Organic Chemistry

with a Biological Emphasis

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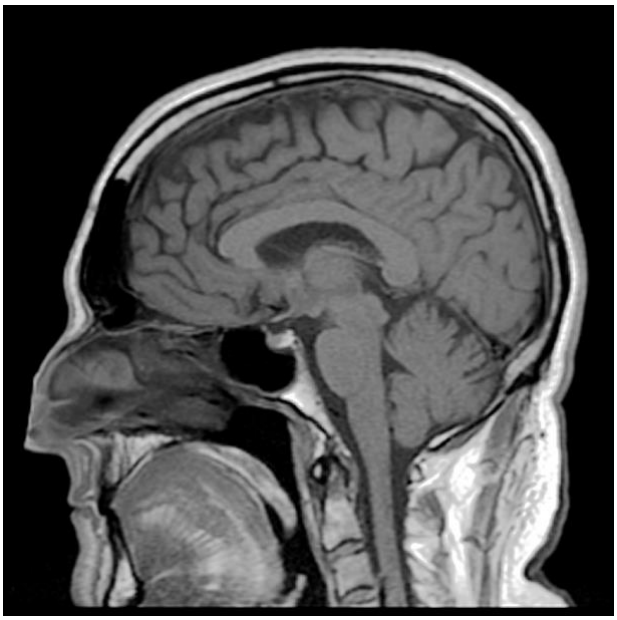
Chapter 5

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 too 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".

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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 into 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 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 to 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.

Learning Outcomes

- Identify groups of chemically equivalent protons in a structure.
- Given an NMR spectrum, explain 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
- predict trends in chemical shifts for protons and carbon atoms in different bonding positions and provide a rationale for the trend.
- estimate the chemical shift of a given proton using a chemical shift chart.
- use proton peak integration values to determine how many protons a particular peak is 'worth'.
- Use the physical basis for spin-spin coupling in 1H-NMR spectra and use the 'n+1 rule' to predict splitting patterns.
- interpret, and draw splitting diagrams for, 1H-NMR spectra with simple coupling.
- Match given structures to their corresponding 1H NMR spectra
- Given a molecular formula (or MS data), you should be confident in your ability to solve an unknown structure based on a 1H- spectrum.

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 observed by NMR spectroscopy, and it is on those techniques that we will focus on 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.

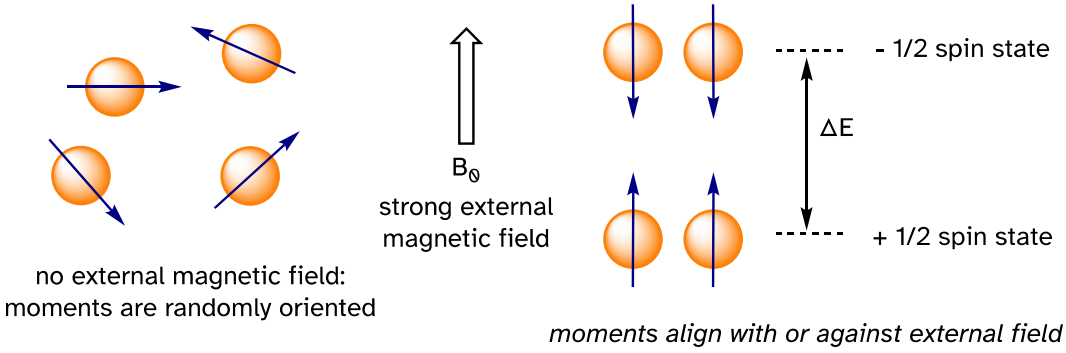
Some examples of magnetic and nonmagnetic nuclei relevant to biological organic chemistry

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 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 $\mathbf{B_0}$) 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.

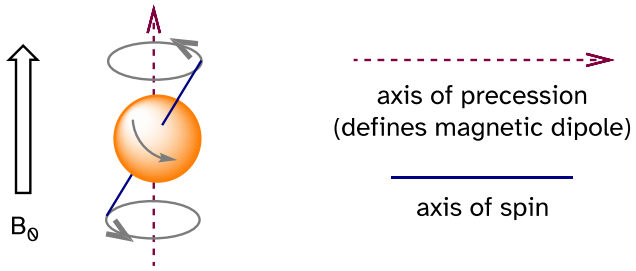
5.1.1



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.

At this point, we need to look a little more closely at how a proton spins in an applied magnetic field. You may recall playing with spinning tops as a child. When a top slows down a little and the spin axis is no longer completely vertical, it begins to exhibit **precessional motion**, as the spin axis rotates slowly around the vertical. In the same way, hydrogen atoms spinning in an applied magnetic field also exhibit precessional motion about a vertical axis. It is this axis (which is either parallel or antiparallel to B_0) that defines the proton's magnetic moment.

5.1.2



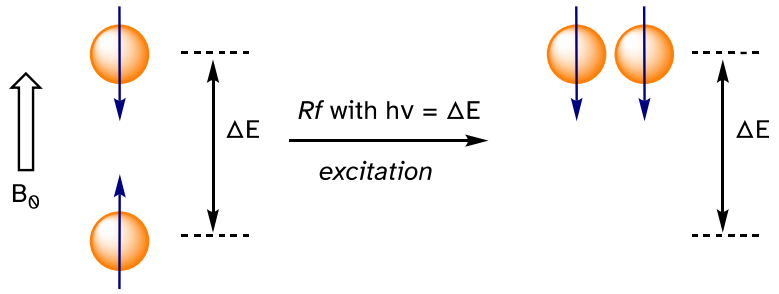
Watch the first minute or so of this video of spinning tops:
look for the precessional motion

The **frequency of precession** (also called the **Larmour frequency,** abbreviated ν_L) is simply the number of times per second that the proton precesses in a

complete circle. A proton`s precessional frequency increases with the strength of B_0.

If a proton that is precessing in an applied magnetic field is exposed to electromagnetic radiation of a frequency ν that matches its precessional frequency ν_L, we have a condition called **resonance**. *In the resonance condition, a proton in the lower-energy +½ spin state (aligned with B_0) will transition (flip) to the higher energy –½ spin state (opposed to B_0) as it absorbs radiation at the resonance frequency corresponds to ΔE, the energy difference between the proton's two spin states*. With the strong magnetic fields generated by the superconducting magnets used in modern NMR instruments, the resonance frequency for protons falls within the radio-wave range, anywhere from 100 MHz to 800 MHz depending on the strength of the magnet.

5.1.3



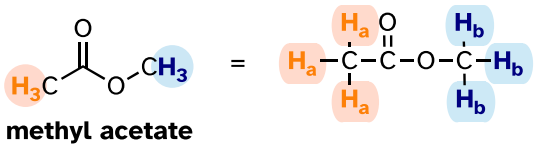
Recall from section 4.2 that photons of electromagnetic radiation of a given frequency correspond to energy (E) given by E = $h\nu$, where *h* is Plank's constant and ν is the frequency in waves per second, or Hz. In NMR, the energy gap ΔE between the +½ and -½ *spin* states of an atomic nucleus in a strong magnetic field corresponds to the energy associated with radiation in the radio frequency (Rf) region of the spectrum. 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 would not be terribly useful for chemists. 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.

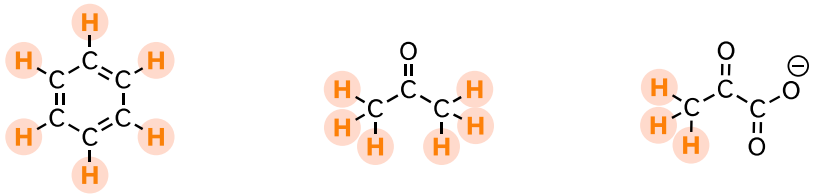
5.2.1



The three methyl acetate protons labeled H_a above (orange) have a different resonance frequency compared to the three H_b protons (blue), because the two sets of protons are in non-identical electronic environments: H_a protons are on a carbon next to a carbonyl carbon, while the H_b protons or on a carbon next to an oxygen atom. 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. Therefore, 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 equivalency 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.

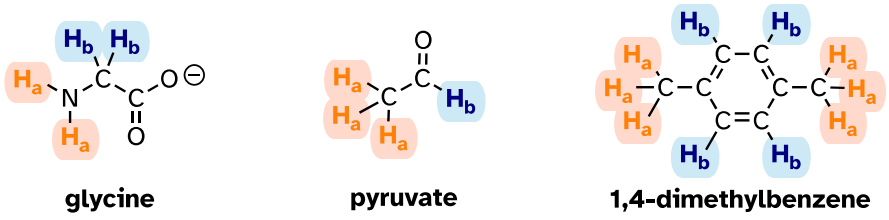
5.2.2



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.

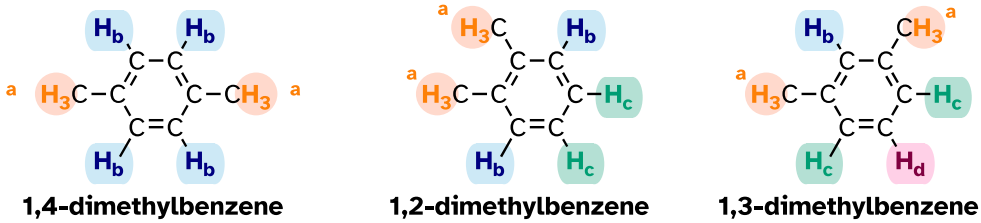
5.2.3



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.

5.2.4



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.

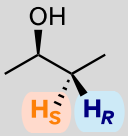
As you have probably already realized, chemical equivalence or non-equivalence in NMR is closely related to *symmetry*. Different planes of symmetry in the three isomers of dimethylbenzene lead to different patterns of equivalence.

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 due to their positions either on the same or opposite side of the ring relative to chlorine.

5.2.5

chloroethene **chlorocyclopropane**

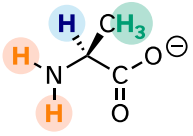
Note: The H_a and H_b protons in the examples above are diastereotopic pairs. In fact, *all* diastereotopic protons are technically non-equivalent in terms of their chemical shift in an NMR experiment. For example, the *pro-R* and *pro-S* protons in (*R*)-2-butanol (below) are diastereotopic, and for reasons that are beyond the scope of our discussion here, it turns out that they are in slightly different electronic environments, and thus have slightly different chemical shifts - they are chemically *non*-equivalent.



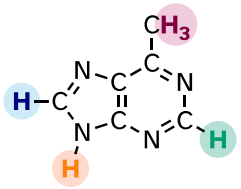
However, in actual practice, the difference in chemical shift between two diastereotopic protons such as these - bonded to the same sp^3-hybridized carbon and *not* part of a ring - is usually so small that they *appear* to be equivalent. There are exceptions, of course, but for the sake of simplicity, in this book we will consider such protons to be chemically equivalent. The chemical non-equivalence of diastereotopic protons usually only becomes pronounced when they are bonded to alkene or ring carbons, as in the previous examples.

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.

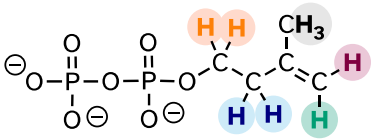
5.2.6



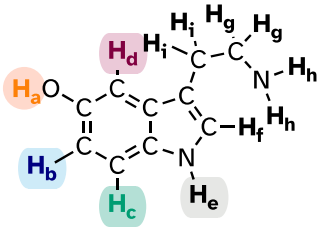
alanine
(an amino acid)



adenine
(a DNA/RNA base)



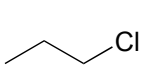
isopentyl diphosphate
(an isoprenoid buliding block)

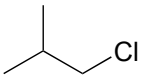


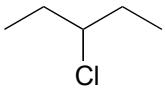
serotonin
(a neurotransmitter)

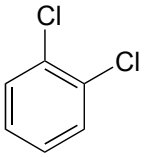
*(Note that protons H_a and H_b in isopentenyl diphosphate, and H_g and H_i in serotonin are all pairs of diastereotopic protons, but for the reason discussed in the shaded box above we are considering them to be chemically equivalent. We **do** consider the diastereotopic protons H_c and H_d in isopentenyl diphosphate, however, to be chemically nonequivalent.)*

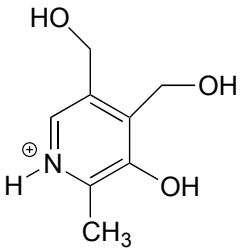
Exercise 5.2: How many sets of equivalent protons do the following molecules contain?



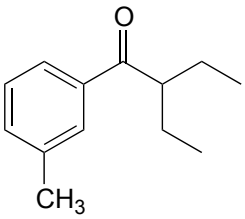




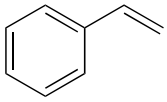




pyridoxine
(vitamin B_6)



DEET
(an insect repellent)

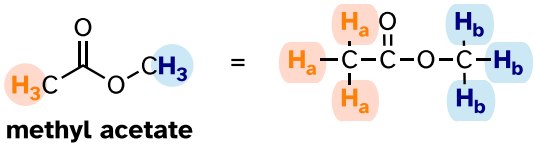


styrene
(used to make polystyrene in styrofoam)

Section 5.3: The ^{1}H-NMR experiment

In an NMR experiment, a sample compound (we will again use methyl acetate as our example) is placed inside a 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 magnetic objects anywhere near them. They are also notorious for erasing the magnetic strips on credit cards!)

5.3.1



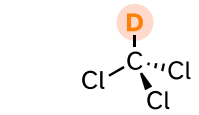
At first, the magnetic moments of (slightly more than) half of the protons in the sample are aligned with B_0, and half are aligned against B_0. Then, the sample is exposed to a range of radio frequencies. Out of all the frequencies which hit the sample, only two - the resonance frequencies for H_a and 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 use of a mathematical technique known as a 'Fourier Transform'.

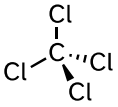
Note: the above description of an NMR experiment is an oversimplification of what is happening in a modern NMR instrument but is adequate for our purpose here. If you take a more advanced course in molecular spectroscopy you will learn about the process in much greater detail.

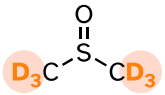
In most cases, a sample being analyzed by NMR is in solution. If we use a common laboratory solvent (diethyl ether, acetone, dichloromethane, ethanol, water, etc.) to dissolve our NMR sample, however, we run into a problem – there many more solvent protons in solution than there are sample protons, so 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.

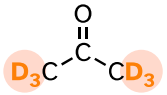
5.3.2

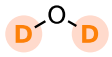
Common NMR solvents


deuterated chloroform
$CDCl_3$


carbon tetrachloride
CCl_4


deuterated dimethylsulfoxide
DMSO-d_6

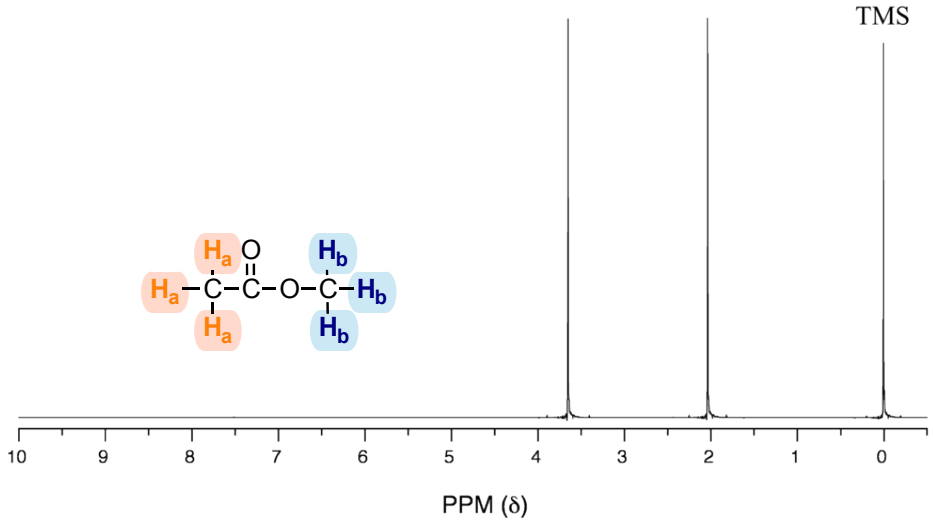

deuterated aceteone
acetone-d_6


deuterated water
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 mysteries will become clear very soon.

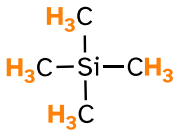
5.3.3

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.

5.3.4



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 each 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 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 chemical shift, *expressed 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 exactly 500 MHz?

b) What is the *actual resonance frequency* (in Hz) of these two protons in that same instrument?

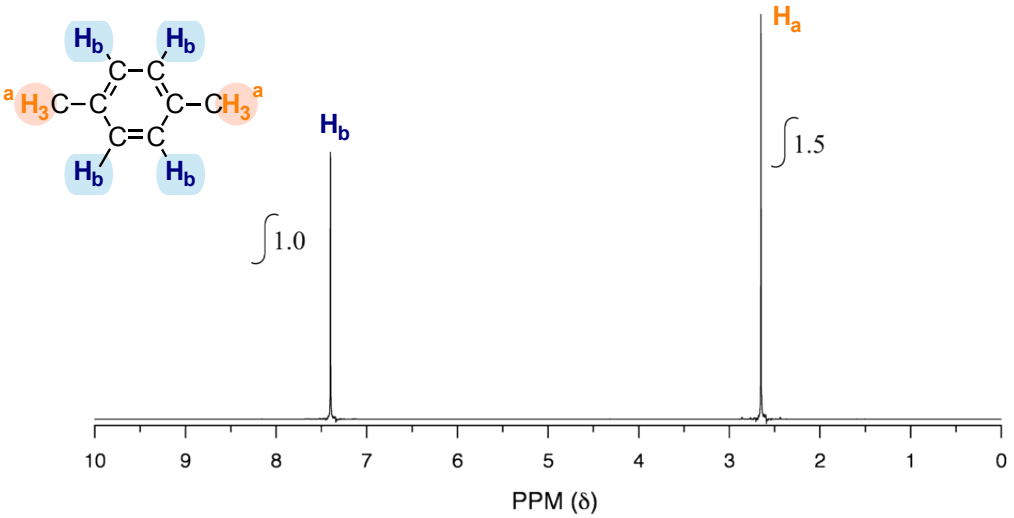
We have already pointed out that, on our spectrum of methyl acetate, there is no *y*-axis scale indicated. With *y*-axis data it is *relative* values, rather than absolute values, that are important in NMR. The computer in an NMR instrument can be instructed to mathematically integrate the area under a signal or group of signals. The **signal integration** process is 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 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:

5.3.5

The ^{1}H-NMR 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 will 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 an 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 cyclopentane 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 to 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.

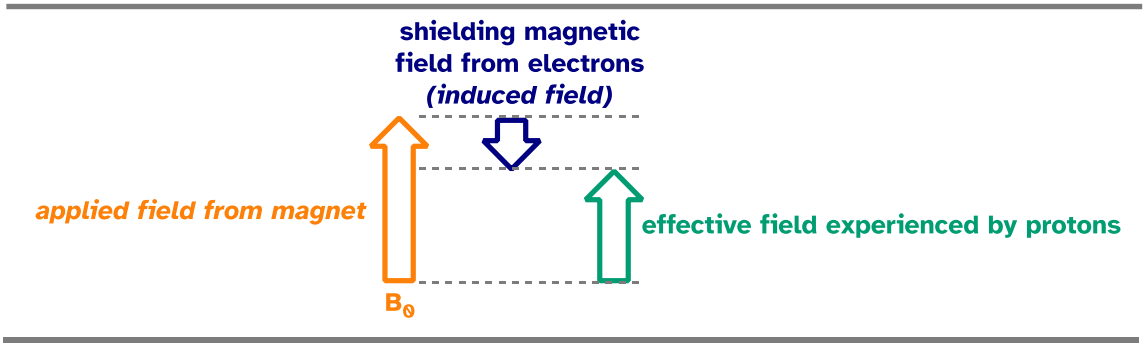
Section 5.4: The basis for differences in chemical shift

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 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.

5.4.1



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 fluorine is the most electronegative and bromine the least).

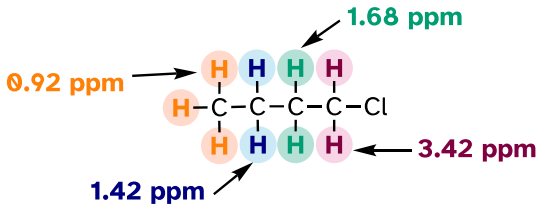
5.4.2

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 (common name chloroform) is, as expected, higher than that of dichloromethane, which is in turn higher than that of chloromethane.

5.4.3

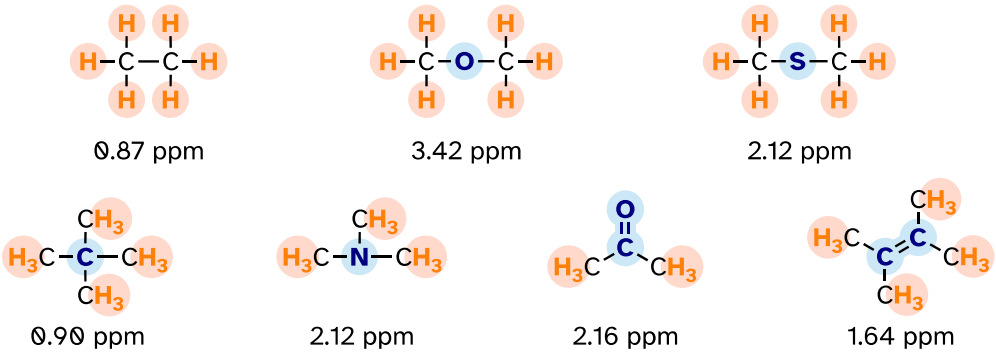
The deshielding effect of an electronegative substituent diminishes sharply with increasing distance:

5.4.4



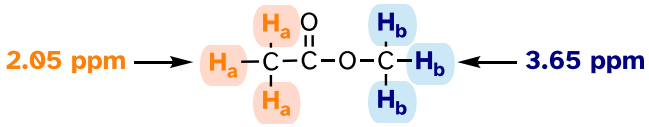
The presence of electronegative oxygen, nitrogen, sulfur, or sp^2-hybridized carbon atoms also tends to shift the NMR signals of nearby protons slightly downfield:

5.4.5



Now we can finally assign the two peaks in 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.

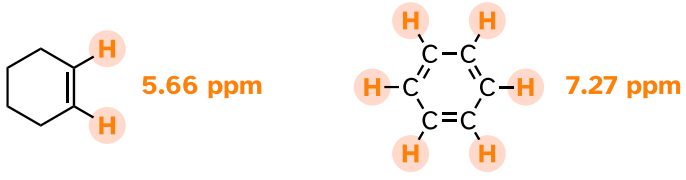
5.4.6



5.4B: Diamagnetic anisotropy

Vinylic protons (those directly bonded to an *sp*^{2}-hybridized 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:

5.4.7

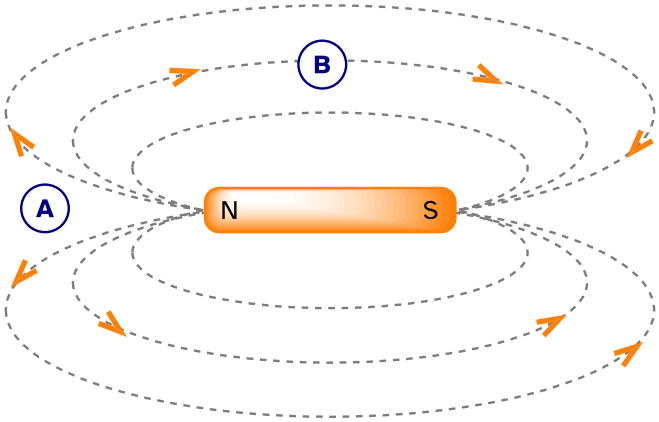


We'll consider the aromatic proton first. Recall that in benzene and many other aromatic structures, 6 π electrons are delocalized around the ring. When the molecule is exposed to B_0, these π electrons begin to circulate in a **ring current**, generating their own induced magnetic field that opposes 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 anisotropic. They start in the 'north' direction, then loop around like a snake biting its own tail.

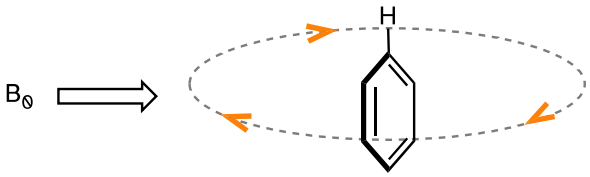
5.4.8



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.

5.4.9



another image

The 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

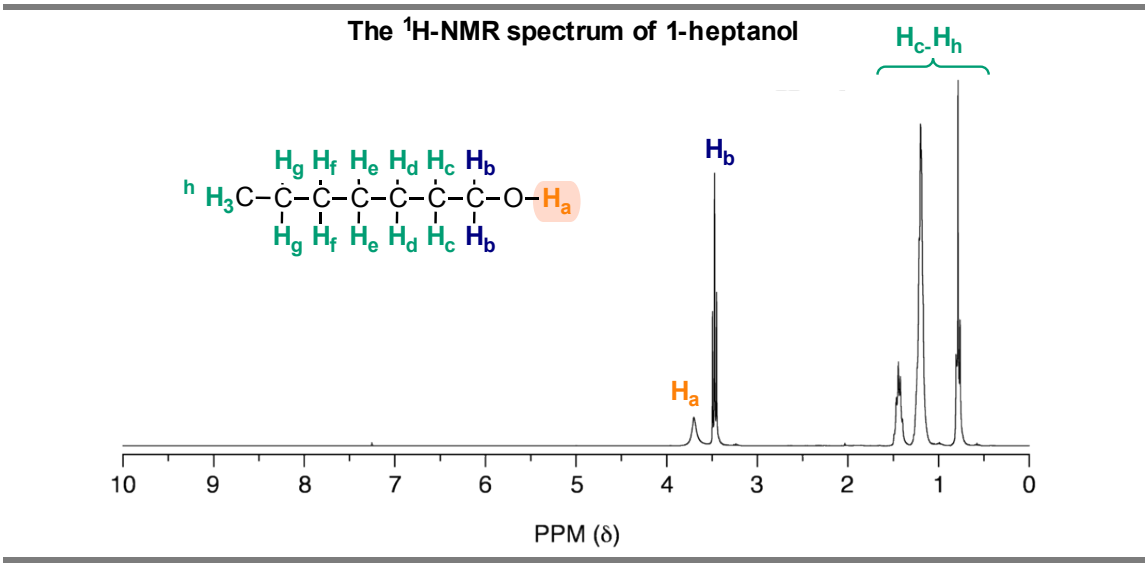
not aromatic and thus do not generate ring currents does benzene, but the π electrons circulate in such a way as to generate a magnetic field that *adds* to B_0 in the regions of space occupied by the protons. Carboxylic acid protons are very far downfield (10-12 ppm) due to the combined influence of 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.

5.4.10

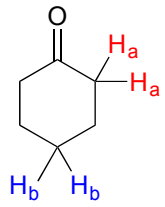


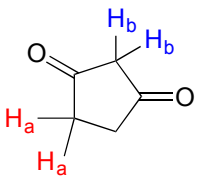
The table below provides a summary of approximate chemical shift ranges for protons in different bonding arrangements. A more detailed table can be found in the appendix.

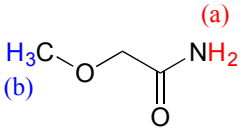
Typical chemical shift ranges in ^{1}H-NMR

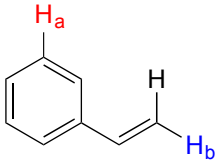
type of proton	chemical shift range (ppm)
bonded to sp^3 carbon	0.5 - 4
bonded to N (amine)	1 - 3
bonded to O (alcohol)	1 - 5
alkene/ vinylic	3.5 - 6.5
terminal alkyne	2 - 3
bonded to N (amide)	5 - 9
aromatic	6 - 9
aldehyde	9.5 - 10
carboxylic acid	10 - 13

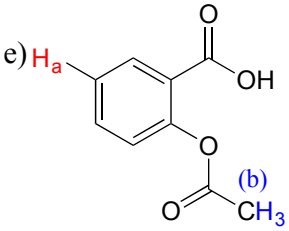
Exercise 5.6: For each pair of protons colored red (H_a) and blue (H_b) in the structures below, state which is expected to have the *higher* chemical shift in ^{1}H-NMR. For some of these it will be helpful to consult Table 2 in the Table Appendix.

a) 

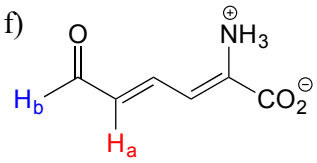
b) 

c) 

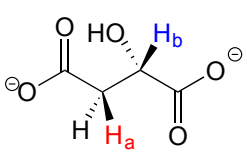
d) 

e) 

acetylsalicyclic acid
(aspirin)

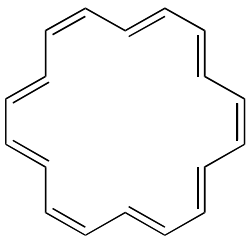
f) 

intermediate in tryptophan degradation

g) 

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.



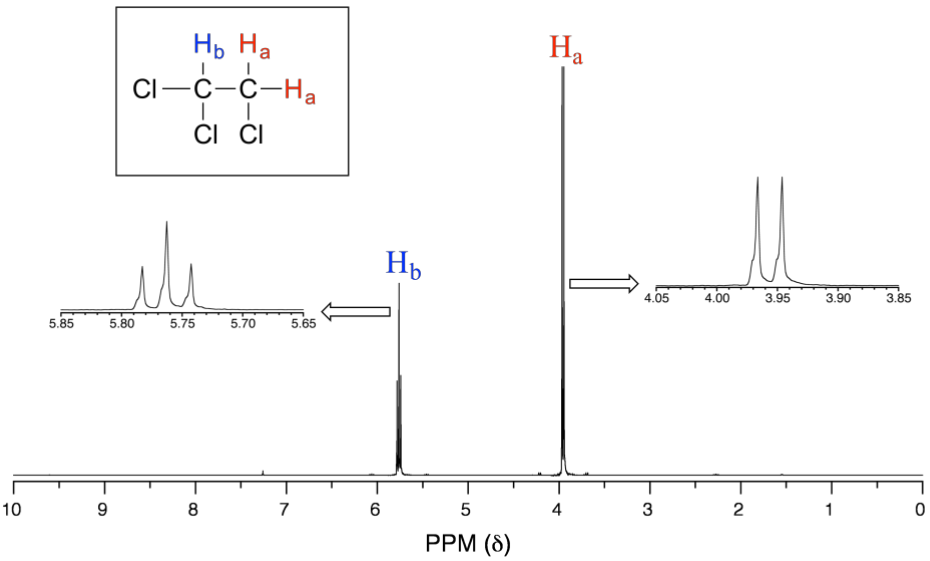
[18] annulene

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 unusual in the sense that in both 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.

5.5.1

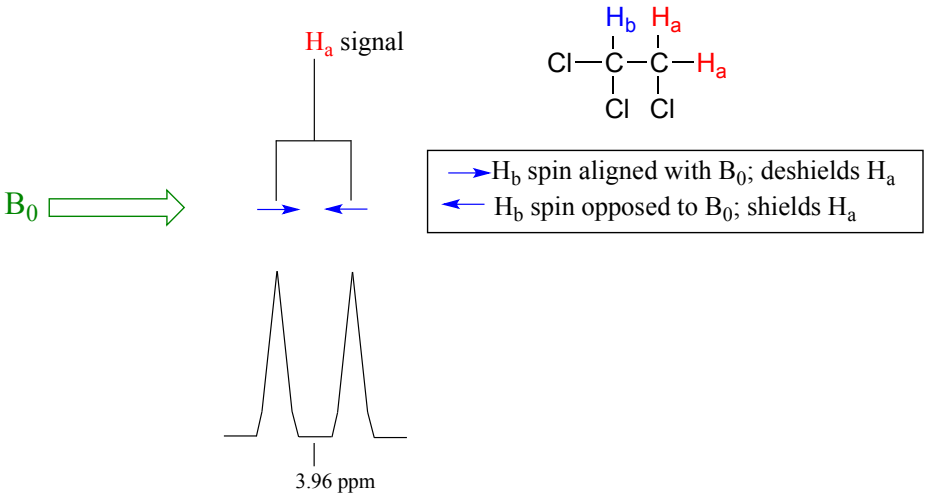


The signal at 3.96 ppm, corresponding to the two H_a protons, is split into two subpeaks of equal height (and area) – this is referred to as a **doublet**. The H_b signal at 5.76 ppm, on the other hand, is split into three sub-peaks, with the middle peak higher than the two outside peaks - if we were 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,

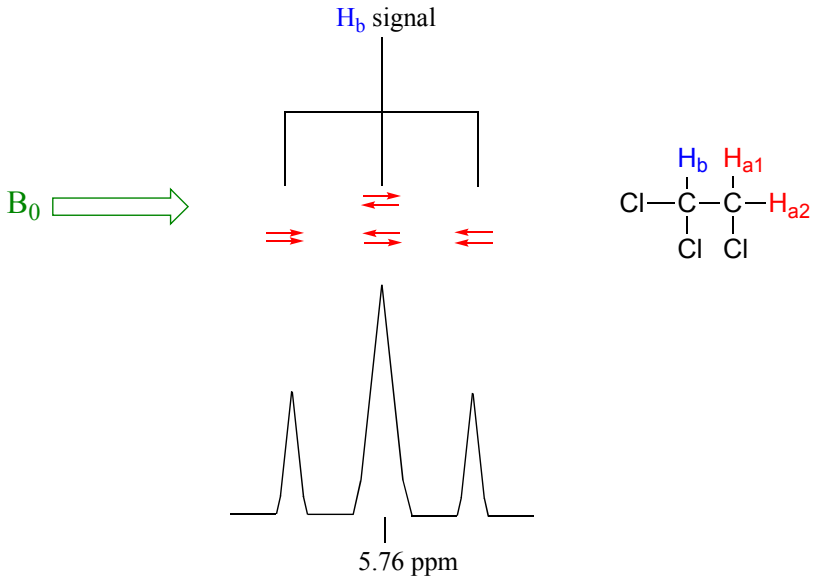
each of the H_a protons is also influenced by the small magnetic field generated by H_b next door (remember, each spinning proton is like a tiny magnet). The magnetic moment of H_b will be aligned *with* B_0 in slightly more than half of the molecules in the sample, while in the remaining molecules it will be opposed to B_0. The B_{eff} 'felt' by H_a is 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 can be illustrated by a **splitting diagram**, as shown below.

5.5.2



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 unchan

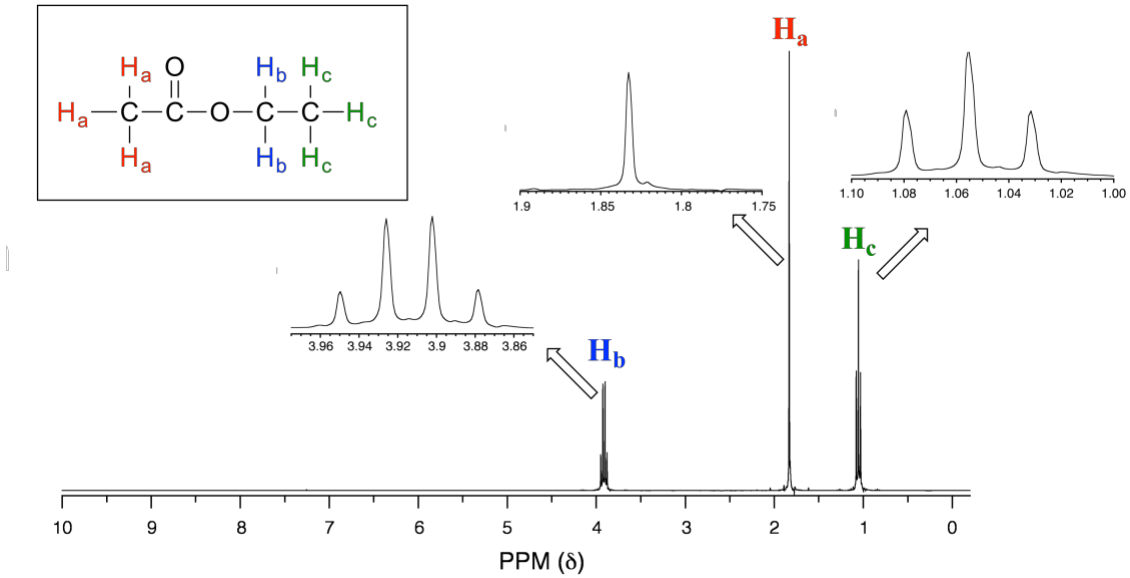
5.5.3



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:

5.5.4



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 than 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 splitting diagram, the possible combinations of nuclear spin states for the H_c protons in ethyl acetate, and why the H_b signal is split into a quartet.

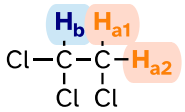
b) The integration ratio of the two 'sub-peaks' in a doublet is 1:1, and in triplets it is 1:2:1. What is the integration ratio of the H_b quartet in ethyl acetate? (Hint – use the illustration that you drew in part a to answer this question.)

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.

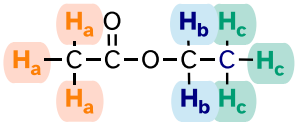
5.5.5



no observed coupling
between equivalent hydrogens

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.

5.5.6



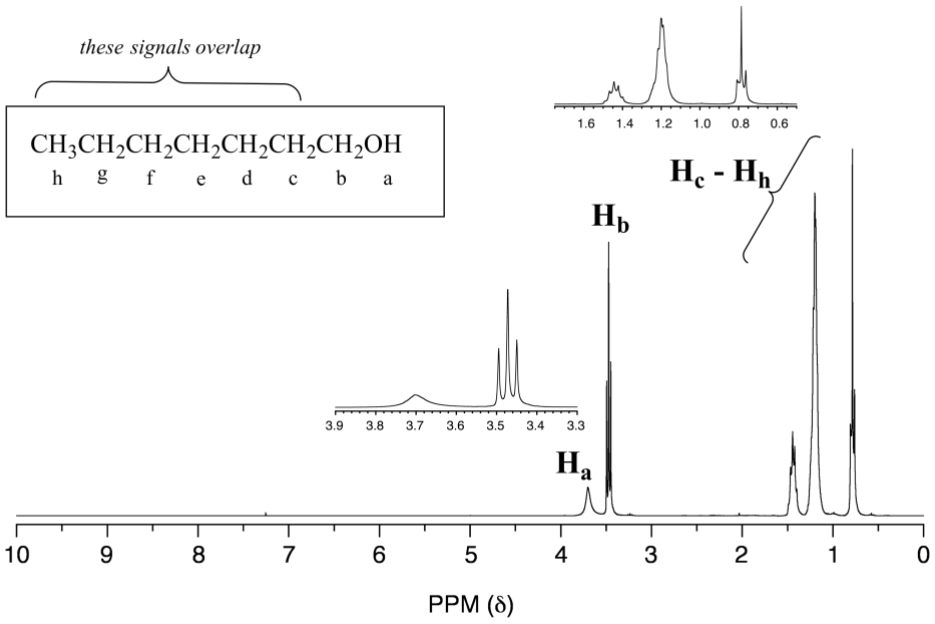
H_a protons are 5 bonds away from H_b
= no observed coupling

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).

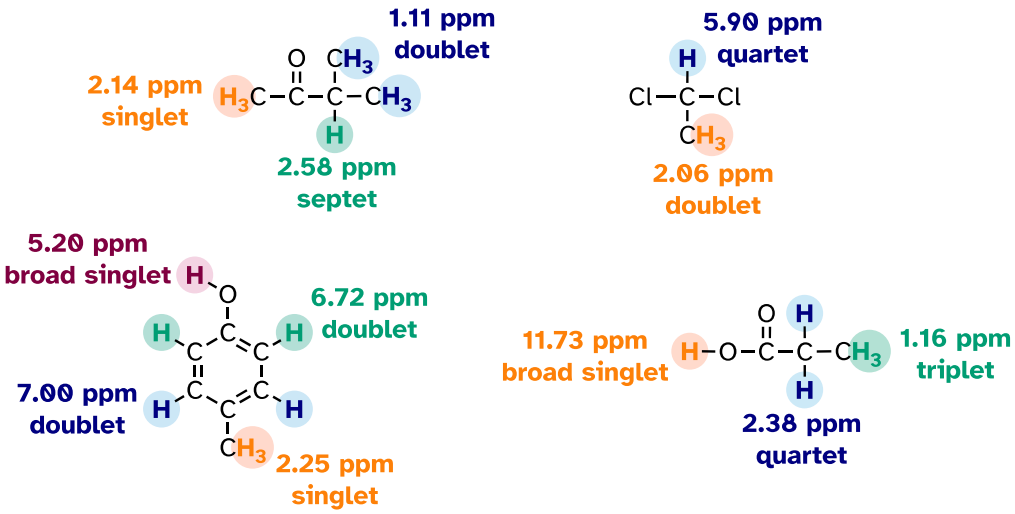
5.5.7



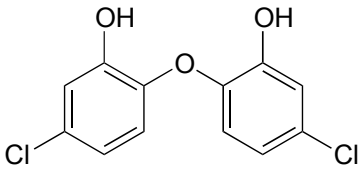
Notice in this spectrum that H_b is a triplet, coupled to the two H_c protons but *not* coupled to H_a. The signals corresponding to H_c through H_h are complex due to overlapping - when this happens (as it often does!), detailed analysis becomes more challenging.

Below are a few more examples of chemical shift and splitting pattern information for some relatively simple organic molecules.

5.5.8

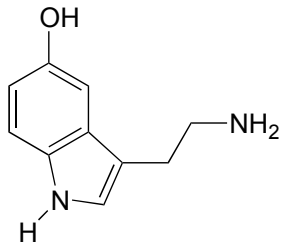


Exercise 5.9: How many proton signals would you expect to see in the ^{1}H-NMR spectrum of triclosan (a common antimicrobial agent in soap)? For each of the proton signals, predict the splitting pattern, assuming that you can see only 3-bond splitting.



Triclosan

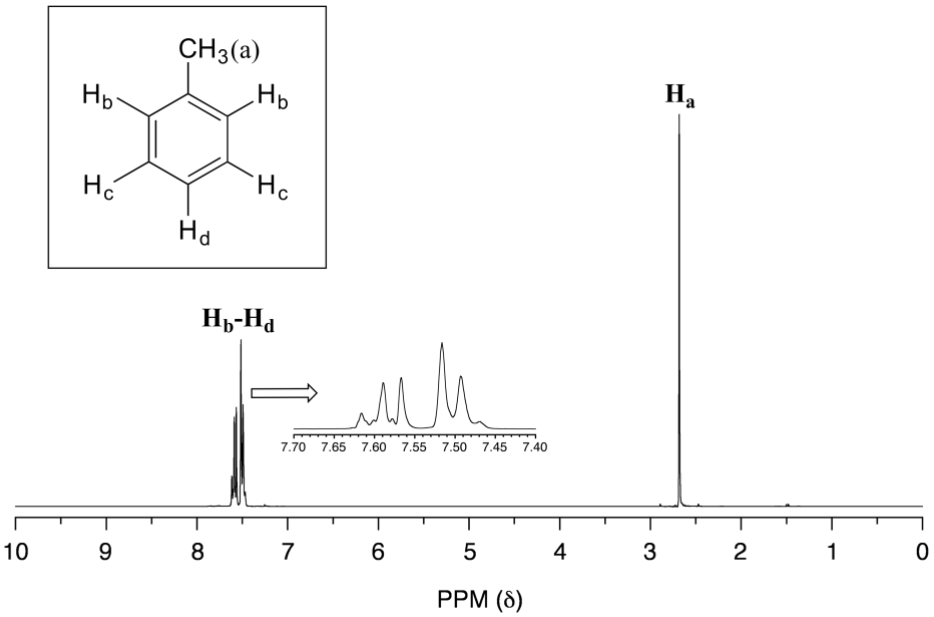
Exercise 5.10: How many proton signals would you expect to see in the ^{1}H-NMR spectrum of the neurotransmitter serotonin? For each of the proton signals, predict the splitting pattern, again assuming only 3-bond splitting.



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.

5.5.9

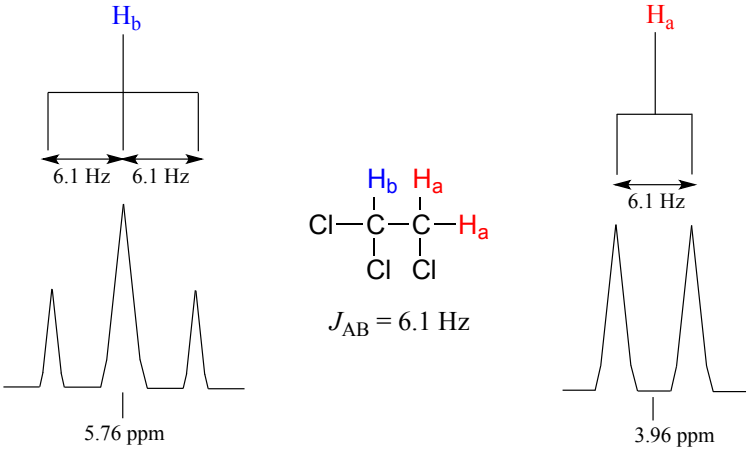


In practice, however, the three aromatic proton sets H_b, H_c and H_d have very similar chemical shifts so their signals overlap substantially, and we cannot recognize doublet or triplet splitting patterns. In this case, we would refer to the aromatic part of the spectrum as a **multiplet**. We can report 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 $^3J_{a\text{-}b}$ = 6.1 Hz.

5.5.10



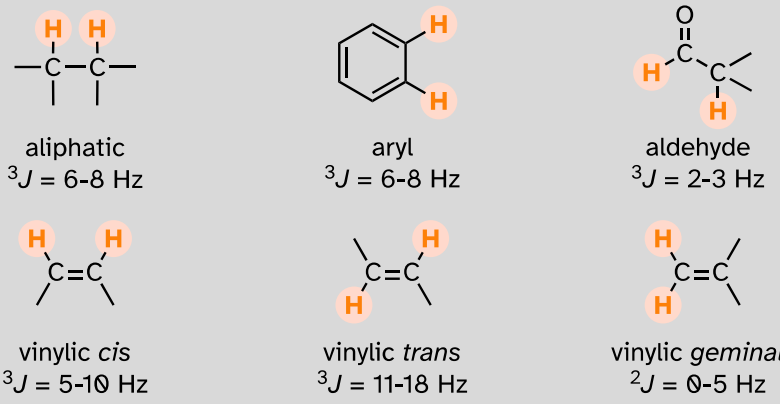
The superscript '3' tells us that this is a three-bond coupling interaction, and the 'a-b' subscript tells us that we are talking about coupling between H_a and H_b. Unlike the chemical shift value, ***the coupling constant, expressed in Hz, is the same regardless of the applied field strength of the 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 $^3J_{a\text{-}b}$ 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
5.5.11

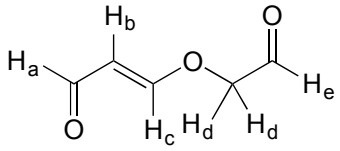
Typical proton-proton coupling constants



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 spectra 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:

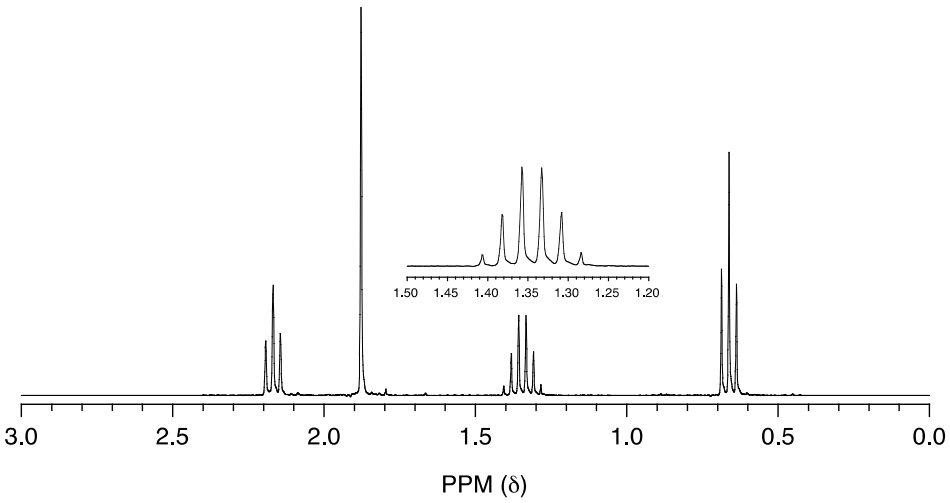
δ (ppm)	splitting	integration
3.92	q	1
1.83	s	1.5
1.06	t	1.5

^{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 the signals.

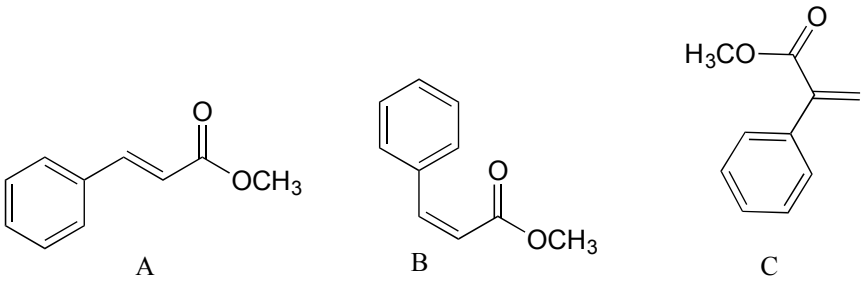


a) cyclopentanone b) 3-pentanone c) butaldehyde
d) 2-pentanone e) 4-heptanone f) 1-butene

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 two triplets?
c) Which would have a spectrum that includes a signal above 8 ppm?

Exercise 5.14: Explain how you could distinguish among the ^{1}H-NMR spectra of the three isomers below.



Video tutorials: proton NMR spectroscopy
Video of an actual NMR experiment

Section 5.6: 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.

To determine 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 92 g. 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 the degrees of unsaturation, or 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:

$$\text{IHD} = \frac{(2n+2) - \text{A}}{2}$$

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.

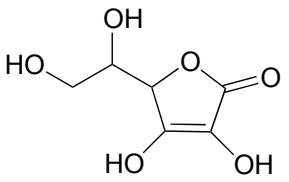
A molecular formula of C_6H_{12}, on the other hand, corresponds to IHD = 1, so a compound with this formula should have one double bond *or* one ring structure. Cyclohexane (one ring structure) and 2-hexene (one double bond) are two possibilities. Benzene (C_6H_6), and methyl benzene (C_7H_8) both have 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:

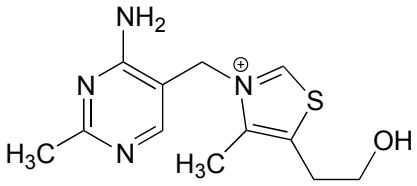
a) What is the IHD that corresponds to a molecular formula $C_6H_{12}O$? Draw the structures of three possible compounds that fit.

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.

^{1}H-NMR:

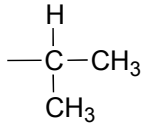
δ (ppm)	splitting	integration
3.38	d	2
1.95	m	1
1.01	d	6

^{13}C-NMR:

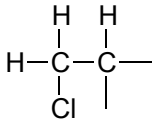
52.49 (CH_2)
31.06 (CH)
20.08 (CH_3)

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 segments 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 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:

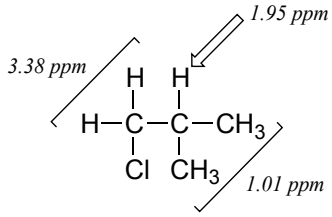


The ^{1}H-NMR signal at 3.38 ppm must be for protons bound to the carbon which is in turn bound to the chlorine (we infer this because this signal is the furthest downfield in the spectrum, due to the deshielding effect of the electronegative chlorine). This signal is for two protons and is a doublet, meaning that there is a single nonequivalent proton on an adjacent carbon.

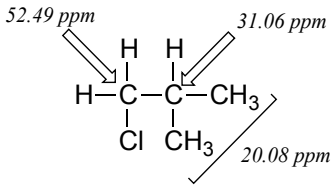


Step 4: Try to put the pieces of the puzzle together and see if everything fits the available data.

At this point, we have accounted for all 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 assign all NMR signals to their corresponding atoms and make sure that our structure fits all 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 assignments.

Compound A: (2-chloro-2-methylpropane)

^{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. 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:

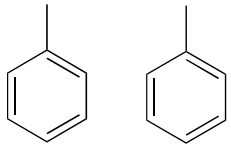
7.5-7.0, 10H, m
5.10, 1H, s
2.22, 3H, s

^{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)

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.

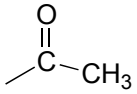
Because we have ten protons with signals in the aromatic region (7.5-7.0 ppm), we are probably dealing with two phenyl groups, each with one substituted carbon. The ^{13}C-NMR spectrum shows only four signals in the range for aromatic carbons, which tells us that the two phenyl groups must be in equivalent electronic environments (if they are in different environments, they would give rise to eight signals).



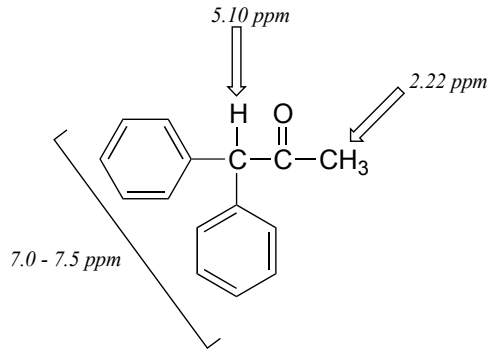
This accounts for 12 carbons, 10 hydrogens, and 8 IHD units. Notice that the carbon spectrum has only six peaks - and only four peaks in the aromatic region! This again indicates that the two phenyl groups are in chemically equivalent positions

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.



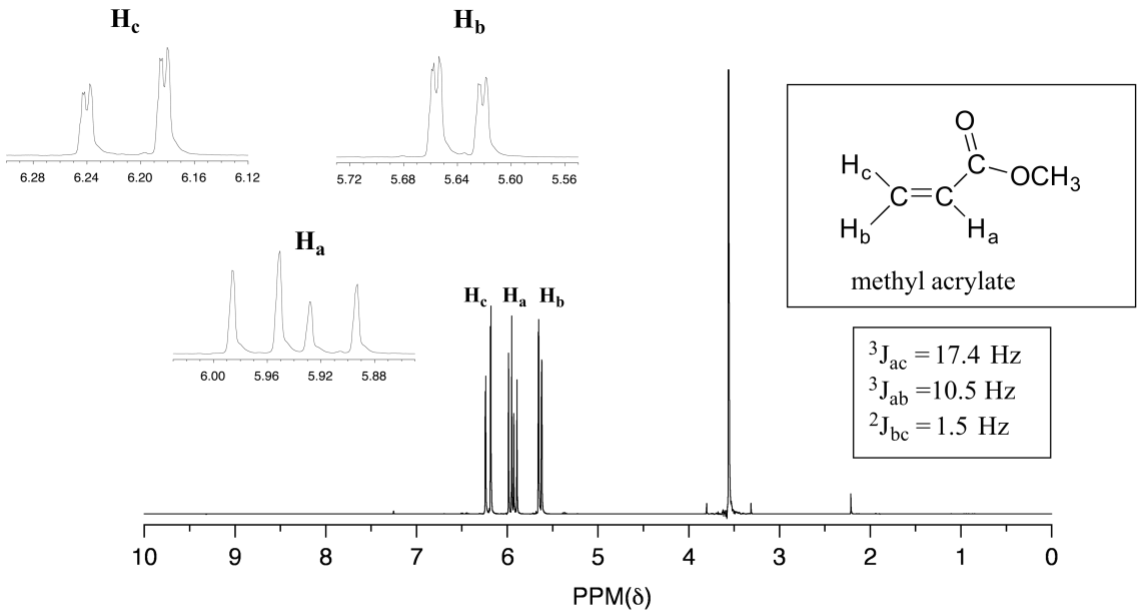
Finally, there is one last proton at 5.10 ppm, also a singlet. Putting the puzzle together, the only possibility that fits is 1,1-diphenyl-2-propanone:



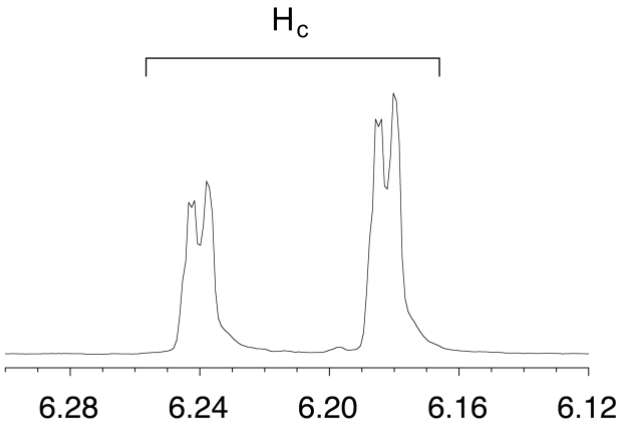
^{1}H-NMR assignments

Section 5.7: Complex coupling in ^{1}H-NMR spectra

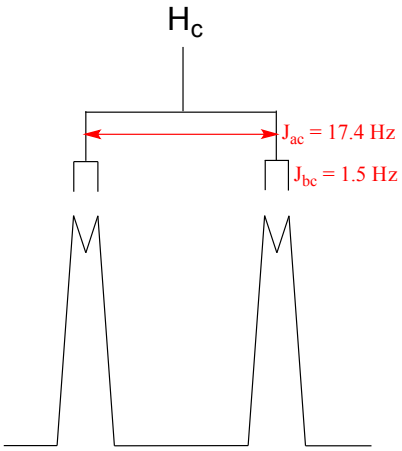
In all the examples of spin-spin coupling we saw in our discussion of proton NMR, the observed splitting resulted from the coupling of one set of protons to *just one* neighboring set of protons. When a set of protons is coupled to *two* sets of nonequivalent neighbors, with significantly different coupling 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 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.

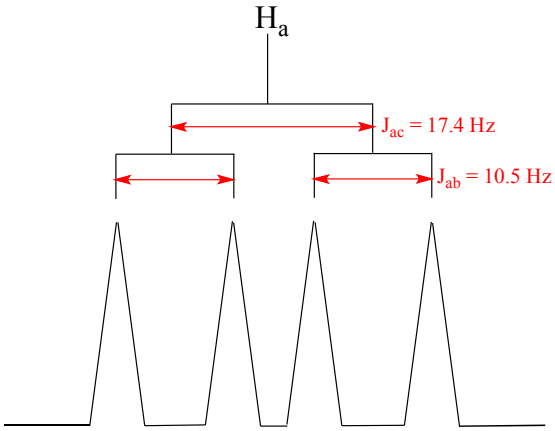


The result of this `double splitting` is a pattern referred to as a **doublet of doublets**, abbreviated `**dd**`.

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 1863.0 Hz. What is the chemical shift, to the nearest 0.1 Hz, of the furthest upfield 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.



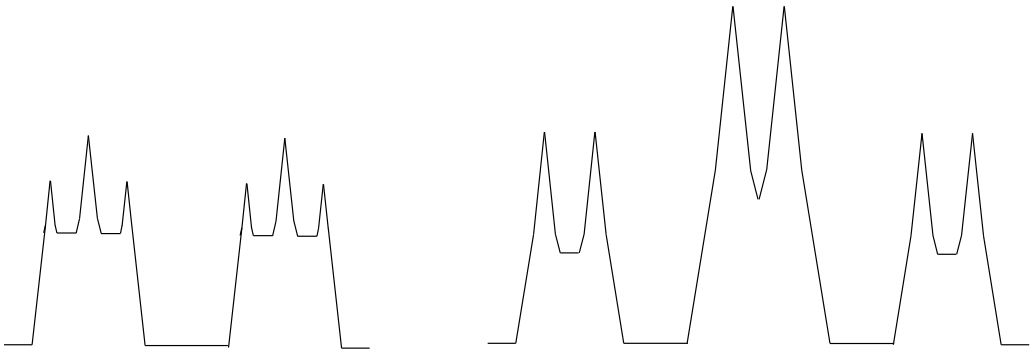
The signal for H_b at 5.64 ppm is split into a doublet by H_a, a *cis* coupling with $^3J_{ab}$ = 10.4 Hz. Each of the resulting sub-peaks is split again by H_c, with the same *geminal* coupling constant $^2J_{bc}$ = 1.5 Hz that we saw previously when we looked at the H_c signal. The overall result is 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.

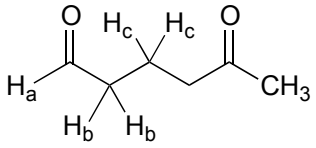
In the methyl acrylate spectrum, the signals for each of the three vinylic protons was a doublet of doublets (abbreviated 'dd'). Other complex splitting patterns are possible: triplet of doublets (td), doublet of triplets (dt), doublet of quartets (dq), and so on. Remember that the broader splitting 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. 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 5.8: 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 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/)

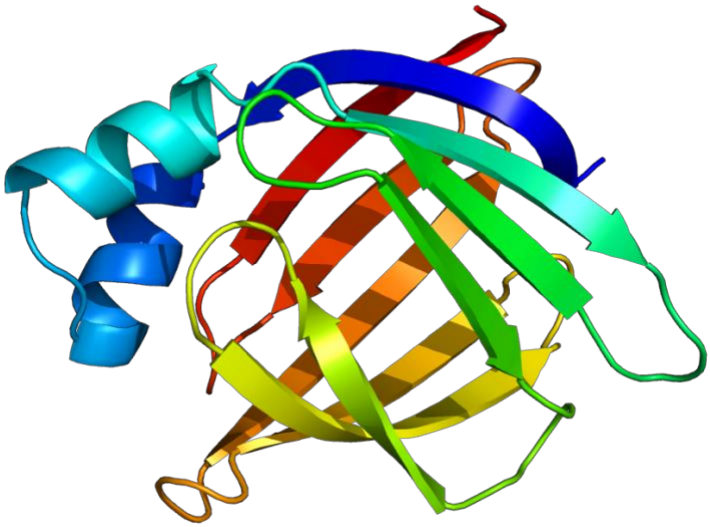
B: NMR of proteins and peptides

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 (i.e. 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 hand, 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.

Note: The Spectral Database of Organic Compounds is a great resource for looking at NMR spectra (both proton and carbon) for a large number of compounds - the more examples you see, the better!

End of Chapter Self-Check List

Check your progress towards success. Verify that after completing this chapter you can:

- ☐ Identify chemically equivalent protons in a structure.
- ☐ Describe the fundamental principles 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.
- ☐ Define chemical shift and its units.
- ☐ Predict trends in chemical shifts for protons in various chemical environments and provide a rationale for the trend.
- ☐ Estimate roughly the chemical shift of a given proton using a chemical shift table.
- ☐ Interpret integration values to determine how many protons a particular peak represents.
- ☐ Describe the physical basis for spin-spin coupling in ^{1}H-NMR spectra and use the 'n+1 rule'.
- ☐ Interpret and draw splitting diagrams for ^{1}H-NMR spectra.
- ☐ Match structures to ^{1}H-NMR spectra.
- ☐ Calculate the Index of Hydrogen Deficiency and interpret the results.

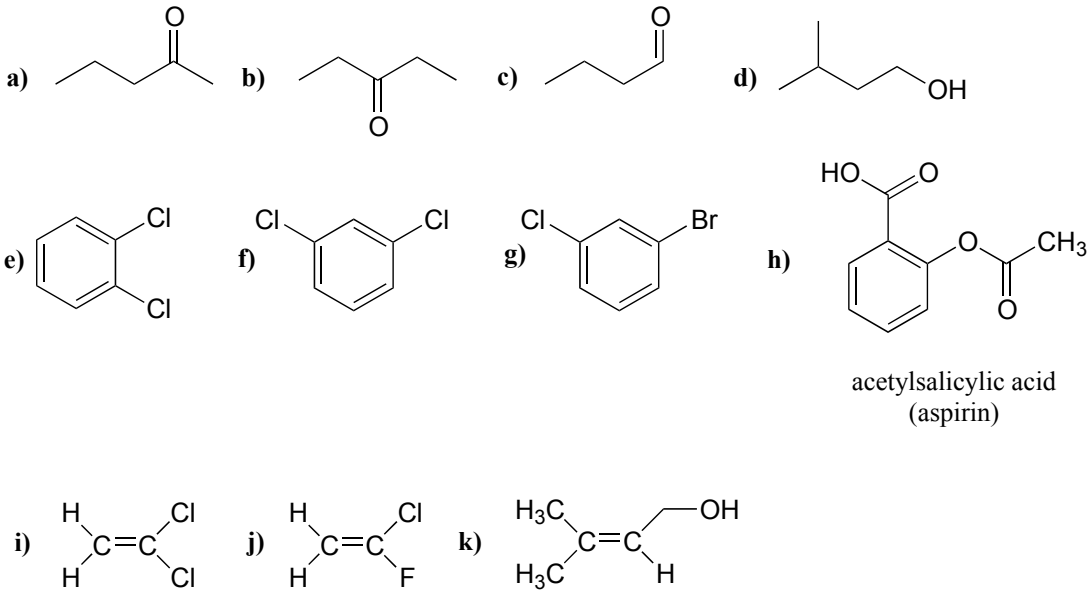
If you didn’t check off all items on this list, practice more and reach out to your instructional team for additional help.

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.

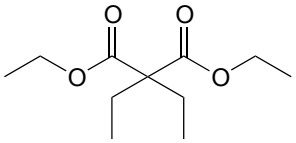


P5.2: For each of the 20 common amino acids, predict the number of signals in the proton-decoupled ^{13}C-NMR spectrum.

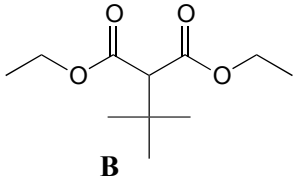
P5.3: Match spectra below to their corresponding structures A-F. Make complete peak assignments for all structures.

(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)

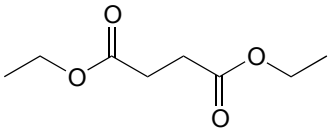
Structures:



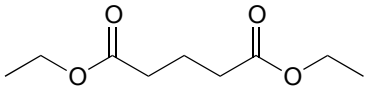
A



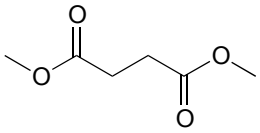
B



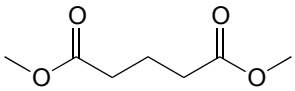
C



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

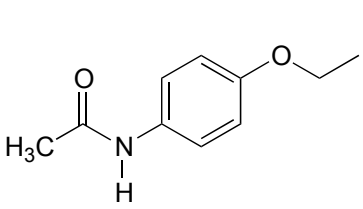
δ (ppm)	splitting	integration
4.18	q	1
1.92	q	1
1.23	t	1.5
0.81	t	1.5

Spectrum 6:

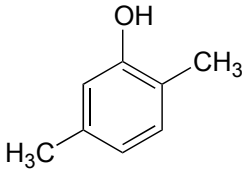
δ (ppm)	splitting	integration
3.69	s	1.5
2.63	s	1

P5.4: Match the ^{1}H-NMR spectra 7-12 to their corresponding structures G-L. Make complete peak assignments for all structures.

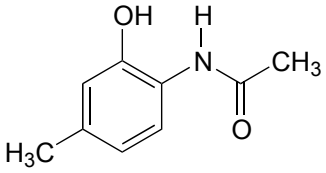
Structures:



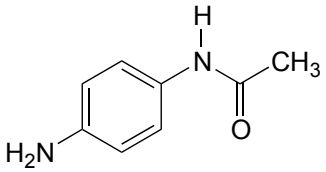
G



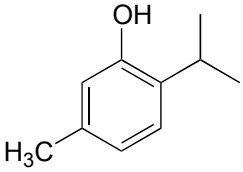
H



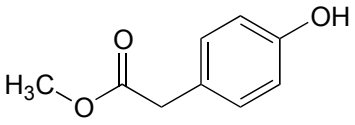
I



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:

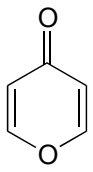
δ (ppm)	splitting	integration
9.49	s	1
7.20	d	2
6.49	d	2
4.82	s	2
1.963	s	3

Spectrum 12:

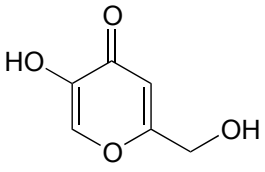
δ (ppm)	splitting	integration
9.58	s(b)	1
9.31	s	1
7.36	d	1
6.67	s	1
6.55	d	1
2.21	s	3
2.11	s	3

P5.5: Match the ^{1}H-NMR spectra 13-18 to their corresponding structures M-R. Make complete peak assignments for all structures.

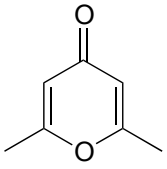
Structures:



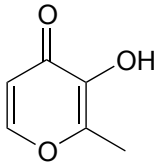
M



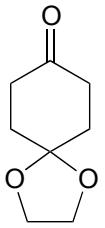
N



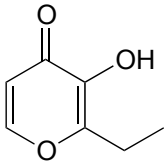
O



P



Q



R

Spectrum 13:

δppm)	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:

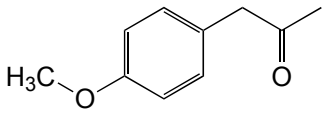
δ (ppm)	splitting	integration
7.76	d	1
7.57	s (b)	1
6.44	d	1
2.78	q	2
1.25	t	3

Spectrum 18:

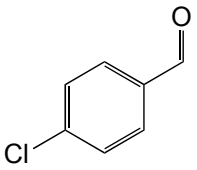
δ (ppm)	splitting	integration
4.03	s	1
2.51	t	1
2.02	t	1

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



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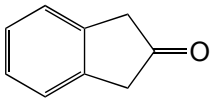

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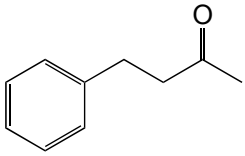

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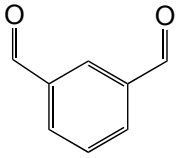

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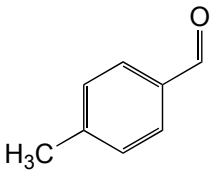


X

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:

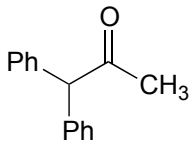
δ (ppm)	splitting	integration
7.10	d	1
6.86	d	1
3.78	s	1.5
3.61	s	1
2.12	s	1.5

Spectrum 24:

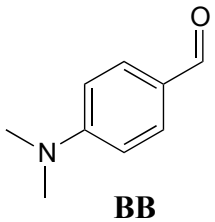
δ (ppm)	splitting	integration
7.23-7.30	m	1
3.53	s	1

P5.7: Match the ^{1}H-NMR spectra 25-30 below to their corresponding structures AA-FF.

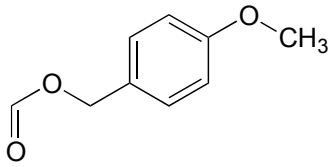
Structures:



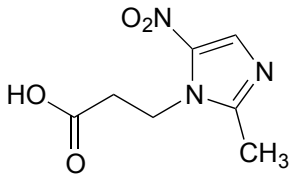
AA



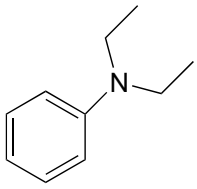
BB



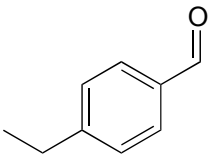
CC



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

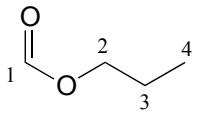
Spectrum 30:

δ (ppm)	splitting	integration
8.32	s	1
4.19	t	2
2.83	t	2
2.40	s	3

P5.8:

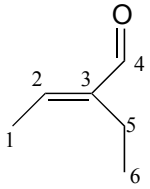
^{13}C-NMR data is given for the molecules shown below. Complete the peak assignment column of each NMR data table.

a)



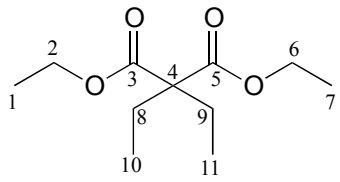
δ (ppm)		**carbon #(s)**
161.12	CH	
65.54	CH_2	
21.98	CH_2	
10.31	CH_3	

b)



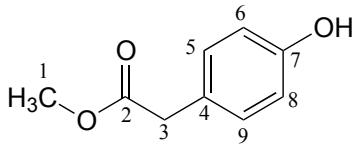
δ (ppm)		**carbon #(s)**
194.72	C	
149.10	C	
146.33	CH	
16.93	CH_2	
14.47	CH_3	
12.93	CH_3	

c)



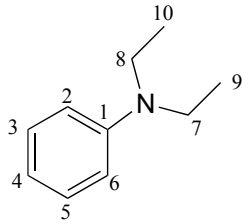
δ (ppm)		carbon #(s)
171.76	C	
60.87	CH_2	
58.36	C	
24.66	CH_2	
14.14	CH_3	
8.35	CH_3	

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

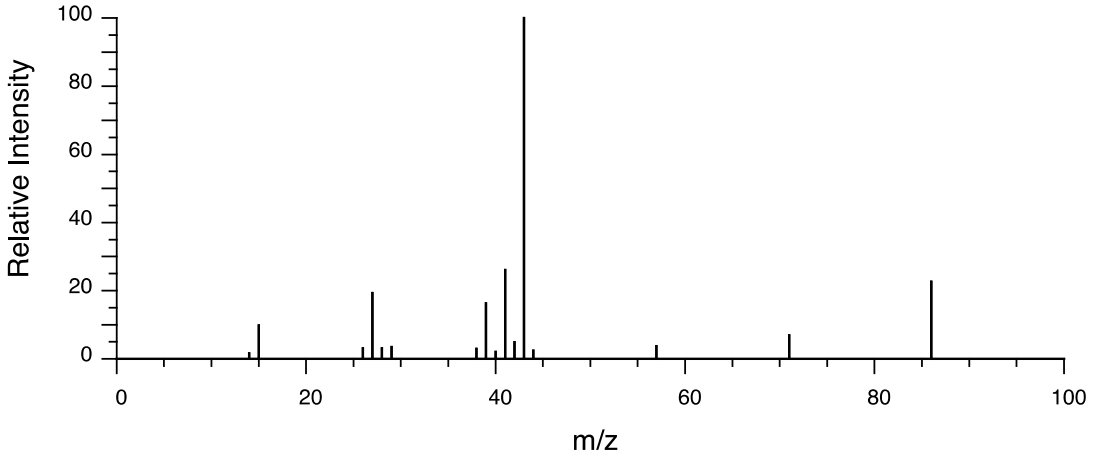
^{13}C-NMR

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

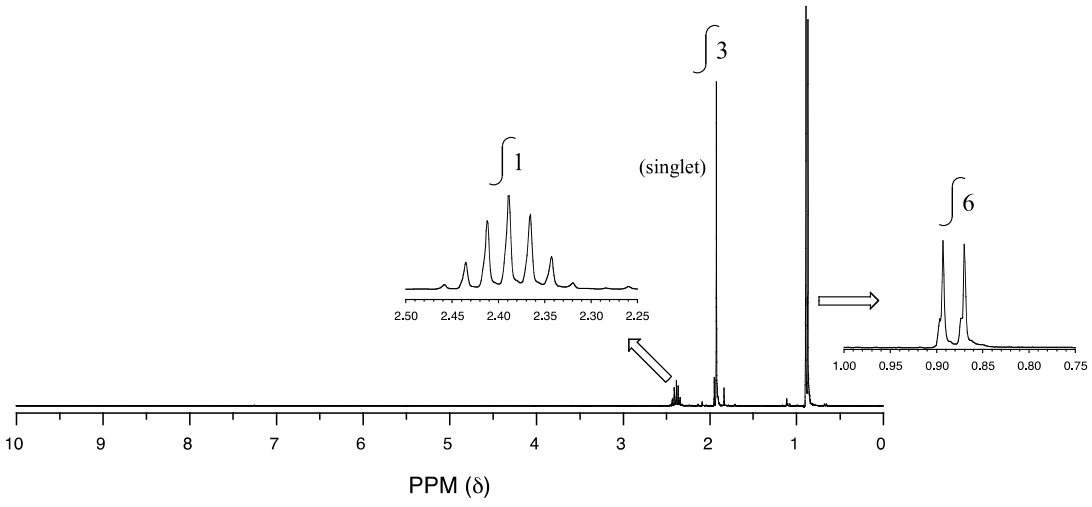
P5.10: You obtain the following data for an unknown sample. Deduce its structure.

Combustion analysis: C (69.7%); H (11.7%)

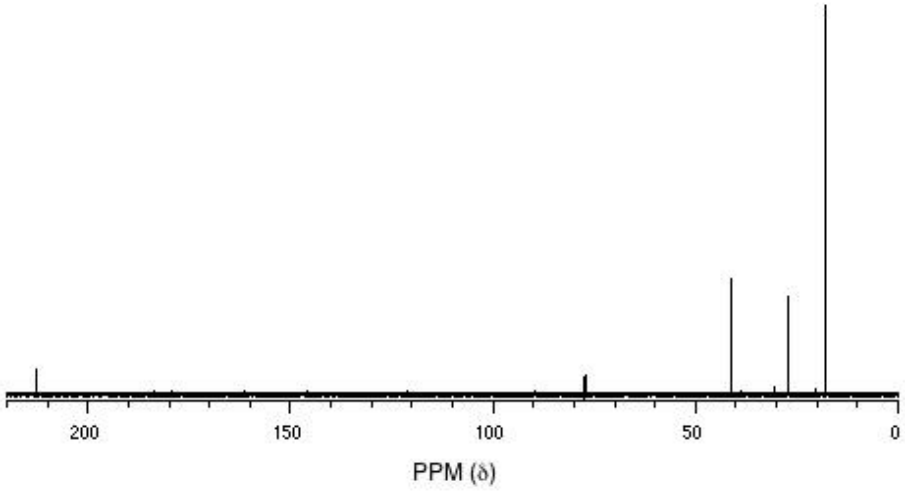
Mass Spectrometry:



^{1}H-NMR:



^{13}C-NMR:



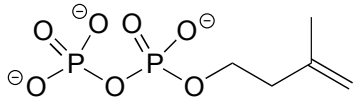
P5.11: You take a ^{1}H-NMR spectrum of a sample that comes from a bottle of 1-bromopropane. However, you suspect that the bottle might be contaminated with 2-bromopropane. The NMR spectrum shows the following peaks:

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 diophosphate

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.