

# 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

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.

1.1

1.2

### 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.



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**

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

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 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.  $0.5\,\mathrm{ppm}$  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 <u>set</u> 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



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)

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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.



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

1. Type edc2

Figure 2.4.

Please specify	data sets 2 and 3	
NAME =	experiment	experiment
EXPNO =	1	2
PROCNO =	3	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)





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 **b** to start the acquisition

### Processing



2.2.4

2.2.3



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



Please specify	data sets 2 and 3	
NAME =	experiment	experiment
EXPNO =	1	2
PROCNO =	3	3
DIR =	D:	D:
USER =	pz	02

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



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 =	3	1
DIR =	D:	D:
USER =	p7	02

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

7. Type xaup

## Adding the F1 projection to the HSQC contour plot





## Creating the plot template

Figure 2.11. 191 + 9 perisent of feering nie 🔤 All 認識 1 Tap - 39 -

## 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.				
1	Edit			2
	Graph   2D Spectr	um   2D Projections   Da	ta Set Basic	
	Position	x 3.000000	у. 1	.000000
	Dimension	x \$5.00000	y. 13	1500000
		Attibutes		
	OK	Cancel	Epply	Help

4. Make the following changes:

Position X	=	3
Dimension X	=	15

- 6. Click on Apply
- 7. Select the '2D Projection' tab
- Figure 2.13.

Graph   2D Spect	rum 20 Proje	ctions   Data S	iet   Basic	
Data Sets				
₩ Tob	Size [	3.00	Select	
E Bottom	Spe	2.00	Select	
₩ Let	Sige	3.00	Select	
E Box	Size [	2.00	Select	
6	ibutes			
	_	. 1		

- 8. Enable 'Data set left'
- 9. Make the following change:

Size = 3 10. Click on Select...









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

Figure 2.18.

Seen As			IP 163
Bare at 10 Incode		- 183	CT 201-
Shekka Shynaka At Shynaka At Shynaka Att Shynaka Att	Chayondo LC Chayondo amili Chayondo attach 10,360,000 10,360,000 10,360,000 10,360,000	THE R. P. LEWIS CO., L	D. PL seep D. PL prop. comp D. PL prop. comp D. PL prot. comp D. PL prot. comp D. PL seek of comp D. photof comp
Physics (20.5cm)	David map	-	1 8++ 1
Same as pare   Tollish	to plan improve (* arrept)	-	Castal



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

24. Change Filename to 2D\_inv\_2proj.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



- 2. Type wrpa 2 on the command line
- 3. Type re 2
- 4. Expand the spectrum from 6ppm to -2ppm

5. Click on 📑 gion	to set the sweep width and the O1 frequency of the displayed re
Figure 3.2.	Set SW, SF01 from current region           Search           SM = 3.9893 ppm           SMH = 1197.318 Hz           01 = 1200.50 Hz           SP01 = 300.1312005 MHz
6. Click on	lose
7. Type td 16	(
8. Type <mark>si 8k</mark>	
9. Click on 10. Type <mark>ef</mark>	to start the acquisition
11. Type <mark>apk</mark>	
12. Type <mark>abs</mark>	
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 parameters4. 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 **b** 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 12. to display the pulse program parameters

13. make the following changes:

GPZ6[%] = 95

- 14. Click on **I** to start the acquisition
- 15. Type <mark>ef</mark>
- 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

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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



to change the acqu dimension

Figure 3.7.



5. Enable 'Change dimension from 1D to 2D'



Figure 3.8.

🤯 dosy	
Enter first gradient amplitude:	
2	
	Cancel

## 9. Enter 2 for first gradient amplitude

-

10. Click on	
Figure 3.9.	
dosy	
Enter final gradient amplitude:	
95	
	OK Cancel

## 11. Enter 95 for final gradient amplitude

12.	Click on	
Fig	ure 3.10.	
	🤄 dosy	2
	Enter number of points:	
	16	
		OK Cancel

## 13. Enter 16 for the number of points

14. <i>Fi</i> c	Click on OK
0	🛃 dosy 🛛 🔀
	ramp type (I/q/e {linear/squared/exponential} ):
	1
	OK Cancel

### 15. Enter I for ramp type

16. Click on 🔽	
Figure 3.12.	
El tory	
s)	Do you want to start acquisition ?
	[28] [2804]

17. Click on to start the

to start the acquisition

### Processing

3.1.4

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







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

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



- 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 100 to extract slice

#### Figure 3.16.



3. (	Click on Spectrum		
Fig	jure 3.17.		
	8		83
	Spectrum slice must be extracted Fin This Spectrum should correspond to All further data preparation will be do	rom the 2d relaxation data. o an experiment with the maximum o one in respect to this spectrum.	or minimum delay time.
	Sice Number =	0	
			QK Cancel

5. Enter 1 for the slice number



8. Click on OK

9. Click on + to define the regions

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



12. Click on

Figure 3.21.

Save	Regions To 'intrng'
Save	Regions To 'reg'
▶ Expo	t Regions To Relaxation Module
Save	& Show List

13. Select 'Export Region To Relaxation Module' by clicking on it



15. In the Guide window, click on 🗮 'Relaxation Window'

Figure 3.22.

Concession of the local division of the loca	
00-+4	LK 997
Fairing Law Contraction Contract	Bruce: Yanana Dasker 
Carson Frank Fairs Real Property	
Delto presentario in dana	•
	÷
	4 4 10 10 20 20 (Dion

## 16. Enable 'Intensity'

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

Presse select th     Presse select th     are to be fitted,     Settings dialog	e function to wi depending on t provides all pos	hich t he ex isibilit	he peak intensities or intr periment which produce les for Relaxation analysi	egrals the resolution data. s adjustment lose <u>Details</u>
18 Click on	ose			
1 iyule 3.24.			-	
	Relaxation pa	ramet	ers 👂	3
	la F		or phase determination	
	1000.0	eff lin	of phase determination	
	-1000.0	Zicht I	mit for baseline correction	
	5 N	lumbe	er of drift points	
	1.0E-5 C	onve	roence limit	
	16 N	lumbr	er of points	
	1 F	irst si	ice	
	1 5	lice in	ncrement	
	Fitting Function			-
	vargrad	•	Function Type	
	1		Number of components	
	difflist	×	List file name	
	0.0010		Increment (auto)	
	Iteration control	l para	meters	
	Setup		Reset	)
	Additional Para	meter	15	
	4257.7		GAMMA(Hz/G)	
	3.6		LITDEL(msec)	
	99.9		BIGDEL(msec)	
	1.0			_
	(	QK	Apply Cancel	]
				_

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



22. Click on Close





23. In the data window, click on <sup>22</sup> 'Calculate fitting parameters for all data points'



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

-Brief Repo	irt
Peak 1 at 5	5.342 ppm
Diff Con.	= 3.529e-010 m2/s
Peak 2 at 5	5.142 ppm
Diff Con.	= 5.746e-010 m2/s
Peak 3 at 4	1.694 ppm
Diff Con.	= 1.961e-009 m2/s

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

## Figure 3.28.

Report				8
		Fitting report		
SIMPIT RESULTS				-
***********				
Dataset : D:/data	/pa/smc/dosy/6/pd	ists/1/ct1t2.t:	(t	
INTENSITY FAR : D	iffusion : Variab	Le Gradient :		
1=1[0]*exp(-D*SQR	(2*F1*gama*01*13	)*{80-LD/3}*1e	(4)	
16 points for Fea	k 1, Prak Point	= 5.342 ppm		
Converged after 6	4 iterations!			
Regults Comp.	1			
r101 =	2.491e-002			
Diff Con. =	3.529e-010 m2/s			
Gazana a	4.250e+003 Hm/G			
Little Delta =	3.600m			
Big Delta =	99.900w			
RSB = 2,660e-	008			
5D = 4.077e-	005			
Foint Gradien	t Rept	Calo	Difference	
1 6,740±-00	1 2.400e-002	2.400e-002	-7.066e-007	
2 2.765e+00	0 2.434e-002	2.431e-002	~3.082e-005	
3 4.855e+00	0 2.313e-002	2,309e-002	~4.208e-005	
4 6.945e+00	0 2.126e-002	2.132e-002	5.564e-005	
5 9.036e+00	0 1.915e-002	1.914e-002	-8.148e-006	8
			Close	um na David
-			Conte   Da	ie an line

25. In the guide window, click on **Print** '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  $\sum$  to set the sweep width and the O1 frequency of the displayed re-

Figure 4.2.

gion

	_		Sea	rch	Match
SM	=	13.0151 ppm			
SMH	=	3906.250 Hz			
01	=	1648.51 Hz			
S₽01	=	300.1316536	MHE		

5. Click on Close

4.1.1

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

7. Make the following changes:

LB = Λ

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 **I** 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 <sup>3</sup> '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

- 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)


## Multiplet assignments

4.1.4

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

Select 'Structure Analysis'

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

Figure 4.8.



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



- 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





- 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 to '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.





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



31. Display all 3 multiplets



	Multiplet Connection Options	
	Maximum of Difference between Couplings Lower Limit for Couplings Change already defined Connections	
		OK Cancel
34. Click	on OK	
34. Click <i>⊏igure 4.:</i>	on OK 23.	
34. Click <i>≓igure 4.:</i> ₽	on OK 23. We tenentlike / Figure t Birt (p	
84. Click Figure 4	ON OK 23. was ferred to / Adjust 4.4970 - 11.39, 2.47, 0 4.4970 - 11.39, 2.47, 0 4.4970 - 11.39, 2.47, 0 4.4970 - 11.39, 2.47, 0 11.39, 0 1	# CN Perfs
4. Click Figure 4.:	ON OK 23. we transition / figure tr #ref (p JHc) M Connection 4.4970 - 1128 - 2 47.0 4.4970 - 1128 - 2 47.0 12860 - 1126 - 2 47.0 1000 T 42.0 1000 T 42.0 1000 S - 2 43.0	# CN Przs Copy
4. Click Figure 4	ON OK 23. We terrent the of Algorit 4.4970 - 1125 - 2.471.01 4.0970 - 1125 - 2.471.01 4.0970 - 1125 - 2.471.01 4.0970 - 1126 - 2.472.01 10.0074 - 2.472.01 2.4964 - 1.026 - 2.472.01 4.0966 - 3.471.11	e Cu Pres Copy Eare
34. Click Figure 4.	ON OK 23. We tree the Affred 4 arrs 7 arrs 7 arrs 3 bea 1128 2 arrs 7 arrs 3 bea 1128 2 arrs 7 3 bea 1128 2 arrs 10 bea 2 arrs	Pres. Copy Enver Table editor



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

35. Click on Ok36. Click on ice 'Return, save multiplets [sret]'

# **19F Experiments**



QNP	19F/31P/13C/1H
ТХО	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

various Bruker systems and probes.



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.

NOTE: Below is a list of hardware options to observe or decoupled Fluorine on

Additional hardware

300 and 400MHz systems

-Internal amplifier BLA-2BB

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5.1.1

5.1.2

-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 B

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-CF<sub>2</sub>-CH<sub>2</sub>-OH

19F observe, no decoupling

5.2.1

5.2

#### Exploratory spectrum

1. Click on 1 and change the following parameters

Figure 5.	1.	
R	New	
P	Prepare for a new nitializing its NMR	experiment by creating a new data set and parameters according to the selected experiment type.
ſ	NAME	f19exp
	EXPNO	1
	PROCNO	1
	DIR	
	Solvent	Acetone 👻
	Experiment	F19 ¥
-	TITLE	
	1-D 19F experim 2,2,3,4,4,4-Hexa	ient no decoupling
		QK Cancel More Info Help
	OK	
2. Click or	n 🛄	
3. Insert t	he sample	e
4. Click or	n 📅 to	display the Lock display
5 In the l	ock displa	av window click on the and select Acetone
6 Tune th	ne probe t	n observe 19F
7 Shim fo	or best ho	mogeneity
8. In the le 9. Select	ock displa the ' <b>Acqı</b>	ay window click on to close the window IPars' tab by clicking on it
10. Click ( 11. In the ' <b>Auto-adj</b>	on <b>b</b> main me <b>just recei</b>	to read in the Prosol parameters enu click on ' <b>Spectrometer</b> ', select ' <b>Adjustment</b> ' and click on ver gain' or type <mark>rga</mark>
12 Click	on 🕨 t	o start the acquisition
13. Proce	ess and Pl	hase correct the spectrum
Figure 5 2	2	
	je ochanij AcquiPanij Tit	e   Pulselhog   Peaks   steegram   stample   structure   Fisl



#### 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:



O1P [PPM] = -140

- 3. Click on 上 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
- 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



#### 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 **I** 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
- 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 **I** 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 **I** 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 Lostart the acquisition
- 13. Process and Phase correct the spectrum







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

## 1H observe, with 19F decoupling using Garp

5.2.5

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

2. Click on to display the pulse program parameters

3. Make the following changes:

CPDPRG2	=	garp
PCPD2	=	70
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

Figure 5.11.



## 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<sub>3</sub>-CFH-CF<sub>2</sub>-CH<sub>2</sub>-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.

	menth		
EXPNO	1		
PROCNO	1		
DIR	D:		
USER	pz		
Solvent		Acetone	
Experiment		F19	
TITLE			
1-D 19F expe	eriment no decoupling		

2. Click on

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

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

to set the sweep width and the O1 frequency of the displayed re-5. Click on gion

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 kto start the acquisition
- 13. Process and Phase correct the spectrum





## 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 1. to set the sweep width and the O1 frequency of the displayed region

Figure	5.1	5.
--------	-----	----



- 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 boost 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:

```
NUC1 = 19F
```





6. Click on Save

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

8. Make the following changes:

PULPROG	=	hfcoqfqn		
SW F2 [ppm]	=	value from step 8 (19F reference spectrum)		
SW F1 [ppm]	=	value from step 10 (19F reference spectrum)		
O1P [ppm]	=	value from step 5 (1H reference spectrum)		
O2P [ppm]	=	value from step 7 (1H reference spectrum)		
SOLVENT	=	Acetone		
9. Click on 📙 to	read i	n the Prosol parameters		
10. Click on 🞵	to disp	lay the pulse program parameters		
11. Make the follow	wing ch	nanges:		
CNST2	=	<b>25</b> = J(FH)		
12. Select the 'Pro	ocPar'	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 ' <b>Title</b> ' tab by clicking on it				
15. Change the titl	e to: 2	-D 1H/19F HETCOR experiment 2,2,3,4,4,4-Hexafluoro-1-Butanol		
16. Select the 'Spo	ectrun	n' tab by clicking on it		
17. In the main me ' <b>Auto-adjust rece</b>	enu cli <b>iver g</b> a	ck on ' <b>Spectrometer</b> ', select ' <b>Adjustment</b> ' and click ain' or type <mark>rga</mark>		
18. Click on 19. Type xfb	to start	the acquisition		
20. Adjust the contour level				

on



## 1-D Selective NOESY

## 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				
Prepare for a ni initializing its NM	ew experiment   /IR parameters	by creating a new da according to the sel	ata set and ected experime	nt type.
NAME	selexp			
EXPNO	1			
PROCNO	1			
DIR	C:			
USER	pz			
Solvent			DMSO	~
Experiment		PROTON		~
TITLE				
reference spe	ectrum			<ul> <li>•</li> <li>•</li> </ul>
	ОК	Cancel M	ore Info	Help



(son)

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 fr	equencies
	SFO1 [MHz] =	300.132548
	O1/2/3 [Hz] =	2548.19
	01 02	O3 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 III 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 boot to start the acquisition
- 4. Type <mark>ef</mark>
- 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 🍒

Comment of the Commen		518
5. 1		(4.)
1.4854 ppe / 2148.2174 Nr / 366.23284	10e	TO THE HER
HT APRICAL PROPERTIES PROVIDENTS OF	217118	
	$\wedge$	

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

Figure 6.7.

🌢 01/02/03	
Define SFO1/O1 fi	requencies
SFO1 [MHz] =	300.132548
01/2/3 [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  $\prod$  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 **b** to start the acquisition
- 3. Type <mark>ef</mark>
- 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.



Selective excitation region set up (example 3)

6.1.8

## Integration region file



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

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

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

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 Modulation acc. to Offset Freq. [manipul offs]
Single Sine Modulation [manipul sinm2]
Single Cosine Modulation [manipul cosm2]
Modulation acc. to Freq. Sweep
Power of Amplitude [manipul power]
Scale Amplitude [manipul scale]
Add constant Phase [manipul addphase]
Time Reversal [manipul trev]
Calc. Shape from Excitation Region [manipul region]
Add Shapes [manipul addshapes]
Expand Shape [manipul expand]







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

Figure 6.14.

Options	Window Help
Set Pa	th to Shape Directory
Define	Parameter Table
Select	associated dataset
Prefere	ences
Remot	e Connection
Admini	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	J
Figure 6.16.		
	4	
	Save as Shape region	
		OK Cancel

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

#### 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 **b** 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  $2 \times 12^{\circ} \times 12^{\circ}$



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		
Prepare for a ne initializing its NM	w experiment by creatin R parameters according	g a new data set and to the selected experiment type.
NAME	seltocsy	
EXPNO	1	
PROCNO	1	
DIR	C:	
USER	pz	
Solvent		DMSO 🔽
Experiment		PROTON V
TITLE		
reference spe	ctrum	
	OK Cano	el More Info Help



from

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.

Define SF01/01 f	requencies
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.



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

Gauss bandw2ry	
180.0	Total rotation [degree]
-Results 0.8820 Def	taOmega*DeltaT factor using -M
Calculator	2000 (2)



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 change	s:	
Length of shaped pulse	=	p12
Power Level of shaped pulse	=	SP2
Name of shaped pulse	=	SPNAM2

**BRUKER BIOSPIN** 

Figure 7.9.





#### Setting up the acquisition parameters

7.1.4

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

2. Click on	л <sub>tc</sub>	o display the pulsprogram parameters
3. Make the	follow	ing 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 **b** to start the acquisition
- 3. Type <mark>ef</mark>
- 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.

7.1.6



- 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  $2^{\circ}$   $2^{\circ}$   $2^{\circ}$   $2^{\circ}$   $2^{\circ}$   $2^{\circ}$  to adjust the spectra *Figure 7.11.*



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



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

#### 1-D selective TOCSY

# **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 and change the following parameters

#### Figure 8.1.

New			×
Prepare for a n initializing its NM	ew experiment by creatin /IR parameters accordin	ng a new data set and g to the selected experiment ty	/pe.
NAME	spdept		
EXPNO	1		
PROCNO	1		
DIR	C:		
USER	pz		
Solvent		CDCI3	~
Experiment		C13DEPT135	~
TITLE			
30 mg Brucin 1-D Dept135	e in CDCI3 using shaped pulse for	180 deg. pulse on f1 channe	<
	OK Can	icel More Info Hel	0



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.

🔄 Open Shape 👘	×
Crp60,0.5,20.1	^
Crp60comp.4	
Crp80,0.5,20.1	
Crp80comp.4	
EBurp1	
G3.256	
G4.256	
G4tr.256	
Gaus1.1000	
Gaus1_180i.1000	
Gaus1_180r.1000	
Gaus1_270.1000	
Gaus1_90.1000	
Gauss.region	
Gauss10Hz	
Gauss_180asf	
Mpf7	
Q3.1000	
Q3_caco.1000	
Q5.1000	×
OK Cancel	

3. Select 'Crp60comp.4'





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.

Length of pulse [usec] 90 deg hard pulse [usec]
90 deg hard pulse [usec]
p rate on resonance [Hz/sec]
maB1(max)/2pi/sqrt(Q) [Hz]
sp. 90 deg square pulse [usec]
ge of power lev comp. to lev of hard pulse [dB]
Q
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

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

2. Click on 1 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 'Spec	trum'	tab by clicking on it

Running the experiment

8.1.4

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

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



# 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	
Prepare for a ne initializing its NM	w experiment by creating a new data set and IR 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 CDCl3
	OK Cancel More Info Help



15

10

(ppm)

- 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



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. **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 the 180 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

**BRUKER BIOSPIN** 

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

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 =	semage	seihisgo
EXPNO =	1	3
PROCNO =	3	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)

OK 3. Click on

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

10.1.1

10.1

1. Click on and change the following parameters

#### Figure 10.1.

🔄 New	
Prepare for a ne initializing its NM	w experiment by creating a new data set and R parameters according to the selected experiment 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





ó

(ppm)

10

15

- 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



Setucity 1 1 IC at		
Torimin ProcPare AccuPare Toe PunePr	og Feana Hotegran   Sample   Structu	ine Fish
	1	
1.		
		1
	1 1 1	
1 1 1	1 1 1	
		1
	4	Ma
	1 A 1	NN 11 A
	NULIN	COUNT UL
		a second second second
		2 (pain)

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:

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

8 Select the 'Title' tab by clicking on it

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

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



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

eile7		
Please specify	/ data sets 2 and 3	
NAME =	termbo	seihmbc
EXPNO =	1	2
PROCNO +	1	2
DIR =	¢.	C.
USER =	pt.	pr.

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

- 3. Click on
- 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 1 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.



4. Make the following changes:

Change size of shape	=	256
Number of cycles	=	3

#### Introduction

# Figure 10.11.

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

#### Figure 10.12.

Save Shape as		
Sinc3 256	▼ F	ile Name
Further Parameters		
90.0	F	itie lip Angle
Excitation	- T	ype of rotation
	OK	Cancel

8. Click on

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

#### Figure 10.13.



10. Make the following changes:

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



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

#### Figure 10.14.



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

13. Click on update parameters

14. In the main menu click on 'Analysis', select 'Integrate Shape'

Figure 10.15.



15. Make the following change:

Total rotation [degree] = 90 16. Press the 'Enter' key

- 17. Make the following change:
- 90 deg. hard pulse [usec] = (p3 from step 8 in 6.1.6 e.g. 8)

18. Press the 'Enter' key

940	∧ nn   % % Id	± ▲			- Health
Sinc Hep2 3714 667 50 9 ResUL 10 01007 98 22103 9922153	Langth of pulse (used) Total rotation (degree) 90 deg hard pulse (used) reing Ratio comp. to square un Corresponding difference (off) Change of power level (off) change of power level (off)	Phone			$\sim$
			-		

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

#### Setting up the acquisition parameters

10.1.8

- 1. Select the 'AcquPars' tab by clicking on it
- 2. Make the following changes:

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

Running the experiment

10.1.9

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



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: