

# 2D NMR SPECTROSCOPY OVERVIEW

## BRUKER

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### 'Essential' <sup>1</sup>H-detected 2D Pulse Sequences:

**COSY** – **C**orrelation **S**pectroscop**Y**, good for determining basic connectivity via J-couplings (through-bond).

**TOCSY** – **T**otal **C**orrelation **S**pectroscop**Y**, same as **COSY**, but also able to generate cross peaks via intermediate spins (mix). Uses a spin lock that produces rf heating of the sample and hence requires many steady state scans (ds).

**NOESY** – **N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**, allows one to see through-space effects, useful for investigating conformation and for determining proximity of adjacent spin systems. Not so useful for MWs in the 1 kDa range due to problems arising from the NMR correlation time.

**ROESY** – **R**otational **O**verhauser **E**ffect **S**pectroscop**Y**, same as **NOESY**, but works for all molecular weights. Has the disadvantage of producing more rf heating, hence it requires more steady state scans.

**HMQC** – **H**eteronuclear **M**ultiple **Q**uantum **C**orrelation, allows one to pair NH or CH resonances. Often uses X-nucleus decoupling and hence gives rise to rf heating, requires reasonably well calibrated pulses and many steady state scans.

**HSQC** – **H**eteronuclear **S**ingle **Q**uantum **C**orrelation, provides the same information as **HMQC**, but gives narrower resonances for <sup>1</sup>H-<sup>13</sup>C correlations. Also requires X-decoupling and hence a large number of steady state scans and is also more sensitive to pulse imperfections.

**HMBC** – **H**eteronuclear **M**ultiple **B**ond **C**orrelation, a variant of the **HMQC** pulse sequence that allows one to correlate X-nucleus shifts that are typically 2-4 bonds away from a proton.

Information of individual 2D pulse sequences can be found in the TopSpin Menu [Help]>[NMR GUIDE]

Experiment	Parameter Set	Pulse Program	Minimum NS
gCOSY	2D-gCOSY-dcif	cosygpqf	1
gNOESY	2D-gNOESY-dcif	noesygpqh	2
ROESY	2D-ROESY-dcif	roesyetgp	8
TOCSY	2D-TOCSY-dcif	mlevetgp	8
gHMQC	2D-gHMQC-dcif	hmqcgpqf	1
gHSQC	2D-gHSQCdcif	hsqcetgpsi2	1
gHMBC	2D-gHMBC-dcif	hmbcgpplndqf	1

Bruker Pulse Program Abbreviations	
qf	absolute value
gp	gradient pulse
ph	phase sensitive
et	phase sensitive (Echo/Anti-Echo TPPI)
si	sensitivity improved
nd	no decoupling
pr	presat

### Summary of Methodology:

1. Set up variable temperature control
2. Tune, Lock and Shim
3. Acquire 1D 1H spectra, set: reference, sweep width, and transmitter frequency. Reacquire 1D 1H spectra with reduced sweep width- determine the number of scans required.
  - Repeat for 1D 13C spectra to run HSQC or HMBC
4. Calibrate the 90 degree pulse for 1H
5. Load 2D parameter set
6. Check 2D pulse program
7. Load prosol parameters and setup: reference, sweep width, transmitter frequency, number of scans, number of points
8. Set receiver gain, acquire,
9. Transform 2D data, phase and load projections.

#### 1. Regulate the temperature. Open temperature controller: **edte**

- a. Select the Carrier Gas: Compressed Air (10-40 °C) or Nitrogen.
  - i. Turn off the compressed Air (may keep 401 magnet legs on compressed air) The valve is closed when the handle is perpendicular to the pipe.
  - ii. Turn on the nitrogen.
- b. Select: **[ Corrections ]** and verify that no correction is applied.
- c. Select: **[ Ramp ]** enter a ramp rate of 2 degrees/min, enable ramp.
- d. Normal Conditions: **[ Main Display ]**
  - i. Sample Temp= 20 °C Thermocouple located below tube.
  - ii. Target Temp= 20 °C
  - iii. Heater= OFF (Set Max = 10% )
  - iv. Gas Flow= 270 L/h
  - v. Cooling= Empty
- e. Increase Gas Flow
  - i. 270 L/h normal, 800 for high/low temp **[ +/- ]**
    - a. Extreme temperatures will need a higher flow rate
  - ii. Turn the heater **[ on ]**
  - iii. Check the maximum heater power **[ Set Max ]** 10%. Increase the heater power if unable to obtain the desired temperature.
- f. Set temperature at 25 °C. The liquid nitrogen dewar is not required for 25 °C
- g. Within the edte window open **[ Monitoring ]**
  - i. Use auto scale for both y-axis':
    - a. Left: Temperature
    - b. Right: Heater Power
  - ii. Let sample equilibrate for 5 to 15 minutes
- h. Open [Self tune], run Self-tune program

## 2. Tune and shim.

- a. Check that the spinning is shut off.
- b. Shim the magnet: X, Y, Z1-Z5
- c. Tune each channel in use during the 2D experiment
  - i. 1H ONLY: COSY, NOESY, TOCSY
  - ii. 1H and 13C: HSQC and HMBC

## 3. Collect a good 1D spectra

### Experiment 1

- a. **Proton**: Acquire a 1D and reference. Zoom in and display all proton signals leaving 0.5 ppm of baseline on each side. Type **.setsw** to set the transmitter offset (o1p) and sweep width (sw). Reacquire “reduced-sweep width spectra” with the number of scans (ns) needed to get good signal to noise and phase. This dataset will become the 1H projection.

Write down the following values:

o1p:\_\_\_\_\_

sw:\_\_\_\_\_

sr:\_\_\_\_\_

ns:\_\_\_\_\_

These values will be used in F2 (direct) dimension

Write these parameters to experiment 10 **wrpa 10** (for pulse calibration)

### Experiment 2

- b. **X Nucleus** (only if acquiring HMQC, HSQC, or HMBC), Acquire a 1D and reference. Zoom in and display all X signals leaving 0.5 ppm of baseline side. Type **.setsw** to set the transmitter offset (o1p) and sweep width (sw). Reacquire “reduced-sweep width spectra”. This dataset will become the X projection.

Write down the following values:

o1p:\_\_\_\_\_

sw:\_\_\_\_\_

sr:\_\_\_\_\_

These values will be used in F1 (indirect) dimension

## DCIF: How to calibrate the $^1\text{H}$ $90^\circ$ Pulse on the Bruker NMRs

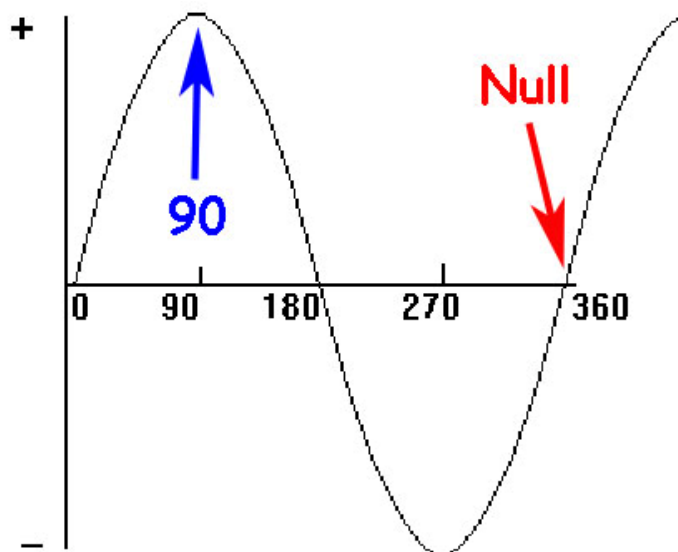
### Background Information:

For many NMR experiments such as DEPT, TOSCY, NOESY, and HMBC, the pulse sequence requires that many specific pulses or a series of pulses ( $90^\circ$ ,  $45^\circ$ ,  $180^\circ$ , etc.) be applied. Without properly calibrated pulses, many of these experiments will yield meaningless results, or most likely, fail outright.

Since each compound (and each nucleus) has a different chemical environment, each had a distinct  $90^\circ$  pulse width ( $p1$ ). The  $90^\circ$  pulse is defined as the duration, in microseconds, that the rf signal must irradiate your sample in order to tilt the magnetizations into the XY-plane,  $90^\circ$  away from the Z-axis of the NMR's magnetic field. Another way to think of it is how long you must pulse in order to tip all the spins into the XY plane. This pulse is often referred to as the  $\pi/2$  pulse.

The  $90^\circ$  pulse width for proton NMR experiments is about 10-20 microseconds on most modern spectrometers. The exact value of the  $90^\circ$  pulse width depends on the sample (nucleus, solvent, etc.) as well as the instrument (probe, transmitter power, etc.). It may be 5 microseconds long, 17 microseconds, or 35 microseconds, or some other number determined experimentally. For this reason, it is necessary to measure the  $90^\circ$  pulse for every sample you need to perform 2D experiments on. Lucky for us, the proton  $90^\circ$  pulse is typically quite similar for all the protons in your sample.

Measuring the  $90^\circ$  pulse width is simple enough. Remember that the  $90^\circ$  pulse tilts the sample magnetization into the XY plane, which contains the detector. A simple pulse sequence of irradiate-observe should show a maximum for the pulse duration corresponding to a  $90^\circ$  pulse. Because it is difficult to discern maximum signal intensities by comparing similarly intense peaks (i.e. comparing an  $89^\circ$ , a  $90^\circ$ , and a  $91^\circ$  pulse.), we look at the  $180^\circ$  or the  $360^\circ$  pulse.



The  $360^\circ$  pulse corresponds to a 'null' – no signal is observed at this irradiation. Searching for this null is easier to determine and has the added advantage of minimizing the time required between pulses due to relaxation issues.

#### 4. Calibrate 90° pulse Experiment 10

##### The Bruker nitty gritty:

- **re 10** and obtain a well-shimmed <sup>1</sup>H spectrum.
- Type **p1**, hit enter and notice the current value for the 90° pulse. Record p1 and pl1
- Type **pulprog zg**. Typically, Bruker uses a 30° pulse (zg30) for a proton 1D. This resets this to a 90° pulse.
- Change parameters (**ns 1; ds 0; d1 60**), reacquire, and phase. The value for d1 should be 5xs T1, hence using a value of 60 here is an estimate. If you have a slow relaxer or know your value for T1, you might want to set d1 to a larger value.
- Fourier transform (**ft**) and phase (**apk**). Type **dpl1** to set the display regions. Type **phmod pk** to use the same phase values for all spectra
- Start the acquisition by executing the AU **popt** program.
  1. Check Optimize button
  2. Enter *parameter* to modify: p1
  3. Choose *optimum* value: zero
  4. Enter *startval* value: 8
  5. Enter *endval* value: 64
  6. Enter the number of experiments (*nexp*) 8
  7. Enter the increment variation mode (*varmod*) lin(ear)
  8. Enter parameter increment (inc) 8
  9. Click [start optimize]

store as 2D data (ser file)  
 The AU program specified in AUNM will be executed  
 Perform automatic baseline correction (ABSF)  
 Overwrite existing files (disable confirmation Message)  
 Run optimisation in background

OPTIMIZE	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC
<input checked="" type="checkbox"/>	p1	ZERO	8	64	8	LIN	8

- In PROCNO 999, the finished array will be displayed, similar to Figure 1.
- On the screen, you should see a series of spectra that start positive, pass through a null at 180°, become negative, and pass through a second null at 360°. Estimate the point where the signal goes from negative values through zero then become positive. This is the location of your 360° pulse. (If you do not see a clear null at 360°, you may need to run **popt** again, adjusting the entered values.)
- Run your array again, to determine the 360° pulse width  $\pm 0.5 \mu\text{s}$  (i.e. array 60 to 63 with an increment of 0.5)
- Calculate the 90° pulse by dividing the p1 value of the null by 4. Use this number for your p1 in your subsequent experiments on this sample.

p1: \_\_\_\_\_ 90° pulse

pl1: \_\_\_\_\_ power level for p1

- Load 2D parameter set  
**Experiment 100**

rpar <PARAMETER SET>

Experiment	Parameter Set	Pulse Program	Minimum NS
gCOSY	2D-gCOSY-dcif	cosygpqf	1
gNOESY	2D-gNOESY-dcif	noesygpqh	2
ROESY	2D-ROESY-dcif	roesyetgp	8
TOCSY	2D-TOCSY-dcif	mleveltgp	8
gHMQC	2D-gHMQC-dcif	hmqcgpqf	1
gHSQC	2D-gHSQCdcif	hsqcetgps2	1
gHMBC	2D-gHMBC-dcif	hmbcgpplndqf	1

- Check pulse program
- Load the prosol parameters type **getprosol**
- Edit the basic parameters based on the information from the 1D experiments

#### Homonuclear Experiments

- **ns** Number of Scans
- **p1** Pulse width (us) (90 degree Pulse)
- **o1p** F2 Transmitter frequency (ppm)
- **sw** Sweepwidth (ppm) Enter the value for F2 and F1 dimensions
- **sr** Reference (Hz) Enter the value for F2 and F1 dimensions

#### Inverse experiments ( 1H vs 13C) require additional parameters

- **p2** Pulse width (us)
- **o2p** F1 Transmitter frequency (ppm)
- **1 sw** F1 Sweepwidth (ppm)
- **1 sr** F1 Reference(Hz)

Check these parameters, loaded during getprosol

- **pl1** Power Level p1 (120dB 1s the default = NO POWER ∴ No signal)
- **pl2** Power Level for p2

- Check Experiment Specific parameter are listed below

- Optional parameter changes

- **2 td** F2 Number of points
- **1 td** F1 Number of points

- Set receiver gain **rga** and acquire **zg**.

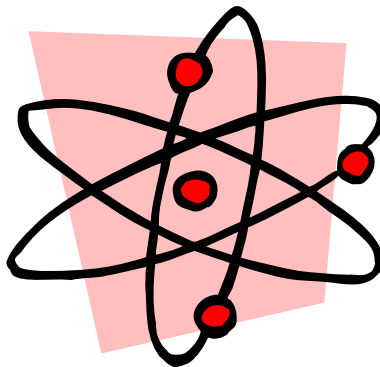
Use **multizg** to start multiple incremental experiments

## 12. Data Processing

- a. Fourier Transform the data: **xfb**
- b. Phase the data.
  - i. Type **ph**
  - ii. Choose manual phasing
  - iii. RMB on three peaks that span your spectrum
  - iv. LBM on the "R" button to start phasing the rows
    1. Click and drag on the "0" and "1" to adjust the zero and first order phasing
    2. When finished, click save and return
  - v. LBM on the "C" button to start phasing the columns
    1. Click and drag on the "0" and "1" to adjust the zero and first order phasing
    2. When finished, click save and return
  - vi. Click return to go back to the spectra
- c. Homonuclear experiments may symmetrize to reduce the noise.  
Type: **sym**

## 13. Load the 1D projections

- a. Type **edc**
  - i. fill out the name, EXPNO and PROCNO information for both F2 and F1.



## Experiment Specific Parameters

### COSY

- **p1** 90 degree pulse
- **pl1** power level for p1 pulse

### TOCSY:

- **d9** mixing time 60ms by default, typically 0.03 to 0.3
- **ds** dummy scans to establish thermal equilibrium

### NOESY:

- **d8** mixing time, 400-500 ms for small molecules, 100-200 ms for larger

### ROESY:

- **d?** mixing time for dipolar or through-space interactions)
- **ds** dummy scans to establish thermal equilibrium

### HSQC

- **d1** Delay time
- **CNST2** (average value for  $^1J(XH)$  will affect  $d24 \frac{1}{(8 * ^1J(XH))}$ )

### HMQC/HSQC:

- **dn** (decoupler nucleus)
- **dof** (decoupler offset)
- **j1xh** (average one-bond H-X J-coupling in Hz, used to determine mixing time, use **j1xh=140** for dn='C13', **j1xh=90** for dn='N15')
- **mbond** (multiple bond correlation flag, set to 'n')
- **null** (enables BIRD nulling, is the time for recovery of resonances following inverting pulse, is set in seconds)
- **pwx** (pulse width for dn/X-nucleus)
- **pwxlvl** (power level for pwx)
- **dm** (decoupler mode, either 'nnn' for off or 'nny' for on)
- **dmm** (decoupler modulation mode, usually 'ccw')
- **dpwr** (decoupler power)
- **dmf** (decoupler modulation frequency)
- **ss** (number of steady state scans, set to 256-512 if dm='nny', otherwise ss=8)
- Set **dof** equal to the value you wrote down for **tof** in the X-nucleus observation.
- Set **sw1** is the value you wrote down for **sw** in the X experiment.
- Set **rfl1** is the value of **rfl**, and **rfp1** is the value of **rfp**.
- If the  $T_1$ 's for your sample are long relative to the recovery time (**at + d1**, type **dps** to display the pulse sequence and view how long the spins have to relax between the read pulse before the FID is acquired and when the next pulse occurs), then you may want to set **sspul='y'** and **PFGflg='y'** (only if you have gradients!), this will randomize the net magnetization prior to the relaxation

delay **d1**, thereby ensuring the net magnetization will be the same at the start of every scan (help minimize artifacts).

#### HMBC:

- **dn** (same as HMQC/HSQC)
  - **dof** (same as HMQC/HSQC)
  - **mbond** (multiple bond correlation flag, set to 'y')
  - **taumb** (mixing time for long-range correlations, usually 0.055 seconds).
    - 2D Sequences with Water Suppression (sometimes start with "tn", e.g., tntocsy)
  - **presat** and **satdly** (length of time to irradiate solvent peak)
  - **satfrq** (offset of solvent resonance, sometimes not used, in which case tof must be on the solvent resonance)
  - **satpwr** (power level to use when irradiating solvent peak)
  - See the end of the above paragraph with regard to setting **dof**, **sw1**, **rfl1**, and **rfl1**.
- 
- Set the size of the 2D data matrix: **fn=2k fn1=2k** (4k can be used, but the processing takes significantly longer)
  - If you are running HMQC or HSQC and **dn='nny'**, then type **np=fn/2**, otherwise use **np=fn**
  - If you are running HMBC, be sure to set **dn='nnn'**
  - Make sure that **d1** is set such that **5\*at** is less than d1. If you need to adjust the d1:at ratio and do not want to increase d1 (doubling d1 will make your experiment run for twice as long), you can decrease at by cutting down np to 1024 or even 768 or 512 (at should be less than 0.2 seconds or extreme sample heating may result).
  - If you are running HMQC, HSQC, or HMBC
    - set **dof** and **sw1** according to the 1D spectrum you already obtained, otherwise **sw1=sw**
    - Also make sure that you have the correct bandpass filter in place in the broadband channel going to the probe (X-nucleus decoupling will kill the wrong filter which costs about \$400). Using no filter will give you garbage for results.
  - If you want to use a squared, shifted sine bell for your apodization function in both dimensions, type **ssb ssb1** (these are MIT macros, otherwise you can use wti after the first slice has been collected). Gaussian functions are also very popular.
  - **Set the relaxation delay d1** to be 1-1.5 seconds.
  - Collect one scan and let the autogain routine set the receiver gain: with **gain='n'** **phase=1 nt=1 ss=1 ni=1 au**
  - Now set the gain back to manual mode, turn it down by 2 dB, turn off the digital signal processing, and collect another scan: **gain='y'** **gain=gain-2 dsp='n'** **au**
  - When the acquisition is complete, type **ddff(1)** to display the data in the FID file, block 1. Look at the text window in the tcl/dg display. Verify that the numbers you

see are in the + or – 10,000 range. If the numbers are low, increase the gain by 1dB, if the numbers are high, decrease the gain. The gain can never drop below 0.

- You can also set the receiver gain by typing **set2dgain** (an MIT macro, where set2dgain: **r1=nt r2=ni r3=ss wexp='set2Dgain2' nt=1 phase=1 ni=1 ss=1 gain='n' au** and set2Dgain2: **nt=r1 ni=r2 ss=r3 phase=1,2 wexp=" gain='y' if (gain > 3) then gain=gain-3 endif**)
- If you are running HMQC, null will load at 0.3 seconds. You may want to adjust this number, as this will allow you increase your receiver gain. You can array null with the rest of the parameters set as above. Type **au** to collect a bunch of FIDs with the different null values (**da** to display these values in the text window). Type **vp=90 ai wft dssh** and find the spectrum whose most intense peak is smallest. Type **da** and find the value of null that corresponds to that spectrum. Go back and find the highest gain setting you can use to give **ddff(1)** values that do not exceed + or – 10000.
- When the gain (and maybe null) is (are) set properly, restore the other parameters, for example: **dsp='i' phase=1,2 nt=16 ss=8 ni=32 au**
- If running TOCSY, ROESY, or HMQC/HSQC with **dn='nny'** set **ss=512 or 256** to allow the sample to reach a steady state temperature because of the rf heating that will occur from either the spin-lock or the decoupling.
- Set **nt** according to how many scans were needed to get a good proton 1D spectrum unless you are running an HMBC spectrum, in which case you should multiply nt by at least four.
- Set **ni**. Use an ni of at least 32, but an ni as large as 256 or 320 may also work. Type **time** will give an approximate estimate of how long the experiment will run.
- Type **au** to start the run. The acquisition status window should give you a good estimate of how long your experiment will run for.
- When the first FID has been collected (the acquisition status window will show FID: 2), transform the first FID by typing **wft(1)**.
- Phase the first slice of the 2D as you normally would phase a 1D spectrum.
- Calculate the first point multiplier by typing **cfpmult**. The value of **fpmult** determined by the **cfpmult** macro helps compensate for the fact that a zero delay cannot exist between pulses with differing phases when the first complex point of the 2D data set is collected. This zero delay corresponds to a zero t1 increment (**d2=0**).
- Set the integrals for all the peaks you see (type **cz** to clear the integral zeroes or resets).
- You can also play with the apodization in the f2 dimension with the **wti** (weight interactively) routine. Simply typing **ssb** (sets **sb=-1\*at, sbs=sb**) will automatically set the apodization to a reasonable value, however. Typing **ssb1** will similarly set the apodization in the f1 dimension to a reasonable value.

- If the ADC overflow light starts flashing, you may need to abort the acquisition and go back and tweak parameters.

### Do preliminary processing with the help of linear prediction.

When FID:33 shows in the acquisition status window, you can begin to look at the first 16 complex points of your 2D data set with the help of linear prediction.

- Type **dolp** (do linear prediction) to predict the remainder of the data set based on the first 32 FIDs (16 complex points). **dolp** makes use of the variable **celem** (completed fid elements) which keeps track of how many completed elements there are in the data set.
- **wft1da** (transform the data set halfway, i.e., only transform the f2 dimension, thereby producing what is termed an interferogram which shows how each FID's individual points vary as a function of the evolution time)
- **bc('f2')** (baseline correct the f2 dimension using the integral resets you set after you transformed the first slice with the **wft(1)** command)
- You will see an interferogram. If you want, you can manually apodize the t1 data points by placing the cursor on an intense peak and typing **wti**. Again, **ssb1** will automatically set a reasonable apodization value for you.
- Typing **wft2da** will transform the spectrum the rest of the way using the linear prediction values set by **lpr1** – to turn linear prediction off, set **proc1='ft'** (instead of 'lp').
- Transforming a completed spectrum. Type **wft1da bc('f2') dolp wft2da**.

### Manipulating the 2D spectrum:

- **dconi** will display to plot contours and make the cursor active (or interactive).
- **dpc** will display the plot contours and leave the cursor active.
- **vs2d** can be adjusted with the middle mouse button in **dconi** mode (but not in **dpc** mode) or **vs2d** can be set by typing in a new value, e.g., **vs2d=200 dconi**

### To phase the 2D spectrum:

- Type **dconi**, click **[full]** and place the cursor on the top-right-most peak.
- Type **r1=[Index]** but do not hit enter. Index is the Index number at the top of the screen. Now position the cursor at the bottom-left-most peak of the spectrum and type **r2=[Index]**. You have just selected two rows with peaks.
- Type **ds(r1)** and click **[phase]**. Only adjust the right side phasing (you have just adjusted the zero order phasing).
- Type **ds(r2)**, click **[phase]**, click on the same spot on the right side of the spectrum BUT DO NOT ADJUST THE PHASE.
- Next, click on the left side of the spectrum and adjust the phase (this adjusts the first order phasing).
- Go back and display row r1 by typing **ds(r1)**. Tweak the right phase if needed.
- Type **ds(r2)** and follow the same row 2 phasing procedure as before (click right but make no adjustments, then click on the left and adjust the phase). Iterate back and forth until you are satisfied with the phasing.
- Type **dconi** to see what should now be a properly phased 2D spectrum. If there are purple and orange vertical streaks in your spectrum, you may have to go back and

adjust the f2 phasing by typing **wft(1)** and readjusting the phase. If you do this, you then need to once again type **wft1da bc('f2') wft2da** to reprocess the data set as you did before.

- Another way to rephase is to set the parameter **pmode** from 'partial' to 'full' (**pmode='full'**), by then changing the trace parameter from 'f1' to 'f2', you can adjust the f2 phasing after the transform has taken place, but your transformed 2D data set will occupy much more memory (i.e., the experiment size will be larger when you type **explib**).

### Setting the reference lines.

- Expanding a peak on the diagonal (if present, i.e., not HMQC, HSQC, HMBC), placing the cursors on top of the peak and typing **rl(7.27p) rl1(7.27p)** (use units of d instead of p (for rl1) if running a heteronuclear 2D, i.e., HMQC, HSQC, HMBC) will set the reference mark in both dimensions.
- **setref2D** should also work to set the reference marks, but small errors may occur.

### Additional manipulations of the 2D data set.

- **bc('f1')** can be run to remove or reduce t1 ridges.
- **foldt** will symmetrize a data set (do not run on any data set where **fn** does not equal **fn1** or where the f1 and f2 axes are not identical, e.g, HMQC).

### Plotting the 2D data set.

- **ppc** will plot, just as **dpc** displays (these are custom macros, the manual version of **ppc** is **pcon(30,1.3) dpcon(30,1.3) dconi('restart')**)
- **th** will adjust how many of the lowest contours are left off when plotting or displaying plot contours. The middle mouse button can adjust **th** in **dconi** mode
- projections can be displayed using the **[proj]** button after typing **dconi**. Adjust the height of the projections with the middle mouse button. Click **[plot]** to send them to the plot buffer
- **wc** is the width of the chart (mm) in the horizontal direction, **wc2** is the width of the chart (mm) in the vertical direction.
- Type **page** to dump the contents of the plot buffer to the plotting device.

Instead of plotting with **pcon**, you can run the **plcosy** macro for homonuclear 2D plots, or the **plhxcor** macro for heteronuclear plots.

- **plcosy** is usually run with three arguments: **plcosy(30,1.5,1)**
  - the first is the number of contour lines to draw
  - the second is the spacing between contour lines (1.5 means that each successive contour line will denote 1.5 times the intensity of the previous line)
  - and the last number will be number of the VNMR experiment where the processed 1D spectrum resides.
  - Note that the experiment with the processed 1D must have the exact same sweep width (**sw**) and transmitter offset (**tof**) in order to line up properly. That is why you collect the 1D and then use the **movefid (mf)** command to move the data including the parameters to another VNMR experiment where you then convert the parameter set to the 2D parameter set.

- Type **svf** to save your file.

### **Finishing up.**

Be sure to turn off temperature regulation when you are done by typing **temp='n' su**. If you changed the temperature of the preconditioner, you will also need to turn the preconditioner temperature back to 20 C.

