BRUKER

• TopSpin 3.x

Introduction to NMR Methods
User Manual

Version 001

NMR Spectroscopy

Innovation with Integrity

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1 Introduction

1.1 General

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

1.2 Disclaimer

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

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

2 Spectrometer Basics

2.1 Introduction

In terms of safety the presence of a relatively strong magnet is what differentiatesNMR spectrometers from most other laboratory equipment. When designing an NMR laboratory, or training personnel who will work in or around the laboratory, no other feature is of greater significance. As long as correct procedures are adhered to, working in the vicinity of superconductive magnets is completely safe and has no known harmful medical side effects. Negligence however can result in serious accidents. It is important that people working in the vicinity of the magnet fully understand the potential hazards. Of critical importance is that people fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet.

The magnet is potentially hazardous due to:

- 1. The large attractive force it exerts on ferromagnetic objects.

- 2. The large content of liquid Nitrogen and Helium.

2.2 Magnetic Safety

A Magnetic Field surrounds the magnet in all directions. This field (known as the stray field) is invisible, hence the need to post warning signs at appropriate locations. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way!

Because the strength of the stray field drops significantly as one moves away from the magnet, it is useful to discuss safety in terms of two broadly defined regions, the inner and outer zone. In terms of organizing a laboratory as well as defining good work practices, the concept of an inner and outer zone is particularly useful.

The physical extent of these two zones will depend on the size of the magnet. The bigger the magnet, the stronger the stray magnetic fields and hence the larger the extent of the two zones. Figure 2.1. shows the concept of the two zones (not drawn to scale). Details of stray fields for various magnets can be found in the Site Planning Guides delivered with the BASH CD.

Figure 2.1

No metal chairs in
magnet room

People with
pacemakers
should not be
allowed near
the magnet

Strap cylinders of
gaseous Helium and
Nitrogen to the wall.
Limit 0.5mT

Don't leave
screwdrivers or bolts
near the magnet

Don't use a ladder
made of iron or steel

The magnetic field can
pass through walls, so
apply these precautions
to neighboring rooms
including rooms above
and below

Label all the
areas affected
by the magnet

Good ventilation
is necessary

Bankers cards
and credit cards

Sensitive mechanical
devices such as watches

Tapes and disks

These items will be damaged if allowed near the magnet
Limit = 1.0mT

2.2.1   Safety Precautions within the Inner Zone

The inner zone extends from the magnet center to the 1mT (10 Gauss) line. Within this region objects may suddenly be drawn towards the magnet center. The attractive force of the magnet can change from barely noticeable to uncontrollable within a very short distance. **Under no circumstances should heavy ferromagnetic objects be located**

or moved within this zone.

Any ladders used when working on the magnet should be made of non-magnetic material such as aluminum. Helium and nitrogen dewars which are used to top up the liquid levels inside the magnet must be made of non-magnetic material.

Do not allow small steel objects (screwdrivers, bolts etc.) to lie on the floor near the magnet. These could cause serious damage if drawn into the magnet bore, especially when no probe is inserted in the magnet.

Mechanical watches may be damaged if worn within the inner zone. Digital watches can be worn safely. Of course, the precautions for the outer zone which will now be discussed must also be adhered to within the inner zone.

2.2.2   Safety precautions within the outer zone

The outer zone extends from the 1mT line to the 0.3mT line. The magnet's stray field does not get blocked by walls, floors or ceilings and the outer zone may well encompass adjoining rooms. The stray field may erase information stored on magnetic tapes or discs. Bank cards, security passes or any devices containing a magnetic strip may be damaged. CD's will not be damaged, although CD drives may contain magnetic parts. When using pressurized gas cylinders made of steel, they should be located well beyond the outer zone (preferably outside the magnet room) and must always be properly fixed to the wall. The color display of computer monitors may suffer some distortion when located too close to the magnet, although permanent damage is unlikely. Once beyond the outer zone any special precautions on account of the magnet stray field are no longer necessary.

2.3   Cryogenic Safety

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

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

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

2.4   Electrical Safety

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

done by trained personnel. Please note that, unless disconnected, cooling fans on the rear panel will continue to run even with the panel removed.

2.5 Chemical Safety

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

2.6 CE Certification

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

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

2.7 AVANCE Architecture Overview

Figure 2.2
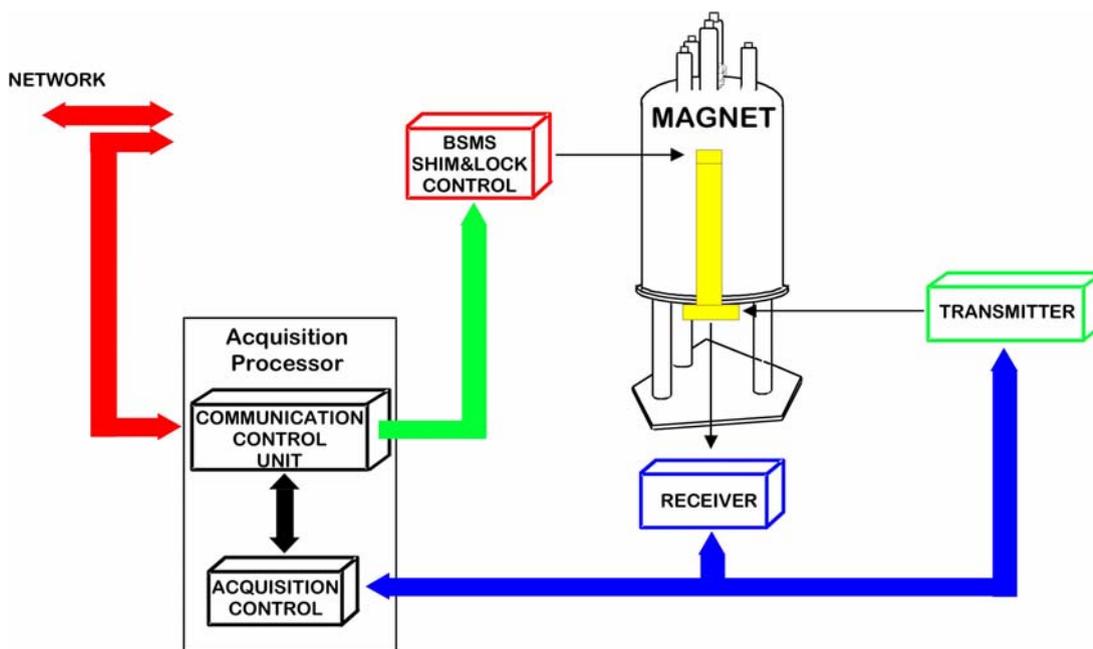
NOTE: Please use the BASH (Bruker Advanced Service Handbook) for further information about the AVANCE system and hardware.

2.8 Sample preparation

- Use clean and dry sample tubes
- Use medium to high quality sample tubes
- Always filter the sample solution
- Always use the same sample volume or solution height
- Filling volume of a 5 mm tubes is 0.6 ml or 5 cm
- Filling volume of a 10 mm tubes is 4 ml or 5 cm
- Use the sample depth gauge to adjust the sample depth (1.8 cm for older style probes, 2.0 cm for newer style probes)
- The sample tube should sit tightly inside the spinner
- Wipe the sample tube clean before inserting into magnet
- Turn on lift air to insert the sample into the magnet

2.9 Inserting the Sample Plus Spinner into the Magnet

The raising and lowering of the sample is controlled by a stream of pressurized air. Be careful never to lift the sample with the plug still inserted at the top of the magnet bore. Newer BOSS-2 shim systems are designed not to enable the LIFT if the magnet bore is still plugged. Furthermore, make sure that the air flow is present (it is quite audible) before placing a sample onto the top of the bore.

Figure 2.3
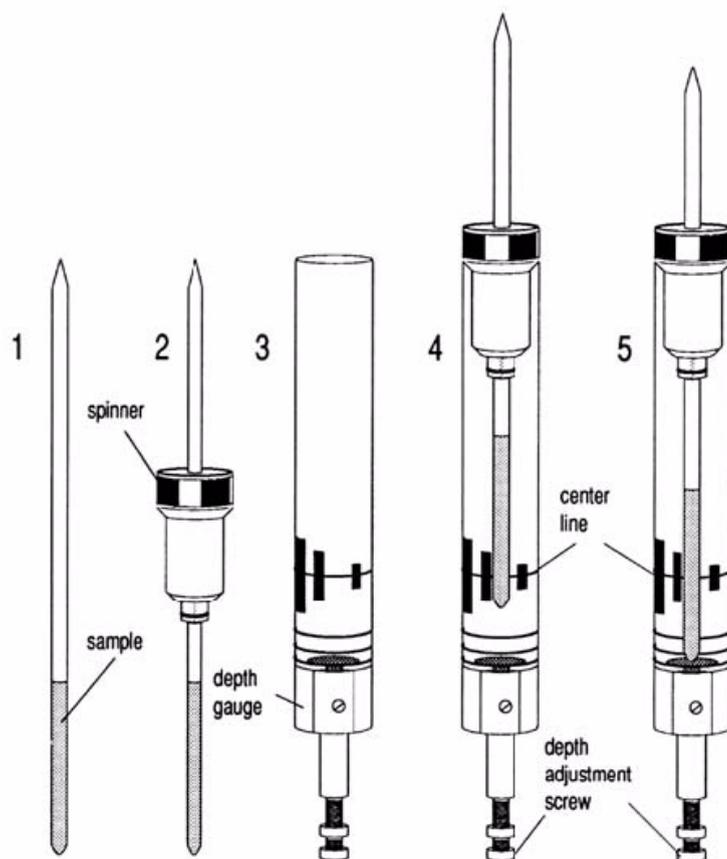
To insert the sample plus spinner into the magnet
use the following procedure:

1. If present, remove the plug from the top of the magnet bore

2. Activate the LIFT button on the BSMS keyboard. A flow of air will be heard
and if a sample is already in the magnet it will be raised and suspended on
a cushion of air at the top of the magnet bore.

3. Remove the old sample and place the new sample onto the air cushion

4. Press the LIFT key again. The sample will gently drop into the magnet and
will settle at a precise position within the probe.

2.10   Spinning the Sample

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

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

Set the spin rate using the following procedure:

1. Open the BSMS display

2. Click on the SPIN button to activate the spinning.

Suggested spin rates are:

20 Hz for a 5 mm probe

12 Hz for a 10 mm probe

2.11 Tuning and Matching the Probe

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry. **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately.

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

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

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

2.11.1 Probes equipped with ATM

2.11.1.1 Automatic tuning

1. Type **edc** and create a new data set

2. Type **atma**

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

2.11.1.2   Manual tuning

1. Type **atmm**

NOTE: The ATM control window appears (see Figure 2.4) and the display will switch automatically to the acquisition window and displays the wobble curve. (Figure 3.2).
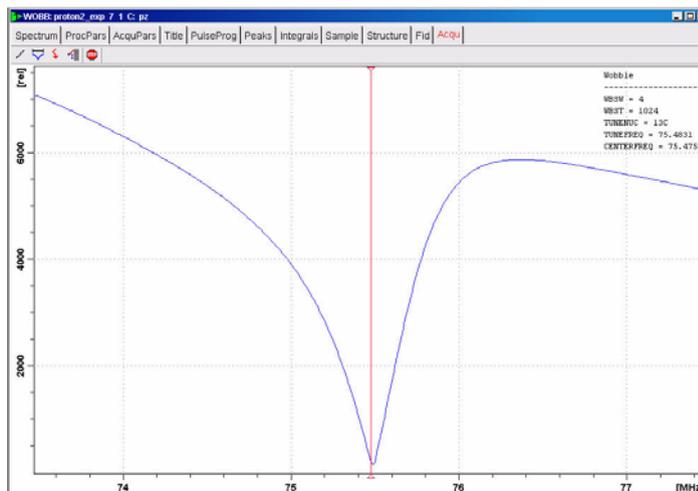
Figure 2.4
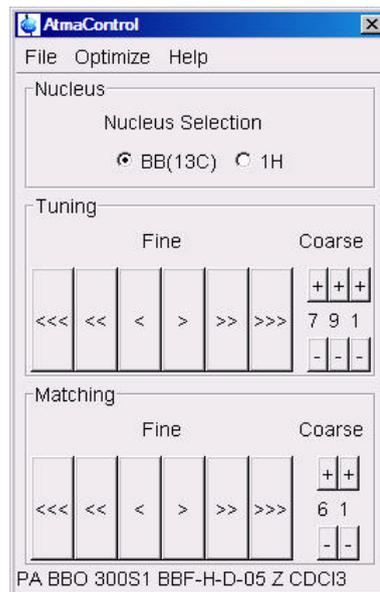
3. Click on the **'Tuning'** buttons in the ATM control window to move the wobble curve in to the center of the display

4. Click on the **'Matching'** buttons in the ATM control window to adjust the dip of the wobble curve to the lowest position

NOTE: Since the Tuning and Matching adjustment interact with each other, a repeat of steps 3 and 4 are necessary for a perfect tune and match (see Figure 2.5). If multiple frequencies are used in a parameter set such as C13CPD, use the **'Nucleus Selection'** radio buttons in the ATM control window to switch to another nucleus and repeat steps 3 and 4.
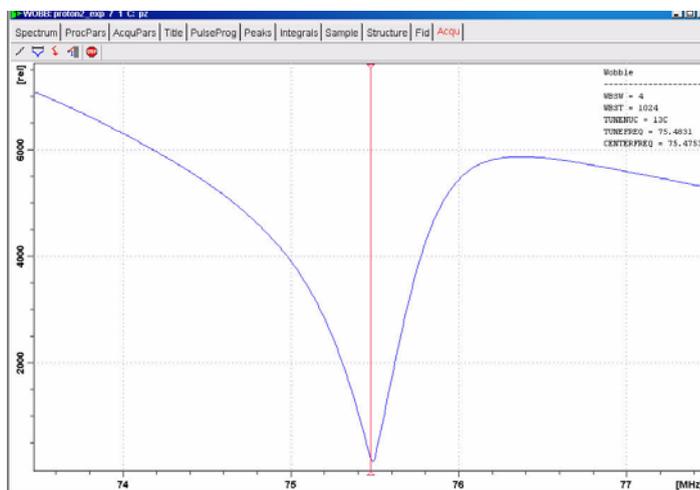
Figure 2.5

2.11.2   Probes without ATM

1. Type **edc** and create a new data set

2. Type **wobb**

NOTE: The display will switch automatically to the acquisition window and displays the wobble curve (see Figure 2.6). If multiple frequencies are used in a parameter set such as C13CPD, HNCACOGP3D etc., wobb will start with the lowest frequency first. The nuclei are selected in the order of increasing frequency. Tuning and Matching rods are color-coded for different nuclei e.g. yellow for 1H, blue for 13C etc.

3. Adjust the Tuning rod marked **T** underneath the probe to move the wobble curve in to the center of the display

4. Adjust the Matching rod marked **M** underneath the probe to adjust the dip of the wobble curve to the lowest position

NOTE: Since the Tuning and Matching adjustment interact with each other, a repeat of steps 3 and 4 are necessary for a perfect tune and match (see Figure 2.6). If multiple frequencies are used in a parameter set and to switch to other frequencies, follow steps 5 through 6 below. and repeat steps 3 and 4.

Figure 2.6

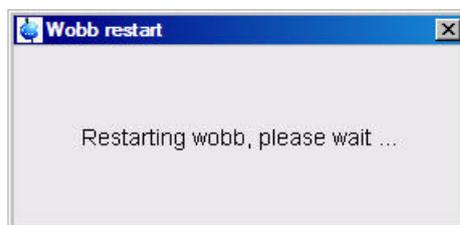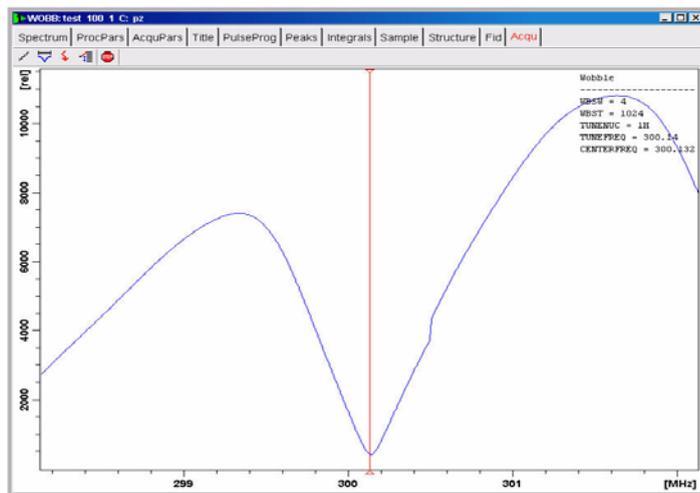5. Click on [icon] in the acquisition window

Figure 2.7

Figure 2.8

6. Click [icon] on to terminate the acquisition

2.12   Locking the sample

To display the lock signal type **lockdisp** on the Topspin command line. This opens a window in which the lock trace appears.

The most convenient way to lock is to use the TopSpin command **lock**. To start the lock-in procedure, enter lock and select the appropriate solvent from the menu. Alternatively, enter the solvent name together with the lock command, e.g., **lock cdcl3**. During lock-in, several parameters such as the lock power, the field value, and the frequency shift for the solvent are set according to the values in the lock table. This table can be edited using the command edlock. Note that the lock power listed in this table is the level used after the sample has been locked. The field-shift mode is then selected and autolock is activated. Once lock-in is achieved, the lock gain is set so that the lock signal is visible in the lock window. At this point the message "lock: finished" appears in the status line at the bottom of the window.

The lock-in procedure outlined above sets the frequency shift to the exact frequency shift value for the given solvent as listed in the edlock table. It also sets the field value to the value listed in the edlock table and then adjusts it slightly to a given ppm value no longer depends on the lock solvent). Following this lock-in procedure, the solvent parameter in the eda table is set automatically, which is important if you wish to use the automatic calibration command sref (see "Spectrum Calibration and Optimization").

The lock-phase adjustment by monitoring the sweep wiggles (i.e., while the field is not locked but is being swept) is recommended each time the probehead is changed, because autolock may fail. If the original phase is reasonably close to the correct value, lock-in can be achieved and the phase can be adjusted using autophase. Note that the lock phase for each probehead is stored in the edlock table. In some cases, the lock power level listed in the edlock table is set too high leading to a saturation of the lock signal. Usually, lock-in can be achieved, but the signal oscillates due to saturation. A quick fix is simply to reduce the lock power manually after lockin. However, it is better to change the power level in the edlock table.

NOTE: that the appropriate lock power level depends on the lock solvent, the field value, and the probehead. Any value changes in the edlock table should only be done by experts.

2.13   Shimming the sample

The following is intended to be a practical guide for adjusting the room temperature shim system (BOSS). The purpose of shimming is to maximize the magnetic field homogeneity, which depends somewhat on probehead and sample geometry. In general, it is necessary to shim the magnetic field after each probehead change, sample change, and occasionally between changes to correct for any system drifts.

Optimal shim settings may vary substantially from probehead to probehead; however, provided the probehead is always positioned the same in the magnet and the sample is always positioned the same with respect to the receiver coil, the shim values for a given probehead will be fairly reproducible. Thus, shimming time can be greatly reduced if the shim settings for each probehead are stored as a shim file on the computer. When the probehead is changed, the shim file for the new probehead can be read in and then final adjustments can be made to these shim values to correct for system drifts, and to

account for the geometry of the particular sample being used.

The BOSS shim system consists of a number of shim coils arranged in the room temperature bore of the magnet. During shimming, the currents in these shim coils are adjusted so that the small magnetic field gradients produced cancel the residual inhomogeneity of the main magnetic field (H0) as completely as possible.

2.13.1 Shimming on the Lock Signal

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

2.13.2 Shimming on the FID (Free Induction Decay)

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

2.13.3 Shimming using the Tune file

This method of shimming is useful when gradients are not available. A simple text file is edited to give the BSMS the instructions to shim the sample automatically. A default shim file "example_bsms" can be edited using the edtune command and then stored with a new name in <TopSpin-home>/exp/stan/nmr/lists/group. The file and can be executed with the command **tune**. Figure 2.9 shows an example of a tune file.

Figure 2.9

```
# SHIMMIT spin
DELAY  3
MAXLOCK      0.4

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

2.13.4 Shimming using TopShim

TopShim is a tool designed for easy and automatic shimming. It requires that the instrument is equipped with a gradient amplifier and a gradient probe. In addition a 2HTX board or any 2H amplifier is necessary for Deuterium gradient shimming.

The core method of TopShim is gradient shimming. This is complemented by a spectrum optimization approach, where a quality criterion for the final lineshape ensures the best results for all possible situations.

Both 1D and 3D shimming modes are provided to adjust only the on-axis or both the on- and off-axis shim functions, respectively. However 3D is restricted to only Proton gradient shimming using a H2O sample.

The acquisition of the B0 field map data can be carried out with 1H or 2H observation, enabling the use of TopShim for protonated as well as deuterated solvents.

Optionally the additional tune functionality can be applied before and/or after gradient shimming in order to adjust low order shims for maximum lock level.

For further information please consult the TopSpin Automatic Shimming Users Manual in the Help section of TopSpin.

2.14 Optimizing Resolution and Lineshape

The standard sample for measuring the proton lineshape and resolution specifications is, CHCL3 in Acetone-d6. The concentration of CHCL3 depends on the field strength of the magnet and the probe and can vary from 3% down to 0.1%.

For measuring the 13C resolution and lineshape test the standard sample ASTM (60% Dioxane in 40% C6D6) sample may be used.

For both tests the line shape is measured at 50%, 055% and 0.11% of the peak. The Bruker standard parameter sets to use for this tests are PRORESOL and C13RESOL.

Figure 2.10 and 2.11 are illustrating the influence of the On-axis shims on the lineshape.

Figure 2.10
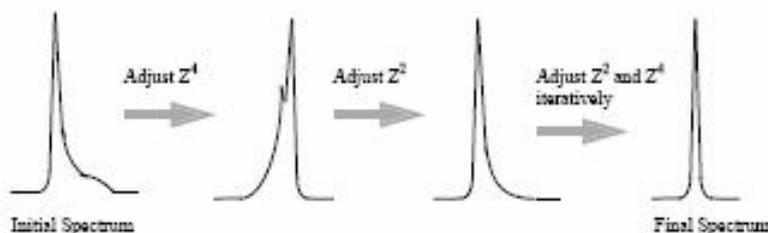
Figure 2.11
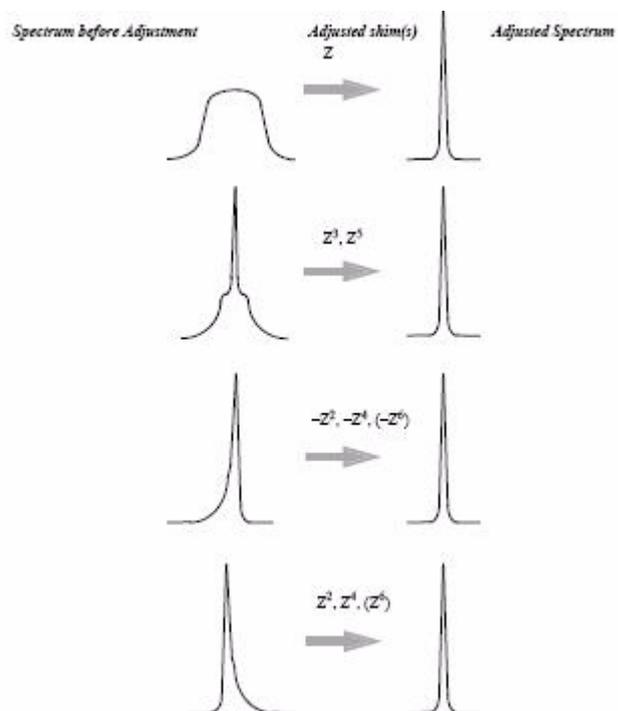
3 1-D Proton experiment

3.1 Sample

A sample of **30mg Menthyl Anthranilate in DMSO-d6** is used for the experiment in this chapter

Figure 3.1
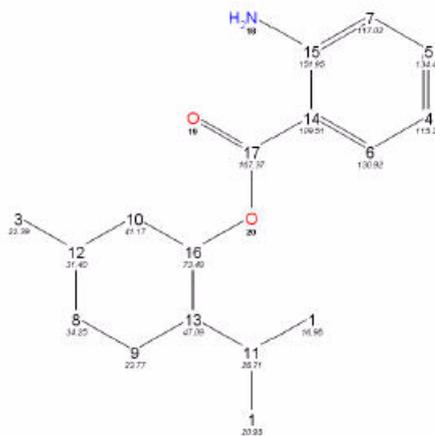
3.2 1-D Proton experiment

3.2.1 Introduction

Section 3.2 describes the acquisition and processing of a one-dimensional 1H NMR spectrum using the standard Bruker parameter set **PROTON**. The pulse sequence **zg30**, Figure 3.2 consists of the recycling delay, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30 degrees. The two parameters, D1 and P1, correspond to the length of the recycle delay, and the length of the 90 degree RF pulse, respectively.

Figure 3.2
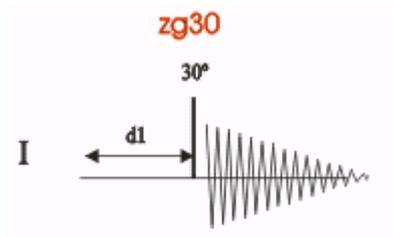
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For

example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

3.2.2   Experiment setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 3.3

2. Select [Create Dataset] by clicking on it

3. Enter the following information in to the 'New' window

Figure 3.4
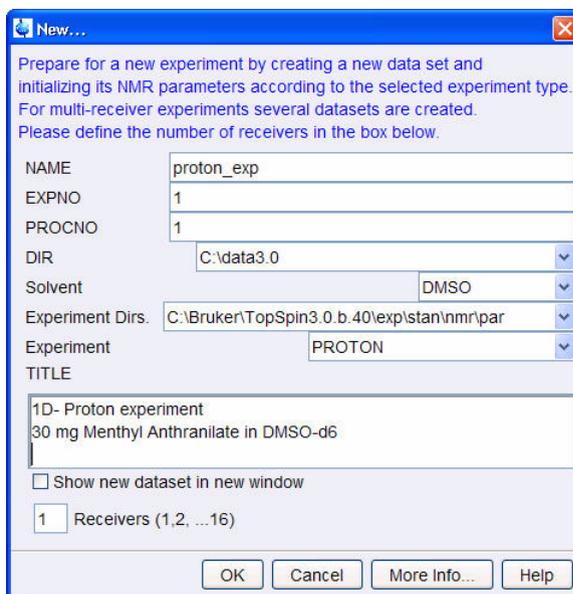
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 3.4 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 3.5

6. Select [Sample ▾] by clicking on it

Figure 3.6

7. Select '**ej**' by clicking on it

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

8. Place the sample on too the top of the magnet

9. Select by clicking on it

Figure 3.7
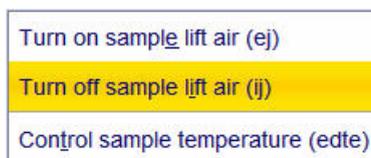
10. Select '**ij**' by clicking on it

NOTE: Wait till the sample is lowered down in to the probe and the lift air is turned off. A licking sound may be heard.

11. Select by clicking on it

Figure 3.8
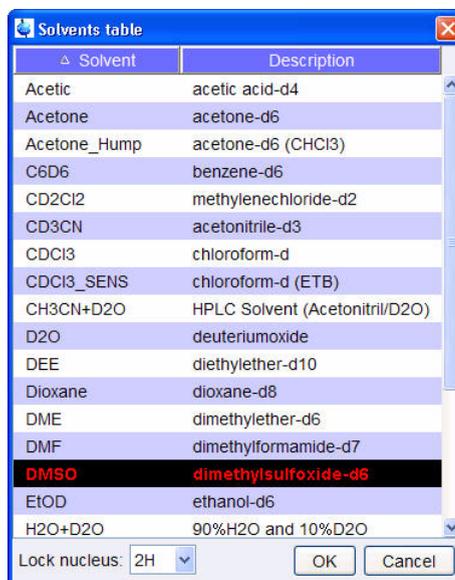
12. Select '**DMSO**' by clicking on it

13. Click on [OK]

14. Select [Ⅴ **Tune** ▽] by clicking on it

NOTE: This performs a '**atma**' (automatic tuning) and requires a probe equipped with a automatic tuning module. Other options can be selected by clicking on the down arrow inside the '**Tune**' button.

15. Select [⚓ **Spin** ▽] by clicking on it

Figure 3.9
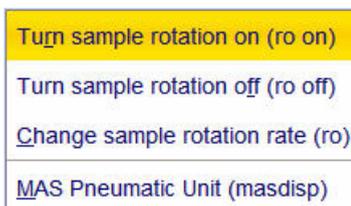
16. Select '**ro on**' by clicking on it

NOTE: Rotation may be turned off for probes such as BBI, TXI, TBI and for small sample probes.

17. Select [⊟ **Shim** ▽] by clicking on it

NOTE: This executes the command '**topshim**'.To select other options. click on the down arrow inside the '**Shim**' button.

18. Select [⌐ **Prosol**] by clicking on it

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

3.2.3   Acquisition

1. Select [⌁ **Gain** ▽] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select [▶ **Go** ▽] by clicking on it

NOTE: Other options are available by clicking on the down arrow inside the '**Go**' button.

3.2.4   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 3.10
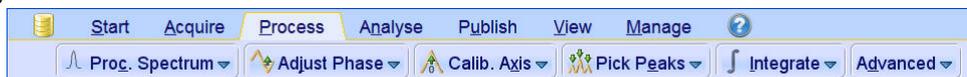
2. Click on   **Proc. Spectrum ▾**

NOTE: 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**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button.

Figure 3.11
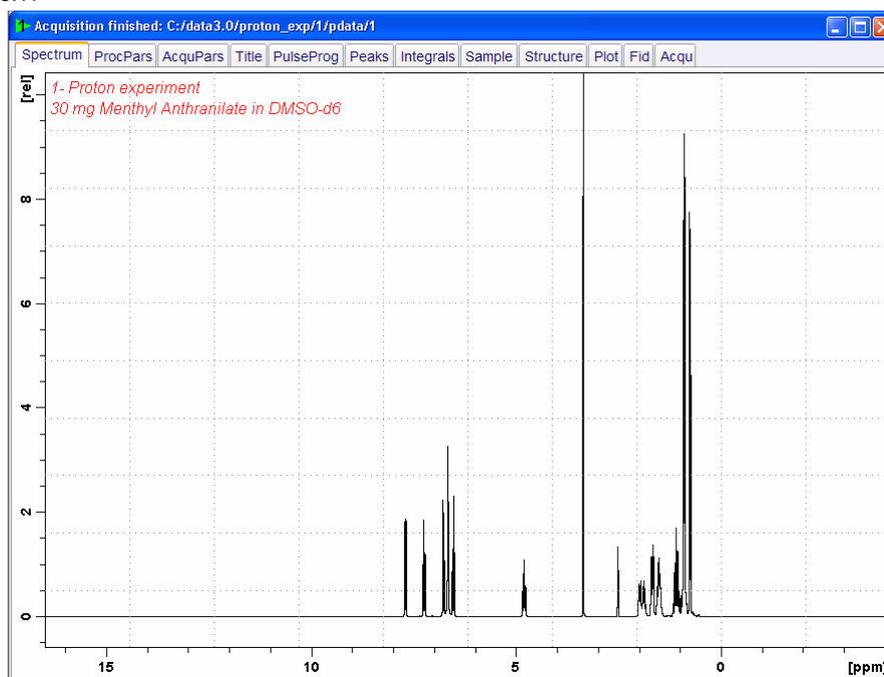
3.2.5   Optimizing the Spectral width

1. Click on ppm

3. Type the following F1 [ppm] values:

From = **9**

To = **-1**

Figure 3.12
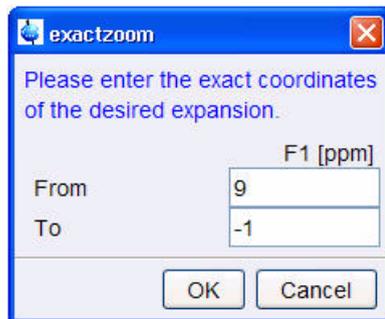
4. Click on [OK]
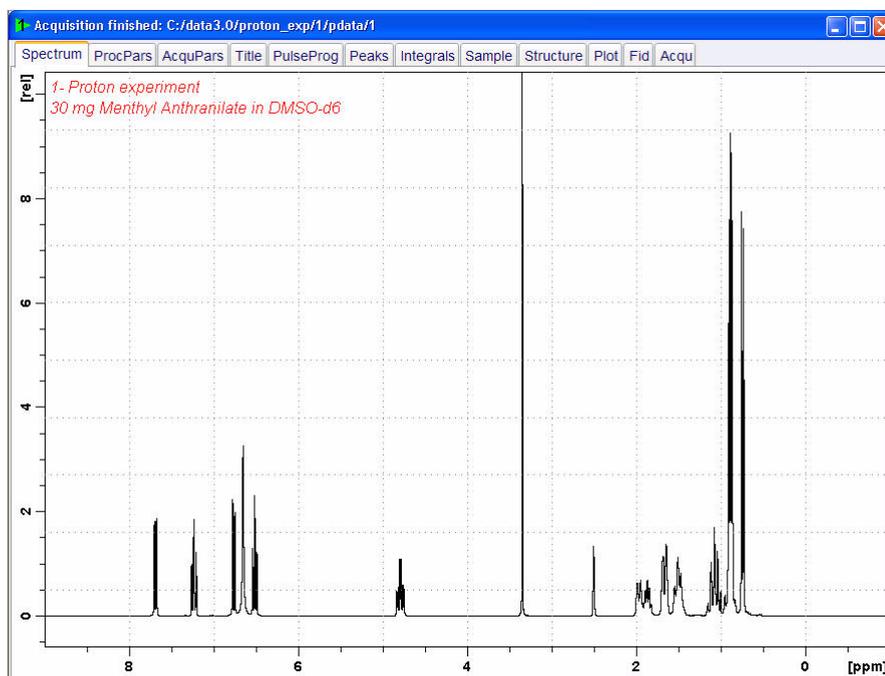
Figure 3.13

4. Click on ⬇ to set the sweep width and the O1 frequency of the displayed region

Figure 3.14

5. Click on [Close]
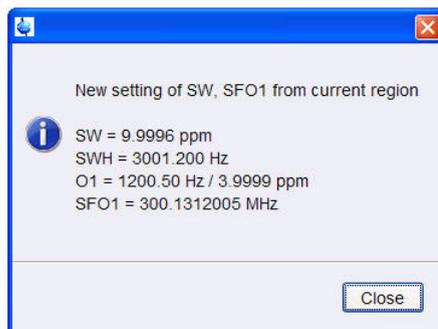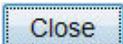
6. Click on [▶] to start the acquisition

7. Click on [∧ Pro<u>c</u>. Spectrum ▽]

8. Expand the spectrum to include all peaks

Figure 3.15
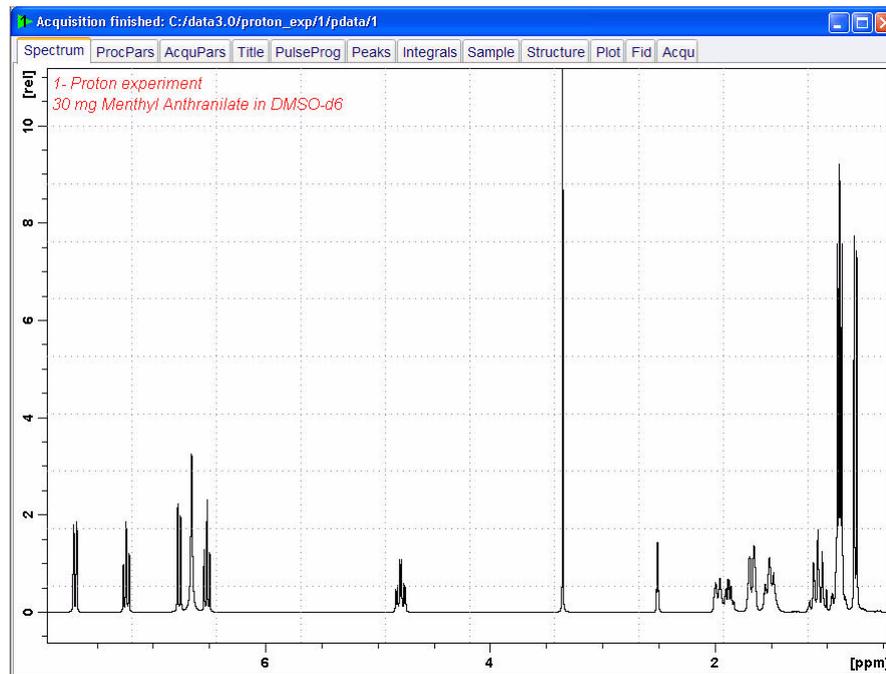
9. Click on [∫ Integrate ▽]

NOTE: This enters the manual Integration mode. Other options are available by clicking on the down arrow inside the '**Integrate**' button.

10. Set the cursor line, starting at the left of the spectrum, to the left of the first peak to be integrated, click the left mouse button and drag the cursor line to the right of the peak, then release the mouse button

Figure 3.16
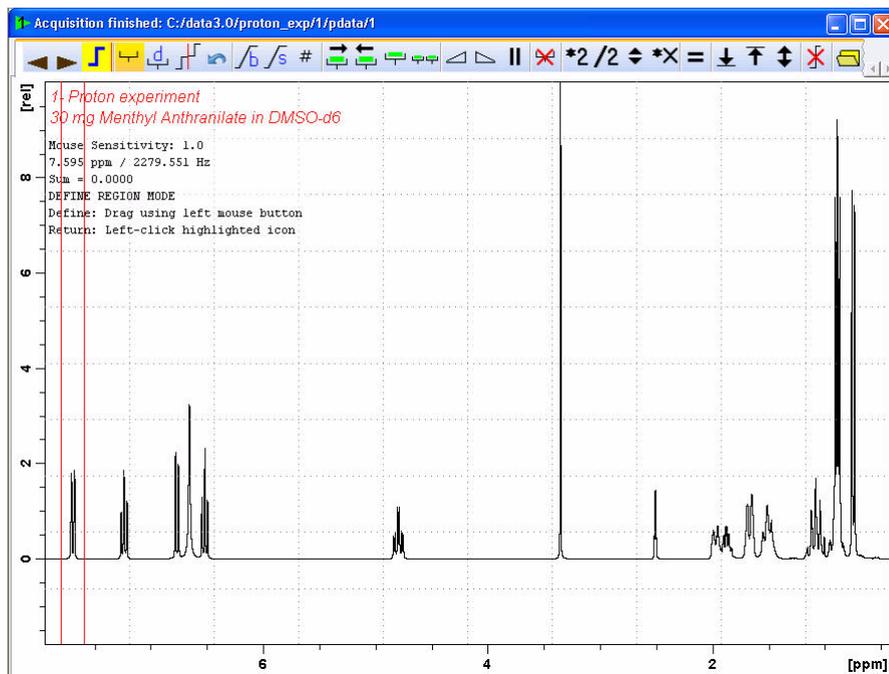
11. Repeat step13 for the remainder of the peaks

Figure 3.17
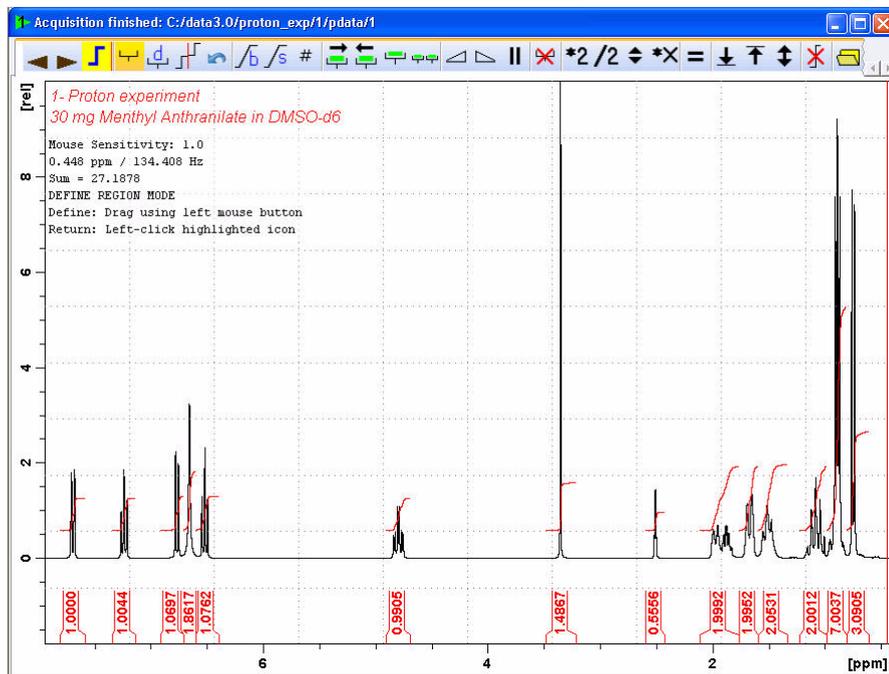
12. Click on 📁 to save the integration regions

3.2.6 Plotting the 1D Proton spectra

1. Expand the spectrum (all peaks in display)

2. Click on [ppm]

3. Type the following F1 [ppm] values:

From = **8.5**

To = **0.5**

Figure 3.18
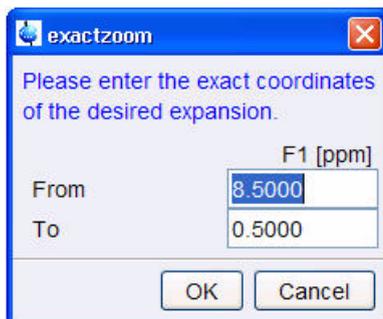
4. Click on OK

5. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 3.19

6. Click on Plot Layout
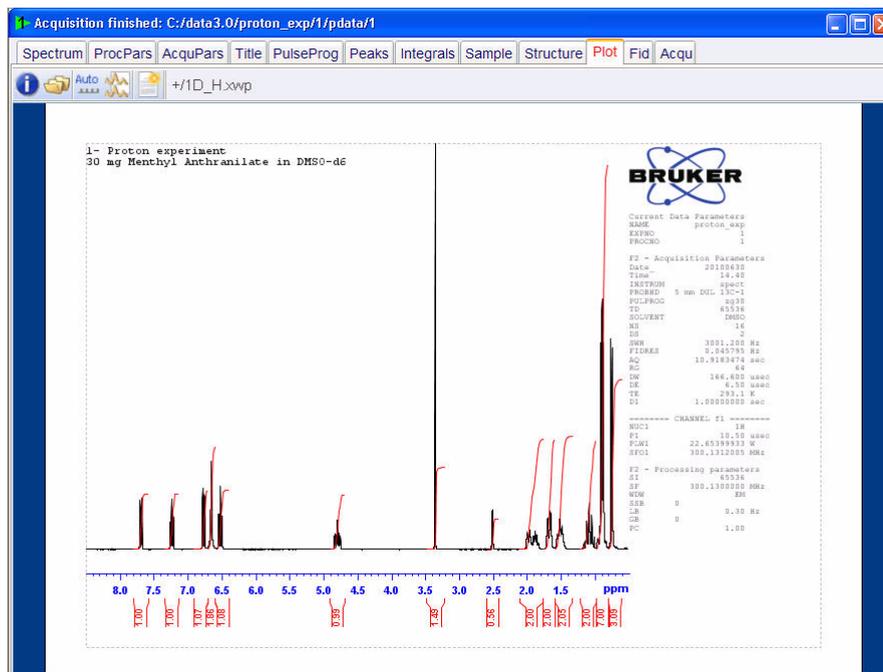
Figure 3.20

NOTE: If desired, any changes can be administered by clicking on the [icon] icon to open the Plot Editor.

7. Click on the [Auto] to plot the spectrum

3.2.7 Observations

4 2-D Homonuclear experiments

4.1 Sample

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

Figure 4.1
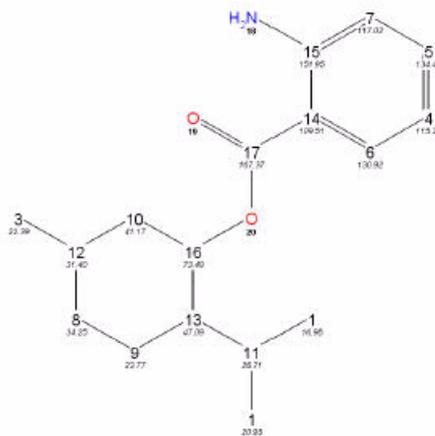
4.2 2-D gradient COSY

4.2.1 Introduction

The COSY experiment relies on the J-coupling to provide spin-spin correlation, and whose cross peaks indicate which 1H's are close to which other 1H's through the bonds of the molecule. Typically proton which are up 3 bonds away can be observed.

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

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

Section 4.2 describes the acquisition and processing of a two-dimensional 1H gradient COSY. The standard Bruker parameter set is **COSYGPSW** and includes the pulse sequence **cosygpppqf** shown in Figure 4.2. It consists of the recycling delay, two radio-

frequency (RF) pulses, separated by the increment delay D0 and the acquisition time during which the signal is recorded. Both pulses have a 90 degrees angle. Two gradient pulses are applied before and after the second pulse in the sequence. Purge pulses are applied before d1.

Figure 4.2
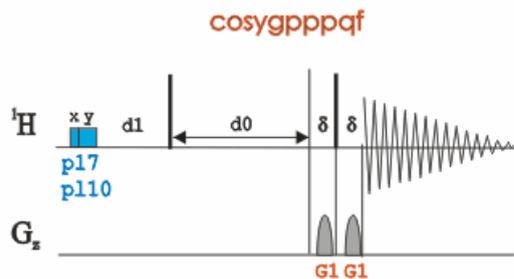
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

4.2.2   Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3, 1-D Proton experiment, Paragraph 3.2.2 Experiment setup** through **3.2.4 Processing**.
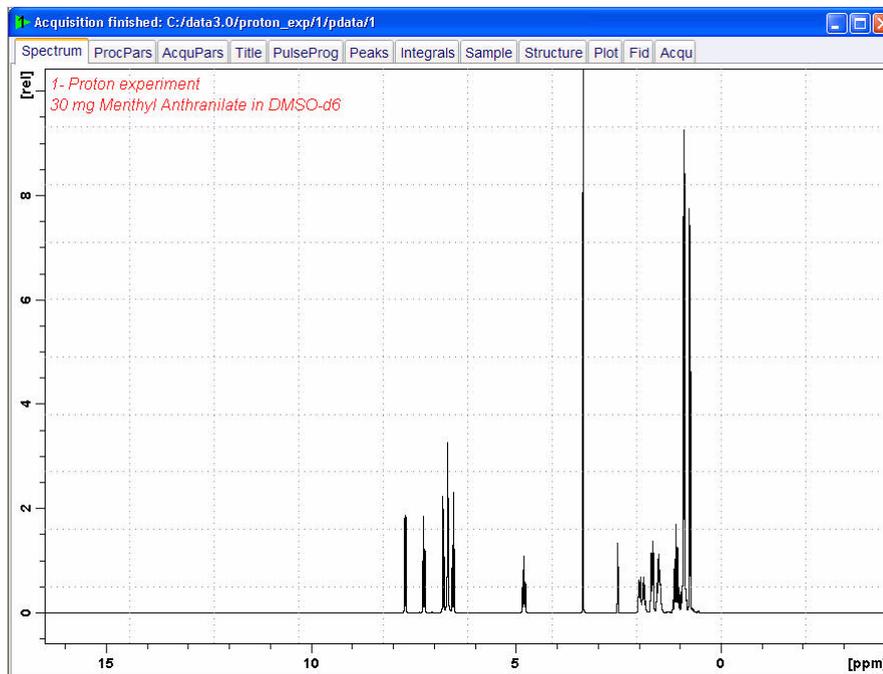
Figure 4.3

4.2.3 Setting up the COSY experiment

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 4.4

2. Select [Create Dataset] by clicking on it

3. Enter the following information in to the 'New' window

Figure 4.5
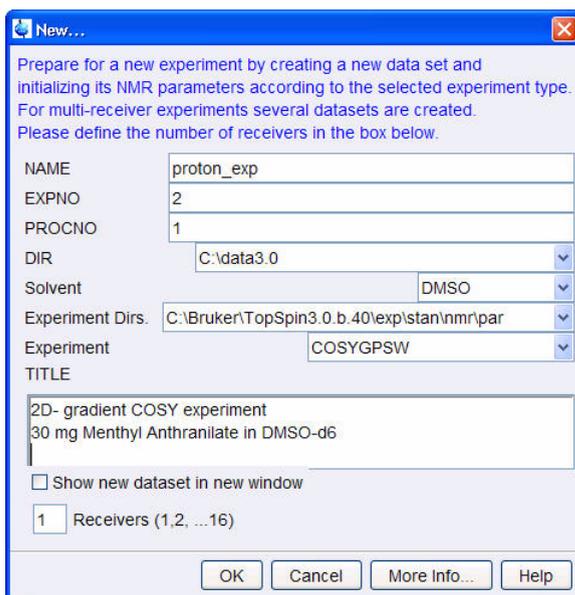
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 4.5 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 4.6
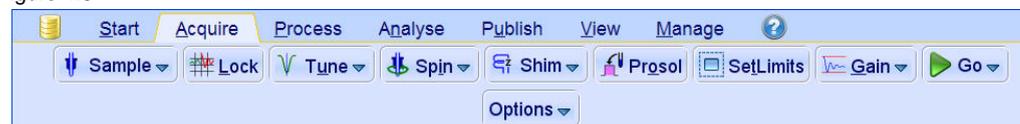
6. Select [Spin] by clicking on it

Figure 4.7
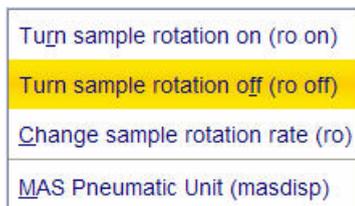
7. Select '**ro off**' by clicking on it

NOTE: 2-D experiments should be run non spinning

8. Select by clicking on it

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

9. Select by clicking on it

Figure 4.8
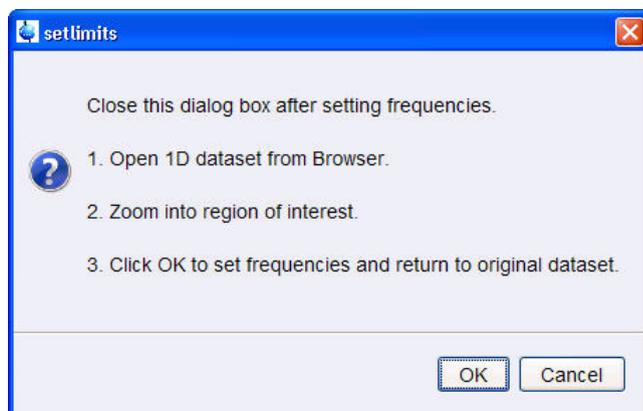
10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

NOTE: 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.

Figure 4.9
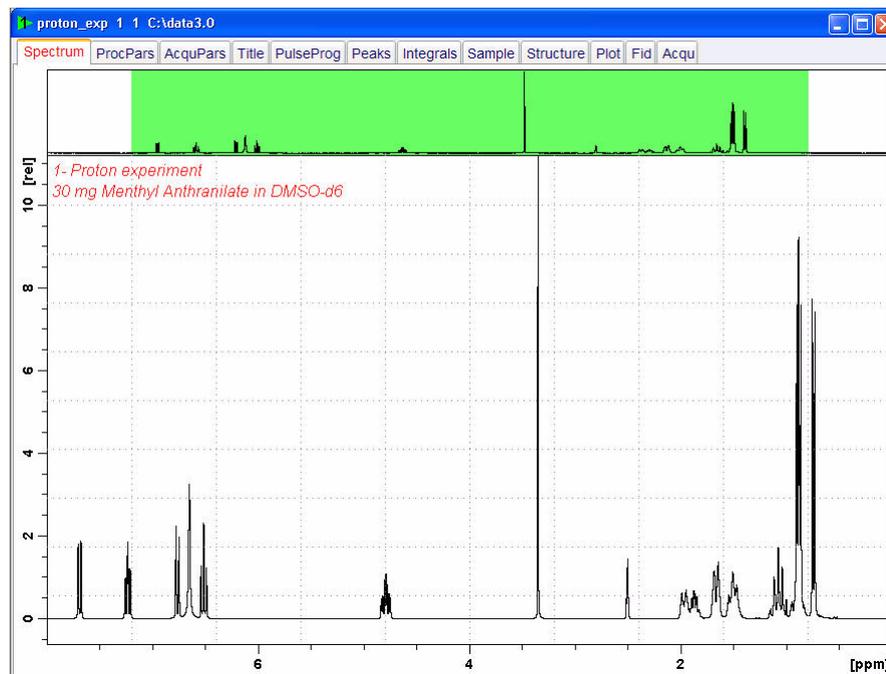
12. Click on [OK] to assign the new limit

Figure 4.10

13. Click on [Close]
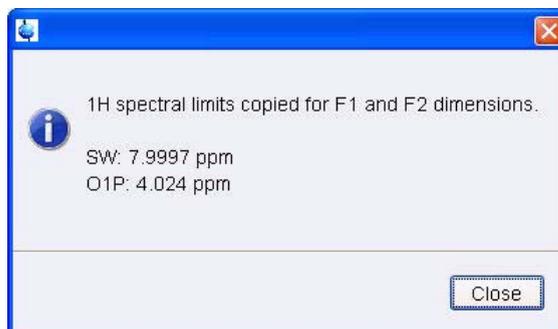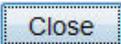
NOTE: The display changes back to the 2D data set.

4.2.4 Acquisition

1. Select [Gain] by clicking on it

2. Select [Go] by clicking on it

4.2.5   Processing

1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 4.11

2. Select by clicking on it

Figure 4.12
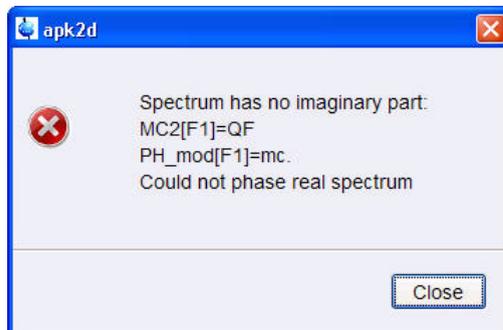
NOTE: This executes a standard processing program **proc2**. The message shown in Figure 4.12 pops up in case of a magnitude 2D experiment and the apk2d option is enabled. To configure the processing program follow steps 4 through 5.
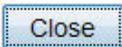
3. Click on

4. Click on the down arrow inside the button

Figure 4.13
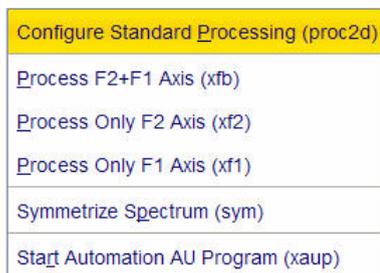
5. Select 'Configure Standard Processing' by clicking on it

Figure 4.14
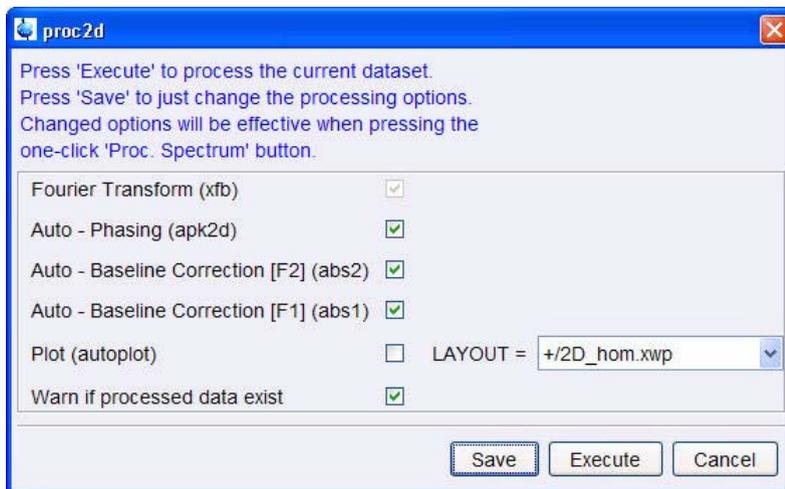
NOTE: To avoid the message shown in Figure 4.12 the option '**Auto-Phasing (apk2d)**' may be disabled for magnitude like 2D experiment.

Figure 4.15
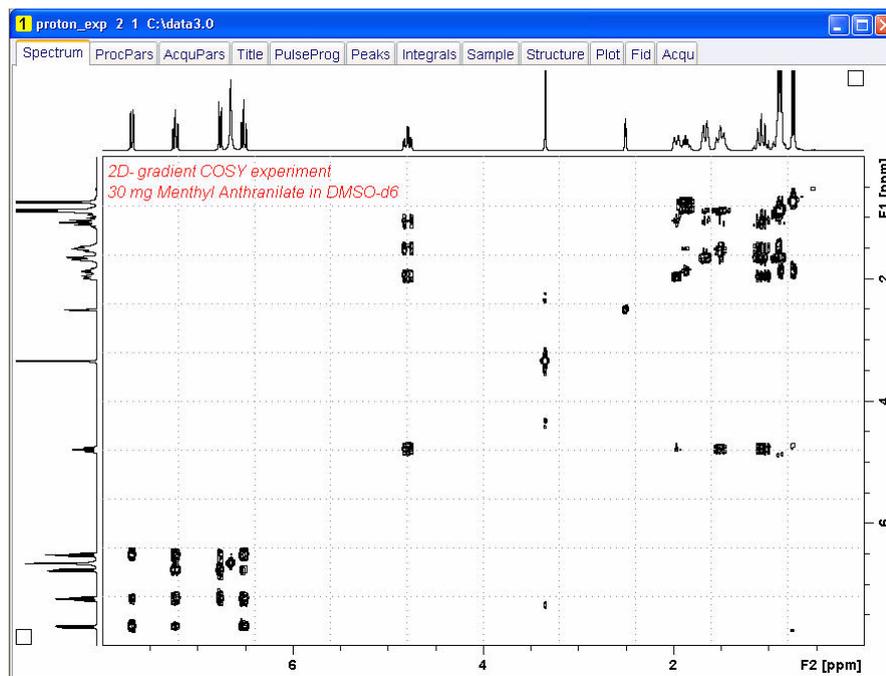
4.2.6   Plotting

1. Use the buttons to adjust for a suitable contour level

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 4.16

3. Click on **Plot Layout ▾**

4. Select the '**Plot**' tab by clicking on it

Figure 4.17
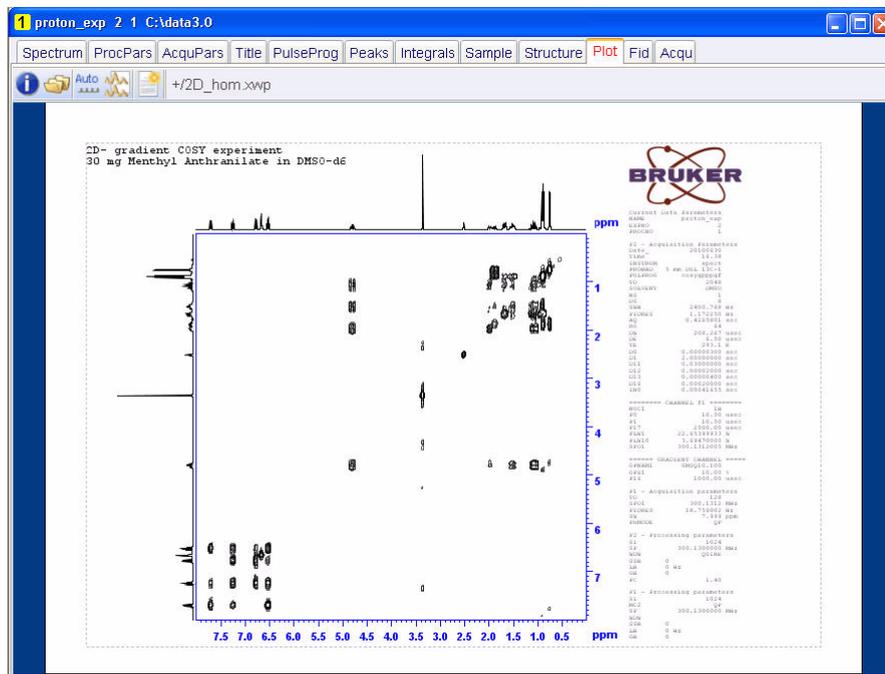
NOTE: If desired, any changes can be administered by clicking on the ⬚ icon to open the Plot Editor.

5. Click on the **Auto** to plot the spectrum

4.2.7 Observations

4.3 2-D gradient NOESY experiment

4.3.1 Introduction

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

The basic NOESY sequence consists of three p/2 pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t1, which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period d8. Note that, for he basic NOESY experiment, d8 is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time t2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t1 and t2.

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

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

Section 4.3 describes the acquisition and processing of a two-dimensional 1H phase sensitive NOESY. The standard Bruker parameter set is **NOESYPHSW** and includes the pulse sequence **noesygpphpp** shown in Figure 4.18. It consists of the recycling delay, three radio-frequency (RF) pulses, separated by the increment delay D0 between the first and second pulse, a mixing time D8 between the second and third pulse and the acquisition time during which the signal is recorded. All three pulses are of 90 degree.

Figure 4.18
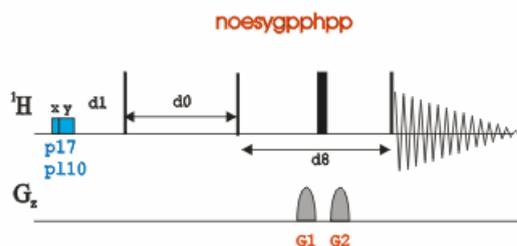
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

4.3.2   Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3, 1-D Proton experiment, Paragraph 3.2.2 Experiment setup** through **3.2.4 Processing**.

Figure 4.19
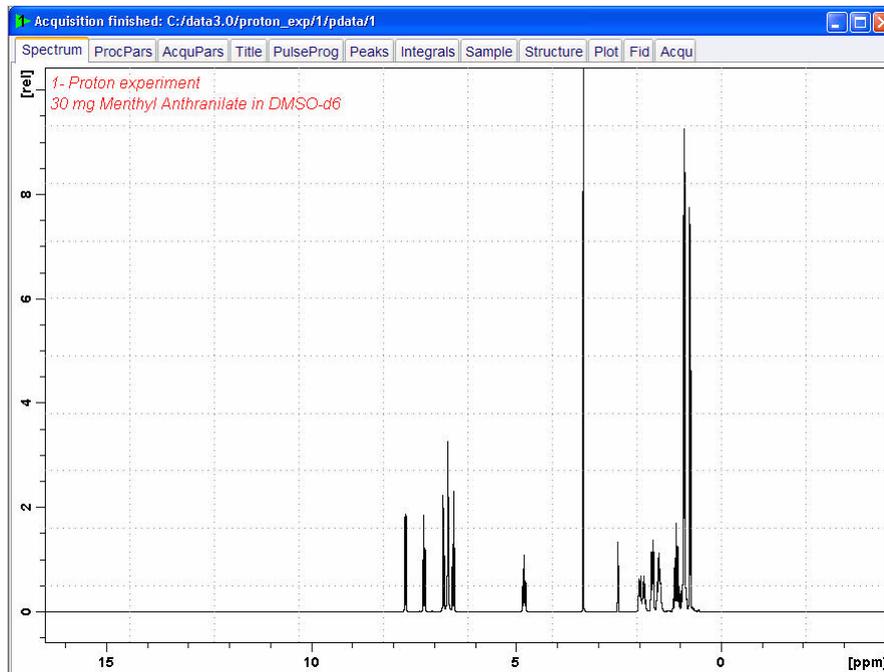
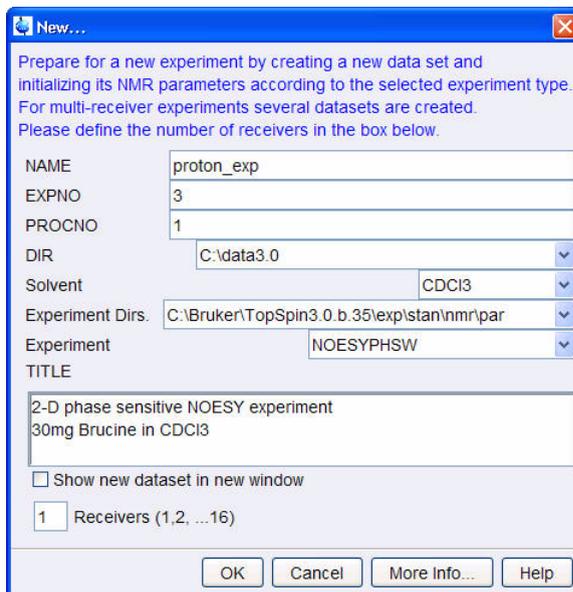## 4.3.3   Setting up the NOESY experiment

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 4.20

2. Select **Create Dataset** by clicking on it
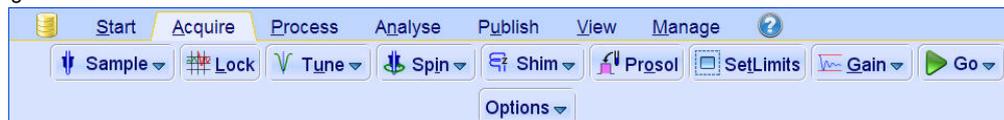3. Enter the following information in to the 'New' window

Figure 4.21

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 4.21 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 4.22

6. Select [Spin] by clicking on it

Figure 4.23
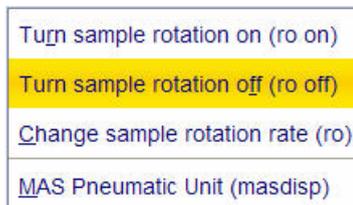
7. Select '**ro off**' by clicking on it

NOTE: 2-D experiments should be run non spinning

8. Select [Prosol] by clicking on it

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

9. Select **SetLimits** by clicking on it

Figure 4.24
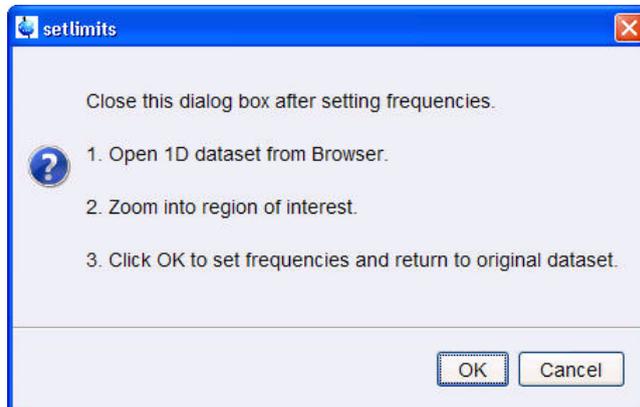
10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

NOTE: 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.

Figure 4.25
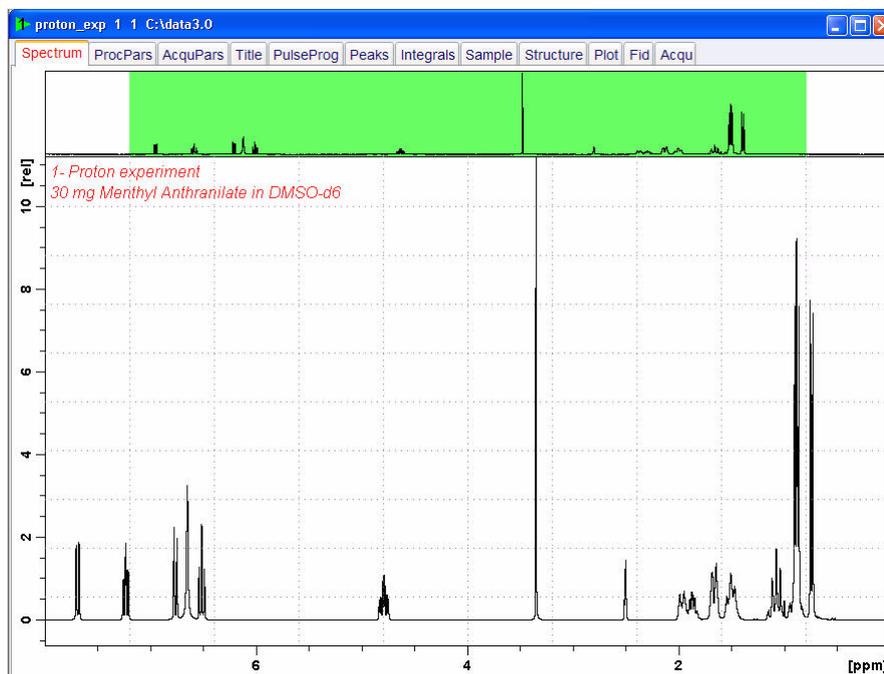
12. Click on [OK] to assign the new limit

Figure 4.26
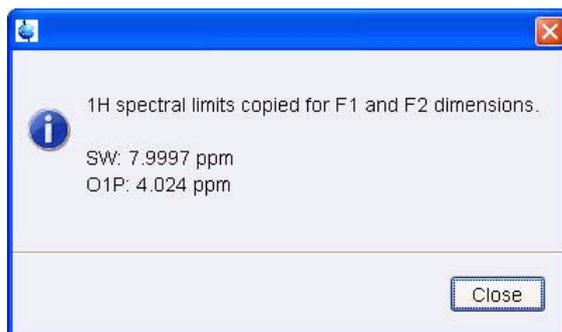
13. Click on [Close]
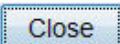
NOTE: The display changes back to the 2D data set.

14. Select the '**AcquPars**' tab by clicking on it

15. Click on [⊓] to display the pulse program parameters

16. Make the following change

Figure 4.27

| D8 [sec] | 0.450 | Mixing time |

NOTE: The mixing time depends on the size of the Molecule. The range for Biomolecules are typically from 0.05 to 0.2 sec., medium size molelecules from 0.1 to 0.5 s ec. and for small molecules 0.5 to 0.9 sec.

17. Select the '**Spectrum**' tab by clicking on it

4.3.4   Acquisition

1. Select [Gain ▾] by clicking on it

2. Select [Go ▾] by clicking on it

4.3.5   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 4.28
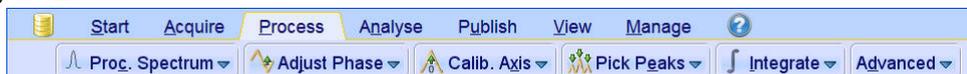
2. Select ![Proc. Spectrum button] by clicking on it

NOTE: This executes a standard processing program **proc2**. To configure this program or select other options, click on the down arrow inside the '**Proc. Spectrum**' button.

Figure 4.29
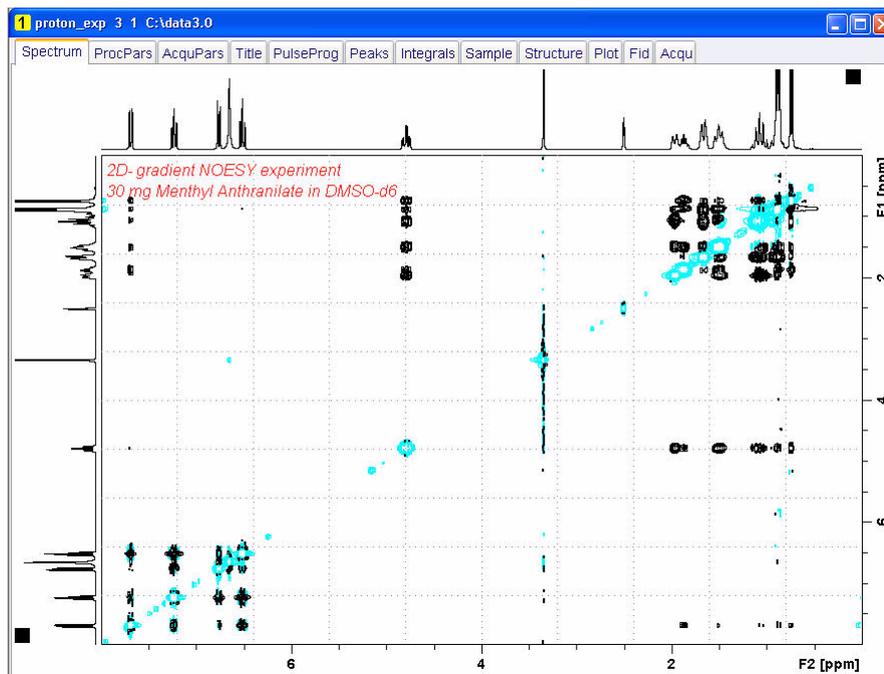
4.3.6 Plotting

1. Use the ![contour buttons] buttons to adjust for a suitable contour level

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 4.30

4. Select the '**Plot**' tab by clicking on it

Figure 4.31

NOTE: If desired, any changes can be administered by clicking on the ![icon] icon to open the Plot Editor.

5. Click on the ![Auto] to plot the spectrum
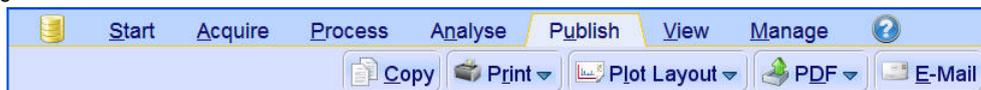
4.3.7 Observations

4.4　2-D phase sensitive TOCSY experiment

4.4.1　Introduction

TOCSY (TOtal Correlation SpectroscopY) provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy in liquids. In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that pure absorption mode spectra with positive intensity peaks are created. In traditional COSY, cross peaks have zero integrated intensity and the coherence transfer is restricted to directly spincoupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as antiphase coherence.

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

Section 4.4 describes the acquisition and processing of a two-dimensional 1H phase sensitive TOCSY. The standard Bruker parameter set is **MLEVPHSW** and includes the pulse sequence **mlevphpp** shown in Figure 4.32. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay D0 and the acquisition time during which the signal is recorded. The first RF pulse is a 90 degree pulse, the second pulse is the mlev spinlock pulse.

Figure 4.32
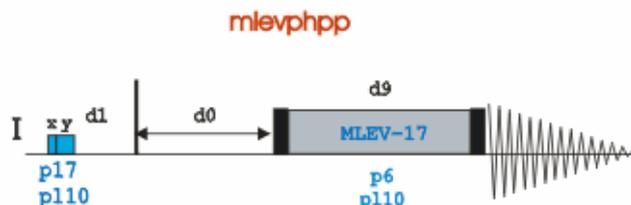
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

4.4.2 Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3, 1-D Proton experiment, Paragraph 3.2.2 Experiment setup** through **3.2.4 Processing**.

Figure 4.33
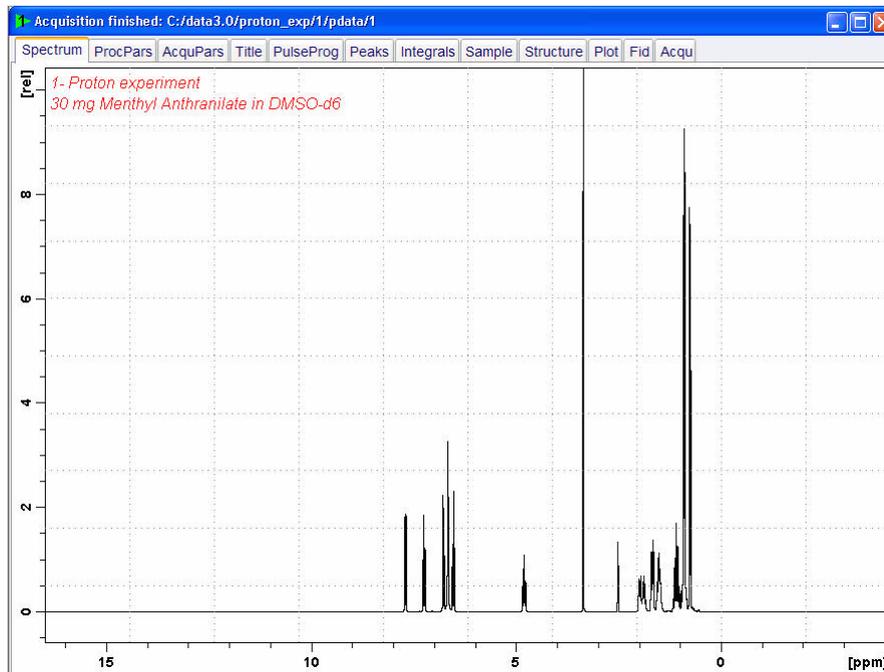
4.4.3 Setting up the TOCSY experiment

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 4.34

2. Select **Create Dataset** by clicking on it

3. Enter the following information in to the 'New' window

Figure 4.35
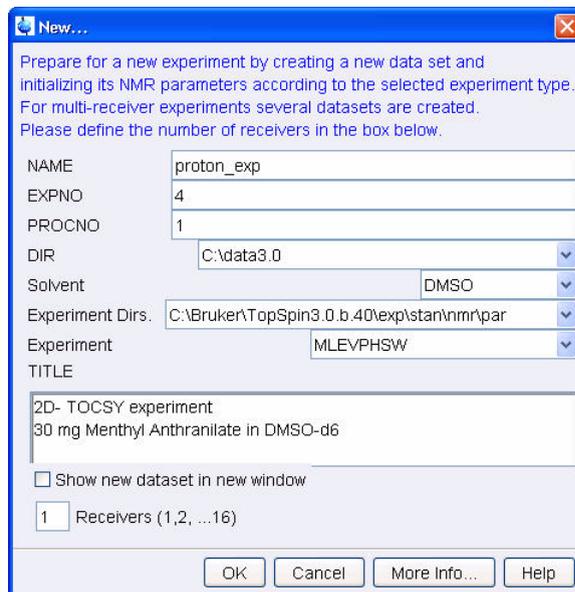
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 4.35 above. Click on the down arrow button to browse for a specific directory.

---

4. Click on

5. Click on the 'Aquire' tab in the TopSpin menu bar

Figure 4.36
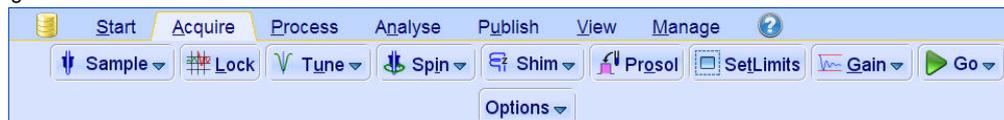
6. Select by clicking on it

Figure 4.37
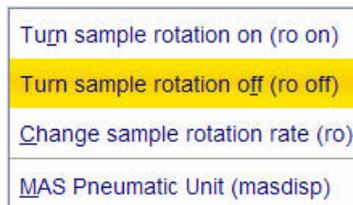
7. Select 'ro off' by clicking on it

NOTE: 2-D experiments should be run non spinning

---

8. Select by clicking on it

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

9. Select [☐ SetLimits] by clicking on it

Figure 4.38
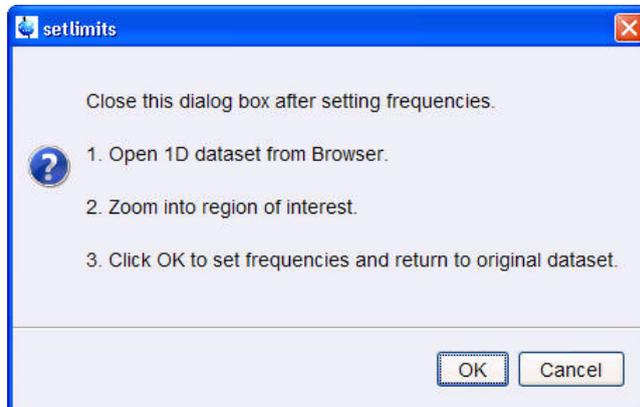
10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

NOTE: 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.

Figure 4.39
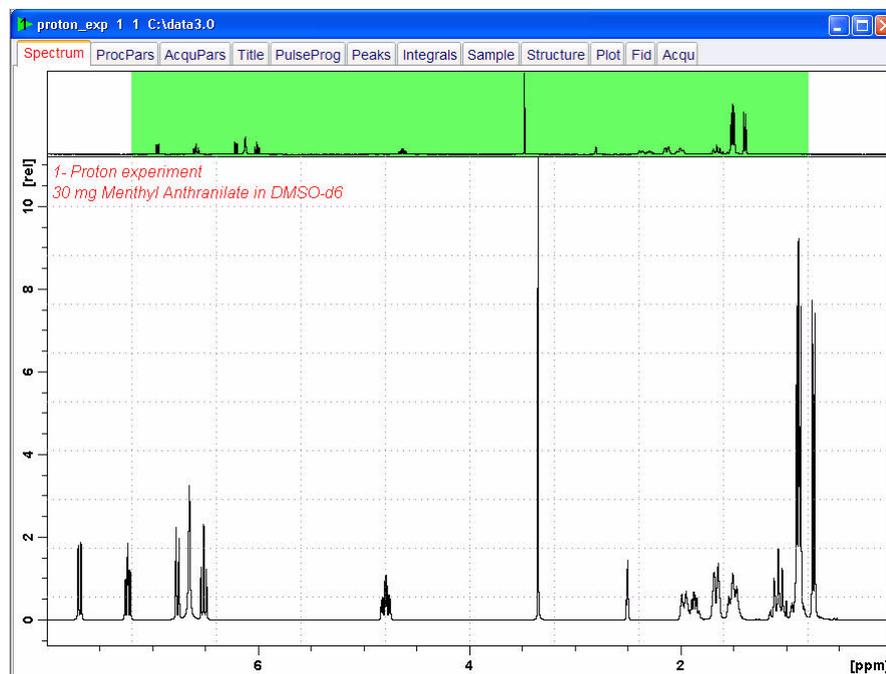
12. Click on [OK] to assign the new limit

Figure 4.40
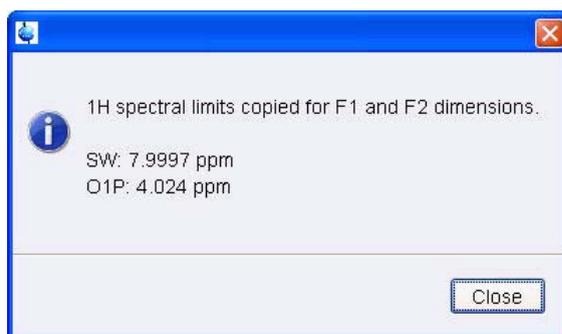
13. Click on [Close]
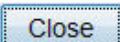
NOTE: The display changes back to the 2D data set.

---

14. Select the '**AcquPars**' tab by clicking on it

15. Click on [icon] to display the pulse program parameters

16. Make the following change

Figure 4.41

D9 [sec]   0.08000000   TOCSY mixing time

NOTE: A mixing time of 0.06 to 0.08 sec. is typically for the TOCSY experiment.

---

17. Select the '**Spectrum**' tab by clicking on it

4.4.4   Acquisition

1. Select [Gain] by clicking on it

2. Select [Go] by clicking on it

4.4.5   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar
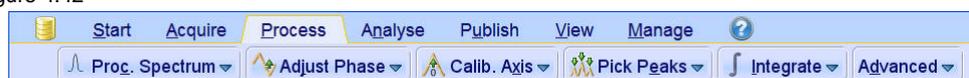
Figure 4.42

Start   Acquire   Process   Analyse   Publish   View   Manage   ?
Proc. Spectrum ▾   Adjust Phase ▾   Calib. Axis ▾   Pick Peaks ▾   ∫ Integrate ▾   Advanced ▾

2. Select [Proc. Spectrum] by clicking on it

NOTE: This executes a standard processing program **proc2**. To configure this program or select other options, click on the down arrow inside the 'Proc. Spectrum' button.
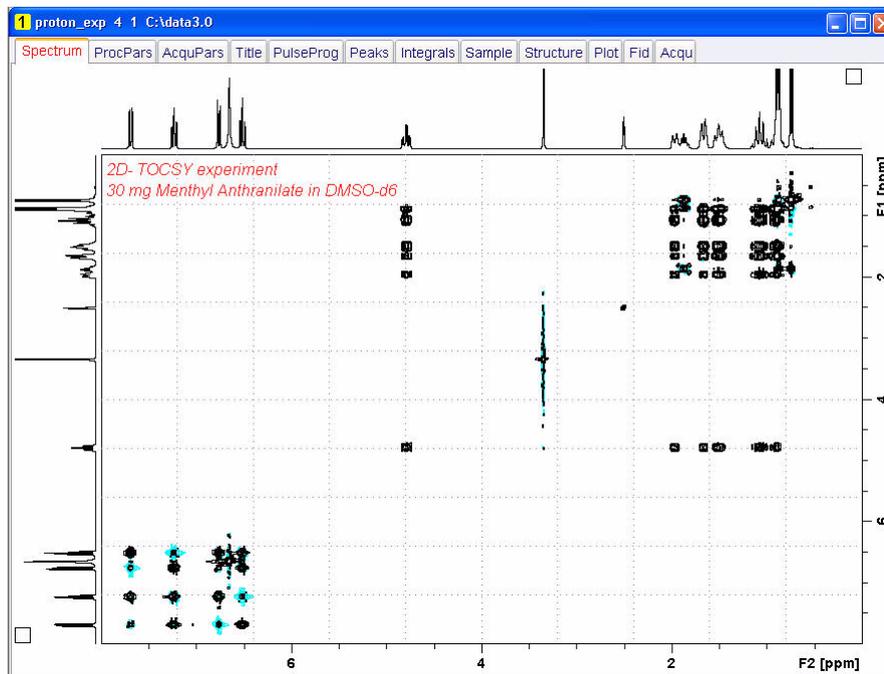
Figure 4.43

4.4.6   Plotting

1. Use the [button] buttons to adjust for a suitable contour level

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 4.44

3. Click on [Plot Layout]
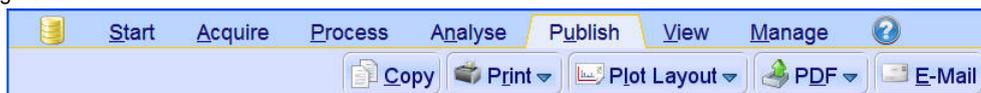
4. Select the '**Plot**' tab by clicking on it

Figure 4.45
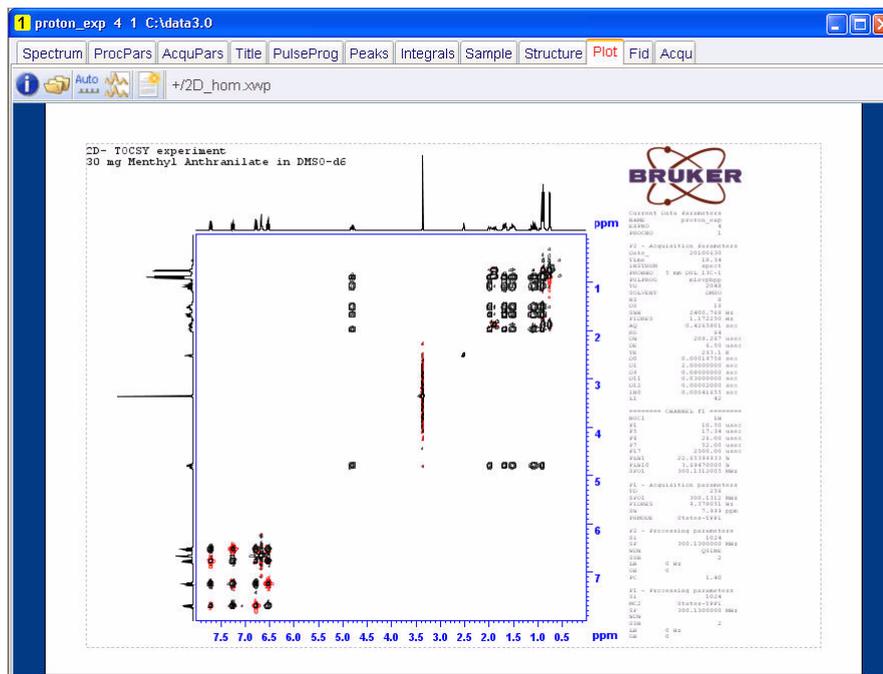
NOTE: If desired, any changes can be administered by clicking on the [icon] icon to open the Plot Editor.

5. Click on the [Auto] to plot the spectrum

4.4.7   Observations

5   1-D Selective experiments

5.1   Sample

A sample of **30mg Menthyl Anthranilate in DMSO-d6** is used for the experiment in this chapter

Figure 5.1
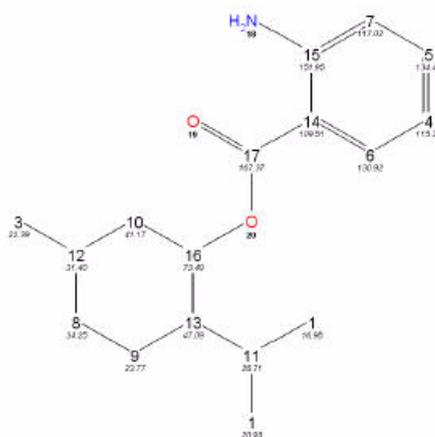
5.2   1-D Selective COSY

5.2.1   Introduction

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

NOTE: 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 5.2 describes the acquisition and processing of a one-dimensional 1H selective gradient COSY experiment. The standard Bruker parameter set is **SELCOGP** and includes the pulse sequence **selcogp** shown in Figure 5.2. 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 degree pulse, followed by a 180 degree shaped pulse, a 180 degree hard pulse and finally a 90 degree pulse. The delay between the 180 and 90 degree pulse is 1/4*J(H,H). The gradient pulses are applied before and after the shape pulse.

Figure 5.2
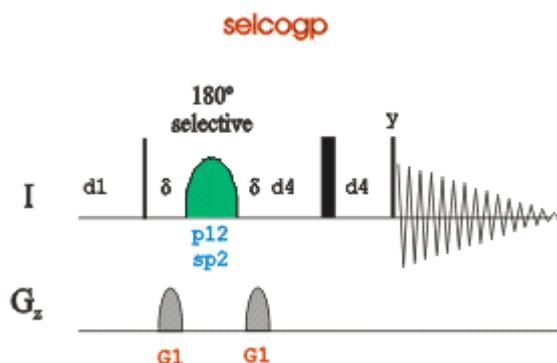
5.2.2   Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3**, **1-D Proton experiment, 3.2.2 Experiment setup,** through **3.2.4 Processing**

Figure 5.3
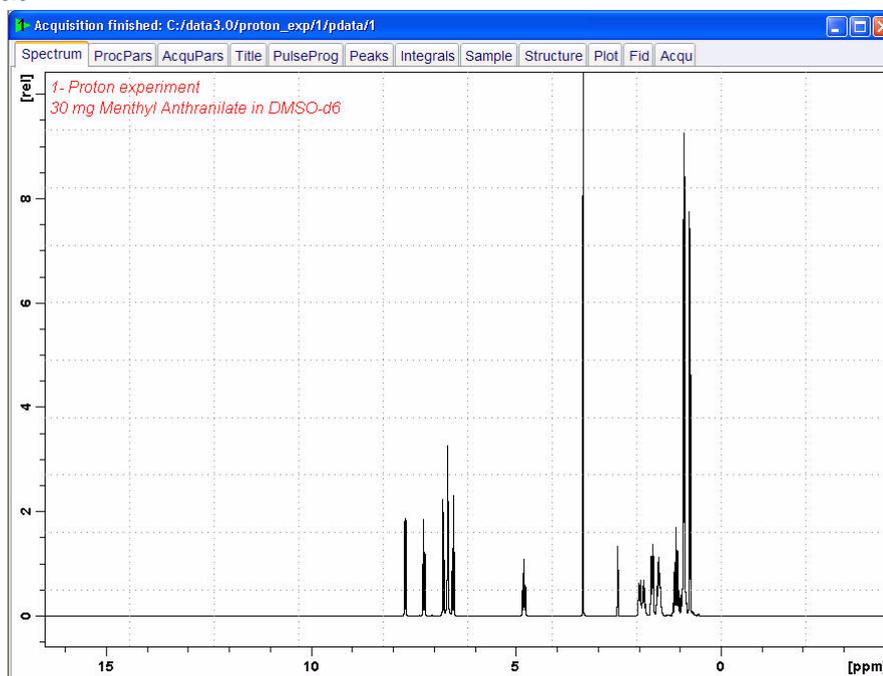
5.2.3   Selective excitation region set up

5.2.3.1   On resonance

NOTE: Make sure 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

1. Type **wrpa**
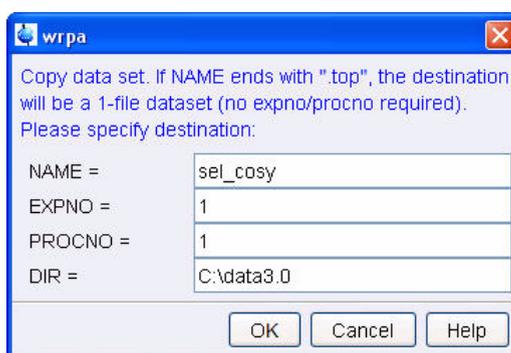
Figure 5.4

2. Change NAME = **sel_cosy**

3. Click on OK

4. Type **re**
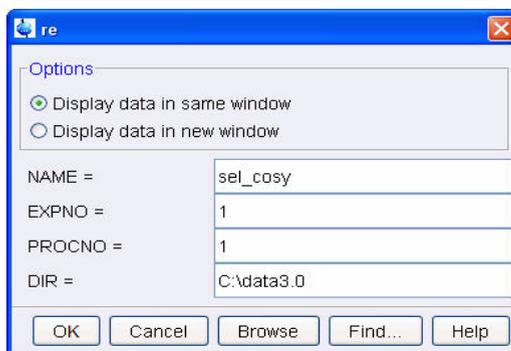
Figure 5.5

5. Change NAME = **sel_cosy**

6. Click on OK

7. Expand peak at 7.7 ppm

8. Click on ⬇ to set the RF from cursor

Figure 5.6
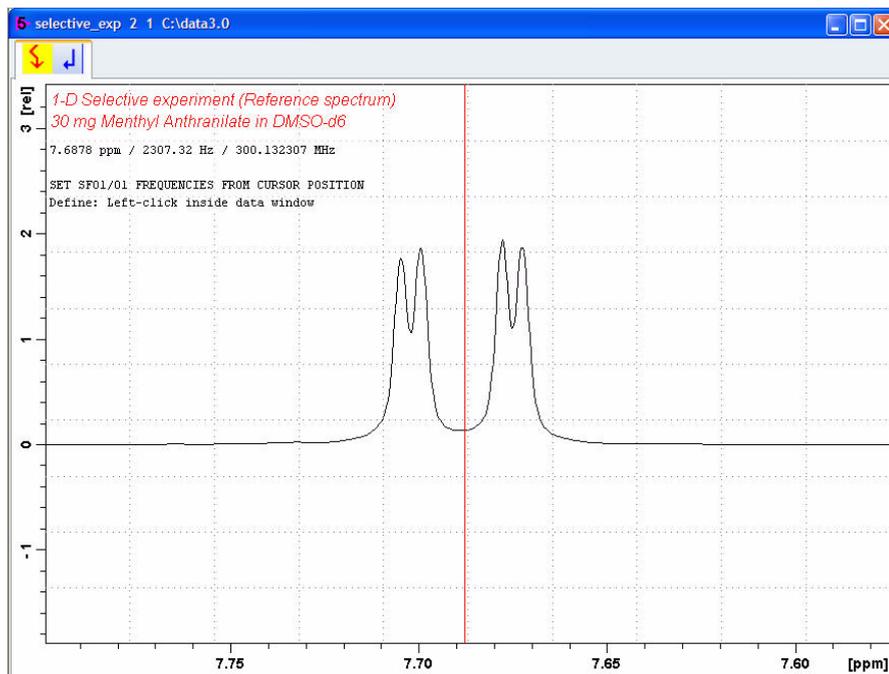
9. Move the cursor line in to the center of the multiplet

10. Click the left mouse button to set the frequency

Figure 5.7
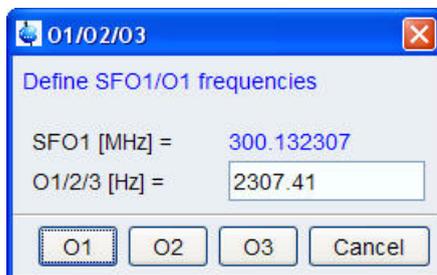
11. Click on O1

5.2.4 Setting up the Selective COSY

1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 5.8

2. Select Read Pars. by clicking on it

Figure 5.9
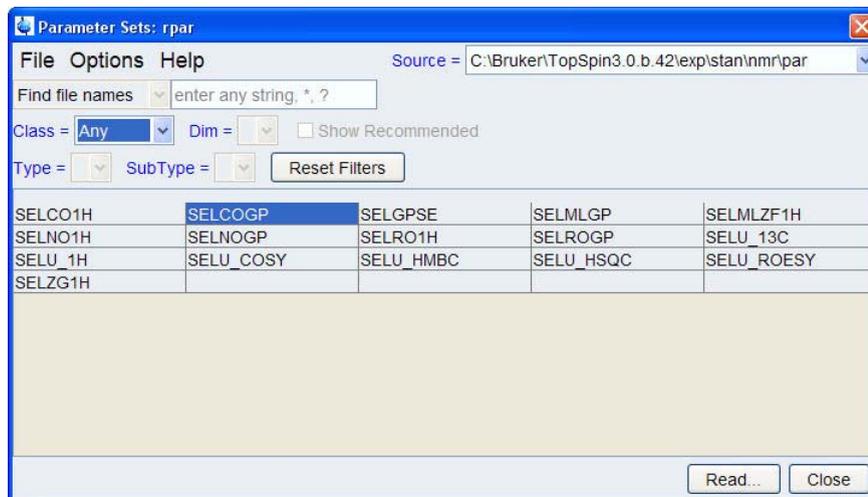
NOTE: Enter **SEL*** in to the '**Find file names**' window and hit '**Enter**' to display all selective parameter sets shown in figure 5.9.

3. Select '**SELCOGP**'

4. Click on Read...
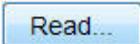
5. Select the **acqu**, **proc** and **outd** parameter options only

6. Click on the down arrow next to the '**Keep the following parameter**' window

7. Select '**P1, O1, PLW1**' from the pull down menu

Figure 5.10
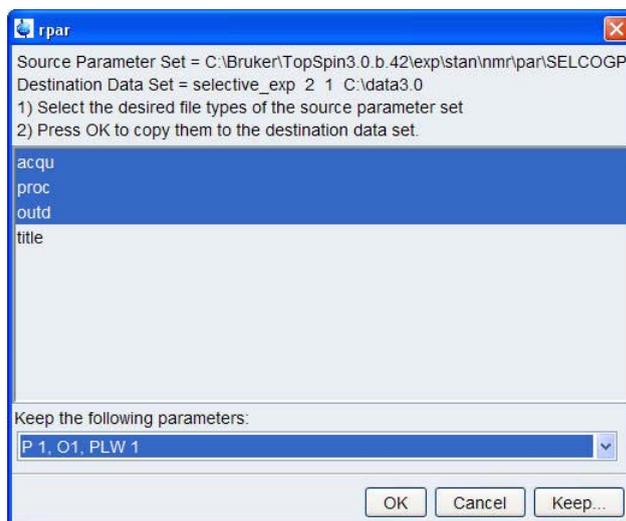
8. Click on OK

9. Select the '**Title**' tab by clicking on it

10. Make the following changes:

1-D Selective gradient COSY experiment
30 mg Menthyl Anthranilate in DMSO-d6

11. Click on [icon] to store the title

12. Select the '**Spectrum**' tab by clicking on it

13. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 5.11

14. Select [Spin icon] by clicking on it

Figure 5.12
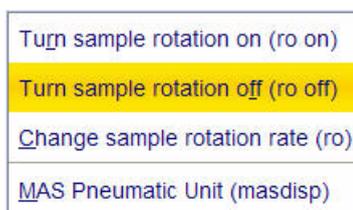
15. Select '**ro off**' by clicking on it

NOTE: 1-D selective experiments should be run non spinning

16. Select [Prosol icon] by clicking on it

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

5.2.5   Acquisition

1. Select [Gain icon] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select [Go icon] by clicking on it

NOTE: Other options are available by clicking on the down arrow inside the '**Go**' button.

5.2.6   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.13
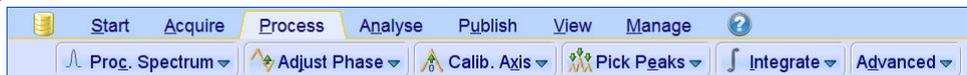
2. Click on the down arrow inside the button

Figure 5.14
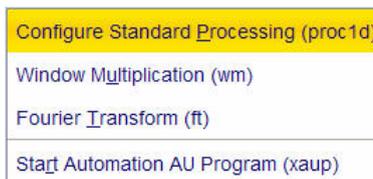
3. Select '**Configure Standard Processing**' by clicking on it

4. Deselect the following options:

> '**Auto-Phasing (apk)**'
> '**Set Spectrum Reference (sref)**'
> '**Auto-Baseline correction (abs)**'
> '**Warn if Processed data exist**'

Figure 5.15
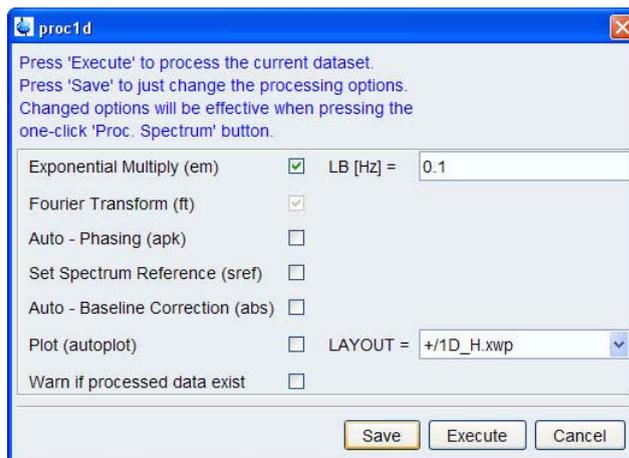
5. Click on
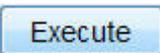
6. Expand the spectrum from 8 ppm to 6 ppm

Figure 5.16
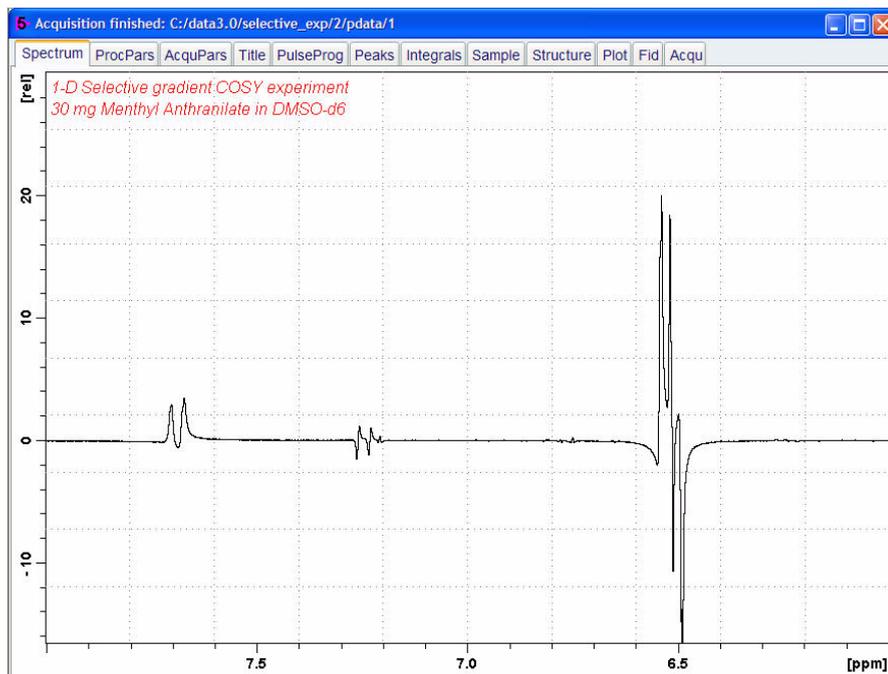
7. Click on

8. Adjust the **0** order phase on the peak at 6.5 ppm to display a antiphase pattern

Figure 5.17
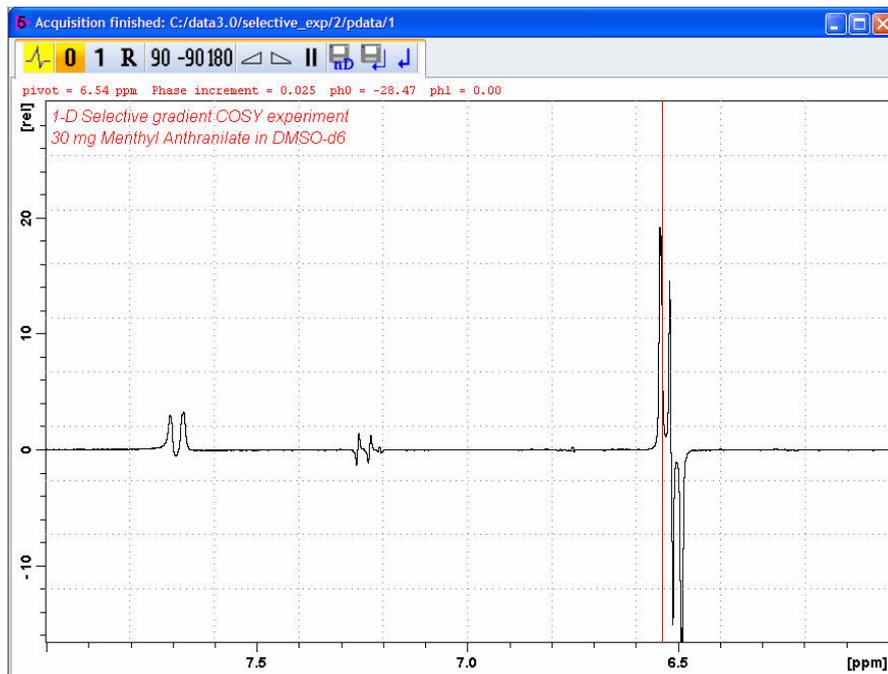
9. Click on to store the phase value

5.2.7 Plotting two spectra on to the same page

1. Display the selective COSY spectrum

2. Click on [icon] to enter the Multiple display option

3. Drag the Reference spectrum in to the spectral window

Figure 5.18
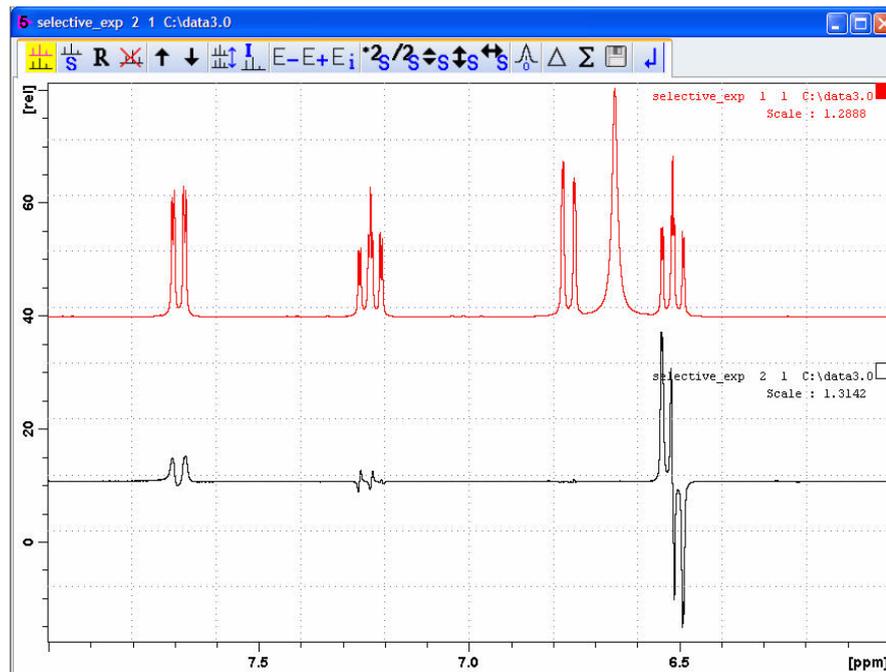
NOTE: To adjust the spectra for best fit, use the [icon] tools

4. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 5.19
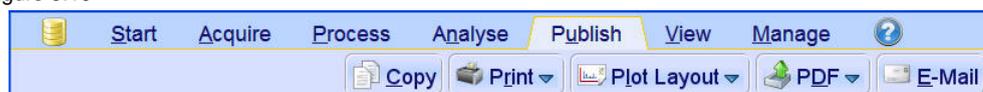
5. Click on the [Print] button to print the active window

5.2.8 Observations

5.3 1-D Selective NOESY

5.3.1 Introduction

Section 5.4 describes the acquisition and processing of a one-dimensional 1H selective gradient NOESY experiment. The standard Bruker parameter set is **SELNOGP** and includes the pulse sequence **selnogp** shown in Figure 5.20. It consists of the recycling delay, five radio-frequency (RF) pulses and the acquisition time during which the signal is recorded. The first RF pulse is a 90 degree pulse, followed by a 180 degree shaped pulse, a 90 degree pulse, a 180 degree pulse and finally a 90 degree pulse. The mixing time **D8** is applied before and after the 180 degree pulse. There are four gradient pulses applied, one each between the RF pulses.

Figure 5.20
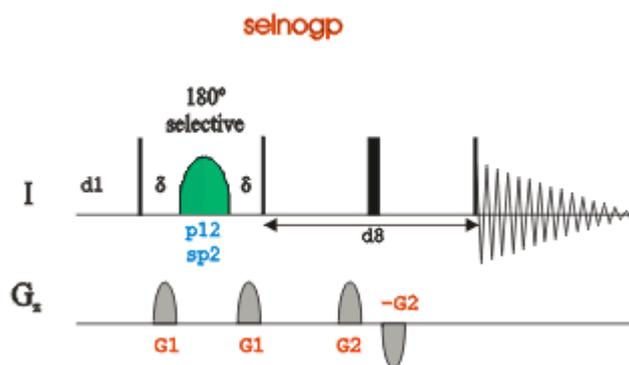
5.3.2 Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3**, **1-D Proton experiment, 3.2.2 Experiment setup,** through **3.2.4 Processing**

Figure 5.21
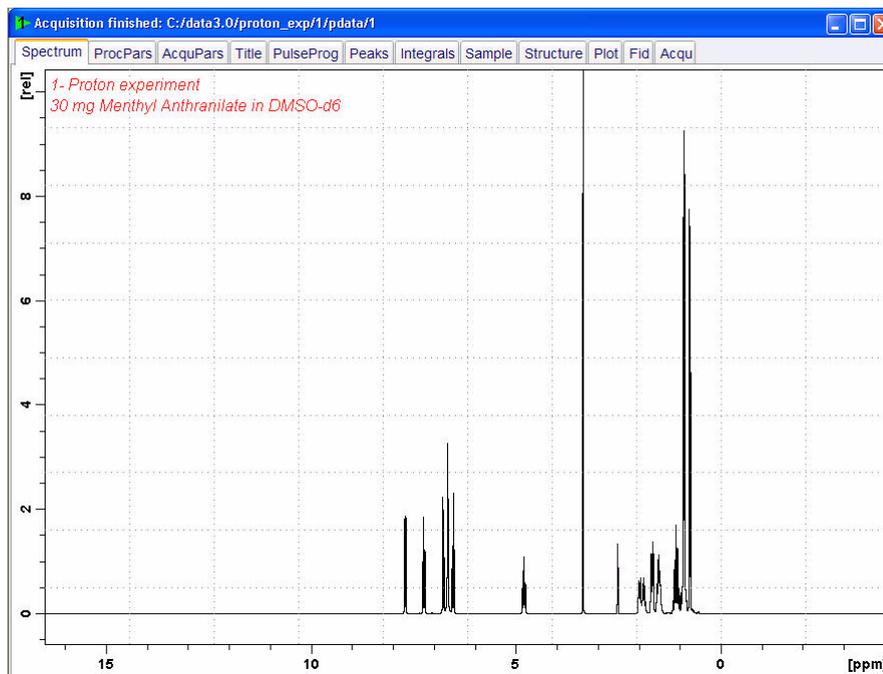
5.3.3   Selective excitation region set up

5.3.3.1   On resonance

NOTE: Make sure 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

1. Type **wrpa**

Figure 5.22
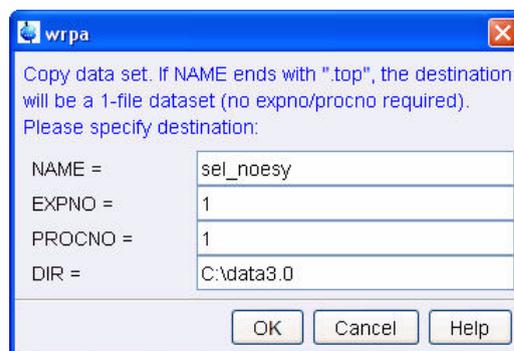
2. Change NAME = **sel_noesy**

3. Click on [OK]

4. Type **re**

Figure 5.23
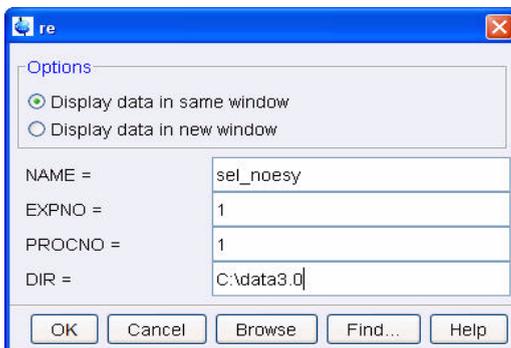
5. Change NAME = **sel_noesy**

6. Click on [OK]

7. Expand peak at 4.8 ppm

8. Click on ⬇ to set the RF from cursor

Figure 5.24
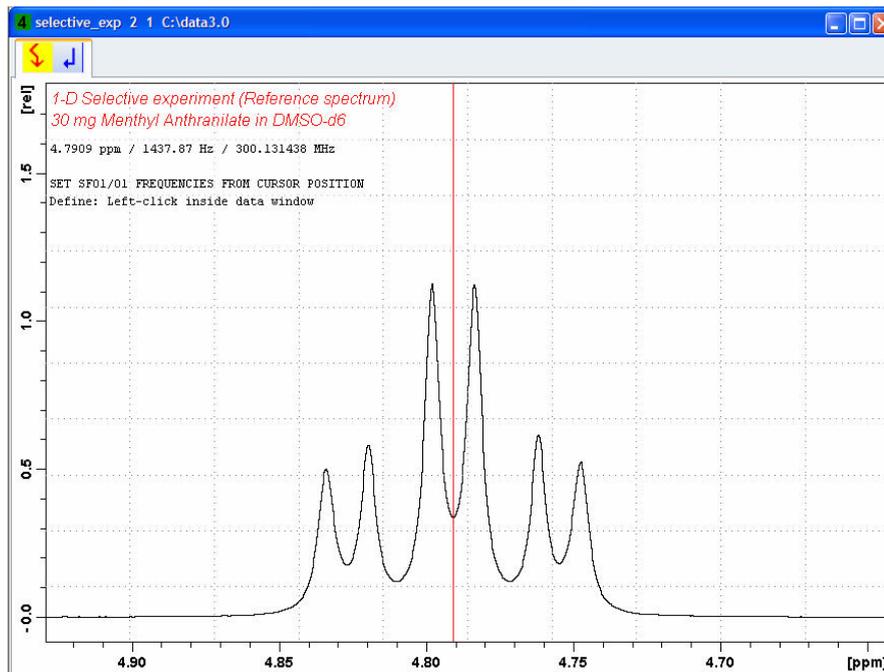
9. Move the cursor line in to the center of the multiplet

10. Click the left mouse button to set the frequency

Figure 5.25
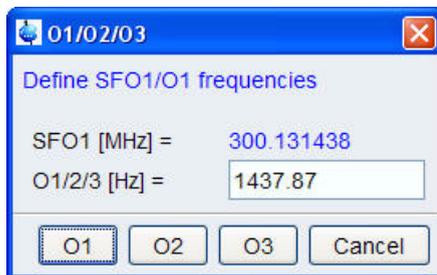
11. Click on

5.3.4   Setting up the Selective NOESY

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 5.26

2. Select by clicking on it

Figure 5.27
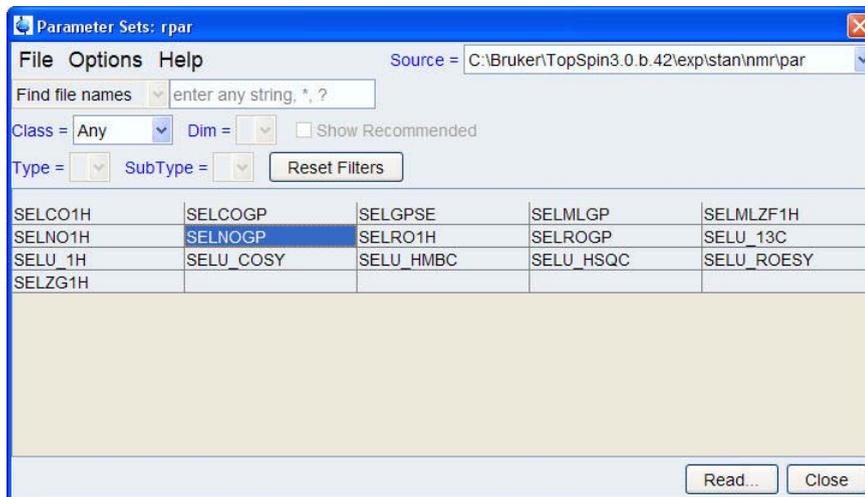
NOTE: Enter **SEL*** in to the '**Find file names**' window and hit '**Enter**' to display all selective parameter sets shown in figure 5.27.

3. Select '**SELNOGP**'

4. Click on

5. Select the following parameter options: **acqu**, **proc**, **outd**

6. Click on the down arrow next to the '**Keep the following parameter**' window

7. Select '**P1, O1, PLW1**' from the pull down menu

Figure 5.28
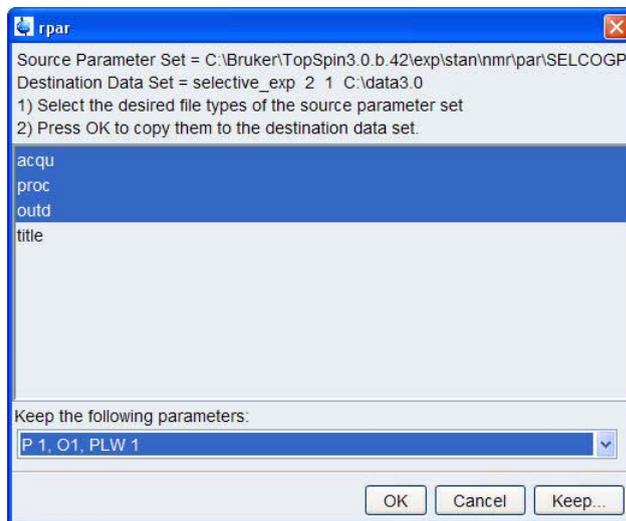
rpar

Source Parameter Set = C:\Bruker\TopSpin3.0.b.42\exp\stan\nmr\par\SELCOGP
Destination Data Set = selective_exp 2 1 C:\data3.0
1) Select the desired file types of the source parameter set
2) Press OK to copy them to the destination data set.

acqu
proc
outd
title

Keep the following parameters:
P 1, O1, PLW 1

OK   Cancel   Keep...

8. Click on [OK]

9. Select the '**Title**' tab by clicking on it

10. Make the following changes:

**1-D Selective gradient NOESY experiment
30 mg Menthyl Anthranilate in DMSO-d6**

11. Click on 🖫 to store the title

12. Select the '**AcquPars**' tab by clicking on it

13. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 5.29

🗄 Start Acquire Process Analyse Publish View Manage ❓
⬍ Sample ▾ ⫛Lock V Tune ▾ ⚓ Spin ▾ ⫤ Shim ▾ ⫶ Prosol ⫸ Gain ▾ ▶ Go ▾ Options ▾

14. Select ⚓ **Spin** ▾ by clicking on it

Figure 5.30

Tu<u>r</u>n sample rotation on (ro on)

Turn sample rotation o<u>f</u>f (ro off)

<u>C</u>hange sample rotation rate (ro)

<u>M</u>AS Pneumatic Unit (masdisp)
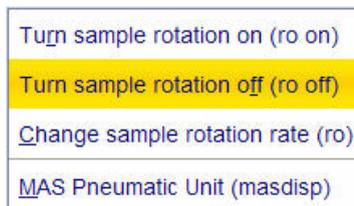
15. Select '**ro off**' by clicking on it

NOTE: 1-D selective experiments should be run non spinning

16. Select [Prosol] by clicking on it

17. Select the '**AcquPars**' tab by clicking on it

18. Make the following parameter changes:

Figure 5.31

| D8 [sec] | 0.44999999 | Mixing time |

19. Select the '**Spectrum**' tab by clicking on it

5.3.5   Acquisition

1. Select [Gain ▽] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select [Go ▽] by clicking on it

NOTE: Other options are available by clicking on the down arrow inside the '**Go**' button.

5.3.6   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.32
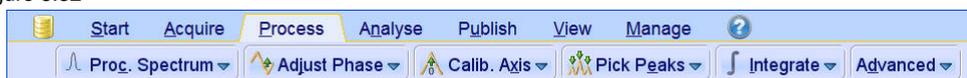
2. Click on the down arrow inside the [Proc. Spectrum ▽] button
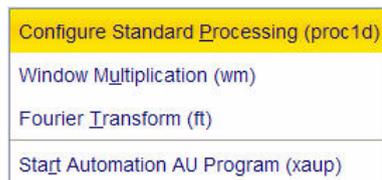
Figure 5.33

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

3. Select '**Configure Standard Processing**' by clicking on it

4. Deselect the following options:
   '**Auto-Phasing (apk)**'
   '**Set Spectrum Reference (sref)**'

'**Auto-Baseline correction (abs)**'

'**Warn if Processed data exist**'

Figure 5.34

5. Click on [Execute]
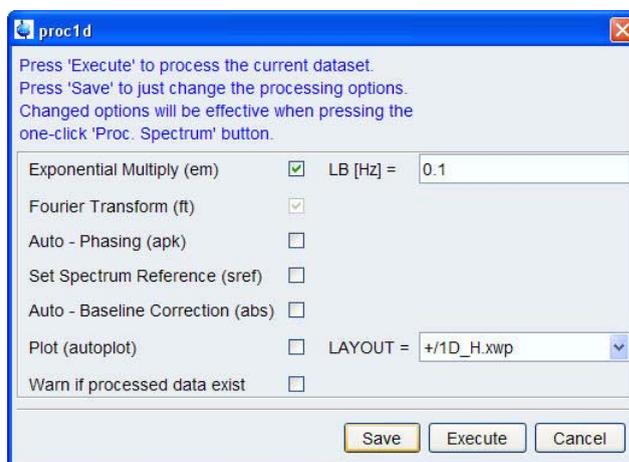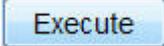
6. Expand the spectrum from 5.5 ppm to 0 ppm

Figure 5.35
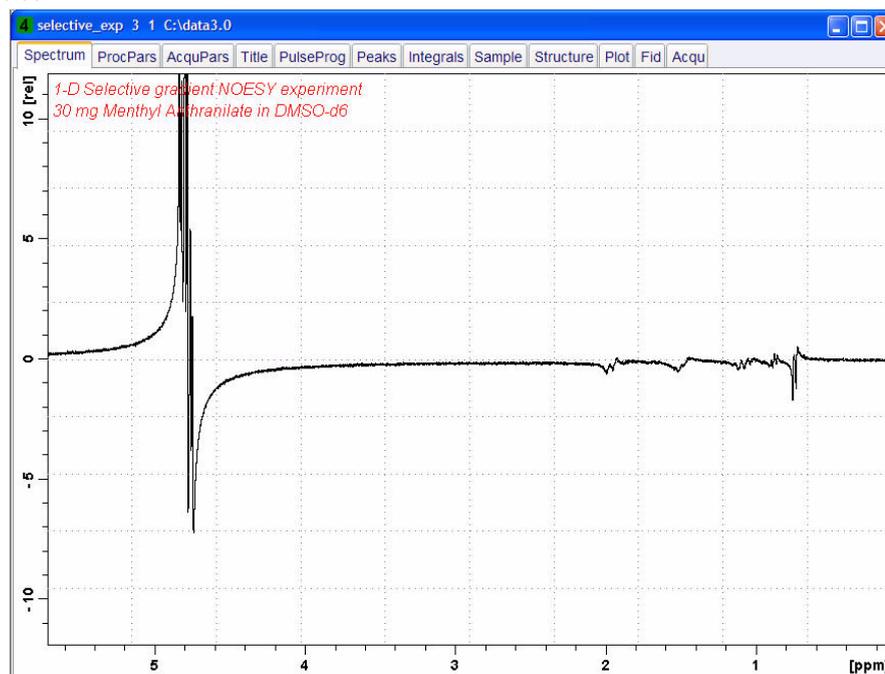
7. Click on [Adjust Phase]

8. Adjust the **0** order phase on the peak at 5 ppm to phase the signal negative, which is the selected exited peak

Figure 5.36
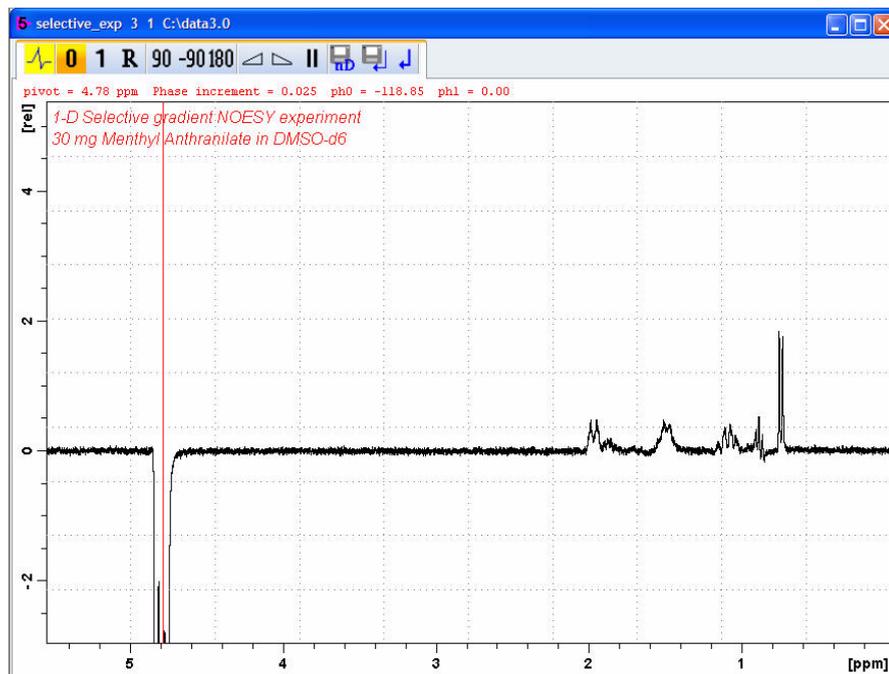
9. Click on [icon] to store the phase value

5.3.7   Plotting two spectra on to the same page

1. Display the selective NOESY spectrum

2. Click on [icon] to enter the Multiple display option

3. Drag the Reference spectrum in to the spectral window

Figure 5.37
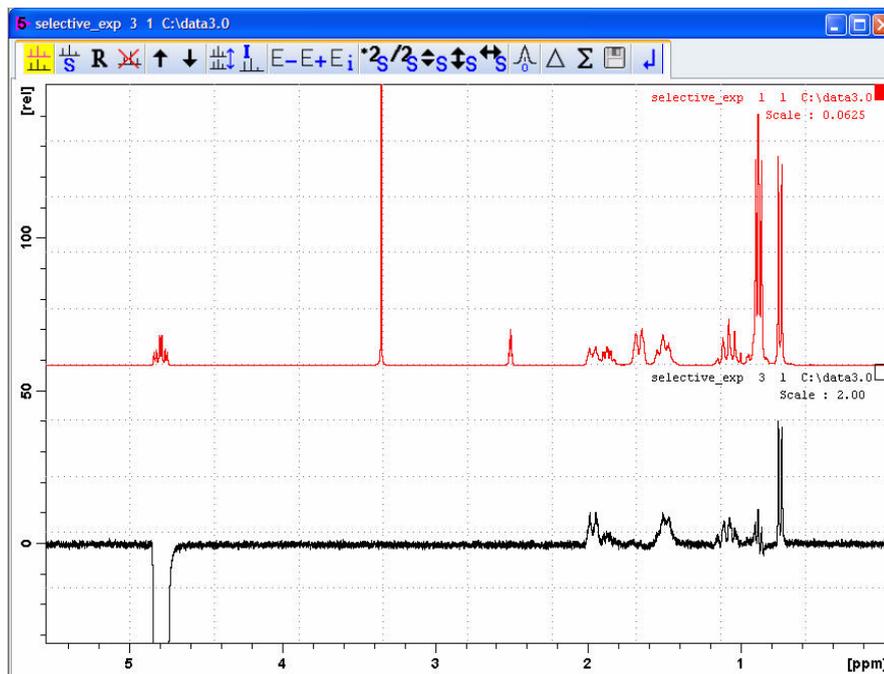
NOTE: To adjust the spectra for best fit, use the tools

4. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 5.38

5. Click on the button to print the active window

5.3.8 Observations

5.4   1-D Selective TOCSY

5.4.1   Introduction

Section 5.4 describes the acquisition and processing of a one-dimensional 1H selective gradient TOCSY experiment. The standard Bruker parameter set is **SELMLGP** and includes the pulse sequence **selmlgp** shown in Figure 5.35. It consists of the recycling delay, a radio-frequency (RF) pulse, a MLEV17 sequence for mixing and the acquisition time during which the signal is recorded.

Figure 5.39
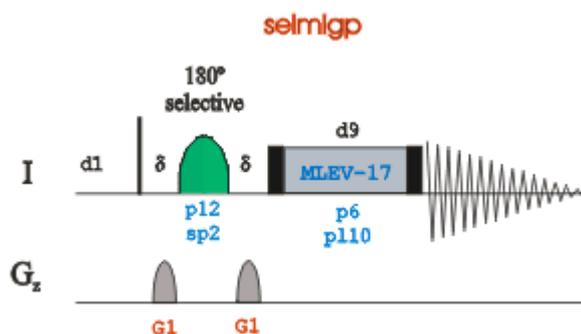
5.4.2   Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **Chapter 3**, **1-D Proton experiment, 3.2.2 Experiment setup,** through **3.2.4 Processing**

Figure 5.40
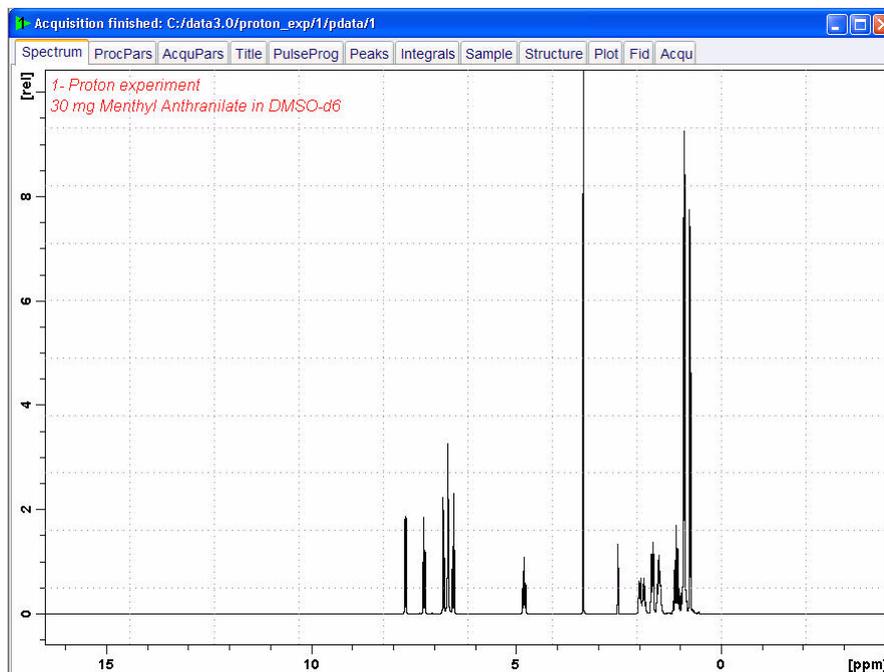
5.4.3 Selective excitation region set up

5.4.3.1 On resonance

NOTE: Make sure 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

1. Type **wrpa**

Figure 5.41
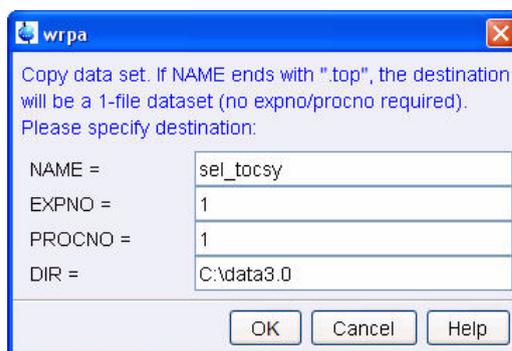
2. Change NAME = **sel_tocsy**

3. Click on OK

4. Type **re**

Figure 5.42
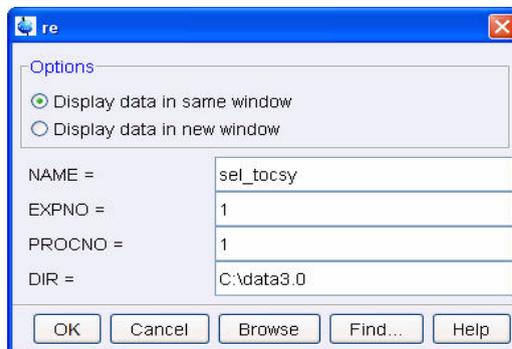
5. Set Change NAME = **sel_tocsy**

6. Click on OK

7. Expand peak at 4.8 ppm

8. Click on 🔽 to set the RF from cursor

Figure 5.43
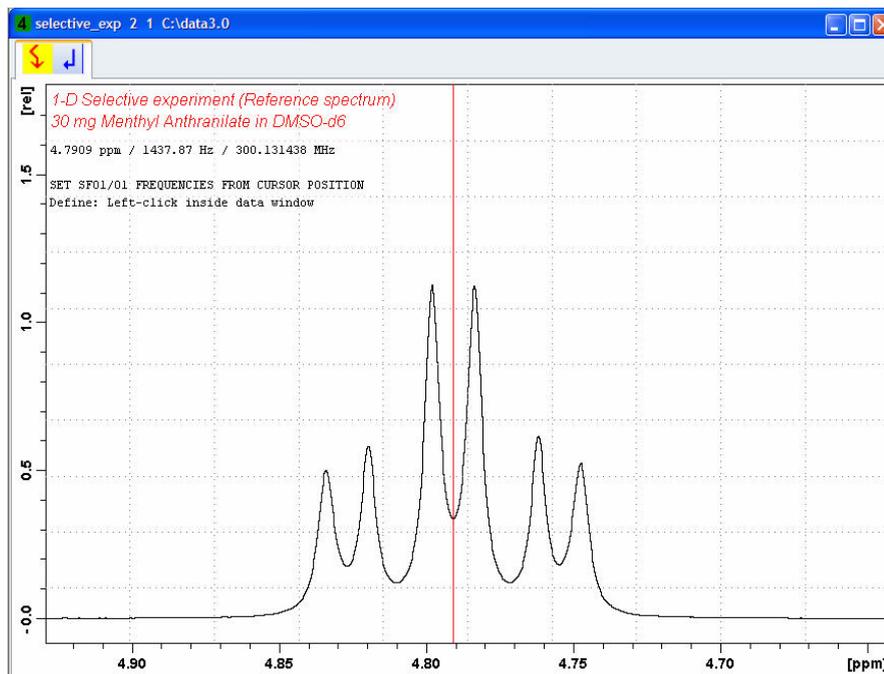
9. Move the cursor line in to the center of the multiplet

10. Click the left mouse button to set the frequency

Figure 5.44
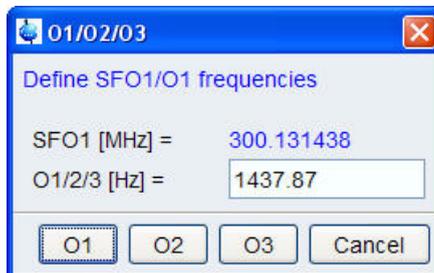
11. Click on [O1]

5.4.4   Setting up the Selective TOCSY

1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 5.45

2. Select [Read Pars.] by clicking on it

Figure 5.46
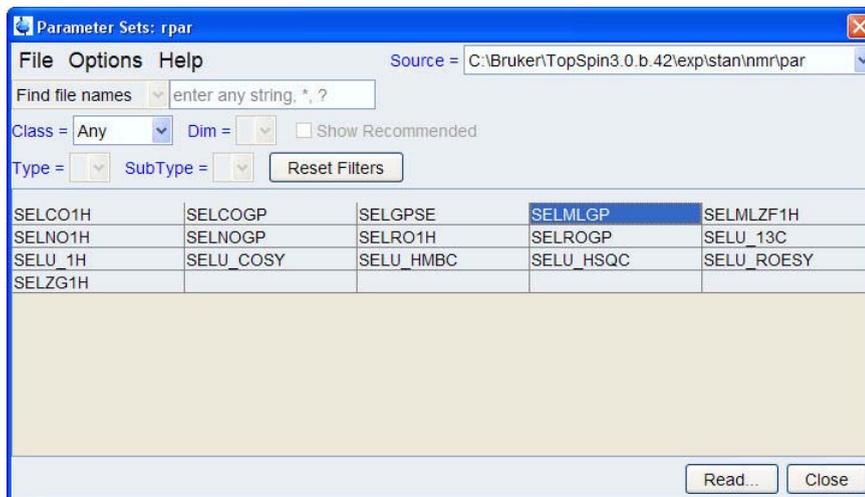
NOTE: Enter **SEL*** in to the '**Find file names**' window and hit '**Enter**' to display all selective parameter sets shown in figure 5.46.

3. Select '**SELMLGP**'

4. Click on [Read...]
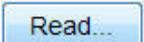
5. Select the following parameter options: **acqu**, **proc**, **outd**

6. Click on the down arrow next to the '**Keep the following parameter**' window

7. Select '**P1, O1, PLW1**' from the pull down menu

Figure 5.47
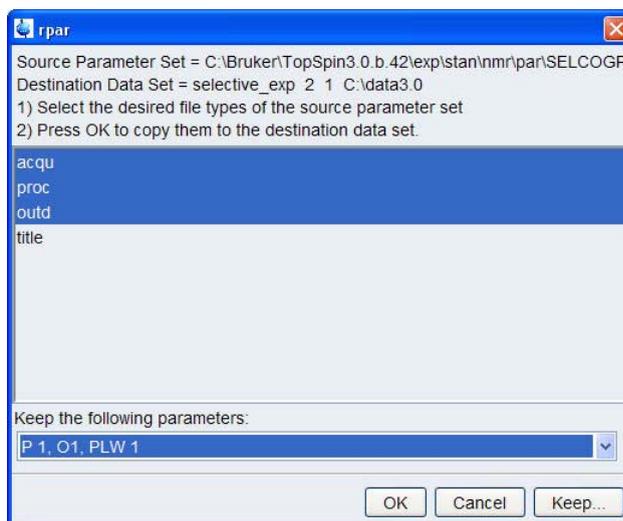
8. Click on [OK]

9. Select the '**Title**' tab by clicking on it

10. Make the following changes:

1-D Selective gradient TOCSY experiment
30 mg Menthyl Anthranilate in DMSO-d6

11. Click on 🖫 to store the title

12. Select the '**AcquPars**' tab by clicking on it

13. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 5.48

14. Select 🔱 Spin ▾ by clicking on it

Figure 5.49
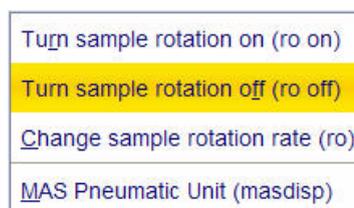
> Turn sample rotation on (ro on)
> Turn sample rotation off (ro off)
> Change sample rotation rate (ro)
> MAS Pneumatic Unit (masdisp)

15. Select '**ro off**' by clicking on it

NOTE: 1-D selective experiments should be run non spinning

16. Select 🔺 Prosol by clicking on it

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

5.4.5   Acquisition

1. Select 〰 Gain ▾ by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select ▶ Go ▾ by clicking on it

NOTE: Other options are available by clicking on the down arrow inside the '**Go**' button.

5.4.6   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.50
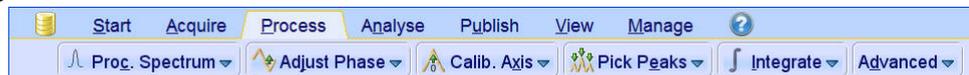
2. Click on the down arrow inside the button
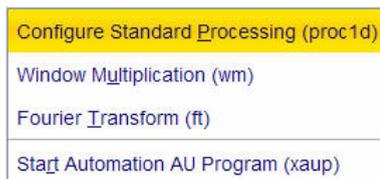
Figure 5.51

3. Select '**Configure Standard Processing**' by clicking on it

4. Diselect the following options:

'**Auto-Phasing (apk)**'
'**Set Spectrum Reference (sref)**'
'**Auto-Baseline correction (abs)**'
'**Warn if Processed data exist**'

Figure 5.52
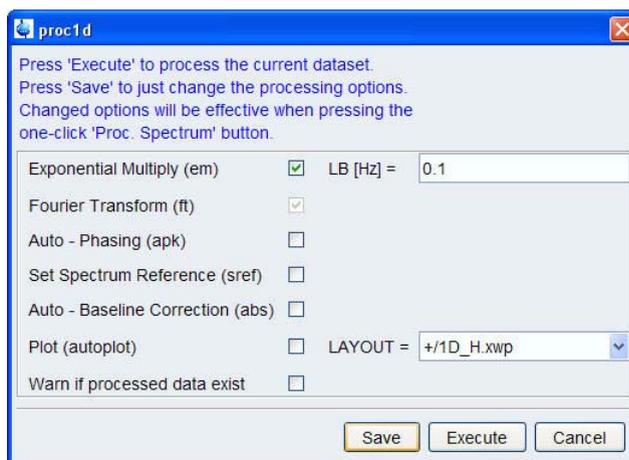
5. Click on

6. Expand the spectrum fom 5.5 ppm to 0 ppm

Figure 5.53

7. Click on **Adjust Phase**
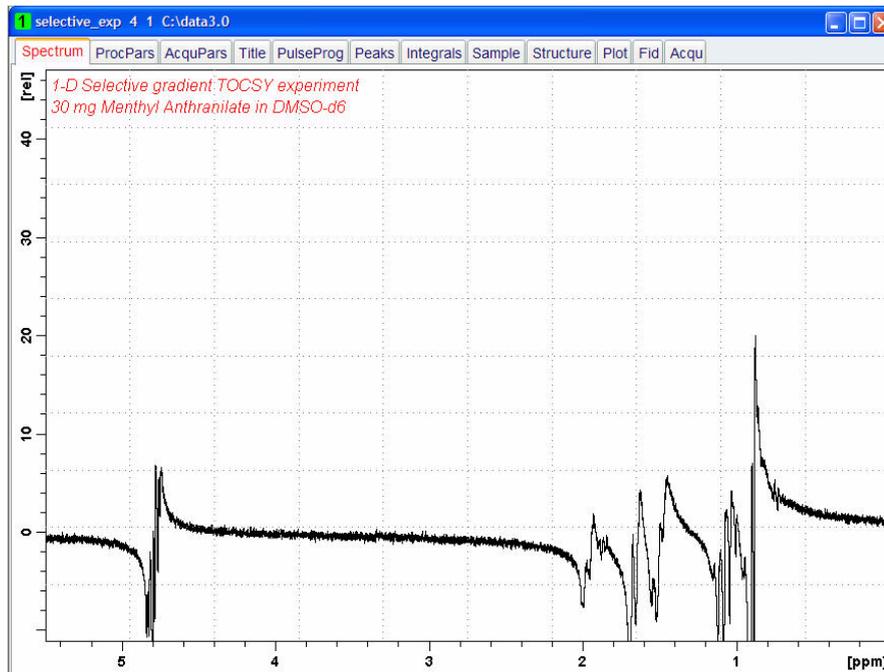
8. Phase all peaks positive

Figure 5.54
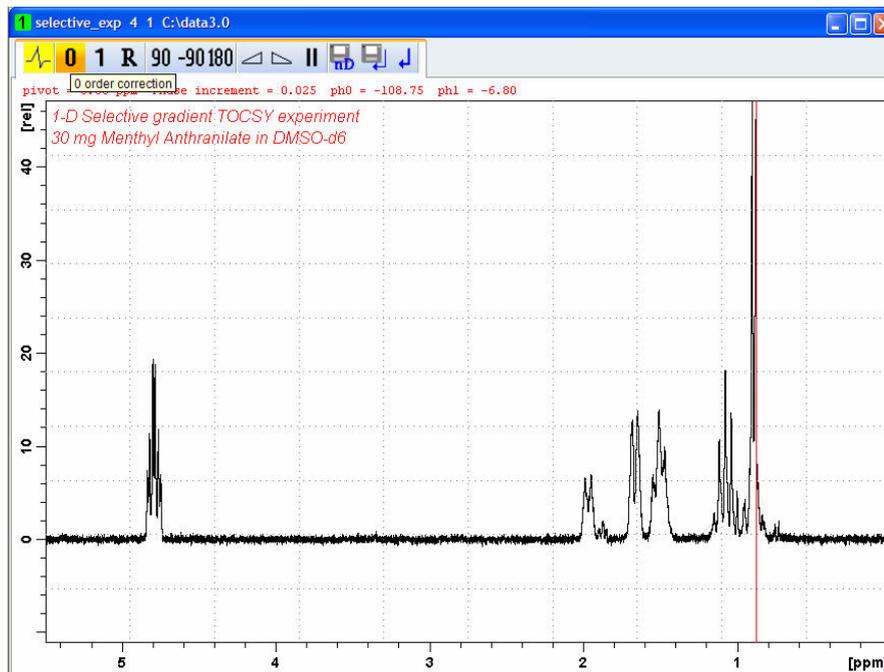
9. Click on to store the phase value

5.4.7 Plotting two spectra on to the same page

1. Display the selectice TOCSY spectrum

2. Click on [icon] to enter the Multiple display option

3. Drag the Reference spectrum in to the spectral window

Figure 5.55
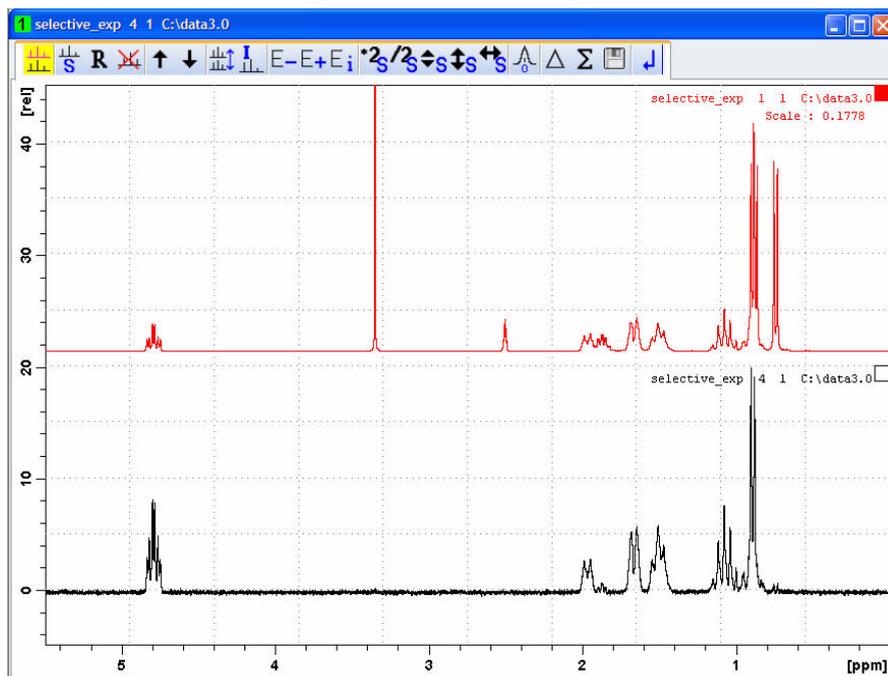
NOTE: To adjust the spectra for best fit, use the [icons] tools

4. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 5.56

5. Click on the [Print icon] button to print the active window

5.4.8 Observations

6 1-D Carbon experiments

6.1 Sample

A sample of **30mg Menthyl Anthranilate in DMSO-d6** is used for the experiment in this chapter

Figure 6.1
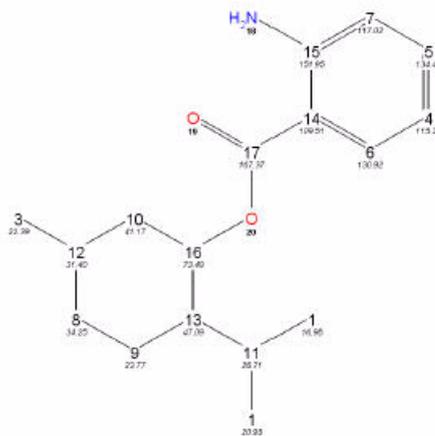
6.2 1-D Carbon Experiment

6.2.1 Introduction

Section 6.2 describes the acquisition and processing of a one-dimensional 13C NMR spectrum. The standard Bruker parameter set **C13CPD**, includes the pulse sequence **zgpg30**, shown in Figure 6.2. The 13C channel consists of the recycling delay, a RF pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30 degrees. The two parameters, D1 and P1, correspond to the length of the recycle delay, and the length of the 90 degree RF pulse, respectively. The 1H channel consists of two decoupling pulses which can be power gated. The first pulse, an NOE build up pulse during the recycle delay may be of lower power then the second pulse on during the acquisition which is the true decoupling pulse. This can be useful to avoid RF heating on salty samples or probes where a higher decoupling power can be problematic.

Figure 6.2
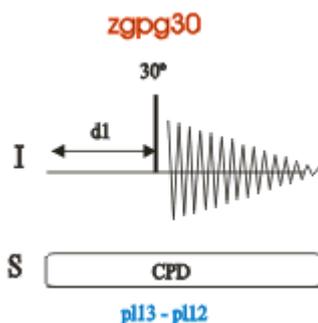
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

6.2.2 Experiment set up

1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 6.3

2. Select by clicking on it

3. Enter the following information in to the 'New' window

Figure 6.4

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 6.4 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Select the '**AcquPars**' tab by clicking on it

6. Make the following change

NS = **256**

7. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 6.5

8. Select [Sample ▾] by clicking on it

Figure 6.6
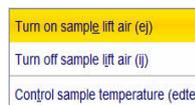
9. Select '**ej**' by clicking on it

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

10. Place the sample on too the top of the bore tube

11. Select [Sample ▾] by clicking on it

Figure 6.7
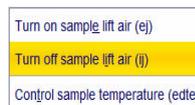
12. Select '**ij**' by clicking on it

NOTE: Wait till the sample is lowered down in to the probe and the lift air is turned off. A licking sound may be heard.

13. Select [Lock] by clicking on it

Figure 6.8
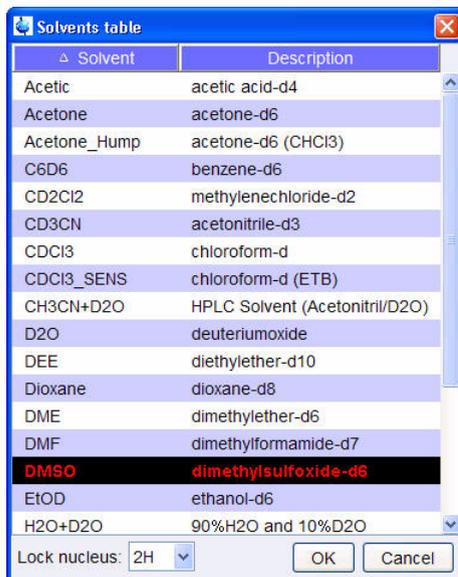
14. Select '**CDCl3**' by clicking on it

15. Click on the down arrow

NOTE: This performs a '**atma**' (automatic tuning) and requires a probe equipped with a automatic tuning module. Other options can be selected by clicking on the down arrow inside the '**Tune**' button.

---

16. Select by clicking on it

Figure 6.9
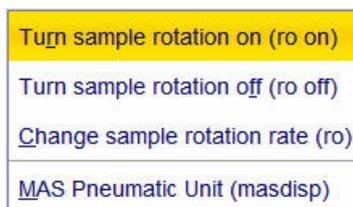
17. Select '**ro on**' by clicking on it

NOTE: Rotation may be turned off for probes such as BBI, TXI, TBI and for small sample probes.

---

18. Select by clicking on it

NOTE: This executes the command '**topshim**'. To select other options. click on the down arrow inside the '**Shim**' button.

---

19. Select by clicking on it

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

6.2.3 Acquisition

1. Select ![Gain icon] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select ![Go icon] by clicking on it

NOTE: Other options are available by clicking on the down arrow inside the '**Go**' button.

6.2.4 Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 6.10
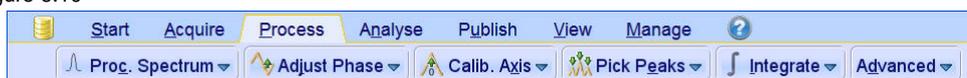
2. Select ![Proc. Spectrum icon] by clicking on it

NOTE: 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**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button.

3. Expand the spectrum to include all peaks

Figure 6.11
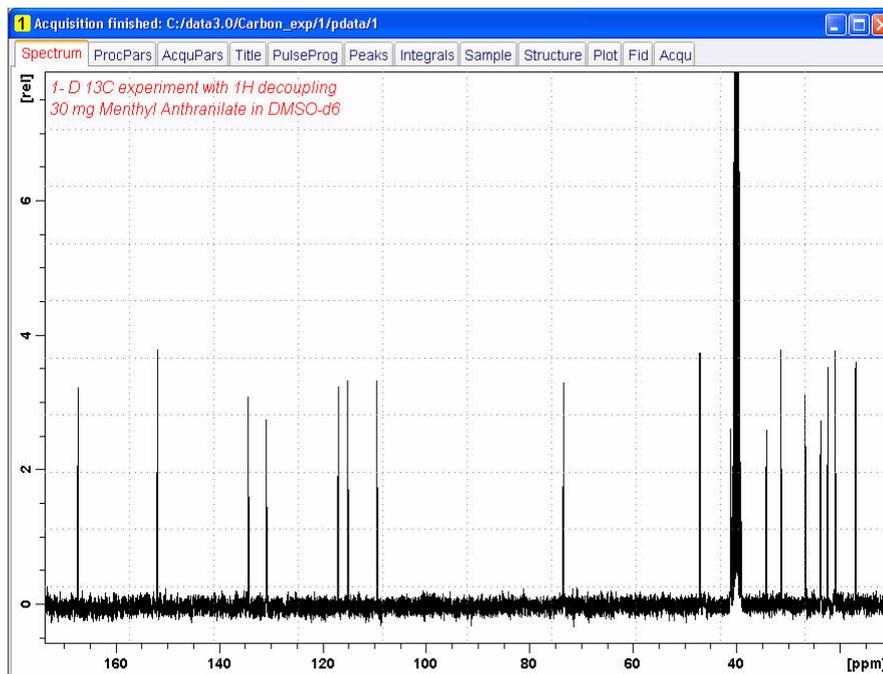
4. Select **Pick Peaks** by clicking on it

NOTE: By default the '**define new peak picking range**' is enabled.

5. Click the left mouse button and drag the cursor line from left to the right side of the spectrum

Figure 6.12
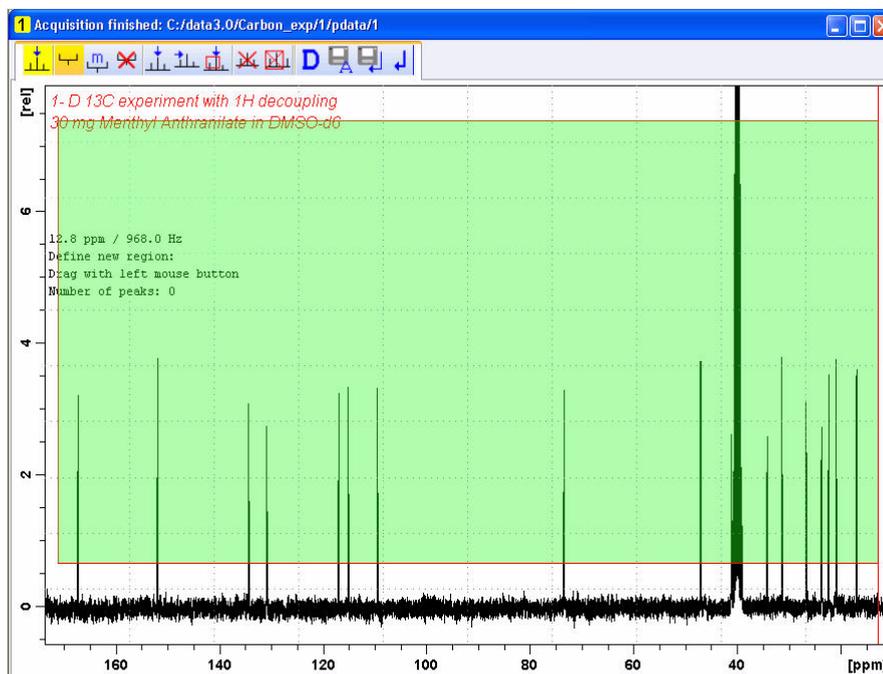
6. Click on [icon] to manually adjust the minimum and maximum intensity levels

7. Click on the bottom line of the region box with the left mouse button and drag the line above the noise level, to set the minimum peak picking level

8. Click on the top line of the region box with the left mouse button and drag the line below unwanted peaks e.g. solvent peaks, to set the maximum peak picking level

Figure 6.13
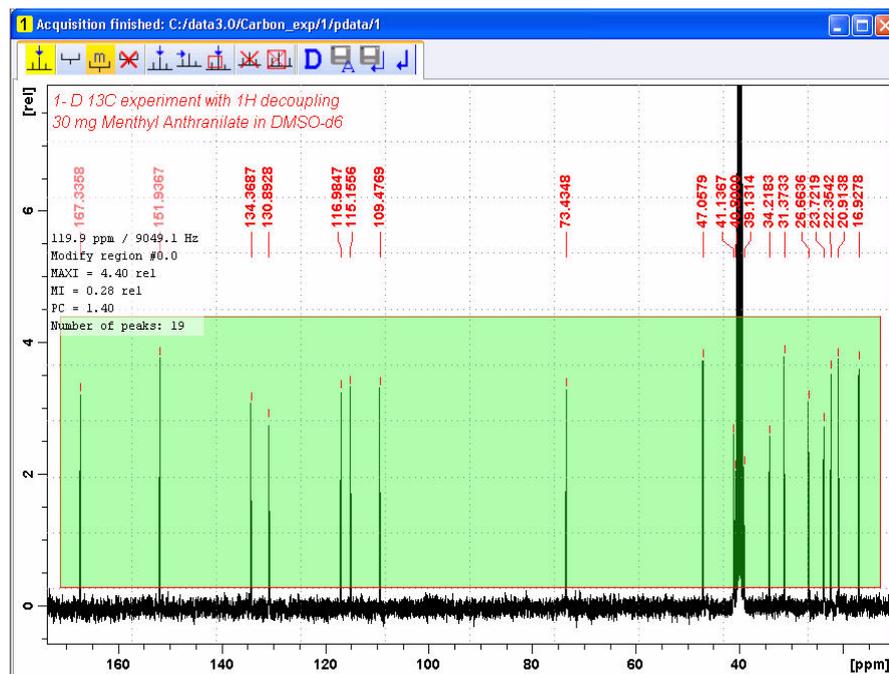
9. Click on [icon] to store the peak picking values

Figure 6.14
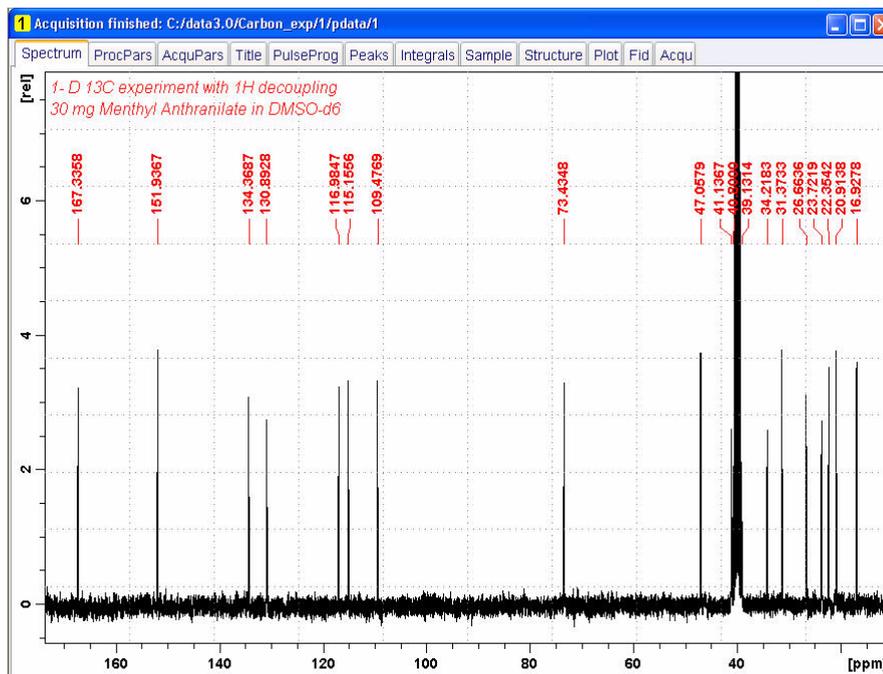
NOTE: To display the peak picking labels, right click inside the spectrum window and select '**Spectra Display Preferences**' by clicking on it. In the '**Spectrum components**' enable '**Peak labels**' and '**Peak annotations**'. Click '**Apply**' and click on '**Close**'

6.2.5   Plotting the 1D Carbon spectrum

1. Expand the spectrum (all peaks in display)

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 6.15

3. Click on the down arrow

Figure 6.16
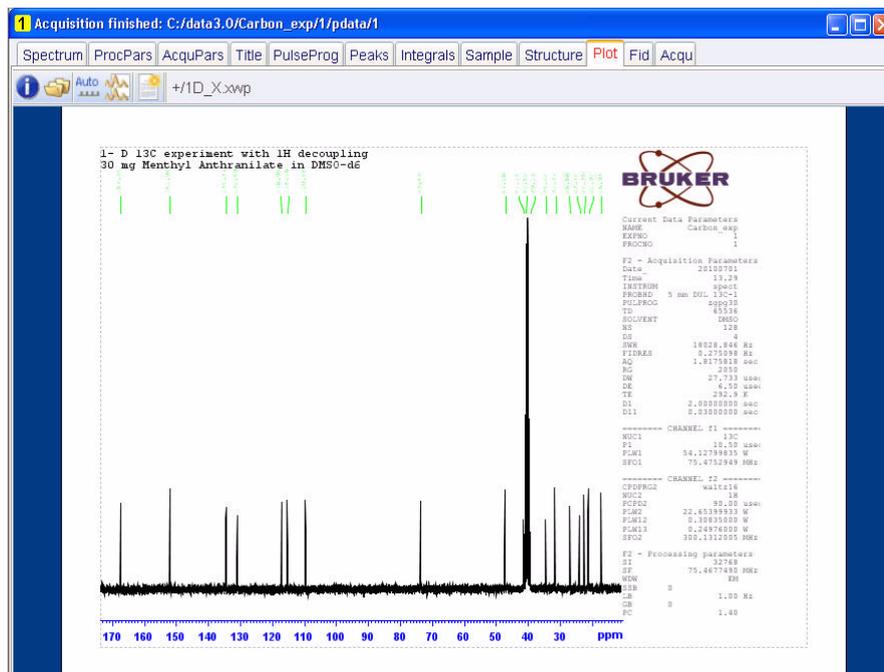
NOTE: If desired, any changes can be administered using the Plot Editor tools.

4. Click on '**File**' and select '**Print**' by clicking on it

6.2.6   Observations

6.3 DEPT-135 Experiment

6.3.1 Introduction

DEPT (Distortion less Enhancement by Polarization Transfer) is a polarization transfer technique used for the observation of nuclei with a small gyro magnetic ratio, which are J-coupled to 1H (most commonly 13C). DEPT is a spectral editing sequence, that is, it can be used to generate separate 13C sub spectra for methyl (CH3), methylene (CH2), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherence to differentiate between the different types of 13C signals. Quaternary carbons are missing a direct bond proton, and as a result are absent from all DEPT spectra.

Section 6.3 describes the acquisition and processing of a one-dimensional 13C-DEPT135 NMR spectrum. The standard Bruker parameter set **C13DEPT135**, includes the pulse sequence **deptsp135**, shown in Figure 6.17. The 13C channel consists of the recycling delay, a 90 degree RF pulse, an editing delay D2 followed by a 180 degree RF pulse and the acquisition time during which the signal is recorded. The editing delay D2 is 1/2*J(XH). The 1H channel consists of two three pulses, a 90 degree, a 180 degree, followed by a 135 degree RF pulse and are separated by the editing delay D2. The final 135 degree 1H pulse selects the CH3, CH2 or CH signals. The protons are decoupled during the acquisition period.

Figure 6.17
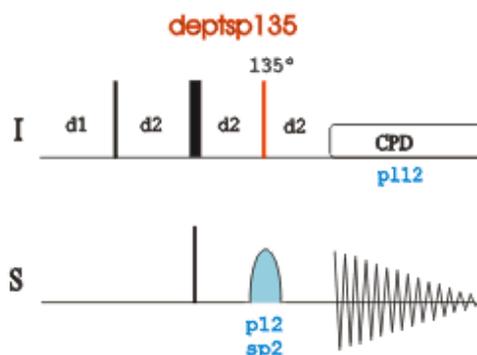
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

6.3.2 Experiment set up

NOTE: This experiment usually follows a regular 1H decoupled 13C experiment. The result of a DEPT-135 experiment shows only the protonated carbons with the CH and CH3 as positive and the CH2 as negative signals.

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 6.18

2. Select by clicking on it

3. Enter the following information in to the 'New' window

Figure 6.19
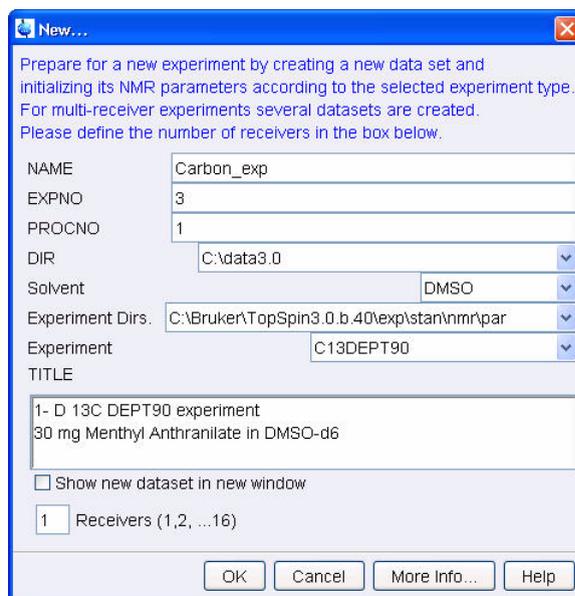
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 6.19 above. Click on the down arrow button to browse for a specific directory.

---

4. Click on

5. Select the '**AcquPars**' tab by clicking on it

6. Make the following change

NS = **128**

7.Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 6.20

8. Select by clicking on it

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

6.3.3 Acquisition

1. Select [Gain] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select [Go] by clicking on it

6.3.4 Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 6.21

2. Select [Proc. Spectrum] by clicking on it

NOTE: 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**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button. Do to the fact that a DEPT135 spectrum contains negative and positive peaks, there is the possibility of getting phase results that are 180 degrees off. In this case, click on the '**Adjust Phase**' button to enter the manual phase routine and reverse the spectrum by clicking on the '**180**' icon.

Figure 6.22
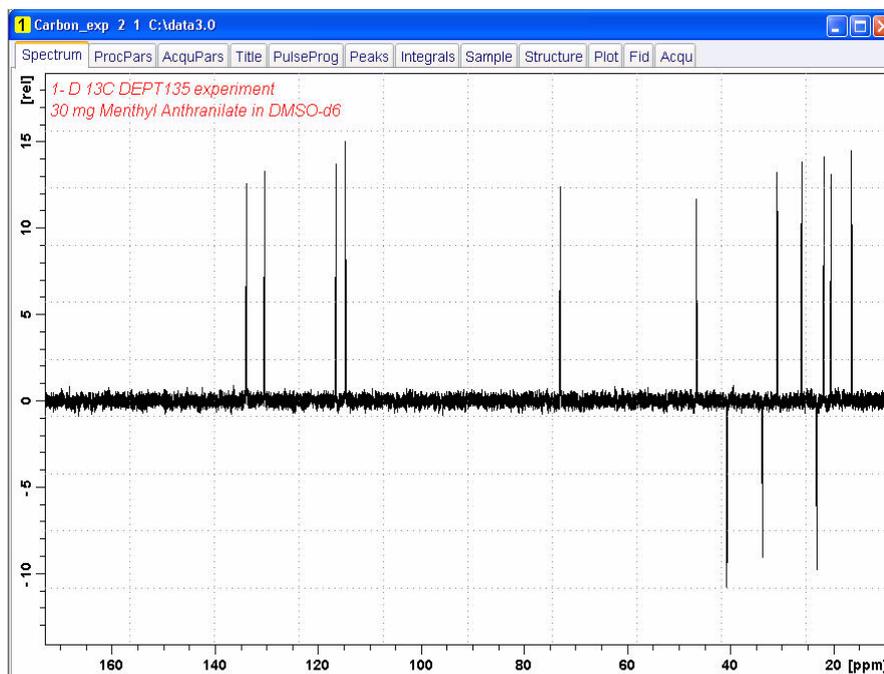
6.3.5 Observations

6.4 DEPT-90 Experiment

6.4.1 Introduction

Section 6.4 describes the acquisition and processing of a one-dimensional 13C-DEPT90 NMR spectrum. The standard Bruker parameter set **C13DEPT90**, includes the pulse sequence **dept90**, shown in Figure 6.23. The 13C channel consists of the recycling delay, a 90 degree RF pulse, an editing delay D2 followed by a 180 degree RF pulse and the acquisition time during which the signal is recorded. The editing delay D2 is 1/2*J(XH). The 1H channel consists of three pulses, a 90 degree, a 180 degree, followed by a 90 degree RF pulse and are separated by the editing delay D2. The final 90 degree 1H pulse selects the CH signals only. The protons are decoupled during the acquisition period.

Figure 6.23
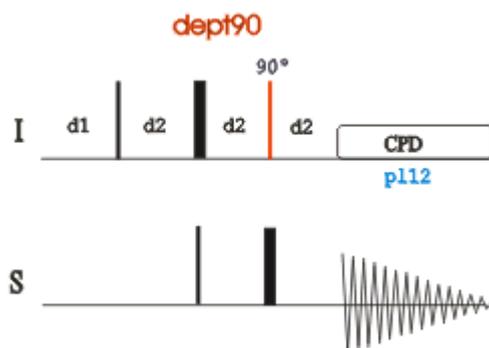
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

6.4.2 Experiment set up

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

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 6.24

2. Select [Create Dataset] by clicking on it

3. Enter the following information in to the 'New' window

Figure 6.25
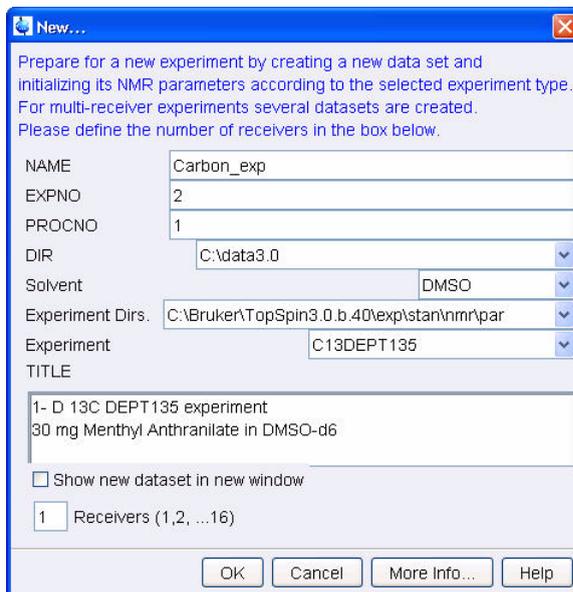
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 6.25 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Select the '**AcquPars**' tab by clicking on it

6. Make the following change

NS = **128**

7. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 6.26

8. Select [**Prosol**] by clicking on it

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

6.4.3   Acquisition

1. Select [**Gain**] by clicking on it

NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select by clicking on it

6.4.4 Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 6.27
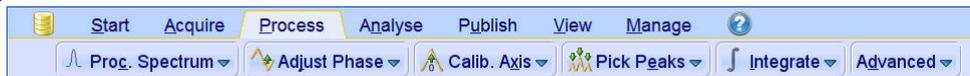
2. Select by clicking on it

NOTE: 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**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button.

Figure 6.28
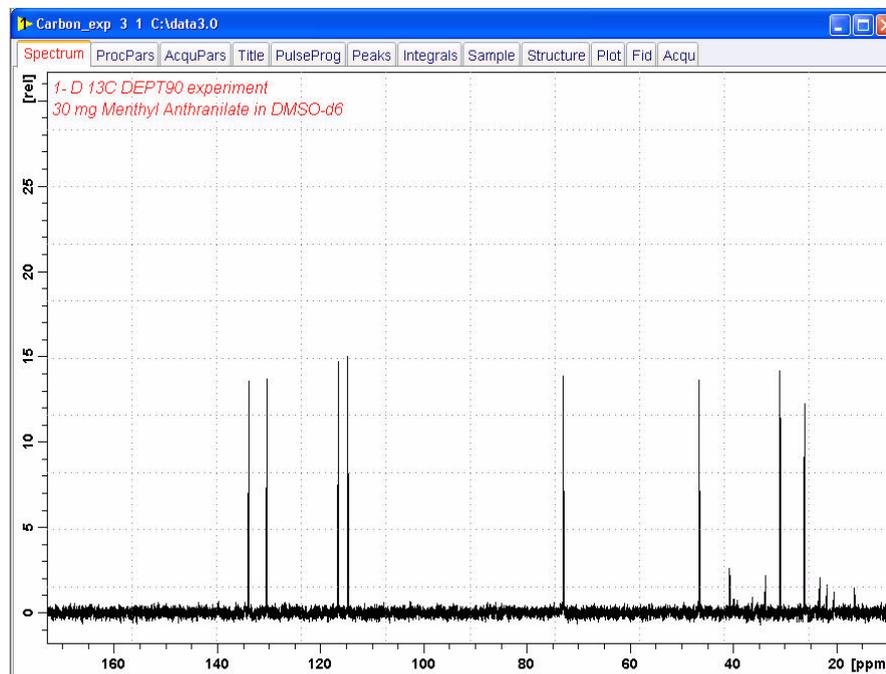
6.4.5 Observations

7 2-D Heteronuclear experiments

7.1 Sample

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

Figure 7.1
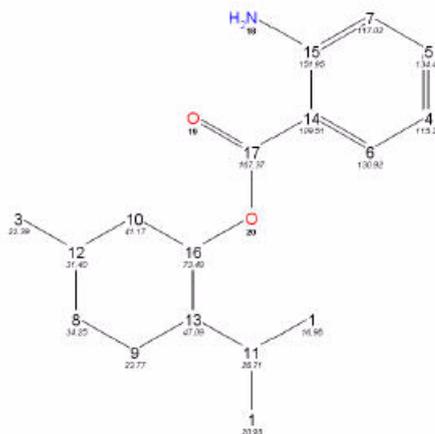
7.2 2D edited HSQC

7.2.1 Introduction

The **HSQC** (Heteronuclear Single Quantum Coherence) experiment performs and H,C-correlation via the 13C chemical shift evolution of the double-quantum coherence. This method is superior to other heteronuclear experiments in the case of a crowded 13C NMR spectrum. In the sequence shown in Figure 7.2., the signals are not broadened by homonuclear H,H coupling in F1. It is possible to obtain a complete editing of inverse -recorded 1-D H,X correlation spectra. This kind of multiplicity determination has been achieved by including an editing period within HSQC, abbreviated as E-HSQC. In the experiment shown here the standard Bruker parameter set **HSQCEDETGP** is used and the graphical display of the pulse program **hsqcedetgp** is shown in Figure 7.2.

Figure 7.2
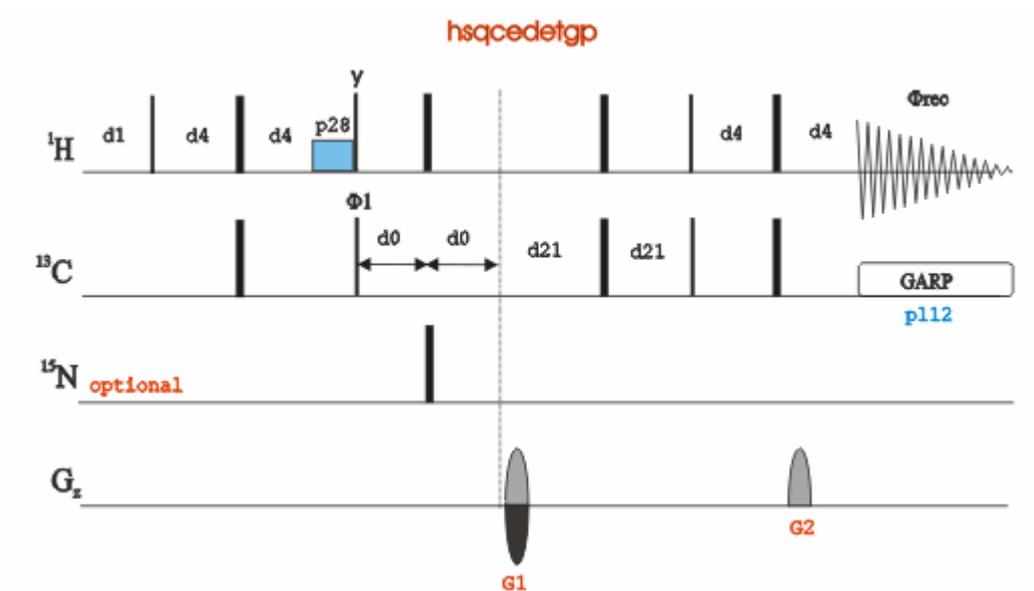
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

7.2.2   Preparation experiment

1. Run a 1D Proton spectrum, following the instructions in Chapter3, 1-D Proton experiment, Paragraph 3.2.2 Experiment setup through 3.2.4 Processing.

Figure 7.3
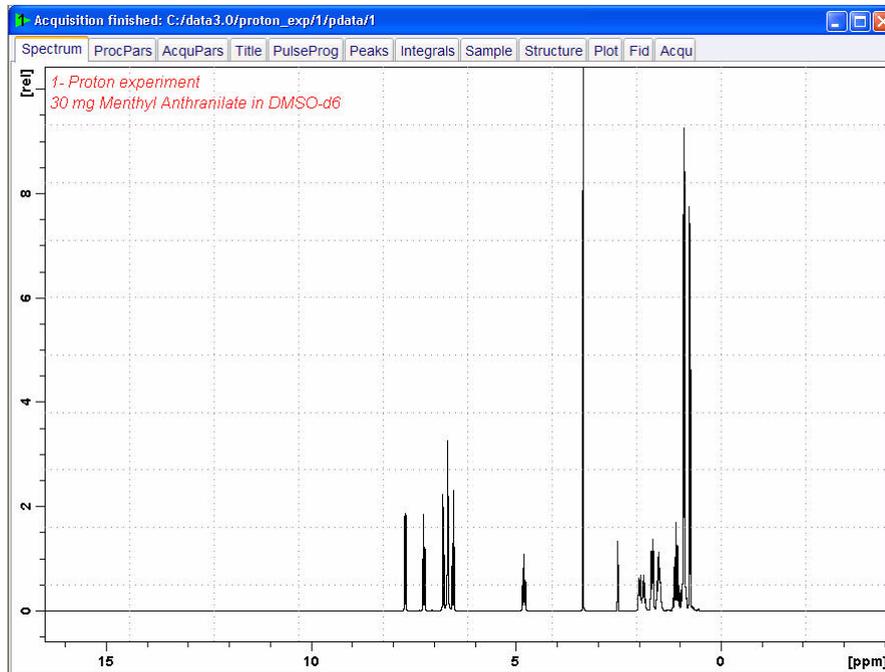
7.2.3 Setting up the HSQC experiment

1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 7.4

2. Select   [Create Dataset]   by clicking on it

3. Enter the following information in to the 'New' window

Figure 7.5
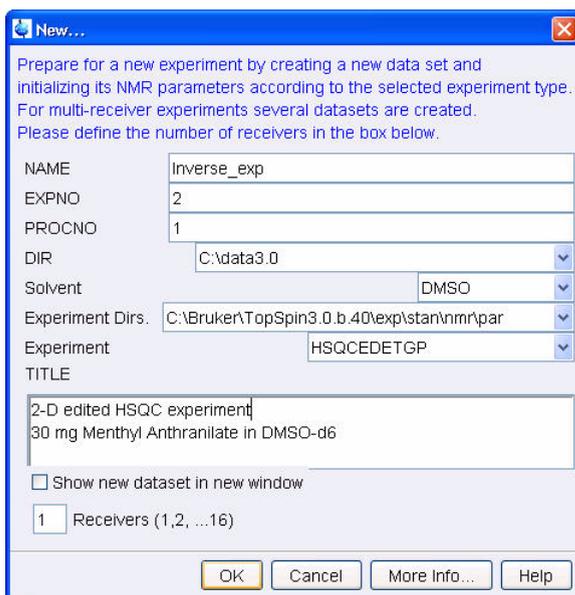
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 7.5 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Click on the 'Aquire' tab in the TopSpin menu bar

Figure 7.6
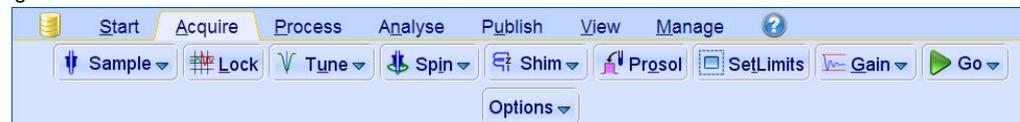
6. Select   [Spin] by clicking on it

Figure 7.7
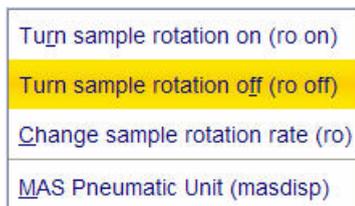
7. Select '**ro off**' by clicking on it

NOTE: 2-D experiments should be run non spinning

8. Select by clicking on it

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

9. Select by clicking on it

Figure 7.8
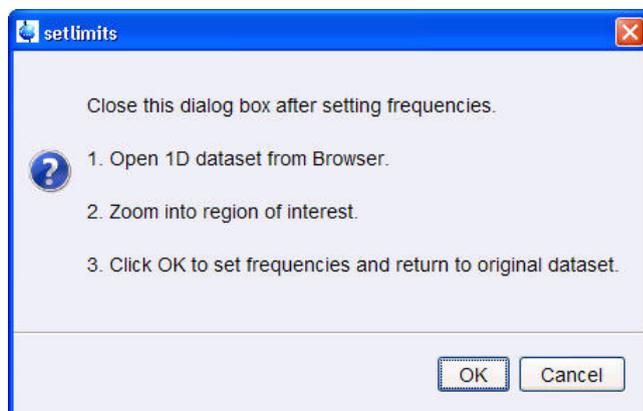
10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. **Inverse_exp 1**) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

NOTE: 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.

Figure 7.9
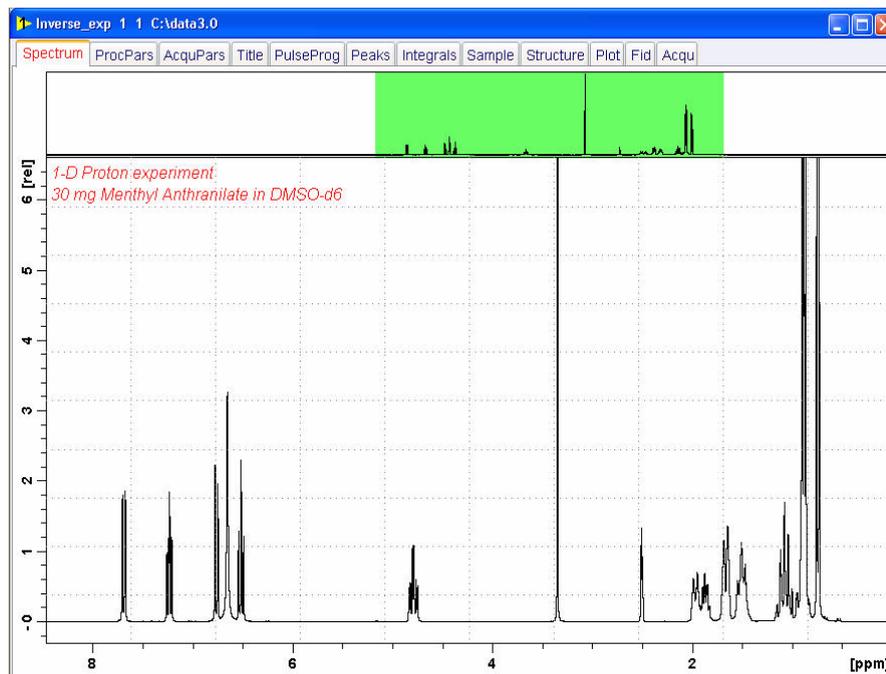
12. Click on [OK] to assign the new limit

Figure 7.10

13. Click on [Close]
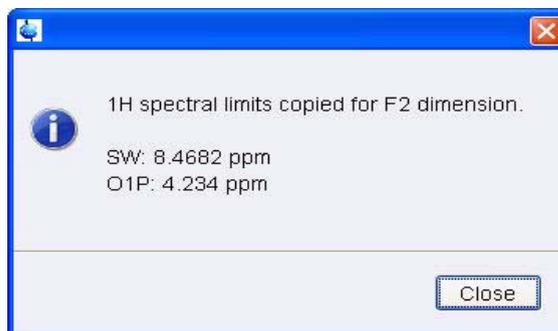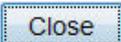
NOTE: The display changes back to the 2D data set. The parameter set **HSQCEDETGP** has a fixed F1 sweep width of 160 ppm and it is big enough to cover the protonated resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the 'Set_limits' button for a second time. In this case a 1-D **C13DEPT45** or **C13DEPT135** experiment on the same sample has to be observed. As an example to set the F1 limit, follow the steps below.

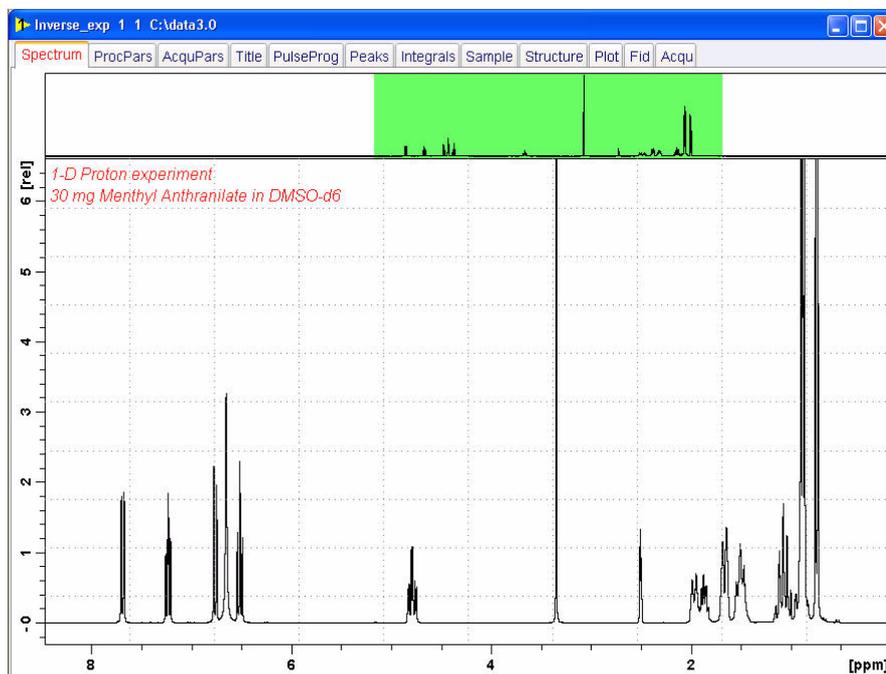14. Select [SetLimits] by clicking on it

Figure 7.11
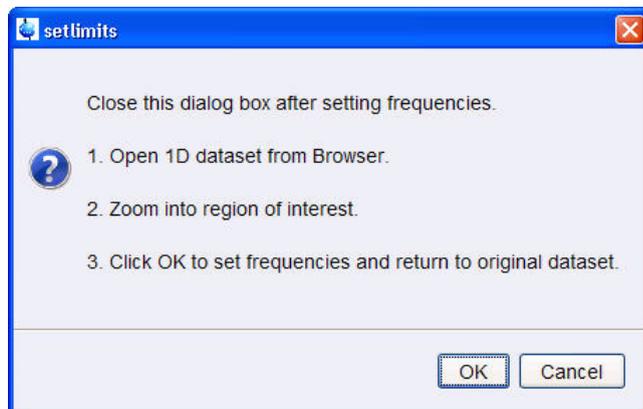
15. To open the 1D C13DEPT spectrum, right click on the dataset name in the browser window (e.g. **Carbon_exp 2**) and select 'Display' or click and hold the left mouse button for dragging the 1D C13DEPT dataset in to the spectrum window

16. Expand the spectrum to display all peaks, leaving ca. 2 ppm of baseline on either side of the spectrum
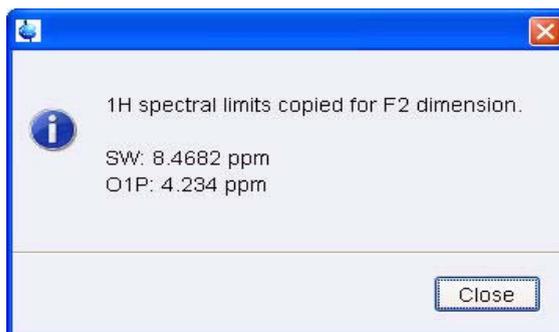
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.
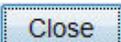
Figure 7.12
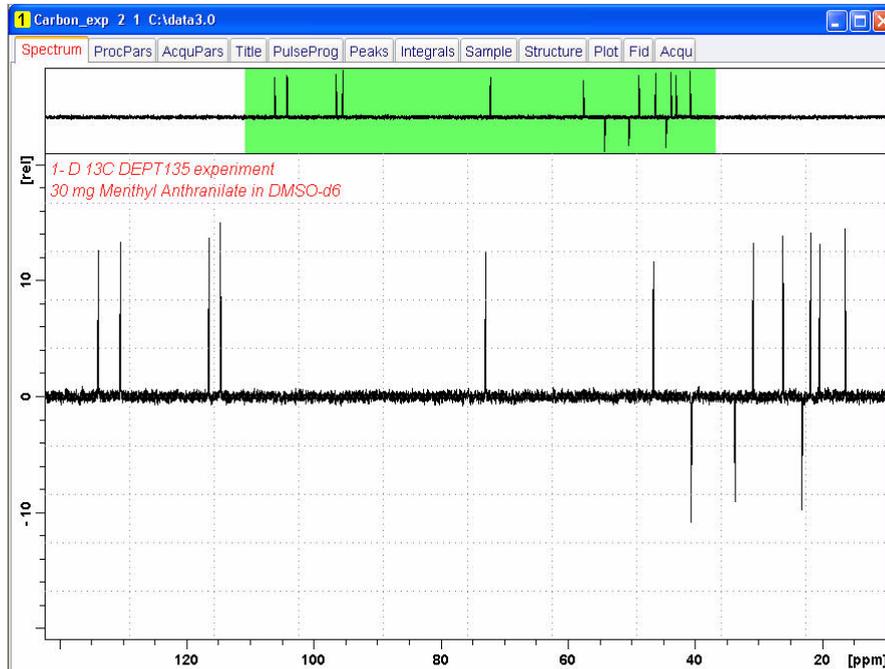
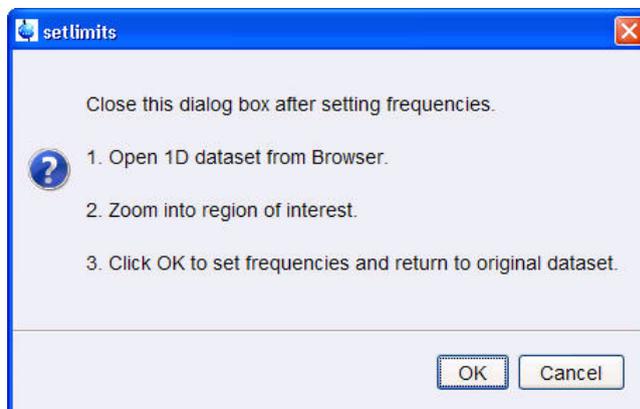17. Click on [OK] to assign the new limit

Figure 7.13

18. Click on [Close]
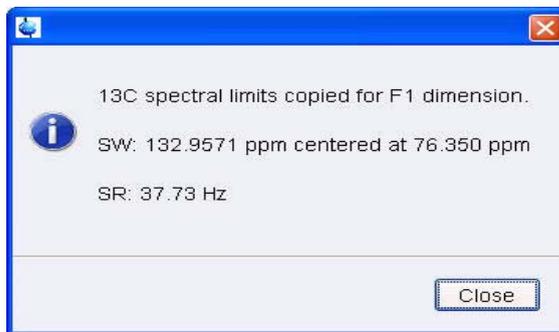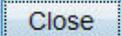
7.2.4   Acquisition

1. Select [Gain ▾] by clicking on it

2. Select [▶ Go ▾] by clicking on it

7.2.5   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 7.14
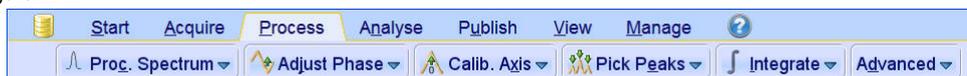
2. Select [Proc. Spectrum ▾] by clicking on it

NOTE: This executes a standard processing program **proc2**. To configure this program or select the right options, click on the down arrow inside the '**Proc. Spectrum**' button. Since this is a phase sensitive experiment the phase correction **apk2d** have to be enabled.

Figure 7.15
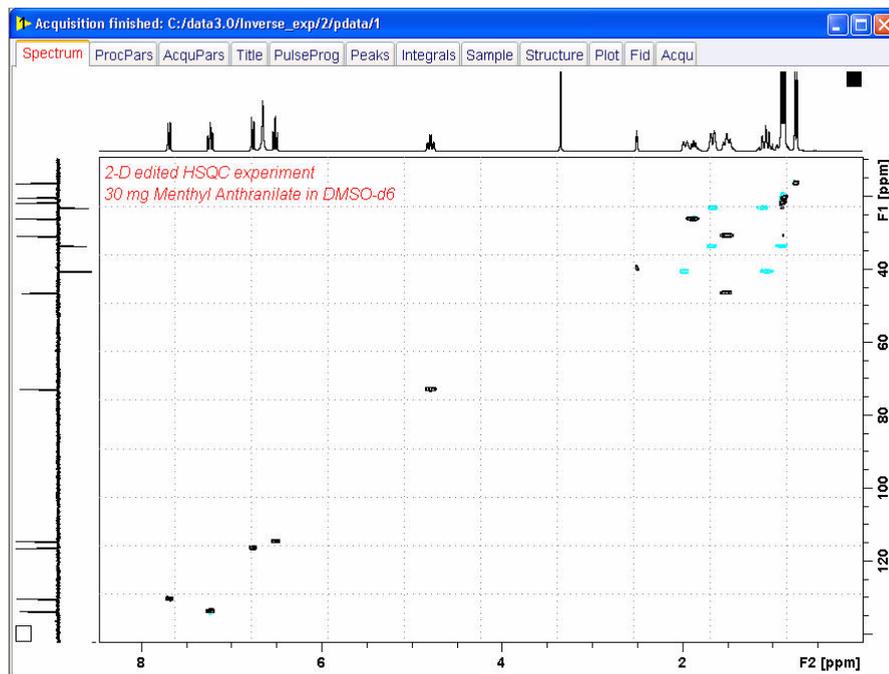
7.2.6   Plotting

1. Use the ⟨⟩ buttons to adjust for a suitable contour level

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 7.16

6. Click on ⟨Print⟩

NOTE: This will print the active window with the colors displayed in the TopSpin window. Using the '**plot**' option starting the plot editor, the default layout is designed not to show the F1 projection (see Figure 7.17 below. A new layout has to be created to add the F1 projection.

Figure 7.17

7.2.7　Observations

7.3   2D HMBC experiment

7.3.1   Introduction

HMBC (Heteronuclear Multiple Bond Correlation) spectroscopy is a modified version of HMQC suitable for determining long-range 1H-13C connectivity. Since it is a long-range chemical shift correlation experiment the pulse program contains a low pass filter to suppress the one bond correlation and is a gradient-selected version which is not phase-sensitive. The experiment is performed without 13C decoupling to distinguish signals coming from the one bond coupling and the standard Bruker parameter set **HMBCGP** is used. The graphical display of the pulse program **hmbcgplpndqf** is shown in Figure 7.18

Figure 7.18
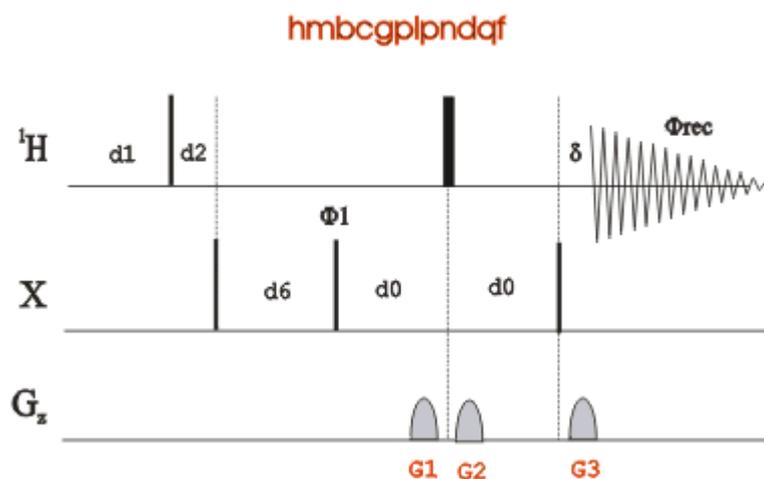
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

7.3.2   Preparation experiment

1. Run a 1D Proton spectrum, following the instructions in Chapter3, 1-D Proton experiment, Paragraph 3.2.2 Experiment setup through 3.2.4 Processing.

Figure 7.19
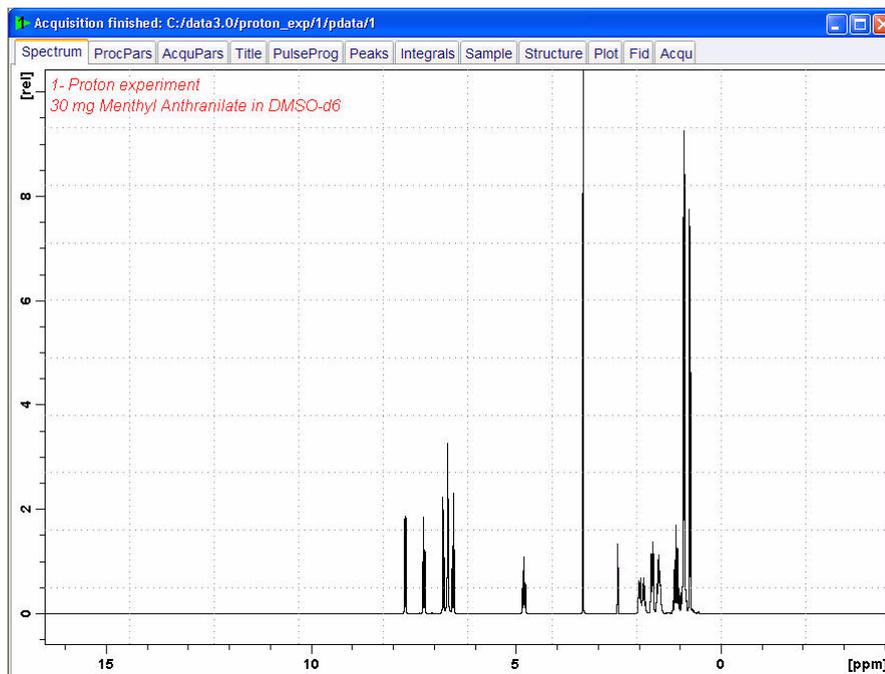
7.3.3 Setting up the HMBC experiment

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 7.20

2. Select by clicking on it

3. Enter the following information in to the 'New' window

Figure 7.21
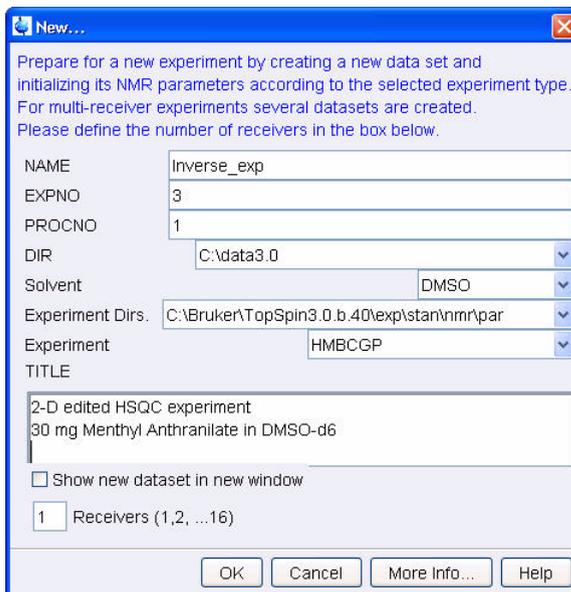
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 7.21 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Click on the 'Aquire' tab in the TopSpin menu bar

Figure 7.22
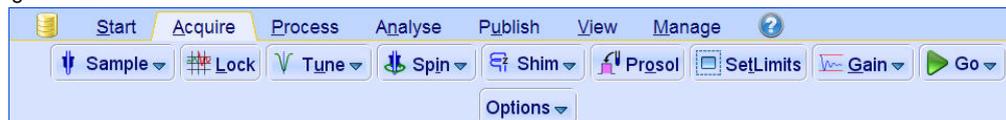
6. Select [Spin] by clicking on it

Figure 7.23
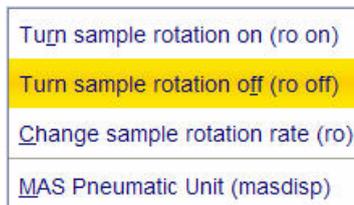
7. Select 'ro off' by clicking on it

NOTE: 2-D experiments should be run non spinning

8. Select [Prosol] by clicking on it

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

9. Select [SetLimits] by clicking on it

Figure 7.24
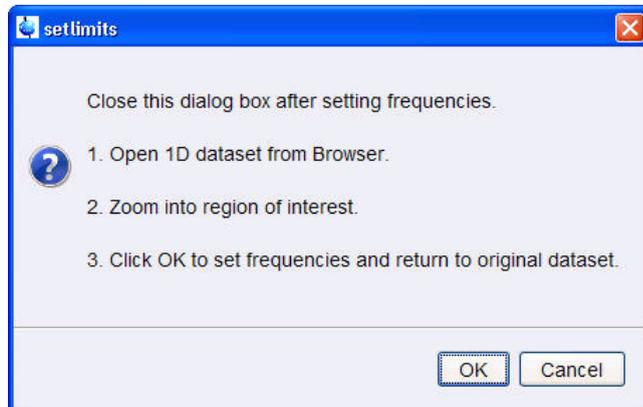
10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. **Inverse_exp 1**) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

NOTE: 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.

Figure 7.25

12. Click on [OK] to assign the new limit

1H spectral limits copied for F2 dimension.

SW: 8.4682 ppm
O1P: 4.234 ppm

Close

13. Click on [Close]

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

14. Select [□ Set_Limits] by clicking on it

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

15. To open the 1D C13DEPT spectrum, right click on the dataset name in the browser window (e.g. **Carbon_exp 1**) and select 'Display' or click and hold the left mouse button for dragging the 1D C13DEPT dataset in to the spectrum window

16. Expand the spectrum to display all peaks, leaving ca. 2 ppm of baseline on either side of the spectrum

NOTE: 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.

Figure 7.28
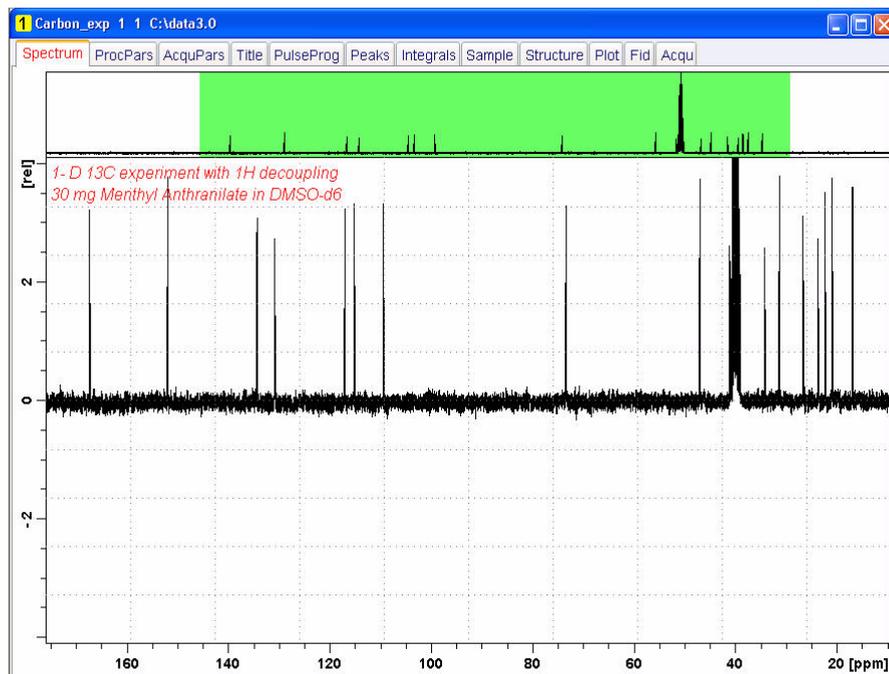
17. Click on [OK] to assign the new limit

Figure 7.29

18. Click on [Close]
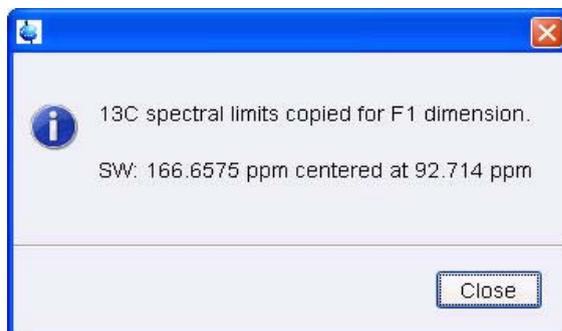
7.3.4   Acquisition

1. Select [Gain] by clicking on it

2. Select [Go] by clicking on it

7.3.5   Processing
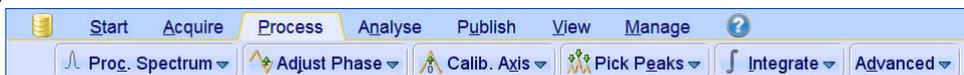
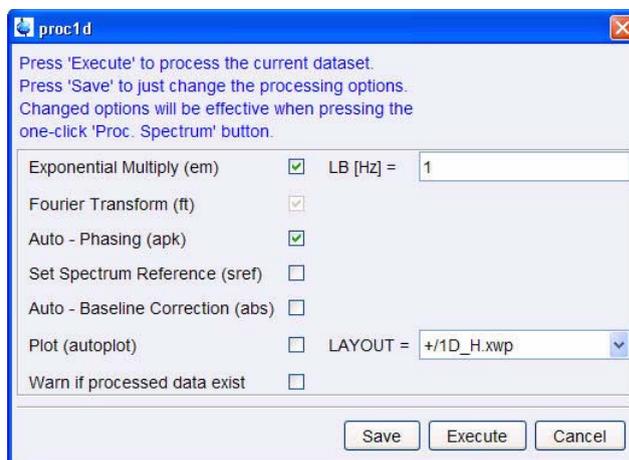1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 7.30
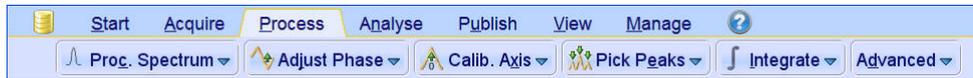
2. Select by clicking on it

Figure 7.31
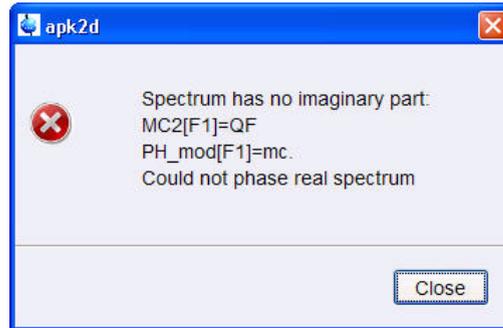
NOTE: This executes a standard processing program **proc2**. The message shown in Figure 7.31 pops up in case of a magnitude 2D experiment and the apk2d option is enabled. To configure the processing program follow steps 4 through 5.

---
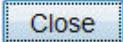
3. Click on

4. Click on the down arrow inside the button

Figure 7.32
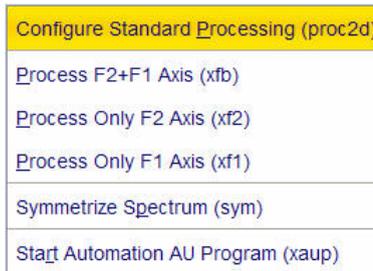
5. Select '**Configure Standard Processing**' by clicking on it

Figure 7.33
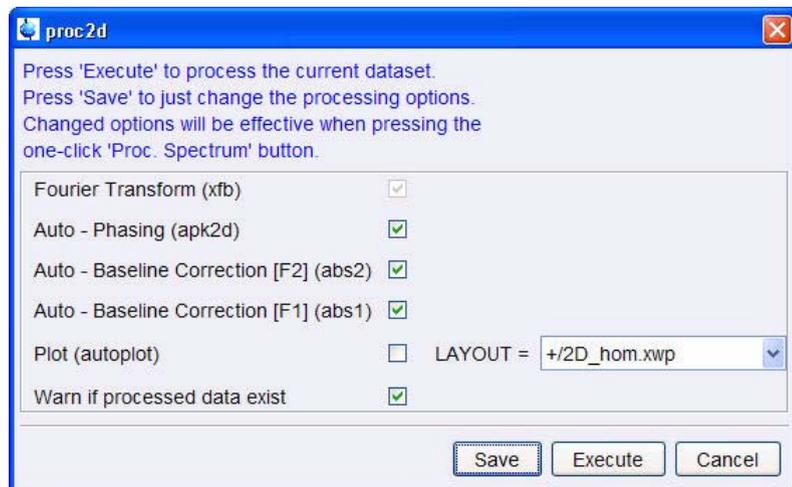
NOTE: To avoid the message shown in Figure 7.31 the option '**Auto-Phasing (apk2d)**' may be disabled for magnitude like 2D experiment.

Figure 7.34
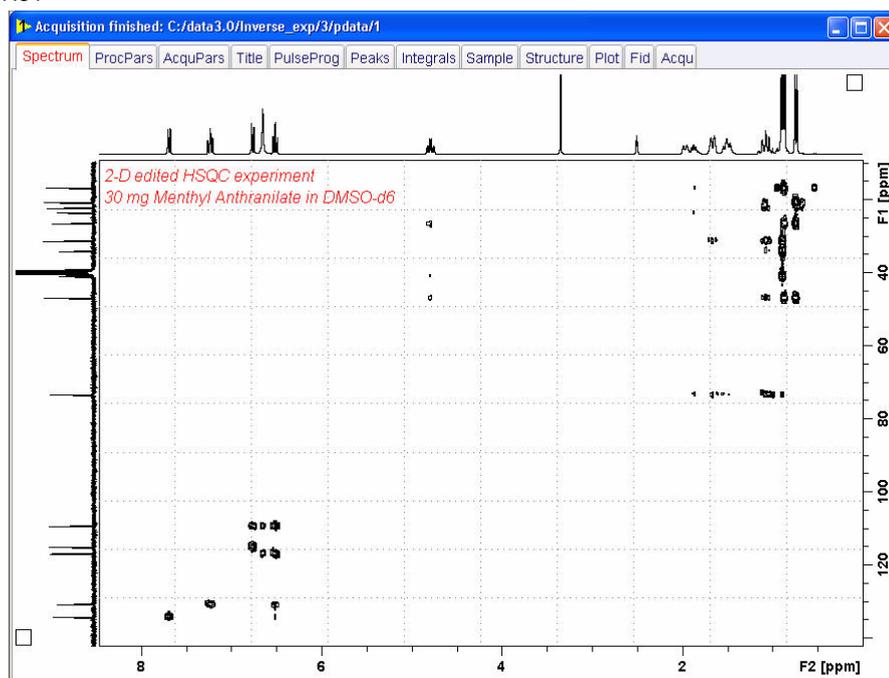
7.3.6   Plotting

1. Use the [icon] buttons to adjust for a suitable contour level

2. Click on the '**Publish**' tab in the TopSpin Menu bar

Figure 7.35

3. Click on   🖶 P<u>r</u>int ▼

NOTE: This will print the active window with the colors displayed in the TopSpin window. Using the '**plot**' option starting the plot editor, the default layout is designed not to show the F1 projection (see Figure 7.36 below. A new layout has to be created to add the F1 projection.

Figure 7.36
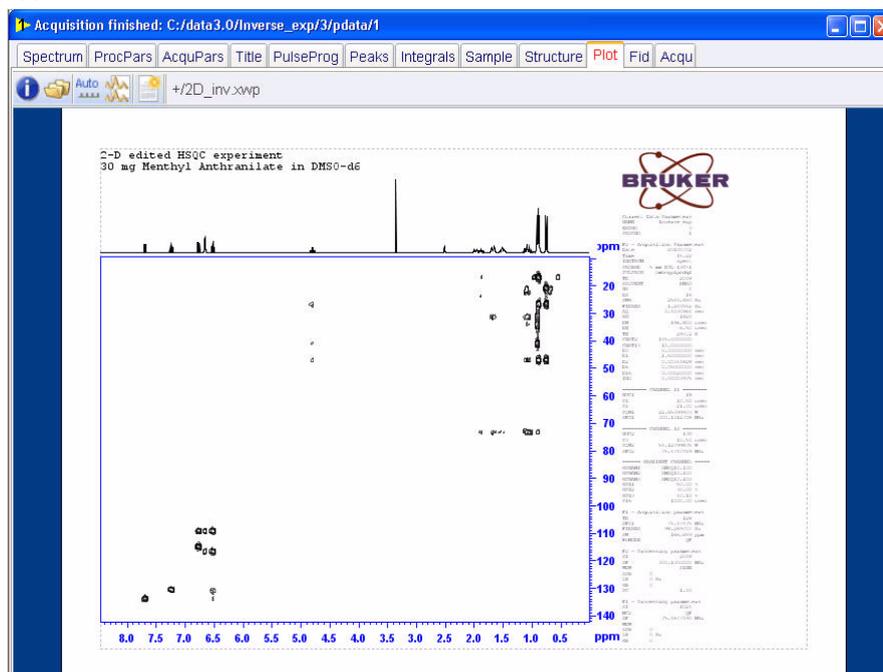
7.3.7   Observations

8 Determination of 90 degree pulses

8.1 Introduction

This chapter describes pulse calibration procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra. Chapter 3 (1-D Proton experiment) and chapter 6 (1-D Carbon experiments).

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

8.2 Proton 90 degree transmitter pulse

Standard Test Sample:

0.1% Ethylbenzene in CDCl3

8.2.1 Parameter setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 8.1

2. Select by clicking on it

3. Enter the following information in to the 'New' window
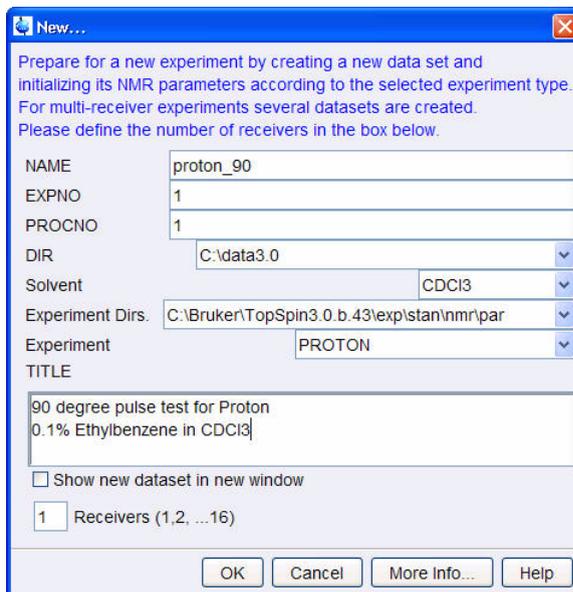
Figure 8.2

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 8.2 above. Click on the down arrow button to browse for a specific directory.

4. Click on **OK**

5. Run a **1D Proton** spectrum, following the instructions in **Chapter 3**, **1-D Proton experiment, Paragraph 3.2.2 Experiment setup, step 5** through **3.2.4 Processing**.

Figure 8.3
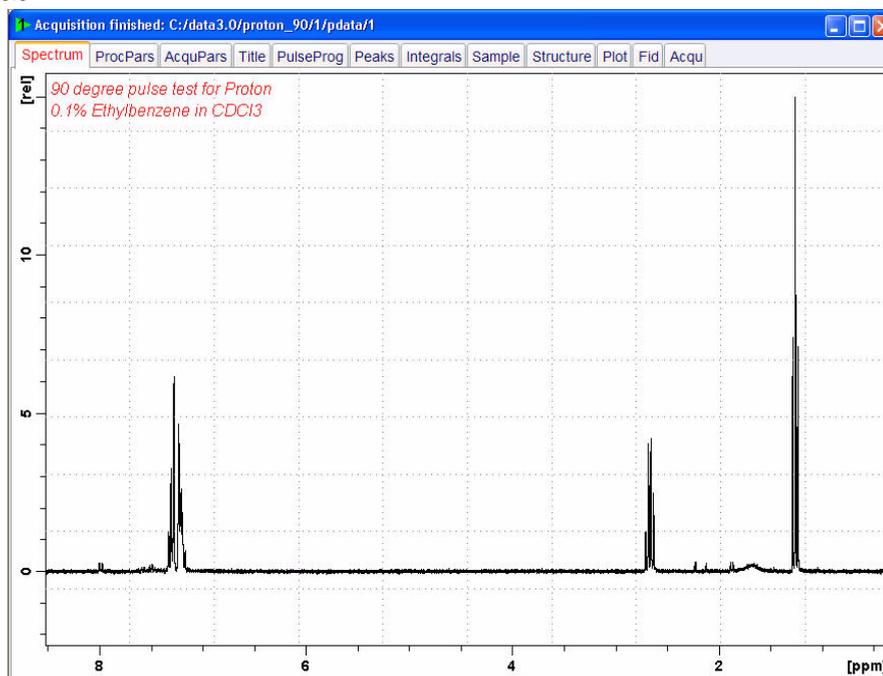
6. Expand peak at 2.7 ppm

7. Click on ↓ to set the RF from cursor

Figure 8.4
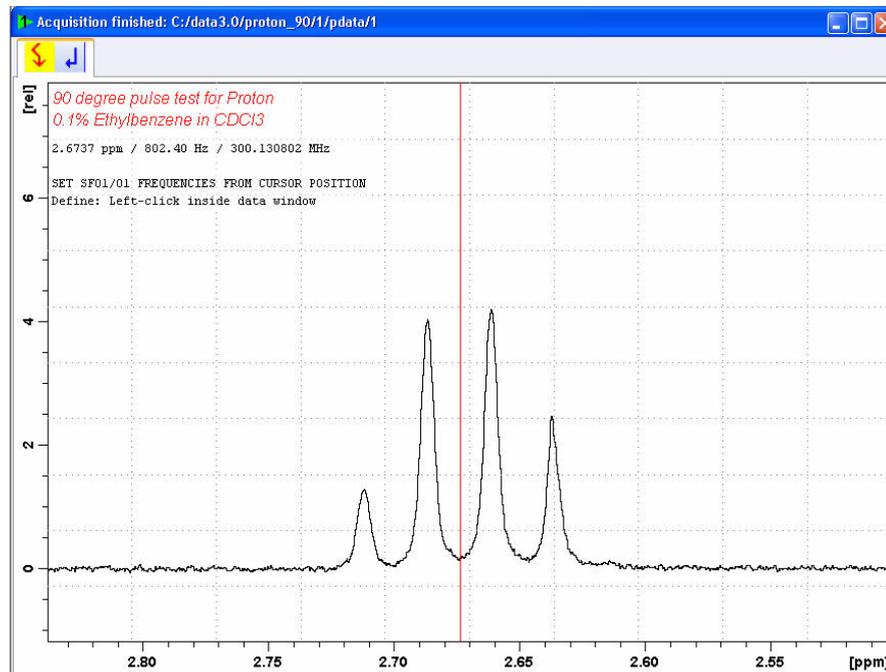
8. Move the cursor line in to the center of the multiplet

9. Click the left mouse button to set the frequency

Figure 8.5
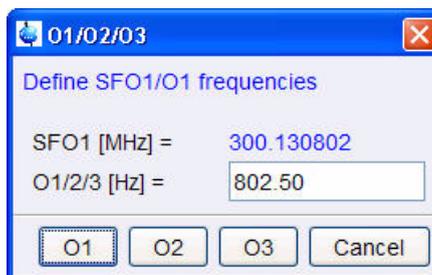
10. Click on O1

11. Select the 'AcquPars' tab by clicking on it

12. Make the following changes:

        PULPROG = **zg**

        TD = **4048**

        SW [Hz] =**300**

        D1 [sec] = **30**

        DS = **0**

        NS = **1**

13. Select the 'ProcPars' tab by clicking on it

14 Make the following changes:

     SI = **2024**

     LB [Hz] = **1**

     PH_mod = select **'pk'**

15. Click on the 'Aquire' tab in the TopSpin menu bar

Figure 8.6
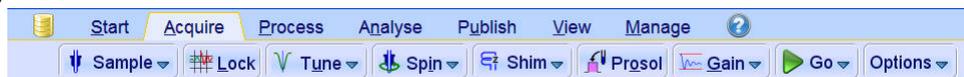
16. Select by clicking on it

Figure 8.7
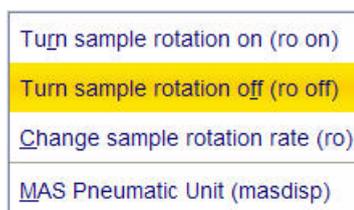
17. Select 'ro off' by clicking on it

NOTE: This test should be run non spinning

8.2.2   Acquisition

1. Select by clicking on it

2. Select by clicking on it

8.2.3   Processing

1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 8.8

2. Click on the down arrow inside the button

Figure 8.9
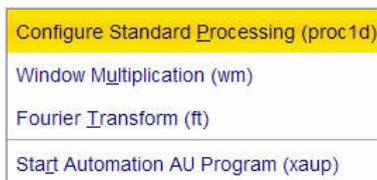
Configure Standard Processing (proc1d)

Window Multiplication (wm)

Fourier Transform (ft)

Start Automation AU Program (xaup)

3. Select '**Configure Standard Processing**' by clicking on it

4. Deselect the following options:

   '**Set Spectrum Reference (sref)**'
   '**Auto-Baseline correction (abs)**'
   '**Warn if Processed data exist**'

Figure 8.10

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] =	1
Fourier Transform (ft)	☑		
Auto - Phasing (apk)	☑		
Set Spectrum Reference (sref)	☐		
Auto - Baseline Correction (abs)	☐		
Plot (autoplot)	☐	LAYOUT =	+/1D_H.xwp
Warn if processed data exist	☐		

Save   Execute   Cancel

5. Click on   Execute
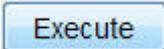
6. Expand the spectrum form 2.8 ppm to 2.5 ppm

Figure 8.11
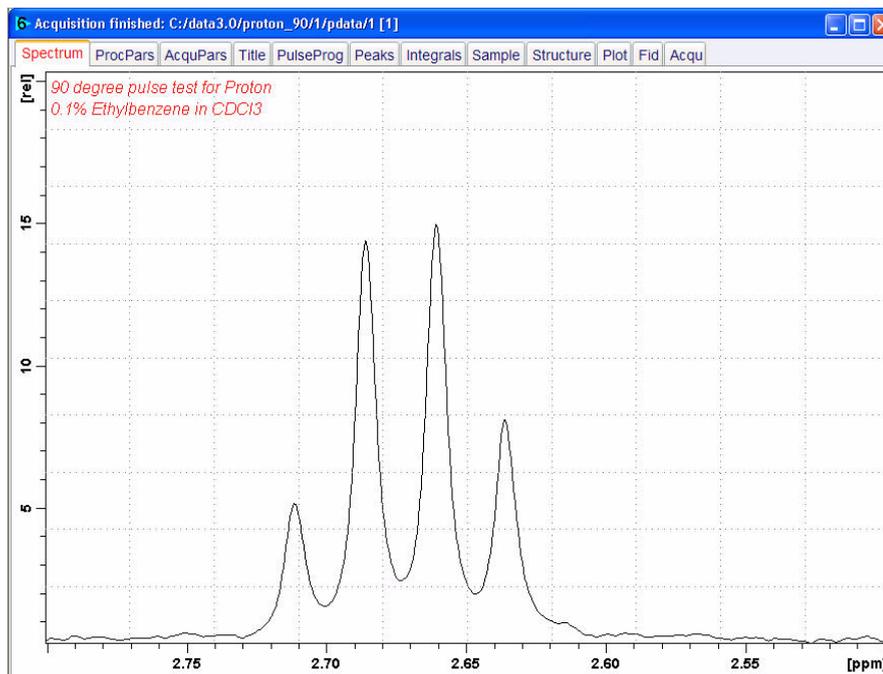
7. Click on the right mouse button inside the spectral window

Figure 8.12
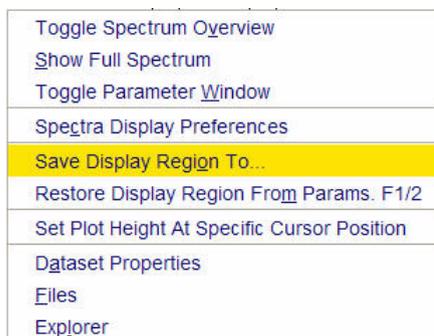
8. Select '**Save Display Region to...**' by clicking on it

Figure 8.13
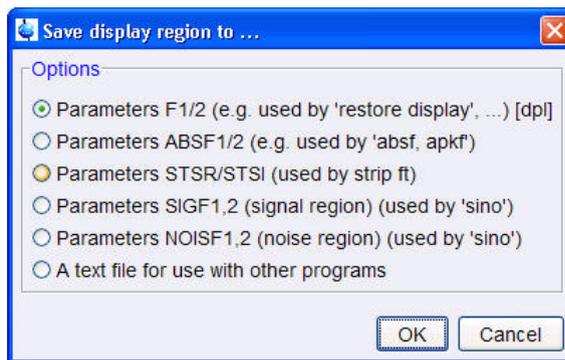
9. **Enable 'Parameters F1/2'**

10. Click on OK

11. Type **wpar** to store the parameter for future use

12. Select the user parameter directory

Figure 8.14

Source = C:\Bruker\TopSpin3.0.b.43\exp\stan\nmr\par\user

Figure 8.15

13. Click on Write New...
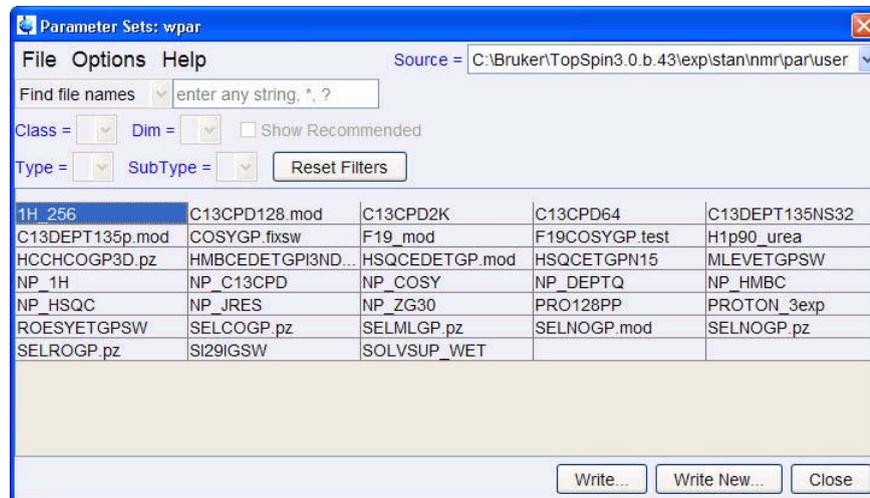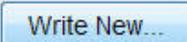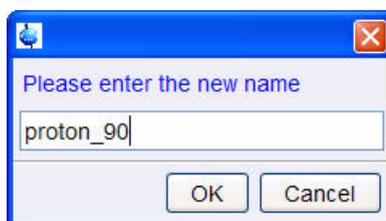
Figure 8.16

14. Type **proton_90** in to the new name window

15. Click on OK
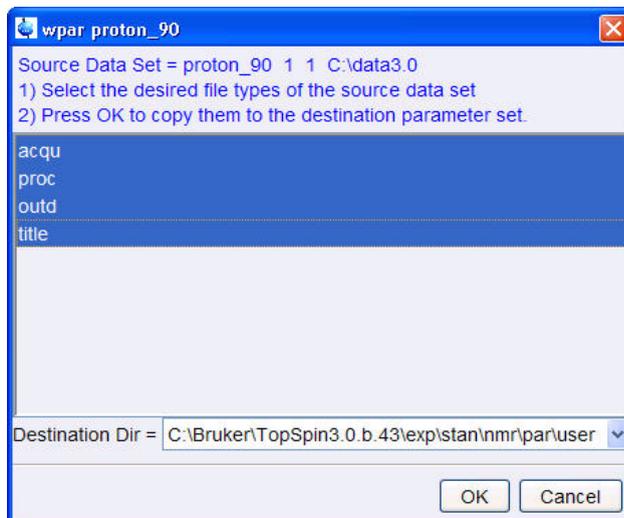
Figure 8.17

16. Select all parameter options

17. Click on OK

Figure 8.18

18. Click on Close
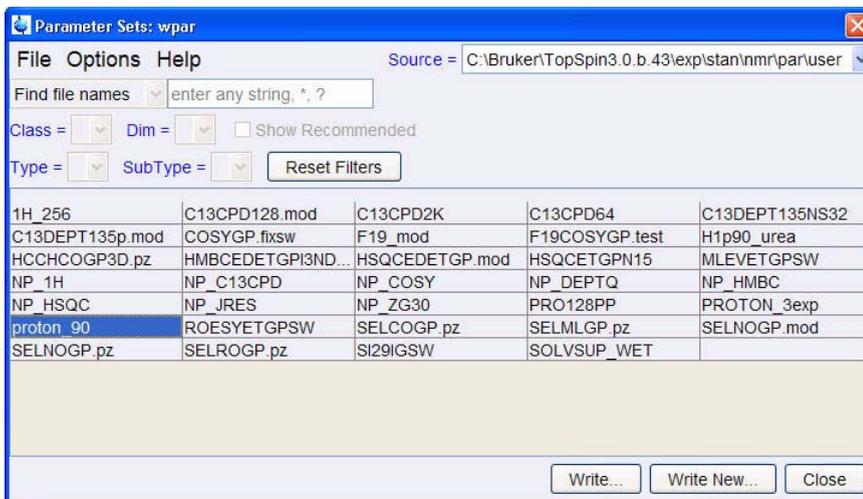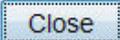
8.2.4   Determine the 90^0 pulse

1. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 8.19

2. Click on the down arrow inside the button

Figure 8.20
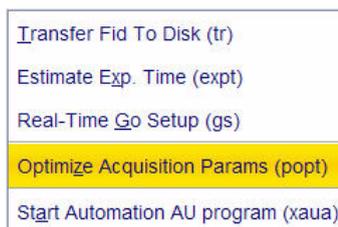
3. Select '**Optimize Acquisition Params (popt)**' by clicking on it

4. Make the following changes:

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

Figure 8.21
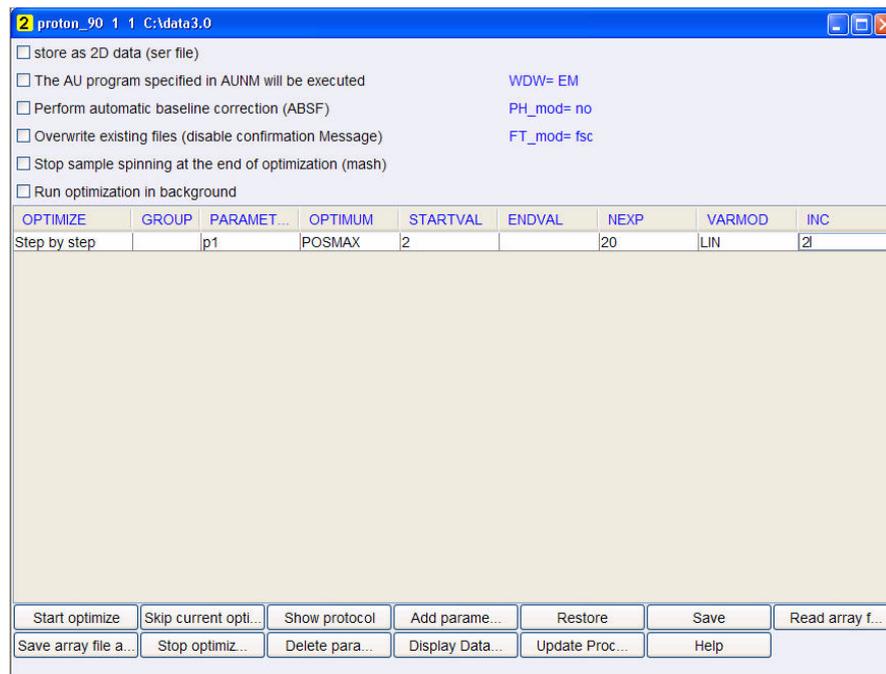
5. Click on
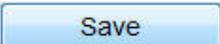
NOTE: The ENDVAL parameter has been updated.
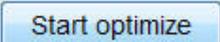
6. Click on

Figure 8.22
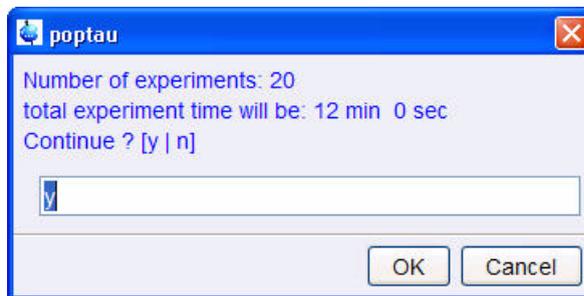
7. Enter **y** in to the poptau window

8. Click on [OK]

NOTE: The parameter optimization starts. The spectrometer acquires and processes 20 spectra with incrementing the parameter p1 from 2 usec by 2 usec to a final value of 40 usec. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file proton_90/1/999 as shown in Figure 8.23.

Figure 8.23
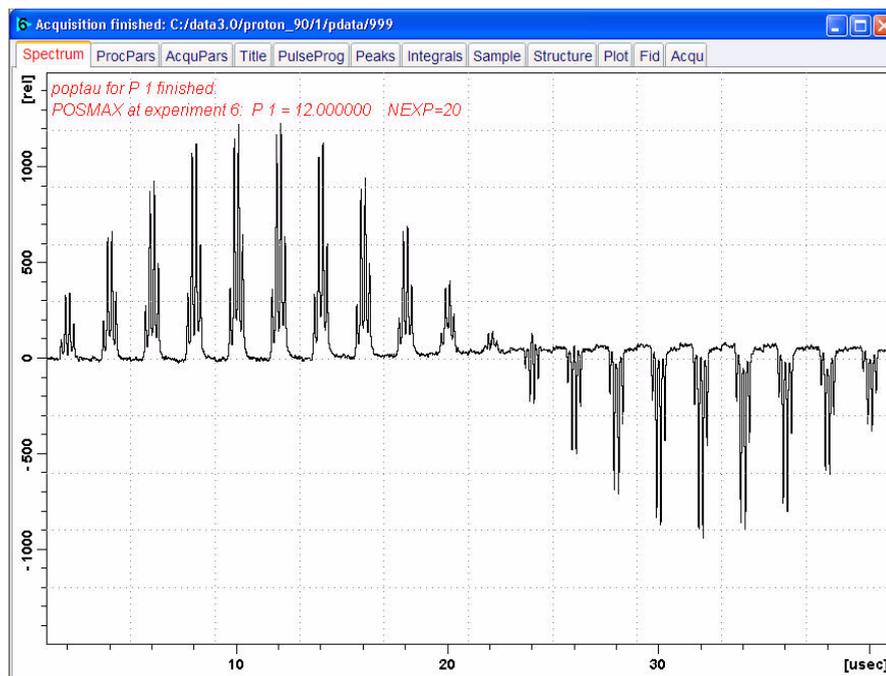
NOTE: The POSMAX value of **p1** is displayed in the title window which is the 90 degree pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90 degree pulse measurement, follow the steps below.

9. Close the popt setup window

10. Type **re 1 1**

11. Type **p1**

12. Enter the value which corresponds to a 360 degree pulse (four times the POSMAX value)

13. Type **zg**

14. Type **efp**

15. Change p1 slightly and repeat steps 13 and 14, until the quartet undergoes a zero crossing as expected for an exact 360 degree pulse.

NOTE: The quartet signal is negative for a pulse angle slightly less then 360 degree and positive when the pulse angle is slightly more then 360 degree.

---

16. Simply divide the determine 360 degree pulse value by 4. This will be the exact 90 degree pulse length for the proton transmitter on the current probe

8.2.5 Observations

8.3   Carbon 90 degree transmitter pulse

Standard Test Sample:

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

8.3.1   Parameter setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 8.24

2. Select [Create Dataset] by clicking on it

3. Enter the following information in to the 'New' window

Figure 8.25

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 8.25 above. Click on the down arrow button to browse for a specific directory.

4. Click on [OK]

5. Run a **1D Carbon** spectrum, following the instructions in **Chapter 6**, **1-D Carbon experiments, Paragraph 6.2.2 Experiment setup, step 5** making the following acquisition parameter changes:

PULPROG = **zg**

DS = **0**

NS = **1**

6. Continue with **6.2.4 Processing**.

Figure 8.26
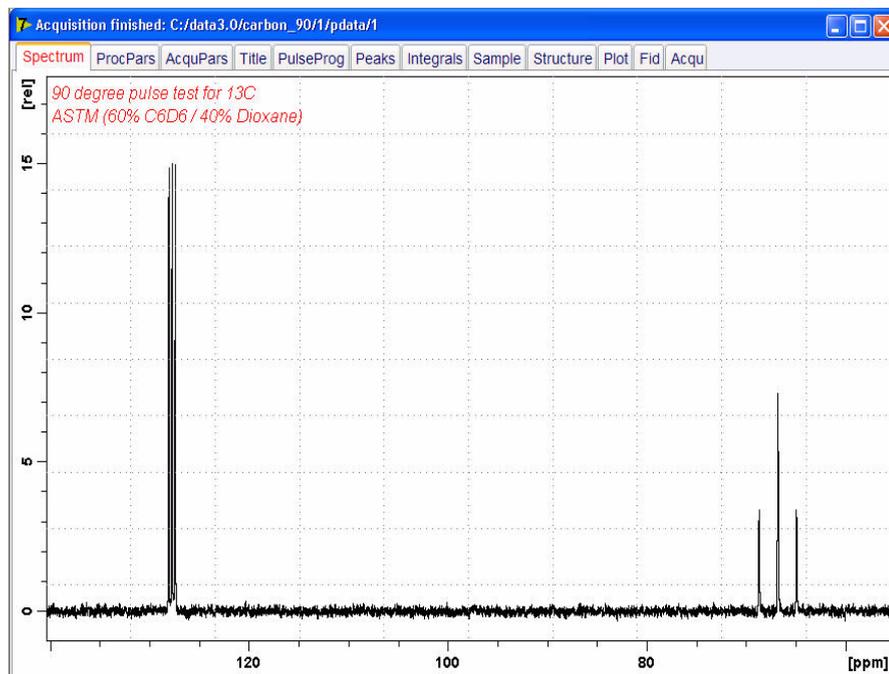
7. Expand peak at 67 ppm

8. Click on [icon] to set the RF from cursor

Figure 8.27
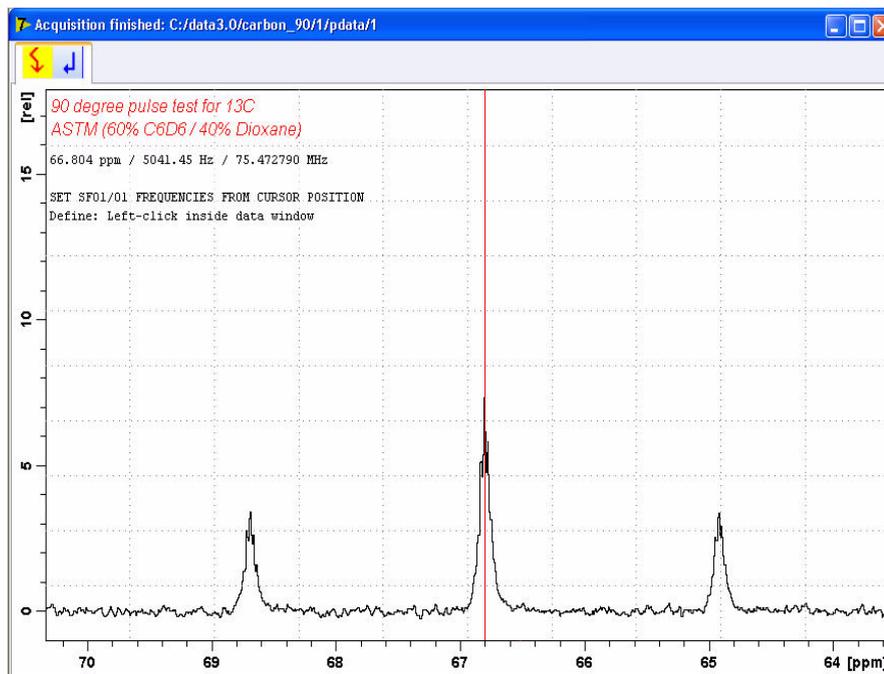
9. Click the left mouse button to set the frequency

Figure 8.28
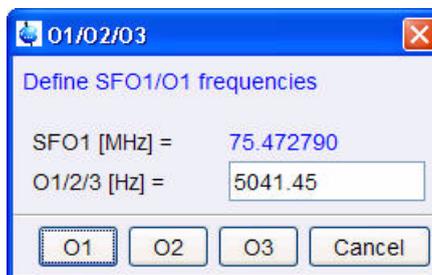
10. Click on [O1]

11. Select the '**AcquPars**' tab by clicking on it

12. Make the following changes:

       TD = **4048**

       SW [Hz] =**20**

       D1 [sec] = **60**

13. Select the '**ProcPars**' tab by clicking on it

14 Make the following changes:

       SI = **2024**

       LB [Hz] = **3.5**

       PH_mod = select '**pk**'

15. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 8.29

Select by clicking on it

Figure 8.30
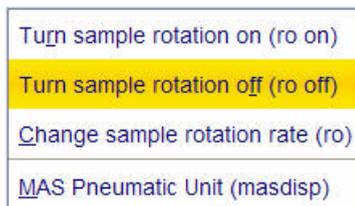
16. Select '**ro off**' by clicking on it

NOTE: This test should be run non spinning

8.3.2   Acquisition

1. Select by clicking on it
2. Select by clicking on it

8.3.3   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 8.31
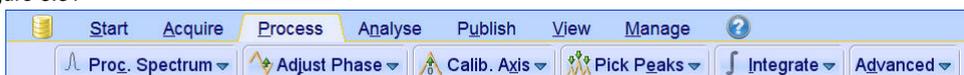
2. Click on the down arrow inside the button

Figure 8.32
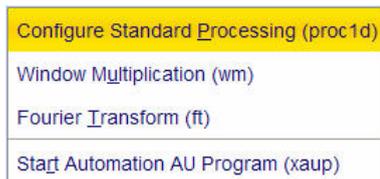
3. Select '**Configure Standard Processing**' by clicking on it
4. Deselect the following options:
    '**Set Spectrum Reference (sref)**'

'**Auto-Baseline correction (abs)**'

'**Warn if Processed data exist**'

Figure 8.33
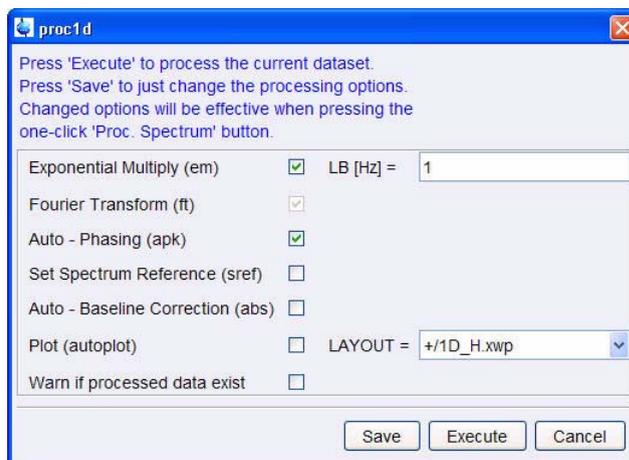
5. Click on [Execute]

6. Expand the spectrum from 71 ppm to 63 ppm

Figure 8.34
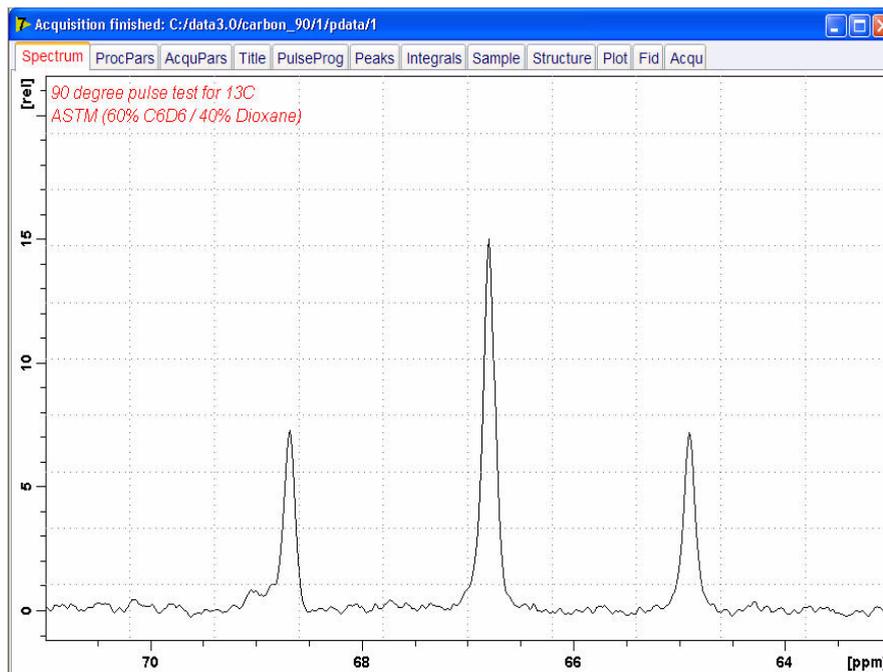
7. Click on the right mouse button inside the spectral window

Figure 8.35

8. Select '**Save Display Region to...**' by clicking on it

Figure 8.36
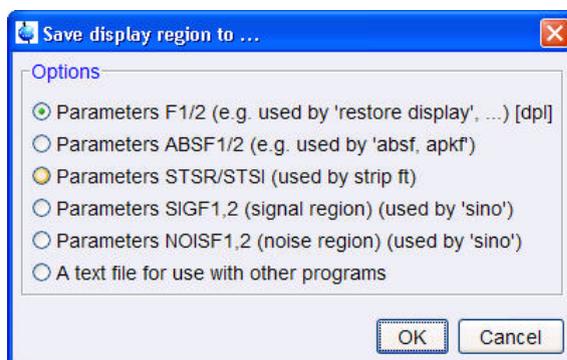
9. **Enable 'Parameters F1/2'**

10. Click on [OK]

11. Type **wpar** to store the parameter for future use

12. Select the user parameter directory

Figure 8.37

Source = C:\Bruker\TopSpin3.0.b.43\exp\stan\nmr\par\user
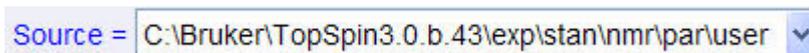
Figure 8.38

13. Click on **Write New...**
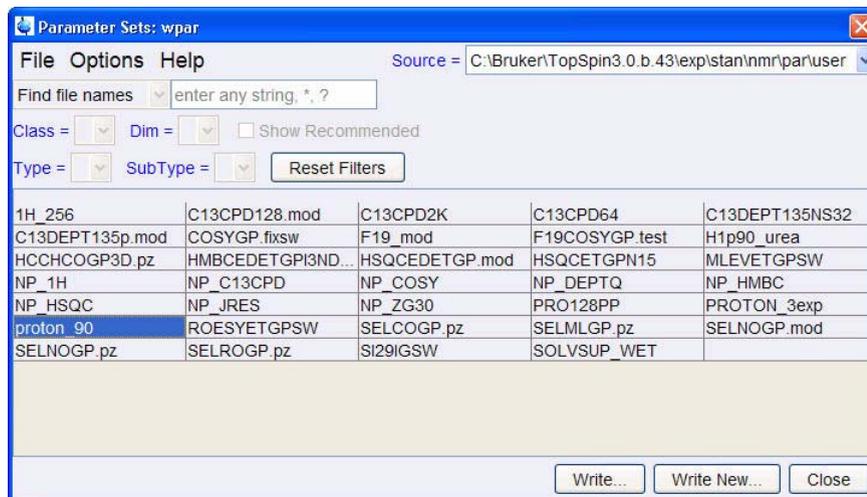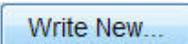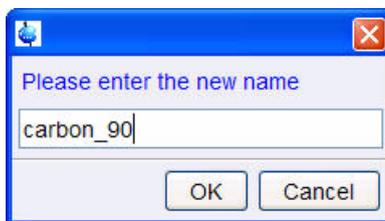
Figure 8.39

14. Type **carbon_90** in to the new name window

15. Click on **OK**
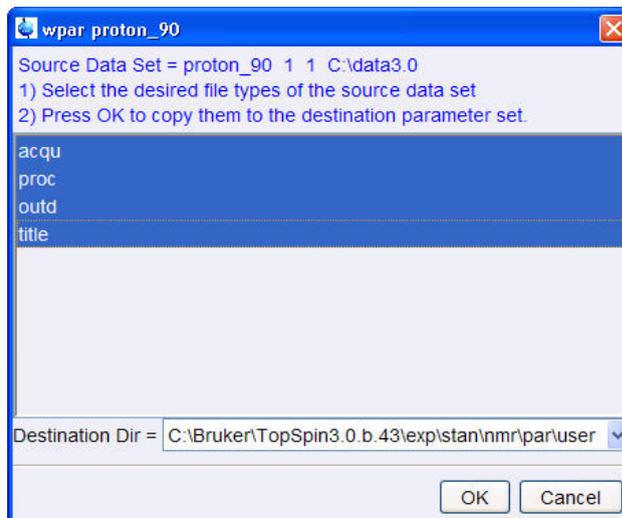
Figure 8.40

16. Select all parameter options

17. Click on [OK]

Figure 8.41

18. Click on [Close]
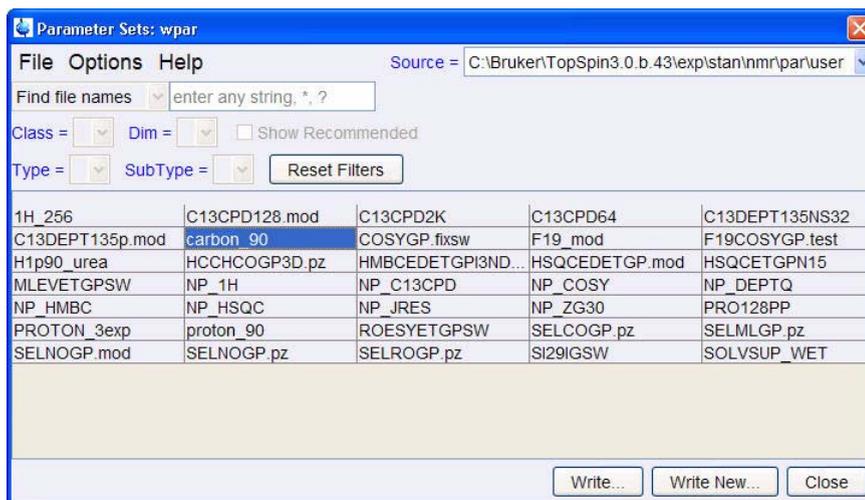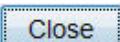
8.3.4   Determine the 90⁰ pulse

1. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 8.42

2. Click on the down arrow inside the [Go▼] button
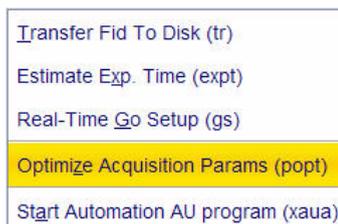
Figure 8.43

3. Select '**Optimize Acquisition Params (popt)**' by clicking on it

4. Make the following changes:

OPTIMIZE = **Step by step**

PARAMETER = **p1**

OPTIMUM = **POSMAX**

STARTVAL = **2**

NEXP = **20**

VARMOD = **LIN**

INC = **2**

Figure 8.44
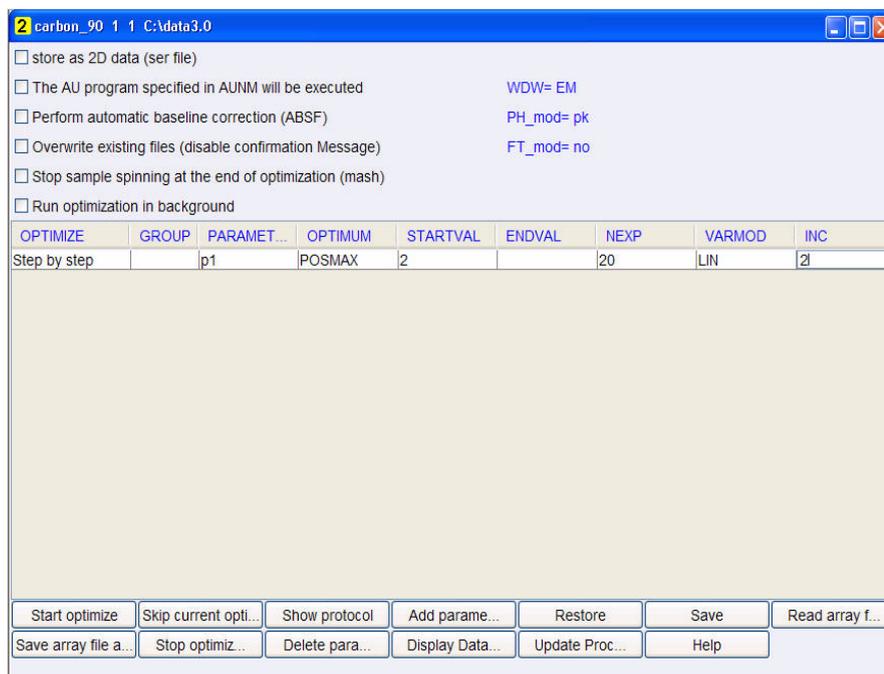
5. Click on
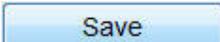
NOTE: The ENDVAL parameter has been updated.

6. Click on

Figure 8.45
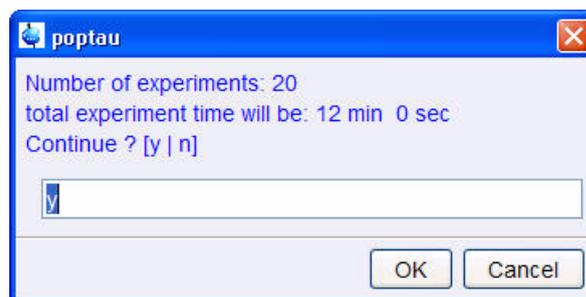
7. Enter **y** in to the poptau window

8. Click on

NOTE: The parameter optimization starts. The spectrometer acquires and processes 20 spectra with incrementing the parameter p1 from 2 usec by 2 usec to a final value of 40 usec. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file carbon_90/1/999 as shown in Figure 8.46.

Figure 8.46
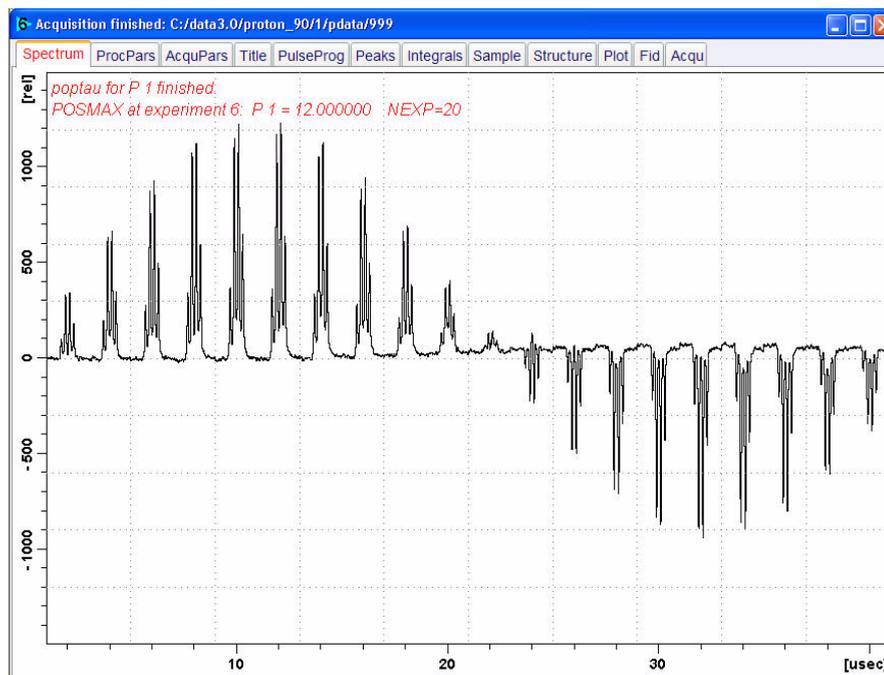
NOTE: The POSMAX value of p1 is displayed in the title which is the 90 degree pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90 degree pulse measurement, follow the steps below.

9. Close the popt setup window

10. Type **re 1 1**

11. Type **p1**

12. Enter the value which corresponds to a 360 degree pulse (four times the POSMAX value)

13. Type **zg**

14. Type **efp**

15. Change **p1** slightly and repeat steps 13 and 14, until the signal undergoes a zero crossing as expected for an exact 360 degree pulse.

NOTE: The signal is negative for a pulse angle slightly less then 360 degree and positive when the pulse angle is slightly more then 360 degree.

16. Simply divide the determine 360 degree pulse value by 4. This will be the exact 90 degree pulse length for the proton transmitter on the current probe

8.3.5 Observations

9 Sensitivity tests

9.1 Introduction

This chapter describes the sensitivity test procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra. Chapter 3 (1-D Proton experiment) and chapter 6 (1-D Carbon experiments). Also the 90 degree pulses have to be properly calibrated, Chapter 8 (Determination of the 90 degree pulses)

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

9.2 1H Sensitivity test

Standard Test Sample:

0.1% Ethylbenzene in CDCl3

9.2.1 Experiment setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 9.1

2. Select [Create Dataset] by clicking on it

3. Enter the following information in to the 'New' window

Figure 9.2
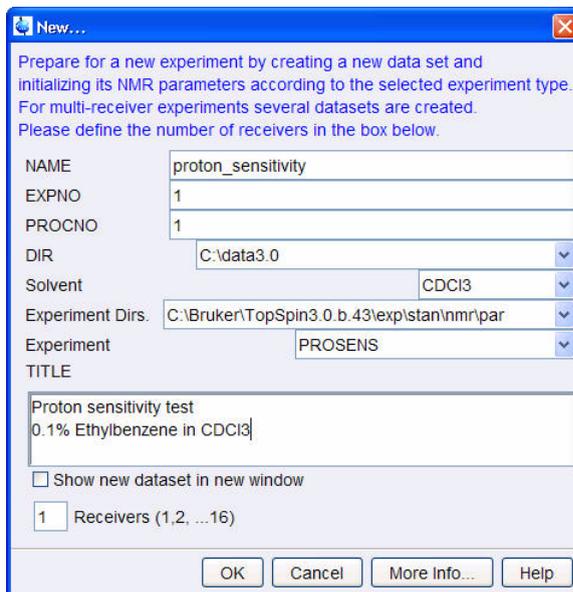
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 9.2 above. Click on the down arrow button to browse for a specific directory.

4. Click on ☐ OK

5. Click on the '**Aquire**' tab in the TopSpin menu bar
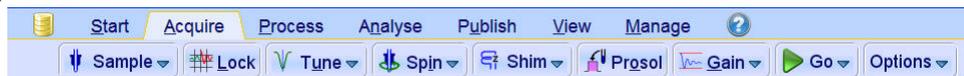
Figure 9.3

6. Select ☐ **Sample ▾** by clicking on it

Figure 9.4

7. Select '**ej**' by clicking on it

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

8. Place the sample on too the top of the magnet

9. Select ☐ **Sample ▾** by clicking on it

Figure 9.5
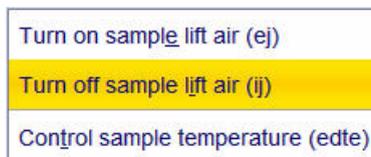
10. Select '**ij**' by clicking on it

NOTE: Wait till the sample is lowered down in to the probe and the lift air is turned off. A licking sound may be heard.

11. Select [Lock] by clicking on it

Figure 9.6
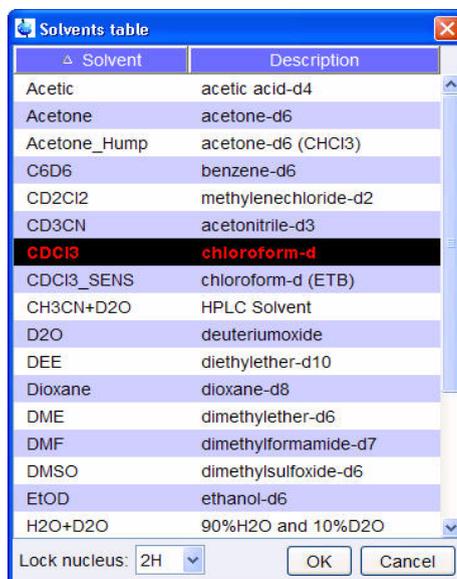
12. Select '**CDCl3**' by clicking on it
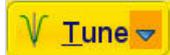
13. Select [Tune ▼] by clicking on it

NOTE: This performs a '**atma**' (automatic tuning) and requires a probe equipped with a automatic tuning module. Other options can be selected by clicking on the down arrow inside the '**Tune**' button.

14. Select [Spin ▼] by clicking on it

Figure 9.7
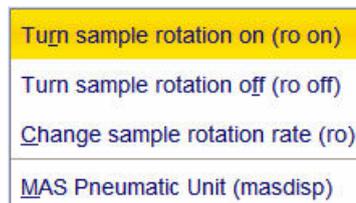
15. Select '**ro on**' by clicking on it

NOTE: Rotation may be turned off for probes such as BBI, TXI, TBI and for small sample probes.

---

16. Select $\boxed{\text{⚙ Shim ▾}}$ by clicking on it

NOTE: This executes the command '**topshim**'. To select other options. click on the down arrow inside the '**Shim**' button.

---

17. Select $\boxed{\text{⚗ Prosol}}$ by clicking on it

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

9.2.2   Acquisition

1. Select $\boxed{\text{〰 Gain ▾}}$ by clicking on it

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

---

2. Select $\boxed{\text{▶ Go ▾}}$ by clicking on it

9.2.3   Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 9.8
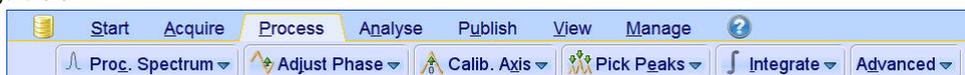
2. Click on $\boxed{\text{∫ Proc. Spectrum ▾}}$

NOTE: 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**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button.

9.2.4   Calculating the Signal to Noise ratio

The signal to noise ratio is determined on the intensity of the quartet lines between 2ppm and 3ppm. It is calculated by AU-program **sinocal** over a range of 2ppm between

2.8ppm and 7ppm. The s/n ratio is strongly dependant on good resolution and line-shape. The splitting between the two central lines of the methylquartet should go lower than 15% (with LB=1Hz), see Figure 9.9.

Figure 9.9
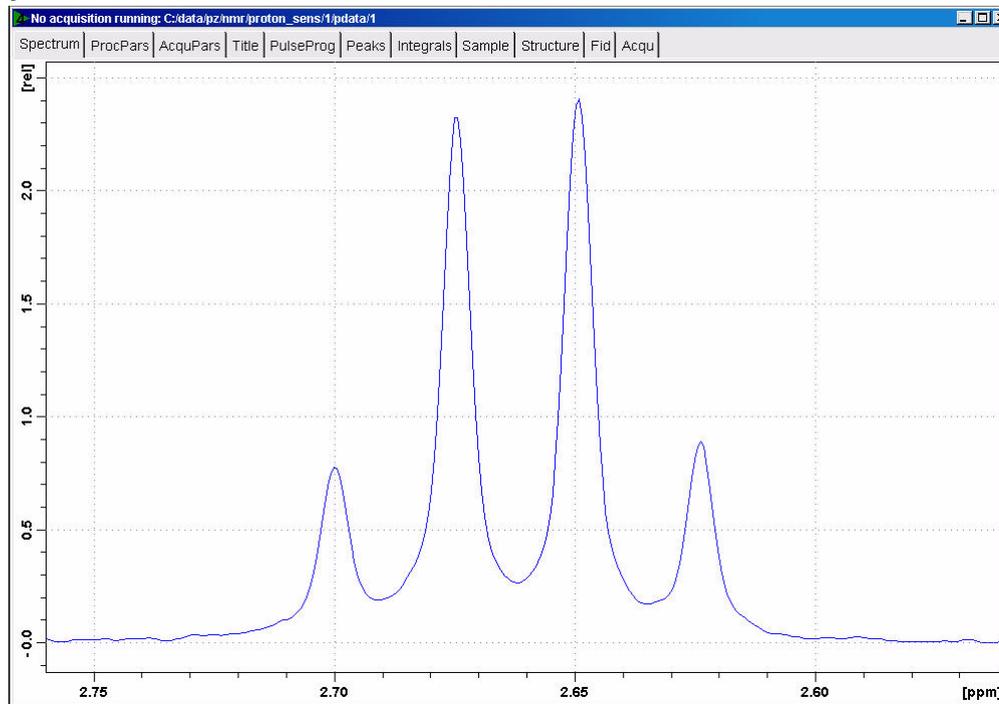
1. Type **sinocal** on the command line

Figure 9.10
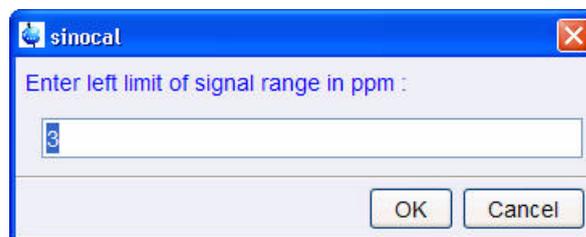
2. Enter **3** for the left limit of the signal range

3. Click on [OK]

Figure 9.11
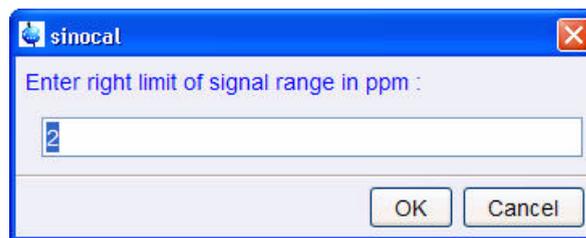
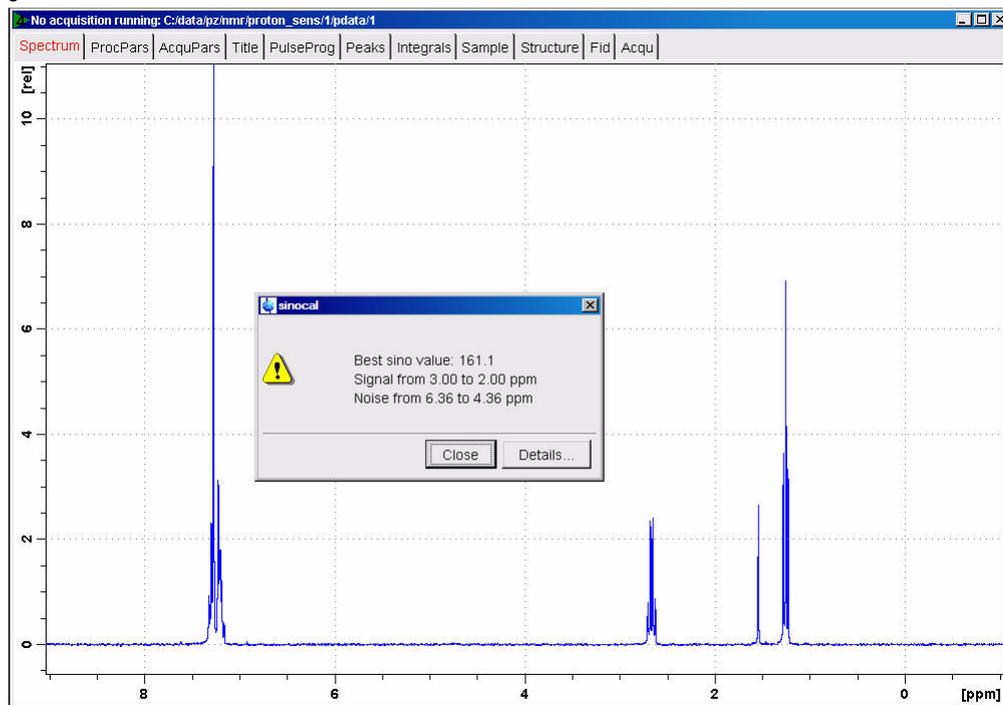4. Enter **2** for the right limit of the signal range

5. Click on [OK]
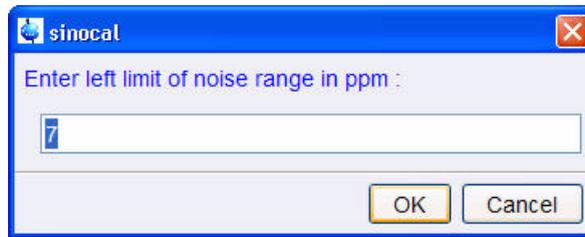
Figure 9.12

6. Enter **7** for the left limit of the noise range

7. Click on [OK]
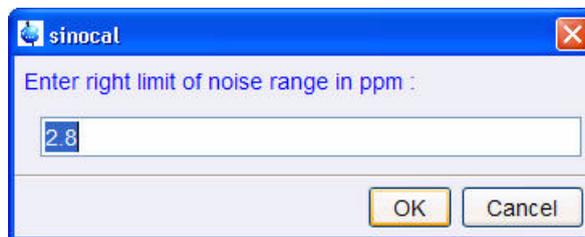
Figure 9.13

8. Enter **2.8** for the right limit of the noise range

9. Click on [OK]
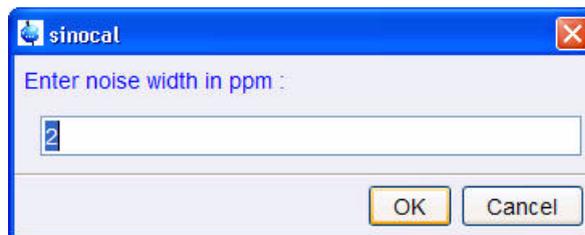
Figure 9.14

10. Enter **2** for the noise width

11. Click on [OK]

Figure 9.15

9.2.5 Observations

9.3   13C Sensitivity test with 1H decoupling

Standard Test Sample:

10% Ethylbenzene in CDCl3

9.3.1   Experiment setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 9.16

2. Select by clicking on it

3. Enter the following information in to the 'New' window

Figure 9.17
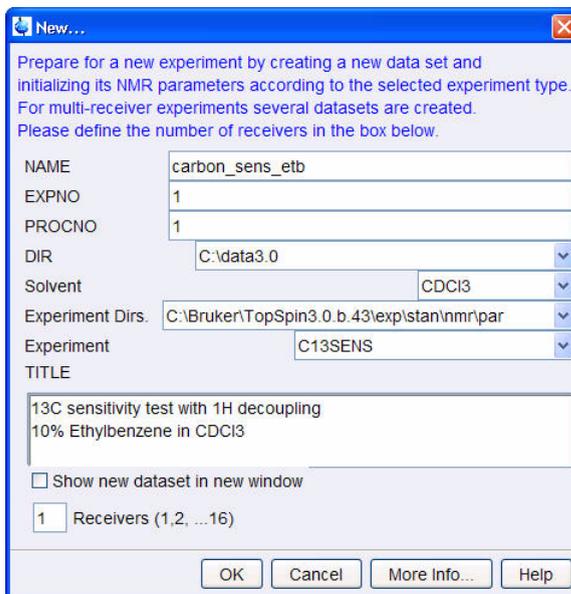
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 9.17 above. Click on the down arrow button to browse for a specific directory.

4. Click on

5. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 9.18

6. Select by clicking on it

Figure 9.19

| Turn on sample lift air (ej) |
| Turn off sample lift air (ij) |
| Control sample temperature (edte) |

7. Select '**ej**' by clicking on it

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

8. Place the sample on too the top of the magnet

9. Select by clicking on it

Figure 9.20

| Turn on sample lift air (ej) |
| Turn off sample lift air (ij) |
| Control sample temperature (edte) |

10. Select '**ij**' by clicking on it

NOTE: Wait till the sample is lowered down in to the probe and the lift air is turned off. A licking sound may be heard.

11. Select by clicking on it

Figure 9.21

△ Solvent	Description
Acetic	acetic acid-d4
Acetone	acetone-d6
Acetone_Hump	acetone-d6 (CHCl3)
C6D6	benzene-d6
CD2Cl2	methylenechloride-d2
CD3CN	acetonitrile-d3
CDCl3	chloroform-d
CDCl3_SENS	chloroform-d (ETB)
CH3CN+D2O	HPLC Solvent
D2O	deuteriumoxide
DEE	diethylether-d10
Dioxane	dioxane-d8
DME	dimethylether-d6
DMF	dimethylformamide-d7
DMSO	dimethylsulfoxide-d6
EtOD	ethanol-d6
H2O+D2O	90%H2O and 10%D2O

Lock nucleus: 2H   OK   Cancel

12. Select '**CDCl3**' by clicking on it

13. Select [Tune] by clicking on it

NOTE: This performs a '**atma**' (automatic tuning) and requires a probe equipped with a automatic tuning module. Other options can be selected by clicking on the down arrow inside the '**Tune**' button.

14. Select [Spin] by clicking on it

Figure 9.22

Turn sample rotation on (ro on)

Turn sample rotation off (ro off)

Change sample rotation rate (ro)

MAS Pneumatic Unit (masdisp)

15. Select '**ro on**' by clicking on it

NOTE: Rotation may be turned off for probes such as BBI, TXI, TBI and for small sample probes.

16. Select [Shim] by clicking on it

NOTE: This executes the command '**topshim**'.To select other options. click on the down arrow inside the '**Shim**' button.

17. Select [Prosol] by clicking on it

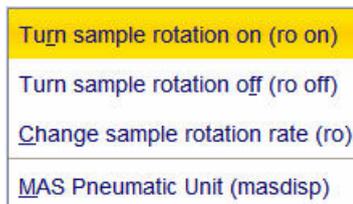NOTE: This will load the pulse width and power levels in to the parameter set.

9.3.2 Acquisition

1. Select [Gain] by clicking on it

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

2. Select [Go] by clicking on it

9.3.3 Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 9.23
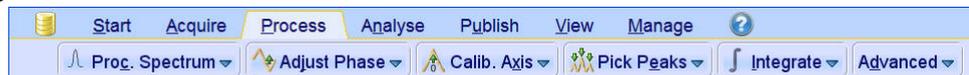
2. Click on

NOTE: 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'. Other options are available by clicking on the down arrow inside the 'Proc. Spectrum' button.

9.3.4   Calculating the Signal to Noise ratio

The signal to noise ratio is determined on the highest peak of the aromatic part between 127ppm and 129ppm see Figure 9.24. below. It is calculated by AU-program **sinocal** over a range of 40ppm between 30ppm and 125ppm. The s/n ratio is strongly dependant on good resolution and line shape.

Figure 9.24
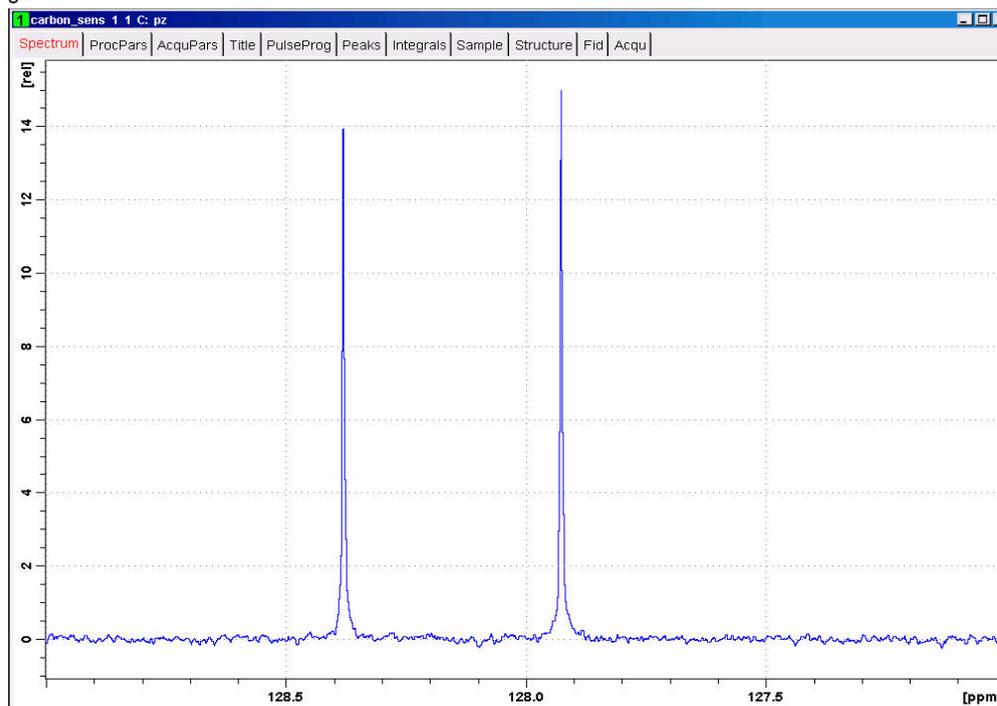
1. Type **sinocal** on the command line

Figure 9.25
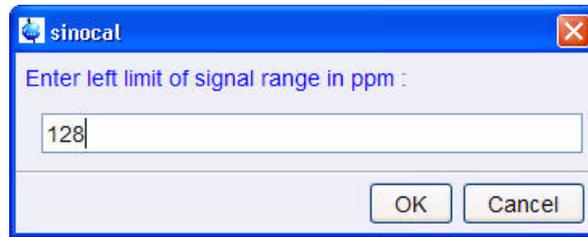
2. Enter **128** for the left limit of the signal range

3. Click on   OK
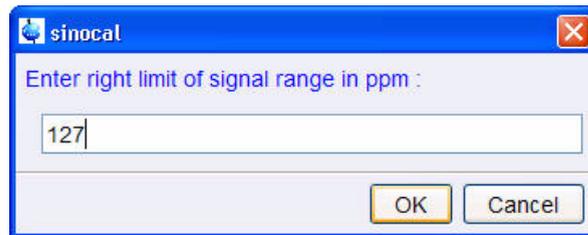
Figure 9.26

4. Enter **127** for the right limit of the signal range

5. Click on   OK
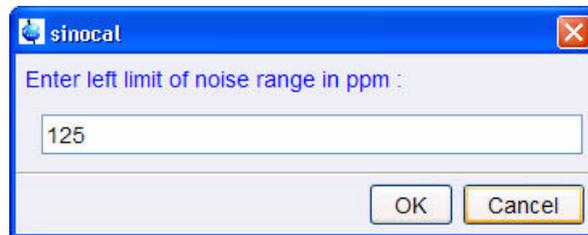
Figure 9.27

6. Enter **125** for the left limit of the noise range

7. Click on   OK
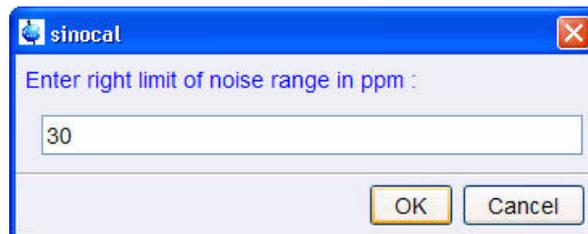
Figure 9.28

8. Enter **30** for the right limit of the noise range

9. Click on   OK
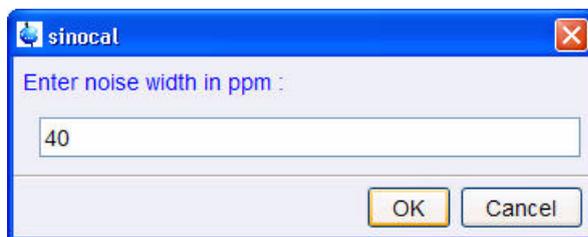
Figure 9.29

10. Enter **40** for the noise width

11. Click on [OK]
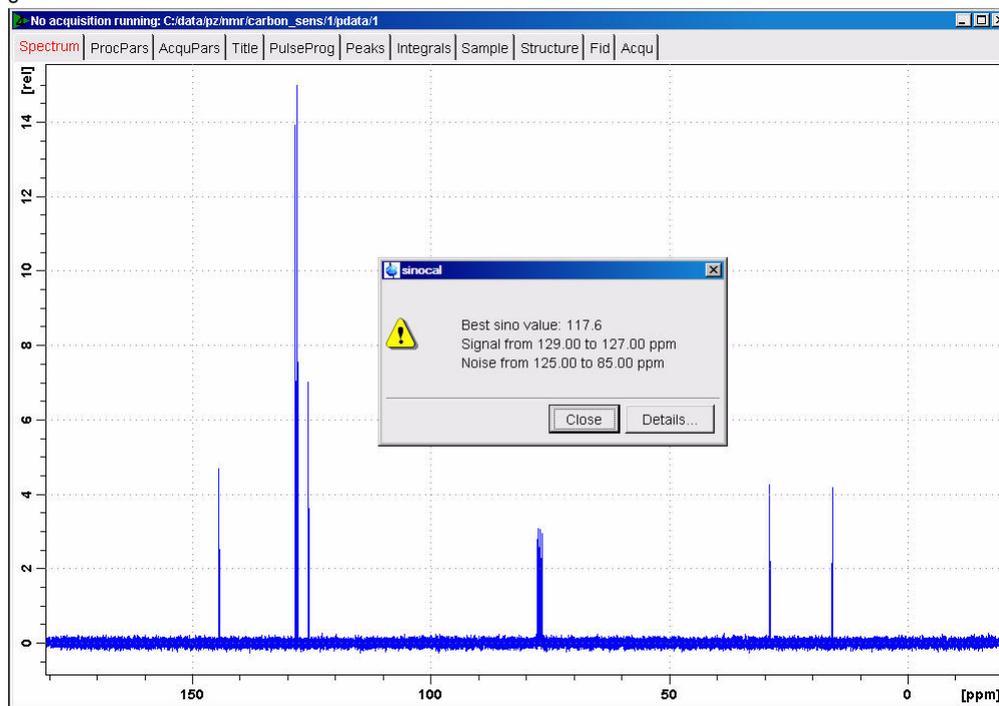
Figure 9.30

9.3.5 Observations

9.4   13C Sensitivity test without 1H decoupling

Standard Test Sample:

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

9.4.1   Experiment setup

1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 9.31

2. Select by clicking on it

3. Enter the following information in to the 'New' window

Figure 9.32
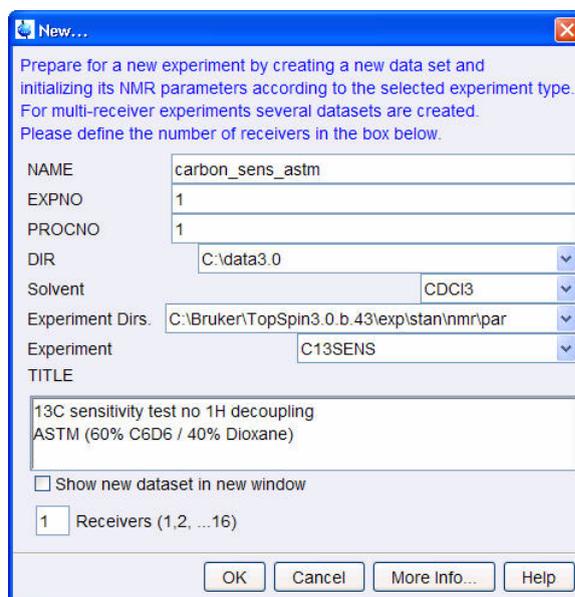
NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 9.32 above. Click on the down arrow button to browse for a specific directory.

4. Click on

5. Click on the '**Aquire**' tab in the TopSpin menu bar

Figure 9.33

6. Select by clicking on it

Figure 9.34

7. Select '**ej**' by clicking on it

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

---

8. Place the sample on too the top of the magnet

9. Select by clicking on it

Figure 9.35
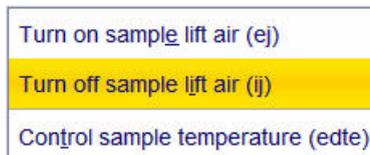
10. Select '**ij**' by clicking on it

NOTE: Wait till the sample is lowered down in to the probe and the lift air is turned off. A licking sound may be heard.

---

11. Select by clicking on it

Figure 9.36
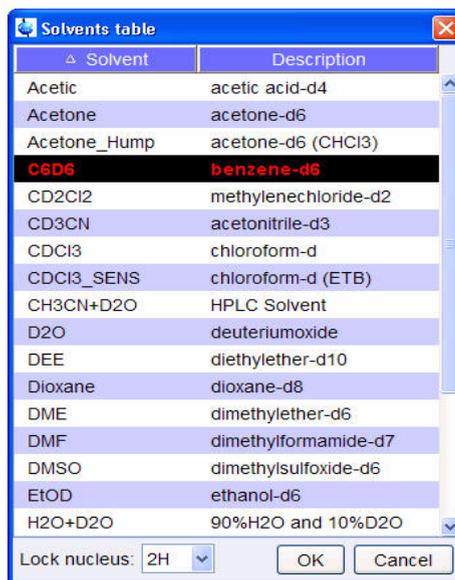
12. Select '**C6D6**' by clicking on it

13. Select [Tune] by clicking on it

NOTE: This performs a '**atma**' (automatic tuning) and requires a probe equipped with a automatic tuning module. Other options can be selected by clicking on the down arrow inside the '**Tune**' button.

---

14. Select [Spin] by clicking on it

Figure 9.37

Turn sample rotation on (ro on)

Turn sample rotation off (ro off)

Change sample rotation rate (ro)

MAS Pneumatic Unit (masdisp)

15. Select '**ro on**' by clicking on it

NOTE: Rotation may be turned off for probes such as BBI, TXI, TBI and for small sample probes.

---

16. Select [Shim] by clicking on it

NOTE: This executes the command '**topshim**'.To select other options. click on the down arrow inside the '**Shim**' button.

---

17. Select [Prosol] by clicking on it

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

---

18. Select the '**AcquPars**' tab by clicking on it

19. Make the following changes:

      PULPROG = **zg**

      TD = **64k**

      SW [ppm] = **200**

      O1p = **100**

20. Select the '**ProcPars**' tab by clicking on it

21. Make the following changes:

      SI = **32k**

      LB [Hz] = **3.5**

22. Click on the '**Aquire**' tab in the TopSpin menu bar

9.4.2 Acquisition

1. Select [⎍ **Gain** ▾] by clicking on it

NOTE: The relaxation time D1 is by default in this parameter set 300 seconds and there-fore the adjustment of the receiver gain will take some time.

2. Select [▶ **Go** ▾] by clicking on it

9.4.3 Processing

1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 9.38
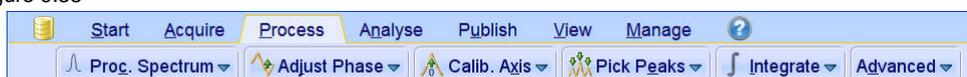
2. Click on [⋀ **Proc. Spectrum** ▾]

NOTE: This executes a processing program including commands such as an exponen-tial window function '**em**', Fourier transformation '**ft**', an automatic phase correction '**apk**' and a baseline correction '**abs**'. Other options are available by clicking on the down arrow inside the '**Proc. Spectrum**' button.

9.4.4 Calculating the Signal to Noise ratio

The signal to noise ratio is determined on the triplet of the deuterated benzene between 127ppm and 129ppm. It is calculated by AU-program **sinocal** over a range of 40ppm between 70ppm and 125ppm. The s/n ratio is strongly dependant on good resolution and line shape. The splitting of the 1:1:1 triplet should go lower than 9% (5mm) see Fig-ure 9.39. 10% (10mm) and 12% (20mm).

Figure 9.39
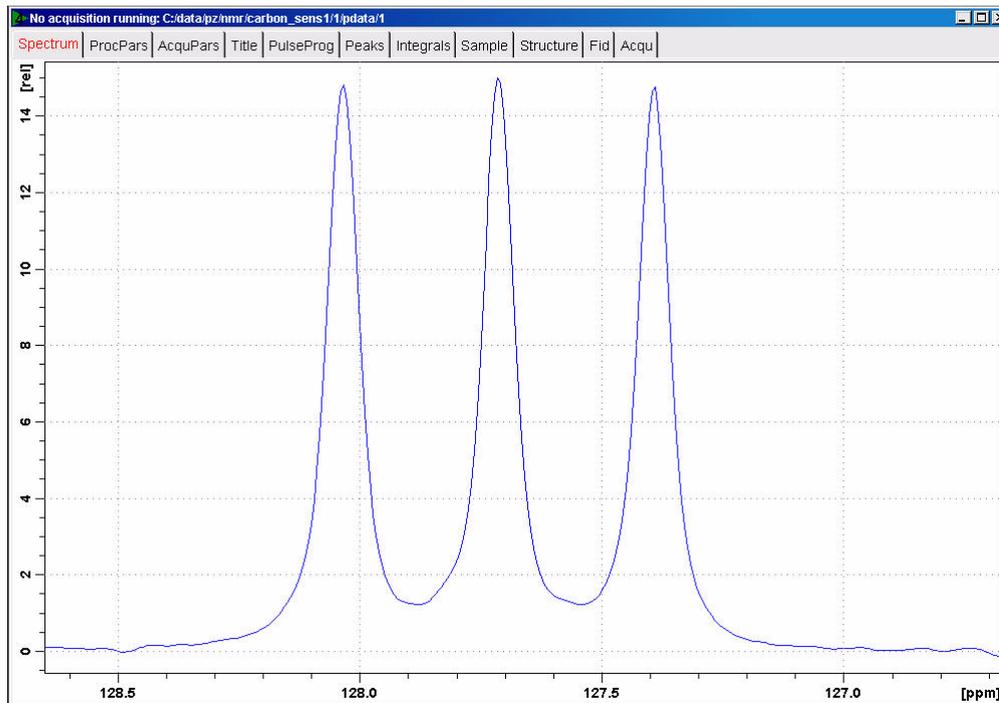
1. Type **sinocal** on the command line

Figure 9.40
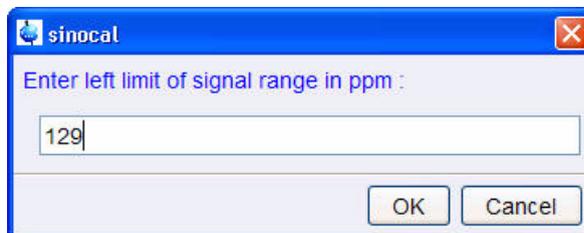
2. Enter **128** for the left limit of the signal range

3. Click on [OK]

Figure 9.41
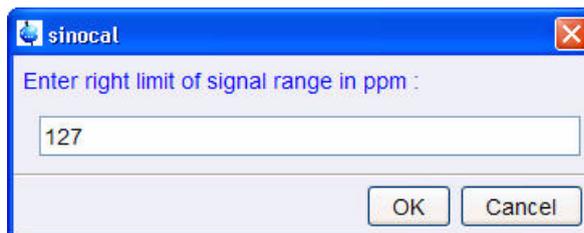
4. Enter **127** for the right limit of the signal range

5. Click on [OK]

Figure 9.42
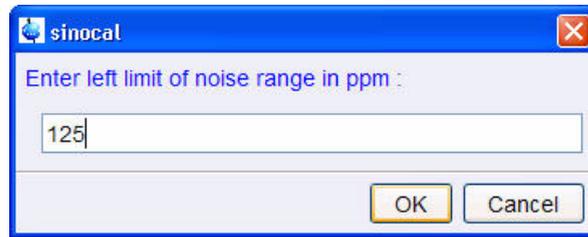
6. Enter **125** for the left limit of the noise range

7. Click on [OK]
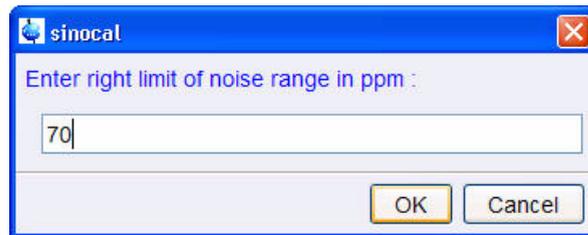
Figure 9.43

8. Enter **30** for the right limit of the noise range

9. Click on [OK]

Figure 9.44

10. Enter **40** for the noise width

11. Click on [OK]

Figure 9.45

B7169_00_01
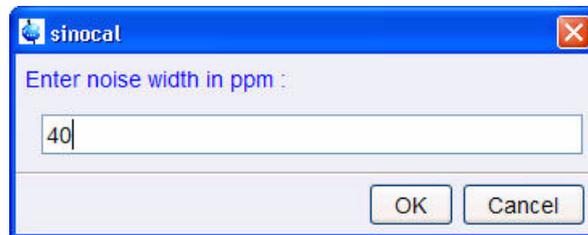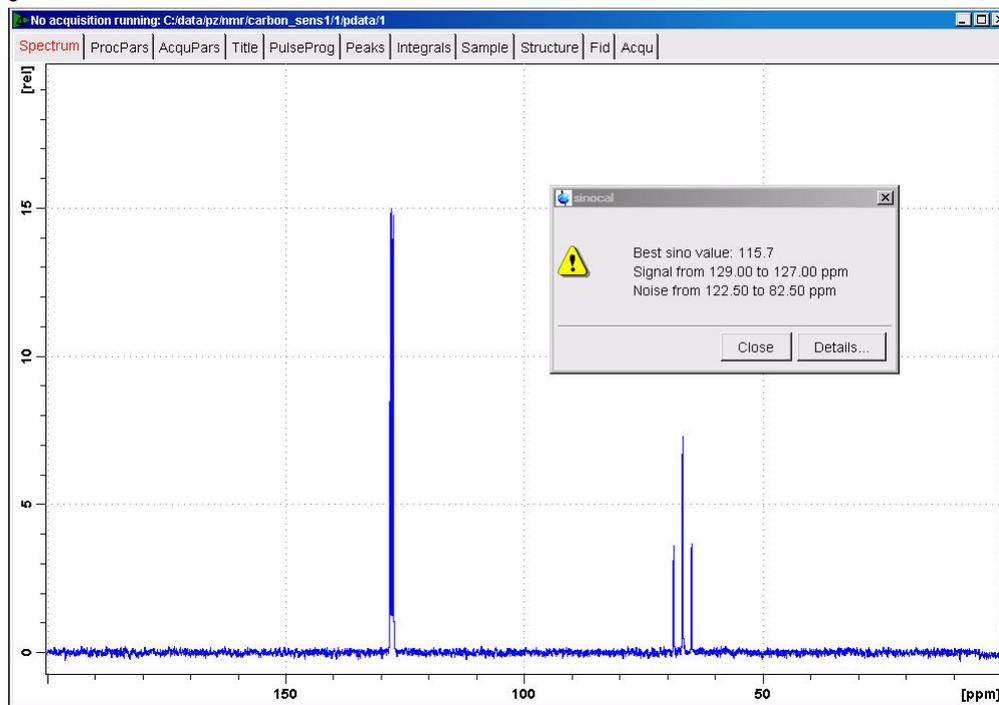
9.4.5 Observations

10 Spectrometer configuration

10.1   Hardware Configuration

1. Click on the 'Manage' tab in the TopSpin Menu bar

Figure 10.1

2. Click on the down arrow inside the [Spectrometer ▼] button

Figure 10.2

3. Select 'Hardware Detection'

4. Select 'Configure Hardware (cf)' by clicking on it

5. Enter the NMR administration password

6. Click on [OK]
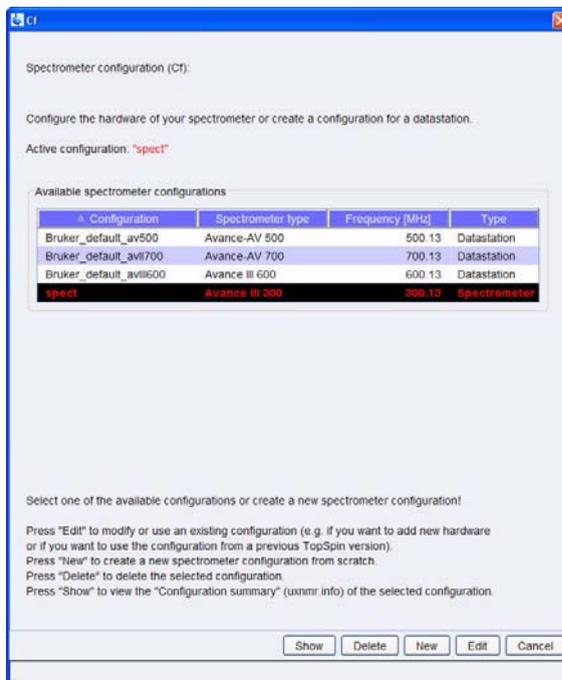
Figure 10.3

7. Select Configuration for **'Spect'** by clicking on it

8. Click on Edit
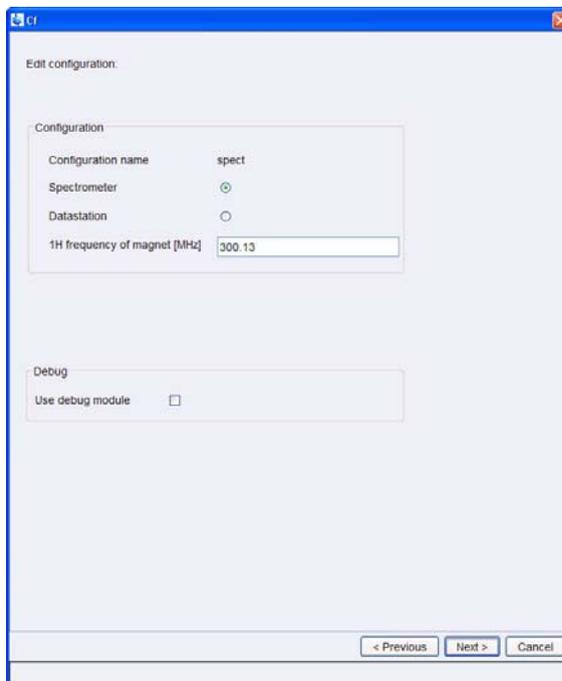
Figure 10.4

9. Click on Next >
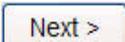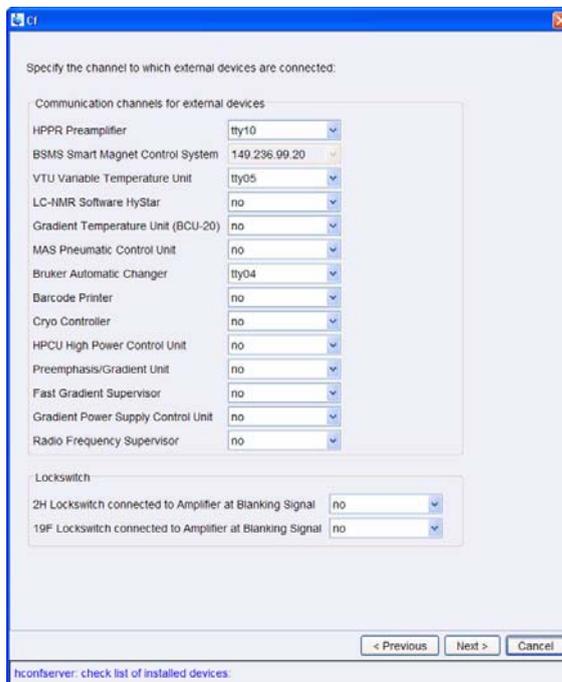
Figure 10.5

10. Enter the RS232 ports for the external devices as shown in Figure 10.5

NOTE: Use the default connection listed on the label on the inside of the console. If a BACS 60 or 120 is used, select the proper RS232 port (normally tty08) and be sure the power of the BACS is on. The sample changer will configure the correct number of sample holders. If a SIXPACK, CASE, MAS or HRMAS sample changer is used, set the BACS port to an unused port number (for example tty20. After a few seconds a message will appear that there is no communication to the sample changer and a default of 60 sample holders is been used. Just click on the 'OK' button to continue with cf. The number of sample holders for different sample changers can be set in the ICONNMR configuration (Default Number of Sample Holders)

11. Click on   Next >
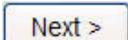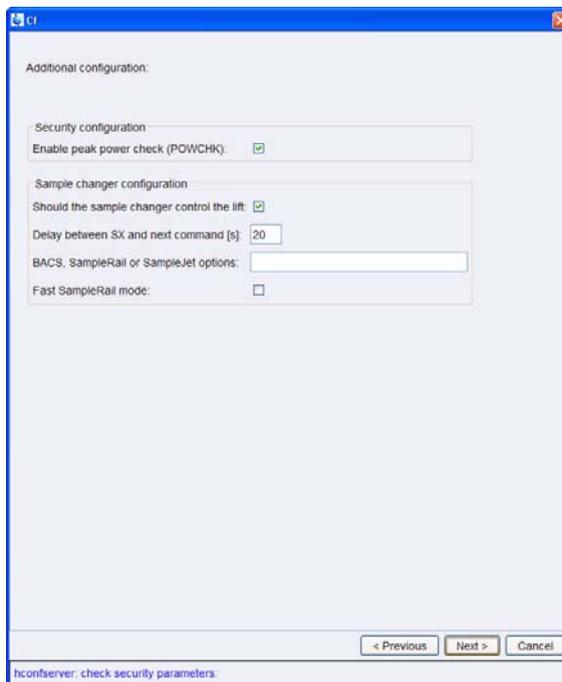
Figure 10.6

NOTE: If the Power check and Cortab have been performed on the system, enable the peak power check (POWCHK). If the Power check has not been performed do not use this option.

12. Click on [Next >]

Figure 10.7

13. Click on   [Restore]
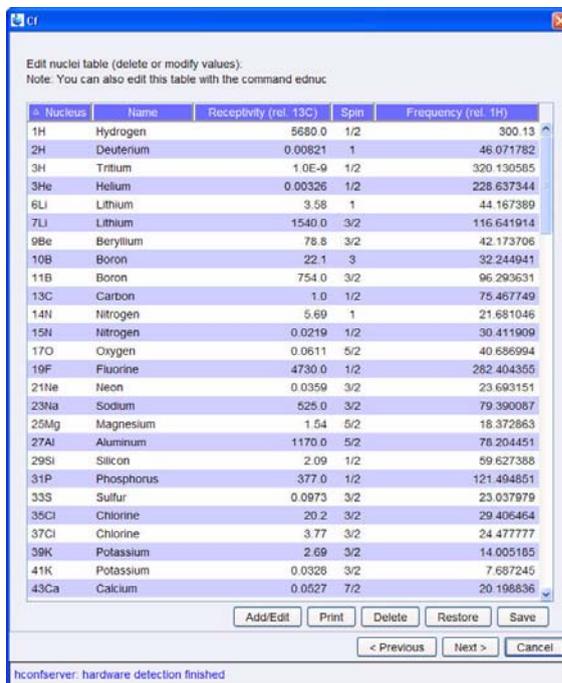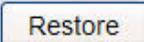
14. Click on   [Save]
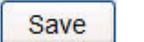
15. Click on   [Next >]
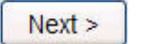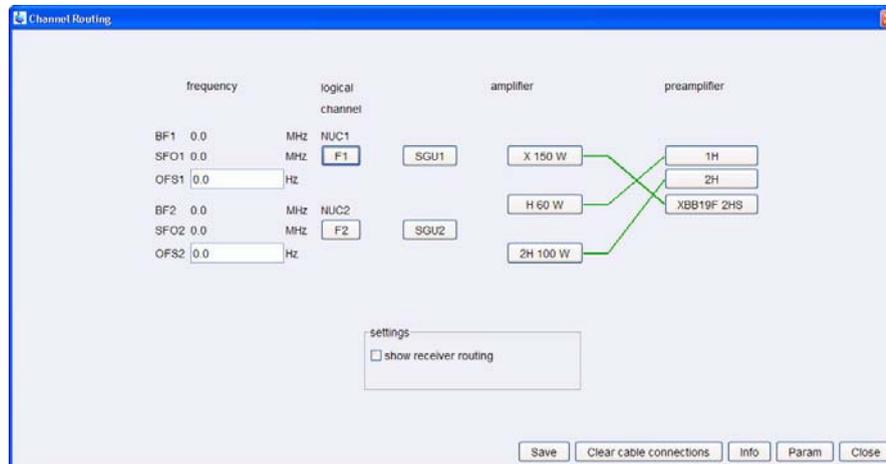
Figure 10.8

NOTE: The edsp window should show the connections from the Amplifiers to the Pream-
plifiers only. If there are incorrect connections, click on 'CLEAR PREAMPLIFIER CON-
NECTIONS' and draw the correct connections.

16. Click on   [Save]
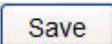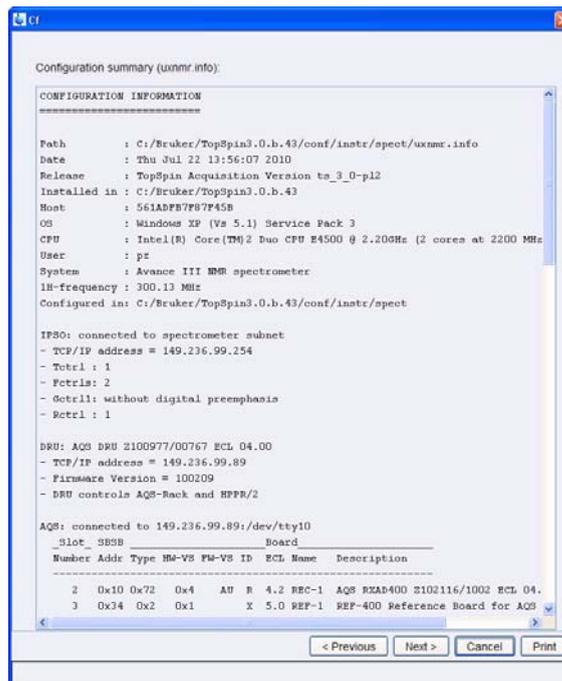
Figure 10.9

NOTE: The configuration information is displayed on the screen. Store the print out of the configuration information with the installation data

17. Click on Print

18. Click on Next >
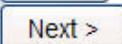
Figure 10.10

10.2   Expinstall

1. Click on Expinstall
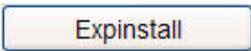
2. Enter the NMR administration password

3. Click on OK

Figure 10.11

4. Click on [Next >]
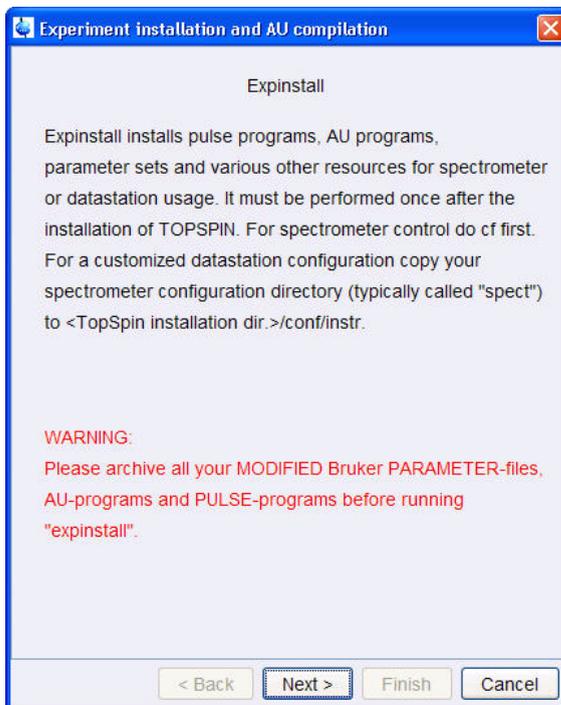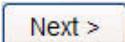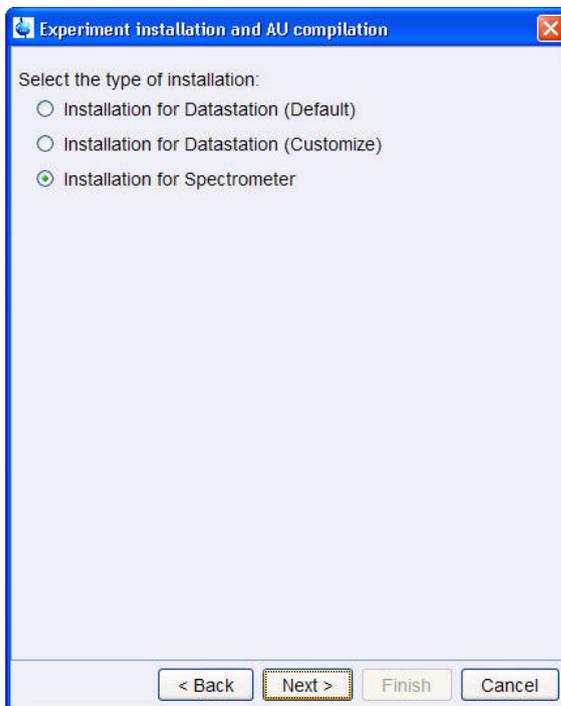
Figure 10.12

5. Select **'Installation for Spectrometer'**

6. Click on Next >
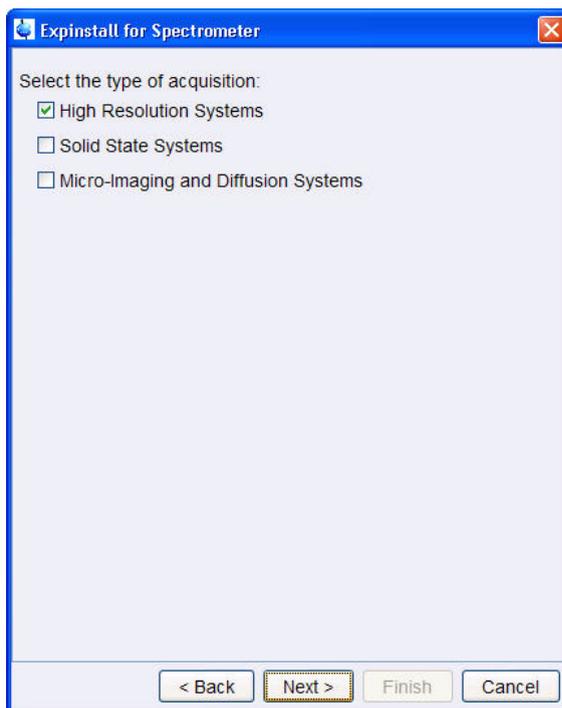
Figure 10.13

7. Select **'High Resolution System'**

8. Click on Next >
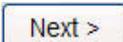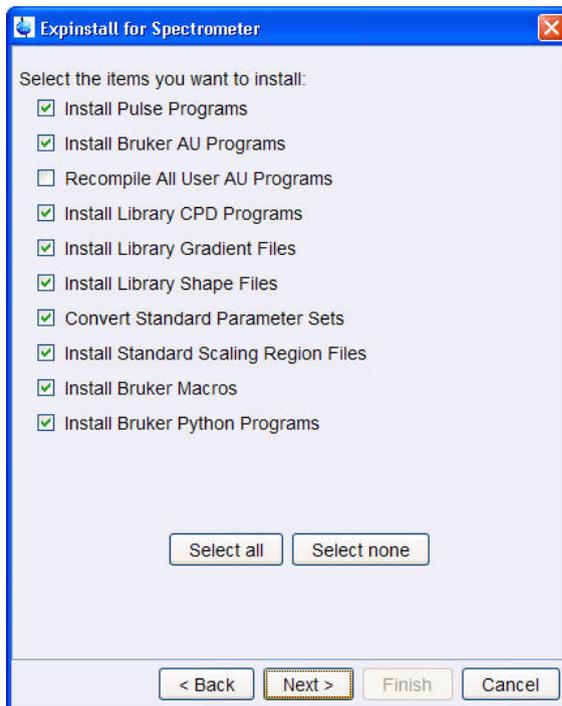
Figure 10.14

9. Click on [Next >]

10. Select Default printer and plotter

11. Select Paper format

12. Click on [Next >]
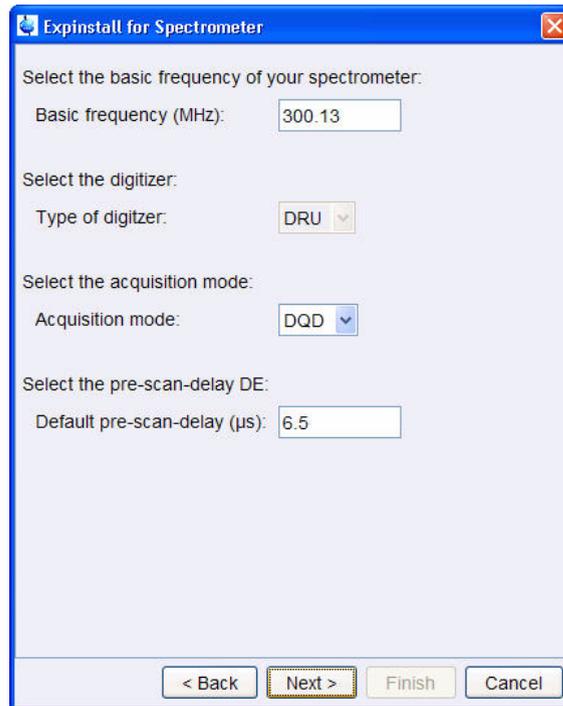
Figure 10.15

13. Click on [Next >]
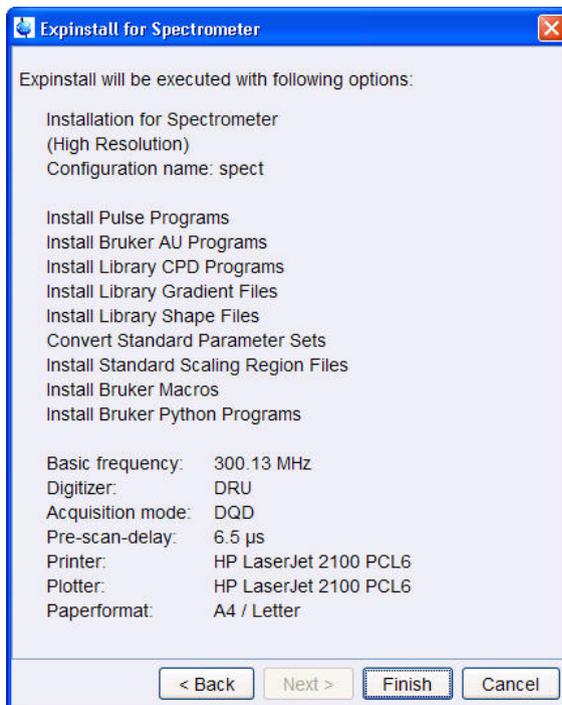
Figure 10.16

14. Click on **Finish**
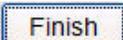
NOTE: expinstall starts now. This process will take approximately. 2 Minutes. On finish the message below appears (Figure 10.17.). To set up a time schedule to perform an NMR_save periodically (recommended) follow the instructions in 10.3 Set up the cron job for NMR_save.
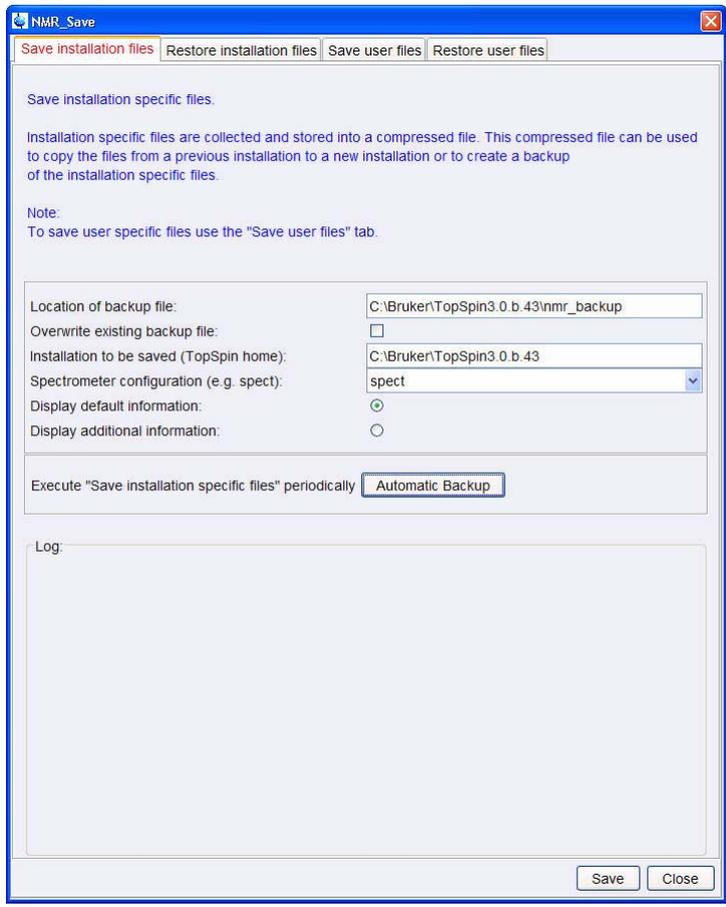
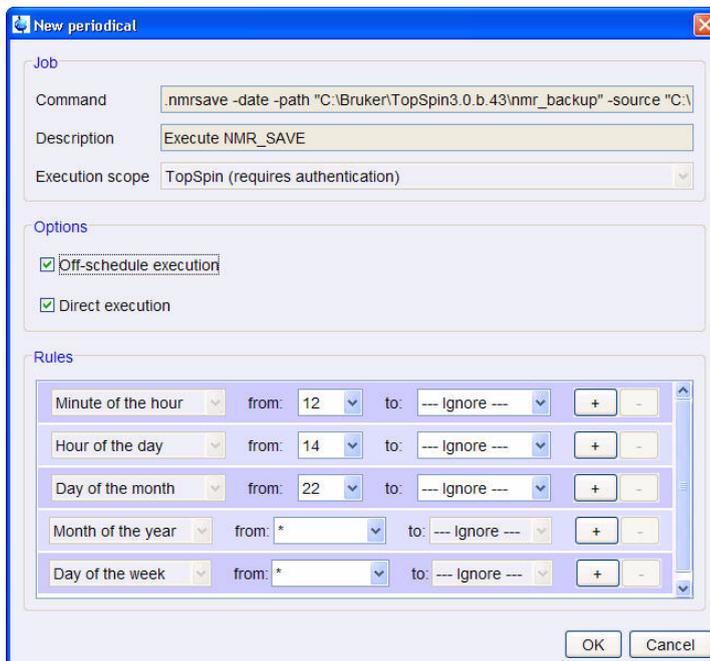---

Figure 10.17
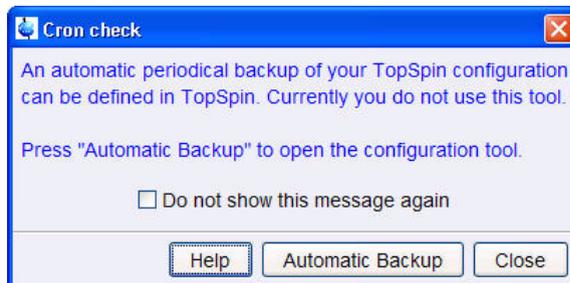
10.3 Set up the cron job for NMR_save

1. Click on **Automatic Backup**
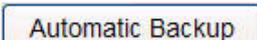
Figure 10.18

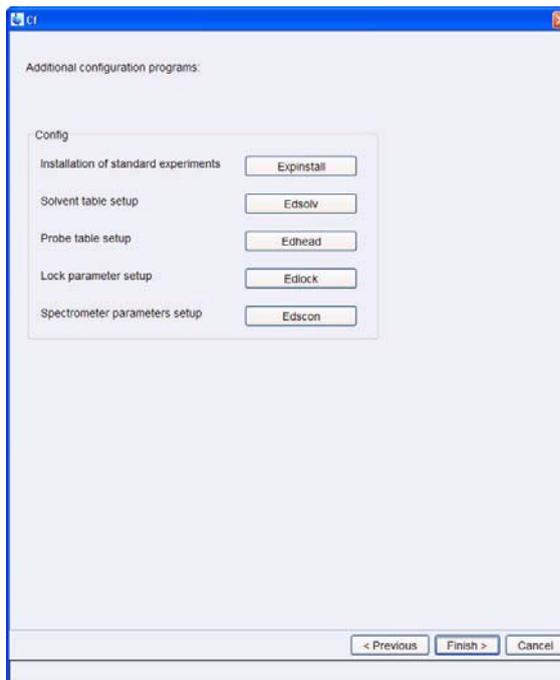2. Click on [Automatic Backup]

Figure 10.19

NOTE: In this example an NMR_save is performed from January to December on the 1st day of the month at 2 o'clock in the morning.

3. Click on   OK

Figure 10.20

10.4 Selection of current Probehead

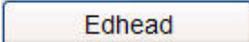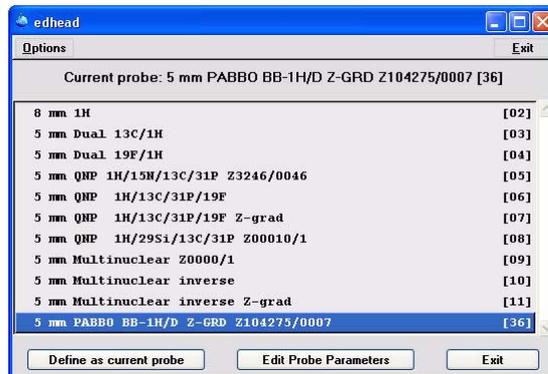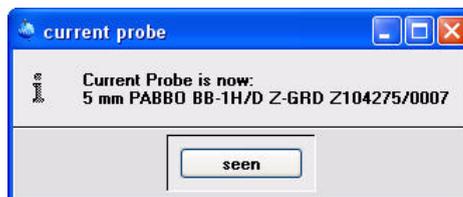### 10.4.1 Current probe equipped with pics:

1. Click on [Edhead]

Figure 10.21

NOTE: The new probe is been automatically added to the probehead list.

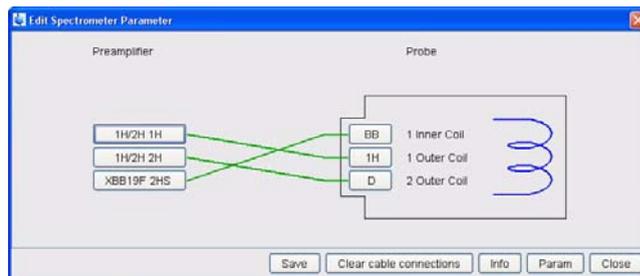2.Click on [Define as current probe]

Figure 10.22

3. Click on [seen]
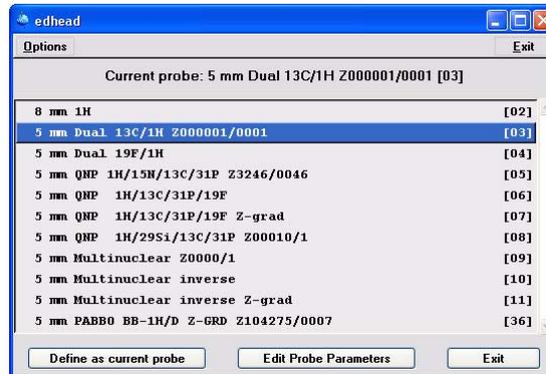
4. Click on [Exit]

Figure 10.23

NOTE: If desired, the connections of the preamplifiers to the probe can be changed

5. Click on [Save]

10.4.2   Current probe not equipped with pics and with probe parameters:

1. Type **edhead**

Figure 10.24

2. Select current probehead from the list by clicking on it

3. Click on [Define as current probe]

Figure 10.25

4. Click on [seen]

5. Click on [Exit]

Figure 10.26

NOTE: If desired, the connections of the preamplifiers to the probe can be changed

6. Click on [Save]

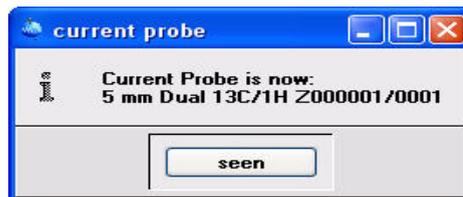## 10.4.3   Current probe not equipped with pics and without probe parameters:

1. Type **edhead**

Figure 10.27

2. Select current probehead from the list by clicking on it
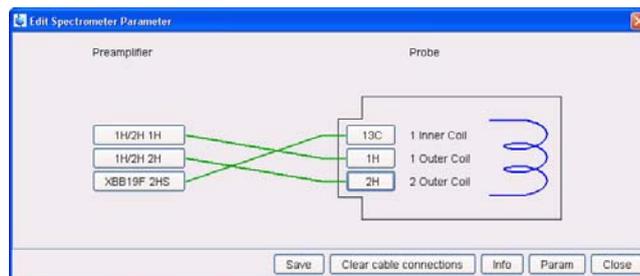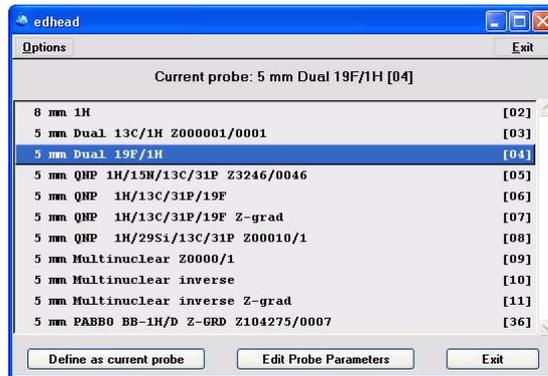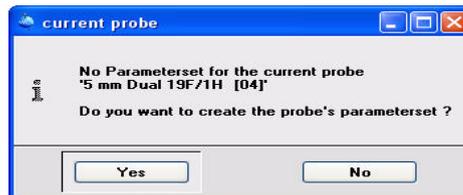
3. Click on [Define as current probe]
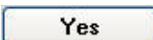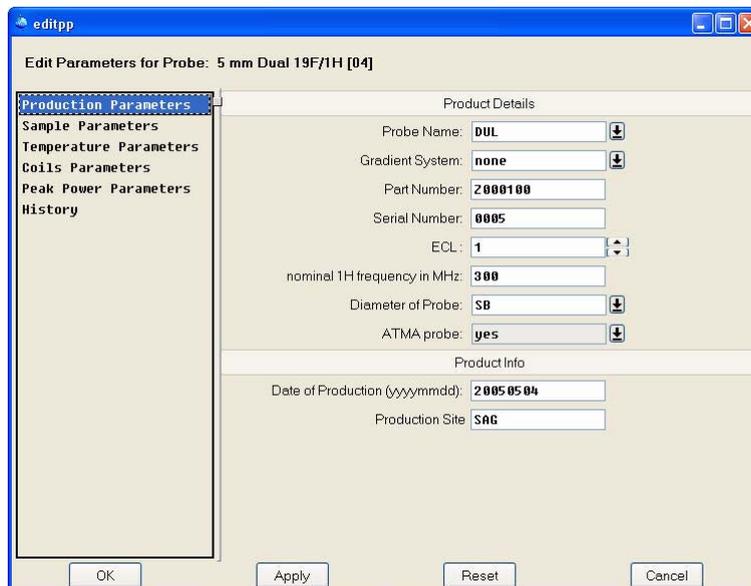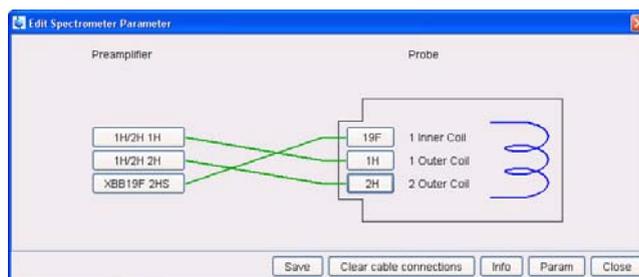
Figure 10.28

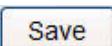
4. Click on [Yes]

Figure 10.29

NOTE: On all new probeheads, most parameters are stored in a chip and are down-loaded through the Pics connection. For older probeheads, fill in all the information.

5. Click on [OK]

6. Click on [Exit]

Figure 10.30

NOTE: If desired, the connections of the preamplifiers to the probe can be changed

7. Click on [Save]

10.5   Lock File setup

10.5.1   Setting the BSMS field

Sample: 0.1% Ethyl benzene in CDCl3 or any sample in CDCl3

NOTE: Do to the magnet drifting, the following procedure should be performed on a regular basis. (e.g. once a month).

1. Insert sample into the magnet

2. Type **lock** and select **'CDCl3'**

NOTE: The system will enter the lock shift value of CDCl3 and automatically lock and adjust the lock gain.
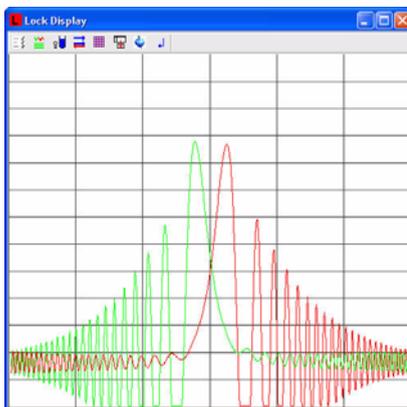
3. Type **bsmsdisp**

Figure 10.31

4. Select the **'Lock/Level'** tab in the BSMS Control Suite window

5. Switch off the lock by clicking on the LOCK **'ON/OFF'** button

6. Click on the Lock **'Field'** button

7. Center the lock trace within the lock window by changing the field value.

NOTE: Set the **'Step size'** to the lowest value to avoid loosing the lock signal.

Figure 10.32

8. Press the **'Lock ON/OFF'** key to lock

9. Shim for best resolution

10. Press **'Phase'** button and adjust the phase for symmetry of the two lock traces

11. Write down the value

12. Type **edlock** at the TopSpin command line

Figure 10.33

13. Click on 〰 to store the new BSMS field value

14. Select the first solvent in the list by clicking on it

15. Enter the new phase value from step 8 into the Lock Phase field

16. Click on ▤ to copy the value of the selected parameter to all solvents

17. Click on ▥ to save the table

18. Enter the NMR Administration password

19. Click on OK

20. Click on ✖ to close the edlock table

10.5.2 Setting the Field compensation

Sample: Tube filled with the solvent Methanol-d4

NOTE: This section describes the procedure to lock on a specific lock signal for solvents with multiple lock signals or deuterated solvents mixtures. The instructions below will guide you through the set up of successfully locking on the right solvent peak during Automation. Deuterated Methanol (CD3OD) is used in this example. To ensure an exact Field compensation value, paragraph 10.5.1. Setting the BSMS field must have been done.

1. Insert sample into the magnet

2. Type **lopo MeOD** at the Topspin command line

NOTE: The system will enter the lock shift value of MeOD in to the BSMS.

3. Type **bsmsdisp**

Figure 10.34

4. Select the **'Lock/Level'** tab in the BSMS Control Suite window

5. Switch off the lock by clicking on the LOCK **'ON/OFF'** button

6. Click on the Lock **'Field'** button

7. Adjust the field to set the desired lock signal exactly on resonance.

NOTE: Methanol d-4 has two deuterium signals. Adjust the field for the more intense signal. Set the **'Step size'** to the lowest value to avoid loosing the lock signal.

Figure 10.35
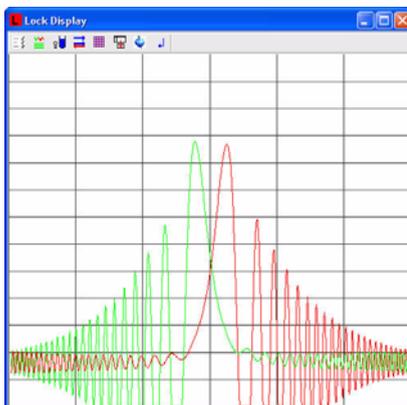
8. Press the **'Lock ON/OFF'** key to lock

9. Write down the value

10. Type **edlock** at the TopSpin command line

Figure 10.36

11. Subtract the BSMS field value for CDCl3 from the field value for MeOD in step 9

12. Enter the difference field value in to the **'Field compensation'** for MeOD

13. Click on to save the table

14. Enter the NMR Administration password

15. Click on

16. Click on to close the edlock table

10.6   Observations

11 Hardware

11.1 Power up procedure for an AV-III console

The console and computer are both off.

First power the console up and just turn the IPSO unit on.

Then boot the computer. This is necessary for Windows computers so the DHCP service is started correctly. If there is no ethernet device on the router when the computer is booted, the Bruker DHCP service will not start correctly.

Once the computer is booted, and you have logged on, reset the IPSO unit so that it boots.

When the POST code gets past the stop at 'C0' and starts to load the IPSO operating system, turn the AQS, BSMS, and amplifiers on. The parts of the console that do not have ethernet connections like VT units, MAS controllers, etc, can be turned on anytime.

If you have the smaller AQS IPSO, then have to turn the AQS on to turn the IPSO on. This seems to work fine too.

When you are finished, the sync lights on all SGU/2's should be green. If not, then go into the DRU with the 'ha' screen, and reset the DRU. This will take about a minute.

Start TopSpin and do an 'ii'. If the sync led's are on for all of the SGU/2's, then you don't need to initialize the DRU again.

11.2 Resetting the ELCB board in the BSMS on a AV-II console

NOTE: Follow the instructions below, in case of a communication problem with the BSMS on a AV-III spectrometer do 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.

1. Type **ha** on the Topspin command line

Figure 11.1
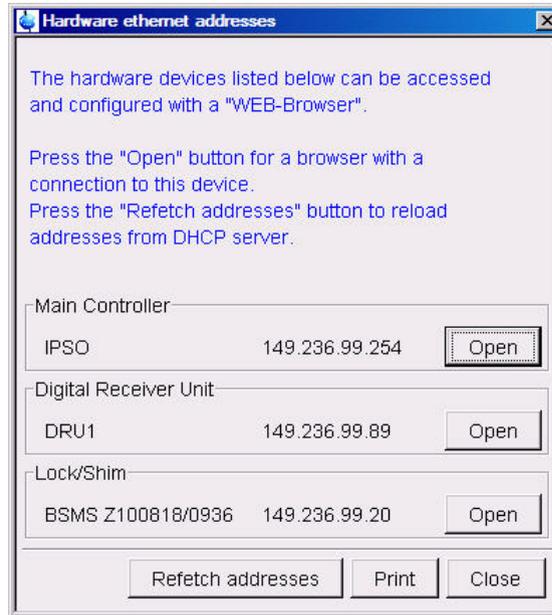
Figure 11.2

2. Click on [Open] in the BSMS '**Lock/Shim**' option

Figure 11.3
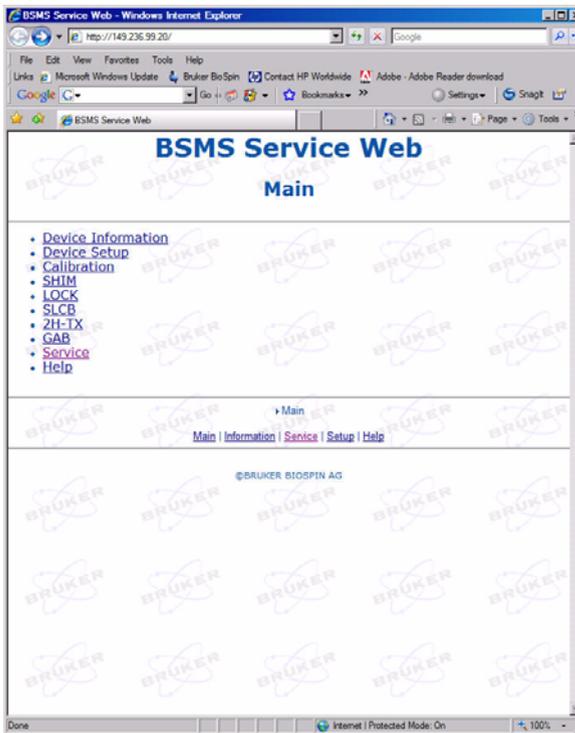
3. Select **'Service'** by clicking on it

Figure 11.4
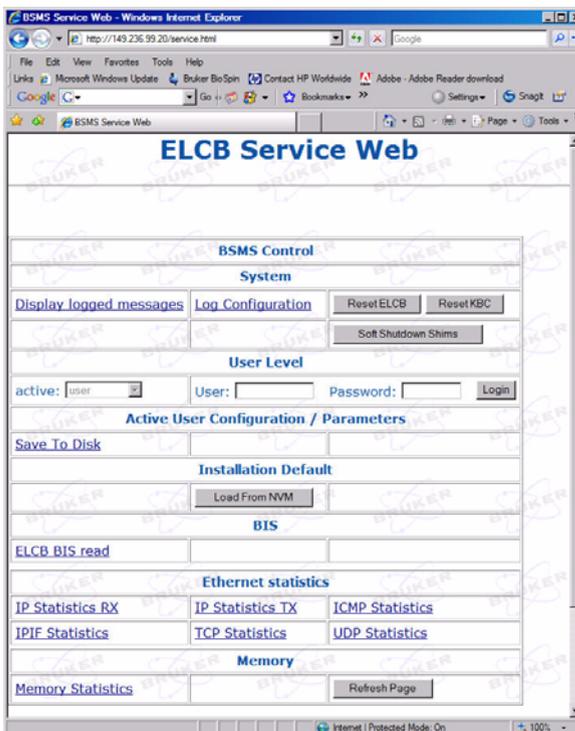
4. Click on [Reset ELCB]

Figure 11.5

Figure 11.6
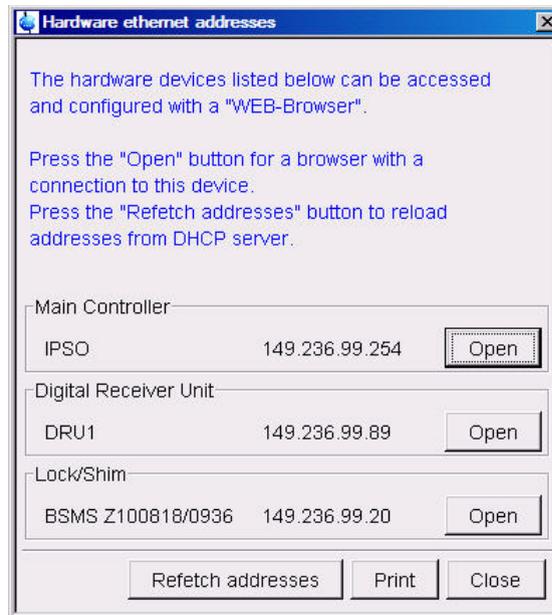
5. Click on [Close]

6. Type **rsh <Name of the last good shim file>**

11.3 Downloading a new DRU Firmware

NOTE: The instructions below are intended for installing a new DRU Firmware in case from a request by Bruker Center or the Application Hotline to fix an problem with the Digital Receiver unit. You would be instructed to down load the Firmware from a ftp site.
If a a message to install a new DRU firmware pops up during a TopSpin software upgrade, follow the instructions showing in the message box.

1. Type **ha** on the Topspin command line

Figure 11.7
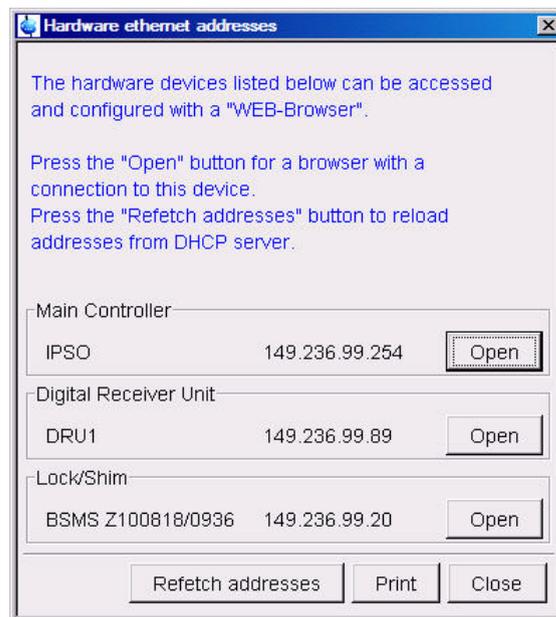
Figure 11.8

2. Click on Open in the DRU1 **'Digital Receiver Unit'** option

Figure 11.9
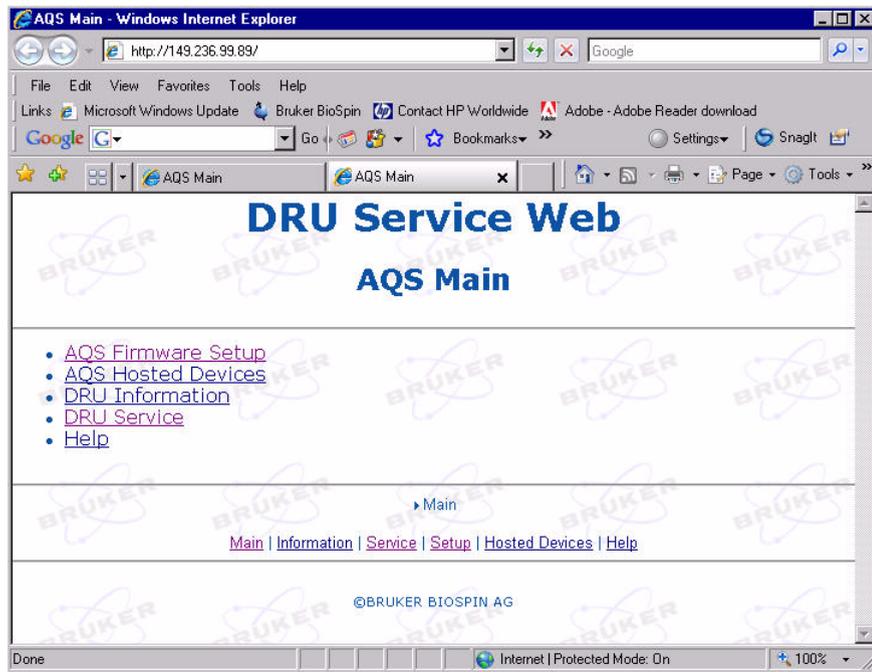
3. Select '**AQS Firmware Setup**' by clicking on it

Figure 11.10
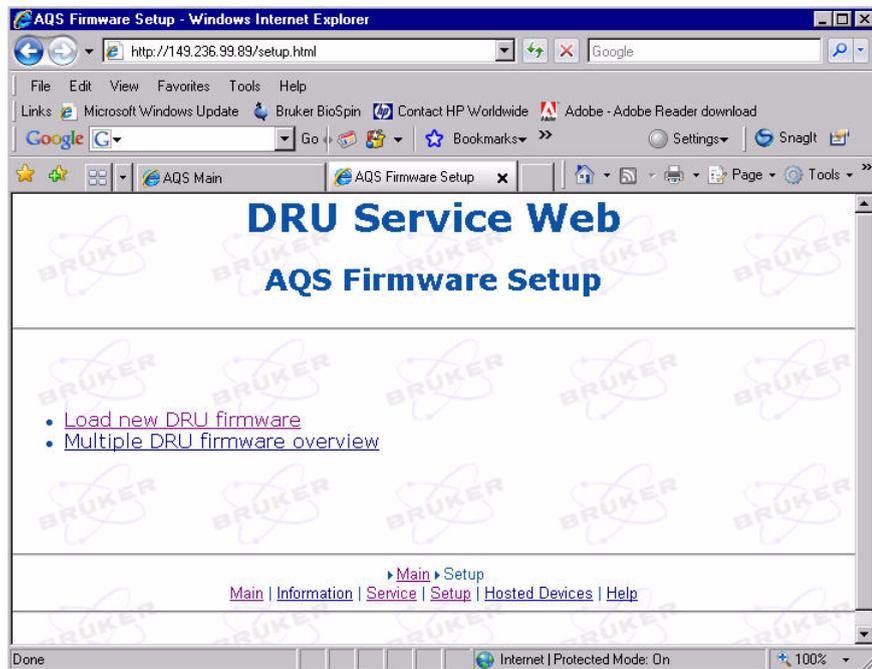
4. Select '**Load new DRU Firmware**' by clicking on it

Figure 11.11
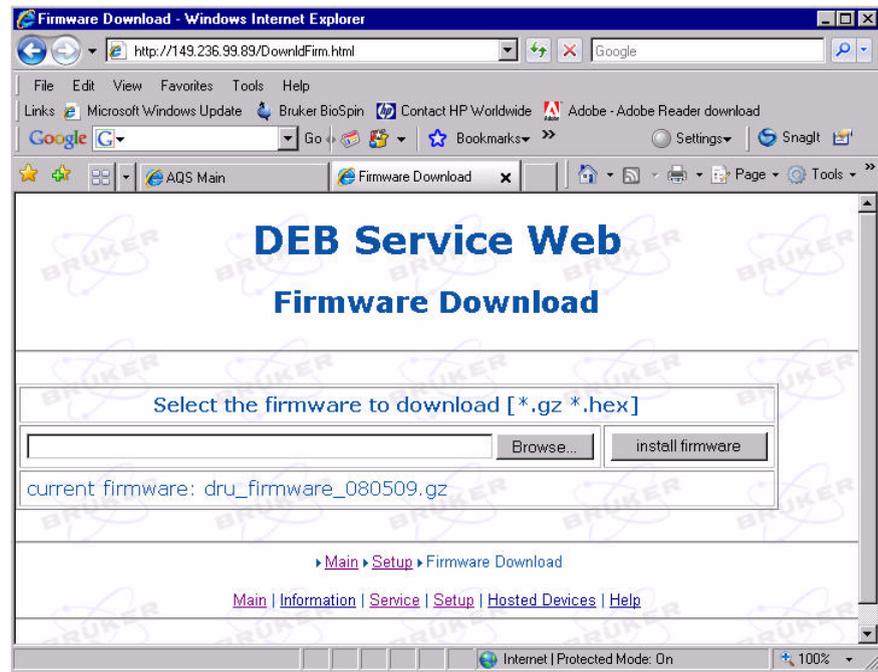
NOTE: The current DRU Firmware version is displayed in this window. Check if the new Firmware has a newer date, then proceed with the steps below. If the new Firmware has the same or older date, no further action is necessary and the Web window can be closed.

5. Click on Browse...

Figure 11.12
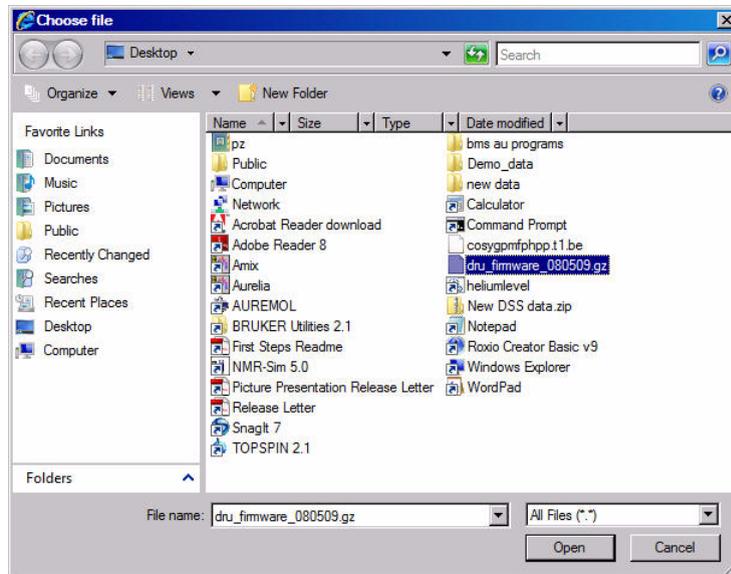
6. Select the Firmware file

7. Click on   Open

8. Click on   install firmware
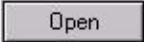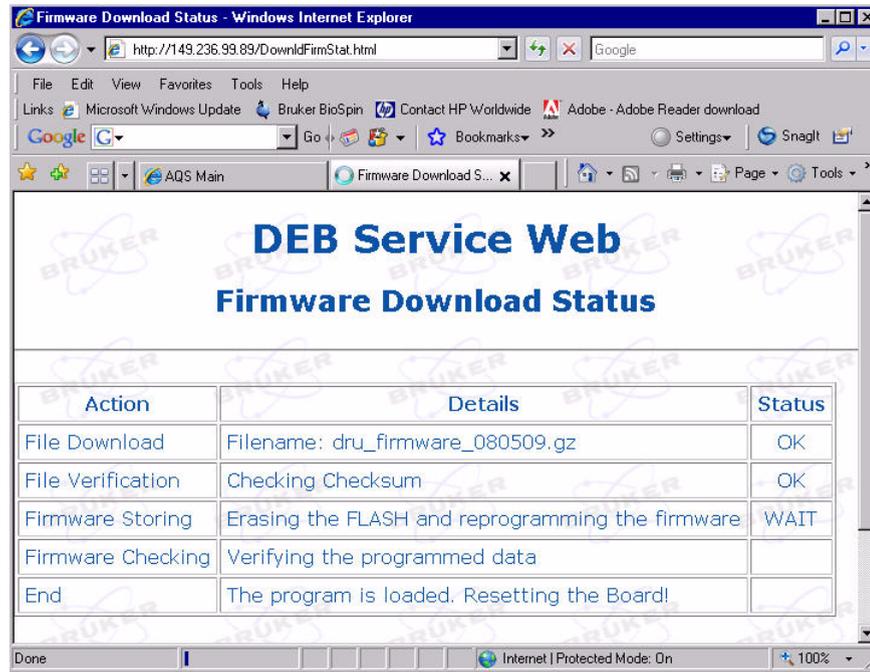
Figure 11.13

NOTE: After the program is loaded and the board is reset the current Web page in Figure 11.13 will close automatically and the Web page in Figure 11.14 is displayed.

Figure 11.14
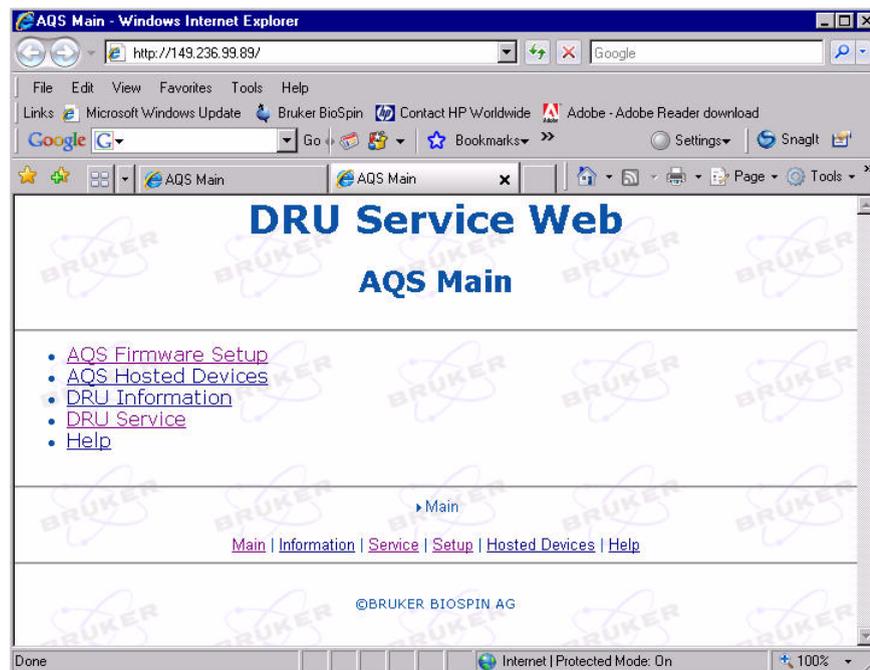
9. Click on ⊠ to close the Web page

Figure 11.15

10. Click on [Close]
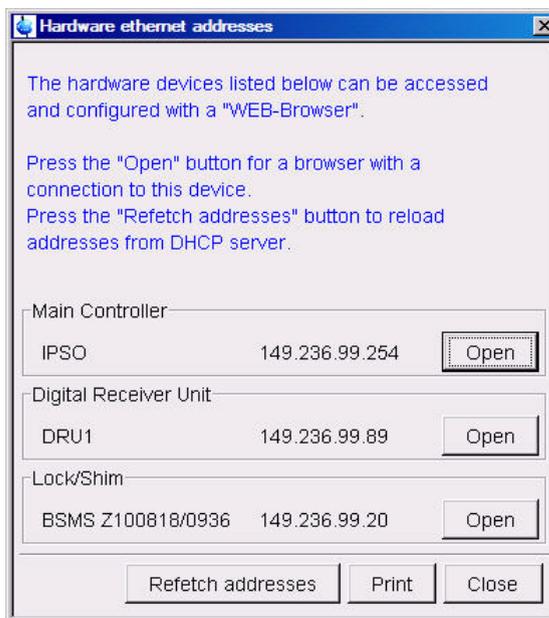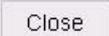
11.4 Observations

Appendix A

A.1 Standard Parameter set list

```
N AL27ND - 27Al exp. no decoupling
N B11ZG - 11B exp. no decoupling
N C13APT - Attached Proton Test using jmod pulse program
N C13CPD - C13 exp. comp. pulse dec. 1024 scans
N C13CPD32 - C13 exp. comp. pulse dec. 32 scans
N C13CPDSN - C13 exp. comp. pulse dec. with signal-to-noise calc.
N C13DE45SN - C13 dept all positive with signal-to-noise calc.
N C13DEPT45 - C13 dept all positive
N C13DEPT90 - C13 dept CH-only
N C13DEPT135 - C13 dept CH,CH3 pos. CH2 neg.
N C13DEPT135p - dept135 with phase of previous C13
N C13GD - C13 exp. gated decoupling
N C13IG - C13 exp. inverse gated decoupling
N C13MULT - 13C automatic multiplicity determination
N C13OFF - C13 exp. off resonance
N C13PPTI - C13 exp. with peak picking in title
N C13HUMP - 13C hump (lineshape) test
N C13RESOL - 13C resolution (half width) test
N C13SENS - 13C sensitivity (SINO) test
N CD111ZG - 111Cd exp. no decoupling
N CD113ZG - 113Cd exp. no decoupling
N CL35ZG - 35Cl exp. no decoupling
N CL37ZG - 37Cl exp. no decoupling
N F19 - 19F exp. no decoupling
N F19CPD - 19F exp. comp. pulse decoupling
N GA71ZG - 71Ga exp. no decoupling
N HG199CPD - 199Hg exp. comp. pulse decoupling
N HMQC1D - 1D version of the HMQC
N LC1D12 - 1H, double presaturation
N LC1DCWPS - 1H, multiple presaturation
N LC1DWTDC - 1H, mult. WET suppr., 13C decoupling
N LCMLCWPS - TOCSY TPPI, mult. presat., 13C decoupling
N N15 - 15N exp. no decoupling
N N15IG - 15N exp. inverse gated
N N15INEPT - 15N exp. inept
N NA23ZG - 23Na exp. no decoupling
N NOEDIFF - 1H noe difference
N O17ZG - 17O exp. no decoupling
N P31 - 31P exp. no decoupling
N P31CPD - 31P exp. comp. pulse decoupling
N PROB11DEC - 1H with B11 decoupling
```

N PROF19DEC - 1H with F19 decoupling
N PROP31DEC - 1H with P31 decoupling
N PROTON - 1H experiment 16 scans
N PROTON128 - 1H experiment 128 scans
N PROTONinfo - 1H experiment with info table
N PROTONCONLF - 1H exp. with conditional low field plot
N PROTONEXP - 1H experiment + expansions
N PROTONLF - 1H experiment + low field plot
N PROTONLFEXP - 1H experiment + low field plot + expansions
N PROTONNR - 1H exp. non spinning
N PROTONNREXP - 1H exp. non spinning + expansions
N PROTONNRLF - 1H exp. non spinning + low field plot
N PRONRLFEXP - 1H exp. non spinning + low field plot + expansions
N PROHOMODEC - 1H homo decoupling experiment
N PROTONT1 - 1H T1 Relaxation measurement
N PROHUMP - 1H hump (lineshape) test
N PRORESOL - 1H resolution (half width) test
N PROSENS - 1H sensitivity (SINO) test
N PT195ZG - 195Pt exp. no decoupling
N RH103ZG - 103Rh exp. no decoupling
N SE77ZG - 77Se exp. no decoupling
N SELCO1H - 1D COSY using sel. excitation w/a shaped pulse
N SELMLZF1H - 1D homo. Hartman-Hahn transfer using MLEV17 and
sel. exc. w/a shaped pulse
N SELNO1H - 1D NOESY using sel. exc. w/a shaped pulse
N SELRO1H - 1D ROESY using sel. exc. w/a shaped pulse
N SELZG1H - 1D sequence using sel. exc. w/a shaped pulse
N SI29IG - 29Si exp. inverse gated decoupling
N SN119IG - 119Sn exp. inverse gated decoupling
N WATERSUP - 1H water supression test
N WATER - water supression
C COSY45SW - sw opt. COSY45 (magn. mode)
C COSY90SW - sw opt. COSY90 (magn. mode)
C COSYDQFPHSW - sw opt. COSY with dq filter (States-TPPI)
C COSYGPDFPHSW - sw opt. COSY with gradients and dq filter
(States-TPPI)
C COSYGPMFSW - sw opt. COSY with gradients and mq filter (magn.
mode)
C COSYGPSW - sw opt. COSY with gradients (magn. mode)
C HCCOSW - sw opt. CH-correlation
C HCCOLOCSW - sw opt. COLOC
C INV4SW - sw opt. HMQC (magn. mode)
C INV4PHSW - sw opt. HMQC (States-TPPI)
C INV4GPSW - sw opt. HMQC with gradients (magn. mode)
C INV4GPMLSW - sw opt. HMQC-TOCSY with gradients (magn. mode)
C INVBSW - sw opt. HMQC using BIRD pulse (magn. mode)
C INVBPHSW - sw opt. HMQC using BIRD pulse (States-TPPI)
C INV4GPLPLRNDSW- - HMBC with gradients and low pass J-filter

```
C INV4GPLRNDSW - sw opt. HMBC with gradients
C INV4LPLRNDSW - sw opt. HMBC with low pass J-filter (magn. mode)
C INVIGPMLPHSW - sw opt. HSQC-TOCSY with gradients (States-TPPI)
C INVIGPPHSW - sw opt. HSQC with gradients (States-TPPI)
C MLEVPHSW - sw opt. TOCSY (States-TPPI)
C NOESYPHSW - sw opt. NOESY (States-TPPI)
C ROESYPHSW - sw opt. ROESY (States-TPPI)
C INVIETGPSW - sw opt. HSQC with gradients (e/a TPPI)
C INVIETGPSISW - sw opt. HSQC sens. improved with gradients (e/a
TPPI)
C INVIETGPMLSW - sw opt. HSQC-TOCSY with gradients (e/a TPPI)
C INVIEDETGPSW - sw opt. edited HSQC with gradients (e/a TPPI)

C INVIEDGPPHSW - sw opt. edited HSQC with gradients (States-TPPI)
```

A.2　Standard Test Samples

1H Lineshape

0.3% Chloroform in Acetone-d6 (CRYO-probes)

1% Chloroform in Acetone-d6 (500MHz and up)

3% Chloroform in Acetone-d6 (up to 500MHz)

1H Sensitivity

0.1% Ethyl benzene in Chloroform-d

1H Solvent Suppression

2mM Sucrose in 90% H2O, 10% D2O

2mM Lisozyme in 90% H2O, 10% D2O

13C Sensitivity

10% Ethyl benzene in Chloroform-d

40% p-Dioxane in 60% Benzene-d6

31P Sensitivity

Triphenylphosphate in Chloroform-d

15N Sensitivity

90% Formamide in Dimethyl Sulfoxide-d6

Calibration of the 13C and 15N 90 degree pulses

0.1M 15N-Urea, 0.1M 13C-Methanol in Dimethyl Sulfoxide-d6

19F Sensitivity

Trifluorotoluene in Chloroform-d

Temperature Calibration

80% Ethylene Glycol in Dimethyl Sulfoxide-d6 (High Temperature)

4% Methanol in 96% Methanol-d (Low Temperature)

1D and 2D Experiments

100mg/mL Cholesteryl Acetate in Chloroform-d

10mg Strychnine in Chloroform-d

50mM Gramicidine in Dimethyl Sulfoxide-d6

B Contact

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