

Instructions for *NOE Difference* NMR experiments

Sample Preparation:

Prepare NMR sample as you would a regular ^1H NMR. The concentration of the sample should be no greater than 5 mg sample/typical sample volume. Insert a stream of N_2 through the sample and allow it to bubble through the NMR sample for a minute or so (I usually use a fresh piece of Teflon tubing connected to my N_2 manifold...be sure it contains no contaminants). This will remove O_2 present in the sample. Remove the bubbler and cap the tube quickly to minimize contamination.

NOE Difference Experiment:

Be sure to have 2 experiments available in your workspace on the instrument. Consult Varian manual on how to create additional experiments (hint: menu options). It is also recommended to use a high-field instrument.

- 1) Acquire ^1H NMR as usual in experiment 1 with lock signal > 60 . The resolution line width of CDCl_3 solvent peak should be < 0.5 (hint: to check, have the solvent peak selected in the ^1H NMR and type '**res**'. This will give the resolution line width). Save as usual ('svf').
- 2) Jump to experiment 2 by typing '**jexp2**'. Then type '**mf(1,2) wft**'. This should move the file from experiment 1 to experiment 2 and display the previously acquired spectrum.
- 3) Box the desired peak that is to be irradiated. Type '**NOESY1D**'. Again, make sure the desired peak is boxed.
- 4) Click on "[**Select**]" in the toolbar.
- 5) Click on "[**Proceed**]" in the toolbar.
- 6) Type '**go**'. This will start the experiment. Each irradiation usually takes about 5 min to acquire.
- 7) Save as usual ('svf').
- 8) Type '**wft process**'. This will show the irradiated peak and any enhancements. Do not phase the NOE difference. To determine the percent enhancement, use the integration tools as usual.

An *NOE difference* experiment can reliably measure enhancements to below 1%. It is generally considered good practice to irradiate in both directions. What that means is that if you think A is in close proximity to B, you may decide to irradiate A and observe an enhancement of B. However, you also need to irradiate B and see if there is an enhancement of A.

Instructions for running GCOSY NMR experiments

- 1) Be sure you have 2 experiments available in your workspace on the instrument. Consult Varian manual on how to create new experiments.
- 2) Shim and set lock signal >70-80 (Be sure to shim Z1-Z3 on the 500Mhz instrument).
- 3) If necessary, adjust non-spinning shims: turn spin off and maximize lock signal with low order X1, Y1 and high order X2Y2
- 4) Turn spin back on and touch up shims
- 5) Acquire ¹H spectrum. Assign reference peak and phase spectrum and save the spectrum.
- 6) Set left red cursor line 1ppm downfield of last peak and right red cursor line 1ppm upfield of TMS peak. If solvent does not contain TMS, set right cursor to around -0.5 – -1ppm.
- 7) Type: movesw
- 8) Reacquire ¹H spectrum.
- 9) Save the spectrum.
- 10) Type: jexp2 to jump to experiment 2.
- 11) Type: gcosy
- 12) Type: ni=256
- 13) Set the number of transients. Type: nt = x, where x is typically equal to 4. For samples with very high concentrations, x can be set to 1-3. For samples with low concentration, x should be set to a higher number, usually a multiple of 4. Time constraints for the experiment should also be considered when setting this value.
- 14) Check pw90 value. Type: pw90? This number should match the pw90 value in the parameter screen.
- 15) Type: dps This number must match the pw value.
- 16) Type: d1=1 This sets the delay time to 1 second.
- 17) Type: pfgon='nny' su This turns the pulse field gradient on.
- 18) Type: time This checks the length of the experiment. To adjust the time, change the number of transients.
- 19) Turn the spin off. Do this in the acquisition window. To be sure the spin stays off, also type: spin=0
- 20) Type: go Look for fluctuation in the lock level. If it is fluctuating, then the experiment should be running correctly.
- 21) Be sure to save your file after the acquisition is complete.

Instructions for working up the GCOSY data

- 1) Move to one of the NMR workup stations.
- 2) Be sure you have two experiments accessible in your workspace on the workstation.
- 3) Load the ¹H NMR spectrum with reduced sw into experiment 1.
- 4) Type: jexp2
- 5) Type: process
- 6) Type: foldt
- 7) Type: vs2d=x where the default is usually x=100. This determines how deep you cut into the spectrum, so x can be increased if a deeper cut is desired.
- 8) Type: dcon1 Now you can expand on the spectrum. The command dcon1 must be typed after entering any workup or printing commands to “regain access” to your spectrum
- 9) Adjust sb and lb to improve spectrum. Type sb = x then lb= x where x usually = 2-12, then reprocess as above (steps 5-7). This will help remove artifacts and reduce “methyl stars.”
- 10) To print, type: plgrid plcosy(14,1.3,1) Usually the first page will print with a grid that is slightly off-center, so print the first page twice. All subsequent pages should print correctly.

Instructions for *Variable Temperature (VT)* NMR experiments

Note: The 400 MHz may be ramped to 80 °C in increments no greater than 11 °C.

- 1) Prepare sample in appropriate solvent (*i.e.* DMSO-*d*₆).
- 2) Acquire ¹H NMR as usual. Save file ('svf').
- 3) Type '**temp**'. In the temperature control panel, click on "**allow temp control in an experiment to go**"
- 4) Begin ramping the temperature by typing '**temp= current temp + 11 su**' where the current temperature is read from the instrument display panel. Continue ramping the instrument in no greater than 11 degree increments until desired temperature is achieved.
- 5) Acquire ¹H NMR as usual. Save file ('svf').
- 6) To ramp down the temperature, type '**temp= current temp - 11 su**'. This will restore the temperature.
- 7) Acquire ¹H NMR as usual. Save file ('svf').

DEPT and INEPT Instructions (Varian)

DEPT:

1. Login and shim.
2. Click **setup**, and then pick nucleus and solvent.
3. Type **nt=64** (you may have to set nt as a larger multiple of 8 for dilute samples)
4. Type **dept ga** (four spectra will be taken)
5. Type the following for each spectrum:
 - ds(1)** -shows all C's with H's attached
 - ds(2)** -shows all CH's
 - ds(3)** -shows all CH₂'s
 - ds(4)** -shows all CH₃'s

NOTE: You will need to take the normal ¹³C separately, the DEPT macro will not give the normal ¹³C spectrum.

INEPT:

1. Login and shim.
2. Click **setup**, and then pick nucleus and solvent.
3. Type **nt=64** (you may have to set nt as a larger multiple of 8 for dilute samples)
4. Type **inept**
5. Here is where the experiment can be customized to your needs:

Multi selects multiplicity

mult=0 gives a normal experiment (dl-pw-at sequence);

mult=2 selects CH's (doublets)

mult=3 gives CH₂'s down, CH's and CH₃'s up; and

mult=4 enhances all protonated carbons.

dm='nny' gives decoupled spectrum; **dm='nnn'** gives coupled spectrum.

dmm is set to '**ccw**' or '**ccf**'.

focus='y' gives normal multiplets in coupled spectra.

6. Type **ga**

2D NOESY

Acquisition

1. Be in experiment 1
2. With spin ON, lock and shim as usual
3. Set lock > 80
4. Turn spin OFF
5. Shim "non-spinning shims": Low order: x1, y1; High order: xy, x2y2 (use +/- 4 button)
6. Turn spin ON
7. Retouch spinning shims. (Z1, Z2, Z3)
8. Take ¹H spectrum. Phase spectrum and assign reference peak
9. Set left cursor 1ppm downfield of last peak and set right cursor 1ppm upfield of first peak
10. Move sweep width: '**movesw**'
11. Take spectrem: '**ga**' – then phase spectrum and assign reference peak
12. Save file: '**svf**'
13. Move file to Experiment 2: '**mf(1,2)**'
14. Move to Experiment 2: '**jexp2**'
15. Type '**noesy**'
16. Set resolution: '**ni=256**'
17. Set # transients '**nt=4**' (or 8)
18. Check pw value: '**pw90?**' – pw value in parameter screen should match pw90 value
19. Check pulse sequence: '**dps**' – pw value shown in graphic should match pw90 value
20. Set delay: '**d1=1**'
21. Set mixing: '**mix=0.5**' (If MW=300-350, set mix=0.4)
22. Type '**pfgon?**'. If value different from 'nny', then type '**pfgon='nny' su**'
23. Check time: '**time**'
24. TURN SPIN OFF
25. Start experiment: '**go**'
26. Lock needle will fluctuate during acquisition
27. After acquisition is complete, save file: '**svf**'

Processing

1. Experiment 1 should contain the first saved spectrum (the 1D spectrum)
2. Experiment 2 should contain the 2D NOESY file. Go to experiment 2: '**jexp2**'
3. Type '**wft2da**'
4. Click on resize to get larger image
5. Type '**foldt**'
6. If machine says: 'fn not equal to fn1' type '**fn=fn1**' then type '**foldt**'
7. Type '**vs2d=100**' (the bigger the #, the deeper the cut)
8. Type '**dcon1**' – Also type this after plotting to get back to the spectrum
9. To expand: click on box; left mouse button sets lower left corner, etc. Then click EXPAND button
10. To plot, type: '**plgrid plcosy(14,1.3,1)**' – plgrid plots grid; the #1 refers to where the saved 1D spectrum is locate (i.e. experiment 1)

Instructions for running 1D solvent suppression experiment with presaturation

Intended for use with Varian Inova 400

- 1) Adjust to Z0 so that you get the highest value while the lock is off
- 2) With the lock 'on' adjust the phase to get the signal as high as you can
- 3) Shim the sample as well as you can using Z1 through Z4. You might also need to adjust the non-spinning shims X1, Y1, and X2Y2. Be sure to turn off the spinner in this case.
- 4) Do a simple 1D scan and focus on the water. It should be a symmetric Gaussian peak. If it is not symmetric, go back and shim some more. The shape of the peak can tell you what shim needs fixing. Broad peak is due to Z1, shoulders are due to Z2/Z4, and Z3/Z5 are responsible for broadening of the base. Note that lock phase can also have an effect on the shape. (see note 1)
- 5) Set the cursor next to the peak and type *nl* to center the cursor on the peak.
- 6) Type *presat* to load the presaturation pulse sequence.
- 7) Type *movetof* to center the spectrum on the water peak.
- 8) Type *satfrq=tof* to set the saturation frequency to that of the water peak.
- 9) Type *dps* to display the pulse sequence
- 10) Set d1 to zero (d1=0).
- 11) Set *satdly* from 2 to 3 sec (*satdly*=2), this is the t_1 of the system. This value should allow for full relaxation of the protons.
- 12) Set the *pw* to a value between 5-8 us. (This depends on the tuning of the probe, assuming it is tuned for CHCl_3).
- 13) Set the *satpwr* to a value greater than zero and less than 30
- 14) Collect the spectrum and work up as a regular 1D.

(In the *dps* window a small white mouse is visible in the upper right hand corner, click this to change the pulse sequence parameters)

Instructions for Running gcosy NMR Experiments

- 1) Be sure the account is setup so a minimal of two experiments are available in the workspace. Consult Varian manuals on how to create new experiments and for understanding commands.
- 2) Lock and shim appropriately. If lock signal is not >60, adjust to >60 using lock gain.
- 3) If necessary, adjust non-spinning shims: turn spin off and maximize lock signal with lower order X1, Y1 and X2Y2 shims.
- 4) Turn spin on and readjust shims as needed.
- 5) Acquire normal ^1H spectrum. Note: The fewer transients need to acquire ^1H spectrum, the less time will be need during gcosy. Slightly concentrated samples desirable
- 6) Box the desired region for gcosy. Make sure boxed region does not have any peaks ± 1 ppm of cursor lines.
- 7) Type: **movesw**
- 8) Reacquire ^1H spectrum.
- 9) Save spectrum.
- 10) Type: **jexp2**
- 11) Type: **mf(1,2)** Type: **wft aph** Only acquisition of desired range from 6).
- 12) Type: **gcosy**
- 13) Type: **ni=xxx** Typical value is 256.
- 14) Set the number of transients needed to acquire acceptable ^1H spectra. Type: **nt=x** For low concentration samples set nt equal to a multiple of 4. Refer to 5) above for x value. Time constraints should also be considered when selecting this value.
- 15) Check pw90 value. Type **pw90?** This number should match the pw90 value in the parameter screen.
- 16) Type: **dps** displays the pulse sequence settings. The pw90 must match the pw value.
- 17) Type: **d1=1** One second delay before first pulse. May reduce d1 for to time considerations.
- 18) Type: **pfgon='nny' su** Turns on z-axis pulse field gradient.
- 19) Type: **time** Check how long acquisition will take before starting.
- 20) Turn spin off through acquisition window. Type: **spin=0** Double check.
- 21) Type: **go** If lock level starts to fluctuate, the experiment should run properly.
- 22) Save file after acquisition is complete. For ni=256 and nt=4 acquisition time is ~20 min.

Instructions for Workup of gcosy NMR Experiments

- 1) Newer workstation does not have macro for gcosy. Load files of ^1H and gcosy in separate experiments. Do not use process button with gcosy.
- 2) Type: **process**
- 3) Type: **foldt**
- 4) Type: **vs2d=x** Default is x=100, adjust to desired depth of cut.
- 5) Type: **dconi** Expansions and adjustment of vs2d are possible.
- 6) Adjust lb and sb to improve spectrum. Type: **sb=x** then **lb=x** Usually x=2-12. Repeat steps (2)-(5). This aids in removal of artifacts and 'methyl stars'
- 7) To print, type: **plgrid plcosy(14,1.3,1)**. The first page with a grid will print with the grid off-centered, so print twice for a centered grid.