


TopSpin

- Basic NMR Experiments
User Manual
Version 005



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Contents

1	Introduction	7
1.1	General	7
1.2	Disclaimer	7
1.3	Policy Statement	7
1.4	Limitation of Liability	7
1.5	Before You Begin	8
1.6	Minimum Qualifications for Operating Personnel	8
1.7	The Bruker Service	8
1.8	Font and Format Conventions	9
2	TopSpin Interface – Details	10
3	Spectrometer Basics	23
3.1	Magnetic Safety	23
3.2	Cryogenic Safety	23
3.3	Electrical Safety	23
3.4	Chemical Safety	23
3.5	CE Certification	23
3.6	AVANCE Architecture Overview	24
3.7	Sample Preparation	24
3.8	Inserting the Sample Plus Spinner into the Magnet	25
3.9	Spinning the Sample	25
3.10	Tuning and Matching the Probe	25
3.10.1	Probes Equipped with ATM	26
3.10.1.1	Automatic Tuning	26
3.10.1.2	Manual Tuning	26
3.11	Locking the Sample	27
3.12	Shimming the Sample	27
3.12.1	Shimming on the Lock Signal	28
3.12.2	Shimming on the FID (Free Induction Decay)	28
3.12.3	Shimming Using the Tune File	28
3.12.4	Shimming Using TopShim	28
3.13	Optimizing Resolution and Line Shape	28
4	1D Proton Experiment	30
4.1	Sample	30
4.2	1D Proton Experiment	30
4.2.1	Experiment Setup	30
4.2.2	Acquisition	34
4.2.3	Processing	34
4.2.4	Integration	35
4.2.5	Plotting the 1D Proton Spectra	37
5	1D Selective Experiments	38
5.1	Sample	38

5.2	1D Selective COSY	38
5.2.1	Reference Spectrum	39
5.2.2	Selective Excitation Region Setup	40
5.2.3	Acquisition.....	42
5.2.4	Processing	42
5.2.5	Plotting Two Spectra on the Same Page	43
5.3	1D Selective NOESY	44
5.3.1	Reference Spectrum	44
5.3.2	Selective Excitation Region Setup	45
5.3.3	Processing	48
5.3.4	Plotting Two Spectra on the Same Page	48
5.4	1D Selective TOCSY	48
5.4.1	Reference Spectrum	49
5.4.2	Selective Excitation Region Set Up	49
5.4.3	Processing	51
5.4.4	Plotting Two Spectra on the Same Page	51
6	2D Homonuclear Experiments	52
6.1	Sample	52
6.2	2D Gradient COSY	52
6.2.1	Preparation Experiment	53
6.2.2	Setting up the COSY Experiment	53
6.2.3	Limit Setting	54
6.2.4	Acquisition.....	55
6.2.5	Processing	55
6.2.6	Plotting the COSY Spectrum	56
6.3	2D Gradient NOESY Experiment.....	57
6.3.1	Preparation Experiment	58
6.3.2	Setting up the NOESY Experiment	58
6.3.3	Acquisition.....	59
6.3.4	Processing	60
6.3.5	Plotting the NOESY Spectrum	60
6.4	2D Phase Sensitive TOCSY Experiment.....	60
6.4.1	Preparation Experiment	61
6.4.2	Setting up the TOCSY Experiment	61
6.4.3	Acquisition.....	62
6.4.4	Processing	63
6.4.5	Plotting the TOCSY Spectrum	63
7	1D Carbon Experiments.....	64
7.1	Sample	64
7.2	1D Carbon Experiment	64
7.2.1	Experiment Setup	65
7.2.2	Acquisition.....	67
7.2.3	Processing	67
7.2.4	Peak Picking	69
7.2.5	Plotting the 1D Carbon Spectrum	70
7.3	DEPT-135 Experiment	71

7.3.1	Experiment Setup	72
7.3.2	Acquisition.....	73
7.3.3	Processing	73
7.3.4	Plotting the DEPT-135 Spectrum.....	73
7.4	DEPT-90 Experiment	74
7.4.1	Experiment Setup	75
7.4.2	Acquisition.....	75
7.4.3	Processing	75
7.4.4	Plotting the DEPT-90 Spectrum.....	76
8	2D Heteronuclear Experiments	78
8.1	Sample.....	78
8.2	2D Edited HSQC.....	78
8.2.1	Preparation Experiment	79
8.2.2	The HSQC Experiment Setup.....	79
8.2.3	Limit Setting	80
8.2.4	Acquisition.....	82
8.2.5	Processing	82
8.2.6	Plotting the 2D HSQC Spectrum.....	83
8.3	2D HMBC Experiment.....	83
8.3.1	Preparation Experiment	84
8.3.2	The HMBC Experiment Setup.....	85
8.3.3	Limit Setting	85
8.3.4	Acquisition.....	87
8.3.5	Processing	87
8.3.6	Plotting the 2D HMBC Spectrum	88
9	Determination of 90 Degree Pulses	89
9.1	Proton 90 Degree Transmitter Pulse	89
9.1.1	Parameter Setup.....	89
9.1.2	Acquisition.....	91
9.1.3	Processing	91
9.1.4	Determine the 90 Degree Pulse.....	94
9.2	Carbon 90 Degree Transmitter Pulse	96
9.2.1	Parameter Setup.....	96
9.2.2	Acquisition.....	98
9.2.3	Processing	98
9.2.4	Determine the 90 Degree Pulse.....	101
10	Sensitivity Tests	103
10.1	^1H Sensitivity Test	103
10.2	Experiment Setup	103
10.3	Acquisition.....	104
10.4	Processing	104
10.5	Calculating the Signal to Noise Ratio.....	104
10.6	^{13}C Sensitivity Test with ^1H Decoupling.....	106
10.6.1	Experiment Setup	106
10.6.2	Acquisition.....	107
10.6.3	Processing	107

10.6.4	Calculating the Signal to Noise Ratio.....	107
10.7	¹³ C Sensitivity Test without ¹ H Decoupling.....	109
10.7.1	Experiment Setup	109
10.7.2	Acquisition.....	110
10.7.3	Processing	110
10.7.4	Calculating the Signal to Noise Ratio.....	111
11	Additional Information	113
11.1	Standard Parameter Set List.....	113
11.2	Pulse Program Information	114
11.3	Standard Test Samples	114
12	Contact	115

1 Introduction

1.1 General

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

1.2 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.

1.3 Policy Statement

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

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

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

1.4 Limitation of Liability

All specifications and instructions in this manual have been compiled taking into account applicable standards and regulations, the current state of technology and the experience and insights we have gained over the years.

The manufacturer accepts no liability for damage due to:

- Failure to observe this manual.
- Improper use.
- Deployment of untrained personnel.
- Unauthorized modifications.
- Use of inadmissible spare parts.

The actual scope of supply may differ from the explanations and depictions in this manual in the case of special designs, take-up of additional ordering options, or as a result of the latest technical modifications.

The undertakings agreed in the supply contract, as well as the manufacturer's Terms and Conditions and Terms of Delivery, and the legal regulations applicable at the time of the conclusion of the contract shall apply.

1.5 Before You Begin

This user manual contains information and safety information that are necessary for the safe operation of the device.

Consider all safety references!

1.6 Minimum Qualifications for Operating Personnel

Type of Task	Personnel	Training and Experience
Routine Use	Appropriately certified and experienced personnel, familiar with use of computers and automation in general	Laboratory technicians or equivalent. Training is usually done in-house. Familiar with MS Windows® environment.
Daily Maintenance		
Setup and optimization of program	Bruker certified personnel only.	Experienced laboratory technician. High degree of knowledge of the relevant application field.
Preventive Maintenance	Bruker certified personnel only.	Technically skilled with a basic understanding of the application.
Servicing	Bruker certified personnel only.	Background and experience in electronics/mechanics with computer knowledge.

Table 1.1: Overview Installation and Operation Requirements for Personnel

1.7 The Bruker Service

Our customer service division is available to provide technical information. See [Contact ► 115](#) for contact details.


In addition, our employees are always interested in acquiring new information and experience gained from practical application; such information and experience may help improve our products.

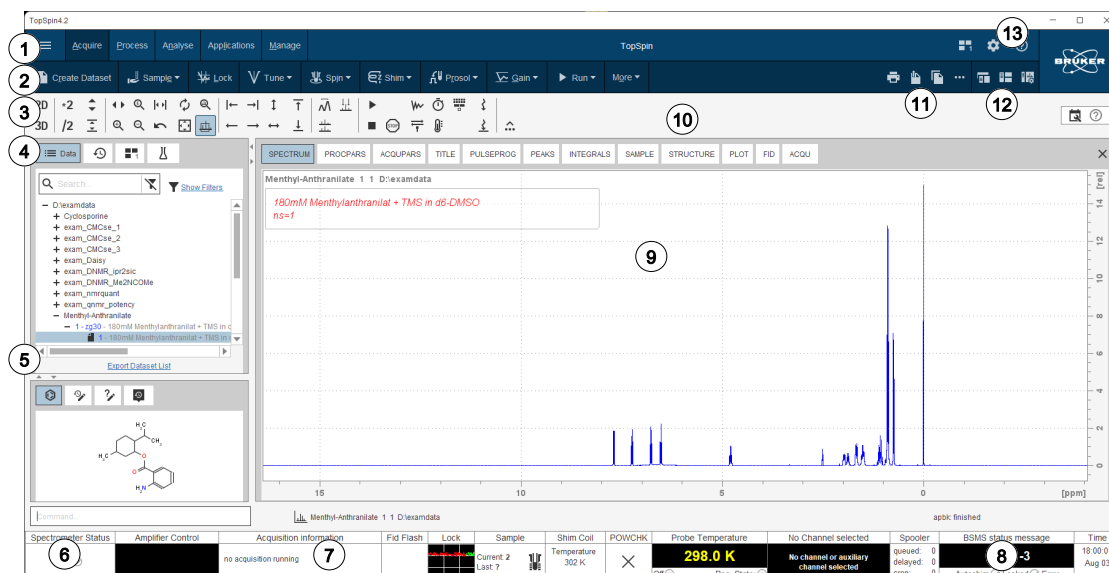
1.8 Font and Format Conventions

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

Table 1.2: Font and Format Conventions

2 TopSpin Interface – Details

Please note the File Icon  in the upper left corner of the Menu Bar. Throughout this manual, we will use the term **File** to indicate this menu icon.

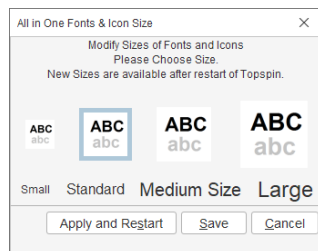


1	Menu Bar	8	Status Display Bar
2	Workflow Button Bar	9	Dataset Window
3	Tool Bar	10	Dataset Window Tabs
4	Browser and Search Window	11	Print, Export, Copy and Publish
5	Structure Window, Command Line History, Status Line History	12	Viewing Options
6	Command Line	13	Window Switcher, Login, Setup Preferences and Help
7	Current Dataset Bar		

TopSpin on High Resolution Screens

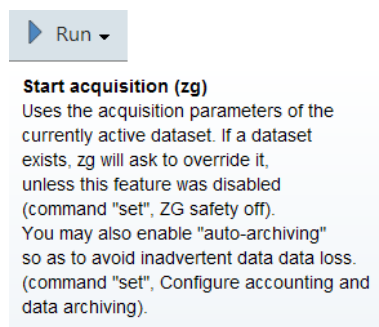
To adapt the font and icon size to a small/standard/medium or large screen resolution

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



Tooltips

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

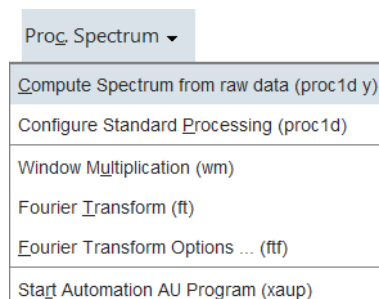


Workflow Button Bar

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.

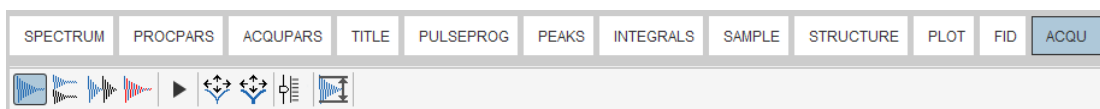
Furthermore, some of the buttons on the Workflow button bar include a **drop-down** arrow. Click the **drop-down** arrow to see more options.



Dataset Toolbar

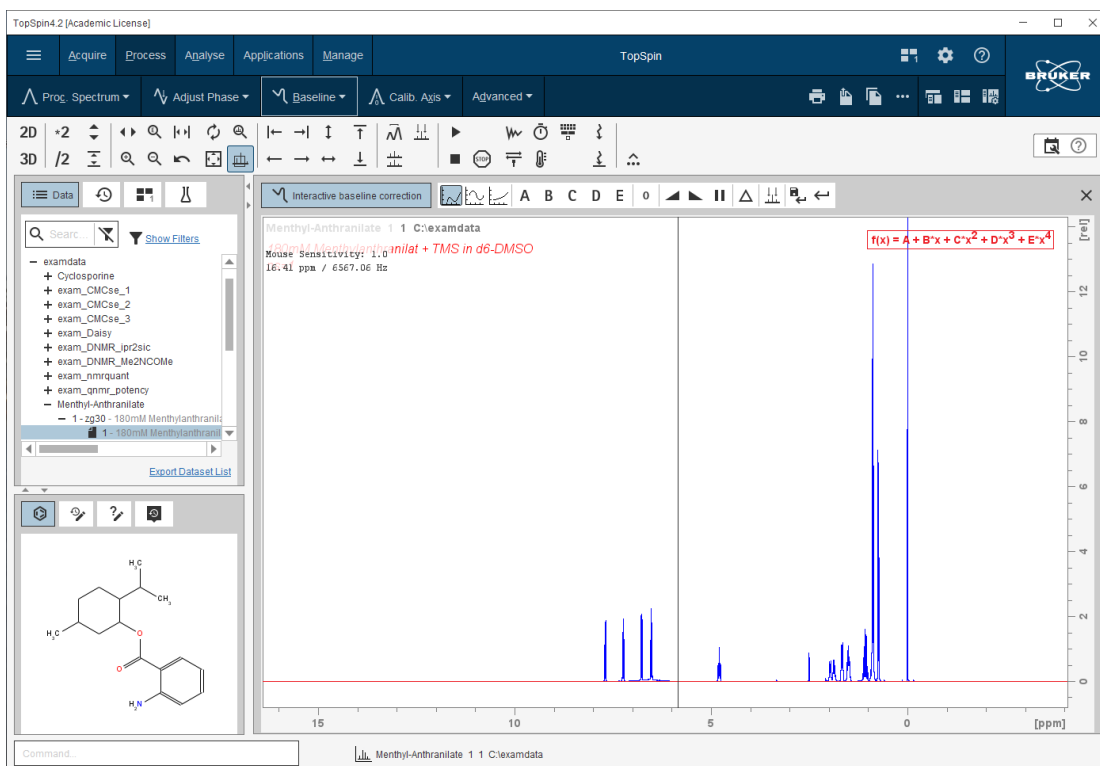
Depending on which dataset window tab is selected, an individual dataset toolbar is displayed, in the example the ACQU toolbar:

TopSpin Interface – Details



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.

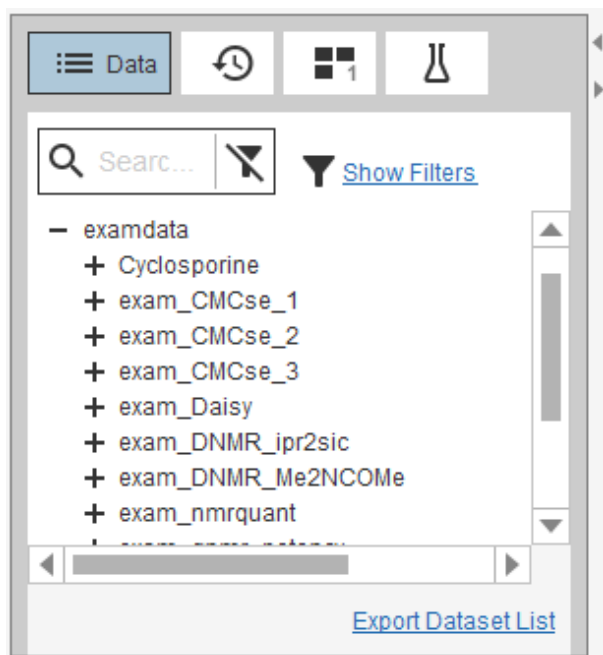
Note: Sometimes the dataset toolbar is displayed without dataset window tabs, e.g. entering a command as **.bas1** will display the baseline correction toolbar:



Browse and Search Window

The Browser window provides tabs as:

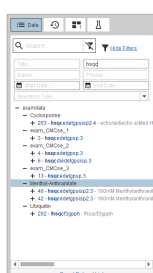
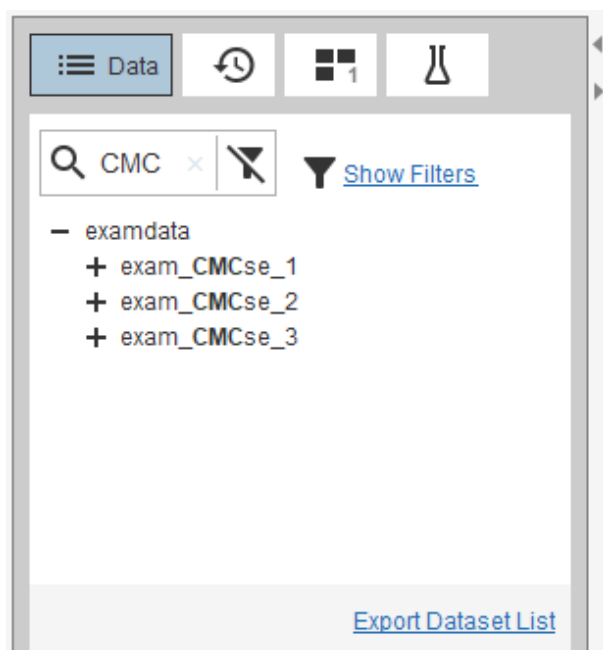
- Data browser and Search
- History browser
- Dataset Switcher
- Experiment Selector library



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

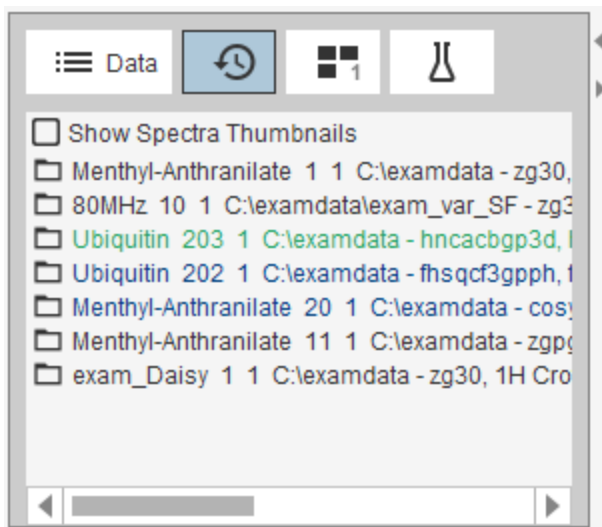


The data tree tab includes a search field. Enter a search phrase, for example:




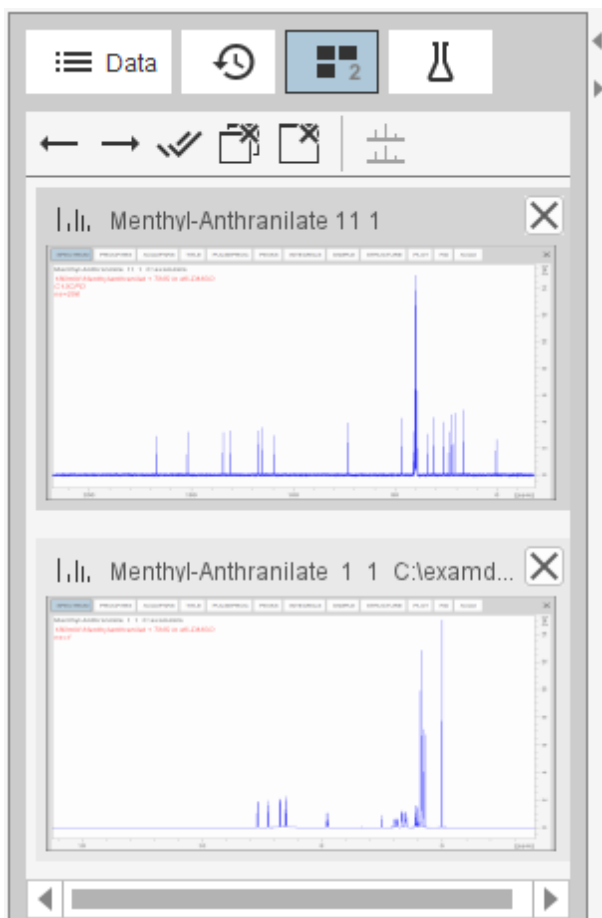
History Tab

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

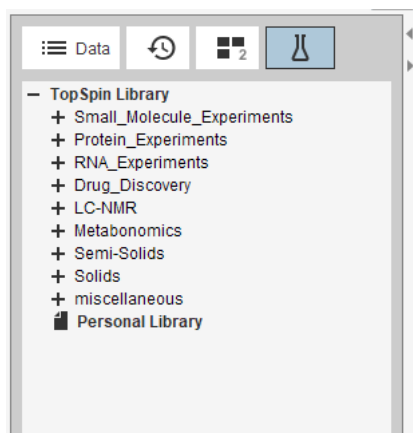


Dataset Switcher Tab

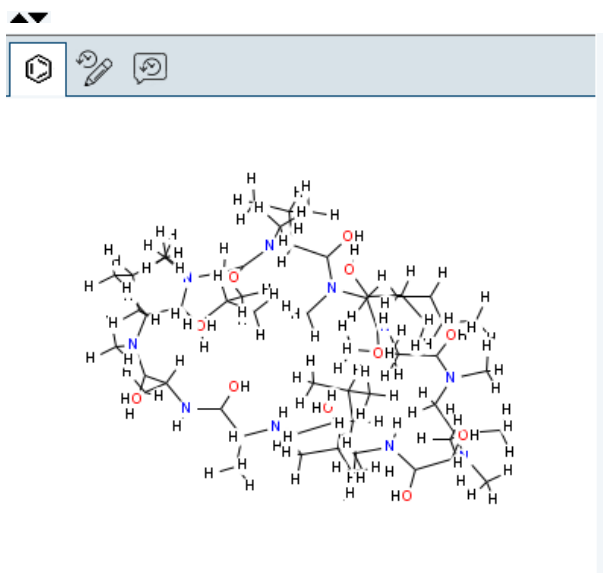
This tab has a similar function as the Window Switcher , see below, but only provides a quick overview of all currently opened datasets.



Experiment Library Tab



Structure Window



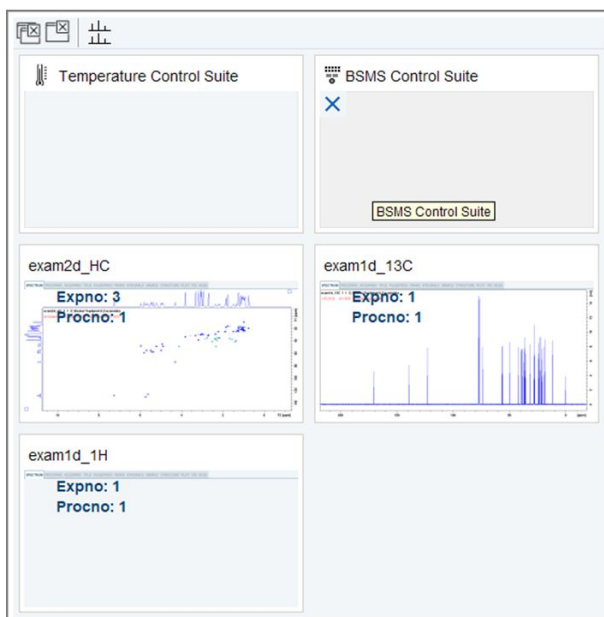
- Drag the vertical or horizontal split bar to resize the structure window.

Window Switcher



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

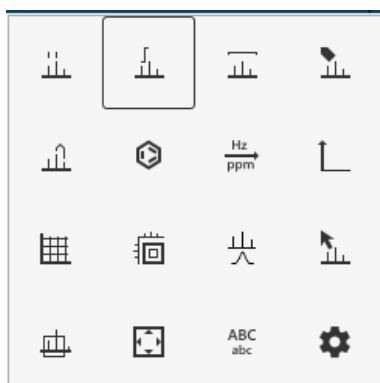
- Dataset windows.
- Lock display window.
- Acquisition display window.
- BSMS display window.
- Temperature unit window.
- Click the **Window Switcher** button to switch between these windows.



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

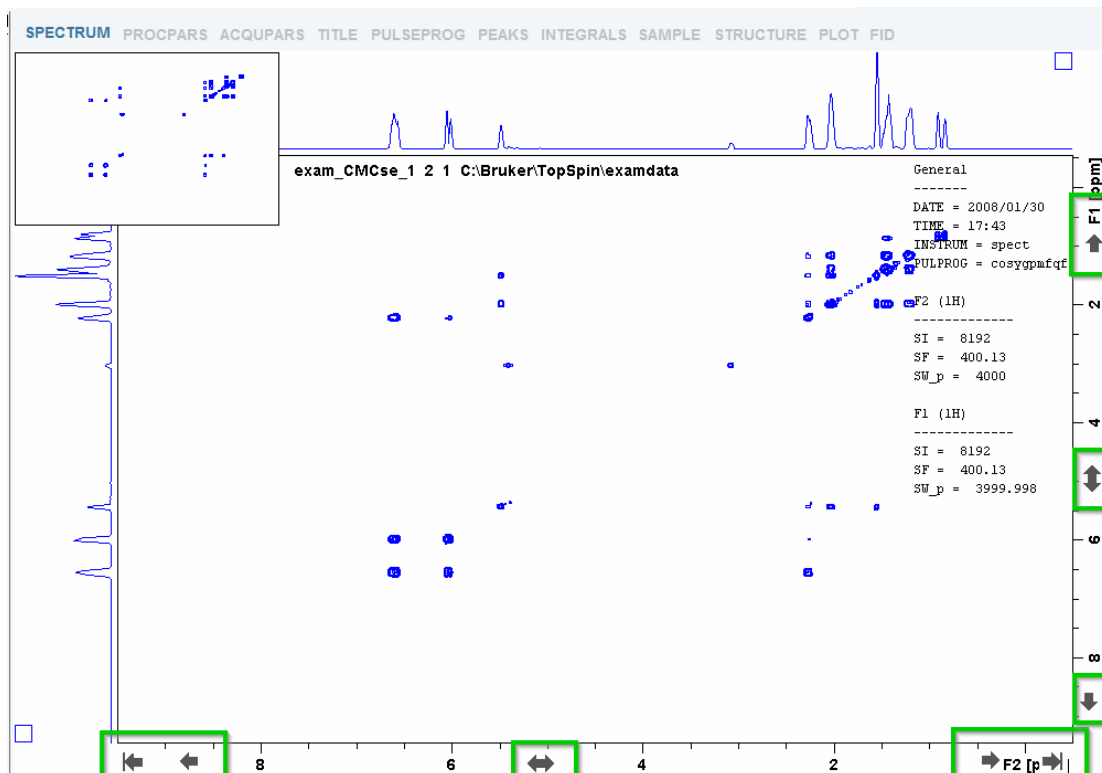
Viewing Integrals, Peaks and other Spectra Components

- 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

- 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

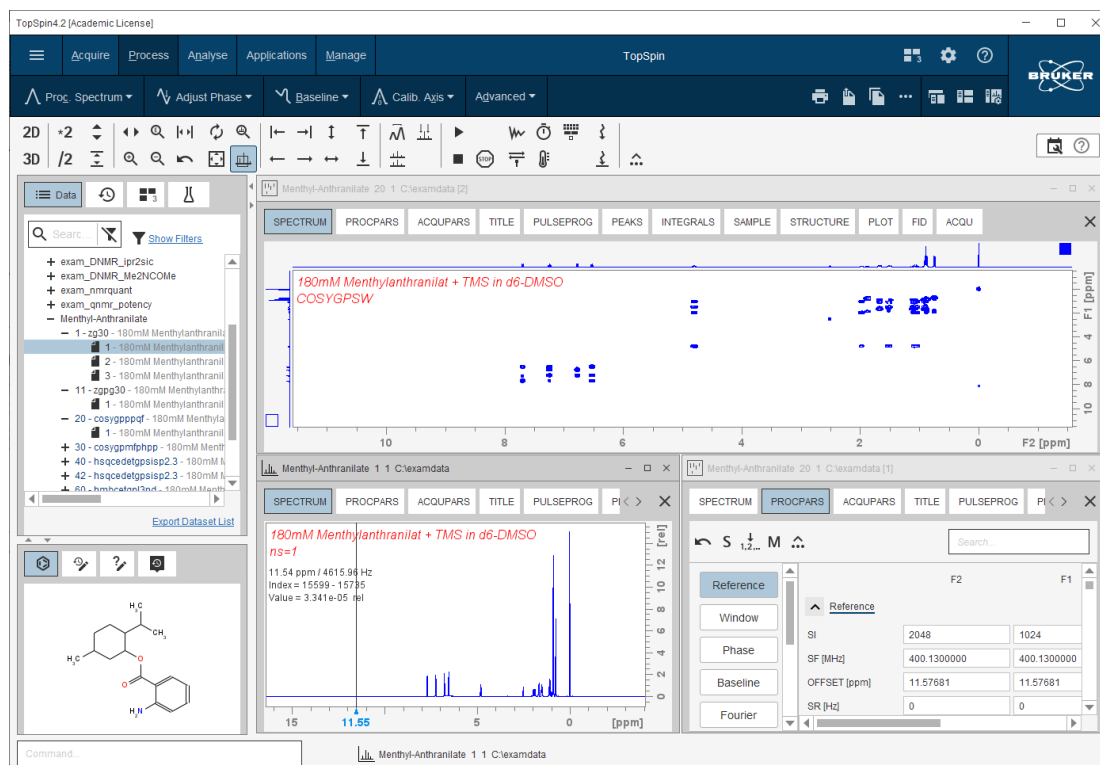
The TopSpin window has a dataset area that may contain multiple dataset windows.

To open multiple dataset windows

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

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

Toggle Perspective button . 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

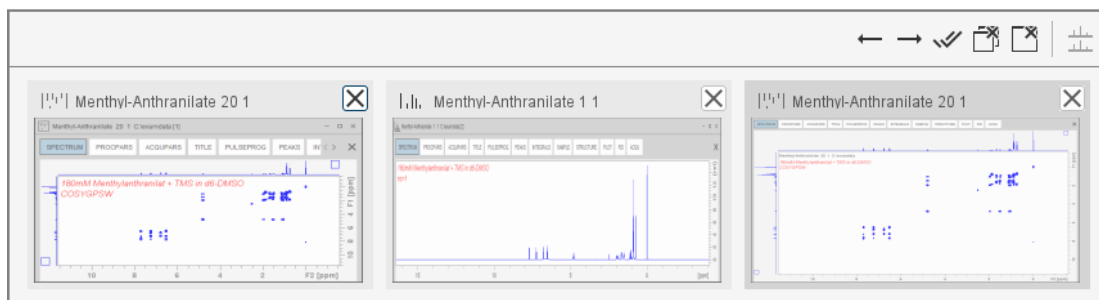
The active dataset window is the window of which the title bar is highlighted. The TopSpin menu, tool bars and command line commands correspond to and act on that window. Only one dataset window is active at a time.

To activate a different dataset window:

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

or

- Click the **Window Switcher** button  to switch between the windows.



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.

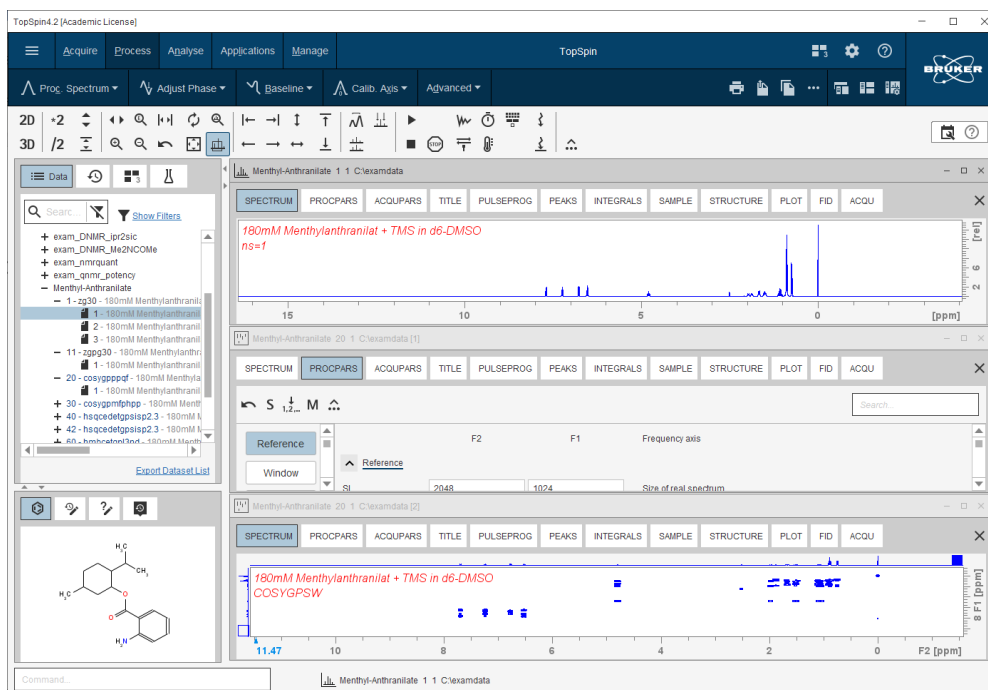
To arrange the dataset windows as a grid:

- 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:

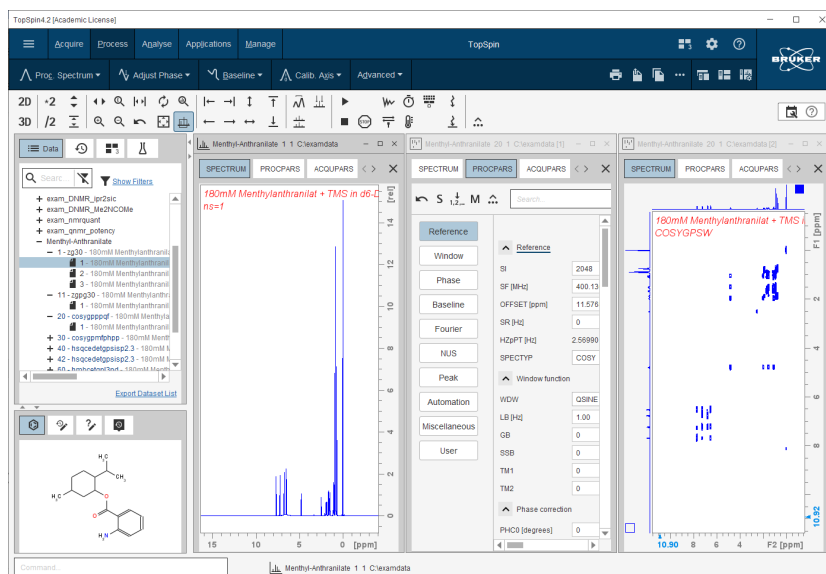
- Click **Show Layout Options**  and .



To arrange dataset windows side by side:

- Click **Show Layout Options**  and .


TopSpin Interface – Details



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 **Ctrl-w**.
or
- Click the  button in the windows title bar.

To close all dataset windows:

- Click **File | Close All Windows** or enter **closeall**.

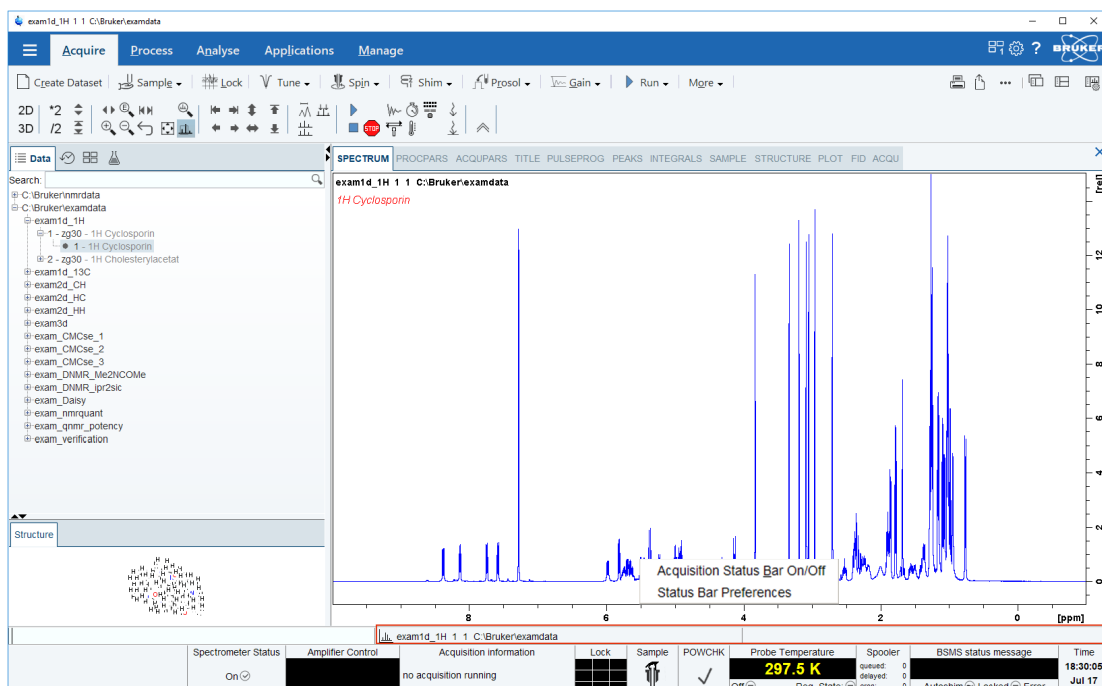
How to Swap Dataset Windows

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 Bar On/Off
Status Bar Preferences

Status Bar Preferences

Acquisition status bar

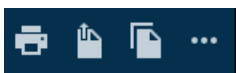
- Include spooler ☒
- Include time ☒
- Include sample temperature ☒
- Include channel or accessory channel temperature ☒
- Include acquisition status ☒
- Include acquisition indicator ☒
- Include lock signal ☒
- Include peak power check (POWCHK) indicator ☒
- Include sample state ☒
- Include shim coil temperature ☒
- Include BSMS status ☒
- Include spectrometer hardware (PDU) indicator ☒
- Include AutoDiagnose status ☒
- Include magnet bore temperature control ☐
- Include amplifier control ☒
- Include ADC control ☒

Apply Back Close

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**.

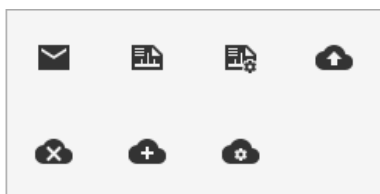
Command:		Methyl-Anthranilate 20 1 D:\examdata									
Spectrometer Status	Acquisition Information	Fid Flash	Lock	Sample	Shim Coil	POWCHK	Probe Temperature	No Channel selected	AutoDiagnose	Spooler	BSMS status mes
On	no acquisition running			Current: 2 Last ?	Temperature 302 K		297.1 K Reg. State:	No channel or auxiliary channel selected		queued: delayed: cron: 0	Δ Z 3 Autoshim Locked

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.



3 Spectrometer Basics

3.1 Magnetic Safety

A Magnetic Field surrounds the magnet in all directions. This field (known as the stray field) is invisible, hence the need to post warning signs at appropriate locations. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way! Of critical importance is that people fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet.

Because the strength of the stray field drops significantly as one moves away from the magnet, it is still useful to discuss safety to work around magnets. Details of stray fields for various magnets can be found in the Site Planning Guides delivered with the BASH CD.

3.2 Cryogenic Safety

The magnet contains relatively large quantities of liquid Helium and Nitrogen. These liquids, referred to as cryogens, serve to keep the magnet core at a very low temperature.

Because of the very low temperatures involved, **gloves, a long sleeved shirt or lab coat and safety goggles** should always be worn when handling cryogens. Direct contact with these liquids can cause frostbite. The system manager should regularly check and make sure that evaporating gases are free to escape from the magnet, i.e. the release valves must not be blocked. Do not attempt to refill the magnet with Helium or Nitrogen unless you have been trained in the correct procedure.

Helium and Nitrogen are non-toxic gases. However, because of a possible **magnet quench**, whereupon the room may suddenly fill with evaporated gases, adequate ventilation must always be provided.

3.3 Electrical Safety

The spectrometer hardware is no more or less hazardous than any typical electronic or pneumatic hardware and should be treated accordingly. Do not remove any of the protective panels from the various units. They are fitted to protect you and should be opened by qualified service personnel only. The main panel at the rear of the console is designed to be removed using two quick release screws, but again, this should only be done by trained personnel.

3.4 Chemical Safety

Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc.

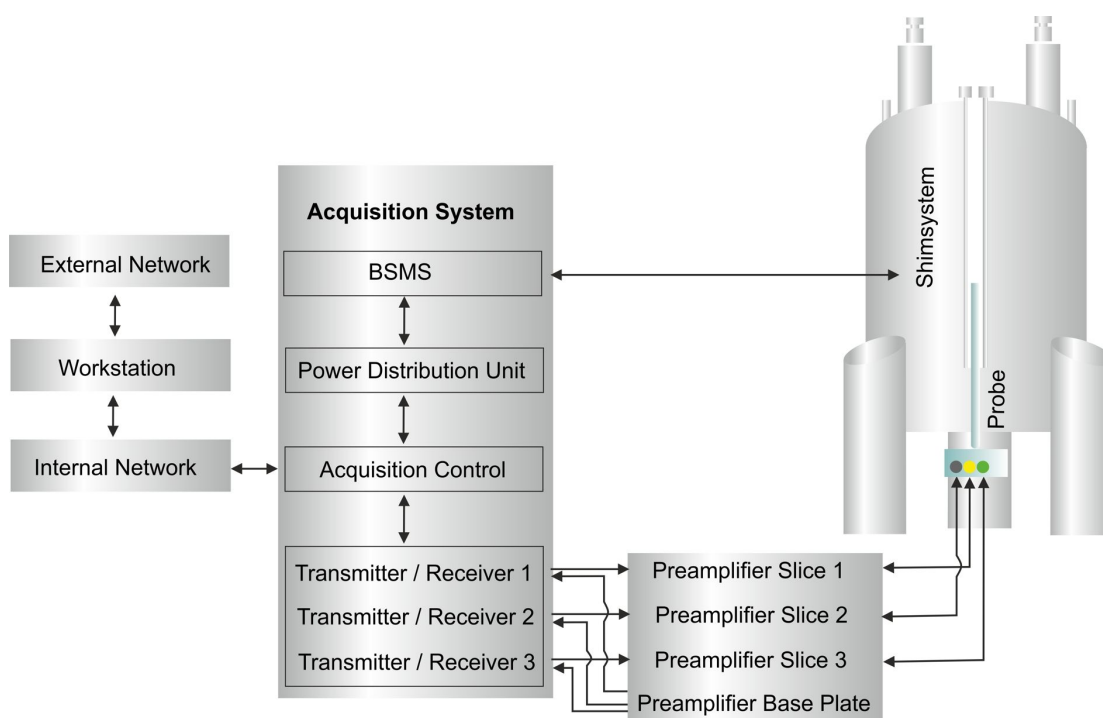
3.5 CE Certification

All major hardware units housed in the AVANCE NEO consoles as well as peripheral units such as the HPPR, shim systems, probe and BSMS keyboards comply with the CE Declaration of Conformity. This includes the level of any stray electromagnetic radiation that might be emitted as well as standard electrical hazards.



To minimize electromagnetic radiation leakage, the doors of the console should be closed and the rear paneling mounted.

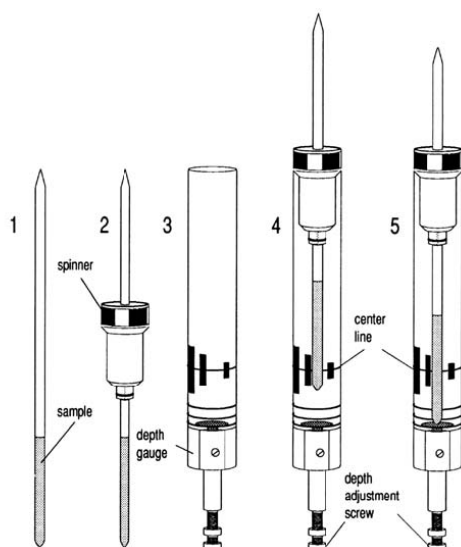
3.6 AVANCE Architecture Overview



Please use the BASH (**B**ruker **A**dvanced **S**ervice **H**andbook) for further information about the AVANCE system and hardware.

3.7 Sample Preparation

- Use clean and dry sample tubes.
- Use medium to high quality sample tubes.
- Always filter the sample solution.
- Always use the same sample volume or solution height.
- Filling volume of a 5 mm tubes is 0.6 ml or 5 cm.
- Filling volume of a 10 mm tubes is 4 ml or 5 cm.
- Use the sample depth gauge to adjust the sample depth (1.8 cm for older style probes, 2.0 cm for newer style probes).



- The sample tube should sit tightly inside the spinner.
- Wipe the sample tube clean before inserting into magnet.
- Turn on lift air to insert the sample into the magnet.

3.8 Inserting the Sample Plus Spinner into the Magnet

The raising and lowering of the sample is controlled by a stream of pressurized air. Make sure that the air flow is present (it is quite audible) before placing a sample onto the top of the bore.

3.9 Spinning the Sample

A second function of pressurized air is to enable the sample to rotate. The spinning of the sample serves to *even-out* some of the inhomogeneities that may exist in the magnetic field at the center of the magnet.



Sample tubes with a diameter of less than 5mm and samples to be investigated using inverse probes are normally not rotated.

Suggested spin rates are:

- 20 Hz for a 5 mm probe
- 12 Hz for a 10 mm probe

3.10 Tuning and Matching the Probe

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry.

Tuning involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately.

If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly. Hence, once the probe has been initially tuned, slight variations in frequency will not warrant retuning. Typically the transmitted frequency would need to be altered by at least 100kHz to warrant retuning. However for broadband probes the frequencies transmitted will vary greatly from nucleus to nucleus and so the probe will need to be tuned each time the selected nucleus is altered.

Whenever a probe is tuned it should also be matched. **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).



Bruker offers two different types of Tuning and Matching adjustments. In addition to the manual adjustments of the tuning and matching capacitors, the probes can be equipped with an Automatic Tuning Module (ATM). Follow the steps below for either option.

3.10.1 Probes Equipped with ATM

3.10.1.1 Automatic Tuning

- Create a new data set, see also [Experiment Setup](#) [► 30].
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Tune**.

or

- On the command line, type **atma**.



The display will switch automatically to the acquisition window and displays the wobble curve. The tuning and matching is performed automatically. If multiple frequencies are used in a parameter set such as C13CPD, HNCACOGP3D etc., ATMA will start adjusting the lowest frequency first and will switch in the order of increasing frequency automatically.

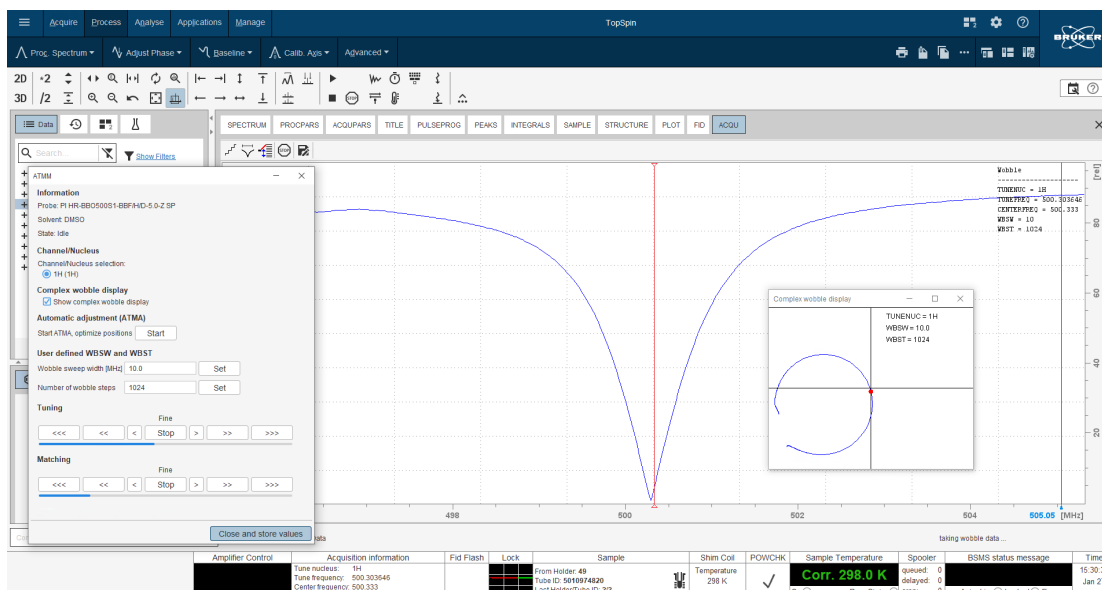
3.10.1.2 Manual Tuning

- Create a new data set, see also [Experiment Setup](#) [► 30].
- On the menu bar, click **Acquire**.
- On the **Tune** button, click the **drop-down** arrow to see more options.
- In the list, select **Tune/match ATM probe manually**.

or

- On the command line, type **atmm**.

The ATMM control window appears and the display will switch automatically to the acquisition window and displays the wobble curve, see the next figure.



- In the ATMM control window, click the **Tuning** buttons to move and display the wobble curve centered.
- In the ATMM control window, click the **Matching** buttons to adjust the dip of the wobble curve to the lowest position.

It is very convenient to look at the complex wobble display. If the circle with the red dot is too big or too small, then adjust matching first. Once the circle runs through the center of the coordinate system, bring the red dot into the center by adjusting the tuning. In essence, the curve tells you in which order to adjust tuning and matching.

Note: The T/M settings will be saved with a click on the **Start** button.



Since the Tuning and Matching adjustment interact with each other, a repeat of all steps is necessary for a perfect tune and match. If multiple frequencies are used in a parameter set such as C13CPD, use the **Nucleus Selection** radio buttons in the ATMM control window to switch to another nucleus and repeat the tuning and matching.

3.11 Locking the Sample

Deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used. The main feature of the Topspin lock routine is that it sets parameters such as the lock power, gain and frequency to a value appropriate to the solvent. With these default values set close to that which would be expected for that solvent, the BSMS can quickly locate and lock onto the solvent signal by sweeping through a range of frequencies or magnetic field values. The solvent dependent parameters are taken from the **edlock** table.

3.12 Shimming the Sample

Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to re-shim each time a probe or sample is changed. The system manager has stored appropriate shim values (in so called shim files) for each probe that will greatly reduce the shimming time required whenever a probe is changed.

3.12.1 Shimming on the Lock Signal

When the spectrometer is locked, the vertical offset of the lock trace on the graphics display corresponds to the amplitude of the lock substance signal, assuming constant lock DC, gain, and power levels. The lock level, then, serves as useful guide for basic shim adjustment. The goal in shimming on the lock signal is to adjust the shims so that the lock trace appears as high on the graphics display as possible. This lock level corresponds to the highest possible lock substance signal amplitude.

3.12.2 Shimming on the FID (Free Induction Decay)

The shape of the FID, and especially the beginning of the FID, indicates the shape of the transformed signal line, while the length of the FID tail is important to the overall resolution. For good line shape and high resolution, the shim controls must be adjusted so that the FID envelope is truly exponential with the longest possible decay time.

3.12.3 Shimming Using the Tune File

This method of shimming is useful when gradients are not available. A simple text file is edited to give the BSMS the instructions to shim the sample automatically. A default shim file *example* can be edited using the **ed tune** command and then stored with a new name in

`<TopSpin-home>/exp/stan/nmr/lists/group.`

The file can be executed with the command **tune**. The figure shows an example of a tune file.

```
# SHIMMIT spin
DELAY 3
MAXLOCK 0.4

TIMEOUT 1800
LOCKDWELL 3
# Shim_name Maximum_Step_Size Number_of_Iterations
Z 30 3
Z2 30 3
Z 5 5
Z2 5 5
Z3 5 5
```

3.12.4 Shimming Using TopShim

This is routine shimming and should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed. Routine shimming involves making fine adjustments to the Z, Z2, Z3, Z4 and Z5 shims. Some higher field magnets may require higher order Z shims. The system administrator has programed TopShim to achieve the best homogeneity on each sample and it is fully automatically.

The core method of TopShim is gradient shimming. A quality criterion for the final line-shape ensures best results for all situations.

TopShim is using for all deuterated solvents the ^2H gradient shimming method and for other solvents especially H_2O , the ^1H gradient shimming method.

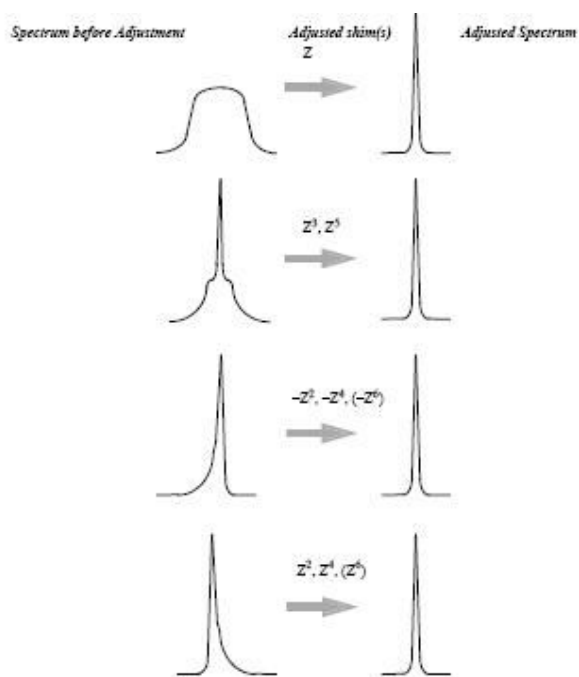
3.13 Optimizing Resolution and Line Shape

The standard sample for measuring the proton line shape and resolution specifications is, CHCl_3 in Acetone- d_6 . The concentration of CHCl_3 depends on the field strength of the magnet and the probe and can vary from 3% down to 0.1%.

For measuring the ^{13}C resolution and line shape test the standard sample ASTM (60% Dioxane in 40% C_6D_6) sample may be used.

For both tests the line shape is measured at 50%, 0.55% and 0.11% of the peak. The Bruker standard parameter sets to use for this tests are PRORESOL and C13RESOL.

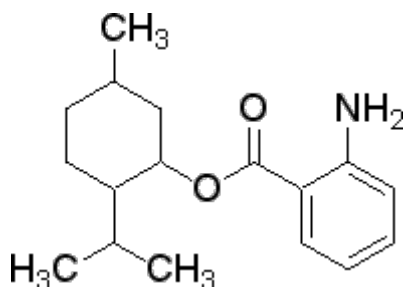
The figure below illustrates the influence of the On-axis shims on the line shape.



4 1D Proton Experiment

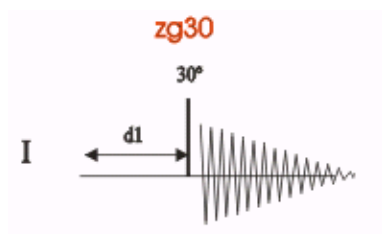
4.1 Sample

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



4.2 1D Proton Experiment

This chapter describes the acquisition and processing of a one-dimensional ^1H NMR spectrum using the standard Bruker parameter set **PROTON**. The pulse sequence **zg30** consists of the recycling delay, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30° . The two parameters, D1 and P1, correspond to the length of the recycle delay and the length of the 90° RF pulse, respectively.



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

4.2.1 Experiment Setup

Create a new dataset

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

Create New Dataset - new

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

Dataset

NAME: Proton_exp

EXPNO: 1

Directory: C:\Data

☐ Open in new window

Parameters

☐ Use current parameters

☒ Read parameterset: PROTON [Select]

☒ Set solvent: DMSO

Additional action

☒ No additional action

☐ Execute gelprosol

☐ Keep parameters: P 1, O1, PLW 1 [Change]

Advanced

Number of datasets (receivers): 1

Title

Menthyl Anthranilate in DMSO
Proton

OK Cancel More Info... Help

- In the Create New Dataset window, enter or select:

NAME = **Proton_exp**

EXPNO = 1

Directory = e.g. C:\Data

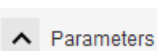
Directory, **NAME** and **EXPNO** define where the new experiment dataset will be stored on the computer:



Directory/NAME/EXPNO/ In the example: C:\Data\proton_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**.

- In the Parameters group



click **Read parameterset** and **Select** to open the rpar window

Parameter Sets: rpar

File Options Help Source Directory: C:\Bruker\TopSpin4.2.0.b.2\explan\nmr\par

Filter by file names: enter any string, *, ? Exclude: Clear

Class = Any Dim = Any ☒ Show Recommended

Type = Any SubType = Any SubTypeB = Any Reset Filters

C13CPD	C13DEPT135	C13DEPTQ135	C13UDEFT	COSYGPDPHSW
COSYGPSW	DIPSIGPZSSW	HMBCEGTL3ND	HMBGCP	HMBGCP_15N
HSQC_TOCSY	HSQC_TOCSY_ADIA	HSQCEDETGPSISP	HSQCEDETGPSISP_...	HSQCETGP_15N
HSQCETGPSISP	HSQCETGPSISP_ADIA	MLEVPHPR	MLEVPHSW	NOESYPHPR
NOESYPHSW	PROTON	ROESYPHEASY	ROESYPHPR	ROESYPHSW
WATER				

Read... Close

- Enable **Show Recommended** to get the list of the most common small molecule experiments.
- Ensure that the source directory is
`<Topspin>\exp\stan\nmr\par`
and not
`<Topspin>\exp\stan\nmr\par\user`
- In the table select **PROTON** as experiment and click **Set selected item in editor**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- On the menu bar, click **Acquire**.

To acquire a spectrum, use the Workflow buttons in the Workflow button bar from left to right (see steps below). Alternatively, commands which are displayed in brackets of the various popup windows, can also be typed at the TopSpin command prompt (e.g. **ej**, **ij**, **edte** etc.).

- On the **Sample** button, click the **drop-down** arrow to see more options.
- In the list, select **Eject sample manually (ej)**.



Wait until the sample lift air is turned on and remove the sample which may be in the magnet.

Load the sample

- Place the sample with the spinner onto the top of the magnet.
- On the **Sample** button, click the **drop-down** arrow to see more options.
- In the list, select **Insert sample manually (ij)**.



Wait until the sample is lowered down into the probe and the lift air is turned off. A clicking sound may be heard.

Lock the solvent

- On the Workflow button bar, click **Lock**.
- In the Solvents table window, select the solvent, e.g. **DMSO**. Click **OK**.

Solvents table		X
^ Solvent	Description	
Acetic	acetic acid-d4	
Acetone	acetone-d6	
C6D6	benzene-d6	
CD2Cl2	dichlormethane-d2	
CD3CN	acetonitrile-d3	
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)	
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)	
CDCl3	chloroform-d	
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)	
CH3OH+D2O	HPLC Solvent (Methanol/D2O)	
D2O	deuteriumoxide	
D2O_salt	deuteriumoxide with salt	
Dioxane	dioxane-d8	
DMF	N,N-dimethylformamide-d7	
DMSO	dimethylsulfoxide-d6	
EtOD	ethanol-d6	
H2O+D2O	90%H2O and 10%D2O	
H2O+D2O_salt	90%H2O and 10%D2O with salt	
HDMSO	90%DMSO and 10%DMSO-d6	
Juice	fruit juice	
MeOD	methanol-d4	
Plasma	blood plasma	
Pyr	pyridine-d5	
T_H2O+D2O+Me4NCl	(CD3)4NCl in 90%H2O and 10%D2O, for NMR thermometer	
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMR thermometer	
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR thermometer	
T_MeOD	methanol-d4, for NMR thermometer	
TFE	trifluoroethanol-d3	
THF	tetrahydrofuran-d8	
Tol	toluene-d8	
Urine	urine	
Lock nucleus: 2H		OK Cancel

Tune and match the probe

- On the Workflow button bar, click **Tune**.

This performs an **atma (automatic tuning and matching)** and requires a probe equipped with an automatic tuning module. For more options, click the **drop-down** arrow on the **Tune** button.

Spin the sample (optional)

- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation on (ro on)**.



Rotation may be turned **OFF** for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

Shim the sample

- On the Workflow button bar, click **Shim**.

This executes the command **topshim**. The shimming starts momentarily and should take less than a minute. On the **Shim** button, click the **drop-down** arrow to see more options.

Load the prosol parameters

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

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

4.2.2 Acquisition

Set the receiver gain

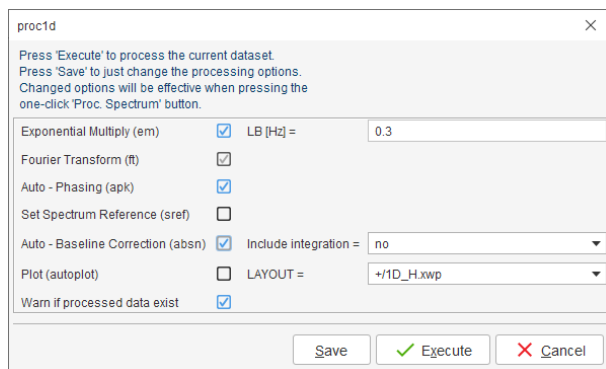
- On the Workflow button bar, click **Gain**.
- or
- On the Gain button, click the drop-down arrow to adjust the receiver gain manually.

Start the data acquisition

- On the Workflow button bar, click **Run**.
- or
- On the **Run** button, click the **drop-down** arrow to see more options.

4.2.3 Processing

- When the acquisition has finished, click **Process** on the menu bar.
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing (proc1d)**.
- In the proc1d window, enable the following options:
 - Exponential Multiply (em)
 - Auto - Phasing (apk)
 - Auto - Baseline Correction (absn)



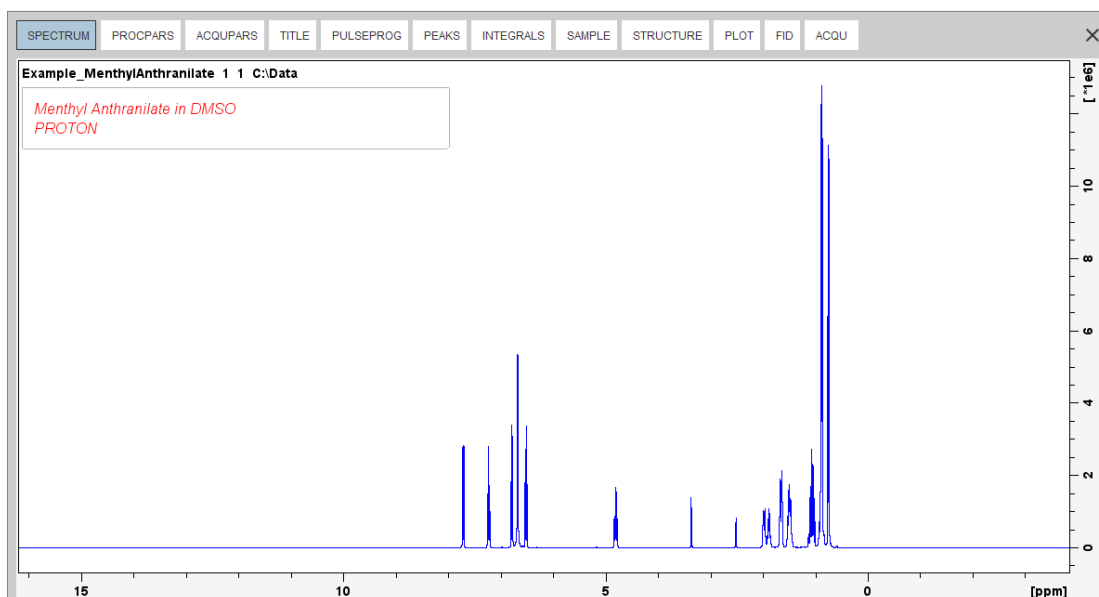
The screenshot shows the 'proc1d' dialog box with the following settings:

Option	Value
Exponential Multiply (em)	<input checked="" type="checkbox"/> LB [Hz] = 0.3
Fourier Transform (ft)	<input checked="" type="checkbox"/>
Auto - Phasing (apk)	<input checked="" type="checkbox"/>
Set Spectrum Reference (sref)	<input type="checkbox"/>
Auto - Baseline Correction (absn)	<input checked="" type="checkbox"/> Include integration = no
Plot (autoplot)	<input type="checkbox"/> LAYOUT = +/-1D_H.xwp
Warn if processed data exist	<input checked="" type="checkbox"/>

Buttons at the bottom: Save, Execute, Cancel.

- If TMS is added to the sample for referencing, enable **Set Spectrum Reference (sref)**.
- In the proc1d window, click **Execute** and then click **Save** to save the selected processing settings.

Now all future datasets can be processed with the defined actions with a click on **Proc Spectrum**.

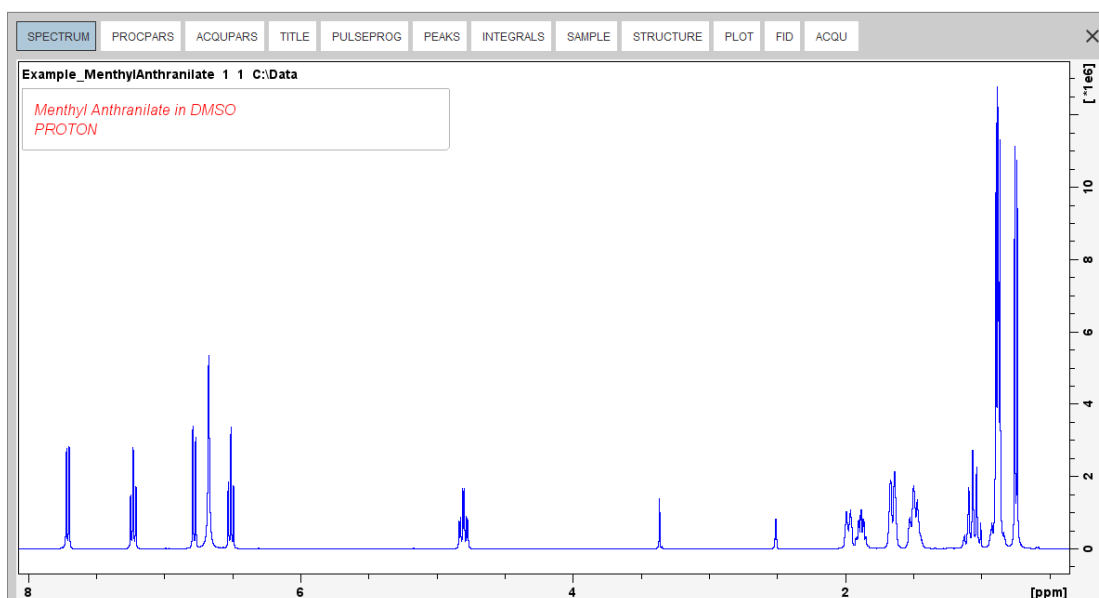


4.2.4 Integration

To quantitatively analyze an observed proton signal, the integrated intensity of the peaks is compared within each other. It is common to integrate a proton spectrum to account for the number of protons in the analyzed molecule.

To get more precise quantitative integration results, please refer to the **Quantitative NMR** manual.


- Expand the spectrum to include all peaks.

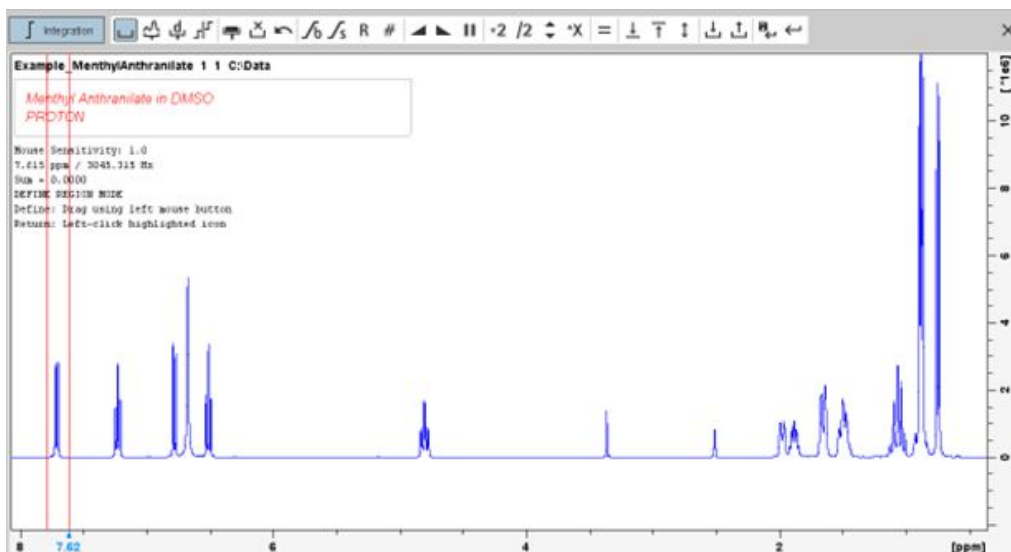


- On the menu, click **Analyse | Integrate**.

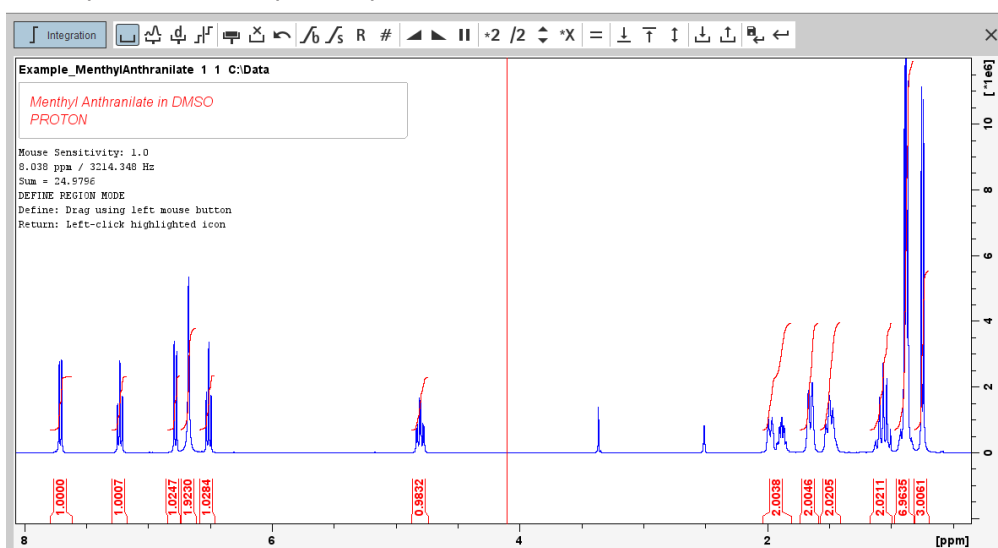
This enters the manual Integration mode. The **Dataset** tabs bar is replaced by the **Integration Tool** bar.

1D Proton Experiment

- Select the **Define new region using cursor** button. 
- Set the cursor line to the left of the first peak to be integrated. Click the left mouse button and drag the cursor line to the right of the peak and then release the mouse button.



- Repeat the last step for all peaks of interest.




- On the Integration Tool bar, click **Return, save region** to save the integration regions.





4.2.5 Plotting the 1D Proton Spectra

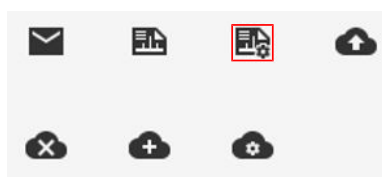
- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**.



The spectrum can be printed as it is displayed in the SPECTRUM tab with a click on **Print active window** .


The spectrum can also be printed with a predefined layout with a click on **Export active data or plot window as PDF** .

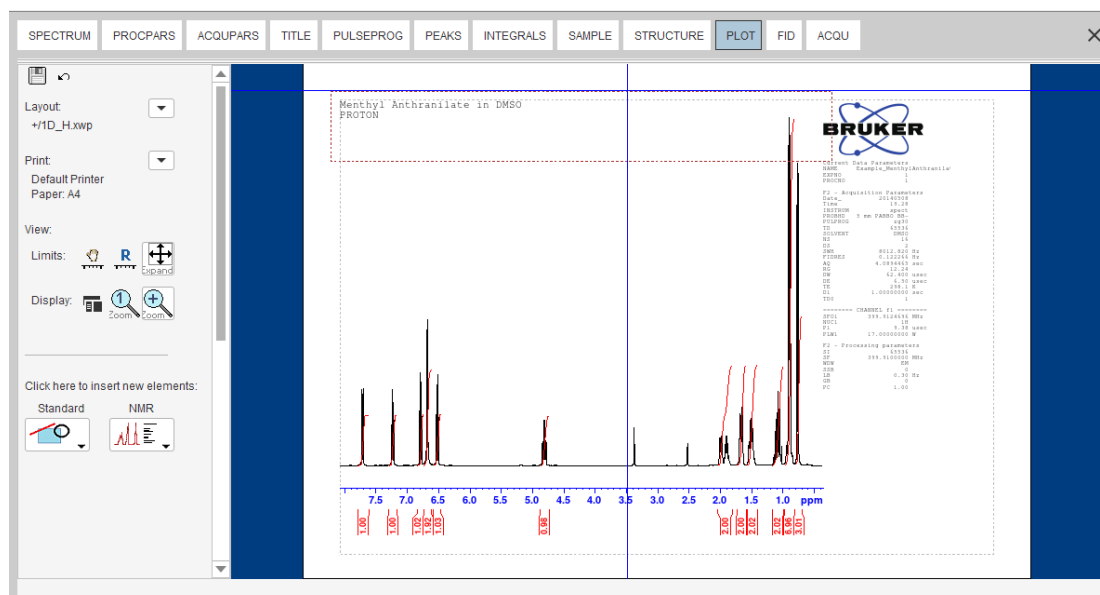
Additional options are available with the **Show more Publish Options** button on the menu bar  and select **Switch to plot editor**.



You may also switch to the interactive plot editor by clicking on the **Plot** tab in the Dataset window tabs bar (see figure below).



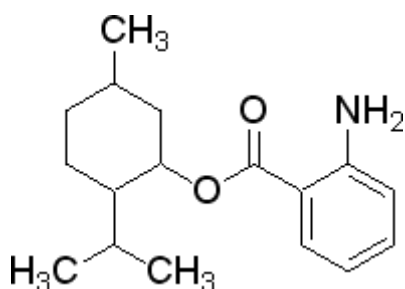
For details of working with the plot editor and modifying layouts see the **Data Publishing** manual in the help menu . Click **Help | Manuals | Automation and Data Publishing | Data Publishing**.



5 1D Selective Experiments

5.1 Sample

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



5.2 1D Selective COSY

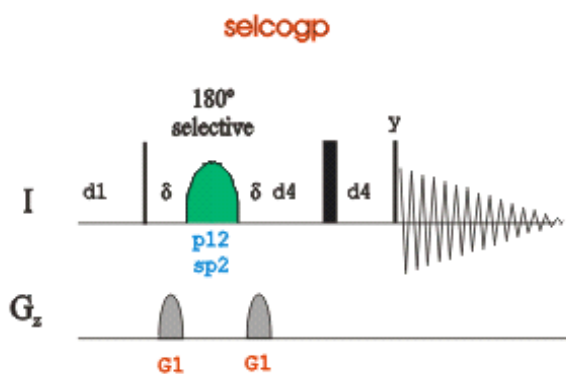
The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ¹H spectrum. Important characteristics of a soft pulse include the shape, the amplitude,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



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

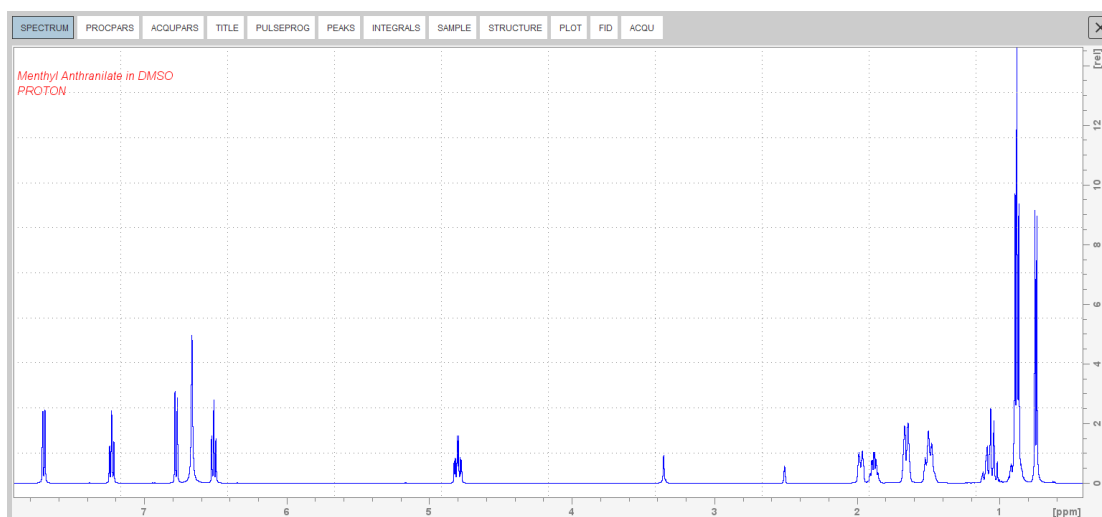
Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments using pulse field gradients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

This chapter describes the acquisition and processing of a one-dimensional ¹H selective gradient COSY experiment. The standard Bruker parameter set is SELCOGP and includes the pulse sequence **selcogp** shown in the next figure. 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 shape pulse.



5.2.1 Reference Spectrum

- Run a **1D Proton** spectrum, following the instructions in the chapter *1D Proton Experiment*, [Experiment Setup](#) [▶ 30] through [Processing](#) [▶ 34].

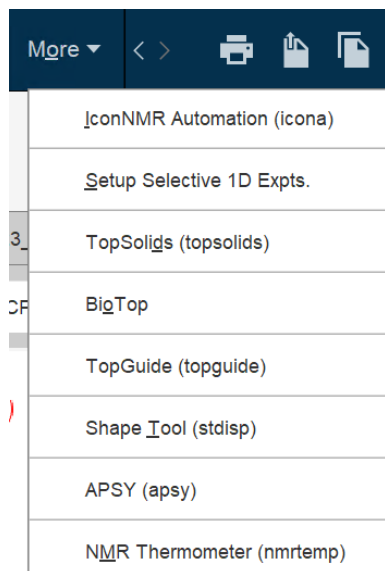


5.2.2 Selective Excitation Region Setup



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.

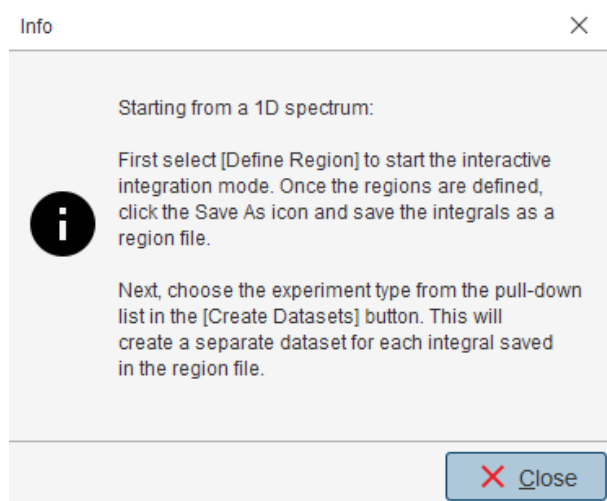
- On the menu bar, click **Acquire**.
- On the **More** button, click the **drop-down** arrow to see more options.



- 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**.



- Expand the peak at **7.2 ppm**.



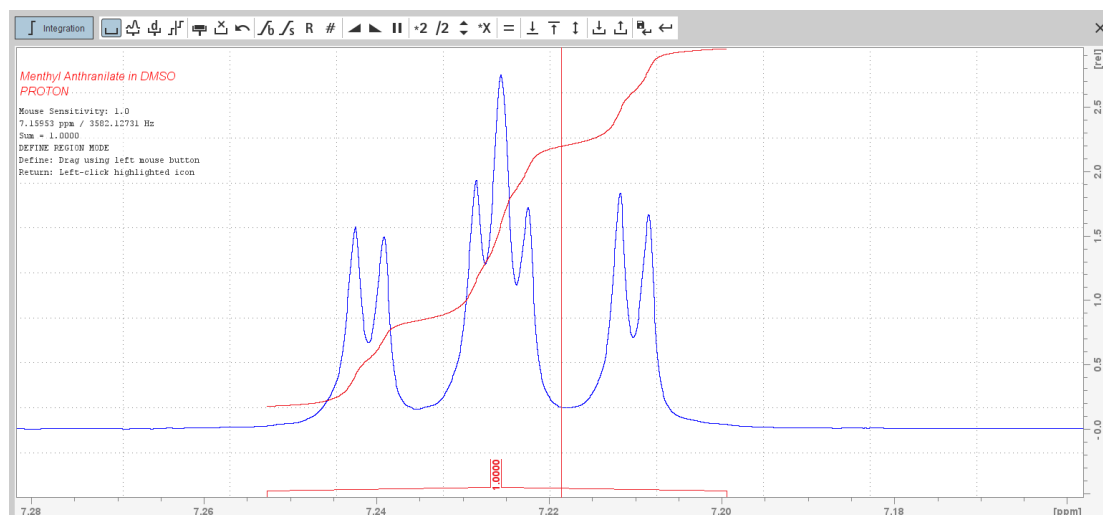
Define Regions

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

The Define Regions toolbar is displayed:



- Integrate the multiplet at **7.2 ppm**.

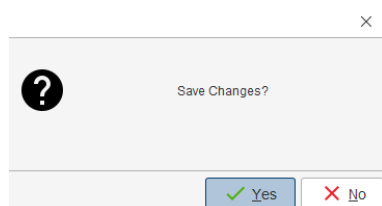


If desired, other peaks can be integrated and a separate dataset will be created for each saved integral.

- On the toolbar, click **Save/Export Integration Region**.
- In the list, select **Save the Region to 'reg'**.
- On the toolbar, click **Return do NOT save regions!** to exit the integration mode.

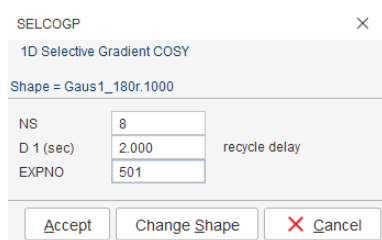


- In the message window, click **No**.



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

To change the **Gaus1_180r.1000** pulse, in the SELCOGP window click **Change Shape**.



SELCOGP

1D Selective Gradient COSY

Shape = Gaus1_180r.1000

NS	8	recycle delay
D 1 (sec)	2.000	
EXPNO	501	

Accept Change Shape Cancel

- In the SELCOGP message window, click **Accept**.

The new dataset is created and all parameters are automatically calculated and set. Cancel and open experiment number 501. Type **ased** in the TopSpin command line and change CNST1 from 1.0 to 7.0.

5.2.3 Acquisition

- On the Workflow button bar, click **Gain**.

or

- On the **Gain** button, click the **drop-down** arrow to adjust **rg** manually.
- On the Workflow button bar, click **Run**.

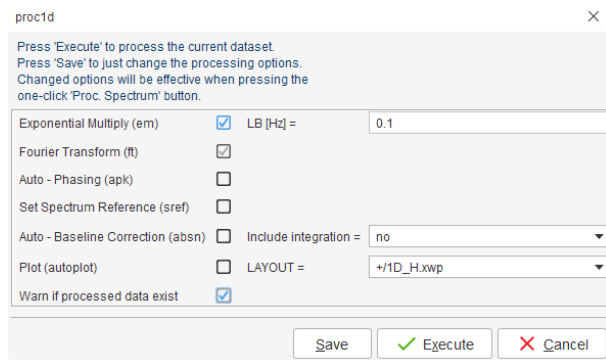
or

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

5.2.4 Processing

When the acquisition is finished:

- 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 (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



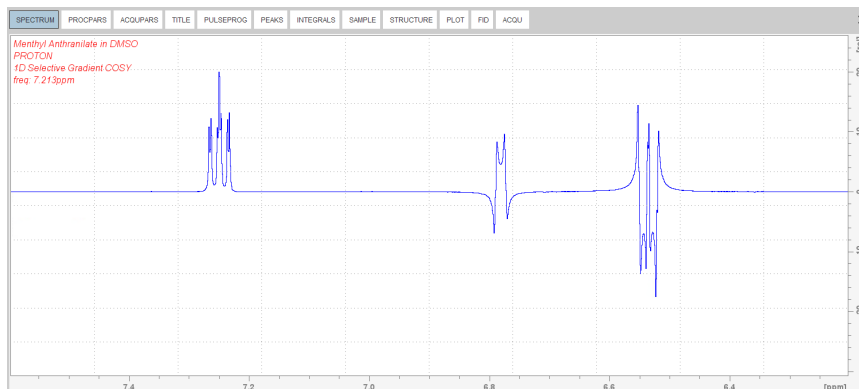
proc1d

Press 'Execute' to process the current dataset.
Press 'Save' to just change the processing options.
Changed options will be effective when pressing the one-click 'Proc. Spectrum' button.

Exponential Multiply (em)	<input checked="" type="checkbox"/>	LB [Hz] =	0.1
Fourier Transform (ft)	<input checked="" type="checkbox"/>		
Auto - Phasing (apk)	<input type="checkbox"/>		
Set Spectrum Reference (sref)	<input type="checkbox"/>		
Auto - Baseline Correction (absn)	<input type="checkbox"/>	Include integration =	no
Plot (autoplot)	<input type="checkbox"/>	LAYOUT =	+1D_H.xwp
Warn if processed data exist	<input checked="" type="checkbox"/>		

Save Execute Cancel

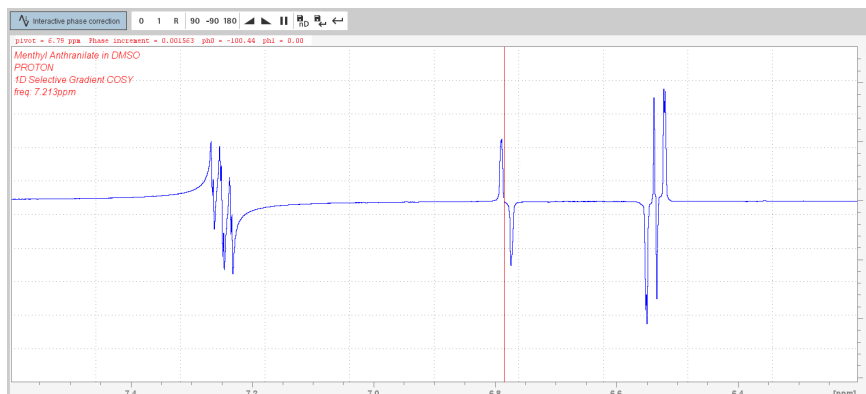
- In the proc1d window, click **Execute**.
- Expand the spectrum from **7.6 ppm** to **6.2 ppm**.



- On the Workflow button bar, click **Adjust Phase**.

The **Dataset tabs** are replaced by the **Adjust Phase** tool bar.

- Adjust the **0** order correction on the peak at **6.8 ppm** to display an antiphase pattern.



- On the toolbar, click **Return & save phased spectrum**.



5.2.5 Plotting Two Spectra on the Same Page

- Display the selective COSY spectrum.

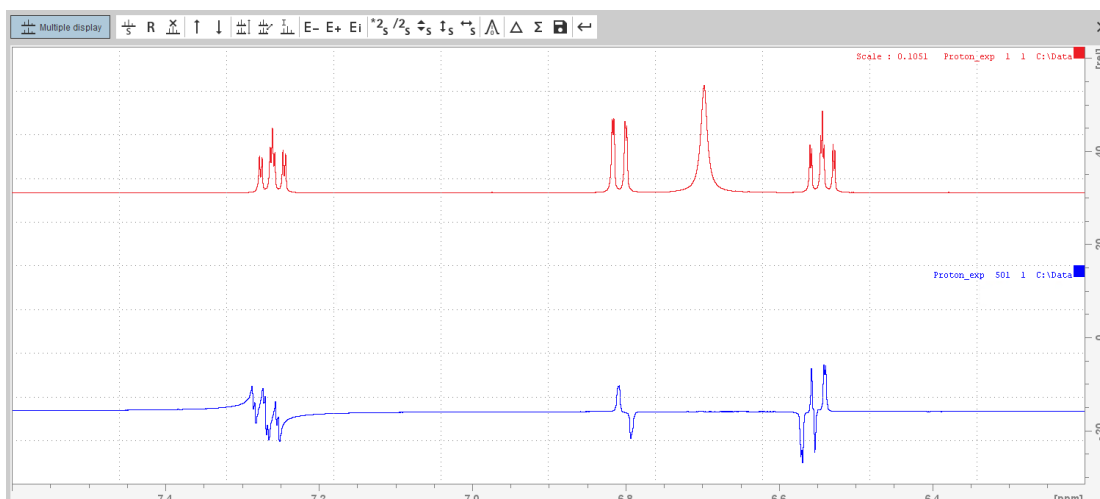
- On the toolbar, click **Multiple display**.



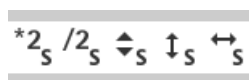
The Multiple display toolbar is displayed:



- Drag the **Reference spectrum** (1D Proton) into the spectral window.



- Click the small box in the upper right corner of the spectrum display to select the reference spectrum.



- Adjust the spectra for best fit with the tools:

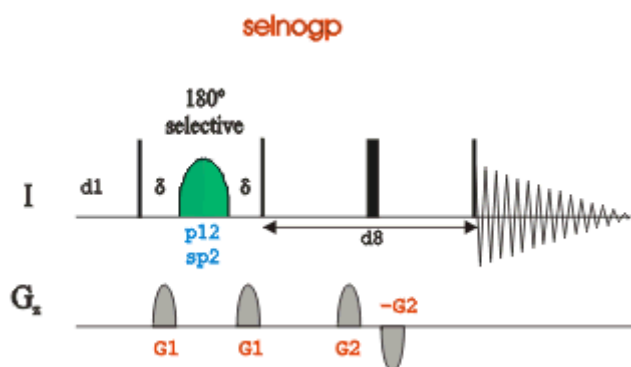


- On the Workflow button bar, click **Print active window**

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

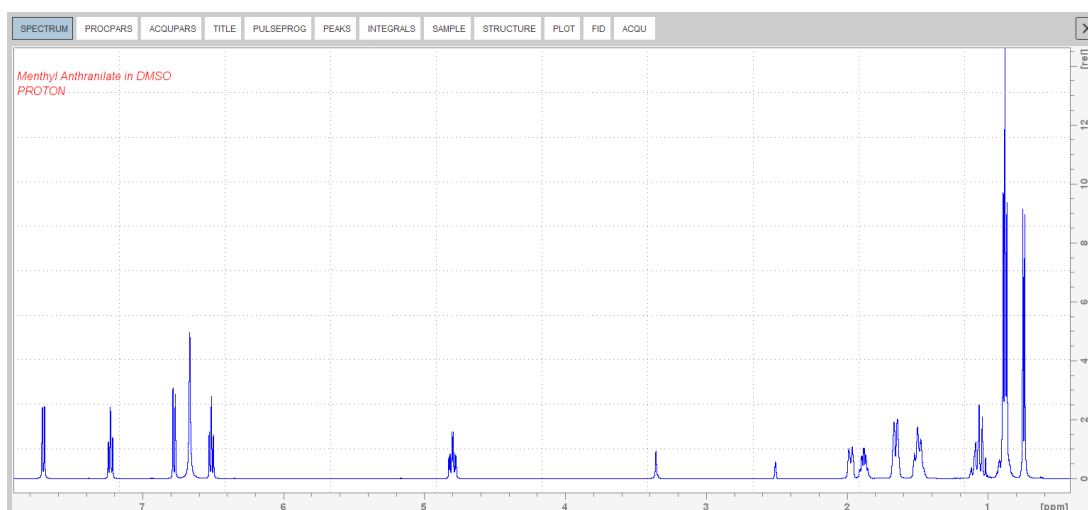
5.3 1D Selective NOESY

This chapter describes the acquisition and processing of a one-dimensional ^1H selective gradient NOESY experiment. The standard Bruker parameter set is SELNOGP and includes the pulse sequence **selnoggp** shown in the next figure. It consists of the recycling delay, five 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 90° degree pulse, a 180° degree pulse and finally a 90° degree pulse. The mixing time **D8** is applied before and after the 180° pulse. There are four gradient pulses applied, one each between the RF pulses.



5.3.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment, Experiment Setup* ▶ 30] through *Processing* ▶ 34].

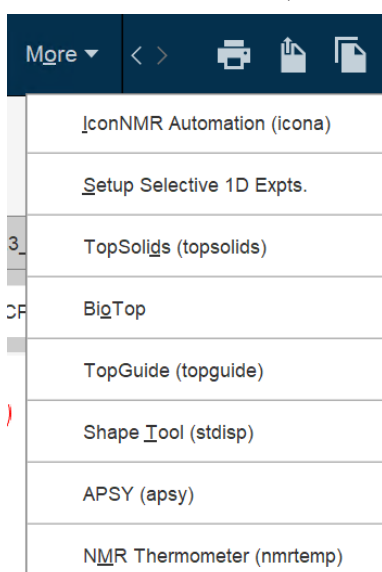


5.3.2 Selective Excitation Region Setup



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.

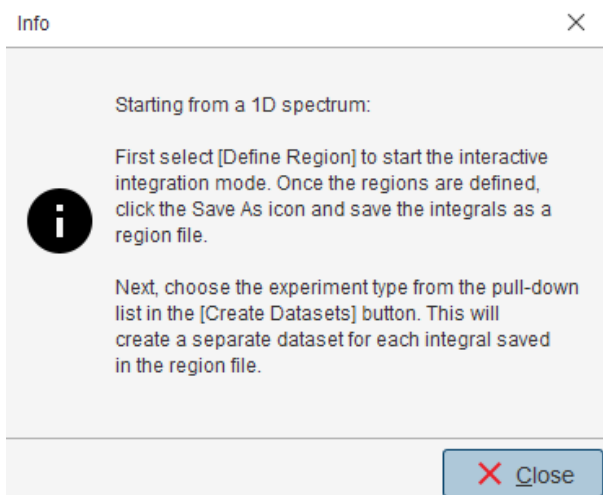


- In the list, select **Setup Selective 1D Expts.**

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**.

1D Selective Experiments



- Expand the peak at **4.8 ppm**.

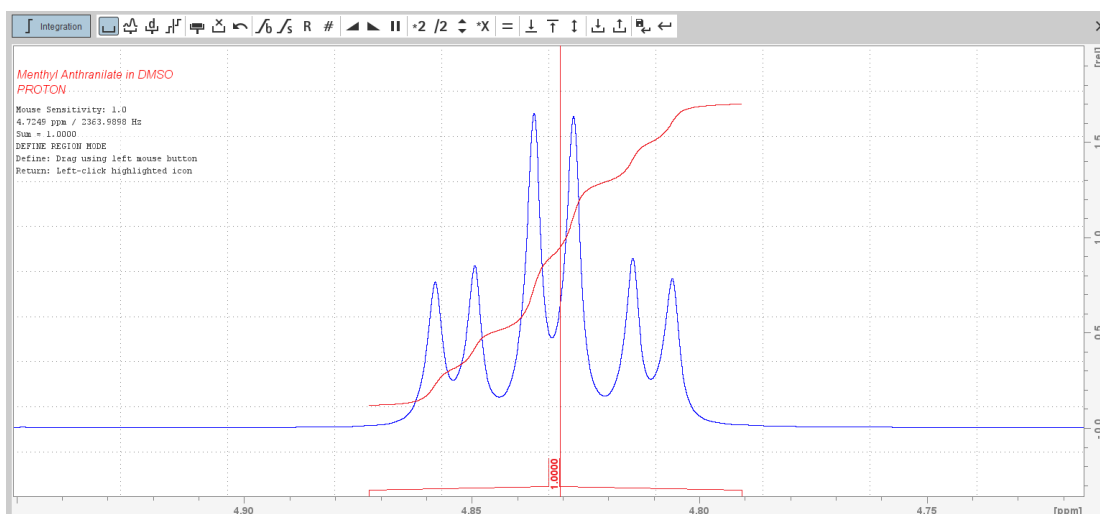
Define Regions

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

The Define Regions toolbar is displayed:

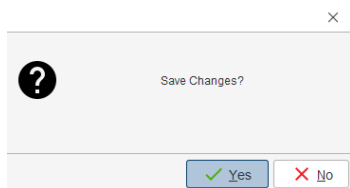


- Integrate the multiplet at **4.8 ppm**.



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

- On the toolbar, click **Save/Export Integration Region**.
- In the list, select **Save the Region to 'reg'**.
- On the toolbar, click **Return do NOT save regions!** to exit the integration mode.
- In the message window, click **No**.



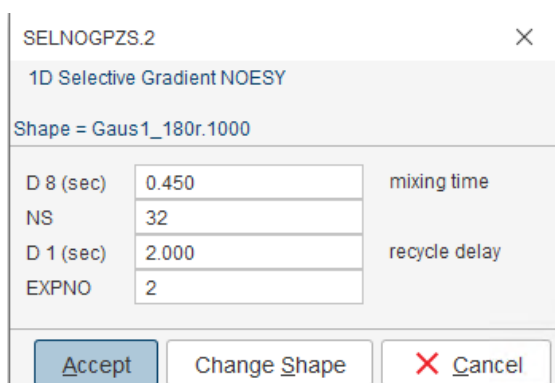
- On the **Create Dataset** button, click the **drop-down** arrow to see more options.
- In the list, select **Selective gradient NOESY**.
- Click **Accept**.

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 field strength. It can vary from **100 ms** to **800 ms** for a large molecule to a small molecule.

- To change the **Gaus1_180r.1000** pulse, in the SELNOGP window click **Change Shape**.
- In the SELNOGP window, enter

D8 = **0.450**

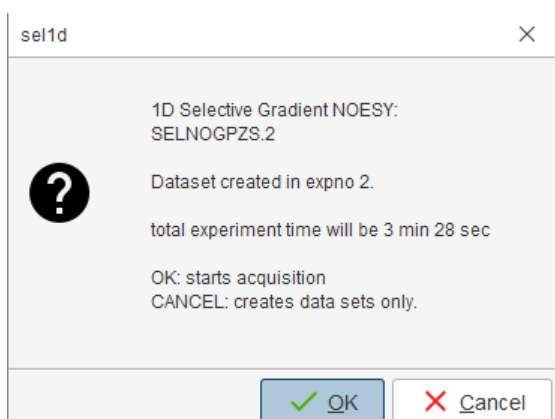
NS = **32**



- In the SELNOGP message window, click **Accept**.

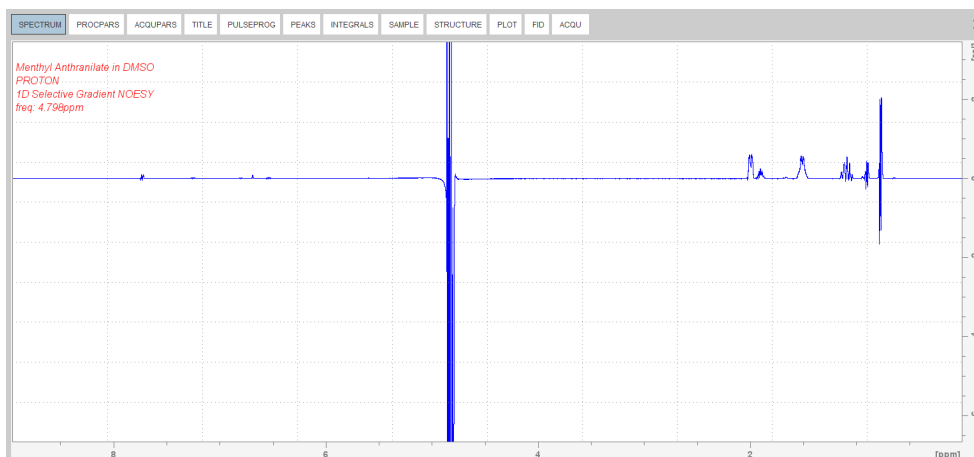
The new dataset is created and all parameters are automatically calculated and set.

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



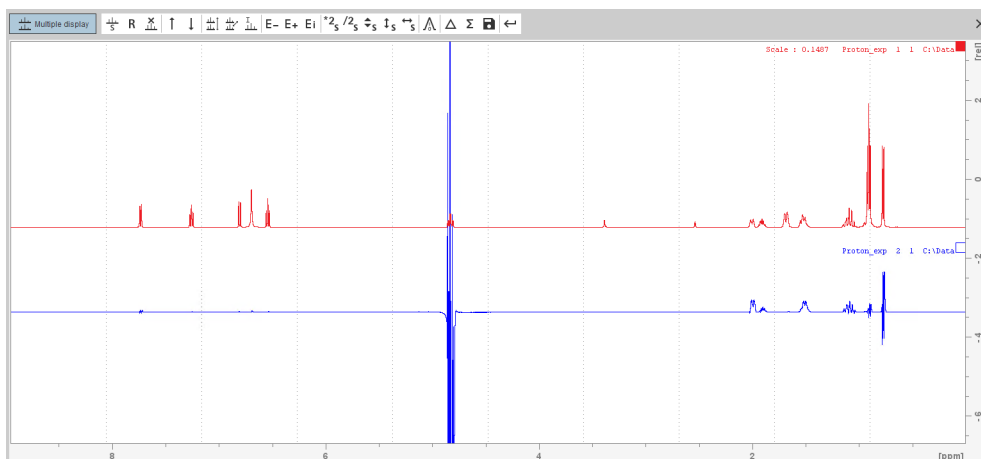
5.3.3 Processing

- Follow the first processing instructions in the chapter *1D Selective Experiments Processing* [42].
- Manually adjust the phase of the irradiation peak at **4.8 ppm** to show negative absorption and phase the peaks between **3 ppm** and **1 ppm** dependent on the field strength, to be either positive or negative.



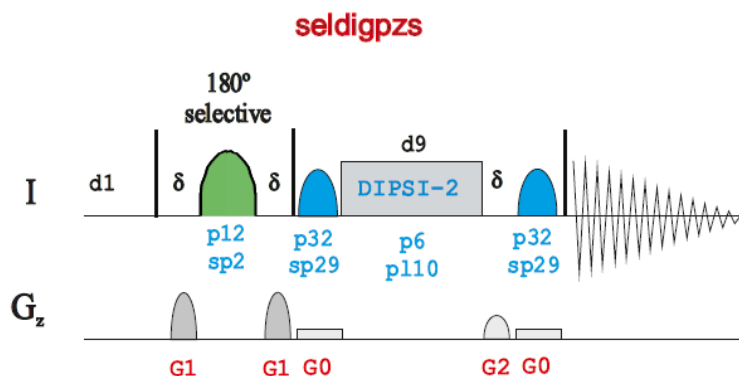
5.3.4 Plotting Two Spectra on the Same Page

- Display the selective NOESY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page* [43] for the Selective COSY.



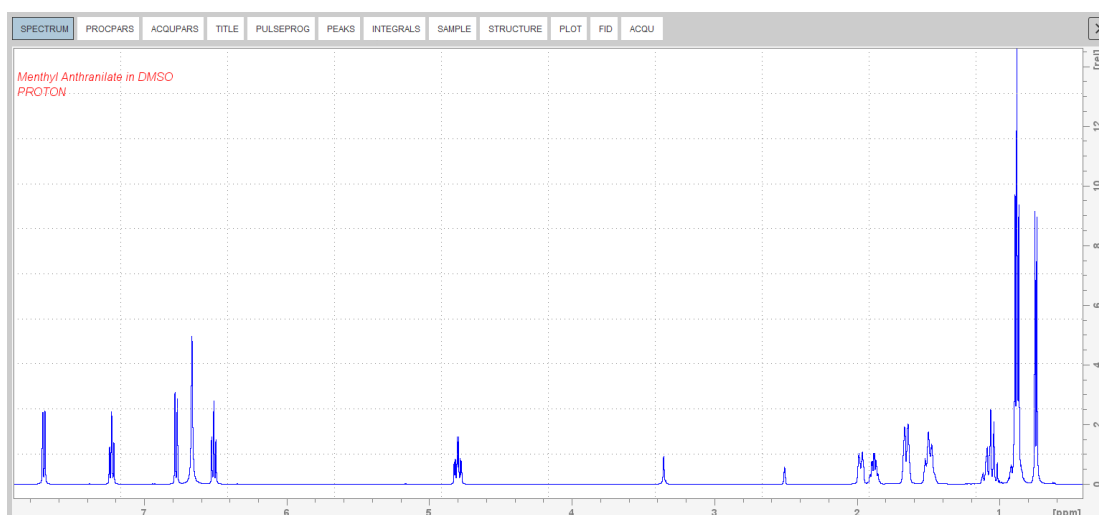
5.4 1D Selective TOCSY

This section describes the acquisition and processing of a one-dimensional ^1H selective gradient TOCSY experiment. The standard Bruker parameter set is SELDIGPZS and includes the pulse sequence **seldigpzs** shown in the figure below. It consists of the recycling delay, a radio-frequency (RF) pulse, a DIPSI2 sequence for mixing and the acquisition time during which the signal is recorded.



5.4.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment, Experiment Setup* [30] through *Processing* [34].



5.4.2 Selective Excitation Region Set Up



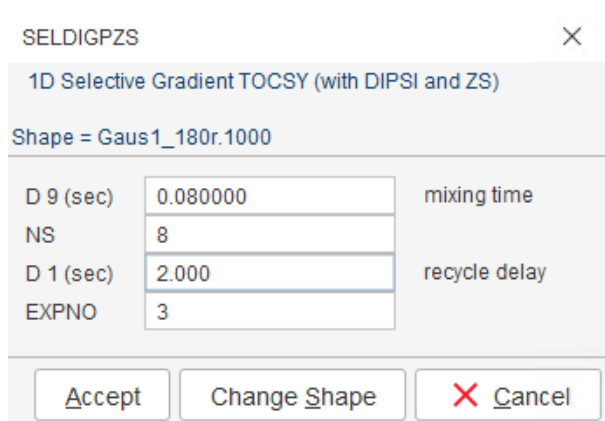
The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

- On the Workflow button bar, click **Define Regions** to define the excitation region. See detailed instructions in chapter *1D Selective Experiments, Selective Excitation Region Setup* [45] up to *Selective Excitation Region Setup* [46].
- On the **Create Dataset** button, click the **drop-down** arrow to see more options.
- In the list, select **Selective gradient TOCSY**.
- Click **Accept**.

The default parameters are taken from the standard parameter set SELDIGPZS. If desired, click **Change Shape** to modify the **Gaus1_180r.1000** pulse. A mixing time of **0.06 s** to **0.08 s** is typical for the TOCSY experiment.

1D Selective Experiments

- In the SELDIGPZS window, enter
D9 = **0.08**
NS = **8**



SELDIGPZS

1D Selective Gradient TOCSY (with DIPSI and ZS)

Shape = Gaus1_180r.1000

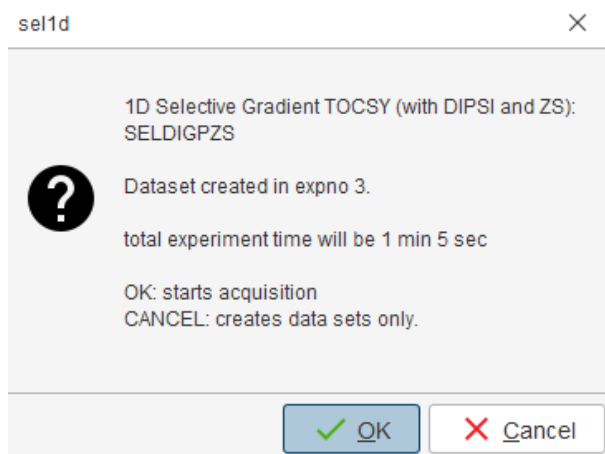
D 9 (sec)	0.080000	mixing time
NS	8	
D 1 (sec)	2.000	recycle delay
EXPNO	3	

Accept Change Shape Cancel

- Click **Accept**.


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

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



sel1d

1D Selective Gradient TOCSY (with DIPSI and ZS):
SELDIGPZS

 Dataset created in expno 3.
total experiment time will be 1 min 5 sec

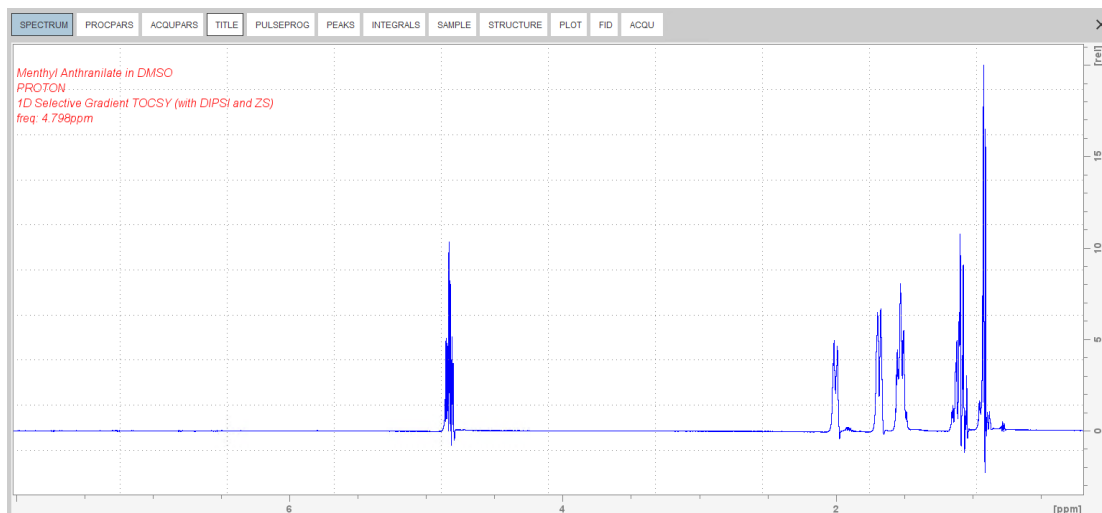
OK: starts acquisition
CANCEL: creates data sets only.

OK Cancel

5.4.3 Processing

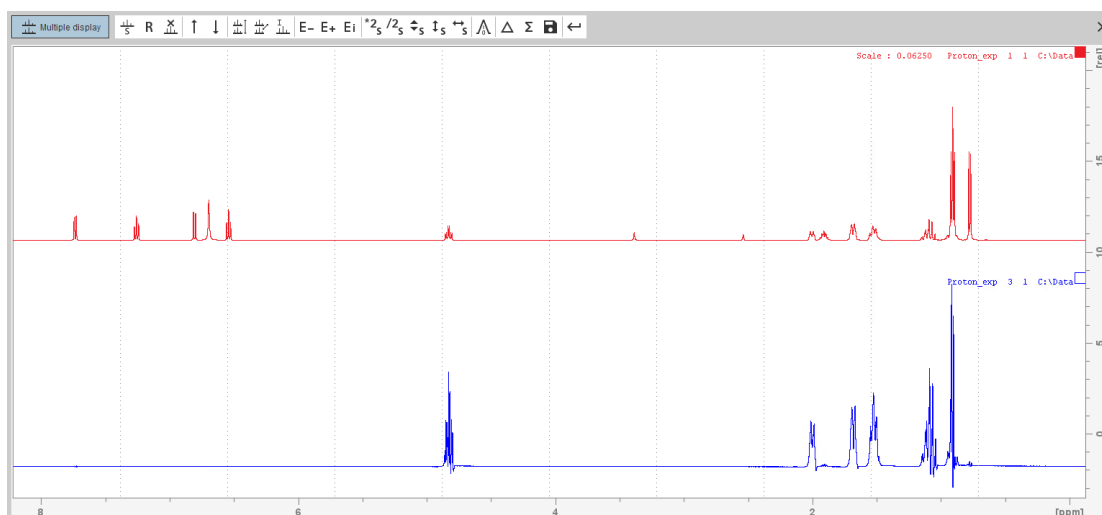
Follow the first processing instructions in the chapter *1D Selective Cosy Processing* [42] up to *Processing* [43].

- Manually adjust the phase on all peaks for positive absorption.



5.4.4 Plotting Two Spectra on the Same Page

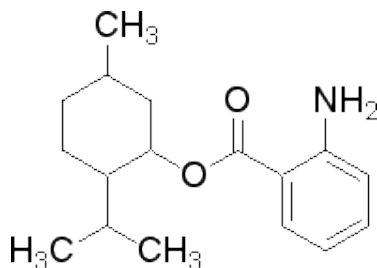
- Display the selective TOCSY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page* [43] for the Selective COSY.



6 2D Homonuclear Experiments

6.1 Sample

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



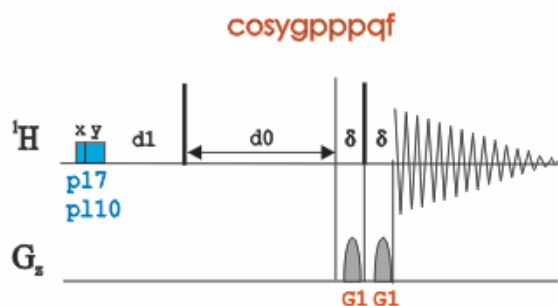
6.2 2D Gradient COSY

The COSY experiment relies on the J-couplings to provide spin-spin correlations, and its cross peaks indicate which ¹H atoms are close to other ¹H atoms through the bonds of the molecule. Typically, protons that are separated by up to 3 bonds can be observed.

The signals acquired with one of these experiments have absorptive and dispersive line shape contributions in both F1 and F2 dimensions. This means that it is impossible to phase the spectrum with all peaks purely absorptive, and, as a consequence, the spectrum must be displayed in magnitude mode. A typical spectral resolution of 3 Hz/pt is sufficient for resolving large scalar couplings. In order to resolve small J-couplings fine digital resolution is required, which significantly increases the experimental time. In general, the DQF-COSY experiment is recommended if a higher resolution is desired.

Using pulsed field gradients (PFG), the coherence pathway selection and the axial peak suppression can be achieved with only one scan per time increment. Thus, if enough substance is available, a typical gradient COSY experiment with 128 time increments can be recorded in 5 minutes.

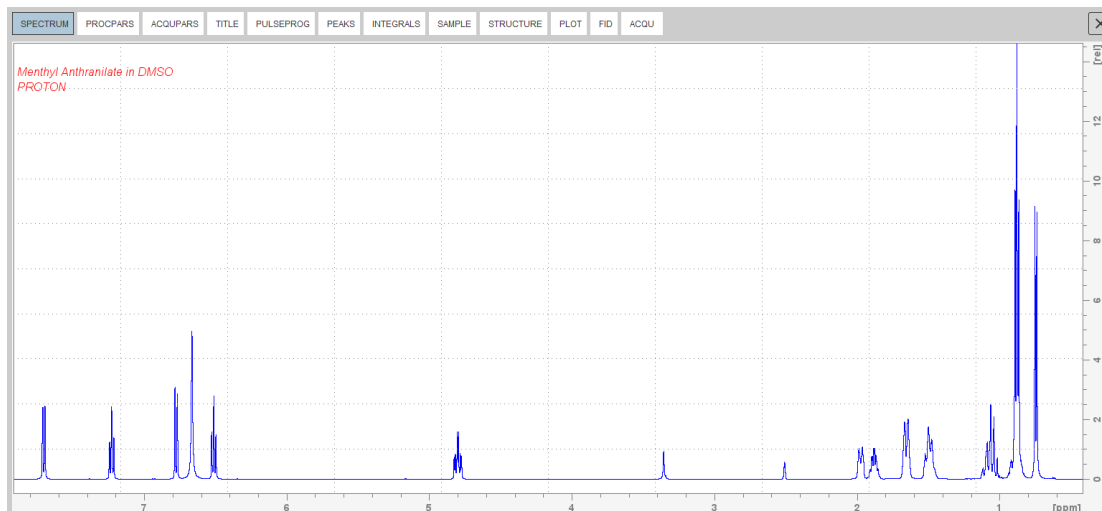
This chapter describes the acquisition and processing of a two-dimensional ¹H gradient COSY. The standard Bruker parameter set is COSYGPSW and includes the pulse sequence **cosygpppqf** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay D0 and the acquisition time during which the signal is recorded. Both pulses have a 90° flip angle. Two gradient pulses are applied before and after the second pulse in the sequence. Purge pulses are applied before d1.



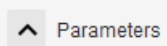
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

6.2.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment, Experiment Setup* [▶ 30] through *Processing* [▶ 34].



6.2.2 Setting up the COSY 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 = **cosy_exp**
EXPNO = **1**
Directory = e.g. **C:\Data**
- In the Parameters group  click **Read parameterset** and select the experiment **COSYGPSW**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation off**.



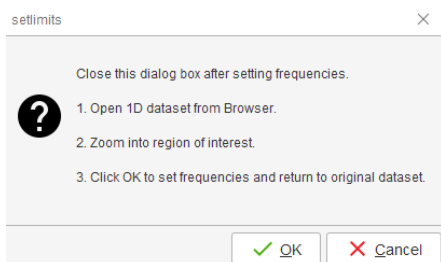
2D experiments should be run non-spinning.

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

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

6.2.3 Limit Setting

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

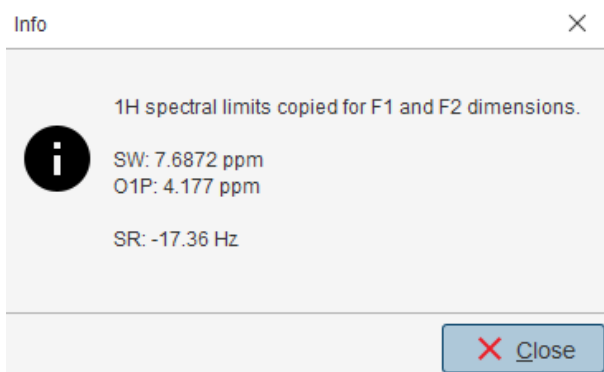


- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or drag the 1D Proton dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.

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



- In the setlimits message window, click **OK** to assign the new limits to the 2D dataset.
- In the message window, click **Close**.



The display changes back to the 2D dataset.

6.2.4 Acquisition

- On the Workflow button bar, click **Gain**.

or

- On the **Gain** button, click the **drop-down** arrow to adjust the receiver gain manually.
- On the Workflow button bar, click **Run**.

or

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

6.2.5 Processing

When the acquisition is finished:

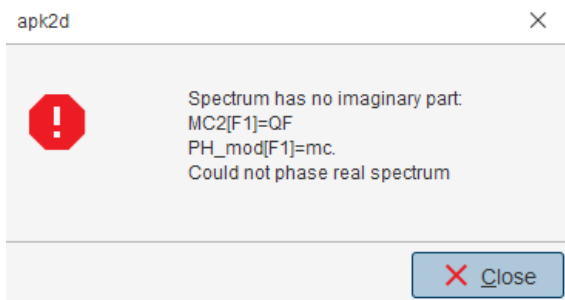
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**.

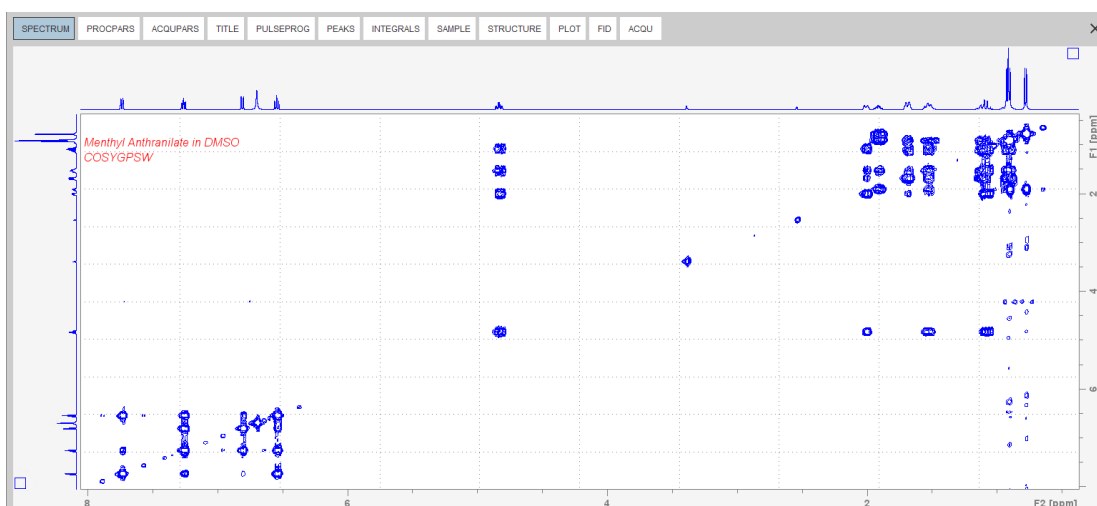
The apk2d message window is displayed in case of a magnitude 2D experiment and when the **apk2d** option is enabled and the processing of the magnitude COSY it not affected.

2D Homonuclear Experiments

- In the apk2d window, just click **Close**.

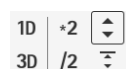


To disable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow to configure the Standard Processing (**proc2d**) program.



6.2.6 Plotting the COSY Spectrum

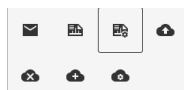
- Use the **Smaller/larger** buttons to adjust for a suitable contour level.

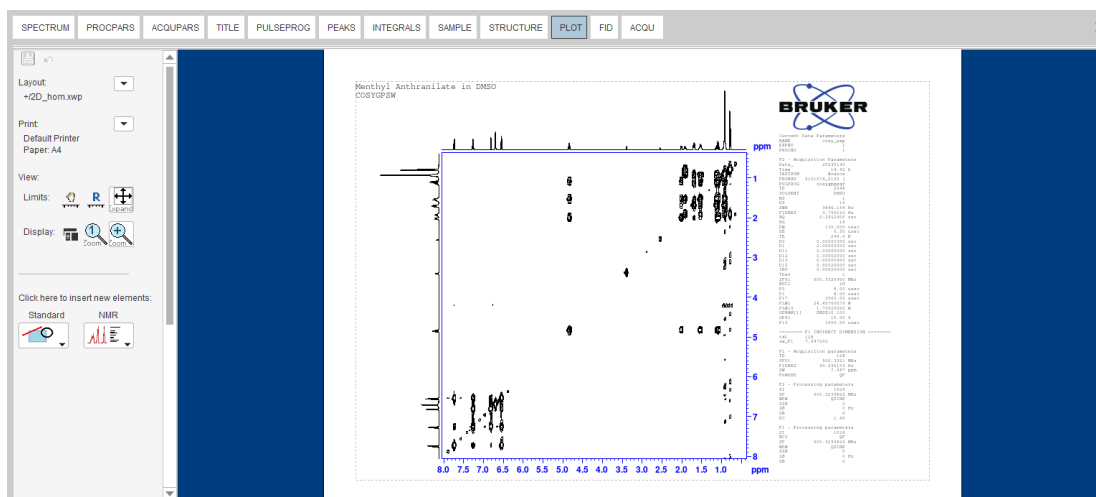


- Type **.ls** or click **Contour levels to disk**.
- On the right side of the Workflow button bar, click **Show more publishing options...**



- Select **Switch to plot editor (plot)**.





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

- Click the **down arrow** button in the left Print section.
- In the list, select **Print ...**

Print



6.3 2D Gradient NOESY Experiment

NOESY (Nuclear Overhauser Effect Spectroscopy) is a 2D spectroscopy method used to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear ^1H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicate which protons are close to each other in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and its cross peaks indicate which ^1H atoms are close to other ^1H atoms through the bonds of the molecule.

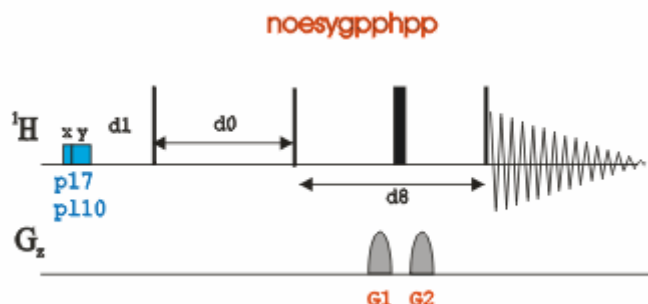
The basic NOESY sequence consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t_1 , which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period d_8 . Note that, for the basic NOESY experiment, d_8 is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time t_2 . The NOESY spectrum is generated by a 2D Fourier transform with respect to t_1 and t_2 .

Axial peaks, which originate from magnetization that has relaxed during t_{mix} , can be removed by the appropriate phase cycling.

NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure.

2D Homonuclear Experiments

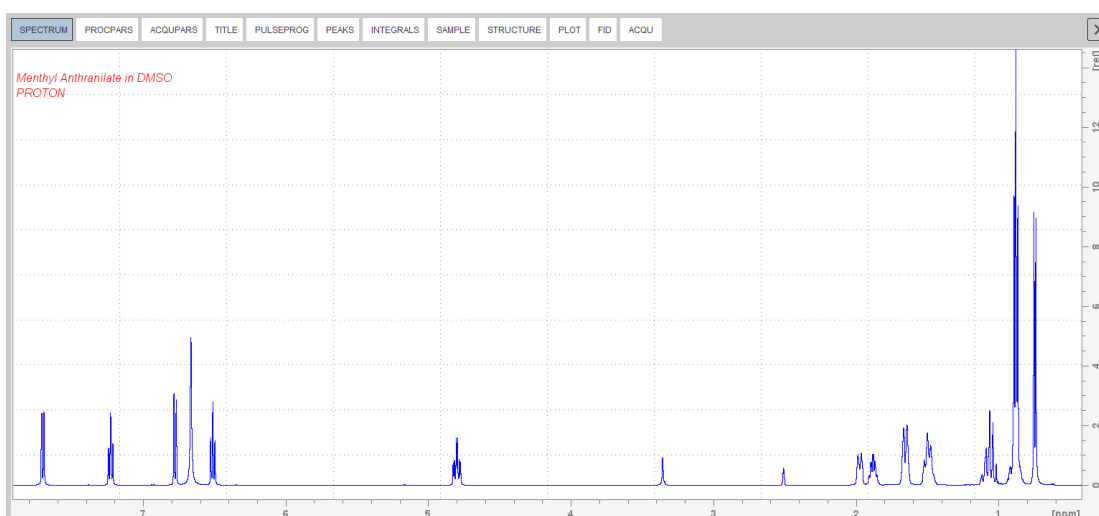
This section describes the acquisition and processing of a two-dimensional ^1H phase sensitive NOESY. The standard Bruker parameter set is NOESYPSHW and includes the pulse sequence **noesygpshpp** shown in the next figure. It consists of the recycling delay, three radio-frequency (RF) pulses, separated by the increment delay $D0$ between the first and second pulse, a mixing time $D8$ between the second and third 90° pulses and the acquisition time during which the signal is recorded. All three pulses are of 90° .



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.

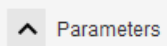
6.3.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, [Experiment Setup](#) [30] through [Processing](#) [34].



6.3.2 Setting up the NOESY Experiment

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New window, enter or select:
NAME = **noesy_exp**
EXPNO = **1**
Directory = e.g. **C:\Data**

- In the Parameters group  click **Read parameterset** and select the experiment **NOESYGPPHSW**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.

Follow the instructions in the chapter [Setting up the COSY Experiment \[53\]](#) for performing **Prosol** and **SetLimits**. If you know what you're doing, this should give you all the necessary information. If you need more details, you're referred to those details from the COSY experiment.

- In the Dataset window, select the **AcquPars** tab.
- In the AcquPars tab toolbar click **Show pulse program parameters**.



- In the Field D8[sec] enter **0.450**.

D8 [sec] Mixing time



The mixing time depends on the size of the molecule. The range for Bio-molecules is typically from **0.05 s** to **0.2 s**, medium size molecules from **0.1 s** to **0.5 s** and for small molecules **0.5 s** to **0.9 s**.

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

6.3.3 Acquisition

- On the Workflow button bar, click **Gain**.

or

- On the **Gain** button, click the **drop-down** arrow to adjust the receiver gain manually.

- On the Workflow button bar, click **Run**.

or

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

6.3.4 Processing

When the acquisition is finished:

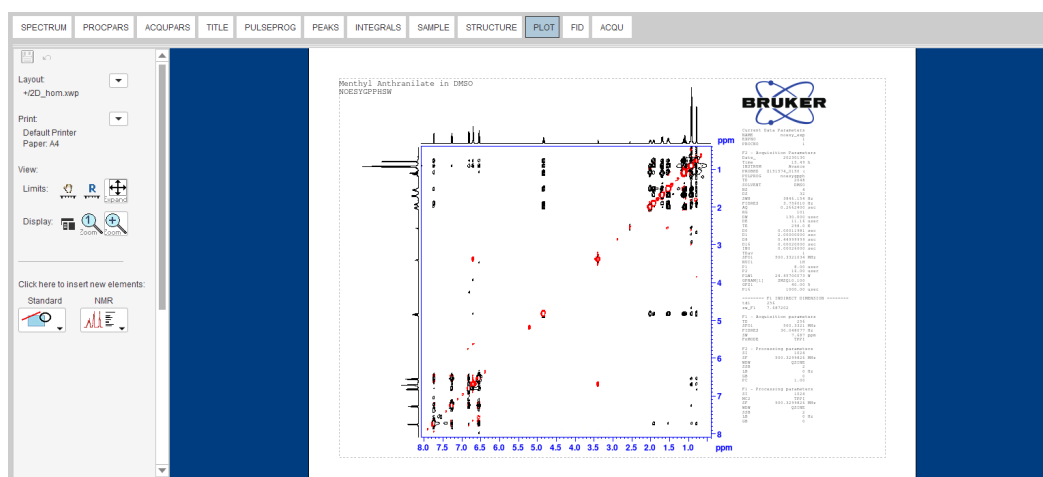
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. The **apk2d** option has to be enabled. To enable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.



6.3.5 Plotting the NOESY Spectrum

- Follow the plotting instructions in the chapter [Plotting the COSY Spectrum](#) [56].



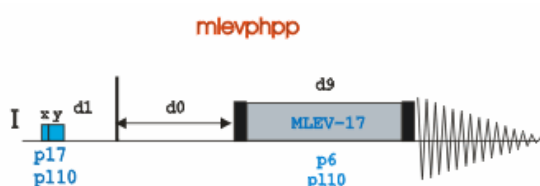
6.4 2D Phase Sensitive TOCSY Experiment

TOCSY (TOtal Correlation Spectroscopy) provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy in liquids. In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that pure absorption mode spectra with positive intensity peaks are created. In traditional COSY, cross

peaks have zero integrated intensity and the coherence transfer is restricted to directly spin coupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as antiphase coherence.

The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how far the spin coupling network will be probed. A general rule of thumb is that $1/(10 \text{ JHH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

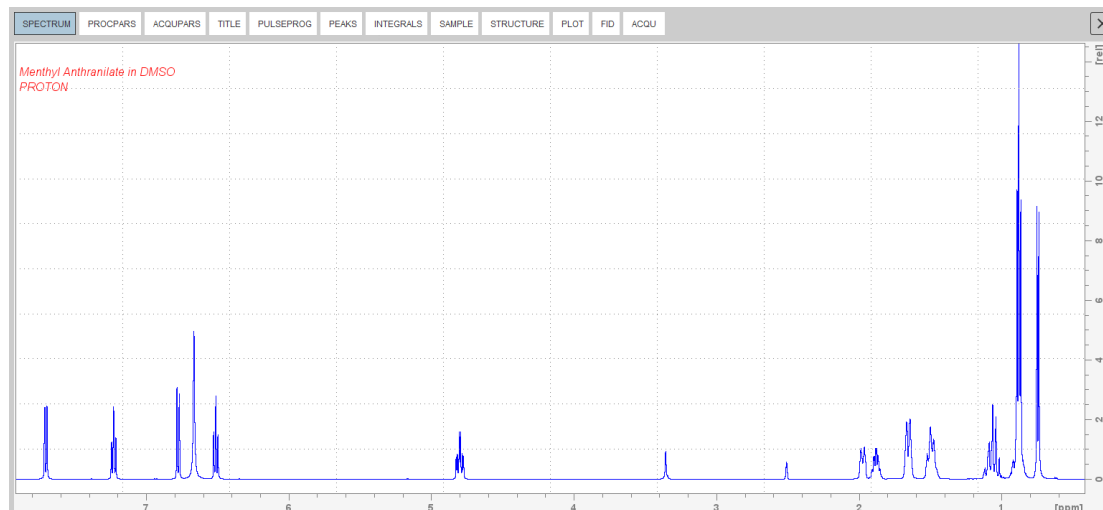
This section describes the acquisition and processing of a two-dimensional ^1H phase sensitive TOCSY. The standard Bruker parameter set is MLEVPHSW and includes the pulse sequence **mlevphpp** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay **D0** and the acquisition time during which the signal is recorded. The first RF pulse is a 90° pulse, the second pulse is the mlev spinlock pulse.



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.

6.4.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment, Experiment Setup* [30] through *Processing* [34].

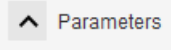


6.4.2 Setting up the TOCSY Experiment

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

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group  click **Read parameterset** and select the experiment **MLEVPHSW**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.

Follow the instructions in the chapter [Setting up the COSY Experiment \[53\]](#) for performing **Prosol** and **SetLimits**. If you know what you're doing, this should give you all the necessary information. If you need more details, you're referred to those details from the COSY experiment.

- In the Dataset window, select the **AcquPars** tab.
- In the AcquPars tab toolbar click **Show pulse program parameters**.



- In the Field D9[sec] enter **0.08**.

D9 [sec]	<input type="text" value="0.08"/>	TOCSY mixing time
----------	-----------------------------------	-------------------



A mixing time of **0.06 s** to **0.08 s** is typical for the TOCSY experiment.

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

6.4.3 Acquisition

- On the Workflow button bar, click **Gain**.

or

- On the **Gain** button, click the **drop-down** arrow to adjust the receiver gain manually.
- On the Workflow button bar, click **Run**.

or

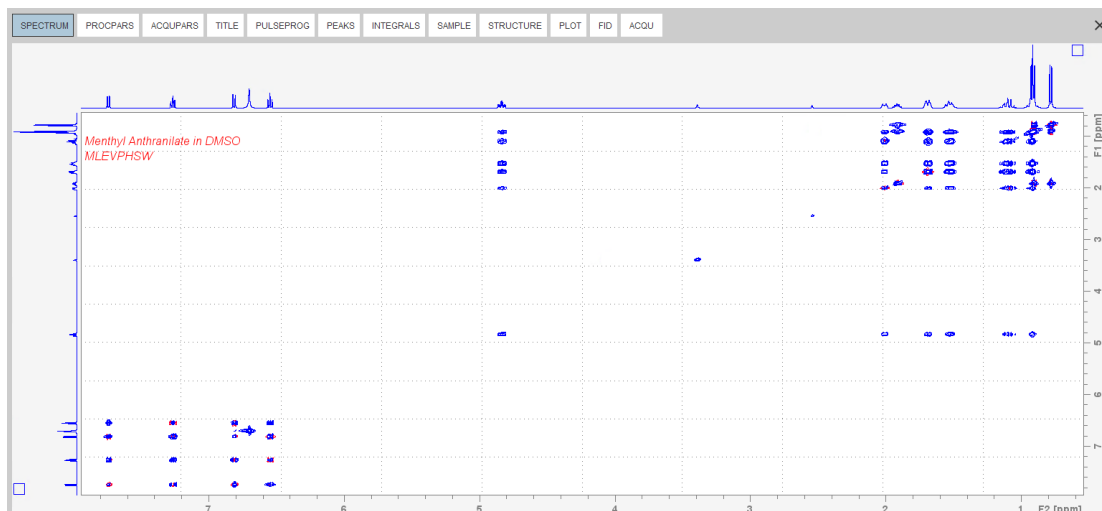
- On the **Run** button, click the **drop-down** arrow to see more options.

6.4.4 Processing

When the acquisition is finished:

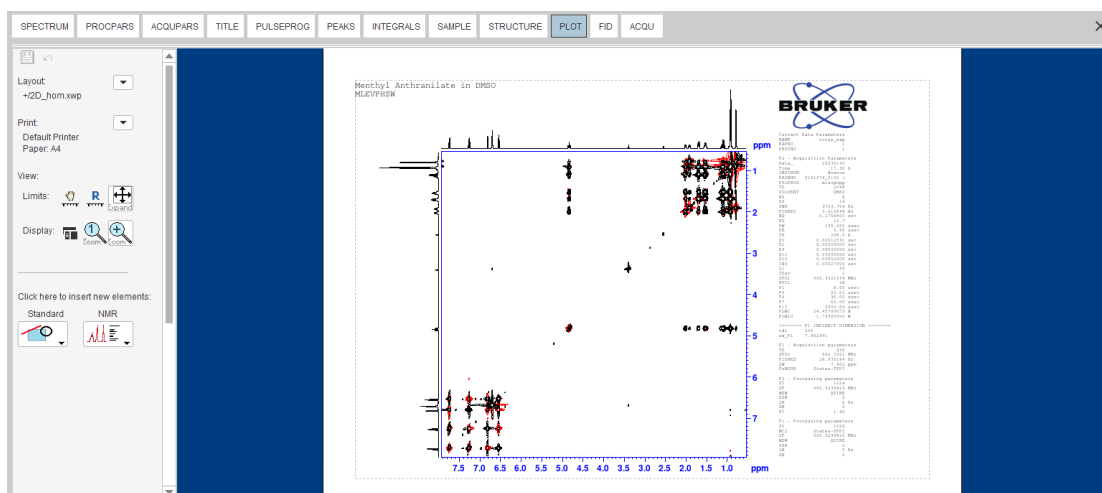
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. The **apk2d** option has to be enabled. To enable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.



6.4.5 Plotting the TOCSY Spectrum

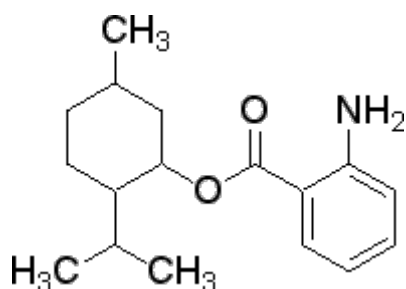
- Follow the plotting instructions in the chapter [Plotting the COSY Spectrum](#) [56].



7 1D Carbon Experiments

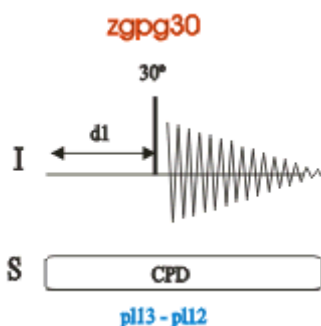
7.1 Sample

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




7.2 1D Carbon Experiment

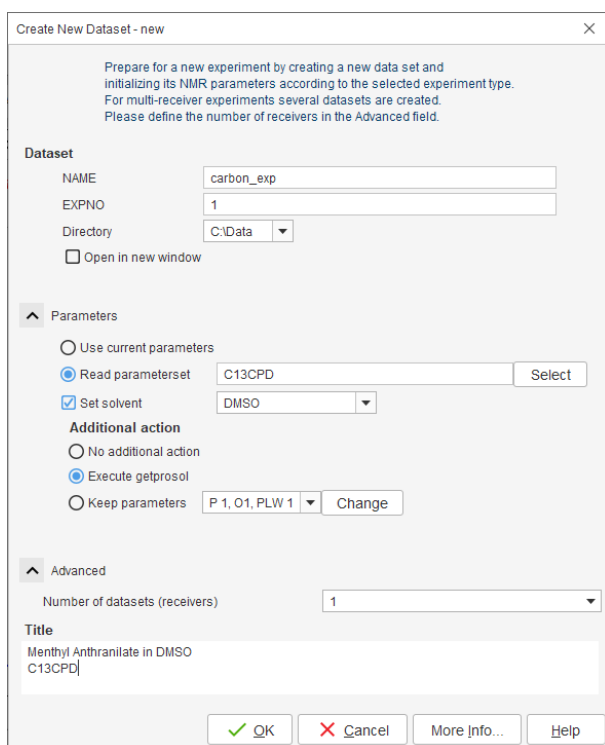
This chapter describes the acquisition and processing of a one-dimensional ¹³C NMR spectrum. The standard Bruker parameter set C13CPD, includes the pulse sequence **zgpg30**, shown in the figure below. The ¹³C channel consists of the recycling delay, a RF pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30°. The two parameters, D1 and P1, correspond to the length of the recycle delay, and the length of the 90° RF pulse, respectively. The ¹H channel consists of two decoupling pulses which can be power gated. The first pulse, an NOE build up pulse during the recycle delay may be of lower power then the second pulse on during the acquisition which is the true decoupling pulse. This can be useful to avoid RF heating on salty samples or probes where a higher decoupling power can be problematic.



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.

7.2.1 Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **carbon_exp**
EXPNO = **1**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **C13CPD**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.



Create New Dataset - new

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

Dataset

NAME: carbon_exp

EXPNO: 1

Directory: C:\Data

☐ Open in new window

Parameters

☐ Use current parameters

☒ Read parameterset: C13CPD [Select]

☒ Set solvent: DMSO

Additional action

☐ No additional action

☒ Execute getprosol

☐ Keep parameters: P 1, O1, PLW 1 [Change]

Advanced

Number of datasets (receivers): 1

Title

Menthyl Anthranilate in DMSO
C13CPD

[OK] [Cancel] [More Info...] [Help]

- As shown in above screenshot, you may also select to execute getprosol directly from the new command. This will make the step of executing prosol described below obsolete.
- 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.
- In the New Dataset window, click **OK**.
- In the Dataset window, select the **AcquPars** tab.
- Make the following change:
NS = **128**
- On the menu bar, click **Acquire**.



To acquire a spectrum, use the Workflow buttons from left to right.

1D Carbon Experiments

- On the **Sample** button, click the **drop-down** arrow to see more options.
- In the list, select **Eject sample manually (ej)**. The sample lift is turned on.



Wait until the sample lift air is turned on and remove any sample which may have been in the magnet.

- Place the sample plus the spinner on top of the magnet bore.
- On the **Sample** button, click the **drop-down** arrow to see more options.
- In the list, select **Insert sample manually (ij)**.



Wait until the sample is lowered down into the probe and the lift air is turned off. A clicking sound may be heard.

- On the Workflow button bar, click **Lock**.
- In the Solvents table list, select **DMSO** and click **OK**.

Solvents table		X
^ Solvent	Description	
Acetic	acetic acid-d4	
Acetone	acetone-d6	
C6D6	benzene-d6	
CD2Cl2	dichlormethane-d2	
CD3CN	acetonitrile-d3	
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)	
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)	
CDCl3	chloroform-d	
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)	
CH3OH+D2O	HPLC Solvent (Methanol/D2O)	
D2O	deuteriumoxide	
D2O_salt	deuteriumoxide with salt	
Dioxane	dioxane-d8	
DMF	N,N-dimethylformamide-d7	
DMSO	dimethylsulfoxide-d6	
EtOD	ethanol-d6	
H2O+D2O	90%H2O and 10%D2O	
H2O+D2O_salt	90%H2O and 10%D2O with salt	
HDMSO	90%DMSO and 10%DMSO-d6	
Juice	fruit juice	
MeOD	methanol-d4	
Plasma	blood plasma	
Pyr	pyridine-d5	
T_H2O+D2O+Me4NCI	(CD3)4NCI in 90%H2O and 10%D2O, for NMR thermometer	
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMR thermometer	
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR thermometer	
T_MeOD	methanol-d4, for NMR thermometer	
TFE	trifluoroethanol-d3	
THF	tetrahydrofuran-d8	
Tol	toluene-d8	
Urine	urine	
Lock nucleus: 2H		<div>OKCancel</div>

- On the Workflow button bar, click **Tune**.



This performs an **atma** (automatic tuning and matching) and requires a probe equipped with an automatic tuning and matching module. The tuning always starts with the lowest frequency, in this case carbon, and then switches over to tune the higher frequencies, in this case proton. On the **Tune** button, click the **drop-down** arrow to see more options.

- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation on (ro on)**.



Rotation may be turned off for probes such as **BBi**, **TXi**, **TBi** and for small sample probes.

- On the Workflow button bar, click **Shim**.

This executes the command **topshim**. On the **Shim** button click the **drop-down** arrow to see more options.

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

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

7.2.2 Acquisition

- On the Workflow button bar, click **Gain**.

or

- On the **Gain** button, click the **drop-down** arrow to adjust the receiver gain manually.
- On the Workflow button bar, click **Run**.

or

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

7.2.3 Processing

When the acquisition is finished:

- 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 (proc1d)**.
- In the proc1d window, select the options:
 - Exponential Multiply (em)
 - Auto - Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto - Baseline Correction (absn)

1D Carbon Experiments

proc1d

Press 'Execute' to process the current dataset.
Press 'Save' to just change the processing options.
Changed options will be effective when pressing the one-click 'Proc. Spectrum' button.

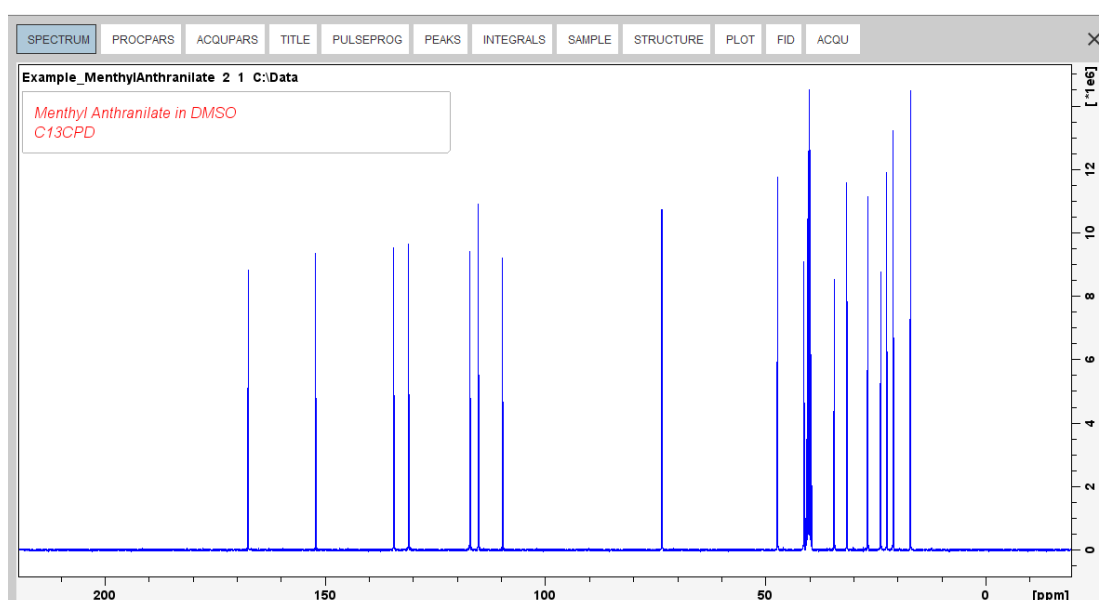
Exponential Multiply (em)	<input checked="" type="checkbox"/>	LB [Hz] =	1
Fourier Transform (ft)	<input checked="" type="checkbox"/>		
Auto - Phasing (apk)	<input checked="" type="checkbox"/>		
Set Spectrum Reference (sref)	<input type="checkbox"/>		
Auto - Baseline Correction (absn)	<input checked="" type="checkbox"/>	Include integration =	no
Plot (autoplot)	<input type="checkbox"/>	LAYOUT =	+1D_X.xwp
Warn if processed data exist	<input checked="" type="checkbox"/>		

Save Execute Cancel

- In the proc1d window, click **Execute**.
- In the proc1d window, click **Save** to save the selected processing settings.

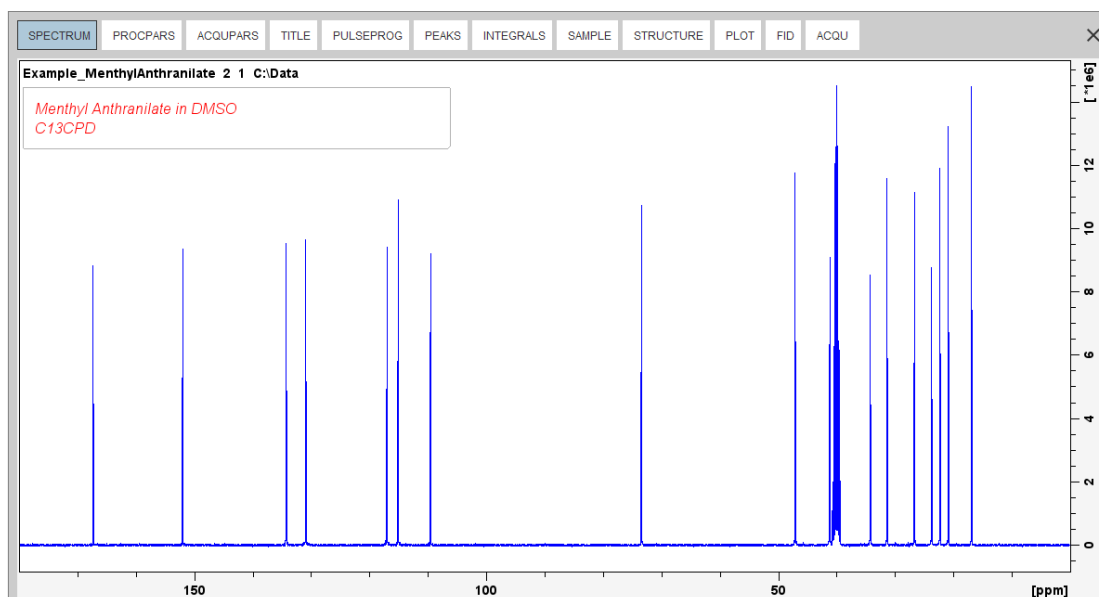


Now all future datasets can be processed with the defined actions with a click on **Proc Spectrum**.



7.2.4 Peak Picking

- Expand the spectrum to include all peaks.



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

or

- On the **Pick Peaks** button, click the **drop-down** arrow to see more options.

This enters the manual peak picking mode.

The **Dataset** tabs are replaced by the **Peak Picking** toolbar.

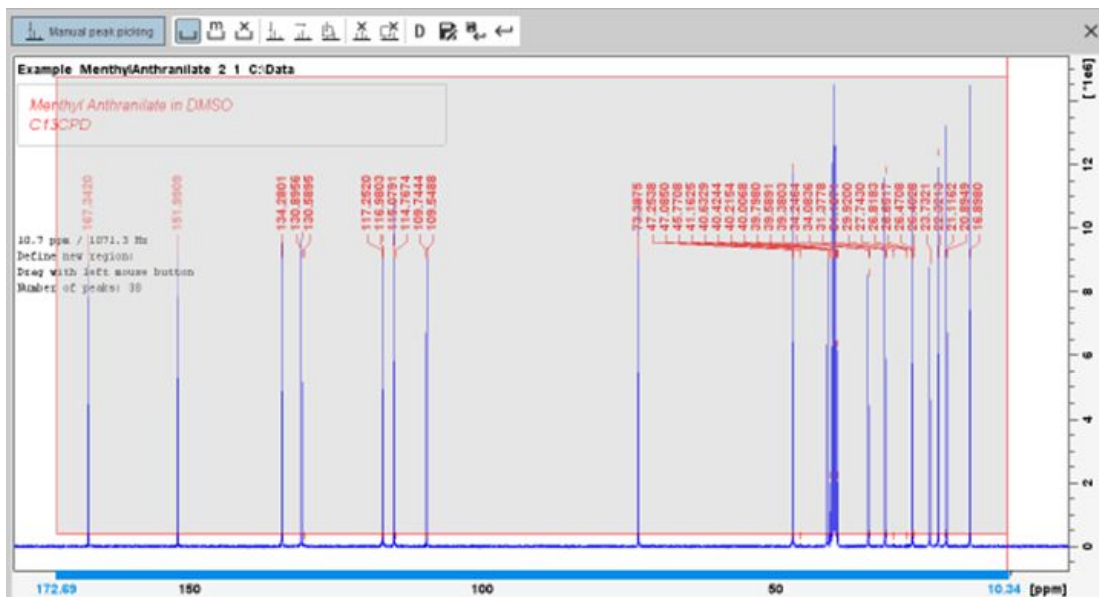



Automatically peak picking is performed with the currently defined peak picking parameters. You may use the peak picking options to modify or redo the peak picking.

- Click left and drag the cursor line from left to the right side of the spectrum, drawing a rectangular box.

Use the **Define new peak picking range** icon to start from scratch. Click left and drag the cursor line from left to the right side of the spectrum, drawing a rectangular box.

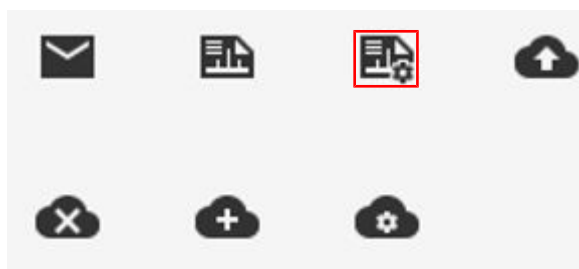
The **Modify existing peak picking range** button allows raising the bottom and/or lowering the top of the peak picking box to exclude noise or solvent.



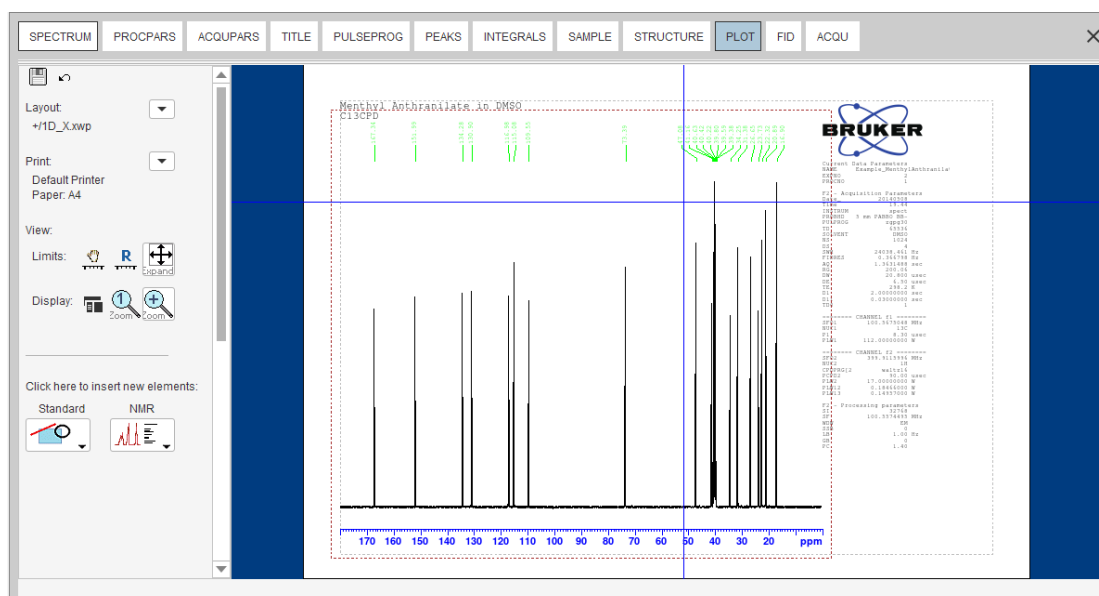
- On the Peak Picking toolbar, click **Return, save region** to store the peak values. 
- To display the peak picking labels, right click in the spectrum window and select **Spectra Display Preferences**. In the Spectrum components enable **Peak labels** and **Peak annotations**. Click **Apply** and **Close**.

7.2.5 Plotting the 1D Carbon Spectrum

- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**.
- On the menu bar, click **Show more Publishing Options**.



- Select **Switch to plot editor** (plot).



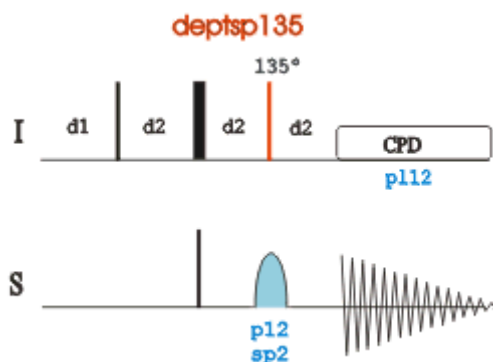
If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select **Print**.

7.3 DEPT-135 Experiment

DEPT (Distortionless Enhancement by Polarization Transfer) is a polarization transfer technique used for the observation of nuclei with a small gyro magnetic ratio, which are J-coupled to ^1H (most commonly ^{13}C). DEPT is a spectral editing sequence, that is, it can be used to generate separate ^{13}C sub spectra for methyl (CH_3), methylene (CH_2), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherence to differentiate between the different types of ^{13}C signals. Quaternary carbons are missing a direct bond proton, and as a result are absent from all DEPT spectra.


This chapter describes the acquisition and processing of a one-dimensional ^{13}C -DEPT135 NMR spectrum. The standard Bruker parameter set C13DEPT135, includes the pulse sequence **deptsp135**, shown in the figure below. The ^{13}C channel consists of the recycling delay, a 90° RF pulse, an editing delay D2 followed by a 180° shaped pulse and the acquisition time during which the signal is recorded. The editing delay D2 is $1/2 \cdot J(\text{XH})$. The ^1H channel consists of three pulses, a 90° , a 180° , followed by a 135° RF pulse and are separated by the editing delay D2. The final 135° ^1H pulse selects the CH_3 , CH_2 or CH signals. The protons are decoupled during the acquisition period.



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.

7.3.1 Experiment Setup

This experiment usually follows a regular ^1H decoupled ^{13}C experiment. The result of a DEPT-135 experiment shows only the protonated carbons with the CH and CH_3 as positive and the CH_2 as negative signals.

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **carbon_exp**
EXPNO = **2**
Directory = e.g. C:\Data
- In the Parameters group  Parameters, click **Read parameterset** and select the experiment **C13DEPT135**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- In the Dataset window, select the **AcquPars** tab.
- Enter:
NS = **64**
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Prosol**.

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

7.3.2 Acquisition

- On the Workflow button bar, click **Gain**.
- or
- To adjust the receiver gain manually, on the **Gain** button click the **drop-down** arrow.
-
- On the Workflow button bar, click **Run**.
- or
- On the **Run** button, click the **drop-down** arrow to see more options.

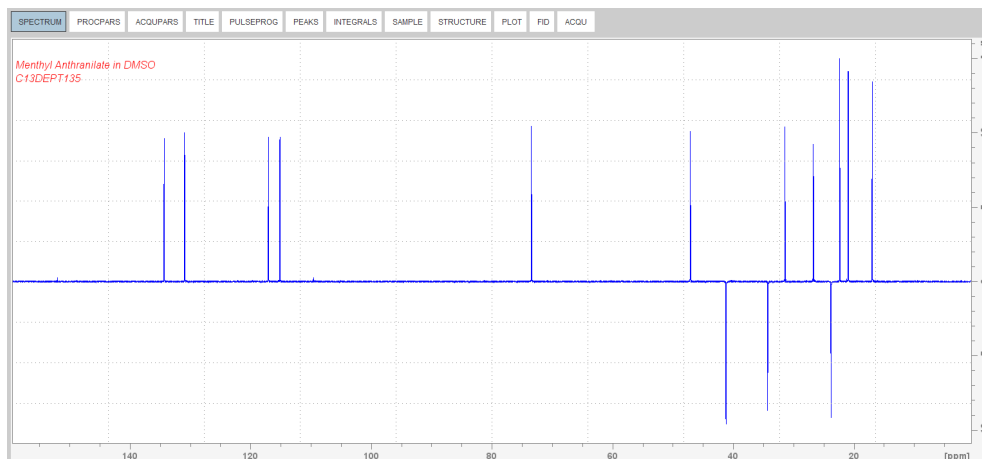
7.3.3 Processing

When the acquisition is finished:

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



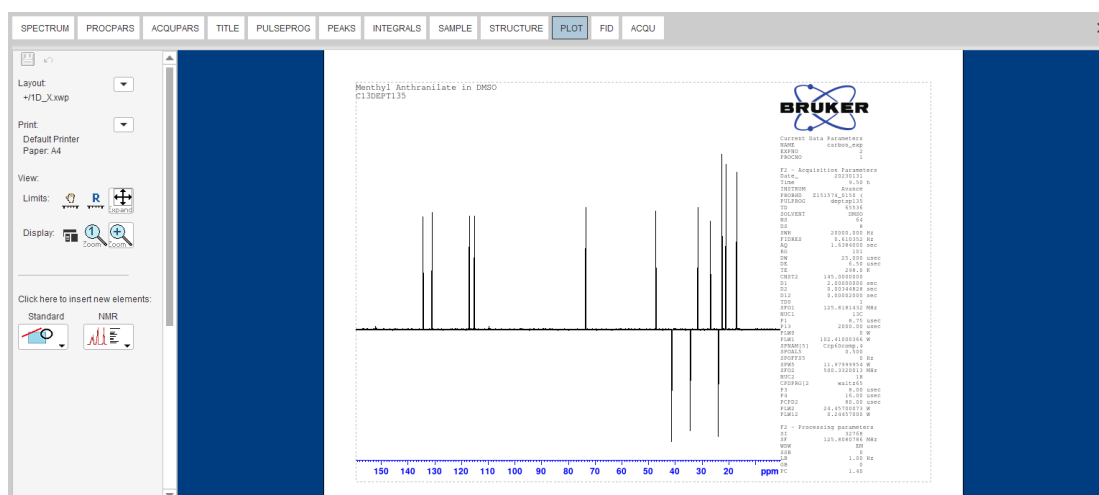
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**. Due to the fact that a DEPT135 spectrum contains negative and positive peaks, there is the possibility of getting phase results that are 180 degrees off from the convention of -CH and -CH₃ peaks phased positively and -CH₂ peaks phased negatively. In this case, click **Adjust Phase** to enter the manual phase routine and reverse the spectrum by clicking on the **180** icon.



7.3.4 Plotting the DEPT-135 Spectrum

- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**.
- On the menu bar, click **Show more Publishing Options**.



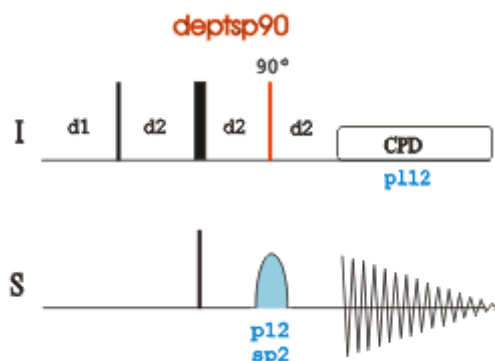


If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select **Print**.

7.4 DEPT-90 Experiment

This section describes the acquisition and processing of a one-dimensional ^{13}C -DEPT90 NMR spectrum. The standard Bruker parameter set C13DEPT90, includes the pulse sequence **dept90**, shown in the next figure. The ^{13}C channel consists of the recycling delay, a 90° RF pulse, an editing delay D2 followed by a 180° shaped pulse and the acquisition time during which the signal is recorded. The editing delay D2 is $1/2 \cdot J(\text{CH})$. The ^1H channel consists of three pulses, a 90° degree, a 180° degree, followed by a 90° RF pulse and are separated by the editing delay D2. The final 90° ^1H pulse selects the CH signals only. The protons are decoupled during the acquisition period. The filtering of the CH_2 and CH_3 resonances is aided by having accurate 90° degree pulse calibrations although small CH_2 and/or CH_3 peaks may *bleed through* because of their J_{CH} value.

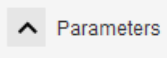


The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

7.4.1 Experiment Setup



The DEPT90 experiment usually follows a regular ^1H decoupled ^{13}C experiment and a DEPT-135 experiment. It is used to assign the methine (CH) signals.

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **carbon_exp**
EXPNO = **3**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **C13DEPT90**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- In the Dataset window, select the **AcquPars** tab.
- Make the following change:
NS = **64**
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Prosol**.

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

7.4.2 Acquisition

- On the Workflow button bar, click **Gain**.
- or
- To adjust the receiver gain manually, on the **Gain** button click the **drop-down** arrow.
 - On the Workflow button bar, click **Run**.
- or
- On the **Run** button, click the **drop-down** arrow to see more options.

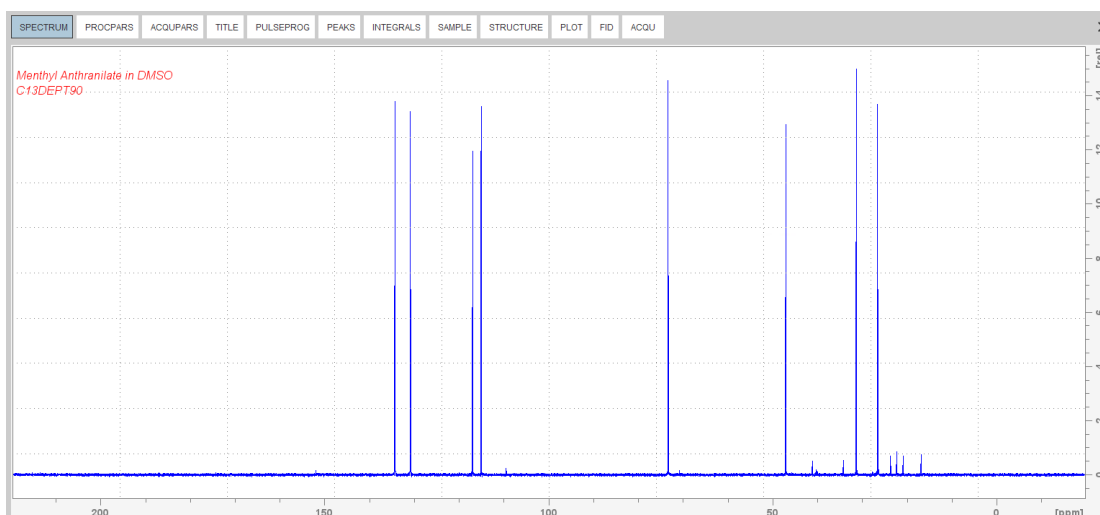
7.4.3 Processing

When the acquisition is finished:

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



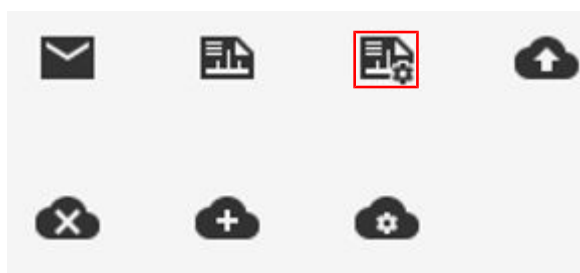
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.



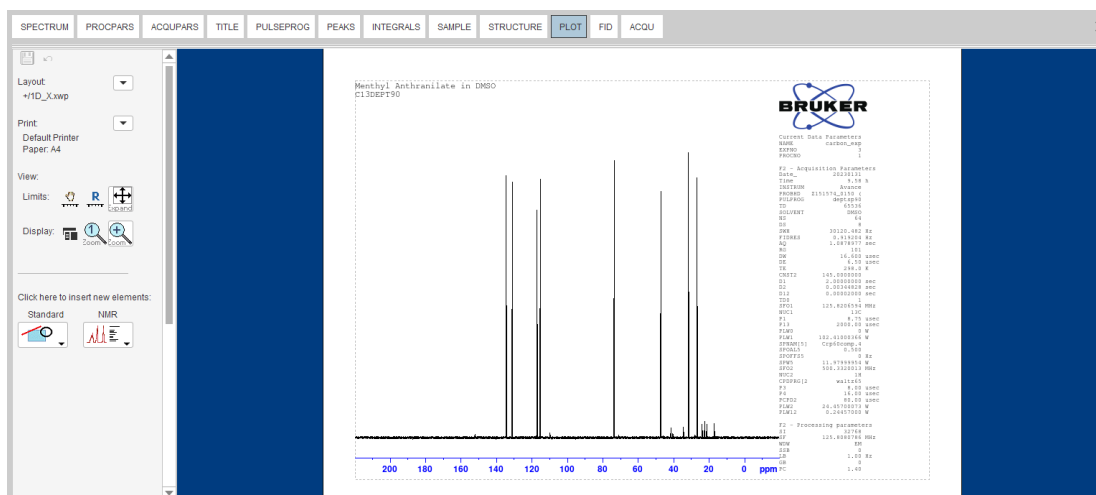
7.4.4 Plotting the DEPT-90 Spectrum

- Expand the spectrum to include all peaks.

- On the toolbar, click **Retain expansion and scale**.
- On the menu bar, click **Show more Publishing Options**.



- Select **Switch to plot editor (plot)**.



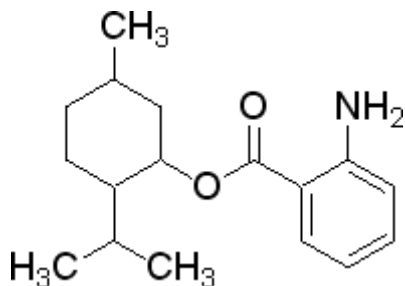
If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select **Print**.

8 2D Heteronuclear Experiments

8.1 Sample

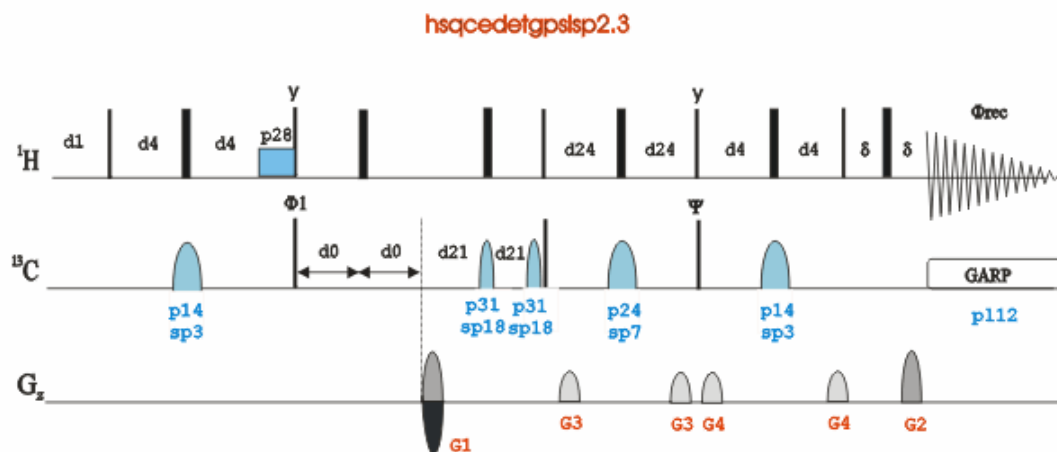
The sample of **30 mg Menthyl Anthranilate in DMSO-d₆** is used for all experiments in this chapter.



8.2 2D Edited HSQC

The **HSQC** (Heteronuclear Single Quantum Coherence) experiment performs an H,C-correlation spectrum via the ¹³C chemical shift evolution of the single-quantum coherence. This method is superior to other heteronuclear experiments in the case of a crowded ¹³C NMR spectrum.

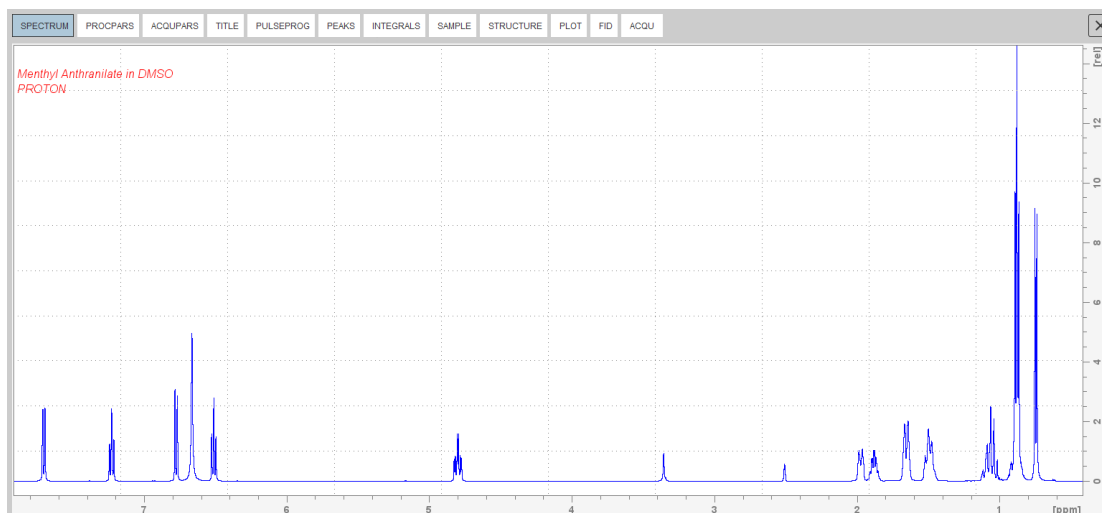
In the sequence shown in the next figure, the signals are not broadened by homonuclear H,H coupling in F1. It is possible to obtain a complete editing of inverse recorded 1D H,X correlation spectra. This kind of multiplicity determination has been achieved by including an editing period within HSQC. In the experiment shown here the standard Bruker parameter set HSQCEDETGPSISP2.3_ADIA is used and the graphical display of the pulse program **hsqcedetgpsisp2.3** is shown in the figure below.



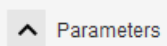
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

8.2.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, [Experiment Setup](#) [▶ 30] through [Processing](#) [▶ 34].



8.2.2 The HSQC Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **hsqc_exp**
EXPNO = **1**
Directory = e.g. **C:\Data**
- In the Parameters group  click **Read parameterset** and select the experiment **HSQCEDETGPSISP2.3_ADIA**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- On the menu bar, click **Acquire**.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation off**.



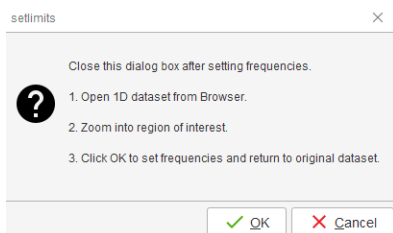
2D experiments should be run non-spinning.

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

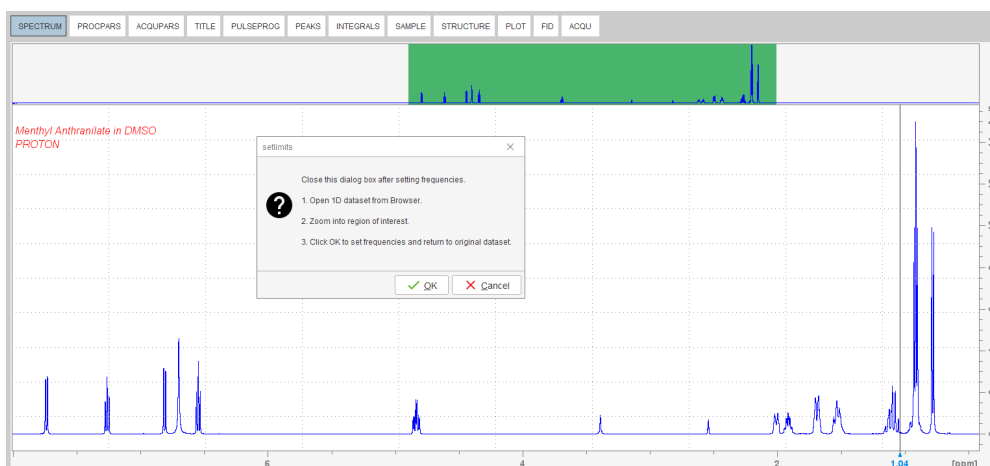
This will load the pulse widths and power levels into the parameter set.

8.2.3 Limit Setting

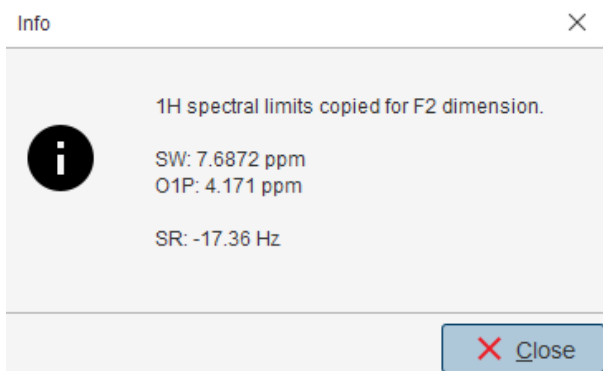
- On the Workflow button bar, click **SetLimits**.



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



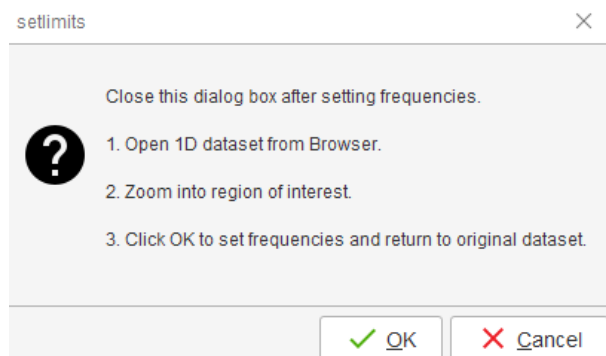
- In the setlimits message window, click **OK** to assign the new limit.
- In the message window, click **Close**.



The display changes back to the 2D dataset.

The parameter set HSQCEDETGPSISP2.3_ADIA has a fixed F1 sweep width of **160 ppm** and it is big enough to cover the protonated resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the **SetLimits** button for a second time. In this case a 1-D **C13DEPT45** or **C13DEPT135** experiment on the same sample has to be observed. Be aware, if the acquisition time is increased do to making the sweep width smaller (e.g. no aromatic peaks), there may be a risk of heating the sample. As an example to set the F1 limit, follow the steps below.

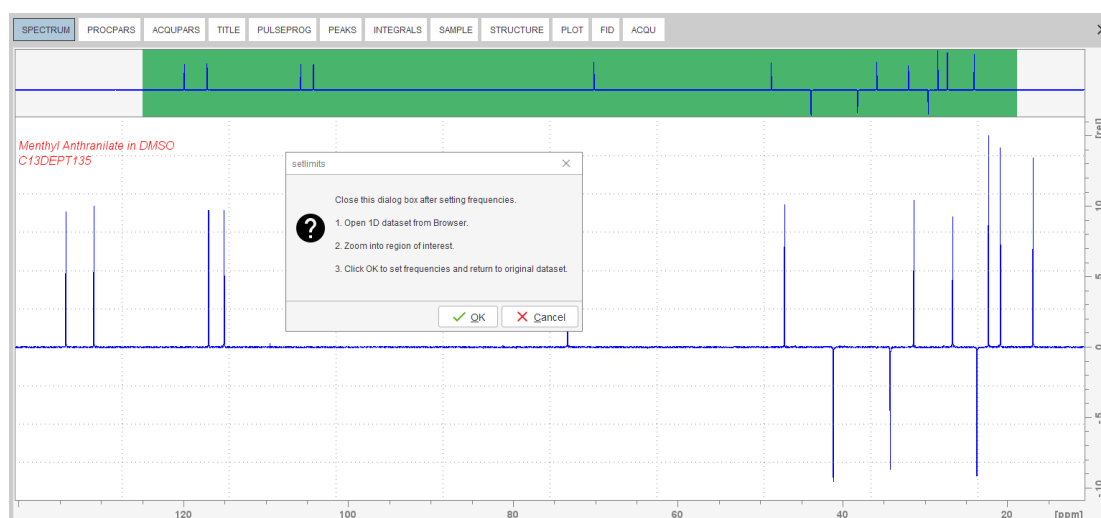
- On the Workflow button bar, click **SetLimits**.



- To open the 1D C13DEPT135 spectrum, right click on the dataset name in the browser window (e.g. **carbon_exp 2**) and select **Display** or drag the 1D C13DEPT135 dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **5 ppm** of baseline on either side of the spectrum.

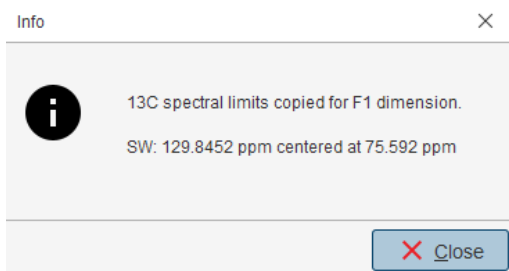


The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the excluded solvent peak will be folded in F1.



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

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



8.2.4 Acquisition

- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click **Run**.

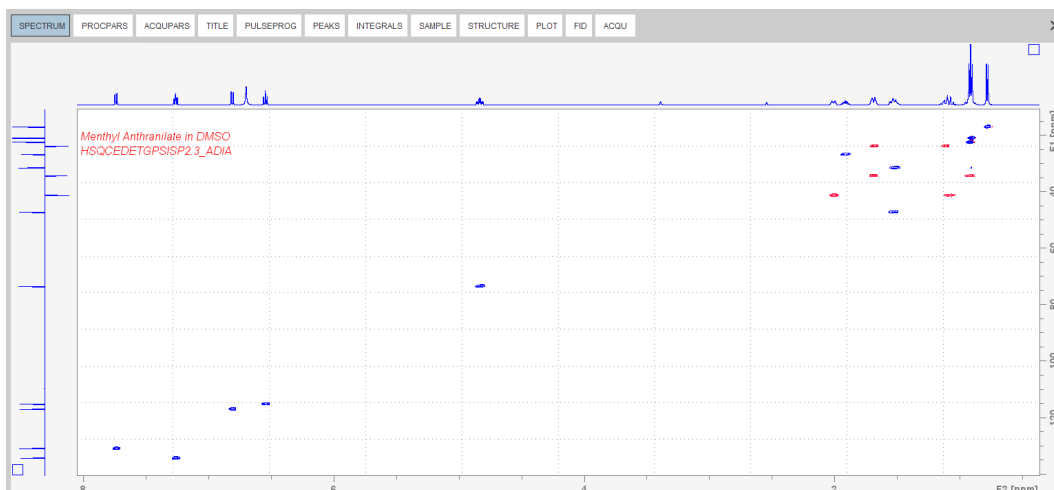
8.2.5 Processing

When the acquisition is finished:






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

This executes a standard processing program **proc2d**.

The **apk2d** option has to be enabled. To enable the **apk2d** option, on the Workflow button bar click the **drop-down** arrow in the Proc. Spectrum button and configure the **Standard Processing (proc2d)** program. By default, the baseline of the F1 projection will be at the bottom, cutting off the negative peaks of the DEPT135 spectrum. Right click inside the F1 projection window and change the setting to display the baseline at the center. The CH and CH₃ groups will phase the same and CH₂ groups will phase oppositely. The convention is to phase CH,CH₃ positive and CH₂ negative. The **apk2d** command may invert this.



8.2.6 Plotting the 2D HSQC Spectrum

- On the toolbar, click **Smaller/larger** and move the mouse up or down to adjust a suitable contour level. 
- Type **.ls** or click **Save the contour levels to disk.** 
- On the menu bar, click **Print active window.**   

This will print the active window with the colors displayed in the TopSpin window showing both the F2 and F1 projections.



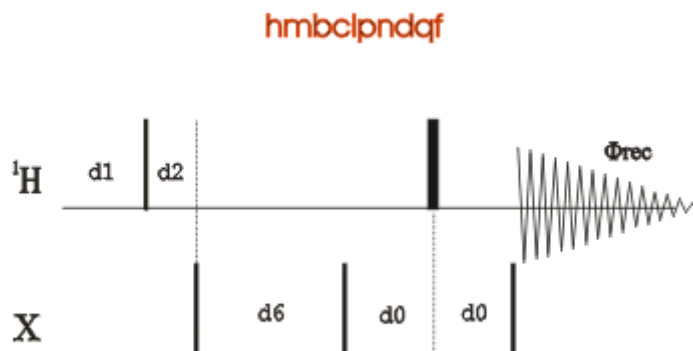
The default layout is designed not to show the F1 projection and the negative peak colors are not assigned. A new layout has to be created to add the F1 projection (in this example, red).

8.3 2D HMBC Experiment

The basic 2D HMBC pulse sequence (see the figure below) is closely related to the HMQC pulse sequence but incorporating the following modifications:

- An optional low-pass J-filter (consisting of a delay- 90° (13C) cluster) can be included after the initial 90° ^1H pulse to minimize direct response.
- The defocusing period is optimized to $1/2 \cdot J(\text{CH})$ (5-10Hz).
- The refocusing period is usually omitted.
- Proton acquisition is performed without X decoupling.

Using this experiment qualitative heteronuclear long-range connectivity, including quaternary carbons or through heteronuclei can be extracted.



The non-gradient 2D HMBC spectrum of Menthyl Anthranilate in DMSO-d6 shows considerable artifacts. Additionally a minimum number of 8 scans is required for the full phase cycling.

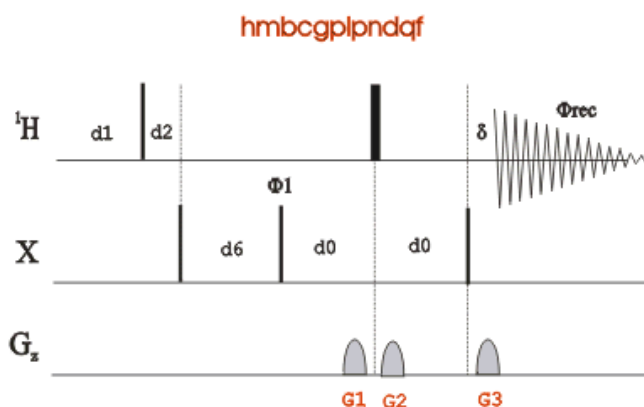
The main advantages of using gradients in high resolution NMR experiments include:

- Coherence selection and frequency-discrimination in the indirect dimension (F1) can be achieved with a single scan per t_1 increment.

2D Heteronuclear Experiments

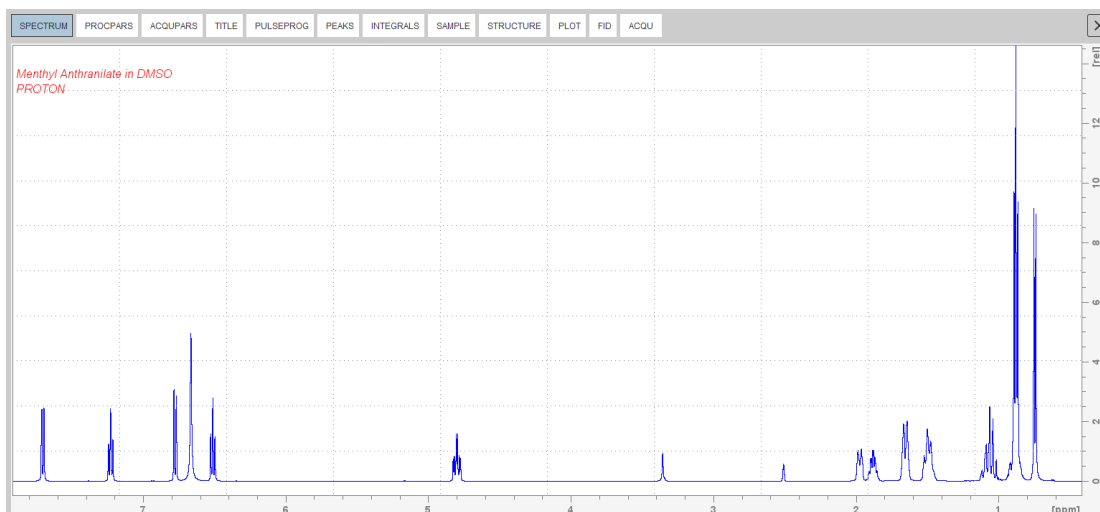
- A reduction in the number of required phase cycle steps for the suppression of undesired coherences.
- An important decrease in the total acquisition times for sufficiently concentrated samples.
- The obtaining of higher quality spectra with an important reduction in T1 noise.
- An efficient suppression of undesired signals such as, for instance, the intense solvent signal in H₂O solution and the ¹H-¹³C (¹H-¹⁴N) magnetization in proton detected heteronuclear experiments at natural abundance. In these inverse experiments, the starting BIRD cluster or spin-lock pulse are no longer needed.
- A much easier data processing and therefore more accurate spectral analysis.
- A decrease of dynamic-range limitation.

The figure below shows the gradient HMBC pulse sequence.

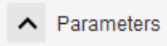


8.3.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, [Experiment Setup](#) [▶ 30] through [Processing](#) [▶ 34].



8.3.2 The HMBC Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New window, enter or select:
NAME = **hmbcgp_exp**
EXPNO = **1**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **HMB CGPND**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- 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.
- In the New Dataset window, click **OK**.
- On the menu bar, click **Acquire**.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation off**.



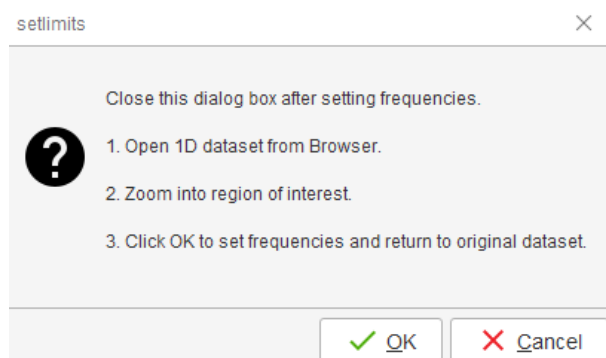
2D experiments should be run non-spinning.

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

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

8.3.3 Limit Setting

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



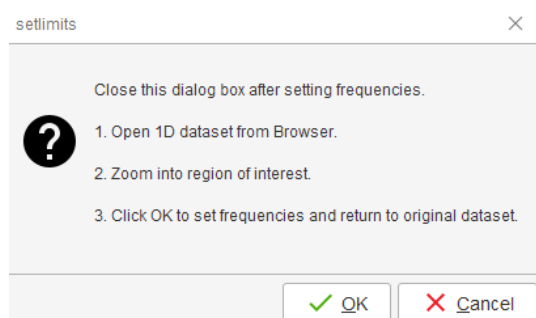
- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. **proton_exp**) and select **Display** or drag the 1D Proton dataset into the spectrum window.

2D Heteronuclear Experiments

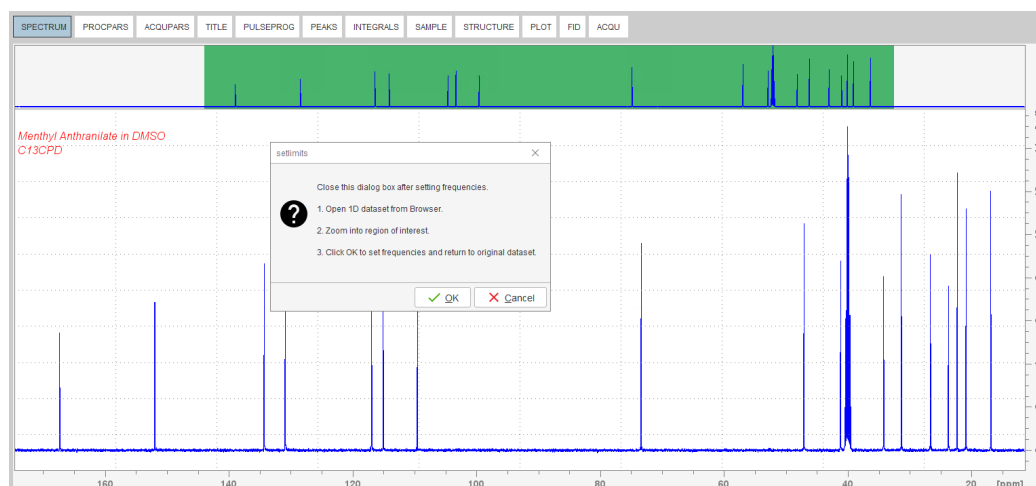
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.
- Click **OK** in the setlimits message window to assign the new limit.

The display changes back to the 2D data set. The parameter set HMBCGP has a fixed F1 sweep width of 222 ppm and it is big enough to cover all Carbon resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done with the **Set_limits** button for a second time. In this case a 1D C13CPD experiment on the same sample has to be selected. As an example to set the F1 limit, follow the steps below.

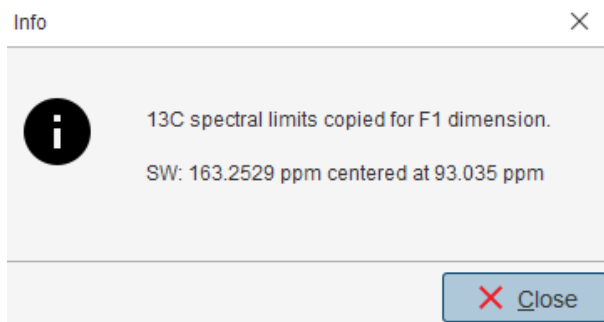
- On the Workflow button bar, click **SetLimits**.



- To open the 1D C13 spectrum, right click on the dataset name in the browser window and select **Display** or drag the 1D C13 dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **5 ppm** of baseline on either side of the spectrum.



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

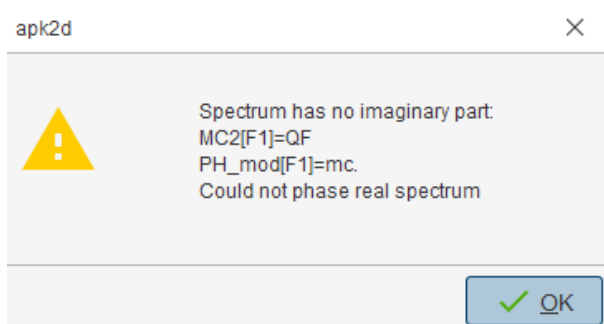


8.3.4 Acquisition

- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click **Run**.

8.3.5 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. The message appears because **apk2d** requires phase sensitive data and the HMBC is a magnitude mode experiment. Nevertheless, the magnitude mode calculation is still performed and the message is just an information message. To disable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.

- In the apk2d message window, click **Close**.



8.3.6 Plotting the 2D HMBC Spectrum

Follow the instructions in chapter [Plotting the 2D HSQC Spectrum](#) [▶ 83].

9 Determination of 90 Degree Pulses

This chapter describes pulse calibration procedures for ^1H and ^{13}C . It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter [1D Proton Experiment \[▶ 30\]](#) and chapter [1D Carbon Experiments \[▶ 64\]](#).



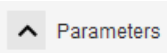
This chapter is intended as a guide for calibrating the 90° pulse of a probe or verifying the values observed using ATP.

9.1 Proton 90 Degree Transmitter Pulse

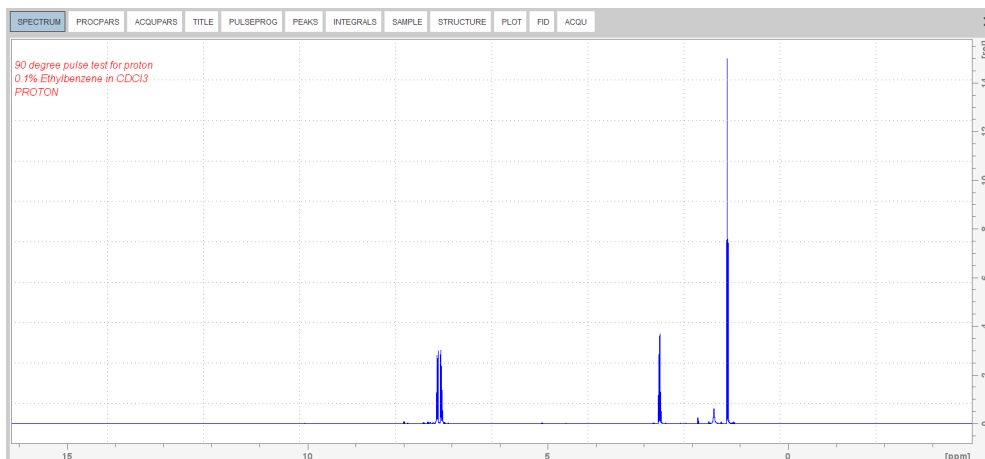
Standard Test Sample:

0.1% Ethylbenzene in CDCl_3

9.1.1 Parameter Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **proton_90**
EXPNO = **1**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **PROTON**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCl_3** .
- 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.
- In the New Dataset window, click **OK**.
- Run a 1D Proton spectrum, following the step [Parameter Setup \[▶ 91\]](#) in chapter 1D Proton Experiment through Processing [Processing \[▶ 34\]](#) described in this manual.

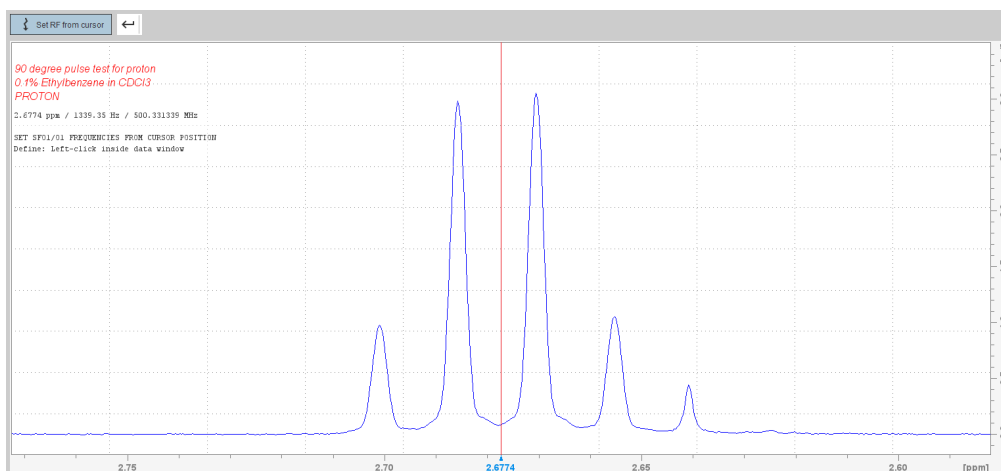
Determination of 90 Degree Pulses



- Expand the peak at **2.7 ppm**.
- On the toolbar, click **Set RF from cursor**.



The Dataset tabs are replaced by the Set RF tool bar.



- Move the cursor line to the center of the multiplet.
- Left click to set the frequency.
- In the O1/O2/O3 window, click **O1**.

O1/O2/O3

Define SFO1/O1 frequencies

SFO1 [MHz] = 500.331340

O1/2/3 [Hz] = 1339.66

O1 O2 O3 Cancel

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

TD = **8192**

SW [Hz] = **1000**

D1 [sec] = **30**

DS = **0**

NS = **1**

- In the Dataset window, select the **ProcPars** tab.

- Enter or select:

SI = **4096**

LB [Hz] = **1**

PH_mod = select **pk**

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



This test should be run non spinning.

9.1.2 Acquisition

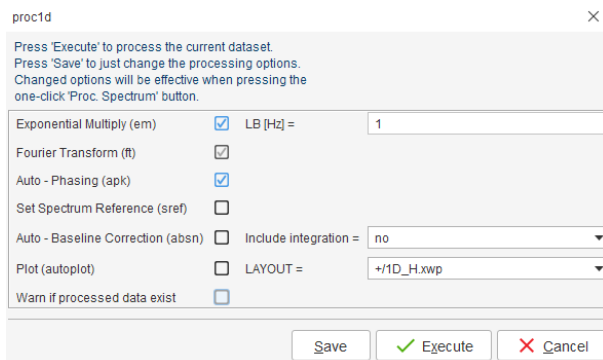
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click **Run**.

9.1.3 Processing

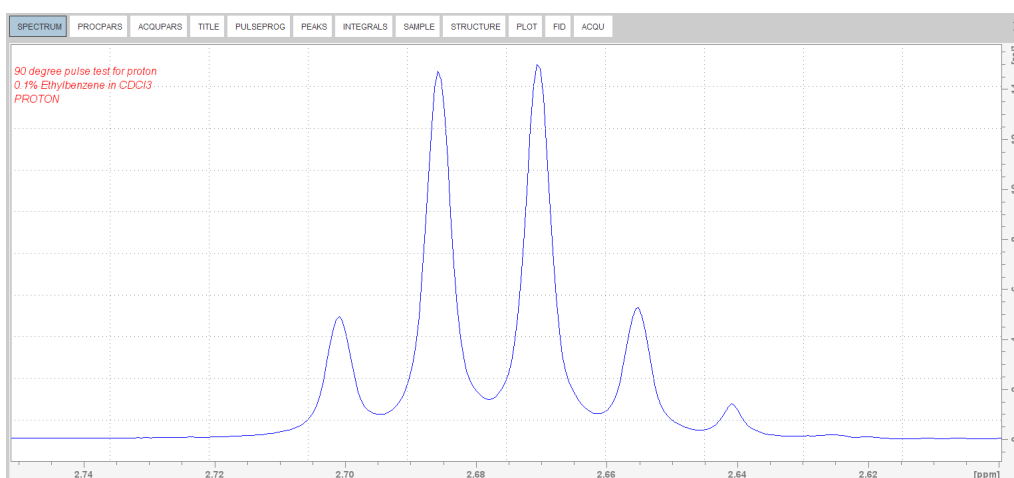
When the acquisition is finished:

- 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 (proc1d)**.
- Enter or select the following options:
 - Exponential Multiplay (em)
 - LB [Hz] = **1**
 - Auto - Phasing (apk)
- Deselect the following options:
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

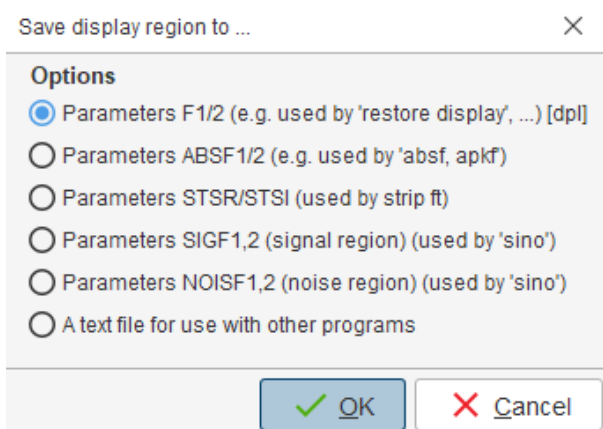
Determination of 90 Degree Pulses



- In the proc1d window, click **Execute**.
- Expand the spectrum from **2.75 ppm** to **2.6 ppm**.



- Right-Click in the spectral window.
- In the list, select **Save Display Region to...**
- In the Save Display Region to... window, select **Parameters F1/2**.
- Click **OK**.

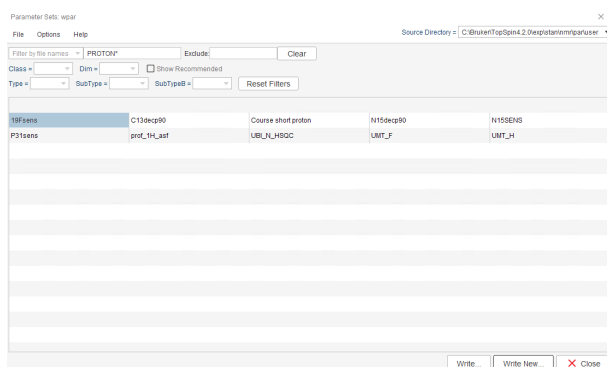


- In the command line, type **wpar** to store the parameter for future use.

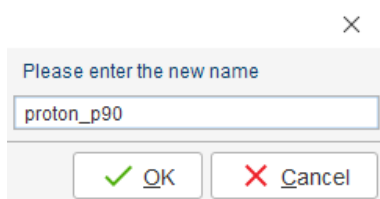
- In the *Parameter Sets: wpar* window, select the user parameter directory.

Source Directory = C:\Bruker\TopSpin4.2.0\exp\stan\nmr\par\user

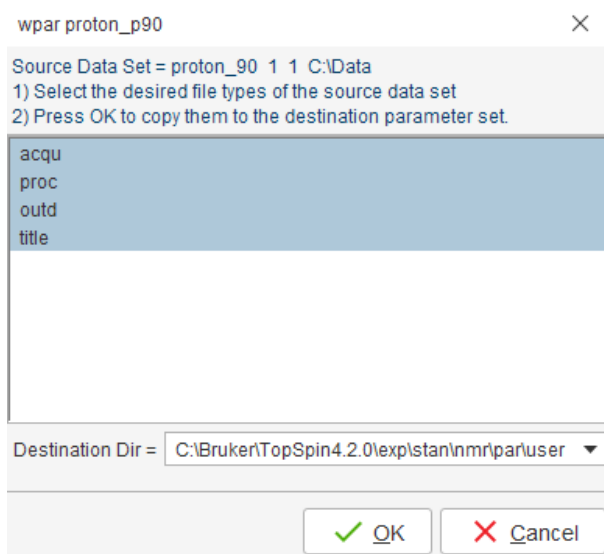
- Click **Write New**.



- In the popup window, type **proton_p90**. Click **OK**.

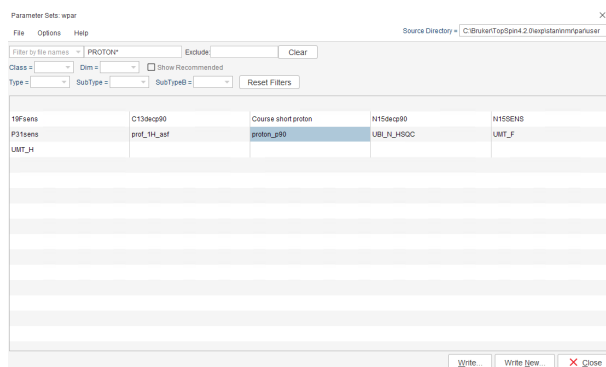


- In the *wpar proton_p90* window, select all parameter options. Click **OK**.



- In the *Parameter Sets: wpar* window, click **Close**.

Determination of 90 Degree Pulses

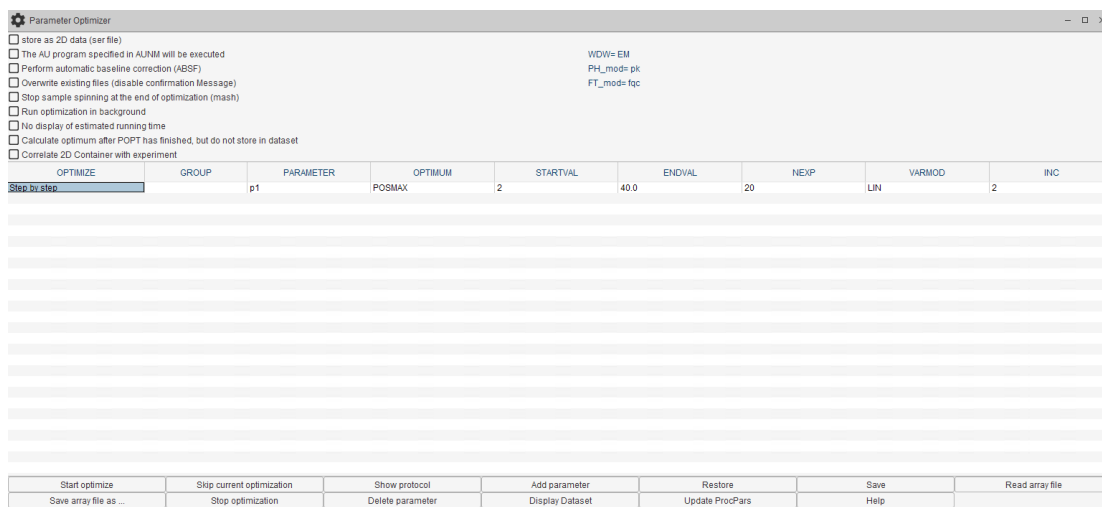


9.1.4 Determine the 90 Degree Pulse

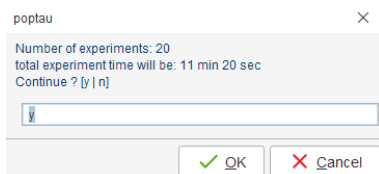
- On the menu bar, click **Acquire**.
- On the **Run** button, click the **drop-down** arrow to see more options.
- In the list, select **Optimize Acquisition Params (popt)**.
- In the proton_90 window, enter:
OPTIMIZE = **Step by step**
PARAMETER = **p1**
OPTIMUM = **POSMAX**
STARTVAL = **2**
NEXP = **20**
VARMOD = **LIN**
INC = **2**
- Click **Save**.

The ENDVAL parameter has been updated.

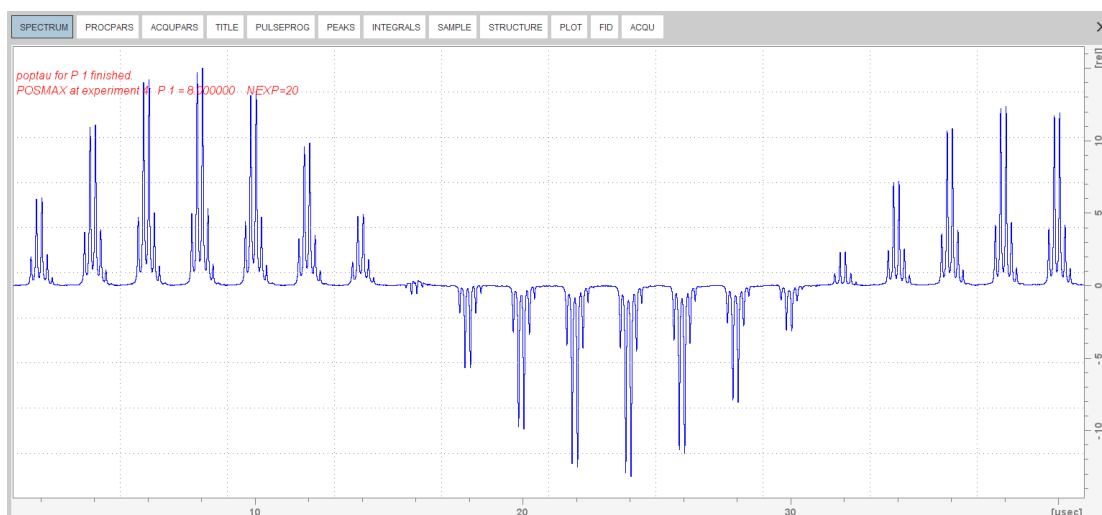
- Click **Start Optimize**.



- In the poptau window, enter **y** and click **OK**.



The parameter optimization starts. The spectrometer acquires and processes 20 spectra with incrementing the parameter p1 from 2 usec by 2 usec to a final value of 40 usec. 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 proton_90/1/999 as shown in the figure below.



The POSMAX value of **p1** is displayed in the title 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:
- Enter **rep 1**. Note, that there is a space between **rep** and **1**.
- Enter **p1**.
- Enter the value which corresponds to a 360° pulse (four times the POSMAX value).
- Enter **zg**.
- Enter **efp**.
- Change **p1** slightly and repeat the last 2 steps, until the quartet undergoes a zero crossing as expected for an exact 360° pulse.



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


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

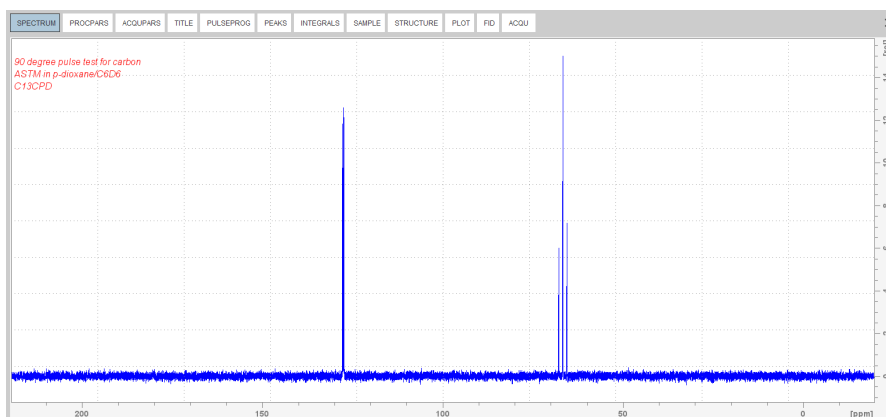
9.2 Carbon 90 Degree Transmitter Pulse

Standard Test Sample:

ASTM (60% C₆D₆ / 40% p-Dioxane)

9.2.1 Parameter Setup

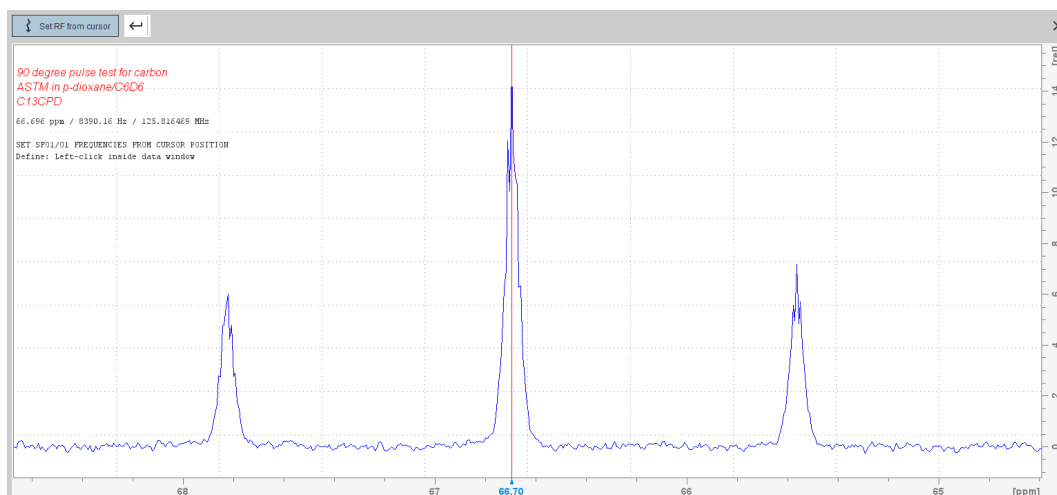
- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **carbon_90**
EXPNO = **1**
Directory = e.g. C:\Data
- In the Parameters group  **Parameters** click **Read parameterset** and select the experiment **C13CPD**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **C₆D₆**.
- 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.
- In the New Dataset window, click **OK**.
- Run a 1D Carbon spectrum, following the instructions in chapter [Experiment Setup \[65\]](#) and chapter [Acquisition \[67\]](#).
- Enter the following acquisition parameters:
 - PULPROG = **zg**
 - DS = **0**
 - NS = **1**
- Continue with chapter [Processing \[67\]](#).



- Expand the peak at **67 ppm**.

- On the toolbar, click **Set RF from cursor**. 

The Dataset tabs are replaced by the Set RF toolbar.




- Move the cursor line into the center peak of the triplet.
- Left click to set the frequency.
- In the O1/O2/O3 window, click **O1**.

O1/O2/O3

Define SFO1/O1 frequencies

SFO1 [MHz] = 125.816469

O1/2/3 [Hz] = 8390.16

O1 O2 O3  Cancel

- In the Dataset window, select the **AcquPars** tab.
- Enter:
 - TD = **8192**
 - D1 [sec] = **60**
- In the Dataset window, select the **ProcPars** tab.
- Enter or select:
 - SI = **4096**
 - LB [Hz] = **3.5**
 - PH_mod = select **pk**
- On the menu bar, click **Acquire**.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select **Turn sample rotation off**.



This test should be run non spinning.

9.2.2 Acquisition

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

9.2.3 Processing

When the acquisition is finished:

- 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 (proc1d)**.
- Select the following options:
 - Exponential Multiplay (em)
 - LB [Hz] = **3.5**
 - Auto - Phasing (apk)
- Deselect the following options:
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

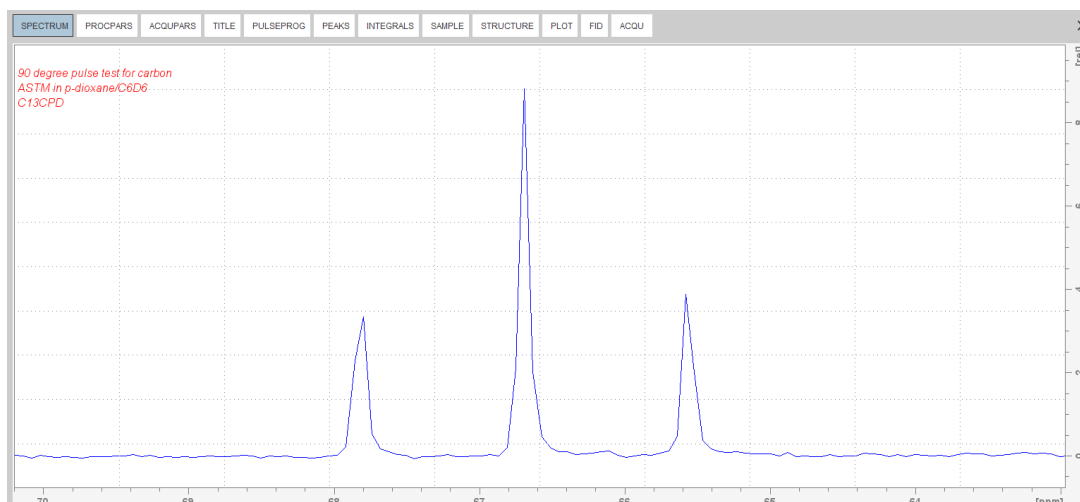
proc1d

Press 'Execute' to process the current dataset.
Press 'Save' to just change the processing options.
Changed options will be effective when pressing the one-click 'Proc. Spectrum' button.

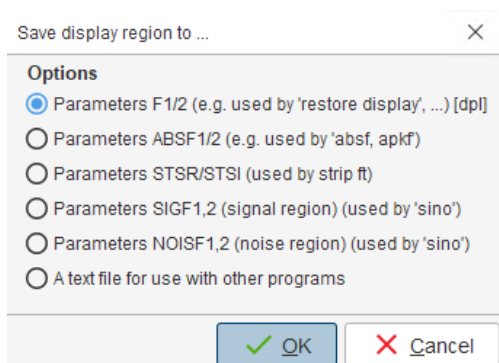
Exponential Multiply (em)	<input checked="" type="checkbox"/>	LB [Hz] =	3.5
Fourier Transform (ft)	<input checked="" type="checkbox"/>		
Auto - Phasing (apk)	<input checked="" type="checkbox"/>		
Set Spectrum Reference (sref)	<input type="checkbox"/>		
Auto - Baseline Correction (absn)	<input type="checkbox"/>	Include integration =	no
Plot (autoplot)	<input type="checkbox"/>	LAYOUT =	+1D_X.xwp
Warn if processed data exist	<input type="checkbox"/>		

Save Execute Cancel

- Click **Execute**.
- Expand the spectrum from **71 ppm** to **63 ppm**.



- In the spectral window click right.
- In the list select **Save Display Region To...**
- In the *Save Display Region To...* window, enable **Parameters F1/2** and click **OK**.



- In the command line, type **wpar** to store the parameter for future use.
- In the *Parameter Sets: wpar* window, select the user source parameter directory.

Source Directory = C:\Bruker\TopSpin4.2.0\exp\stan\nmr\par\user

- Click **Write New**.

Determination of 90 Degree Pulses

Parameter Settings

File Options Help

Source Directory : C:\baker\TopSpin4.2\exp\stanimm\pcr\user

Filter by file names : C13CPG* Exclude Clear

Class : Dem : ☐ Show Recommended

Type : SubType : Reset Filters

13C Peaks	C13cpq0	Course short proton	H15cpq0	H15SE10
F3peaks	prot_H1_asf	protein_gly	URL_H1HSGC	URL_f
URL_H1				

Write Write new Close

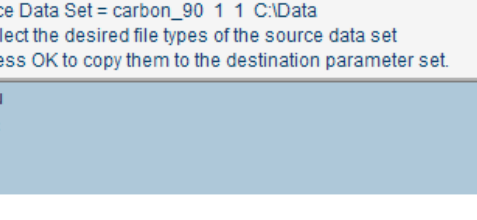
- In the popup window, enter **carbon_p90** and click **OK**.

Please enter the new name

carbon_p90

OK Cancel

- In the *wpar carbon_p90* window, select all parameter options and click **OK**.



wpar carbon_p90

Source Data Set = carbon_90 1 1 C:\Data

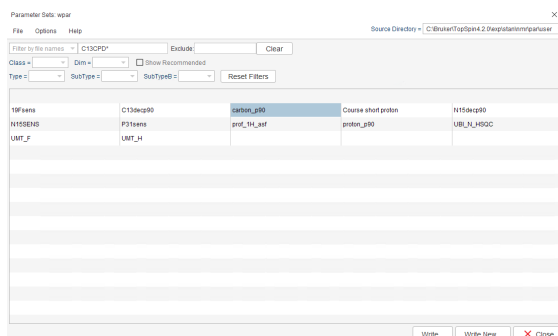
1) Select the desired file types of the source data set
2) Press OK to copy them to the destination parameter set.

acqu
proc
outd
title

Destination Dir = C:\Bruker\TopSpin4.2.0\exp\stan\nmr\par\user

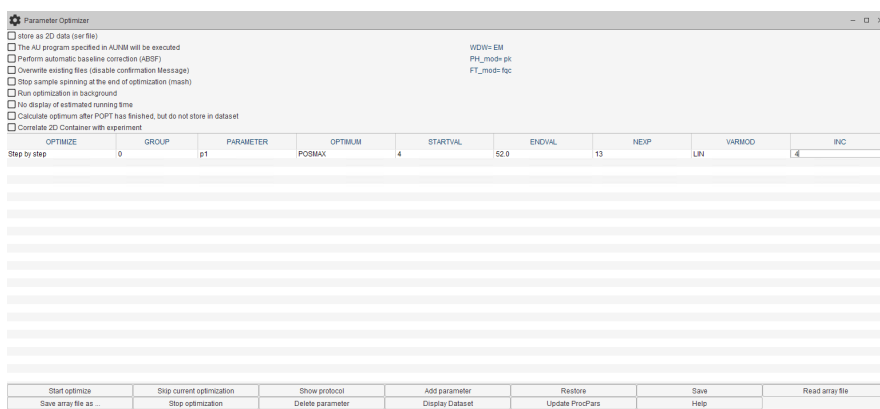
OK Cancel

- In the *Parameter Sets: wpar* window click **Close**.



9.2.4 Determine the 90 Degree Pulse

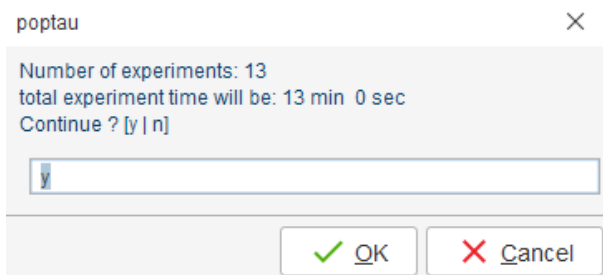
- On the menu bar, click **Acquire**.
- On the **Run** button, click the **drop-down** arrow to see more options.
- In the list, select **Optimize Acquisition Params (popt)**.
- In the carbon_90 window, enter:
 OPTIMIZE = **Step by step**
 PARAMETER = **p1**
 OPTIMUM = **POSMAX**
 STARTVAL = **4**
 NEXP = **13**
 VARMOD = **LIN**
 INC = **4**
- Click **Save**.



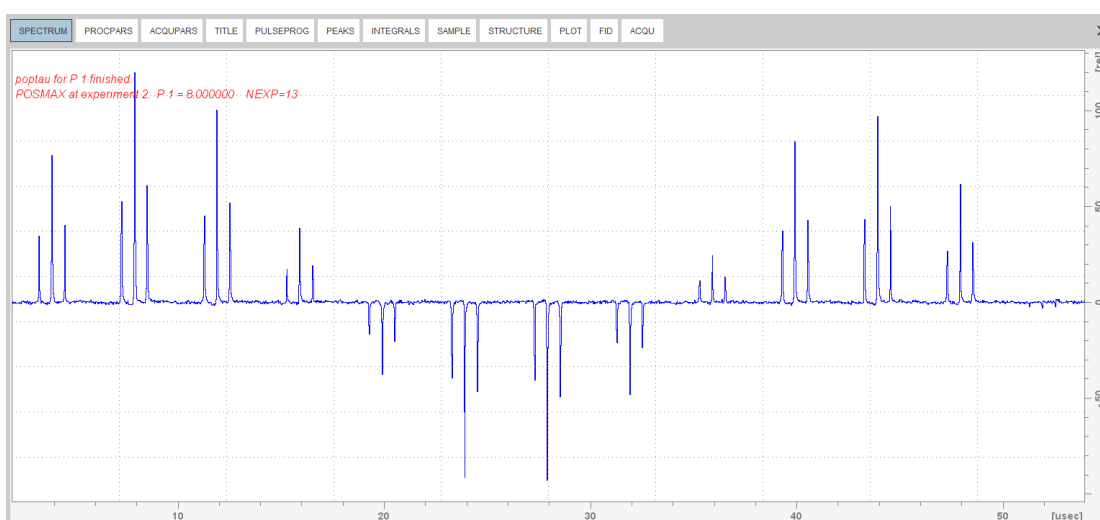
The ENDVAL parameter has been updated.

- Click **Start optimize**.
- In the poptau window, enter **y** and click **OK**.

Determination of 90 Degree Pulses



The parameter optimization starts. The spectrometer acquires and processes 13 spectra with incrementing the parameter p1 from 4 usec by 4 usec to a final value of 40 usec. For each of the 13 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file carbon_90/1/999 as shown in the figure below.



The POSMAX value of **p1** is displayed in the title 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:
- Enter **rep 1**. Note, that there is a space between **rep** and **1**.
- Enter **p1**.
- Enter the value which corresponds to a 360° pulse (four times the POSMAX value).
- Enter **zg**.
- Enter **efp**.
- Change **p1** slightly and repeat the last 2 steps, until the quartet undergoes a zero crossing as expected for an exact 360° pulse.



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

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

10 Sensitivity Tests

This chapter describes the sensitivity test procedures for ^1H and ^{13}C . It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter [1D Proton Experiment \[▶ 30\]](#) and chapter [1D Carbon Experiments \[▶ 64\]](#) in this manual.

Also the 90° pulses have to be properly calibrated, see chapter [Determination of 90 Degree Pulses \[▶ 89\]](#).




This chapter is intended as a guide for running the ^1H and ^{13}C Signal to Noise test on a probe or verifying the values observed using ATP.

10.1 ^1H Sensitivity Test

Standard Test Sample:

0.1% Ethylbenzene in CDCl_3

10.2 Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **proton_sensitivity**
EXPNO = **1**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **PROSENS**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCl_3** .
- 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.
- 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 **CDCl_3** solvent.
- To tune the probe, click **Tune**.
- Click **Spin** and select **Turn sample rotation on**.



The Proton sensitivity test should be run with the sample spinning. Rotation may be turned off for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

- On the Workflow button bar, click **Shim**.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

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

10.3 Acquisition

To adjust the receiver gain:

- On the Workflow button bar, click **Gain**.



The relaxation time **D1** is by default in this parameter set **60 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

- On the Workflow button bar, click **Run**.

10.4 Processing

When the acquisition has finished:

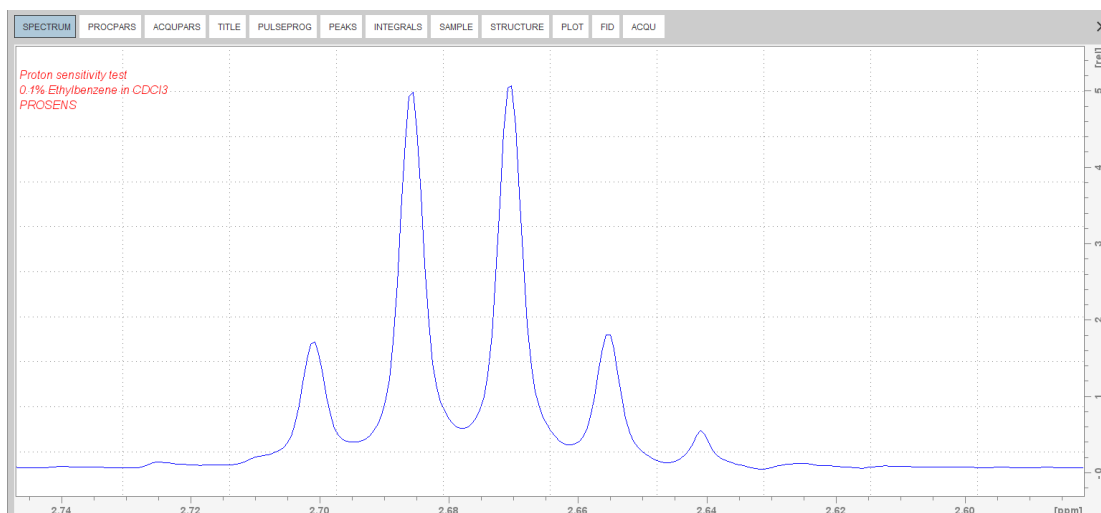
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.



Proc. Spectrum 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)**.

10.5 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the intensity of the **quartet** lines between **2 ppm** and **3 ppm**. It is calculated by AU-program sinocal over a range of **2 ppm** between **2.8 ppm** and **7 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting between the two central lines of the methyl quartet should go lower than 15% of the signal height (with LB=1Hz), see the figure below.



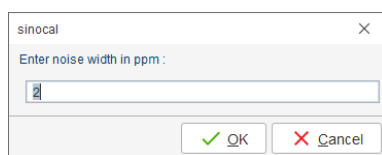
- At the command prompt, type **sinocal**.
- In the sinocal window, enter **3** for the left limit of the signal range. Click **OK**.

- In the sinocal window, enter **2** for the right limit of the signal range. Click **OK**.

- In the sinocal window, enter **7** for the left limit of the noise range. Click **OK**.

- In the sinocal window, enter **2.8** for the right limit of the noise range. Click **OK**.

- In the sinocal window, enter **2** for the noise width. Click **OK**.




10.6 ^{13}C Sensitivity Test with ^1H Decoupling

Standard Test Sample:

10% Ethylbenzene in CDCl_3

10.6.1 Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **Carbon_sensitivity_ETB**
EXPNO = **1**
Directory = e.g. *C:\Data*
- In the Parameters group  click **Read parameterset** and select the experiment **C13SENS**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCl_3** .
- 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.
- 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 **CDCl₃** solvent.
- To tune the probe, click **Tune**.
- Click **Spin** and select **Turn sample rotation on**.



The Carbon sensitivity test should be run with the sample spinning. Rotation may be turned off for some probes. Please refer to the probe specification sheet for more details.

- On the Workflow button bar, click **Shim**.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

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

10.6.2 Acquisition

To adjust the receiver gain:

- On the Workflow button bar, click **Gain**.



The relaxation time **D1** is by default in this parameter set **300 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

- On the Workflow button bar, click **Run**.

10.6.3 Processing

When the acquisition has finished:

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

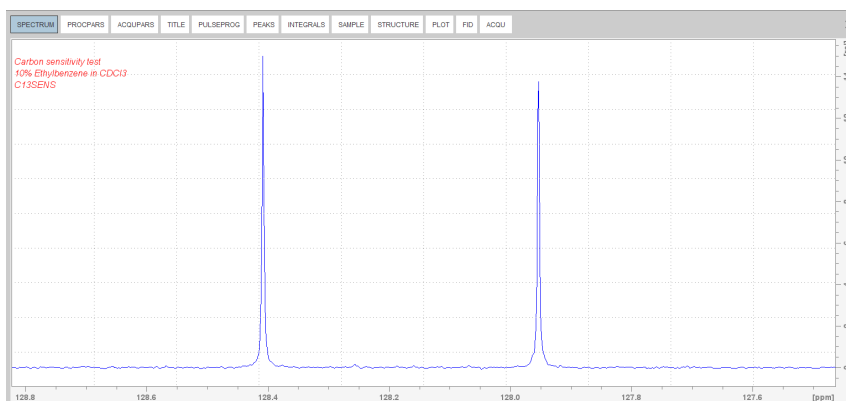


Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.

10.6.4 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the highest peak of the **aromatic** part between **127 ppm** and **129 ppm**, see the figure below. It is calculated by AU-program sinocal over a range of **40 ppm** between **30 ppm** and **125 ppm**. The s/n ratio is strongly dependant on good resolution and line shape.

Sensitivity Tests



- At the command prompt, type **sinocal**.
- In the sinocal window, enter **128** for the left limit of the signal range. Click **OK**.

sinocal

Enter left limit of signal range in ppm :

128

OK Cancel

- In the sinocal window, enter **127** for the right limit of the signal range. Click **OK**.

sinocal

Enter right limit of signal range in ppm :

127

OK Cancel

- In the sinocal window, enter **125** for the left limit of the noise range. Click **OK**.

sinocal

Enter left limit of noise range in ppm :

125

OK Cancel

- In the sinocal window, enter **30** for the right limit of the noise range. Click **OK**.

sinocal

Enter right limit of noise range in ppm :

30

OK Cancel

- In the sinocal window, enter **40** for the noise width. Click **OK**.

sinocal

Enter noise width in ppm :

40

OK Cancel




10.7 ¹³C Sensitivity Test without ¹H Decoupling

Standard Test Sample:

ASTM (60% C₆D₆ / 40% p-Dioxane)

10.7.1 Experiment Setup

- On the menu bar, click **Acquire | Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:
NAME = **Carbon_sensitivity_ASTM**
EXPNO = 1
Directory = e.g. C:\Data
- In the Parameters group  click **Read parameterset** and select the experiment **C13SENS**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **C6D6**.
- 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.
- 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 **C6D6** solvent.
- To tune the probe, click **Tune**.
- Click **Spin** and select **Turn sample rotation on**.



The Carbon sensitivity test should be run with the sample spinning. Rotation may be turned off for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

- On the Workflow button bar, click **Shim**.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

- On the Workflow button bar, click **Prosol**.
- In the Dataset window, select the **AcquPars** tab.
- Make the following changes:
PULPROG = **zg**
TD = **65536**
SW [ppm] = **200**
O1p = **100**
- In the Dataset window, select the **ProcPars** tab.
- Make the following changes:
SI = **32768**
LB [Hz] = **3.5**
- In the Dataset window, select the **Spectrum** tab.

10.7.2 Acquisition

To adjust the receiver gain:

- On the Workflow button bar, click **Gain**.



The relaxation time **D1** is by default in this parameter set **300 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

- On the Workflow button bar, click **Run**.

10.7.3 Processing

When the acquisition has finished:

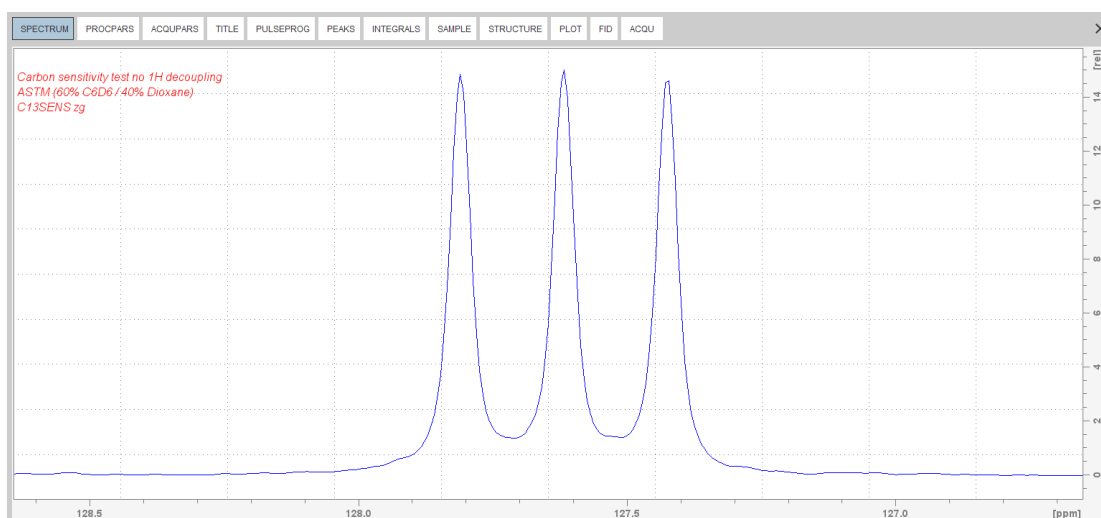
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.



Proc. Spectrum 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)**.

10.7.4 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the triplet of the **deuterated benzene** between **127 ppm** and **129 ppm**. It is calculated by AU-program **sinocal** over a range of **40 ppm** between **70 ppm** and **125 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting of the 1:1:1 triplet should go lower than 9% of the signal height for **5mm** probes and 10% for **10mm** probes, see the figure below.



- At the command prompt, type **sinocal**.
- In the **sinocal** window, enter **129** for the left limit of the signal range. Click **OK**.

sinocal

Enter left limit of signal range in ppm :

129

OK Cancel

- In the **sinocal** window, enter **127** for the right limit of the signal range. Click **OK**.

sinocal

Enter right limit of signal range in ppm :

127

OK Cancel

- In the **sinocal** window, enter **125** for the left limit of the noise range. Click **OK**.

Sensitivity Tests

sinocal

Enter left limit of noise range in ppm :

125

OK Cancel

- In the sinocal window, enter **70** for the right limit of the noise range. Click **OK**.

sinocal

Enter right limit of noise range in ppm :

70

OK Cancel

- In the sinocal window, enter **40** for the noise width. Click **OK**.

sinocal

Enter noise width in ppm :

40

OK Cancel

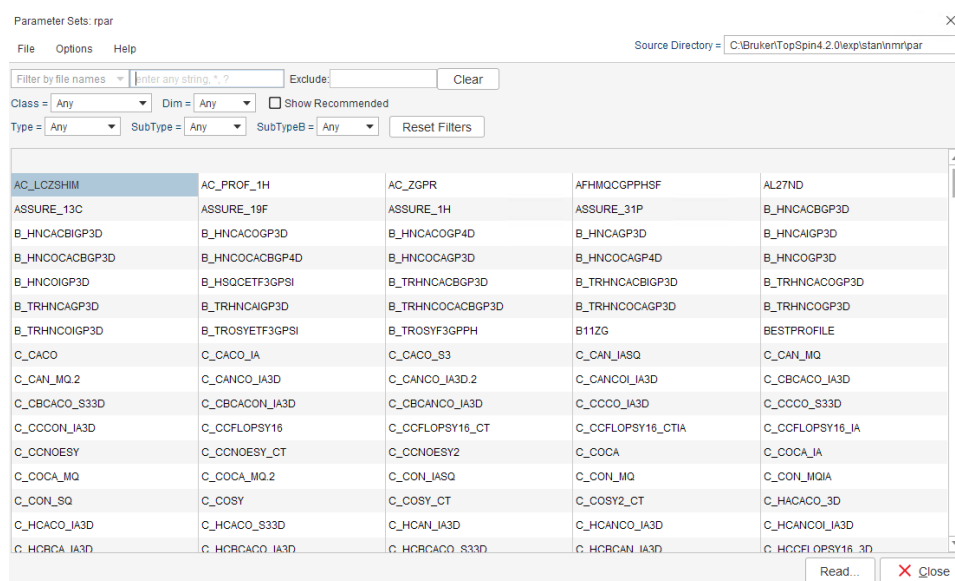


11 Additional Information

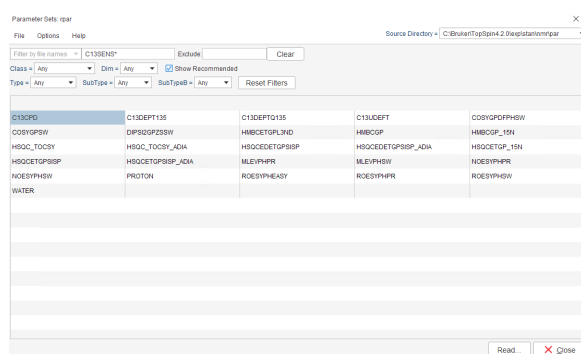
A list of all standard parameter sets is available with the command **rpar**, see figure below. The list varies according to the number of channels of your spectrometer.

11.1 Standard Parameter Set List

- On the command line, enter **rpar**.



- Click **Show Recommended** to reduce the list to the most frequently used parameter sets as recommended by Bruker.

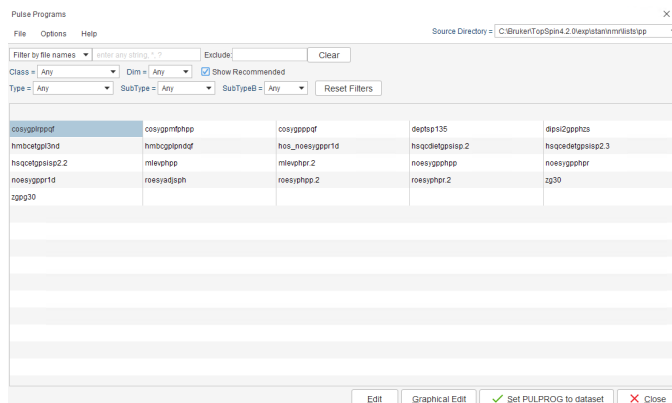


- Ensure that the source directory is
`<Topspin>\exp\stan\nmr\par`
 and not `<Topspin>\exp\stan\nmr\par\user`

11.2 Pulse Program Information

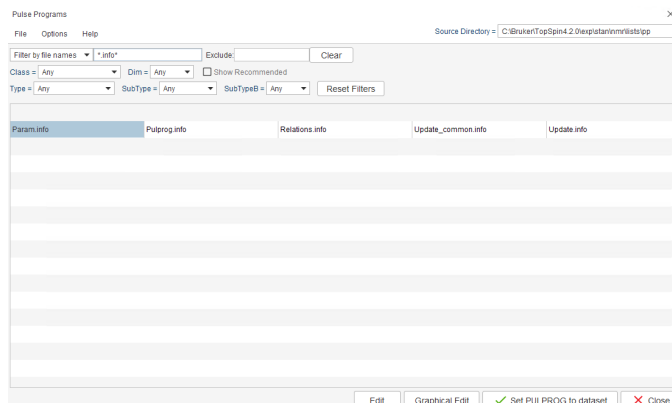
A list of all standard pulse programs is available with the command **edpul**, see figure below.

- The list can be reduced by specifying text fragments as argument for the **edpul** command, for example **edpul hmbc***.
- Click **Show Recommended** to reduce the list to the most frequently used pulse programs as recommended by Bruker.



Find further information about pulse programs in the following files which can be accessed with the command **edpul *.info** :

- *Pulprog.info* (Topspin two letter code naming conventions)
- *Param.info* (Standard parameter assignments consistent with prosol & relations files)
- *Relations.info* (Prosol to parameter translation files)
- *Update.info* (Additions, changes, corrections to sequences)



11.3 Standard Test Samples

For a list of all commercially available standard reference samples go to the Bruker webshop <https://bruker-labscape.store/>

12 Contact

Manufacturer

Bruker BioSpin GmbH
Rudolf-Plank-Str. 23
D-76275 Ettlingen
Germany

Contact form: <https://www.bruker.com/en/services/service/magnetic-resonance.html>

Website: <http://www.bruker.com>

WEEE DE43181702

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/en/services/support.html>



Bruker Corporation

nmr-support@bruker.de
www.bruker.com

