases nucleophilicity. For example, methoxide ion (CH_3O^-) is a stronger nucleophile than acetate ion.

In addition:

Steric effects: less sterically hindered nucleophiles are more powerful. For example, ethanol is less hindered and more nucleophilic than *tert*-butyl alcohol.

The vertical periodic trend *in protic solvent* (water or alcohol) is opposite the trend in basicity: for example, thiols are more nucleophilic than alcohols.

<u>Electrophiles</u>

Electrophiles are electron poor atoms: for our present purposes this means a carbon bonded to an electronegative atom.

Less hindered electrophiles will react faster in S_N2 reactions: for example chloromethane is a better electrophile than a primary alkyl chloride.

Leaving groups

The trends in leaving groups parallel trends in basicity. *A good leaving group is a weak base.*

Common laboratory leaving groups are halides and para-toluenesulfonate (abbreviated tosyl, or OTs).

Common biochemical leaving groups are phosphates and sulfide.

Carbocation stability

Understand that, in general, factors which destabilize a negative charge will stabilize a positive charge.

More substituted carbocations are more stable: for example, a tertiary carbocation is more stable than a secondary carbocation.

Allylic and benzylic carbocations, in which the positive charge is delocalized by resonance, are relatively stable.

The presence of electron-withdrawing groups (by inductive or resonance effects) decreases carbocation stability.

The presence of electron-donating groups (by inductive or resonance effects) increases carbocation stability.

The presence of a heteroatom can stabilize a nearby carbocation by the resonance-based electron donating effect. Otherwise, heteroatoms act as weakly electron withdrawing carbocation-destabilizing groups by inductive effects.

General concepts and skills

Be able to predict whether a given substitution reaction is likely to proceed by S_N2 or S_N1 mechanisms, based on the identity of the nucleophile, the electrophile, and the solvent.

S_N2 reactions involve strong nucleophiles and unhindered electophiles, and are accelerated by the use of polar, aprotic solvents.

SN1 reactions involve weaker nucleophiles relatively stable carbocations, and are accelerated by protic solvents.

Given a nucleophile and electrophile, be able to predict the product(s) of a nonenzymatic substitution reaction, and predict a mechanisms (S_N1 or S_N2). Be able to predict different regiochemical and stereochemical outcomes leading to formation of more than one product.

Be able to 'think backwards' to show the starting compounds in a substitution reaction, given a product or products.

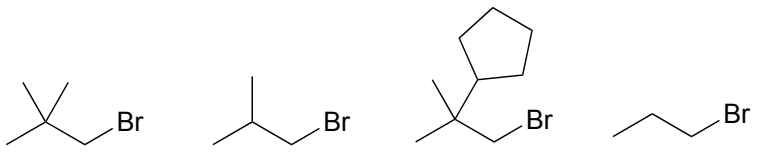
Be able to recognize and draw a complete mechanism for a biochemical nucleophilic substitution reaction. Be able to evaluate the nucleophile, electrophile, and leaving group in the reaction, and predict whether the reaction is likely to have more S_N2 or S_N1 character.

Understand how S-adenosylmethionine (SAM) acts as a methyl group donor in biochemical S_N2 reactions.

Be able to select appropriate alkyl halide and alcohol starting compounds to synthesize a given ether product, using the Williamson ether synthesis procedure.

Problems

P8.1: Rank the following molecules in order of how fast they would be expected to react with CH_3SNa in acetone. (CH_3SNa is simply the sodium salt of CH_3S^-. Na^+ is a spectator ion.)

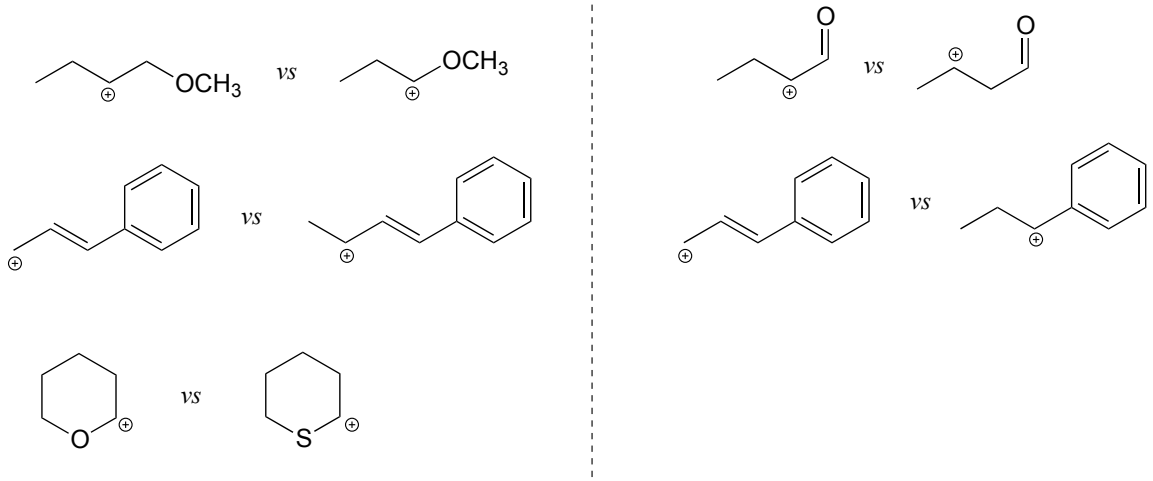


A B C D

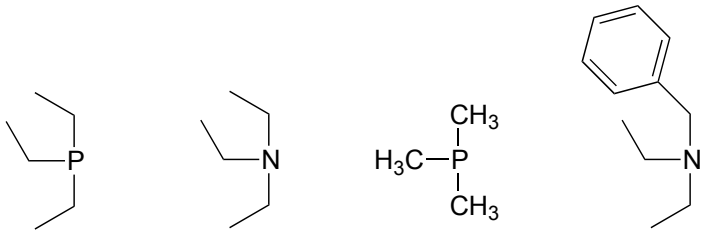
P8.2: Draw line structures representing the *most stable* cation with the given molecular formula:

a) $C_3H_7^+$ b) $C_4H_9^+$ c) $C_3H_8N^+$ d) $C_4H_7^+$

P8.3: For each pair of carbocations below, choose the one that is more stable, and explain your reasoning.



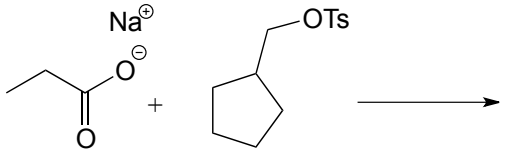
P8.4: Arrange the following species in order of increasing nucleophilicity in protic solvent:



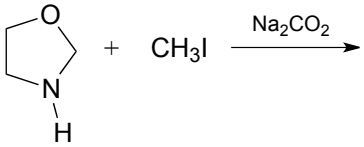
A B C D

P8.5: Predict the organic products of the following nucleophilic substitution reactions, all of which are carried out in polar aprotic solvent. Show stereochemistry at chiral carbons. Hints: Na_2CO_3, sodium carbonate, is a weak base. For part (f): What is the conjugate acid of NH_2^-? What is the pKa of this conjugate acid, and what is the pKa of a terminal alkyne?

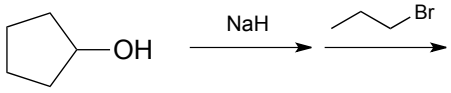
a)



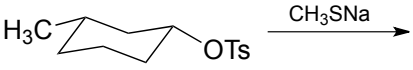
b)



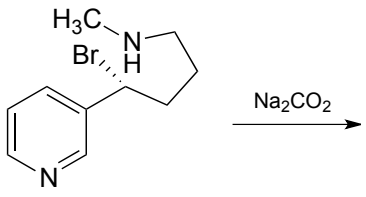
c)



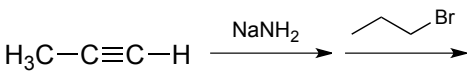
d)



e)



f)



P8.6: Which of the reactions in the previous problem has a *unimolecular* rate determining step? Explain.

P8.7: From the following pairs, select the compound that would react more rapidly with bromomethane in acetone solvent.

a) water or hydroxide ion

b) CH_3S^- or CH_3OH

c) CH_2S^- or CH_3SH

d) acetate ion or hydroxide ion

e) diethyl sulfide or diethyl ether

f) dimethylamine or diethylether

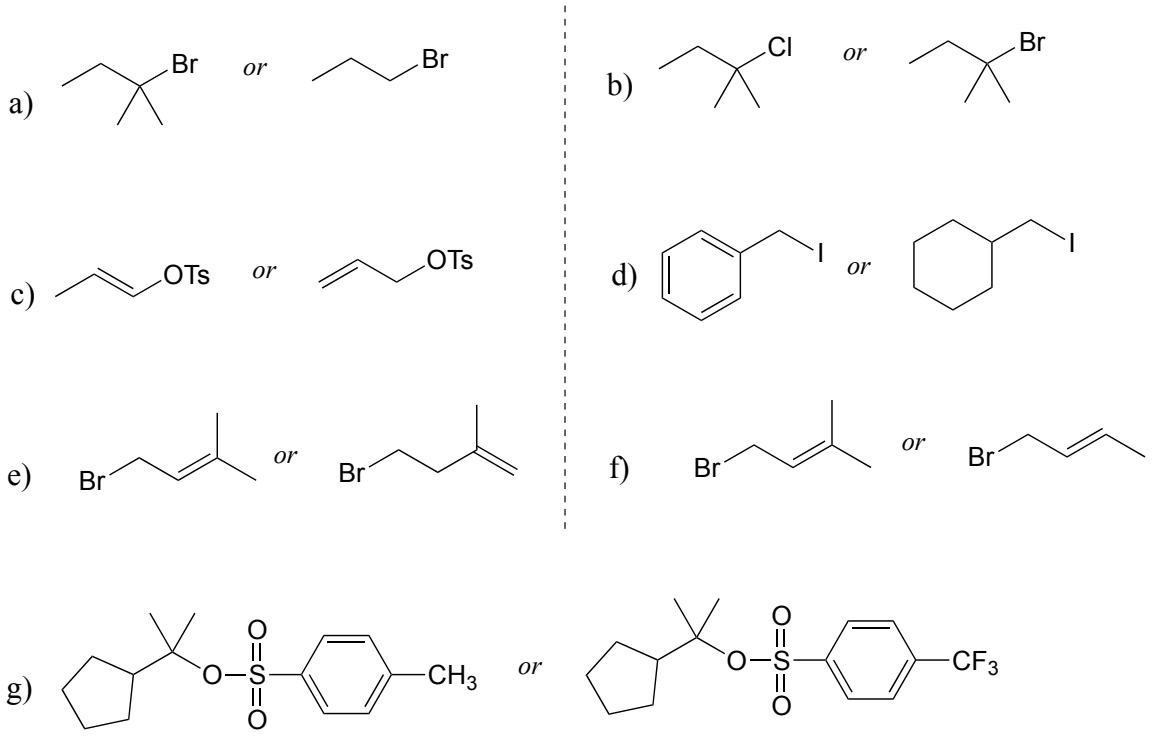
g) trimethylamine or 2,2-dimethylpropane

P8.8: Methyl iodide (0.10 mole) is added to a solution that contains 0.10 mole $NaOCH_3$ and 0.10 mole $NaSCH_3$.

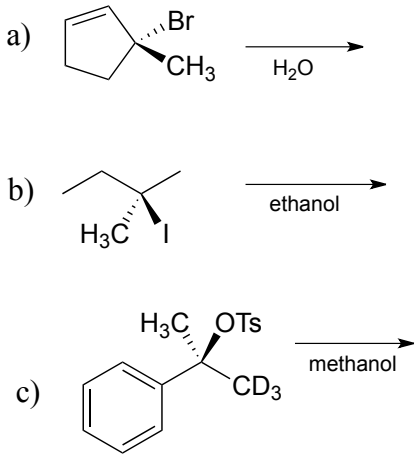
a) Predict the most abundant neutral organic product that would form, and explain your reasoning.

b) Assume that you isolate a mixture the major product (which you predicted in part) along with a smaller amount of a different nucleophilic substitution product. Explain briefly but specifically how you could use ^{1}H-NMR to determine the ratio of the two products in the mixture.

P8.9: For each pair of compounds, predict which will more rapidly undergo solvolysis in methanol solution.

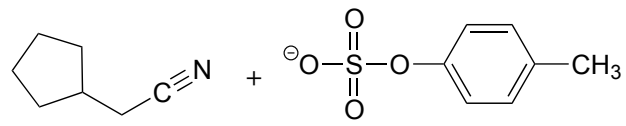


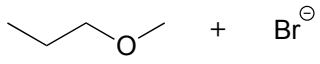
P8.10: Predict the solvolysis product(s) of each of the reactions below. Consider both regiochemistry and stereochemistry.



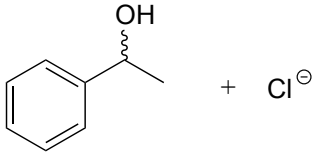
e) Draw a complete curved-arrow mechanism for the formation of the secondary allylilc alcohol product in part (a).

P8.11: Show starting compounds that would lead to the following products through nucleophilic substitution reactions.

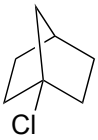
a) 

b) 

c) 

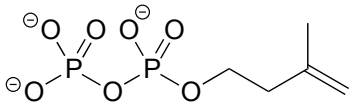
c) 

P8.12: The fused ring compound shown below is very unreactive to nucleophilic substitution, even with a powerful nucleophile.. Explain. (Hint – consider bond geometry - a model will be very helpful!)



P8.13 Laboratory synthesis of isopentenyl diphosphate - the 'building block' molecule used by nature for the construction of isoprenoid molecules (section 1.3A) - was accomplished by first converting isopentenyl alcohol into an alkyl tosylate then displacing the tosylate group with an inorganic pyrophosphate nucleophile. Based on this verbal description, draw a mechanism for the second (nucleophilic substitution) step,

showing starting and ending compounds for the step and curved arrows for electron movement



isopentenyl diphosphate

P8.14: Choline, an important neutotransmitter in the nervous system, is formed from 2-(*N*,*N*-dimethylamino)ethanol:

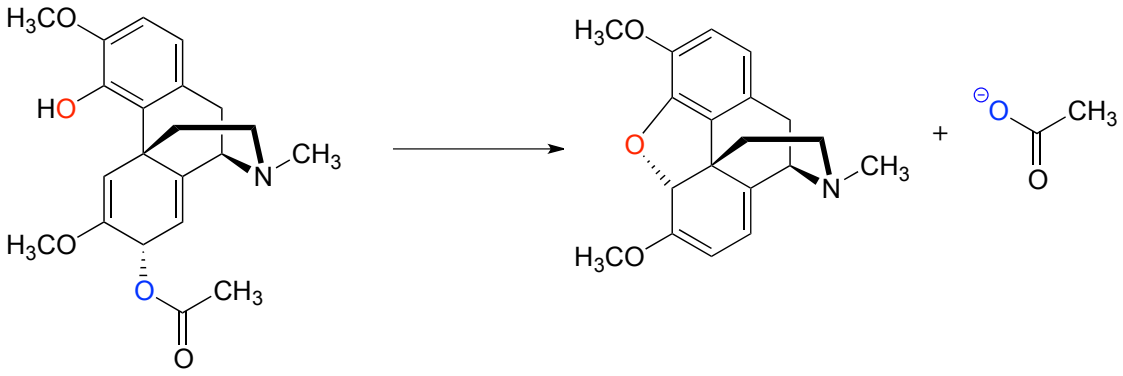
choline

a) Besides the enzyme and the starting compound, what other important biomolecule do you expect plays a part in the reaction?

b) Draw a mechanism for the reaction.

c) Briefly explain how ^{1}H-NMR could be used to distinguish between the substrate and the product of this reaction.

P8.15 The following is a reaction in the biosynthesis of morphine in opium poppies.



a)Draw a complete mechanism, assuming an S_N1 pathway.

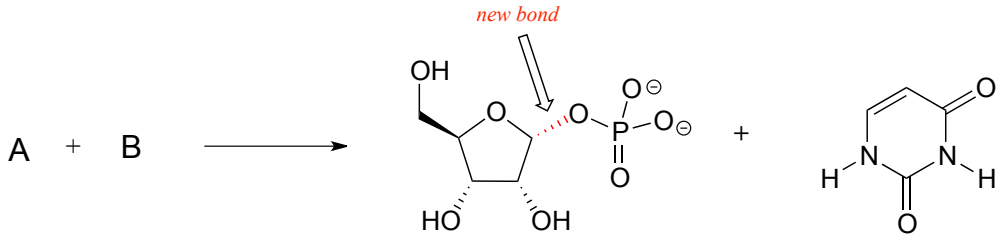
b) What would you expect to be the most noticeable difference between the IR spectrum of the product and that of the substrate?

c) This reaction is an example of the regiospecificity of enzymatic nucleophilic substitution reactions noted earlier in the chapter. Draw two alternate nucleophilic, ring-closing steps for this reaction (leading to different products from what is shown above), and explain why these alternate pathways are both less favorable than the actual reaction catalyzed by the enzyme.

P8.16: The enzymatic reaction below, which is part of the metabolism of nucleic acids, proceeds by an S_N1 mechanism. The new bond formed in the substitution is indicated.

a) Predict the structures of the two substrates A and B.

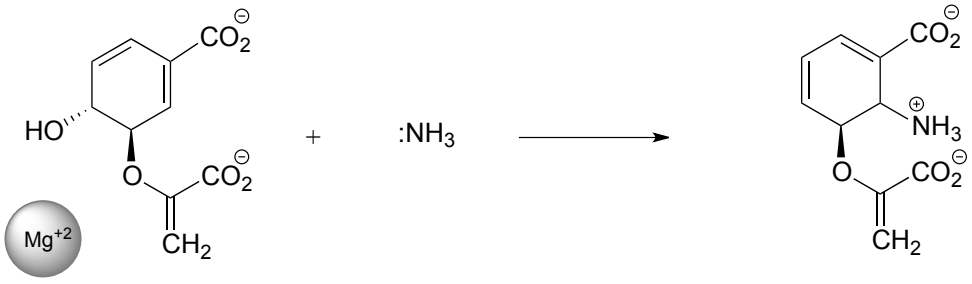
b) Draw a complete mechanism, and use resonance drawings to illustrate how both the carbocation intermediate and the leaving group are stabilized.



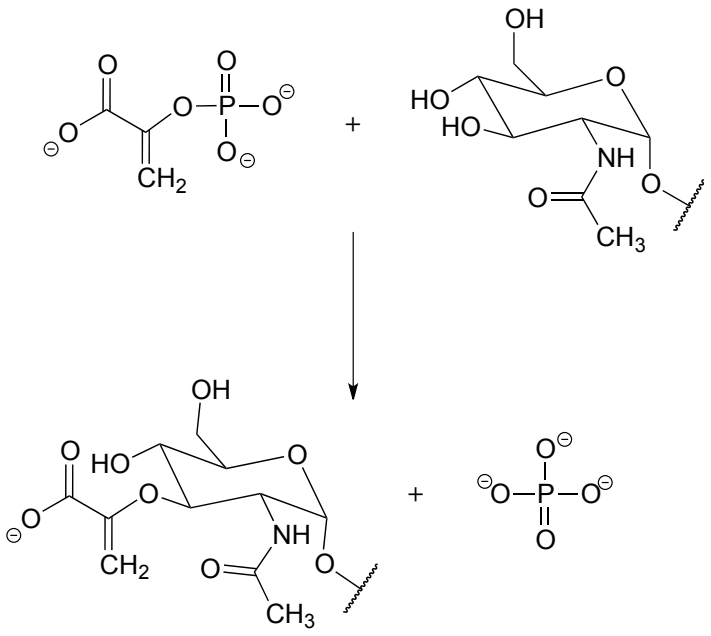
P8.17: Below is the first step of the reaction catalyzed by anthranilate synthase, an enzyme involved in biosynthesis of the amino acid tryptophan.

a) This reaction is somewhat unusual in that the leaving group is a hydroxide anion, which is of course is normally thought to be a very poor leaving group. However, studies show that an Mg^{+2} ion is bound in the active site close to the hydroxide. Explain how the presence of the magnesium ion contributes to the viability of hydroxide as a leaving group.

b) Draw a complete mechanism for the reaction, assuming an S_N1 pathway.

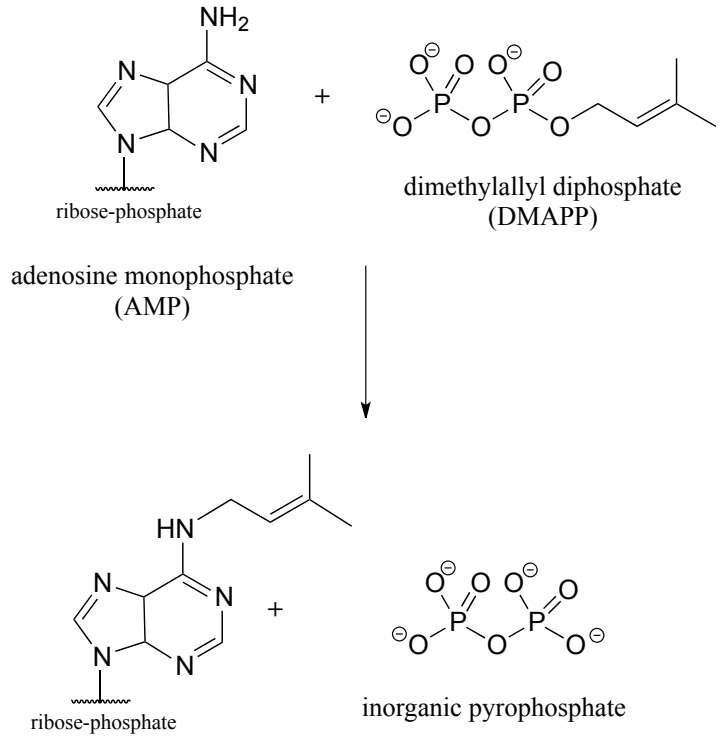


P8.18: The reaction below is part of the biosynthesis of peptidoglycan, a major component of bacterial cell walls. Is it likely to proceed by a nucleophilic substitution mechanism? Explain.

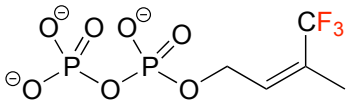


P8.19: Compare the reaction below, catalyzed by the enzyme AMP-DMAPP transferase, to the protein prenyltransferase reaction we learned about in section 8.8C, the mechanism of which, as we discussed, is thought to be *mostly* S_N2-like with some S_N1-like character.

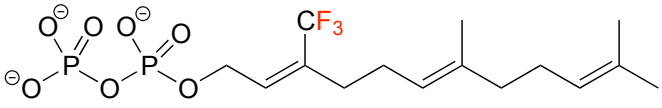
a) Is the AMP-DMAPP transferase reaction below likely to have more or less S_N1-like character compared to the protein prenyltransferase reaction? Explain.



b) Given your answer to part (a), which reaction is likely to be more dramatically slowed down when a fluorinated isoprenoid substrate analog is substituted for the natural substrate? Explain.



substrate analog for AMP-DMAPP transferase



substrate analog for protein prenyltransferase

P8.20: In a classic experiment in physical organic chemistry, (*R*)-2-iodooctane was allowed to react (non-enzymatically) with a radioactive isotope of iodide ion, and the researchers monitored how fast the radioactive iodide was incorporated into the alkane (the rate constant of incorporation, k_i) and also how fast optical activity was lost (the rate constant of racemization, k_r). They found that the rate of racemization was, within experimental error, equal to twice the rate of incorporation. Discuss the significance of this result - what does it say about the actual mechanism of the reaction?

Tables

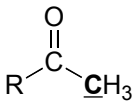
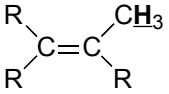
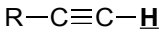
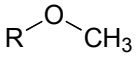
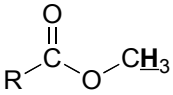
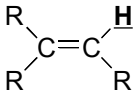
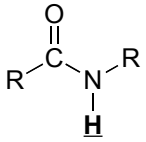
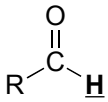
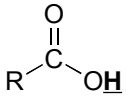
List of tables:

Table 1: Some characteristic absorption frequencies in IR spectroscopy

Bond type	**frequency (cm^{-1})**
C-H alkanes	2950 – 2850
C-H alkenes	3080 – 3020
C-H aldehyde	~2900
C-H alkyne	~3300
alkyne triple bond	2250 – 2100 (s)
alkene double bond	1680 - 1620(s)
carbonyl, ketone	1725 – 1700 (s)
carbonyl, aldehyde	1740 – 1720 (s)
carbonyl, ester	1750 – 1730 (s)
carbonyl, acid	1725 – 1700 (s)
carbonyl, amide	1690 – 1650 (s)
O-H, alcohols	3600 – 3200 (s, broad)
O-H, acids	3000 – 2500 (broad)
C-O, alcohols, esters, ethers	1300 - 1000

s = strong absorbance

Table 2: Typical values for ^{1}H-NMR chemical shifts

Hydrogen type	Chemical shift (ppm)
$RC\underline{\mathbf{H}}_3$	0.9 - 1.0
$RC\underline{\mathbf{H}}_2R$	1.2 - 1.7
$R_3C\underline{\mathbf{H}}$	1.5 – 2.0
	2.0 – 2.3
	1.5 – 1.8
$RN\underline{\mathbf{H}}_2$	1 - 3
$ArC\underline{\mathbf{H}}_3$	2.2 – 2.4
	2.3 – 3.0
	3.7 – 3.9
	3.7 – 3.9
$RO\underline{\mathbf{H}}$	1 - 5
	3.7 – 6.5
	5 - 9
$Ar\underline{\mathbf{H}}$	6.0 – 8.7
	9.5 – 10.0
	10 - 13

Chemical shift values are in parts per million (ppm) relative to tetramethylsilane.

Table 3: Typical values for ^{13}C-NMR chemical shifts

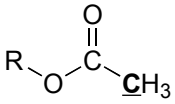
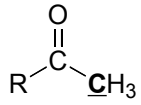
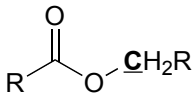
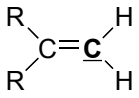
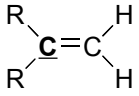
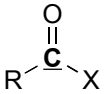
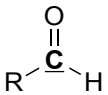
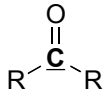
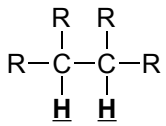
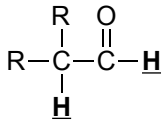
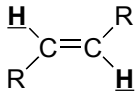
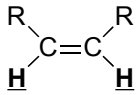
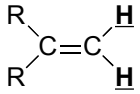
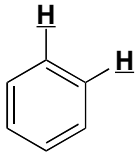
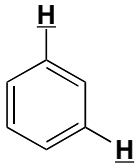
Carbon type	Chemical shift (ppm)
R**C**H$_3$	13 - 16
R**C**H$_2$R	16 - 25
R$_3$**C**H	25 - 35
	18 - 22
	28 - 32
R**C**H$_2$NHR	35 - 45
R**C**H$_2$OH	50 - 65
R—C≡**C**—R	65 - 70
RO**C**H$_2$R	50 - 75
	50 - 75
	115 - 120
	125 - 140
aromatic carbon	125 - 150
 (carboxylic acid derivatives)	165 - 185
	190 - 200
	200 - 220

Table 4: Typical coupling constants in NMR

H-H coupling	J (Hz)
	6 -8
	2 - 3
	12 - 18
	6 - 12
	0 - 2
	6 - 10
	1- 3

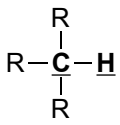
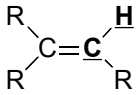
C-H coupling	J (Hz)
	125 - 130
	150 - 170

Table 5: The 20 common amino acids

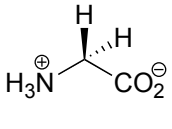
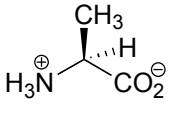
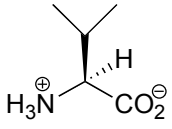
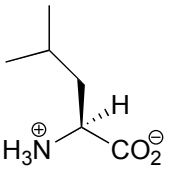
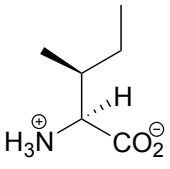
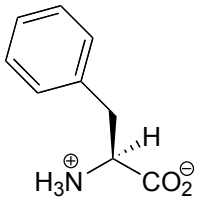
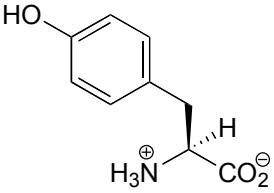
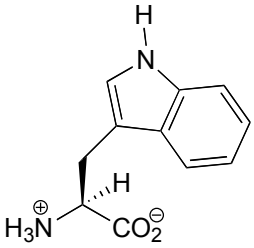
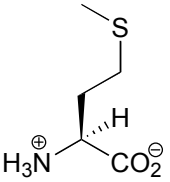
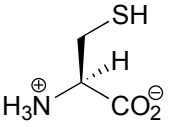
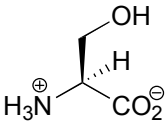
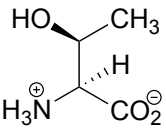
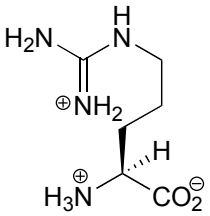
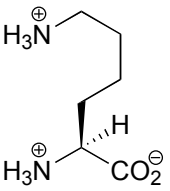
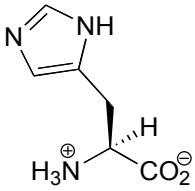
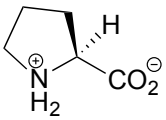
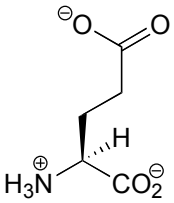
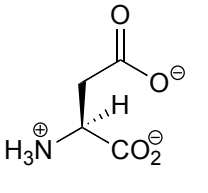
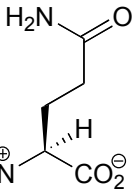
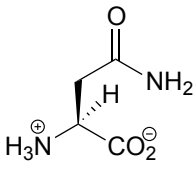
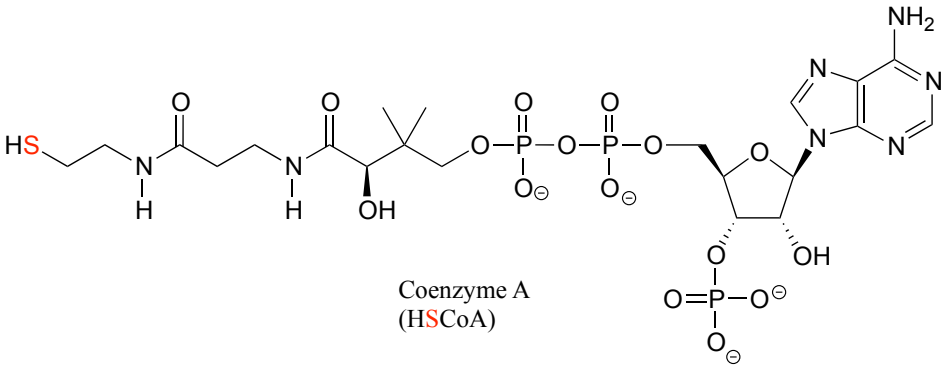
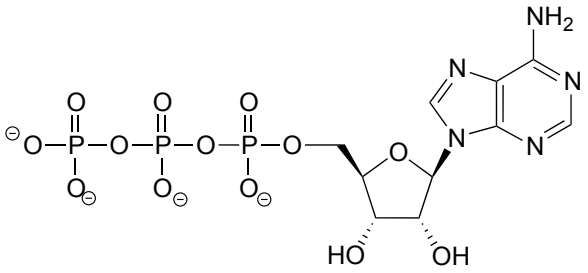
				
Glycine (Gly, G)	**Alanine** (Ala, A)	**Valine** (Val, V)	**Leucine** (Leu, L)	**Isoleucine** (Ile, I)
				
Phenylalanine (Phe, F)	**Tyrosine** (Tyr, Y)	**Tryptophan** (Trp, W)	**Methionine** (Met, M)	**Cysteine** (Cys, C)
				
Serine (Ser, S)	**Threonine** (Thr, T)	**Arginine** (Arg, R)	**Lysine** (Lys, K)	**Histidine** (His, H)
				
Proline (Pro, P)	**Glutamate** (Glu, E)	**Aspartate** (Asp, D)	**Glutamine** (Gln, Q)	**Asparagine** (Asn, N)

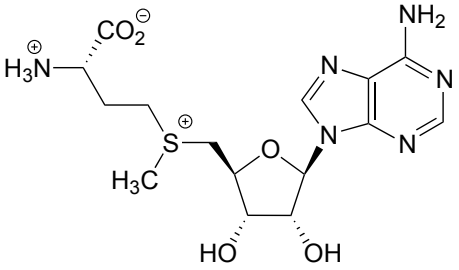
Table 6: Structures of common coenzymes



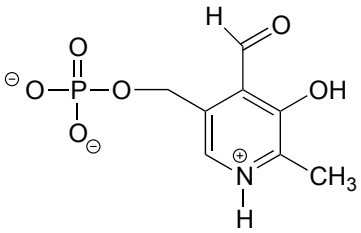
Coenzyme A
(HSCoA)



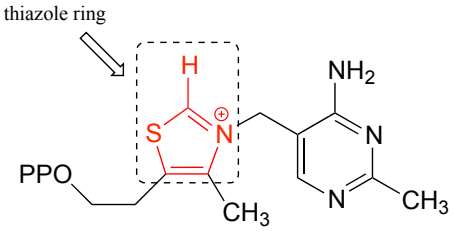
adenosine triphosphate
(ATP)



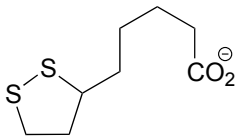
S-adenosyl methionine
(SAM)



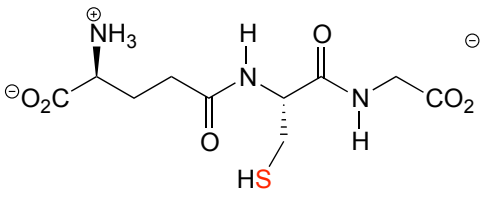
pyridoxal phosphate
(PLP)



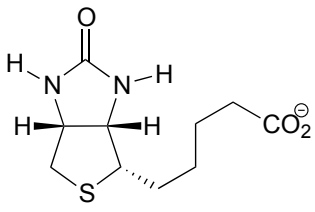
thiamine diphosphate
TPP)



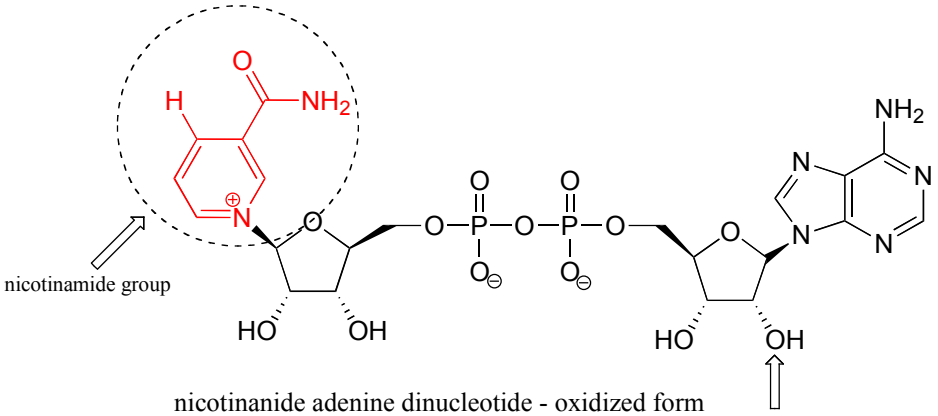
lipoate



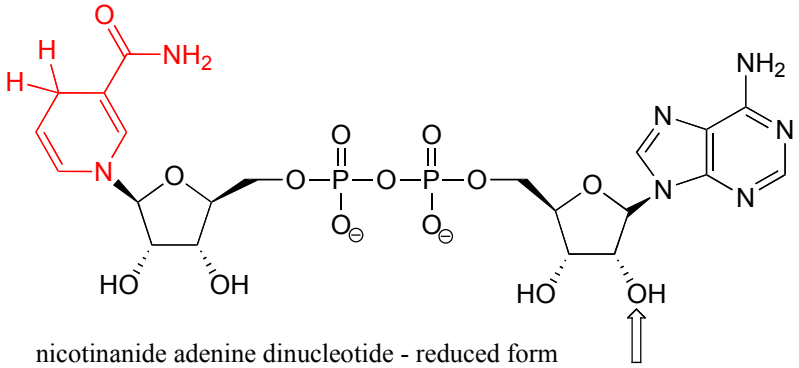
Glutathione
(GSH)



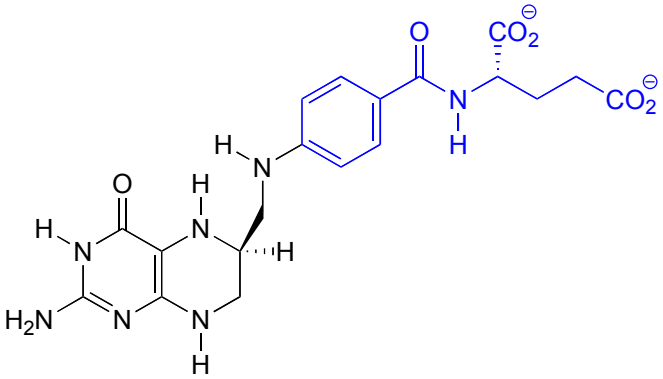
biotin



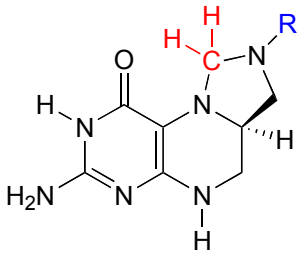
nicotinanide adenine dinucleotide - oxidized form
(NAD$^+$, or NADP$^+$ if phosphorylated at arrow position)



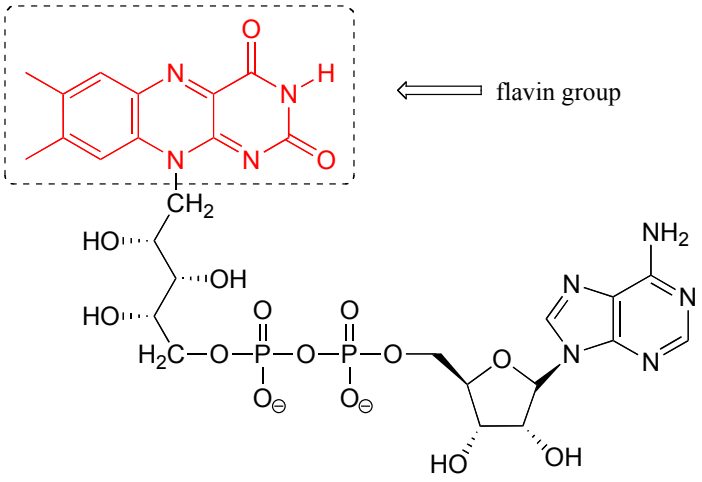
nicotinanide adenine dinucleotide - reduced form
(NADH, or NADPH if phosphorylated at arrow position)



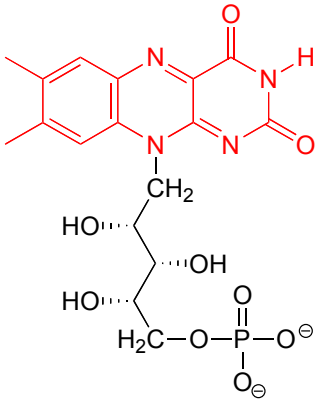
tetrahydrofolate (THF)



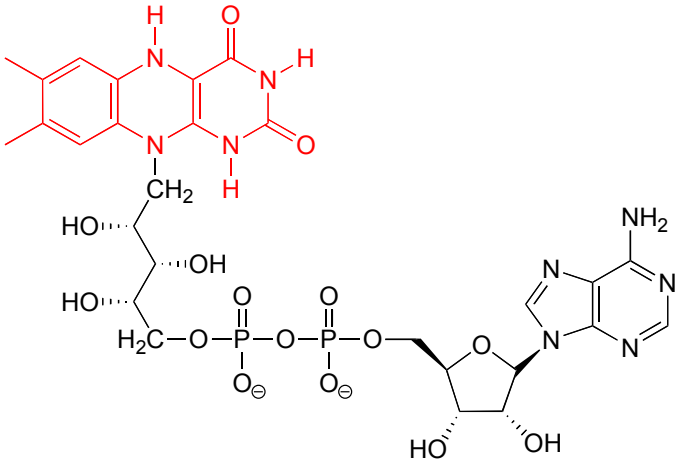
5,10-methylenetetrahydrofolate



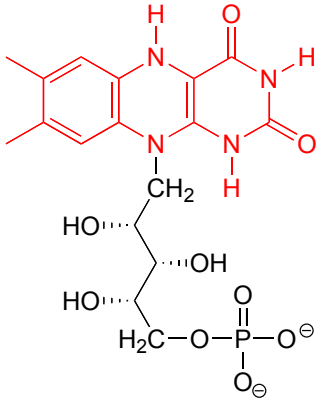
flavin adenine dinucleotide, oxidized form
(FAD)



flavin mononucleotide, oxidized form
(FMN)

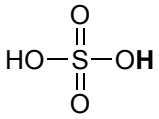
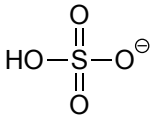
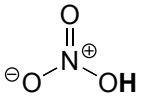
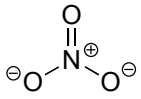
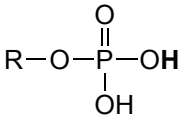
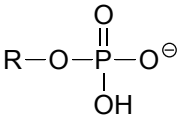
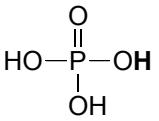
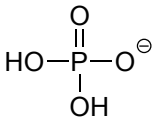
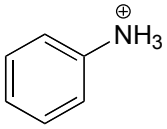
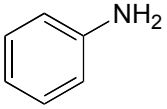
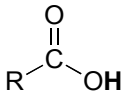
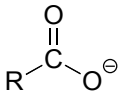
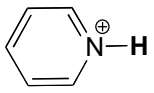
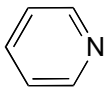


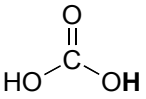
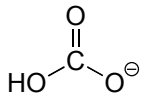
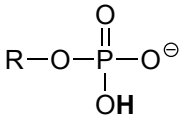
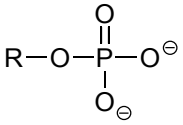
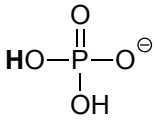
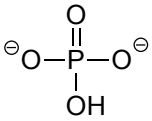
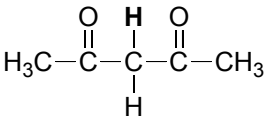
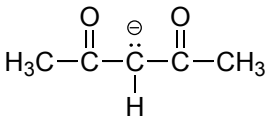
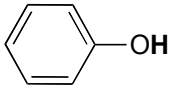
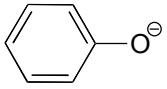
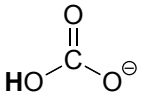
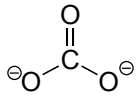
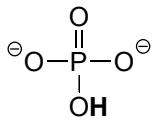
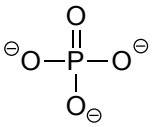
flavin adenine dinucleotide, reduced form
($FADH_2$)

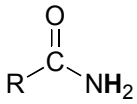
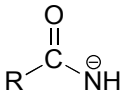
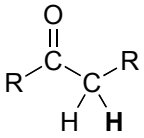
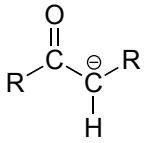


flavin mononucleotide,reduced form
($FMNH_2$)

Table 7: Representative acid constants.

acid	pK_a	conjugate base
 sulfuric acid	-10	
HCl	-7	Cl^-
H_3O^+	-1.7	H_2O
 nitric acid	-1.4	
	1.0[i]	
 phosphoric acid	2.2[ii]	
HF	3.2	F^-
	4.6	
	4-5	
 pyridinium	5.3[ii]	 pyridine

acid	pK_a	conjugate base
 carbonic acid	6.4	 bicarbonate
	6.5[i]	
	7.2[ii]	
	9.0	
HCN	9.2	CN^-
 ammonium	9.2	NH_3 ammonia
 phenol	9.9[ii]	 phenolate
 bicarbonate	10.3[ii]	 carbonate
RS**H**	10-11	RS^-
RNH_3^+	10 -11	RNH_2
	12.3[i]	
H_2O	15.7	OH^-

acid	pK_a	conjugate base
	17	
$RCH_2O\mathbf{H}$	16	RCH_2O^-
	19-20	
RCC**H** terminal alkyne	25	RCC^-
H_2	35	H^-
NH_3 ammonia	38	NH_2^-

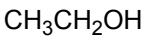
All pK_a values, unless otherwise noted, are taken from March, Jerry, Advanced Organic Chemistry, Fourth Edition, Wiley, New York, 1992.

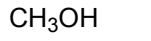
(i) Silva, J.J.R. Fraústo da, The Biological Chemistry of the Elements: the Inorganic Chemistry of Life, 2nd Edition, Oxford, New York, 2001.

(ii)Lide, David R. (ed.) The CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1995.

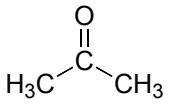
Table 8: Some common laboratory solvents, acids, and bases

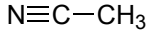
Solvents

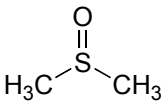

ethanol

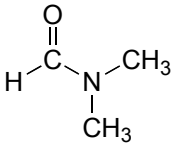

methanol

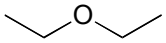

water


acetone

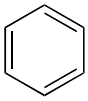

acetonitrile

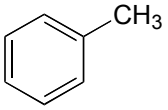

dimethyl sulfoxide (DMSO)

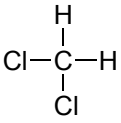

dimethylformamide

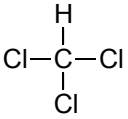

diethylether

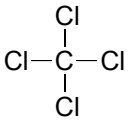

tetrahydrofuran (THF)


benzene

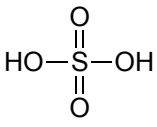

toluene

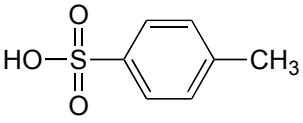

dichloromethane (methylene chloride)

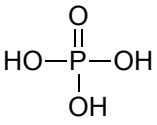

chloroform


carbon tetrachloride

Acids


sulfuric acid

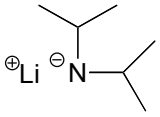

p-toluenesulfonic acid (TsOH)


phosphoric acid

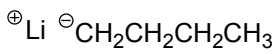

hydrochloric acid

Bases

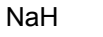
very strong bases:



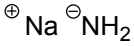
lithium diisopropylamide
(LDA)



N-butyllithium

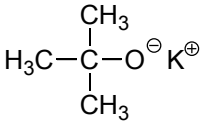


sodium hydride

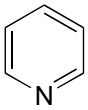


sodium amide

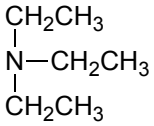
weaker bases:



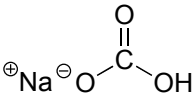
potassium *tert*-butoxide



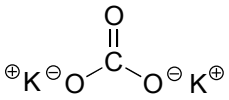
pyridine



triethylamine



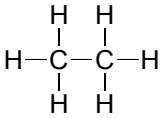
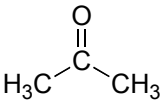
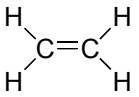
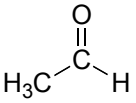
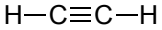
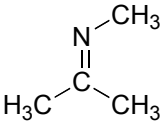
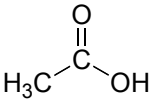
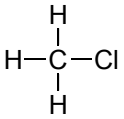
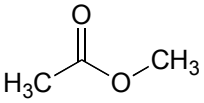
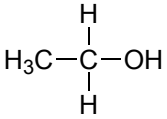
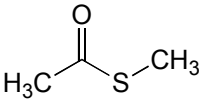
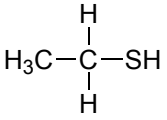
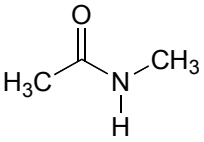
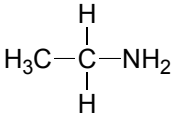
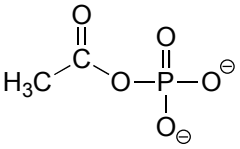
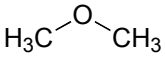
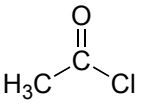
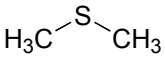
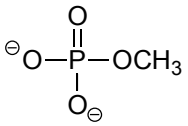
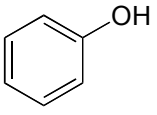
sodium bicarbonate
$NaHCO_3$



potasium carbonate
K_2CO_3

sodium hydroxide

Table 9: Examples of common functional groups in organic chemistry

alkane		**ketone**	
alkene		**aldehyde**	
alkyne		**imine (Schiff base)**	
aromatic hydrocarbon		**carboxylic acid**	
alkyl halide		**ester**	
alcohol		**thioester**	
thiol		**amide**	
amine		**acyl phosphate**	
ether		**acid chloride**	
sulfide		**phosphate ester**	
phenol		**phosphate diester**