1D NMR Techniques 1

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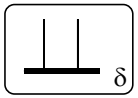
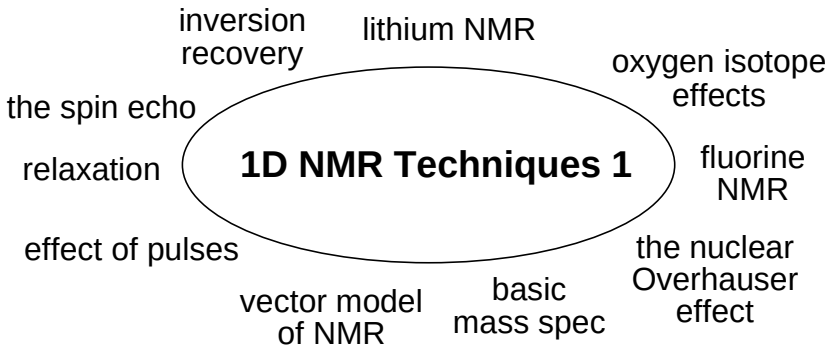
February 2, 2012.



Scope of Lecture



Helpful References

1. Understanding NMR Spectroscopy (2nd Ed.) Keeler, J. Wiley, **2010**. (Chapters 4 and 5)

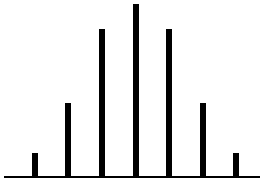
2. NMR Spectroscopy Explained: Simplified Theory... Jacobsen, N.E. Wiley, **2007**. (Chapter 3)

3. The ABCs of FT-NMR Roberts, J.D. University Science Books, **2000**. (Chapters 3 and 5)

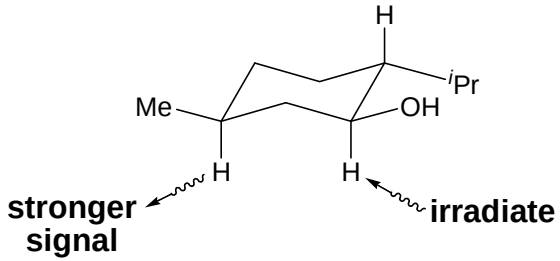
4. High-Resolution NMR Techniques in Organic Chemistry (2nd Ed.) Claridge, T.D.W. Elsevier, **2009**. (Chapter 3)

Key Questions

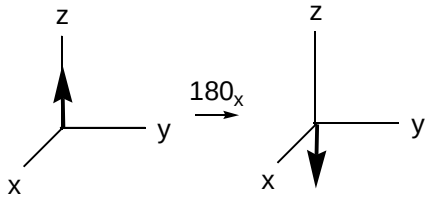
(1) What are the applications of heteronuclear NMR?



(2) What's the nuclear Overhauser effect?



(3) What's a simple model to explain what's going on in NMR experiments?



(4) What is relaxation?

$$\frac{dM_z}{dt} = \frac{-(M_0 - M_z)}{T_1}$$

I thank Dr. Neil Jacobsen (Arizona) for providing many of the beautiful diagrams in this lecture.

Lithium NMR

"Modern NMR Spectroscopy of Organolithium Compounds." Gunther, H.; Moskau, D.; Bast, P.; Schmalz, D. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1212-1220. (review)

Lithium has two naturally occuring isotopes: lithium-6 and lithium-7. Of these, lithium-6 is more useful. It is used in the nuclear industry for producing tritium, absorbing neturons in fission processes, and nuclear weapons. Thus, it is rather cheap to obtain commercially: $95/gram from Cambridge Isotope Labs (January 2011). Here are its NMR properties:

Nuc.	Abundance	I	γ	Q	ν_0	Recept.
^{1}H	99.9%	1/2	26.7	--	100	5 680
^{13}C	1.1%	1/2	6.7	--	25	1
^{6}Li	**7.4%**	**1**	**3.9**	**-8 x 10^{-4}**	**15**	**4**
^{7}Li	92.6%	3/2	10.4	-4.5 x 10^{-2}	39	1 540

Receptivity is relative to carbon-13 at natural abundance.

Lithium-6 is more useful than lithium-7, despite the higher abundance and larger gyromagnetic ratio of lithium-7 because of its small quadrupolar moment. It has relatively long relaxation times which make it possible to measure couplings between protons or carbons and lithium, giving a wealth of structural information.

- NMR spectra are referenced to LiCl in D_2O.

- Because the lithium-6 resonance frequency is close that of deuterium (59 vs 61 MHz), lithium-6 decoupling is readily available for carbon-13 spectra.

- Measuring couplings by a 2D NMR method, *J*-resolved spectroscopy, is popular.

- Heteronuclear ^{6}Li, ^{13}C through-bond or through-space correlation experiments are also possible.

Chemical shifts for lithium seem to be highly medium- and condition-dependent, and are not very informative for structural investigations. However, **coupling constants** *are* useful and form the basis of this problem (Problem 4.27 in Lambert and Mazzola).

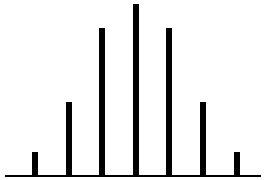
Q: The ^{7}Li{^{1}H} spectrum of $[^{6}Li^{13}CMe_3]_x$ shows this quartet:

***J* = 14.3 Hz**

(The nucleus in the braces is being broadband-decoupled.) How many nearest neighbor *tert*-butyl groups does lithium have?

A: Three. Carbon-13 is an I=1/2 nucleus, so a 1:3:3:1 quartet pattern indicates a coupling from lithium to three equivalent carbons. The fact that lithium-7 has an I of 3/2 is irrelevant.

Q: The ^{13}C{^{1}H} spectrum of $[^{6}Li^{13}CMe_3]_x$ at –88 °C is a septet with relative intensities of 1:3:6:7:6:3:1 with equal line spacings of 5.4 Hz:



How many nearest neighbor lithiums does carbon have?

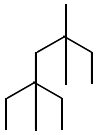
A: This is more complicated. Imagine carbon had one nearest neighbor lithium-6 (I=1). It would then have a 1:1:1 pattern, since I=1 implies three spin states:

What would it look like if carbon had two nearest neighbor lithiums?

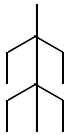
Lithium NMR

This is where a coupling tree comes in handy. With one neighbor, the coupling tree has one level to it (all the lithiums are identical, so the couplings are all degenerate):

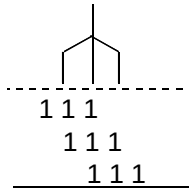
If there are two neighbors, there are two levels. Considering only the leftmost branch:



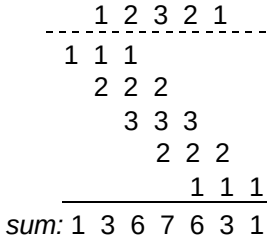
Considering only the middle one:



Because all of the degeneracy, the branches overlap. It's easier to draw this with numbers:



This doesn't match what we have. Going for three levels:

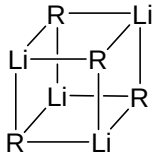


Why didn't I just have 1 1 1 on each line? Because the peak intensities from the last level must be nested into the next level. This is the desired pattern.

A: Carbon has three nearest-neighbor lithiums.

Q: Please suggest a structure for *tert*-butyllithium.

A: A cubic tetramer is one possibility:

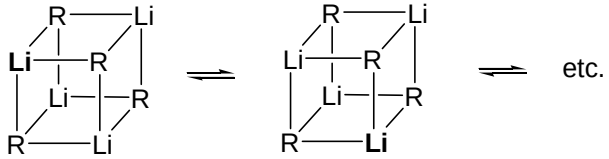


$^{1}J_{LiC}$ = 5.4 Hz
(at –88 °C)

These data imply no multiple-bond coupling from one lithium to another. Other structures can be drawn.

Q: Above –5 °C, the septet is replaced by a nonet (nine line multiplet) is formed with an equal line spacing of 4.1 Hz. What happened to the structure of *tert*-butyllithium? What are the relative peak intensities?

A: This is a manifestation of *intra-aggregate exchange.* At low temperatures, the lifetime of a single cube is long relative to the NMR timescale. Thus, every lithium sees three nearest neighbor carbons and vice versa. At high temperatures, every cube becomes fluxional, and there are now *four* nearest neighbors, resulting in line intensities of 1:4:10:16:20:16:10:4:1.



Oxygen Isotope Effects

Risley, J.M.; Van Etten, R.L. Isotope Effects in NMR Spectroscopy in *NMR: Basic Principles and Progress* Vol. 22, Springer-Verlag, **1990** (Chapter 3).

Oxygen is a terrible NMR nucleus: it has a large quadrupolar moment, low natural abundance, and is extremely expensive. The prices from Cambridge Isotope Labs (January 2010) are:

water, 70% ^{17}O, $1 375 for 0.5 g

Its NMR properties are:

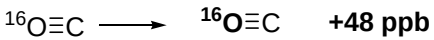
Nuc.	Abundance	I	γ	Q	ν_0	Recept.
^{1}H	99.9%	1/2	26.7	--	100	5 680
^{13}C	1.1%	1/2	6.7	--	25	1
^{16}O	99.8%	0	--	--	--	0
^{17}O	**0.04%**	**5/2**	**-3.6**	**-2.6**	**14**	**0.06**
^{18}O	0.2%	0	--	--	--	0

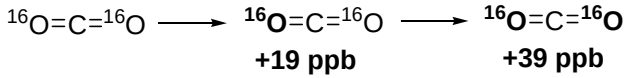
Receptivity is relative to carbon-13 at natural abundance.

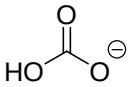
However, this does not mean it's useless in NMR! Note that oxygen-18 is much cheaper (Aldrich 2010 prices), perhaps because of its use as a precursor for PET imaging agents:

water, 95% ^{18}O, $163 for 1.0 g

This is useful because the presence of an oxygen atom always increases the chemical shift of adjacent ^{13}C atoms a bit. Here are three examples:







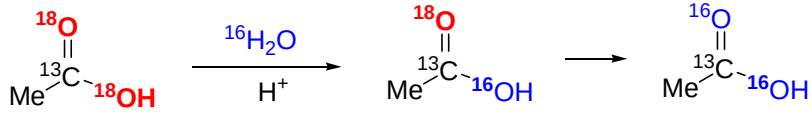
+7 ppb per label

Thus, the effect is essentially additive. The effect is larger for more substituted alcohols:

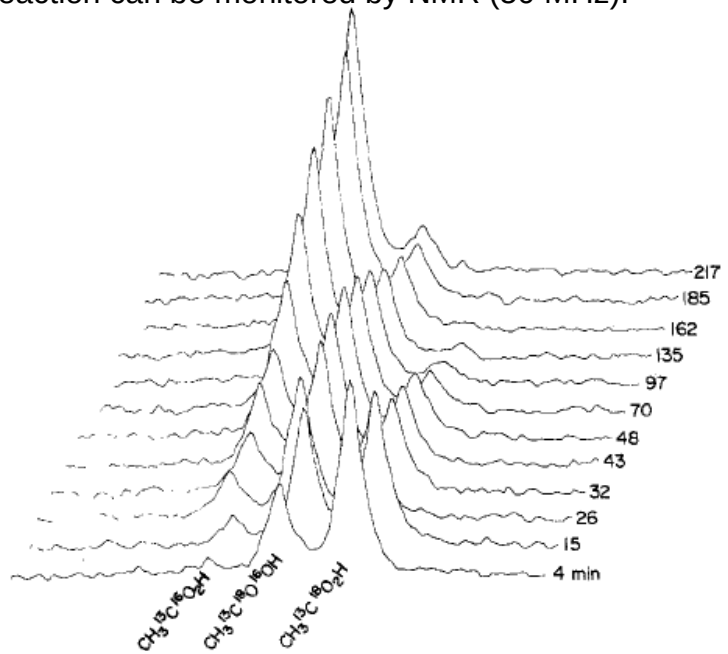
phenol (+10-16) < 1° ROH (+18-21) < 2° ROH (+23-26) < 3° ROH (+30-35)

Note that for historic reasons, a *positive* shift corresponds to movement of the signal *upfield* (i.e., the labeled compounds have smaller chemical shifts and resonance frequencies). The change seems to be related to the reduced mass of the bond the oxygen is attached to; secondary effects like hybridization, conjugation, substituent groups, etc. don't play much of a role.

The degenerate "hydrolysis" of carboxylic acids has been studied this way (Risley *JACS* **1981** *103* 4389):

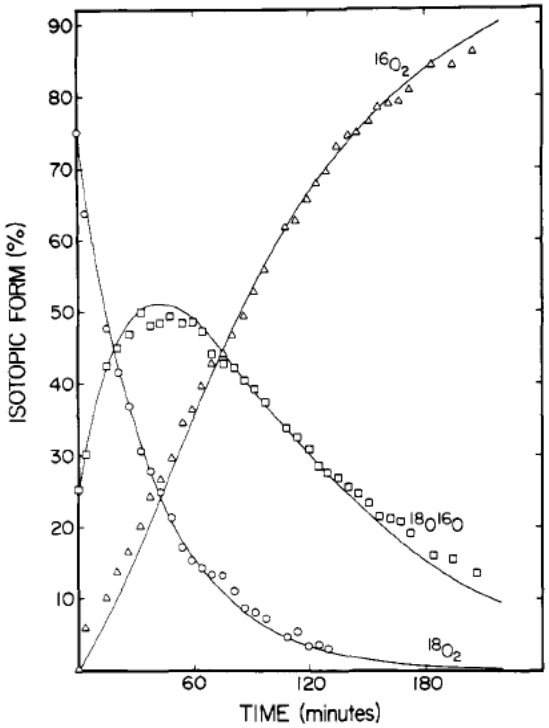


The reaction can be monitored by NMR (50 MHz):



Oxygen Isotope Effects

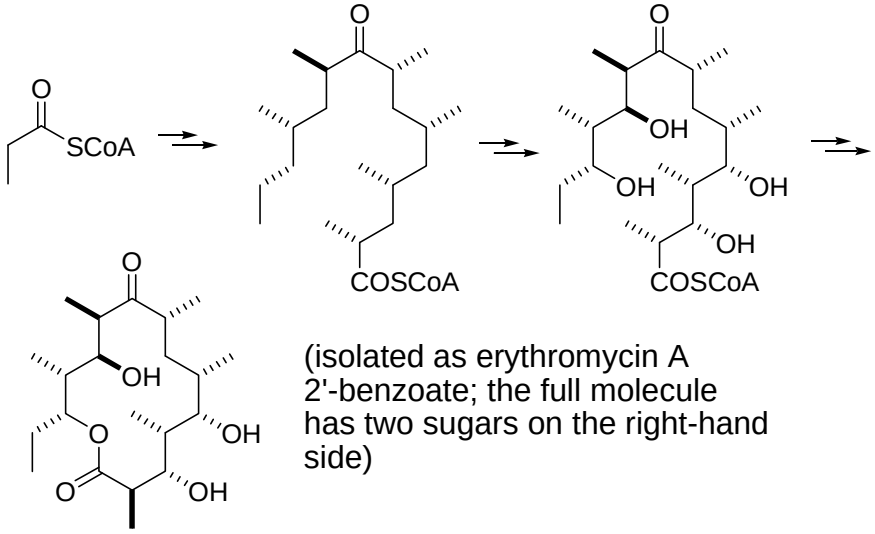
These are broadband-decoupled spectra, which usually cannot be integrated. However, it is assumed that labeling has no effect on the relaxation times, so that the *relative* peak areas should correspond to concentration. A plot of the integrals over time leads to data amenable to kinetic analysis:



This sort of technique has been used to study a wide variety of organic reaction mechanisms. It is also useful in elucidating biosynthetic pathways. For example, the biosynthetic origin of the oxygens in erythromycin was determined by feeding the organism, *Streptomyces erythreus* (Cane *JACS* **1981** *103* 5960; *Tetrahedron* **1983** *39* 3449). Erythromycin is a broad spectrum antibiotic we now know is a type I polyketide (see Lecture 27, Chem 106 notes).

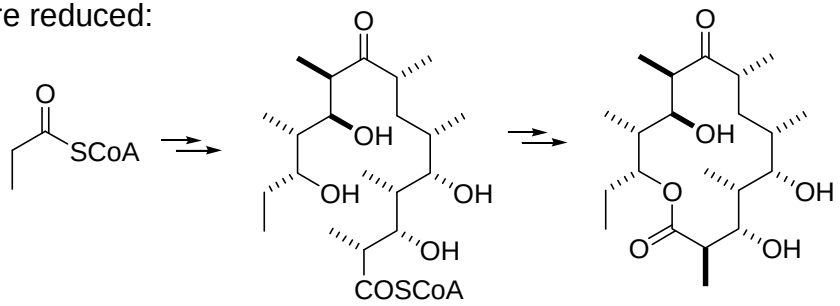
Q: Where do the oxygens in its aglycone come from?

Option A: A fatty acid chain is produced, and then oxidized:

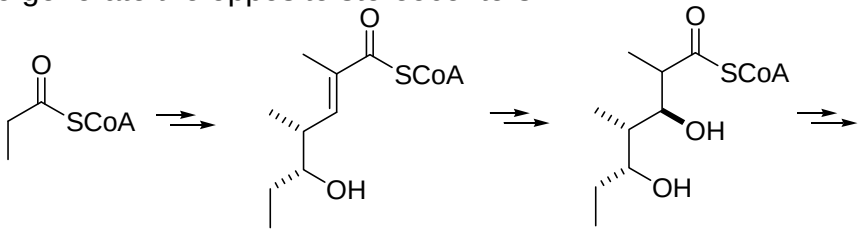


(isolated as erythromycin A 2'-benzoate; the full molecule has two sugars on the right-hand side)

Option B: A polypropionate chain is formed by sequential Claisen-type condensations; intermediate β-ketothioesters are reduced:



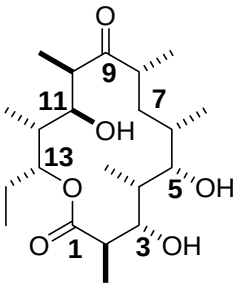
Option C: Reductions at every stage proceed with the same stereochemistry, with a dehydration-rehydration sequence to generate the opposite stereocenters:



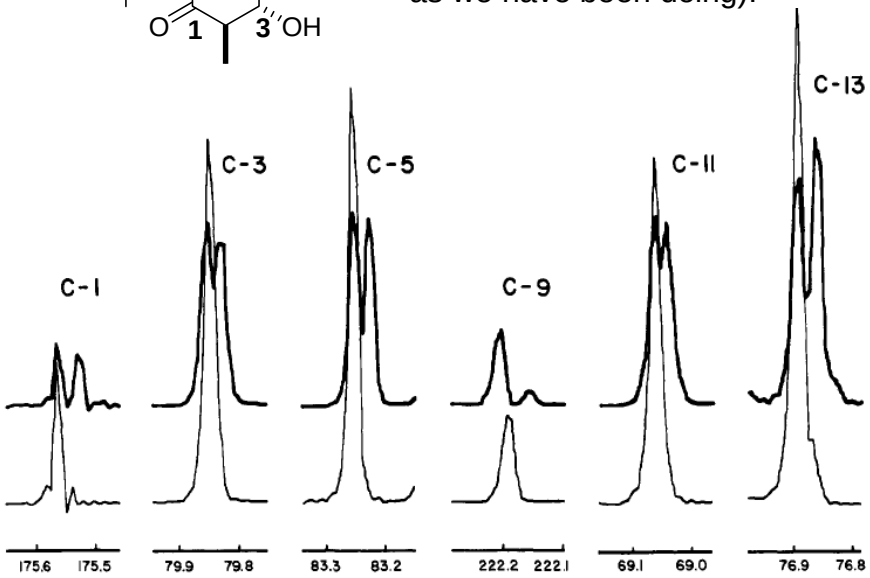
Oxygen Isotope Effects

Q: How would you use a feeding study to distinguish between these proposals?

Top spectrum: organism fed [1-$^{18}O_2$,1-^{13}C]propionate
Bottom spectrum: organism fed [1-^{13}C]propionate



Here, the numbering is based on the structure of the molecule (rather than the chemical shift of the peaks, as we have been doing).



Q: Which pathway is important?

A: Pathway B. The "doublet" patterns for every carbon mean that every oxygen came from the triply-labeled propionate. It is now known that type I polyketide biosynthesis is performed by a multimodular enzyme which has ketoreductase, dehydratase, and enoyl reducatase components which can modify the chain at every stage.

Fluorine NMR

Guide to Fluorine NMR for Organic Chemists Dolbier, W.R. Jr. Wiley, **2009**.

"Fluorine-19 NMR" in the *Encylopedia of Magnetic Resonance* Brey, W.S.; Brey, M.L. (Grant, D.M.; Harris, R.K., eds.)

Fluorine is almost as good an NMR nucleus as proton is:

Nuc.	Abundance	I	γ	Q	ν_0	Recept.
^{1}H	99.9%	1/2	26.7	--	100	5 680
^{13}C	1.1%	1/2	6.7	--	25	1
^{19}F	**100%**	**½**	**25.2**	**--**	**94**	**4 716**

Receptivity is relative to carbon-13 at natural abundance.

Referencing. In the old days, spectra were referenced to trifluoroacetic acid, but reactivity and safety issues have led to the common standard compound $CFCl_3$. Unlike TMS, which is upfield of most resonances, $CFCl_3$ is *downfield* of most peaks, so that most shifts are negative. For reference:

$CFCl_3$: 0 ppm
CF_3CO_2H: -76.2 ppm
hexafluorobenzene: -162.2 ppm
trifluoromethylbenzene: -63.2 ppm
ethyl trifluoroacetate: -75.8 ppm

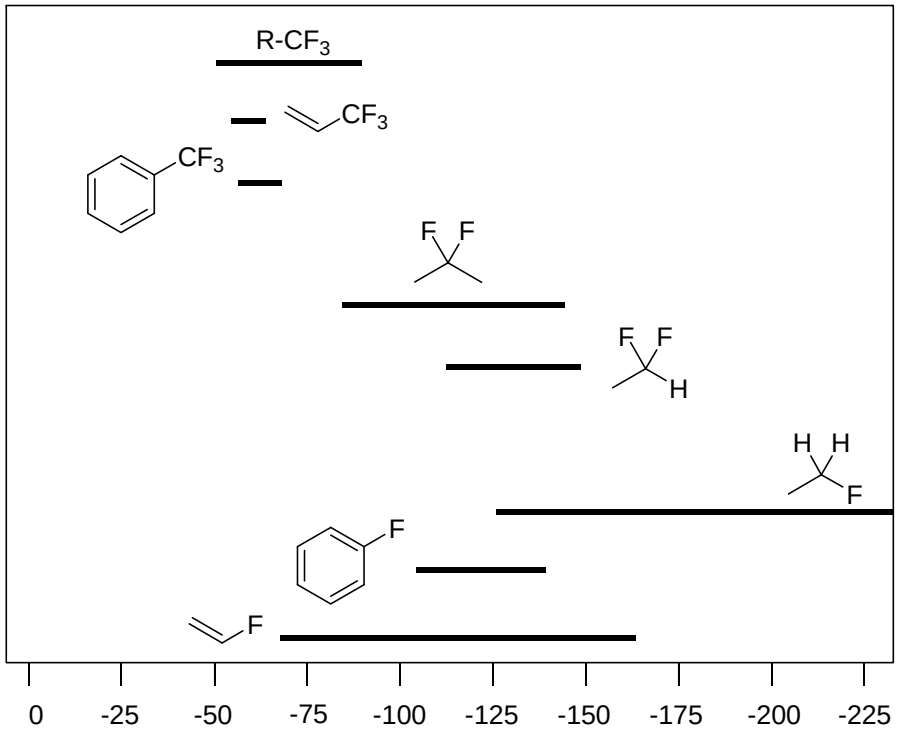
With poor resolution $CFCl_3$ appears as a single peak, but with higher resolution, there are four lines, corresponding to the presence of one, two, three, and four ^{37}Cl nuclides. However, **the chemical shift range of fluorine is enormous: over 500 ppm**, so the minor uncertainties created by this are irrelevant. The wide chemical shift range actually presents some technical challenges; sometimes, spectra need to be acquired in several parts to cover the entire range.

Q: What are typical chemical shifts for fluorine?

As usual, chemical shifts are affected by a complex interplay of diamagnetic, paramagnetic, and anisotropic effects.

Fluorine NMR

The chemical shifts of fluorine nuclei follow the same trends as those of carbon nuclei:

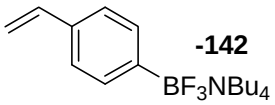


Inorganic fluorides have some more exotic chemical shifts and demonstrate significant contributions from paramagnetic effects:

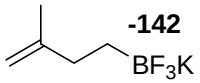
Compound	F_2	NF_3	BF_3	SF_2	PF_3	SiF_4
Shift (ppm)	+423	+143	-126	-167	-32	-160

(positive shifts are *very* deshielded)

Boron fluorides have found substantial use in synthesis, so here are some chemical shifts:



-142
BF_3NBu_4

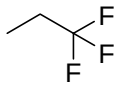


-142
BF_3K

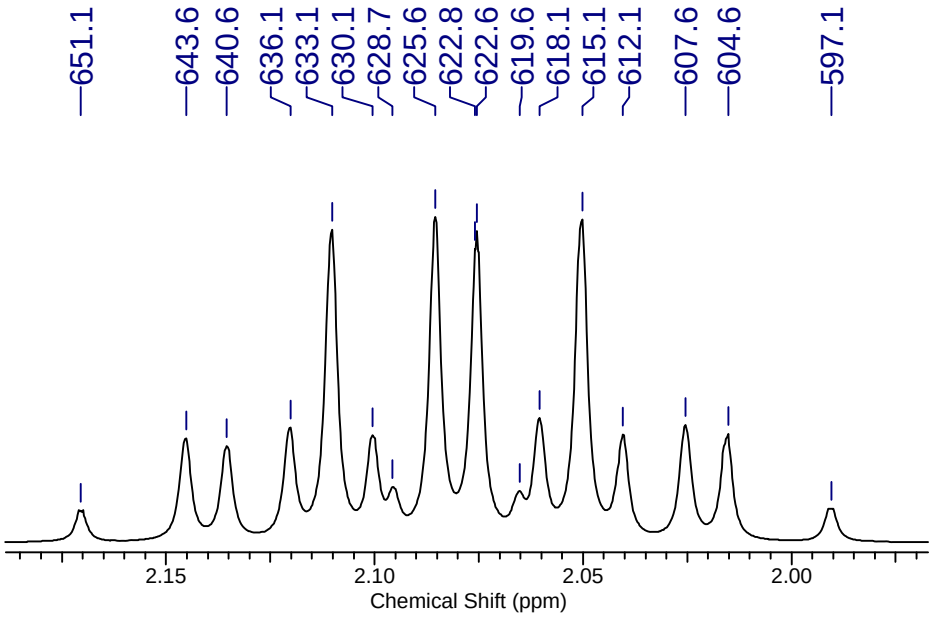
-154
BF_3OEt_2

-150
$BF_4^{\ominus}$

Of course, both homo- and heteronuclear **fluorine coupling constants** are important. For example, this is the ^{19}F spectrum of 1,1,1-trifluoropropane (the following spectra are taken from the book by Dolbier):



Here is the corresponding proton spectrum:

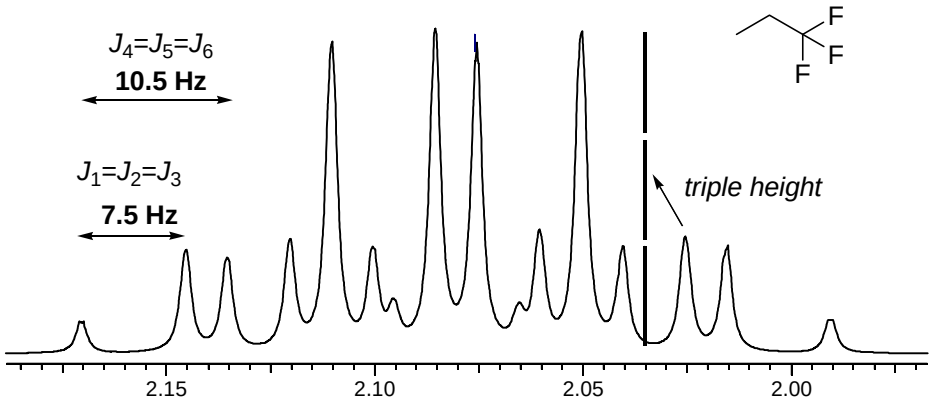


Q: Is this first-order or second-order?

Q: What are the coupling constants?

Fluorine NMR

This is a first-order spectrum of a quartet of quartets where the couplings correspond to $^3J_{HF}$ and $^3J_{HH}$. (This means three couplings each to the methyl group and the trifluoromethyl group, making for 2^6=64 components total.) Applying Hoye's method:

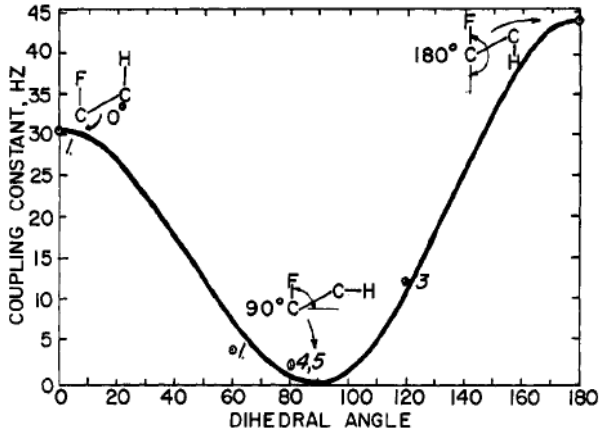


				19		32
				18		31
				17		30
				16		29
				15		28
				14		27
	(4)	(7)	10	13	22	26
	(3)	(6)	9	12	21	25
1	(2)	(5)	8	11	20 23	24

this side is just a mirror image of the other side

Assigning components is a bit tricky here (I only wrote down the left half above). If you wanted to cheat here, you would have just looked at the compound, and recognized it's a qq, and that 10.5 is not a multiple of 7.5, so no components should be removed from consideration, and therefore those must be the two distinct coupling constants).

As it turns out, basically all coupling constants corresponding to vicinal X-C-C-Y relationships obey Karplus-type curves. Vicinal proton-fluorine couplings are no exception:



However, they do seem to be larger in magnitude. There are significant subtituent effects on these, with values decreasing with substituent electronegativity (Dolbier, pg 18):

	$^3J_{FH}$ (Hz)		$^3J_{FH}$ (Hz)		$^3J_{FF}$ (Hz)
CH_3–CH_2F	27	CF_3–CH_3	13	CF_3–CH_2F	16
CH_3–CHF_2	21	CF_3–CH_2–Cl	8.5	CF_3–CHF_2	3
CH_3–CF_3	13	CF_3–$CHCl_2$	4.7	CF_3–CF_2–CR_3	~0
CH_2F–CH_2F	17			CF_3–CF_2–O–R	~0
CHF_2–CHF_2	3			CF_3–CF_2–S–R	3
CF_3–CHF_2	3				

Fluorine decoupling *is* possible, but is tricky and needs special equipment, since the resonance frequencies of proton and fluorine are so close together.

Fluorines can also couple to each other, but are generally larger than similar proton-proton couplings:

$^2J_{FF}$: 220-290 Hz (diastereotopic sp^3 CF_2)
$^2J_{FF}$: 14-110 Hz (sp^2 CF_2) $^3J_{FF}$: 15-16 Hz

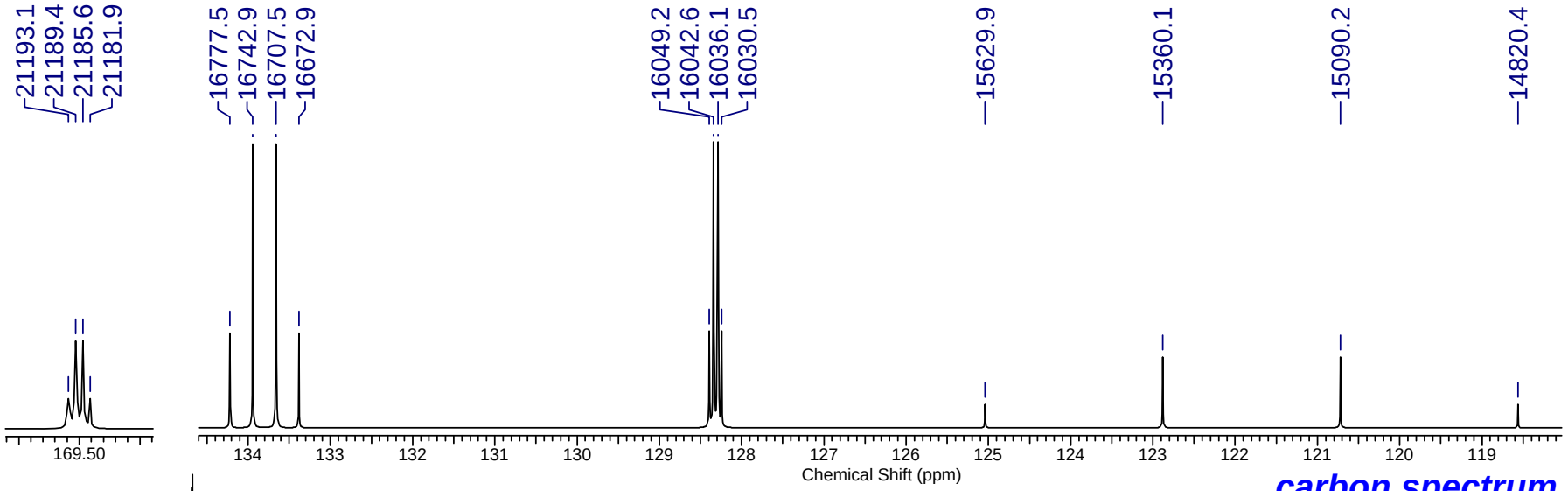
These also decrease with electronegativity.

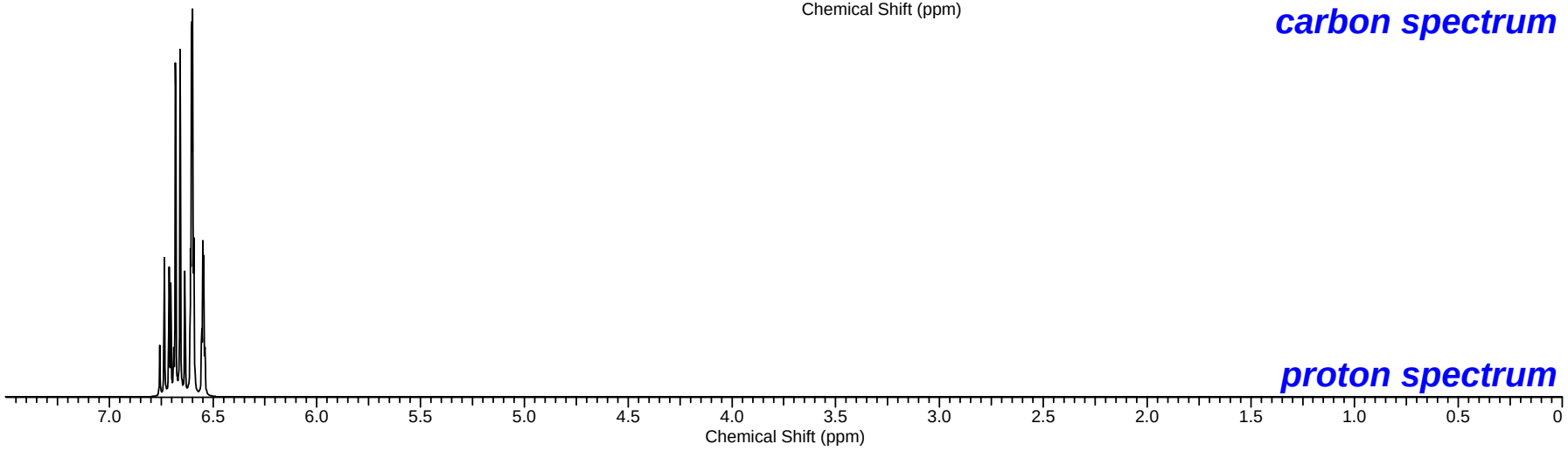
Fluorine NMR

Here's a another practice problem.

Q: The compound whose spectra are shown below has the molecular formula $C_4H_3F_3O_2$. Please determine the structure of the unknown, assigning relative stereochemistry if necessary. Determine all of the coupling constants and assign all the peaks.

fluorine NMR: there are two peaks at -66.5 ppm with spaced 6.4 Hz apart.



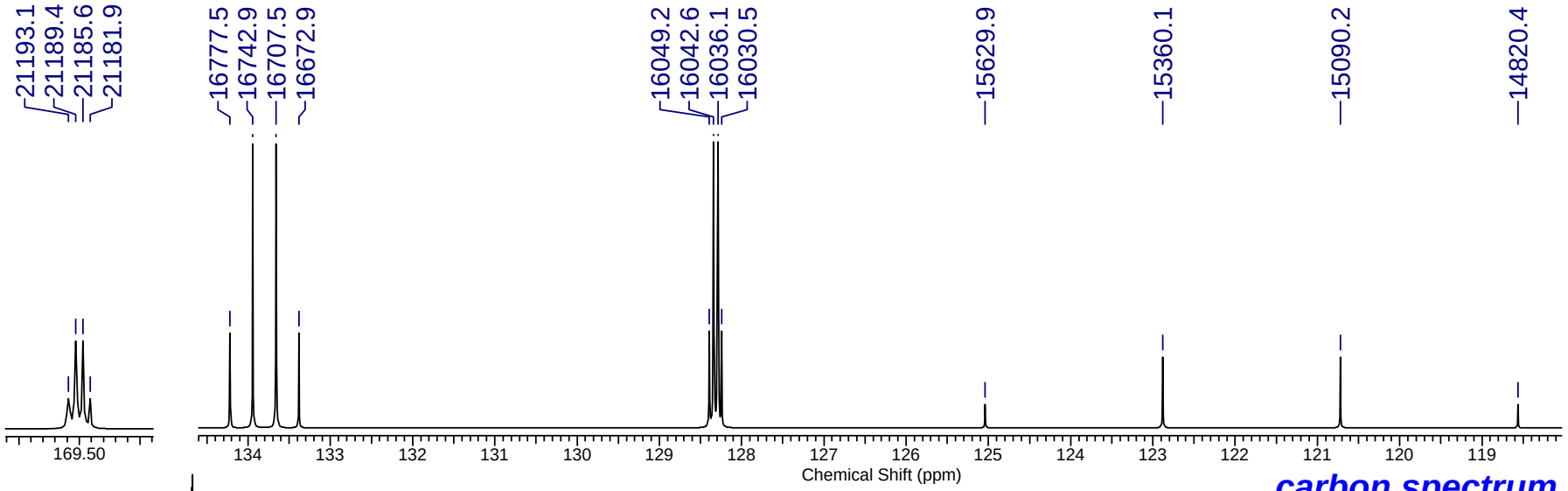


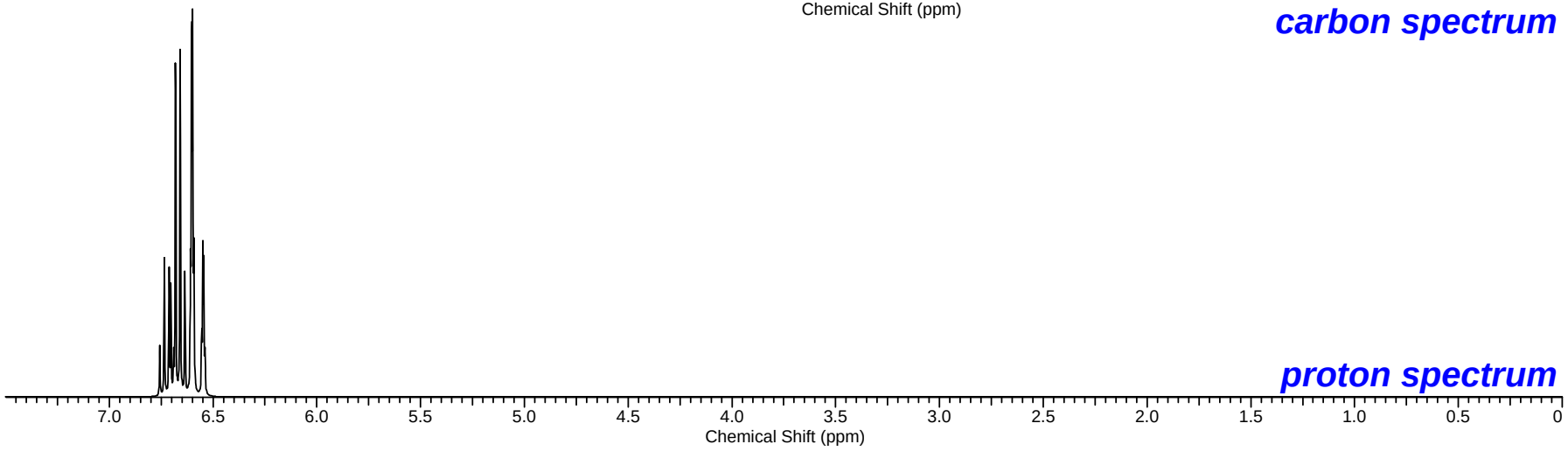
Fluorine NMR

(1) The unsaturation number is 4 + 1 - (3+3)/2 = 2.

(2) There seems to be a carbonyl group here. The remaining unit is probably an olefin.

(3) The is probably only one unique fluorine here, given the doublet in the fluorine spectrum.



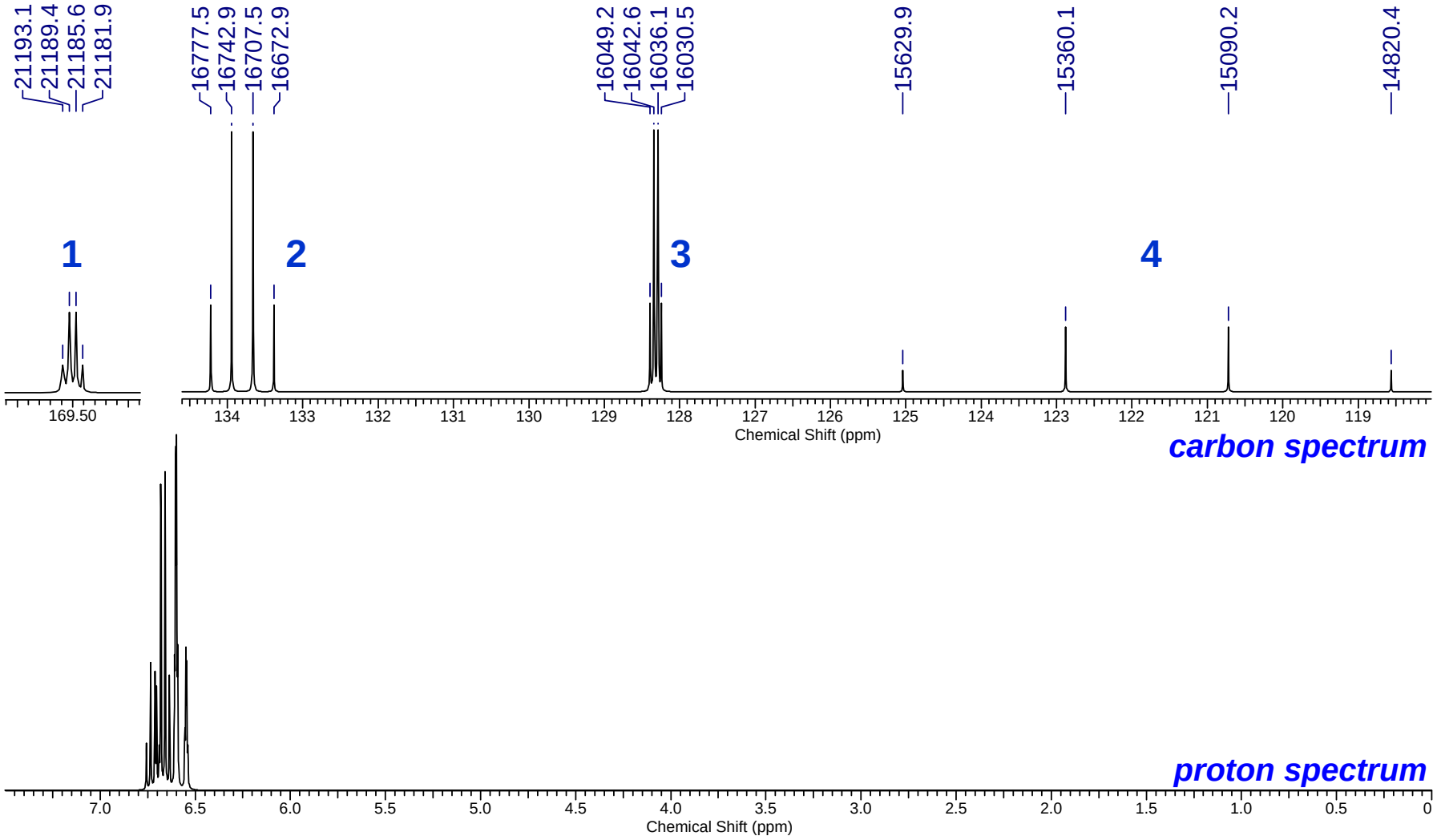


Fluorine NMR

(4) There are four unique carbons. Carbon **4** is a quartet. This must be directly attached to three fluorines.

(5) Carbon **2** has geminal couplings to fluorine and carbons **1** and **2** have vicinal or long-range couplings to fluorine.

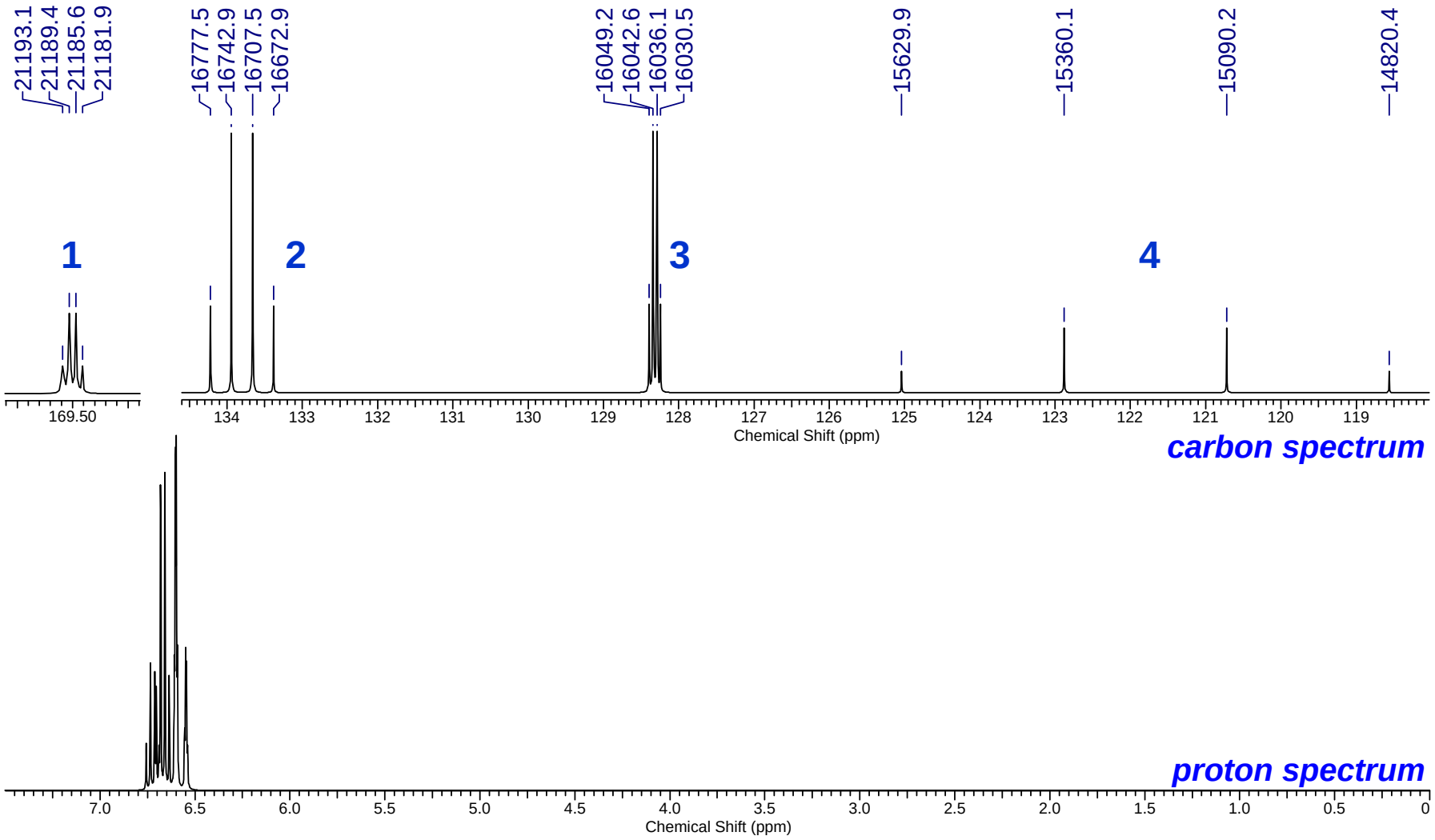
(6) The proton, however, is a jumble. It looks second-order.



Fluorine NMR

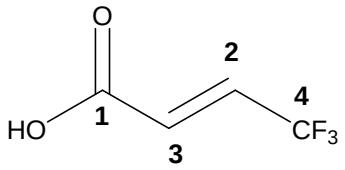
(7) It looks like there are three olefin carbons, but **4** is a CF_3 group, so it has a very high shift. Therefore, **2** and **3** are an olefin. We now have an olefin, a carbonyl group, and CF_3 fragments.

(8) Note that the couplings in peak **3** are 3x6.6 Hz, which correspond to the fluorine-19 splittings.



Fluorine NMR

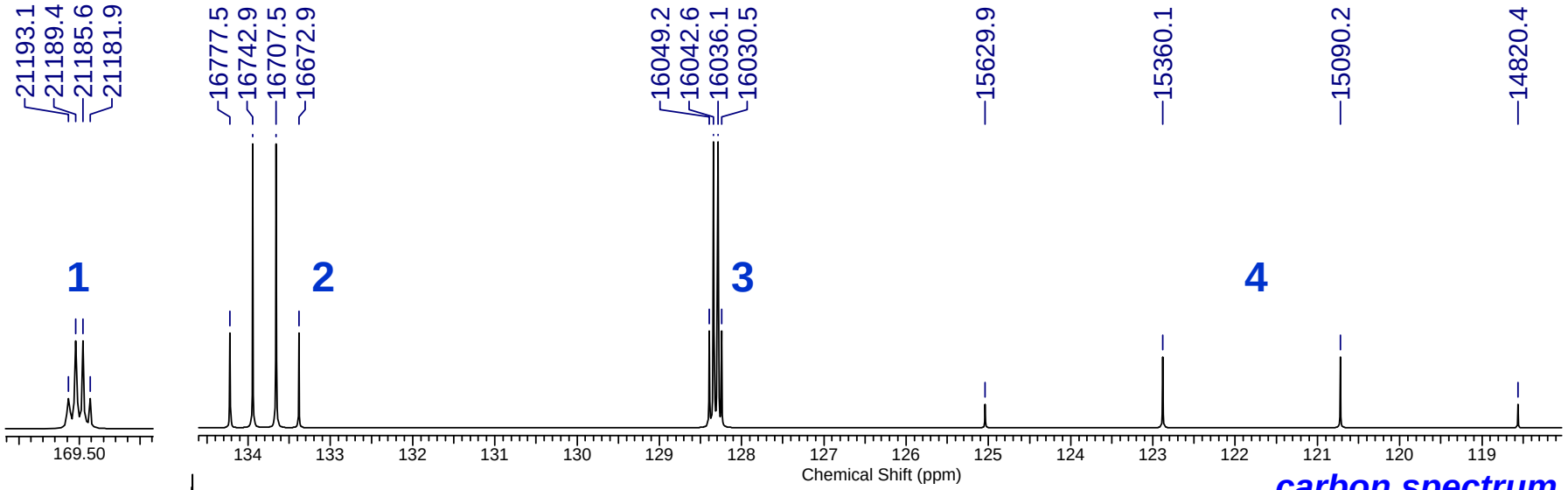
The answer is:

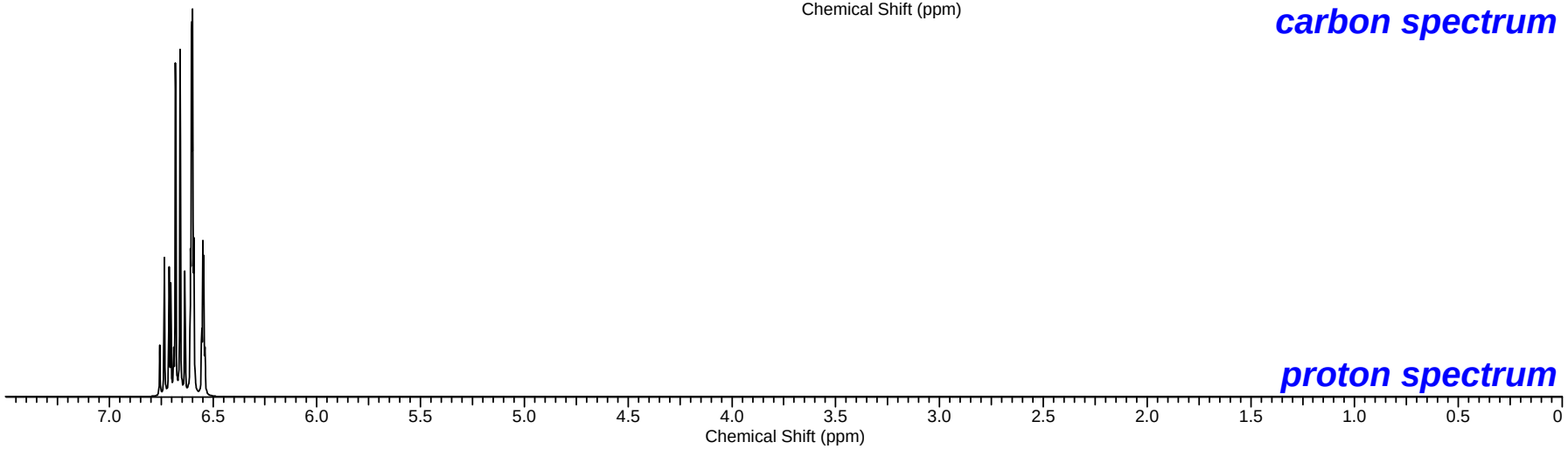


$^{1}J_{CF}$ = 270 Hz
$^{2}J_{CF}$ = 35 Hz
$^{3}J_{CF}$ = 6.4 Hz
$^{4}J_{CF}$ = 4 Hz

How would you determine the stereochemistry?

The proton spectrum is clearly first-order and needs spectral deconvolution. Alternatively, the three-bond C-F coupling is diagnostic.





The Nuclear Overhauser Effect

There are two kinds of interactions in NMR:

scalar couplings: these are through-bond couplings *J*

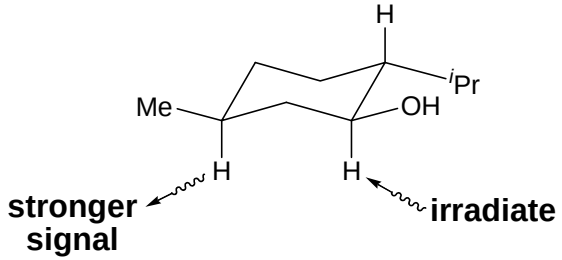
dipolar couplings: these are through-space couplings *D*

The **nuclear Overhauser effect (nOe)** deals with through-space interactions. Suppose a molecule has two spins, I and S. If the intensity of spin I changes when the populations of spin S are perturbed from equilibrium, there is said to be an nOe from S to I. More generally, the nOe enhancement is defined as:

$$n_I\{S\} = \frac{I - I_0}{I_0} \times 100\%$$

where I is the intensity of spin I after the perturbation and I_0 is the equilibrium intensity.

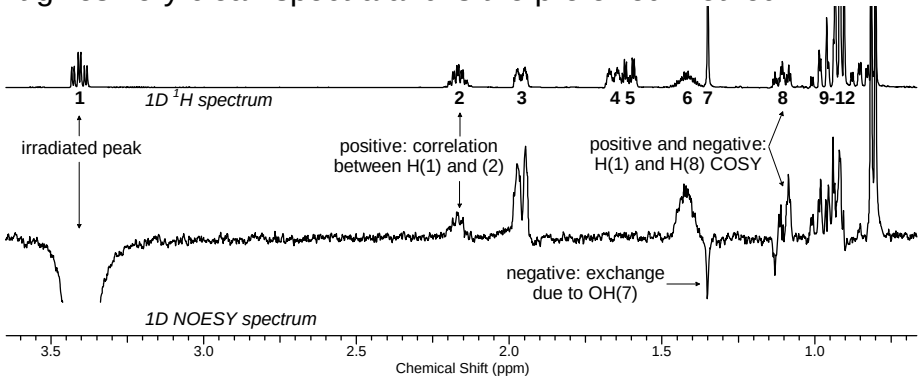
The nOe is all about **magnetization transfer**. Here is a concrete example using menthol. Suppose I want to know the relative configuration of the methyl and hydroxyl stereocenters:



The idea is that when one proton is irradiated, protons near it in space will give stronger signals, while others will not be changed. The nOe has a strong $1/r^6$ distance dependence, so it is very sensitive to different stereochemistries and molecular conformations in general.

At the moment, we are no position to understand how the nOe works. For now, I will just say that it is intimiately related to **relaxation**, the process by which spins return to their equilibrium states.

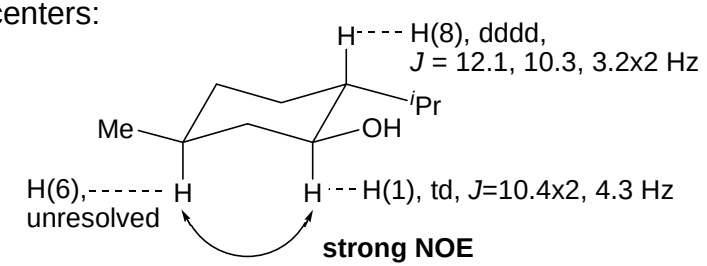
These days, the most common kind of nOe experiment has the tongue-twisting name: double pulsed field gradient spin echo nuclear Overhauser effect spectroscopy (DPFGSE-NOESY). It gives very clean spectra and is the preferred method:



By convention, nOe peaks are positive, while the irradiated proton is negative. Peaks due to chemical exchange (EXSY) are negative. Peaks due to COSY (*J*-coupling) have a distorted anti-phase appearance.

Q: What does this mean to you?

The main application of the nOe is the determination of relative stereochemistry. In a cyclohexane ring, coupling constants $^3J_{HH}$ tell you stereochemical relationships between adjacent stereocenters through the Karplus relationship while the nOe tells you about stereochemical relationships between vicinal stereocenters:



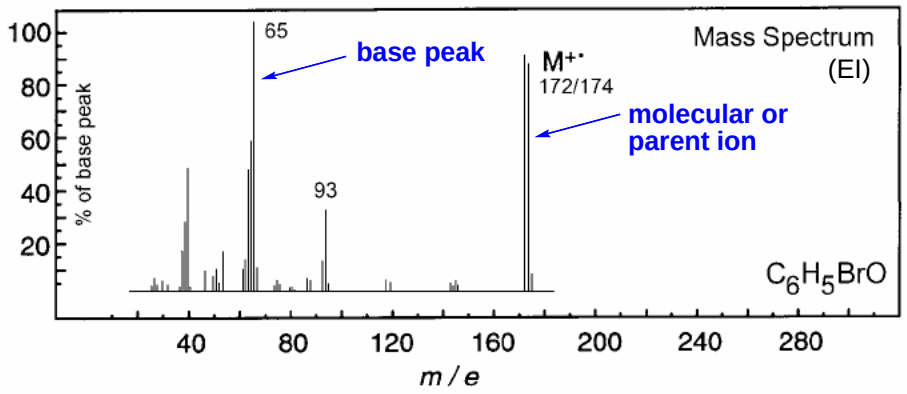
Mass Spectrometry: The Bare Essentials

There will be one lecture at the end of this class on mass spectrometery, but here are the bare essentials of what you need to function in the lab.

(1) Mass spectrometry weighs ions. Your molecule gets ionized with a variety of methods, and then **mass to charge ratio (*m/z*) is determined** by a fancy, expensive instrument. For small molecules, the charge is usually +1 or -1.

(2) The most common ionization type is electrospray (ESI). In the old days, you would shoot electrons at your compound and this would generate radical cations which would fly apart, producing characteristic patterns. This is called *electron impact* (EI). A somewhat milder method is *chemical ionization* (CI), which is where a reagent gas is ionized, and then used to ionize the molecule.

As an organic chemist, however, you are probably more interested in just the weight of the molecule itself. In electrospray ionization, the sample is sprayed through a fine tip with a strong electric field. As the aerosol evaporates, charged particles form, which fly apart and are collected by the detector. This is gentle enough to avoid fragmentation a lot of the time.



(3) The weight of a compound can be converted to a molecular formula.

In low-resolution spectra, peaks are accurate to the nearest integer (probably better than this). According to the *nitrogen rule*, **molecular ions with even weights have an even number of nitrogens**. Similarly, odd weights mean an odd number of nitrogens. (This is just because nitrogen is trivalent and all the common atomic masses except hydrogen are even.)

High-resolution spectra give molecular weights accurately to three or four decimal places (in Da). Because the weights of the elements are also known very accurately, this means a molecular formula can be determined from just an accurate mass. Additional information comes from the splitting pattern of the molecular ion, which arises from the presence of more than one isotopomer as natural abundance.

(4) Electrospray Ions Are Usually Adducts

The electrospray process is not strong enough to make radical cations and instead ionizes molecules as positively or negatively charged adducts using ions already present in the aqueous buffer vehicle. As such, molecules with basic groups like amines (ionized to ammonium) or acidic groups like carboxylic acids (ionized to carboxylate) give strong peaks. Here are some common adducts:

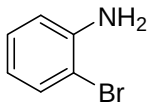
positive adducts (ESI+)	**negative adducts (ESI-)**
$M+H^+$ (+1)	$M-H^+$ (-1)
$M+Na^+$ (+23)	$M+Cl^-$ (-35)
$M+K^+$ (+39)	$M+Br^-$ (-79)
$M+NH_4^+$ (+18)	$M+H_2O-H^+$ (-19)
$M+MeCN+H^+$ (+42)	$M+TFA-H^+$ (+113)
$M+MeOH+H^+$ (+33)	
$M+i\text{-}PrOH+H^+$ (+61)	
$M+DMSO+H^+$ (+79)	

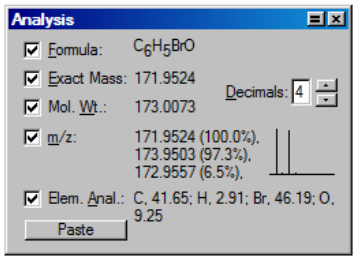
Mass Spectrometry: The Bare Essentials

There are computer programs that can help with the analysis. I like **Molecular Weight Calculator**, which is available at http://www.alchemistmatt.com. (Like a lot of software for chemistry, this only works for the PC.)

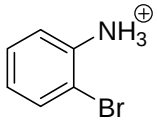
Suppose I've prepared 2-bromoaniline, and I want to verify its presence by ESI-MS. This compound has an amine, so it's reasonable to use positive mode. I can use ChemDraw to give me some key information:

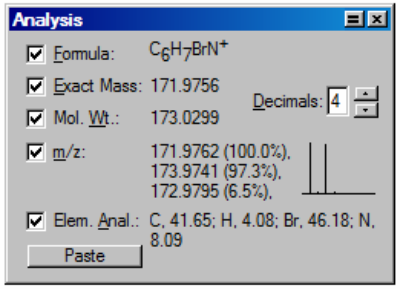
View...Show Analysis Window





However, this is not what I want, because in ESI+, I'll probably get the ammonium ion. So I draw that:

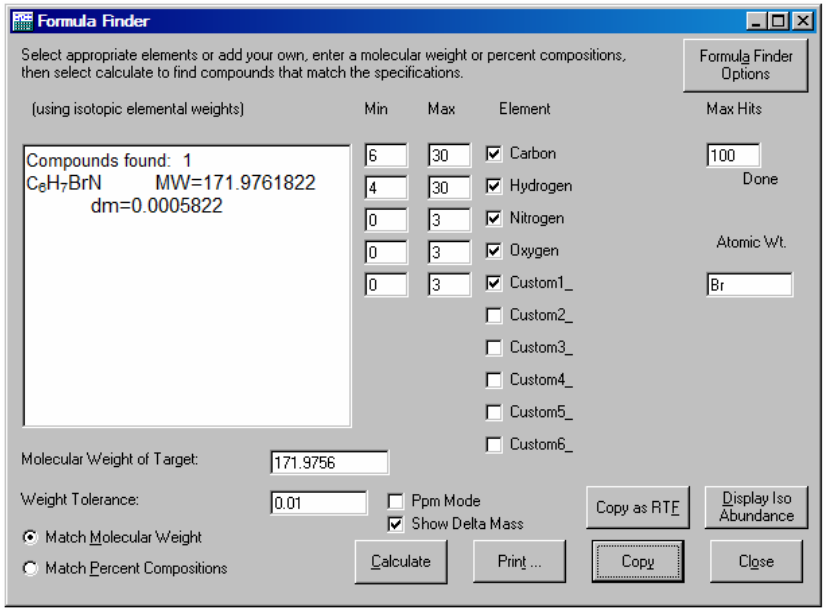




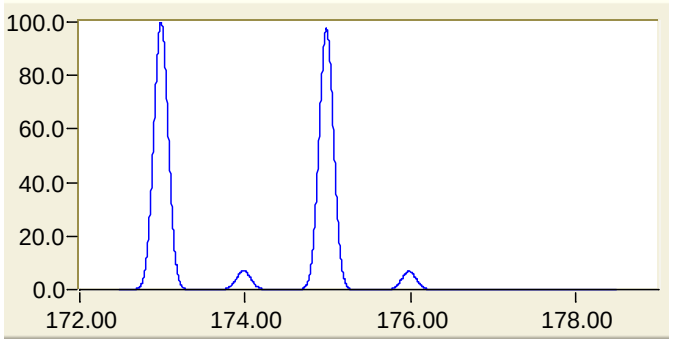
Note that I want "exact mass," not molecular weight, which is a natural abundance-averaged number. If the high-resolution mass spectrum gives a number that's very close to this (varies in the fourth decimal place), I have a "hit."

I can also get backwards from the splitting pattern to the molecular formula. Suppose I get the weight 171.9756, and I want to know what molecular formula it is.

Usually, one has some information on what elements might be present, so I tell Molecular Weight Calculator there could be carbon, hydrogen, oxygen, nitrogen, or bromine in the molecule:



In this relatively simple case, it found only one possibility. For more complex cases, there will be more hits. The program can also calculate the expected splitting pattern (Tools...Show Isotopic Distribution for Current Formula). This pattern can be compared against the observed pattern. Here, the complex pattern arises from the natural abundance of ^{79}Br (50.7%) and ^{81}Br (49.3%).



The Vector Model

Now that you have some idea of how to interpret NMR spectra, we can start talking a bit about how NMR works. There are three canonical treatments:

(1) The vector model is a semi-classical model. The individual nuclear spins are treated quantum-mechanically and have quantized angular momentum. However, the entire system is treated as an ensemble of spins which leads to a net magnetization vector. The effect of pulses and magnetic fields on this vector are treated classically

This is enough to explain any behavior involving single-quantum coherence: processes which only involve a single spin flipping at a time. E.g.: inversion recovery, the NOE, HSQC, etc.

This gives a lot of physical intuition, and we'll spend a lot of time thinking about it.

(2) The product operator formalism is a simplified, but fully quantum-mechanical model. It can explain the behavior of all the NMR pulse sequences we deal with, including those that involve multiple-quantum coherence. E.g., COSY.

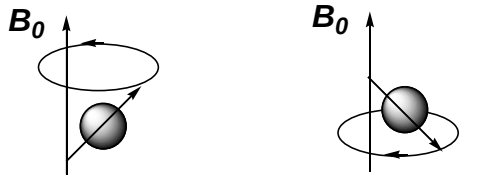
This is much more complicated, however, and I defer a full treatment of it till the end of the course.

(3) The density matrix formulation is a more general version of what's described by the product operator formalism. However, it is very cumbersome, and doesn't really lead to any physical insights you wouldn't get from the product operator formalism. Thus, I will not deal with it.

2D NMR: Density Matrix and Product Operator Treatment
Mateescu, G.D.; Valeriu, A.

http://www.case.edu/artsci/chem/faculty/mateescu/2dnmr/

In the vector model, we view each nucleus as a postively charged sphere which is magnetic and spinning. (Actually, the nucleus is *not* actually physically spinning, but we pretend it is.) By convention, the external magnetic field B_0 is represented on the z-axis:



***m* = +1/2 (α)** ***m* = -1/2 (β)**

The gyromagnetic ratio γ tells you how magnetic each sphere is. Each spin can align with or against the field, but only certain quantized angles between the nuclear spin moment and the external field are allowed. For a spin with a positive γ, the +1/2 or α state is lower in energy than the β or -1/2 state.

The nucleus never stops spinning and has angular momentum. The presence of the field causes it to precess at the **Larmor frequency:**

$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

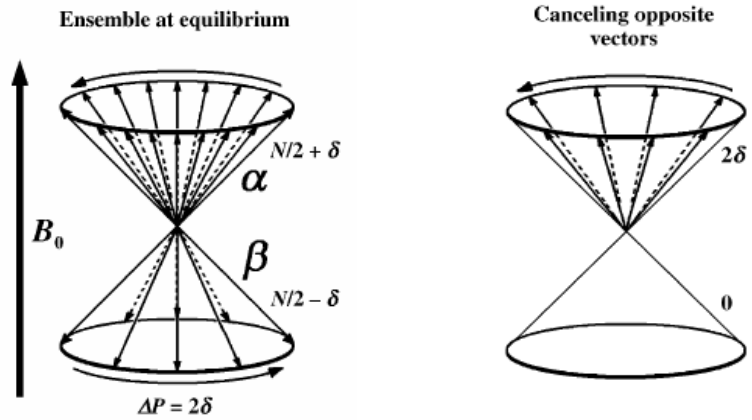
(This is in frequency units: Hz. For the angular frequency, do not divide by 2π.) When we say a magnet has a frequency of 500 MHz, we mean that protons in the magnetic field will precess at 500 MHz. Carbon has a γ which is one quarter that of proton, so a 500 MHz magnet for proton is also a 125 MHz magnet for carbon.

For this discussion, let's assume the nuclei are all protons. Proton is I=1/2, so only 45° angles between the spheres and the +z axis are allowed. Now, at equilibrium, the α state is slightly more stable than the β state.

Q: What is the bulk magnetization vector at equilibrium?

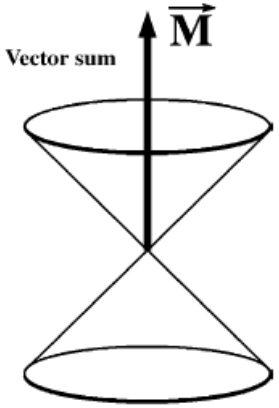
The Vector Model

The key here is that quantum mechanics doesn't say anything about the x- and y-components of the individual magnetization vectors. **If we assume the phases are randomly distributed at equilibrium, we have this diagram (Jacobsen, pg 158):**



(1) We start out with a slight excess of the spins in the α state. (They may be pointing in the +z or top part of the diagram, but they're lower in energy.) The excess is very small and has been exaggerated.

(2) Most of the top vectors have counterparts in the bottom cone with the same x- and y-components, but opposite z components. These components cancel.



(3) The x- and y-phases are randomly distributed, so the sum is pointing up towards +z. This is the **bulk magnetization vector.**

Despite the fact that there is a net vector at equilibrium, the sample doesn't produce any signal. For a signal to be picked up, there needs to be an oscillation, and nothing is moving yet.

Q: How big is this excess population?

The answer is given by the **Boltzmann distribution**, which gives this ratio:

$$\frac{N_\alpha}{N_\beta} = e^{-\Delta E/k_B T}$$

Here, N_α is the population of the a energy level. For now, you can think of it as how many spins are in the α state. However, later you will see this is not quite correct--in quantum mechanical language, we should really be dealing with superpositions, not eigenstates. ΔE is the energy difference between the spins, which depends on the magnetic field strength. k_B is Boltzmann's constant, and T is the absolute temperature.

At 750 MHz (really powerful), the population difference is only 0.012%:

$$N_\alpha - N_\beta \approx 10^{-4}$$

In a moment, we'll see that the signal strength depends on this population difference. If the population difference is so small, then why is NMR useful at all?

- the signal is weak, but there's not much background noise, so the contrast is quite high

- the lines are very narrow because the lifetimes of the states is long (energy-time uncertainty principle)

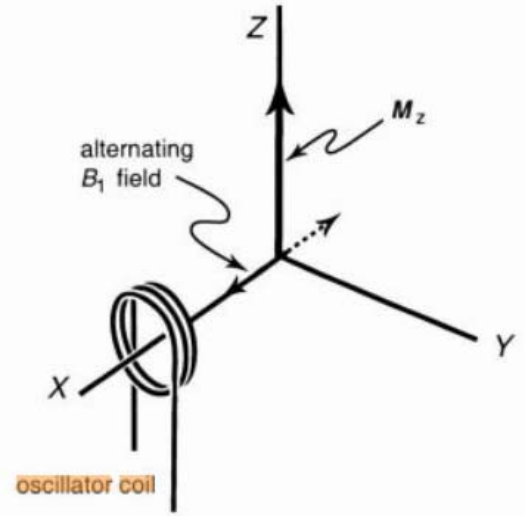
The Vector Model

This brings up an important point: **NMR is a "slow" method.** The states have lifetimes of at least seconds, so the "NMR timescale" is no faster than this. All translational, vibrational, rotational, and electronic motions are averaged. (Actually, there is no specific NMR timescale. Exactly how "fast" a particular experiment is will depend on exactly what is being observed.) Thus, NMR can only see ground states (in both a traditional electronic sense and the organic chemistry reaction coordinate sense).

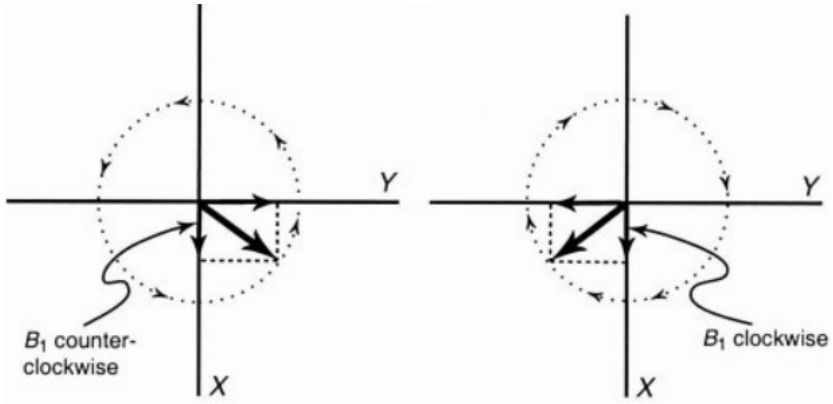
Now, we apply a pulse. The fundamental condition is that **a pulse will only affect the net magnetization if it has the same frequency as the spins**. In other words, the pulse has to be on resonance with the Larmor frequency.

Q: What happens when pulses are applied?

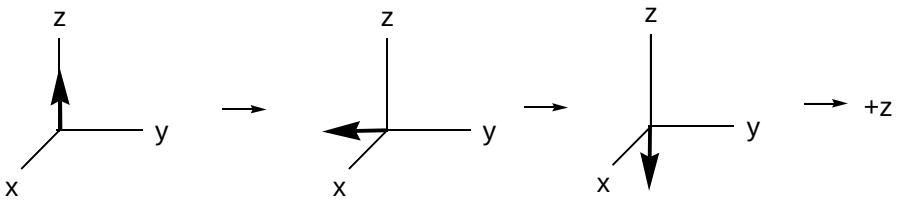
For this lecture, let us assume the pulses are being generated by an oscillator coil at +x (Roberts, page 18):



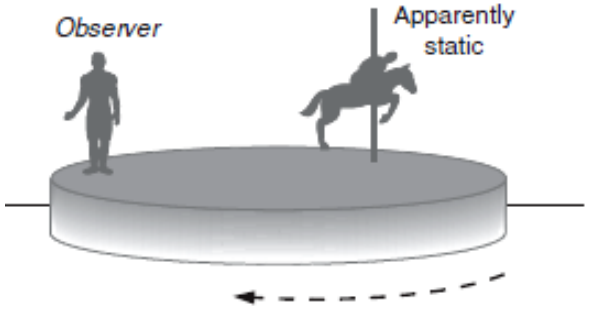
The pulse produces an oscillating magnetic field along the x-axis, which, by convention, is denoted B_1. This can be pictured as the sum of two counter-rotating vectors:



The application of a magnetic field in the xy-plane will cause the bulk magnetization vector to tip away from the z-axis. **In this course, we will follow the right-hand rule: pulses give counterclockwise rotations.** (Picture your thumb along B_1, your forefinger along M_z, the bulk vector, and your curled fingers as directing you counterclockwise.) The result is to rotate M_z through -y, -z, +y, and back to +z:

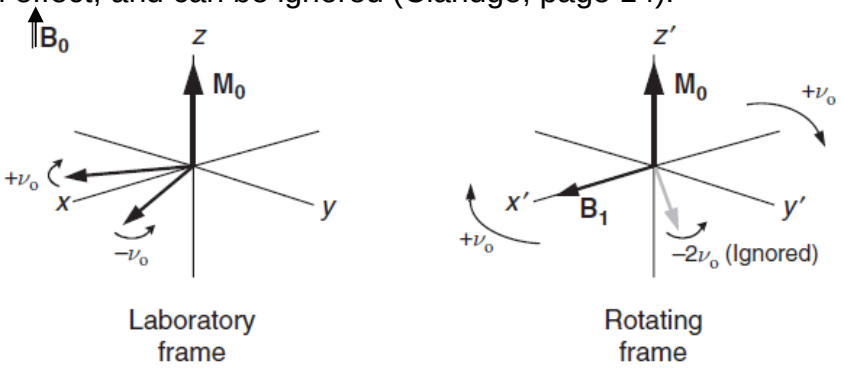


Note that there is a constant phase relationship between the B_1 field and the rotating bulk magnetization vector. This is easier to see in the **rotating frame** (Claridge, page 14):

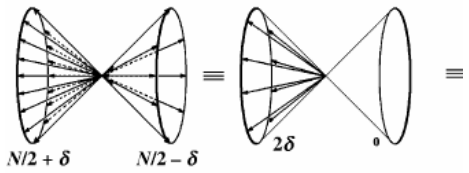


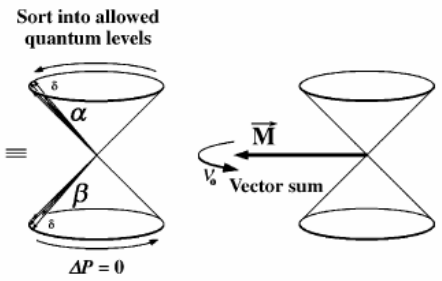
The Vector Model

If we set the rotating frame at the frequency of one of the B_1 component vectors, then one will be frozen on resonance, while the other one will be moving at twice the resonance frequency. This latter vector is too far from resonance to have an effect, and can be ignored (Claridge, page 14):



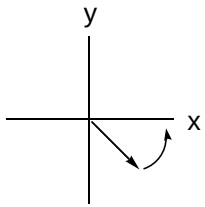
What does this look like in the "cone" picture? First, we rotate all the vectors by 90°. Then, we cancel the opposing components. At this point, we realize that some of these are not in quantum-mechanically allowed angles. We "sort these" and get the same result. (This is a bit of hocus pocus, but one has to expect some formal defects in a semi-classical model) Here is the picture (Jacobsen, page 161):



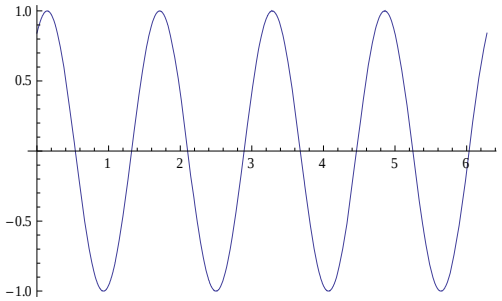


Notice that these bunched up vectors no longer have randomly disposed x- and y-phases. This is what we mean by **phase coherence**.

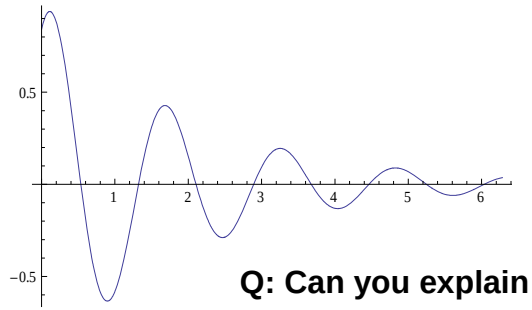
Once the bulk vector has moved away from equilibrium, it starts precessing in the total magnetic field. To see this, just apply the same right hand rule thinking to the B_0 field. Previously, the B_0 field had no effect, since rotation of a +z vector along the +z axis has no effect:



This, too, is a counterclockwise rotation about +z. If we put a detector at +y, we will get a sine wave (or some phase-shifted version of one):



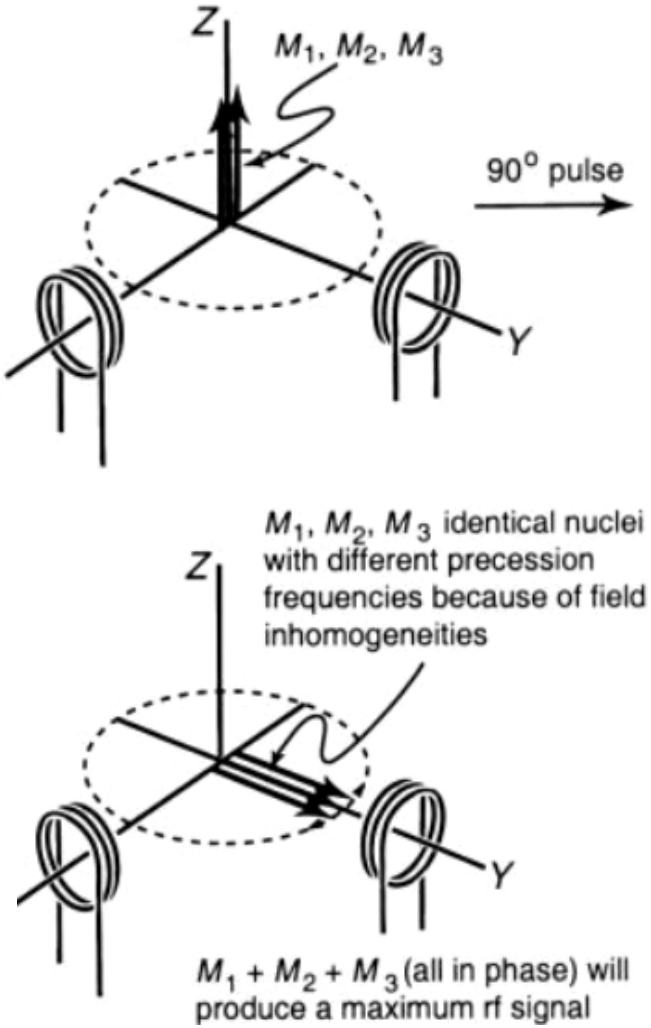
In real life, however, what is *actually* observed is something like this:



Q: Can you explain this?

Transverse Relaxation

This is **relaxation** at work. Suppose, for the sake of argument, that there is *no* z-magnetization and we can ignore the z-dimension. Imagine we have three separate nuclei: M_1, M_2, and M_3. But the magnetic field is not quite homogeneous, so they experience some slightly different magnetic fields, and therefore, have slightly different Larmor precession frequencies. The picture is (Roberts, page 36):



What happens is that the phase coherence will be lost. In the picture above, we are in the rotating frame, set at the frequency of M_2. This is a kind of **phase decoherence.** Empirically, it is found that this **transverse relaxation** (i.e., in the x-y plane) can be described by:

$$\frac{dM_{xy}}{dt} = \frac{-M_{xy}}{T_2^*}$$

This is classic first-order exponential decay where M_{xy} is Sqrt[M_x^2 + M_y^2] and T_2* is the characteristic time. Now, it turns out that in addition to relaxation due to magnetic inhomog-eneities, there are some intrinsic sample-related factors. For standard organic samples, T_2^{Bfield} is the most important:

$$\frac{1}{T_2^*} = \frac{1}{T_2^{sample}} + \frac{1}{T_2^{Bfield}}$$

There are several sources of T_2 relaxation:

(1) **Molecular motions:** As the nuclei move around in solution, they experience different chemical shifts depending on their orientation relative to other molecules. Although the *average* shifts are isotropic due to tumbling, there is still a spread of shifts that causes decoherence.

(2) **Chemical exchange:** This is a related process where a chemical process shuttles protons between sites of different chemical shift (like the alcohol on a substrate molecule and residual water in the solvent).

(3) **Spin exchange:** When two nuclei get close to each other, they can exchange spin orientations, and this can cause phase changes.

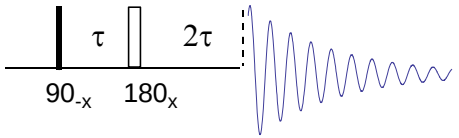
As it turns out, these *molecular-level* inhomogenities become more significant in more viscous solutions as the rate of tumbling decreases. (Transverse = spin-spin relaxation.)

The Carr-Purcell and CPMG Sequences

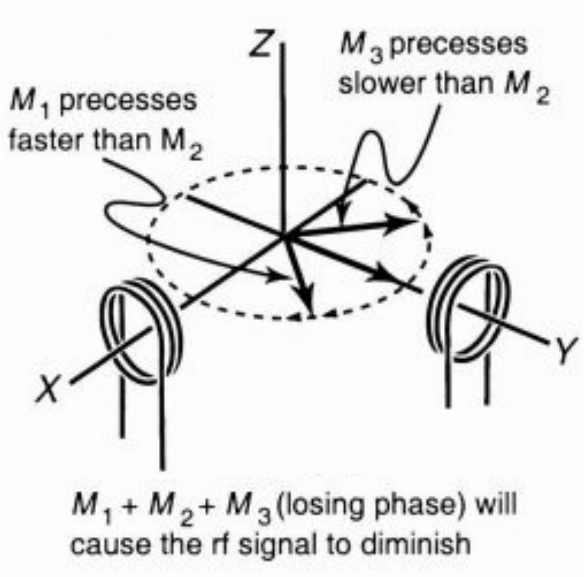
Q: How would you measure T_2^{sample}?

The answer is not very interesting from a strictly organic chemistry perspective, but the *method* we use *is* important. They are based on the **spin echo** concept, which we will see again and again in virtually every NMR experiment.

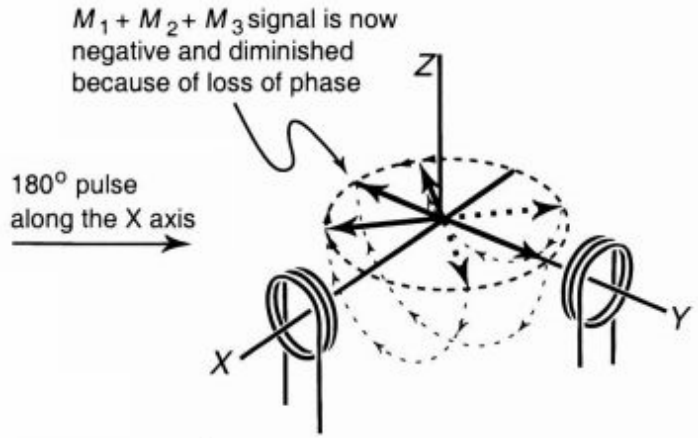
The **Carr-Purcell pulse sequence** is:



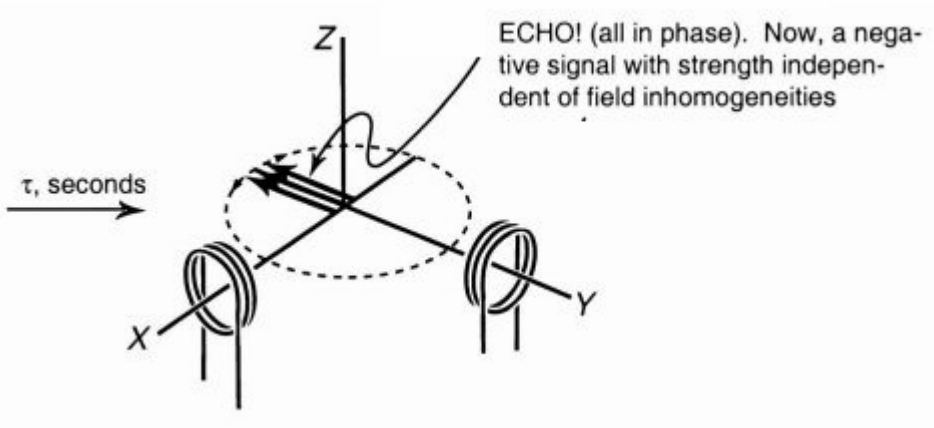
We'll be analyzing a lot of different pulse sequences. We'll follow the convention that thin, solid bars mean 90° pulses, while wider, open rectangles mean 180° pulses. The phase of the pulse is indicated by the subscript. The dashed line is just a marker; no pulse is implied. The oscillating signal represents the **free induction decay (FID)**, and is the time period during which data are being acquired. (Roberts, page 33.)



Let's take this step by step. After the first pulse, we generate vectors along +y. After some time τ, they fan out in the xy plane. The key is that when the 180_x pulse is applied, the vectors get reflected about the x-axis, but *maintain their direction*.

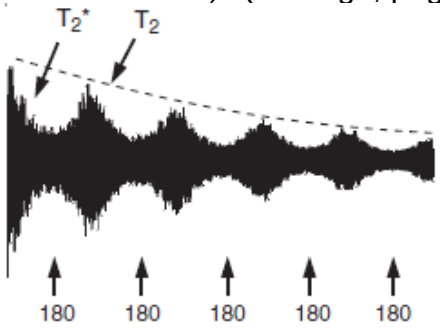


We are in the rotating frame at the frequency of M_2. The frequency differences between the vectors, or off*sets,* have not changed, so they continue their rotation in the same direction as before. M_1 is precessing faster than M_2, so it looks like it is speeding ahead (counterclockwise) in the rotating frame; M_3 is precesssing slower, and is going clockwise.



The Carr-Purcell and CPMG Sequences

After another period of τ, the vectors all get lined up again, but at the -y axis. This is a spin echo (a negative one). **The τ-180-τ sequence produces spin echoes.** The magnetic field inhomogeneities have been canceled out. The envelope of the overall decay represents T_2^{sample}, while the envelope of each fast decay is related to T_2* (Claridge, page 26):



For technical reasons, the negative echoes are inconvenient, so it is customary to apply the **Carr-Purcell-Meiboom-Gibb (CPMG) sequence** instead: 90_{-x}-τ-[$\mathbf{180_y}$-2τ]$_n$.

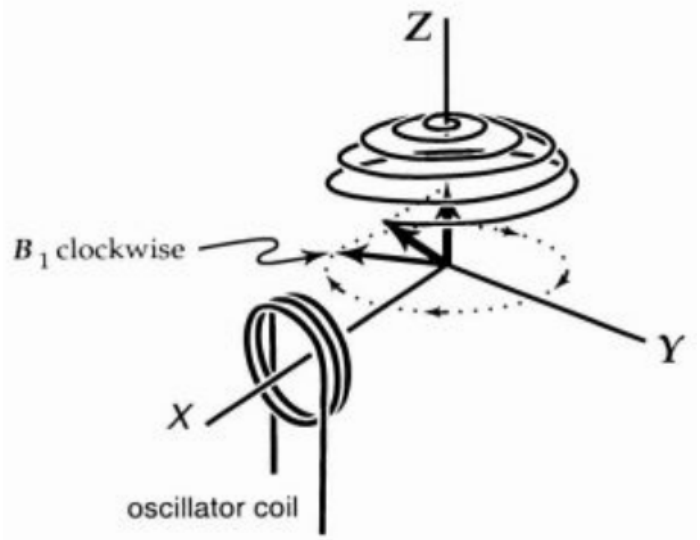
A key point to remember is that **the shorter T_2^* is, the faster relaxation is, and the broader lines are.** In general, the full width at half-maximum (FWHM) of a peak is $1/\pi T_2$*. You will see why this is in Lecture 5, when we talk about the Fourier transform.

Longitudinal Relaxation

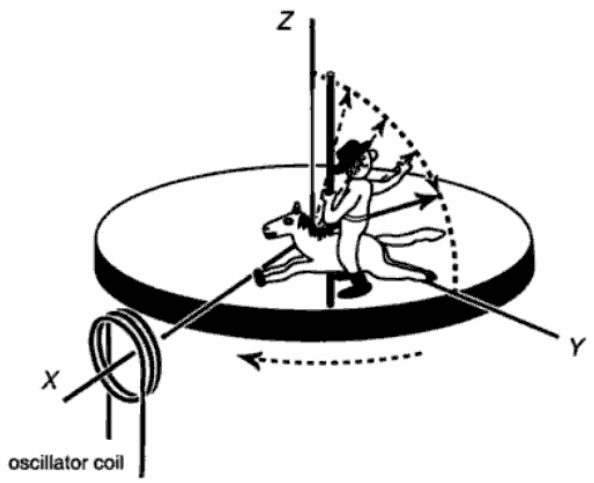
It turns out that z-magnetization also decays exponentially, but with a time constant T_1:

$$\frac{dM_z}{dt} = \frac{-(M_0 - M_z)}{T_1}$$

In general T_1 and T_2 are independent quantities, with T_2 being dominant for organic samples. In the laboratory frame, this means that as the vector precesses counterclockwise, its z-component is getting shorter and shorter. This gives rise to this picture (Roberts, page 22):



In the rotating frame, the vector just seems to move smoothly up to the +z axis. (It's turning back into M_0, the equilibrium magnetization vector.) (For some reason, Roberts follows a left-hand rule.)



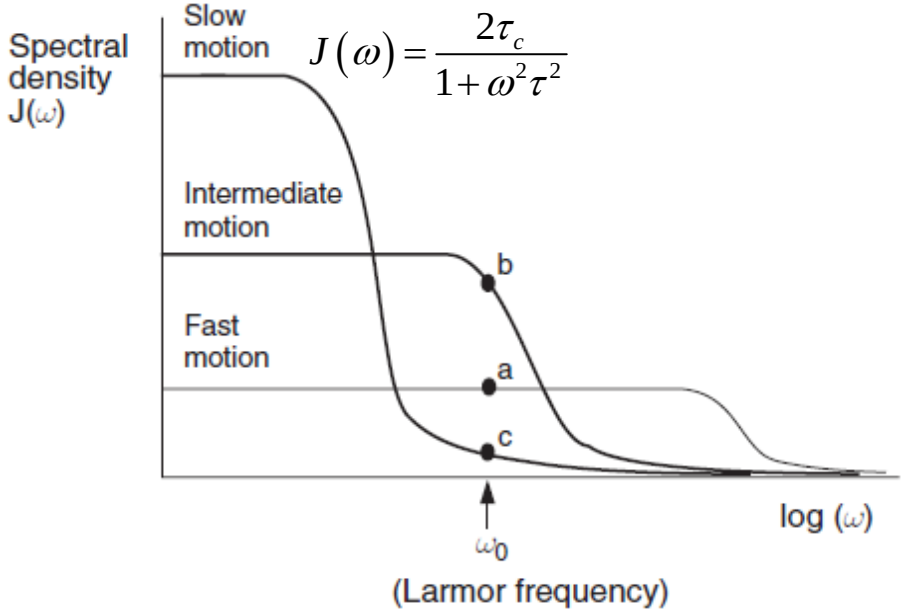
Q: What causes T_1?

Q: How can T_1 be measured?

Longitudinal Relaxation

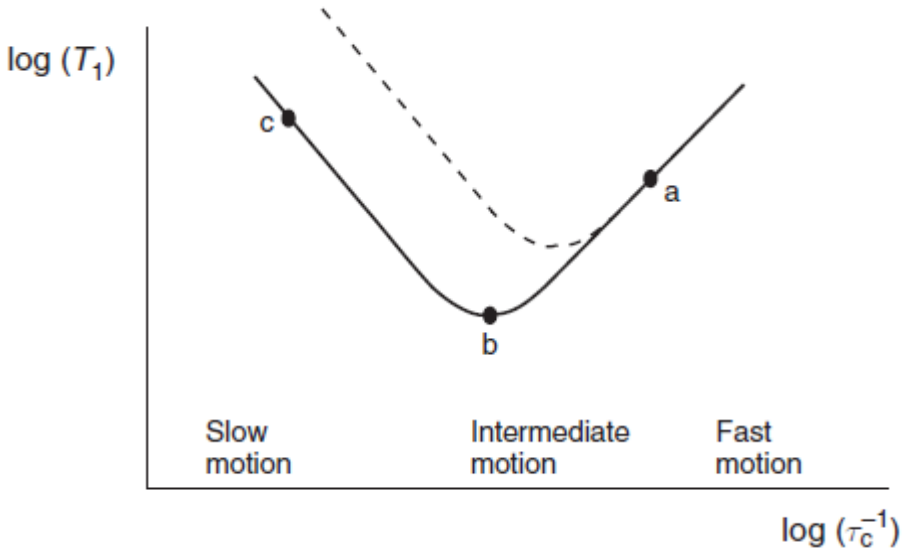
The fundamental requirement for relaxation is a fluctuating magnetic field **at the Larmor frequency**. A key concept is that of **correlation time**, the average time it takes to rotate a molecule through one radian as it tumbles in solution.

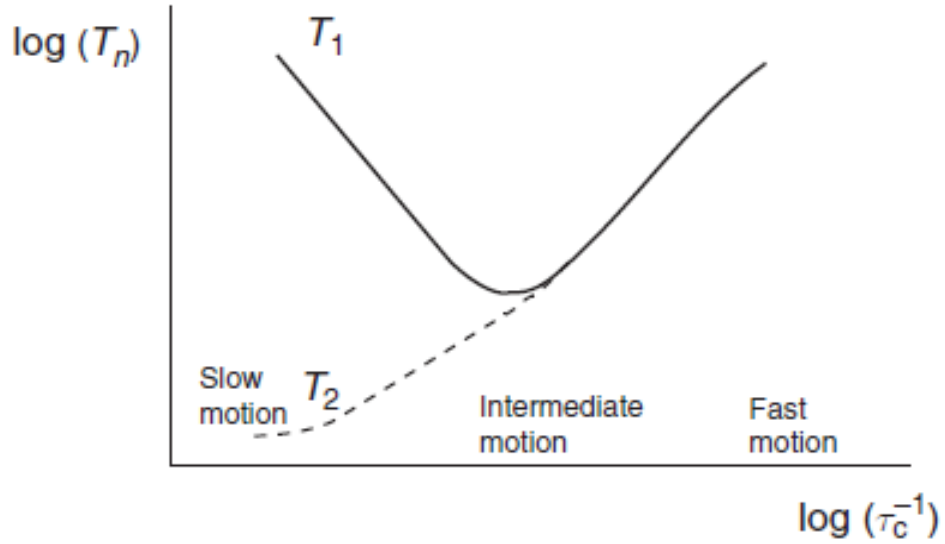
The **spectral density** tells you how much energy there is at every frequency. It depends on how fast the molecules are tumbling (Claridge, page 29):



Other molecular motions, like vibrations, rotations, etc. are too fast to be useful. The spectral density depends on the frequency of molecular collisions. Later, we'll derive this behavior and see why this is the case. The key conclusion is that **intermediate molecules have the narrowest lines**. This is shown on the facing panel. Note that the minimum moves to the right (smaller molecules) at higher field strengths (dashed line) since the Larmor frequency is different.

For small molecules, T_1 and T_2 are comparable, but for large molecules, $T_2 << T_1$ (proteins):





Q: What are the mechanisms for T_1 relaxation?

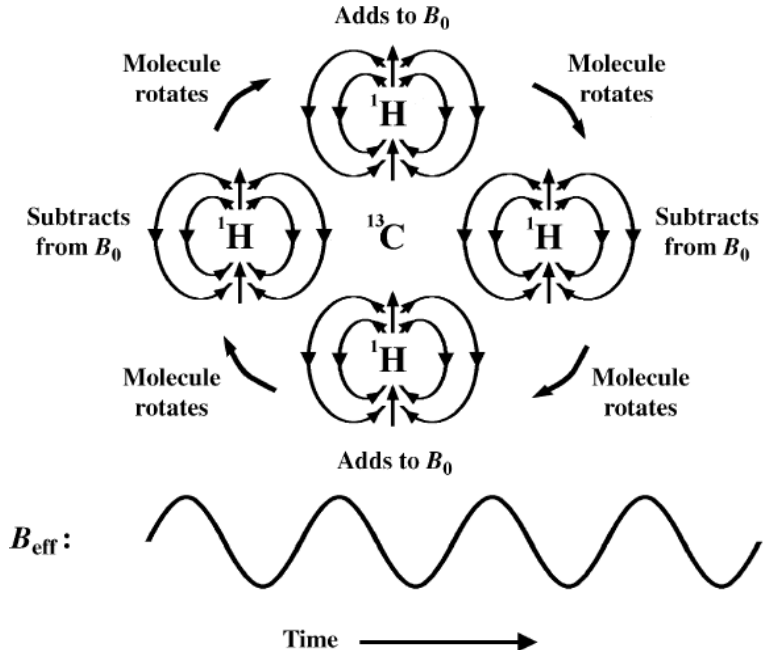
(1) dipole-dipole; (2) chemical shift anisotropy (CSA); (3) spin-rotation; (4) quadrupolar; (5) paramagnetic

Longitudinal Relaxation

We'll talk more about this in detail later, but here is a summary.

(1) Dipole-Dipole Relaxation

This is the most impotant kind of relaxation for small organic molecules. For example, consider a methane (Jacobsen, page 171). As the molecule tumbles, the protons around the carbon alternately add to and subtract from the local magnetic field felt by the carbon.



Just how good the relaxation is depends on "how magnetic" the surrounding dipoles are. Unpaired electrons are *very* magnetic (about 1000 times more than protons), so they give very effective **paramagnetic relaxation**. This is why $Cr(acac)_3$ is sometimes used as a relaxation agent to increase the rate of relaxation of carbons. Remember, quaternary carbons don't have as many surrounding dipoles and relax slowly, giving smaller lines.

(2) Chemical Shift Anisotropy (CSA) Relaxation

The electorn distribution in bonds is not symmetrical, so the effective field felt by the nuclei depends on the orientation of the bond with respect to the external field. This mechanism is most important for heteronuclei, which have a much wider chemical shift range.

CSA relaxation depends on the B_o^2.

Thus, it is actually possible to get better spectra at lower field strengths occasionally!

(3) Spin Rotation

This is essentially dipole-dipole relaxation, but between molecular dipoles and local dipoles, rather than two local dipoles. This can be identified if relaxation rates increase with molecular tumbling (the other mechanisms are less effective with faster tumbling).

(4) Quadrupolar Relaxation

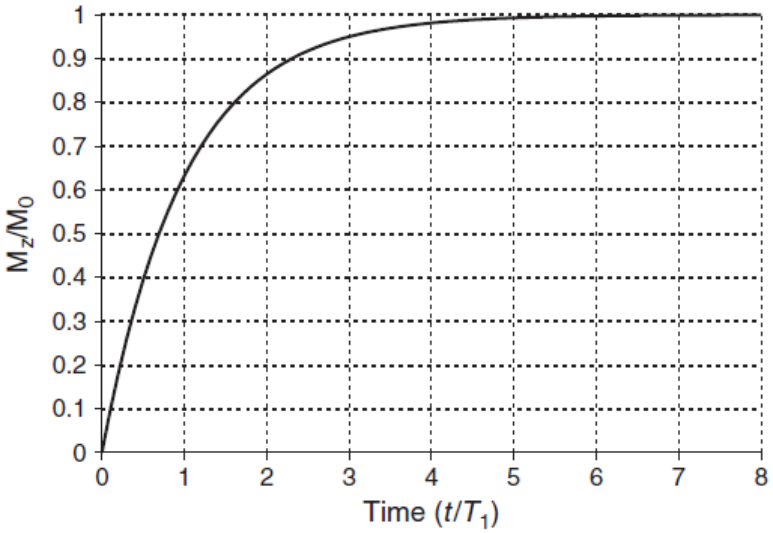
This is for nuclei with I > 1/2. The distribution of magnetic moments in quadrupolar nuclei is not symmetric, so the nuclear charge behaves as if it were spinning in an asymmetric manner. When the nucleus is surrounded by an asymmetric electric field, as the molecule it's in tumbles, the nucleus will pass through an electric field gradient. The nucleus will the reorient and its magnetic quantum number will change, causing relaxation. Thus, in symemtrical environments, this mechanism is less important.

Scalar couplings to quadrupolar nuclei get lost when the quadrupolar nuclei are in asymmetric chemical environments. So no chlorine splittings are seen in $CDCl_3$, even though Cl-35 and Cl-37 are I=5/2 nuclei.

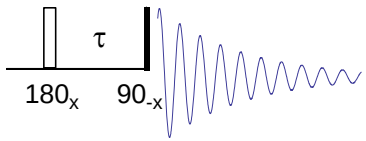
Inversion Recovery

Q: Why does this matter?

The exponential recovery of T_1 means that to get accurate integrations between spectra, one must wait at least $5T_1$ between scans (99.33% recovery):

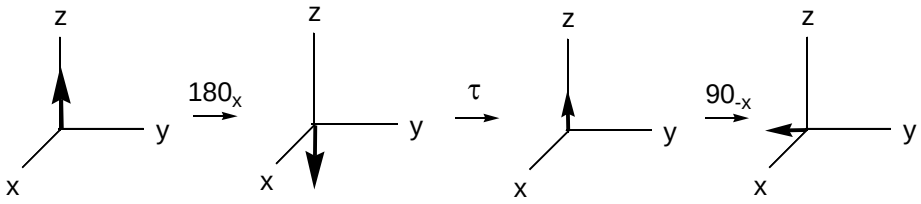


The obvious follow-up question is: well, how long is T_1? It varies depending on the chemical environment of each proton. In general, the more protons near the proton, the faster the relaxation. These can be measured easily using the **inversion recovery sequence:**

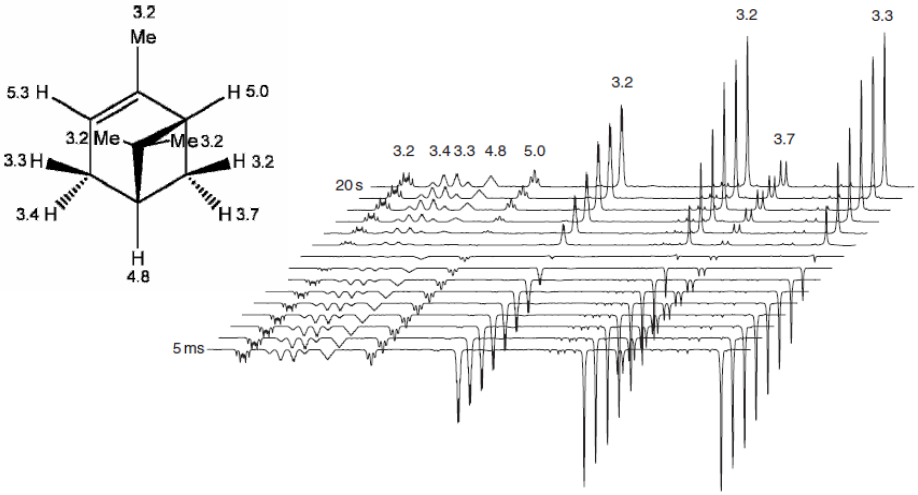


This places magnetization on -z, allows it to recover for a time τ, and then converts the longitudinal magnetization to transverse magnetization for observation. The intensity is:

$$M(\tau) = M_0\left(1 - 2e^{\tau/T_1}\right)$$



Typically, one runs the experiment for various times τ to make a stacked plot (Claridge, page 23):



The different phases represent different recovery times. The recovery to equilibrium can either be fitted to the above equation (more accurate) using a non-linear least squares method, or the "null time" (the τ it takes to null a signal) can be used (faster):

$$0 = M_0\left(1 - 2e^{\tau_{null}/T_1}\right)$$

$$1 = 2e^{\tau_{null}/T_1}$$

$$T_1 = \frac{\tau_{null}}{\ln 2}$$

In the spectrum above, you can see that the olefin protons take longer to relax.