Diffusion Ordered Spectroscopy (DOSY)

Step-by-step procedure:

Before running 2D DOSY, parameters should be optimized with 1D DOSY acquisition parameters (**ledbpgs2s1d**). This is bipolar gradient pulse sequence which help in eliminating any background gradients that would lead to additional unwanted echoes.

Note: make sure at least 8 (or multiple of 8) scans are used for this experiment

Note: Initial estimate for d20 (diffusion time, big Delta) should be set to 100 ms and p16 (gradient pulse, little Delta) should be set to 1 ms.

1. Run a standard 1D proton spectrum and process data.

2. Setup the next experiment by keeping the same file name but different experiment number.

3. Type **rpar up_dosy1d all** to load 1D DOSY experiment. Fill in all std. 1D parameters such as **sw**, **o1p**, **td**, **d1**, **p1**, **pl1**, **rg** in appropriate window (**ased**).

4. Set initial value for **d20** and **p16**.

5. Type **cnst21** and enter **2** in new dialog box.

6. Run the experiment (**zg**) and make sure the intensity is nearly same compared to std. proton spectrum at exact same number of scans. This 1d spectrum is used as reference.

Note: If signal is attenuated a lot compared to 1D ^{1}H NMR spectrum, manipulate d20 and p16 to achieve nearly identical signal height.

7. Setup the next experiment by keeping the same file name but different experiment number.

8. Increase the value of **cnst21** to **95** from 2. Run the spectrum. The remaining signal intensity should be approximately 2-5% of the original (***reference***) signal intensity.

9. Note: If this is not the case, then adjust acquisition parameters (**d20** and **p16**) accordingly until above criteria are matched.

10. The first variable to try adjusting is **p16**. For bipolar gradients **p16** can be < **2.5 ms**. The second variable that can be modified is **d20**, the diffusion time.

11. Once the parameters have been adjusted accordingly, you may proceed with the 2D DOSY acquisition.

Diffusion Ordered Spectroscopy (DOSY)

12. Type **rpar up_dosy all** to load 2D DOSY experiment.

13. In the **ased** menu edit the appropriate values for the pulse widths, delays, constants and sweep width (optimized from above steps).

14. Execute the au program dosy by typing xau dosy. You will be asked following question:
    1. Enter the first gradient amplitude: **2**
    2. Enter the final gradient amplitude: **95**
    3. Enter the number of points: **16** or **32**
    4. Do you want to begin the acquisition: **y**

Note: Typing xau dosy 2 95 16 y in the command line will have the same effect as answering the questions above.

5. Process data by typing **xf2** (after acquisition finished message appears).

6. Rest of the DOSY processing is performed in Topspin (transfer data to PC loaded with Topspin) and open the dataset.

7. Type **eddosy** to open windows to set up DOSY processing parameters.

8. Type **xlist** = **vdlist**, enter appropriate value for **gdist** (big Delta) and **glen** (little Delta) from DOSY experiment, enter **gamma** = **4258**.

9. Type **dosy2d** to perform fitting and at the end it displayed 2D DOSY plot.

Multiple Peak Suppression

Multiple peak suppression is based on the similar principle of presaturation method of solvent suppression routinely used in NMR spectroscopy. Instead of suppressing one peak, in this method more than one peak can be suppressed.

There are many different methods available in modern NMR spectrometer that can perform similar tasks of suppressing multiple peaks. Some of the commonly used methods are as follows:

> **1.** Classical Approach: Shaped pulse for off-resonance presaturation (zgps)
> **2.** Gradient Approach: 1D WATERGATE (zggpwg)
>   1D excitation sculpting (ss-zgesgp)
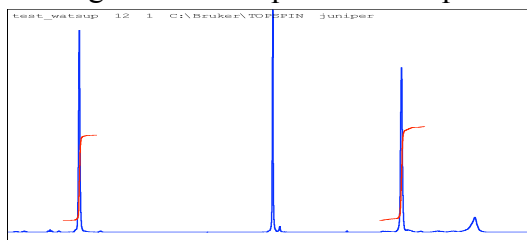>   1D WET (ss-wet)

1. Classical Approach:
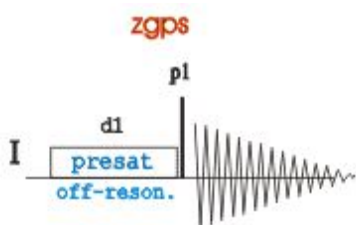
In the above pulse sequence, off-resonance pulse is a multiple lobe shaped pulse and should be created at the beginning depending on the requirement.
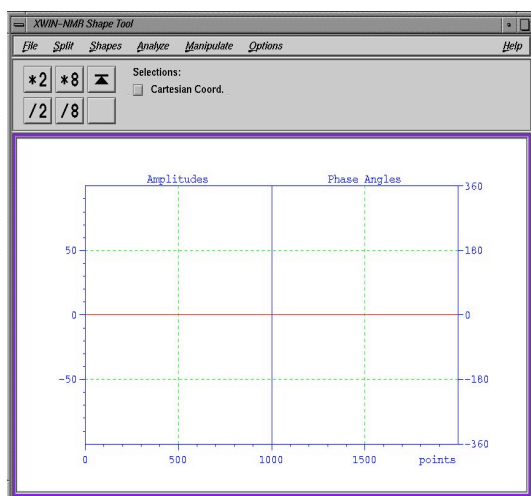
Step-by-step Instructions:

1. Record routine ^{1}H NMR spectrum with standard proton parameter.

2. Select the individual peaks and/or peak regions to be suppressed from the ^{1}H NMR spectrum.

3. Integrate selected peaks and/or peak regions and save the integration.

Note: To obtain better suppression, try to keep the transmitter offset to the middle of the peak regions to be suppressed.
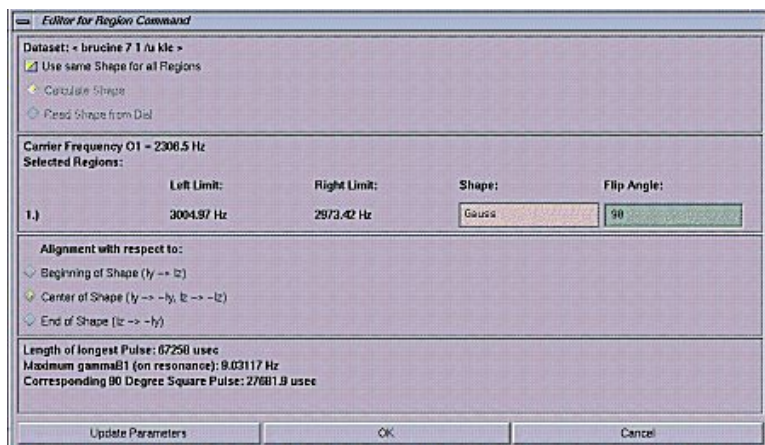
4. Open "**Shape Tool**" from **windows** menu of **xwinnmr**. A new window will appear as below.

Multiple Peak Suppression

5. Select the desired shape from **Shape menu**, e.g. "**Gauss**". This shape works for most of the routine multiple peak suppression.

6. Select "**Calc. shape from excitation region**" from the **Manipulate menu**. A new window will appear as follows.

Note: Number of selected regions in the middle panel should be equal to number of peaks and/or peak regions to be suppressed.

7. Enter the chosen **shape** in step v in **Shape window** dialog box and **flip angle** (in this case: **180** (degree)) in the **flip angle window** dialog box. Also choose middle radio button "**Center of shape**" in **alignment with respect to** panel. Click **OK**.

8. After getting all three of the parameters in the bottom panel, click on "**Update Parameters**". A new dialog box will appear with a dialog, **save as:**. Give a unique shape name and note down

Multiple Peak Suppression

the name as you need this for setting up the multiple peak suppression experiment. A new shape file will create and stored at "/u/exp/stan/nmr/lists/wave/".

9. Now close all **Shape Tool** windows and return back to **xwinnmr** window.

10. Note down the value of **p11** (in msec) and **sp1** (in dB) from the current data set.

11. Increment experiment number to create a new experiment with same file name.

12. Change the **pulse program** in the **eda** window, to "**zgps**".

13. Type **ased** and enter previously noted values for **sp6** (same as **sp1** from step 10), **p18** (same as **p11** from step 10) and **SPNAM6** (shape name from step 8).

14. Save and return to **xwinnmr**.

14. Adjust receiver gain by typing **rga**.

15. Minimum of **8** scans is required for this experiment, so make **ns** multiple of 8 depending on the desired signal-to-noise ratio.

16. Type **zg** and after acquisition is over, process as routine 1D ^{1}H NMR spectrum.
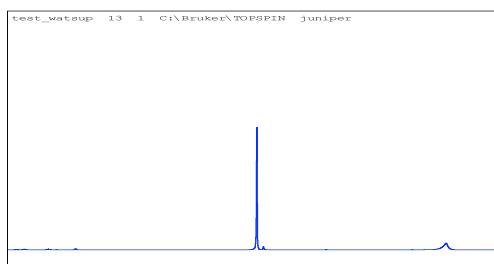
If every step correctly followed, it is possible to completely suppress desired peaks as seen in the above spectrum compared to the normal ^{1}H NMR spectrum shown above.

If peaks are not suppressed as desired, different shapes such as **BURP** or **Gaussian cascades (such as G3 or Q3)** can also be used for better suppression especially in case of multiplets. Alternatively, for more effective suppression, gradient methods can also be implemented.

Multiple Peak Suppression

2. Gradient Approach
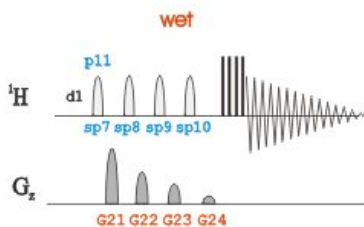
Step-by-step Instructions:

1. Record routine ^{1}H NMR spectrum with standard proton parameter.

2. Select the individual peaks and/or peak regions to be suppressed from the ^{1}H NMR spectrum.

3. Integrate selected peaks and/or peak regions and save the integration.
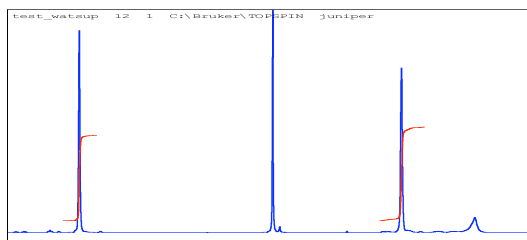
Note: To obtain better suppression, try to keep the transmitter offset to the middle of the peak regions to be suppressed.

4. Open "**Shape Tool**" from **windows** menu of **xwinnmr**. A new window will appear as below.

Multiple Peak Suppression

5. Select the desired shape from **Shape menu**, e.g. "**Gauss**". This shape works for most of the routine multiple peak suppression.

Note: In original paper "**SEDUCE**" shape was used to suppress multiple solvent peaks. It is advisable to create a "**SEDUCE**" shape if available in pulse shape menu for multiple solvent suppression.

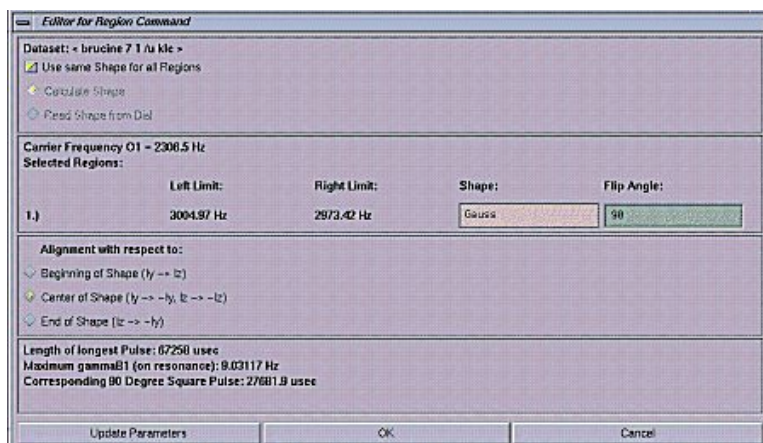6. Select "**Calc. shape from excitation region**" from the **Manipulate menu**. A new window will appear as follows.

Note: Number of selected regions in the middle panel should be equal to number of peaks and/or peak regions to be suppressed.

7. Enter the chosen **shape** in step v in **Shape window** dialog box and **flip angle** (in this case: 180 (degree)) in the **flip angle window** dialog box. Also choose middle radio button "**Center of shape**" in **alignment with respect to** panel. Click **OK**.

8. After getting all three of the parameters in the bottom panel, click on "**Update Parameters**". A new dialog box will appear with a dialog, **save as:**. Give a unique shape name and note down the name as you need this for setting up the multiple peak suppression experiment. A new shape file will create and stored at "/u/exp/stan/nmr/lists/wave/".

9. Now close all **Shape Tool** windows and return back to **xwinnmr** window.

10. Note down the value of **p11** (in msec) and **sp1** (in dB) from the current data set.

11. Increment experiment number to create a new experiment with same file name.

Multiple Peak Suppression

12. Change the **pulse program** in the **eda** window, to "**ss_wet**".

13. Type **ased** and enter previously noted values for **sp1** (same as **sp1** from step 10), **p11** (same as **p11** from step 10) and **SPNAM6** (shape name from step 8).

14. Save and return to **xwinnmr**.

15. Adjust receiver gain by typing **rga**.

16. Minimum of **8** scans is required for this experiment, so make **ns** multiple of 8 depending on the desired signal-to-noise ratio.

17. Type **zg** and after acquisition is over, process as routine 1D ^{1}H NMR spectrum.

Proton Homonuclear Decoupling

1. Run the conventional proton NMR spectrum using the standard parameters.

2. Process and phase the proton spectrum.

3. Type wrpa <new experiment number> to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "xyz_123" and the experiment number "1", typing wrpa 2 will copy "xyz_123 / 1" to "xyz_123 / 2".

4. Type re <new experiment number> to go to the new dataset. Following the example above, type re 2 to go to experiment number 2.

5. In the new dataset, type e.g. rpar protonhomodec all to read all the parameters for the homode-coupling experiment (pulse sequence: zghd/zg0hd).

*** Make sure DIGMOD is set to "homodecoupling-digital" ***

6. SET THE DECOUPLER FREQUENCY: Expand the spectrum about the peak you want to decouple. Click with the LMB on the utilities button to the left of the spectrum. Then click on the o2 button with the LMB. Then move the mouse pointer to the spectrum area (the pointer will jump to the spectrum) and place the cursor on the center of the multiplet you want to decouple. Click the MMB to define this frequency as the decoupler frequency (parameter O2). Then click the return button on the lower left of the screen to return to the normal spectrum display.

7. Type **rga** to set the receiver gain. After "**rga:finished**" displayed in the remarks line, type **zg** to run the homodecoupled spectrum. Process data in normal fashion.

8. Expand the decoupled peak to check for complete decoupling. If decoupling is not complete, adjust the decoupler power. To do this, type pl14 and enter a new value that is **3** units lower numerically than the current value (i.e., if the current value is 45, enter 42).

Note: Do not enter pl14 value less (numerically lower) than 30! Repeat the experiment to check decoupling.

9. COMPARING SPECTRA USING THE DUAL DISPLAY MODE: To compare the decoupled and undecoupled spectra, type **dual** to enter the dual display mode. A menu will pop up containing fields to enter the information needed to define the second data set. Click the **expno2** button and enter the experiment number of the reference (undecoupled) spectrum - probably this will be **1**. Click the **procno2** button and enter **1**. Then click the **done** button on the lower left of the pop-up window, and the spectrum display will now have the homodecoupled spectrum in green and the reference (undecoupled) spectrum in purple displayed together. The buttons on the upper left can be used to expand and manipulate the display. Click the **return** button on the lower left when you are finished with the dual display mode.

Proton Homonuclear Decoupling

10. Repeat steps 6 and 7, above, to decouple other peaks as needed.

1D NOE Difference

Sample Preparation: Sample for this experiment should be dilute (1% (v/v), ~5 mg in 700 µl) and degassed (freeze-pump-thaw cycle)

1. Run the conventional proton NMR spectrum using the standard parameters.

   Note: Don't spin your sample for this experiment.

2. Process (**use lb 2Hz**) and phase the proton spectrum as normal.

3. Type **wrpa <new experiment number>** to copy the current dataset to a new dataset with the requested experiment number. For example, if the current dataset has the name "xyz_123" and the experiment number "1", typing **wrpa 2** will copy "xyz_123 / 1" to "xyz_123 / 2".

4. In the new dataset, type e.g. **rpar noediff all** to read all the parameters for the NOEDIFF experiment (pulse sequence: **zgf2pr**).
   * NOE experiment is recorded without sample spinning *

5. Type **edasp**, make sure both NUC1 and NUC2 are set to ^{1}H, otherwise set both to ^{1}H and click on **SAVE** tab.

6. Type **rga** to set the receiver gain. Type **zg** to run the reference spectrum (depending on your sample concentration, adjust number of scans (**ns**) accordingly in this step).

7. SET THE NOE FREQUENCY LIST: Click with the LMB on the **utilities** button to the left of the spectrum. Then click on the **frqlist** button with the LMB answer the questions as shown below.

   * Please enter type of list (f1, f2, f3) : **f1** (this should be **f2** for AMX spectrometer)
   * Please enter name of the f1 list : **in_noelist_123**
   * Write name of f1 list to acqu parameters? : **n**

The following option appears if a f1 frequency list with the same name already exists:

   * Frequency list exists, append (a), overwrite (o) or quit (q) :

At this stage cursor will be tied to the baseline of the spectrum. First select a specific frequency near -2 ppm by clicking MMB (a green arrow designate your selection). Next move the cursor to the center of the multiplet to be irradiated, click MMB to select the multiplet. You may choose as many frequencies as you want (remember each frequency will be a new experiment). When finished, click LMB.

1D NOE Difference

8. Type eda, navigate to FQ1LIST field, select newly created f1 frequency list file from the pop up menu (remember it is f2list for AMX spectrometer).

9. Type **noediff** and a message box will appear which states you are about to run an NOEDIFF experiment. Click **OK** and answer the questions as shown below.

 * Base Name of all frequency lists : **in_noelist_123**
 * # of irradiation points : number of listed frequency in the above file
 * # of cycles through each list: at least 8-10 (so total number of scans for each experiment = 8-10 * ns)

Now the au program executes all the experiments automatically.

10. Type **re 2 1** to activate first reference spectrum. Process and phase the spectrum as normal. Type **multiefp** and answer the questions as shown below.

 * Enter first experiment number to process : **2**
 * Enter number of experiments : **# number of irradiation points**

11. COMPARING SPECTRA USING THE DUAL DISPLAY MODE: To compare spectra, type **re 3 1** and type **dual** to enter the dual display mode. A menu will pop up containing fields to enter the information needed to define the second data set. Click the **expno2** button and enter the experiment number of the reference spectrum - probably this will be 2. Click the **procno2** button and enter **1**. Then click the **done** button on the lower left of the pop-up window, and the spectrum display will now have the noe spectrum in green and the reference spectrum in purple displayed together. The buttons on the upper left can be used to expand and manipulate the display. Clicking on **diff** tab will subtract second spectrum from the first (reference). Click the **return** button followed by **save and return** and you will see a message that states the difference spectrum will be saved in expno. 3 and procno. 2. Click **OK**.

 * You now have an NOE difference spectrum which (in an ideal case) will have one large negative peak corresponding to the signal you irradiated, and several properly phased positive peaks (dispersive signals are due to imperfect subtraction) corresponding to protons which experience an NOE with the irradiated peak.

12. Continue step 10 changing expno to get difference spectrum for other irradiated frequencies.

DPFGSE NOE (GOESY)
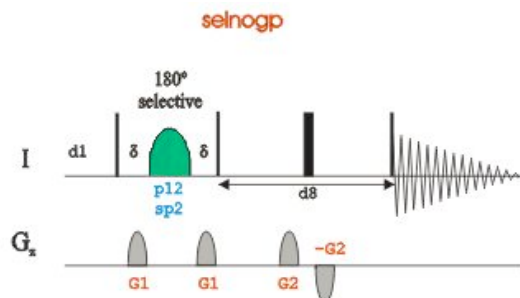
DPFGSE-NOESY (Improved version of GOESY)

This pulse sequence gives nearly identical results to conventional NOE difference spectrum, but is free from the subtraction artifacts.

Step-by-step procedure:
1. Run a standard 1D proton spectrum and process data.

2. In the integrate mode, integrate just the peak to selectively excite and save the integration.

3. Open Shape tool from windows menu.

4. In the shape tool window, click on the **shape menu** and select the desired shape type e.g. **Gauss** (Gauss 180 degree shape works well).

5. In shape parameter editor dialog box, enter **size of shape 1000** and **truncation level 5**.

6. After loading the basic shape, click on the **manipulate** menu and select -> **Calc. Shape from excitation region**.

7. The region you have selected in the integrate mode will be used to define the region to selectively excite with the selective pulse.

8. Make sure **Flip Angle** is set to **180**, **Alignment with respect to:** is set to **Center of Shape** and **Type of 180 degree pulse:** is set to **Refocusing**.

9. In the window that comes up, click on **OK** to execute the calculation with the already defined region.

10. Next, click on **Update Parameters** and close the window. A new **Save As** dialog box appears, with option to enter **Name of Shape:** provide a new name unique to your shape and click **OK.**

DPFGSE NOE (GOESY)

11. Close the shape tool window.

12. If all the above steps are correct, now you can see the updated parameters for shape length (**p11**) and shape power (**sp1**).

13. Setup the next experiment by keeping the same file name but different experiment number.

14. In acquisition window, change the pulse sequence name to **selnogp3** (alternatively you can use single spin echo version of the same sequence, **selnogp2**)

15. Type **ased** to edit necessary acquisition parameters directly related to the above pulse sequence. Among others, optimize **d8** (NOESY mixing time) and **d1** (recycle delay) depending on the nature of the sample being analyzed.

16. Type the value of **p12** (180 degree shaped pulse) as determined from step 11 (same as **p11**).

17. Similarly type the value of **sp2** as determined from step 11, **sp1**.

18. Type the name of the new shaped file created in step 9, for the parameter **spname2**.

19. Type **edcpul**, check the values of gradient level as well as gradient shape and update in the current dataset.

20. Type **rga** to adjust receiver gain.

21. Type **zg**, and acquire 8*n number of scans for desired signal-to-noise.

22. Process data with **efp** command and phase correct spectrum.

Gradient edited HSQC

This version of 2D HSQC (heteronuclear single quantum coherence) pulse sequence (pulse program: **invietedetgp**/**hsqcedetgp**) provides one bond CH correlation ($^1J_{CH}$) along with DEPT 135 type spectral editing information e.g. CH and CH_3 are oppositely phased compared to CH_2 contours.

Step-by-step procedure:

1. Run the conventional proton NMR spectrum using the standard parameters.

   Note: Don't spin your sample for this experiment.

2. Make sure proton 90° pulse is set correctly or measure proton 90° pulse for your sample.

3. Reduce proton spectral window (**sw**) so that transmitter frequency is in the middle of the desired resonances. Click on **utilities**, click on **O1**, click MMB on the desired position of the ^1H NMR spectrum. Reduce value of **sw**, if necessary by typing **sw**, and entering new value in ppm inside pop-up window. Rerun proton NMR spectrum.

4. Note down the value of **sw** and **o1p** from **eda** window.

5. Increment the experiment number to create a new experiment with same file name.

6. Type **rpar up_ghsqced all** to load parameters for ghsqced experiment and follow on screen instructions.

7. Type **eda**, change the value of **sw** and **o1p** in the F2 column from step 4. Optimize **sw** in F1 column (spectral width for ^{13}C window) to keep it as small as possible to get best resolution (if possible take the advantage of folding aromatic peaks by reducing ^{13}C spectral window).

8. Set number of scans (**ns** in F2 column) (should be multiple of 4) and number of increments (**td** in F1 column) (e.g. 128, 256, 512, 1024) based on ^{13}C spectral window, experimental time (can be measured by typing **expt**) and signal-to-noise requirement.

9. Type **rga** to adjust receiver gain.

10. Type **zg** to start data acquisition. Type **acqu** to switch to FID window.

11. Optional: After first increment finished, type **rser 1** to extract first row to a Temp window and type **efp** to process extract row. Make sure all intense peaks from ^1H NMR spectrum are visible. If you cannot see any peaks, increase number of scans (**ns**) or ask NMR Manager for some help in set up.

Gradient edited HSQC

12. Before data processing, set the value of **1 si** (**si** in F1 dimension) to 2-4 times of number of increments (**1 td**).

13. Set-up linear prediction parameter by typing edp, setting **ME_MOD** to **Lpfc**, **NCOEF** to 3 times number of cross-peaks in the spectrum (usually 64-128), **LPBIN** to **2*td** in F1.

14. After acquisition is over, type **xfb** to process 2D data.

    Note: Make sure you see both positive and negative contours in the spectrum, otherwise click +/- button couple of times till you see both sets of contours. Click on DefPlot button, hit enter key on your keyboard without altering anything 4 times.

15. Click on phase button, click on **row** button, click MMB on middle of the top-right contour (you will see an intense peak, dispersed if improperly phased), click on **mov-1** button to extract row to the screen on right (marked **1**), then click on **row** button again, click MMB on the middle of the bottom-left contour, click on **mov-2** button to extract row to the screen on right (marked **2**). Click on **1** screen. Adjust **ph0** and **ph1** to properly phase peaks. Click on **return**, click on **save & return**. Click on **OK** tab when prompt with message "**start xf2p?**".

16. Select a known peak (e.g. solvent peak) for calibration. Click on **calibrate** tab, then click MMB on middle of contour (solvent peak), enter ^{1}H chemical shift of the peak in new dialog box, followed by ^{13}C chemical shift.

17. Click on **contours** tab to display 2D spectrum for analysis.

H2BC Correlation Experiment

This version of 2D H2BC pulse sequence (pulse program: **ss_h2bc_ctea12**) provides two bond CH correlation.

Step-by-step procedure:

1. Run the conventional proton NMR spectrum using the standard parameters.

Note: Don't spin your sample for this experiment.

2. Increment the experiment number to create a new experiment with same file name.

3. Type **rpar up_h2bc all** to load parameters for H2BC experiment and follow on screen instructions.

4. Type **eda**, change the value of **sw** and **o1p** in the F2 column from step 4. Optimize **sw** in F1 column (spectral width for ^{13}C window) to keep it as small as possible to get best resolution.

5. Type ased, set **cnst6** to **125** Hz, **cnst8** to **165** Hz and **cnst15** to **20** ms.

6. Adjust **sw** and/or **cnst15** until the calculated parameter **TD1MAX** is less or equal to the current **td1** (= number of increments of t1 × 2).

7. Set number of scans (**ns** in F2 column) (should be multiple of 4) and number of increments (**td** in F1 column) (e.g. 128, 256, 512, 1024) based on ^{13}C spectral window, experimental time (can be measured by typing **expt**) and signal-to-noise requirement.

8. Type **rga** to adjust receiver gain.

9. Type **zg** to start data acquisition. Type **acqu** to switch to FID window.

10. Optional: After first increment finished, type **rser 1** to extract first row to a Temp window and type **efp** to process extract row. Make sure all intense peaks from ^1H NMR spectrum are visible. If you cannot see any peaks, increase number of scans (**ns**) or ask NMR Manager for some help in set up.

11. Before data processing, set the value of **1 si** (**si** in F1 dimension) to 2-4 times of number of increments (**1 td**).

12. Set-up linear prediction parameter by typing edp, setting **ME_MOD** to **Lpfc**, **NCOEF** to 3 times number of cross-peaks in the spectrum (usually 64-128), **LPBIN** to **2*td** in F1.

13. After acquisition is over, type **xfb** to process 2D data.

H2BC Correlation Experiment

Note: Make sure you see both positive and negative contours in the spectrum, otherwise click +/- button couple of times till you see both sets of contours. Click on DefPlot button, hit enter key on your keyboard without altering anything 4 times.

14. Click on phase button, click on **row** button, click MMB on middle of the top-right contour (you will see an intense peak, dispersed if improperly phased), click on **mov-1** button to extract row to the screen on right (marked **1**), then click on **row** button again, click MMB on the middle of the bottom-left contour, click on **mov-2** button to extract row to the screen on right (marked **2**). Click on **1** screen. Adjust **ph0** and **ph1** to properly phase peaks. Click on **return**, click on **save & return**. Click on **OK** tab when prompt with message "**start xf2p?**".

15. Select a known peak (e.g. solvent peak) for calibration. Click on **calibrate** tab, then click MMB on middle of contour (solvent peak), enter ^{1}H chemical shift of the peak in new dialog box, followed by ^{13}C chemical shift.

16. Click on **contours** tab to display 2D spectrum for analysis.

gHSQMBC

This version of 2D HSQMBC pulse sequence (pulse program: **ss_hsqcetgpjclrndbi**) provides CH correlation to measure two and three bond CH couplings ($^2J_{CH}$ and $^3J_{CH}$).

Step-by-step procedure:

1. Run the conventional proton NMR spectrum using the standard parameters.

   Note: Don't spin your sample for this experiment.

2. Make sure proton 90° pulse is set correctly or measure proton 90° pulse for your sample.

3. Reduce proton spectral window (**sw**) so that transmitter frequency is in the middle of the desired resonances. Click on **utilities**, click on **O1**, click MMB on the desired position of the ^{1}H NMR spectrum. Reduce value of **sw**, if necessary by typing **sw**, and entering new value in ppm inside pop-up window. Rerun proton NMR spectrum.

4. Note down the value of **sw** and **o1p** from **eda** window.

5. Increment the experiment number to create a new experiment with same file name.

6. Type **rpar up_ghsqmbc all** to load parameters for ghsqmbc experiment and follow on screen instructions.

7. Type **eda**, change the value of **sw** and **o1p** in the F2 column from step 4. Optimize **sw** in F1 column (spectral width for ^{13}C window) to keep it as small as possible to get best resolution.

8. Set number of scans (**ns** in F2 column) (should be multiple of 4) and number of increments (**td** in F1 column) (e.g. 128, 256, 512, 1024) based on ^{13}C spectral window, experimental time (can be measured by typing **expt**) and signal-to-noise requirement.

9. Type **rga** to adjust receiver gain.

10. Type **zg** to start data acquisition. Type **acqu** to switch to FID window.

11. Before data processing, set the value of **1 si** (**si** in F1 dimension) to 2-4 times of number of increments (**1 td**).

12. Set-up linear prediction parameter by typing **edp**, setting **ME_MOD** to **Lpfc**, **NCOEF** to 3 times number of cross-peaks in the spectrum (usually 64-128), **LPBIN** to **2*td** in F1.

13. Optional: After first increment finished, type **rser 1** to extract first row to a Temp window and type **efp** to process extract row. Make sure all intense peaks from ^{1}H NMR spectrum are

gHSQMBC

visible. If you cannot see any peaks, increase number of scans (**ns**) or ask NMR Manager for some help in set up.

14. After acquisition is over, type **xfb** to process 2D data.

> *Note: Make sure you see both positive and negative contours in the spectrum, otherwise-click +/- button couple of times till you see both sets of contours. Click on DefPlot button, hit enter key on your keyboard without altering anything 4 times.*

15. Click on phase button, click on **row** button, click MMB on middle of the top-right contour (you will see an intense peak, dispersed if improperly phased), click on **mov-1** button to extract row to the screen on right (marked **1**), then click on **row** button again, click MMB on the middle of the bottom-left contour, click on **mov-2** button to extract row to the screen on right (marked **2**). Click on **1** screen. Adjust **ph0** and **ph1** to properly phase peaks. Click on **return**, click on **save & return**. Click on **OK** tab when prompt with message "**start xf2p?**".

16. Select a known peak (e.g. solvent peak) for calibration. Click on **calibrate** tab, then click MMB on middle of contour (solvent peak), enter ^{1}H chemical shift of the peak in new dialog box, followed by ^{13}C chemical shift.

17. Click on **contours** tab to display 2D spectrum for analysis.

Selective COSY/TOCSY

<u>Step-by-step procedure for selective COSY (gradient version):</u>

1. Run a standard 1D proton spectrum and process data.

2. In the integrate mode, integrate just the peak/multiplet to selectively excite and save the integration.

3. Open Shape tool from windows menu.

4. In the shape tool window, click on the **shape menu** and select the desired shape type e.g. **Gauss** (Gauss 180 degree shape works well).

5. In shape parameter editor dialog box, enter **size of shape 1000** and **truncation level 5**.

6. After loading the basic shape, click on the **manipulate** menu and select -> **Calc. Shape from excitation region**.

7. The region you have selected in the integrate mode will be used to define the region to selectively excite with the selective pulse.

8. Make sure **Flip Angle** is set to **180**, **Alignment with respect to:** is set to **Center of Shape** and **Type of 180 degree pulse:** is set to **Refocusing**.

9. In the window that comes up, click on **OK** to execute the calculation with the already defined region.

10. Next, click on **Update Parameters** and close the window. A new **Save As** dialog box appears, with option to enter **Name of Shape:** provide a new name unique to your shape and click **OK.**

Note: The pulse shape have now been modified to excite the region you have selected (the file is saved under /HOME/exp/stan/nmr/lists/wave/..).

11. Close the shape tool window.

12. If all the above steps are correct, now you can see the updated parameters for shape length (**p11**) and shape power (**sp1**).

13. Setup the next experiment by keeping the same file name but different experiment number.

14. In acquisition window, change the pulse sequence name to **selcogp**.

Selective COSY/TOCSY

15. Type **ased** to edit necessary acquisition parameters directly related to the above pulse sequence. Among others, optimize **d4** ($\sim 1/4 J_{HH}$) and **d1** (recycle delay) depending on the nature of the sample being analyzed.

16. Type the value of **p12** (180 degree shaped pulse) as determined from step 11 (same as **p11**).

17. Similarly type the value of **sp2** as determined from step 11, **sp1**.

18. Type the name of the new shaped file created in step 9, for the parameter **spname2**.

19. Type **edcpul**, check the values of gradient level as well as gradient shape and update in the current dataset.

20. Type **rga** to adjust receiver gain.

21. Type **zg**, and acquire 8*n number of scans for desired signal-to-noise.

22. Process data with **efp** command and phase correct spectrum.

Note: Peaks will be all in dispersive mode. Don't try to phase them all positive.

Step-by-step procedure for selective TOCSY (gradient version):

Note: For selective TOCSY, proceed exactly as selective COSY up to step 13.

1. In acquisition window, change the pulse sequence name to **selmlgp**.

2. Type **ased** to edit necessary acquisition parameters directly related to the above pulse sequence. Among others, optimize **d9** (mixing time 40-100 ms) and **d1** (recycle delay) depending on the nature of the sample being analyzed.

3. Type the value of **p12** (180 degree shaped pulse) as determined from step 11 (same as **p11**).

4. Similarly type the value of **sp2** as determined from step 11, **sp1**.

5. Type the name of the new shaped file created in step 9, for the parameter **spname2**.

6. Type **edcpul**, check the values of gradient level as well as gradient shape and update in the current dataset.

Selective COSY/TOCSY

7. Get the value of **p6** and corresponding power level (**pl10**) from either regular TOCSY experiment or calculate from previous experiment number for **p6** (~36-38 μs) using **pulse** command.

8. Type **rga** to adjust receiver gain.

9. Type **zg**, and acquire 8*n number of scans for desired signal-to-noise.

10. Process data with **efp** command and phase correct spectrum.