Nuclear Magnetic Resonance

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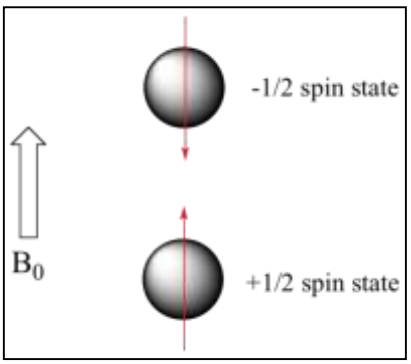
1. NMR-Active nuclei

The basis for nuclear magnetic resonance is the observation that many atomic nuclei spin about an axis and generate their own magnetic field, or **magnetic moment (Induced field)**. But only those nuclei with an odd number of protons and/or neutrons have a magnetic moment. Fortunately for chemists, several common nuclei, including 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 are essentially invisible in NMR. Other nuclei such as deuterium (^{2}H) and nitrogen (^{14}N) have magnetic moments and are NMR-active, but the nature of their magnetic moments is such that these nuclei are more difficult to analyze by NMR. In practice it is **^{1}H, ^{13}C, ^{19}F, and ^{31}P that are most often** observed by NMR spectroscopy.

In this chapter, we will develop our understanding of the principles behind NMR spectroscopy by focusing our attention first on the detection of protons in 1H-NMR experiments (in discussion about NMR, the terms 'hydrogen' and 'proton' are used interchangeably). Much of what we learn, however, will also apply to the detection and analysis of other NMR-active nuclei, and later in the chapter we will shift our attention to NMR experiments involving 13C atoms.

2. Nuclear precession, spin states, and the resonance condition



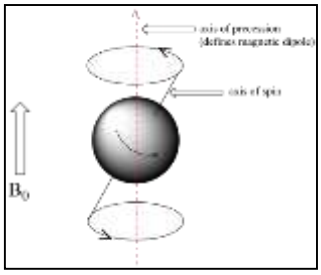
When a sample of an organic compound is sitting in a flask on a laboratory benchtop, the magnetic moments of its hydrogen atoms are randomly oriented. When the same sample is placed within the field of a very strong magnet in an NMR instrument (this field is referred to by NMR spectroscopists as the **applied field**, abbreviated **B0**) each hydrogen will assume one of two possible **spin states**. In what is referred to as the +½ spin state, the hydrogen's magnetic moment is aligned *with* the direction of B0, while in the -½ spin state it is aligned *opposed to* the direction of B0.

Because the +½ spin state is slightly lower in energy, in a large population of organic molecules slightly more than half of the hydrogen atoms will occupy this state, while slightly less than half will occupy the –½ state. *The difference in energy between the two spin states increases with increasing strength of B0.* This last statement is in italics because it is one of the key ideas in NMR spectroscopy, as we shall soon see.

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 B0) that defines the proton's magnetic moment. In the figure below, the proton is in the +1/2 spin state.

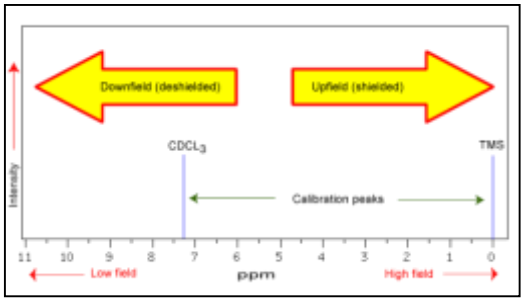
The **frequency of precession** (also called the **Larmour frequency** abbreviated **ωL**) is simply the number of times per second that the proton precesses in a complete circle. A proton`s precessional frequency increases with the strength of B0.



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 ωL, we have a condition called **resonance**. *In the resonance condition, a proton in the lower-energy +½ spin state (aligned with B0) will transition (flip) to the higher energy –½ spin state (opposed to B0). In doing so, it will absorb radiation at this resonance frequency* **ν** = **ω**L. This frequency, as you might have already guessed, corresponds to 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.

3. Diamagnetic shielding and deshielding – Chemical Shift

We come now to the question of *why* nonequivalent protons have different chemical shifts. The chemical shift of a given proton is determined primarily by its immediate electronic environment. Consider the methane molecule (CH4), in which the protons have a chemical shift of 0.23 ppm. The valence electrons around the methyl carbon, when subjected to B0, are induced to circulate and thus generate their own very small magnetic field that *opposes* B0. This **induced field**, to a small but significant degree, *shields* the nearby protons from experiencing the full force of B0, an effect known as **local diamagnetic shielding**. The methane protons therefore do not experience the full force of B0 - what they experience is called Beff, or the **effective field**, which is slightly weaker than B0.



Therefore, their resonance frequency is slightly lower than what it would be if they did not have electrons nearby to shield them.

The position on the plot at which the nuclei absorbs is called the chemical shift. Since this has an arbitrary value a standard reference point must be used. The two most common standards are TMS (tetramethylsilane, (Si(CH3)4) which has been assigned a

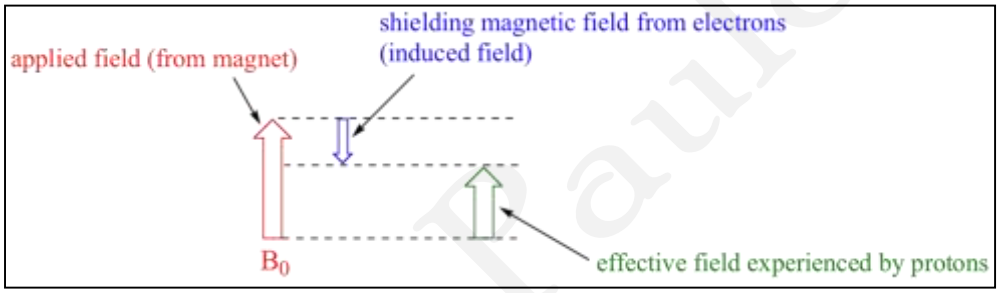
chemical shift of zero, and $CDCl_3$ (deuterochloroform) which has a chemical shift of 7.26 for 1H NMR and 77 for 13C NMR.

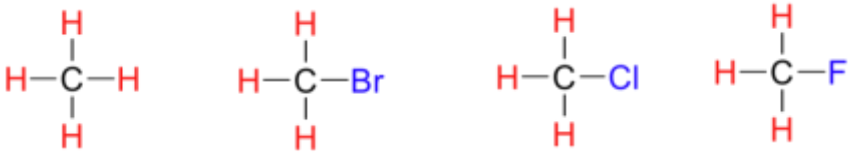
The scale is commonly expressed as parts per million (ppm) which is independent of the spectrometer frequency. The scale is the delta (δ) scale.

$$\delta = \frac{\text{frequency of signal - frequency of standard}}{\text{spectrometer frequency}} \times 10^6$$

The range at which most NMR absorptions occur is quite narrow. Almost all 1H absorptions occur downfield within 10 ppm of TMS. For 13C NMR almost all absorptions occurs within 220 ppm downfield of the C atom in TMS.

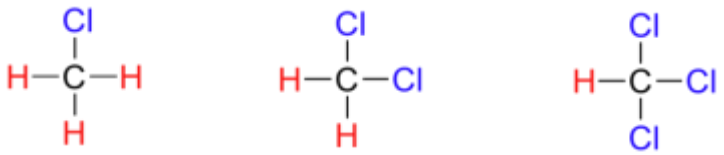
Now consider methyl fluoride, CH_3F, in which the protons have a chemical shift of 4.26 ppm, significantly higher than that of methane. This is caused by something called the deshielding effect. Because fluorine is more electronegative than carbon, it pulls valence electrons away from the carbon, effectively decreasing the electron density around each of the protons. For the protons, lower electron density means less diamagnetic shielding, which in turn means a greater overall exposure to B0, a stronger Beff, 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 B0. 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).





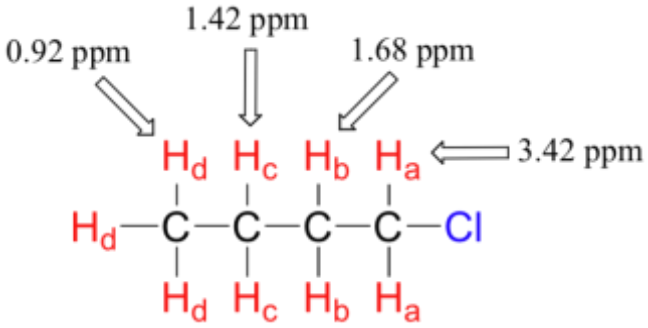
0.23 ppm | 2.68 ppm | 3.05 ppm | 4.26 ppm

To a large extent, then, we can predict trends in chemical shift by considering how much deshielding is taking place near a proton. The chemical shift of trichloromethane is, as expected, higher than that of dichloromethane, which is in turn higher than that of chloromethane.

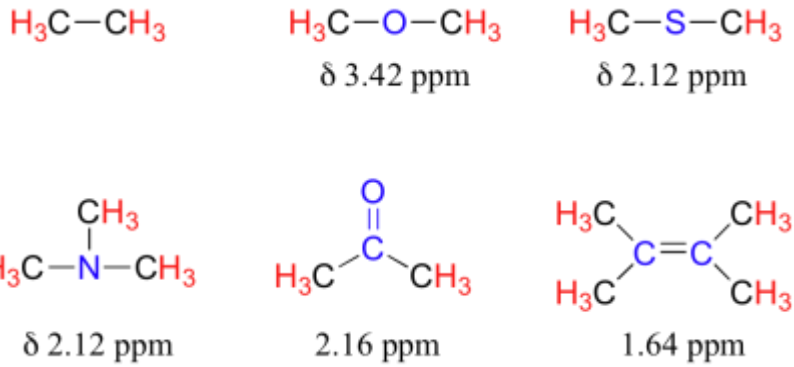


3.05 ppm | 5.30 ppm | 7.27 ppm

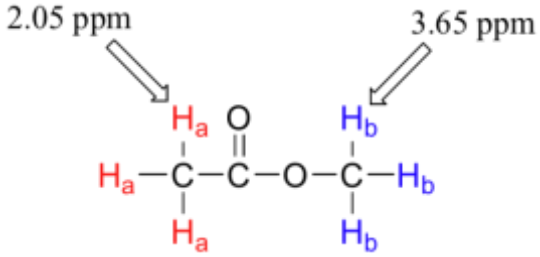
The deshielding effect of an electronegative substituent diminishes sharply with increasing distance:



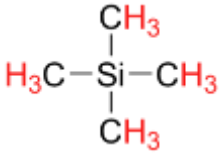
The presence of an electronegative oxygen, nitrogen, sulfur, or sp2-hybridized carbon also tends to shift the NMR signals of nearby protons slightly downfield:



Armed with this information, we can finally assign the two peaks in the the 1H-NMR spectrum of methyl acetate that we saw a few pages back. The signal at 3.65 ppm corresponds to the methyl ester protons (Hb), which are deshielded by the adjacent oxygen atom. The upfield signal at 2.05 ppm corresponds to the acetate protons (Ha), which is deshielded - but to a lesser extent - by the adjacent carbonyl group.



Finally, a note on the use of TMS as a standard in NMR spectroscopy: one of the main reasons why the TMS proton signal was chosen as a zero-point is that the TMS protons are highly shielded: silicon is slightly *less* electronegative than carbon, and therefore *donates* some additional shielding electron density. Very few organic molecules contain protons with chemical shifts that are negative relative to TMS.



3.1. Factors Affecting Chemical Shift

3.1.1 Electronegativity and Inductive Effect

The electrons that surround the nucleus are in motion so they created their own electromagnetic field. This field opposes the the applied magnetic field and so reduces the field experienced by the nucleus. Thus the electrons are said to **shield** the nucleus. Since the magnetic field experienced at the nucleus defines the energy difference between spin states it also defines what the chemical shift will be for that nucleus. Electron with-drawing groups can decrease the electron density at the nucleus, deshielding the nucleus and result in a larger chemical shift. Compare the data in the table below.

Compound, CH_3X	**CH_3F**	**CH_3OH**	**CH_3Cl**	**CH_3Br**	**CH_3I**	**CH_4**	**$(CH_3)_4Si$**
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift δ (ppm)	4.26	3.4	3.05	2.68	2.16	0.23	0

As can be seen from the data, as the electronegativity of X increases the chemical shift, δ increases. This is an effect of the halide atom pulling the electron density away from the methyl group. This exposes the nuclei of both the C and H atoms, "deshielding" the nuclei and shifting the peak downfield.

These **inductive effects** are not only felt by the immediately adjacent atoms, but the deshielding can occur further down the chain, i.e.

NMR signal	$-CH_2-CH_2-CH_2Br$
δ (ppm)	1.25 1.69 3.30

3.2.1 Magnetic Anisotropy: π Electron Effects

The word "anisotropic" means "non-uniform". So magnetic anisotropy means that there is a "non-uniform magnetic field". Electrons in π systems (e.g. aromatics, alkenes, alkynes, carbonyls etc.) interact with the applied field which induces a magnetic field that causes the anisotropy. As a result, the nearby protons will experience 3 fields: the applied field, the shielding field of the valence electrons and the field due to the π system. Depending on the position of the proton in this third field, it can be either shielded (smaller d) or deshielded (larger d), which implies that the energy required for, and the frequency of the absorption will change.

Anisotropy: Benzene and many other aromatic structures, a sextet of pi-lectrons is delocalized around the ring. When the molecule is exposed to B_0, these pi-lectrons 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 pi-lectrons does not shield the benzylic 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.

The **18 π-electron bridged annulene** is an **aromatic (4n + 2) system**, and has the same anisotropy as benzene. Nuclei located over the face of the ring are shielded, and those on the periphery are deshielded. The **ring hydrogens** give resonance signals in the range **8.0 to 8.7 δ**, as expected from their **deshielded location** (note that there are three structurally different hydrogens on the ring). But hydrogen on the **periphery give** resonance signals in the range **-1.8 to 1.9 δ.** The negative chemical shifts noted here indicate that the resonances occurs at a higher field than the TMS reference signal.

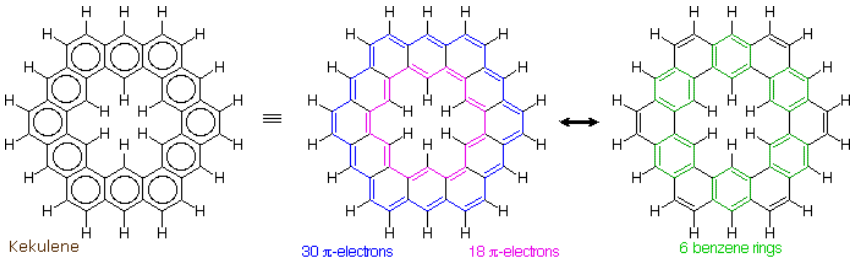
A remarkable characteristic of annulenes is that **antiaromatic 4n π-electron (16)** systems are **anisotropic in the opposite sense as their aromatic counterparts**. A dramatic illustration of this fact is provided by the dianion derivative of the above bridged annulene. In the NMR spectrum, the **ring hydrogens resonate at high field** (they are shielded), and the hydrogens of the **periphery** are all shifted **downfield** (deshielded).

Compounds in which two or more benzene rings are fused together include examples such as naphthalene, anthracene and phenanthrene, shown in the following diagram, present interesting insights into aromaticity and reactivity. The resonance stabilization of these compounds, calculated from heats of hydrogenation or combustion, is given beneath each structure.

Unlike benzene, the structures of these compounds show measurable double bond localization, which is reflected in their increased reactivity both in substitution and addition reactions. However, the ^{1}HNMR spectra of these aromatic hydrocarbons do not provide much insight into the distribution of their pi-electrons. As expected, naphthalene displays two equally intense signals at δ 7.46 & 7.83 ppm. Likewise, anthracene shows three signals, two equal intensity multiplets at δ 7.44 & 7.98 ppm and a signal half as intense at δ 8.4 ppm. Thus, the influence of double bond localization or competition between benzene and higher annulene stabilization cannot be discerned.

The much larger $C_{48}H_{24}$ fused benzene ring cycle, named "kekulene" by Heinz Staab and sometimes called "superbenzene" by others, serves to probe the relative importance of benzenoid

versus annulenoid aromaticity. A generic structure of this remarkable compound is drawn on the left below, together with two representative Kekule contributing structures on its right. There are some 200 Kekule structures that can be drawn for kekulene, but these two canonical forms represent extremes in aromaticity. The central formula has two [4n+2] annulenes, an inner [18]annulene and an outer [30]annulene (colored pink and blue respectively). The formula on the right has six benzene rings (colored green) joined in a ring by meta bonds, and held in a planar configuration by six cis-double bond bridges.



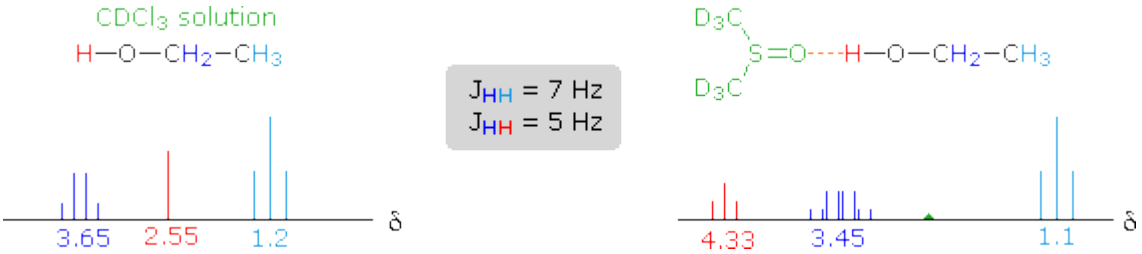
The coupled annulene contributor in the center has an energetically equivalent canonical form in which the single and double bonds making up the annulenes are exchanged. If these contributors dominate the aromatic character of kekulene, the 6 inside hydrogens should be shielded by the ring currents, and the 18 hydrogens on the periphery should be deshielded. Furthermore, the C:C bonds composing each annulene ring should have roughly equal lengths. If the benzene contributor on the right (and its equivalent Kekule form) dominate the aromaticity of kekulene, all the benzene hydrogens will be deshielded, and the six double bond links on the periphery will have bond lengths characteristic of fixed single and double bonds

3.3.1 Hydrogen Bonding

Hydrogen bonding of hydroxyl and amino groups not only causes large variations in the chemical shift of the proton of the hydrogen bond, but also influences its coupling with adjacent C-H groups. As shown on the right, the 60 MHz proton NMR spectrum of pure (neat) methanol exhibits two signals, as expected. At 30° C these signals are sharp singlets located at δ 3.35 and 4.80 ppm, the higher-field methyl signal (magenta) being three times as strong as the OH signal (orange) at lower field. When cooled to -45 ° C, the larger higher-field signal changes to a doublet (J = 5.2 Hz) having the same chemical shift. The smaller signal moves downfield to δ 5.5 ppm and splits into a quartet (J = 5.2 Hz). The relative intensities of the two groups of signals remains unchanged. This interesting change in the NMR spectrum, which will be illustrated by clicking the "Cool the Sample" button, is due to increased stability of hydrogen bonded species at lower temperature. **Since hydrogen bonding not only causes a resonance shift to lower field, but also decreases the rate of intermolecular proton exchange, the hydroxyl proton remains bonded to the alkoxy group for a sufficient time to exert its spin coupling influence.**

The solvent effect shown above suggests a useful diagnostic procedure for characterizing the OH resonance signals from alcohol samples. For example, a solution of ethanol in chloroform-d displays the spectrum shown on the left below, especially if traces of HCl are present (otherwise broadening of the OH and CH_2 signals occurs). Note that the chemical shift of the OH signal (red) is less than that of the methylene group (blue), and no coupling of the OH proton is apparent. The vicinal coupling (J = 7 Hz) of the methyl and methylene hydrogens is typical of ethyl groups. In DMSO-d_6 solution small changes of chemical shift are seen for the methyl and methylene group hydrogens, but a dramatic downfield shift of the hydroxyl signal takes place because of hydrogen

bonding. Coupling of the OH proton to the adjacent methylene group is evident, and both the coupling constants can be measured. Because the coupling constants are different, the methylene signal pattern is an overlapping doublet of quartets (eight distinct lines) rather than a quintet. Note that residual hydrogens in the solvent give a small broad signal near δ 2.5 ppm.



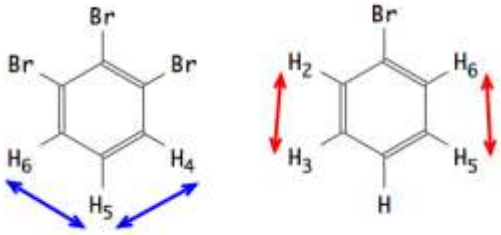
For many alcohols in dilute chloroform-d solution, the hydroxyl resonance signal is often broad and obscured by other signals in the δ 1.5 to 3.0 region. The simple technique of using DMSO-d_6 as a solvent, not only shifts this signal to a lower field, but permits 1°-, 2 °- & 3 °-alcohols to be distinguished. Thus, the hydroxyl proton of 2-propanol generates a doublet at δ 4.35 ppm, and the corresponding signal from 2-methyl-2-propanol is a singlet at δ 4.2 ppm. The more acidic OH protons of phenols are similarly shifted – from δ 4 to 7 in chloroform-d to δ 8.5 to 9.5 in DMSO-d

3.2. Magnetically and Chemically Equivalent

When two equivalent nuclei have identical relations with the same identical partners, they are known as "magnetically equivalent". Only in this case it's possible to define them as a group and not individually.

Two nuclei are magnetically equivalent when they have:

- ✓ **The same chemical shift**
- ✓ **The same coupling constants...**
- ✓ **Interact with the same partners !**

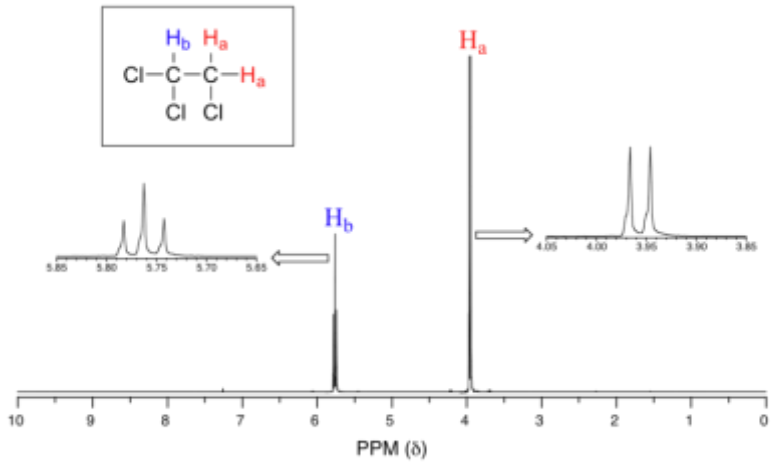


At left, H4 and H6 are magnetically equivalent, because they are both coupled with H5 and with no other. At right, H2 and H6 are related by symmetry, therefore they have the same chemical shift and the same coupling constants. Their partners, however, are different: H2 is coupled with H3, while H6 is not (or not with the same intensity). In conclusion. H2 and H6 are NOT magnetically equivalent since the third assumption is not valid but chemically equivalent.

4. Spin-Spin Coupling

^{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 actually provides us with more information about our sample molecule.

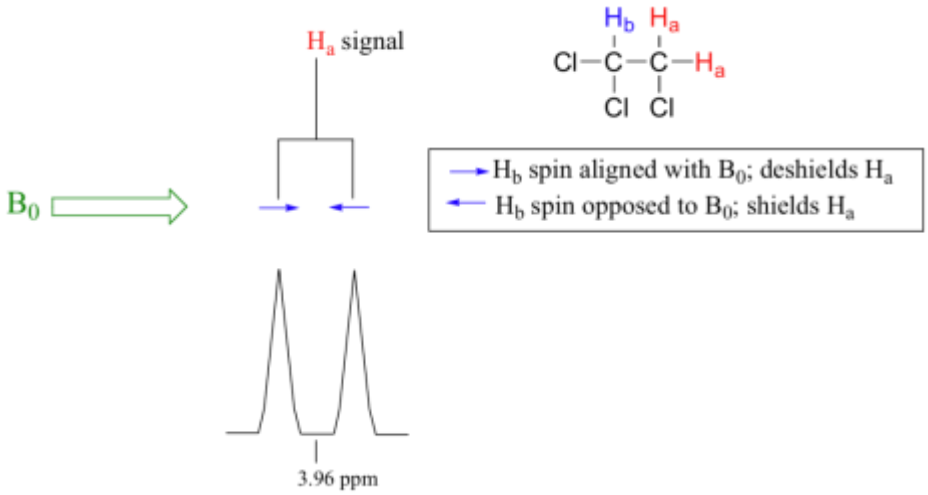
Consider the spectrum for **1, 1, 2-trichloroethane.** In this and in many spectra to follow, we show enlargements of individual signals so that the signal splitting patterns are recognizable.



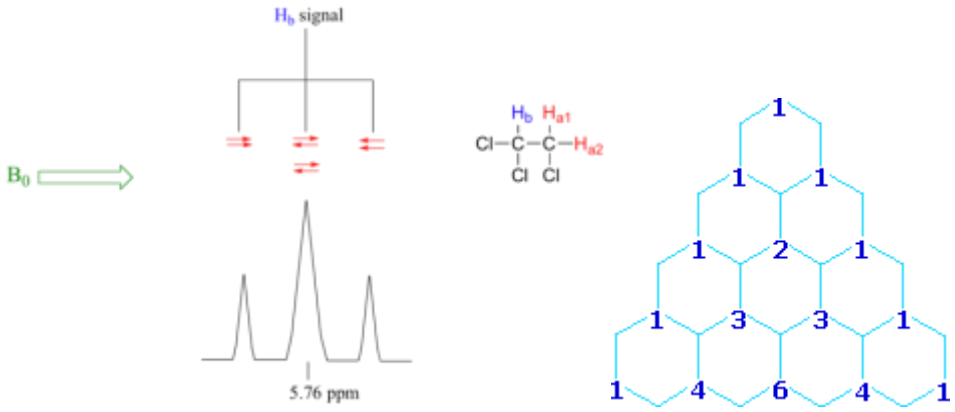
The signal at **3.96 ppm**, corresponding to the two Ha protons, is split into two subpeaks of equal height (and area) – this is referred to as a **doublet.** The H_b signal at **5.76 ppm**, on the other hand, is split into three sub-peaks, with the middle peak higher than the two outside peaks - if 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**. 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 Ha signal: in addition to being shielded by nearby valence electrons, each of the Ha 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 half of the molecules it will be opposed to B0. The Beff 'felt' by Ha is a slightly weaker if Hb is aligned against B_0, or slightly stronger if H_b is aligned with B_0. In other words, in half of the molecules Ha is shielded by H_b (thus the NMR signal is shifted slightly upfield) and in the other half Ha is deshielded by H_b (and the NMR signal shifted slightly downfield). What would otherwise be a single Ha peak has been split into two sub-peaks (a doublet), one upfield and one downfield of the original signal. These ideas an be illustrated by a splitting diagram, as shown below.



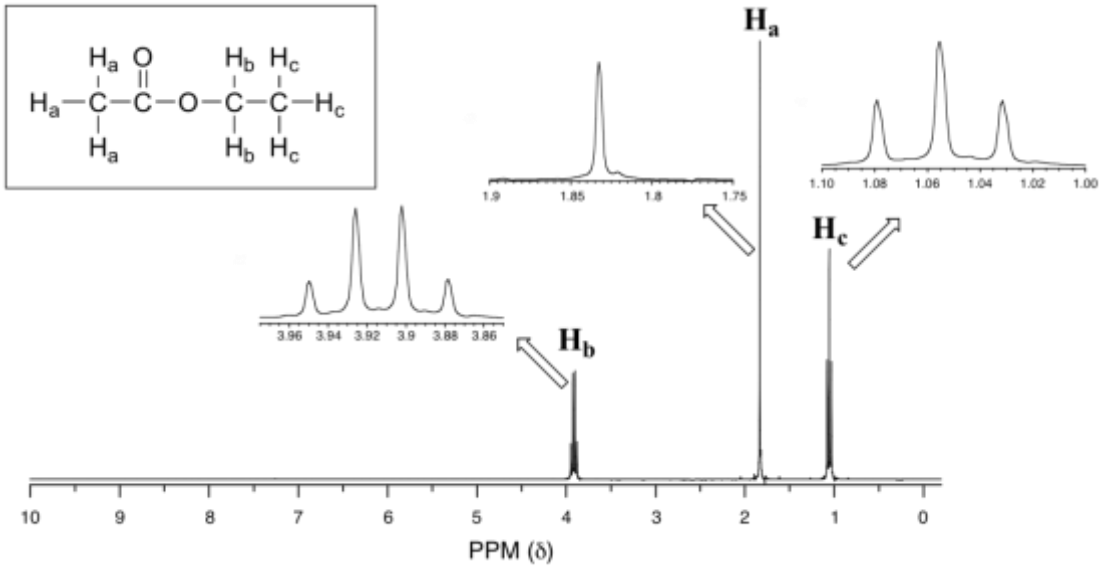
Now, let's think about the H_b signal. The magnetic environment experienced by H_b is influenced by the fields of both neighboring Ha protons, which we will call Ha1 and Ha2. There are four possibilities here, each of which is equally probable. First, the magnetic fields of both Ha1 and Ha2 could be aligned with B0, which would deshield Hb, shifting its NMR signal slightly downfield. Second, both the Ha1 and Ha2 magnetic fields could be aligned opposed to B_0, which would shield H_b, shifting its resonance signal slightly upfield. Third and fourth, Ha1 could be with B0 and Ha2 opposed, or Ha1opposed to B_0 and Ha2 with B_0. In each of the last two cases, the shielding effect of one Ha proton would cancel the deshielding effect of the other, and the chemical shift of H_b would be unchanged.



So in the end, the signal for H_b is a **triplet**, with the middle peak twice as large as the two outer peaks because there are two ways that Ha1 and Ha2 can cancel each other out.

Now, consider the spectrum for **ethyl acetate:**

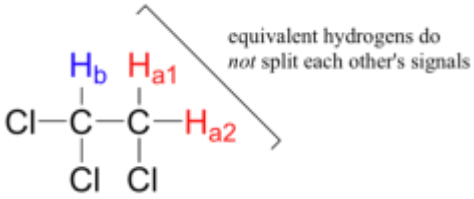
We see an unsplit 'singlet' peak at **1.833 ppm** that corresponds to the acetyl (Ha) hydrogens – this is similar to the signal for the acetate hydrogens in methyl acetate that we considered earlier. **This signal is unsplit because there are no adjacent hydrogens on the molecule**. The signal at 1.055 ppm for the Hc hydrogens is split into a triplet by the two H_b hydrogens next door. The explanation here is the same as the explanation for the triplet peak we saw previously for 1,1,2-trichloroethane.



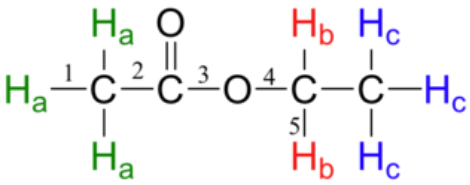
The Hb hydrogens give rise to a **quartet** signal at **3.915 ppm** – notice that the two middle peaks are taller then the two outside peaks. This splitting pattern results from the spin-coupling effect of the three Hc hydrogens next door, and can be explained by an analysis similar to that which we used to explain the doublet and triplet patterns.

By now, you probably have recognized the pattern which is usually referred to as **the *n* + 1 rule: if a set of hydrogens has *n* neighboring, non-equivalent hydrogens, it will be split into *n* + 1 subpeaks.** Thus the two Hb hydrogens in ethyl acetate split the Hc signal into a triplet, and the three Hc hydrogens split the Hb signal into a quartet. 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 hydrogen or set of hydrogens 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.

Three important points need to be emphasized here. First, signal splitting only occurs between non-equivalent hydrogens – in other words, Ha1 in 1,1,2-trichloroethane is *not* split by Ha2, and vice-versa.



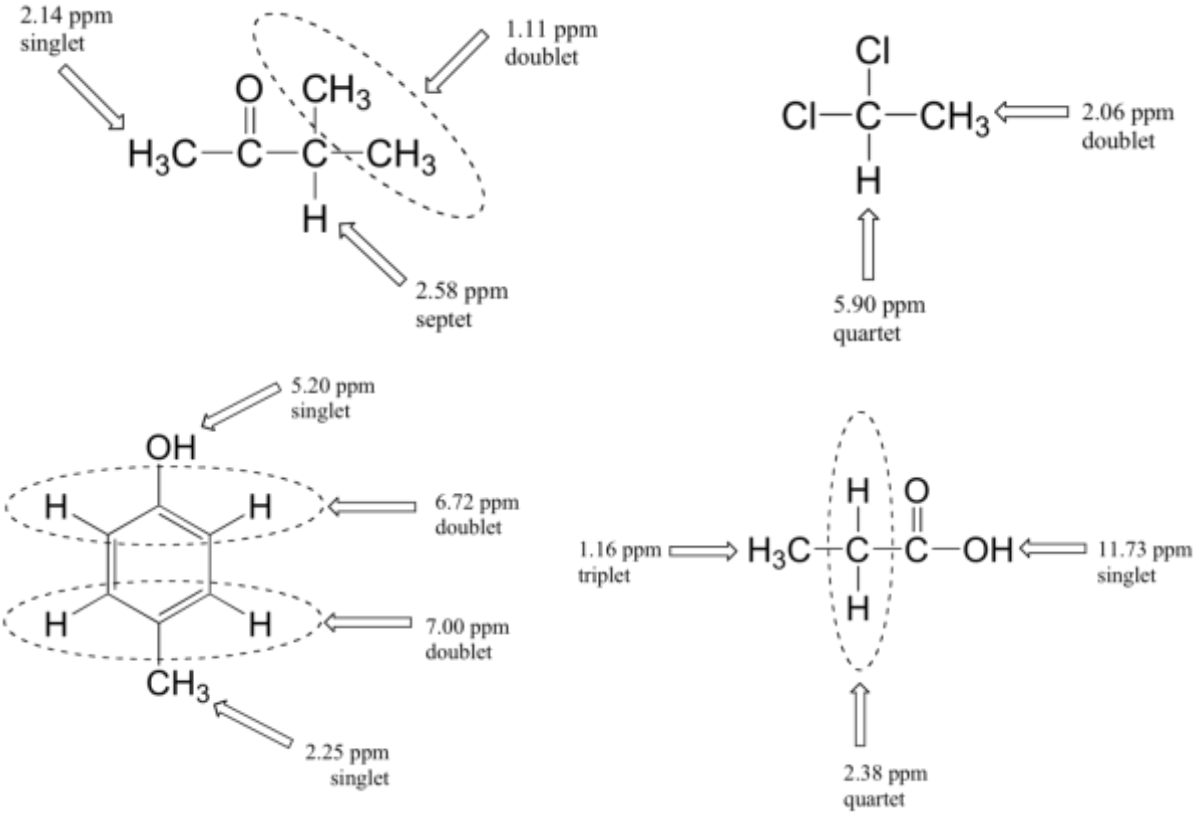
Second, splitting occurs primarily between hydrogens that are separated by three bonds. This is why the Ha hydrogens in ethyl acetate form a singlet– the nearest hydrogen neighbors are five bonds away, too far for coupling to occur.



H_a protons are 5 bonds away from H_b - no coupling

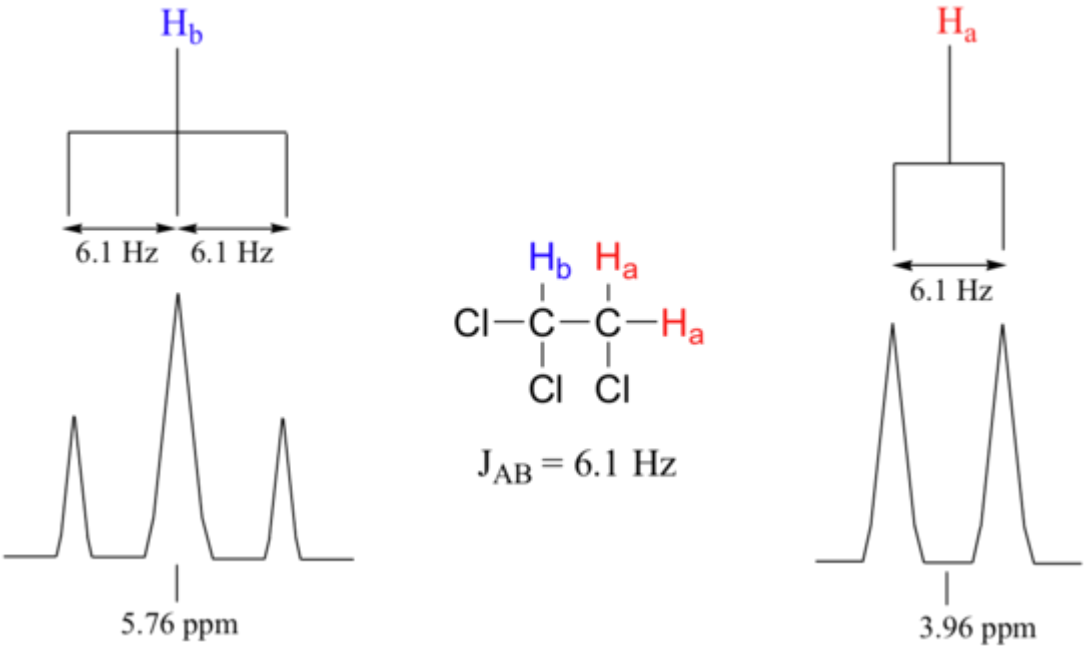
Occasionally we will see four-bond and even 5-bond splitting, but in these cases the magnetic influence of one set of hydrogens on the other set is much more subtle than what we typically see in three-bond splitting. Finally, splitting is most noticeable with hydrogens bonded to carbon. Hydrogens that are bonded to heteroatoms (alcohol or amino hydrogens, for example) are coupled weakly - or not at all - to their neighbors. This has to do with the fact that these protons exchange rapidly with solvent or other sample molecules.

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



3.4.1 Coupling constants

Chemists quantify the spin-spin coupling effect using something called the **coupling constant,** which is 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. For our doublet in the 1,1,2-trichloroethane spectrum, for example, the two subpeaks are separated by 6.1 Hz, and thus we write $^{3}J_{a\text{-}b} = 6.1$ Hz.



The superscript 3 tells us that this is a three-bond coupling interaction, and the a-b subscript tells us that we are talking about coupling between Ha and Hb. Unlike the

chemical shift value, the coupling constant, expressed in Hz, is the same **regardless of the applied field strength of the NMR magnet. This is because 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.**

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. This is an important concept! The coupling constant $^3J_{a\text{-}b}$ quantifies the magnetic interaction between the Ha and Hb hydrogen sets, and this interaction is of the same magnitude in either direction. In other words, Ha influences Hb to the same extent that Hb influences Ha. When looking at more complex NMR spectra, this idea of reciprocal coupling constants can be very helpful in identifying the coupling relationships between proton sets.

Coupling constants between proton sets on neighboring sp^3-hybridized carbons is typically in the region of 6-8 Hz. With protons bound to sp^2-hybridized carbons, coupling constants can range from 0 Hz (no coupling at all) to 18 Hz, depending on the bonding arrangement.

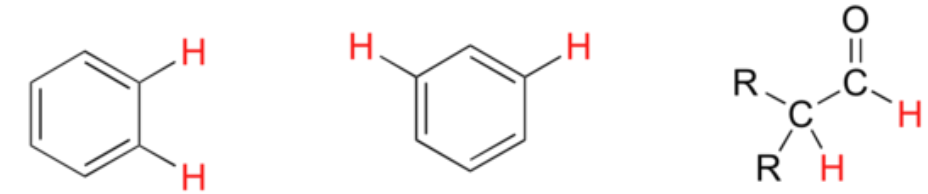
^3J = 6-8 Hz

vinylic *trans*:
^3J = 11-18 Hz

vinylic *cis*:
^3J = 6-15 Hz

vinylic *gem*:
^2J = 0-5 Hz

For vinylic hydrogens in a *trans* configuration, we see coupling constants in the range of ^3J = 11-18 Hz, while *cis* hydrogens couple in the ^3J = 6-15 Hz range. The 2-bond coupling between hydrogens bound to the same alkene carbon (referred to as geminal hydrogens) is very fine, generally 5 Hz or lower. *Ortho* hydrogens on a benzene ring couple at 6-10 Hz, while 4-bond coupling of up to 4 Hz is sometimes seen between *meta* hydrogens.



ortho-benzylic:
^3J = 6-10 Hz

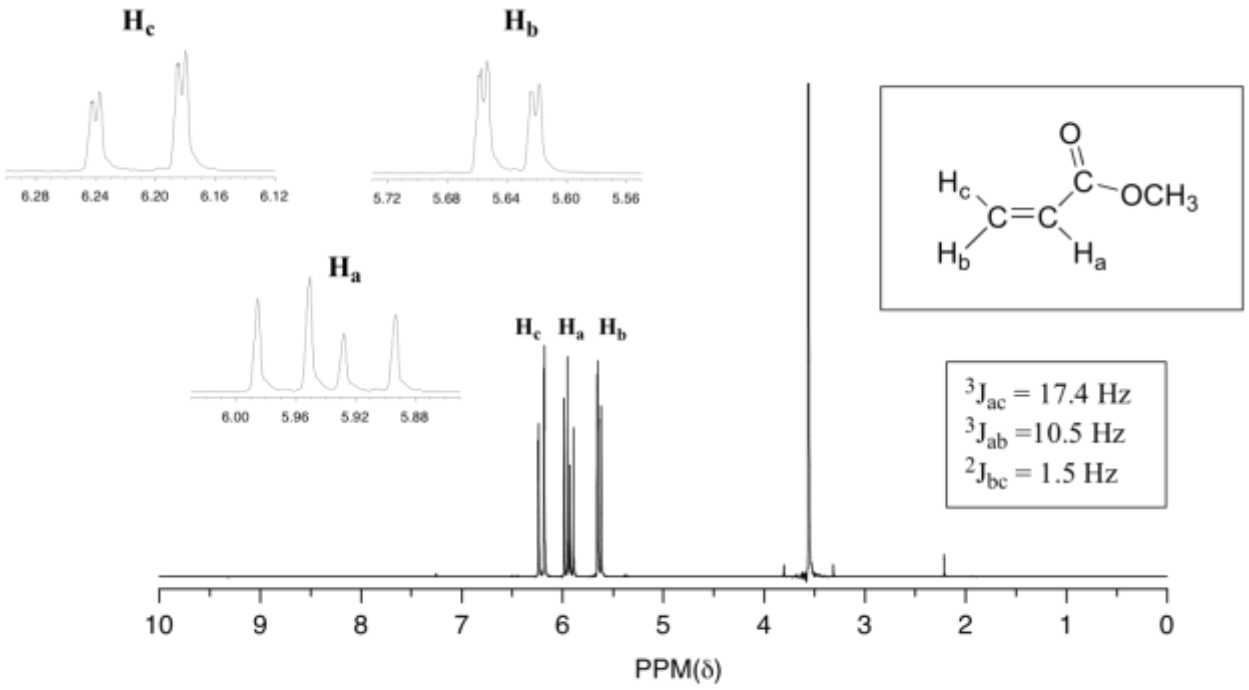
meta-benzylic:
^4J = 0-4 Hz

aldehyde:
^3J = 2-3 Hz

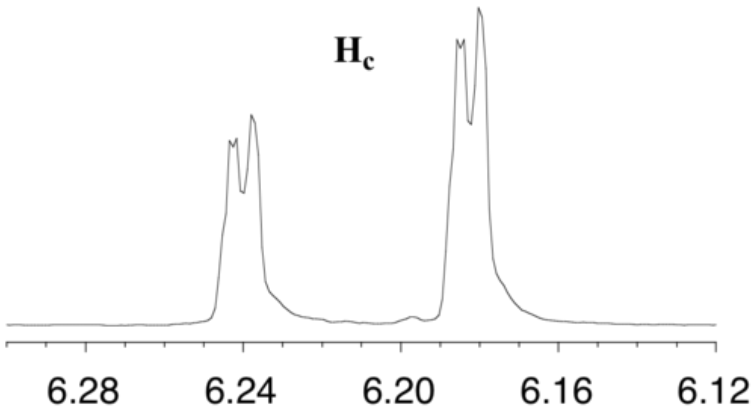
Fine (2-3 Hz) coupling is often seen between an aldehyde proton and a three-bond neighbor. Table 4 lists typical constant values.

3.5.1 Complex coupling

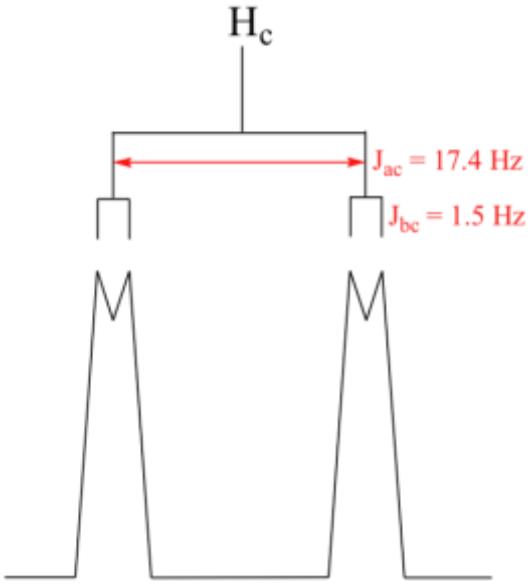
In all of the examples of spin-spin coupling that we have seen so far, the observed splitting has resulted from the coupling of one set of hydrogens to *just one* neighboring set of hydrogens. When a set of hydrogens is coupled to *two or more* sets of nonequivalent neighbors, the result is a phenomenon called **complex coupling**. A good illustration is provided by the 1H-NMR spectrum of methyl acrylate:



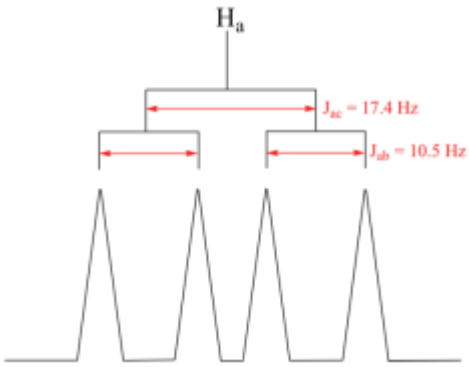
First, let's first consider the H_c signal, which is centered at 6.21 ppm. Here is a closer look:



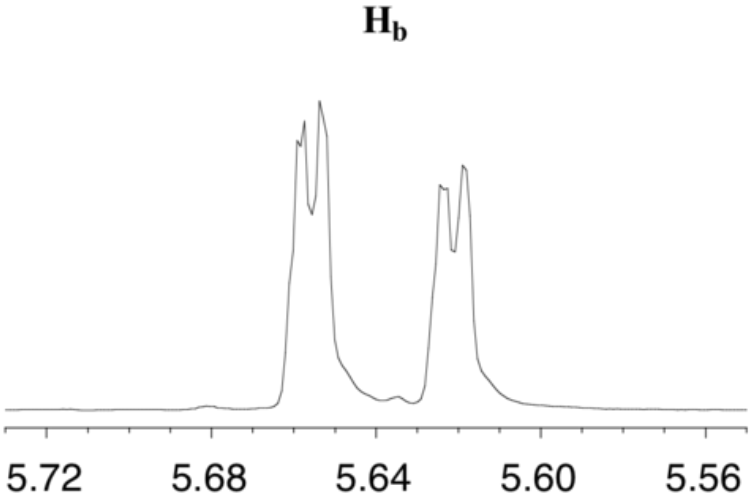
With this enlargement, it becomes evident that the Hc signal is actually composed of four sub-peaks. Why is this? Hc is coupled to both Ha and Hb , but with *two different coupling constants.* Once again, a splitting diagram can help us to understand what we are seeing. Ha is *trans* to Hc across the double bond, and splits the Hc signal into a doublet with a coupling constant of $^3J_{ac}$ = 17.4 Hz. In addition, each of these Hc doublet sub-peaks is split again by Hb (*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 signal for Ha at 5.95 ppm is also a doublet of doublets, with coupling constants $^{3}J_{ac}$ = 17.4 Hz and $^{3}J_{ab}$ = 10.5 Hz.

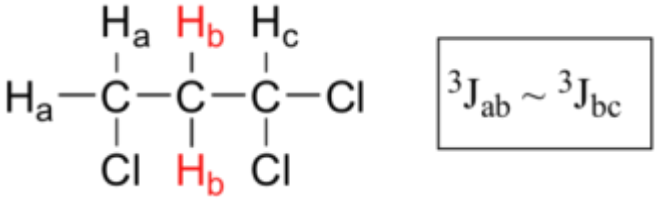


The signal for Hb at 5.64 ppm is split into a doublet by Ha, a *cis* coupling with $^{3}J_{ab}$ = 10.4 Hz. Each of the resulting sub-peaks is split again by Hc, with the same *geminal* coupling constant $^{2}J_{bc}$ = 1.5 Hz that we saw previously when we looked at the Hc signal. The overall result is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

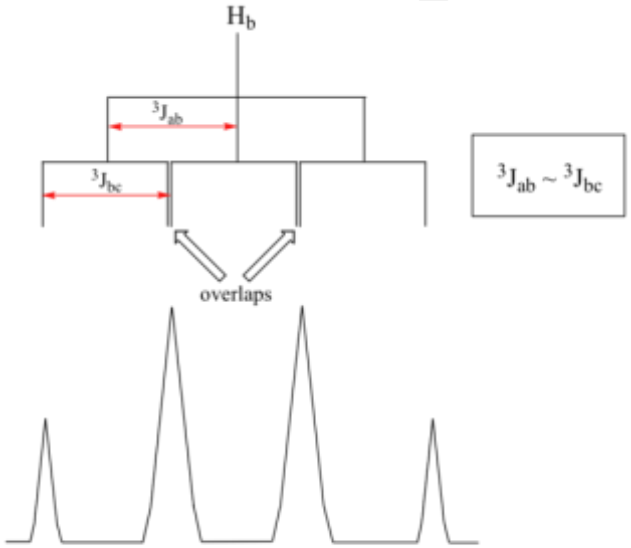


When constructing a splitting diagram to analyze complex coupling patterns, it is usually easier to show the larger splitting first, followed by the finer splitting (although the reverse would give the same end result).

When a proton is coupled to two different neighboring proton sets with identical or very close coupling constants, the splitting pattern that emerges often appears to follow the simple `$n + 1$ rule` of non-complex splitting. In the spectrum of 1,1,3-trichloropropane, for example, we would expect the signal for Hb to be split into a triplet by Ha, and again into doublets by Hc, resulting in a 'triplet of doublets'.

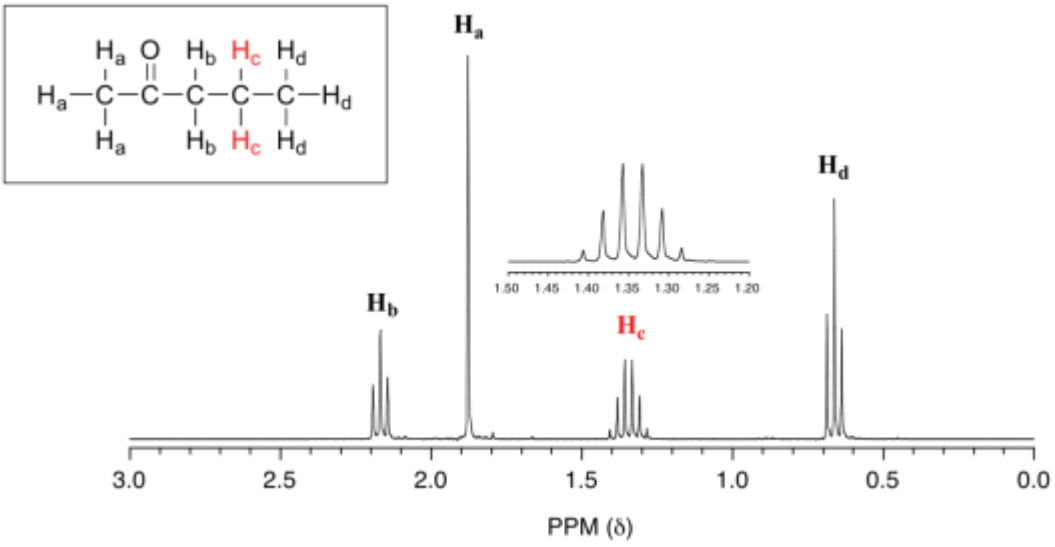


Ha and Hc are not equivalent (their chemical shifts are different), but it turns out that 3Jab is very close to 3Jbc. If we perform a splitting diagram analysis for Hb, we see that, due to the overlap of sub-peaks, the signal appears to be a quartet, and for all intents and purposes follows the $n + 1$ rule.



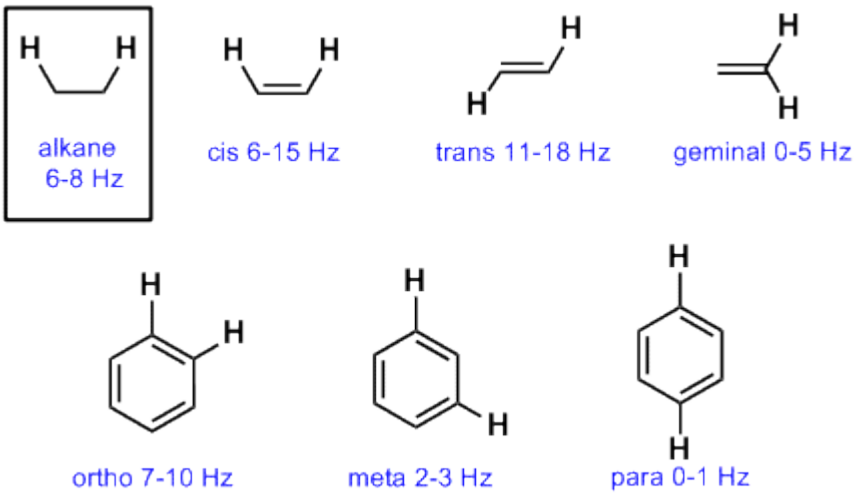
triplet of doublets becomes a quartet
when coupling constants are close

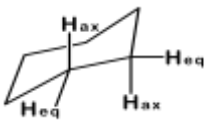
For similar reasons, the Hc peak in the spectrum of 2-pentanone appears as a sextet, split by the five combined Hb and Hd protons. Technically, this 'sextet' could be considered to be a 'triplet of quartets' with overlapping sub-peaks.



In practice, however, all three aromatic proton groups have very similar chemical shifts and their signals overlap substantially, making such detailed analysis difficult. In this case, we would refer to the aromatic part of the spectrum as a **multiplet**.

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!).





Hax,Hax = 8 to 14
Hax,Heq = 0 to 7
Heq,Heq = 0 to 5

3.6.1 Factors affecting coupling constant

1. Number of bonds between interacting nuclei
2. Hybridization of the atoms involved in coupling
3. Bond and torsional angles
4. Bond lengths
5. The presence of neighboring π bonds
6. The effects of neighboring lone-pair electrons
7. The substituent effect

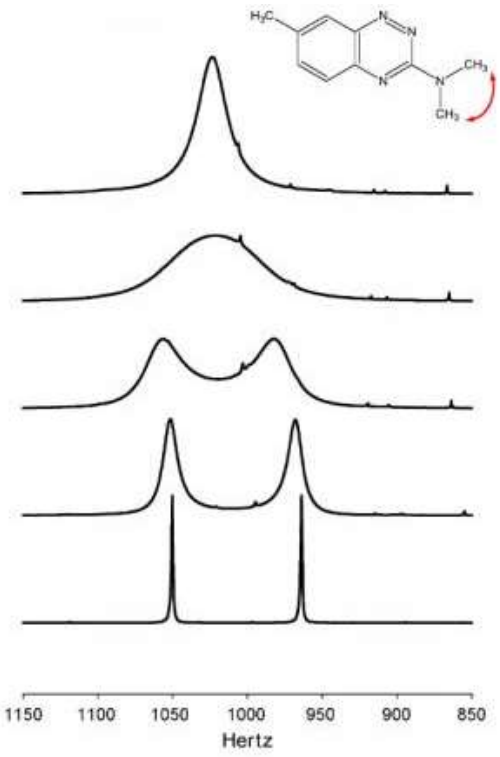


Figure 1: The N-methyl region from a 300 MHz ^{1}H NMR spectrum of a azapropzaone derivate recorded at 223 (lowest), 243, 253, 263 and 273 (top) K. The figure is adapted from [Bain, 2003].

5. Chemical Exchange

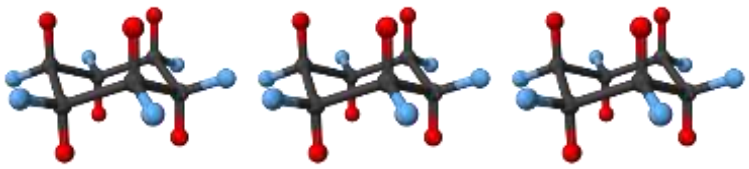
The chemical shift of a nucleus is extremely sensitive to the surrounding chemical environment. Hence the chemical shift of a nucleus changes when the chemical environment around it changes. In a molecule, this can occur either due to a chemical reaction or due to conformational changes (isomerization reactions).

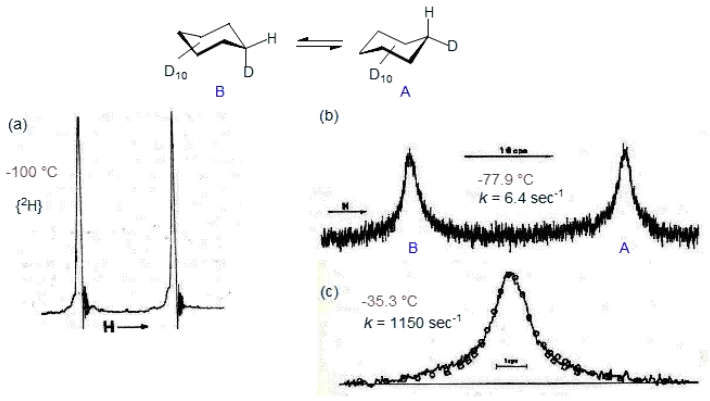
During these processes, the nuclei are exchanging between different chemical environments which leads to this phenomenon being called" **chemical exchange"** in the NMR literature. As solution NMR usually records the chemical shifts of the various nuclei in a molecule, chemical exchange affects the solution NMR spectrum of the molecule. Consider the case of the azapropazone derivative shown in figure 1. **Rotation about the C-N bond can interconvert the positions of the two methyl groups which have different chemical environments.** At very low temperatures (223K bottom of figure 1) when this interconversion is nonexistent the ^{1}H spectrum of the N-methyl groups consists of two sharp peaks arising from each of the methyl groups. As the temperature is increased and the rate of interconversion becomes faster, the peaks first broaden, then merge 2 into a single broad peak and finally give rise to a **narrow peak at the average position (of the two peaks).** This is called **coalescence.** This

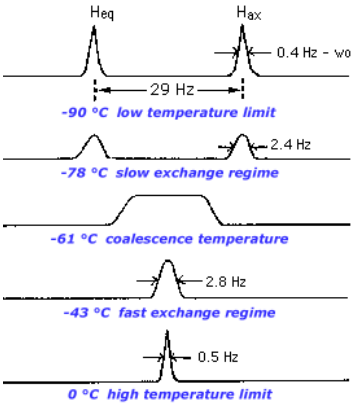
sensitivity of the NMR spectrum to chemical exchange makes NMR a very powerful method to follow the kinetics and study the dynamics of molecules in solution.

A particularly impressive example of the use of NMR to characterize dynamics in solution is the study of the isomerization between the two boat forms of cyclohexane. Hasha, Eguchi, and Jonas [Hasha et al., 1982] monitored the effect of pressure on the isomerization reaction by NMR. Interestingly they found that initially, the rate of interconversion increases with pressure although the volume of the transition state is slightly larger than the two boat forms. Contrary to popular belief which assumed that condensed phase reactions lie in the diffusive regime of the Kramer's model, this showed that the reaction lies in the inertial regime of the Kramer's model although it occurs in solution. The initial studies of chemical exchange were confined to small molecules. However, due to significant developments in NMR methodology over the last ten years, it is now possible to study conformational exchange in large macromolecules like proteins and to detect excited states which are transiently populated for short amounts of time, even though these cannot be observed in conventional NMR spectra.

The aim is to look at how chemical exchange affects NMR spectra and how we can derive information about the kinetics from the NMR spectra.





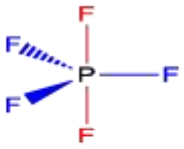


6. Fluxional molecules

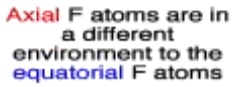
Fluxional molecules are molecules that undergo dynamics such that some or all of their atoms interchange between symmetry-equivalent positions. Because virtually all molecules are fluxional in some respects, e.g. bond rotations in most organic compounds, the term fluxional depends on the context and the method used to assess the dynamics. Often, a molecule is considered fluxional if its spectroscopic signature exhibits line-broadening (beyond that dictated by the Heisenberg uncertainty principle) due to chemical exchange.

The structure of PF_5 has been found to be trigonal bipyramidal. As stated before the trigonal bipyramidal polyhedron has two distinct sites, the axial site and the equatorial site. The 19F NMR spectrum of this compound shows a doublet with the separation between the lines corresponding to the coupling constant J(P-F). However, for a trigonal bipyramidal structure, we anticipate a more complex pattern as the axial fluorine atoms belong to one chemically equivalent set and the equatorial fluorines belong to another chemically equivalent set. Therefore the spectrum should have been more complicated as shown below.

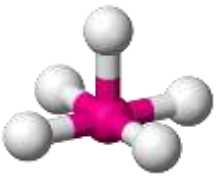
Why is only a doublet seen then?

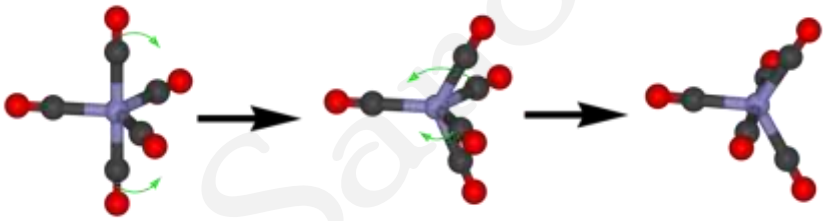


The answer lies in the fact that the molecule is stereochemically non-rigid and is continuously changing its structure. This is known as fluxional behaviour. The mechanism that accounts for the behaviour of PF_5 is known as **Berry Pseudo Rotation** and is shown below:



Pseudo Rotation: it's actually not rotating but looks like that





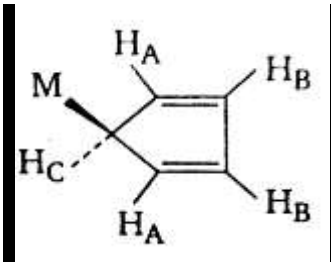
Square

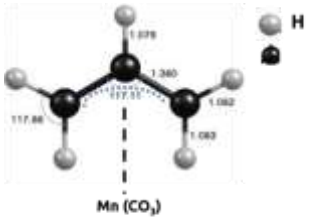
In structure 1a the angle F1-P-F2 is 180° and the F3-P-F4 angle is 120°. Imagine that the former is shrinking and the latter is expanding so that both the angles become nearly equal and structure 2, a square pyramid, is obtained. If this process is continued further such that the former F1-P-F2 angle now becomes 120° and the former F3-P-F4 angle becomes 180° we get the second trigonal bipyramidal structure 1b. Thus in reaching 1b from 1a through 2, we have essentially exchanged a pair of axial fluorine atoms with a set of equatorial fluorine atoms without breaking any bonds. In this whole process, one PF_5 does not change. If this process takes place faster than the NMR time scale in the NMR experiment only one set of fluorines is seen.

Keep in mind that we single out one interchange path here, while in reality, all atoms will rapidly occupy all possible positions in a very dynamic way. Having bulky substituents such as chlorine or substituents that do not prefer to occupy axial positions can slow this process down. Accordingly in PCl_2F_3, although at room temperature all the fluorine atoms are still equivalent and only a doublet is seen in the fluorine NMR spectrum, at -143° C the exchange is stopped and

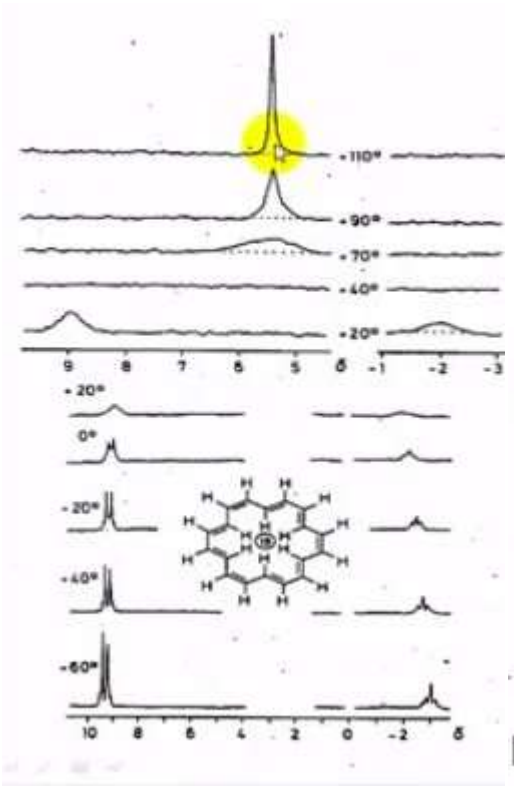
one sees two sets of peaks (a doublet of doublet for the two axial fluorine atoms and a doublet of triplets for the equatorial fluorine atom).

Examples:

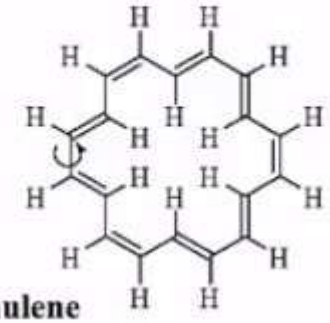




3 signals at a low temperature -70°C at 2:2:1
At high temperature 2 signals 4:1



Fluxional behaviour of large annulenes

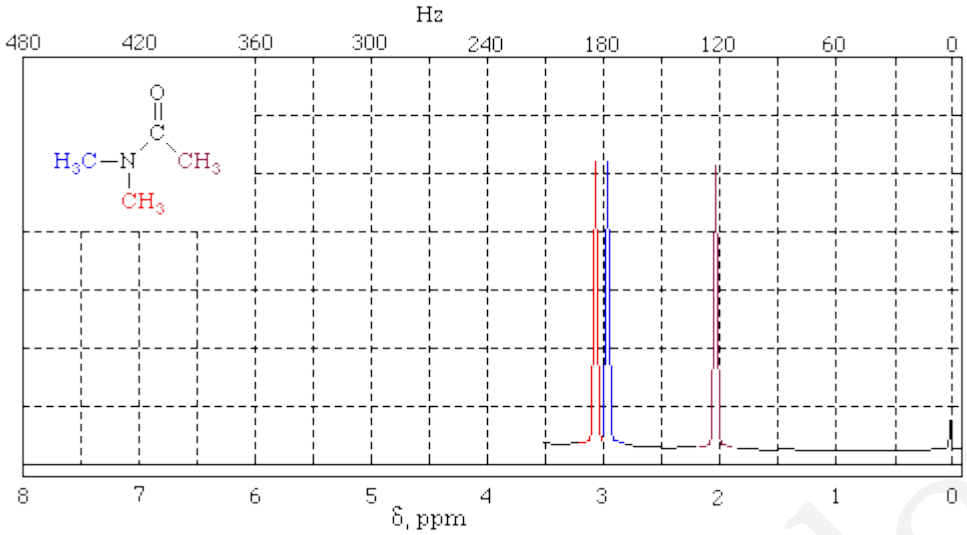


[18]annulene

7. Hindered Rotation

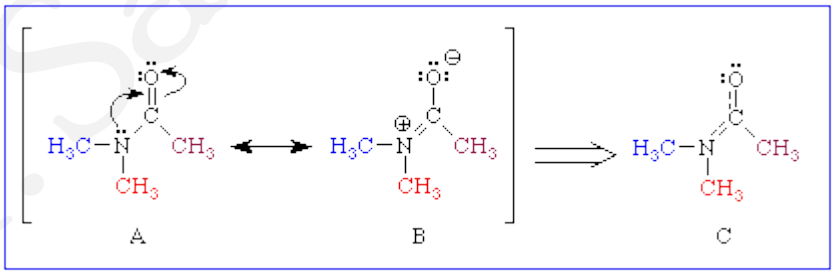
In Molecular Motions we saw that free rotation was a property of sigma bonds. We also learned that the adjective "free" has a qualified meaning, implying a potential energy barrier that is small compared to the amount of energy available at room temperature. We're now going to look at situations where structural features raise that barrier, thereby restricting free rotation.

Consider the colour-coded structure and 1H-NMR spectrum of N, N-dimethylacetamide showed in Figure



You know much about NMR to realize that the presence of **three signals of equal intensity** suggests that the three methyl groups in N, N-dimethylacetamide are different. If the formula in the inset represents the true structure of N, N-dimethylacetamide, you should expect free rotation around the sigma bond between the nitrogen atom and the carbonyl carbon. This **rotation would make the two methyl groups attached to the nitrogen atom identical.** In that case, the 1H-NMR spectrum of N, N-dimethylacetamide should **contain two signals**, one for the methyl group attached to the carbonyl carbon, and a second for the two methyl groups attached to the nitrogen. The intensity of the second signal should be twice that of the first. Clearly, that is not the case. **Something must be restricting the rotation around the C-N bond**.

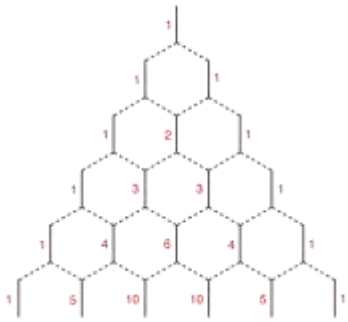
In resonance theory, we learned that one of the structural features required for resonance involved a non-bonded pair of electrons adjacent to a pi bond. This situation occurs in amides, where the lone pair of electrons on the nitrogen atom interacts with the pi bond of the carbonyl group. This interaction is shown in Figure for N, N-dimethylacetamide.



Structure C in Figure represents the resonance hybrid that is formed by mixing structures A and B. The partial double bond in the hybrid structure C implies a barrier to rotation around the bond between the nitrogen atom and the carbonyl carbon atom. If that barrier is great enough, the two methyl groups attached to the nitrogen atom would be non-identical; one of them would always be adjacent to the methyl group attached to the carbonyl carbon, while the other would be next to the carbonyl oxygen atom. Then the ^{1}H-NMR spectrum of **N N-dimethylacetamide should contain three signals**, one for the methyl group attached to the carbonyl carbon, and one for each methyl group attached to the nitrogen. The intensities of the three signals should be the same. Clearly, that's the case. **The restricted rotation observed in N, N-dimethylacetamide is characteristic of amides found proteins, i.e. polypeptides.**

8. First order and non first order spectra

First-order splitting pattern



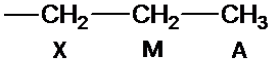
splitting pattern	the ratio of heights of lines in the peak
singlet	1
doublet	1:1
triplet	1:2:1
quartet	1:3:3:1
quintet	1:4:6:4:1
sextet	1:5:10:10:5:1

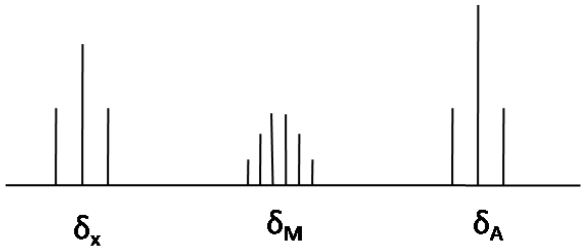
The chemical shift difference in Hertz between coupled protons in Hertz is much larger than the JJ coupling constant:

$$\Delta\nu/J \geq 10$$

Where $\Delta\nu$ is the difference of chemical shift. In other word, the proton is only coupled to other protons that are far away in chemical shift. The spectrum is called *first-order spectrum*. The splitting pattern depends on the magnetic field. The second-order splitting at the lower field can be resolved into first-order splitting pattern at the high field. The first-order splitting pattern is allowed to multiplicity rule (N+1) and Pascal's triangle to determine splitting pattern and intensity distribution.

The note is that structure system is A3M2X2. Ha and Hx has the triplet pattern by Hm because of N+1 rule. The signal of Hm is split into six peaks by Hx and Ha (Figure3) The First order pattern easily is predicted due to separation with equal splitting pattern.





9. Simplification of Second-order spectra

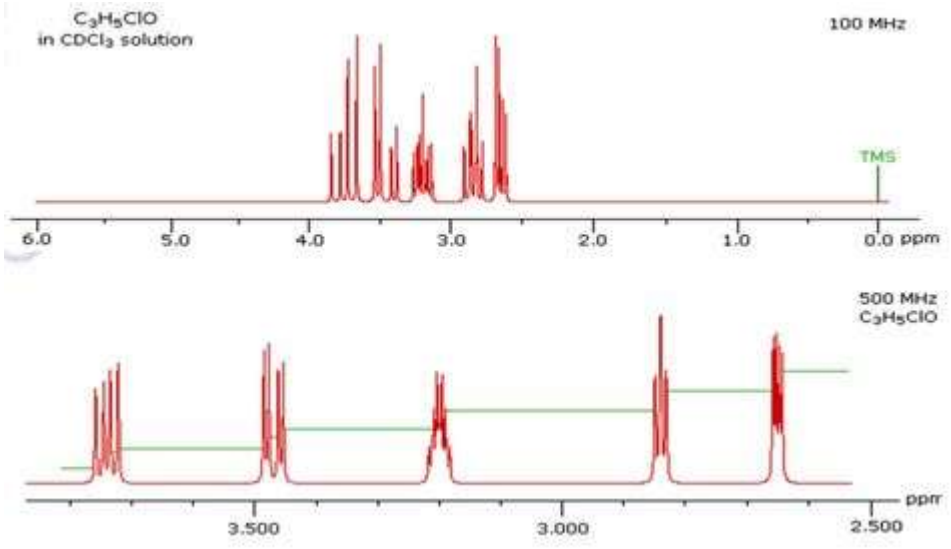
The complete analysis of a NMR spectrum becomes difficult when signals overlap and hence useful information is buried due to complexity of the spectrum. For example, if several closely related methylene groups are present in a molecule, their signals may overlap and the signals would not be clearly recognized. There are some important methods to simplify a NMR spectrum to get the maximum information.

8.1 High field strength
8.2 Solvent effect
8.3 Deuterium exchange reactions
8.4 Spin-spin decoupling (Double irradiation or double resonance)
8.5 Use of Lanthanide shift reagents
8.6 Chiral resolving agents
8.7 Nuclear Overhauser effect (NOE)
8.8 Variable temperature NMR

High field strength

As we know that the chemical shift measured in Hertz is directly proportional to the applied magnetic field, whereas the coupling constant (Hz) remains the same. Thus on increasing the field strength, the value of $\Delta\nu/J$ will increases which further gives the more separation between individual multipl ts, overlapped at lower field strength. Thus the NMR instruments with higher field strength give better resolution and therefore easily interpretable spectra.

In the figure given below, NMR spectra of C3H5ClO have shown at 100 and 500 MHz NMR. instruments. It is clear that at higher field strength, the multiplets are having larger separation and thus giving higher resolution.



Solvent Effects

Chemical shift depends on the solvent used to record NMR spectra. The interaction between the solvent molecules and the solute molecule may shift the resonance position of a proton. If the solvent is non-polar such as hydrocarbons, there will only beweak (van der Walls or London forces) interactions between solvent and solute molecules and hence the chemical shift of the protons in a molecule will remain same or very minute changes will occur.

If the solvent is polar in nature such as acetone, chloroform, DMSO etc., strong dipole interactions between solvent and solute molecules would be there especially when the solute molecules also contains polar bonds. Because of these strong interactions, the observed chemical shift will shift to a greater extent with respect to that taken in non-polar solvent.

If the solvent has a strong diamagnetic anisotropy (e.g., benzene, pyridine) the interaction between the compound and the anisotropic field of the solvent may give rise to a remarkable shifting in the resonance position.

It is observed that the solvents, such as benzene or pyridine cause the shifting of observed resonance to the lower δ value, whereas other solvents such as acetonitrile results in shifting to higher δ value. This difference is believed to be dependent on the shape of the solvent molecules, which affect the nature of the solute-solvent interaction in the solution. Aromatic solvents are flat molecules while acetonitrile has a rod-like shape.

Thus, we can use these solvent shift effects to resolve the complex spectra to some extent. By adding a small amount of deuterated benzene or pyridine to the NMR sample can cause a dramatic difference in the appearance of the NMR spectrum. The overlapping multiplets can be separated from one another and hence can be analyzed more accurately.

Deuterium Exchange Reactions

Deuterium exchange is the most elementary and very useful technique for assigning the protons of OH, NH2, SH etc groups which are exchangeable with deuterium.

Due to the above reactions, the peak due to the alcoholic hydrogen will disappear and a signal due to HOD proton will appear in the spectrum.

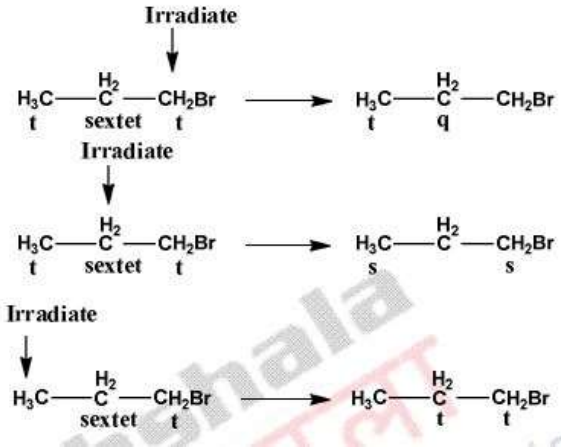
For detecting the protons exchangeable with D2O, either the spectrum is recorded in D2O or a few drops of D2O are added to the sample and the sample tube is shook properly before recording the NMR spectrum. The deuterium is not detected in the 1H NMR as it absorbs at different field strength and hence there will not be any coupling/splitting due to the proton which has been exchanged with deuterium

Spin-spin Decoupling (Double Irradiation or Double Resonance)

The spin-spin coupling between neighboring nuclei results in to the signals splitting causing different multiplets, however in certain cases, the splitting patterns are very complex. The spin- spin decoupling technique is used to simplify of the NMR spectra.

In spin decoupling, a second strong radiofrequency is applied while the spectrum is scanned in usual fashion. This radiofrequency can be set to irradiate a particular group of equivalent hydrogens in the molecule. Irradiation with high energy radio waves causes rapid transition among their nuclear spin states. Thus the neighboring protons cannot see different spin states of the irradiated protons and consequently any spin-spin interactions are effectively removed and the NMR spectrum is simplified.

Consider an example of 1-bromopropane to understand the concept of spin-spin decoupling.

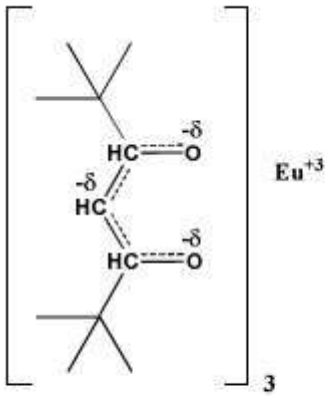


The above figure shows the effects of double irradiation of the α-CH2, β-CH2 and γ-CH2 protons. If the protons at α-carbon are irradiated, the signal due to these protons will be collapsed and hence no coupling will be observed due to these protons. Therefore the protons at β-carbon will be observed as quartet due to coupling with methyl protons only, and the methyl protons will appear as a triplet due to coupling with adjacent methylene protons. The irradiation of the β-CH2 and γ-CH2 protons and the changes in the signals due to other protons can be explained in a similar way.

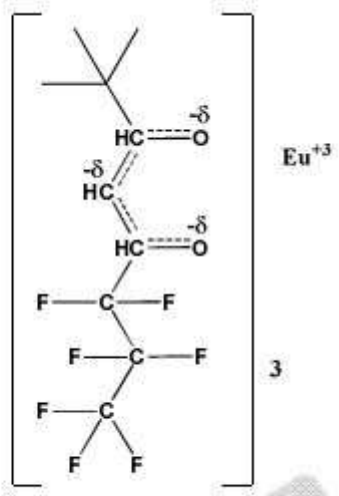
Use of Lanthanide Shift Reagents

The lanthanide shift reagents were first introduced by Hinckley in 1969. When such reagents are added to the compounds under investigation, there is a remarkable shifts in the resonance positions of the protons in the molecule without increasing the strength of the applied magnetic field i.e., a second order spectrum is converted to first order spectrum.

The lanthanide shift reagents are β-dicarbonyl complexes of rare earth metals (lanthanides). The commonly used lanthanides shift reagents are Tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane- 4,6-dionato) europium, Eu(fod)3; Tris-(dipivaloylmethanato) europium, Eu(dpm)3.



Eu(dpm)3

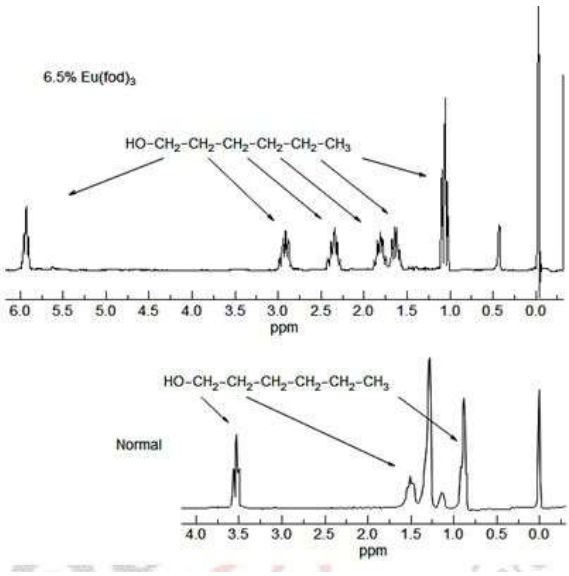


Eu(fod)3

The lanthanide shift reagents are mild Lewis acids, which attach themselves to the sites which have lone pairs such as amines, hydroxyl, aldehydes, ketoses, thiols, ethers etc. The amount of shift in resonance position depends on the lanthanide metal ion, proton under investigation and concentration of the shift reagent in the solution. More is the availability of the lone pair, greater is the complexation. The strength of the complex formation is in the following order, provided the complexation site of the molecule is not sterically hindered.

$$NH_2 > OH > C{=}O > —O— > —COOR > CN$$

The simplification of complex spectra by the addition of shift reagent can be explained by the example of 1-hexanol. In the absence of shift reagent, the spectra of 1-hexanol look like as shown in figure given below. We can clearly see only the signals due to the terminal methyl protons (a triplet at δ 0.9 ppm) and due to the methylene group next to the hydroxyl group (triplet at δ 3.8 ppm). The remaining methylene protons come into the broad and unresolved peak between δ 1.2-



1.8. On adding the shift reagent, the signals due to the methylene protons closer to the hydroxyl group are shifted downfield resulting in clearly observed signals. The methylene group which is relatively more closer to the OH group is shifted more downfield and as the distance increase from the OH group, shifting of the peak is less pronounced. Thus, on addition of shift reagent, spectrum is simplified to almost to the first order.

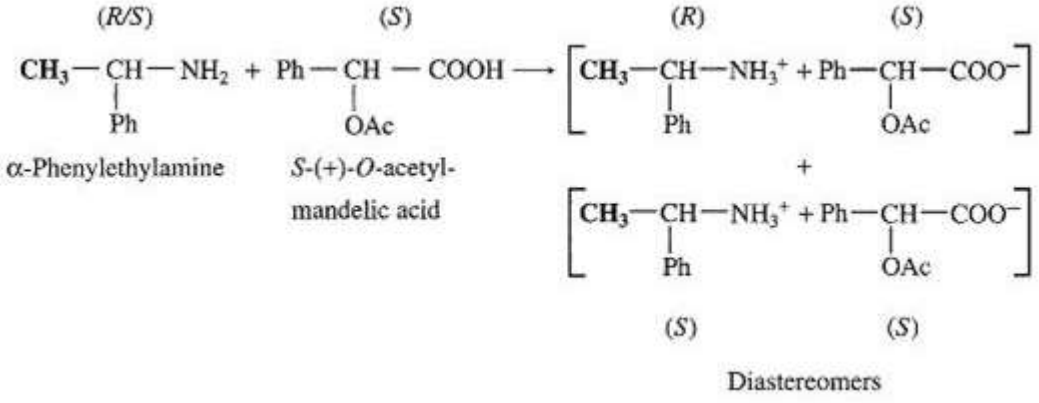
The ^1H NMR of 1-hexanol with and without 6.5% $Eu(fod)_3$

Chiral Resolving Agents

The NMR spectra of the enantiomers are identical. In some cases, however, an optically active solvent may cause chemical shift difference between resonance positions of the two enantiomers but the amount of shift is often too small to be utilized for the analysis of complex spectrum. The enantiomers can be distinguished by running their spectrum in the presence of chiral shift reagent.

When a racemic mixture (pair of enantiomers) is treated with an optically pure chiral resolving reagent, a mixture of diastereomers is obtained. Thus the protons will become chemically non- equivalent (diastereotopic protons have different chemical shift).

Suppose we have a mixture of R and S isomers of α-phenylethylamine. When an optically pure (S)-(+)-O-acetylmandelic acid is mixed with the mixture, two diastereomers (RS, SS) are formed.



Now the methyl group has become diastereomeric and the chemical shifts of the methyl protons in both the diastereomers will be different. Here in this particular case, two doublets (each for one diastereomer) will be observed due to the methyl protons. The integration of the two doublets can determine the percentage of the R and S isomers in the mixture.

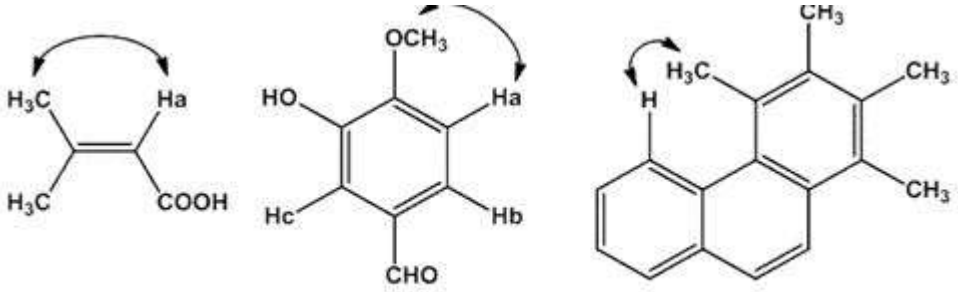
Nuclear Overhauser Effect (NOE)

The Nuclear Overhauser Effect was first introduced by Overhauser in 1953. This effect is a change in the intensity of a signal due to a particular proton on double irradiation of nearby protons within the molecule. In this case less intense radiation is used than the spin-spin decoupling or double irradiation. An increase of few percent in the intensity of absorption is observed between protons which are in close proximity. It is generally observed over very short distances, 2-4 Å. NOE and spin-spin coupling are two different concepts. NOE is the interaction of the nuclei through space and the number of intervening bonds between the concerned protons has no significance, whereas spin-spin coupling takes place via the bonds separating the nuclei.

NOE is associated with dipolar relaxation mechanism. A spin excited nucleus may undergo spin relaxation via the transfer of its spin energy to the nuclei in close proximity. The efficiency of this process is directly related to the distance between the two nuclei. If we irradiate one of the nuclei, the intensity of the nucleus closet in space to the irradiated nucleus will be enhanced in comparison to those far removed. Thus NOE may be useful in studying the stereochemical relationship between molecules.

To understand the phenomenon of NOE let's take an example of 3-methyl-2-butanoic acid. If the vinyl proton Hc is irradiated, the intensity (integral value) of the cis-methyl group becomes greater than that due to the trans-methyl group. Similarly on irradiation of the cis-methyl group, the intensity of the Hc increases, whereas the irradiation of the trans methyl group, which is at

more distant, does not affect the intensity of the Hc signal.



Double headed arrow showing NOE interaction

In isovanillin, irradiation of OCH_3 protons, the integral of the Ha proton (doublet) is increased due to NOE. In example III, on irradiation of CH_3 protons, the intensity of the H as depicted in figure increases.