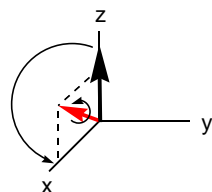


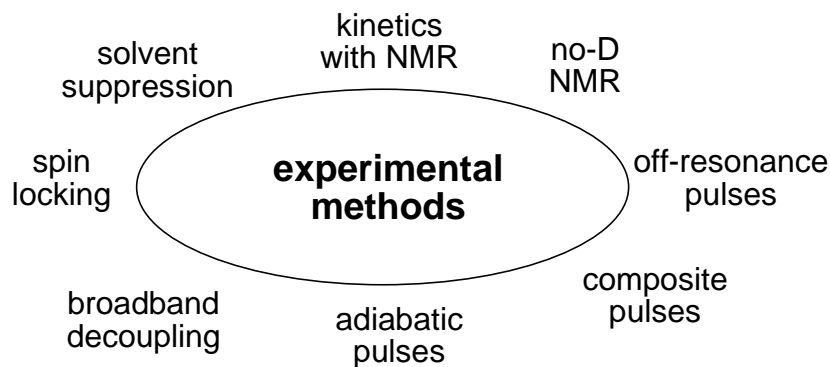
## Experimental Methods

Eugene E. Kwan

March 29, 2011.



### Scope of Lecture



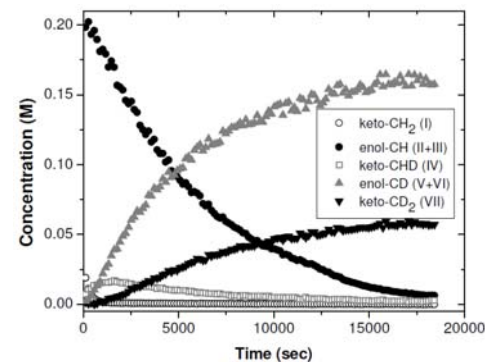
### Key References

1. Understanding NMR Spectroscopy (2nd Ed.) Keeler, J. Wiley, 2010.
2. NMR Spectroscopy Explained: Simplified Theory... Jacobsen, N.E. Wiley, 2007. (Chapter 8)
3. The ABCs of FT-NMR Roberts, J.D. University Science Books, 2000.
4. High-Resolution NMR Techniques in Organic Chemistry (2nd Ed.) Claridge, T.D.W. Elsevier, 2009. (**Chapters 10, 11**)

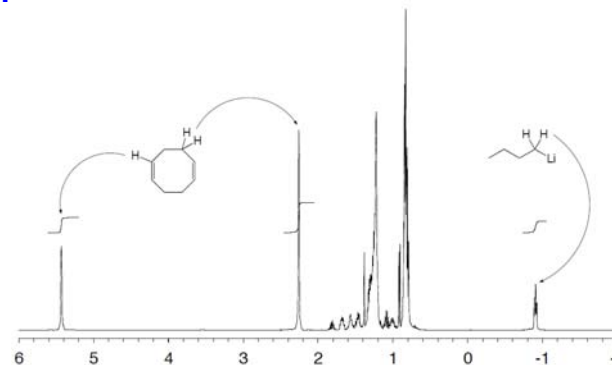
Many figures are from references 2 and 4.

### Key Questions

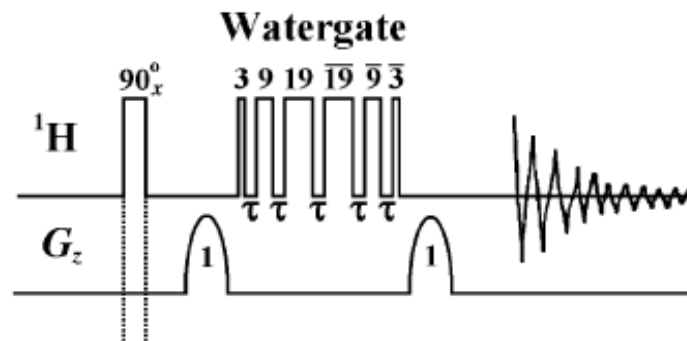
(1) How can NMR be used to obtain kinetic data?



(2) How can NMR spectra be obtained in non-deuterated solvents? Why is this useful?



(3) What are composite pulses? How can they be used to suppress solvent signals?



### Obtaining Kinetic Data with NMR Spectroscopy

For reactions that are slower than the timescale of a standard pulse-acquire experiment, NMR is one of the best ways to get reliable, structure-specific data about a chemical reaction. As it turns out, this generally means reactions that have half-lives of tens of minutes to hours. Why is NMR so great?

- different peaks correspond to starting material, product, etc. (as opposed to UV-vis or HPLC, where the detector's response is not necessarily related to any particular functional group)
- it's easy -- just tell the spectrometer to acquire spectra at regular intervals; the data are already digital
- temperature control is built right into the spectrometer
- degenerate reactions (starting material = product) can be studied with chemical exchange methods

Of course, not all reactions are suitable for NMR analysis. Reactions that must be performed at low temperature and that are sensitive to air or moisture can be problematic (although painstaking experimental technique can sometimes get around this). Reactions that are too fast are hard to study (rapid injection techniques are available with special hardware).

The fundamental idea is one you already know: **the observed spectrum is the concentration-weighted average of the starting material and product.** (This can be a bit of an approximation if there are any aggregation effects, but for most of us, this is good enough.) By measuring the integrals of key, diagnostic signals at periodic intervals, one can follow the progress of the reaction. Typically, these are referenced to an internal standard to correct for various changes over the course of the experiment.

Acquisition is generally done in the proton dimension, but high-sensitivity heteronuclear acquisition is possible. Ideally, samples should give good S/N on one scan.

### Typical Protocol

- (1) Prepare sample containing most of the reagents for the reaction, including the internal standard. The concentrations of every reagent should be accurately prepared.
- (2) Have the final reagent(s) ready in a syringe. Calculate the volumes such that a 5 mm tube will have a final volume of 0.7-0.8 mL.

**Note:** Solvents can boil or freeze! Stay at least 10 to 20 °C away from these phase transitions to avoid the ignominy of being the one to damage the probe.

- (3) Ready the spectrometer (have the lock values set in the right range, recall the default shims, have the commands for the kinetics run ready to go). If the reaction will not be run at room temperature, it may make sense to have both your reagents and a dummy NMR sample of similar composition thermally equilibrated at the desired temperature. The dummy sample can be locked and shimmed on to minimize the amount of time spent before acquiring any data.

**Note:** The variable temperature (VT) control is not accurate. The temperature setting must be calibrated beforehand. For details, see: *J. Mag. Res.* **1982**, 46, 319 (MeOH or ethylene glycol); *J. Mag. Res.* **1998**, 36, S118 (tris(TMS) methane).

- (4) Inject the final reagent and begin the run.

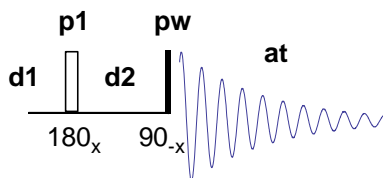
**Note:** You can spin the sample to enhance mixing. Heterogeneous reactions are generally *not* suitable for this technique. Of course, *never* put a stir bar in the magnet. (Duh.) That could have hilarious, but unfortunate results.

- (5) Collect scans at pre-determined intervals and use a computer program to turn this into rate vs. time data.

## Obtaining Kinetic Data with NMR Spectroscopy

### Q: What about $T_1$ ?

As you know, if there is insufficient time between scans, the integrals will not be accurate due to differential  $T_1$  relaxation. One can measure  $T_1$  values by inversion recovery (for details, see the end of Lecture 4). On Varian spectrometers, this can be performed by using the standard two pulse experiment (s2pul). That is, loading the parameters for a standard pulse-acquire experiment will give this pulse sequence (use **dps** to see this):



The observed signal is given by:

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

where  $\tau$  is d2. When this sequence nulls a particular signal, its  $T_1$  is approximately  $d2_{\text{null}} / \ln 2$ . For the purposes of kinetics, this null method is good enough. The goal is to wait at least  $5T_1$  between scans.

Ideally, good S/N should be obtained in one scan; otherwise, the many  $>5T_1$  delays may force the interval between data points to be rather large. (Despite the fact that many people seem to insist on many scans, there is no advantage to this for typical proton samples.) Recall from Lecture 5, slide 7 that under the default parameters, an "autogain" is performed before every scan. This means that a series of dummy scans with varying gain until the ADC is appropriately "filled up." (I.e., gain is reduced to prevent overflow or increased to fill up the entire dynamic range.)

These dummy scans will cause undesirable spin saturation and generally waste time since the required gain should remain the

same over the course of the experiment. Therefore, it makes sense to pre-determine what the gain should be before starting the kinetics experiment.

Here is how to calibrate the pulse width and perform a  $T_1$  inversion recovery experiment:

- (1) prepare sample; lock and shim
- (2) nt=1 bs=1 d1=1 ss=0 pw=pw90 ga
- (3) gain? (note the returned value)
- (4) gain = xx (xx is whatever the value was)
- (5) ga (acquire the spectrum)
- (6) wft ai (display in absolute intensity mode to allow direct comparisons between spectra)
- (7) pw=4\*pw90 (set the pulse width to the approximate 360° value; pw is the value that will be used in the experiment while pw90 is a default number set for your solvent and nucleus)
- (8) adjust pw to give a null spectrum (if the peaks are negative, increase pw and vice versa)
- (9) once the null value has been found, reacquire a spectrum with pw=pw/4, which will give the 90° pulse width
- (10) pw90=pw (tell the spectrometer what the correct 90° pulse width is)
- (11) p1=2\*pw90 (set the 180° pulse for inversion recovery)
- (12) d2=2 ga (a useful starting point for small molecules)
- (13) adjust d2 to null most of the peaks
- (14) compute the approximate  $T_1$  as  $1.4*d2$

$T_1$  values will undoubtedly vary amongst the nuclei of the molecule. Consider the peaks you are integrating. Use their *maximum* (rather than average)  $T_1$  value for calculating the  $5T_1$  delay time.

Although I have given procedure here for reference, pulse calibrations are not critical in standard kinetics experiments; as long as each pulse in the experiment is the same length, there is no trouble. In contrast, the **ernst(xx)** command, which sets the tip angle to give maximum S/N for a given  $T_1$  time of xx seconds,

### Obtaining Kinetic Data with NMR Spectroscopy

is **not** appropriate. Remember, this is for experiments with  $d1=0$ . This means pulses are applied very quickly, with only enough time being given between scans to collect the FID and let  $T_2^*$  take away the transverse magnetization. For maximum S/N, it makes sense to use a pulse width that is close to  $90^\circ$  (since we are waiting  $>5T_1$  between scans anyways). For the inversion recovery experiment, however, an accurate pulse width *is* useful.

Once the spectrometer is properly calibrated and your reaction starts running, you will need to tell the spectrometer to acquire spectra at regular intervals using the **pad** parameter. This is the "preacquisition delay"--the time the spectrometer waits between each scan.

You can use the "array" command to set up pad. Let's say you want to record scans at exactly regular intervals. Here's what happens when you type that into VNMR:

Parameter to be arrayed? pad  
 Number of increments? [this is how many data points you need]  
 Enter starting value: [this is the time between data points, in seconds]  
 Enter array increment: 0 [this means there will be a constant delay between scans]

*Caution:* The array increment is not the delay between scans.

This generates an array called pad. You can see the elements of the array by typing `pad[1]?`. That will tell you what is in the first element of pad. Set `pad[1]=0` to tell the spectrometer to begin acquiring data immediately. All of this can be done before starting your reaction.

For a direct comparison between your spectra, you will want to use absolute intensity mode (**ai**). Here are some useful commands:

`ds(n)`: display spectrum  $n$

`dssa`: display all spectra vertically  
`dssh`: display all spectra horizontally

If you select a region in a particular spectrum (in `ds(n)` mode) with the mouse cursors and click on expand, then the same region will be expanded in `dssa` or `dssh` mode. This can be useful for monitoring the height of a peak as the experiment progresses. To return the display to the full range of the spectral window, type "full." This is analogous to the "f" command.

Set all the other acquisition parameters. For a one-scan per data point protocol: `at=2, d1=0, bs=1, ss=0`. At the conclusion of your experiment, save your FID as usual with the **svf** command. Processing is conveniently done with ACD/NMR Processor (free for academics). If you want to learn the details of how it works, try doing the tutorial provided in the manual.

This will automatically integrate the peaks in your spectra. If you have too many spectra, this could take a very long time even with automation, so keep the number of data points under 200.

- (1) Tools...Quantalyst [brings up a dialog box]
- (2) Click on "Insert..." to define a quantitation variable.
- (3) Give the variable an appropriate name and check "keep as result."
- (4) Under "object" select "integral" and under property select "abs value"
- (5) Click on "options" to indicate the range of chemical shifts you want to integrate over. Repeat this process for every peak of interest.
- (6) Click "run" to perform the analysis and "close" to exit the dialog box.
- (7) Tools...Quantitation Graph. Right click to bring up a properties box and click on "select data."
- (8) Under "select x-axis," choose "d2."
- (9) Select the appropriate variables under "user data" and display the graph.
- (10) To export this data, right click on the graph and click on

### Obtaining Kinetic Data with NMR Spectroscopy

- (10) "table of data."  
 (11) Right click on the table and choose "export table..." This data can be imported into Excel or your favorite data analysis program.

For spectra with high S/N, the use of integrals is the most accurate method. For spectra with more noise, peak height is more accurate. In the latter case, be sure to pick the peaks of every signal of interest in each spectrum of the spectral series using the peak picking tool mode. Additionally, it will be necessary to have two variables for every peak whose intensity you want to record.

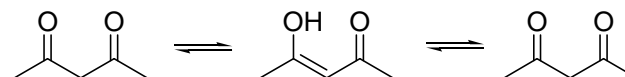
For example, if you want to record the height of a peak at 1.00 ppm, use this procedure:

- (1) Pick the peak at 1 ppm in every spectrum using the manual peak picking command
- (2) Enter the quantitation dialog box as before
- (3) Call the first variable "p1" and select "peak" as the object type and "position" as the property. (Do not keep this as a result.) Under options, select 1.00 ppm and enter in a tolerance.
- (4) Call the second variable "p2" and select "peak" as the object type and "absheight" as the property." (Keep this as a result.) Under options, type in p1.
- (5) Return to the main quantitation dialog box and ensure that p2 appears below p1 using the "up" and "down" buttons.

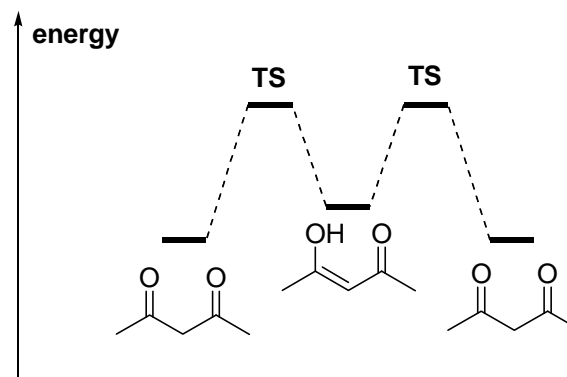
Use the rest of the procedure given above for using integrals to complete the analysis. You can correct the signal areas or intensities to that of the internal standard in your spreadsheet program. The rate data themselves can be analyzed in many ways: slope of log [concentration] vs. time for a pseudo-first-order reaction; non-linear least squares fit to a rate law; reaction progress kinetics; etc. These rates can then be obtained at different temperatures to get an activation barrier via the Eyring equation. However, a detailed discussion of these techniques is outside the scope of this lecture.

"Kinetic and Mechanistic Studies of the Deuterium Exchange in Classical Keto-Enol Tautomeric Equilibrium Reactions." Nichols, M.A.; Waner, M.J. *J. Chem. Ed.* **2010**, *87*, 952-955.

For reactions at equilibrium, NMR is amazing in that it can look at both the difference in equilibrium energies and transition state energies! For example, consider the classic case of keto-enol tautomerism in 1,3-diketones:

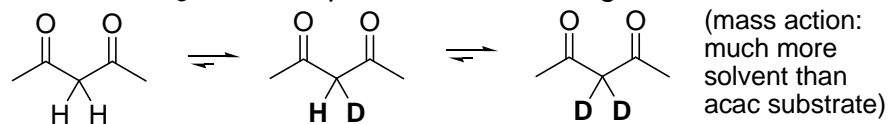


Now, the details of exactly how this occurs may well involve solvent or some other more complicated mechanism, but for our purposes, it can be thought of as this double well potential:



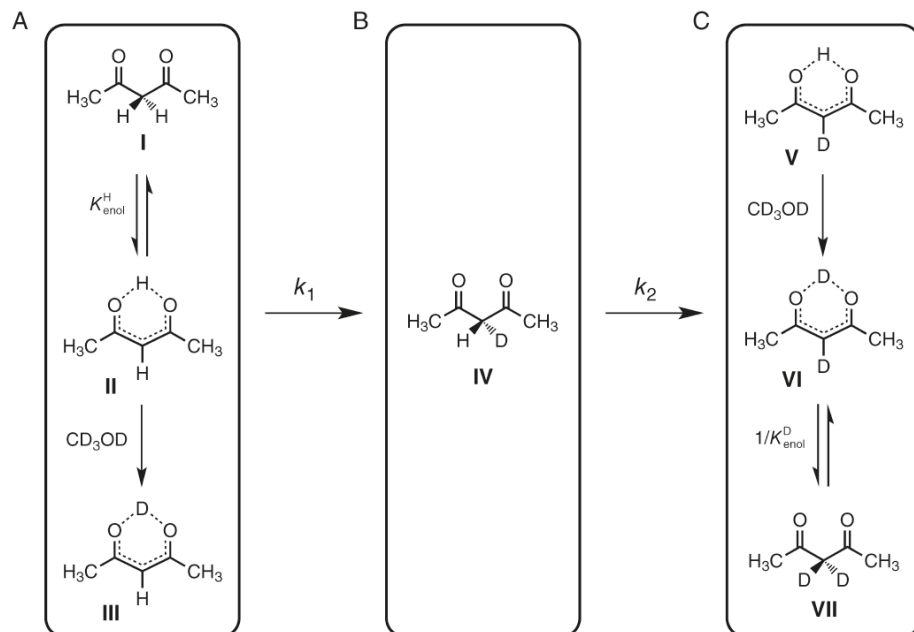
Now, depending on the rate, we could try to study this by selective inversion, lineshape analysis, or CPMG. These methods are pretty awesome. Why? The integrals of the peaks for the keto and enol forms gives the ground state energy difference--i.e., the equilibrium constant. The rates give the barrier heights between the *degenerate* ground states!

There is another clever way to do this. Imagine that we put acac into CD<sub>3</sub>OD. The protons will exchange out:



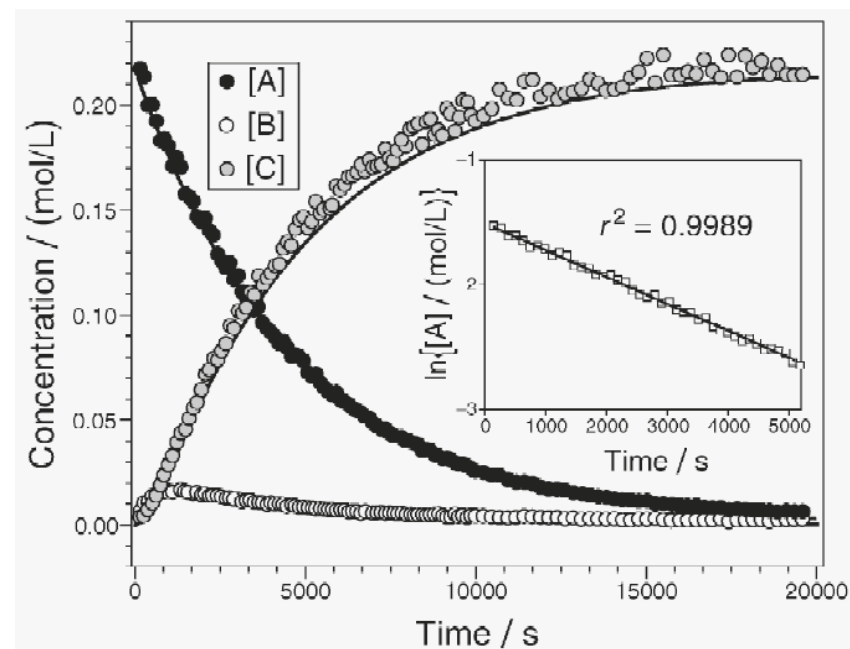
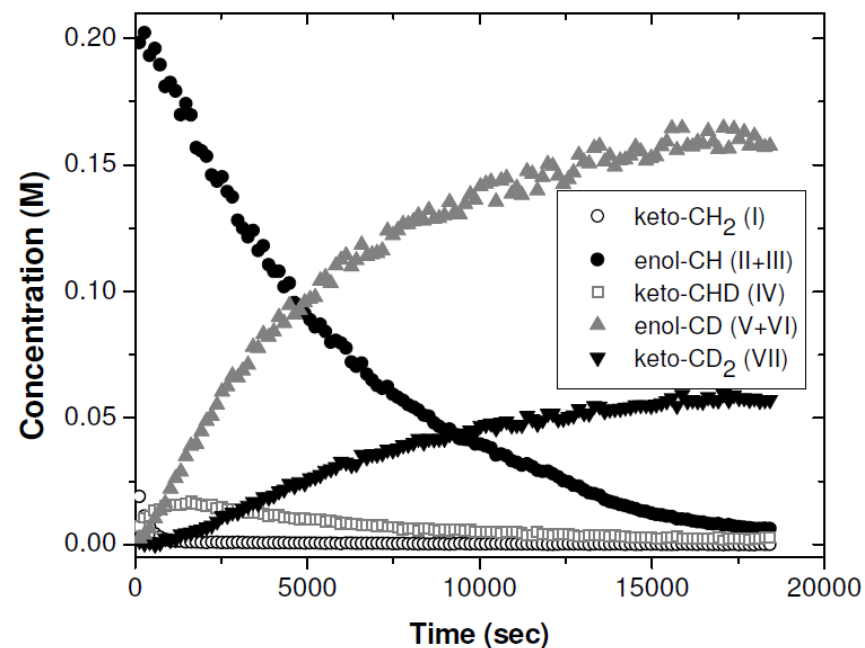
### Obtaining Kinetic Data with NMR Spectroscopy

(As you know, the mono-deutero acac will show a 1:1:1 triplet at the methine between the ketones, while the di-deutero acac will have no proton signal at all between the ketones.) What isotopomers might be in the reaction? Here is the analysis:



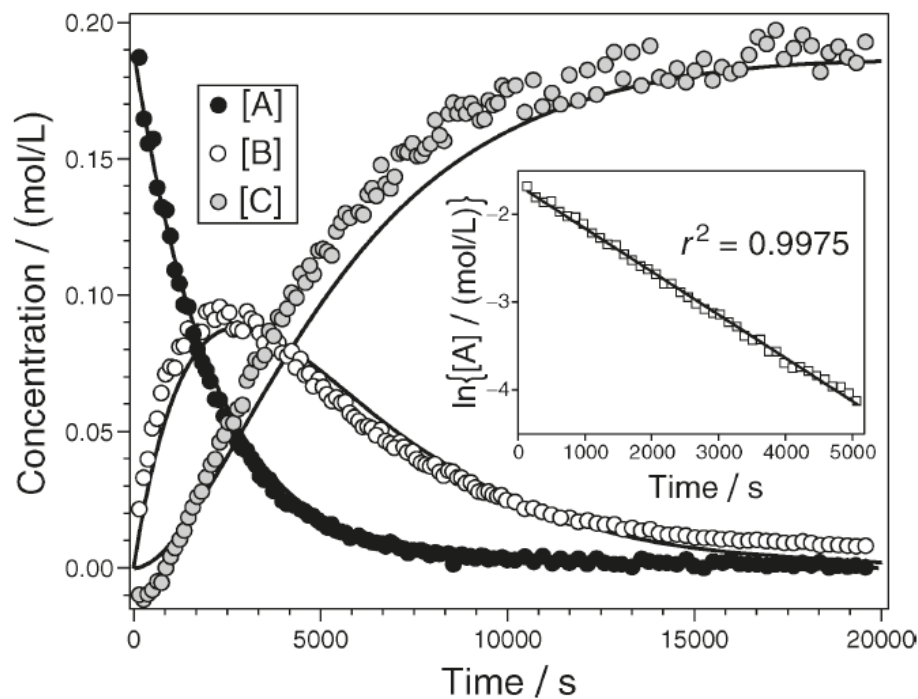
Neither the protio-enol isotopomers **II** and **III** nor the deuterio-enol isotopomers **V** and **VI** can be distinguished by NMR (as I mentioned before, isotopic substitution generally has a small effect on chemical shifts). Furthermore, the species that can be distinguished have a complicated kinetic behavior (see facing panel). Therefore, **I**, **II**, and **III** are combined as reactant **A**; **IV** is shown as the intermediate **B**; and **V**, **VI**, and **VII** are shown as product **C**.

A plot of concentration vs. time shows the "reaction progress kinetics." Starting material **A** decays in a pseudo-first-order fashion. The intermediate **B** builds up for a time, and then goes away. The product **C** builds up as equilibrium is reached.



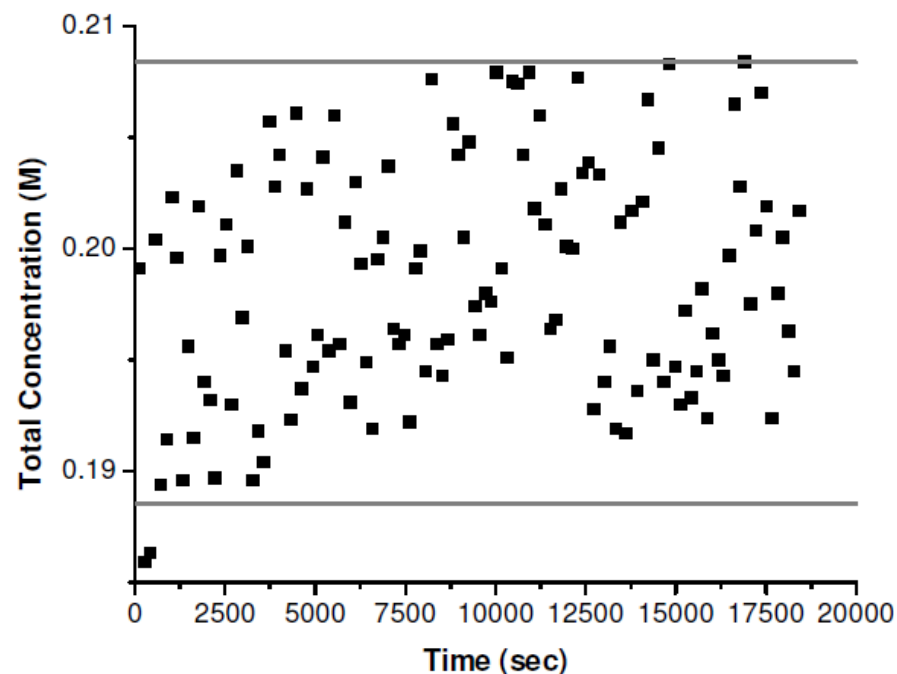
### Obtaining Kinetic Data with NMR Spectroscopy

Note that the enol is much more stable with acac than with ethyl acetoacetate (80% vs. 10% enol). With ethyl acetoacetate, the intermediate **B** is clearly not in a steady state:



A detailed rate analysis shows that these rates are on the order of  $1 \text{ s}^{-1}$  (A 20 000 second reaction means about 6 hours--perfect for an overnight NMR experiment.)

In this experiment, an internal standard was used. A sealed capillary of ca. 0.5 mM benzene in  $\text{CDCl}_3$  was used. This capillary was calibrated before the experiment to a solution containing known masses of THF and  $\text{CH}_2\text{Cl}_2$ . This was a bit elaborate. For most applications, a "direct" internal standard placed directly in the reaction medium will be sufficient. One can analyze the errors in the normalized integral data by plotting the total mass balance over time. In this case, it was good to within 5%. This gives a reproducibility in these  $k$  values of two decimal places--enough for quantitative work.



### No-D NMR

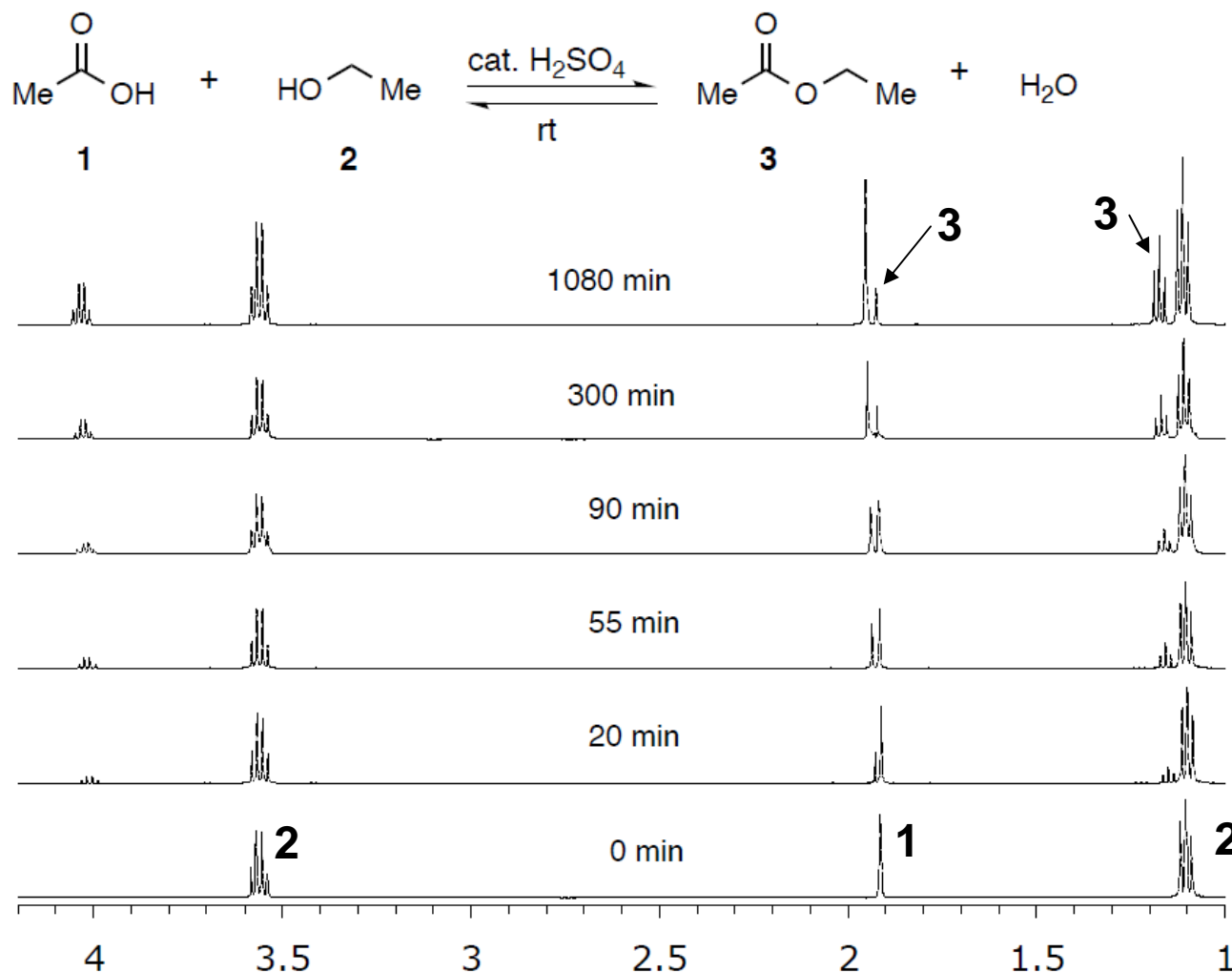
**Q: How can reactive intermediates be analyzed?**

"No-D NMR (No-Deuterium Proton NMR) Spectroscopy..."  
Hoye, T.R. et al. *Org. Lett.* **2004**, 6 953-956.

Most organic chemists take their NMR spectra in a deuterated solvent. This is because the solvent is usually much more concentrated than the substrate; if the solvent had observable proton signals, they might overwhelm the substrate signals. However, modern analog-to-digital converters are now 16-bit, meaning they can easily handle the dynamic range of relatively concentrated reactions. For example, consider THF. It has a density of 0.89 and a MW of 72, which works out to about 12 M. If you run a reaction at 0.01 M in THF, then the dynamic range is of the order of 1200:1. This is well below the range given by a 16-bit ADC:  $2^{15}-1:1 = 65\,535:1$ . (In reality, solvent peaks can be broad, which can still pose a problem. Nonetheless, for relatively concentrated reactions, this is good enough.)

**No-D NMR**

Now, of course, if dynamic range is not a problem for relatively concentrated solutions, what about the field lock? Typically, the stability of the magnetic field ( $B_0$ ) is maintained by a feedback loop on the deuterium channel. If there's no deuterated solvent, then is this a problem? It turns out that it isn't. Modern NMR spectrometers are quite stable, and if one doesn't have to do a lot of signal averaging (which you wouldn't if the sample was of an appropriate concentration), then the lock is basically unnecessary. Thus, the conclusion is that it is appropriate to acquire the NMR spectra of reagents, reaction mixtures, or other relatively concentrated (0.01-1 M) solutions without using deuterated solvents. Here are spectra from the Hoye report for an esterification:

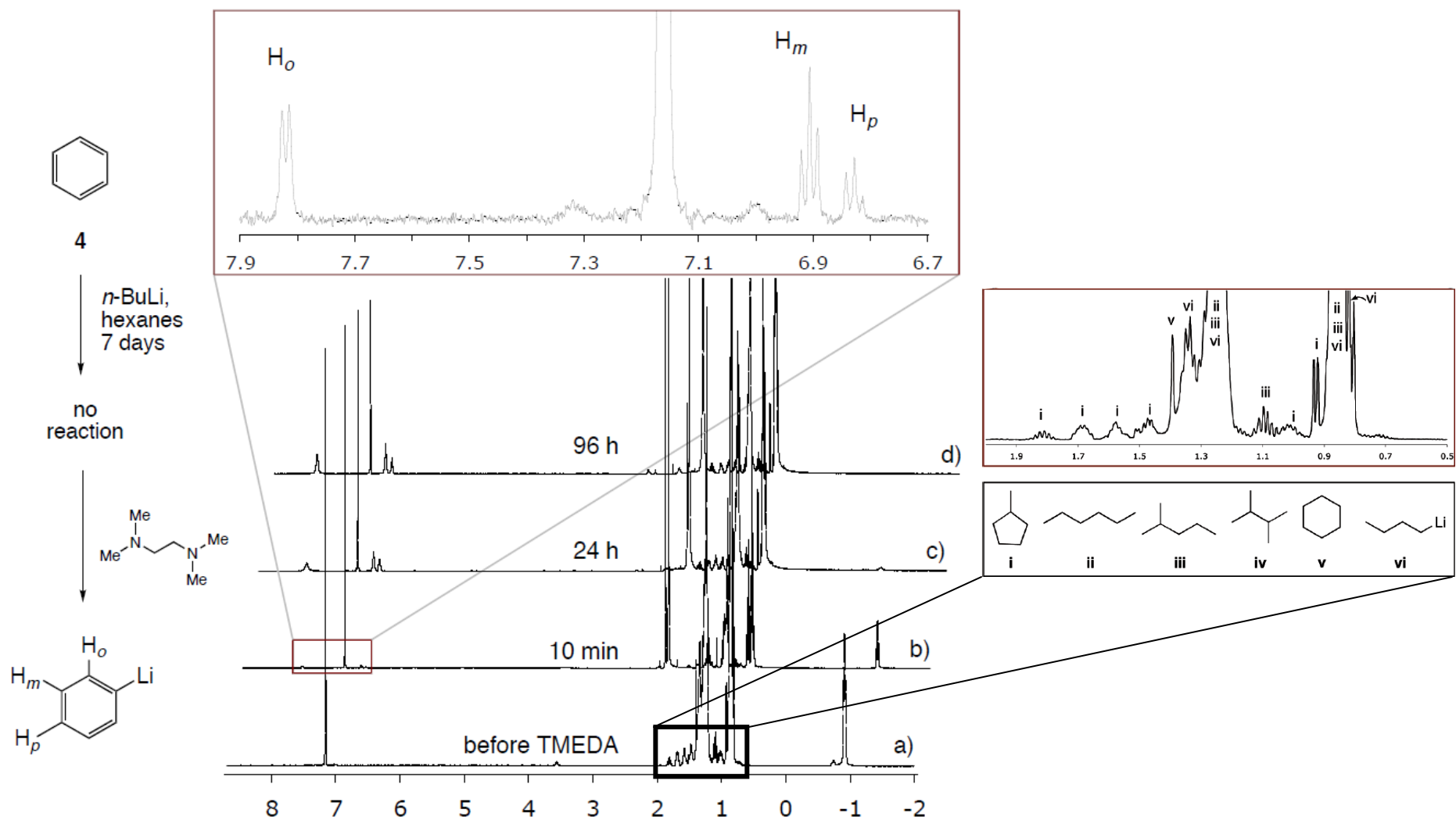


4:1 molar ratio of EtOH/AcOH  
with 1 mol% H<sub>2</sub>SO<sub>4</sub>



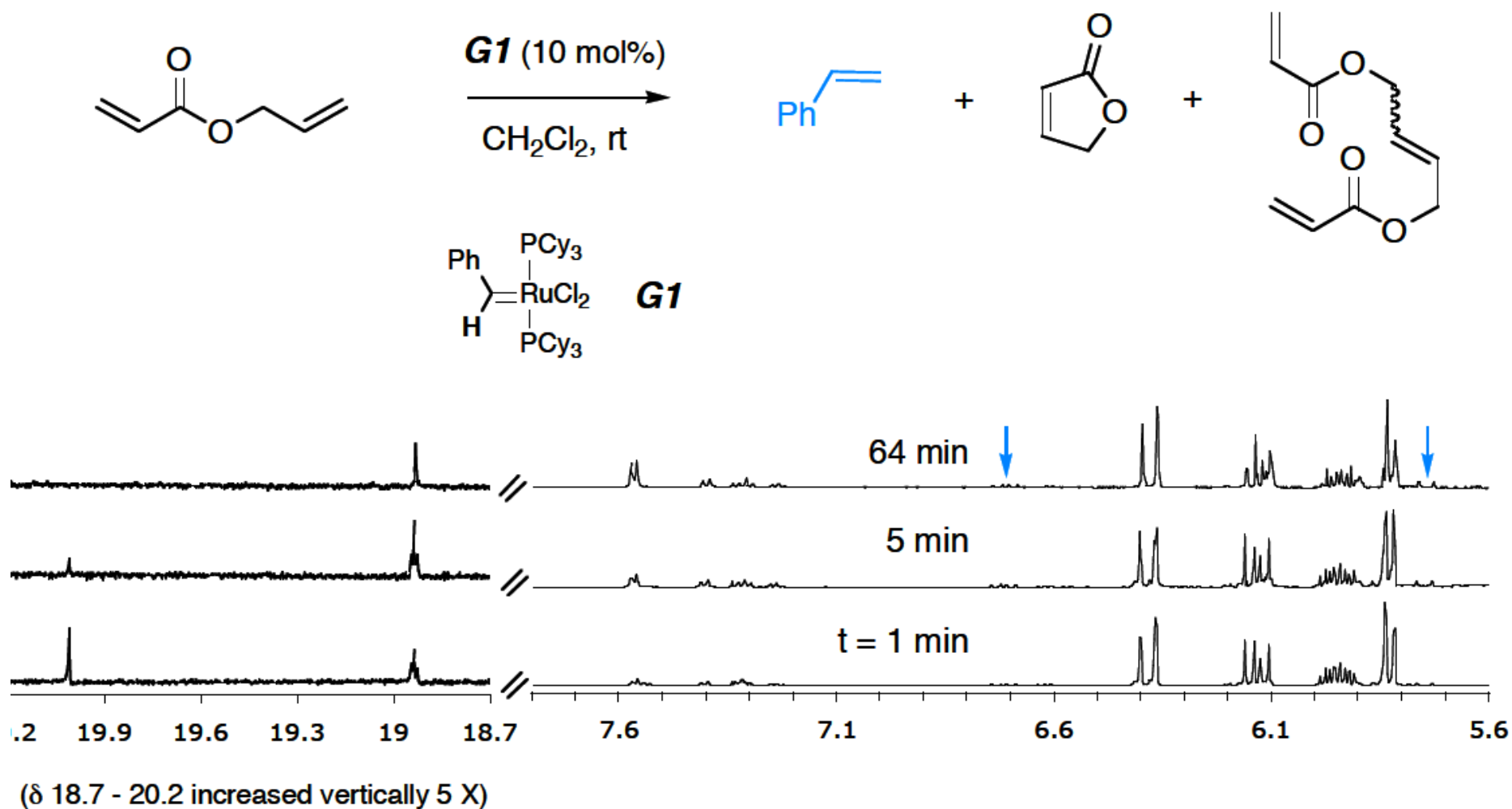
## No-D NMR

Here's another example. Mixing benzene and *n*-butyllithium only gives lithiation if TMEDA is added. In the "before TMEDA" spectrum, you can clearly see the various components of hexanes:



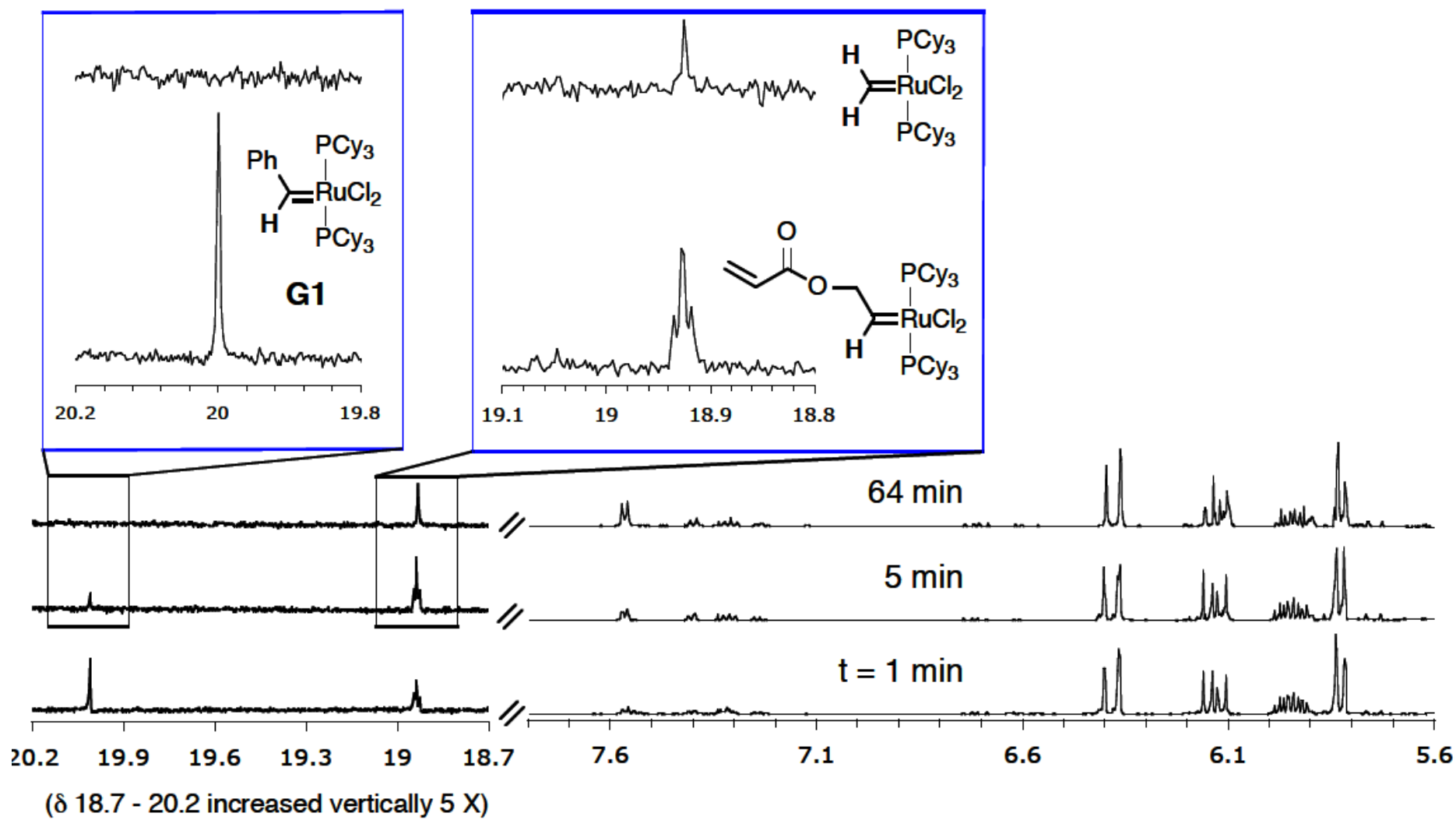
**No-D NMR**

Here's a more sophisticated example involving a ring-closing metathesis. The carbene protons of ruthenium alkylidenes are very downfield. At the beginning of the reaction, the Grubbs first-generation pre-catalyst is clearly visible. Another carbene signal, corresponding to the ethylene and substrate carbenes can be seen growing in. The butenolide signals are growing in as well.



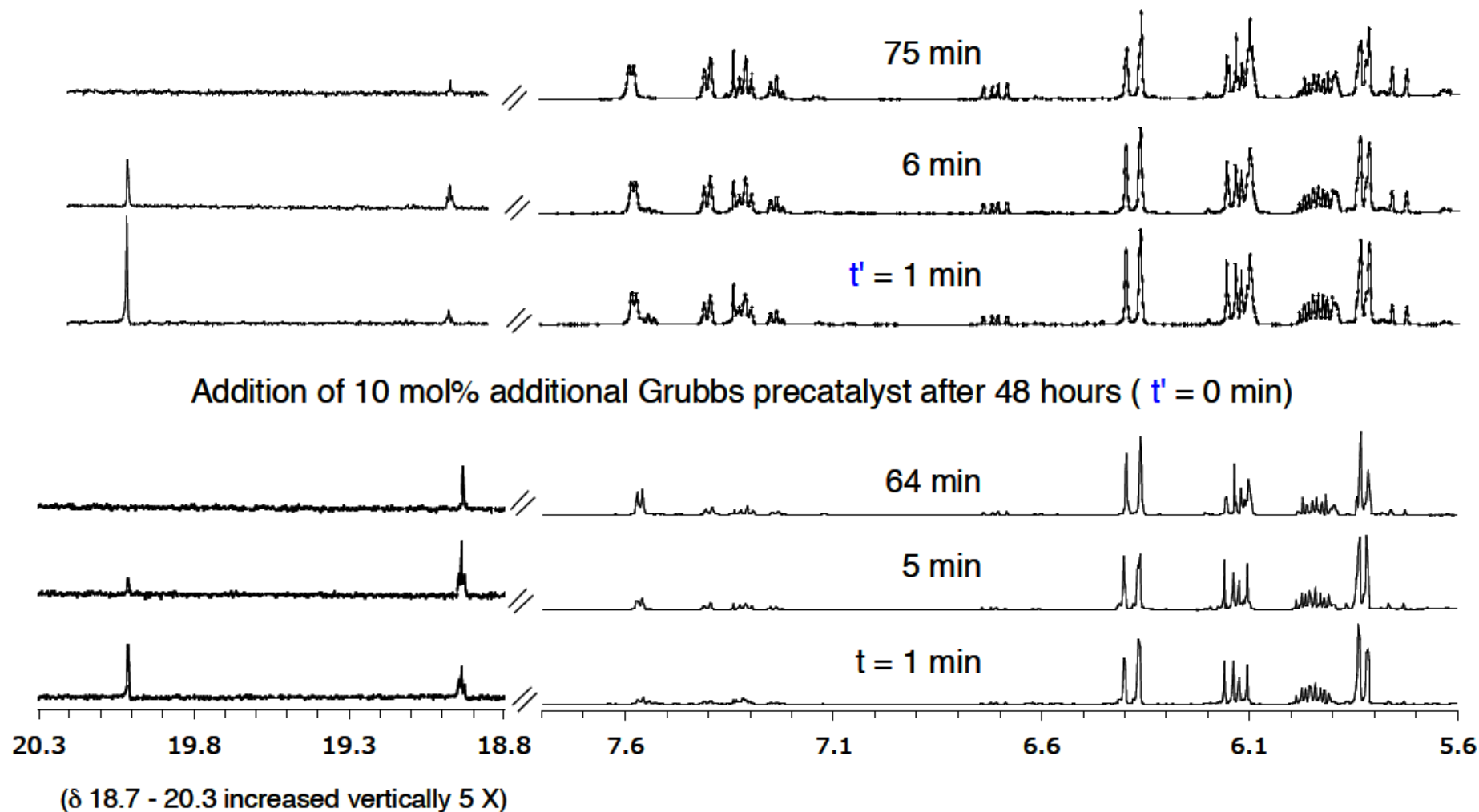
**No-D NMR**

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**No-D NMR**

After 3 hours, the reaction stalls out. The spectrum shows no carbene signals at all, so apparently the catalyst has been eaten up by something. Adding more catalyst continues the reaction. So this can be a good way to optimize a reaction qualitatively, or even obtain quantitative kinetics information.



**No-D NMR****Q: What about shimming?**

Of course, in a No-D NMR, there's no deuterium, so you can neither use deuterium gradient shimming nor shim on the lock signal of the deuterium channel. You have several options:

- (1) Just shim another tube that's very similar but does contain deuterated solvent or use the default shims. For "rough work" this is good enough.
- (2) Use a capillary insert containing a deuterated solvent. This is annoying from a practical perspective.
- (3) Shim on the FID or shim on the spectrum.

**FID shimming** is where you acquire an FID every few seconds and adjust the shims to make the ringing last as long as possible. Remember that magnetic field inhomogeneity is the dominant factor behind linewidths in organic molecules, so the better the shims, the narrower the lines. Narrow lines (frequency domain) correspond to longer ringing (time domain). Here is a procedure for taking a no-D NMR on a Varian instrument:

- (1) Load parameters for  $^1\text{H}$ ,  $\text{CDCl}_3$  (the exact solvent doesn't really matter, since none of the protonated solvents will be pre-programmed).
- (2) nt=1 bs=1 d1=1 ss=0 gain=2 pw=1

*Note:* The signals from No-D NMR spectra can be very intense and overwhelm the detector if the gain is set too high. A low gain is required to avoid errors like "ADC overflow" or "FIFO error."

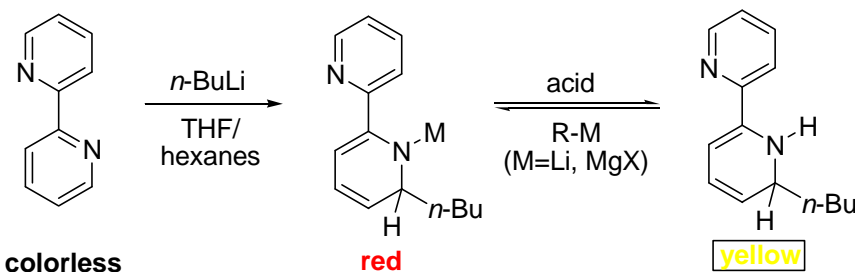
- (3) load default shims (on our systems, this is rts('cdcl3') su)
- (4) click on "acqi" button
- (5) turn lock off and spin on
- (6) click on the FID button at the top to enter FID shimming mode

- (7) Click the up and down buttons to adjust the vertical scale of the FID display to a reasonable value.
- (8) Adjust Z1 and Z2 in large increments (like 32 or 64) to make the FID last as long as possible. The FID is acquired with the settings set in step (2), and so it will refresh every few seconds. Therefore, make changes slowly. There is no need to be very accurate--we just want the shims to be approximately right.
- (9) Acquire your spectrum with ga and phase as usual.

**Titration Organometallic Solutions**

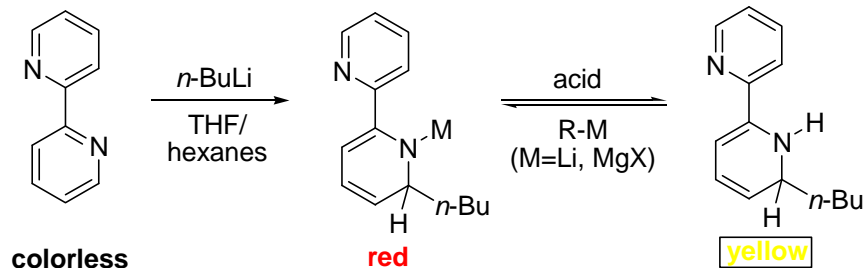
A great application of this is to assess the titers of common organometallic reagents. Before I tell you how the no-D NMR method works, it is useful to review how some reagents are normally titrated. There is a good review of organolithium titration methods here: Kamienski, C.W. *FMC Lithium Link*, Winter 1994, *Titration Methods for Commercial Organolithium Compounds*. <http://www.fmclithium.com>, Technical Solutions. (I have provided a copy of this paper on the course website; access is restricted to the class list, since this is copyrighted material.)

The Ireland method (*JOC* **1991** 56 4566) is a colorimetric titration that is appropriate for lithium amide bases, and now, as Hoyer shows, R-Li and R-MgX carbanions:



First, a few mg of indicator (bipyridine or phenanthroline) are added to some THF or ether. Then, a known amount of some acid, like 2-butanol, is added to the solution.

### Titration Organometallic Solutions



Then, one performs a "dummy run." *n*-butyllithium is added with a volumetric syringe. The 2-butanol gets used up as more and more *n*-BuLi is added until it's all gone. At that point, the next drop of *n*-BuLi attacks the indicator, forming a blood red, metallated adduct. This is the endpoint of the dummy run. This is advantageous because regardless of the reagent being titrated, the same indicator species is being used. Furthermore, any moisture in the flask is destroyed by the *n*-BuLi and cannot interfere with further results.

Data are collected by successively performing titration cycles in which a known volume of 2-butanol is added and then titrated to the red endpoint with the organometallic reagent. This means that multiple measurements can be made using the same flask. Usually, an average of 5 runs is taken.

The No-D NMR titration method does not use an indicator. Instead, a known volume of solution is mixed with a known volume of inert internal standard, and the relative integral areas are used to infer the concentration. Because there is no need to use deuterated solvents, the organometallic reagents can be titrated directly.

Typically, a tared 5 mm tube (with cap) is prepared with:

- about 100  $\mu\text{L}$  of 1,5-cyclooctadiene or cyclooctene; exact amount is determined by weighing by difference
- exactly 600  $\mu\text{L}$  of organometallic solution

There is no need to flush the tube with nitrogen, but it should be dry (i.e. have been stored in a desiccator). Because the organometallic solution is quite concentrated and the volume of air in the tube is quite small, there just isn't enough oxygen and moisture in the tube to affect the reading very much.

The exact amount of 1,5-COD is known. Accurate (one-scan) integrals give the ratio of 1,5-COD to the reagent. The volume of the reagent is known, so the concentration can be calculated.

The results are very good. Colorimetric and No-D NMR agree on what the titers of many reagents to within a few percent:

species	NMR		colorimetric	
<i>n</i> -BuLi	2.50	2.50	2.45	2.50
<i>s</i> -BuLi	1.32	1.34	1.33	1.36
<i>t</i> -BuLi	1.63	1.68	1.71	1.71
LDA	1.22	1.23	1.18	1.20
MeLi	1.41	1.42	1.53	1.54
EtMgCl	0.86	0.87	0.88	0.89
allylMgCl	2.04	2.05	1.96	1.97
vinylMgBr	1.25	1.25	1.25	1.27
Et <sub>2</sub> Zn	0.94	1.01	not performed	

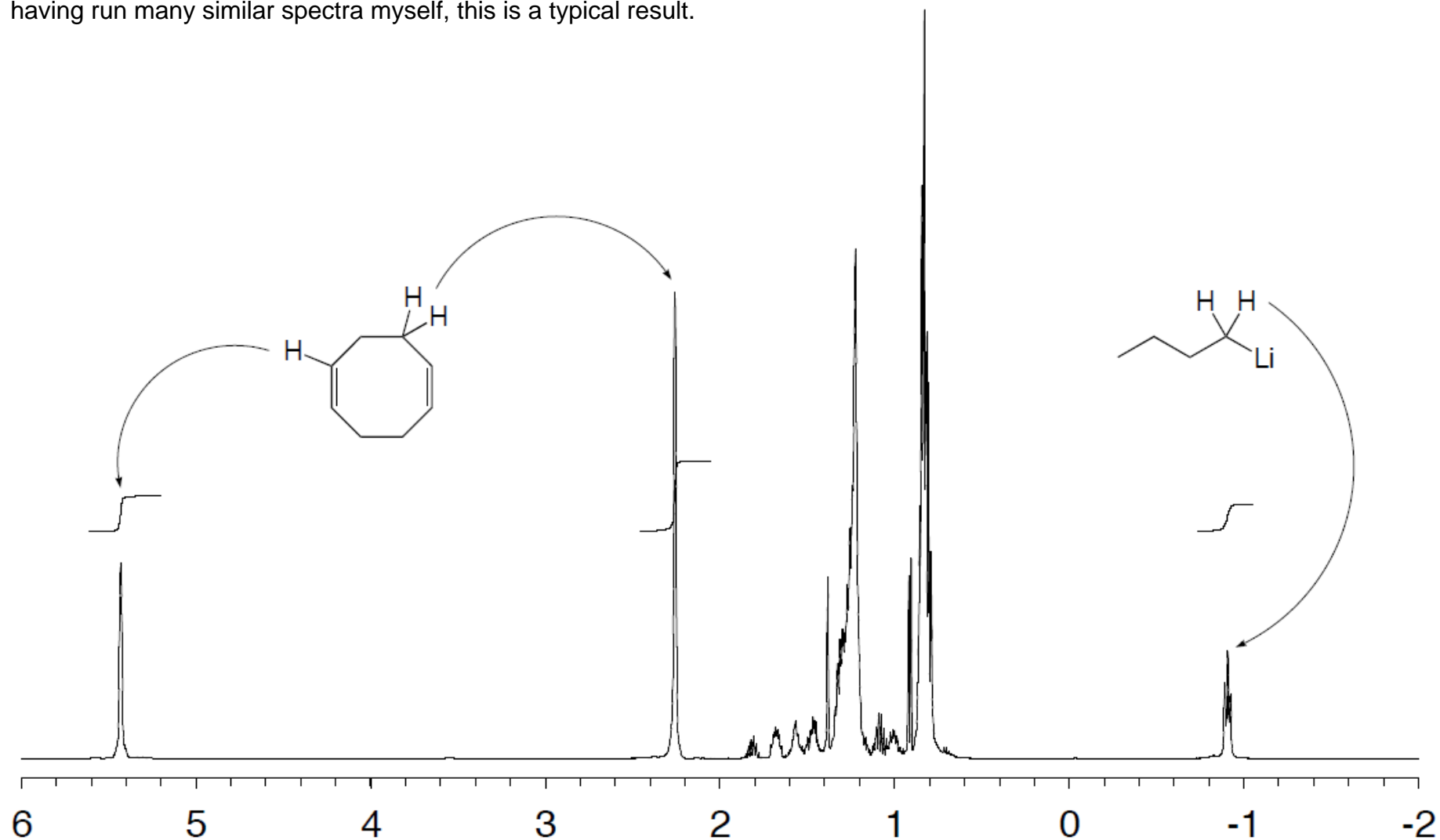
<sup>a</sup> Average difference in the two sets of measurements is 0.21%, while the average unsigned difference is 2.9%.

The method is advantageous because it's very fast--it takes about ten minutes to prepare a sample and ten minutes to take an NMR spectrum and work out the numbers. Compare that to flame-drying a flask under vacuum, preparing a solution of indicator, drawing up multiple volumes of 2-butanol and reagent solution, etc. Another advantage is that you can actually assess the condition of the organometallic in the bottle. In a colorimetric titration, all you get is the concentration of base in the molecule. In No-D NMR, you can directly see if the reagent is contaminated or has decomposed.

**No-D NMR**

"No-D NMR Spectroscopy as a Convenient Method for Titering Organolithium...Solutions." Hoyer, T.R. et al. *Org. Lett.* **2004**, 6, 2567-2570.

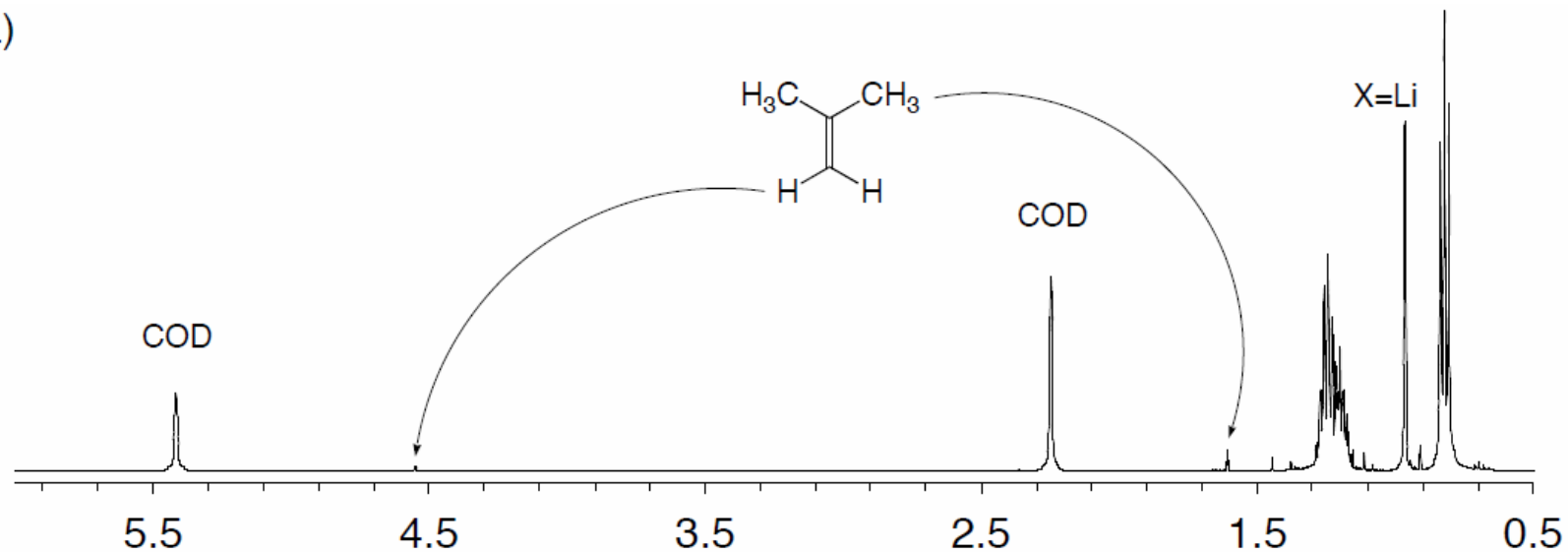
This is *n*-butyllithium. The results are spectacular. Although this spectrum is from the Hoyer report, I can truthfully say that, having run many similar spectra myself, this is a typical result.



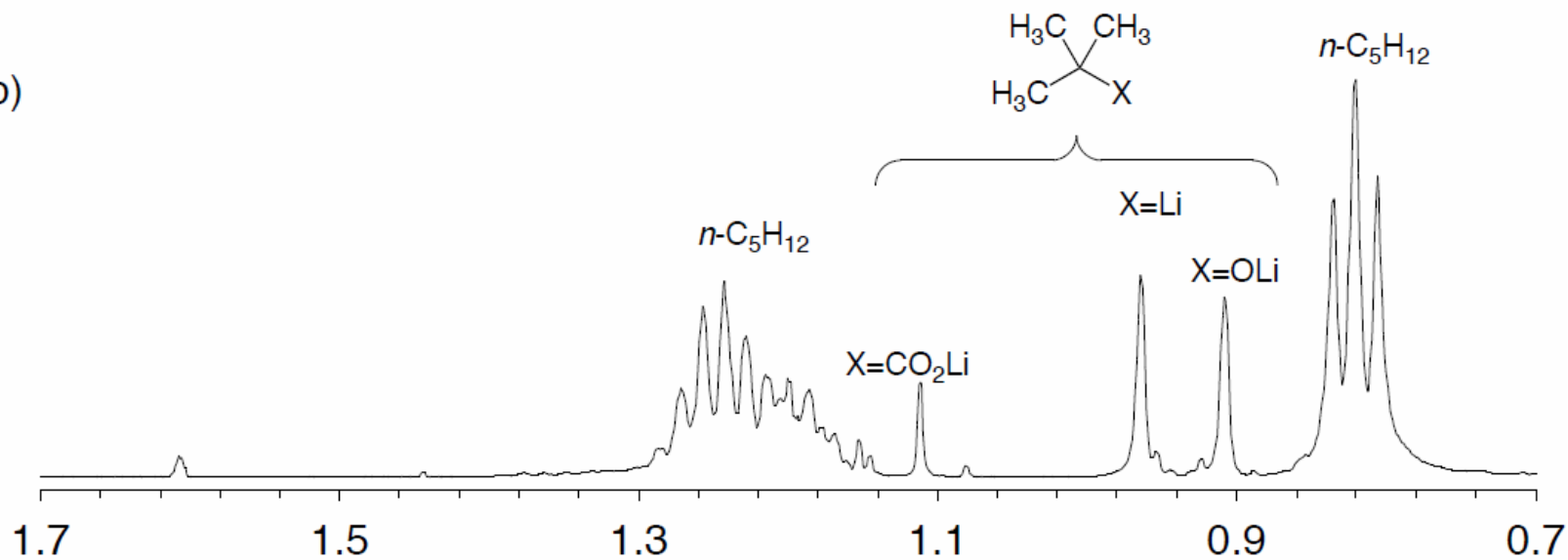
**No-D NMR**

This is **tert-butyl lithium**, which can be annoying to titrate because of its sensitivity to air and moisture. (a) is high-titer solution while (b) is a low-titer solution. The alkoxide and carboxylated byproducts are visible.

a)



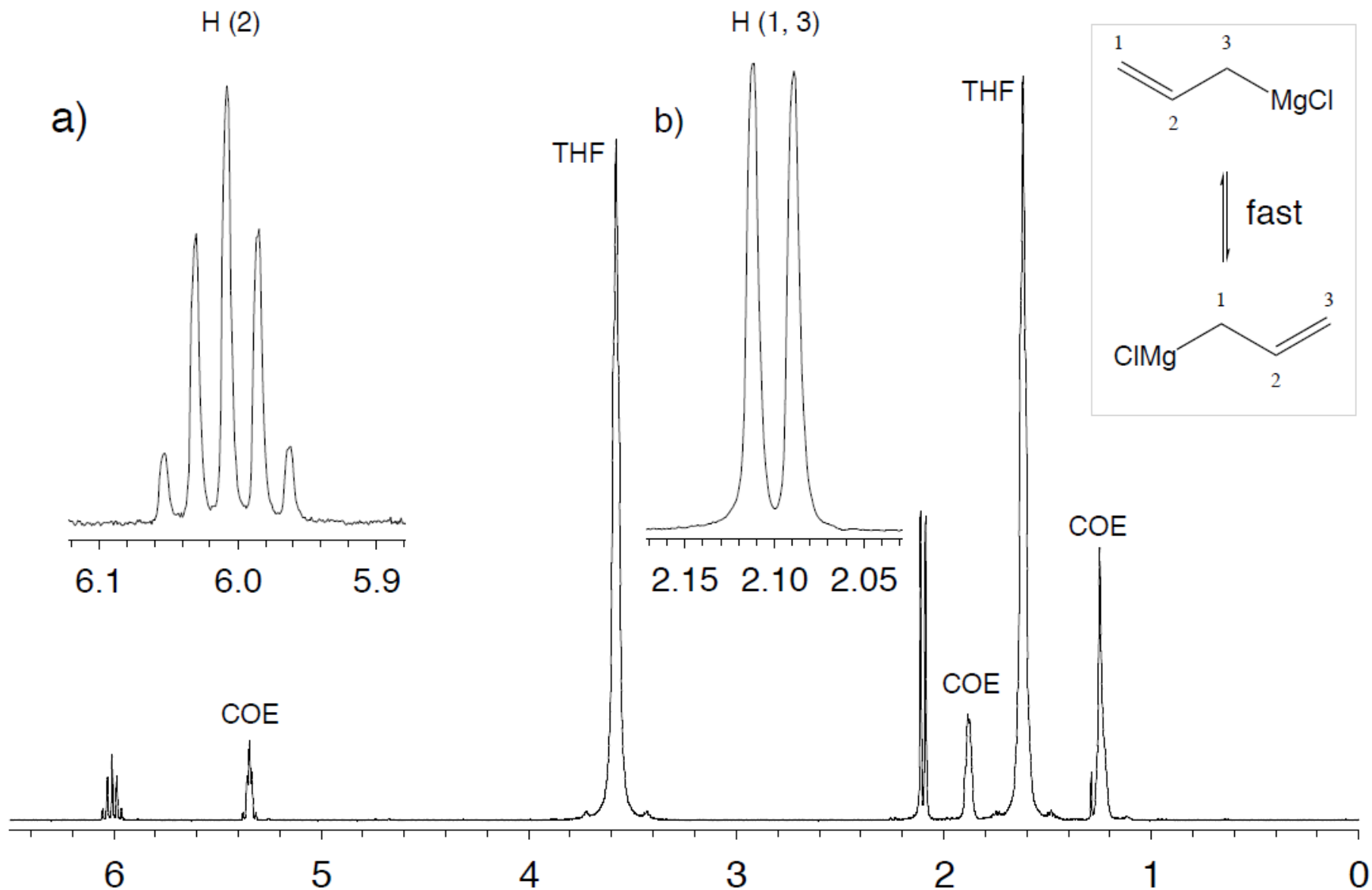
b)





**No-D NMR**

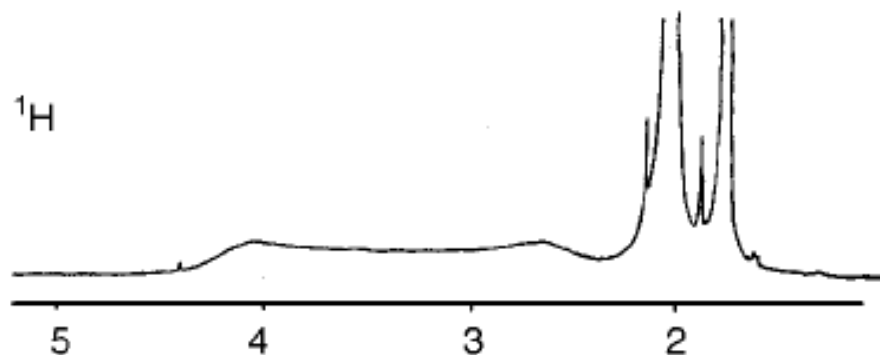
This is **allyl Grignard**. It has an interesting spectrum because it is well on its way to fast exchange at room temperature, so the reagent signal has an averaged chemical shift. Clearly, very reactive solutions can be quantitated reliably.



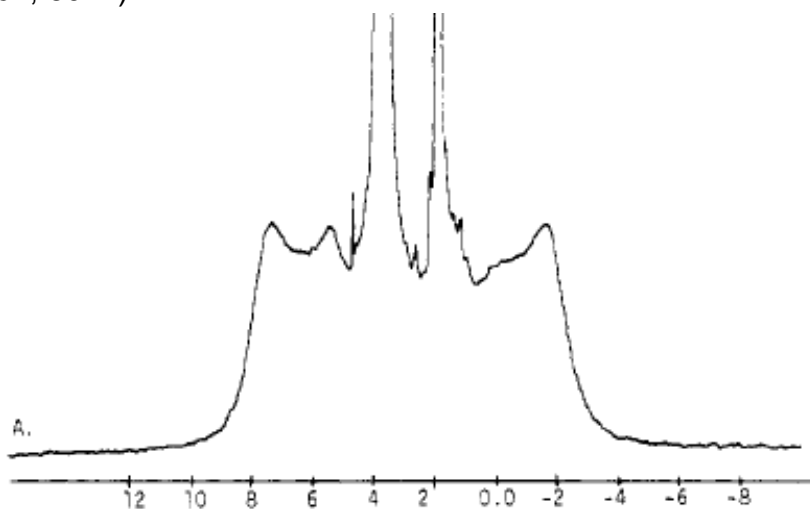
### Titration Hydride Reagents

"Reaction Titration: A Convenient Method for Titering Hydride Agents..." Hoye, T.R. et al. *Org. Lett.* **2005**, 7, 2205.

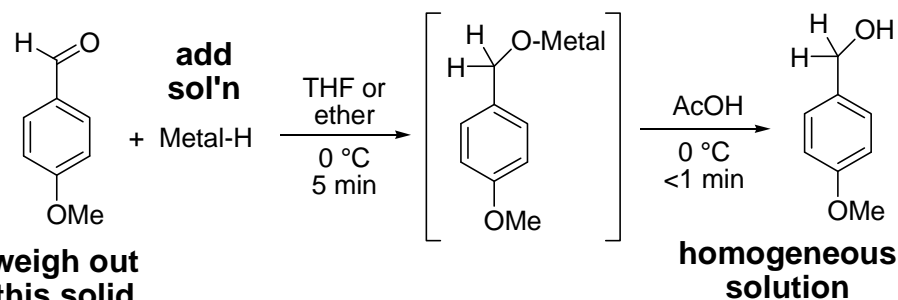
Unfortunately, titrating organometallic hydrides is not as easy because the hydride resonances are not always well-resolved. This is probably due to chemical exchange effects. Here is a proton spectrum of  $[\text{Li}(\text{TMEDA})(\text{AlH}_4)_2]_2$  in benzene (Smith, *J. Chem. Soc. Dalton Trans.* **1998**, 249):



This spectrum is of  $\text{LiAlH}_4$  itself in  $d_8$ -THF at 90 MHz. It would look better at higher field, but not by much (Horne, *JACS* **1980**, 102, 6011):



Fortunately, there is a simple modification of the No-D NMR procedure that works in these cases. The strategy is to combine reducing agents like LAH with a stable, easily reduced solid, *p*-anisaldehyde. If LAH is the limiting reagent, then the remaining *p*-anisaldehyde and the product alcohol, *p*-methoxybenzyl alcohol, act as the internal standard:



This takes about twenty minutes. This is performed in a reaction flask, with the NMR analysis being performed on a small aliquot. Your alternative is to conduct a volumetric protonolysis--that is, add an acid that generates hydrogen gas, which you trap and measure the volume of (Aldrich Technical Bulletin AL-123). There are also colorimetric methods that work. But I think this is clearly faster. Here are some results:

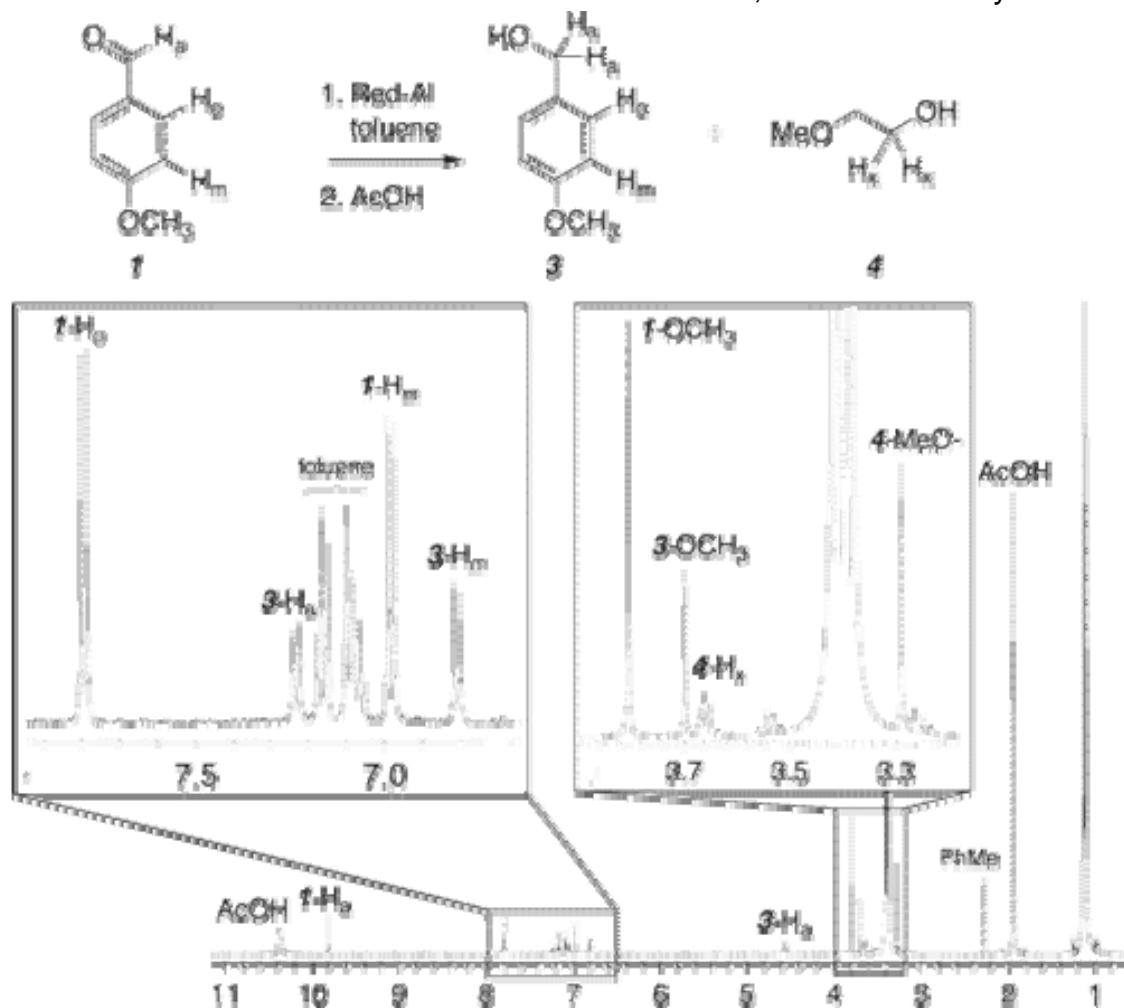
met-H species	[nominal]	concentration (M)
Red-Al (PhMe) <sup>a</sup>	3.2 M	3.19 (±0.09)
LiAlH <sub>4</sub> (Et <sub>2</sub> O) <sup>b</sup>	1.5 M	0.81 (±0.01)
DIBAL-H (PhMe) <sup>b</sup>	1.5 M	1.39 (±0.02)
DIBAL-H (hexanes) <sup>c</sup>	1.5 M, aged	0.54 (±0.02)
L-Selectride (THF) <sup>d</sup>	1.0 M	1.00 (±0.01)
L-Selectride (THF) <sup>d,e</sup>	1.0 M "direct"	1.04 (±0.01)

Interestingly, LAH seems to mostly react via its "first hydride." Clearly, DIBAL-H is not a very stable reagent in solution. In my experience, neat DIBAL-H is stable in the fridge, but solutions degrade over weeks to months. L-selectride can be observed directly by No-D NMR and gives similar results to this method.

### Titration of Hydride Reagents

This **Red-Al**<sup>®</sup>, or sodium bis(2-methoxyethoxy)aluminum hydride, a common reducing reagent. It has a similar reactivity profile to  $\text{LiAlH}_4$ , but is soluble in non-etheral solvents. In that sense, it's like the difference between  $\text{LiHMDS}$  and  $\text{LDA}$ . In the protocol, there is a roughly 2:1 ratio of anisaldehyde to Red-Al, so the starting aldehyde is still visible. Acetic acid is visible; it was needed to protonate the metal alkoxides into something soluble. This is a real advantage, since it avoids an aqueous workup.

The "activity" of LAH is notoriously unreliable. Generally, LAH reacts with water to produce  $\text{LiAlO}_2$  and hydrogen gas. This is what the gray, insoluble bits are. Solutions in ether are better than ones in THF, since the density of THF is close to that of the insoluble impurities.



### Titrating Hydride Reagents

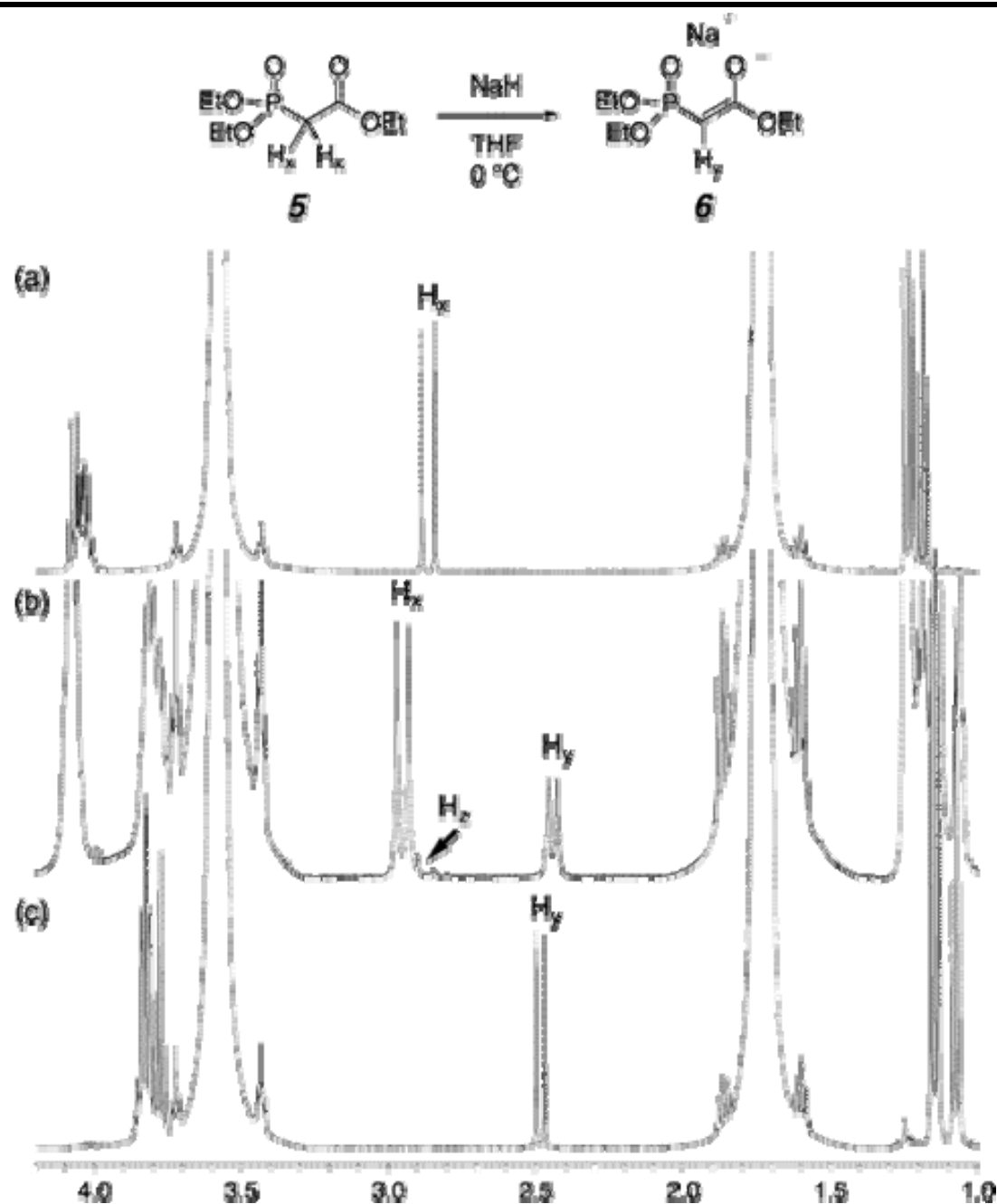
Of course, some common hydrides are not reducing agents like sodium hydride and potassium hydride. They are also relatively insoluble in most organic solvents. For example, NaH is sparingly soluble in THF and is generally used as a suspension. The larger the counterion, the more reactive. It is not uncommon to see bubbling (hydrogen evolution) when NaH is added to THF.

Typically, NaH is available as a suspension in mineral oil. You take up some oily NaH suspension with a fat pipette and put it into a tared, nitrogen-flushed flask. It is then rinsed successively with hexanes. It also comes as a 95% dry powder. It's advisable to weigh out portions of dry NaH in a glovebox. KH, to my knowledge, is only available as a suspension; it's just too reactive.

The direct observation of NaH suffers from the same problems as other hydrides (broad signals, chemical exchange). Fortunately, there is an analogous reagent it reacts with quite easily: ethyl diethylphosphonoacetate. (You may recognize these conditions as being identical to those of Horner-Wadsworth-Emmons reactions.)

- (a) just the phosphonoacetate
- (b) 0.5 equiv of NaH added
- (c) 1.5 equiv of NaH added

H<sub>z</sub> is probably sodium hydroxide-related.



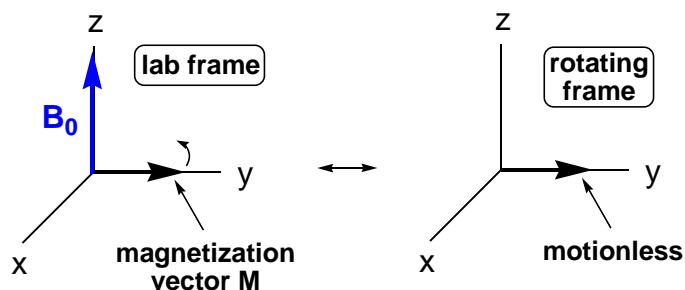
### Composite Pulses

**Q: What if the solvent signal is just too big and has to be suppressed?**

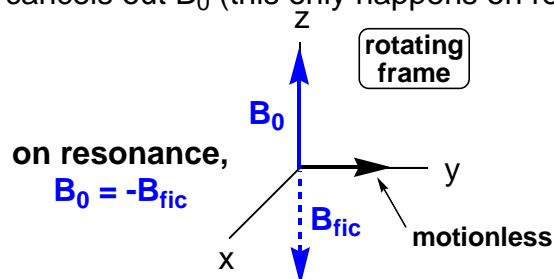
It turns out that, surprisingly, the answer to this question is related to this one:

**Q: What happens when pulses are mis-calibrated or are not applied on-resonance?**

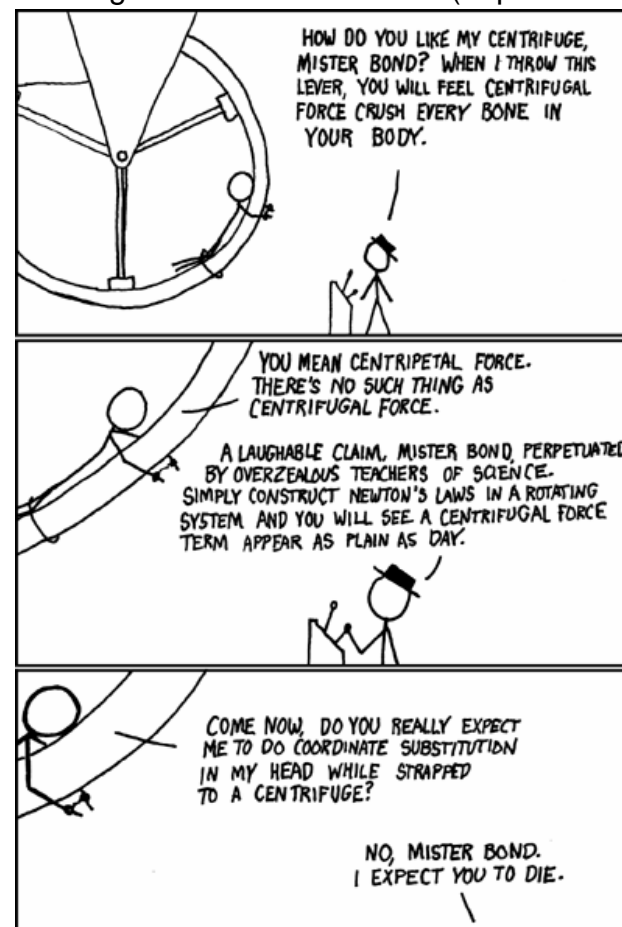
First, let's consider what happens when a perfectly calibrated pulse is applied **on resonance** (i.e., the transmitter frequency matches the Larmor frequency of the spin exactly). In the lab frame of reference, we have a stationary  $B_0$  field at +z, about which transverse magnetization precesses. If we set the rotating frame frequency to this precession frequency, the  $B_0$  vector disappears.



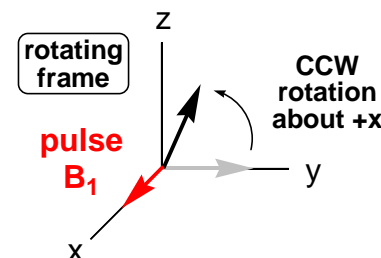
But *why* does the field disappear? It turns out that in classical mechanics, the use of an accelerating frame of reference (or "non-inertial" frame) gives rise to fictitious forces like the Coriolis force. In this case, it gives rise to a " $B_{fic}$ " field that exactly cancels out  $B_0$  (this only happens on resonance):



There's an amusing xkcd comic about this (<http://xkcd.com/123/>):

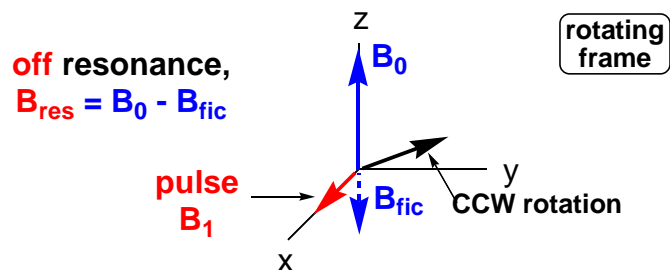


Now, suppose we apply a pulse via  $B_1$  with a phase of +x. For the whole duration, we have this (rotating frame) vector diagram:

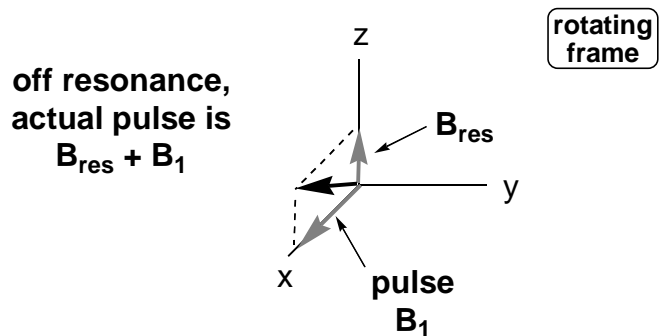


### Composite Pulses

The trouble starts when we are *not* on resonance. As before, we choose the rotating frame reference frequency to be the frequency of the pulse. As before,  $B_1$  lies motionless at  $+x$ . However, now we have incomplete cancellation between  $B_0$  and  $B_{fic}$ , leading to a new field,  $B_{residual}$ . The sign of this depends on whether the reference frequency is less than or greater than the Larmor frequency  $\nu_0$ . Here, the reference frequency is smaller than the Larmor frequency:

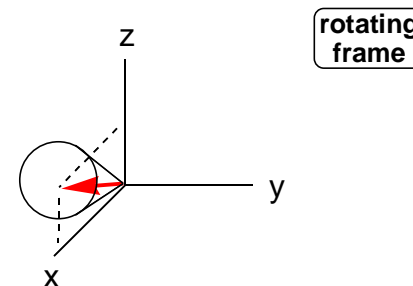


This means that  $B_{res}$  will "drag"  $B_1$  away from its intended phase,  $+x$  and add in some undesired  $+z$  phase. Here's the sum of  $B_1$  and  $B_{res}$ :



Because  $B_{res}$  lies along  $+z$  and  $B_1$  lies along  $+x$ , the sum lies in the  $xz$ -plane, and hence, pulses cause counterclockwise rotation around a different axis than expected. (In general,  $B_1$  lies along whatever axis corresponds to the phase the user sets the pulse to.  $B_{res}$  always lies along  $+z$  or  $-z$ . But the result is the same: if  $B_{res}$  is significant, then the pulse will not rotate the vector about the intended axis. You can work out all the angles with trigonometry.)

instead of rotating  
CCW about  $+z$ ,  
rotation about  
 $B_1 + B_{res}$

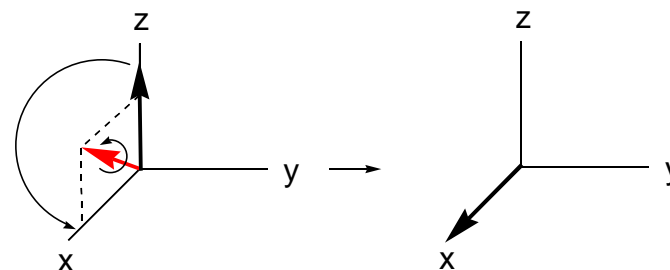


(Note that because rotations in three dimensions do not, in general, commute, you cannot just rotate about  $+z$  and then rotate about  $B_{res}$ .) Here's a scenario to get you thinking:

Let's say the vector just spins about on the unit sphere. (This is like saying there's no  $T_1$  or  $T_2$  relaxation.) Start at equilibrium such that  $M = (0,0,1)$ . Apply a  $180_x$  pulse. On resonance, this takes us to  $(0,0,-1)$ .

**Q: If  $B_{res} = B_1$ , what happens?**

The effective rotation is about  $(1,0,1)$  instead of  $(1,0,0)$  now. This actually takes you to  $(1,0,0)$  instead of  $(0,0,-1)$ !



Clearly, if you wanted a  $180_x$  pulse, you are not going to be happy! This sort of scenario comes up a lot in heteronuclear experiments, where the spectral window is very wide. This means that resonances far from the middle of the window are very much off-resonance.

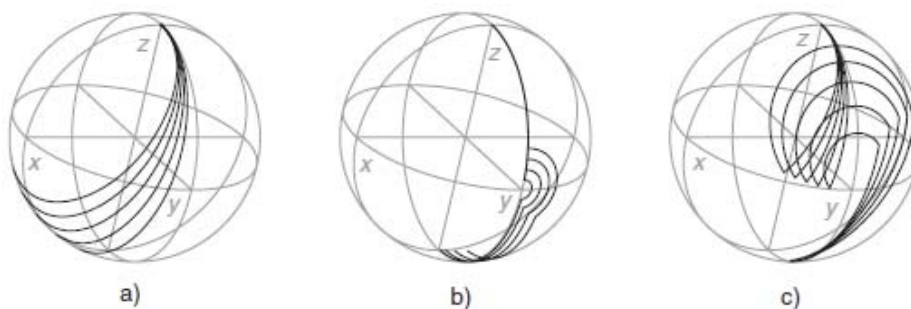
One common solution to this problem is to use **composite pulses** -- groups of smaller pulses.

### Composite Pulses

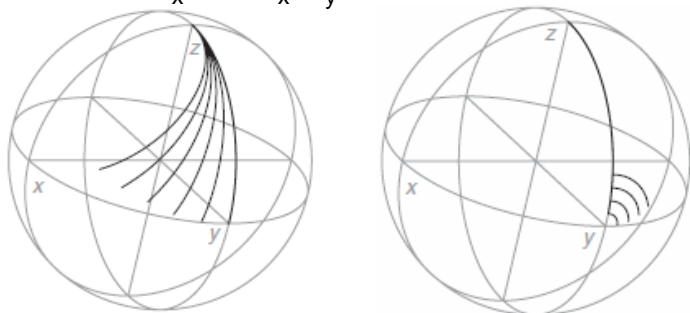
The math is complicated, but has been worked out. If you want to play around with it yourself, check out the Mathematica notebooks by Malcolm Levitt (see "mPackages" at <http://www.mhl.soton.ac.uk/public/Main/index.html>).

Here are diagrams from Claridge (pg 336). The  $90_x-180_y-90_x$  sequence compensates for miscalibrated pulses (and  $B_1$  field inhomogeneity) as well as resonance offsets. The middle pulse corrects for any errors in the first and last pulses by spinning the residual magnetization in a tight cone about the y-axis (see Jacobsen, page 295 for details).

- (a) effect of resonance offset on a single  $180_x$  pulse  
 (b) compensation for miscalibration  
 (c) compensation for resonance offset



This turns out to be five times better in terms of bandwidth than a simple  $180$  pulse, which is a pretty good improvement. People have invented all kinds of composite pulses to replace simple pulses. Here is  $90_x$  vs.  $90_x90_y$ :

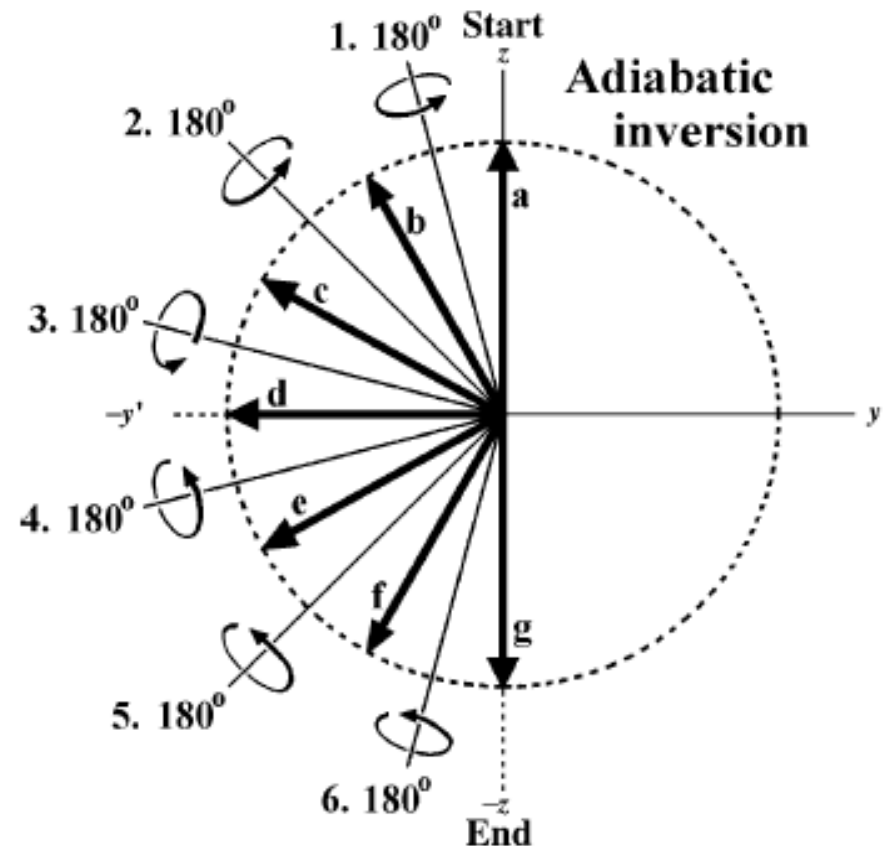


Another strategy is to use **adiabatic pulses**. Think of a spin-lock. It normally keeps all the vectors along the y-axis. Now, imagine starting the phase of the spin-lock at  $+z$ , and then slowly moving it from  $+z$  to  $-z$ . As it comes into contact with vectors of different frequencies, it will collect them and drag them to the  $-z$  axis.

More formally, we must sweep the frequency slow enough to satisfy the adiabatic condition:

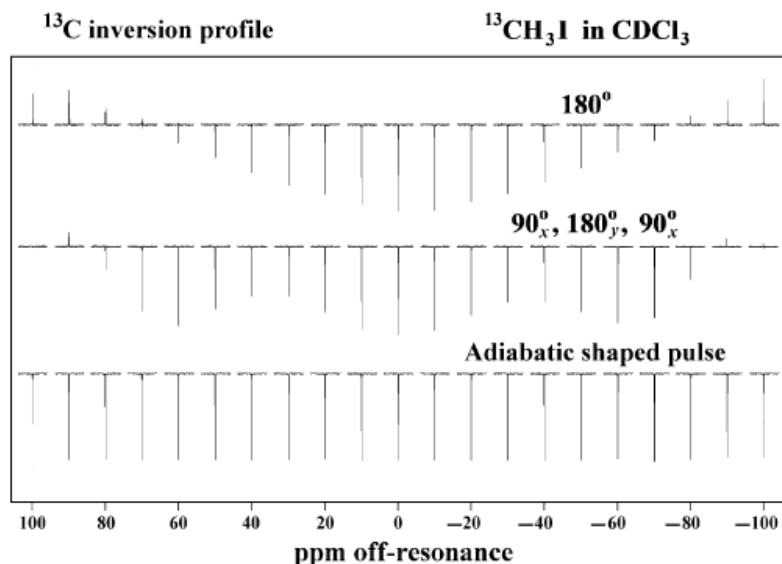
$$\left| \frac{d\theta}{dt} \right| \ll \omega_{eff} = \gamma B_{eff}$$

but fast enough to avoid relaxation (**adiabatic fast passage**):

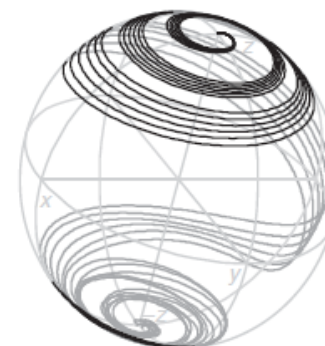
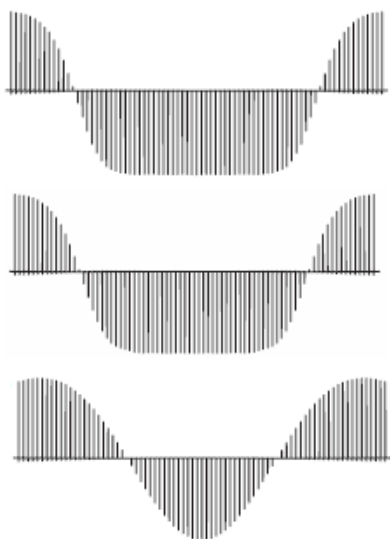


## Composite Pulses

The performance of these adiabatic pulses is terrific:



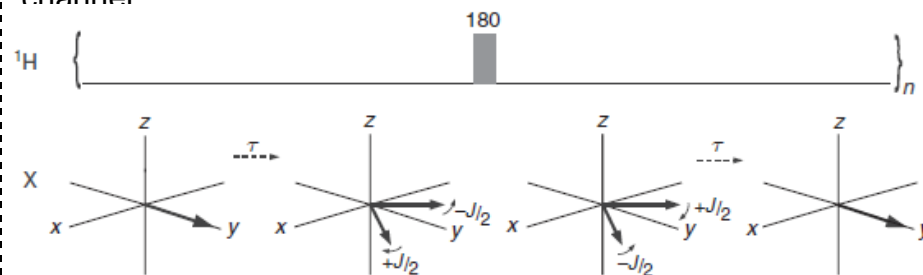
The CHIRP and WURST sequences are popular adiabatic pulses. Here is their performance: (a) CHIRP, (b) WURST-20, (c) 25 us hard 180 pulse (d) trajectories for WURST-20



Although there are many different composite pulses in the literature, only a few of them get used a lot. In general, each composite will correct for  $B_1$  inhomogeneity or resonance offset, but not both at once. Very fancy sequences that compensate for both involve a lot of pulses, which start to be problematic from a relaxation standpoint.

### Q: What are some applications?

Broadband decoupling of carbon-13 spectra requires that continuous irradiation be applied to the protons. You can think of this as a continuous train of 180 pulses applied to the proton channel:



This is a heteronuclear spin-echo, with pulses being applied to only one nucleus, which means that both chemical shifts and couplings are refocused. From the above discussions, it is clear that simple 180 pulses are unlikely to be effective. In the limit that the spin echo delays become infinitely short, this is a long duration time-domain signal, which necessarily has a small frequency-domain bandwidth.



### Composite Pulses

Now, you might think the logical answer is to just replace the 180 pulses with composite 180 pulses, like  $90_x 180_y 90_x$ . Let us define such a composite as "Q." Thus, QQ would mean:

$$90_x 180_y 90_x \text{ then } 90_x 180_y 90_x$$

In principle, this would just send things from +z to -z and back to +z. Unfortunately, each pulse element is not itself perfect, so this leads to some errors. If enough of these Qs are put together, then the errors will accumulate. Instead, a better combination is "QR" where R is the same as Q, but with opposite phase:

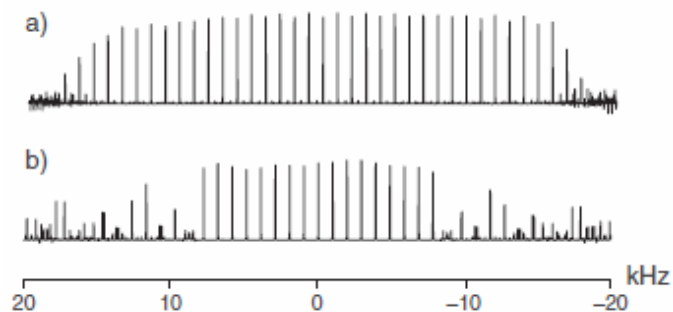
$$Q = 90_{-x} 180_{-y} 90_{-x}$$

The "magic" combination turns out to be QQRR. This is called MLEV-4. Even better is **MLEV-16**, which is a nested version of MLEV-4 cycles:

$$QQRR \text{ RQQR } RRQQ \text{ RQQR}$$

This is a common **composite pulse decoupling** sequence that we use for our routine carbon-13 spectra. Others include things like WALTZ-16 ( $90_x 180_x 270_x$ ) and GARP. These have various bandwidths, power requirements, etc. WALTZ-16 gives smaller residual linewidths than MLEV-16, while GARP is designed to decouple carbon nuclei during 2D NMR experiments like HSQC.

$^1\text{H}\{^{13}\text{C}\}$  profiles for CHIRP (top) vs. GARP (bottom):



TOCSY requires spin locks as well. A slightly modified version of MLEV-16, called MLEV-17, incorporates a 60 pulse at the end of each cycle to compensate for errors that accumulate during extended mixing. Even better is **DIPSI-2** (this is the sequence we use on our spectrometers), which uses a complicated repeating unit:

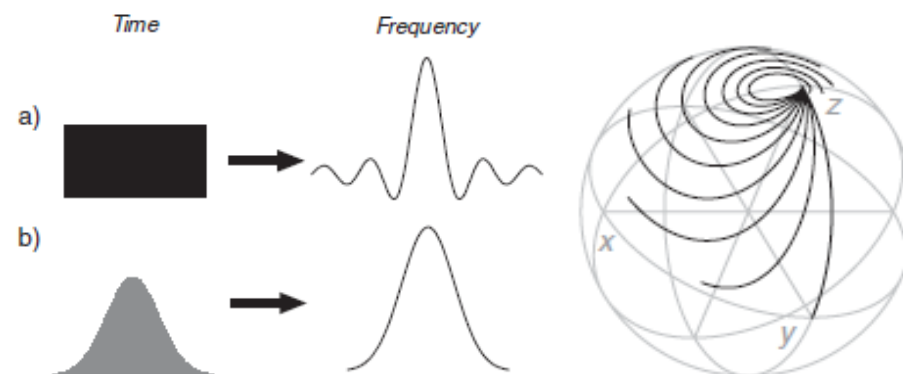
$$320_x 420_{-x} 290_x 285_{-x} 30_x 245_{-x} 375_x 265_{-x} 370_x$$

This was developed by computer modeling to maximize TOCSY transfer with the smallest amount of sample heating. It's not clear at all where this comes from theoretically.

### Selective Pulses

Sometimes, we would like to have a very *narrow* excitation profile instead of a very wide one. This is done with **soft pulses**. Remember, very short pulses (time domain) mean exciting a lot of different frequencies (frequency domain). So the question is: *what kind of time-domain pulse will give a narrow frequency excitation without phase distortions?*

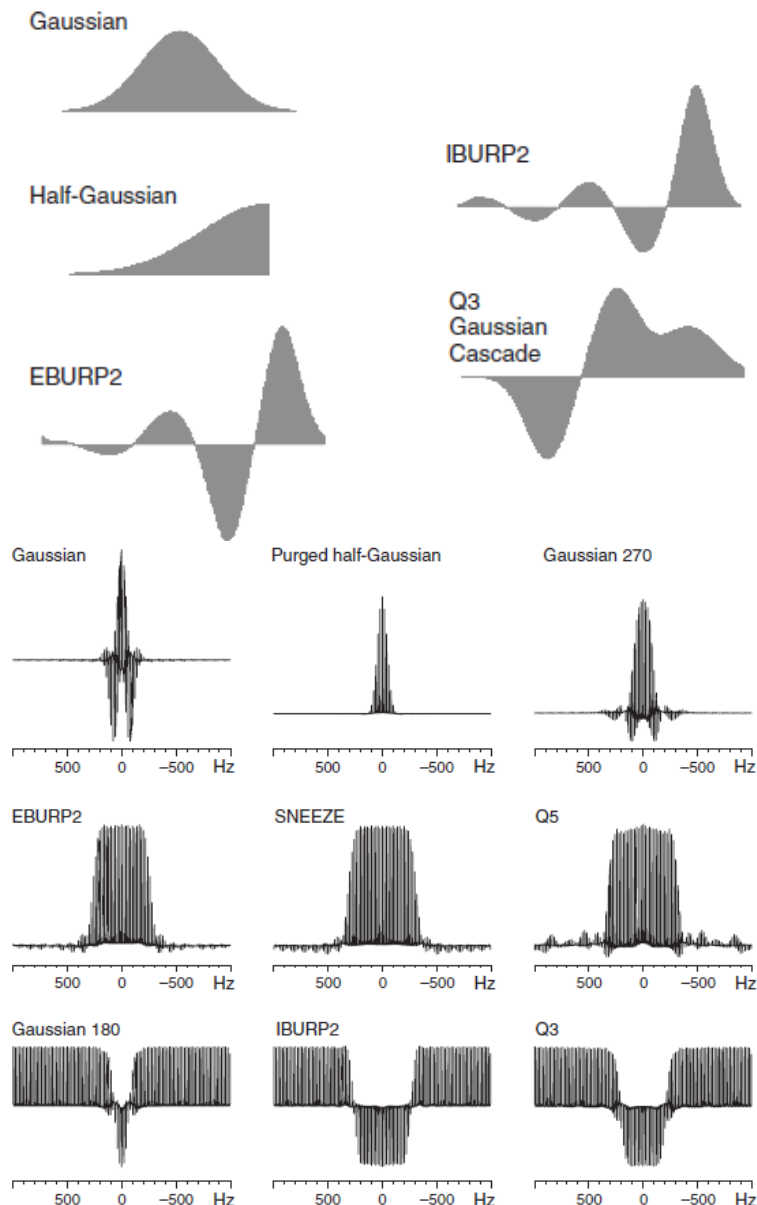
Like composite pulses, there are a lot of options. The simplest is a Gaussian. This is conceptually nice because the Fourier transform of a Gaussian is another Gaussian:



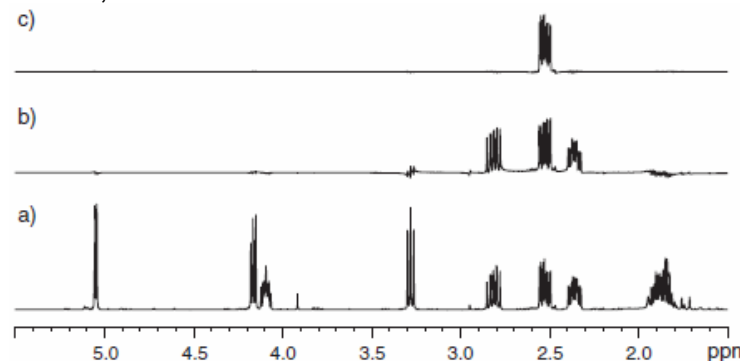
Unfortunately, as this shows, the Gaussian does not have very desirable phase properties as a function of resonance offset.

### Selective Pulses

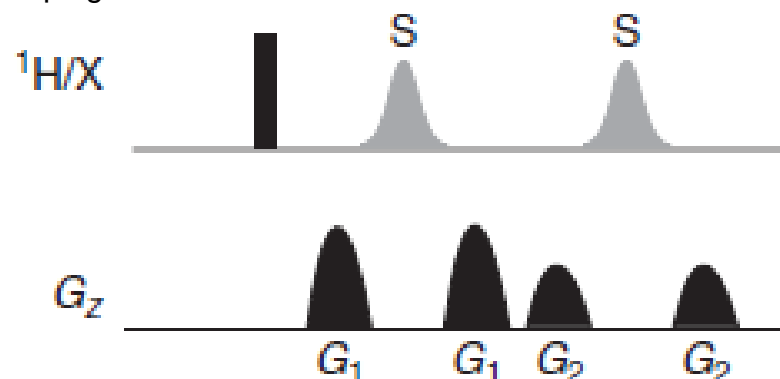
Here are time- and frequency-domain pictures of many of the possibilities (Claridge, page 347):



Amusingly, the BURP family of pulses (band-selective, uniform response, pure-phase) are very popular. The crucial thing is that the spectrometer must have properly calibrated pulse widths. Note that each pulse generally only does one thing well. For example, I've used the IBURP2 pulse before, which is only good for inverting resonances. The longer the pulse, the more effective it is: (a) regular  $^1\text{H}$ ; (b) EBURP2, 14 ms, (c) EBURP2, 100 ms:



This is precisely the sort of thing that is needed for excitation sculpting:



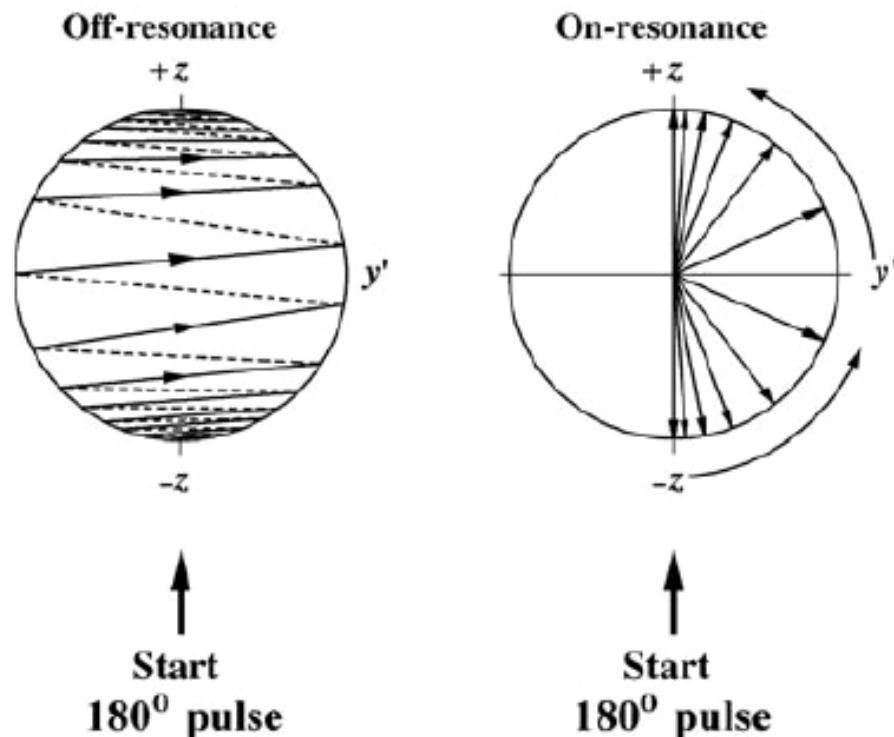
Recall that only the resonances that are inverted between gradients get refocused. If the selective pulse accidentally inverts some other resonances as well, they will be refocused by the gradient echo as well.

**Q: How can solvent signals be suppressed?**

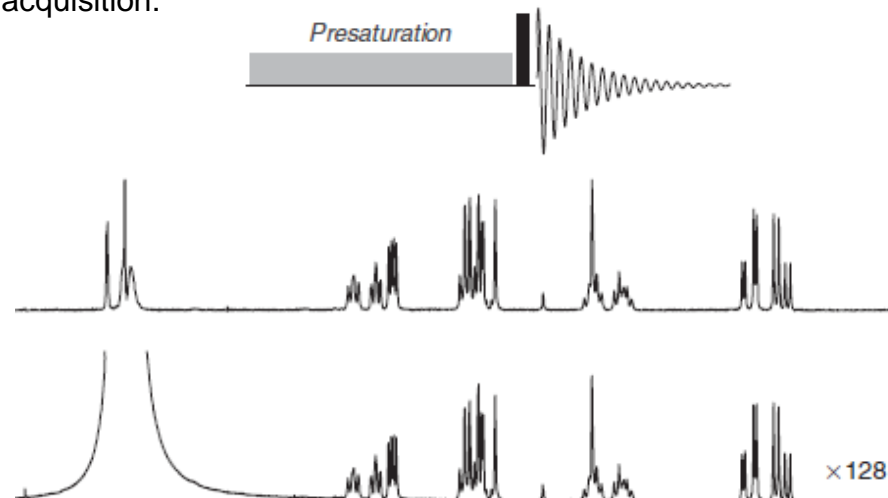
### Solvent Suppression

The most important are where this comes up is in biological samples, which contain water. The water resonance is probably the most annoying signal in all of NMR spectroscopy. It is very intense in a 90% H<sub>2</sub>O/10% D<sub>2</sub>O mixture (50 M; this mixture is required to avoid exchanging out important amide protons).

Imagine you decide to put the water signal along -z with a selective inversion pulse. In principle, it ought to decay smoothly and invisibly along the z axis while not affecting anything else. This doesn't work for many reasons. One big problem is called **radiation damping**. Pulses aren't perfect, so there's always a bit of transverse magnetization. The signal is so intense that the signal in the coil actually acts as a B<sub>1</sub> pulse that pushes the magnetization vector away from the z-axis in a feedback loop. A full explanation is given in Jacobsen on page 567:



If we can't just invert water, can we just equalize its population? Yes--this is called **presaturation**. In its simplest form, you just apply low power RF irradiation to the water resonance before acquisition:



Now, we can see the rest of the signals. However, a bit of water is still left. For some samples this is not a sufficient level of suppression. Fancier versions of presaturation like FLIPSY have been developed (see Claridge, page 355), but the bigger problem is that of **saturation transfer**. Since amide protons are in chemical exchange with the water protons, over the course of the experiment, the amide protons will get saturated, too.

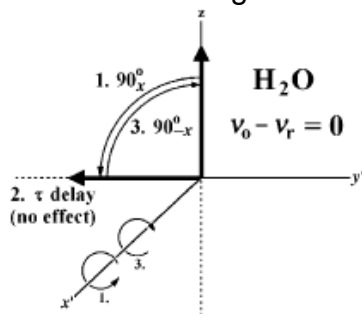
Another strategy which gets around this problem is called **zero-excitation**--the idea being that we won't have a problem with observing the water signal if we don't excite the water resonance to start with. The simplest of these is called **jump-return**:

$$90_x - \tau - 90_{-x}$$

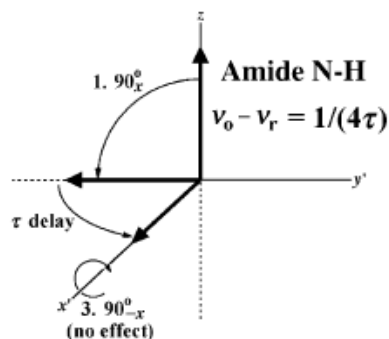
Q: What does this pulse sequence do?

### Solvent Suppression

Imagine putting the water resonance in the center of the spectral window. It is exactly on resonance, so all that happens is it gets sent from +z to -y and back to +z again:

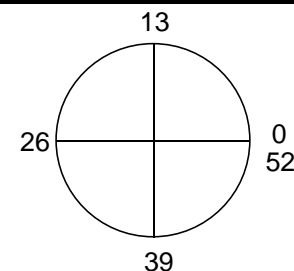


Its effect on the other resonances will depend on where they are in the spectrum. For an offset of  $1/4\tau$ :

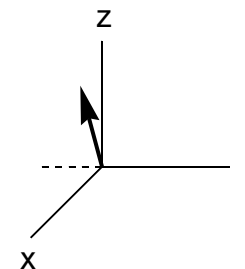


The first pulse takes the protons from +z to -y. The delay sends them to +x. The final pulse does nothing at all, since its phase is along the x-axis. So for these two cases, this sequence does what is advertised: water isn't excited at all, while some other protons are.

Unfortunately, at other offsets, the performance isn't nearly as good. For example, there are periodic nulls at  $n\pi/\tau$ . A better sequence is  $\underline{3}$ - $\underline{9}$ - $\underline{19}$ - $\underline{19}$ - $\underline{9}$ - $\underline{3}$ . Each number represents a rotation of  $3 \times 90/13$  degrees (about +x for the ones without underlines and about -x for the ones with underlines). Let us divide the unit circle into 13 equal parts per quadrant:



Let's use this as a shorthand for what happens. (Implicit in the notation is a constant delay of  $\tau$  between pulses.) For the water peak, the first pulse does this:



This a partial CCW rotation away from +z to something else in the yz plane. If we imagine the circle above as lying in the yz plane, we have a rotation from 13 to 16. Now the rest of the rotations are trivial--they are equally balanced between CCW and CW rotations, so basically nothing happens.

The same is not true for other resonances. If the offset is  $1/2\tau$ , then the delay  $\tau$  means a CCW rotation of  $2\pi \times \tau \times 1/2\tau = \pi$  every time. For the first half, which is  $\underline{3}$  -  $\underline{9}$  -  $\underline{19}$  -  $\underline{19}$  -  $\underline{9}$  -  $\underline{3}$ :

$$13 \xrightarrow{\underline{3}} 16 \xrightarrow{\underline{9}} 10 \xrightarrow{\underline{19}} 19 \xrightarrow{\underline{19}} 7 \xrightarrow{\underline{9}} 26 \xrightarrow{\underline{3}} 0$$

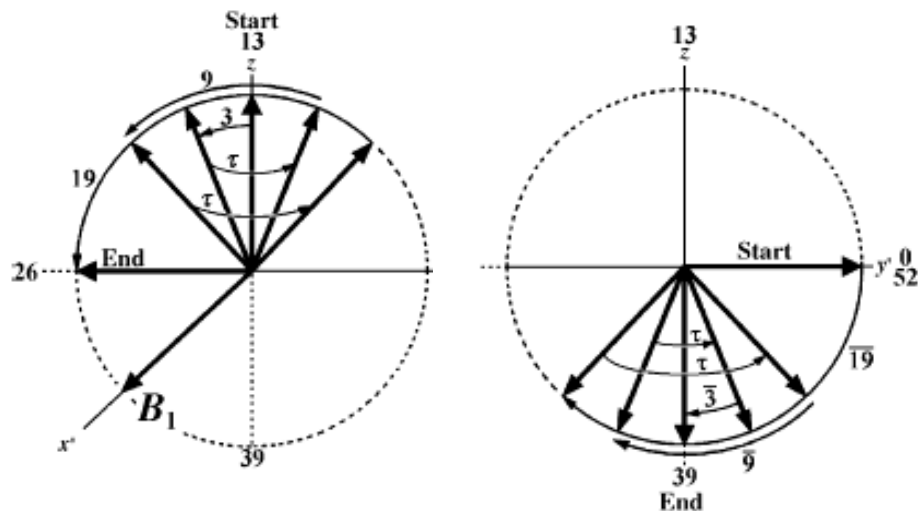
For the second half, which is  $\underline{19}$  -  $\underline{9}$  -  $\underline{19}$  -  $\underline{9}$  -  $\underline{3}$ , we have:

$$0 \xrightarrow{\underline{19}} 33 \xrightarrow{\underline{9}} 45 \xrightarrow{\underline{19}} 36 \xrightarrow{\underline{9}} 42 \xrightarrow{\underline{3}} 39$$

The result of 39 means magnetization ends up on the -z axis. Thus, this is a suitable element for inverting the sense of the coherence helix in a PFGSE experiment.

**Solvent Suppression**

Here is the diagram from Jacobsen, page 313:



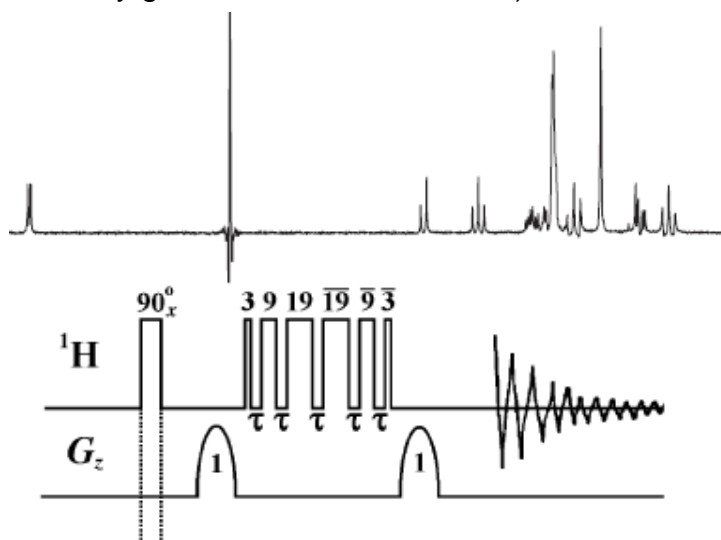
First half:  $3 - \tau - 9 - \tau - 19$ :

$13 \rightarrow 16 \rightarrow 10 \rightarrow 19 \rightarrow 7 \rightarrow 26$

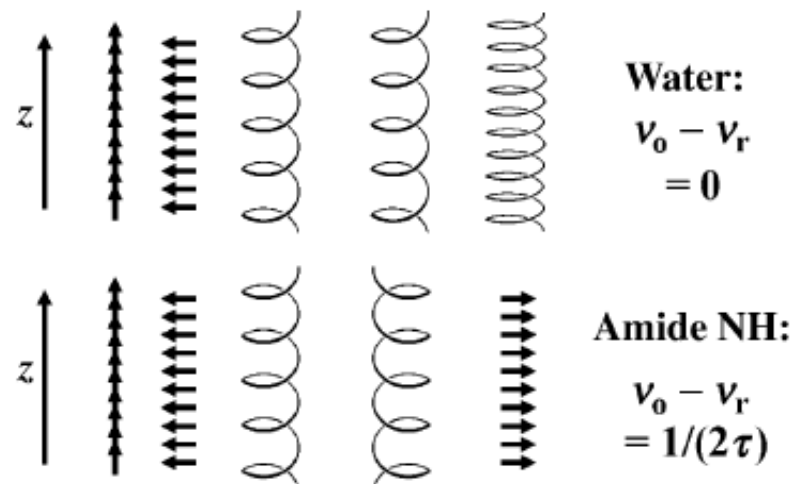
Second half:  $\bar{19} - \tau - \bar{9} - \tau - \bar{3}$ :

$0 \rightarrow 33 \rightarrow 45 \rightarrow 36 \rightarrow 42 \rightarrow 39$

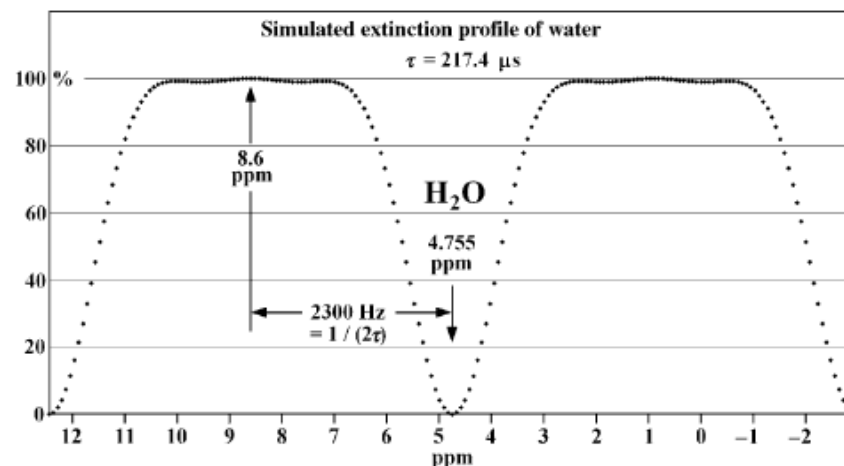
The use of this in a PFGSE is called **WATERGATE** (water suppression by gradient-tailored excitation).



Only the inverted (namely, non-water) resonances are recovered in the spin echo, so water is essentially destroyed:



A real advantage to this is that at the end of this sequence, the length of water's magnetization vector is still large. At any particular height in the NMR tube, the water molecules still possess coherence. Thus, there is no saturation transfer problem. Here is the "extinction profile" for WATERGATE:



There are fancier versions of this like the "W3" and "W5" binomial sequences.