

TopSpin

Guide Book
 Advanced NMR Experiments
 Version 002

Innovation with Integrity

NMR

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1 About This Manual

This manual enables safe and efficient handling of the device.

This manual is an integral part of the device, and must be kept in close proximity to the device where it is permanently accessible to personnel. In addition, instructions concerning labor protection laws, operator regulations tools and supplies must be available and adhered to.

Before starting any work, personnel must read the manual thoroughly and understand its contents. Compliance with all specified safety and operating instructions, as well as local work safety regulations, are vital to ensure safe operation.

The figures shown in this manual are designed to be general and informative and may not represent the specific Bruker model, component or software/firmware version you are working with. Options and accessories may or may not be illustrated in each figure.

1.1 Policy Statement

It is Bruker's policy to improve products as new techniques and components become available. Bruker reserves the right to change specifications at any time.

Every effort has been made to avoid errors in text and Figure presentation in this publication. In order to produce useful and appropriate documentation, we welcome your comments on this publication. Field Service Engineers are advised to check regularly with Bruker for updated information.

Bruker is committed to providing customers with inventive, high-quality, environmentallysound products and services.

1.2 Symbols and Conventions

Safety instructions in this manual and labels of devices are marked with symbols. .

The safety instructions are introduced using indicative words which express the extent of the hazard.

In order to avoid accidents, personal injury or damage to property, always observe safety instructions and proceed with care.



DANGER indicates a hazardous situation which, if not avoided, will result in death or serious injury.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.



WARNING indicates a hazardous situation, which, if not avoided, could result in death or serious injury.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.



CAUTION indicates a hazardous situation, which, if not avoided, may result in minor or moderate injury or severe material or property damage.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.

NOTICE

NOTICE indicates a property damage message.

This is the consequence of not following the notice.

- 1. This is a safety condition.
- ► This is a safety instruction.

SAFETY INSTRUCTIONS

SAFETY INSTRUCTIONS are used for control flow and shutdowns in the event of an error or emergency.

This is the consequence of not following the safety instructions.

- 1. This is a safety condition.
- ► This is a safety instruction.



This symbol highlights useful tips and recommendations as well as information designed to ensure efficient and smooth operation.

1.3 Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All what you can enter"	Arial bold	Type or enter fromjdx zg
Button, Tab, Pane and Menu Names "All what you can click"	Arial bold, initial letters capitalized	Use the Export To File button. Click OK . Click Processing
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names Data Path Variables Table Column Names Field Names (within Dialog Windows)	Arial Italics	\$tshome/exp/stan/nmr/ lists expno, procno,
Parameters	Arial in Capital Letters	VCLIST
Program Code Pulse and AU Program Names Macros Functions Arguments Variables	Courier	go=2 au_zgte edmac CalcExpTime() XAU(prog, arg) disk2, user2
AU Macro	Courier in Capital Letters	REX PNO

Table 1.1: Font and Format Conventions

2 Introduction

2.1 General

This manual was written for AVANCE systems running **TopSpin version 3.5 including patches** and should be used as a guide through the set up process for some experiments. The success of running the experiments in this manual is under the assumption that all parameters have been entered in to the prosol table.

This manual features various advanced procedures for ¹H, ¹³C, ¹⁵N and ³¹P experiments. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, chapter *1D Proton Experiment* and chapter *1D Carbon Experiments* described in the TopSpin Guide Book *Basic NMR Experiments*.

2.2 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, especially 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 trained in the operation of the AVANCE systems should operate the unit.

NOTICE

Material Damage Due to Excessive Power

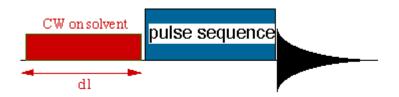
The NMR probe can be severely damaged if too much power or power over a too long time is applied.

Always start to optimize pulses with low power values and short pulses. Respect the pulse and power limits as programmed into the PICS data of the probe.

3 1D Solvent Suppression Experiments

3.1 Introduction

Many experiments on samples dissolved in protonated solution require some method to minimize the strong resonance belonging to the solvent. This suppression can be performed in several ways, depending on the number of signals to suppress, and on which part of the pulse sequence can be modified. Solvent suppression can be applied during the relaxation period just prior to the conventional pulse sequence as outlined in the figure below. This is referred to as Pre-saturation.



However, pre-saturation can also reduce the signal intensities of exchangeable protons. For this reason, other schemes, as the WATERGATE, WET and Excitation Sculpting schemes, can be used to overcome this problem and are discussed in this chapter.

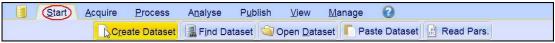
In HPLC-NMR applications it is mandatory to suppress multiple-solvent resonances. The incorporation of specific multiple-solvent suppression schemes into pulse sequences is made in analogy with classical methods.

3.2 Samples

2 mM Raffinose in 90% H2O + 10% D2O 2 mM Lysozyme in 90% H2O + 10% D2O

3.3 **Preparation Experiment**

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



• In the New Dataset window,enter or select:

NAME = solvent_suppression_exp EXPNO = 1 PROCNO = 1 Experiment: Select **PROTON** Set Solvent: Select **H2O+D2O**

1D Solvent Suppression Experiments

🖕 New					
Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the Options.					
NAME	solvent_suppression_exp				
EXPNO	1				
PROCNO	1				
O Use current parameters					
Experiment PROTON	Select				
 Options 					
Set solvent:	H2O+D2O -				
Execute "getprosol	n.				
Keep parameters:	P 1, O1, PLW 1 Change				
DIR	C:\Data 🗸				
🖾 Show new dataset	in new window				
Receivers (1,2,16) 1				
1-D Proton	solvent suppression experiment				
	OK Cancel More Info Help				

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click OK.
- On the menu bar, click Aquire.



For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select H2O + D2O solvent.
- To tune the probe, click **Tune**.
- Click Spin and select Turn sample rotation off.



Solvent suppression experiments should be run non-spinning.

- To autoshim the sample with TopShim and best homogeneity, click **Shim**.
- To load the probe/solvent depended parameters, click **Prosol**.

3.3.1 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.3.2 Processing

• On the menu bar, click **Process**.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0		
	A Proc. Sp	ectrum 🗢	Adjust Pha	ase 🗢 🕅 🧥	Calib. A <u>x</u> is	N Pick P	eaks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Workflow button bar, click **Proc Spectrum**.

This executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the drop-down arrow to see more options.

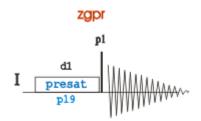
- 2 No acquisition running: C:/Bruker/TOPSPIN/data/nmrsu/nmr/1d_solvent_suppression/1/pdata/1 - • 🛛 Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Fid Acqu [le] solvent suppression experiment 2mM Raffinose in 90% H2O+10% D2O 0.5 0.4 0.3 0.2 0.1 0.0 5 15 10 ò [ppm]
- In the list, select Configure Standard Processing (proc1d).

Ensure that the SW is large enough to cover the entire spectrum accounting for the position of O1. The pre-saturation is applied on resonance (at the O1 position). The power level for pre-saturation has to be known and entered into the Prosol parameters.

3.4 1D Solvent Suppression with Pre-saturation

Pre-saturation is the most common procedure to minimize and suppress the intense solvent resonance when ¹H spectra are recorded in protonated solutions. This experiment is performed by applying a low-power continuous wave irradiation on the selected resonance during the pre-scan delay:

W



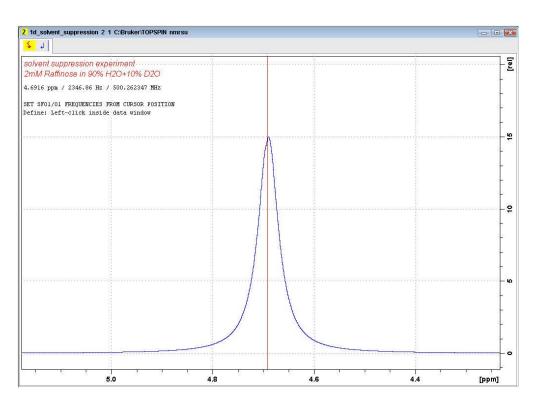
3.4.1 Parameter Setup

· At the command prompt, type:

wrpa 2

re 2

- Expand the peak at 4.7ppm.
- On the toolbar, click Set RF from cursor.



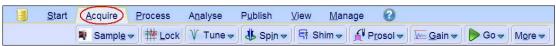
• Move the cursor line to the center of the peak and press the mouse button.



- In the O1/O2/O3 window, click O1.
- In the Dataset window, select the AcquPars tab.
- Enter: PULPROG = zgpr TD = 16384 NS = 8 DS = 4 SW[ppm] = 10 (for the Raffinose sample) SW[ppm] = 14 (for the Lysozyme sample) D1 [s] = 2
- In the Dataset window, select the **ProcPars** tab.
- Enter:
 SI = 8192
- In the Dataset window, select the **Spectrum** tab.

3.4.2 Fine Tuning

• On the menu bar, click Acquire.



• On the Workflow button bar, click Gain.

	Start Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u>	lanage (2	
	💐 Sampl <u>e</u> 🔻	tock	V Tune 🗢	& Sp <u>i</u> n →	Shim -	Prosol	▼	● Go マ More マ

• On the Go button, click the drop-down arrow to see more options.

Start Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>I</u>	<u>M</u> anage 🤇	3		
💐 Sampl <u>e</u> 🔻	tock	V Tune ▼	∄ Sp <u>i</u> n ▼	Shim -	Prosol	▼ <u>G</u> ain ▼	D Go 💦	M <u>o</u> re ▼

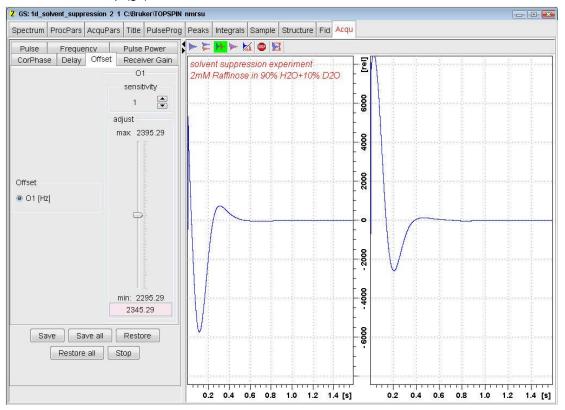
• In the list, select Real-Time Go Setup (gs).

<u>T</u> ransfer Fid To Disk (tr)	
Estimate Exp. Time (expt)	
Real-Time <u>G</u> o Setup (gs)	
Optimize Acquisition Params (popt	t)
Start Automation AU program (xau	ia)

• In the Dataset tool bar, select Unshuffle data on display.



• In the Go Setup (gs) window, select the Offset tab.



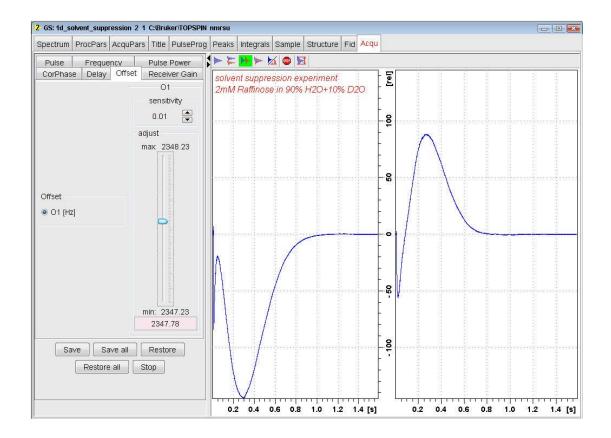
• Change the O1 value by clicking just below or above the adjust slider.



For smaller changes, adjust the **sensitivity** inside the gs window to a smaller value.

- Observe the fid area in the Acquisition information window for a smaller integration value and the FID to become a single line.
- In the Go Setup (gs) window, click Save.
- In the Go Setup (gs) window, click Stop.

1D Solvent Suppression Experiments



• In the Save changed GS parameters window, click OK.

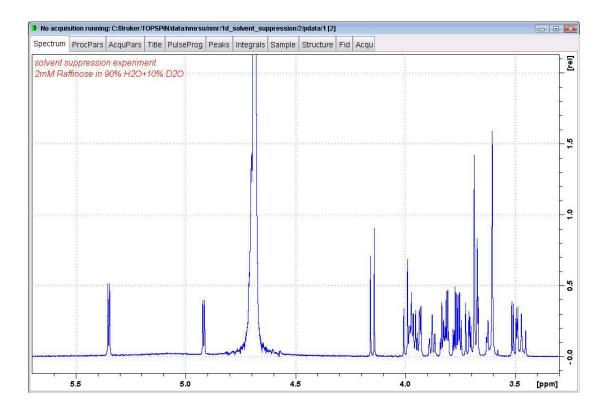


3.4.3 Acquisition

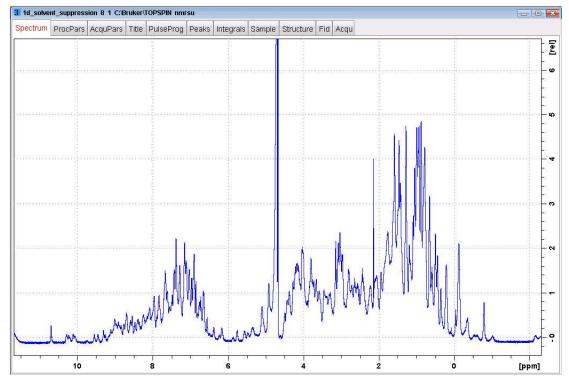
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.4.4 Processing

• Process and phase correct the spectrum.

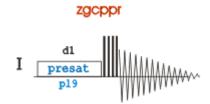


The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



3.5 1D Solvent Suppression with Presaturation and Composite Pulses

This experiment is performed by applying a low-power continuous wave irradiation on the water resonance during the pre-scan period, followed by a rapid succession of four 90° pulses to further reduce the residual hump of the water signal:



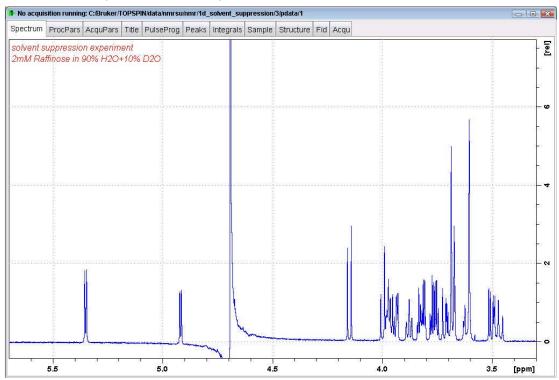
3.5.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter *1D Solvent Suppression with Pre-saturation* [▶ 18].
- In the Dataset window, select the AcquPars tab.
- Enter:
 - PULPROG = zgcppr
- In the Dataset window, select the Spectrum tab.

3.5.2 Acquisition

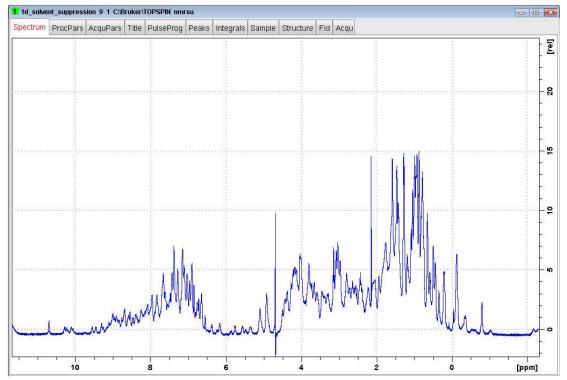
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.5.3 Processing



• Process and phase correct the spectrum.

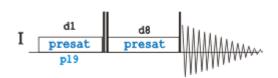
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The following figure shows the 1D spectrum of the Lysozyme sample:



3.6 1D Solvent Suppression Using the Noesy Sequence

This experiment is performed by using the 1D version of the noesyphpr sequence applying a low-power continuous wave irradiation on the water resonance during the pre-scan and during the mixing time period of the NOESY sequence:

noesypr1d



3.6.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter *1D Solvent Suppression with Pre-saturation* [▶ 18].
- In the Dataset window, select the AcquPars tab.
- Enter:

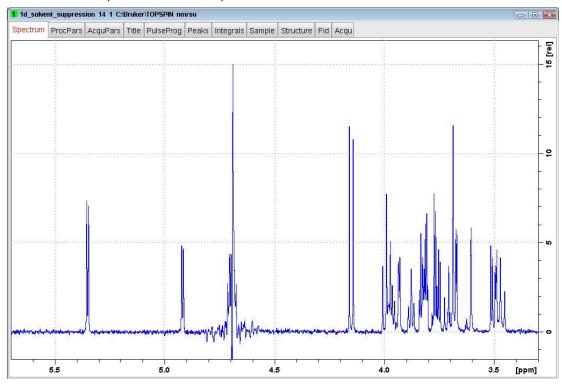
PULPROG = noesypr1d D8[s] = 0.1

• In the Dataset window, select the **Spectrum** tab.

3.6.2 Acquisition

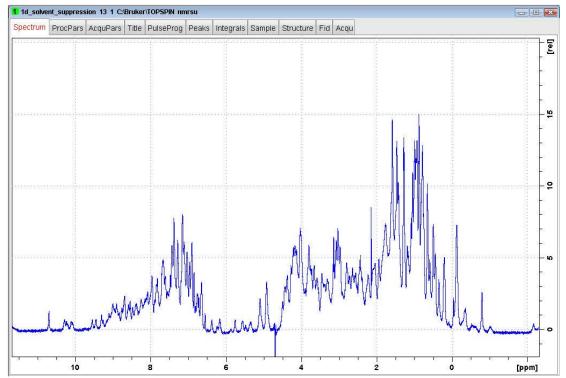
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.6.3 Processing



• Process and phase correct the spectrum.

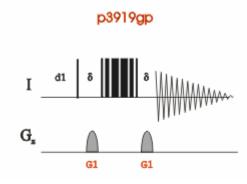
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



3.7 1D Solvent Suppression with WATERGATE

The WATERGATE (**WATER** suppression by **GrA**dient **T**ailored **E**xcitation) technique, which uses pulsed field gradients, is claimed to be independent of line-shape, yielding better suppression compared with other methods. Exchangeable protons are not affected and there is no phase jump at the water resonance, although signals very close to the water resonance are also suppressed.

The sequence is in principle, a spin-echo experiment in which the 180 degree pulse is embedded between two pulsed field gradients. After excitation by the first pulse p1 the field gradient G1 dephases all coherence. The selective inversion element consists of a symmetrical 3-9-19 pulse sequence 3a-t-9a-t-19a-t-9a-t-3a, with 26a=180°, as shown in the figure below. Additional suppression appears at different sidebands (1/t).



3.7.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter 1D Solvent Suppression with Pre-saturation [▶ 18].
- In the Dataset window, select the AcquPars tab.
- · Enter or select:

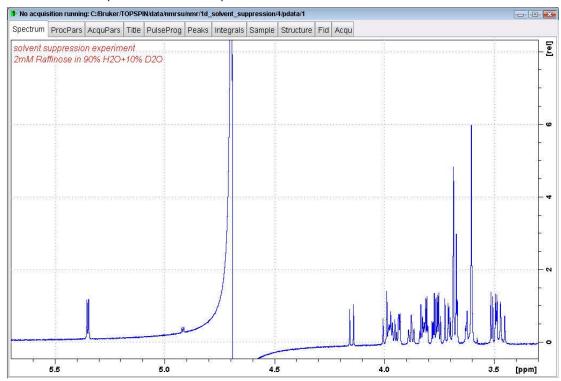
PULPROG = **p3919gp** D19 [s] = **0.00015** = 1/(2*d) where d = distance to next null in Hz GPNAM1 = **SMSQ10.100** GPZ1 [%] = **20**

• In the Dataset window, select the Spectrum tab.

3.7.2 Acquisition

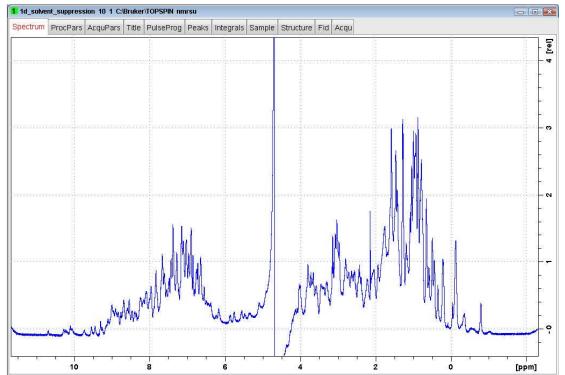
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.7.3 Processing

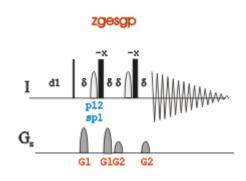


• Process and phase correct the spectrum.

The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



3.8 1D Solvent Suppression with Excitation Sculpting



3.8.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter *1D Solvent Suppression with Pre-saturation* [▶ 18].
- In the Dataset window, select the AcquPars tab.
- Enter or select:

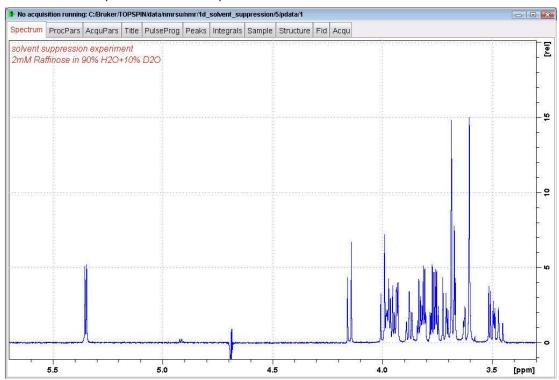
PULPROG = zgesgp P12 [us] = 2000 SPdB1 = 44.5 GPNAM1 = SMSQ10.100 GPNAM2 = SMSQ10.100 SPNAM1 = Squa100.1000 GPZ1 [%] = 31 GPZ2 [%] = 11

• In the Dataset window, select the **Spectrum** tab.

3.8.2 Acquisition

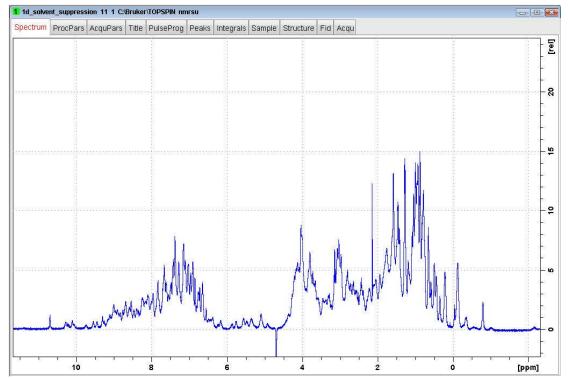
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.8.3 Processing



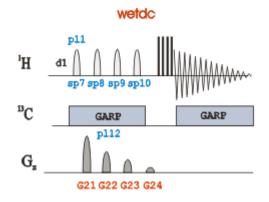
• Process and phase correct the spectrum.

The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



3.9 1D Solvent Suppression with WET

This pulse sequence uses a shaped, selective pulse and pulse field gradients to suppress one or more solvent signals. The option of carbon decoupling is available for suppression of solvent signals with large ¹³C satellites. It provides very efficient suppression with excellent selectivity.



3.9.1 Sample

2 mg Sucrose in Acetonitrile and D2O

3.9.2 Preparation Experiment

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



 In the New Dataset window, enter or select: NAME = wet_solvent_suppression_exp EXPNO = 1 PROCNO = 1 Experiment: Select LC1DWTDC

Set Solvent: Select CH3CN+D2O

1D Solvent Suppression Experiments

🖕 New					
Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the Options.					
NAME	wet_solvent_suppression_exp				
EXPNO	1				
PROCNO	1				
O Use current parameters					
Experiment LC1DWTDC	Select				
 Options 					
Set solvent:	CH3CN+D2O -				
Execute "getprosol"					
Keep parameters:	P 1, O1, PLW 1 Change				
DIR	C:\Data 🗸				
🖾 Show new dataset in	n new window				
Receivers (1,2,16)	1				
	olvent suppression experiment using WET e in Acetonitril and D2O				
	OK Cancel More Info Help				

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click OK.
- On the menu bar, click Aquire.



For the following steps, use the Workflow button bar.

- Click Sample and Insert.
- Click Lock and select the CH3CN+D2O solvent.
- Click **Tune** to tune the probe.
- Click Spin and select Sample rotation off.



Solvent suppression experiments should be run non-spinning.

- To autoshim the sample with TopShim and best homogeneity, click Shim.
- In the Dataset window, select the AcquPars tab.

 Enter: PULPROG = zg30 DS = 0 NS = 1

• To load the probe/solvent depended parameters, click Prosol.

3.9.3 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

3.9.4 Processing

• On the menu bar, click **Process**.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> bli	ish <u>V</u> iew	<u>M</u> anage	0		
	Pro <u>c</u> . Sp	ectrum 🗢	Adjust Ph 🔶	ase 🔻	Å Calib. A <u>x</u> i	s 🎊 Pick F	P <u>e</u> aks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Workflow button bar, click **Proc Spectrum**.

This executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the drop-down arrow to see more options.

• In the list, select Configure Standard Processintg (proc1d).

Acquisition finished: C:/Bruker/data/rek/WET_AVB/1/pda					00
	g Peaks Integrals Sample Structure Plot Fid Acq	ęu.			
WET 4.574 ppm / 2623.912 Hz Index = 30659 - 30664					Let Let
Index = 38659 - 38664 Value = -0.002518 rel					-
					ľ.
					- 2
					5
					- 9
					Ī
					-
4					- 4
					-
				, A ,]
4.5	4.0	3.5	3.0	2.5	(mpg)

1D Solvent Suppression Experiments

3.9.5 Selective Excitation Region Setup

• On the menu bar, click Acquire.



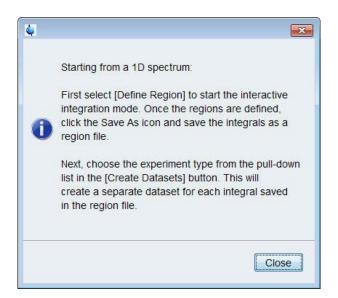
- On the More button, click the drop-down arrow to see more options.
- In the list, select Setup Selective 1D Expts.



The Workflow button bar changes for setting up the 1D selective experiment.

• On the Workflow button bar, click **1D Selective Experiment Setup**.

<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G Back		1D Se	elective Expe	r[ment Setup	🕹 De	fine <u>R</u> egions	🖹 Create <u>D</u> atasets 🛡	



There is no other function to this button then the instruction displayed above.

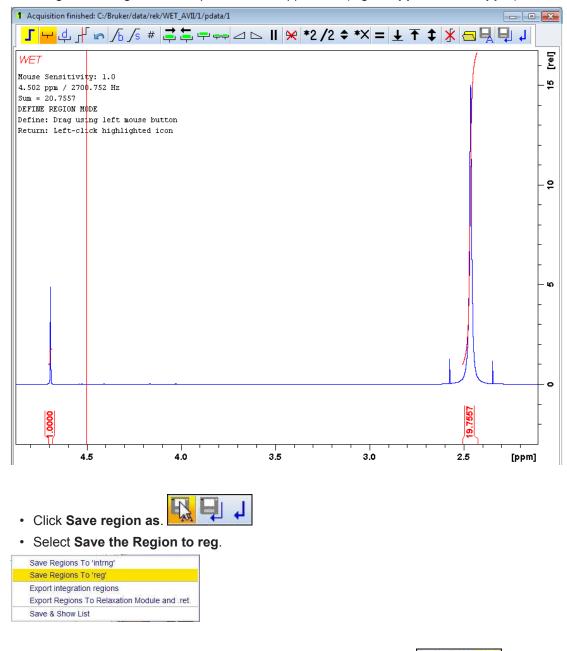
• In the message window, click Close.

1D Solvent Suppression Experiments

• On the Workflow button bar, click **Define Regions**.

Start Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0
Back	1D Se	elective Expe	riment Setup	De	fine <u>R</u> egions	Create Datasets 🗢

Integrate the regions on the peaks to be suppressed (e.g. 4.7 ppm and 2.45 ppm).



To exit the integration mode, click Return do not save regions!

Ы

• In the Save Changes window, click No.



• On the Create Dataset button, click the drop-down arrow to see more options.

	<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0		
G <u>B</u> ack	ĸ		1D Se	elective Expe	riment Setup	🕹 De	fine <u>R</u> egions	H CI	reate <u>D</u> atasets 🟠	

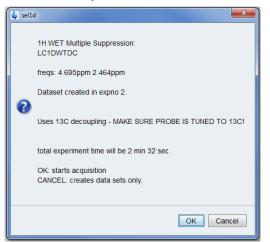
• In the list, select Mult. Solvent Suppr./WET.

Selective gradient 1H
Selective gradient COSY
Selective gradient NOESY
Selective gradient TOCSY
Selective gradient ROESY
1H Homonuclear Decoupling
Selective 1H
Selective COSY
Selective NOESY
Selective TOCSY
Selective ROESY
Mult. Solvent Suppr./presat
Mult. Solvent Suppr./WET
2D Selective HMBC

- In the LC1DWTDC window, enter NS = 16
- In the LC1DWTDC window, click Accept.



· Check the parameters in the information window.



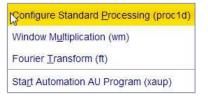
• In the sel1d window, click **OK** to start the acquisition.

3.9.6 Processing

• On the menu bar, click **Process**.

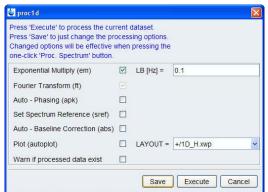
<u>S</u> tart	<u>A</u> cquire	Process	>	A <u>n</u> alyse	P <u>u</u> b	lish	<u>V</u> iew	<u>M</u> anage	0			
	A Proc. Sp	oectrum 🗢	4	Adjust Pha	ase 🔻	^ (Calib. A <u>x</u> is	NR Pick P	<u>e</u> aks ▼	∫ <u>I</u> nte	grate 🗢	A <u>d</u> vanced ▼

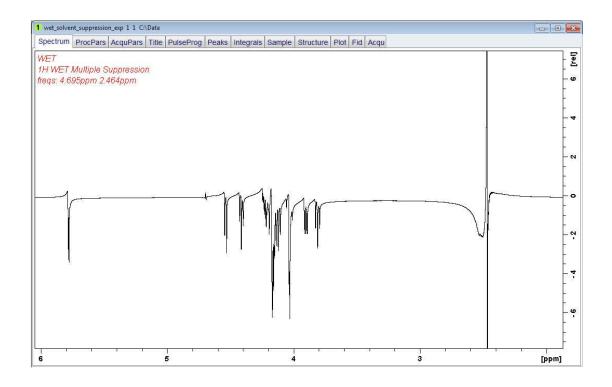
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- Select Configure Standard Processing (proc1d).



- · Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

• In the proc1d window, click Execute.





• On the Workflow button bar, click Adjust Phase.

Start Acquire Pro	cess Analyse	P <u>u</u> blish	<u>∨</u> iew	<u>M</u> anage	0	
A Pro <u>c</u> . Spectrum ▼						A <u>d</u> vanced ▼

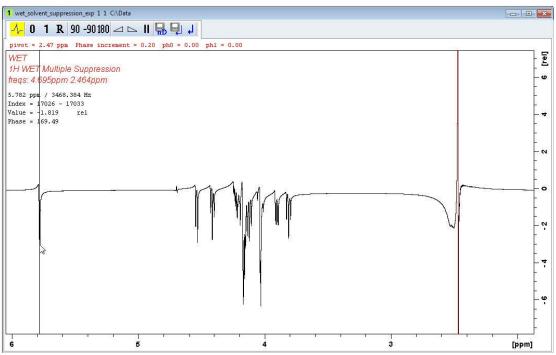
The Dataset window tabs are replaced with the **Adjust Phase** toolbar.



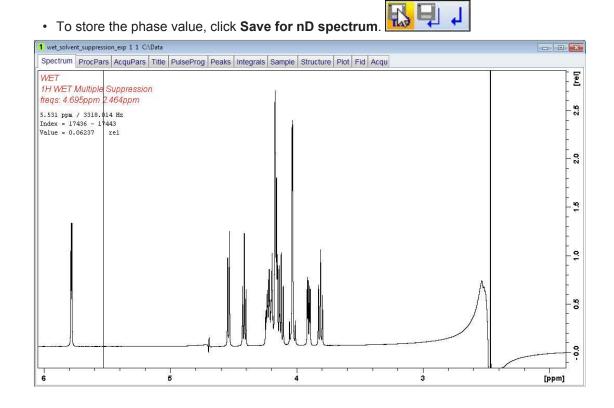
- Move the cursor line on top of the peak at 5.7 ppm and right-click.
- On the shortcut menu, select Set Pivot Point.

Set Pivot Point Calculate ph0

- Adjust the **0** order phase on the selected pivot point to positive absorption.
- Adjust the 1st order phase on the peaks between 4.6 ppm and 3.6 ppm.



The peak at **4.7 ppm** and **2.4 ppm** are the suppressed solvent peaks and those will appear out of phase.



4 2D Gradient Experiments

4.1 Introduction

The vital importance of NMR in chemistry and biochemistry relies on the direct relationship between any given NMR experiment and the molecular information that can be extracted from it. Thus, every experiment is based on some NMR parameter, usually coupling constants or NOE, which is related to a specific molecular parameter (through-bond or through-space connectivity, chemical exchange, molecular motion...). The quantitative measurement of such NMR parameters allows us to obtain valuable information about structural parameters such as dihedral angles, intermolecular distances, relaxation and exchange rates. etc... For this reason, the development of new and/or improved NMR methodologies is a key factor to be considered. Since the 90's when the gradients were introduced as a useful tool to incorporate them in to NMR applications, the suite of NMR experiments available to researchers has grown. A large percentage of them are using pulse field gradients.

Gradient enhanced NMR spectroscopy is widely used in liquid state spectroscopy for coherence pathway selection, solvent suppression, artifact reduction, and diffusion weighting and has had a tremendous impact by improving the quality of NMR spectra.

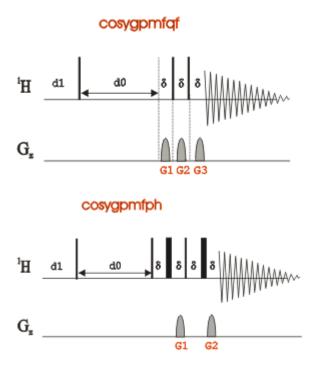
Thus, all advantages offering the incorporation of PFG as a powerful elements into highresolution NMR pulse sequences, combined with the advanced software tools available at the present time to acquire and process multidimensional NMR experiments with great simplicity, has dramatically changed the concept of routine work in NMR for chemists.

4.2 2D Multiple Quantum Filtered COSY Experiment

The COSY Multiple-Quantum Filtered (COSY-MQF) experiment is an alternative version of the COSY experiment, in which a multiple-quantum filter is inserted to allow the detection of signals from all coupled spin systems but suppresses signals arising of lower coherence levels. Thus, a COSY with a double-quantum filter (2D COSY-DQF experiment) experiment efficiently suppress single-quantum coherence from singlet uncoupled signals as, for instance, those of methyl groups or solvents. The COSY-DQF experiment can be performed in magnitude or phase-sensitive mode by selecting the appropriate phase programs and transform algorithm. However, phase-sensitive data is usually recommended.

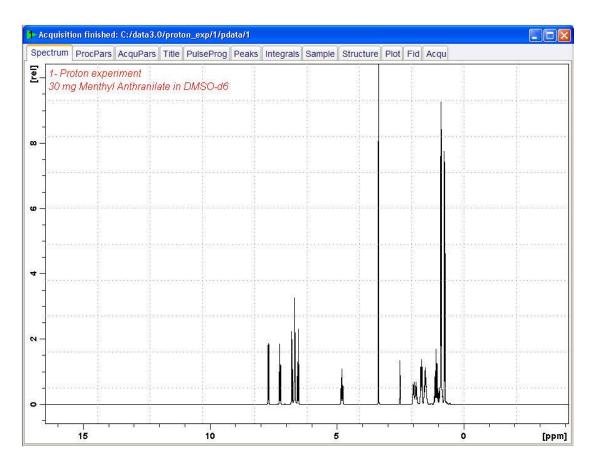
In spectrometers equipped with gradient technology, gradient-based COSY versions are highly recommended.

The gp-2D COSY-MQF experiment allows to obtain a 2D COSY-MQF spectrum with a single scan per t1 increment provided that the S/N ratio is adequate. The main advantage of such approach is the large reduction in the total acquisition time compared with a conventional phase-cycled 2D COSY-MFQ experiment. Magnitude-mode (**cosygpmfqf**) or phase-sensitive (**cosygpmfph**) data is obtained depending of the selected pulse sequence and acquisition/processing procedure. The COSY-MQF experiment permits to trace out through-bond proton-proton connectivity via the homo nuclear JHH coupling constant.



4.2.1 Preparation Experiment

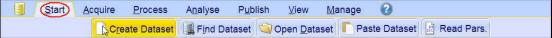
Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



4.2.2 Setting up the MQF-COSY Experiment

The steps below assume that the sample remains in the magnet after observing the proton spectrum.

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



 In the New Dataset window,enter or select: NAME = cosydqf_exp EXPNO = 1 PROCNO = 1 Experiment: select COSYGPDFPHSW Set Solvent: select DMSO

🎍 New	-					
Prepare for a new experim initializing its NMR parame For multi-receiver experim Please define the number	ters according to ents several data	the selected experiment type. asets are created.				
NAME	cosydqf_e	exp				
EXPNO	2					
PROCNO	1					
O Use current parameters	5					
Experiment COSYGPD	FPHSW	Select				
 Options 						
Set solvent:		DMSO -				
C Execute "getprose	ol"					
Keep parameters		P 1, O1, PLW 1 Change				
DIR		C:\Data 🔹				
🖾 Show new datase	t in new window					
Receivers (1,2,10	5)	1				
2-D gadient DQF-COSY experiment 30 mg Menthyl Antranilate in DMSO d-6						
	ОК	Cancel More Info Help				

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

• In the New Dataset window, click OK.

• On the menu bar, click Aquire.

	<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> a	nage 🕜			
		Sampl <u>e</u> 🗢	Lock	Tune 🚽	👃 Sp <u>i</u> n 🚽	🖙 Shim 🚽	Prosol -	Gain 🔻	▶ Go - N	∕l <u>o</u> re ▼

For the following steps, use the Workflow button bar.

- Click **Tune** to tune the probe.
- · Click Spin and select Sample rotation off.



2D experiments should be run non-spinning.

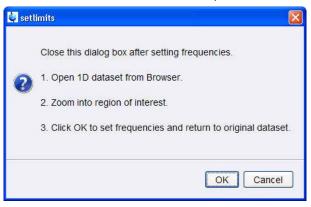
- To autoshim the sample with TopShim and best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.

4.2.3 Limit Setting

• On the Workflow button bar, click SetLimits.

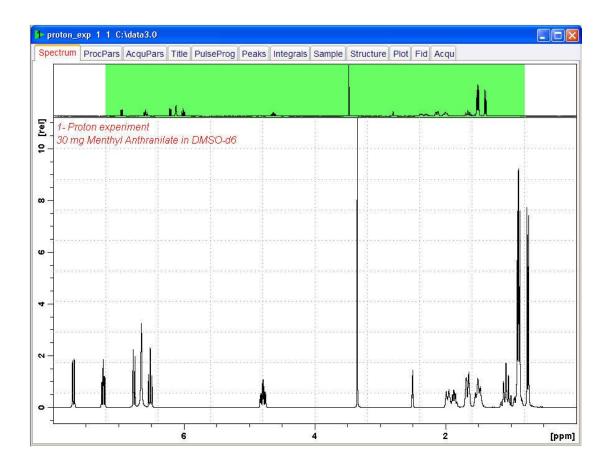
Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish <u>V</u> i	ew <u>M</u> anage	0			
Sampl <u>e</u> 🚽 🗮 Loc	k V Tune	🗸 🕹 Spin 🗢	🗧 🛱 Shim 🗢	f Prosol マ	SetLimits 🗸	<u> </u>	▶ Go 🗢	M <u>o</u> re ▼

 To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.



 Expand the spectrum to display all peaks, leaving ca. 0.2 ppm of baseline on either side of the spectrum.

The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak will be folding in F1.



- In the setlimits message window, click OK to assign the new limit.
- In the message window click **Close**.

۵.	
0	1H spectral limits copied for F1 and F2 dimensions. SW: 7.9997 ppm O1P: 4.024 ppm
	Close

The display changes back to the 2D dataset.

4.2.4 Acquisition

The first increment of the DQF-COSY experiment has a low signals to noise ratio and the signals grow as the experiment is progressing. It is therefore not advisable to use the automatic receiver gain adjustment **rga** since it adjusts the receiver gain on the first increment. In this case an AU program **au_zgcosy** is available. Executing this AU program changes the pulse program to **zg** and performs an **rga** and then changes back again to **cosygpmfph** and then starts the acquisition.

• At the command prompt, type **au_zgcosy**.

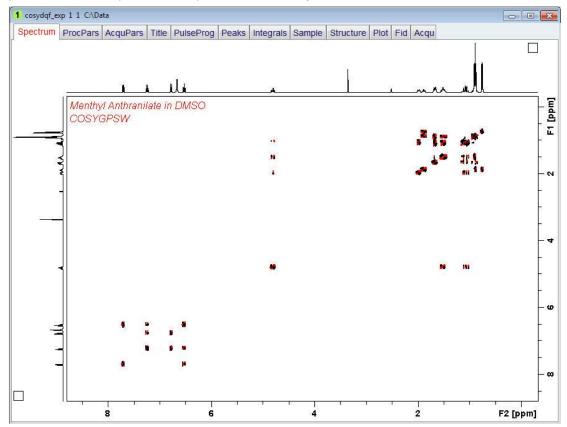
4.2.5 Processing

• On the menu bar, click **Process**.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blis	sh <u>V</u> iew	<u>M</u> anage	0		
	_ Pro <u>c</u> . Sp	ectrum v	Adjust Ph 🔶	ase▼	Å Calib. A <u>x</u> is	N Pick P	<u>e</u> aks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. To configure this program or select the right options, click the down arrow inside the **Proc. Spectrum** button. Since this is a phase sensitive experiment the phase correction **apk2d** should to be enabled.



4.2.6 Plotting

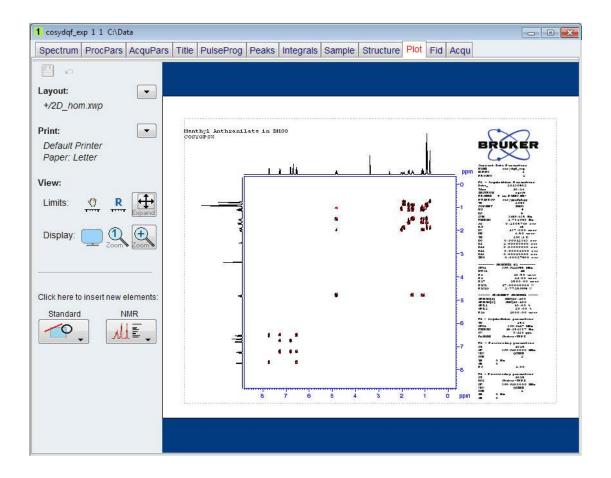
• Use the Smaller/larger buttons to adjust for a suitable contour level.



i

- Type .ls or click on the Contour levels to disk button.
- On the menu bar, click **Publish** and on the Workflow button bar, click **Plot Layout**.

<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse (Publish Vi	ew <u>M</u> anage	e 🕜	
		C opy	Print 🗢	<mark>⊡</mark> [<mark>₿l</mark> ot Layou	t 🔌 P <u>D</u> F 🔻	🚨 <u>E</u> -Mail	Mo <u>b</u> ile ▼





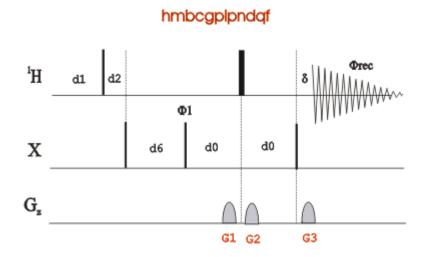
If desired, any changes can be administered by using the tools on the left side of the display.

• In the Print section, click the **down** arrow button and select **Print**.

Layout:	•					
+/1D_H.xwp						
Print:	-					
Default Printer	<u>P</u> rint					
Paper: A4	Set target printer					
View:	<u>M</u> ulti-print					
	Page <u>s</u> etup					
Limits: 🖑 🖳	Split page setting					
Display:	+ oom					
Click here to insert new elements: Standard NMR						

4.3 2D ¹H/³¹P Gradient HMBC Experiment

The **2D** gradient HMBC experiment records qualitative heteronuclear long-range connectivity, including through hetero nuclei. This section of the manual will guide you through the set up of a ¹H/X gradient experiment using the standard Bruker HMBCGP parameter set. In addition of changing the nucleus in F1 from ¹³C to another X-nucleus, the gradient ratio for the new X-nucleus also have to be calculated. The HMBC pulse sequence is shown in the figure below.



The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

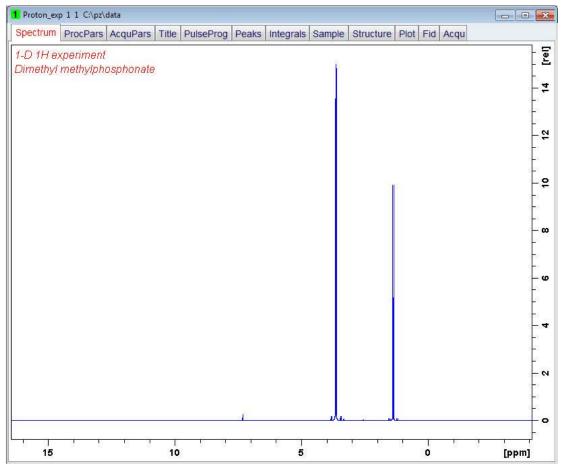
4.3.1 Sample

30mg Dimethyl methylphosphonate in CDCl₃

This ³¹P nucleus in this sample does not have any direct proton attached The long range coupling from the 3 methyl protons to ³¹P is **17 Hz**, where the other 6 methyl protons through the additional oxygen nuclei show a J-value of **11 Hz**. The J-values can be easily obtained from the proton spectrum (see chapter ¹H Reference Experiment [> 49]).

4.3.2 ¹H Reference Experiment

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book Basic NMR experiments, chapter 1D Proton Experiment, Paragraph Experiment Setup through Processing using CDCl₃ as a lock solvent.



4.3.3 ³¹P Reference Experiment

The steps below assume that the sample remains in the magnet after observing the proton spectrum.

• On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.

 In the New Dataset window, enter or select: NAME = 31P_exp EXPNO = 1

PROCNO = 1 Experiment: select P31CPD

Set Solvent: select CDCI3

🧅 New	1. 18 Care 1					
Prepare for a new experin initializing its NMR param For multi-receiver experin Please define the numbe	eters according to nents several data	the selected experiment type. asets are created.				
NAME	31P_exp					
EXPNO	1					
PROCNO	1					
O Use current parameter	rs					
Experiment P31CPD		Select				
 Options 						
V Set solvent:		CDCI3				
C Execute "getpros	sol"					
Keep parameter	S.	P 1, O1, PLW 1 Change				
DIR		C:\pz\data ▼				
🖾 Show new datas	et in new window					
Receivers (1,2,1	16)	1				
	experiment methylphosphona	te				
	OK	Cancel More Info Help				

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

• In the New Dataset window, click OK.

• On the menu bar, click Acquire.

	<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> a	anage	2	 	
		💐 Sampl <u>e</u> 🗢							Þ Go 🚽	M <u>o</u> re ▼

For the following steps, use the Workflow button bar.

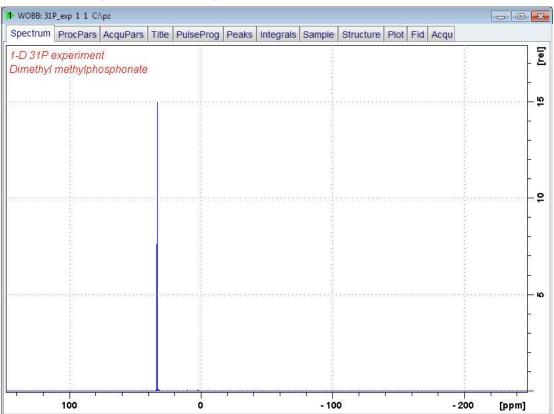
- Click **Tune** to tune the probe.
- Click Spin and select Sample rotation on.
- Click Shim for best homogeneity use TopShim.
- To autoshim the sample with TopShim and best homogeneity, click Shim.
- Click Prosol to load the probe/solvent depended parameters.

4.3.4 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

4.3.5 Processing

· Process and phase correct the spectrum.



4.3.6 Setting up the HMBC Experiment

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



• In the New Dataset window, enter or select:

NAME = 1H_31P_hmbc_exp EXPNO = 1 PROCNO = 1 Experiment: select HMBCGP Set Solvent: select CDCI3

🖕 New	
initializing its NMR paramete	nt by creating a new data set and rs according to the selected experiment type. Its several datasets are created. I receivers in the Options.
NAME	1H_31P_hmbc_exp
EXPNO	1
PROCNO	1
O Use current parameters	
Experiment HMBCGP	Select
 Options 	
Set solvent:	CDCI3 -
Execute "getprosol"	
Keep parameters:	P 1, O1, PLW 1 Change
DIR	C:\pz\data 🗸
C Show new dataset in	n new window
Receivers (1,2,16)	1
	HMBC experiment hylphosphonate in CDCI3
	OK Cancel More Info Help

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

• In the New Dataset window, click OK.

• On the menu bar, click Acquire.

	<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> ar	nage 🕜			
		💐 Sampl <u>e</u> 🔻	tock	V Tune 🗸	👃 Spin 🚽	🖙 Shim 🚽	Prosol v	▶ <u> </u>	▶ Go → Mor	e▼

- In the Dataset window, select the **AcquPars** tab.
- Click the Set nuclei and routing button to display the routing window.



🖕 Channe	el Routing	A REAL PROPERTY OF			×
Fr	requency	Logical Channel	Amplifier	Preamplifier	
SFO1 3 OFS1 2 BF2 1	00.13247 M 2469.6 H 21.494851 N 21.494851 M	IHZ NUC2 IHZ F2 SGU2	H 60 W	1H 2H XBB19F 2HS	
:	cable wiring possible RF routing cortab available	1	 settings show selected routing show receiver wiring show probe wiring show RF routing 	show receiver routing show power at probe in	
Save	e and Close S	witch F1/F2 Switch F1/F3	Add logical channel Rem	ove logical channel Defau	It Info Param Close

 Change the following parameter: NUC2 = 31P

•
č.,
U

Do not modify the routing!

- Click the Save and Close button inside the Channel Routing window.
- Scroll down to the Nucleus 1 section in the AcquPars window.

NUC1	1H	Edit	31P	Observe nucleus
O1 [Hz]	1200.68	5570.91		Transmitter frequency offset
O1P [ppm]	4.001		45.853	Transmitter frequency offset
SFO1 [MHz]	300.1312007	7	121.5004219	Transmitter frequency
BF1 [MHz]	300.1300000)	121.4948510	Basic transmitter frequency

- Change the following parameter: NUC1 [F2] = 31P
- Scroll down to the Program parameter section in the AcquPars window.
- Click the CNST Edit button (Constant used in pulse programs).

Program paramet	ers			
L	Edit	Loop counter		
CNST	Edit 🔓	Constant used in pulse programs		
CPDPRG	Edit Set constants used	in pulse programs imposite pulse decoupling program (cpd)		
PHCOR [degree]	Edit	Correction angle for phase program		
SUBNAM	Edit	Name of subroutine		
ZGOPTNS		Acquisition (zg) options		

 Change the following parameter: CNST13 = 14 (J 31P/1H long range)

	sed in pulse	programa					
CNST[0]	1	CNST[16]	1	CNST[32]	1	CNST[48]	1
CNST[1]	1	CNST[17]	1	CNST[33]	1	CNST[49]	1
CNST[2]	145	CNST[18]	1	CNST[34]	1	CNST[50]	1
CNST[3]	1	CNST[19]	1	CNST[35]	1	CNST[51]	1
CNST[4]	1	CNST[20]	1	CNST[36]	1	CNST[52]	1
CNST[5]	1	CNST[21]	1	CNST[37]	1	CNST[53]	1
CNST[6]	1	CNST[22]	1	CNST[38]	1	CNST[54]	1
CNST[7]	1	CNST[23]	1	CNST[39]	1	CNST[55]	1
CNST[8]	1	CNST[24]	1	CNST[40]	1	CNST[56]	1
CNST[9]	1	CNST[25]	1	CNST[41]	1	CNST[57]	1
CNST[10]	1	CNST[26]	1	CNST[42]	1	CNST[58]	1
CNST[11]	1	CNST[27]	1	CNST[43]	1	CNST[59]	1
CNST[12]	1	CNST[28]	1	CNST[44]	1	CNST[60]	1
СИЗТ[13]	14	CNST[29]	1	CNST[45]	1	CNST[61]	1
CNST[14]	1	CNST[30]	1	CNST[46]	1	CNST[62]	1
CNST[15]	1	CNST[31]	1	CNST[47]	1	CNST[63]	1

The CNST13 long range J value of **14 Hz** is an average value of the two coupling constants **11 Hz** and **17 Hz**, see chapter Sample [\triangleright 49].

• On the **Spin** button, click the **drop-down** arrow to see more options.

Sta	irt	Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0			
		🕴 Sampl <u>e</u> 🗢	tock	∛ Tune 🗢	\rm Spin 🎝	Shim -	Pros	iol 🗢	<u> G</u> ain ▼	Þ Go 🗢	M <u>o</u> re ▼

• In the list, select ro off.

Turn sample rotation on (ro on) Turn sample rotation off (ro off) Change sample rotation rate (ro) MAS Pneumatic Unit (masdisp) Start MAS Spinning (masg) Stop MAS Spinning (mash) Get MAS Spinning Rate (masrget) Set MAS Spinning Rate (masrset)



2D experiments should be run non-spinning.

• On the Workflow button bar, click **Prosol**.

Start Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew <u>N</u>	<u>A</u> anage 🕜			
💐 Sampl <u>e</u> 🗢	tock	V Tune →	l Sp <u>i</u> n →	🛱 Shim 🗸	Prosol -	<u>I∽ G</u> ain ▼	● Go マ More マ	

This will load the pulse width and power levels in to the parameter set.

• At the command prompt, enter gradratio.

🖕 gradratic	X
8	pulse program = hmbcgplpndqf
	nucleus1 = 1H nucleus2 = 31P gpz1=70.0 gpz2=30.0 gpz3=80.5
	Close

The command executes the AU_program **gradratio** to calculate the gradient ratio **GPZ1**, **GPZ2** and **GPZ3** for the nucleus ³¹**P**. To check if the correct gradient ratio values has been entered in the **AcquPars**, follow the steps below.

Power		
PLW [W]	Edit	Power level in Watt
PLdB	Edit	Power level in dB
PLSTRT [dB]	-6	First step for PL switching
PLSTEP	0.1	Step width for PL switching
SHAPE	Edit	Shaped pulse parameter
GRADIENT	Edit	Gradient parameters
CAGPARS	Edit	Parameters for gradient calculation
AMP [%]	Edit	Amplitude of pulse

• In the AcquPars window, scroll down to the Power section.

· Click GRADIENT Edit (Gradient parameters).

radient	parameters				
Index	GPX [%] (GPX)	GPY [%] (GPY)	GPZ [%] (GPZ)	Filename (GPNAM)	
0	0	0	0		E
1	0	0	70	SMSQ10.100	E
2	0	0	30	SMSQ10.100	E
3	0	0	80.4807	SMSQ10.100	E

• In the Dataset window, select the **Spectrum** tab.

4.3.7 Limit Setting

• On the Workflow button bar, click SetLimits.

Start Acquire	Process ,	A <u>n</u> alyse P	ublish <u>V</u> iev	w <u>M</u> anage	0			
💐 Sampl <u>e</u> 🗢 🛛 🇱 I	_ock 📝 Tune 🚽	👃 Spin 🗢	ि Shim	ff Prosol マ	SetLimits 🗸	<u> G</u> ain ▼	Þ Go 🚽	M <u>o</u> re ▼

 Open 1D dataset from Browser. Zoom into region of interest. Click OK to set frequencies and return to original dataset. 	 Zoom into region of interest. 		Close this dialog box after setting frequencies.
		2	1. Open 1D dataset from Browser.
3. Click OK to set frequencies and return to original dataset.	3. Click OK to set frequencies and return to original datase		2. Zoom into region of interest.
			3. Click OK to set frequencies and return to original dataset.

- To open the 1D Proton spectrum, right-click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.

The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak will be folding in F1.

pectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Plot	Fid	Acqu		
						2							1
									in the second se				
						10			a an				
D 1H ex	<i>cperiment</i>			÷.			÷					3	
methvl	methvlph	osphonate					1						
							********	• • • • • • • • • • • •		a (• • a)	• • • • • • •		
							1						
		xxxxxXX x x x x x x x x x x x x x x x x											
							i.						1
	sarýtarstar T	ara) catarat		neon Ganeo T	a sa a a sa a		arearea fr			11		kara tara t T	ana ang ang a
							-			-			
												Į	
	1						1						Ē
							1						-
• • • • • • • • • •	····	••••		•••••	+ • • • • • • • •		********	•••••					
										11			
													E
	i.	1					÷						F
					01.20.001.20.00							1	
		IA.					1						
		-M_u	<u> </u>							M		<u>.</u>	ľ
				1			Ĩ					1	
r 1		1	15 8		1 1	1	3. 1	16	L:	Q	18	18	

- In the setlimits message window, click **OK** to assign the new limit.
- In the message window, click Close.

4	
	1H spectral limits copied for F2 dimension.
•	SW: 3.9926 ppm O1P: 2.499 ppm
	Close

The display changes back to the 2D dataset. Follow the steps below to set the limit in the F1 dimension.

• On the Workflow button bar, click SetLimits.

Start Acquire	<u>P</u> rocess A	A <u>n</u> alyse P	<u>u</u> blish <u>V</u> ie	w <u>M</u> anage	0			
🛊 Sampl <u>e</u> 🗢 🗰 Lo	ck 🕅 Tune 🗢	👃 Sp <u>i</u> n マ	🛱 Shim 🔻	f¶ P <u>r</u> osol ▼	SetLimits 🗸	<u> G</u> ain ▼	Þ Go 🚽	More 🗢

	Close this dialog box after setting frequencies.
2	1. Open 1D dataset from Browser.
	2. Zoom into region of interest.
	3. Click OK to set frequencies and return to original dataset.

To open the 1D ³¹P spectrum, right click on the dataset name in the browser window (e.g. ³¹P_exp 1) and select **Display** or click and hold the left mouse button for dragging the 1D ³¹P dataset into the spectrum window.

• Expand the spectrum to display all peaks.

Spectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Plot F	Fid Acqu	
1-D 31P e	experimer	nt .		1							
Jimethyl	methylph	osphonate									
			2001200	1					120120		
	:										
										1	-
	········										
	:										
	:									-	
	1									Ì	
	1					1					-
1 1			-	i i î î 34		33		32	î î	31	[ppm]

- In the setlimits message window, click OK to assign the new limit.
- In the message window, click **Close**.

The display changes back to the 2D dataset.

4.3.8 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

4.3.9 Processing

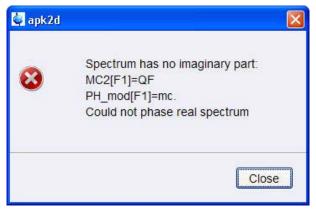
• On the menu bar, click **Process**.

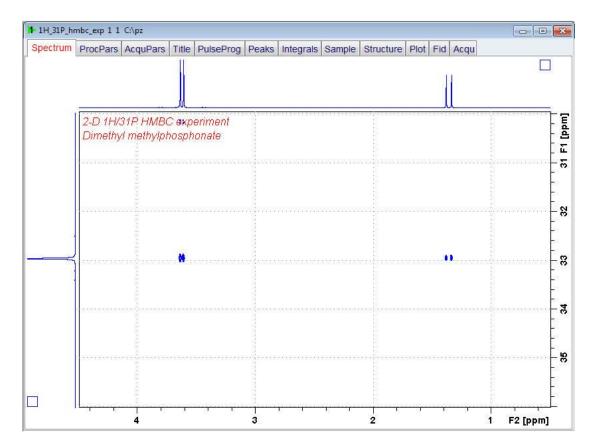
<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blis	sh <u>V</u> iew	<u>M</u> anage	0		
	Proc. Sp	oectrum 🗢	Adjust Ph	ase 🔻 🗍	\Lambda Calib. A <u>x</u> is	N Pick P	<u>e</u> aks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. The message shown in the figure above pops up in case of a magnitude 2D experiment and the **apk2d** option is enabled. To disable the **apk2d** option, click the **down arrow** in the **Proc. Spectrum** button in the Workflow button bar and configure the Standard Processing (**proc2d**) program.

• In the apk2 message window, click Close.





4.3.10 Plotting

- Use the Smaller/larger buttons to adjust for a suitable contour level.
- <mark>}}</mark> ₹ +⁄-@
- Enter .Is or click the Contour levels to disk button.
- On the menu bar, click **Publish** and on the Workflow button bar, click **Plot Layout**.

<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse (Publish Vie	ew <u>M</u> anage	• 🕜	
		Copy	♥ P <u>r</u> int ▼	<mark>⊫∄[Şl</mark> ot Layout	🦂 P <u>D</u> F マ	😅 <u>E</u> -Mail	Mo <u>b</u> ile ▼

I

1 1H_31P_hmbc_exp 1 1 C:\pz\data		- 0 ×
Spectrum ProcPars AcquPars	Title PulseProg Peaks Integrals Sample Structure Plot Fid Acqu	
<u>е</u> ю		
Layout:		
+/2D_inv.xwp		
Print:	2-D 1H/31P HMBC experiment Dimethyl methylphosphonate	\sim
Default Printer	Dimethyl methylphosphonate	BRUKER
Paper: Letter	1	
View:		
Limits: 🔨 R Expand		Family Strategy and
Display:		31.0
Zoom Zoom		31.5
		32.5
Click here to insert new elements:		33.0
Standard NMR		- 33.5
		a - alleration families
		a second particular and a seco
		35.0
		an tart
	4.0 3.5 3.0 2.5 2.0 1.5 1.0	- 36.0

If desired, any changes can be administered by using the tools on the left side of the display.

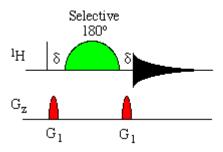
• In the Print section, click the **down** arrow button and select **Print**.

Layout: +/1D_H.xwp	•					
Print:	-					
Default Printer	<u>P</u> rint					
Paper: A4	Set target printer					
View:	Multi-print					
Page setup						
Limits: R R Split page setting						
Display:						
Click here to insert new eler Standard NMR						

5 1D Experiments using Shaped Pulses

5.1 Introduction

Selective homonuclear 1D experiments usually start from the selective ¹H excitation of a given resonance followed by a mixing process. When PFG's are available, the SPFGE scheme is highly recommended as a selective excitation scheme. The SPFGE or **S**ingle **P**ulsed **F**ield **G**radient **E**cho scheme is a single echo experiment in which the central selective 180° pulse is flanked by two gradient pulses. It is used for efficient selective excitation purposes.

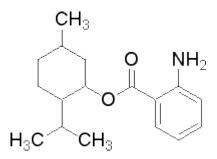


Selective 1D experiments can be easily derived by adding the corresponding mixing process between the SPFGE block and the acquisition period.

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. **Example 1** and **2** show how to manually set the excitation region using the on- and off-resonance options. **Example 3** uses the Flow bar tools to automatically calculate the excitation region. All 3 examples can be applied to the 1D selective experiments, such as **SELCOGP**, **SELNOGP** and **SELMLGP**.

5.2 Sample

A sample of **30mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



5.3 Example 1: 1D Selective COSY Experiment using the On-Resonance Option

5.3.1 Introduction

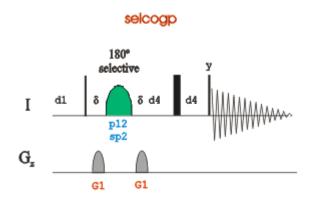
The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ¹H spectrum. Important characteristics of a soft pulse include the shape, the amplitude, and the length. The selectivity of a pulse is measured by its ability to excite a certain resonance (or group of resonances) without affecting near neighbors. Since the length of the selective pulse affects its selectivity, the length is selected based on the selectivity desired and then the pulse amplitude (i.e., power level) is adjusted to give a 90° (or 270°) flip angle.



The transmitter offset frequency of the selective pulse must be set to the frequency of the desired resonance. This transmitter frequency does not have to be the same as o1p (the offset frequency of the hard pulses), but for reasons of simplicity, they are often chosen to be identical.

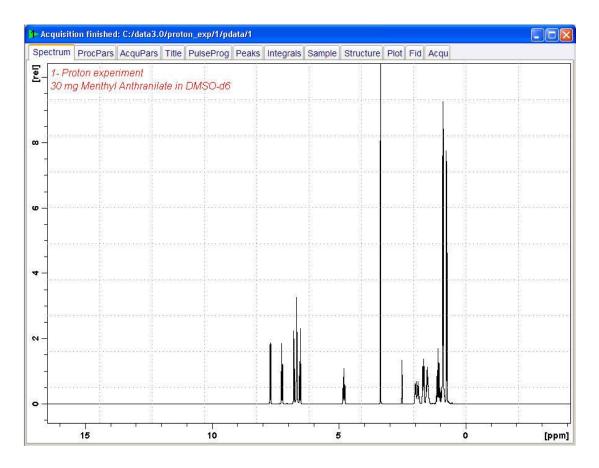
Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments using pulse field gradients and non-spinning.

Section *Example 1: 1D Selective COSY Experiment using the On- Resonance Option* [▶ 62] describes the acquisition and processing of a one-dimensional ¹H selective gradient COSY experiment, using the on-resonance option. The standard Bruker parameter set is SELCOGP and includes the pulse sequence **selcogp** shown in the figure below. It consists of the recycling delay, four radio-frequency (RF) pulses and the acquisition time during which the signal is recorded. The first RF pulse is a 90° pulse, followed by a 180° shaped pulse, a 180° hard pulse and finally a 90° pulse. The delay between the 180° and 90° pulse is 1/4*J(H,H). The gradient pulses are applied before and after the shaped pulse.



5.3.2 Reference Spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



5.3.3 Selective Excitation Region Set Up

5.3.3.1 On Resonance

Ensure 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.

• At the command prompt, type wrpa.

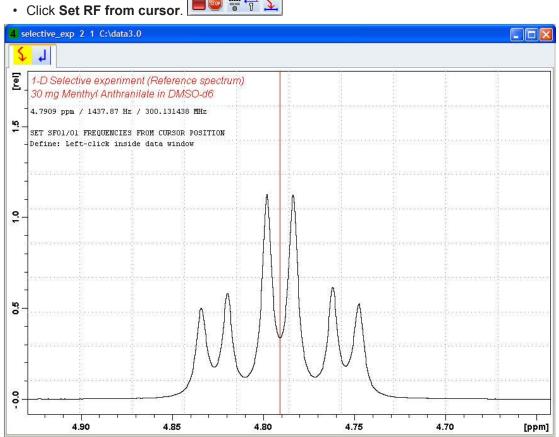
	NAME ends with ".top", the destination ataset (no expno/procno required). lestination:
NAME =	sel_cosy
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
	OK Cancel Help

- Change NAME = **sel_cosy**.
- In the wrpa window, click OK.
- At the command prompt, type **re** and hit **Enter**.

😂 re	
Options	
NAME =	sel_cosy
EXPNO = PROCNO =	1
DIR =	C:\data3.0
OK Canci	el Browse Find Help

- Change NAME = **sel_cosy**.
- In the re window, click **OK**.
- Expand peak at 4.8 ppm.





- Move the cursor line into the center of the multiplet.
- To set the frequency, click left.

• In the O1/O2/O3 window, click O1.

🍓 01/02/03	
Define SF01/01	frequencies
SFO1 [MHz] =	300.131438
O1/2/3 [Hz] =	1437.87
01 02	O3 Cancel

5.3.4 Setting Up the Selective COSY

• On the menu bar, click Start.

<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	⊻iew	<u>M</u> anage	2		
	C <u>r</u> eate Da	ataset 🖳 F	ind Dataset	🔄 Open 🛙	ataset	Paste Dat	taset	Read Pars.	

- On the Workflow button bar, click Read Pars.
- In the Find file names field, enter SEL* to display all selective parameter sets as shown in the figure below.

🖕 Parameter Sets: rpar					×
File Options Hel	р	S	Source = C:\Bruker\TopSpi	n3.5pl5\exp\stan\nmr\par	~
Find file names s	el*	Exclude:	Clear		
Class = Any ~	Dim = Any 🗸 🗌 S	how Recommended			
Type = Any ~	SubType = Any 🗸	SubTypeB = Any ~	Reset Filters		
SELCO1H	SELCOGP	SELGPSE	SELMLGP	SELMLZF1H	
SELNO1H	SELNOGP	SELRO1H	SELROGP	SELZG1H	
				Read Clo	ose
				Read Cit	ose

- Select SELCOGP.
- In the Parameter Sets: rpar window, click Read.
- · Select the acqu, proc and outd parameter options only.
- In the Keep parameters list of values, select P1, O1, PLW1.
- Enable the Keep parameters option.
- In the rpar window, click OK.

		×
Destination Data Set = 1) Select the desired f	I = C:\Bruker\TopSpin3.5pi5\exp\stan\nmr\p Example_MenthylAnthranilate 1 1 C:\Dat The types of the source parameter set nem to the destination data set.	
acqu proc outd		
title		
Set solvent: DMS		
hannan		

- In the Dataset window, select the Title tab and enter:
 1D Selective COSY experiment
 30 mg Menthyl Anthranilate in DMSO-d6
- To store the title, click Save.
- In the Dataset window, select the **Spectrum** tab.
- On the menu bar, click Acquire.



For the following steps, use the Workflow button bar.

· Click Spin and select Sample rotation off.



1D selective experiments should be run non-spinning.

• To load the probe/solvent depended parameters, click Prosol.

5.3.5 Acquisition

• To start the acquisition, click Go.

5.3.6 Processing

• On the menu bar, click Process.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> bli	sh <u>V</u> iew	<u>M</u> anage	0		
	A Proc. Sp	ectrum 🗢	Adjust Ph	ase 🔻	A Calib. A <u>x</u> is	NR Pick P	<u>e</u> aks ▼	∫ Integrate →	A <u>d</u> vanced ▼

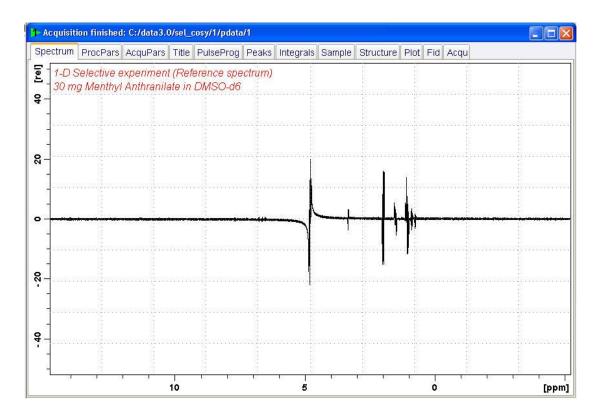
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- · In the list, select Configure Standard Processing.

Configure Standard Processing (proc1d)
Window Multiplication (wm)
Fourier <u>T</u> ransform (ft)
Eourier Transform Options (ftf)
Start Automation AU Program (xaup)

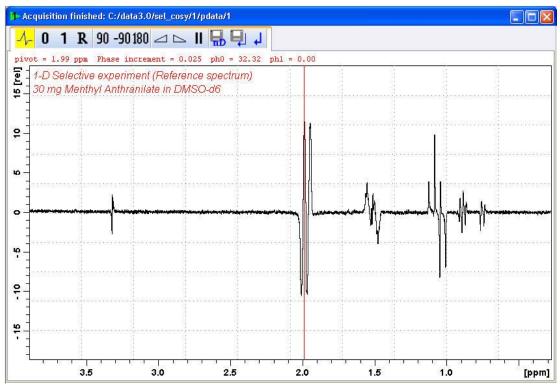
- · Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

• In the proc1d window, click Execute.

Press 'Execute' to process the curre Press 'Save' to just change the pro Changed options will be effective will one-click 'Proc. Spectrum' button.	essi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)			
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp
Warn if processed data exist			



• Expand the spectrum from **4 ppm** to **0.5 ppm**.



• To display an antiphase pattern, adjust the **0** order phase on the peak at **2.0 ppm**.

• To store the phase value, click Return & save phased spectrum.

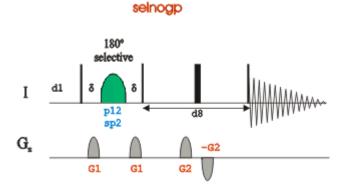
Follow the instructions in chapter *Plotting Two Spectra on the Same Page* [82] to plot two spectra on the same page.

5.4 Example 2: 1D Selective NOESY Experiment using the Off-Resonance Option

5.4.1 Introduction

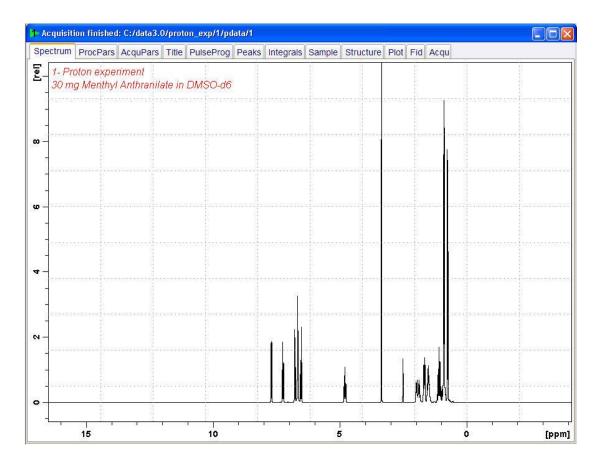
This experiment consist of three parts:

- **Selective excitation** of the selected resonance using the SPFGE block.
- **Mixing period** consisting of the basic 90°(¹H)-delay-90°(¹H) block in phase polarization transfer to other spins via NOE. Purging gradients are usually applied during the mixing period in order to remove any residual transverse magnetization.
- Proton detection as usual.



5.4.2 Reference Spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



1D Experiments using Shaped Pulses

5.4.3 Selective Excitation Region Set Up



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.

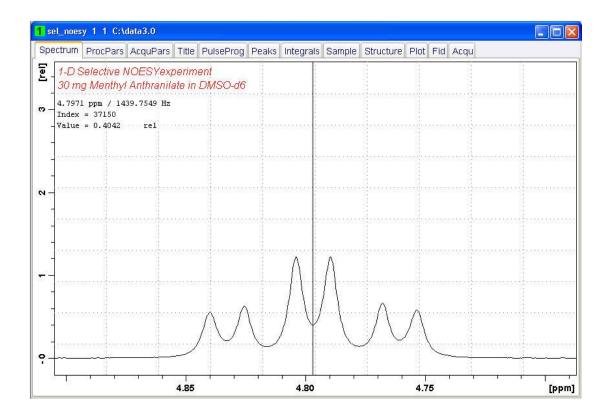
- At the command prompt, type wrpa.
- Change NAME = **sel_noesy**.
- In the wrpa window, click OK.

🥥 wrpa	X
States and the state of the state of the	AME ends with ".top", the destination set (no expno/procno required). tination:
NAME =	sel_noesy
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
	OK Cancel Help

• At the command prompt, type re.

🚑 re	
Options	
NAME =	sel_noesy
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
OK Cancel	Browse Find Help

- Change NAME = **sel_noesy**.
- In the re window, click **OK**.
- Expand the peak at 4.8 ppm.



- · Move the cursor line to the center of the peak.
- Step 1: Write down the cursor offset frequency value displayed in the upper left of the spectrum window (e.g. 1439.75).



To display the cursor information, right-click inside the spectrum window and select **Spectra Display Preferences** and enable **Cursor information** in the Spectra Display Preferences window.

• Step 2: At the TopSpin command prompt, type O1.



- Step 3: Write down the current value (e.g. 1853.43).
- Step 4: Calculate the difference of step 1 and 3 (e.g. -413.68).
- In the O1 window, click Cancel.



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.

1D Experiments using Shaped Pulses

5.4.4 Setting Up the Selective NOESY

• On the menu bar, click Start.

3	<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew	<u>M</u> anage	0		
		C <u>r</u> eate Da	ataset [😹 Fi	ind Dataset	🕥 Open [ataset	Paste Da	taset	Read Pars.	

- On the Workflow button bar, click Read Pars.
- In the **Find file names** field, enter **SEL*** to display all selective parameter sets as shown in the figure below.

🍦 Parameter Sets: rpar					×
File Options Help			Source = C:\Bruke	er\TopSpin3.5pl5\exp\stan\nmr\par	×
Find file names 🔗 sel	×	Exclude:	Clear		
Class = Any v [Dim = Any ~	Show Recommende	d		
Type = Any ~	SubType = Ar	ny ~ SubTypeB = Any	y V Reset Filters		
SELCO1H	SELCOGP	SELGPSE	SELMLG	P SELMLZF1H	
SELNO1H	SELNOGP	SELRO1H	SELROG	P SELZG1H	

- Select SELNOGP.
- In the Parameter Sets: rpar window, click Read.
- · Select the acqu, proc and outd parameter options only.
- In the Keep parameters list of values, select P1, O1, PLW1.
- Enable the Keep parameters option.
- In the rpar window, click OK.

🤹 rpar	×
Source Parameter Set = C:\Bruker\TopSpin3.5pl5\exp\stan\nmr' Destination Data Set = Example_MenthylAnthranilate 1 1 C:\D 1) Select the desired file types of the source parameter set 2) Press OK to copy them to the destination data set.	 State Western State and Stat State and State and Stat
acqu proc	
outd title	
Set solvent: DMSO	
 ✓ Set solvent: DMSO ✓ Execute 'getprosol' 	

• In the Dataset window, select the Title tab.

- Make the following changes:
 1D Selective NOESY experiment
 30 mg Menthyl Anthranilate in DMSO-d6
- To store the title, click Save.
- In the Dataset window, select the Spectrum tab.
- On the menu bar, click Acquire.

	<u>S</u> tart	Acquire Process		A <u>n</u> alyse	A <u>n</u> alyse P <u>u</u> blish		<u>V</u> iew <u>M</u> anage 🕜				
		💐 Sampl <u>e</u> 🗢	teck	V Tune 👻	l Sp <u>i</u> n マ	🗧 Shim 🔻	f¶ P <u>r</u> osol マ	<u> G</u> ain ▼	Þ Go 🗢	M <u>o</u> re ▼	

For the following steps, use the Workflow button bar.

· Click Spin and select Sample rotation off.



1D selective experiments should be run non-spinning.

- To load the probe/solvent depended parameters, click **Prosol**.
- In the Dataset window, select the AcquPars tab.
- Make the following changes:

PULPROG = selnogp D8 = 0.450 DS = 8 NS = 64 SPNAM2 = Gaus1_180r.1000

SPOFF2 = value from *Step 4: Calculate the difference of step 1 and 3* in chapter *Selective Excitation Region Set Up* [▶ 71].



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

5.4.5 Acquisition

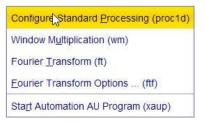
• To start the acquisition, click Go.

5.4.6 Processing

• On the menu bar, click **Process**.

3	<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0		
		A Proc. Sp	oectrum 🗢	Adjust Ph	ase 🗢 🕅 🧥 🤇	Calib. A <u>x</u> is	Pick P	<u>e</u> aks ▼	∫ Integrate →	Advanced 🛩

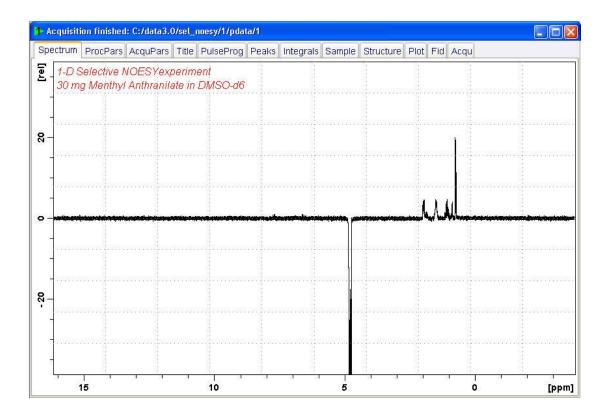
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing.



- · Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist
- In the proc1d window, click Execute.

Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective will one-click 'Proc. Spectrum' button.	cessi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)			
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp
Warn if processed data exist			

- Expand the spectrum from 4 ppm to 0.5 ppm.
- Manually adjust the phase of the selective peak at 4.8 ppm to show negative absorption to assure the correct phasing of the NOE peaks between 3 ppm and 1 ppm. Dependent on the field strength the peaks could be either positive or negative.



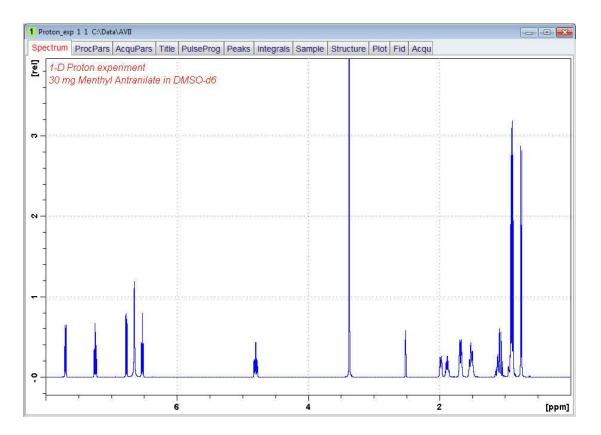
• To store the phase value, click Return & save phased spectrum.

Follow the instructions in chapter *Plotting Two Spectra on the Same Page* [82] to plot two spectra on the same page.

5.5 Example 3: 1D Selective-COSY, -NOESY and -TOCSY Experiments using the Flow Bar Tools

5.5.1 Reference Spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



5.5.2 Selective Excitation Region Set Up

The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

• On the menu bar, click Acquire.



• On the More button, click the drop-down arrow to see more options.

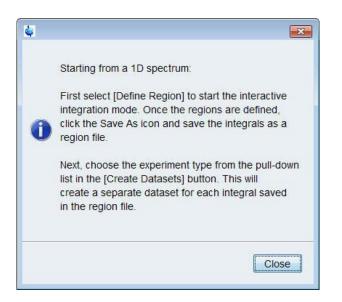
• In the list, select Setup Selective 1D Expts.

IconNMR Automation (icona)
Setup Selective 1D Expts.
TopSolids
TopGuide (topguide)
One-Click Experiments
Shape <u>T</u> ool (stdisp)
APSY (apsy)
NMR Thermometer (nmrtemp)

The Workflow button bar changes for setting up the 1D selective experiment.

• On the Workflow button bar, click **1D Selective Experiment Setup**.

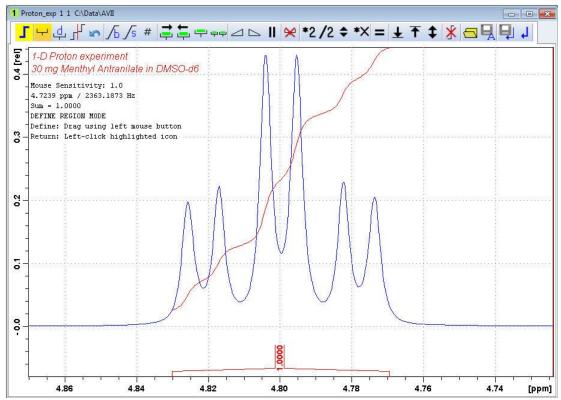
Start Acquire Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G M BACK_M	1DLSelective	Experiment	Setup	₩ Define <u>R</u> e	gions	🗷 Create <u>D</u> atasets 🗢



This button is only used for the instruction displayed above.

- In the message window, click Close.
- Expand the peak at 4.8 ppm.
- On the Workflow button bar, click **Define Regions**.

<u>Start</u> <u>Acquire</u>	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0
G Back	1D Se	elective Expe	riment Setup	A De	fine <u>R</u> egions	🖹 Create Datasets 🗢



• Integrate the multiplet at 4.8 ppm.



If desired, other peaks can be integrated and a separate dataset will be created for each integral saved in the region file.

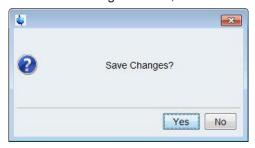
• On the Integration toolbar, click **Save region as**.



• In the list, select Save the Region to 'reg'.



- On the toolbar, click Return do NOT save regions!
- In the message window, click No.



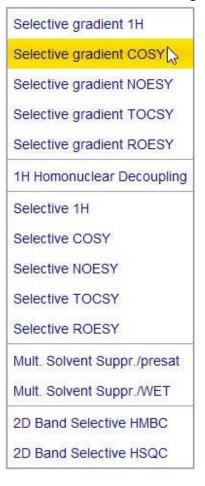


1D Experiments using Shaped Pulses

• On the Create Dataset button, click the drop-down arrow to see more options.

	<u>S</u> tart	Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	⊻iew	<u>M</u> anage	0	
🔾 м в,	ACK_M			1D Selective	Experiment	Setup	₩ Define <u>R</u> e	gions	🗄 Create Datasets 🗸

· In the list, select Selective gradient COSY.



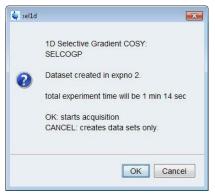
The default parameters are taken from the standard parameter set **SELCOGP**. If desired, the **Gaus1_180r.1000** pulse can be changed by clicking on the **Change Shape** button in the above window.

• In the SELCOGP window, click Accept.

SELCOGP	Gradient COSY	X
Shape = Gaus	s1_180r.1000	
D 4 (sec)	0.031250	mixing time
NS	8	
	2	-

The new dataset is created and all parameters are automatically set.

• In the sel1d window, click **OK** to start the acquisition.



5.5.3 Processing

• On the menu bar, click Process.

<u>S</u> tart	Acquire Proces	3	A <u>n</u> alyse	P <u>u</u> bl	ish <u>V</u> iew	Manag	je 🕜		
	_	1	[∿] ∲ Adjust Ph	ase 🔻	Å Calib. A <u>x</u> i	s 🎊 Pic	k P <u>e</u> aks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Proc Spectrum button, click the drop-down arrow to see more options.

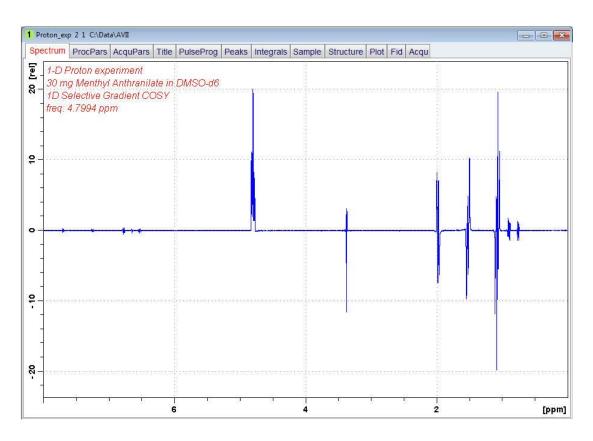




- · Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist
- In the proc1d window, click Execute.

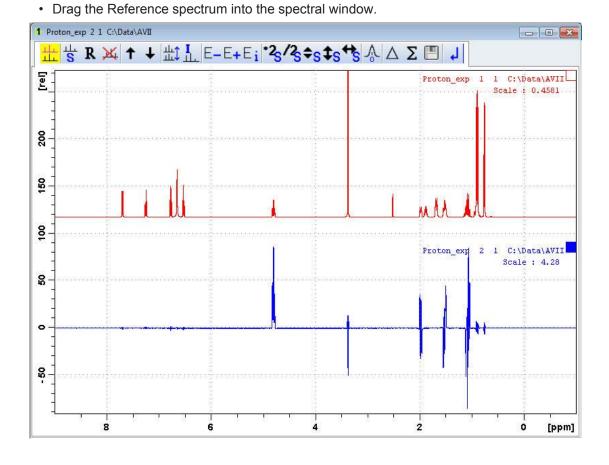
Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective will one-click 'Proc. Spectrum' button.	essi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)	V		
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp
Warn if processed data exist			

• Manually adjust the phase of the peaks between **3 ppm** and **1 ppm** for an antiphase pattern and if desired the selective peak at **4.8 ppm** can be phased positive.



5.5.4 Plotting Two Spectra on the Same Page

- Display the selective COSY spectrum.
- On the toolbar, click Multiple display.



To adjust the spectra for best fit, use the $\frac{23}{3}$ tools.

• On the menu bar, click **Publish** and on the Workflow button bar, click **Print**.

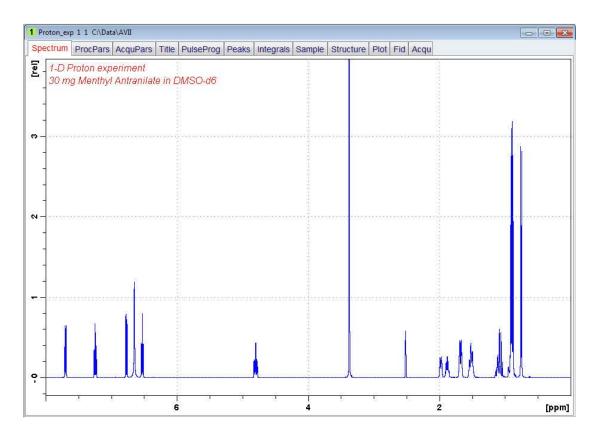
-	3	<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	Publish	<u>V</u> iew	<u>M</u> anage	0	
				Copy	€ P <u>rint</u> →	Plot La	yout 🎒	₽ <u>D</u> F →	📑 <u>E</u> -Mail	Mo <u>b</u> ile ▼

This will print the active window with the colors displayed in the TopSpin window.

5.6 1D Selective NOESY

5.6.1 Reference Spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



5.6.2 Selective Excitation Region Set Up

The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

• On the menu bar, click Acquire.

				A <u>n</u> alyse							
	-	Sampl <u>e</u> ▼	tock	V Tune →	& Sp <u>i</u> n →	Shim	n → <u>f</u> P <u>r</u> o	sol 🗢	<u>I∽</u> <u>G</u> ain ▼	Þ Go 🗢	More 42

• On the More button, click the drop-down arrow to see more options.

• In the list, select Setup Selective 1D Expts.

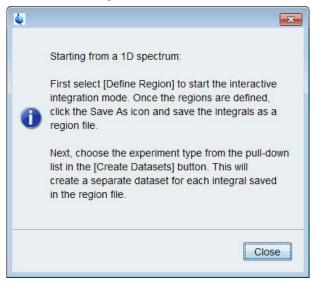
IconNMR Automation (icona)	
Setup Selective 1D Expts.	
TopSolids	
TopGuide (topguide)	
One- <u>Click Experiments</u>	
Shape <u>T</u> ool (stdisp)	
APSY (apsy)	
NMR Thermometer (nmrtemp))

The Workflow button bar changes for setting up the 1D selective experiment.

• On the Workflow button bar, click **1D Selective Experiment Setup**.

Start Acquire Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G M BACK_M	1DLSelective	Experiment	Setup	M Define <u>R</u> e	gions	🖹 Create <u>D</u> atasets v

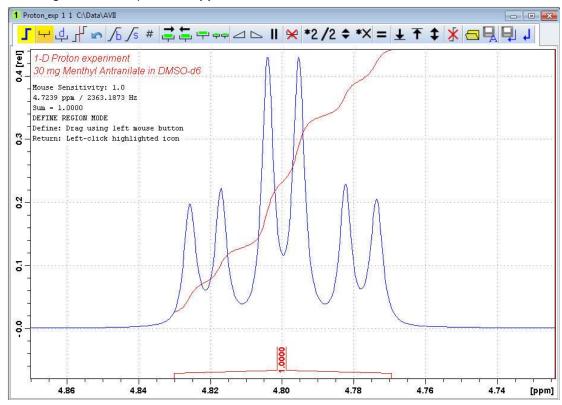
• In the message window, click Close.



There is no other function to this button then the instruction displayed above.

- Expand the peak at 4.8 ppm.
- On the Workflow button bar, click **Define Regions**.

Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew	<u>M</u> anage	0
G Back	1D Sel	lective Expe	riment Setup	👆 De	fine <u>R</u> egions	Create <u>D</u> atasets ▼



• Integrate the multiplet at 4.8 ppm.

If desired, other peaks can be integrated and a separate dataset will be created for each integral saved in the region file.

- • On the toolbar, click Save Region as.
- · In the list, select Save the Region to 'reg'.

Save Regions To 'intrng' Save Regions To 'reg' Export integration regions Export Regions To Relaxation Module and ret. Save & Show List

On the toolbar, click Return do NOT save regions! .



• In the message window, click No.



• On the Create Dataset button, click the drop-down arrow to see more options.

Start Acquire Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G M BACK_M	1D Selective	Experiment	Setup	Å Define <u>R</u> e	gions	⊯ ¢reate <u>D</u> atasets ⊽

- Selective gradient 1H Selective gradient COSY Selective gradient NOESY Selective gradient TOCSY Selective gradient ROESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective NOESY Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC 2D Band Selective HSQC
- In the list, select Selective gradient NOESY.

The default parameters are taken from the standard parameter set **SELNOGP**. The mixing time **D8** is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from **100 ms** to **800 ms**. If desired, the **Gaus1_180r.1000** pulse can be changed by clicking on the **Shape** button in the above window.

- Enter:
 - D8 = 0.450 NS = 32
- In the SELNOGP window, click Accept.

🖕 SELNOGP		X		
1D Selective	e Gradient NOESY			
Shape = Gaus	s1_180r.1000			
D 8 (sec)	0.450	mixing time		
NS	32			
first EXPNO	2			
Accept	Change Shape	Cancel		

The new dataset is created and all parameters are automatically set.

• In the sel1d window, click **OK** to start the acquisition.

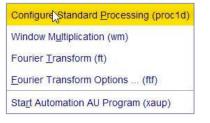
🤹 sel1d	•••
	1D Selective Gradient NOESY: SELNOGP
2	Dataset created in expno 2.
	total experiment time will be 4 min 25 sec
	OK: starts acquisition
	CANCEL: creates data sets only.
	OK Cancel
	OK Cancel

5.6.3 Processing

• On the menu bar, click **Process**.

🥑 <u>S</u> ta	t <u>A</u> cquire	Process	An	alyse	P <u>u</u> bli	sh	<u>V</u> iew	<u>M</u> anage	0			
	B Proc. S	pectrum v	Ad 🔶	ljust Pha	se 🔻	A Ca	alib. A <u>x</u> is	N Pick F	eaks ▼	ſ	Integrate 🗢	A <u>d</u> vanced ▼

- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select Configure Standard Processing.

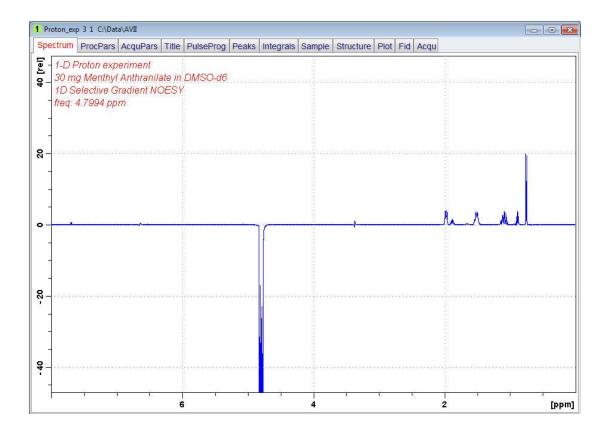


- · Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

• In the proc1d window, click **Execute**.

ress 'Execute' to process the curre ress 'Save' to just change the proc hanged options will be effective will	essi	ng options.	
ne-click 'Proc. Spectrum' button. Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)	V		
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp
Warn if processed data exist			

• Manually adjust the phase of the selective peak at **4.8 ppm** to show negative absorption to assure the correct phasing of the NOE peaks between **3 ppm** and **1 ppm**. Dependent on the field strength the peaks could be either positive or negative.

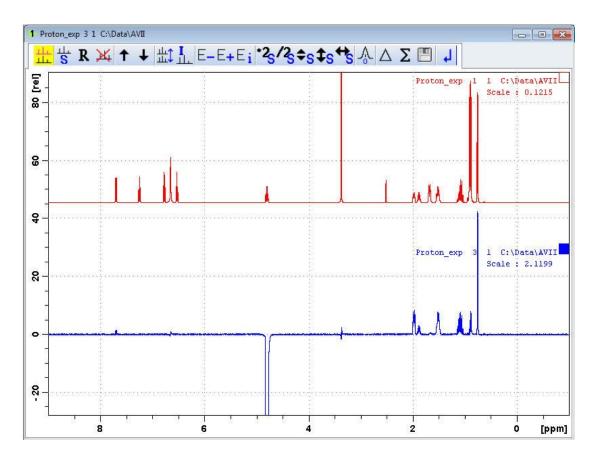


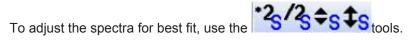
5.6.4 Plotting Two Spectra on the Same Page

· Display the selective TOCSY spectrum.



- On the toolbar, click Multiple display.
- Drag the Reference spectrum in to the spectral window.





• On the menu bar, click **Publish** and on the Workflow button bar, click **Print**.

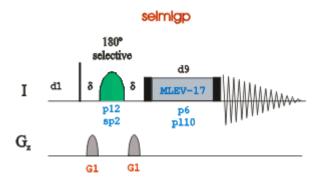
<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	Publish Vie	w <u>M</u> anag	le 🕜	
		Copy	€ P <u>r</u> int ▼	Plot Layout	🦂 P <u>D</u> F マ	E-Mail	Mo <u>b</u> ile ▼

This will print the active window with the colors displayed in the TopSpin window.

5.7 1D Selective Gradient TOCSY

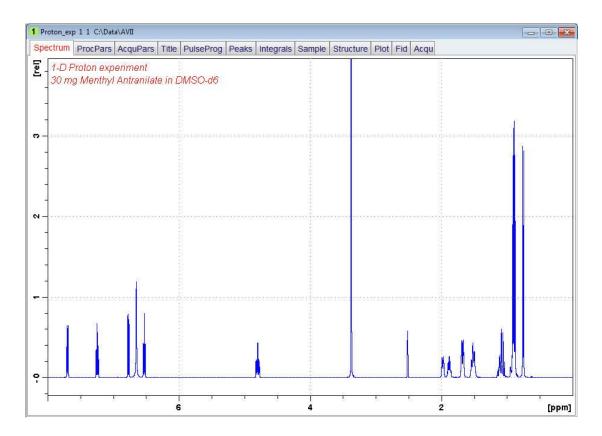
This experiment consist of three parts:

- Selective excitation of the selected resonance using the SPFGE block.
- **Mixing period** to achieve in phase polarization transfer to other spins. This is usually achieved by applying some isotropic mixing sequence like MLEV, WALTZ or DIPSI pulse trains. This in-phase transfer avoids possible cancellation when the coupling is poorly resolved.
- Proton detection as usual.



5.7.1 Reference Spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



5.7.2 Selective Excitation Region Set Up

The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

• On the menu bar, click Acquire.

Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew <u>N</u>	<u>l</u> anage 🕜			
💐 Sampl <u>e</u> 🔻	tock	V Tune →	ll Sp <u>i</u> n →	🖙 Shim 🗸	¶ P <u>r</u> osol ▼	<u>I∽ G</u> ain ▼	Þ Go 🗢	More 42

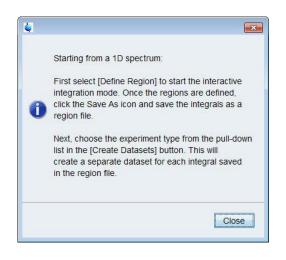
- On the More button, click the drop-down arrow to see more options.
- In the list, select Setup Selective 1D Expts.

IconNMR Automation (icona)
Setup Selective 1D Expts.
TopSolids
TopGuide (topguide)
One-Click Experiments
Shape Tool (stdisp)
APSY (apsy)
NMR Thermometer (nmrtemp)

The Workflow button bar changes for setting up the 1D selective experiment.

On the Workflow button bar, click 1D Selective Experiment Setup.

<u>Start Acquire Process</u>	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G M BACK_M	1DDSelective	Experiment	Setup	Å Define <u>R</u> e	gions	🖹 Create <u>D</u> atasets 🔻

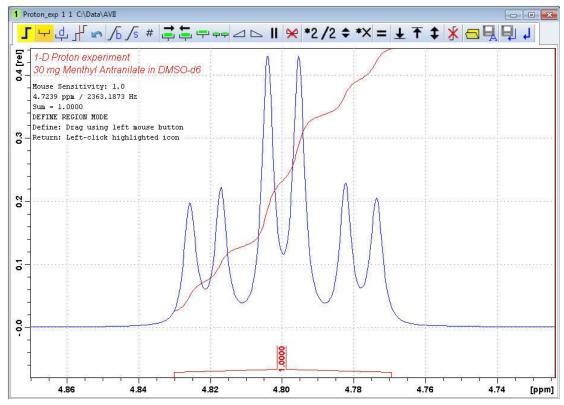


This button is only used for the instruction displayed above.

- In the message window, click **Close**.
- Expand the peak at 4.8 ppm.
- On the Workflow button bar, click **Define Regions**.

	Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
3 Back		1D Se	elective Expe	eriment Setup	b De	efine <u>R</u> egions	Create Datasets	-

• Integrate the multiplet at 4.8 ppm.



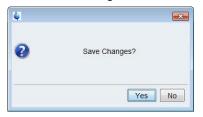
If desired, other peaks can be integrated and a separate dataset will be created for each integral saved in the region file.

- On the Integration toolbar, click **Save region as**.
- · In the list, select Save the Region to 'reg'.

Save R	egions To 'intrng'
Save R	egions To 'reg'
Export	integration regions
Export	Regions To Relaxation Module and .ret
Save &	Show List

On the toolbar, click Return do NOT save regions! .

• In the message window, click No.



• On the Create Dataset button, click the drop-down arrow to see more options.

Start Acquire Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
G M BACK_M	1D Selective	Experiment	Setup	Å Define <u>R</u> e	gions	🗟 Create Datasets 🗢

• In the list, select Selective gradient TOCSY.



The default parameters are taken from the standard parameter set **SELMLGP**. If desired, the **Gaus1_180r.1000** pulse can be changed by clicking on the **Shape** button in the above window. A mixing time of **0.06 s** to **0.08 s** is typically for the **TOCSY** experiment.

Enter:

D9 = **0.08** NS = **8** • In the SELMLGP window, click Accept.

SELMLGP 1D Selective	e Gradient TOCSY	×
Shape = Gaus	s1_180r.1000	
D 9 (sec)	0.080	mixing time
NS	8	
first EXPNO	2	
Accept	Change Shape	Cancel

The new dataset is created and all parameters are automatically set.

• In the sel1d window, click **OK** to start the acquisition.

	1D Selective Gradient TOCSY:
	SELMLGP
0	Dataset created in expno 2.
	total experiment time will be 1 min 14 sec
	OK: starts acquisition
	CANCEL: creates data sets only.
	OK Cancel

5.7.3 Processing

• On the menu bar, click Process.

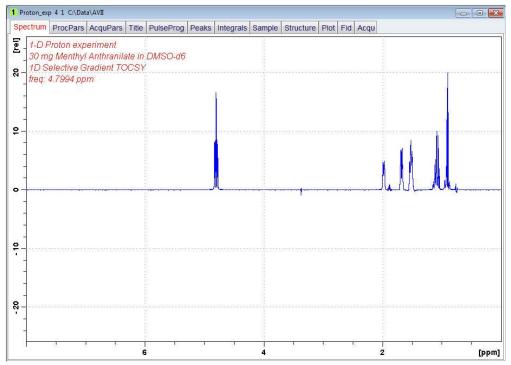


- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select Configure Standard Processing.



- Deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist
- In the proc1d window, click **Execute**.

Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective will one-click 'Proc. Spectrum' button.	cessi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)			
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp
Warn if processed data exist			



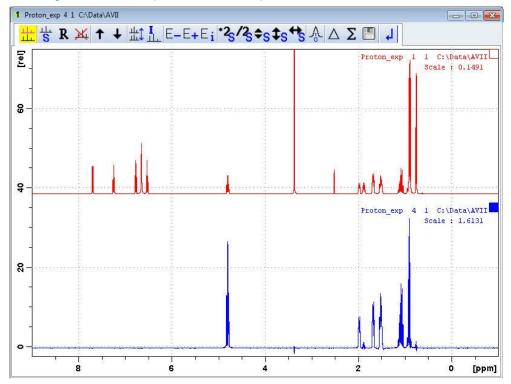
· Manually phase all peaks for positive absorption.

5.7.4 Plotting Two Spectra on the Same Page

- · Display the selective TOCSY spectrum.



On the toolbar, click Multiple display.
Drag the reference • Drag the reference spectrum into the spectral window.



To adjust the spectra for best fit, use the tools.

• On the menu bar, click **Publish** and on the Workflow button bar, click **Print**.

<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	Publish	<u>V</u> iew	<u>M</u> anage		
		Copy	P <u>rint</u> マ	Plot Layo	out 🦂	P <u>D</u> F ▼	📑 <u>E</u> -Mail	Mo <u>b</u> ile 🗢

Le

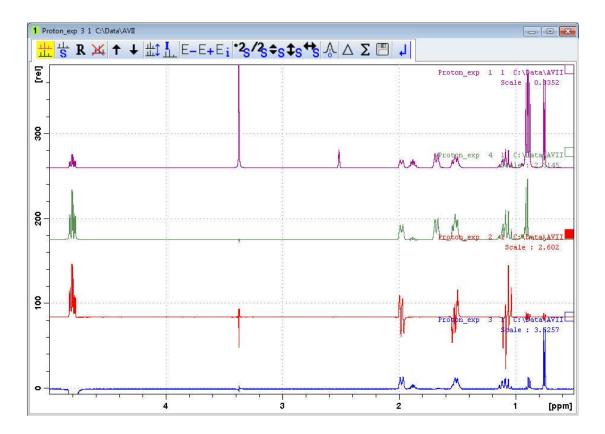
This will print the active window with the colors displayed in the TopSpin window.

5.7.5 Plotting All 4 Experiments on the Same Page

· Display the selective NOESY spectrum.



- · Drag the selective COSY spectrum into the spectral window
- · Drag the selective TOCSY spectrum into the spectral window
- Drag the Reference spectrum into the spectral window.



• On the menu bar, click Publish and on the Workflow button bar, click Print.

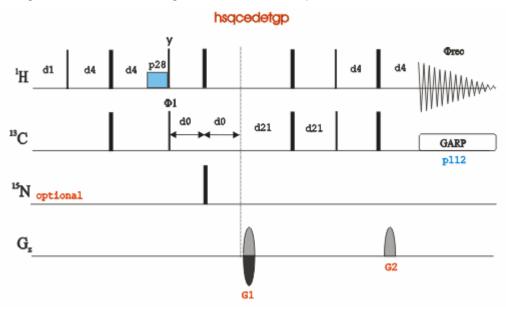
<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	Publish V	iew <u>M</u> anage	e 🕜	
		Copy	€ P <u>r</u> int ▼	Plot Layou	ut 🦂 P <u>D</u> F 🔻	🖾 <u>E</u> -Mail	Mo <u>b</u> ile ▼

6 2D Experiments using Shaped Pulses

6.1 2D Edited HSQC Experiment with Adiabatic Pulses

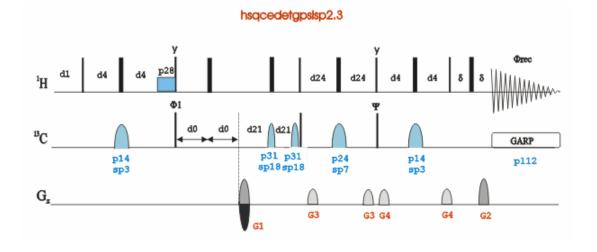
6.1.1 Introduction

The HSQC experiment is the method of choice for a very well resolved H,C correlation. However, in contrast to the HMQC this experiment uses 180^o pulses, which causes problems if the 180^o pulses become to long (e.g.TXI probes) and have to cover a very wide spectral range. This leads to phasing problems for high field instruments above 500 MHz. To work around this problem is to apply frequency-swept adiabatic 180^o pulses which can cover the large ¹³C spectral width.

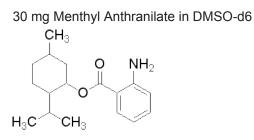


The figure below shows the regular edited HSQC sequence:

The edited HSQC sequence using shaped pulses for all 180° pulses on f2-channel with gradients in back-inept. For improvement of the phasing the pulse sequence using matched sweep adiabatic pulses **hsqcedetgpsisp2.3** is used in this chapter (see figure below). This pulse sequence is used in the recommended Bruker parameter set HSQCEDETGPSPSISP_ADIA. If desired the sequence **hsqcedetgpsisp2.4** can be used to suppress the COSY peaks.

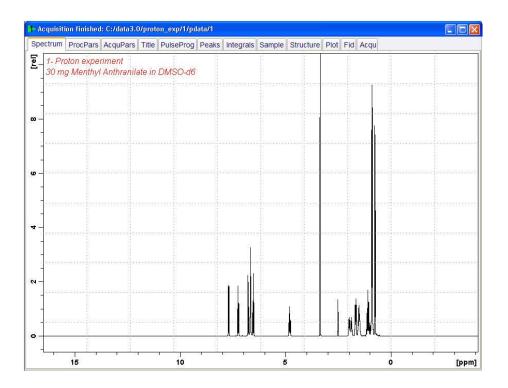


6.1.2 Sample



6.1.3 Reference spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.



The reference spectrum is necessary to adjust the spectral limits of the sweep width in the **F2** dimension and to use it for the projection. The HSQCEDETGPSP_ADIA parameter set has a default sweep width in the **F1** dimension of **165 ppm**, If a Carbon DEPT135 or DEPT45 spectrum of the same sample is available, the F1 sweep width can be further reduced using the **setlimit** AU-program.

6.1.4 Setting up the HSQC experiment

The steps below assume that the sample remains in the magnet after observing the proton spectrum.

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.

Start)	<u>A</u> cquire <u>P</u> rocess	A <u>n</u> alyse P <u>u</u> blish	<u>V</u> iew <u>M</u> anage	0
/	Create Datase	<mark>t</mark> [Find Dataset	Open <u>D</u> ataset	aste Dataset 🔡 Read Pars.

• In the New Dataset window, enter or select:

NAME = shape_hsqc_exp EXPNO = 1 PROCNO = 1

Experiment select HSQCEDETGPSP_ADIA

Set Solvent select DMSO

🖕 New	-		3
Prepare for a new experiment initializing its NMR paramete For multi-receiver experiment Please define the number of	rs according to	the selected experiment type. sets are created.	
NAME	shape_hs	qc_exp	
EXPNO	1		
PROCNO	1		
O Use current parameters			
Experiment HSQCEDET	GPSP_ADIA	Select	
 Options 			
Set solvent:		DMSO -	
Execute "getprosol"	6		
Keep parameters:		P 1, O1, PLW 1 Change	
DIR		C:\Data 👻	
🖾 Show new dataset i	n new window		
Receivers (1,2,16)		1	
	nyl Antranilate in		
	OK	Cancel More Info Help	

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click OK.
- On the menu bar, click Aquire.

<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> a	nage	0			
	Sampl <u>e</u> 🗢	tock	V Tune 🗸	l Sp <u>i</u> n ◄	🖙 Shim 🚽	<mark>∫∫[↓] P<u>r</u>o</mark>	sol 🗢	<u> G</u> ain ▼	De 🗕	M <u>o</u> re ▼

• To tune the probe, click Tune.



The last step is necessary to tune the X-channel which is in this case ¹³C. This performs an **atma** (automatic tuning) and requires a probe equipped with an automatic tuning module. Other options can be selected by clicking on the down arrow inside the **Tune** button.

· Click Spin and select Turn sample rotation off.

2D experiments should be run non-spinning.

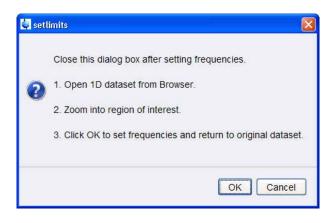
- To autoshim the sample with TopShim and best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click **Prosol**.

6.1.4.1 Limit Setting

· On the Workflow button bar, click SetLimits.

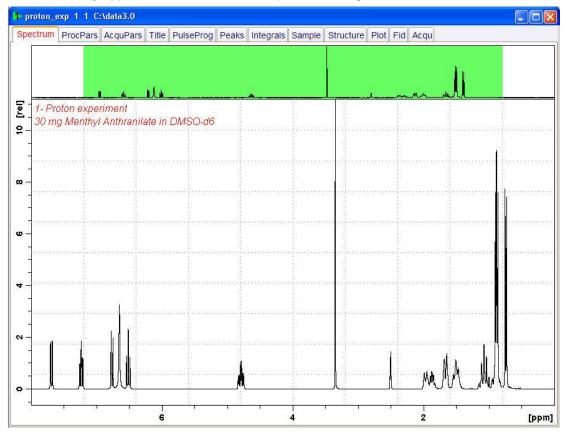
 Start
 Acquire
 Process
 Analyse
 Publish
 View
 Manage
 Manage

 Image
 Image



- To open the 1D Proton spectrum, right-click on the dataset name in the browser window (e.g. proton_exp 1) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.

The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.



• In the setlimits message window, click OK to assign the new limit.

۵	
0	1H spectral limits copied for F1 and F2 dimensions. SW: 7.9997 ppm O1P: 4.024 ppm
	Close

• In the message window, click Close.

The display changes back to the 2D dataset.

6.1.4.2 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

6.1.4.3 Processing

The steps below will guide you through the processing and the manual phase correction on the edited HSQC experiment.

• On the menu bar, click **Process**.

<u>S</u> tart	<u>A</u> cquire	Process	Analyse	P <u>u</u> blis	⊧h <u>V</u> iew	<u>M</u> anage	0		
	<mark>♪ Proc</mark> . Spectrum ▼		Adjust Ph	nase 🔻 🗍	A Calib. A <u>x</u> is	t Pick F	eaks ▼	∫ Integrate →	Advanced 🕶

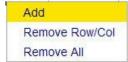
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Process F2+F1 (xfb), or at the command prompt, type xfb.

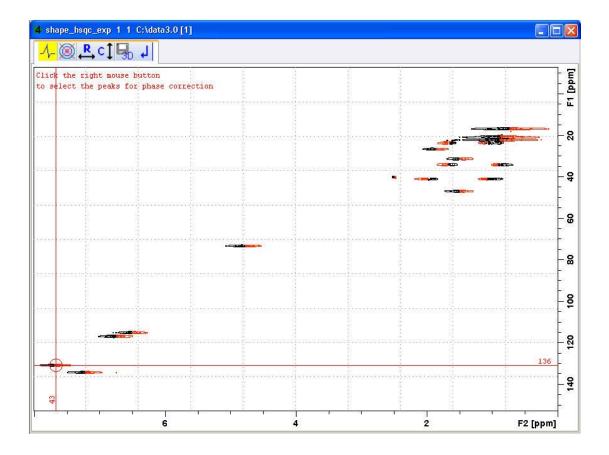
Configure Standard Processing (proc2d)
Process F2+F1 Axis (xfb)
Process Only F2 Axis (xf2)
Process Only F1 Axis (xf1)
Symmetrize Spectrum (sym)
Start Automation AU Program (xaup)

• On the Workflow button bar, click Adjust Phase.

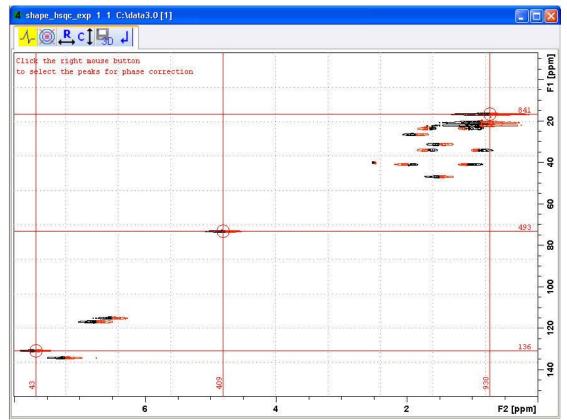
<u>Start</u> <u>A</u> cquire Pro	cess A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
Λ Pro <u>c</u> . Spectrum ▼	🔨 Adjust Phase 🗸	👌 Calil	o. A <u>x</u> is ▼	tick Pe	aks 🗢	∫ Integrate ▼ Advanced ▼

- Select the peak at 7.7 ppm/130.9 ppm.
- Right-click and on the shortcut menu, select Add.



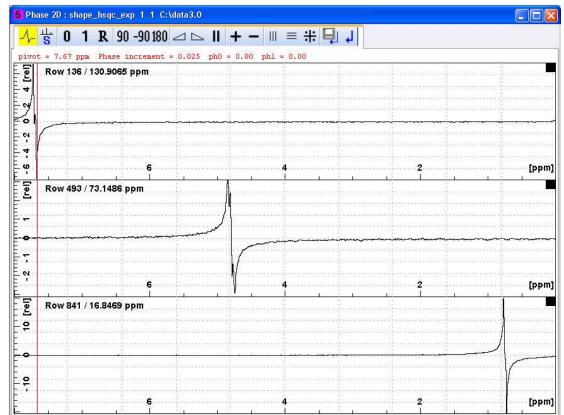


• Repeat the last step for the peaks at 4.8 ppm/73.2 ppm and 0.76 ppm/16.8 ppm.



2D Experiments using Shaped Pulses

Click Start the phase correction on rows.



• Adjust the **0** and 1st order phase.

<u>∕_ @ _R cî </u> ↓

Ph	e 2D : shape_hsqc_exp 1 1 C:\data3.0	
A	$\frac{1}{5}$ 0 1 R 90 -90 180 \bigtriangleup \bowtie II + - III = $\#$ \square	
	= 7.67 ppm Phase increment = 0.025 ph0 = 85.77 ph1 = 6.60	
E	Row 136 / 130.9065 ppm	
4		
0	ana faanaanaa dyna	
0		
20	a na frantsa na higa na mana na frantsa na mana na frantsa na mana higa na mana na frantsa na higa na mana na f	
4		
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[le]	Row 493 / 73.1486 ppm	
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		en e
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1,535		
•		
9		
	6	[ppi
8		- Lbbi

• To store the phase values, click **Return and Save phased spectrum**.

- Click Start the phase correction on columns.
- Adjust the **0** and 1st order phase.

2D Experiments using Shaped Pulses

5 Phase 2D : shape	_hsqc_exp 1 1 (C:\data3.0						
<mark>-√-</mark> ╬ 0 1	R 90 -90 18		+ - III	≡ # 📮	L			
pivot = 130.91 pp		ent = 0.025 p	n0 = -5.04 pl	al = 7.70				
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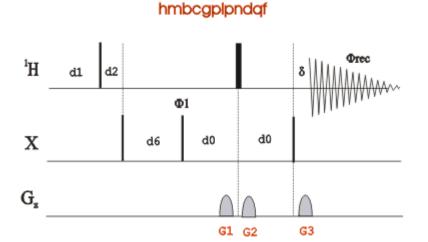
- To store the phase values, click **Return and Save phased spectrum**.
- To exit the phase window, click the **Return** button. <mark>↗ இ ヱ c ţ 啺 ℷ</mark>

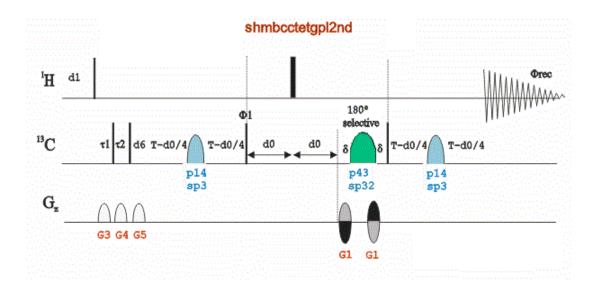
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2D- editeo 30 mg Me	d HSQC experir enthyl Anthranil	nent with adibati ate in DMSO-d6	c pulses exper	iment			Ē
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6.2 2D Selective HMBC experiment

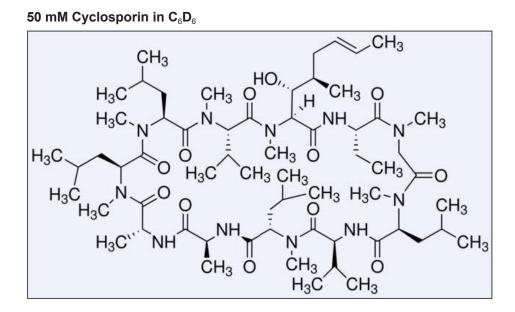
6.2.1 Introduction

The **Semi-selective 2D HMBC experiment** is a simple modification of the 2D HMBC pulse sequence shown in the first figure below in which one of the two carbon 90° pulses is applied selectively on a specified region, see second figure below. The main purpose is to achieve better resolution in the indirect dimension and therefore is recommended when high overlapped carbon spectra precludes an easy resonance assignment. There are three ways to set this experiment up. Each one will be covered separately below. Before running any one of these methods, you need at least a **Proton** or either a **2D HMBC** or a **1D Carbon spectrum** if possible.





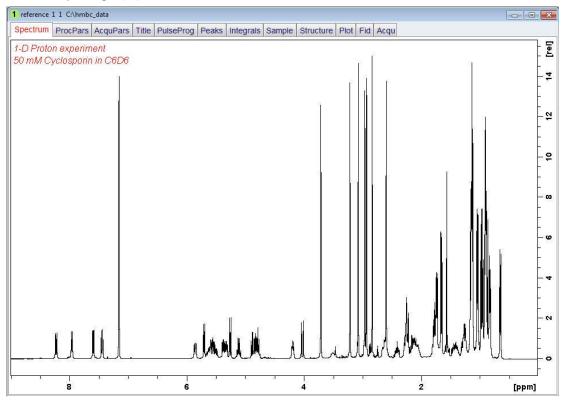
6.2.2 Sample



6.2.3 Preparation Experiments

6.2.3.1 1D Proton Experiment

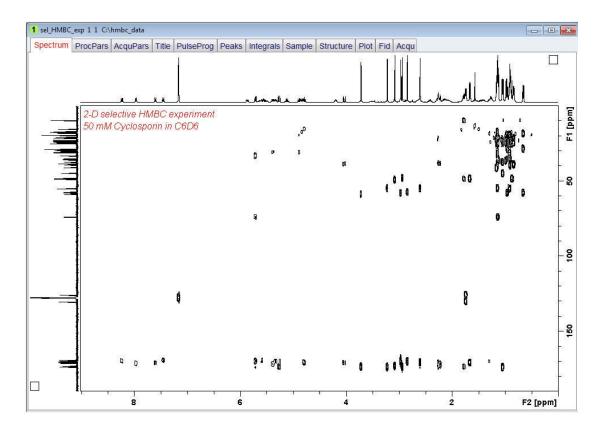
Run a **1D Proton** spectrum of Cyclosporin, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter *1D Proton Experiment*, *Experiment Setup* through *Processing* using C_6D_6 as the solvent.



6.2.3.2 2D HMBC Experiment

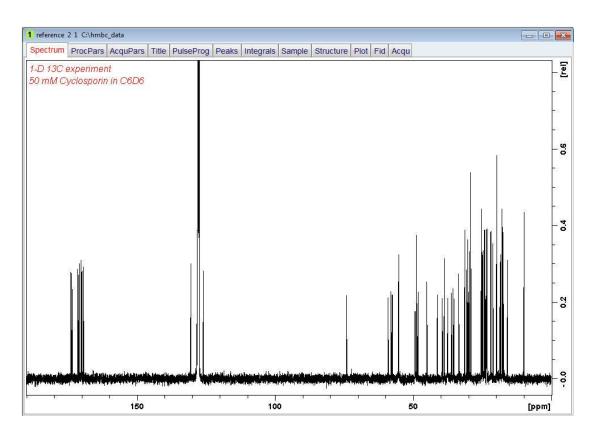
The steps below assume that the sample remains in the magnet after observing the proton spectrum.

Run a **2D HMBC** experiment of Cyclosporin following the instructions in TopSpin Guide Book *Basic NMR experiments*, chapter 2D HMBC experiment using C_6D_6 as the solvent.



6.2.3.3 1D Proton Decoupled Carbon Experiment

Run a **1D Carbon** spectrum of **Cyclosporin**, following the instructions the TopSpin Guide Book *Basic NMR Experiments*, Chapter *1D Carbon experiment*, Paragraph *Experiment Setup* through *Processing* using C_6D_6 as the solvent.

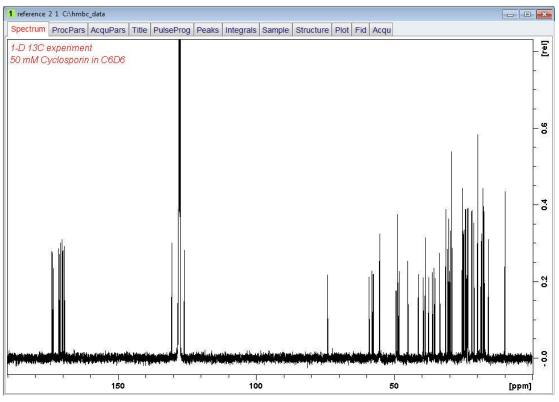


The carbon spectrum is necessary for method 1 but not for method 2, if the sample concentration is to low to get a spectrum in a reasonable time frame.

6.2.3.4 Method 1 for Setting Up the Selective HMBC Experiment

This method requires a 1D Proton decoupled ¹³C spectrum, if it can be obtained with a reasonable number of scans.

• Display the carbon spectrum as observed in the last chapter 1D Proton Decoupled Carbon Experiment [▶ 112].



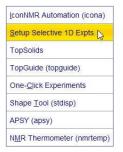
6.2.3.5 Selective Excitation Region Setup

The selective pulse region is set up same way as the 1D selective experiments using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

• On the menu bar, click Acquire.

Start Acquire									
💐 Sampl <u>e</u> 🗢	teck	V Tune →	& Sp <u>i</u> n →	Shim .	✓ <u>∫</u> Pro	osol 🗢	<u> G</u> ain ▼	Þ Go 🗢	More 42

- On the More button, click the drop-down arrow to see more options.
- In the list, select Setup Selective 1D Expts.

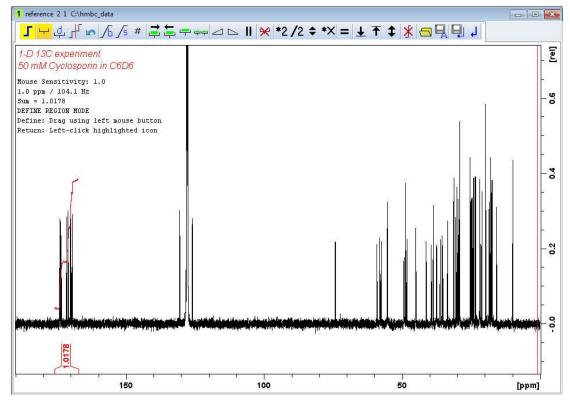


The Aquire Workflow button bar changes for setting up the 1D selective experiment.

• On the Workflow button bar, click **Define Regions**.

Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0
Back	1D Se	elective Expe	eriment Setu	De 🔥 De	fine <u>R</u> egions	🛃 Create <u>D</u> atasets 🗢

• Integrate the region from 175 ppm to 167 ppm.



- Click Save region as.
 And in the list, save Region to reg.
 Save Regions To 'intrng'
 Save Regions To 'reg'
 Export Integration regions
 Export Regions To Relaxation Module and .ret.
 Save & Show List
- To exit from the integration mode, click Return do not save regions!.



• In the message window, click **No**.



• On the **Create Dataset** button, click the **drop-down** arrow to see more options.

<u>Start</u> <u>Acquire</u>	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0
Back	1D Se	elective Expe	riment Setur	De 🔬 De	fine <u>R</u> egions	📴 Create Datasets 🏡

• In the list, select 2D Selective HMBC.

Selective gradient 1H
Selective gradient COSY
Selective gradient NOESY
Selective gradient TOCSY
Selective gradient ROESY
1H Homonuclear Decoupling
Selective 1H
Selective COSY
Selective NOESY
Selective TOCSY
Selective ROESY
Mult. Solvent Suppr./presat
Mult. Solvent Suppr./WET
2D Selective HMBC

• In the SHMBCCTETGPL2ND window, click Accept.

Shape = Q3.100	00	
CNST 13 (Hz)	8.000	J(XH) long range
NS	4	
EXPNO	2	

All parameters are automatically calculated and stored as an increment in the next free experiment number of the dataset.

• In the sel1d window, click **OK** to start the acquisition.

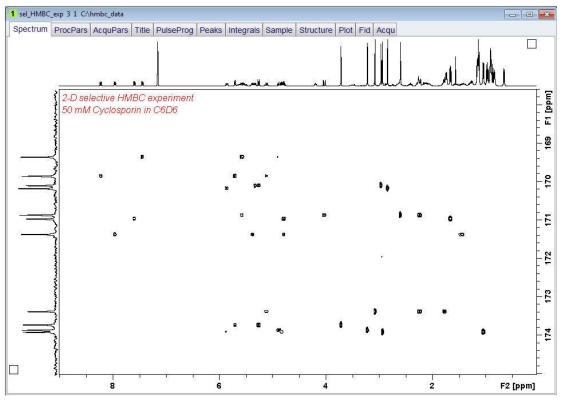
sel1d 🤤	8
	Selective 2D HMBC: SHMBCCTETGPL2ND
?	Dataset created in expno 3.
	total experiment time will be 16 min 37 sec
	OK: starts acquisition
	CANCEL: creates data sets only.
	OK Cancel
10	

The acquisition starts momentarily.

6.2.3.6 Processing

The pulse program **shmbcctetgpl2nd** is a phase sensitive program. However the data should be processed in magnitude mode. Do not use the **Proc. Spectrum flow** button, rather follow the steps below for the processing.

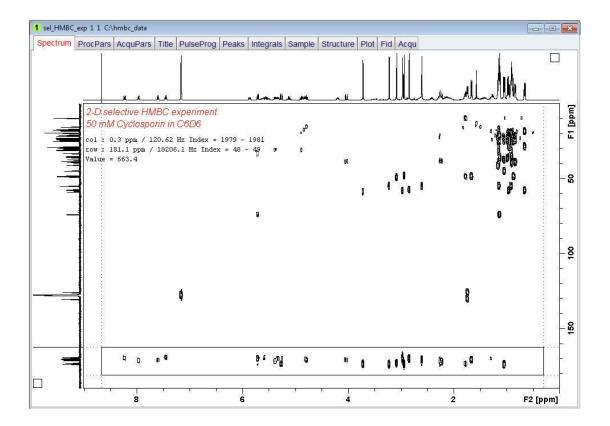
- At the command prompt, type **xfb** to process the data in both dimensions.
- At the command prompt, type xf2m to calculate magnitude spectrum in F2.

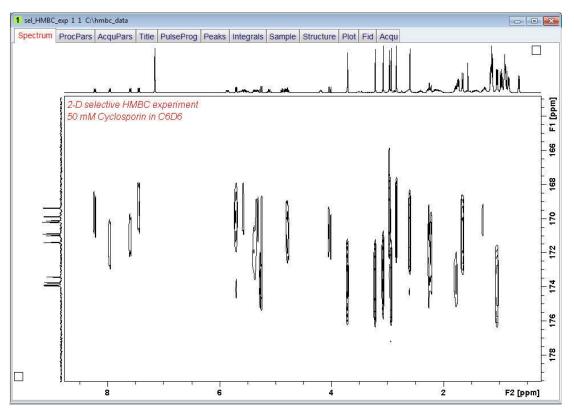


6.2.3.7 Method 2 for Setting Up the Selective HMBC Experiment

This method is using a regular **2D HMBC** acquired spectrum for setting up the **2D selective HMBC** experiment. In this example, the **1D Proton decoupled carbon** spectrum is only used to display the F1 projection and is not necessary to obtain the **2D selective HMBC**.

- Display the HMBC spectrum as observed in chapter 2D HMBC Experiment [> 111].
- Expand the region including all cross peaks (e.g. 163 ppm to 179 ppm).





• On the menu bar, click Acquire.

Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>I</u>	<u>M</u> anage	0			
💐 Sampl <u>e</u> 🔻	tock	V Tune ◄	l Sp <u>i</u> n マ	Shim -	Pros	sol 🗢 🛛	<u>∽ G</u> ain ▼	Þ Go 🗢	More 💫

- On the More button, click the drop-down arrow to see more options.
- In the list, click the arrow in the selection **Setup Selective 2D Excpts**. and on the shortcut menu, select **Band Selective 2D HMBC**.

IconNMR Automation (iconnmr)	Band Selective 2D HMBC
Setup Selective 2D Expts. 🔓 👂	Band Selective 2D HSQC
TopSolids	
TopGuide (topguide)	
One- <u>Click Experiments</u>	
Shape Tool (stdisp)	
APSY (apsy)	
NMR Thermometer (nmrtemp)	

• In the SHMBCCTETGPL2ND window, click Accept.

		×				
2D selective H	IMBC					
Shape = Q3.100	00					
CNST 13 (Hz)	8.000	J(XH) long range				
NS	4					
EXPNO	2					
Acce	ept Change	e Shape Cancel				

All parameters are automatically calculated and stored as an increment in next free experiment number of the dataset.

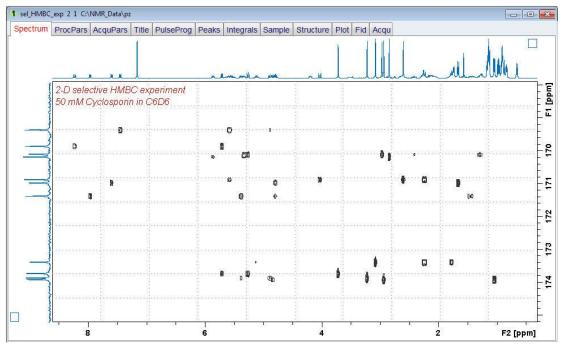
🤹 selhmbc 🗾
Acquisition parameters for 2D selHMBC: SHMBCCTETGPL2ND Dataset created in EXPNO: 2 SW(F1) = 16.8174 ppm / 1691.28 Hz O2 = 171.5711 ppm / 17252.75 Hz TD(F1) = 128 AQ(F1) = 0.0378 sec shape pulse (SPNAM 32): Q3.1000 shape pulse duration (P 43): 2822.72 us power level (SPW 32): 0.166779 W Experiment time: 16 min 8 sec OK: starts acquisition CANCEL: creates data sets only.
OK

• To start the acquisition, click **OK**.

6.2.3.8 Processing

The pulse program **shmbcctetgpl2nd** is a phase sensitive program however the data should be processed in magnitude mode. Do not use the **Proc. Spectrum flow** button, rather follow the steps below for the processing.

- At the command prompt, type **xfb** to process the data in both dimensions.
- At the command prompt, type **xf2m** to calculate magnitude spectrum in F2.



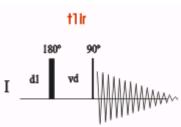
The **Selective HMBC** has a significantly higher ¹³C resolution compared to the standard **HMBC** experiment.

7 T1 Experiment

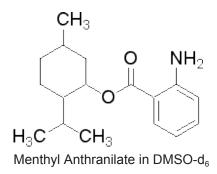
7.1 Introduction

The inversion-recovery experiment allows to measure longitudinal or spin-lattice T1 relaxation times of any nucleus.

The basic pulse sequence consists of a 180° pulse that inverts the magnetization to the -z axis. During the following delay, relaxation along the longitudial plane takes place. Magnetization comes back to the original equilibrium z-magnetization. A 90° pulse creates transverse magnetization. The experiment is repeated for a series of delay values taken from a variable delay list. A 1D spectrum is obtained for each value of vd and stored in a 2D dataset. The relaxation time d1 must be set to 5*T1. A rough estimation of the T1 value can be calculated from the null-point value by using T1=tnull/ln(2).



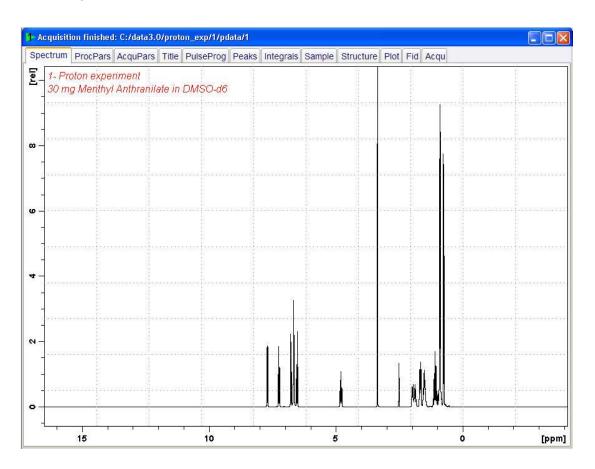
7.2 Sample



7.3 **Proton Inversion-Recovery T1 Experiment**

7.3.1 Preparation Experiment

Run a 1D Proton spectrum, following the instructions in the *TopSpin Guide Book Basic NMR Experiments*, Chapter 1D Proton experiment, Paragraph Experiment Setup through Processing.



The reference spectrum is necessary to adjust the spectral limits of the sweep width to gain more data points.

7.3.2 Setting up the T1 Experiment

The steps below assume that the sample remains in the magnet after observing the proton spectrum.

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.

```
      Start
      Acquire
      Process
      Analyse
      Publish
      View
      Manage
      Manage

      Create
      Dataset
      Dataset
      Open
      Dataset
      Paste
      Dataset
      Read
      Pars.
```

In the New Dataset window, enter or select:
 NAME = t1_exp
 EXPNO = 1

PROCNO = 1 Experiment: select PROTONT1 Set Solvent: select DMSO

Prepare for a new experimer initializing its NMR parameter For multi-receiver experimen Please define the number of	s according to t ts several datas	he selected experiment type. ets are created.							
NAME	t1_exp								
EXPNO	1								
PROCNO	1								
○ Use current parameters									
Experiment PROTONT1		Select							
 Options 									
Set solvent:		DMSO -							
Execute "getprosol"									
Keep parameters:		P 1, O1, PLW 1 Change							
DIR		C:\Data							
🕅 Show new dataset ir	new window								
Receivers (1,2,16)		1							
Proton T1 experiment 30 mg Menthyl Antranilate in DMSO-d6									
	OK	OK Cancel More Info Help							

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

×

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click **OK**.
- On the menu bar, click Acquire.

<u>S</u> tart	Acquire F	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> a	inage	2		
	💐 Sampl <u>e</u> 🔻	the Lock	¥ Tune ◄	& Sp <u>i</u> n →	Shim -	Pro	osol 🗢 🛛 🚾 🤆	ain 🚽 Þ Go	▼ M <u>o</u> re ▼

For the following steps, use the Workflow button bar.

- To tune the probe, click **Tune**.
- · Click Spin and select Sample rotation off.

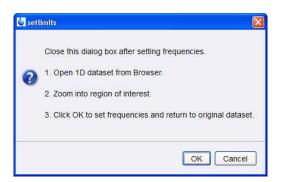
T1 experiments should be run non-spinning.

- To autoshim the sample with TopShim and best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.

7.3.3 Limit Setting

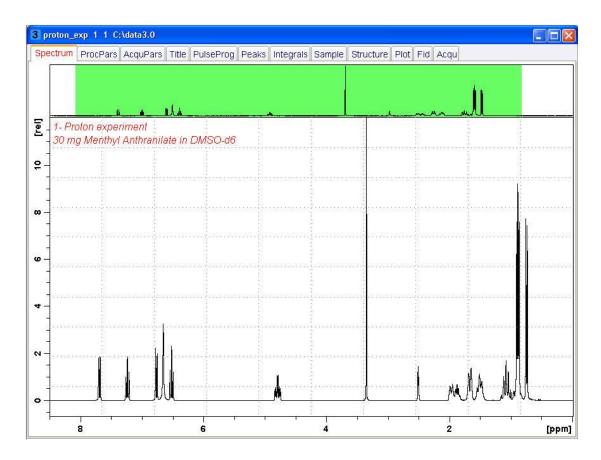
• On the Workflow button bar, click SetLimits.



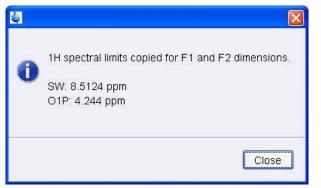


- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select Display or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window.
- Expand the spectrum to display all peaks, leaving about **0.5 ppm** of baseline on either side of the spectrum.

The solvent peak may be excluded if it falls outside of the region of interest.



· Click OK in the setlimits message window to assign the new limit.



• In the message window, click Close.

The display changes back to the 2D dataset.

- In the Dataset window, select the AcquPars tab.
- · Click Show pulse program parameters.
- Make the following changes:

- D1 = 15

- VDLIST = t1delay

VDLIST Tridelay Ex Variable delay	VDLIST	t1delay	Uariable delay l
-----------------------------------	--------	---------	------------------

• Click Edit variable delay list right of the VDLIST name box.

JC S

12

File	e <mark>E</mark> dit Searc	:h
1	0.01	^
2	0.050	
3	0.100	
4	0.25	
5	0.500	
6	1	
7	2	
8	4	
9	8	
10	15	
11		~

- Enter the variable delay values as shown in the figure above.
- Click File and Save.
- Click File and Close.
- In the Dataset window, select the Spectrum tab.

7.3.4 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

7.3.5 Processing

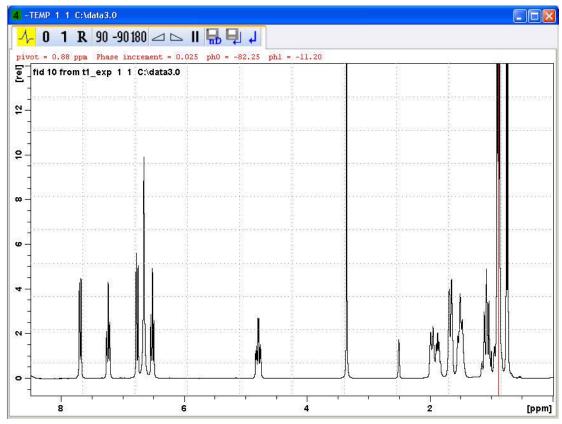
• On the menu bar, click Process.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
Λ Pro <u>c</u> . S	pectrum v	Adjust P	hase 🗢 📝	Calib. A <u>x</u> is 🗟	• 👯 P	ick P <u>e</u> aks →	∫ <u>I</u> ntegrate →	A <u>d</u> vanced ▼

- At the command prompt, type **rser 10**.
- At the command prompt, type ef.
- On the Workflow button bar, click Adjust Phase.

<u>S</u> tart <u>A</u> cquire	Process Analyse	P <u>u</u> blish <u>V</u> iew	<u>M</u> anage 🕜	
A Pro <u>c</u> . Spectr	um 🗢 🔼 Adjust Phase	Calib. A <u>x</u> is 🛪	✓ Nick Peaks マ	∫ Integrate ▼ A <u>d</u> vanced ▼

• Adjust the phase manually.

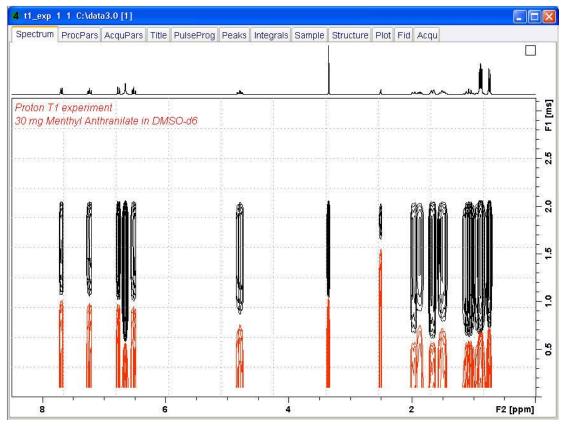


- On the Adjust Phase toolbar, click Save for nD spectrum.
- On the toolbar, click Return, do NOT save phased spectrum.

The spectrum will go back to the un-phased view since the phase correction values were stored only for the 2D spectrum.

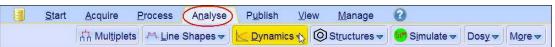


- On the toolbar, click Last 2D data to go back to the 2-D spectrum display.
- At the command prompt, type xf2 to process only the F2 axis.

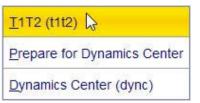


7.3.6 T1 Calculation

• On the menu bar, click Analyze.



- On the **Dynamics** button, click the drop-down arrow to see more options.
- In the list, select T1/T2 Module.



The flow buttons change to determine the T1 / T2 relaxation times:

T1 Experiment

<u>S</u> tart <u>A</u> cquire	Process Analyse Publish	<u>V</u> iew <u>M</u> anage	0
G Back	🔓 Eid 🕼 Peaks/Ranges 🖾 Relax	ation Fitting	Seculation Report

While executing the steps below, message windows will be displayed. Please read each message thoroughly and follow the instructions.

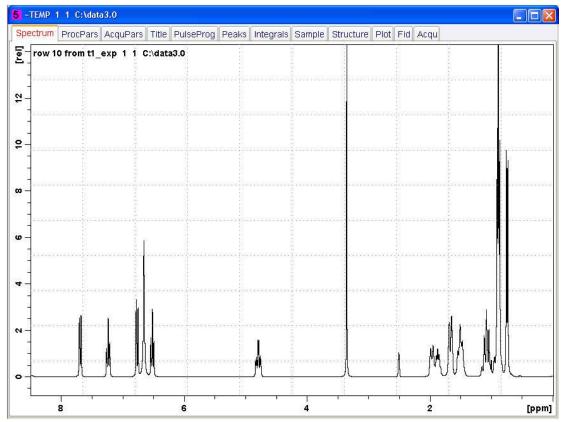
- On the Workflow button bar, click Fid.
- In the Extract a row from 2d data window, click **Spectrum**.

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

• Enter Slice Number = 10.

4		X
Spectrum slice must be extracted Fri This Spectrum should correspond to All further data preparation will be do Slice Number =	an experiment with one in respect to this	the maximum or minimum delay time.

• In the message window, click **OK**.

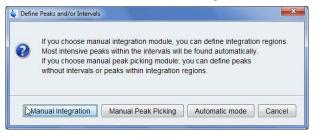


T1 Experiment

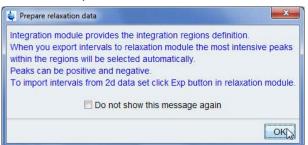
• On the Workflow button bar, click **Peaks/Ranges**.

<u>S</u> tart	<u>A</u> cquire <u>P</u> roc	ess Analyse	P <u>u</u> blish \	/iew	<u>M</u> anage	0	
G Back	<u>₩ </u> <u>F</u> id	A Peaks/Ranges	Relaxatio	on 📐	Fitting	Calculation	Report

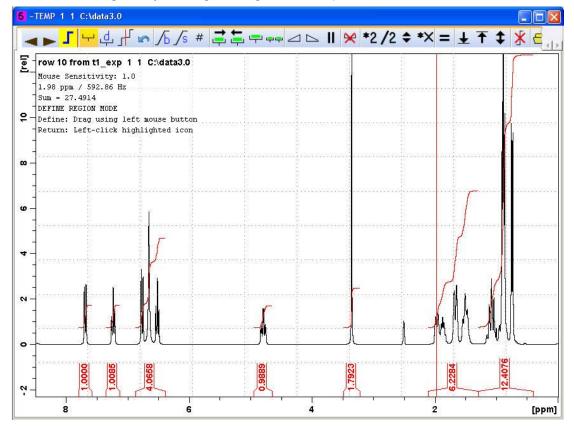
• In the Define Peaks and/or Integrals window, click Manual Integration.



• In the Prepare relaxation data window, click OK.



· Define the regions by drawing an integral over the peaks of interest.



- On the Integration toolbar, click **Save region as**.
- In the list, select Export Region To Relaxation Module.

Save	Regions To 'intrng'
Save	Regions To 'reg'
Ехро	rt integration regions
Expo	rt Regions To Relaxation Module and .ret
Save	& Show List

• On the Workflow button bar, select Relaxation.

<u>S</u> tart	<u>A</u> cquire <u>P</u> rocess	Analyse	P <u>u</u> blish <u>\</u>	<u>∕</u> iew <u>M</u> an	age 🕜
G Back	<u>₩ E</u> id <u>& P</u> e	aks/Ranges	Relaxation	Fitting	<u>Calculation</u> <u>Report</u>

• In the Relaxation parameters window, click OK.

🖕 Relaxation	parameter	5	X			
General P	arameters	÷	Ì			
10	FID # f	or phase determir	nation			
1000.0	Left lim	mit for baseline correction				
-1000.0	Right li	mit for baseline co	orrection			
5	Numbe	r of <mark>drift</mark> points				
1.0E-5	Conver	rgence limit				
16	r o <mark>f points</mark>					
1	First sli	ice				
1	Slice in	crement				
1.0	Peak s	ensitivity				
Fitting Function						
uxnmrt1 - Function Type						
1		Number of components				
vdlist	*	List file name				
0.001		Increment (auto)				
pd	•	to pick data points				
Iteration co	ontrol para	ameters				
	Guesse	s j	Reset			
Additional	Paramete	rs				
10000.0	(GAMMA(Hz/G)				
10.0	1	LITDEL(msec)				
100.0	ł	BIGDEL(msec)				
1.0	(GRADIEN(G/cm)				
	OK	Apply	ancel			

• On the Workflow button bar, select Fitting.

<u>S</u> tart	<u>A</u> cquire <u>F</u>	Process Anal	yse P <u>u</u> blish	<u>V</u> iew <u>M</u>	anage 🕜	
G Back	<u>₩~ </u> <u>F</u> id	I <u>↓</u> <u>P</u> eaks/Ran	ges 🛃 Relaxa	tion 🔽 Fi <u>ttin</u>	g 📀 <u>C</u> alculation	Report

• In the message window, click Close.

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

• In the Relaxation parameters window, click **OK**.

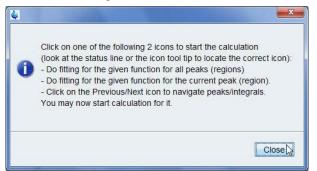
🖕 Relaxation p	parameters 🛛 🕅	J			
10	FID # for phase determination				
1000.0	Left limit for baseline correction				
-1000.0	Right limit for baseline correction				
5	Number of drift points				
1.0E-5	Convergence limit				
10	Number of points	Number of points			
1	First slice				
1	Slice increment				
1.0	Peak sensitivity				
uxnmrt1	 Function Type 				
1	Number of components				
vdlist	 List file name 				
0.001	Increment (auto)				
pd	✓ to pick data points				
	Guesses Reset				
10000.0	GAMMA(Hz/G)				
10.0 LITDEL(msec)					
100.0	00.0 BIGDEL(msec)				
1.0	GRADIEN(G/cm)				
	OK Apply Cancel				

5 Relaxation ForPZ 42 1 C:	Data	
<mark>≫ > as</mark> – + +	🗄 🛿 🔢 🔄 🔄 🔛 🧃 🚺	
Fitting type Intensity Area 	T1 I[[]=I[0]+P*exp(-t/T1) Region 1 from 7.835 to 7.629 ppm	0.1 0 [*1 e9]
Current Integral	o	-
Brief Report Data preparation is dor	o 0	- - - - - -
	o	-
	o.	_ 0; - - -
	o o	- - - - -
	0 0 0	-10.
4 III +	-0 1 2 3 4	

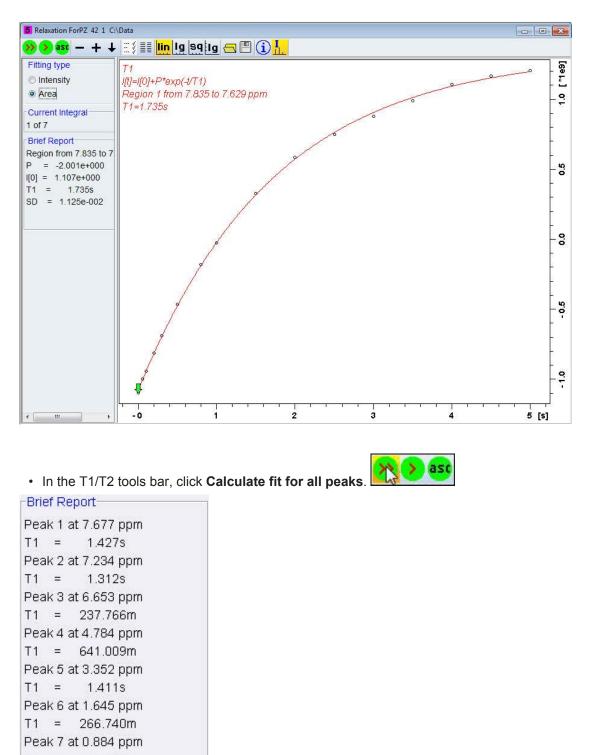
• On the Workflow button bar, select Calculation.

<u>S</u> tart	<u>A</u> cquire	<u>P</u> rocess	Analyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> a	inage 🕜	
G Back	//// <u>E</u> i	d <u> </u>	aks/Ranges	Relaxation			Report

• In the message window, click Close.



• Select Area for fitting type.



T1 = 413.572m

T1 Experiment

• On the Workflow button bar, select **Report**.

<u>S</u> tart	<u>A</u> cquire <u>P</u>	rocess Analyse	P <u>u</u> blish <u>V</u>	iew <u>M</u> an	age 🕜	
G Back		<u> </u>	Relaxation	Fitting	Calculation	E Report

Fit	ting report		
File	Edit Search	1	
1	Dataset :		
2	C:/data3.0/	t1 exp/1/pdata/1	
3	INTENSITY fi		
4	I[t]=I[0]+P	*exp(-t/T1)	
5		2	
6	10 points fo	Peak 1, Peak Point at 7.677 ppm	
7	Results	Comp. 1	
8			
9	I[0] = 9.	389e-001	
10	P = -1	.856e+000	
11	T1 =	1.427s	
12	SD = 8.	522e-003	
13			
14	tau p	m integral intensity	
15			
16	10.000m	7.677 -1.5504e+009 -5.6827e+007	
17	50.000m	7.677 -1.4565e+009 -5.3222e+007	
18	100.000m	7.677 -1.3444e+009 -4.8986e+007	
19	250.000m	7.677 -1.0229e+009 -3.7122e+007	
20	500.000m	7.677 -5.6068e+008 -2.0265e+007	
21	1.000s	7.676 1.4819e+008 4.9657e+006	
22	2.000s	7.677 9.9172e+008 3.4448e+007	
23	4.000s	7.677 1.6315e+009 5.6781e+007	
24	8.000s	7.677 1.8712e+009 6.4937e+007	
25	15.000s	7.677 1.9011e+009 6.5935e+007	
26			
27	1		1:1

8 **Pulse Calibration**

8.1 Introduction

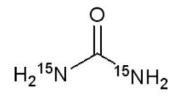
This chapter describes the pulse calibration procedures for determining the 90° transmitter pulse of ¹H, ¹³C and ¹⁵N nuclei.



Note: If your system has been cortabed, it is always a good practice to obtain spectra with the power check turned on.

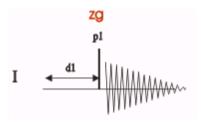
8.2 Sample

Mixture **0.1** M each of ${}^{15}N$ enriched Urea and ${}^{13}C$ enriched methanol in **DMSO-d**₆, see the next figures:



¹³CH3-OH

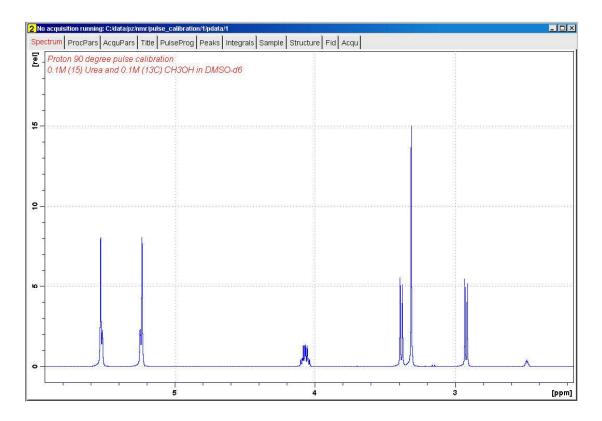
8.3 ¹H 90° Transmitter Pulse



The pulse program **zg** is used to determine the ¹H **90**° transmitter pulse. The sequence consists of one channel **f1** with a recycle delay **d1**, a ¹H pulse **p1**, followed by the ¹H signal detection. The signal has maximum intensity if **p1** is a **90**° pulse and 2 nulls at a **180**° and **360**° pulse. A methanol signal region from **3.5 ppm** to **2.8 ppm** is used for this experiment.

8.3.1 **Preparation Experiment**

Run a **1D Proton** spectrum of urea/methanol in DMSO-d₆, following the instructions from the *TopSpin Guide Book Basic NMR Experiments*, chapter *1D Proton Experiment*, paragraphs *Experiment Setup* through *Processing*.



8.3.2 Parameter Setup

• At the command prompt, type wrpa.

🎃 wrpa	
	f NAME ends with ".top", the destination ataset (no expno/procno required). lestination:
NAME =	p90_proton
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
	OK Cancel Help

- In the field *Name*, enter **p90_proton**.
- Click OK.

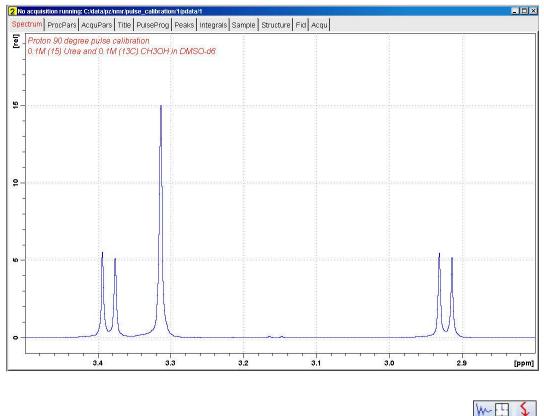
• At the command prompt, type re.

🍓 re	
 Options Oisplay data in s ○ Display data in r 	
NAME =	p90_proton
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
OK Cancel	Browse Find Help

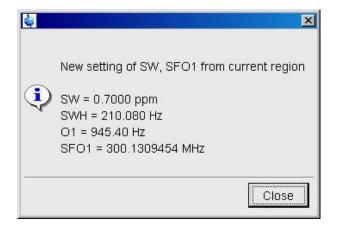
- In the field *Name*, enter **p90_proton**.
- Click OK.

Normally a single on-resonance peak is used to determine the 90° transmitter pulse. For practical reasons the methanol signal region from **3.5 ppm** to **2.8 ppm** is used to measure the ¹H 90° transmitter pulse, since the same signals will also be used in determining the ¹³C 90° decoupler pulse.

• Expand the spectrum for the region between **3.5 ppm** and **2.8 ppm**.



On the toolbar, click Set sw to current region and SFO1 to center of region.



- In the pop up window, click Close.
- In the Dataset window, select the AcquPars tab.
- Enter: PULPROG = zg TD = 4096 NS = 1 DS = 0 D1 = 10
- In the Dataset window, select the ProcPars tab.
- Enter:
 - SI = 2048

```
PH_mod = pk
```

• In the Dataset window, select the **Spectrum** tab.

8.3.3 Acquisition

• On the Workflow button bar, click Gain.



Alternatively type **rga** at the TopSpin command prompt. To adjust the receiver gain manually, click the **drop-down** arrow on the **Gain** button.

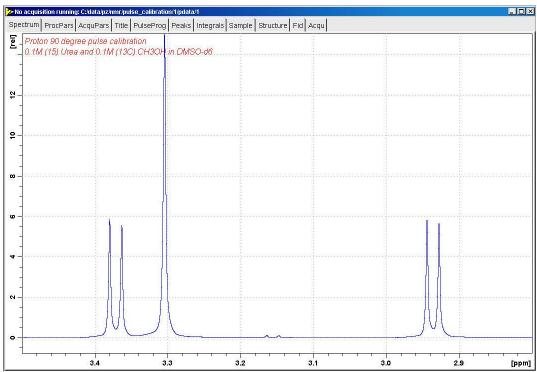
• On the Workflow button bar, click Go.



Alternatively type **go** at the TopSpin command prompt. On the **Go** button, click the **drop-down** arrow to see more options.

8.3.4 Processing

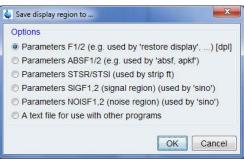
- Process and phase correct the spectrum.
- Display the full spectrum.



- Right-click in the spectrum window.
- In the list, select Save Display Region To...



• Select Parameters F1/2 (e.g. used by restore display, ...) [dpl].



- Click OK.
- At the command prompt, type wpar H1p90_urea all to store the parameter set for future use.

8.3.5 Determine the ¹H 90°Transmitter Pulse

- At the command prompt, type **popt** to display the **P**arameter **OPT**imization window.
- · Enter or select from the list boxes:

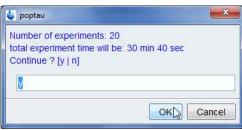
OPTIMIZE = Step by step PARAMETER = p1 OPTIMUM = POSMAX STARTVA = 2 NEXP = 20 VARMOD = LIN INC= 2

· Click Save.

	Data								
store as 2D o	data (ser file))							
The AU prog	ram specifie	d in AUNM will be	e executed		WDW= EM				
Perform auto	matic baseli	ne correction (Al	BSF)		PH_mod= pk				
🖉 Overwrite exi	sting files (d	isable confirmati	on Message)		FT_mod= fs	5			
Stop sample	spinning at t	he end of optimi	ization (mash)						
🗐 Run optimiza	tion in backg	round							
No display of	estimated ru	unning time							
Calculate op	timum after F	OPT has finishe	ed, but do not	store in					
Correlate 2D									
OPTIMIZE	GROUP	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC	
						1 A STATE OF			
Step by step	<u> </u>	p1	POSMAX	2		20	LIN	2	
Step by step		<u> p1</u>	POSMAX	2		20	LIN	2	
Step by step	e Skip cu	2	POSMAX	2 Add parame) Rest		LIN Save 🎝	2 Read an	ray f

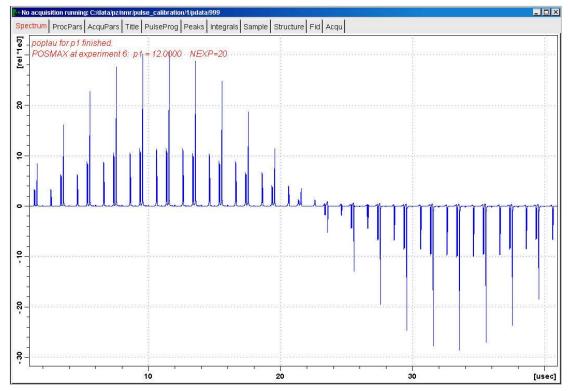
The ENDVAL parameter has been updated.

- In the popt window, click Start optimize to display the poptau window.
- Enter y.



• Click OK.

The parameter optimization starts. The spectrometer acquires and processes 20 spectra by incrementing the parameter p1 from $2 \mu s$ by $2 \mu s$ to a final value of $40 \mu s$. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file *pulse_calibration/2/999* as shown:



• In the Dataset window, select the Title tab.

2 No acquisition running: C:/data/pz/nmr/pulse_calibration/1/pdat						
Spectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	
n 🛛 🖉	E					
	r p1 finishe					
POSMAX :	at experime	ent 6: p1 =	12.000	0 NEXP=:	20	

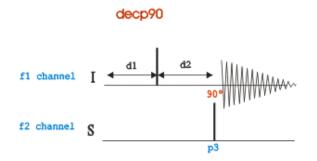
The POSMAX value of p1 is displayed in the title tab window which is the 90° pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90° pulse measurement, follow the steps below:

- Close the popt setup window.
- At the command prompt, type re 1 1
- At the command prompt, type **p1**
- Enter the value which corresponds to a 360° pulse (the second zero crossing in the popt spectrum, which should be approximately 4 times the POSTMAX value).
- Step 1: At the command prompt, type **zg** to start the acquisition.
- Step 2: At the command prompt, type efp
- Change p1 slightly and repeat steps 1 and 2, until the signal undergoes a zero crossing as expected for an exact 360° pulse.

The signals are negative for a pulse angle slightly less than 360° and positive when the pulse angle is slightly more than 360° .

• Divide the determined 360° pulse value by 4. This will be the exact 90° pulse length for the proton transmitter on the current probe.

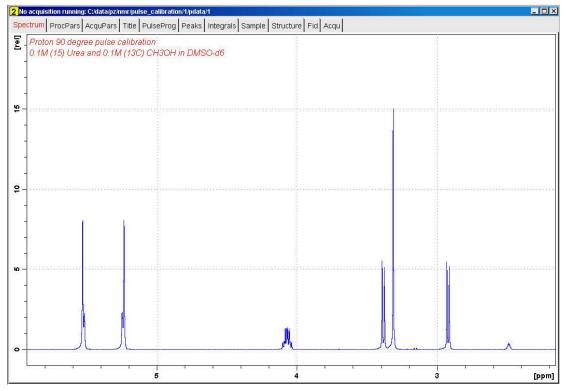
8.4 ¹³C 90° Decoupler Pulse



The pulse program used in this procedure is the **decp90** sequence shown in the figure above. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for ¹H and f2 to ¹³C. Channel f1 shows a recycle delay **d1** followed by a 90^o pulse and a delay **d2 = 1/(2JXH)** for the creation of antiphase magnetization. A ¹³C pulse on channel f2 is been executed after the delay **d2** and then the ¹H signal is detected. When the ¹³C pulse is exactly 90^o, the ¹H signals will go through a null. The methanol signal region from **3.5 ppm** to **2.8 ppm** is used for this experiment.

8.4.1 **Preparation Experiment**

Run a **1D** proton spectrum of urea/methanol in DMSO-d6, following the instructions the *TopSpin Guide Book Basic NMR Experiments*, Chapter 1D Proton Experiment, Experiment Setup through Processing.



8.4.2 Parameter Setup

- At the command prompt, type wrpa and press Enter.
- Change NAME = **p90_carbon**.
- In the wrpa window, click **OK**.

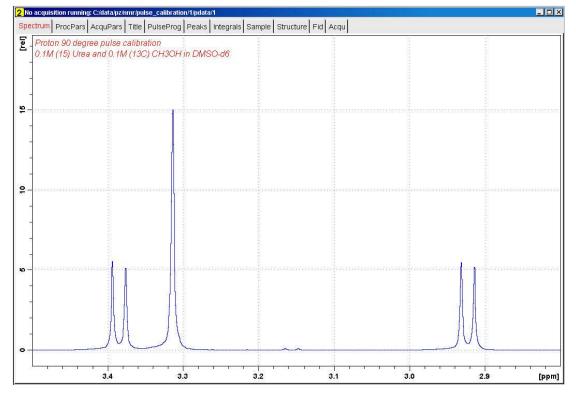
🍯 wrpa	
The second second second second second second	NAME ends with ".top", the destination aset (no expno/procno required).
NAME =	p90_carbon
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
	OK Cancel Help

- At the command prompt, type **re** and press **Enter**.
- Change NAME = **p90_carbon**

• In the re window, click **OK**.

🚑 re	
Options	
NAME =	p90_carbon
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
OK Cance	Browse Find Help

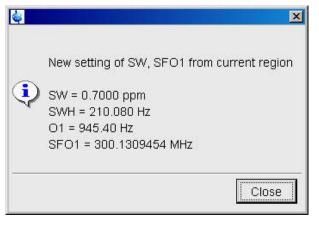
• Expand the spectrum for the region between **3.5 ppm** and **2.8 ppm**.



			Ł
On the toolbar, click Set sw to current region and SFO1 to center of region	. 0	1	

W

• In the pop up window, click Close.



- In the Dataset window, select the **AcquPars** tab.
- Enter:

PULPROG = decp90

TD = 4096

NS = 1

DS = 0

• In the Nucleus2 section of the AcquPars, click Edit next to NUC2.

Vucleus 2		
NUC2	off Edit	2nd nucleus
O2 [Hz]	1853.43	Frequency offset of 2nd nucleus
O2P [ppm]	6.175	Frequency offset of 2nd nucleus
SFO2 [MHz]	300.1318534	Frequency of 2nd nucleus
BF2 [MHz]	300.1300000	Basic frequency of 2nd nucleus

• Select ¹³C for NUC2.

Edit Spectrometer Parameter			×
frequency	logical channel	amplifier	preamplifier
BF1 300.13	MHz NUC1		
SFO1 300.130945	MHz F1	SGU1 X 150 W	1H/2H 1H
OFS1 945.4	Hz IH		1н/2н 2н
BF2 300.13	MHZ NUC2	H 60 W	XBB19F 2HS
SFO2 300.13	MHz F2	SGU2	
OFS2 0.0	Hz off	2H 20 W	
	3He A 6Li 7Li		
: cable wiring	s9Be		
: possible RF routing	11B		
🗢 💠 cortab available	Γ <mark>13C</mark> ting Γ <mark>14N ∎t</mark> probe i	n	
Save Switch F1/F2 Switch	F1/F3 Add a logical chan	nel Remove a logical channel	Default Info Param Close

	frequency	logi	ical channel	amplifier	preamplifier
BF1	300.13	MHz NUC	C1		
SFO	I 300.130945	MHz	F1 / SGU1	X 150 W	1H/2H 1H
OFS	945.4	Hz H			1н/2н 2н
BF2	75.467749	MHZ NUC	C2	H 60 W	XBB19F 2HS
SFO	2 75.475295	MHz	F2 SGU2		
OFS:	2 7546.3	Hz 13	c 🔻	2H 20 W	
	: cable wiring : possible RF routing	j ⊑ setti	ngs how receiver routing how RF routing		

• In the Edit Spectrometer Parameter window, click **Default** to set the routing.

- In the Edit Spectrometer Parameter window, click Save.
- In the AcquPars enter: O2[ppm] = 49.5 D1 = 10 CNST2 = 139 P3 = 3
- On the Workflow button bar, click **Prosol**.



- In the Dataset window, select the ProcPars tab.
- Enter:

SI = 2048

- In the Dataset window, select the **Spectrum** tab.
- At the command prompt, type wpar C13p90_urea all to store the parameter set for future use.

8.4.3 Determine the ¹³C 90° Decoupler Pulse

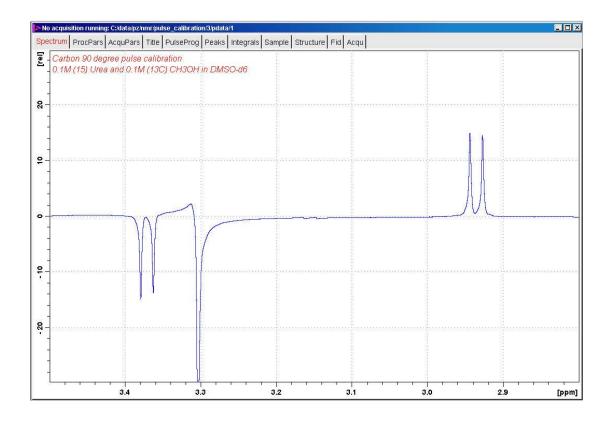
• On the Workflow button bar, click Tune.

```
Start Acquire Process Analyse Publish ⊻iew Manage 
Sample → 排 Lock VLTune → ♣ Spin → 록 Shim → ♣ Prosol → Gain → ▶ Go → More →
```

• On the Workflow button bar, click Go.

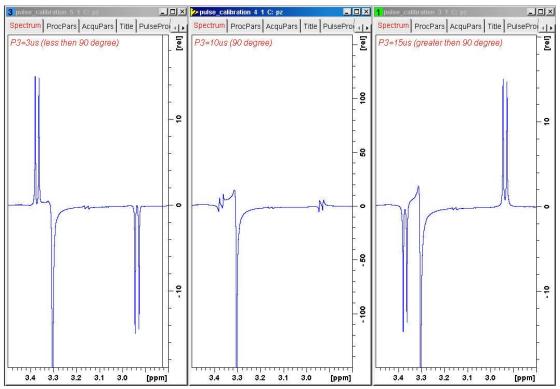
Start Acquire	Process	A <u>n</u> alyse	P <u>u</u> blish	⊻iew	Manage	0			
💐 Sample 👻 🛱	Lock V -	Tune 🗸 🐇 :	Sp <u>i</u> n 👻 🗧	🕈 Shim 🔫	f¶ Prosol マ	<u> G</u> ain ▼	Go -	More 🗢	

· Process and phase correct the spectrum.

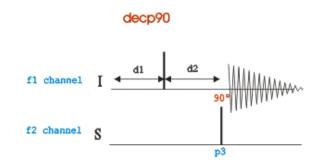


Phase the left doublet negative and the right doublet positive. The Water peak at **3.3 ppm** can be ignored and does not have to be in phase.

Increase p3 in increments of 1 or 2 μs, execute zg followed by the command efp until the signals go through a null or a phase change. This will be the ¹³C 90⁰ decoupler pulse.

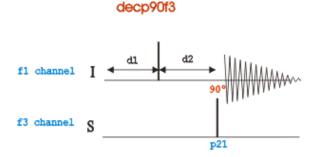


8.5 ¹⁵N 90° Decoupler Pulse



The pulse program used in this procedure is the **decp90** sequence shown in the figure above. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for ¹H and f2 to ¹⁵N. Channel f1 shows a recycle delay **d1** followed by a 90^o pulse and a delay **d2 = 1/(2JXH)** for the creation of antiphase absorption. A ¹⁵N pulse on channel f2 is executed after the delay **d2** and then the ¹H signal is detected. When the ¹⁵N pulse is exactly 90^o, the ¹H signals will go through a null. The urea signal region from **5.6 ppm** to **5.1 ppm** is used for this experiment.

If your system is equipped with a 3rd channel for ¹⁵N observation, you can still follow the same instructions in this chapter with the exceptions of using the pulse sequence **decp90f3** shown in the figure below and the routing which is illustrated in the next section.



8.5.1 Parameter Setup

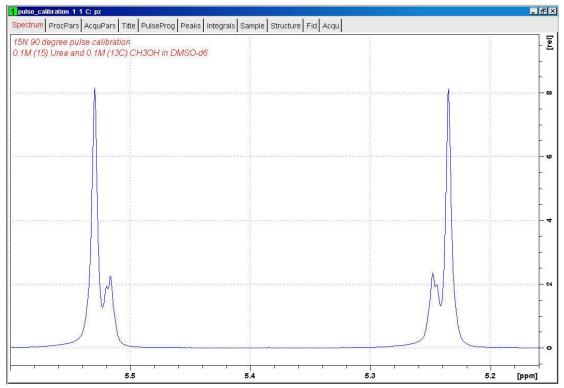
- At the command prompt, type wrpa and press Enter.
- Change NAME = p90_nitrogen
- In the wrpa window, click OK.

💐 wrpa	X
The second state of the second s	NAME ends with ".top", the destination taset (no expno/procno required). estination:
NAME =	p90_nitrogen
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0
	OK Cancel Help

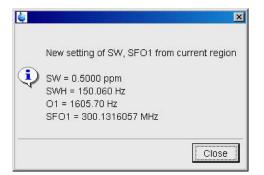
- At the command prompt, type **re** and press **Enter**.
- Change NAME = **p90_nitrogen**.
- In the re window, click **OK**.

🦉 re	×
Options	
NAME =	p90_nitrogen
EXPNO = PROCNO =	1
DIR =	C:\data3.0
OK Cance	el Browse Find Help

• Expand the spectrum for the region between **5.6 ppm** and **5.1 ppm**.



On the toolbar, click Set sw to current region and SFO1 to center of region.



- In the pop up window, click **Close**.
- In the Dataset window, select the AcquPars tab.
- Make the following changes:
 - PULPROG = decp90

TD = **4096**

- NS = 1
- DS = 0
- In the Nucleus2 section of the AcquPars, click Edit next to NUC2.

Vucleus 2		
NUC2	off Edit	2nd nucleus
O2 [Hz]	1853.43	Frequency offset of 2nd nucleus
O2P [ppm]	6.175	Frequency offset of 2nd nucleus
SFO2 [MHz]	300.1318534	Frequency of 2nd nucleus
BF2 [MHz]	300.1300000	Basic frequency of 2nd nucleus

8.5.1.1 Two Channel System

• Select ¹⁵N for NUC2.

😸 Edit Spectrometer Parameter			×
frequency	logical channel	amplifier	preamplifier
BF1 300.13 MHz SF01 300.131853 MHz OFS1 1853.43 Hz BF2 300.13 MHz SF02 300.131853 MHz OFS2 1853.43 Hz : cable wiring : : possible RF routing : cortab available	NUC1 F1 SGU1 1H ▼ NUC2 F2 off ▼ 6Li ▲ 7Li 98e \$10B F1 118 r routing 13C ing 14N ✓ at probe in	X 150 W H 60 W 2H 20 W	1H/2H 1H 1H/2H 2H XBB19F 2HS
Save Switch F1/F2 Switch F1/F3	Add a logical channel Remo	ve a logical channel Default	Info Param Close

	frequency	logica	l channel	amplit	ler	preamplifier
BF1	300.13	MHZ NUC1				
SFO	1 300.131606	MHz	F1 / S	IGU1	X 150 W	1H/2H 1H
OFS	1 1605.7	Hz 1H	\Box \vee \Box		$ \times$	1H/2H 2H
BF2	30.411909	MHZ NUC2			H 60 W	XBB19F 2HS
SFO	2 30.419455	MHz	F2	iguz		
OFS	2 7546.3	Hz 15N	-		2H 20 W	
	: cable wiring : possible RF routing	setting	s w receiver routing			

• In the Edit Spectrometer Parameter window, click **Default** to set the routing.

• In the Edit Spectrometer Parameter window, click **Save**.

8.5.1.2 Three Channel System

• Select ¹⁵N for NUC2.

🔄 Edit Spectromete	r Parameter				
2	frequency	logical channel		amplifier	preamplifier
BF1	399.87 1	MHZ NUC1			
SF01		MHz F1	SGU1	X 150 W	1H/2H 1H
OFS1	2469.36	Hz 1H 🗸	\backslash		1H/2H 2H
BF2	399.87	MHz NUC2	$\langle \cdot \rangle$	H 60 W	XBB19F 2HS
		MHZ F2	SGU2		
			5602		/
OFS2	2469.36	Hz off 🖌 🖌		X 300 W	
BF3	399.87 1	MHZ NUC3			
SF03	399.872469	viHz F3	SGU3	2H 20 W	/
OFS3	2469.36	Hz Off 🔽			
		6Li 📩			
		7LI 🧮			
	: cable wiring	S9Be			
	: possible RF routing	C ^{10B}	r routing		
1.00		11B 13C	ing		
•	: cortab available		at probe in		
		15N 🗸			
Save Swit	tch F1/F2 Switch I	E1/E3 Add a log	ical channel Rem	ve a logical channel	Default Info Param Close

• In the Edit Spectrometer Parameter window, click **Default** to set the routing.

	frequency	logica	al channel	ar	nplifier	preamplifier	
BF1	399.87	MHz NUC1				C	_
SFO1	399.872469	MHz	F1 S	GU1	— X 150 W —	1H/2H 1H	
OFS1	2469.4	Hz 1H	✓ \ /			1H/2H 2H	
BF2	399.87	MHz NUC2	X		- H 60 W -	XBB19F 2HS	7
	399.87			302	· /		
				302 r			
OFS2	0.0	Hz off	✓ /		X 300 W		
BF3	40.518475	MHZ NUC3	i /				
SF03	40.525975	MHz	F3 S	GU3	2H 20 W		
OFS3	7499.8	Hz 15N	~				
	: cable wiring : possible RF routin : cortab available	sho	is w receiver routing w RF routing w power at probe in				

- In the Edit Spectrometer Parameter window, click Save.
- In the AcquPars make the following change: O2[ppm] = 76 D1 = 10 CNST2 = 88.5 P3 = 6
- On the Workflow button bar, click **Prosol**.



- · In the Dataset window, select the ProcPars tab.
- Make the following change:

SI = 2048

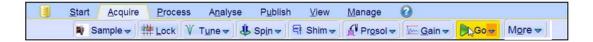
- In the Dataset window, select the **Spectrum** tab.
- At the command prompt, type wpar N15p90_urea all to store the parameter set for future use.

8.5.2 Determine the ¹⁵N 90° Decoupler Pulse

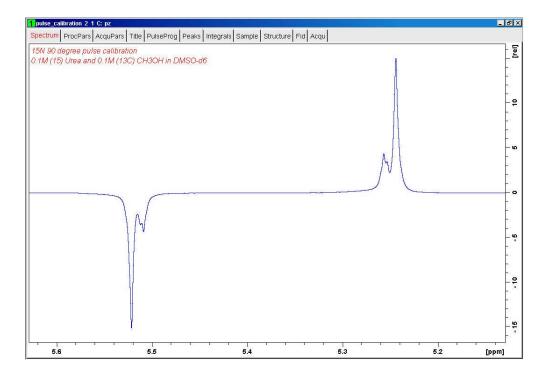
• On the Workflow button bar, click Tune.



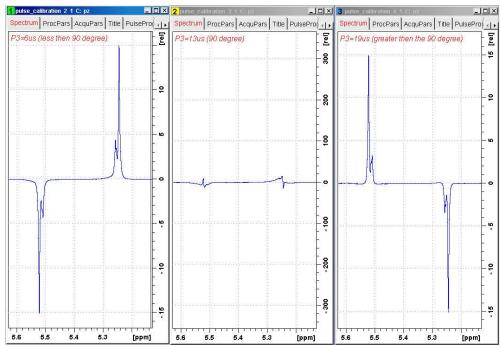
• On the Workflow button bar, click Go.



- Process and phase correct the spectrum.
- · Phase the left side signal negative and the right side signal positive!

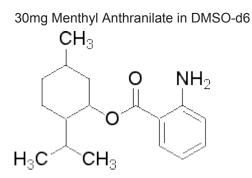


Increase p3 in increments of 1 or 2 μs, execute zg followed by the command efp until the signals go through a null or a phase change. This will be the ¹⁵N 90⁰ decoupler pulse.



9 ¹H Homonuclear Decoupling

9.1 Sample



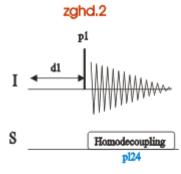
9.2 ¹H Homonuclear Decoupling Experiment

9.2.1 Introduction

The homonuclear decoupling (homodecoupling) allows to simplify multiplet structures by irradiating a specific ¹H resonance. In ambiguous assignments and measurement of ¹H-¹H coupling constants can be performed by analyzing the resulting residual multiplets.

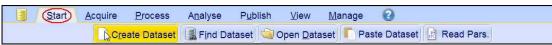
During an homo-decoupling experiment, a conventional ¹H spectrum is recorded. From a second channel, low-power irradiation is applied on a predefined frequency during the acquisition period.

Important parameters to consider are the offset and the power level of the irradiation. It is useful to have a calibration of the field strength delivered from the decoupler in order to optimize the required selectivity and to minimize Bloch-Siegert shift effects.



9.2.2 Preparation Experiment

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



 In the New Dataset window, enter or select: NAME = homodec
 EXPNO = 1

PROCNO = 1 Experiment: select **PROHOMODEC** Set Solvent: select **DMSO**

🍯 New				
initializing its NMR parameters For multi-receiver experime	ent by creating a new data set and ters according to the selected experiment type. ents several datasets are created. of receivers in the Options.			
NAME	homodec			
EXPNO	1			
PROCNO	1			
O Use current parameters				
Experiment PROHOMO	DEC Select			
 Options 				
Set solvent:	DMSO -			
Execute "getprose) "			
Keep parameters:	P 1, O1, PLW 1 - Change			
DIR	C:\Data			
🕅 Show new dataset	in new window			
Receivers (1,2,16	5) 1			
Reference 30 mg Met	spectrum hyl Antranilate in DMSO-d6			
	OK Cancel More Info Help			

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

• In the New Dataset window, click **OK**.

• On the menu bar, click Aquire.

<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>I</u>	<u>M</u> anage	0			
	💐 Sampl <u>e</u> 🔻	tock	V Tune 🗸	lu Sp <u>i</u> n →	독 Shim	1 ▼ <u>∫</u> F	rosol 🗢	🚾 <u>G</u> ain 🔫	Þ Go 🚽	M <u>o</u> re 🗢

For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select DMSO solvent.
- To tune the probe, click **Tune**.
- · Click Spin and select Turn sample rotation off.



Homonuclear experiments should be run non-spinning.

- To autoshim the sample with TopShim and best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.
- In the Dataset window, select the **AcquPars** tab.
- Make the following change:
 PULPROG = zg30
- In the Dataset window, select the Spectrum tab.

9.2.3 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click **Go**.

9.2.4 Processing

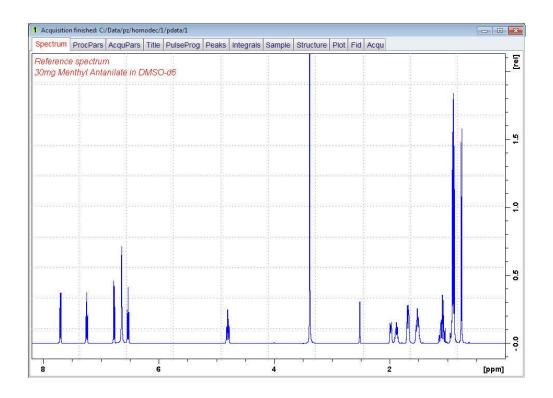
• On the menu bar, click **Process**.



• On the Workflow button bar, click **Proc Spectrum**.

This executes a processing program including commands such as an exponential window function em, Fourier transformation ft, an automatic phase correction apk and a baseline correction abs.

To configure the commands, click the drop-down arrow on the **Proc Spectrum** button and select **Configure Standard Processing**.

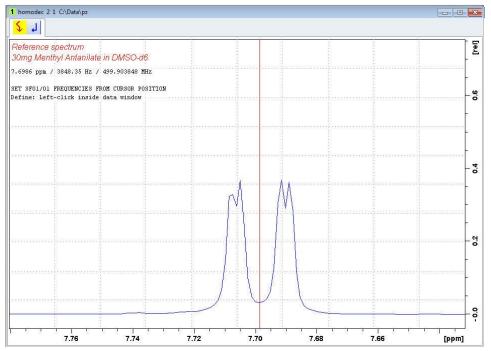


9.2.5 Setting up the Homonuclear Experiment Parameters

At the command prompt, type:
 wrpa 2
 re 2



- Expand the multiplet at **7.7 ppm**.
- On the Tool bar, click Set RF from cursor.
- · Move the cursor line into the center of the multiplet.



· Use the mouse and click to set the frequency.

6 01/02/03	×
Define SFO1/O1 1	irequencies
SFO1 [MHz] =	499.903848
O1/2/3 [Hz] =	3848.35
01 02	O3 Cancel

- In the O1/O2/O3 window, click O2.
- In the Dataset window, select the AcquPars tab.
- Make the following change:
 PULPROG = zghd.2
- In the Dataset window, select the Title tab.
- Change the title to:
 1H Homonuclear decoupling experiment irradiation at 7.7 ppm
 30 mg Menthyl Anthranilate in DMSO-d6
- In the Dataset window, select the Spectrum tab.
- On the menu bar, click Acquire.

<u>S</u> tart	Acquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew <u>M</u> ar	nage	0			
	💐 Sampl <u>e</u> 🗢	Lock	V Tune 🚽	👃 Sp <u>i</u> n →	🖙 Shim 🚽	A Pro	osol 🗢	<u>Iv⊷</u> <u>G</u> ain ▼	Þ Go 🚽	M <u>o</u> re ▼

9.2.6 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

9.2.7 Processing

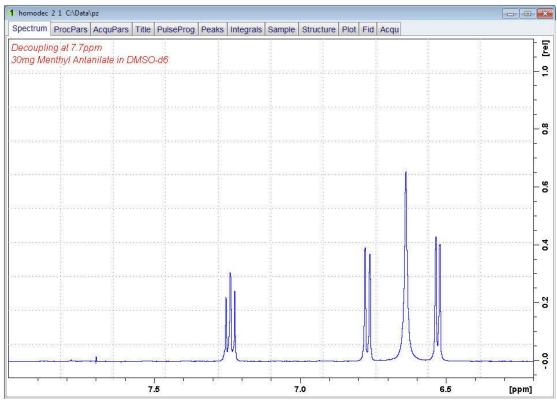
• On the menu bar, click **Process**.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> bl	ish <u>V</u> iew	<u>M</u> anage	0		
	J Proc. Sp	oectrum 🗢	Adjust Pł	nase 🔻	🔥 Calib. A <u>x</u> is	Nr Pick P	eaks ▼	∫ Integrate →	A <u>d</u> vanced ▼

• On the Workflow button bar, click **Proc Spectrum**.

This executes a processing program including commands such as an exponential window function em, Fourier transformation ft, an automatic phase correction apk and a baseline correction abs.

To configure the commands, click the drop-down arrow on the **Proc Spectrum** button and select **Configure Standard Processing**.



• Expand the region from 8 ppm to 6.2 ppm.

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The multiplet at **6.4 ppm** should collapse from a triplet in to doublet. If the triplet is partially collapsed, increase the decoupling power pl24 and repeat the steps in chapter *Acquisition* [\triangleright 159] and *Processing* [\triangleright 159].

NOTICE

Material Damage Due to Excessive Power

The NMR probe can be severely damaged if too much power or power over a too long time is applied.

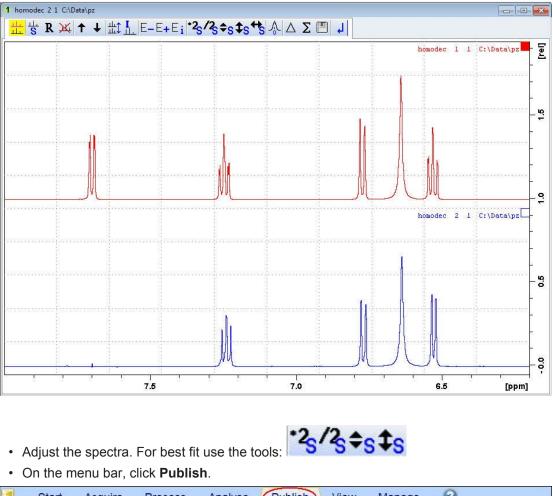
Always start to optimize pulses with low power values and short pulses. Respect the pulse and power limits as programmed into the PICS data of the probe.

9.2.8 Plotting Two Spectra on the Same Page

• Display the decoupled spectrum.



• Drag the Reference spectrum (1D proton) into the spectral window.



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- On the Workflow button bar, click **Print**.
- This will print the active window with the colors displayed in the TopSpin window.

10 Proton DOSY Experiment

10.1 Introduction

The **DOSY** (Diffusion-Ordered Spectroscopy) **experiment** provides accurate, noninvasive, molecular diffusion measurements on biofluids, complex chemical mixtures and multi component solutions. In DOSY spectra, chemical shift is along the detected F2 axis and diffusion coefficient is along the other F1 axis.

Molecules in the solution state move. This translational motion is known as Brownian molecular motion and is often simply called diffusion or self diffusion. Molecular diffusion depends on a lot of physical parameters like size and shape of the molecule, temperature and viscosity.

Pulsed field gradient NMR spectroscopy can be used to measure translational diffusion. By use of a gradient pulse, molecules can be spatially labeled. After this encoding gradient pulse (δ), molecules move during the diffusion time (Δ). Their new position can be decoded by a second gradient pulse. This encoding/decoding procedure results in an attenuation of the NMR signal which can be described by the following equation:

$$I(g) = I(o)exp\left[-(\gamma g\delta)^2 D\left(\Delta - \frac{\delta}{3}\right)\right]$$

Where I is the observed intensity, **D** is the diffusion coefficient, γ is the gyro magnetic ratio of the encoded nucleus, **g** is the gradient strength, δ is the length of the gradient pulse, and Δ as mentioned previously is the diffusion time.

The diffusion experiment described below records a series of 1D 1 H spectra at increasing gradient strengths (g) and then fits the signal intensity decay to the above equation to obtain **D**.

10.2 Sample

Mixture of Ibuprofen, Pamoic acid and Pinene in DMSO-d6.



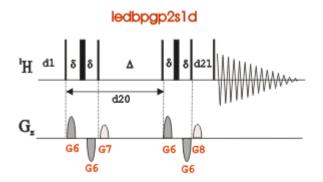
The experimental parameters of δ (pl30) and Δ (d20) described here are for this sample. If using a different sample, they will likely be different.

10.3 DOSY Experiment

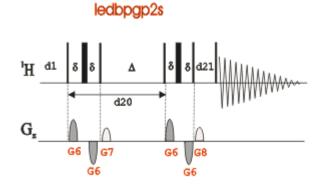
10.3.1 Pulse Programs

The DOSY pulse program used in this chapter is a Stimulated spin-echo experiment using bipolar gradients and an additional delay just prior to detection for the ring-down of any possible eddy currents (led).

The figure below is a 1D version of the pulse program and is used to optimize parameters, see the chapter *Parameter Setup* [▶ 167] and.



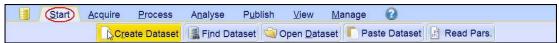
The pulsprogram in the figure below is used for the DOSY experiment, see the chapter *Running the Experiment* [\triangleright 172]. The difference between these 2 pulse sequences is that the one shown in the figure below is a pseudo 2D sequence, and includes the code to automatically increment the gradient strength.



To run this experiment the instrument has to be equipped with the hardware to run gradient experiments.

10.3.2 Preparation Experiment

• On the menu bar, click Start and on the Workflow button bar, click Create Dataset.



· In the New Dataset window, enter or select:

NAME = **DOSY_exp** EXPNO = **1** PROCNO = **1** Experiment: select **PROTON** Set Solvent: select **DMSO**

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EXPNO	1		
PROCNO	1		
O Use current parameters			
Experiment PROTON		Select	
 Options 			
Set solvent:		DMSO •	
Execute "getprosol"			
⊘ Keep parameters:		P 1, O1, PLW 1 Change	
DIR		C:\Data	•
🖾 Show new dataset i	n new window		
Receivers (1,2,16)		1	
DOSY exper Micture of Ib		acid and Pinene in DMSO-d6	
	OK	Cancel More Info	Help

DIR

The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click the drop-down arrow to browse for a specific directory.

Title

In the TITLE window enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.

- In the New Dataset window, click **OK**.
- On the menu bar, click Aquire.



For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select DMSO solvent.
- To tune the probe, click **Tune**.
- · Click Spin and select Turn sample rotation off.



DOSY experiments should be run non-spinning.

- To autoshim the sample with TopShim and best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.

10.3.3 Acquisition

- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

10.3.4 Processing

· Process and phase correct the spectrum.

10.3.5 Limit Settings



Changing the sweep width to a smaller value increases the resolution.

 At the command prompt, type: wrpa 2

re 2

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Expand the spectrum from 9 ppm to 0 ppm.



On the toolbar, click Set sw to current region and SFO1 to center of region.

· Click Close.



Spectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Plot Fid	Acqu			
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10.3.6 Parameter Setup

For an accurate DOSY experiment, certain parameters need to be calibrated for each sample to ensure that the observed signal decay is appropriate. This section will walk you through this process.

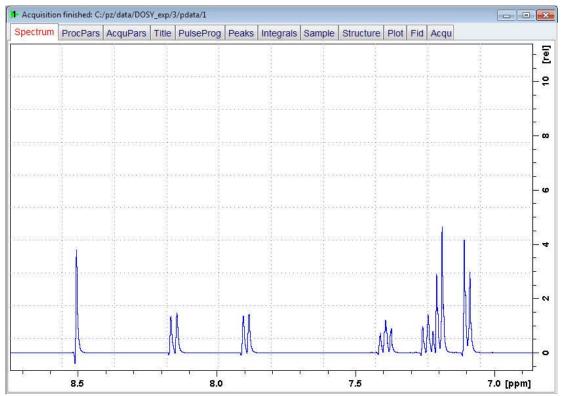
🔊 🞵 S

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- At the command prompt, type iexpno.
- In the Dataset window, select the AcquPars tab.
- · Click Show pulse program parameters.

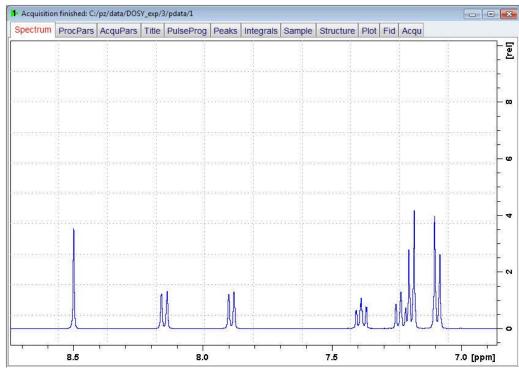
- Make the following changes: PULPROG = ledbpgp2s1d D20[s] = 0.1 D21[s] = 0.005 GPNAM6 = SMSQ10.100 GPNAM7 = SMSQ10.100 GPNAM8= SMSQ10.100 GPZ6[%] = 2 GPZ7[%] = -17.13 GPZ8[%] = -13.17 P30[us] = 1400
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.
- Process and phase correct the spectrum.
- Expand the spectrum to display all peaks.



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The asymmetry of the peaks is an artifact which is caused by non-optimal lock settings. The problem can be fixed by applying a loop adjust of the lock especially that of the lock phase.

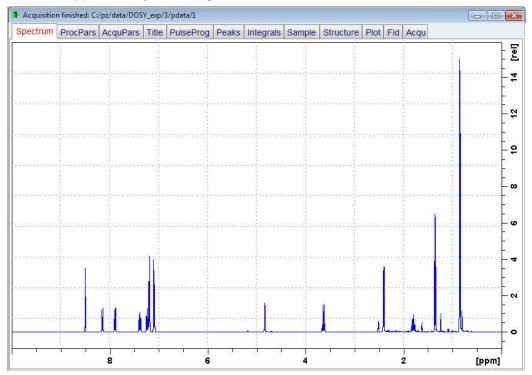
- At the command prompt, type loopadj and confirm with OK.
- To start the acquisition, click Go.



• Process and phase correct the spectrum.

• Display the full spectrum.

This is the first spectrum (2%). Because the DOSY experiment is fitting the signal as it decays, it is crucial that there be enough signal in this first experiment that it can decay by a factor of about 95%, but still have good enough signal to noise so that these attenuated values are still reasonably error free. Thus it is recommended that this first experiment have an S/N of approximately 100:1 or greater. If this is not the case, increase the NS.



The steps described below are necessary to make sure the attenuation level is sufficient at the final (95%) gradient strength. In this second (95% gradient strength) experiment, there should still be signal, but it should be attenuated by a factor of 90-95% as compared to that of the first (2% gradient strength). If there is only noise, then p30 and or d20 need to be reduced. If there is less than 90-95% attenuation, p30 and or d20 need to be increased.

For both the cases of increasing or decreasing the gradient pulse/delays adjusting p30 will have more of an effect than adjusting d20.

Keep in mind that the recommended safety limit for p30 is 2.5 ms, after this limit has been reached, further attenuation must be achieved through increasing d20.

If large changes to p30 and d20 are necessary, then it is recommended to re-run both experiments because these values will affect the intensity of not only the attenuation in the 2nd spectrum, but the starting intensity in the 1st spectrum.

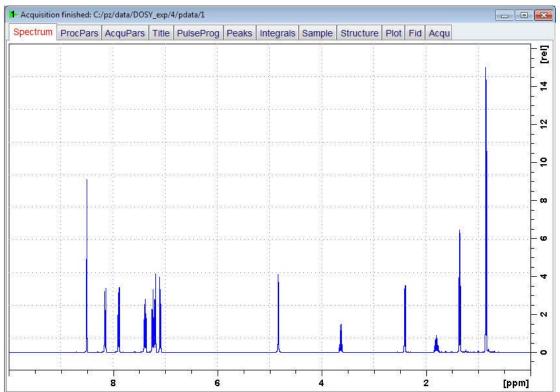
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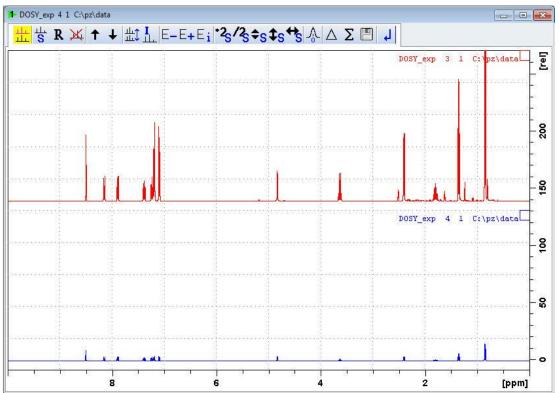
- At the command prompt, type iexpno.
- In the Dataset window, select the AcquPars tab.
- · Click Show pulse program parameters.
- Make the following change:
 - GPZ6[%] = **95**
- To start the acquisition, click Go.
- · Process and phase correct the spectrum.





If there are no signals present, then p30 and/or d20 need to be reduced.

- On the toolbar, click Multiple display.
 Drag the provide · Drag the previous experiment into the multiple display window (in this example it is experiment # 3) or type re 3.





As described above, there need to be signal remaining in the 2nd experiment, but the intensity difference of the two spectra should be a factor of 90-95%. If neither of these are true, it is necessary to change p30, and d20 accordingly.

• To exit the multiple display, click **Return**.

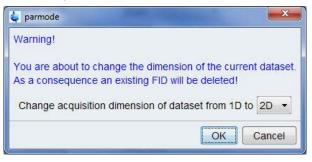


10.3.7 Running the Experiment

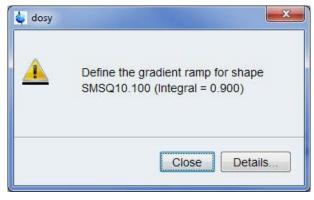
- At the command prompt, type **iexpno**.
- In the Dataset window, select the AcquPars tab.
- Make the following change:
 PULPROG = ledbpgp2s
- · Click Change acquisition dimension of current dataset.



In the parmod window, select 2D and click OK.



- Make the following changes: TD[F1] = 25 FnMODE = QF
- At the command prompt, type **dosy**.
- In the dosy window, click Close.



• Enter 2 for first gradient amplitude and click OK.

🧅 dosy	X
Enter first gradient a	mplitude:
2	
	OK Cancel

• In the dosy window, enter 95 for final gradient amplitude and click OK.

	×
OK	Cancel
	ОК

• In the dosy window, enter 25 for the number of points and click OK.

🤹 dosy		×
Enter number of points:		
25		12 12 12 12 12
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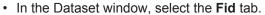
• In the dosy window, enter I for the ramp type and click **OK**.

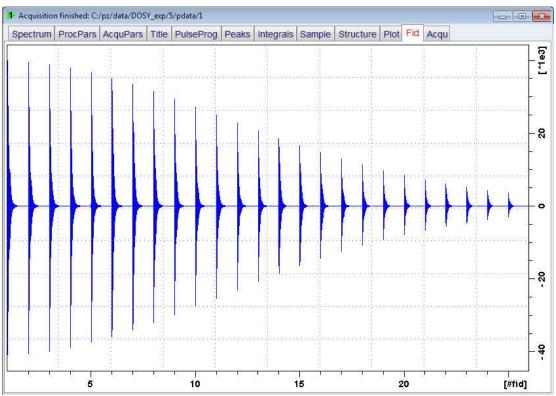
ared/exponential}):
OK Cancel

• To start the acquisition, click **OK**.



10.3.8 Processing





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This step is only used to illustrate the DOSY experiment as a decay function.

- In the Dataset window, select the ProcPars tab.
- Make the following changes:
 - SI [F1] = 256
 - PH_mod [F1] = no
 - PH_mod [F2] = **pk**
- At the command prompt, type rser 1 to read in the first serial file of the 2D experiment.
- At the command prompt, type **em** to apply the window function.
- At the command prompt, type ft.
- On the menu bar, click **Process**.
- On the Workflow button bar, click Adjust Phase.

Start Acquire Pro	cess A <u>n</u> alyse	P <u>u</u> blish	⊻iew	<u>M</u> anage	2	
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- · Process and phase correct the spectrum.
- On the Adjust Phase toolbar, click **Save for nD spectrum**.

On the toolbar, click Return, do NOT save phased spectrum.

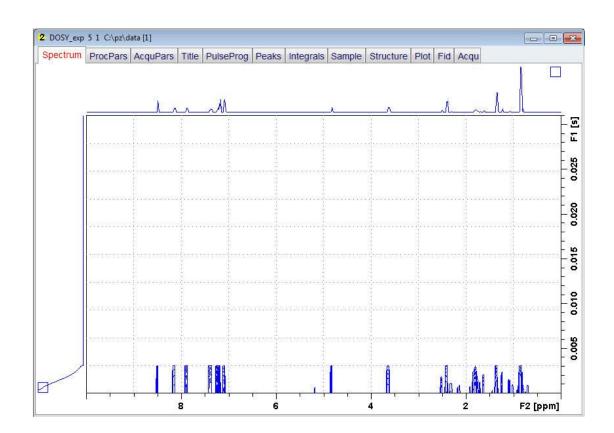


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The spectrum will go back to the un-phased view since the phase correction values were stored only for the 2D spectrum.

- On the toolbar, click Last 2D data to go back to the 2-D spectrum display.
- At the command prompt, type xf2.
- At the command prompt, type **abs2**.
- At the command prompt, type setdiffparm.

This command transfers experimental parameters into the values used for fitting the data.



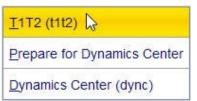
10.3.9 Calculating the Diffusion Coefficient

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

- On the menu bar, click Analyze.
- On the Dynamics button, click the drop-down arrow to see more options.

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• In the list, select T1/T2.



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The flow buttons change to the mode: Determination of the T1 / T2 relaxation times.

· On the Workflow button bar, click Fid.





While executing the next steps, message windows will pop up. Please read each message thoroughly and follow the instructions in it.

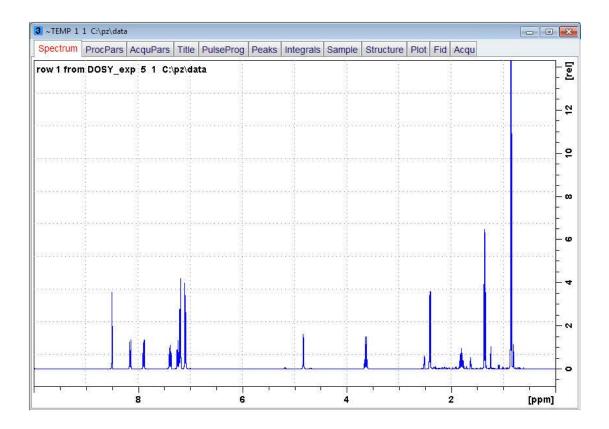
• In the Extract a row from 2d data window, click **Spectrum**.



• In the field Slice Number, enter 1 and in the message window click OK.

Spectrum slice must be extracted From the 2d relaxati	on data.
This Spectrum should correspond to an experiment wil All further data preparation will be done in respect to the Slice Number =	
	OK Cancel

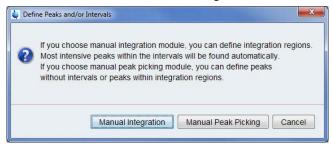
Proton DOSY Experiment



• On the Workflow button bar, click **Peaks/Ranges**.

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3 Back	W- <u>F</u> id	A Peaks/Ranges	Relaxatio	n K Fitting	Seleculation Report

• In the Define Peaks and/or Integrals window, click Manual Integration.



• In the Prepare relaxation data window, click OK.

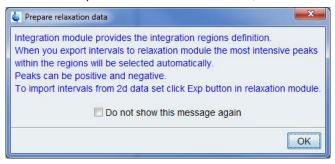
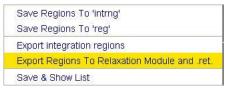


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	8	6	4	2	[ppm]

• Define the regions by drawing an integral over the peaks of interest.

- On the Integration toolbar, click **Save region as**.
- From the drop-down list, select Export Region To Relaxation Module.



• On the Workflow button bar, click Relaxation.

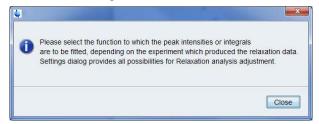


- 3 Relaxation DOSY_exp 5 1 C:\pz\data <mark>≫ > 🚥 – + ↓</mark> 🗉 🔝 🛄 Ig S9 Ig 📥 🖱 🛈 🚹 [*1e9] Fitting type Diffusion : Variable Gradient I=I[0]*exp(-D*SQR(2*PI*gamma*Gi*LD)*(BD-LD/3)*1e4) Intensity Region 1 from 8.568 to 8.445 ppm Area Current Integral 0 1 of 5 0 Brief Report-Data preparation is done. 0 0.6 ö 0 6 0.4 0 0 ò 0 0.2 ó 0 0 0 10 20 30 40 [G/cm]
- In the group box Fitting type, select **Area**.

• On the Workflow button bar, click Fitting.

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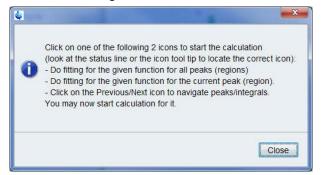
• In the message window, click Close.



• In the Fitting Function group box, select vargrad and difflist and click OK.

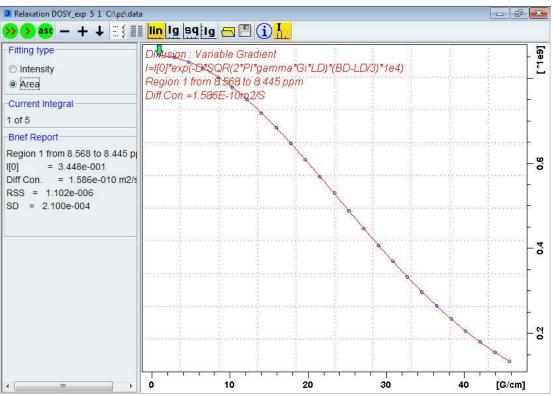
🆕 Relaxation	paramete	ers			X			
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0.0	Right	Right limit for baseline correction						
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1.0E-5	Conve	er	gence limit					
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1.0 GRADIEN(G/cm)								
OK Apply Cancel								

- On the Workflow button bar, click Calculation.
- In the message window, click Close.



> asc

On the T1/T2 toolbar, click Calculate fit for all peaks.





All calculated values are displayed in the Brief Report group box of the data window.

Degion 1	from	8.610 to 8.446 ppm
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		1.585e-010 m2/s
		3.740 to 3.539 ppm
		2.415e-010 m2/s
Region 3	from	1.293 to 1.205 ppm
Diff Con.	=	3.853e-010 m2/s

• On the Workflow button bar, click Report.

<u>S</u> tart	<u>A</u> cquire <u>P</u> r	rocess Analyse	P <u>u</u> blish <u>V</u>	<u>/</u> iew <u>M</u> ar	nage 🕜	
(3) Back	<u>₩ </u> <u>F</u> id	<u>Å</u> <u>P</u> eaks/Ranges	Relaxation	Fitting	Calculation	🗏 🔀eport

• In the Fitting report window click File and Print to print the report.

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File	Edit Search				
1	SIMFIT RESULTS				
2					
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4	Dataset : C:/pz/	data/DOSY_exp/5/p	lata/1/ct1t2.t	xt	
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7	T T [0] + (D+CO)	R(2*PI*qamma*Gi*Ll		- 4 \	
9	1=1[0].exb(-p.20	K(Z"PI"gamma"61"LI), (C/UJ-UJ), (e4)	
10	25 points for To	terral 1 Interra	al Region from	8.610 to 8.446 ppm	
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12	Converged after	49 iterations!			
13					
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15					
16	I[0] =				
17	Diff Con. =				
18	Gamma =				
19 20	Little Delta =	2.800m			
20	Big Delta =	99.900m			
22	RSS = 1.348e	-005			
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28	2 2.829e+0		9.748e-001	6.746e-004	
29	3 4.695e+0		9.629e-001	5.530e-004	
30	4 6.560e+0		9.453e-001	6.885e-004	
31	5 8.426e+0		9.223e-001	6.356e-005	
32 33	6 1.029e+0		8.944e-001	1.490e-004	
33	7 1.216e+0 8 1.402e+0		8.620e-001 8.257e-001	1.291e-004 3.019e-004	
35	9 1.589e+0		7.861e-001	-2.347e-005	
36	10 1.775e+0		7.439e-001	2.300e-004	
37	11 1.962e+0		6.996e-001	-1.402e-004	
38	12 2.149e+0		6.539e-001	-2.506e-004	
39	13 2.335e+0	01 6.095e-001	6.075e-001	-1.988e-003	
40	14 2.522e+0	01 5.610e-001	5.609e-001	-4.750e-005	
41	15 2.708e+0	01 5.140e-001	5.148e-001	7.867e-004	
42	16 2.895e+0	01 4.686e-001	4.695e-001	9.776e-004	
43	17 3.082e+0	01 4.250e-001	4.257e-001	6.155e-004	
44	18 3.268e+0		3.835e-001	2.036e-005	
45	19 3.455e+0	01 3.428e-001	3.434e-001	6.505e-004	

• In the Fitting report window click File and select Close.

10.3.10 Displaying the DOSY Plot

• On the Workflow button bar, click Back.



• On the Workflow button bar, click **Dosy**.

<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew	<u>M</u> anage	0		
사 다고	/lul <u>t</u> iplets	Line Shapes ⊽		Spin T <u>1</u> /T2 N	/lodule 🗢	O Structu	res 🔻	Simulate 🗢	Dosy 4

• From the drop-down list select Setup Parameters.

Setup Parameters (eddosy) Start Processing (dosy2d)

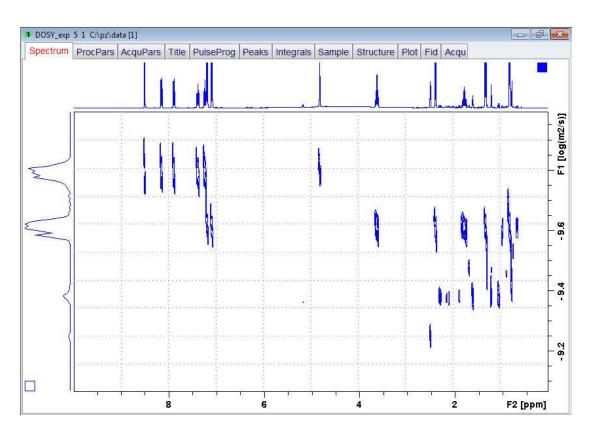
Proton DOSY Experiment

	5 1 C:\pz\data		Title D	ula e Dra e	Deal			Otructur		A	
	ProcPars A	awaran i	Title P	uiseProg	Реак	s integr	ais sample	structur	e Plot Fla	Acqu	
PG			<i>P</i> 2								
General First	Gene	eral									
Second	Method		expon	ential	•	Proces	sing method	t 🗧			
Third	ExpVar		Gradie	ent	-	Variabl	e <mark>paramet</mark> e	r			
Baseline	Xlist		difflist			Variabl	e paramete	r values file	e name		
Contin	Nstart		0			Start of	input point	s			
	Ndata		25			Numbe	r of input po	oints (TD)			
	Maxiter		100			Maximu	im <mark>number o</mark>	of iteration:	5		
	EPS		1			Tolerar	nce				
	Nexp		1			Numbe	r of compon	ents to fit			
	Noise		0			Noise le	evel (S_DE	/)			
	PC		4			Noise s	ensitivity fa	ctor			
	SpiSup		1			Spike s	uppression	factor			
	F1mode		Peaks		Ŧ	F1 outp	out data mo	de			
	Imode		Integra	al	•	Fitted in	ntensity me	aning			
	Scale		Logari	ithmic	•	Scaling					
	LWF		1			Line wi	dth factor				
	DISPmin		-10			Lower	display limit				
	DISPmax		-8.022	28		Upper of	display limit				
	Npars		7			Numbe	r of parame	ters			

Make the following change:

Scale = Logarithmic

On the toolbar, click Start fitting. ■ PG L I → C ▼



11 Multiplet Analysis

11.1 Introduction

This analysis tool can be used to define multiplets and deduce chemical shifts, coupling constants, multiplicities and connections.

11.2 Sample

100 mg 2, 3,-Dibromopropionic acid in CDCl₃

11.3 Multiplet Assignments

11.3.1 Preparation Experiment

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter 1D Proton Experiment, Experiment Setup through Processing.

11.3.2 Limit Settings

Changing the Sweep width to a smaller value will increase the resolution.

• At the command prompt, type:

wrpa 2

re 2

- Expand the spectrum from 10.6 ppm to -0.5 ppm.
- On the toolbar, click Set sw to current region and SFO1 to center of region.



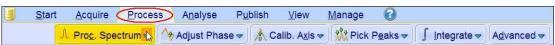


11.3.3 Acquisition

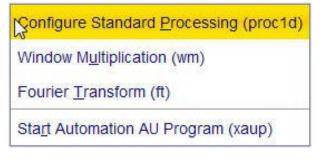
- To adjust the receiver gain, click Gain.
- To start the acquisition, click Go.

11.3.4 Processing

• On the Workflow button bar, click Process.

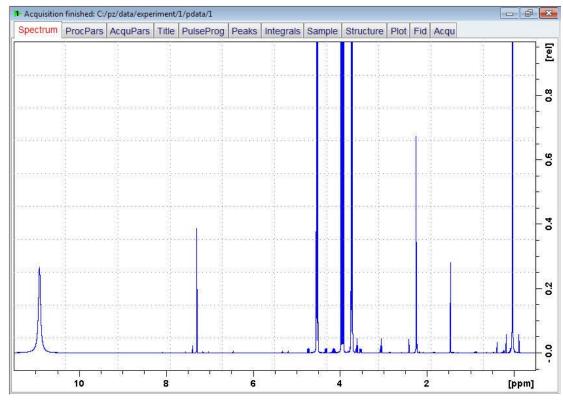


- On the Proc Spectrum button, click the drop-down arrow to see more options.
- Select Configure Standard Processing (proc1d).



- · Enable the following options:
 - Exponential Multiplay (em)
 - Auto Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto Baseline Correction (abs)
- Change LB [Hz] = 0

🤹 procld			×
Press 'Execute' to process the cur Press 'Save' to just change the pri Changed options will be effective v one-click 'Proc. Spectrum' button.	ocess	sing options.	8
Exponential Multiply (em)		LB [Hz] =	0
Fourier Transform (ft)			
Auto - Phasing (apk)	V		
Set Spectrum Reference (sref)			
Auto - Baseline Correction (abs)			
Plot (autoplot)		LAYOUT =	+/1D_H.xwp 🔹
Warn if processed data exist			
		Save	e Execute Cancel



• In the proc1d window, click Execute.

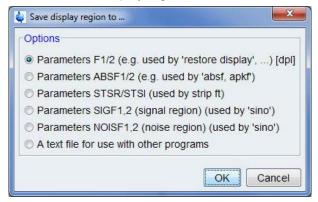
- On the toolbar, click Exact Zoom.
- In the exactzoom window enter the following parameters:
 - From = **4.6**
 - To = 3.6
- In the exactzoom window, click **OK**.

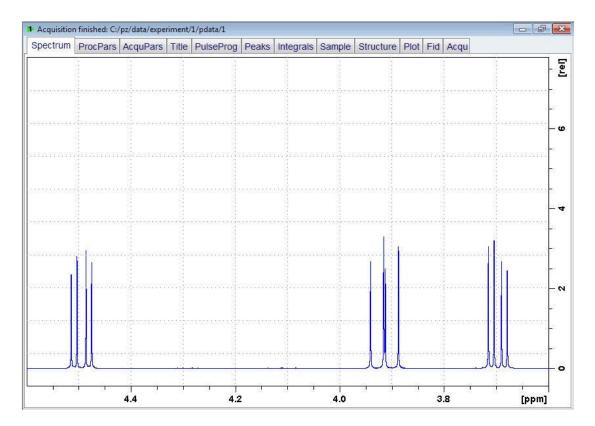
🖕 exactzoom	
Please enter of the desired	the exact coordinates I expansion.
	F1 [ppm]
From	4.6
То	3.6
	OK Cancel

• Right-click the spectrum window and in the shortcut menu select **Save Display Region To**.

T	oggle Spectrum Overview
SI	now Full Spectrum
T	oggle Parameter <u>W</u> indow
S	be <u>c</u> tra Display Preferences
Sa	ave Display Regi <u>o</u> n To
R	estore Display Region From Params. F1/2
Se	et Plot Height At Specific Cursor Position
D	ataset Properties
Ei	les
E)	plorer

- Enable the option Parameters F1/2 [dp1].
- In the Save display region window, click OK.





11.3.5 Peak Picking

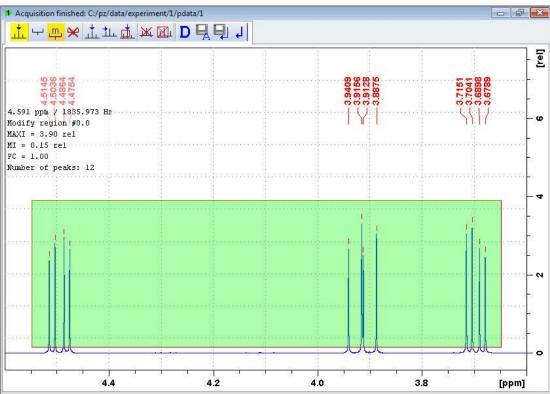
• On the Workflow button bar, click Peak Peaks.



This enters the manual peak picking mode. The Dataset tabs are replaced by the Peak Picking Tool bar.

By default the **Define new peak picking range** button is enabled.

- Click and draw a rectangle over all multiplets up to 3.7 ppm.
- On the Peak Picking toolbar, click **Modify existing peak picking range** to manually adjust the minimum and maximum intensity levels.
- 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).



11.3.6 Assigning the Multiplets

- Expand the multiplet at 4.5 ppm.
- On the menu bar, click Analyze.
- On the Workflow button bar, click Enter multiplet analysis.

<u>S</u> tart <u>A</u> cqu	ire <u>P</u> rocess	Analyse Publi	sh <u>V</u> iew	<u>M</u> anage	0		
rh Multolets マ	Line Shapes ▼	TopSpin T1/T2	2 Module 🔻	O Structures 🔻	Simulate	Quantify	T <u>o</u> ols ▼

This enters the multiplet analysis mode. The Dataset tabs bar is replaced by the Multiplet analysis button bar.

▝▋┓┓╚╗╲╸∝╳╳╴╴┇╓╴╔╗┍	M AN H H A	王母祖王		î. l. <u>1 × </u> ¥ ⊭	ן 🗗 🖪 🖪 ५ी 🎊 🗠 י
---------------------	------------	------	--	-----------------------	------------------

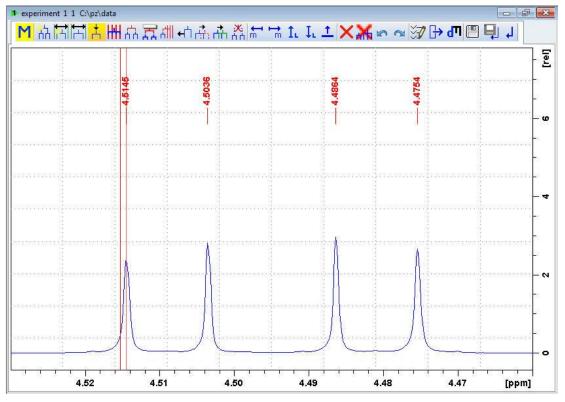
- Click Define Multiplets Manually. It should be highlighted in yellow.
- · Place the cursor lines to the left of the first peak of the multiplet.

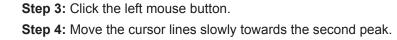
Multiplet Analysis

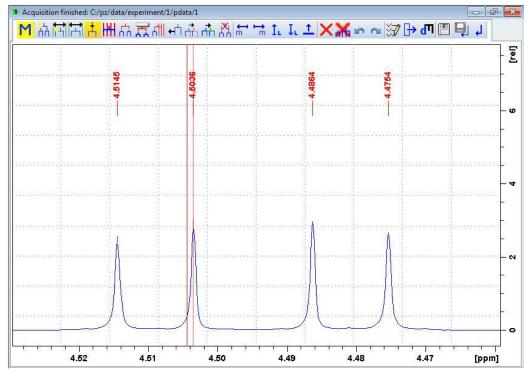
There will be 2 cursor lines displayed. Use the right cursor line to select a peak.

Step 1: Move the cursor lines slowly towards the first peak.

Step 2: The right cursor line will stop when it gets in to the center of the peak.



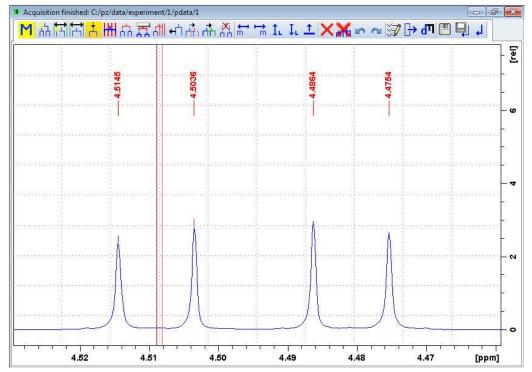




Step 5: The right cursor line will stop when it gets in to the center of the peak.

Step 6: Click the left mouse button.

A small marker is placed above the top of the two peak.

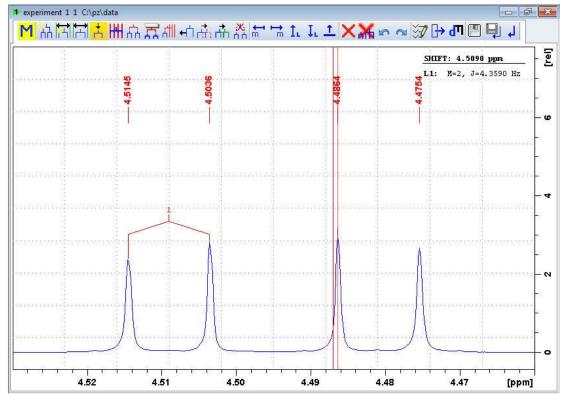


Step 7: Move the cursor lines in to the center of the two marked peaks.

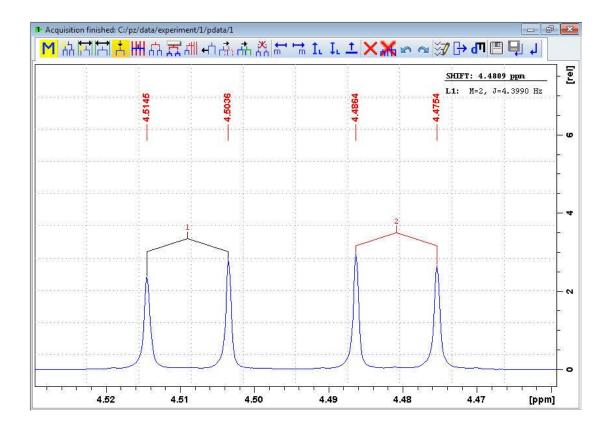
Step 8: Right-click to open the shortcut menu.

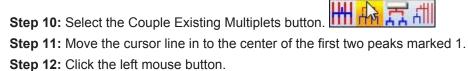
Define Multiplet
Finish Current Mode
Automatically Define Multiplet
Automatically Define Multiplet By Region
Define Multiplet By Region
Define Multiplet Manually
Define Multiplet By Free Grid
Couple Existing Multiplets
Define Multiplet By Coupled Grid

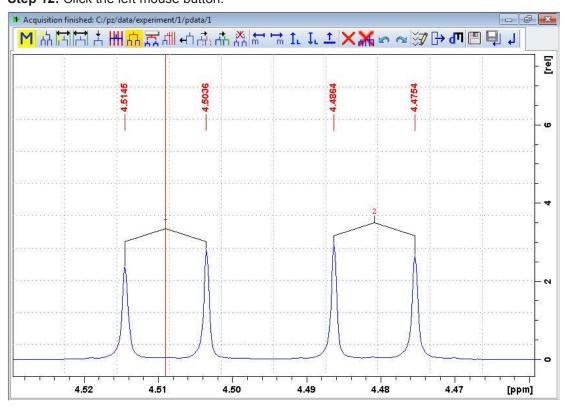
Step 9: Select Define Multiplet.



• Repeat steps 1 - 9 starting with the third peak and ending with the fourth peak.







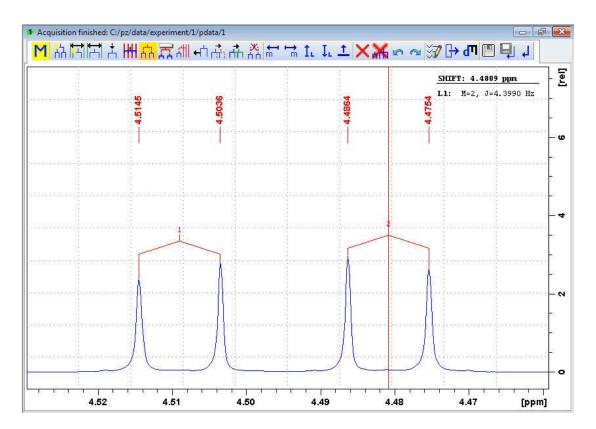
Step 13: Move the cursor line in to the center of the second two lines marked 2.

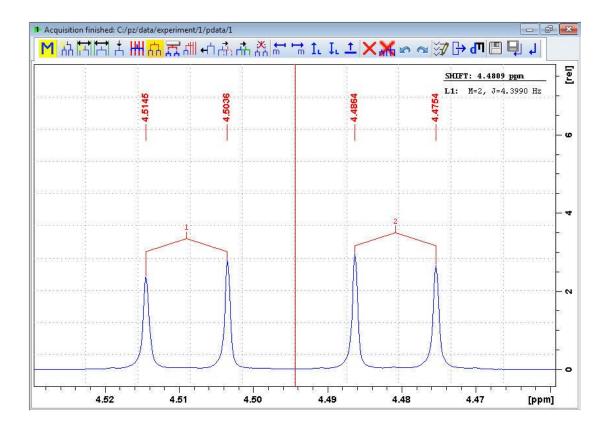
j

While executing the next 2 steps, the colors of the brackets over the peaks 1 and 2 change from black to red.

Step 14: Click the left mouse button.

Step 15: Move the cursor into the center of the displayed multiplet.

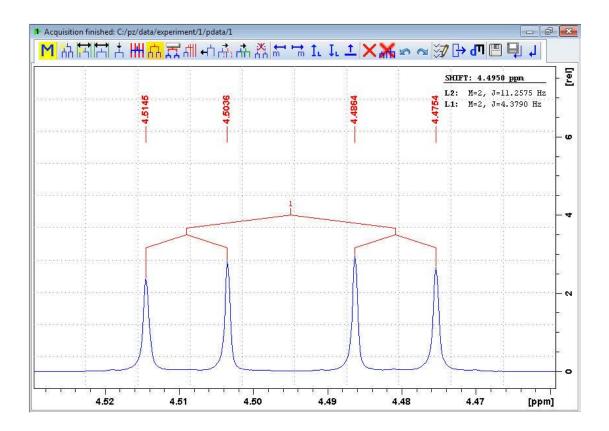




Step 16: Right-click to open the shortcut menu.



Step 17: Select Define Multiplet.

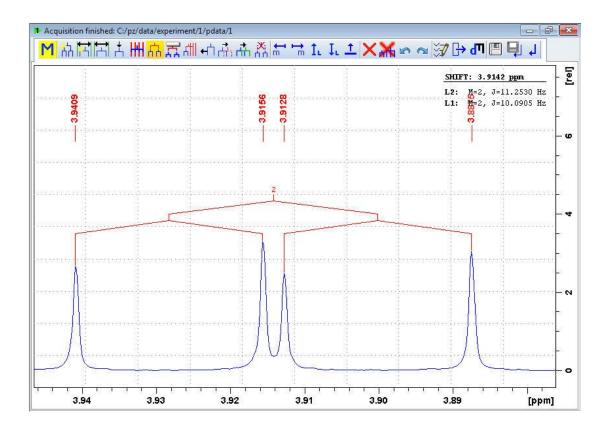


Step 18: Right-click in the spectrum window to open the shortcut menu.

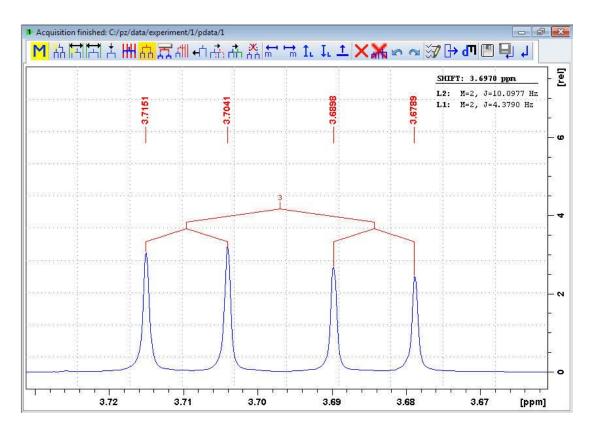
Step 19: Select Finish Current Mode.

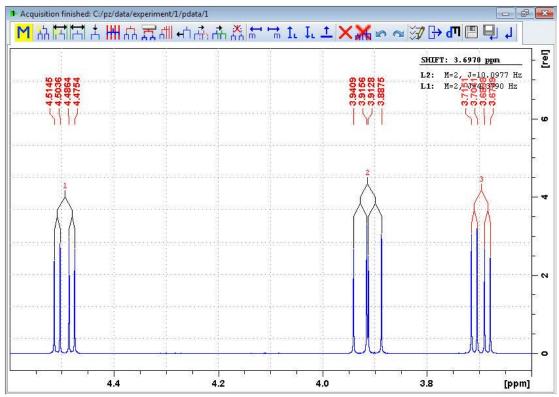


- Expand the multiplet at **3.9 ppm**.
- Repeat steps 1 19 for this multiplet.



- Expand the multiplet at 3.7 ppm.
- Repeat steps 1-19 for this multiplet.

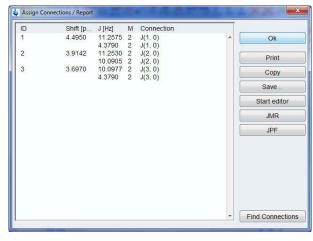




• Display all 3 multiplets.



• In the Assign Connections / Report window, click Find Connections.



• In the Multiplet Connection options window, click **OK**.

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

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

• In the Assign Connections / Report window, click **OK**.

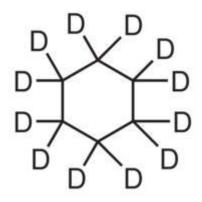
ID	Shift [p	J [Hz]	М	Connection		
	4.4950	11.2575	2	J(1, 2)	^	Ok
2	3.9142	4.3790 11.2530	2	J(1, 3)		
2	3.3142	10.09	2	J(2, 3)		Print
3	3.6970	10.09	2			Сору
		4.5790	2	3(3, 1)		Save
						Start editor
						JMR
						JPF
					-	Find Connections

Click Return, save multiplets [sret].

12 Adding a New Solvent

12.1 Introduction

This chapter describes the procedure how to add a new solvent to the solvent list. As an example, the solvent C_6D_{12} , Cyclohexane-d12 is used.



12.2 Adding Cyclohexane-d12 to the Solvent List

- At the command prompt, type edsolv.
- In the Edsolv window, select the Solvents tab.

🖕 Edsolv		x
Solvents Edit	BSMS Help	
Lock Nucleus	Current probe	
● 2H ○ 19F	Current probe: 5 mm DUL 13C-1H/D Z-GRD Z111650/0002	
Solvents Lock	Spectrum Reference Properties	
≙ Solvent	Description	
Acetic	acetic acid-d4	-
Acetone	acetone-d6	E
C6D6	benzene-d6	
CD3CN	acetonitrile-d3	
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)	
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)	
CDCI3	chloroform-d	-
	с	lose

• Right-click on the C₆D₆ solvent and on the shortcut menu, select Add new solvent.

Add new solvent
Edit solvent
Delete solvent
Copy and paste solvent
Hide current solvent
Сору
Export
Import
Print
Print preview
Table properties

- In the Password request window, type the password.
- In the Password request window, click **OK**.

🖕 Password	request	X
Please ent password:		administration
	•••	
	OK	Cancel

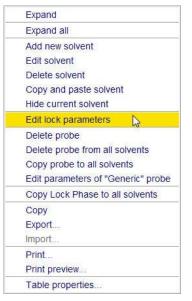
- Add the following Solvent parameters: Solvent name = C₆D₁₂
 Solvent description = Cyclohexane-d12 enable Lock Solvent
 Melting Point [K] = 279
 Boiling Point [K] = 354
- In the Create solvent window, click **OK**.

🧅 Create solvent	
Create a new solvent	
Solvent parameters	
Solvent name:	C6D12
Solvent description:	Cyclohexane-d12
Lock Nucleus:	2Н
Lock Solvent:	
Hidden:	
Auto Phase:	
Melting Point [K]:	279
Boiling Point [K]:	354
Solvent class:	
	OK Cancel

• In the Edsolv window, select the Lock tab.

Solvents Eult	BSMS Hel	p						
Lock Nucleus	Current probe							
● 2H ◎ 19F 0	Current probe:	5 mm DUL 13C-1	H/D Z-GRD Z111650/000	2				
Solvents Lock :	Spectrum Refe	erence Properties						
Solvent	V Probe	Lock Power	Lock Power Instep	Loop Gain	Loop Time	Loop Filter	Lock Phase	Shift [ppm]
Acetic	Generic	-38	10	-10	0.1	100	-1	2.03
Acetone	Generic	-38	10	-2	0.1	200	-1	2.04
C6D12	Generic	-20	10	-10	0.4	100	-1	
C6D6	Generic	-26	10	-0	0.2	300	-1	7.16
E CD3CN	Generic	-38	10	-2	0.1	200	-1	1.93
CD3CN_SPE	Generic	-20	10	-10	0.1	100	-1	1.93
	Generic	-25	10		0 1	100	_1	33
<				m				Clos

• Right-click on the C₆D₁₂ solvent and on the shortcut menu, select **Edit lock parameters**.



Add the following Lock parameters: Probe description = Default probe Lock power = -30 Loop gain = -0 Loop time = 0.4 Loop filter = 200 Lock phase = -1 Lock power instep = 10 Shift [pm] = 1.38 Relative intensity = 1 Type = Lock

Lock parameters	Generic			
Probe description:				
Lock power:				
Loop gain:	0			
Loop time:	0.4			
Loop filter:	200			
Lock Phase:	-1			
Lock power instep:	10			
Signals Signal Shift [ppm] R 1 1.38	elative intensity	Type	Description	Delete
	Add Signal	Delete Sign	al	_

• In the Edit lock parameters window, click **OK**.

The Lock parameters will be stored for the current probe and for the selected probe only. This current probe (as defined in edhead) will then also appear in the Lock parameters list. Parameters for the other probes will remain unchanged and remain visible as probe type Generic.

Lock Nucleus	Current probe							
2H 19F	Current probe:	5 mm DUL 13C-1H	/D Z-GRD Z111650/000:	2				
Solvents Lock	Spectrum Refe	erence Properties						
Solvent	V Probe	Lock Power	Lock Power Instep	Loop Gain	Loop Time	Loop Filter	Lock Phase	Shift [ppm]
HDMSO	Generic	-25	10	-9.4	0.464	50	-1	2.49
🕀 Pyr	Generic	-25	10	-15	0.1	100	-1	8.71
Acetone	Generic	-38	10	-2	0.1	200	-1	2.04
CD3CN	Generic	-38	10	-2	0.1	200	-1	1.93
C6D6	Generic	-26	10	-0	0.2	300	-1	7.16
H2O+D2O	Generic	-18	10	-5	0.35	100	-1	4.7
C6D12	5 mm D	-30	10	-0	0.4	200	-1	1.38
< [m				

• In the Edsolv window, select the Spectrum Reference tab.

Solvents Edit	BSMS He	elp			
Lock Nucleus	Current probe	9			
🖲 2H 🔘 19F	Current prob	e: 5 mm DUL 13C-1H/D Z-GF	RD Z111650/0002		
Solvents Lock	Spectrum Re	ference Properties			
			Nucleus: 1H	•	
A Solvent	Noise	Reference Shift [ppm]	Search Width [ppm]		
Acetic	10	0	0.5		
Acetone	10	0	0.5		
C6D12					
C6D6	10	0	0.5		
CD3CN	10	0	0.5		
CD3CN SPE	10	1.93	0.2		

• In the Nucleus list, select 1H.

1H	-
1H	
2H	
ЗH	
ЗНе	
6Li	
7Li	
9Be	
10B	-

• Right-click on the C₆D₁₂ solvent and on the shortcut menu, select **Edit spectrum** reference parameters.

Ad	d new solvent
Edi	it solvent
De	lete solvent
Co	py and paste solvent
Hid	e current solvent
Edi	t spectrum reference parameters
Со	ру
Exp	port
Imp	port
Pri	nt
Pri	nt preview
Tal	ble properties

 In the Edit spectrum reference parameters window, add the following Spectrum reference parameters for C₆D₁₂:

Noise factor = 10

Reference shift [ppm] = 0

Search Width [ppm] = 0.5

- Click Add solvent regions.
- Add the following regions:

Region 1, Lower limit [ppm] **15**, Upper limit [ppm] **1.42** Region 2, Lower limit [ppm] **1.35**, Upper limit [ppm] **0.2** Region 3, Lower limit [ppm] **-0.2**, Upper limit [ppm] **-3**

	eference parameters		1
	Noise fac	tor: 10	
	Reference shift [pp	m]: 0	
	Search Width [pp	m]: 0.5	
15	0.2	Region 1 Region 2	
1 95	0.2	Region 2	
1.35 -0.2	-3	Region 3	

• In the Edit spectrum reference parameters window, click **OK**.

Solvents Edit					
Lock Nucleus	Current probe	9			
● 2H ◎ 19F	Current prob	e: 5 mm DUL 13C-1H/D Z-GF	RD Z111650/0002		
Solvents Lock	Spectrum Re	ference Properties			
			Nucleus: 1H	•	
△ Solvent	Noise	Reference Shift [ppm]	Search Width [ppm]		
Acetic	10	0	0.5		
Acetone	10	0	0.5		
C6D12	10	0	0.5		
C6D6	10	0	0.5		
CD3CN	10	0	0.5		
CD3CN SPE	10	1.93	0.2		

• In the Edsolv window, select Nucleus = 13C.

13C	-
13C N	-
14N	-
15N	
170	
19F	
21Ne	
23Na	
25Mg	-

• Right-click on the C₆D₁₂ solvent and on the shortcut menu, select **Edit spectrum** reference parameters.

	Add new solvent
	Edit solvent
	Delete solvent
	Copy and paste solvent
	Hide current solvent
	Edit spectrum reference parameters
	Сору
	Export
	Import
	Print
	Print preview
	Table properties
_	

- Add the following Spectrum reference parameters for C₆D₁₂ Noise factor = 10 Reference shift [ppm] = 0 Search Width [ppm] = 5
- Add the following regions:

Region 1, Lower limit [ppm] **200**, Upper limit [ppm] **27.5** Region 2, Lower limit [ppm] **25.5**, Upper limit [ppm] **1** Region 3, Lower limit [ppm] **-1**, Upper limit [ppm] **-5**

Spectrum refer	ence parameters			
	Noise fa	ctor:	10	
	Reference shift [p	pm]:	0	
	Search Width [p	pm]:	5	
200 25.5	m] Upper limit [ppn 27.5 1	R	Description egion 1 egion 2	Delete
-1	-5	R	egion 3	
Add	Solvent Region	D	elete Solvent Reg	ion

• In the Edit spectrum reference parameters window, click OK.

• In the Edsolv window, click **Close**.

Solvents Edi	t BSINS He	пр			
Lock Nucleus	Current probe	9			
● 2H © 19F	Current probe	e: 5 mm DUL 13C-1H/D Z-GF	RD Z111650/0002		
Solvents Lock	Spectrum Re	ference Properties			
			Nucleus: 13C	•	
A Solvent	Noise	Reference Shift [ppm]	Search Width [ppm]		
Acetic	10	0	5		
Acetone	10	0.9	5		
C6D12	10	0	5		
C6D6	10	0.22	5		
CD3CN	10	0	5		
CD3CN SPE	10	0	5		

12.3 TopShim Solvent Parameters

Follow the instructions in the *TopShim Automatic Shimming Users Manual*, chapter *Solvents* to configure the TopShim solvent parameters.

13 Troubleshooting

13.1 Resetting the ELCB Board on a AV-III Console

Follow the instructions below, in case of a communication problem with the BSMS on a AV-III spectrometer due to a power glitch or during a console boot up. It is always essential in case of a BSMS problem to have stored a good shim file on a regular base.

• At the command prompt, type ha.

	ises		>
The hardware devices lis and configured with a "W			cessed
Press the "Open" button		er with a	
connection to this device			
Press the "Refetch addre		n to reloa	be
addresses from DHCP si	erver.		
Main Controller			
IPSO	149 236 9	9 254	Open
1 00	110.200.0		Open
Digital Receiver Unit	110.200.0		
	149.236.9	9.89	Open
Digital Receiver Unit		9.89	I I I I I I I I I I I I I I I I I I I
Digital Receiver Unit	149.236.9		Open
Digital Receiver Unit	149.236.9		I I I I I I I I I I I I I I I I I I I

• In the BSMS Lock/Shim group, click Open.

149.236.99.20	Open
	149.236.99.20

• In the BSMS Service Web window, click Service.

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• In the BSMS Service Web window, click ResetELCB.

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• In the Hardware ethernet addresses window, click Close.



• At the TopSpin command prompt, type rsh <Name of the last good shim file>.

13.1.1 Downloading a New DRU Firmware

The instructions below show how to install new DRU Firmware. This would be needed e.g. in case from a request made by Bruker Center or the Application Hotline. It is also possible that during a software upgrade a message appears with a request to install new DRU firmware. Firmware may be e-mailed or can be downloaded from an ftp-site (e.g. *ftp.bruker.ch/NMR/ download/servtools/firmware*). It is recommended to move the firmware into the appropriate directory:

<TopSpin-home>/conf/instr/servtool/dru

It can be uploaded to the DRU service web as follows:

• At the TopSpin command prompt, type ha.

🝓 Hardware ethernet addres	ses	×
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Press the "Open" button connection to this device Press the "Refetch addro		
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DRU1	149.236.99.89	Open
_Lock/Shim-		5
BSMS Z100818/0936	149.236.99.20	Open
Refetch ac	Idresses Prir	it Close

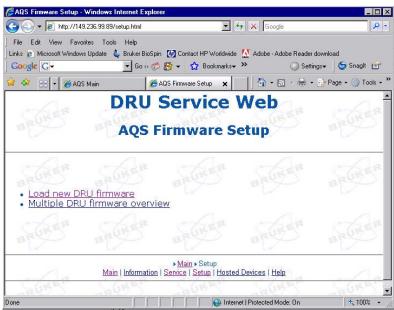
• In the DRU1 Digital Receiver Unit group, click **Open**.

Digital Receiver Unit
DRU1 149.236.99.89 Open

In the AQS Main window, click AQS Firmware Setup.

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• Click Load new DRU Firmware.



The current DRU Firmware version is displayed in this window. If no firmware newer than the current one is available, simply close the window. You may want to discuss any further action with a Bruker Center.

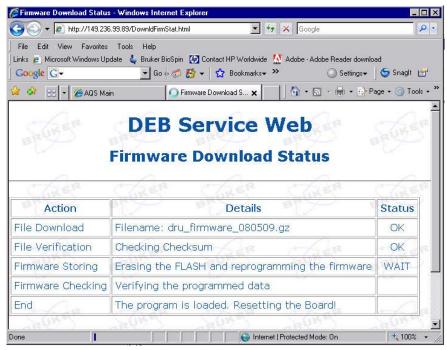
• In the Firmware Download window, click Browse.

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· Double-click the newest firmware file.

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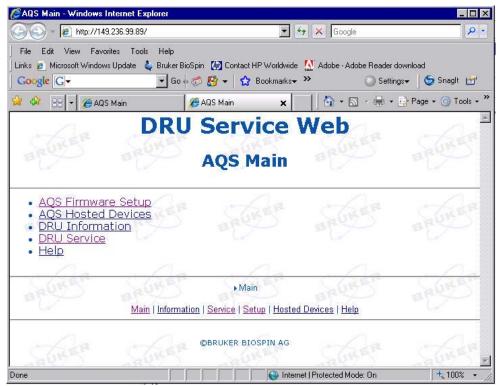
• Click Install firmware.





After the program is loaded and the board is reset the current Web page will close automatically and the Web page in the figure below is displayed.

· Close the Web page.



• In the Hardware ethernet addresses window, click Close.

🔄 Hardware ethernet addre	ISSES	<u>×</u>
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Press the "Refetch add addresses from DHCP s		d
Main Controller		
IPSO	149.236.99.254	Open
Digital Receiver Unit		
DRU1	149.236.99.89	Open
Lock/Shim		
BSMS Z100818/0936	149.236.99.20	Open
Refetch a	ddresses Print	Close

14 Contact

Manufacturer

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WEEE DE43181702

NMR Hotlines

Contact our NMR service centers.

Bruker BioSpin NMR provides dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

Please select the NMR service center or hotline you wish to contact from our list available at:

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Phone: +49 721-5161-6155 E-mail: nmr-support@bruker.com

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