13. Nuclear Magnetic Resonance (NMR) Spectroscopy

A. Basic Principles

Nuclear magnetic resonance (NMR) spectroscopy is one of the most important and widely used methods for determining the structure of organic molecules. NMR allows one to deduce the carbon-hydrogen connectivity in a molecule. The following experiment provides a general overview of NMR theory and interpretation. For a more extensive discussion, you should consult your textbook.

The nucleus of a hydrogen atom (^{1}H) as well as certain other nuclei (^{13}C, ^{15}N, ^{31}P, etc.) possess a property called nuclear spin. Any nucleus that has an odd mass number and/or an odd atomic number has a nuclear spin. Nuclear spins are oriented randomly in space, however, in the presence of an external magnetic field ($\mathbf{B_{ext}}$) the spins align. Some spins align parallel to $\mathbf{B_{ext}}$ (α spin state) while others align antiparallel to $\mathbf{B_{ext}}$ (β spin state). The nuclear spins that align parallel to $\mathbf{B_{ext}}$ are slightly lower in energy. (Figure 1)
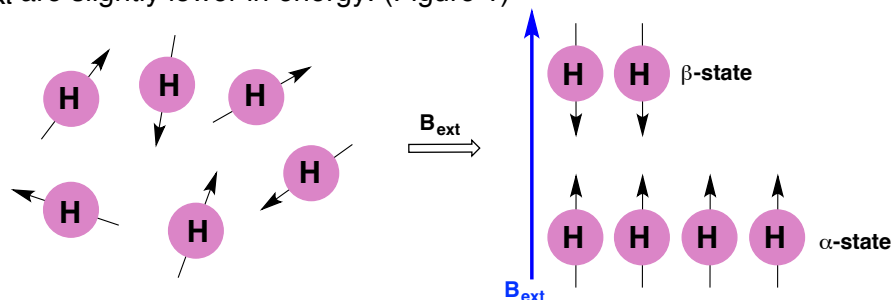

Figure 1. Nuclear Spins in the Presence of B$_{ext}$

When the nuclear spins in the α and β states are irradiated with electromagnetic energy from the radio frequency (RF), energy is absorbed and some of the nuclear spins in the α state are "spin-flipped" to the higher energy β state. At this stage, the nuclei are said to be in resonance with the applied RF radiation. (Figure 2)

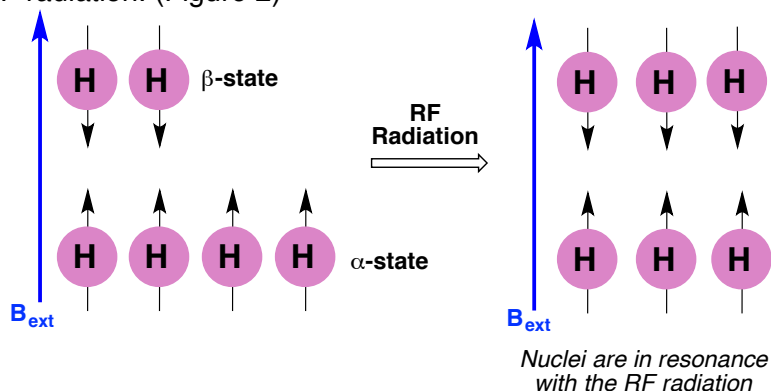*Nuclei are in resonance with the RF radiation*

Figure 2. Nuclei Irradiated with RF Radiation

Over time, the flipped spins slowly fall back down to the higher energy state. The signal decay is detected over time producing a raw signal called a free induction decay (FID). A mathematical function called Fourier Transform converts the FID into the NMR spectrum. (Figure 3)
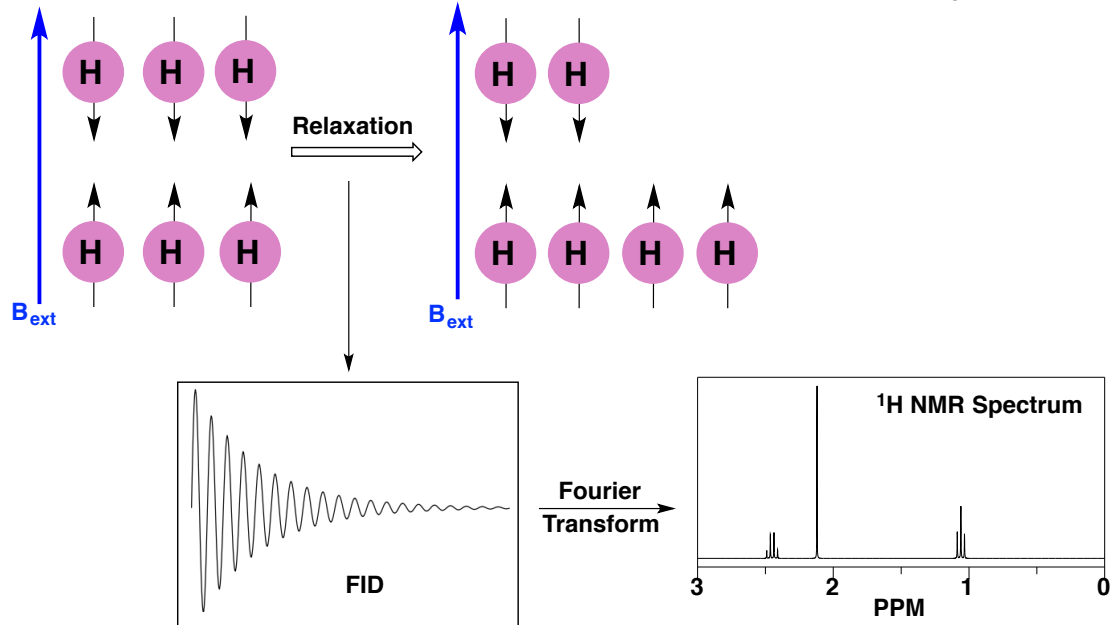

Figure 3. Relaxation and Generation of the NMR Spectrum

Every distinct proton in a molecule will require a specific RF frequency to promote the "spin-flip." Thus, the energy difference between the α and β states for each proton is distinct. Depending on the energy difference (ΔE), the absorption of each proton on the NMR spectrum will appear at different locations.

B. The NMR Instrument

The NMR spectrometer contains a very strong magnet, which generates an extremely strong, uniform magnetic field. The magnet poses no health hazard, however, you should not bring certain items such as watches, credit cards, and cellphones close to it. The NMR instrument in our laboratory is a 400 MHz instrument, which corresponds to a magnetic field of 9.4 Tesla. A higher magnetic field will produce a higher resolution NMR spectrum, however, the instrument cost rises steeply with magnetic field strength.
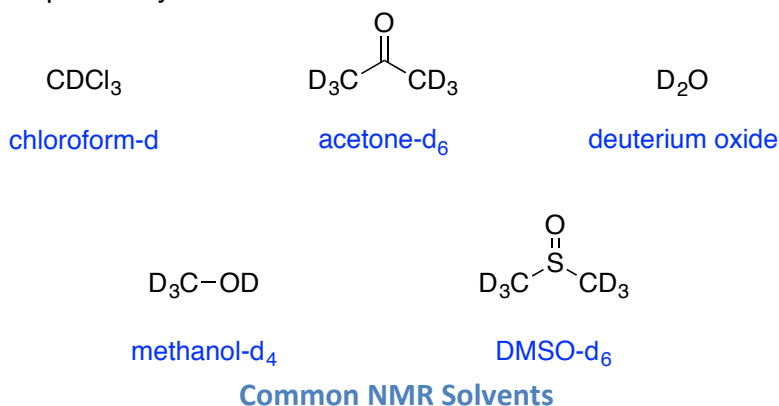
The sample is placed in an NMR tube, which is inserted into the center of the instrument. The tube is spun rapidly to improve homogeneity resulting in a better spectrum. A radio frequency (RF) console is attached to the spectrometer; this console sends an intense pulse of RF energy to the sample. The RF pulse covers a wide enough range to excite all of the protons in the molecule. A computer collects the resulting FID and converts it to the NMR spectrum, which can be printed and analyzed.
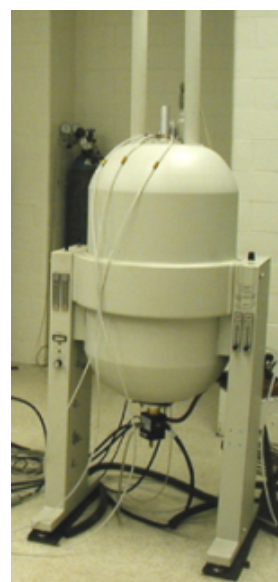
NMR Spectrometer

C. Sample Preparation

NMR samples are typically prepared as a dilute solution in a deuterated solvent. A typical sample size is 5-10 mg of sample dissolved in 0.5 – 1.0 mL of deuterated solvent. Less than 1 mg of sample can be used, however, this will necessitate a longer NMR acquisition time. Deuterated solvents are ones in which all of the hydrogen in the molecule have been replaced with the deuterium isotope. Deuterium does not show up in the range examined for ^{1}H NMR, thus the deuterated solvent is effectively NMR transparent. Some common NMR solvents are shown below. The most commonly used NMR solvent is chloroform-d, where the single hydrogen in chloroform is replaced by deuterium.

$CDCl_3$ D_3C—C(=O)—CD_3 D_2O

chloroform-d   acetone-d_6 deuterium oxide

D_3C—OD D_3C—S(=O)—CD_3

methanol-d_4 DMSO-d_6

Common NMR Solvents

Sometimes, an NMR solvent is spiked with tetramethylsilane (TMS), which provides a reference signal that can be used to calibrate the spectrum. The TMS signal is set at exactly 0 ppm and every other signal is based off of that reference point.

NMR Tube

NMR samples are placed inside a specially designed NMR tube, which is a 5 mm precision glass tube that fits perfectly inside the instrument. Once inside the tube, the diluted sample should have a height of 2-4 cm. NMR tubes are reusable and should be washed out with acetone and left to dry thoroughly before the next use.

Great care should be taken when inserting the sample tube into the spectrometer. If a sample tube breaks inside the instrument, a great deal of time is required to remove the broken glass and to get the instrument back up and running.

D. Interpretation of NMR Spectra

1. Basics of the ^{1}H NMR Spectrum

The interpretation of ^{1}H NMR spectra involves three basic concepts: **chemical shift**, **peak integration**, and **coupling**. Each of these items provides important information regarding the molecular structure. Combined, this information can be used to elucidate the structure of an unknown organic molecule.

Figure 4 shows a sample ^{1}H NMR spectrum. The x-axis is the **chemical shift (δ)** expressed in parts per million (ppm). The typical chemical shift scale for proton NMR is 0-12 ppm. The chemical shift provides information regarding the chemical environment of the corresponding proton. The area under each peak is know as the **integration**. The integration for a signal is

proportional to the number of hydrogen corresponding to that particular signal. Finally, you will notice that some peaks are split (b and c, figure 4). Peak splitting is also known as **coupling**. The splitting for an individual signal provides information as to the number of hydrogen present on the adjacent carbon atoms.
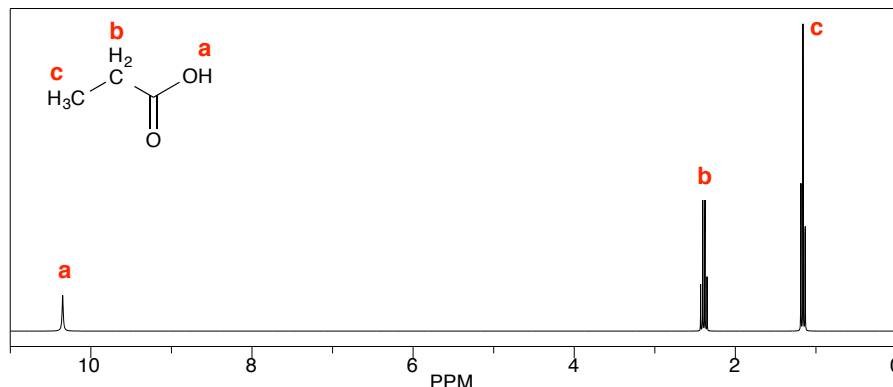

Figure 4. Sample Proton NMR Spectrum

2. Chemically Equivalent and Distinct Hydrogen

Every chemically distinct type of hydrogen in a molecule gives rise to an individual signal in the proton NMR spectrum. This is in contrast to chemically equivalent hydrogen, which cannot be differentiated and will be represented by a single signal. For example, methane (figure 5A) has four protons. These four protons cannot be distinguished and are thus all chemically equivalent. Since there is only one type of hydrogen in methane, only one proton NMR signal will result. In ethanol (figure 5B), there are three distinct types of hydrogen: the OH proton, the CH_2 group, and the CH_3 group. Note that the two protons in the CH_2 group are equivalent and the three protons in the CH_3 group are equivalent. When analyzing a molecule for chemically equivalent or distinct atoms, pay special attention to molecular symmetry. Butane (figure 5D), for example, has a plane of symmetry, thus there are two equivalent ethyl (CH_2CH_3) groups in the molecule.
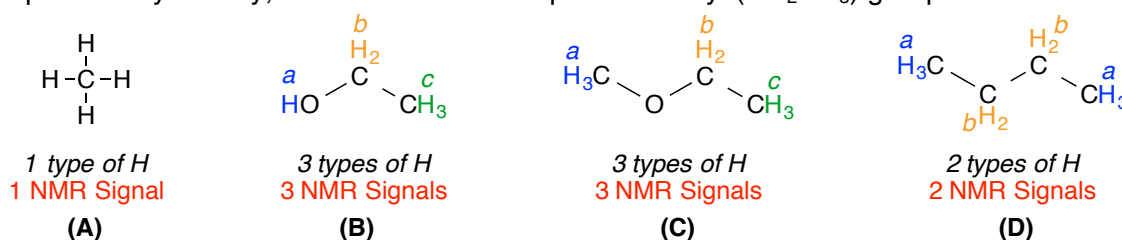

Figure 5. Examples of Chemically Equivalent and Distinct Protons

Chiral Centers and Diastereotopic Protons

In an achiral molecule, the two hydrogen in a methylene -CH_2- group are typically chemically equivalent (see figure 5 B-D). If however, a chiral center (*) is present, the two methylene protons are no longer equivalent and will each have its own distinct NMR signal. In this type of situation, the methylene protons are said to be **diastereotopic**. For example, the molecule in figure 6A does not have a chiral center and the two highlighted hydrogen of the CH_2 group are chemically equivalent. The molecule in figure 6B has a chiral center, which makes the two hydrogen in the CH_2 group diastereotopic and chemically distinct. One special case is that shown in figure 6C where there is not a chiral center, yet the two hydrogen in each methylene group are diastereotopic and chemically distinct. This can be rationalized by thinking three dimensionally. Considering group Z as an "out" group on a wedge, reveals the spatial difference between each methylene hydrogen and Z. One hydrogen is "out" in a closer spatial orientation

to Z, while the other hydrogen is "back" and is further away from Z. These two hydrogen reside in different chemical environments and will have different NMR shifts. Diastereotopic hydrogen give rise to complex NMR signals that can often be difficult to interpret.
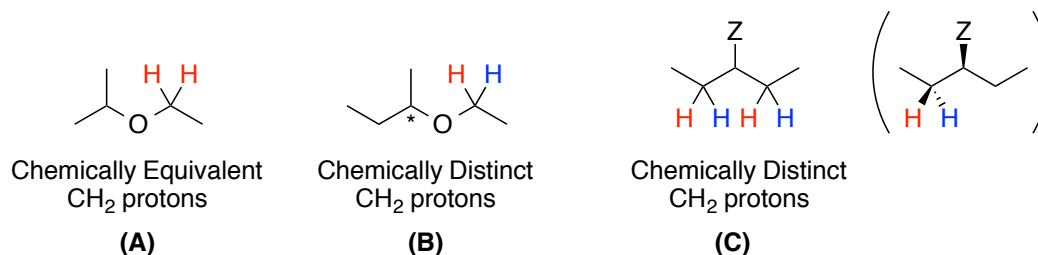

Chemically Equivalent CH_2 protons	Chemically Distinct CH_2 protons	Chemically Distinct CH_2 protons
(A)	(B)	(C)

Figure 6. Examples of Diastereotopic Protons

3. Chemical Shift

The chemical shift of a proton gives us insight into the chemical environment of that proton – i.e. what type of groups are nearby. Chemical shift increases from right to left on the NMR spectrum. The right end of the spectrum is considered **upfieled** and protons in this region are said to be **shielded**. The left end of the spectrum is considered **downfield** and protons in this region are said to be **deshielded**. (Figure 7)
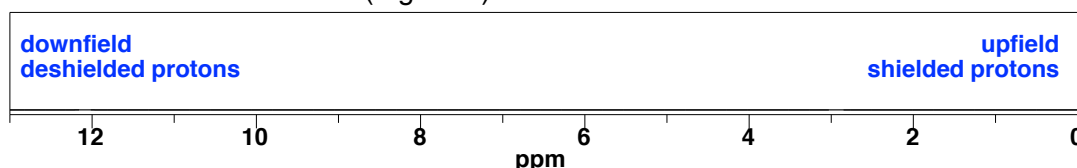

Figure 7. Chemical Shift Terminology

All proton NMR chemical shifts are relative to the signal produced by tetramethylsilane (TMS), which has a chemical shift that is defined at $\delta = 0$ ppm. TMS is often used as an internal standard and the spectrum can be referenced to the chemical shift of this standard.

Chemical shifts are remarkably predictable and knowing a few base values along with some simple trends will allow you analyze a variety of NMR spectra. Some typical proton NMR regions are shown in figure 8.
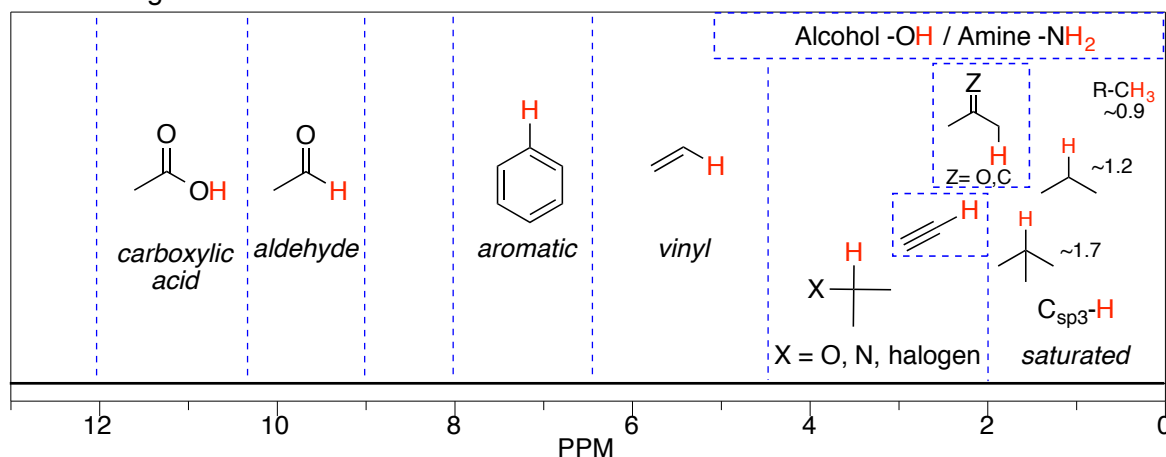

**Figure 8. Typical ^{1}H NMR Chemical Shift Regions**

Chemical Shift Origin

Examination of figure 8 reveals that protons in different chemical environments display NMR resonances (signals) at different chemical shifts. The protons in a molecule are each shielded by the nearby electrons. In the presence of an external magnetic field (B_{ext}) the molecule's electrons produce small local magnetic fields (B_{local}) that opposes the applied field from the instrument. In effect, the proton is *shielded* from the external magnetic field by the opposing local magnetic field. The overall magnetic field experienced by an individual proton is the effective magnetic field ($B_{effective}$) A smaller $B_{effective}$, means that due to shielding, a proton experiences less of the external magnetic field resulting in a lower chemical shift.

$$B_{effective} = B_{ext} - B_{local}$$

Protons in more electron deficient environments are less shielded from the external magnetic field and resonate at higher chemical shifts. Protons at higher chemical shifts are said to be deshielded.
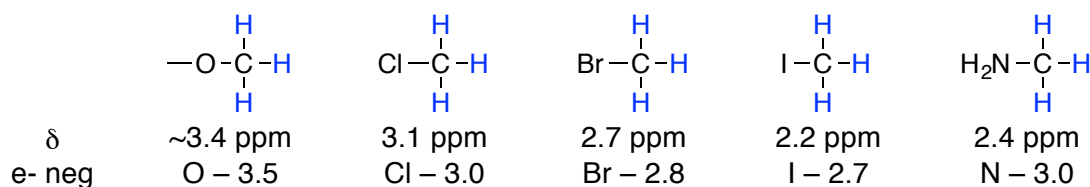
Chemical Shift Predictions

Chemical shift values are typically additive. Starting with a base value, a chemical shift value can be approximated by incrementally adding the chemical shift effect from each attached group. Base values for the methyl, methylene, and methine groups are shown below.

Methyl	Methylene	Methine
~0.9 ppm	~1.2 ppm	~1.7 ppm
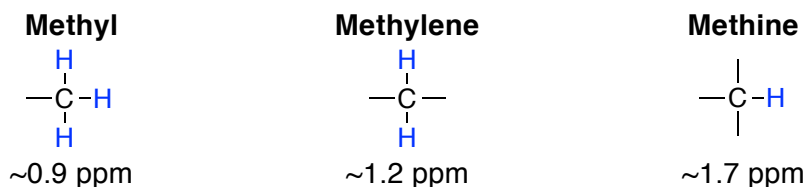
For example, adding an electronegative chlorine increases a proton's chemical shift by ~2.2 ppm. Thus, chloromethane has proton NMR signal at 0.9 + 2.2 = 3.1 ppm. Chloroethane, on the other hand, has a proton NMR signal at 1.2 + 2.2 = 3.4 ppm corresponding to the methylene group.

$Cl-CH_3$ $Cl-\underset{H_2}{C}-CH_3$

3.1 ppm

3.4 ppm
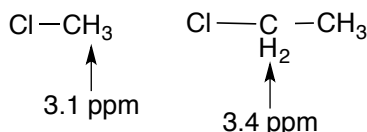
The presence of one or more electronegative atoms will deshield a nearby proton by the inductive effect. The more electronegative the attached atom, the stronger the deshielding effect and the higher the chemical shift. Some representative examples are shown below.

	$-O-CH_2-H$	$Cl-CH_2-H$	$Br-CH_2-H$	$I-CH_2-H$	H_2N-CH_2-H
δ	~3.4 ppm	3.1 ppm	2.7 ppm	2.2 ppm	2.4 ppm
e- neg	O – 3.5	Cl – 3.0	Br – 2.8	I – 2.7	N – 3.0

Additionally, as the number of electronegative atoms near a particular proton increases, the chemical shift also increases.

CH_3CH_3	CH_3Cl	CH_2Cl_2	$CHCl_3$
0.9 ppm	3.1 ppm	5.3 ppm	7.3 ppm

Alcohol and Amine Protons

The OH group of an alcohol and NH/NH_2 group of an amine typically gives a broad singlet in the 0-5 ppm range. In most cases, OH and NH protons are not split by protons on neighboring atoms.

Alkene and Aromatic Protons

Protons with nearby π-electrons, specifically vinyl and aryl protons, have unusually high chemical shifts. This is due to an effect known as magnetic anisotropy. In these systems, the local magnetic field aligns with the external magnetic field, causing the proton to have a larger than expected effective magnetic field.

Vinylic protons typically appear in the 4.5-6.5 ppm region. Terminal alkene protons tend to have a slightly lower chemical shift than an internal alkene proton (4.9 vs 5.4 ppm).

Aryl protons typically appear in the 6.5-8 ppm region. A phenyl group $-C_6H_5$ has three distinct types of protons, however, the signals for these protons frequently overlap and show up as a single line corresponding to 5 hydrogen or a messy grouping of peaks corresponding to 5 hydrogen. When the aromatic ring is di or tri substituted, the aryl proton signals are typically better resolved, especially when one of the substituents is an electronegative atom or electron withdrawing group.

4. Integration

It is important to recognize that the area under an NMR signal is proportional to the number of protons corresponding to that particular signal. Modern NMR spectrometers have the ability to automatically integrate each signal providing relative peak areas with ease.

For example, the integrated NMR spectrum of 2-butanone is shown in figure 9. The area under each integral is proportional to the number of H represented by that particular signal. Methyl group *a*, has three equivalent hydrogen. This is represented in the NMR spectrum by an integration for 3H. The methylene group *b* has two equivalent hydrogen and therefore integrates for 2H. Finally, methyl group *c* has three equivalent hydrogen and integrates for 3H.
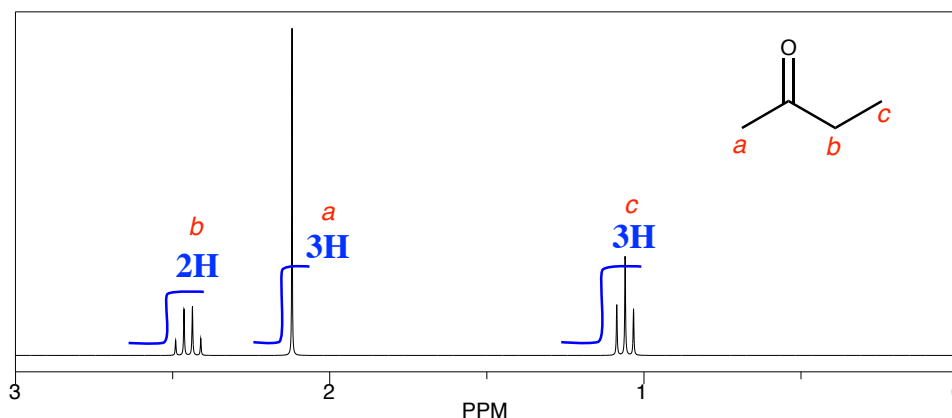

Figure 9. NMR Spectrum of 2-Butanone

In some cases, you will have integration values that need to be normalized based on the smallest integration value. This process is further explored in pre-lab question 4.

5. Spin-Spin Splitting (Coupling)

The signal corresponding to a particular proton will split as a result of the protons on neighboring carbon atoms. Basic splitting follows the **n+1 rule** where *n* is the number of protons on the adjacent carbon.

Looking at the NMR spectrum of 2-butanone (figure 9), you will see that signal **b** is split into four lines and signal **c** is split into three lines. The reason for this observed splitting is due to coupling with the protons on the adjacent carbon atoms. Figure 10 shows 2-butanone along with the observed splitting for each proton in the molecule. The signal corresponding to **a** appears as a single line (singlet). This is because it is not coupled to any other hydrogen. The adjacent carbon does not have any hydrogen for **a** to couple with. The signal corresponding to **b** on the other hand is split into four lines (quartet). This is due to one of the adjacent carbons having three protons attached. Following the *n + 1 rule*, with *n* = 3, *3 + 1* = 4 (quartet). Finally, the signal corresponding to **c** is a triplet because the adjacent carbon has two protons (2 + 1 = 3).
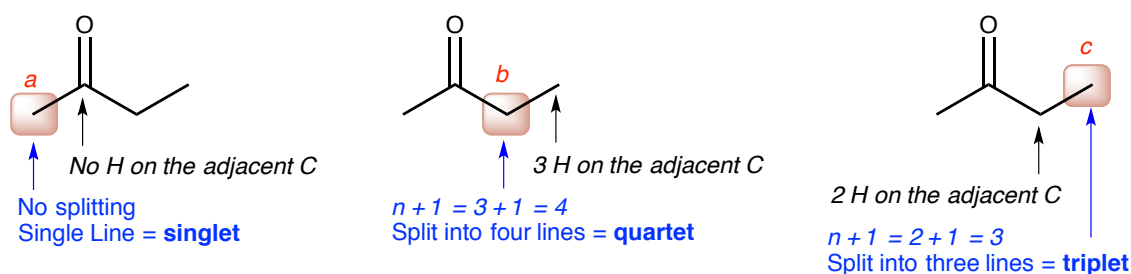

Figure 10. Splitting in 2-Butanone

The most common splitting patterns, also known as multiplicities, are shown in figure 11. Once a peak is split into more than seven lines, it is commonly referred to as a multiplet, m.
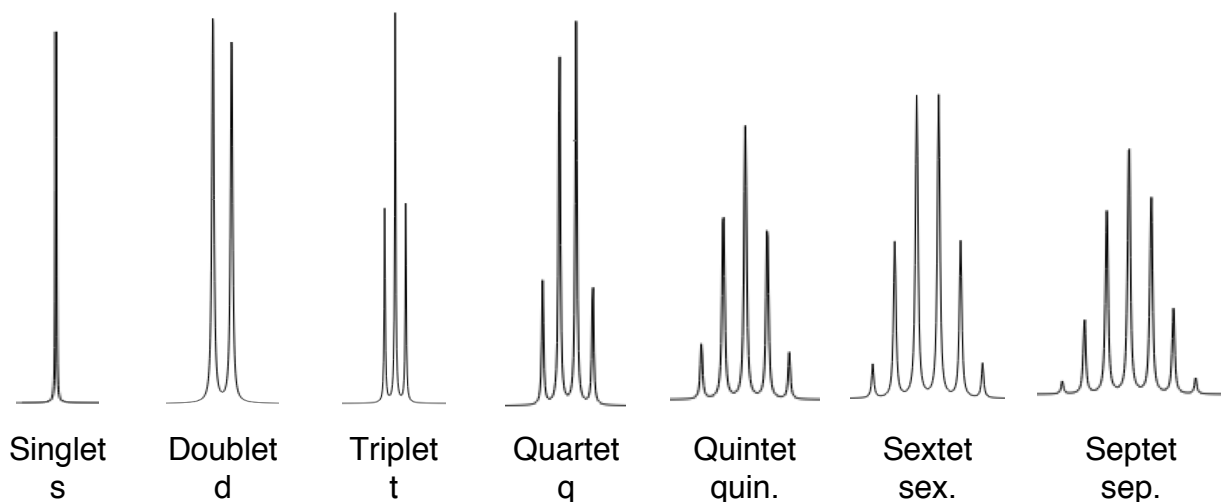

| Singlet | Doublet | Triplet | Quartet | Quintet | Sextet | Septet |
| s | d | t | q | quin. | sex. | sep. |

Figure 11. Common Multiplicities

Complex Splitting

When a proton is coupled to two or more sets of not equivalent hydrogen, the splitting becomes more complex and follows the *(m + 1)(n + 1)* splitting pattern. Consider for example the alkene shown in figure 12a. The carbon highlighted in the green box is coupled with two different types of hydrogen. Coupling with the alkene H, splits the signal into a doublet. Coupling with the CH_2 hydrogens further splits the signal into a triplet providing a doublet of triplets. A splitting tree

(figure 12b) is used to show how the signal splits into a double of triplets. Think of the initial signal first splitting into two (doublet) then each of these two lines further splitting into three lines (triplet). In reality, the peaks often overlap which results in the observation of fewer than expected peaks (figure 12c).
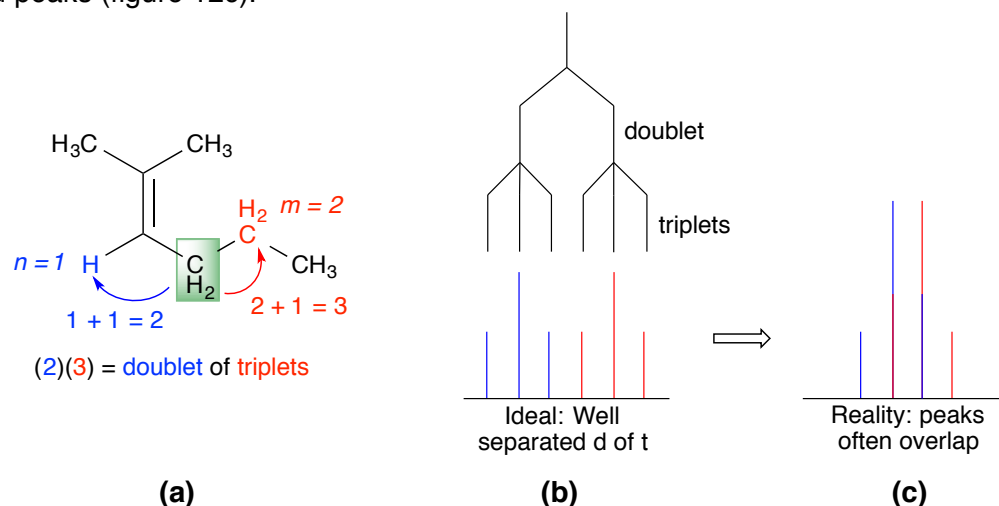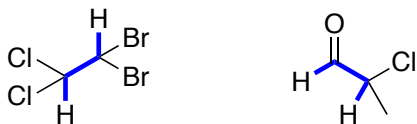

Figure 12: Complex Splitting

Coupling Summary
Several important features regarding coupling are listed below.
1. The most common coupling occurs between non-equivalent protons that are separated by three bonds.


2. Coupling will be observed between protons separated by two bonds if the two protons are chemically distinct.

   Each alkene hydrogen appears as a doublet because they are chemically distinct and coupled to one another.
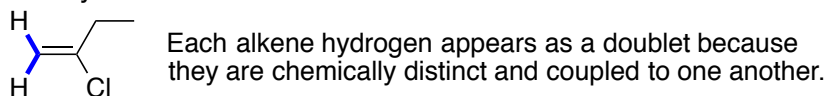
3. Coupling is typically not observed through heteroatoms such as oxygen and nitrogen.
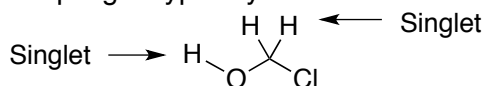
4. Chemically equivalent protons do not couple.

   Both CH_2 groups are chemically equivalent. They show up as a singlet integrating for 4H
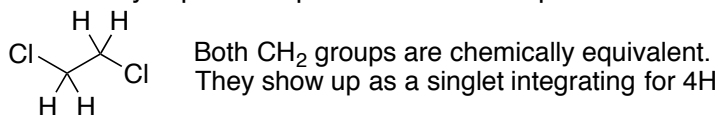
The Coupling Constant (*J – Values*)
The coupling constant (aka *J* - value) is the distance between two peaks in a split signal. If two protons are coupled to one another, they will have identical coupling constants. When analyzing complex spectra, the coupling constant is a convenient piece of data to help determine whether or not two signals are coupled to one another. The coupling constant for a particular signal is calculated by subtracting the chemical shift of two neighboring peaks in a signal and multiplying that number by the frequency of the spectrometer. The NMR below was taken on a 300 MHz instrument. The coupling constant of the quartet at ~3.4 ppm is: (3.48 – 3.45) x 300 = 9 Hz. Now

prove that this quartet is coupled to the triplet by calculating its coupling constant. Tables of common coupling constants can be found in most organic textbooks and online.
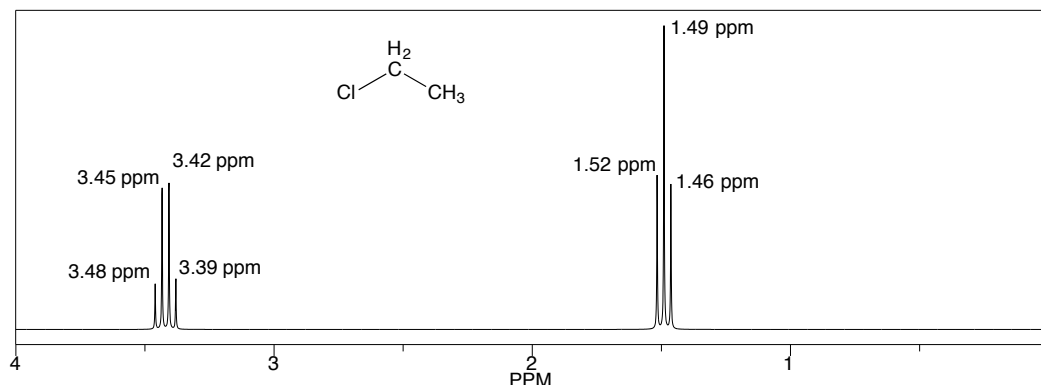

E. Experimental Procedure

A set of unknown IR and NMR spectra for a variety of organic compounds will be available in the laboratory. A list of possible structures will be provided separately. Your TA will assign you two of the numbered unknowns. You will analyze the IR and NMR data and match your spectra to one of the possible structures. In your laboratory notebook, you should identify each of your unknowns. You should include a list of the pertinent IR absorptions and NMR signals along with the identity of the group that each signal corresponds to. You should also include a brief explanation of how you identified your unknown based on piecing together this data.
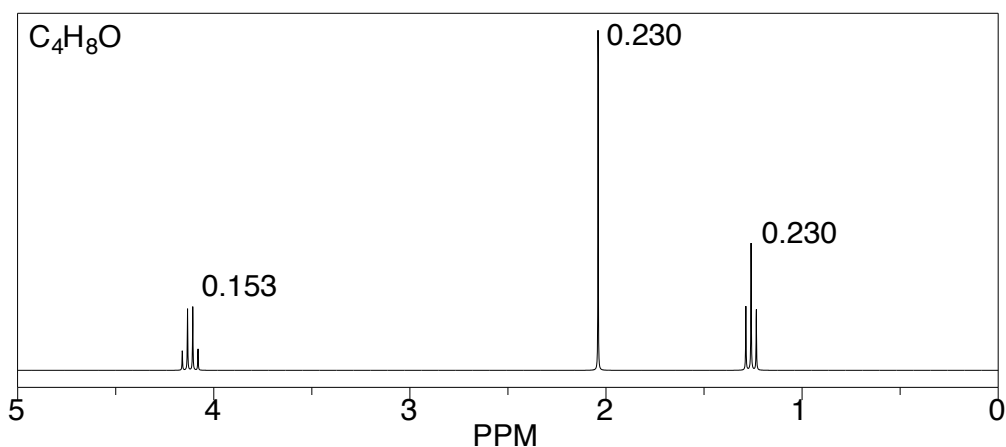
F. Pre-Lab Questions

1. The structure of morphine is shown below. How many chemically distinct types of hydrogen are present in this molecule?
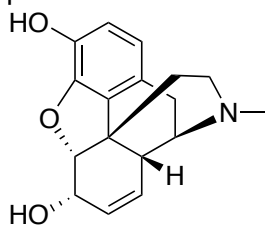

2. Predict the expected multiplicity (singlet, doublet, etc.) for every distinct set of hydrogen in each molecule below.
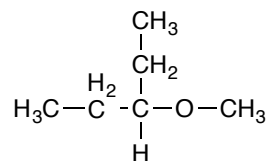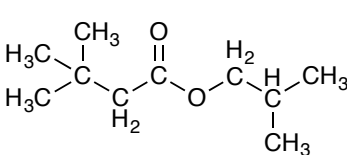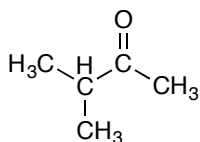
3. Consider the NMR spectrum shown below. The integration values are not normalized. Normalize each integration by dividing each integration value by the lowest integration value. Do your values add up for the correct number of hydrogen? If not, multiply each value by a common number to get the correct total number of hydrogen represented.

C₄H₈O ... (NMR spectrum with peaks labeled 0.230, 0.230, 0.153)

C$_4$H$_8$O

0.230

0.230

0.153

PPM

4. Calculate the coupling constant *(J)* for the NMR signal at δ 6.4 ppm shown below. A 500 MHz instrument was used to record the spectrum. You will find that this is a typical coupling constant for *trans*-alkenes.

6.42   6.39

PPM
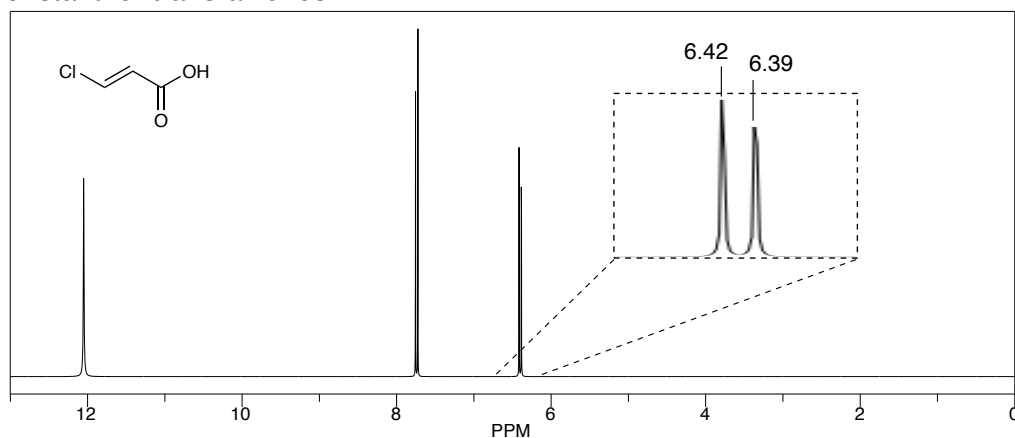
G. Post-Lab Questions

1. A careless student mistakenly dissolved his sample in non-deuterated chloroform (CHCl$_3$). What do you think resulted in the corresponding NMR spectrum that he recorded?

2. For each compound shown below, predict the expected IR and NMR data. Record the NMR data in the form (chemical shift, multiplicity, integration). You chemical shift values and IR absorption values only need to be a rough approximation. The first molecule is already assigned to provide an example.

4.0 ppm, q, 2H

1.4 ppm, t, 2H

0.9 ppm, s, 9H

1.2 ppm, t, 3H

2.3 ppm, t, 2H
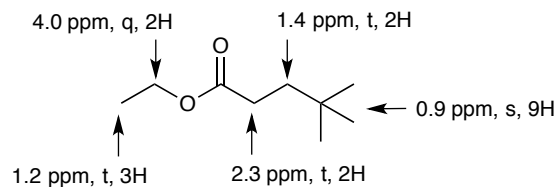
IR Data: C=O, 1740 cm⁻¹
Csp³-H, 2930-2990 cm⁻¹
Csp²-O, 1250 cm⁻¹
Csp³-O, 1050 cm⁻¹