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

Advanced Experiments User Guide

Version 003

This manual was written by

Peter Ziegler

Billerica, Massachusetts, USA

P/N: B4722
DWG-Nr.: 003

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-biospin.com

2-D Inverse Experiments

2

2D edited HSQC

2.1

Sample:

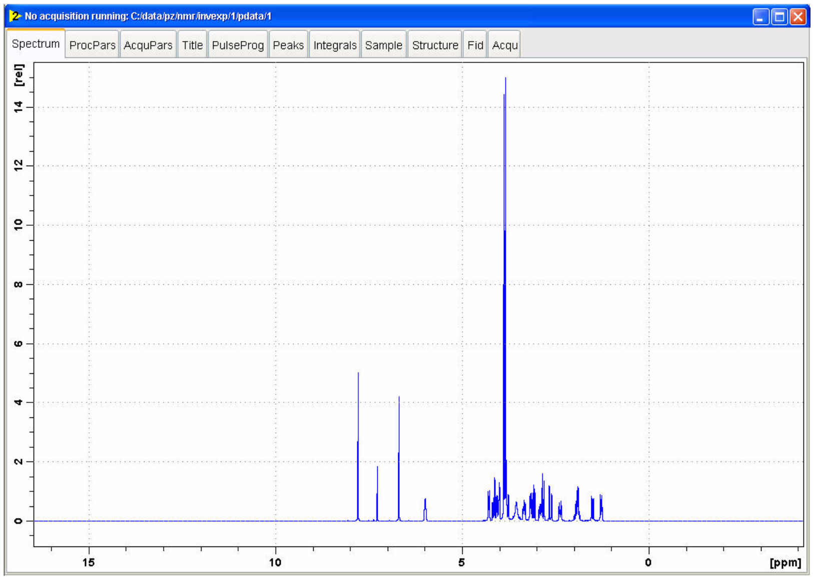
30 mg Brucine in CDCl3

Reference 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.



No acquisition running: C:/data/pz/nmr/invexp/1/pdata/1

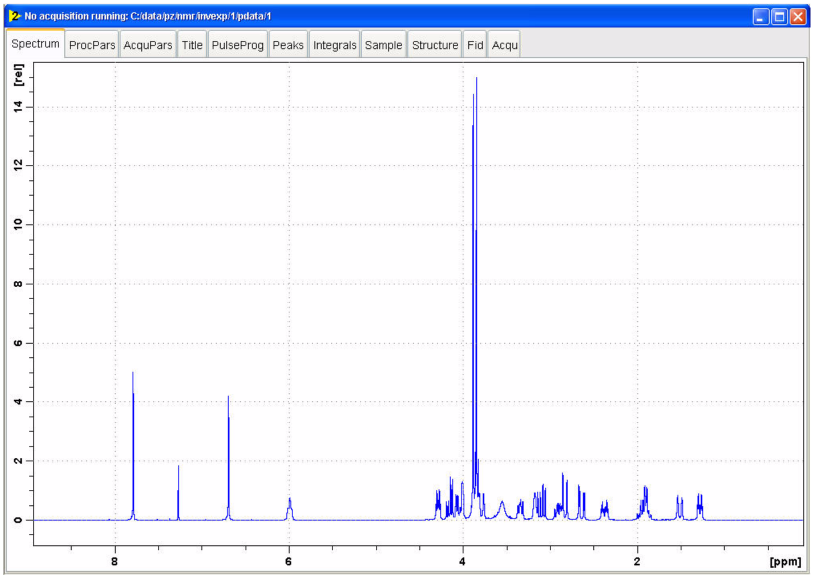
2. Type **wrpa 2** on the command line

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

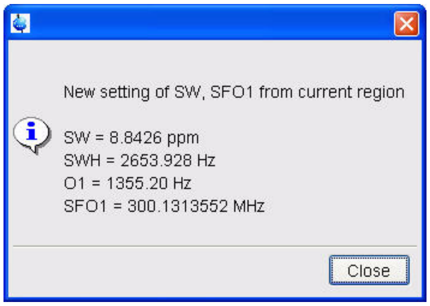
NOTE: You may exclude the solvent peak, if it falls outside of the region of interest.

Figure 2.2.



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

Figure 2.3.



New setting of SW, SFO1 from current region

SW = 8.8426 ppm
SWH = 2653.928 Hz
O1 = 1355.20 Hz
SFO1 = 300.1313552 MHz

6. Write down the value of SW, rounding off to the nearest 1/10th of a ppm (e.g. **8.8** ppm)

7. Write down the value of O1, rounding off to the nearest Hz (e.g. **1355** 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 (e.g. **8.8**)

O1 [Hz] = value from step 7, Preparation experiment 2.1.1 (e.g. **1355**)

SOLVENT = **CDCl3**

All Bruker 2D inverse parameter sets use 13C in the F1 dimension. Sweep width and O1 are 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 (e.g. **0**)

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. Type **rga**

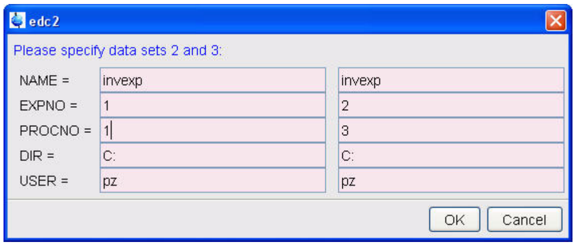
2. Type **zg** to start the acquisition

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. For executing the processing AU program the command xaup may be used.

1. Type **edc2**

Figure 2.4.



Please specify data sets 2 and 3:

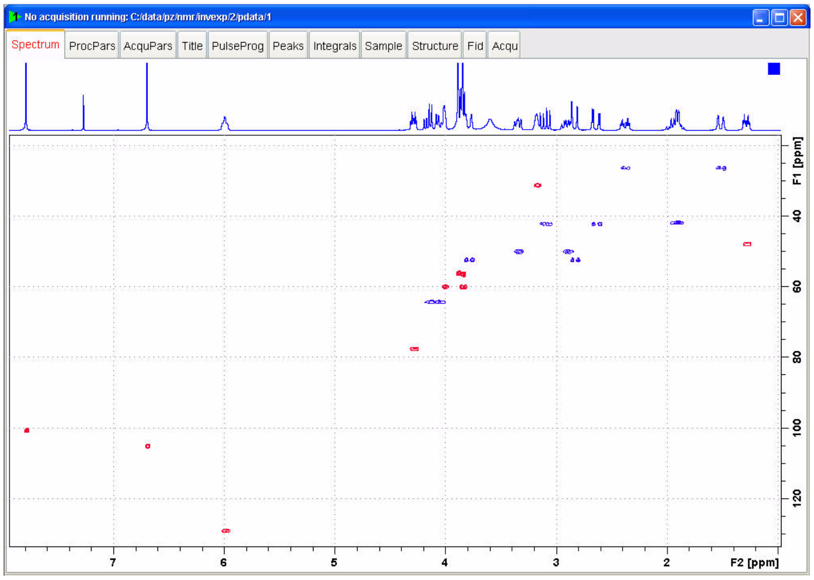
NAME =	invexp	invexp
EXPNO =	1	2
PROCNO =	1	3
DIR =	C:	C:
USER =	pz	pz

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.



No acquisition running: C:/data/pz/nmr/invexp/2/pdata/1

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*

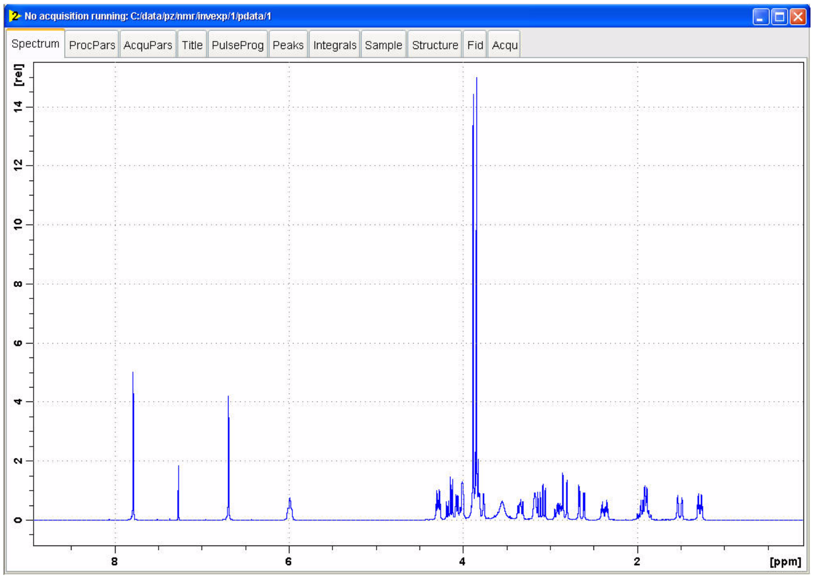
Sample:

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 HMBCGP 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 (e.g. **8.8**)
O1 [Hz] = value from step 7, Preparation experiment 2.1.1 (e.g. **1355**)
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 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. Type **rga**

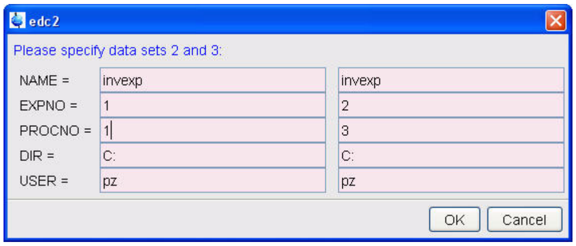
2. Type **zg** to start the acquisition

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. For executing the processing AU program the command xaup may be used.

1. Type **edc2**

Figure 2.7.



edc2

Please specify data sets 2 and 3:

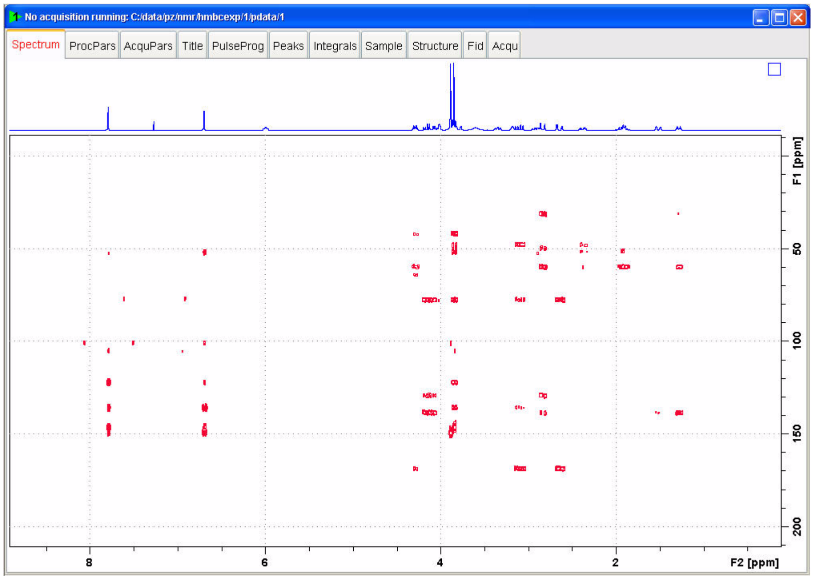
NAME =	invexp	invexp
EXPNO =	1	2
PROCNO =	1	3
DIR =	C:	C:
USER =	pz	pz

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.



No acquisition running: C:/data/pz/nmr/hmbcexp/1/pdata/1

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, then 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.

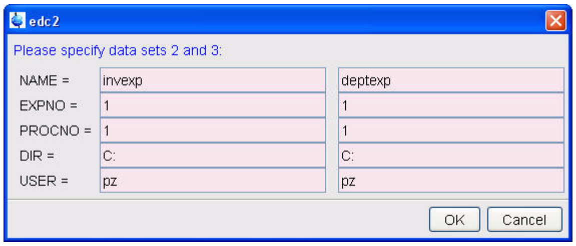
Creating the external projection spectrum *2.3.1*

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.



Please specify data sets 2 and 3:

NAME =	invexp	deptexp
EXPNO =	1	1
PROCNO =	1	1
DIR =	C:	C:
USER =	pz	pz

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

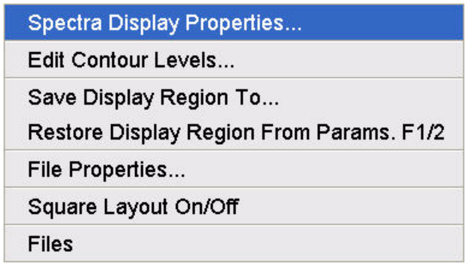
5. Enter the EXPNO and PROCNO of the DEPT135 spectrum into the second column (data set 3)

6. Click on OK

7. Type **xaup**

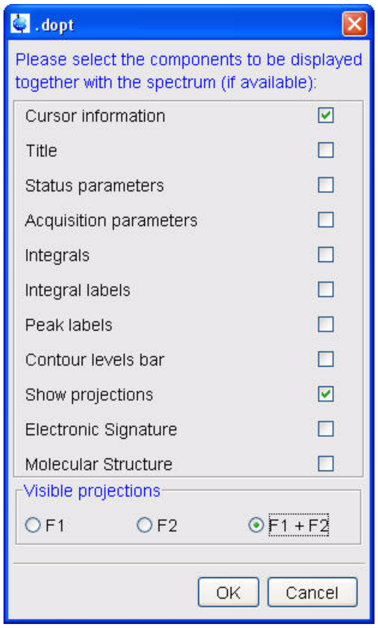
8. Right click inside the spectrum window

Figure 2.10.



9. Select **'Spectra Properties'** by clicking on it

Figure 2.11.



.dopt

Please select the components to be displayed together with the spectrum (if available):

- Cursor information ☑
- Title ☐
- Status parameters ☐
- Acquisition parameters ☐
- Integrals ☐
- Integral labels ☐
- Peak labels ☐
- Contour levels bar ☐
- Show projections ☑
- Electronic Signature ☐
- Molecular Structure ☐

Visible projections

○ F1 ○ F2 ● F1 + F2

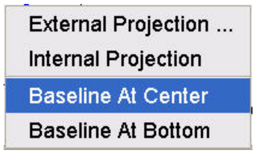
10. Enable **'Show projections'**

11. Enable Visible projections **'F1 + F2'**

12. Click on OK

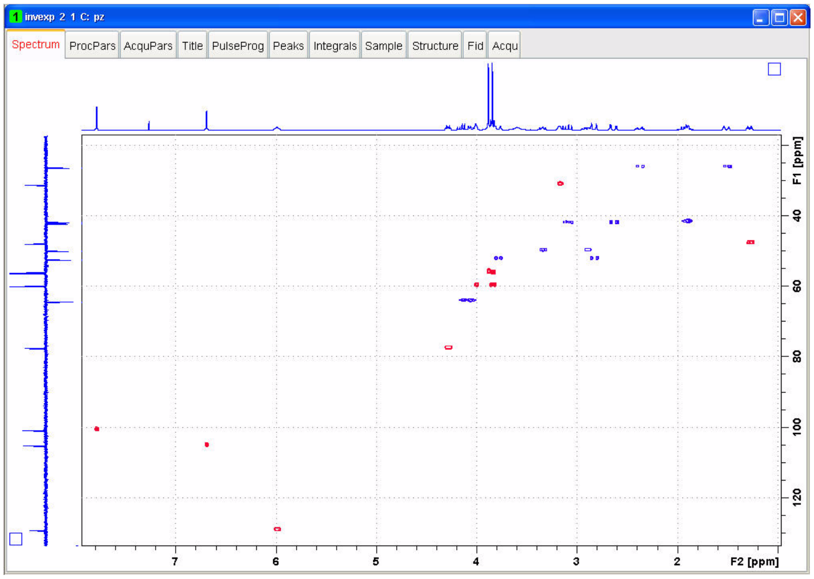
13. Right click on the F1 projection

Figure 2.12.



14. Select **'At Center'** by clicking on it

Figure 2.13.

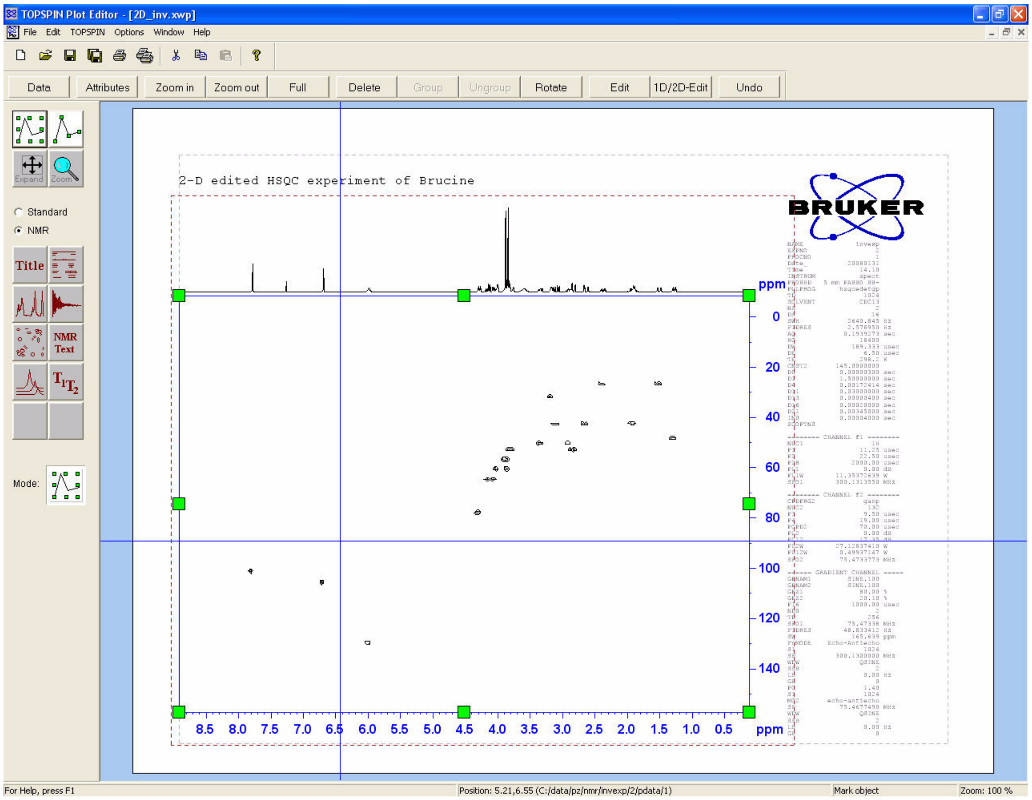


NOTE: Discard the plot

Creating the plot template 2.3.2

1. Type **viewxwinplot**

Figure 2.14.

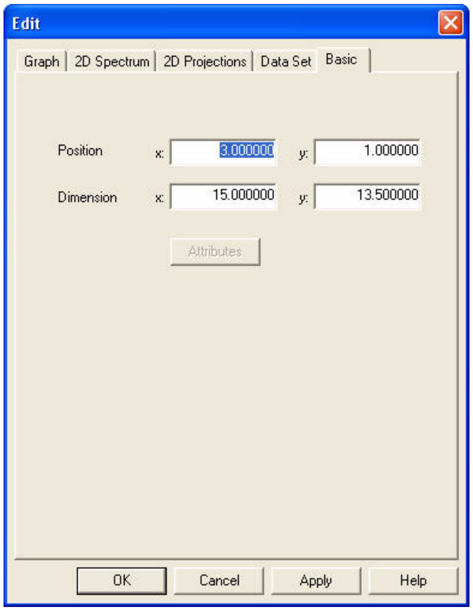


2. Click inside the spectrum window to display the green handles

3. Click on Edit

4. Select the ‘**Basic**’ tab

Figure 2.15.



Position x: 3.000000 y: 1.000000

Dimension x: 15.000000 y: 13.500000

4. Make the following changes:

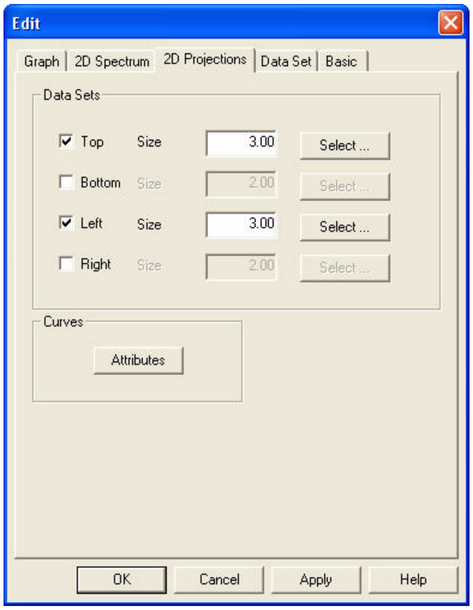
Position X = **3**

Dimension X = **15**

6. Click on Apply

7. Select the ‘**2D Projection**’ tab

Figure 2.16.



☑ Top Size 3.00

☐ Bottom Size 2.00

☑ Left Size 3.00

☐ Right Size 2.00

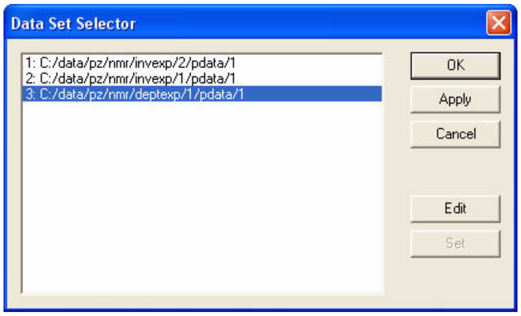
8. Enable ‘**Data set left**’

9. Make the following change:

Size = **3**

10. Click on Select ...

Figure 2.17.



1: C:/data/pz/nmr/invexp/2/pdata/1
2: C:/data/pz/nmr/invexp/1/pdata/1
3: C:/data/pz/nmr/deptexp/1/pdata/1

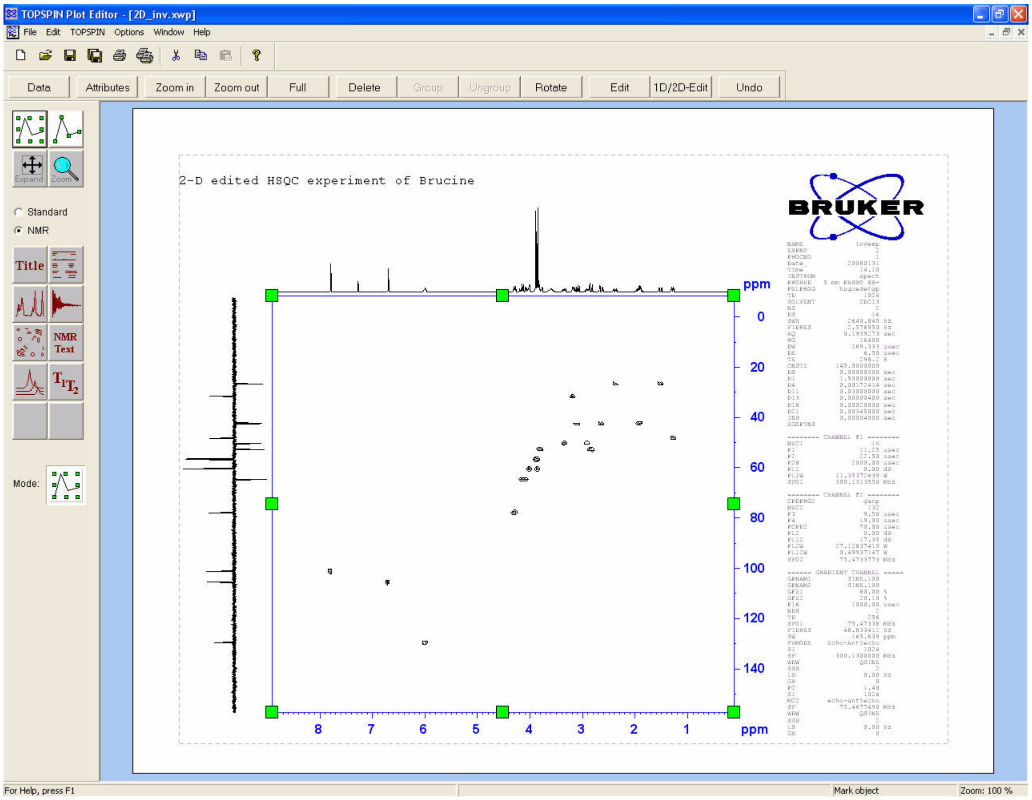
11. Select the DEPT data set path

12. Click on OK

13. Click on Apply

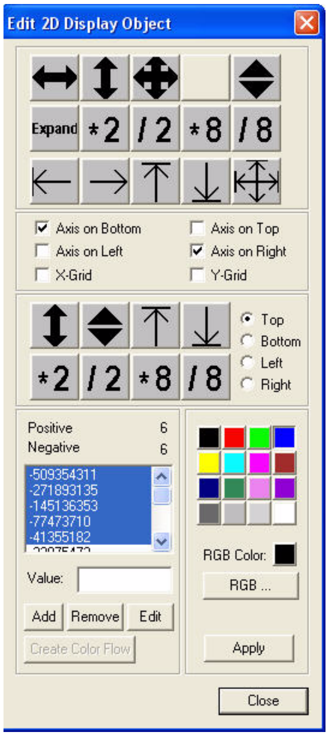
14. Click on OK

Figure 2.18.



15. Click on 1D/2D-Edit

Figure 2.19.



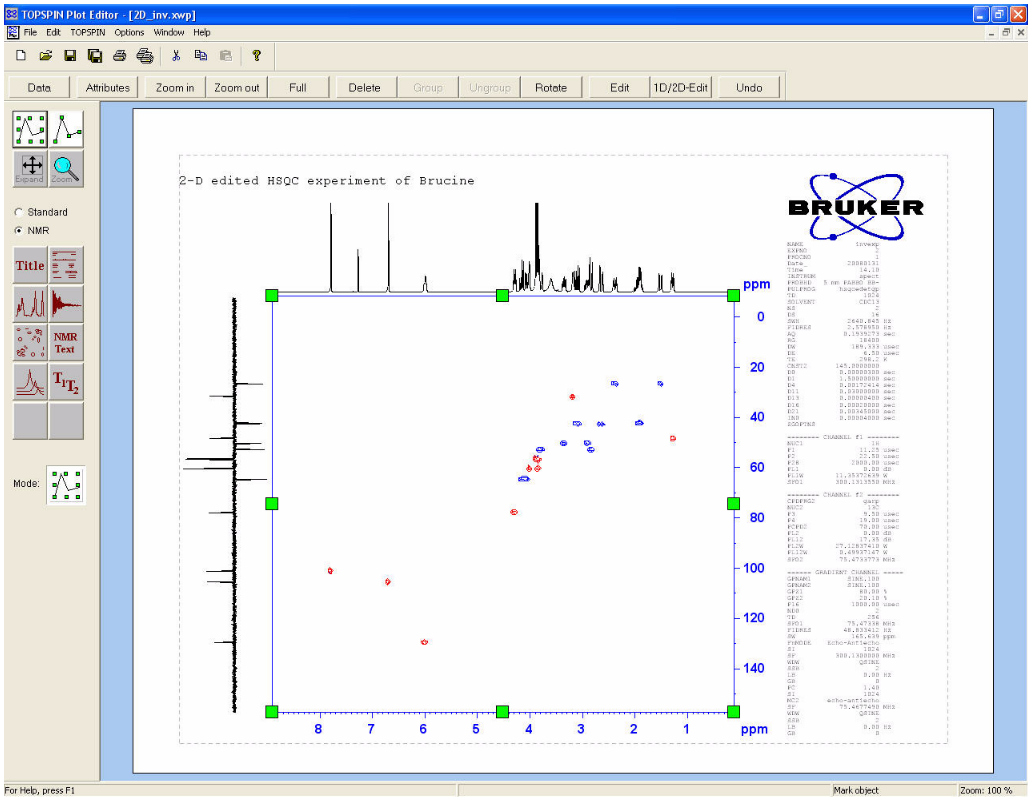
☑ Axis on Bottom
☐ Axis on Top
☐ Axis on Left
☑ Axis on Right
☐ X-Grid
☐ Y-Grid

● Top
○ Bottom
○ Left
○ Right

-509354311
-271893135
-145136353
-77473710
-41355182

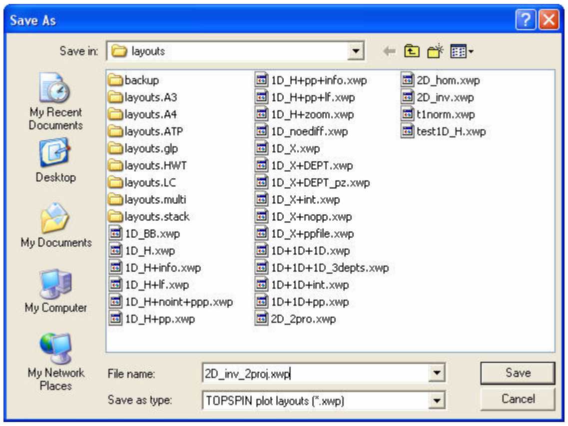
16. Select all negative contour levels
17. Click in the blue color button
18. Click on Apply
19. Select all positive levels
20, Click on the red color button
21. Click on Apply
22. Adjust the contour level using the *2 /2 or ▲▼ buttons
23. Click on Close

Figure 2.20.



24. Click on ‘**File**’ in the main menu bar and select ‘**Save as**’

Figure 2.21.



File name: 2D_inv_2proj.xwp

Save as type: TOPSPIN plot layouts (*.xwp)

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

---

25. Change Filename to **2D_inv_2proj.xwp**

26. 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.

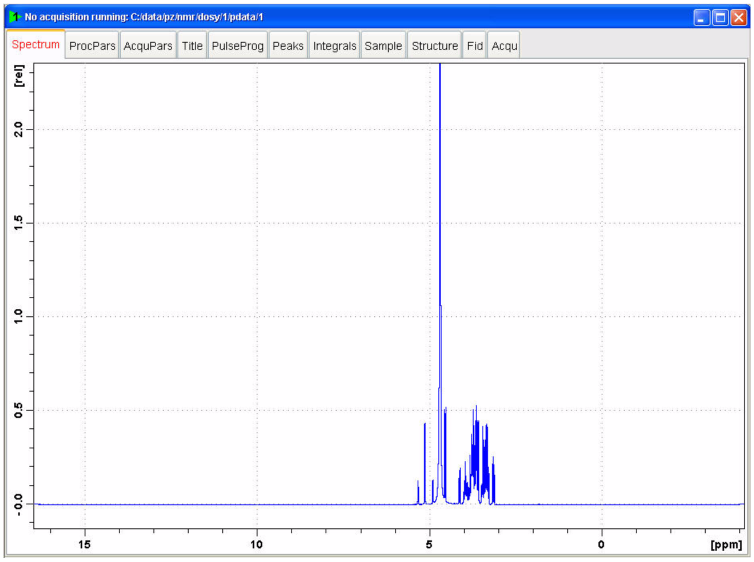
Sample:

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.



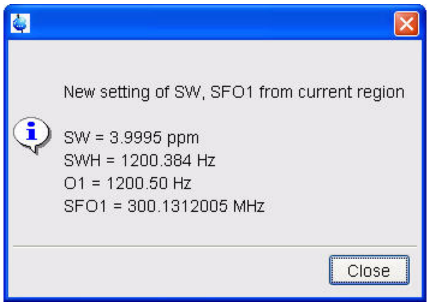
2. Type **wrpa 2** on the command line

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.



New setting of SW, SFO1 from current region

SW = 3.9995 ppm
SWH = 1200.384 Hz
O1 = 1200.50 Hz
SFO1 = 300.1312005 MHz

6. Click on Close

7. Type **td 16k**

8. Type **si 8k**

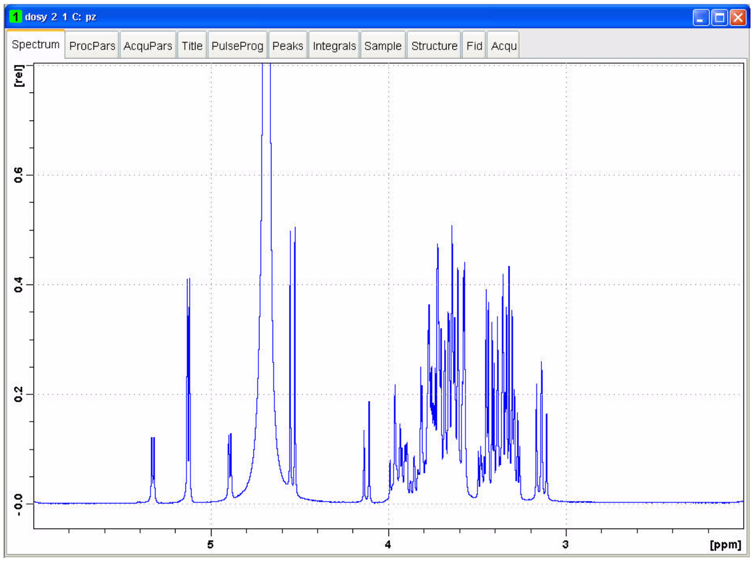
9. Type **zg**

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**

D20[s] = **0.1**

GPZ6[%] = **2**

GPZ7[%] = **-17.13**

P30[us] = **1800**

5. Type **rga**

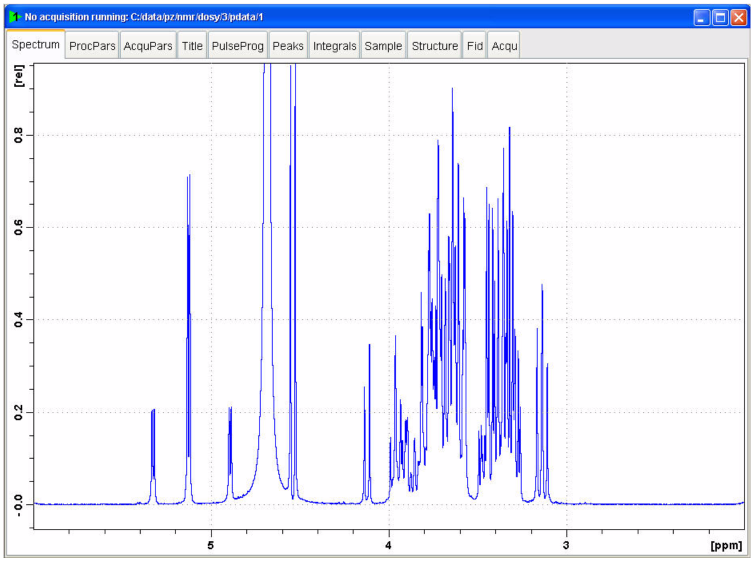
6. Type **zg**

7. Type **ef**

8. Type **apk**

9. Type **abs**

Figure 3.4.



No acquisition running: C:/data/pz/nmr/dosy/3/pdata/1

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**

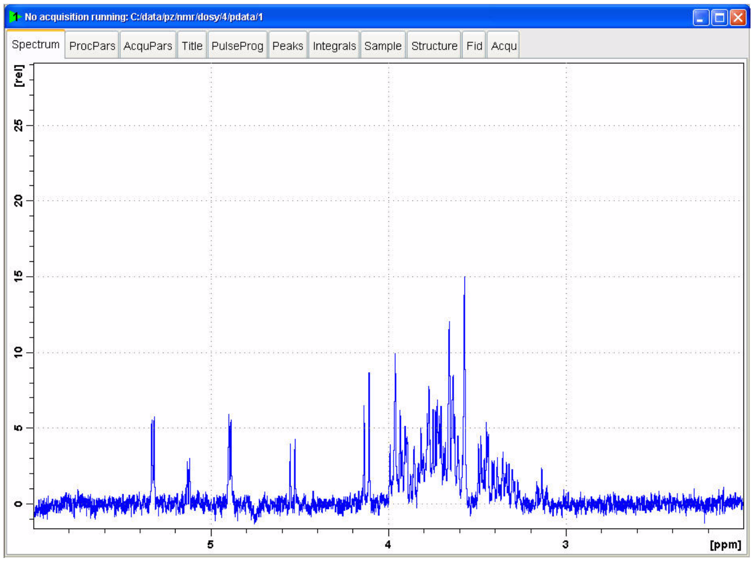
14. Type **zg**

15. Type **ef**

16. Type **apk**

17. Type **abs**

Figure 3.5.

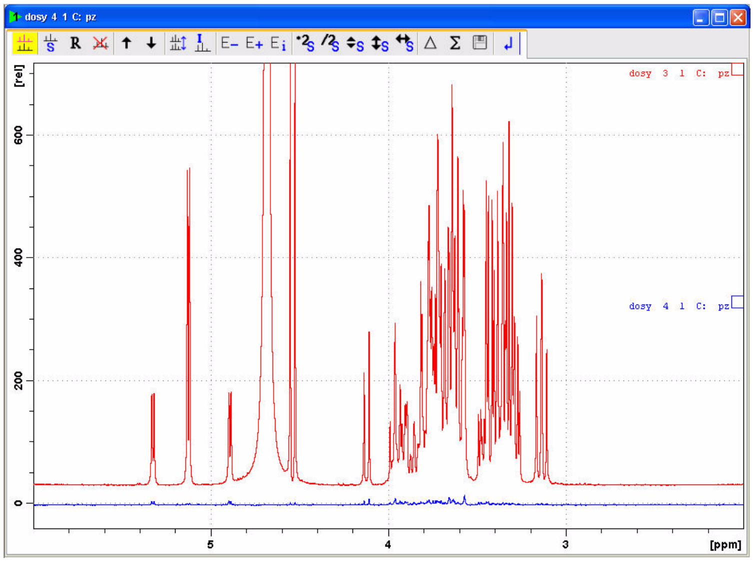


No acquisition running: C:/data/pz/nmr/dosy/4/pdata/1

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 ~95. If the difference is less then 50, change P30 and or D20 in both data sets.

20. Click on ↲ to exit the multiple display

Acquisition 3.1.3

1. Type **iexpno**

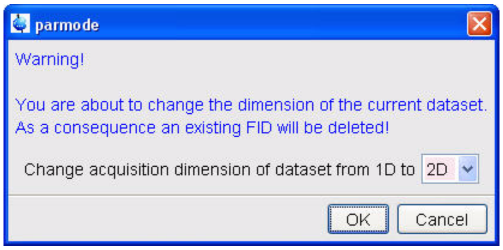
2. Select the ‘**AcquPars**’ tab by clicking on it

3. Make the following changes:

PULPROG = **stebpgp1s**

4. Click on ⇣12 to change the acqu dimension

Figure 3.7.



Warning!

You are about to change the dimension of the current dataset.
As a consequence an existing FID will be deleted!

Change acquisition dimension of dataset from 1D to 2D

5. select ‘**2D**’

6. Click on OK

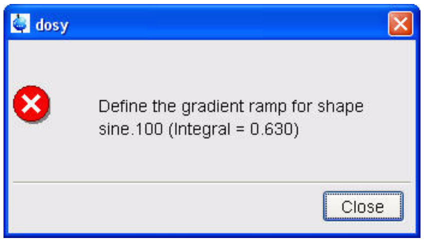
7. Make the following changes:

TDF1 = **16**

FnMODE = **QF**

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

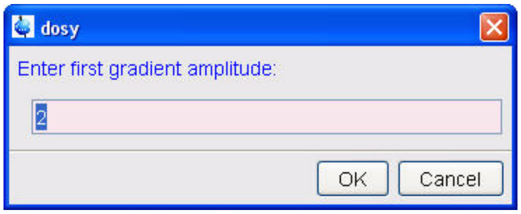
Figure 3.8.



Define the gradient ramp for shape
sine.100 (Integral = 0.630)

9. Click on Close

Figure 3.9.



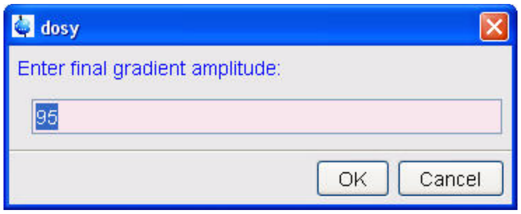
Enter first gradient amplitude:

2

10. Enter **2** for first gradient amplitude

11. Click on OK

Figure 3.10.



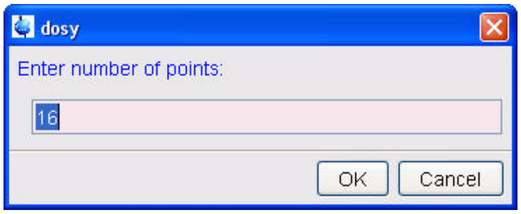
Enter final gradient amplitude:

95

12. Enter **95** for final gradient amplitude

13. Click on OK

Figure 3.11.



Enter number of points:

16

14. Enter **16** for the number of points

15. Click on OK

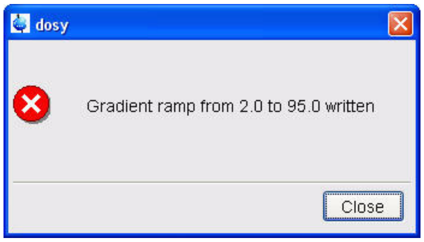
Figure 3.12.

ramp type (l/q/e {linear/squared/exponential}):

16. Enter **l** for ramp type

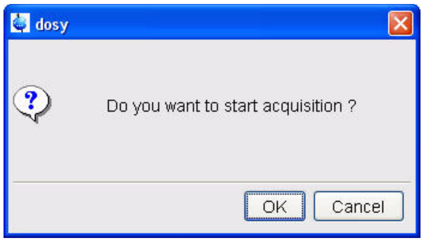
17. Click on OK

Figure 3.13.



Gradient ramp from 2.0 to 95.0 written

Figure 3.14.



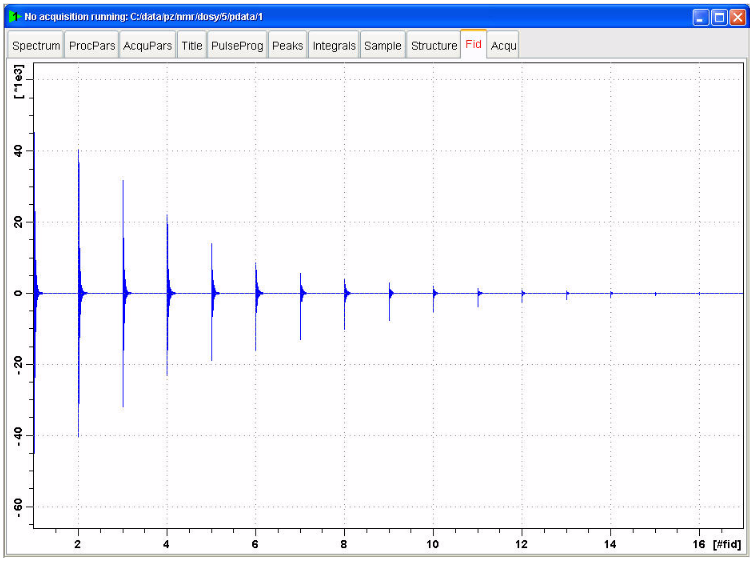
Do you want to start acquisition ?

18. Click on OK to start the acquisition

Processing *3.1.4*

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

Figure 3.15.



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. Make the following changes:

SI [F1] = **16**

PH_mod [F1] = **no**

PH_mod [F2] = **pk**

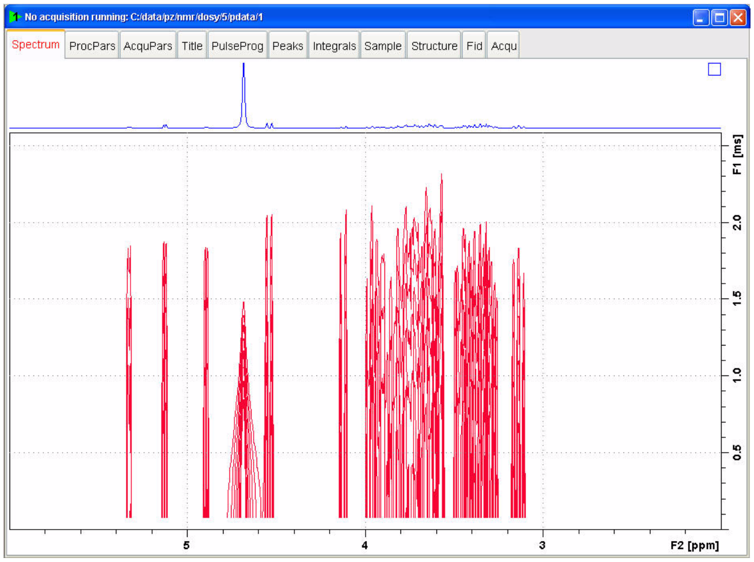
4. Type **xf2** on the command line

5. Type **abs2** on the command line

6.Type **setdiffparm** on the command line

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

Figure 3.16.

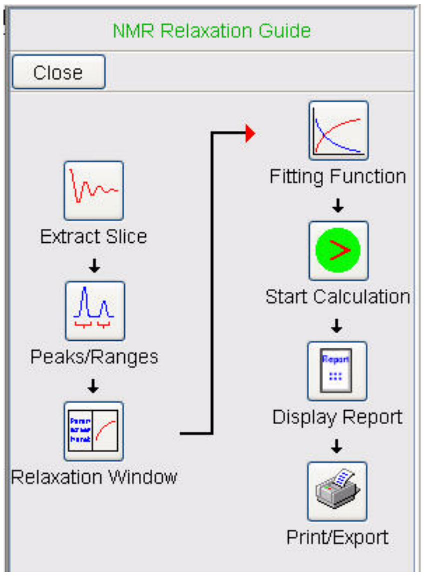


Calculating the diffusion coefficient 3.1.5

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

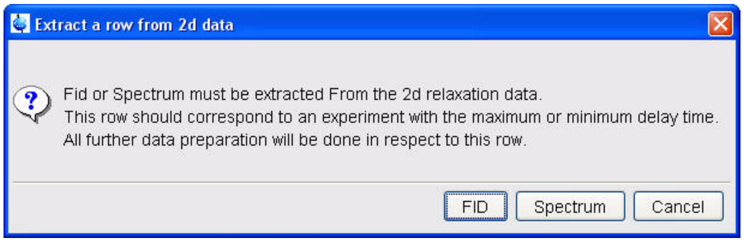
1. Click on ‘**Analysis**’ in the main menu
2. Select ‘**T1/T2 Relaxation**’

Figure 3.17.



3. Click on to extract slice

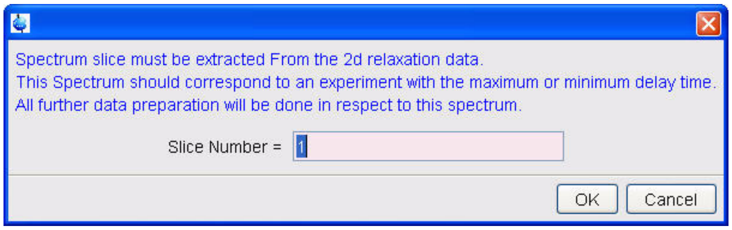
Figure 3.18.



Fid or Spectrum must be extracted From the 2d relaxation data.
This row should correspond to an experiment with the maximum or minimum delay time.
All further data preparation will be done in respect to this row.

4. Click on Spectrum

Figure 3.19.



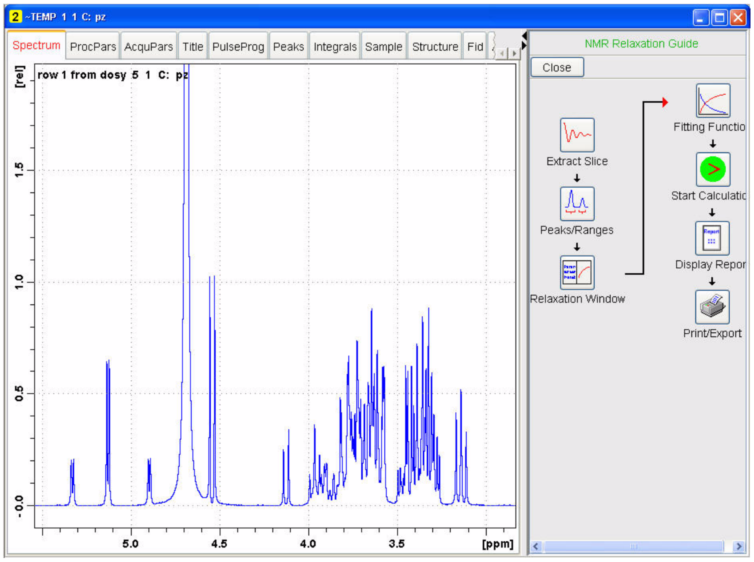
Spectrum slice must be extracted From the 2d relaxation data.
This Spectrum should correspond to an experiment with the maximum or minimum delay time.
All further data preparation will be done in respect to this spectrum.

Slice Number = 1

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

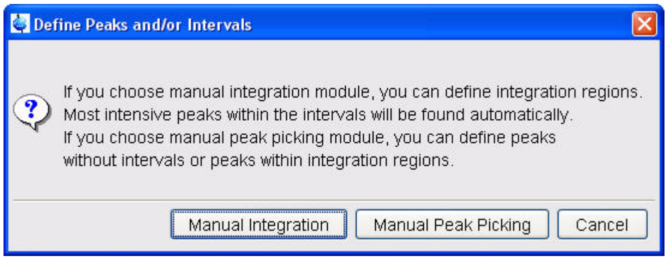
6. Click on OK

Figure 3.20.



7. Click on to define ranges

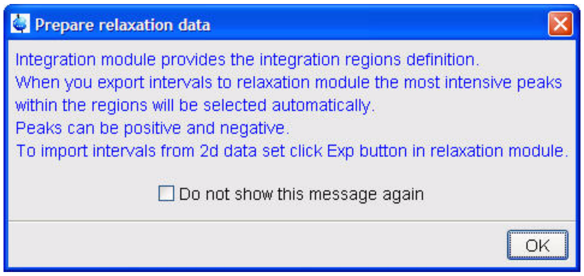
Figure 3.21.



If you choose manual integration module, you can define integration regions.
Most intensive peaks within the intervals will be found automatically.
If you choose manual peak picking module, you can define peaks
without intervals or peaks within integration regions.

8. Click on Manual Integration

Figure 3.22.



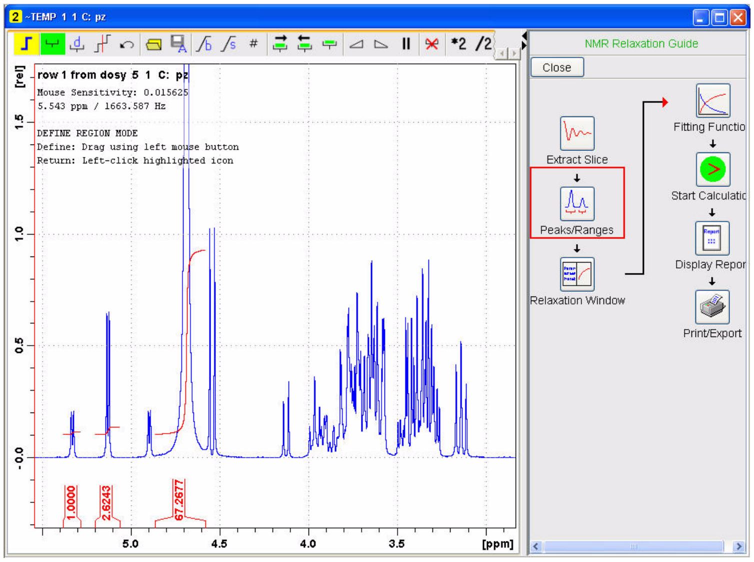
Integration module provides the integration regions definition.
When you export intervals to relaxation module the most intensive peaks
within the regions will be selected automatically.
Peaks can be positive and negative.
To import intervals from 2d data set click Exp button in relaxation module.

☐ Do not show this message again

9. Click on OK

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

Figure 3.23.



```
row 1 from dosy 5 1 C: pz
Mouse Sensitivity: 0.015625
5.543 ppm / 1663.587 Hz

DEFINE REGION MODE
Define: Drag using left mouse button
Return: Left-click highlighted icon
```

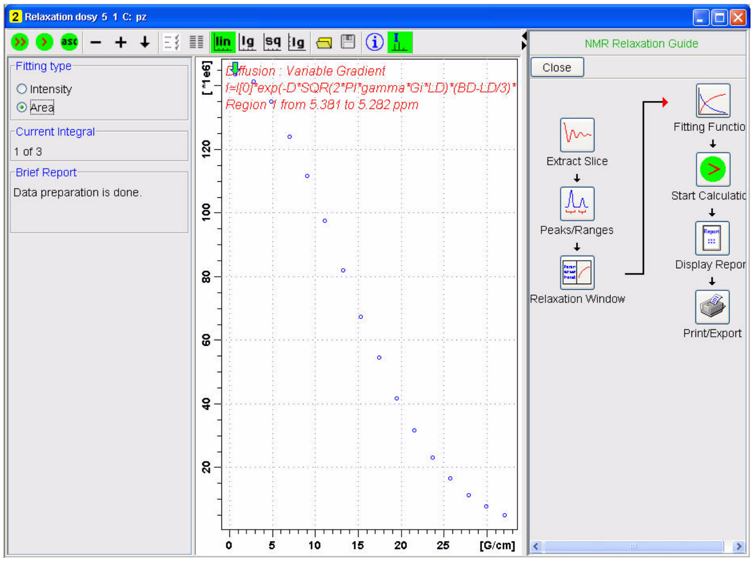
11. Click on

Figure 3.24.

12. Select ‘**Export Region To Relaxation Module**’ by clicking on it

13. In the Guide window, click on ‘Relaxation Window’

Figure 3.25.



1 of 3

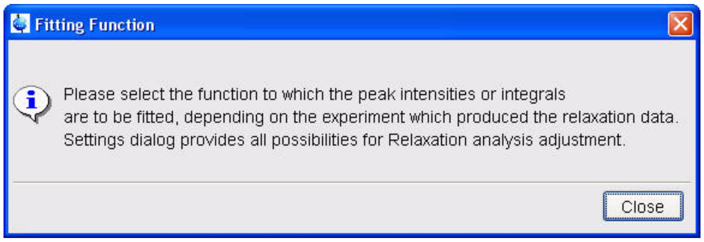
Data preparation is done.

Diffusion : Variable Gradient
f=I[0]*exp(-D*SQR(2*PI*gamma*Gi*LD)*(BD-LD/3)*
Region 1 from 5.381 to 5.282 ppm

14. Enable ‘**Area**’

15. In the guide window, click on ‘Fitting Function’

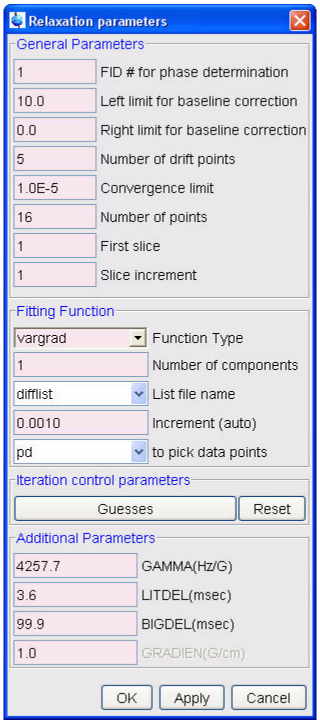
Figure 3.26. 1



Please select the function to which the peak intensities or integrals are to be fitted, depending on the experiment which produced the relaxation data. Settings dialog provides all possibilities for Relaxation analysis adjustment.

16. Click on Close

Figure 3.27.



1 FID # for phase determination
10.0 Left limit for baseline correction
0.0 Right limit for baseline correction
5 Number of drift points
1.0E-5 Convergence limit
16 Number of points
1 First slice
1 Slice increment

vargrad Function Type
1 Number of components
difflist List file name
0.0010 Increment (auto)
pd to pick data points

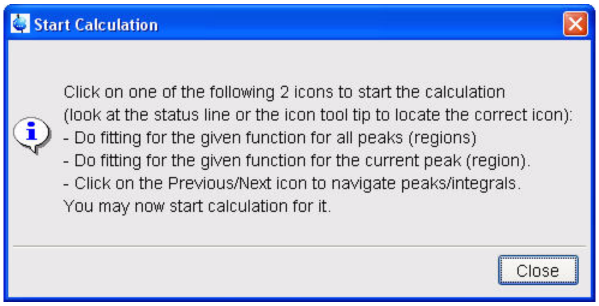
4257.7 GAMMA(Hz/G)
3.6 LITDEL(msec)
99.9 BIGDEL(msec)
1.0 GRADIEN(G/cm)

17. In the ‘**Fitting Function**’ section, select ‘**vargrad**’ and ‘**difflist**’

18. Click on OK

19. In the guide window, click on > ‘Start Calculation’

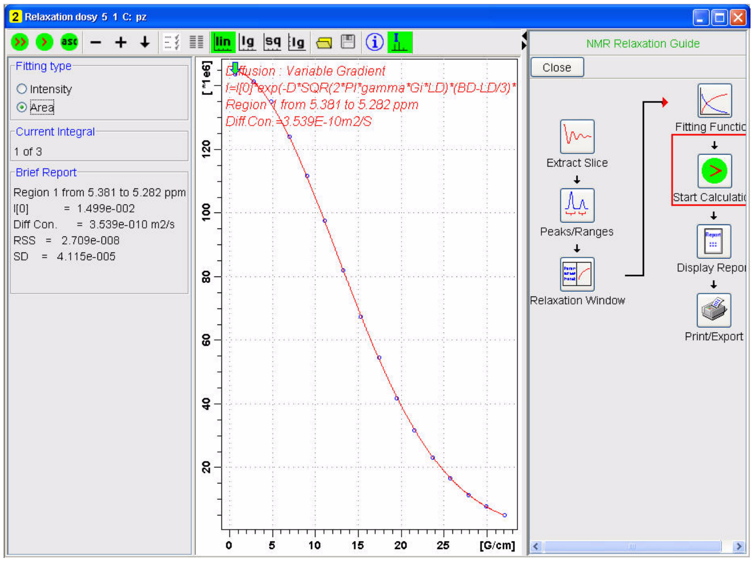
Figure 3.28.



Click on one of the following 2 icons to start the calculation
(look at the status line or the icon tool tip to locate the correct icon):
- Do fitting for the given function for all peaks (regions)
- Do fitting for the given function for the current peak (region).
- Click on the Previous/Next icon to navigate peaks/integrals.
You may now start calculation for it.

20. Click on Close

Figure 3.29.



Fitting type

○ Intensity

◉ Area

Current Integral

1 of 3

Brief Report

Region 1 from 5.381 to 5.282 ppm

I[0] = 1.499e-002

Diff Con. = 3.539e-010 m2/s

RSS = 2.709e-008

SD = 4.115e-005

Diffusion : Variable Gradient

I=I[0]*exp(-D*SQR(2*PI*gamma*Gi*LD)*(BD-LD/3)*

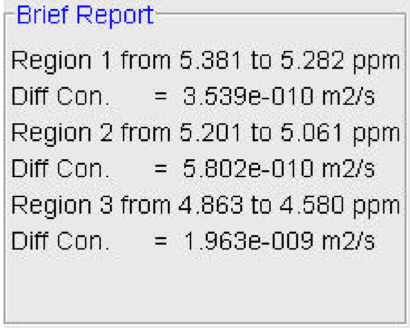
Region 1 from 5.381 to 5.282 ppm

Diff.Con.=3.539E-10m2/S

21. 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 3.30.



Brief Report

Region 1 from 5.381 to 5.282 ppm

Diff Con. = 3.539e-010 m2/s

Region 2 from 5.201 to 5.061 ppm

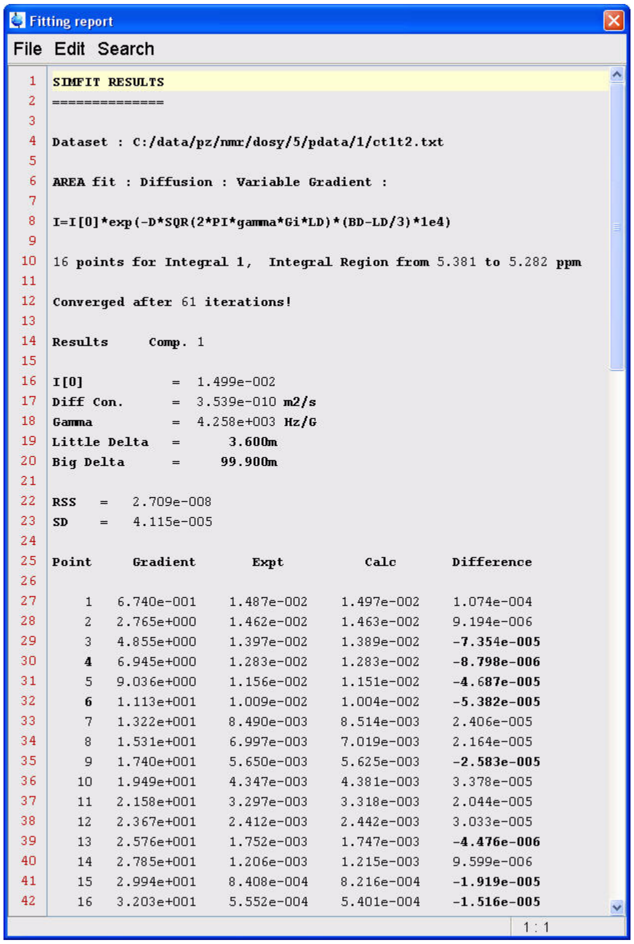
Diff Con. = 5.802e-010 m2/s

Region 3 from 4.863 to 4.580 ppm

Diff Con. = 1.963e-009 m2/s

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

Figure 3.31.



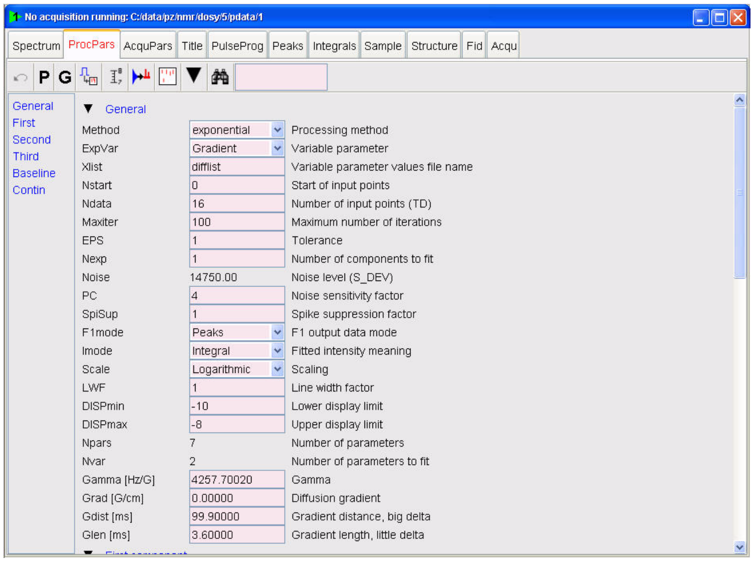
```
SIMFIT RESULTS
==============

Dataset : C:/data/pz/nmr/dosy/5/pdata/1/ct1t2.txt

AREA fit : Diffusion : Variable Gradient :

I=I[0]*exp(-D*SQR(2*PI*gamma*Gi*LD)*(BD-LD/3)*1e4)

16 points for Integral 1,  Integral Region from 5.381 to 5.282 ppm

Converged after 61 iterations!

Results      Comp. 1

I[0]            =   1.499e-002
Diff Con.       =   3.539e-010 m2/s
Gamma           =   4.258e+003 Hz/G
Little Delta    =       3.600m
Big Delta       =      99.900m

RSS    =   2.709e-008
SD     =   4.115e-005

Point      Gradient       Expt          Calc        Difference

    1   6.740e-001    1.487e-002    1.497e-002    1.074e-004
    2   2.765e+000    1.462e-002    1.463e-002    9.194e-006
    3   4.855e+000    1.397e-002    1.389e-002   -7.354e-005
    4   6.945e+000    1.283e-002    1.283e-002   -8.798e-006
    5   9.036e+000    1.156e-002    1.151e-002   -4.687e-005
    6   1.113e+001    1.009e-002    1.004e-002   -5.382e-005
    7   1.322e+001    8.490e-003    8.514e-003    2.406e-005
    8   1.531e+001    6.997e-003    7.019e-003    2.164e-005
    9   1.740e+001    5.650e-003    5.625e-003   -2.583e-005
   10   1.949e+001    4.347e-003    4.381e-003    3.378e-005
   11   2.158e+001    3.297e-003    3.318e-003    2.044e-005
   12   2.367e+001    2.412e-003    2.442e-003    3.033e-005
   13   2.576e+001    1.752e-003    1.747e-003   -4.476e-006
   14   2.785e+001    1.206e-003    1.215e-003    9.599e-006
   15   2.994e+001    8.408e-004    8.216e-004   -1.919e-005
   16   3.203e+001    5.552e-004    5.401e-004   -1.516e-005
```

23. In the guide window, click on ‘Print Report

Displaying the DOSY plot *3.1.6*

1. Click on ☒
2. Click on Close to close the NMR Relaxation Guide
3. Type **eddosy**

Figure 3.32.



General

Method	exponential	Processing method
ExpVar	Gradient	Variable parameter
Xlist	difflist	Variable parameter values file name
Nstart	0	Start of input points
Ndata	16	Number of input points (TD)
Maxiter	100	Maximum number of iterations
EPS	1	Tolerance
Nexp	1	Number of components to fit
Noise	14750.00	Noise level (S_DEV)
PC	4	Noise sensitivity factor
SpiSup	1	Spike suppression factor
F1mode	Peaks	F1 output data mode
Imode	Integral	Fitted intensity meaning
Scale	Logarithmic	Scaling
LWF	1	Line width factor
DISPmin	-10	Lower display limit
DISPmax	-8	Upper display limit
Npars	7	Number of parameters
Nvar	2	Number of parameters to fit
Gamma [Hz/G]	4257.70020	Gamma
Grad [G/cm]	0.00000	Diffusion gradient
Gdist [ms]	99.90000	Gradient distance, big delta
Glen [ms]	3.60000	Gradient length, little delta

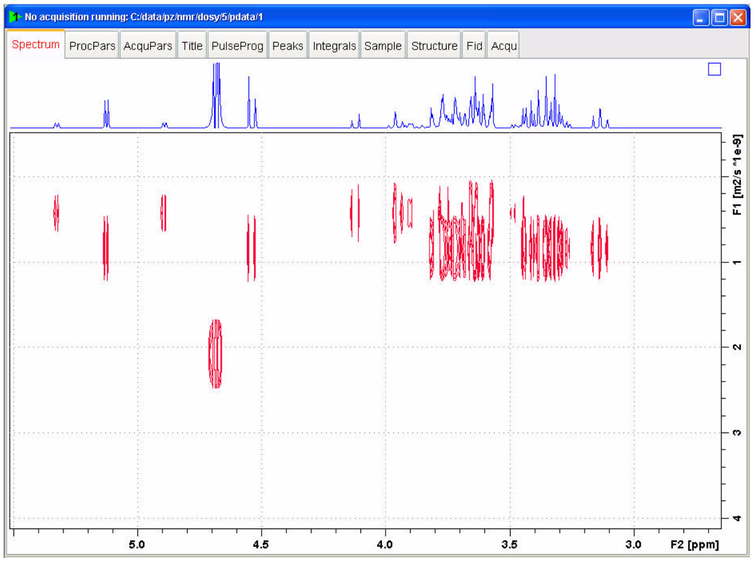
4. Make the following change:

Scale = **Linear**

5. Click on to set the upper and lower limits

6. Click on

Figure 3.33.



Multiplet Analysis

4

Multiplet assignments 4.1

Sample:

100 mg 2, 3,-Dibromopropionic acid in CDCl3

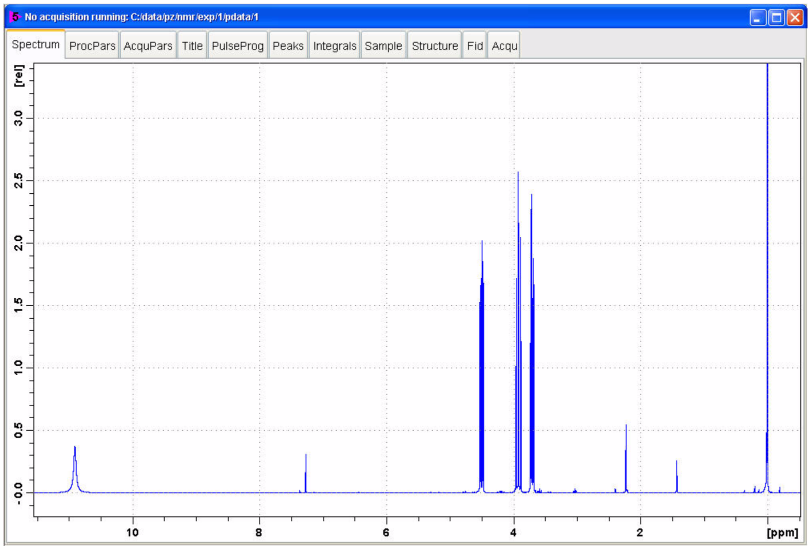
Preparation experiment 4.1.1

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

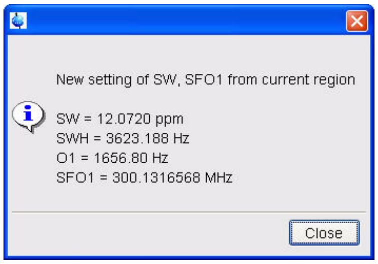
3. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

Figure 4.1.



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

Figure 4.2.



New setting of SW, SFO1 from current region

SW = 12.0720 ppm
SWH = 3623.188 Hz
O1 = 1656.80 Hz
SFO1 = 300.1316568 MHz

5. Click on Close

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. Type **rga**
2. Type **zg** 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.

---

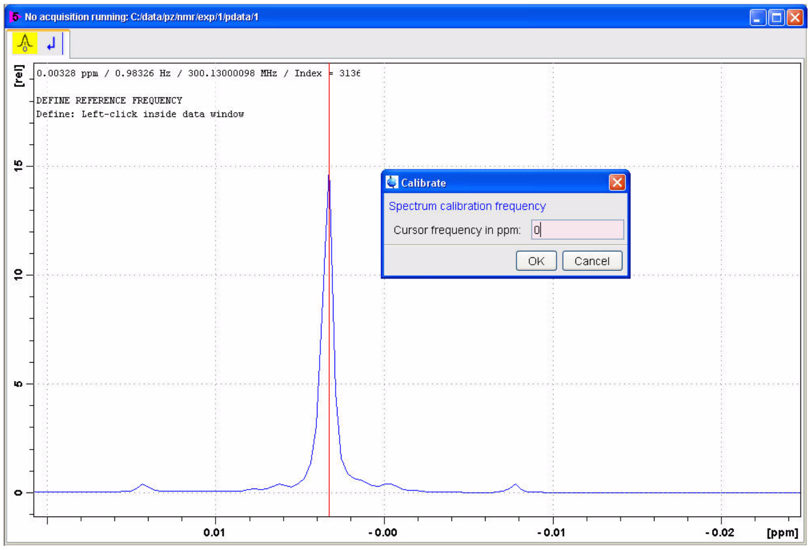
4. Expand the TMS peak

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.



No acquisition running: C:/data/pz/nmr/exp/1/pdata/1

```
0.00328 ppm / 0.98326 Hz / 300.13000098 MHz / Index = 3136

DEFINE REFERENCE FREQUENCY
Define: Left-click inside data window
```

Calibrate

Spectrum calibration frequency

Cursor frequency in ppm: 0

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

9. Click OK

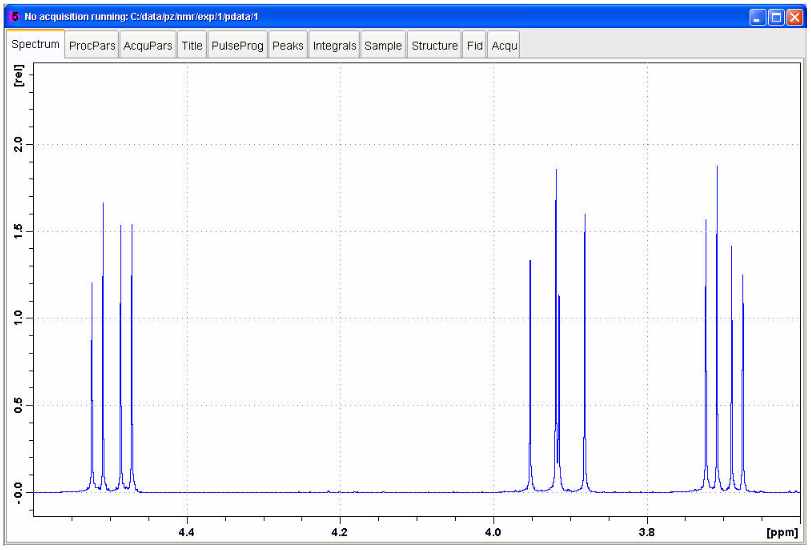
10. Expand the spectrum from 3.6 ppm to 4.6 ppm

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]**’

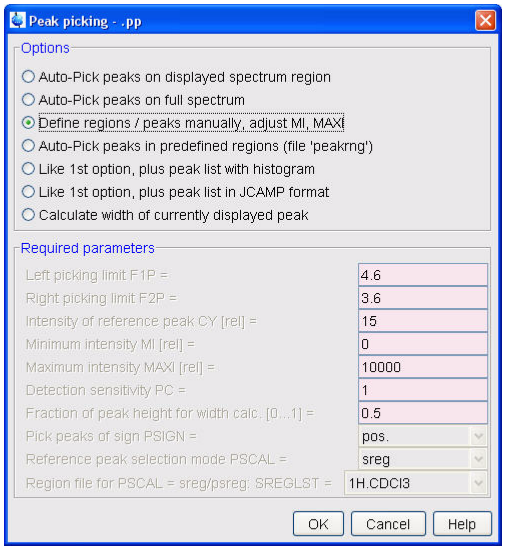
Figure 4.4.



14. Click on ‘**Analysis**’ in the main menu bar

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

Figure 4.5.



Peak picking - .pp

Options

○ Auto-Pick peaks on displayed spectrum region
○ Auto-Pick peaks on full spectrum
● Define regions / peaks manually, adjust MI, MAXI
○ Auto-Pick peaks in predefined regions (file 'peakrng')
○ Like 1st option, plus peak list with histogram
○ Like 1st option, plus peak list in JCAMP format
○ Calculate width of currently displayed peak

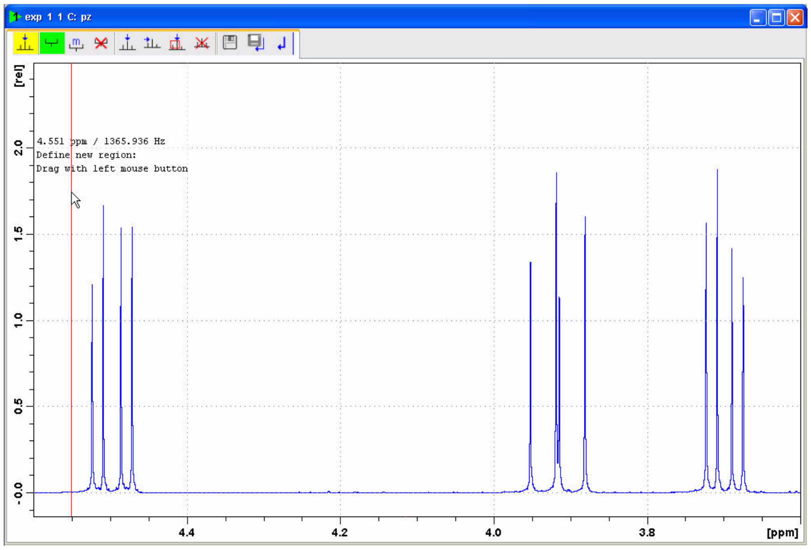
Required parameters

Left picking limit F1P = 4.6
Right picking limit F2P = 3.6
Intensity of reference peak CY [rel] = 15
Minimum intensity MI [rel] = 0
Maximum intensity MAXI [rel] = 10000
Detection sensitivity PC = 1
Fraction of peak height for width calc. [0...1] = 0.5
Pick peaks of sign PSIGN = pos.
Reference peak selection mode PSCAL = sreg
Region file for PSCAL = sreg/psreg: SREGLST = 1H.CDCl3

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

17. Click on OK

Figure 4.6.



4.551 ppm / 1365.936 Hz
Define new region:
Drag with left mouse button

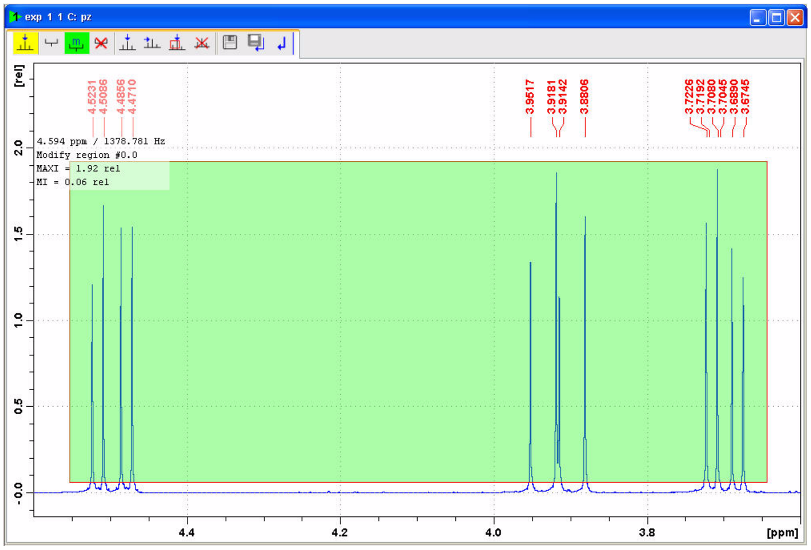
18. Move the cursor line to the left of the multiplet at 4.5 ppm

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.



4.594 ppm / 1378.781 Hz
Modify region #0.0
MAXI = 1.92 rel
MI = 0.06 rel

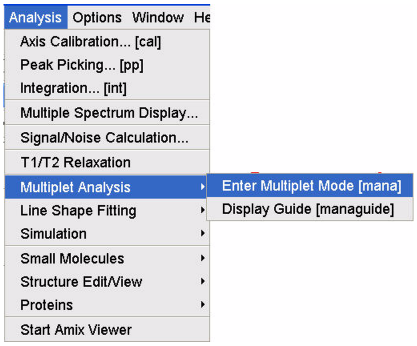
22. Click on

Multiplet assignments *4.1.4*

1. Expand the multiplet at 4.5 ppm

2. Click on ‘**Analysis**’ in the main menu bar

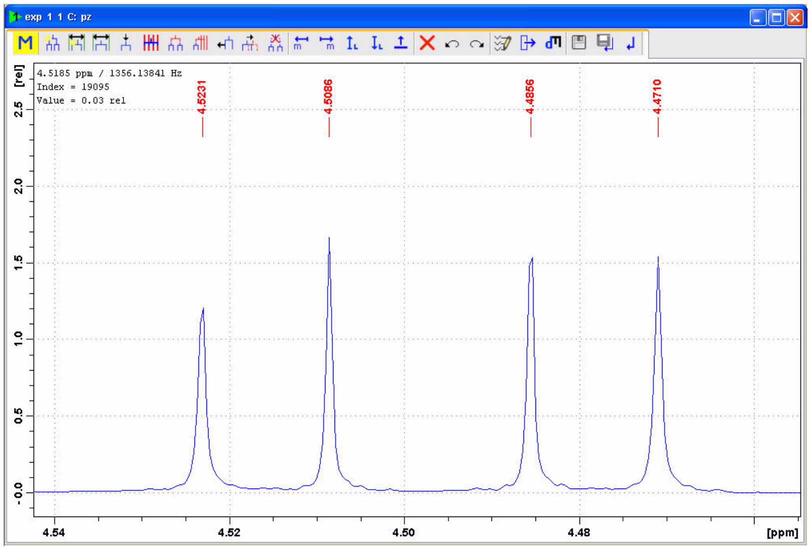
Figure 4.8.



3. Select ‘**Multiplet Analysis**’

4. Select ‘**Enter Multiplet Mode [mana]**’ by clicking on it

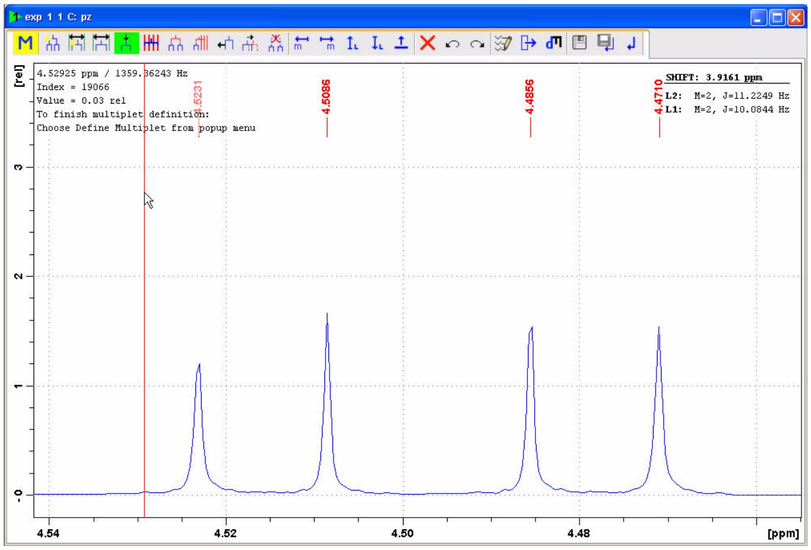
Figure 4.9.



5. Click on ‘Define Multiplets Manually’

6. Place the cursor line to the left of the first peak of the multiplet

Figure 4.10.

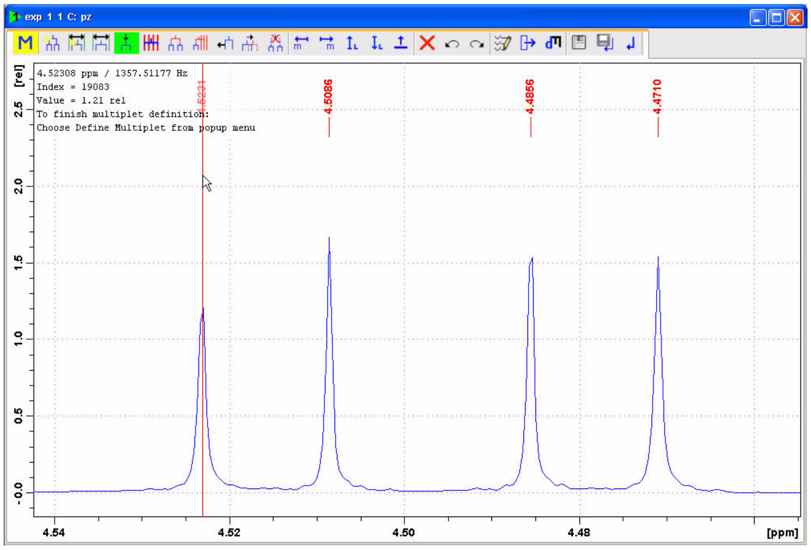


```
4.52925 ppm / 1359.36243 Hz
Index = 19066
Value = 0.03 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 3.9161 ppm
L2:  M=2, J=11.2249 Hz
L1:  M=2, J=10.0844 Hz
```

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 4.11.



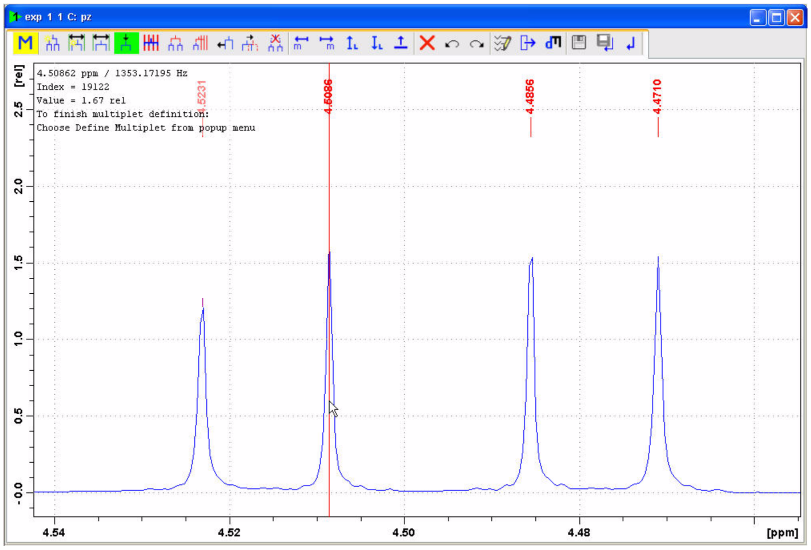
```
4.52308 ppm / 1357.51177 Hz
Index = 19083
Value = 1.21 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

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

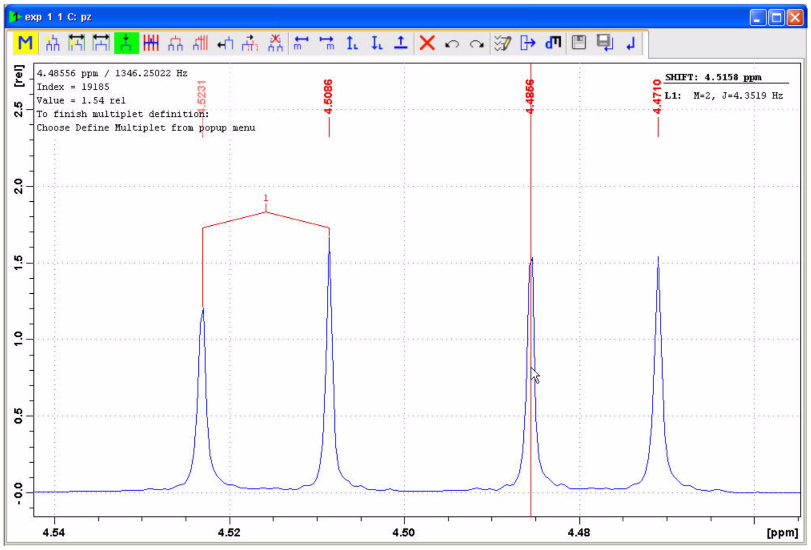
Figure 4.12.



```
4.50862 ppm / 1353.17195 Hz
Index = 19122
Value = 1.67 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

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 4.13.

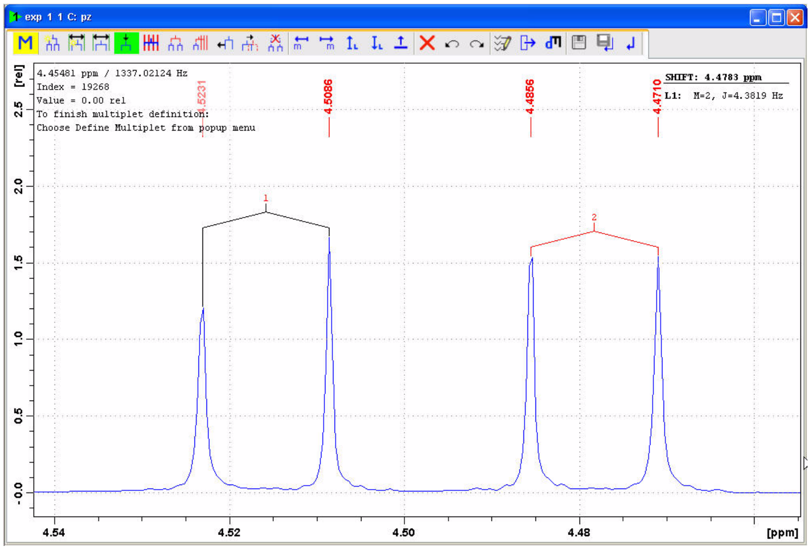


```
4.48556 ppm / 1346.25022 Hz
Index = 19185
Value = 1.54 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 4.5158 ppm
L1:  M=2, J=4.3519 Hz
```

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

Figure 4.14.



4.45481 ppm / 1337.02124 Hz
Index = 19268
Value = 0.00 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu

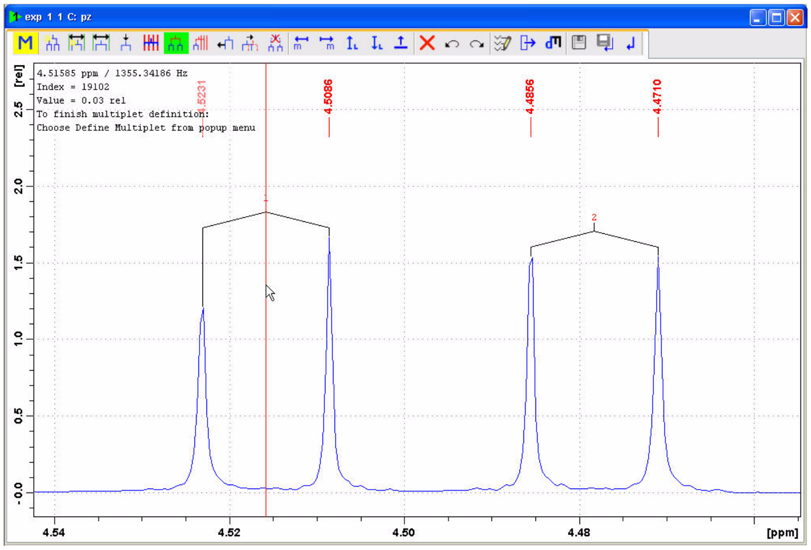
SHIFT: 4.4783 ppm
L1: M=2, J=4.3819 Hz

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 4.15.

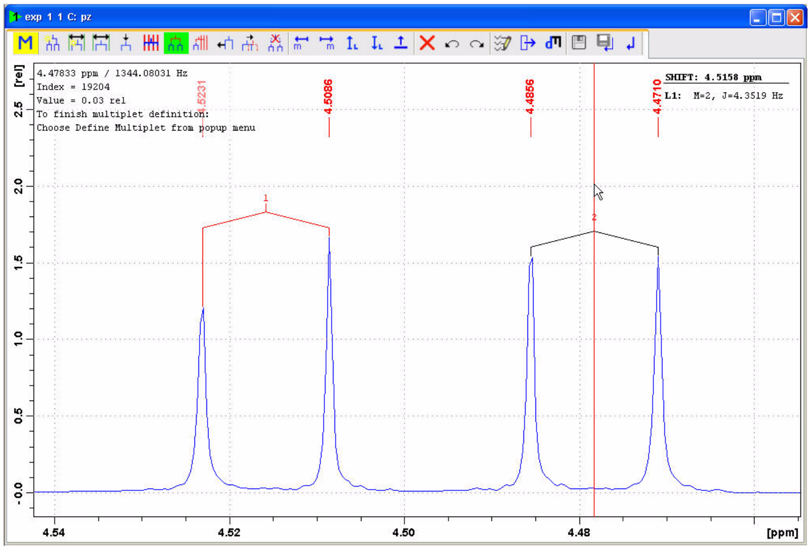


4.51585 ppm / 1355.34186 Hz
Index = 19102
Value = 0.03 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu

20. Move the cursor line in to the center of the second two lines marked 2

21. Click the left mouse button

Figure 4.16.



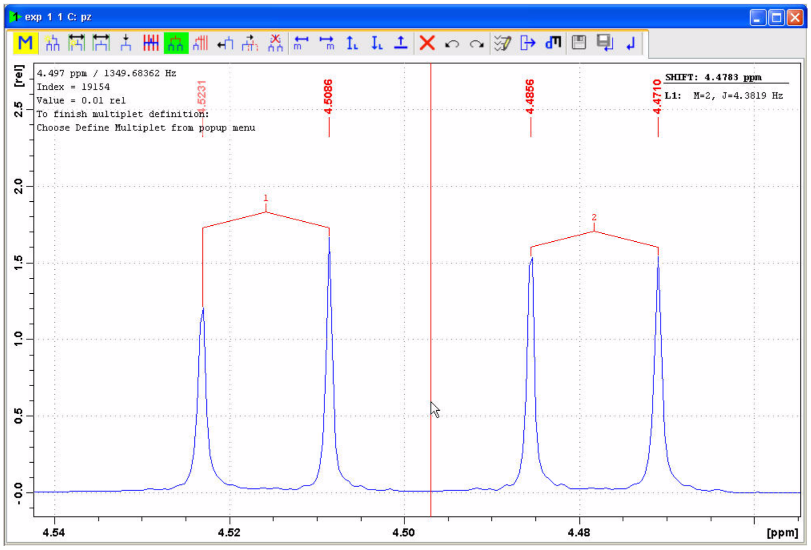
```
4.47833 ppm / 1344.08031 Hz
Index = 19204
Value = 0.03 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 4.5158 ppm
L1:   M=2, J=4.3519 Hz
```

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 4.17.



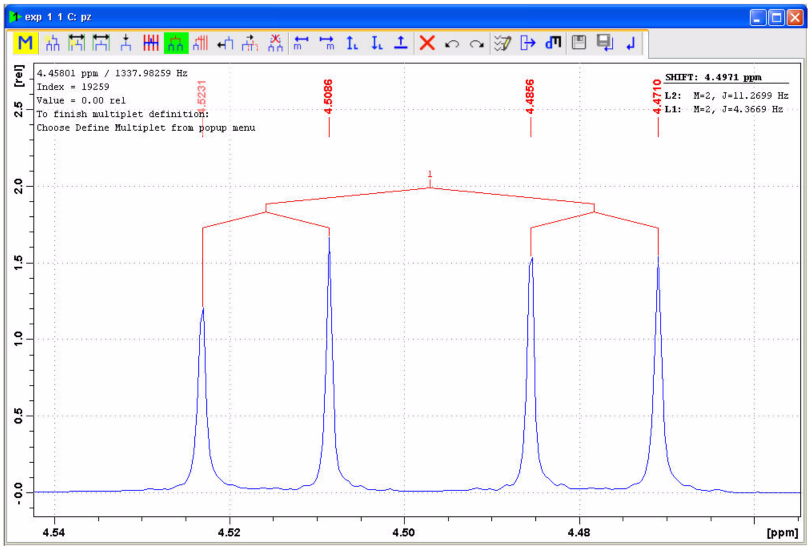
```
4.497 ppm / 1349.68362 Hz
Index = 19154
Value = 0.01 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 4.4783 ppm
L1:   M=2, J=4.3819 Hz
```

23. Click the right mouse button

24. Select ‘**Define Multiplet**’ by clicking on it

Figure 4.18.

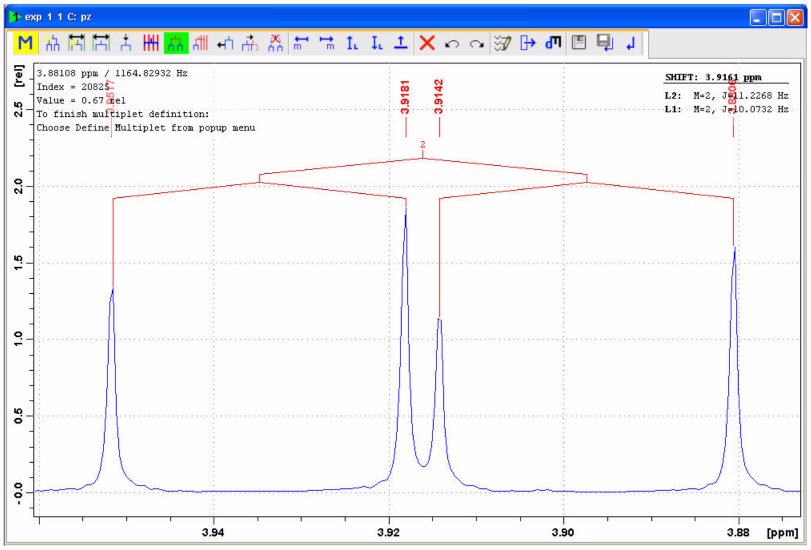


```
4.45801 ppm / 1337.98259 Hz
Index = 19259
Value = 0.00 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 4.4971 ppm
L2:  M=2, J=11.2699 Hz
L1:  M=2, J=4.3669 Hz
```

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 4.19.

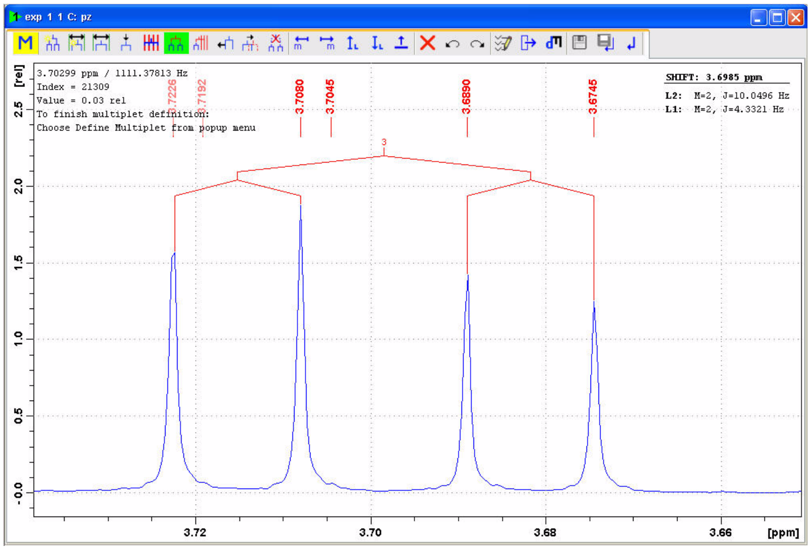


```
3.88108 ppm / 1164.82932 Hz
Index = 20825
Value = 0.67 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 3.9161 ppm
L2:  M=2, J=11.2268 Hz
L1:  M=2, J=10.0732 Hz
```

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

Figure 4.20.

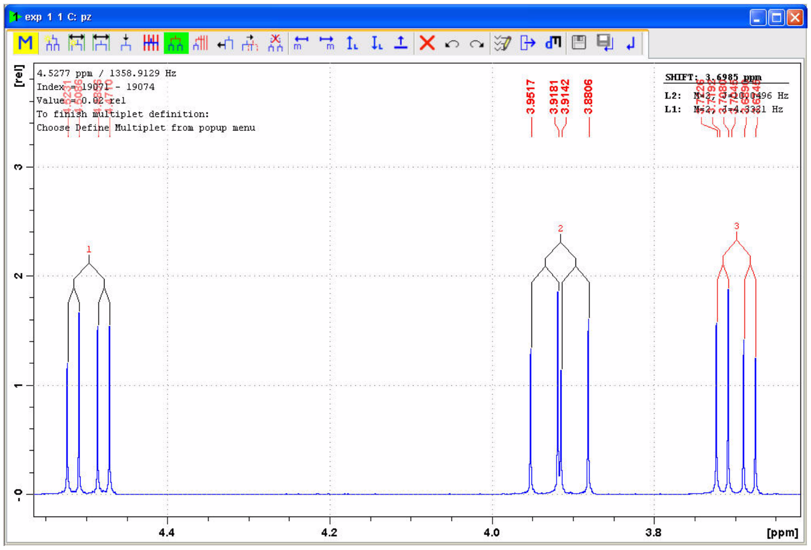


```
3.70299 ppm / 1111.37813 Hz
Index = 21309
Value = 0.03 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 3.6985 ppm
L2:  M=2, J=10.0496 Hz
L1:  M=2, J=4.3321 Hz
```

31. Display all 3 multiplets

Figure 4.21.

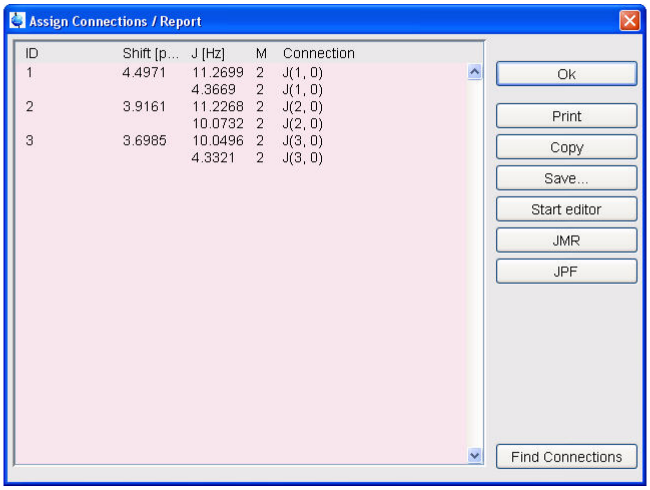


```
4.5277 ppm / 1358.9129 Hz
Index = 19071 - 19074
Value = 0.02 rel
To finish multiplet definition:
Choose Define Multiplet from popup menu
```

```
SHIFT: 3.6985 ppm
L2:  M=2, J=10.0496 Hz
L1:  M=2, J=4.3321 Hz
```

32. Click on ⇥ ‘Show Multiplet Report’

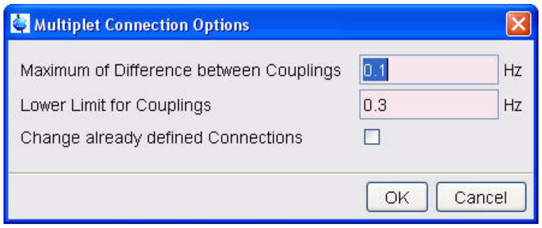
Figure 4.22.



ID	Shift [p...	J [Hz]	M	Connection
1	4.4971	11.2699	2	J(1, 0)
		4.3669	2	J(1, 0)
2	3.9161	11.2268	2	J(2, 0)
		10.0732	2	J(2, 0)
3	3.6985	10.0496	2	J(3, 0)
		4.3321	2	J(3, 0)

33, Click on Find Connections

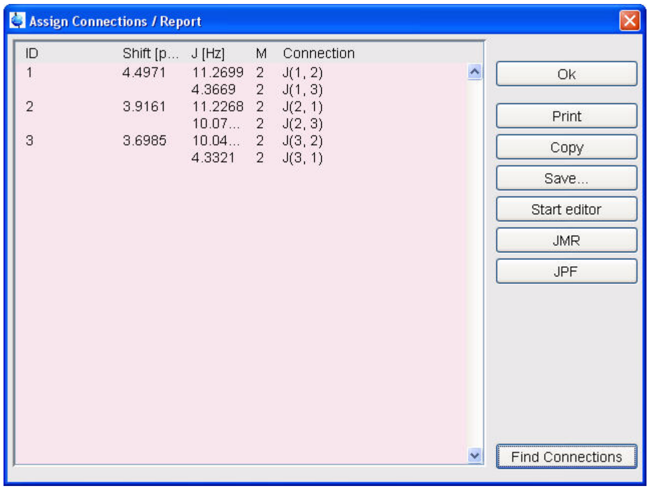
Figure 4.23.



Maximum of Difference between Couplings 0.1 Hz
Lower Limit for Couplings 0.3 Hz
Change already defined Connections ☐

34. Click on OK

Figure 4.24.



ID	Shift [p...	J [Hz]	M	Connection
1	4.4971	11.2699	2	J(1, 2)
		4.3669	2	J(1, 3)
2	3.9161	11.2268	2	J(2, 1)
		10.07...	2	J(2, 3)
3	3.6985	10.04...	2	J(3, 2)
		4.3321	2	J(3, 1)

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 observe, without 1H decoupling *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:

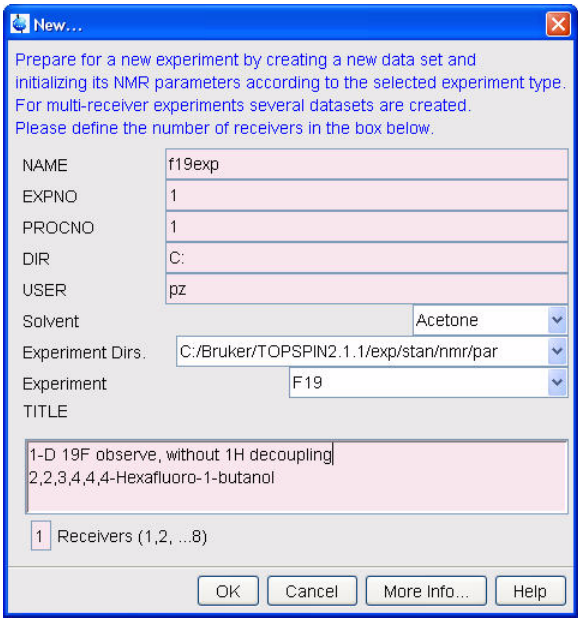
2,2,3,4,4,4-Hexafluoro-1-butanol in Acetone-d6

CF_3-CFH-CF_2-CH_2-OH

Exploratory spectrum *5.2.1*

1.Type **edc** and change the following parameters

Figure 5.1.



New...

Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME: f19exp
EXPNO: 1
PROCNO: 1
DIR: C:
USER: pz
Solvent: Acetone
Experiment Dirs.: C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment: F19
TITLE:
1-D 19F observe, without 1H decoupling
2,2,3,4,4,4-Hexafluoro-1-butanol

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select Acetone

5. Shim for best homogeneity

6. Tune the probe

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

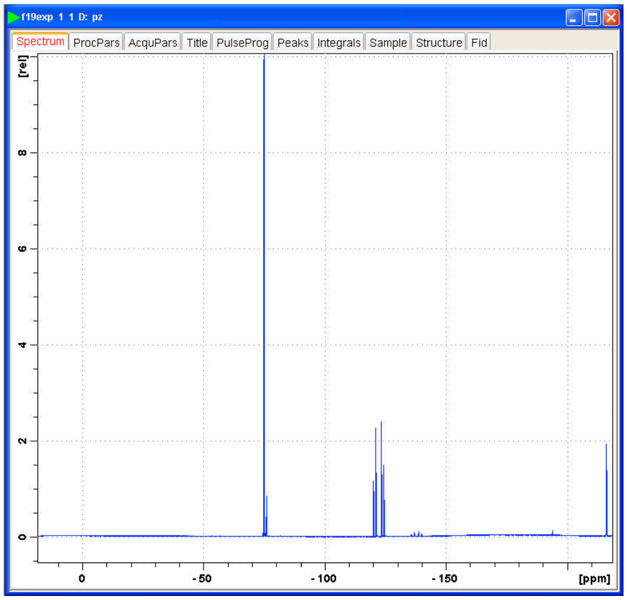
8. Click on to read in the Prosol parameters

9. Type **rga**

10. Type **zg** to start the acquisition

11. Process and Phase correct the spectrum

Figure 5.2.



Optimizing the sweep width *5.2.2*

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:

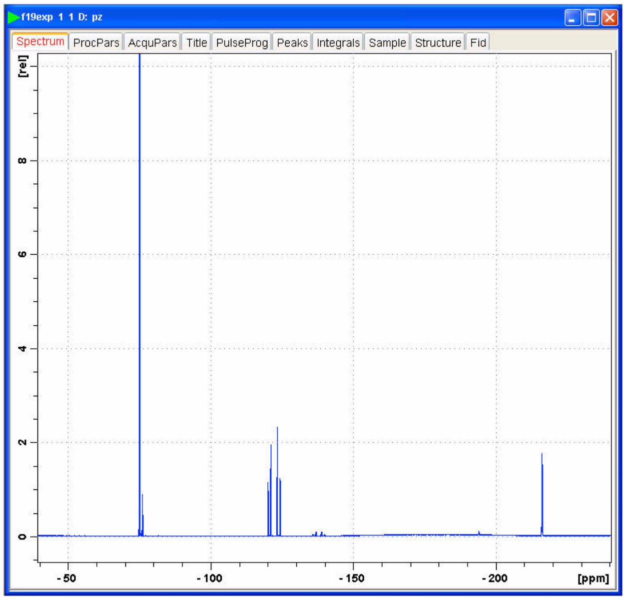
SW [PPM] = **200**

O1P [PPM] = **-140**

3.Type **zg** to start the acquisition

4. Process and Phase correct the spectrum

Figure 5.3.

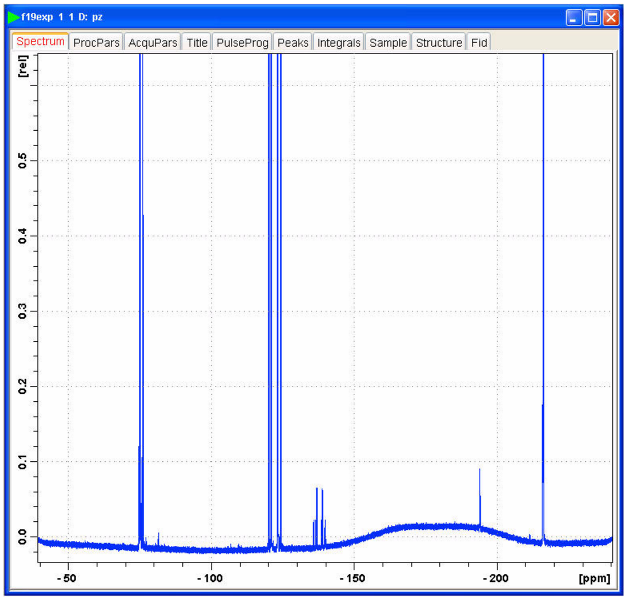


Baseline correction *5.2.3*

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.

Enter new EXPNO for fid: 2

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**

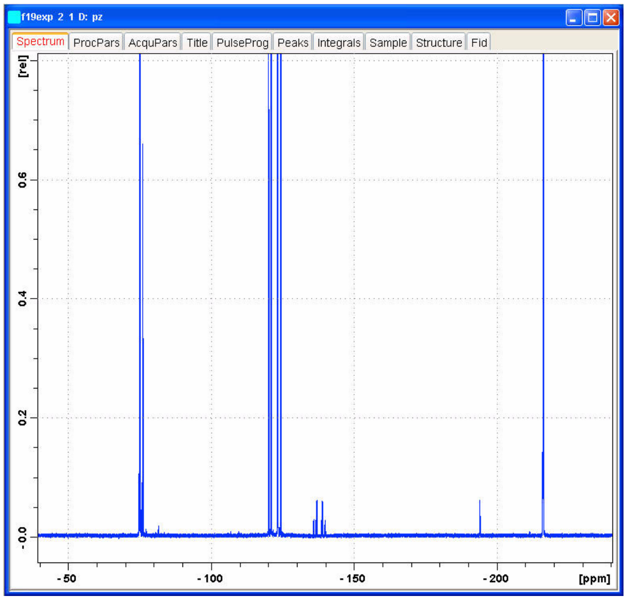
NCOEF = **32**

TDoff = **16**

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

10. Type **abs**

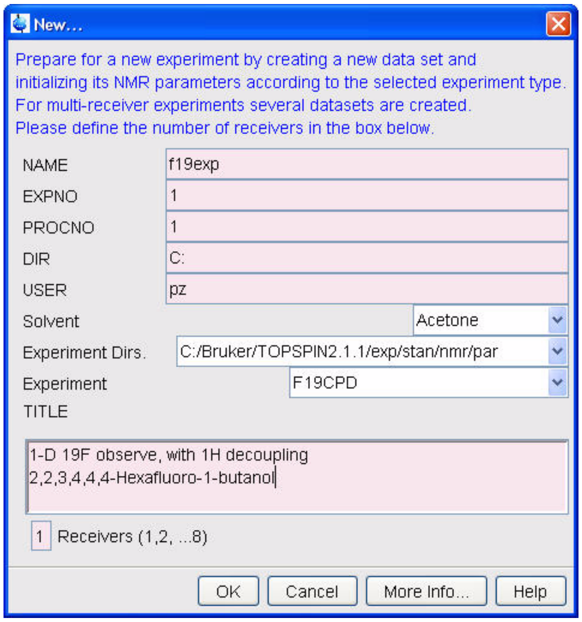
Figure 5.6.



19F observe, with 1H decoupling 5.3

1.Type **edc** and change the following parameters

Figure 5.7.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME f19exp
EXPNO 1
PROCNO 1
DIR C:
USER pz
Solvent Acetone
Experiment Dirs. C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment F19CPD
TITLE

1-D 19F observe, with 1H decoupling
2,2,3,4,4,4-Hexafluoro-1-butanol

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select Acetone

5. Shim for best homogeneity

6. Tune the probe

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

8. Change the following parameters:

SW [PPM] = **200**

O1P [PPM] = **-140**

9. Click on to read in the Prosol parameters

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

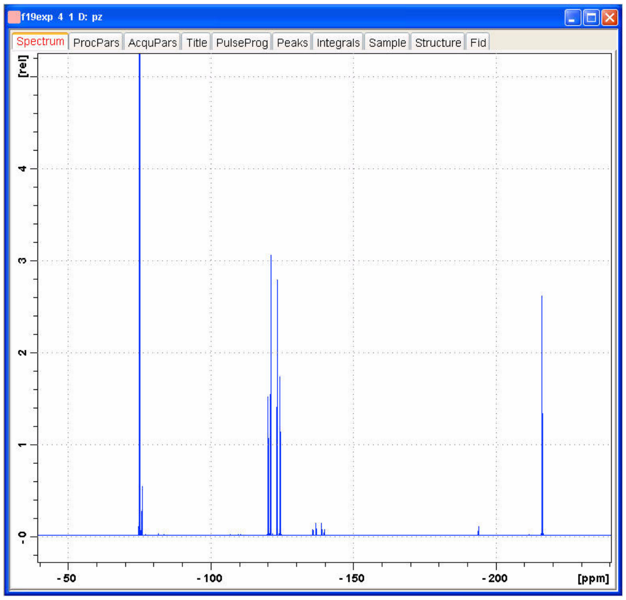
11. Type **rga**

12. Type **zg** to start the acquisition

13. Process and Phase correct the spectrum

14. To get rid of the background signal, follow the instructions in 5.2.3, Baseline correction, steps 1 through 9

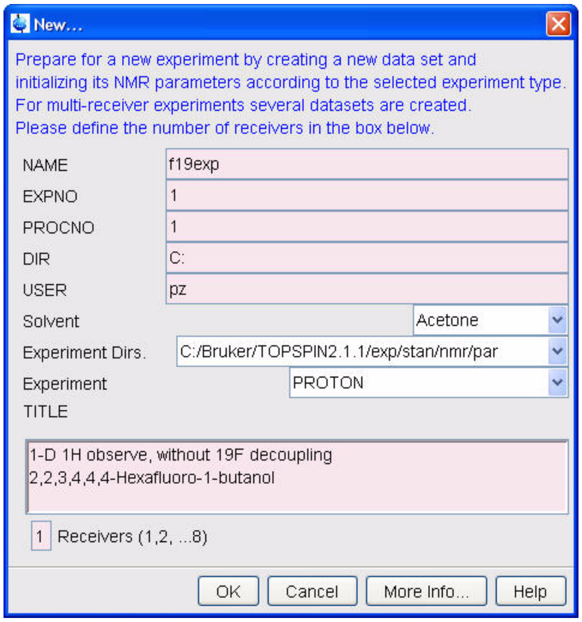
Figure 5.8.



1H observe, without 19F decoupling *5.3.1*

1.Type **edc** and change the following parameters

Figure 5.9.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME f19exp
EXPNO 1
PROCNO 1
DIR C:
USER pz
Solvent Acetone
Experiment Dirs. C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment PROTON
TITLE
1-D 1H observe, without 19F decoupling
2,2,3,4,4,4-Hexafluoro-1-butanol
1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select Acetone

5. Shim for best homogeneity

6. Tune the probe

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

8. Click on to read in the Prosol parameters

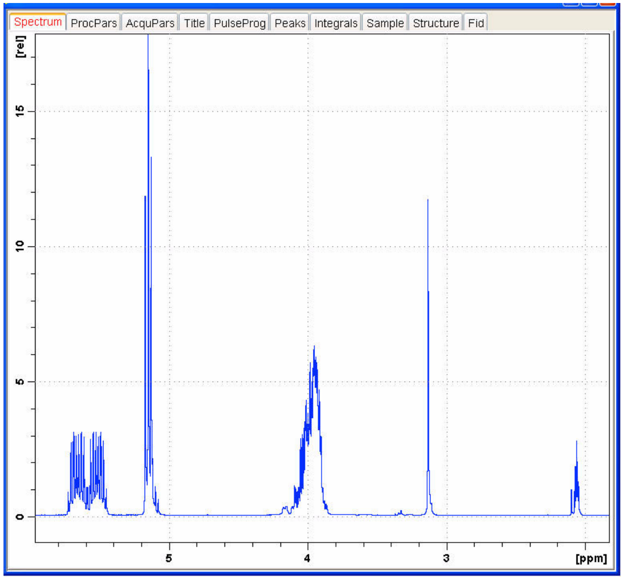
9. Select the '**Spectrum**' tab by clicking on it

10. Type **rga**

11. Type **zg** to start the acquisition

12. Process and Phase correct the spectrum

Figure 5.10.



1H observe, with 19F decoupling using WALTZ 5.3.2

1. Type **iexpno**

NOTE: The experiment number is incremented by one (e.g. EXPNO = 2)

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 using WALTZ16**
2,2,3,4,4,4-Hexafluoro-1-Butanol

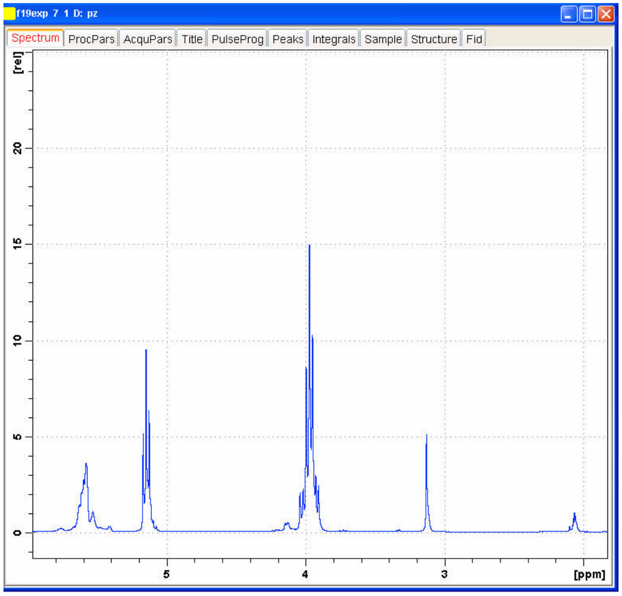
10. Select the ‘**Spectrum**’ tab by clicking on it

11. Type **rga**

12. Type **zg** to start the acquisition

13. Process and Phase correct the spectrum

Figure 5.11.



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 *5.3.3*

1. Type **iexpno** in the command line

NOTE: The experiment number is incremented by one (e.g. EXPNO = 3)

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

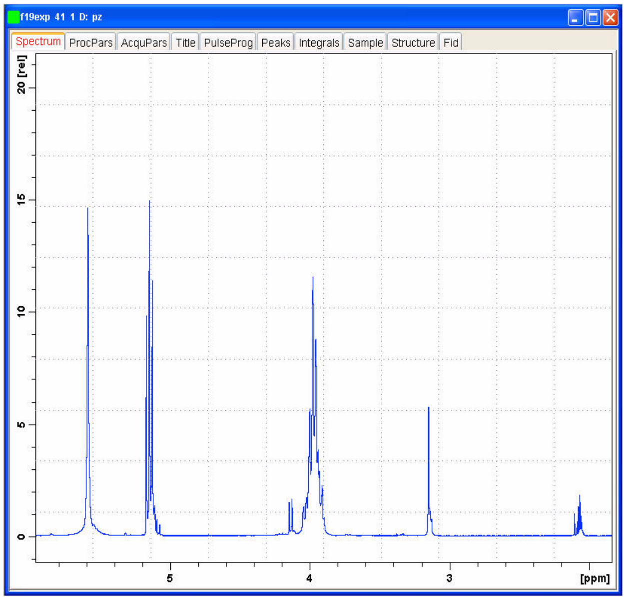
CPDPRG2 = **garp**

PCPD2 = **70**

Pl12 = **calculate the power level in the prosol table**

5. Type **rga**
6. Type **zg** to start the acquisition
7. Process and Phase correct the spectrum

Figure 5.12.



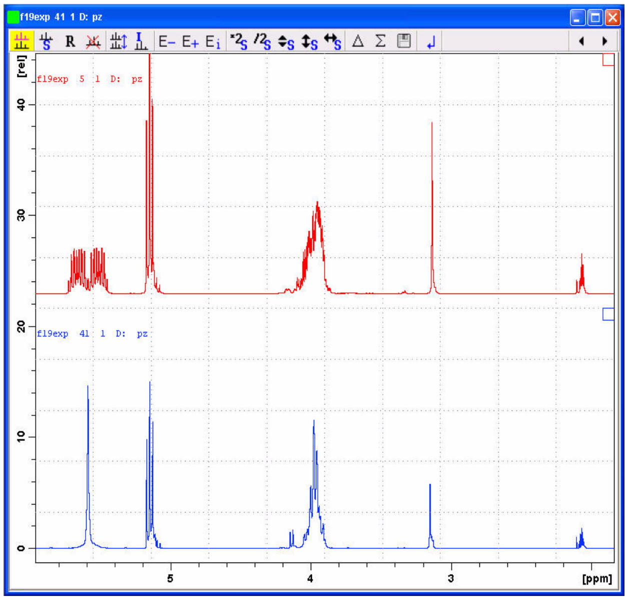
Plotting the 19F coupled and decoupled 1H spectrum on to the same page. *5.3.4*

1. Type **re 3** to display the 1H observe with 19F decoupled spectrum

2.. Click on

3. Type **re 1** on the command line (1H observe without 19F decoupled spectrum)
4. Click on to separate the two spectra
5. Using the display tools to adjust the spectra

Figure 5.13.



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 7, Homo-decoupling, 7.1.6 Plotting the reference and decoupled spectra on the same page, steps 1 through 21.

2-D 19F/1H Hetero correlated experiment *5.4*

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

Sample:

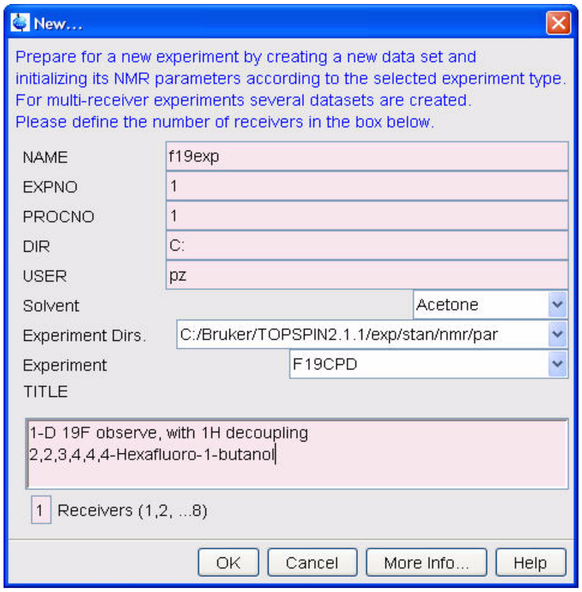
2,2,3,4,4,4-Hexafluoro-1-butanol in Acetone-d6

CF_3-CFH-CF_2-CH_2-OH

1-D 19F reference spectrum *5.4.1*

1. Type **edc** and change the following parameters

Figure 5.14.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME f19exp
EXPNO 1
PROCNO 1
DIR C:
USER pz
Solvent Acetone
Experiment Dirs. C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment F19CPD
TITLE
1-D 19F observe, with 1H decoupling
2,2,3,4,4,4-Hexafluoro-1-butanol

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select Acetone

5. Shim for best homogeneity

6. Tune the probe

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

8. Click on to read in the Prosol parameters

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

10. Type **rga**

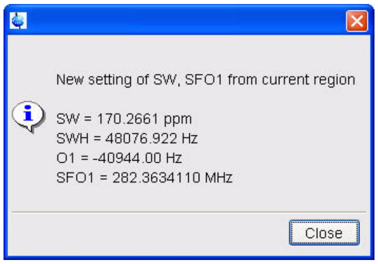
11. Type **zg** to start the acquisition

12. Process and Phase correct the spectrum

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

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

Figure 5.15.



New setting of SW, SFO1 from current region

SW = 170.2661 ppm
SWH = 48076.922 Hz
O1 = -40944.00 Hz
SFO1 = 282.3634110 MHz

15. Write down the SW value, rounding off to the nearest ppm (e.g. **170**)

16. Write down the O1 value, rounding off to the nearest Hz (e.g. **-40944**)

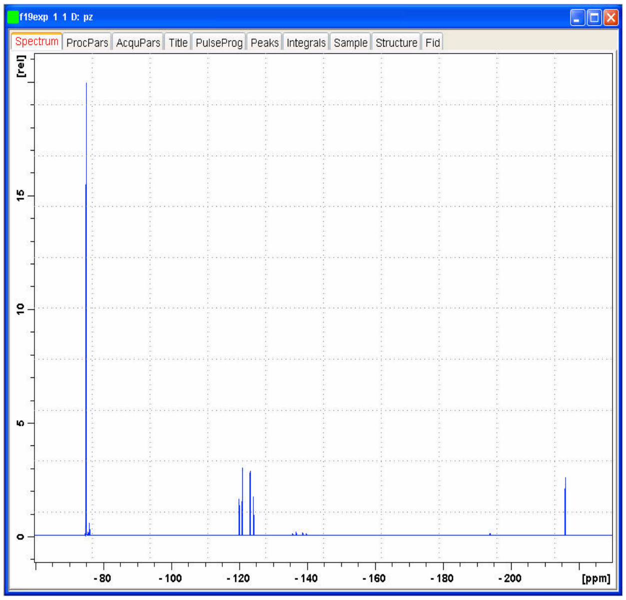
17. Click on Close

18. Type **sr** and write down the exact value (e.g. **0**)

19. Type **zg** to start the acquisition

20. Process and Phase correct the spectrum

Figure 5.16.



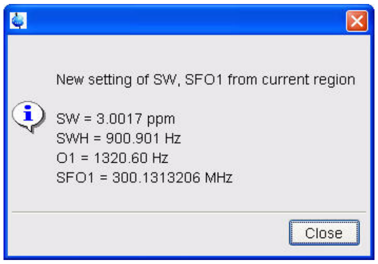
1-D 1H reference spectrum *5.4.2*

1. Type **iexpno**

NOTE: The experiment number is incremented by one (e.g. EXPNO = 2)

2. Type **rpar PROTON all**
3. Select the ‘**AcquPars**’ tab by clicking on it
4. Click on to read in the Prosol parameters
5. Select the ‘**Spectrum**’ tab by clicking on it
6. Type **rga**
7. Type **zg** to start the acquisition
8. Process and Phase correct the spectrum
9. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum
10. Click on to set the sweep width and the O1 frequency of the displayed region

Figure 5.17.

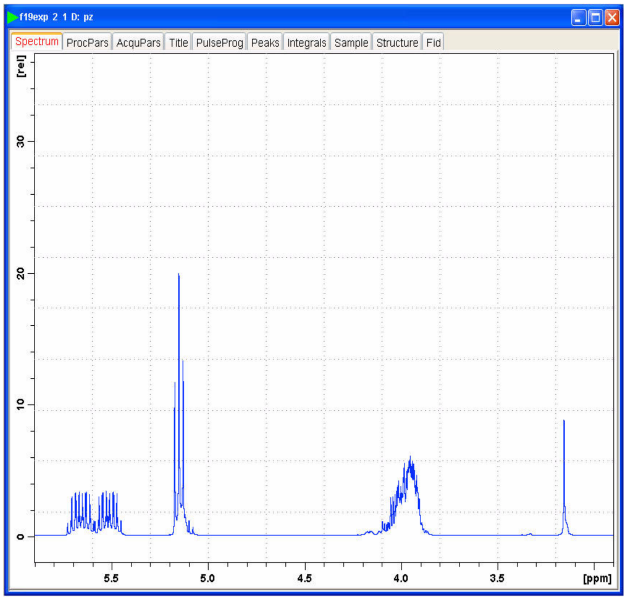


New setting of SW, SFO1 from current region

SW = 3.0017 ppm
SWH = 900.901 Hz
O1 = 1320.60 Hz
SFO1 = 300.1313206 MHz

11. Write down the SW value, rounding off to the nearest 1/10th of a ppm (e.g. **3**)
12. Write down the O1 value, rounding off to the nearest Hz (e.g. **1320**)
13. Click on Close
14. Type **sr** and write down the exact value (e.g. **0**)
15. Type **zg** to start the acquisition
16. Process and Phase correct the spectrum

Figure 5.18.



Setting up of the 2-D HETCOR experiment 5.4.3

1. Type **iexpno**

NOTE: The experiment number is incremented by one (e.g. EXPNO = 3)

---

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

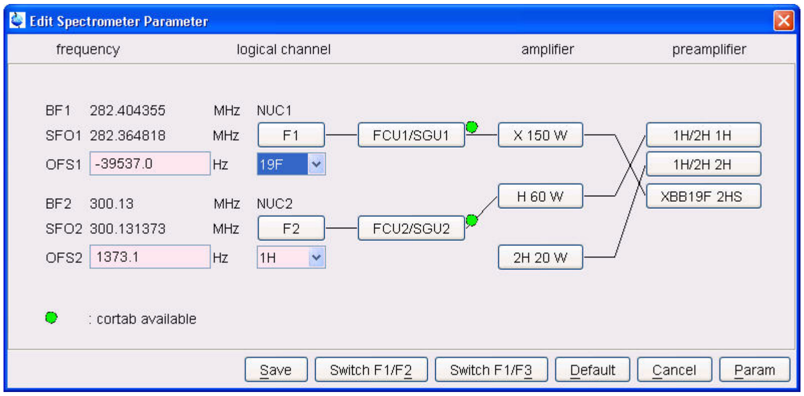
NOTE: 2-D experiments should be run non spinning

---

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

NUC1 = 19F

Figure 5.19.



BF1 282.404355 MHz
SFO1 282.364818 MHz
OFS1 -39537.0 Hz
NUC1 F1 19F

BF2 300.13 MHz
SFO2 300.131373 MHz
OFS2 1373.1 Hz
NUC2 F2 1H

6. Click on Save

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

8. Make the following changes:

PULPROG = **hfcoqfqn**

SW F2 [ppm] = e.g. **170** (19F reference spectrum 5.4.1, step 15)

SW F1 [ppm] = e.g. **3** (1H reference spectrum 5.4.2, step 11)

O1 [Hz] = e.g. **-40944** (19F reference spectrum 5.4.1, step 16)

O2 [Hz] = e.g. **1320** (1H reference spectrum 5.4.2, step 12)

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 = e.g. **0** (19F reference spectrum 5.4.1, step 18)

SR F1 = e.g. **0** (1H reference spectrum 5.4.2, step 14)

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. Type **rga**

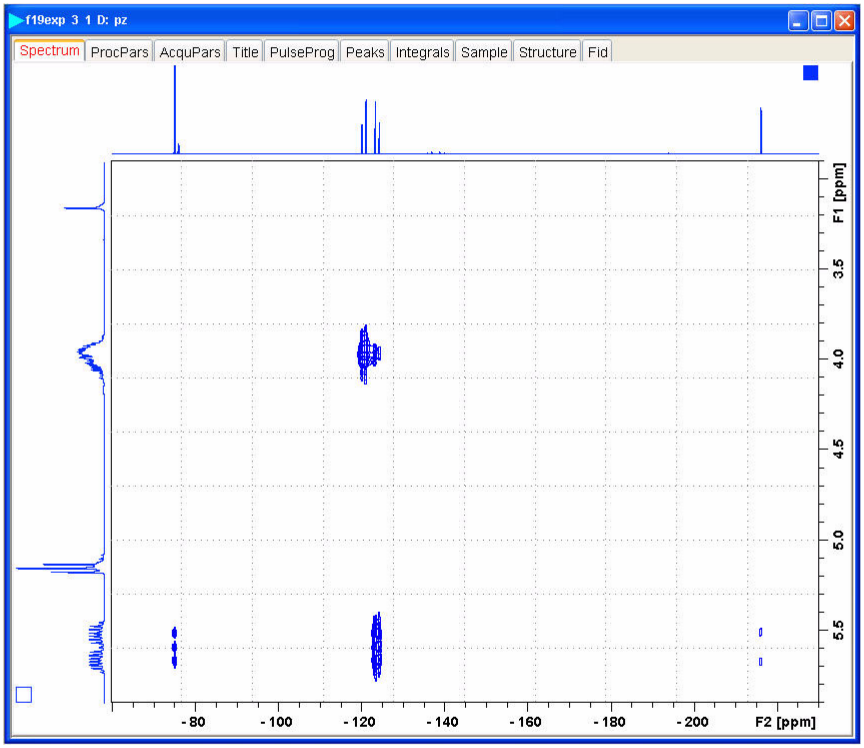
18. Type **zg** to start the acquisition

19. Type **xfb**

20. Select the reference spectra as the external projections

21. Adjust the contour level

Figure 5.20.



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.

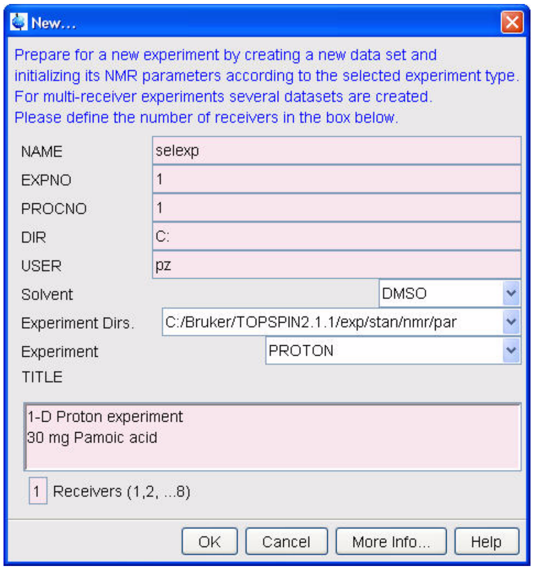
Sample:

30 mg Paomic acid in DMSO

Reference spectrum 6.1.1

1. Type **edc** and change the following parameters

Figure 6.1.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME	selexp
EXPNO	1
PROCNO	1
DIR	C:
USER	pz
Solvent	DMSO
Experiment Dirs.	C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment	PROTON

TITLE

1-D Proton experiment
30 mg Pamoic acid

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select DMSO

5. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

6. Shim for best homogeneity

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

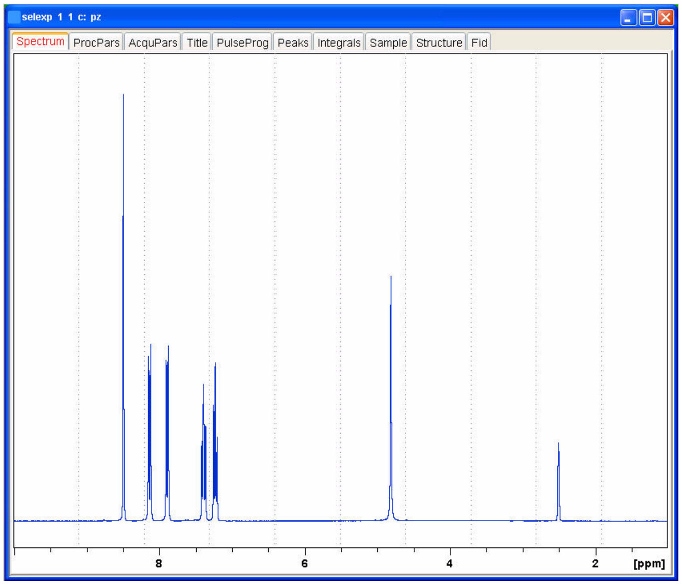
8. Click on to read in the Prosol parameters

9. Tune the probe

10. Type **rga**

11. Process and Phase correct the spectrum

Figure 6.2.



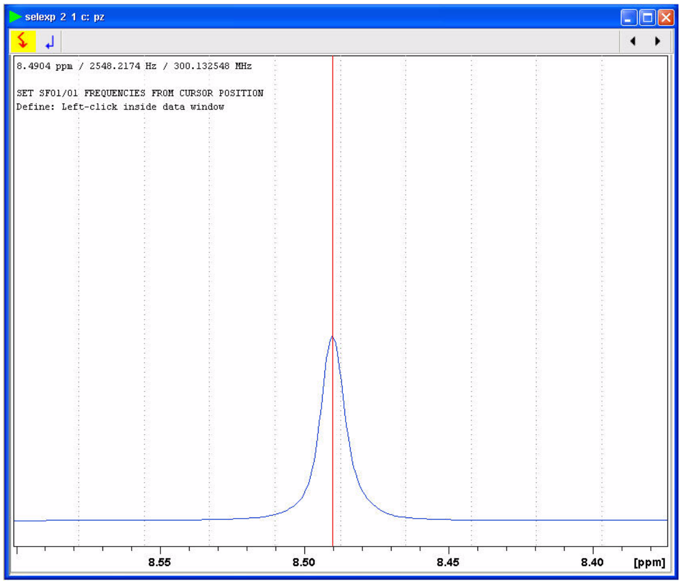
Selective excitation region set up (example 1) 6.1.2

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 ↯

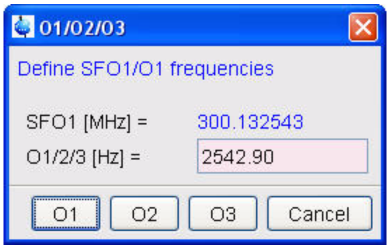
Figure 6.3.



```
8.4904 ppm / 2548.2174 Hz / 300.132548 MHz

SET SF01/01 FREQUENCIES FROM CURSOR POSITION
Define: Left-click inside data window
```

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

Figure 6.4.



O1/O2/O3

Define SFO1/O1 frequencies

SFO1 [MHz] = 300.132543

O1/2/3 [Hz] = 2542.90

9. Click on O1

Setting up the acquisition parameters *6.1.3*

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

2. Click on ⎍ to display the pulse program parameters

3. Make the following changes:

PULPROG = **selnogp**
NS = **64**
DS = **8**
D1 = **2**
D8 = **0.700**
SPNAM2 = **Gaus1.1000**
SPOFF2 = **0**
GPNAM1 = **sine.100**
GPNAM2 = **sine.100**
GPZ1 = **15**
GPZ2 = **40**

NOTE: The mixing time D8 is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from 100 ms to 800 ms.

Running the experiment *6.1.4*

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

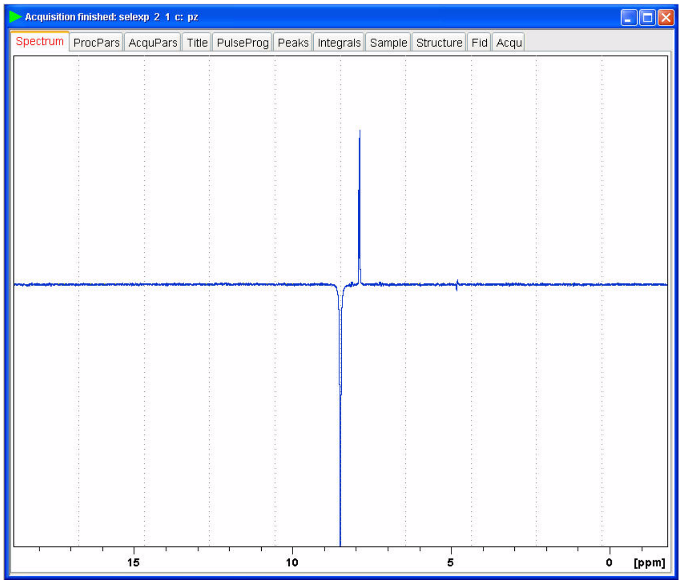
3. Type **zg** to start the acquisition

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 6.1.1 Reference Spectrum in this Chapter.

2. Type **wrpa 2**

3. Type **re 2**

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

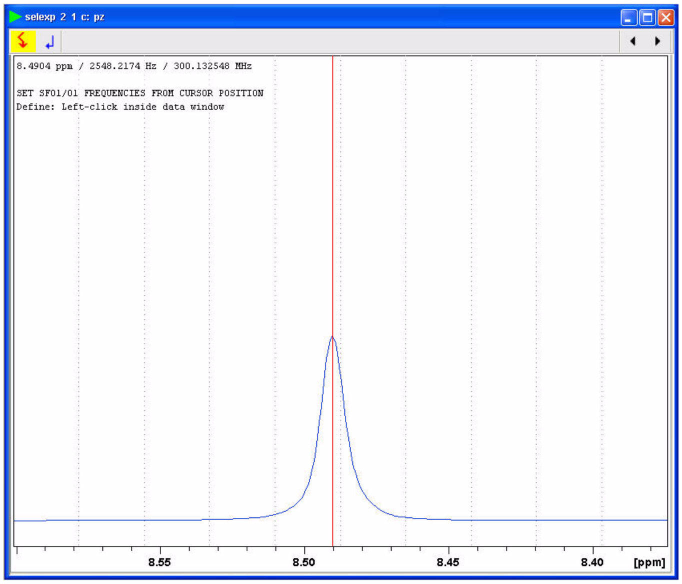
5. Change the title to: **Selective NOESY experiment**

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

7. Expand the signal region at 8.5 ppm

8. Click on

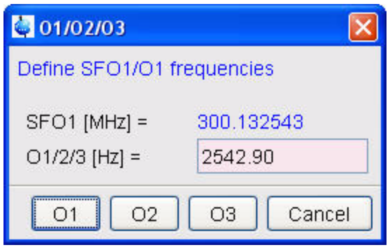
Figure 6.6.



```
8.4904 ppm / 2548.2174 Hz / 300.132548 MHz

SET SF01/01 FREQUENCIES FROM CURSOR POSITION
Define: Left-click inside data window
```

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

Figure 6.7.



Define SFO1/O1 frequencies

SFO1 [MHz] = 300.132543

O1/2/3 [Hz] = 2542.90

10. Write down the O1/2/3 (Hz) value showing in the Info window (e.g. **2542.9**)

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. **689.47**)

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 pulse program parameters
3. Make the following changes:

PULPROG = **selnogp**

NS = **64**

DS = **8**

D1 = **2**

D8 = **0.700**

SPNAM2 = **Gaus1.1000**

SPOFF2 = value from 6.1.5, step 14 (e.g. **689.47**)

GPNAM1 = **sine.100**

GPNAM2 = **sine.100**

GPZ1 = **15**

GPZ2 = **40**

NOTE: The mixing time D8 is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from 100 ms to 800 ms.

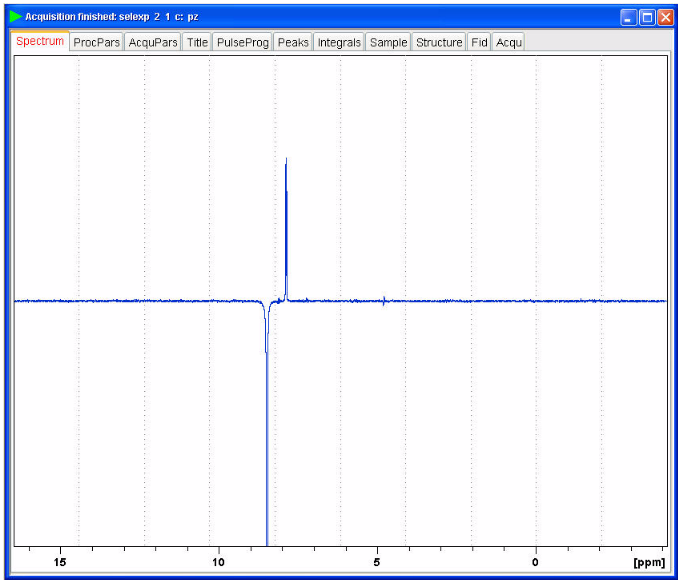
Running the experiment *6.1.7*

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

2. Type **zg** 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.



Acquisition finished: selexp 2 1 c: pz

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.

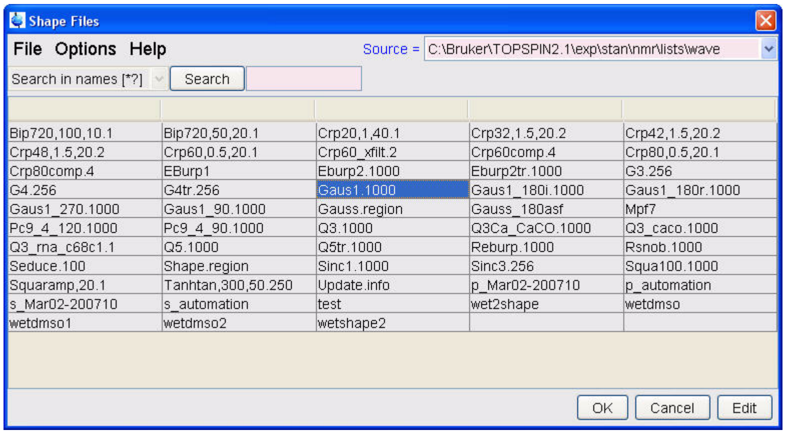
1. Run a Reference spectrum, following the instructions in 6.1.1 Reference Spectrum in this Chapter.
2. Type **wrpa 2**
3. Type **re 2**
4. Select the '**Title**' tab by clicking on it
5. Change the title to: **Selective NOESY experiment**
6. Select the '**Spectrum**' tab by clicking on it
7. Type **pulprog selnogp** in the command line

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 '**Calculate the excitation profiles**' option in the shaped tool program. Other method of calculating the pulse width and power level can be used, see Chapter 7, 1-D Selective TOCSY, Bandwidth region file, in this manual, or use the Prosol parameters to run this experiment.

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 **'Shape'**

Figure 6.9.

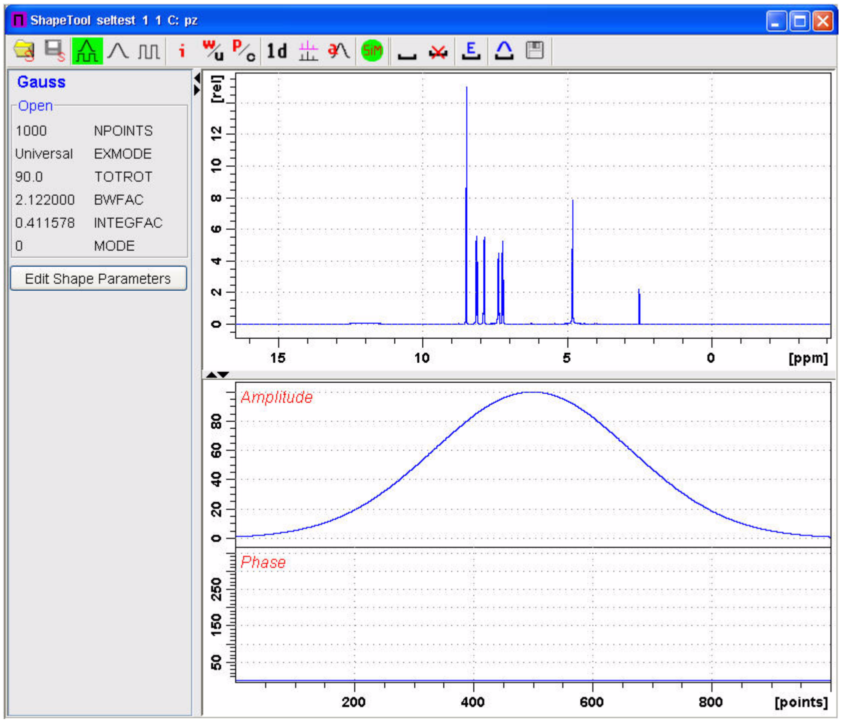


Source = C:\Bruker\TOPSPIN2.1\exp\stan\nmr\lists\wave

Bip720,100,10.1	Bip720,50,20.1	Crp20,1,40.1	Crp32,1.5,20.2	Crp42,1.5,20.2
Crp48,1.5,20.2	Crp60,0.5,20.1	Crp60_xfilt.2	Crp60comp.4	Crp80,0.5,20.1
Crp80comp.4	EBurp1	Eburp2.1000	Eburp2tr.1000	G3.256
G4.256	G4tr.256	Gaus1.1000	Gaus1_180i.1000	Gaus1_180r.1000
Gaus1_270.1000	Gaus1_90.1000	Gauss.region	Gauss_180asf	Mpf7
Pc9_4_120.1000	Pc9_4_90.1000	Q3.1000	Q3Ca_CaCO.1000	Q3_caco.1000
Q3_rna_c68c1.1	Q5.1000	Q5tr.1000	Reburp.1000	Rsnob.1000
Seduce.100	Shape.region	Sinc1.1000	Sinc3.256	Squa100.1000
Squaramp,20.1	Tanhtan,300,50.250	Update.info	p_Mar02-200710	p_automation
s_Mar02-200710	s_automation	test	wet2shape	wetdmso
wetdmso1	wetdmso2	wetshape2		

3. Select '**Gaus1.1000**'
4. Click on OK

Figure 6.10.

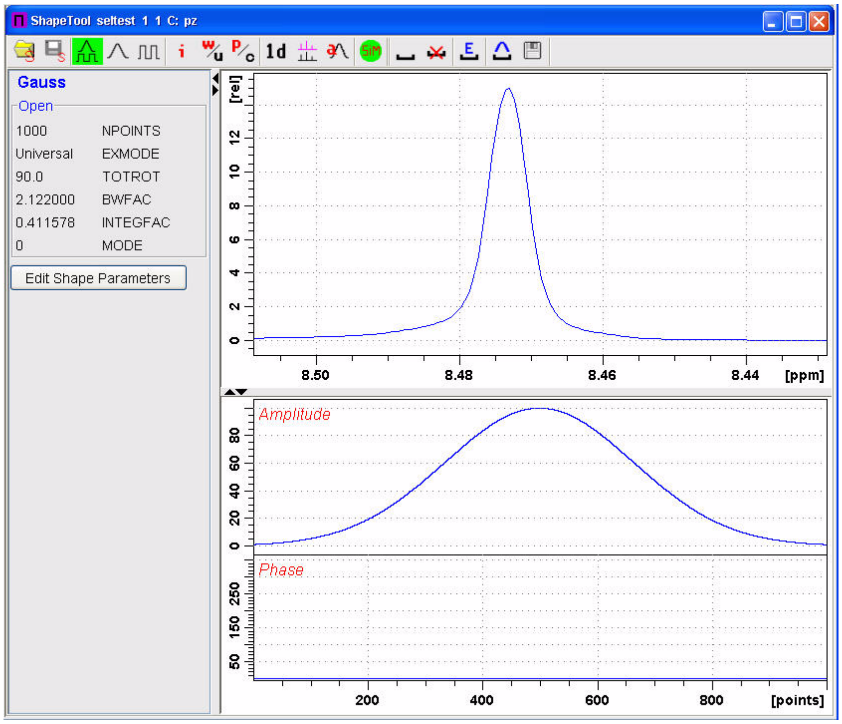


Gauss
Open

1000	NPOINTS
Universal	EXMODE
90.0	TOTROT
2.122000	BWFAC
0.411578	INTEGFAC
0	MODE

5. In the shapetool window expand the signal region around 8.5 ppm

Figure 6.11.



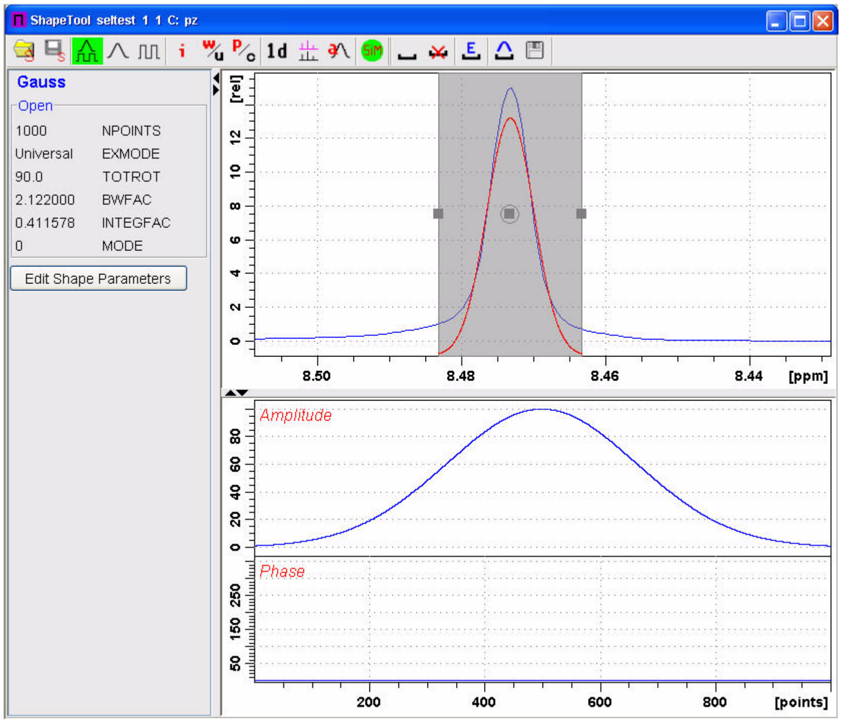
Gauss
Open

1000	NPOINTS
Universal	EXMODE
90.0	TOTROT
2.122000	BWFAC
0.411578	INTEGFAC
0	MODE

6. Click on ⎵ ‘New excitation region’

7. Adjust the excitation region to cover the full width of the peak

Figure 6.12.



ShapeTool seltest 1 1 C: pz

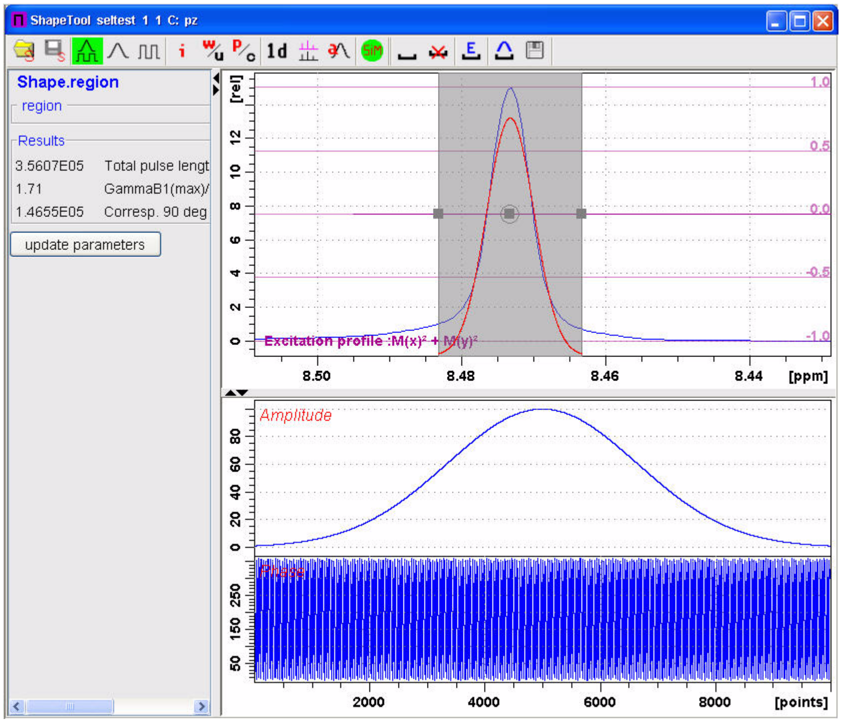
Gauss

Open

1000	NPOINTS
Universal	EXMODE
90.0	TOTROT
2.122000	BWFAC
0.411578	INTEGFAC
0	MODE

8. Click on △ ‘Calculate the excitation profiles”

Figure 6.13.



ShapeTool seltest 1 1 C: pz

Shape.region

region

Results

3.5607E05	Total pulse lengt
1.71	GammaB1(max)/
1.4655E05	Corresp. 90 deg

9. In the main menu click on ‘**Options**’ and select ‘**Define Parameter Table**’ by clicking on it

Figure 6.14.

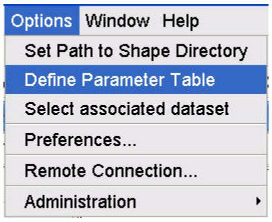
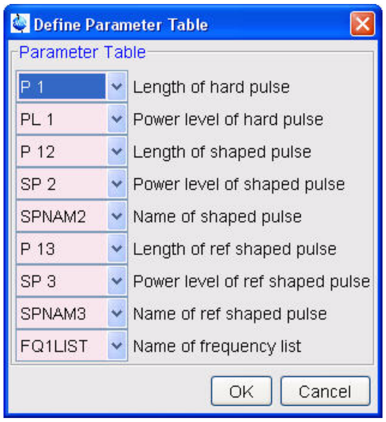


Figure 6.15.



P 1	Length of hard pulse
PL 1	Power level of hard pulse
P 12	Length of shaped pulse
SP 2	Power level of shaped pulse
SPNAM2	Name of shaped pulse
P 13	Length of ref shaped pulse
SP 3	Power level of ref shaped pulse
SPNAM3	Name of ref shaped pulse
FQ1LIST	Name of frequency list

10. Make the following changes:

Length of shaped pulse = **p12**

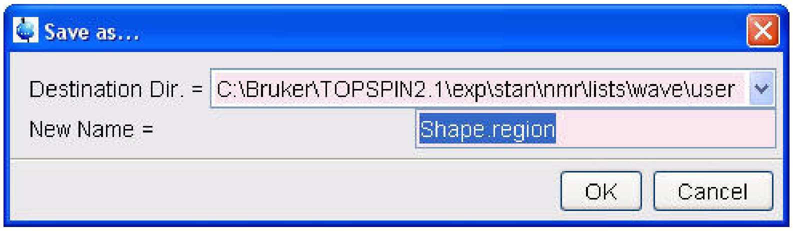
Power Level of shaped pulse = **SP2**

Name of shaped pulse = **SPNAM2**

11. Click on OK

12. Click on update parameters

Figure 6.16.



Destination Dir. = C:\Bruker\TOPSPIN2.1\exp\stan\nmr\lists\wave\user

New Name = Shape.region

13. Select a new name or use the default name displayed

14. Click on OK

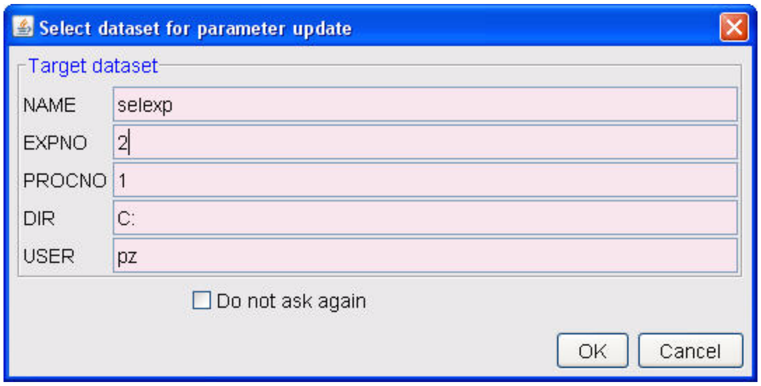
NOTE: If the shape region file already exists, a overwrite message will appear like the one below.

Figure 6.17.

'C:/Bruker/TOPSPIN2.1/exp/stan/nmr/lists/wave/user/Shape.region' exists.
OK to overwrite?

15. Click on OK

Figure 6.18.



Select dataset for parameter update

Target dataset

NAME selexp
EXPNO 2
PROCNO 1
DIR C:
USER pz

☐ Do not ask again

NOTE: The Target data set window above is to verify the correct data set and can be switched off by enable the ‘Do not ask again’ option.

16. Click on OK

17. 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 pulse program parameters
3. Make the following changes:

NS = **64**
DS = **8**
D1 = **2**
D8 = **0.700**
GPNAM1 = **sine.100**
GPNAM2 = **sine.100**
GPZ1 = **15**
GPZ2 = **40**

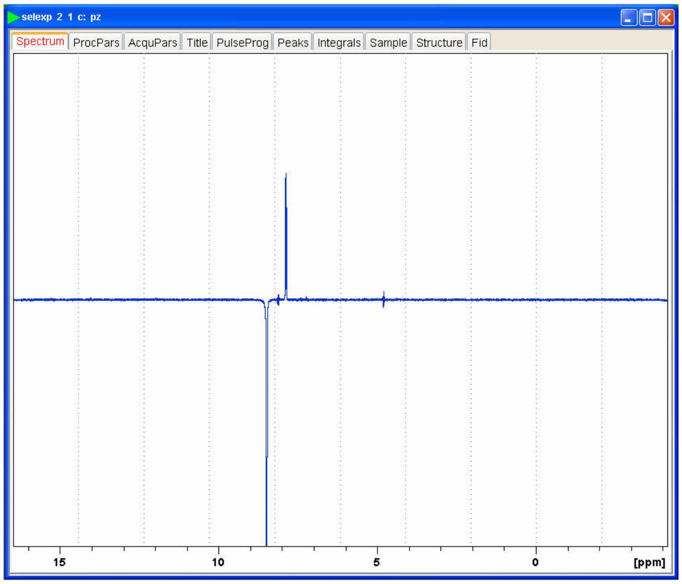
NOTE: The mixing time D8 is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from 100 ms to 800 ms.

Running the experiment *6.1.11*

1. Select the ‘**Spectrum**’ tab by clicking on it
2. Type **zg** 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.

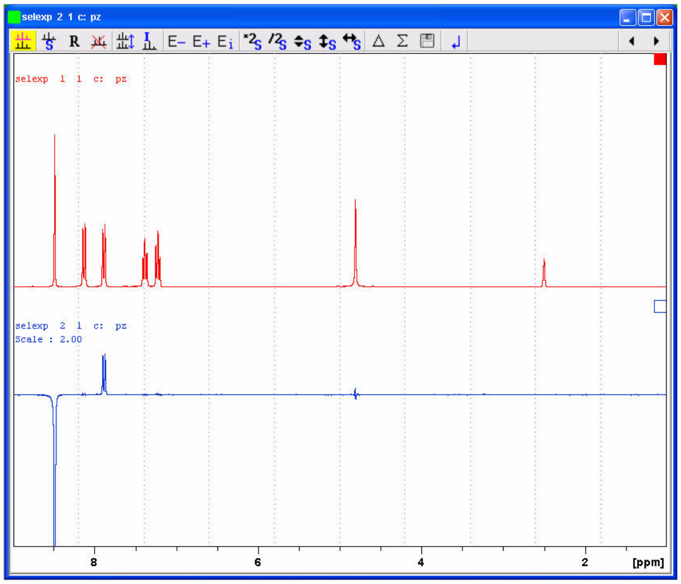
Figure 6.19.



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 6.20.



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 7, Homodecoupling, 7.1.6 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.

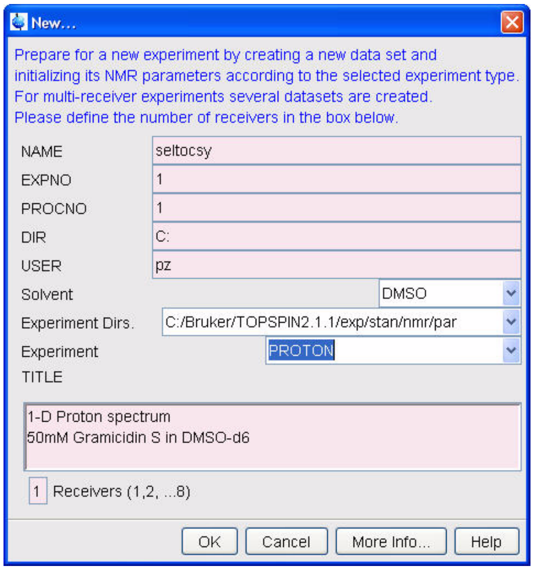
Sample:

50 mM Gramicidin S in DMSO

Reference spectrum 7.1.1

1. Type **edc** and change the following parameters

Figure 7.1.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME	seltocsy
EXPNO	1
PROCNO	1
DIR	C:
USER	pz
Solvent	DMSO
Experiment Dirs.	C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment	PROTON

TITLE

1-D Proton spectrum
50mM Gramicidin S in DMSO-d6

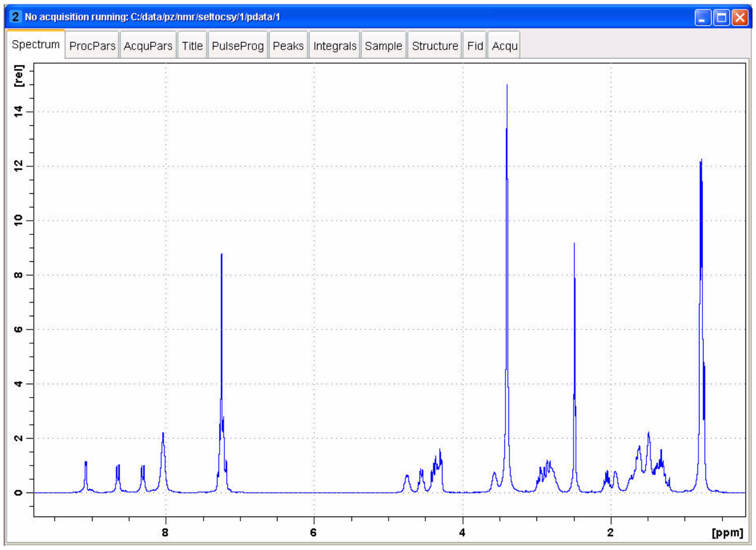
1 Receivers (1,2, ...8)

2. Click on OK
3. Insert the sample
4. Type **lock** and select DMSO
5. Turn the spinner off

NOTE: selective excitation experiments should be run non spinning

6. Shim for best homogeneity
7. Select the ‘**AcquPars**’ tab by clicking on it
8. Click on to read in the Prosol parameters
9. Tune the probe
10. Type **rga**
11. Process and Phase correct the spectrum

Figure 7.2.



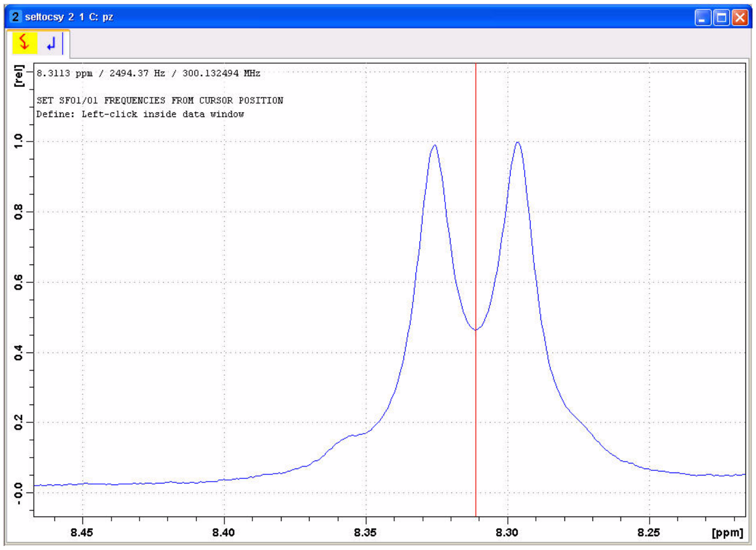
Selective excitation region set up *7.1.2*

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 ↯

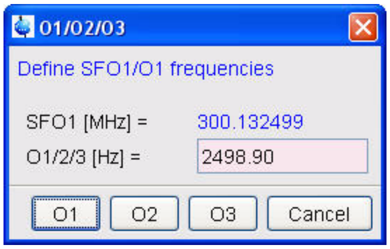
Figure 7.3.



```
8.3113 ppm / 2494.37 Hz / 300.132494 MHz

SET SF01/01 FREQUENCIES FROM CURSOR POSITION
Define: Left-click inside data window
```

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

Figure 7.4.



01/02/03

Define SFO1/O1 frequencies

SFO1 [MHz] = 300.132499

O1/2/3 [Hz] = 2498.90

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*

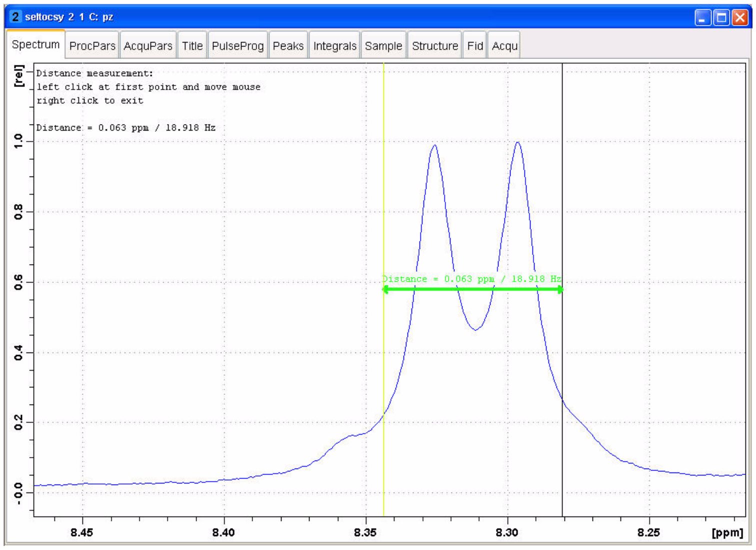
In this example the shaped pulse width and power level are determine using the **'Calculate Bandwidth'** option in the shaped tool program. Other method of calculating the pulse width and power level can be used, see Chapter 2, 1-D Selective NOESY, integration region file, in this manual, or use the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



Distance measurement:
left click at first point and move mouse
right click to exit

Distance = 0.063 ppm / 18.918 Hz

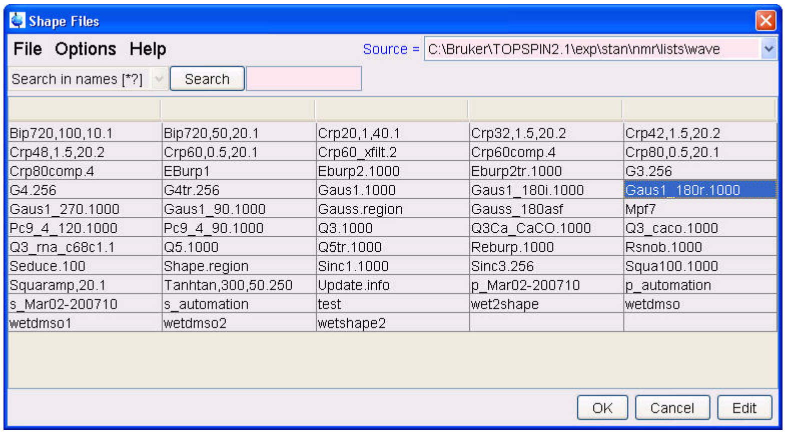
4. Write down the value for the distance between the two cursor lines (e.g. **19**)

5. Type **pulprog selmlgp**

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

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

Figure 7.6.



Source = C:\Bruker\TOPSPIN2.1\exp\stan\nmr\lists\wave

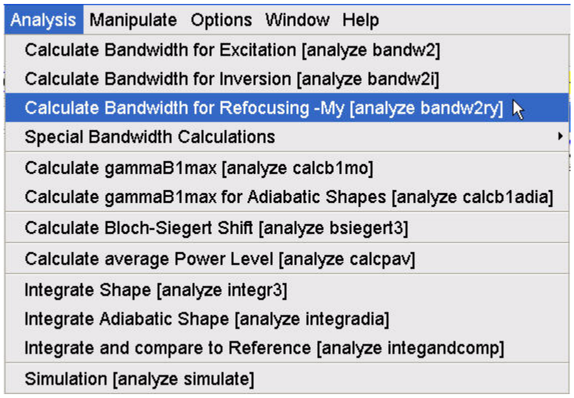
Bip720,100,10.1	Bip720,50,20.1	Crp20,1,40.1	Crp32,1.5,20.2	Crp42,1.5,20.2
Crp48,1.5,20.2	Crp60,0.5,20.1	Crp60_xfilt.2	Crp60comp.4	Crp80,0.5,20.1
Crp80comp.4	EBurp1	Eburp2.1000	Eburp2tr.1000	G3.256
G4.256	G4tr.256	Gaus1.1000	Gaus1_180i.1000	Gaus1_180r.1000
Gaus1_270.1000	Gaus1_90.1000	Gauss.region	Gauss_180asf	Mpf7
Pc9_4_120.1000	Pc9_4_90.1000	Q3.1000	Q3Ca_CaCO.1000	Q3_caco.1000
Q3_rna_c68c1.1	Q5.1000	Q5tr.1000	Reburp.1000	Rsnob.1000
Seduce.100	Shape.region	Sinc1.1000	Sinc3.256	Squa100.1000
Squaramp,20.1	Tanhtan,300,50.250	Update.info	p_Mar02-200710	p_automation
s_Mar02-200710	s_automation	test	wet2shape	wetdmso
wetdmso1	wetdmso2	wetshape2		

8. Select '**Gaus1_180r.1000**'

9. Click on OK

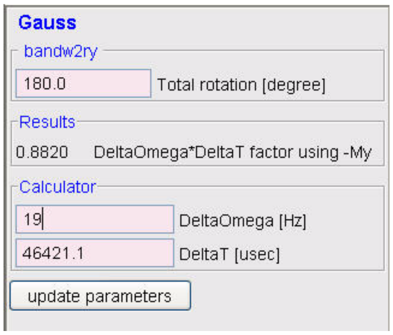
10. In the main menu click on '**Analysis**' and select '**Calculate Bandwidth for Refocusing -My'**

Figure 7.7.



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

Figure 7.8.



Gauss

bandw2ry

180.0 Total rotation [degree]

Results

0.8820 DeltaOmega*DeltaT factor using -My

Calculator

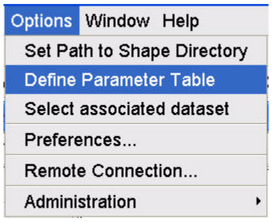
19 DeltaOmega [Hz]

46421.1 DeltaT [usec]

NOTE: The value for ‘Delta T [usec]’ is calculated after executing step 11.

12. In the main menu click on ‘**Options**’ and select ‘**Define Parameter Table**’

Figure 7.9.



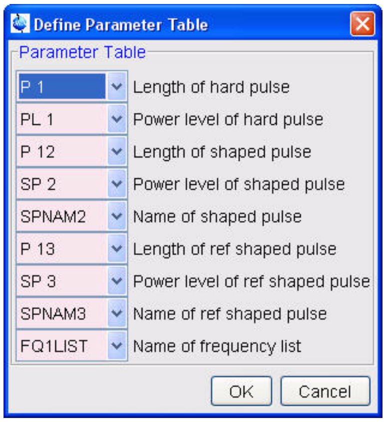
13. Make the following changes:

Length of shaped pulse = **p12**

Power Level of shaped pulse = **SP2**

Name of shaped pulse = **SPNAM2**

Figure 7.10.

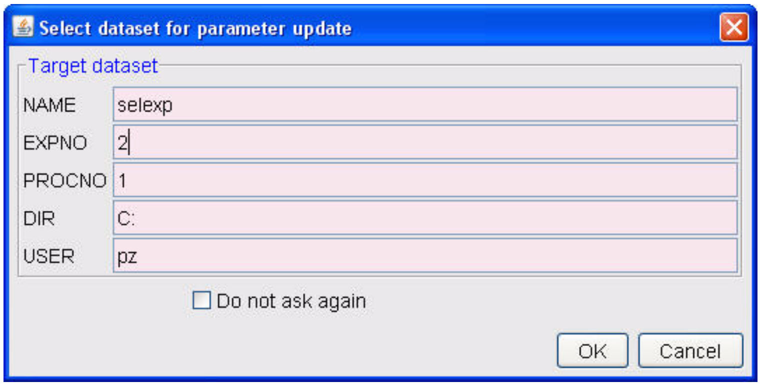


P 1	Length of hard pulse
PL 1	Power level of hard pulse
P 12	Length of shaped pulse
SP 2	Power level of shaped pulse
SPNAM2	Name of shaped pulse
P 13	Length of ref shaped pulse
SP 3	Power level of ref shaped pulse
SPNAM3	Name of ref shaped pulse
FQ1LIST	Name of frequency list

14. Click on OK

15. Click on update parameters

Figure 7.11.



NAME selexp
EXPNO 2
PROCNO 1
DIR C:
USER pz

☐ Do not ask again

NOTE: The Target data set window above is to verify the correct data set and can be switched off by enable the ‘Do not ask again’ option.

---

16. Click on OK

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 pulse program parameters
3. Make the following changes:

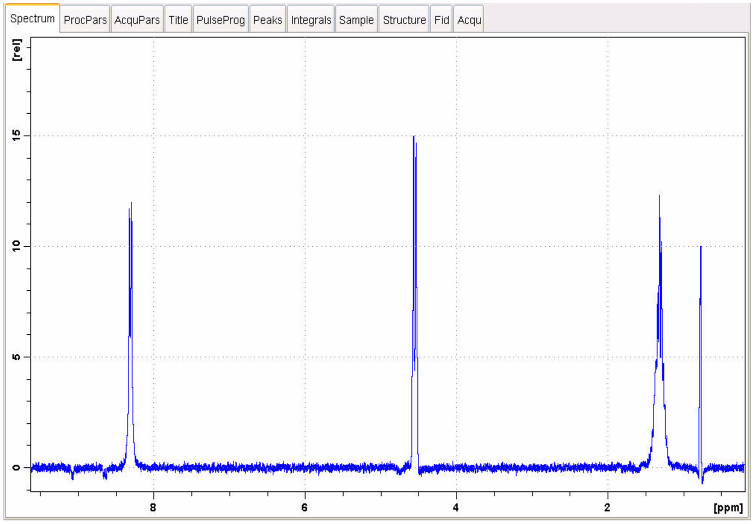
NS = **64**
DS = **8**
D1 = **2**
D9 = **0.075**
SPOFF2 = value from 7.1.5, step 14 (e.g. **645.47**)
GPZ1 = **15**

Running the experiment *7.1.5*

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

NOTE: All peaks should be phased positive.

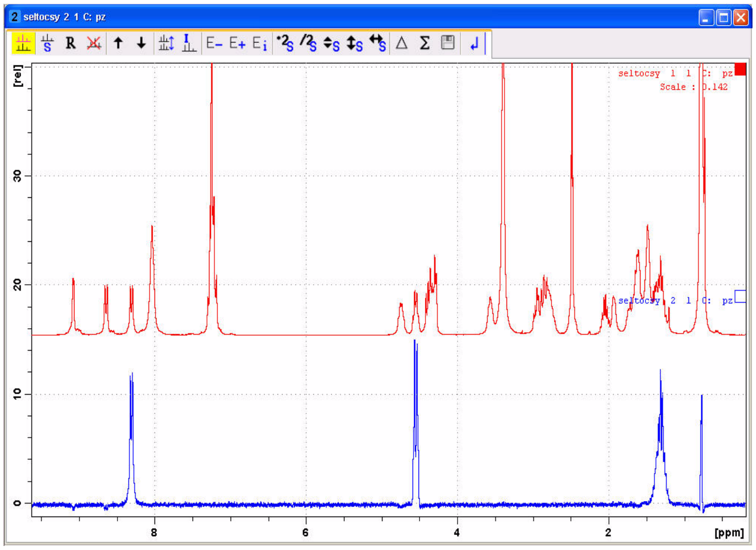
Figure 7.12.



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 7.13.



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 7, Homodecoupling, 7.1.6 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.

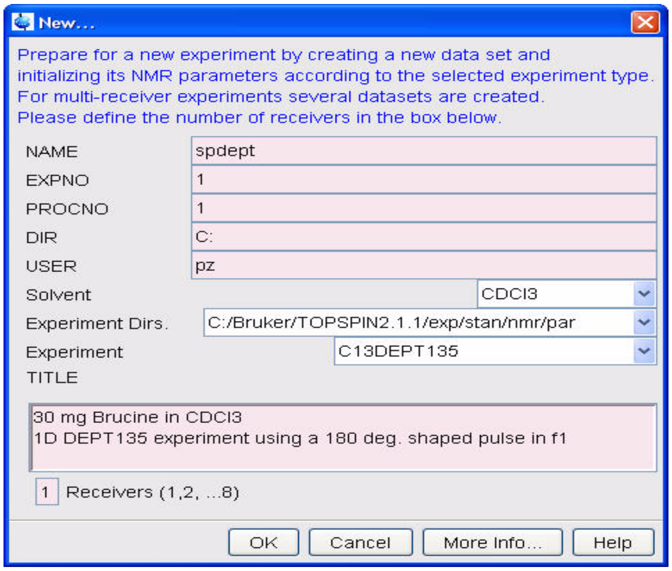
Sample:

30 mg Brucine in CDCl3

Experiment set up 8.1.1

1. Type **edc** and change the following parameters

Figure 8.1.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME	spdept
EXPNO	1
PROCNO	1
DIR	C:
USER	pz
Solvent	CDCl3
Experiment Dirs.	C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment	C13DEPT135

TITLE

30 mg Brucine in CDCl3
1D DEPT135 experiment using a 180 deg. shaped pulse in f1

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select CDCl3

5. Shim for best homogeneity

6. Tune the probe

7. Type **pulprog deptsp135** in the command line

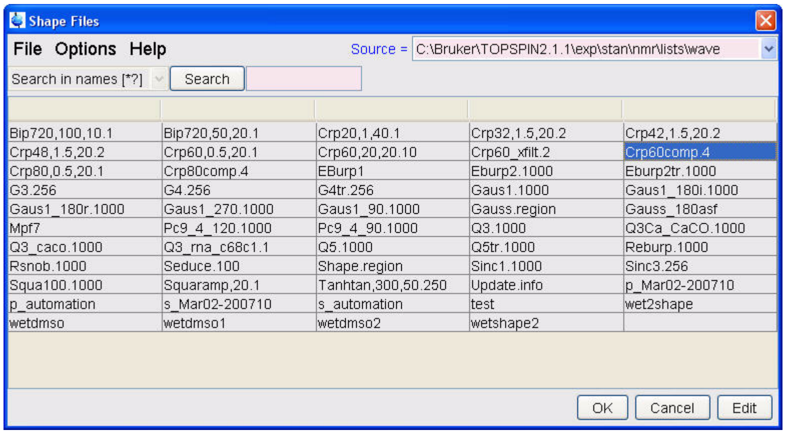
8. Type **getprosol** in the command line

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.



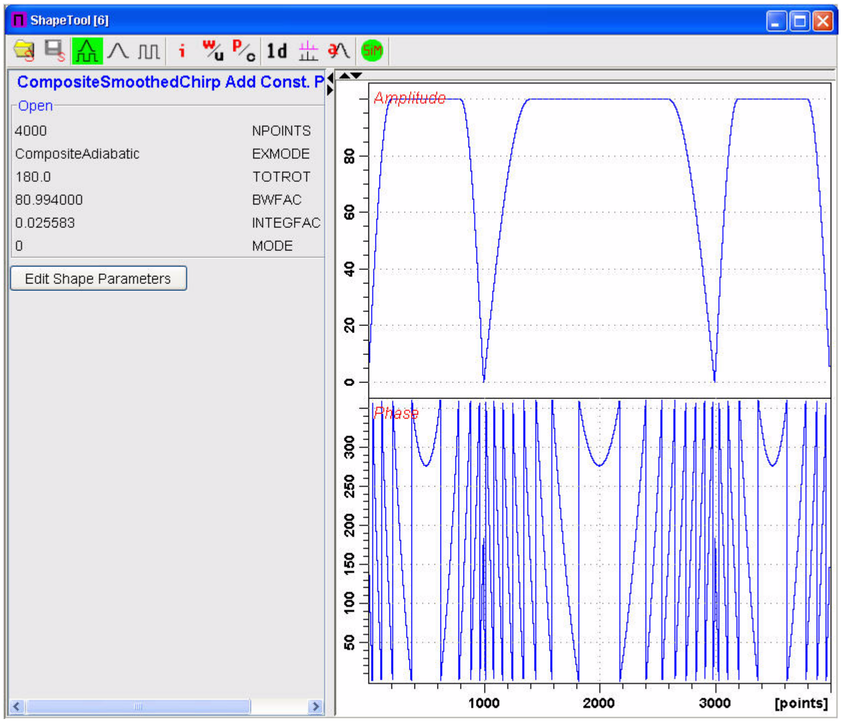
Source = C:\Bruker\TOPSPIN2.1.1\exp\stan\nmr\lists\wave

Bip720,100,10.1	Bip720,50,20.1	Crp20,1,40.1	Crp32,1.5,20.2	Crp42,1.5,20.2
Crp48,1.5,20.2	Crp60,0.5,20.1	Crp60,20,20.10	Crp60_xfilt.2	Crp60comp.4
Crp80,0.5,20.1	Crp80comp.4	EBurp1	Eburp2.1000	Eburp2tr.1000
G3.256	G4.256	G4tr.256	Gaus1.1000	Gaus1_180i.1000
Gaus1_180r.1000	Gaus1_270.1000	Gaus1_90.1000	Gauss.region	Gauss_180asf
Mpf7	Pc9_4_120.1000	Pc9_4_90.1000	Q3.1000	Q3Ca_CaCO.1000
Q3_caco.1000	Q3_rna_c68c1.1	Q5.1000	Q5tr.1000	Reburp.1000
Rsnob.1000	Seduce.100	Shape.region	Sinc1.1000	Sinc3.256
Squa100.1000	Squaramp,20.1	Tanhtan,300,50.250	Update.info	p_Mar02-200710
p_automation	s_Mar02-200710	s_automation	test	wet2shape
wetdmso	wetdmso1	wetdmso2	wetshape2	

3. Select '**Crp60comp.4**'

4. Click on OK

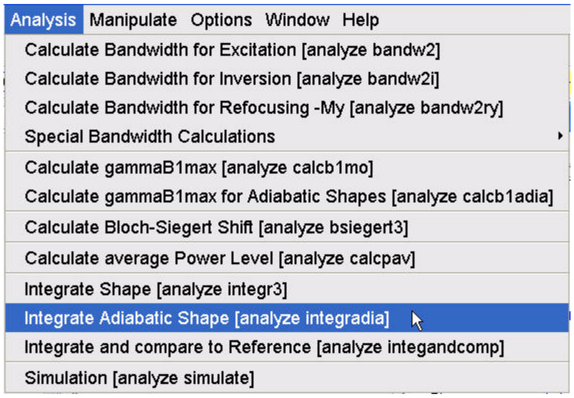
Figure 8.3.



4000 NPOINTS
CompositeAdiabatic EXMODE
180.0 TOTROT
80.994000 BWFAC
0.025583 INTEGFAC
0 MODE

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

Figure 8.4.

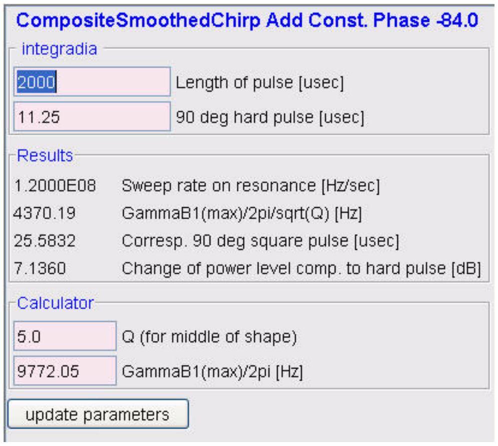


6. Make the following change:

Length of pulse [usec] = **2000**

7. Press the ‘Enter’ key

Figure 8.5.



CompositeSmoothedChirp Add Const. Phase -84.0

integradia

2000	Length of pulse [usec]
11.25	90 deg hard pulse [usec]

Results

1.2000E08	Sweep rate on resonance [Hz/sec]
4370.19	GammaB1(max)/2pi/sqrt(Q) [Hz]
25.5832	Corresp. 90 deg square pulse [usec]
7.1360	Change of power level comp. to hard pulse [dB]

Calculator

5.0	Q (for middle of shape)
9772.05	GammaB1(max)/2pi [Hz]

NOTE: The value for ‘change of power lev comp. to lev of hard pulse’ is calculated after executing Figure 8.5.

---

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

Figure 8.6.

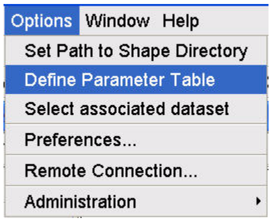
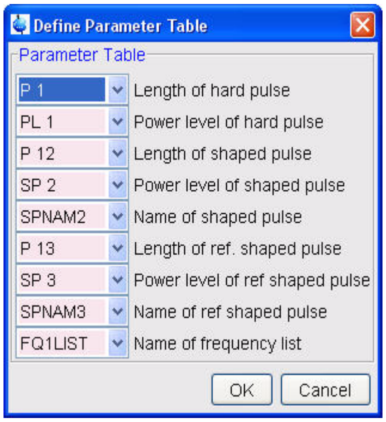


Figure 8.7.



Define Parameter Table

Parameter Table

P 1	Length of hard pulse
PL 1	Power level of hard pulse
P 12	Length of shaped pulse
SP 2	Power level of shaped pulse
SPNAM2	Name of shaped pulse
P 13	Length of ref. shaped pulse
SP 3	Power level of ref shaped pulse
SPNAM3	Name of ref shaped pulse
FQ1LIST	Name of frequency list

9. Make the following changes:

Length of hard pulse = **P1**

Power level of hard pulse = **PL1**

Length of shaped pulse = **p12**

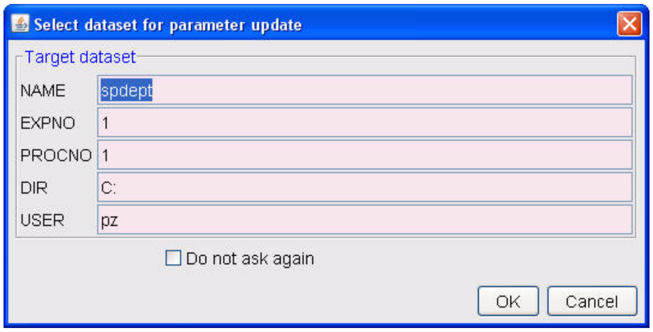
Power Level of shaped pulse = **SP2**

Name of shaped pulse = **SPNAM2**

10. Click on OK

11. Click on update parameters

Figure 8.8.



Select dataset for parameter update

Target dataset

NAME spdept

EXPNO 1

PROCNO 1

DIR C:

USER pz

☐ Do not ask again

12. Click on OK

13. Click on ☒ to close the Shape Tool window

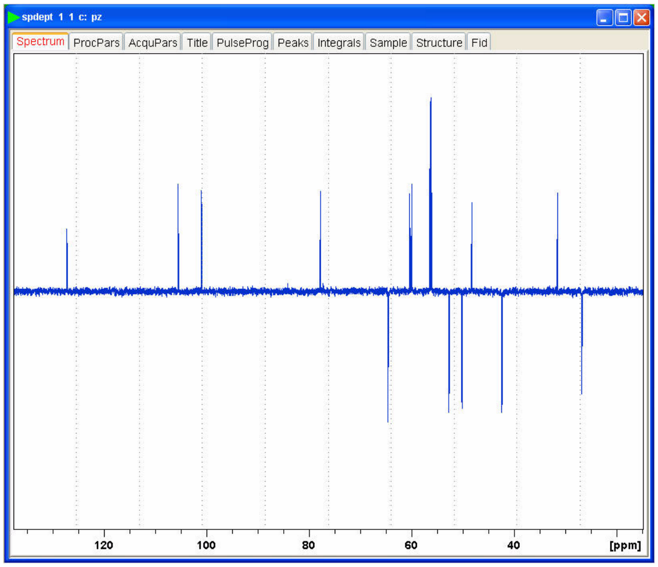
Running the experiment 8.1.3

1. Type **rga**

2. Type **zg** to start the acquisition

3. Process and Phase correct the spectrum

Figure 8.9.



2-D HSQC using a shaped 13C pulse

9

Introduction 9.1

Using this experiment will yield a higher Signal to noise compared 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.

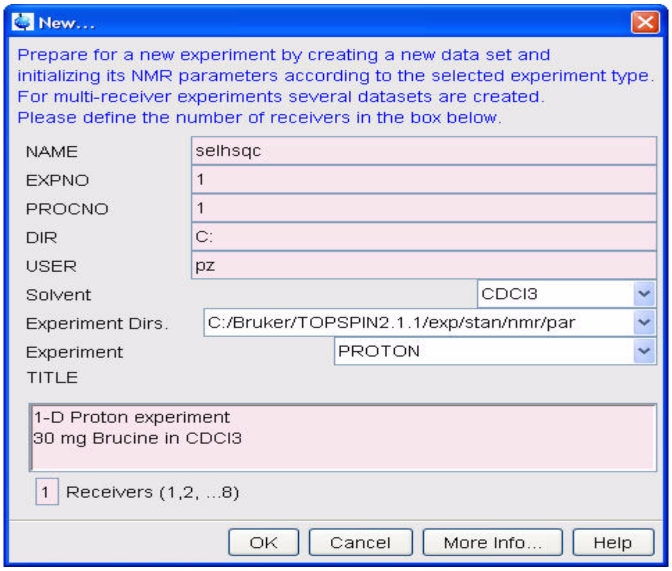
Sample:

30mg Brucine in CDCl3

Reference spectrum 9.1.1

1. Type **edc** and change the following parameters

Figure 9.1.



New...

Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

NAME	selhsqc
EXPNO	1
PROCNO	1
DIR	C:
USER	pz
Solvent	CDCl3
Experiment Dirs.	C:/Bruker/TOPSPIN2.1.1/exp/stan/nmr/par
Experiment	PROTON

TITLE

1-D Proton experiment
30 mg Brucine in CDCl3

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample

4.Type **lock** and select CDCl3

5. Shim for best homogeneity

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

7. Click on to read in the Prosol parameters

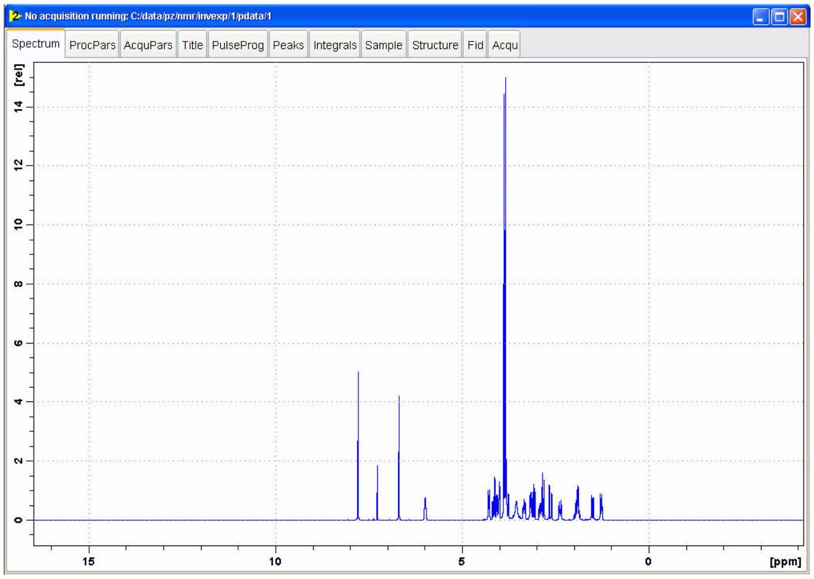
8. Tune the probe

9. Type **rga**

10. Type **zg** to start the acquisition

11. Process and Phase correct the spectrum

Figure 9.2.



No acquisition running: C:/data/pz/nmr/invexp/1/pdata/1

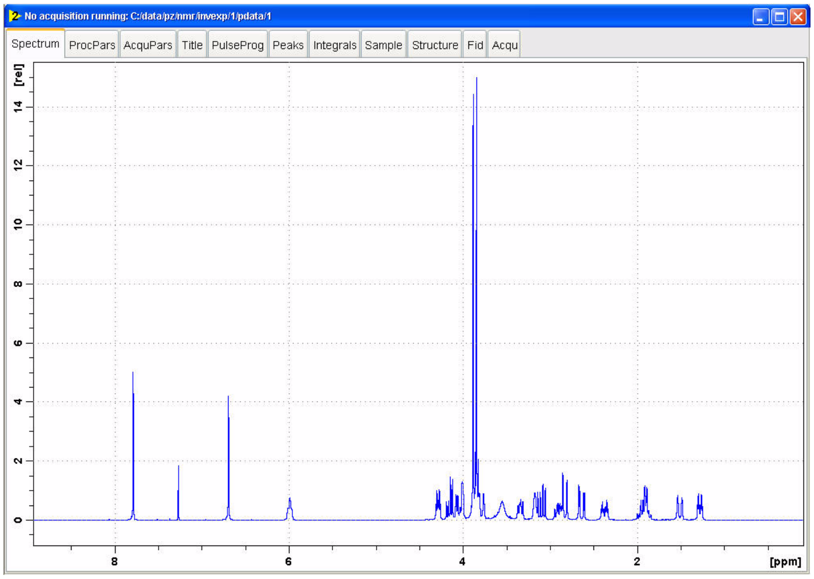
Limit setting 9.1.2

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

2. Type **re 2**

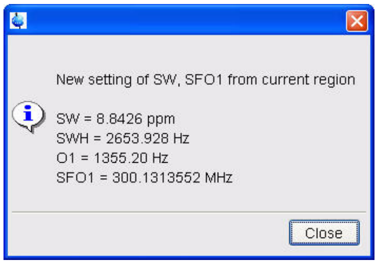
3. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

Figure 9.3.



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

Figure 9.4.



New setting of SW, SFO1 from current region

SW = 8.8426 ppm
SWH = 2653.928 Hz
O1 = 1355.20 Hz
SFO1 = 300.1313552 MHz

5. Write down the SW value, rounding off to the nearest 1/10th of a ppm (e.g. **8.8**)

6. Write down the O1 value, rounding off to the nearest Hz (e.g. **1355**)

7. Click on Close

8. Type **sr** on the command line 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:

SW F2 [ppm] = e.g. 8.8 (Limit setting 9.1.2, step 5)
O1 [Hz] = e.g. 1355 (Limit setting 9.1.2, step 6)
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 = e.g. 0 (Limit setting 9.1.2, step 8)

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*

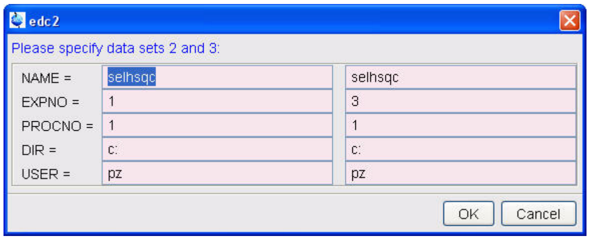
1. Type rga

2. Type zg to start the acquisition

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.



Please specify data sets 2 and 3:

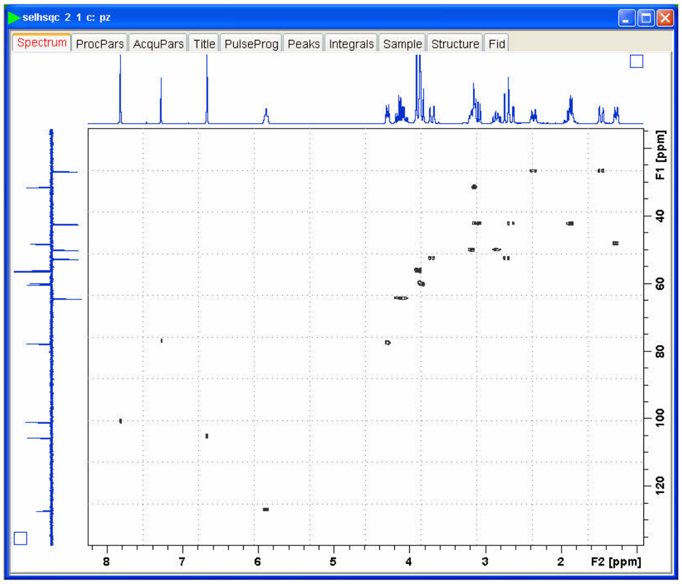
NAME =	selhsqc	selhsqc
EXPNO =	1	3
PROCNO =	1	1
DIR =	c:	c:
USER =	pz	pz

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 and probe has to be equipped with the Gradient hardware.

Sample:

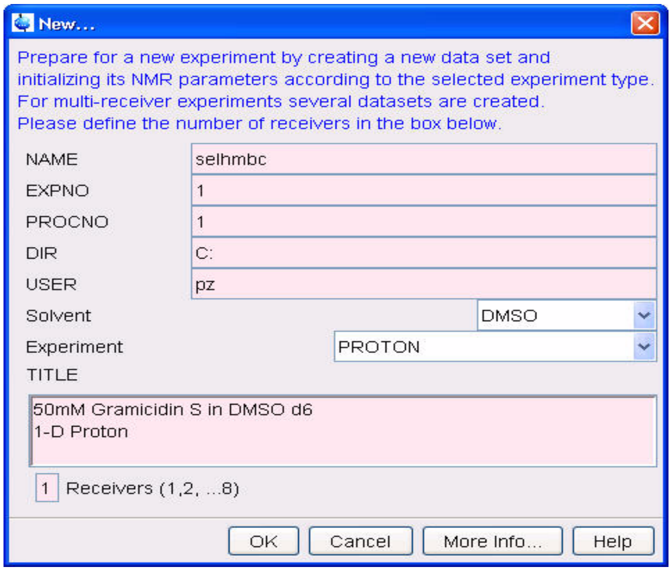
50 mM Gramicidin S in DMSO

Reference spectrum

10.1.1

1. Type **edc** and change the following parameters

Figure 10.1.



Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the box below.

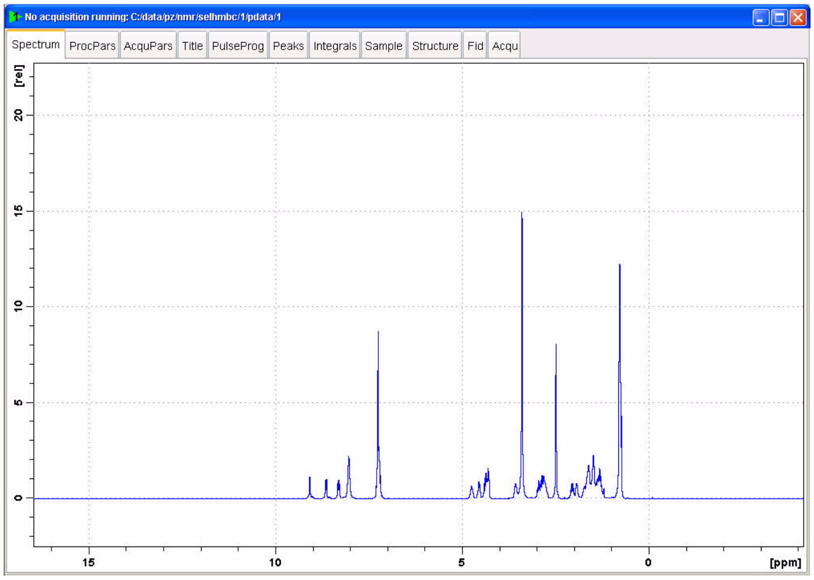
NAME: selhmbc
EXPNO: 1
PROCNO: 1
DIR: C:
USER: pz
Solvent: DMSO
Experiment: PROTON
TITLE:
50mM Gramicidin S in DMSO d6
1-D Proton

1 Receivers (1,2, ...8)

2. Click on OK

3. Insert the sample
4.Type **lock** and select DMSO
5. Shim for best homogeneity
6. Select the ‘**AcquPars**’ tab by clicking on it
7. Click on to read in the Prosol parameters
8. Tune the probe
9. Type **rga**
10. Type **zg** to start the acquisition
11. Process and Phase correct the spectrum

Figure 10.2.

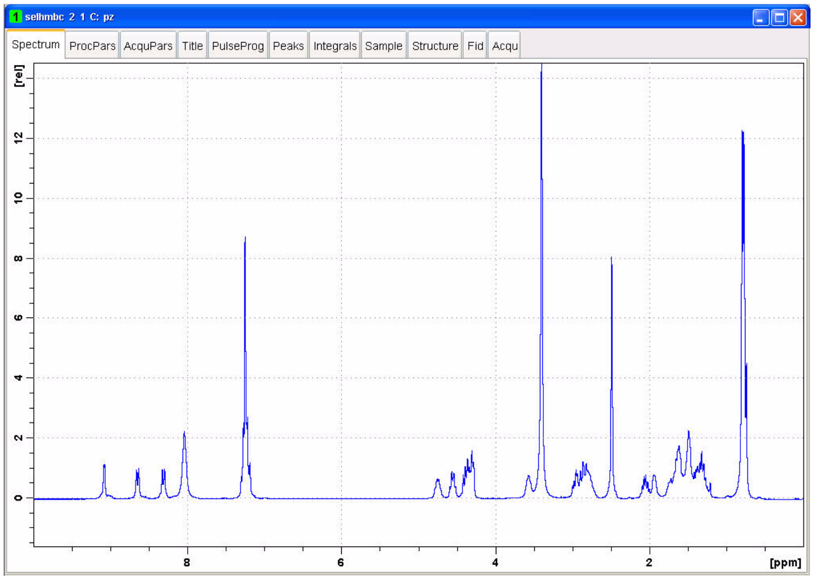


No acquisition running: C:/data/pz/nmr/selhmbc/1/pdata/1

Limit setting 10.1.2

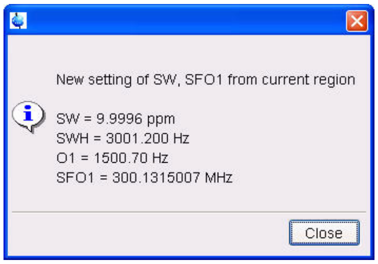
1. Type **wrpa 2** on the command line
2. Type **re 2**
3. Expand the spectrum to include all peaks (e.g. 0 ppm to 10 ppm

Figure 10.3.



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

Figure 10.4.



New setting of SW, SFO1 from current region

SW = 9.9996 ppm
SWH = 3001.200 Hz
O1 = 1500.70 Hz
SFO1 = 300.1315007 MHz

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 **o1p** on the command line and write down the value of O1p, rounding off to the nearest 1/10th of a ppm (e.g. **5 ppm**)

8. Type **sr** on the command line and write down the exact value (e.g. **0 Hz**)

Running a 2-D HMBC experiment *10.1.3*

1. Type **rpar HMBCGP 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:

F2 SW [ppm] = value from step 6, Limit setting 10.1.2 (e.g. **10**)

O1P [Hz] = value from step 7, Limit setting 10.1.2 (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 10.1.2 (e.g. **0**)

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

9. Change the title to: **50 mM Gamicidin S in DMSO-d6, 2-D HMBC**

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

Acquisition *10.1.4*

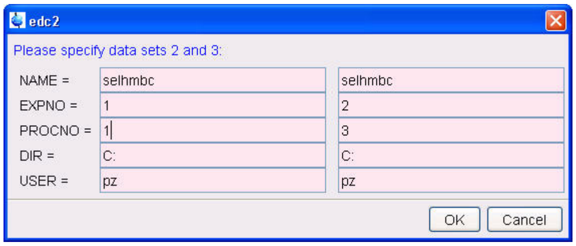
1. Type **rga**

2. Type **zg** to start the acquisition

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.



Please specify data sets 2 and 3:

NAME =	selhmbc	selhmbc
EXPNO =	1	2
PROCNO =	1	3
DIR =	C:	C:
USER =	pz	pz

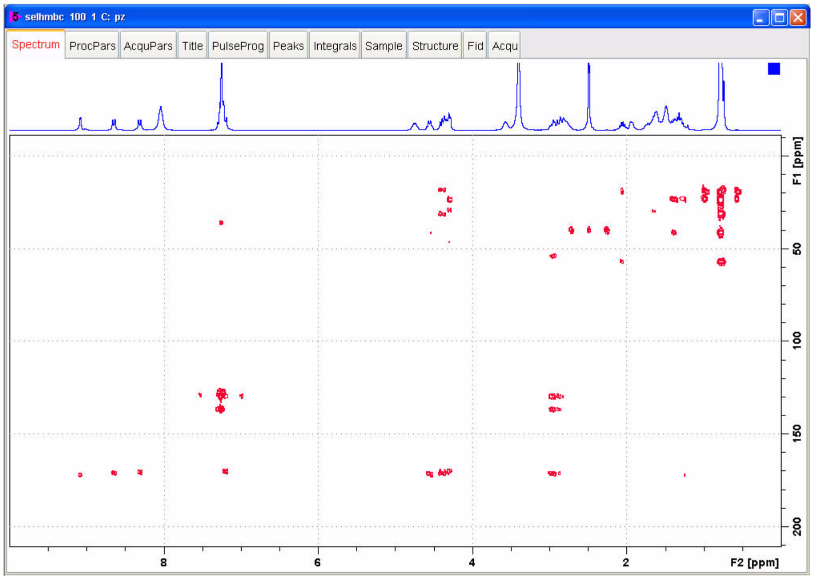
2. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

3. Click on OK

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.

4. Type **xaup**

Figure 10.6.



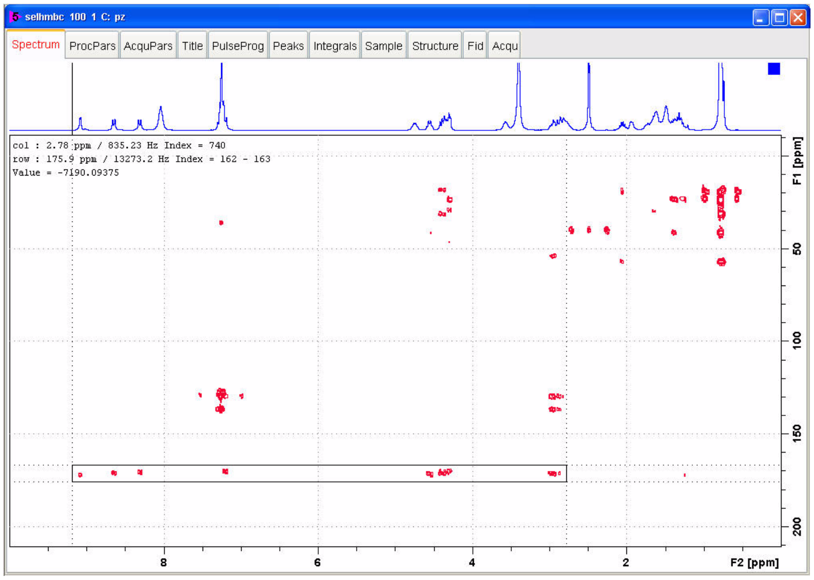
Optimizing the parameters on the carbonyl region 10.1.6

1. Type **wrpa 3** on the command line

2. Type **re 3**

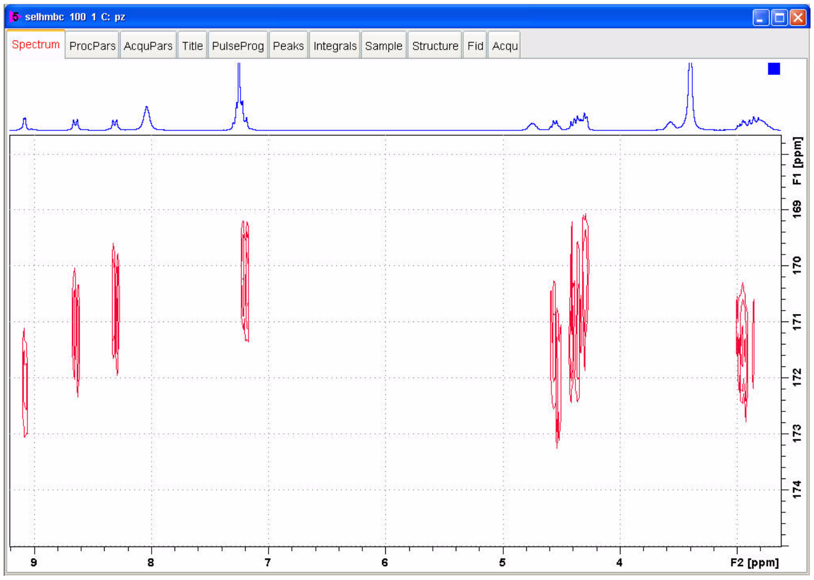
3. Expand the carbonyl region including all cross peaks (e.g. 168 ppm to 178 ppm)

Figure 10.7.



```
col : 2.78 ppm / 835.23 Hz Index = 740
row : 175.9 ppm / 13273.2 Hz Index = 162 - 163
Value = -7190.09375
```

Figure 10.8.



4. Write down the expanded F1 sweep width in ppm (e.g. **10 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 pulse program parameters

8. Write down the value for P3 [us] (e.g. **9.5 us**)

9. Write down the value for PL2 [dB] (e.g. **0 dB**)

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

11. Change the title to: **50 mM Gamicidin S in DMSO-d6, selective 2-D HMBC**

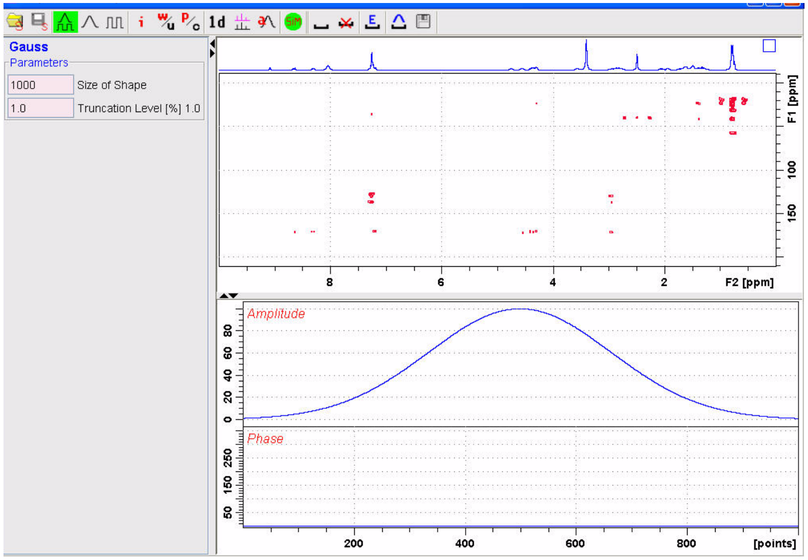
12. Select the '**Spectrum**' tab by clicking on it

Set up the selective pulse *10.1.7*

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.



Gauss

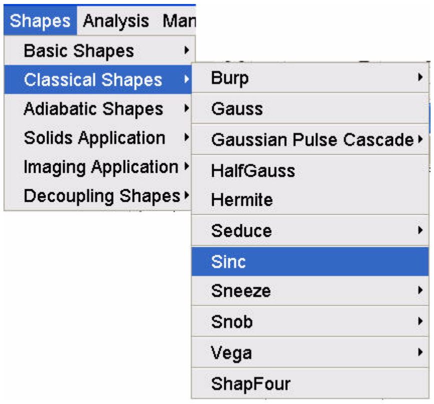
Parameters

1000 Size of Shape

1.0 Truncation Level [%] 1.0

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

Figure 10.10.

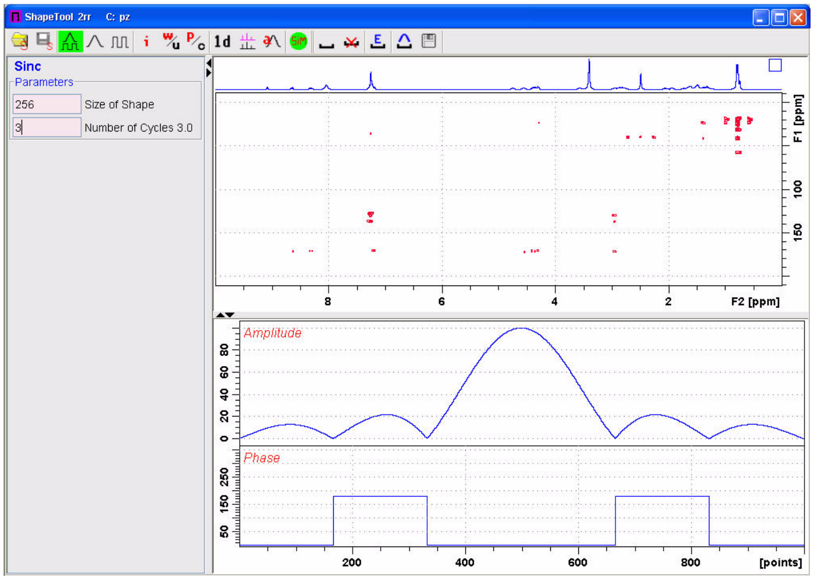


4. Make the following changes:

Change size of shape = **256**

Number of cycles = **3**

Figure 10.11.



256 Size of Shape

3 Number of Cycles 3.0

5. Click on

6. Click on ‘**Save Shape**’

7. Make the following changes:

File Name = **Sinc3.256**

Figure 10.12.

sinc3.256 Title

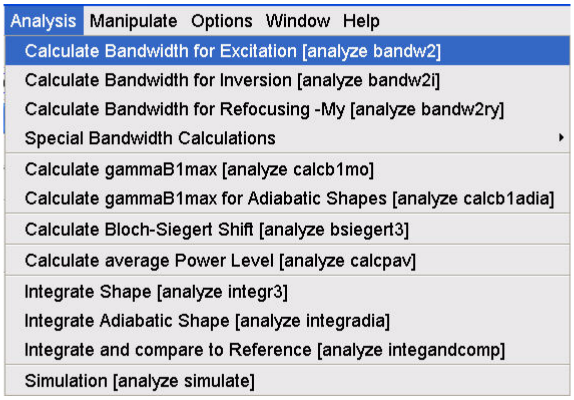
90.0 Flip Angle

Excitation Type of rotation

8. Click on OK

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

Figure 10.13.



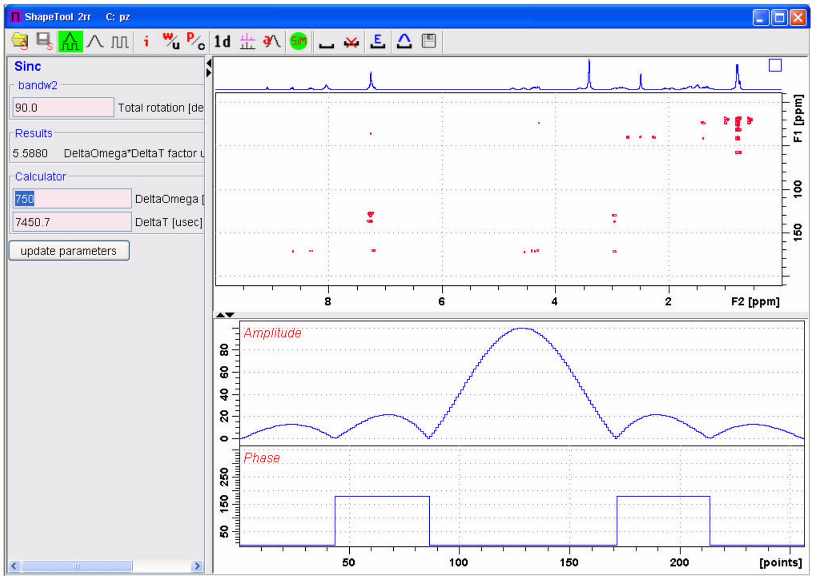
10. Make the following changes:

DeltaOmega [Hz] = **750** (e.g. SW 10 ppm from step 4 in 10.1.6)

11. Press the ‘Enter’ key

NOTE: The value of Delta T [usec] is being calculated. (e.g. 7450.7 usec)

Figure 10.14.



90.0 Total rotation [de

5.5880 DeltaOmega*DeltaT factor

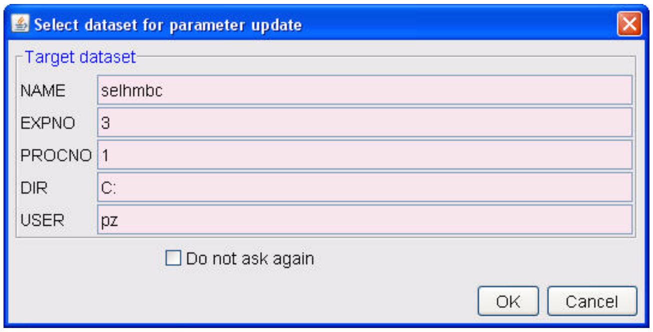
750 DeltaOmega [

7450.7 DeltaT [usec]

12. Write down the Delta T value [usec] (e.g. **7450.7 usec**)

13. Click on update parameters

Figure 10.15.



NAME selhmbc

EXPNO 3

PROCNO 1

DIR C:

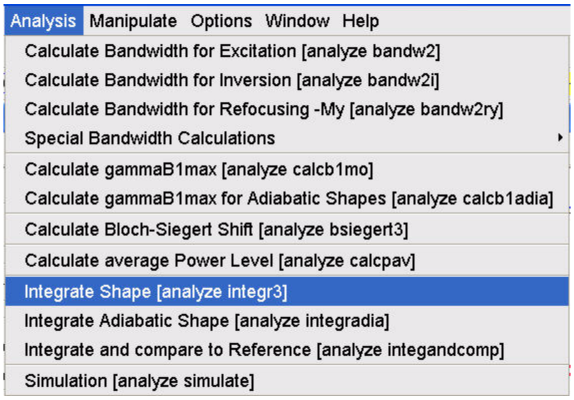
USER pz

☐ Do not ask again

14. Click on OK

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

Figure 10.16.



16. Make the following change:

Length of pulse [usec] = value from step 12 in 10.1.7 (e.g.**7450.7**)

17. Press the 'Enter' key

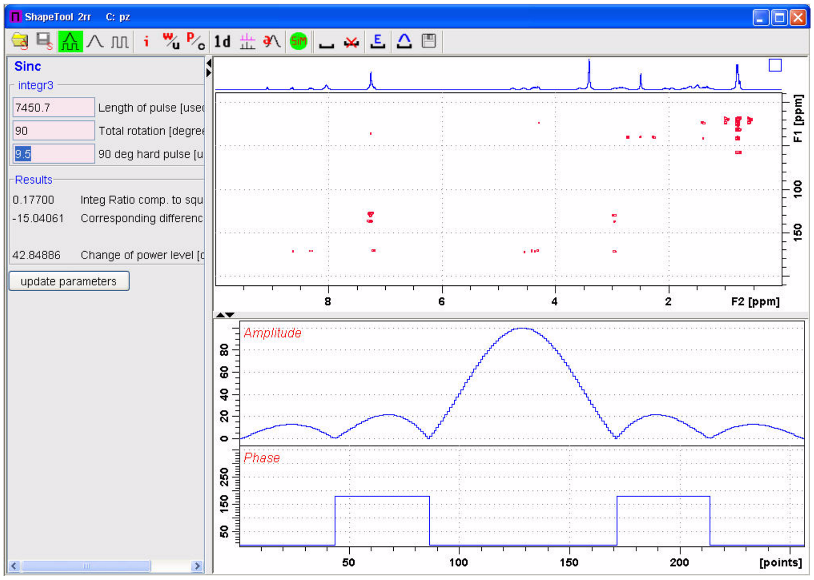
Total rotation [degree] = **90**

18. Press the 'Enter' key

90 deg. hard pulse [usec] = p3 from step 8 in 10.1.6 e.g. **9.5**)

19. Press the 'Enter' key

Figure 10.17.



7450.7 Length of pulse [usec
90 Total rotation [degree
9.5 90 deg hard pulse [u

0.17700 Integ Ratio comp. to squ
-15.04061 Corresponding differenc
42.84886 Change of power level [c

20. Write down the change of power level [dB] value (e.g. **42.85**)

21. 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 10.1.6 (e.g. **10**)

O2P [ppm} = value from step 5 in 10.1.6 (e.g. **172**)

3. Click on ⎍ to display the pulse program parameters

4. Make the following changes:

P13 [us] = value from step 12 in 10.1.7 (e.g. **7450.7**)

SP14 [dB] = (value from step 19 in 10.1.7) + (PL2) (e.g. **42.85**)

Running the experiment *10.1.9*

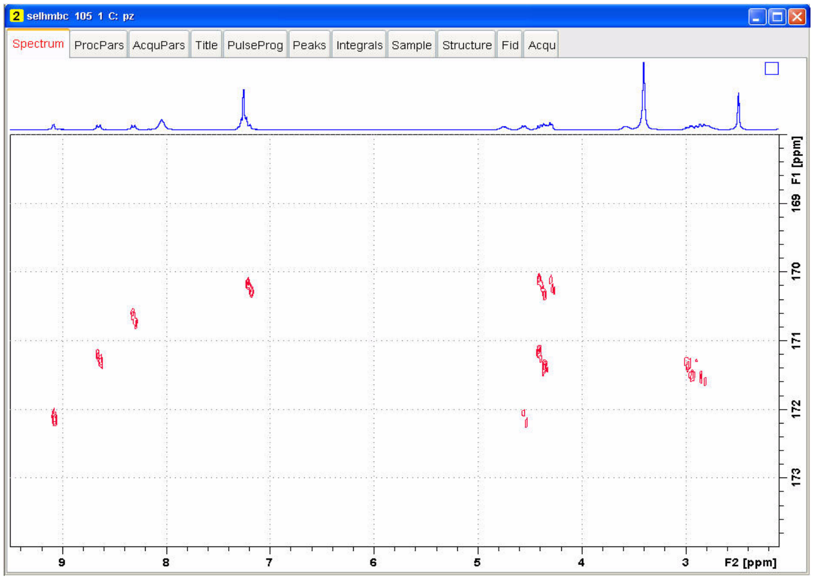
1. Select the '**Spectrum**' tab by clicking on it

2. Type **zg** to start the acquisition

3. Type xfb to process the 2-D data

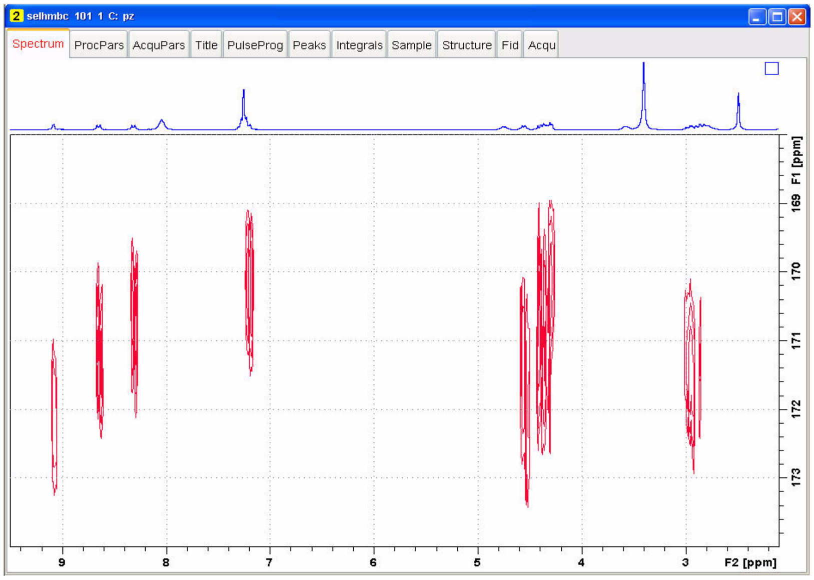
4. Expand the 2-D spectrum

Figure 10.18.



5. Compare the result of the selective HMBC against the regular HMBC in 10.1.3

Figure 10.19.



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: