TopSpin

Basic NMR Experiments

User Manual

Version 004

Innovation with Integrity

NMR

Document Number: 10000036683

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1 About This Manual

This manual enables safe and efficient handling of the device.

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

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

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

1.1 Policy Statement

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

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

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

1.2 Symbols and Conventions

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

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

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

⚠ DANGER

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

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

⚠ WARNING

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

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

⚠ CAUTION

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

This is the consequence of not following the warning.

1. This is the safety condition.

▶ This is the safety instruction.

NOTICE

NOTICE indicates a property damage message.

This is the consequence of not following the notice.

1. This is a safety condition.

▶ This is a safety instruction.

SAFETY INSTRUCTIONS

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

This is the consequence of not following the safety instructions.

1. This is a safety condition.

▶ This is a safety instruction.

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

1.3 Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All 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	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST

Program Code
Pulse and AU Program Names
Macros
Functions
Arguments
Variables
	Courier	`go=2`
		`au_zgte`
		`edmac`
		`CalcExpTime()`
		`XAU(prog, arg)`
		`disk2, user2`

AU Macro	Courier in Capital Letters	`REX` `PNO`

Table 1.1: Font and Format Conventions

About This Manual

2 Introduction

2.1 Limitation of Liability

All specifications and instructions in this manual have been compiled taking account of 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.
- Technical modifications.
- Use of unauthorized 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.

2.2 Copyright

2.3 Warranty Terms

The warranty terms are included in the manufacturer's Terms and Conditions.

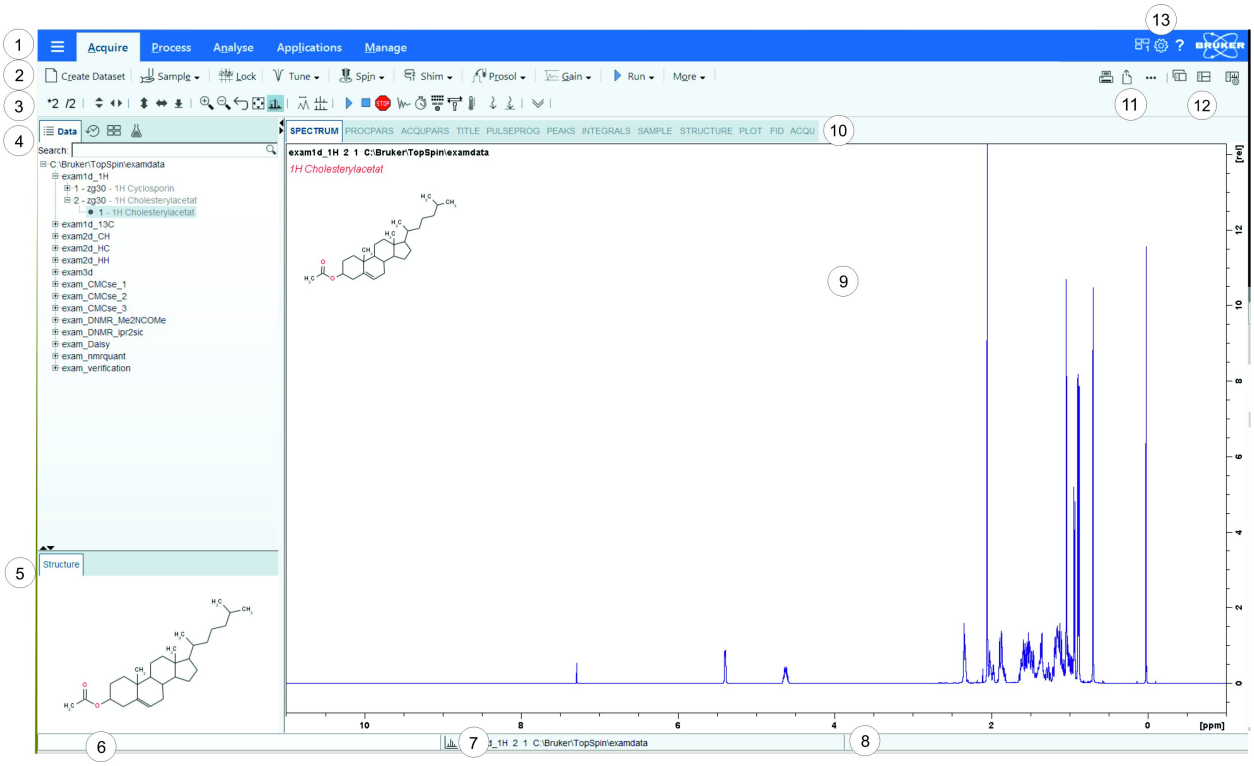
2.4 Customer Service

Our customer service division is available to provide technical information. See the chapter *Contact [▷ 127]* for contact information.

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.

Introduction

3 The TopSpin Interface



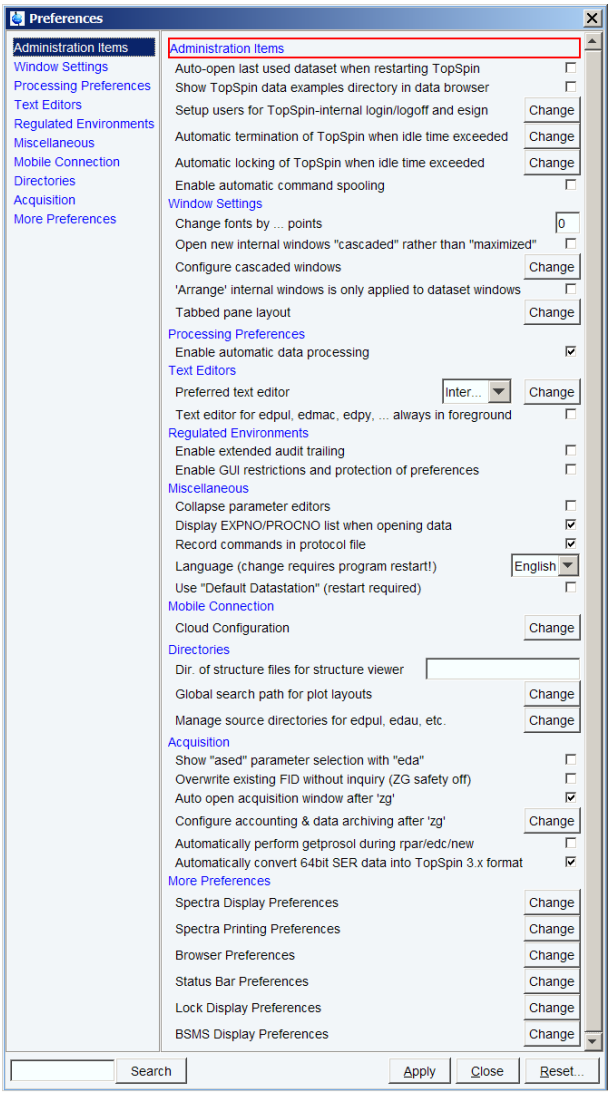
1	Menu bar	8	Status display bar
2	Workflow button bar	9	Dataset window
3	Tool bar	10	Dataset window tabs
4	Browser and search window	11	Print, export and publish
5	Structure window	12	Viewing options
6	Command line	13	Window switcher, setup preferences and help
7	Current dataset bar		

Setup Preferences 

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

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

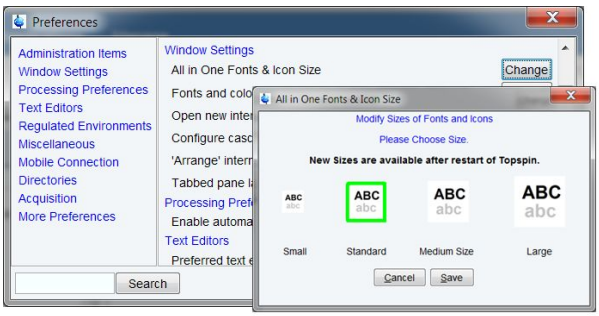
A dialog box will appear with, at the left side, the categories that can be tailored. Click the category you want to view or change. It will become highlighted and the corresponding objects will be displayed at the right part of the dialog box.



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**.



Help/About TopSpin/Version and Licence Information 

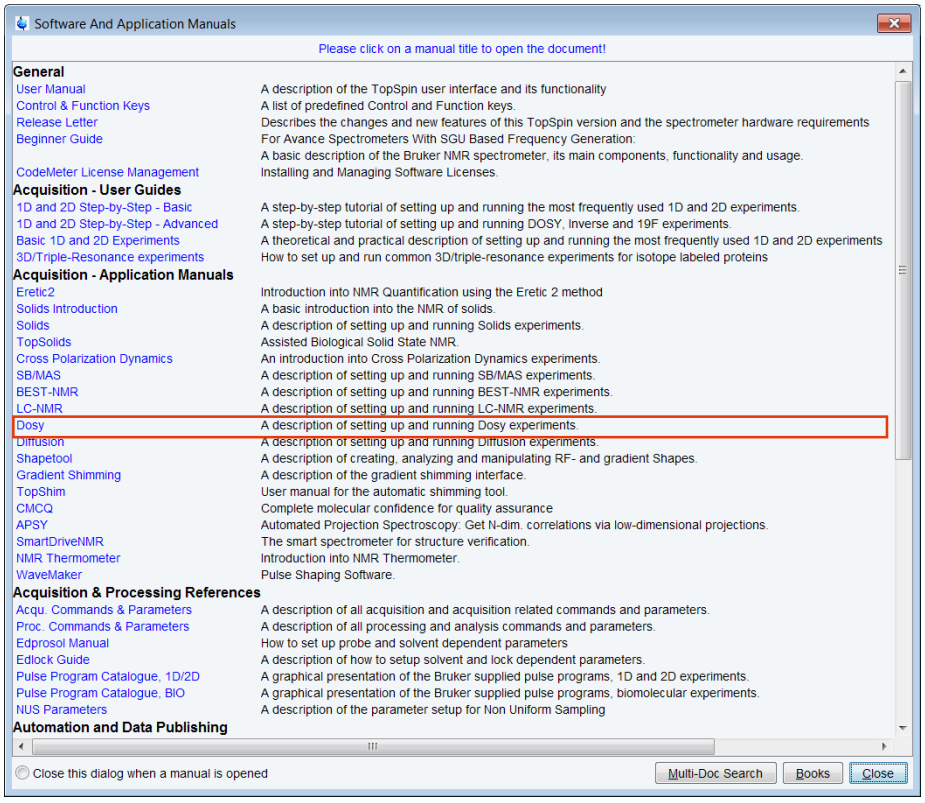
This button gives information about the TopSpin documentation, software version and licence.

A hint in the manuals like

- Click **Help | Manuals | Acquisition Application Manuals | Dosy**

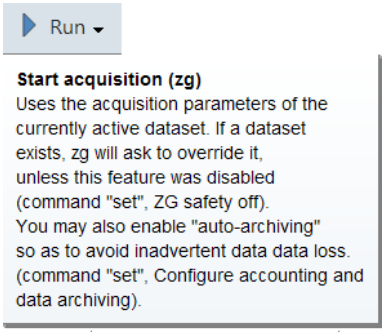
will open the *DOSY_and_Diffusion.pdf* document.

Alternatively enter **help** or **docs** on the command line.



Tooltips

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

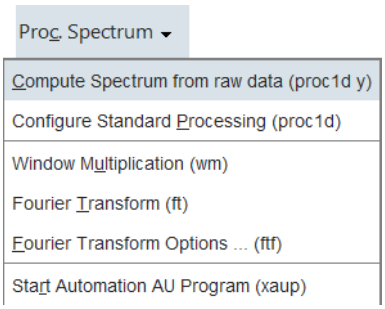


Workflow Button Bar

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

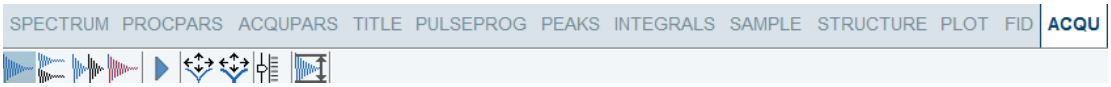
Clicking one of the menu buttons opens the corresponding workflow. It contains an horizontal feature list which stays open and provides all functionality for this workflow with one mouse-click.

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

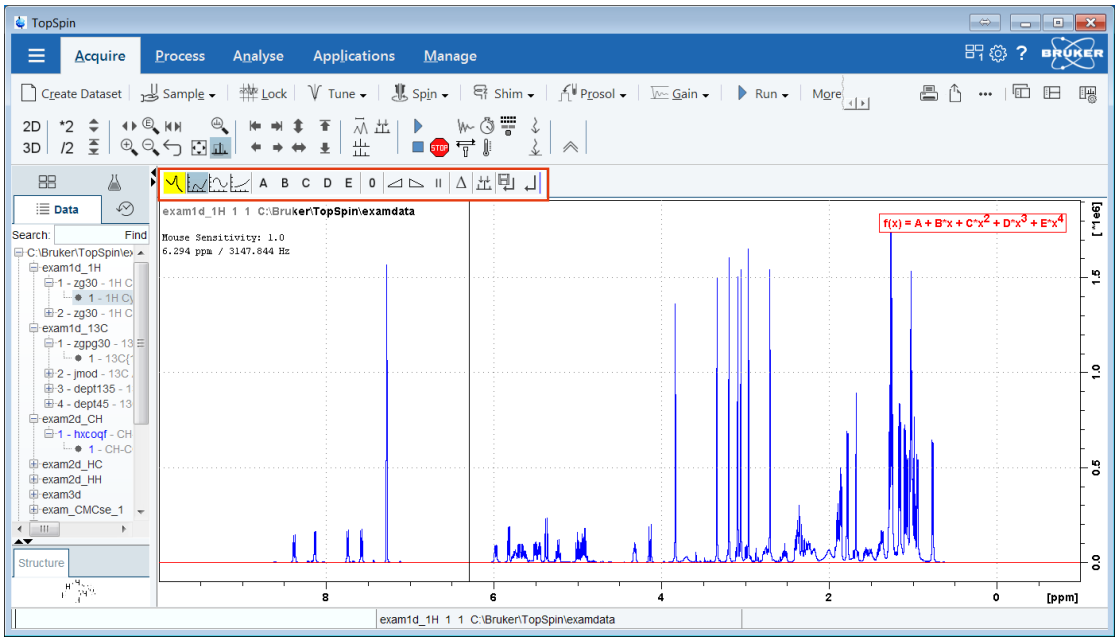


Dataset Toolbar

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



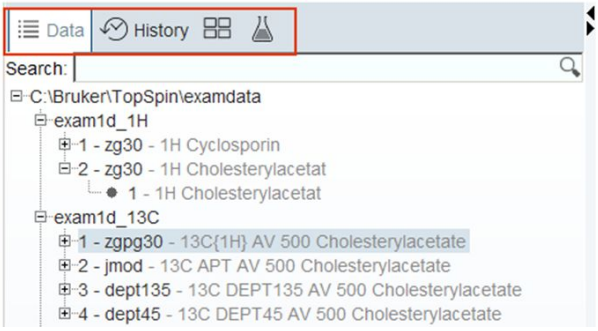
Note: Sometimes the dataset toolbar is displayed without dataset window tabs, e.g. entering a command as **.basl** will display the baseline correction toolbar**:**



Browse and Search Window

The Browser window provides tabs as:

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

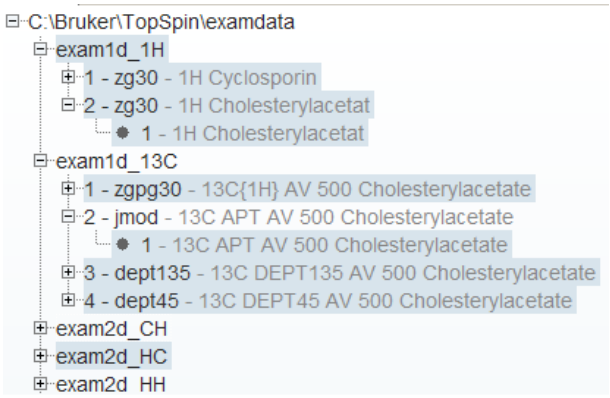


The window can be toggled On or Off with a click on the black **left** or **right** arrow.

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

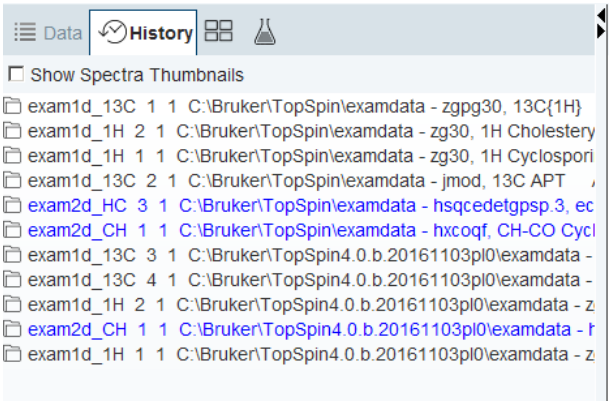
The data tree tab includes a the search field. Enter a search phrase, for example:

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



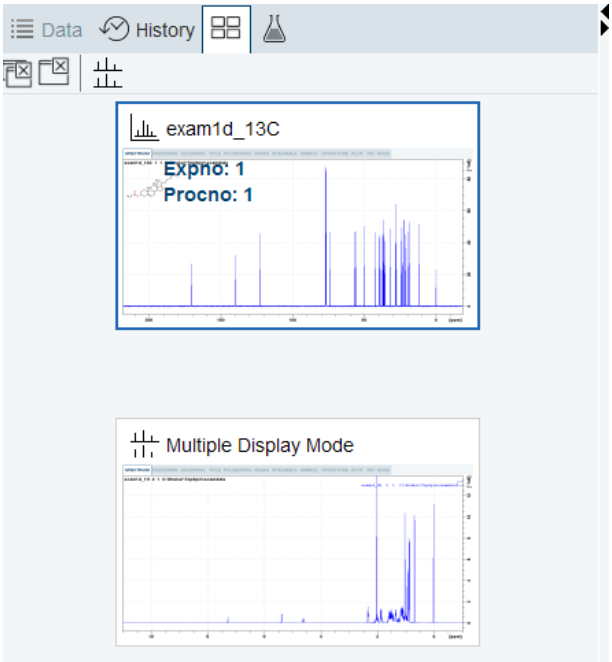
History Tab

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



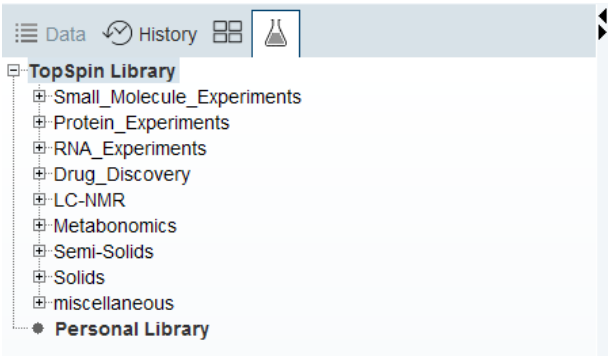
Dataset Switcher Tab

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



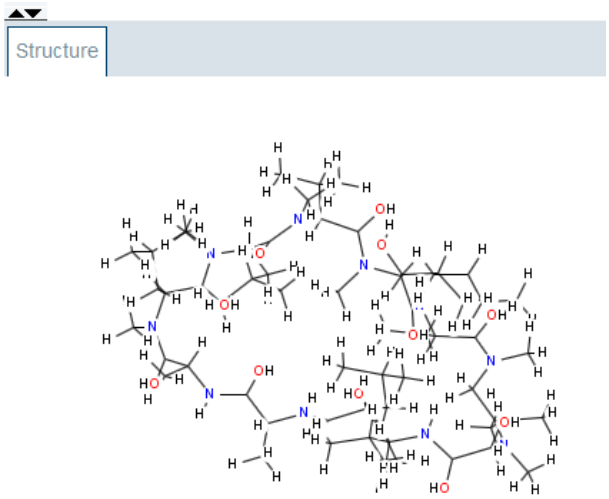
Experiment Library Tab

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

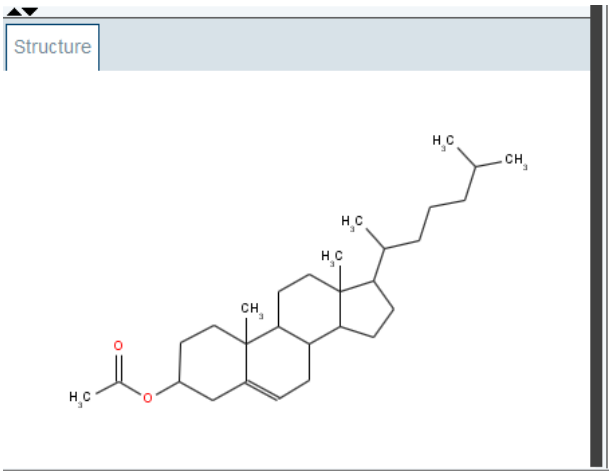


Structure Window

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



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



Window Switcher 

The **Window Switcher** button indicates the number of all opened dataset windows plus all opened acquisition control windows like the BSMS Control Suite and the Temperature Control Suite.

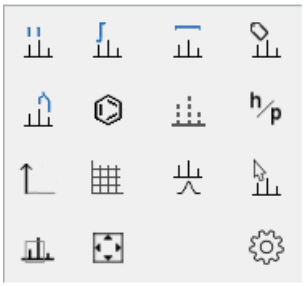
- Click the **Window Switcher** button to switch between these windows.

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

Viewing Options

- Switch the perspective, e.g. full screen or side-by-side, compare open datasets or change spectra view options in the Viewing Options bar.

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



Print, Export and Publish

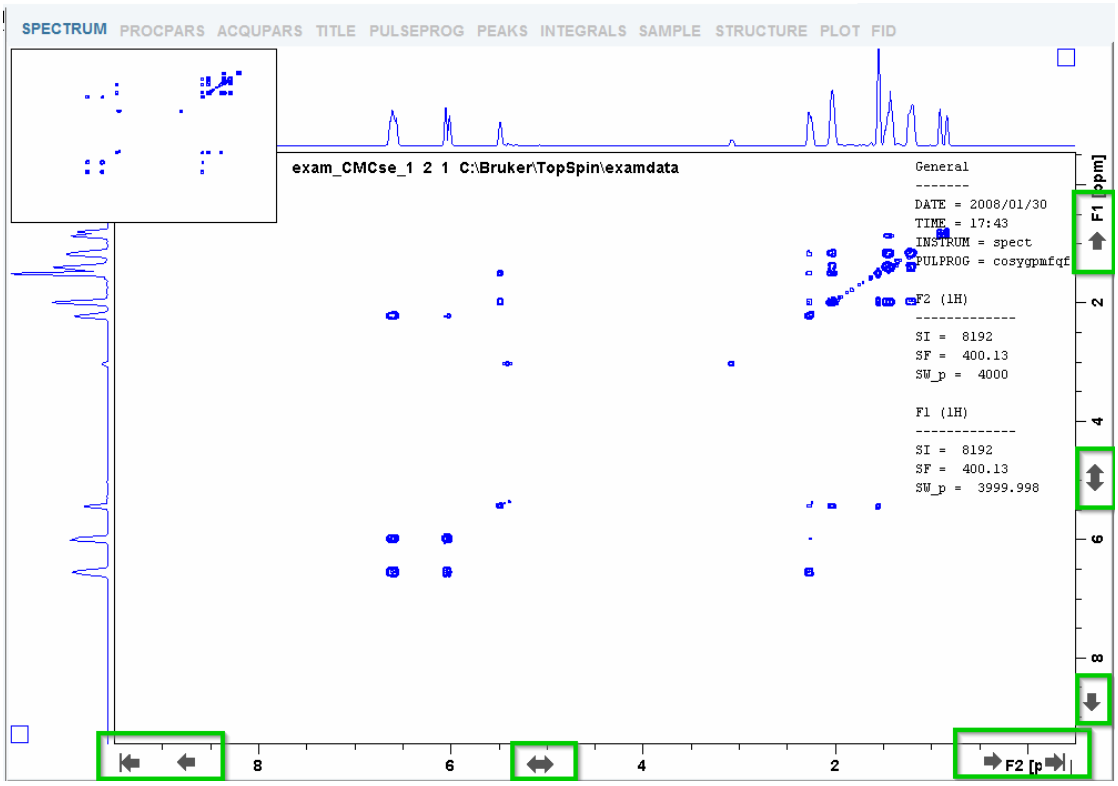
The work can be shared from every point in the working routine with colleagues, sent to printer, export in optional data formats.

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

Shift, Scroll and Zoom Spectra Axes

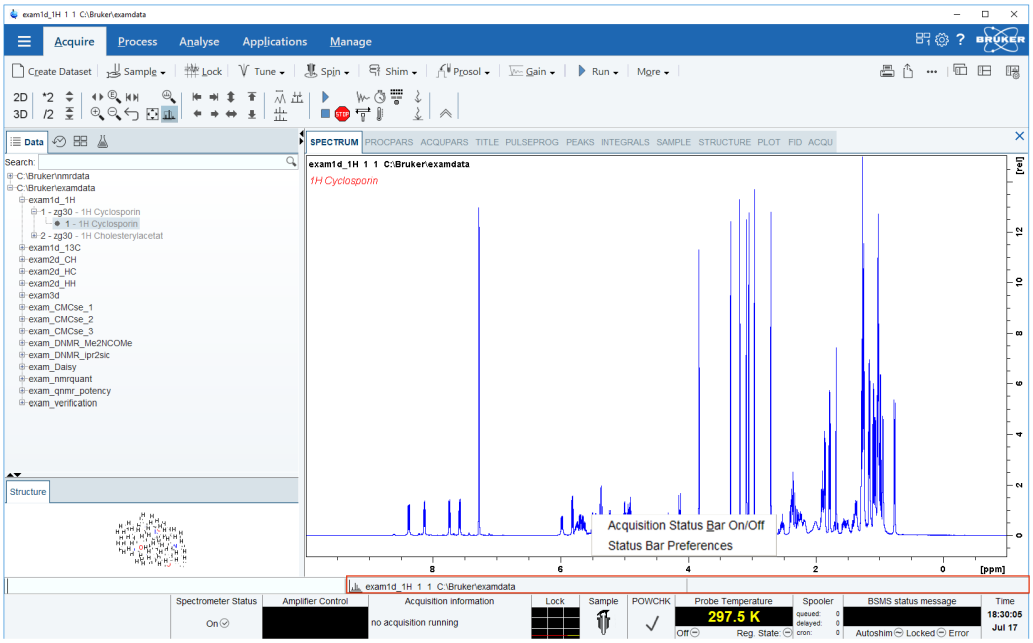
Clicking and dragging the spectrum axis allows intuitive scrolling through the spectrum.

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

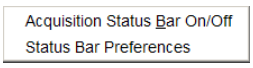


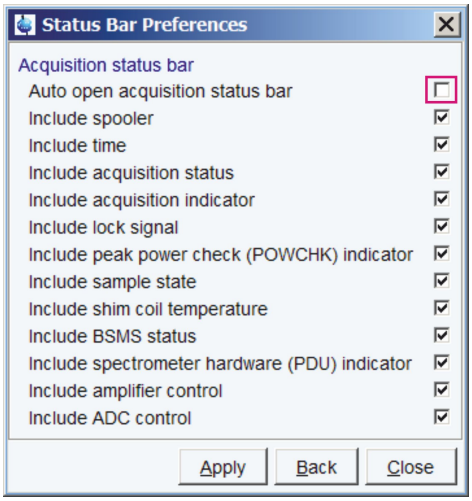
Acquisition Status Bar

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

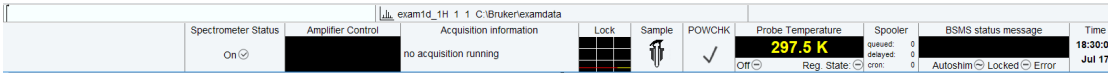


- Click **Status Bar Preferences** to set the *Auto-open the acquisition status bar* option as default.





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**.



4 Spectrometer Basics

4.1 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.

4.2 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.

4.3 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.

4.4 Chemical Safety

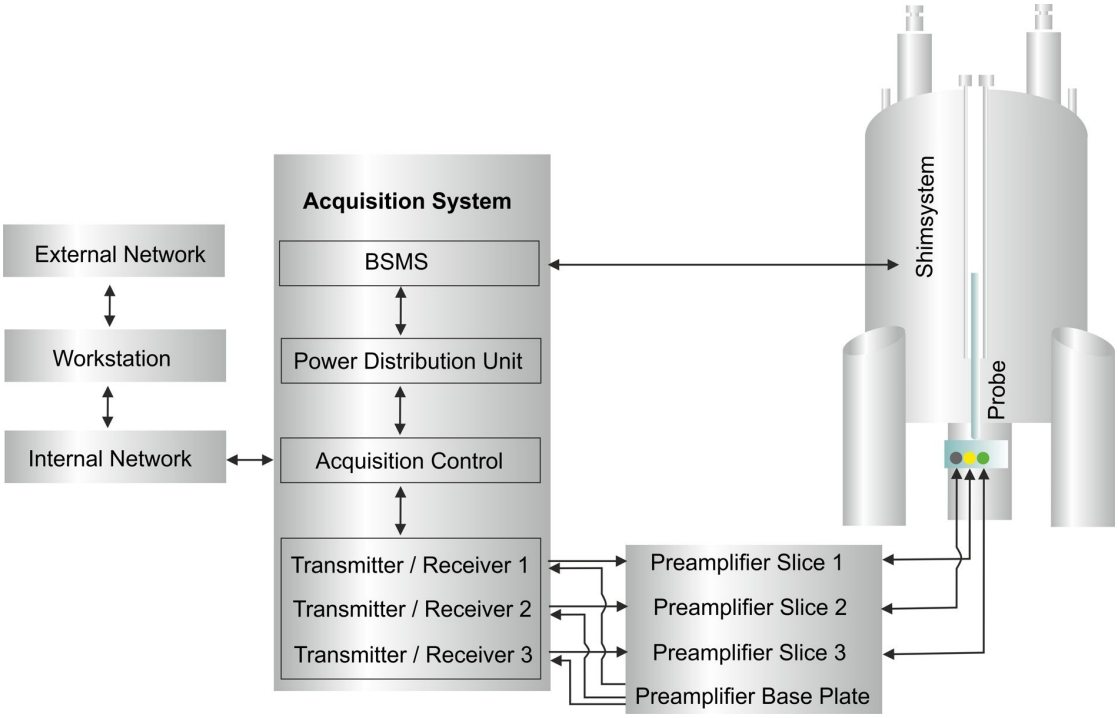
Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc.

4.5 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.

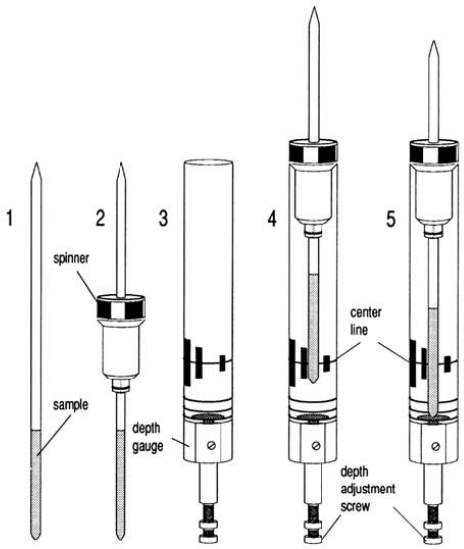
4.6 AVANCE Architecture Overview



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

4.7 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.

4.8 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.

4.9 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 then 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

4.10 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.

4.10.1 Probes Equipped with ATM

4.10.1.1 Automatic Tuning

- Create a new data set, see also *Experiment Setup [31]*.
- 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.

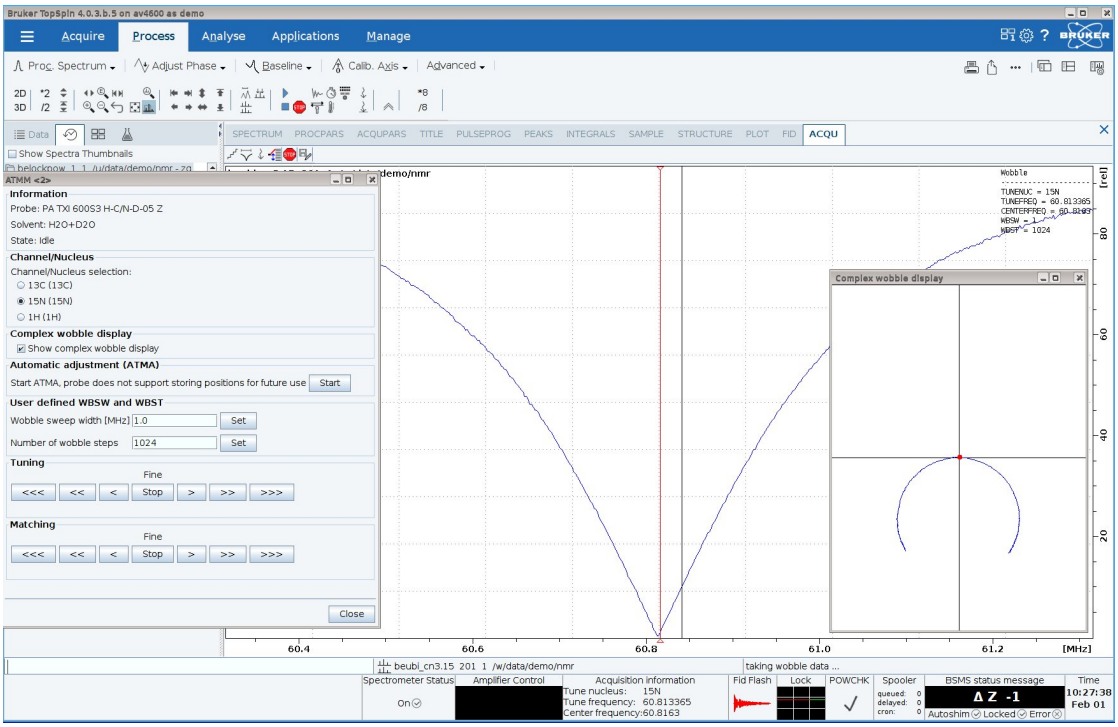
4.10.1.2 Manual Tuning

- Create a new data set, see also *Experiment Setup [31]*.
- 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.

4.11 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.

4.12 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.

4.12.1 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.

4.12.2 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.

4.12.3 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 **edtune** 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
```

4.12.4 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 ^{2}H gradient shimming method and for other solvents especially H_2O, the ^{1}H gradient shimming method.

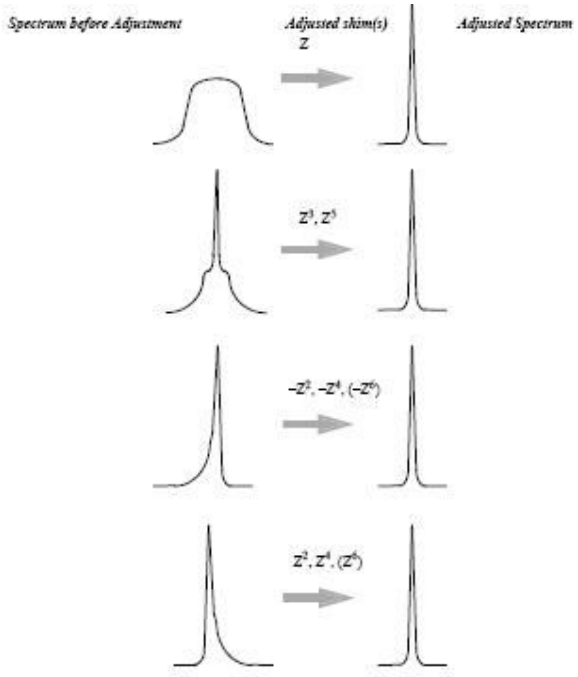
4.13 Optimizing Resolution and Line Shape

The standard sample for measuring the proton line shape and resolution specifications is, $CHCl_3$ in Acetone-d6. 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% C6D6) 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.

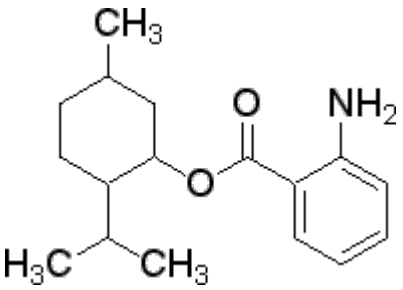
The figure below illustrates the influence of the On-axis shims on the line shape.



5 1D Proton Experiment

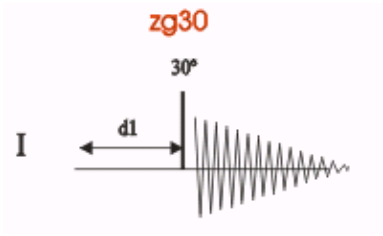
5.1 Sample

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



5.2 1D Proton Experiment

This chapter describes the acquisition and processing of a one-dimensional ^{1}H 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^{0}. The two parameters, D1 and P1, correspond to the length of the recycle delay and the length of the 90^{0} RF pulse, respectively.

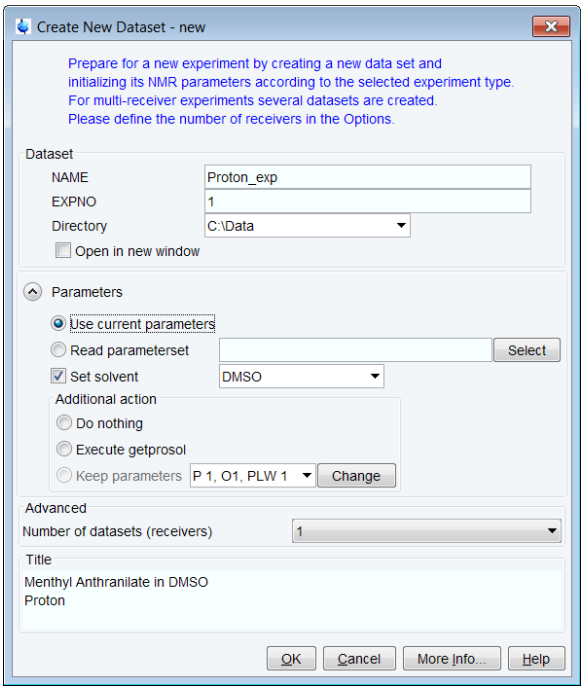


The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

5.2.1 Experiment Setup

Create a new dataset

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



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

  NAME = **Proton_exp**

  EXPNO = **1**

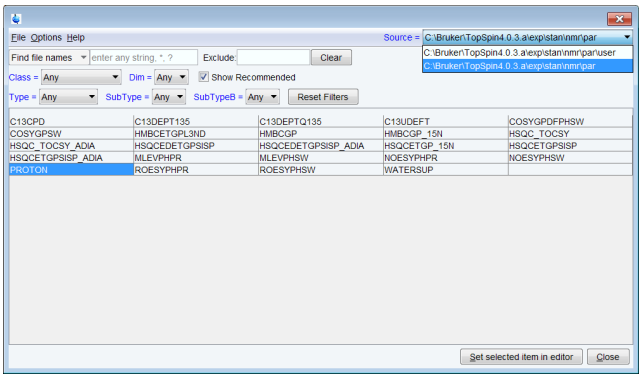
  Directory = e.g. *C:\Data*

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



- 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.

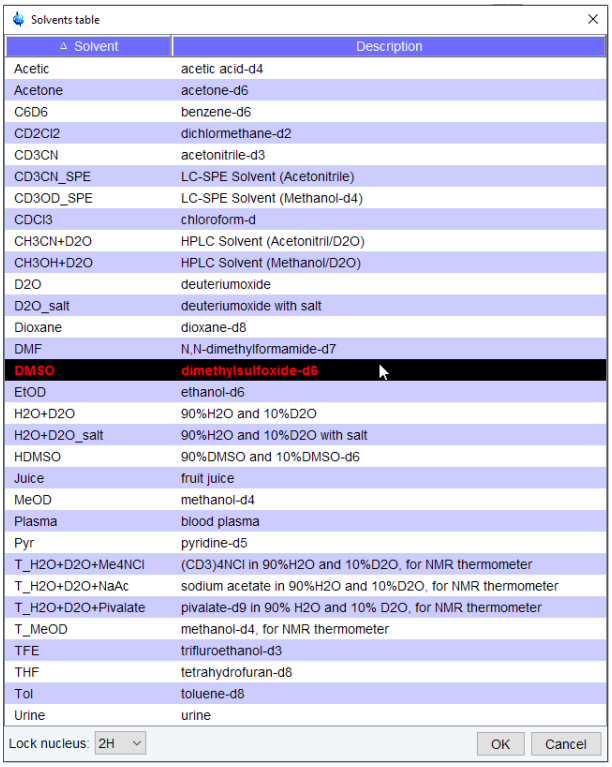
Load the sample

- 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**.



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 then 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.

5.2.2 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

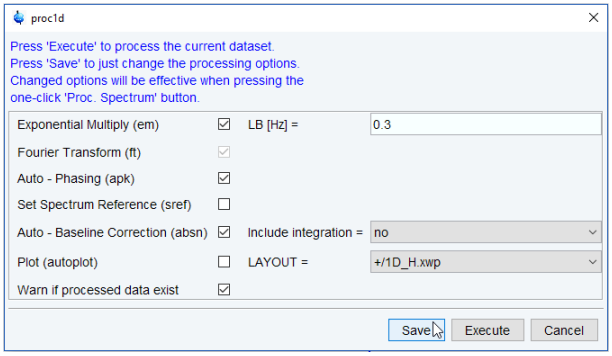
- On the Workflow button bar, click **Run**.

or

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

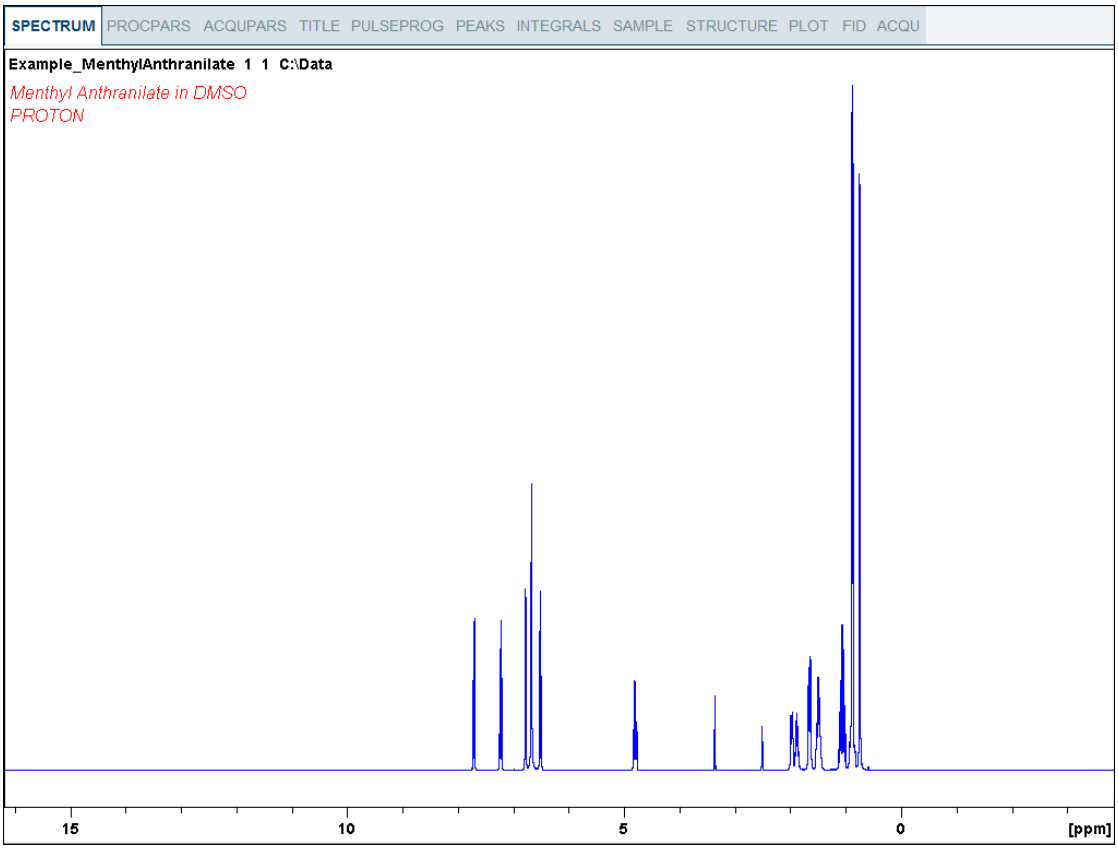
5.2.3 Processing

- 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)



- 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.**

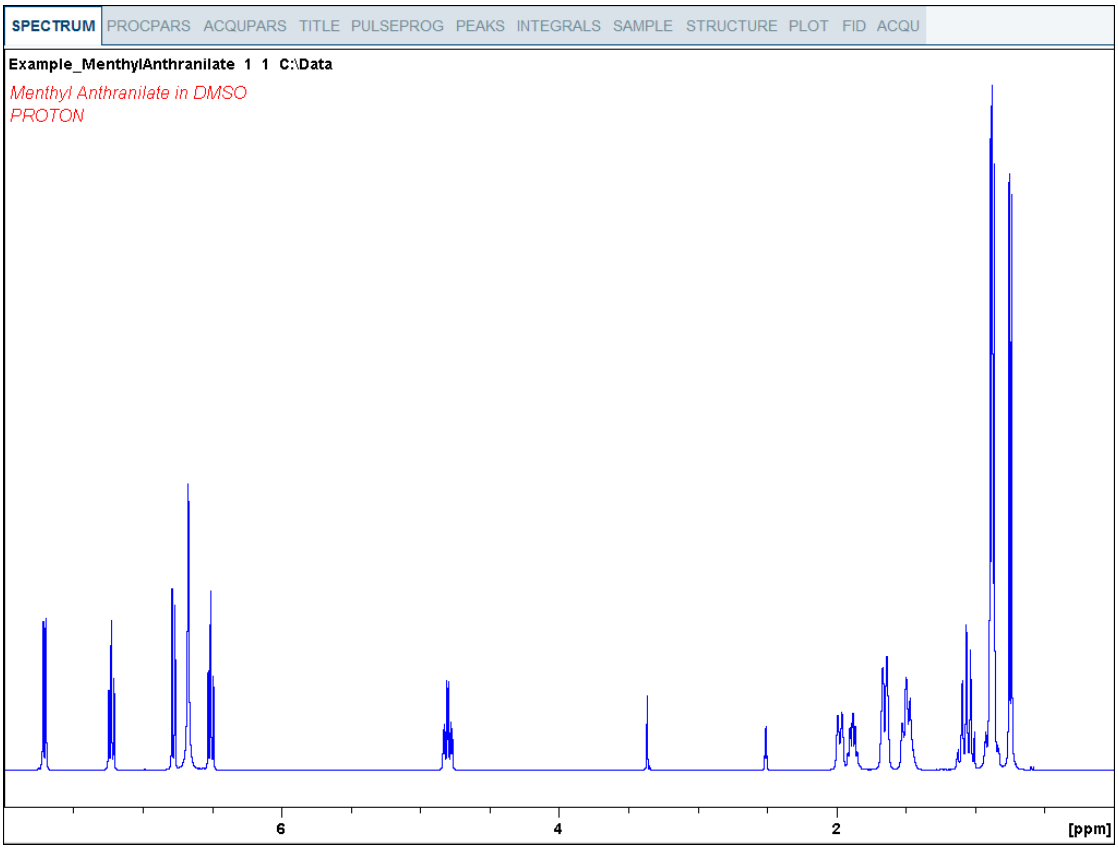


5.2.4 Integration

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**.**

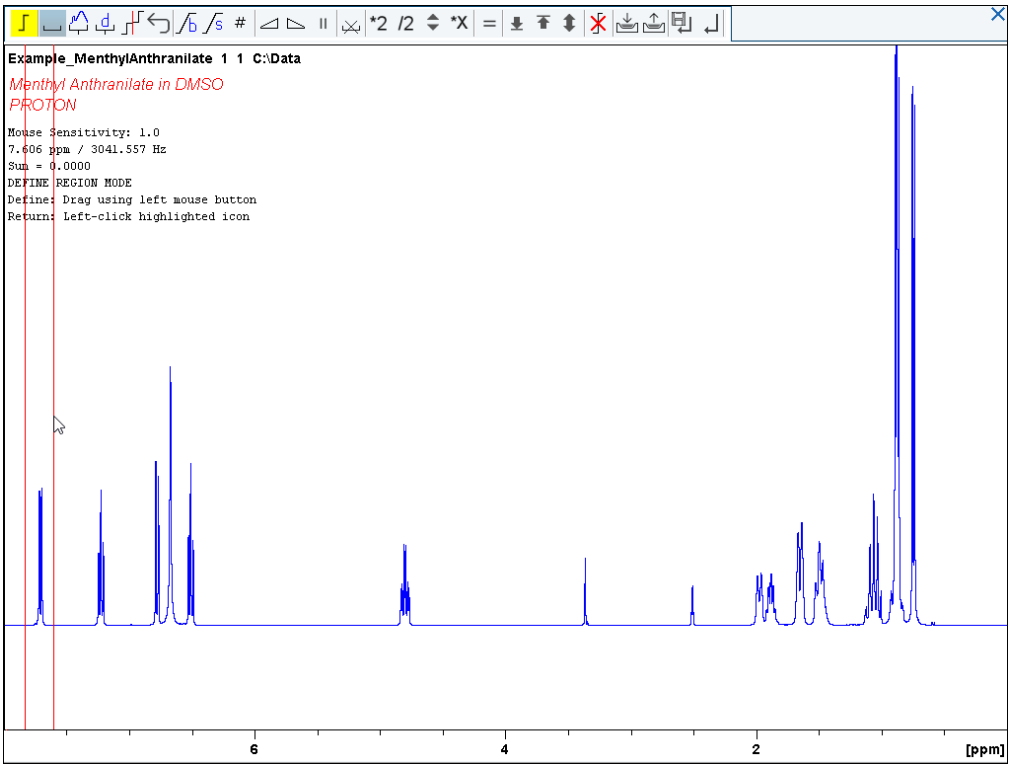
- Expand the spectrum to include all peaks.



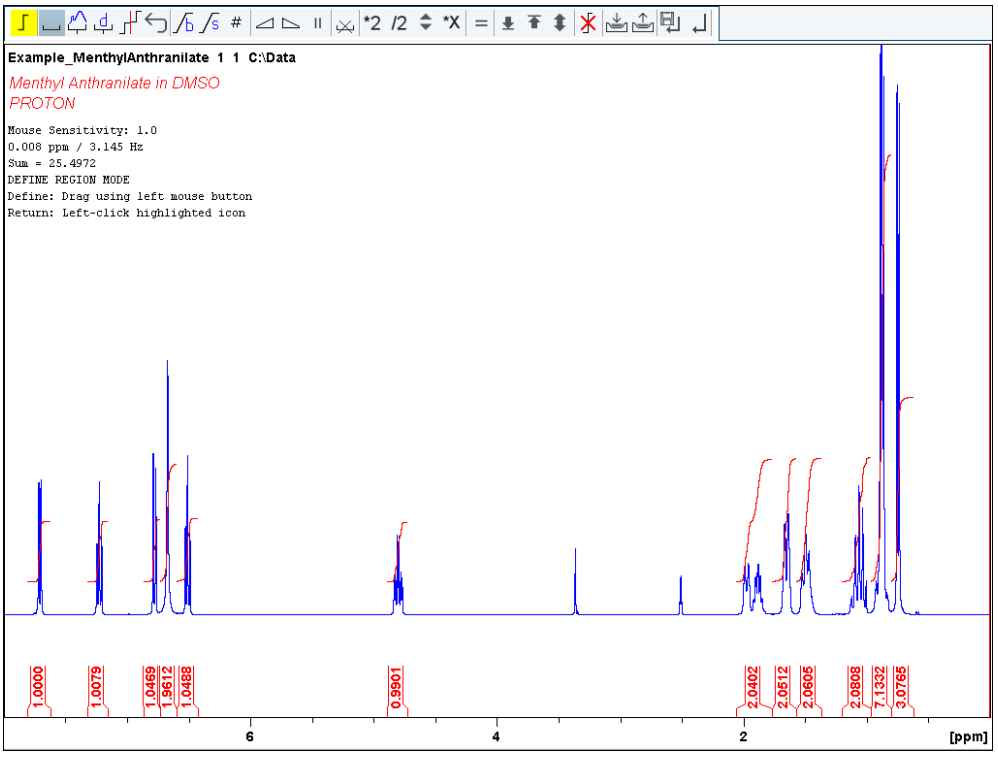
- On the menu, click **Analyse** | **Integrate**.

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

- 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.

5.2.5 Plotting the 1D Proton Spectra

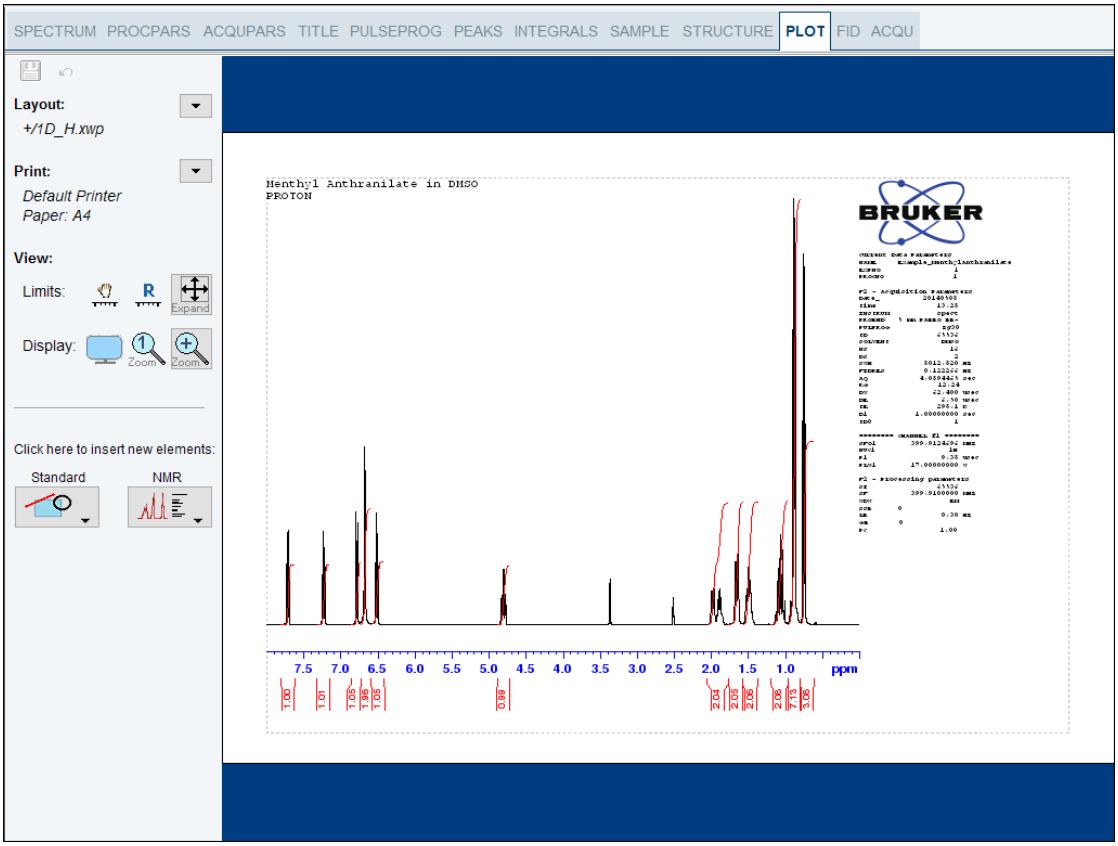
- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**. 

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 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**.

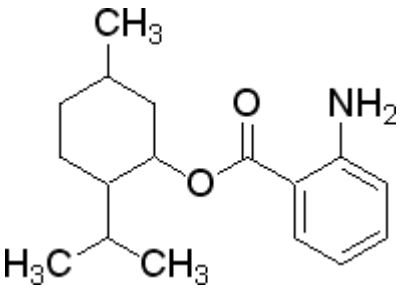


1D Proton Experiment

6 1D Selective Experiments

6.1 Sample

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



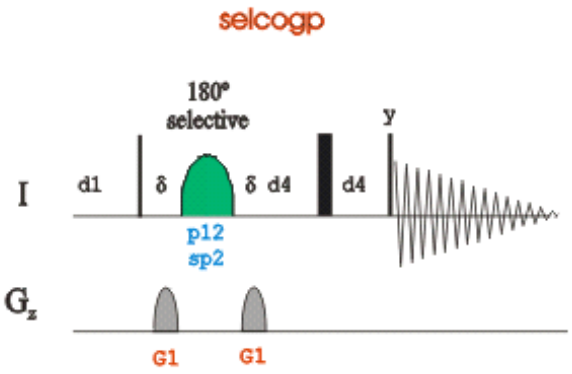
6.2 1D Selective COSY

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 ^{1}H spectrum. Important characteristics of a soft pulse include the shape, the amplitude, and the length. The selectivity of a pulse is measured by its ability to excite a certain resonance (or group of resonances) without affecting near neighbors. Since the length of the selective pulse affects its selectivity, the length is selected based on the selectivity desired and then the pulse amplitude (i.e., power level) is adjusted to give a 90° (or 270°) flip angle.

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

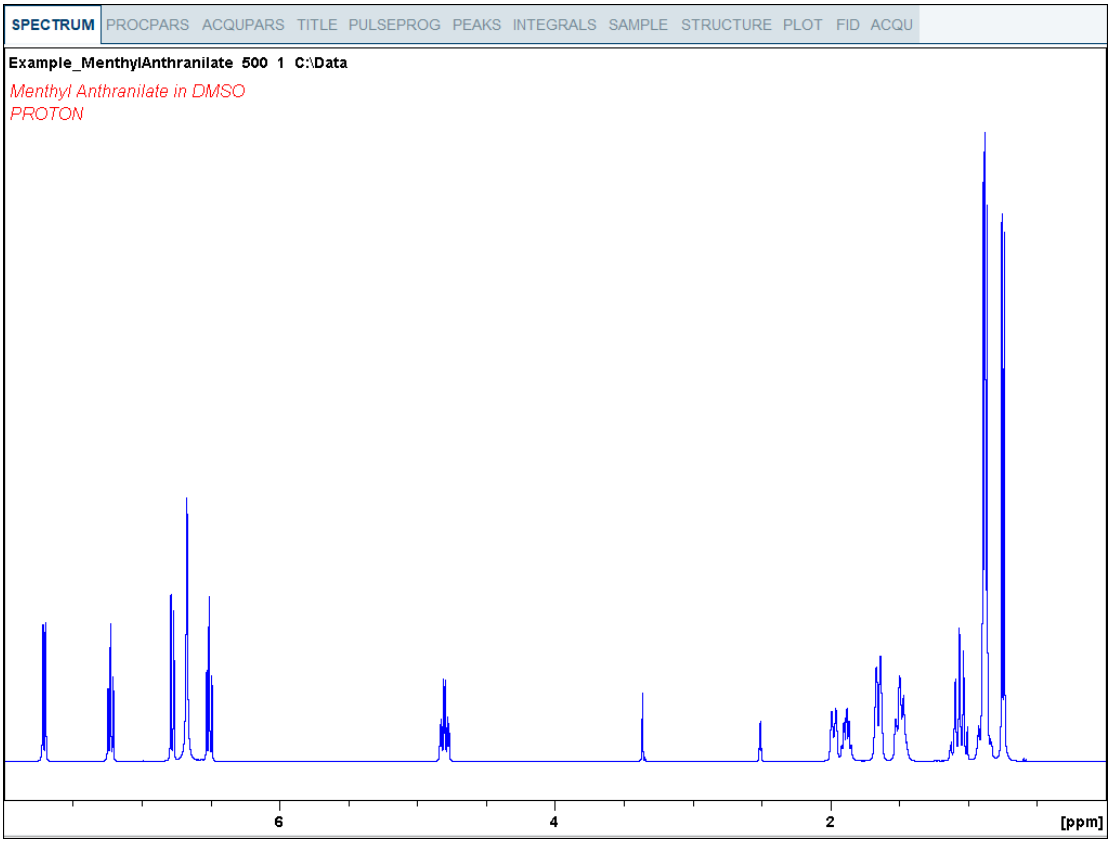
Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments using pulse field gradients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

This chapter describes the acquisition and processing of a one-dimensional ^{1}H 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.



6.2.1 Reference Spectrum

- Run a **1D Proton** spectrum, following the instructions in the chapter *1D Proton Experiment*, *Experiment Setup [▷ 31]* through *Processing [▷ 35]*.



6.2.2 Selective Excitation Region Setup

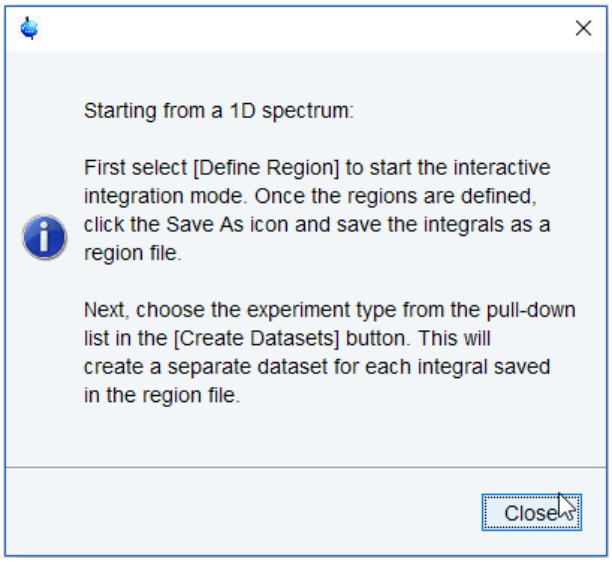
The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

- On the menu bar, click **Acquire**.

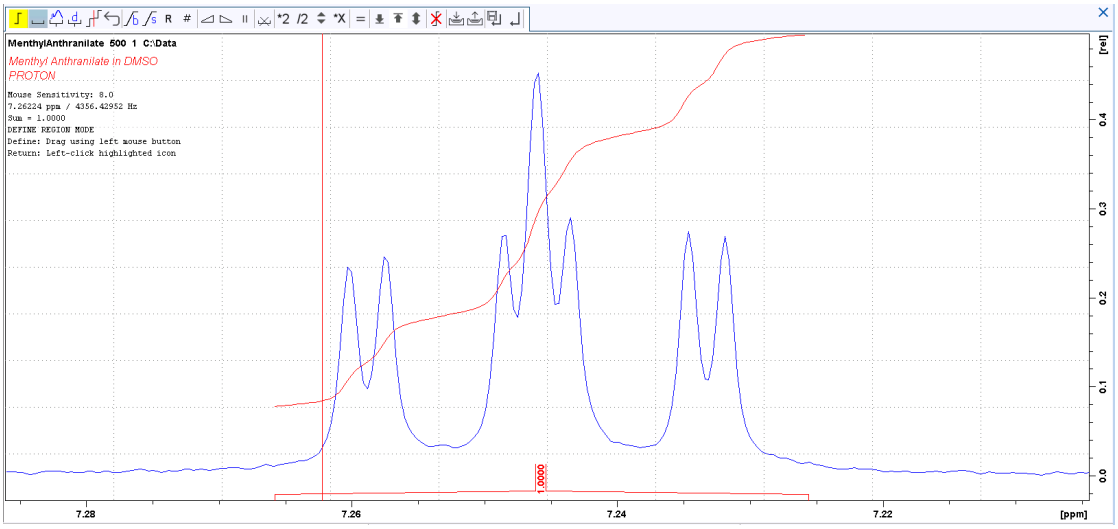
- On the **More** button, click the **drop-down** arrow to see more options.
- In the list, select **Setup Selective 1D Expts**.

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

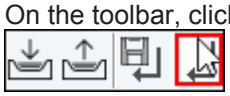
- On the Workflow button bar, click **1D Selective Experiment Setup.**
- In the message window, click **Close**.

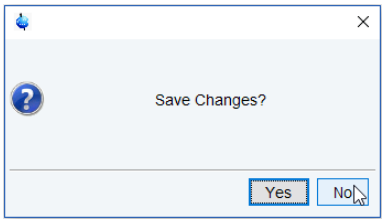


- Expand the peak at **7.2 ppm**.
- On the Workflow button bar, click **Define Regions**.
- Integrate the multiplet at **7.2 ppm**.



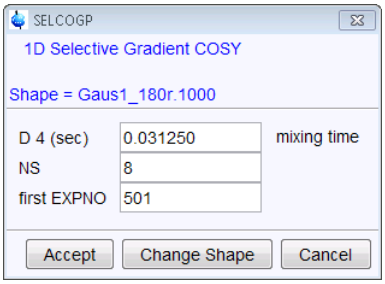
If desired, other peaks can be integrated and a separate dataset will be created for each saved integral.

- 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**.

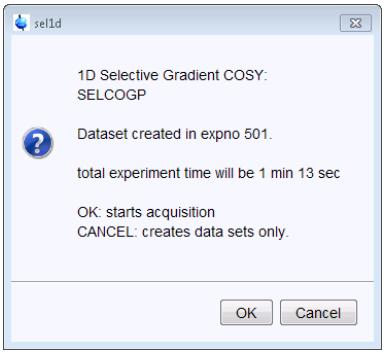
To change the **Gaus1_180r.1000** pulse, in the SELCOGP window click **Change Shape**.



- In the SELCOGP 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.



6.2.3 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**.

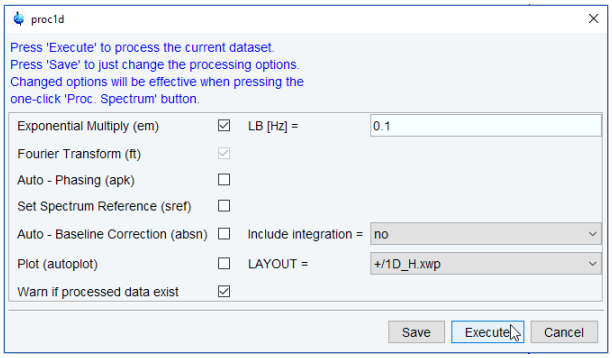
or

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

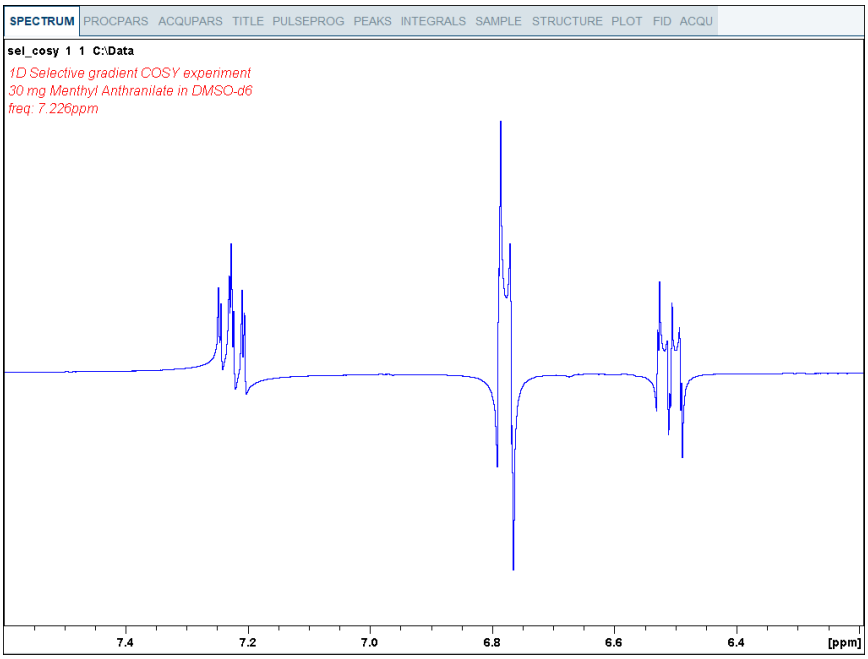
6.2.4 Processing

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



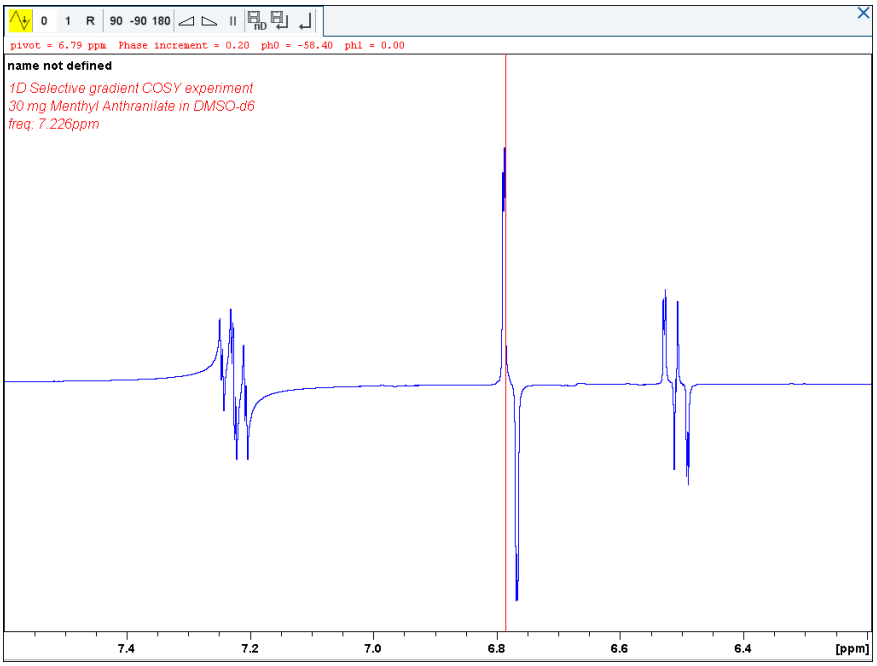
- In the proc1d window, click **Execute**.
- Expand the spectrum from **7.6 ppm** to **6.2 ppm**.



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

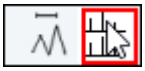
The **Dataset tabs** are replaced by the **Adjust Phase** tool bar.

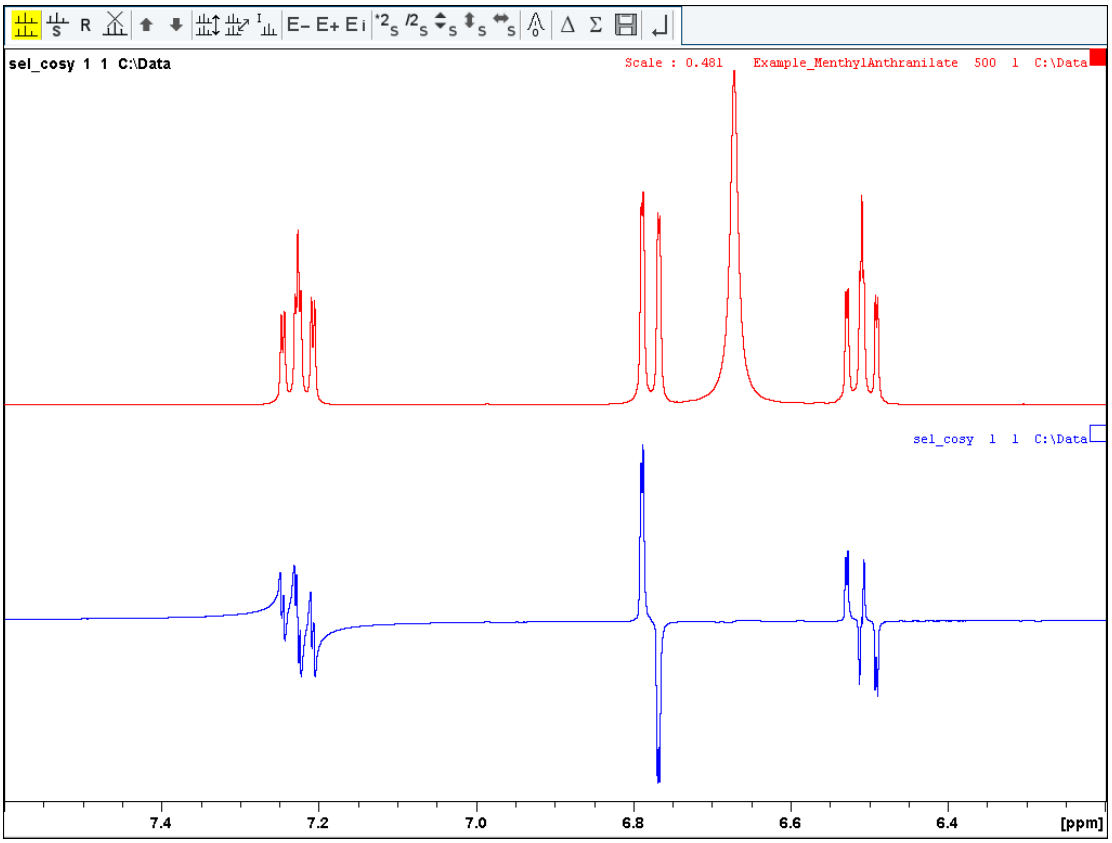
- Adjust the **0** order correction on the peak at **6.8 ppm** to display an antiphase pattern.

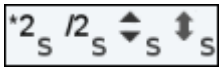


- On the toolbar, click **Return & save phased spectrum**. 

6.2.5 Plotting Two Spectra on the Same Page

- Display the selective COSY spectrum.
- On the toolbar, click **Multiple display**. 
- Drag the **Reference spectrum** (1D Proton) into the spectral window.

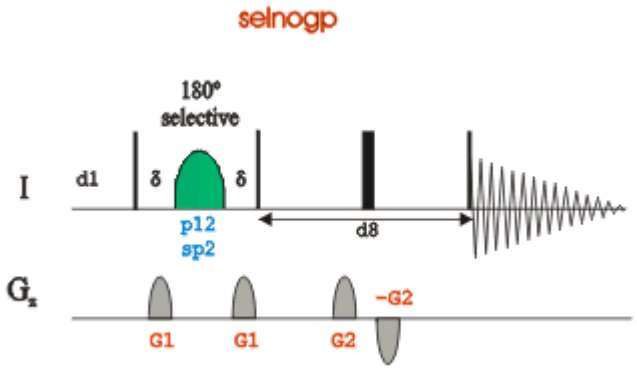


- Click the small box in the upper right corner of the spectrum display to select the reference spectrum.
- Adjust the spectra for best fit with the tools: 
- On the Workflow button bar, click **Print active window**  .

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

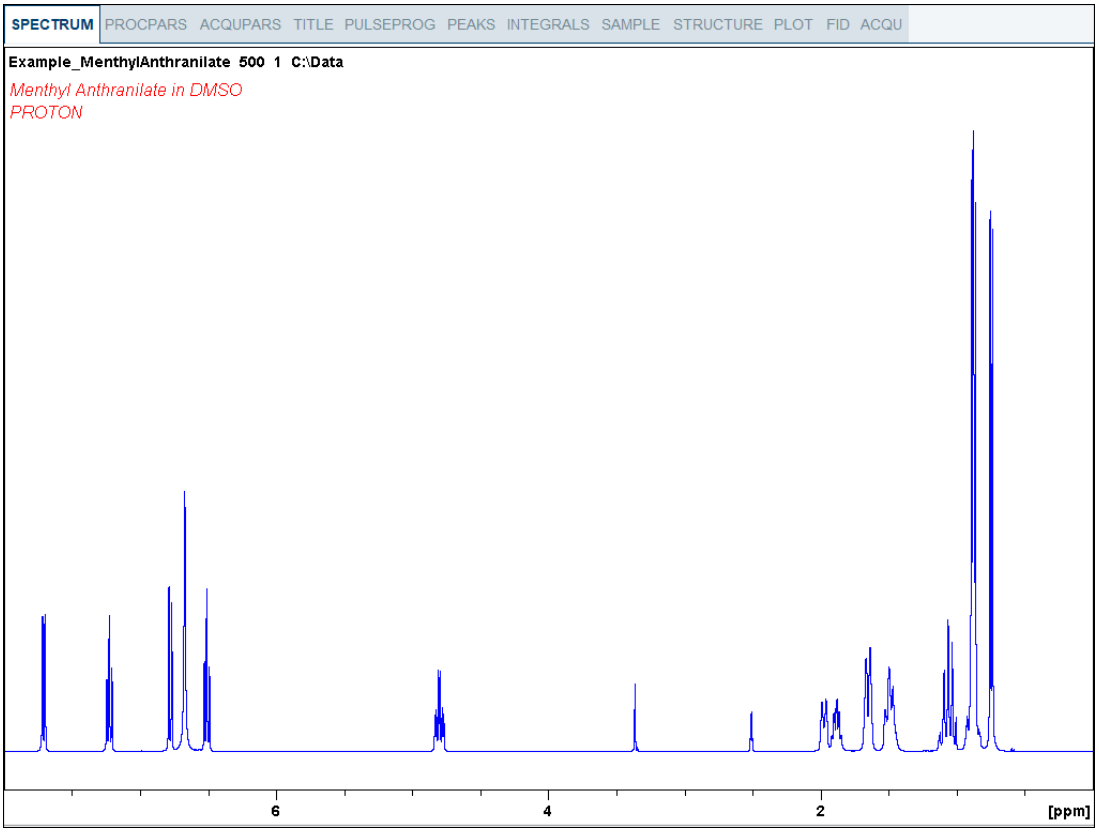
6.3 1D Selective NOESY

This chapter describes the acquisition and processing of a one-dimensional ^{1}H selective gradient NOESY experiment. The standard Bruker parameter set is SELNOGP and includes the pulse sequence **selnogp** 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.



6.3.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup [▷ 31]* through *Processing [▷ 35]*.



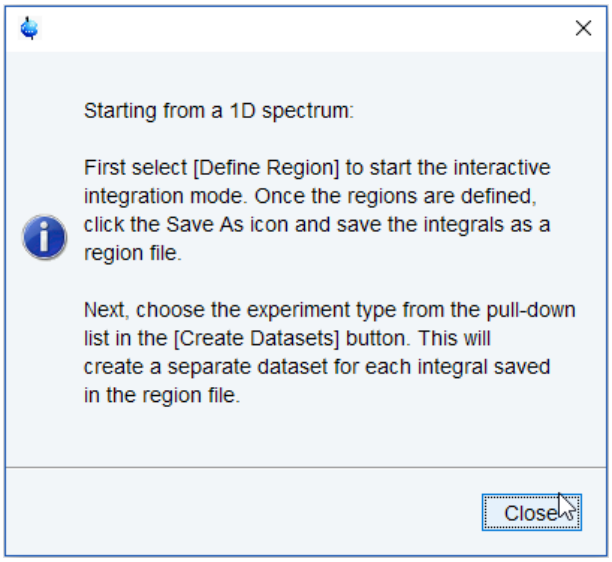
6.3.2 Selective Excitation Region Setup

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.

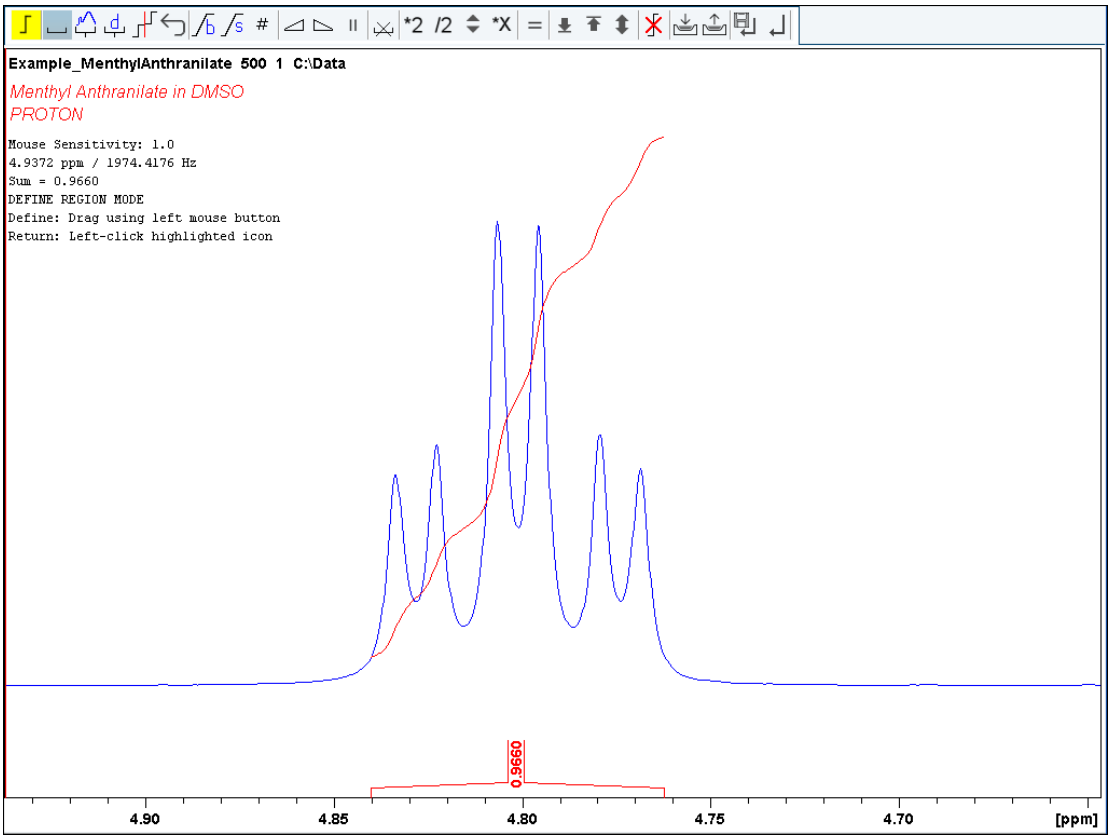
- On the menu bar, click **Acquire**.
- On the **More** button, click the **drop-down** arrow to see more options.
- In the list, select **Setup Selective 1D Expts**.

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**.

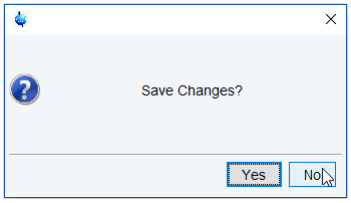


- Expand the peak at **4.8 ppm**.
- On the Workflow button bar, click **Define Regions**.
- Integrate the multiplet at **4.8 ppm**.



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

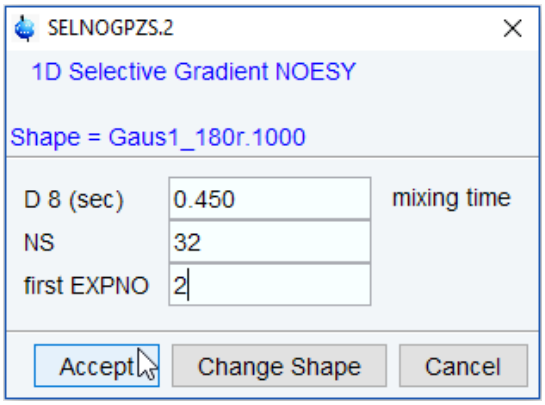
- On the toolbar, click **Save/Export Integration Region**. 
- In the list, select **Save the Region to 'reg'**.
- On the toolbar, click **Return do NOT save regions!** to exit the integration mode. 
- In the message window, click **No**.



- 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.

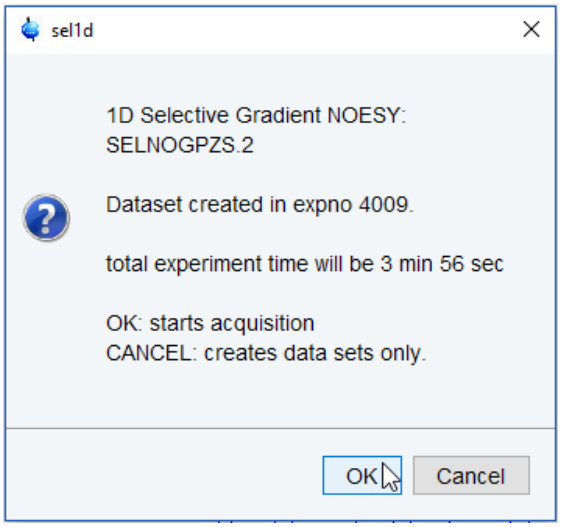
- To change the **Gaus1_180r.1000** pulse, in the SELNOGP window click **Change Shape**.
- In the SELNOGP window, enter
  D8 = **0.450**
  NS = **32**



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

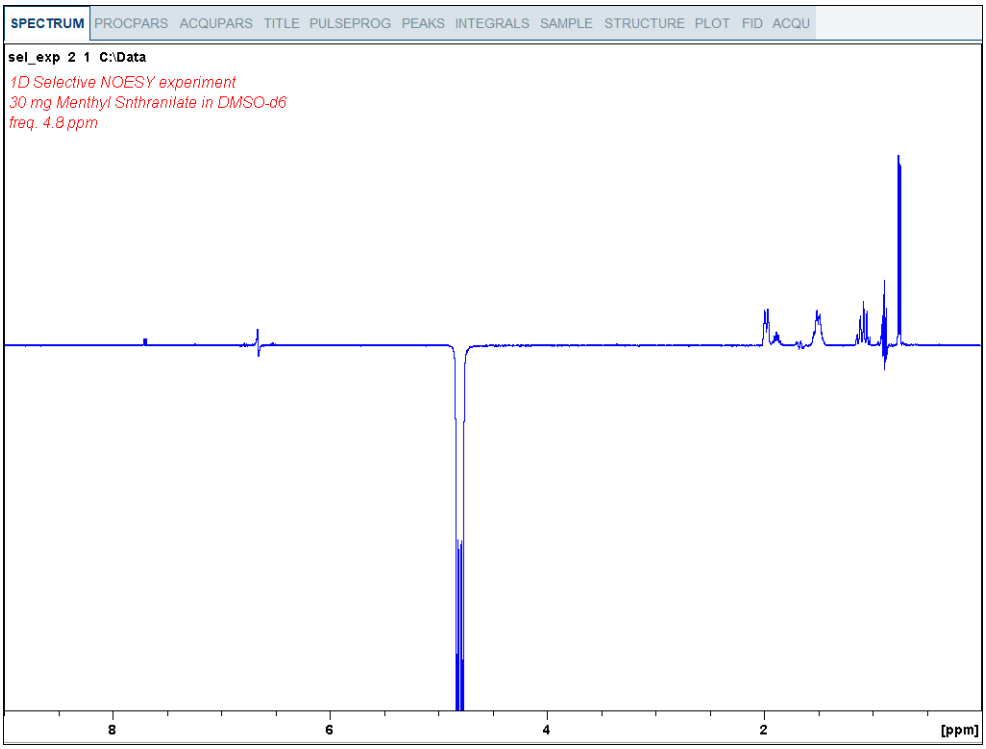
The new dataset is created and all parameters are automatically calculated and set.

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



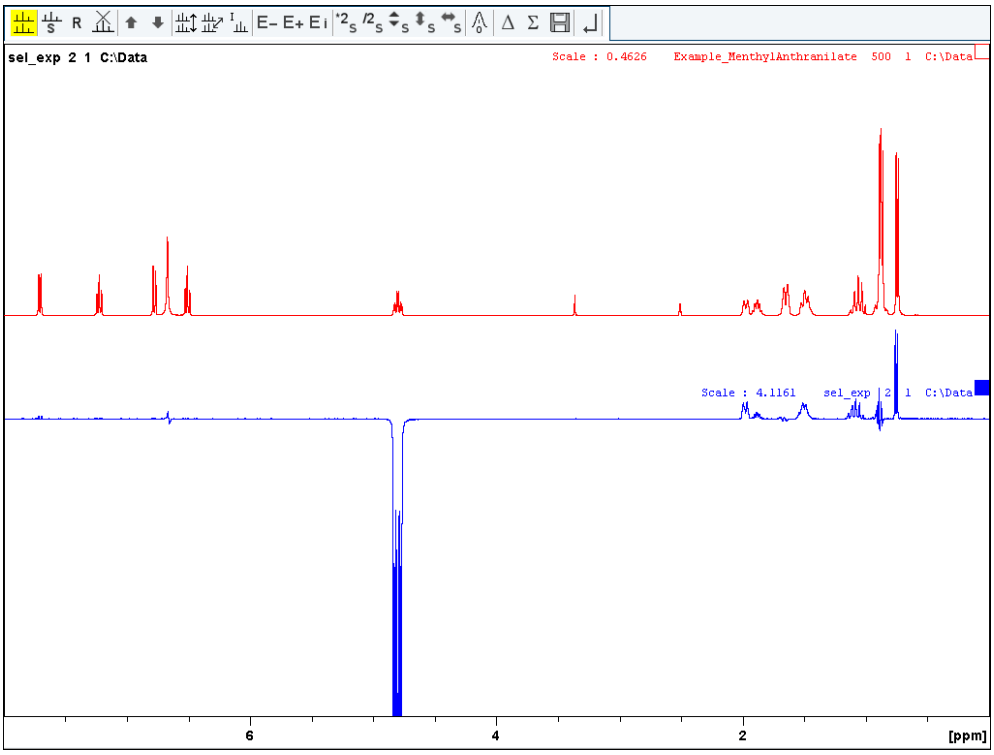
6.3.3 Processing

- Follow the first processing instructions in the chapter *1D Selective Experiments Processing [▶ 45]*.
- 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.



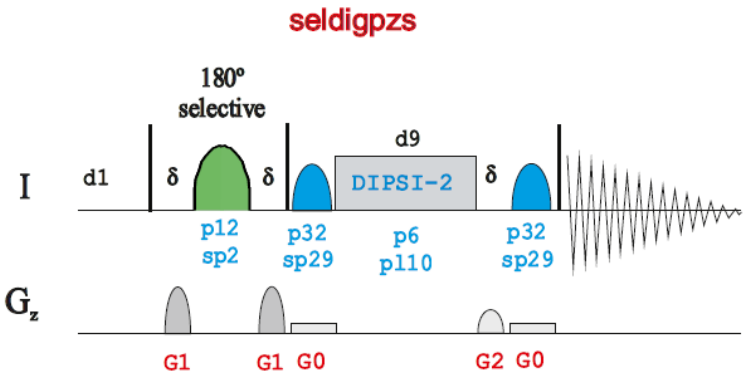
6.3.4 Plotting Two Spectra on the Same Page

- Display the selective NOESY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page [▷ 47]* for the Selective COSY.



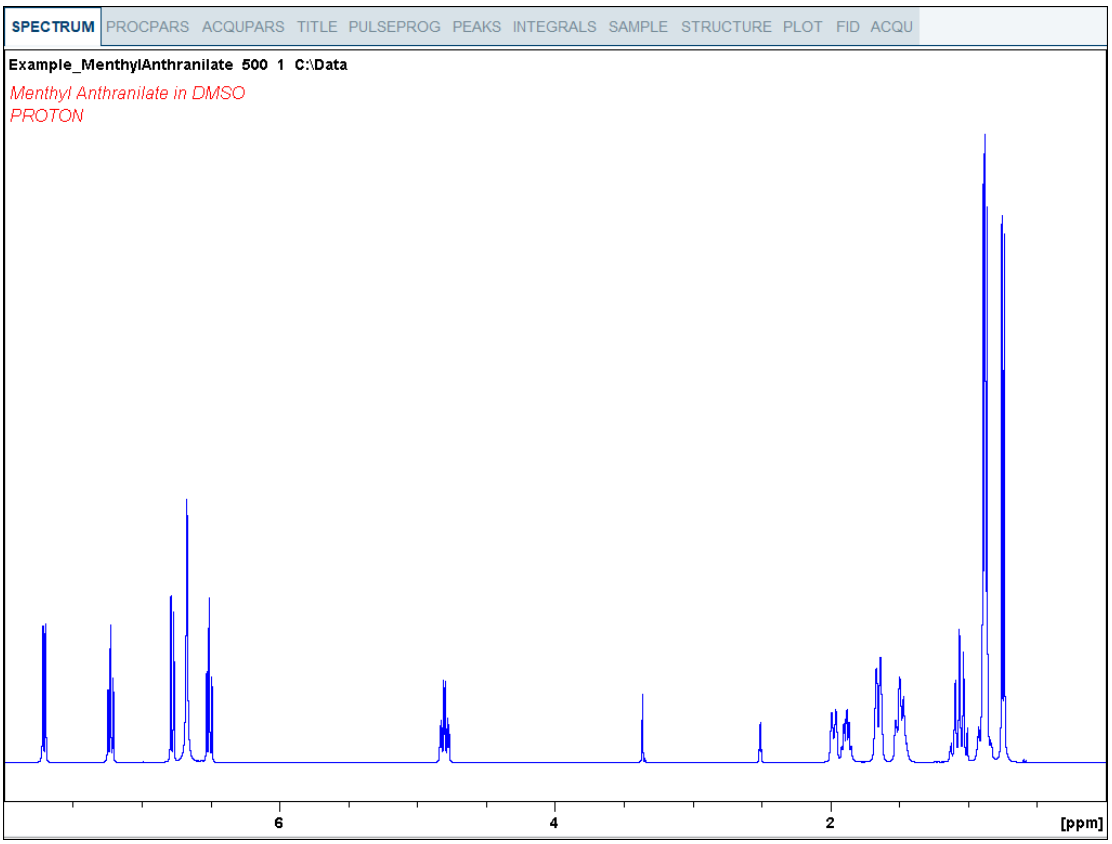
6.4 1D Selective TOCSY

This section describes the acquisition and processing of a one-dimensional ^{1}H 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.



6.4.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup [31]* through *Processing [35]*.



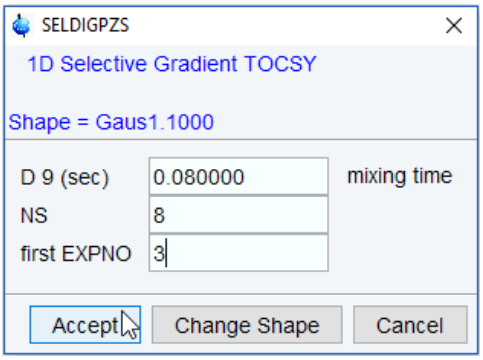
6.4.2 Selective Excitation Region Set Up

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 [▶ 49]* up to *Selective Excitation Region Setup [▶ 50]*.
- 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.

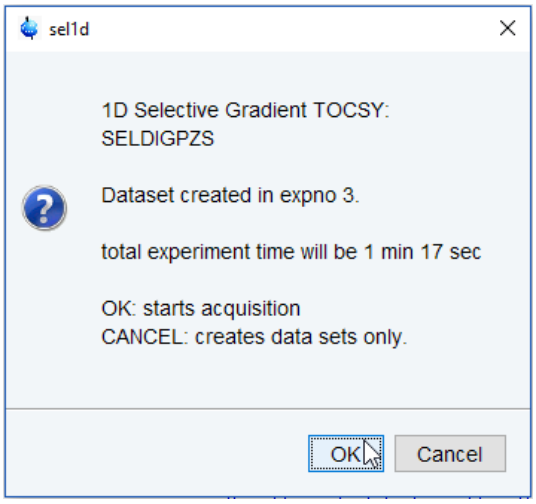
- In the SELDIGPZS window, enter
  D9 = **0.08**
  NS = **8**



- Click **Accept**.

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

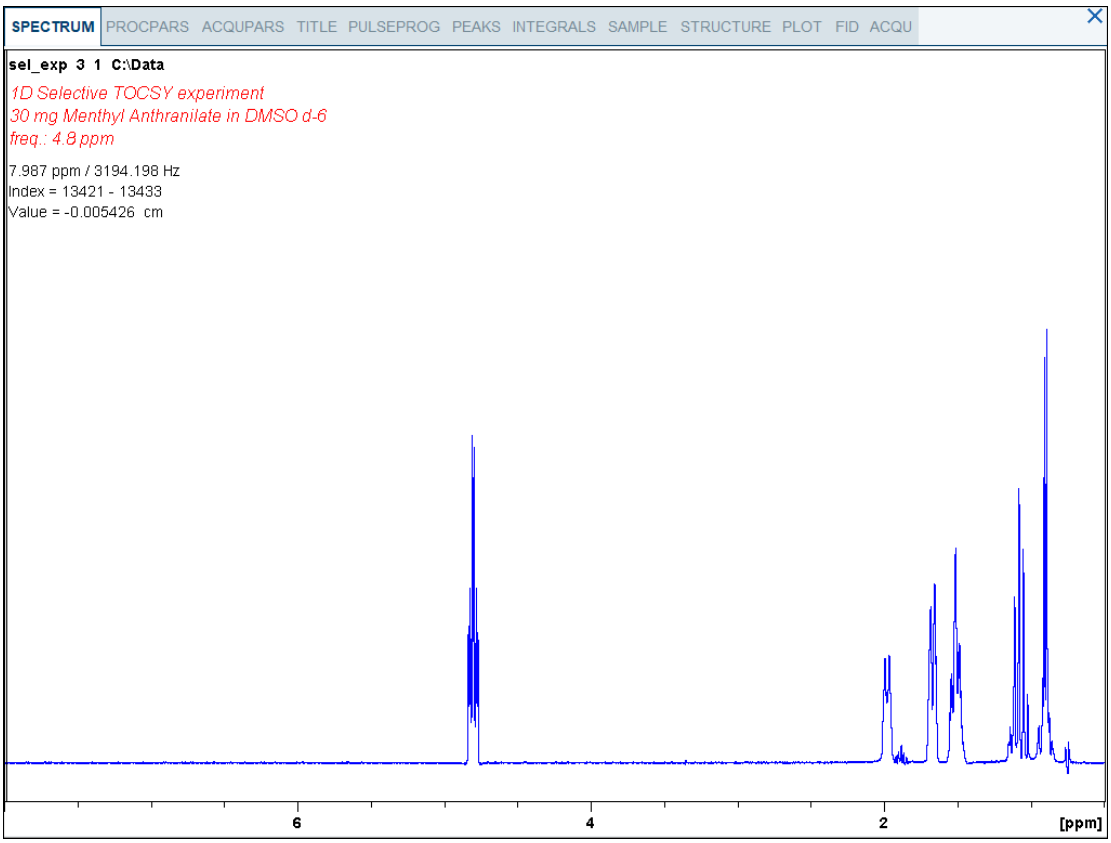
- In the sel1d message window, click **OK** to start the acquisition.



6.4.3 Processing

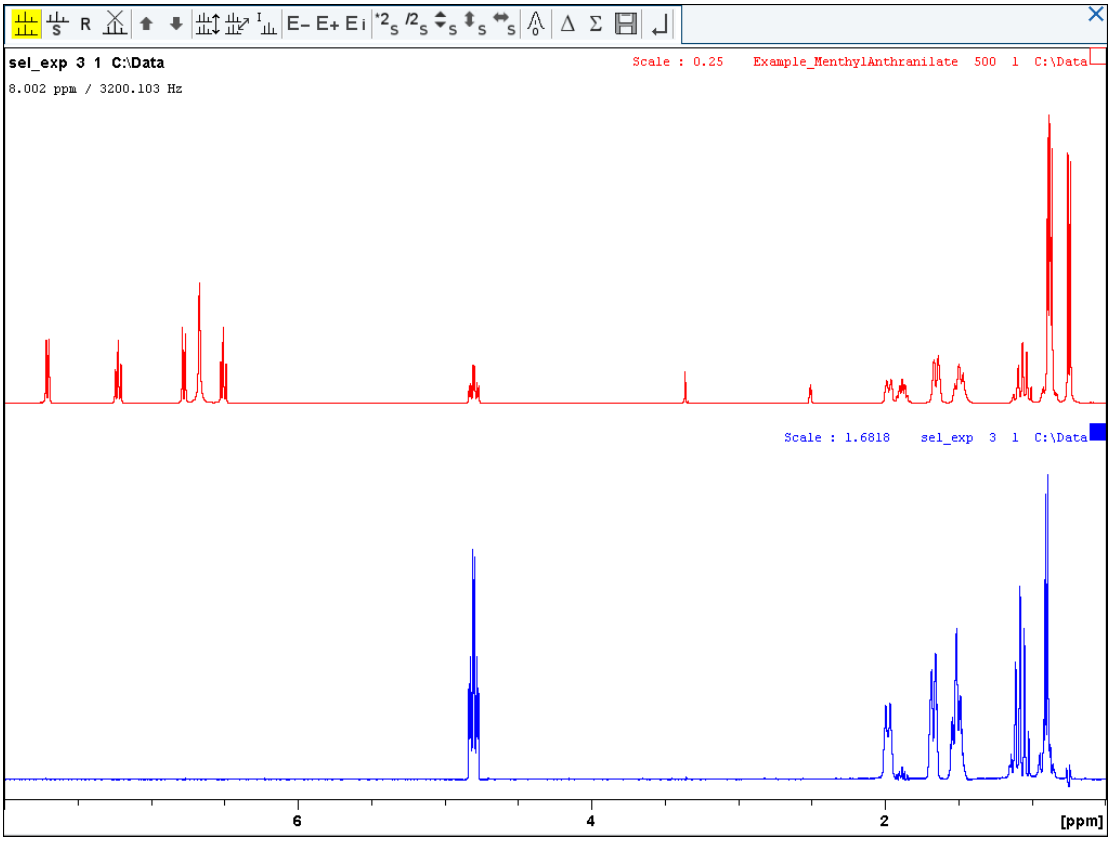
Follow the first processing instructions in the chapter *1D Selective Cosy Processing [▶ 45]* up to *Processing [▶ 45]*.

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



6.4.4 Plotting Two Spectra on the Same Page

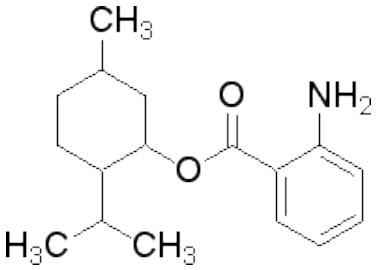
- Display the selective TOCSY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page [▷ 47]* for the Selective COSY.



7 2D Homonuclear Experiments

7.1 Sample

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



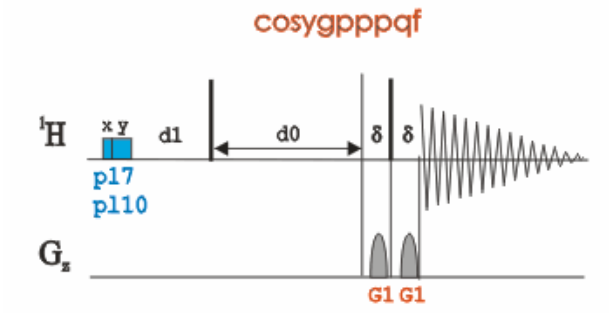
7.2 2D Gradient COSY

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.

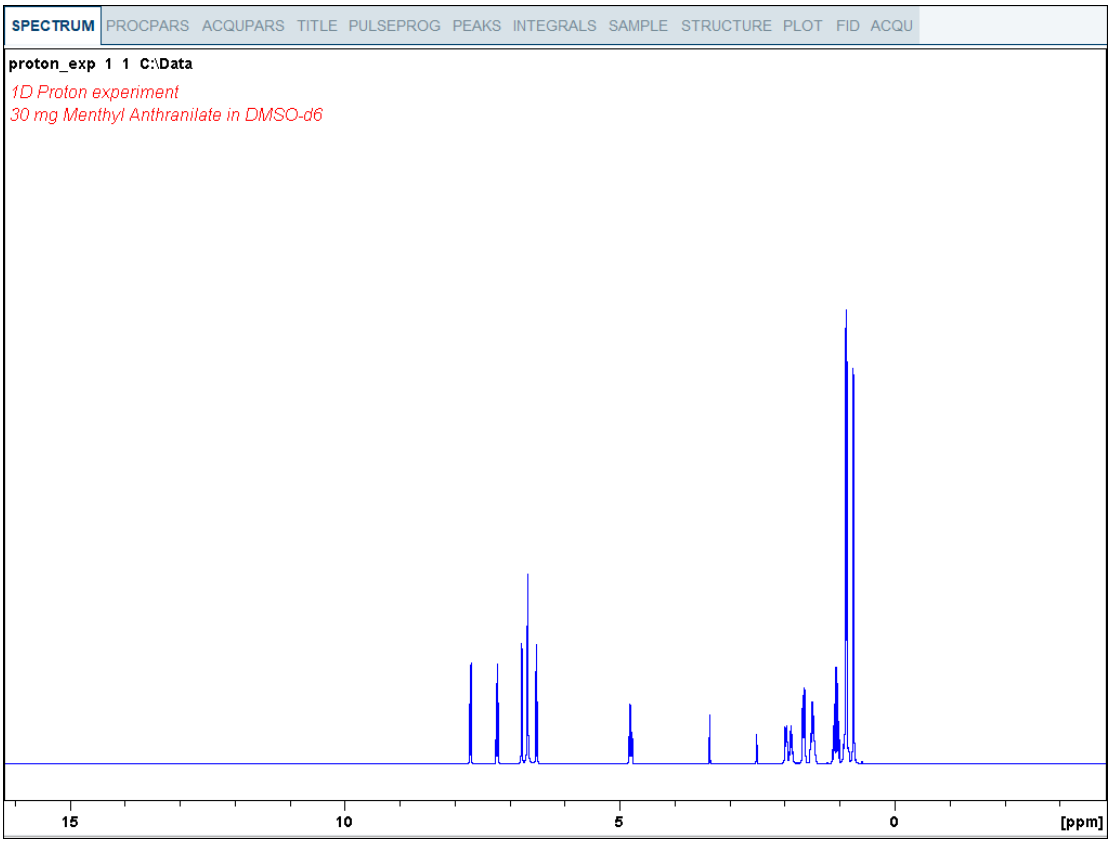
This chapter describes the acquisition and processing of a two-dimensional 1H gradient COSY. The standard Bruker parameter set is COSYGPSW and includes the pulse sequence **cosygpppqf** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay D0 and the acquisition time during which the signal is recorded. Both pulses have a 90^0 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.

7.2.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup [31]* through *Processing [35]*.

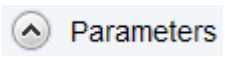


7.2.2 Setting up the COSY Experiment

- 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**.

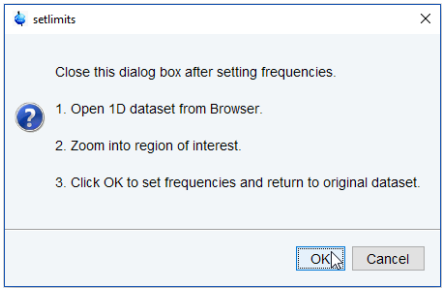
2D experiments should be run non-spinning.

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

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

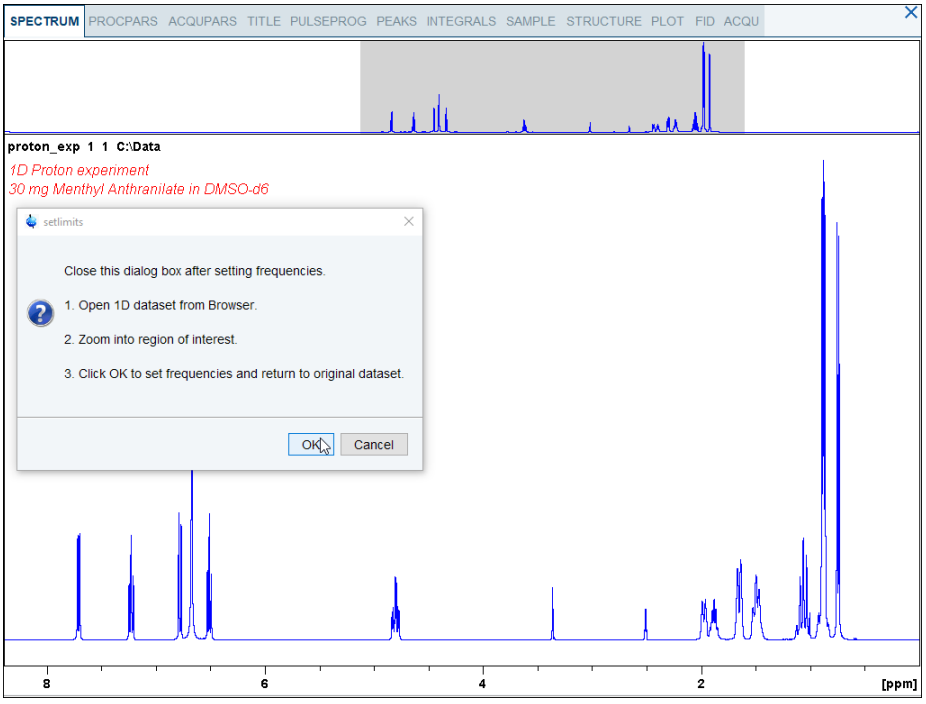
7.2.3 Limit Setting

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

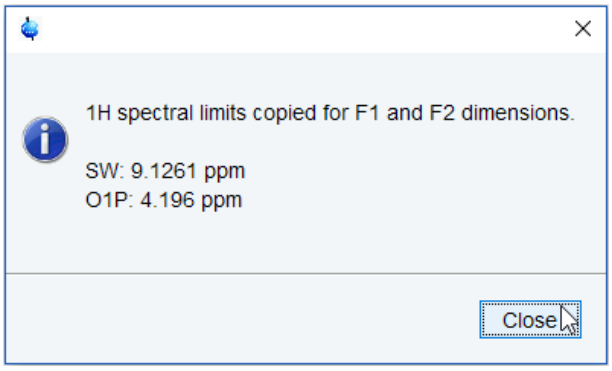


- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or drag the 1D Proton dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.

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.

7.2.4 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.

7.2.5 Processing

When the acquisition is finished:

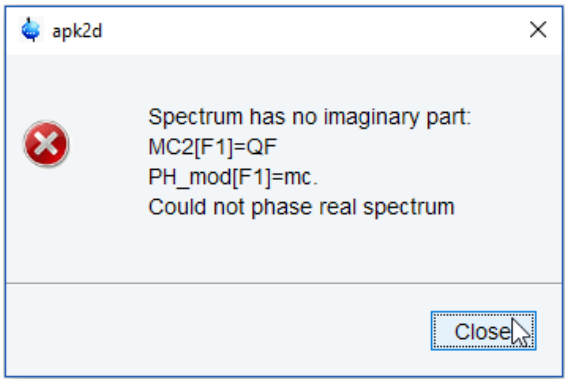
- On the menu bar, click **Process**.

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

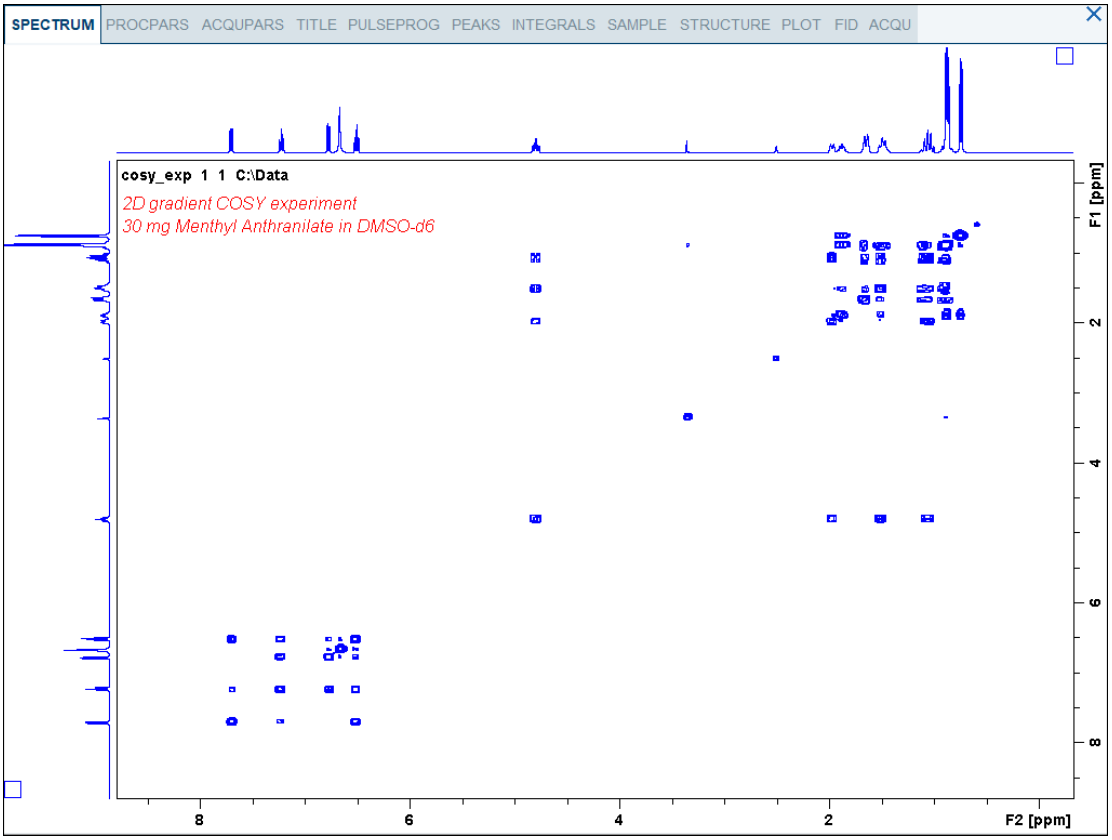
This executes a standard processing program **proc2d**.

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.

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



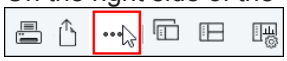
To disable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow to configure the Standard Processing (**proc2d**) program.

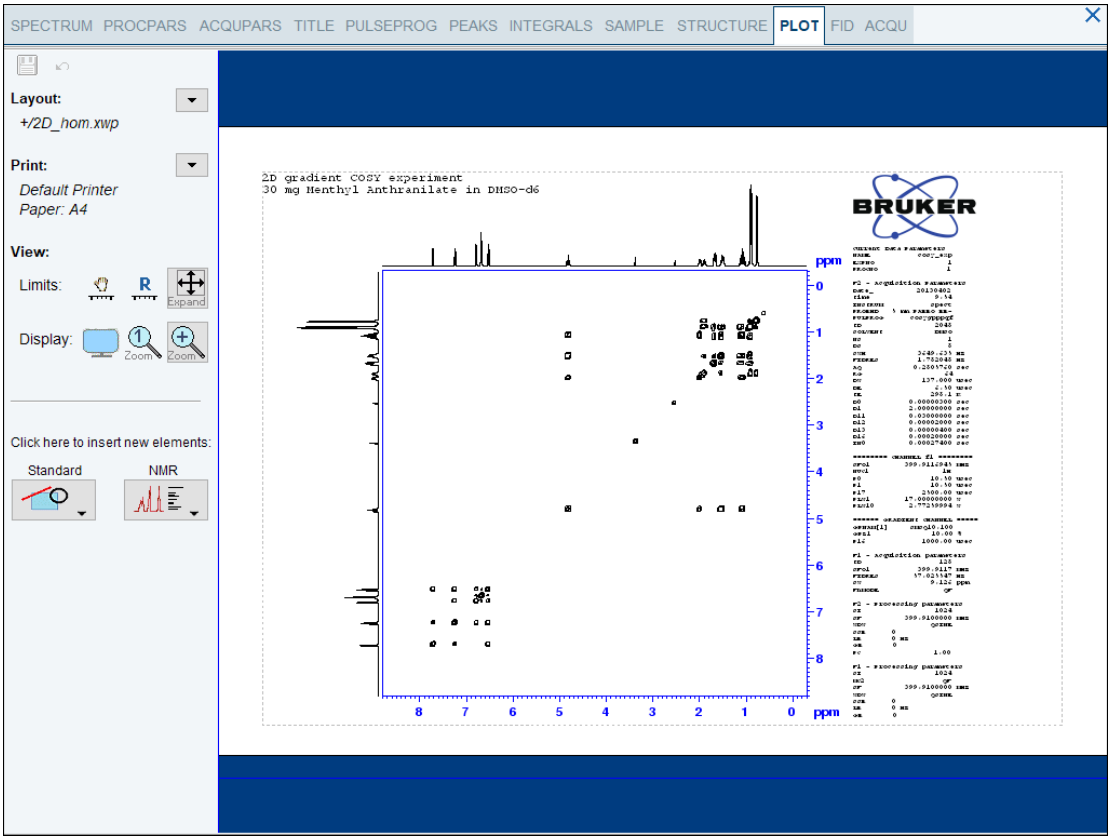


7.2.6 Plotting the COSY Spectrum

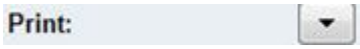
- Use the **Smaller/larger** buttons to adjust for a suitable contour level. 

- 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** ...

7.3 2D Gradient NOESY Experiment

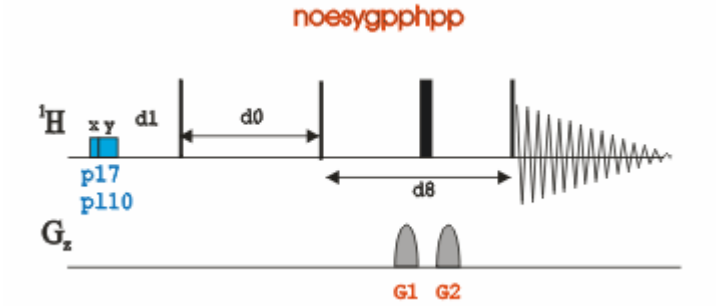
NOESY (Nuclear Overhauser Effect SpectroscopY) is a 2D spectroscopy method used to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear 1H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, 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 π/2 pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t1, 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 d8. Note that, for the basic NOESY experiment, d8 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 t2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t1 and t2.

Axial peaks, which originate from magnetization that has relaxed during tmix, 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.

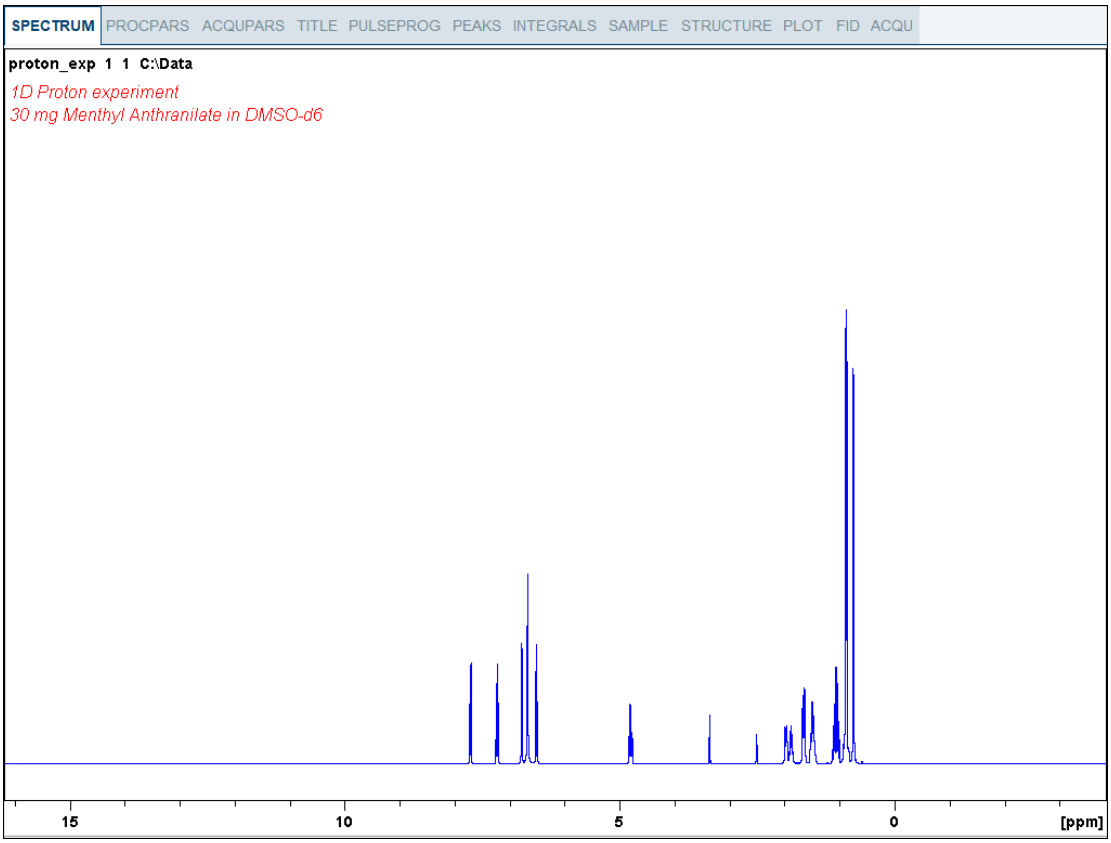
This section describes the acquisition and processing of a two-dimensional 1H phase sensitive NOESY. The standard Bruker parameter set is NOESYPHSW and includes the pulse sequence **noesygpphpp** shown in the next figure. It consists of the recycling delay, three radio-frequency (RF) pulses, separated by the increment delay D0 between the first and second pulse, a mixing time D8 between the second and third 90 degree pulses and the acquisition time during which the signal is recorded. All three pulses are of 90^0.



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 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup [31]* through *Processing [35]*.

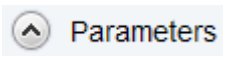


7.3.2 Setting up the NOESY Experiment

- 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 [▶ 58]* 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**.

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.

7.3.3 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

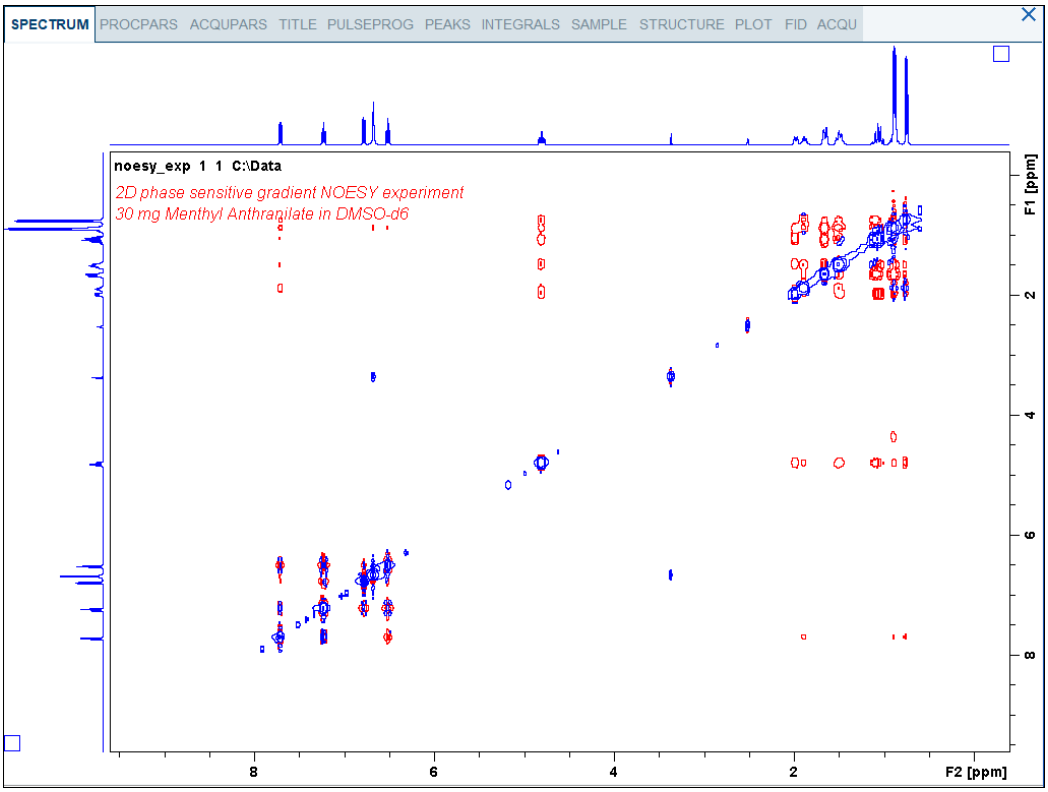
- On the **Run** button, click the **drop-down** arrow to see more options.

7.3.4 Processing

When the acquisition is finished:

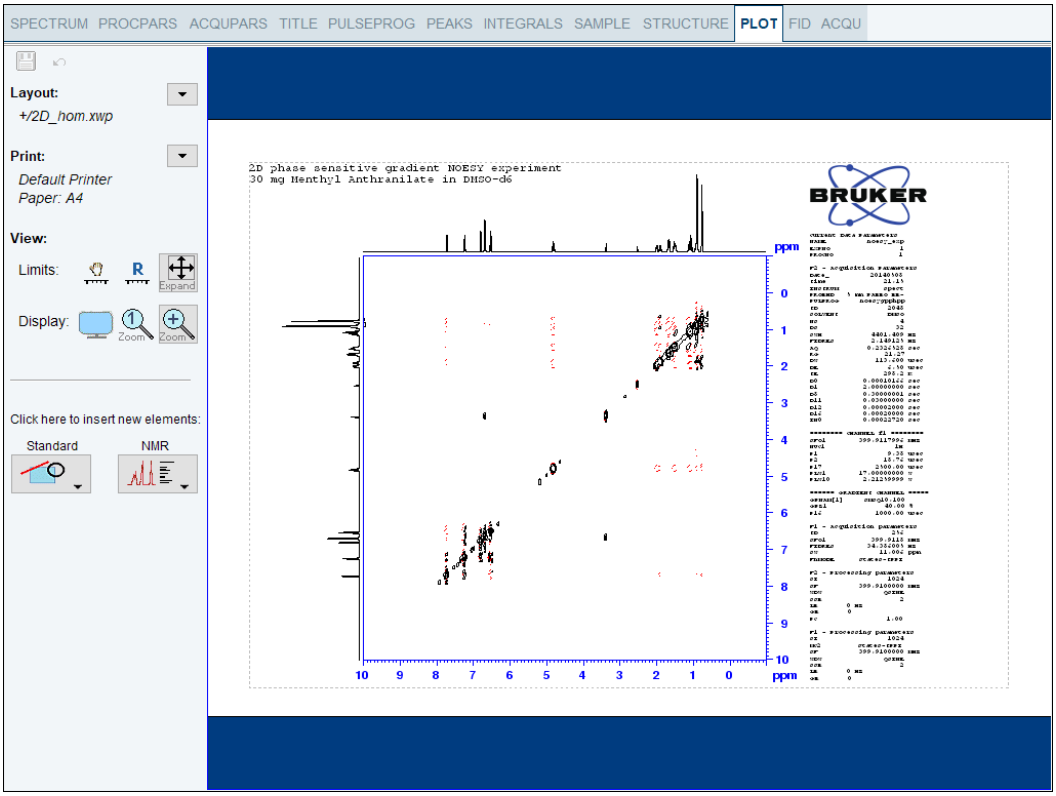
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

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.



7.3.5 Plotting the NOESY Spectrum

- Follow the plotting instructions in the chapter *Plotting the COSY Spectrum [▶ 62]*.

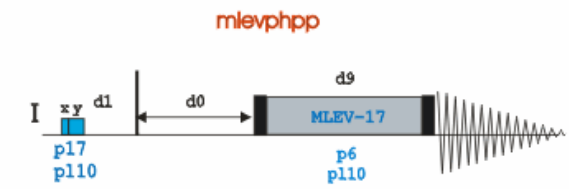


7.4 2D Phase Sensitive TOCSY Experiment

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 JHH) should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

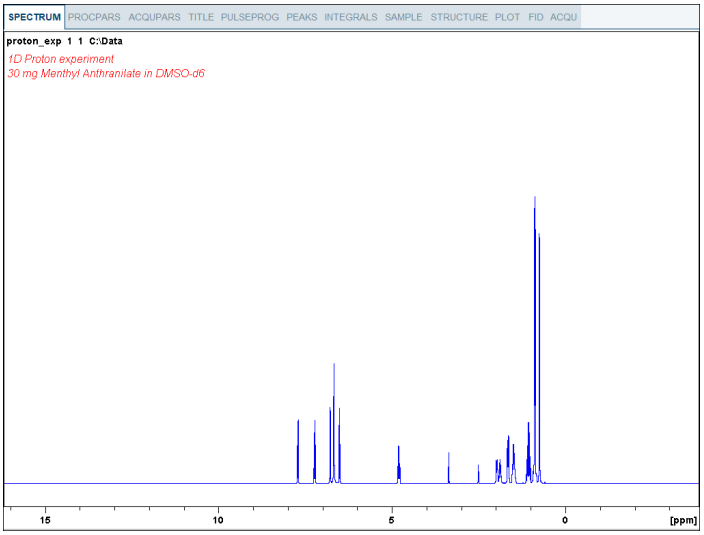
This section describes the acquisition and processing of a two-dimensional ^{1}H phase sensitive TOCSY. The standard Bruker parameter set is MLEVPHSW and includes the pulse sequence **mlevphpp** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay **D0** and the acquisition time during which the signal is recorded. The first RF pulse is a 90^{0} 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.

7.4.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup [▶ 31]* through *Processing [▶ 35]*.

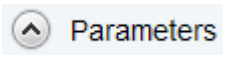


7.4.2 Setting up the TOCSY Experiment

- 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 [▶ 58]* 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**.

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.

7.4.3 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.

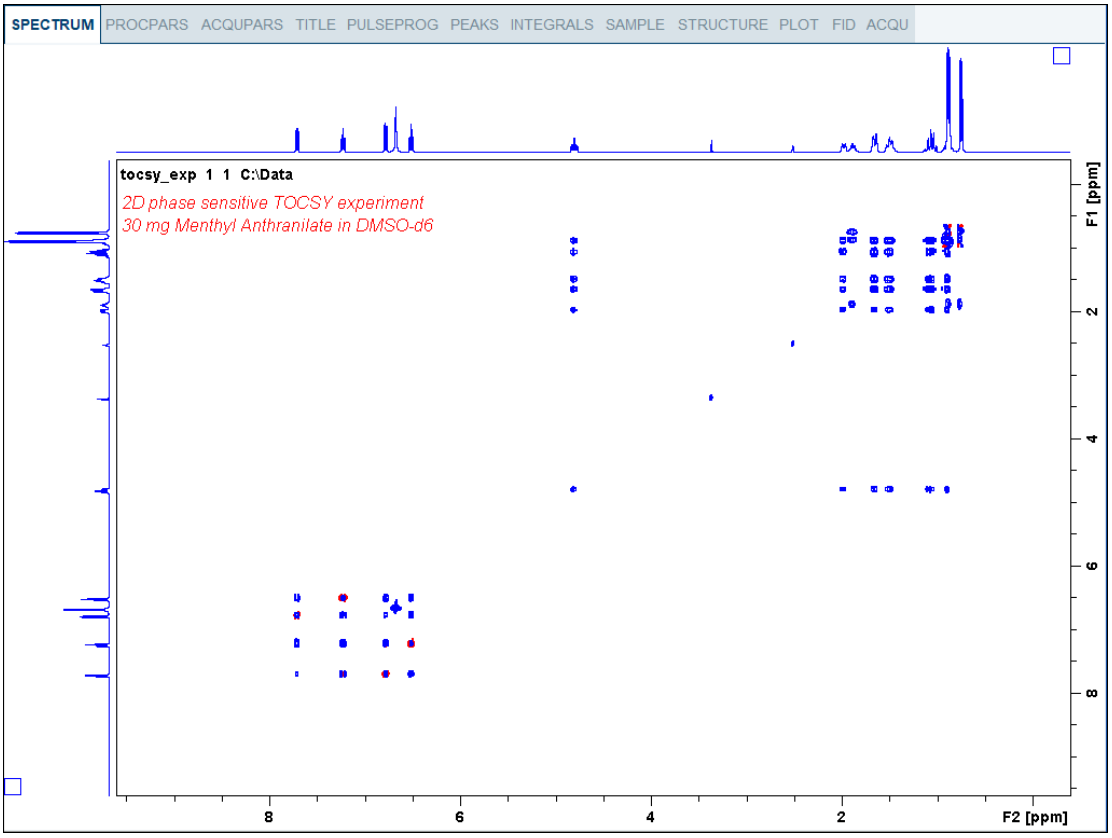
7.4.4 Processing

When the acquisition is finished:

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

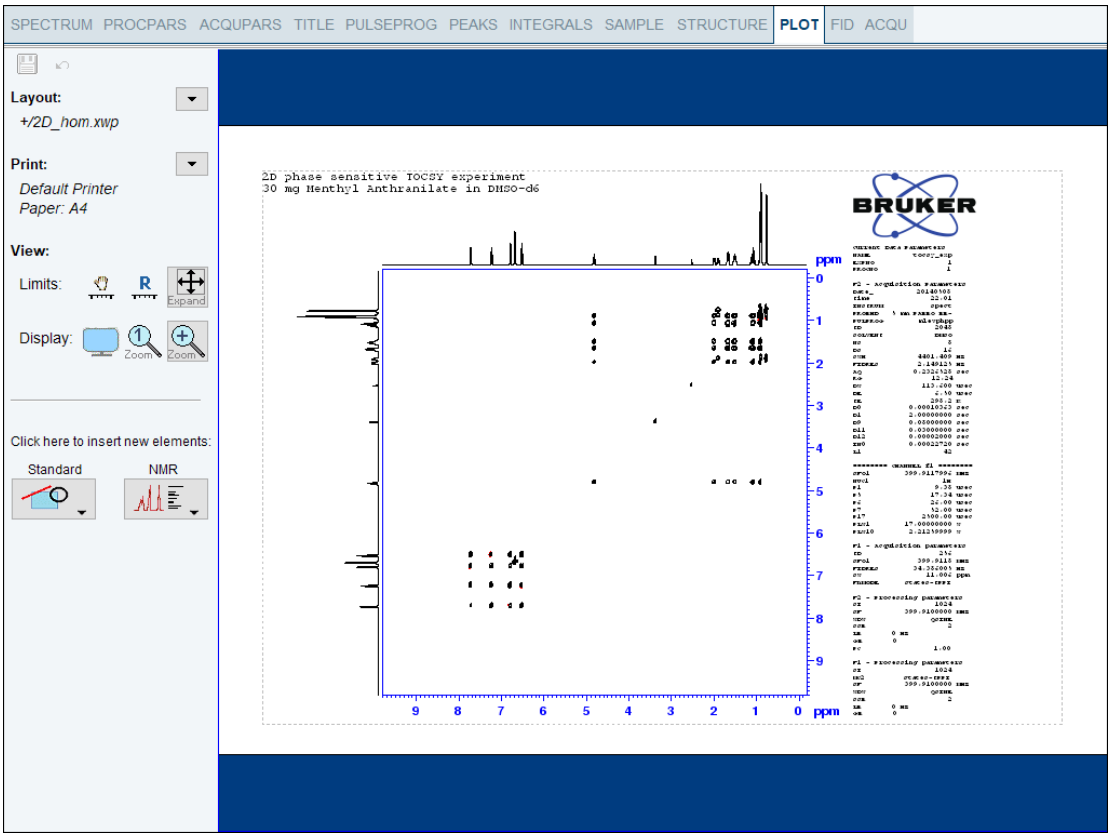
- On the Workflow button bar, click **Proc Spectrum**.

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.



7.4.5 Plotting the TOCSY Spectrum

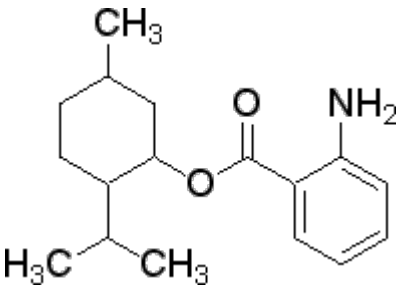
- Follow the plotting instructions in the chapter *Plotting the COSY Spectrum [▶ 62]*.



8 1D Carbon Experiments

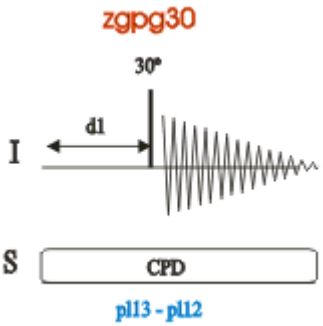
8.1 Sample

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



8.2 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 ^{13}C 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 ^{1}H 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.



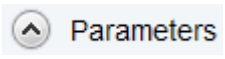
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

8.2.1 Experiment Setup

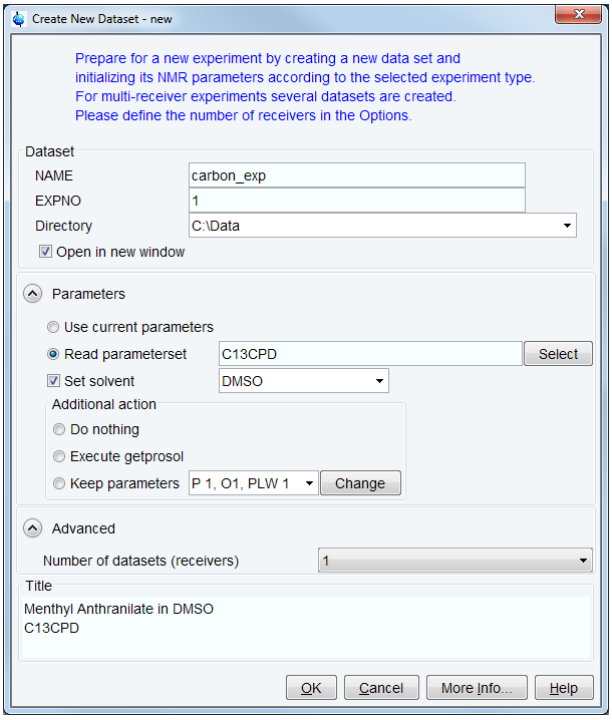
- On the menu bar, click **Acquire** | **Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:

  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**.



- 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 aquire a spectrum, use the Workflow buttons from left to right.

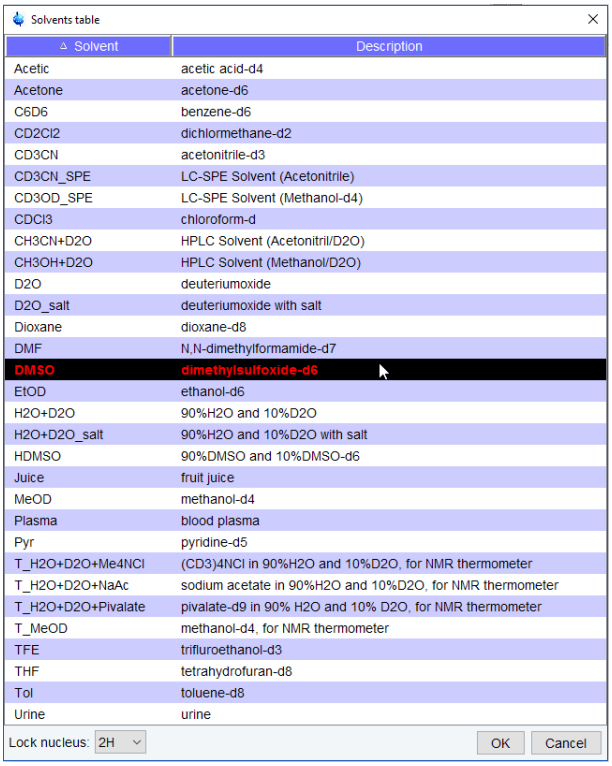
- On the **Sample** button, click the **drop-down** arrow to see more options.
- In the list, select **Eject sample manually (ej)**. The sample lift is turned on.

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**.



- 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.

8.2.2 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.

8.2.3 Processing

When the acquisition is finished:

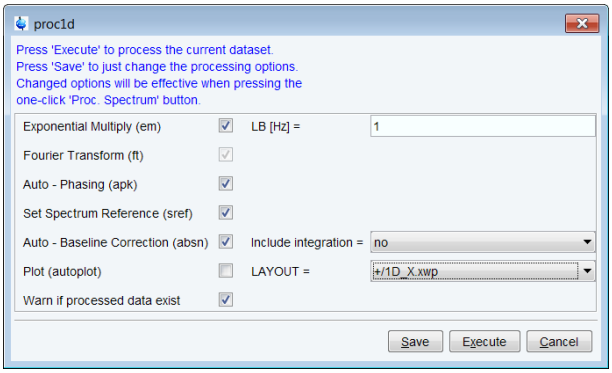
- On the menu bar, click **Process**.
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing (proc1d)**.
- In the proc1d window, select the options:

  Exponential Multiply (em)

  Auto - Phasing (apk)

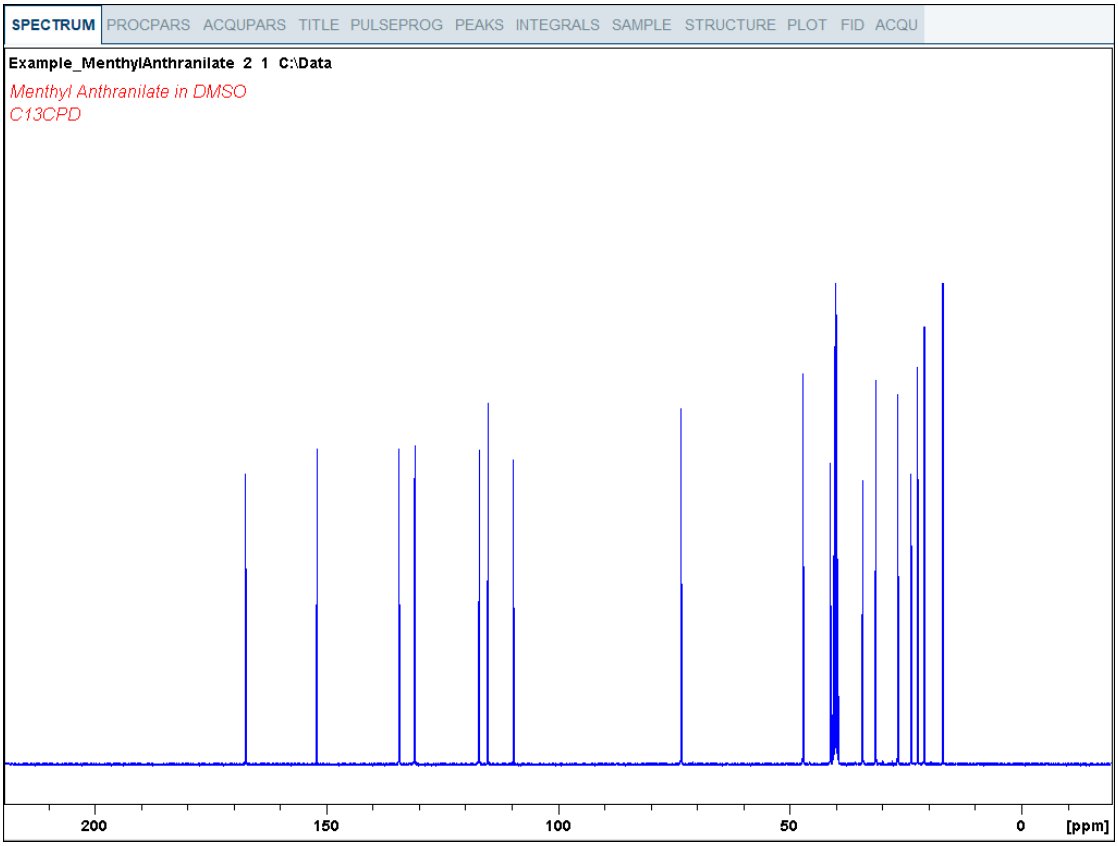
  Set Spectrum Reference (sref)

  Auto - Baseline Correction (absn)



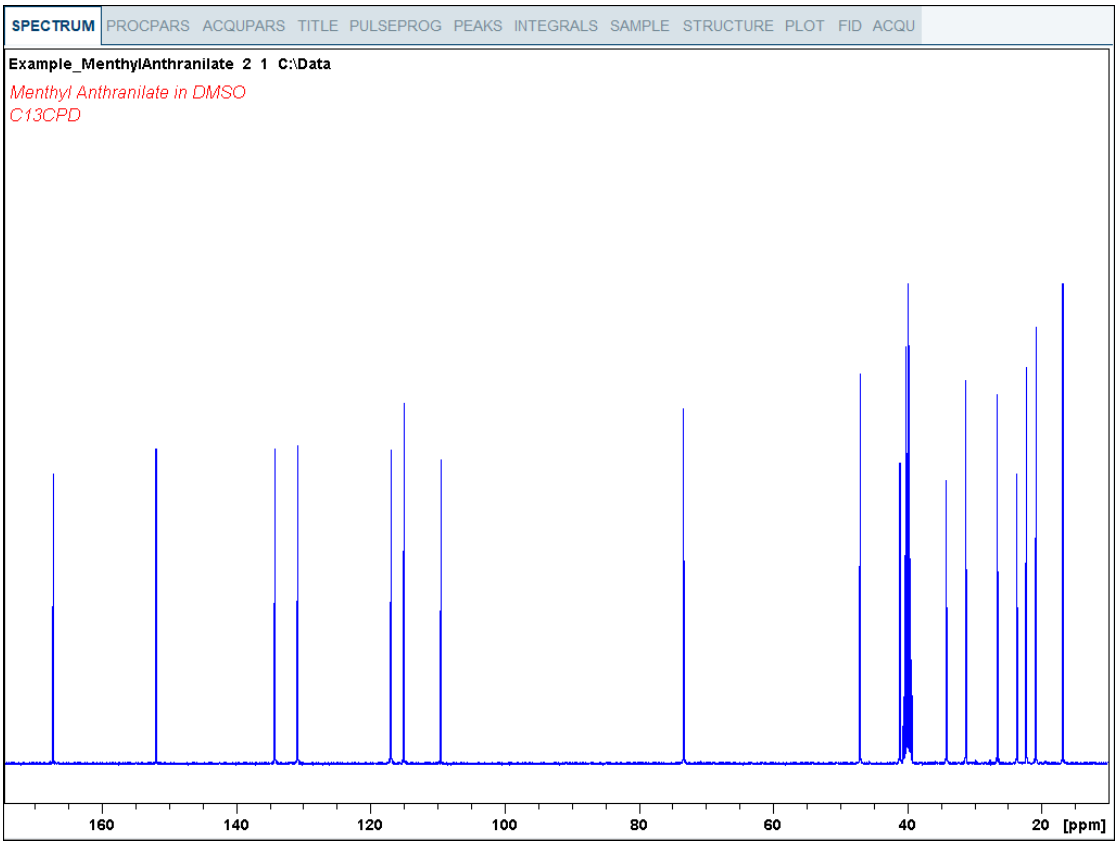
- In the proc1d window, click **Execute**.
- In the proc1d window, click **Save** to save the selected processing settings.

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



8.2.4 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.

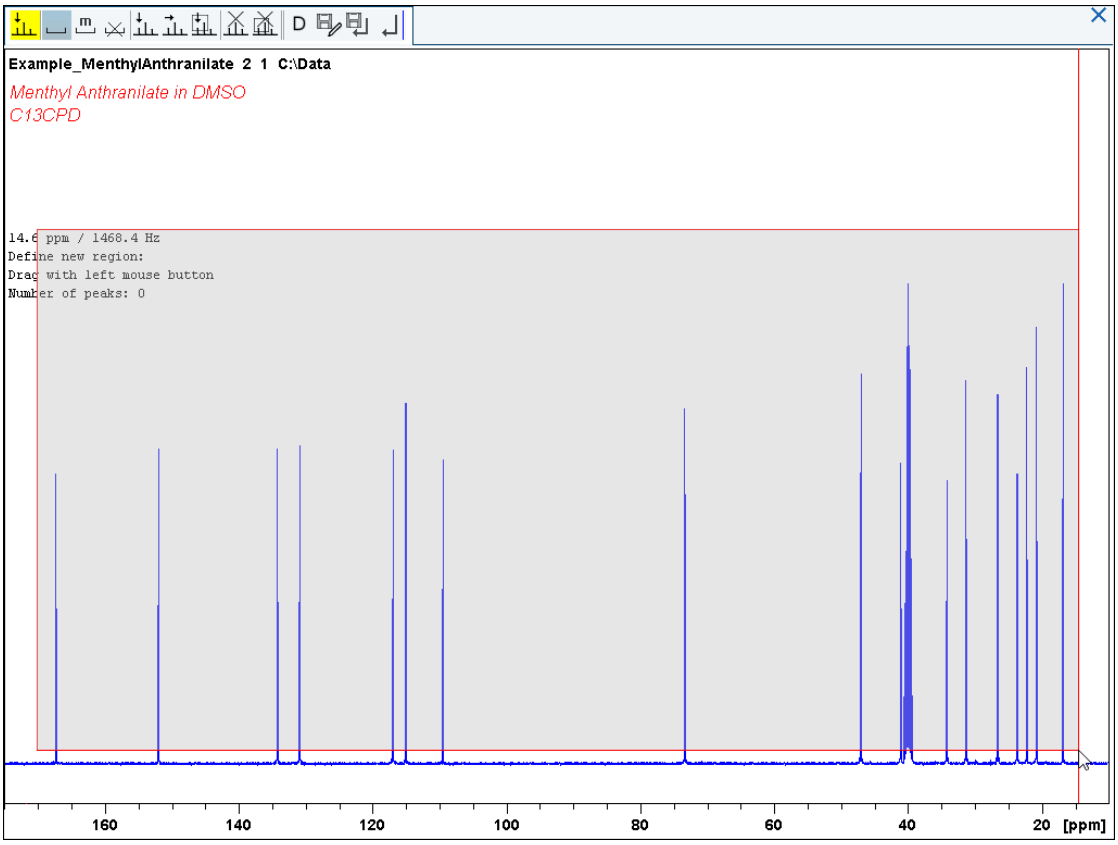
This enters the manual peak picking mode.

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

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

- 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.

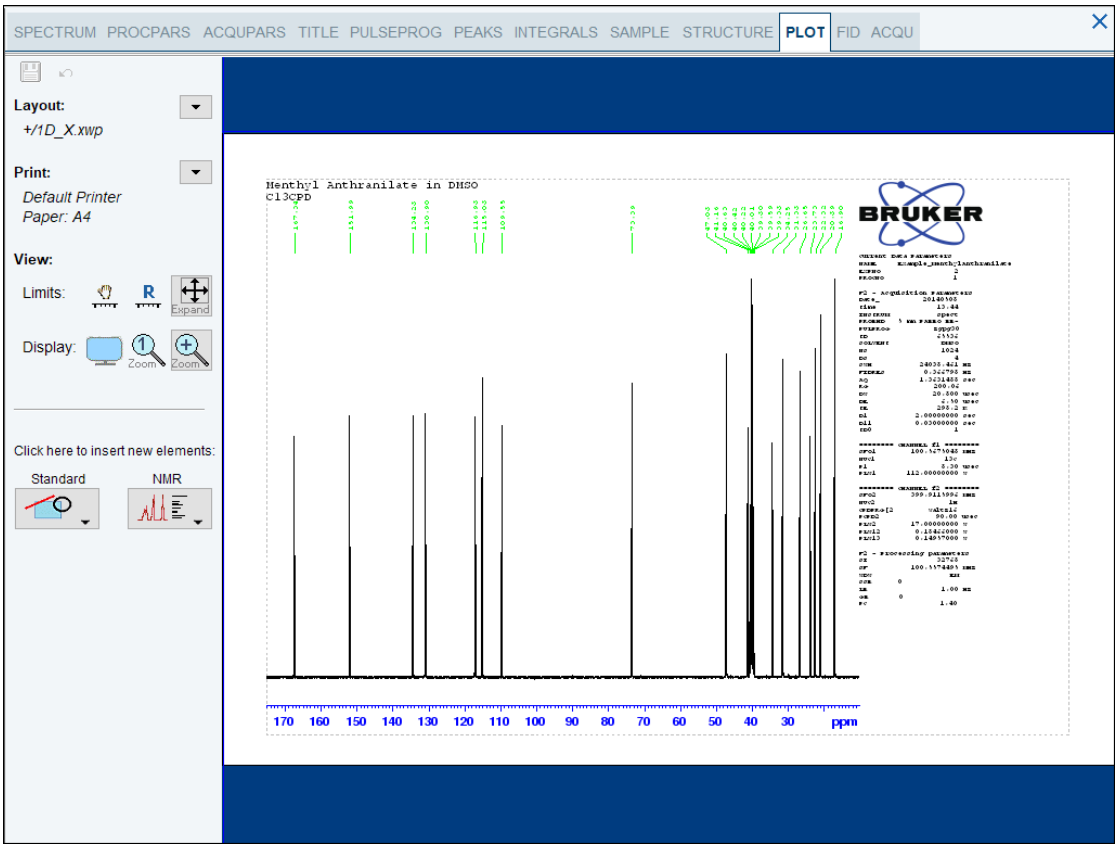
- To display the peak picking labels, right click in the spectrum window and select **Spectra Display Preferences**. In the Spectrum components enable **Peak labels** and **Peak annotations**. Click **Apply** and **Close**.

8.2.5 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).



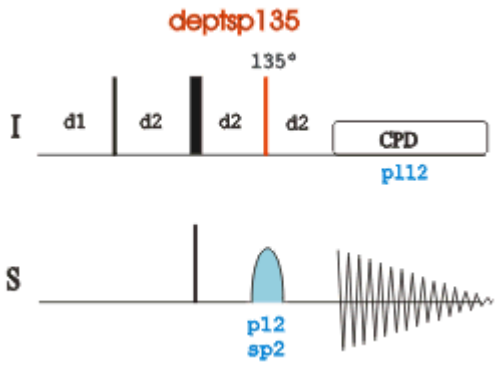
If desired, any changes can be administered with the tools on the left side of the display.

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

8.3 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 13C). DEPT is a spectral editing sequence, that is, it can be used to generate separate 13C 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 13C 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 13C-DEPT135 NMR spectrum. The standard Bruker parameter set C13DEPT135, includes the pulse sequence **deptsp135**, shown in the figure below. The 13C 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*J(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.

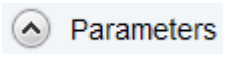
8.3.1 Experiment Setup

This experiment usually follows a regular ^{1}H 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 

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.

8.3.2 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

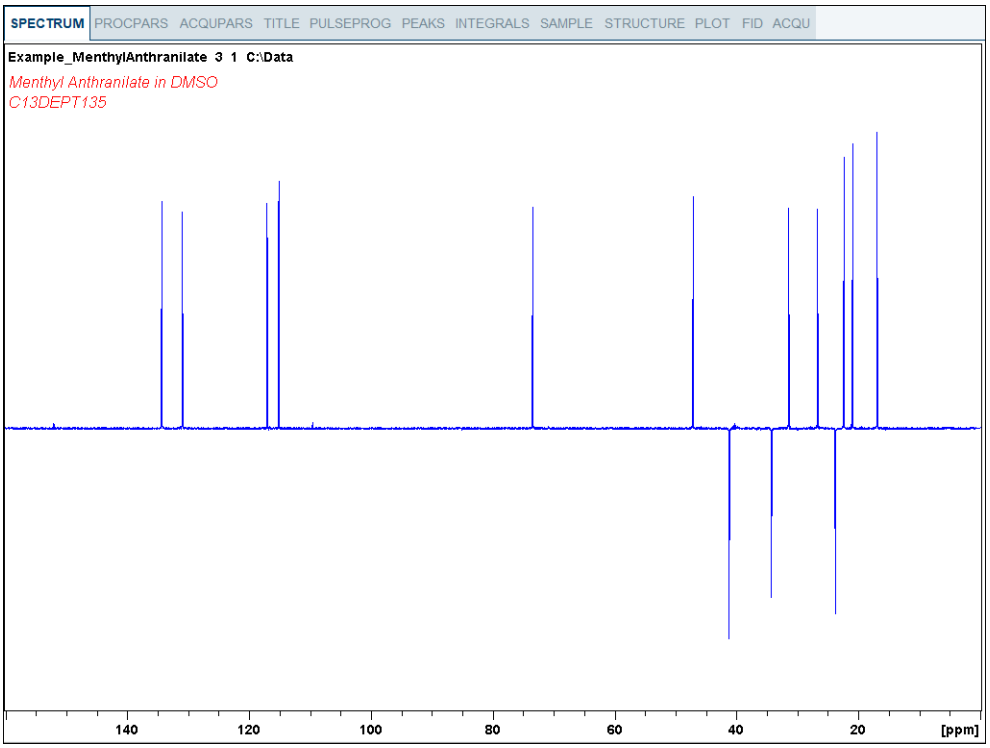
- On the **Run** button, click the **drop-down** arrow to see more options.

8.3.3 Processing

When the acquisition is finished:

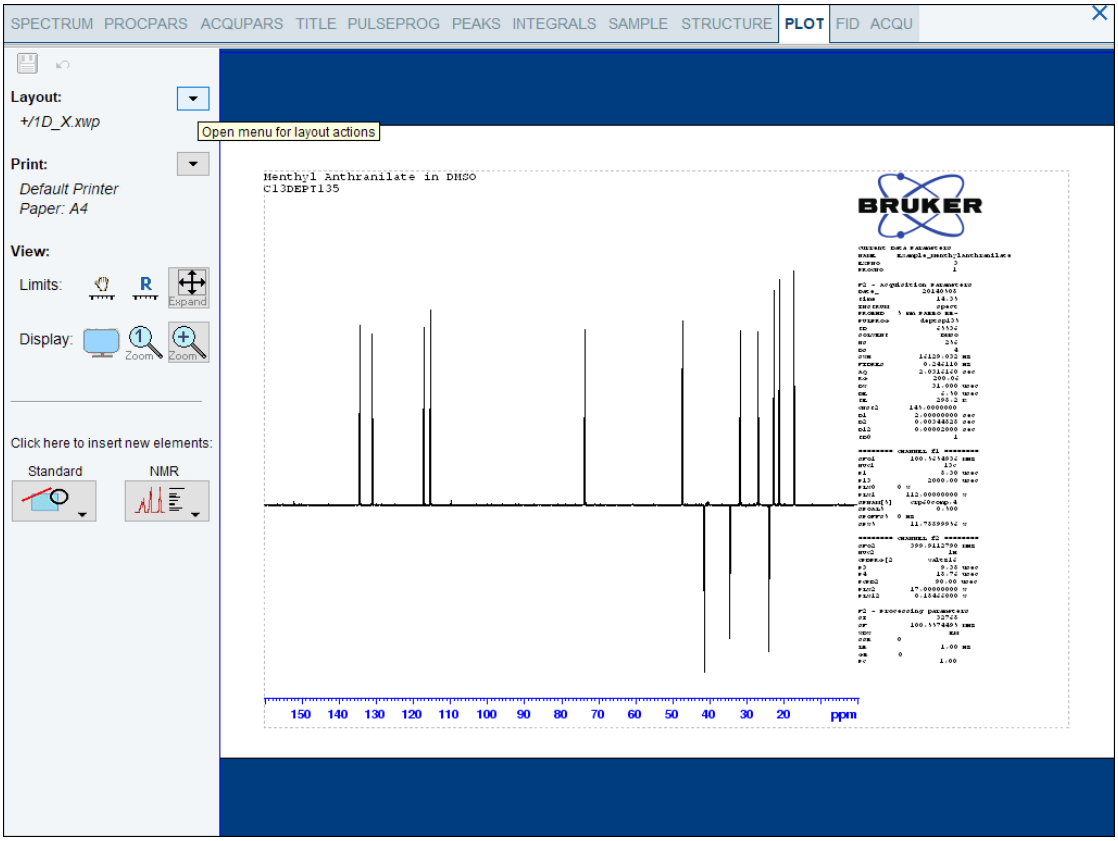
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

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 -CH_3 peaks phased positively and -CH_2 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.



8.3.4 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**. 



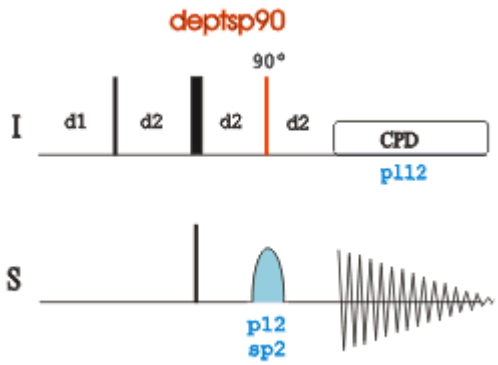
If desired, any changes can be administered with the tools on the left side of the display.

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

8.4 DEPT-90 Experiment

This section describes the acquisition and processing of a one-dimensional 13C-DEPT90 NMR spectrum. The standard Bruker parameter set C13DEPT90, includes the pulse sequence **dept90**, shown in the next figure. The 13C 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*J(XH). 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.

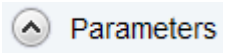
8.4.1 Experiment Setup

The DEPT90 experiment usually follows a regular ^{1}H 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.

8.4.2 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

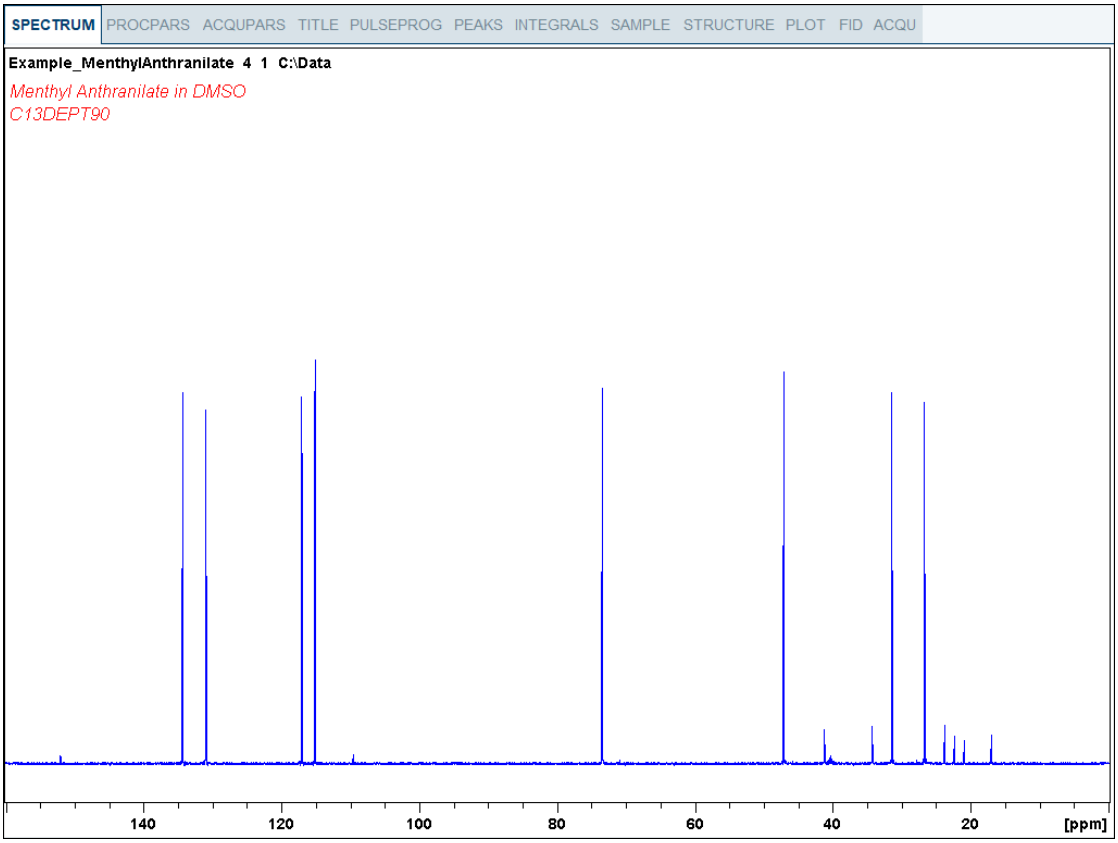
- On the **Run** button, click the **drop-down** arrow to see more options.

8.4.3 Processing

When the acquisition is finished:

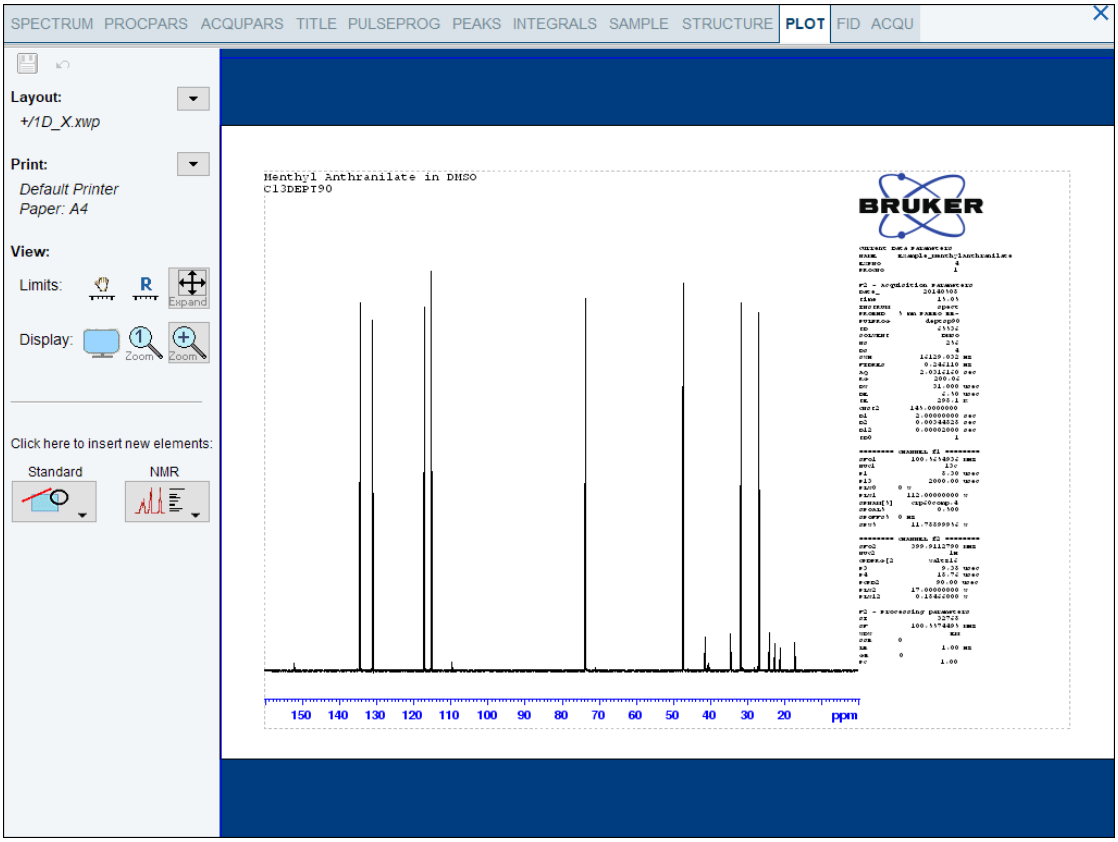
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

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)**.



8.4.4 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). 



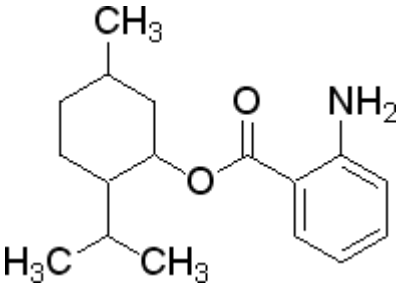
If desired, any changes can be administered with the tools on the left side of the display.

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

9 2D Heteronuclear Experiments

9.1 Sample

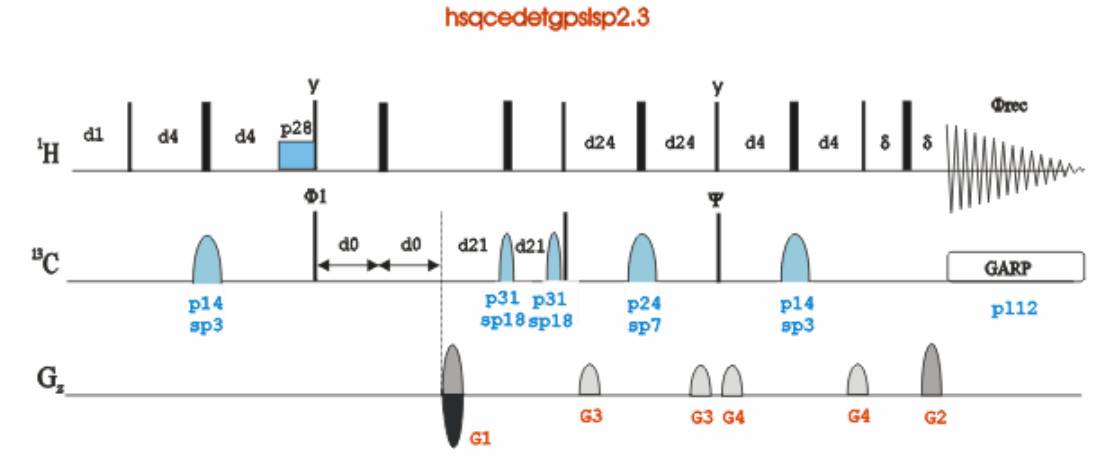
The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



9.2 2D Edited HSQC

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

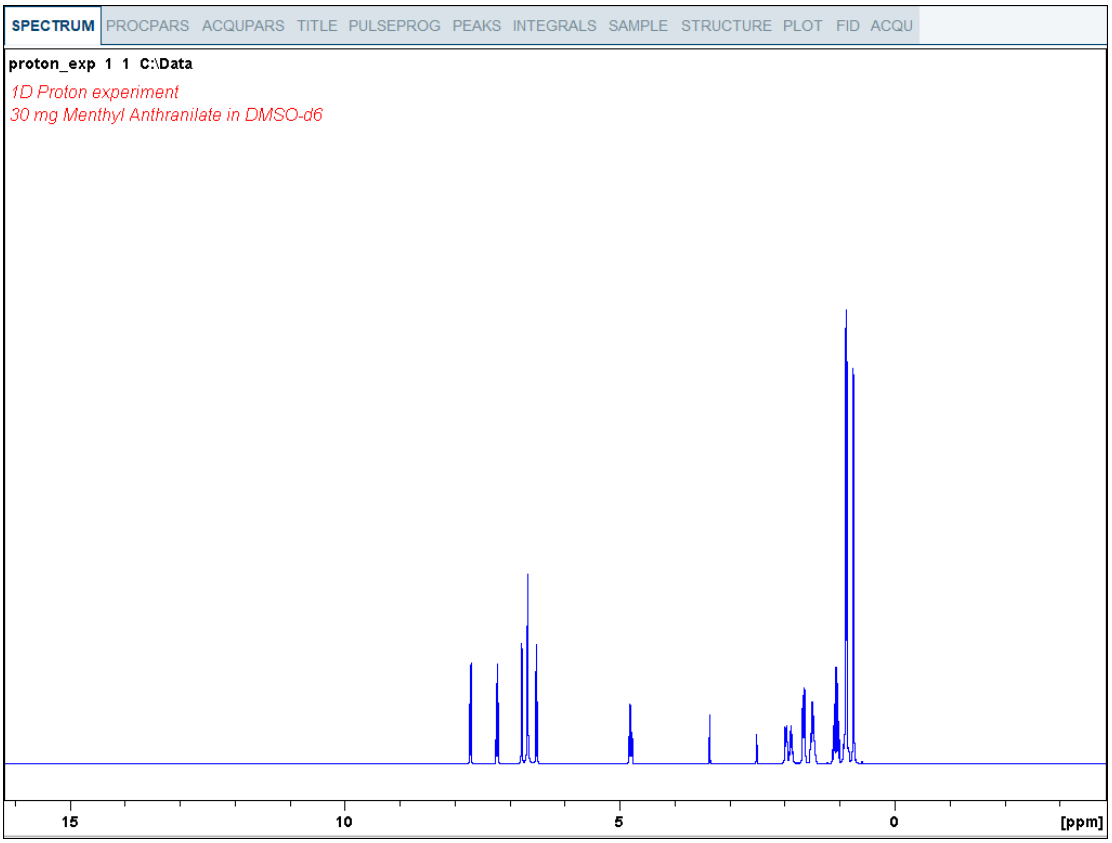
In the sequence shown in the next figure, the signals are not broadened by homonuclear H,H coupling in F1. It is possible to obtain a complete editing of inverse recorded 1D H,X correlation spectra. This kind of multiplicity determination has been achieved by including an editing period within HSQC. 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.

9.2.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, *Experiment Setup [▷ 31]* through *Processing [▷ 35]*.

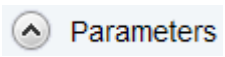


9.2.2 The HSQC Experiment Setup

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

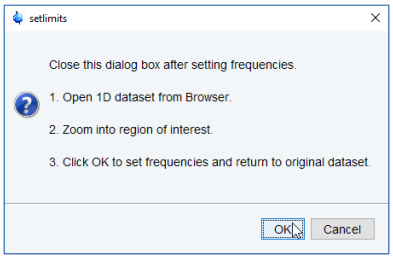
2D experiments should be run non-spinning.

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

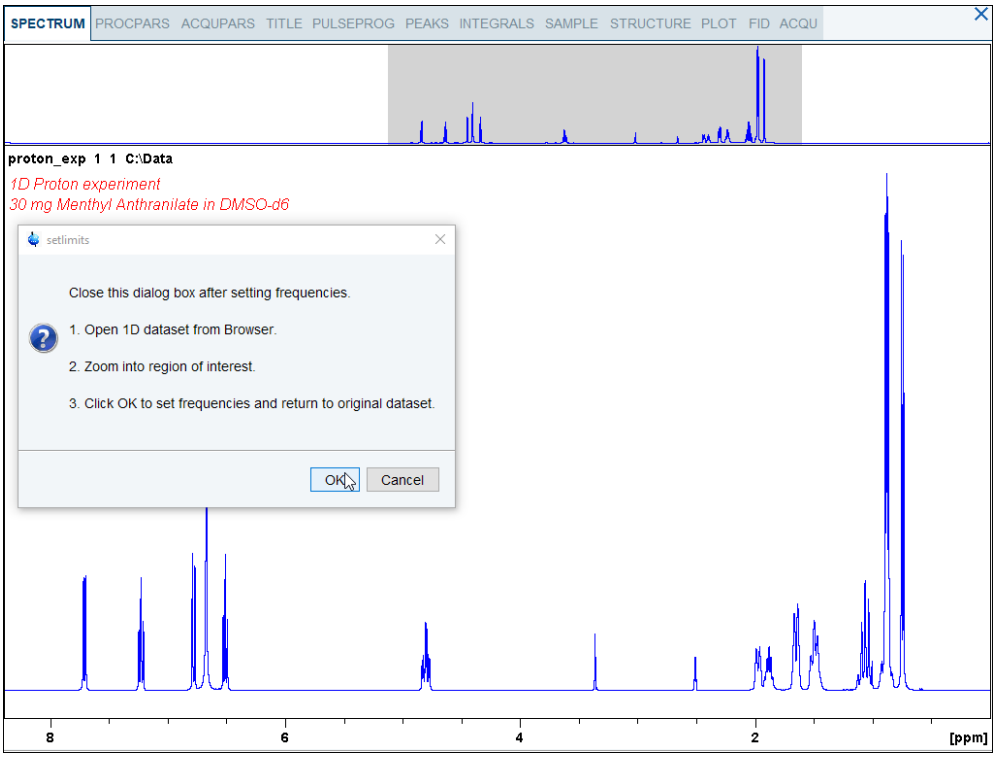
This will load the pulse widths and power levels into the parameter set.

9.2.3 Limit Setting

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

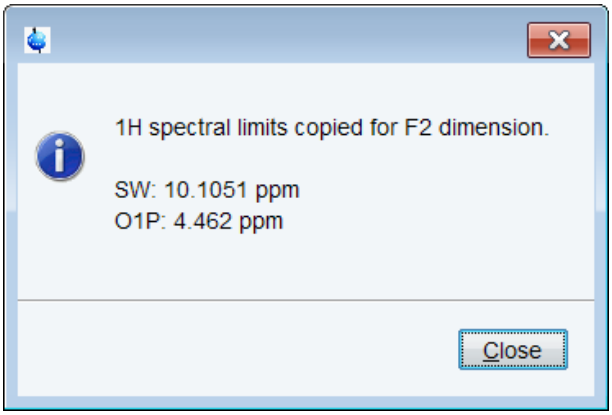


- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or drag the 1D Proton dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.



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

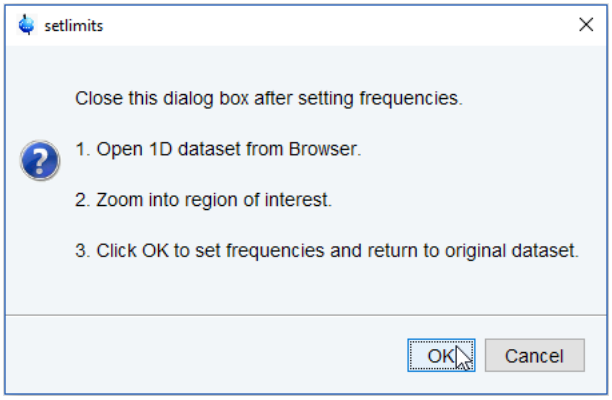
- In the message window, click **Close**.



The display changes back to the 2D dataset.

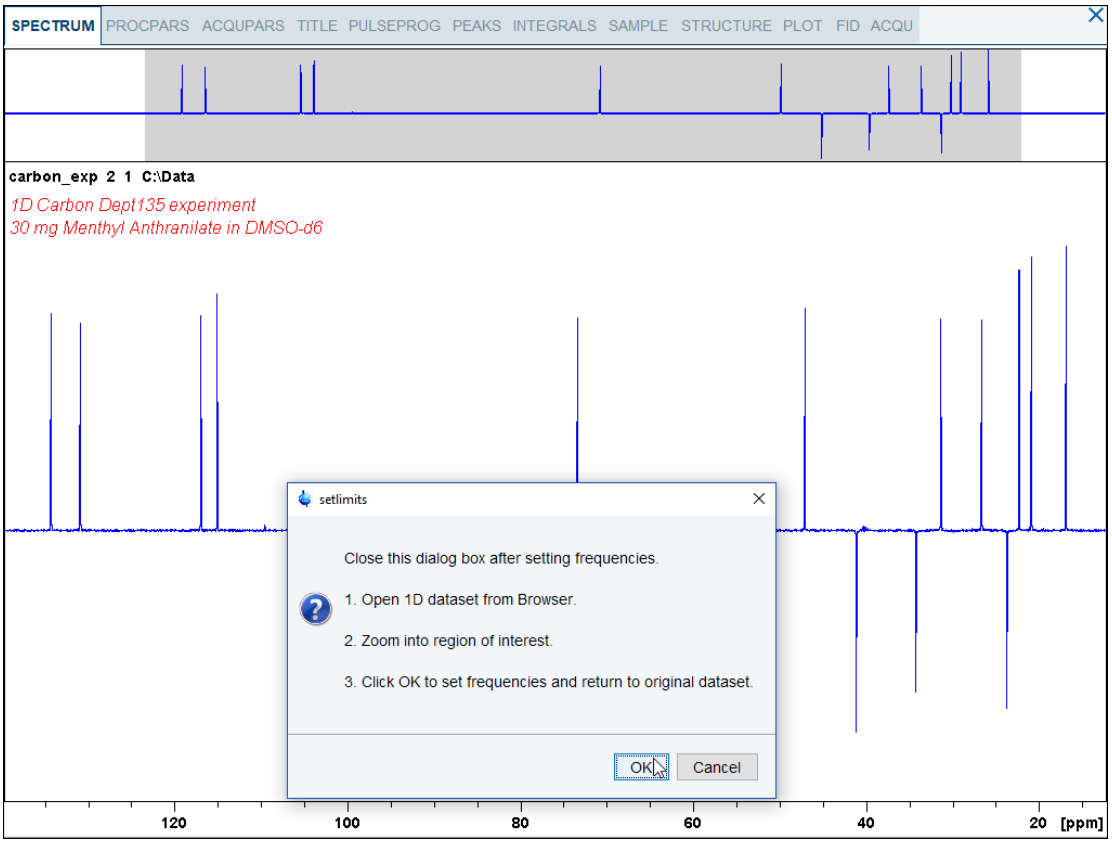
The parameter set HSQCEDETGPSISP2.3_ADIA has a fixed F1 sweep width of **160 ppm** and it is big enough to cover the protonated resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the **SetLimits** button for a second time. In this case a 1-D **C13DEPT45** 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**.

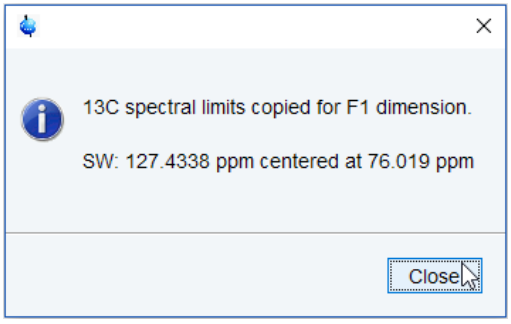


- To open the 1D C13DEPT135 spectrum, right click on the dataset name in the browser window (e.g. **carbon_exp 2**) and select **Display** or drag the 1D C13DEPT135 dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **5 ppm** of baseline on either side of the spectrum.

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**.



9.2.4 Acquisition

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

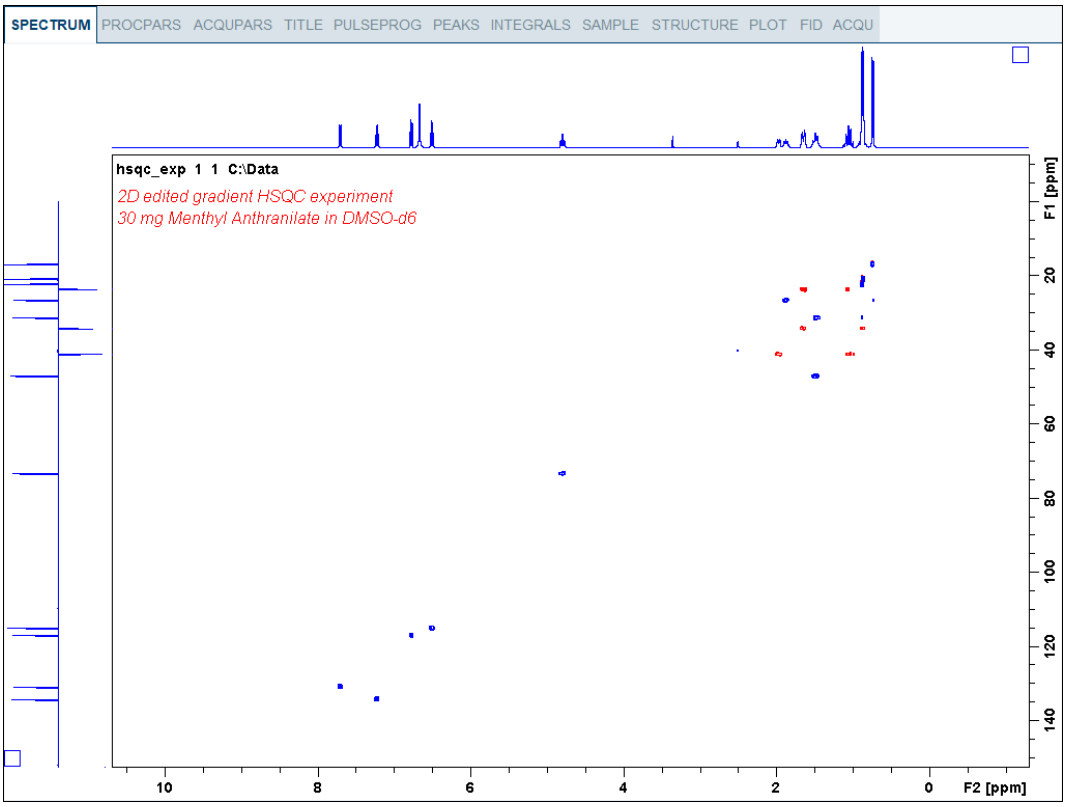
9.2.5 Processing

When the acquisition is finished:

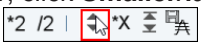
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

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 CH_3 groups will phase the same and CH_2 groups will phase oppositely. The convention is to phase CH,CH_3 positive and CH_2 negative. The **apk2d** command may invert this.



9.2.6 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.

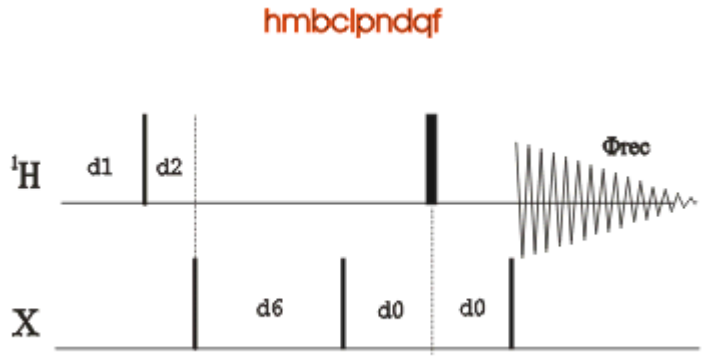
The default layout is designed not to show the F1 projection and the negative peak colors are not assigned. A new layout has to be created to add the F1 projection (in this example, red).

9.3 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-90^0(13C) cluster) can be included after the initial 90^0 1H pulse to minimize direct response.
- The defocusing period is optimized to 1/2*^{n}J(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.

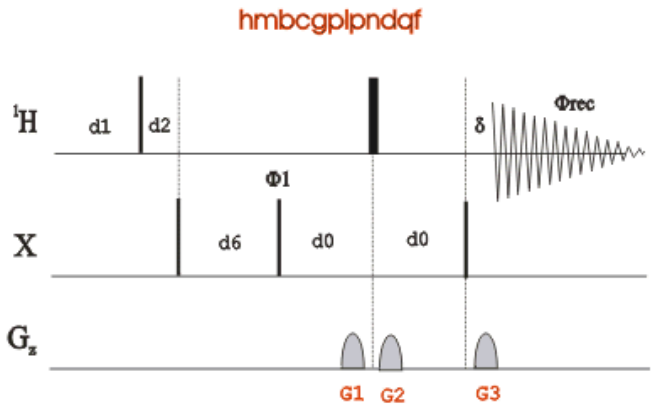


The non-gradient 2D HMBC spectrum of Menthyl Anthranilate in DMSO-d6 shows considerable artifacts. Additionally a minimum number of 8 scans is required for the full phase cycling.

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

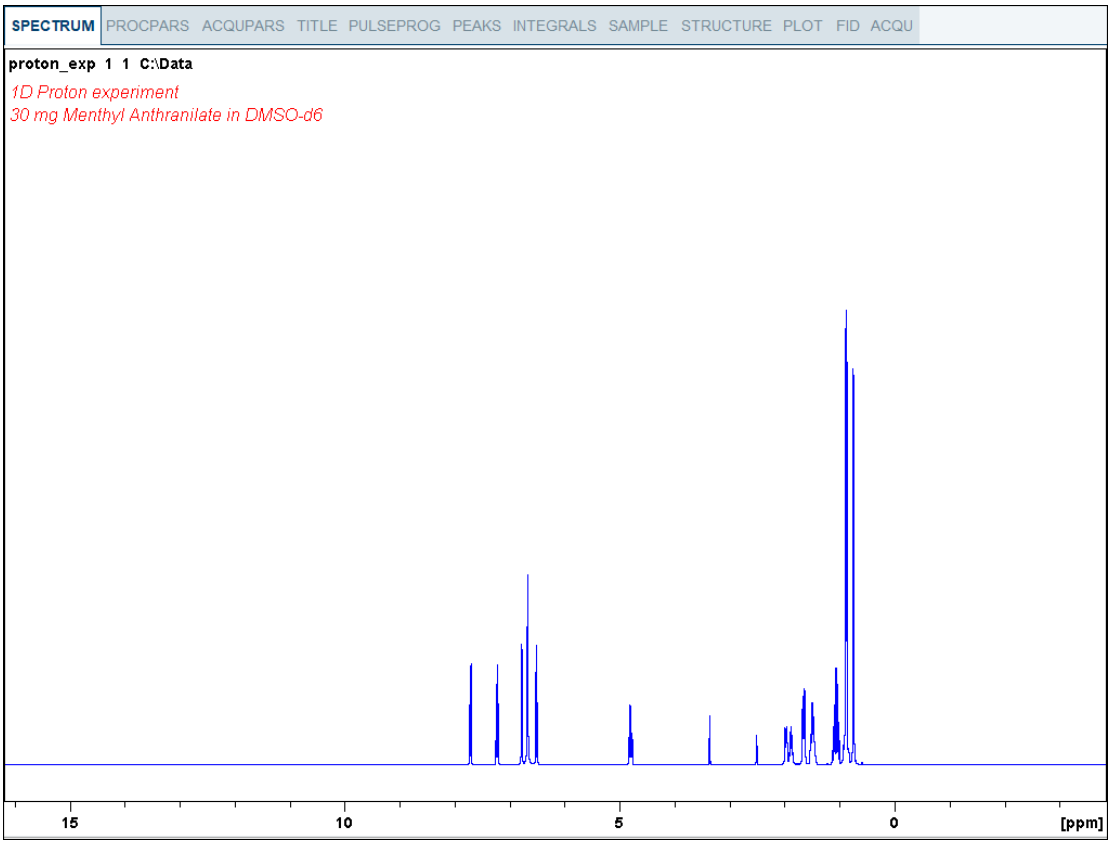
- Coherence selection and frequency-discrimination in the indirect dimension (F1) can be achieved with a single scan per t1 increment.
- A reduction in the number of required phase cycle steps for the suppression of undesired coherences.
- An important decrease in the total acquisition times for sufficiently 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-12C (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.



9.3.1 Preparation Experiment

- Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, *Experiment Setup [▶ 31]* through *Processing [▶ 35]*.

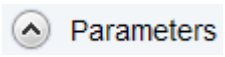


9.3.2 The HMBC Experiment Setup

- 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 **HMBCGPND**
- 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**.

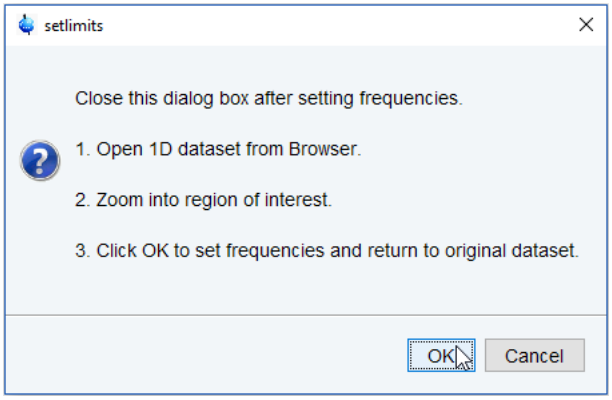
2D experiments should be run non-spinning.

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

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

9.3.3 Limit Setting

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

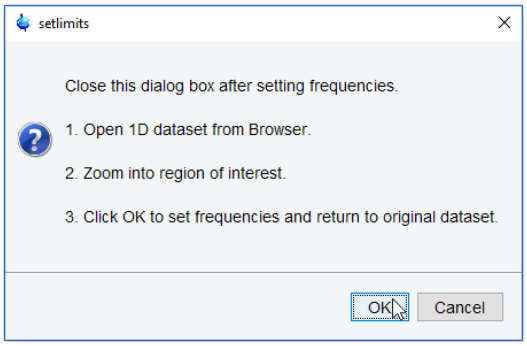


- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. **proton_exp**) and select **Display** or drag the 1D Proton dataset into the spectrum window.

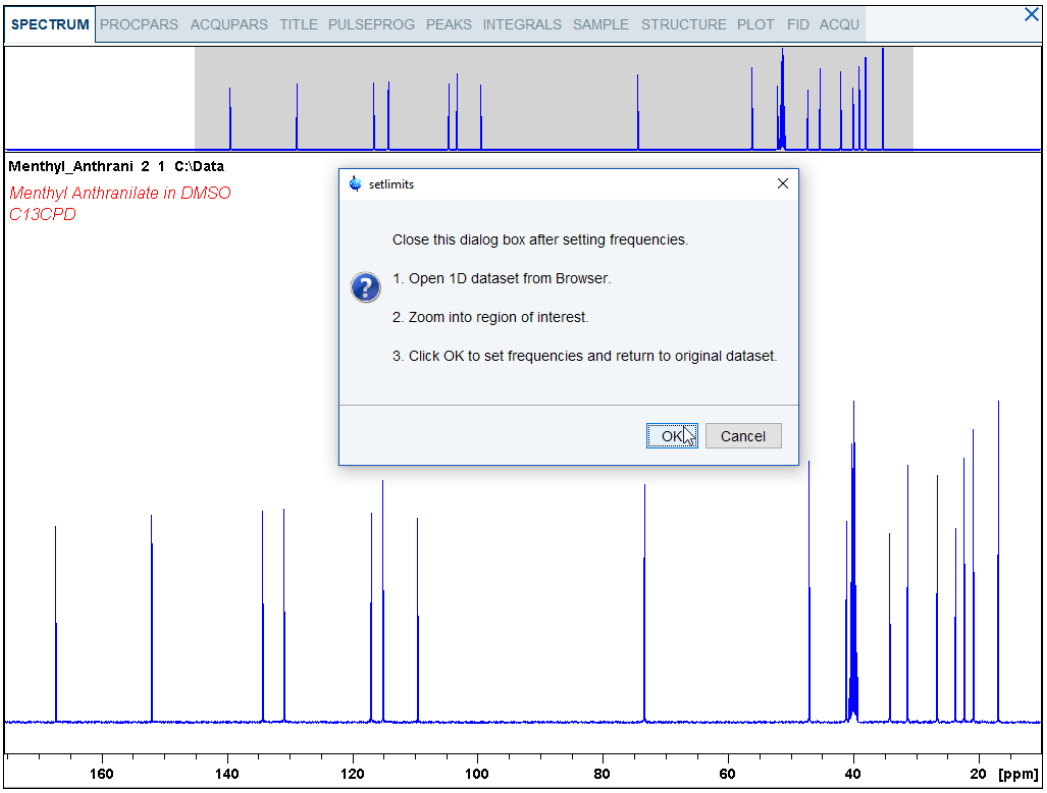
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.
- Click **OK** in the setlimits message window to assign the new limit.

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.

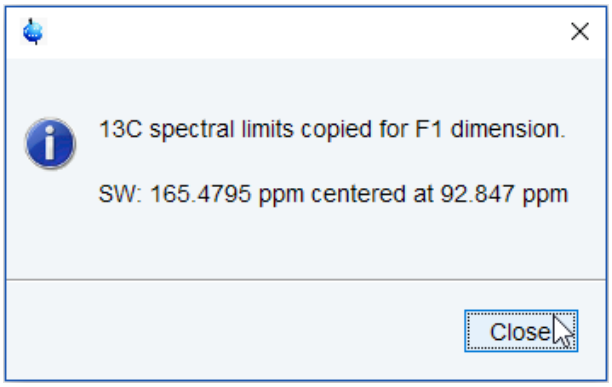
- On the Workflow button bar, click **SetLimits**.



- 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**.

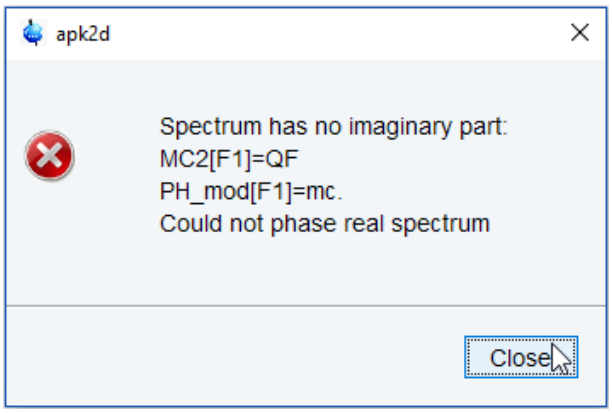


9.3.4 Acquisition

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

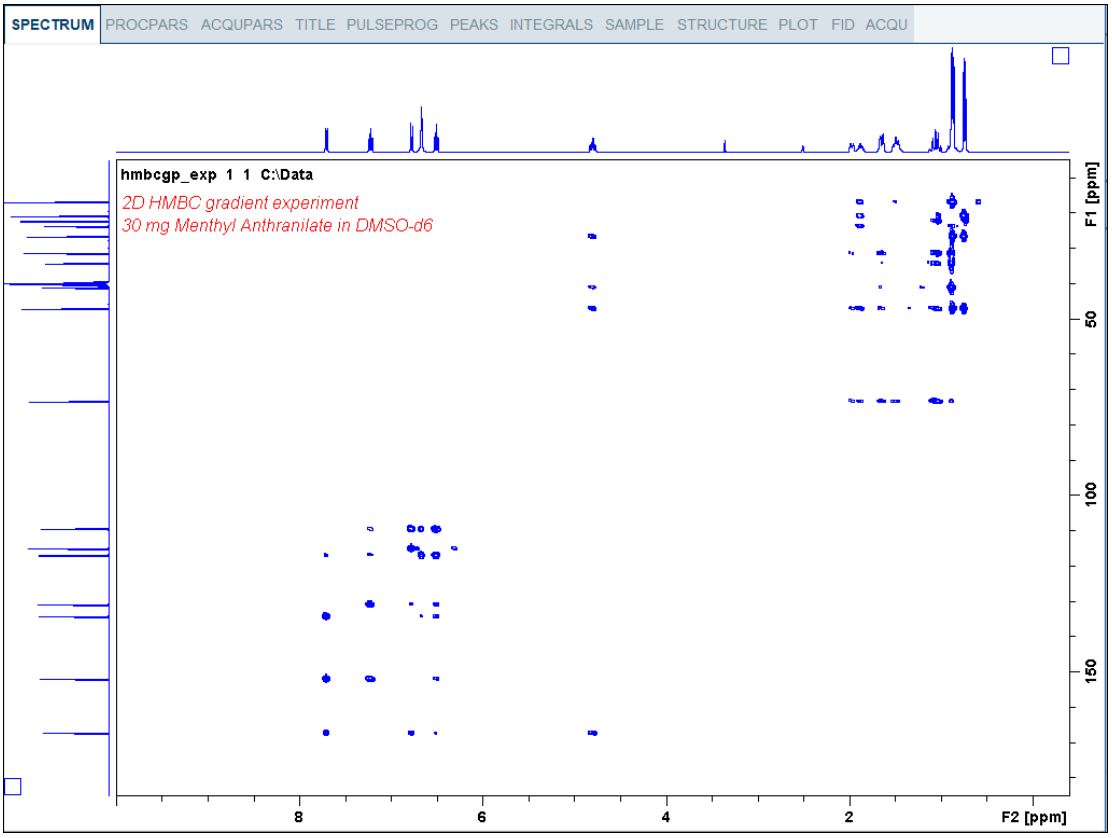
9.3.5 Processing

- 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**.



9.3.6 Plotting the 2D HMBC Spectrum

Follow the instructions in chapter *Plotting the 2D HSQC Spectrum [▶ 90]*.

10 Determination of 90 Degree Pulses

This chapter describes pulse calibration procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter *1D Proton Experiment [▶ 31]* and chapter *1D Carbon Experiments [▶ 71]*.

This chapter is intended as a guide for calibrating the 90^0 pulse of a probe or verifying the values observed using ATP.

10.1 Proton 90 Degree Transmitter Pulse

Standard Test Sample:

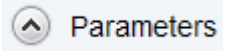
0.1% Ethylbenzene in CDCl3

10.1.1 Parameter Setup

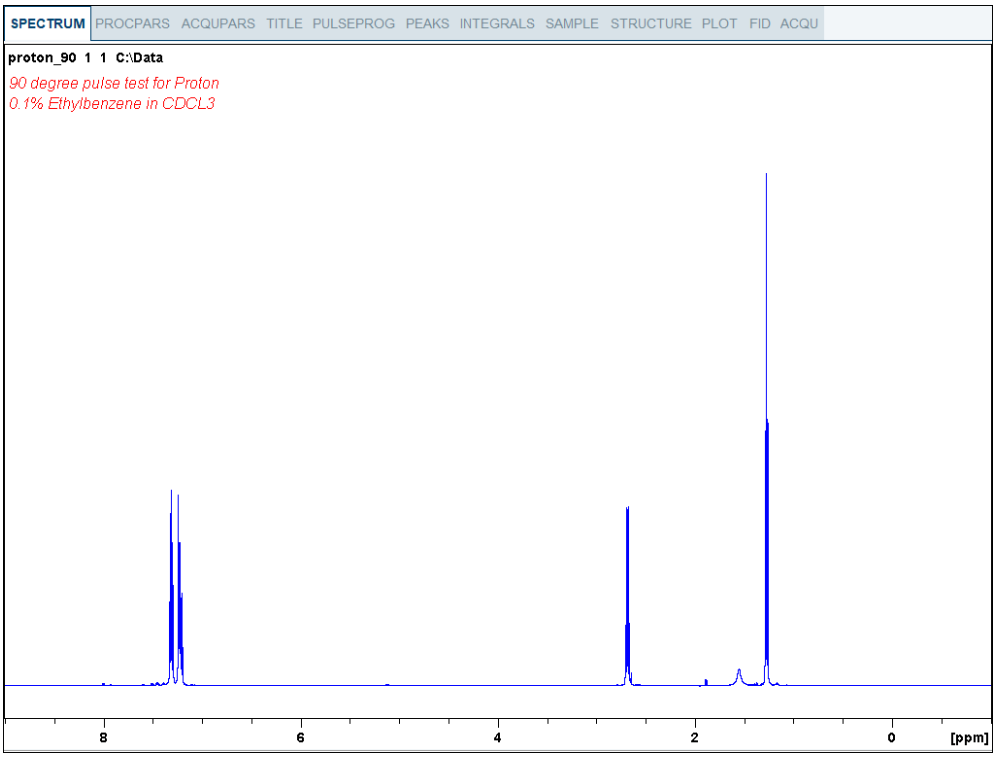
- On the menu bar, click **Acquire** | **Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:

  NAME = **proton_90**

  EXPNO = **1**

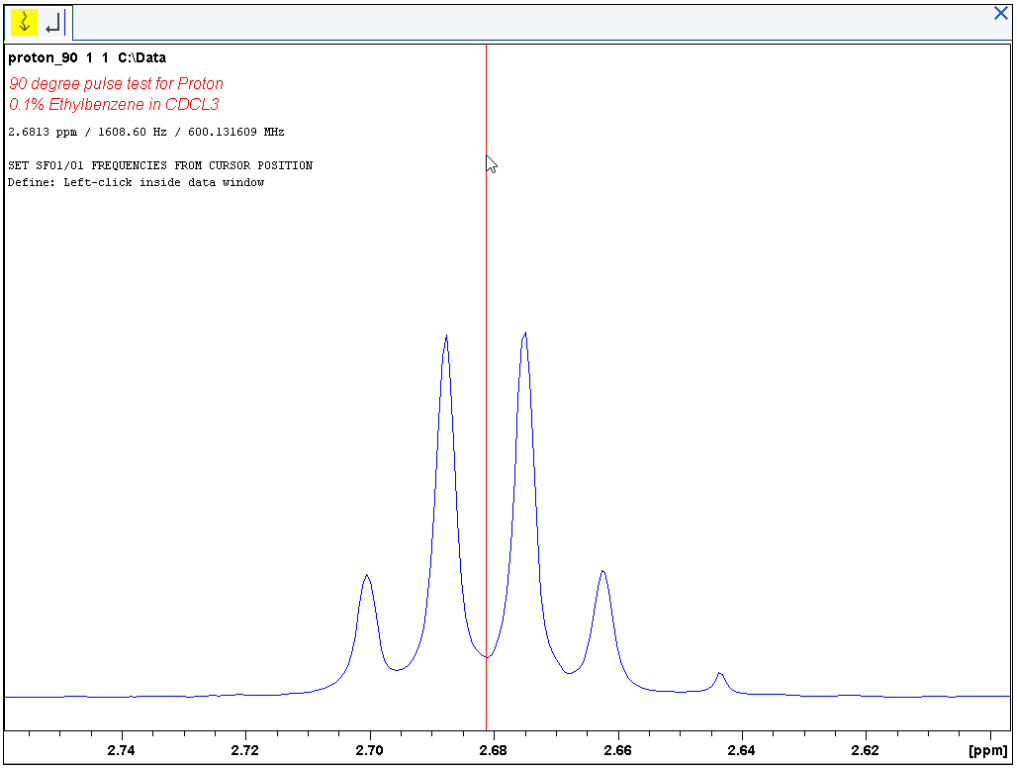
  Directory = e.g. *C:\Data*
- In the Parameters group 

click **Read parameterset** and select the experiment **PROTON**
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCl3**.
- 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 [▶ 99]* in chapter 1D Proton Experiment through Processing *Processing [▶ 35]* described in this manual.

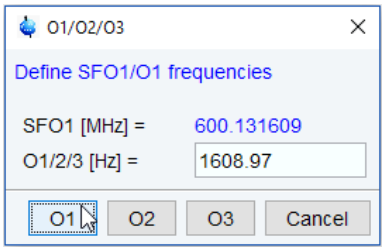


- Expand the peak at **2.7 ppm**.
- On the toolbar, click **Set RF from cursor**. 

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**.



- 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.

10.1.2 Acquisition

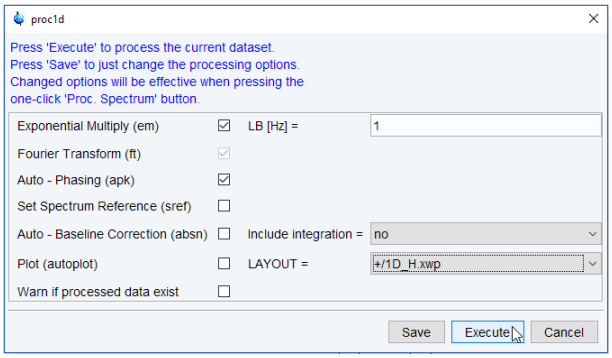
- On the menu bar, click **Acquire**.
- On the Workflow button bar, click **Gain**.
- On the Workflow button bar, click **Run**.

10.1.3 Processing

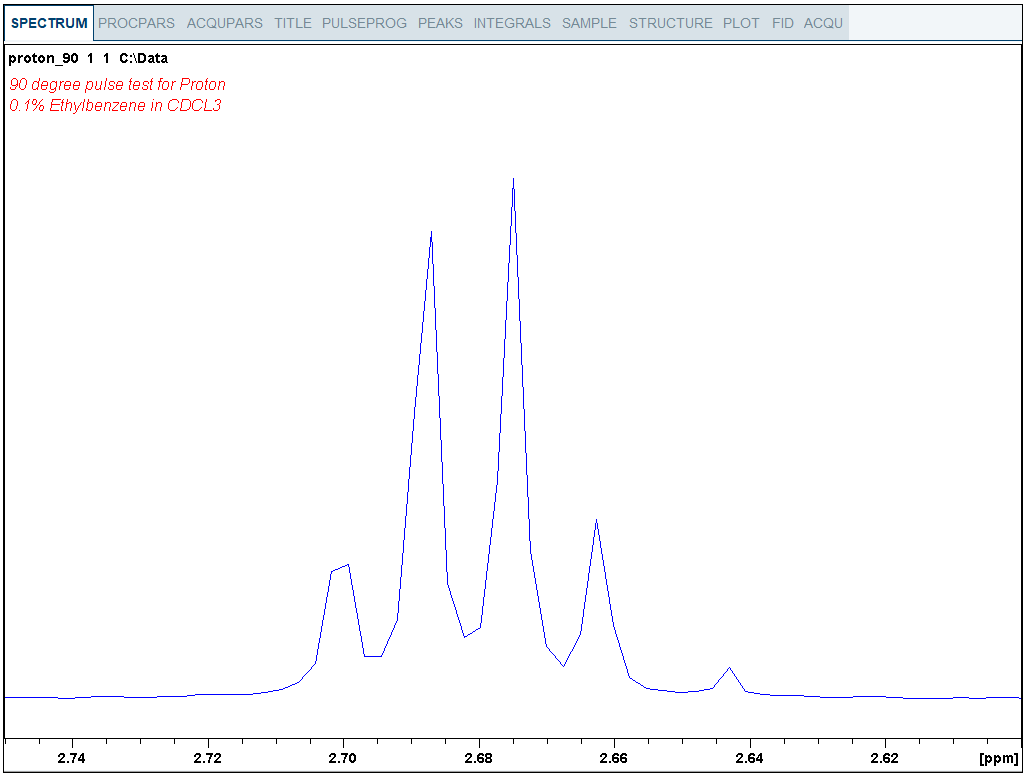
When the acquisition is finished:

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

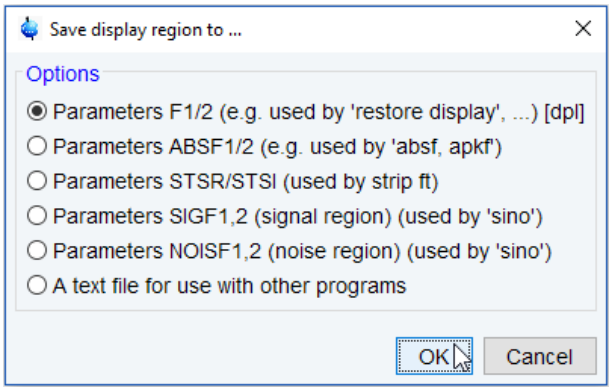
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing (proc1d)**.
- Enter or select the following options:
  - Exponential Multiplay (em)
  - LB [Hz] = **1**
  - Auto - Phasing (apk)
- Deselect the following options:
  - Set Spectrum Reference (sref)
  - Auto-Baseline correction (abs)
  - Warn if Processed data exist



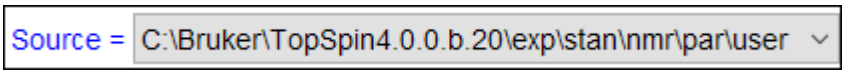
- In the proc1d window, click **Execute**.
- Expand the spectrum from **2.75 ppm** to **2.6 ppm**.



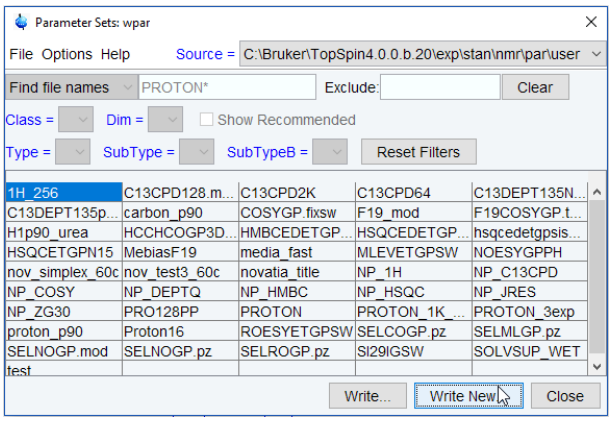
- Right-Click in the spectral window.
- In the list, select **Save Display Region to...**
- In the Save Display Region to... window, select **Parameters F1/2**.
- Click **OK**.



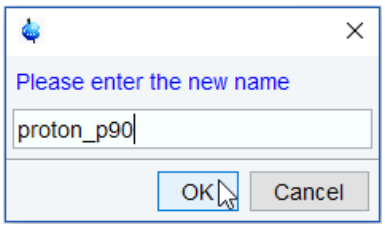
- In the command line, type **wpar** to store the parameter for future use.
- In the *Parameter Sets: wpar* window, select the user parameter directory.



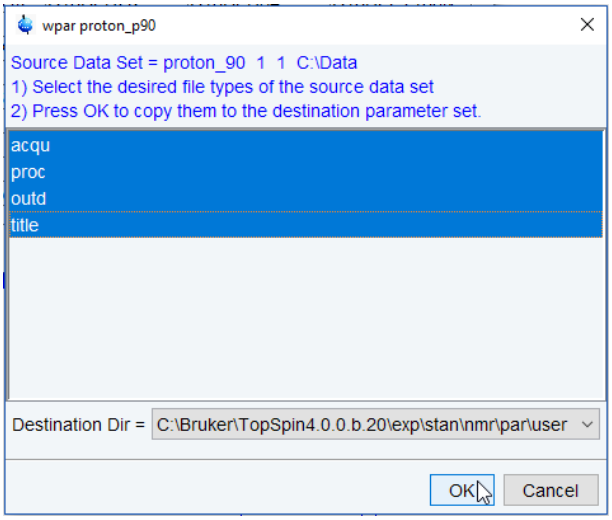
- Click **Write New**.



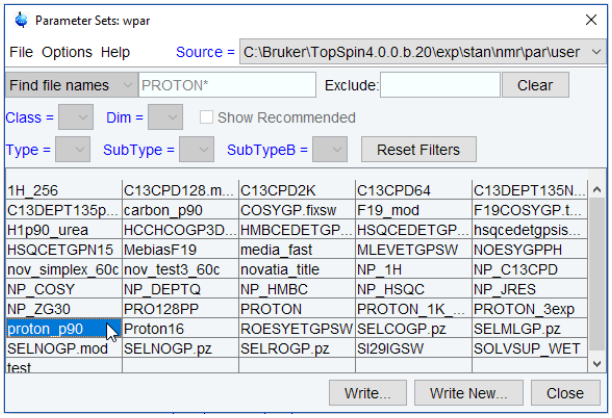
- In the popup window, type **proton_p90**. Click **OK**.



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



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



10.1.4 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**

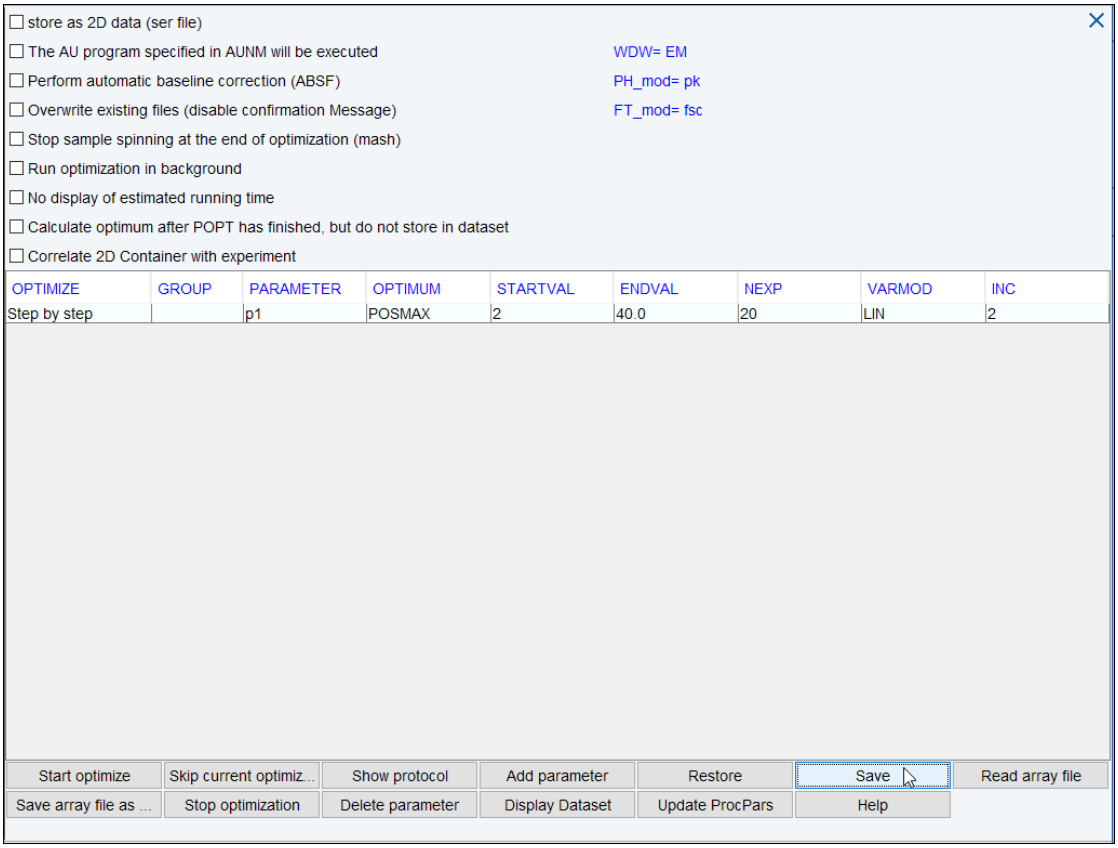
  VARMOD = **LIN**

  INC = **2**

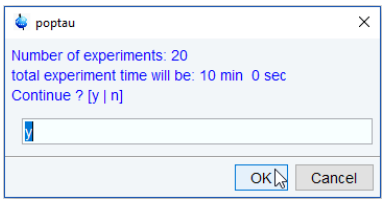
- Click **Save**.

The ENDVAL parameter has been updated.

- Click **Start Optimize**.

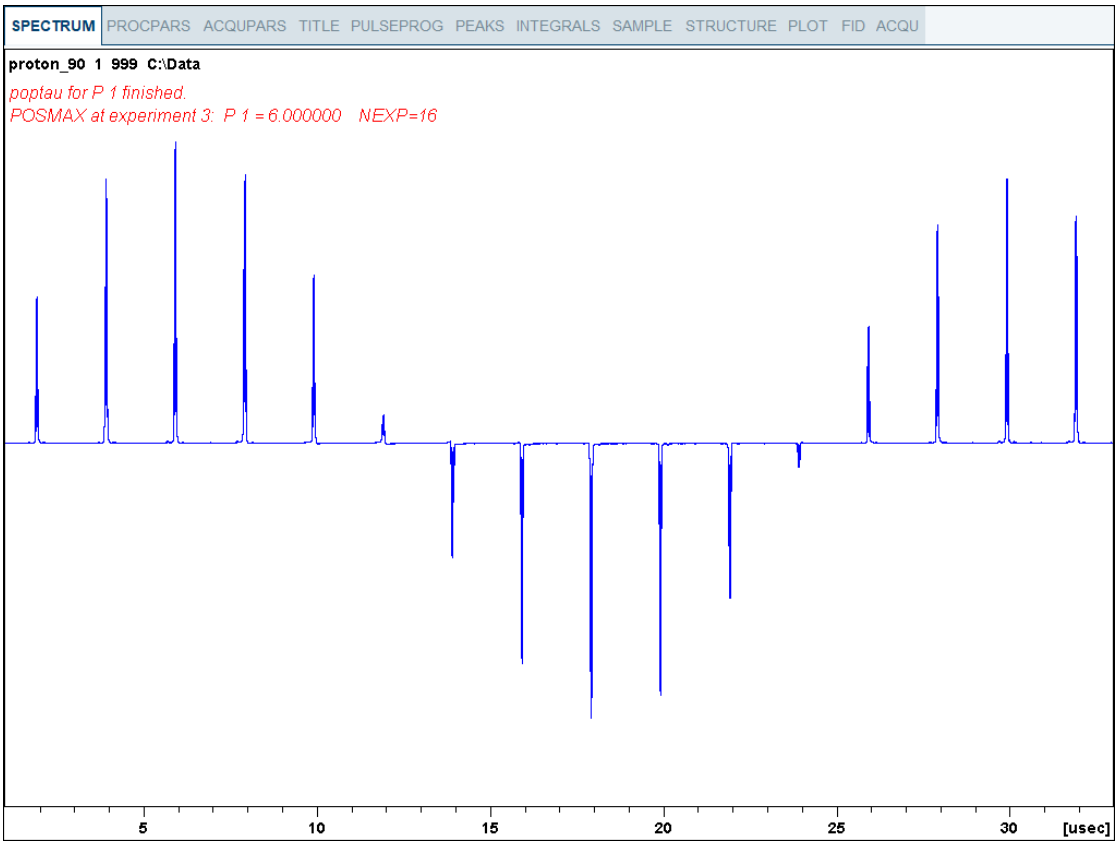


- In the poptau window, enter **y** and click **OK**.



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.

Determination of 90 Degree Pulses



The POSMAX value of **p1** is displayed in the title window which is the 90^0 pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90^0 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 360^0 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 360^0 pulse.

The quartet signal is negative for a pulse angle slightly less then 360^0 and positive when the pulse angle is slightly more then 360^0.

- Simply divide the determined 360^0 pulse value by 4. This will be the exact 90^0 pulse length for the proton transmitter on the current probe.

10.2 Carbon 90 Degree Transmitter Pulse

Standard Test Sample:

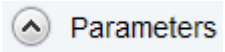
ASTM (60% C6D6 / 40% p-Dioxane)

10.2.1 Parameter Setup

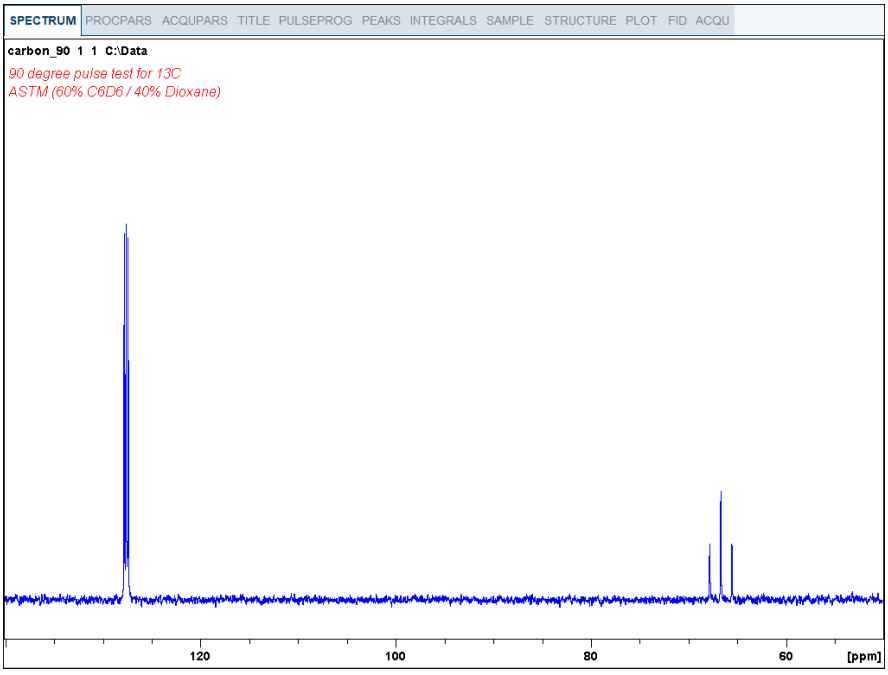
- On the menu bar, click **Acquire** | **Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:

  NAME = **carbon_90**

  EXPNO = **1**

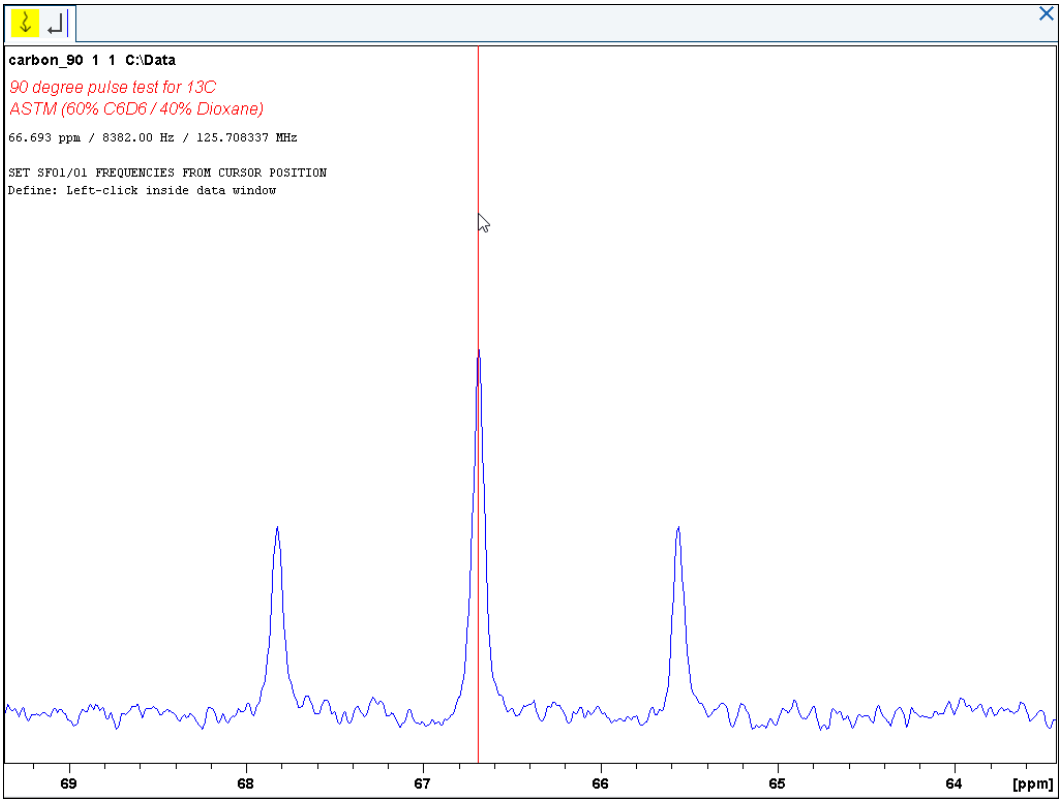
  Directory = e.g. *C:\Data*
- In the Parameters group 

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 [▶ 72]* and chapter *Acquisition [▶ 74]*.
- Enter the following acquisition parameters:
  - PULPROG = **zg**
  - DS = **0**
  - NS = **1**
- Continue with chapter *Processing [▶ 74]*.

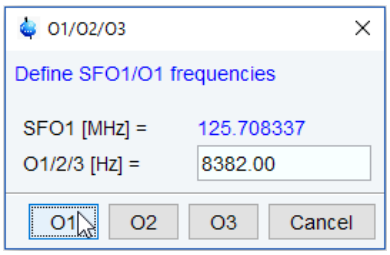


- Expand the peak at **67 ppm**.
- On the toolbar, click **Set RF from cursor**. 

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**.



- 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.

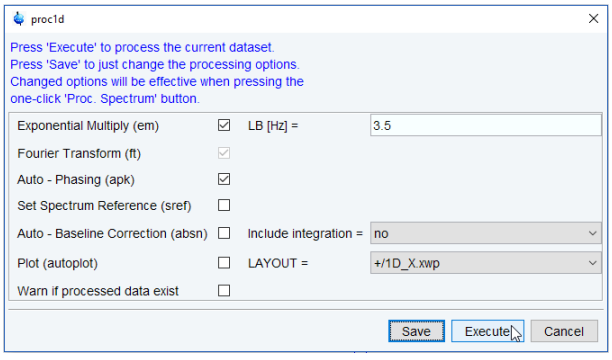
10.2.2 Acquisition

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

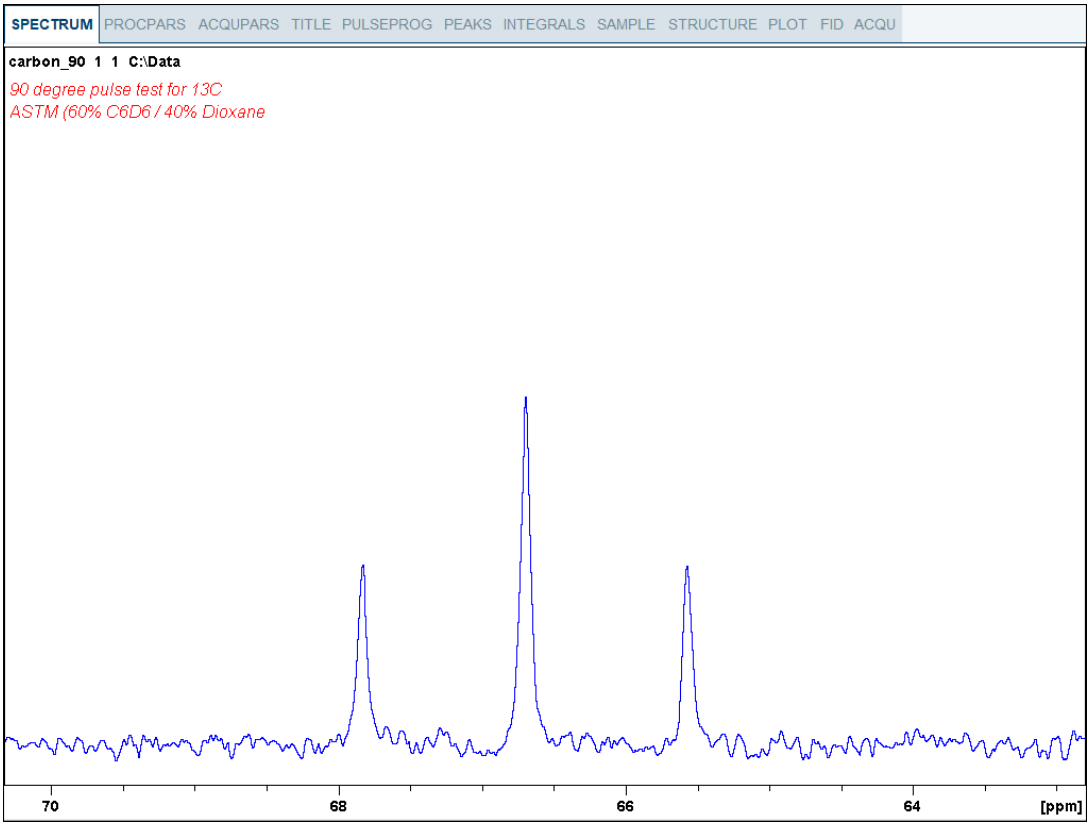
10.2.3 Processing

When the acquisition is finished:

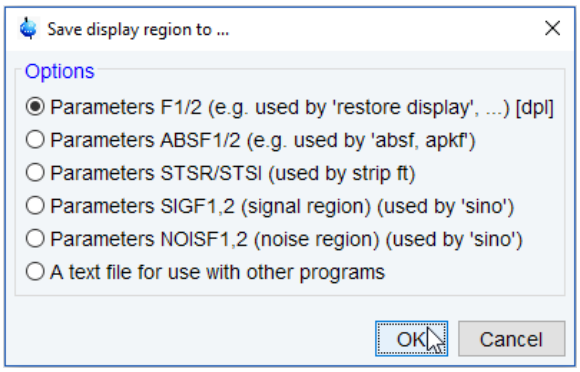
- On the menu bar, click **Process**.
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing (proc1d)**.
- Select the following options:
  - Exponential Multiplay (em)
  - LB [Hz] = **3.5**
  - Auto - Phasing (apk)
- Deselect the following options:
  - Set Spectrum Reference (sref)
  - Auto-Baseline correction (abs)
  - Warn if Processed data exist



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

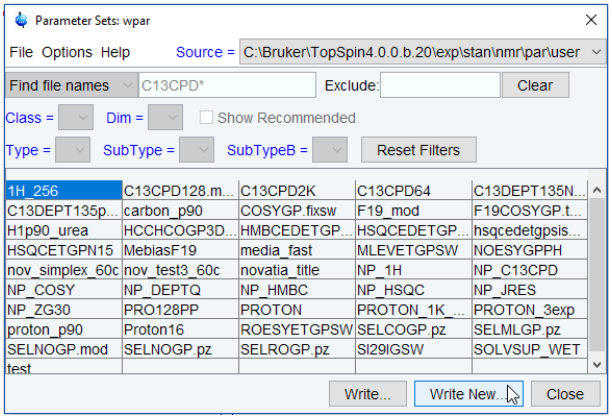


- In the spectral window click right.
- In the list select **Save Display Region To…**
- In the *Save Display Region To…* window, enable **Parameters F1/2** and click **OK**.

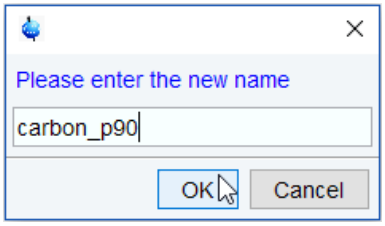


- In the command line, type **wpar** to store the parameter for future use.
- In the *Parameter Sets: wpar* window, select the user source parameter directory.

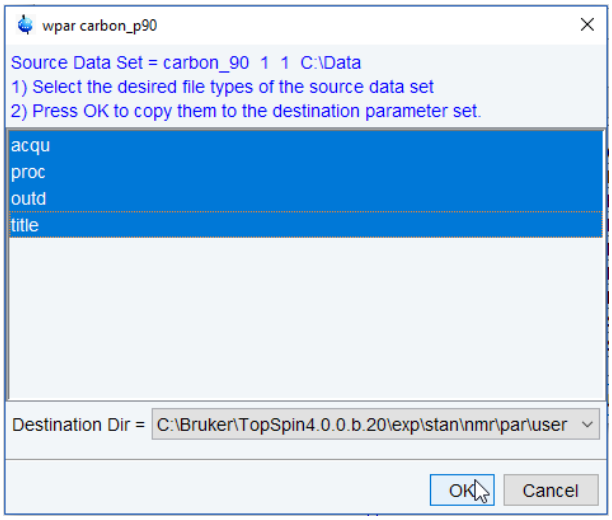
- Click **Write New**.



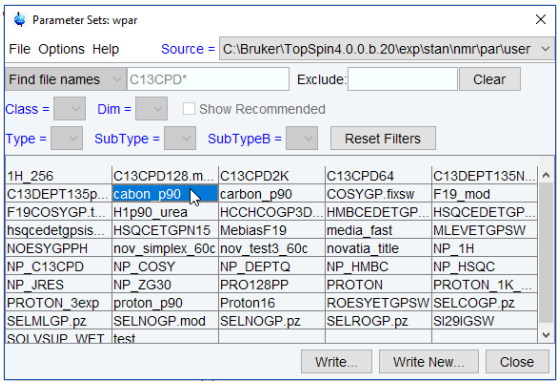
- In the popup window, enter **carbon_p90** and click **OK**.



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



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



10.2.4 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**

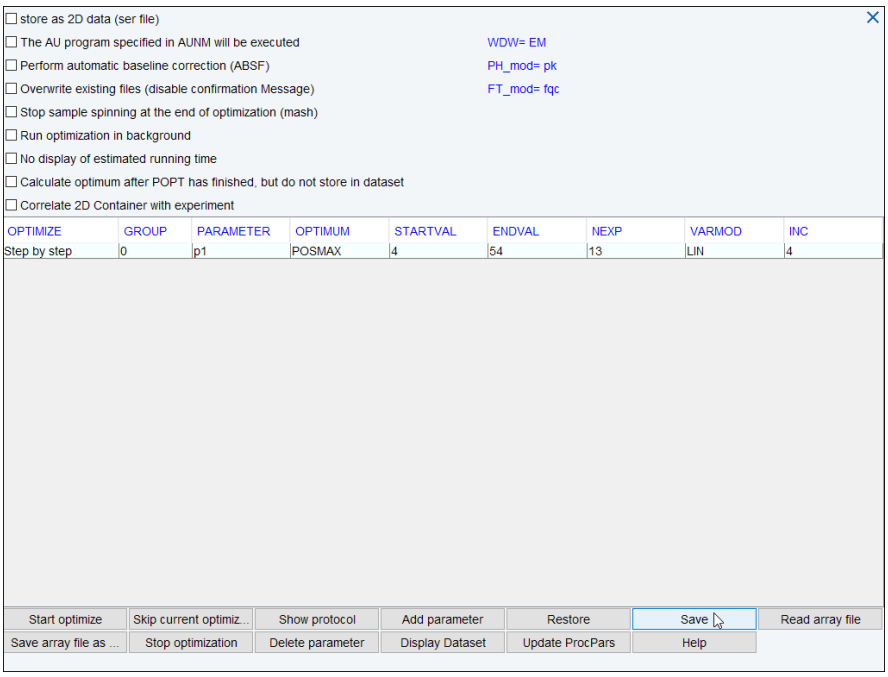
  STARTVAL = **2**

  NEXP = **13**

  VARMOD = **LIN**

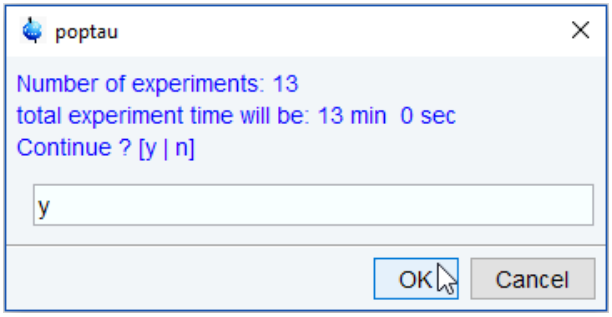
  INC = **2**

- Click **Save**.

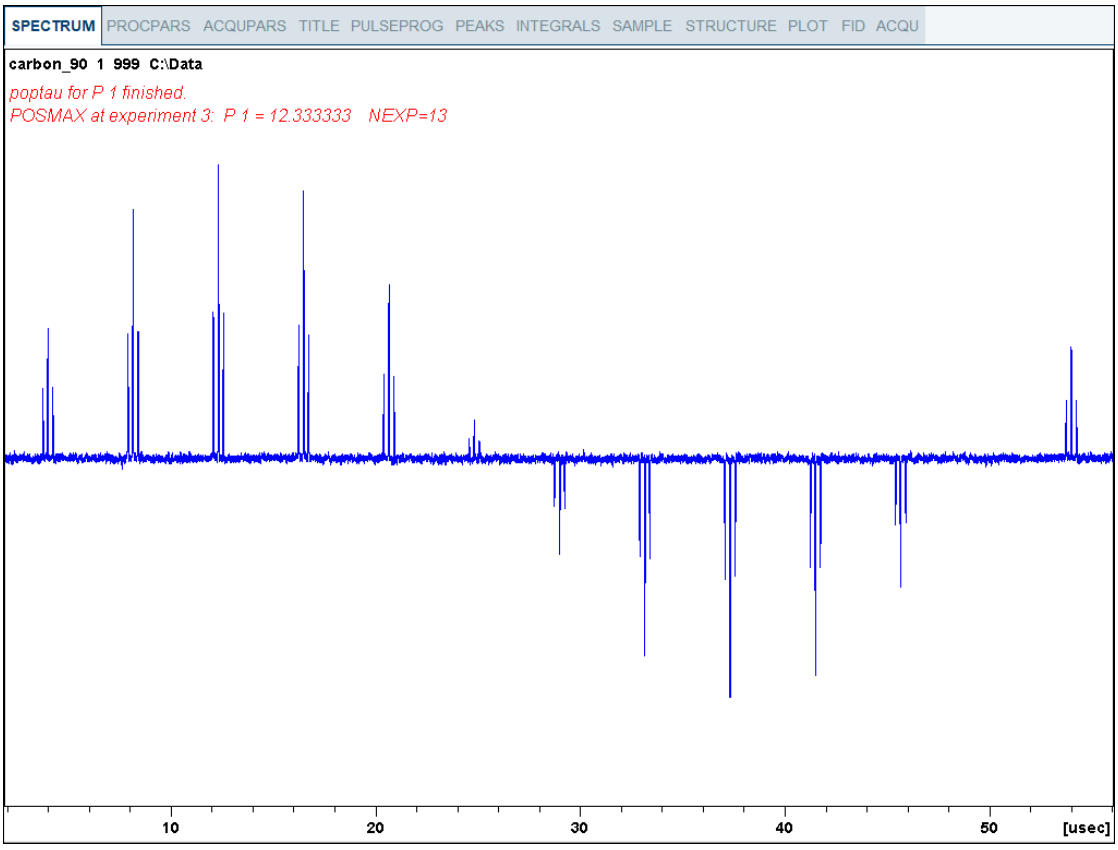


The ENDVAL parameter has been updated.

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



The parameter optimization starts. The spectrometer acquires and processes 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.



Determination of 90 Degree Pulses

The POSMAX value of **p1** is displayed in the title window which is the 90^0 pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90^0 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 360^0 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 360^0 pulse.

The quartet signal is negative for a pulse angle slightly less then 360^0 and positive when the pulse angle is slightly more then 360^0.

- Simply divide the determined 360^0 pulse value by 4. This will be the exact 90^0 pulse length for the proton transmitter on the current probe.

11 Sensitivity Tests

This chapter describes the sensitivity test procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter *1D Proton Experiment [▶ 31]* and chapter *1D Carbon Experiments [▶ 71]* in this manual.

Also the 90^0 pulses have to be properly calibrated, see chapter *Determination of 90 Degree Pulses [▶ 97]*.

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

11.1 ^{1}H Sensitivity Test

Standard Test Sample:

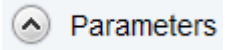
0.1% Ethylbenzene in CDCl3

11.2 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 **CDCl3**.
- 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 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**.

11.3 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**.

11.4 Processing

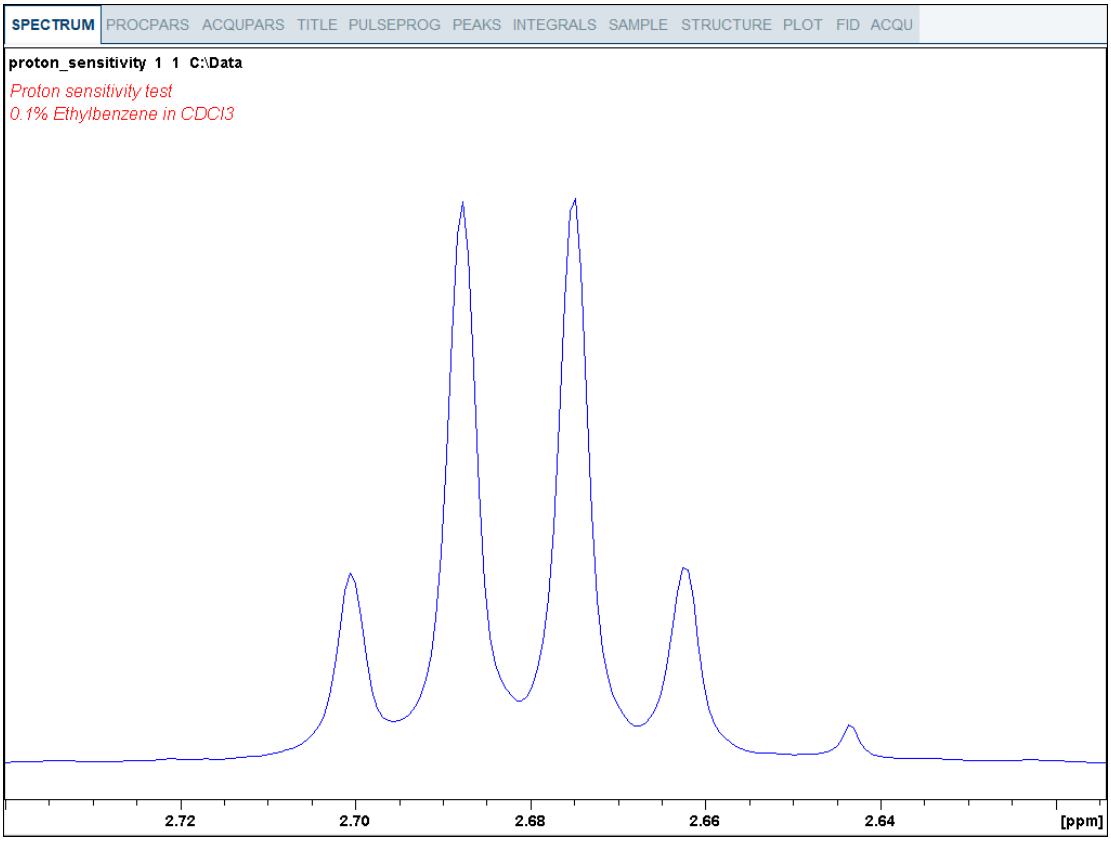
When the acquisition has finished:

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

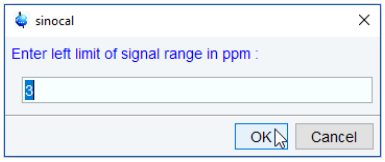
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In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

11.5 Calculating the Signal to Noise Ratio

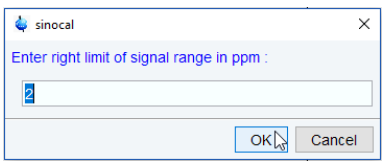
The signal to noise ratio is determined on the intensity of the **quartet** lines between **2 ppm** and **3 ppm**. It is calculated by AU-program sinocal over a range of **2 ppm** between **2.8 ppm** and **7 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting between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



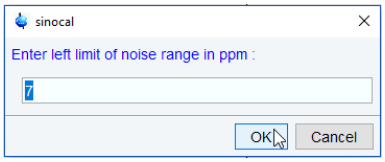
- At the command prompt, type **sinocal**.
- In the sinocal window, enter **3** for the left limit of the signal range. Click **OK**.



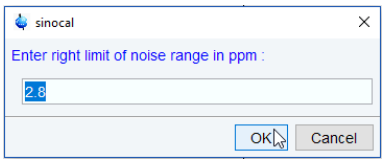
- In the sinocal window, enter **2** for the right limit of the signal range. Click **OK.**



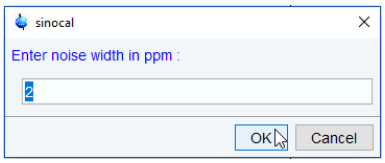
- In the sinocal window, enter **7** for the left limit of the noise range. Click **OK**.

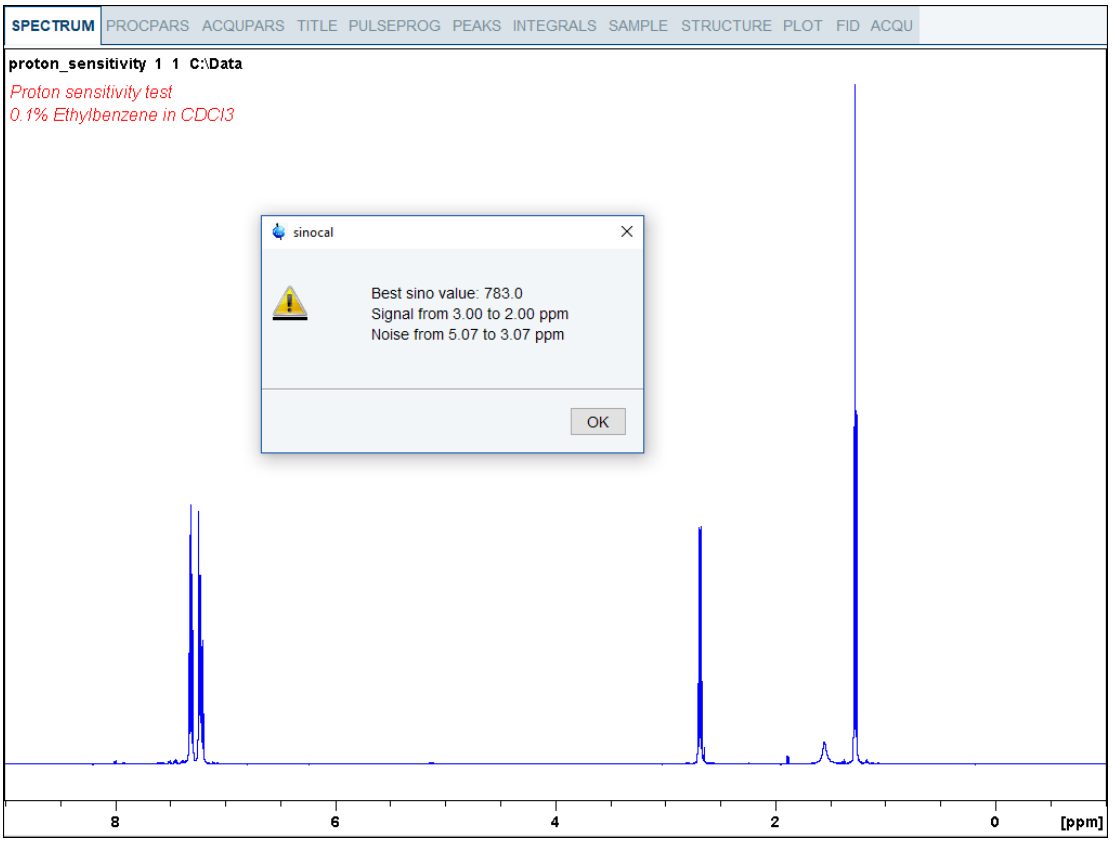


- In the sinocal window, enter **2.8** for the right limit of the noise range. Click **OK**.



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





11.6 ^{13}C Sensitivity Test with ^{1}H Decoupling

Standard Test Sample:

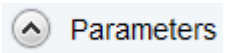
10% Ethylbenzene in CDCl3

11.6.1 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 **CDCl3**.
- 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 **Aquire**.

For the following steps, use the Workflow button bar.

- Click **Sample** and eject the sample, if there is one inserted, and insert the new sample.
- Click **Lock** and select **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**.

11.6.2 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**.

11.6.3 Processing

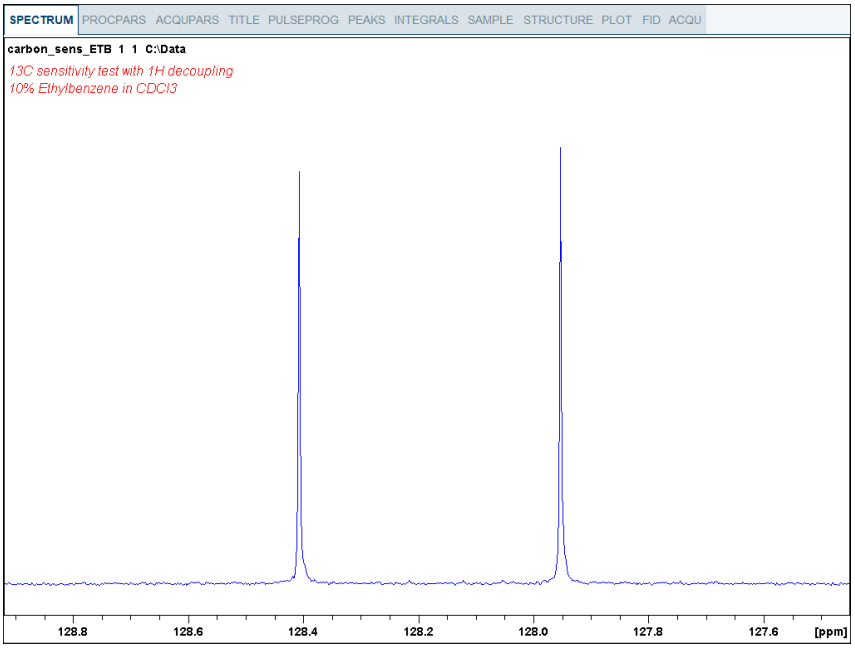
When the acquisition has finished:

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

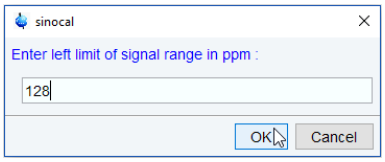
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In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

11.6.4 Calculating the Signal to Noise Ratio

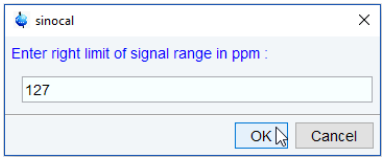
The signal to noise ratio is determined on the highest peak of the **aromatic** part between **127 ppm** and **129 ppm**, see the figure below. It is calculated by AU-program sinocal over a range of **40 ppm** between **30 ppm** and **125 ppm**. The s/n ratio is strongly dependant on good resolution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



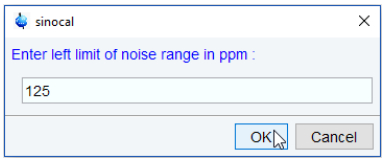
- At the command prompt, type **sinocal**.
- In the sinocal window, enter **128** for the left limit of the signal range. Click **OK**.



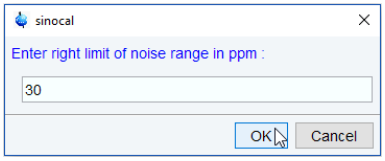
- In the sinocal window, enter **127** for the right limit of the signal range. Click **OK**.



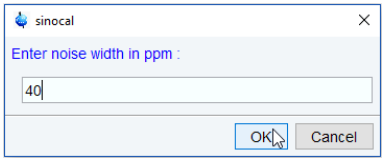
- In the sinocal window, enter **125** for the left limit of the noise range. Click **OK**.

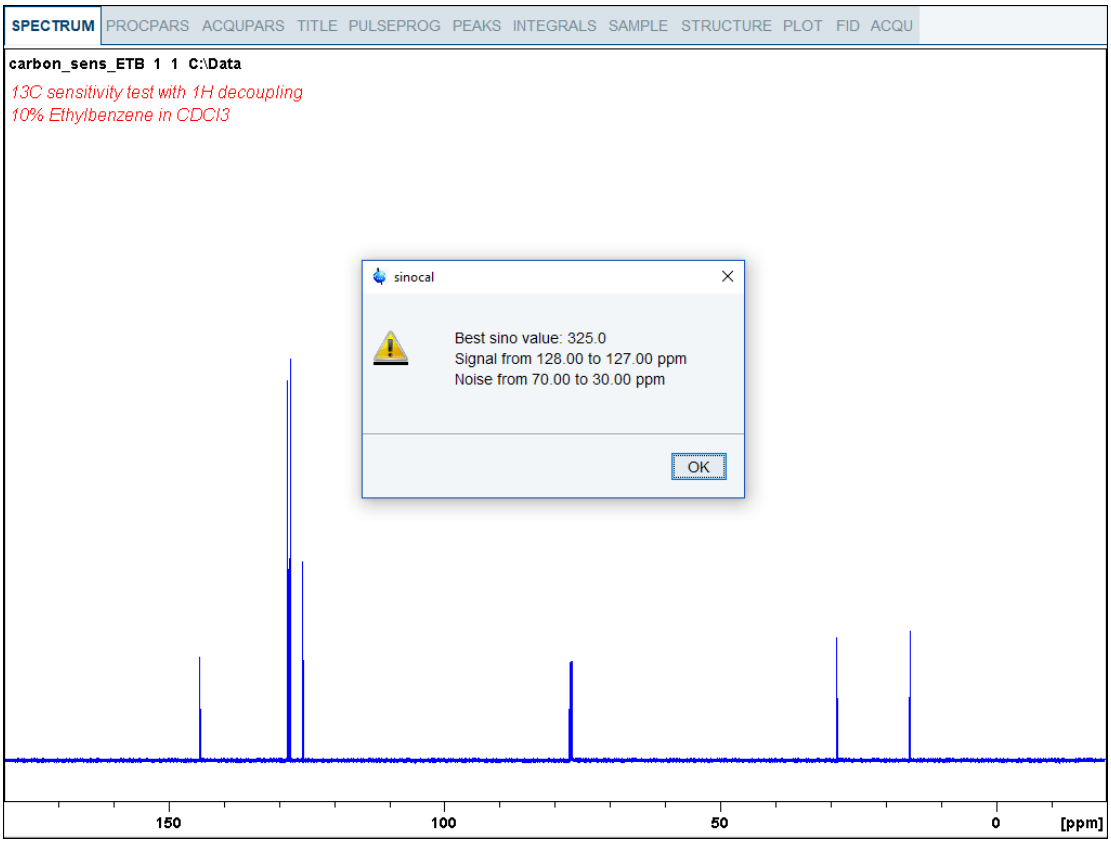


- In the sinocal window, enter **30** for the right limit of the noise range. Click **OK**.



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





11.7 ^{13}C Sensitivity Test without ^{1}H Decoupling

Standard Test Sample:

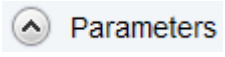
ASTM (60% C6D6 / 40% p-Dioxane)

11.7.1 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 **Aquire**.

For the following steps, use the Workflow button bar.

- Click **Sample** and eject the sample, if there is one inserted, and insert the new sample.
- Click **Lock** and select **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.

11.7.2 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**.

11.7.3 Processing

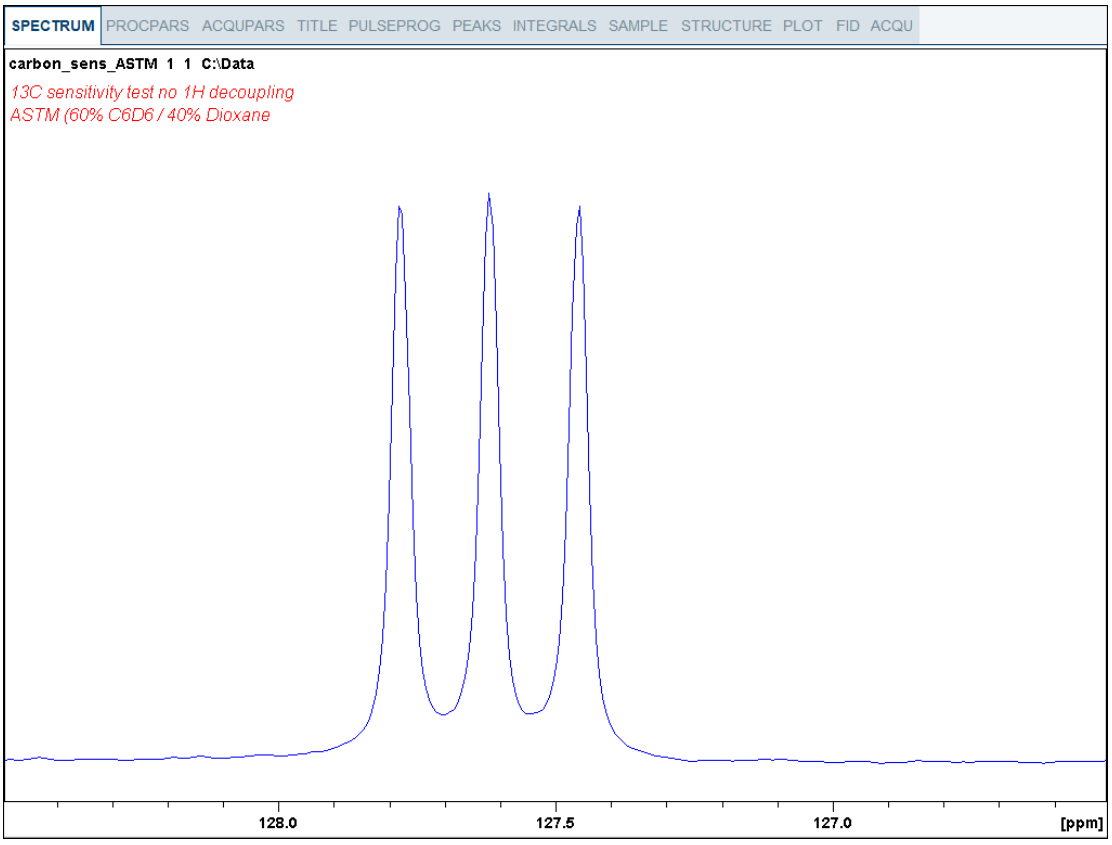
When the acquisition has finished:

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

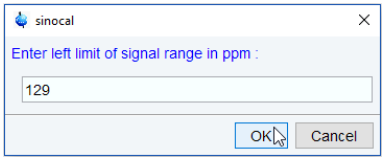
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In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

11.7.4 Calculating the Signal to Noise Ratio

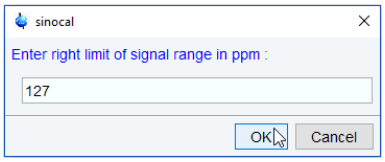
The signal to noise ratio is determined on the triplet of the **deuterated benzene** between **127 ppm** and **129 ppm**. It is calculated by AU-program sinocal over a range of **40 ppm** between **70 ppm** and **125 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting 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.



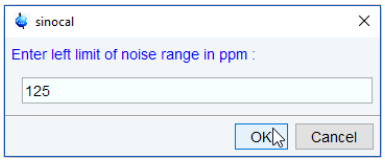
- At the command prompt, type **sinocal**.
- In the sinocal window, enter **129** for the left limit of the signal range. Click **OK**.



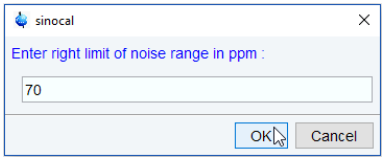
- In the sinocal window, enter **127** for the right limit of the signal range. Click **OK**.



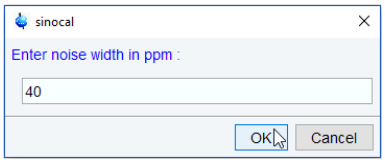
- In the sinocal window, enter **125** for the left limit of the noise range. Click **OK**.



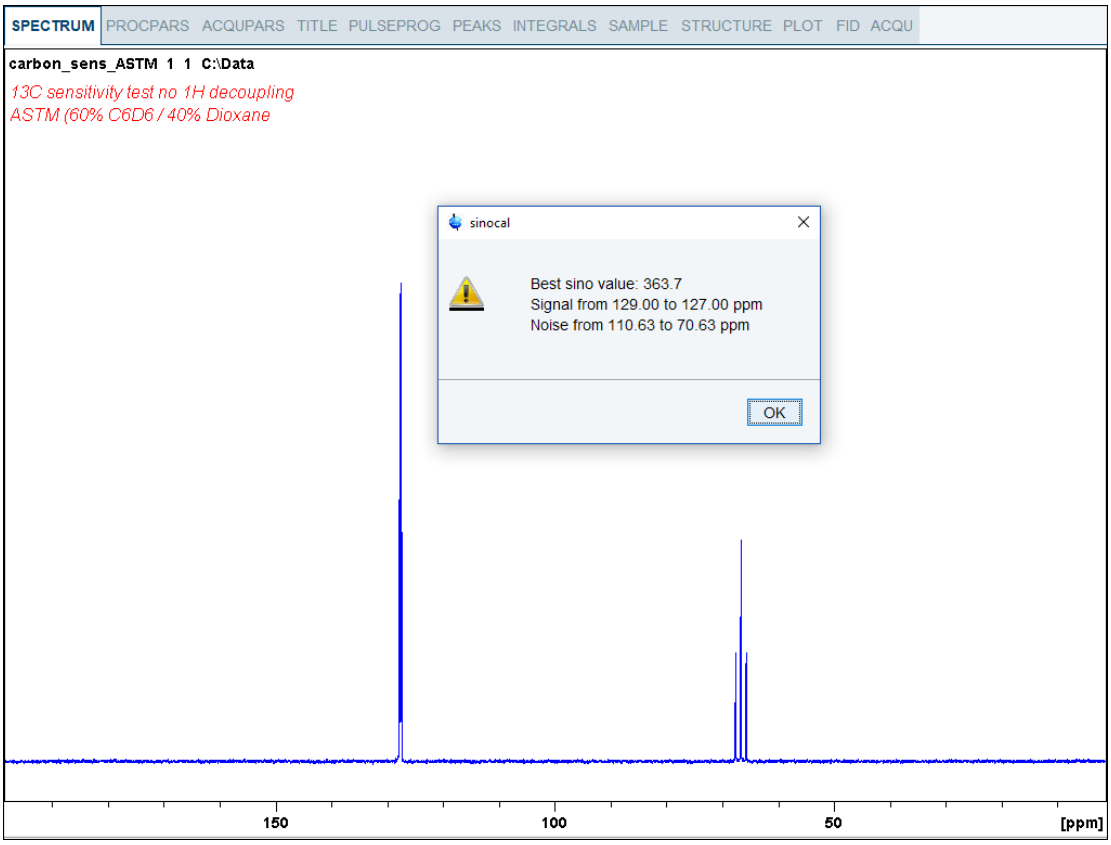
- In the sinocal window, enter **70** for the right limit of the noise range. Click **OK**.



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



Sensitivity Tests

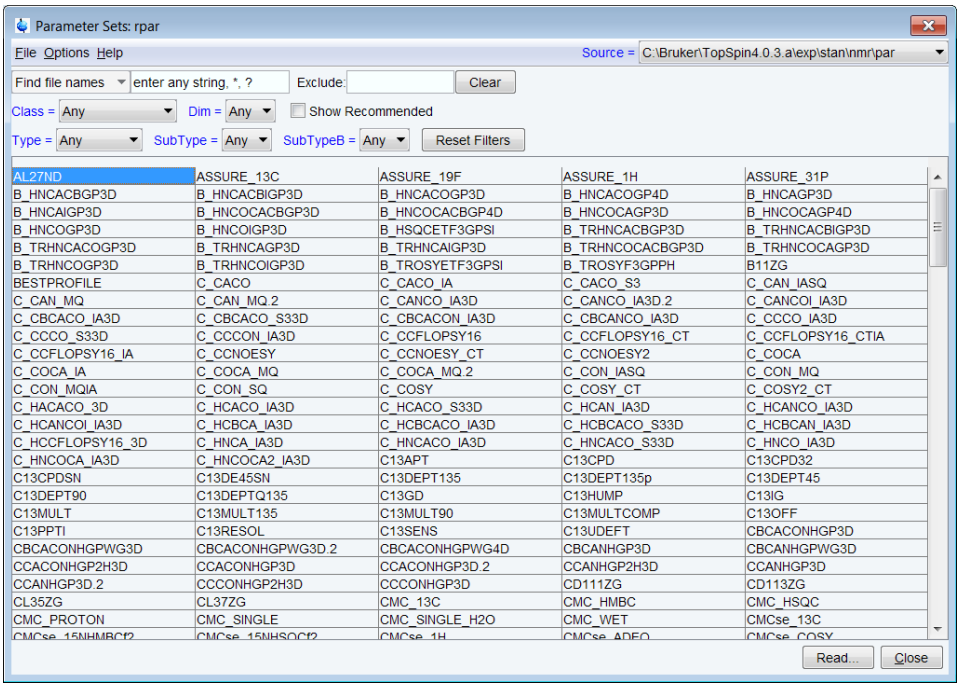


12 Additional Information

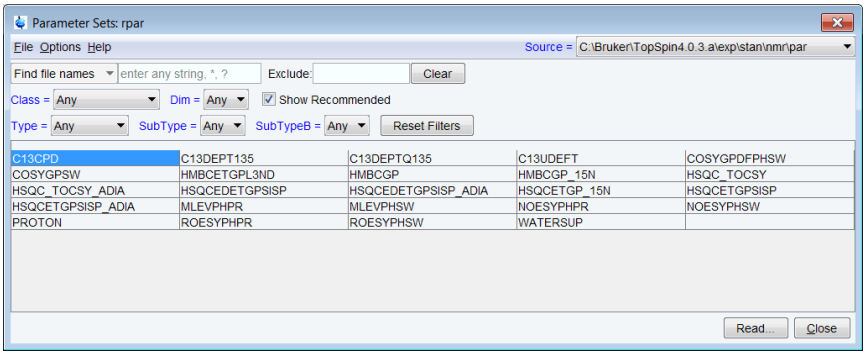
A list of all standard parameter sets is available with the command **rpar**, see figure below. The list varies according to the number of channels of your spectrometer.

12.1 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.

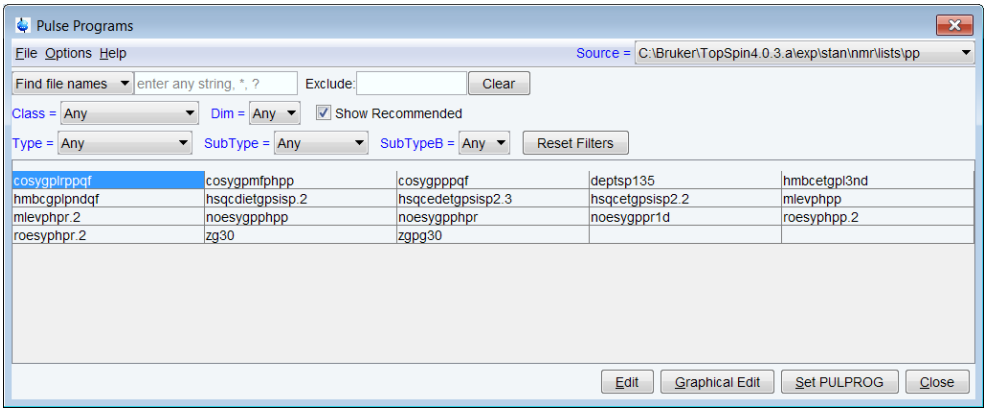


- Ensure that the source directory is
  <Topspin>\exp\stan\nmr\par
  and not *<Topspin>\exp\stan\nmr\par\user*

12.2 Pulse Program Information

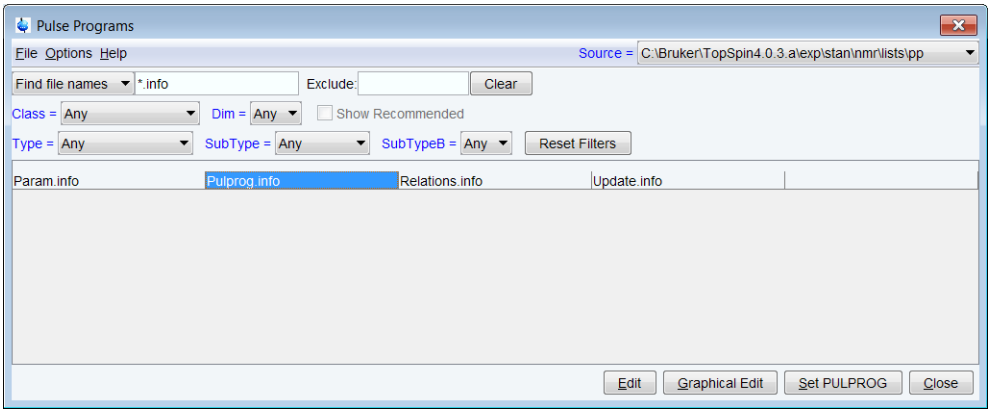
A list of all standard pulse programs is available with the command **edpul**, see figure below.

- 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)



12.3 Standard Test Samples

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

13 Contact

Manufacturer

Bruker BioSpin GmbH
Silberstreifen 4
D-76287 Rheinstetten
Germany
http://www.bruker.com

WEEE DE43181702

NMR Hotlines

Contact our NMR service centers.

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

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