

# TopSpin

Advanced NMR Experiments
 User Manual
 Version 005

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 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 that, if not avoided, will result in death or serious injury. This signal word is limited to the most extreme situations.

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 that, 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 that, if not avoided, could result in minor or moderate injury.

This is the consequence of not following the warning.

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

## NOTICE

## NOTICE: Indicates information considered important, but not hazard-related (e.g. messages relating to property damage).

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.

j

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

## 2 Introduction

### 2.1 General

This manual was written for AVANCE systems running **TopSpin version 4.x 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 into the prosol table.

This manual features various advanced procedures for <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N and <sup>31</sup>P 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 The TopSpin Interface

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	examidaa ■ Example_MenthylAnthranilate ■ exam1d_1H ■ exam1d_13C ■ 1 - zgp30 - 13C(1H) AV 500 Cl ■ 1 - 13C(1H) AV 500 Choles ■ 999 - 13C(1H) AV 500 Chol ■ 2 - jmod - 13C APT AV 500 Chol	examtd_13C 1 1 C/l 13C/1H) AV 500 Unegned			9		General         F           JATE + 2607/90/18         Y           TATE + 2607/90/18         Y           TATE + 2607/90/18         Y           TATE + 2607/90/18         Y           FIL (10)         F           TI = 30268         SF - 125/28           SF - 125/28         W           SF - 23761.905         W           G         G
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1	Menu Bar	8	Status Display Bar
2	Workflow Button Bar	9	Dataset Window
3	Tool Bar	10	Dataset Window Tabs
4	Browser and Search Window	11	Print, Export, Copy and Publish
5	Structure Window, Command Line History, Status Line History	12	Viewing Options
6	Command Line	13	Window Switcher, Login, Setup Preferences and Help
7	Current Dataset Bar		



For all changes in the TopSpin appearance use the global **Setup preferences** button in the menu bar.

TopSpin can be tailored to your preference in many respects. This ranges from startup options to spectrum objects, menu settings, remote connections, colors and fonts etc. Every standard user can create his own set of preferences.

A dialog box will appear with, at the left side, the categories that can be tailored. Click the category you want to view or change. It will become highlighted and the corresponding objects will be displayed at the right part of the dialog box.

Preferences		×
Administration Items	Administration Items	
Window Settings	Auto-open last used dataset when restarting TopSpin	
Processing Preferences	Show TopSpin data examples directory in data browser	$\checkmark$
Text Editors	Setup users for TopSpin-internal login/logoff and esign	Change
Regulated Environments	Automatic termination of TopSpin when idle time exceeded	Change
Miscellaneous Mobile Connection	Automatic locking of TopSpin when idle time exceeded	Change
Directories	Enable automatic command spooling	
Acquisition	Window Settings	
More Preferences	All in One Fonts & Icon Size	Change
	Fonts and colors	Change
	'Arrange' internal windows is only applied to dataset windows	
	Tabbed pane layout	Change
	Processing Preferences	Change
	Enable automatic data processing	$\checkmark$
	Text Editors	
	Preferred text editor Internal ~	Change
	Text editor for edpul, edmac, edpy, always in foreground	
	Regulated Environments	
	Enable extended audit trailing	$\checkmark$
	Enable GUI restrictions and protection of preferences	
	Miscellaneous	
	Collapse parameter editors	
	Display EXPNO/PROCNO list when opening data	$\checkmark$
	Record commands in protocol file	$\checkmark$
		nglish ~
	Use "Default Datastation" (restart required)	
	Mobile Connection	
	Cloud Configuration	Change
	Directories	
	Dir. of structure files for structure viewer	
	Global search path for plot layouts	Change
	Manage source directories for edpul, edau, etc.	Change
	Acquisition	
	Show "ased" parameter selection with "eda"	
	Overwrite existing FID without inquiry (ZG safety off)	
	Auto open acquisition window after 'zg'	
	Configure accounting & data archiving after 'zg'	Change
	Automatically perform getprosol during rpar/edc/new	
	Automatically convert 64bit SER data into TopSpin 3.x format	$\checkmark$
	Scale acquired FID according to receiver gain	
	Scale acquired FID according to number of scans	
	More Preferences	
	Spectra Display Preferences	Change
	Spectra Printing Preferences	Change
	Browser Preferences	Change
	Status Bar Preferences	Change
	Lock Display Preferences	Change
	BSMS Display Preferences	Change
Sea	rch Apply Close	Reset

#### **TopSpin on High Resolution Screens**

To adapt the font and icon size to a small/standard/medium or large screen resolution

• click Setup Preferences and in the category Window Settings | All In One Fonts & Icon Size click Change.

🍬 All in One F	🖕 All in One Fonts & Icon Size 🛛 🗙					
Modify Sizes of Fonts and Icons Please Choose Size. New Sizes are available after restart of Topspin.						
ABC abc	ABC abc	ABC abc	ABC abc			
Small	Standard	Medium Size	Large			
		Apply and Restart	Save Cancel			

## Help/About TopSpin/Version and License Information ?

This button gives information about the TopSpin documentation, software version and license.



Clicking **Manuals (docs)** or entering **docs** on the command line will open the list of all manuals delivered with TopSpin.

 Click Help | Manuals | Acquisition Application Manuals | Dosy will open the DOSY manual, for example.

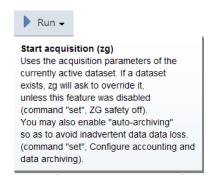
		×
	Please click on a manual title to open the document!	
Seneral		
Jser Manual	A description of the TopSpin user interface and its functionality	
Control & Function Keys	A list of predefined Control and Function keys.	
Release Letter	Describes the changes and new features of this TopSpin version and the spectrometer hardware requirements	
Software License Agreement	Software Copyright / License Documents.	
Beginner Guide	For AVANCE NEO spectrometers:	
-	A basic description of the Bruker NMR spectrometer, its main components, functionality and usage.	
Beginner Guide (other languages)	Versions of the Beginners Guide in other languages are available on the Bruker Web	
CodeMeter License Management	Installing and Managing Software Licenses.	
Acquisition - User Guides		
Basic NMR Experiments	A step-by-step tutorial of setting up and running the most frequently used 1D and 2D experiments.	
Advanced NMR Experiments	A step-by-step tutorial of setting up and running DOSY, Inverse and 19F experiments.	
3D/Triple-Resonance experiments	How to set up and run common 3D/triple-resonance experiments for isotope labeled proteins	
Acquisition - Application Manuals		
Fretic2	Introduction into NMR Quantification using the Eretic 2 method	
Solids Introduction	A basic introduction into the NMR of solids.	
Solids	A description of setting up and running Solids experiments.	
TopSolids	Assisted Biological Solid State NMR.	
Cross Polarization Dynamics	An introduction into Cross Polarization Dynamics experiments.	
SB/MAS	A description of setting up and running SB/MAS experiments.	
C-NMR	A description of setting up and running LC-NMR experiments.	
Dosv	A description of setting up and running Dosy experiments.	
Diffusion	A description of setting up and running Diffusion experiments.	
Shapetool	A description of creating, analyzing and manipulating RF- and gradient Shapes.	
TopShim	User manual for the automatic shimming tool.	
CNCQ	Complete molecular confidence for quality assurance	
APSY	Automated Projection Spectroscopy: Get N-dim. correlations via low-dimensional projections.	
SmartDriveNMR	The smart spectrometer for structure verification.	
NMR Thermometer	Introduction into NMR Thermometer.	
NaveMaker	Pulse Shaping Software.	
cquisition & Processing Referen	nces	
Acou. Commands & Parameters	A description of all acquisition and acquisition related commands and parameters.	
Proc. Commands & Parameters	A description of all processing and analysis commands and parameters.	
Edprosol Manual	How to set up probe and solvent dependent parameters	
Edlock/Edsolv Guide	A description of how to setup solvent and lock dependent parameters.	
Pulse Program Catalogue, 1D/2D	A graphical presentation of the Bruker supplied pulse programs, 1D and 2D experiments.	
Pulse Program Catalogue, BIO	A graphical presentation of the Bruker supplied pulse programs, biomolecular experiments.	
NUS Parameters	A description of the parameter setup for Non Uniform Sampling	
<		>
		-

Enter **help** to get information for an individual command. Three different sources can be selected for the search:

🖕 help	×
Options Search in comma Search in NMR G Display Comman	uide knowledge base
Command or Search	<u>O</u> K <u>C</u> ancel

#### **Tooltips**

Pointing to a button with the mouse in the various menus opens a tooltip that describes the button functionality. Example:



#### **Workflow Button Bar**

The workflow-based interface with its arrangement of all working processes allows the user to control the workflow intuitively.

Clicking one of the menu buttons opens the corresponding workflow. It contains a horizontal feature list which stays open and provides all functionality for this workflow with one mouse-click.

Furthermore, some of the buttons on the Workflow button bar include a **drop-down** arrow. Click the **drop-down** arrow to see more options.

Pro <u>c</u> . Spectrum 👻
<u>C</u> ompute Spectrum from raw data (proc1d y)
Configure Standard Processing (proc1d)
Window Multiplication (wm)
Fourier <u>T</u> ransform (ft)
Eourier Transform Options (ftf)
Start Automation AU Program (xaup)

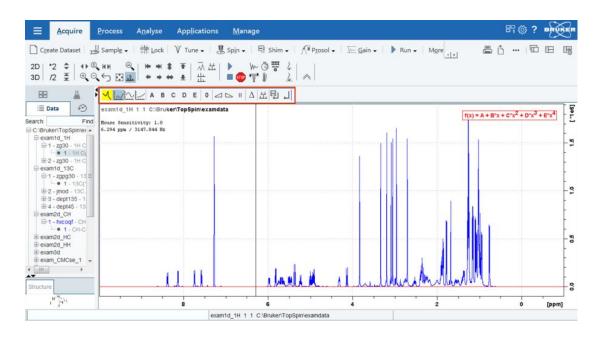
#### **Dataset Toolbar**

Depending on which dataset window tab is selected, an individual dataset toolbar is displayed, in the example the ACQU toolbar:



**Note:** The ACQU window tab is only displayed when TopSpin controls a spectrometer (noticeable through the Acquire tab in the TopSpin menu). When TopSpin is installed for processing-only, the Acquire tab is also not displayed.

**Note:** Sometimes the dataset toolbar is displayed without dataset window tabs, e.g. entering a command as **.basl** will display the baseline correction toolbar:



#### Browse and Search Window

The Browser window provides tabs as:

- Data browser and Search
- · History browser
- Dataset Switcher
- Experiment Selector library



The window can be toggled On or Off with a click on the black left or right arrow.

Alternatively, the **Toggle Perspective** button on the top-right of the TopSpin window can be clicked.

The data tree tab includes a search field. Enter a search phrase, for example:

Search: pulprog=hsqc

X

To limit the search results, select or deselect the data trees with SHIFT click or CTRL click.



#### **History Tab**

The History tab displays the last opened datasets in a list.



#### **Dataset Switcher Tab**

This tab has a similar function as the Window Switcher a quick overview of all currently opened datasets.

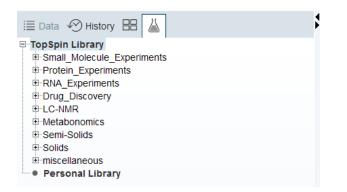


see below, but only provides

<u>الله</u> exam1d_13C
Expno: 1
H Multiple Display Mode
· · · · · · · · · · · · · · · · · · ·

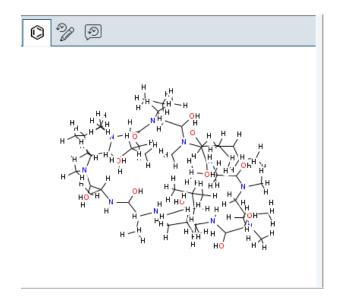
#### **Experiment Library Tab**

The Experiment library tab provides easy access to the vast experiment library of standard experiments that is available in the standard release of TopSpin. It is also a tool that allows the user to personalize his most frequently used experiments into a separate library.



#### **Structure Window**

Molecular structures as *.mol* or *.pdb* files can be displayed here and are freely resizable. The structure window can be toggled On or Off with a click on the black **up** or **down** arrow **\_**.



• Drag the vertical or horizontal split bar to resize the structure window.



The **Window Switcher** button indicates the number of all opened dataset windows plus all opened TopSpin window types like

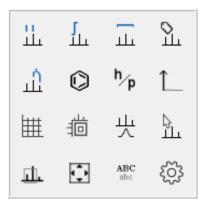
- · Dataset windows.
- · Lock display window.
- Acquisition display window.
- BSMS display window.
- Temperature unit window.
- Click the Window Switcher button to switch between these windows.

I Temperature Control Suite	BSMS Control Suite
exam2d_HC Expno: 3	exam1d_13C
exam1d_1H Expno: 1 Procno: 1	

A dataset can be closed with the **Close** button

#### Viewing Integrals, Peaks and other Spectra Components

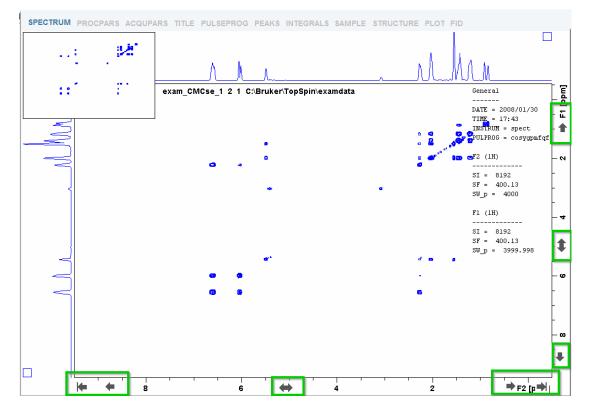
• Click **Spectrum display properties** to toggle the visibility of integrals, peaks and other spectra components. They can only be displayed when available.



#### Shift, Scroll and Zoom Spectra Axes

Clicking and dragging the spectrum axis allows intuitive scrolling through the spectrum.

- Single Clicks on the end regions of the axes or a click and dragging the mouse shift through the axes depending on the mouse position, see the next figure.
- Double click in the middle of the spectra axes switches to full axes region.
- Double click in the middle of the spectra itself maximizes all axes regions.
- Click on axes to shift left, right, up or down.
- Turn mouse wheel to zoom in or out.



#### **Dataset Windows**

The TopSpin window has a dataset area that may contain multiple dataset windows.

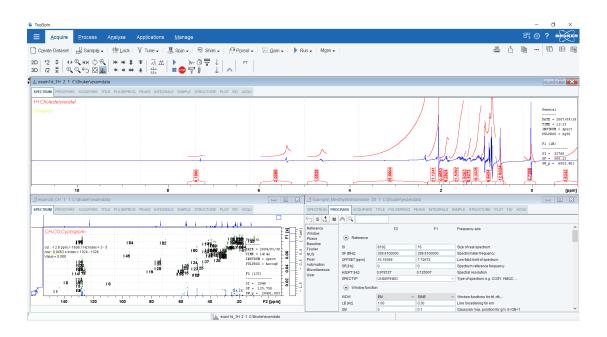
To open multiple dataset windows

• Right-click in the Browser window and in the list, select **Display in new window**.

Note, that selecting **Display** will override a current dataset.

The size of the data area depends on the overall size of the TopSpin window and on presence of the Browser. Note, that the Browser window can be toggled On or Off with the **Tog-**

**gle Perspective** button <sup>1</sup> The following figure shows the TopSpin window without the Browser and three dataset windows.



Note, that the three dataset windows show different data objects: a 1D spectrum, a 2D spectrum and 1D processing parameters.

#### How to Move a Dataset Window

• Click-hold the title bar and move the mouse.

#### How to Resize a Dataset Window

- Move the cursor to the window edge until it becomes a double-headed arrow.
- Click-hold that position and move the mouse.

Depending on the position of the double-headed arrow, you can change the window height, width or both.

#### How to Select (activate) a Dataset Window

The active dataset window is the window of which the title bar is highlighted. The TopSpin menu, tool bars and command line commands correspond to and act on that window. Only one dataset window is active at a time.

To activate a different dataset window:

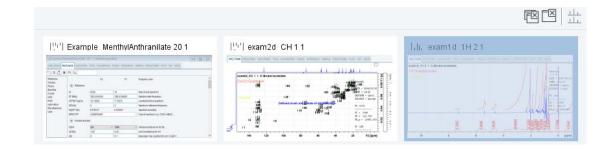
Click the Window Switcher button

• Click in the desired dataset window or click its title bar.

or



to switch between the windows.



## The TopSpin Interface

#### How to Arrange Dataset Windows

If the data area contains multiple dataset windows, you can arrange them in various ways. All the arrange commands arrange the windows left to right and/or top to bottom in the order in which the windows have been active. The currently active dataset window will therefore be positioned at the top and/or left of the data area.

To arrange the dataset windows as a grid:

- Click Show Layout Options and Show as Grid
- Depending on the number of windows, they will be arranged vertically and/or horizontally.

To arrange dataset windows in stack:

• Click Show Layout Options and .

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To arrange dataset windows side by side:

Click Show Layout Options
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	A married 31 2 1 C Enderlearnide			

To display a dataset windows as full screen:

Click Show Layout Options and or click the full screen windows button

To close the active dataset window:

 Click File | Close Active Window or enter Crtl-w. or • Click the 🗙 button in the windows title bar.

To close all dataset windows:

• Click File | Close All Windows or enter closeall.

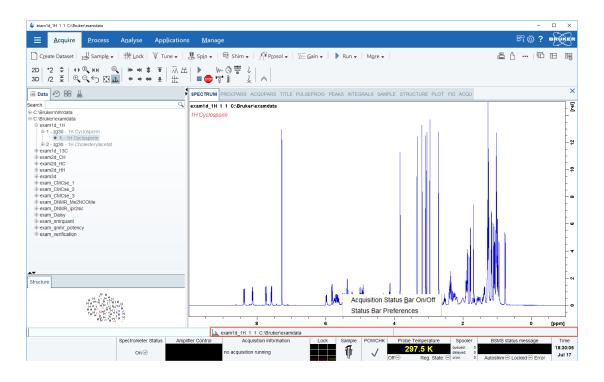
#### How to Swap Dataset Windows

Within a certain layout, you can easily swap two TopSpin windows with the command **swin**. If the data area contains exactly two windows, **swin** simple swaps their position and geometry.

If it contains more than two dataset windows, **swin** opens a list from which you can select any window to be swapped with the currently selected (active) window.

#### **Acquisition Status Bar**

• Right-click the Status display or Current dataset bar to toggle the Acquisition status bar On or Off.



• Click **Status Bar Preferences** to set the *Auto-open the acquisition status bar* option as default.

Acquisition Status <u>B</u>ar On/Off Status Bar Preferences

🛶 Status Bar Preferences	×
Acquisition status bar	
Auto open acquisition status bar	<b>v</b>
Include spooler	-
Include time	-
Include sample temperature	-
Include channel or accessory channel temperature	<b>v</b>
Include acquisition status	-
Include acquisition indicator	<b>v</b>
Include lock signal	-
Include peak power check (POWCHK) indicator	<b>v</b>
Include sample state	<b>v</b>
Include shim coil temperature	<b>v</b>
Include BSMS status	<b>v</b>
Include spectrometer hardware (PDU) indicator	<b>v</b>
Include amplifier control	<b>v</b>
Include ADC control	<ul> <li>Image: A start of the start of</li></ul>
Apply Back Clo	se

The acquisition status bar contains the new Spectrometer Status area to turn the spectrometer On and Off. This functionality is also available in the menu with a click on **Manage | Spectrometer | Spectrometer power On/Off** or the command **pdudisp**.

1										
	Spectrometer Status	Amplifier Control	Acquisition information	Lock	Sample	POWCHK	Probe Temperature	Spooler	BSMS status message	Time
	On⊗		no acquisition running		Ð	$\checkmark$		queued: 0 delayed: 0 cron: 0	Autoshim C Locked Error	18:30:0 Jul 17

#### Print, Export and Publish

Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

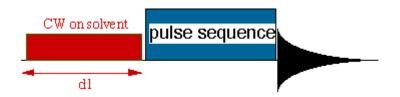
• Click **Show More publishing options**, e.g. to copy and paste, E-mail or use shared cloud directories.



## 4 1D Solvent Suppression Experiments

### 4.1 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.

#### 4.2 Samples

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

### 4.3 **Preparation Experiment**

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the Create New Dataset window, enter or select:

NAME = solvent\_suppression\_exp EXPNO = 1

Directory = e.g. C:\DMB

**Directory**, **NAME** and **EXPNO** define where the new experiment dataset will be stored on the computer:



Directory/NAME/EXPNO/ In the example: C:\DMB\solvent\_suppression\_exp\1

**Directory** can be selected in the drop-down list, or a new location can be typed in the text field. **NAME** is a subdirectory of **Directory**, and **EXPNO** is a subdirectory of **NAME**. **EXPNO** must be a positive integer. It provides a way to store multiple related datasets under the same **NAME**.

	xperiment by creating a new data set and arameters according to the selected experiment type.
	periments several datasets are created.
	umber of receivers in the Options.
Dataset	
	solvent_suppression_exp
EXPNO	1
Directory	C:\DMB ~
Open in new window	w
<ul> <li>Parameters</li> </ul>	
O Use current paramet	ters
Read parameterset	ZGPR Select
Set solvent	H2O+D2O ~
Additional action	
Do nothing	
<ul> <li>Do nothing</li> <li>Execute getprosol</li> </ul>	
	P 1, 01, PLW 1 V Change
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Execute getprosol     Keep parameters     Advanced	eivers) 1

- In the Parameters group Parameters check **Read parameterset** and enter **ZGPR** or click **Select** to open the Experiment Table window.
- Select the experiment **ZGPR** from the table. If **ZGPR** is not in the list, uncheck **Show rec**ommended.

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TRHNCOGP2H3D	TRHNCOGP3D	TRHNCOGPHB3D	TRHNCOGPRC3D1	TRHNCOGPRC3D2	
TRHNCOGPRC3D3	TRHNCOGPRC3D4	TRHNCOGPRC3D5	TRHNCOGPRC3D6	TRNOEETF3GPSI3D.3	
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TROSYF3GPPH19	TROSYF3GPPHSI19	TROSYF3GPPHSI19.2	TRT1ETF3GPSI	TRT1ETF3GPSITC3D.3	
TRT2ETF3GPSI	TRTRETF3GPSITC3D.3	WATER	WATERSUP	WLOGSY_PREP	
XCTB	ZGCPPR	ZGESGP	ZGGPWG	ZGPR	
ZN67ZG					

- Click Set selected item in editor and Close.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **H2O+D2O**.
- In the TITLE field 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, e.g. **1-D Proton solvent suppression experiment**.
- In the Create New Dataset window, click OK.

For the following steps, use the Workflow button bar from left to right.

- 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 for best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.

#### 4.3.1 Acquisition

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

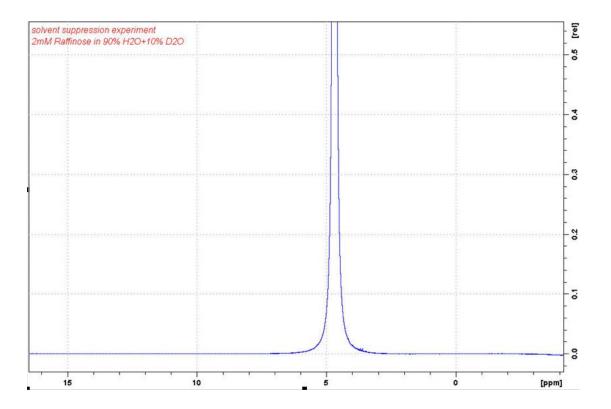
#### 4.3.2 Processing

- 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**.

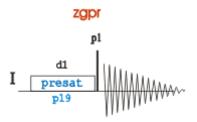
- On the Proc. Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).

🖕 procld			×
Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective wh one-click 'Proc. Spectrum' button.	essin	g options.	
Exponential Multiply (em)	<b>v</b>	LB [Hz] =	0.3
Fourier Transform (ft)	$\checkmark$		
Auto - Phasing (apk)	<b>v</b>		
Set Spectrum Reference (sref)			
Auto - Baseline Correction (absn)		Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_H.xwp 💌
Warn if processed data exist	<b>v</b>		
			Save Execute Cancel



### 4.4 1D Solvent Suppression with Pre-saturation

**Pre-saturation** is the most common procedure to minimize and suppress the intense solvent resonance when <sup>1</sup>H 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:



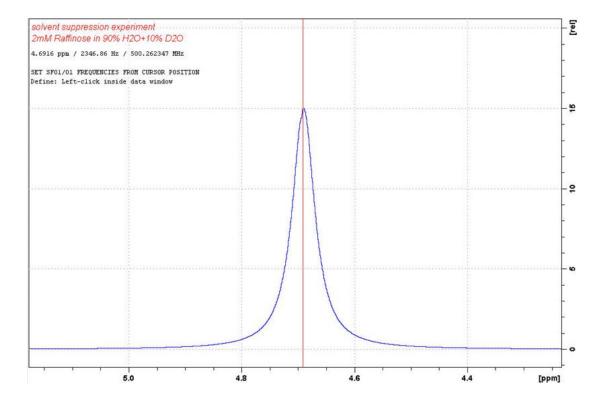
#### 4.4.1 Parameter Setup

 On the command line, type: wrpa 2

re 2

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

## **1D Solvent Suppression Experiments**



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

O1/O2/O3	×						
Define SFO1/O1 frequencies							
SFO1 [MHz] =	500.262345						
01/2/3 [Hz] =	2345.29						
01 02	03 Cancel						

- In the O1/O2/O3 window, click O1.
- In the Dataset window, select the **Spectrum** tab.

#### 4.4.2 Fine Tuning

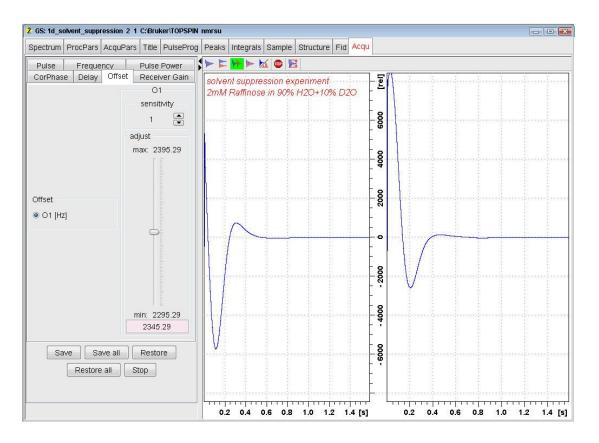
- On the menu bar, click Acquire.
- On the Workflow button bar, click Gain.
- On the Run button, click the drop-down arrow to see more options.
- In the list, select Real-Time Go Setup (gs).

Start Aquistion (zg)
Start acquisition, add to existing data (go)
Transfer Fid To Disk (tr)
Estimate Exp. Time (expt)
Real-Time Go <u>S</u> etup (gs)
Optimize Acquisition Params (popt)
Start Automation AU program (xaua)

Click 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.

i

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

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

## **1D Solvent Suppression Experiments**

2 GS: 1d_solvent_suppression 2 1 C:\Bruker\TOPSPIN r	nmrsu 🛛 🕞 🖬	×
Spectrum ProcPars AcquPars Title PulseProg	Peaks Integrals Sample Structure Fid Acqu	
CorPhase Delay Offset Receiver Gain Of sensitivity 0.01	solvent suppression experiment 2mM Raffinose in 90% H2O+10% D2O	
adjust		
Offset Offset Offset Offset Offset Max. 2348.23 max. 2348.23 min. 2347.23 2347.78 Save Restore all Stop		
	0.2 0.4 0.6 0.8 1.0 1.2 1.4 [s] 0.2 0.4 0.6 0.8 1.0 1.2 1.4	[s]

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

🤹 Save changed GS parameters	
Select the modified GS parameters to be saved:	
SFO1 [500.26234778 MHz]	
Select all	Select none OK Cancel

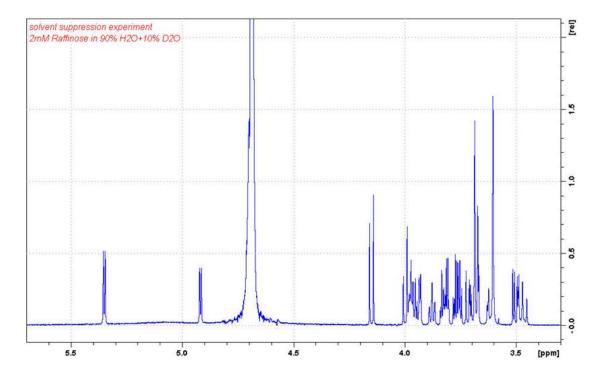
#### 4.4.3 Acquisition

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

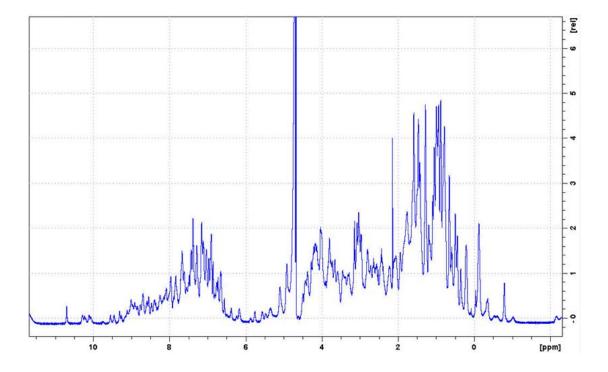
#### 4.4.4 Processing

• Process and phase correct the spectrum.

## **1D Solvent Suppression Experiments**

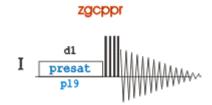


The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



### 4.5 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<sup>o</sup> pulses to further reduce the residual hump of the water signal:



#### 4.5.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter 1D Solvent Suppression with Pre-saturation [▶ 30].
- In the Dataset window, select the AcquPars tab.
- Enter:

PULPROG = zgcppr



There is also a standard parameter set **ZGCPPR**.

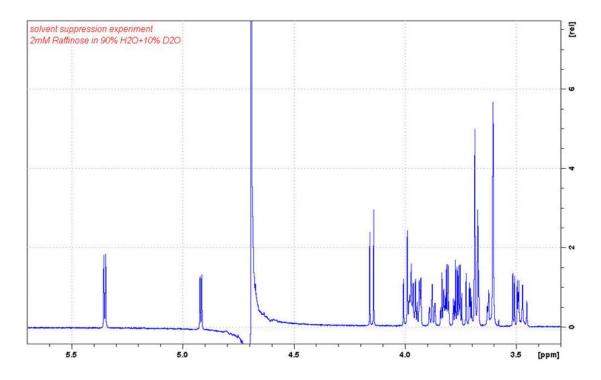
• In the Dataset window, select the Spectrum tab.

#### 4.5.2 Acquisition

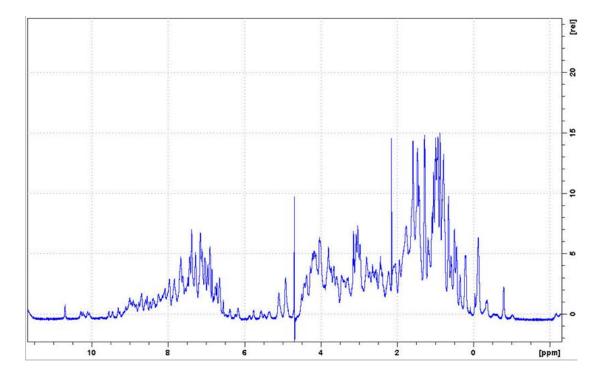
- To adjust the receiver gain, click Gain.
- To start the acquisition, click **Run**.

#### 4.5.3 Processing

• Process and phase correct the spectrum.

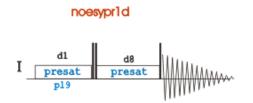


The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The following figure shows the 1D spectrum of the Lysozyme sample:



## 4.6 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:



## 4.6.1 Parameter Setup

 On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.

Enter or select

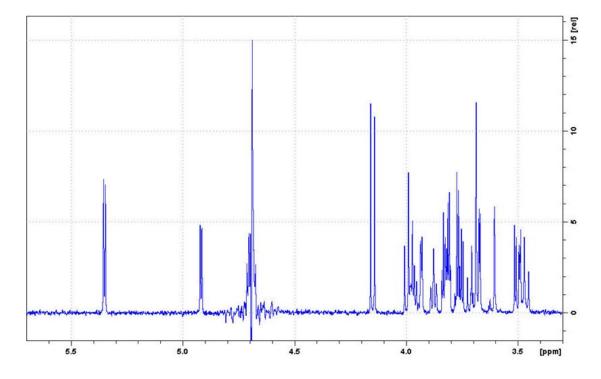
Name: noesypr1d EXPNO: 1 Read parameterset: NOESYPR1D Set solvent: H2O+D2O

initializing its For multi-re	a new experiment by s NMR parameters a ceiver experiments s ne the number of rec	cording to the sele everal datasets are	ected experiment type e created.	
Dataset				
NAME	noesypr1d			
EXPNO	1			
Directory	C:\DMB			•
Copen in new wind	W			
<ul> <li>Use current parame</li> <li>Read parameterset</li> <li>Set solvent</li> </ul>		-		Select
Set solvent Additional action	noconno	•		Select
<ul> <li>Do nothing</li> <li>Execute getprosol</li> </ul>				
Keep parameters	P 1, 01, PLW 1 👻	Change		
Advanced				
Number of datasets (re	ceivers)	1		
Title pre-sat NOESY1D		100		

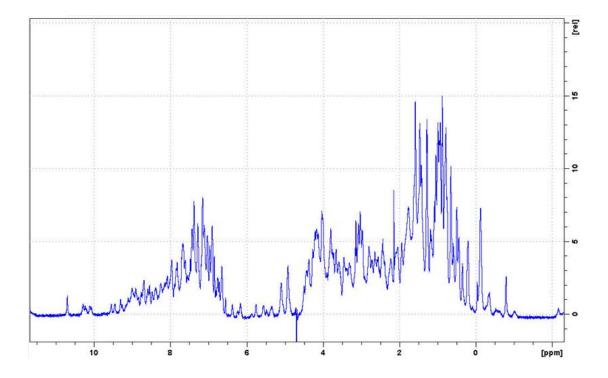
- On the Workflow button bar, click **Lock** and select **H2O+D2O**.
- On the Workflow button bar, click **Tune**.
- On the Workflow button bar, click Shim.
- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click **Run**.

# 4.6.2 Processing

• Process and phase correct the spectrum.



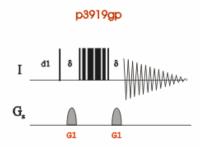
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



# 4.7 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).



## 4.7.1 Parameter Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · Enter or select

Name: **p3919gp** 

EXPNO: 1

Read parameterset: P3919GP

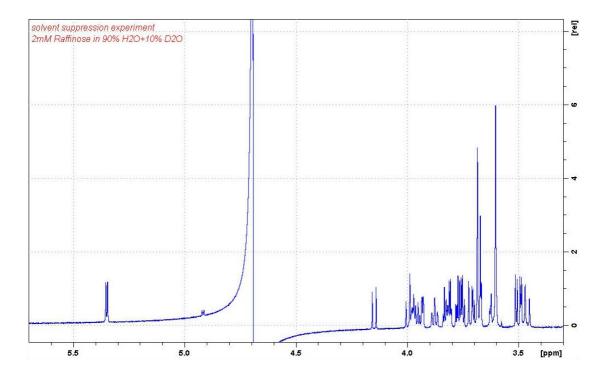
Set solvent: H2O+D2O

initializing I For multi-re	r a new experiment by creating a new data set and ts NMR parameters according to the selected experiment type, ecciver experiments several datasets are created. line the number of receivers in the Options.
Dataset	
NAME	p3919gp
EXPNO	1
Directory	C:\DMB •
Copen in new wind	dow
Set solvent Additional action Do nothing Execute getprosol	H2O+D2O •
	P 1, O1, PLW 1  Change
<ul> <li>Advanced</li> <li>Number of datasets (re</li> </ul>	eceivers) 1
Number of datasets (re	eceivers)
Advanced     Number of datasets (re Title 3-9-19 Watergate	eceivers) 1

- On the Workflow button bar, click **Lock** and select **H2O+D2O**.
- On the Workflow button bar, click **Tune**.
- On the Workflow button bar, click Shim.
- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click Run.

## 4.7.2 Processing

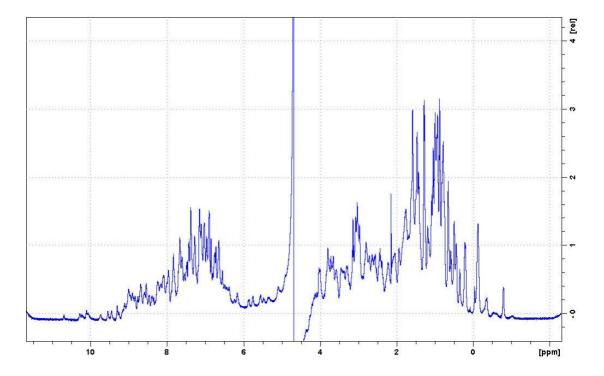
· Process and phase correct the spectrum.



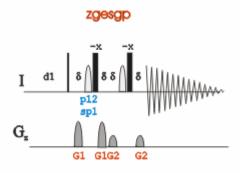
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



The final pulse, p0, of the 3-9-19 pulse train can be optimized (shortened) in gs mode to eliminate the dispersive tail on the HOD peak.



# 4.8 1D Solvent Suppression with Excitation Sculpting



# 4.8.1 Parameter Setup

 On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.

Enter or select
 Name: zgesgp
 EXPNO: 1
 Read parameterset: ZGESGP
 Set solvent: H2O+D2O

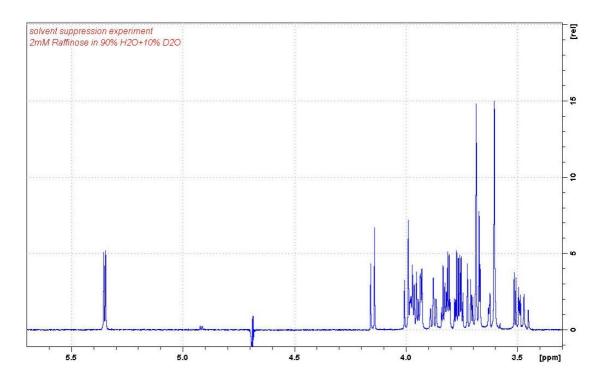
# **1D Solvent Suppression Experiments**

Create New Dataset - new		
initializing it For multi-re	a new experiment by creating a new data set and NMR parameters according to the selected experim ceiver experiments several datasets are created, the number of receivers in the Options.	ient type.
Dataset		
NAME	zgesgp	
EXPNO	1	
Directory	C:\DMB	•
🖾 Open in new wind	ow	
Read parameterset	ZGESGP	Select
O Use current parame	ters	
Survey and a second post second		Select
Set solvent	H2O+D2O *	
Additional action		
<ul> <li>Do nothing</li> <li>Execute getprosol</li> </ul>		
Keep parameters	P 1, 01, PLW 1 - Change	
Advanced		
Number of datasets (re	ceivers) 1	-
Title	1.255%.151# 703	
Excitation sculpting		
	OK Cancel	More Info Help
		Contraction of the second

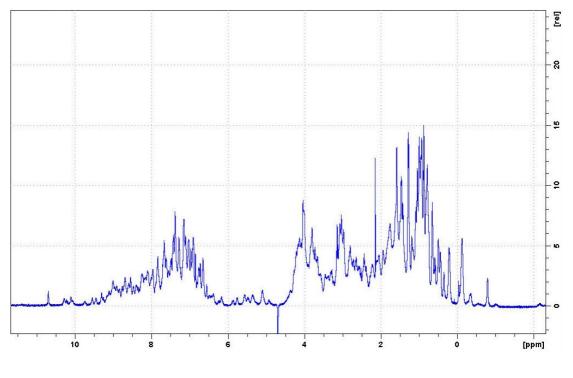
- On the Workflow button bar, click Lock and select H2O+D2O.
- On the Workflow button bar, click **Tune**.
- On the Workflow button bar, click Shim.
- On the Workflow button bar, click Gain.
- On the Workflow button bar, click Run.

# 4.8.2 Processing

· Process and phase correct the spectrum.

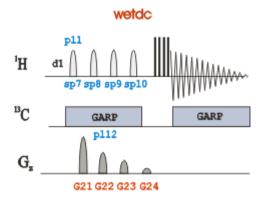


The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.



# 4.9 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 <sup>13</sup>C satellites. It provides very efficient suppression with excellent selectivity.



# 4.9.1 Sample

2 mg Sucrose in Acetonitrile and D2O

## 4.9.2 **Preparation Experiment**

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the Create New Dataset window, enter or select: NAME = wet\_solvent\_suppression\_exp EXPNO = 1 Experiment: Select PROTON Set Solvent: Select CH3CN+D2O
- In the New Dataset window, click OK.
- On the menu bar, click Acquire.

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 for best homogeneity, click **Shim**.
- To load the probe/solvent depended parameters, click **Prosol**.

#### 4.9.3 Acquisition

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

## 4.9.4 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**.

- On the Proc. Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processintg (proc1d).

WE7 4.374 ppm / 2623.912 Hs Index = 30659 - 30664 Value = -0.002518 zel				- -
				-2
				-
				- - 9 -
				-
				- <del>1</del>
4.5	4.0	 	25	(ppm)

#### 4.9.5 Selective Excitation Region Setup

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

IconNMR Automation (icona)
Setup Selective 1D Expts.
TopSoli <u>d</u> s (topsolids)
Ві <u>о</u> Тор
TopGuide (topguide)
Shape Tool (stdisp)
APSY (apsy)
NMR Thermometer (nmrtemp)

• 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**.

Selective Experiment Setup	↓ Define <u>R</u> egions	🔡 Create <u>D</u> atasets 🗸
----------------------------	--------------------------	-----------------------------

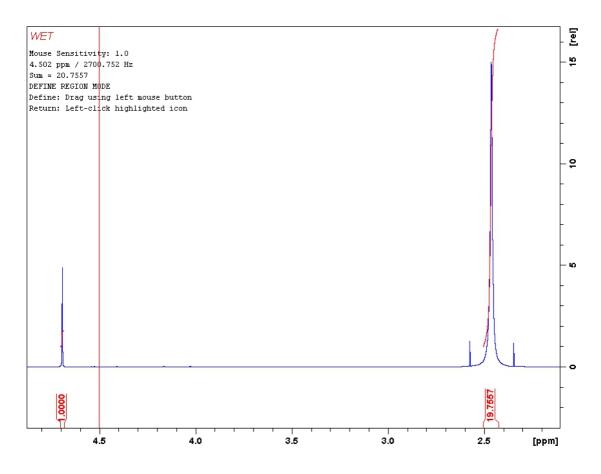


There is no other function to this button then the instruction displayed above.

- In the message window, click Close.
- On the Workflow button bar, click **Define Regions**.

The Define Regions toolbar is displayed:

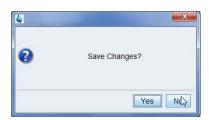
• Integrate the regions on the peaks to be suppressed (e.g. 4.7 ppm and 2.45 ppm).



- Click Save / Export integration regions.
- Select Save Regions to 'reg'.

Save Regions	To 'intrng'
Save Regions	To 'reg'
Export integra	tion regions
Export Regior	is To Relaxation Module and .ret.
Save & Show	List

- To exit the integration mode, click **Return do NOT save regions**!
- In the Save Changes window, click No.



- On the **Create Dataset** button, click the **drop-down** arrow to see more options.
- In the list, select Mult. Solvent Suppr./WET.

🔣 Create <u>D</u>atasets 🗸

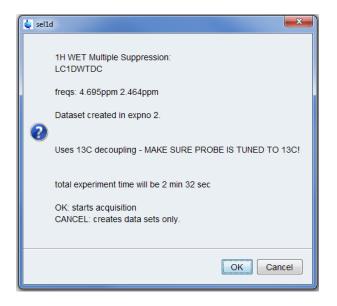
Selective gradient 1H
Selective gradient COSY
Selective gradient NOESY
Selective gradient TOCSY
Selective gradient ROESY
Selective gradient STEP-NOESY
1H Homonuclear Decoupling
Selective 1H
Selective COSY
Selective NOESY
Selective TOCSY
Selective ROESY
Mult. Solvent Suppr./presat
Mult. Solvent Suppr./WET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

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

# **1D Solvent Suppression Experiments**



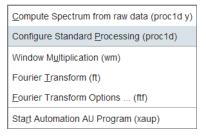
· Check the parameters in the information window.



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

#### 4.9.6 Processing

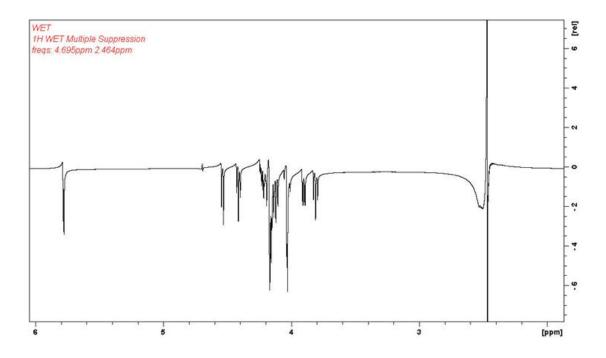
- On the menu bar, click Process.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- · Select Configure Standard Processing (proc1d).



- In the proc1d window deselect the following options:
  - Auto-Phasing (apk)
  - Set Spectrum Reference (sref)
  - Auto-Baseline correction (abs)
  - Warn if Processed data exist

🝦 proc1d			×
Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective will one-click 'Proc. Spectrum' button.	essin	g options.	
Exponential Multiply (em)	<b>v</b>	LB [Hz] =	0.3
Fourier Transform (ft)	$\checkmark$		
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	<ul> <li>Image: A start of the start of</li></ul>		
			Save Execute Cancel

• In the proc1d window, click Execute.

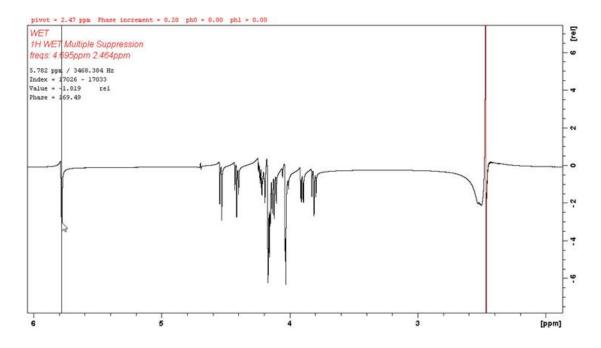


• On the Workflow button bar, click Adjust Phase.

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

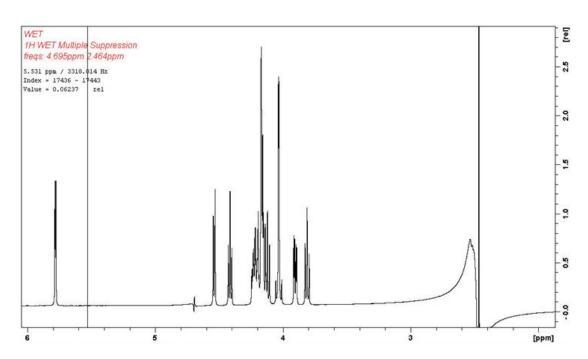
$\wedge$	0	1	R	90	-90 18	0 🖂	$\square$	Ш		Ļ			
• M	love tl	he c	urso	r line	on top	of the	peak	at	5.7 ppm a	and ri	ight-	click.	
• 0	n the	sho	rtcut	men	u, sele	ot <b>Set</b>	Pivot	Po	int.				
5	Set Piv	ot Po	oint										
(	Calcula	ate pl	n0										

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



The peak at **5 ppm** and **2.4 ppm** are the suppressed solvent peaks and those will appear out of phase.

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



# **5 2D Gradient Experiments**

# 5.1 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 1990's when the gradients were introduced as a useful tool to incorporate them into 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 (Pulsed Field Gradients) as a powerful element into high-resolution 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.

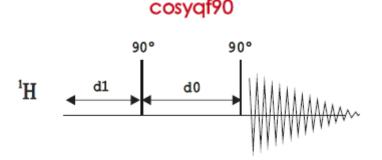
# 5.2 Setting up the COSY Experiment



This section illustrates the need for phase cycling to eliminate spectral artifacts in a nongradient experiment.

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

The 2D COSY (**CO**rrelated **S**pectroscop**Y**) is a two dimensional homonuclear experiment that correlates through bond coupled protons by tracing out connectivity via the homonuclear  $J_{HH}$  coupling constant. The COSY experiment can be acquired with or without PFG. The PFG selects coherences for observation and eliminates spectral artifacts, whereas in the non-gradient version, this is accomplished by phase cycling of the RF pulses in the sequence.



- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
  - In the New Dataset window, enter: NAME = cosyqf\_exp
  - EXPNO = 1
  - Experiment: select COSY90SW
  - Set Solvent: select DMSO
  - Set Title: non-gradient, magnitude mode COSY, NS=1
  - In the New Dataset window, click **OK**.
  - Set TD(F1) to **128**.
  - Set NS to 1.
- Under the Acquire menu bar, 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.

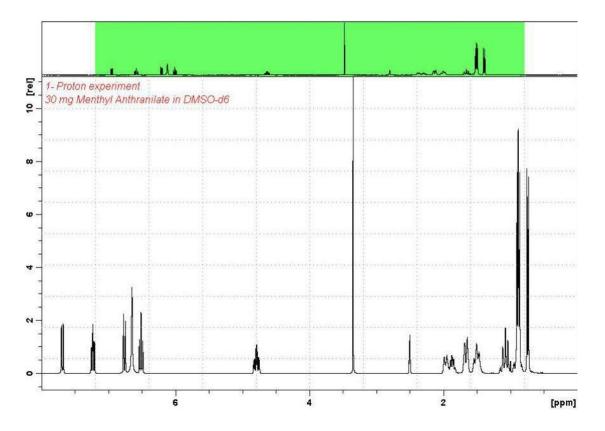
- To autoshim the sample with TopShim for best homogeneity, click Shim.
- To load the probe/solvent dependent parameters, click **Prosol**.

#### 5.2.1 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) 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. 1.0 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.

<b>e</b>	
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.

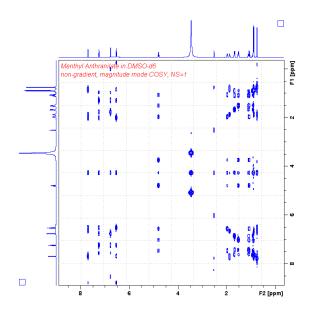
# 5.2.2 Acquisition

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

# 5.2.3 Processing

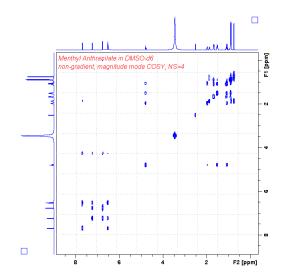
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

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 magnitude mode experiment the phase correction **apk2d** should be disabled.



To eliminate the anti-diagonal and axial peaks using the phase cycling coded in the pulse program, the number of scans needs to be increased.

- Type the command **iexpno** on the command line to copy the parameters of the current dataset to a new dataset whose EXPNO is increased by 1.
- Set NS to 4.
- Set Title: non-gradient, magnitude mode COSY, NS=4
- On the menu bar, click Acquire | Run.
- On the menu bar, click **Process**.
- On the Workflow button bar, click Proc Spectrum.



# 5.3 Setting up the COSYGP Experiment

This section illustrates the recording of N- or P-type coherence depending upon the gradient values.

 $G_{z} \xrightarrow{G_{1} G_{1}} G_{1}$ 

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

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select: NAME = cosygp\_exp
  - EXPNO = 1
  - Experiment: select COSYGPSW
  - Set Solvent: select DMSO
  - Set Title: gradient, magnitude mode COSY, N-type
- In the New Dataset window, click OK.
- On the menu bar, click **Aquire**.

For the following steps, use the Workflow button bar.

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



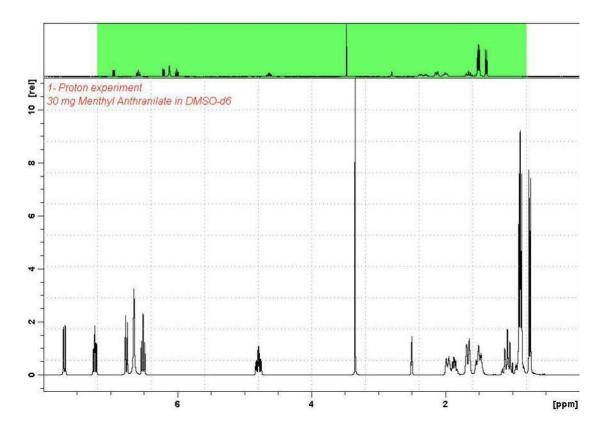
2D experiments should be run non-spinning.

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

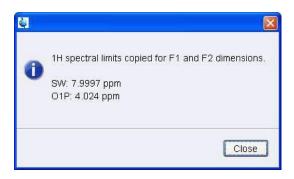
## 5.3.1 Limit Setting

- 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) 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. **1.0 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.



The display changes back to the 2D dataset.

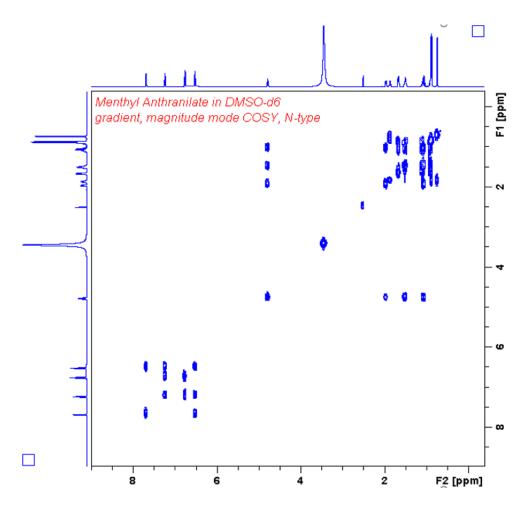
# 5.3.2 Acquisition

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

## 5.3.3 Processing

- On the menu bar, click Process.
- 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 magnitude mode experiment the phase correction apk2d should be disabled.

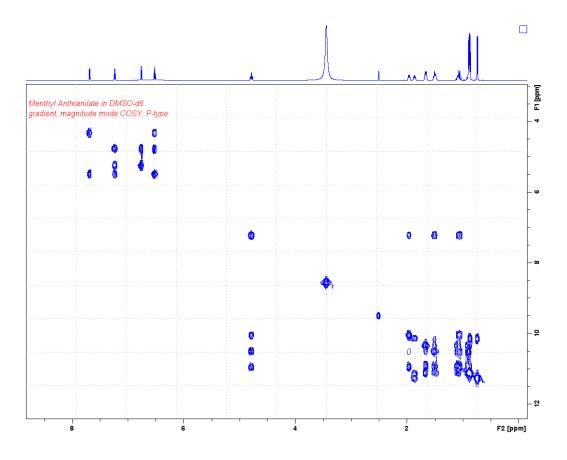


# 5.3.4 N- to P-Type Coherence Selection

To select the P-type coherence pathway instead of the N-type, the two Z gradient pulses in the pulse program need to have opposite sign.

• Type the command **iexpno** on the command line to copy the parameters of the current dataset to a new dataset whose EXPNO is increased by 1.

- Edit the pulse program **cosygpppqf** by changing the second occurrence of p16:gp1 in the sequence to p16:gp1\*-1. Save the edited pulse program to **cosygpppqf.Ptype** in the user subdirectory of the standard pulse program library.
- Change the PULPROG to cosygpppqf.Ptype.
- · Set Title: gradient, magnitude mode COSY, P-type
- On the menu bar, click Acquire | Run
- On the menu bar, click Process.
- On the Workflow button bar, click **Proc Spectrum**.



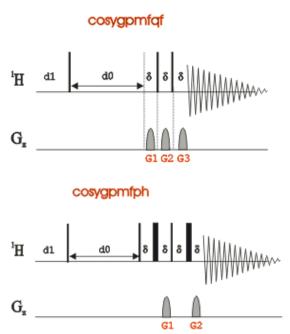
# 5.4 Setting up the Multiple-Quantum Filtered COSY Experiment

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 from 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.

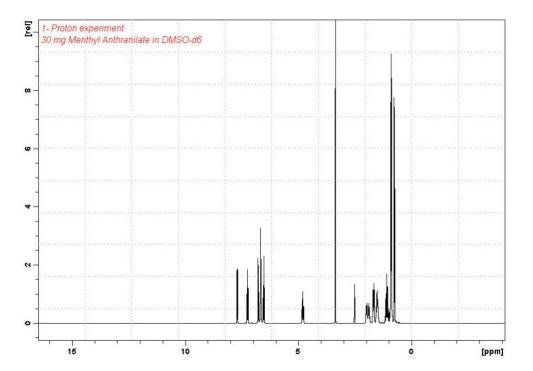
The gp-2D COSY-MQF experiment yields a 2D COSY-MQF spectrum with a single scan per t1 increment provided that the S/N ratio is adequate. The main advantage of such an approach is the large reduction in the total acquisition time compared with a conventional phase-cycled 2D COSY-MFQ experiment. Magnitude-mode (**cosygpmfqf**) or phase-sensi-

tive (**cosygpmfph**) data is obtained depending on the selected pulse sequence and acquisition/processing procedure. The COSY-MQF experiment traces out through-bond proton-proton connectivity via the homonuclear  $J_{HH}$  coupling constant.



## 5.4.1 **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.



# 5.4.2 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 Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:
  - NAME = cosydqf\_exp
  - EXPNO = 1
  - Experiment: select COSYGPDFPHSW
  - Set Solvent: select DMSO
- In the New Dataset window, click OK.
- On the menu bar, click Acquire.

For the following steps, use the Workflow button bar.

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

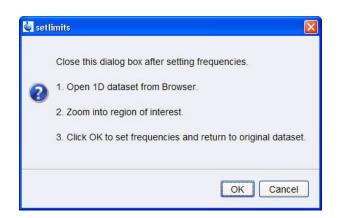


2D experiments should be run non-spinning.

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

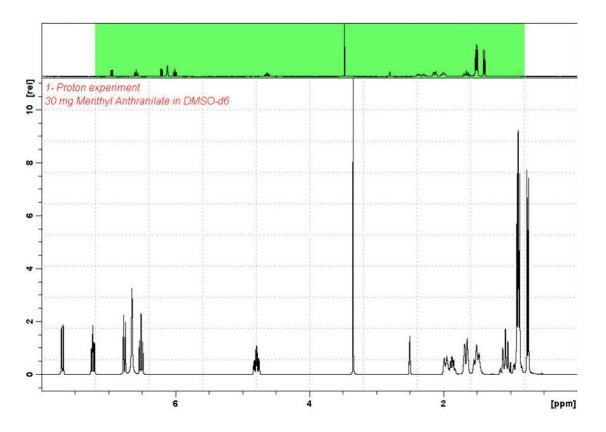
# 5.4.3 Limit Setting

- 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) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.

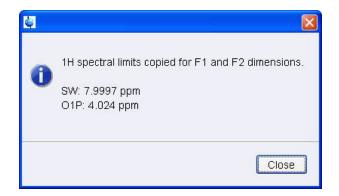


• Expand the spectrum to display all peaks, leaving ca. **1.0 ppm** of baseline on either side of the spectrum.

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.



The display changes back to the 2D dataset.

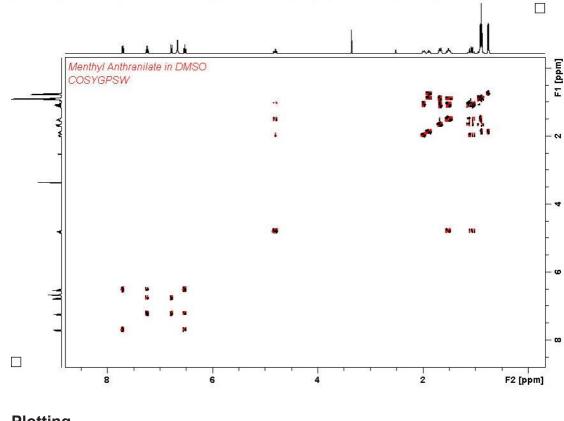
#### 5.4.4 Acquisition

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**.

#### 5.4.5 Processing

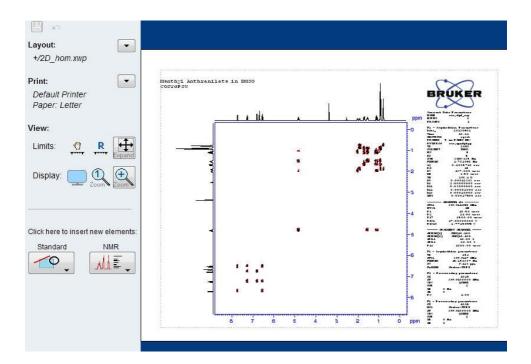
- On the menu bar, click Process.
- On the Workflow button bar, click **Proc Spectrum**.

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 be enabled.



## 5.4.6 Plotting

Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.





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

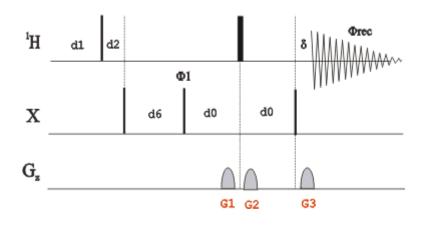
• In the Print section, click the **down** arrow button and select **Print**.

<u>е</u> о	·
Layout:	•
+/2D_inv.xwp	
Print:	
Default Printer	Print
Paper: A4	Set target printer
View:	Multi-print
Limits: 🖑 R	Page <u>s</u> etup
	Split page setting
Display: Display:	+ oom
	_
Click here to insert new eler	ments:
Standard NMF	2
1 - MI	- 

# 5.5 Setting up the 13C-HMBCGP 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 setup of an <sup>1</sup>H/X gradient experiment using the standard Bruker HMBCGP parameter set. In addition to changing the nucleus in F1 from <sup>13</sup>C to another X-nucleus, the gradient ratio for the new X-nucleus also must be calculated. The HMBC pulse sequence is shown in the figure below.

## hmbcgplpndqf



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.

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select: NAME = 13Chmbcgp\_exp
  - EXPNO = 1
  - Experiment: select HMBCGP
  - Set Solvent: select DMSO
  - Set Title: gradient, magnitude mode HMBC
- In the New Dataset window, click OK.
- On the menu bar, click Aquire.

For the following steps, use the Workflow button bar.

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



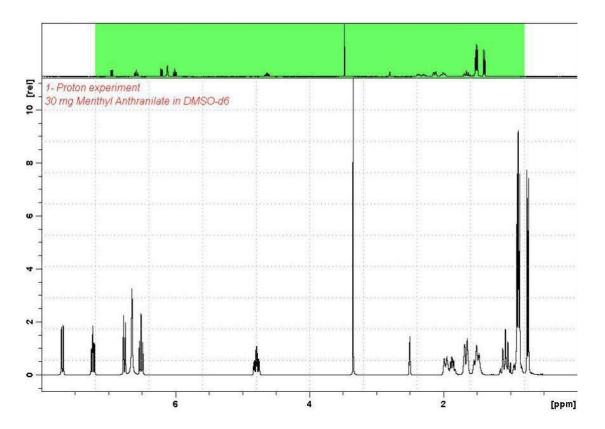
2D experiments should be run non-spinning.

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

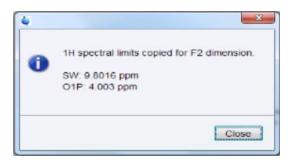
## 5.5.1 Limit Setting

- 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) 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. 1.0 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.



The display changes back to the 2D dataset.

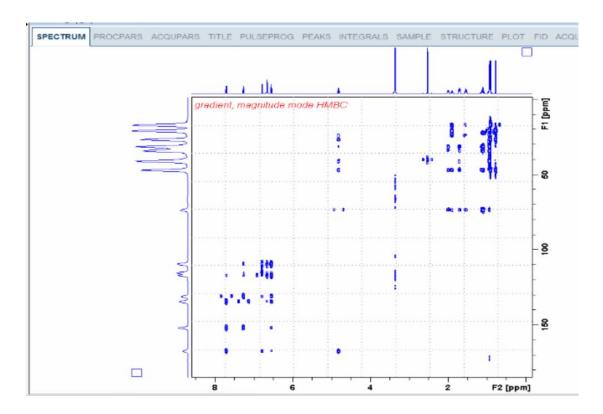
## 5.5.2 Acquisition

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

## 5.5.3 Processing

- On the menu bar, click Process.
- 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 magnitude mode experiment the phase correction **apk2d** should be disabled.



# 5.6 Setting up the 15N-HMBCGP from the 13C-HMBCGP

Although there is a dedicated standard parameter set for acquiring a 15N-HMBC, HM-BCGP\_15N, in this section one will be created starting from the previously acquired 13C-HMBC. In the new dataset, the X nuclei will be changed from 13C to 15N. This exercise illustrates that in the gradient HMBC experiment the gradients are used to select the coherence corresponding to the HX nuclei defined in the dataset. These steps can be used to set up the HMBC for other heteronuclei combinations, e.g. 1H/31P, for which there is no dedicated parameter set in the library.

## 5.6.1 Limit Setting

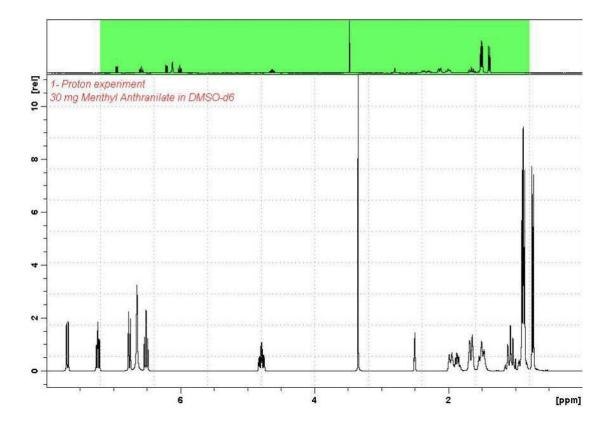
• Type the command **iexpno** on the command line to copy the parameters of the current dataset to a new dataset whose EXPNO is increased by 1.

- Change the Title to 15Nhmbcgp\_exp
- 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) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.

	limits 🛛 🔀
	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.
	OK

• Expand the spectrum to display all peaks, leaving ca. **1.0 ppm** of baseline on either side of the spectrum.

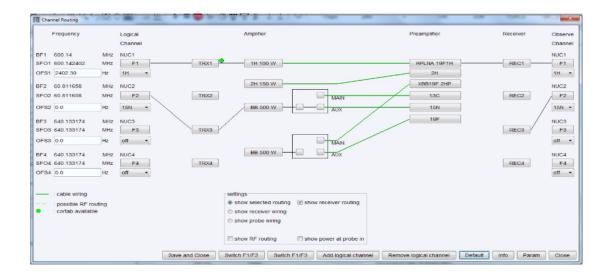
The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak will be folding in F1.



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

# 5.7 Setting up the 15N-HMBC Experiment

- In the Dataset window, select the AcquPars tab.
- Click the **Set nuclei and routing** button to display the routing window.
- Change the following parameter using the pull-down arrow:
   NUC2 = 15N



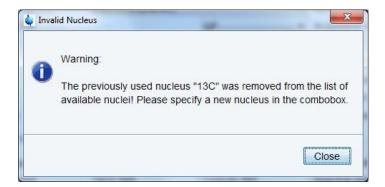
After switching NUC2 from 13C to 15N, click Default and then Save and Close.



#### Do not modify the routing!

After closing the Channel Routing window, two windows will appear to alert the user to update some experimental parameters in the AcquPars tab of the dataset:

~	Parameter value(s) "Out of range"! inconsistent parameter relation:
U	O1P=O1/BF1<==== out of range
	Please fix the value(s).
1 nev	v warnings in history



- In the AcquPars tab scroll down to the Nucleus 2 section. Either ensure that 15N is defined as the 2<sup>nd</sup> nucleus or select it using the Edit button.
- Set O2P [ppm] to 150.

Nucleus 2		
NUC2	15N Edit	2nd nucleus
O2 [Hz]	9121.75	Frequency offset of 2nd nucleus
O2P [ppm]	150.000	Frequency offset of 2nd nucleus
SFO2 [MHz]	60.8207797	Frequency of 2nd nucleus
BF2 [MHz]	60.8116580	Basic frequency of 2nd nucleus

- Click on the getprosol icon at the top of the AcquPars tab to update the 15N 90 degree pulse:
- · Change the following parameters:

```
PULPROG = hmbcgpndqf
D1 = 2 sec
```

CNST13 = **5 Hz** 

SW [ppm] in F1 = **400 ppm** 

 To calculate the gradient ratio necessary for 1H-15N detection, type gradratio on the command line. The correct gradient ratios will appear in a pop-up window and will be updated in the dataset automatically.

🖕 gradra	tio
8	pulse program = hmbcgpndqf nucleus1 = 1H nucleus2 = 15N GPZ1=70.0 GPZ2=30.0 GPZ3=50.1
	Close

• Under the Acquire menu, click on **Tune** to tune and match the probe to 15N.

## 5.7.1 Acquisition

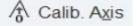
- To auto-adjust the receiver gain, click Gain.
- To start the acquisition, click Run.

#### 5.7.2 Processing

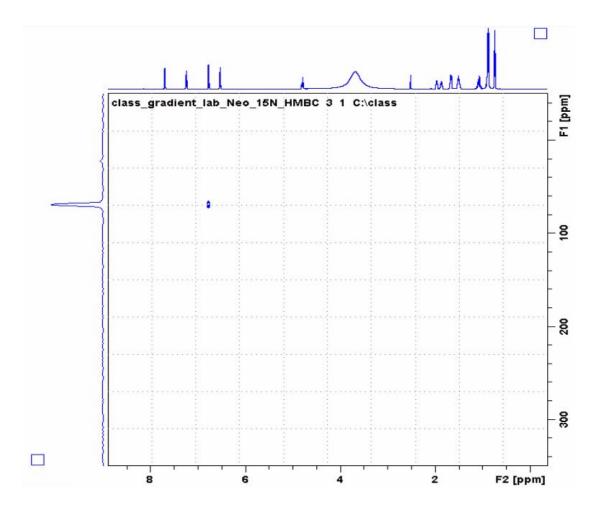
- On the menu bar, click Process.
- On the Workflow button bar, click **Proc Spectrum**.

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 magnitude mode experiment the phase correction **apk2d** should be disabled.

• To correct the F1 axis for 15N, click on Calib. Axis under the Process menu.

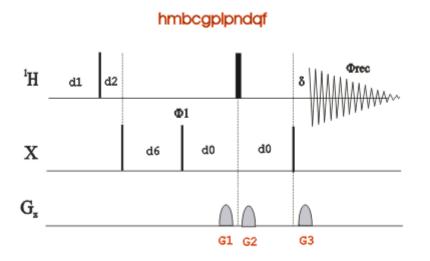


• At the top left in the dataset window, click on **C** to Calibrate to center of spectrum. Set F1[ppm] to 150 and click **OK**.



# 5.8 2D <sup>1</sup>H / <sup>31</sup>P 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 <sup>1</sup>H/X gradient experiment using the standard Bruker HMBCGP parameter set. In addition to changing the nucleus in F1 from <sup>13</sup>C to another X-nucleus, the gradient ratio for the new X-nucleus also has 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.

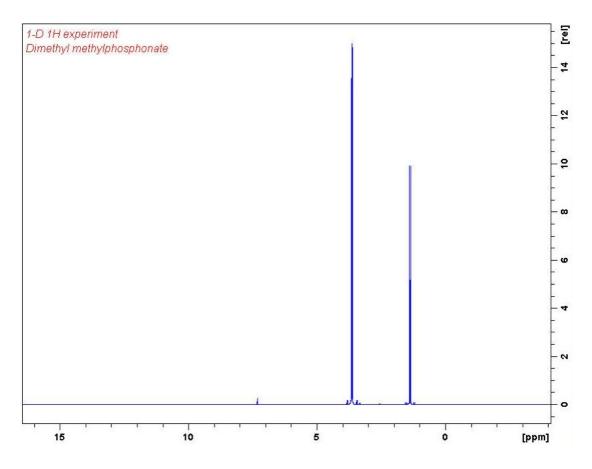
#### 5.8.1 Sample

30mg Dimethyl methylphosphonate in CDCl₃

This <sup>31</sup>P nucleus in this sample does not have any direct proton attached The long range coupling from the 3 methyl protons to <sup>31</sup>P 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 <sup>1</sup>*H* Reference Experiment [ $\triangleright$  71]).

#### 5.8.2 <sup>1</sup>H 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 CDCl<sub>3</sub> as a lock solvent.



## 5.8.3 <sup>31</sup>P Reference Experiment

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

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = **31P\_exp** EXPNO = **1** Experiment: select **P31CPD** Set Solvent: select **CDCI3** 

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

For the following steps, use the Workflow button bar.

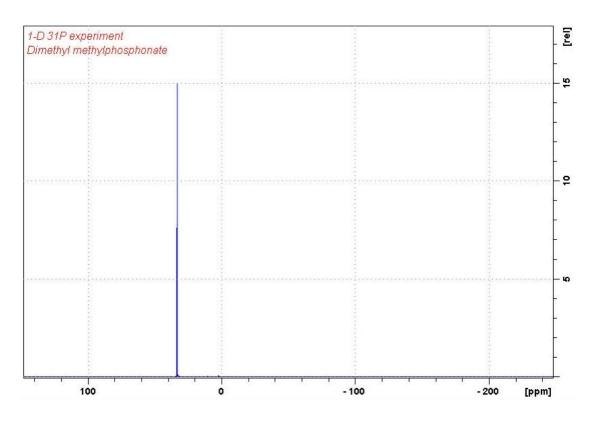
- Click **Tune** to tune the probe.
- Click Spin and select Sample rotation on.
- Click Shim for best homogeneity use TopShim.
- To autoshim the sample with TopShim for best homogeneity, click Shim.
- Click Prosol to load the probe/solvent depended parameters.

#### 5.8.4 Acquisition

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

#### 5.8.5 Processing

• Process and phase correct the spectrum.

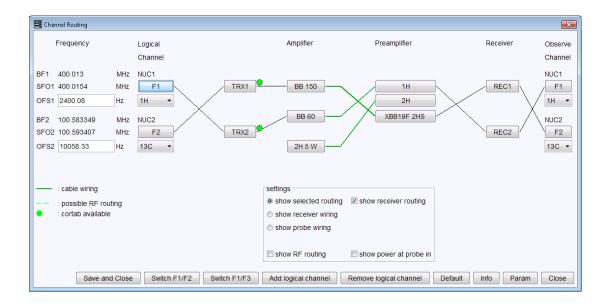


#### 5.8.6 Setting up the HMBC Experiment

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = **1H\_31P\_hmbc\_exp** EXPNO = **1** Experiment: **HMBCGP** Set Solvent: **CDCI3** 

- In the New Dataset window, click **OK**.
- On the menu bar, click **Acquire**.
- In the Dataset window, select the AcquPars tab.
- Click the Set nuclei and routing button to display the routing window.



· Change the following parameter: NUC2 = 31P



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	/	121.5004219	Transmitter frequency
BF1 [MHz]	300.1300000	)	121.4948510	Basic transmitter frequency

- · Change the following parameter:
  - NUC1 [F1] = 31P
- · Scroll down to the Program parameter section in the AcquPars window.
- Click the CNST Edit button (Constant used in pulse programs).

Program paramet	010	
L	Edit	Loop counter
CNST	Edit	Constant used in pulse programs
CPDPRG	EditSet constants used	in pulse programs mposite 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)

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
เกรฐ13]	14	CNST[29]	1	CNST[45]	1	CNST[61]	1
CNST[14]		CNST[30]	1	CNST[46]	1	CNST[62]	1
CNST[15]	1	CNST[31]	1	CNST[47]	1	CNST[63]	1

#### Constant used in pulse programs

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* [▶ 71].

- On the **Spin** button, click the **drop-down** arrow to see more options.
- · In the list, select Turn sample rotation off.



2D experiments should be run non-spinning.

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

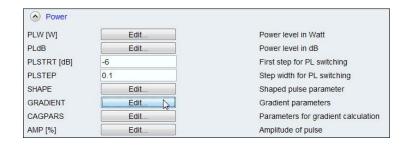
This will load the pulse width and power levels into the parameter set.

• At the command prompt, enter gradratio.

🎍 gradratio	
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 <sup>31</sup>**P**. To check if the correct gradient ratio values has been entered in the **AcquPars**, follow the steps below.

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



• Click **GRADIENT Edit** (Gradient parameters).

ndex	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.

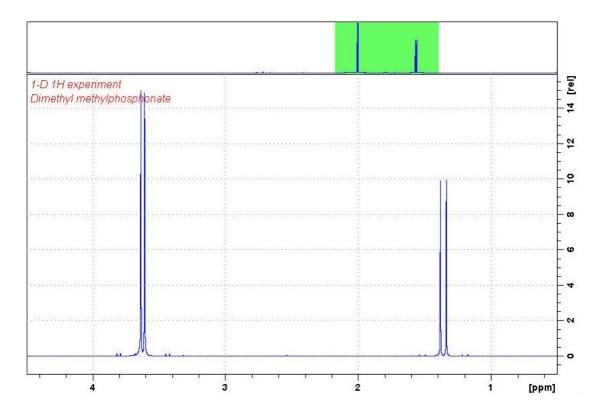
#### 5.8.7 Limit Setting

• On the Workflow button bar, click SetLimits.

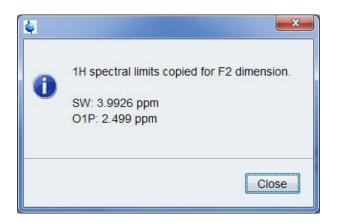
🦉 setl	imits 🛛
?	Close this dialog box after setting frequencies. 1. Open 1D dataset from Browser. 2. Zoom into region of interest. 3. Click OK to set frequencies and return to original dataset.
	OK Cancel

 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. **1.0 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.

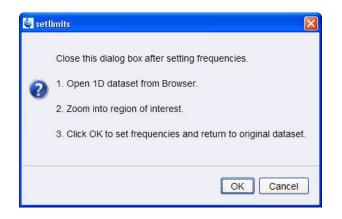


- In the setlimits message window, click **OK** to assign the new limit.
- In the message window, click **Close**.

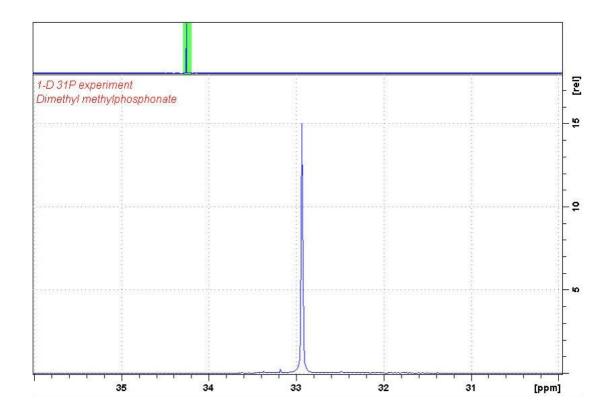


The display changes back to the 2D dataset. Follow the steps below to set the limit in the F1 dimension.

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



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



• Expand the spectrum to display all peaks.

- 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.

#### 5.8.8 Acquisition

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

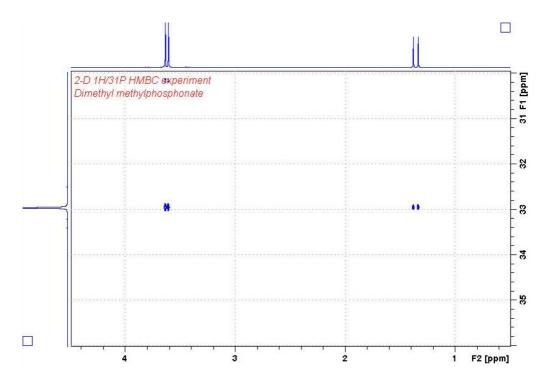
#### 5.8.9 Processing

- On the menu bar, click Process.
- 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.

🍓 apk2d	
8	Spectrum has no imaginary part: MC2[F1]=QF PH_mod[F1]=mc. Could not phase real spectrum
	Close

• In the apk2 message window, click Close.



### 5.8.10 Plotting

5 h ...

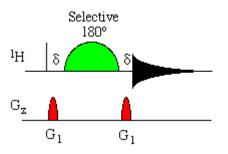
Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

Layout: +/2D_inv.xwp								
Print: Default Printer Paper: Letter	2-D 1H/31 Dimethyl	P HMBC emper methylphospl	iment monate			ļ		BRUKER
View:	-							ppm states
Limits: $\begin{tabular}{lllllllllllllllllllllllllllllllllll$								30.5 31.0 31.0 32.0 32.0
Click here to insert new elements:						**		33.0
Standard NMR								33.5 34.0 35.0 35.0 35.5
	4	0 3.5	3.0	2.5	2.0	1.5	1.0	

# 6 1D Experiments Using Shaped Pulses

## 6.1 Introduction

Selective homonuclear 1D experiments usually start from the selective <sup>1</sup>H 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.

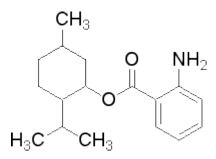


Selective 1D experiments can be easily derived by adding the corresponding mixing process between the SPFGE block and the acquisition period.

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. Sections 6.3 - 6.5 show how to use the flow bar tools to automatically set up selective excitation regions for selective COSY, NOESY and, TOCSY experiments respectively. Sections 6.6 and 6.7 illustrate how to manually set up selective excitation regions using the on- and off-resonance options.

## 6.2 Sample

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



## 6.3 1D Selective COSY Using the Flow Bar Tools

#### 6.3.1 Introduction

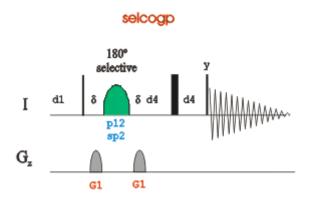
The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a <sup>1</sup>H spectrum. Important characteristics of a soft pulse include the shape, the 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.

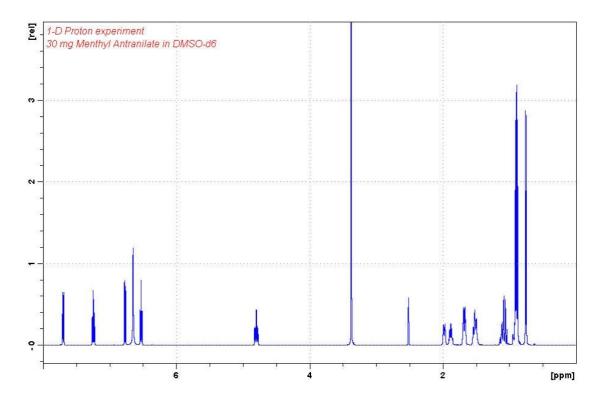
Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments using pulse field gradients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

Section 1D Selective COSY Experiment Using the On- Resonance Option [ 101] describes the acquisition and processing of a one-dimensional <sup>1</sup>H 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.



#### 6.3.2 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.



### 6.3.3 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.

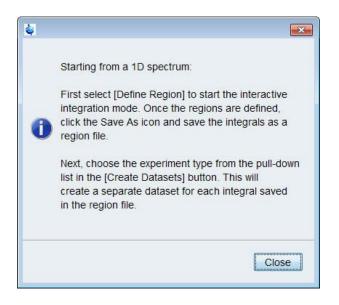


• In the list, select Setup Selective 1D Expts.

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

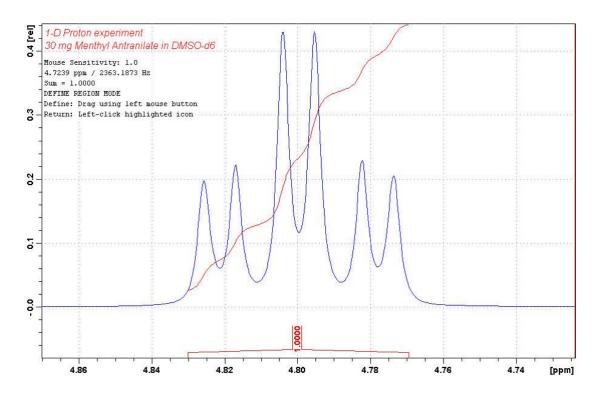


• On the Workflow button bar, click **1D Selective Experiment Setup**.



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**.
- Integrate the multiplet at **4.8 ppm**.



## **1D Experiments Using Shaped Pulses**



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/export integration regions**
- 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.

4	<b></b>
0	Save Changes?
	Yes

- On the Create Dataset button, click the drop-down arrow to see more options.
- In the list, select Selective gradient COSY.

E Crea	te <u>D</u> atasets 🗸
	Selective gradient 1H
	Selective gradient COSY
	Selective gradient NOESY
	Selective gradient TOCSY
	Selective gradient ROESY
	Selective gradient STEP-NOESY
	1H Homonuclear Decoupling
	Selective 1H
	Selective COSY
	Selective NOESY
	Selective TOCSY
	Selective ROESY
	Mult. Solvent Suppr./presat
	Mult. Solvent Suppr./WET
	2D Band Selective HMBC
	2D Band Selective HSQC

## **1D Experiments Using Shaped Pulses**

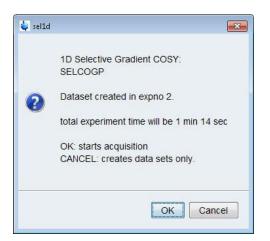
The default parameters are taken from the standard parameter set **SELCOGP**. If desired, the **Gaus1\_180r.1000** pulse can be changed by clicking on the **Change Shape** button in the above window.

• In the SELCOGP window, click Accept.

1D Selective	e Gradient COSY	
Shape = Gaus	s1_180r.1000	
D 4 (sec)	0.031250	mixing time
NS	8	

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

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



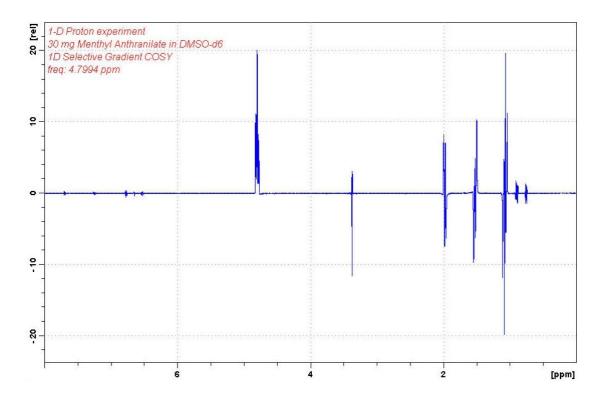
#### 6.3.4 Processing

- 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.

ress 'Execute' to process the curre ress 'Save' to just change the proc hanged options will be effective wh ne-click 'Proc. Spectrum' button.	essi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)	<b>V</b>		
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			

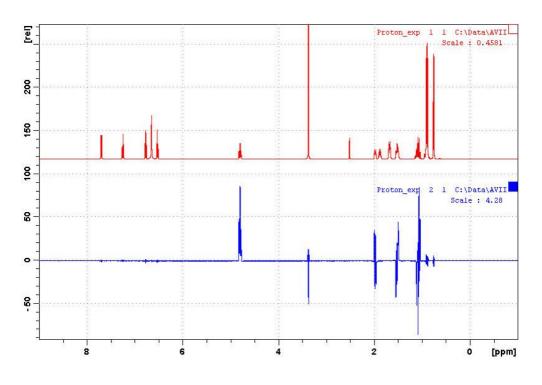
• Manually adjust the phase of the peaks between **3 ppm** and **1 ppm** for an antiphase pattern and if desired the selective peak at **4.8 ppm** can be phased positive.



#### 6.3.5 Plotting Two Spectra on the Same Page

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

The Multiple display toolbar is displayed:



• Drag the Reference spectrum into the spectral window.

• To adjust the spectra for best fit, use the <sup>\*2</sup><sub>s</sub> <sup>/2</sup><sub>s</sub> <sup>+</sup><sub>s</sub> <sup>+</sup><sub>s</sub> <sup>+</sup><sub>s</sub> toolbar buttons.

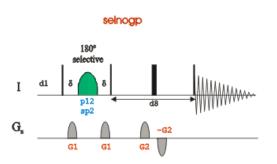
Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

## 6.4 1D Selective NOESY Using the Flow Bar Tools

#### 6.4.1 Introduction

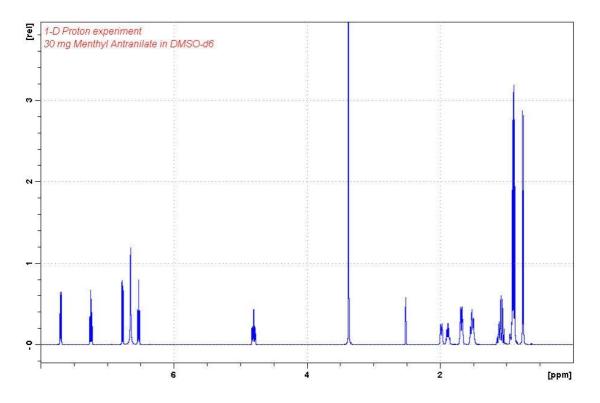
This experiment consists of three parts:

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



#### 6.4.2 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.



#### 6.4.3 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.

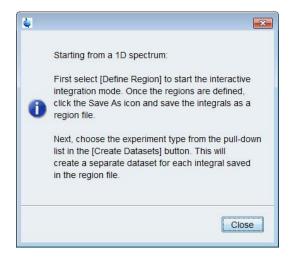
- On the menu bar, click Acquire.
- On the More button, click the drop-down arrow to see more options.



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

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

- On the Workflow button bar, click 1D Selective Experiment Setup.
- In the message window, click Close.



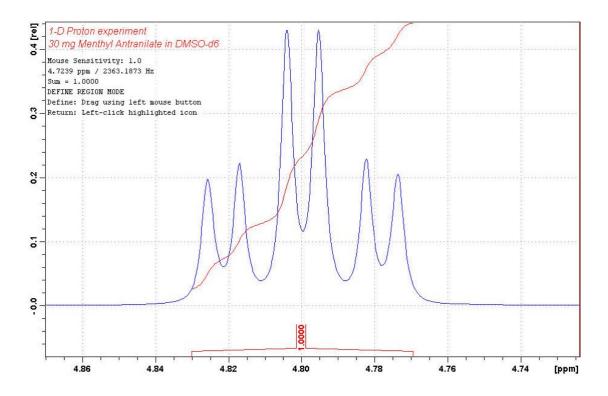
There is no other function to this button then the instruction displayed above.

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

The Define Regions toolbar is displayed:



• 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/export regions
- · In the list, select Save Regions to 'reg'.
- On the toolbar, click Return do NOT save regions!
- In the message window, click **No**.

é.	
0	Save Changes?
	Yes No

- On the Create Dataset button, click the drop-down arrow to see more options.
- In the list, select Selective gradient NOESY.
- Create Datasets -

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

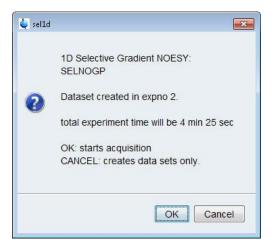
The default parameters are taken from the standard parameter set **SELNOGP**. The mixing time **D8** is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from **100 ms** to **800 ms**. If desired, the **Gaus1\_180r.1000** pulse can be changed by clicking on the **Shape** button in the above window.

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

	×
Gradient NOESY	
s1_180r.1000	
0.450	mixing time
32	
2	
r	Cancel
	0.450

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

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



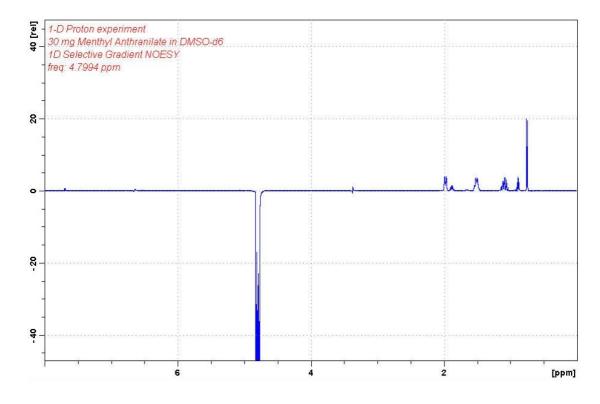
#### 6.4.4 Processing

- 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.
- In the proc1d window, 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.

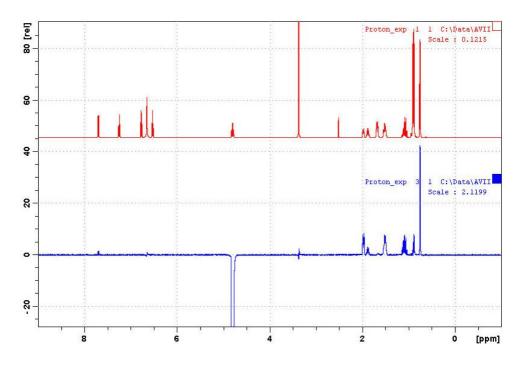
ress 'Execute' to process the curre ress 'Save' to just change the proc hanged options will be effective will ne-click 'Proc. Spectrum' button.	essi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)			
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline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

 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.



- 6.4.5 Plotting Two Spectra on the Same Page
  - Display the selective TOCSY spectrum.
  - On the toolbar, click **Multiple display**.

The Multiple display toolbar is displayed:



• Drag the Reference spectrum into the spectral window.

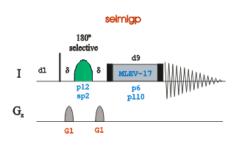
• To adjust the spectra for best fit, use the <sup>\*2</sup><sub>s</sub> <sup>/2</sup><sub>s</sub> <sup>+</sup><sub>s</sub> <sup>+</sup><sub>s</sub> toolbar buttons.

Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

## 6.5 1D Selective TOCSY Using the Flow Bar Tools

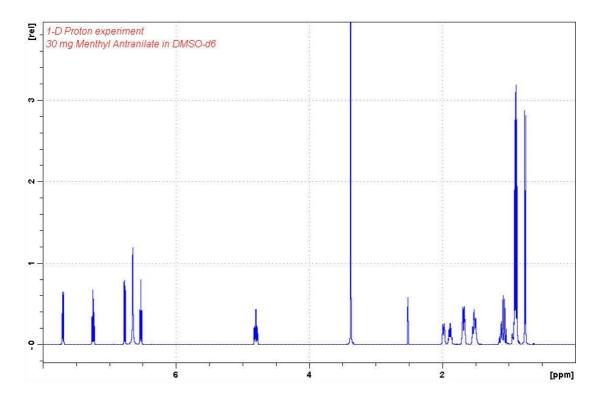
This experiment consists of three parts:

- 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.



#### 6.5.1 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.



#### 6.5.2 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.

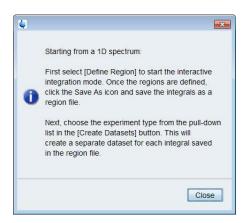
More -

IconNMR Automation (icona)			
Setup Selective 1D Expts.			
TopSoli <u>d</u> s (topsolids)			
Ві <u>о</u> Тор			
TopGuide (topguide)			
Shape <u>T</u> ool (stdisp)			
APSY (apsy)			
NMR Thermometer (nmrtemp)			

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

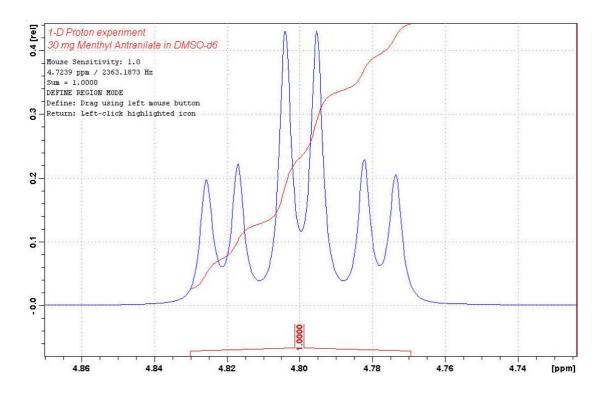
The Workflow button bar changes for setting up the 1D selective experiment.

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



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.
- 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/export integration regions
- · In the list, select Save the Region to 'reg'.

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

ē.	<b>X</b>
0	Save Changes?
	Yes No

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

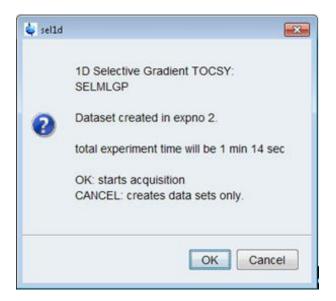
D9 = **0.08** NS = **8** 

• In the SELMLGP window, click Accept.

SELMLGP		X
1D Selective	e Gradient TOCSY	
Shape = Gaus	1_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.



#### 6.5.3 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.
- In the proc1d window, 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**.

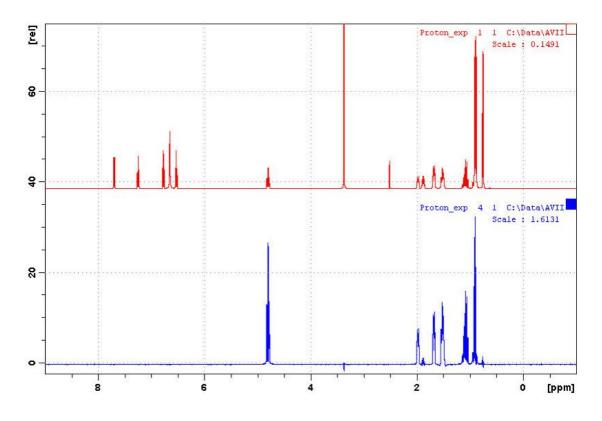
ress 'Execute' to process the curre ress 'Save' to just change the proo hanged options will be effective wh ne-click 'Proc. Spectrum' button.	essi	ng options.	
Exponential Multiply (em)		LB [Hz] =	0.3
Fourier Transform (ft)			
Auto - Phasing (apk)			
Set Spectrum Reference (sref)			
Auto - Baseline Correction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

- Image: Sector Structure
   Image: Sector Structure</t
- · Manually phase all peaks for positive absorption.

## 6.5.4 Plotting Two Spectra on the Same Page

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

The Multiple display toolbar is displayed:



• Drag the reference spectrum into the spectral window.

• To adjust the spectra for best fit, use the <sup>\*2</sup>s <sup>/2</sup>s <sup>+</sup>s <sup>\*</sup>s toolbar buttons.

Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

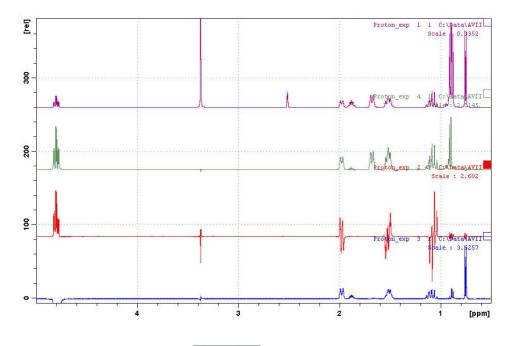
#### 6.5.5 Plotting All 4 Experiments on the Same Page

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

The Multiple display toolbar is displayed:

<u>₩</u> ₩ R <u>M</u> + |±±±<sup>1</sup><sub>μ</sub> E- E+ E i |<sup>\*2</sup>s <sup>2</sup>s <sup>\$</sup>s <sup>\$</sup>s <sup>\$</sup>s | || Δ Σ [|| ]

- 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.

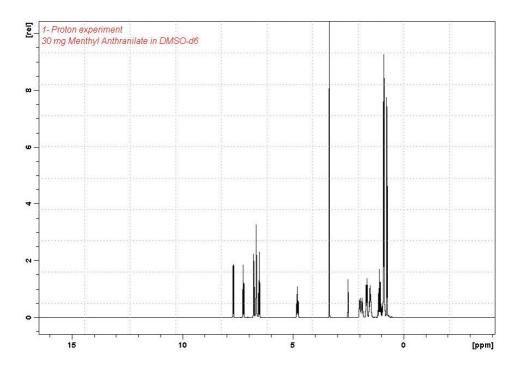


Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

## 6.6 1D Selective COSY Experiment Using the On- Resonance Option

#### 6.6.1 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.



#### 6.6.2 Selective Excitation Region Set Up



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 must be known and entered in the Prosol parameter table.

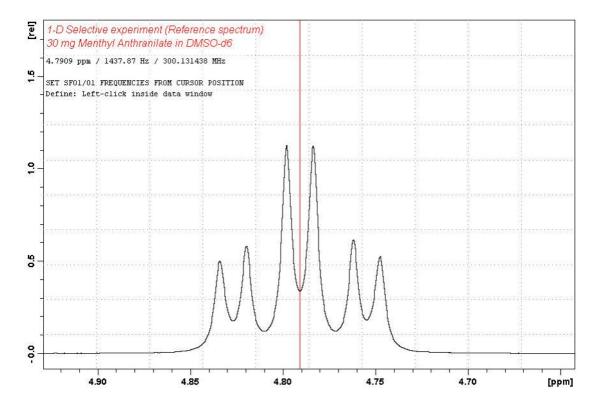
• At the command prompt, type wrpa.

🧔 wrpa		
	NAME ends with ".top", the destination staset (no expno/procno required). estination:	
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 =	1
PROCNO =	1
DIR =	C:\data3.0
OK Cancel	Browse Find Help

- Change NAME = sel\_cosy.
- In the re window, click **OK**.
- Expand peak at 4.8 ppm.
- On the toolbar, click Set RF from cursor.



- 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 f	requencies
SFO1 [MHz] =	300.131438
O1/2/3 [Hz] = 1437.87	
01 02	O3 Cancel

#### 6.6.3 Setting Up the Selective COSY

- On the menu bar, click Start.
- 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.

## **1D Experiments Using Shaped Pulses**

File Options Help			Source = C:\Bruker\TopSpin3.5pl5\exp\stan\nmr\par ~		
Find file names verse	<b>*</b>	Exclude:	Clear		
Class = Any ~	Dim = Any 🗸 🗌 Sh	now Recommended			
Type = Any ~	SubType = Any ~	SubTypeB = Any ~	Reset Filters		
SELCO1H	SELCOGP	SELGPSE	SELMLGP	SELMLZF1H	
SELNO1H	SELNOGP	SELRO1H	SELROGP	SELZG1H	

- 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**.

🖕 rpar	×
Source Parameter Set = C:\Bruker\TopSpin3.5pl5\exp\stan\nmr\pai Destination Data Set = Example_MenthylAnthranilate 1 1 C:\Data 1) Select the desired file types of the source parameter set 2) Press OK to copy them to the destination data set.	
acqu proc outd	
title	
Set solvent: DMSO ~	
O Execute 'getprosol'	
Keep parameters: P 1, O1, PLW 1      Change	
OK	Cancel

- 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.

#### 6.6.4 Acquisition

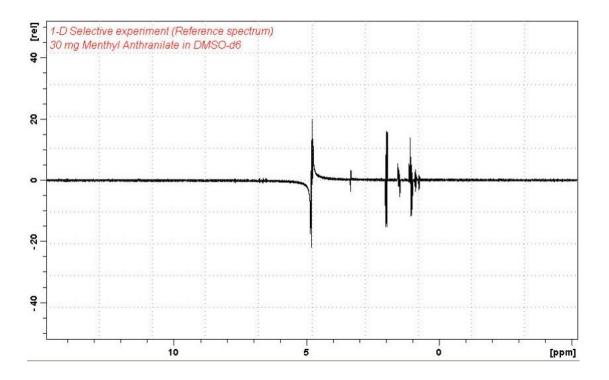
• To start the acquisition, click **Run**.

#### 6.6.5 Processing

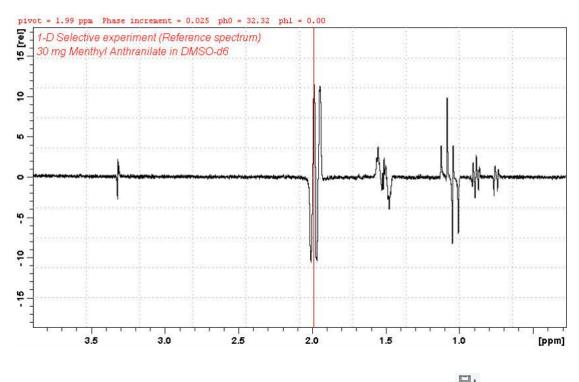
- On the menu bar, click **Process**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing.
- In the proc1d window, deselect the following options:
  - Auto-Phasing (apk)
  - Set Spectrum Reference (sref)
  - Auto-Baseline correction (abs)
  - Warn if Processed data exist
- In the proc1d window, click Execute.

🧔 procld			×
Press 'Execute' to process the curre Press 'Save' to just change the pro 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			
		Save	Execute Cancel

## **1D Experiments Using Shaped Pulses**



- 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**.



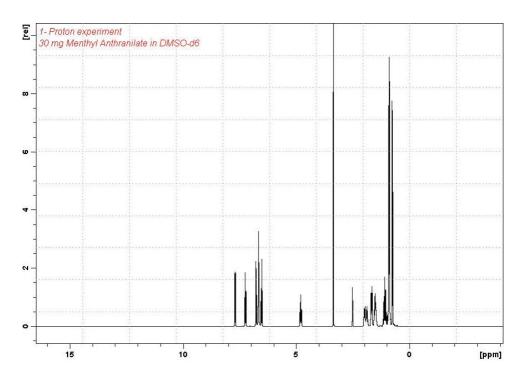
To store the phase values, click Return and Save phased spectrum.

Follow the instructions in chapter *Plotting Two Spectra on the Same Page* [ 87] to plot two spectra on the same page.

## 6.7 1D Selective NOESY Experiment Using the Off- Resonance Option

#### 6.7.1 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.



## 6.7.2 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.

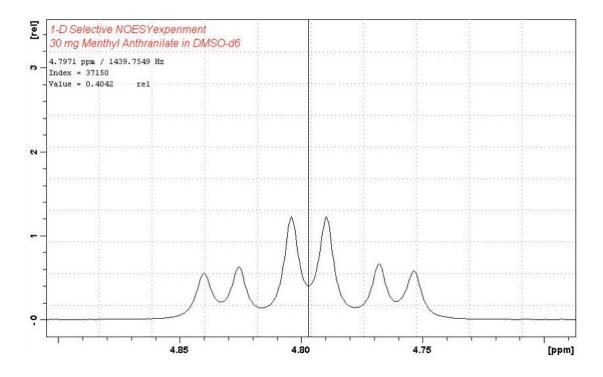
- At the command prompt, type wrpa.
- Change NAME = **sel\_noesy**.
- In the wrpa window, click OK.

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

• 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.

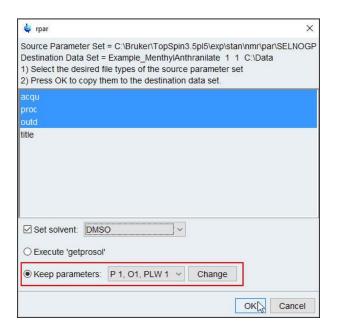
# 6.7.3 Setting Up the Selective NOESY

- On the menu bar, click Start.
- 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:\Bruker\TopSp	in3.5pl5\exp\stan\nmr\par ~
Find file names sel*		Exclude:	Clear	
Class = Any V Dim =	Any V Sho	w Recommended		
Type = Any ~ Sub	Type = Any ~ S	subTypeB = Any ~	Reset Filters	
SELCO1H SE	LCOGP	SELGPSE	SELMLGP	SELMLZF1H
SELNO1H SE	LNOGP	SELRO1H	SELROGP	SELZG1H
	- 1992 (			Read Close

- 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.



- 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.

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* [▶ 109].



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**.

#### 6.7.4 Acquisition

• To start the acquisition, click Run.

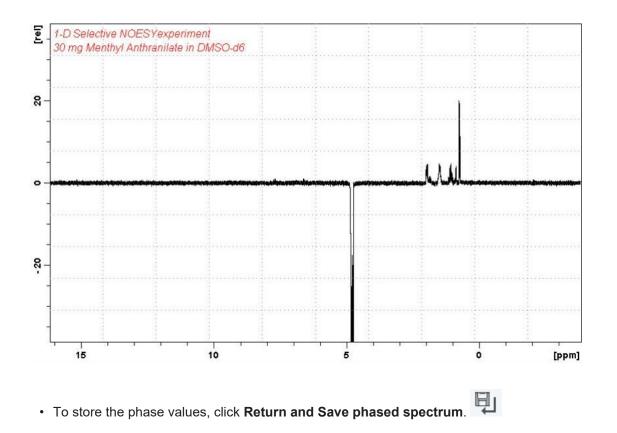
#### 6.7.5 Processing

- 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.
- In the proc1d window, 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.

🧔 procld			
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			
		Save	Execute Cancel

- 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.

# 1D Experiments Using Shaped Pulses



Follow the instructions in chapter *Plotting Two Spectra on the Same Page* [> 87] to plot two spectra on the same page.

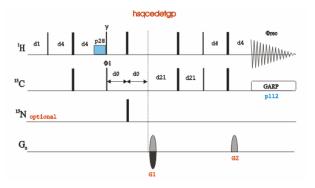
# 7 2D Experiments using Shaped Pulses

# 7.1 2D Edited HSQC Experiment with Adiabatic Pulses

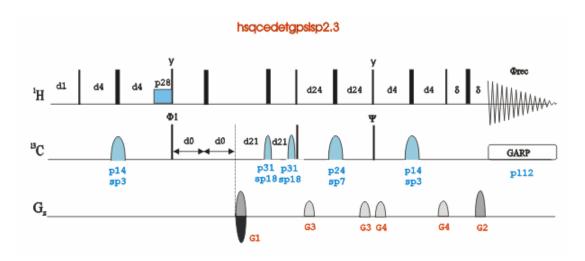
# 7.1.1 Introduction

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 <sup>13</sup>C 180<sup>o</sup> pulses, which causes problems if the 180<sup>o</sup> pulses are too long in duration (e.g.TXI probes) to cover a very wide spectral range. This leads to phasing problems for high field instruments above 500 MHz. This problem is avoided by applying <sup>13</sup>C frequency-swept adiabatic 180<sup>o</sup> pulses which can cover the large <sup>13</sup>C spectral width.

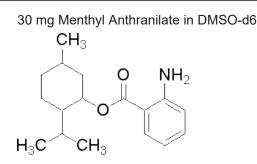
The figure below shows the edited HSQC sequence using hard <sup>13</sup>C pulses:



The edited HSQC sequence using shaped pulses for all 180<sup>o</sup> pulses on the f2-channel with gradients in the back-inept is shown below. For improvement of the phasing the pulse sequence using matched sweep adiabatic pulses **hsqcedetgpsisp2.3** is used in this chapter. This pulse sequence is used in the recommended Bruker parameter set HSQCEDETGPSP-SISP\_ADIA. If desired the sequence **hsqcedetgpsisp2.4** can be used to suppress the COSY peaks.

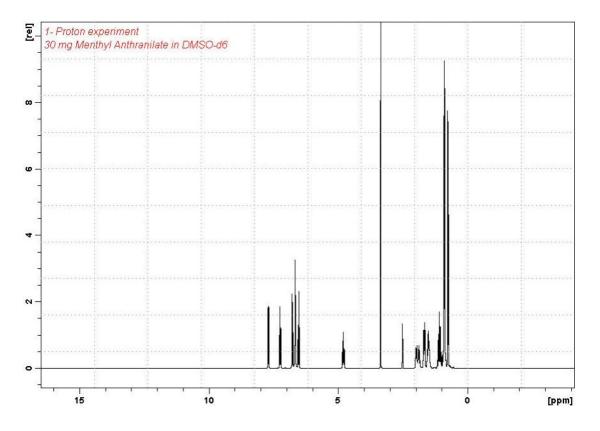


# 7.1.2 Sample



# 7.1.3 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 **setlimits** AU-program.

# 7.1.4 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 Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = shape\_hsqc\_exp EXPNO = 1 Experiment select HSQCEDETGPSP\_ADIA Set Solvent select DMSO

- In the New Dataset window, click OK.
- On the menu bar, click Acquire.
- To tune the probe, click **Tune**.



The last step is necessary to tune the X-channel which is in this case <sup>13</sup>C. 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.

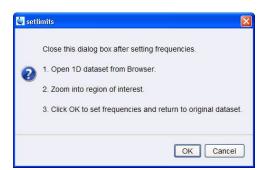
· Click Spin and select Turn sample rotation off.

2D experiments should be run non-spinning.

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

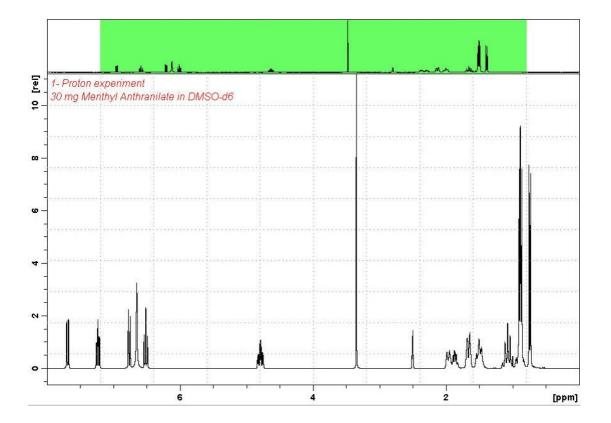
#### 7.1.4.1 Limit Setting

• 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 into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **1.0 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 fold in F1.



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

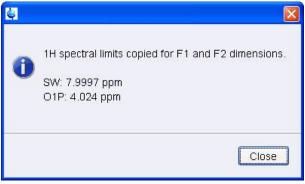


Figure 7.1:

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

The display automatically changes back to the 2D dataset.

## 7.1.4.2 Acquisition

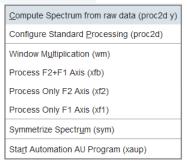
- To adjust the receiver gain, click Gain.
- To start the acquisition, click **Run**.

#### 7.1.4.3 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**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Process F2+F1 Axis (xfb), or at the command prompt, type xfb.

∫ A. Pro<u>c</u>. Spectrum →

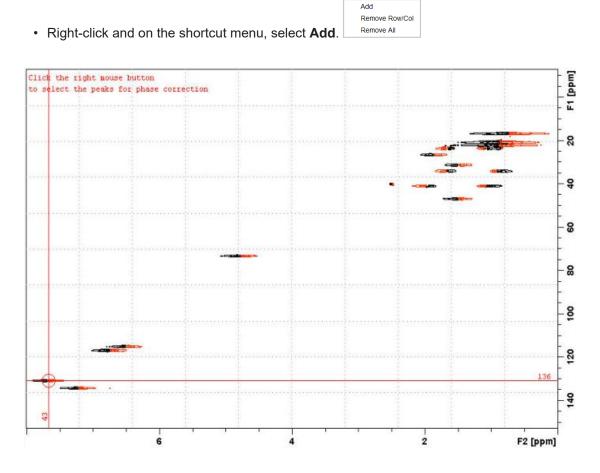


• On the Workflow button bar, click Adjust Phase.

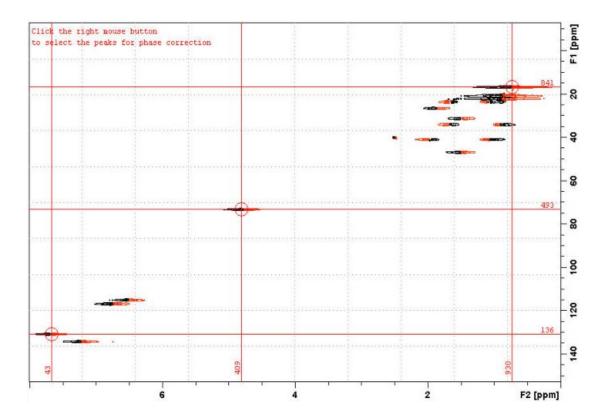
The Adjust phase toolbar is displayed.

• Select the peak at 7.7 ppm/130.9 ppm.

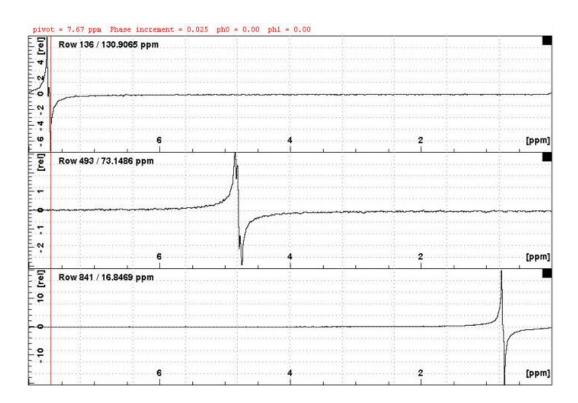
# 2D Experiments using Shaped Pulses



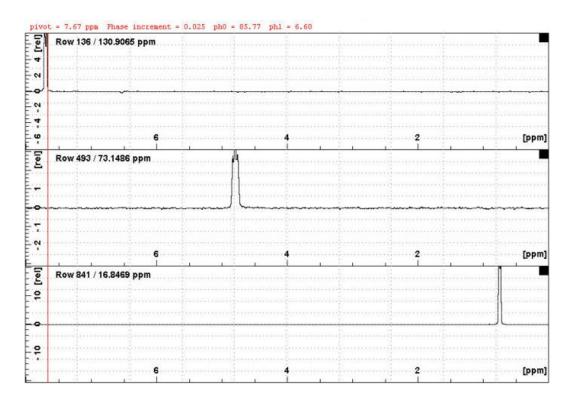
• Repeat the last step for the peaks at 4.8 ppm/73.2 ppm and 0.76 ppm/16.8 ppm.



Click Start the phase correction on rows.



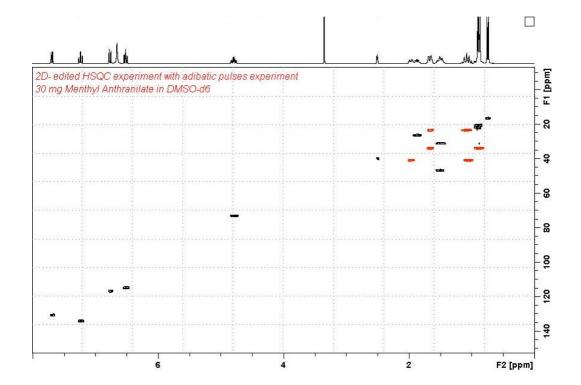
• Adjust the **0** order phase on the peak at 7.7 ppm and the 1<sup>st</sup> order phase on the peak at 0.76 ppm.



- To store the phase values, click Return and Save phased spectrum.  $\blacksquare$
- Click Start the phase correction on columns. C1
- Adjust the **0** and 1<sup>st</sup> order phase.

= 130.91 ppm Phase increment = 0.025 ph0 = -5.04 ph1 = 7.70 pivot [lel] Column 43 / 7.6745 ppm N 2 140 120 100 80 60 40 20 0 [ppm] 4 [rel] Column 409 / 4.8118 ppm 0.5 20 -1.0 20 140 120 100 80 60 40 0 [ppm] [rel] Column 930 / .7368 ppm 40 0 - 40 120 100 60 40 140 80 20 0 [ppm]

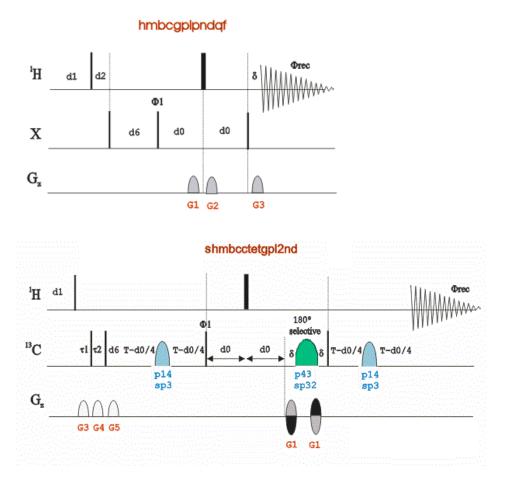
- To store the phase values, click Return and Save phased spectrum.  $\blacksquare$
- To exit the phase window, click the **Return** button.



# 7.2 2D Selective HMBC Experiment

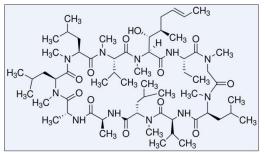
## 7.2.1 Introduction

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 highly 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.



# 7.2.2 Sample

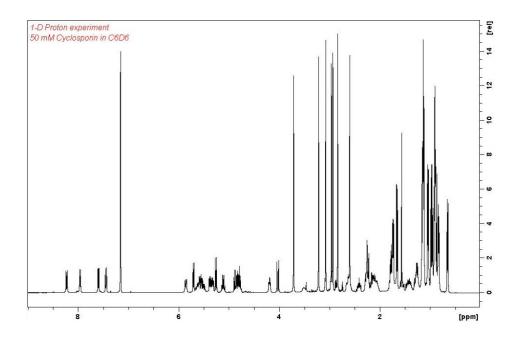
# 50 mM Cyclosporin in $C_6D_6$



# 7.2.3 Preparation Experiments

#### 7.2.3.1 1D Proton Experiment

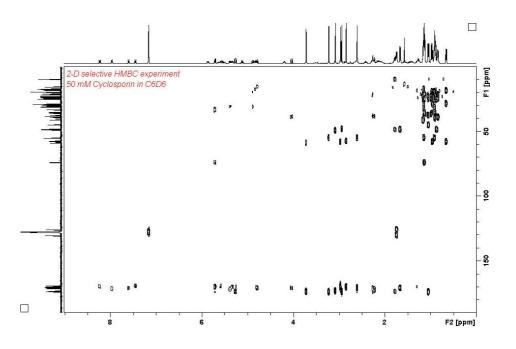
Run a **1D Proton** spectrum of Cyclosporin, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter *1D Proton Experiment*, *Experiment Setup* through *Processing* using  $C_6D_6$  as the solvent.



#### 7.2.3.2 2D HMBC Experiment

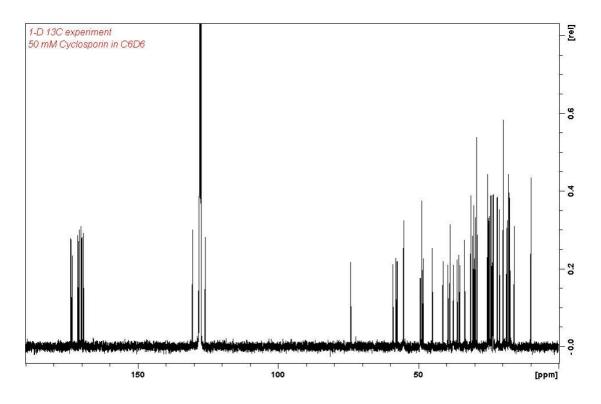
The steps below assume that the sample remains in the magnet after observing the proton spectrum.

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.



#### 7.2.3.3 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.

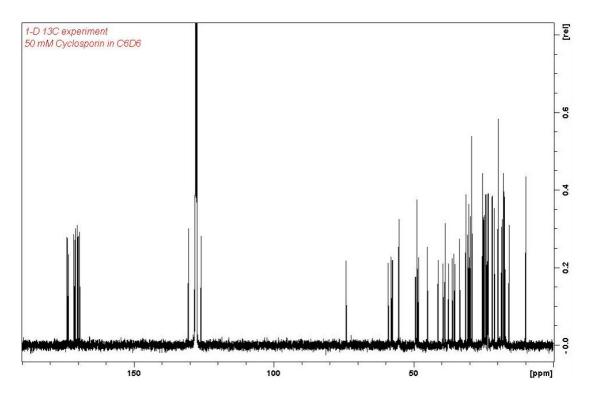


The carbon spectrum is necessary for method 1 but not for method 2, because the sample concentration is too low to get a <sup>13</sup>C spectrum in a reasonable time frame.

#### 7.2.3.4 Method 1 for Setting Up the Selective HMBC Experiment

This method requires a 1D Proton decoupled <sup>13</sup>C spectrum, if it can be obtained with a reasonable number of scans for adequate S/N (Signal to noise).

• Display the carbon spectrum as observed in the last chapter 1D Proton Decoupled Carbon Experiment [▶ 123].



#### 7.2.3.5 Selective Excitation Region Setup

The selective pulse region is set up same way as the 1D selective experiments using the Workflow button bar. 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.

More -

IconNMR Automation (icona)
Setup Selective 1D Expts.
TopSoli <u>d</u> s (topsolids)
Ві <u>о</u> Тор
TopGuide (topguide)
Shape <u>T</u> ool (stdisp)
APSY (apsy)
NMR Thermometer (nmrtemp)

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

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

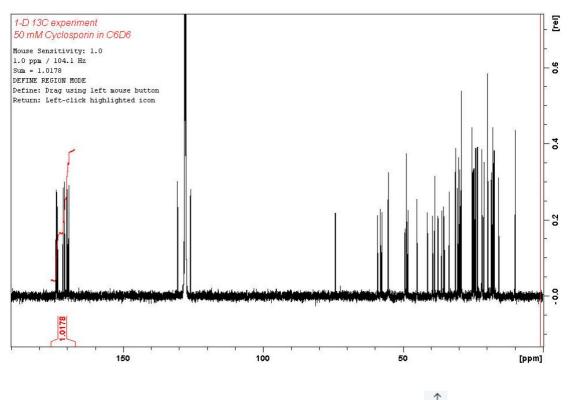
Ġ <u>B</u> ack	1D Selective Experiment Setup	♣ Define <u>R</u> egions	1H DC	Create <u>D</u> atasets -
----------------	-------------------------------	--------------------------	----------	---------------------------

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

The Define Regions toolbar is displayed:



• Integrate the region from 175 ppm to 167 ppm.



On the Integration toolbar, click Save/export integration regions <sup>1</sup>/<sub>2</sub>.

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

• In the list, select Save Regions to reg.

To exit from the integration mode:

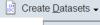
On the toolbar, click Return do NOT save regions!

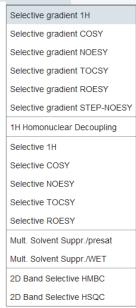


• In the message window, click No.

# 2D Experiments using Shaped Pulses

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





- In the list, select 2D Selective HMBC.
- In the SHMBCCTETGPL2ND window, click Accept.

2D selective H	IMBC		
Shape = Q3.10	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.

	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

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

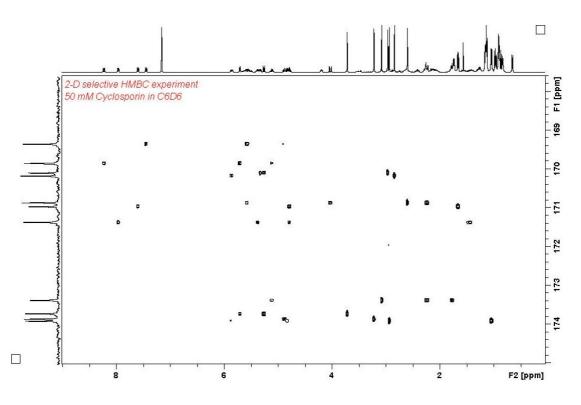
The acquisition starts momentarily.

#### 7.2.3.6 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.

Note: These instructions are at the bottom of the comments in the pulse program file.

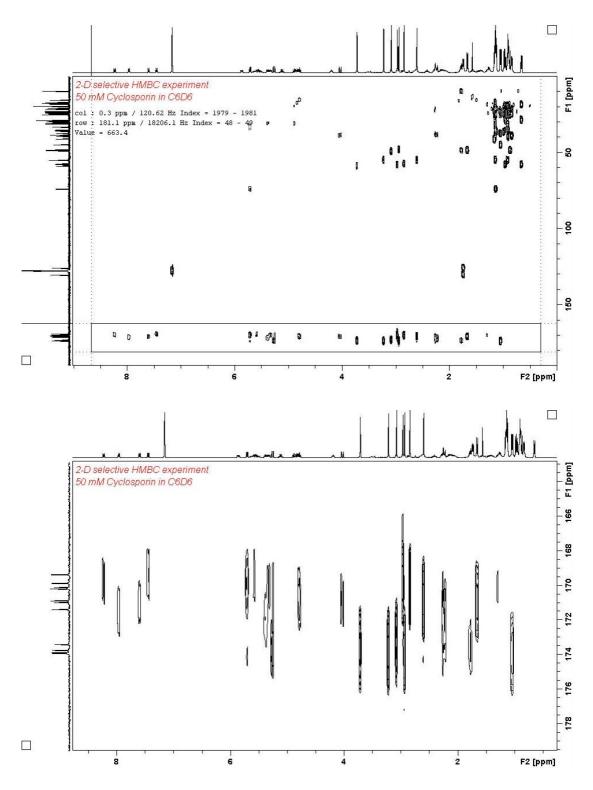
- At the command prompt, type **xfb** to process the data in both dimensions.
- At the command prompt, type **xf2m** to calculate magnitude spectrum in F2.



#### 7.2.3.7 Method 2 for Setting Up the Selective HMBC Experiment

This method uses 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 [> 122].

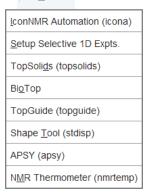


• Expand the region including all cross peaks (e.g. **163 ppm** to **179 ppm**).

• On the menu bar, click **Acquire**.

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

More -



• 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
TopSoli <u>d</u> s (topsolids)	
Ві <u>о</u> Тор	
TopGuide (topguide)	
Shape <u>T</u> ool (stdisp)	
APSY (apsy)	
NMR Thermometer (nmrtemp)	

• In the SHMBCCTETGPL2ND window, click Accept.

🧅 SHMBCCTETGPL2ND 🧮						
2D selective HMBC						
Shape = Q3.100	00					
CNST 13 (Hz)	8.000	J(XH) long range				
NS	4					
EXPNO	2					
Acce	pt Change S	Shape Cancel				

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

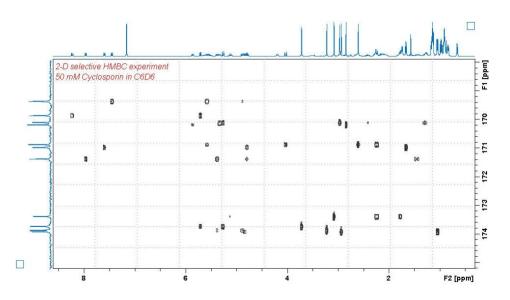
🤤 selhn	nbc 💽
	Acquisition parameters for 2D selHMBC: SHMBCCTETGPL2ND
	Dataset created in EXPNO: 2
?	SW(F1) = 16.8174 ppm / 1691.28 Hz O2 = 171.5711 ppm / 17252.75 Hz TD(F1) = 128 AQ(F1) = 0.0378 sec
	shape pulse (SPNAM 32): Q3.1000 shape pulse duration (P 43): 2822.72 us power level (SPW 32): 0.166779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

• To start the acquisition, click OK.

#### 7.2.3.8 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.



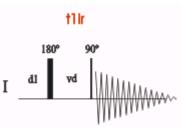
The **Selective HMBC** has a significantly higher <sup>13</sup>C resolution compared to the standard **HMBC** experiment.

# 8 T1 Experiment

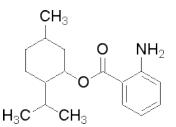
# 8.1 Introduction

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

The basic pulse sequence consists of a  $180^{\circ}$  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  $90^{\circ}$  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 pseudo 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).



# 8.2 Sample

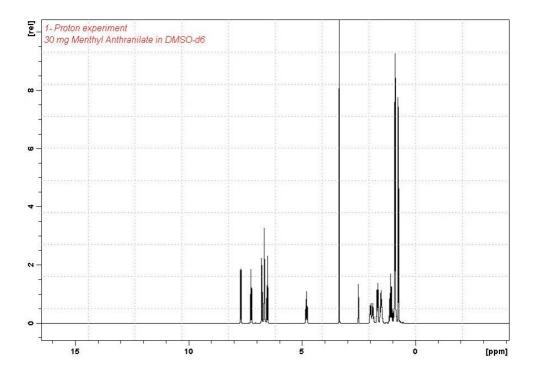


Menthyl Anthranilate in DMSO-d<sub>6</sub>

# 8.3 Proton Inversion-Recovery T1 Experiment

# 8.3.1 **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.

# 8.3.2 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 Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = t1\_exp EXPNO = 1 Experiment: select PROTONT1 Set Solvent: select DMSO

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

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 for best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click Prosol.

# 8.3.3 Limit Setting

• On the Workflow button bar, click SetLimits.

set	inins 🔼
	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.
	OK

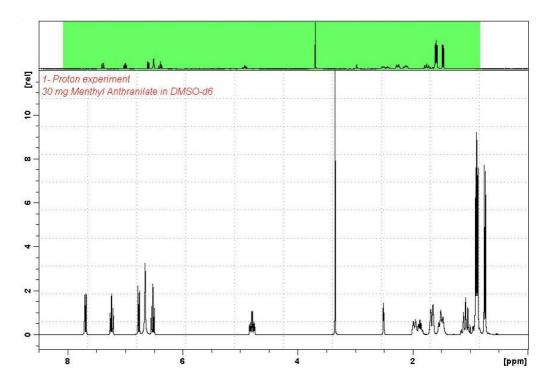
To open the 1D Proton spectrum

 Right click on the dataset name in the browser window (e.g. proton\_exp 1) and select Display

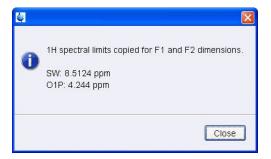
or

- Click and hold the left mouse button and drag the 1D Proton dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving about **1.0 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\_MA

VDLIST t1delay\_MA .....E Variable delay list

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

🖕 t1delay_MA (C:\Bruker\Top 🗾				
<u>F</u> ile	e <u>E</u> dit <u>S</u> earch			
1	0.001			
2	0.050			
3	0.100			
4	0.200			
5	0.300			
6	0.500			
7	0.800			
8	1			
9	1.5			
10	-			
11	2.5			
12	3			
13				
14	-			
15				
16	5 👻			
	19 : 1			

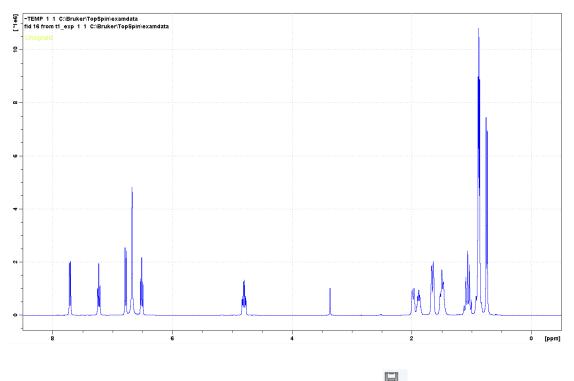
- Enter the variable delay values as shown in the figure above.
- Click File and Save.
- Click File and Close.
- In the Dataset window, select the **Spectrum** tab.

# 8.3.4 Acquisition

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

# 8.3.5 Processing

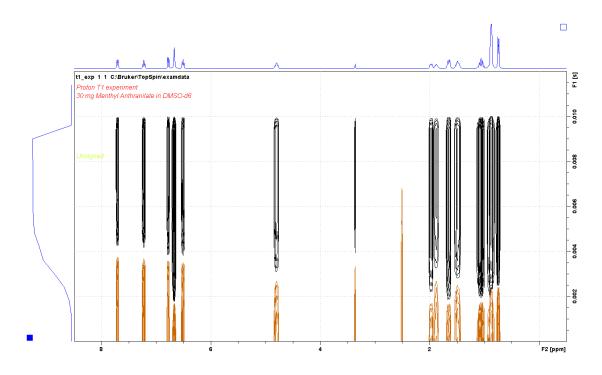
- On the menu bar, click Process.
- At the command prompt, type **rser 10**.
- At the command prompt, type ef.
- On the Workflow button bar, click Adjust Phase.
- · Adjust the phase manually or enter apk for automatic phase correction.



- 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 **To Last 2D data** to go back to the 2-D spectrum display. 2D
- At the command prompt, type **xf2** to process only the F2 axis.
- Type **abs2** to baseline correct the rows.



# 8.3.6 T1 Calculation

- On the menu bar, click **Applications**.
- On the **Dynamics** button, click the drop-down arrow to see more options and in the list, select **T1/T2 Module**.

K	Dynamics 🗸
	<u>T</u> 1T2 (t1t2)
	Prepare for Dynamics Cente
	Dynamics Center (dync)

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

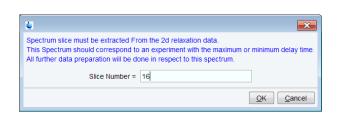
🌀 <u>B</u> ack   🛛 🗠 <u>F</u> id   🔬 <u>P</u> eaks/Ranges	Relaxation	K Fitting	>	Calculation	Beport <u>R</u> eport
---	------------	-----------	---	-------------	-----------------------

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

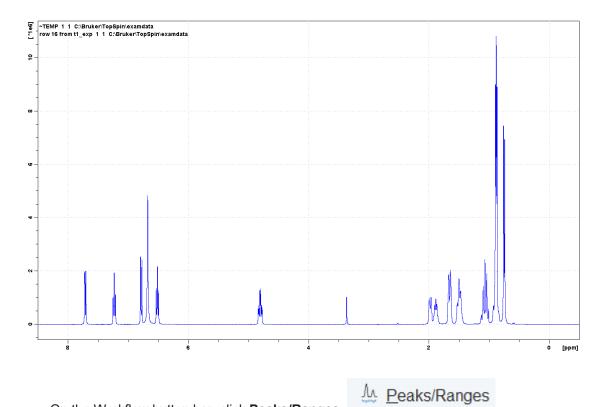
- On the Workflow button bar, click Fid.
- In the Extract a row from 2d data window, click **Spectrum**.

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.



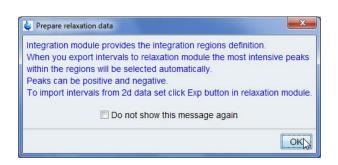
• In the message window, click OK.



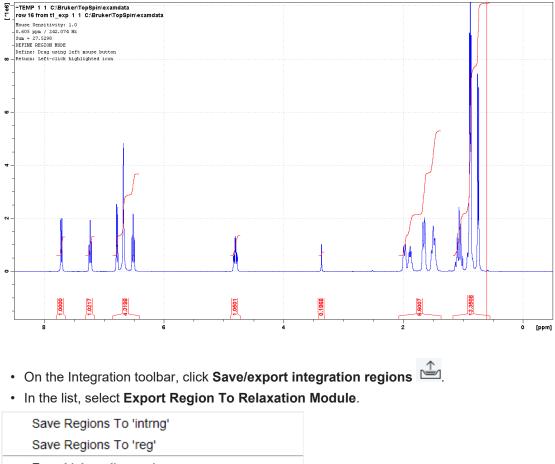
- On the Workflow button bar, click Peaks/Ranges.
- In the Define Peaks and/or Integrals window, click Manual Integration.

?	If you choose manual integration module, you can define integration regions. Most intensive peaks within the intervals will be found automatically. If you choose manual peak picking module, you can define peaks without intervals or peaks within integration regions.
	Ianual Integration Manual Peak Picking Automatic mode Cancel

• In the Prepare relaxation data window, click **OK**.



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



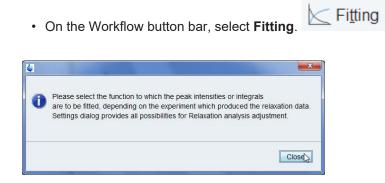
- Export integration regions
- Export Regions To Relaxation Module and .ret.

Save & Show List

• On the Workflow button bar, select Relaxation.

Relaxation

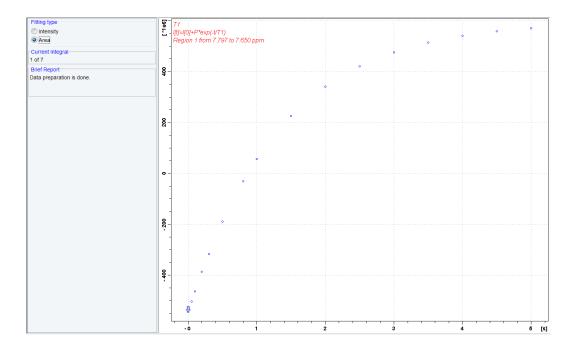
By default, the selected areas are peak-picked, and the first peak is displayed in the Relaxation window.



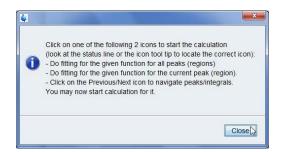
• In the message window, click Close.

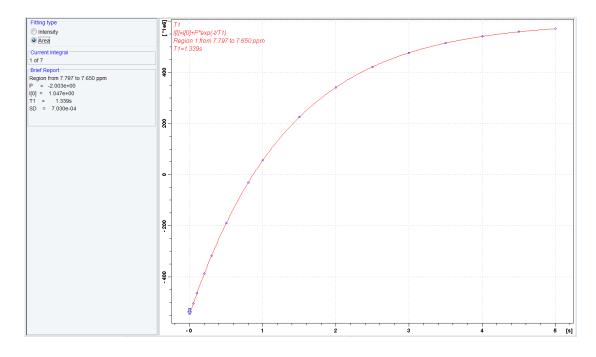
kelaxation parameters			
General Parameters			
16	FID # for phase determ	nination	
1000.0	Left limit for baseline c	orrection	
-1000.0	Right limit for baseline	correction	
5	Number of drift points		
1.0E-5	Convergence limit		
16	Number of points		
1	First slice		
1	Slice increment		
1.0	Peak sensitivity		
Fitting Function			
uxnmrt1			
1	Number of cor	mponents	
vdlist	<ul> <li>List file name</li> </ul>		
0.001	Increment (au	to)	
pd	pd  v to pick data points		
Iteration control parameters			
Guesses Reset			
Additional Parameters			
10000.0	GAMMA(Hz/G)		
10.0	LITDEL(msec)		
100.0	BIGDEL(msec)		
1.0	GRADIEN(G/cm	)	
OK Apply Cancel			

• In the Relaxation parameters window, click **OK** and select **Area** as Fitting type.



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





In the T1/T2 tools bar, click Calculate fit for all peaks.

# Brief Report Region 1 from 7.797 to 7.650 ppm T1 = 1.339s Region 2 from 7.313 to 7.159 ppm T1 = 1.294s Region 3 from 6.860 to 6.413 ppm T1 = 555.498m Region 4 from 4.891 to 4.725 ppm T1 = 644.916m Region 5 from 3.414 to 3.321 ppm T1 = 1.110s Region 6 from 2.076 to 1.372 ppm T1 = 398.815m Region 7 from 1.171 to 0.552 ppm T1 = 378.896m

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

	tting report	×
lie i	Edit Search	
1	Pataset :	-
2	C:/Bruker/TopSpin/examdata/t1_exp/1/pdata/1	
3	AREA fit :	=
4	I[t]=I[0]+P*exp(-t/T1)	1
5		
6	16 points for Integral 1, Integral Region from 7.797 to 7.650 ppm	
7	Results Comp. 1	
8		
9	I[0] = 1.047e+00	
10	P = -2.003e+00	
11 12	T1 = 1.339s SD = 7.030e-04	
12	SD = 7.030e - 04	
13	tau ppm integral intensity	
15	tau ppm integral intensity	
16	1.000m 7.698 -5.4489e+08 -6.8373e+07	
17	50.000m 7.698 -5.0393e+08 -6.3011e+07	
18	100.000m 7.698 -4.6377e+08 -5.7767e+07	
19	200.000m 7.698 -3.8748e+08 -4.7869e+07	
20	300.000m 7.698 -3.1673e+08 -3.8814e+07	
21	500.000m 7.698 -1.8933e+08 -2.298e+07	
22	800.000m 7.698 -3.0687e+07 -3.7791e+06	
23	1.000s 7.698 5.6667e+07 6.6579e+06	
24	1.500s 7.698 2.2514e+08 2.658e+07	
25	2.000s 7.698 3.4075e+08 4.0165e+07	
26	2.500s 7.698 4.2051e+08 4.9459e+07	
27	3.000s 7.698 4.7569e+08 5.5909e+07	
28	3.500s 7.698 5.1425e+08 6.042e+07	
29	4.000s 7.698 5.406e+08 6.3606e+07	
30	4.500s 7.698 5.5853e+08 6.5961e+07	
31	5.000s 7.698 5.7096e+08 6.7579e+07	
32	1:1	-

# 9 **Pulse Calibration**

# 9.1 Introduction

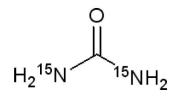
This chapter describes the pulse calibration procedures for determining the  $90^{\circ}$  transmitter pulse of <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N nuclei.



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

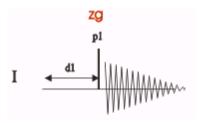
# 9.2 Sample

Mixture **0.1 M** each of <sup>15</sup>N enriched Urea and <sup>13</sup>C enriched methanol in **DMSO-d**<sub>6</sub> (NMR pulse calibration reference standard at *https://bruker-labscape.store*)



<sup>13</sup>CH3-OH

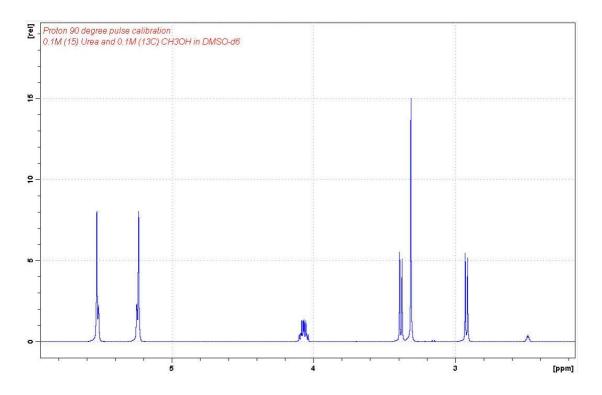
# 9.3 <sup>1</sup>H 90° Transmitter Pulse



The pulse program **zg** is used to determine the <sup>1</sup>H **90**° transmitter pulse. The sequence consists of one channel **f1** with a recycle delay **d1**, a <sup>1</sup>H pulse **p1**, followed by the <sup>1</sup>H 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.

# 9.3.1 Preparation Experiment

Run a **1D Proton** spectrum of urea/methanol in DMSO-d<sub>6</sub>, following the instructions from the *TopSpin Guide Book Basic NMR Experiments*, chapter *1D Proton Experiment*, paragraphs *Experiment Setup* through *Processing*.



# 9.3.2 Parameter Setup

• At the command prompt, type wrpa.

🖉 wrpa			
Copy data set. If NAME ends with ".top", the destination will be a 1-file dataset (no expno/procno required). Please specify destination:			
NAME =	p90_proton		
EXPNO =	1		
PROCNO =	1		
DIR =	C:\data3.0		
	OK Cancel Help		

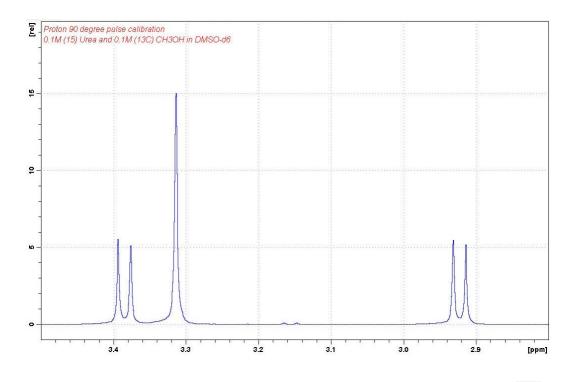
- In the field *Name*, enter **p90\_proton**.
- Click OK.
- At the command prompt, type re.

🔄 re	
<ul> <li>Options</li> <li>Oisplay data in s</li> <li>○ Display data in r</li> </ul>	
NAME = EXPNO =	p90_proton
PROCNO =	1
DIR =	C:\data3.0 Browse Find Help

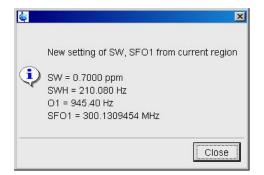
- In the field *Name*, enter **p90\_proton**.
- Click OK.

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

# 9.3.3 Acquisition

- On the menu bar, click Acquire.
- On the Workflow button bar, click Gain.

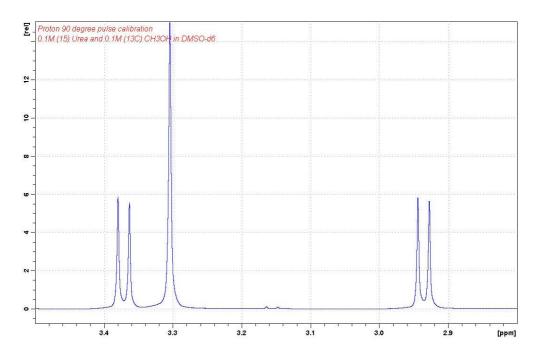
Alternatively type **rga** at the TopSpin command prompt. To adjust the receiver gain manually, click the **drop-down** arrow on the **Gain** button.

• On the Workflow button bar, click Run.

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

## 9.3.4 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...

To	ggle Spectrum Overview
<u>S</u> h	ow Full Spectrum
To	ggle Parameter <u>W</u> indow
Sp	e <u>c</u> tra Display Preferences
Sa	ve Display Regi <u>o</u> n To 🍃
	store Display Region From Params. F1/2
Set	t Plot Height At Specific Cursor Position
Da	taset Properties
<u>F</u> ile	es
Exp	blorer

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

Optio	ons
• Pa	arameters F1/2 (e.g. used by 'restore display',) [dpl]
O P	arameters ABSF1/2 (e.g. used by 'absf, apkf')
O P	arameters STSR/STSI (used by strip ft)
O Pa	arameters SIGF1,2 (signal region) (used by 'sino')
O P	arameters NOISF1,2 (noise region) (used by 'sino')
© A	text file for use with other programs
	,,,

- Click OK.
- At the command prompt, type wpar H1p90\_urea all to store the parameter set for future use.

# 9.3.5 Determine the <sup>1</sup>H 90°Transmitter Pulse

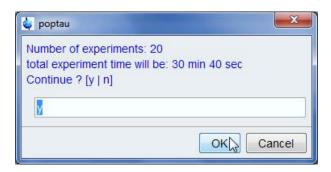
- At the command prompt, type **popt** to display the **P**arameter **OPT**imization window.
- Enter or select from the list boxes:

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

	ata							
store as 2D d	ata (ser file)							
The AU progr	in AUNM will be	e executed	WDW= EM					
Perform autor	natic baselin	e correction (Al	BSF)		PH_mod= pH	k		
Overwrite exis	ting files (di	sable confirmati	on Message)		FT_mod= fs	с		
Stop sample s	spinning at th	ne end of optimi	ization (mash)					
Run optimizat	ion in backg	round						
No display of	estimated ru	nning time						
Calculate opti	mum after P	OPT has finishe	ed, but do not	store in				
Correlate 2D	Container w	th experiment						
OPTIMIZE	GROUP		OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC
OTTIVIZE	GROOF	TANAMETER						
Step by step		p1	POSMAX	2		20	LIN	2
and the second		The second second second		2		20	LIN	2
		p1		2 Add parame.	Rest		LIN	2 Read array f

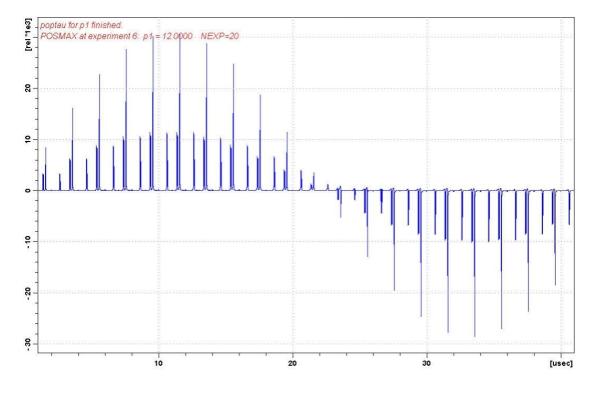
The **ENDVAL** parameter has been updated.

- 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 *p90\_proton/1/pdata/999* as shown:



• In the Dataset window, select the Title tab.

Spectrum ProcPars AcquPars Title PulseProg Peaks

POSMAX at experiment 6: p1 = 12.0000 NEXP=20

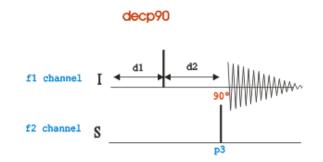
The POSMAX value of p1 is displayed in the title tab window which is the 90° pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90° pulse measurement, follow the steps below:

- · 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.

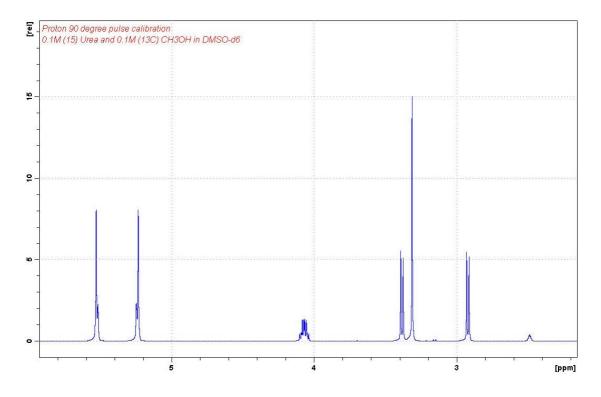
# 9.4 <sup>13</sup>C 90° Decoupler Pulse



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 <sup>1</sup>H and f2 to <sup>13</sup>C. Channel f1 shows a recycle delay **d1** followed by a 90<sup>0</sup> pulse and a delay **d2 = 1/(2JXH)** for the creation of antiphase magnetization. A <sup>13</sup>C pulse on channel f2 is been executed after the delay **d2** and then the <sup>1</sup>H signal is detected. When the <sup>13</sup>C pulse is exactly 90<sup>0</sup>, the <sup>1</sup>H signals will go through a null. The methanol signal region from **3.5 ppm** to **2.8 ppm** is used for this experiment.

#### 9.4.1 **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.



# 9.4.2 Parameter Setup

• At the command prompt, type **wrpa** and press **Enter**.

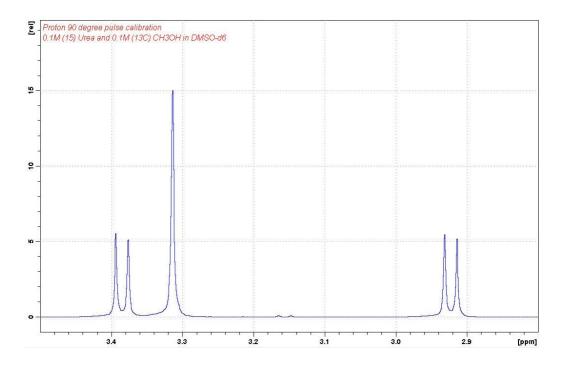
- Change NAME = **p90\_carbon**.
- In the wrpa window, click **OK**.

🛎 wrpa	
In state of the second second second	f NAME ends with ".top", the destination ataset (no expno/procno required). Jestination:
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**.

e re	
Options	
<ul> <li>Display data in</li> <li>Display data in</li> </ul>	
NAME =	p90_carbon
EXPNO =	1
PROCNO =	1
DIR =	C:\data3.0

• 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 message window, click **Close**.



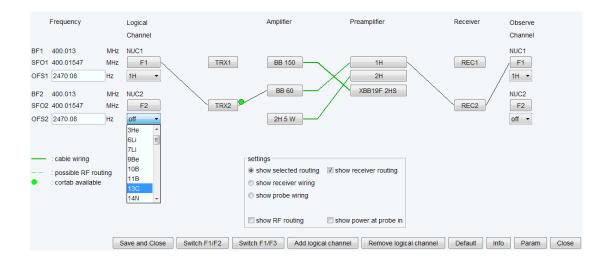
- In the Dataset window, select the **AcquPars** tab.
- Enter:

```
PULPROG = decp90
TD = 2048
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 <sup>13</sup>C for NUC2.



- Frequency Logical Amplifier Preamplifier Receiver Observe Channel Channel BF1 400.013 MHz NUC1 NUC1 SFO1 400.01547 MHz TRX1 BB 150 REC1 F1 1H F1 OFS1 2470.08 Hz 2H 1H 🔻 1H 🔻 BB 60 XBB19F 2HS BF2 100.583349 MHz NUC2 NUC2 SFO2 100.583349 MHz F2 TRX2 REC2 F2 OFS2 0.0 2H 5 W Hz 13C 🔹 off 🔹 : cable wiring settings possible RF routing cortab available Show receiver wiring show probe wiring show RF routing show power at probe in 
   Save and Close
   Switch F1/F2
   Switch F1/F3
   Add logical channel
   Remove logical channel
   Default
   Info
   Param
   Close
- 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** 

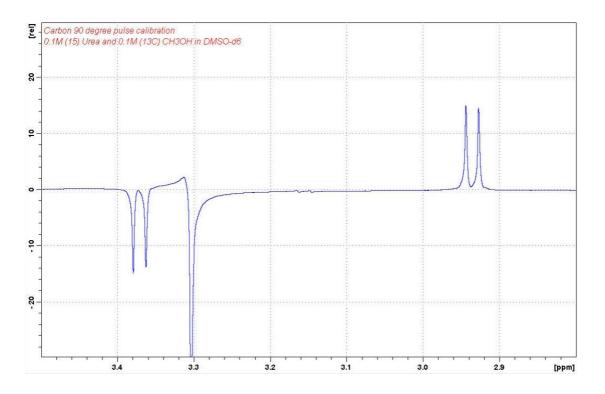
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Prosol**.
- In the Dataset window, select the ProcPars tab.
- Enter:

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.

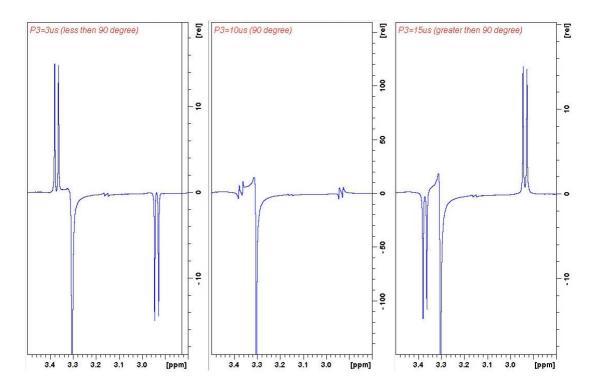
## 9.4.3 Determine the <sup>13</sup>C 90° Decoupler Pulse

- On the menu bar, click Acquire.
- On the Workflow button bar, click Tune.
- On the Workflow button bar, click Run.
- Process and phase correct the spectrum.

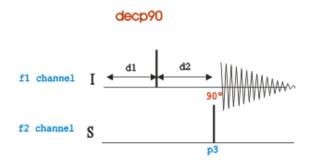


Phase the left doublet negative and the right doublet positive. The water peak at **3.3 ppm** can be ignored and does not have to be in phase.

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 <sup>13</sup>C 90<sup>0</sup> decoupler pulse.

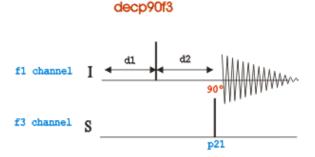


# 9.5 <sup>15</sup>N 90° Decoupler Pulse



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 <sup>1</sup>H and f2 to <sup>15</sup>N. Channel f1 shows a recycle delay **d1** followed by a 90<sup>o</sup> pulse and a delay **d2 = 1/(2JXH)** for the creation of antiphase absorption. A <sup>15</sup>N pulse on channel f2 is executed after the delay **d2** and then the <sup>1</sup>H signal is detected. When the <sup>15</sup>N pulse is exactly 90<sup>o</sup>, the <sup>1</sup>H 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 <sup>15</sup>N 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.



## 9.5.1 Parameter Setup

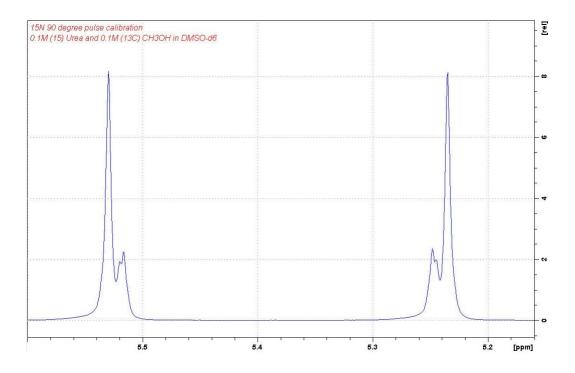
- At the command prompt, type wrpa and press Enter.
- Change NAME = p90\_nitrogen
- In the wrpa window, click OK.

Register and the second statement	f NAME ends with ".top", the destination ataset (no expno/procno required). destination:
NAME =	p90_nitrogen
EXPNO =	1
PROCNO =	1
	C:\data3.0

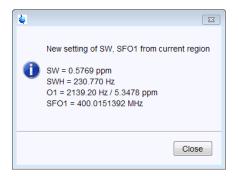
- At the command prompt, type **re** and press **Enter**.
- Change NAME = **p90\_nitrogen**.
- In the re window, click OK.

same window new window
p90_nitrogen
1 1
C:\data3.0

• 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.  $\clubsuit$ 



- In the message window, click Close.
- In the Dataset window, select the AcquPars tab.
- Enter:

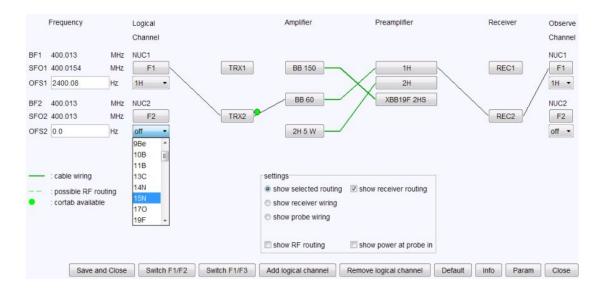
PULPROG = decp90 TD = 2048 NS = 1 DS = 0

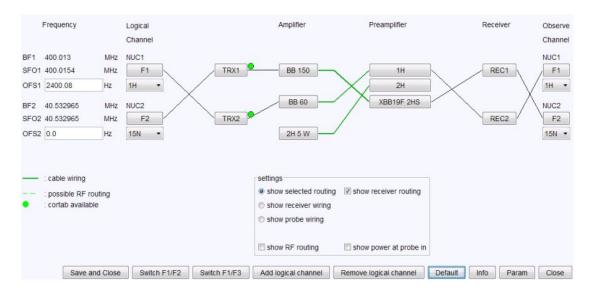
• In the Nucleus2 section of the AcquPars, click Edit next to NUC2.

Vucleus 2				
NUC2	off Edit	2nd nucleus		
O2 [Hz]	1853.43	Frequency offset of 2nd nuclei		
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		

# 9.5.1.1 Two Channel System

• Select <sup>15</sup>N for NUC2.



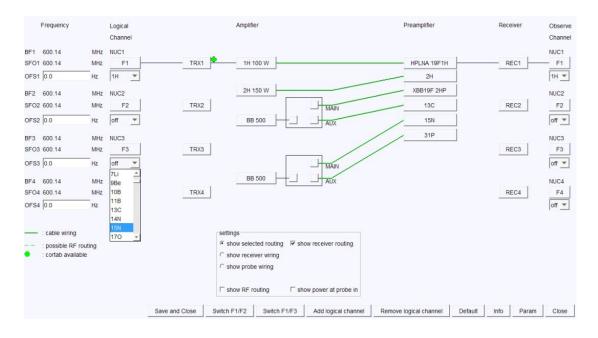


• In the Edit Spectrometer Parameter window, click Default to set the routing.

• In the Edit Spectrometer Parameter window, click Save.

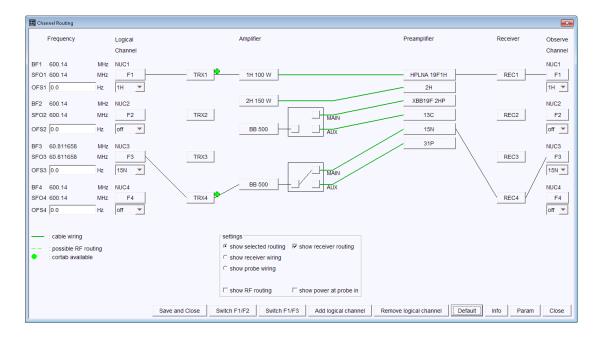
#### 9.5.1.2 Three Channel System

Select <sup>15</sup>N for NUC2.



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

# **Pulse Calibration**



- In the Edit Spectrometer Parameter window, click Save.
- In the AcquPars make the following change:

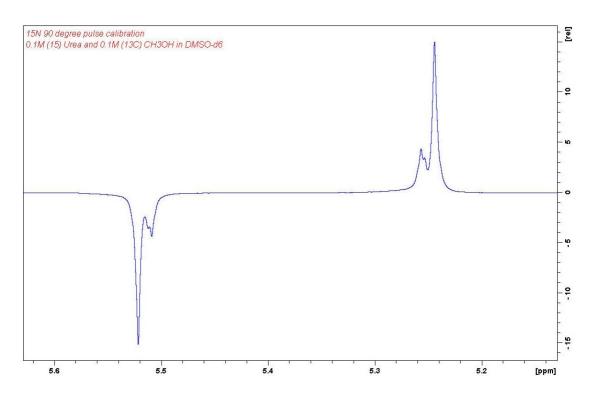
O2[ppm] = **76** D1 = **10** CNST2 = **88.5** P3 = **6** 

- 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.

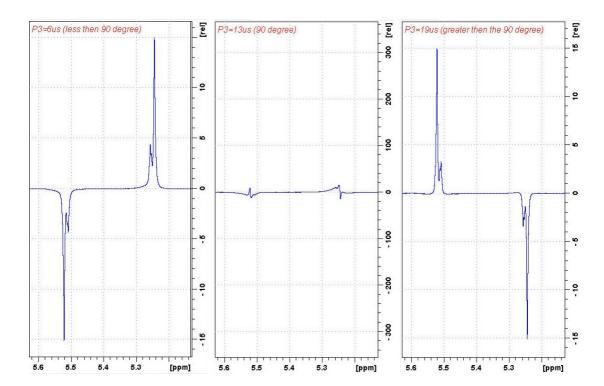
# 9.5.2 Determine the <sup>15</sup>N 90° Decoupler Pulse

- On the Workflow button bar, click **Tune**.
- On the Workflow button bar, click Run.
- Process and phase correct the spectrum.

• Phase the left side signal negative and the right-side signal positive!

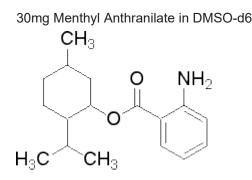


Increase p3 in increments of 1 or 2 μs, execute zg followed by the command efp until the signals go through a null or a phase change. This will be the <sup>15</sup>N 90<sup>0</sup> decoupler pulse.



# **10** <sup>1</sup>H Homonuclear Decoupling

# 10.1 Sample



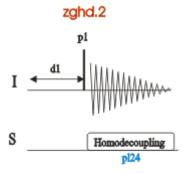
# 10.2 <sup>1</sup>H Homonuclear Decoupling Experiment

## 10.2.1 Introduction

The homonuclear decoupling (homodecoupling) simplifies multiplet structures by irradiating a specific <sup>1</sup>H resonance. Unambiguous assignments and measurement of <sup>1</sup>H-<sup>1</sup>H coupling constants can be performed by analyzing the resulting residual multiplets.

During an homo-decoupling experiment, a conventional <sup>1</sup>H 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.



## 10.2.2 Acquisition

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.

The steps below assume that the sample remains in the magnet after acquiring the proton experiment.

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

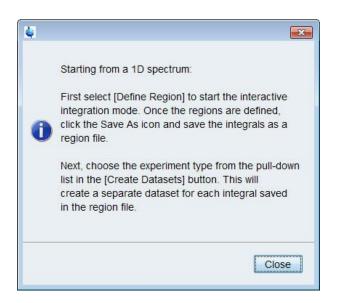
More ✓ [conNMR Automation (icona) Setup Selective 1D Expts. TopSolids (topsolids) BioTop TopGuide (topguide) Shape Tool (stdisp) APSY (apsy) NMR Thermometer (nmrtemp)

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

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

G <u>B</u> ack	1D Selective Experiment Setup	♣ Define <u>R</u> egions	HI DC	Create <u>D</u> atasets $\bullet$
----------------	-------------------------------	--------------------------	----------	-----------------------------------

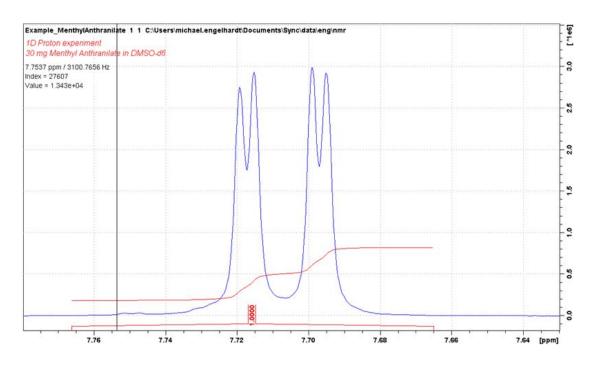
• On the Workflow button bar, click 1D Selective Experiment Setup.



This button is only used for the instruction displayed above.

- In the message window, click Close.
- Expand the peak at 7.7 ppm.
- On the Workflow button bar, click Define Regions.

• Integrate the multiplet at 7.7 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/export integration regions**
- In the list, select Save the Region to 'reg'.



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

¢.	<b>•</b>
0	Save Changes?
	Yes

- On the Create Dataset button, click the drop-down arrow to see more options.
- In the list, select 1H Homonuclear Decoupling.

🛃 Create <u>D</u>atasets 🗸

Selective gradient 1H Selective gradient COSY Selective gradient NOESY Selective gradient TOCSY Selective gradient ROESY Selective gradient STEP-NOESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective ROESY Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./wET 2D Band Selective HBOC	
Selective gradient NOESY Selective gradient TOCSY Selective gradient TOCSY Selective gradient ROESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective ROESY Selective ROESY Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective gradient 1H
Selective gradient TOCSY Selective gradient ROESY Selective gradient RTEP-NOESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective ROESY Selective ROESY Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective gradient COSY
Selective gradient ROESY Selective gradient STEP-NOESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective ROESY Selective ROESY Mult. Solvent Suppr/presat Mult. Solvent Suppr/WET 2D Band Selective HMBC	Selective gradient NOESY
Selective gradient STEP-NOESY 1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective ROESY Mult. Solvent Suppr/presat Mult. Solvent Suppr/WET 2D Band Selective HMBC	Selective gradient TOCSY
1H Homonuclear Decoupling Selective 1H Selective COSY Selective NOESY Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective gradient ROESY
Selective 1H Selective COSY Selective NOESY Selective ROESY Mult: Solvent Suppr./presat Mult: Solvent Suppr./WET 2D Band Selective HMBC	Selective gradient STEP-NOESY
Selective COSY Selective NOESY Selective NOESY Selective ROESY Mult: Solvent Suppr./presat Mult: Solvent Suppr./WET 2D Band Selective HMBC	1H Homonuclear Decoupling
Selective NOESY Selective TOCSY Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective 1H
Selective TOCSY Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective COSY
Selective ROESY Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective NOESY
Mult. Solvent Suppr./presat Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective TOCSY
Mult. Solvent Suppr./WET 2D Band Selective HMBC	Selective ROESY
2D Band Selective HMBC	Mult. Solvent Suppr./presat
	Mult. Solvent Suppr./WET
2D Band Selective HSOC	2D Band Selective HMBC
20 Dana Gelective Holdo	2D Band Selective HSQC

• In the PROHOMODEC window, click Accept.

🖕 PROHOMODEC 🛛 🔀		
1H Homonuclear Decoupling		
NS	32	
first EXPNO	100001	
Accept	Cance	

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

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

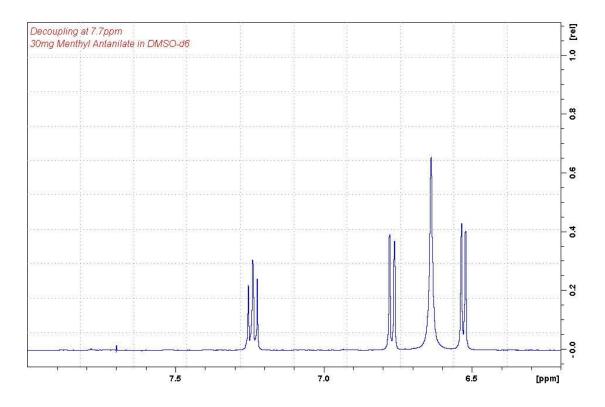
🛶 sel1d	ß
0	1H Homonuclear Decoupling: PROHOMODEC Dataset created in expno 100000. total experiment time will be 3 min 16 sec OK: starts acquisition CANCEL: creates data sets only.
	OK Cancel

# 10.2.3 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**.



• Expand the region from 8 ppm to 6.2 ppm.



The multiplet at **6.4 ppm** should collapse from a triplet to a doublet. If the triplet is partially collapsed, increase the decoupling power pl24 and repeat the steps in chapter Acquisition and *Processing* [> 164].

# 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.

# **10.2.4 Plotting Two Spectra on the Same Page**

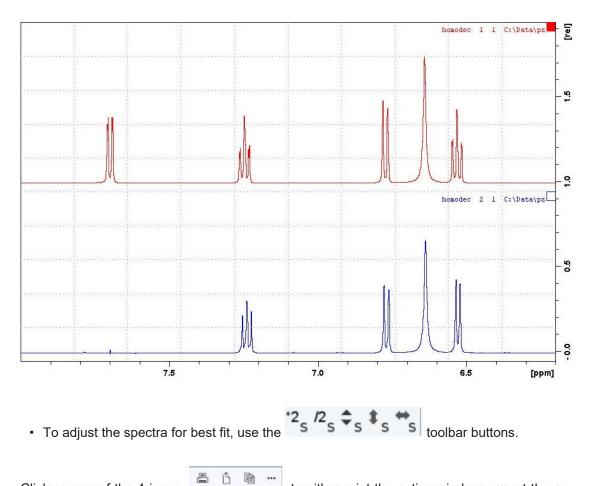
• Display the decoupled spectrum.

• On the toolbar, click **Multiple display**.

The Multiple display toolbar is displayed:



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



Click on any of the 4 icons to either print the active window, export the active window to a PDF file, copy the active window to the clipboard or, show more publishing options such as E-mailing a dataset, sending a dataset to cloud storage, etc.

# **11 Proton DOSY Experiment**

# 11.1 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 ( $\delta$ ), molecules move during the diffusion time ( $\Delta$ ). 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:

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

Where I is the observed intensity, **D** is the diffusion coefficient,  $\gamma$  is the gyro magnetic ratio of the encoded nucleus, **g** is the gradient strength,  $\delta$  is the length of the gradient pulse, and  $\Delta$  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**.

# 11.2 Sample

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

i

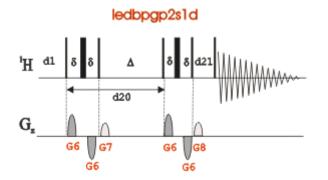
The experimental parameters of  $\delta$  (pl30) and  $\Delta$  (d20) described here are for this sample. If using a different sample, they will likely be different.

# 11.3 DOSY Experiment

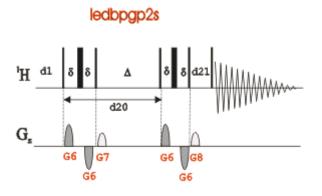
# 11.3.1 Pulse Programs

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* [▶ 171] and.



The pulse program in the figure below is used for the DOSY experiment, see the chapter *Running the Experiment* [> 175]. 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.

# 11.3.2 **Preparation Experiment**

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = **DOSY\_exp** EXPNO = **1** Experiment: select **PROTON** Set Solvent: select **DMSO** 

Description for a second	a supervise and the supervised and a supervised state and the supervised state of the supervised state	
	v experiment by creating a new data set and a parameters according to the selected experiment type.	
	experiments several datasets are created.	
Please define the	number of receivers in the Options.	
Dataset		
NAME	DOSY_exp	
EXPNO	1	
Directory	C:\DMB ~	
Open in new wind	dow	
<ul> <li>Parameters</li> </ul>		
	notoro	
O Use current paran		
Read parameters	et PROTON Selec	ŧ.,
		•
Set solvent	DMSO ~	L
Additional action	DMSO ~	
Additional action Do nothing		
Additional action		
Additional action Do nothing     Execute getprose		
Additional action	ol	L
Additional action	ol	
Additional action	ol s P 1, O1, PLW 1 V Change	
Additional action	ol s P 1, O1, PLW 1 V Change	
Additional action © Do nothing O Execute getprose O Keep parameters Advanced Number of datasets (r Title DOSY experiment	ol s P 1, O1, PLW 1 V Change	
Additional action © Do nothing O Execute getprose O Keep parameters Advanced Number of datasets (r Title DOSY experiment	ol s P 1, O1, PLW 1 V Change	
Additional action © Do nothing O Execute getprose O Keep parameters Advanced Number of datasets (r Title DOSY experiment	ol s P 1, O1, PLW 1 V Change	

#### 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.

#### 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 for best homogeneity, click Shim.
- To load the probe/solvent depended parameters, click **Prosol**.

## 11.3.3 Acquisition

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

## 11.3.4 Processing

· Process and phase correct the spectrum.

# 11.3.5 Limit Settings

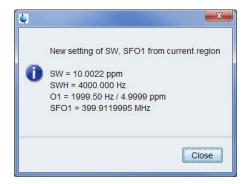
Changing the sweep width to a smaller value increases the resolution.

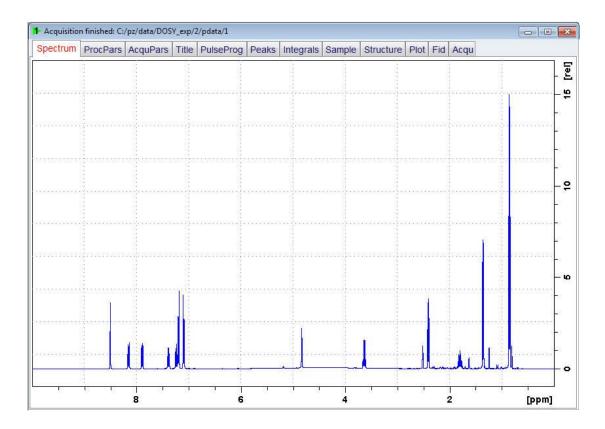
• On the command line, type:

wrpa 2

re 2

- 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.





# 11.3.6 Parameter Setup

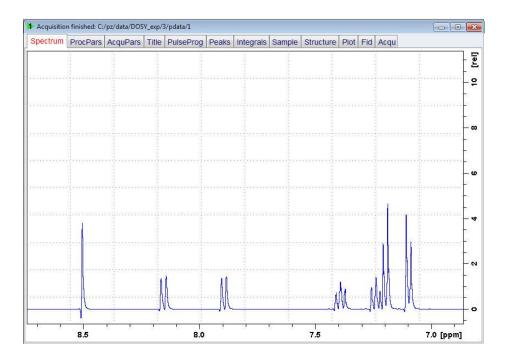
For an accurate DOSY experiment, certain parameters need to be calibrated for each sample to ensure that the observed signal decay is appropriate. This section will walk you through this process.

- On the command line, type **iexpno**.
- In the Dataset window, select the AcquPars tab.
- Click Show pulse program parameters.
- Enter:

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 **Run**.
- Process and phase correct the spectrum.

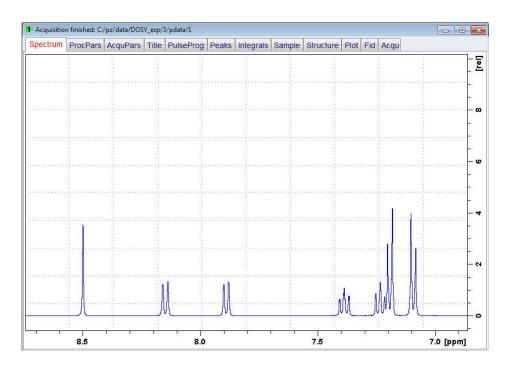
• Expand the spectrum to display all peaks.





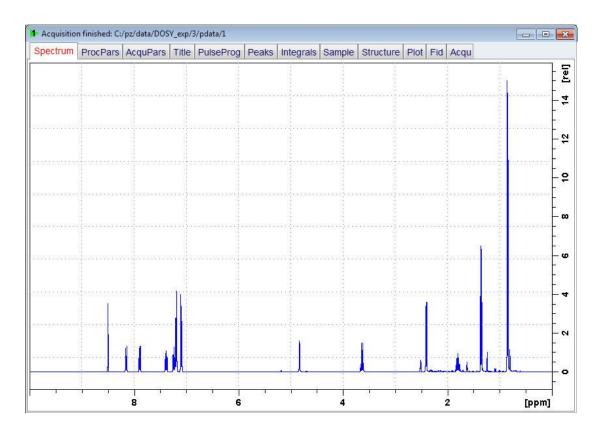
The asymmetry of the peaks is an artifact which is caused by non-optimal lock settings. The problem can be fixed by applying a loop adjust of the lock especially that of the lock phase.

- At the command prompt, type **loopadj** and confirm with **OK**.
- To start the acquisition, click **Run**.
- · 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 a 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.

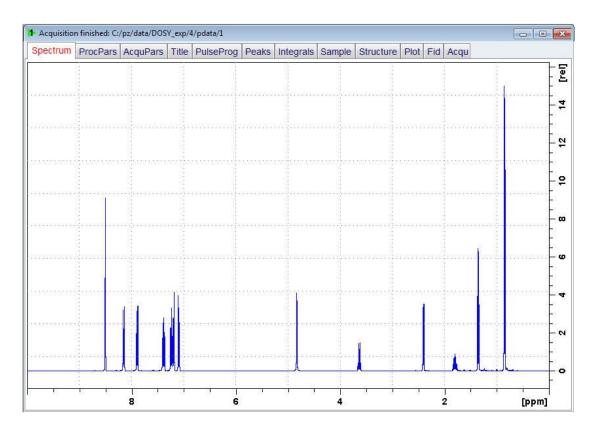
If large changes to p30 and d20 are necessary, then it is recommended to re-run both experiments because these values will affect the intensity of not only the attenuation in the 2nd spectrum, but the starting intensity in the 1st spectrum.

- On the command line, type **iexpno**.
- In the Dataset window, select the AcquPars tab.
- Click Show pulse program parameters.

Enter:

GPZ6[%] = **95** 

- To start the acquisition, click **Run**.
- Process and phase correct the spectrum.



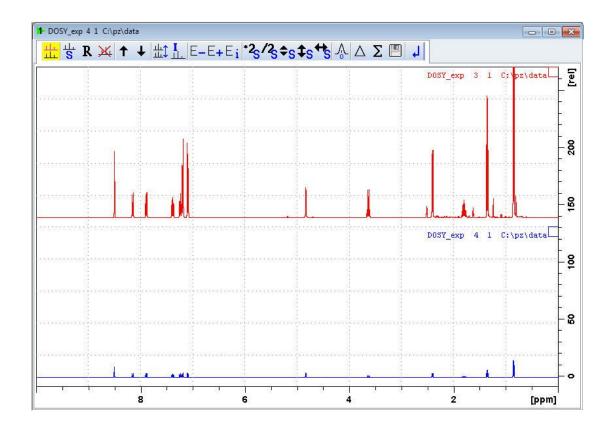


If there are no signals present, then p30 and/or d20 need to be reduced.

• On the toolbar, click **Multiple display**.

The Multiple display toolbar is displayed:

• 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.

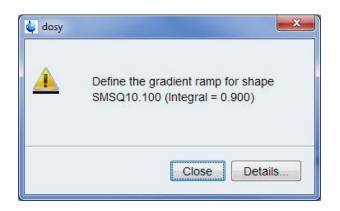
• To exit the multiple display, click Return.

# 11.3.7 Running the Experiment

- On the command line, type **iexpno**.
- In the Dataset window, select the AcquPars tab.
- Enter:
  - PULPROG = ledbpgp2s
- Click Change acquisition dimension of current dataset.

🦕 parmode 📃 🔜
Warning!
You are about to change the dimension of the current dataset. As a consequence an existing FID will be deleted! Change acquisition dimension of dataset from 1D to 2D -
OK Cancel

- In the parmod window, select **2D** and click **OK**.
- Enter: TD[F1] = 25 FnMODE = QF
- At the command prompt, type **dosy**.



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

X
ude:
OK Cancel

• Enter 2 for first gradient amplitude and click OK.

OK	Cancel
	OK

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

🖕 dosy		×
Enter number of points:		
25		
	ОК	Cancel

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

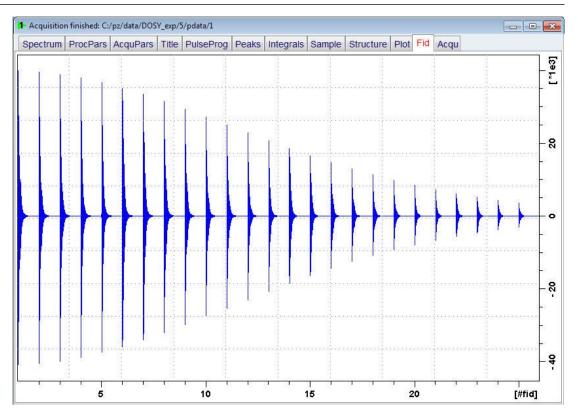
🖕 dosy	X
ramp type (I/q/e {line	ear/squared/exponential} ):
l	
Б <u>.</u>	
	OK Cancel

• In the dosy window, enter I for the ramp type and click **OK**.

🧅 dosy	
0	Do you want to start acquisition ?
	OK Cancel

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

# 11.3.8 Processing



• In the Dataset window, select the Fid tab.



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

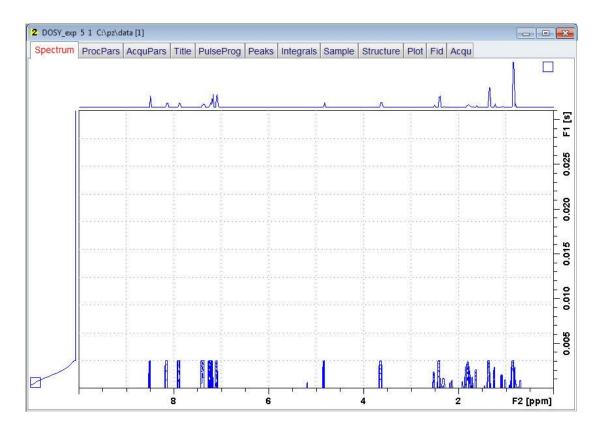
- In the Dataset window, select the ProcPars tab.
- Make the following changes: SI [F1] = 256
  - PH\_mod [F1] = **no**
  - PH\_mod [F2] = **pk**
- At the command prompt, type rser 1 to read in the first serial file of the 2D experiment.
- At the command prompt, type **em** to apply the window function.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



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 **To Last 2D data** to go back to the 2-D spectrum display. 2D
- 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.



# 11.3.9 Calculating the Diffusion Coefficient

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

• On the menu bar, click Applications.

×

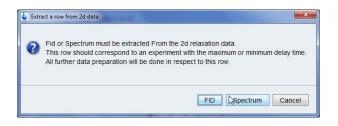
- On the **Dynamics** button, click the **drop-down** arrow to see more options.
- In the list, select **T1/T2**.

ζ	Dynamics -
	<u>T</u> 1T2 (t1t2)
	Prepare for Dynamics Center
	Dynamics Center (dync)

i	The Workflow buttons change to the mode: Determination of the <b>T1</b> / <b>T2</b> relaxation times.		
	General Section → Eid → Peaks/Ranges → Elevent Relaxation → Elevent Section → Elevent Report     Section → Elevent Alevent Alevent → Elevent Alevent Alevent → Elevent Alevent → Elevent →		
	• On the Workflow button bar, click <b>Fid</b> .		

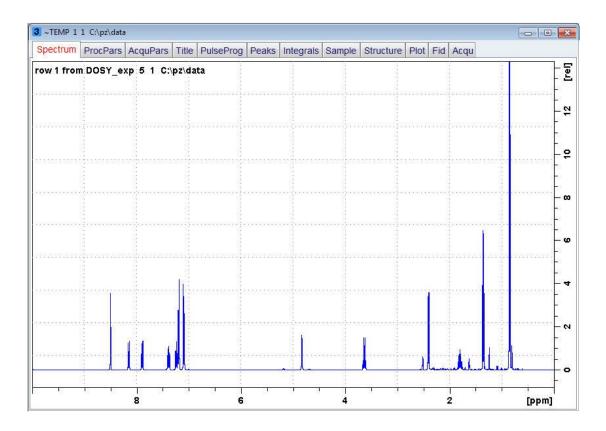
While executing the next steps, message windows will pop up. Please read each message thoroughly and follow the instructions in it.

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

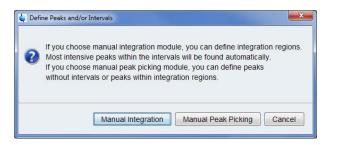


• In the field Slice Number, enter 1 and in the message window click OK.

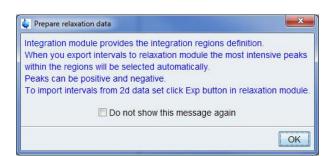
é	×
Spectrum slice must be extracted From the 2d relaxa This Spectrum should correspond to an experiment w All further data preparation will be done in respect to Slice Number =	with the maximum or minimum delay time.
	OK Cancel



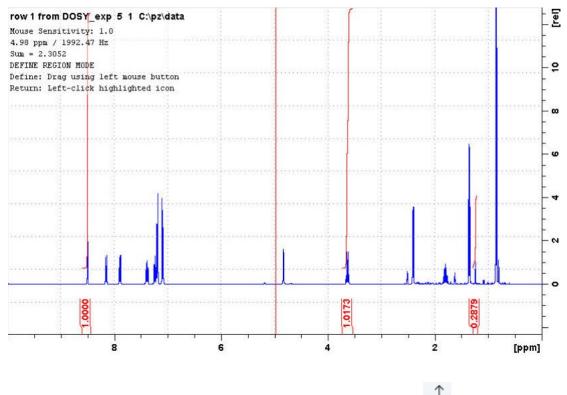
- On the Workflow button bar, click Peaks/Ranges.
- In the Define Peaks and/or Integrals window, click Manual Integration.



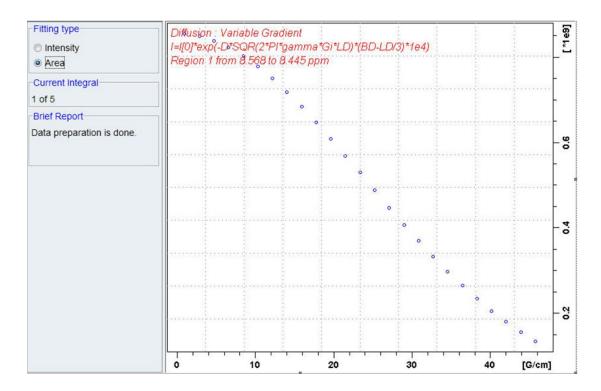
• In the Prepare relaxation data window, click **OK**.



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



- On the Integration toolbar, click Save/export integration regions
- From the drop-down list, select Export Region To Relaxation Module.
- On the Workflow button bar, click **Relaxation**.
- In the group box Fitting type, select Area.



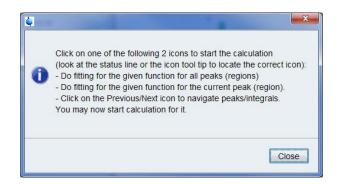
- On the Workflow button bar, click **Fitting**.
- In the message window, click **Close**.

0	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.
	Ciose

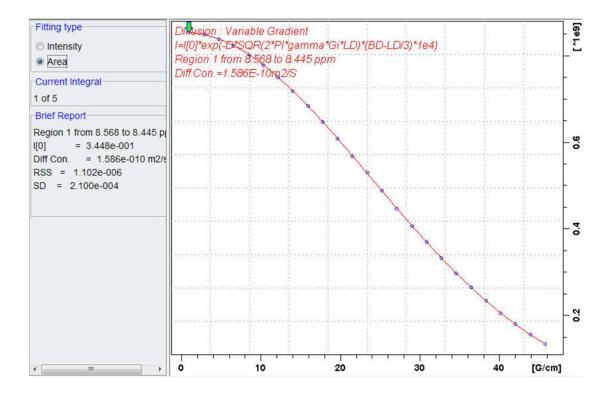
• In the Fitting Function group box, select vargrad and difflist and click OK.

🍓 Relaxation	parameter	s	x			
1	1 FID # for phase determination					
10.0	Left limit for baseline correction					
0.0	Right li	mit for baseline	correction			
5	Numbe	r of drift points				
1.0E-5	Conve	rgence limit				
25	Numbe	r of points				
1	First sl	ice				
1	Slice in	crement				
1.0	Peak s	ensitivity				
vargrad	vargrad • Function Type					
1 Number of components						
difflist   List file name						
0.001	0.001 Increment (auto)					
pd	pd   to pick data points					
Guesses Reset						
4257.7		GAMMA(Hz/G)				
2.8	1	LITDEL(msec)				
99.9	1	BIGDEL(msec)				
1.0	(	GRADIEN(G/cm	)			
	OK Apply Cancel					

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



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



```
j
```

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

# Brief Report Region 1 from 8.610 to 8.446 ppm Diff Con. = 1.585e-010 m2/s Region 2 from 3.740 to 3.539 ppm Diff Con. = 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.

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

🖕 Fitt	ing report				x
File	Edit Search				
1	SIMFIT RESULTS				
2	PHETI RESOLIS				
3					
4	Dataset : C:/pz/data	Junsy evo/5/nd	lata/1/ct1t2 to	vt	
5	bataset . c./pz/data	(1 1031_6xp) 3/ pa	laca) 1) ccicz. cz		
6	AREA fit : Diffusion	· Variable Gr	adient :		
7			uurene i		
8	I=I[0]*exp(-D*SQR(2*	PI*gamma*Gi*LD	) * (BD-LD/3) *1	e4)	Е
9		,			
10	25 points for Integr	al 1, Integra	l Region from	8.610 to 8.446 ppm	
11	. ,		2		
12	Converged after 49 i	terations!			
13					
14	Results Comp. 1				
15					
16	I[0] = 9.	817e-001			
17	Diff Con. = 1.	585e-010 m2/s			
18	Gamma = 4.	258e+003 Hz/G			
19	Little Delta =	2.800m			
20	Big Delta =	99.900m			
21					
22	RSS = 1.348e - 005				
23	SD = 7.343e-004				
24					
25	Point Gradient	Expt	Calc	Difference	
26 27	1 9.630e-001	9.830e-001	9.809e-001	0.051.000	
28	1 9.630e-001 2 2.829e+000	9.830e-001 9.742e-001	9.748e-001	-2.054e-003 6.746e-004	
29	2 2.829e+000 3 4.695e+000	9.623e-001	9.629e-001	5.530e-004	
30	4 6.560e+000	9.446e-001	9.453e-001	6.885e-004	
31	5 8.426e+000	9.222e-001	9.223e-001	6.356e-005	
32	6 1.029e+001	8.942e-001	8.944e-001	1.490e-004	
33	7 1.216e+001	8.619e-001	8.620e-001	1.291e-004	
34	8 1.402e+001	8.254e-001	8.257e-001	3.019e-004	
35	9 1.589e+001	7.862e-001	7.861e-001	-2.347e-005	
36	10 1.775e+001	7.437e-001	7.439e-001	2.300e-004	
37	11 1.962e+001	6.998e-001	6.996e-001	-1.402e-004	
38	12 2.149e+001	6.542e-001	6.539e-001	-2.506e-004	
39	13 2.335e+001	6.095e-001	6.075e-001	-1.988e-003	
40	14 2.522e+001	5.610e-001	5.609e-001	-4.750e-005	
41	15 2.708e+001	5.140e-001	5.148e-001	7.867e-004	
42	16 2.895e+001	4.686e-001	4.695e-001	9.776e-004	
43	17 3.082e+001	4.250e-001	4.257e-001	6.155e-004	
44	18 3.268e+001	3.835e-001	3.835e-001	2.036e-005	
45	19 3.455e+001	3.428e-001	3.434e-001	6.505e-004	+
				1:1	

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

#### 11.3.10 Displaying the DOSY Plot

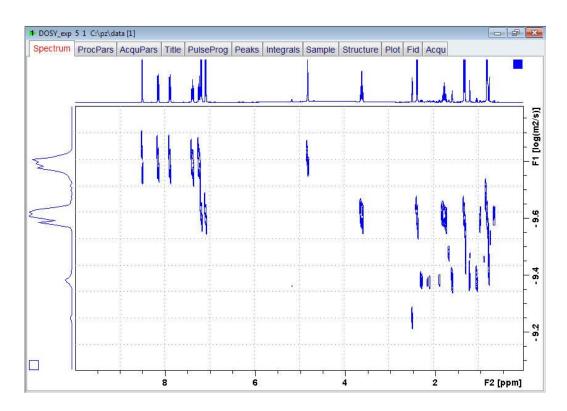
- On the Workflow button bar, click **Back**.
- On the command line, type **re 5**.
- On the menu bar, click Analyse.
- On the Workflow button bar, click **Dosy**.

• From the drop-down list select Setup Parameters.

Setup Parameters (eddosy) Start Processing (dosy2d)

General First	General			
Second	Method	exponential	-	Processing method
Third	ExpVar Xlist	Gradient	+	Variable parameter
Baseline		difflist		Variable parameter values file name
Contin	Nstart	0		Start of input points
	Ndata	25		Number of input points (TD)
	Maxiter	100		Maximum number of iterations
	EPS	1		Tolerance
	Nexp	1		Number of components to fit
	Noise	0		Noise level (S_DEV)
	PC	4		Noise sensitivity factor
	SpiSup	1		Spike suppression factor
	F1mode	Peaks	-	F1 output data mode
	Imode	Integral	-	Fitted intensity meaning
	Scale	Logarithmic	•	Scaling
	LWF	1		Line width factor
	DISPmin	-10		Lower display limit
	DISPmax	-8.02228		Upper display limit
	Npars	7		Number of parameters

- Make the following change: Scale = Logarithmic
- On the toolbar, click **Start fitting**.



# **12 Multiplet Analysis**

## 12.1 Introduction

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

#### 12.2 Sample

100 mg 2, 3,-Dibromopropionic acid in CDCI<sub>3</sub>

### **12.3 Multiplet Assignments**

#### 12.3.1 **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.

#### 12.3.2 Limit Settings

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

• On the command line, 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.



#### 12.3.3 Acquisition

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

#### 12.3.4 Processing

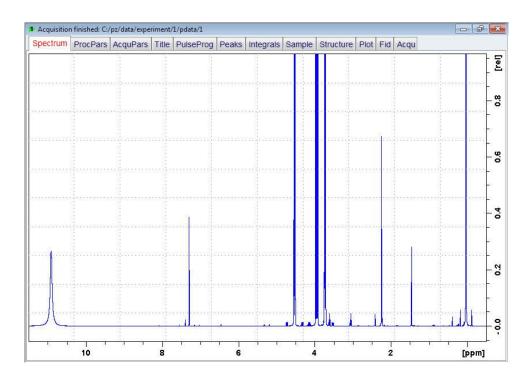
- On the Workflow button bar, click **Process**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).



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



• In the proc1d window, click Execute.



- E
- On the toolbar, click Exact Zoom.
- In the exactzoom window enter the following parameters:

From = **4.6** 

To = **3.6** 

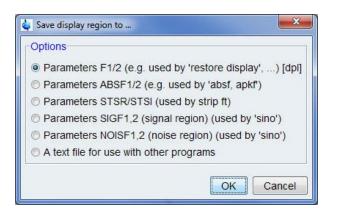
• In the exactzoom window, click OK.

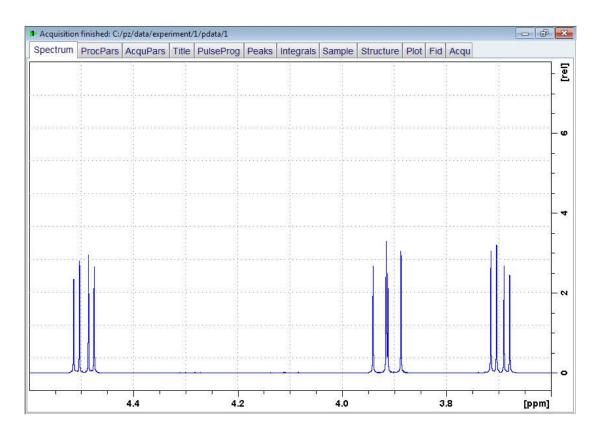
🖕 exactzoom	×
Please enter t of the desired	the exact coordinates expansion.
	F1 [ppm]
From	4.6
То	3.6
	OK Cancel

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



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





#### 12.3.5 Peak Picking

- On the menu bar, click Analyse.
- On the Workflow button bar, click **Pick Peaks**.

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

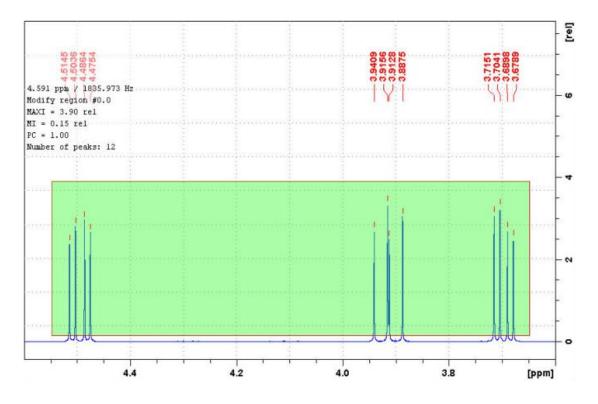
بلب	ட்	ж Ш	шħ	灭璞	D	副司	$\downarrow$
-----	----	--------	----	----	---	----	--------------

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 ad-

just 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).



#### 12.3.6 Assigning the Multiplets

- Expand the multiplet at 4.5 ppm.
- On the menu bar, click Analyse.
- On the Multiplets button, click the drop-down arrow to see more options.
- In the list, select Enter multiplet analysis.

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

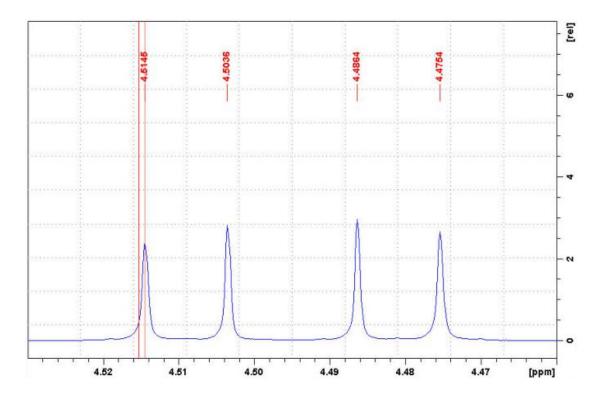
■「「●□」をしくてメメーナはは、「※「●□」、「●□」、「●□」を使用し、

- Click Define Multiplets Manually.
- · 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.

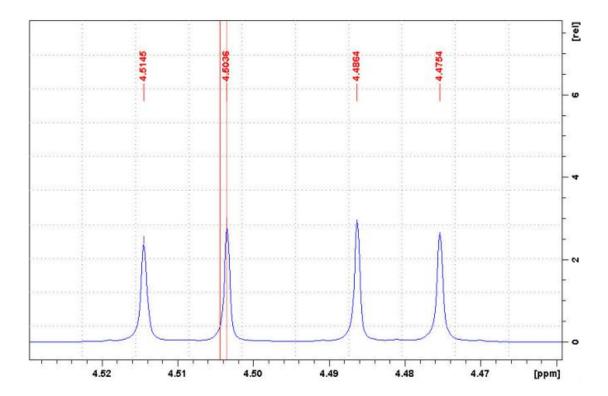
**Step 2:** The right cursor line will stop when it gets into the center of the peak.





Step 4: Move the cursor lines slowly towards the second peak.

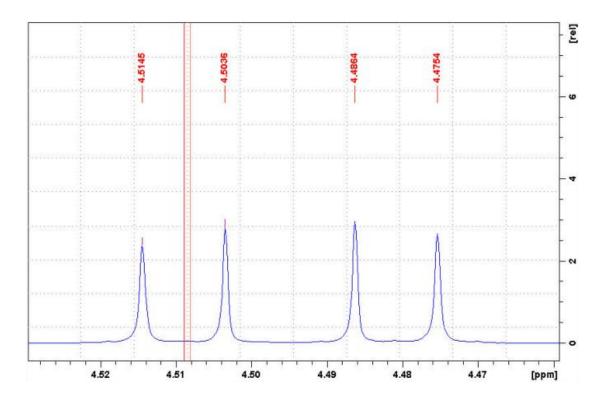
**Step 5:** The right cursor line will stop when it gets into the center of the peak.



Step 6: Click left.

#### A small marker is placed above the top of the two peaks.

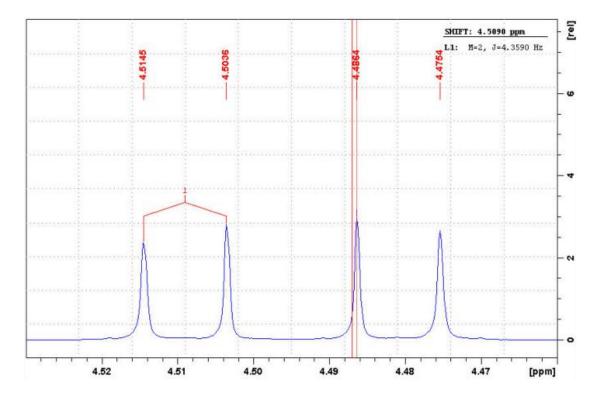
Step 7: Move the cursor lines into the center of the two marked peaks.



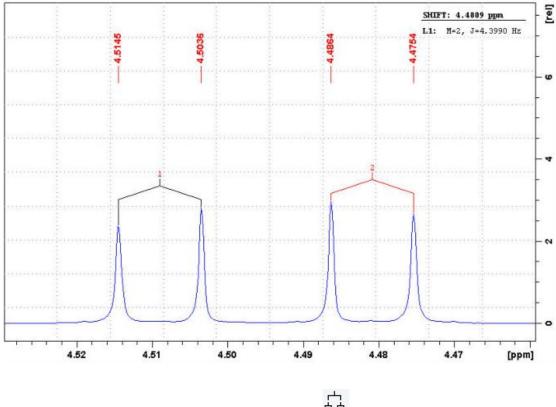
Step 8: Right-click to open the shortcut menu.



Step 9: In the list, select Define Multiplet.



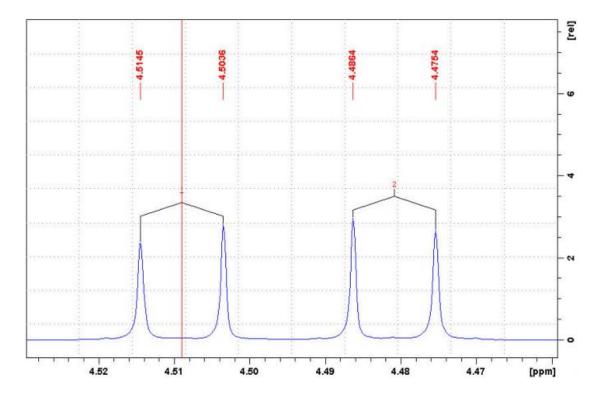
• Repeat steps 1 - 9 starting with the third peak and ending with the fourth peak.



 Step 10: Select the Couple Existing Multiplets button.

 Step 11: Move the cursor line into the center of the first two peaks marked 1.

 Step 12: Click left.



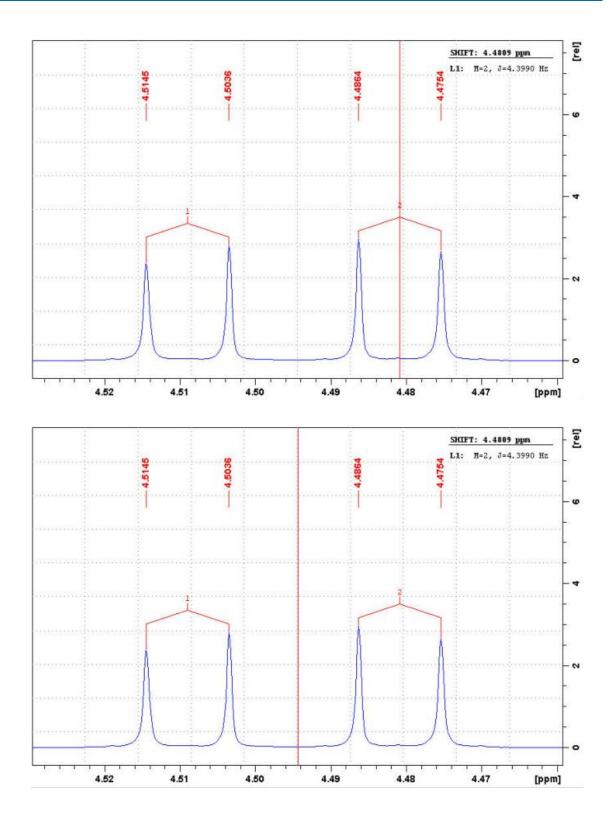
Step 13: Move the cursor line into the center of the second two lines marked 2.



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 left.

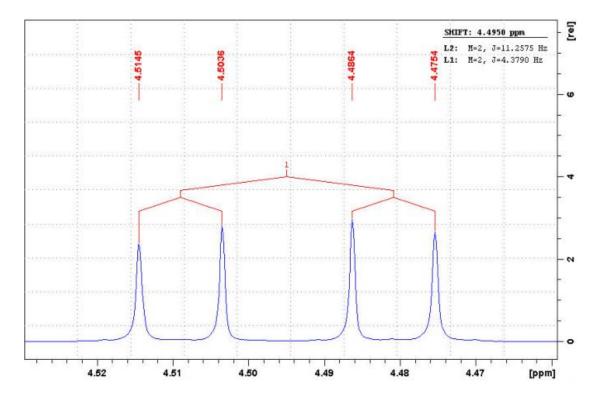
Step 15: Move the cursor into the center of the displayed multiplet.



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

Automatically Define Multiplet	
Automatically Define Multiplet By Region	
Define Multiplet By Region	
Define Multiplet Manually	
Define Multiplet By Free Grid	۲
Couple Existing Multiplets	
Define Multiplet By Coupled Grid	۲
Shift Single Line	
Shift Multiplet Tree Horizontally	
Decouple Multiplet	
Renumber multiplets	
Define Multiplet Identifier	
Define Multiplet Integral label	
Delete Multiplet	
Designate Multiplet	
Connect Multiplets	
Disconnect Multiplets	
Molecule Operations	•

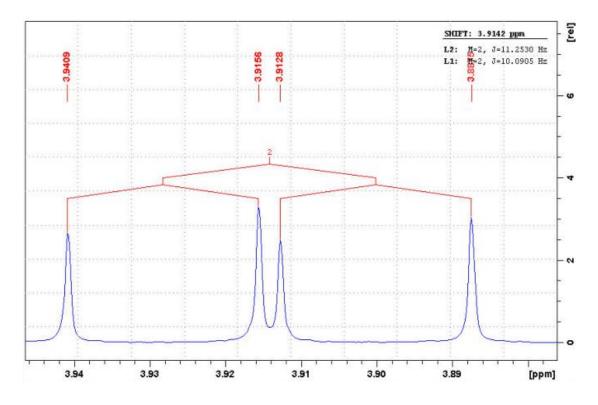
#### Step 17: Select Couple Existing Multiplets.



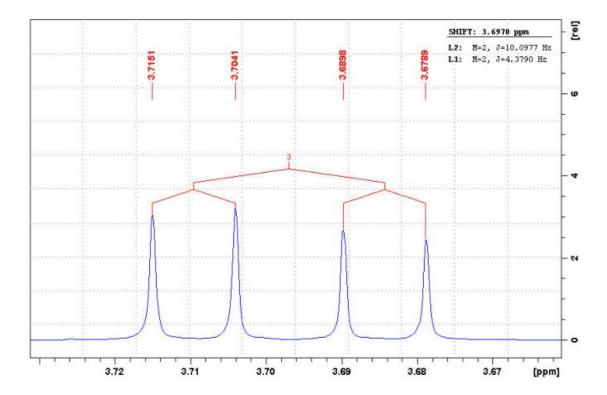
Step 18: Right-click in the spectrum window to open the shortcut menu.Step 19: Select Finish Current Mode.

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

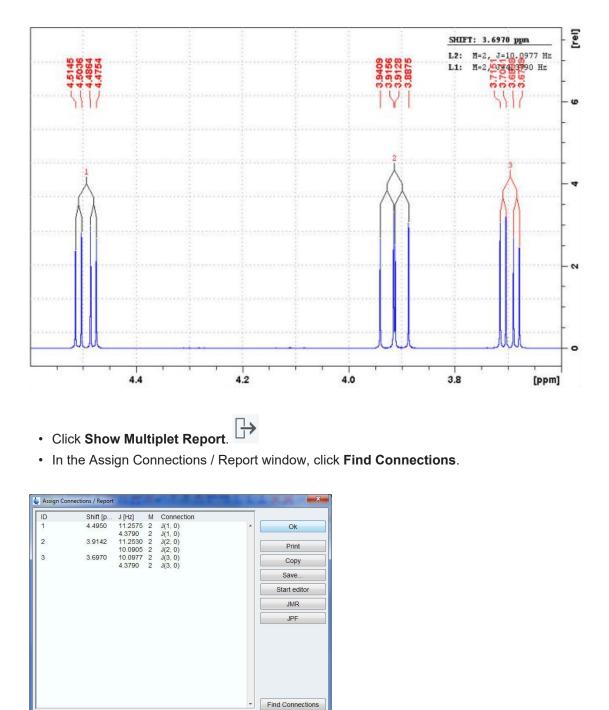
- Expand the multiplet at **3.9 ppm**.
- Repeat steps 1 19 for this multiplet.



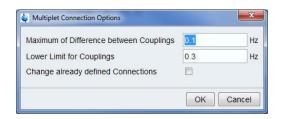
- Expand the multiplet at **3.7 ppm**.
- Repeat steps 1-19 for this multiplet.



• Display all 3 multiplets.



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



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

Bj

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

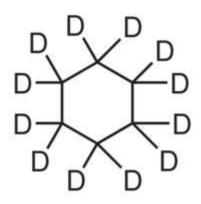
Assign Co	nnections / Report		<b>a</b> .	1445	The second	
ID	Shift [p	J [Hz]	М	Connection		
1	4.4950	11.2575	2		*	Ok
		4.3790				
2	3.9142	11.2530 10.09				Print
3	3.6970	10.09	2	J(3, 2)		Сору
		4.3790	2	J(3, 1)		
						Save
						Start editor
						JMR
						JPF
					~	Find Connections
			_			

• Click Return, save multiplets [sret].

# 13 Adding a New Solvent

### 13.1 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.



# 13.2 Adding Cyclohexane-d12 to the Solvent List

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

Solvents Edit	BSMS Help				
Ock Nucleus ● 2H  ○ 19F	Current probe 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	- 20			
CD3CN	acetonitrile-d3				
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)				
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)				
CDCI3	chloroform-d	-			

• Right-click on the C<sub>6</sub>D<sub>6</sub> solvent and on the shortcut menu, select Add new solvent.



• In the Password request window, enter the password and click OK.



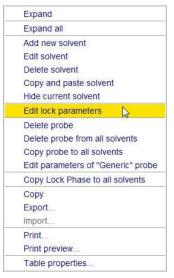
- Add the following Solvent parameters: Solvent name = C<sub>6</sub>D<sub>12</sub> Solvent description = Cyclohexane-d12 Enable Lock Solvent Melting Point [K] = 279 Boiling Point [K] = 354
- In the Create solvent window, click OK.

🖕 Create solvent	- >	<
Create a new solvent.		
Solvent parameters		
Solvent name:	C6D12	
Solvent description:	Cyclohexane-d12	
Lock Nucleus:	2H	
Lock Solvent:		
Hidden:		
Field Offset:	0	
Field Update:		
Auto Lock:		
Melting Point [K]:	279	
Boiling Point [K]:	354	
Solvent class:		
	OK Cancel	]

• In the Edsolv window, select the Lock tab.

	t BSMS Help							
Lock Nucleus	Current probe							
🧿 2H 🔘 19F	Current probe:	5 mm DUL 13C-1	H/D Z-GRD Z111650/0002	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
CD3CN	Generic	-38	10	-2	0.1	200	-1	1.93
. CD3CN_SP	E Generic	-20	10	-10	0.1	100	-1	1.93
	Ceneric	-25	10	_5	0.1	100	_1	33

• Right-click on the C<sub>6</sub>D<sub>12</sub> solvent and on the shortcut menu, select **Edit lock parameters**.



Add the following Lock parameters: Probe description = Default probe Lock power = -30 Loop gain = -10 Loop time = 0.4 Loop filter = 200 Lock phase = -1 Lock power instep = 10 Temperature lock power = -20 Shift [ppm] = 1.38 Relative intensity = 1 Type = Lock

🖕 Edit lock parameters						
Edit lock parameters for solvent "C6D12".						
Lock parameters						
Probe:	Z126282_0002					
Probe description:	Default probe					
Lock power:	-30					
Loop gain:	-10					
Loop time:	0.4					
Loop filter:	200					
Lock Phase:	-1					
Lock power instep:	10					
Temperature lock power:	-20					
Signals						
Signal Shift [ppm] Relati	ve intensity Type Description Delete					
1 1.38	1 Lock -					
Add Signal Delete Signal						
	Add temperature/shift values					
	OK Cancel					

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

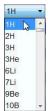
The Lock parameters will be stored for the current probe and for the selected probe only. This current probe (as defined in edhead) will then also appear in the Lock parameters list. Parameters for the other probes will remain unchanged and remain visible as probe type Generic.



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

Solvents Edi	t BSMS He	lp						
Lock Nucleus	Current probe							
● 2H © 19F	Current prob	Current probe: 5 mm DUL 13C-1H/D Z-GRD 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								
C6D6	10	0	0.5					
CD3CN	10	0	0.5					
CD3CN SPE	10	1.93	0.2					

• In the Nucleus list, select 1H.



• Right-click on the C<sub>6</sub>D<sub>12</sub> solvent and on the shortcut menu, select **Edit spectrum refer**ence 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

 In the Edit spectrum reference parameters window, add the following Spectrum reference parameters for C<sub>6</sub>D<sub>12</sub>:

Noise factor = 10

Reference shift [ppm] = 0

Reference shift correction [ppm] = 1 Search Width [ppm] = **5** 

🖕 Edit spectrum reference parameters 🛛 🗙						
Edit "1H" spectrum reference parameters for solvent "C6D12".						
Spectrum reference parameters						
Noise factor: 10						
Reference shift [ppm]: 0						
Reference shift correction [ppm]: 0.1						
Search Width [ppm]: 0.5						
Add signal regions						
<u>O</u> K <u>C</u> ancel						

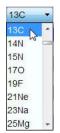
 Click Add signal regions and add the following regions: Region 1, Lower limit [ppm] 15, Upper limit [ppm] 1.42 Region 2, Lower limit [ppm] 1.35, Upper limit [ppm] 0.2 Region 3, Lower limit [ppm] -0.2, Upper limit [ppm] -3

🖕 Edit spect	trum reference parameters		×					
Edit "1H" sp	ectrum reference paran	neters for solvent "C6D"	12".					
Spectrum	reference parameters							
Noise factor: 10								
	Reference shift [ppm]:	0						
Reference	e shift correction [ppm]:	0.1						
	Search Width [ppm]:	0.5						
Signal Reg	gions							
Lower lim	it [ppm] Upper limit [ppn	n] Description	Delete					
15	1.42	Region 1						
1.35	0.2	Region 2						
-0.2	-3	Region 3						
Add Signal Region Delete Signal Region								
		<u>O</u> K	<u>C</u> ancel					

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

🖕 Edsolv									
Solvents Edit BSMS H	lelp								
Auto Phase	Auto pha	se algorithm	Current probe						
Auto phase during lock	Auto phase during lock Auto phase algorithm: Spectrum Current probe: TCI H-F 002 (Z126282_0002)								
Solvents Lock Spectrum R	eference p	Properties							
			Nucleus: 1	3C -					
A Solvent	Noise	Reference Shift [ppm]	Reference Shift Corr. [ppm]	Search Width [ppm]	Signal Regions [ppm]				
Acetic	10	0	0	5	1000/180, 176/21, 19/1, -1/-1000	A			
Acetone	10	0.9	0	5	1000/207, 205/30.8, 28.8/1, -1/-1000				
@-C6D12	10	0	1	5	200/27.5, 25.5/1, -1/-5				
· C6D6	10	0.22	0	5	1000/129, 127/1, -1/-1000				

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



• Right-click on the C<sub>6</sub>D<sub>12</sub> solvent and on the shortcut menu, select **Edit spectrum refer**ence parameters.

Ad	d new solvent
Ed	it solvent
De	elete solvent
Co	ppy and paste solvent
Hic	de current solvent
Ed	it spectrum reference parameter
Co	рру
Ex	port
Im	port
Pri	int
Pri	int preview
	ble properties

 Add the following Spectrum reference parameters for C<sub>6</sub>D<sub>12</sub> Noise factor = 10 Reference shift [ppm] = 0 Reference shift correction [ppm] = 1 Search Width [ppm] = 5

👹 Edit spectrum reference parameters 🛛 🗙
Edit "13C" spectrum reference parameters for solvent "C6D12"
Spectrum reference parameters
Noise factor: 10
Reference shift [ppm]: 0
Reference shift correction [ppm]: 1
Search Width [ppm]: 🧧
Add signal regions
OK Cancel

 Click Add signal regions and 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

🖕 Edit spectrum referer	ice parameters	<b>—</b> ×						
Edit "13C" spectrum	reference pa	arameters for solvent "C6D12".						
Spectrum reference parameters								
	Noise factor	pr: 10						
Referen	ice shift [ppm]	n]: O						
Reference shift co	rrection [ppm]	າ]: 1						
Searc	h Width [ppm]	ı]: 5						
Signal Regions	Signal Regions							
Lower limit [ppm]	Upper limit [pp	opm] Description Delete						
200	27.5	Region 1						
25.5	1	Region 2						
-1	-5	Region 3						
Add Signal Region Delete Signal Region								
		OK Cancel						

- In the Edit spectrum reference parameters window, click OK.
- In the Edsolv window, click Close.

🖕 Edsolv						
Solvents Edit BSMS H	lelp					
Auto Phase	Auto pha	se algorithm	Current probe			
Auto phase during lock	Auto pha	se algorithm: Spectrum	Current probe: TCI H	-F 002 (Z126282_0002)		
Solvents Lock Spectrum R	eference	Properties				
			Nucleus: 1	н •		
△ Solvent	Noise	Reference Shift [ppm]	Reference Shift Corr. [ppm]	Search Width [ppm]	Signal Regions [ppm]	
-Acetic	10	0	0	0.5	1000/12.2, 10.8/2.5, 1.5/0.2, -0.2/-1000	
Acetone	10	0	0	0.5	1000/2.24, 1.84/0.2, -0.2/-1000	
C6D12	10	0	0.1	0.5	15/1.42, 1.35/0.2, -0.2/-3	
	10	0	0	0.5	1000/7.35. 6.95/0.20.2/-1000	

# 13.3 TopShim Solvent Parameters

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

# 14 Contact

#### Manufacturer

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#### **Bruker BioSpin Hotlines**

Contact our Bruker BioSpin service centers.

Bruker BioSpin 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 service center or hotline you wish to contact from our list available at:

https://www.bruker.com/service/information-communication/helpdesk.html





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