

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

Advanced Experiments User Guide

Version 002



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Contents

Introduction

General

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

1.1

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

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.



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

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

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

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

2-D Inverse Experiments

2D edited HSQC

Sample:

20 mg Brucine in CDCl3

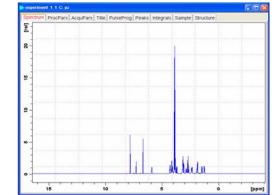
Preparation experiment

2.1.1

2.1

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

Figure 2.1.



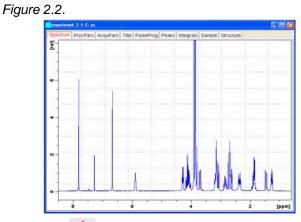
2. Type wrpa 2 on the command line

3. Type re 2

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

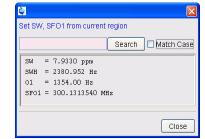


NOTE: You may exclude the solvent peak, if it falls outside of the region of interest.



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





6. Write down the value of SW, rounding off to the nearest 1/10th of a ppm

- 7. Write down the value of O1, rounding off to the nearest Hz
- 8. Click on Close
- 9. Type sr and write down the exact value

Setting up the HSQC experiment

2.1.2

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



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)

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O1 [Hz]	=	value	from step 7 (Preparation experiment 2.1.1)
SOLVENT		=	CDCI3



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

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

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

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

2. Click on **b** to start the acquisition

Processing

2.1.4

2.1.3



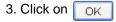
The standard Bruker parameter sets are optimized to run under complete automation through the use of AU programs. The name of the AU program is entered in the acquisition (eda) and processing (edp) parameter lists, as AUNM. To start the acquisition, the command xaua may be used. For executing the processing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

1. Type edc2

Figure 2.4.

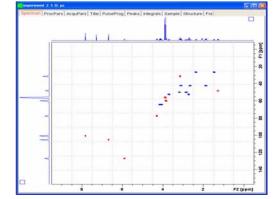
Please specify	data sets 2 and 3:	
NAME =	experiment	experiment
EXPNO =	1	2
PROCNO =	1	3
DIR =	D:	D:
USER =	pz	pz

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



4. Type xaup

Figure 2.5.





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

2D HMBC experiment

2.2

Sample:

20 mg Brucine in CDCl3

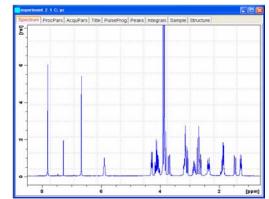
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Preparation experiment

2.2.1

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





Setting up the HMBC experiment

2.2.2

1. Type rpar HMBCLPND all

2. Turn the spinner off



NOTE: 2-D experiments should be run non spinning

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

4. Make the following changes:

SW [F2]	=	value from step 6 (Preparation experiment 2.1.1)
O1 [Hz]	=	value from step 7 (Preparation experiment 2.1.1)
SOLVENT		= CDCI3



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

- 5. Click on 📘 to read in the Prosol parameters
- 6. Select the 'ProcPar' tab by clicking on it
- 7. Make the following changes:
- SR [F2] = value from step 9 (Preparation experiment 2.1.1)

8 Select the 'Title' tab by clicking on it

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

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

Acquisition

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

2. Click on 🕨 to start the acquisition

Processing



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 acquisition (eda) and processing (edp) parameter lists, as AUNM. To start the acquisition, the command xaua may be used. For executing the processing AU program the command xaup may be used.

1. Type edc2

2.2.3

2.2.4

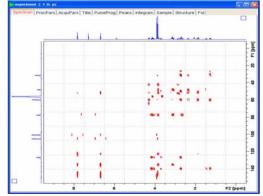
Figure 2	2.7
----------	-----

lease specify	data sets 2 and 3:	
NAME =	experiment	experiment
EXPNO =	1	2
PROCNO =	1	3
DIR =	D:	D:
USER =	ρz	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.





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

Adding the F1 projection to the HSQC contour plot

2.3



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

If one wishes to add the F1 projection to the plot, an additional 13C spectrum has

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

Creating the external projection spectrum

2.3.1

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

- 2. Open the HSQC experiment
- 3. Type edc2

Figure 2.9.

Please specify	data sets 2 and 3:	
NAME =	experiment	experiment
EXPNO =	1	5
PROCNO =	1	1
DIR =	D:	D:
USER =	07	pz

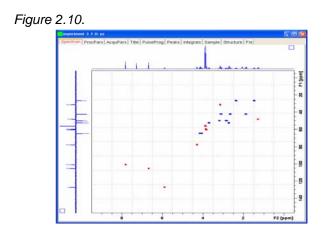
4. Enter the EXPNO and PROCNO of the 1D Proton spectrum into the first column (data set 2)

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

6. Click on OK

7. Type xaup

Adding the F1 projection to the HSQC contour plot





Creating the plot template

Figure 2.11. Dote MA + Q HSQC experiment of Brucine RUKER C Stand Title 🗮 All none d'a Teur A. T.1. Mode: 120 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 05 00 ppn

1. Type viewxwinplot

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

3. Click on Edit

2.3.2

4. Select the 'Basic' tab

Figure 2.12.	
Edit	

Position	×	3.000000	y:	1.000000
Dimension	x. 🕅	<u> 15.000000</u>	y:	13.500000
		Attributes		

4. Make the following changes:

Position X	=	3
Dimension X	=	15

- 6. Click on Apply
- 7. Select the '2D Projection' tab

Figure 2.13.

✓ Iop	<u>S</u> ize	3.00	Select
✓ Left	Size	3.00	Select
∏ <u>R</u> ight	Siz <u>e</u>	2,00	Select
<u>_A</u> t	tributes		

8. Enable 'Data set left'

9. Make the following change:

Size = 3 10. Click on Select...

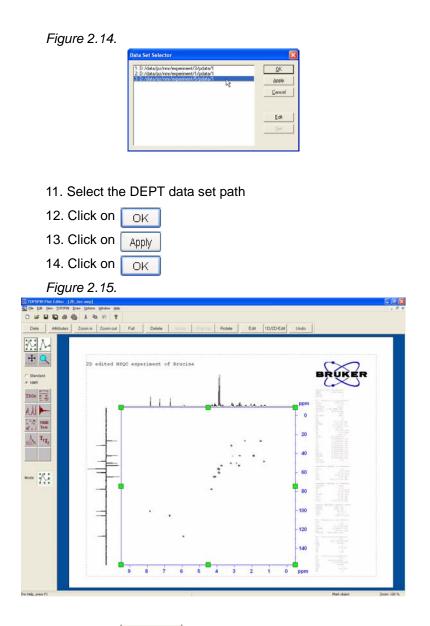




Figure 2.16.



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

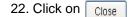
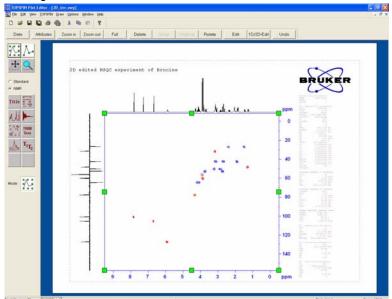


Figure 2.17.



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

Figure 2.18.

Save in: 🙆 layouts		- 60 0	- 111 -
backup layouts.A3 layouts.A4 layouts.ATP layouts.glp layouts.HWT	layouts.LC layouts.multi layouts.stack lb_BB.xwp lb_BB_170.xwp lb_BB_170.xwp lb_BB_pz.xwp	10 10 10 10 10	_H.xwp _H_exp.xwp _H_pz.xwp _H_water.xwp _H+info.xwp _H+If.xwp
<			>
File name: 2D_inv_	2pro l xwp		Save
Save as type: TOPSPII	v plot lavouts (".xwp)	-	Cancel



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

24.	Change	Filename	to 2D_	inv_	2proj	i.xwp)
-----	--------	----------	--------	------	-------	-------	---

25. Click on Save

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Diffusion Experiment

3.1

Introduction



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.

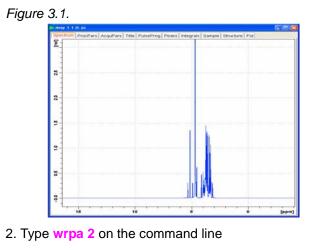
Sample:

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



- 3. Type re 2
- 4. Expand the spectrum from 6ppm to -2ppm

5. Click on <u>s</u> to set the sweep width and the O1 frequency of the displayed re- gion
Figure 3.2.
6. Click on Close
7. Type <mark>td 16k</mark>
8. Type <mark>si 8k</mark>
9. Click on 🕨 to start the acquisition
10. Type <mark>ef</mark>
11. Type <mark>apk</mark>
12. Type abs
Figure 3.3.
Convert 2: 10: path Image: Second S

Parameter set up



- 1. Type iexpno
- 2. Select the 'AcquPars' tab by clicking on it
- 3. Click on \prod to display the pulse program parameters

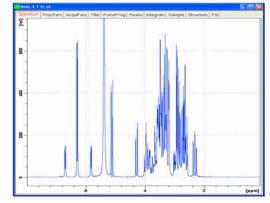
4. make the following changes:

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

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

- 6. Click on 🕨 to start the acquisition
- 7. Type <mark>ef</mark>
- 8. Type apk
- 9. Type abs

Figure 3.4.



- 10. Type iexpno
- 11. Select the 'AcquPars' tab by clicking on it
- 12. Click on **I** to display the pulse program parameters

13. make the following changes:

GPZ6[%] = 95

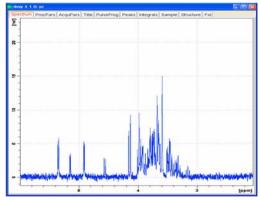
14. Click on 🕨 to start the acquisition

15. Type ef

16. Type apk

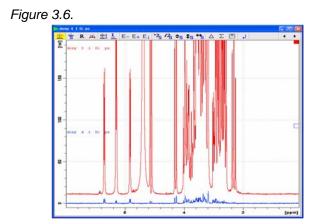
17. Type abs

Figure 3.5.



18. Click on 🏪 to open the multiple display window

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





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

Acquisition

3.1.3

- 1. Type iexpno
- 2. Select the 'AcquPars' tab by clicking on it
- 3. Make the following changes:
- PULPROG = stebpgp1s

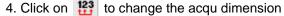


Figure 3.7.



- 5. Enable 'Change dimension from 1D to 2D'
- 6. Click on OK
 7. Make the following changes:
 TDF1 = 16
 FnMODE = QF
- 8. Type dosy on the command line

🥘 dosy	
Enter first gradient amplitude:	
2	
	OK <u>C</u> ancel

9. Enter 2 for first gradient amplitude

10.	Click on OK	
Fig	ure 3.9.	
	🦉 dosy	
	Enter final gradient amplitude:	
	95	
		OK Cancel

11. Enter 95 for final gradient amplitude

12.	Click on	С	ΙK)		
Figu	ıre 3.10.					
	🧼 dosy					

🔤 dosy	
Enter number of points:	
16	
	OK Cancel

13. Enter 16 for the number of points

14.	Click on OK
Fig	ure 3.11.
	🛃 dosy 🛛 🔀
	ramp type (I/q/e (linear/squared/exponential)):
	OK Cancel

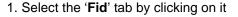
15. Enter I for ramp type

16. Click on 🚺	ĸ	
Figure 3.12.		
	🛃 dosy	×
	Ų	Do you want to start acquisition ?

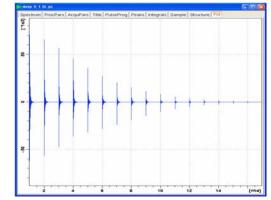
17. Click on OK to start the acquisition

Processing

3.1.4









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

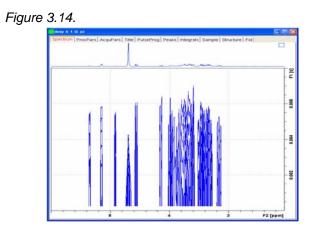
- 2. Select the 'ProcPars' tab by clicking on it
- 3. Click on **P** to display the processing parameters

16

4. Make the following changes:



- $PH_mod [F1] = no$
- PH_mod [F2] = pk
- 5. Type xf2 on the command line
- 6. Type abs2 on the command line
- 7. Type setdiffparm on the command line
- 8. Select the 'Spectrum' tab by clicking on it



Calculating the diffusion coefficient

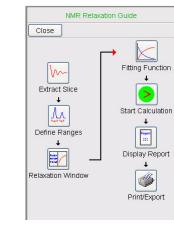
3.1.5



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

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

Figure 3.15.



3. Click on 🚾 to extract slice

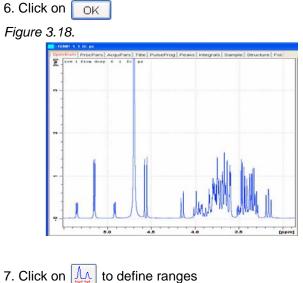
Figure 3.16.

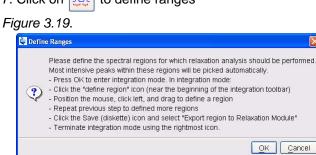


3. Click on Spectrum

Figure 3.17.					
a					
Spectrum slice must b This Spectrum should All further data prepar Sil	correspond to	an experin	nent with the	maximum or n	ninimum delay time.
					OK Cancel

5. Enter 1 for the slice number

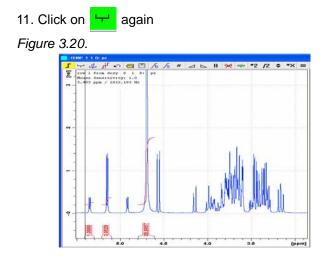




8. Click on OK

9. Click on 4 to define the regions

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



12. Click on 🔳

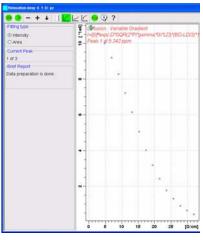
Figure 3.21.



- 13. Select 'Export Region To Relaxation Module' by clicking on it
- 14. Click on 斗

15. In the Guide window, click on <a>['Relaxation Window'

Figure 3.22.



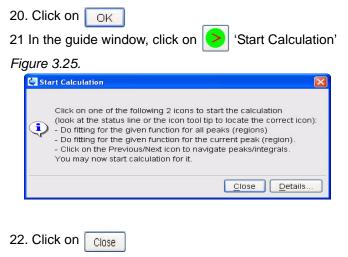
16. Enable 'Intensity'

17. In the guide window, click on Fit

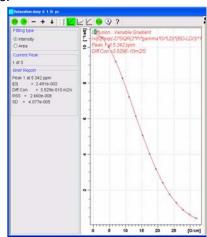
'Fitting Function'

Fig	ure 3.23.	1	
	🥞 Fitting Functi	• 🛛	
	are to be	ect the function to which the peak intensities or integrals tted, depending on the experiment which produced the relaxation data, alog provides all possibilities for Relaxation analysis adjustment. Close Details	
18.	Click on	Close	
	ure 3.24.		
гıу	ure 3.24.		
		Relaxation parameters	
		FID # for phase determination	
		1000.0 Left limit for baseline correction	
		-1000.0 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	
		Fitting Function	
		Vargrad Function Type	
		1 Number of components	
		difflist 🛛 List file name	
		0.0010 Increment (auto)	
		Iteration control parameters	
		Setup	
		Additional Parameters	
		4257.7 GAMMA(Hz/G)	
		3.6 LITDEL(msec)	
		99.9 BIGDEL(msec)	
		1.0 GRADIEN(G/cm)	
		OK Apply Cancel	

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







23. In the data window, click on 🔌 'Calculate fitting parameters for all data points'



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

-Brief Repo	t			
Peak 1 at 5	.342 ppm			
Diff Con.	= 3.529e-010 m2/s			
Peak 2 at 5.142 ppm				
Diff Con.	= 5.746e-010 m2/s			
Peak 3 at 4.694 ppm				
Diff Con.	= 1.961e-009 m2/s			
	Peak 2 at 5 Diff Con. Peak 3 at 4			

24. In the guide window. click on i bisplay Report'

Figure 3.28.

🔄 Report				
		Fitting report		
SIMFIT RESULTS				^
Dataset : D:/data/p	z/nmr/dosy/6/pc	lata/1/ct1t2.tx	t	
INTENSITY fit : Dif	fusion : Variak	le Gradient :		111
I=I[0]*exp(-D*SQR(2	*PI*gamma*Gi*LI)*(BD-LD/3)*1e	4)	
16 points for Peak	1, Peak Point	= 5.342 ppm		
Converged after 64	iterations!			
Results Comp. 1				
I[0] = 2	.491e-002			
Diff Con. = 3	.529e-010 m2/s			
Gamma = 4	.258e+003 Hz/G			
Little Delta =	3.600m			
Big Delta =	99.900m			
RSS = 2.660e-00	8			
SD = 4.077e-00	5			
Point Gradient	Expt	Calc	Difference	
1 6.740e-001	2.488e-002	2.488e-002	-7.066e-007	
2 2.765e+000	2.434e-002	2.431e-002	-3.082e-005	
3 4.855e+000	2.313e-002	2.309e-002	-4.208e-005	
4 6.945e+000	2.126e-002	2.132e-002	5.564e-005	
5 9.036e+000	1.915e-002	1.914e-002	-8.148e-006	×
			Close	Save as Print

25. In the guide window, click on Pint 'Print Report

Multiplet Analysis

Multiplet assignments

Sample:

100 mg 2, 3,-Dibromopropionic acid in CDCI3

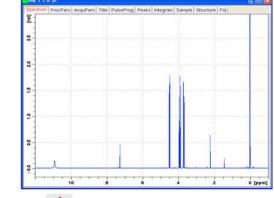
Preparation experiment

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 <u>set the sweep width and the O1 frequency of the displayed region</u>

Figure 4.2.

			Search	🗌 🗌 Match Case
SW	=	13.0151 ppm		-
SWH	=	3906.250 Hz		
01	=	1648.51 Hz		
SF01	=	300.1316536 MH	(z	

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

2. Click on 🕨 to start the acquisition

Processing

4.1.3

- 1. Type ft
- 2. Type apk



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

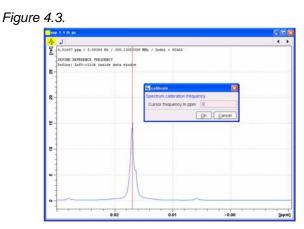
3. Type abs



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

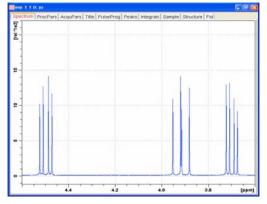
- 4. Expand the TMS peak
- 5. Click on 🙏 'Spectrum Calibration'
- 6. Move the cursor line into the center of the TMS peak

7. Click the left mouse button

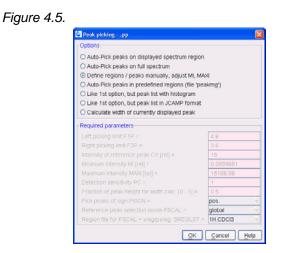


- 8. Change the value of the cursor frequency in ppm = 0
- 9. Click OK
- 10. Expand the spectrum from 3.6 ppm to 4.6 ppm
- 11. Click with the right mouse button inside the spectrum window
- 12. Select 'Save Display Region To'
- 13. Enable the option 'Parameters F1/2 [dp1]'

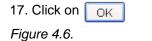
Figure 4.4.

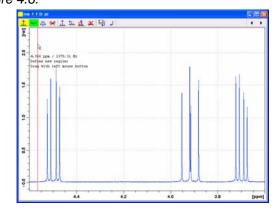


- 14. Click on 'Analysis' in the main menu bar
- 15. Select 'Peak Picking [pp]' by clicking on it



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



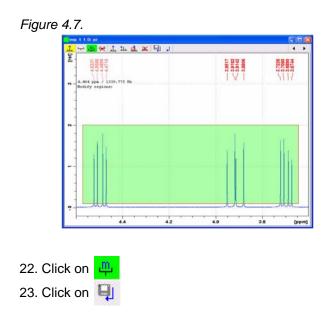


18. Move the cursor line to the left of the multiplet at 4.5 ppm

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)

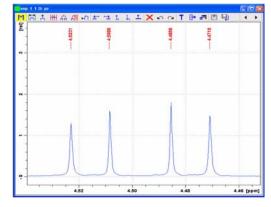


4.1.4

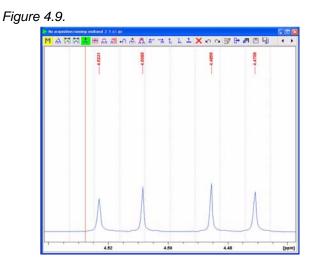
- 1. Expand the multiplet at 4.5 ppm
- 2. Click on 'Analysis' in the main menu bar

Select 'Structure Analysis'

- 4. Select 'Multiplet Definition [mana]' by clicking on it
- Figure 4.8.

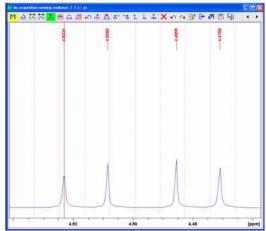


- 5. Click on 📩 'Define Multiplets Manually'
- 6. Place the cursor line to the left of the first peak of the multiplet



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

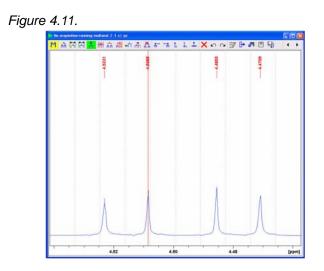
Figure 4.10.



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

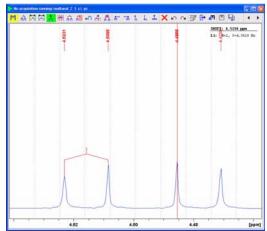


NOTE: A small marker is placed above the top of the first peak

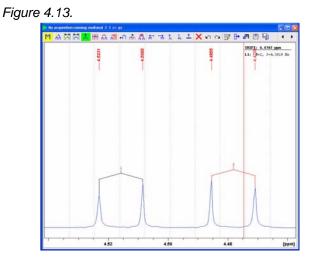


- 13. Move the cursor line in to the center of the two marked peaks
- 14. Click the right mouse button
- 15. Select 'Define Multiplet' by clicking on it

Figure 4.12.

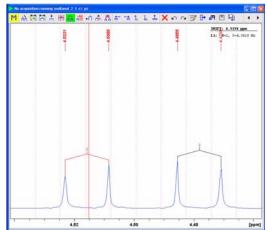


16. Repeat steps 6 through 15 starting with the third peak and ending with the fourth peak

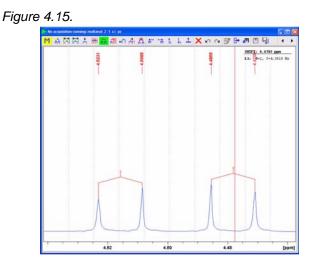


- 17. Click on 📅 'Couple Existing Multiplets'
- 18. Move the cursor line in to the center of the first two peaks marked 1
- 19. Click the left mouse button

Figure 4.14.



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





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

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

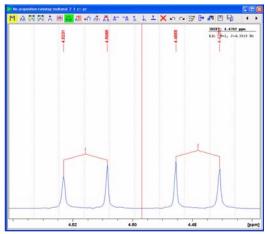
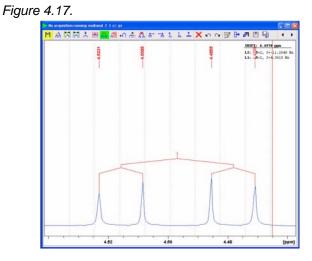


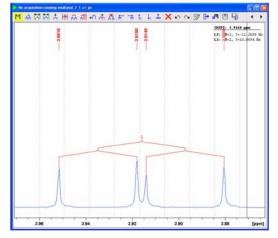
Figure 4.16.

- 23. Click the right mouse button
- 24. Select 'Define Multiplet' by clicking on it

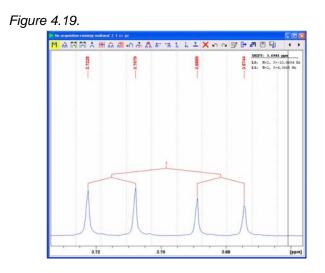


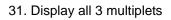
- 25. Click the right mouse button inside the spectrum window
- 26. Select 'Finish Current Mode' by clicking on it
- 27. expand the multiplet at 3.9 ppm
- 28. Repeat steps 6 through 26 for this multiplet

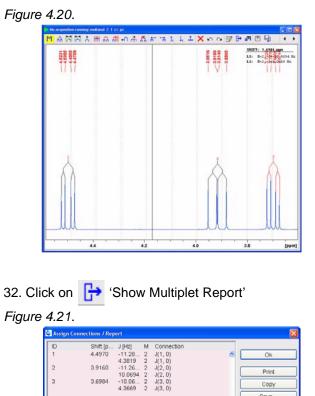
Figure 4.18.



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







33, Click on

Find Connections

Copy Save... Start edito JMR JPF

Find Connections

Figure 4.2	22.	
	Multiplet Connection Options	
	Maximum of Difference between Couplings Lower Limit for Couplings Change already defined Connections	0.1 Hz 0.3 Hz
		OK Cancel
34. Click Figure 4.2	23. stgn Connections / Report Shift [p J [Hz] M Connection	X
1 2 3	4.4970 -11.282 J(1.0) 4.3619 2 J(1.3) 3.9160 -11.262 J(2.0) 10.0694 2 J(3.0) 3.6964 -10.062 J(3.0) 4.3669 2 J(3.1)	Ok Print Copy Save Start editor JMR JPF*
		Find Connections



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



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

Probes

 -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)

 -BB11H/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



5.1.1

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

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



NOTE: By default amplifier 1 is connected to the X-BB preamplifier and amplifier 2 is connected to the 1H preamplifier. Each amplifier delivers 150 Watts from 10Mhz to the 31P frequency and 60 Watts above 31P to the 1H frequency and this will include the 19F frequency.

Standard pulse programs such as zg. zgdc etc. can be used to observe 19F.

500MHz and above

-external amplifiers BLAXH (less then 1.5 years old)

BLA(R)H, BLAX combinations

- -external QNP accessory unit for RF routing
- -19F pass filter for doing observe 13C and 19F decoupling experiments.

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



NOTE: The 19F signal is generated on the 1H stage of the amplifier and the QNP accessory unit is designed to route the 19F frequency either to the 19F selective or X-QNP output. In addition it switches between the 1H and 19F for decoupling either of the nuclei.

Pulse programs have to include the routing and switching statements such as QNP_X, QNP_F, SWITO_F, SWITO_H.

Older AV systems

-external amplifier BLAXH (more then 1.5 years old)

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

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

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



NOTE: The 19F signal is generated on the 1H stage of the amplifier and the QNP accessory unit is designed to route the 19F frequency either to the 19F selective or X-QNP output. In addition it switches between the 1H and 19F for decoupling either of the nuclei.

Pulse programs have to include the routing and switching statements such as QNP_X, QNP_F, SWITO_F, SWITO_H.

1-D 19F experiments

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: $CFCI_3$ 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-CF₂-CH₂-OH

19F observe, no decoupling

5.2.1

5.2

Exploratory spectrum

1. Click on and change the following parameters

Figure 5	5.1.			
	New		×	
		w experiment by creating a new data set a R parameters according to the selected e>		
	NAME	f19exp		
	EXPNO PROCNO	1		
	DIR	D:		
	USER	pz		
	Solvent	Aceto		
	Experiment TITLE	F19	<u> </u>	
		iment no decoupling		
	2,2,3,4,4,4-He	xafluoro-1-butanol		
		OK Cancel More Info.		
2. Click	on OK]		
3. Insert	t the samp	le		
4. Click	on 😴 to	o display the Lock disp	lay	
5. In the	lock displ	lay window click on 👔	and select	Acetone
6. Tune	the probe	to observe 19F		
7. Shim	for best he	omogeneity		
8. In the	e lock displ	lay window click on 🚽	to close the	e window
9. Selec	t the ' Acq	uPars' tab by clicking	on it	
10. Clicl	k on 📙	to read in the Prosol p	arameters	
		enu click on ' Spectro eiver gain' or type rga	meter', select	'Adjustment' and click on
12. Clicl	k on 🕨	to start the acquisition		
13. Proc	cess and F	hase correct the spec	trum	
Figure 5	5.2.			
Spectrum		Title PulseProg Peaks Integrals Sample Structu	re Eid	
Ξ	i i dei dio [redoi dio] i	The Protectog Product Integrated complete or deco		
1				
-				

- 50

6

- 100

- 150

[ppm]

Optimizing the sweep width



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

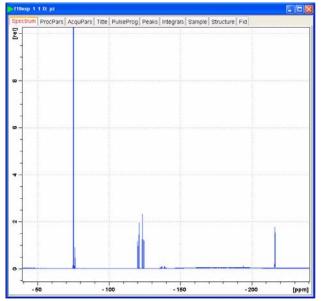
- 1. Select the 'AcquPars' tab by clicking on it
- 2. Change the following parameters:

SW [PPM]	=	200
----------	---	-----

O1P [PPM] = -140

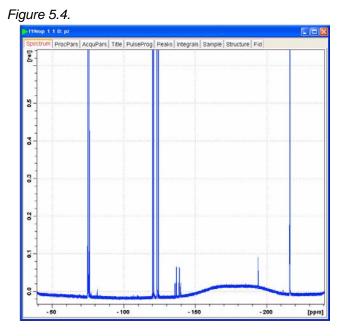
- 3. Click on 🕨 to start the acquisition
- 4. Process and Phase correct the spectrum





Baseline correction

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

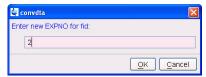




If a Fluorine background signal is present, a simple abs will not straighten the baseline and a linear prediction calculation may be necessary. See steps below.

3. Type convdta

Figure 5.5.



4. Type 2 into the convdta window

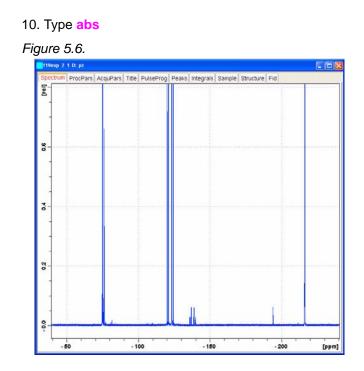
5. Click on OK

- 6. Select the 'Procpar' tab by clicking on it
- 7. Change the following parameters:

ME_mod	=	LPbc
NCOEF	=	32
TDoff	=	16

8. Type ef

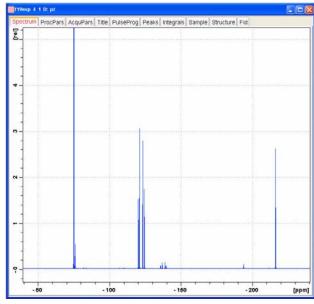
9. Phase correct the spectrum



19F observe, with 1H decoupling

5.2.2

- 1. Type iexpno
- 2. Type rpar F19CPD all
- 3. Tune the probe for 19F and 1H
- 4. Select the 'AcquPars' tab by clicking on it
- 5. Change the following parameters:
- SW [PPM] = 200
- O1P [PPM] = -140
- SOLVENT = Acetone
- 6. Click on 🔰 to read in the Prosol parameters
- 7. Select the 'Title' tab by clicking on it
- 8. Change the title to:1-D 19F experiment with 1H decoupling 2,2,3,4,4,4-Hexafluoro-1-Butanol
- 9. Select the 'Spectrum' tab by clicking on it
- 10. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**
- 11. Click on 🕨 to start the acquisition
- 12. Process and Phase correct the spectrum
- 13. To get rid of the background signal, follow the instructions in 5.2.1, Baseline correction, steps 1 through 9



1H observe, no 19F decoupling

5.2.3

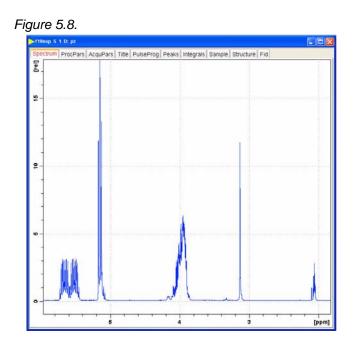
1. Type iexpno

Figure 5.7.

- 2. Type rpar PROTON all
- 3. Tune the probe for 1H
- 4. Select the 'AcquPars' tab by clicking on it
- 5. Make the following changes:
- SOLVENT = Acetone
- 6. Click on 📙 to read in the Prosol parameters
- 7. Select the 'Title' tab by clicking on it
- 8. Change the title to:1-D 1H experiment no 19F decoupling 2,2,3,4,4,4-Hexafluoro-1-Butanol
- 9. Select the 'Spectrum' tab by clicking on it

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

- 11. Click on 🕨 to start the acquisition
- 12. Process and Phase correct the spectrum



1H observe, with 19F decoupling using WALTZ

5.2.4

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

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

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

Change the following parameter:

SI = 32k

7. Click on 📋 to read in the Prosol parameters

- 8. Select the 'Title' tab by clicking on it
- 9. Change the title to:1-D 1H experiment with 19F decoupling 2,2,3,4,4,4-Hexafluoro-1-Butanol
- 10. Select the 'Spectrum' tab by clicking on it

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

- 12. Click on 🕨 to start the acquisition
- 13. Process and Phase correct the spectrum

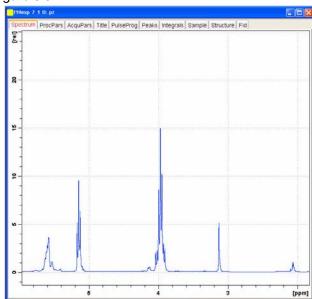


Figure 5.9.



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

1H observe, with 19F decoupling using Garp

5.2.5

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

2. Click on **I** to display the pulse program parameters

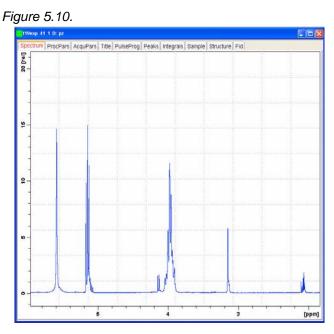
3. Make the following changes:

CPDPRG2	=	garp
PCPD2	=	70
PI12	=	calculate the power level in the prosol table

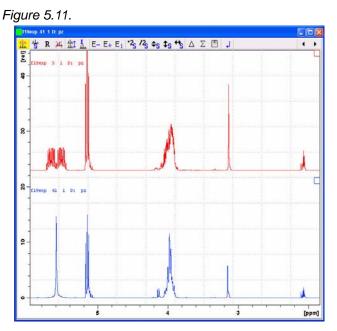
4. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

5. Click on 🕨 to start the acquisition

19. Process and Phase correct the spectrum



- 6. Click on 7. Drag the 19F coupled proton spectrum in to the display window



2-D 19F experiments



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

Sample:

2,2,3,4,4,4-Hexafluoro-1-butanol in Acetone-d6 CF_3 -CFH-CF₂-CH₂-OH

2-D Heteronuclear 1H/19F shift correlation

5.3.1

1-D 19F reference experiment

1. Click on and change the following parameters

Figure 5.12.

EXPNO	1	
PROCNO	1	
DIR	D:	
USER	pz	
Solvent	8	Acetone
Experiment		F19
TITLE		

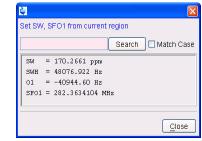
2. Click on OK

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

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

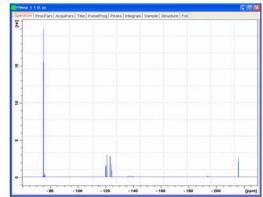
5. Click on 5 to set the sweep width and the O1 frequency of the displayed region

Figure 5.13.



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

Figure 5.14.



b) 1-D 1H reference experiment

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

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

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

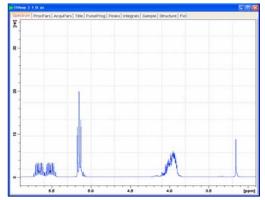
1 igui c 0. i 0.	Figure	5.	15.
------------------	--------	----	-----



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

10. Process and Phase correct the spectrum





- b) Set up of the 2-D HETCOR experiment
- 1. Type expno
- 2. Type rpar HCCOSW all
- 3. Turn the spinner off



NOTE: 2-D experiments should be run non spinning

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

58 (111)

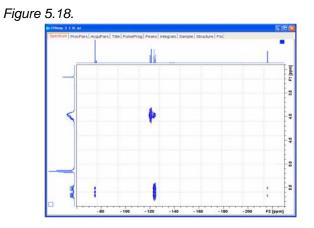
BRUKER BIOSPIN

Figure 5	I dit Spectrometer Param	itar		
	frequency	logical channel	amplifier	preamplifier
	8F1 282.404355 8F01 282.364818 0F31 -39537.0 8F2 300.13 8F02 300.131373 0F52 1373.1	Metry NUC1 Metry F1 FCU1/S0 Hr 100000 Metry F2 Metry F2 FCU2/S0 Hr 110000 F2 FCU2/S0	HEOW	1H/2H 1H 1H/2H 2H X8819F 2HS

6. Click on Save

7.Select the 'Acq	uPars	' tab by clicking on it		
8. Make the follow	wing cł	nanges:		
PULPROG	=	hfcoqfqn		
SW F2 [ppm]	=	value from step 8 (19F reference spectrum)		
SW F1 [ppm]	=	value from step 10 (19F reference spectrum)		
O1P [ppm]	=	value from step 5 (1H reference spectrum)		
O2P [ppm]	=	value from step 7 (1H reference spectrum)		
SOLVENT	=	Acetone		
9. Click on 📙 t	o read	in the Prosol parameters		
10. Click on <u> </u>				
11. Make the follo	owing o	changes:		
CNST2	=	25 = J(FH)		
12. Select the ' ProcPar ' tab by clicking on it				
13. Make the following changes:				
SR F2	=	value from step 11 (19F reference spectrum)		
SR F1	=	value from step 8 (1H reference spectrum)		
WDW F2	=	SINE		
WDW F1	=	SINE		
SSB F2	=	2		
SSB F1	=	2		
14. Select the 'Title' tab by clicking on it				
15. Change the title to: 2-D 1H/19F HETCOR experiment 2,2,3,4,4,4-Hexafluoro-1-Butanol				
16. Select the ' Spectrum ' tab by clicking on it				
17. In the main menu click on ' Spectrometer ', select ' Adjustment ' and click on ' Auto-adjust receiver gain ' or type rga				
18. Click on 🕨	to sta	rt the acquisition		
19. Type <mark>xfb</mark>				
20. Adjust the co	ntour le	evel		

Param



1-D Selective NOESY

6.1

Introduction



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

Sample:

30 mg Pamoic acid in DMSO

Reference spectrum

6.1.1

1. Click on 📋 and change the following parameters

Figure 6.1.

New			
A CARLES AND SHOULD DEPOSITE	w experiment by creating a new data set and R parameters according to the selected experiment type.		
NAME	selexp		
EXPNO	1		
PROCNO	1		
DIR	C:		
USER	pz		
Solvent	DMSO 🗸		
Experiment			
TITLE			
reference spectrum			
	OK Cancel More Info Help		

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



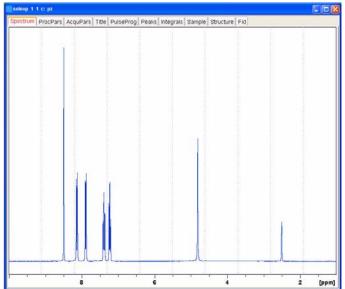
NOTE: selective excitation experiments should be run non spinning

- 7. Shim for best homogeneity
- 8. In the lock display window click on 🤳 to close the window
- 9. Select the 'AcquPars' tab by clicking on it
- 10. Click on 📋 to read in the Prosol parameters
- 11. Tune the probe

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

- 13. Click on 🕨 to start the acquisition
- 14. Process and Phase correct the spectrum

Figure 6.2.



6.1.2

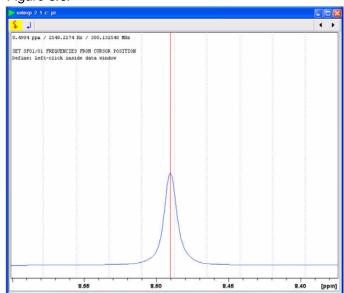
On resonance



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

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

Figure 6.3.



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

Figure 6.4.	
	01/02/03
	Define SF01/01 frequencies
	SFO1 [MHz] = 300.132548 O1/2/3 [Hz] = 2548.19
	01 02 03 Cancel
9. Click on	01

Setting up the acquisition parameters

6.1.3

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

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

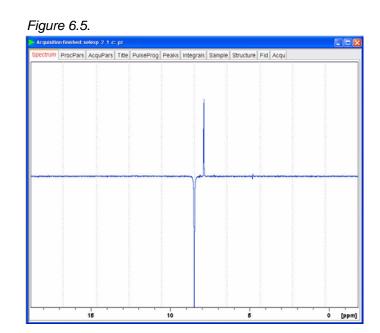
Running the experiment

6.1.4

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



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



Selective excitation region set up (example 2)

6.1.5

Off resonance



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

1. Run a Reference spectrum, following the instructions in 2.1.1 Reference Spectrum in this Chapter.

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

Figure 6.6.					
selexp 2 1 c: pz					
له <mark>ال</mark> ه					4 >
8.4904 ppm / 2548.2174 Hz / 300.132548 MHz					
SET SF01/01 FREQUENCIES FROM CURSOR POSITION Define: Left-click inside data window					
8.55	8.50	8.4	15	8.40	[ppm]

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

Figure 6.7.

🍓 01/02/03	X
Define SFO1/O1 fi	requencies
SFO1 [MHz] =	300.132548
O1/2/3 [Hz] =	2548.19
01 02	O3 Cancel

- 10. Write down the O1/2/3 (Hz) value showing in the Info window (e.g. 2548.19)
- 12. Click on Cancel
- 13. Type O1 and write down the current value (e.g. 1853.43)
- 14. Calculate the difference of step 9 and 11 (e.g. 694.55)
- 15. Click on Cancel



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

Setting up the acquisition parameters

6.1.6

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

BRUKER BIOSPIN

2. Click on **I** to display the pulsprogram parameters

3. Make the following changes:

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

Running the experiment

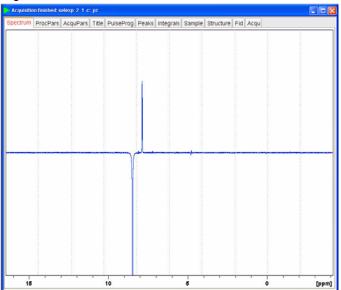
6.1.7

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



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

Figure 6.8.



Selective excitation region set up (example 3)

6.1.8

Integration region file



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

1. Run a Reference spectrum, following the instructions in 2.1.1 Reference Spectrum in this Chapter.

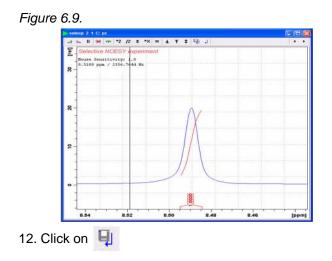
- 2. Type wrpa 2
- 3. Type **re 2**
- 4. Select the 'Title' tab by clicking on it
- 5. Change the title to: Selective NOESY experiment
- 6. Select the 'Spectrum' tab by clicking on it
- 7. Expand the signal region at 8.5 ppm
- 8. Click on 了
- 9. In the Integration menu bar click on 🖵 to define a integration region

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



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





Calculating the selective pulse width and power level

6.1.9



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

1. Type pulprog selnogp in the command line

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

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



Figure 6.10.

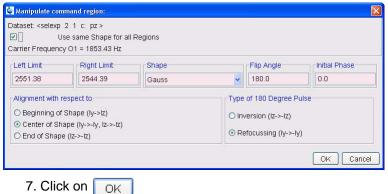
- 4. Select 'Gaus1.1000'
- 5. Click on OK

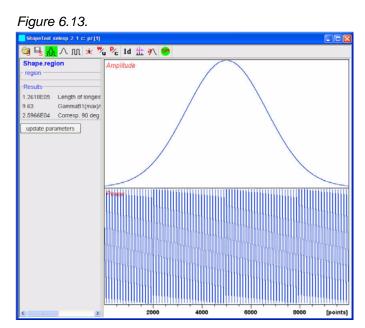
6. In the main menu click on 'Manipulate' and select 'Calc. Shape from Excitation Region' by clicking on it

Figure 6.11.

Manipulate	Options	Window	Help
Phase M	dulation a	icc. to Off	set Freq. [manipul offs]
Single Si	ne Modula	tion [mani	pul sinm2]
Single Co	sine Modu	ulation [ma	anipul cosm2]
Modulatio	on acc. to F	Freq. Swe	ер
Power of	Amplitude	[manipul	power]
Scale Am	plitude [ma	anipul sca	le]
Add cons	tant Phase	e (manipul	addphase]
Time Rev	rersal [mar	nipul trev]	
Calc. Sha	pe from E	xcitation F	Region [manipul region]
Add Shap	es (manip	ul addsha	ipes]
Expand S	hape [mar	nipul expa	nd]





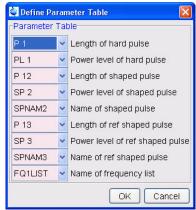


8. In the main menu click on '**Options**' and select '**Define Parameter Table**' by clicking on it

Figure 6.14.

Window Help
th to Shape Directory
Parameter Table
associated dataset
ences
e Connection
stration 🔸





9. Make the following changes:

Length of shaped pulse	=	p12
Power Level of shaped pulse	=	SP2
Name of shaped pulse	=	SPNAM2
10. Click on OK		

11. Click on	update parameters	
Figure 6.16.		
(ê.	
	Save as Shape.region	
		DK Cancel
-		

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

Setting up the acquisition parameters

6.1.10

- 1. Select the 'AcquPars' tab by clicking on it
- 2. Click on **I** to display the pulsprogram parameters

3. Make the following changes:

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

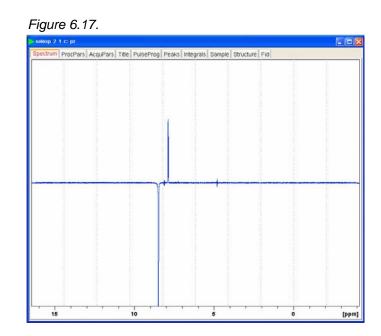
Running the experiment

6.1.11

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



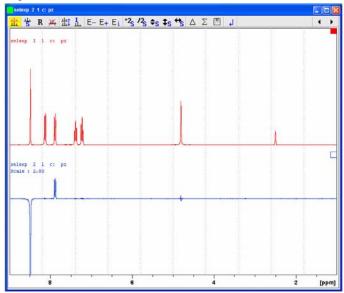
NOTE: Phase the selective exited peak negative to a sure the correct phase of the noe peaks.



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 $2 \times 12^{\circ} \times 12^{\circ} \times 12^{\circ} \times 12^{\circ}$ to adjust the spectra

Figure 6.18.



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



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

1-D selective TOCSY

Introduction



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

Sample:

50 mM Gramicidin S in DMSO

Reference spectrum

7.1.1

1. Click on 📋 and change the following parameters

Figure 7.1.

New		
	w experiment by creating a new data set and R parameters according to the selected experim	nent type.
NAME	seltocsy	
EXPNO	1	
PROCNO	1	
DIR	C:	
USER	pz	
Solvent	DMSO	~
Experiment	PROTON	~
TITLE		
reference spect	trum	<
	OK Cancel More Info	Help

7.1

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



NOTE: selective excitation experiments should be run non spinning

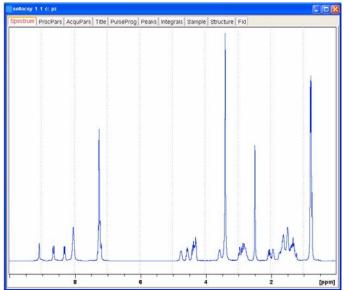
- 7. Shim for best homogeneity
- 8. In the lock display window click on 🤳 to close the window
- 9. Select the 'AcquPars' tab by clicking on it
- 10. Click on 📙 to read in the Prosol parameters

11. Tune the probe

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

- 13. Click on 🕨 to start the acquisition
- 14. Process and Phase correct the spectrum

Figure 7.2.



76 (111)

7.1.2

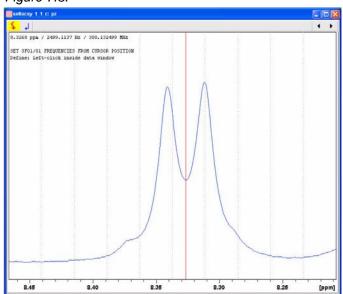
Off resonance



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

Figure 7.4.					
	🔄 01/02/03 🛛 🔀				
	Define SF01/01 fr	requencies			
	SFO1 [MHz] =	300.132499			
	O1/2/3 [Hz] =	2498.90			
	01 02	O3 Cancel			

- 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



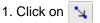
NOTE: If the signal is down field of O1, a positive value must be entered for spoff. If the signal is up field of O1, spoff will have a negative value.

Calculating the selective pulse width and power level

7.1.3

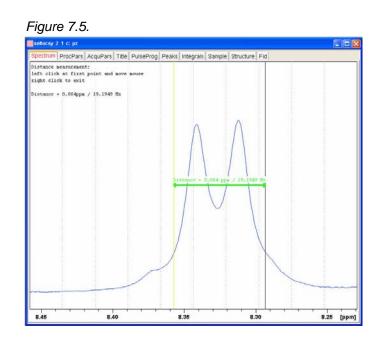


In this example the shaped pulse width and power level are determine using the **'Calculate Bandwidth'** option in the shaped tool program. Other method of calculating the pulse width and power level can be used, see Chapter 2, 1-D Selective NOESY, integration region file, in this manual, or use the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



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

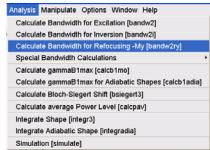


- 4. Write down the value for the distance between the two cursor lines (e.g. 19)
- 5. Type pulprog seimigp
- 6. Type getprosol

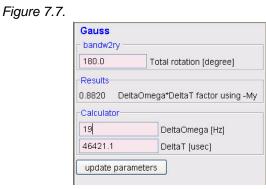
7. In the main menu click on 'Spectrometer' and select 'Shape Tool' or type stdisp

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





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

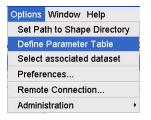




NOTE: The value for 'Delta T [usec]' is calculated after executing step 9.

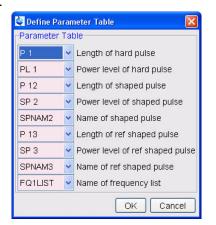
10. In the main menu click on '**Options**' and select '**Define Parameter Table**'

Figure 7.8.



11. Make the following changes:

Length of shaped pulse	=	p12
Power Level of shaped pulse	=	SP2
Name of shaped pulse	=	SPNAM2
Figure 7.9.		





Setting up the acquisition parameters

1. Select th	ie ' Acqı	Pars '	tab by	clicking on it	

2. Click on	Л	to display the pulsprogram parameters
-------------	---	---------------------------------------

3.	Make	the	following	changes:
----	------	-----	-----------	----------

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

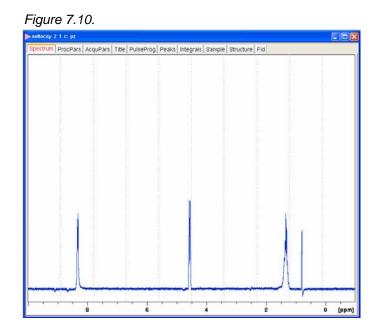
Running the experiment

7.1.5

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



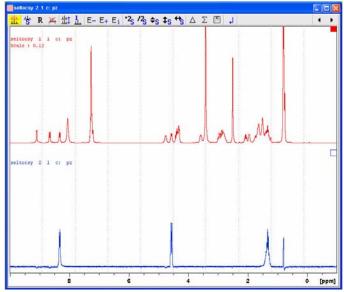
NOTE: All peaks should be phased positive.



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

- 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 $2 \times 12^{\circ} \times 12^{\circ} \times 12^{\circ} \times 12^{\circ}$ to adjust the spectra





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



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

1-D DEPT using a shaped 13C pulse

8.1

Introduction



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

Sample:

30 mg Brucine in CDCl3

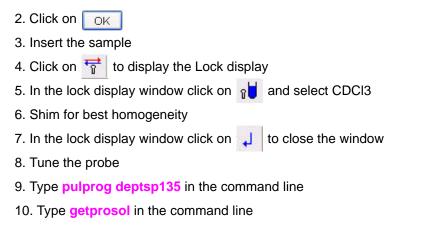
Experiment set up

8.1.1

1. Click on 1 and change the following parameters

Figure 8.1.

New			×
		ating a new data set and ing to the selected experiment typ	e.
NAME	spdept		
EXPNO	1		
PROCNO	1		
DIR	C:		
USER	pz		
Solvent	- Ar	CDCI3	~
Experiment		C13DEPT135	-
TITLE			
30 mg Brucin 1-D Dept135		or 180 deg. pulse on f1 channel	
	ок с	ancel More Info Help	



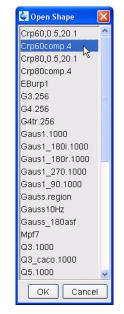
Calculating the shaped pulse power level

8.1.2

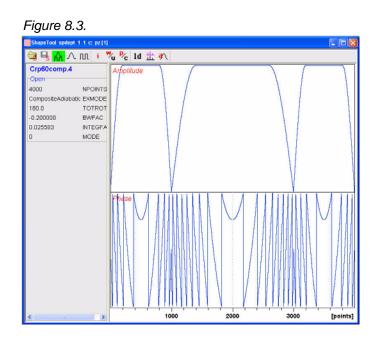
1. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp** in the command line

2. In the shape tool menu bar click on 🤤 and select 'Open Shape'

Figure 8.2.

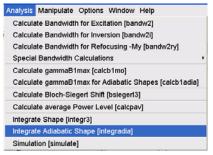


- 3. Select 'Crp60comp.4'
- 4. Click on OK



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





6. Make the following change:

Length of pulse [usec] = 2000

7. Press the 'Enter' key

Figure 8.5.

2000.0		Length of pulse [usec]			
8.0		90 deg hard pulse [usec]			
Results					
1.2000E08	Sweep r	ate on resonance [Hz/sec]			
4370.19	Gammal	B1(max)/2pi/sqrt(Q) [Hz]			
25.5832	Corresp	. 90 deg square pulse [usec]			
10.0973 Change		of power lev comp. to lev of hard pulse [dB]			
Calculator					
5.0]a			
9772.05		GammaB1(max)/2pi [Hz]			



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

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

9. Click on 🔀 to close the Shape Tool window

Setting up the acquisition parameters

8.1.3

8.1.4

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

2. Click on **I** to display the pulsprogram parameters

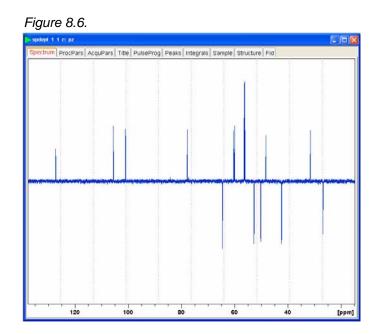
3. Make the following changes:

PL2 [us]	=	2000
SP2 [dB]	=	value of step 8 in 4.1.2 + PL1 (e.g. 7.3)
SPNAM2	=	Crp60comp.4
4. Select the 'Spe	ctrum'	tab by clicking on it

Running the experiment

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

- 2. Click on 🕨 to start the acquisition
- 3. Process and Phase correct the spectrum



1-D DEPT using a shaped 13C pulse

2-D HSQC using a shaped 13C pulse

9.1

Introduction



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

Sample:

30mg Brucine in CDCl3

Reference spectrum

9.1.1

1. Click on 📋 and change the following parameters

Figure 9.1.

New	
A CANADA CARACTERIA CONCERNING	w experiment by creating a new data set and R parameters according to the selected experiment type.
NAME	shape
EXPNO	1
PROCNO	1
DIR	C:
USER	pz
Solvent	CDCI3 🔽
Experiment	PROTON
TITLE	
30mg Brucine 1-D Proton	in CDCI3
	OK Cancel More Info Help

- 2. Click on OK
- 3. Insert the sample
- 4. Click on 📅 to display the Lock display
- 5. In the lock display window click on 100 and select CDCI3
- 6. Turn the spinner off



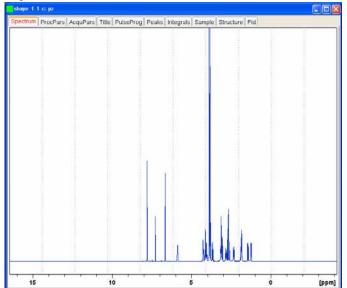


- 7. Shim for best homogeneity
- 8. In the lock display window click on 🤳 to close the window
- 9. Select the 'AcquPars' tab by clicking on it
- 10. Click on 📋 to read in the Prosol parameters
- 11. Tune the probe

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

- 13. Click on 🕨 to start the acquisition
- 14. Process and Phase correct the spectrum





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

hape 2 1 c: pz										. [[
pectrum ProcPars	AcquPars	Title PulsePre	og Peaks	Integrals	Sample	Structure	Fid			
i.	1	1	ŝ.	3		á		8		_
					1					
					1					
					1					
1					1					
1	1									
1	1									
1					- 1		T.			
1					1					
0.00				1	n e	1		T		
1		1		1	N a		11		11.11	
		Λ			M.	N		A		
	1 1	- P - P			- T			-	1 1	_

4. Click on <u>set</u> to set the sweep width and the O1 frequency of the displayed region



			Searc	:h	🛄 Match (
SW	=	7.9991 ppm			
SWH	=	2400.768 Hz			
01	=	1350.60 Hz			
SF01	=	300.1313506	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. **8** ppm)

7. Type **o1** on the command line and write down the value of O1, rounding off to the nearest 1/10th of a ppm (e.g. **4.5 ppm**)

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

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

1. Type rpar HSQCETGPSISP all

2. Turn the spinner off



NOTE: 2-D experiments should be run non spinning

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

F1 SW [ppm	=	value from step 6, Limit setting 5.1.2 (e.g. 8)
O1 [Hz]	=	value from step 7, Limit setting 5.1.2 (e.g. 4.5)
SOLVENT	=	CDCI3



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

5. Click on 🔰 to read in the Prosol parameters



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

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

7. Make the following changes:

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

8 Select the 'Title' tab by clicking on it

9. Change the title to: 30 mg Brucine in CDCI3, 2D HSQC using a 180 deg adiabatic inversion shaped pulse in F1

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

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

2. Click on 🕨 to start the acquisition

Processing

9.1.5



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

1. Type edc2

Figure 9.5.

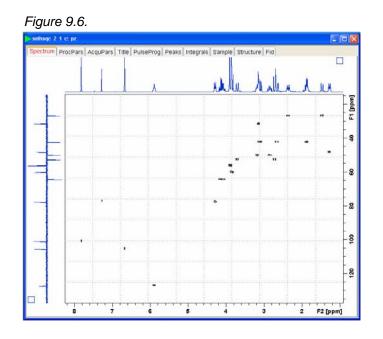
Please specify	/ data sets 2 and 3:	
NAME =	selhsqc	selhsqc
EXPNO =	1	3
PROCNO =	1	1
DIR =	C:	C:
USER =	pz	pz

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

2-D HSQC using a shaped 13C pulse





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

2-D Selective HMBC

Introduction



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

1. Click on 📋 and change the following parameters

Figure 10.1.

New		
A CONTRACTOR OF STREET, STREET	ew experiment by creating a new data set and IR parameters according to the selected experime	nt type.
NAME	selhmbc	
EXPNO	1	
PROCNO	1	
DIR	C:	
USER	pz	
Solvent	DMSO	~
Experiment	PROTON	~
TITLE		
50mM Gramic 1-D Proton	idin S in DMSO d6	<
	OK Cancel More Info	Help

10.1.1

10

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



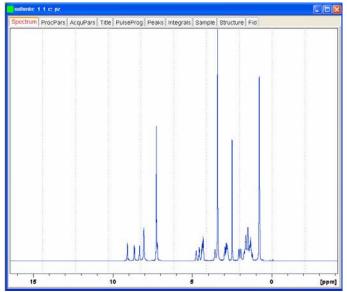
NOTE: selective excitation experiments should be run non spinning

- 7. Shim for best homogeneity
- 8. In the lock display window click on 🤳 to close the window
- 9. Select the 'AcquPars' tab by clicking on it
- 10. Click on 📋 to read in the Prosol parameters
- 11. Tune the probe

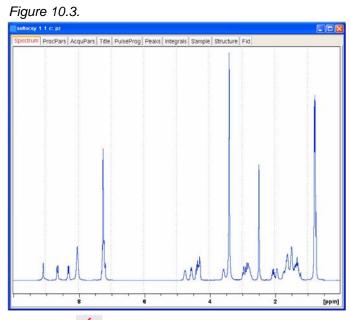
12. In the main menu click on '**Spectrometer**', select '**Adjustment**' and click on '**Auto-adjust receiver gain**' or type **rga**

- 13. Click on 🕨 to start the acquisition
- 14. Process and Phase correct the spectrum

Figure 10.2.



- 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



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





5. Click on Close

6. Type **sw** on the command line and write down the value of SW, rounding off to the nearest 1/10th of a ppm (e.g. **10 ppm**)

7. Type **o1** on the command line and write down the value of O1, rounding off to the nearest 1/10th of a ppm (e.g. **5** ppm)

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

Running a 2-D HMBC experiment

10.1.3

1. Type rpar HMBCGPND all

2. Turn the spinner off



NOTE: 2-D experiments should be run non spinning

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

F1 SW [ppm]	=	value from step 6, Limit setting 6.1.2 (e.g. 10)
O1 [Hz]	=	value from step 7, Limit setting 6.1.2 (e.g. 5)
Solvent	=	DMSO



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

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

8 Select the 'Title' tab by clicking on it

- 9. Change the title to: 50 mM Gamicidin S in DMSO, 2-D HMBC
- 10. Select the 'Spectrum' tab by clicking on it



10.1.4

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

2. Click on 🕨 to start the acquisition



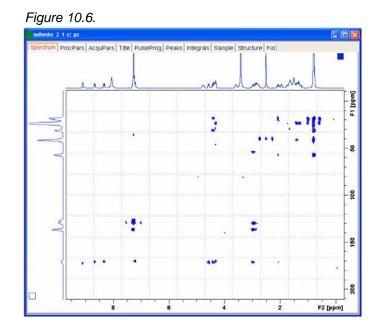
The standard Bruker parameter sets are optimized to run under complete automation. One of the processing parameters is an AU program for processing the data, which can be executed with the command 'xaup'. The next steps assures to use the external spectrum of Gramicidin for the F2 projection.

1. Type edc2

💐 edc 2		
Please specify	/ data sets 2 and 3:	
NAME =	selhmbc	selhmbc
EXPNO =	1	2
PROCNO =	1	2
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





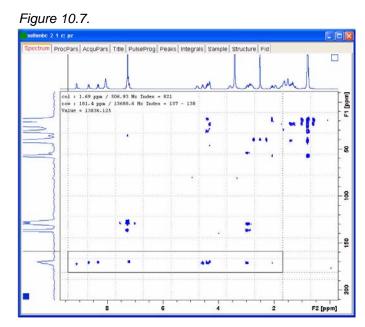
The processing AU program includes the 2D Fourier transform, baseline correction and plotting of the data. The HMBC experiment uses magnitude mode for processing and shows only positive peaks.

Optimizing the parameters on the carbonyl region

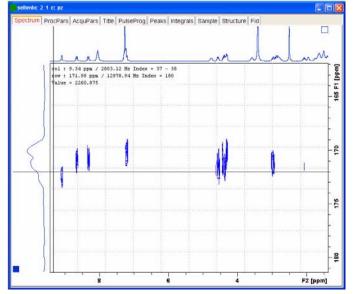
10.1.6

- 1. Type wrpa 3 on the command line
- 2. Type re 3

3. Expand the carbonyl region including all cross peaks (e.g. 162 ppm to 182 ppm)







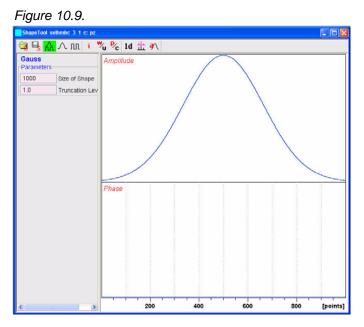
4. Write down the expanded F1 sweep width in ppm (e.g. 20 ppm)

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

- 6. Select the 'AcquPars' tab by clicking on it
- 7. Click on **I** to display the pulsprogram parameters
- 8. Write down the value for P3 [us] (e.g. 8 us)
- 9. Write down the value for PL2 [dB] (e.g. -2.8 dB)
- 10. Select the 'Title' tab by clicking on it
- 11. Change the title to: 50 mM Gamicidin S in DMSO, selective 2-D HMBC
- 10. Select the 'Spectrum' tab by clicking on it

1. 1. Type pulprog shmbcgpnd in the command line

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



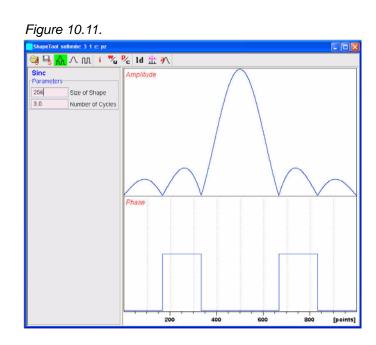
3. In the main menu click on 'Shapes', select 'Classical' and select 'Sinc' by clicking on it

Figure 10.10.

Shapes Ana	lysis Ma	nipulate Options Window H	10
Basic Shap	es	• 🗄 🕨 🖿 💷 🥯 🚧 📅	
Classical S	hapes	Burp	,
Adiabatic S	hapes	 Gauss 	
Solids App	ication	Gaussian Pulse Cascade	,
Imaging Ap	plication	HalfGauss	
Decoupling		' Hermite	
	1000	Seduce	,
	1.0	Sinc	
		Sneeze	,
		Snob	,
		Vega	•
		ShapFour	

4. Make the following changes:

Change size of shape	=	256
Number of cycles	=	3



- 5. Click on 🖳
- 6. Click on 'Save Shape'
- 7. Make the following changes:
- File Name = Sinc3.256

Figure 10.12.

🥪 Save Shape as	Σ
Sinc3.256	🔽 File Name
Further Parameters:	
	Title
90.0	Flip Angle
Excitation	Flip Angle Type of rotation
	OK Cancel

8. Click on OK

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

Figure 10.13.

Analysis	Manipulate	Options	Window	Help	
Calcula	te Bandwidti	h for Excil	ation [ban	idw2]	
Calcula	te Bandwidti	h for Invei	sion [ban	dw2i]	
Calcula	te Bandwidt	h for Refo	cusing -M	y [bandw2ry]	
Special	Bandwidth	Calculatio	ns		•
Calcula	te gammaB1	max [calo	:b1mo]		
Calcula	te gammaB1	max for A	diabatic S	hapes [calcb1adia]
Calcula	te Bloch-Sie	gert Shift	[bsiegert3	5]	
Calcula	te average F	ower Lev	/el [calcpa	v]	
Integrat	e Shape [int	egr3]			
Integrat	e Adiabatic	Shape [inf	legradia]		
Simulat	ion [simulate	1			

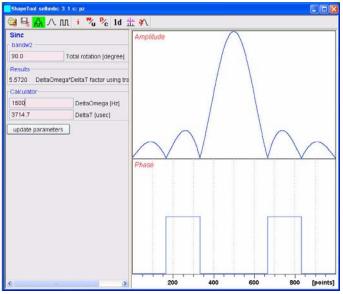
10. Make the following changes:

```
DeltaOmega [Hz] = 1500 (e.g. SW 20 ppm from step 4 in 6.1.6)
11. Press the 'Enter' key
```



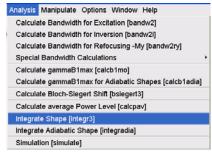
NOTE: The value of Delta T [usec] is being calculated. (e.g. 3714.7 usec)

Figure 10.14.



- 12. Write down the Delta T value [usec] (e.g. 3714.7 usec)
- 13. Click on update parameters
- 14. In the main menu click on 'Analysis', select 'Integrate Shape'

Figure 10.15.



15. Make the following change:

Total rotation [degree] = 90 16. Press the 'Enter' key 17. Make the following change:

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

18. Press the 'Enter' key

Figure 1	0.16.					
ShapeTool selhr						
🔄 号 <u>ん</u> /	∿ւող i 💑 🇞 1d ։	壯 🄊				
Sinc integr3 3714.667 90 8 0.17752 15.01507 Cord	Length of pulse [usec] Totai rotation (degree] 90 deg hard pulse [usec] teg Ratio comp. to square on prresponding difference (dB) nange of power level (dB)	Amplitude				
<	>	200	400	600	800	[points]

19. Write down the change of power level [dB] value (e.g. 38.32156 dB)20. Click on to close the Shape Tool window

Setting up the acquisition parameters

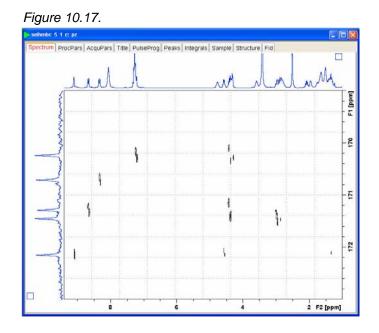
1. Select the 'AcquPars' tab by clicking on it

2. Make the following changes:				
NS	=	32		
F1 SW [ppm]	=	value from step 4 in 6.1.6 (e.g. 20)		
O2P [ppm}	=	value from step 5 in 6.1.6 (e.g. 172)		
3. Click on $\boxed{\mathbf{\Pi}}$ to display the pulsprogram parameters				
4. Make the following changes:				
P13 [us]	=	value from step 12 in 6.1.7 (e.g. 3714.7)		
SP14 [dB]	=	(value from step 19 in 6.1.7) + (PL2) (e.g. 35.42)		

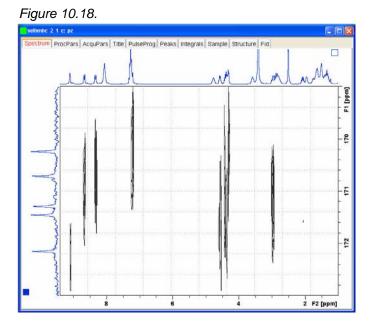
Running the experiment

10.1.9

- 1. Select the 'Spectrum' tab by clicking on it
- 2. Click on 🕨 to start the acquisition
- 3. Type xfb to process the 2-D data
- 4. Expand the 2-D spectrum



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





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.

2-D Selective HMBC

Notes: