

Practicum 1, Spring 2004

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Collecting a basic 1d spectrum.

We will go through basic steps in maintaining your account, loading a sample, locking, shimming, collecting a simple 1d spectrum, processing it, saving it ejecting the sample and leaving the machine for the next user. When you have to click on a 'button' the button's name is outlined, when you have to type a command the command is in **bold** and when I refer to a parameter it will be underlined.

Log On.

Log on to the Computer. Our course account name is che555 and the password is nmr4you. Don't change the password please.

Open a terminal shell.

Type **ls** to see the contents of your 'home' directory. (bold face text is a command to be typed in, followed by the return key).

cd data to enter the data subdirectory where you should store all the spectra you wish to save.

mkdir <yourname> to create a subdirectory of data for your saved spectra (the <> brackets indicate that you should replace the brackets with your name or initials, i.e. actually type 'mkdir afm'. We ask each of you to store your spectra in your own data subdirectory, in order to reduce the confusion that arises from many saved spectra with different naming systems. Establish a naming system for yourself that facilitates identification of stored spectra with notes and sample identities. For example include a reference to the sample and the date in the file name.

cd .. takes you back up one level in the directory tree to the common data directory.

Put away the terminal window.

Launch vnmr by clicking on the icon. It will open in the last experiment that was active, which may not be the same as the last experiment YOU ran.

Get Oriented

explib to list the contents of all the experiments available to you.

jexp4 to join experiment number 4 if that one is set up to do approximately what you want to do, or contains a no-longer-necessary experiment which you can over-write with what you want to do. (4 is just given here as an example.) Since we will all share the same class account save anything important to you. experiments will be overwritten regularly.

dps (display pulse sequence) to display the pulse sequence currently set up and **dg** to 'display group' display the group of parameters to be used.

Load a Sample

acqi (click on the acqi button) to activate the interactive acquisition window.

Check the condition of your sample, tube etc. Only put intact, clean tubes in the spectrometer. Check that your solvent is compatible with the temperature of the probe.

eject to eject the current sample, go and get that sample.

Dry the outside of your tube. Mount your sample in the spinner, ensuring that it is securely held, and positioned where the probe is most sensitive (using the depth gauge). You are encouraged to always try to have the same volume of sample eg. 600 μ l or 750 μ l (5 cm or more is ideal), so that your sample height and thus shimming will be consistent. Remember that at least 10% of your solvent must be deuterated at a single position.

Place your sample at the top of the magnet so that it floats in the lift air stream.

insert to lower your sample into the magnet.

Lock and Shim Crudely on your Sample

lock Adjust the zo to minimize the frequency of the beats between the actual ^2H resonance frequency and the ^2H lock transmitter.

Adjust lock power to approach but not reach saturation, for maximum shimming responsiveness. A decrease of the power by 4 should be compensated well by an increase in the gain by 4.

shim adjust the z1 correction to the field such as to maximize the 'lock level'. Then proceed to z2, return to z1 and alternate until the optimum combination is obtained. In the bad old days you would then proceed to include z3 and z4 in the optimization cycle, then z5 and z6 as well (see Claridge's book).

close out of the acqi window.

Check alock and wshim. these should be set to 'n' unless you wish the instrument to automatically override your settings and find new ones.

Begin Setting Up

In your chosen experiment, set up an NMR experiment the simple way by loading Varian's default parameters (a good place to start, not perfect but 'good enough for government work'). Click on main menu, set up, nucleus, solvent, H1, CDCl_3 . The different spectrometers have capabilities of observing different nuclei, but all can collect ^1H spectra. One of the Gemini also collects ^{13}C and thing 1 is set up for ^{19}F and ^{31}P as well. For more options you will have to use Thing 2 with John Layton's help.

Check that the temp parameter is turned off by typing **temp ?** to 'ask' VNMR what temp's current setting is. The response should be 'n'. If it is not set temp to no (temperature regulation off): **temp = 'n'**.

Type **su** to 'set up' i.e. send the parameters on your computer screen to the NMR console.

type **gshmsr** (gradient shimming start) to initiate the gradient shimming process. This launches a small program of automated events (a macro) that will optimize the z shims for most samples and most conditions. Special cases may require more user intervention, and will be covered later.

Click on the button autoshim on z

When the computer obtains shims it finds acceptable, it will tell you so and stop making further adjustments.

Type **gshmend** (gradient shimming end) to quit the gradient shimming routine and return to the experiment you are setting up.

Having done this, make sure that alock = 'n' and wshim='n', so the machine will not attempt to redo your shimming etc.

Check Experimental Parameters

dps to check that the pulse sequence to be executed is indeed what you want. ALWAYS do this before starting an experiment. It takes 2 seconds and can save a \$30,000 probe. We intend to apply a single short 'observe' or 'probe' pulse that should not exceed pw = 30 μs . This should be followed by an acquisition period (acquisition time, at) during which the detection coils will measure and the computer will digitize the magnitude of signal, as a function of time. For 'small' molecules of molecular weights less than ≈ 1000 Da at should be more than 1 s long. All of this is preceded by a relaxation delay 'd1'. We most often want to signal-average over several scans to improve signal-to-noise and cancel spectral artifacts (a later lecture). The d1 interval serves to allow magnetization excited by one pulse to recover before the next is collected. Note that because we are performing Fourier transform NMR we are not in fact scanning at all, but the term persists from the days of 'cw' field-swept NMR. For at = 1 set lb = 1, for at = 2, set lb = 0.5 etc.

dg to display the other parameters that will control your experiment.

nt indicates the number of transients that will be averaged together. We have the luxury of a nice pure concentrated sample, so we only need nt = 1.

ss is the number of times the pulse sequence will be executed beforehand, without any data being collected. Such 'warm-up' runs establish an equilibrium for the magnetization in the context of your pulse sequence. This will become more important later. For simple observation of a single ^1H spectrum ss can be 0.

It is worth taking a minute to check that the transmit nucleus 'tn' is in fact the nucleus you requested, i.e. H1.

'tof', the transmitter offset, determines the frequency at which your spectrum will be centered, like the setting on your radio dial. 'sw', the sweep width, indicates how wide a frequency window will be observed. For a first look at a new sample, sw = 6000 is a good choice on a 400 MHz spectrometer, sw = 3000 is a good starting point on a 200 MHz spectrometer. These are chosen to be much wider than the spectrum is likely to be, so that no signal will be missed. The sw can be optimized later, once you have a spectrum to look at.

Check the gain being used to amplify the signal prior to digitization by typing **gain** ? If you have a protiated solvent you must set gain = 0 until you have solvent suppression working (later lecture). With a deuterated solvent and a strong (concentrated) sample, gain = 20 is a good starting point.

Finally, for the good of the nation (the spectrometer probes) check that the decoupler is off. This was also evident in the dps window, but the parameter responsible, 'dm' or decoupler mode, should be set to dm = 'n'.

Collect a trial Spectrum and adjust its Phase

Type **ga** to execute the experiment. You should see a green light flash indicating that a pulse was successfully executed. You should see the yellow 'acquire' light come on for the 2 second acquisition period. You should NOT see the red overload light come on (ever). If it does come on, decrease the gain (gain = 10) before trying again.

Use of the **ga** command automatically results in weighted transformation of your data into a spectrum (Fourier transformation will be discussed next week and weighting will be discussed thereafter).

The functions of the three mouse buttons depend on what menu you are working under, and are shown below the spectral window. When a spectrum is being displayed in interactive mode, depressing the middle mouse button allows you to adjust the vertical scale of the spectrum. A short click will adjust the scale to bring the spectrum's height to the position of the mouse at the frequency of the cursor. Depressing and dragging the mouse up and down expands and shrinks the spectrum vertically. clicking at the left-hand edge repositions the spectrum vertically. Practice makes close-to-perfect. The left-most mouse button places and moves a cursor, allowing you to read spectral amplitude. The right mouse button allows you to define the width of a 'box' or spectral region. The screen can be toggled between box and cursor mode (two cursors and one) using a menu button.

Your spectrum may be dispersive. You can have the computer correct this by issuing the **aph** command (automatic phase adjustment), which works pretty well. You should however also be comfortable with adjusting the phase manually. Click on the **phase** button in the menu, this changes the functions of the left and right mouse buttons, but not the middle one. Move the cursor to the right-most resonance in your spectrum. Depress the left button and drag up or down to untwist the signal and produce an absorptive line with a symmetric 'balanced' baseline on either side. Release the mouse button and move the cursor to a signal near the left end of the spectrum. Depress the left mouse button again and adjust the phase of this signal. When you are done click on the **phase** button again or on the **box** button. The right mouse button acts similarly to the left mouse button but applies finer control to the phase.

To zoom in on the spectral region containing your spectrum place cursors on either side of it and click on **expand**.

Many commands can be used to modify the display and annotate it (below). A few particularly useful ones are **f** (show the full spectrum), **full** (use the full screen width), **s1** (s2, s3, . . . , save the current expansion), **r1** (return to the expansion saved with s1).

The vertical expansion of your spectrum is achieved via the middle button of the mouse. Either click one at the height you would like a particular position of the spectrum to have, or 'grab' a position on the spectrum and drag it up (with the mouse button depressed). Alternatively, you can specify the value of the vertical scale (**vs**) parameter, eg. **vs** = 100, and use mathematical commands to increase or decrease the amplitude of the spectrum (eg. **vs** = 4 * **vs**). **vp** controls the vertical position (offset). You can enter a numerical value (**vp** = 30) or modify it interactively by dragging the mouse with the middle button depressed and the cursor at the left edge of the screen. Before doing this though, type **dc**, to introduce a drift correction to the data and in essence place it at a **vp** of 0.

dscale displays the scale, **dscale(0)** displays it at a vertical height of 0, or whatever you entered in the parentheses. The scale will be in ppm if **axis** = 'p' or in Hz if **axis** = 'h'. To calibrate the scale place the cursor on the top of a line of known chemical shift (eg. TMS at 0 ppm) and type **rl**. If the reference line chosen is not at 0 ppm, for example it is at 10, you have to specify that using **rl(10p)** instead of just **rl**. Now **dscale** again to see the correct chemical shifts of all your lines.

To collect a spectrum containing only the spectral regions containing your spectrum expand around your spectrum so that it fills the display with $\approx 10\%$ of the spectral width allowed for baseline on either side. Then type **movesw** (move sweep width). The computer will calculate new values for **tof** and **sw**, while retaining spectral calibration information.

Collect a 'Good' Spectrum and Obtain Peak Positions and Integrals.

Set **nt** = 8 **ss** = 2 and **ga** to collect a new spectrum of the correct width. You will have to adjust the phase again, as above. Even if you don't need 8 scans for sensitivity reasons, averaging 8 scans will cause a couple of artifacts to cancel out of the spectrum (a later lecture). This doesn't take much time but is good practice.

Correct the baseline: display the spectrum in interactive mode (**ds**) and click the **full/partial/no integrals** button until it you are displaying partial integrals. **cz** to clear any pre-existing selection points. Choose the menu option **resets**. Working from left to right, place the cursor on the baseline immediately before the first signal and left-click once, then move the cursor just past the signal and click again. You have now delimited that signal. Repeat for each of the signals in the spectrum. You may choose to group some clustered signals together, especially if they represent a single split signal. You can remove a misplaced selection point by clicking with the right mouse button.

You should now have dotted green integral line between signals and a solid green integral line arching up at each signal. Typing **bc** (baseline correct) will cause the computer to use regions between signals as the basis for calculating a baseline, which it then subtracts from the spectrum to yield a spectrum vertically positioned with the baseline at 0. (Alternately you can **dc** (drift correct) to position the spectrum at 0 without manipulating the baseline.)

The heights of the individual integral lines now represent the areas under the peaks to which they correspond. These areas can be displayed several ways. **dli** lists them **dpir** presents them in the display **dpirn** does so and normalizes to the largest signal in the spectrum. To use the latter two options the spectrum must be positioned vertically with **vp** = 12 or more, so that the program has space to print the integral values beneath the axis. Very small integrals will simply appear as 0.00. set **ins** to a large value, use normalized integrals, and **list** (**dll** or **pll**) instead of *displaying* integrals to get values for small ones. If you want to use a particular integral as the reference, and have its value be a particular value, then do **dpirn**, note the actual value of the reference integral and set **ins** = **ins** * **<the desired value of the integral>/<the current value of the integral>**. (If the integral currently has a value of .06 but you want it to have a value of 1, type **ins** = **ins***1/0.06). **ds** and **dpirn** again and the reference integral should have the value 1 (or the desired value). Recall that the

resonance of one proton may be split into several lines by J coupling !! Integral display can be turned off again using the `full/partial/no` integral button.

Other useful commands: `dpr`

Peak positions can also be listed or displayed, either before or after correcting the baseline and integrating. In interactive display mode, click on the `threshold` button. A horizontal line will appear. Position this using the mouse so that it is below the tops of signals you wish to list but above the tops of baseline teeth and rolls (and signals of impurities you do not want to draw to your advisor's attention). Typing `dpr` (display peak frequencies) will now result in all the taller-than-threshold signals being identified with the chemical shift (when axis = 'p') or frequency (when axis = 'h'). `dll` lists peak frequencies. As for the integral display commands, there are lots of variants of these, and you will have to experiment to find the one you prefer. John Layton has written macros that provide custom options that are convenient for both purposes. Line lists have many uses, to be discussed later.

Annotate and Print Your Spectrum

Annotate your spectrum using the parameter/command text and the command `atext`. Type `text('my very first spectrum at UK')` to give the string parameter `text` the value my very first spectrum at UK. You can now record things like the identity of the sample, or interesting observations in the text line. More text can be entered using `atext('baseline wavy because I collected these data while PPD was using a jack-hammer 2 feet away')`. (Please call John Layton, Dr. Meier or Dr. Miller immediately if anyone ever suggests they will try this or other similar lunacy.)

To print your spectrum type `pl`. Most commonly you will type a string of printer-related commands to plot the spectrum (`pl`), also plot its ppm axis (`pscale`), include the integrals (`pir` or `pirn`), include line positions (`ppf`) and parameter information (`pap` or `ppa` provide two different formats). All of this goes to a virtual page called a print buffer but is not actually set to paper until you issue the command `page`. Thus, a typical print order might be `pl pscale(0) pap(200,160) page`. John Layton has written macros to simplify your life in this area too, `plt` plots the spectrum and the axis to a page. We advise against using `pll` on the same page as `pl` etc, as this can result in congestion.

Save, Save, Save

If you ever want to see your spectrum again, for example to include it in a document, save it as a file in your data directory. Click on the `main menu` button (whenever you need to get back to the top of the command tree). Then click on `file` to display the contents of the current directory. The `data` button will take you straight to the data directory and display all the subdirectories. Select yours and click on `change directory` to enter it. Now save your file: `svf('a_useful_filename')`. Personally, I like to include a date and information about the sample and pulse sequence if it is variable. To get out of a directory, click `main menu`, `file`, `change directory`, `parent`.

Leave the Spectrometer Locked for the Next User

Replace your sample with the lock sample, and leave the machine locked and at least roughly shimmed (z1, z2) as above.

Exit VNMR using `main menu`, `more`, `exit`, or by typing `exit`. Give the computer several seconds to shut down VNMR, then log out by clicking `exit` in the SUN tool bar.

Before leaving either of the 400 MHz spectrometers, BE SURE to fill out the log book with your name, the solvent you used and any observations you had related to spectrometer performance. These are very valuable to John Layton when he is trying to diagnose weird, and especially sporadic, behaviour.

Remember when handling spinners to not touch the black belt around the top. Getting gunk on this reduces the contrast between the black background and the white dots, and makes it more difficult for the spinner's tachometer to read.

IMPORTANT

Everyone breaks a sample in the machine at some time. IT IS VERY IMPORTANT THAT YOU REPORT THIS to John Layton (preferred) or Dr. Miller RIGHT AWAY. Breakage of a tube is regarded as human error, unless it happens regularly, but failure to report a problem or worse yet attempts to disguise one, are regarded as unprofessional abuse of our very fine and expensive shared research resource. Abusive users will not be allowed to use the spectrometers. Please report problems right away so they can be fixed while they are small, and before they result in more, avoidable damage.

Problem set 1

(Please, for all problem sets, STAPLE together all pages and put your name on the top one. Also highlight or otherwise indicate important parameters.)

- 1 Go to the data subdirectory in the course directory (not the one in vnmrsys) and make a directory for your work. Give it a name which I can recognize as your name.
- 2 Collect a simple ^1H 1d spectrum of your sample with a wide spectral width to show all possible signals. Make sure that you describe the sample in the attached text file. Turn in a printout that includes the parameters and text, using the **pap** command. Throughout this course, I will want spectra to be accompanied by all relevant parameters, as well as information about the sample. Get in the habit of writing a little bit about your sample in the text string and printing parameters with your spectrum using **pap**.
- 3 Collect a second ^1H 1d containing only the signals and showing the ppm axis, the resonance positions and integrals, on the printout, as well as the parameters. Save this spectrum in your directory with a useful name.

Practicum 2, Spring 2004

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Optimizing the sensitivity of a 1d -part 1.

R.B.'s coffee, dried and redissolved in $^2\text{H}_2\text{O}$

Today we will perform a 'smart' 1d experiment. Here the objective will be to gain some understanding of the experimental parameters and make intelligent choices for them with the objective of collecting the most intense and artifact-free spectrum. In the description that follows procedures that have already been detailed are not detailed again, and when you have to click on a 'button' the button's name is outlined, when you have to type a command the command is in **bold** and when I refer to a parameter it will be underlined.

Clean the sample, load the sample, check hardware configuration

Lock (don't saturate), preshim Z1 and Z2, set you spinning speed and confirm temp = 'n'.

Gradient shim. (For fun some day, collect a 1d before and after gradient shimming to see the improvement shimming well makes.)

When you have a set of shims you like you can save these as a shim file with the **svs** command (go to the shims directory first please). Similarly, parameter sets can be saved (in parlib) with **svp**. Alternately, both can be obtained from an older experiment in which they were used by reloading that .fid directory, setting **load = 'y'** so that your current shims will be overwritten and then **su**. The default value for load is 'n', for obvious reasons.

Type **s2pul** to load basic parameters and this basic pulse sequence.

S2pul is one of many macros intrinsic to VNMR that will execute series of VNMR commands and modify parameters. Among other things, this macro (and John Layton's gshmend macro) will reset a couple of hidden parameters which you should therefore be aware of. spin? reports whether the spinner is on or off, but unfortunately does not give you control over it. To turn on/off the spinner you have to open acqi and then **lock**. The spin speed should be 20 Hz when on and should be off for all 2D and higher experiments (you will find that I tend to work with it off out of habit, whereas 'small molecule' people will tend to automatically have it on, so check the parameters of your experiment instead of assuming that it is still as you left it.) Also check that wshim='n' and alock='n' otherwise when you launch your experiment with a **ga** or **go** command the machine will automatically proceed to reestablish lock and reshim, overwriting your own shims.

The name of the pulse sequence to be executed resides in the parameter seqfil (pulse sequence file). Thus, after executing the **s2pul** macro, seqfil='s2pul'. The s2pul (or std1h) sequence encodes the most basic, and frequently used 1 pulse sequence consists of an excitation pulse, a period during which an FID is digitized and then a delay during which magnetization is allowed to recover (relax) before the sequence is repeated. Even this very simple sequence of events encompasses a number of decisions (adjustable parameters) that can affect the quality of the spectrum that results.

pw: pulse tip angle A 90 degree pulse will produce the largest signal in a single scan, but not necessarily the most signal for a fixed amount of time, when a pulse sequence is repeated many times. Nonetheless, it is good to know what tip angle is being used, or equivalently the duration of a pulse effecting a 90 degree tip. This value depends on the power (tpwr) used and is typically stored as the value of pw90. This value is best determined by measuring the pw360 and dividing by four (guess why). Note that most pulse sequences do not actually use pw90, it is just stored in the experimental parameter set as a convenience to you.

tpwr embodies the power used during the pulse (the size of the H_1 magnetic field tipping the magnetization). Large values signify high powers, but the units used are those of attenuation: db. The decibell scale is logarithmic. a 1 db decrease in tpwr will decrease the size of H_1 by 12% and thus require that pw be 12% longer to achieve the same tip angle. The easiest relation to remember is that a 6 db decrease in tpwr can be compensated for by a 2-fold increase in pw. The preceding refers to ideal probe behaviour, or linear behaviour. However, at the high end of the power scale, the probe's power handling capability is less efficient and eventually saturates. This is called the compression regime and it means that you have to push harder and harder to shorten your pw90 at high powers. It also indicates that you are approaching the probe's limits (yellow light). For this reason, we often check probe linearity before proceeding with an aggressive experiment. If you ever encounter probe arcing (below), stop whatever you are doing. This is the red light. tpwr can take integer values between -16 and 63, but again, don't use top power unless you need it AND it is not stressing the probe.

Finding the pw90:

Choose a reasonable tpwr, such as **tpwr=57** for ^1H . Set pw to a value you are confident is much smaller than a 180° pulse. **pw=2**. This will set pw to 2 μs , the default unit for pulse widths. Take a second and **dps** before launching the experiment. The different time units are colour-coded, always be sure that your pulses are in μs (or occasionally in ms), NOT s. Note that changes in tpwr will alter the height of the pulses shown in dps. Compare the results of **tpwr=60** and **tpwr=10**. **ga** to launch the experiment and automatically produce the Fourier transformed spectrum. Phase it up.

Now compare the results of using an increasingly long pw. This is accomplished in a single experiment by setting up an array of pw values, trying each of them and displaying the resulting spectra side-by-side for easy comparison. Type **array**, then respond to the prompts with **pw, 10, 5, 5**, for comparison of the effects of 10 pw values beginning at 5 μs and spaced by 5 μs . Before interpreting the results be sure that the absolute spectral intensities are displayed (as opposed to internally normalized intensities), by typing **ai**. (Normalized intensities are obtained by typing **nm.**, but don't do that now). **dgai?** gets the machine to tell you whether it is in ai or nm mode.

This should correspond to tipping magnetization further and further around the axis of the H_1 field applied during the pulse (eg. -x). Thus magnetization should appear along y, then shrink along y as it grows along -z, then grow along -y as it shrinks along -z, then shrink a long -y as it grow along z. Once it returns to maximum along z and zero along y it will have executed one complete precession of 360° and the length of pw required to accomplish this is the pw360. (Note in the preceding that the only magnetization we detect is the $\pm y$ magnetization.) Calculate the pw90 from the pw360/4.

If your first array did not include the pw360, create a new array with longer pw values and possibly smaller spacing for a more accurate pw90. If you are checking for probe linearity, determine pw90 again for a tpwr 6 db smaller than the one you just used. It should be twice as long. Write your results in the log book, to help John Layton keep track of spectrometer and probe performance (thank you).

In cases such a pulse width calibration when the results of a series of scans are to be compared, it is useful to be able to display all the spectra simultaneously. **dss** will overlay them all. **dssa** will stack them one above the other vertically, with the first member of the array at the bottom. **dssh** is what we used, to display stacked spectra in a horizontal row with the first spectrum on the left (display

stacked spectra horizontally). To plot the whole stack of spectra use the `pl` command with the option `all: pl('all')`. To view the array of values which your arrayed parameter takes, type `da`.

If you ever see weird or random behaviour in response to a smoothly increasing `pw`, this may be probe arcing. IMMEDIATELY abort the experiment by typing `aa`. You can also use the `abort acquisition` button or type `stop`.

Assuming the probe is linear, enter the higher value of `tpwr` and the corresponding `pw90` as `pw` in your experiment.

Spectral width

Correct identification of `solvent` is necessary for `tof` to be meaningful and reproducible. Make sure this is set correctly before proceeding.

`sw`: spectral width (or sweep width in the old days of CW spectroscopy) This is the width of the frequency domain spectrum that is to be obtained after Fourier transformation of the data. Since spins with a frequency very different from the carrier frequency precess very rapidly in the rotating frame, data points must be collected very rapidly in order to determine their frequency. The Nyquist condition defines the relation between the "dwell time" between acquisition of complex data points (`dw`) and the maximum frequency (relative to the center of the spectrum, `sw/2`) that will be properly contained in the spectrum.

$$dw = 1/sw$$

Resonances outside the spectral window will be wrapped in and attenuated by filters.

Begin by collecting an `nt=1` spectrum with a wide `sw` you are certain will contain all your resonances (30 ppm is tons for most purposes). The resulting spectrum will be extra noisy from the noise of all the extra data points collected, but will allow you to choose a spectral window that does not cut anything out. Place cursors around your spectrum, expand and type `movesw`. This will move the `tof` as well as the `sw` to give the requested spectral window. `tof` denotes the center of the spectral window frequency (transmitter offset).

Note that if you have one very strong signal, for example that of residual water, your best bet is to center the spectrum on that signal, even though this may require you to use a slightly larger `sw` to include all of your resonances. The reason is that if the strong signal is very strong then artifacts associated with it may be sufficiently strong to contaminate your spectrum. In this case, don't adjust `sw` first. In your wide trial spectrum place the cursor on the one strong signal and type `nl` (nearest line) to have the computer optimize the cursor position to the very top of the line, then type `movetof` to place the `tof` at that frequency. `ga` to collect another spectrum at the correct `tof` and place cursors on the farthest-flung line and the strong central line. You will want `sw = 2` times this width plus ten percent for baseline correction purposes later. Set `axis='h'`, then `sw=2.2*delta`. The former is necessary because `sw` is in Hz unless it is entered with a 'p'.

We will look at aliases produced by improper choices of `sw` and `tof`. Other macros that will help you choose a `sw` fast include `minsw`, `sw(down,up)`.

Acquisition time

The duration of the acquisition time determines the maximum possible spectral resolution, as only frequency differences $\Delta \nu > 1/at$ will be resolvable. With a strong sample, you can always start with a long `at`, and collect 4 or 8 scans. Process without line broadening (later lecture) and evaluate the width of your sharpest lines. This is the limit determined by the quality of your shimming, NMR

tube, sample contents . . . There is no point in digitizing to higher resolution than these afford. For an observed limiting line width at half height $\Delta\nu$, set $\text{at}=2/\Delta\nu$ for maximum resolution. This provides for the possibility of resolution enhancement (later).

If you cut at too short, you can truncate the fid and produce sinc wiggles called truncation artefacts (which we will explain in lecture). These wiggles look a bit like feet. Weighting functions were devised to shape the fid, squeezing it down to zero at the end *as if* it had fully decayed. Subsequent Fourier transformation produces broader but normal-looking signals (without feet) so application of the weighting function is called apodization.

d1: relaxation delay

When insufficient time is allowed for relaxation each successive scan produces less signal, the phase cycle fails to cancel artifacts effectively and signals that relax slowly are preferentially suppressed. We will deal with this in more detail next time. For small molecules, allow several seconds. Optimal sensitivity after a 90° pulse is obtained when $\text{at} + \text{d1} = 1.3 \cdot T_1$. $\text{at} + \text{d1} = 3.7$ is a good choice. The default units for d1 (and all delays) is seconds (s). Set d1 to some value and then in dps , check the colour coding of the delay.

Others

gain, make sure that you make good use of the spectrometer's dynamic range by using a gain of 30 or higher whenever possible (*i.e.* without having the receiver overload light come on). You can have the spectrometer choose a good value of gain by setting $\text{gain} = 0$ $\text{gain} = 'n'$ before acquiring the spectrum. The spectrometer will then collect single shots of your spectrum with a series of different, increasing gains until it starts to approach the capacity of the receiver. You can then learn what it chose using $\text{gain}?$ or take back control of gain without changing it by typing $\text{gain} = 'y'$.

If your gain is set too high, you can damage the receiver by subjecting it to too much power. You can also damage your spectra. When the amplitude of the incoming fid exceeds the dynamic range of the receiver, the highest possible value is recorded but this will be less than the actual value the amplitude should have had. The fid will have its top 'clipped' off. Fourier transformation of this flat-topped fid produces sinc wiggles, but due to their different origin they cannot be apodized away. You have to decrease gain and collect your data all over again. Better to save time (and the receiver) by optimizing the gain first on single shots before launching a long run. NOTE that certain 2Ds like COSY will produce small signals in early increments and much stronger signal in later increments, so the gain should be optimized on a scan set up to look like a mid-run increment.

Choice of nt, bs affect the effectiveness of the phase cycle, and whether or not data are stored in the event of premature termination of the experiment. For long experiments, set bs = some multiple of 8 that consumes ≈ 5 minutes. Then you will never lose more than 5 min. of data, and you will be able to see updates in the spectrum after reasonable (significant) numbers of scans have been added to it. Set nt to some integer multiple of the phase cycle, which is commonly 8 for the experiments we will be doing at first. eg. **bs=8 nt=16**.

Be sure $\text{dp} = 'y'$ for double precision in digitizing your data (this is the default).

For a truly gourmet baseline, and before collecting 2d spectra:

Set tof and sw, and rephase, then optimize the gating delays rof2 and alfa

rof2 is allowed for the power delivered in the pulse to decay to zero before the (very sensitive) receiver is turned on. alfa is allowed to allow the amplifiers involved in signal detection to stabilize before the first data point is collected. When dsp is set to 'r' (digital signal processing in real time) data points are collected at very close intervals, and errors in the synchronization of the pulse timetable and that of the receiver produce baseline roll and first-order phase errors. First get the

computer to reset rof2 based on your current phases by typing **crof2**. Then optimize alfa keeping the sum of the two constant and arraying alfa. This pair of values should then be applicable to essentially all experiments. The commands to use to find an optimum pair of rof2 and alfa values are: **array='(rof2,alfa)', rof2= x, y, z, alfa= a, b, c**. Where a,b,c,x,y,z are in μ s and $a+x = b+y = c+z =$ the sum of the rof2 and alfa that were obtained upon typing **crof2**

Collect a gourmet spectrum (**dps** first). Save your parameter set.

Show an example of spinning side bands, especially when XY shims are poor.
Distinguish from ^{13}C satellites.

Reading for next week:

Chapter 3.1 and 3.2 (3.4.4 optional) 3.5.1 pages 94- 97 (top 2/3) from "High Resolution NMR Techniques in Organic Chemistry" .

Second assignment, Due 28 Jan. 04 at the start of class

- 1 (/4) Turn in a spectrum with a **strong** resonance in it and show me a quadrature artifact. Note that it really is best to have one or just a few very strong signals, and place it (them) off centre by several hundred Hz. Circle or indicate with arrows visible quadrature artifacts.. Change one parameter, recollect the spectrum and show me that it (they) is (are) now gone (turn in another spectrum). Print out each of the spectra with all the parameters using **pl pscale pap page**. Underline or highlight relevant parameters.

- 2 (/4) Turn in a spectrum with too small a sw and circle or identify with arrows the artifacts of having done so. Describe how you might recognize these artifacts in future. Turn in a spectrum with the problem corrected. Print out each of the spectra with all the parameters using **pl pscale pap page**. Underline or highlight relevant parameters.

- 3 (/4) Collect two spectra with gains that differ by 6 db. Plot these both with the same vs, after typing **ai**. Measure the height of a resonance in both the spectra and comment on the results. Print out each of the spectra with all the parameters using **pl pscale pap page**. Underline or highlight relevant parameters.

Practicum 3, Spring 2004

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Optimizing the sensitivity of a 1d -part 2. (ethyl crotonate in CDCl₃)

IMPORTANT: know the ways to stop a run immediately, and the times when you should do so. How to: enter **stop**, enter **aa**. Click on the **abort acquisitions** button under main menu. When to: whenever the receiver overflow light is on. Whenever you even suspect that a pulse is too long or a power is too high, if the machine loses lock, the temperature goes off, you hear anything unusual smell anything suspicious etc. etc etc. You can always restart a machine that is not damaged. However, once damage is done, it can be weeks before the machine can be used again. Whenever you set up long runs in a queue, make sure you launch each one and let it run into the sequence proper (past the ss scans) to be sure you are not overloading anything. Then stop and set up your queue.

TODAY we will perform a quantitative 1d experiment. The objective will be to collect a spectrum in which the integrated areas of the individual resonances are really proportional to the proton concentrations responsible. This involves taking into account the different relaxation rates of the different hydrogen atoms in a molecule. Knowledge of these relaxation rates also enables one to obtain the highest signal-to-noise per unit time, which is valuable for spectra of dilute compounds or rare nuclei (¹³C, ¹⁵N).

In the description that follows procedures that have already been detailed are not detailed again, and when you have to click on a 'button' the button's name is outlined, when you have to type a command the command is in **bold** and when I refer to a parameter it will be underlined.

Upon starting vnmr: type **explib** to list the text and pulse sequences of all your experiments. This will allow you to choose an experiment which is just what you want, or one you are willing to overwrite. The **text** command and **atext** provide for entering text, 'c~~text~~' clears the text. 'seqfil' is the name of the parameter that is set to the name of the pulse sequence to be used.

Set up an experiment using the pulse sequence name macro: **s2pul**. Disable wshim and alock. Load a clean sample, lock, shim, calibrate the pw at tpwr=57, choose the gain, sw and at. Collect a decent 1d.

Recall from last time, and your problem set, that phase cycling with nt = 4 or 8 eliminates centerband glitch and quad images. Collecting with an adequate sw prevents resonances from 'folding in'.

Common phase notation describes a pulse along the x axis as having a phase of 0, a y pulse has phase = 1, a -x has 2, a -y has a phase of 3. The phase notation applied to the receiver refers to the phase a simple 90° pulse would have had to produce the observed magnetization: although an x pulse (0) produces -y (3) magnetization, the receiver phase is defined as 0.

Measurement of the relaxation times T₂ and T₁.

T₁ and T₂ are the 'characteristic times' or longitudinal (parallel to z) and transverse (perpendicular to z) relaxation. Mathematically, they are the times required for 1/e of the spins of a given type to return to their equilibrium distribution. Just as all systems spontaneously return to equilibrium with their environment ('thermal bath' in physics-speak), the magnetization we placed in the X-Y plane with our pulse will eventually return to the Z axis. This involves two phenomena: the magnetization disappears from the XY plane *and* it reappears along the Z axis, parallel to the static field. Recall that we are considering NET magnetization. Just as hot coffee cools rapidly at first and then increasingly slowly as its temperature approaches that of the room (its bath), recovery of Z magnetization is rapid

at first but only reaches 99% complete recovery after a surprisingly long relaxation time. The recovery is exponential (see your book). Of course the magnetization restored along Z came from somewhere: the XY plane. However, the NET magnetization in XY can disappear faster than it reappears along Z. This is because although the total number of nuclear magnetic moments in the sample is unchanged, they are to some extent aligned together right after the pulse, so they produce a NET moment. After the pulse they precess freely in the static field, so if some spins are subject to a stronger static field than others they will get out of phase with the others and eventually cancel the others to some extent. Thus, NET magnetization decreases by additional mechanisms than those that mediate T_1 relaxation (recovery along Z). Similarly, the spins of protons that 'change identity' via chemical exchange will alternate between different precession frequencies and tend to cancel out faster than the spins whose identities are fixed. The above explain why poor shimming (a non-uniform static field) and molecular and chemical dynamics cause short T_2 values. In lecture, we will give a mathematical proof that short T_2 values are associated with large line widths. The apparent T_2 including the effects of poor shims is called T_2^* while the use of ' T_2 ' is correctly reserved for the intrinsic T_2 that is a property of the molecule and its spins only.

Now for the practical aspects: to choose good values for pw and d1, you should know T_1 , and to choose at you should know T_2 . These relaxation times will be even more important in the intelligent choice of parameters used in setting up 2-dimensional experiments (later).

To measure T_1 , obtain a nice 1D, calibrate the pw90, type **dot1** (do T_1), you will be prompted for guesstimates of the T_1 s and the amount of time you are willing to spend measuring them. You are free to overwrite these with your own choice of six or more values: **d2=a, b, c, d, e, f**. Also note the nt value the software suggests, and change it to what you want. A 180° p1 pulse is used to invert all magnetization and followed by a delay of d2. By monitoring the recovery of magnetization as a function of d2, using a 90° pulse you can fit the recovery profile and obtain T_1 , for different signals. Work with the last spectrum (which should be fully upright). If you had eight spectra type **ds(8)**. Phase it up and set th and peak pick using **dpf** or **dll**. **fp(x,y,z)** to tabulate the heights of resonances x,y,z in the peak list. **t1s** calculates T_1 s and **expl** displays the fits (**pexpl** plots them).

To measure T_2 , use **cpmgt2** (invented by Carr, Purcell, Meiboom and Gill for the measurement of T_2). You must know the 90 pulse length (set pw90). Using pw = pw90, p1 = 2^*pw90 and a d1 of at least 3^*T_1 . In order to measure *bona-fide* transverse relaxation, not just static field inhomogeneity, we will refocus magnetization at intervals of d2 during the total relaxation interval of bt. set **d2 = .01** for small molecules, **d2 = .001** for macromolecules, bt = values ranging from 4^*d2 to $\approx 2^*T_2$ (your best guess, eg. from line widths, in seconds). Display the first spectrum, set th, execute **dll** and choose lines for analysis: **fp(x,y,z)**. **t2s**, **expl** and **pexpl** function as above. Note that in addition to T_2 relaxation, 1H spins are subject to J coupling with other 1H spins during the bt interval. This will cause lines to cycle through antiphase and in-phase forms as they decay, and it can be a bit tricky to separate the two effects. For decent estimates of T_1 and T_2 , note that T_1 or T_2 can be estimated from the time needed for a signal to relax half the way, $t_{1/2}$: $T = t_{1/2}/.7 = 1.44^*t_{1/2}$ (try to figure out where the factor of 0.7 comes from).

Compare the CPMG T_2 with the apparent T_2 : $T_2^* = 1/(\Delta^* \Delta)$ obtained from the line width (Δ) which includes field inhomogeneity, recall that Δ is frequency in Hz. Also, for a good line width measurement, use a long at, little or no apodization (ie lb = 'n', gf = 'n', sb = 'n'). Shim, shim, shim your magnet, shim the lines up tight . . . (to the tune of 'row, row row your boat')

Use of T₁ information

Many molecules' T₁s are longer than the amount of time we want to commit to at+d1. If you must use a 90° excitation pulse you should allow $(d1+at) = 1.3 \cdot T_1$ for the longest-T₁ signal in the spectrum (eg. as in all our favourite 2Ds). Otherwise, for maximum sensitivity, you are MUCH BETTER OFF to use a pw << pw90. The best tip angle for signals with a given T₁ is called the Ernst angle, α_e , where

$$\cos(\alpha_e) = e^{-\text{del}/T_1}$$

del is the total relaxation time to be allowed (d1 + at) and T₁ is the longitudinal relaxation time of the spin of interest (Ernst & Sternlicht, 1972, J. Magn. Reson. 6: 167-182). Using an Ernst angle pulse gets you optimal signal per hour of machine time (when sensitivity matters!). Use the 'ernst' macro. **ernst(T₁,pw90)** or **ernst(T₁)** if pw90 has been calibrated and the correct value set as pw90.

If you want to be able to interpret signal integrals in terms of spin concentrations (numbers of identical hydrogens), you must be sure that *all* the hydrogen nuclei are equally relaxed at the beginning of each scan. Therefore you must set $(at+d1) > 3 \cdot T_1$, at least, where T₁ is the longest T₁ in your molecule. $(at+d1) > 5 \cdot T_1$ is slightly better.

Use of T₂ information

Weighting functions -1

The signals' line widths reflect their T₂s: $\Delta\nu = 1/\Delta T_2$, in Hz. Broad signal line widths obscure potentially informative couplings, but it is pointless to try to acquire data with a higher resolution than the resonances themselves have. Moreover, if the spectra are noisy, the noise may make it difficult to measure and interpret couplings. Line broadening is applied to the fid (free induction decay) before Fourier transformation, to emphasize the early portion of the fid, in which the signal is strongest compared to the noise. This however has the effect of broadening resonances. For best signal-to-noise apply a 'matched filter' of exponential decay with **lb=x** where $x = \Delta\nu \Delta T_2$ and $\Delta\nu$ in Hz is the line width of the resonance in question (the sharpest one, to be conservative).

For a start, **lb = 1/at** is a good conservative choice which will somewhat reduce the noise and not broaden anything more than it is already by the size of the data set. For sine-bell weighting, a first choice might be **sb = 1*at**, **sbs = -1*at**. For Gaussian weighting try **gf = at/2**. For all of these, you can use **wti** to view your weighting function and its effect on the spectrum.

Reading from text: section 2.4

2 February 2004 Quiz: (#1)

/2 List two different ways of stopping an experiment and distinguish between them.

/1 In what units are pulse widths defined ?

/1 In what units are delays defined ?

/1 Name the VNMR parameter used to specify the duration of the delay allowed between repeats of a pulse sequence.

/1 Name the VNMR parameter used to specify the power used for a standard proton pulse.

/1 What subdirectory is all the vnmr stuff in, inside each user's directory ?

/2 List two things you should not take near the magnet and say why.

/1 What does FID stand for ?

/2 Why do we include in each sample some deuterated solvent ? Why do we use a solvent with only one NMR line ?

Reading for next week (Feb 11 2004): Skim Chapter 4.1 and 9.3 and 9.4 (we will not cover all of this, we will focus on the 'easy stuff') from "High Resolution NMR Techniques in Organic Chemistry".

Third assignment, Due 18 Feb. 04 at the start of class

NOTE that you must be checked out on a 400 MHz NMR by 11 Feb. 04

From Feb 11 on, you should perform your practical assignments with the numbered sample assigned to you. Corollary: if you want to use a sample of your own choice, bring it in to Mr. Layton on/before Feb. 9 so he can run a few tests on it to ascertain suitability.

1 Photocopy for me a chart showing different chemical functionalities and their ^1H chemical shifts. Remember to include a complete citation of the source. Do the same for ^{13}C .

2 How many Hz are there in 1 ppm on a 200 MHz NMR ? 5 ppm?

3 at Using a sample that does not contain CrAcAc and that you have shimmed up sufficiently to obtain 1.2 Hz line width at half height or better, collect a spectrum with $\text{d1}=0$, $\text{at}=3.7$ and $\text{lb}=\text{'n'}$, plot an expanded region with the axis in Hz to show that your line is sufficiently sharp. Now recollect the spectrum with $\text{d1}=4.2$, $\text{at}=.5$. Measure the line width at half height in both cases and compare these values. Using a simple equation, explain the basis of your observation. Use this sample for problem 5 below.

(To measure the line width at half height, first apply drift correction or baseline correction to your spectrum (dc or choose reset values and type bc). Zoom in on the resonance of interest, so that it uses at least 20% of the screen width, also be sure that you have sufficient fn . Activate threshold and set it at the top of your resonance. Type $\text{th} = \text{th}/2$ to move the threshold to half height, then activate box and place the cursors at the intersection of the resonance and the threshold line, on each side of the resonance. Now, delta is the width at half height. Use $\text{axis} = \text{'h'}$ to get delta in Hz.)

4 recycle time Using a sample that does not contain a relaxation agent, collect a series of $\text{nt}=8$ spectra using $\text{pw} = \text{pw90}$, $\text{at} = 0.5$ and $\text{lb}=2$. Vary d1 from a very small value up to 10 seconds. Do not do this using an array, since you will then be forced to use the same step increment between all steps. In fact you need small increments between small values of d1 and a longer increment between long d1 values. Do this by typing $\text{d1}=\text{a, b, c, d, e}$ where a, b, c, d and e are the durations of your choices of d1 , in seconds. Print out the series of spectra in ai mode and comment on the result.

5 Relaxation times Using the sample you used for problem 3, that does not contain a relaxation agent and that you have shimmed up sufficiently to obtain 1.2 Hz line width at half height, measure the T_1 s and T_2 s of two signals, chosen to be as different as possible. Compare the T_2 s obtained with the T_2^* s estimated from the line widths. (show exponential fits to resonance intensities with good exponential behaviour).

resonance ppm	T_1	T_2	T_2^*	comments

7 peak intensities Using $\text{d1}+\text{at} = 5*T_1$, compare the areas obtained with $\text{lb}=0.5$ and $\text{lb}=2$. Comment on the result.

Using $\text{d1}+\text{at} = 5*T_1$, compare the areas obtained with $\text{nt}=8$ and $\text{nt}=16$. Comment on the result.

8 amplifier linearity Measure pw_{90} at $\text{tpwr}=57$ and again at $\text{tpwr}=45$. report the pw_{90} value you get in each case. What factor shorter do you expect $\text{pw}_{90_{57}}$ to be compared to $\text{pw}_{90_{45}}$ under ideal (linear) conditions? Obtain an $\text{nt}=8$ spectrum using the pw_{90} for each of these powers. Does one give better sensitivity (signal-to-noise) than the other? Are there any other differences you might expect given that one spectrum was obtained with a longer pulse width?

Practicum 4, Spring 2004

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Selective 1 d methods using simple square pulses and shaped pulses.
Strychnine with Acac in CDCl₃.

Quick good-citizen notes:

Saving files to diskette: open two file manager windows, drag and drop. Do this often, you can use PC formatted diskettes.

If you have collected a spectrum using continuous decoupling, please turn off the decoupler before logging out.

Set up

Lock, shim, calibrate pw90 at $tpwr=60, 54, 48$. Choose a good gain, sw and at for your sample.

Note that if the gain is too high you may 'clip' off the top of the FID. Because this has the effect of making the FID look square, the FID's Fourier transform takes on the appearance of sinc-shaped lines. You will see ripples, or fuzz at the base of all resonances, but most visibly at the bases of strong ones. You can display the FID **df** to look for clipping, or use of **ddff(1)** to see if data points are approaching but not exceeding 32k.

Another source of similar 'feet' on signals is truncation of the FID with too short an at. This has the same effect because it too makes the FID look a bit square. You can use linear prediction to extend the FID and then a weighting function to decrease the contribution of the calculated points to the final spectrum. This will have the dual benefit of eliminating the feet (hence the name 'apodization' functions for weighting functions), while increasing the spectral resolution obtained from that data set.

(note that you can always increase digital resolution by 'zero filling' or increasing the number of points in the frequency domain.)

Linear prediction (lp)

parlp creates the parameters for a 1D, **parlp(1)** for first indirect, etc.

dglp displays them.

You can use backward **lp** to fix early points, forward **lp** to extend the FID and mitigate the effects of the weighting function. The latter is especially important for 2Ds and higher, where you may only be able to collect relatively few points in the indirect dimension (ni complex, see later), and therefore may have a severely truncated interferogram in t1 (later). Set **proc = 'lp'** to activate linear prediction. (or **proc1 = 'lp'**) Set **proc = 'ft'** to inactivate linear prediction, **proc** stands for processing.

parameters you might change include:

lpopt f or b. forwards or backwards

lpfilt 8 to 64 at most. \approx the number of signals in terms of which the FID should be simulated, 8 is good for a typical 2D's indirect dimension whereas a typical 1D might be dominated by 10 - 30 strong signals. **lpfilt** must be $\leq 0.5 * \text{lpnupts}$.

lpnupts ni to 512. The number of complex points in the direct dimension is $\text{np}/2$, so this is your upper limit in 1-d linear prediction. For linear prediction in multi-d spectra, use all ni indirect dimension complex points. **lpnupts** must be $\geq 2 * \text{lpfilt}$, at least, and it is better to use 4* or more.

strtlp For forward prediction, use **lpnupts** (1d work) or **ni** (multi-d) For backward LP, use the first good point (look at the FID to see when the good points start).

When linear predicting backwards the reference point is the point separating later (good) points which will be used as the basis for the lp and the earlier points which will be replaced (recreated).

When linear predicting forwards the reference point is the last point in the (indirect) FID that will be used as the basis for the LP, since all the preceding points will be the basis for the lp.

lpext $ni - 3*ni$ or the number of bad points.

When linear predicting backwards, use either **strtlp-1** (replace just bad points) or **strtlp** (to replace **strtlp** with the fitter version too), a matter of taste.

When linear predicting forward you can add as many calculated points as you want, but it is common to add ni or $np/2$, to 'double' your dataset, or add $2ni$ or np , to triple it.

strtext For backward LP, use **strtlp** or **strtlp-1** as decided above.

For forward LP, in 1-d spectra, use $np/2 + 1$ for 1d spectra (the first calculated point comes right after the last data point, and points are complex points hence the $np/2$). For multi-d forward LP use $ni+1$.

This is the first point (in the direction b or f) which will be replaced by the lp value).

fn $\geq 2*(lpext+np/2)$ or $\geq 2*(ni + lpext)$, rounded up to the next power of 2.

The 'Fourier number' is the number of points used for your spectrum after Fourier transformation. This will determine the digital resolution, the number of Hz per point. Since you would like digital resolution better or equal to your spectral resolution (that is to say a smaller number of Hz / point), make **fn** large enough that the digital resolution is a smaller number than 1/2 your sharpest line's width at half height. These values can be checked on a line by placing the cursor on the line and typing **dres**. You don't need to collect new data if **fn** is too small, just increase it and Fourier transform again (**wft**).

Example, for a 1-d spectrum, collected with $np = 728$ and $at = .1$

$lpopt = f$, $lpfilt = 16$, $lpnupts = 364$, $strtlp = 364$, $strtext = 365$. This doubles the FID, so **gf** was reset from $gf = 0.05$ to $gf = .1$ (double the window function to go with the doubled FID).

Note about LP up to a power of 2 instead of zero filling, use appropriate factor extension of **gf.

DO NOT FORGET TO SET **proc = 'lp'** in order to implement linear prediction.

Amplifier linearity: a 6 db decrease in amplifier power should require a pulse 2^* as long to achieve the same tip angle. For example if the pw_{90} at $tpwr = 51$ is 25 μ s, the pw_{90} at $tpwr = 45$ should be 50 μ s. It is convenient to work in the linear regime when possible, this allows calculation of desired pulse widths, instead of tedious recalibration at all the powers of for all the pulse lengths to be used. A 1 db decrease in power increases pw_{90} by a factor of 1.12. The reason we care is that we may need to use a long pulse to achieve selectivity.

Selectivity: a second incidence of the inverse relation between the time domain and the frequency domain. The longer the pulse, the narrower its excitation profile. Example of 180° pulses of different lengths. Next week we will see how a composite pulse can provide improved band width, especially for 180° pulses.

You can array **tof** to visualize a pulse's excitation profile.

Thus, for selective decoupling, we will use long pulses and low power. Set **homo = 'y'**, **dn = 'H1'** (or whatever nucleus you are observing). Collect a 1D, set cursor on line to be decoupled, type **sd**, use two cursors to measure the splitting to be covered by the selective decoupling. For waltz decoupling (**dmm = 'w'**) you will need **dmf = 2^* delta** and therefore an effective decoupler pw_{90} of $1/dmf$ in μ s. Equivalently, decoupler $pw_{90} = 1000,000/2^* delta$, or 35.7 ms to decouple a line split by

28 Hz. Calculate the dpwr required to satisfy this condition using the 6 db rule and set dmf to 28. Try a couple of dpwr / dmf combinations to optimize the completeness of decoupling (do not use too much power, keep dpwr < 20) in combination with the selectivity (improved by low power). You can do continuous on-resonance decoupling with dmm set to 'c'. Note that this is by definition narrow-band decoupling and 'on resonance' is set at the frequency dof.

Selective inversion pulse

Recall the parameter set 'selinv' using the load button. You will need to update the pulse widths to ensure that the p1 pulse really is a 180 degree pulse at the power used. Also update tof, sw, pw, tpwr. The longer the duration of the inversion pulse, the more selective it is. Note that when you are using a simple 'square' pulse, the inversion pulse is delivered at the carrier frequency tof. This means it is centered there. You can, however, choose tof to be right on the resonance you want to invert. Just be sure that your sw is adequate so that the entire spectrum is still collected properly without folding.

The making of shaped pulses is described in the next exercise.

Problem set 4 due Feb25 2004**1, /3 points**

Look up J coupling / splitting in a book and then draw the signal expected of a proton which is split by a 11 Hz coupling to one other proton as well as 4 Hz coupling to three identical protons.

2, /10 + 4 points

Experiment with Gaussian line broadening and resolution enhancement: Using a spectrum with relatively high inherent signal-to-noise turn off Lorentz broadening and sine-bell functions (**lb = 'n'** **sb = 'n'**). Zoom in on a region of the spectrum containing a resonance with small splitting. Estimate the splitting using the cursors (set **axis='h'**, place one cursor on one line of a multiplet and the other on its neighbour, **delta** will be the splitting between them.) Also identify a spectral region with a strong peak and a good stretch free of signals (just noise amplitude), measure the peak height and the noise height (or use the VNMR **dsn** macro). Present a table demonstrating how the splitting you can resolve varies with choice of **gf** and **gfs** (Gaussian line broadening and Gaussian shift) and also providing some measure of the signal-to-noise ratio as a function of **gf** and **gfs**. In all, try five different combinations of **gf** and **gfs**. For each, give a qualitative description of how the window function produced by the chosen **gf** and **gfs** compares with the fid. Examples: "wdwfcn matches fid", "wdwfcn half as long as fid", "wdwfcn max is half way down fid" . . .

Suggestions: do a 'control' with **gf = 'n'** (no Gaussian), a set with no **gfs** (shift) but various widths of Gaussian, another set with a constant **gf** but various values of **gfs**.

gf and gfs	smallest resolvable splitting	signal-to-noise	describe the weighting function

Write a sentence describing trends you see associated with the choice of **gf**.

Write another sentence describing trends you see associated with the choice of **gfs**.

3 1 point each for a total of 4 points

Turn in a NICE spectrum for each of four different samples, remember to supply me with all the parameters, in the form of a **pap** printout or the printout of **printon dg printoff**.

Useful note:

saving a good shim set also saves you lots of time later. When you get shims you like, get into your shims directory and then type **svs('nameofyourshims')**. Alternately there is a button for the same operation. When you want to use those shims get into the shims directory and type **rts('nameofyourshims')**. Alternately, navigate the file system, get into the shims directory, select the desired shim set file and click the load shims button. NOTE that the shims in you experiment will not be actually overwritten by the retrieved ones unless you also type **load = 'y'**. Then **su**.

Checking probe linearity: the pw90 at some power tpwr1 should be twice the pw90 at a higher tpwr = tpwr1+6. For example if you get pw90 = 11.25 at tpwr = 60, you can be satisfied with pw90 = 23 at tpwr = 54, because 23 is $\approx 2 \times 11.25$. If you EVEN find that the probe is not linear, 1- don't use it and 2- report your observation. It is a good idea, when proposing to use high powers, to check probe linearity, as it often fails at the highest powers, and this is an indication that you should avoid those powers.

Solvent suppression

When the solvent is protonated, this represents a very concentrated sample, of up to 100 M. Such a situation arises for samples with exchangeable protons which must be suspended in protonated solvent (eg. proteins in water) but also when in-line NMR is being used to monitor HPLC effluent, considering the expense of using fully deuterated HPLC solvents.

Presaturation

This is one of the 'traditional methods'. It still has its place but should no longer be used as the default, because it also saturates both resonances near solvent and protons that exchange with solvent. It works because the small solvent molecules normally have a much longer T_1 than the sample, so that when the solvent has a single line that can be saturated, it will remain saturated for the time needed to collect an FID.

First, use the normal s2pul experiment with **gain = 0** and observe the solvent itself to calibrate tpwr and pw, and to measure the tof for the solvent resonance (place the cursor on the solvent and type **movetof**).

Type **presat** to load the basic presaturation experiment. Then correct the pw, sw, tpwr. Shim up to the best lineshape with the narrowest line base possible (this is crucial). Set satfrq equal to the tof you previously determined for the solvent, **d1=0**, **satdly=2**, **satmode='ynn'**, use at least **ss=2**, **nt=2**. Higher satpwr will give more complete saturation, but will begin obliterating and deforming lines near that of the solvent. Do not exceed 12. Array satfrq over a 20 Hz range in 1 Hz steps in 'av' mode (Since the above tof value represents the top of the resonance, not its center of mass, you may need to array tof over 10 Hz to find the value that gives the best presaturation.). Go back to 'ph' mode when you have finished. Shim, shim, shim and try to get gain up to 30 without overloading the receiver.

Counter-selective excitation

A composite excitation pulse can be used for frequency counter-selectivity: binomial pulse trains and the Sklenar-Bax sequence are two easy examples. The latter is easily understood as a long and thus very selective pulse that tips a particular (solvent) spin into the XY plane (eg. along the Y axis) and an immediately-following short (hard, non-selective) pulse that tips everything in the opposite direction by 90° with the effect that the magnetization along Y tips back up to Z (where it should not contribute to the spectrum) and the magnetization initially along Z is tipped down into the XY plane along the -Y axis, where it can be observed.

Sklennar-Bax

Retrieve my SKBax.par parameter set and update the tpwr, pw, tof. Calculate values for selpw and tpwrse1, the duration and power of the selective (long, soft) pulse. You will have to optimize a tiny delay between the pulses that effectively adjusts the relative phases of the two pulses. rof1 is defined in \square s.

A composite 180 degree pulse, will give a larger bandwidth of inversion.

Binomial

This is a combination of 'hard' pulses and delays that is counter-selective. Have a calibrated pw90 value present and then type '**binom**'. If you want on-resonance suppression set **offset**= a positive integer, the larger it is the wider the window of excitation. To suppress a line 1500 Hz off resonance set **offset**=-1500, maximum excitation will be at tof. The macro will calculate good values for the d2 delay accordingly (if you set **offset**=0 you will have to calculate d2 yourself, $d2 = 0.5/\text{offset}$ of maximum excitation). Choose the type of composite pulse, type **seq=1331**. set **gain='n'** and **ga**. Collect a generous number of scans because you may have to work on the phase. This sequence does not give uniform excitation so choose offset to fully encompass your signals of interest. Signals very near the solvent will be weak and signals near offset Hz away from solvent will be weak again. Offset will be the frequency of maximal excitation.

Shaped pulses, sh2pul, pbox

Again, the starting point is a good 1D (emphasizing the importance of your ability to collect one). For greatest ease of later use, you should use a 1D that has the same solvent, sw and tof as you plan to use for the experiment utilizing your shaped pulse. The pbox software makes the generation and calibration of shaped pulses completely painless. However you will need a correctly referenced spectrum in hand (since you should not change tof later), and you will need a calibrated pw90 and related tpwr in the linear regime ('ref_pw90' appropriate for the 'ref_pwr') either set these in your experiment if they already exist, or just launch pbox and pbox will prompt for them and create them if they do not yet exist.. (Check to learn whether ref_pw90 and ref_tpwr are present before you initiate pbox, because pbox will not give you a chance to correct wrong values of these parameters).

Type **ds**, click on **pbox** in the second menu bar (Pandora's box). Choose the type of pulse you want, eg. 90°, set up the cursors to specify the spectral region to which the pulse should apply, choose the shape to be used (eg. eburp, = excitation band universal rotor pulse), **close**, **name** and give the computer a name for your pulse (an informative one for yourself), **close**. To see your pulse click **bloch**, **y close**. The computer will tell you what pulse width and power to use for it. WRITE THESE DOWN ! The files for the possible shapes are stored as wave files in vnmrsys/wavelib.

Make sure that **ref_pw90** and **ref_pwr**, if they exist in your experiment, are correctly set.

If you haven't got a spectrum at hand to put cursors on, you can use the **options** button: To generate a 90 to dispose of water (at tof) click on **pbox**, **90**, **options**, **offset**, enter '0' and return, click on **bandwidth** and enter '600' and return. Click on return, **e-Burp1**, **close**, **name**, enter the name you choose (e.g. killwater) and return, **close**. Give the software **ref_pw90** and **ref_pwr** when prompted (if prompted). WRITE DOWN the pulse width and pulse power returned by the macro (7.5 ms and 27 dB).

wet and water sequences for solvent suppression

Type **wet1d**, set **tof** correctly, if necessary make an excitation pulse that simultaneously excites all the solvent lines, Use this for **wetshape** (ie enter **wetshape** = 'the_name_of_your_pulse'), use pbox's suggestions for **wetpwr**, **pwwet**. Note that **c13wet** is by default set to 'y' and ¹³C is decoupled throughout. This is unnecessary unless you have a ¹³C labelled sample. Set **c13wet='n'**. **ssfilter** is also automatically set to 30, which will process out signals within 30 Hz of **tof**. Initially set this to **ssfilter='n'**. Set gain to at least 8 dB less. Set **gzlwlw** = 20000 (the default value in the parameter set is unnecessarily high).

1- Array **tof** over 20 Hz as above, and choose the value that minimizes the solvent signal.

2- Array the power for the shaped pulse **wetpwr**

3- Array the duration of the pulse **pwwet** over $\pm 20\%$ in ≈ 7 steps.

repeat the cycle if needed.

When you think you are doing pretty well, set **gain='n'** and let the system set its gain value. If your solvent is suppressed well, gain should come out to be above 30.

Problem set 5

1 For a 1D that has a wavy baseline, what command(s) should you use to determine how the FID is flawed ?

Provide realistic values for LP parameters needed to fix the baseline, based on your conclusions from above.

lpopt
lpfilt
lpnupts
strtlp
lpext
strtext

3 The following soft-hard pulse combination is frequency counter-selective. Explain how it works and draw vectors of the magnetization that results before and after each pulse.

d1- low power 2ms long 90° pulse (phase 0) - high power 10 μ s short 90° pulse (phase 2) - observe (phase 2).

Quiz (#2)

Circle one

- 1 A short pulse will excite a (narrow / wide) spectral range.
- 2 A short pulse will require a (large / small) value of tpwr.
- 3 (T1/T2) relaxation pertains to Z magnetization.
- 4 Sinc wiggles result from too big a (gain / at).
- 5 a large value of lb produces better (signal-to-noise / resolution).
- 6 a large value of gf produces better (signal-to-noise / resolution).

/4 When trying to 'lock' on a sample, we adjust the value of z_0 which corresponds to the sliding bar at the: top / second / third down / fourth (circle one). You will be watching for large 'waves' or oscillations in the line in the display. As you adjust z_0 , you try to make the oscillations become _____ (fill in with a few words).
 When you first begin, the lock signal may be very weak, you can make it larger by increasing one or both of two parameters, what are they ?

Finally, describe (briefly) a way of knowing whether you have too much lock power.

/2 In the event of a complete power outage or fire when you are at the NMR in the middle of an NMR experiment, what should you take care of ?

/5 The following parameter list appears with printed spectra, circle 5 parameters and say what they mean.

```

ACQUISITION
sfrq      199.925
tn        H1
at        1.994
np        11968
sw        3000.3
fb        1500
bs        16
tpwr      57
pw        13
dl        2
tof       0
p1        0
d2        0
nt        4
ct        4

```


Practicum 6 **Spring 2004** © **Anne-Frances Miller, 2004**
2d spectra I: gDQCOSY of Strychnine in CDCl₃ with CrAcAc (T₂ ≈ 90 ms, T₁ ≈ 200 ms)

Obtaining a respectable 2D is based entirely on having a nice 1D and good calibrations. Know pw90 (in the linear power regime if possible), have a good idea of your sample's T_2 s and T_1 s.

In all 2Ds, each point in the indirect dimension is acquired at the cost of a whole additional spectrum in the direct spectrum. Thus although large np and resolution cost only disk space in the direct dimension ('f2') **each ni costs approximately a minute in a traditional 2D**. A typical small molecule 2D used to take 4 hours. Using gradient coherence pathway selection to suppress artifacts and linear prediction to extend indirect dimension data set size, it is now possible to get very nice 2Ds in 10 minutes with sufficiently concentrated samples. However, we still have to compromise on the digital resolution possible in the indirect dimension (f1).

COSY: Correlation spectroscopy

COSY is the 'original' 2D, but not the easiest to understand. It nonetheless illustrates important points and is an excellent method for identifying coupled resonances and thus walking through a spin system. However Varian's COSY pulse sequence produces a magnitude mode COSY, which is low in resolution.

DQCOSY vs. gDQCOSY

We will use **gradient Double-Quantum-filtered COSY (gDQCOSY)** because it is phase sensitive and the double quantum filter suppresses the uninformative diagonal signals and reduces their tendency to spread across the spectrum. Thus, it is preferred over even COSYps (phase sensitive) despite the theoretical loss of half the signal. Non-gradient DQCOSY relies on phase cycling (i.e. $\text{nt} =$ some multiple of 8) to cancel out artefacts. gDQCOSY uses gradients to eliminate artefacts in the first place, so there is nothing to cancel and nt can be set to 1. This is a big advantage if you have a concentrated sample that doesn't need $\text{nt} > 1$ for sensitivity reasons, and gets around the problem that artefacts (or anything else) don't substrate out cleanly unless the machine, room, sample etc are absolutely stable over the course of the experiment. Our spectrometers are excellent, but the probes are so sensitive that even small variations in temperature etc result in non-cancellation. However, the use of the gradient-based artefact suppression will cost you half the signal intensity, and this may not be tolerable, depending on the sample. I still advise the use of gDQCOSY, because even with lower signal, the S/N is so much better that the signal you have is much more useful.

High resolution is required in all phase-sensitive COSY spectra, in order that antiphase resonance components do not cancel (cf. TOCSY).

Finally, because magnetization transfer is mediated by J coupling in a COSY, and antiphase components of crosspeaks are separated by J, you will need to have a value of J in mind when you set up your COSY. Your choice will depend on what couplings are resolved in your spectrum (use a J value \geq the smallest resolved value) and the types of couplings that characterize the spin systems that interest you most. I often use a compromise value of $J = 7$ Hz.

With a good 1d spectrum already in an experiment (including pw90 , tof , sw , solvent and phases set), type **gDQCOSY**. You may want to make changes in at (if you double it, double fn also) and ni , but recall that resolution will be bought at the cost of S/N, because at the long at and ni values needed, there will be much less signal in the FID. Similarly, d1 should be $= 1.3 \times T_1$, at least. In fact, for COSY spectra, spurious diagonals appear when d1 is less than $3 T_1$ and purge pulses are not used between scans. Always use $\text{sspul}='y'$ for all COSYs without fail. This is also a good idea for multidimensional experiments in general.

Understanding the indirect dimension

During direct detection we monitor magnetization as it precesses and measure its amplitude along two axes (X and Y = real and imaginary) in 'real time' *i.e.* as it is happening. However, just as the fast reactions of colourless compounds can only be monitored by taking our samples and analyzing them, later, to yield concentration of each reactant and product as functions of the amount of time they had been allowed to react before the sample in question was taken, the indirect dimensions in multidimensional NMR experiments are collected one 'sample' at a time, and each 'sample' is a 1d spectrum representing the state of the 'reaction' after a given amount of time had been allowed to elapse, $\Delta t = (\text{number of increments}) \cdot (\text{time per increment}) = n_i \cdot (1/\text{sw}_1)$. sw_1 is the sweep width of the indirect dimension and as you recall from earlier lectures, and the Nyquist criterion, short (*small*) time increments of $1/\text{sw}_1$ must be used to collect data if high (*large*) frequencies $\text{sw}_1/2$ are to be distinguished from smaller ones (another incidence of time Δt /frequency). Thus for a large sw_1 a small time increment = $1/\text{sw}_1$ is used. (The factor of two results from the carrier frequency being in the center of the spectrum so the biggest frequency offset is $\text{sw}_1/2$).

If you were to collect one hundred samples from your reaction, 5 seconds apart, you would have sampled the reaction until 500 seconds. You would not be able to distinguish from baseline processes happening faster than $1/(5 \text{ sec})$, or with rates $> 0.2 \text{ Hz}$. You would also not be able to detect at all processes happening more slowly than $\approx 1/(500 \text{ sec})$. Thus, in NMR parlance, your sweep width would be limited to .2 Hz (faster processes would fall outside your spectrum), and your spectral resolution would be .002 Hz (slower processes would look like they weren't happening at all, and would not be resolved from 0 Hz). Finally, your total acquisition time '*at*' would be 500 sec. Since we are talking about the first indirect dimension, I will refer to the longest time point sampled as *at*₁, by analogy with *at*, and recognizing that *at*₁ is related to sw_1 (not *sw*). Although such a parameter is not defined in VNMR, it is very useful. In the example above, *at*₁=500 sec. In your standard VNMR data set, *at*₁ = n_i/sw_1 .

In VNMR all the parameters pertaining to the first indirect dimension are labelled with a 1. sw_1 , *lpext*₁ are the analogs of *sw* and *lpext* for the first indirect dimension. Similarly, in 3d and 4d spectra, the second and third indirect dimension's parameters are identified with 2 and 3. The exception is n_i , which is the number of complex data points (samples) you will collect in the first indirect detection, n_{i2} and n_{i3} are the analogs for the second and third indirect dimensions.

In brief: two properties of the F1 dimension are its width, sw_1 , which you set $\text{sw}_1 = \text{sw}$, and its resolution, also in Hz. Each is related to the inverse of a different time. sw_1 is related to $1/\text{increment time used in extending } t_1$ (*d2*). The resolution is related to the length of the indirect detection delay, just as direct dimension resolution is related to *at*. Thus the longest time point used in *t*₁ is equivalent to *at*₁ and *at*₁ = number of steps * step size = $n_i \cdot (1/\text{sw}_1)$.

Choosing parameters pertaining to the indirect dimension

sw_1 is usually set for you, $\text{sw}_1 = \text{sw}$. You may decide to double *at*, in which case also double *fn*. Make sure *d*₁ is sufficiently long (above) and *sspul* = 'y'.

n_i

In choosing our *at* in a 1d spectrum we weight the loss of signal amplitude due to T₂ against gain in resolution in proportion with $1/\text{at}$. You have seen that one can in fact collect a modest *at* of data and double it by linear prediction before applying a weighting function to emphasize the actual data while forcing the FID to decay all the way to zero in order to avoid sinc wiggles around the lines of slowly-relaxing resonances. In addition, zero filling by a factor of two ($\text{fn} \geq \text{np}$) improves resolution.

Similar considerations apply in the generic 2d (special COSY considerations follow). T₂ relaxation proceeds during the period of sampling (sampling Δ 'indirectly taking data'). This period is

called t_1 in texts and pulse sequences but d_2 in the VNMR parameter sets. (A second indirect detection period is called t_2 in the literature but d_3 in your parameter sets.) Each successive 1d (sample) taken in your 2d will be collected after the t_1 (d_2) has grown a bit longer, and the signal has decayed a bit more due to T_2 . Therefore it is generally a waste to let at_1 be bigger than $2T_2$. For typical samples $2T_2$ is a good limit. The cost of restraining t_1 (d_2) is that you will be placing a limit on your resolution in the frequency dimension corresponding to t_1 : F_1 . Since the maximum t_1 before sampling is our ' at_1 ' parameter = ni/sw_1 , and the resolution limit is $1/at_1$, we are limited to sw_1/ni resolution. Check that this does indeed have units of Hz. This number is in essence the Hz per indirect dimension point. So your indirect dimension resolution improves as sw_1/ni ($\propto 1/ni$) but your signal decays more with larger ni ($\propto ni/sw_1$). The other disadvantage of choosing the large ni (long at_1) you might be most comfortable with, is that in the indirect dimension it takes you up to a minute to sample each time point (you need another whole 1d). This is in sharp contrast to the direct dimension where a longer at comes at little or no cost in time since d_1 often compensates for it. However, the need for resolution is much less severe in a 2d, since your points will be spread out in a plane, not just a line. Therefore, the best strategy is to collect relatively low resolution (low ni) indirect dimensions and linear predict and weight as necessary to suppress sinc wiggles. It will be very rare to collect $ni > 512$, and this is usually only done for phase sensitive COSY spectra.

One additional factor enters into your choice of ni for a COSY. In this case only, the amplitude of the cross peaks (the guys you want) grows with t_1 as $\sin(\pi J t_1)$. Therefore you will get your strongest contributions from the samples (1ds) taken with $t_1 \approx 1/2J$. In practice, it is nice to go out 50% further to $at_1 = 3/4J$ or $ni = 3sw_1/4J$.

Processing

Initially, set **pmode='full'** (allows phase correction in both dimensions after the FT) and **proc='ft'**. The first increment of a COSY has very little signal in it, instead process a later odd-numbered increment. For example, **wft(201)**. Set up the window function in **wti** as usual. For gDQCOSY, Varian adopts the luxury of using a Gaussian, because there are so few artifacts. (Later we will also employ the sine-bell indicated by theory to best emphasize COSY crosspeaks). For now, set **gf = .5 * at**. Also, for a COSY there is no need to linear predict beyond $at = 1/J$, since this is where the sine bell will cut off the data (see below). Our typical at are $> 1/J$ without linear prediction, so leave **proc = 'ft'**.

Process the first dimension as a block by typing **wft1da**. This will produce a whole stack of 1ds whose frequency axes are along f_2 . To see these, set **trace='f2'** and **dconi** to redraw, then activate **trace** (a button) and drag the cursor from the bottom of the box (where the first of the 1D spectra is) up, and watch the trace to see how each successive spectrum has signals in the very same places, but with growing amplitudes and with changing phases. Set **trace='f1'** to use the t_1 axis as the X axis, **dconi** again, place the cursor on a streak and type **ds** to show it as a 1D of the amplitude of the selected peak vs time in t_1 . This is in effect an indirect dimension FID (subject to Fourier transformation, like any other FID). **wti**, and set up a window function, as usual. Again for gDQCOSY you can get away with a Gaussian just as for standard 1ds, for now. **gf1 = 0.5 at1**, or more correctly **gf1 = 0.5 * ni/sw1**. Note that a '1' follows the gf parameter name to indicate that it pertains to the first indirect dimension.

Recall that the **gf1** chosen at this point does not take into account forward linear prediction. Set up linear prediction and **proc1='lp'**. For COSY data, you would like your data (actual and calculated) to extend to $1/J$ in t_1 . Calculate how many points ('n') that would entail. You know that $at_1 = n*(1/sw_1)$, and in this case you want $at_1 = 1/J$, so $n = sw_1/J$. (You can confirm for yourself that you will get $n = 4ni/3$ or $n = ni + ni/3$ if you used the recipe above for $ni = 3 sw_1/4J$). Since you want n

points and you have n_i points, you need to predict $n-n_i$ points, so $lpext1 = n-n_i$, (or $n_i/3$ if you used the recipe for n_i above). Use $lpopt1 = 'f'$, $lpfilt1 = 12$ (a bit more than the number of crosspeaks in a typical row). Use all your data by setting $lpnupts1 = n_i$ and $strtlp1 = n_i$ $strtlp1 = n_i+1$. Check that you have $fn1 > 2 * (n_i+lpext1)$ so there is room for all the actual and calculated data points to fit into the Fourier transform. Adjust the window function if needed. Then type **wft2da** to complete the 2d transform.

Phase up your spectrum, in each of the two dimensions, one at a time. It is best to work on a strong and simple cross peaks, which you know should have positive intensity in two diagonal corners (eg. North-East and South-West) and negative intensity in the other two diagonal corners (eg. North-West and South-East). Beginning with the f_1 dimension, which may be what is already horizontal (if not, type **trace = 'f1' dconi**); place the horizontal cursor line across the peak and trough that make up the top half of your cross peak (begin with one in the right-hand side of the spectrum), and type **ds** to get a 1d. Phase this to get a negative peak followed by a positive one of equal magnitude, and flat level baseline on both sides using zero-order phase ($rp1$) (it shouldn't need much). Do this without releasing the mouse button or repositioning the mouse, you ONLY want to adjust $rp1$. Then choose another simple strong cross peak in the left portion of your spectrum and adjust the first order phase ($lp1$). As before, place your cursor across the top half of the peak and type **ds**. Click once on the **phase** button then click ONCE on the right-hand side of the spectrum WITHOUT CHANGING THE rp . Then move the mouse to the cross peak you selected, press down on the mouse button and adjust lp WITHOUT MOVING THE CURSOR ANOTHER TIME. Then click out of **phase** mode.

Select and correct f_2 phasing if necessary by setting **trace = 'f2' dconi** and repeating the above exercise for rp and lp . Again, your objective is to produce peaks that are antiphase absorptive in both dimensions. They should be little squares with positive amplitude at the top right and bottom left, and negative amplitude at the bottom right and top left.. If you just need to phase f_1 , **pmode='partial'** will suffice but if you want to phase both dimensions you will need to set **pmode = 'full'** before you begin the Fourier transformations. For gDQCOSY, theoretical phases are $rp=0$, $lp=0$, $rp1=90$, $lp1=0$, but 15 degree deviations from these are not unusual after optimization. Once the phases are optimized, set **pmode = ''** and repeat the **wft2da** to produce a much smaller data set which now lacks all the imaginary numbers you don't see anyways (the data you collected are all saved, only the Fourier transformed spectrum is culled).

If your gDQCOSY was based on a 1d that already had a referenced chemical shift axis, and you used a macro to convert your 1d parameters into a gDQCOSY parameter set, then the f_2 axis will retain the chemical shift calibration. This may or may not be transferred cleanly onto the f_1 axis. The parameters responsible for the chemical shift calibration are rfl and rfp for the f_2 axis. Typing will **rfl1 = rfl** and **rfp1 = rfp** apply the same calibration to the f_1 axis. You can also calibrate a spectrum based on a line of known chemical shift. With **trace = 'f2'**, place the vertical cursor on the line (in the centre of a COSY cross peak) and type **rl(4.7p)** for the example of a known chemical shift of 4.7 ppm. Note that you will use the **rl1** command instead of **rl** to set a reference chemical shift when calibrating the f_1 axis..

To get a pretty looking contour plot, analogous to the printout but in colour, type **dconi('dpcon')**. **dconi('dpcon', 10, 1.3)** plots the spectrum with 10 contours spaced by 1.3

plcosy(10,1.3,2) page plots the COSY using 10 contours spaced by 1.3, and the spectrum in experiment 2 along one edge. This macro does not require that the sequence used actually be a COSY.

Special treatment for COSY spectra

For COSY-type spectra, the diagonal peaks contribute intensity to the FID even at early times, but the cross peaks grow in with $\sin(\pi J t)$. Thus, in order to emphasize the all-important crosspeaks, it is customary to use sine bell window functions. For the J value you want to emphasize, set $\underline{sb} = 1/2J$ and $\underline{sb1} = 1/2J$, and turn off all other window functions. You can look in wti to see what this function looks like on your data, to be sure that you have a long enough \underline{at} (likely) and your linear prediction results in a long enough set of data in t1. You will need $\underline{at} \geq 1/J$ and $n/\underline{sw1} \geq 1/J$ where n is the total number of points in t1, including actual and predicted ones (above). With sine bell windows in place, repeat the wft2da. The overall intensity of the spectrum will decrease, but this will mainly correspond to loss of diagonal peak intensity. The result is more emphasis on the (informative) crosspeaks. (Another less optimal but operationally simple choice of sine bell filters is $\underline{sb} = -\underline{at}$, $\underline{sb1} = -1*\underline{at1}$, where the - sign indicates the use of a squared sine instead of a simple sine, which suppresses f1 noise a bit more.)

Read chapter 5 of Claridge's book for this time, chapter 8 for next time.

Be prepared for a quiz question requiring you to predict the COSY spectrum for a spin system, and the corresponding TOCSY.

2d spectra II: gDQCOSY vs. DQCOSY, and TOCSY

Strychnine in CDCl₃ with CrAcAc (T₂ ≈ 90 ms, T₁ ≈ 200 ms)TOCSY: total correlation spectroscopy.

In this method magnetization on each spin in a network of spins connected by J coupling can 'flow' to other spins in the network ('spin system'). This is made possible by aligning all the spins along the X (or Y) axis and holding them there with a series of pulses called a 'spin lock'. While they are there, all aligned together and held together, it is as if they all have the same chemical shift (none is precessing away from the others). Thus, the strong coupling limit applies and the spins exchange magnetization via J coupling. Complete transfer of all the magnetization from isolated spin A to coupled but otherwise isolated spin B would take $1/2J$ (time). In practice every spin B that receives magnetization is also passing it on (to C, D, ... and back to A), and T₂ relaxation* is continuously eroding the total net magnetization on each spin, so that magnetization builds and decays (and builds again) with different delays at different spins, during the spin lock. (* actually rotating frame T₁ relaxation).

To set up a TOCSY start with your nice 1D, a tof and sw that allows approximately 10% extra on each side of the last resonance (for base line correction and axial peaks), pw90, lp = 0, and type **TOCSY**. That's it !! The computer will suggest values of the mixing time, mix, the d1 delay and the number of complex points in the f1 dimension ni.

lp = 0 is obtained by phasing up and typing **crof2**. The computer uses your value of lp to recalculate rof2, a delay that follows the excitation pulse, to in effect synchronize the excitation hardware with the detection hardware, so that there will be no net spin precession in the detection frame relative to the excitation frame. You have to collect your 1D one more time to see the effect. Also query lp and alfa (another delay after the pulse). If your baseline has a serious smile or frown, you should optimize alfa too (with a constant value of rof2 + alfa). In this case, for the example of lp = 3, alfa = 6 (sum = 9): **array=(rof2,alfa)', alfa = 3, 4, 5, 6, 7, 8, 9 rof2 = 6, 5, 4, 3, 2, 1, 0. da** should indicate a 7 value array. Choose the alfa/lp combination that gives the flattest baseline.

mix, slpwr and slpw

Varian will suggest a spin-lock field strength in the form of a slpwr (spin lock power) and the corresponding slpw (spin lock pulse width). However they tend to err on the aggressive side (they recommend a 15 ppm spin lock). Your spin lock field will have to be strong enough to overcome the resonances' tendency to precess away from the X axis, so in units of Hz (cycles per second), it should be $\gg sw$. Using sw as the spin lock field strength, $1/sw$ will be the period (cycle time) and since a cycle is 360° the corresponding pw90 will be a quarter that, or $1/(4*sw)$. Make sure you calculate this slpw in μ s though, not sec. This is a TARGET VALUE, not what you will actually use, because you will have to use a pulse width that really is a 90° pulse at an integer power. Use your (linear) calibrated pw90 at tpwr, and calculate the pw90s you would get at a series of lower powers, with powers extending down until you calculate pw90s as close as possible to the target slpw. Use the calculated pw90 that is closest to but smaller than the target slpw. Enter this value as slpw and enter the corresponding power as slpwr.

You should make sure that mix is close to half of $1/J$ for the smallest J coupling you wish to be effective in magnetization transfer. For example if you are using steps of three-bond couplings on the order of $J \approx 7$ Hz, choose mix = 0.07 s. In consideration of T₂ relaxation however, you will have very little signal left to observe if you use a mix > $3*T_2$. Signal decays by a factor of $e=2.7$ for every interval of T₂, so after $3*T_2$ you can expect a FID only 5% as strong as the one you got after a simple

90° pulse. Thus how long a mix you can get away with depends on your sample concentration and T_2 s. It also depends on the magnitude of the spin lock power. This is rarely an issue for ^1H TOCSY where the power should be <50 . The **max** safe spin lock is **mix** = 100 ms, **max** safe power is **slpwr** = 50.

ni and at

You may want to increase ni (or at) larger for better resolution. However the cost is that this will cause the experiment to continue increasing the indirect t_1 delay (called d2 by Varian) to a maximum value of $\text{at1} = \text{ni}/\text{sw1}$. If this is longer than $3 \cdot T_2$ you will only have 5% of your starting S/N left in the latter increments of the $2d$, and you will be merely wasting spectrometer time. It is better to collect fewer ni and linear predict based on these (stronger) points.

d1 and sspul

Ideally d1 should be at least $1.3 \cdot T_1$. Because we use an 'SS' pulse purge sequence to kill magnetization left over from one scan and shorten the delay before the next (sspul='y'), you must allow $\text{d1} = 1.3T_1$ for maximum sensitivity, not $(\text{d1} + \text{at}) = 1.3T_1$.

For long 2Ds, get trained to use variable temperature control, set temp = 25, shim up well and have a strong lock signal but without too much lock power (lock level ≈ 60). Reduce ALL instabilities.

Last but not least

Before actually launching ANY 2D, please first check that it will not be hard on the hardware by doing a trial mini-run. set ss=0, ni=1, phase=1, and retain the nt you think you will need for the real deal (this is a good time to check it, rather than learn at the end that your data are not quite strong enough to support your analysis). **ga** and watch for rcvr overflow, ADC overflow etc. If you are a real keener (and/or a member of the Miller group) set ss = 128, **ga**, and immediately upon completion of the run open acqi and optimize z1 before the sample cools. Thus your shims will be better suited to the steady state level of heating produced by the spin lock (or decoupling for heteronuclear experiments).

If all is well, set up ss = 64 (or 128), phase = 1,2 (so you get both real and imaginary numbers in t_1 , in support of a full hypercomplex data set), ni = whatever-it-was-you-chose. Check **da** to make sure you are getting all the data you intend. Type **time** to have the computer calculate how long your run will take, and adjust ni and nt to conform to the time you have allocated. **ga**

Relaxation during internal delays:

T_2 relaxation is operative during the mix time of a TOCSY (actually rotating frame T_1). The benefit of a longer mix is longer-range couplings and more magnetization steps linking more distant Hs in a spin system. (Note that the short-range TOCSY transfer cross peaks may get weaker and even disappear when mix is long.)

T_1 relaxation is operative during the mix time of a NOESY. The benefit of a longer mix is strong long-distance nOes, because these take longer to build up than short-range nOes.

T_2 relaxation is operative during the mix time of a ROESY (actually rotating frame T_1). The benefit of a longer mix is strong long-distance nOes, because these take longer to build up than short-range nOes.

Processing and linear prediction

Type **wft(1)** and set up the weighting and linear prediction you want to use for the first dimension, as if it were a 1d. If you want to linear predict in f_2 (normally not necessary) and no linear prediction parameters are in the data set, create them by typing **parlp**. You will then also have to set

proc = 'lp', set up the linear prediction, and modify the window function accordingly. These operations are described below for the f1 dimension, which is where linear prediction is most often used.

Process the whole first dimension: **wft1da**. Display the resulting interferogram with the t₁ axis along the bottom: **trace='f1' dconi**. Place the cross hair on the line corresponding to a signal of interest's behaviour in t₁. Display this signal's t₁ time dependence with **ds**. This is that signal's FID in t₁ (**d2**). Use **wti** to choose processing parameters as usual. Note that the window function parameters for t₁ are identified with '1's as belonging to the indirect dimension (**gf1**, **lb1** . . .). The window function you have just made is based on actual data only and will have to be scaled to accommodate linear prediction. Now set up linear prediction (COSY was discussed in Practicum 6). For TOCSY, NOESY, HSQC etc, linear prediction is often used in the f1 dimension to increase resolution. To double your data set **lpnupts1=ni**, **strtlp1=ni**, **lpext1=ni**, **strtext1=ni+1**. Choose **lpfilt1** based on the number of strong signals you expect in an individual row of the finished 2d, this number will be much less than the number of signals in the whole spectrum, e.g. 8, in which case you might use **lpfilt1** = 12. How would you triple your data? Double or triple the corresponding processing parameters **gf1**, **lb1** etc consistent with the extension of your FID (if you are going to double your number of data points in f1 (i.e. using **lpext1** = **ni**) then you should double **gf1** or **sb1** and **sbs1**). Make sure that **fn1** is large enough to allow for 2 or 3* **ni** points, times two, plus zero filling. Eg. for **ni** = 200 and **lpext1**=400 (tripling) **fn1**=2048. You could now go ahead and type **wft2da** to complete the 2-dimensional Fourier transformation, if your spectra have no further artifacts.

You may, however, need to correct the FID's dc offsets with the t2dc option in the wft2da command (type **wft2da('t2dc','t1dc')**). A first point correction is often required for the Fourier transformation of the f1 dimension. Either set **fpmult** based on the first increment: (type **wft(1)**, **dc** place the cursor at the right edge of the base line, **cdc**, if the spectrum rises try a lower **fpmult**, if it drops try a higher one, repeat until you have an **fpmult** value that produces a spectrum with no dc offset.). Alternately, type **wft(1)** and then just type **cfpmult** and VNMR will calculate **fpmult** for you. A good value of **fpmult** reduces the amount of T1 noise in the spectrum. Alternately perform a baseline correction on each spectrum produced in the first FT before doing the second one, by using **wft2da('bc')**. **You must have already chosen the regions of the spectrum to be treated as baseline, using **cz** followed by the **resets** button.**

You can deal with ugly baselines after the fact too, using **bc2d('f1')** or **bc2d('f2')**.

Phasing the spectrum.

If you want to be able to correct phases in your 2d, it must have been processed with **pmode = 'full'**. If you are good at phasing the first increment (obtained by typing **wft(1)**) you can then set **pmode = 'partial'** and only phase the f1 dimension in the 2d itself.

Unlike gDQCOSY, which produces peaks consisting of two positive and two negative components, paired across the diagonal, TOCSY cross peaks are in-phase absorptive, and this greatly simplifies the spectrum as well as its processing. Work with the diagonal (strong and known to not be artifactual). With trace = 'f2', place the crosshairs on a diagonal peak at the right-hand side of the spectrum and type **ds** to generate the corresponding 1d slice. Phase ONLY the **rp** (do not click a second time), based on the diagonal peak. Then return to the 2D with **dconi**. Place the crosshairs on a diagonal peak at the left-hand side and type **ds**. Phase **lp**. To do this go into **phase** mode, click once on the right-hand side to step past **rp** optimization (which you have already done) then move the mouse over to the diagonal peak and hold down the left or right mouse button while phasing it up (the usual way). Just do this once, do not move the mouse and click again to rephase elsewhere in the spectrum. **dconi** to return to the 2d, then change trace to turn the spectrum on its side and phase the other dimension (**trace='f1' dconi** if you just did f2 and now want to do f1). As before, once your

phases are optimized, set **pmode = "** and **wft2da** once more time so you will not be carrying around all your imaginary data.

TOCSY1D

To view a buildup curve showing how magnetization decays from a source position, builds and then decays at destination positions, or simply to observe the spin system to which a chosen resonance belongs, load parameters for TOCSY1D onto your nice proton 1d by typing (you guessed it) **TOCSY1D**. As always, temper the strength of the spin lock. Shoot for $1/(4*\underline{slpw}) \approx \underline{sw}$ (although Varian recommends at 15 ppm spin lock regardless of sw). You should know the origin of this rule of thumb by now. You may want a longer-than-suggested $\underline{}$. Take your T₂ into account in choosing the maximum duration of spin lock (which is often called 'mix'), Take your T₁ into account in choosing d1. For clean results with this sequence, I suggest nt = 8 or multiples thereof.

The selectivity of the TOCSY1D sequence is based on a pair of gradient echoes employing selective inversion pulses that will invert the resonance of interest, so that it is rephased by the second of each pair of gradients. All other resonances will experience accumulating dephasing by the sum of all four gradients. Make a selective inversion pulse using **pbox**, based on your gourmet 1d. Insert its name, power, pulse width into TOCSY1D 's parameter set. Confirm that the desired selectivity is achieved by setting mix = 0 and collecting 8 scans. You should see ONLY the desired resonance. Now array mix to watch the time course of magnetization transfer to other resonances in the spin system. Note that different mix times are optimal for different transfers.

NOESY and ROESY

As for TOCSY, the most important determinant of the quality of your 2d is the quality of your reference 1d, one that is well phased, has good sw and tof and is associated with a good pw90 and knowledge of the T_1 and T_2 .

Once you have such a calibrated 1d, save it (svf) and move the parameters, or the parameters plus data to other experiments in which you plan to set up the NOESY and ROESY. Type **mp(destination expt #)** if you want to move the parameters from the experiment you are currently in. Alternately **mp(from expt #, to expt #)**. For example mp(2) or mp(1,2), or mf(4). jexp2 (or jexp4) to go into experiment 2 and type NOESY to convert your parameter set to a set for a NOESY.

As for TOCSY, you can watch the buildup and decay of NOESY magnetization transfer from a resonance of interest, using **NOESY1D**, this time via T_1 cross relaxation, as mix is extended.

NOESY spectrum.

In an experiment containing your good 1d, type **NOESY**. Your sw will be used for sw1 too (to generate a square spectrum).

The value of at is a compromise between resolution (obtained with a long at) and loss of signal due to T_2 relaxation.

ni will determine the length of $at_1 = ni / sw_1 = \text{maximum value } t_1 \text{ (d2) will have}$. For better resolution in the indirect dimension you will need to pay against losses due to T_2 relaxation, with a large ni. However you probably need less resolution than you think in F1, because you have two dimensions. Thus it is better to collect high resolution in F2 with a long at and save time and disk space with a lower ni. You can always linear predict more indirect dimension data. I advise against $ni / sw_1 \geq 2T_2$.

Choose nt depending on the nt needed for a good 1d. The same value or half that value are both good choices. Make sure that **sspul=y**, and leave a pretty generous d1 because NOESY is a cross relaxation method, each scan should start with a relaxed, or at the very least, equally relaxed.

For quantitative distance measurements of distances based on nOe cross peaks d1 should be $\approx 3T_1$ and the mix should be $< T_1$, often as short as 15 ms (macromolecules). However for qualitative information, such as which H is close to which, mix $\approx T_1$ works well. For your first look at a molecule, this is a good choice.

ROESY spectrum.

This experiment, like NOESY, measures cross relaxation through space, but it does so in the rotating frame. Spins are trapped in the rotating frame by a spin lock. Artfactual peaks due to TOCSY magnetization transfer (through bonds) are a pitfall to be avoided in ROESY. ROESY and TOCSY cross peaks can be distinguished by their signs, unless you have the severe bad luck to have them perfectly cancel each other. TOCSY cross peaks have the same sign as the diagonal. ROESY cross peaks have the opposite sign**. ROESY is useful for intermediate sized molecules which may have nOes close to zero because of cancellation between stimulated emission and stimulated absorptive mechanisms (see the textbook). Molecular weights on the order of 1000 are a size range where things might be complicated, and a ROESY might be a better choice than a NOESY.

As for NOESY, in an experiment containing your good 1d, type **ROESY**.

In order to do this we use a weaker spin lock. We want $\beta_1 \approx sw / 2$ (not sw). β_1 is set by your choice of slpwr and slpw, as for TOCSY. We will also implement a parameter called ratio which introduces delays between the pulses in the string used to achieve spin locking. When ratio=1 the duration of the delays is equal to the length of the pulses, so the spin lock is effectively diluted by a factor of two. We will test a range of ratio values and choose one that is large enough that TOCSY

transfer is avoided. The duration of the spin lock has to be sufficient for cross relaxation to occur, i.e. $\underline{\text{mix}} \approx T_2$ for small molecules. For large molecules (proteins) rOes build up twice as fast as in a NOESY so $\underline{\text{mix}}$ should be $\approx T_2/2$ for qualitative information. For quantitative distances, a series of $\underline{\text{mix}}$ values are used, all shorter than T_2 , to sample the 'nOe buildup' period. This is beyond our current consideration.

Process data as for TOCSY. Note that the in-phase nature of NOESY and ROESY cross peaks greatly simplifies the spectrum, and its processing. However, for small molecules the cross peaks will have the opposite sign from those of the diagonal, opposite to the case in TOCSY. For large molecules NOESY cross peaks have the same sign as the diagonal but ROESY cross peaks have the opposite sign**.

Practicum 9 Spring 2004
2Ds with solvent suppression

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Aside: for those who will need to suppress solvent, a set of 2Ds with the wet presequence are available as wetdqcosy, wetgcosy, wetghmqc, wetghsqc, wetnoesy, wetntocsy to name the most popular. These are good for cases where there are multiple resonances to be suppressed (mixed solvent systems) or protons that exchange quickly with solvent and therefore will become saturated during long solvent saturation periods due to exchange. If neither of the former hold, you can also use presaturation which is robust and often easier to interleave into some sequences. For example solvent saturation can be continued during the t1 and mix times in some sequences.

For the case of water suppression (one solvent peak) you need to keep tof on the water resonance in order that quadrature artifacts will lie under the resonance itself. Therefore, you should first calibrate pw90 on water using gain = 0. Then use a small tip angle pulse and nt = 4 (to eliminate quadrature artifacts), centre the cursor on water then type **movetof** (NOT **movesw**). This will make the tof choice the overriding consideration and you will then have to do some simple calculations to optimize sw (without changing tof).

Note that if you are using presaturation, you will have to set **presat = 'y'**, and you may have the option of setting satfrq (saturation frequency) to something other than tof. Array satfrq over tof ± 10 Hz to get the best value, as the water line is often not symmetric, so that tof (the top of the line) is not the centre of mass of the line. (The better your shims are, the closer tof generally is to satfrq.) For successful presaturation, excellent shimming is crucial.

For the example of wetntocsy, under mainmenu, click on file, setdirectory, parent, parent ... until you see vnmr6.1B2. select that and Set Directory, select parlib and Set Directory. Click on 'Click HERE for more elements' and select wetntocsy.par. Then load these parameters.

Note that **p1lv1** is the high power pulse, enter the value you had in tpwr when you first calibrated pw90. Similarly enter pw90 for **p1**. The spin lock is implemented with tpwr and pw. tpwr should be around 44 and no higher than 50. Recall that the spin lock field should be approximately two times as strong as the biggest shift from carrier frequency in your spectrum (i.e. ≈ sw). To 'lock' a resonance 2000 Hz from tof use $4 * pw = 2 / 2000$ or $pw = 1 / 4000$, = 62.5 μs. This is clearly nothing like what is in the standard data set. The latter knows nothing about your prior calibrations, solvent, sweep width or anything. Once you get an experiment to run well, save your own parameter set !!

Also, you will have to put in your solvent selective 90 pulse as the wetshape with its pulse width (us) and power (db) as pwwet and wetpwr. Turn down gzlv1w from 32k to 20k, please.

Set **c13wet='n'**.

Recall that for a 2D you will need **ni** increments (eg. ni=256) and **phase = 1,2**. **time** calculates an estimate of the experiment duration.

Displaying 2Ds, your best reference is the manual.

Problem Assignment.

On your own sample (or one of the course's samples), collect a DQCOSY, a short-mix TOCSY, a long mix TOCSY, a NOESY and a ROESY. Use identical spectral parameters such as sweep width, ni, nt, tpwr and pw90. Process them each appropriately and turn in a printout of each along with one short paragraph describing how each spectrum is better or worse than the others, and how the information content differs. Also include a plot on which all the parameters appear, and supply me

with the linear prediction parameters, please. This can be accomplished by typing **rinton dglp printoff**.

Quiz # 3

'Walk' the DQCOSY below and identify the signals in the accompanying 1d according to which of two spin systems they belong to (A or B) and their position in the spin system (1, 2, 3, 4...). For clarity either use different colours of pen for the two spin systems or draw the walk for one above the diagonal and the walk for the other below it.

1D methods for ¹³C

The effect of the ¹H nOe vs. the effect of decoupling, calibration of decoupling using strychnine in CDCl₃ with CrAcac.

The maximum noe (extreme narrowing condition for two isolated spins) is

$$\rho_I\{S\} = \frac{I_S}{I_I} = \frac{I_S I_0}{I_0^2}$$

This is the amount by which ¹³C signal intensity is increased when ¹H is saturated (I), over the ¹³C signal intensity when ¹H is not saturated (I₀). Signals are also stronger-looking if they are not split by J coupling to their attached protons, thus, sensitivity gains are obtained by acquiring ¹³C spectra with ¹H decoupling.

We will collect ¹³C spectra without the noe vs. with noe by setting the ¹H decoupling mode to dm = 'n' vs. 'y' during d1. We will also collect ¹³C spectra with and without ¹H decoupling during acquisition, again via dm. The d1 interval is the A status period in the s2pul pulse sequence, the at is the C status period and the B status period is essentially irrelevant because we have d2=0. vs. noe (dm = 'yyy', 'yyn', 'nny', 'nnn'). Thus, for ¹H saturation only during d1 set dm = 'ynn', for decoupling only during at set dm = 'nny', for both set dm = 'yyy' (the usual) and for neither set dm = 'nnn'. ¹H decoupling also permits faster recycling between scans. To see the benefits of these different devices, compare spectra obtained with dm = 'yyy', 'yyn', 'nny', 'nnn'. Be sure that at is short (≈0.2 s) and d1 is long (≈3 s) to maintain good saturation with dm = 'yyn' and to not get an accidental noe when dm = 'nny'. Maintain **dmm = 'w'** for waltz decoupling, which roughly doubles the bandwidth you achieve for a given decoupling field. For best results, the ¹H should be calibrated (see below).

Calibrating the ¹H decoupler to be used when observing ¹³C

Collect a ¹H 1D as well as a ¹³C 1D in advance. From the ¹H spectrum determine the frequency of the highest line in the spectrum and the lowest, using 'sd' (write the frequencies down), use the same 'solvent' as you will use in the decoupling calibration. From the ¹³C spectrum determine the ¹J_{CH} (C-H coupling constant) of the line whose decoupling you will assess (use the same line throughout), also calibrate the pw90 for ¹³C, by direct observation of a strong resonance (such as solvent) while arraying pw.

In your ¹³C experiment, set 'dof = (lowest frequency), (highest frequency)', ie and array with two variables, for example **dof = -900, 800**. To be safe you could even use frequencies a bit beyond the spectra limits. Set dmm = 'c' and dm = 'yyy' or 'nny'. set dpwr to the value for which you want a calibration, for example **dpwr=41**. You may need nt = 16 or more, depending on your sample. **ga** to collect the two 1Ds. **ds(1)** to view the first, place the cursors on the two components of the ¹³C line you are using to calibrate decoupling (use the same line throughout), and write down the splitting in Hz, **ds(2)** and do the same again. Type in **h2cal**. You will be prompted for the apparent coupling constants in each of ds(1) and ds(2), the dof values for each (the low frequency is requested first), and the true coupling constant for your ¹³C line (obtained earlier from the ¹³C spectrum. The h2cal program will then return values for the strength of the decoupling field, the pw90 for the decoupler at the dpwr employed and the third value will be the dof value that would be right on resonance for decoupling the ¹H attached to the ¹³C peak you are observing.

Note that **dmm='c'** is used for so-called CW decoupling which at low dpwr (and low decoupling field or band width) can be very selective.

Now that you have calibrated the decoupler, use **dmm='www'** for waltz decoupling over a spectral window ≈ 2* as wide as the decoupling field strength (half of dof). dof is defined as 1/pw90, dof is in Hz but pw90 is in us, so you will enter dof=1000000/<pw90> where <pw90> is the

value you just got from h2cal. Because $\text{dmf} = 1/\text{pw90}$, not $1/\text{pw360}$, dmf is actually 4* the decoupler field strength.

If the above sounds too hairy, put the dioxane sample in the spectrometer (or ask Mr. Layton to do so), and load parameters for ^1H decoupling calibration. Use the File, Set Directory, Parent, select 'vnmr_6.1B2', change, select 'test' change, select 'gamah2.par' return, load. Correct pw and tpwr are not necessary but decent values for dof are. The values in the parameter set should be good for dioxane. One value of dof must be above the frequency of the proton to be decoupled and one must be below. Collect the two ^{13}C 1ds using these parameters, then invoke h2cal as above.

DEPT, on strychnine

DEPT detection permits increased sensitivity detection of ^{13}C coupled to ^1H . By modifying the tip angle of the last pulse one can also use the sequence for spectral editing, i.e. to learn which Cs have 1 H bound, which have 2. The tip angle of the last pulse is set to $90^\circ * \text{mult}$, where mult is a parameter usually set to 0.5, 1.0 or 1.5. $\text{mult} = 1.0$ is ideal for CH groups (CH_2 and C_3 groups = 0), $\text{mult} = 0.5$ is best for CH_2 (CH, CH_2 and CH_3 are all positive), $\text{mult} = 1.5$ gives positive C signals for CH and CH_3 groups and negative signals for CH_2 Cs. The selectivity of the $\text{mult} = 1$ spectrum reveals how close the pp ^1H pulse is to a true pw90.

Set up a nice ^{13}C 1D spectrum and type 'dept'.

You should retain the parameters you chose for a nice ^{13}C spectrum, including tof , sw tpwr and pw , based on direct observation of ^{13}C . $\text{tn}='C13'$ and $\text{dn}='H1'$. You can use pw and tpwr from a direct calibration of ^{13}C pulse widths (collect a simple ^{13}C 1d, array pw and watch the solvent ^{13}C resonance.) *The ^1H pulses however will have to be appropriate for the decoupler channel.*

^1H pulse calibration on the decoupler channel using d2pul

Because your values for pp and pplvl (proton pulses and proton pulse level) will be used for the decoupler channel, they have to be based on a different calibration than the one in which $\text{tn}='H1'$. the h2cal routine above satisfies this criterion, however, since it involves decoupling over the duration of the at, it would be damaging to use this to calibrate high dpwr powers. Instead, either use h2cal for a low dpwr and calculate the equivalent pw90 at a higher power (+6 db gives a factor of 2 shorter pw), or use the d2pul pulse sequence.

d2pul is the decoupler channel analog of s2pul. It is invoked by typing **d2pul**. Make sure that **homo='n'**, **dn='H1'** and **tn='H1'**. Set dof to the value you had for tof in a standard ^1H 1d. Array pw at a chosen tpwr (which now controls the decoupler). Check the dps at a high *vs.* a low tpwr , to confirm this latter point. Array pw to find the pw360 and divide the value obtained by four to determine the 90° pulse width for the dpwr value used. These become pp and pplvl in the DEPT sequence, ie the length and power for a high power ^1H pulse (as delivered by the decoupler channel). (Decent performance is obtained using the pw90 and related tpwr you obtained by directly calibrating ^1H in a ^1H 1D spectrum for pp and pplvl , but you will probably be ≈ 1 db off.)

Other DEPT parameters:

dpwr is the ^1H decoupling power, chosen based on calibrations using 'h2cal', above such that the decoupler field obtained is $\approx 1/2$ the width of the ^1H spectrum you need to decouple (alternately, dmf is 2* the ^1H spectral width), dof is the centre of the ^1H spectrum (important) and dmf is $1/(\text{pw90 that applies at the stated dpwr})$.

The ^{13}C parameters (such as tof and sw) should be those used to collect a nice 1D, EXCEPT that you must use a **true 90° pulse for pw** . Also, do not forget to phase up your spectrum nicely. The easy one to do this on is with j set and $\text{mult} = 0.5$

You should use **nt** = a multiple of 4 and a multiple of 16 is suggested. **You must use dm='nny'**. Set **j1xh** = an average of the C-H one-bond coupling constants in effect in your molecule (look at a coupled ^{13}C spectrum for guidance). I recommend you use **satdly=0**.

ga to acquire data (using a good value of gain). **ds(1)** and set the **th** to be well above noise but still catch all *bona-fide* peaks. Type '**adept**' to analyze the **dept**, **pldept** to plot the results. The original spectra can be recovered by typing **wft**, and plotted as usual. **padept** analyzes and plots the results.

INEPT ^{13}C spectrum, and indirect ^{13}C pulse calibration.

Like DEPT, this is ^{13}C -detected experiment, but magnetization is derived from excitation of ^1H . Thus, **tn='C13'** and **dn='H1'**. Other aspects of the set up are as for DEPT.

INEPT transfer of ^1H magnetization to ^{13}C .

Collect a decent ^{13}C spectrum (in practice this may be an old ^{13}C of a related sample). It serves mainly to provide **tof** and **sw**.

Calibrate ^{13}C **pw** at a high **tpwr**. You may do this by observing the strong signal of solvent.

In the ^{13}C experiment, type **inept**.

You can provide your own one-bond J value and let the computer calculate delays for you. In that case, set **j=140** (or whatever is a better compromise). For the first half of the INEPT, the two delays are set to $1/4J$. This works well regardless of how many ^1H s are coupled to a given ^{13}C .

Set **pp** to the decoupler channel's proton **pw90**, for the decoupler power **pplvl**. These values can be calculated from the 90° pulse width given at a lower power by the **h2cal** macro (previous demo). Alternately, the decoupler ^1H pulses can be calibrated directly using **d2pul** (above).

If **focus='y'** a refocussed INEPT will be performed, and there will be a second pair of delays. The best duration for these depends on the number of ^1H s coupled to a ^{13}C . With a value given for **j**, you don't get to set the delays yourself, however you can choose whether you would like an INEPT optimized for CH groups (doublets, set **mult=2**), for discrimination between CH_2 and the other possibilities (CH_2 s negative, CH and CH_3 positive, **mult=3**) or a compromise enhancement of all Cs attached to Hs (**mult=4**).

You can override Varian's choices by setting **j=0** and doing your own calculations of how long the delays should be. The first pair of delays together are **d3** (each delay is **d3/2**) and the second pair of delays is **d2** (**d2/2** each). Thus you can set **d3=1/2J** for ideal performance or somewhat shorter for a sample with a very short T_2 ($T_2 \approx 1/2J$). Your choice for **d2** will determine which type of C is best emphasized, and with what sign. For best observation of CH with no enhancement of CH_2 or CH_3 , **d2=1/2J**. For negative CH_2 s, positive CH and CH_3 **d2=3/4J**. For best observation of (positive) CH_2 **d2=1/4J**. For best observation of CH_3 **d2=1/5J** and for a good compromise spectrum **d2=1/3.3J**.

Indirect observation of ^{13}C and its correlations with ^1H , by gHSQC. Strychnine in CDCl_3 with CrAcAc

From Eq. 4.2 of your text, signal-to-noise, S/N increases with the static field strength, H_0 , the gyromagnetic ratio of the nucleus from which magnetization derives, γ_{exc} , and the gyromagnetic ratio of the nucleus detected, γ_{obs} .

$$S/N \propto \frac{NA}{T} H_0^{3/2} \gamma_{\text{exc}} \gamma_{\text{obs}}^{3/2} T_2^* \sqrt{nt} ,$$

where N is the number of molecules in the sample, A is the abundance of the isotope being observed, T is the absolute temperature, T_2^* is the effective transverse relaxation time and nt is the number of transients averaged together.

The enhancement available from the heteronuclear nOe is $\rho_{I(S)} = \frac{\gamma_S}{2\gamma_I}$ so there is an nOe of 2 and the ^{13}C signal is three times larger in the presence of the ^1H nOe. This, is however not as good as a whole factor of $\frac{\gamma_S}{\gamma_I}$ obtained by switching the roles of the I and S nuclei.

INEPT transfer enables you to exploit the high γ_{exc} of ^1H but still relies on the lower γ_{obs} of ^{13}C for detection. Thus the next step up in sensitivity is to use ^1H for both γ_{exc} and γ_{obs} . This is the strategy implemented by HSQC, HMQC and HMBC. The cost involved in the first two is that only C directly bonded to ^1H is observed (these are single bond methods). The HMBC (MB = multiple bonds) allows observation of Cs two bonds from a ^1H , and thus most quaternary compounds.

We will obtain gHSQC and gHMQC spectra of menthol (concentrated, with short T_1 and T_2). Whenever possible, gradient versions of experiments are used, because the results they give are cleaner.

preamble to HSQC

As usual, first obtain a nice ^1H 1d with good choices of sw, tof, gain and a calibrated pw90. (We had tpwr=59, pw90 = 11.8 on thing 2)

You will also need to know about the spectral range covered by your ^{13}C resonances. In the real world when we often don't have strong enough samples to obtain a ^{13}C spectrum directly, we usually start with standard parameters that contain a built-in overestimate of the sw.

You will need calibrated high power (short) and low power (long) pulses for ^{13}C . The former will be used for broad-band excitation and inversion pulses. The latter will be used for decoupling ^{13}C during ^1H detection. Since at is many ms long, the decoupling power must be kept low, this is especially important for decoupling of heteronuclei such as ^{13}C and ^{15}N . You could do these calibrations directly observing ^{13}C , which would get you the right values for our 400 MHz spectrometers. However, for other machines, and for conservative practise, you should calibrate in accordance with the mode in which a channel will be used. Since tn will be ^1H and ^{13}C will be dn, for the HSQC, we will use the same assignments in calibrating. This is accomplished using **pwxcals** ('X nucleus pw calibration').

pwxcals for calibration of ^{13}C pulses

In an experiment that has your good ^1H 1d parameters, type **pwxc**. You can override the defaults of calibrating ^{13}C with channel 2, but we won't.

Set the ^1H pw and tpwr to result in a 90° pulse and set the J value correctly ($j\text{C13} = 151$ for methyl iodide). To determine a good value, look at either a ^1H coupled ^{13}C spectrum, or the ^{13}C satellites in your ^1H spectrum. Make your decision based on either the J value for important resonances, or a compromise between the different Js represented by typical resonances. J is the separation in Hz between lines in a multiplet, or between the two ^{13}C satellites. Set dof to be near the ^{13}C resonances in your spectrum. This is especially important for low power calibrations, where dof needs to be within 500 Hz of the ^{13}C coupled to the ^1H under observation. In practice, you could use the TMS satellite signals, or resort to a separate calibration sample, such as ^{13}C enriched methyl iodide.

Set dpwr to 57 for high power pulses. Array pw1 from 1 to 15. **ga**. The satellite signals ONLY should decrease in amplitude and then invert as the ^{13}C spins to which they are attached are tipped off the Z axis through the XY plane and then through to the negative Z axis. The spectrum in which the satellites are at a null represents a ^{13}C 90° pulse. Write down that pulse length and the associated dpwr (7 μs at dpwr=57 on thing 2). **MAKE SURE THAT dm = 'nnn' or 'n' !**

Set dpwr to a low value (40) and repeat the above, with pw1 arrayed between 40 and 70. Write down the low power pw1 which gives a null for the satellites, this is the low power pw90 (pw90 = 50 μs for 40 = dpwr). (At this lower power, a poor choice of dof may result in failure of the satellites to invert. If you encounter this problem, take the pw1 value that has the strongest effect on the satellites and array dof over 20,000Hz in 1,000 Hz steps. The value at which the satellites are best inverted should be used as dof in another array of pw1, where the value that gives a null for the satellites is the pw90).

Return to HSQC

In a third experiment, set up with the good ^1H 1d parameters, type **gHSQC**. This will install parameters for a decent gHSQC. Confirm that you have correct ^1H pw90 and tpwr etc. in place. Enter the high ^{13}C power and associated pw1 for pw1vl and pw1. You will also have to enter a good value for the H-C J coupling, j1xh. This is used to calculate the INEPT transfer delays. Finally, note that sw1 is not the same as sw. This is because you are now observing different nuclei on the different axes. Your old ^1H sw will appear as the new sw, because you are still directly detecting ^1H . However you are indirectly detecting ^{13}C in the t1 (d2) interval. Thus, you will need a decent estimate of the sw of your ^{13}C spectrum (only those ^{13}C coupled to at least one ^1H), for sw1. When in doubt, overestimate, you can always trim back. Similarly, you will need a decent estimate for dof, the ^{13}C centre frequency, from the tof of a ^{13}C 1d of a related sample. For now, set **dm='nnn'** and **dmm='w'**.

Confirm that the experiment works using **phase=1, ni=1, nt=1, pw1=0, <your value for pw1>**
ga. The second spectrum should show only the ^{13}C coupled peaks right way up. The first spectrum should show nothing (gHSQC) (but if you don't have gradients and you are running HSQC, the first spectrum will include all peaks, inverted. Check for cancellation of ^1H coupled to ^{12}C with $nt=2$ vs. $nt=1$, using your value for pw1). The use of gradients suppresses protons attached to ^{12}C even better than the phase cycle, and a 'TANGO' presequence also suppresses them, and can be activated with **nullflg='y'**.

Decoupling of ^{13}C must be done with extreme care for the hardware. Ideally you would like to decouple over the full sw1. When using Waltz decoupling this would imply you should set dmf to $2*\text{sw1}$. In practice, we can rarely afford this. Your choice of dmf implies a choice of dpwr because $1/\text{dmf}$ is the 90° pulse and dpwr must be the corresponding power. In the relatively easy case of menthol/benzene, sw1 = 8000, so the ideal dmf=16000 which implies a 61.25 μs decoupling 90° and

therefore $\underline{dpwr} \approx 39$. Even this is not tolerable for $\underline{at} > .15$ s. We therefore compromise at $\underline{dmf} \approx \underline{sw1}$, and also keep \underline{at} short, $\approx .15$ s. (for menthol in benzene, $\underline{dof}=-5424$, $\underline{j1xh}=131$).

Parameters drawn from the ^1H spectrum and their use in the gHSQC

tof -> tof
sw -> sw

Parameters drawn from the ^{13}C spectrum and their use in the gHSQC

tof -> dof
sw -> sw1
tpwr -> pwxlvl
pw -> pwx

For \underline{dpwr} and \underline{dmf} , see above.

Others: the proton power/pulse calibration must be done in such a way that the high-band channel performance is being calibrated, NOT the full band channel that normally functions in $\underline{s2pul}$ or $\underline{std1h}$. To trick the machine into using the high band channel you can run one of those two experiments and calibrate $\underline{pw90}$ as usual as long as $\underline{dm} = \text{'C13'}$ (then \underline{su}). Alternatively, calibrate ^1H using $\underline{d2pul}$ or $\underline{h2cal}$, as described in previous exercises.

From the h2cal method

$\underline{dpwr} / \underline{pw90} \rightarrow \underline{tpwr} / \underline{pw}$ calculate a high power \underline{tpwr} for ^1H (h2cal is performed at low \underline{dpwr} , such as 40, to calculate the $\underline{pw90}$ that would result for $\underline{dpwr} = 58$ take the obtained $\underline{pw90}$ and divide by 8 (you should be able to figure out why it is a factor of 8).

from the d2pul method

$\underline{tpwr} / \underline{pw} \rightarrow \underline{tpwr} / \underline{pw}$ calibrate as usual by arraying \underline{pw} , \underline{tpwr} controls the decoupler in this pulse sequence.

From the s2pul method

$\underline{tpwr} / \underline{pw} \rightarrow \underline{tpwr} / \underline{pw}$ Set $\underline{dm} = \text{'C13'}$ then \underline{su} , then calibrate as usual by arraying \underline{pw} , \underline{tpwr} now controls the high band because whenever C13 is invoked it is given the full band channel *on our 400 MHz spectrometers*. The same is not necessarily true on other machines, only in cases where there is a single full band. If in doubt either use one of the methods above or ask someone who is familiar with the hardware.

\underline{PFGflg} sets up gradient-90-gradient suppression of left-over magnetization from the previous scan, set = 'y'.

$\underline{nullflg}$ sets TANGO suppression of resonances of ^1H coupled to ^{12}C .

$\underline{null} \neq 0$ implements BIRD suppression of resonances of ^1H coupled to ^{12}C . TANGO is recommended on the 400s, BIRD will work well without gradients (i.e. on the gemini).

Processing is similar to that for TOCSY. All cross peaks should be absorptive and positive. Use $\underline{rl(3p)}$ for the direct dimension and $\underline{rl1(70d)}$ for the ^{13}C dimension, with 3 and 70 chosen simply as examples.

gctHSQCafm2

AFM's gradient constant-time HSQC, which distinguishes between CH₂ groups and Cs with odd numbers of attached Hs (CH and CH₃).

For this one, load a parameter set or data set used previously for this pulse sequence, such as HSQC_CT_afm7nov01.fid. Also make sure that the pulse sequence itself is present in the psglib directory of your account (copy gctHSQCafm2.c over if the pulse sequence is not present, then, in vnmr, type **seqgen(seqfil)** to compile the pulse sequence in your own account). You will now have to change relevant parameters to your choice of values: sw, tof, tpwr, pw for ¹H; sw1, dof, pwxlvl, pwX for ¹³C. Keep at short, <<0.15 s, unless you turn off ¹³C decoupling during acquisition (**dm='nnn'**). Set j1xh to the one-bond HC J coupling constant that typifies your Cs of interest. Set **nullflg='y'** for suppression of H coupled to ¹²C (recommended). If you want your CH₂ signals to have the opposite phase to the CH and CH₃s, set **mltflg='y'** (multiplicity flag). If you are using this for an H-C 2d, you don't need to touch the gradients. However if you are doing HN, you will (not covered). Use **sspul='y'** and d1 = 1 - 1.3* the T₁ of your Hs (note that this is much shorter than the T₁ of your Cs, another advantage of getting the Cs via the Hs). As for the HSQC, the maximum value at1 of the indirect detection delay, t1, will be ni/sw1. Calculate this value and make sure that BigT is longer by at least 1 ms (**BigT = .001+(ni/sw1)**). As always, the cost of a large ni (and BigT) is loss of signal to T₂ relaxation.

Long range ^{13}C - ^1H correlations with ^1H , by gHMBC. Strychnine with Cr Acac.

We will look at the benefits of composite pulses, useful for covering broad spectral widths (like ^{13}C).

Setup for the HMBC is very like that for HSQC. Start with a good ^1H 1d Also know the ^{13}C pw and corresponding (high) pwxlvl.

Type **gHMBC**.

Keep **dm='nnn'** The ^1H is antiphase with respect to ^{13}C at the beginning of at, so it will be cancelled out by decoupling. This is similar to the situation for DQCOSY, and likewise requires that a non-shifted sine bell be used for the window function in f2. **sb=at/2** and **sbs=0**. If you double at by forward linear prediction, also double sb.

Check in dg1 that you are in absolute value mode: or type **av** to be sure.

j1xh should be the average one bond C-H coupling constant ≈ 140 Hz and jnxh is your choice of target long range coupling constant. One is advised to err on the large side for this one because shooting for very small coupling constants is tantamount to choosing a very long delay, during which you will lose signal due to T_2 relaxation. **jnxh = 8** is a common starting-point. If your spectrum is strong but you are missing peaks, you can then run another spectrum with a smaller jnxh. If the spectrum is too weak and $1/(2*\text{jnxh})$ is $\geq T_2$, you may have to settle for a larger jnxh. Choose nt to be 4 or 8 times the nt required to get a good HSQC, the HMBC is a weaker spectrum.

For the ^{13}C spectral window, you will need to choose dof and sw1 so as to include not only the resonances you can see in the HSQC, but also quarternary Cs, carbonyls and ketones. Since the latter are at shifts as great as 220 ppm, allow for such signals unless you are sure that your compound does not have such functionalities.

Choose d1 as usual, and do use **sspul='y'**.

Dynamics, below the coalescence temp, DMA in D2O.

When a nucleus spends time in two different environments, the signal it displays depends its life times in each of the two environments and the separation in Hz between the the signals that would arise from each of the two species in the absence of exchange.

You are probably most familiar with the phenomenon of coalescence, which is when the resonances representing the inter-converting species merge. Spectra are collected at a range of temperatures. At the temperature at which coalescence occurs, $k_c = \Delta\nu/\sqrt{2}$ where k_c is the rate of inter-conversion at the coalescence temperature T_c $\Delta\nu$ is the separation between the lines a low temperature (in the absence of inter-conversion), assuming isolated two-site exchange (see 150+ experiments pg 144. . . and references therein).

At temperatures well above coalescence one observes a single signal whose resonant frequency and line width are averages of those of the two individual species' signals. One can measure the rate of inter-conversion between the two species nonetheless via accurate line width measurements (typically digital simulation of the line shape) or $T_{1\rho}$ measurement (see 150+ experiments pg 150 and references therein). The latter is especially useful because $T_{1\rho}$ depends on the strength of the spin lock field in which $T_{1\rho}$ is measured and is extremely sensitive to dynamics on the time scale of $k_c \approx 2\rho\Delta\nu$, where ρ is the strength of the spin lock field in Hz. Thus, one commonly measures $T_{1\rho}$ at a range of ρ values (called B_1 fields in practice, but still quoted in Hz). With knowledge of the T_1 in hand, k_c and $\Delta\nu$ can then be calculated from

$$1/T_{1\rho} - 1/T_1 = \Delta\nu^2 \frac{(1/k_c)}{1 + (2\rho\Delta\nu/k_c)^2}, \text{ which applies only in the absence of spin coupling and}$$

assumes that you are well above coalescence.

For the purposes of our course, in which we do not wish to bake any probes, and I want to use samples I can buy for cheap, we will have to work between room temperature and 70 °C. If you know of any samples for which this is well above coalescence or spans coalescence, I would love to know about them. In the mean time, we will learn how to measure k_c in cases where you are well below coalescence temperature.

Below coalescence one can nonetheless document and characterize chemical exchange via magnetization transfer. We will study bond rotation in N,N-dimethyl acetamide by saturation transfer. Thus, we will saturate one of the N methyl carbons and measure the extent to which this C hops into the other position by the extent to which the other N methyl gets saturated. This can also be done by saturating the methyl protons, but at the risk of complicating nOes, so we will use ^{13}C at natural abundance.

We will measure the ^{13}C pw90, measure the T_1 of one methyl, while saturating the other (and ^1H) and then measure the extent of magnetization transfer, all using the 'presat' pulse sequence. The combination of the T_1 of the ^{13}C in question, the intensity of the ^{13}C in question when the other is not being saturated (I_0) and the intensity of the ^{13}C in question when the other is being saturated (I) yield the rate constant k characterizing chemical exchange of the C in question from its position to the other position (i.e. in this instance the rate constant for amide group flips).

$$k_{\text{ex}} = \frac{(I_0 - I)}{I} * \text{Error!}$$

where $T_{1,\text{obs}}$ is measured for the ^{13}C in question while the other ^{13}C resonance is being saturated (i.e. under magnetization transfer conditions).

For example for C A, $T_{1,\text{obs}}$ is measured using standard inversion-recovery using presat with satfrq set to C B's resonance frequency, **satmode='yyn'** and the *minimum* satpower sufficient satpower to saturate B. **p1=2*pw90, pw=pw90** for ^{13}C (calibrate these). **dm='yyy'** for continuous ^1H decoupling at previously calibrated dpwr, dof, dmm. Array d2, as for the determination of $T_{1\text{s}}$ for ^1H . process and analyze as for ^1H , to determine C A's $T_{1,\text{obs}}$.

Now measure I and I_0 : revert to a standard presat experiment: **p1=0**. set a good long d1, 3 to 5* T_1 (as above). **ss=4**. **satmode='yyn','nnn'**. **nt=16**, or whatever is needed to get good signal:noise. integrate the resonance of A in both spectra. The first gives I and the second gives I_0 . I should be < I_0 .

Use the above equations to calculate k_{ex} .

The above would normally be done at each of a series of temperatures. The Eyring plot of $\log(k/T)$ vs. T then yields the activation energy for the motion or chemical exchange process.

Measurement of k_{ex} for faster rates

Three dynamic regimes:

Slow exchange: $\Delta\nu \gg k$

the line width of each separated resonance gives the rate of exchange ; $1/T_2^* = 1/T_2 + k_{\text{exch}}$, where $1/T_2^*$ is determined by simulation of the resonances to obtain $\Delta\nu = 1/\Delta T_2^*$ and T_2 is obtained, similarly, from a resonance observed at a low temperature at which exchange is negligible (k_{ex} is very small).

and, by magnetization transfer, $k_{\text{ex}} = \frac{(I_0 - I)}{I}$ *Error!

where $T_{1,\text{obs}}$ is measured for the ^{13}C in question while the other ^{13}C resonance is being saturated (i.e. under magnetization transfer conditions).

Approaching coalescence: $\Delta\nu > k$

Lines begin to broaden and approach one another

Individual lines' shapes now reflect both the separation $\Delta\nu_0$ of the original non-exchanging lines observed at low temperature and their line widths : $R_{2,0} = 1/T_{2,0}$.

$I(\nu) =$

Error!

Problem set, due Dec 5 (remember that the exam is scheduled for Dec 11)

1 Spin Echos of all kinds:

For a H-C fragment, draw the vector description of net magnetization at various points in the pulse sequence, for each sequence below. Thus conclude whether or not chemical shift is refocussed, and whether or not J coupling is refocussed, in each case.

2 Using Strychnine with CrAcAc, perform DEPT editing of the ^{13}C spectrum, as we did in class. Hand in the set of edited spectra plus the conventional ^{13}C 1d.

3 Using strychnine with CrAcAc and the INEPT pulse sequence, with refocussing, set $J=0$ so you get control over the delays. What is the optimal d_3 delay for a typical HC INEPT, and why?

Collect ^{13}C rINEPT spectra using a series of different d_2 values and comment on the result for each of the following: CH, CH_2 and CH_3 groups. Choose at least 4 different values of d_2 and have these span $1/6J$ to $1/J$.

Repeat the above with the Strychnine sample without CrAcac. How do the results differ from those above? why? How would you choose the delays for optimum detection of CH, CH_2 and CH_3 in this case?

4 On your sample, collect a gHSQC, a gctHSQC and a gHMBC.

Note that you now have a complete set of the spectra normally required to assign the ^1H and ^{13}C signals of your molecules. Start looking at them and see what you can assign.

5 Calibrate a ^{13}C pulse using pwxcal and the methyl iodide sample. Turn in a plot of the result of dssh with the null spectrum circled. What tip angle of pwx1 does this correspond to?