1D and 2D Experiments Step-by-Step Tutorial

Advanced Experiments User Guide

Version 002

The information in this manual may be altered without notice.

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This manual was written by

Peter Ziegler

Billerica, Massachusetts, USA

P/N: B4722
DWG-Nr.: 002

Contents

Contents

Introduction 1

General 1.1

This manual was written for AVANCE systems running TopSpin and should be used as a guide through the set up process for some experiments. The successful completion of the experiments in this manual presumes that all parameters have been entered in to the prosol table.

Disclaimer 1.2

This guide should only be used for its intended purpose as described in this manual. Use of the manual for any purpose other than that for which it is intended is taken only at the users own risk and invalidates any and all manufacturer warranties.

Some parameter values, specially power levels suggested in this manual may not be suitable for all systems (e.g. Cryo probes) and could cause damage to the unit. Therefore only persons schooled in the operation of the AVANCE systems should operate the unit.

Warnings and Notes 1.3

There are two types of information notices used in this manual. These notices highlight important information or warn the user of a potentially dangerous situation. The following notices will have the same level of importance throughout this manual.

Note: Indicates important information or helpful hints

WARNING: Indicates the possibility of severe personal injury, loss of life or equipment damage if the instructions are not followed.

Contact for Additional Technical Assistance 1.4

For further technical assistance on the BPSU36-2 unit, please do not hesitate to contact your nearest BRUKER dealer or contact us directly at:

BRUKER BioSpin Corporation
19 Fortune Drive, Manning Park
Billerica, MA 01821
USA

Phone: (978) 667-9580
FAX: (978) 667-2955
Email: applab@bruker-biospin.com
Internet: www.bruker.com

2-D Inverse Experiments

2

2D edited HSQC 2.1

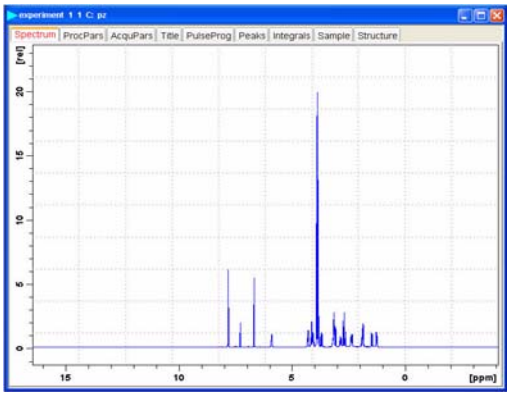
Sample:

20 mg Brucine in CDCl3

Preparation experiment 2.1.1

1. Run a 1D Proton spectrum, following the instructions in the Step-by-Step Tutorial, Basic Experiments User Guide, 1-D Proton Experiment, 2.2

Figure 2.1.



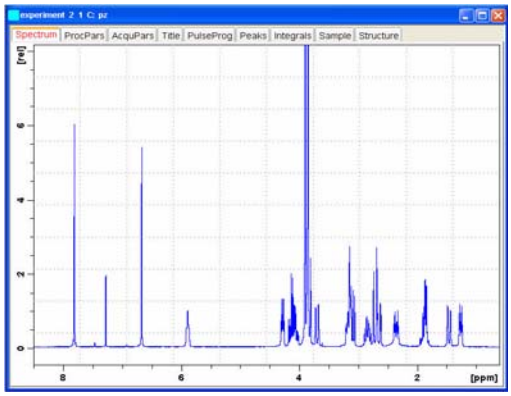
2. Type **wrpa 2** on the command line

3. Type **re 2**

4. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

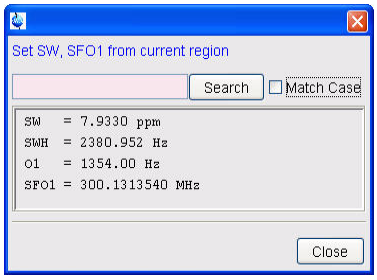
NOTE: You may exclude the solvent peak, if it falls outside of the region of interest.

Figure 2.2.



5. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 2.3.



6. Write down the value of SW, rounding off to the nearest 1/10th of a ppm
7. Write down the value of O1, rounding off to the nearest Hz
8. Click on Close
9. Type **sr** and write down the exact value

Setting up the HSQC experiment *2.1.2*

1. Type **rpar HSQCEDETGP all**
2. Turn the spinner off

NOTE: 2-D experiments should be run non spinning

3. Select the '**AcquPars**' tab by clicking on it
4. Make the following changes:

SW [F2] = value from step 6 (Preparation experiment 2.1.1)

O1 [Hz] = value from step 7 (Preparation experiment 2.1.1)

SOLVENT = **CDCl3**

All Bruker 2D inverse parameter sets use 13C in the F1 dimension. Sweep width and O1are optimized to include all Carbon peaks of interest. For HSQC and HMQC experiments the SW is optimized to 164 ppm.

5. Click on  to read in the Prosol parameters

6. Select the '**ProcPar**' tab by clicking on it

7. Make the following changes:

SR [F2] = value from step 9 (Preparation experiment 2.1.1)

8 Select the '**Title**' tab by clicking on it

9. Change the title to: **2-D edited HSQC experiment of Brucine**

10. Select the '**Spectrum**' tab by clicking on it

Acquisition *2.1.3*

1. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

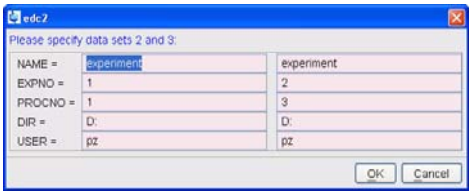
2. Click on  to start the acquisition

Processing *2.1.4*

The standard Bruker parameter sets are optimized to run under complete automation through the use of AU programs. The name of the AU program is entered in the acqusition (eda) and processing (edp) parameter lists, as AUNM. To start the acquisition, the command xaua may be used. For executing the processing AU program the command xaup may be used.

1. Type **edc2**

Figure 2.4.

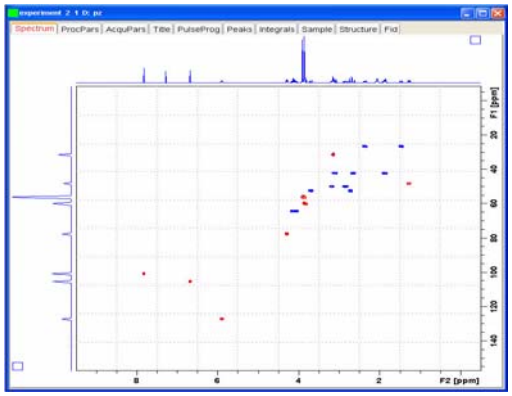


2. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

3. Click on OK

4. Type **xaup**

Figure 2.5.



The processing AU program includes the 2D Fourier transform, phase correction, baseline correction and plotting of the data. The HSQC experiment is phase sensitive and it shows positive (red) peaks representing the CH and CH3 correlation and negative peaks (blue) shows the CH2.

2D HMBC experiment *2.2*

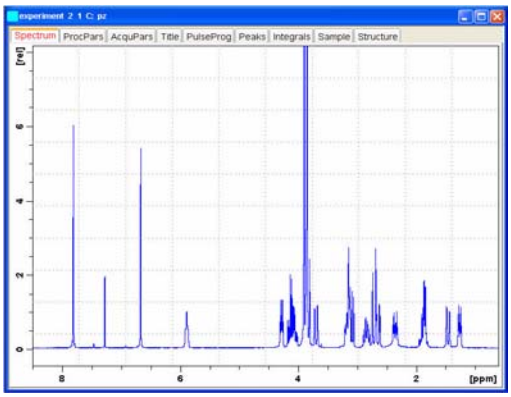
Sample:

20 mg Brucine in CDCl3

Preparation experiment *2.2.1*

1. Follow the instructions in the previous HSQC experiment 2.1.1 Preparation experiment step 1 through 9

Figure 2.6.



Setting up the HMBC experiment *2.2.2*

1. Type **rpar HMBCLPND all**
2. Turn the spinner off

NOTE: 2-D experiments should be run non spinning

3. Select the '**AcquPars**' tab by clicking on it
4. Make the following changes:

SW [F2] = value from step 6 (Preparation experiment 2.1.1)

O1 [Hz] = value from step 7 (Preparation experiment 2.1.1)

SOLVENT = **CDCl3**

All Bruker 2D inverse parameter sets use 13C in the F1 dimension and the sweep width and O1are optimized to include all Carbon peaks of interest. For HMBC experiments the SW is optimized to 220 ppm.

5. Click on  to read in the Prosol parameters
6. Select the '**ProcPar**' tab by clicking on it
7. Make the following changes:

SR [F2] = value from step 9 (Preparation experiment 2.1.1)

8 Select the '**Title**' tab by clicking on it

9. Change the title to: **2-D HMBC experiment of Brucine**

10. Select the '**Spectrum**' tab by clicking on it

Acquisition *2.2.3*

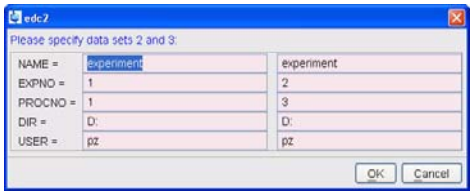
1. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
2. Click on  to start the acquisition

Processing *2.2.4*

The standard Bruker parameter sets are optimized to run under complete automation trough the use of AU programs. The name of the AU program is entered in the acqusition (eda) and processing (edp) parameter lists, as AUNM. To start the acquisition, the command xaua may be used. For executing the processing AU program the command xaup may be used.

1. Type **edc2**

Figure 2.7.

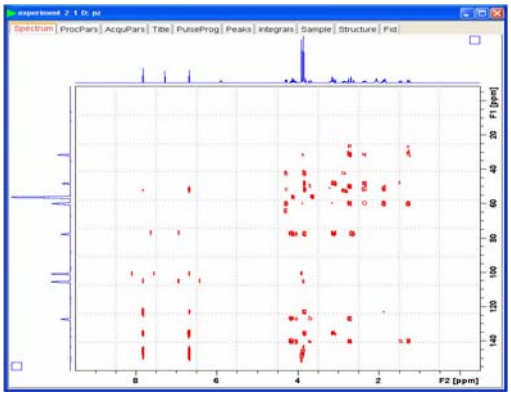


2. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

3. Click on OK

4. Type **xaup**

Figure 2.8.



The processing Au program includes the 2D Fourier transform, baseline correction and plotting of the data. The HMBC experiment uses magnitude mode for processing and shows only positive peaks.

Adding the F1 projection to the HSQC contour plot *2.3*

All Bruker 2D inverse parameter sets use the nucleus 13C in the F1 dimension. The sweep width and O1 frequency are set to include all Carbon peaks of interest. In most cases it is not necessary to run a Carbon spectrum to optimize the parameters. In the default plot template the F1 projection is disabled and therefor is not plotted.
If one wishes to add the F1 projection to the plot, an additional 13C spectrum has

to be obtained and a new plot template has to be created. For HMQC, HSQC type of experiments a DEPT45 or DEPT135 is best suited and for HMBC experiments a normal proton decoupled carbon spectrum should to be used.

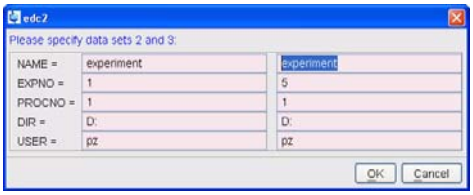
Creating the external projection spectrum *2.3.1*

1. Run a DEPT135 experiment following the instructions in the Step-by-Step Tutorial, Basic Experiments User Guide, DEPT135 Experiment 2.4.

2. Open the HSQC experiment

3. Type **edc2**

Figure 2.9.



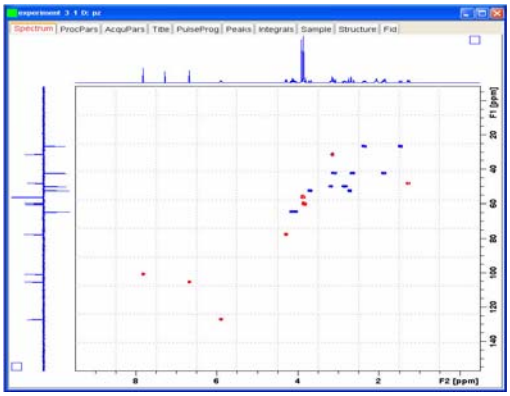
4. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

5. Enter the EXPNO and PROCNO of the DEPT135 spectrum into the second column (data set 3)

6. Click on OK

7. Type **xaup**

Figure 2.10.

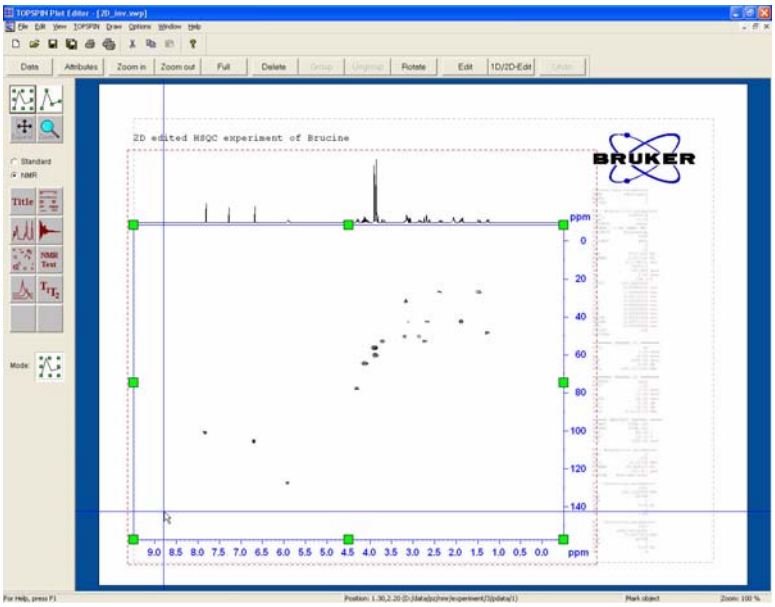


NOTE: Discard the plot

Creating the plot template *2.3.2*

1. Type **viewxwinplot**

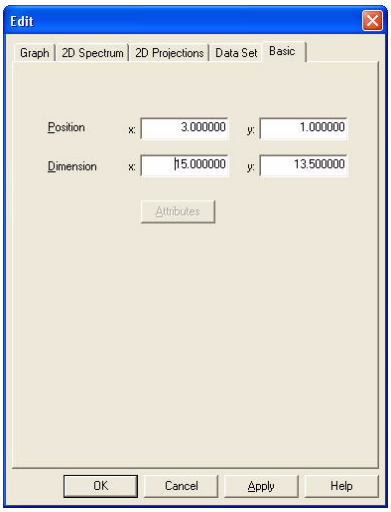
Figure 2.11.



2. Click inside the spectrum window to display the green handles
3. Click on Edit

4. Select the '**Basic**' tab

Figure 2.12.



4. Make the following changes:

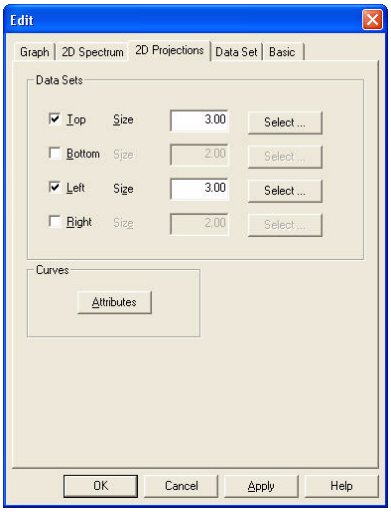
Position X = **3**

Dimension X = **15**

6. Click on Apply

7. Select the '**2D Projection**' tab

Figure 2.13.



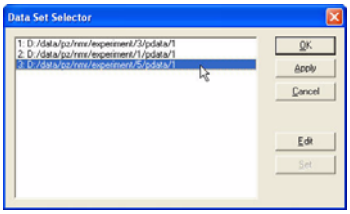
8. Enable '**Data set left**'

9. Make the following change:

Size = **3**

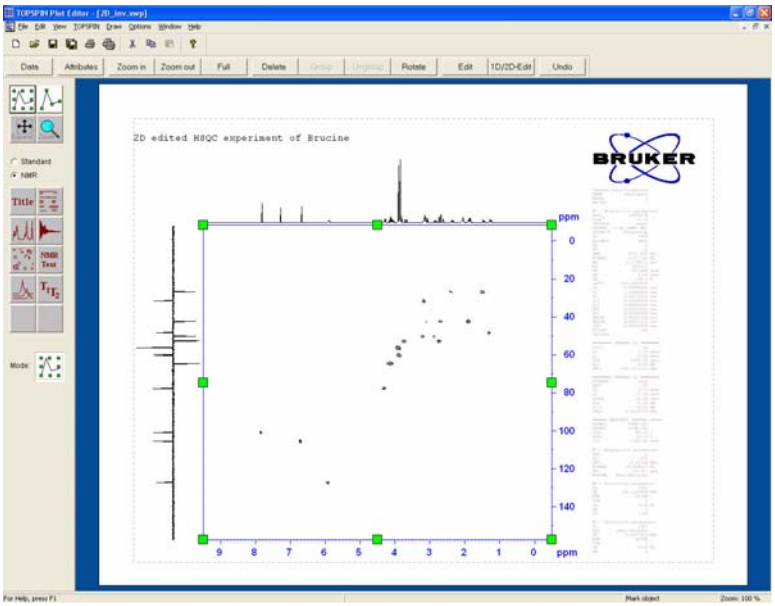
10. Click on Select ...

Figure 2.14.



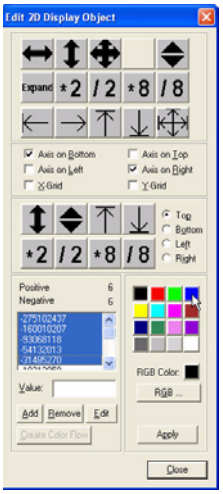
11. Select the DEPT data set path
12. Click on OK
13. Click on Apply
14. Click on OK

Figure 2.15.



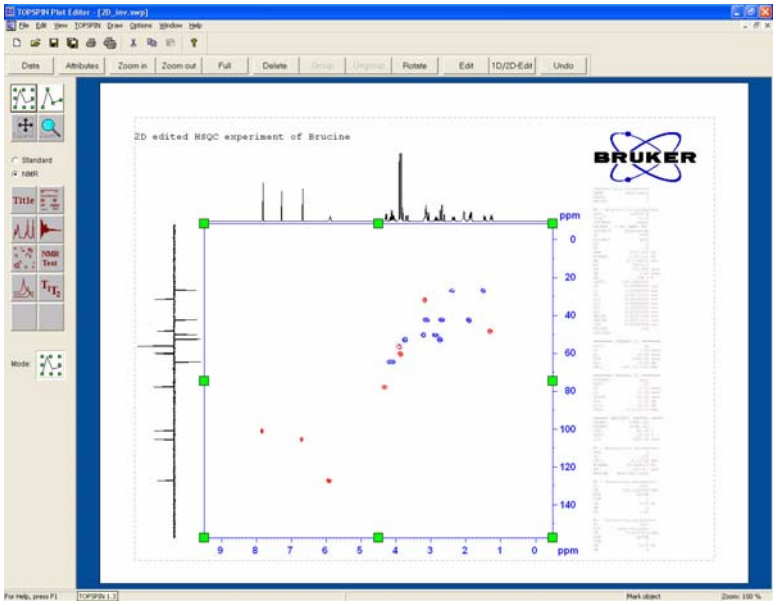
15. Click on 1D/2D-Edit

Figure 2.16.



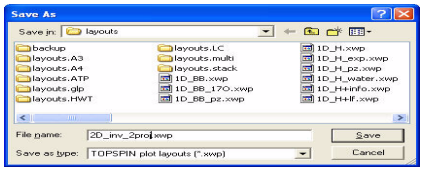
16. Select all negative contour levels
17. Click in the blue color button
18. Click on Apply
19. Select all positive levels
20, Click on the red color button
21. Adjust the contour level using the *2 /2 or ▲▼ buttons
22. Click on Close

Figure 2.17.



23. Click on '**File**' in the main menu bar and select '**Save as**'

Figure 2.18.



NOTE: Make sure to be in the directory [TopSpin home]\plot\layouts

24. Change Filename to **2D_inv_2proj.xwp**
25. Click on Save

Diffusion Experiment

3

Introduction 3.1

NOTE: To run this experiment the instrument has to be equipped with the hardware to run Gradient experiments. Pulse field gradient NMR spectroscopy can be used to measure translational diffusion of molecules. The example in this chapter uses a mixture of two sugars dissolved in D2O.

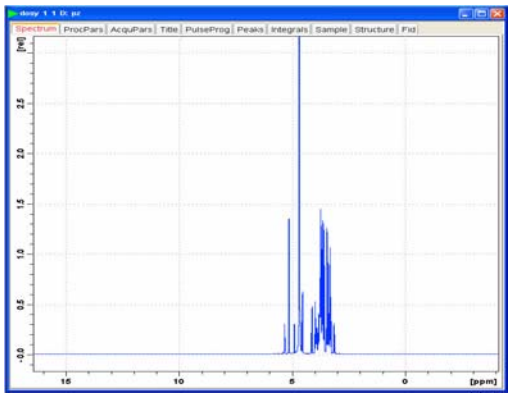
Sample:

Mixture of Glucose and Raffinose each 20mg in D2O

Preparation experiment 3.1.1

1. Run a 1D Proton spectrum, following the instructions in the Step-by-Step Tutorial, Basic Experiments User Guide, 1-D Proton Experiment, 2.2

Figure 3.1.



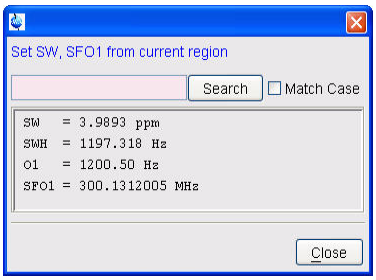
2. Type **wrpa 2** on the command line

3. Type **re 2**

4. Expand the spectrum from 6ppm to -2ppm

5. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 3.2.



6. Click on Close

7. Type **td 16k**

8. Type **si 8k**

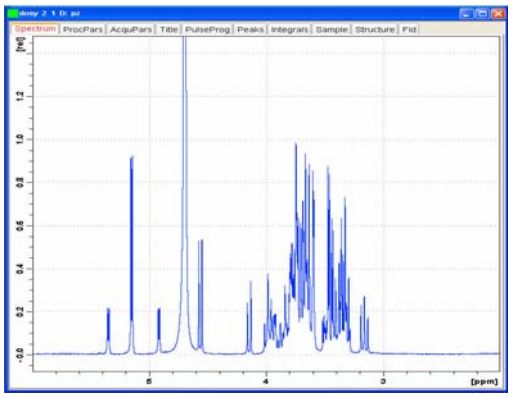
9. Click on  to start the acquisition

10. Type **ef**

11. Type **apk**

12. Type **abs**

Figure 3.3.



Parameter set up *3.1.2*

1. Type **iexpno**

2. Select the '**AcquPars**' tab by clicking on it

3. Click on  to display the pulse program parameters

4. make the following changes:

PULPROG	=	**stebpgp1s1d**
GPZ6[%]	=	**2**
GPZ7[%]	=	**-17.13**
D20[s]	=	**0.1**
P30[us]	=	**1800**

5.In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

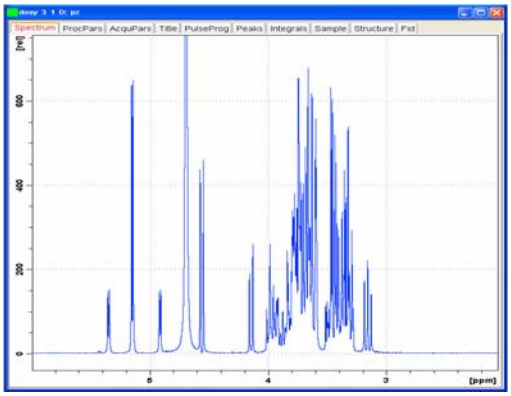
6. Click on  to start the acquisition

7. Type **ef**

8. Type **apk**

9. Type **abs**

Figure 3.4.



10. Type **iexpno**

11. Select the '**AcquPars**' tab by clicking on it

12. Click on  to display the pulse program parameters

13. make the following changes:

GPZ6[%] = **95**

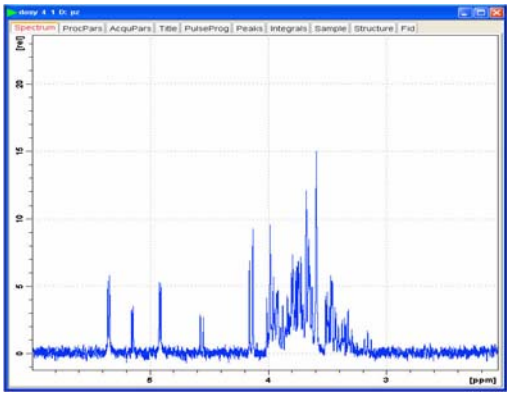
14. Click on  to start the acquisition

15. Type **ef**

16. Type **apk**

17. Type **abs**

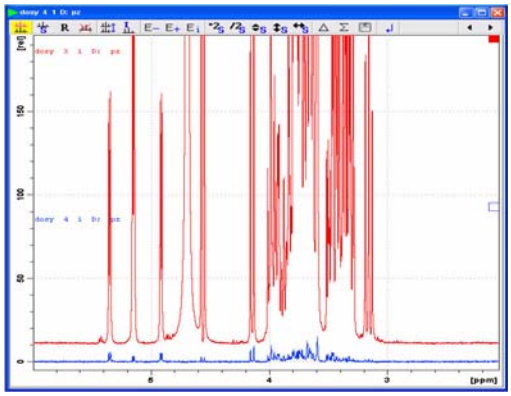
Figure 3.5.



18. Click on  to open the multiple display window

19. Drag the previous experiment into the multiple display window (in this example it is experiment # 3) or type **re 3**

Figure 3.6.



NOTE: The intensity difference of the two spectra should be a factor of ~50. If the difference is less then 50, change P30 and or D20 in both data sets.

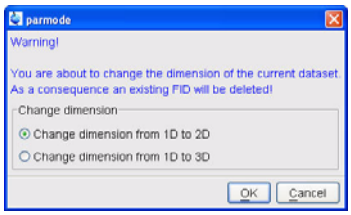
Acquisition *3.1.3*

1. Type **iexpno**
2. Select the '**AcquPars**' tab by clicking on it
3. Make the following changes:

PULPROG = **stebpgp1s**

4. Click on  to change the acqu dimension

Figure 3.7.



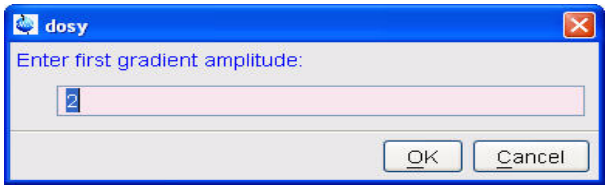
5. Enable '**Change dimension from 1D to 2D**'
6. Click on 
7. Make the following changes:

TDF1 = **16**

FnMODE = **QF**

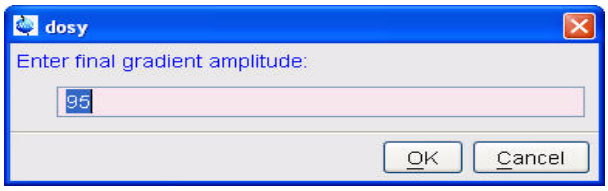
8. Type **dosy** on the command line

Figure 3.8.



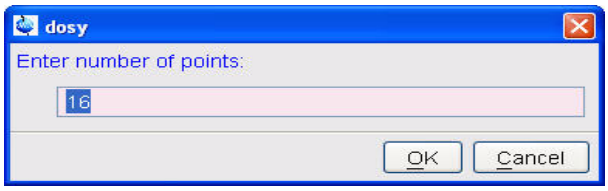
9. Enter **2** for first gradient amplitude
10. Click on OK

Figure 3.9.



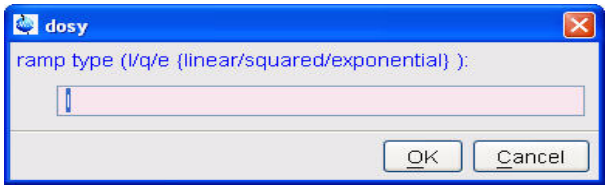
11. Enter **95** for final gradient amplitude
12. Click on OK

Figure 3.10.



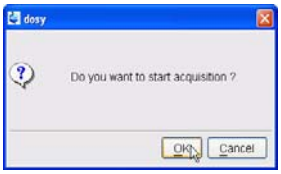
13. Enter **16** for the number of points
14. Click on OK

Figure 3.11.



15. Enter **l** for ramp type
16. Click on OK

Figure 3.12.

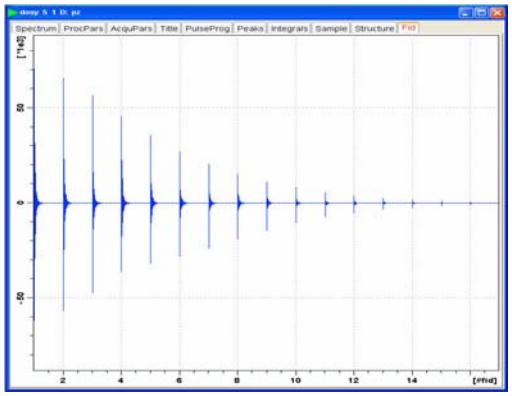


17. Click on OK to start the acquisition

Processing *3.1.4*

1. Select the '**Fid**' tab by clicking on it

Figure 3.13.



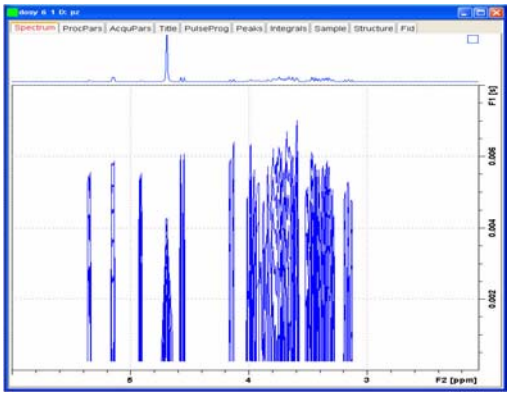
NOTE: Step 1 is only used to illustrate the DOSY experiment as a decay function.

2. Select the '**ProcPars**' tab by clicking on it
3. Click on P to display the processing parameters
4. Make the following changes:

SI [F1]	=	**16**
PH_mod [F1]	=	**no**
PH_mod [F2]	=	**pk**

5. Type **xf2** on the command line
6. Type **abs2** on the command line
7.Type **setdiffparm** on the command line
8. Select the '**Spectrum**' tab by clicking on it

Figure 3.14.

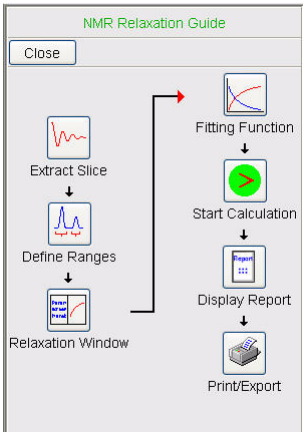


Calculating the diffusion coefficient *3.1.5*

NOTE: As you follow the steps below, message windows with important instructions will pop up. Please read this instructions very carefully.

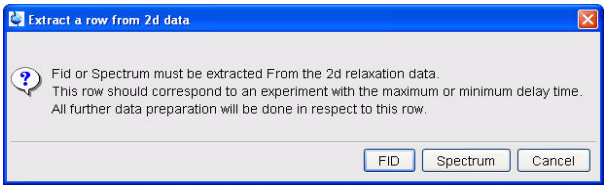
1. Click on '**Analysis**' in the main menu
2. Select '**T1/T2 Relaxation**'

Figure 3.15.



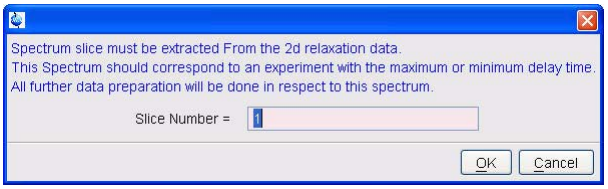
3. Click on  to extract slice

Figure 3.16.



3. Click on Spectrum

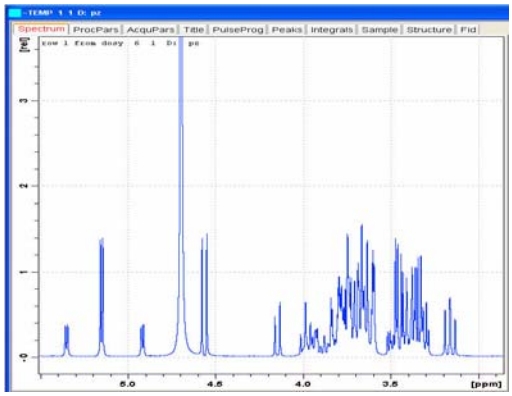
Figure 3.17.



5. Enter **1** for the slice number

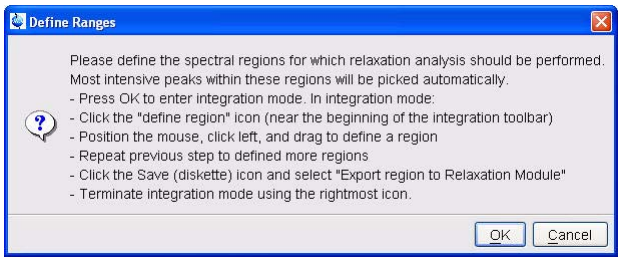
6. Click on OK

Figure 3.18.



7. Click on  to define ranges

Figure 3.19.



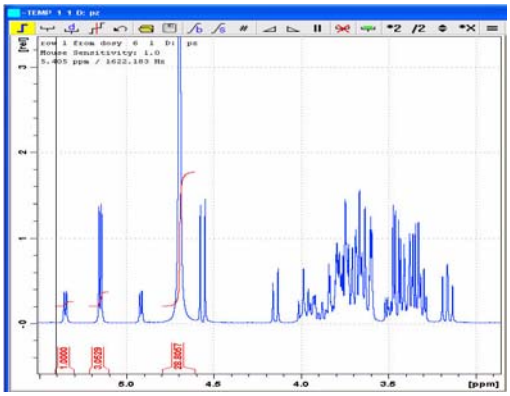
8. Click on OK

9. Click on  to define the regions

10. Define the regions by clicking the left mouse button and the use of the cursor lines

11. Click on  again

Figure 3.20.

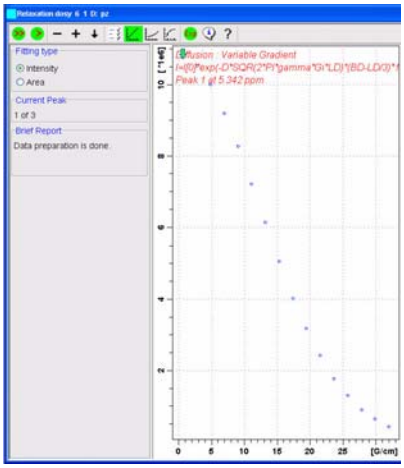


12. Click on 

Figure 3.21.

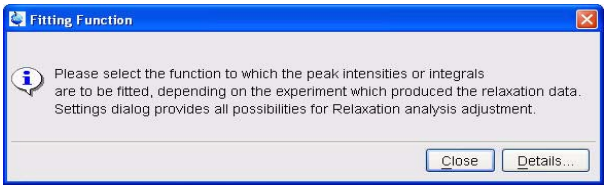
13. Select '**Export Region To Relaxation Module**' by clicking on it
14. Click on 
15. In the Guide window, click on  'Relaxation Window'

Figure 3.22.



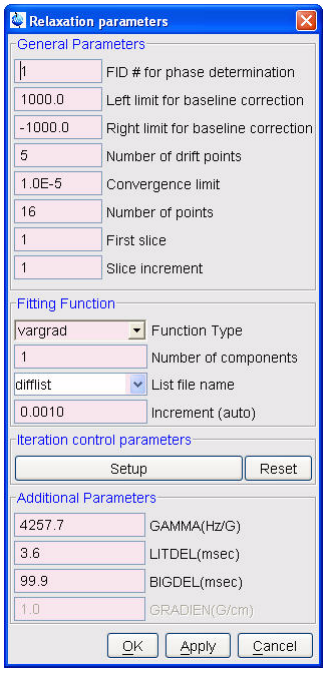
16. Enable '**Intensity**'
17. In the guide window, click on  'Fitting Function'

Figure 3.23. 1



18. Click on Close

Figure 3.24.

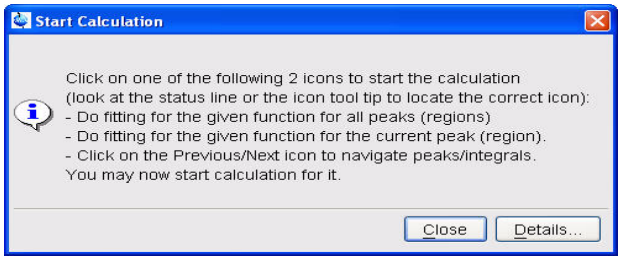


19. In the '**Fitting Function**' section, select '**vargrad**' and '**difflist**'

20. Click on OK

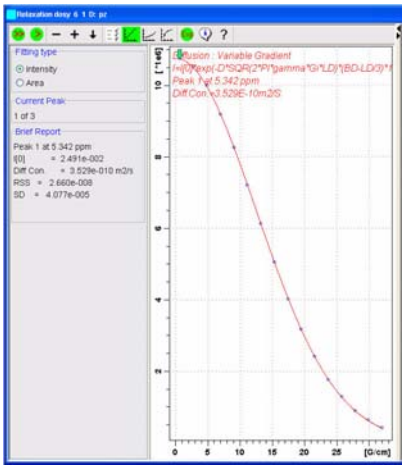
21 In the guide window, click on  'Start Calculation'

Figure 3.25.



22. Click on Close

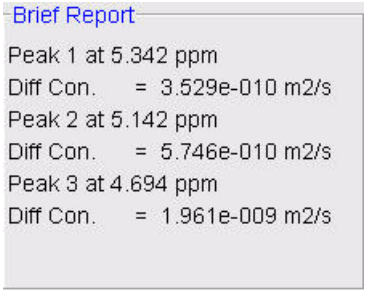
Figure 3.26.



23. In the data window, click on  ‘Calculate fitting parameters for all data points’

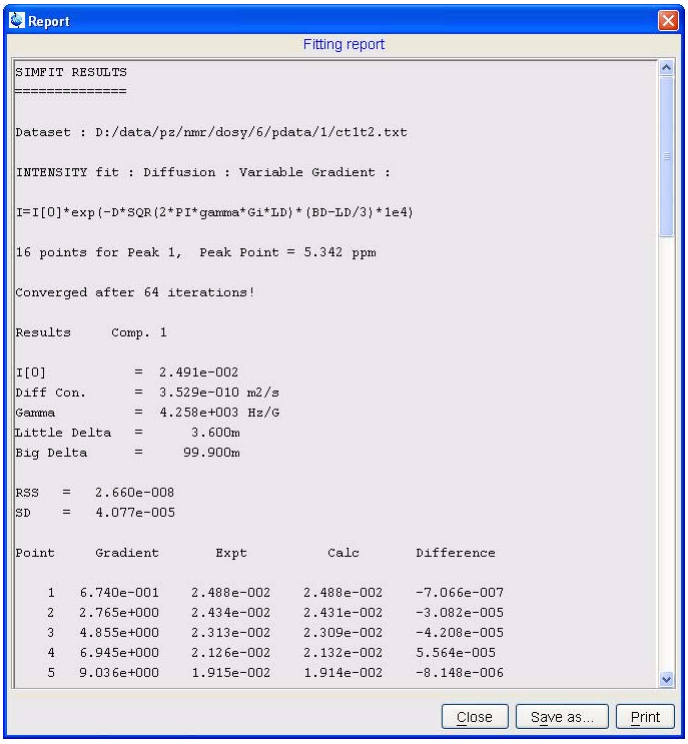
NOTE: All calculated values are displayed in the ‘Brief Report’ section of the data window.

Figure 3.27.



24. In the guide window. click on  ‘Display Report’

Figure 3.28.



25. In the guide window, click on 

‘Print Report

Multiplet Analysis 4

Multiplet assignments 4.1

Sample:

100 mg 2, 3,-Dibromopropionic acid in CDCl3

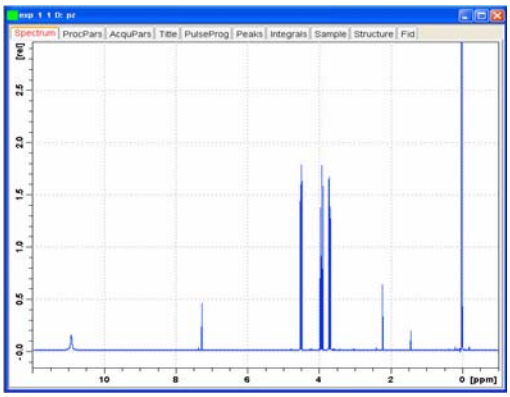
Preparation experiment 4.1.1

1. Run a 1D Proton spectrum, following the instructions in the Step-by-Step Tutorial, Basic Experiments User Guide, 1-D Proton Experiment, 2.2

2. Type **iexpno** on the command line

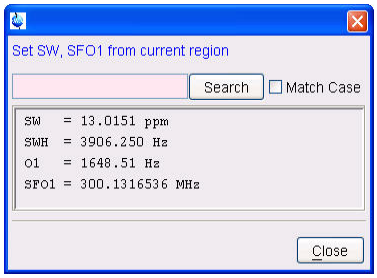
3. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

Figure 4.1.



4. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 4.2.



5. Click on 

6. Select the '**ProcPar**' tab by clicking on it

7. Make the following changes:

LB = **0**

Select the '**Title**' tab by clicking on it

8. Change the title to: **1D Proton spectrum of 2, 3-Dibromopropionic acid**

9. Select the '**Spectrum**' tab by clicking on it

Acquisition *4.1.2*

1. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

2. Click on  to start the acquisition

Processing *4.1.3*

1. Type **ft**

2. Type **apk**

NOTE: It may be necessary do a additional manual phase correction for a perfect phased spectrum.

3. Type **abs**

NOTE: If an internal reference such as TMS is added to the sample, a manual calibration should be done to the spectrum to assume a correct chemical shift of the peaks. This may not be important for the multiplicity analysis, but for any spin simulation programs you may be using.

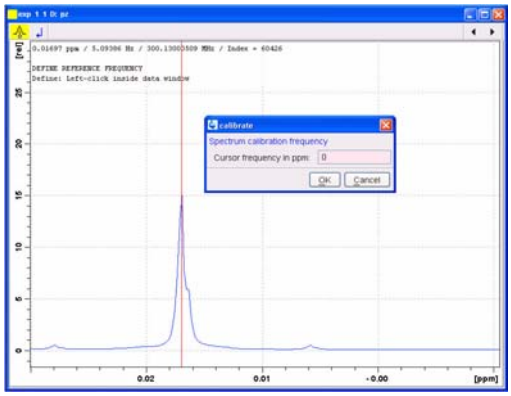
4. Expand the TMS peak

5. Click on  'Spectrum Calibration'

6. Move the cursor line into the center of the TMS peak

7. Click the left mouse button

Figure 4.3.



8. Change the value of the cursor frequency in ppm = **0**

9. Click 

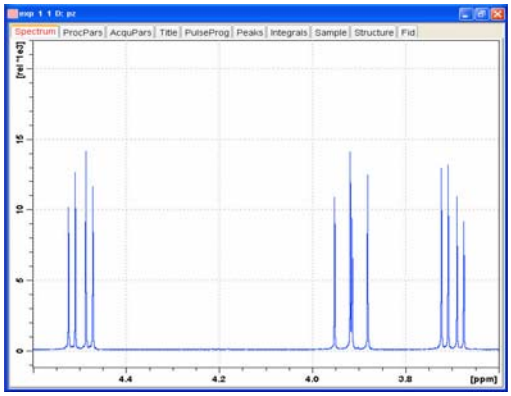
10. Expand the spectrum from 3.6 ppm to 4.6 ppm

11. Click with the right mouse button inside the spectrum window

12. Select '**Save Display Region To**'

13. Enable the option '**Parameters F1/2 [dp1]**'

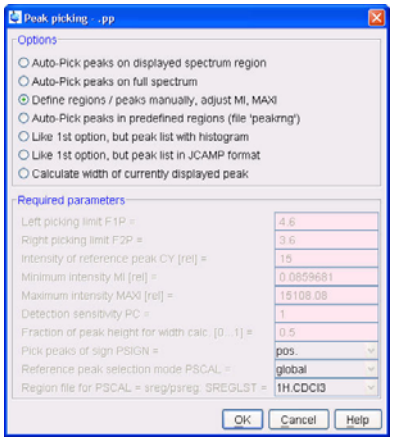
Figure 4.4.



14. Click on '**Analysis**' in the main menu bar

15. Select '**Peak Picking [pp]**' by clicking on it

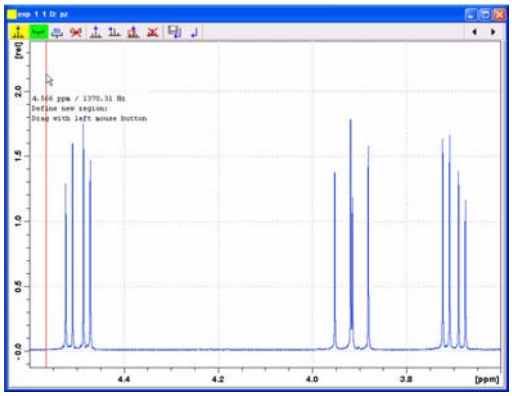
Figure 4.5.



16. Enable '**Define regions/peaks manually, adjust MI, MAXI**'

17. Click on 

Figure 4.6.



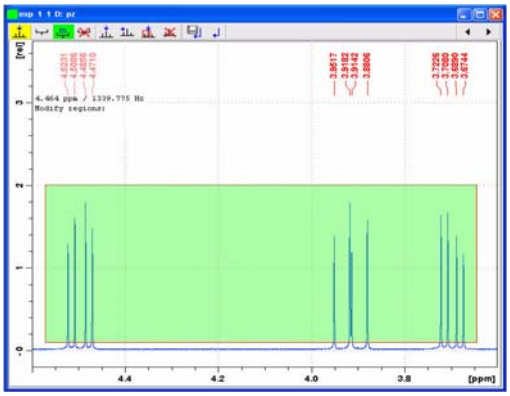
18. Move the cursor line to the left of the multiplet at 4.5 ppm

19. Click and hold the left mouse button and drag the cursor across the spectrum to the right of the multiplet at 3.7 ppm to draw a box over all multiplets

20. Click on  'Modify existing peak picking range'

21. Adjust the bottom line of the box to be above the baseline (Minimum intensity) and the top line above the highest peak of all multiplets (Maximum intensity)

Figure 4.7.



22. Click on 
23. Click on 

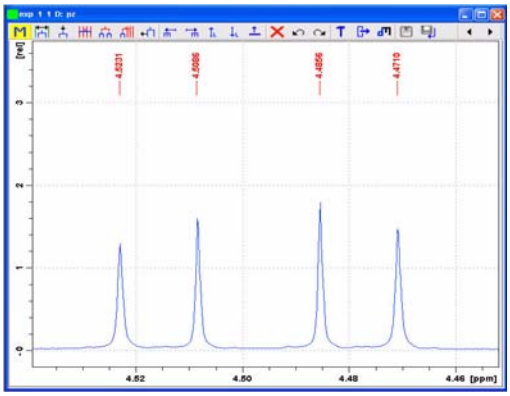
Multiplet assignments *4.1.4*

1. Expand the multiplet at 4.5 ppm
2. Click on '**Analysis**' in the main menu bar

Select '**Structure Analysis**'

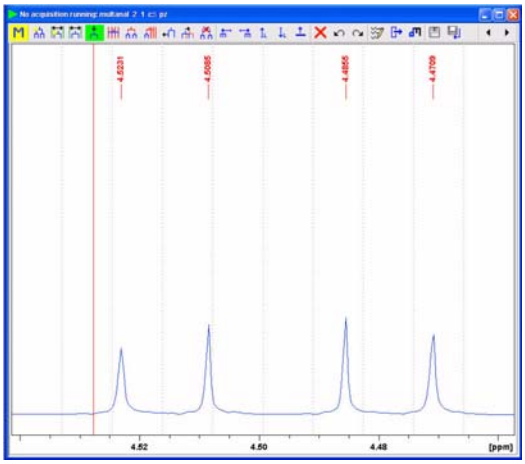
4. Select '**Multiplet Definition [mana]**' by clicking on it

Figure 4.8.



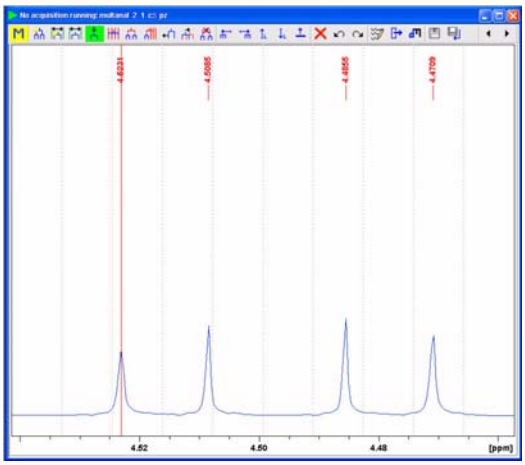
5. Click on  'Define Multiplets Manually'
6. Place the cursor line to the left of the first peak of the multiplet

Figure 4.9.



7. Move the cursor line slowly towards the first peak
8. The cursor line will stop when it gets in to the center of the peak
9. Click the left mouse button

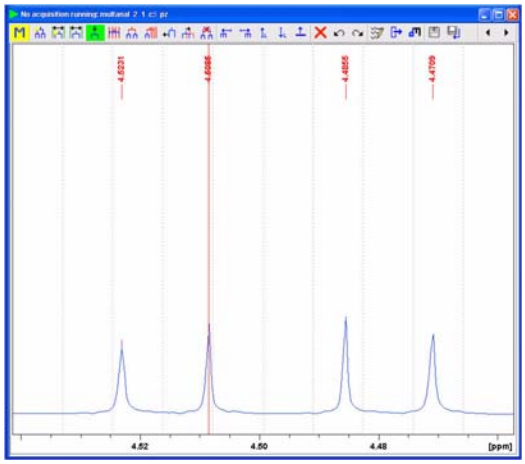
Figure 4.10.



10. Move the cursor line slowly towards the second peak
11. The cursor line will stop when it gets in to the center of the peak
12. Click the left mouse button

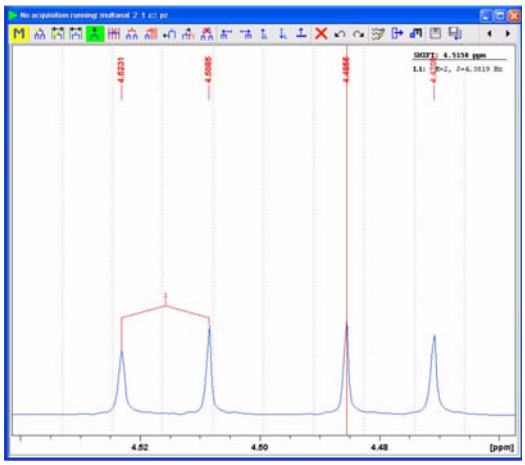
NOTE: A small marker is placed above the top of the first peak

Figure 4.11.



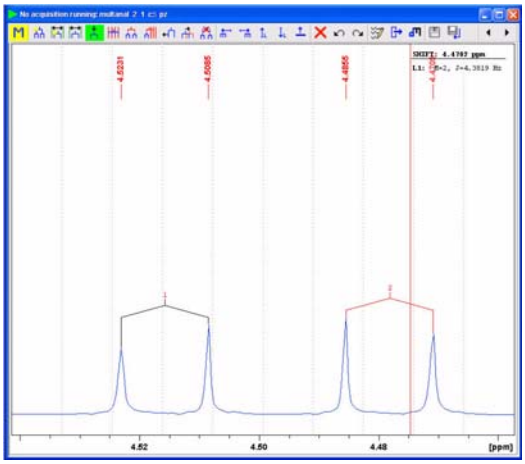
13. Move the cursor line in to the center of the two marked peaks
14. Click the right mouse button
15. Select '**Define Multiplet**' by clicking on it

Figure 4.12.



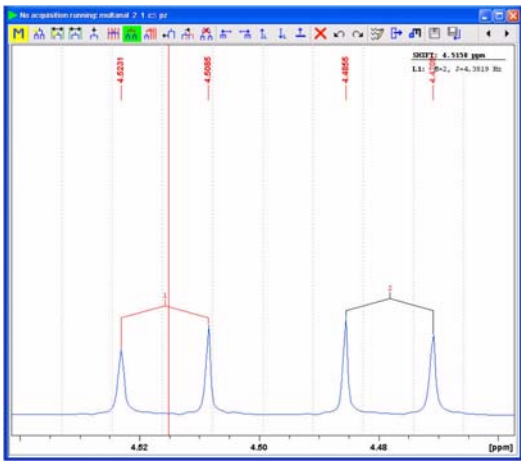
16. Repeat steps 6 through 15 starting with the third peak and ending with the fourth peak

Figure 4.13.



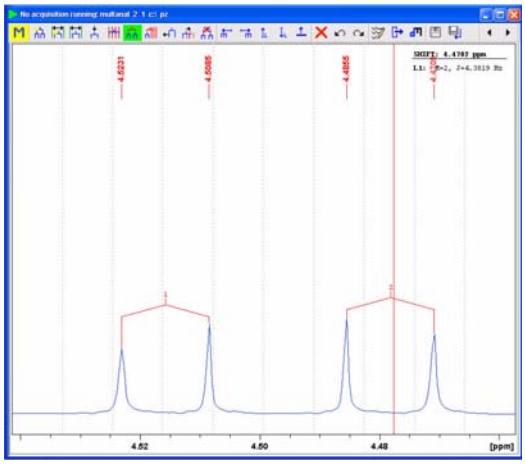
17. Click on  ‘Couple Existing Multiplets’
18. Move the cursor line in to the center of the first two peaks marked 1
19. Click the left mouse button

Figure 4.14.



20. Move the cursor line in to the center of the second two lines marked 2
21. Click the left mouse button

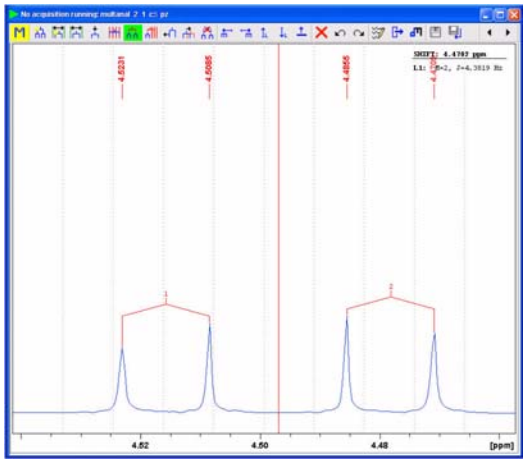
Figure 4.15.



NOTE: While executing steps 20 trough 21, the color of the brackets over the peaks 1 and 2 turn from black to red.

22. Move the cursor into the center of the displayed multiplet

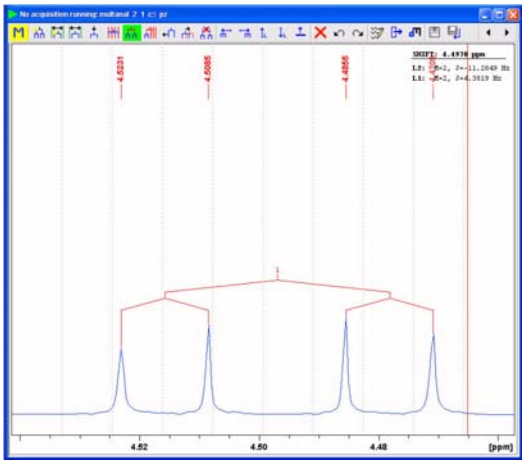
Figure 4.16.



23. Click the right mouse button

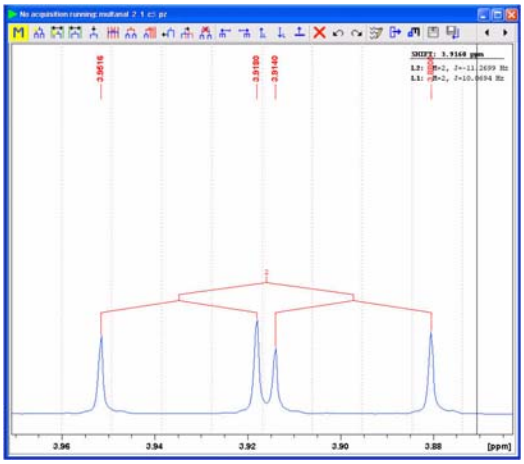
24. Select '**Define Multiplet**' by clicking on it

Figure 4.17.



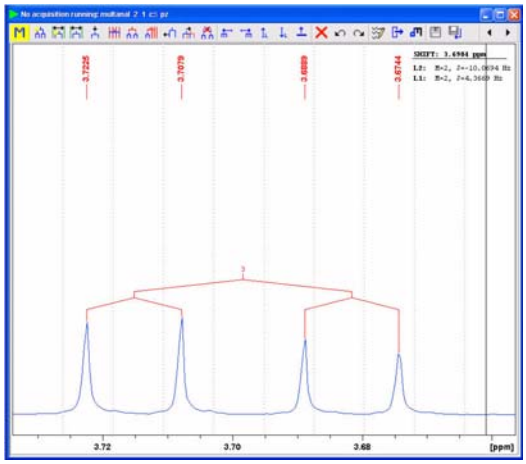
25. Click the right mouse button inside the spectrum window
26. Select '**Finish Current Mode**' by clicking on it
27. expand the multiplet at 3.9 ppm
28. Repeat steps 6 through 26 for this multiplet

Figure 4.18.



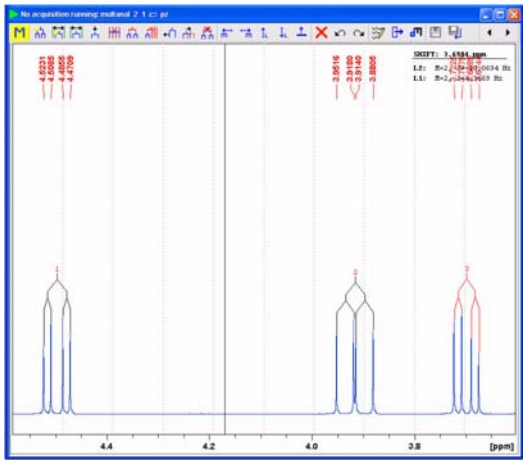
29. Expand the multiplet at 3,7 ppm
30. Repeat steps 6 through 26 for this multiplet

Figure 4.19.



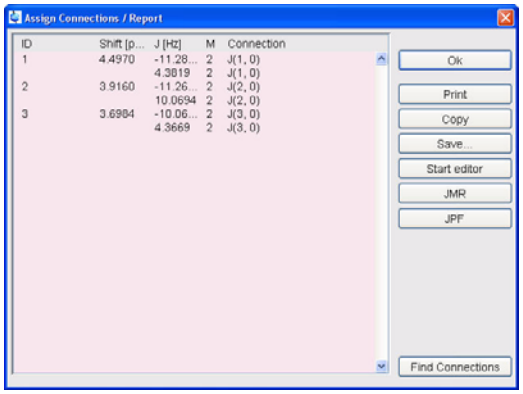
31. Display all 3 multiplets

Figure 4.20.



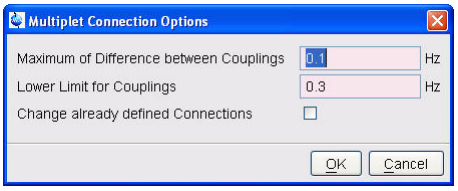
32. Click on  'Show Multiplet Report'

Figure 4.21.



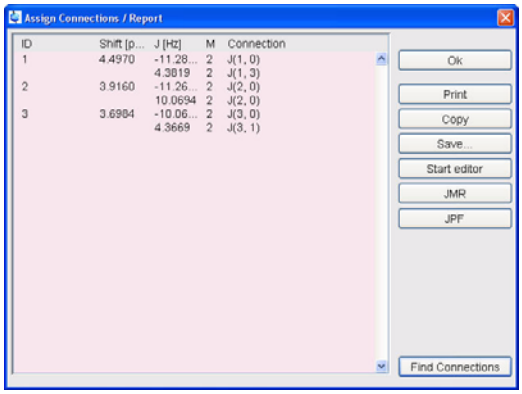
33, Click on 

Figure 4.22.



34. Click on OK

Figure 4.23.



NOTE: The connections are now assigned and the report can be printed.

35. Click on Ok

36. Click on  'Return, save multiplets [sret]'

19F Experiments 5

Hardware necessary to observe 19F 5.1

NOTE: Below is a list of hardware options to observe or decoupled Fluorine on various Bruker systems and probes.

Probes 5.1.1

-QNP	19F/31P/13C/1H
-TXO	13C/1H/19F
-BBFO	BB/19F/1H (300 and 400MHz systems only)
-BBO	BB/1H (1H coil may be tunable to 19F)
-BBI1H/BB	(1H coil may be tunable to 19F)
-DUAL	1H/19F

NOTE: The probes listed above will have a Fluorine background with the exception of the Dual probe which is made Fluorine free. The BBO and BBI probes can only observe 19F without 1H decoupling. On the other hand, observing 13C and decoupling 19F is possible.

Additional hardware 5.1.2

300 and 400MHz systems

-Internal amplifier BLA-2BB

-19F pass filter for doing observe 13C and 19F decoupling experiments.

-Other filters are built in to the preamplifiers (HPPR/2)

NOTE: By default amplifier 1 is connected to the X-BB preamplifier and amplifier 2 is connected to the 1H preamplifier. Each amplifier delivers 150 Watts from 10Mhz to the 31P frequency and 60 Watts above 31P to the 1H frequency and this will include the 19F frequency.
Standard pulse programs such as zg. zgdc etc. can be used to observe 19F.

500MHz and above

-external amplifiers BLAXH (less then 1.5 years old)

BLA(R)H, BLAX combinations

-external QNP accessory unit for RF routing

-19F pass filter for doing observe 13C and 19F decoupling experiments.

-Other filters are built in to the preamplifiers (HPPR/2)

NOTE: The 19F signal is generated on the 1H stage of the amplifier and the QNP accessory unit is designed to route the 19F frequency either to the 19F selective or X-QNP output. In addition it switches between the 1H and 19F for decoupling either of the nuclei.
Pulse programs have to include the routing and switching statements such as QNP_X, QNP_F, SWITO_F, SWITO_H.

Older AV systems

-external amplifier BLAXH (more then 1.5 years old)

-The QNP switch unit is built in to the amplifier and the functions are the same as the above QNP accessory unit.

-19F pass filter for doing observe 13C and 19F decoupling experiments.

-Additional filters such as ‘Band Pass X, 19F//Band Stop 1H’ and ‘Band Pass 1H// Band Stop 19F’ are necessary if a HPPR/1 is in use.

NOTE: The 19F signal is generated on the 1H stage of the amplifier and the QNP accessory unit is designed to route the 19F frequency either to the 19F selective or X-QNP output. In addition it switches between the 1H and 19F for decoupling either of the nuclei.
Pulse programs have to include the routing and switching statements such as QNP_X, QNP_F, SWITO_F, SWITO_H.

1-D 19F experiments *5.2*

The 19F chemical shift range is rather large and covers approximately from +100ppm to -300ppm. The default sweep width of the Bruker standard 19F parameter sets may not cover the whole chemical shift range and adjustment may be needed. A common reference standard is: $CFCl_3$ at 0ppm. Others standards such as CF_3COOH and C_6F_6 may also be used.

Sample:

2,2,3,4,4,4-Hexafluoro-1-butanol in Acetone-d6

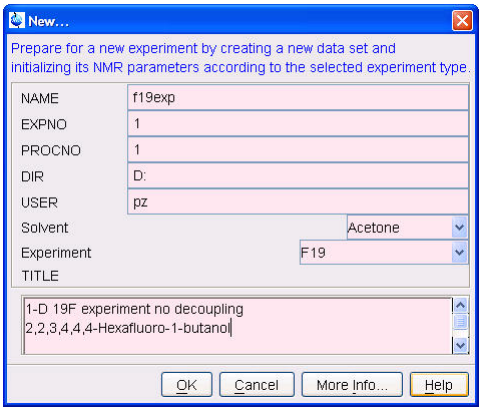
CF_3-CFH-CF_2-CH_2-OH

19F observe, no decoupling *5.2.1*

Exploratory spectrum

1. Click on  and change the following parameters

Figure 5.1.



2. Click on 

3. Insert the sample

4. Click on  to display the Lock display

5. In the lock display window click on  and select Acetone

6. Tune the probe to observe 19F

7. Shim for best homogeneity

8. In the lock display window click on  to close the window

9. Select the '**AcquPars**' tab by clicking on it

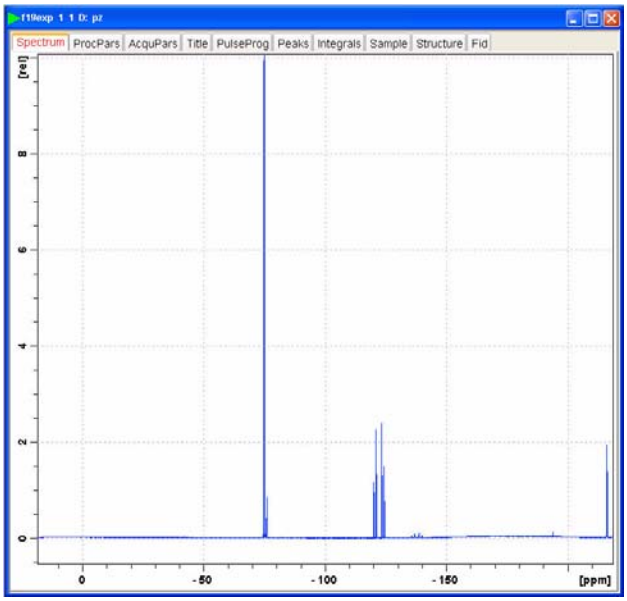
10. Click on  to read in the Prosol parameters

11. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

12. Click on  to start the acquisition

13. Process and Phase correct the spectrum

Figure 5.2.



Optimizing the sweep width

In this example, the right most peak at ca. 220ppm is to close to the edge and may be distorted by the digital filtering. In this case, the SW and O1P should to be adjusted.

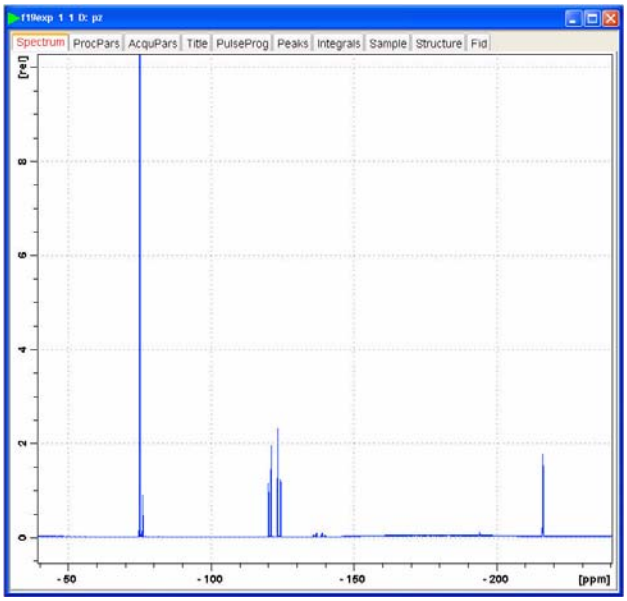
1. Select the '**AcquPars**' tab by clicking on it
2. Change the following parameters:

SW [PPM] = **200**

O1P [PPM] = **-140**

3. Click on  to start the acquisition
4. Process and Phase correct the spectrum

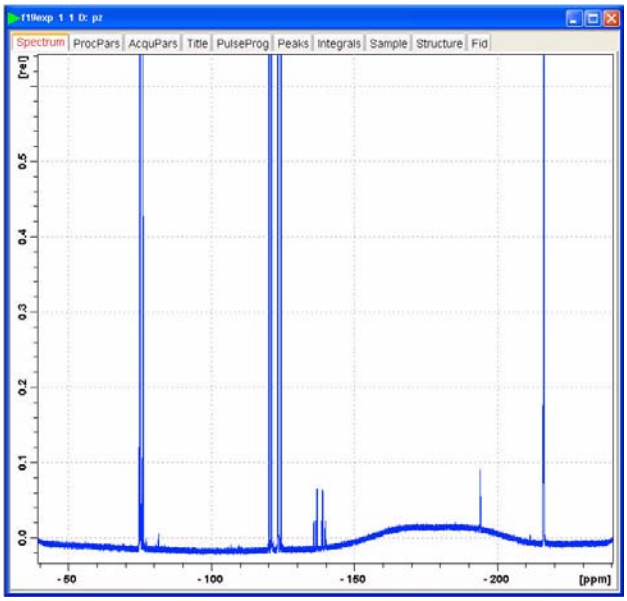
Figure 5.3.



Baseline correction

1. Display the full spectrum
2. Expand the spectrum vertically

Figure 5.4.



If a Fluorine background signal is present, a simple abs will not straighten the baseline and a linear prediction calculation may be necessary. See steps below.

3. Type **convdta**

Figure 5.5.

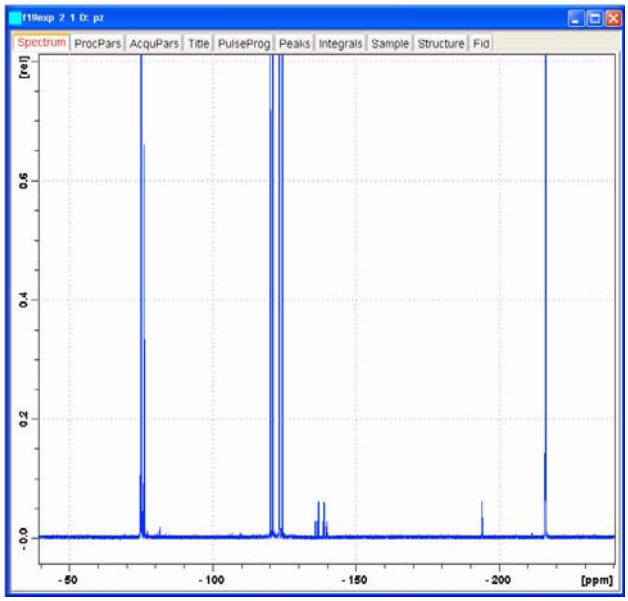
4. Type **2** into the convdta window
5. Click on OK
6. Select the '**Procpar**' tab by clicking on it
7. Change the following parameters:

ME_mod	=	**LPbc**
NCOEF	=	**32**
TDoff	=	**16**

8. Type **ef**
9. Phase correct the spectrum

10. Type **abs**

Figure 5.6.



19F observe, with 1H decoupling *5.2.2*

1. Type **iexpno**
2. Type rpar **F19CPD all**
3. Tune the probe for 19F and 1H
4. Select the '**AcquPars**' tab by clicking on it
5. Change the following parameters:

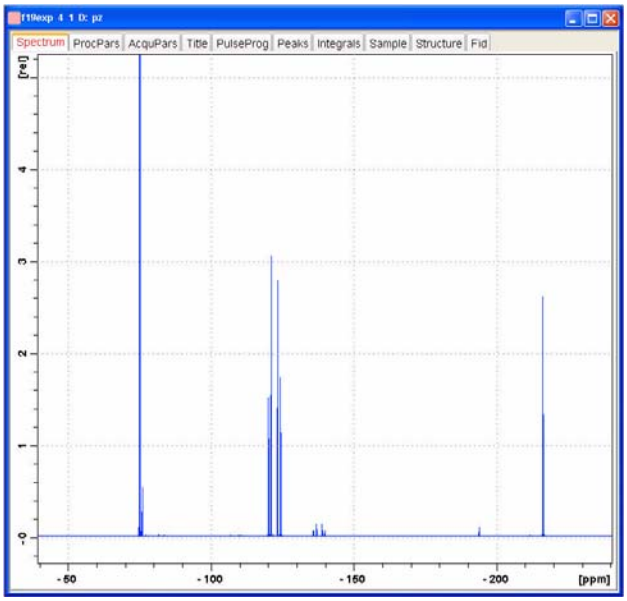
SW [PPM] = **200**

O1P [PPM] = **-140**

SOLVENT = **Acetone**

6. Click on  to read in the Prosol parameters
7. Select the '**Title**' tab by clicking on it
8. Change the title to:**1-D 19F experiment with 1H decoupling**
   2,2,3,4,4,4-Hexafluoro-1-Butanol
9. Select the '**Spectrum**' tab by clicking on it
10. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
11. Click on  to start the acquisition
12. Process and Phase correct the spectrum
13. To get rid of the background signal, follow the instructions in 5.2.1, Baseline correction, steps 1 through 9

Figure 5.7.



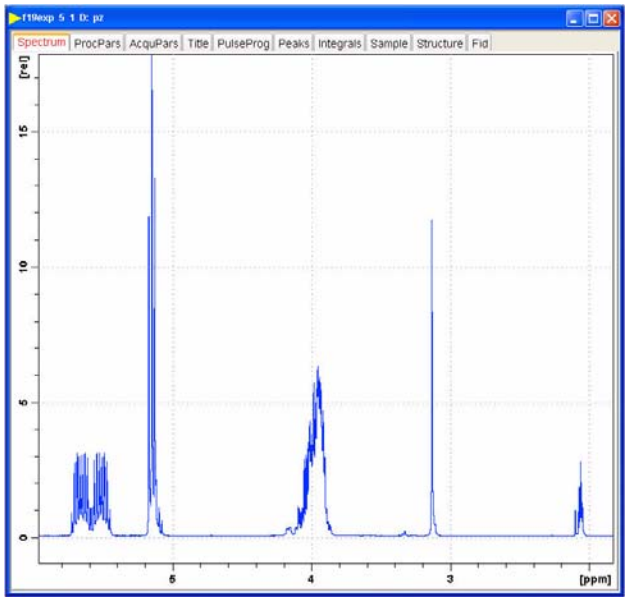
1H observe, no 19F decoupling *5.2.3*

1. Type **iexpno**
2. Type rpar **PROTON all**
3. Tune the probe for 1H
4. Select the '**AcquPars**' tab by clicking on it
5. Make the following changes:

SOLVENT = **Acetone**

6. Click on  to read in the Prosol parameters
7. Select the '**Title**' tab by clicking on it
8. Change the title to:**1-D 1H experiment no 19F decoupling**
   2,2,3,4,4,4-Hexafluoro-1-Butanol
9. Select the '**Spectrum**' tab by clicking on it
10. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
11. Click on  to start the acquisition
12. Process and Phase correct the spectrum

Figure 5.8.



1H observe, with 19F decoupling using WALTZ *5.2.4*

1. Type **iexpno**
2. Type **rpar PROF19DEC all**
3. Tune the probe for 19F and 1H
4. Select the '**AcquPars**' tab by clicking on it
5. Change the following parameters:

TD	=	**64k**
DS	=	**10**
O2P [PPM]	=	**-180**
SOLVENT	=	**Acetone**

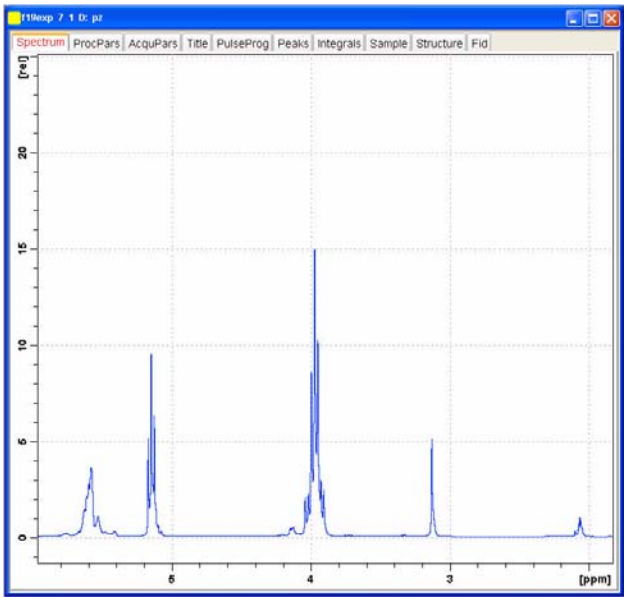
6. Select the '**ProcPars**' tab by clicking on it

Change the following parameter:

SI	=	**32k**

7. Click on  to read in the Prosol parameters
8. Select the '**Title**' tab by clicking on it
9. Change the title to:**1-D 1H experiment with 19F decoupling**
   2,2,3,4,4,4-Hexafluoro-1-Butanol
10. Select the '**Spectrum**' tab by clicking on it
11. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
12. Click on  to start the acquisition
13. Process and Phase correct the spectrum

Figure 5.9.



The Bruker standard parameter set PROF19DEC is using WALTZ for decoupling 19F. This may not be sufficient of a bandwidth to cover the 19F chemical shift range of some of the 19F spectra. In this example the 19F signals covers a sweep width of 200 ppm. To decouple all the 19F peaks, two approaches can be applied. Using the WALTZ decoupling the O2 frequency would have to be adjusted for the various 19F resonances which results in multiple proton spectra. Using garp or adiabatic pulses widens the decoupling range. Below is a example using garp decoupling.

1H observe, with 19F decoupling using Garp *5.2.5*

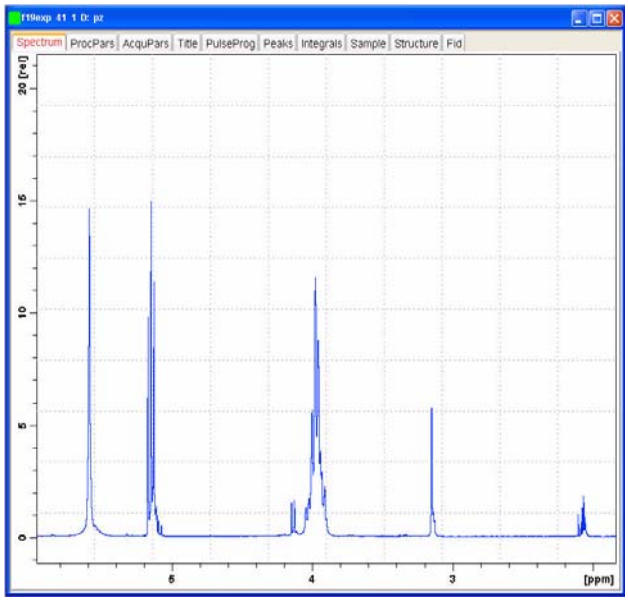
1. Select the '**AcquPars**' tab by clicking on it
2. Click on  to display the pulse program parameters
3. Make the following changes:

CPDPRG2	=	**garp**
PCPD2	=	**70**
Pl12	=	**calculate the power level in the prosol table**

4. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
5. Click on  to start the acquisition

19. Process and Phase correct the spectrum

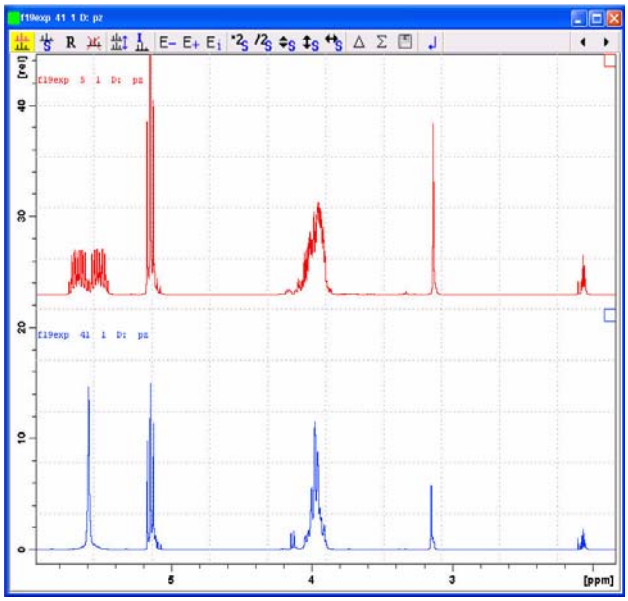
Figure 5.10.



6. Click on 

7. Drag the 19F coupled proton spectrum in to the display window

Figure 5.11.



2-D 19F experiments 5.3

There are currently no standard parameter sets for 19F 2-D experiments. The instructions below will guide you through the creation of some of the 19F 2-D parameter sets and running the experiments.

Sample:

2,2,3,4,4,4-Hexafluoro-1-butanol in Acetone-d6

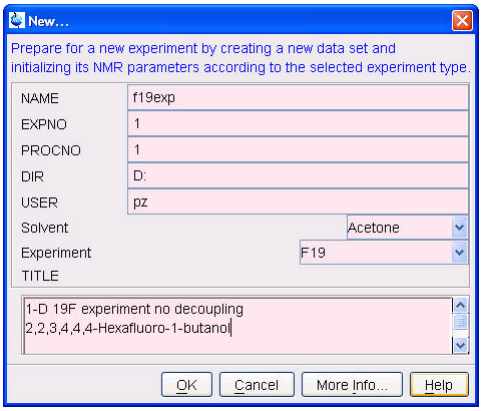
CF_3-CFH-CF_2-CH_2-OH

2-D Heteronuclear 1H/19F shift correlation 5.3.1

1-D 19F reference experiment

1. Click on  and change the following parameters

Figure 5.12.



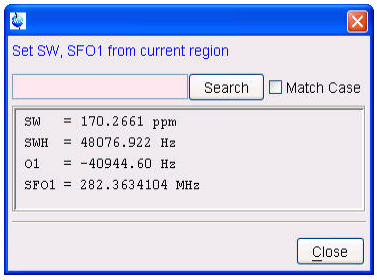
2. Click on 

3. Run a 1D 1H decoupled 19Fspectrum, following the instructions in this chapter, 19F observe with 1H decoupling, 5.2.2

4. Expand the spectrum to display all peaks, leaving ca. 15ppm of baseline on either side of the spectrum

5. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 5.13.



6. Click on Close

7. Type **sw**, set the value rounding off to the nearest 1/10th of a ppm

8. Write the value down

9. Type **o1p**, set the value rounding off to the nearest Hz

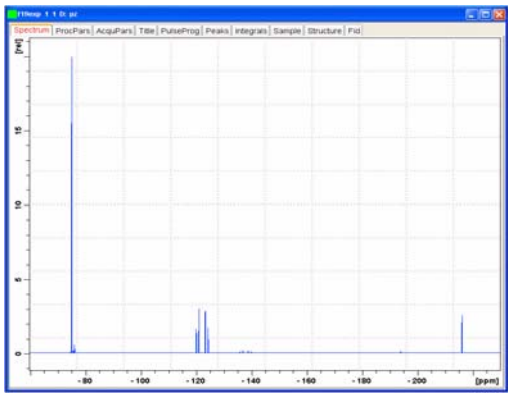
10. Write the value down

11. Type **sr** and write down the exact value

12. Click on  to start the acquisition

13. Process and Phase correct the spectrum

Figure 5.14.



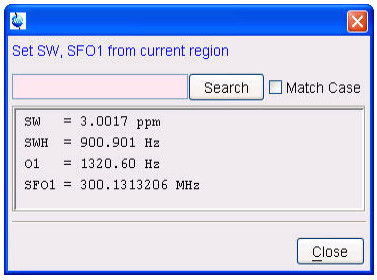
b) 1-D 1H reference experiment

1. Run a 1D 1H spectrum, following the instructions in this chapter, 1H observe no 19F decoupling, 5.2.3

2. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

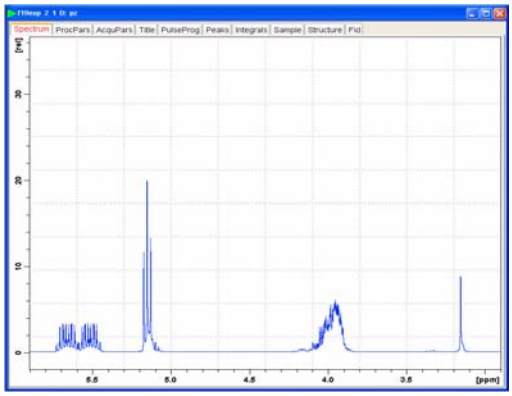
3. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 5.15.



4. Type **sw**, set the value rounding off to the nearest 1/10th of a ppm
5. Write the value down
6. Type **o1p**, set the value rounding off to the nearest Hz
7. Write the value down
8. Type **sr** and write down the exact value
9. Click on  to start the acquisition
10. Process and Phase correct the spectrum

Figure 5.16.



b) Set up of the 2-D HETCOR experiment

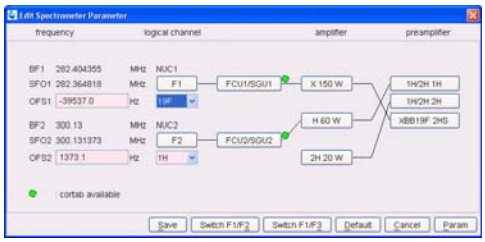
1. Type **expno**
2. Type **rpar HCCOSW all**
3. Turn the spinner off

NOTE: 2-D experiments should be run non spinning

4. Type **edasp**
5. Make the following change:

NUC1 = 19F

Figure 5.17.



6. Click on Save

7.Select the '**AcquPars**' tab by clicking on it

8. Make the following changes:

PULPROG = **hfcoqfqn**

SW F2 [ppm] = value from step 8 (19F reference spectrum)

SW F1 [ppm] = value from step 10 (19F reference spectrum)

O1P [ppm] = value from step 5 (1H reference spectrum)

O2P [ppm] = value from step 7 (1H reference spectrum)

SOLVENT = **Acetone**

9. Click on to read in the Prosol parameters

10. Click on to display the pulse program parameters

11. Make the following changes:

CNST2 = **25** = J(FH)

12. Select the '**ProcPar**' tab by clicking on it

13. Make the following changes:

SR F2 = value from step 11 (19F reference spectrum)

SR F1 = value from step 8 (1H reference spectrum)

WDW F2 = **SINE**

WDW F1 = **SINE**

SSB F2 = **2**

SSB F1 = **2**

14. Select the '**Title**' tab by clicking on it

15. Change the title to: **2-D 1H/19F HETCOR experiment**
2,2,3,4,4,4-Hexafluoro-1-Butanol

16. Select the '**Spectrum**' tab by clicking on it

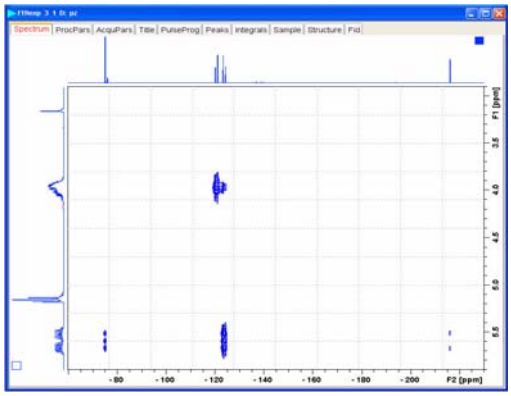
17. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

18. Click on to start the acquisition

19. Type **xfb**

20. Adjust the contour level

Figure 5.18.


Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Fid
F1 [ppm]
3.5
4.0
4.5
5.0
5.5
-80
-100
-120
-140
-160
-180
-200
F2 [ppm]

1-D Selective NOESY

6

Introduction 6.1

NOTE: To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients. Three different ways to run this experiment are discussed in this chapter and can also be applied to other selective experiments such as SELCOSY, SELROESY and SELTOCSY.

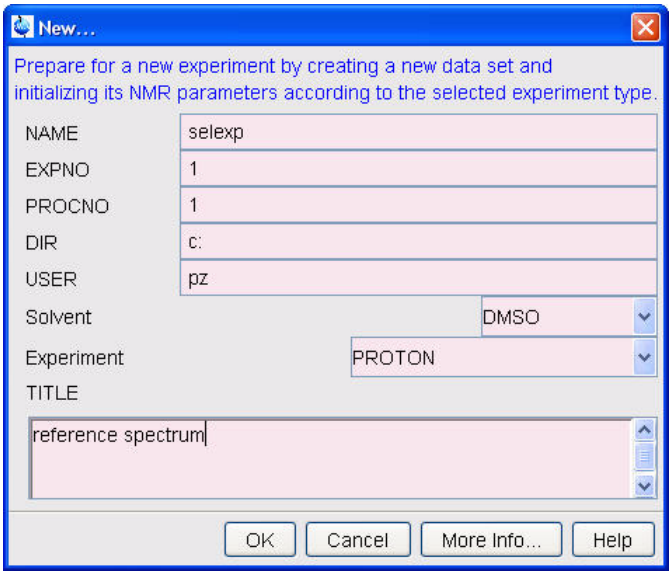
Sample:

30 mg Pamoic acid in DMSO

Reference spectrum 6.1.1

1. Click on  and change the following parameters

Figure 6.1.

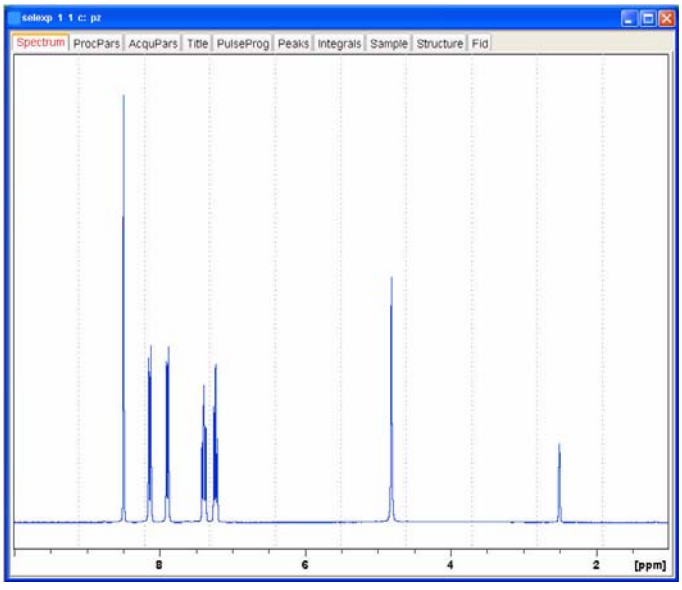


2. Click on OK
3. Insert the sample
4. Click on  to display the Lock display
5. In the lock display window click on  and select DMSO
6. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

7. Shim for best homogeneity
8. In the lock display window click on  to close the window
9. Select the '**AcquPars**' tab by clicking on it
10. Click on  to read in the Prosol parameters
11. Tune the probe
12. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
13. Click on  to start the acquisition
14. Process and Phase correct the spectrum

Figure 6.2.



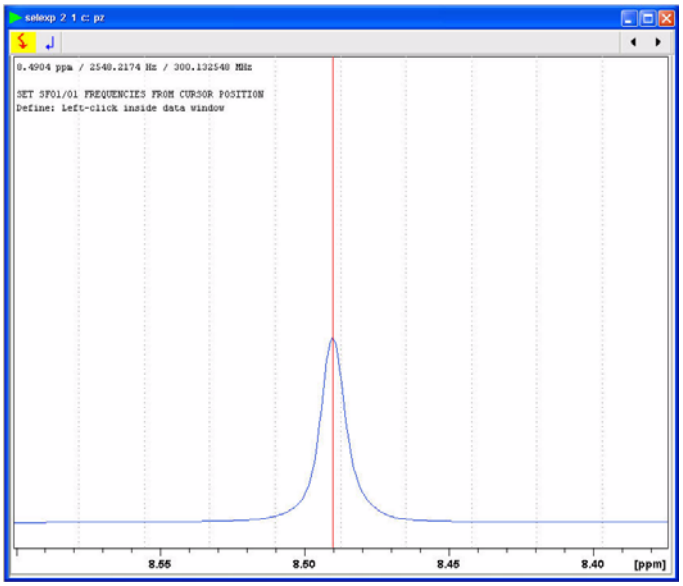
Selective excitation region set up (example 1) *6.1.2*

On resonance

NOTE: Make sure that the SW is large enough to cover the entire Spectrum accounting for the position of O1. The shaped pulse is applied on resonance (at the o1 position) The power level and width of the excitation pulse have to be known and entered into the Prosol parameter table

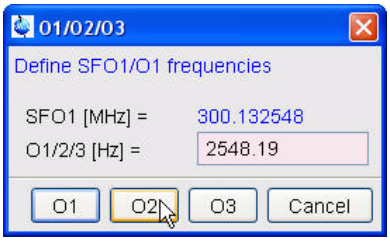
1. Type **wrpa 2**
2. Type **re 2**
3. Select the '**Title**' tab by clicking on it
4. Change the title to: **Selective NOESY experiment**
5. Select the '**Spectrum**' tab by clicking on it
6. Expand the signal region at 8.5 ppm
7. Click on 

Figure 6.3.



8. Move the cursor line to the center of the peak and click the left mouse button

Figure 6.4.



9. Click on 

Setting up the acquisition parameters *6.1.3*

1. Select the '**AcquPars**' tab by clicking on it
2. Click on  to display the pulsprogram parameters
3. Make the following changes:

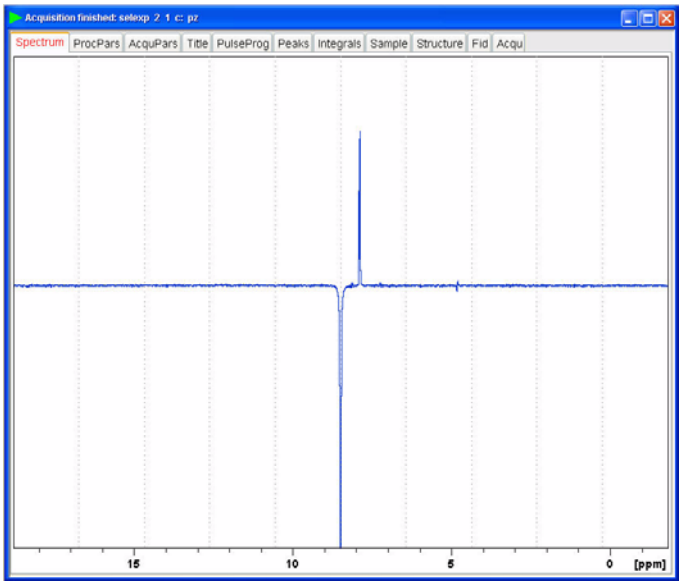
PULPROG	=	**selnogp**
NS	=	**64**
DS	=	**8**
D1	=	**2**
D8	=	**0.750**
SPNAM2	=	**Gaus1.1000**
SPOFF2	=	**0**
GPNAM1	=	**sine.100**
GPNAM2	=	**sine.100**
GPZ1	=	**15**
GPZ2	=	**40**

Running the experiment *6.1.4*

1. Select the '**Spectrum**' tab by clicking on it
3. Click on  to start the acquisition
4. Type **ef**
5. Phase the spectrum using the manual phase adjust

NOTE: Phase the selective exited peak negative to a sure the correct phase of the noe peaks.

Figure 6.5.



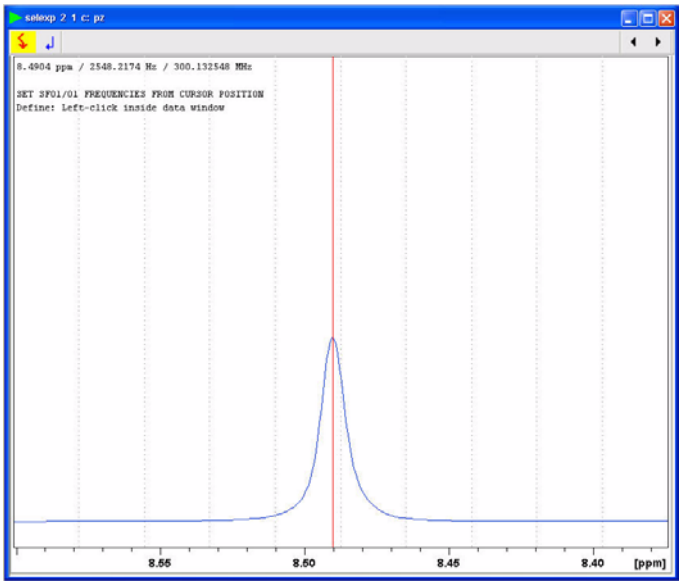
Selective excitation region set up (example 2) *6.1.5*

Off resonance

NOTE: This method does not require a large SW. The shaped pulse is applied off resonance (not on the O1 position). The power level and pulse width of the excitation pulse have to be known and entered into the Prosol parameters.

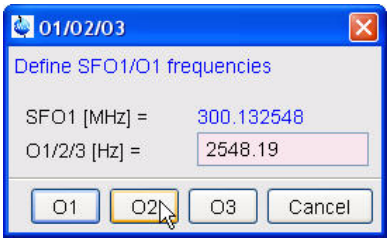
1. Run a Reference spectrum, following the instructions in 2.1.1 Reference Spectrum in this Chapter.
2. Type **wrpa 2**
3. Type **re 2**
4. Select the '**Title**' tab by clicking on it
5. Change the title to: **Selective NOESY experiment**
6. Select the '**Spectrum**' tab by clicking on it
7. Expand the signal region at 8.5 ppm
8. Click on 

Figure 6.6.



9. Move the cursor line to the center of the peak and click the left mouse button

Figure 6.7.



10. Write down the O1/2/3 (Hz) value showing in the Info window (e.g. **2548.19**)

12. Click on Cancel

13. Type **O1** and write down the current value (e.g. **1853.43**)

14. Calculate the difference of step 9 and 11 (e.g. **694.55**)

15. Click on Cancel

NOTE: If the signal is down field of O1, a positive value must be entered for spoff. If the signal is up field of O1, spoff will have a negative value.

Setting up the acquisition parameters *6.1.6*

1. Select the '**AcquPars**' tab by clicking on it

2. Click on  to display the pulsprogram parameters

3. Make the following changes:

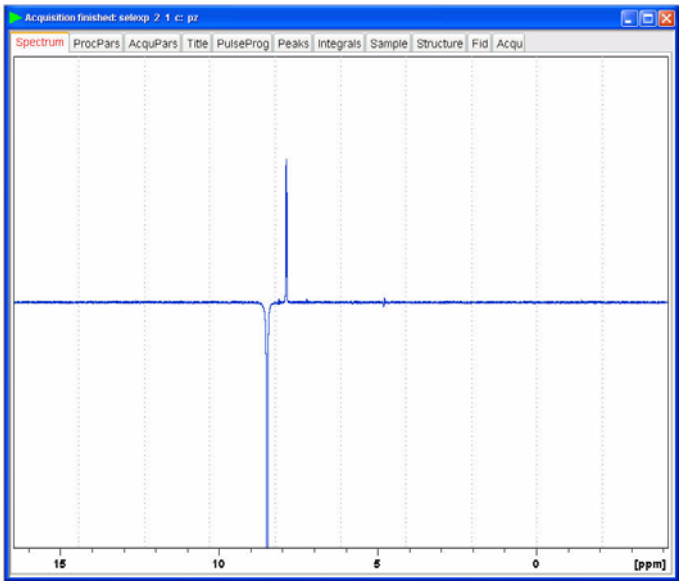
PULPROG	=	**selnogp**
NS	=	**64**
DS	=	**8**
D1	=	**2**
D8	=	**0.750**
SPNAM2	=	**Gaus1.1000**
SPOFF2	=	**694.55**
GPNAM1	=	**sine.100**
GPNAM2	=	**sine.100**
GPZ1	=	**15**
GPZ2	=	**40**

Running the experiment *6.1.7*

1. Select the '**Spectrum**' tab by clicking on it
2. Click on  to start the acquisition
3. Type **ef**
4. Phase the spectrum using the manual phase adjust

NOTE: Phase the selective exited peak negative to a sure the correct phase of the noe peaks.

Figure 6.8.



Selective excitation region set up (example 3) *6.1.8*

Integration region file

NOTE: In this example the shaped pulse is applied at a position determine using a integration region file and therefor does not require a large SW. This method calculates the precise shaped pulse for the selected peak using the 90 degree hard pulse and the Shape Tool program.

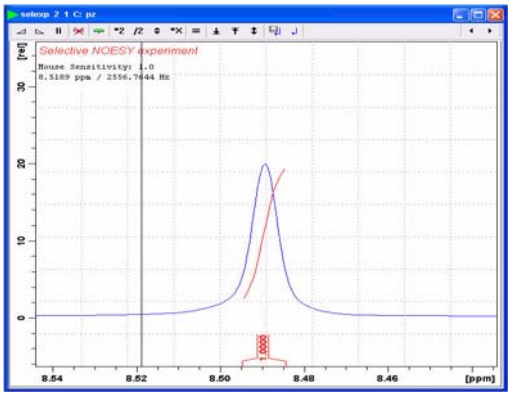
1. Run a Reference spectrum, following the instructions in 2.1.1 Reference Spectrum in this Chapter.
2. Type **wrpa 2**
3. Type **re 2**
4. Select the '**Title**' tab by clicking on it
5. Change the title to: **Selective NOESY experiment**
6. Select the '**Spectrum**' tab by clicking on it
7. Expand the signal region at 8.5 ppm
8. Click on 
9. In the Integration menu bar click on  to define a integration region

10. Define the regions by clicking the left mouse button and the use of the cursor lines

NOTE: Place the integral inside of the peak, from and to about 1/5th up from the base line.

11. Click on  again

Figure 6.9.



12. Click on 

Calculating the selective pulse width and power level *6.1.9*

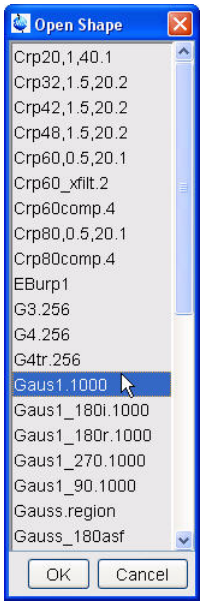
In this example the shaped pulse width and power level are determine using the '**Calc. Shape from Excitation Region**' option in the shaped tool program. Other method of calculating the pulse width and power level can be used, see Chapter 3, 1-D Selective TOCSY, Bandwidth region file, in this manual, or use the Prosol parameters to run this experiment.

1. Type **pulprog selnogp** in the command line

2. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp** in the command line

3. In the shape tool menu bar click on  and select **'Open Shape'**

Figure 6.10.



4. Select '**Gaus1.1000**'

5. Click on OK

6. In the main menu click on '**Manipulate**' and select '**Calc. Shape from Excitation Region**' by clicking on it

Figure 6.11.

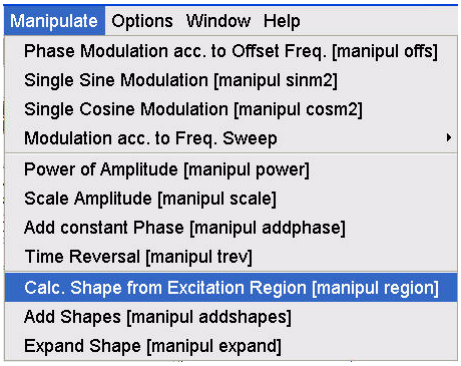
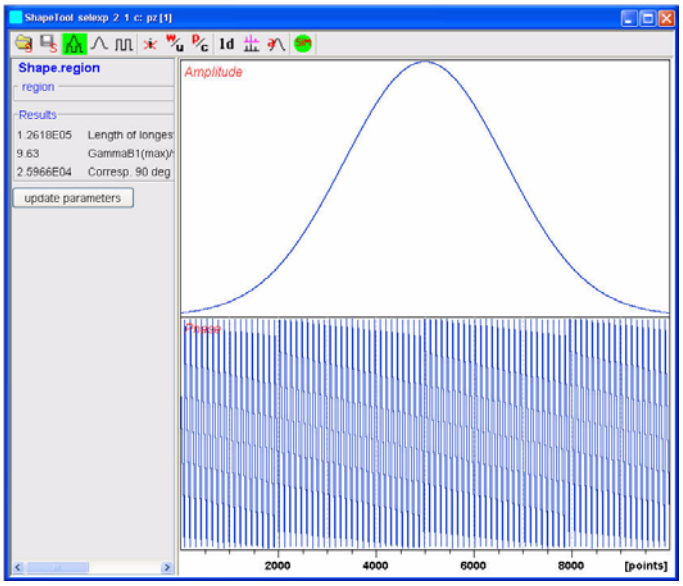


Figure 6.12.

7. Click on OK

Figure 6.13.



8. In the main menu click on '**Options**' and select '**Define Parameter Table**' by clicking on it

Figure 6.14.

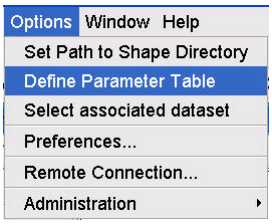
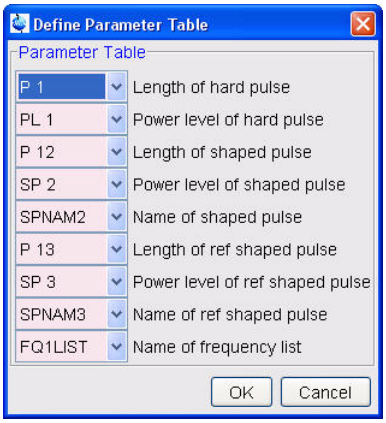


Figure 6.15.



9. Make the following changes:

Length of shaped pulse = **p12**

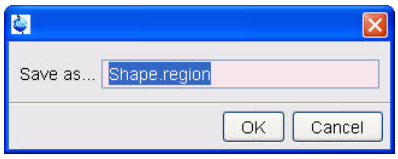
Power Level of shaped pulse = **SP2**

Name of shaped pulse = **SPNAM2**

10. Click on OK

11. Click on update parameters

Figure 6.16.



12. Select a new name
13. Click on OK
14. Click on to close the Shape Tool window

Setting up the acquisition parameters *6.1.10*

1. Select the '**AcquPars**' tab by clicking on it
2. Click on to display the pulsprogram parameters
3. Make the following changes:

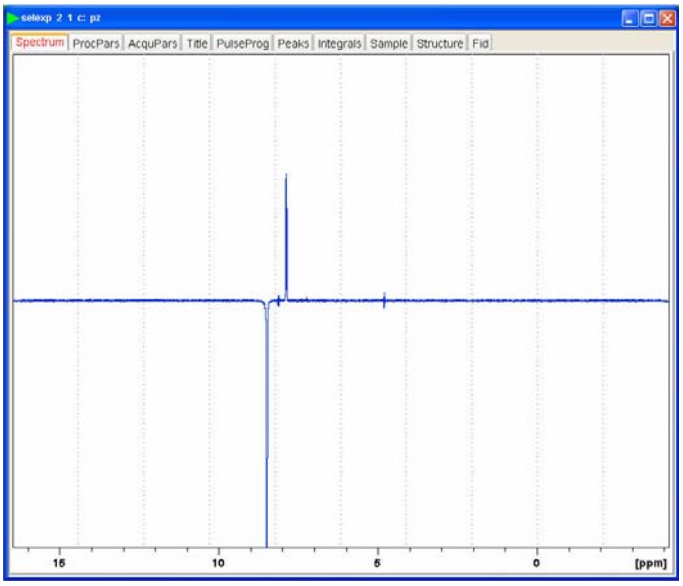
NS	=	**64**
DS	=	**8**
D1	=	**2**
D8	=	**0.750**
GPNAM1	=	**sine.100**
GPNAM2	=	**sine.100**
GPZ1	=	**15**
GPZ2	=	**40**

Running the experiment *6.1.11*

1. Select the '**Spectrum**' tab by clicking on it
2. Click on to start the acquisition
3. Type **ef**
4. Phase the spectrum using the manual phase adjust

NOTE: Phase the selective exited peak negative to a sure the correct phase of the noe peaks.

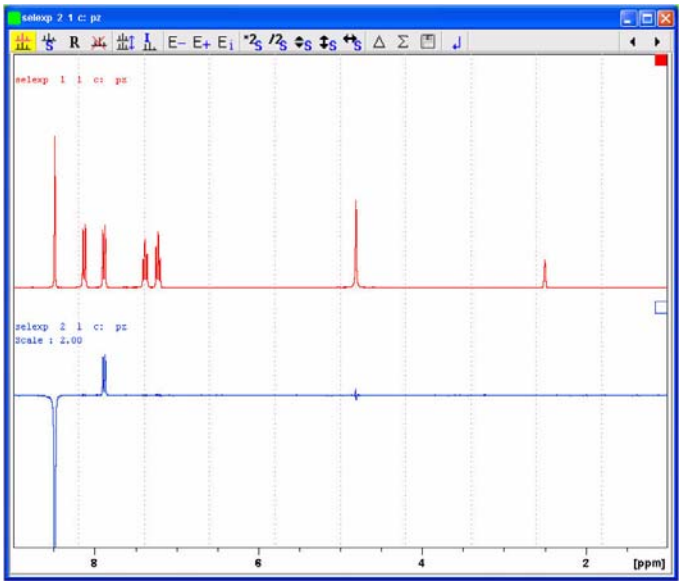
Figure 6.17.



Plotting the reference and the selective NOESY spectra on the same page 6.1.12

1. Type **re 2** to display the selective NOESY spectrum
2. Click on 
3. Type **re 1** on the command line (reference spectrum)
4. Click on  to separate the two spectra
5. Using the display tools  to adjust the spectra

Figure 6.18.



6. Type **prnt** on the command line to print the active window

NOTE: To plot the two spectra using the plot editor, follow the instructions in the manual Step-by-Step Tutorial, Basic Experiments Users Guide, Chapter 8, Homo-decoupling, 8.1.3 Plotting the reference and decoupled spectra on the same page, steps 1 through 21.

1-D selective TOCSY

7

Introduction 7.1

NOTE: To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients. The method to determine the pule width and power level for the selective pulse in this chapter, can also be used for other selective experiments such as SELCOSY, SELROESY and SELNOESY.

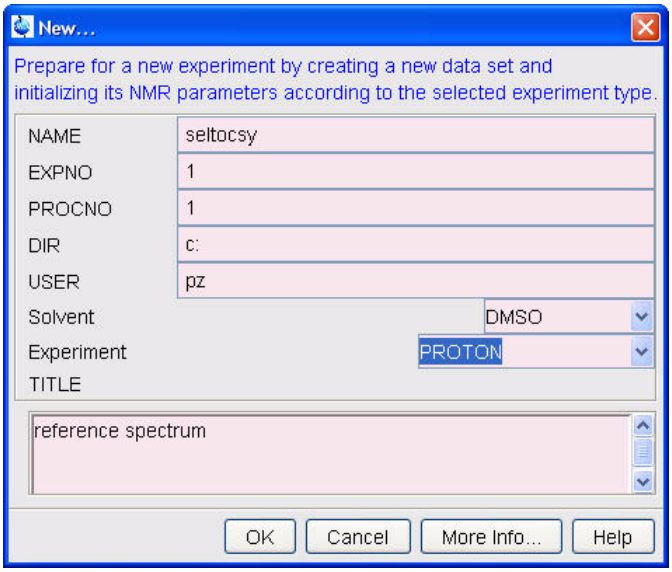
Sample:

50 mM Gramicidin S in DMSO

Reference spectrum 7.1.1

1. Click on  and change the following parameters

Figure 7.1.

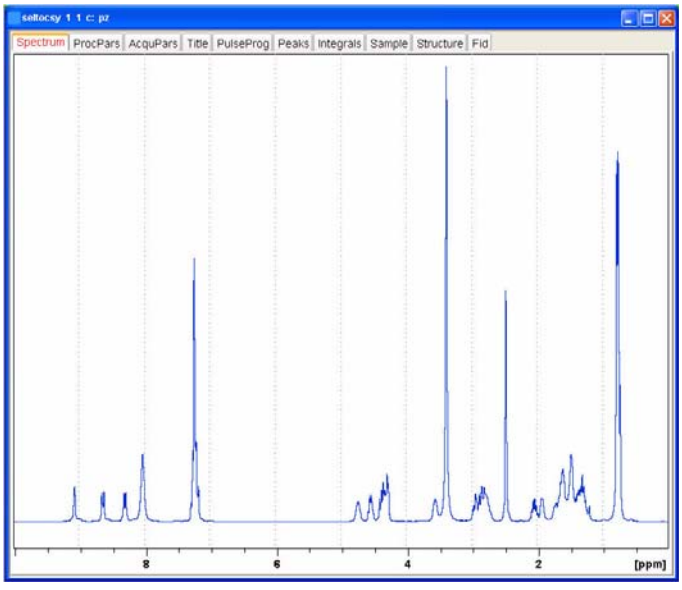


2. Click on OK
3. Insert the sample
4. Click on  to display the Lock display
5. In the lock display window click on  and select DMSO
6. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

7. Shim for best homogeneity
8. In the lock display window click on  to close the window
9. Select the '**AcquPars**' tab by clicking on it
10. Click on  to read in the Prosol parameters
11. Tune the probe
12. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
13. Click on  to start the acquisition
14. Process and Phase correct the spectrum

Figure 7.2.

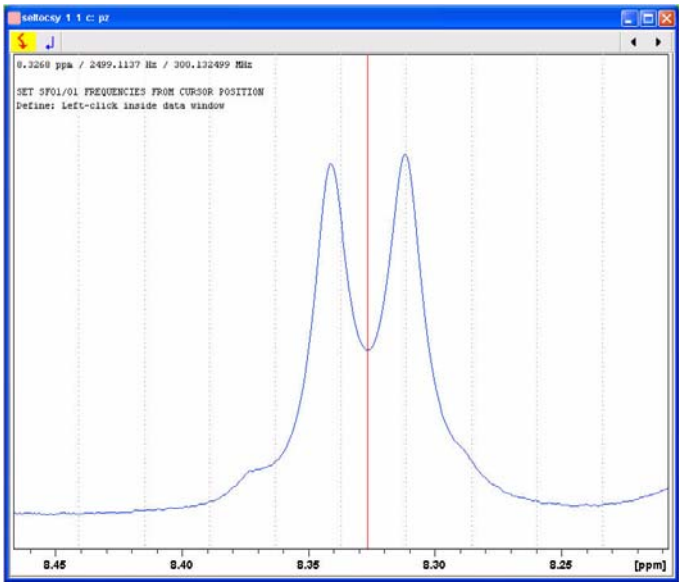


Off resonance

NOTE: In this example the shaped pulse is applied at the off resonance position and therefor does not require a large SW. Other excitation region set up method can be used to run this experiment, see Chapter 2, 1-D Selective NOESY in this manual.

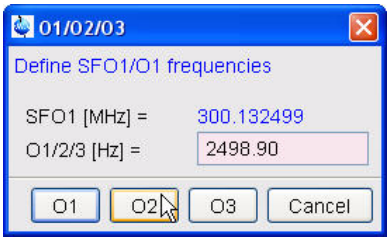
1. Type **wrpa 2**
2. Type **re 2**
3. Select the '**Title**' tab by clicking on it
4. Change the title to: **Selective TOCSY experiment**
5. Select the '**Spectrum**' tab by clicking on it
6. Expand the amid peak of Leucine at 8.3 ppm
7. Click on 

Figure 7.3.



8. Move the cursor line to the center of the peak and click the left mouse button

Figure 7.4.



9. Write down the O1/2/3 (Hz) value showing in the Info window (e.g. **2498.9**)

10. Click on Cancel

11. Type **O1** and write down the current value (e.g. **1853.43**)

12. Calculate the difference of step 9 and 11 and write down the value, (e.g. **645.47** Hz)

14. Click on Cancel

NOTE: If the signal is down field of O1, a positive value must be entered for spoff. If the signal is up field of O1, spoff will have a negative value.

Calculating the selective pulse width and power level 7.1.3

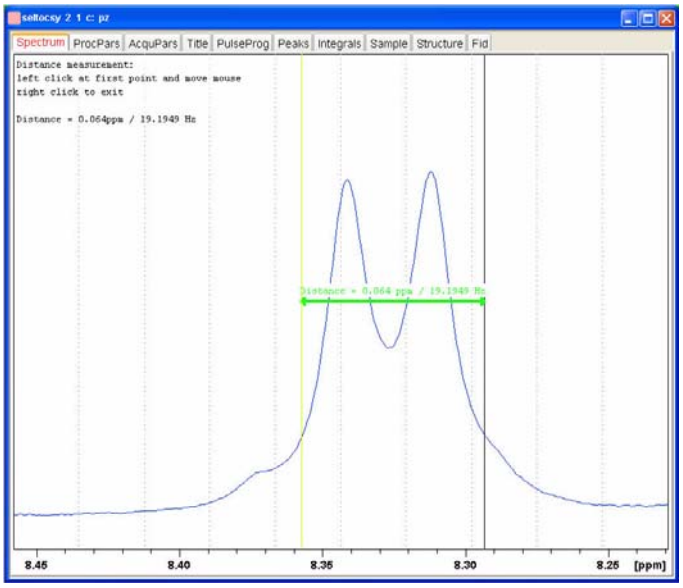
In this example the shaped pulse width and power level are determine using the **'Calculate Bandwidth'** option in the shaped tool program. Other method of calculating the pulse width and power level can be used, see Chapter 2, 1-D Selective NOESY, integration region file, in this manual, or use the Prosol parameters to run this experiment.

1. Click on 

2. Position the cursor line at the left side of the peak, up 1/5 from the baseline

3. Click the left mouse button and drag the cursor line to the right side of the peak, up 1/5 from the baseline

Figure 7.5.



4. Write down the value for the distance between the two cursor lines (e.g. **19**)

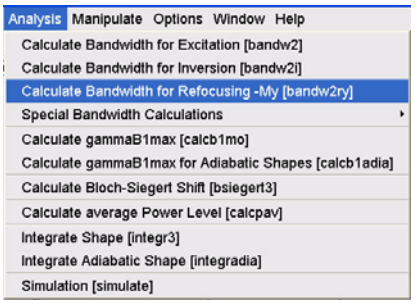
5. Type **pulprog selmlgp**

6. Type **getprosol**

7. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp**

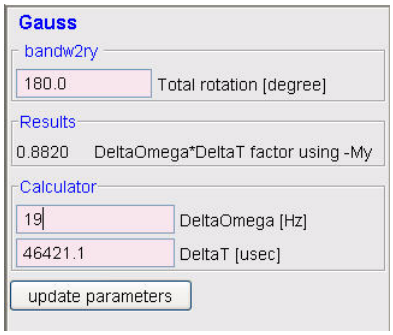
8. In the main menu click on '**Analysis**' and select '**Calculate Bandwidth for Refocusing -My'**

Figure 7.6.



9. Type the value from step 4 (e.g. **19**) in to the Calculator window 'Delta Omega [Hz] and hit the Enter key

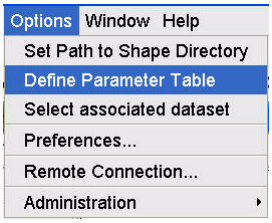
Figure 7.7.



NOTE: The value for 'Delta T [usec]' is calculated after executing step 9.

10. In the main menu click on '**Options**' and select '**Define Parameter Table**'

Figure 7.8.



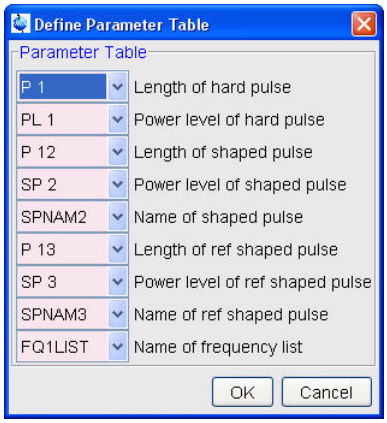
11. Make the following changes:

Length of shaped pulse = **p12**

Power Level of shaped pulse = **SP2**

Name of shaped pulse = **SPNAM2**

Figure 7.9.



12. Click on OK
13. Click on update parameters
14. Click on to close the Shape Tool window

Setting up the acquisition parameters *7.1.4*

1. Select the '**AcquPars**' tab by clicking on it
2. Click on to display the pulsprogram parameters
3. Make the following changes:

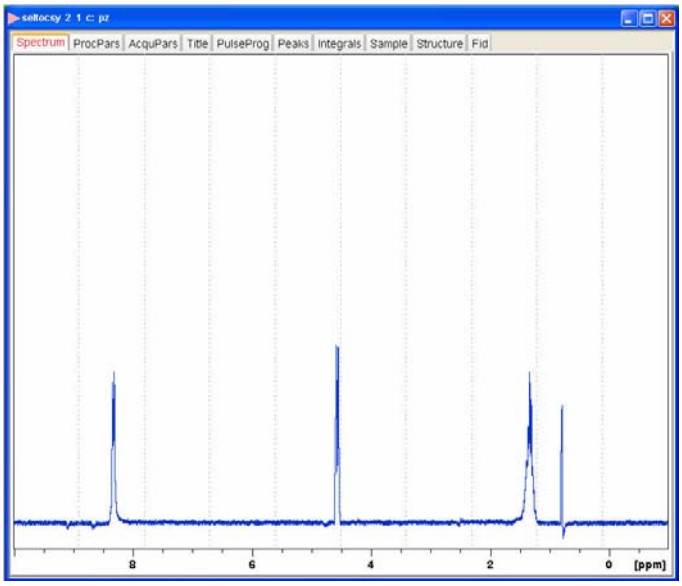
NS	=	**64**
DS	=	**8**
D1	=	**2**
D6	=	**0.075**
SPOFF2	=	(value from step 12, Determine the value for SPOFF) e.g.**694.55**
GPZ1	=	**15**

Running the experiment *7.1.5*

1. Select the '**Spectrum**' tab by clicking on it
2. Click on to start the acquisition
3. Type **ef**
4. Phase the spectrum using the manual phase adjust

NOTE: All peaks should be phased positive.

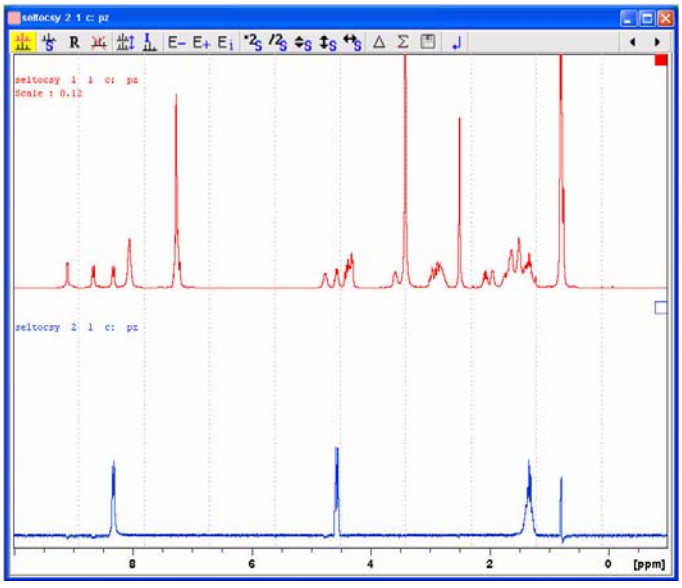
Figure 7.10.



Plotting the reference and the TOCSY spectrum on to the same page. *7.1.6*

1. Type **re 2** to display the selective TOCSY spectrum
2. Click on 
3. Type **re 1** on the command line (reference spectrum)
4. Click on  to separate the two spectra
5. Using the display tools  to adjust the spectra

Figure 7.11.



6. Type **prnt** on the command line to print the active window

NOTE: To plot the two spectra using the plot editor, follow the instructions in the manual Step-by-Step Tutorial, Basic Experiments Users Guide, Chapter 8, Homo-decoupling, 8.1.3 Plotting the reference and decoupled spectra on the same page, steps 1 through 21.

1-D DEPT using a shaped 13C pulse

8

Introduction 8.1

Using this experiment will yield a higher Signal to noise compared with the conventional DEPT135. It is more noticeable on higher field instrument using a larger sweep width. To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses.

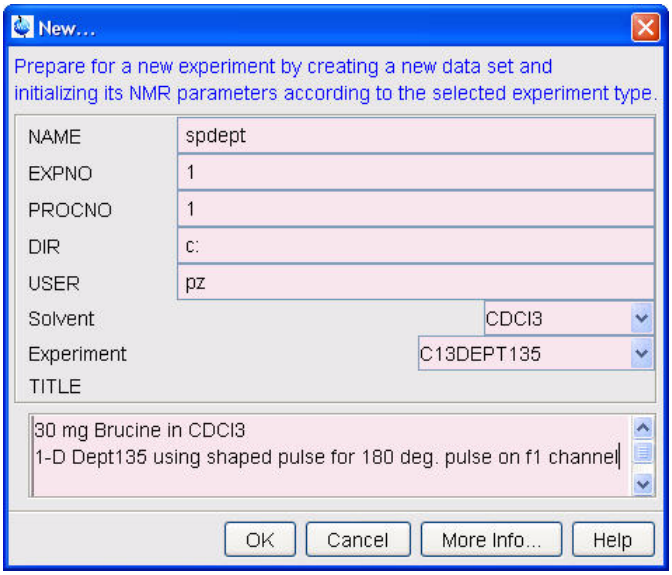
Sample:

30 mg Brucine in CDCl3

Experiment set up 8.1.1

1. Click on  and change the following parameters

Figure 8.1.

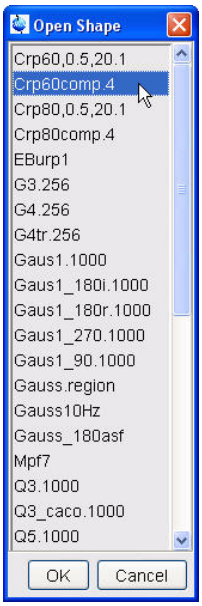


2. Click on OK
3. Insert the sample
4. Click on  to display the Lock display
5. In the lock display window click on  and select CDCl3
6. Shim for best homogeneity
7. In the lock display window click on  to close the window
8. Tune the probe
9. Type **pulprog deptsp135** in the command line
10. Type **getprosol** in the command line

Calculating the shaped pulse power level *8.1.2*

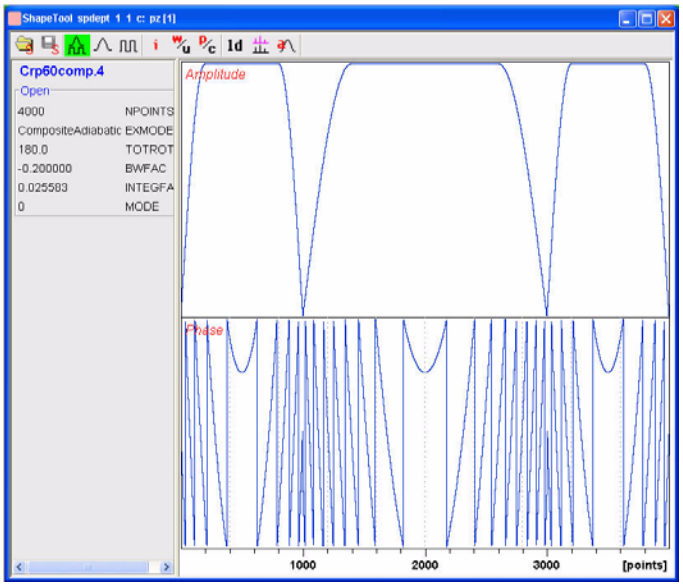
1. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp** in the command line
2. In the shape tool menu bar click on  and select **'Open Shape'**

Figure 8.2.



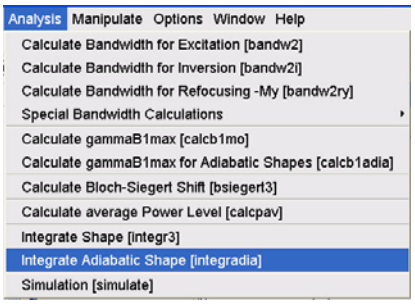
3. Select '**Crp60comp.4**'
4. Click on OK

Figure 8.3.



5. In the main menu click on '**Analysis**' and select '**Integrate Adiabatic Shape'**

Figure 8.4.

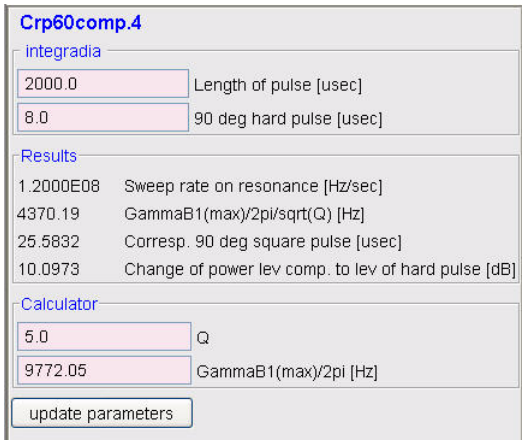


6. Make the following change:

Length of pulse [usec] = 2000

7. Press the 'Enter' key

Figure 8.5.



NOTE: The value for ‘change of power lev comp. to lev of hard pulse’ is calculated after executing step 7.

8. Write down the value of ‘change of power lev comp. to lev of hard pulse [dB] (e.g. **10.0973 dB**)

9. Click on  to close the Shape Tool window

Setting up the acquisition parameters *8.1.3*

1. Select the ‘**AcquPars**’ tab by clicking on it

2. Click on  to display the pulsprogram parameters

3. Make the following changes:

PL2 [us]	=	**2000**
SP2 [dB]	=	value of step 8 in 4.1.2 + PL1 (e.g. **7.3**)
SPNAM2	=	**Crp60comp.4**

4. Select the ‘**Spectrum**’ tab by clicking on it

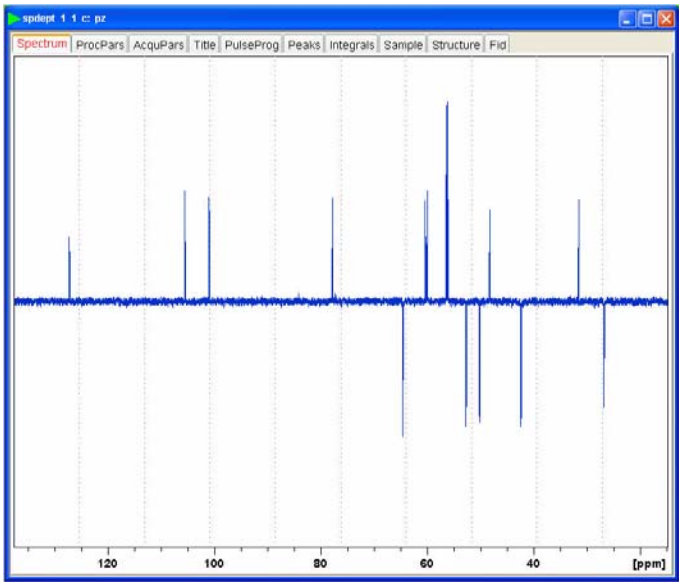
Running the experiment *8.1.4*

1. In the main menu click on ‘**Spectrometer**’, select ‘**Adjustment**’ and click on ‘**Auto-adjust receiver gain**’ or type **rga**

2. Click on  to start the acquisition

3. Process and Phase correct the spectrum

Figure 8.6.


spdept 1 1 c: pz
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Fid
120
100
80
60
40
[ppm]

1-D DEPT using a shaped 13C pulse

2-D HSQC using a shaped 13C pulse

9

Introduction 9.1

Using this experiment will yield a higher Signal to noise compaired with the conventional HSQCETGP. It is more noticeable on higher field instrument using a larger sweepwidth. To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients.

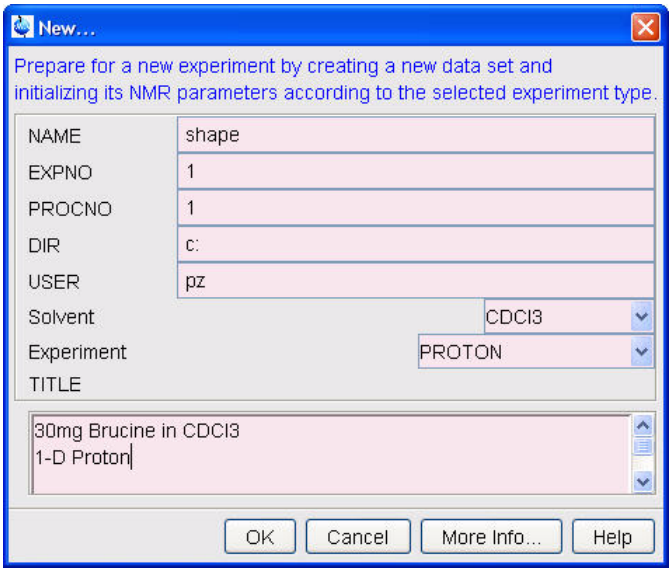
Sample:

30mg Brucine in CDCl3

Reference spectrum 9.1.1

1. Click on  and change the following parameters

Figure 9.1.

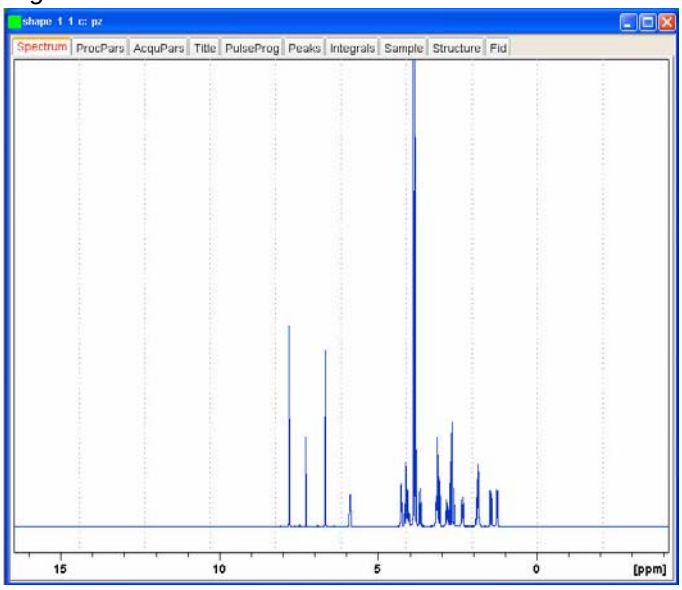


2. Click on OK
3. Insert the sample
4. Click on  to display the Lock display
5. In the lock display window click on  and select CDCl3
6. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

7. Shim for best homogeneity
8. In the lock display window click on  to close the window
9. Select the '**AcquPars**' tab by clicking on it
10. Click on  to read in the Prosol parameters
11. Tune the probe
12. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
13. Click on  to start the acquisition
14. Process and Phase correct the spectrum

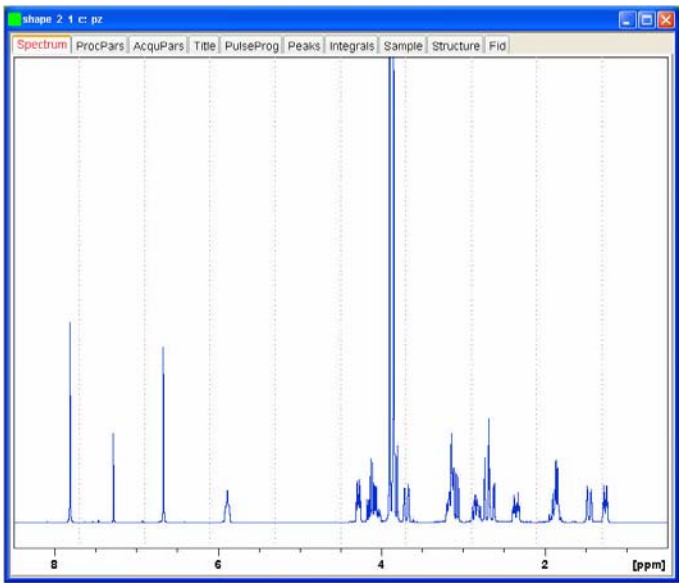
Figure 9.2.



Limit setting *9.1.2*

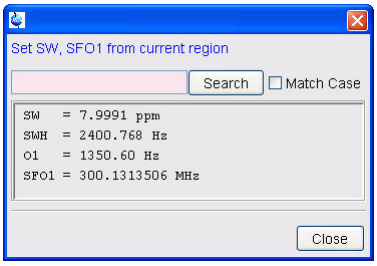
1. Type **wrpa 2** on the command line
2. Type **re 2**
3. Expand the spectrum to include all peaks (e.g. 0.5 ppm to 8.5 ppm

Figure 9.3.



4. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 9.4.



5. Click on Close
6. Type **sw** on the command line and write down the value of SW, rounding off to the nearest 1/10th of a ppm (e.g. **8 ppm**)
7. Type **o1** on the command line and write down the value of O1, rounding off to the nearest 1/10th of a ppm (e.g. **4.5 ppm**)
8. Type **sr** and write down the exact value (e.g. **0 Hz**)

Running the 2-D HSQC using a 180 adiabatic inversion shaped pulse in F1 *9.1.3*

1. Type **rpar HSQCETGPSISP all**

2. Turn the spinner off

NOTE: 2-D experiments should be run non spinning

3. Select the '**AcquPars**' tab by clicking on it

4. Make the following changes:

F1 SW [ppm = value from step 6, Limit setting 5.1.2 (e.g. **8**)

O1 [Hz] = value from step 7, Limit setting 5.1.2 (e.g. **4.5**)

SOLVENT = CDCl3

All Bruker 2D inverse parameter sets use 13C in the F1 dimension and the sweep width and O1 are optimized to include all Carbon peaks of interest. For HSQC experiments the sw is optimized to 160 ppm.

5. Click on  to read in the Prosol parameters

The values for the pulse length and power level of the180 deg. adiabatic inversion shaped pulse (crp60,0.5.20.1) have to be entered in to the prosol table.

6. Select the '**ProcPar**' tab by clicking on it

7. Make the following changes:

SR [F2] = value from step 8, Limit setting 5.1.2 (e.g. **0**)

8 Select the '**Title**' tab by clicking on it

9. Change the title to: **30 mg Brucine in CDCl3, 2D HSQC using a 180 deg adiabatic inversion shaped pulse in F1**

10. Select the '**Spectrum**' tab by clicking on it

Acquisition *9.1.4*

1. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

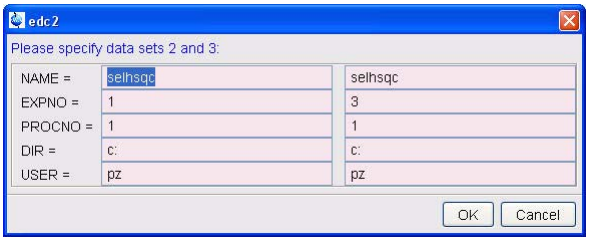
2. Click on  to start the acquisition

Processing *9.1.5*

The standard Bruker parameter sets are optimized to run under complete automation. One of the processing parameters is an AU program for processing the data, which can be executed with the command 'xaup'. The next steps assures to use the external spectrum of Brucine for the F2 and F1 projections.

1. Type **edc2**

Figure 9.5.

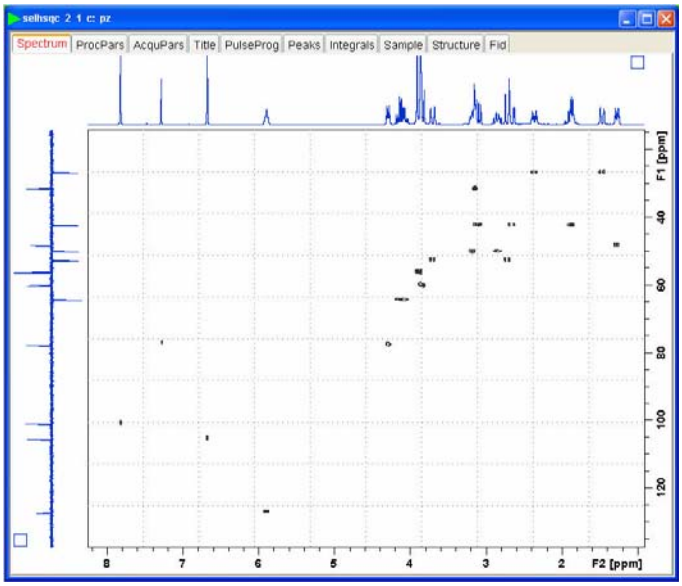


2. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

3. Click on OK

4. Type **xaup**

Figure 9.6.



The processing AU program includes the 2D Fourier transform, baseline correction and plotting of the data.

2-D Selective HMBC

10

Introduction 10.1

NOTE: To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients. The method to determine the pule width and power level for the selective pulse in this chapter, can also be used for other selective experiments such as SELCOSY, SELROESY and SELNOESY.

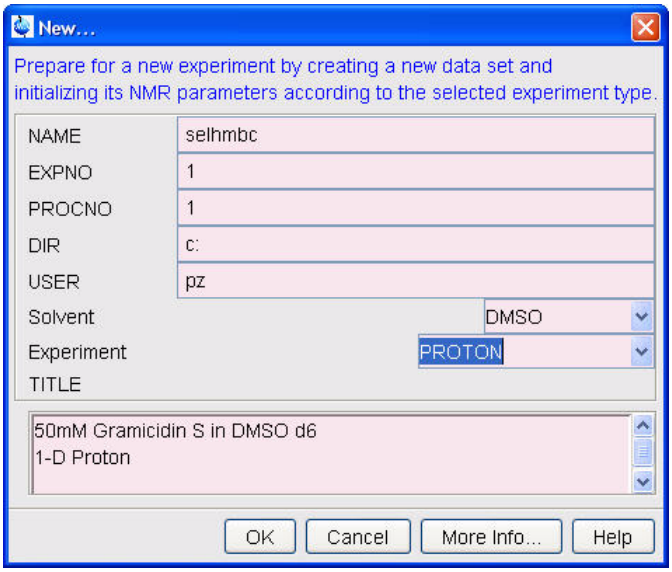
Sample:

50 mM Gramicidin S in DMSO

Reference spectrum 10.1.1

1. Click on  and change the following parameters

Figure 10.1.

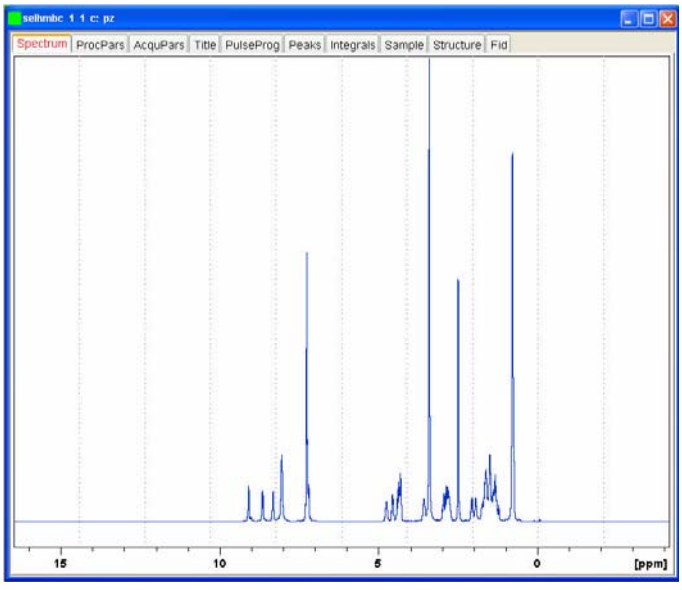


2. Click on OK
3. Insert the sample
4. Click on to display the Lock display
5. In the lock display window click on and select DMSO
6. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

7. Shim for best homogeneity
8. In the lock display window click on to close the window
9. Select the '**AcquPars**' tab by clicking on it
10. Click on to read in the Prosol parameters
11. Tune the probe
12. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
13. Click on to start the acquisition
14. Process and Phase correct the spectrum

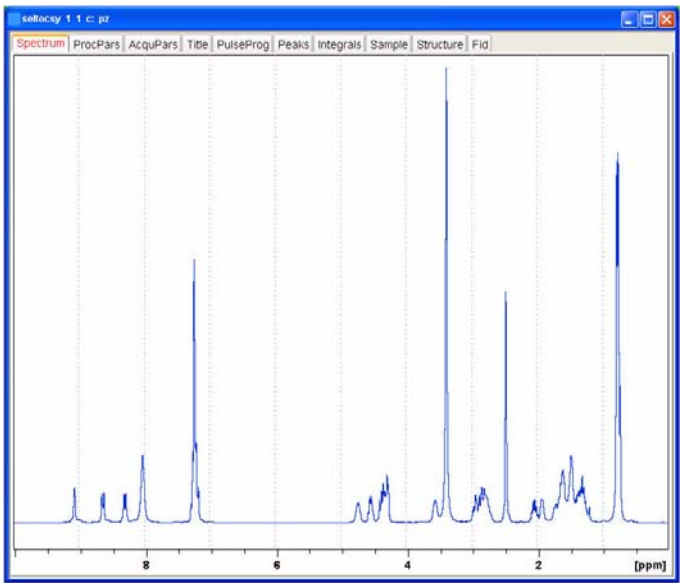
Figure 10.2.



Limit setting 10.1.2

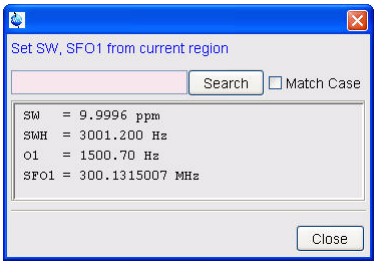
1. Type **wrpa 2** on the command line
2. Type **re 2**
3. Expand the spectrum to include all peaks (e.g. 0 ppm to 10 ppm

Figure 10.3.



4. Click on  to set the sweep width and the O1 frequency of the displayed region

Figure 10.4.



5. Click on Close
6. Type **sw** on the command line and write down the value of SW, rounding off to the nearest 1/10th of a ppm (e.g. **10 ppm**)
7. Type **o1** on the command line and write down the value of O1, rounding off to the nearest 1/10th of a ppm (e.g. **5 ppm**)
8. Type **sr** and write down the exact value (e.g. **0 Hz**)

Running a 2-D HMBC experiment 10.1.3

1. Type **rpar HMBCGPND all**

2. Turn the spinner off

NOTE: 2-D experiments should be run non spinning

3. Select the '**AcquPars**' tab by clicking on it

4. Make the following changes:

F1 SW [ppm] = value from step 6, Limit setting 6.1.2 (e.g. **10**)

O1 [Hz] = value from step 7, Limit setting 6.1.2 (e.g. **5**)

Solvent = DMSO

All Bruker 2D inverse parameter sets use 13C in the F1 dimension and the sweep width and O1 are optimized to include all Carbon peaks of interest. For HMBC experiments the sw is optimized to 220 ppm.

5. Click on  to read in the Prosol parameters

6. Select the '**ProcPar**' tab by clicking on it

7. Make the following changes:

SR [F2] = value from step 8, Limit setting 6.1.2 (e.g. **0**)

8 Select the '**Title**' tab by clicking on it

9. Change the title to: **50 mM Gamicidin S in DMSO, 2-D HMBC**

10. Select the '**Spectrum**' tab by clicking on it

Acquisition *10.1.4*

1. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

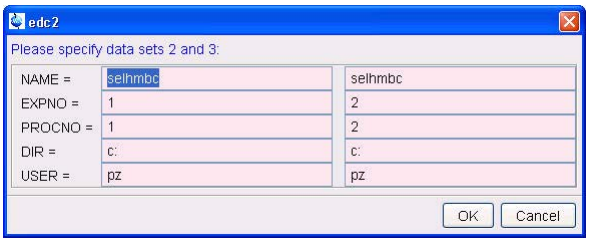
2. Click on  to start the acquisition

Processing *10.1.5*

The standard Bruker parameter sets are optimized to run under complete automation. One of the processing parameters is an AU program for processing the data, which can be executed with the command ‘xaup’. The next steps assures to use the external spectrum of Gramicidin for the F2 projection.

1. Type **edc2**

Figure 10.5.

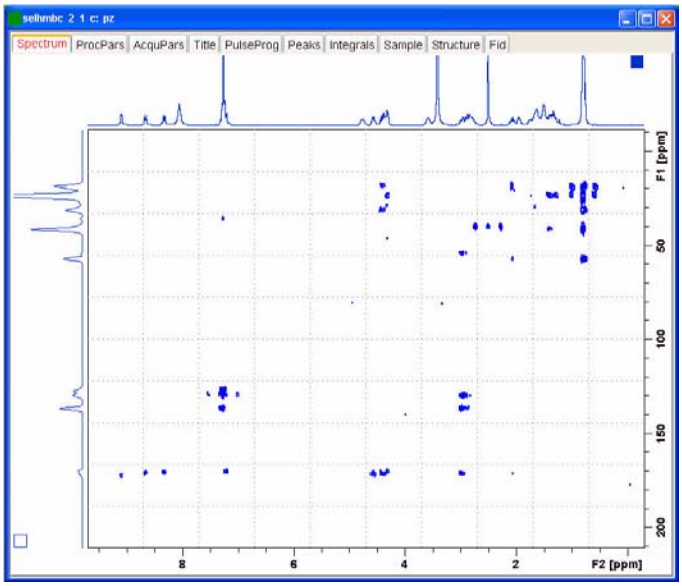


2. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

3. Click on OK

4. Type **xaup**

Figure 10.6.



The processing AU program includes the 2D Fourier transform, baseline correction and plotting of the data. The HMBC experiment uses magnitude mode for processing and shows only positive peaks.

Optimizing the parameters on the carbonyl region *10.1.6*

1. Type **wrpa 3** on the command line
2. Type **re 3**
3. Expand the carbonyl region including all cross peaks (e.g. 162 ppm to 182 ppm)

Figure 10.7.

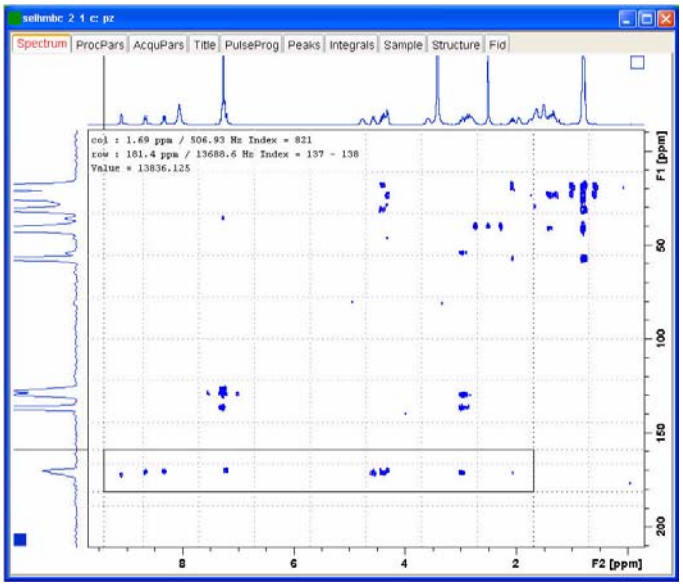
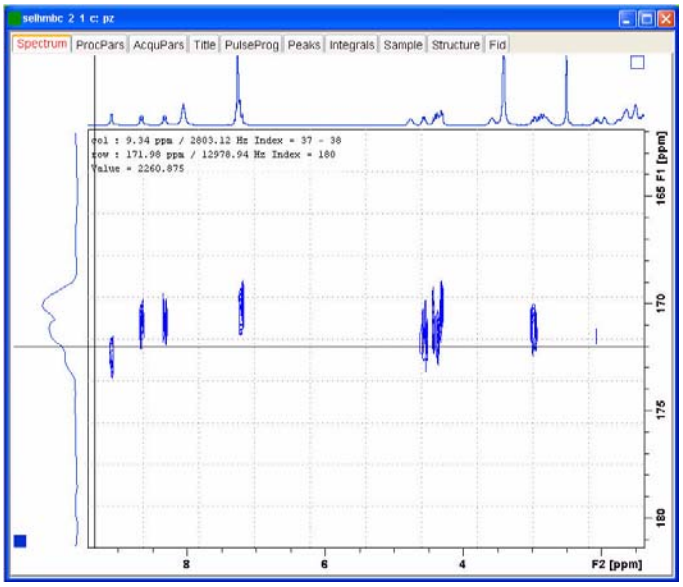


Figure 10.8.



4. Write down the expanded F1 sweep width in ppm (e.g. **20 ppm**)

5. Write down the center frequency (O2) of the expanded F1 sweep width in ppm (e.g. **172 ppm**)

6. Select the '**AcquPars**' tab by clicking on it

7. Click on  to display the pulsprogram parameters

8. Write down the value for P3 [us] (e.g. **8 us**)

9. Write down the value for PL2 [dB] (e.g. **-2.8 dB**)

10. Select the '**Title**' tab by clicking on it

11. Change the title to: **50 mM Gamicidin S in DMSO, selective 2-D HMBC**

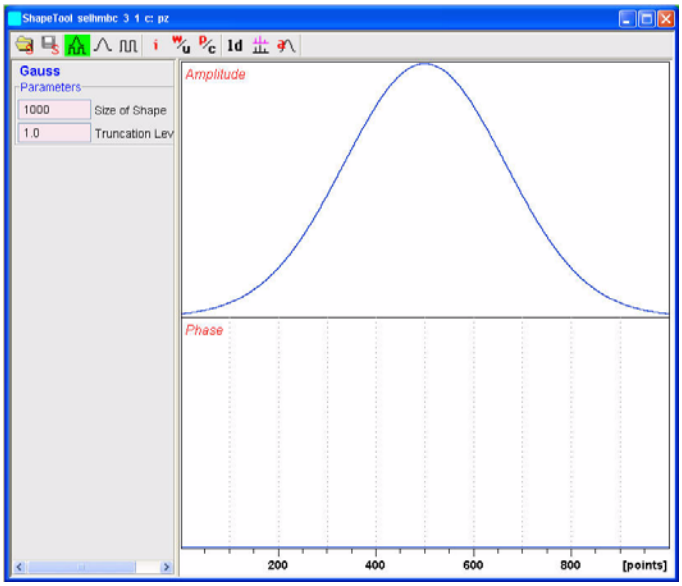
10. Select the '**Spectrum**' tab by clicking on it

Set up the selective pulse *10.1.7*

1. 1. Type **pulprog shmbcgpnd** in the command line

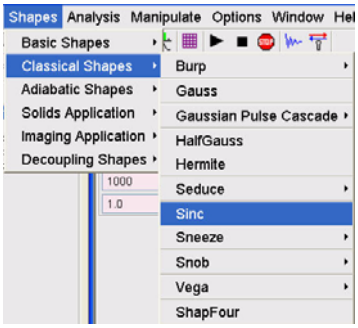
2. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp** in the command line

Figure 10.9.



3. In the main menu click on '**Shapes**', select '**Classical**' and select '**Sinc**' by clicking on it

Figure 10.10.

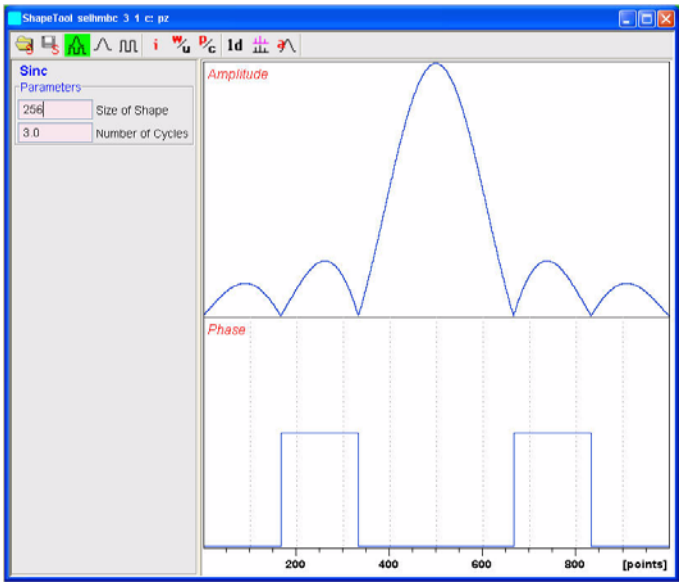


4. Make the following changes:

Change size of shape = **256**

Number of cycles = **3**

Figure 10.11.



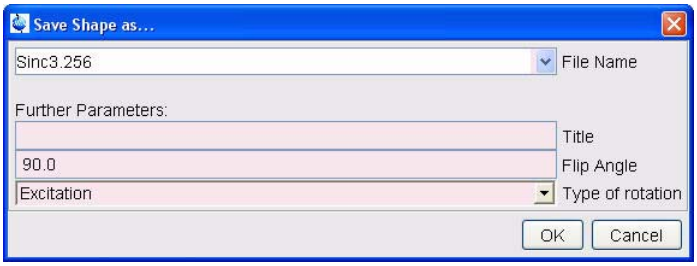
5. Click on 

6. Click on '**Save Shape**'

7. Make the following changes:

File Name = **Sinc3.256**

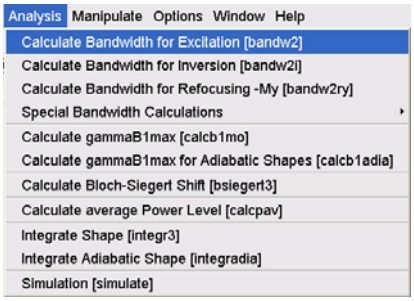
Figure 10.12.



8. Click on 

9. In the main menu click on '**Analysis**', select '**Calculate Bandwidth for Excitation**'

Figure 10.13.



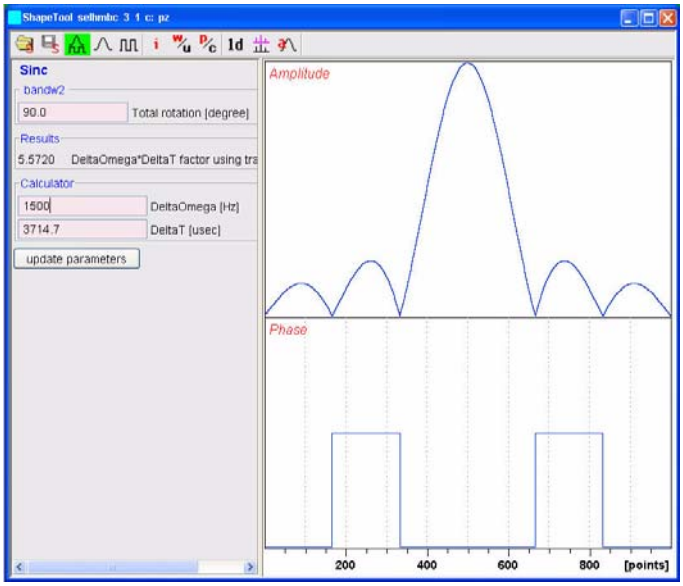
10. Make the following changes:

DeltaOmega [Hz] = **1500** (e.g. SW 20 ppm from step 4 in 6.1.6)

11. Press the ‘Enter’ key

NOTE: The value of Delta T [usec] is being calculated. (e.g. 3714.7 usec)

Figure 10.14.

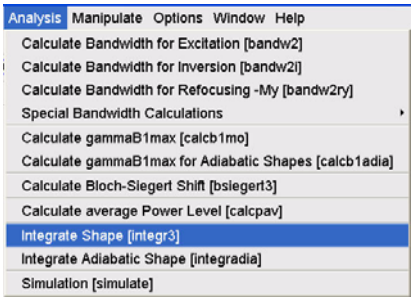


12. Write down the Delta T value [usec] (e.g. **3714.7 usec**)

13. Click on update parameters

14. In the main menu click on ‘**Analysis**’, select ‘**Integrate Shape**’

Figure 10.15.



15. Make the following change:

Total rotation [degree] = **90**

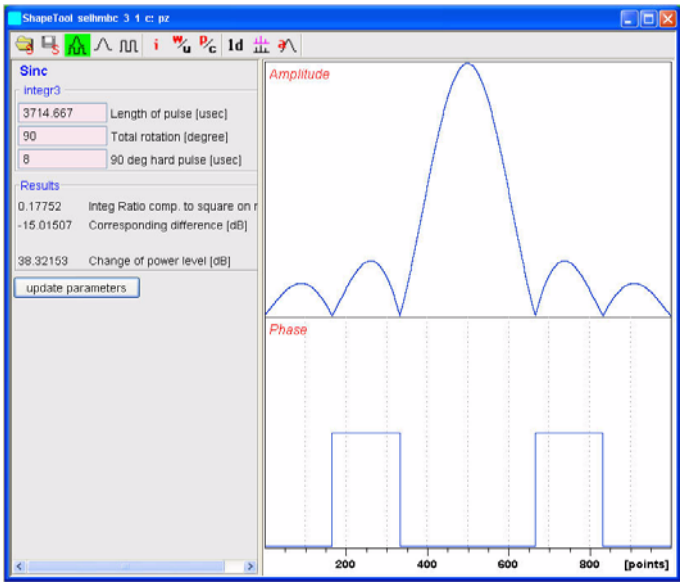
16. Press the ‘Enter’ key

17. Make the following change:

90 deg. hard pulse [usec] = (p3 from step 8 in 6.1.6 e.g. 8)

18. Press the 'Enter' key

Figure 10.16.



19. Write down the change of power level [dB] value (e.g. 38.32156 dB)

20. Click on  to close the Shape Tool window

Setting up the acquisition parameters *10.1.8*

1. Select the '**AcquPars**' tab by clicking on it

2. Make the following changes:

NS = 32

F1 SW [ppm] = value from step 4 in 6.1.6 (e.g. 20)

O2P [ppm} = value from step 5 in 6.1.6 (e.g. 172)

3. Click on  to display the pulsprogram parameters

4. Make the following changes:

P13 [us] = value from step 12 in 6.1.7 (e.g. 3714.7)

SP14 [dB] = (value from step 19 in 6.1.7) + (PL2) (e.g. 35.42)

Running the experiment *10.1.9*

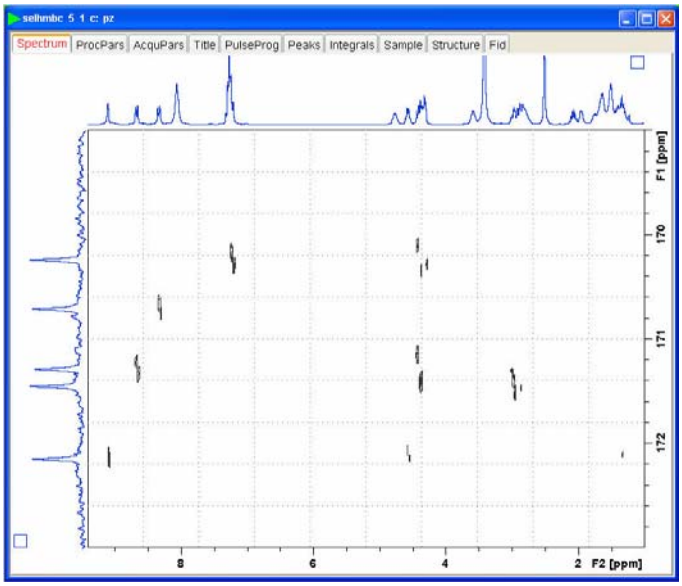
1. Select the '**Spectrum**' tab by clicking on it

2. Click on  to start the acquisition

3. Type xfb to process the 2-D data

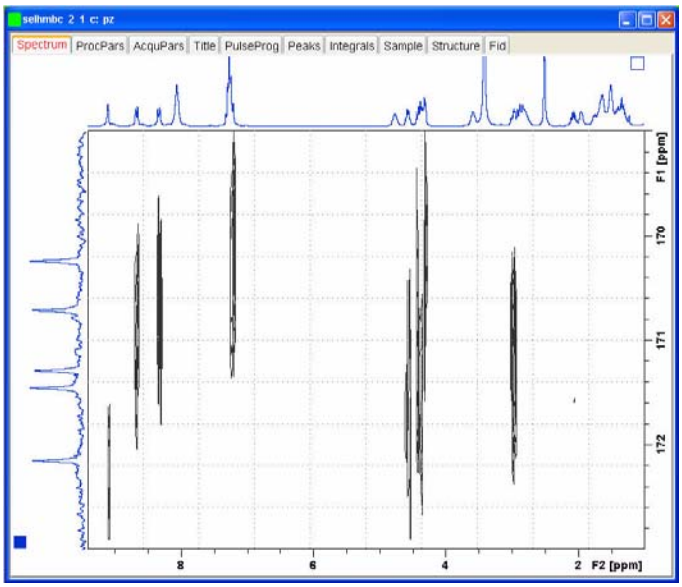
4. Expand the 2-D spectrum

Figure 10.17.



5. Compare the result of the selective HMBC against the regular HMBC in 6.1.3

Figure 10.18.



NOTE: The cross peaks in the selective HMBC show nice separation do to the increased resolution in F1, compared to the regular HMBC. The projections are external high resolution spectra.

Notes: