TopSpin

● Guide Book

Advanced NMR Experiments

Version 002

NMR

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Contents

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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.

About This Manual

⚠ 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 what you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All what you can click"	Arial bold, initial letters capitalized	Use the **Export To File** button. Click **OK**. Click **Processing**…
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names Data Path Variables Table Column Names Field Names (within Dialog Windows)	Arial Italics	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST

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

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

Table 1.1: Font and Format Conventions

2 Introduction

2.1 General

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

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

2.2 Disclaimer

This guide should only be used for its intended purpose as described in this manual. Use of the manual for any purpose other than that for which it is intended is taken only at the users own risk and invalidates any and all manufacturer warranties.

Some parameter values, especially power levels suggested in this manual may not be suitable for all systems (e.g. Cryo probes) and could cause damage to the unit. Therefore only persons trained in the operation of the AVANCE systems should operate the unit.

| *NOTICE* |
|---|
| **Material Damage Due to Excessive Power** |
| The NMR probe can be severely damaged if too much power or power over a too long time is applied. |
| ▶ Always start to optimize pulses with low power values and short pulses. Respect the pulse and power limits as programmed into the PICS data of the probe. |

3   1D Solvent Suppression Experiments

3.1   Introduction

Many experiments on samples dissolved in protonated solution require some method to minimize the strong resonance belonging to the solvent. This suppression can be performed in several ways, depending on the number of signals to suppress, and on which part of the pulse sequence can be modified. Solvent suppression can be applied during the relaxation period just prior to the conventional pulse sequence as outlined in the figure below. This is referred to as Pre-saturation.
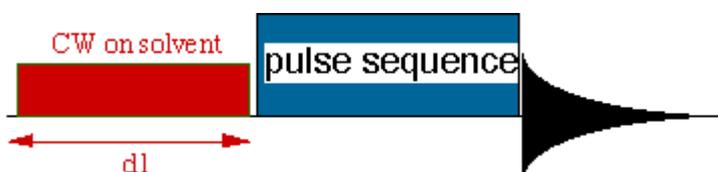
However, pre-saturation can also reduce the signal intensities of exchangeable protons. For this reason, other schemes, as the WATERGATE, WET and Excitation Sculpting schemes, can be used to overcome this problem and are discussed in this chapter.

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

3.2   Samples

2 mM Raffinose in 90% H_2O + 10% D_2O

2 mM Lysozyme in 90% H_2O + 10% D_2O

3.3   Preparation Experiment

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
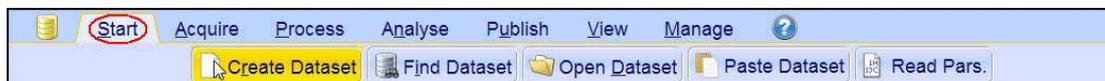
- In the New Dataset window, enter or select:

  NAME = **solvent_suppression_exp**

  EXPNO = **1**

  PROCNO = **1**

  Experiment: Select **PROTON**

  Set Solvent: Select **H2O+D2O**

DIR

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

Title

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

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

For the following steps, use the Workflow button bar.

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

ℹ   Solvent suppression experiments should be run non-spinning.

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

3.3.1 Acquisition

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

3.3.2 Processing

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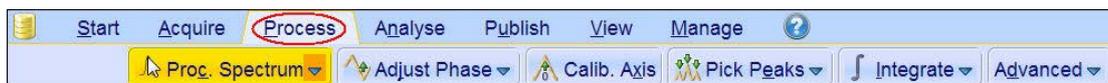
- On the Workflow button bar, click **Proc Spectrum**.

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

- In the list, select **Configure Standard Processing (proc1d)**.

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

3.4   1D Solvent Suppression with Pre-saturation

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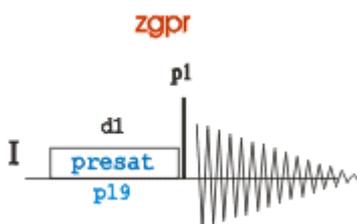

3.4.1   Parameter Setup

- At the command prompt, type:

   wrpa 2

   re 2

- Expand the peak at **4.7ppm**.

- On the toolbar, click **Set RF from cursor**.
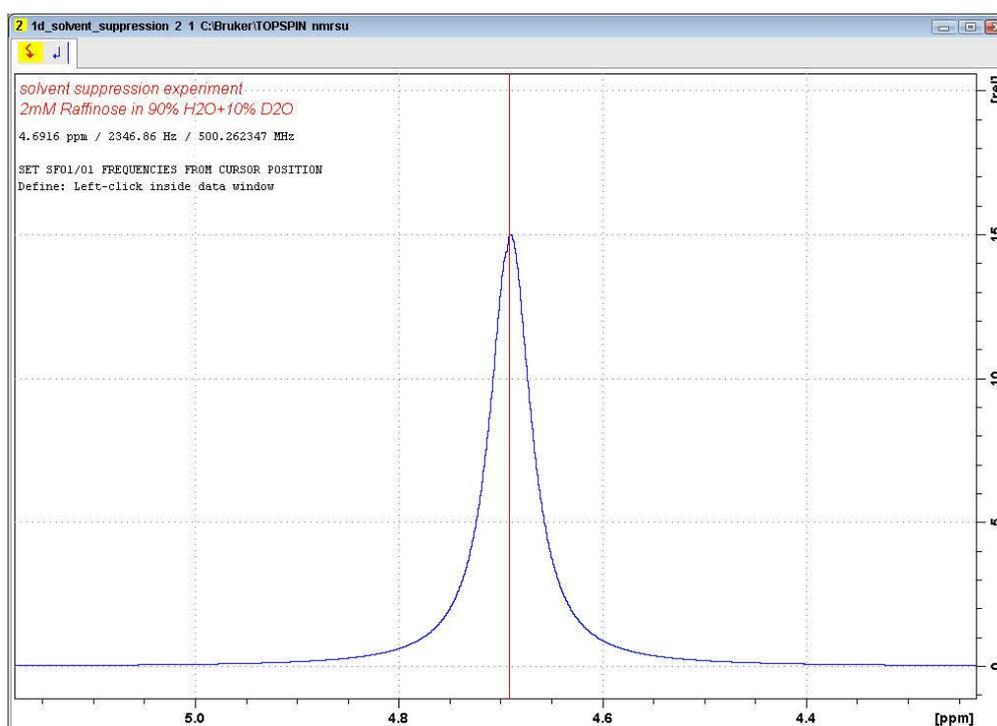
- Move the cursor line to the center of the peak and press the mouse button.
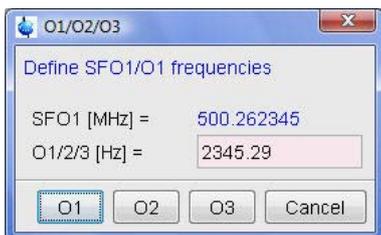
- In the O1/O2/O3 window, click **O1**.
- In the Dataset window, select the **AcquPars** tab.
- Enter:

    PULPROG = **zgpr**

    TD = **16384**

    NS = **8**

    DS = **4**

    SW[ppm] = **10** (for the Raffinose sample)

    SW[ppm] = **14** (for the Lysozyme sample)

    D1 [s] = **2**

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

    SI = **8192**

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

3.4.2   Fine Tuning

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

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

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

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

- In the Dataset tool bar, select **Unshuffle data on display**.
- In the Go Setup (gs) window, select the **Offset** tab.
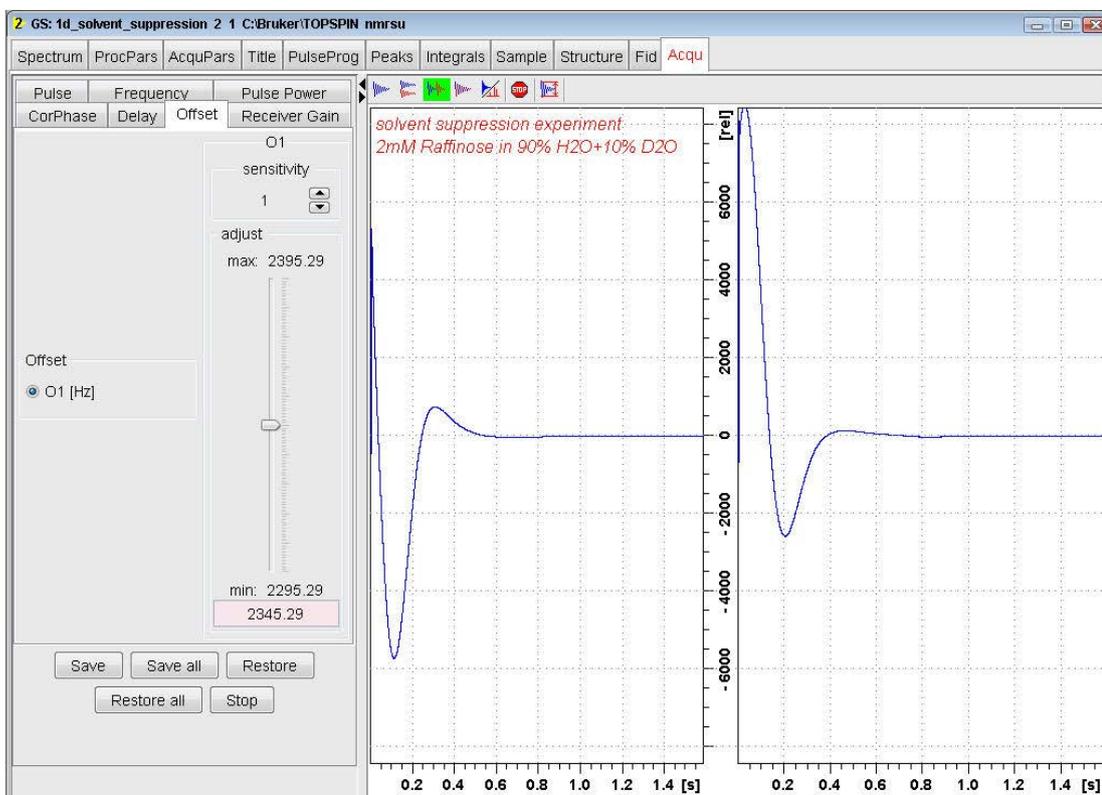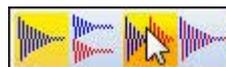
- Change the **O1** value by clicking just below or above the adjust slider.

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

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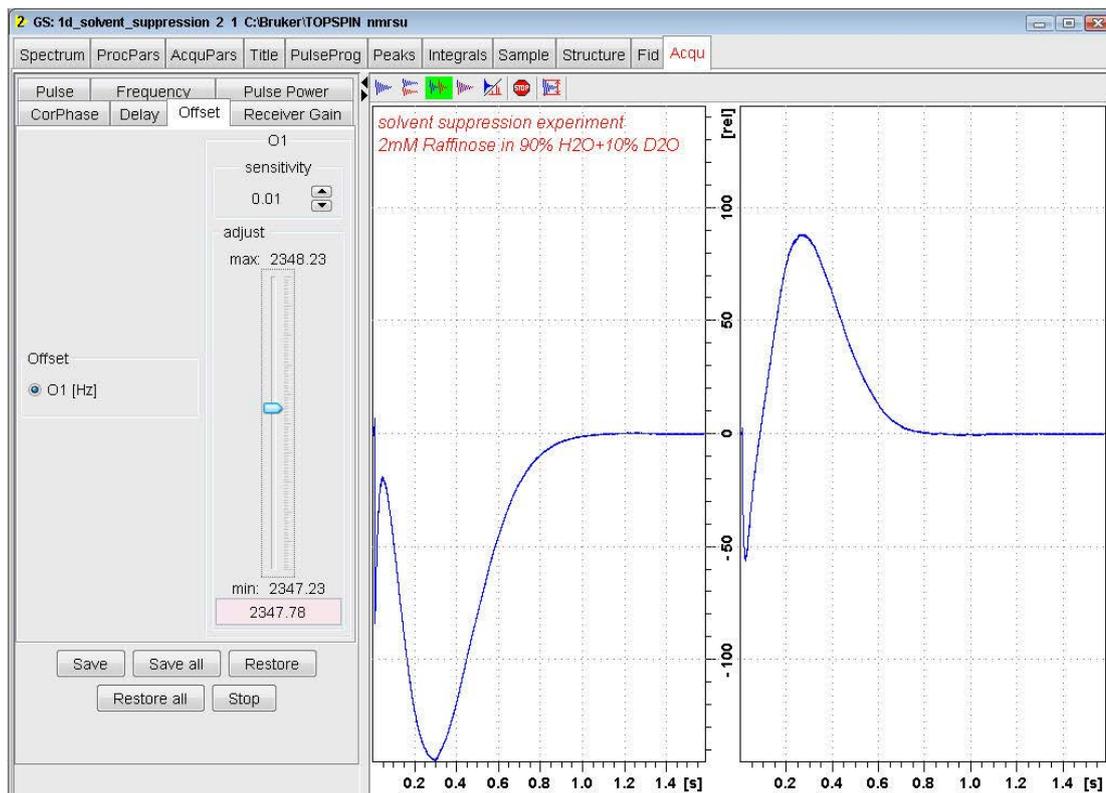
- In the Save changed GS parameters window, click **OK**.


3.4.3   Acquisition

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

## 3.4.4   Processing

• Process and phase correct the spectrum.
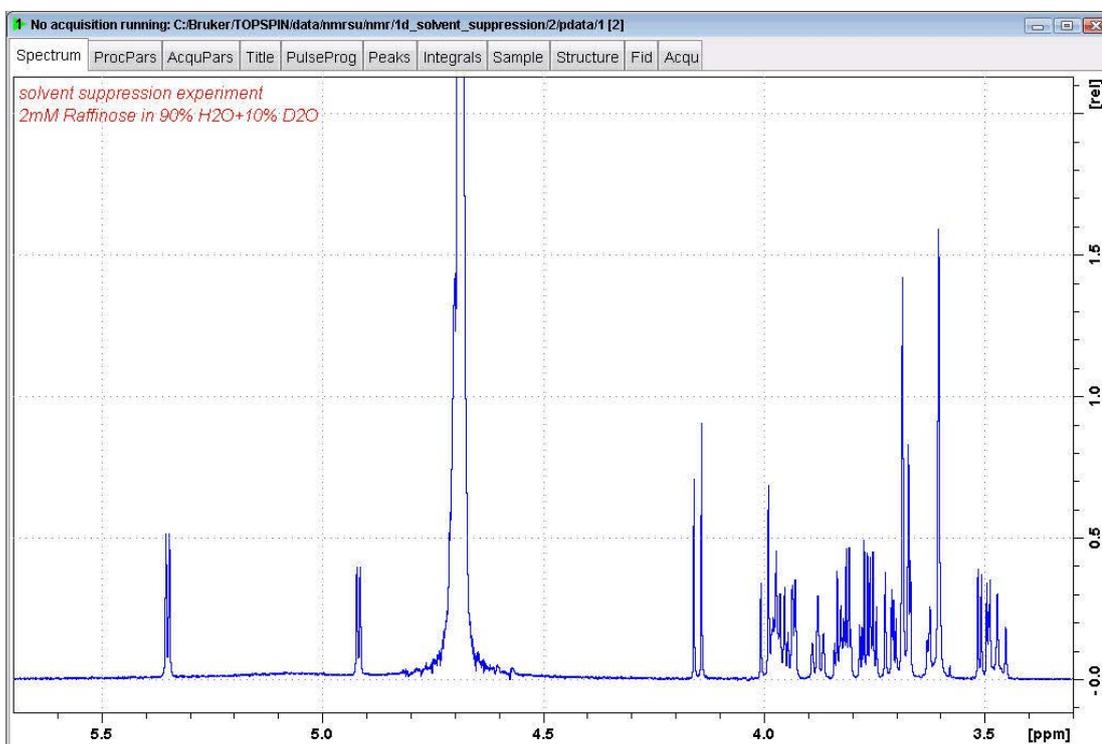
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.
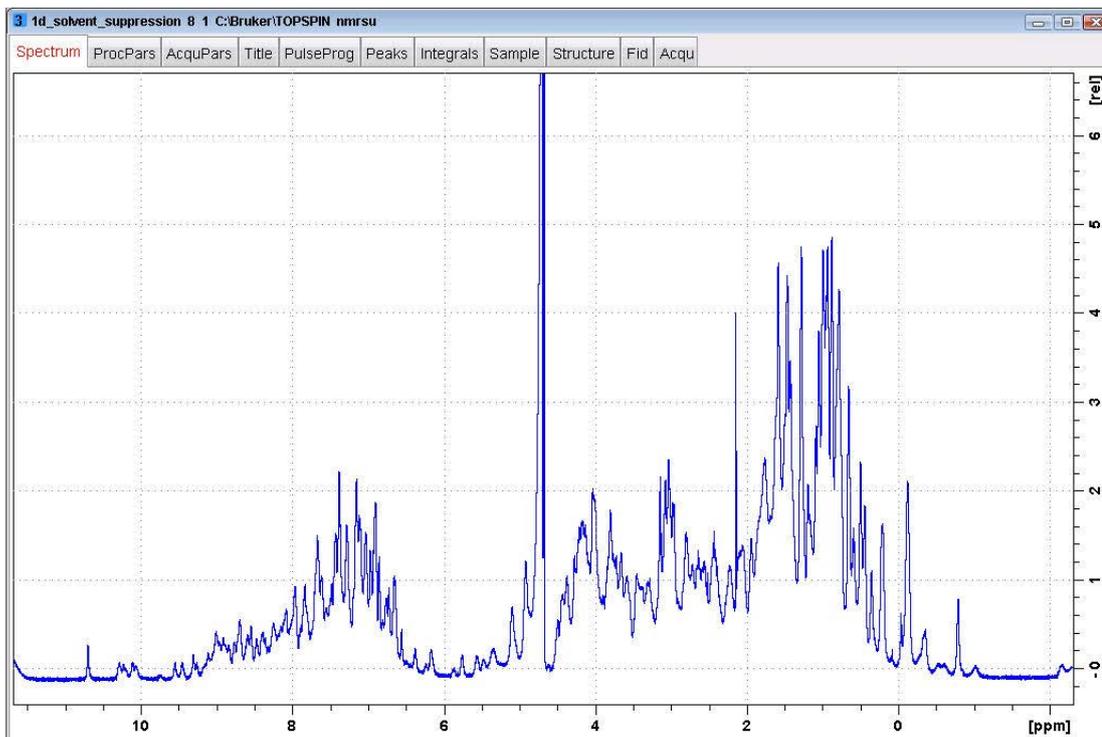
3.5   1D Solvent Suppression with Presaturation and Composite Pulses

This experiment is performed by applying a low-power continuous wave irradiation on the water resonance during the pre-scan period, followed by a rapid succession of four 90^0 pulses to further reduce the residual hump of the water signal:


3.5.1   Parameter Setup

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

3.5.2   Acquisition

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

3.5.3 Processing

- Process and phase correct the spectrum.
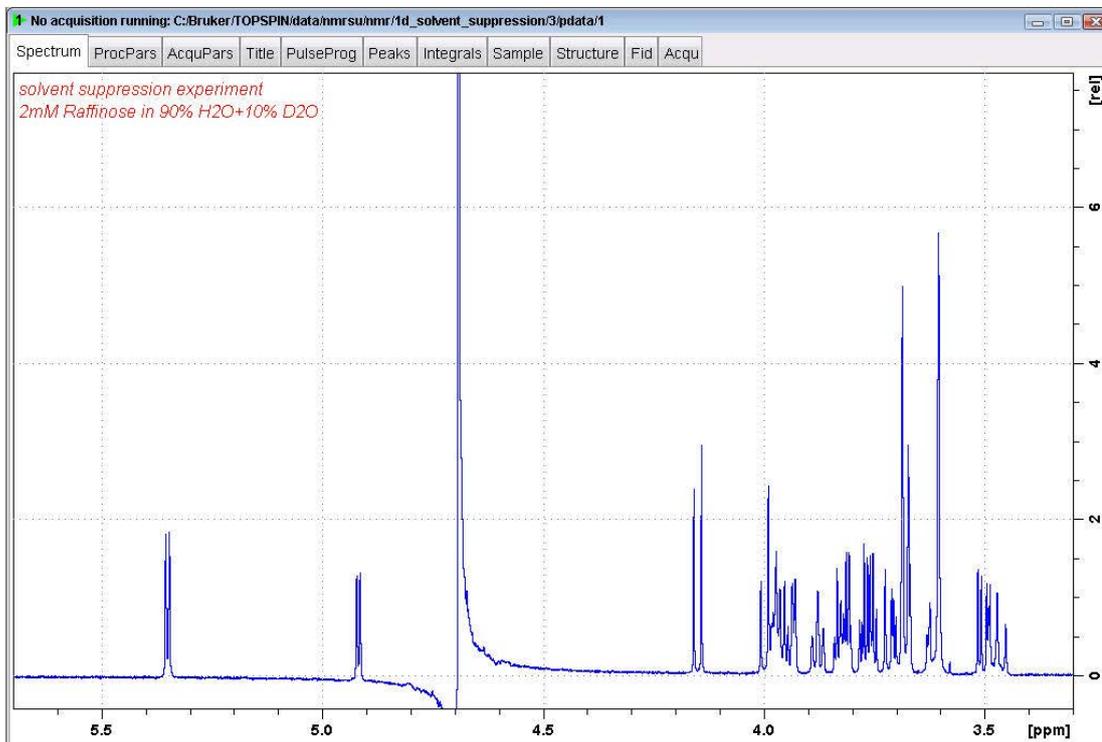
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The following figure shows the 1D spectrum of the Lysozyme sample:
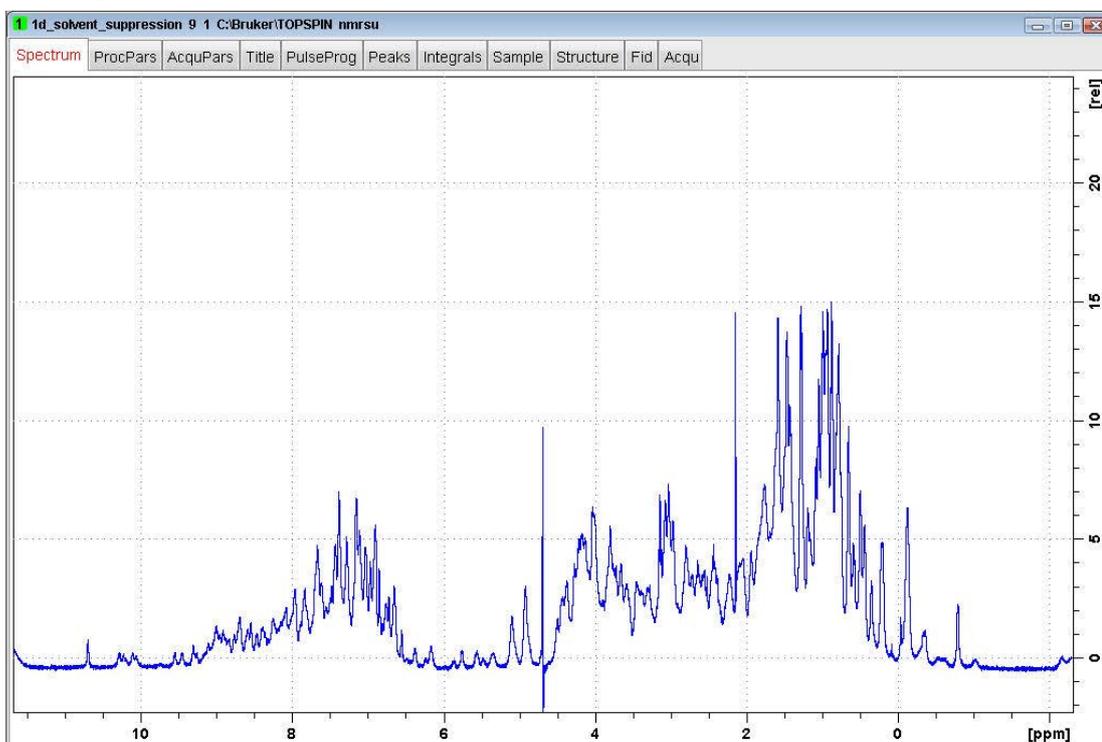
3.6 1D Solvent Suppression Using the Noesy Sequence

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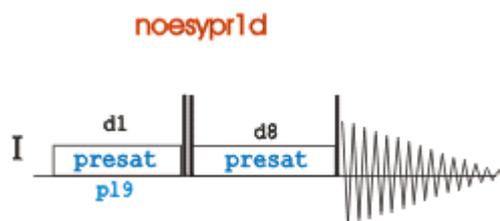
3.6.1 Parameter Setup

- Follow the instructions *Parameter Setup* and *Fine Tuning* in the chapter *1D Solvent Suppression with Pre-saturation [▷ 18]*.
- In the Dataset window, select the **AcquPars** tab.
- Enter:
  PULPROG = **noesypr1d**
  D8[s] = **0.1**

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

3.6.2 Acquisition

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

### 3.6.3   Processing

• Process and phase correct the spectrum.
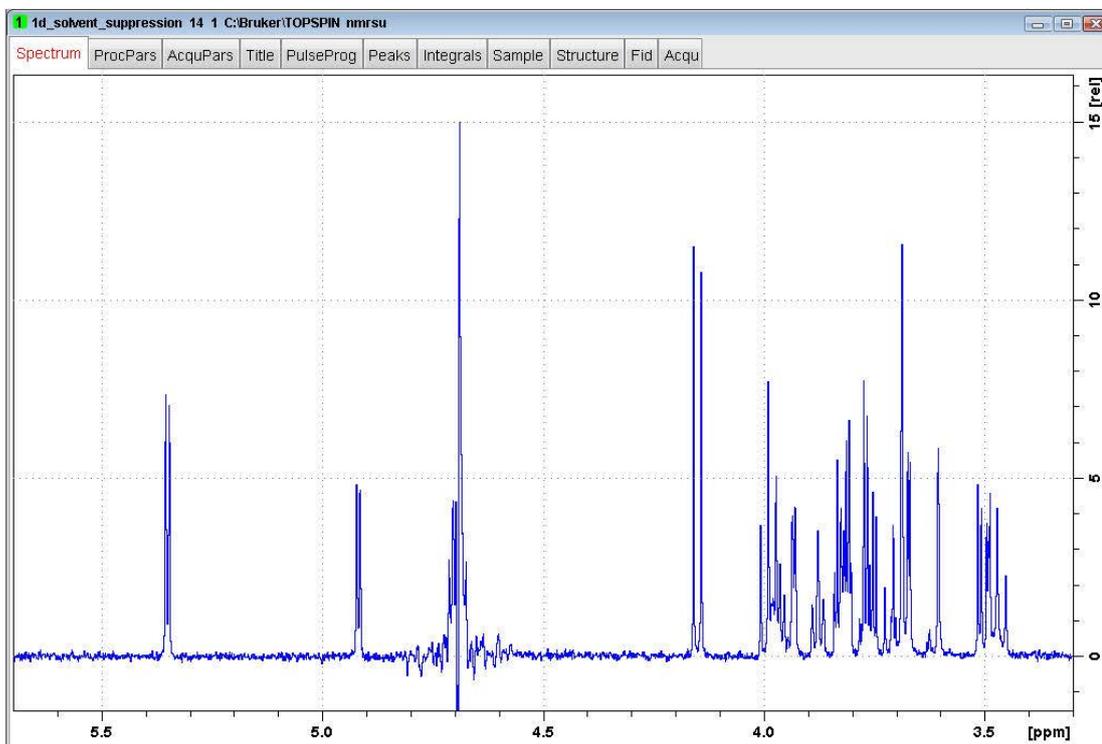
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.
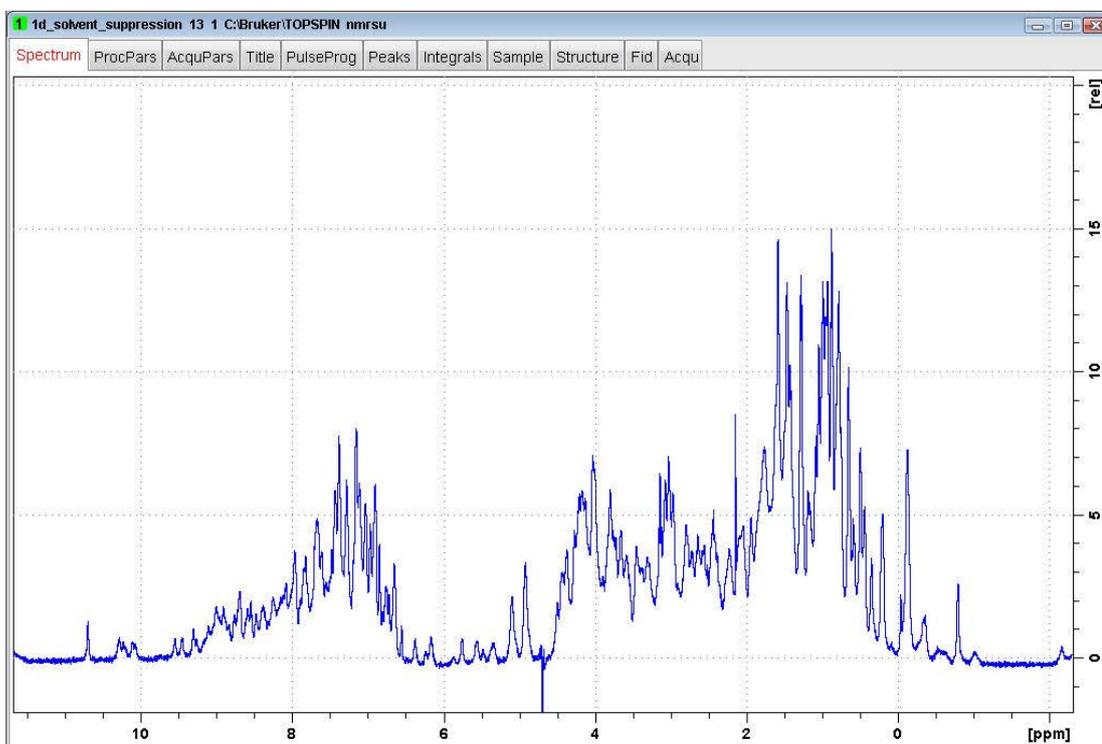
3.7 1D Solvent Suppression with WATERGATE

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

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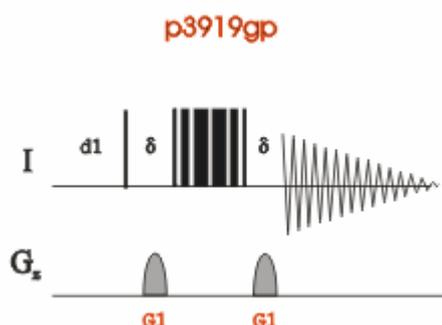
3.7.1 Parameter Setup

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

  PULPROG = **p3919gp**

  D19 [s] = **0.00015** = 1/(2*d) where d = distance to next null in Hz

  GPNAM1 = **SMSQ10.100**

  GPZ1 [%] = **20**

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

3.7.2 Acquisition

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

3.7.3   Processing

• Process and phase correct the spectrum.
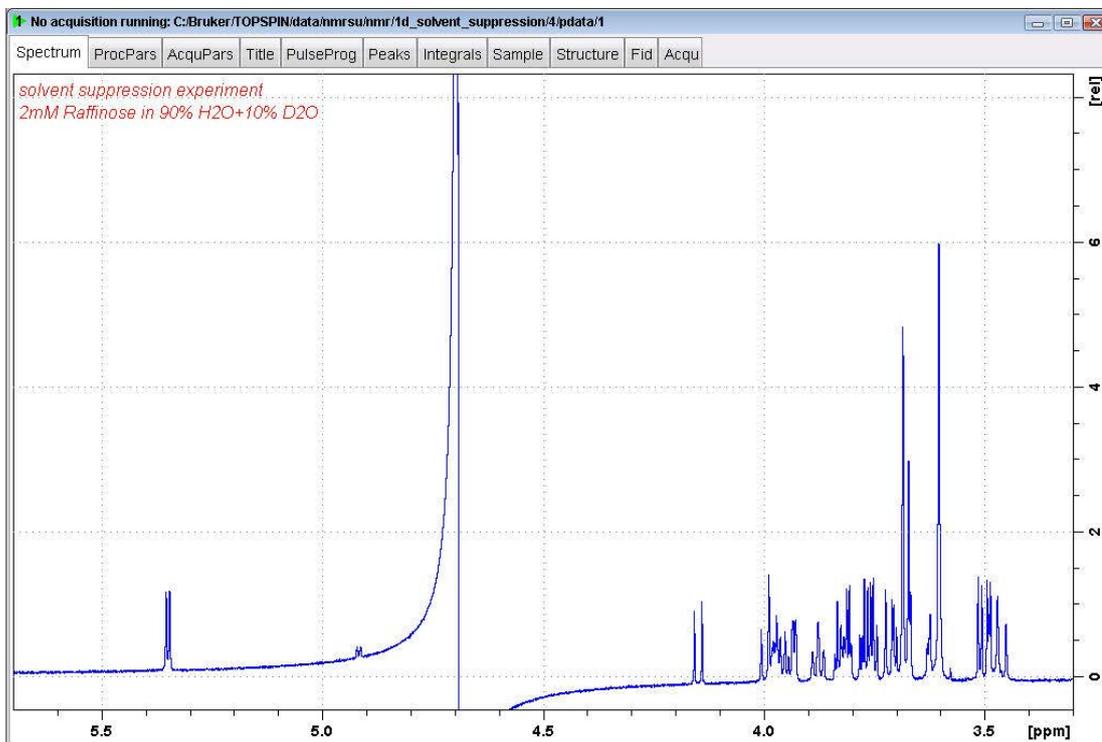
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.
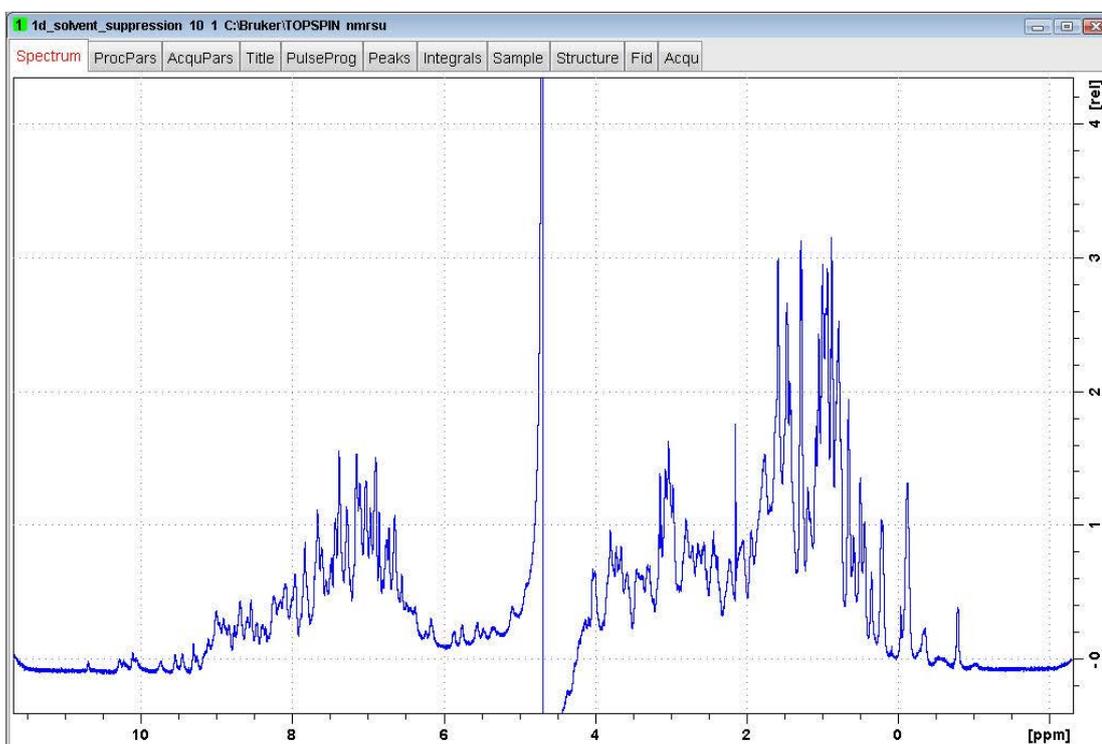
3.8   1D Solvent Suppression with Excitation Sculpting

3.8.1   Parameter Setup

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

  PULPROG = **zgesgp**

  P12 [us] = **2000**

  SPdB1 = **44.5**

  GPNAM1 = **SMSQ10.100**

  GPNAM2 = **SMSQ10.10**0

  SPNAM1 = **Squa100.1000**

  GPZ1 [%] = **31**

  GPZ2 [%] = **11**

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

3.8.2   Acquisition

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

3.8.3 Processing

- Process and phase correct the spectrum.
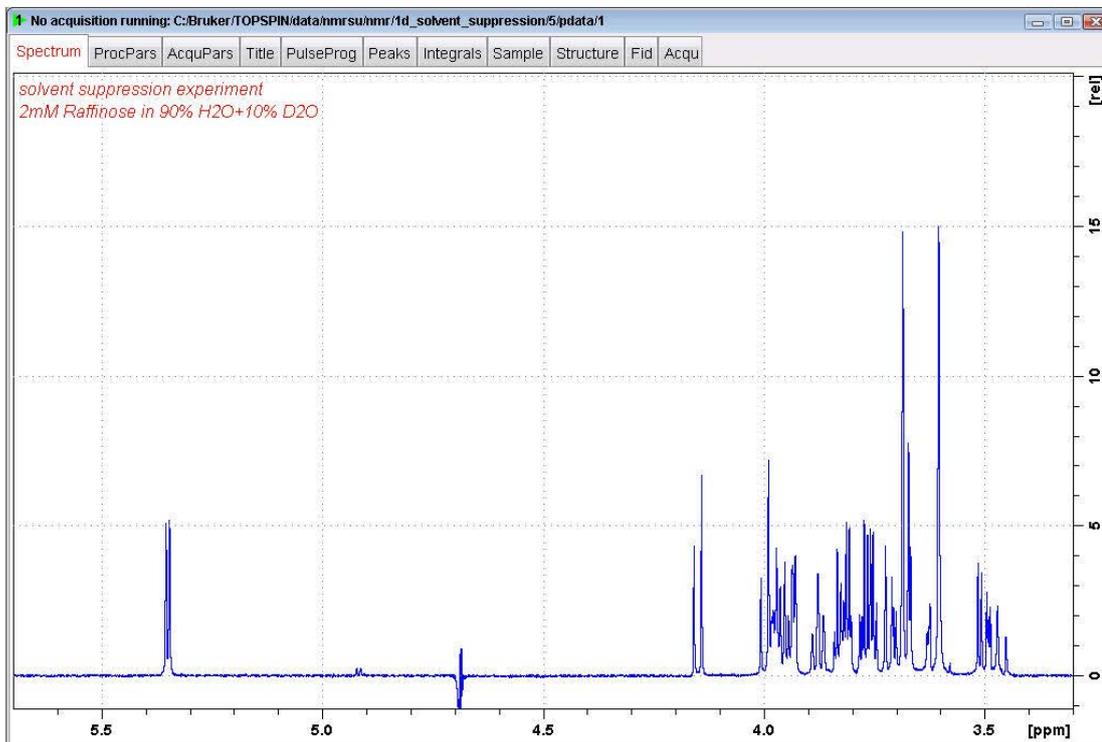
The figure above shows the solvent suppressed 1D spectrum of the Raffinose sample. The figure below shows the 1D spectrum of the Lysozyme sample.
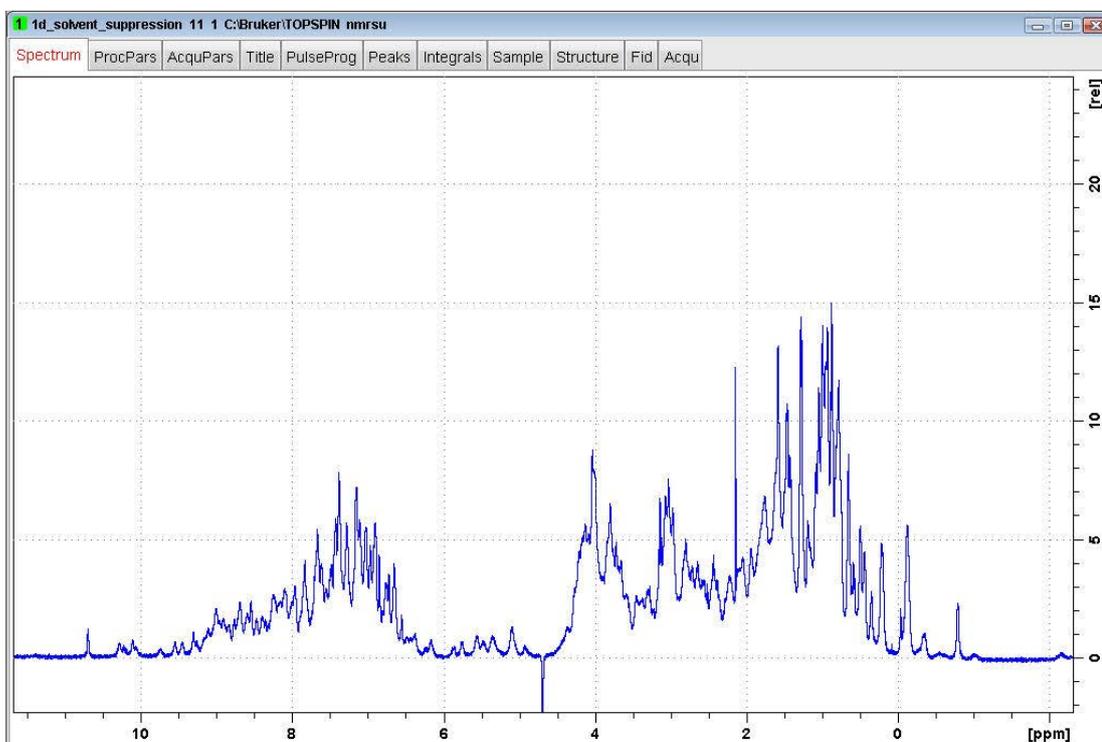
3.9 1D Solvent Suppression with WET

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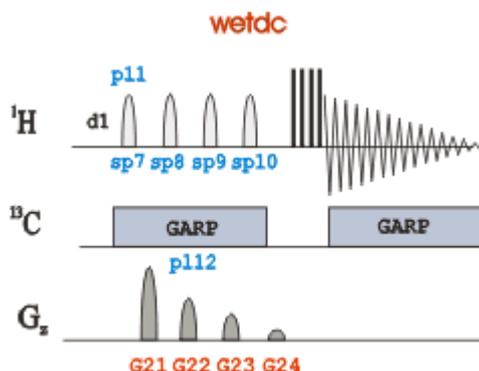
3.9.1 Sample

2 mg Sucrose in Acetonitrile and D2O

3.9.2 Preparation Experiment

• On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
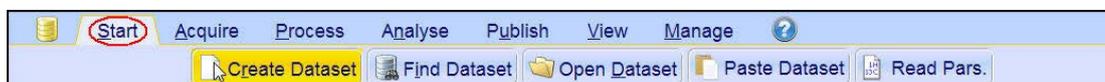
• In the New Dataset window, enter or select:

NAME = **wet_solvent_suppression_exp**

EXPNO = **1**

PROCNO = **1**

Experiment: Select **LC1DWTDC**

Set Solvent: Select **CH3CN+D2O**

DIR

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

Title

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

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

For the following steps, use the Workflow button bar.
- Click **Sample** and **Insert**.
- Click **Lock** and select the **CH3CN+D2O** solvent.
- Click **Tune** to tune the probe.
- Click **Spin** and select **Sample rotation off**.

i   Solvent suppression experiments should be run non-spinning.

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

  PULPROG = **zg30**

  DS = **0**

  NS = **1**

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

3.9.3 Acquisition

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

3.9.4 Processing

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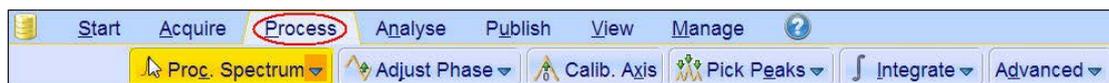
- On the Workflow button bar, click **Proc Spectrum**.

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

- In the list, select **Configure Standard Processintg (proc1d)**.
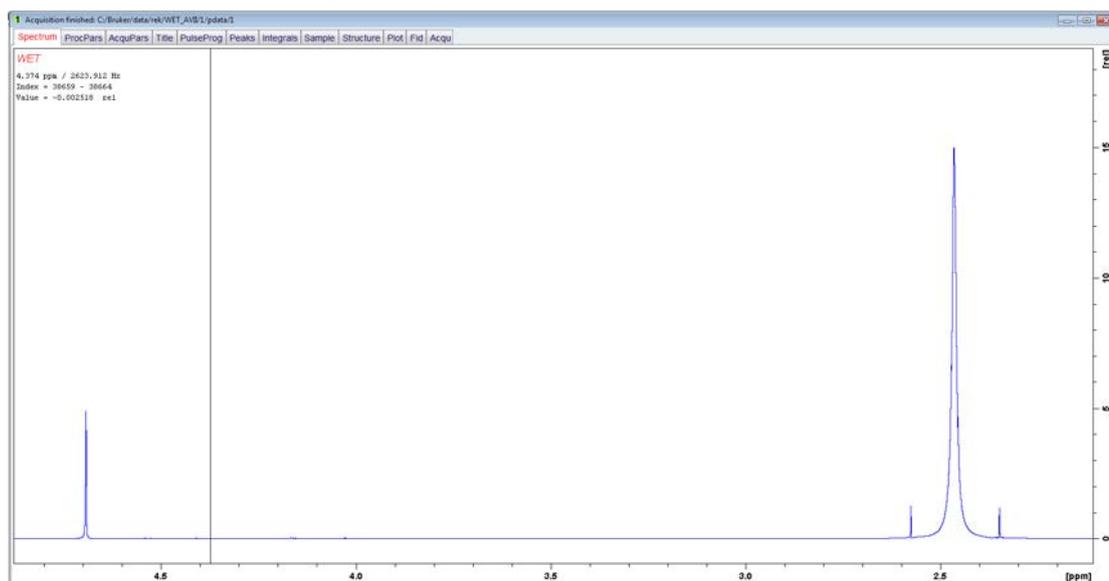
3.9.5 Selective Excitation Region Setup

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

- On the **More** button, click the **drop-down** arrow to see more options.
- In the list, select **Setup Selective 1D Expts**.
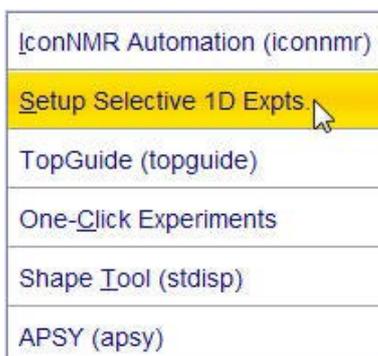
The Workflow button bar changes for setting up the 1D selective experiment.

- On the Workflow button bar, click **1D Selective Experiment Setup**.
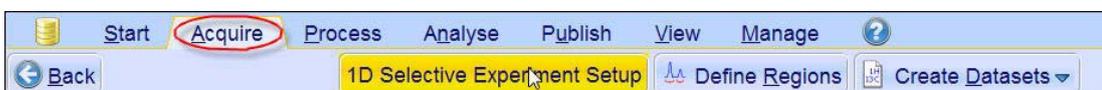
There is no other function to this button then the instruction displayed above.

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

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

- Integrate the regions on the peaks to be suppressed (e.g. **4.7 ppm** and **2.45 ppm**).
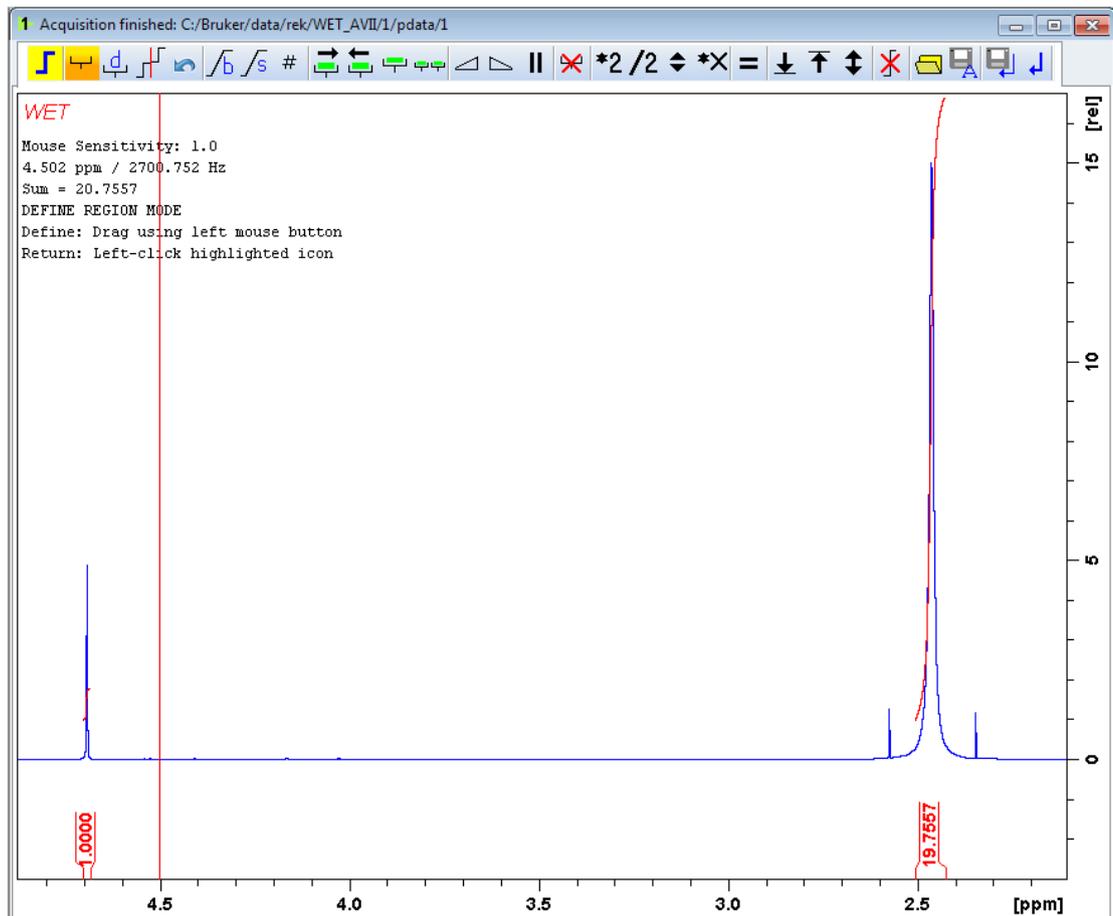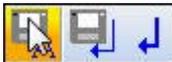
- Click **Save region as**.
- Select **Save the Region to reg**.

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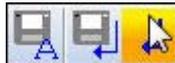
- In the Save Changes window, click **No**.

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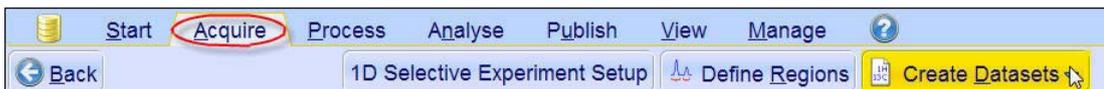
- In the list, select **Mult. Solvent Suppr./WET**.

- In the LC1DWTDC window, enter NS = **16**
- In the LC1DWTDC window, click **Accept**.
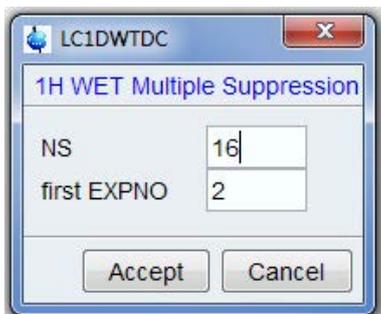
- Check the parameters in the information window.
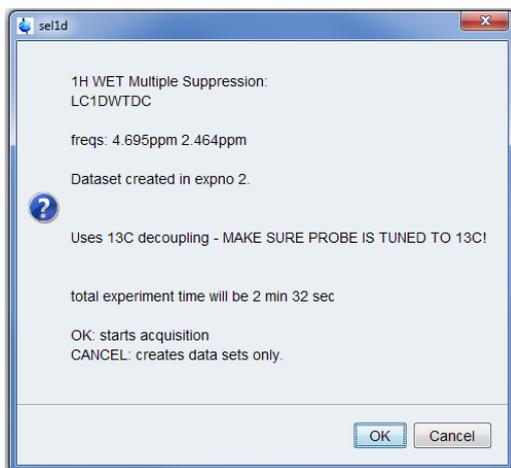
- In the sel1d window, click **OK** to start the acquisition.

3.9.6   Processing

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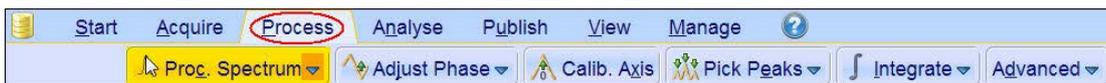
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- Select **Configure Standard Processing (proc1d)**.
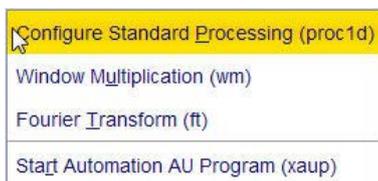
- Deselect the following options:
  - **Auto-Phasing (apk)**
  - **Set Spectrum Reference (sref)**
  - **Auto-Baseline correction (abs)**
  - **Warn if Processed data exist**

- In the proc1d window, click **Execute**.
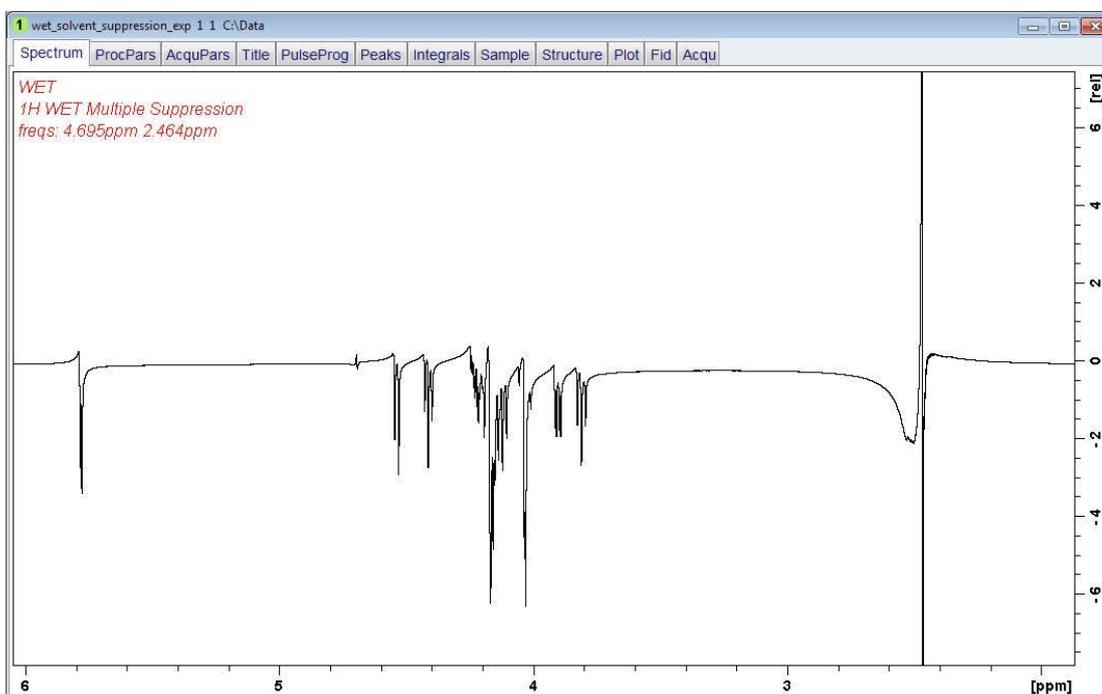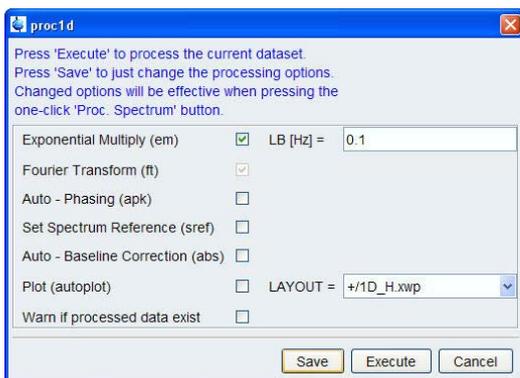
- On the Workflow button bar, click **Adjust Phase**.
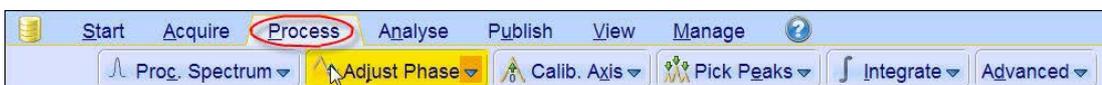
The Dataset window tabs are replaced with the **Adjust Phase** toolbar.


- Move the cursor line on top of the peak at **5.7 ppm** and right-click.
- On the shortcut menu, select **Set Pivot Point**.
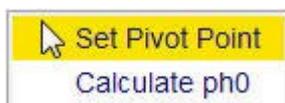
- Adjust the **0** order phase on the selected pivot point to positive absorption.
- Adjust the **1st** order phase on the peaks between **4.6 ppm** and **3.6 ppm**.
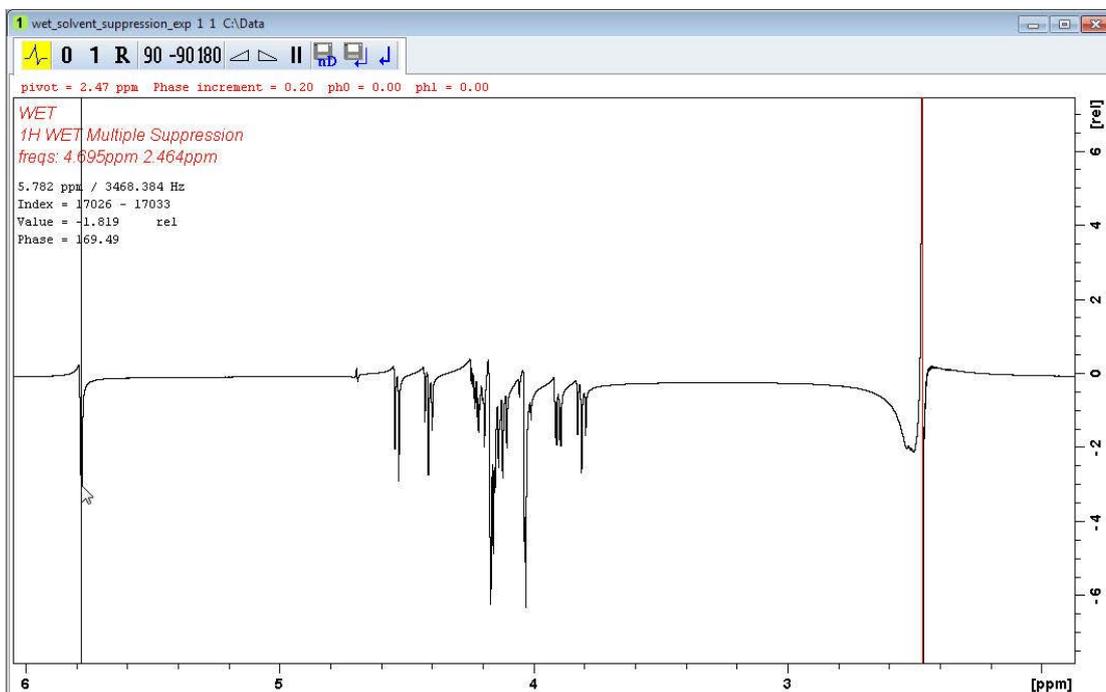
The peak at **4.7 ppm** and **2.4 ppm** are the suppressed solvent peaks and those will appear out of phase.

- To store the phase value, click **Save for nD spectrum**.
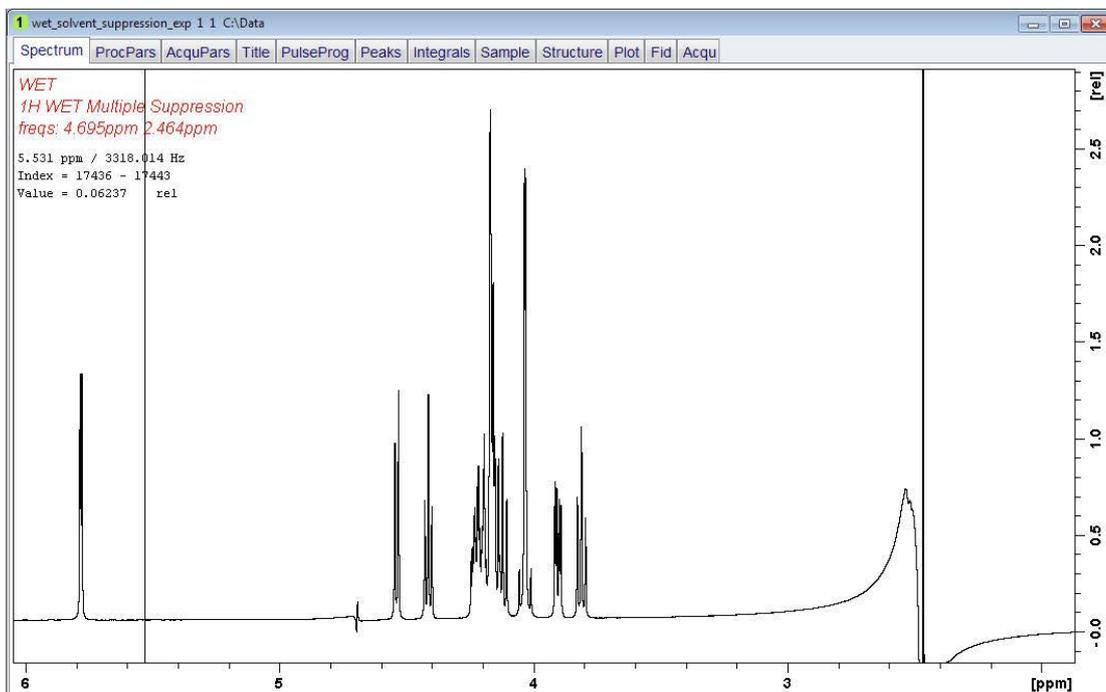
4 2D Gradient Experiments

4.1 Introduction

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

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

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

4.2 2D Multiple Quantum Filtered COSY Experiment

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

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

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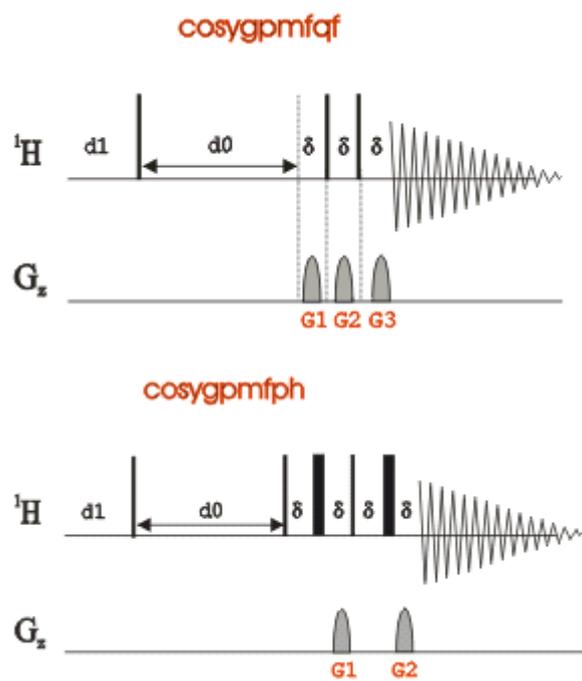
4.2.1 Preparation Experiment

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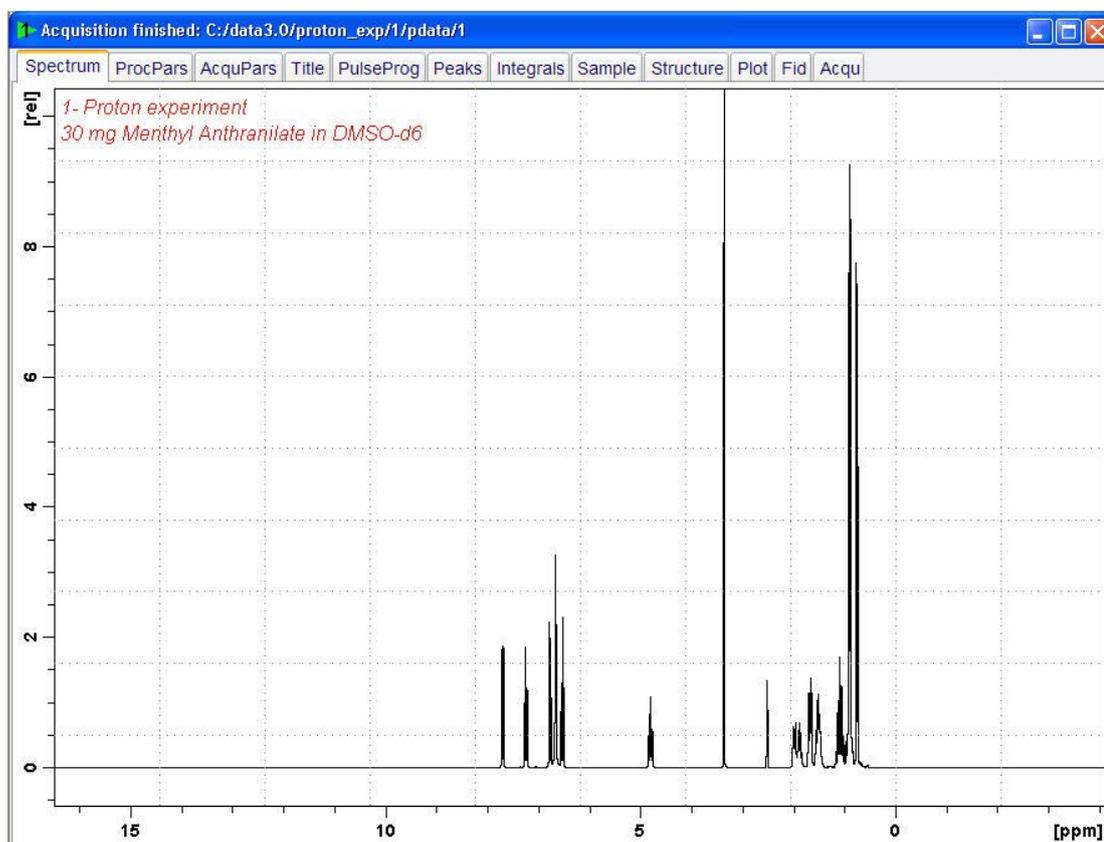
4.2.2 Setting up the MQF-COSY Experiment

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

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
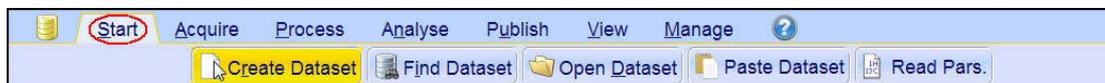
- In the New Dataset window, enter or select:

  NAME = **cosydqf_exp**

  EXPNO = **1**

  PROCNO = **1**

  Experiment: select **COSYGPDFPHSW**

  Set Solvent: select **DMSO**

DIR

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

Title

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

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

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

For the following steps, use the Workflow button bar.
- Click **Tune** to tune the probe.
- Click **Spin** and select **Sample rotation off**.

> **i** 2D experiments should be run non-spinning.

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

4.2.3   Limit Setting

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

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

setlimits

Close this dialog box after setting frequencies.

1. Open 1D dataset from Browser.

2. Zoom into region of interest.

3. Click OK to set frequencies and return to original dataset.

OK   Cancel

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

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

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

The display changes back to the 2D dataset.

4.2.4 Acquisition

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

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

4.2.5 Processing

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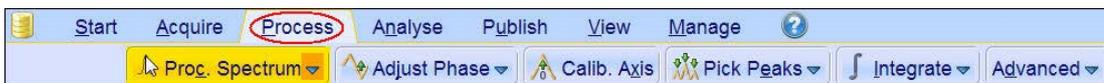
- On the Workflow button bar, click **Proc Spectrum**.

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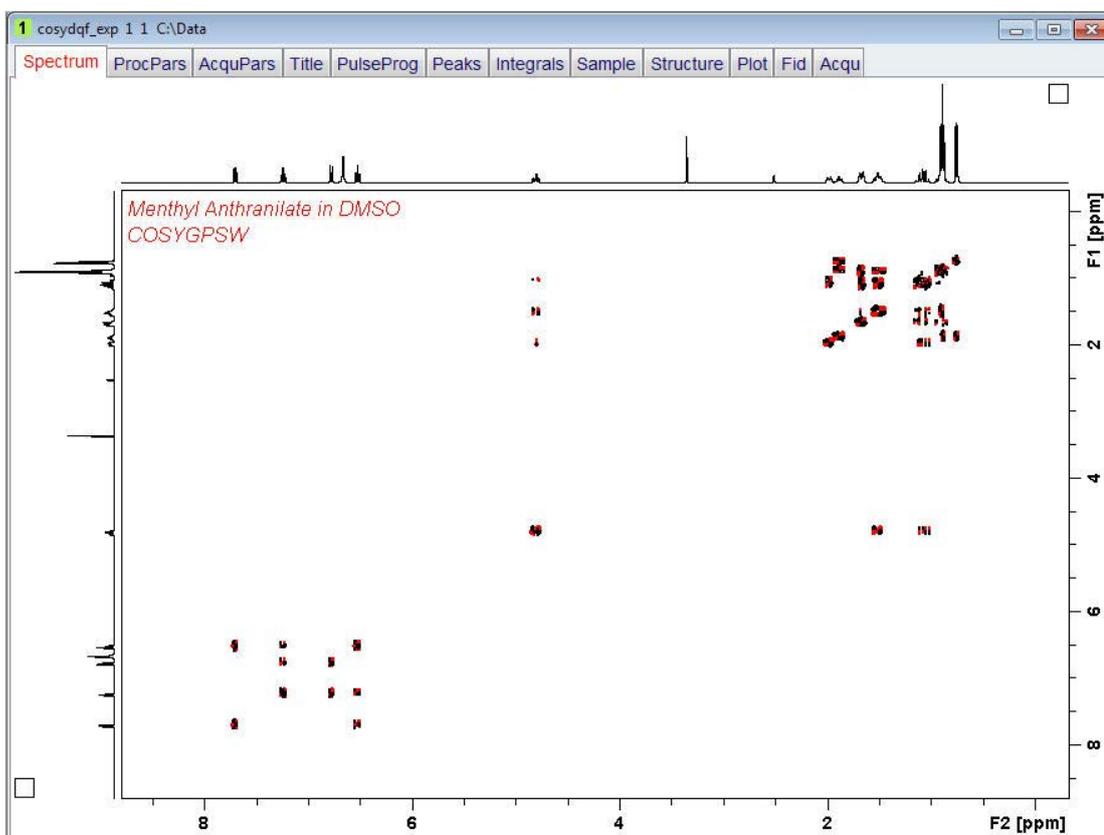
4.2.6 Plotting

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

- Type **.ls** or click on the **Contour levels to disk** button.

- On the menu bar, click **Publish** and on the Workflow button bar, click **Plot Layout**.
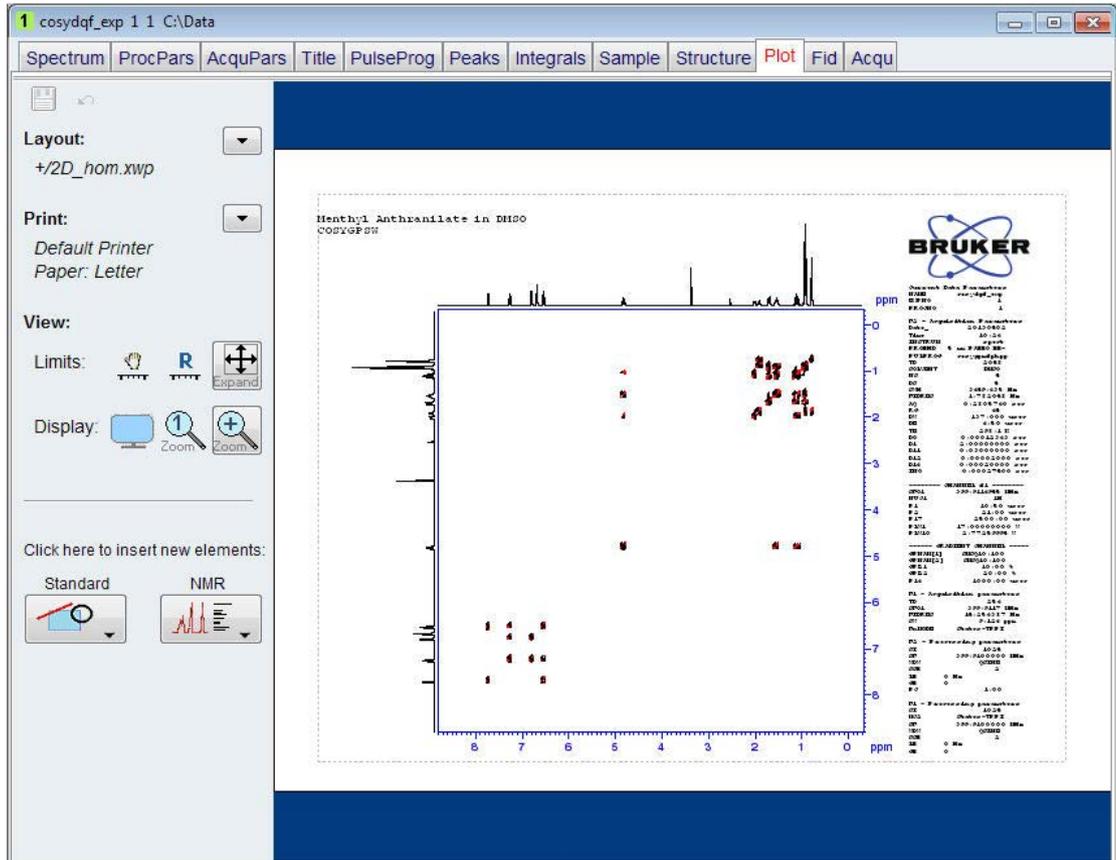
If desired, any changes can be administered by using the tools on the left side of the display.

- In the Print section, click the **down** arrow button and select **Print**.
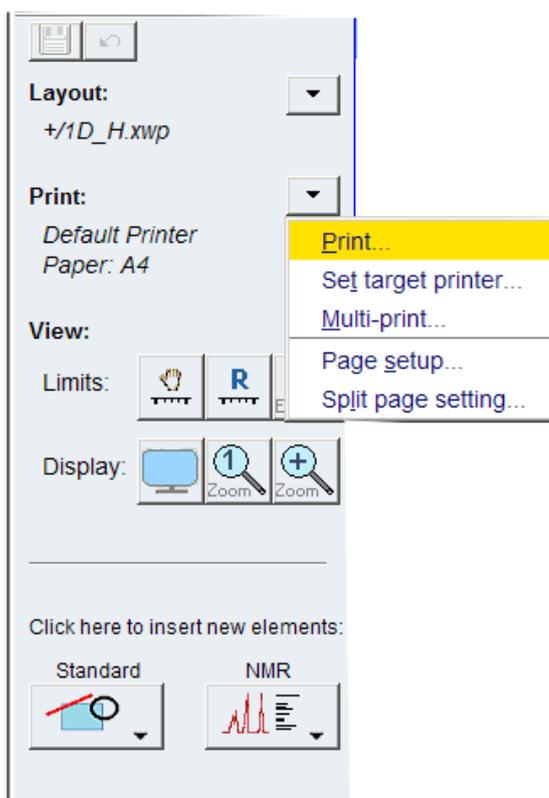
4.3   2D ¹H/³¹P Gradient HMBC Experiment

The **2D gradient HMBC** experiment records qualitative heteronuclear long-range connectivity, including through hetero nuclei. This section of the manual will guide you through the set up of a ¹H/X gradient experiment using the standard Bruker HMBCGP parameter set. In addition of changing the nucleus in F1 from ¹³C to another X-nucleus, the gradient ratio for the new X-nucleus also have to be calculated. The HMBC pulse sequence is shown in the figure below.
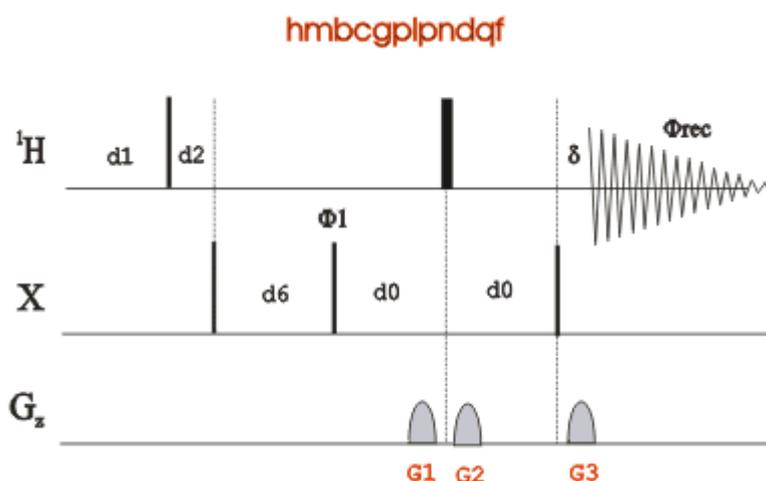
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

4.3.1 Sample

30mg Dimethyl methylphosphonate in CDCl$_3$
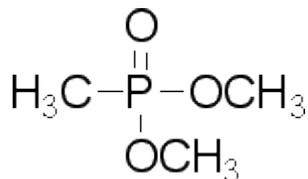
This ^{31}P nucleus in this sample does not have any direct proton attached The long range coupling from the 3 methyl protons to ^{31}P is **17 Hz**, where the other 6 methyl protons through the additional oxygen nuclei show a J-value of **11 Hz**. The J-values can be easily obtained from the proton spectrum (see chapter *¹H Reference Experiment [▷ 49]*).

4.3.2 ¹H Reference Experiment

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book Basic NMR experiments, chapter *1D Proton Experiment*, Paragraph *Experiment Setup* through *Processing* using CDCl$_3$ as a lock solvent.
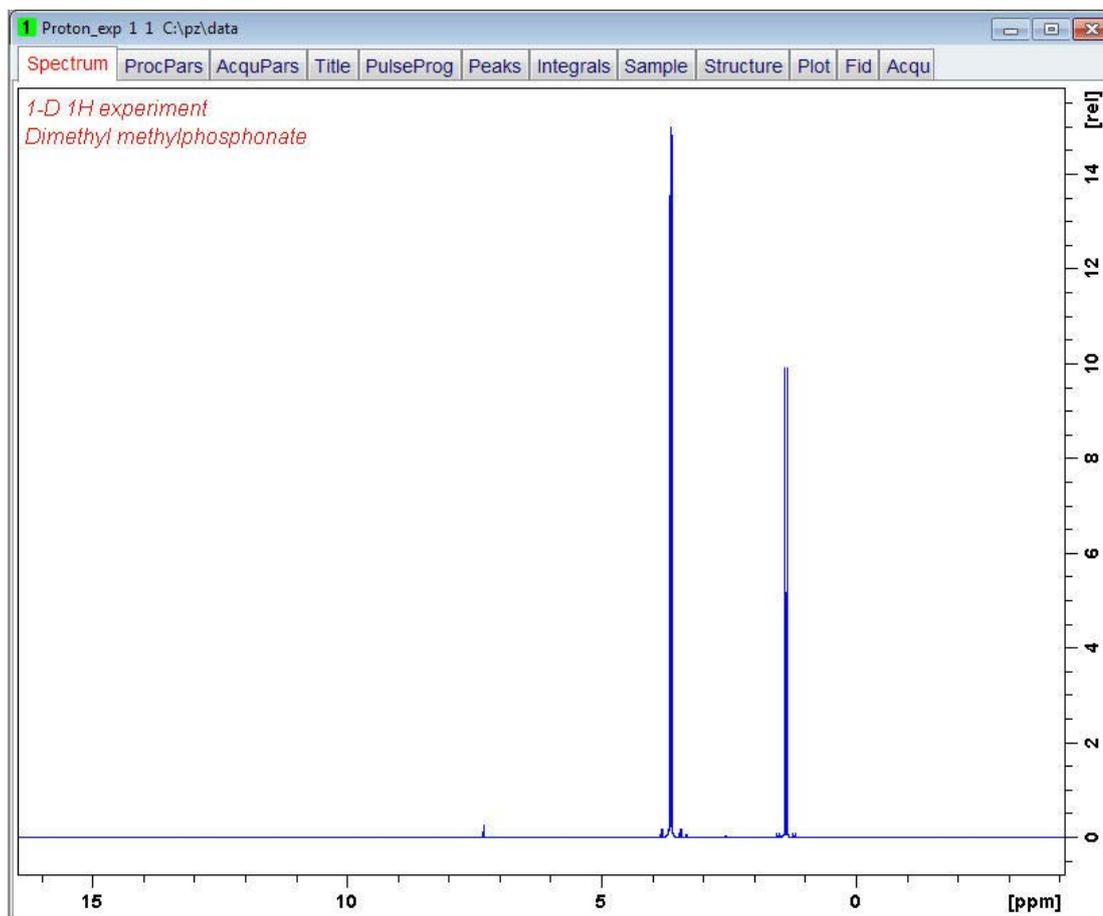
4.3.3   ³¹P Reference Experiment

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

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

- In the New Dataset window, enter or select:

   NAME = **31P_exp**

   EXPNO = **1**

   PROCNO = **1**

   Experiment: select **P31CPD**

   Set Solvent: select **CDCl3**

DIR

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

Title

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

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

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

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

4.3.4 Acquisition

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

4.3.5 Processing

- Process and phase correct the spectrum.

2D Gradient Experiments
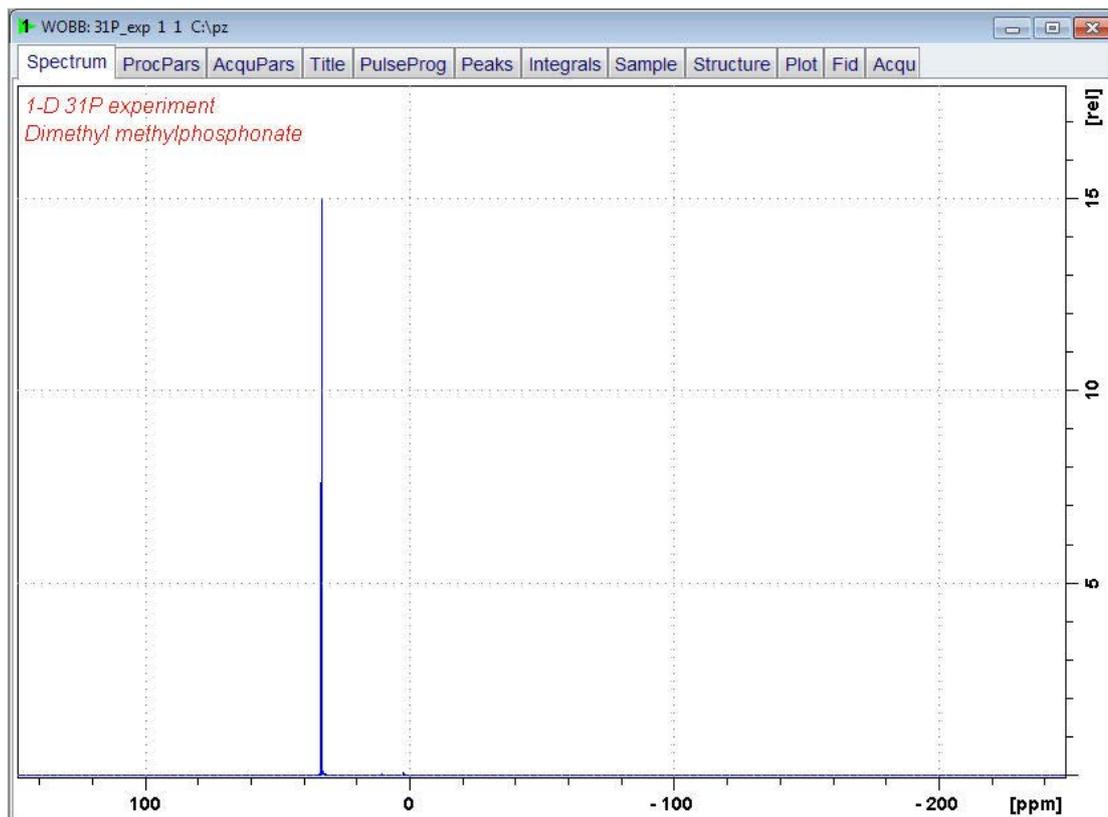
4.3.6   Setting up the HMBC Experiment

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
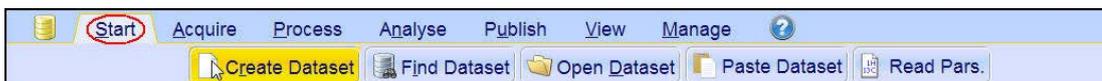
- In the New Dataset window, enter or select:

NAME = **1H_31P_hmbc_exp**

EXPNO = **1**

PROCNO = **1**

Experiment: select **HMBCGP**

Set Solvent: select **CDCl3**
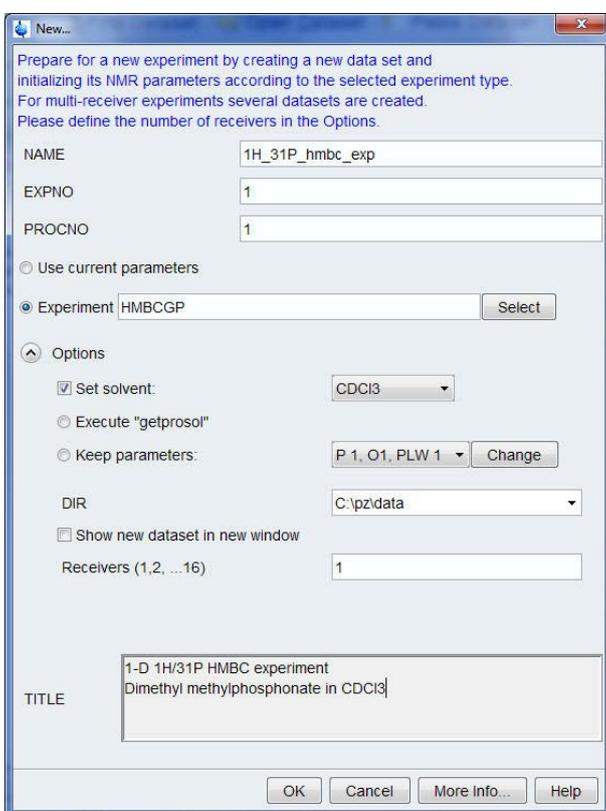
DIR

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

Title

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

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

• On the menu bar, click **Acquire**.

• In the Dataset window, select the **AcquPars** tab.
• Click the **Set nuclei and routing** button to display the routing window.
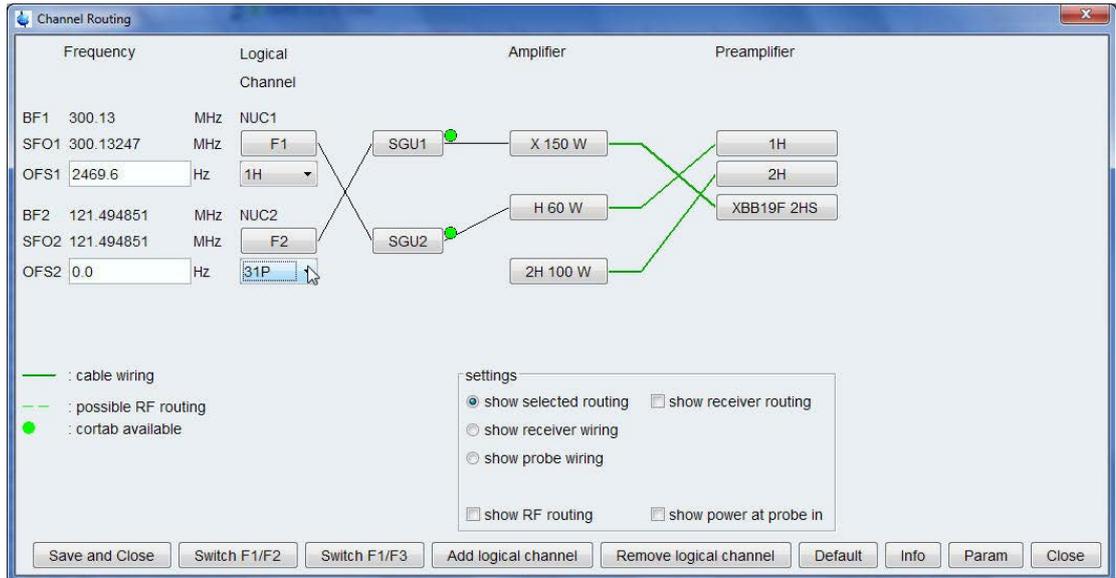
• Change the following parameter:

NUC2 = **31P**

ℹ️   Do not modify the routing!

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

• Change the following parameter:

NUC1 [F2] = **31P**

• Scroll down to the Program parameter section in the AcquPars window.
• Click the CNST **Edit** button (Constant used in pulse programs).

- Change the following parameter:

  CNST13 = **14** (J 31P/1H long range)

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

- On the **Spin** button, click the **drop-down** arrow to see more options.
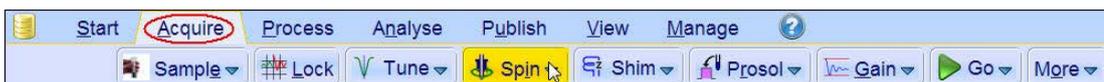
- In the list, select **ro off**.
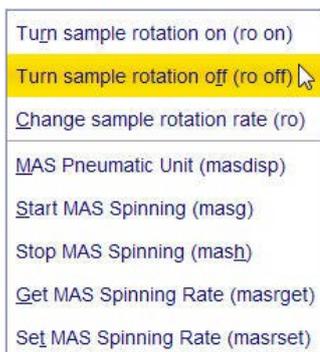
> **i**   2D experiments should be run non-spinning.

- On the Workflow button bar, click **Prosol**.
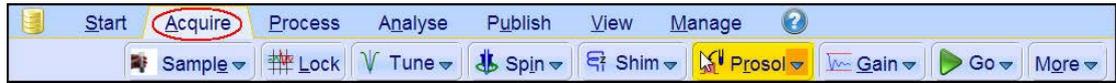
This will load the pulse width and power levels in to the parameter set.

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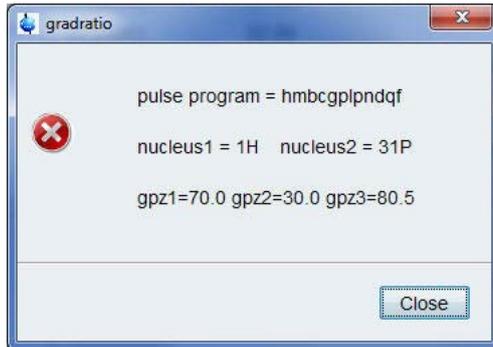
The command executes the AU_program **gradratio** to calculate the gradient ratio **GPZ1**, **GPZ2** and **GPZ3** for the nucleus **31P**. To check if the correct gradient ratio values has been entered in the **AcquPars**, follow the steps below.

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

- Click **GRADIENT Edit** (Gradient parameters).
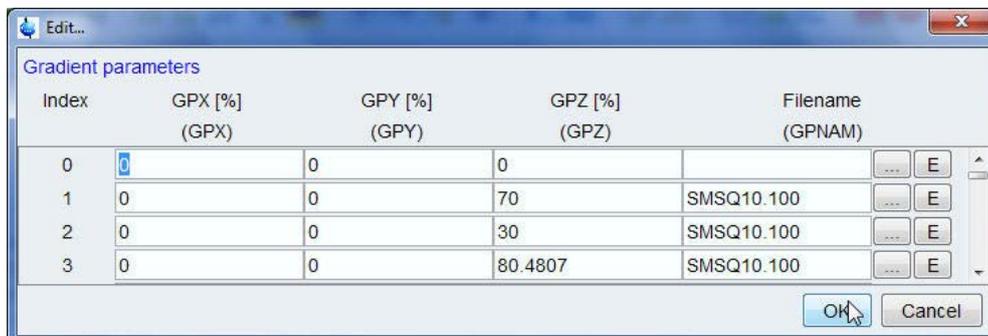
- In the Dataset window, select the **Spectrum** tab.

4.3.7 Limit Setting

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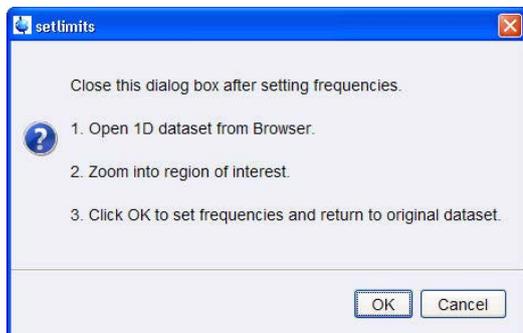
- To open the 1D Proton spectrum, right-click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of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

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

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

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

- On the Workflow button bar, click **SetLimits**.
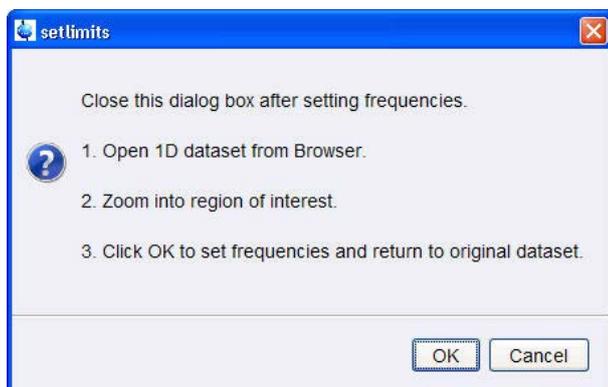
- To open the 1D ^{31}P spectrum, right click on the dataset name in the browser window (e.g. ^{31}**P_exp 1**) and select **Display** or click and hold the left mouse button for dragging the 1D ^{31}P dataset into the spectrum window.
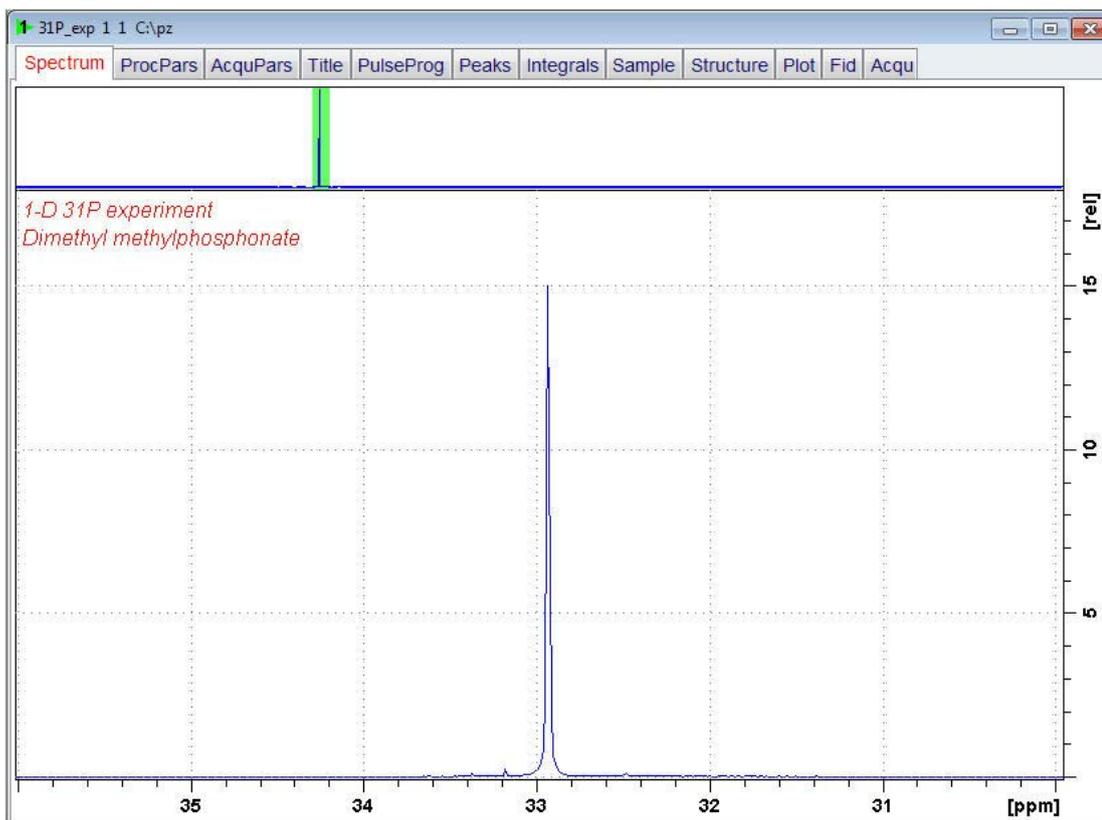
- Expand the spectrum to display all peaks.

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

The display changes back to the 2D dataset.

4.3.8 Acquisition

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

4.3.9 Processing

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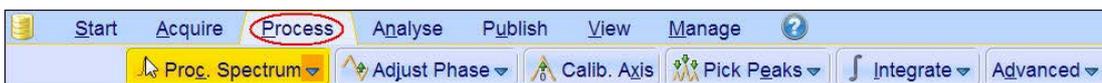
- On the Workflow button bar, click **Proc Spectrum**.

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

- In the apk2 message window, click **Close**.
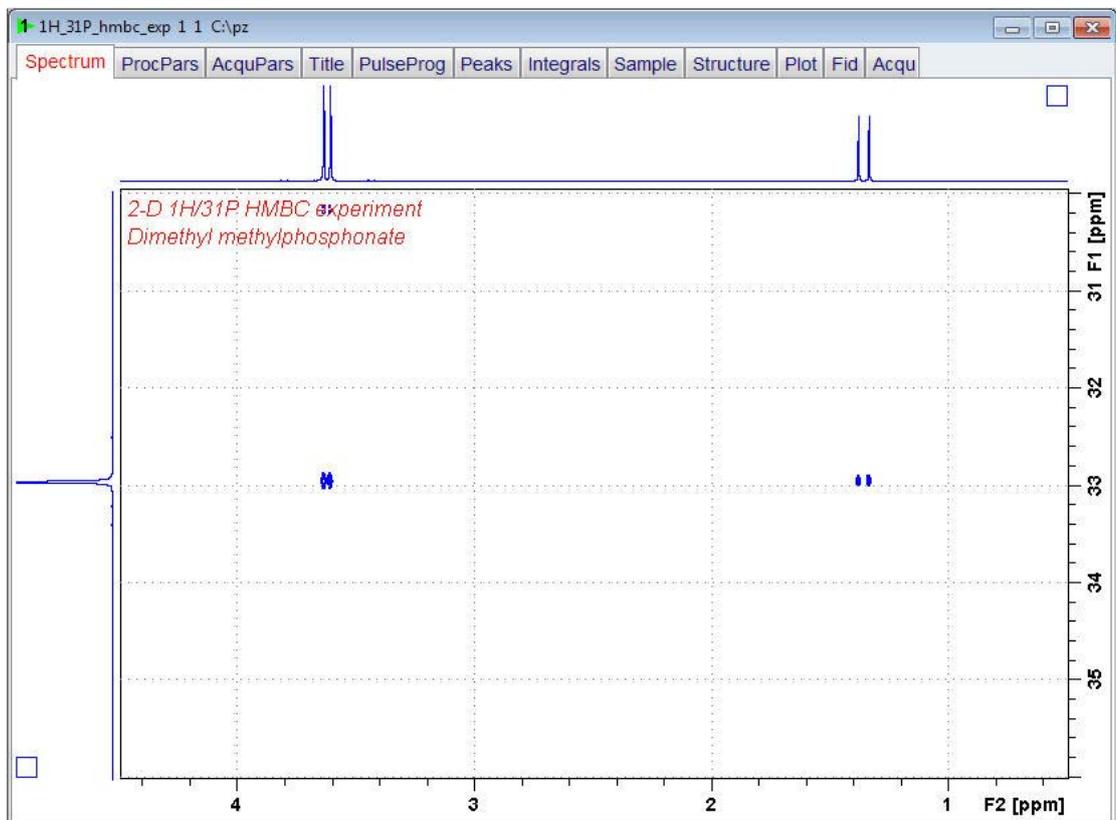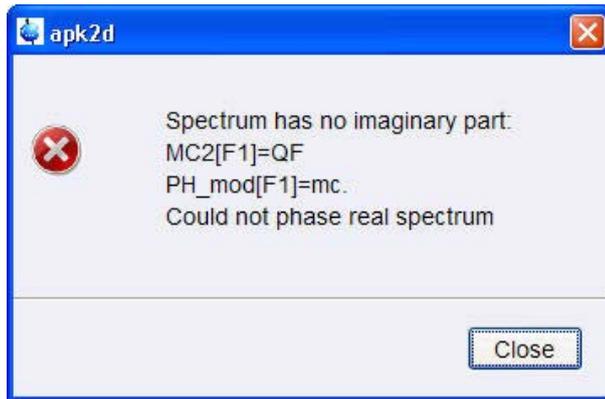
4.3.10 Plotting

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

- Enter **.ls** or click the **Contour levels to disk** button.

- On the menu bar, click **Publish** and on the Workflow button bar, click **Plot Layout**.
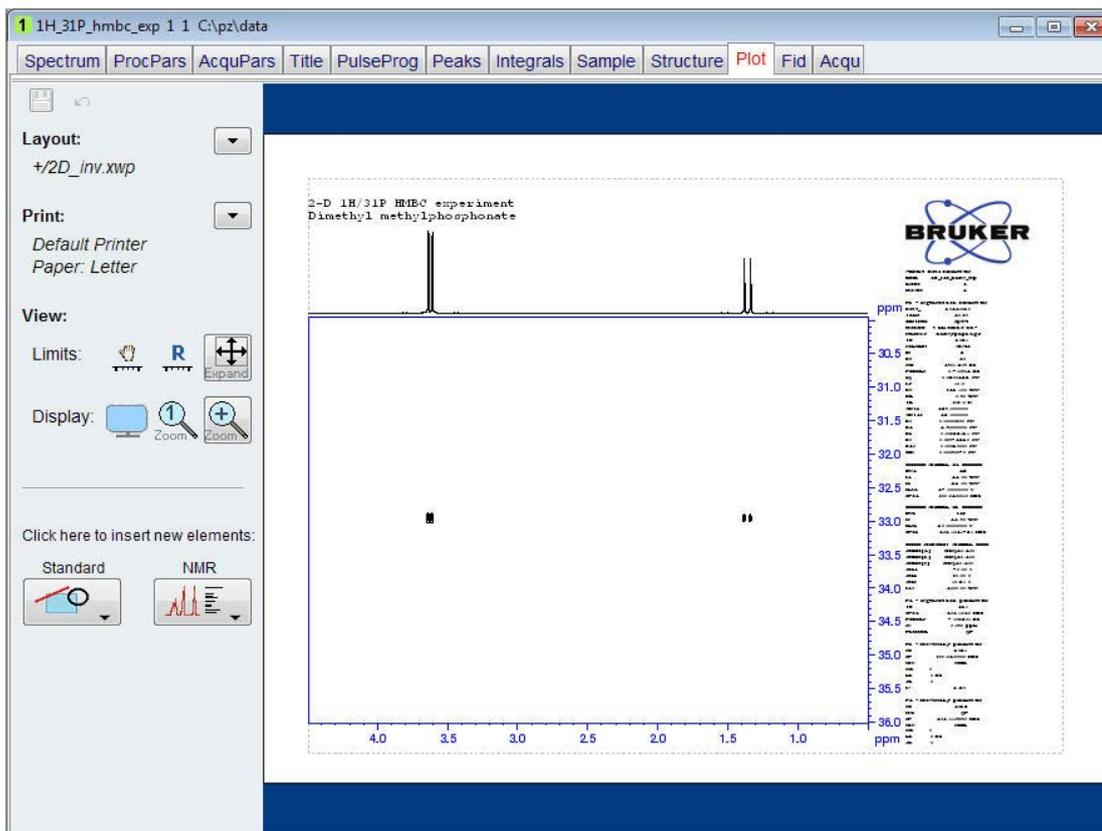
If desired, any changes can be administered by using the tools on the left side of the display.

- In the Print section, click the **down** arrow button and select **Print**.
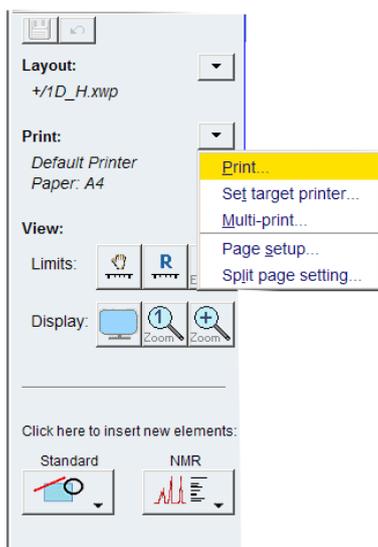
5 1D Experiments using Shaped Pulses

5.1 Introduction

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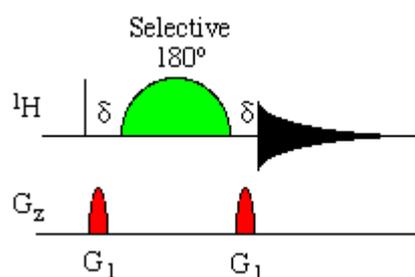

Selective 1D experiments can be easily derived by adding the corresponding mixing process between the SPFGE block and the acquisition period.

To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients. Three different ways to run this experiment are discussed in this chapter. **Example 1** and **2** show how to manually set the excitation region using the on- and off-resonance options. **Example 3** uses the Flow bar tools to automatically calculate the excitation region. All 3 examples can be applied to the 1D selective experiments, such as **SELCOGP**, **SELNOGP** and **SELMLGP**.

5.2 Sample

A sample of **30mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.
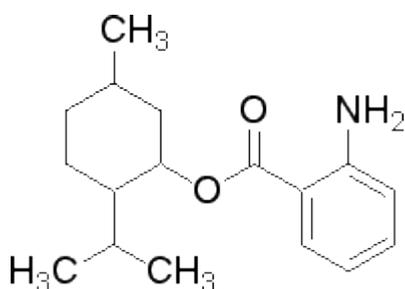
5.3 Example 1: 1D Selective COSY Experiment using the On-Resonance Option

5.3.1 Introduction

The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ^{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.

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

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

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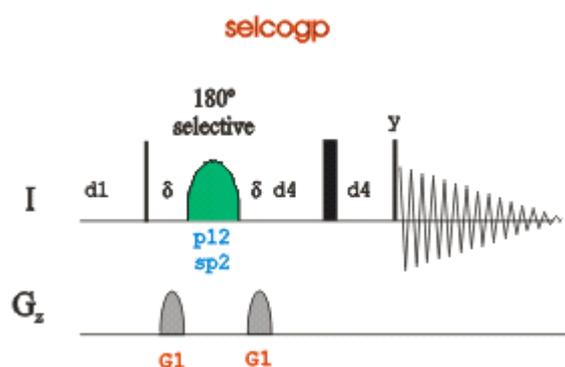
5.3.2   Reference Spectrum

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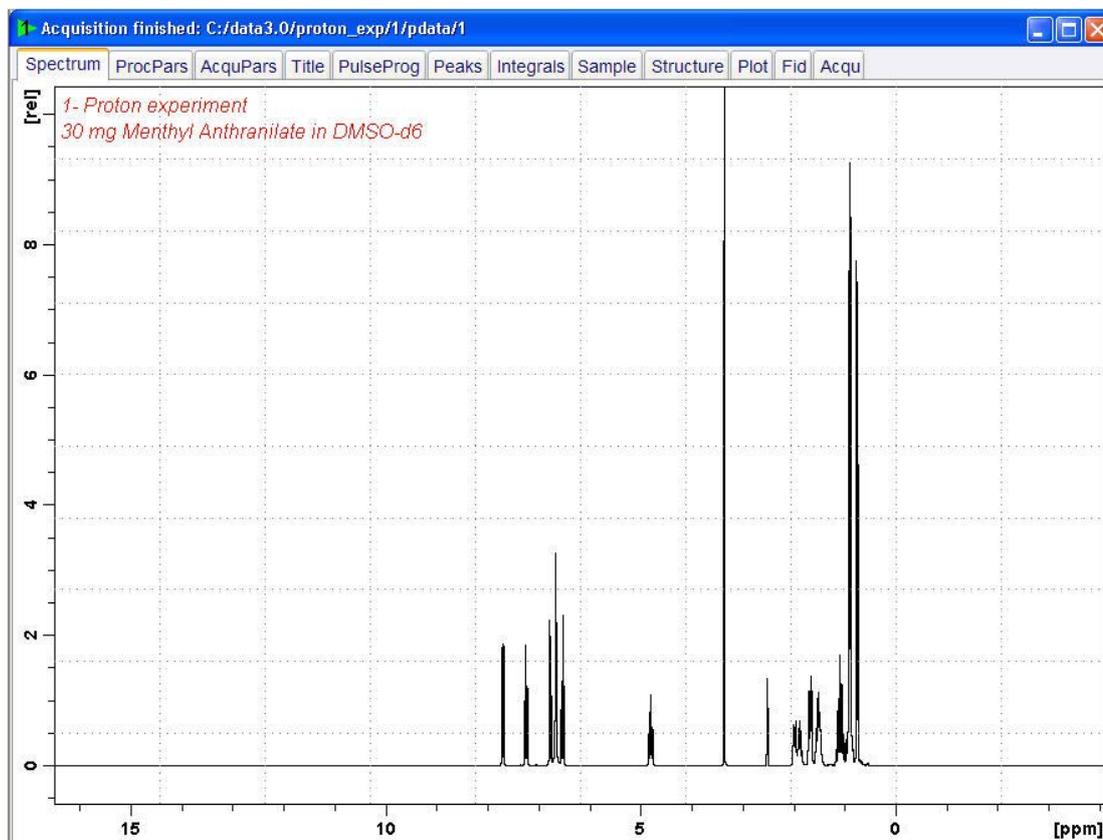
5.3.3   Selective Excitation Region Set Up

5.3.3.1   On Resonance

Ensure that the SW is large enough to cover the entire Spectrum accounting for the position of O1. The shaped pulse is applied on resonance (at the O1 position). The power level and width of the excitation pulse have to be known and entered into the Prosol parameter table.

• At the command prompt, type **wrpa.**
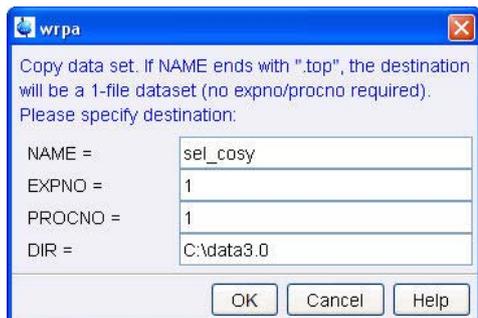
- Change NAME = **sel_cosy**.
- In the wrpa window, click **OK**.
- At the command prompt, type **re** and hit **Enter**.
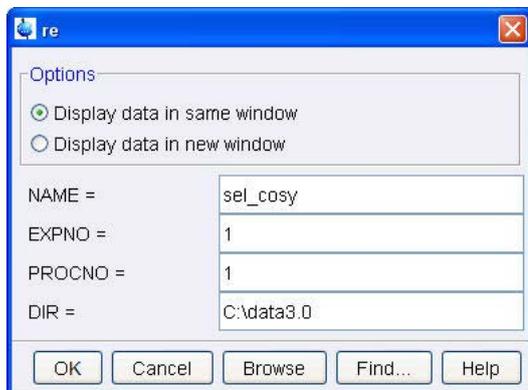
- Change NAME = **sel_cosy**.
- In the re window, click **OK**.
- Expand peak at **4.8 ppm**.

- Click **Set RF from cursor**.
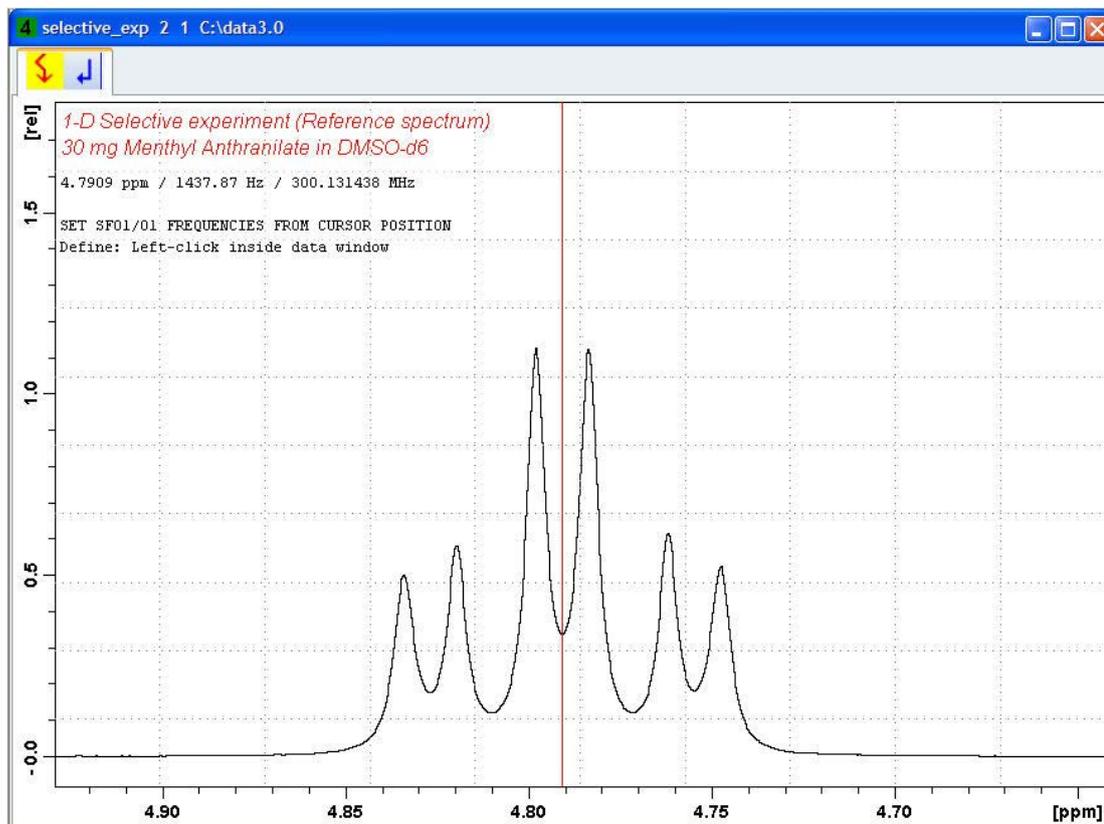
- Move the cursor line into the center of the multiplet.
- To set the frequency, click **left**.

- In the O1/O2/O3 window, click **O1**.
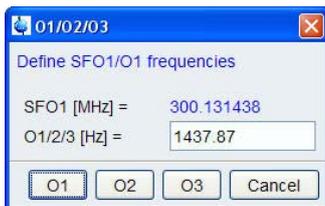
5.3.4 Setting Up the Selective COSY

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

- On the Workflow button bar, click **Read Pars**.
- In the **Find file names** field, enter **SEL*** to display all selective parameter sets as shown in the figure below.
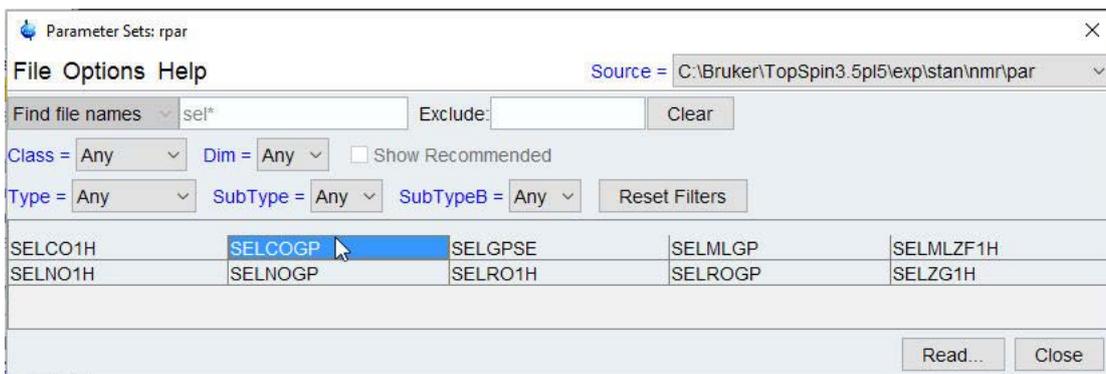
- Select **SELCOGP**.
- In the Parameter Sets: rpar window, click **Read**.
- Select the acqu, proc and outd parameter options only.
- In the **Keep parameters** list of values, select **P1, O1, PLW1**.
- Enable the **Keep parameters** option.
- In the rpar window, click **OK**.
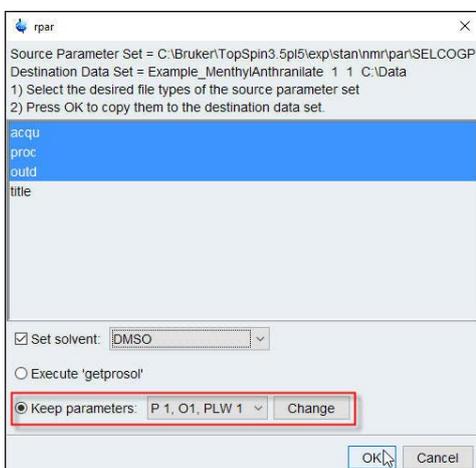
- In the Dataset window, select the **Title** tab and enter:

  1D Selective COSY experiment

  30 mg Menthyl Anthranilate in DMSO-d6

- To store the title, click **Save**.
- In the Dataset window, select the **Spectrum** tab.
- On the menu bar, click **Acquire**.

For the following steps, use the Workflow button bar.

- Click **Spin** and select **Sample rotation off**.

> **i** 1D selective experiments should be run non-spinning.

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

5.3.5 Acquisition

- To start the acquisition, click **Go**.

5.3.6 Processing

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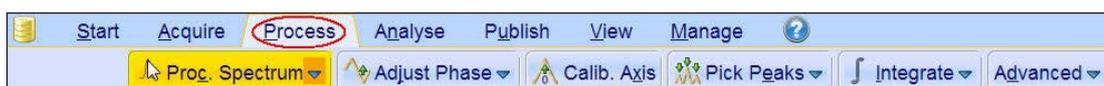
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing**.

  Configure Standard Processing (proc1d)
  Window Multiplication (wm)
  Fourier Transform (ft)
  Fourier Transform Options ... (ftf)
  Start Automation AU Program (xaup)

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

- In the proc1d window, click **Execute**.
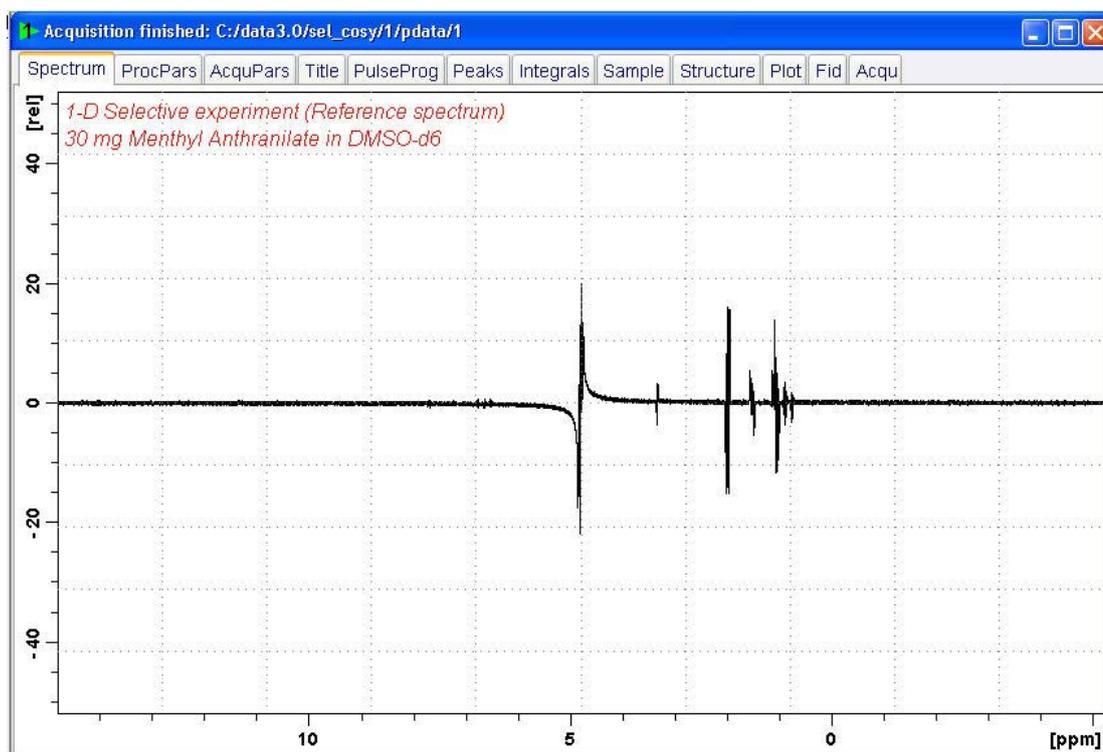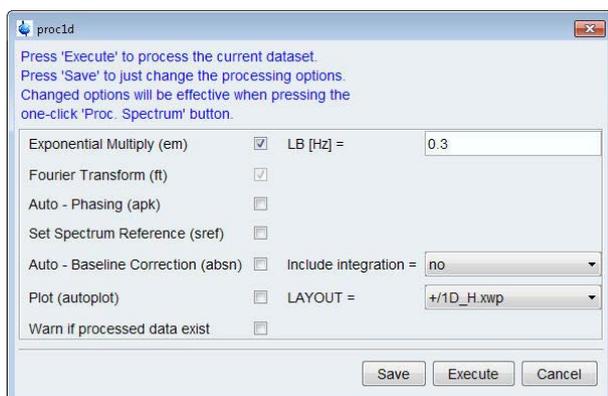
- Expand the spectrum from **4 ppm** to **0.5 ppm**.

- To display an antiphase pattern, adjust the **0** order phase on the peak at **2.0 ppm**.
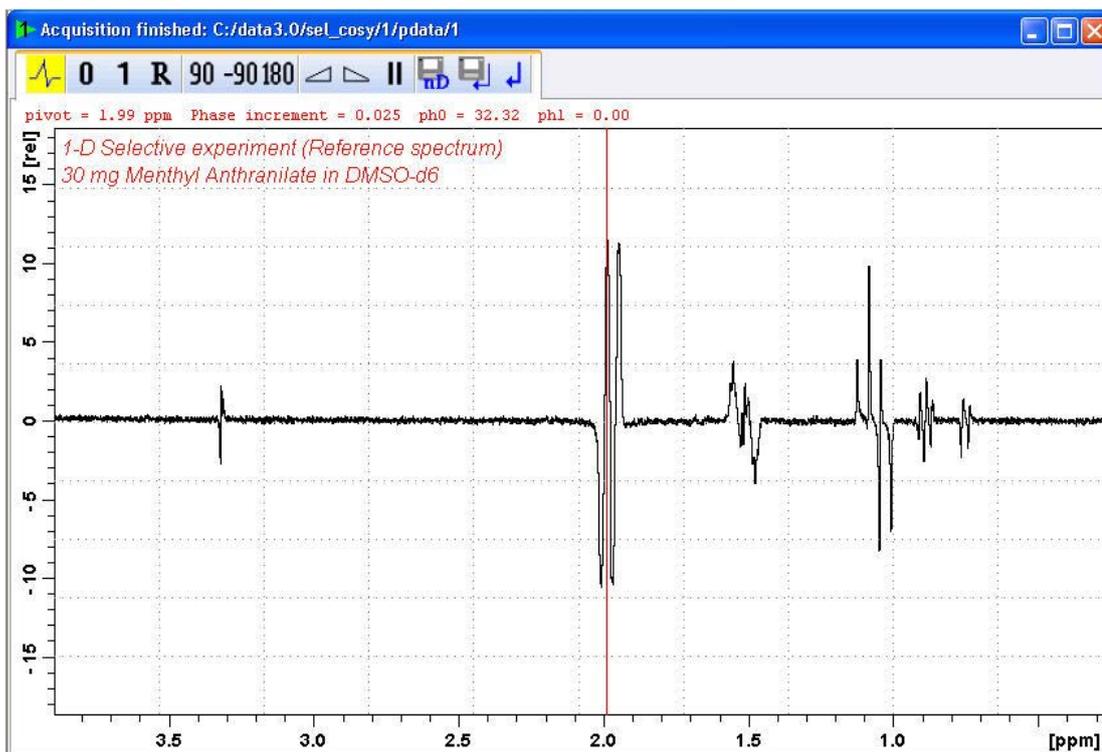
- To store the phase value, click **Return & save phased spectrum**.

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

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

5.4.1 Introduction

This experiment consist of three parts:

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

selnogp
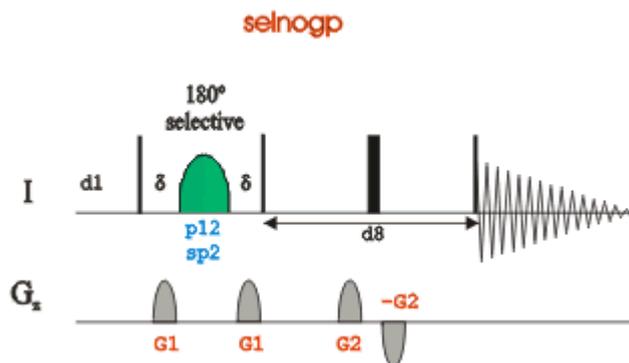
5.4.2   Reference Spectrum

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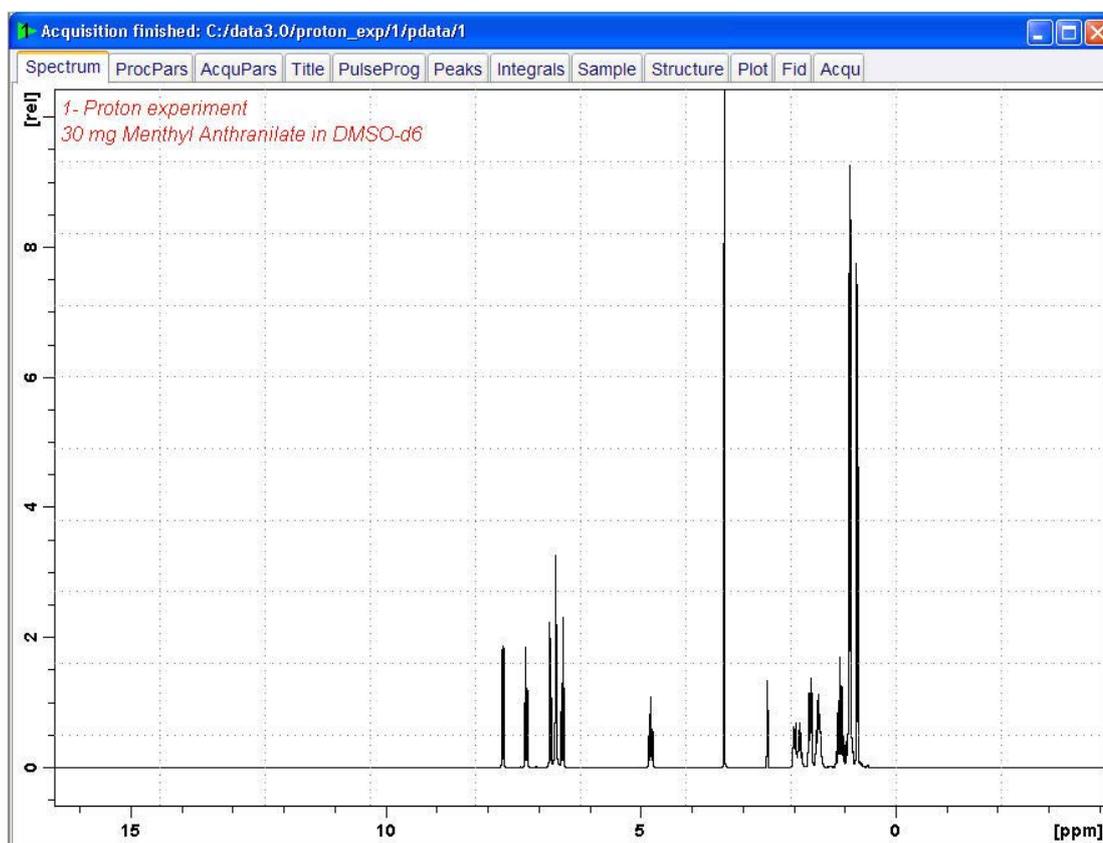
5.4.3   Selective Excitation Region Set Up

ℹ️ This method does not require a large SW. The shaped pulse is applied off resonance (not on the O1 position). The power level and pulse width of the excitation pulse have to be known and entered into the Prosol parameters.

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

- At the command prompt, type **re**.

- Change NAME = **sel_noesy**.
- In the re window, click **OK**.
- Expand the peak at **4.8 ppm**.
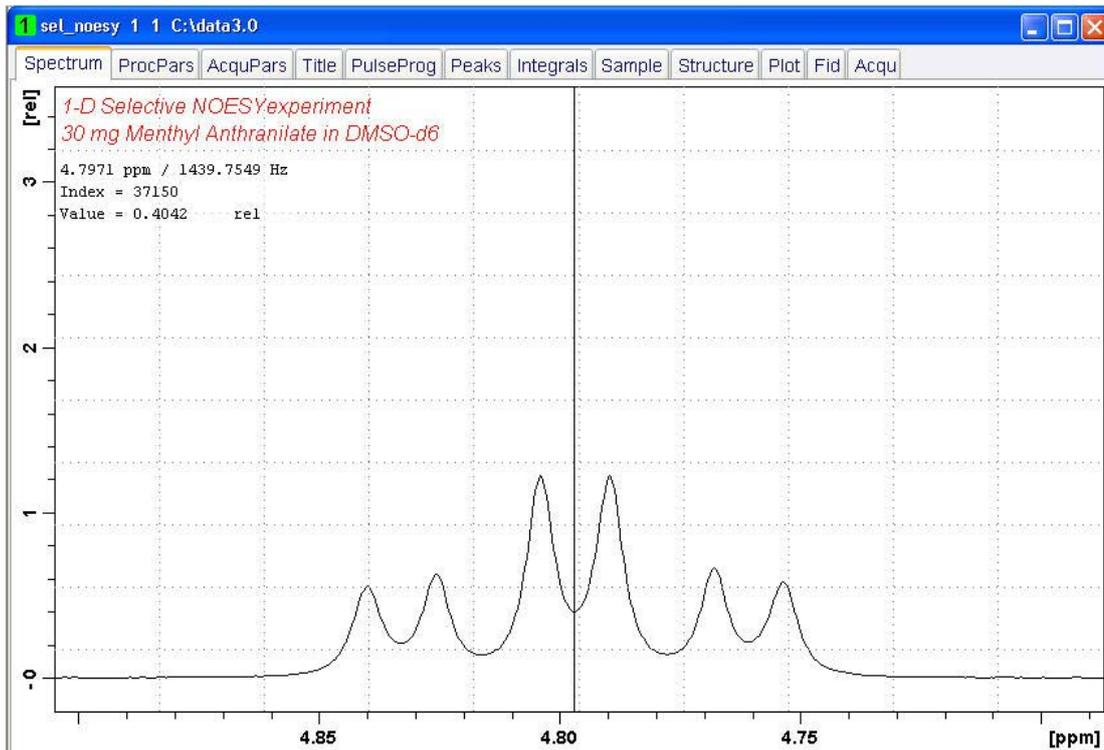
- Move the cursor line to the center of the peak.
- **Step 1:** Write down the cursor offset frequency value displayed in the upper left of the spectrum window (e.g. **1439.75**).

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

- **Step 2:** At the TopSpin command prompt, type **O1**.
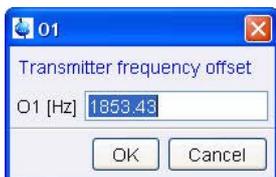
- **Step 3:** Write down the current value (e.g. **1853.43**).
- **Step 4:** Calculate the difference of step 1 and 3 (e.g. **-413.68**).

- In the O1 window, click **Cancel**.

ℹ If the signal is down field of O1, a positive value must be entered for spoff. If the signal is up field of O1, spoff will have a negative value.

5.4.4   Setting Up the Selective NOESY

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

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

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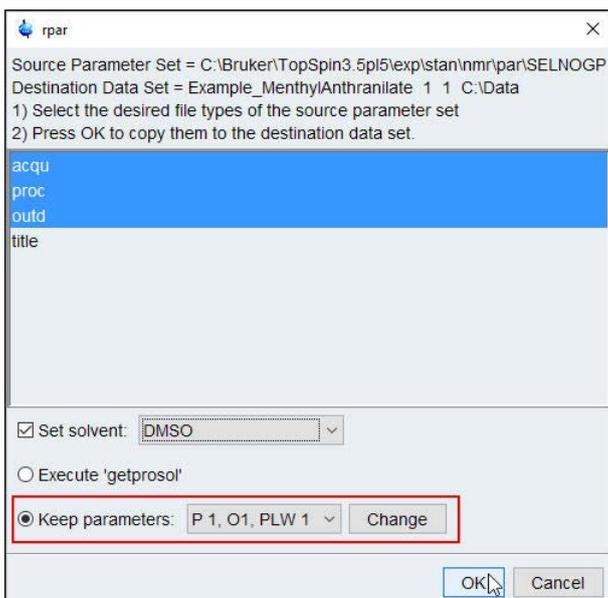
- In the Dataset window, select the **Title** tab.

- Make the following changes:

  1D Selective NOESY experiment

  30 mg Menthyl Anthranilate in DMSO-d6

- To store the title, click **Save**.

- In the Dataset window, select the **Spectrum** tab.
- On the menu bar, click **Acquire**.
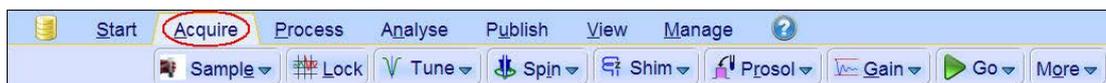
For the following steps, use the Workflow button bar.

- Click **Spin** and select **Sample rotation off**.

ⓘ 1D selective experiments should be run non-spinning.

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

  PULPROG = **selnogp**

  D8 = **0.450**

  DS = **8**

  NS = **64**

  SPNAM2 = **Gaus1_180r.1000**

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

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

5.4.5 Acquisition

- To start the acquisition, click **Go**.

5.4.6 Processing

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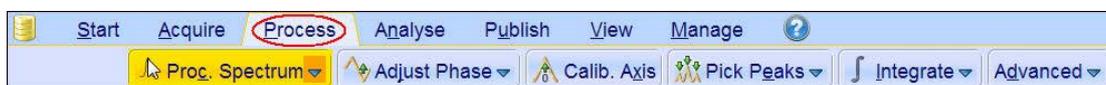
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing**.

Configure Standard Processing (proc1d)
Window Multiplication (wm)
Fourier Transform (ft)
Fourier Transform Options ... (ftf)
Start Automation AU Program (xaup)

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

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

proc1d

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

Exponential Multiply (em)	☑	LB [Hz] =	0.3
Fourier Transform (ft)	☑		
Auto - Phasing (apk)	☐		
Set Spectrum Reference (sref)	☐		
Auto - Baseline Correction (absn)	☐	Include integration =	no
Plot (autoplot)	☐	LAYOUT =	+/1D_H.xwp
Warn if processed data exist	☐		

Save   Execute   Cancel
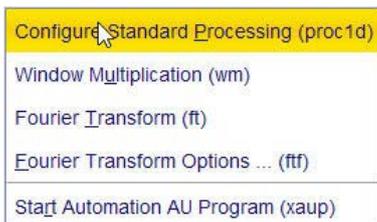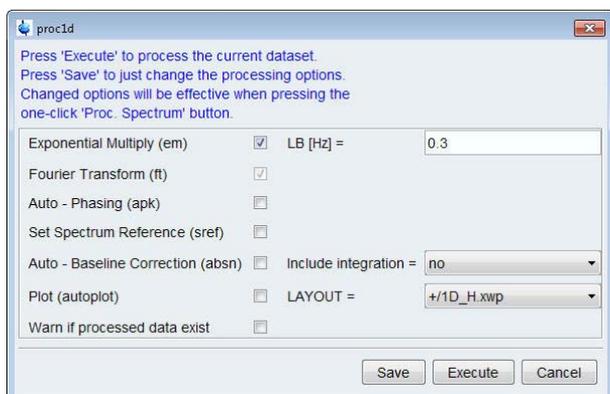
- Expand the spectrum from **4 ppm** to **0.5 ppm**.
- Manually adjust the phase of the selective peak at **4.8 ppm** to show negative absorption to assure the correct phasing of the NOE peaks between **3 ppm** and **1 ppm**. Dependent on the field strength the peaks could be either positive or negative.
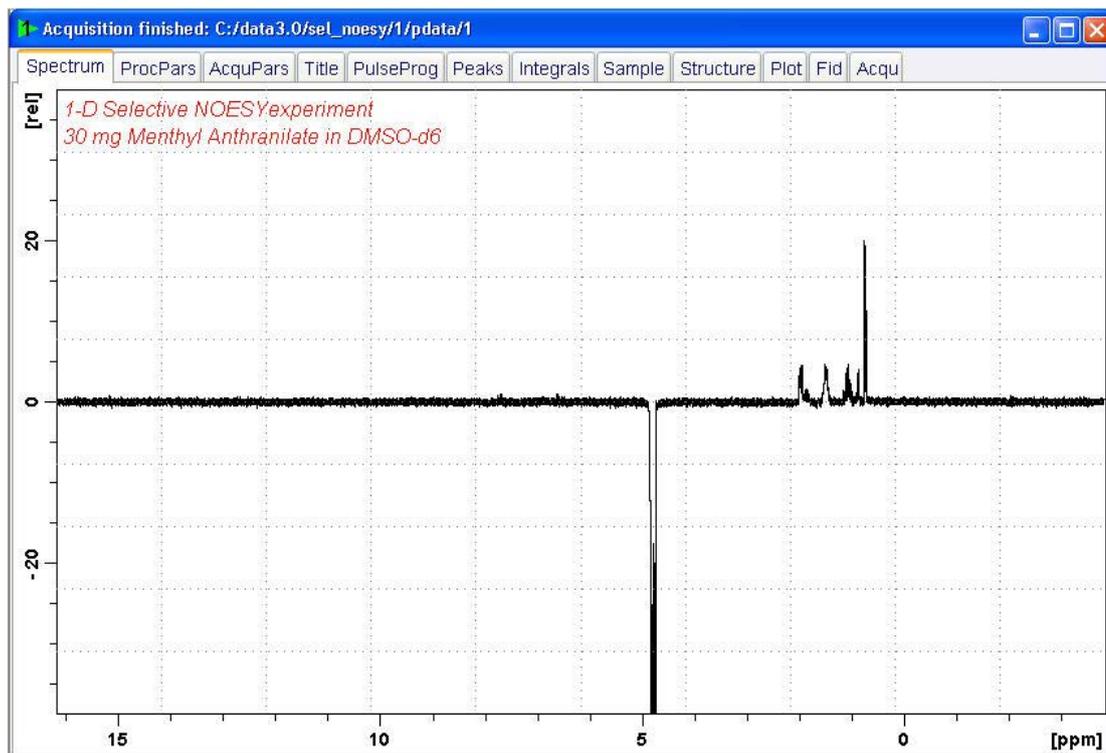
- To store the phase value, click **Return & save phased spectrum**.

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

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

5.5.1 Reference Spectrum

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

5.5.2 Selective Excitation Region Set Up

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

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

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

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

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

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

This button is only used for the instruction displayed above.

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

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

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

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

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

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

	Start	Acquire	Process	Analyse	Publish	View	Manage	?
M BACK_M				1D Selective Experiment Setup	Define Regions		Create Datasets	

- In the list, select **Selective gradient COSY**.

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

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

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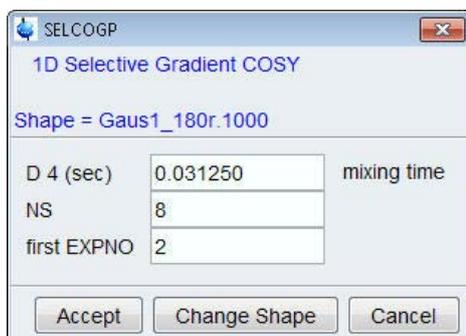
SELCOGP

1D Selective Gradient COSY

Shape = Gaus1_180r.1000

D 4 (sec)	0.031250	mixing time
NS	8	
first EXPNO	2	

Accept   Change Shape   Cancel

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

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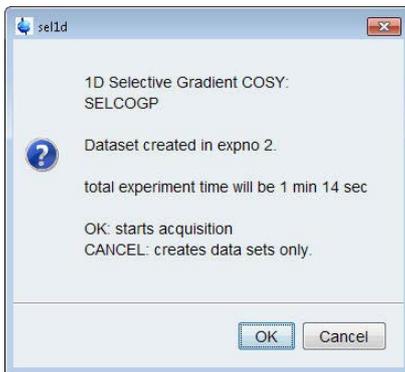
5.5.3   Processing

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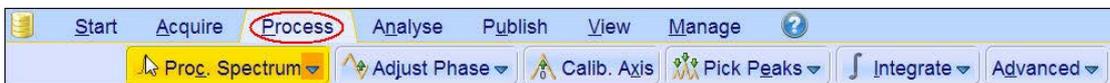
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Configure Standard Processing**.

- 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**.
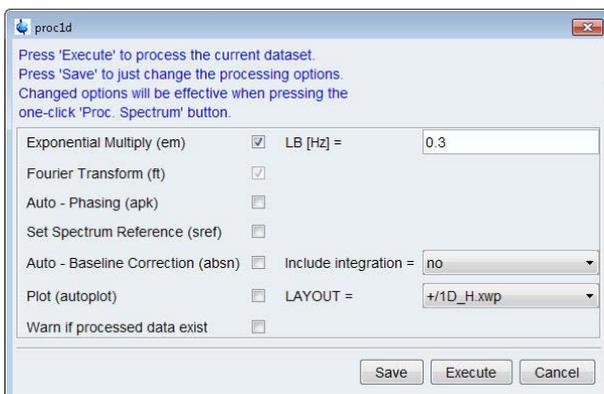
- Manually adjust the phase of the peaks between **3 ppm** and **1 ppm** for an antiphase pattern and if desired the selective peak at **4.8 ppm** can be phased positive.
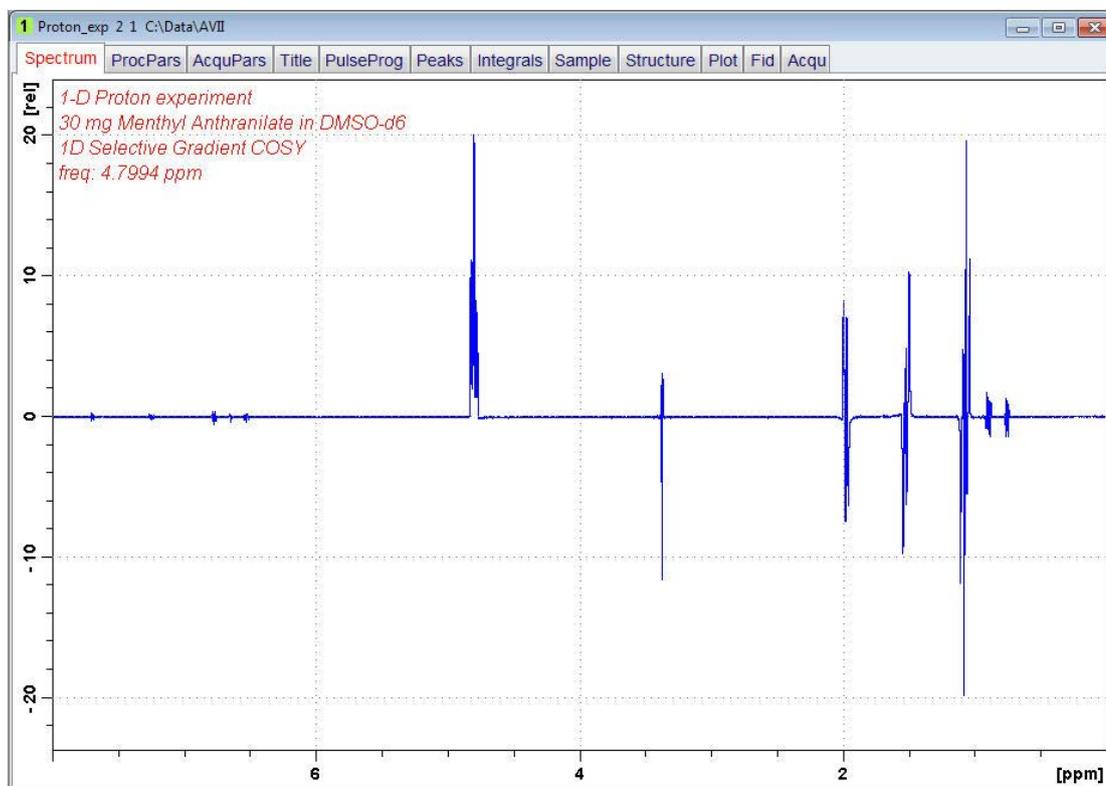
### 5.5.4 Plotting Two Spectra on the Same Page

- Display the selective COSY spectrum.

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

- Drag the Reference spectrum into the spectral window.
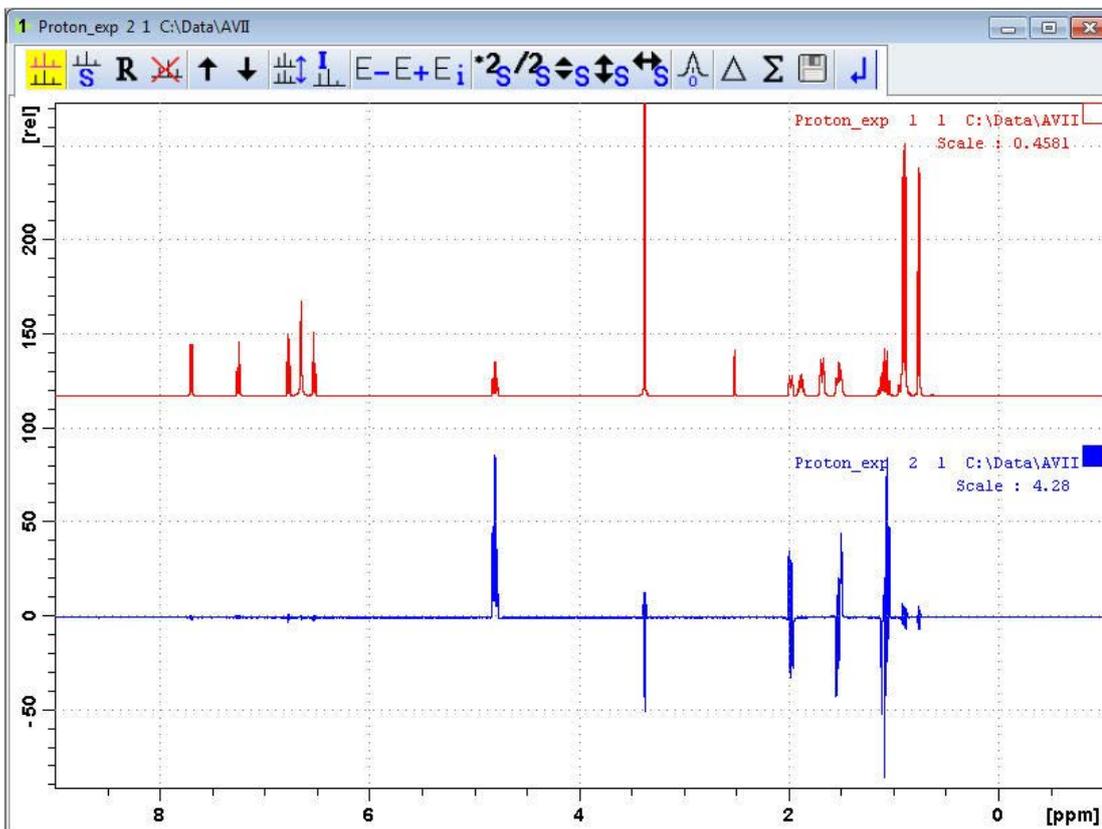
To adjust the spectra for best fit, use the tools.

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

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

5.6   1D Selective NOESY

5.6.1   Reference Spectrum

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

5.6.2   Selective Excitation Region Set Up

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

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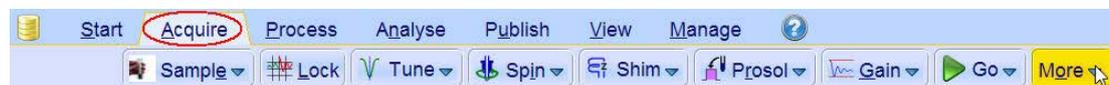
- On the **More** button, click the **drop-down** arrow to see more options.

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

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

- On the Workflow button bar, click **1D Selective Experiment Setup**.
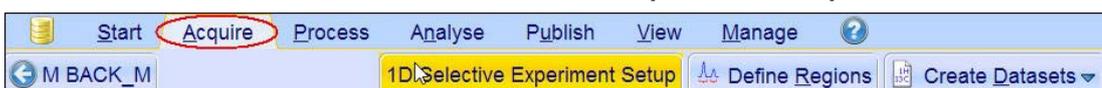
- In the message window, click **Close**.
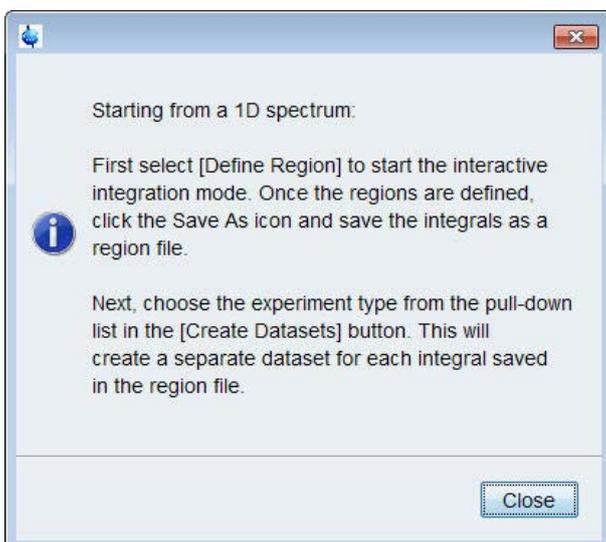
There is no other function to this button then the instruction displayed above.

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

- Integrate the multiplet at **4.8 ppm**.
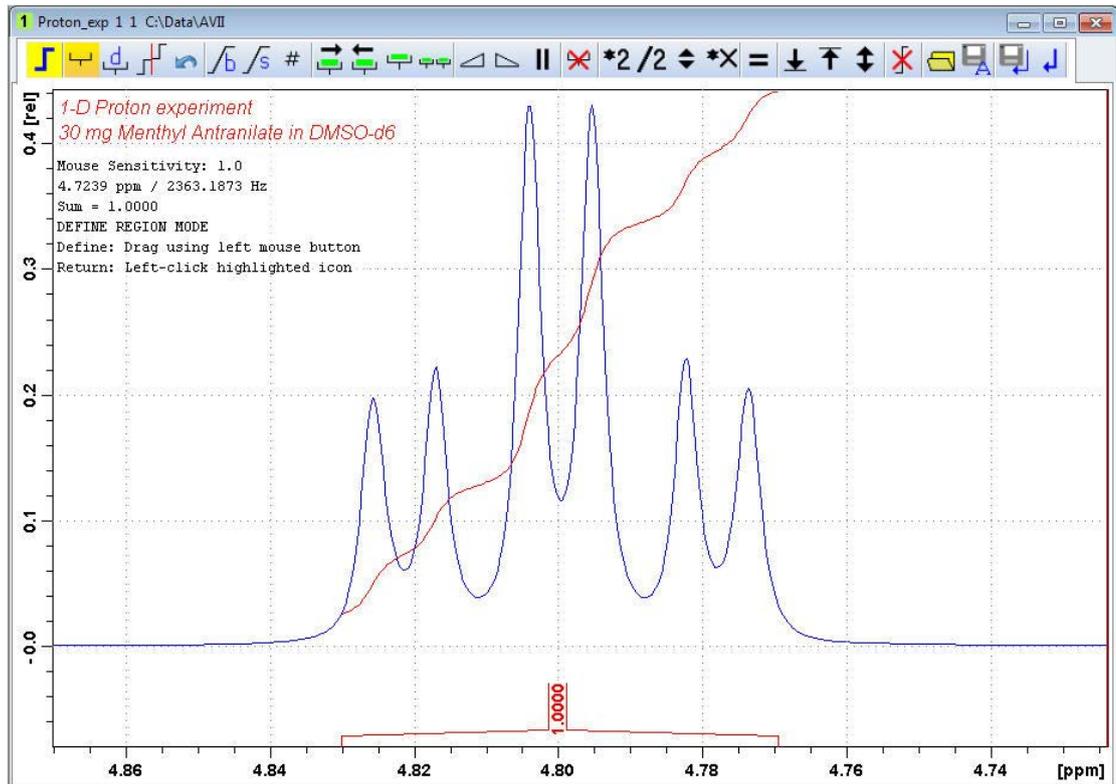
If desired, other peaks can be integrated and a separate dataset will be created for each integral saved in the region file.

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

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

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

- In the list, select **Selective gradient NOESY**.

Selective gradient 1H
Selective gradient COSY
Selective gradient NOESY
Selective gradient TOCSY
Selective gradient ROESY
1H Homonuclear Decoupling
Selective 1H
Selective COSY
Selective NOESY
Selective TOCSY
Selective ROESY
Mult. Solvent Suppr./presat
Mult. Solvent Suppr./WET
2D Band Selective HMBC
2D Band Selective HSQC
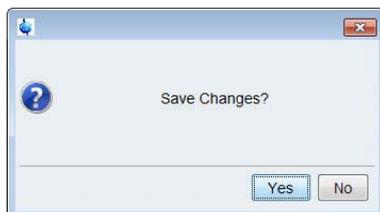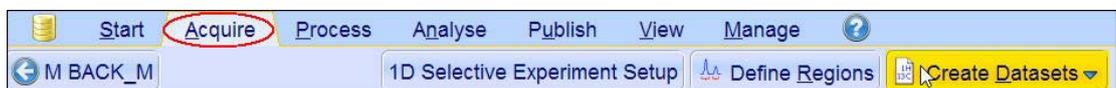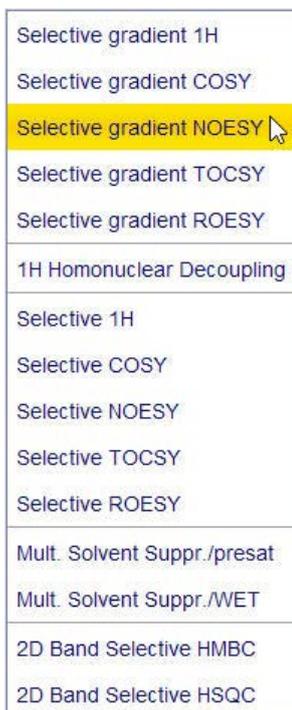
The default parameters are taken from the standard parameter set **SELNOGP**. The mixing time **D8** is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from **100 ms** to **800 ms**. If desired, the **Gaus1_180r.1000** pulse can be changed by clicking on the **Shape** button in the above window.

- Enter:

    D8 = **0.450**

    NS = **32**

- In the SELNOGP window, click **Accept**.
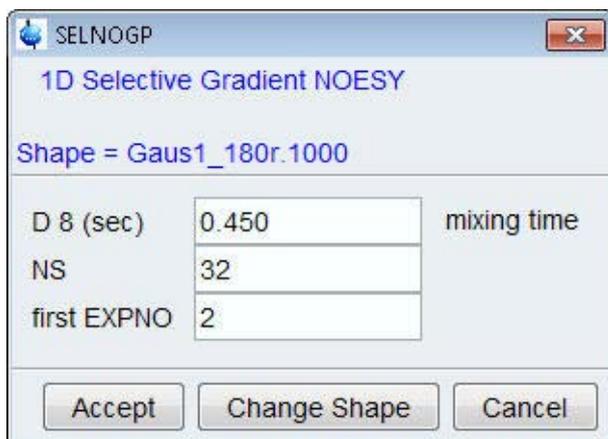
The new dataset is created and all parameters are automatically set.

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

5.6.3 Processing

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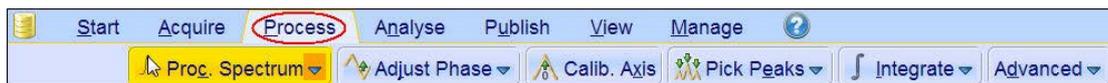
• On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
• In the list, select **Configure Standard Processing**.

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

- In the proc1d window, click **Execute**.
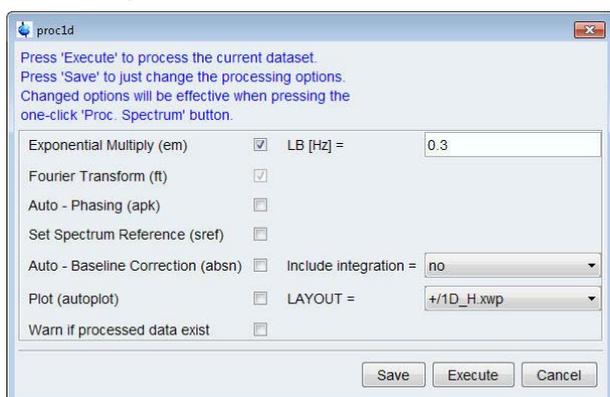
- Manually adjust the phase of the selective peak at **4.8 ppm** to show negative absorption to assure the correct phasing of the NOE peaks between **3 ppm** and **1 ppm**. Dependent on the field strength the peaks could be either positive or negative.

5.6.4 Plotting Two Spectra on the Same Page

- Display the selective TOCSY spectrum.

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

- Drag the Reference spectrum in to the spectral window.
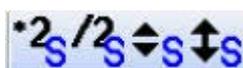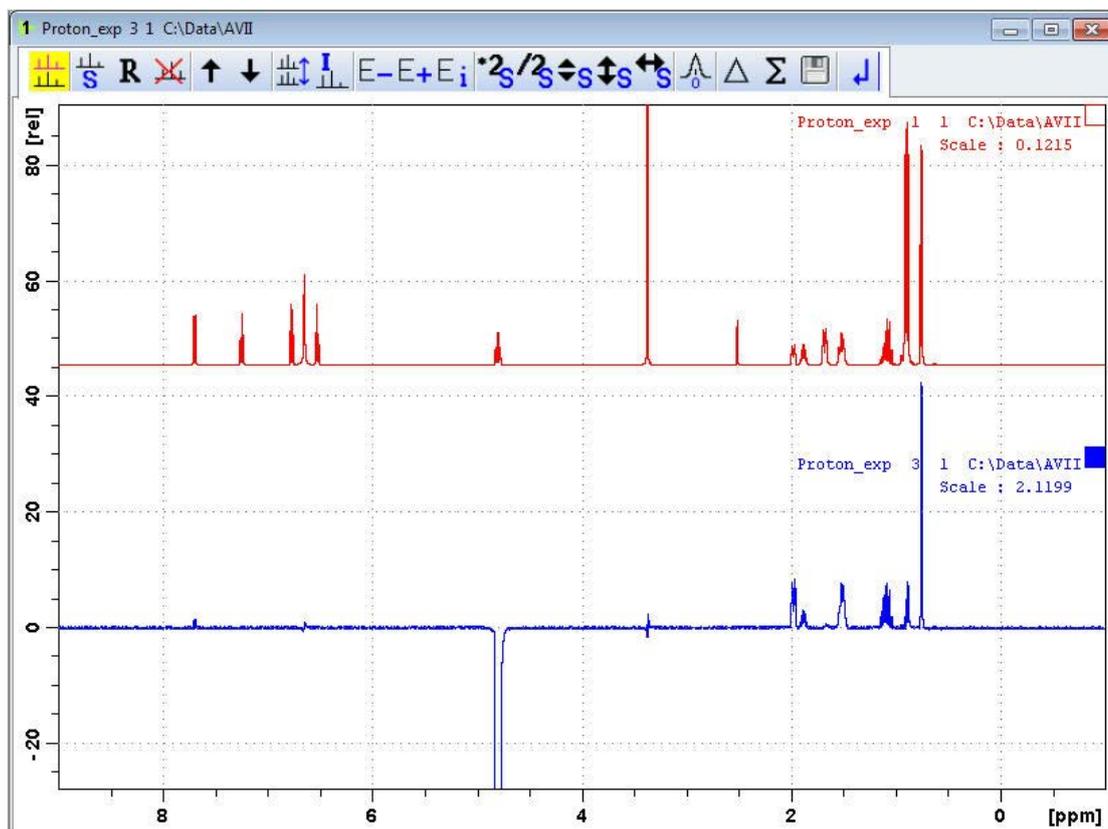
To adjust the spectra for best fit, use the ![tools] tools.
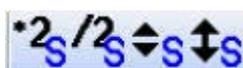
- On the menu bar, click **Publish** and on the Workflow button bar, click **Print**.

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

5.7   1D Selective Gradient TOCSY

This experiment consist of three parts:

- **Selective excitation** of the selected resonance using the SPFGE block.

- **Mixing period** to achieve in phase polarization transfer to other spins. This is usually achieved by applying some isotropic mixing sequence like MLEV, WALTZ or DIPSI pulse trains. This in-phase transfer avoids possible cancellation when the coupling is poorly resolved.

- **Proton detection** as usual.
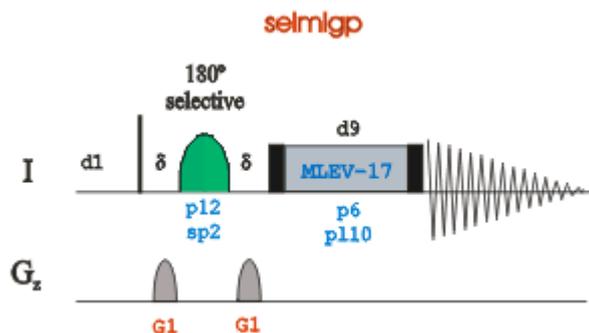
5.7.1   Reference Spectrum

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

5.7.2   Selective Excitation Region Set Up

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

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

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

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

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

This button is only used for the instruction displayed above.

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

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

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

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

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

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

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

• In the list, select **Selective gradient TOCSY**.

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

• Enter:
  D9 = **0.08**
  NS = **8**

- In the SELMLGP window, click **Accept**.
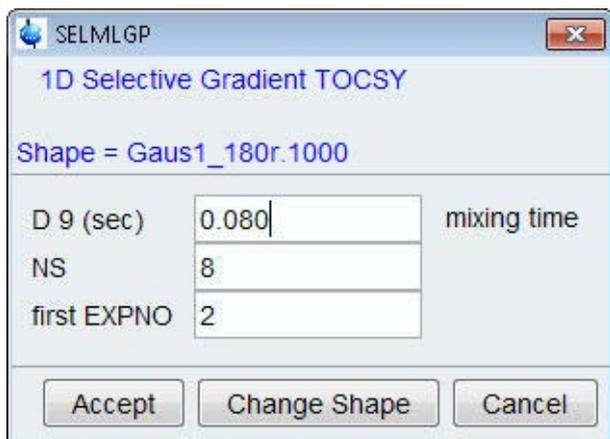
SELMLGP

1D Selective Gradient TOCSY

Shape = Gaus1_180r.1000

D 9 (sec)	0.080	mixing time
NS	8	
first EXPNO	2	

Accept   Change Shape   Cancel

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

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

sel1d

1D Selective Gradient TOCSY:
SELMLGP

Dataset created in expno 2.

total experiment time will be 1 min 14 sec

OK: starts acquisition
CANCEL: creates data sets only.

OK   Cancel

5.7.3   Processing

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

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

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

- In the proc1d window, click **Execute**.
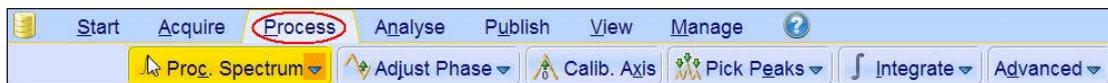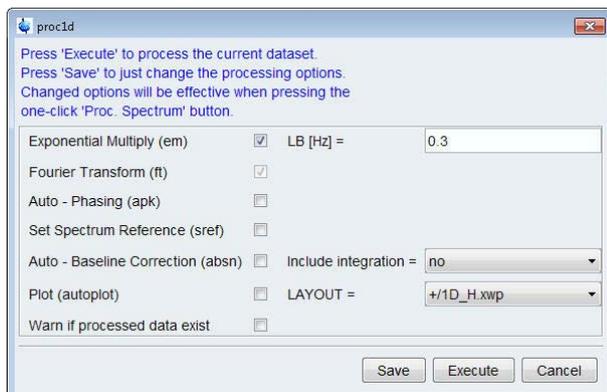
- Manually phase all peaks for positive absorption.

5.7.4　Plotting Two Spectra on the Same Page

- Display the selective TOCSY spectrum.

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

- Drag the reference spectrum into the spectral window.

To adjust the spectra for best fit, use the ![tools] tools.

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

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

5.7.5 Plotting All 4 Experiments on the Same Page

- Display the selective NOESY spectrum.

- On the toolbar, click **Multiple display**. ![icon]
- Drag the selective COSY spectrum into the spectral window
- Drag the selective TOCSY spectrum into the spectral window
- Drag the Reference spectrum into the spectral window.

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

6 2D Experiments using Shaped Pulses

6.1 2D Edited HSQC Experiment with Adiabatic Pulses

6.1.1 Introduction

The HSQC experiment is the method of choice for a very well resolved H,C correlation. However, in contrast to the HMQC this experiment uses 180^0 pulses, which causes problems if the 180^0 pulses become to long (e.g.TXI probes) and have to cover a very wide spectral range. This leads to phasing problems for high field instruments above 500 MHz. To work around this problem is to apply frequency-swept adiabatic 180^0 pulses which can cover the large ^{13}C spectral width.

The figure below shows the regular edited HSQC sequence:
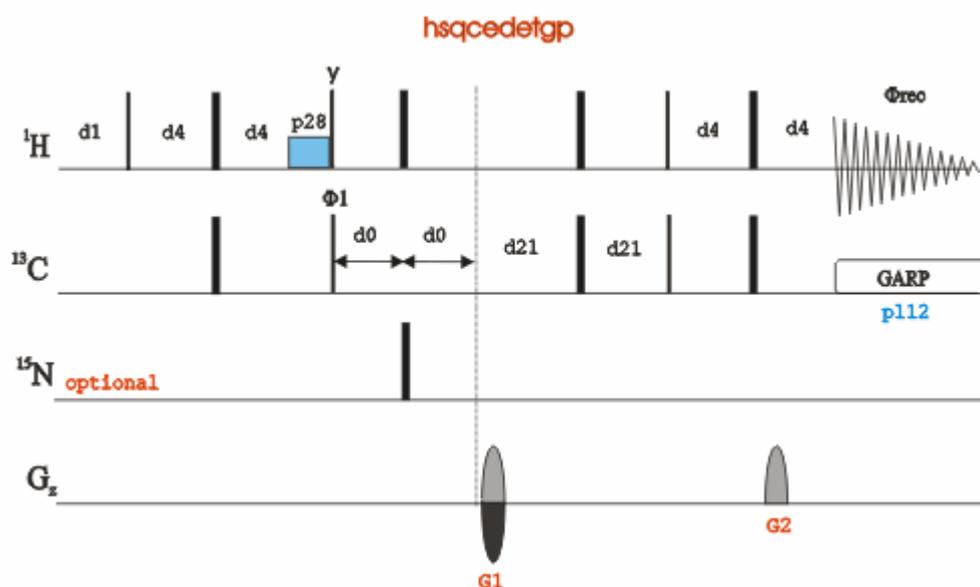
The edited HSQC sequence using shaped pulses for all 180^0 pulses on f2-channel with gradients in back-inept. For improvement of the phasing the pulse sequence using matched sweep adiabatic pulses **hsqcedetgpsisp2.3** is used in this chapter (see figure below). This pulse sequence is used in the recommended Bruker parameter set HSQCEDETGPSPSISP_ADIA. If desired the sequence **hsqcedetgpsisp2.4** can be used to suppress the COSY peaks.

hsqcedetgpslsp2.3
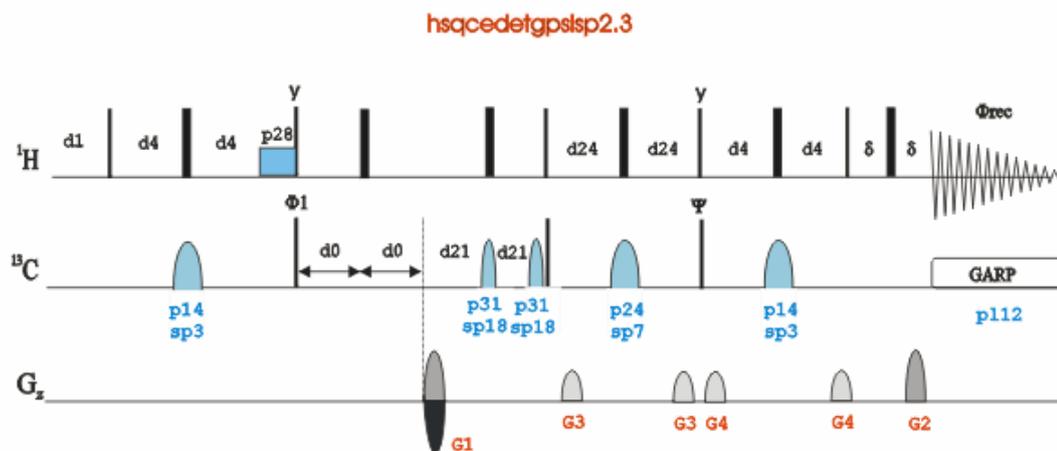
6.1.2   Sample

30 mg Menthyl Anthranilate in DMSO-d6
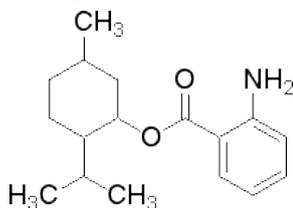
6.1.3   Reference spectrum

Run a 1D Proton spectrum, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter *1D Proton Experiment*, *Experiment Setup* through *Processing*.
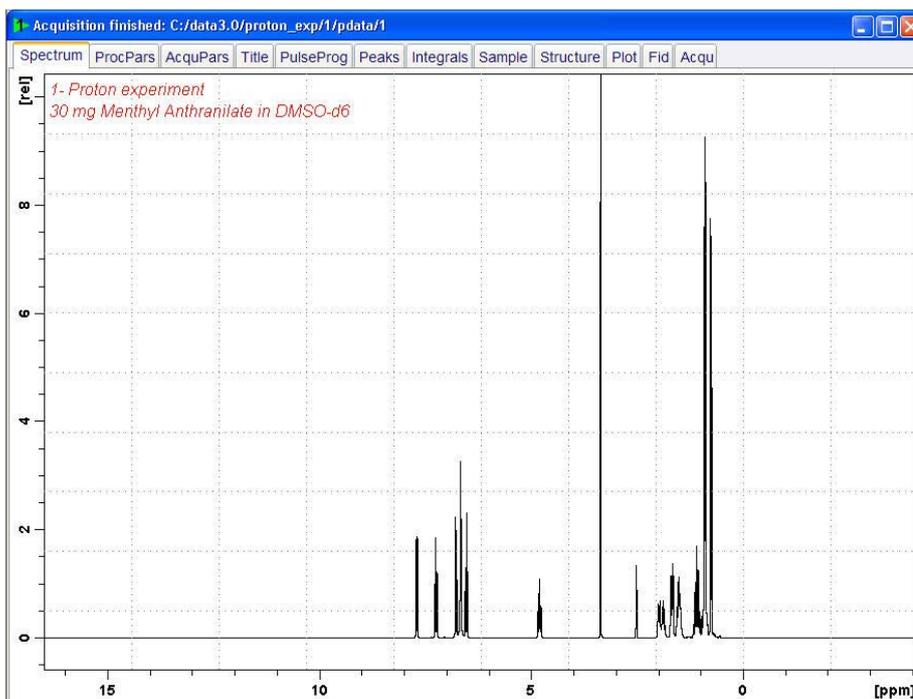
The reference spectrum is necessary to adjust the spectral limits of the sweep width in the **F2** dimension and to use it for the projection. The HSQCEDETGPSP_ADIA parameter set has a default sweep width in the **F1** dimension of **165 ppm**, If a Carbon DEPT135 or DEPT45 spectrum of the same sample is available, the F1 sweep width can be further reduced using the **setlimit** AU-program.

6.1.4   Setting up the HSQC experiment

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

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
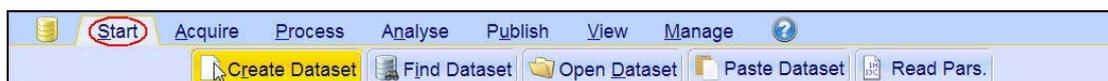
- In the New Dataset window, enter or select:

    NAME = **shape_hsqc_exp**

    EXPNO = **1**

    PROCNO = **1**

    Experiment select **HSQCEDETGPSP_ADIA**

    Set Solvent select **DMSO**
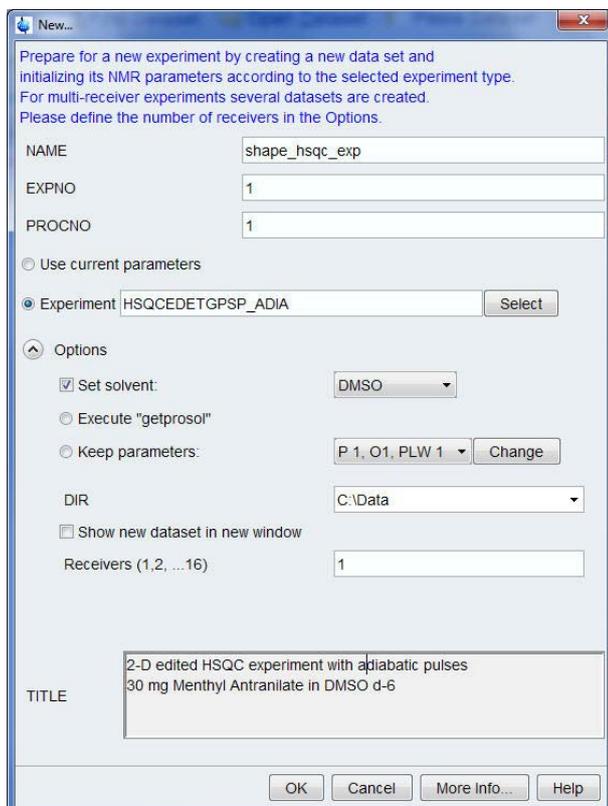
DIR

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

Title

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

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

- To tune the probe, click **Tune**.

---

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

- Click **Spin** and select **Turn sample rotation off**.

2D experiments should be run non-spinning.

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

6.1.4.1   Limit Setting

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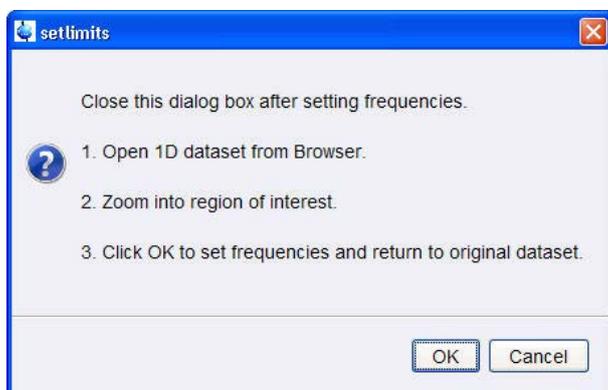
setlimits

Close this dialog box after setting frequencies.

1. Open 1D dataset from Browser.

2. Zoom into region of interest.

3. Click OK to set frequencies and return to original dataset.

OK   Cancel

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

The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.
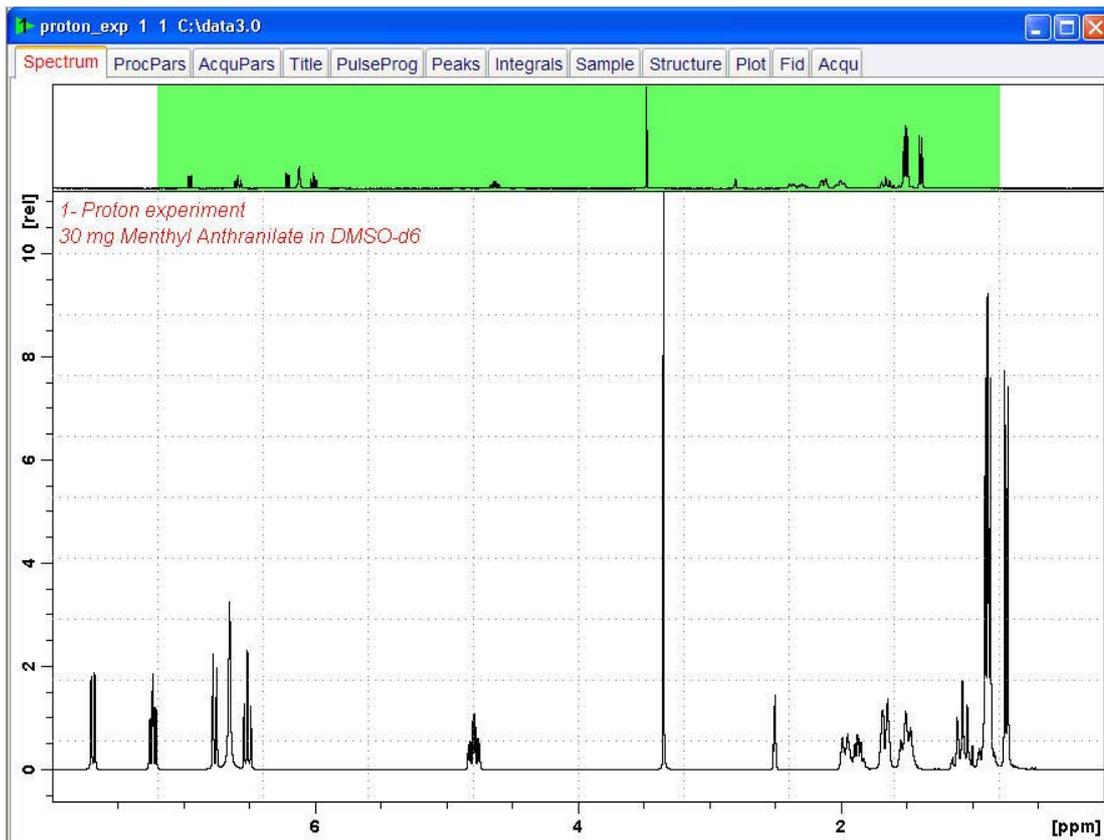
- In the setlimits message window, click **OK** to assign the new limit.
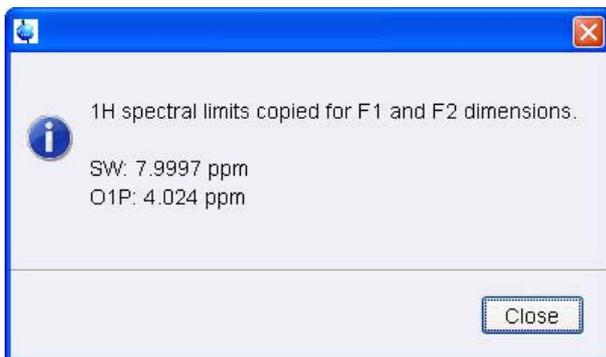
- In the message window, click **Close**.

The display changes back to the 2D dataset.

6.1.4.2   Acquisition

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

6.1.4.3   Processing

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

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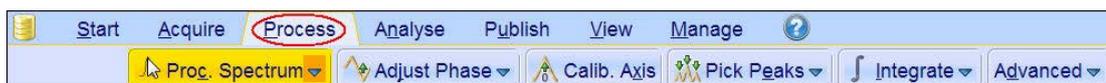
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- In the list, select **Process F2+F1 (xfb)**, or at the command prompt, type **xfb**.

| Configure Standard Processing (proc2d) |
| Process F2+F1 Axis (xfb) |
| Process Only F2 Axis (xf2) |
| Process Only F1 Axis (xf1) |
| Symmetrize Spectrum (sym) |
| Start Automation AU Program (xaup) |
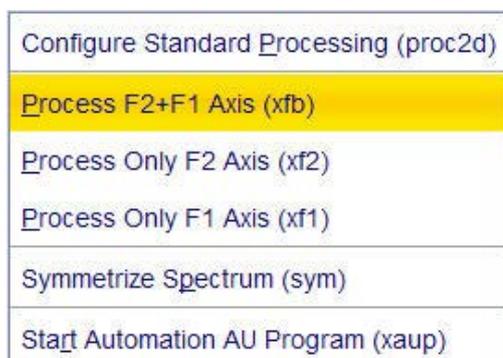
- On the Workflow button bar, click **Adjust Phase**.
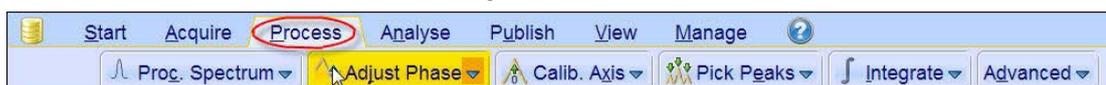
- Select the peak at **7.7 ppm/130.9 ppm**.
- Right-click and on the shortcut menu, select **Add**.

| Add |
| Remove Row/Col |
| Remove All |
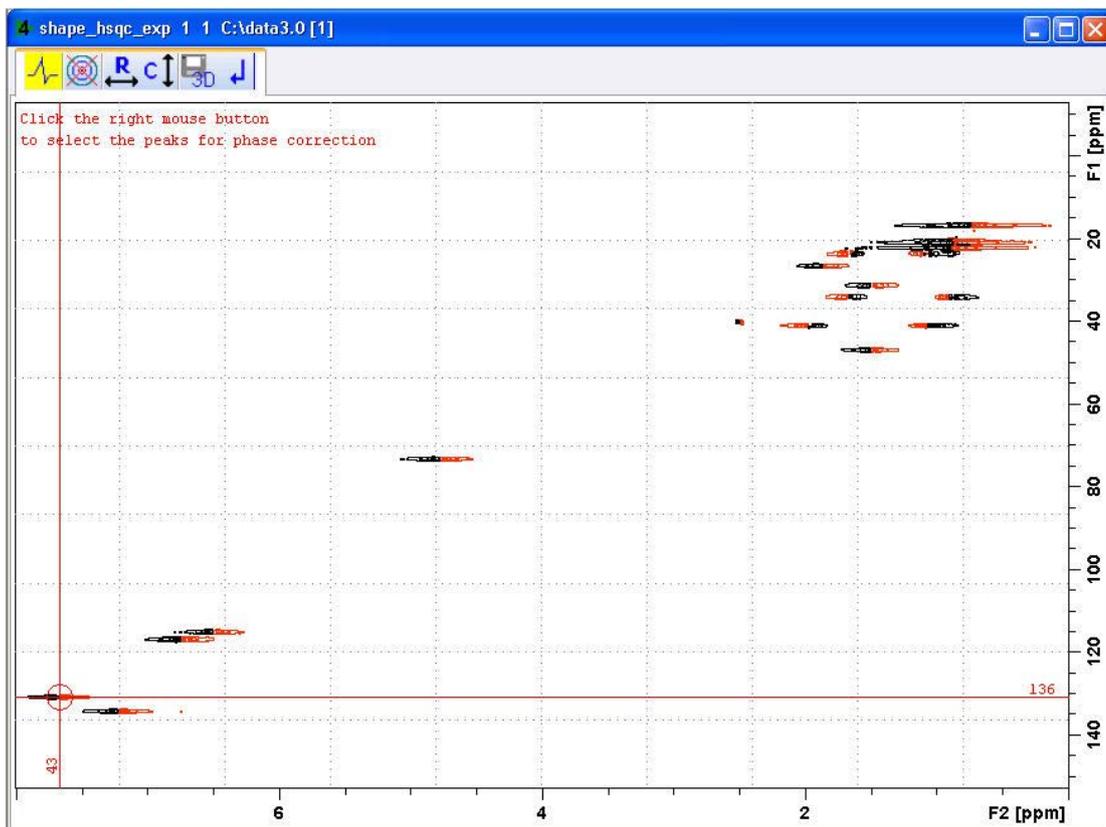
• Repeat the last step for the peaks at **4.8 ppm**/**73.2 ppm** and **0.76 ppm**/**16.8 ppm**.
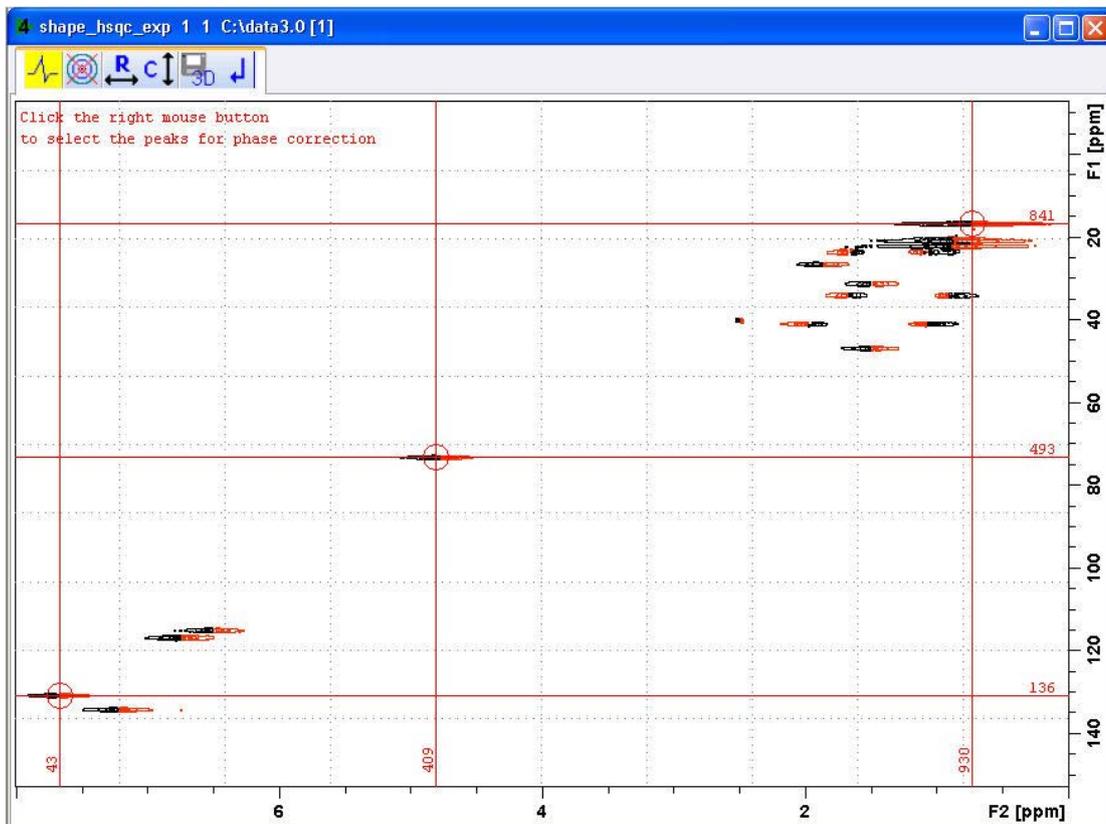
- Click **Start the phase correction on rows**.
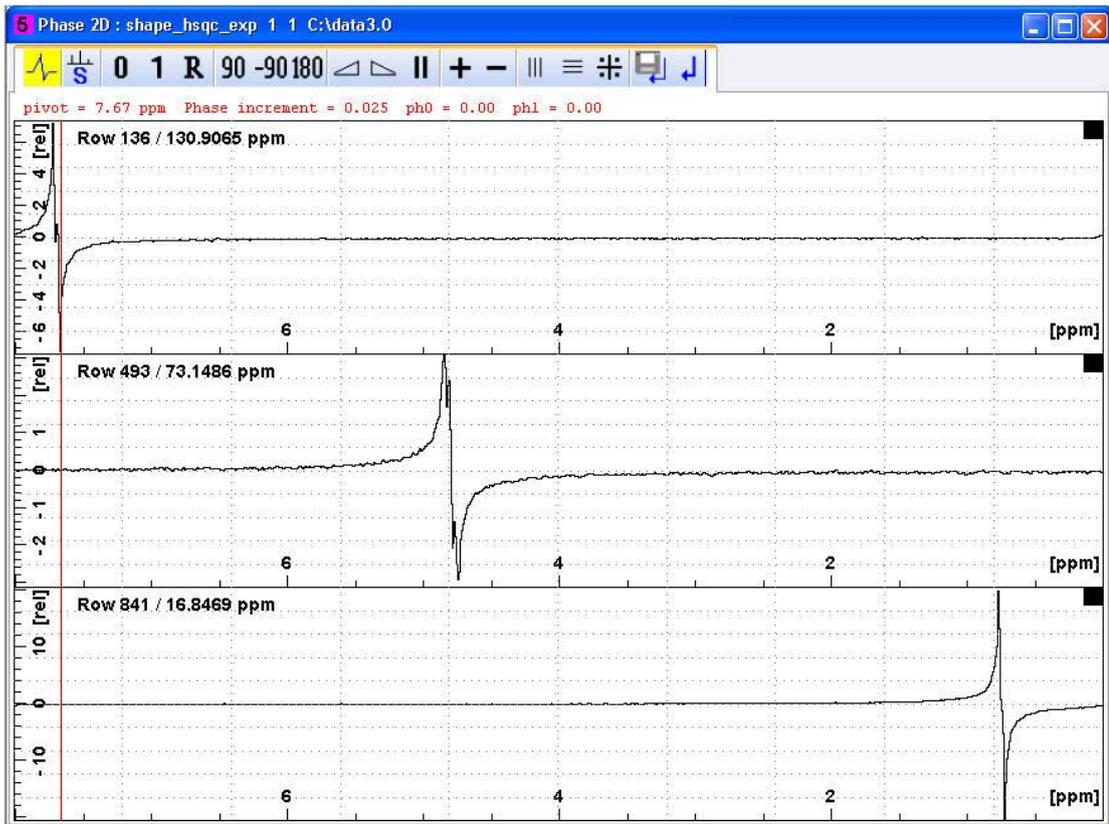
- Adjust the **0** and 1st order phase.
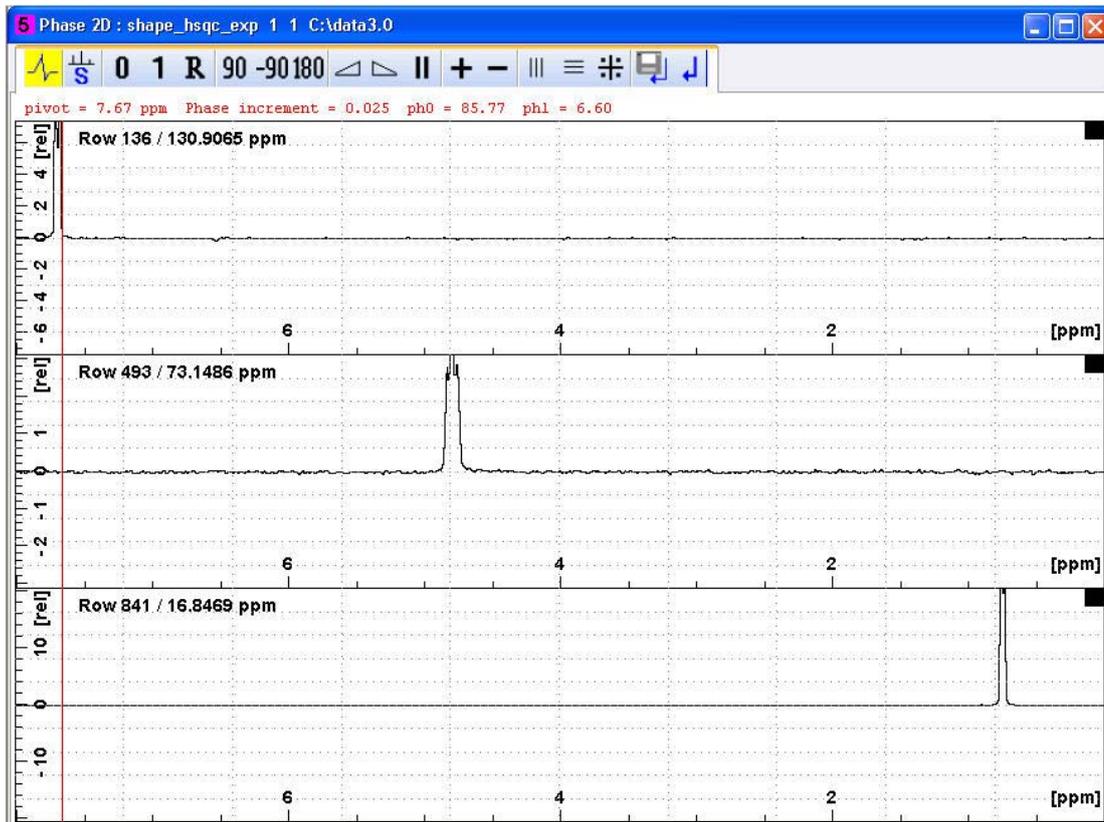
- To store the phase values, click **Return and Save phased spectrum**.
- Click **Start the phase correction on columns**.
- Adjust the **0** and 1st order phase.
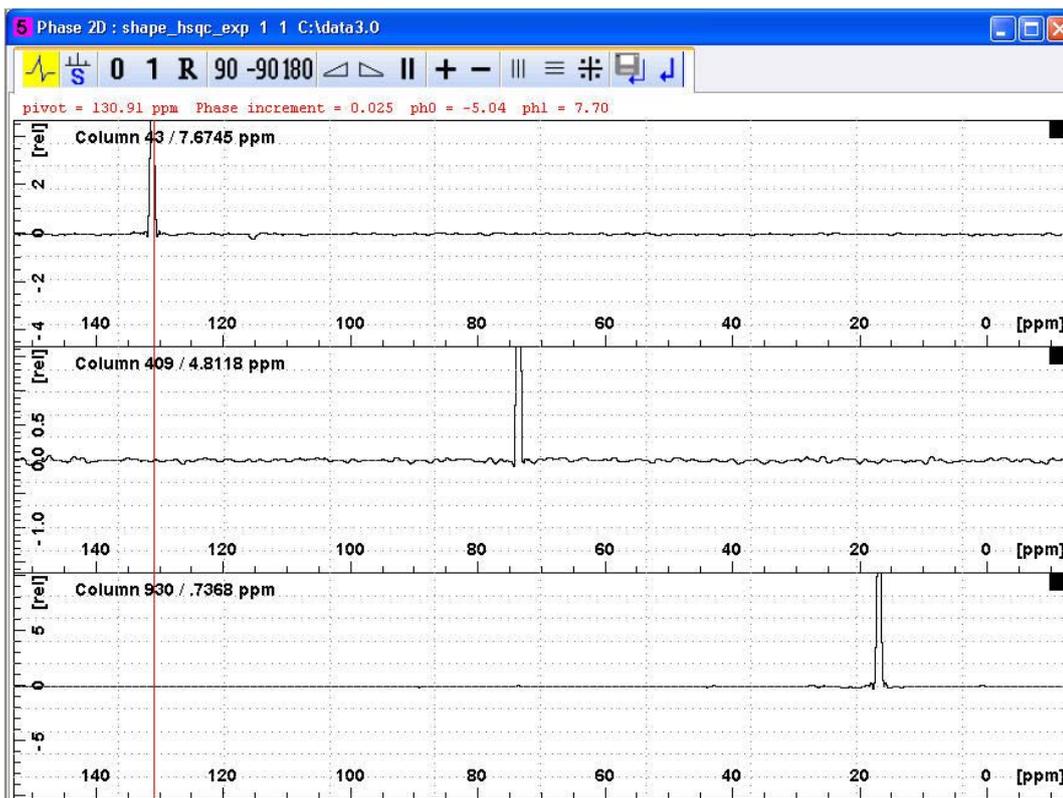
- To store the phase values, click **Return and Save phased spectrum**.

- To exit the phase window, click the **Return** button.
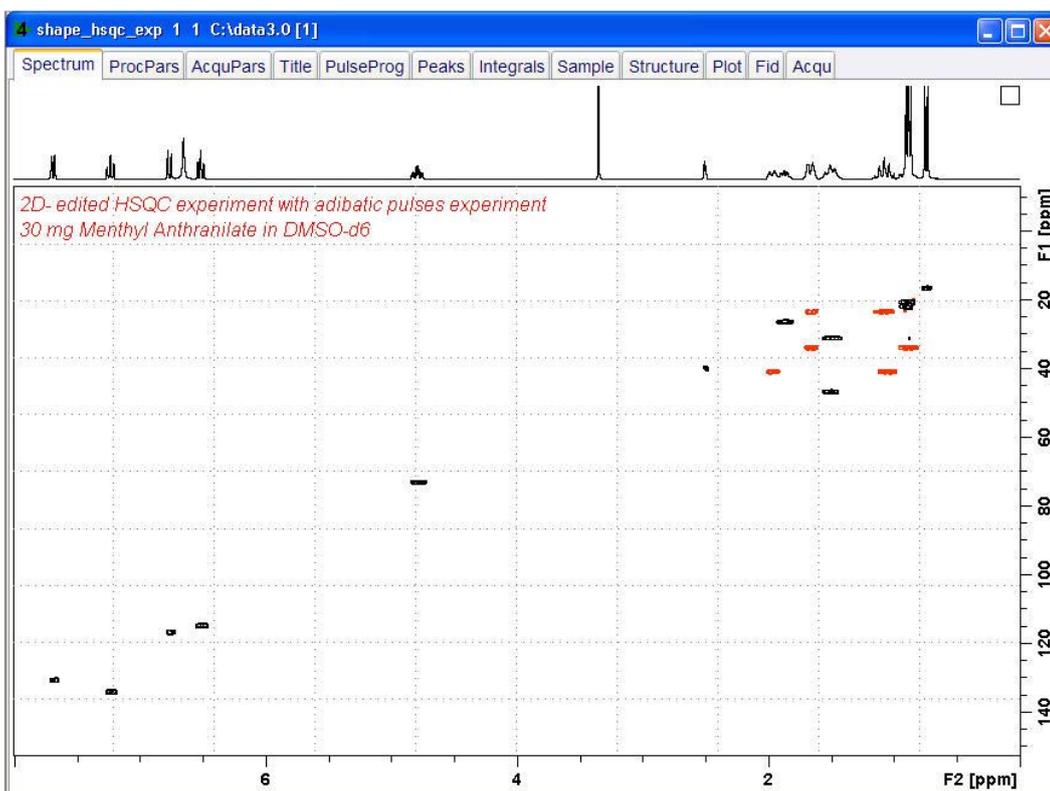
6.2   2D Selective HMBC experiment

6.2.1   Introduction

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

6.2.2   Sample

50 mM Cyclosporin in C_6D_6

6.2.3   Preparation Experiments

6.2.3.1   1D Proton Experiment

Run a **1D Proton** spectrum of Cyclosporin, following the instructions in the TopSpin Guide Book *Basic NMR Experiments*, chapter *1D Proton Experiment*, *Experiment Setup* through *Processing* using C_6D_6 as the solvent.
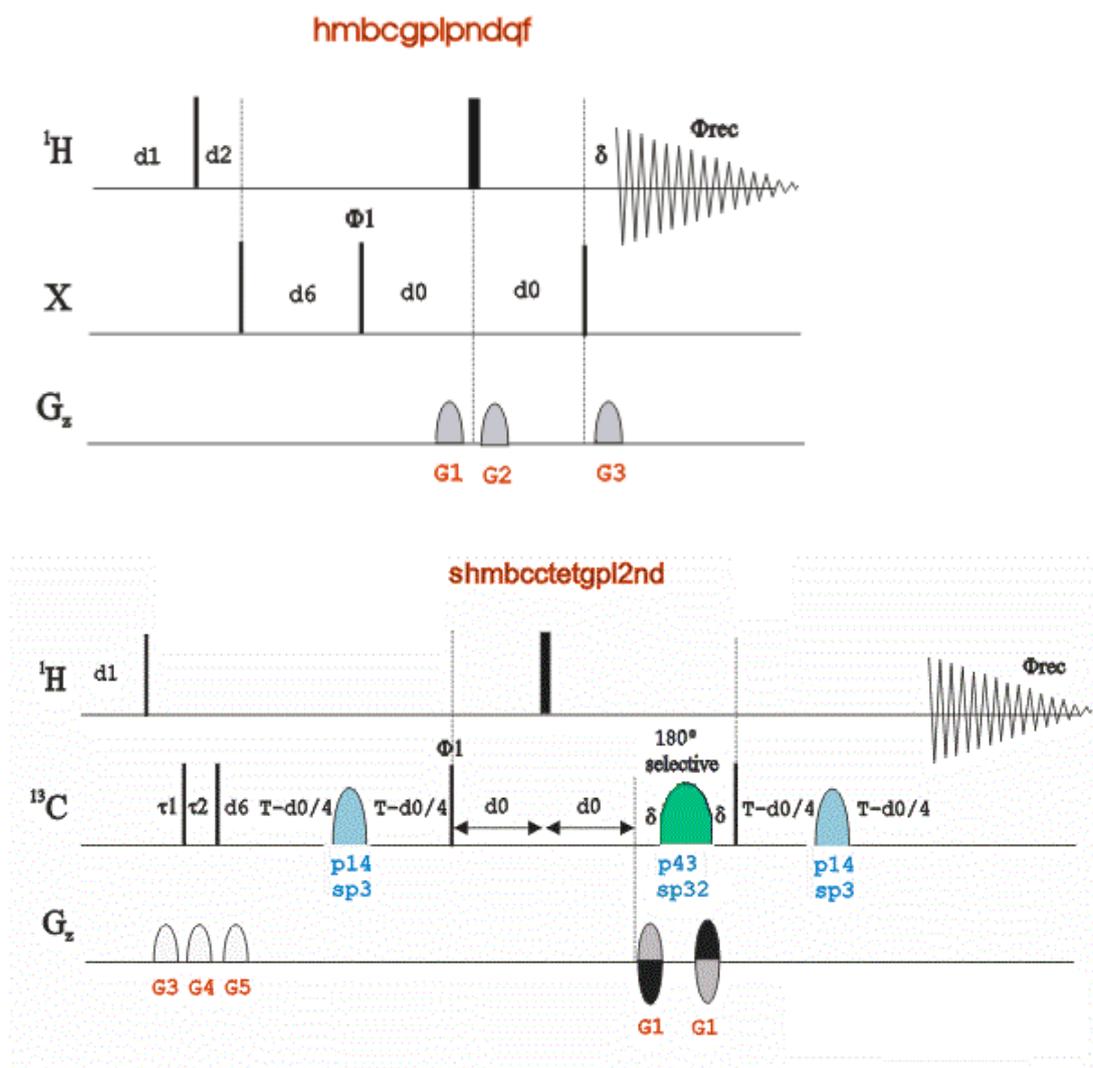
6.2.3.2 2D HMBC Experiment

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

Run a **2D HMBC** experiment of Cyclosporin following the instructions in TopSpin Guide Book *Basic NMR experiments*, chapter *2D HMBC experiment* using C_6D_6 as the solvent.
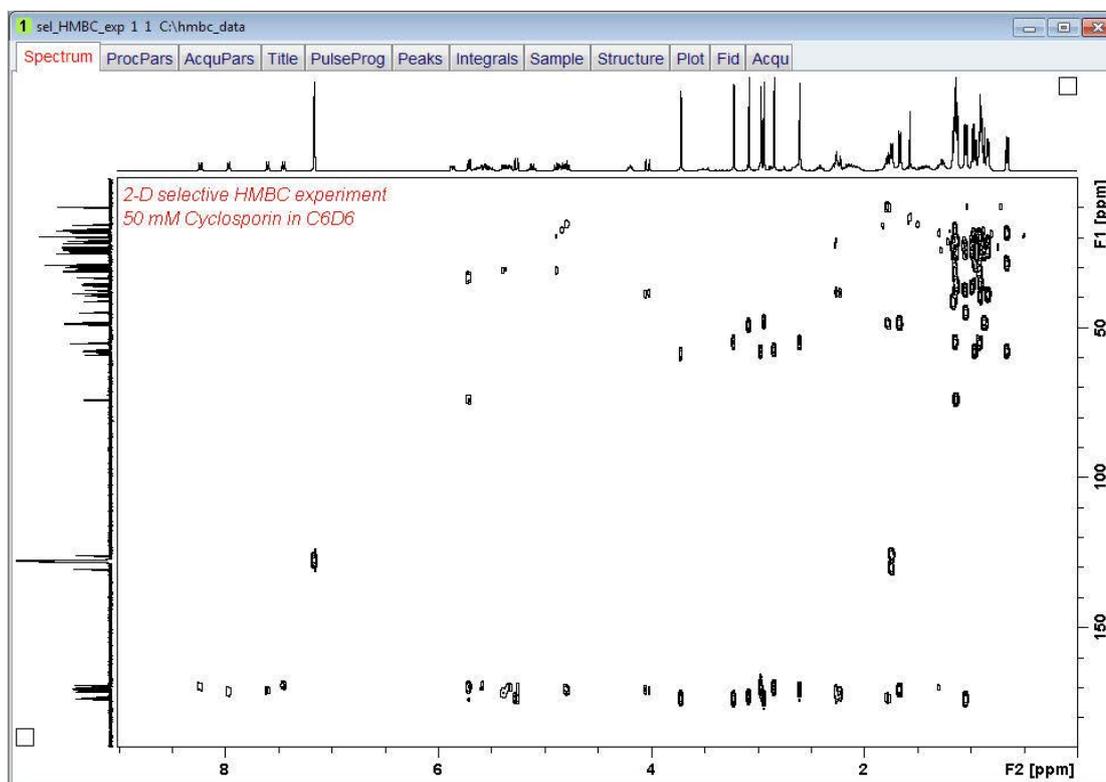
6.2.3.3   1D Proton Decoupled Carbon Experiment

Run a **1D Carbon** spectrum of **Cyclosporin**, following the instructions the TopSpin Guide Book *Basic NMR Experiments*, Chapter *1D Carbon experiment*, Paragraph *Experiment Setup* through *Processing* using C_6D_6 as the solvent.
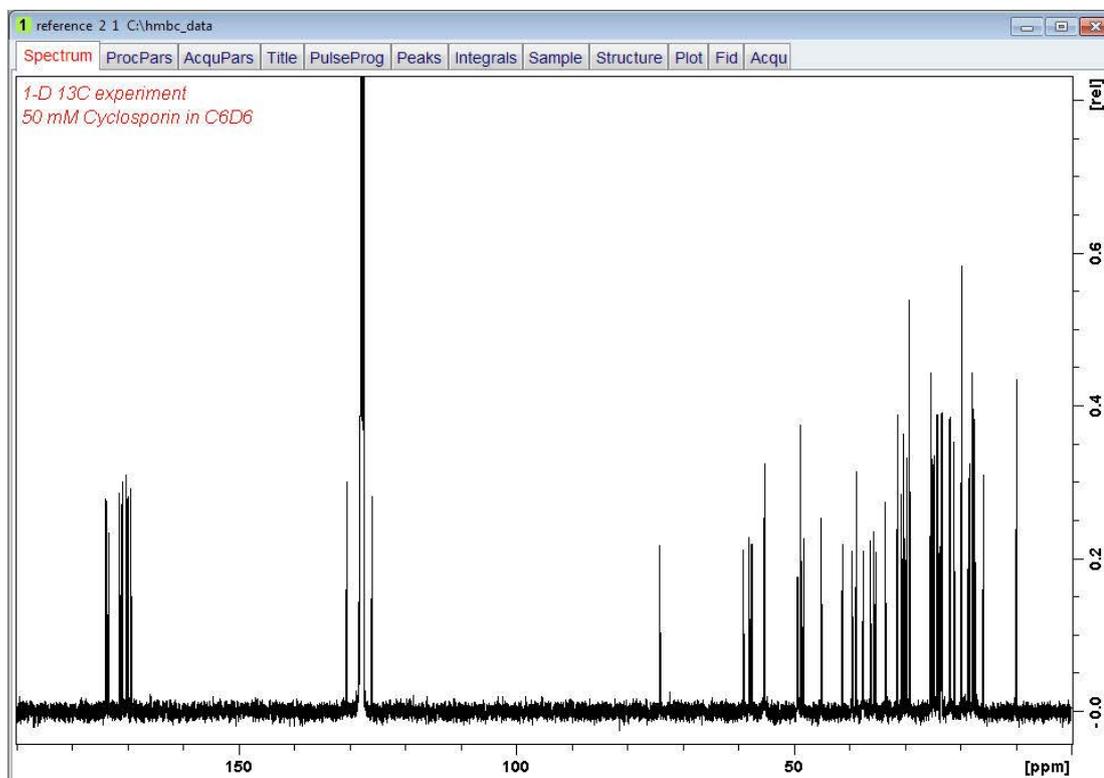
The carbon spectrum is necessary for method 1 but not for method 2, if the sample concentration is to low to get a spectrum in a reasonable time frame.

6.2.3.4   Method 1 for Setting Up the Selective HMBC Experiment

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

- Display the carbon spectrum as observed in the last chapter *1D Proton Decoupled Carbon Experiment [▷ 112]*.
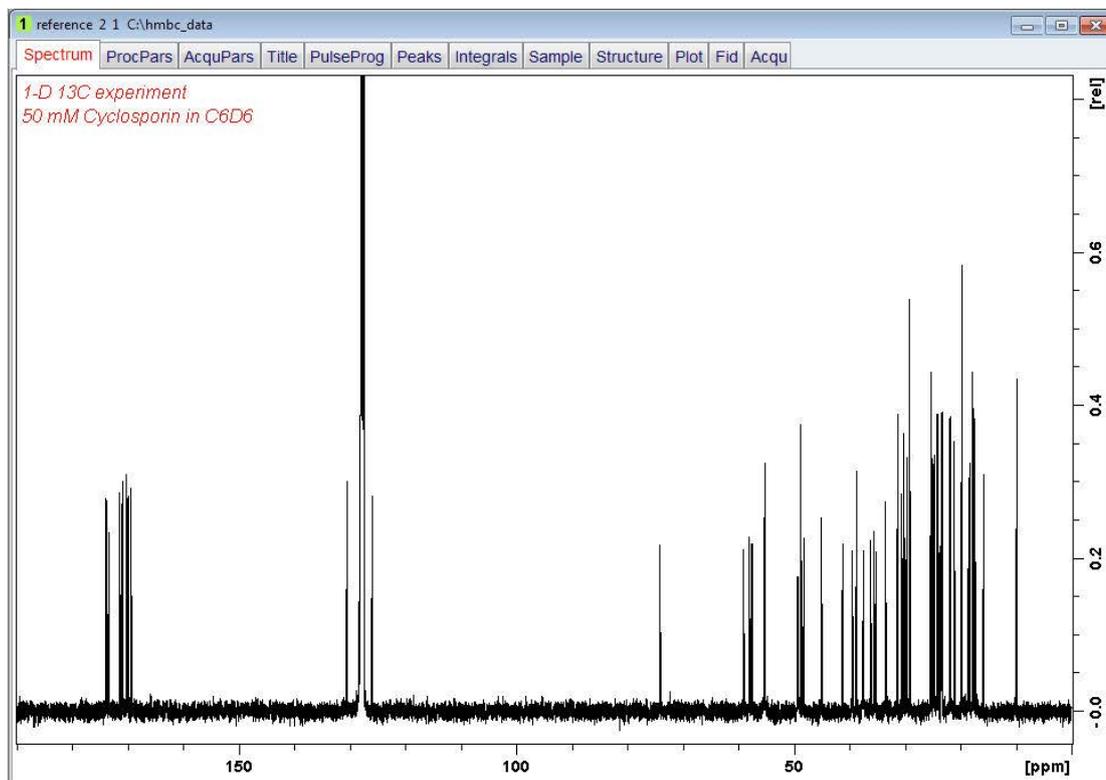
6.2.3.5   Selective Excitation Region Setup

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

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

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

2D Experiments using Shaped Pulses

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

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

- Integrate the region from **175 ppm** to **167 ppm**.
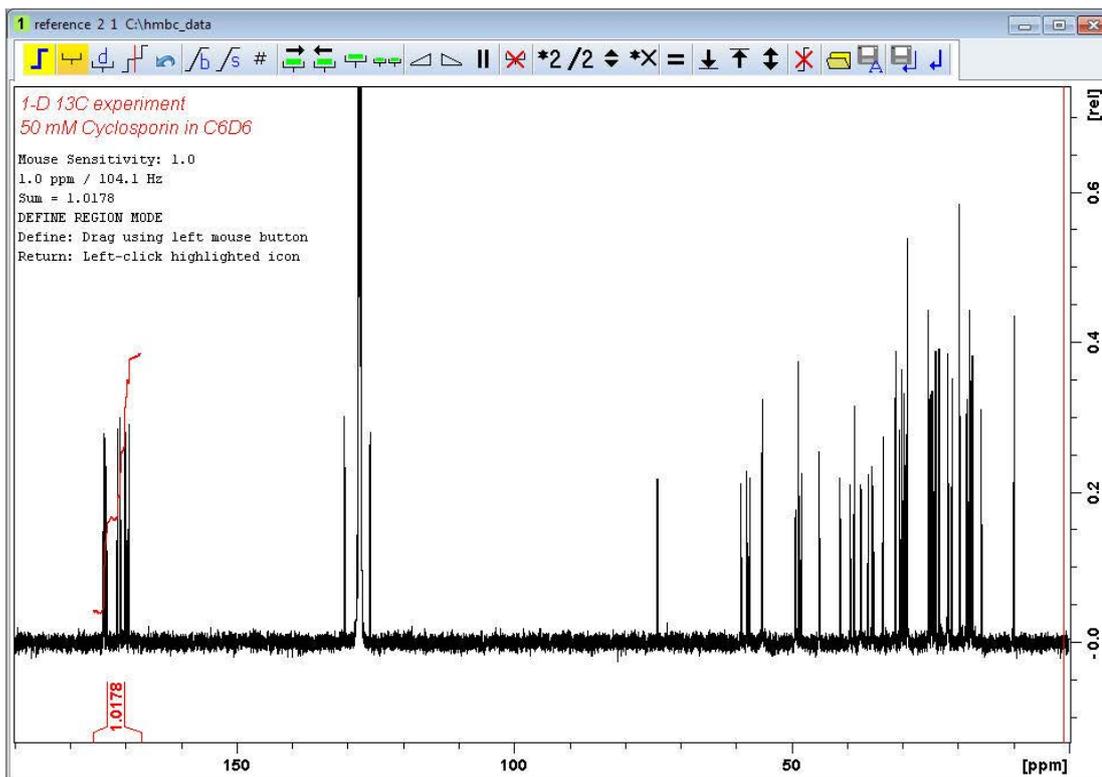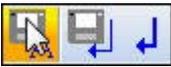
- Click **Save region as**. and in the list, **save Region to reg**.

- To exit from the integration mode, click **Return do not save regions!**.
- In the message window, click **No**.

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

- In the list, select **2D Selective HMBC**.

- In the SHMBCCTETGPL2ND window, click **Accept**.
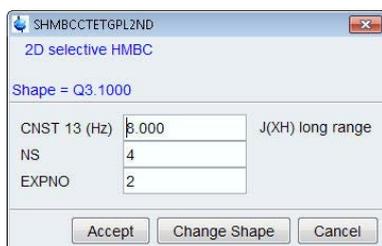
All parameters are automatically calculated and stored as an increment in the next free experiment number of the dataset.

- In the sel1d window, click **OK** to start the acquisition.
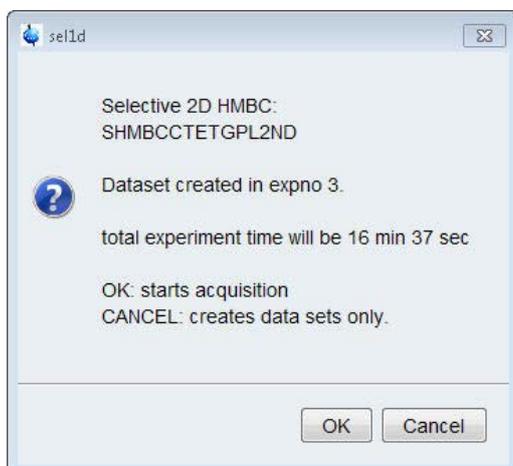
The acquisition starts momentarily.

6.2.3.6   Processing

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

- At the command prompt, type **xfb** to process the data in both dimensions.
- At the command prompt, type **xf2m** to calculate magnitude spectrum in F2.
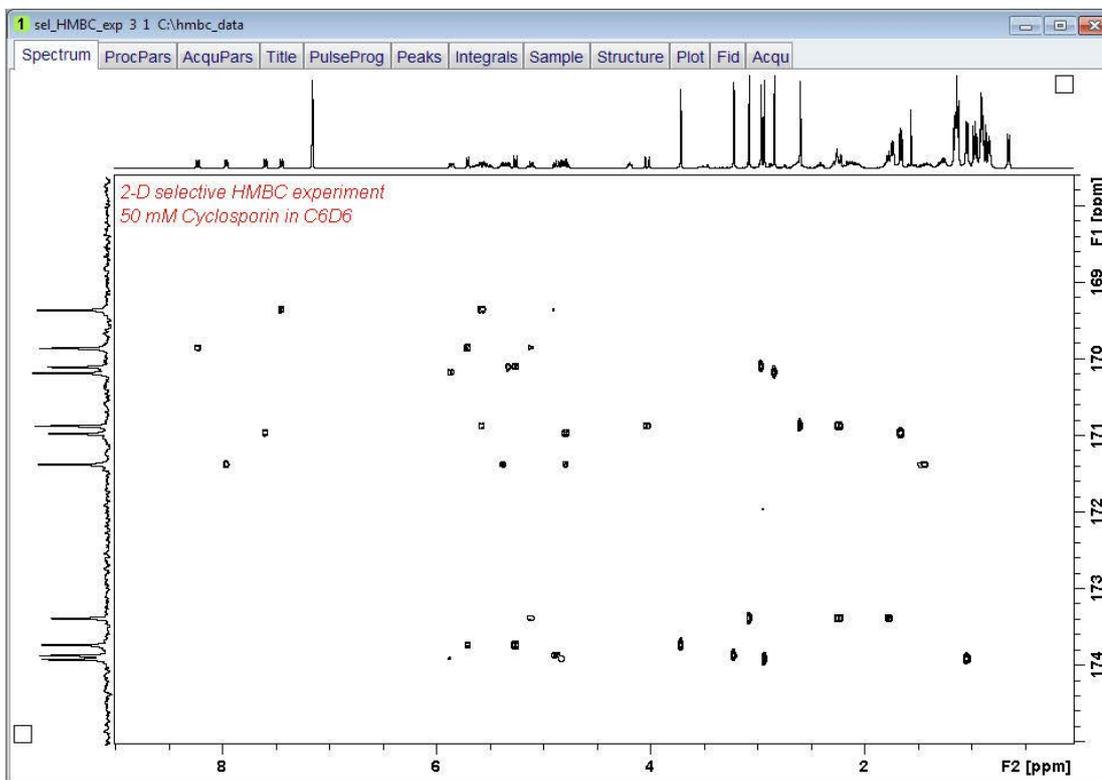
6.2.3.7   Method 2 for Setting Up the Selective HMBC Experiment

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

- Display the **HMBC** spectrum as observed in chapter *2D HMBC Experiment [▸ 111]*.
- Expand the region including all cross peaks (e.g. **163 ppm** to **179 ppm**).
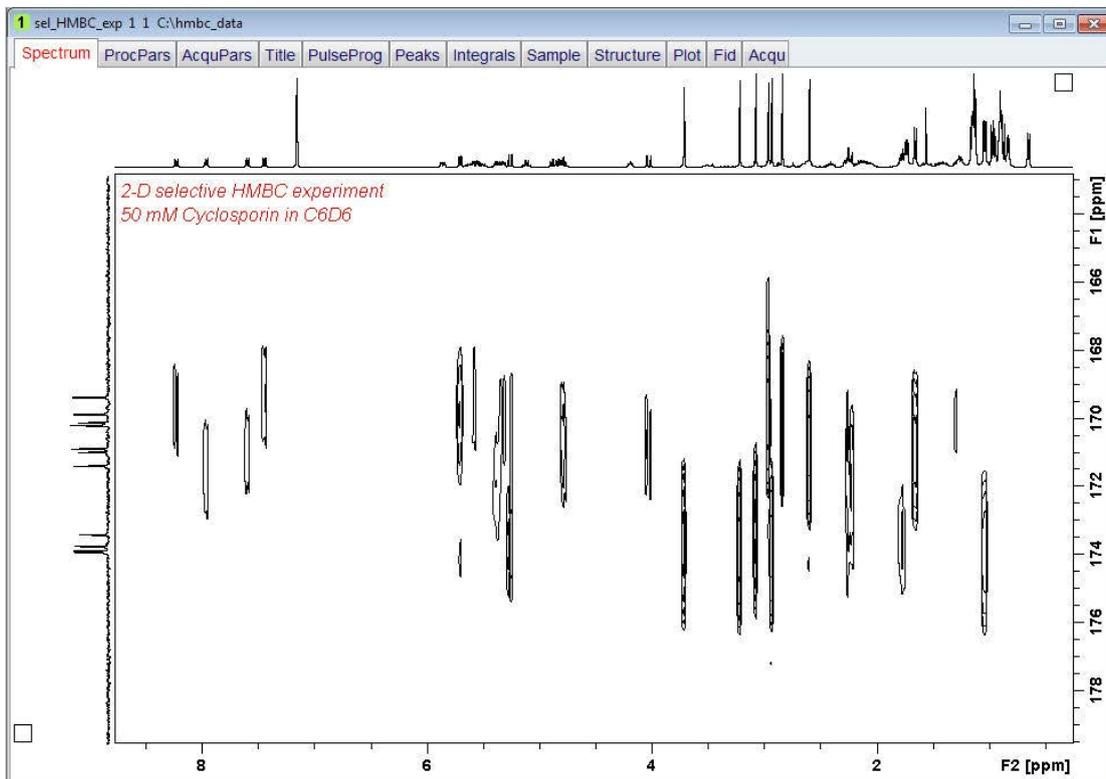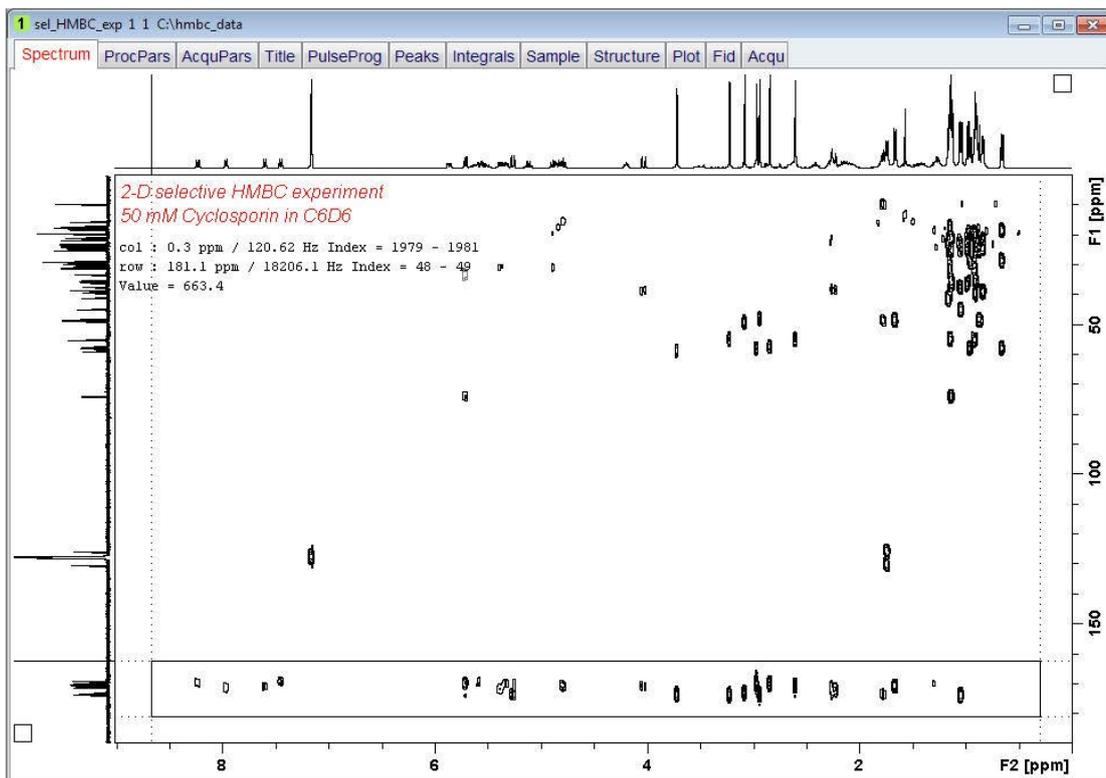
• On the menu bar, click **Acquire**.

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

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

- In the SHMBCCTETGPL2ND window, click **Accept**.

SHMBCCTETGPL2ND

2D selective HMBC

Shape = Q3.1000

CNST 13 (Hz)	8.000	J(XH) long range
NS	4	
EXPNO	2	

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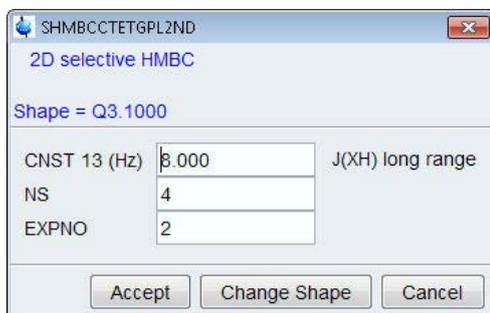
All parameters are automatically calculated and stored as an increment in next free experiment number of the dataset.

selhmbc

Acquisition parameters for 2D selHMBC:
SHMBCCTETGPL2ND

Dataset created in EXPNO: 2

SW(F1) =   16.8174 ppm / 1691.28 Hz
O2   =   171.5711 ppm / 17252.75 Hz
TD(F1) =   128
AQ(F1) =   0.0378 sec

shape pulse (SPNAM 32):   Q3.1000
shape pulse duration (P 43): 2822.72 us
power level (SPW 32):   0.166779 W

Experiment time: 16 min 8 sec

OK: starts acquisition
CANCEL: creates data sets only.

OK   Cancel
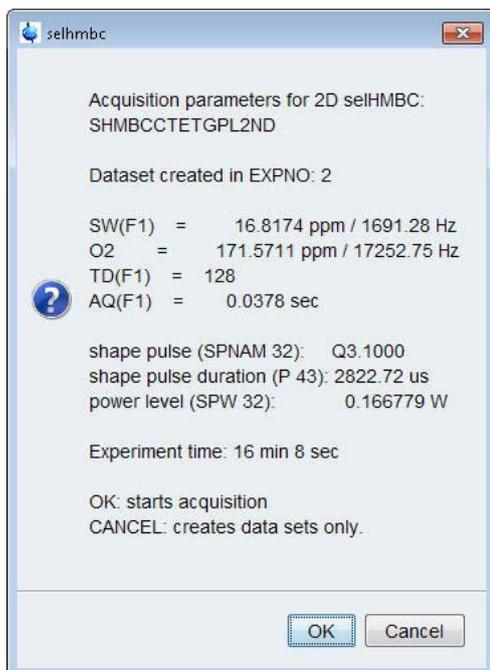
- To start the acquisition, click **OK**.

6.2.3.8   Processing

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

- At the command prompt, type **xfb** to process the data in both dimensions.
- At the command prompt, type **xf2m** to calculate magnitude spectrum in F2.
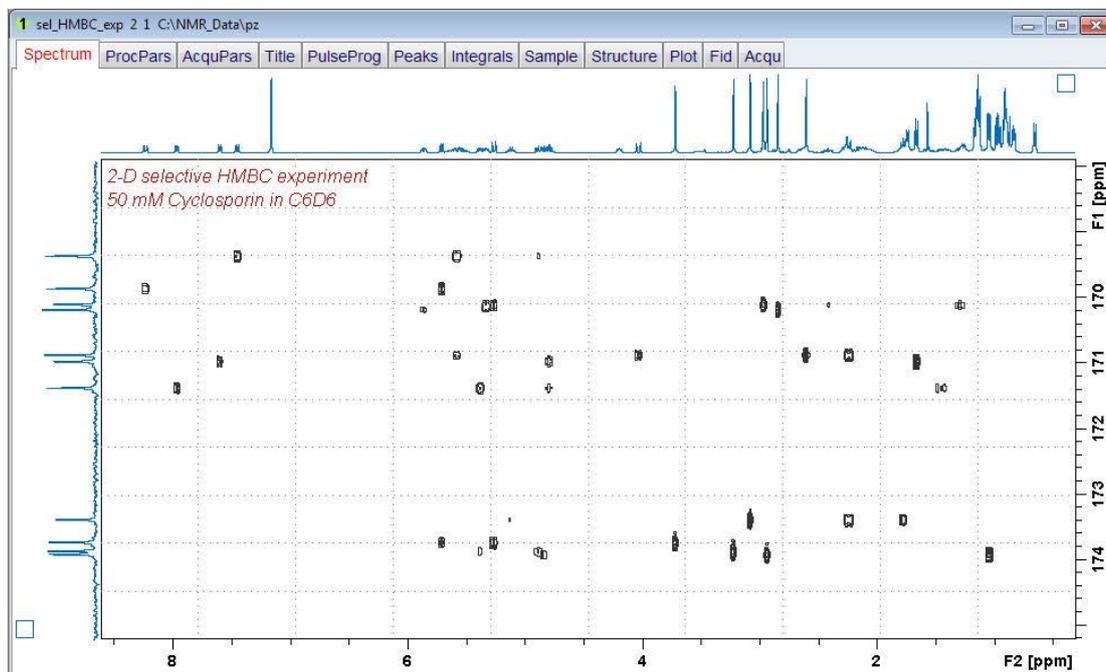
The **Selective HMBC** has a significantly higher ^{13}C resolution compared to the standard **HMBC** experiment.

7 T1 Experiment

7.1 Introduction

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

The basic pulse sequence consists of a 180^0 pulse that inverts the magnetization to the -z axis. During the following delay, relaxation along the longitudial plane takes place. Magnetization comes back to the original equilibrium z-magnetization. A 90^0 pulse creates transverse magnetization. The experiment is repeated for a series of delay values taken from a variable delay list. A 1D spectrum is obtained for each value of vd and stored in a 2D dataset. The relaxation time d1 must be set to 5*T1. A rough estimation of the T1 value can be calculated from the null-point value by using T1=tnull/ln(2).

7.2 Sample

Menthyl Anthranilate in DMSO-d_6
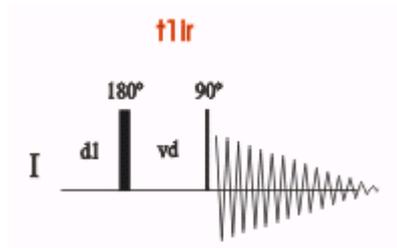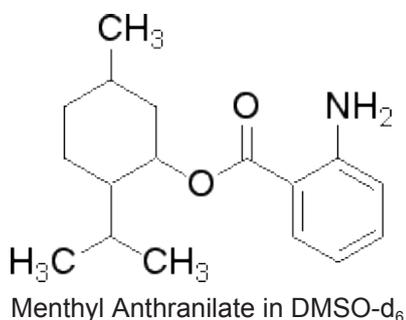
7.3   Proton Inversion-Recovery T1 Experiment

7.3.1   Preparation Experiment

Run a 1D Proton spectrum, following the instructions in the *TopSpin Guide Book Basic NMR Experiments*, Chapter *1D Proton experiment*, Paragraph *Experiment Setup* through *Processing*.
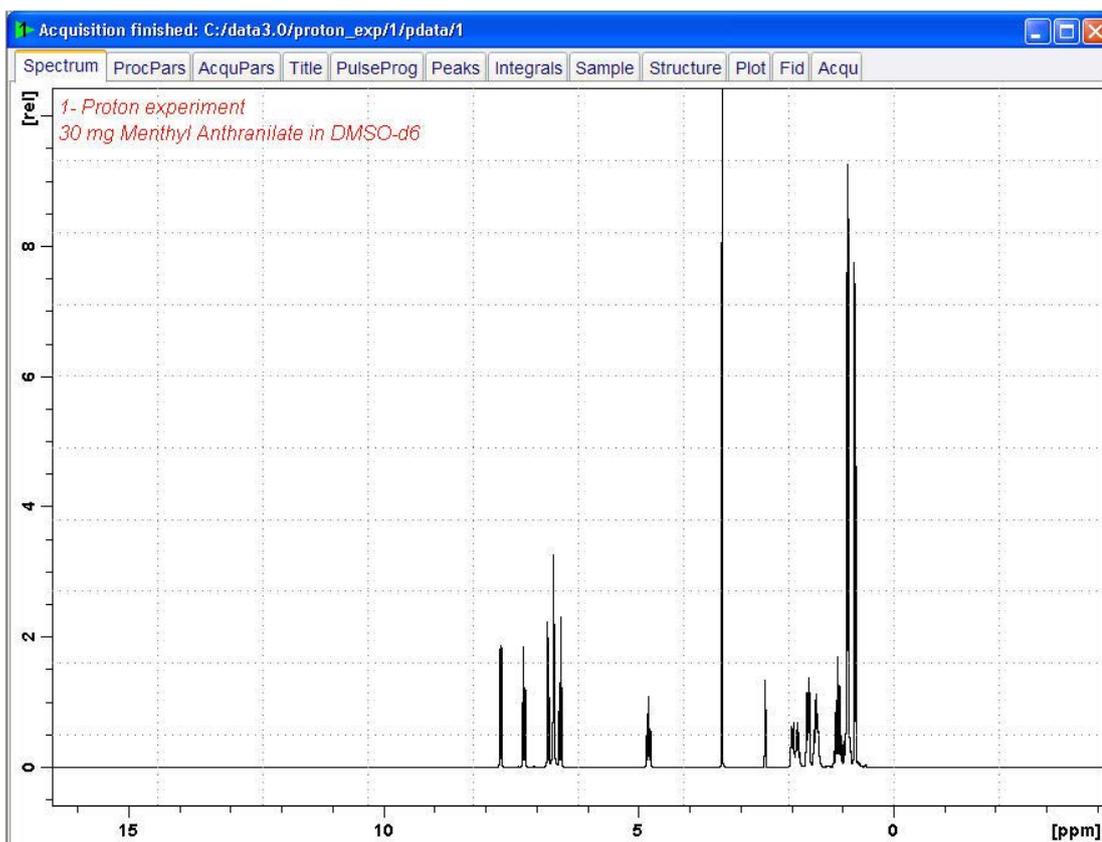
The reference spectrum is necessary to adjust the spectral limits of the sweep width to gain more data points.

7.3.2   Setting up the T1 Experiment

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

• On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
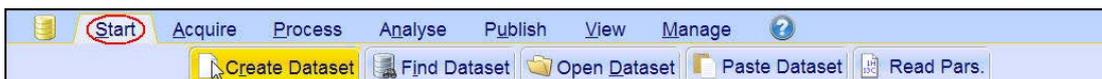
• In the New Dataset window, enter or select:

NAME = **t1_exp**

EXPNO = **1**

PROCNO = **1**

Experiment: select **PROTONT1**

Set Solvent: select **DMSO**

DIR

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

Title

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

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

For the following steps, use the Workflow button bar.

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

T1 experiments should be run non-spinning.

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

7.3.3 Limit Setting

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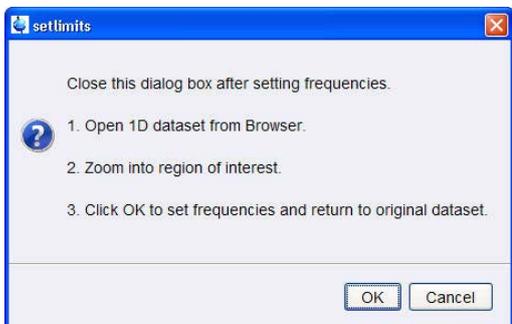
- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. **proton_exp 1**) and select **Display** or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window.
- Expand the spectrum to display all peaks, leaving about **0.5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

The solvent peak may be excluded if it falls outside of the region of interest.
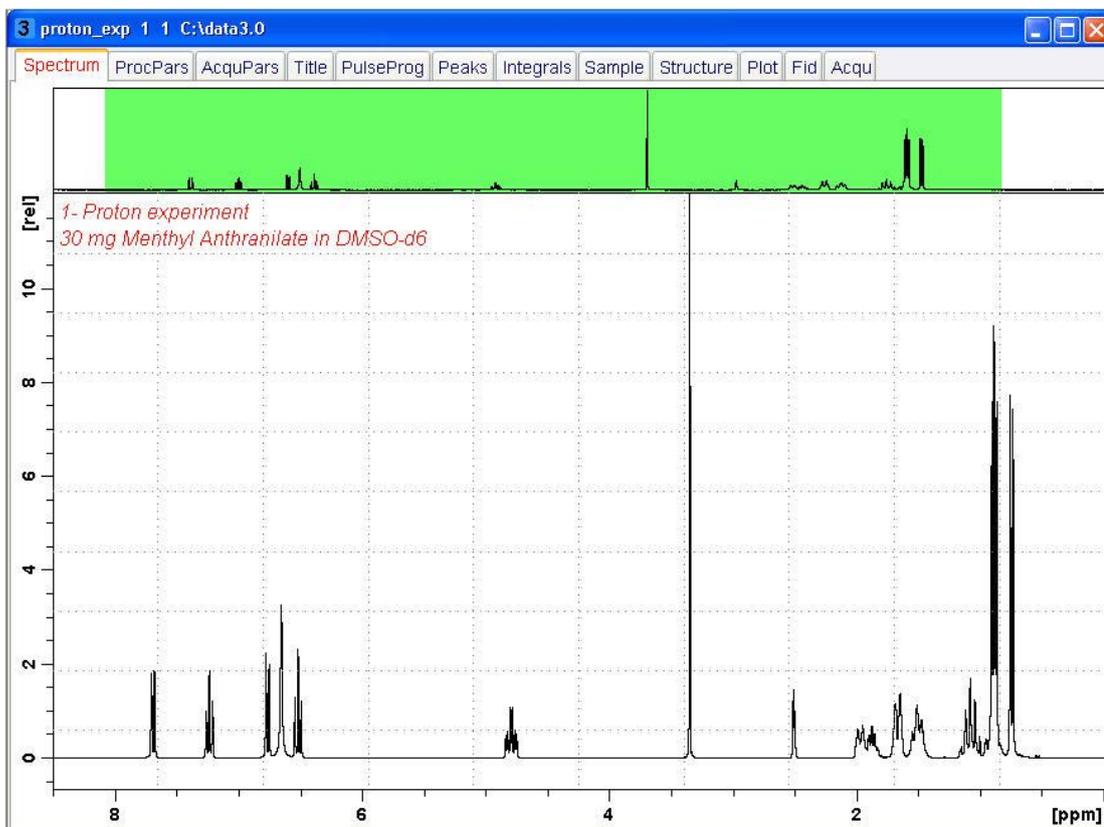
- Click **OK** in the setlimits message window to assign the new limit.
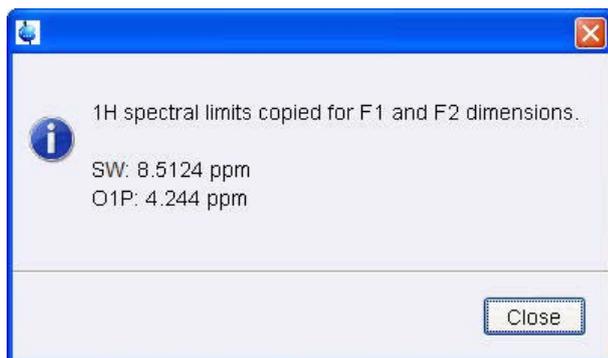
- In the message window, click **Close**.

The display changes back to the 2D dataset.

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

- Click **Show pulse program parameters**.
- Make the following changes:
  - – D1 = **15**
  - – VDLIST = **t1delay**

- Click **Edit variable delay list** right of the VDLIST name box.
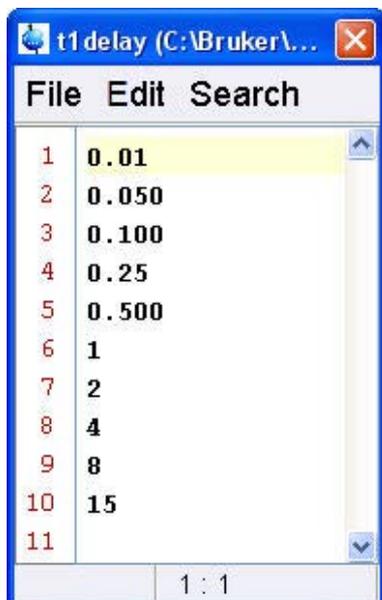
- Enter the variable delay values as shown in the figure above.
- Click **File** and **Save**.
- Click **File** and **Close**.
- In the Dataset window, select the **Spectrum** tab.

7.3.4 Acquisition

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

7.3.5 Processing

- On the menu bar, click **Process**.
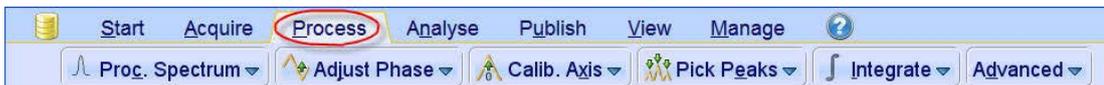
- At the command prompt, type **rser 10**.
- At the command prompt, type **ef.**
- On the Workflow button bar, click **Adjust Phase**.
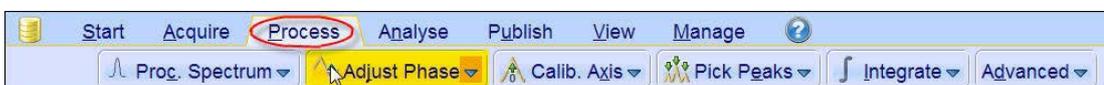
- Adjust the phase manually.
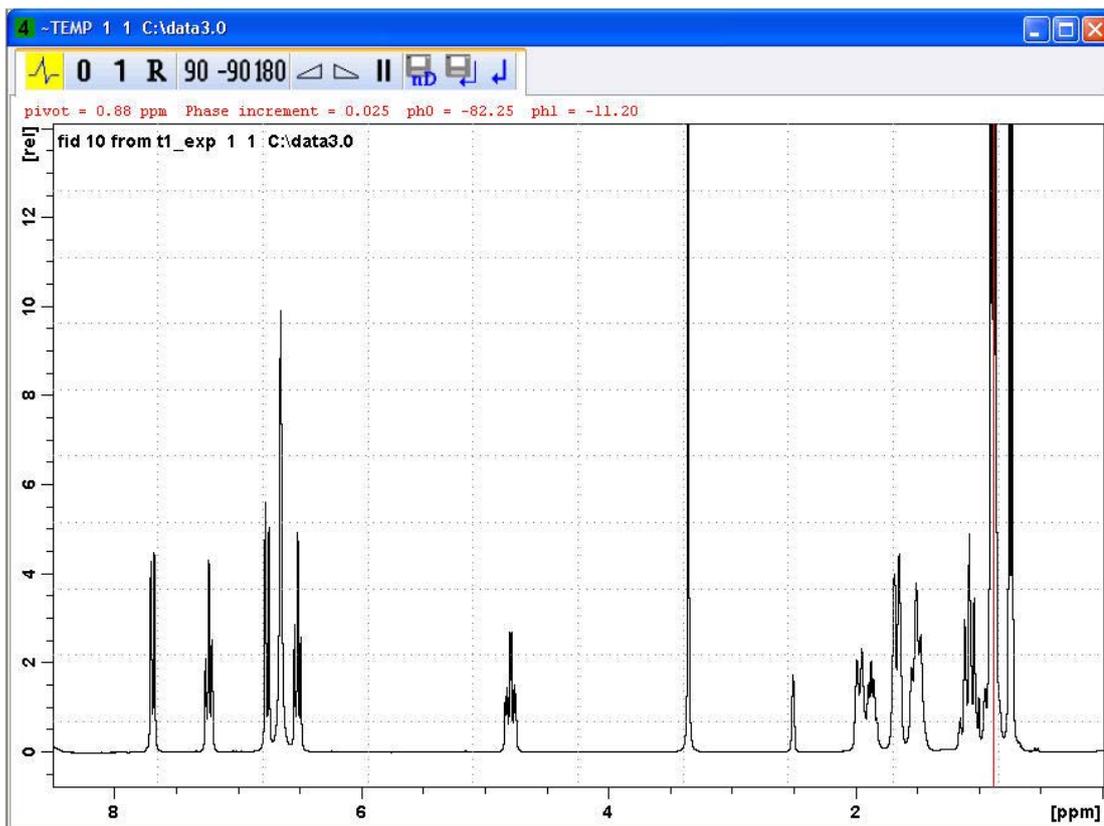
- On the Adjust Phase toolbar, click **Save for nD spectrum**.
- On the toolbar, click **Return, do NOT save phased spectrum**.
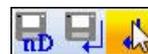
The spectrum will go back to the un-phased view since the phase correction values were stored only for the 2D spectrum.

- On the toolbar, click **Last 2D data** to go back to the 2-D spectrum display.
- At the command prompt, type **xf2** to process only the F2 axis.
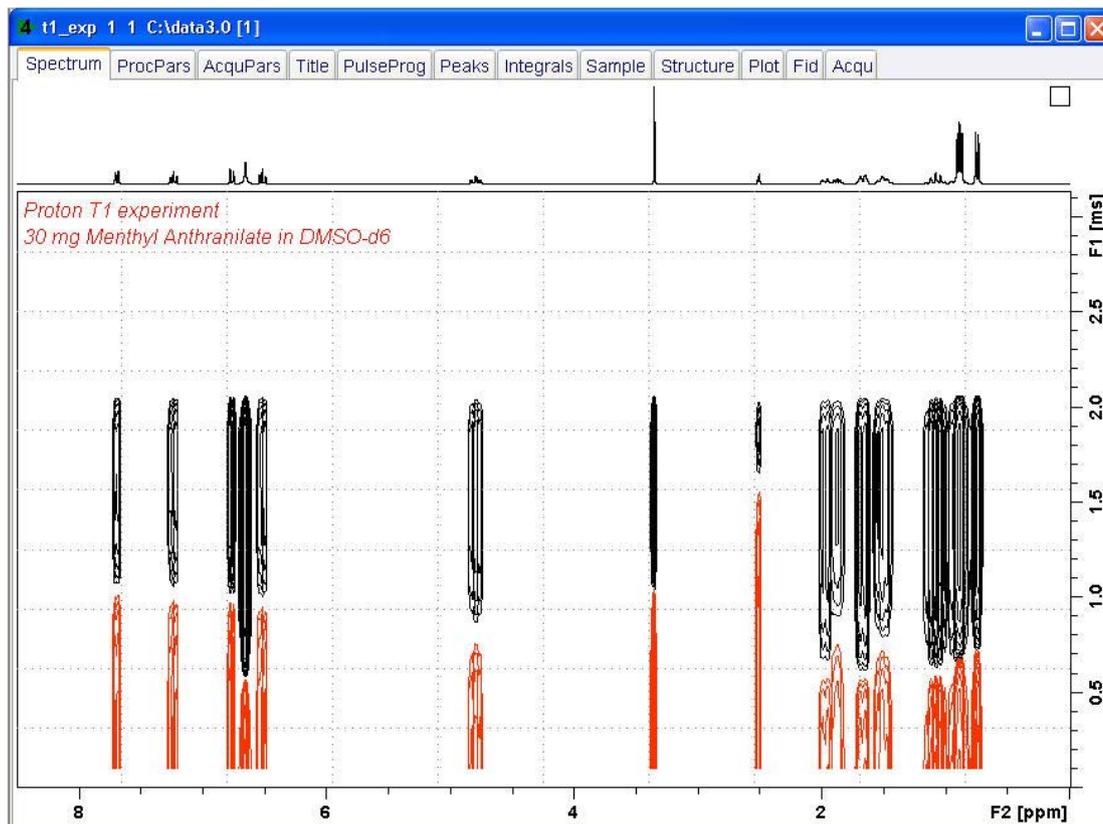
7.3.6   T1 Calculation

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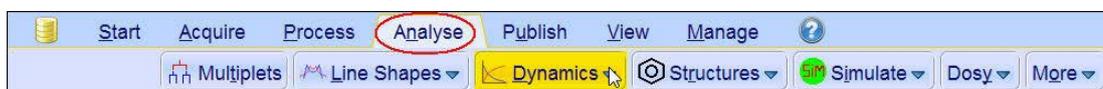
- On the **Dynamics** button, click the drop-down arrow to see more options.
- In the list, select **T1/T2 Module**.

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

T1 Experiment

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

- On the Workflow button bar, click **Fid**.
- In the Extract a row from 2d data window, click **Spectrum**.
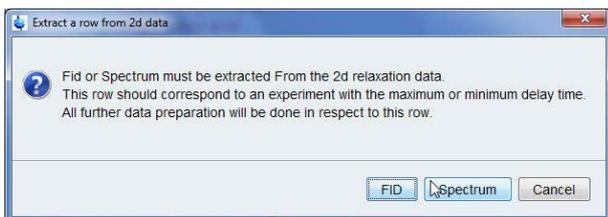
- Enter Slice Number = **10**.
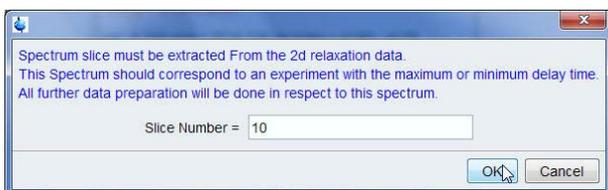
- In the message window, click **OK**.
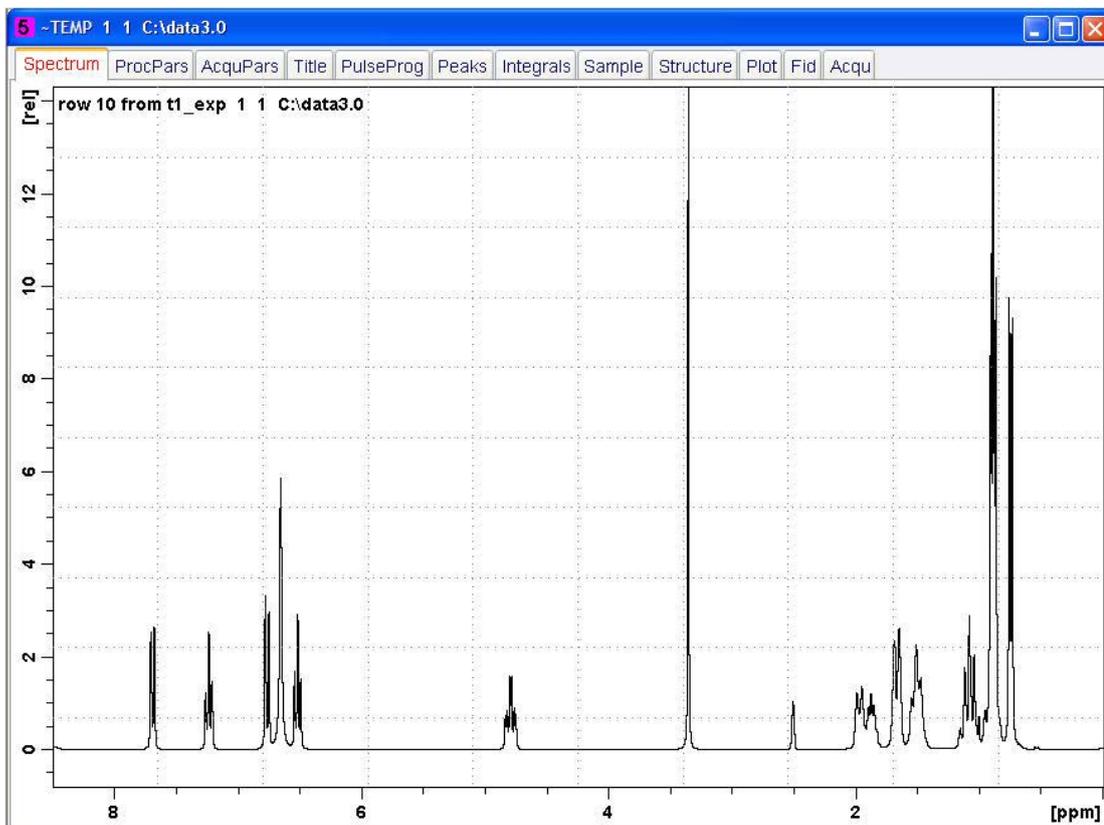
• On the Workflow button bar, click **Peaks/Ranges**.

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

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

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

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

- In the list, select **Export Region To Relaxation Module**.

Save Regions To 'intrng'
Save Regions To 'reg'
Export integration regions
Export Regions To Relaxation Module and .ret.
Save & Show List
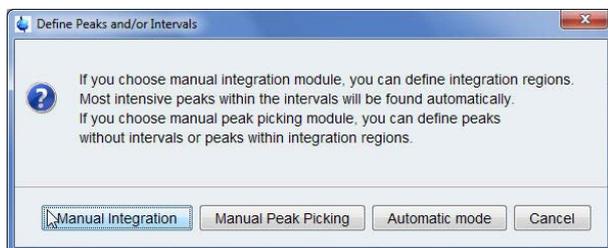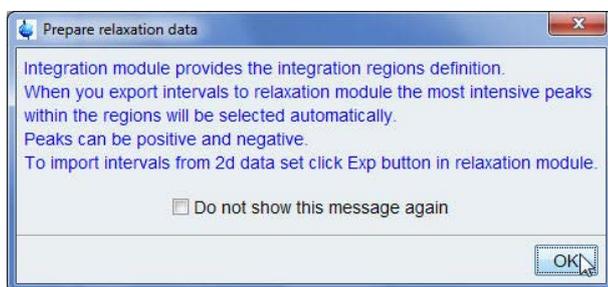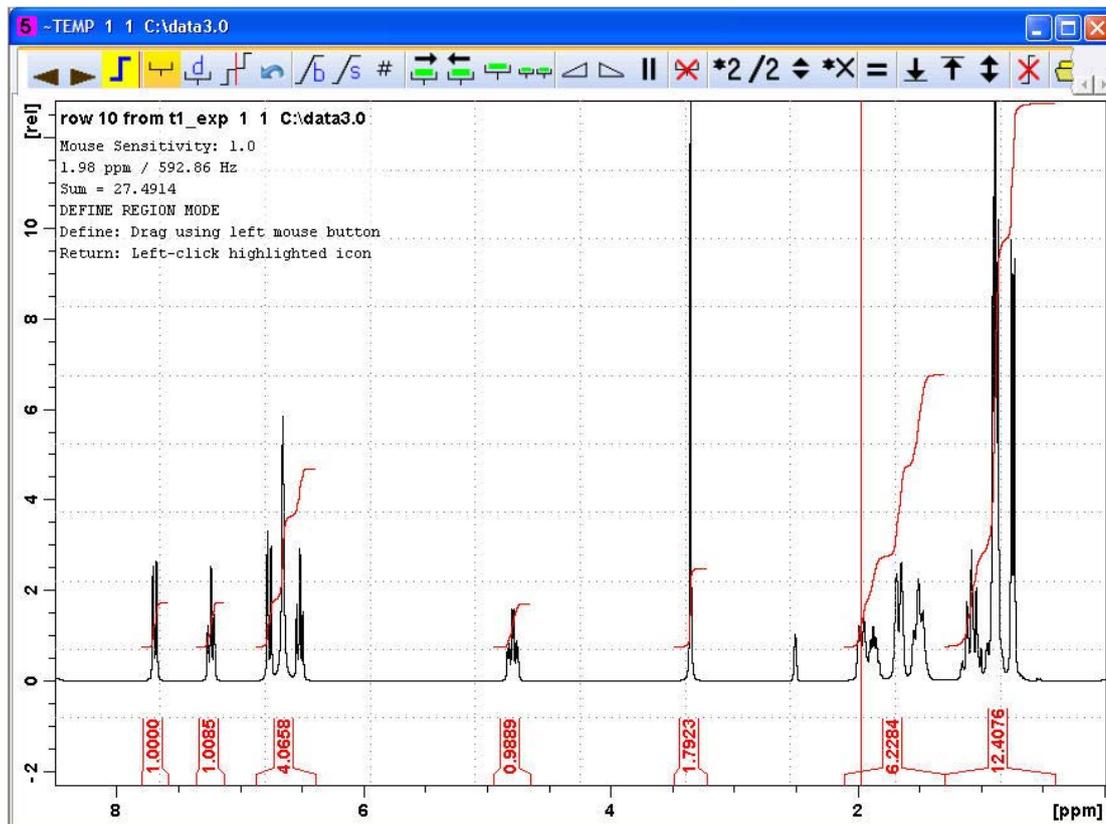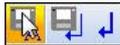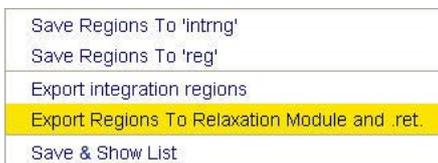
- On the Workflow button bar, select **Relaxation**.

- In the Relaxation parameters window, click **OK**.
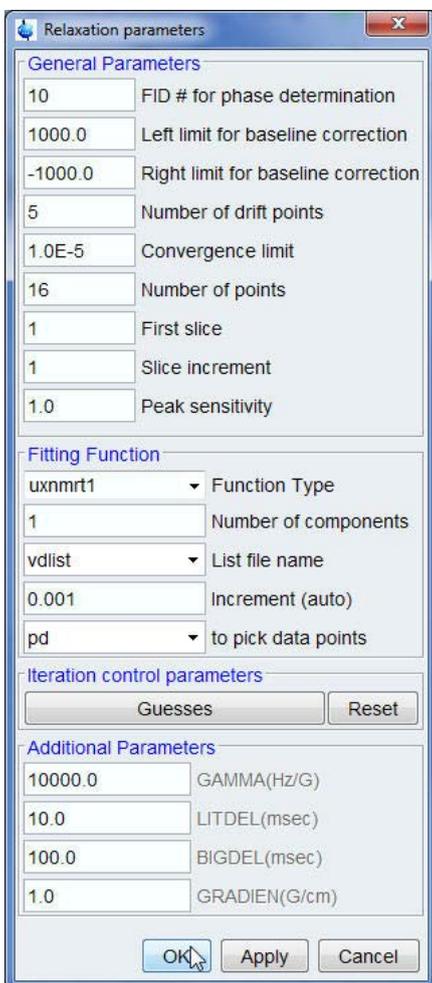
- On the Workflow button bar, select **Fitting**.

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

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

Close

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

Relaxation parameters

10	FID # for phase determination
1000.0	Left limit for baseline correction
-1000.0	Right limit for baseline correction
5	Number of drift points
1.0E-5	Convergence limit
10	Number of points
1	First slice
1	Slice increment
1.0	Peak sensitivity

uxnmrt1	Function Type
1	Number of components
vdlist	List file name
0.001	Increment (auto)
pd	to pick data points

Guesses   Reset

10000.0	GAMMA(Hz/G)
10.0	LITDEL(msec)
100.0	BIGDEL(msec)
1.0	GRADIEN(G/cm)

OK   Apply   Cancel

- On the Workflow button bar, select **Calculation**.

- In the message window, click **Close**.
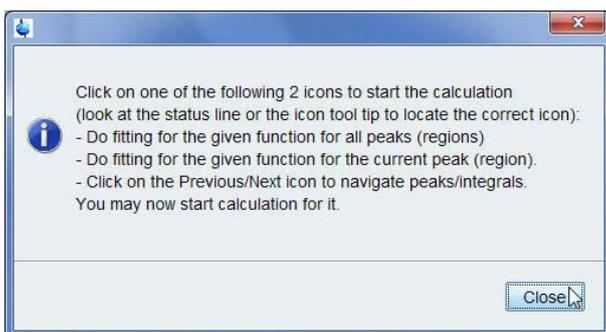
• Select **Area** for fitting type.
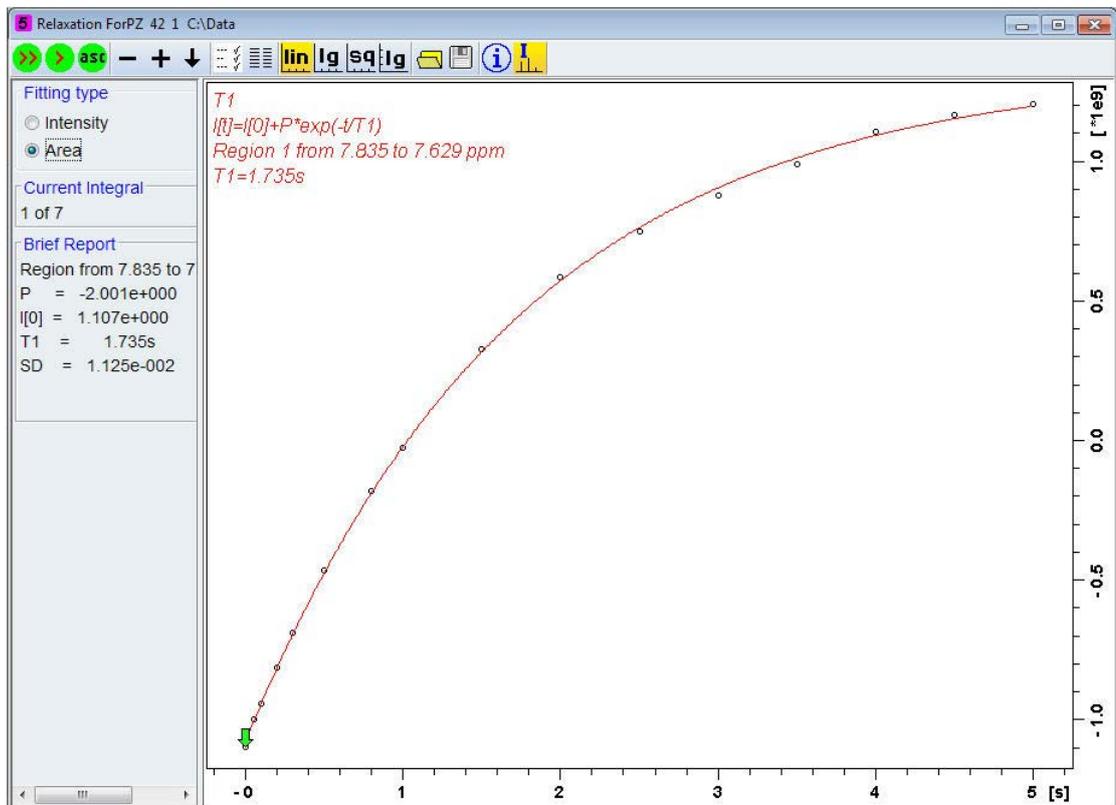
• In the T1/T2 tools bar, click **Calculate fit for all peaks**.

Brief Report

Peak 1 at 7.677 ppm
T1   =   1.427s
Peak 2 at 7.234 ppm
T1   =   1.312s
Peak 3 at 6.653 ppm
T1   =   237.766m
Peak 4 at 4.784 ppm
T1   =   641.009m
Peak 5 at 3.352 ppm
T1   =   1.411s
Peak 6 at 1.645 ppm
T1   =   266.740m
Peak 7 at 0.884 ppm
T1   =   413.572m

- On the Workflow button bar, select **Report**.
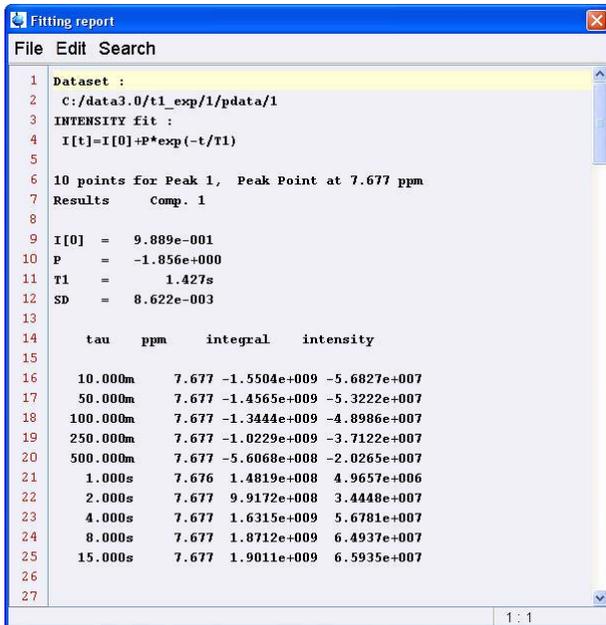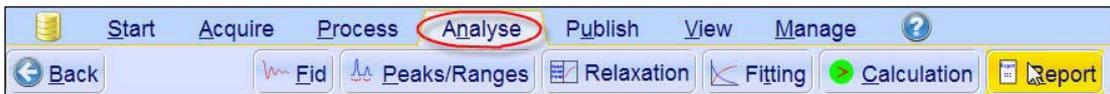
8   Pulse Calibration

8.1   Introduction

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

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

8.2   Sample

Mixture **0.1 M** each of **¹⁵N** enriched Urea and **¹³C** enriched methanol in **DMSO-d$_6$**, see the next figures:
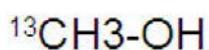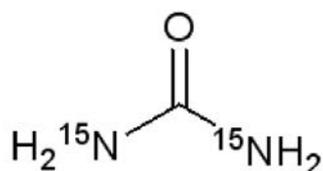
8.3   ¹H 90° Transmitter Pulse
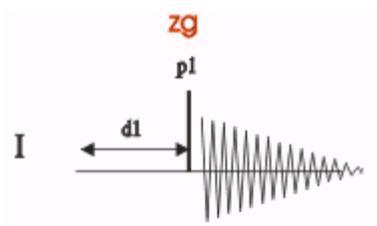
The pulse program **zg** is used to determine the **¹H 90°** transmitter pulse. The sequence consists of one channel **f1** with a recycle delay **d1**, a **¹H** pulse **p1**, followed by the **¹H** signal detection. The signal has maximum intensity if **p1** is a **90°** pulse and 2 nulls at a **180°** and **360°** pulse. A methanol signal region from **3.5 ppm** to **2.8 ppm** is used for this experiment.

8.3.1 Preparation Experiment

Run a **1D Proton** spectrum of urea/methanol in DMSO-d$_6$, following the instructions from the *TopSpin Guide Book Basic NMR Experiments*, chapter *1D Proton Experiment*, paragraphs *Experiment Setup* through *Processing*.
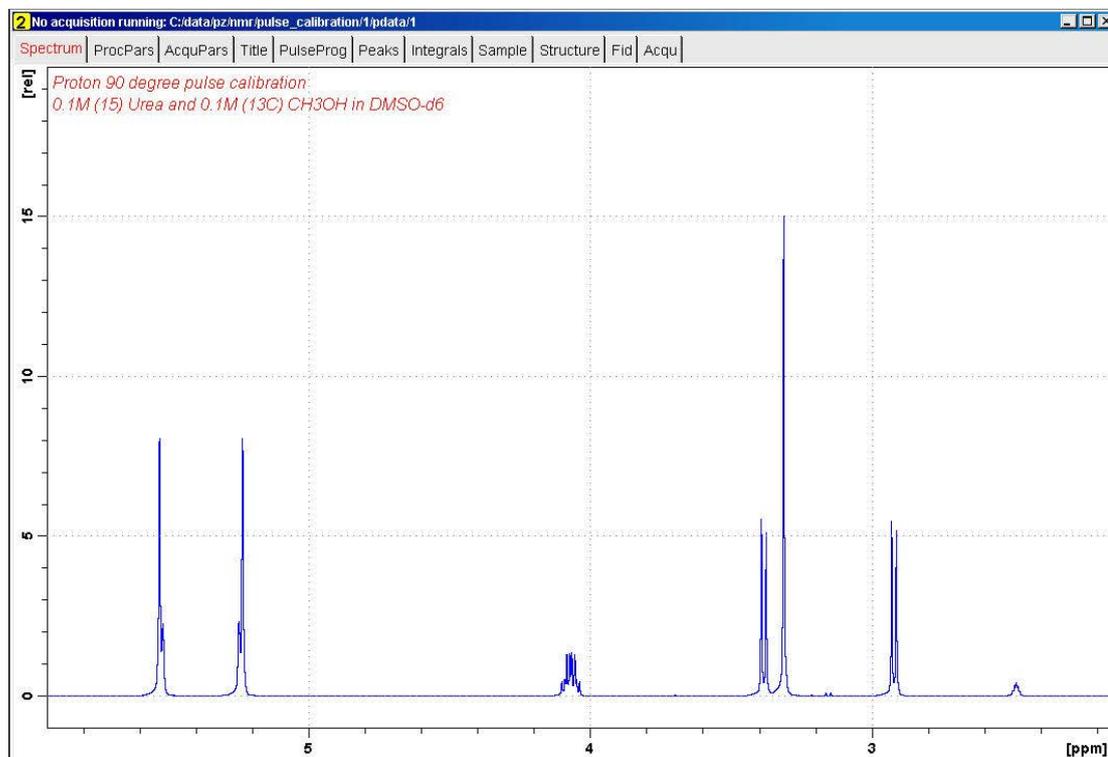
8.3.2 Parameter Setup

- At the command prompt, type **wrpa**.
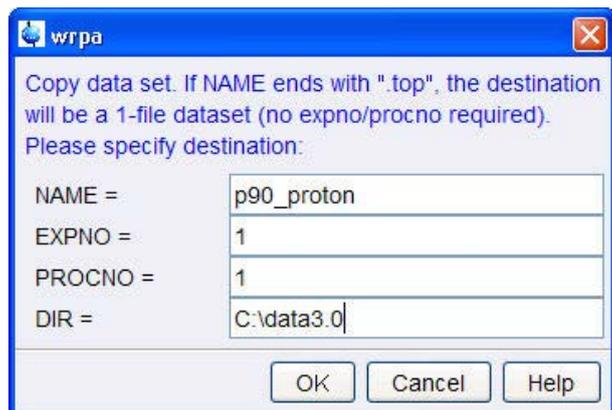
- In the field *Name*, enter **p90_proton**.
- Click **OK**.

- At the command prompt, type **re**.
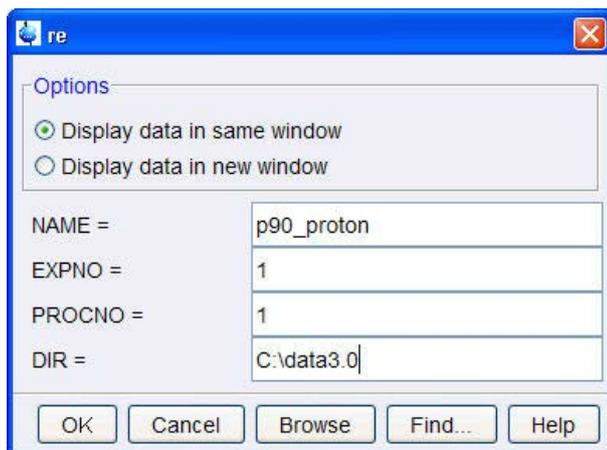
- In the field *Name*, enter **p90_proton**.
- Click **OK**.

Normally a single on-resonance peak is used to determine the 90^0 transmitter pulse. For practical reasons the methanol signal region from **3.5 ppm** to **2.8 ppm** is used to measure the ^{1}H 90° transmitter pulse, since the same signals will also be used in determining the ^{13}C 90° decoupler pulse.

- Expand the spectrum for the region between **3.5 ppm** and **2.8 ppm**.
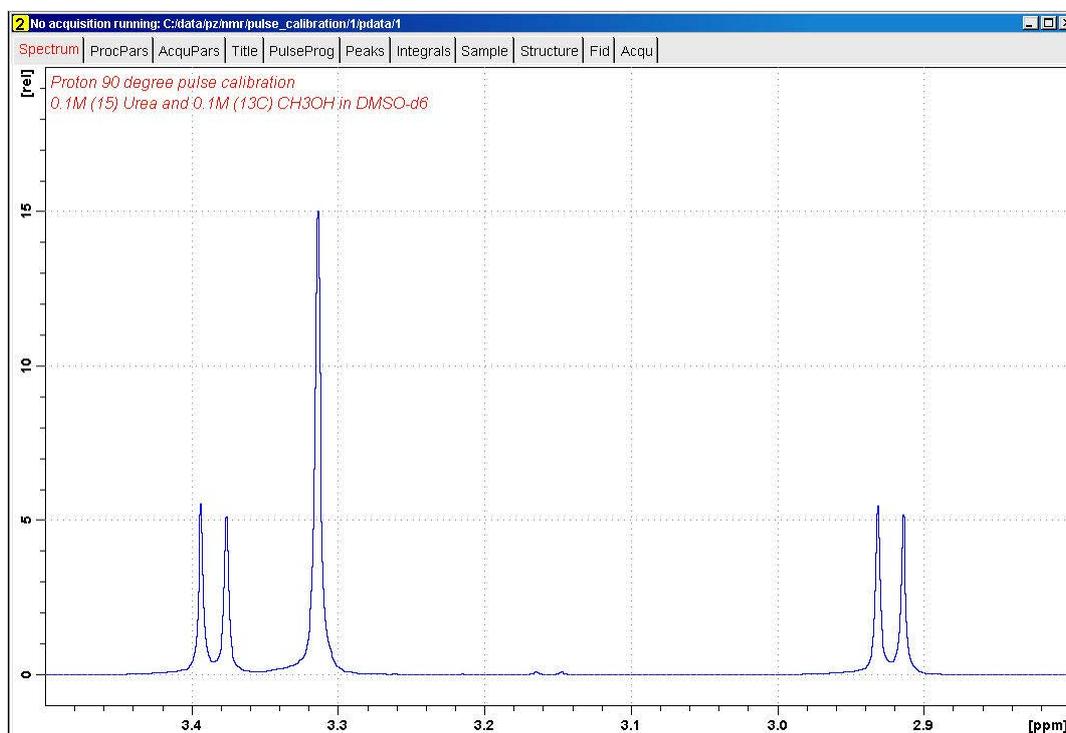
- On the toolbar, click **Set sw to current region** and **SFO1 to center of region**.
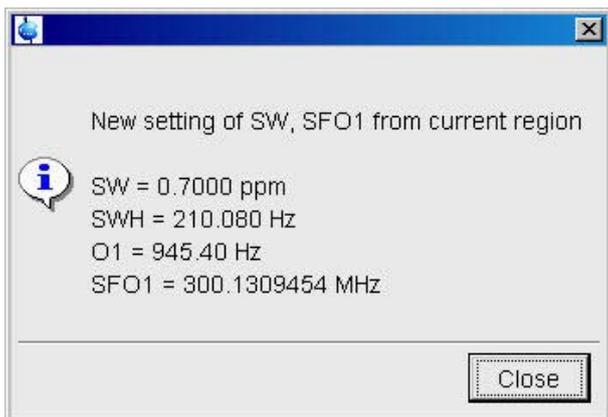
- In the pop up window, click **Close.**
- In the Dataset window, select the **AcquPars** tab.
- Enter:

  PULPROG = **zg**

  TD = **4096**

  NS = **1**

  DS = **0**

  D1 = **10**

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

  SI = **2048**

  PH_mod = **pk**

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

8.3.3   Acquisition

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

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

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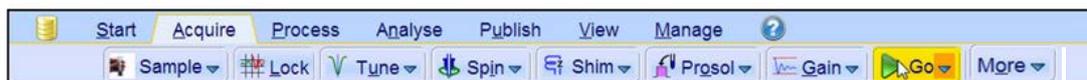
Alternatively type **go** at the TopSpin command prompt. On the **Go** button, click the **drop-down** arrow to see more options.

8.3.4   Processing

- Process and phase correct the spectrum.
- Display the full spectrum.
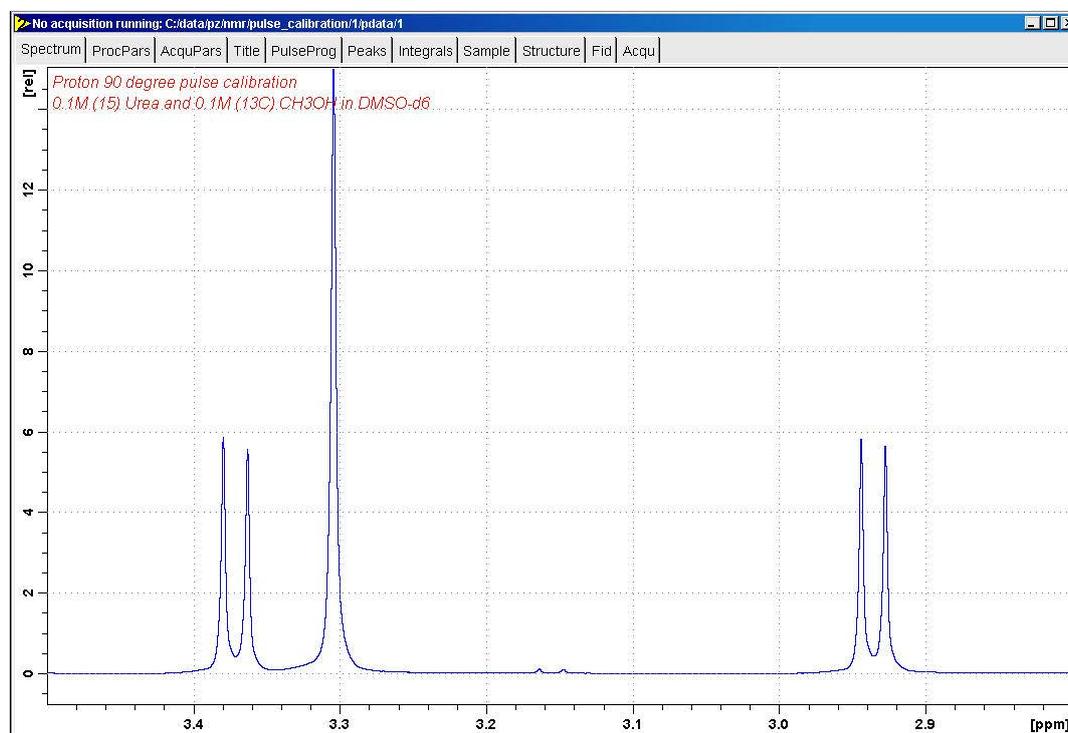
- Right-click in the spectrum window.
- In the list, select **Save Display Region To...**
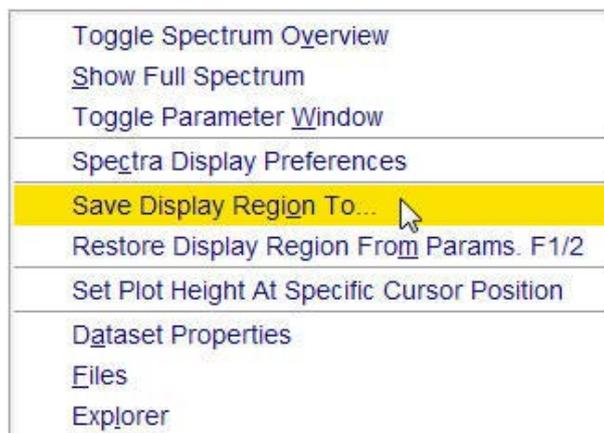
- Select **Parameters F1/2 (e.g. used by restore display, ...) [dpl].**
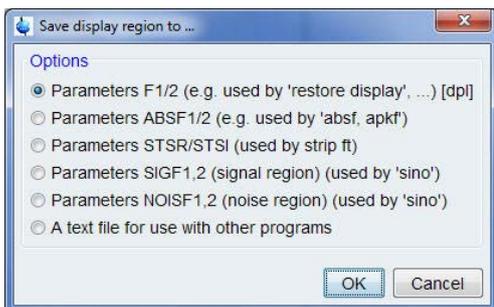
- Click **OK**.
- At the command prompt, type **wpar H1p90_urea all** to store the parameter set for future use.

8.3.5   Determine the ¹H 90°Transmitter Pulse

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

OPTIMIZE = **Step by step**

PARAMETER = **p1**

OPTIMUM = **POSMAX**

STARTVA = **2**

NEXP = **20**

VARMOD = **LIN**

INC= **2**

- Click **Save**.
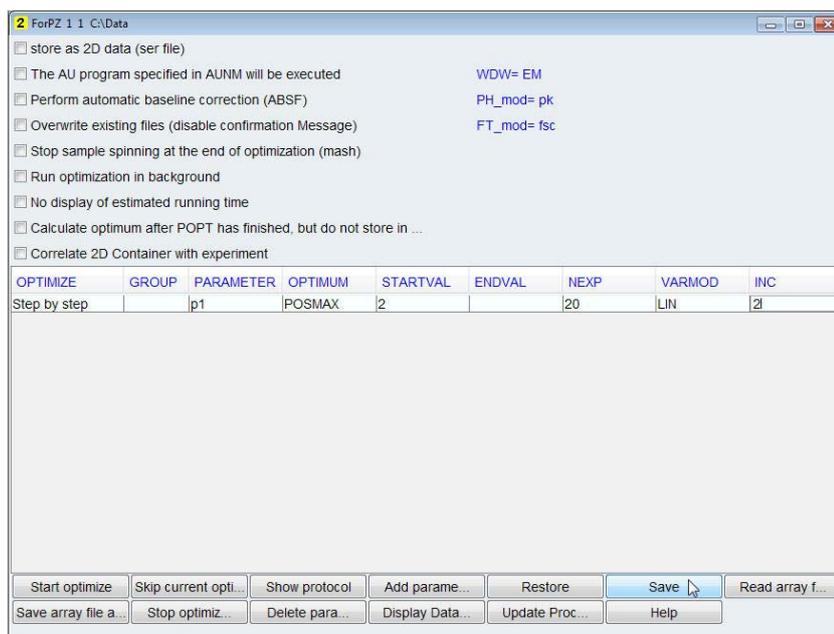
The **ENDVAL** parameter has been updated.

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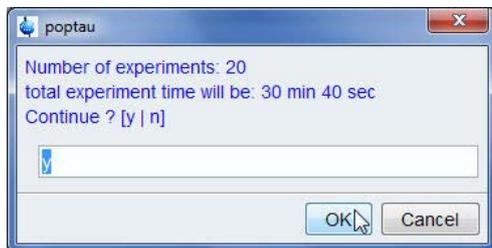
- Click **OK**.

The parameter optimization starts. The spectrometer acquires and processes 20 spectra by incrementing the parameter p1 from **2 µs** by **2 µs** to a final value of **40 µs**. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file *pulse_calibration/2/999* as shown:
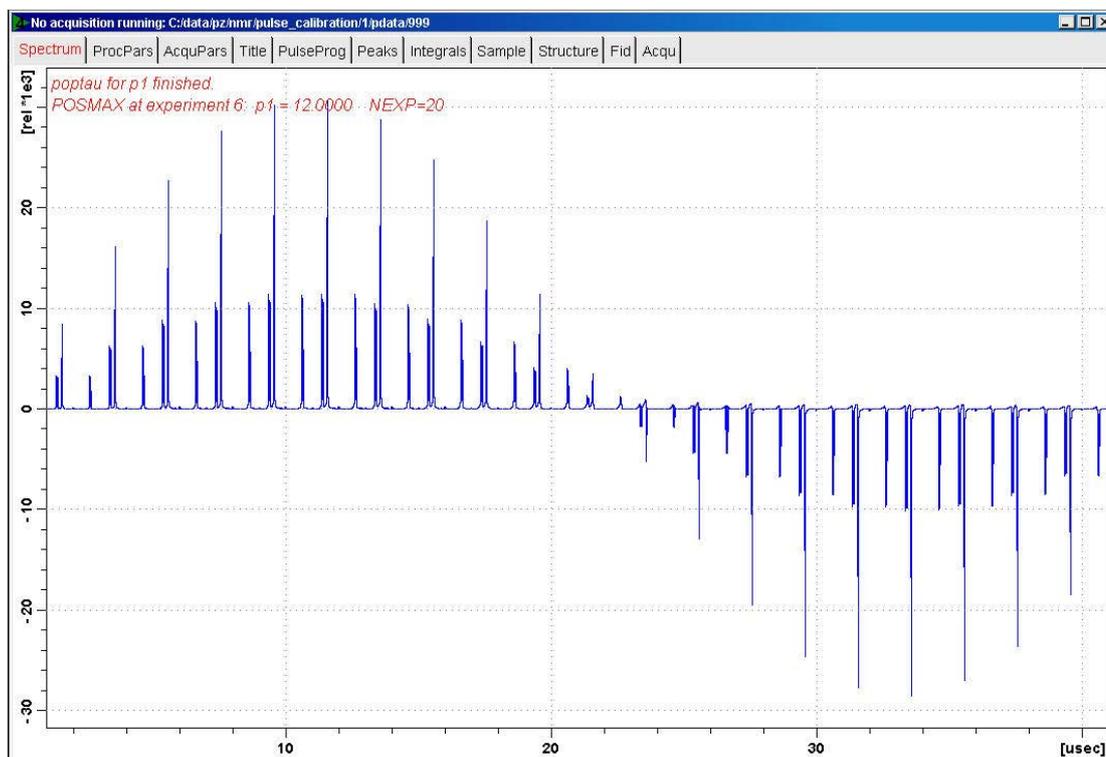
- In the Dataset window, select the **Title** tab.
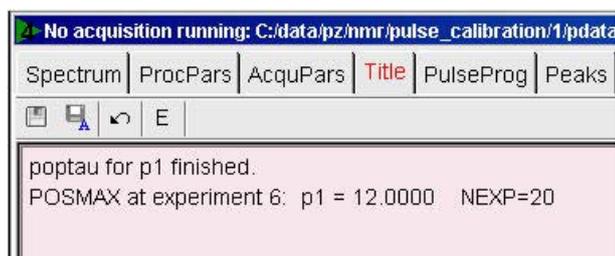
The POSMAX value of p1 is displayed in the title tab window which is the 90° pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90° pulse measurement, follow the steps below:

- Close the popt setup window.
- At the command prompt, type **re 1 1**
- At the command prompt, type **p1**
- Enter the value which corresponds to a 360^0 pulse (the second zero crossing in the popt spectrum, which should be approximately 4 times the POSTMAX value).
- Step 1: At the command prompt, type **zg** to start the acquisition.
- Step 2: At the command prompt, type **efp**
- Change p1 slightly and repeat steps 1 and 2, until the signal undergoes a zero crossing as expected for an exact 360^0 pulse.

The signals are negative for a pulse angle slightly less than 360° and positive when the pulse angle is slightly more than 360^0.

- 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.

8.4 ^{13}C 90° Decoupler Pulse
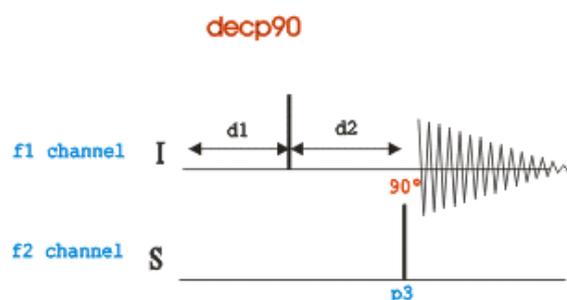
The pulse program used in this procedure is the **decp90** sequence shown in the figure above. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for ^{1}H and f2 to ^{13}C. Channel f1 shows a recycle delay **d1** followed by a 90^0 pulse and a delay **d2 = 1/(2JXH)** for the creation of antiphase magnetization. A ^{13}C pulse on channel f2 is been executed after the delay **d2** and then the ^{1}H signal is detected. When the ^{13}C pulse is exactly 90^0, the ^{1}H signals will go through a null. The methanol signal region from **3.5 ppm** to **2.8 ppm** is used for this experiment.

8.4.1 Preparation Experiment

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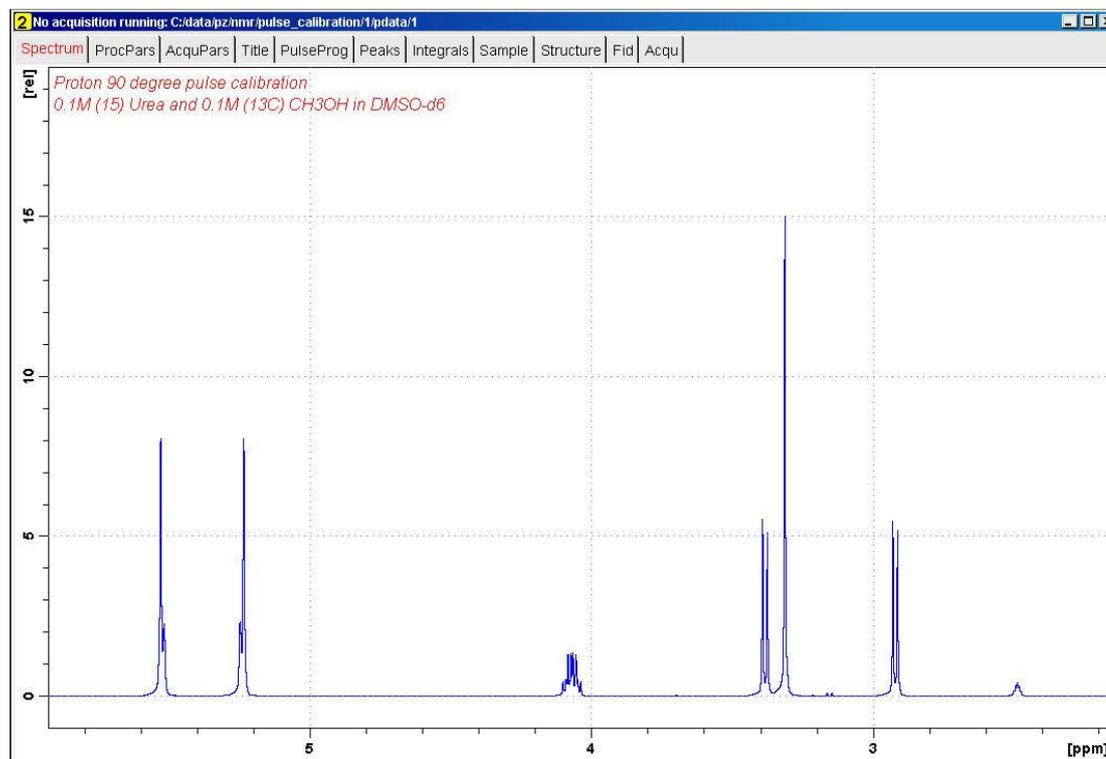
8.4.2 Parameter Setup

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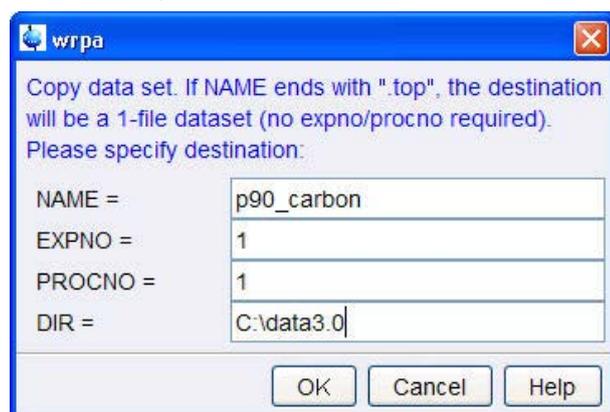
- At the command prompt, type **re** and press **Enter**.
- Change NAME **= p90_carbon**

- In the re window, click **OK**.

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

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

- In the pop up window, click **Close**.

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

  PULPROG = **decp90**

  TD = **4096**

  NS = **1**

  DS = **0**

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

- Select ^{13}C for **NUC2**.

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

- In the Edit Spectrometer Parameter window, click **Save**.
- In the **AcquPars** enter:

  O2[ppm] = **49.5**

  D1 = **10**

  CNST2 = **139**

  P3 = **3**

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

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

  SI = **2048**

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

8.4.3   Determine the ¹³C 90° Decoupler Pulse

- On the Workflow button bar, click **Tune**.
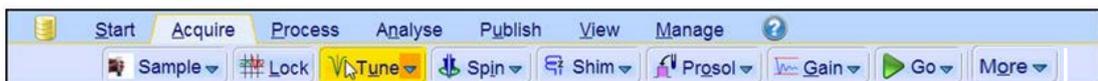
- On the Workflow button bar, click **Go**.
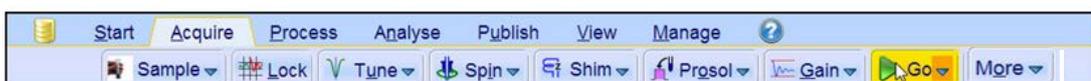
- Process and phase correct the spectrum.
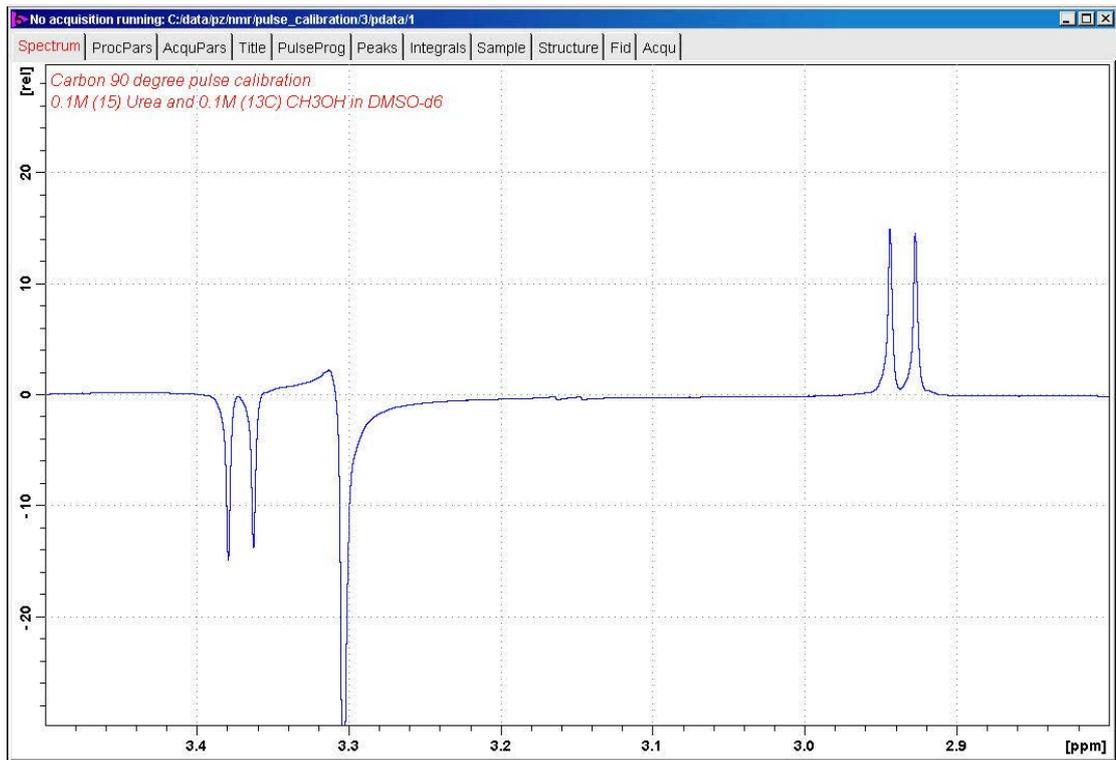
Phase the left doublet negative and the right doublet positive. The Water peak at **3.3 ppm** can be ignored and does not have to be in phase.

- Increase **p3** in increments of **1** or **2 µs**, execute **zg** followed by the command **efp** until the signals go through a null or a phase change. This will be the ^{13}C 90^0 decoupler pulse.
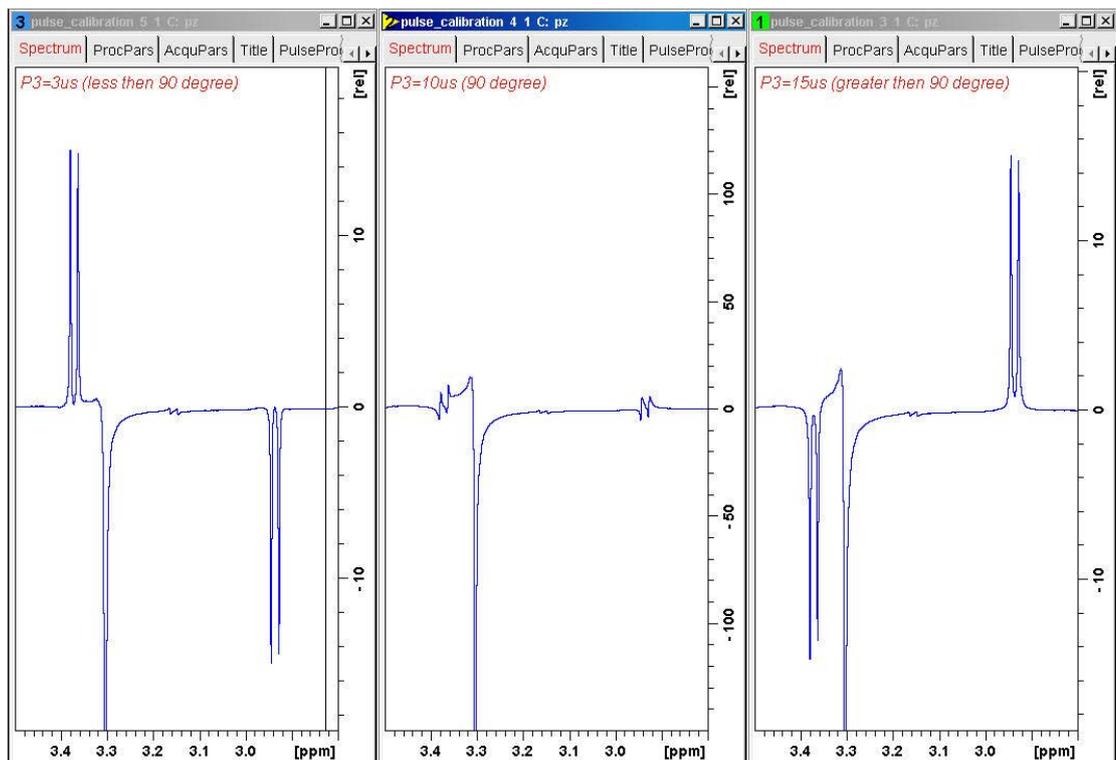
8.5 ^{15}N 90° Decoupler Pulse
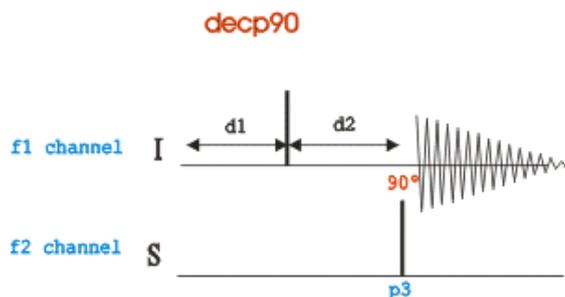
decp90

The pulse program used in this procedure is the **decp90** sequence shown in the figure above. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for ^{1}H and f2 to ^{15}N. Channel f1 shows a recycle delay **d1** followed by a 90^0 pulse and a delay **d2 = 1/(2JXH**) for the creation of antiphase absorption. A ^{15}N pulse on channel f2 is executed after the delay **d2** and then the ^{1}H signal is detected. When the ^{15}N pulse is exactly 90^0, the ^{1}H signals will go through a null. The urea signal region from **5.6 ppm** to **5.1 ppm** is used for this experiment.

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

decp90f3
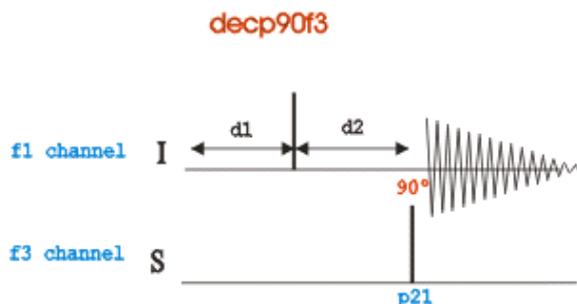
8.5.1   Parameter Setup

- At the command prompt, type **wrpa** and press **Enter**.
- Change NAME = **p90_nitrogen**
- In the wrpa window, click **OK**.
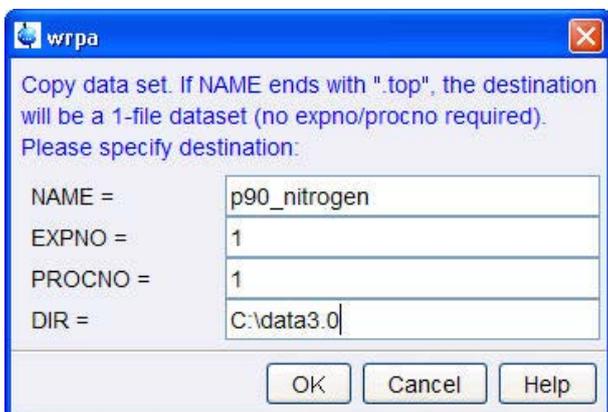
- At the command prompt, type **re** and press **Enter**.
- Change NAME = **p90_nitrogen**.
- In the re window, click **OK**.
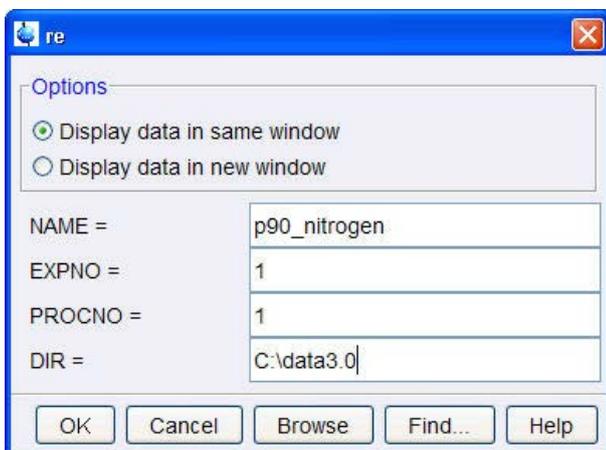
- Expand the spectrum for the region between **5.6 ppm** and **5.1 ppm**.
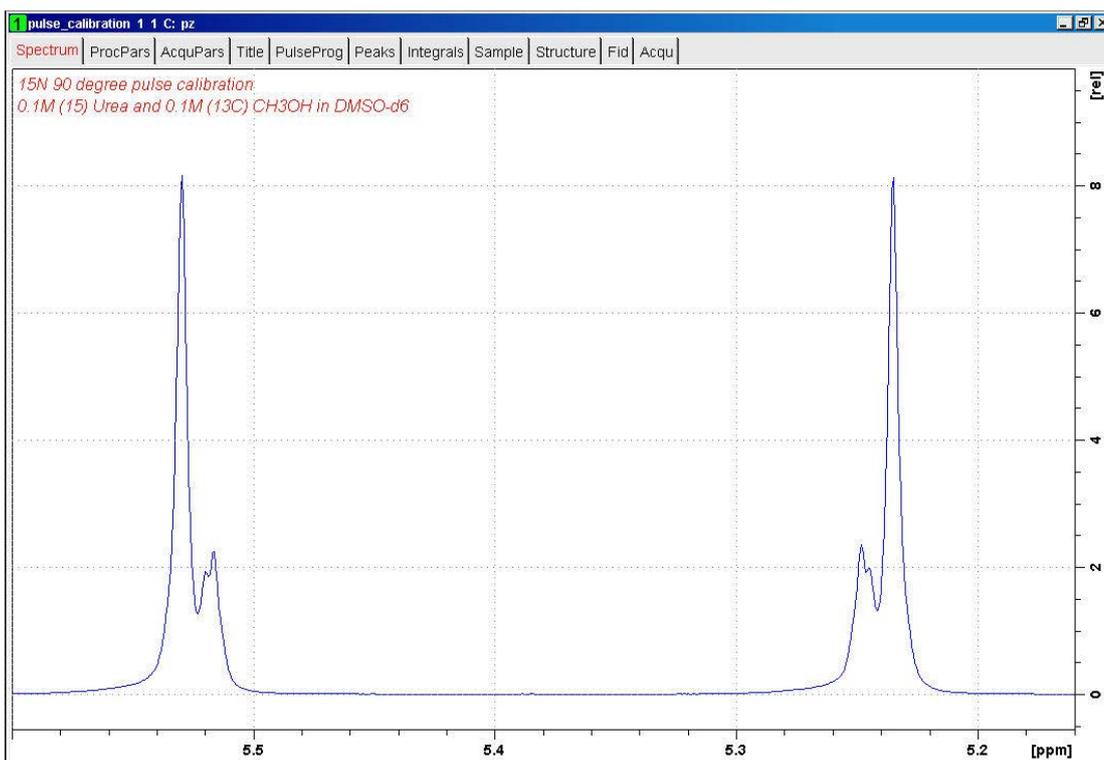
- On the toolbar, click **Set sw to current region** and **SFO1 to center of region**.
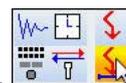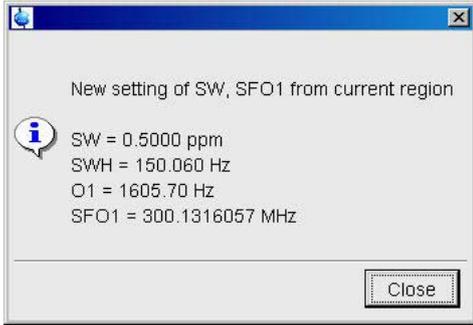
New setting of SW, SFO1 from current region

SW = 0.5000 ppm
SWH = 150.060 Hz
O1 = 1605.70 Hz
SFO1 = 300.1316057 MHz

Close

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

  PULPROG = **decp90**

  TD = **4096**

  NS = **1**

  DS = **0**

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

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

8.5.1.1 Two Channel System

- Select ^{15}N for **NUC2**.
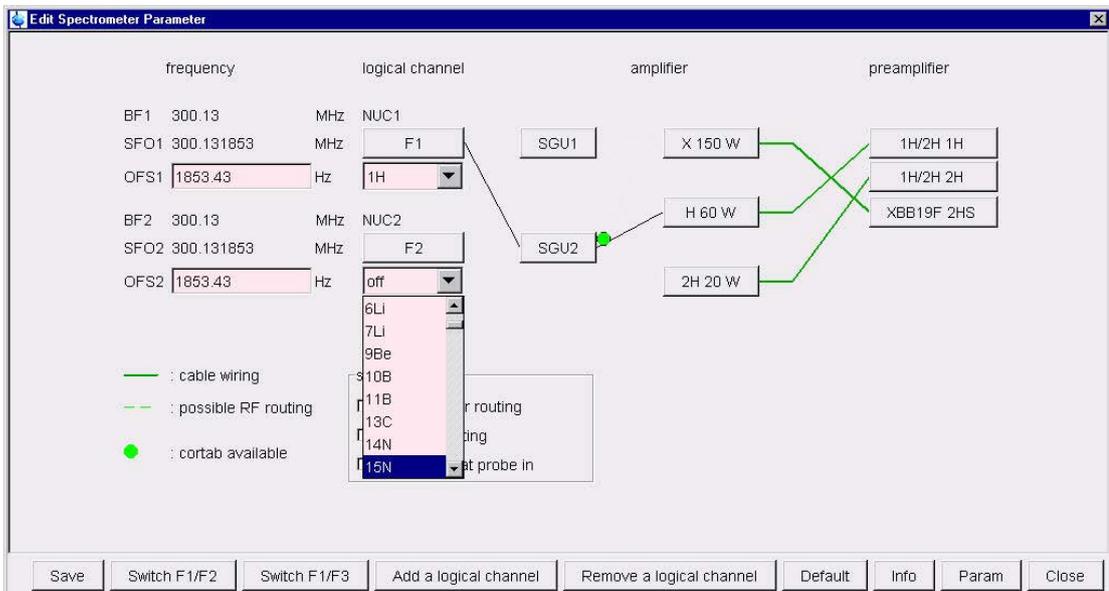
• In the Edit Spectrometer Parameter window, click **Default** to set the routing.
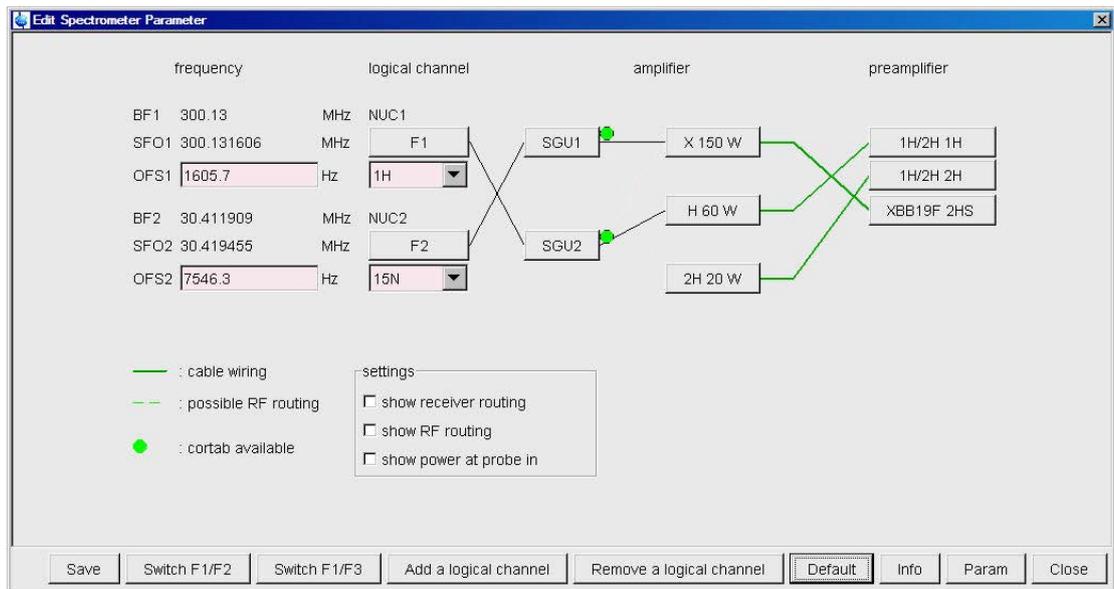
• In the Edit Spectrometer Parameter window, click **Save**.

8.5.1.2 Three Channel System

• Select ^{15}N for **NUC2**.
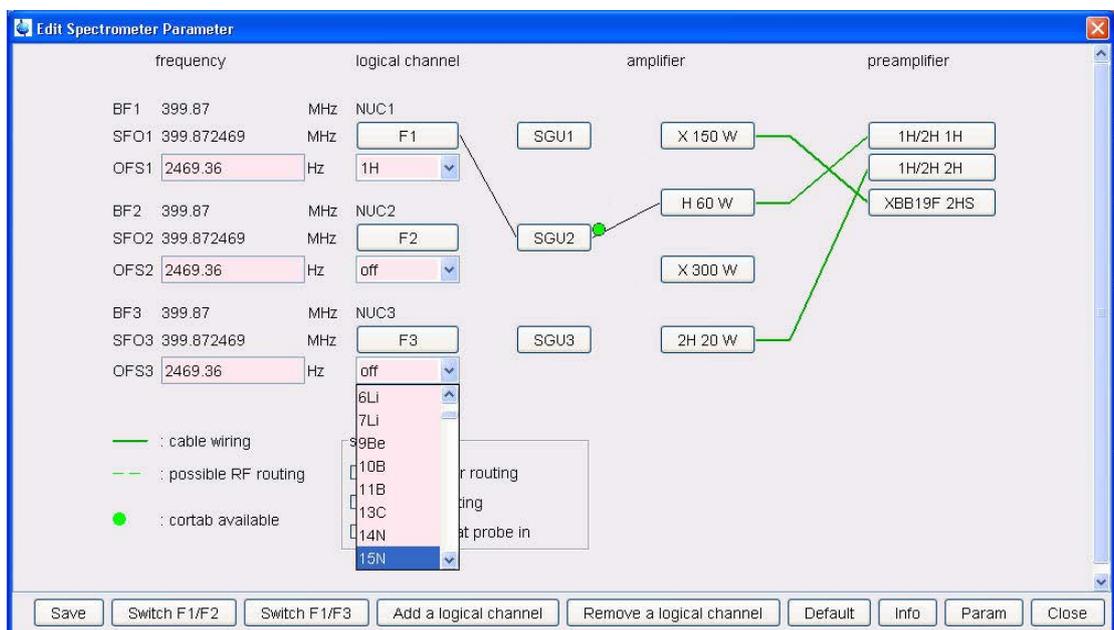
• In the Edit Spectrometer Parameter window, click **Default** to set the routing.
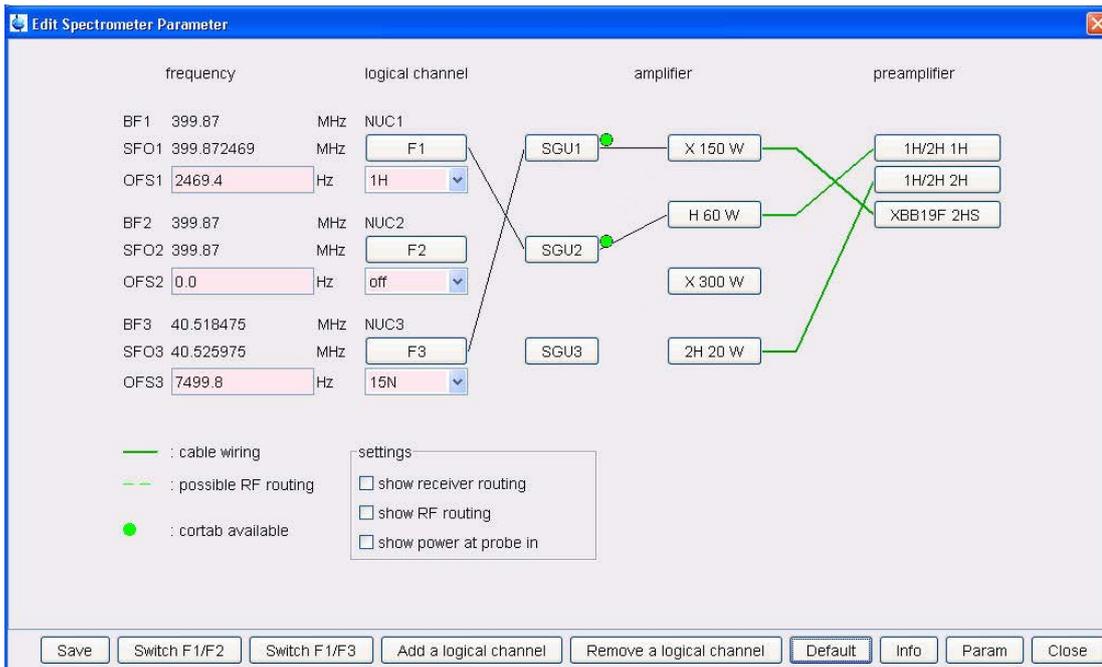
- In the Edit Spectrometer Parameter window, click **Save**.
- In the AcquPars make the following change:

    O2[ppm] = **76**

    D1 = **10**

    CNST2 = **88.5**

    P3 = **6**

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

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

    SI = **2048**

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

8.5.2   Determine the ^{15}N 90° Decoupler Pulse

- On the Workflow button bar, click **Tune**.
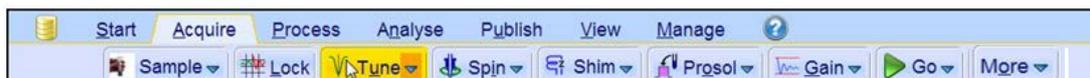
- On the Workflow button bar, click **Go**.
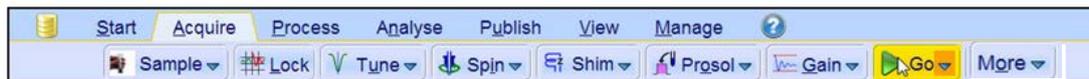
- Process and phase correct the spectrum.
- Phase the left side signal negative and the right side signal positive!
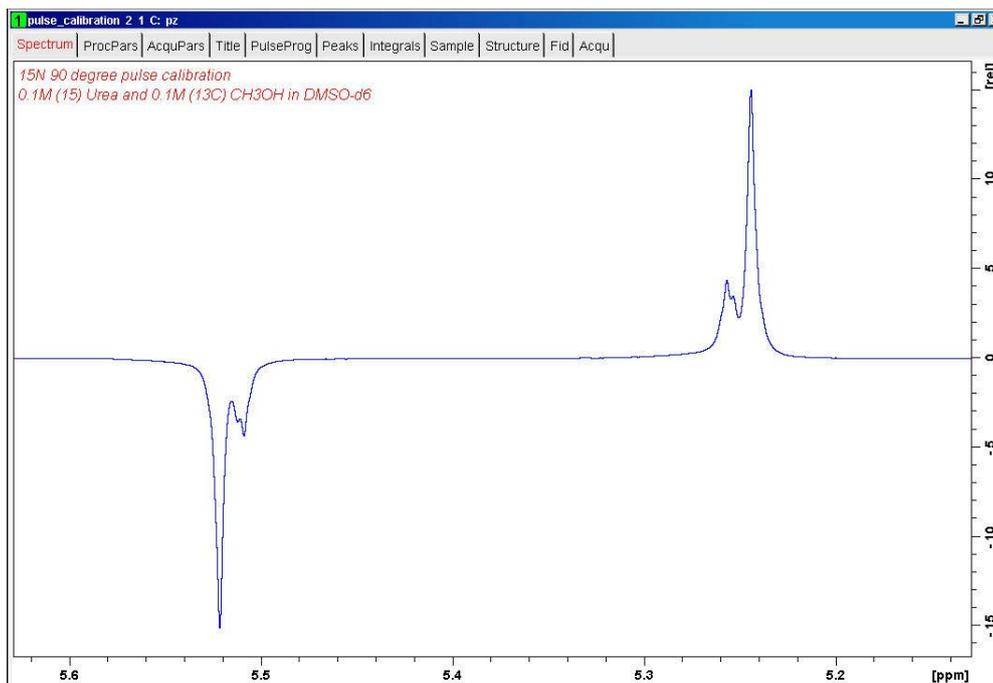
- Increase **p3** in increments of **1** or **2 µs**, execute **zg** followed by the command **efp** until the signals go through a null or a phase change. This will be the ^{15}N 90^0 decoupler pulse.

Pulse Calibration
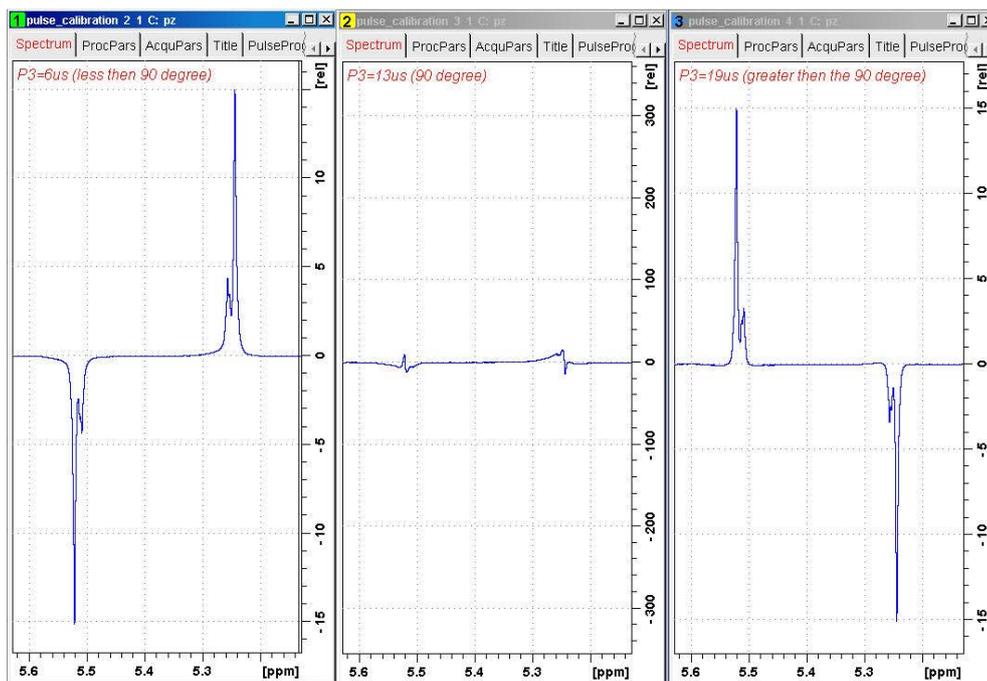
9 ¹H Homonuclear Decoupling

9.1 Sample

30mg Menthyl Anthranilate in DMSO-d6
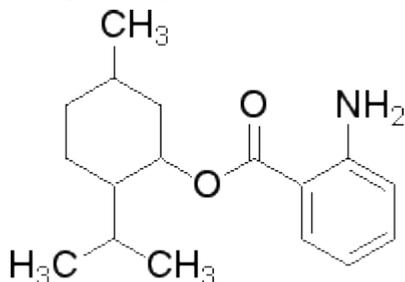
9.2 ¹H Homonuclear Decoupling Experiment

9.2.1 Introduction

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

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

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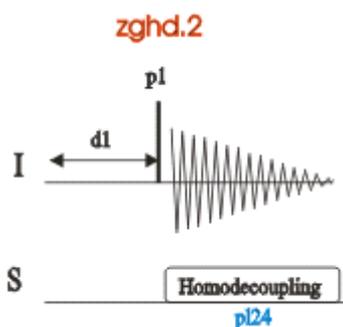
9.2.2   Preparation Experiment

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
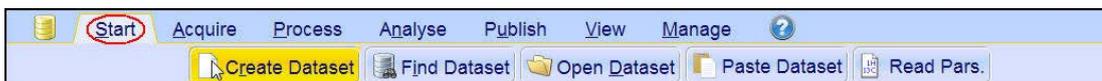
- In the New Dataset window, enter or select:

  NAME = homodec

  EXPNO = **1**

  PROCNO = **1**

  Experiment: select **PROHOMODEC**

  Set Solvent: select **DMSO**

DIR

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

Title

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

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

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

For the following steps, use the Workflow button bar.
- Click **Sample** and eject the sample, if there is one inserted, and insert the new sample.
- Click **Lock** and select **DMSO** solvent.
- To tune the probe, click **Tune**.
- Click **Spin** and select **Turn sample rotation off**.

---

i   Homonuclear experiments should be run non-spinning.

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

  PULPROG = **zg30**
- In the Dataset window, select the **Spectrum** tab.

9.2.3   Acquisition

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

9.2.4   Processing

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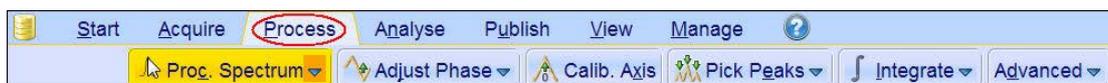
- On the Workflow button bar, click **Proc Spectrum**.

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

To configure the commands, click the drop-down arrow on the **Proc Spectrum** button and select **Configure Standard Processing**.
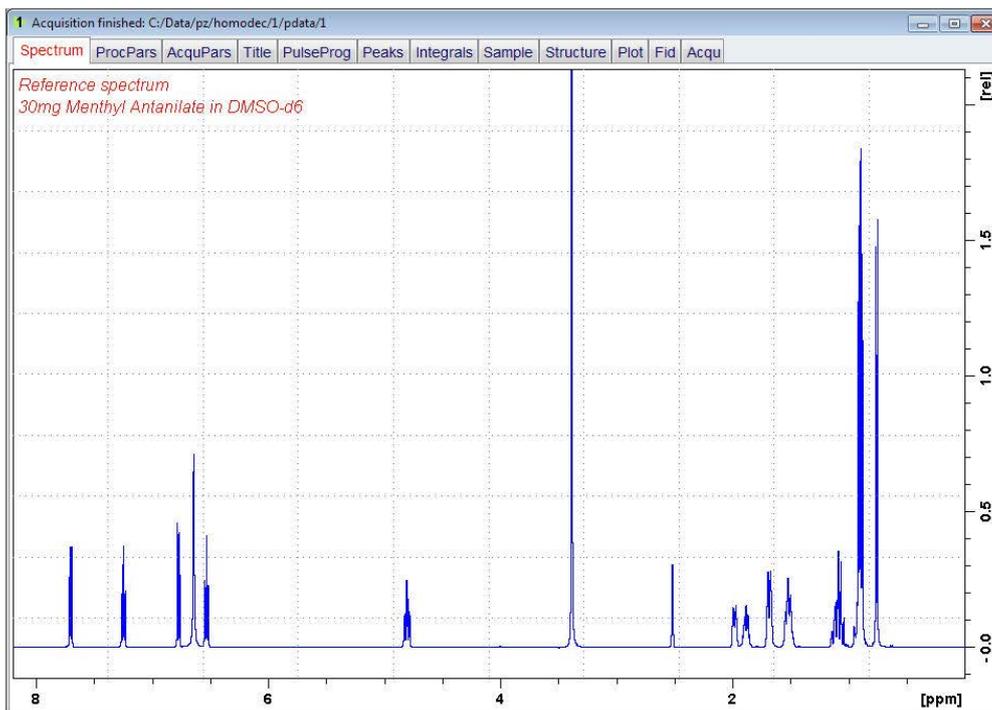
9.2.5 Setting up the Homonuclear Experiment Parameters

- At the command prompt, type:

  wrpa 2

  re 2

- Expand the multiplet at **7.7 ppm**.
- On the Tool bar, click **Set RF from cursor**.
- Move the cursor line into the center of the multiplet.
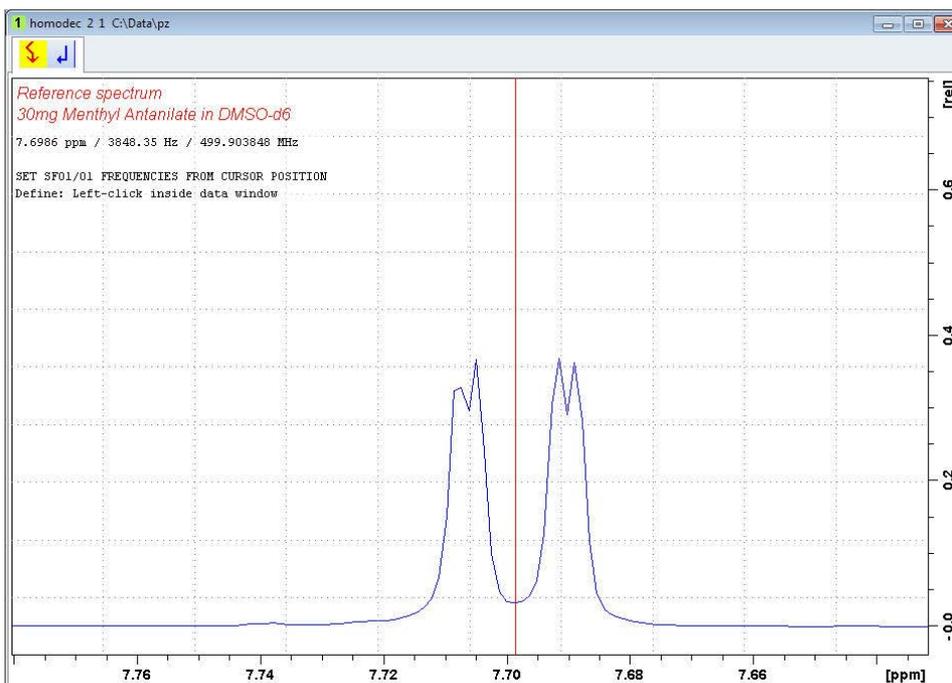
- Use the mouse and click to set the frequency.
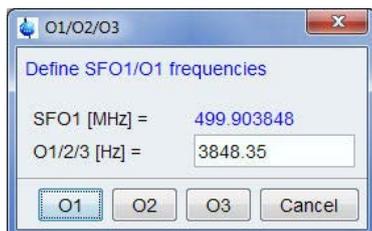
- In the O1/O2/O3 window, click **O2**.
- In the Dataset window, select the **AcquPars** tab.

- Make the following change:
  PULPROG = **zghd.2**
- In the Dataset window, select the **Title** tab.

- Change the title to:
  1H Homonuclear decoupling experiment irradiation at 7.7 ppm
  30 mg Menthyl Anthranilate in DMSO-d6
- In the Dataset window, select the **Spectrum** tab.
- On the menu bar, click **Acquire**.

9.2.6 Acquisition

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

9.2.7 Processing

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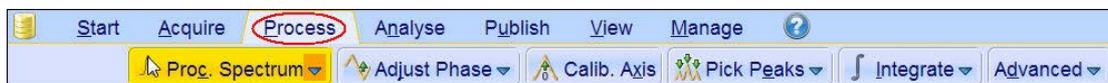
- On the Workflow button bar, click **Proc Spectrum**.

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

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

- Expand the region from 8 ppm to 6.2 ppm.
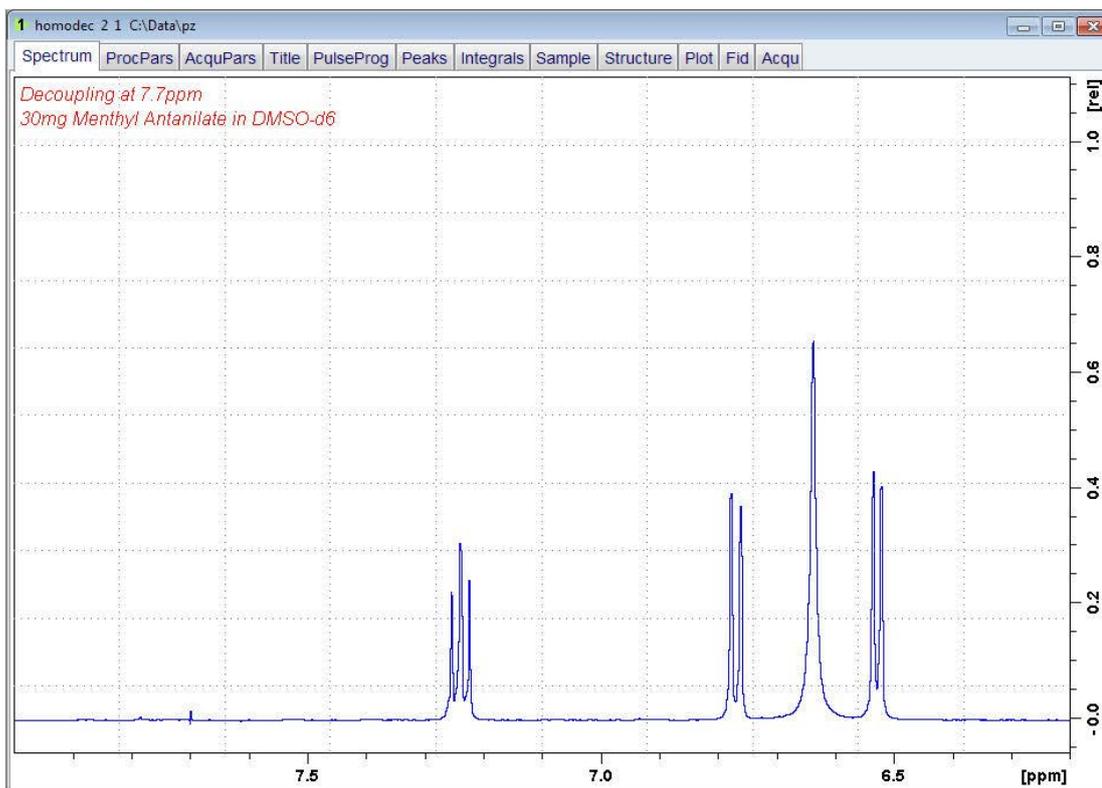
The multiplet at **6.4 ppm** should collapse from a triplet in to doublet. If the triplet is partially collapsed, increase the decoupling power pl24 and repeat the steps in chapter *Acquisition [▷ 159]* and *Processing [▷ 159]*.

NOTICE

Material Damage Due to Excessive Power

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

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

9.2.8   Plotting Two Spectra on the Same Page

- Display the decoupled spectrum.

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

- Drag the Reference spectrum (1D proton) into the spectral window.
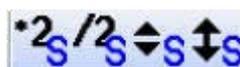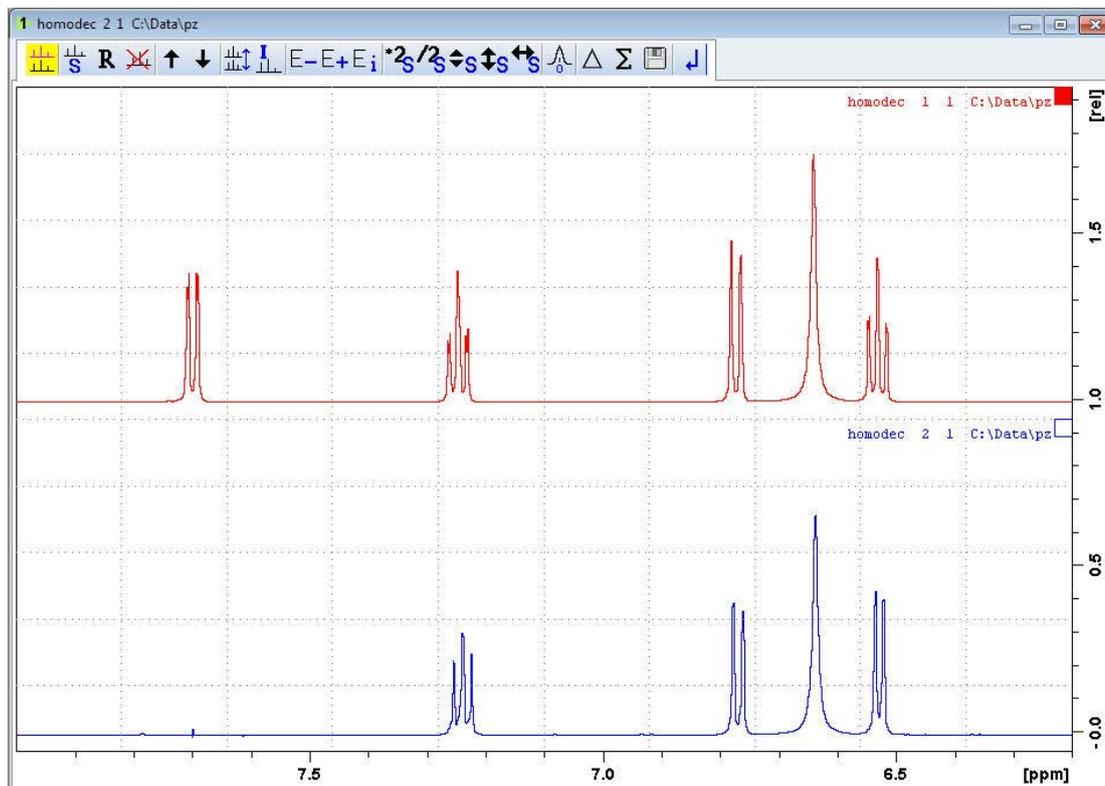
- Adjust the spectra. For best fit use the tools:

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

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

¹H Homonuclear Decoupling

10   Proton DOSY Experiment

10.1   Introduction

The **DOSY** (**D**iffusion-**O**rdered **S**pectroscop**y**) **experiment** provides accurate, noninvasive, molecular diffusion measurements on biofluids, complex chemical mixtures and multi component solutions. In DOSY spectra, chemical shift is along the detected F2 axis and diffusion coefficient is along the other F1 axis.

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

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

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

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

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

10.2   Sample

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

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

10.3 DOSY Experiment

10.3.1 Pulse Programs

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

The figure below is a 1D version of the pulse program and is used to optimize parameters, see the chapter *Parameter Setup [▷ 167]* and.
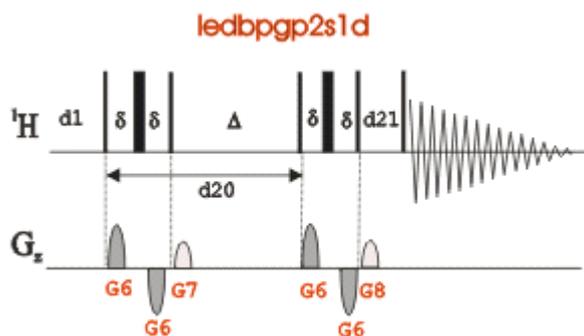
The pulsprogram in the figure below is used for the DOSY experiment, see the chapter *Running the Experiment [▷ 172]*. The difference between these 2 pulse sequences is that the one shown in the figure below is a pseudo 2D sequence, and includes the code to automatically increment the gradient strength.
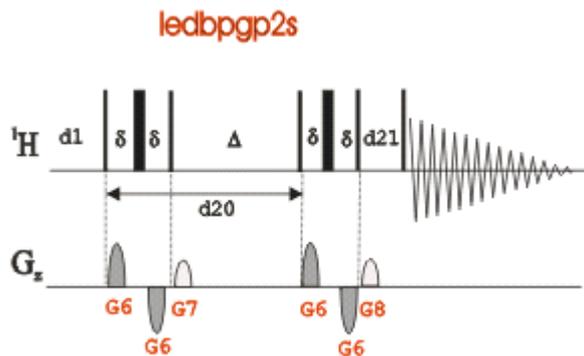
> **i** To run this experiment the instrument has to be equipped with the hardware to run gradient experiments.

10.3.2 Preparation Experiment

- On the menu bar, click **Start** and on the Workflow button bar, click **Create Dataset**.
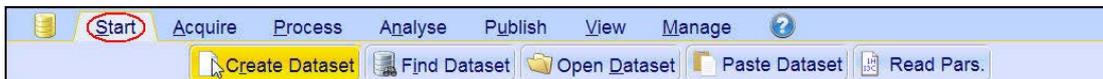
- In the New Dataset window, enter or select:

NAME = **DOSY_exp**

EXPNO = **1**

PROCNO = **1**

Experiment: select **PROTON**

Set Solvent: select **DMSO**

DIR

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

Title

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

Proton DOSY Experiment

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

For the following steps, use the Workflow button bar.

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

> **i** DOSY experiments should be run non-spinning.

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

10.3.3   Acquisition

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

10.3.4   Processing

- Process and phase correct the spectrum.

10.3.5   Limit Settings

> **i** Changing the sweep width to a smaller value increases the resolution.

- At the command prompt, type:

  wrpa 2
  re 2
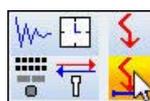
- Expand the spectrum from **9 ppm** to **0 ppm**.
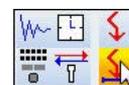
- On the toolbar, click **Set sw to current region** and **SFO1 to center of region**.

• Click **Close**.

New setting of SW, SFO1 from current region

SW = 10.0022 ppm
SWH = 4000.000 Hz
O1 = 1999.50 Hz / 4.9999 ppm
SFO1 = 399.9119995 MHz

Close
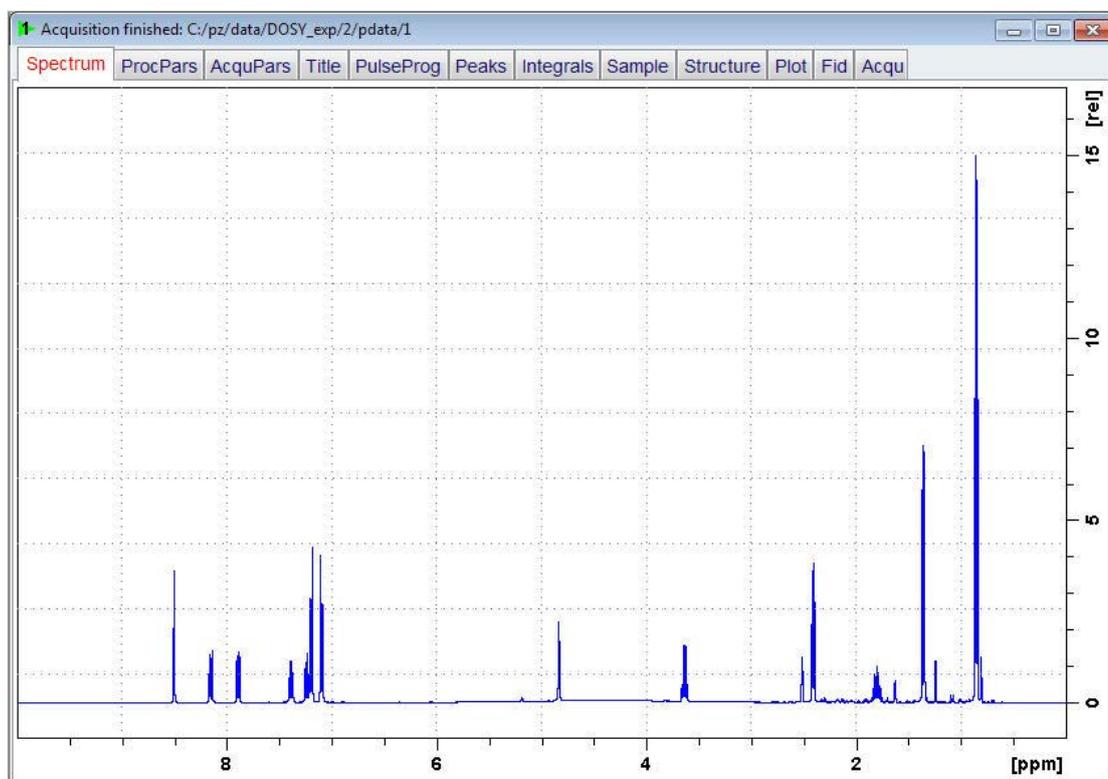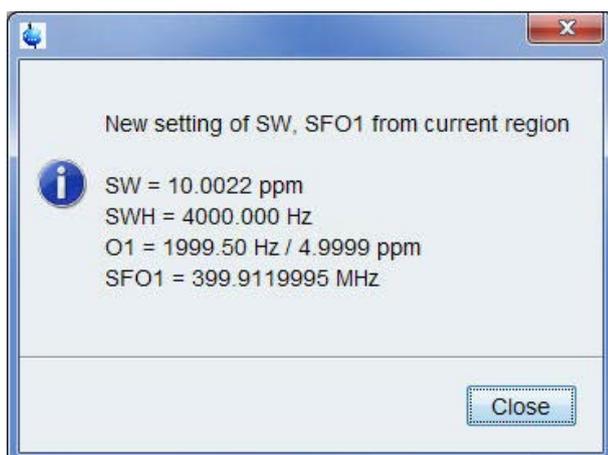
10.3.6　Parameter Setup

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

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

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

• Click **Show pulse program parameters**.

Proton DOSY Experiment

- Make the following changes:

  PULPROG = **ledbpgp2s1d**

  D20[s] = **0.1**

  D21[s] = **0.005**

  GPNAM6 = **SMSQ10.100**

  GPNAM7 = **SMSQ10.100**

  GPNAM8= **SMSQ10.100**

  GPZ6[%] = **2**

  GPZ7[%] = **-17.13**

  GPZ8[%] = **-13.17**

  P30[us] = **1400**

- To adjust the receiver gain, click **Gain**.
- To start the acquisition, click **Go**.
- Process and phase correct the spectrum.
- Expand the spectrum to display all peaks.
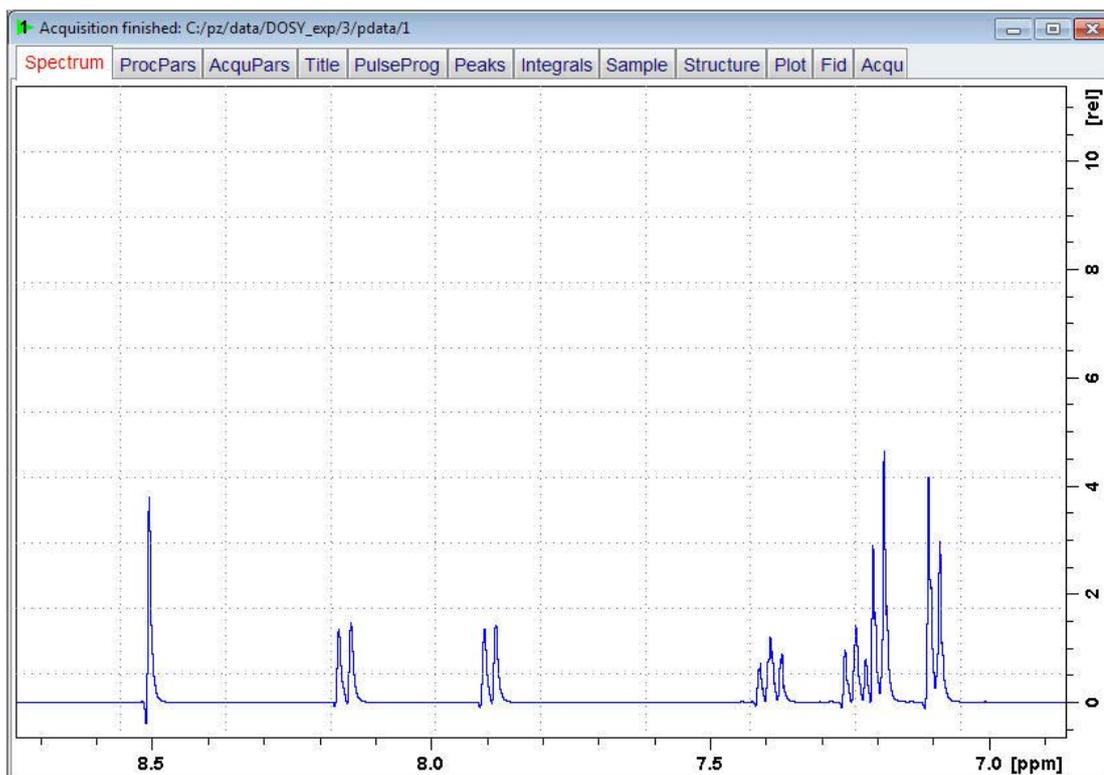
> ℹ The asymmetry of the peaks is an artifact which is caused by non-optimal lock settings. The problem can be fixed by applying a loop adjust of the lock especially that of the lock phase.

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

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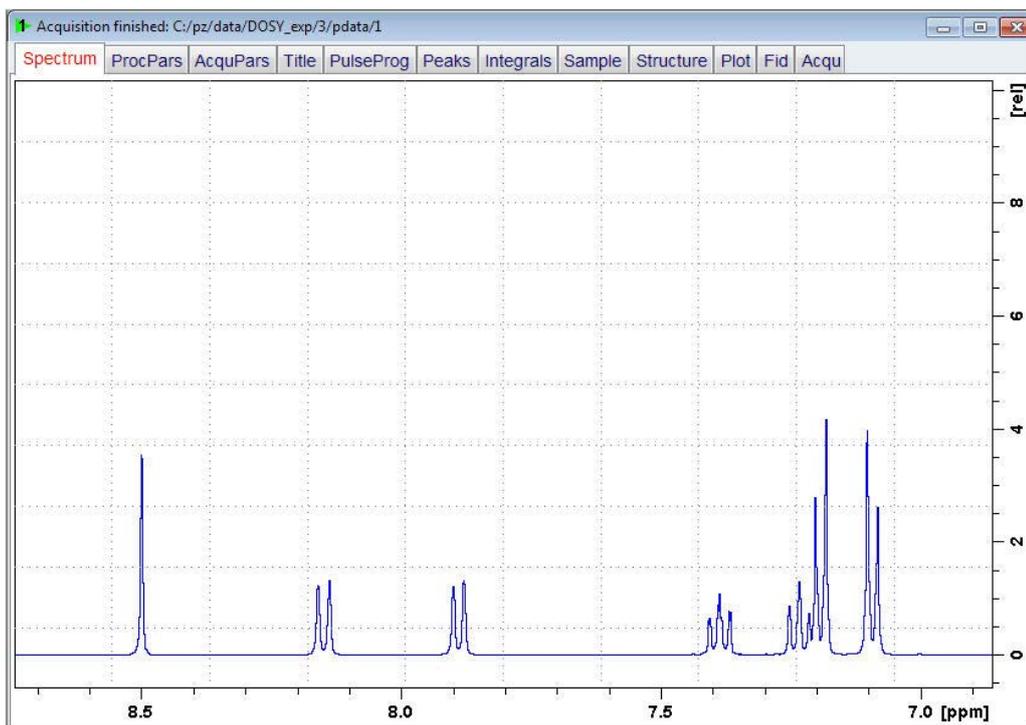
- Display the full spectrum.

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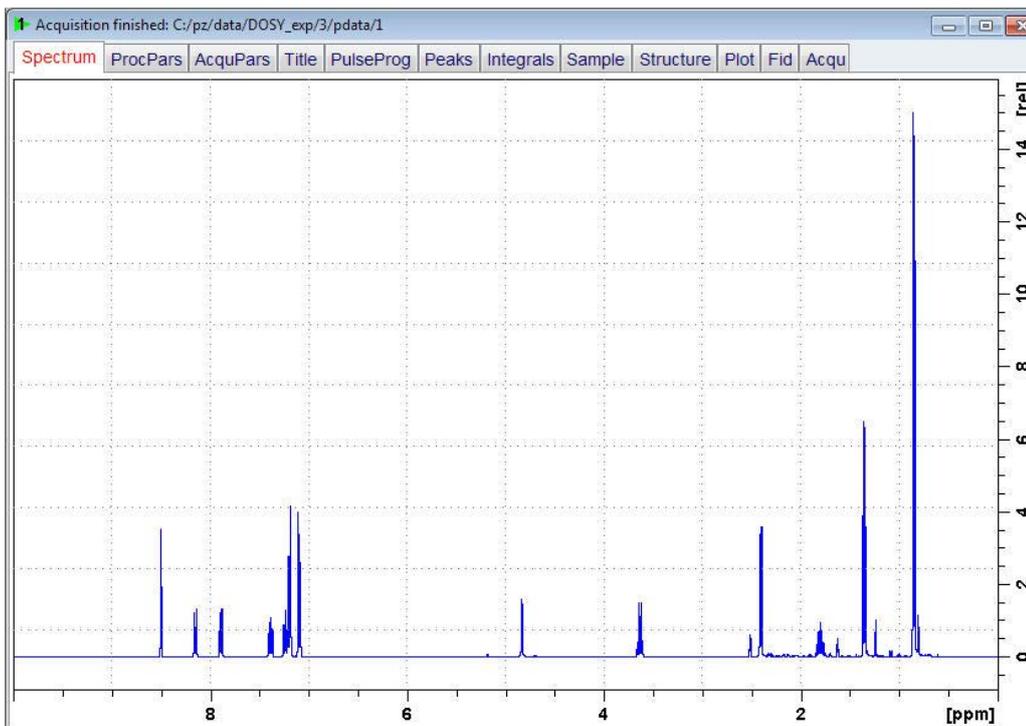
Proton DOSY Experiment

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

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

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

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

- At the command prompt, type **iexpno**.
- In the Dataset window, select the **AcquPars** tab.
- Click **Show pulse program parameters**.
- Make the following change:
  GPZ6[%] = **95**
- To start the acquisition, click **Go**.
- Process and phase correct the spectrum.
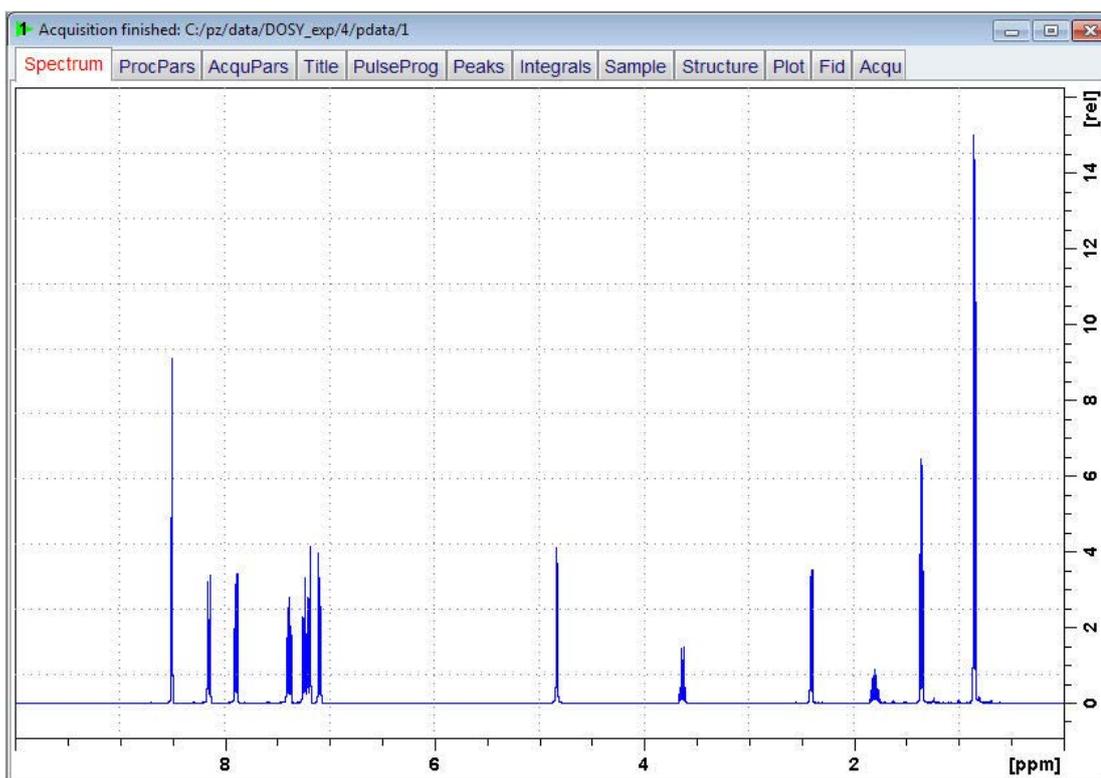
If there are no signals present, then p30 and/or d20 need to be reduced.

- On the toolbar, click **Multiple display**.
- Drag the previous experiment into the multiple display window (in this example it is experiment # 3) or type **re 3**.
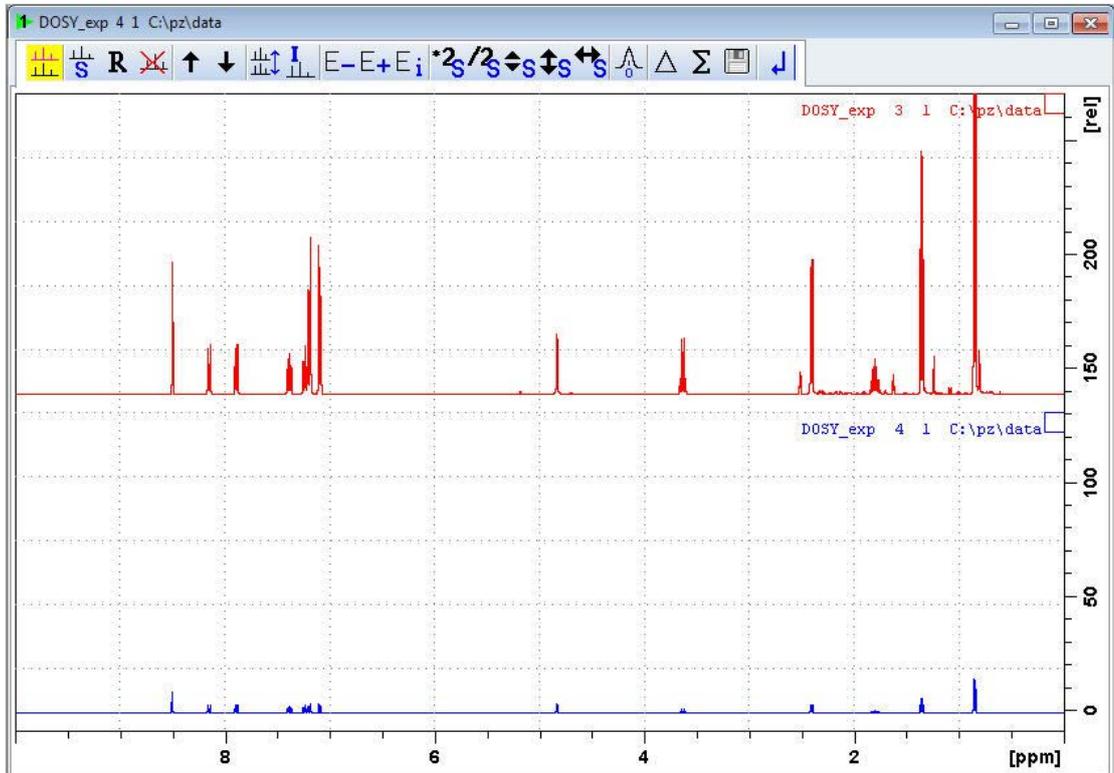
ℹ️ As described above, there need to be signal remaining in the 2nd experiment, but the intensity difference of the two spectra should be a factor of 90-95%. If neither of these are true, it is necessary to change p30, and d20 accordingly.

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

10.3.7   Running the Experiment

- At the command prompt, type **iexpno**.
- In the Dataset window, select the **AcquPars** tab.
- Make the following change:

    PULPROG = **ledbpgp2s**
- Click **Change acquisition dimension of current dataset**.

- In the parmod window, select **2D** and click **OK**.
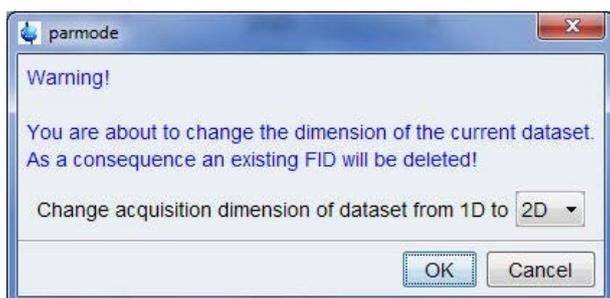
- Make the following changes:

    TD[F1] = **25**

    FnMODE = **QF**

- At the command prompt, type **dosy**.
- In the dosy window, click **Close**.
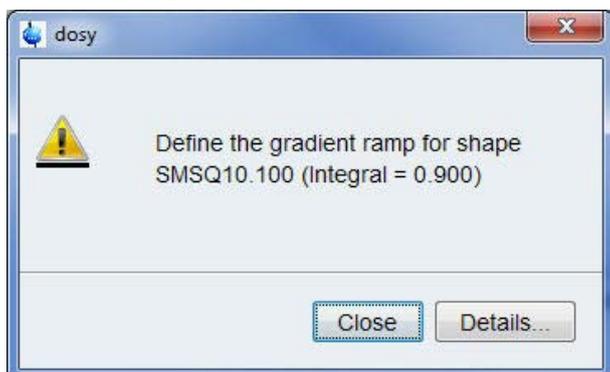
- Enter **2** for first gradient amplitude and click **OK**.
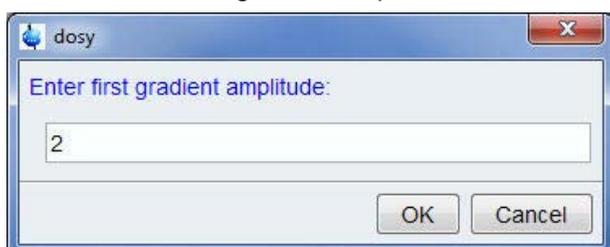
- In the dosy window, enter **95** for final gradient amplitude and click **OK**.
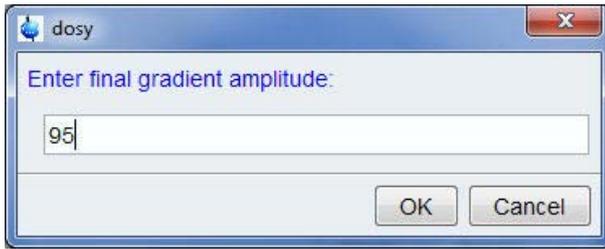
- In the dosy window, enter **25** for the number of points and click **OK**.
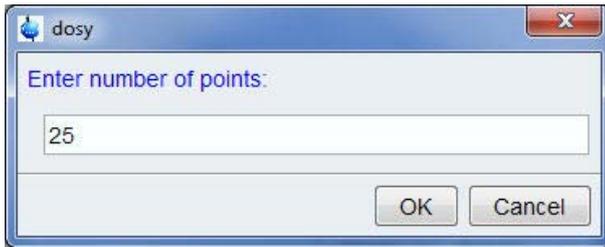
- In the dosy window, enter **I** for the ramp type and click **OK**.
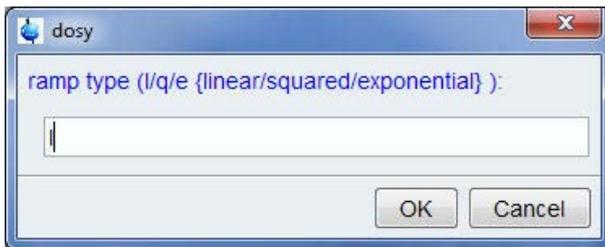
- To start the acquisition, click **OK**.
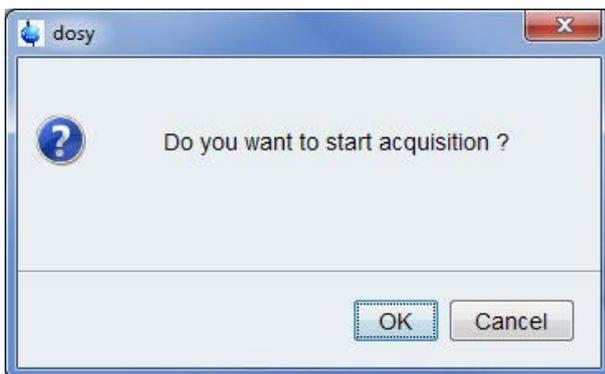
10.3.8   Processing

- In the Dataset window, select the **Fid** tab.
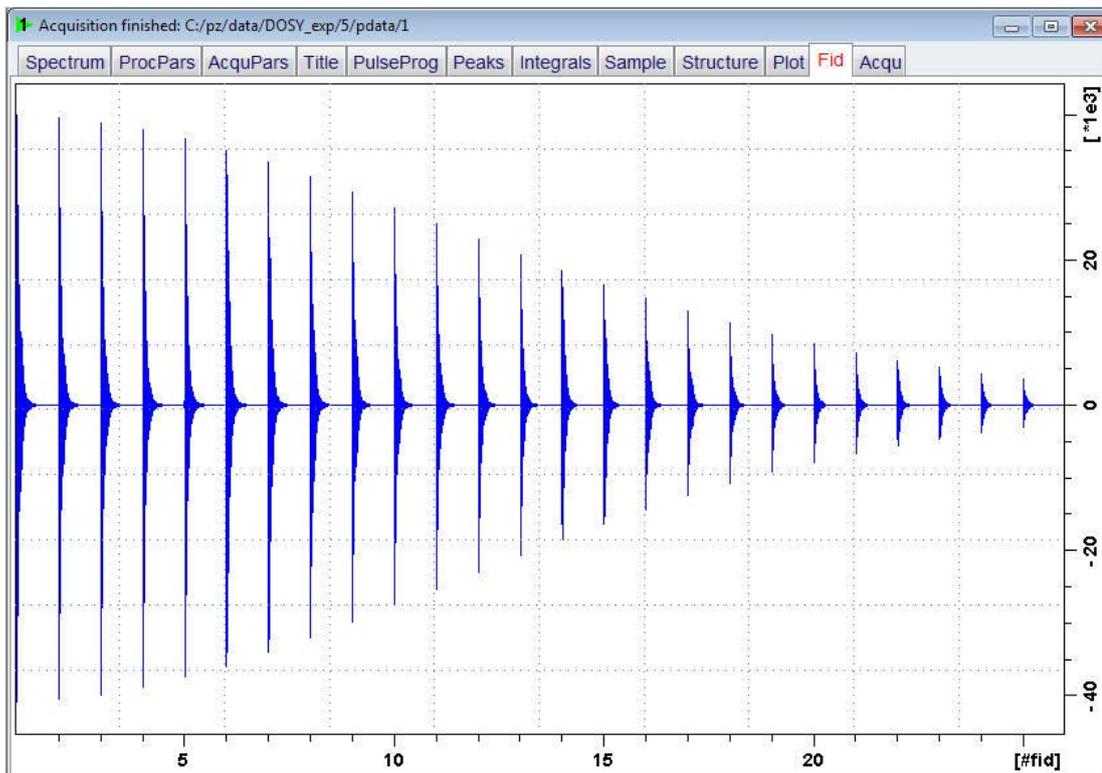
> **i** This step is only used to illustrate the DOSY experiment as a decay function.

- In the Dataset window, select the **ProcPars** tab.
- Make the following changes:

    SI [F1] = **256**

    PH_mod [F1] = **no**

    PH_mod [F2] = **pk**

- At the command prompt, type **rser 1** to read in the first serial file of the 2D experiment.
- At the command prompt, type **em** to apply the window function.
- At the command prompt, type **ft**.
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Adjust Phase**.
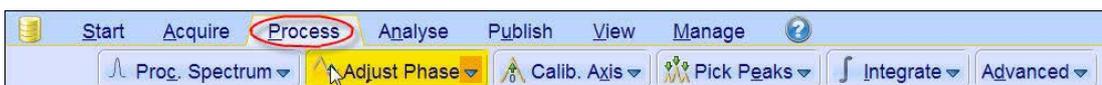
- Process and phase correct the spectrum.

- On the Adjust Phase toolbar, click **Save for nD spectrum**.

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

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

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

ℹ This command transfers experimental parameters into the values used for fitting the data.
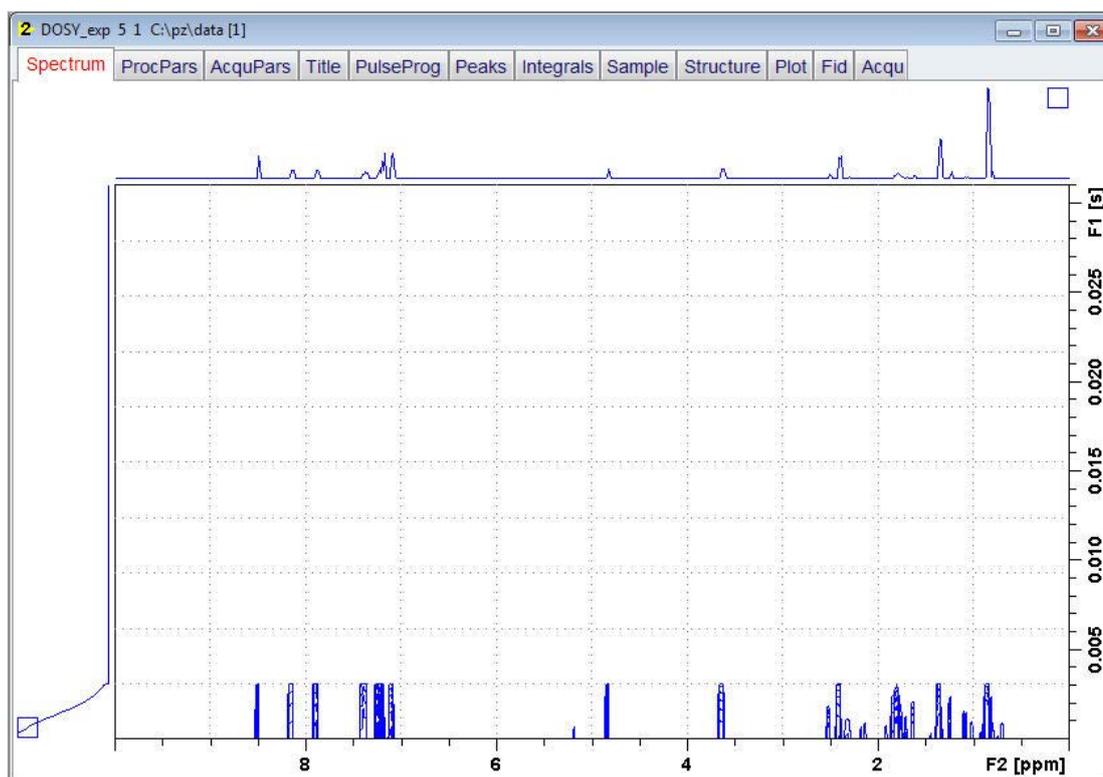
10.3.9 Calculating the Diffusion Coefficient

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

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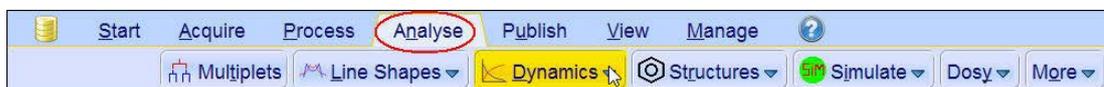
- In the list, select **T1/T2**.

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

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

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

- In the Extract a row from 2d data window, click **Spectrum**.
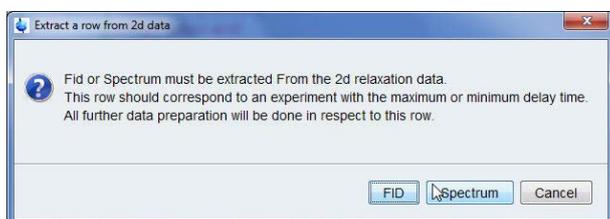
- In the field Slice Number, enter **1** and in the message window click **OK**.
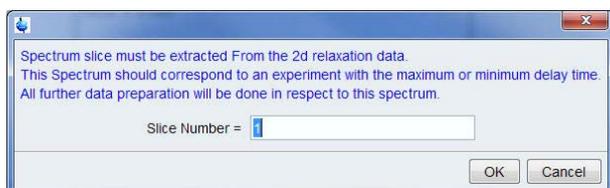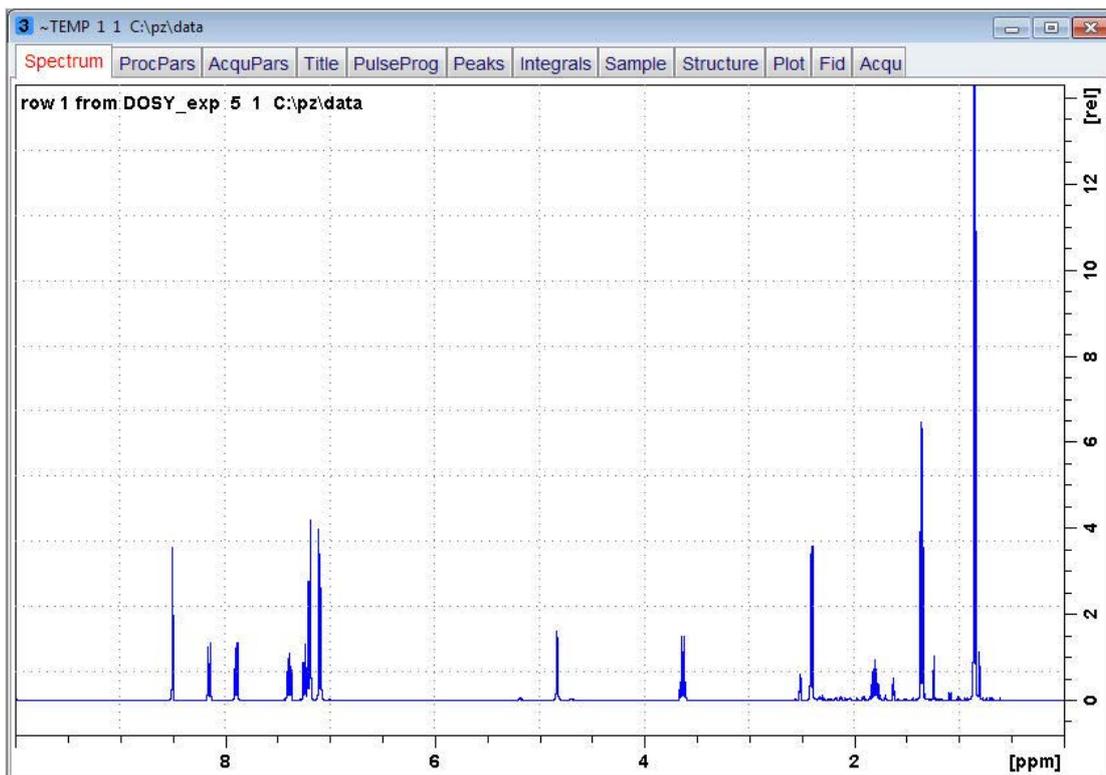
- On the Workflow button bar, click **Peaks/Ranges**.

- In the Define Peaks and/or Integrals window, click **Manual Integration**.
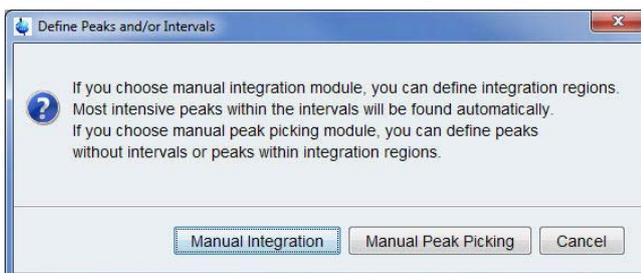
- In the Prepare relaxation data window, click **OK**.
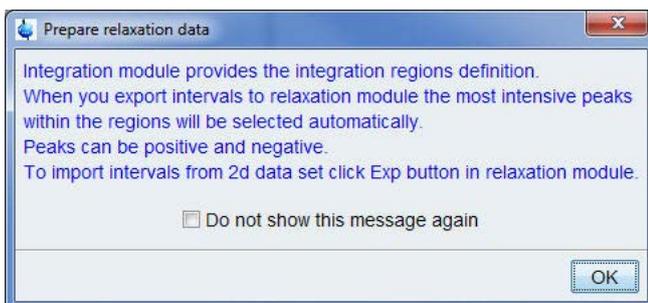
- Define the regions by drawing an integral over the peaks of interest.
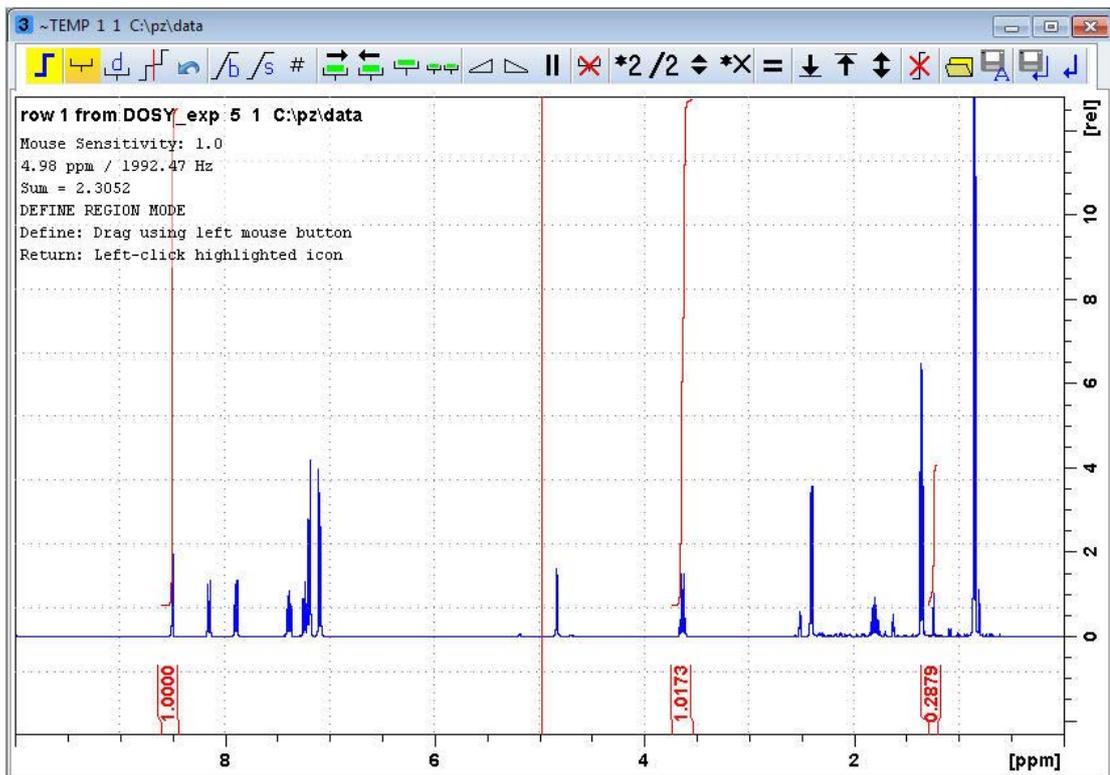
- On the Integration toolbar, click **Save region as**.
- From the drop-down list, select **Export Region To Relaxation Module**.

Save Regions To 'intrng'
Save Regions To 'reg'
Export integration regions
Export Regions To Relaxation Module and .ret.
Save & Show List
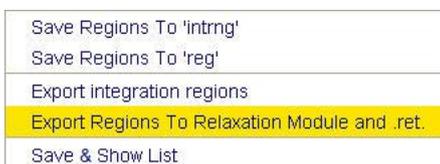
- On the Workflow button bar, click **Relaxation**.

• In the group box Fitting type, select **Area**.

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

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

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

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

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

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

Brief Report

Region 1 from 8.610 to 8.446 ppm
Diff Con.   = 1.585e-010 m2/s
Region 2 from 3.740 to 3.539 ppm
Diff Con.   = 2.415e-010 m2/s
Region 3 from 1.293 to 1.205 ppm
Diff Con.   = 3.853e-010 m2/s

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

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

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

10.3.10 Displaying the DOSY Plot

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

- On the Workflow button bar, click **Dosy**.
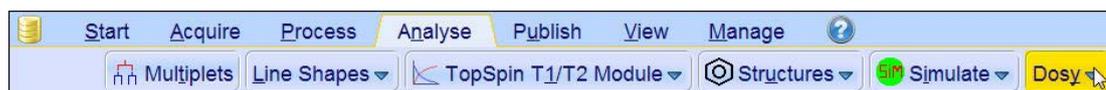
- From the drop-down list select **Setup Parameters**.
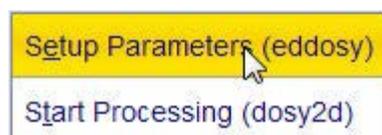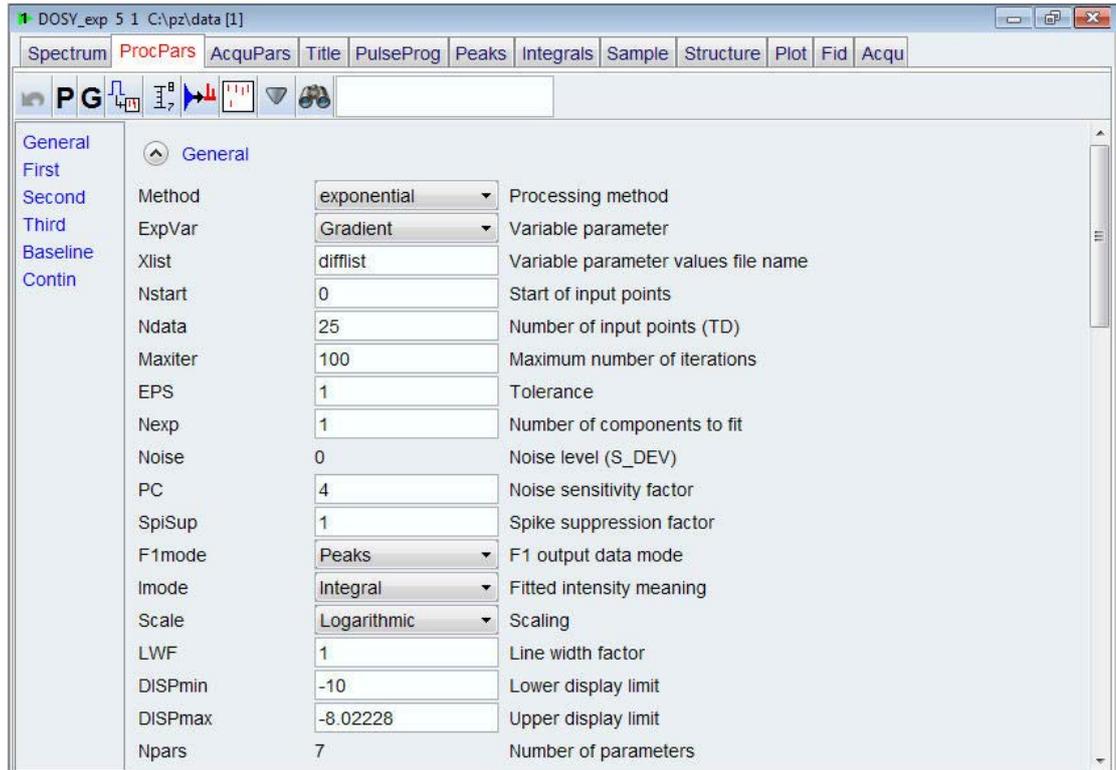
- Make the following change:

  Scale = **Logarithmic**

- On the toolbar, click **Start fitting**.
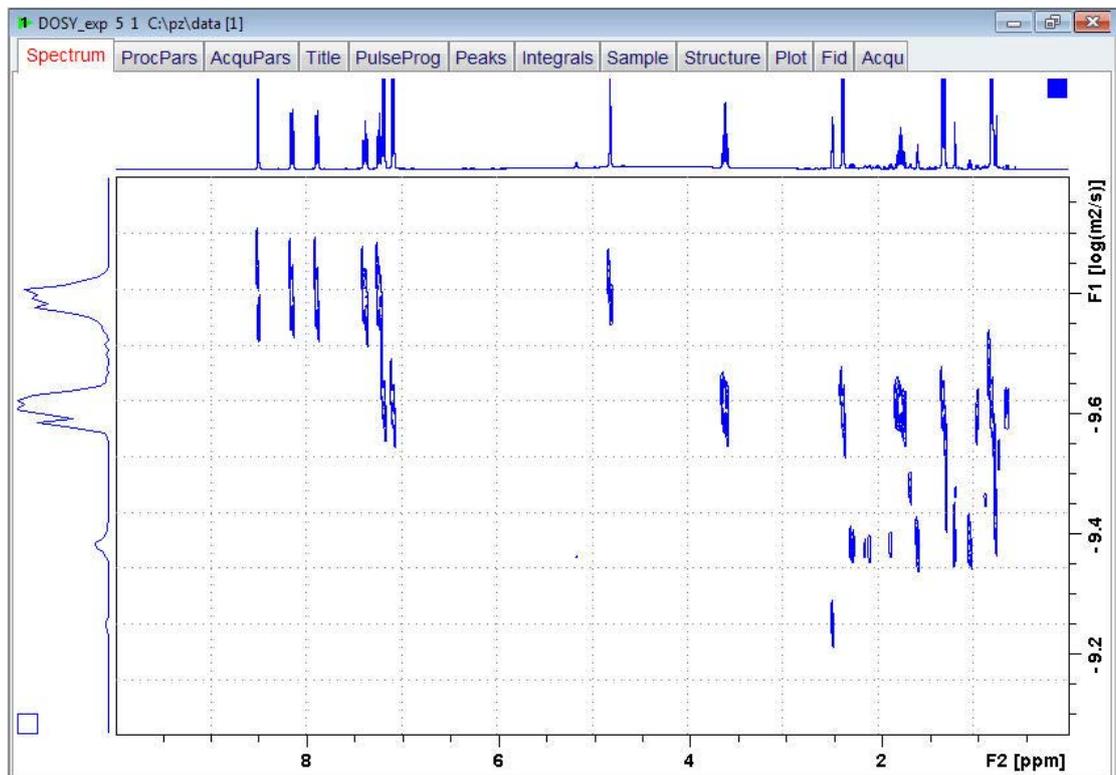
11 Multiplet Analysis

11.1 Introduction

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

11.2 Sample

100 mg 2, 3,-Dibromopropionic acid in CDCl$_3$

11.3 Multiplet Assignments

11.3.1 Preparation Experiment

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

11.3.2 Limit Settings

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

- At the command prompt, type:

  wrpa 2

  re 2

- Expand the spectrum from **10.6 ppm** to -**0.5 ppm**.

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

New setting of SW, SFO1 from current region

SW = 11.9911 ppm
SWH = 4795.396 Hz
O1 = 2199.50 Hz / 5.5000 ppm
SFO1 = 399.9121995 MHz

Close
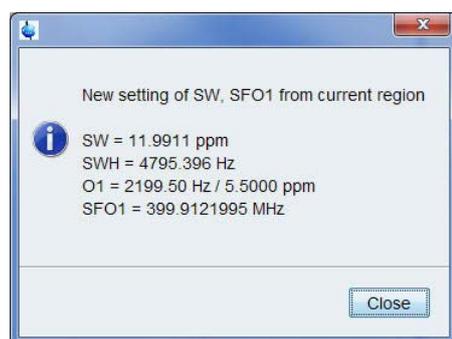
11.3.3 Acquisition

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

11.3.4   Processing

- On the Workflow button bar, click **Process**.
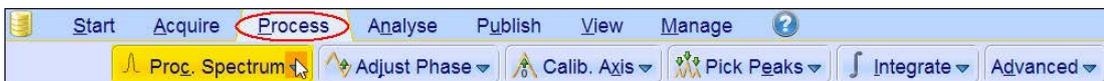
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- Select **Configure Standard Processing (proc1d)**.
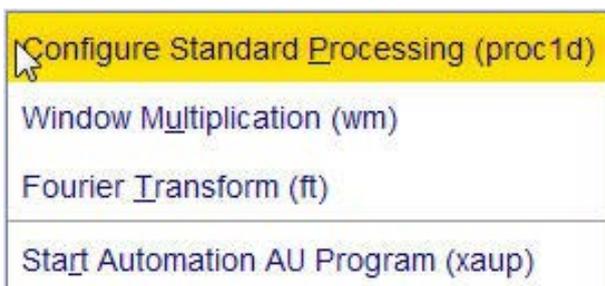
- Enable the following options:
  - Exponential Multiplay (em)
  - Auto - Phasing (apk)
  - Set Spectrum Reference (sref)
  - Auto Baseline Correction (abs)
- Change LB [Hz] = 0
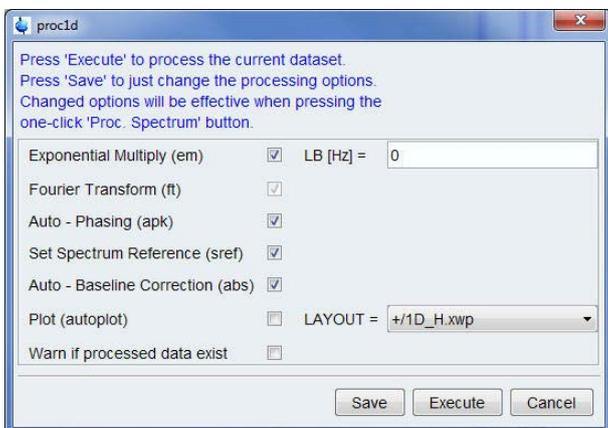
- In the proc1d window, click **Execute**.
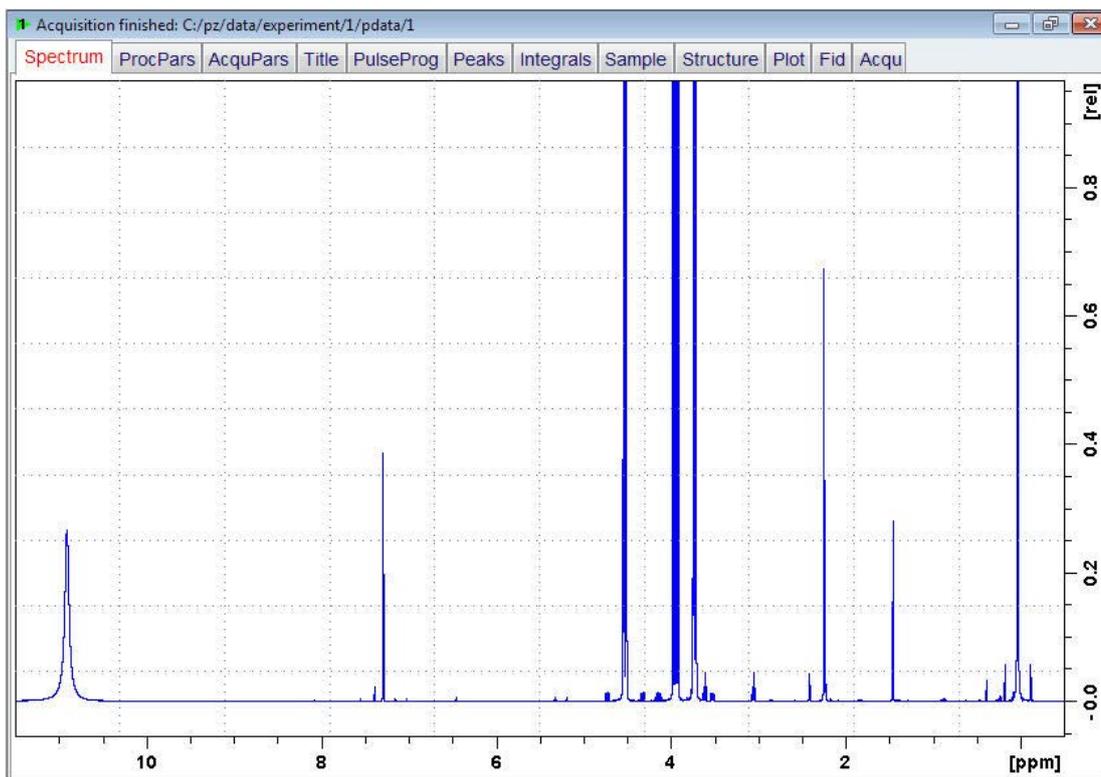
- On the toolbar, click **Exact Zoom**.

- In the exactzoom window enter the following parameters:

  From = **4.6**

  To = **3.6**

- In the exactzoom window, click **OK**.
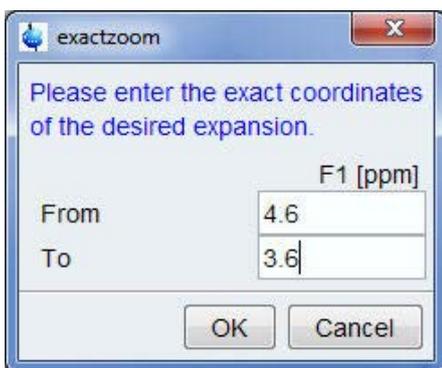
- Right-click the spectrum window and in the shortcut menu select **Save Display Region To**.

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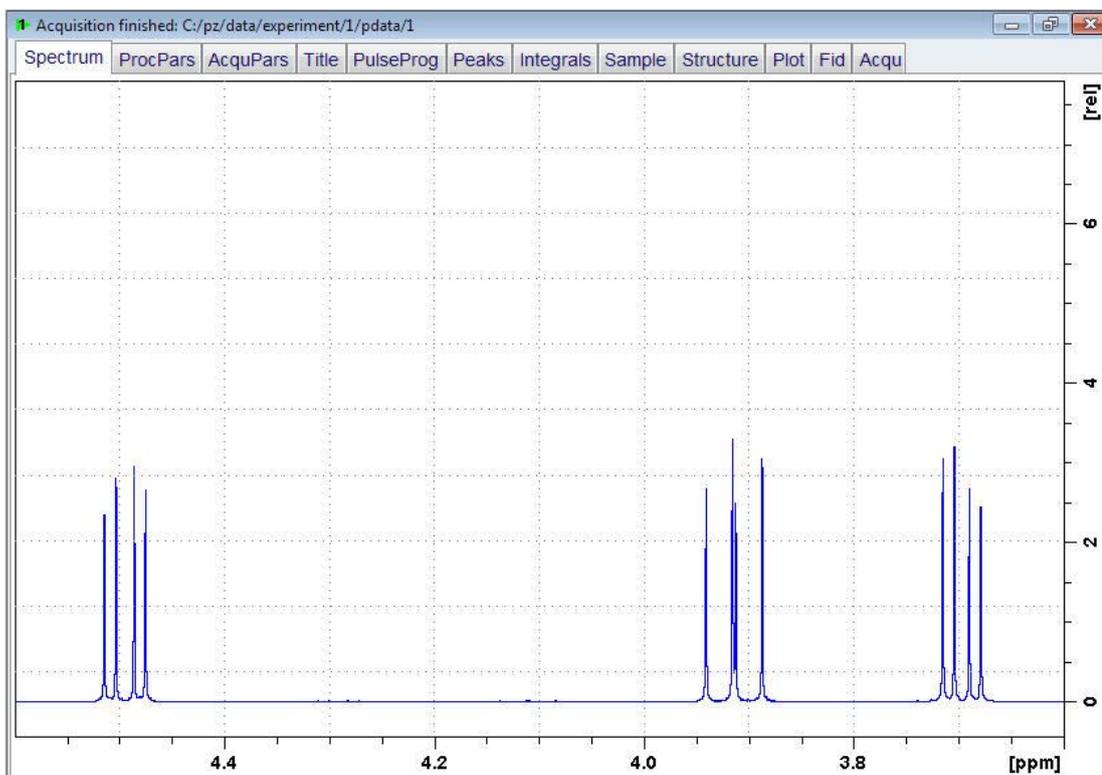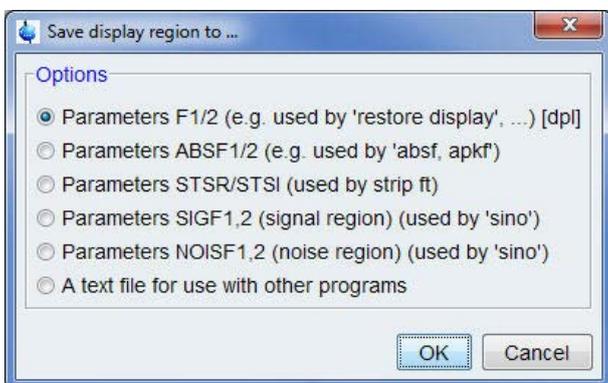
11.3.5   Peak Picking

- On the Workflow button bar, click **Peak Peaks**.
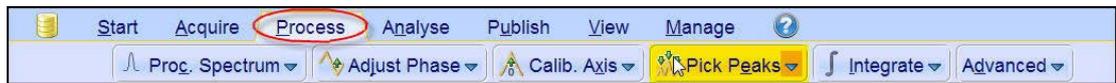
This enters the manual peak picking mode. The Dataset tabs are replaced by the Peak Picking Tool bar.
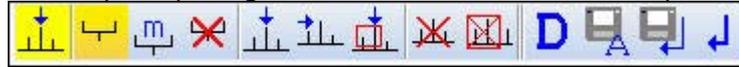
By default the **Define new peak picking range** button is enabled.

- Click and draw a rectangle over all multiplets up to 3.7 ppm.
- On the Peak Picking toolbar, click **Modify existing peak picking range** to manually adjust the minimum and maximum intensity levels.
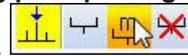
- Adjust the bottom line of the box to be above the baseline (Minimum intensity) and the top line above the highest peak of all multiplets (Maximum intensity).
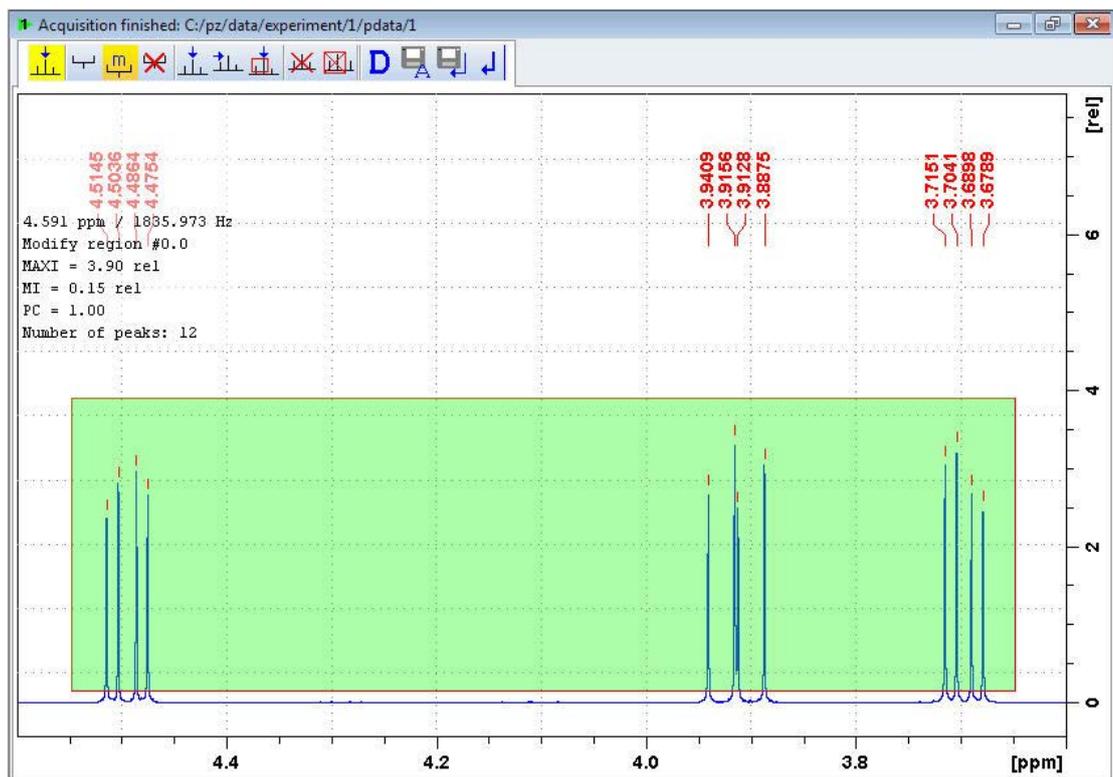
- On the Peak Picking toolbar, click **Return, save region** to store the peak values.

11.3.6 Assigning the Multiplets

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

This enters the multiplet analysis mode. The Dataset tabs bar is replaced by the Multiplet analysis button bar.
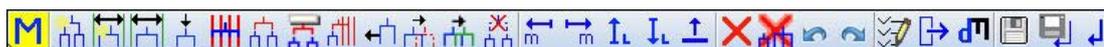
- Click **Define Multiplets Manually**. It should be highlighted in yellow.
- Place the cursor lines to the left of the first peak of the multiplet.
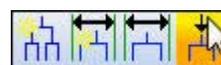
Multiplet Analysis

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

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

Step 2: The right cursor line will stop when it gets in to the center of the peak.
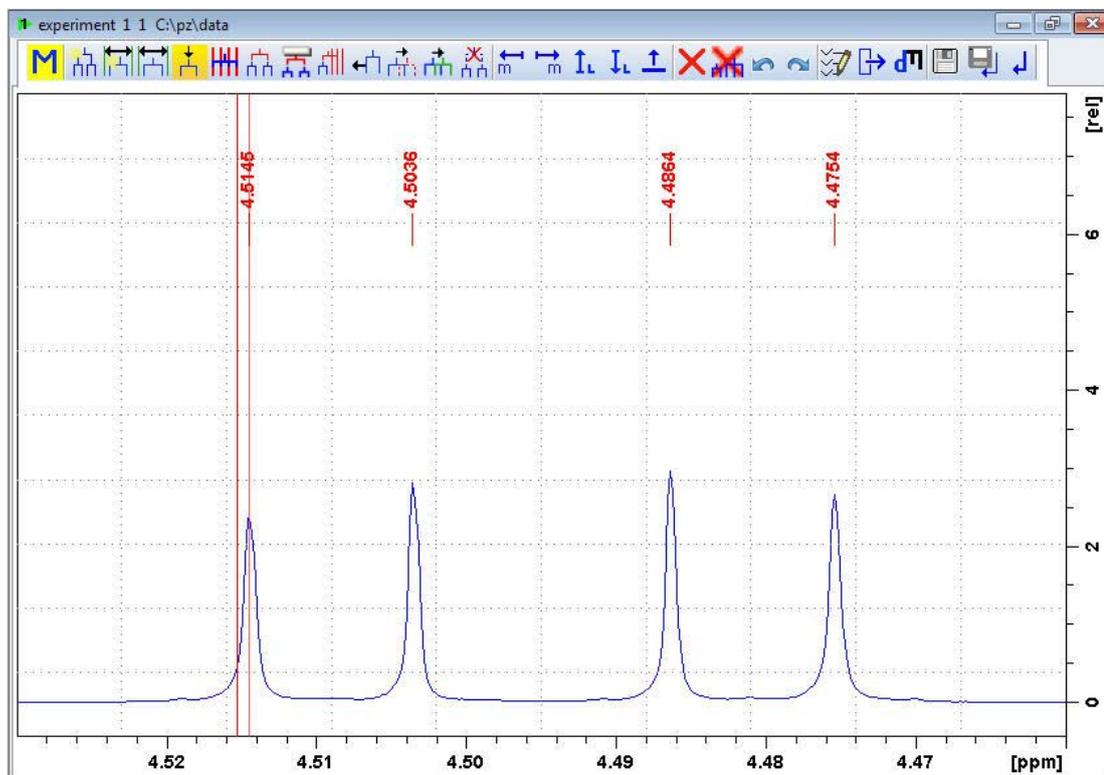
Step 3: Click the left mouse button.

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

Step 5: The right cursor line will stop when it gets in to the center of the peak.
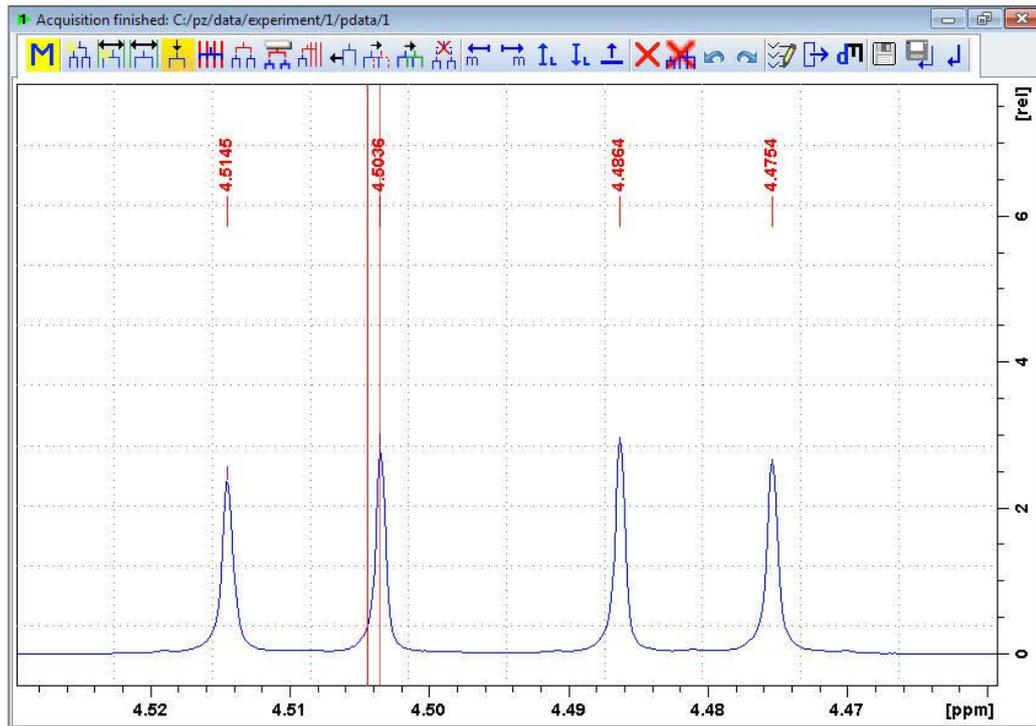
Step 6: Click the left mouse button.

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

Step 7: Move the cursor lines in to the center of the two marked peaks.
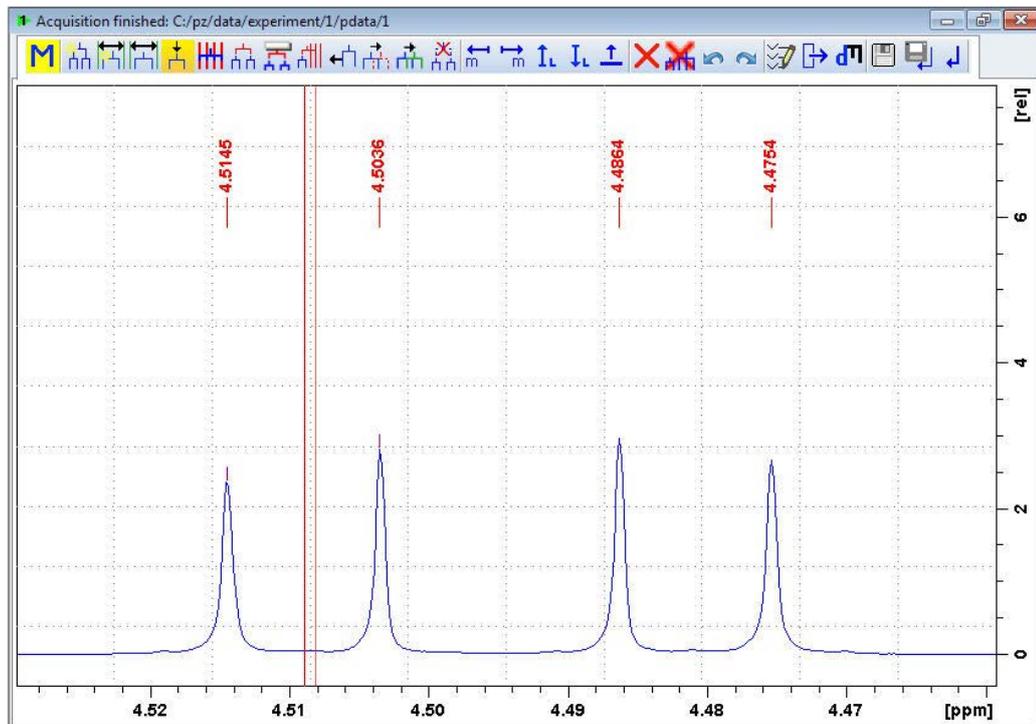
Step 8: Right-click to open the shortcut menu.

Define Multiplet
Finish Current Mode
Automatically Define Multiplet
Automatically Define Multiplet By Region
Define Multiplet By Region
Define Multiplet Manually
Define Multiplet By Free Grid
Couple Existing Multiplets
Define Multiplet By Coupled Grid
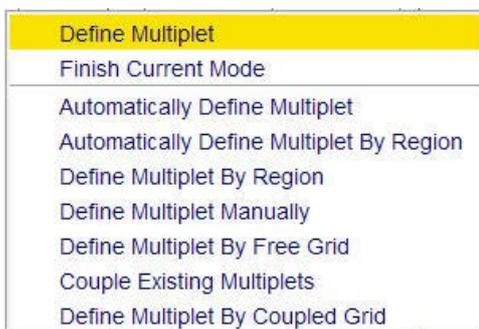
Step 9: Select **Define Multiplet**.
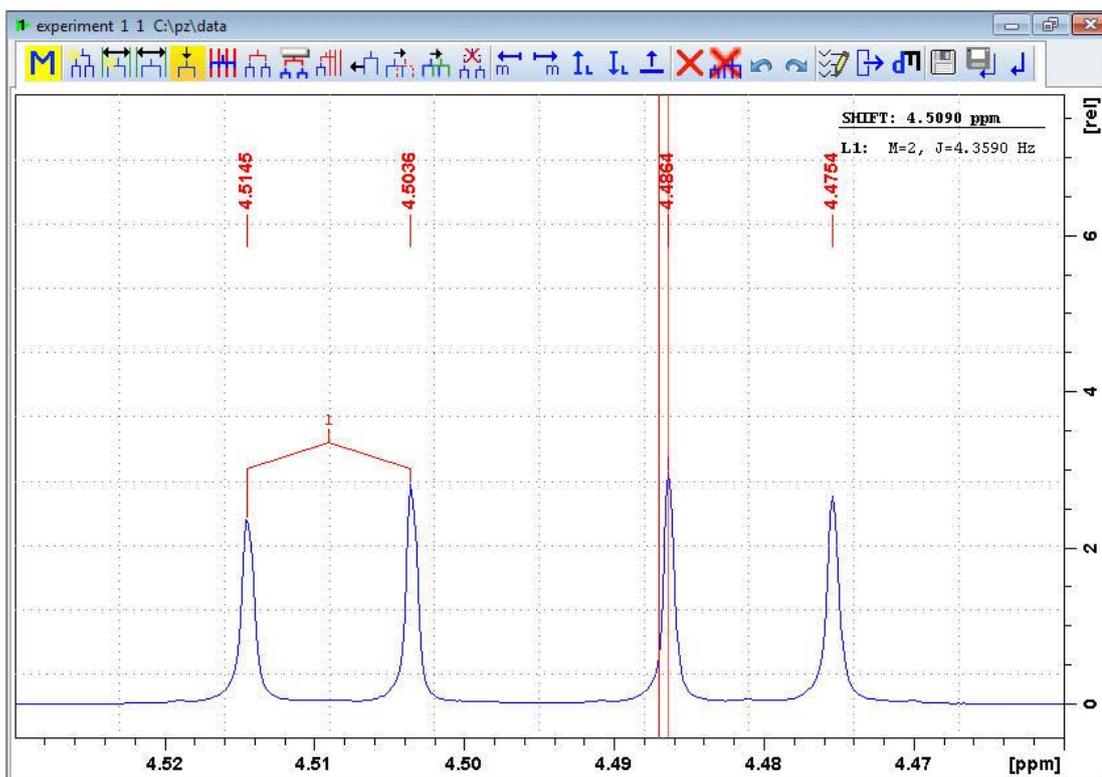
- Repeat steps 1 - 9 starting with the third peak and ending with the fourth peak.
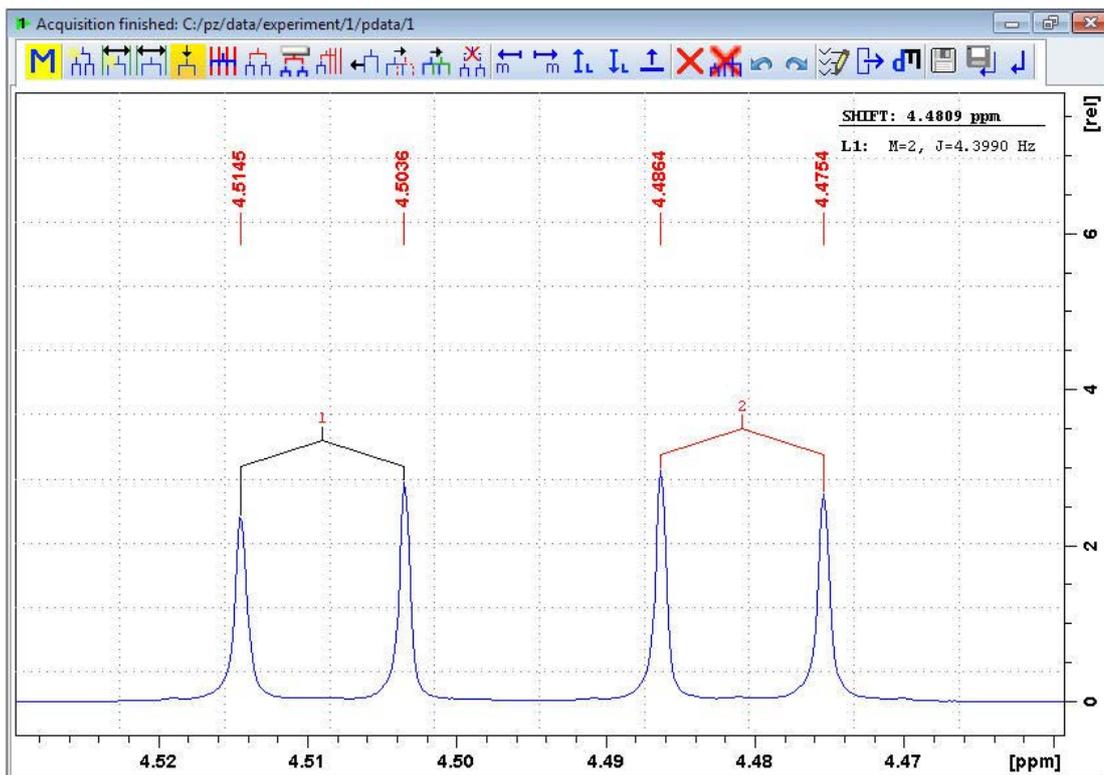
Step 10: Select the Couple Existing Multiplets button.

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

Step 12: Click the left mouse button.
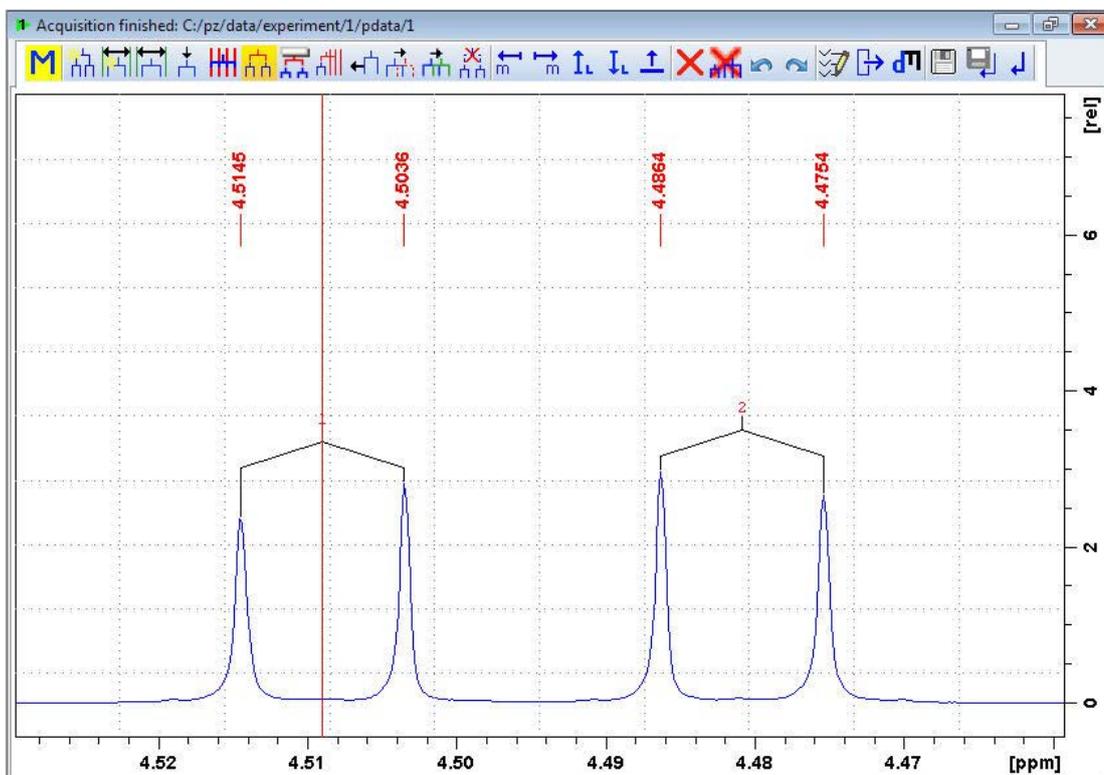
Multiplet Analysis

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

---

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

Step 14: Click the left mouse button.

Step 15: Move the cursor into the center of the displayed multiplet.
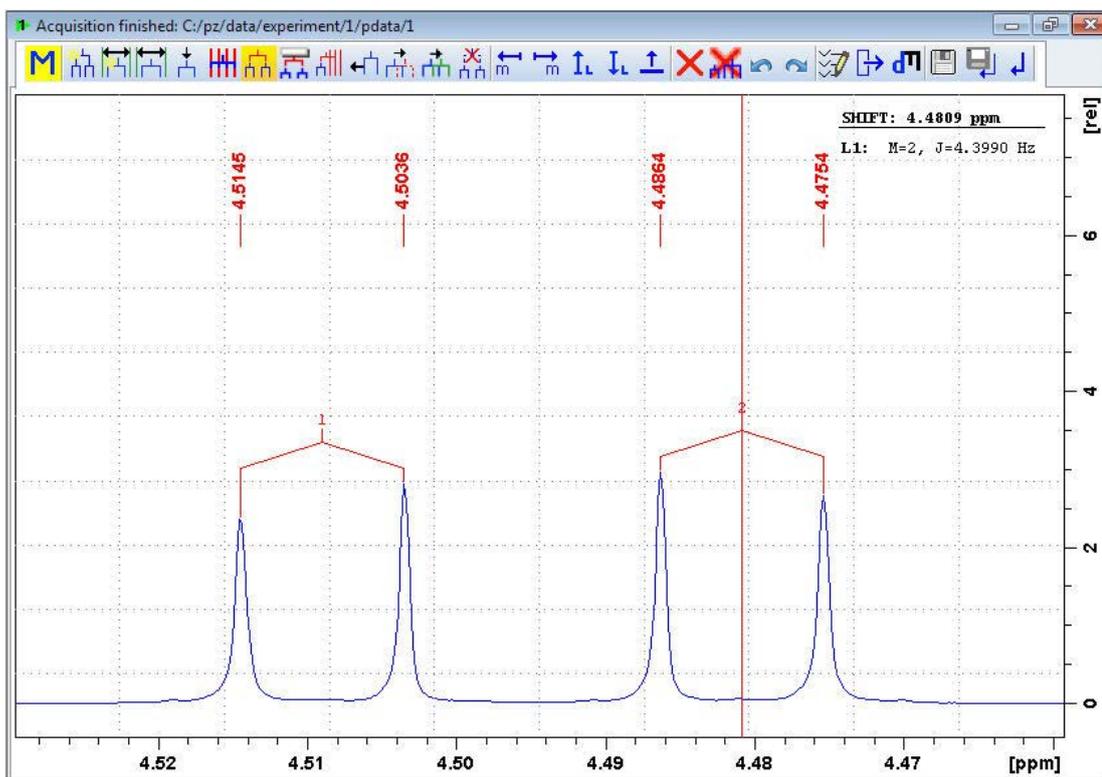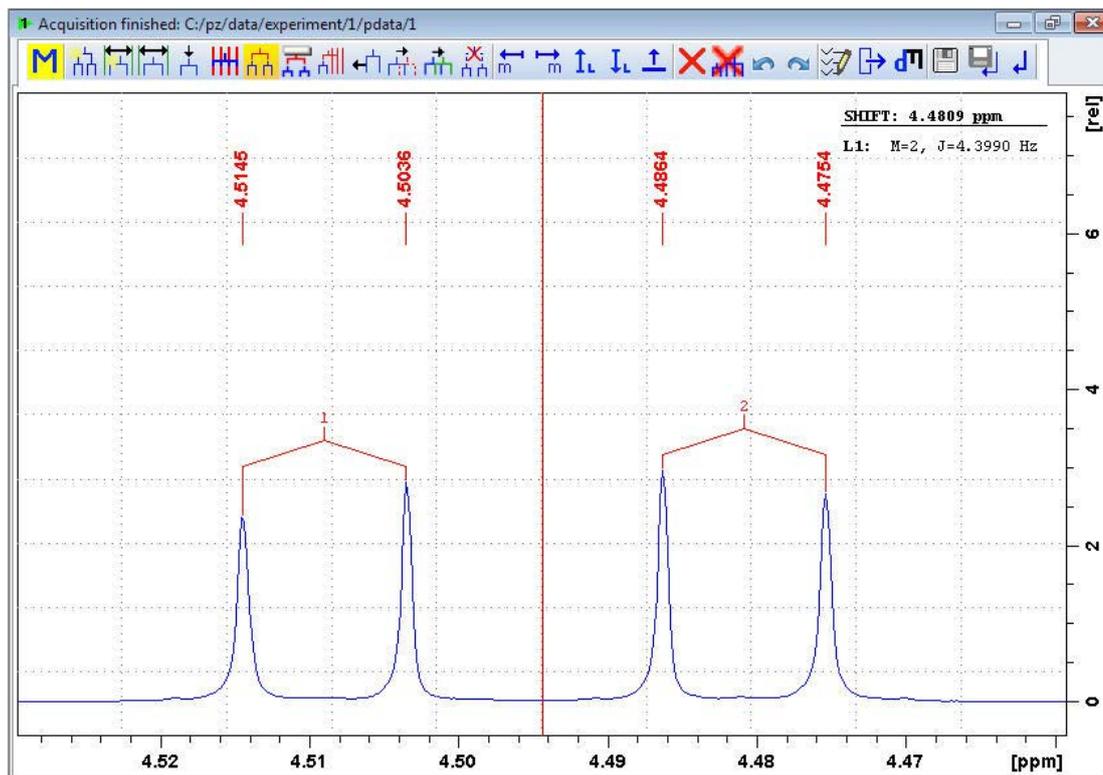
Step 16: Right-click to open the shortcut menu.

Step 17: Select **Define Multiplet**.

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

Step 19: Select **Finish Current Mode**.
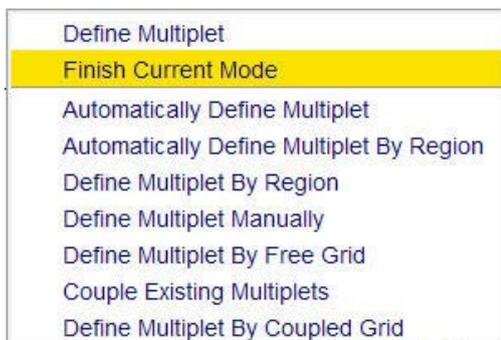
- Expand the multiplet at **3.9 ppm**.
- Repeat steps 1 - 19 for this multiplet.
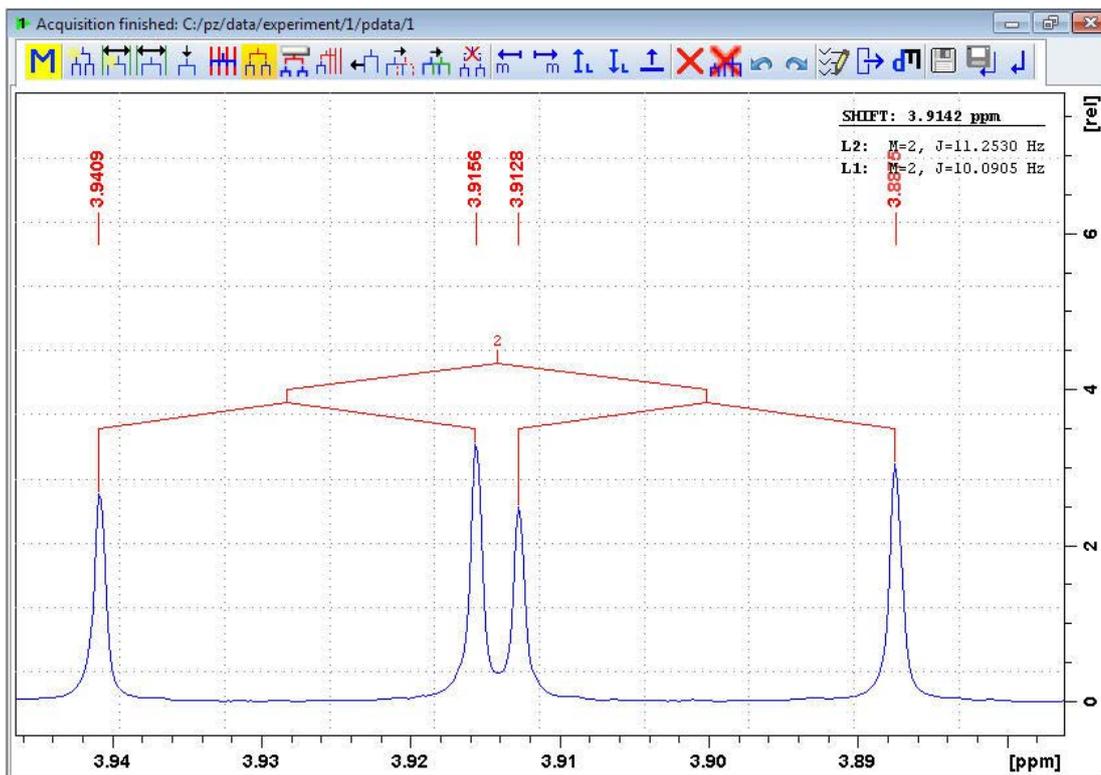
- Expand the multiplet at **3.7 ppm**.
- Repeat steps 1-19 for this multiplet.
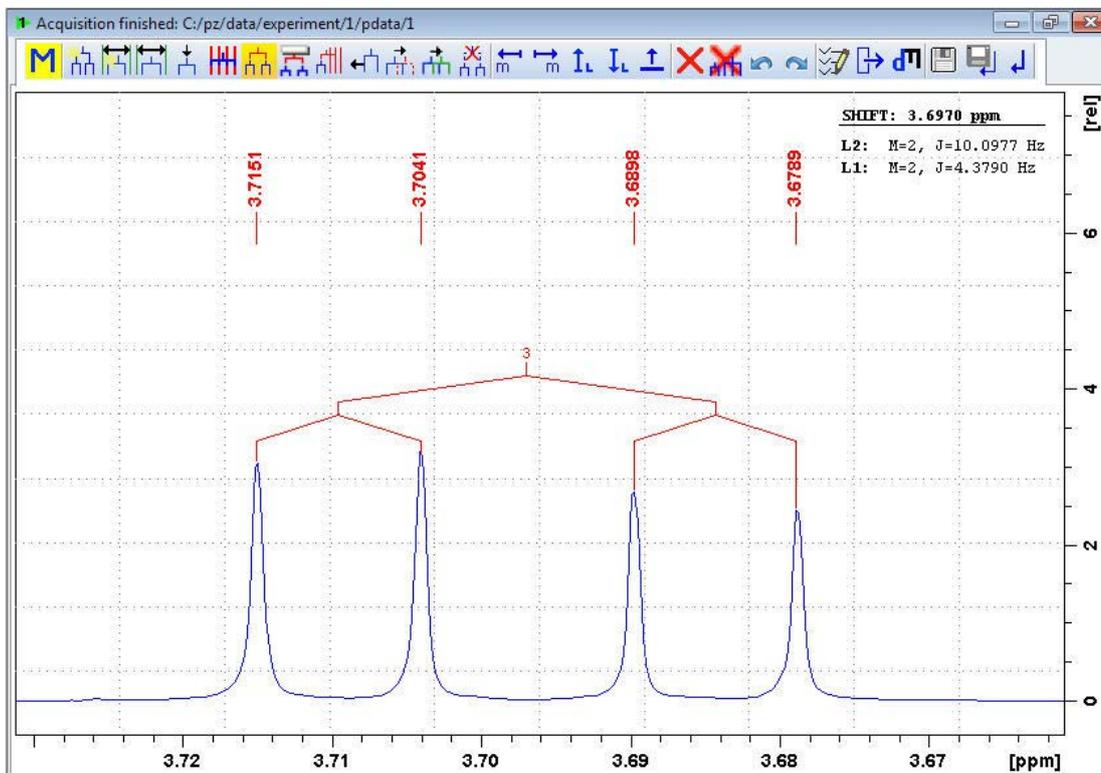
- Display all 3 multiplets.
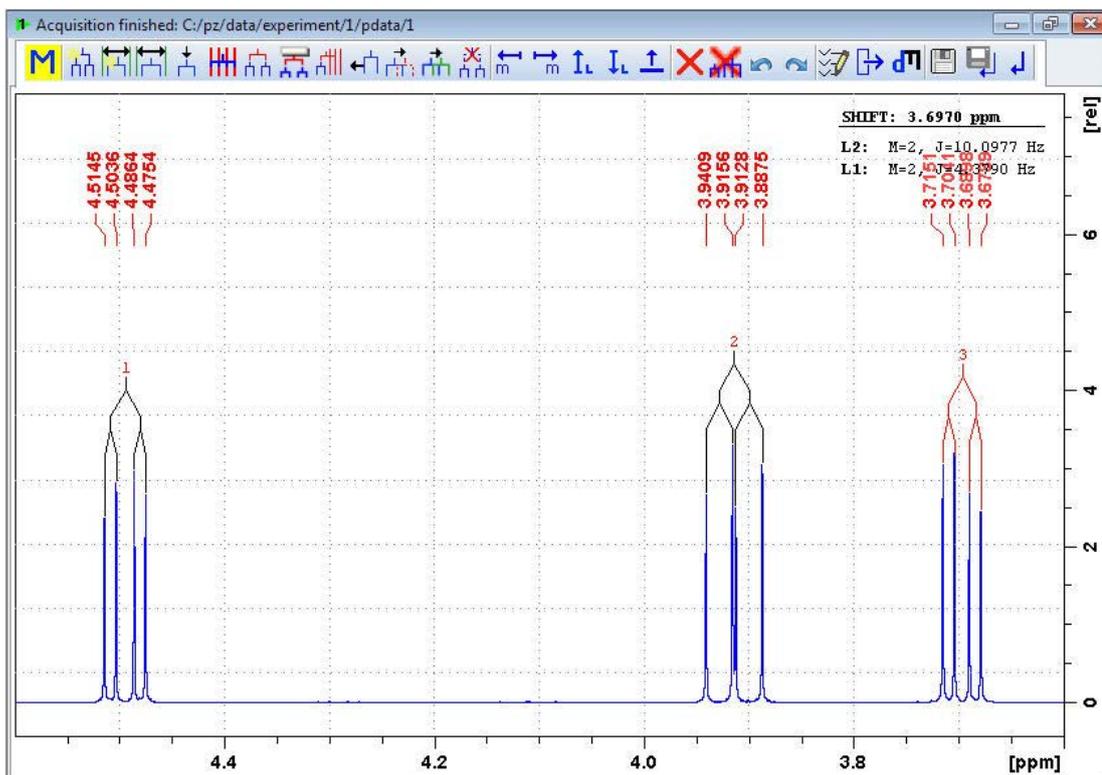
- Click **Show Multiplet Report**.
- In the Assign Connections / Report window, click **Find Connections**.

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

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

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

ID	Shift [p...	J [Hz]	M	Connection
1	4.4950	11.2575	2	J(1, 2)
		4.3790	2	J(1, 3)
2	3.9142	11.2530	2	J(2, 1)
		10.09...	2	J(2, 3)
3	3.6970	10.09...	2	J(3, 2)
		4.3790	2	J(3, 1)

Ok
Print
Copy
Save...
Start editor
JMR
JPF
Find Connections
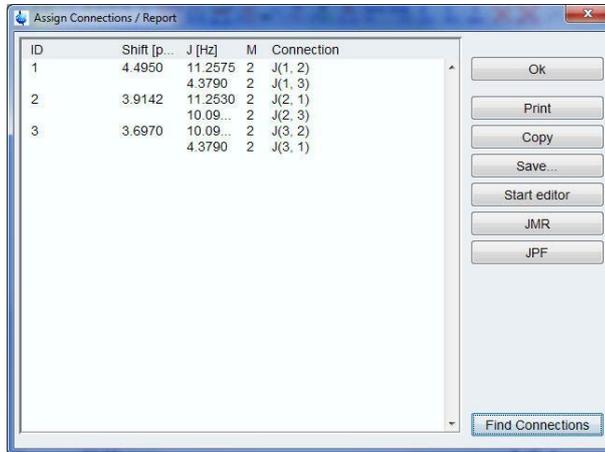
- Click **Return, save multiplets [sret]**.

12   Adding a New Solvent

12.1   Introduction

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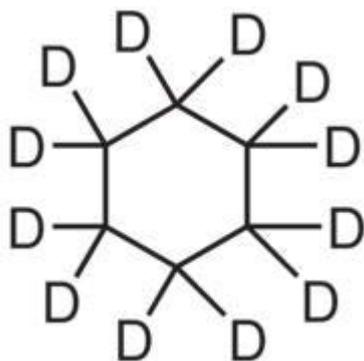
12.2   Adding Cyclohexane-d12 to the Solvent List

- At the command prompt, type **edsolv**.
- In the Edsolv window, select the **Solvents** tab.
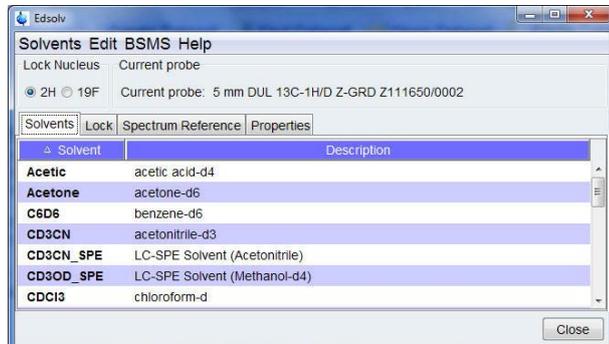
- Right-click on the C_6D_6 solvent and on the shortcut menu, select **Add new solvent**.

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

- Add the following Solvent parameters:

  Solvent name = C_6D_{12}

  Solvent description = **Cyclohexane-d12**

  enable Lock Solvent

  Melting Point [K] = **279**

  Boiling Point [K] = **354**

- In the Create solvent window, click **OK**.
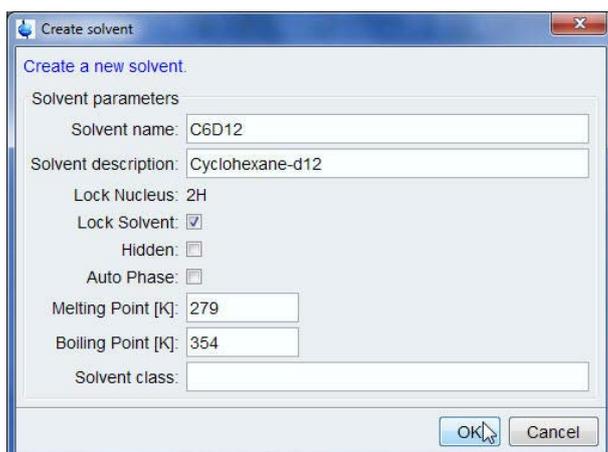
- In the Edsolv window, select the **Lock** tab.

- Right-click on the C_6D_{12} solvent and on the shortcut menu, select **Edit lock parameters**.
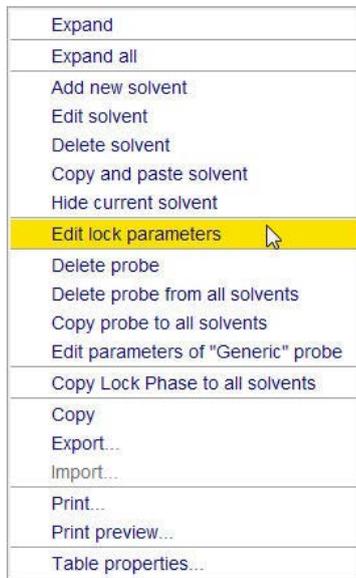
- Add the following Lock parameters:

  Probe description = **Default probe**

  Lock power = **-30**

  Loop gain = **-0**

  Loop time = **0.4**

  Loop filter = **200**

  Lock phase = **-1**

  Lock power instep = **10**

  Shift [pm] = **1.38**

  Relative intensity = **1**

  Type = **Lock**
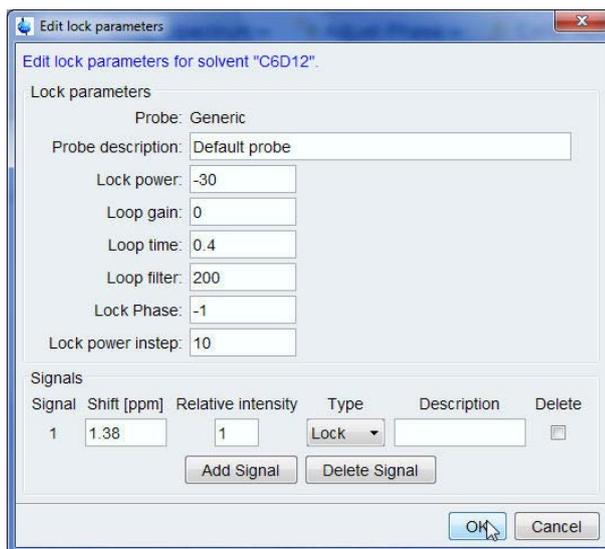
- In the Edit lock parameters window, click **OK**.

Adding a New Solvent

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

- In the Edsolv window, select the **Spectrum Reference** tab.
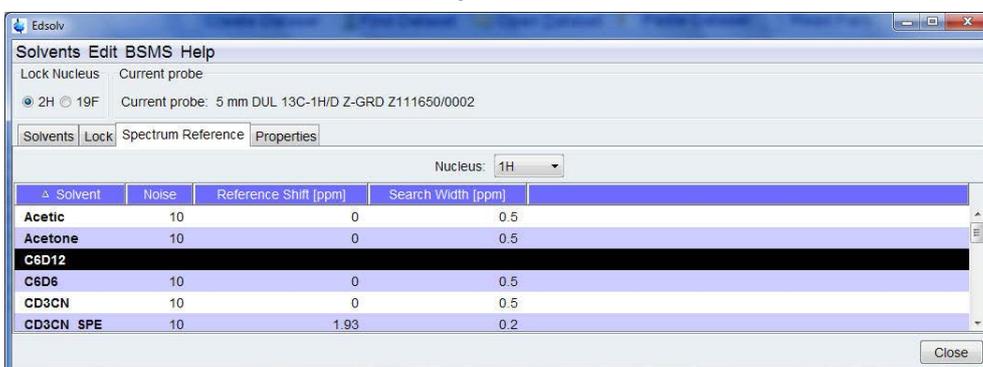
- In the Nucleus list, select **1H**.
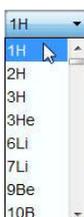
- Right-click on the C_6D_{12} solvent and on the shortcut menu, select **Edit spectrum reference parameters**.

- In the Edit spectrum reference parameters window, add the following Spectrum reference parameters for C_6D_{12}:

  Noise factor = **10**

  Reference shift [ppm] = **0**

  Search Width [ppm] = **0.5**
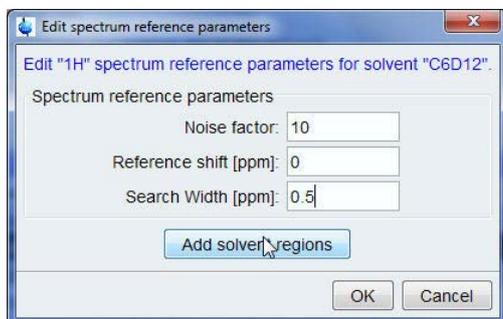
- Click **Add solvent regions**.
- Add the following regions:

  Region 1, Lower limit [ppm] **15**, Upper limit [ppm] **1.42**

  Region 2, Lower limit [ppm] **1.35**, Upper limit [ppm] **0.2**

  Region 3, Lower limit [ppm] **-0.2**, Upper limit [ppm] **-3**
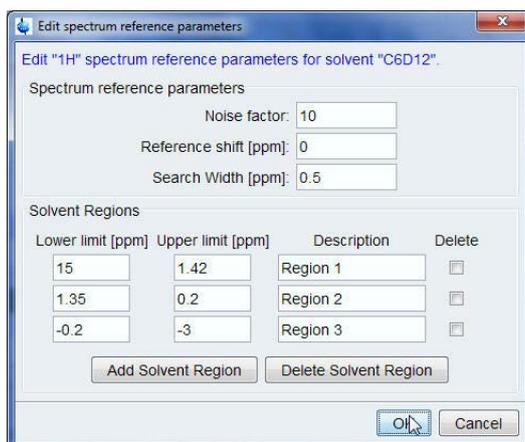
- In the Edit spectrum reference parameters window, click **OK**.
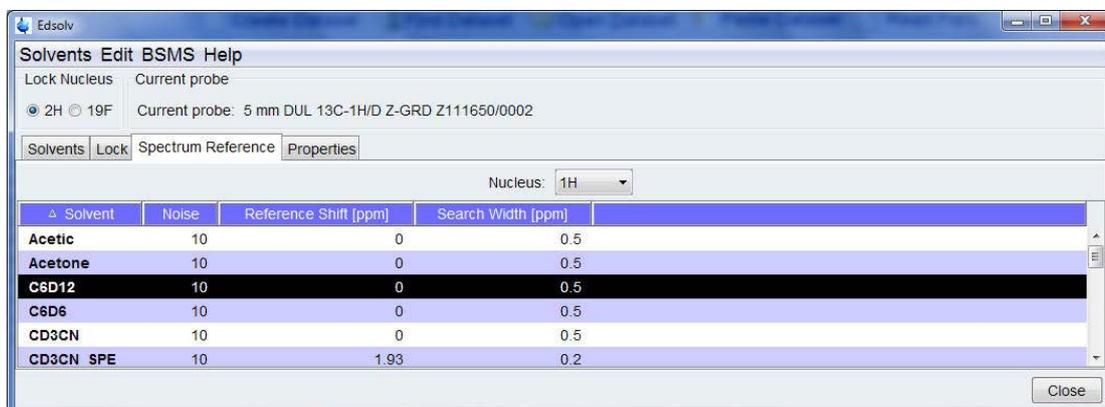
- In the Edsolv window, select Nucleus = **13C**.
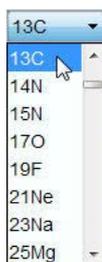
- Right-click on the C_6D_{12} solvent and on the shortcut menu, select **Edit spectrum reference parameters**.

- Add the following Spectrum reference parameters for C_6D_{12}

  Noise factor = **10**

  Reference shift [ppm] = **0**

  Search Width [ppm] = **5**

- Add the following regions:

  Region 1, Lower limit [ppm] **200**, Upper limit [ppm] **27.5**

  Region 2, Lower limit [ppm] **25.5**, Upper limit [ppm] **1**

  Region 3, Lower limit [ppm] **-1**, Upper limit [ppm] **-5**
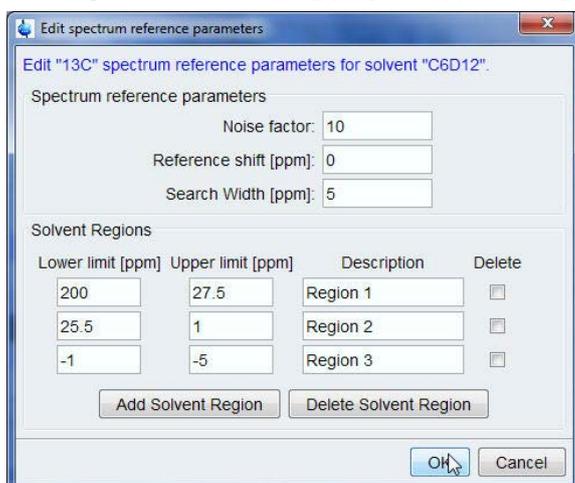
- In the Edit spectrum reference parameters window, click **OK**.

• In the Edsolv window, click **Close**.
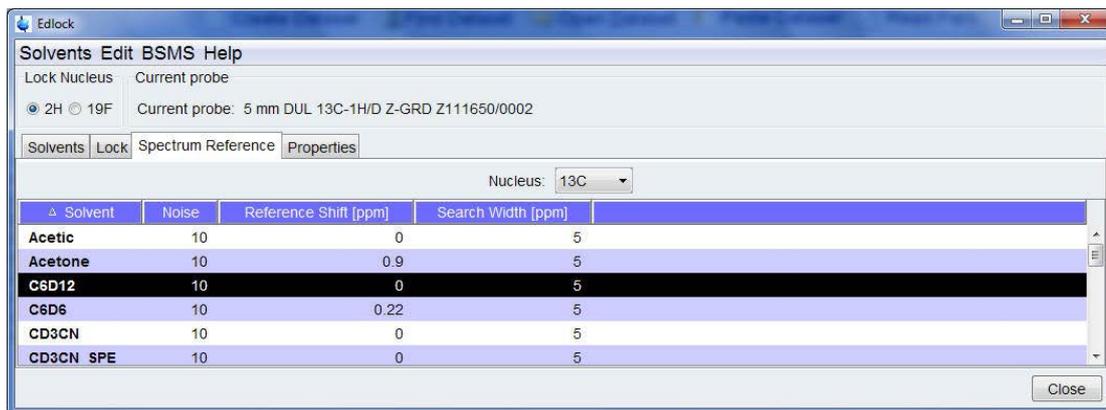
12.3   TopShim Solvent Parameters

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

13 Troubleshooting

13.1 Resetting the ELCB Board on a AV-III Console

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

• At the command prompt, type **ha**.

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

• In the BSMS Service Web window, click **Service**.
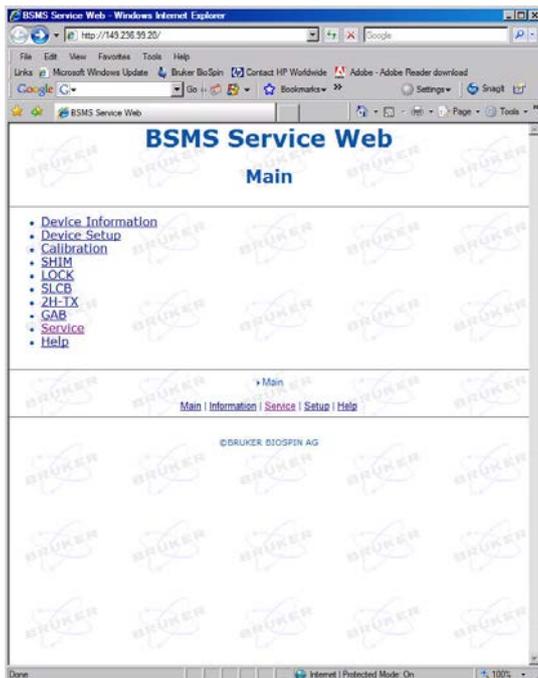
- In the BSMS Service Web window, click **ResetELCB**.
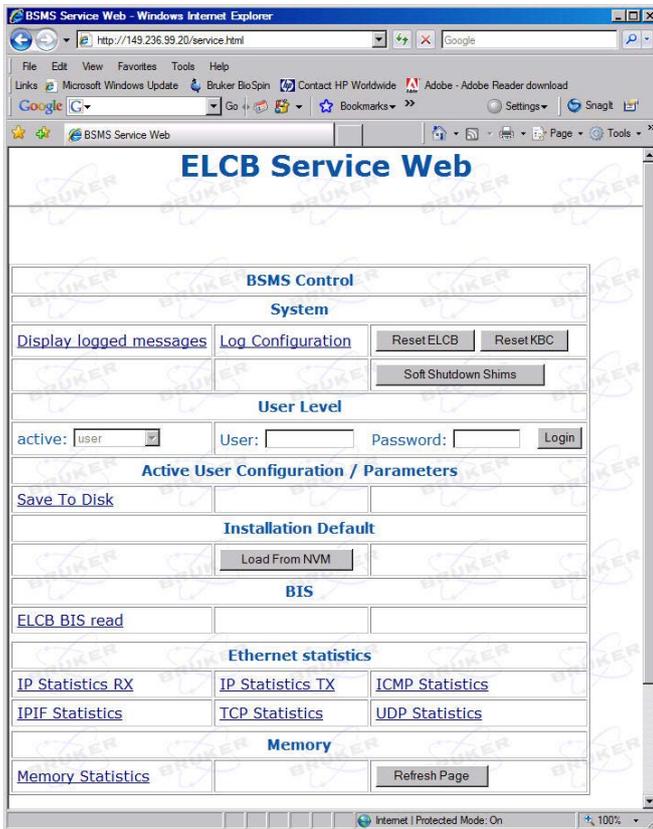
- In the Hardware ethernet addresses window, click **Close**.

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

13.1.1 Downloading a New DRU Firmware

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

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

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

• At the TopSpin command prompt, type **ha**.
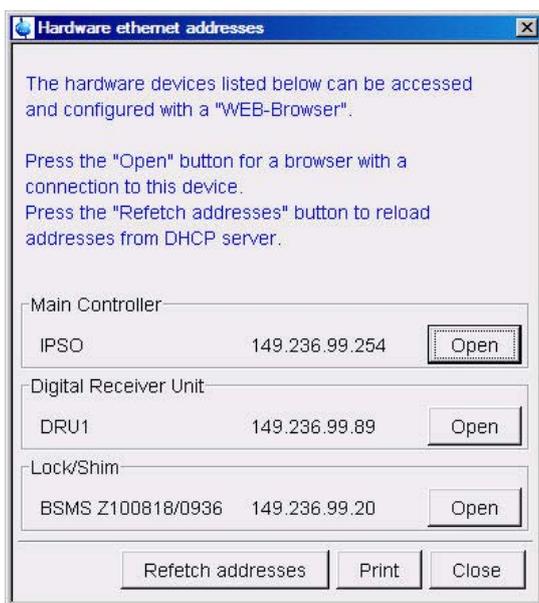
• In the DRU1 Digital Receiver Unit group, click **Open**.

In the AQS Main window, click **AQS Firmware Setup**.
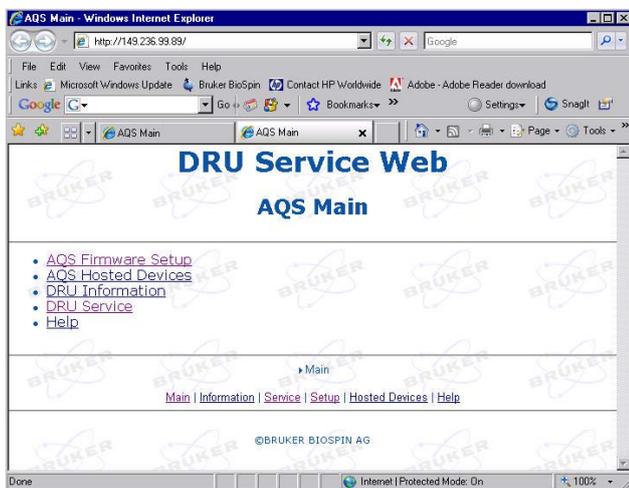
- Click **Load new DRU Firmware**.
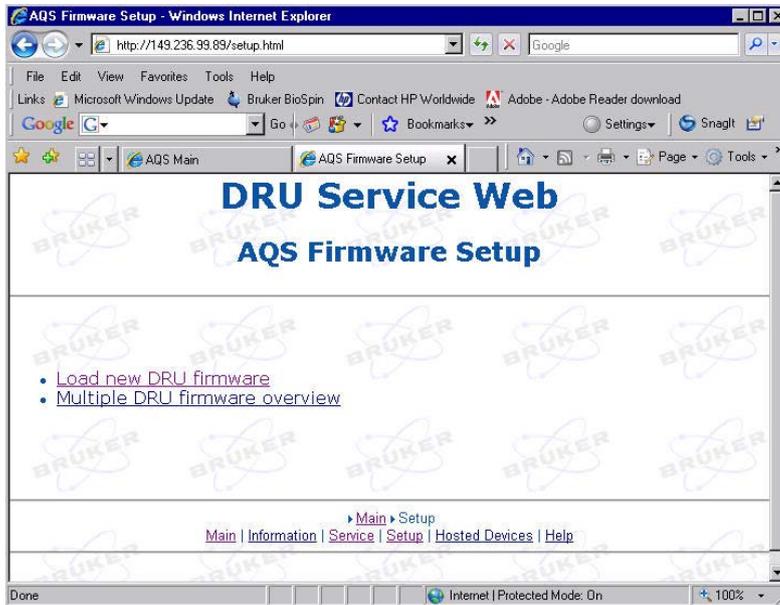
The current DRU Firmware version is displayed in this window. If no firmware newer than the current one is available, simply close the window. You may want to discuss any further action with a Bruker Center.

- In the Firmware Download window, click **Browse**.
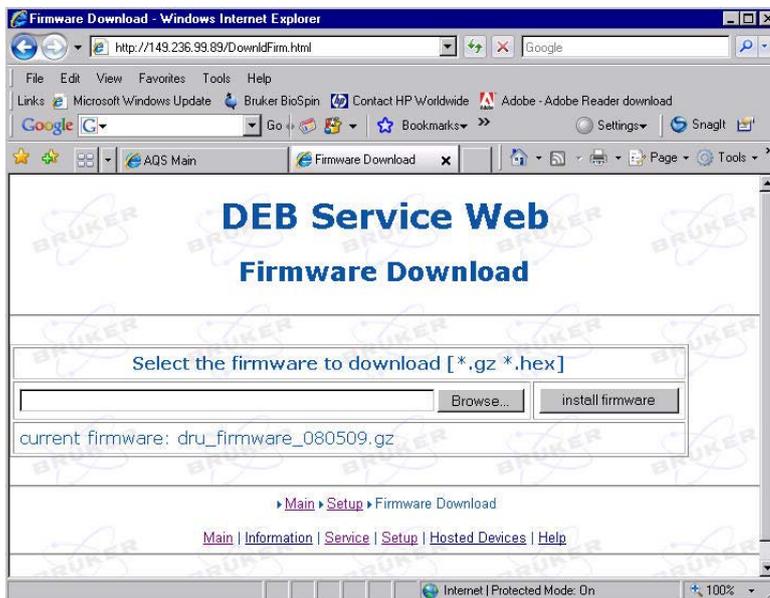
- Double-click the newest firmware file.
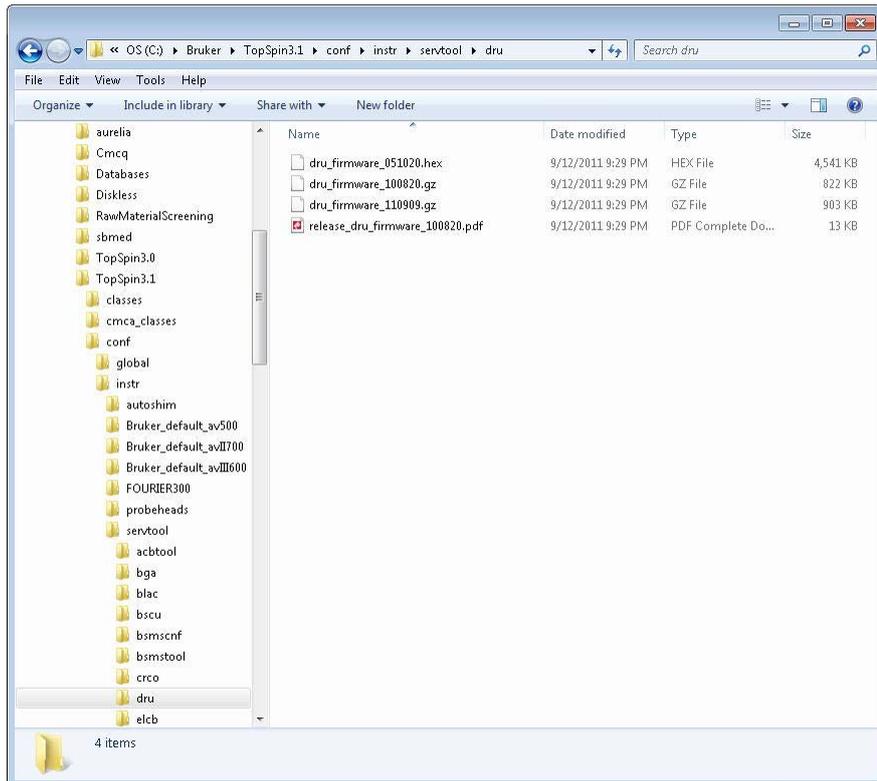
- Click **Install firmware**.
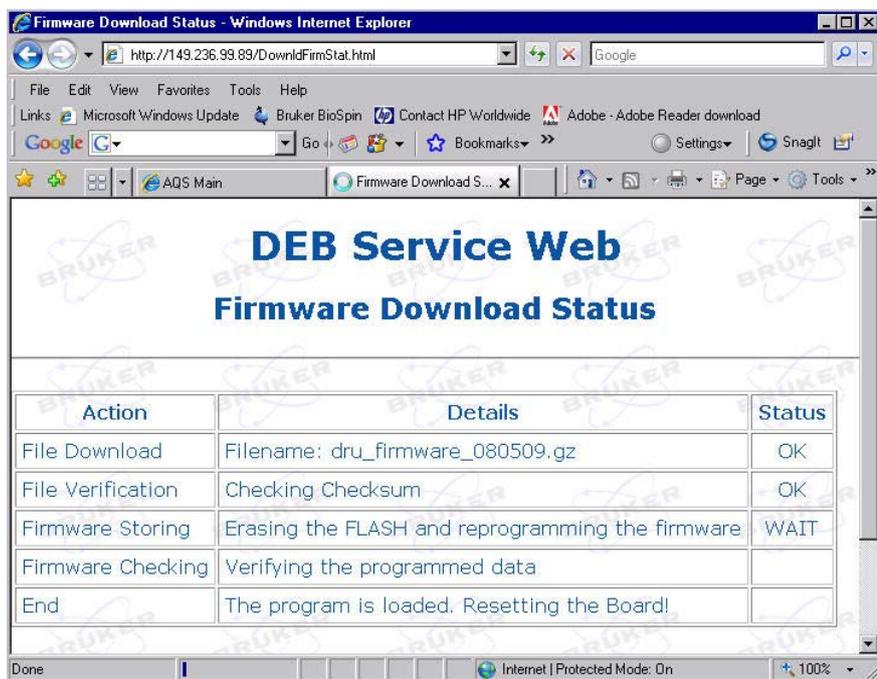
| ℹ | After the program is loaded and the board is reset the current Web page will close automatically and the Web page in the figure below is displayed. |

- Close the Web page.
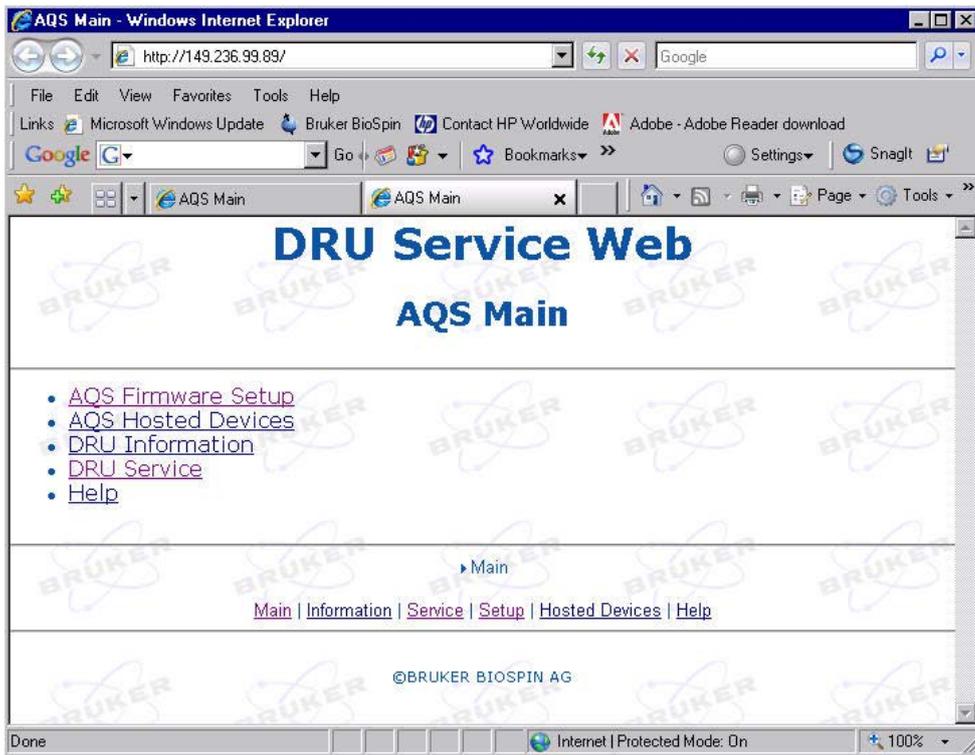
- In the Hardware ethernet addresses window, click **Close**.

14 Contact

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NMR Hotlines

Contact our NMR service centers.

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

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

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

Phone: +49 721-5161-6155

E-mail: *nmr-support@bruker.com*

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