

- **TopSpin 3.x**

Advanced NMR Methods
User Manual

Version 001

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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 Pulse calibration

2.1 Introduction

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

- ▶ **Warning: It is always a good practice to obtain spectra with the power check turned on, if your system has been cortabed.**

2.2 Sample

Mixture 0.1M each of 15N enriched Urea (Figure 2.1) and 13C enriched Methanol (Figure 2.2) in DMSO-d6.

Figure 2.1

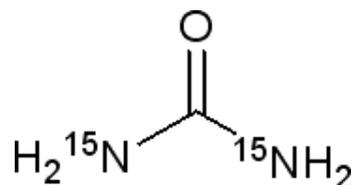
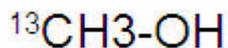
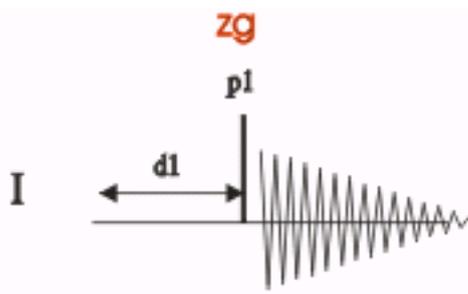


Figure 2.2



2.3 1H 90 deg. transmitter pulse

Figure 2.3



The pulse program **zg** is used to determine the **1H 90°** transmitter pulse. The sequence consists of one channel **f1** with a recycle delay **d1**, a **1H** pulse **p1**, followed by the **1H** signal detection. The signal has maximum intensity if **p1** is a 90° pulse and 2 Nulls at a

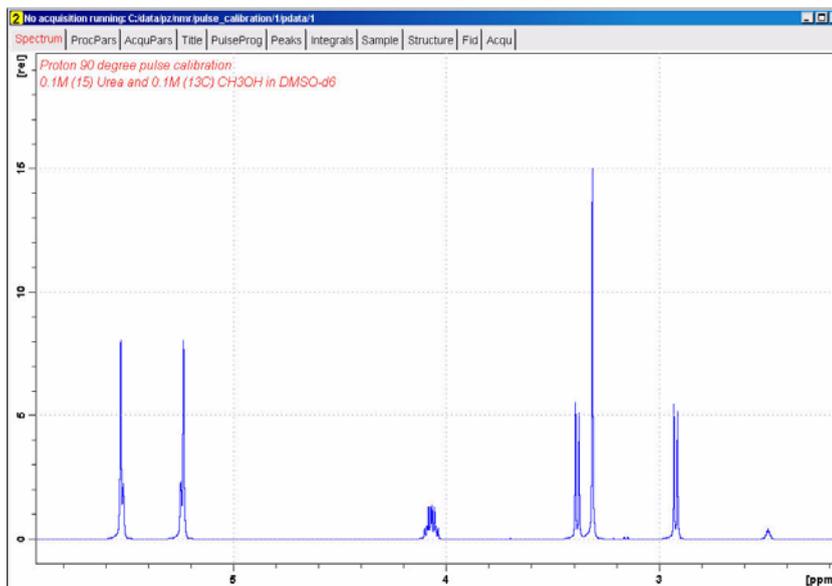
180° and 360° pulse.

The Methanol signal region from 3.5ppm to 2.8ppm is used for this experiment.

2.3.1 Preparation experiment

1. Run a **1D Proton** spectrum of Urea / Methanol in DMSO-d6, following the instructions in **1-D Proton experiment, Chapter 3**

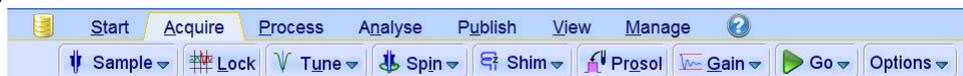
Figure 2.4



2.3.2 Parameter set up

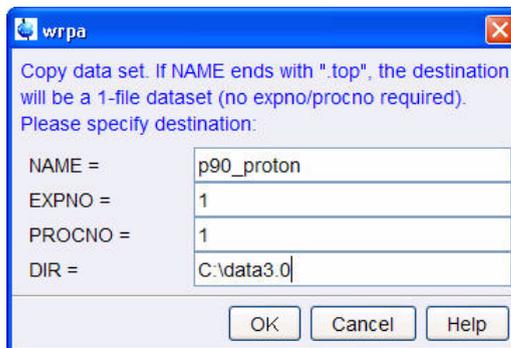
1. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 2.5



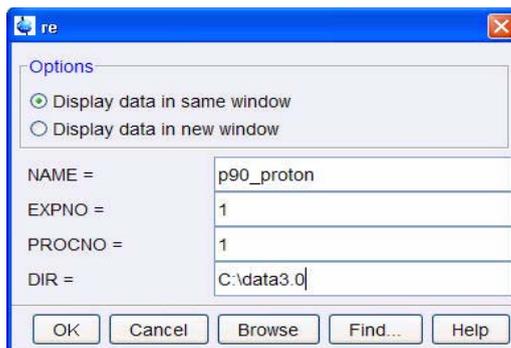
2. In the command line type **wrpa** and hit 'Enter'

Figure 2.6



3. Change **NAME** = p90_proton
4. Click on 
5. In the command line type **re** and hit 'Enter'

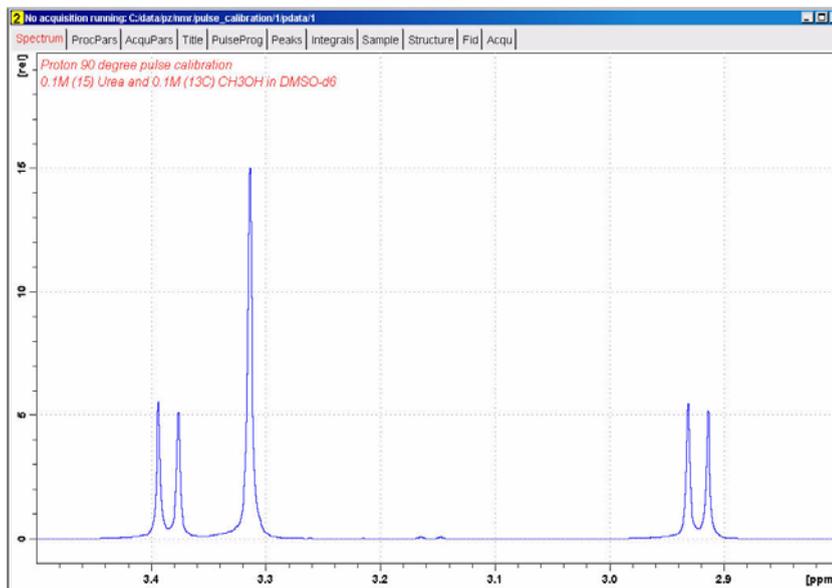
Figure 2.7



6. Change **NAME** = p90_proton
7. Click on 
8. Expand the region between 3.5ppm and 2.8ppm

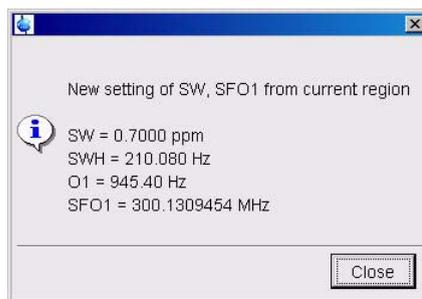
NOTE: Normally a single peak set to on resonance is used to determine the 90⁰ transmitter pulse. For practical reason the Methanol signal region from 3.5ppm to 2.8ppm is used to measure the 1H 90⁰ transmitter pulse, since the same signals will also be used in determining the 13C 90⁰ decoupler pulse.

Figure 2.8



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

Figure 2.9



10. Click on 

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

12. Make the following changes:

PULPROG = **zg**

TD = **4K**

NS = **1**

DS = **0**

D1 = **10**

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

14. Make the following changes:

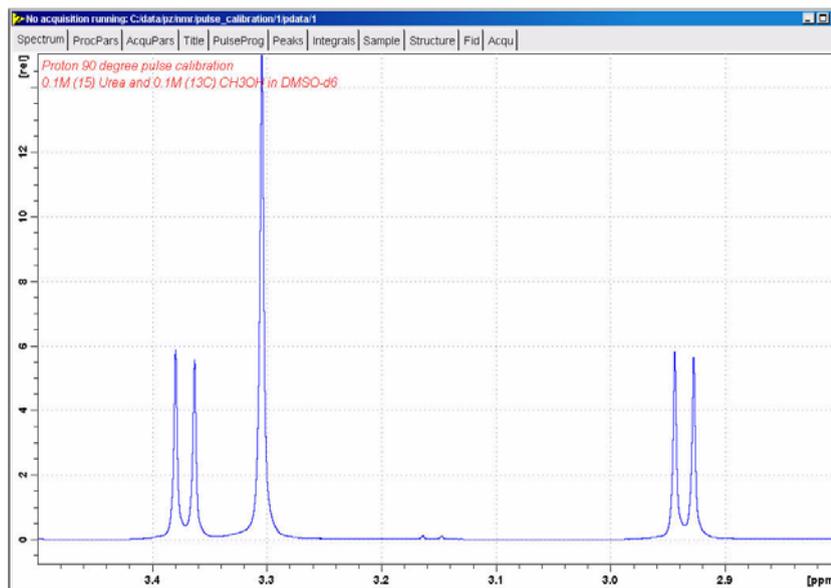
SI = **2K**

PH_mod = **pk**

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

16. Select  by clicking on it
17. Select  by clicking on it
18. Process and Phase correct the spectrum
19. Display the full spectrum

Figure 2.10

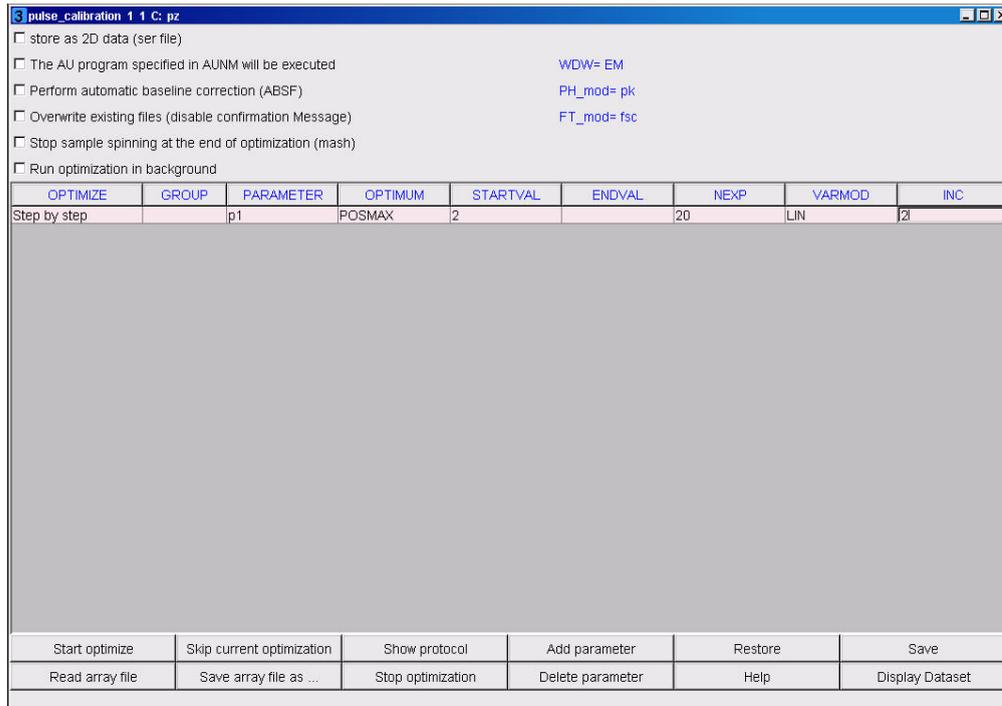


20. In the command line type **dpl** to save the region to parameter F1P/F2P
21. In the command line type **wpar H1p90_urea all** to store the parameter set for future use

2.3.3 Determine the 1H 90 deg. transmitter pulse

1. In the command line type **popt**
2. Make the following changes:
 - OPTIMIZE = Step by step**
 - PARAMETER = p1**
 - OPTIMUM = POSMAX**
 - STARTVA = 2**
 - NEXP = 20**
 - VARMOD = LIN**
 - INC= 2**

Figure 2.11

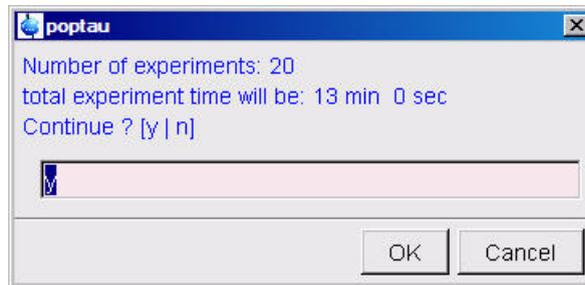


3. Click on 

NOTE: The ENDVAL parameter has been updated

4. Click on 

Figure 2.12

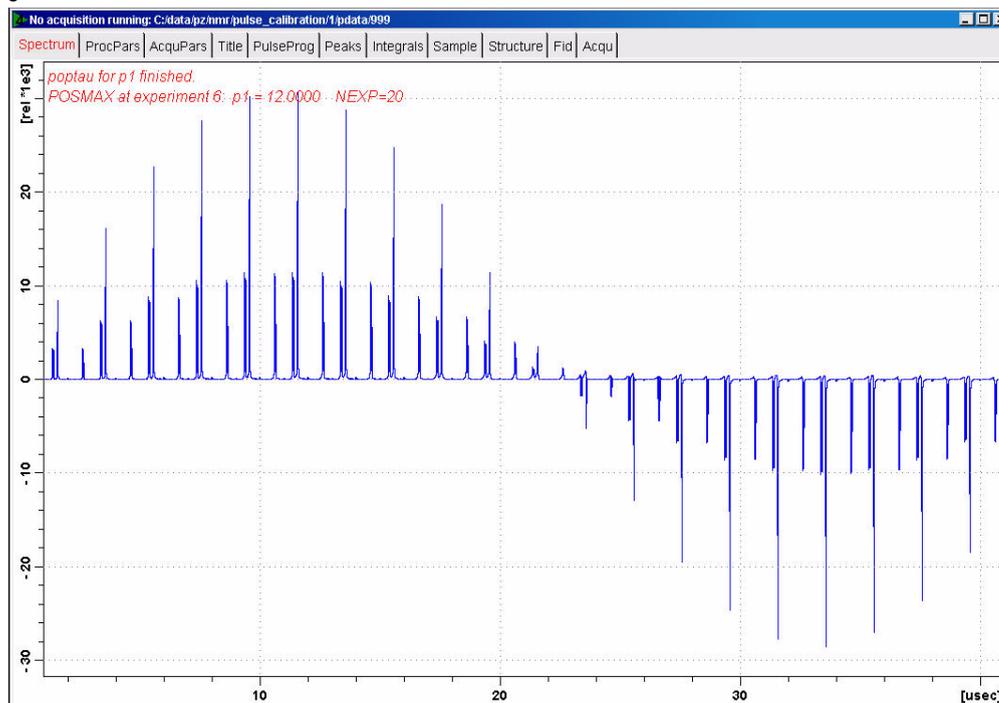


5. Type **y** into the 'poptau' window

6. Click on 

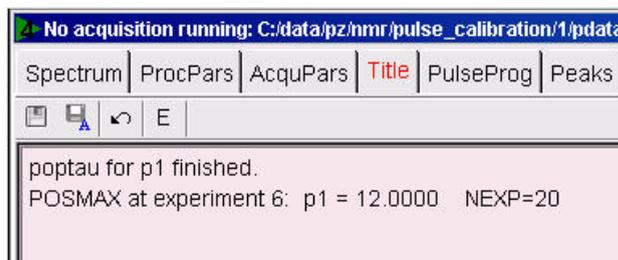
NOTE: The parameter optimization starts. The spectrometer acquires and processes 20 spectra with incrementing the parameter p1 from 2 us by 2 us to a final value of 40 us. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file pulse_calibration/2/999 as shown in Figure 2.13.

Figure 2.13



7. Select the 'Title' tab by clicking on it

Figure 2.14



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.

8. Close the popt setup window
9. In the command line type **re 2 1**
10. In the command line type **p1**
11. Enter the value which corresponds to a 360 degree pulse (the second zero crossing in the popt spectrum, which should be approximately 4 times the POSTMAX value)
12. Select  by clicking on it
13. In the command line type **efp**

14. Change p1 slightly and repeat steps 12 and 13, until the signals undergoes a zero crossing as expected for an exact 360 degree pulse.

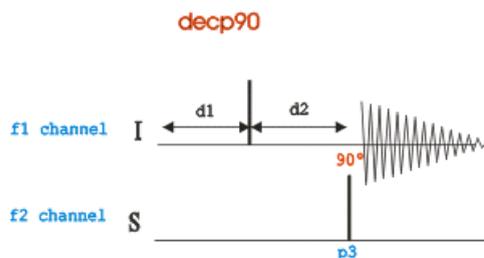
NOTE: The signals are negative for a pulse angle slightly less than 360 degree and positive when the pulse angle is slightly more than 360 degree.

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

2.4 Observations

2.5 13C 90 deg. decoupler pulse

Figure 2.15



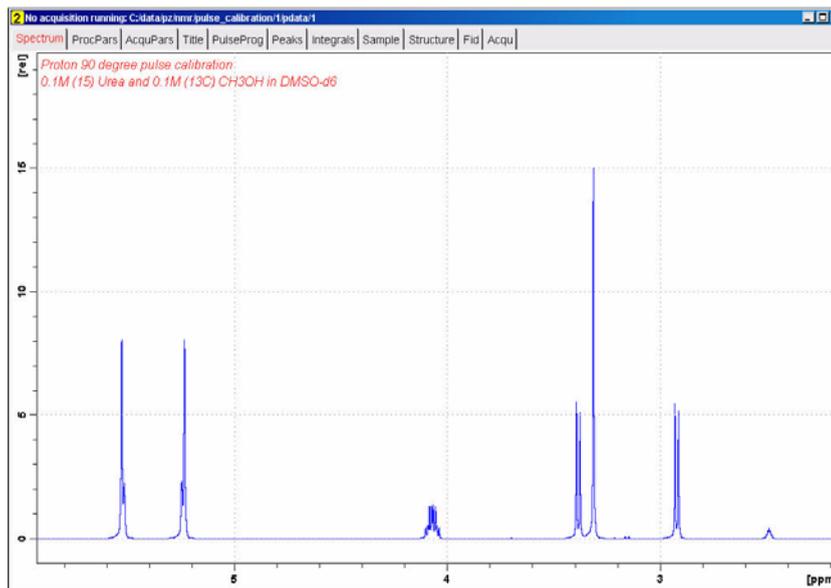
The pulse program used in this procedure is the decp90 sequence shown in Figure 2.15. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for 1H and f2 to 13C. Channel f1 shows a recycle delay d1 followed by a 90° pulse and a delay d2 = 1/(2J_{CH}) for the creation of antiphase magnetization. A 13C pulse on channel f2 is executed after the delay d2 and then the 1H signal is detected. When the 13C pulse is exactly 90°, the 1H signals will go through a null.

The Methanol signal region from 3.5ppm to 2.8ppm is used for this experiment.

2.5.1 Preparation experiment

1. Run a **1D Proton** spectrum of Urea / Methanol in DMSO-d6, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 2.16



2.5.2 Parameter set up

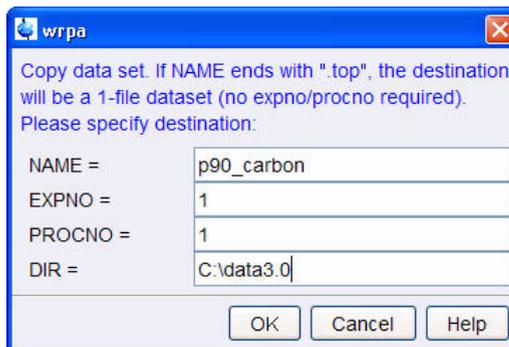
1. Click on the **'Acquire'** tab in the TopSpin menu bar

Figure 2.17



2. In the command line type **wrpa** and hit 'Enter'

Figure 2.18

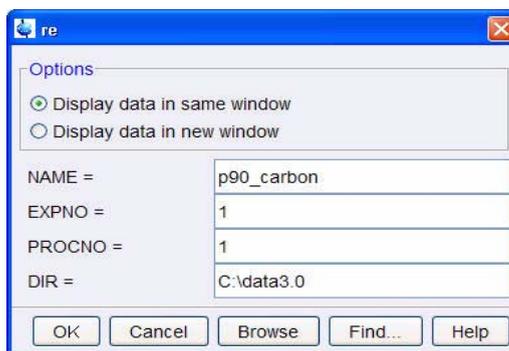


3. Change **NAME = p90_carbon**

4. Click on **OK**

5. In the command line type **re** and hit 'Enter'

Figure 2.19



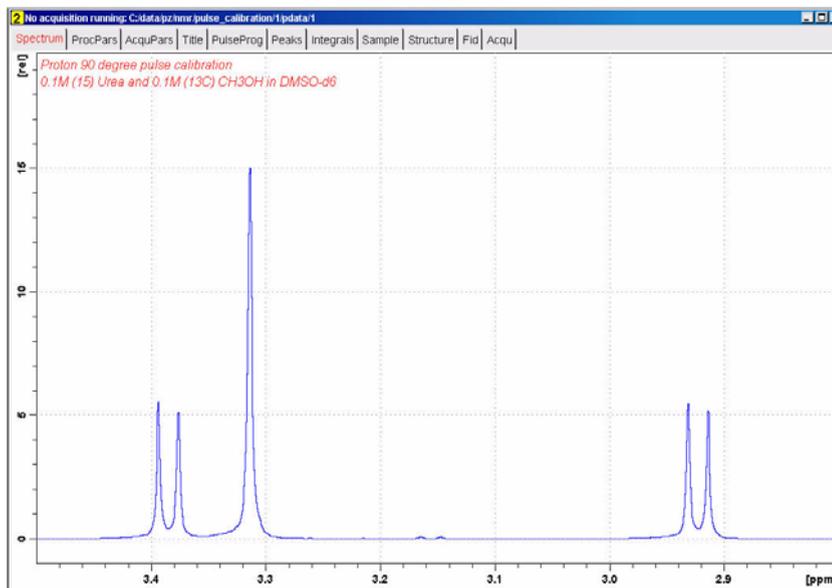
6. Change **NAME = p90_carbon**

7. Click on **OK**

8. Expand the region between 3.5ppm and 2.8ppm

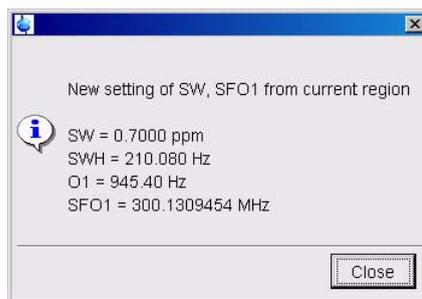
NOTE: Normally a single peak set to on resonance is used to determine the 90^0 transmitter pulse. For practical reason the Methanol signal region from 3.5ppm to 2.8ppm is used to measure the ^{13}C 90^0 transmitter pulse, since the same signals will also be used in determining the ^1H 90^0 decoupler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

Figure 2.20



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

Figure 2.21



10. Click on

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

12. Make the following changes:

PULPROG = decp90

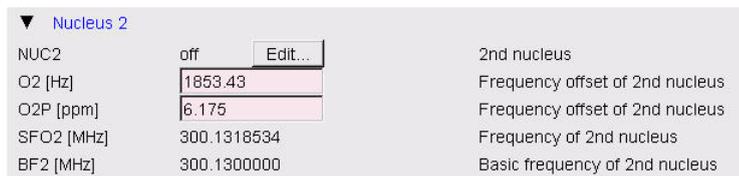
TD = 4K

NS = 1

DS = 0

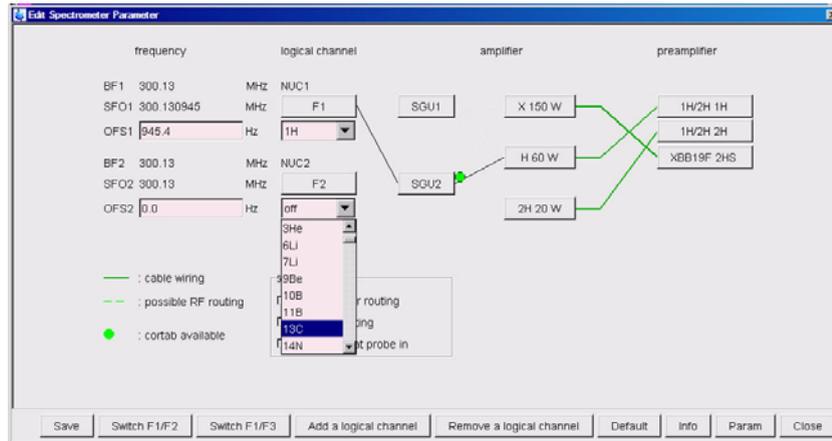
13. Click on next to NUC2 in the Nucleus2 section of the '**AcquPars**'

Figure 2.22



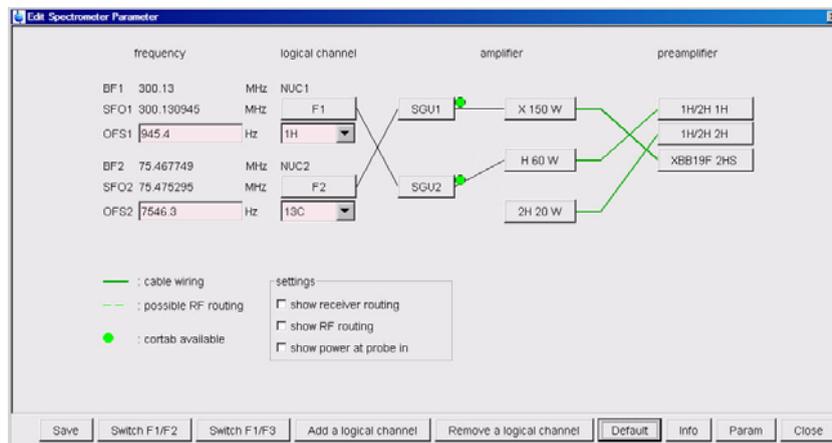
14. Select **13C** for **NUC2**

Figure 2.23



15. Click on **Default** to set the routing

Figure 2.24



16. Click on **Save**

17. In the **'AcquPars'** make the following change:

O2[ppm] = 49

D1 = 10

CNST2 = 130

P3 = 3

18. Select **Prosol** by clicking on it, to read in the Prosol parameters

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

20. Make the following changes:

SI = 2K

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

22. In the command line type **wpar C13p90_urea all** to store the parameter set for

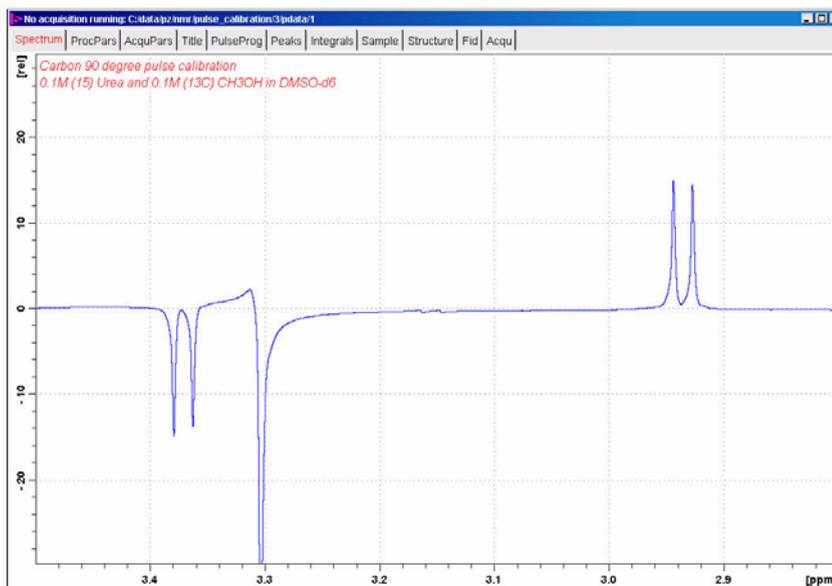
future use

2.5.3 Determine the ^{13}C 90 deg. decoupler pulse

1. Select  by clicking on it
2. Select  by clicking on it
3. Process and Phase correct the spectrum

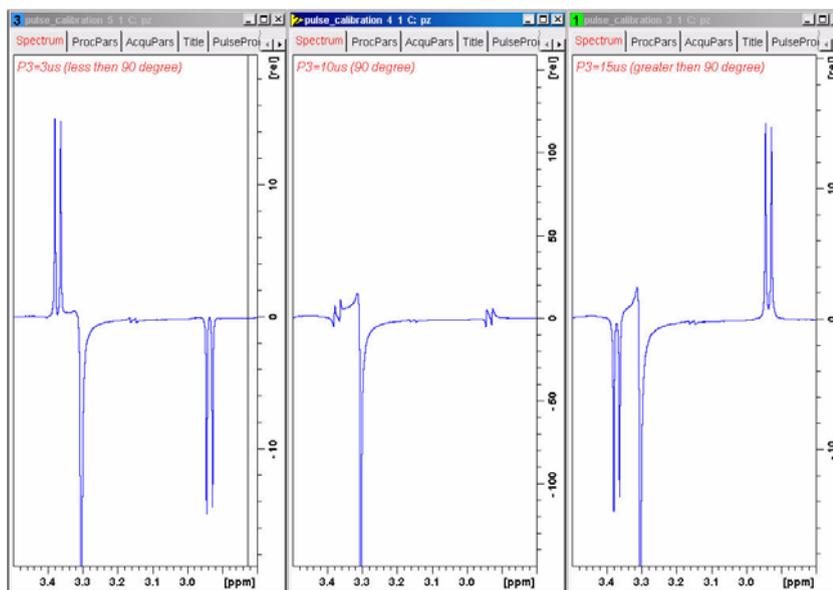
NOTE: Phase the left doublet negative and the right doublet positive. The Water peak at 3.3 ppm can be ignored and does not have to be in phase.

Figure 2.25



4. Increase **p3** in increments of 1 or 2us, execute **zg** followed by the command **efp** until the signals go through a null or a phase change. This will be the ^{13}C 90⁰ decoupler pulse

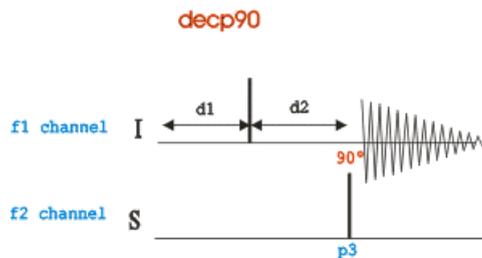
Figure 2.26



2.6 Observations

2.7 15N 90 deg. decoupler pulse

Figure 2.27

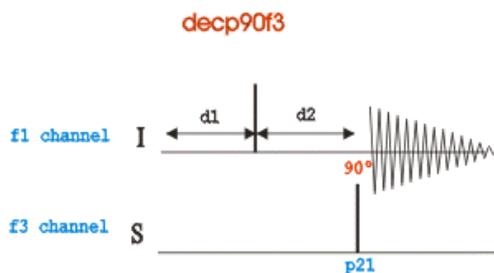


The pulse program used in this procedure is the decp90 sequence shown in Figure 2.27. The sequence consists of two channels f1 (I) and f2 (S), where in this case f1 is set for 1H and f2 to 15N. Channel f1 shows a recycle delay d1 followed by a 90° pulse and a delay $d2 = 1/(2J_{\text{XH}})$ for the creation of antiphase magnetization. A 15N pulse on channel f2 is executed after the delay d2 and then the 1H signal is detected. When the 15N pulse is exactly 90°, the 1H signals will go through a null.

The Urea signal region from 5.6ppm to 5,1ppm is used for this experiment.

If your system is equipped with a 3rd channel for 15N observation, you can still follow the same instructions in this chapter with the exceptions of using the pulse sequence decp90f3 shown in Figure 2.28 and the routing which is illustrated in the section, Parameter set up 2.4.2, Figure 2.29 and Figure 2.31.

Figure 2.28



2.7.1 Parameter set up

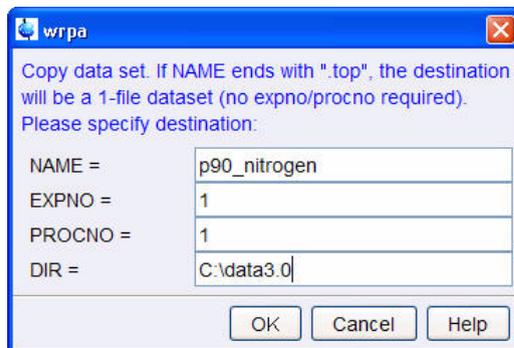
1. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 2.29



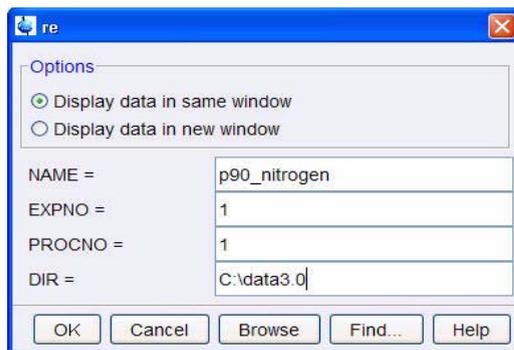
2. In the command line type **wrpa** and hit 'Enter'

Figure 2.30



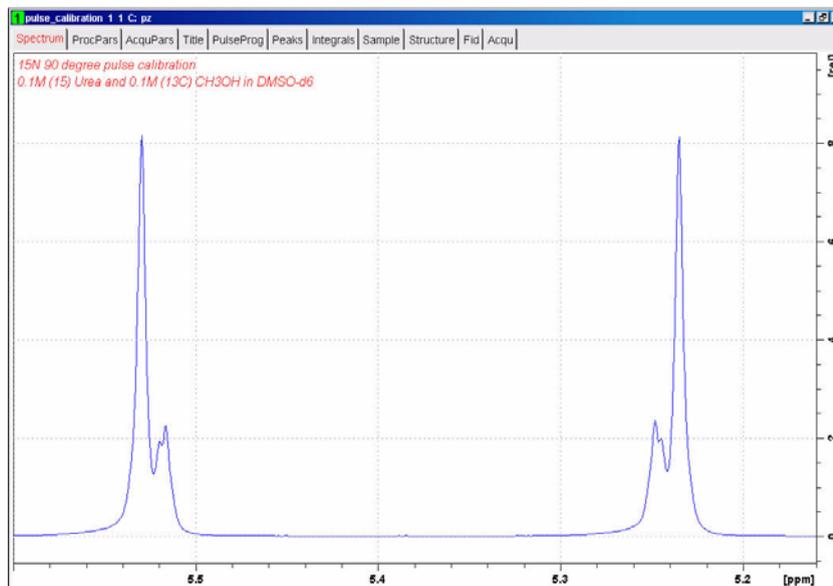
3. Change **NAME** = p90_nitrogen
4. Click on
5. In the command line type **re** and hit 'Enter'

Figure 2.31



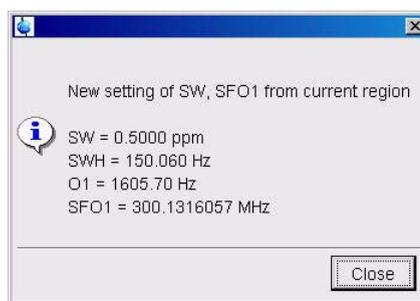
6. Change **NAME** = p90_nitrogen
7. Click on
8. Expand the region between 5.6ppm and 5.1ppm

Figure 2.32



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

Figure 2.33



10. Click on 

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

12. Make the following changes:

PULPROG = decp90

TD = 4K

NS = 1

DS = 0

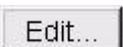
13. Click on  next to NUC2 in the Nucleus2 section of the 'AcquPars'

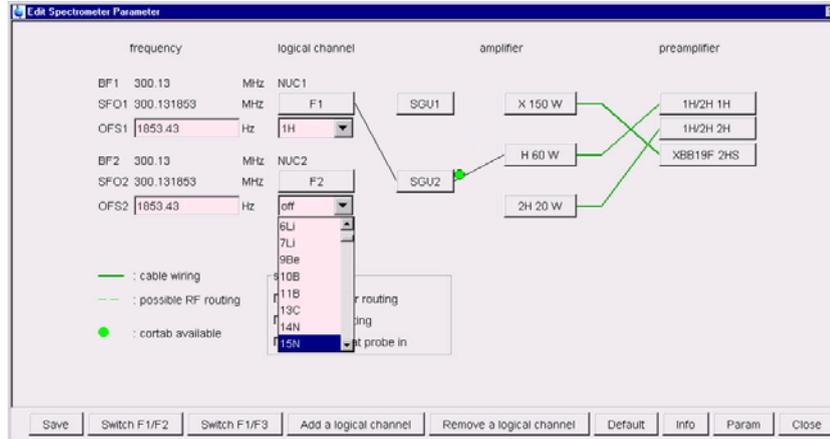
Figure 2.34

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

2.7.1.1 Two channel system

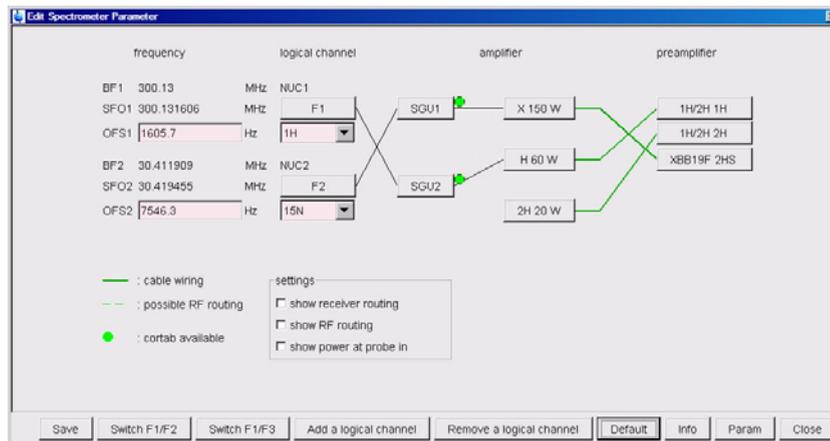
14. Select **15N** for **NUC2**

Figure 2.35



15. Click on **Default** to set the routing

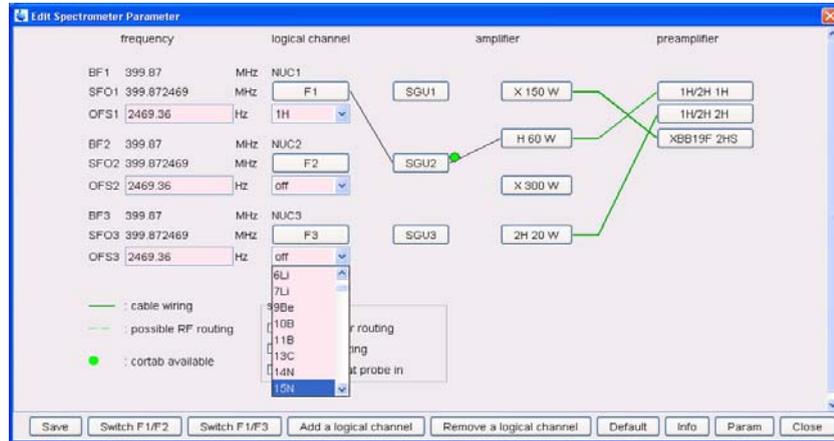
Figure 2.36



2.7.1.2 Three channel system

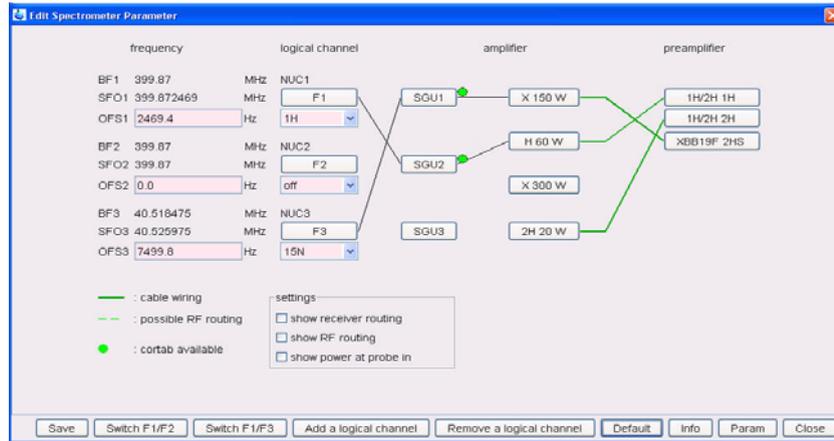
14. Select **15N** for **NUC2**

Figure 2.37



15. Click on **Default** to set the routing

Figure 2.38



16. Click on **Save**

17. In the 'AcquPars' make the following change:

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

18. Select **Prosol** by clicking on it, to read in the Prosol parameters

19. Select the 'ProcPar' tab by clicking on it

20. Make the following changes:

SI = 2K

21. Select the 'Spectrum' tab by clicking on it

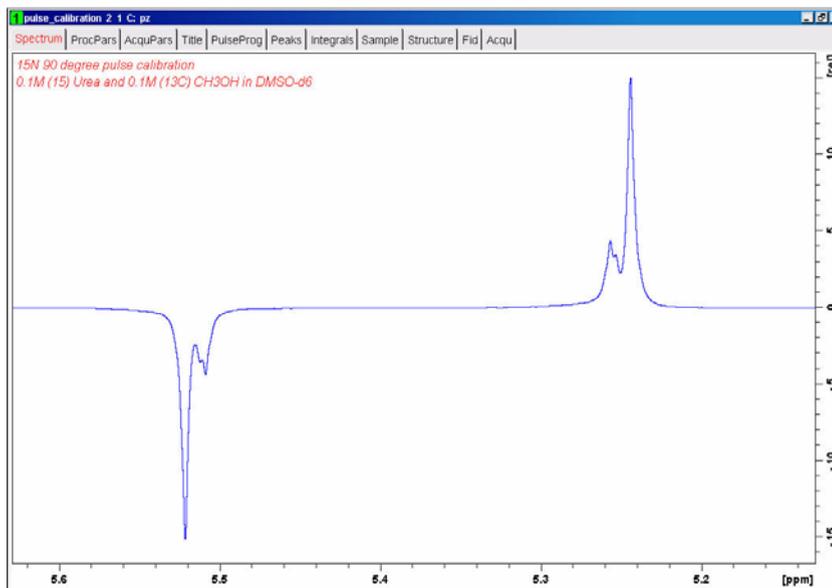
22. In the command line type **wpar N15p90_urea all** to store the parameter set for future use

2.7.2 Determine the ^{15}N 90 deg. decoupler pulse

1. Select  by clicking on it
2. Select  by clicking on it
3. Process and Phase correct the spectrum

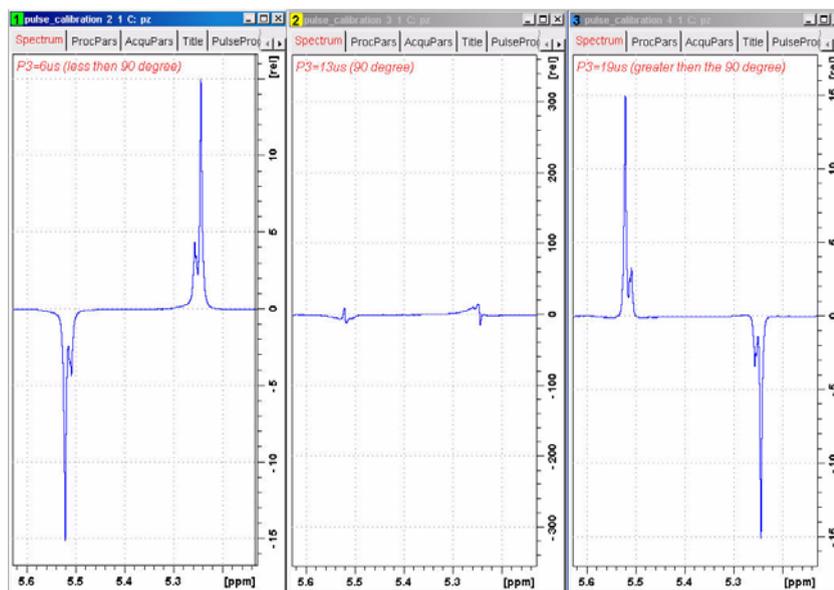
NOTE: Phase the left side signal negative and the right side signal positive.

Figure 2.39



4. Increase **p3** in increments of 1 or 2us, execute zg followed by the command efp until the signals go through a null or a phase change. This will be the ^{15}N 90⁰ decoupler pulse

Figure 2.40



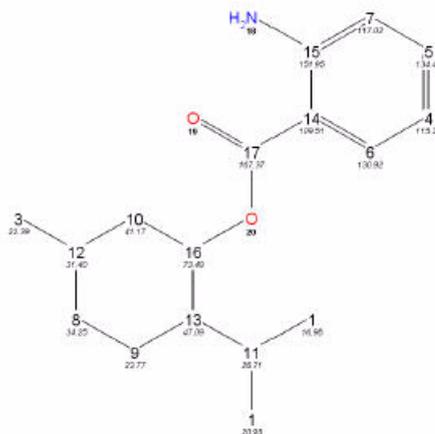
2.8 Observations

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

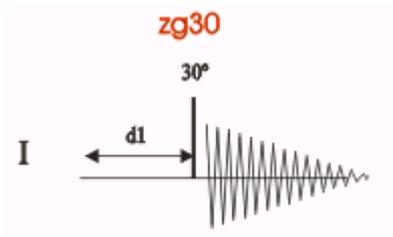


3.2 1-D Proton experiment

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  by clicking on it
3. Enter the following information in to the 'New' window

Figure 3.4

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

NAME	proton_exp
EXPNO	1
PROCNO	1
DIR	C:\data3.0
Solvent	DMSO
Experiment Dirs.	C:\Bruker\TopSpin3.0.b.40\exp\stan\nmr\par
Experiment	PROTON
TITLE	1D- Proton experiment 30 mg Menthyl Anthranilate in DMSO-d6

Show new dataset in new window

1 Receivers (1,2, ...16)

OK Cancel More Info... Help

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

Figure 3.5



6. Select  by clicking on it

Figure 3.6



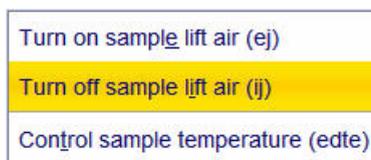
7. Select 'ej' by clicking on it

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 top of the magnet

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

Figure 3.7

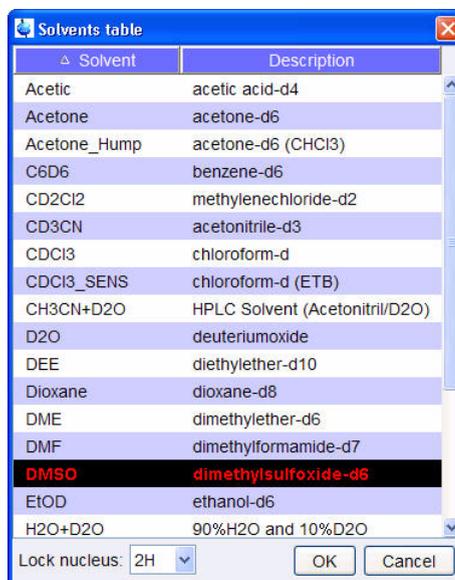


10. Select 'ij' by clicking on it

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  **Lock** by clicking on it

Figure 3.8



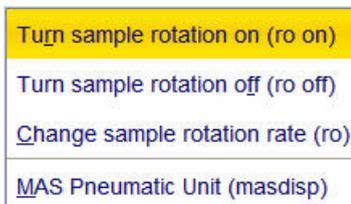
12. Select **DMSO** by clicking on it

13. Select  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  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  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  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  by clicking on it

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

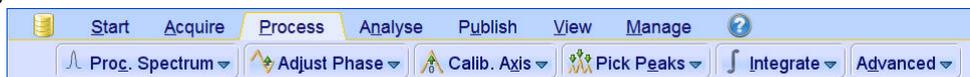
2. Select  by clicking on it

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

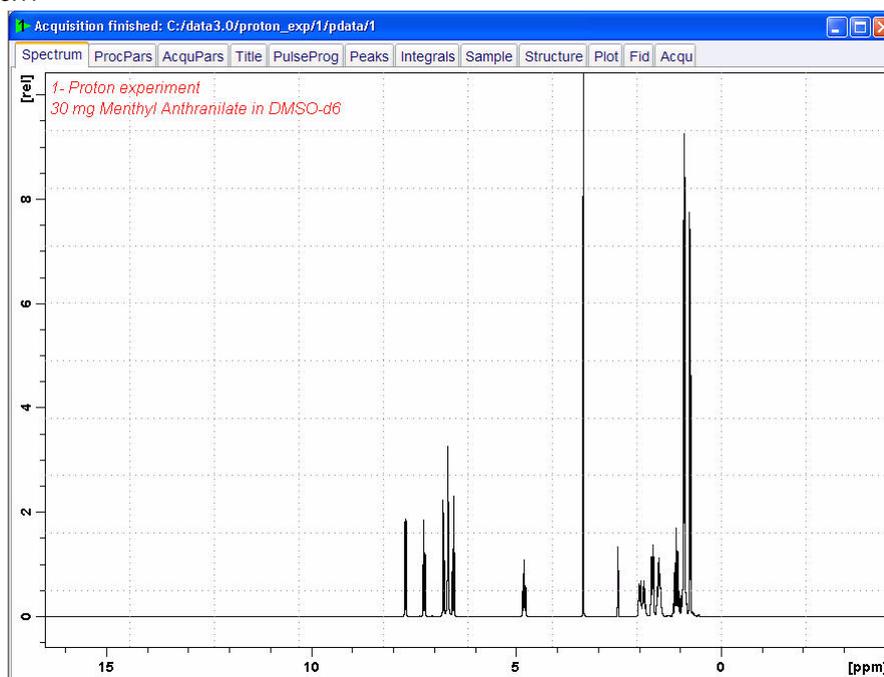
Figure 3.10



2. Click on 

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



3.3 Observations

4 2-D gradient experiments

4.1 Introduction

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

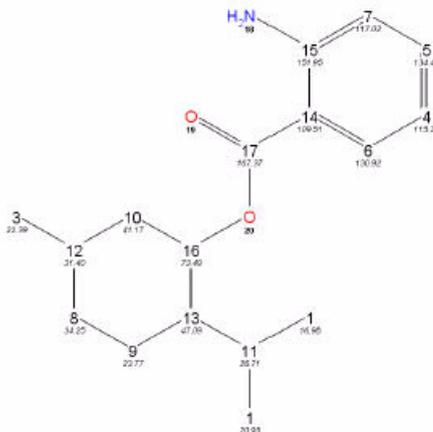
Gradient enhanced NMR spectroscopy is widely used in liquid state spectroscopy for coherence pathway selection, solvent suppression, artifact reduction, and diffusion weighting and has had a tremendous impact by improving the quality of NMR spectra.

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

4.2 Sample

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

Figure 4.1



4.3 2-D gradient COSY

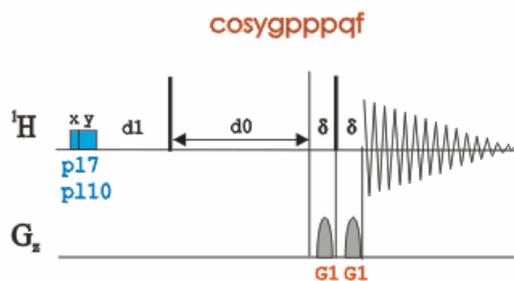
Several simple two-pulse programs can be used to record a magnitude mode COSY spectrum, e.g., cosy, cosy45, and cosy90. These vary with respect to the angle of the final pulse. Any value between 20° and 90° may be chosen for the final pulse angle. However, a pulse angle of 45° is recommended because this yields the best signal-to-noise ratio together with a simple cross peak structure in the final spectrum. A minimum of 8 scans have to be acquired do to the quadrature phase cycle.

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. As well, the DQF-COSY experiment reduces the intensity of the diagonal, allowing for analyses of peaks close in chemical shift.

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 4.3 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

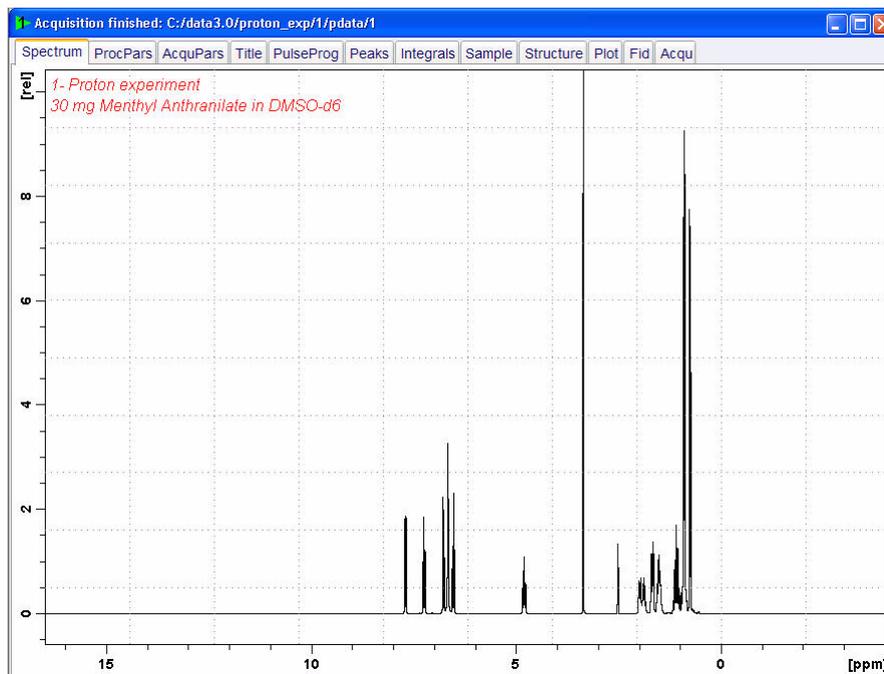


The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, $d1$ is typically a few seconds while $p1$ is typically a few microseconds in length.

4.3.1 Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 4.3



4.3.2 Setting up the COSY experiment

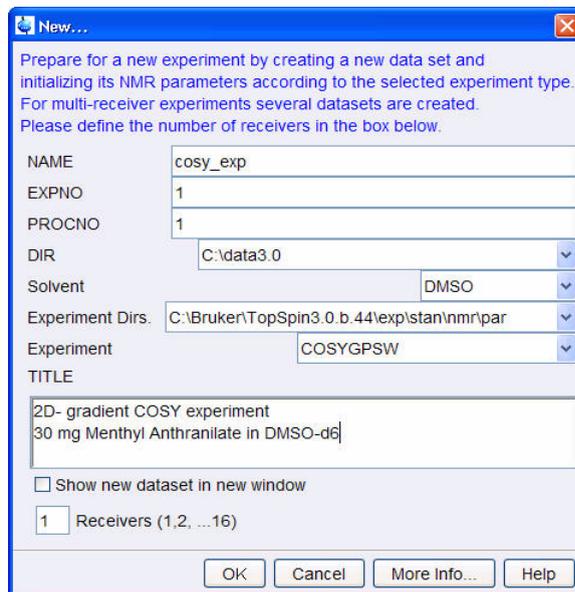
1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 4.4



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

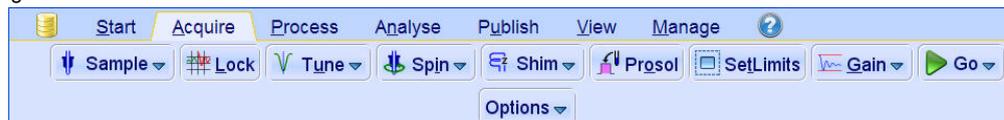
Figure 4.5



NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 3.5 above. Click on the down arrow button to browse for a specific directory.

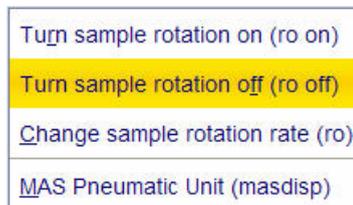
4. Click on 
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 4.6



6. Select  by clicking on it

Figure 4.7



7. Select 'ro off' by clicking on it

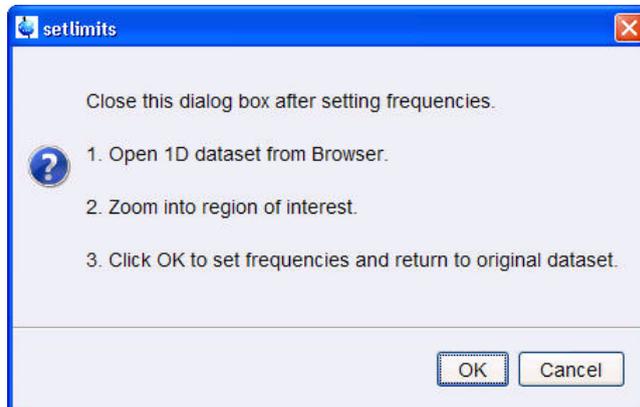
NOTE: 2-D experiments should be run non spinning

8. Select  by clicking on it

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

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

Figure 4.8

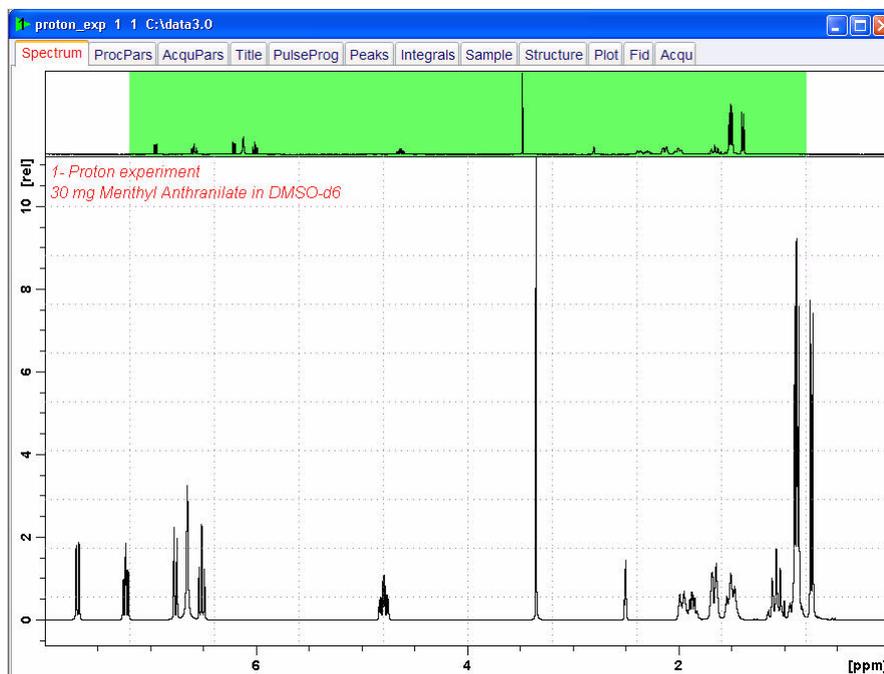


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

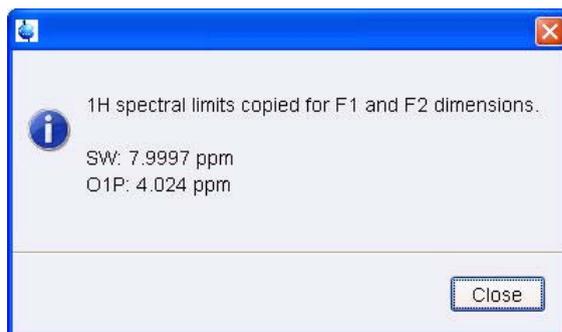
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

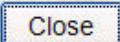
Figure 4.9



12. Click on  to assign the new limit

Figure 4.10



13. Click on 

NOTE: The display changes back to the 2D data set.

4.3.3 Acquisition

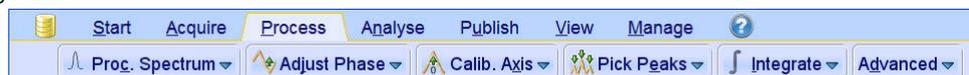
1. Select  by clicking on it

2. Select  by clicking on it

4.3.4 Processing

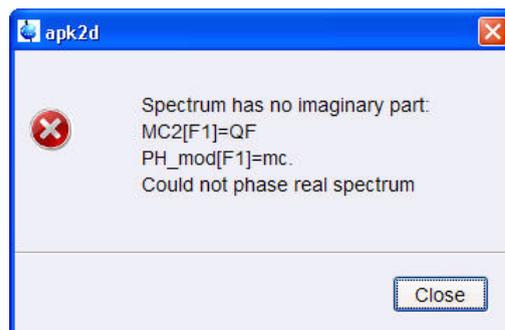
1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 4.11



2. Select  by clicking on it

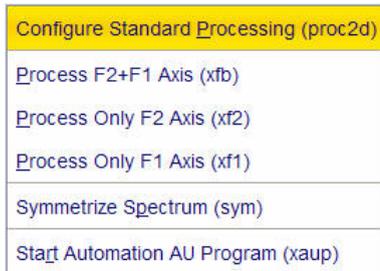
Figure 4.12



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 the steps below.

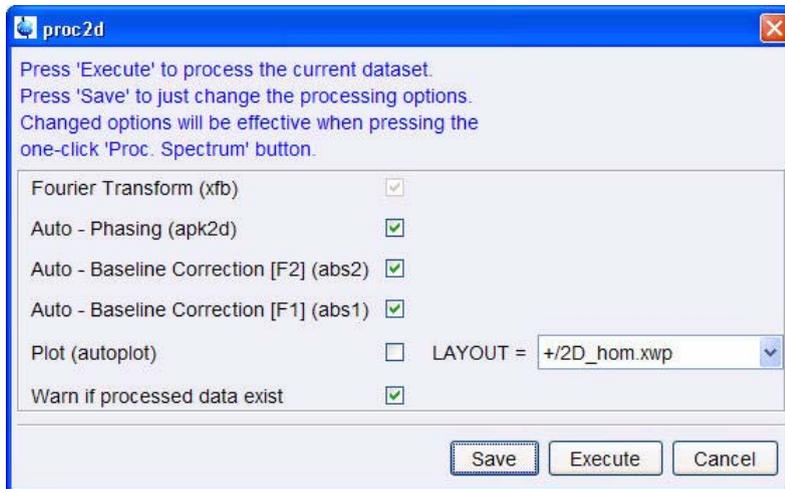
3. Click on the down arrow inside the  button

Figure 4.13



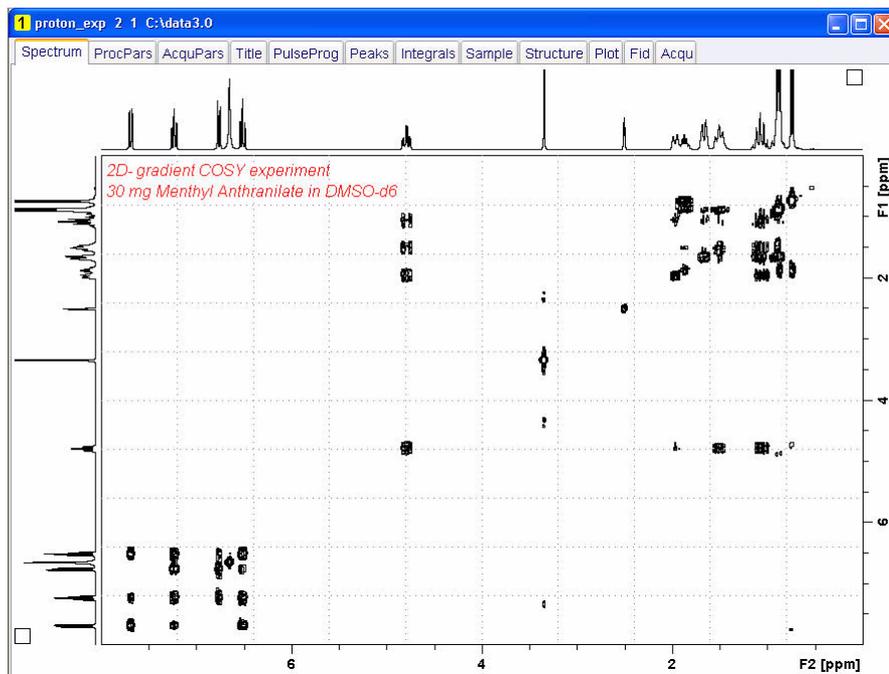
4. Select 'Configure Standard Processing' by clicking on it

Figure 4.14



NOTE: To avoid the message shown in Figure 3.14 the option 'Auto-Phasing (apk2d)' may be disabled for magnitude like 2D experiment.

Figure 4.15



4.3.5 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

4.4 Observations

4.5 2-D Multiple Quantum Filtered COSY experiment

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

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

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

Figure 4.18

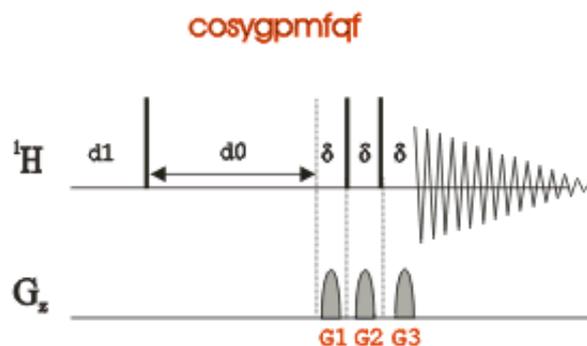
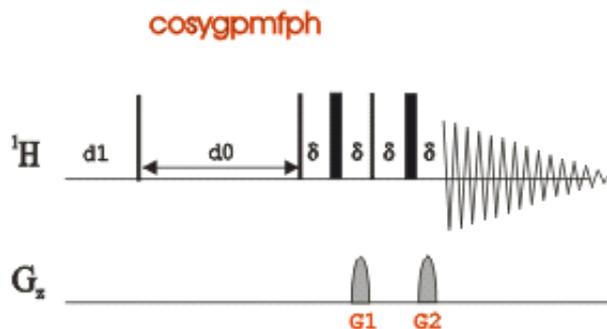


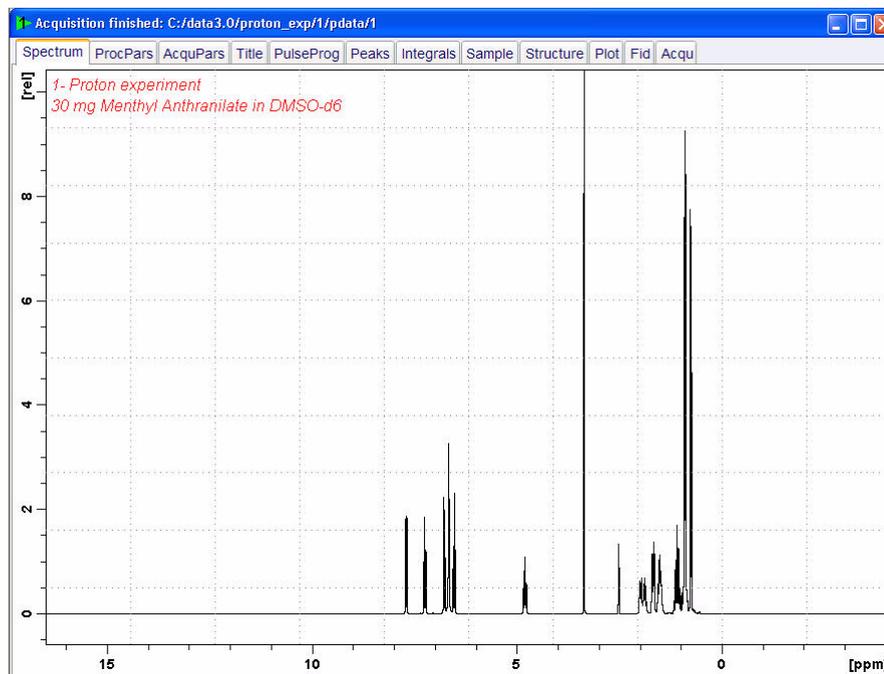
Figure 4.19



4.5.1 Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 4.20



4.5.2 Setting up the MFQ-COSY experiment

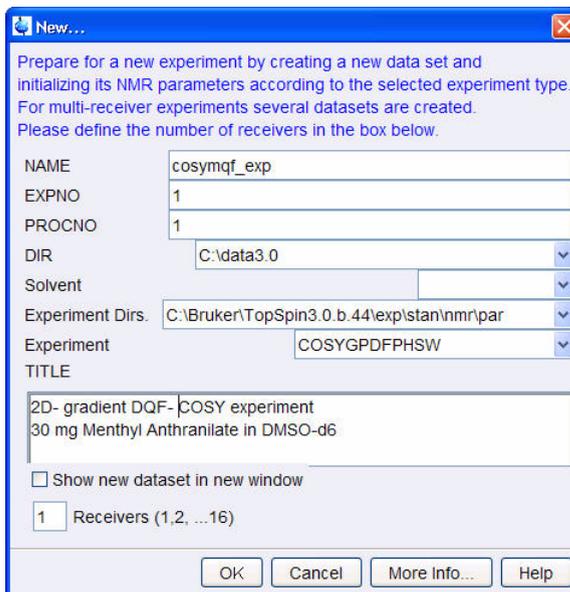
1. Click on the **'Start'** tab in the TopSpin Menu bar

Figure 4.21



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

Figure 4.22

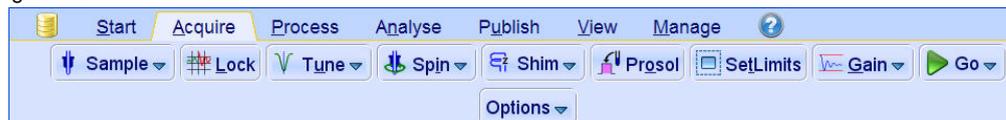


NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 3.22 above. Click on the down arrow button to browse for a specific directory.

4. Click on 

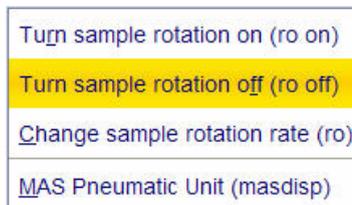
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 4.23



6. Select  by clicking on it

Figure 4.24



7. Select 'ro off' by clicking on it

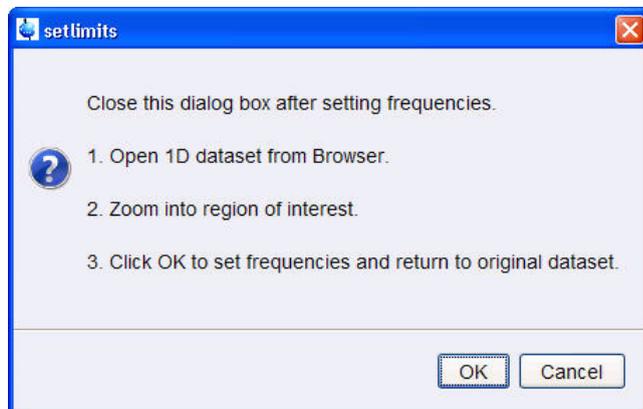
NOTE: 2-D experiments should be run non spinning

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 4.25

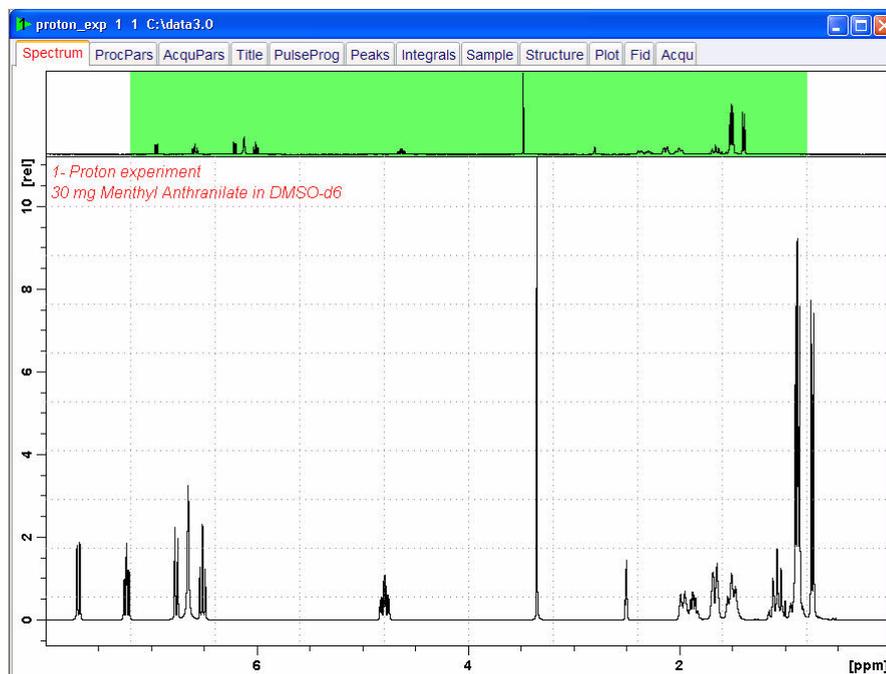


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

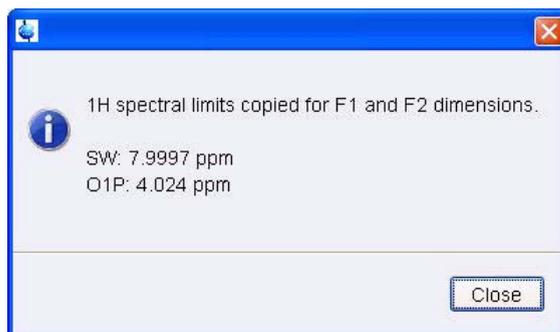
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

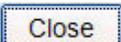
Figure 4.26



12. Click on  to assign the new limit

Figure 4.27



13. Click on 

NOTE: The display changes back to the 2D data set.

4.5.3 Acquisition

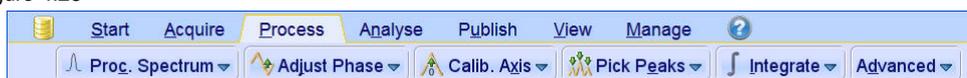
NOTE: The first increment of the DQF-COSY experiment has a low signals to noise ratio and the signals grow as the experiment is progressing. It is therefore not advisable to use the automatic receiver gain adjustment 'rga' since it adjusts the receiver gain on the first increment. In this case an AU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

1. Type **au_zgcosy** on the command line

4.5.4 Processing

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

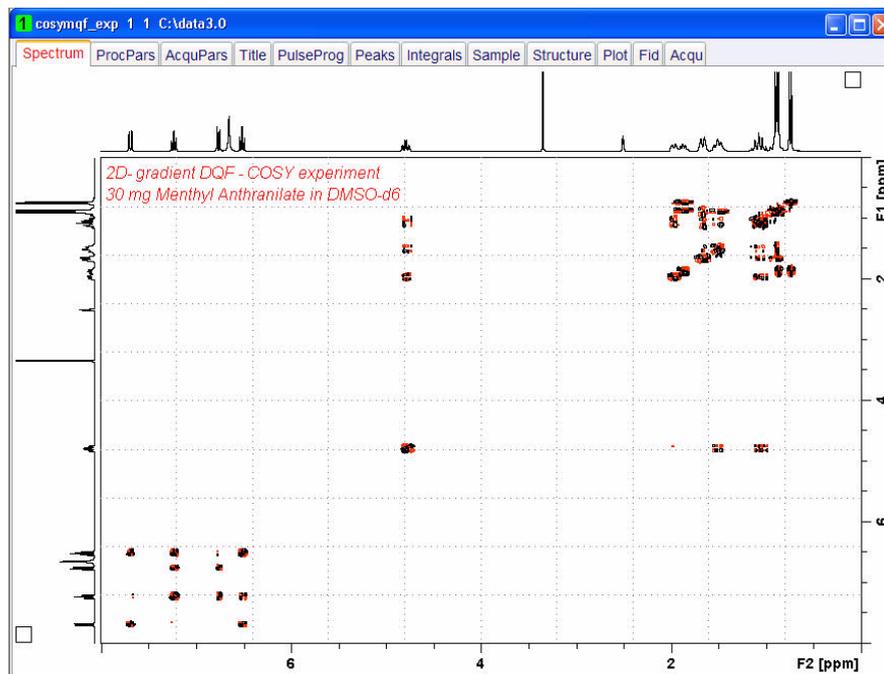
Figure 4.28



2. Select  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 4.29



4.5.5 Plotting

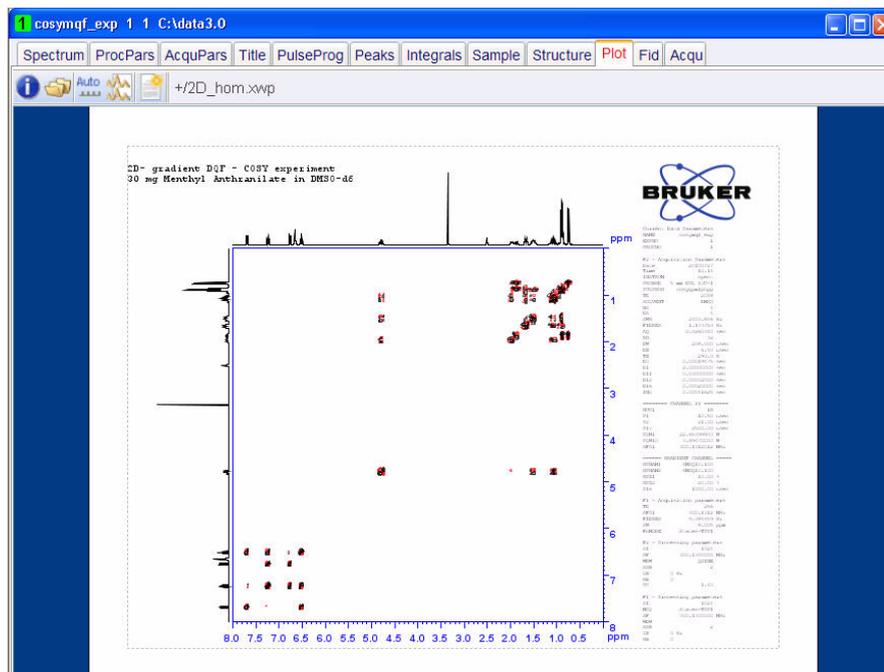
1. Use the  buttons to adjust for a suitable contour level
2. Click on the **'Publish'** tab in the TopSpin Menu bar

Figure 4.30



3. Click on  **Plot Layout**
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 to open the Plot Editor.

5. Click on the  to plot the spectrum

4.6 Observations

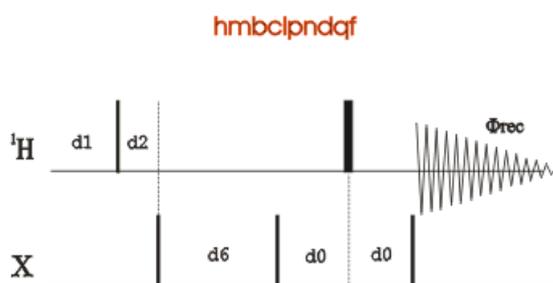
4.7 2-D HMBC experiment

The basic 2D HMBC pulse sequence (see Figure 4.32) is closely related to the HMQC pulse sequence but incorporating the following modifications:

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

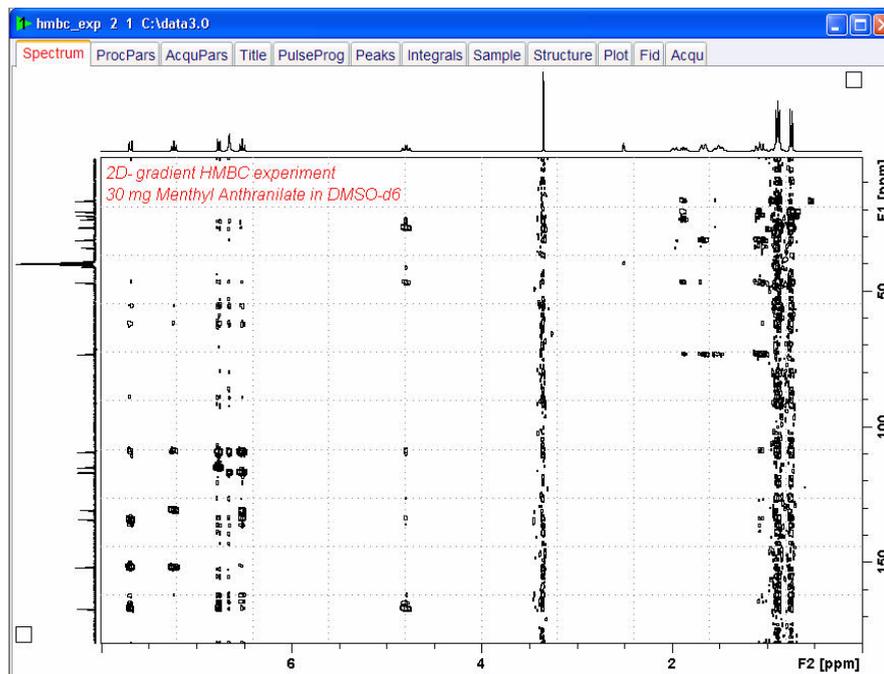
Using this experiment qualitative heteronuclear long-range connectivity, including quaternary carbons or through heteronuclei can be extracted.

Figure 4.32



The non gradient 2D HMBC spectrum of Menthyl Anthranilate in DMSO- d_6 is illustrated in Figure 3.33, showing considerable artifacts. Additionally a minimum number of 8 scans had to be used for the full phase cycling.

Figure 4.33

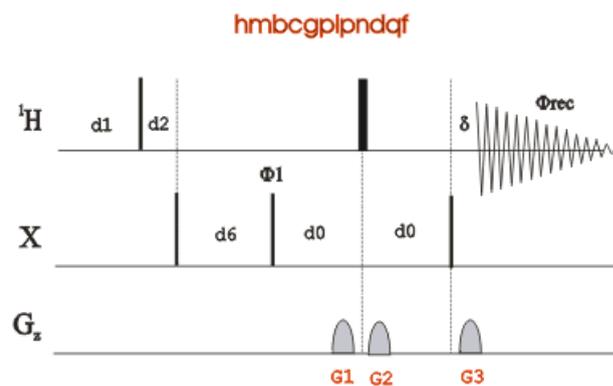


The main advantages of using gradients in high resolution NMR experiments include:

- Coherence selection and frequency-discrimination in the indirect dimension (F1) can be achieved with a single scan per T1 increment.
- A reduction in the number of required phase cycle steps for the suppression of undesired artifacts.
- An important decrease in the total acquisition times for sufficiently concentrated samples.
- The obtaining of higher quality spectra with an important reduction in T1 noise.
- An efficient suppression of undesired signals such as, for instance, the intense solvent signal in H₂O solution and the ¹H-¹²C (¹H-¹⁴N) magnetization in proton detected heteronuclear experiments at natural abundance. In these inverse experiments, the starting BIRD cluster or spin-lock pulse are no longer needed.
- A much easier data processing and therefore more accurate spectral analysis.
- A decrease of dynamic-range limitation.

Figure 4.34 shows the gradient HMBC pulse sequence and the instructions below will guide you through the set up of the experiment.

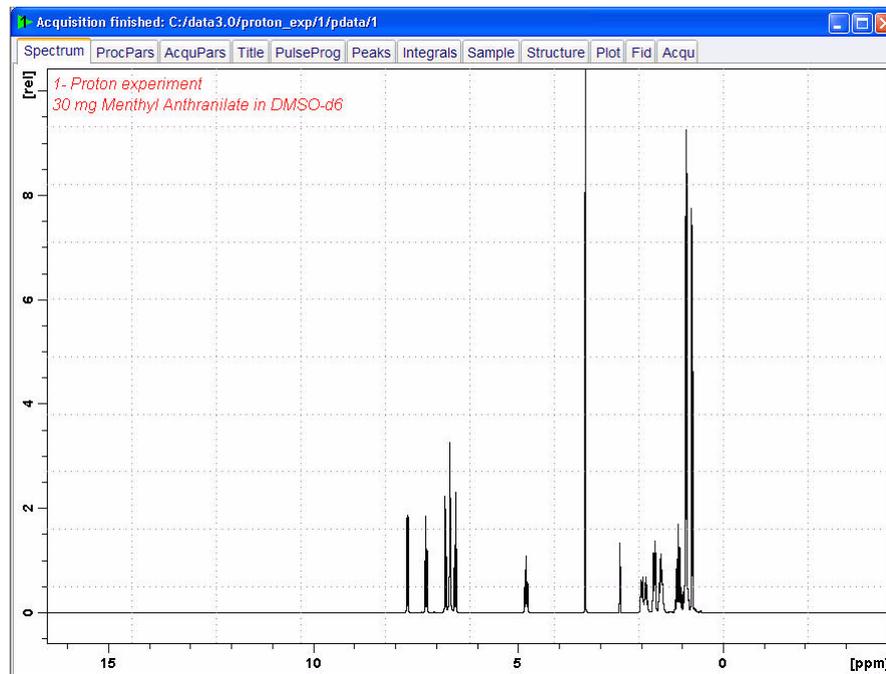
Figure 4.34



4.7.1 Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 4.35



NOTE: The reference spectrum is necessary to adjust the spectral limits of the sweep width in the **F2** dimension and to use it for the projection. The **HMBCGP** parameter set has a default sweep width in the **F1** dimension of 222ppm. If a regular carbon spectrum of the same sample is available, the F1 sweep width can be limited using the 'setlimit' AU-program. The steps in 4.7.2 Setting up the HMBC experiment illustrate the limit setting in both, the F2 and F1 dimensions.

4.7.2 Setting up the HMBC experiment

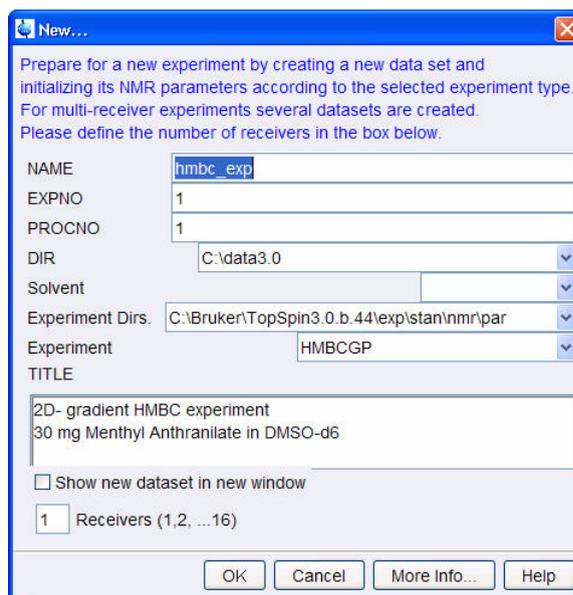
1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 4.36



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

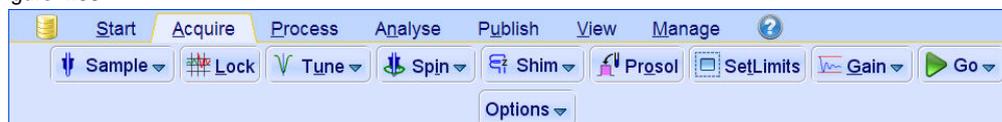
Figure 4.37



NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 4.37 above. Click on the down arrow button to browse for a specific directory.

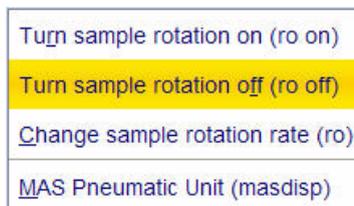
4. Click on 
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 4.38



6. Select  by clicking on it

Figure 4.39



7. Select 'ro off' by clicking on it

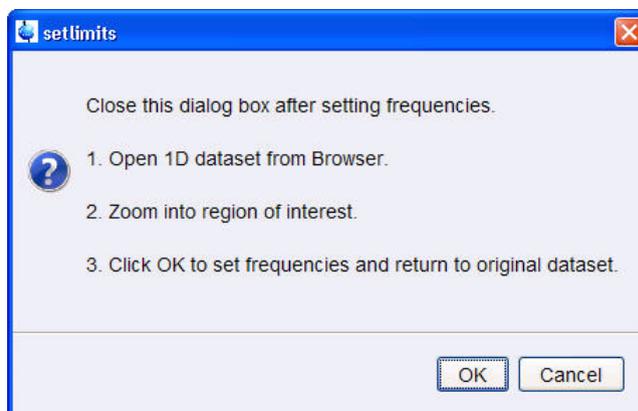
NOTE: 2-D experiments should be run non spinning

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 4.40

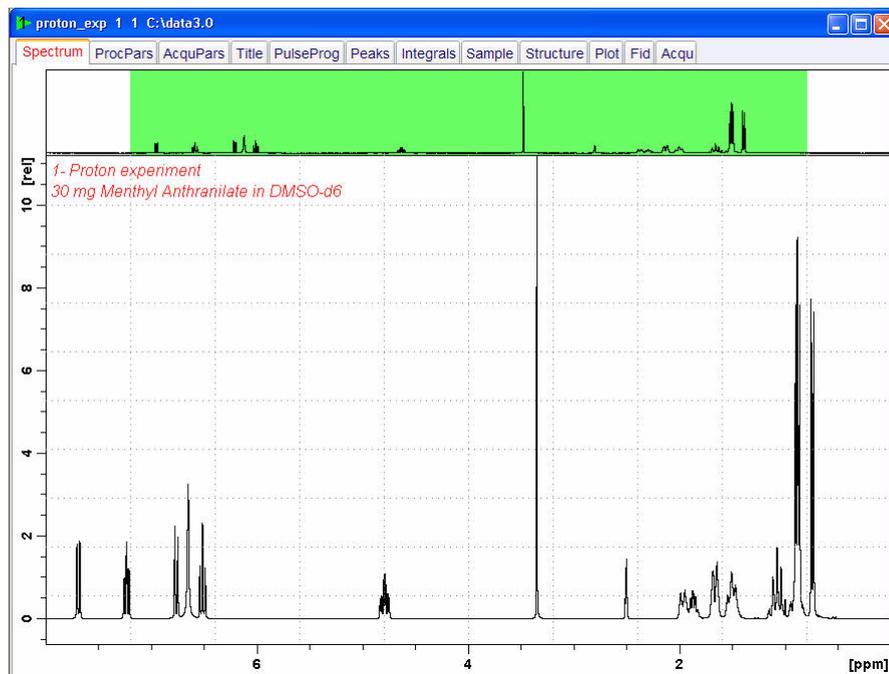


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

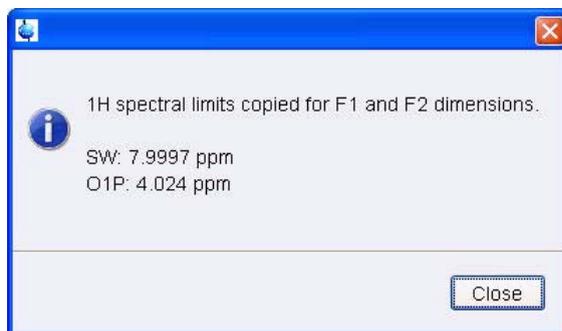
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

Figure 4.41



12. Click on to assign the new limit

Figure 4.42

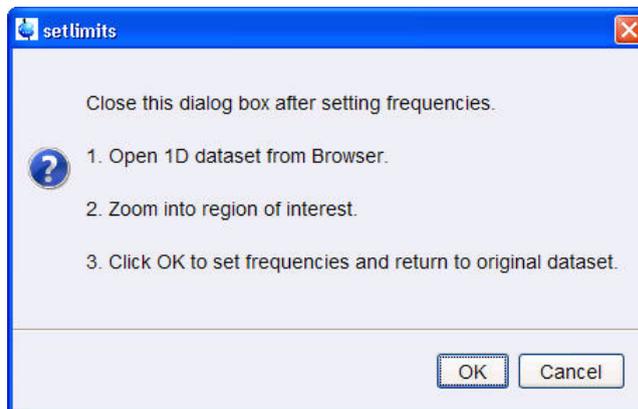


13. Click on

NOTE: The display changes back to the 2D data set. To set the limits in the F1 dimension, follow the steps below.

14. Select by clicking on it

Figure 4.43

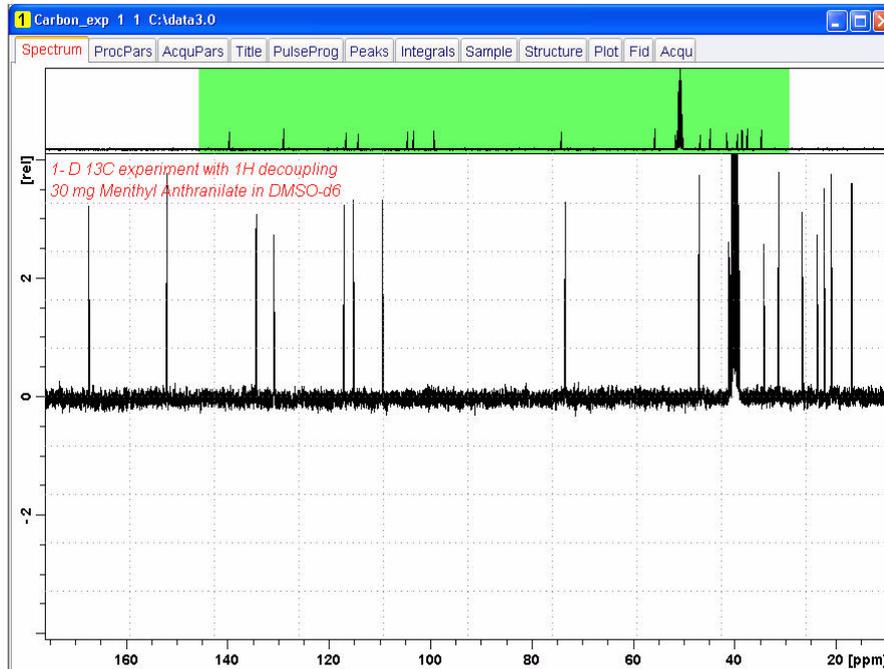


15. To open the 1D C13DEPT spectrum, right click on the dataset name in the browser window (e.g. **Carbon_exp 1**) and select 'Display' or click and hold the left mouse button for dragging the 1D C13DEPT dataset in to the spectrum window

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

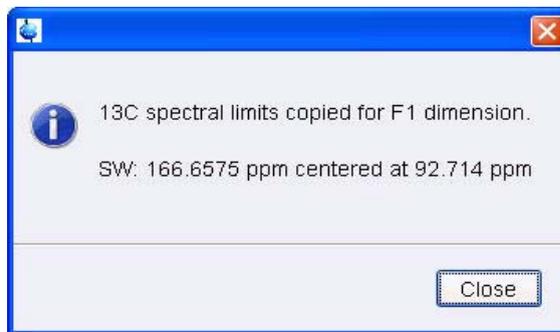
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

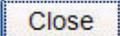
Figure 4.44



17. Click on to assign the new limit

Figure 4.45



18. Click on 

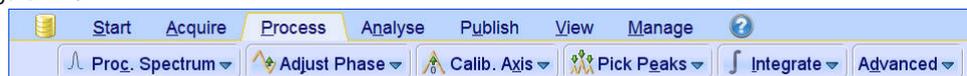
4.7.3 Acquisition

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

4.7.4 Processing

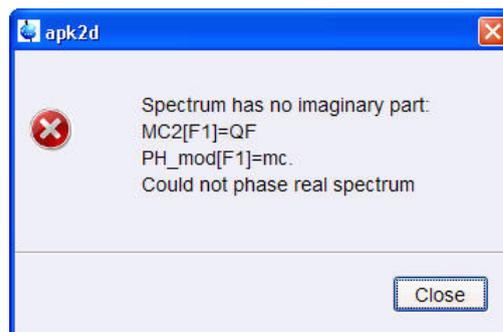
1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 4.46



2. Select  by clicking on it

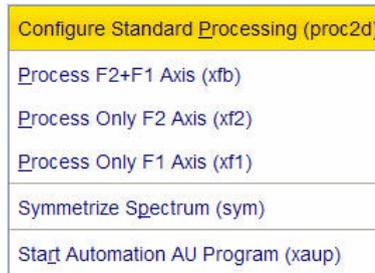
Figure 4.47



NOTE: This executes a standard processing program **proc2**. The message shown in Figure 4.47 pops up in case of a magnitude 2D experiment and the **apk2d** option is enabled. To configure the processing program follow the steps below.

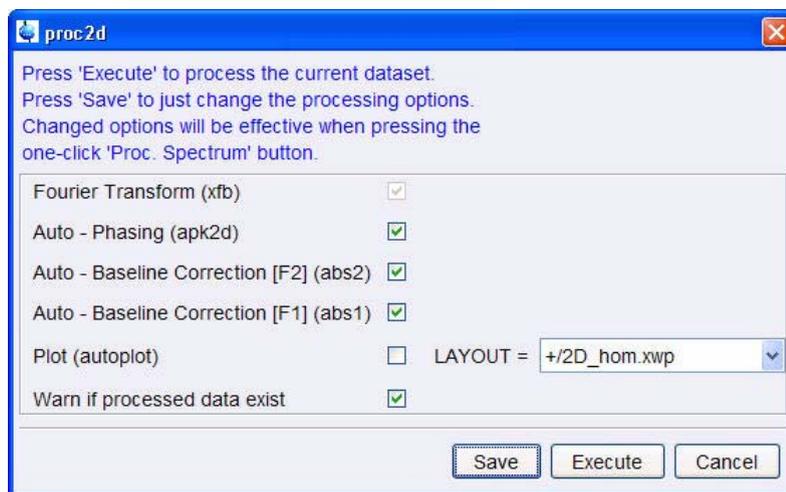
3. Click on the down arrow inside the  button

Figure 4.48



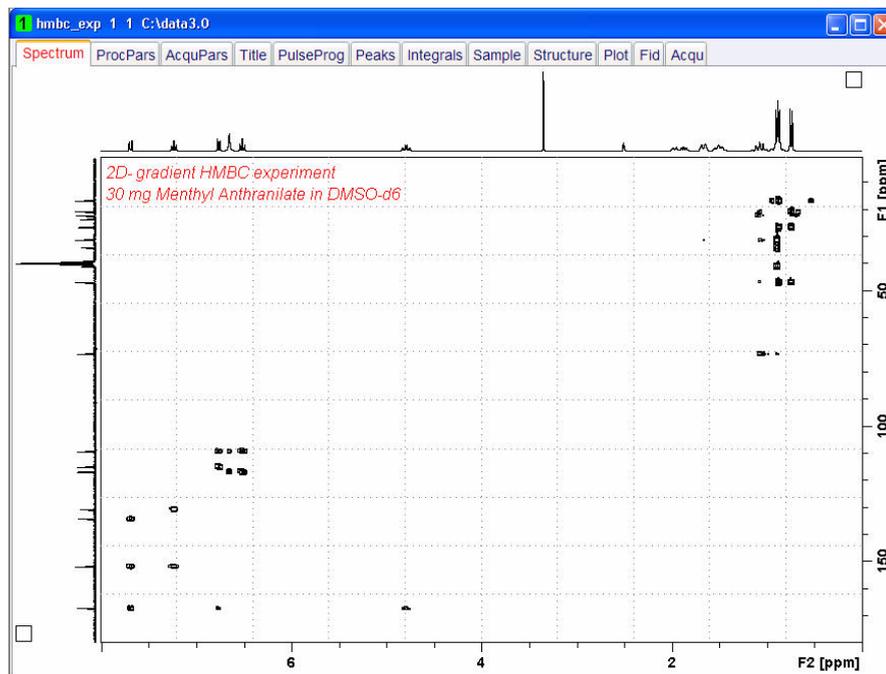
4. Select '**Configure Standard Processing**' by clicking on it

Figure 4.49



NOTE: To avoid the message shown in Figure 4.47 the option '**Auto-Phasing (apk2d)**' may be disabled for magnitude like 2D experiment.

Figure 4.50



4.7.5 Plotting

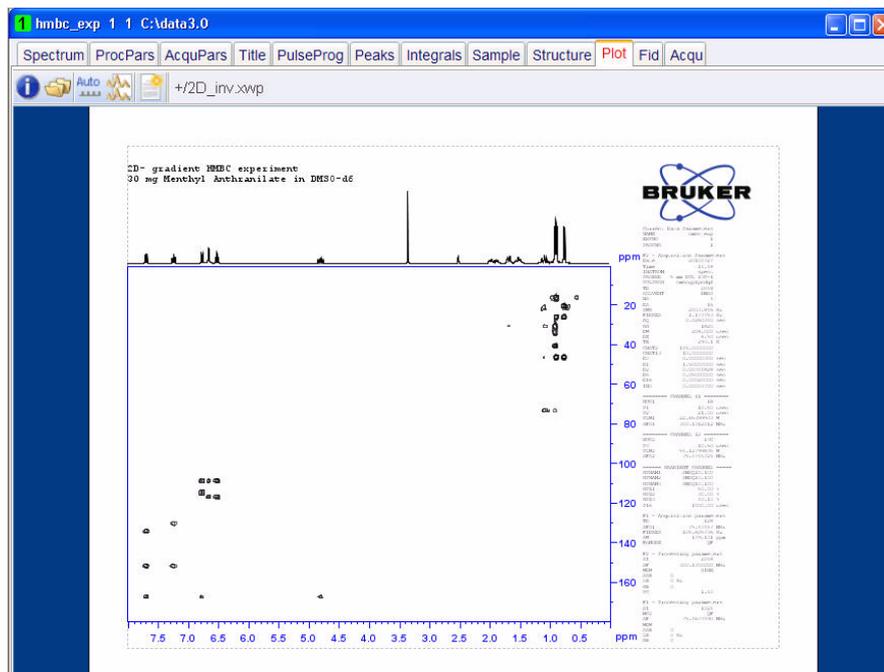
1. Use the  buttons to adjust for a suitable contour level
2. Click on the **'Publish'** tab in the TopSpin Menu bar

Figure 4.51



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

Figure 4.52



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

5. Click on the  to plot the spectrum

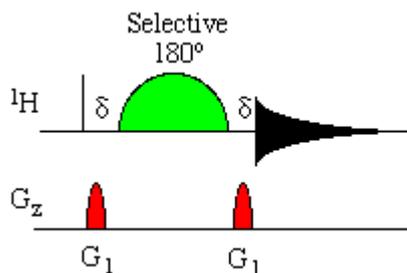
4.8 Observations

5 1-D experiments using shaped pulses

5.1 Introduction

Selective homonuclear 1D experiments usually start from the selective ^1H excitation of a given resonance followed by a mixing process. When PFG's are available, the SPFG scheme is highly recommended as a selective excitation scheme. The SPFG or **Single Pulsed Field Gradient Echo** scheme is a single echo experiment in which the central selective 180 degree pulse is flanked by two gradient pulses. It is used for efficient selective excitation purposes.

Figure 5.1



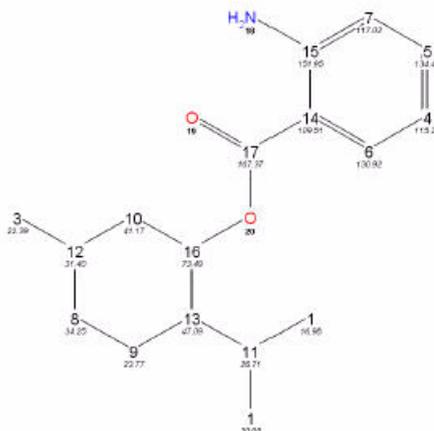
Selective 1D experiments can be easily derived by adding the corresponding mixing process between the SPFG block and the acquisition period.

NOTE: To run this experiment the instrument has to be equipped with the hardware to do Shaped Pulses and Gradients. Three different ways to run this experiment are discussed in this chapter and can also be applied to other selective experiments such as SELCOSY, SELROESY and SELTOCSY.

5.2 Sample

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

Figure 5.2



5.3 1-D Selective COSY

5.3.1 Introduction

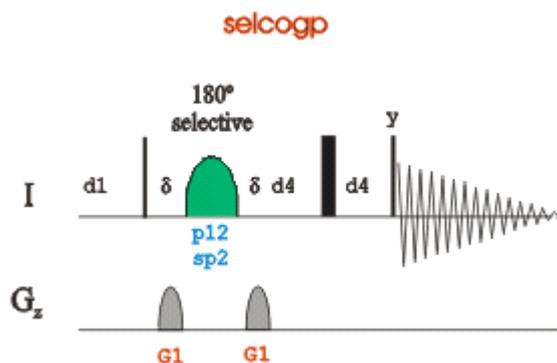
The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ^1H spectrum. Important characteristics of a soft pulse include the shape, the amplitude, 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 5.3 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 5.3. 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 $1/4 \cdot J(\text{H,H})$. The gradient pulses are applied before and after the shape pulse.

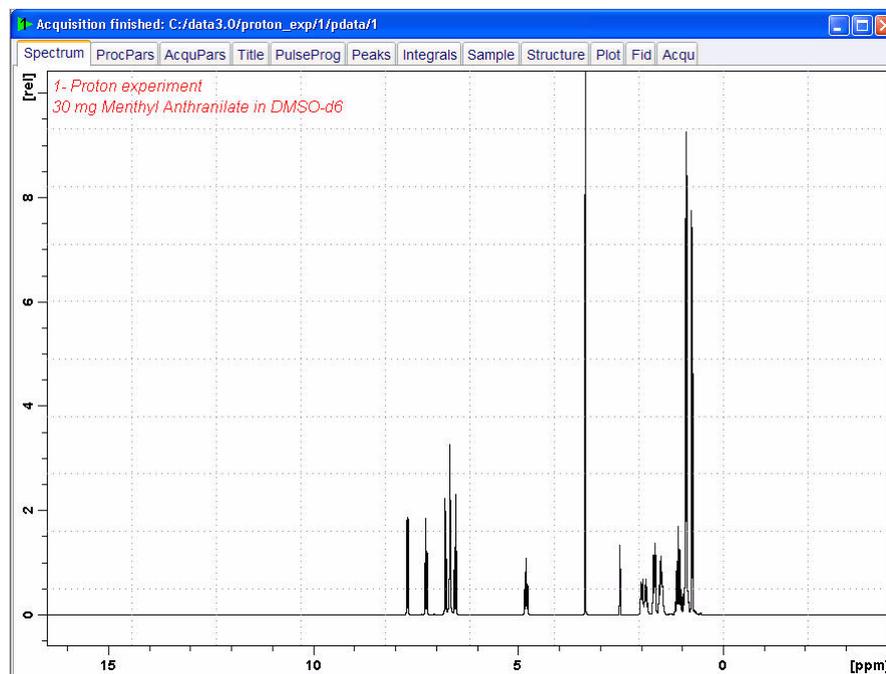
Figure 5.3



5.3.2 Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 5.4



5.3.3 Selective excitation region set up

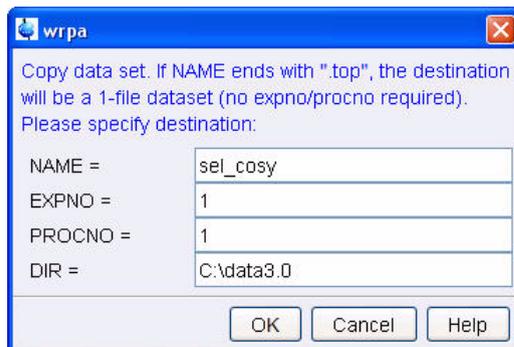
5.3.3.1 On resonance

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

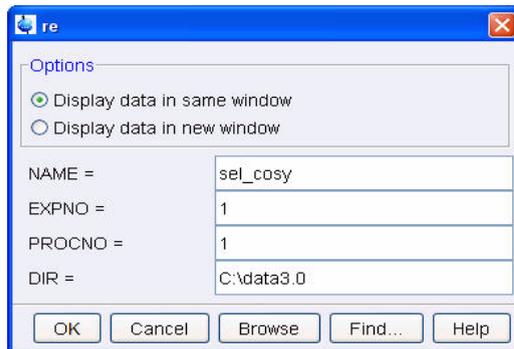
1. In the command line type **wrpa** and hit **'Enter'**

Figure 5.5



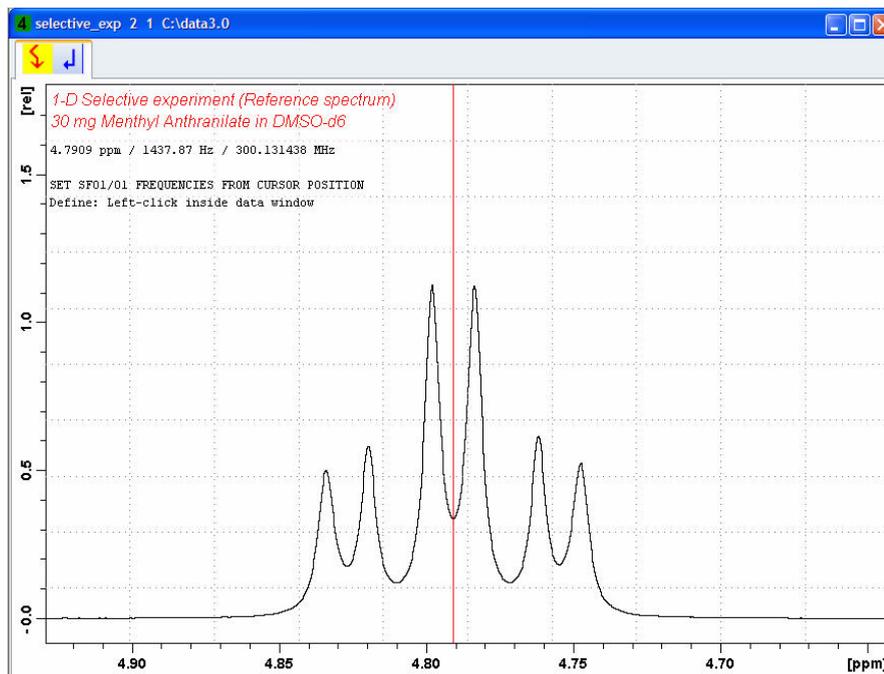
2. Change NAME = **sel_cosy**
3. Click on 
4. In the command line type **re** and hit **'Enter'**

Figure 5.6



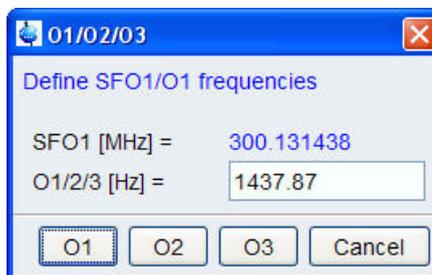
5. Change NAME = **sel_cosy**
6. Click on 
7. Expand peak at 4.8ppm
8. Click on  to set the RF from cursor

Figure 5.7



9. Move the cursor line in to the center of the multiplet
10. Click the left mouse button to set the frequency

Figure 5.8



11. Click on 

5.3.4 Setting up the Selective COSY

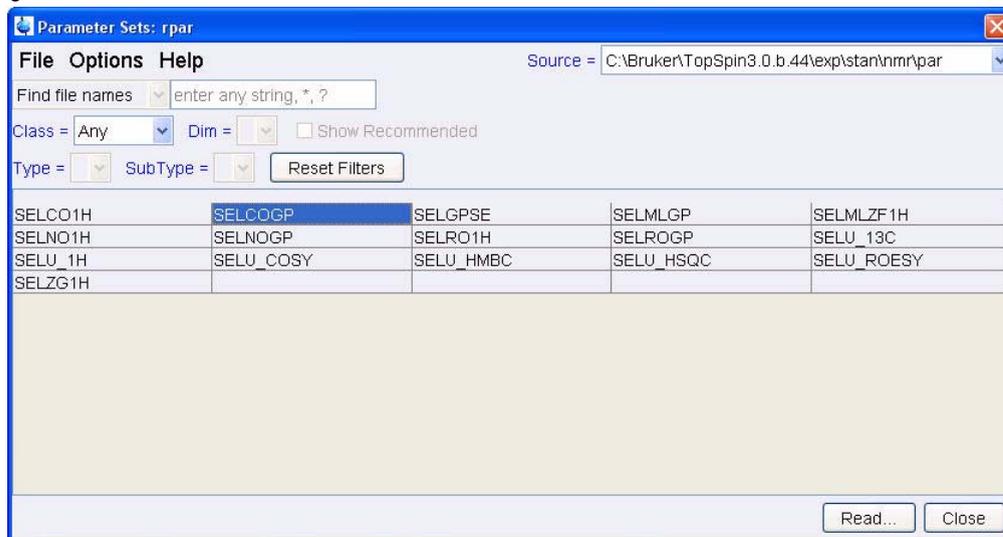
1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 5.9



2. Select  by clicking on it

Figure 5.10



NOTE: Enter **SEL*** in to the 'Find file names' window and hit 'Enter' to display all selective parameter sets shown in figure 5.10.

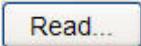
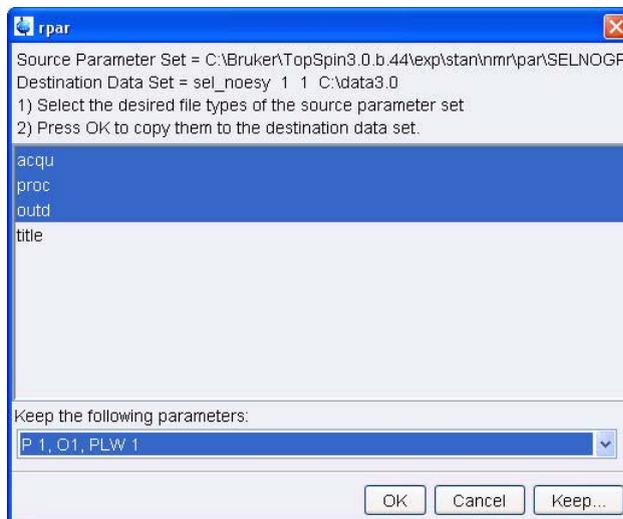
3. Select '**SELCOGP**'
4. Click on 
5. Select the **acqu**, **proc** and **outd** parameter options only
6. Click on the down arrow next to the '**Keep the following parameter**' window
7. Select '**P1, O1, PLW1**' from the pull down menu

Figure 5.11



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

10. Make the following changes:

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

11. Click on  to store the title

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

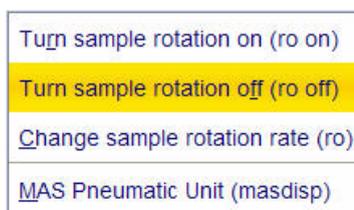
13. Click on the '**Acquire**' tab in the TopSpin menu bar

Figure 5.12



14. Select  by clicking on it

Figure 5.13



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

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

16. Select  by clicking on it

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

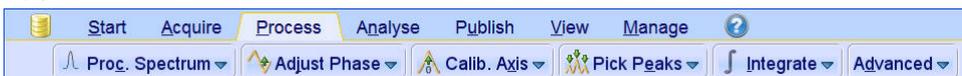
5.3.5 Acquisition

1. Select  by clicking on it

5.3.6 Processing

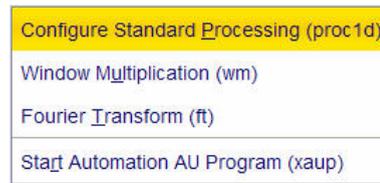
1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.14



2. Click on the down arrow inside the  button

Figure 5.15



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

4. Deselect the following options:

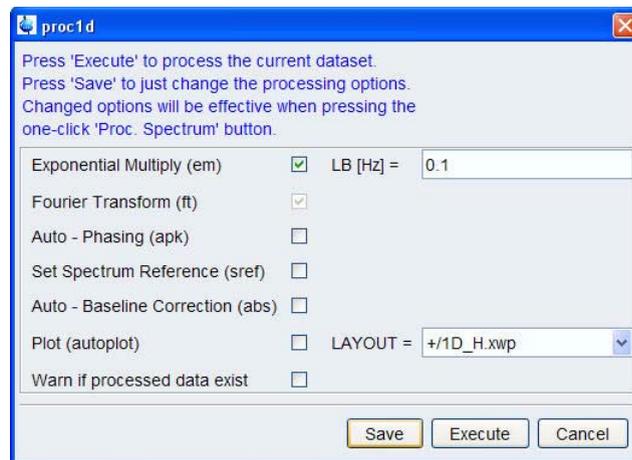
'Auto-Phasing (apk)'

'Set Spectrum Reference (sref)'

'Auto-Baseline correction (abs)'

'Warn if Processed data exist'

Figure 5.16



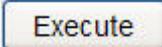
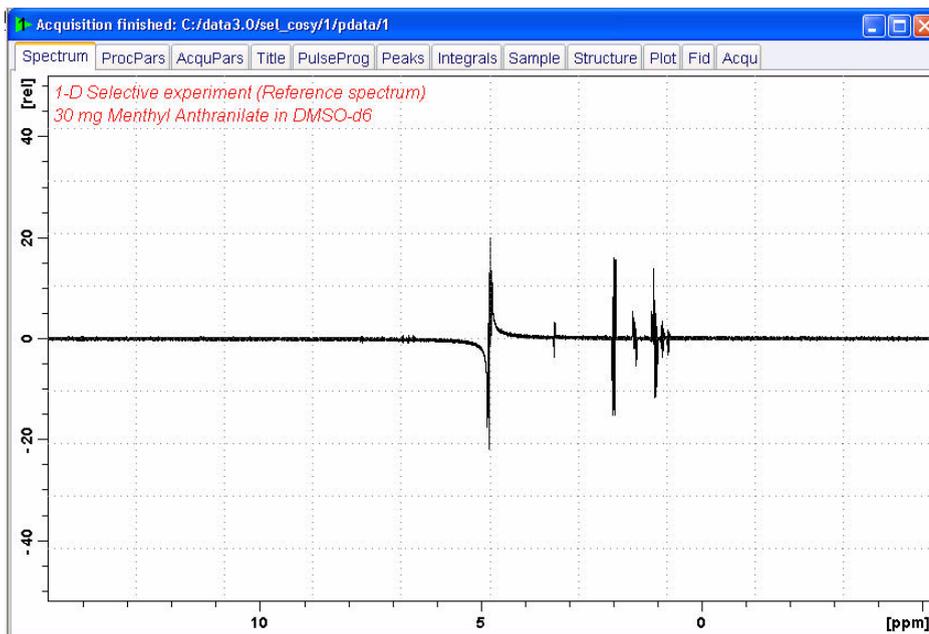
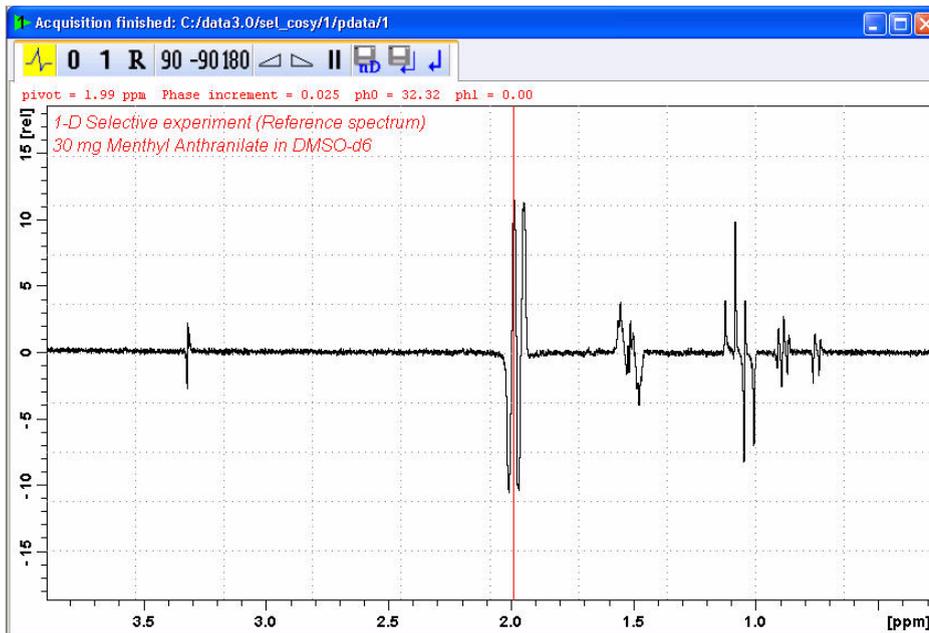
5. Click on 

Figure 5.17



6. Expand the spectrum from 4 ppm to 0.5 ppm
7. Click on 
8. Adjust the **0** order phase on the peak at 2.0 ppm to display a antiphase pattern

Figure 5.18

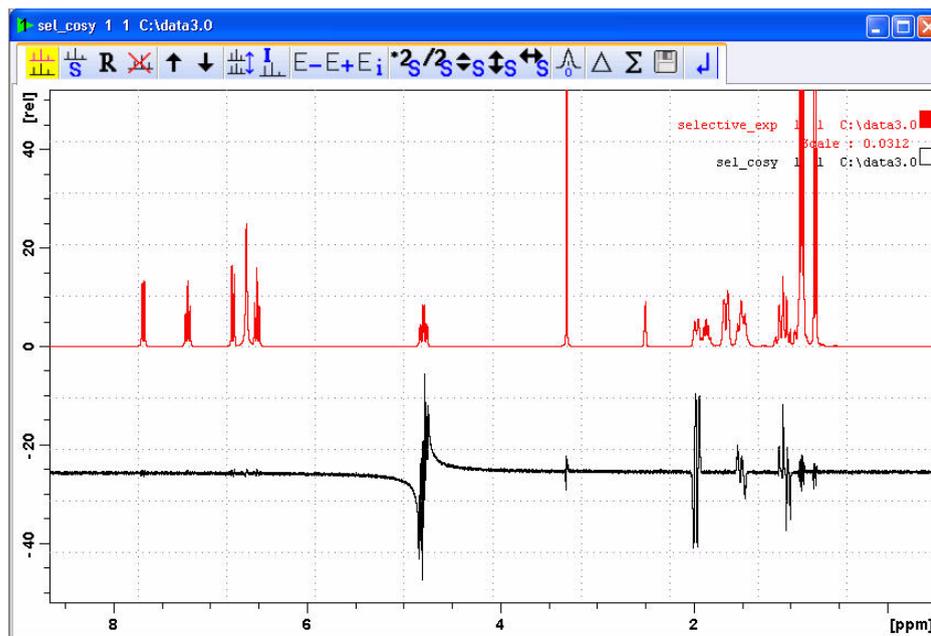


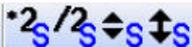
9. Click on  to store the phase value

5.3.7 Plotting two spectra on to the same page

1. Display the selective COSY spectrum
2. Click on  to enter the Multiple display option
3. Drag the Reference spectrum in to the spectral window

Figure 5.19



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

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

Figure 5.20



5. Click on the  button to print the active window

5.4 Observations

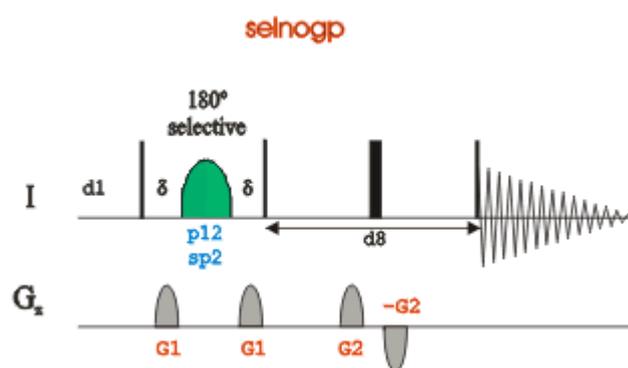
5.5 1-D Selective NOESY

5.5.1 Introduction

This experiment consist of three parts:

- **Selective excitation** of the selected resonance using the SPFG block.
- **Mixing period** consisting of the basic $90^0(1H)$ -delay- $90^0(1H)$ block in phase polarization transfer to other spins via NOE. Purging gradients are usually applied during the mixing period in order to remove any residual transverse magnetization.
- **Proton detection** as usual.

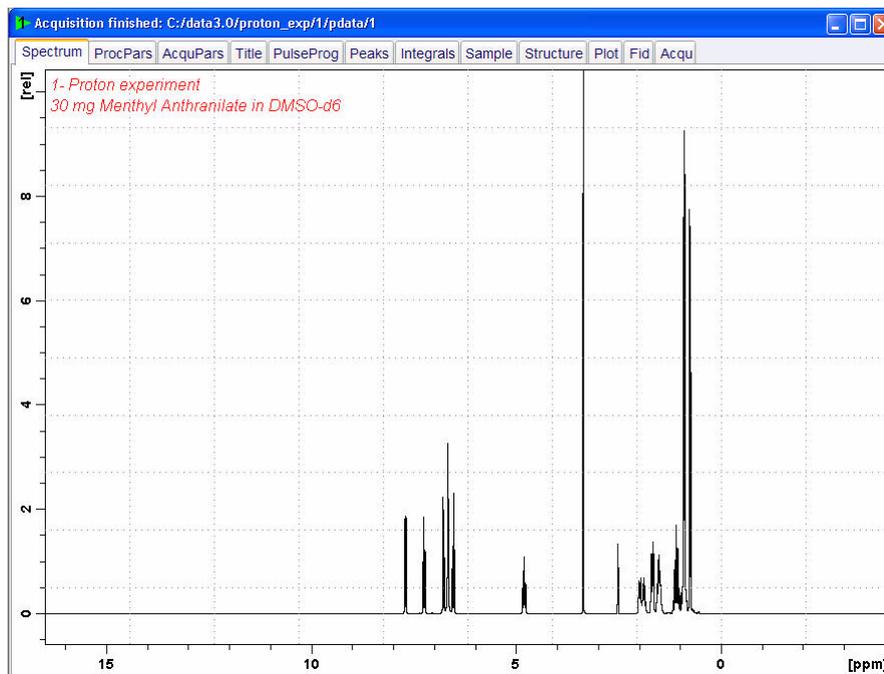
Figure 5.21



5.5.2 Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 5.22



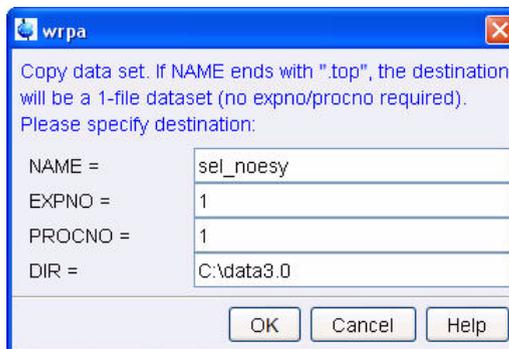
5.5.3 Selective excitation region set up

5.5.3.1 Off resonance

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

1. In the command line type **wrpa** and hit 'Enter'

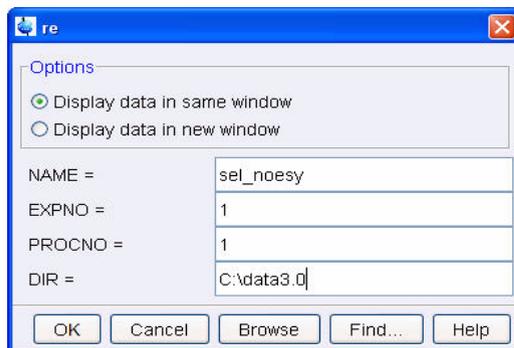
Figure 5.23



2. Change NAME = **sel_noesy**

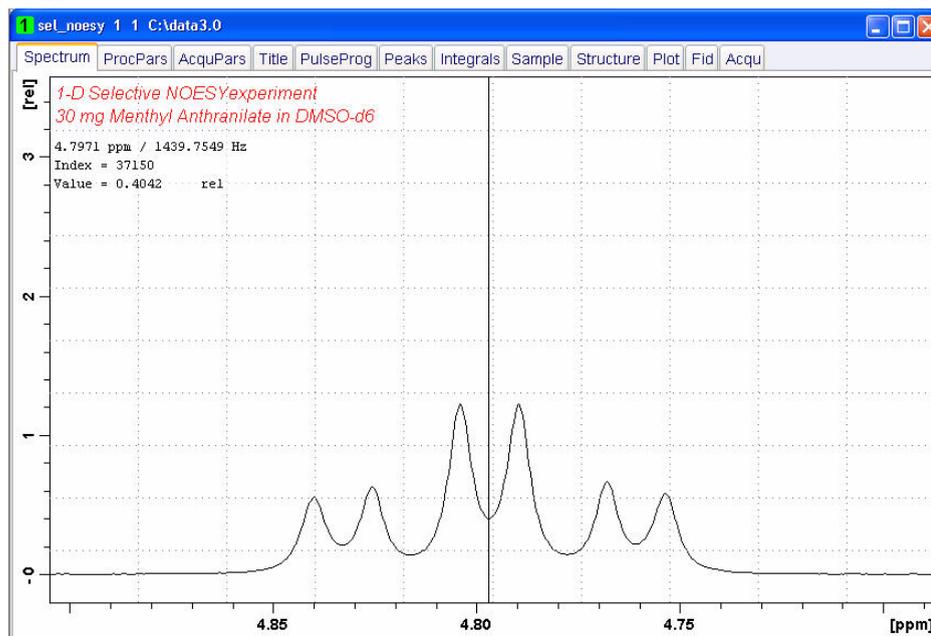
3. Click on
4. In the command line type **re** and hit 'Enter'

Figure 5.24



5. Change NAME = **sel_noesy**
6. Click on
7. Select the '**Spectrum**' tab by clicking on it
8. Expand peak at 4.8ppm

Figure 5.25

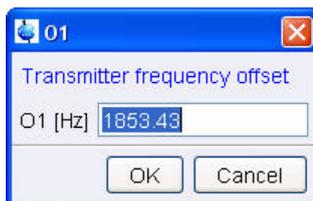


9. Move the cursor line to the center of the peak
10. Write down the cursor offset frequency value displayed in the upper left of the spectrum window (e.g. **1439.75**)

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

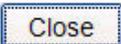
11. Type **O1**

Figure 5.26



12. Write down the current value (e.g. **1853.43**)

13. Calculate the difference of step 10 and 11 (e.g. **-413.68**)

14. Click on 

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

5.5.4 Setting up the Selective NOESY

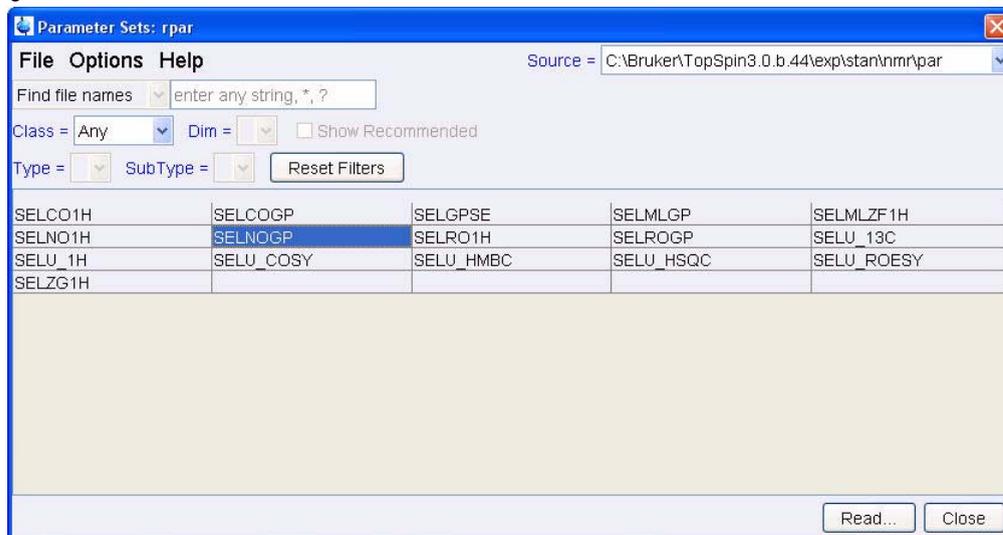
1. Click on the '**Start**' tab in the TopSpin Menu bar

Figure 5.27



2. Select  by clicking on it

Figure 5.28



NOTE: Enter **SEL*** in to the **'Find file names'** window and hit **'Enter'** to display all selective parameter sets shown in figure 5.28.

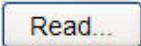
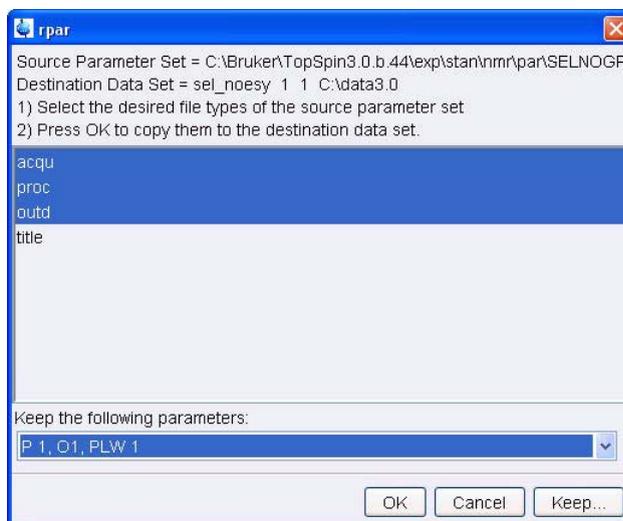
3. Select **'SELNOGP'**
4. Click on 
5. Select the **acqu**, **proc** and **outd** parameter options only
6. Click on the down arrow next to the **'Keep the following parameter'** window
7. Select **'P1, O1, PLW1'** from the pull down menu

Figure 5.29



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

10. Make the following changes:

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

11. Click on  to store the title

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

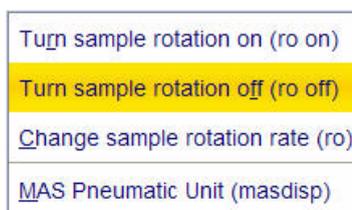
13. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 5.30



14. Select  by clicking on it

Figure 5.31



15. Select 'ro off' by clicking on it

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

16. Select  by clicking on it

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

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

18. Make the following changes:

PULPROG = selnogp

D8 = 0.450

DS = 8

NS = 64

SPNAM2 = Gaus1_180r.1000

SPOFF2 = value from 5.5.3, step 13 (e.g. -413.68)

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

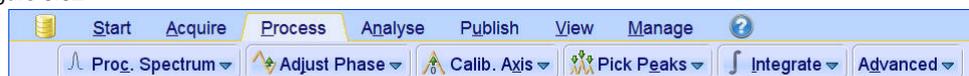
5.5.5 Acquisition

1. Select  by clicking on it

5.5.6 Processing

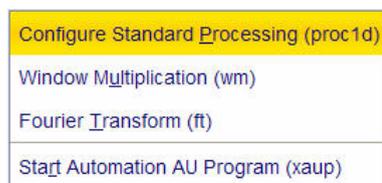
1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.32



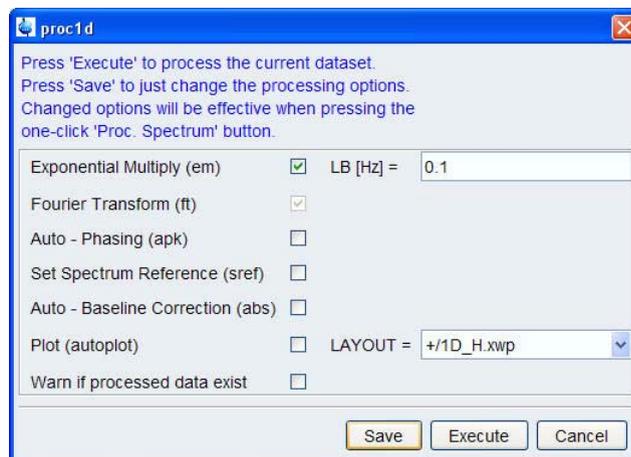
2. Click on the down arrow inside the  button

Figure 5.33



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

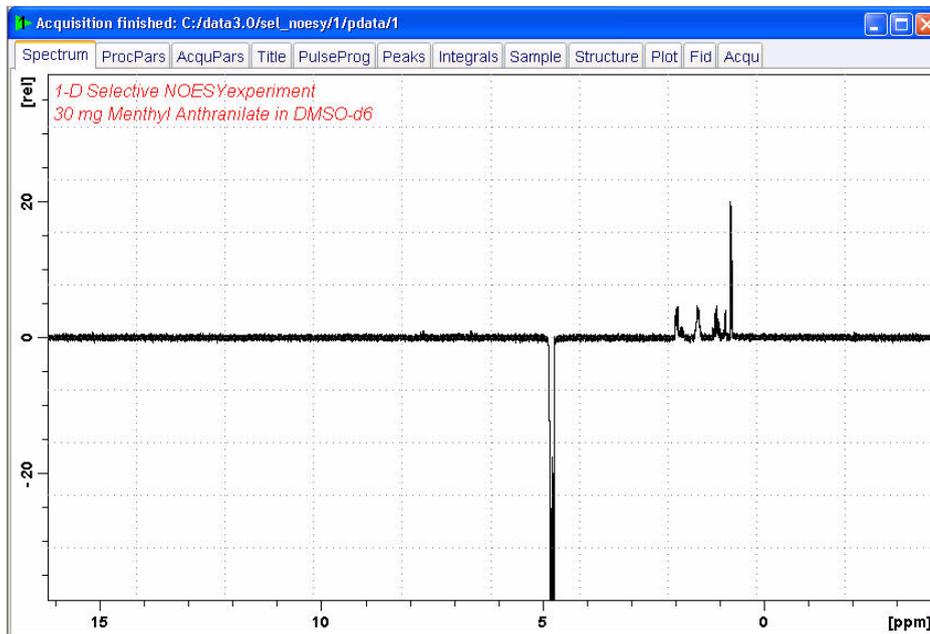


5. Click on 
6. Expand the spectrum from 4 ppm to 0.5 ppm

7. Click on  Adjust Phase

8. To assure the correct phasing of the NOE peaks, phase the signal at 4.8 ppm negative

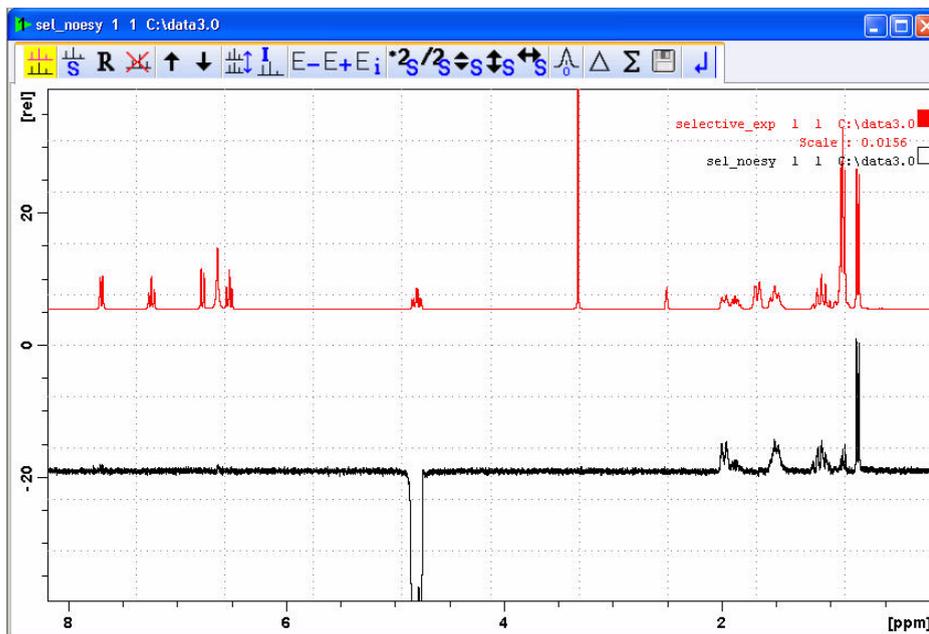
Figure 5.35



5.5.7 Plotting two spectra on to the same page

1. Display the selective NOESY spectrum
2. Click on  to enter the Multiple display option
3. Drag the Reference spectrum in to the spectral window

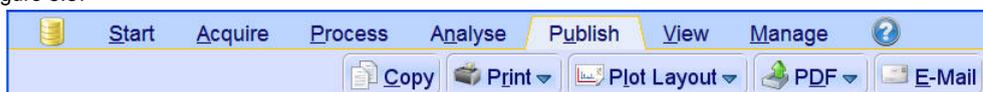
Figure 5.36



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

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

Figure 5.37



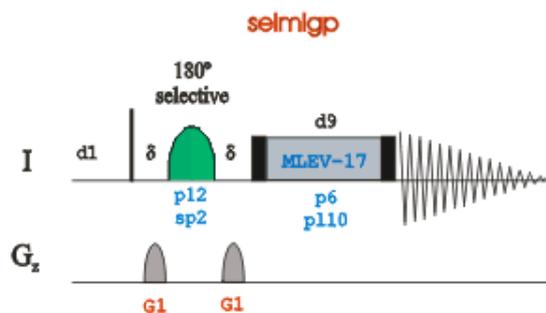
5. Click on the  button to print the active window

5.6 Observations

5.7 1-D selective gradient TOCSY

- This experiment consist of three parts:
- **Selective excitation** of the selected resonance using the SPFGGE block.
- **Mixing period** to achieve in phase polarization transfer to other spins. This is usually achieved by applying some isotropic mixing sequence like MLEV, WALTZ or DIPSI pulse trains. This in-phase transfer avoids possible cancellation when the coupling is poorly resolved.
- **Proton detection** as usual.

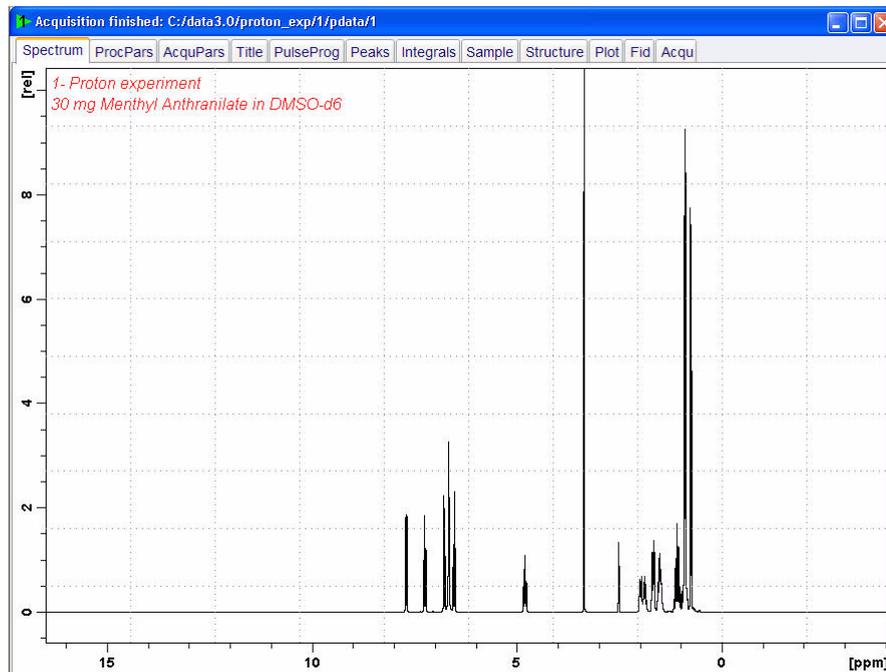
Figure 5.38



5.7.1 Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 5.39



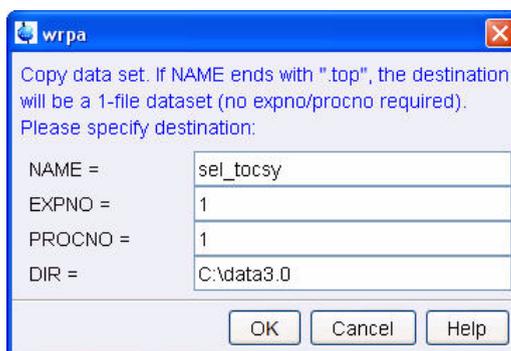
5.7.2 Selective excitation region set up

5.7.2.1 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

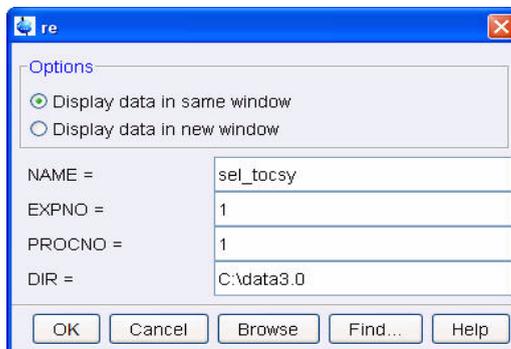
1. In the command line type **wrpa** and hit 'Enter'

Figure 5.40



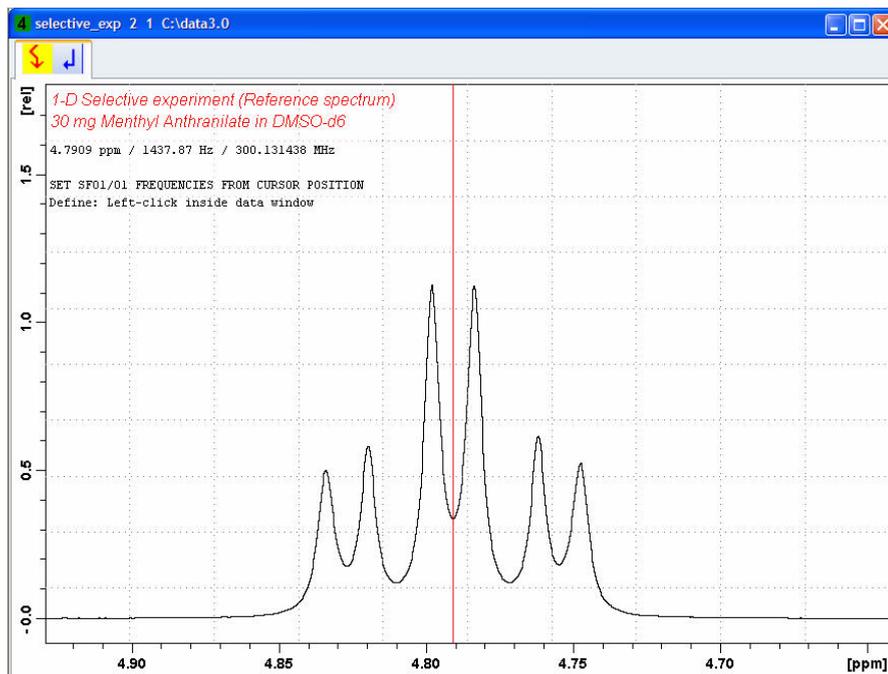
2. Change NAME = **sel_tocsy**
3. Click on 
4. In the command line type **re** and hit 'Enter'

Figure 5.41



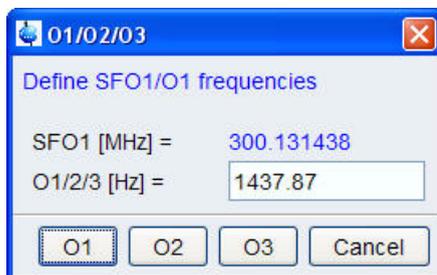
5. Change NAME = **sel_tocsy**
6. Click on 
7. Expand peak at 4.8ppm
8. Click on  to set the RF from cursor

Figure 5.42



9. Move the cursor line in to the center of the multiplet
10. Click the left mouse button to set the frequency

Figure 5.43



11. Click on

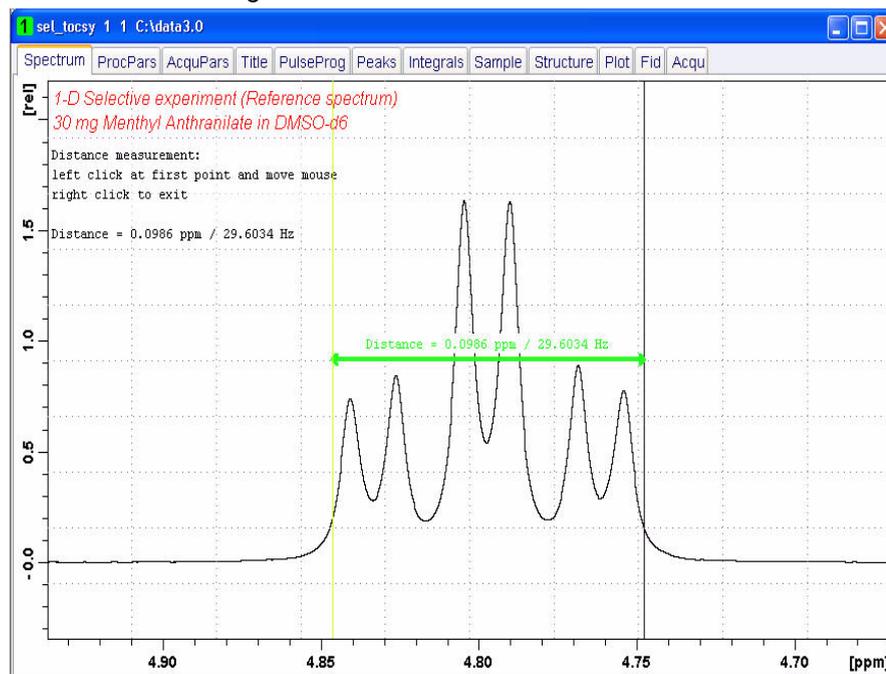
5.7.3 Calculating the selective pulse width and power level

NOTE: In this example the shaped pulse width and power level are determine using the 'Calculate Bandwidth' option in the shaped tool program. Other method of calculating the pulse width and power level can be used.

1. Click on  to start distance measurement
2. Position the cursor line at the left side of the peak, up 1/5 from the baseline
3. Click the left mouse button and drag the cursor line to the right side of the multiplet, up

1/5 from the baseline

Figure 5.1.



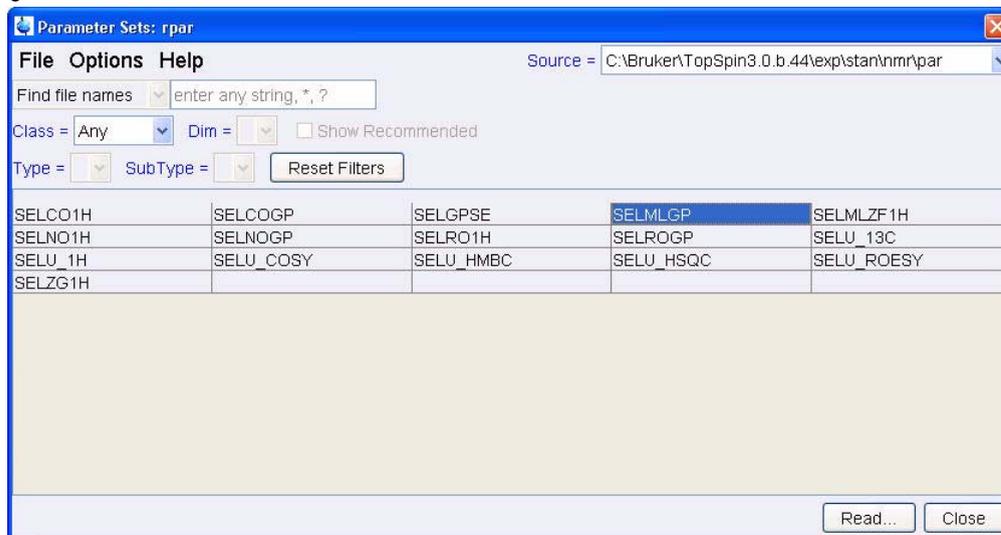
4. Write down the value in Hz for the distance between the two cursor lines (e.g. 29.6)
5. Click on the 'Start' tab in the TopSpin Menu bar

Figure 5.44



6. Select  **Read Pars.** by clicking on it

Figure 5.45



NOTE: Enter **SEL*** in to the 'Find file names' window and hit 'Enter' to display all selective parameter sets shown in figure 5.45.

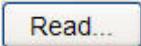
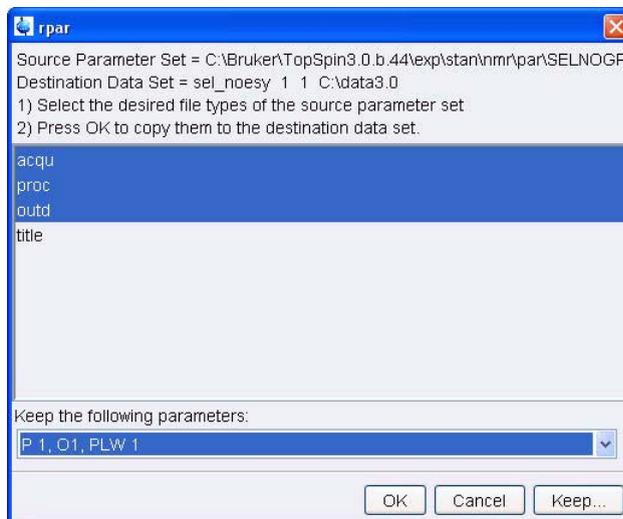
7. Select '**SELMLGP**'
8. Click on 
9. Select the **acqu**, **proc** and **outd** parameter options only
10. Click on the down arrow next to the '**Keep the following parameter**' window
11. Select '**P1, O1, PLW1**' from the pull down menu

Figure 5.46



12. Click on 
13. Select the '**Title**' tab by clicking on it

14. Make the following changes:

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

15. Click on  to store the title

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

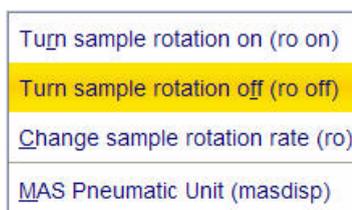
17. Click on the '**Acquire**' tab in the TopSpin menu bar

Figure 5.47



18. Select  by clicking on it

Figure 5.48



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

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

20. Select  by clicking on it

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

21. Click on the down arrow inside the  button

Figure 5.49



22. In the shape tool menu bar click on  and select '**Shape**' by clicking on it

Figure 5.50

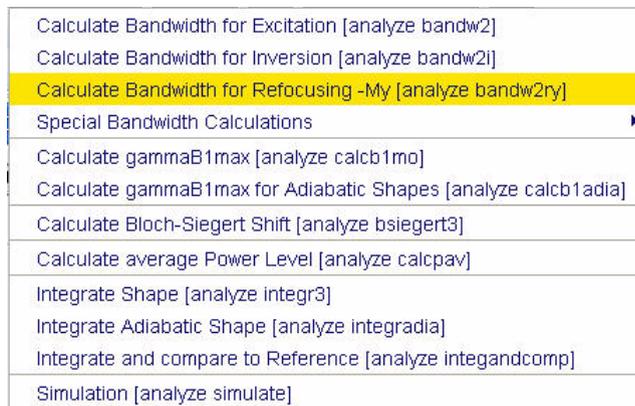


23. Select '**Gaus1_180r.1000**'

24. Click on 

25. In the main menu click on 

Figure 5.5112



26. Type the value from step 4 (e.g. **29.6**) in to the Calculator window 'Delta Omega [Hz]' and hit the Enter key

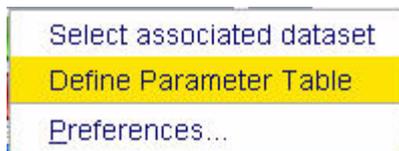
Figure 5.52



NOTE: The value for 'Delta T [usec]' is calculated after executing step 11.

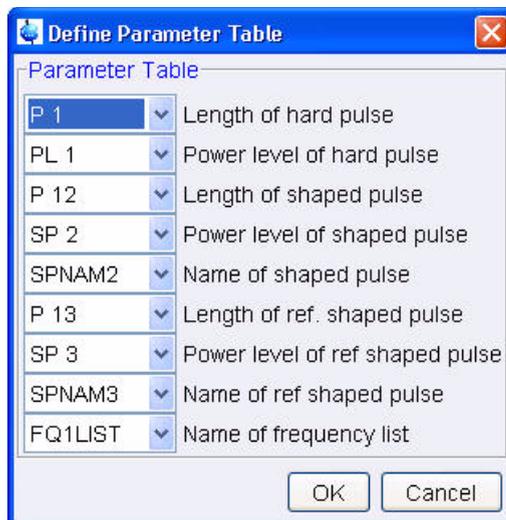
27. Click on the down arrow inside the  button

Figure 5.53



28. Select '**Define Parameter Table**' by clicking on it

Figure 5.54



29. Make the followin

Length of shaped pulse = p12

Power Level of shaped pulse = SP2

Name of shaped pulse = SPNAM2

30. Click on Click on 

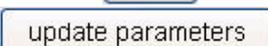
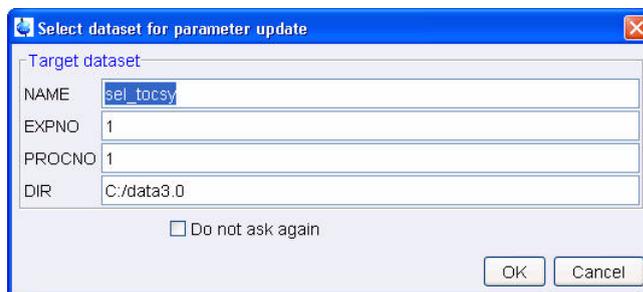
31. Click on 

Figure 5.55



NOTE: The Target data set window above is to verify the correct data set and can be switched off by enable the 'Do not ask again' option.

32. Click on 

33. Use the **Ctrl/w** keys to close the Shape Tool window

5.7.4 Setting up the acquisition parameters

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

2. Make the following changes:

NS = 64

DS = 8

D9 = 0.080

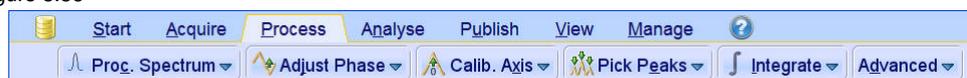
5.7.5 Acquisition

1. Select  by clicking on it

5.7.6 Processing

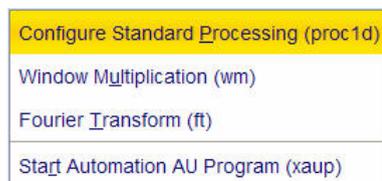
1. Click on the '**Process**' tab in the TopSpin Menu bar

Figure 5.56



2. Click on the down arrow inside the  button

Figure 5.57



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

4. Deselect the following options:

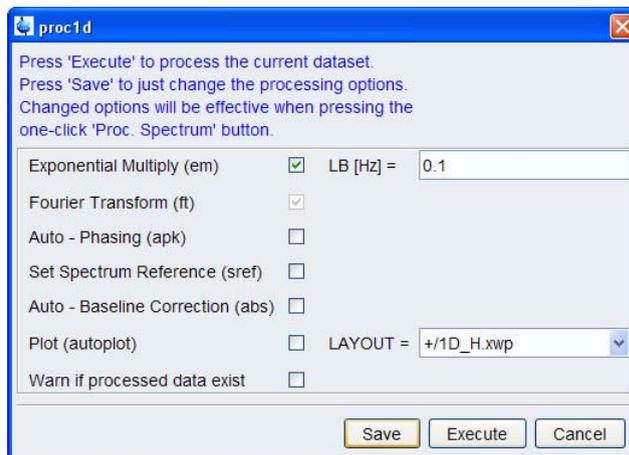
'**Auto-Phasing (apk)**'

'**Set Spectrum Reference (sref)**'

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

'**Warn if Processed data exist**'

Figure 5.58



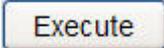
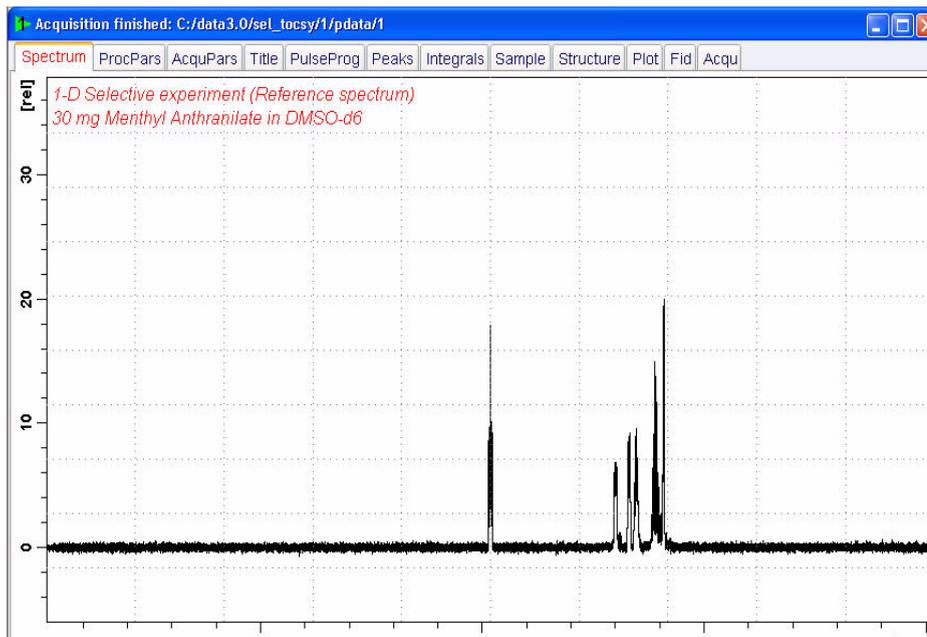
5. Click on 
6. Expand the spectrum from 4 ppm to 0.5 ppm
7. Click on 
8. All peaks should be phased for positive absorption

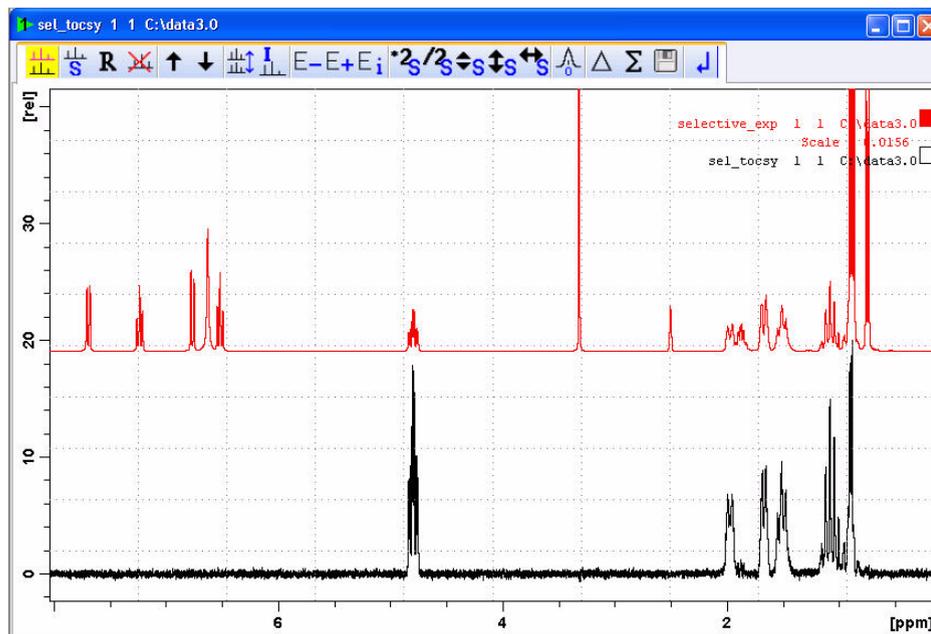
Figure 5.59



5.7.7 Plotting two spectra on to the same page

1. Display the selective TOCSY spectrum
2. Click on  to enter the Multiple display option
3. Drag the Reference spectrum in to the spectral window

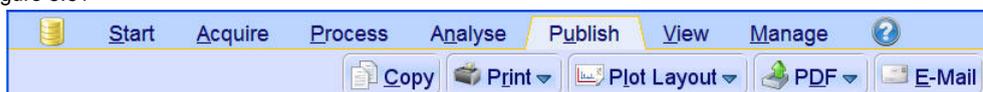
Figure 5.60



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

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

Figure 5.61

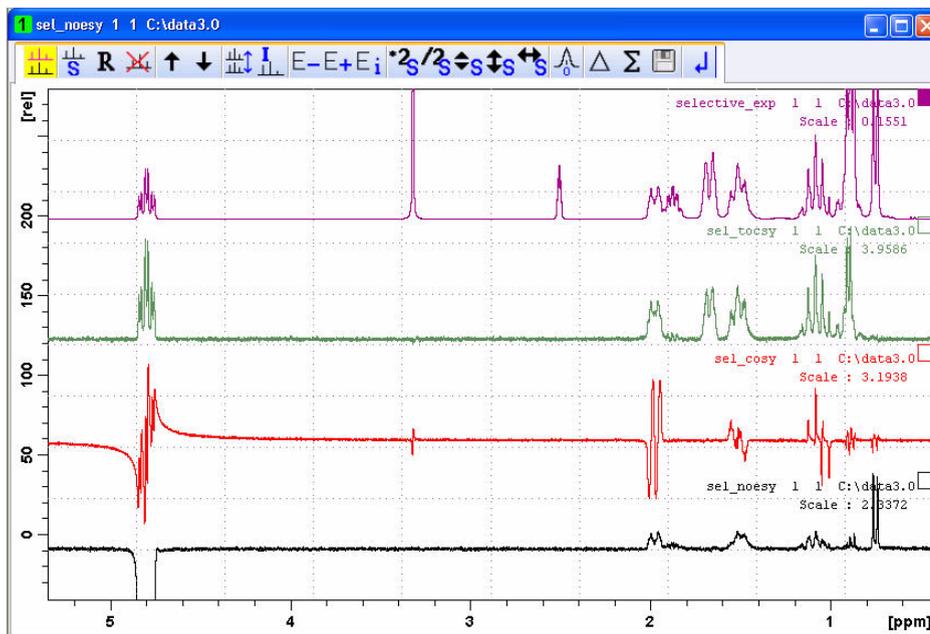


5. Click on the  button to print the active window

5.7.8 Plotting all 4 experiment on to the same page

1. Display the selective NOESY spectrum
2. Click on  to enter the Multiple display option
3. Drag the selective COSY spectrum in to the spectral window
4. Drag the selective TOCSY spectrum in to the spectral window
5. Drag the Reference spectrum in to the spectral window

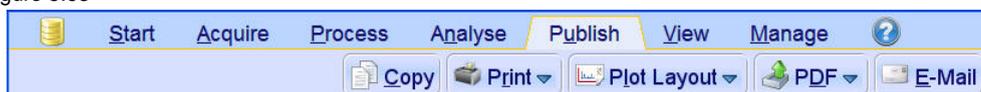
Figure 5.62



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

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

Figure 5.63



7. Click on the  **Print** button to print the active window

5.8 Observations

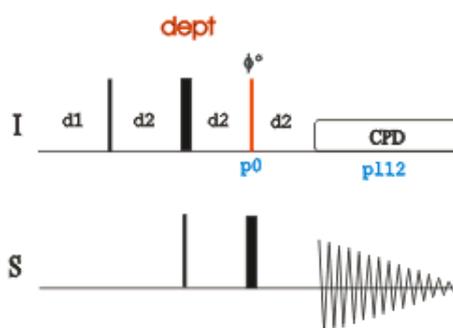
5.9 1-D Carbon DEPT experiment using a shaped ^{13}C pulse

5.9.1 Introduction

The basic DEPT pulse sequence consists of the following steps:

- **Relaxation period** (d_1) to achieve a pre-equilibrium state.
- **90° ^1H pulse** (p_1) to create transverse ^1H magnetization (I_y).
- An **evolution delay** optimized to $1/2J(\text{XH})$ to achieve antiphase proton magnetization (I_xS_z).
- **Simultaneous 180° ^1H and 90° ^1H pulses.** The proton pulse will allow to refocus ^1H chemical shift evolution while the carbon pulse creates multiple quantum coherence.
- During a **second delay** (also optimized to $1/2J(\text{XH})$) heteronuclear coupling is not evolving.
- **Simultaneous Y° ^1H and 90° ^1H pulses.** The carbon pulse refocus ^{13}C chemical shift evolution while the Y proton pulse creates a different functional dependence as a function of carbon multiplicity:
 - $\text{CH } 2I_z\text{Sysin}(Y)$
 - $\text{CH}_2 \ 4I_zI'_z\text{Sysin}(Y)\cos(Y)$
 - $\text{CH}_3 \ 8I_zI'_zI''_z\text{Sysin}(Y)\cos^2(Y)$
- A **final evolution delay** (also optimized to $1/2J(\text{XH})$) to achieve in-phase ^{13}C magnetization.
- **^{13}C acquisition** is performed under broadband proton decoupling.

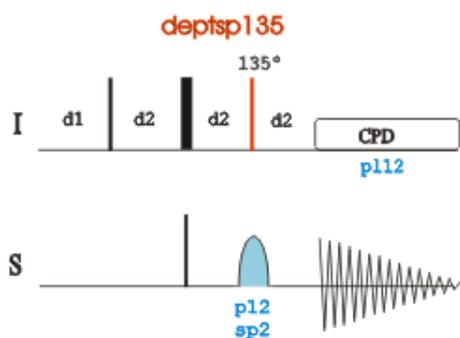
Figure 5.64



The 90°X pulse can be replaced with a *adiabatic shaped pulse* to achieve better phasing over the whole spectrum range. This is specially useful on higher field instruments where the phasing of a normal DEPT spectrum can be a problem.

Figure 5.65 below shows the selective pulse DEPT-135 sequence using a proton ^{135}O pulse

Figure 5.65



5.9.2 Experiment setup

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

Figure 5.66



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

Figure 5.67

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

NAME	deptsp
EXPNO	1
PROCNO	1
DIR	C:\data3.0
Solvent	DMSO
Experiment Dirs.	C:\Bruker\TopSpin3.0.b.44\exp\star\nmr\par
Experiment	C13DEPT135
TITLE	1-D Selective 13C DEPT135 experiment 30 mg Menthyl Anthranilate in DMSO-d6

Show new dataset in new window

1 Receivers (1,2, ...16)

OK Cancel More Info... Help

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 5.67 above. Click on the down arrow button to browse for a specific directory.

4. Click on 
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 5.68



6. Select  by clicking on it

Figure 5.69



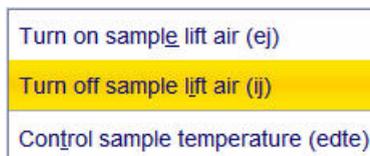
7. Select 'ej' by clicking on it

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 5.70

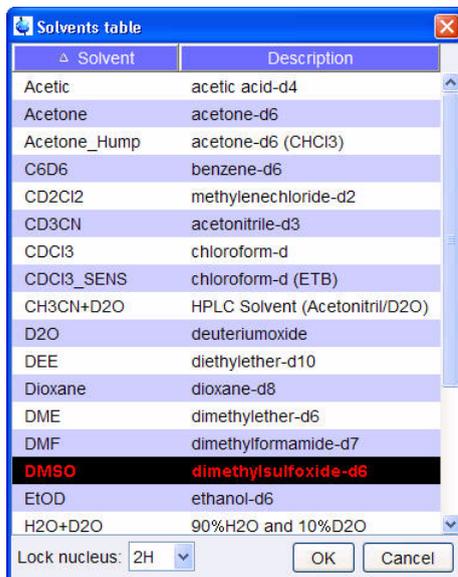


10. Select 'ij' by clicking on it

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 5.71



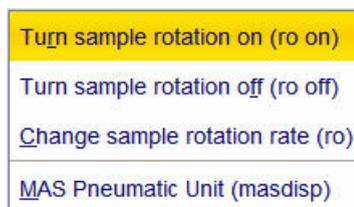
12. Select '**DMSO**' by clicking on it

13. Select  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  by clicking on it

Figure 5.72



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  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  by clicking on it

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

5.9.3 Acquisition

1. Select  by clicking on it

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

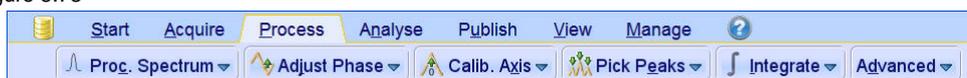
2. Select  by clicking on it

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

5.9.4 Processing

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

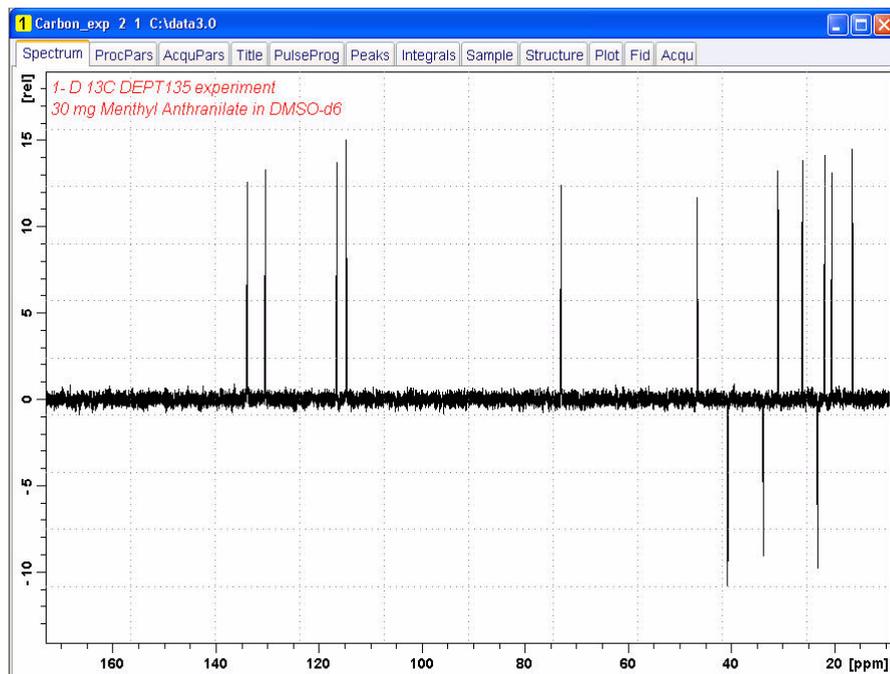
Figure 5.73



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. 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 5.74



5.10 Observations



6 2-D experiments using shaped pulses

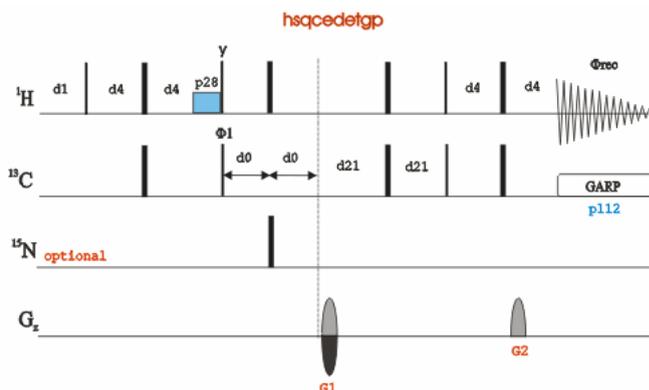
6.1 2-D edited HSQC experiment with Adiabatic pulses

6.1.1 Introduction

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

Figure 5.1 shows the regular edited HSQC sequence and Figure 6.1 the edited HSQC sequence using shaped pulses for all 180° pulses on f2-channel with gradients in back-inept

Figure 6.1



hsqcetgpsisp: shaped pulses for inversion on f2

hsqcetgpsisp.2: shaped pulses for inversion and refocusing on f2

hsqcetgpsisp2: shaped pulses for inversion on f2, gradients in back INEPT

hsqcetgpsisp2.2: shaped pulses for inversion and refocusing on f2 gradients in back INEPT

hsqcetgpsisp

hsqcetgpsisp.2

hsqcetgpsisp2

hsqcetgpsisp2.2

hsqcetgpsp: shaped pulses for inversion on f2

hsqcetgppsp.2: shaped pulses for inversion and refocusing on f2

hsqcetgppsp.3: shaped pulses for inversion and refocusing on f2, for ^{13}C -labeled molecules

hsqcdiedetgppsisp.1: shaped pulses for all 180° pulses on f2, multiplicity editing during selection,

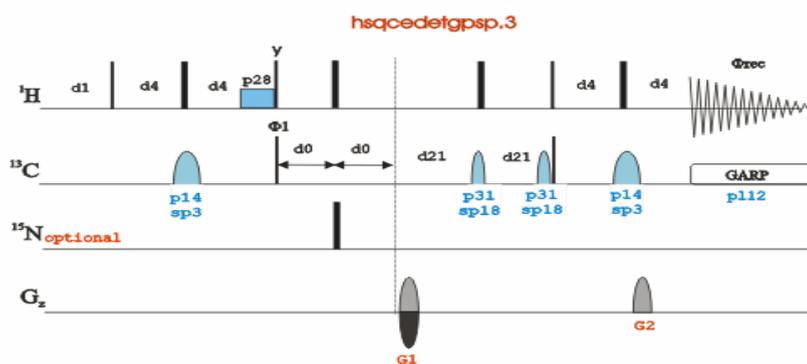
hsqcdiedetgppsisp.2: shaped pulses for all 180° pulses on f2, inversion of directly coupled protons

hsqcdiedetgppsisp.3: shaped pulses for all 180° pulses on f2, multiplicity editing during selection,

6.1.2 2-D edited HSQC experiment using adiabatic pulses

For improvement of the phasing the pulse sequence using matched sweep adiabatic pulses, Figure 6.2. is used in this chapter. If desired the sequence **hsqcedetgppsisp2.4** can be used to suppress the COSY peaks.

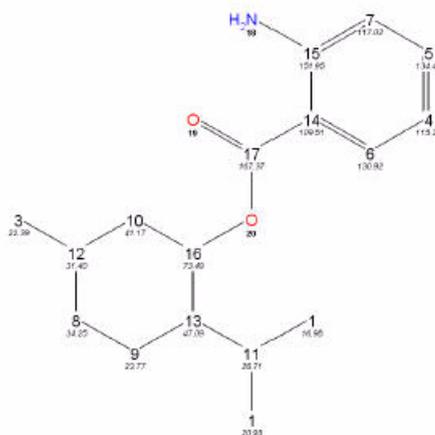
Figure 6.2



6.1.3 Sample

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

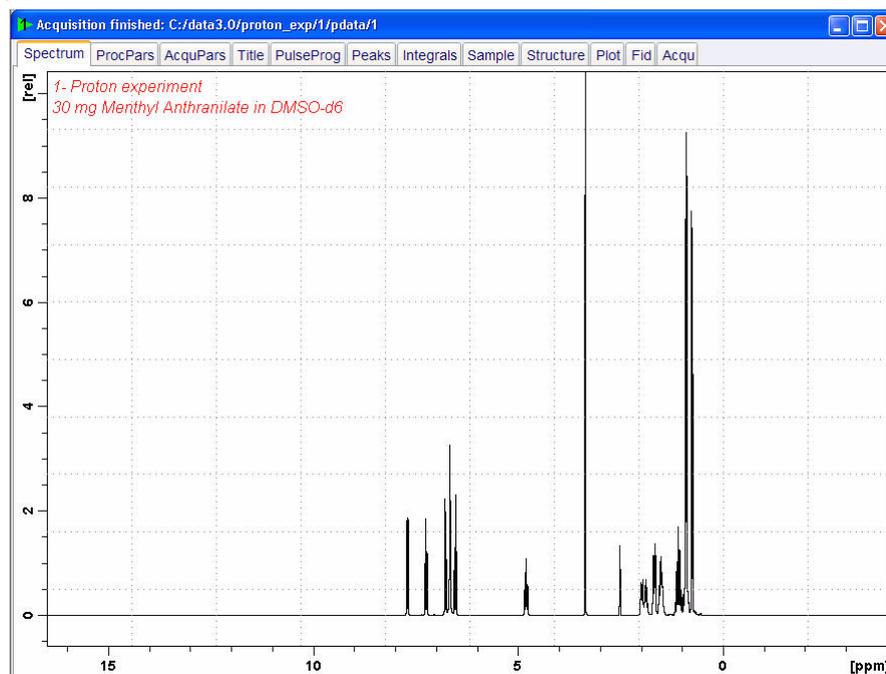
Figure 6.3



6.1.4 Reference spectrum

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 6.4



NOTE: The reference spectrum is necessary to adjust the spectral limits of the sweep width in the **F2** dimension and to use it for the projection. The **HSQCEDETGSP.3** parameter set has a default sweep width in the **F1** dimension of 165ppm, If a carbon DEPT135 or DEPT45 spectrum of the same sample is available, the F1 sweep width can

be limited using the 'setlimit' AU-program.

6.1.5 Setting up the HSQC experiment

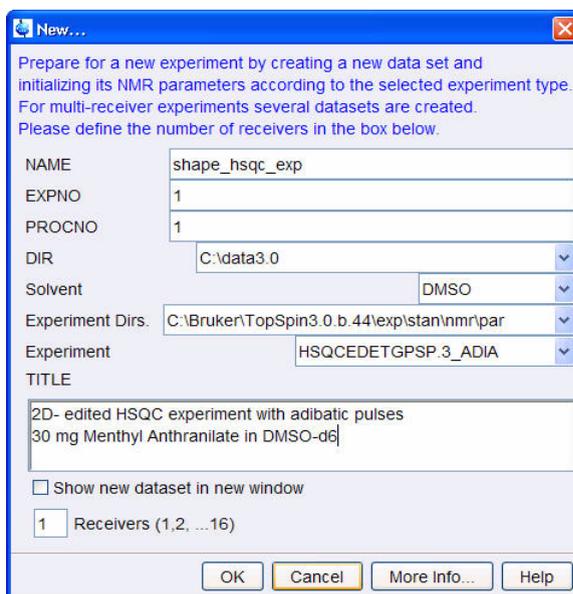
1. Click on the 'Start' tab in the TopSpin Menu bar

Figure 6.5



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

Figure 6.6

The image shows the 'New...' dialog box in TopSpin. It contains the following fields and values:

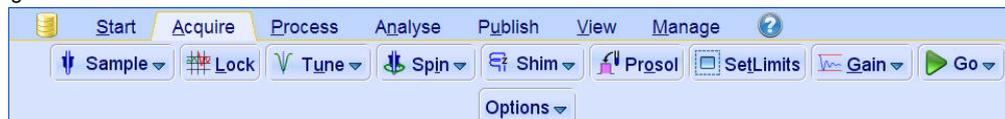
- NAME: shape_hsqc_exp
- EXPNO: 1
- PROCNO: 1
- DIR: C:\data3.0
- Solvent: DMSO
- Experiment Dirs.: C:\Bruker\TopSpin3.0.b.44\exp\stan\nmr\par
- Experiment: HSQCEDETGSP.3_ADIA
- TITLE: 2D- edited HSQC experiment with adiabatic pulses
30 mg Menthyl Anthranilate in DMSO-d6
- Show new dataset in new window
- 1 Receivers (1,2, ...16)

Buttons at the bottom include 'OK', 'Cancel', 'More Info...', and 'Help'.

NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 6.6 above. Click on the down arrow button to browse for a specific directory.

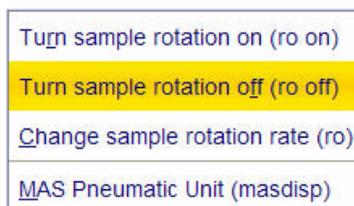
4. Click on 
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 6.7



6. Select  by clicking on it

Figure 6.8



7. Select 'ro off' by clicking on it

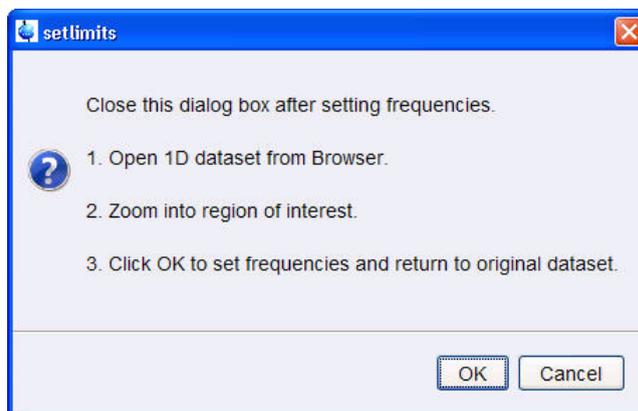
NOTE: 2-D experiments should be run non spinning

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 6.9

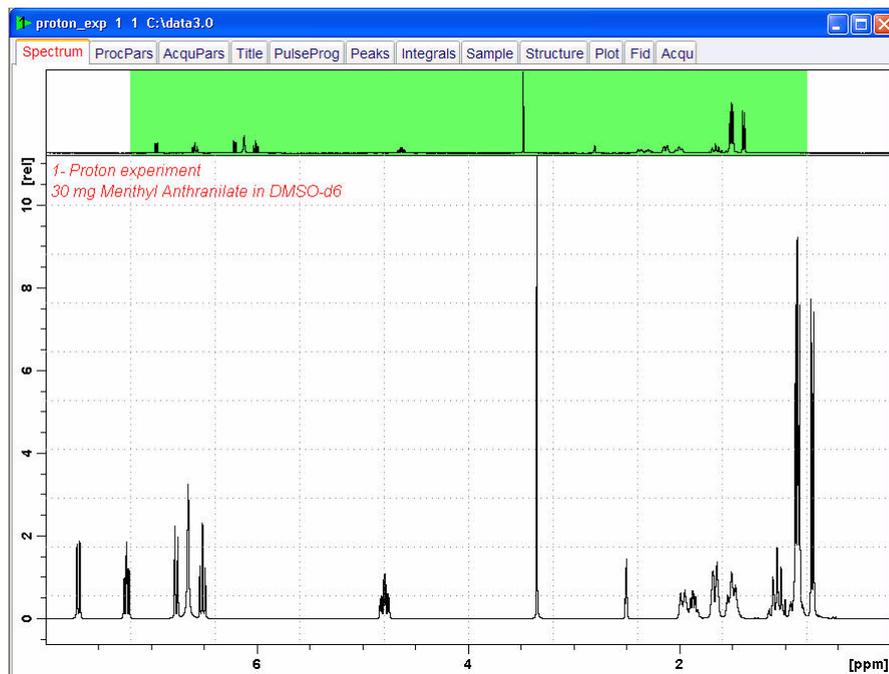


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

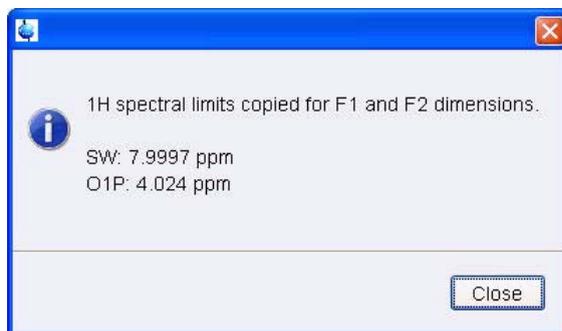
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

Figure 6.10



12. Click on to assign the new limit

Figure 6.11



13. Click on

NOTE: The display changes back to the 2D data set.

6.1.6 Acquisition

1. Select by clicking on it

2. Select by clicking on it

6.1.7 Processing

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

Figure 6.12

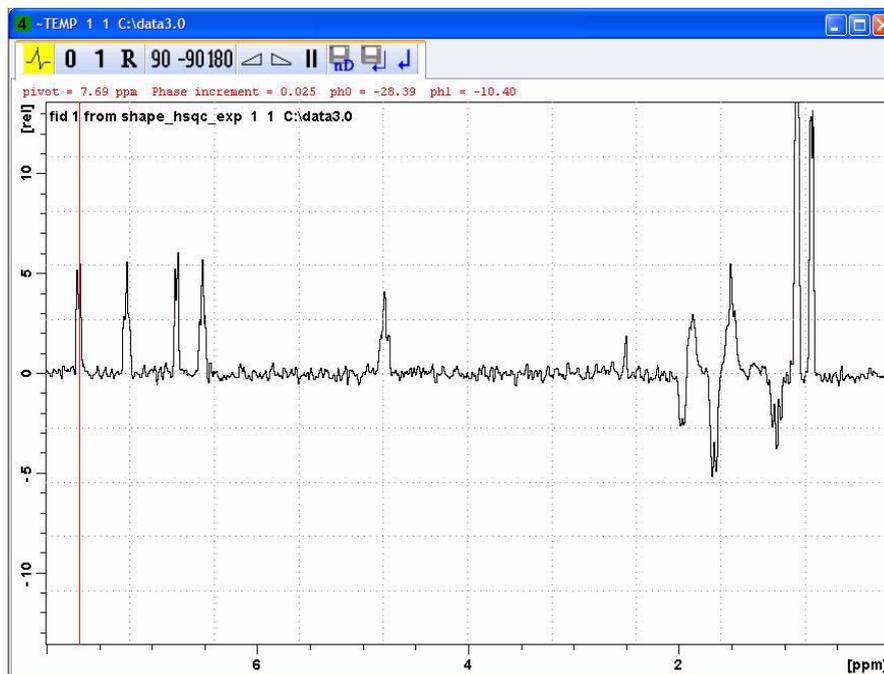


NOTE: The steps below will guide you through a manually phase correct a phase sensitive 2-D spectrum.

2. In the command line type **rser 1** (read in the first increment)
3. In the command line type **qsin** (executing the window function)
4. In the command line type **ft**
5. Click on 
6. Adjust the phase manually

NOTE: The spectrum will have positive and negative peaks showing the CH and CH3 as positive where the CH2 will be negative. To assure the right phase, correct the Aromatic peaks (7 - 9 ppm) positive.

Figure 6.13



7. Click on  to store the 2-D phase values
8. Click on 

NOTE: The spectrum will go back to the unphased view since the phase correction values were stored only for the 2-D spectrum.

9. Click on  (going back to the 2-D spectrum display)

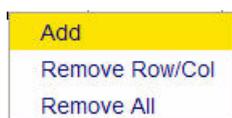
10. Type **xfb** (fourier transform the 2-D spectrum)

11. Click on 

12. Select the peak at **7.7ppm/130.9ppm**

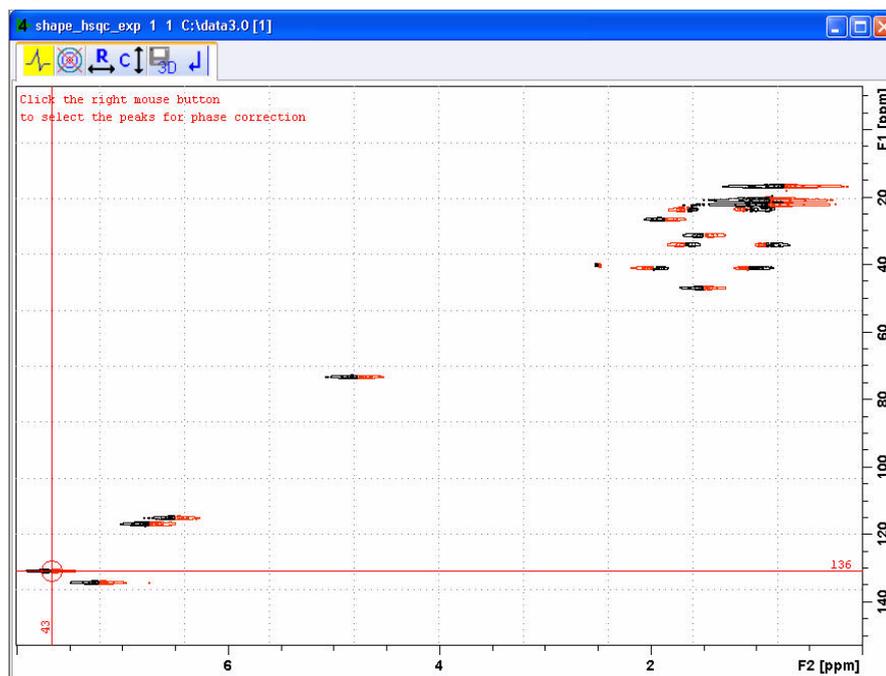
13. Click the left mouse button

Figure 6.14



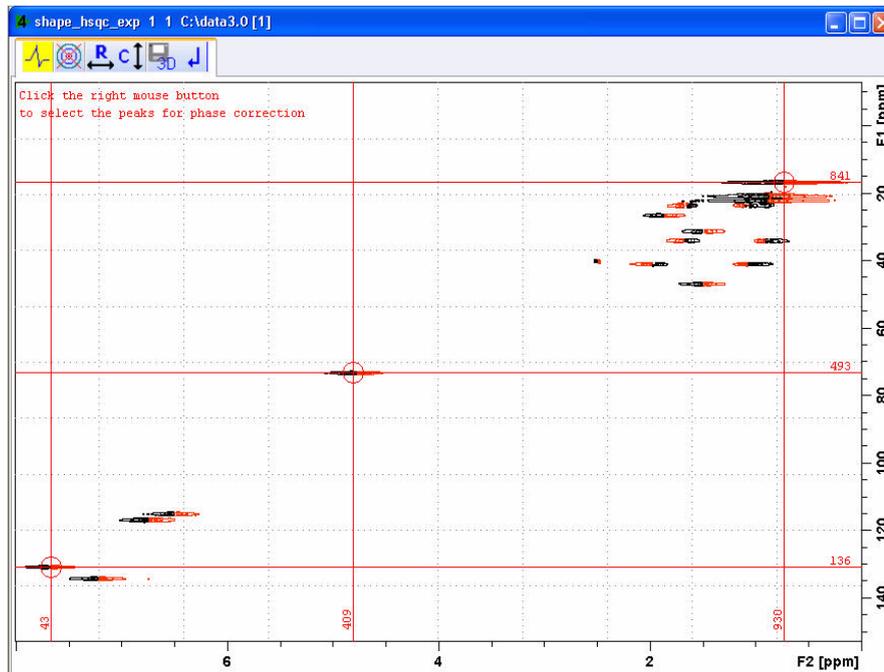
14. Select '**Add**'

Figure 6.15



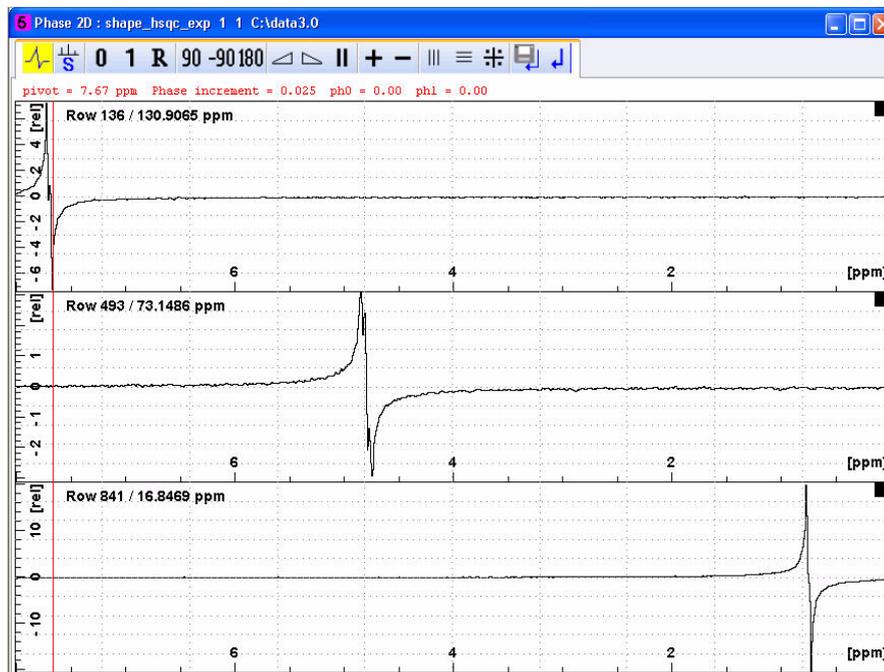
15. Repeat steps 13 and 14 for the peaks at **4.8ppm / 73.2ppm** and **0.76ppm / 16.8ppm**

Figure 6.16



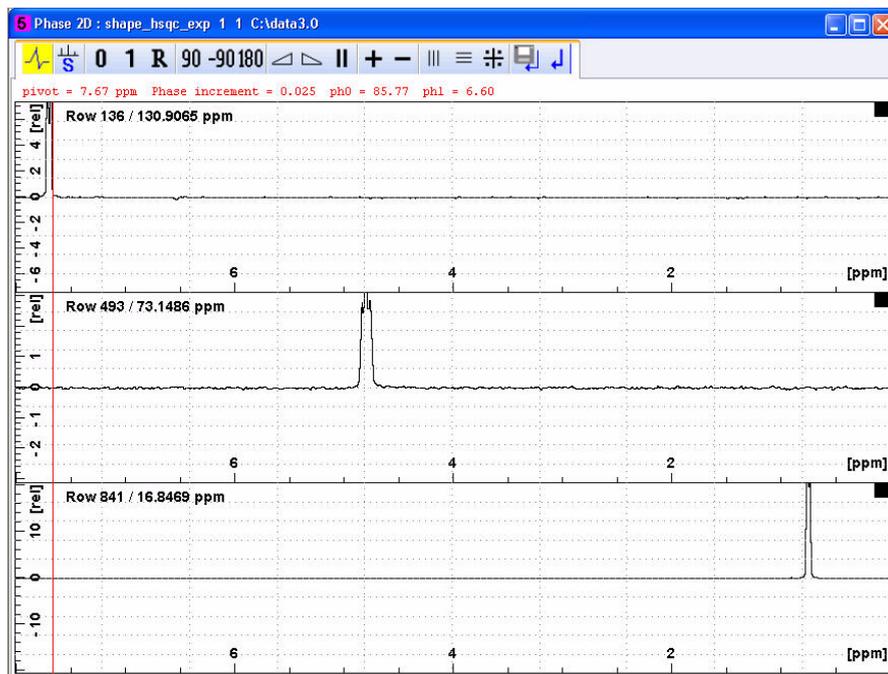
16. Click on 

Figure 6.17



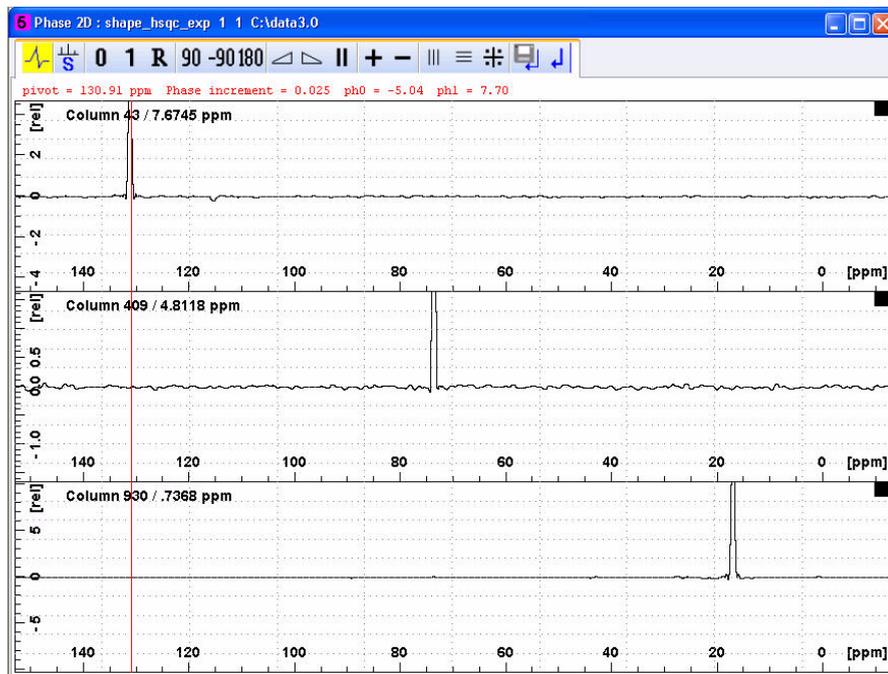
17. Adjust the phase using the  and  buttons

Figure 6.18



- 18. Click on 
- 19. Click on 

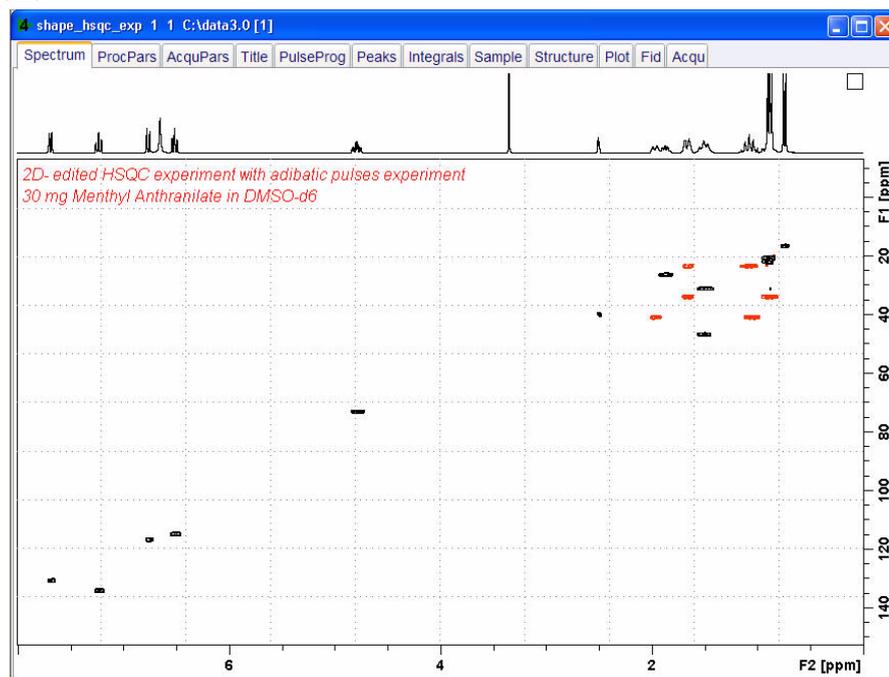
Figure 6.19



- 20 Adjust the phase if necessary using the **0** and **1** buttons
- 21. Click on 

22. Click on 

Figure 6.20



6.2 Observations

6.3 2-D Selective HMBC experiment

6.3.1 Introduction

The **Semi-selective 2D HMBC experiment** is a simple modification of the 2-D HMBC pulse sequence (Figure 6.21) in which one of the two carbon 90 degree pulses is applied selectively on a specified region (Figure 6.22). The main purpose is to achieve better resolution in the indirect dimension and therefore is recommended when high overlapped carbon spectra precludes an easy resonance assignment.

Figure 6.21

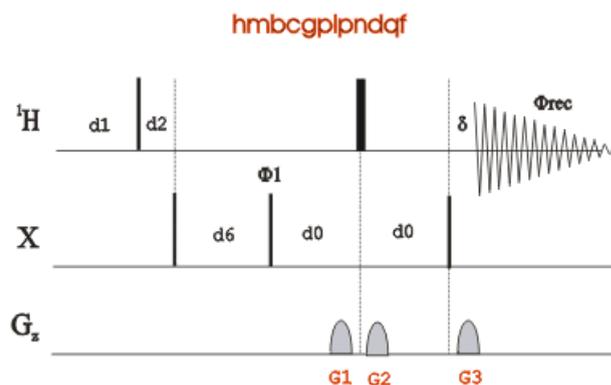
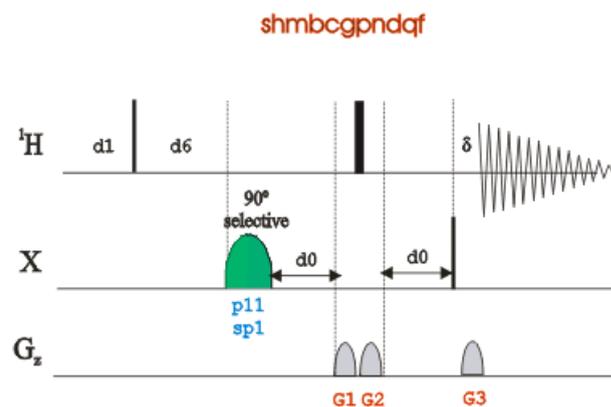


Figure 6.22



6.3.2 Sample

50mM Gramicidin-S in DMSO-d6

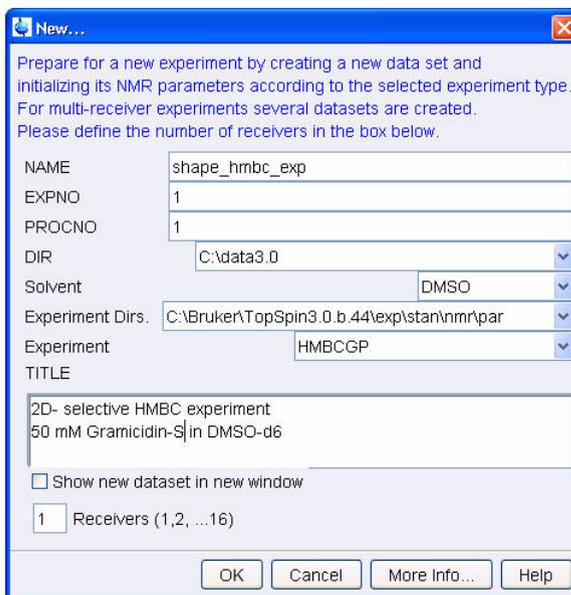
1. Click on the **'Start'** tab in the TopSpin Menu bar

Figure 6.25



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

Figure 6.26



NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 6.26 above. Click on the down arrow button to browse for a specific directory.

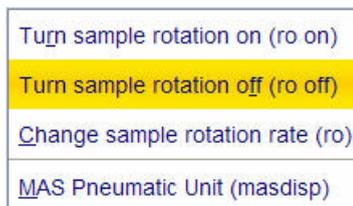
4. Click on **OK**
5. Click on the **'Acquire'** tab in the TopSpin menu bar

Figure 6.27



6. Select **Spin** by clicking on it

Figure 6.28



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

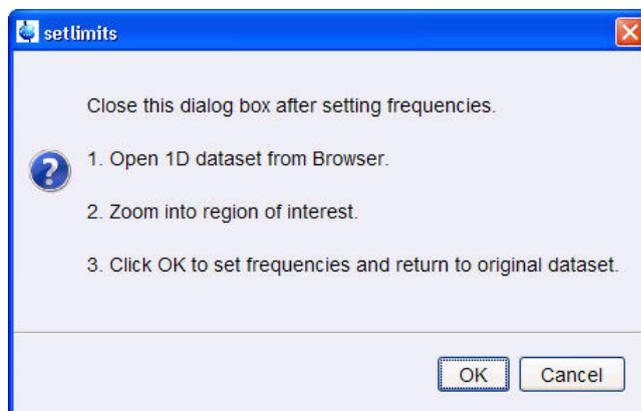
NOTE: 2-D experiments should be run non spinning

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 6.29

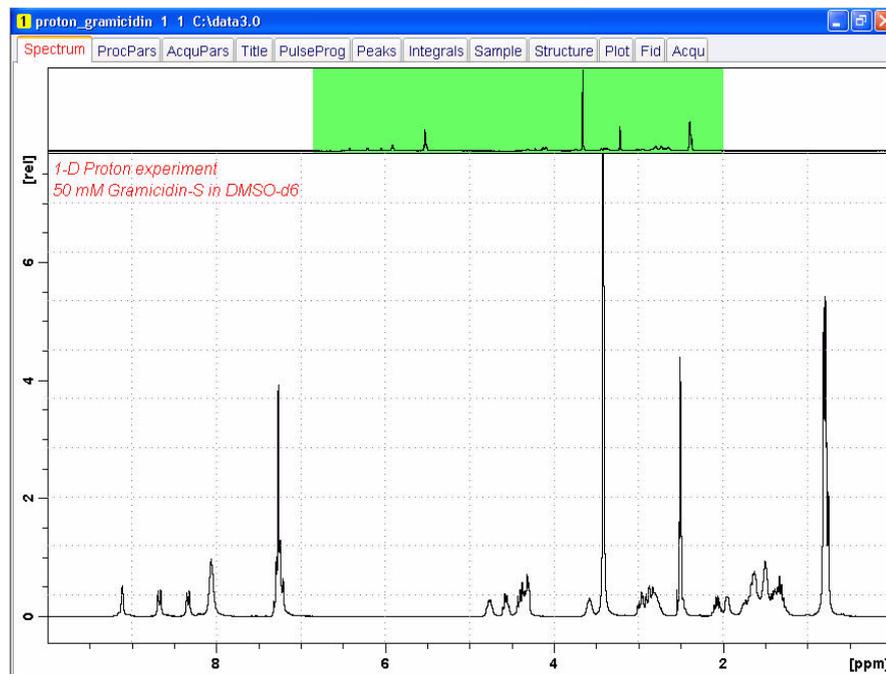


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_gramicidin) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

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

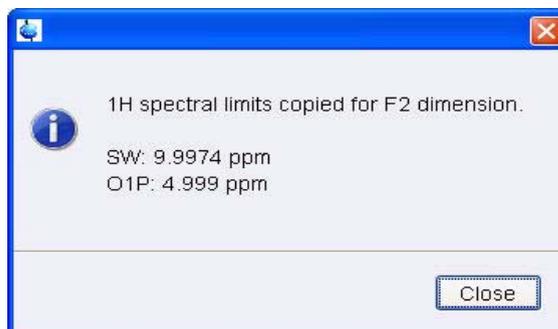
NOTE: The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak is folding in F1.

Figure 6.30



12. Click on to assign the new limit

Figure 6.31



13. Click on

NOTE: The display changes back to the 2D data set.

6.3.4 Acquisition

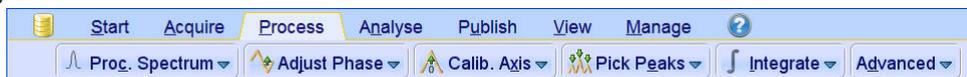
1. Select by clicking on it

2. Select by clicking on it

6.3.5 Processing

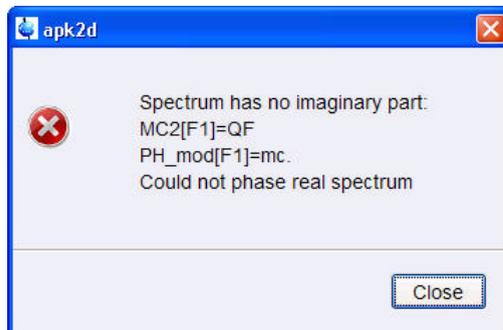
1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 6.32



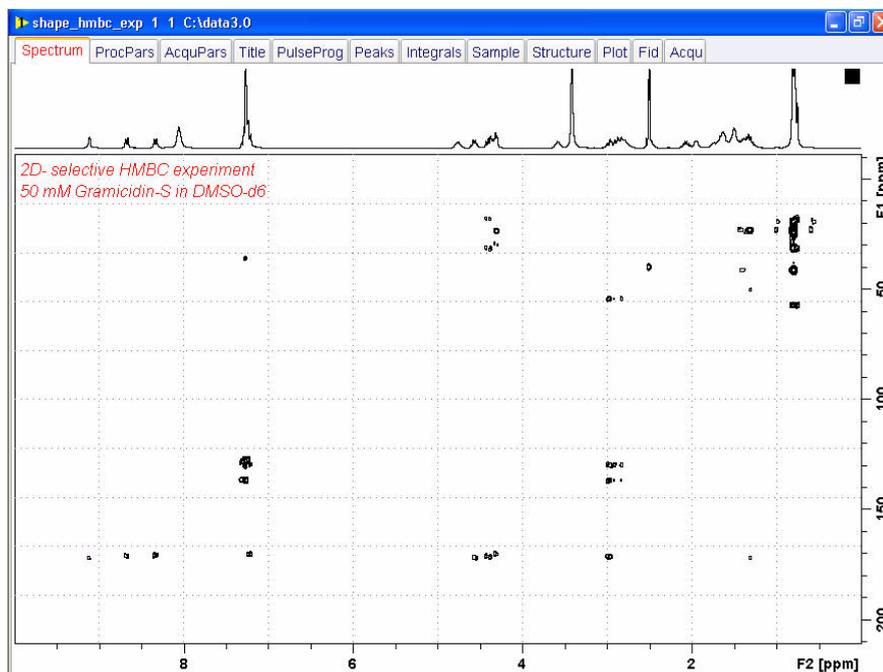
2. Select  by clicking on it

Figure 6.33



NOTE: This executes a standard processing program **proc2**. The message shown in Figure 6.33 pops up in case of a magnitude 2D experiment and the **apk2d** option is enabled. To configure the processing program follow the steps below. To avoid the message shown in Figure 6.33 the option '**Auto-Phasing (apk2d)**' may be disabled for magnitude like 2D experiment.

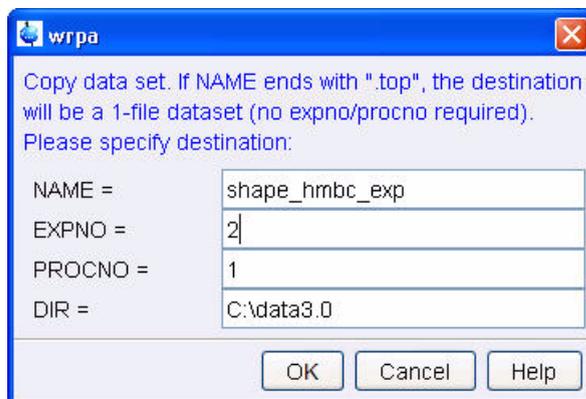
Figure 6.34



6.3.6 Optimizing the parameters on the carbonyl region

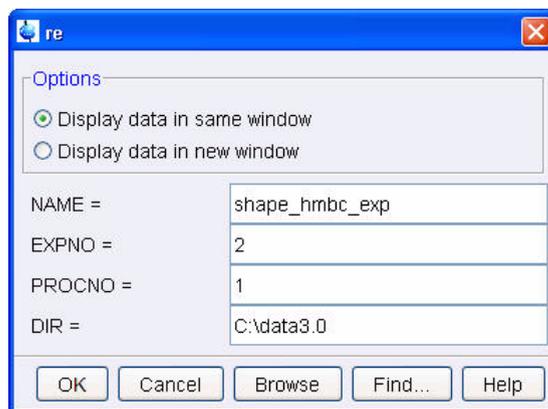
1. Type **wrpa** on the command line

Figure 6.35



2. Change the **EXPNO** to **2**
3. Type **re** on the command line

Figure 6.36



4. Change the **EXPNO** to **2**
5. Expand the carbonyl region including all cross peaks (e.g. 168 ppm to 178 ppm)

Figure 6.37

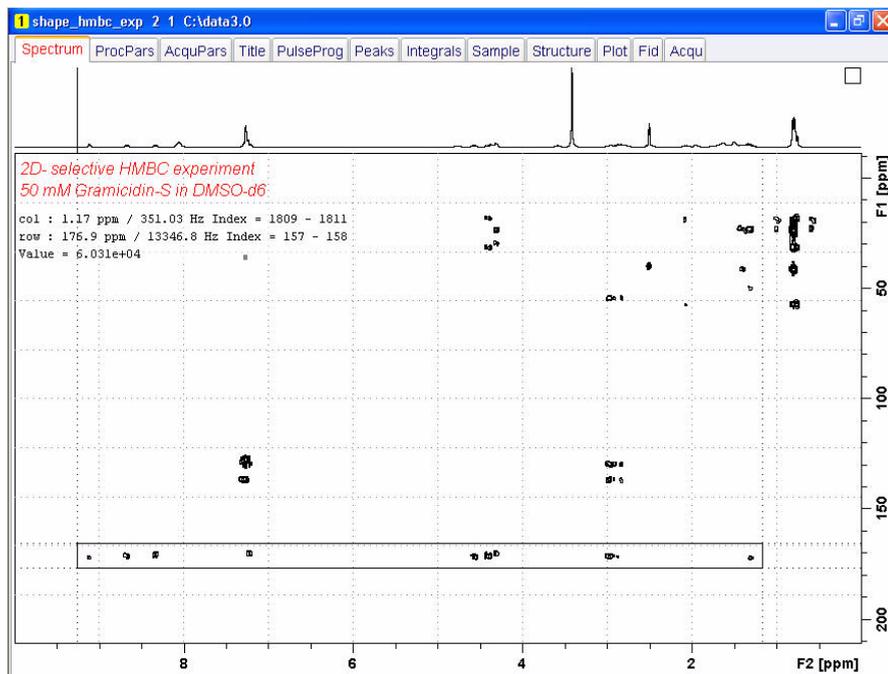
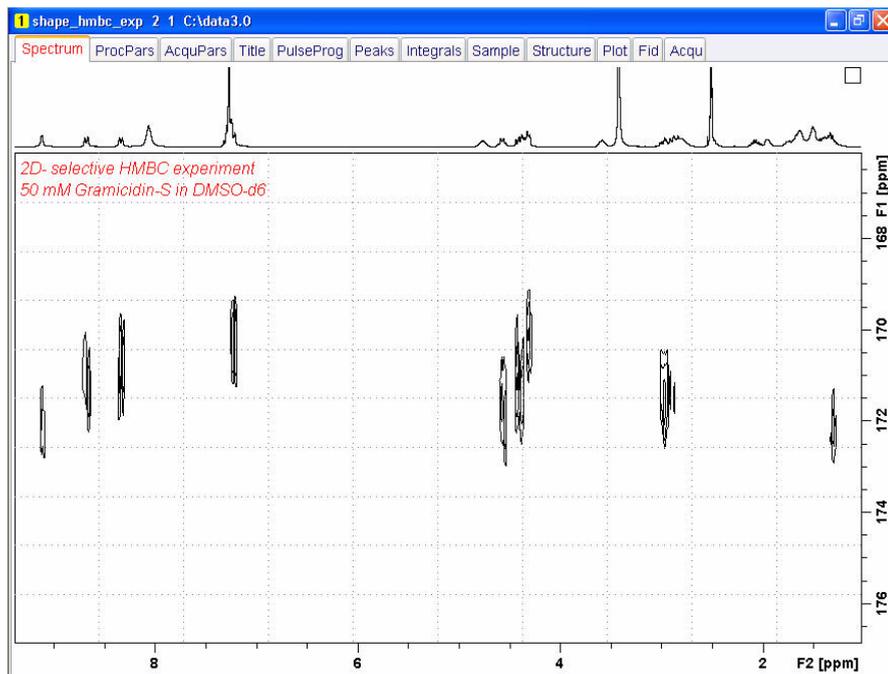


Figure 6.38



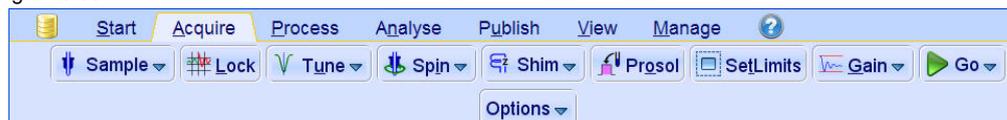
6. Write down the expanded F1 sweep width in ppm and Hz (e.g. **10 ppm, 750 Hz**)
7. Write down the center frequency (O2) of the expanded F1 sweep width in ppm (e.g. **172 ppm**)
8. Select the '**AcquPars**' tab by clicking on it

9. Select the pulse program parameters view
10. Write down the value for **P3** [us] (e.g. **10.5** us)
11. Write down the value for **PL2** [dB] (e.g. **-17.33** dB)
12. Change the **PULPROG** to **shmbcgpndqf**
13. Select the '**Spectrum**' tab by clicking on it

6.3.7 Set up the selective pulse

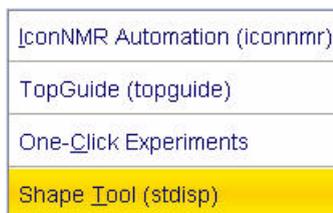
1. Click on the '**Acquire**' tab in the TopSpin menu bar

Figure 6.39



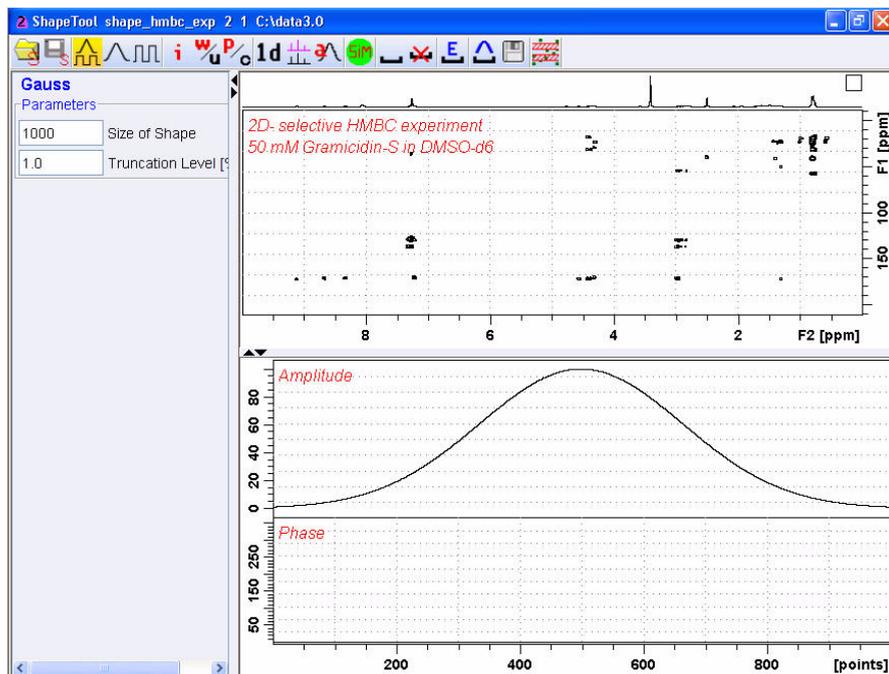
2. Click on the down arrow inside the **Options** button

Figure 6.40



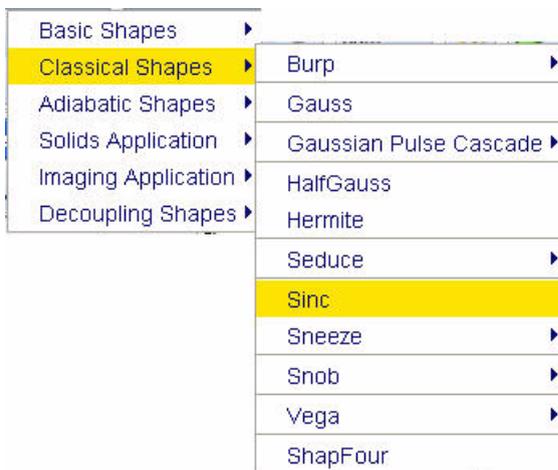
3. Select '**Shape Tool (spdisp)**' by clicking on it

Figure 6.41



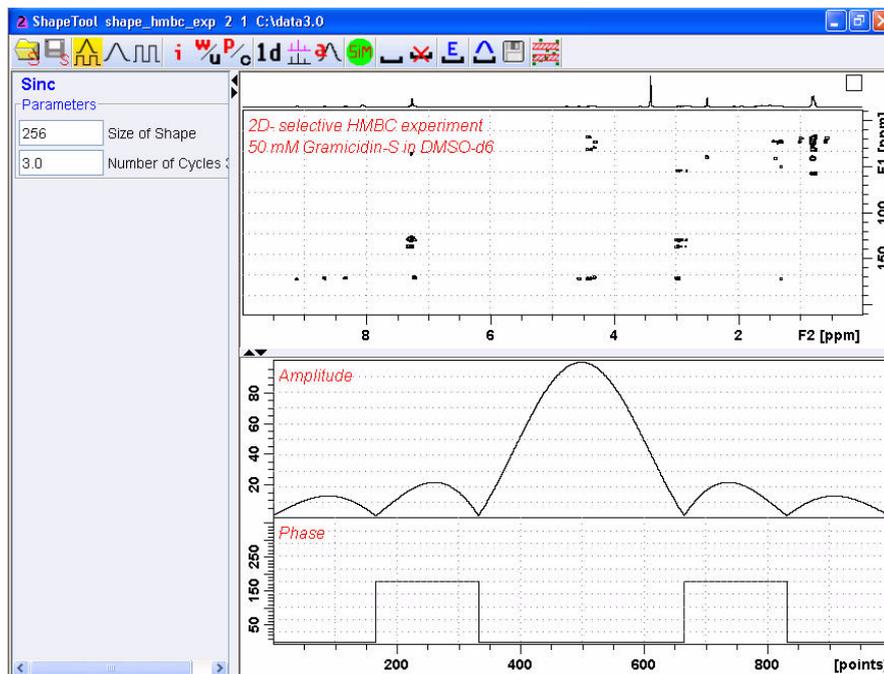
4. In the main menu click on **Shapes**

Figure 6.42



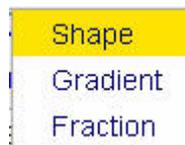
5. Select '**Classical Shapes**'
6. Select '**Sinc**' by clicking on it
7. Make the following changes:
 - Size of Shape = 256**
 - Number of cycle = 3**

Figure 6.43



8. Click on 

Figure 6.44



9. Select 'Shape' by clicking on it

Figure 6.45



10. Make the following changes:

Title = Sinc3.256

Flip Angle = 90

Type of Rotation = Excitation

11. Click on 

Figure 6.46



12. Make the following changes:

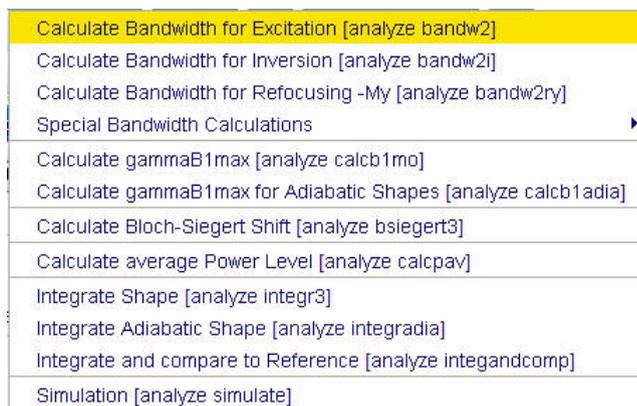
Destination Dir = <TOPSPIN HOME>\exp\stan\nmr\lists\wave\user

New Name = **Sinc3.256**

13. Click on 

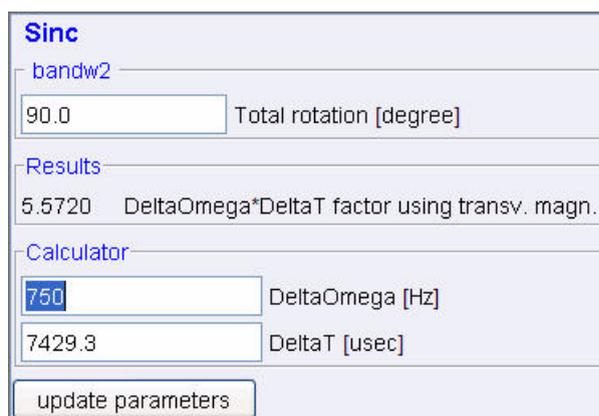
14. In the main menu click on 

Figure 6.4712



15. Select 'Calculate Bandwidth for Excitation' by clicking on it

Figure 6.48



16. Make the following changes:

DeltaOmega [Hz] = **750** (e.g. SW value in Hz from 6.3.6 step 6)

17. Press the 'Enter' key

NOTE: The value of Delta T [usec] is being calculated. (e.g. 7429.3 usec)

18. Write down the Delta T value [usec] (e.g. **7429.3**)

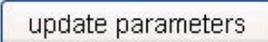
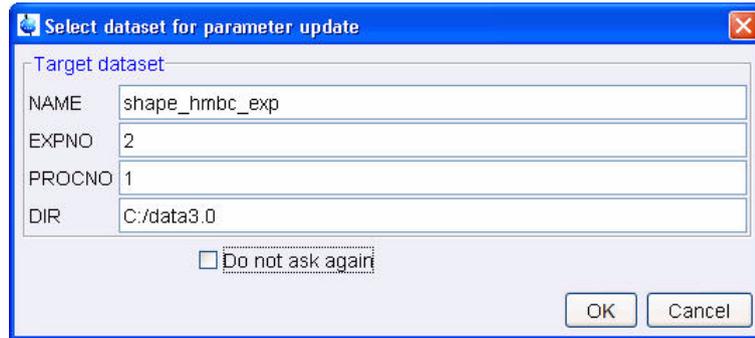
19. Click on 

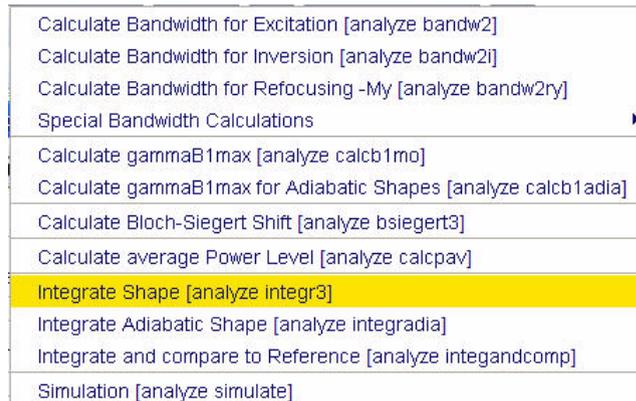
Figure 6.49



20. Click on 

21. In the main menu click on 

Figure 6.5012



22. Select '**Integrate Shape [analyze integr3]**' by clicking on it

Figure 6.51

The screenshot shows a software window titled "Sinc" with a sub-tab "integr3". It contains three input fields: "Length of pulse [usec]" with the value 7429.3, "Total rotation [degree]" with the value 90, and "90 deg hard pulse [usec]" with the value 10.5. Below these is a "Results" section with three rows of data: "Integ Ratio comp. to square on res." (0.17752), "Corresponding difference [dB]" (-15.01507), and "Change of power level [dB]" (41.98010). At the bottom is an "update parameters" button.

Parameter	Value
Length of pulse [usec]	7429.3
Total rotation [degree]	90
90 deg hard pulse [usec]	10.5

Result	Value
Integ Ratio comp. to square on res.	0.17752
Corresponding difference [dB]	-15.01507
Change of power level [dB]	41.98010

23. Make the following changes:
Length of pulse [usec] = value from in 6.3.7 step 14 (e.g. 7429.3)
24. Press the 'Enter' key
Total rotation [degree] = 90
25. Press the 'Enter' key
90 deg. Hard pulse [usec] = value from in 6.3.6 step 10 (e.g. 10.5)
26. Press the 'Enter' key
27. Write down the change of power level [dB] value (e.g. 41.9801)
28. Click on  to close the Shape Tool window

6.3.8 Setting up the acquisition parameters

1. Select the 'AcquPars' tab by clicking on it
2. Select all parameters view
3. Make the following changes:
TD (F1) = 64
NS = 32
SW [ppm] (F1) = value from 6.3.6 step 6 (e.g. 10)
O2P [ppm] = value from in 6.3.6 step 7 (e.g. 172)
4. Select the pulse program parameters display
5. Make the following changes:
P13 [us] = value from in 6.3.7 step 18 (e.g. 7429.3)
SP14 [dB] = (value from in 6.3.6 step 11) + (value from 4.3.7 step 27) = (e.g. 24.6501)
SPNAM14 = Sinc3.256

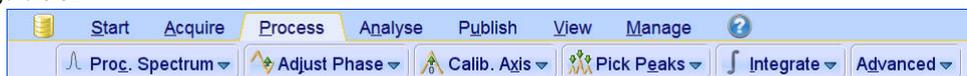
6.3.9 Running the experiment

1. Select the 'Spectrum' tab by clicking on it
2. Select  by clicking on it

6.3.10 Processing

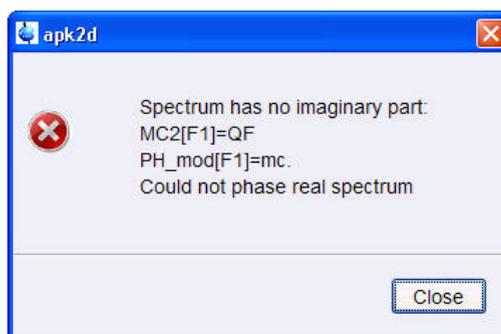
1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 6.52



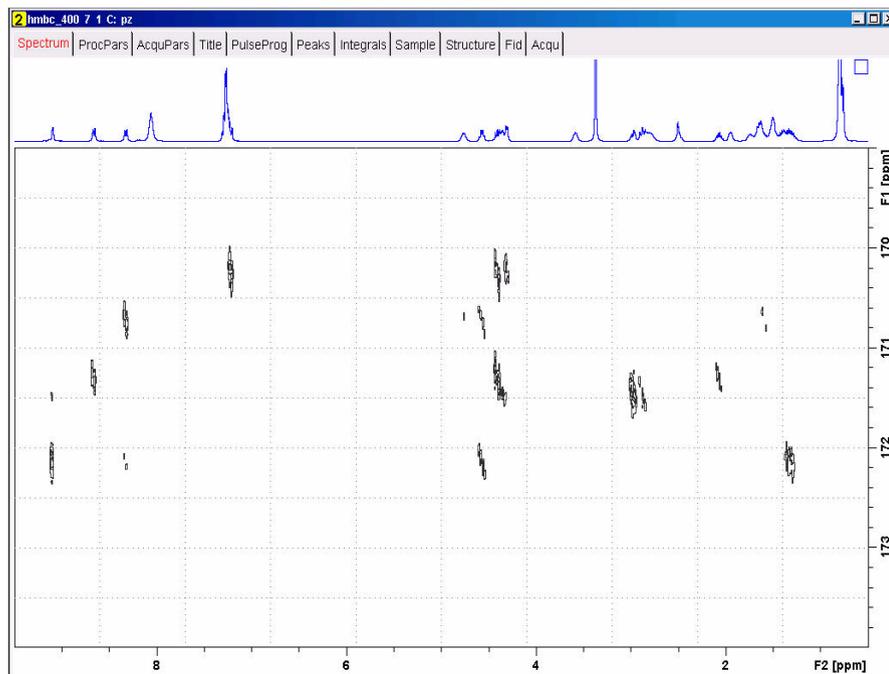
2. Select  by clicking on it

Figure 6.53



NOTE: This executes a standard processing program **proc2**. The message shown in Figure 6.53 pops up in case of a magnitude 2D experiment and the **apk2d** option is enabled. To configure the processing program follow the steps below. To avoid the message shown in Figure 6.53 the option '**Auto-Phasing (apk2d)**' may be disabled for magnitude like 2D experiment.

Figure 6.54



NOTE: The cross peaks in the selective HMBC show nice separation do to the increased resolution in F1, compared to the regular HMBC. The projections are external high resolution spectra.

6.4 Observations

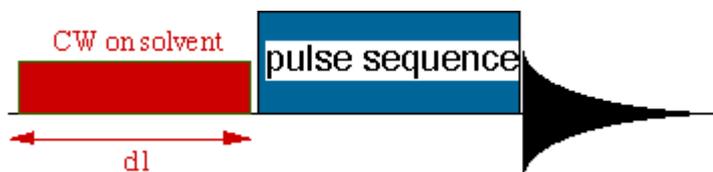


7 1-D Solvent suppression experiments

7.1 Introduction

Many experiments on samples dissolved in protonated solution require some method to minimize the strong resonance belonging to the solvent. This suppression can be performed in several ways, depending on the number of signals to suppress and depending on which part of the pulse sequence can be modified. Solvent suppression can be applied during the relaxation period just prior to the conventional pulse sequence as outlined in Figure 7.1 below. This is referred to as Presaturation.

Figure 7.1



However, presaturation can also reduce the signal intensities of exchangeable protons. For this reason, other schemes, as the WATERGATE, WET and Excitation Sculpting schemes, can be used to overcome this problem and are discussed in this chapter.

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

7.1.1 Samples

2mM Raffinose in 90% H₂O + 10% D₂O

2mM Lysozyme in 90% H₂O + 10% D₂O

7.1.2 Preparation experiment

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

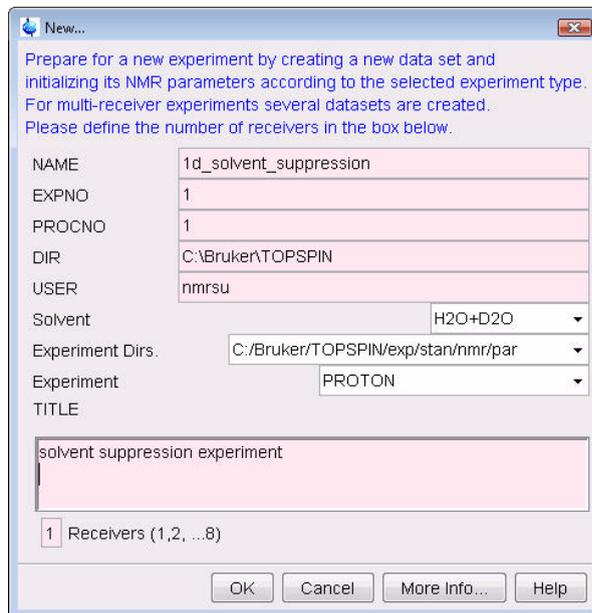
Figure 7.2



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

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

Figure 7.3



NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 7.3 above. Click on the down arrow button to browse for a specific directory.

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

Figure 7.4



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

Figure 7.5

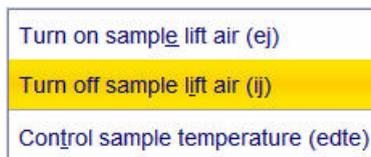


7. Select **'ej'** by clicking on it

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 7.6

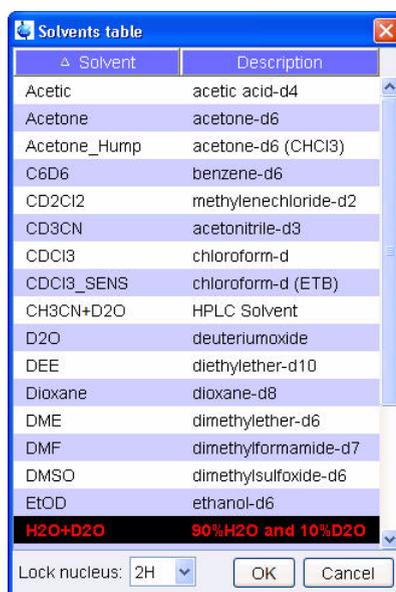


10. Select 'ij' by clicking on it

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 7.7



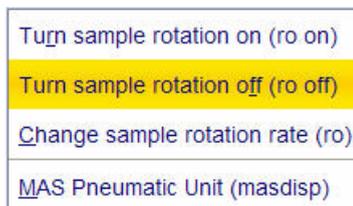
12. Select 'H2O+D2O' by clicking on it

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.

15. Select  Spin by clicking on it

Figure 7.8



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

NOTE: Solvent suppression experiments should be run non spinning

16. Select  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  by clicking on it

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

7.1.3 Acquisition

1. Select  by clicking on it

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

2. Select  by clicking on it

7.1.4 Processing

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

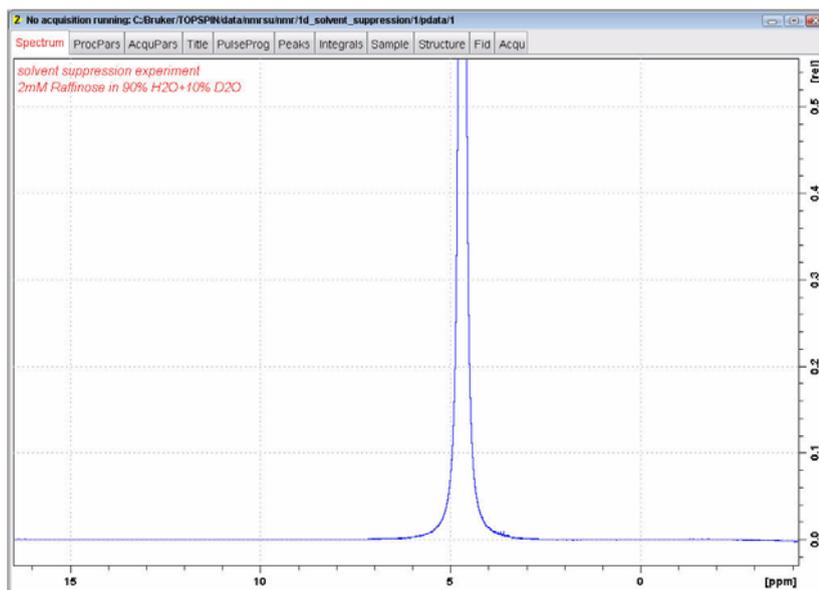
Figure 7.9



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.

Figure 7.10

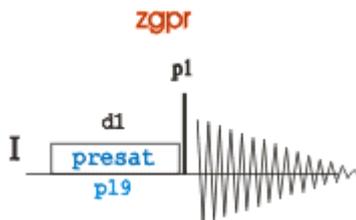


NOTE: Make sure that the SW is large enough to cover the entire Spectrum accounting for the position of O1. The presaturation is applied on resonance (at the O1 position) The power level for presaturation has to be known and entered into the Prosol parameters.

7.2 1-D Solvent suppression with Presaturation

Presaturation is the most common procedure to minimize and suppress the intense solvent resonance when ^1H spectra are recorded in protonated solutions. This experiment is performed by applying a low-power continuous wave irradiation on the selected resonance during the pre-scan delay, see Figure 7.1

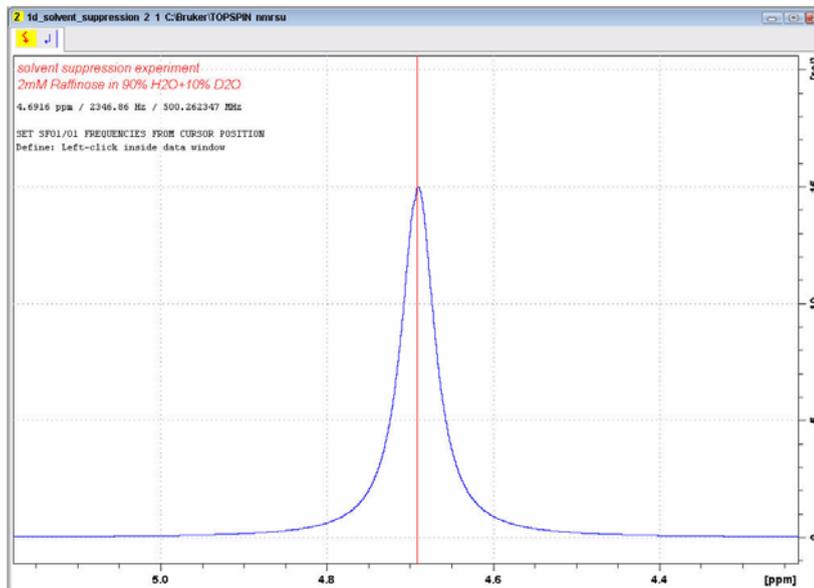
Figure 7.11



7.2.1 Parameter set up

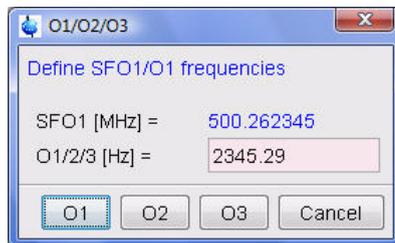
1. Type **wrpa 2** on the command line
2. Type **re 2** on the command line
3. Expand the Water signal at 4.8 ppm
4. Click on 

Figure 7.12



5. Move the cursor line to the center of the peak and click the left mouse button

Figure 7.13



6. Click on 
7. Select the 'AcquPars' tab by clicking on it
8. Make the following changes:
 - PULPROG = **zgpr**
 - TD = **16k**
 - NS = **8**
 - DS = **4**
 - SW[ppm] = **10** (for the Raffinose sample)
 - SW[ppm] = **14** (for the Lysozyme sample)
 - D1 [s] = **2**
9. Select the 'ProcPar' tab by clicking on it
10. Make the following changes:
 - SI = **8k**
11. Select the '**Spectrum**' tab by clicking on it

7.2.2 Fine tuning

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

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

2. Click on the down arrow inside the  **Go** button

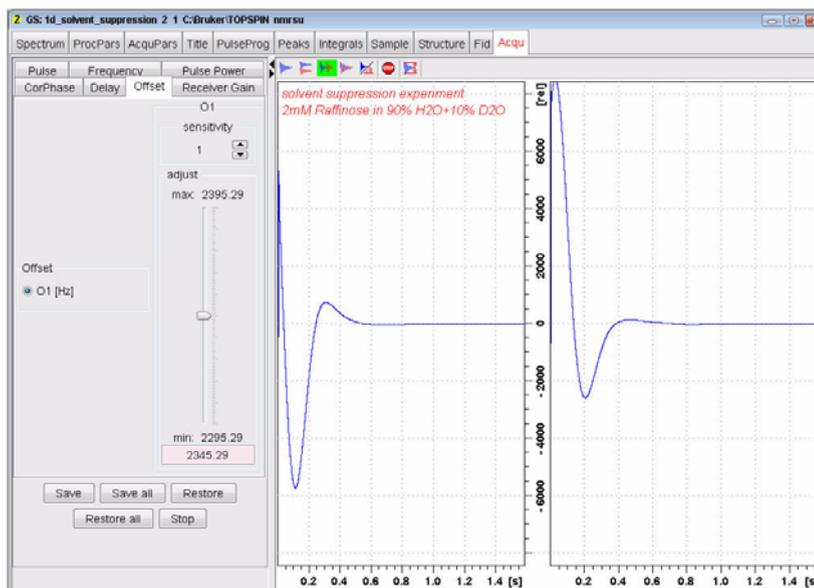
Figure 7.14



3. Select '**Real-Time Go setup (gs)**' by clicking on it
4. Click on 

5. Select the 'Offset' tab

Figure 7.15

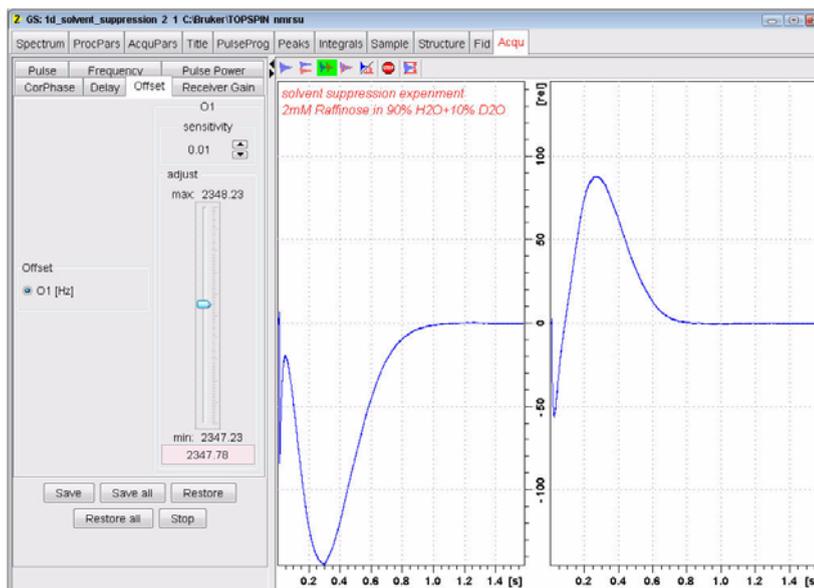


6. Change the O1 value by clicking just below or above the adjust slider

NOTE: For smaller changes, adjust the 'sensitivity' to smaller values.

7. Observe the fid area in the Acquisition information window for a smaller integration value and the FID to become a single line

Figure 7.16



8. Click on 

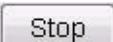
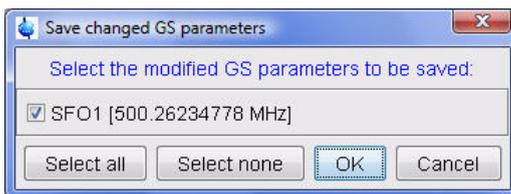
9 Click on 

Figure 7.17



10. Click on 

7.2.3 Acquisition

1. Select  by clicking on it

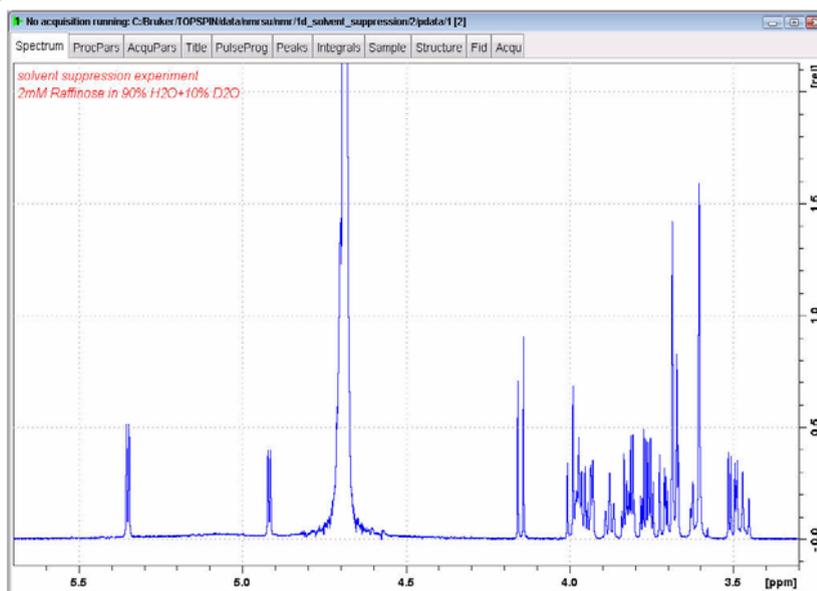
NOTE: To adjust rg manually, click on the down arrow inside the 'Gain' icon

2. Select  by clicking on it

7.2.4 Processing

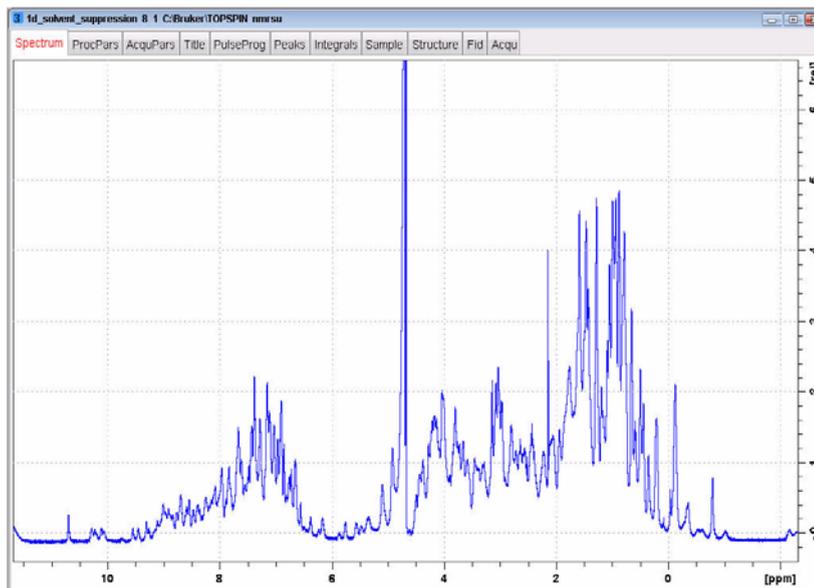
1. Process and phase correct the spectrum

Figure 7.18



NOTE: Figure 7.18 above shows the solvent suppressed 1-D spectrum of the Raffinose sample and Figure 7.19 below shows the 1-D spectrum of the Lysozyme sample.

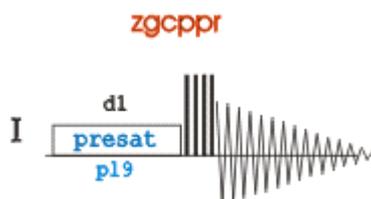
Figure 7.19



7.3 1-D Solvent suppression with Presaturation and Composite Pulses

This experiment is performed by applying a low-power continuous wave irradiation on the water resonance during the pre-scan period, followed by a rapid succession of four 90 degree pulses to further reduce the residual hump of the water signal, see Figure 7.20

Figure 7.20



7.3.1 Parameter set up

1. Follow the instructions in paragraphs 7.2.1 through 7.2.2 step 10 in this chapter
2. Select the '**AcquPars**' tab by clicking on it
3. Make the following changes:
PULPROG = zgcprr
4. Select the '**Spectrum**' tab by clicking on it

7.3.2 Acquisition

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

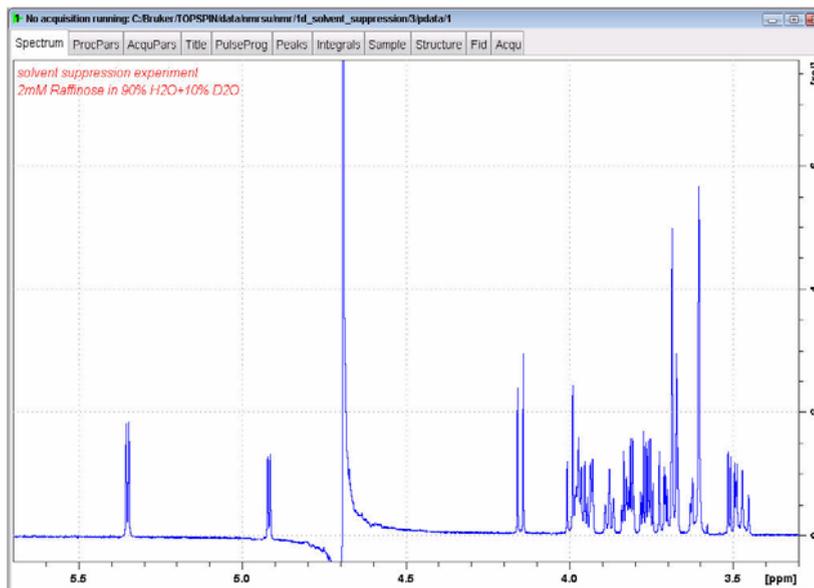
NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select  **Go** by clicking on it

7.3.3 Processing

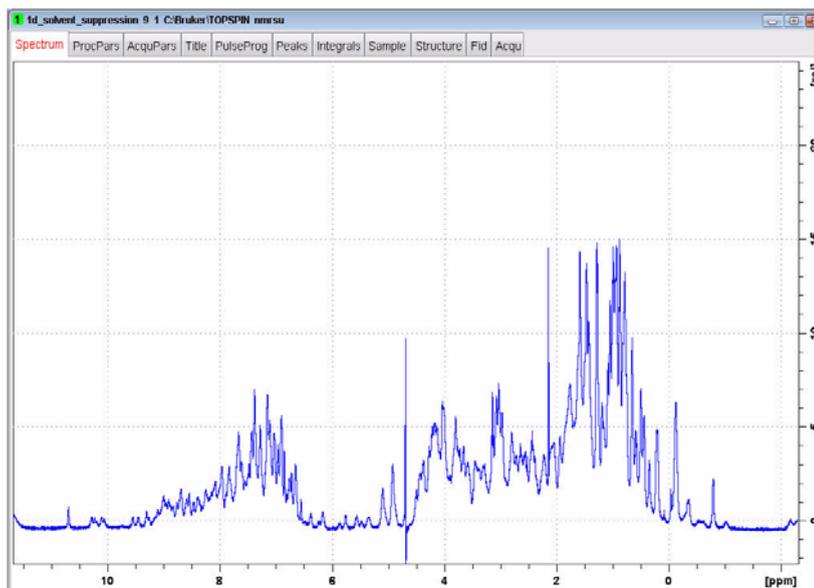
1. Process and phase correct the spectrum

Figure 7.21



NOTE: Figure 7.21 above shows the solvent suppressed 1-D spectrum of the Raffinose sample and Figure 7.22 below shows the 1-D spectrum of the Lysozyme sample.

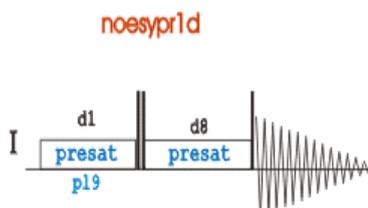
Figure 7.22



7.4 1-D Solvent suppression using the noesy sequence

This experiment is performed by using the 1-D version of the noesyphpr sequence applying a low-power continuous wave irradiation on the water resonance during the pre-scan and the during the mixing time period of the NOESY sequence, see Figure 7.23

Figure 7.23



7.4.1 Parameter set up

1. Follow the instructions in paragraphs 7.2.1 through 7.2.2 step 10 in this chapter
2. Select the '**AcquPars**' tab by clicking on it
3. Make the following changes:
PULPROG = noesypr1d
D8[s] = 0.1
4. Select the '**Spectrum**' tab by clicking on it

7.4.2 Acquisition

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

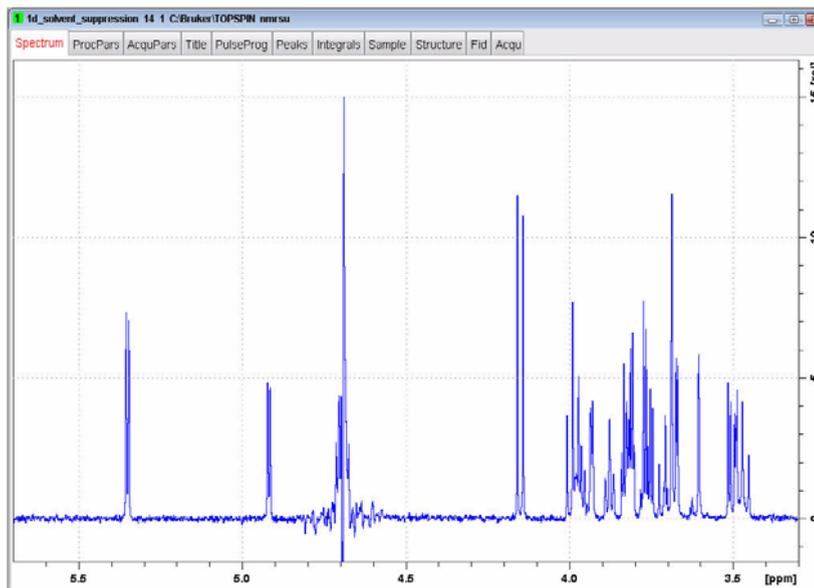
NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select  **Go** by clicking on it

7.4.3 Processing

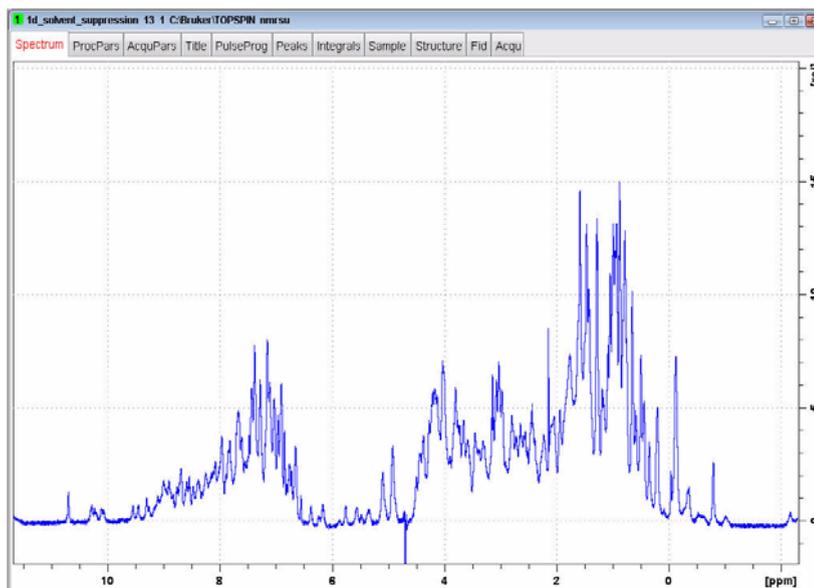
1. Process and phase correct the spectrum

Figure 7.24



NOTE: Figure 7.24 above shows the solvent suppressed 1-D spectrum of the Raffinose sample and Figure 7.25 below shows the 1-D spectrum of the Lysozyme sample.

Figure 7.25

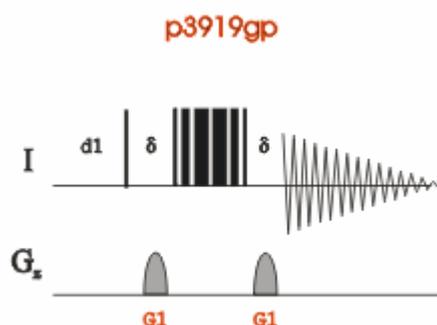


7.5 1-D Solvent suppression with WATERGATE

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

The sequence is in principle, a spin-echo experiment in which the 180 degree pulse is embedded between two pulsed field gradients. After excitation by the first pulse p1 the field gradient G1 dephases all coherence. The selective inversion element consists of a symmetrical 3-9-19 pulse sequence 3a-t-9a-t-19a-t-19a-t-9a-t-3a, with $26a=180^\circ$ degree (Figure 7.26). Additional suppression appears at different sidebands ($1/t$).

Figure 7.26



7.5.1 Parameter set up

1. Follow the instructions in the paragraphs 7.2.1 through 7.2.2 step 10
2. Select the '**AcquPars**' tab by clicking on it
3. Make the following change
 - PULPROG = p3919gp**
 - D19 [s] = 0.00015** = $1/(2*d)$ where d = distance to next null in Hz
 - GPZ1 [%] = 20**
4. Select the '**Spectrum**' tab by clicking on it

7.5.2 Acquisition

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

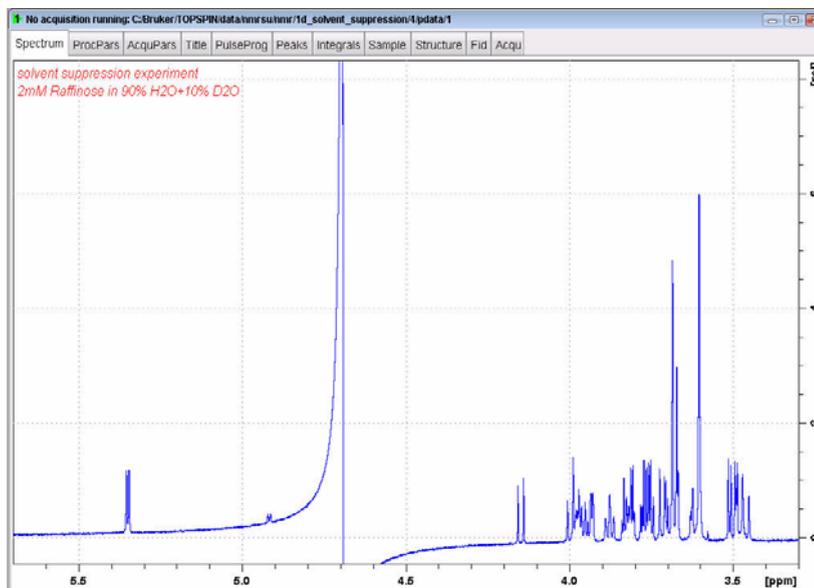
NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select  **Go** by clicking on it

7.5.3 Processing

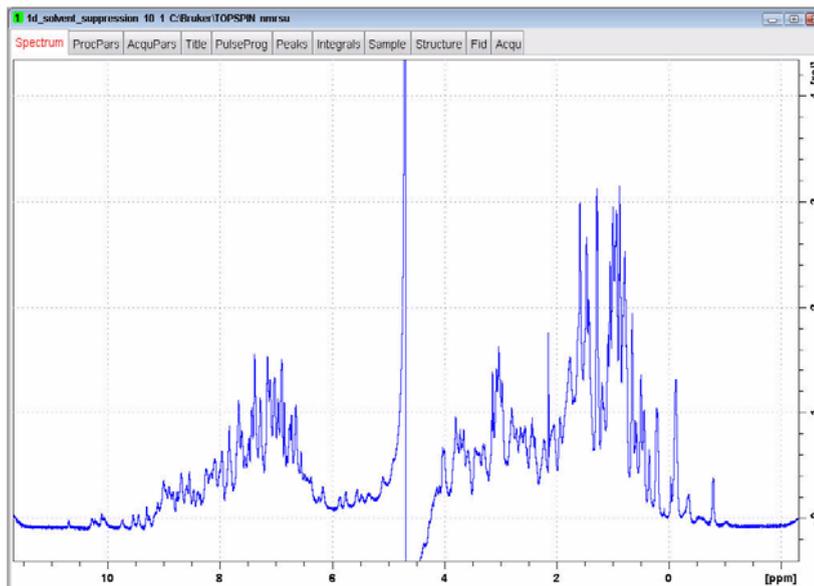
1. Process and phase correct the spectrum

Figure 7.27



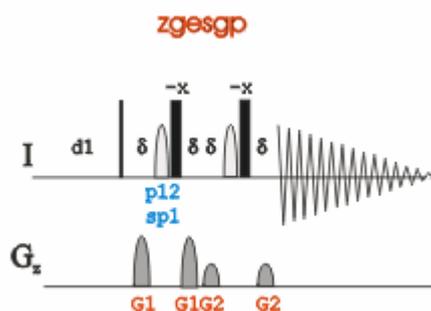
NOTE: Figure 7.27 above shows the solvent suppressed 1-D spectrum of the Raffinose sample and Figure 7.28 below shows the 1-D spectrum of the Lysozyme sample.

Figure 7.28



7.6 1-D Solvent suppression with excitation sculpting

Figure 7.29



7.6.1 Parameter se up

1. Follow the instructions in the paragraphs 7.2.1 through 7.2.2 step 10
2. Select the '**AcquPars**' tab by clicking on it
3. Make the following changes:
 - PULPROG** = **zgesgp**
 - P12** [us] = **2000**
 - SP1** [dB] = calculate using the AU-program 'pulse' and subtract 6dB since this is a 180⁰ pulse(e.g. **44.5**)
 - SPNAM1** = **Squa100.1000**
 - GPZ1** [%] = **31**
 - GPZ2** [%] = **11**
4. Select the '**Spectrum**' tab by clicking on it

7.6.2 Acquisition

1. Select  by clicking on it

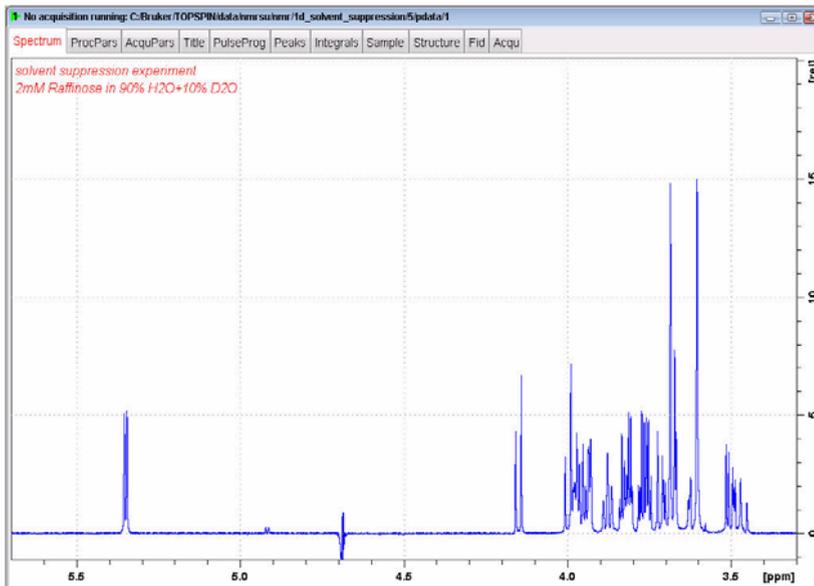
NOTE: To adjust rg manually, click on the down arrow inside the '**Gain**' icon

2. Select  by clicking on it

7.6.3 Processing

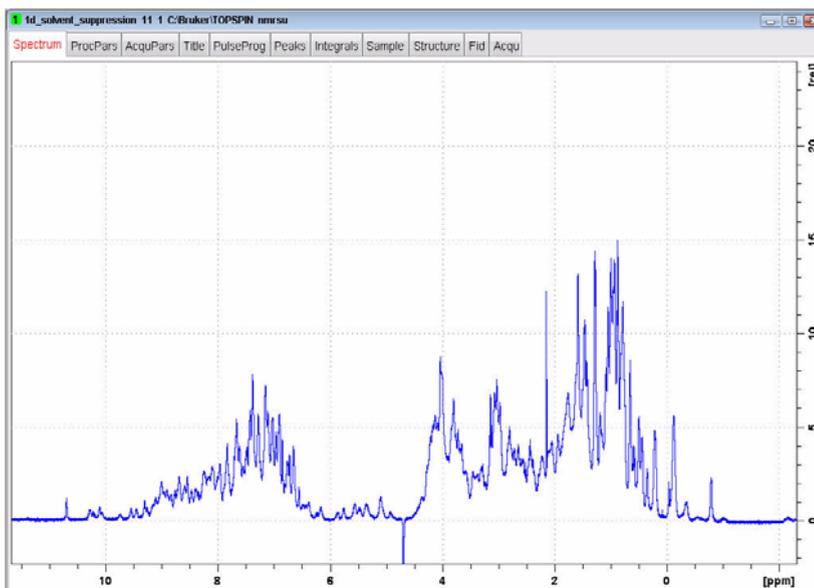
1. Process and phase correct the spectrum

Figure 7.30



NOTE: Figure 7.30 above shows the solvent suppressed 1-D spectrum of the Raffinose sample and Figure 7.31 below shows the 1-D spectrum of the Lysozyme sample.

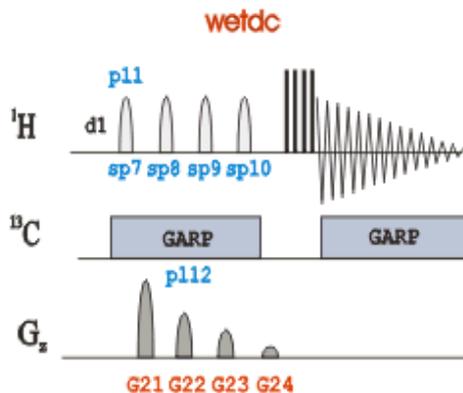
Figure 7.31



7.7 1-D Solvent suppression with WET

This pulse sequence uses a shaped, selective pulse and pulse field gradients to suppress one or more solvent signals. The option of carbon decoupling is available for suppression of solvent signals with large C13 satellites. It provides very efficient suppression with excellent selectivity.

Figure 7.32



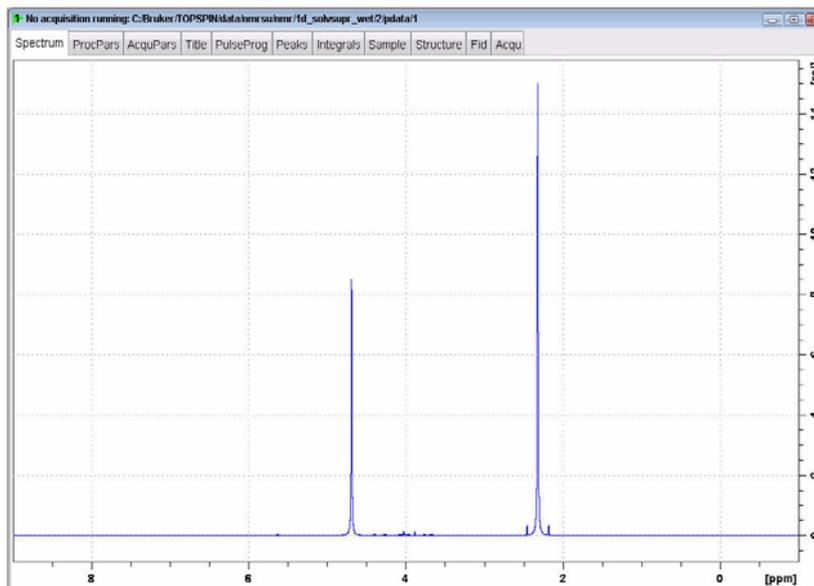
7.7.1 Sample:

2mg Sucrose in Acetonitril and D2O

7.7.2 Preparation experiment

1. Run a 1D Proton spectrum, following the instructions in **7.1.2 Preparation experiment** in this Chapter

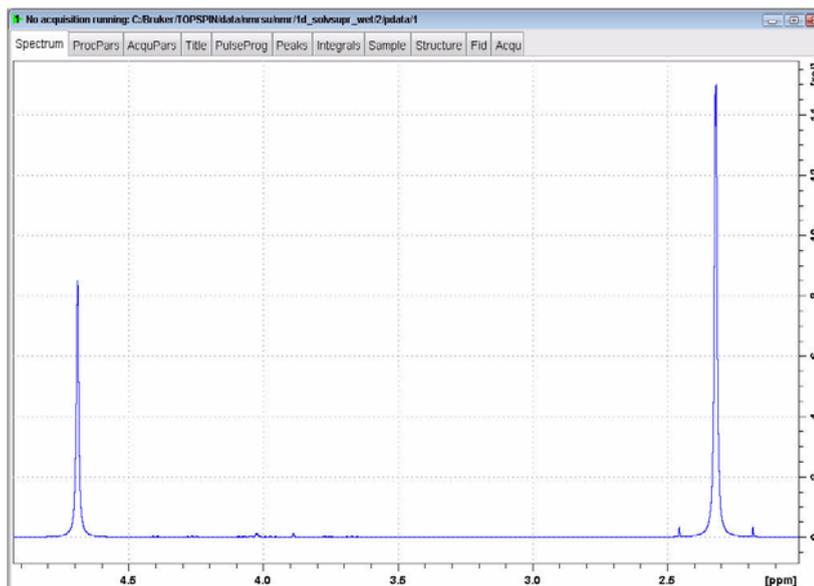
Figure 7.33



7.7.3 Frequency list set up

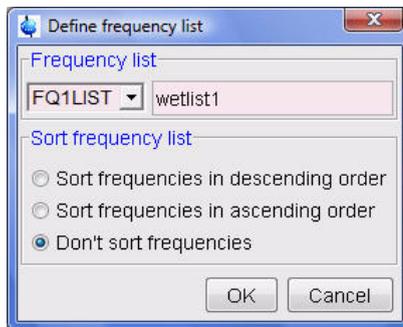
1. Type **wrpa 2** on the command line
2. Type **re 2** on the command line
3. Expand the spectrum to include both peaks for suppression

Figure 7.34



4. Click on 

Figure 7.35



5. Select '**FQ1LIST**' and type a frequency list name (e.g. **wetlist1**)

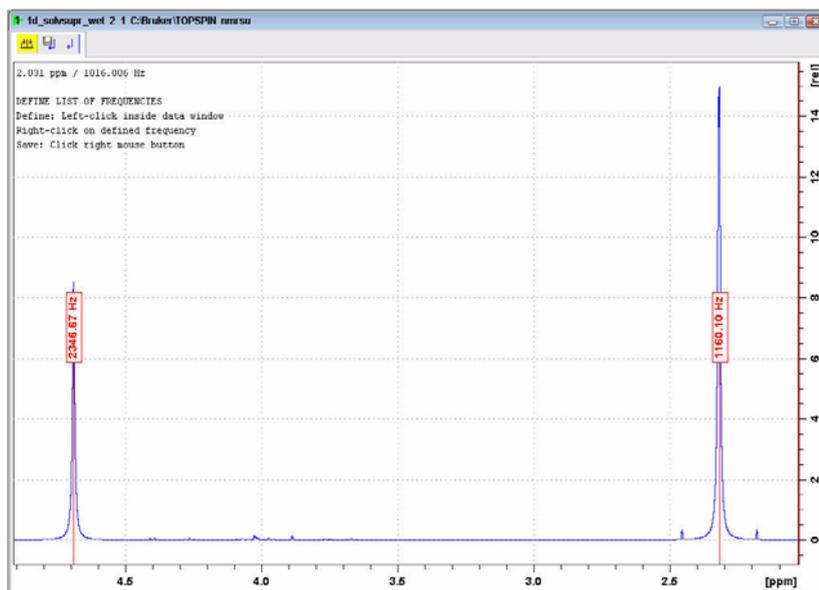
6. Enable '**Don't sort frequencies**'

7. Click on 

8. Move the cursor line to the center of the Water peak at 4.7 ppm and click the left mouse button

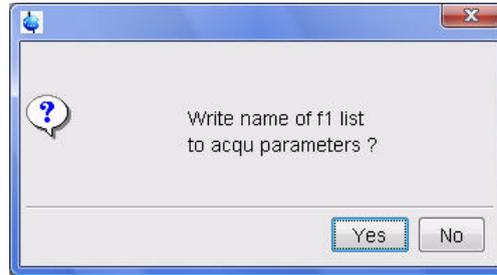
9. Move the cursor line to the center of the Acetonitril peak at 2.3 ppm and click the left mouse button

Figure 7.36



10. Click on  to save the frequency list

Figure 7.37

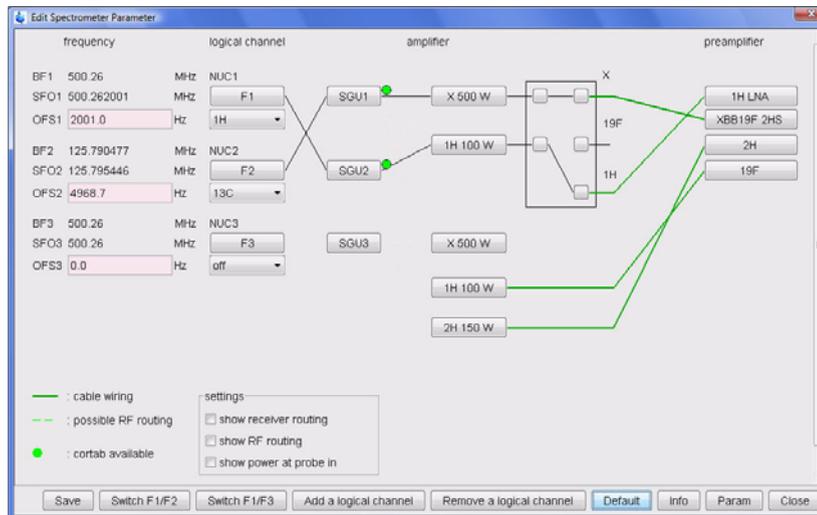


11. Click on 

7.7.4 Setting up the acquisition parameters

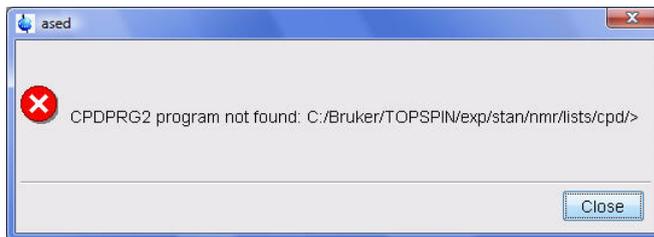
1. Select the 'AcquPars' tab by clicking on it
2. Change the following parameter:
PULPROG = wtdw
3. Click on  to display the routing
4. Select '13C' for 'F2'
5. Click on 

Figure 7.38



6. Click on 
7. Click on  to display the pulse-program parameters

Figure 7.39



NOTE: The message in Figure 7.39 appears if there is no decoupling program entered in the CPDPRG2 parameter.

8. Click on 

NS = 16

DS = 16

CPDPRG2 = garp

GPZ21 = 80

GPZ22 = 40

GPZ23 = 20

GPZ24 = 10

9. Click on  to read in the Prosol parameters

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

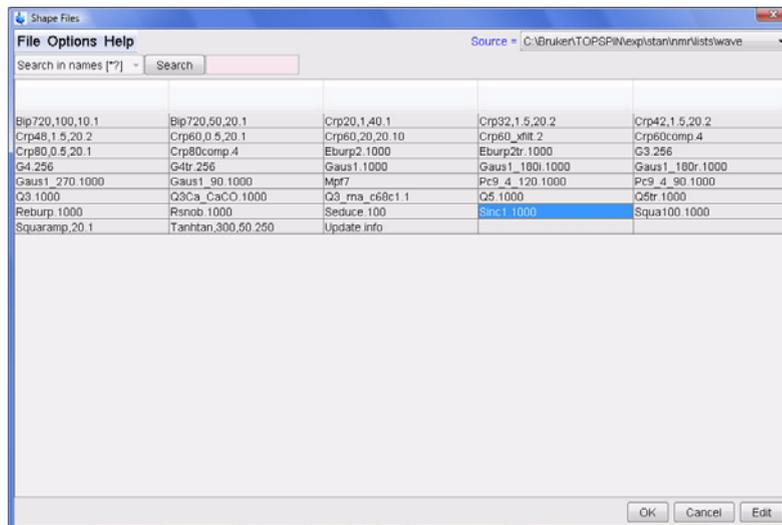
7.7.5 Selective pulses set up

NOTE: One shaped pulse is created and can be tailored to select for a single or multiple resonances.

1. In the main menu click on '**Spectrometer**' and select '**Shape Tool**' or type **stdisp** in the command line

2. In the shape tool menu bar click on  and select '**Shape**'

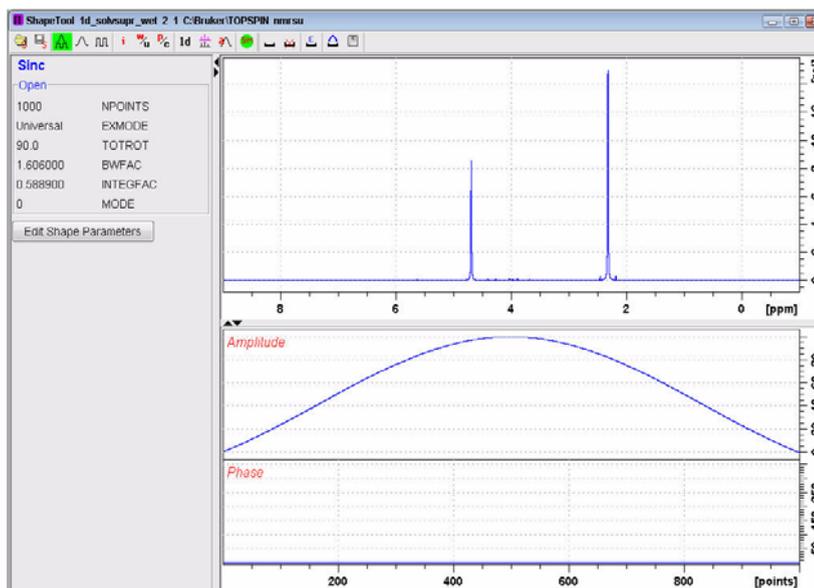
Figure 7.40



3. Select 'Sinc1.1000'

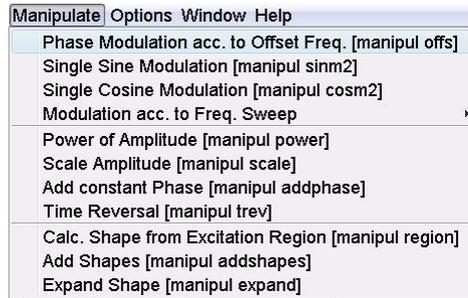
4. Click on 

Figure 7.41



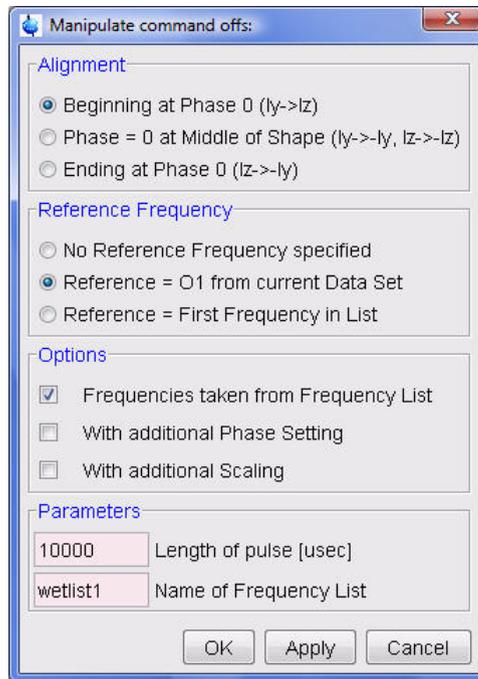
5. In the main menu click on 'Manipulate' and select 'Phase Modulation acc. to Offset Freq.' by clicking on it

Figure 7.42



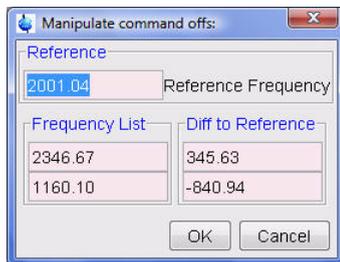
6. Enable **'Beginning at Phase 0 (ly->Iz)'**
7. Enable **'Reference = O1from current Data Set'**
8. Enable **'Frequencies taken from Frequency List'**
9. Change Parameters:
 - Length of pulse (usec) = 10000
 - Name of Frequency List = wetlist1

Figure 7.43



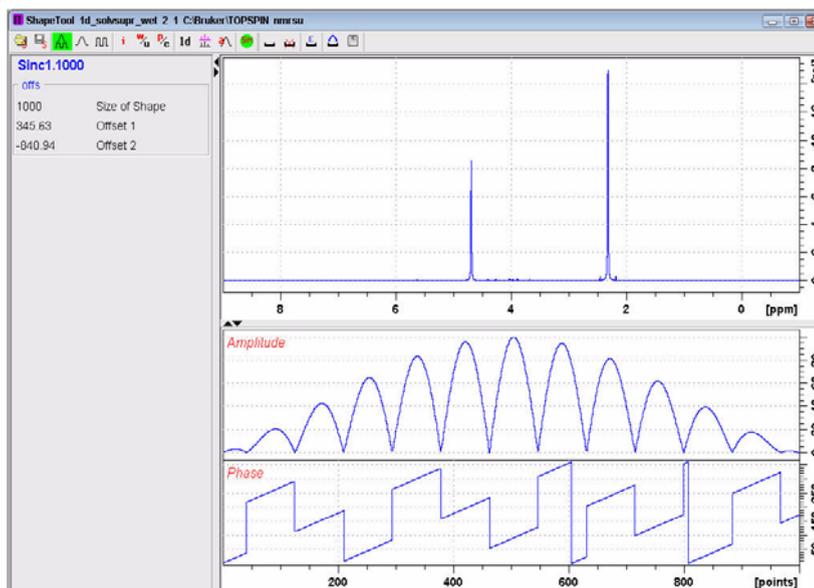
10. Click on 

Figure 7.44



11. Click on 

Figure 7.45



12. In the main menu click on '**Options**' and select '**Define Parameter Table**' by clicking on it

Figure 7.46

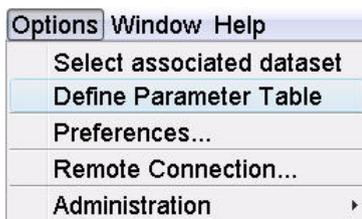
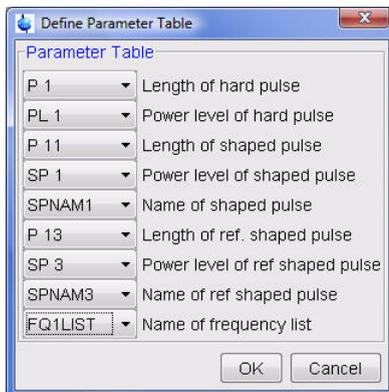


Figure 7.47



13. Make the following changes:

Length of shaped pulse = p11

Power Level of shaped pulse = SP1

Name of shaped pulse = SPNAM1

14. Click on 

15. Click on  and select 'Shape'

Figure 7.48



16. Type **wetshape1** in the 'File Name' window

17. Click on 

Figure 7.49



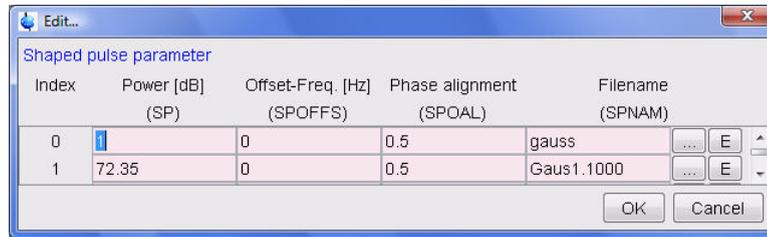
18. Type **wetshape1** in the 'New Name' window

19. Click on 

20. Click on  to close the Shape Tool window

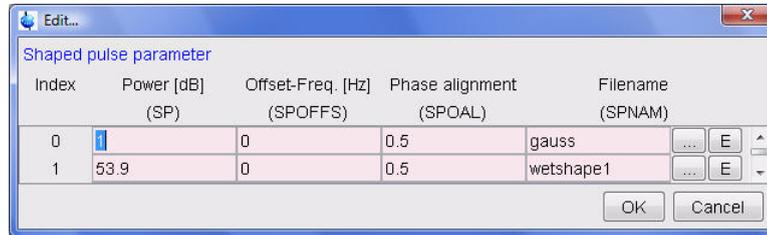
21. Type **shape** in the command line

Figure 7.50



22. Click on  to select SPNAM 1
23. Select the user directory in the 'Source' window
24. Select 'wetshape1' from the list
25. Click on 

Figure 7.51



- SP1** = power level adjusted to account for the number of frequency positions (see list below)
- 1 frequency = calibrated power level, e.g. 59.9db
 - 2 frequencies = calibrated level minus 6 dB, e.g. 53.9db
 - 3 frequencies = calibrated level minus 9.5 db, e.g. 50.4db
 - 4 frequencies = calibrated level minus 12 db, e.g. 47.9db

NOTE: In this example the power level SP1 for 2 frequencies is used (e.g. 53.9dB)

26. Click on 

7.7.6 Running the experiment

1. Type **lcwetsset** in the command line
 2. Tune the probe
-

NOTE: Step 2 is necessary for tuning the F2 frequency which is used to decouple 13C coupling

3. Select  by clicking on it

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

4. Select  by clicking on it

7.7.7 Processing

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

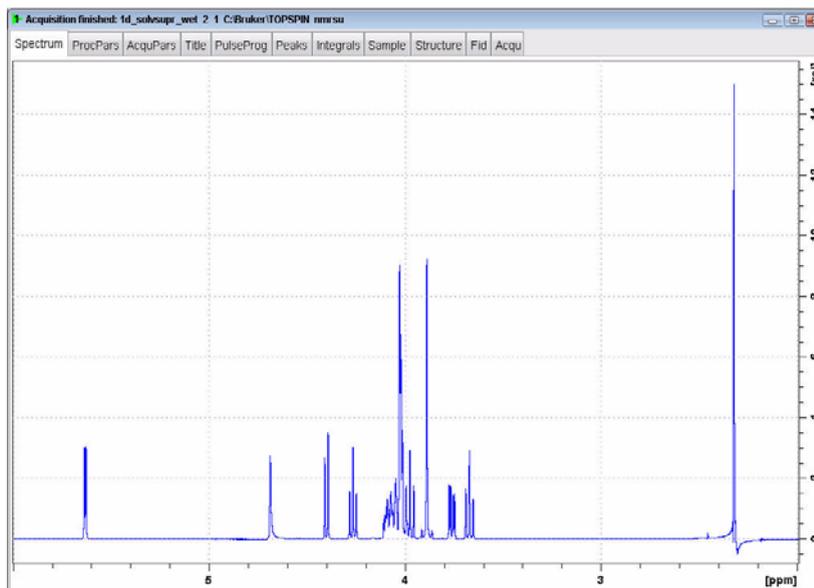
Figure 7.52



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.

Figure 7.53





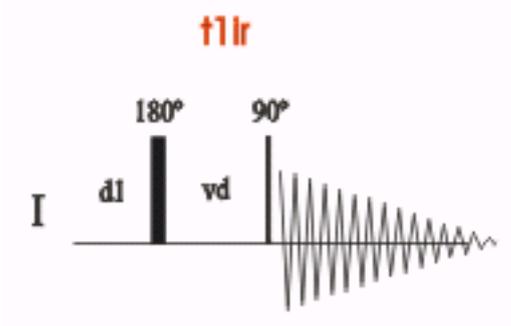
8 T1 experiment

8.1 Introduction

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

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

Figure 8.1

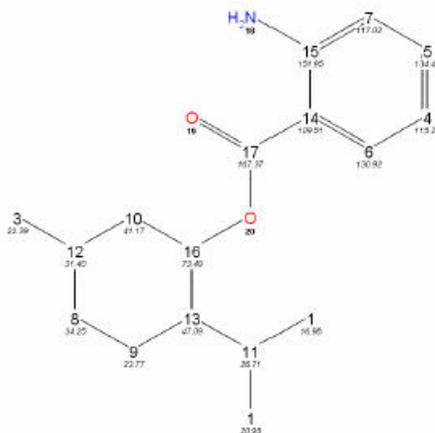


8.2 Proton Inversion-Recovery T1 experiment

8.2.1 Sample

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

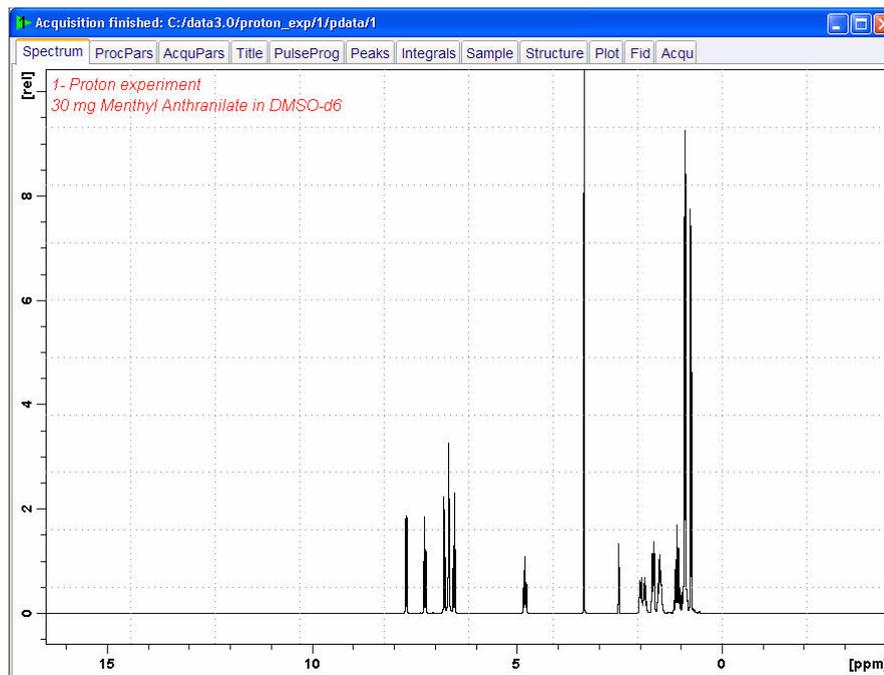
Figure 8.2



8.2.2 Preparation experiment

1. Run a **1D Proton** spectrum, following the instructions in **1-D Proton experiment, Chapter 3**

Figure 8.3



NOTE: The reference spectrum is necessary to adjust the spectral limits of the sweep width to gain more data points.

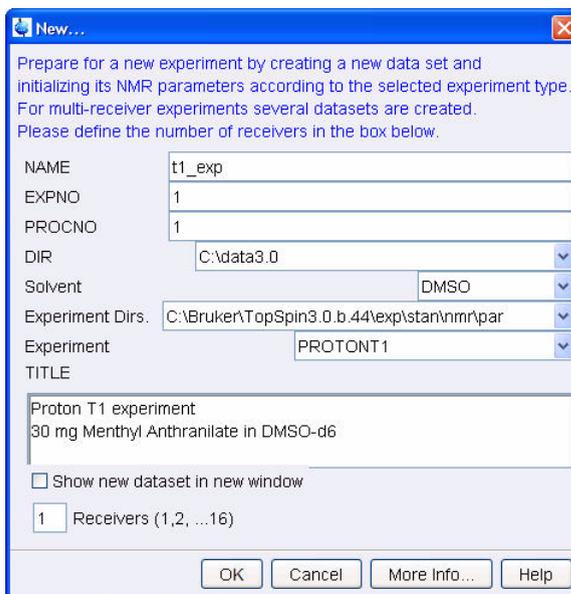
1. Click on the **'Start'** tab in the TopSpin Menu bar

Figure 8.4



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

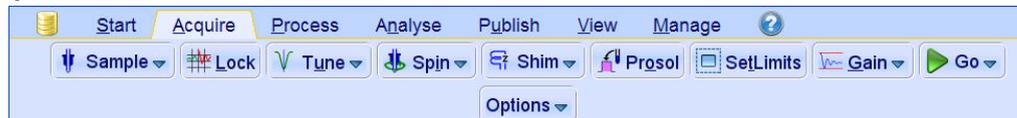
Figure 8.5



NOTE: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in Figure 8.5 above. Click on the down arrow button to browse for a specific directory.

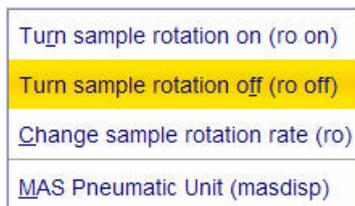
4. Click on 
5. Click on the 'Acquire' tab in the TopSpin menu bar

Figure 8.6



6. Select  by clicking on it

Figure 8.7



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

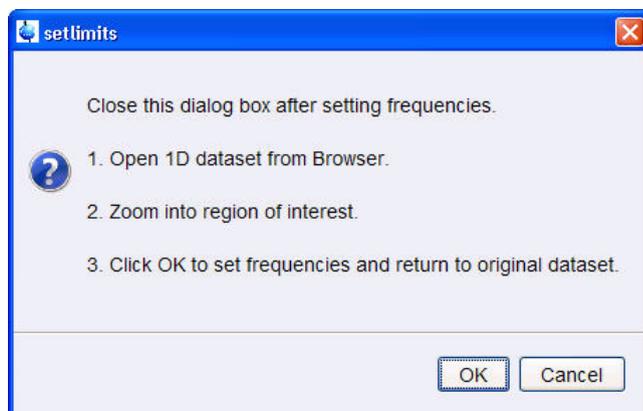
NOTE: T1 experiments should be run non spinning

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 8.8

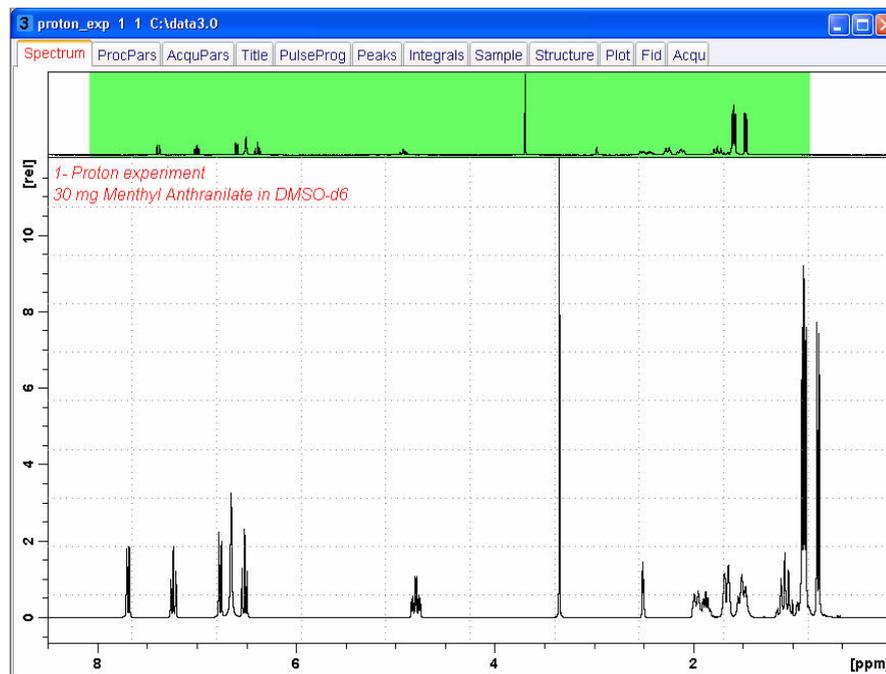


10. To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp 1) and select 'Display' or click and hold the left mouse button for dragging the 1D Proton dataset in to the spectrum window

11. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum

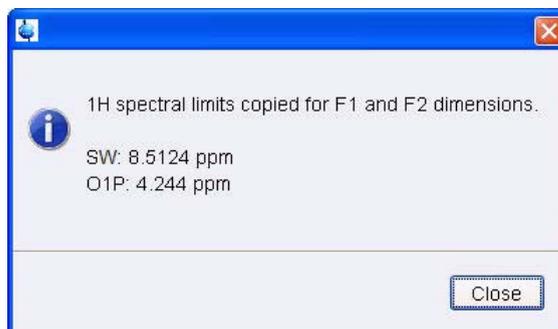
NOTE: The solvent peak may be excluded if it falls outside of the region of interest.

Figure 8.9



12. Click on to assign the new limit

Figure 8.10



13. Click on

NOTE: The display changes back to the 2D data set.

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

15. Select the pulse program parameters view

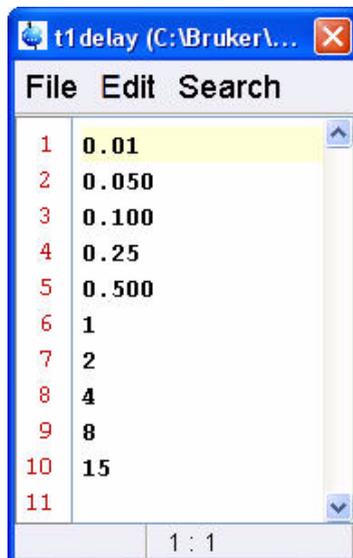
16. Make the following changes:

D1 = 15

VDLIST = t1delay

17. Click on to right of the VDLIST name box

Figure 8.11



18. Enter the variable delay values as shown in Figure 8.11 above
19. Click on 'File' and select 'Save' by clicking on it
20. Click on 'File' and select 'Close' by clicking on it
21. Select the 'Spectrum' tab by clicking on it

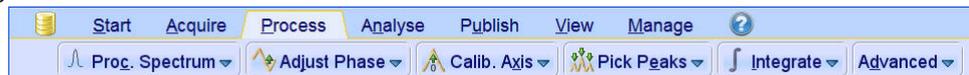
8.2.3 Acquisition

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

8.2.4 Processing

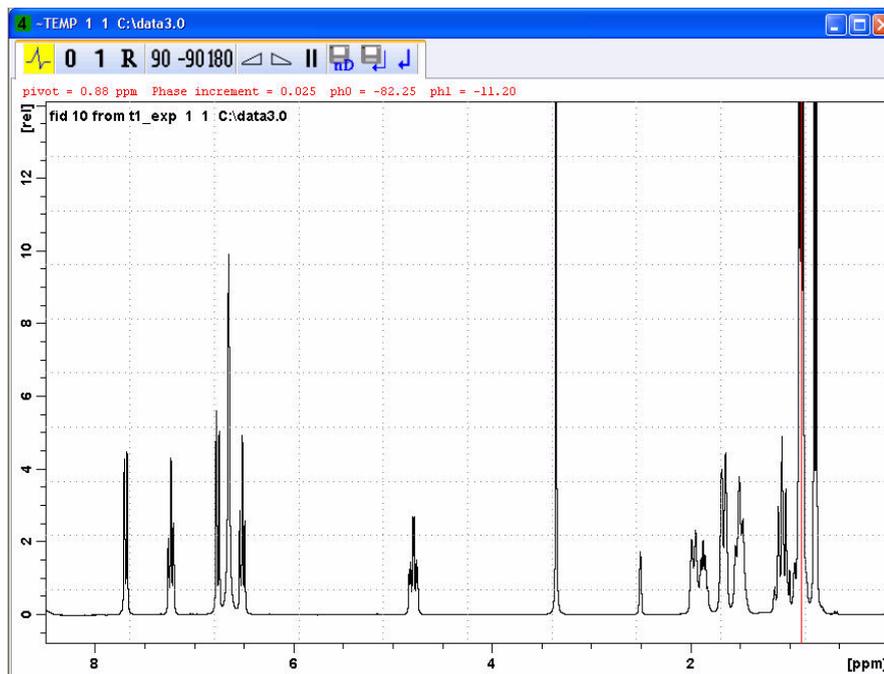
1. Click on the 'Process' tab in the TopSpin Menu bar

Figure 8.12



1. In the command line type **rser 10**
2. In the command line type **ef**
3. Click on 
4. Adjust the phase manually

Figure 8.13



5. Click on  to store the 2-D phase values

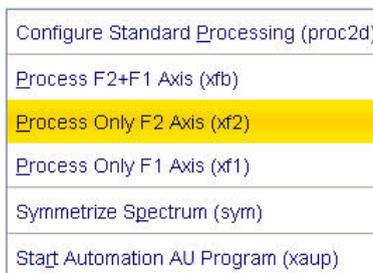
6. Click on 

NOTE: The spectrum will go back to the unphased view since the phase correction values were stored only for the 2-D spectrum.

7. Click on  (going back to the 2-D spectrum display)

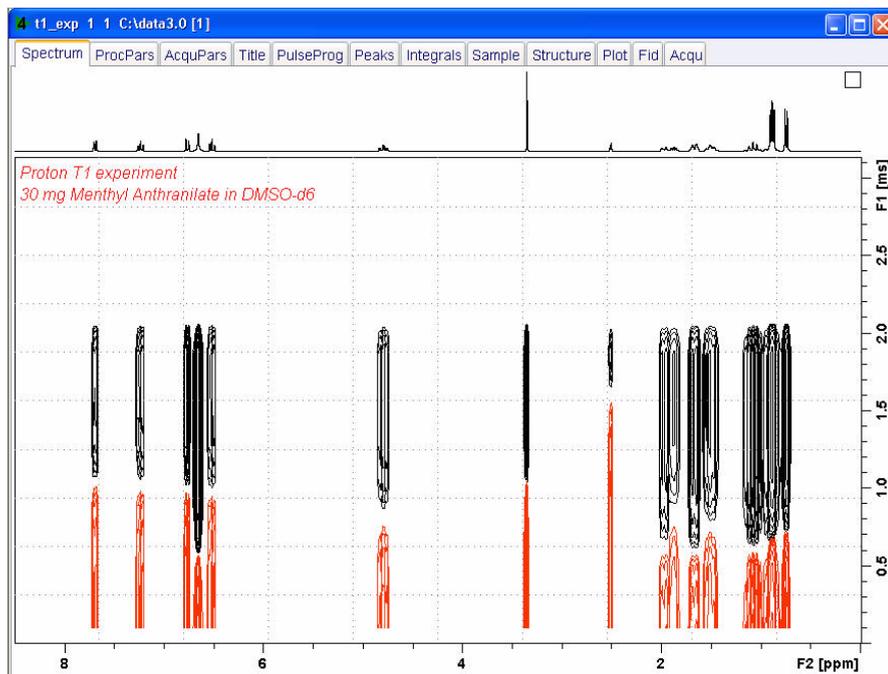
8. Click on the down arrow inside the  **Proc. Spectrum** button

Figure 8.14



9. Select 'Process Only F2 Axis (xf2)' by clicking on it

Figure 8.15



8.2.5 T1 calculation

1. Click on the **Analyse** tab in the TopSpin Menu bar

Figure 8.16



2. Click on 

NOTE: The flow buttons change for determine the T1 / T2 relaxation times, see Figure 8.17 below.

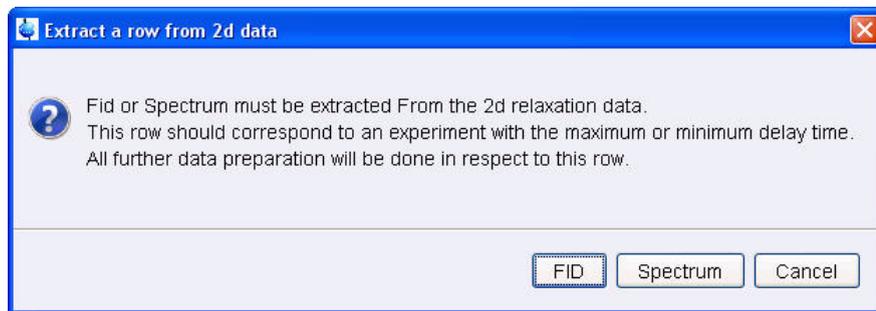
Figure 8.17



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

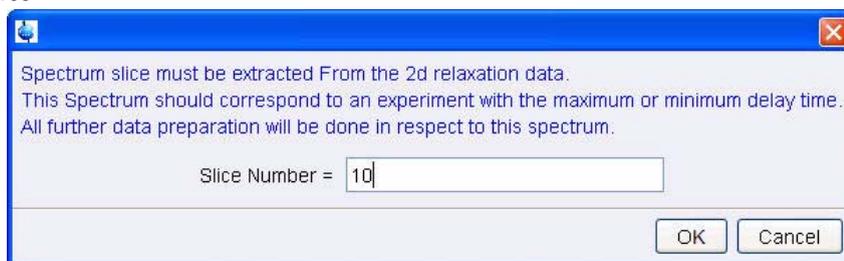
3. Click on  **'Extract Slice'**

Figure 8.18



4. Click on

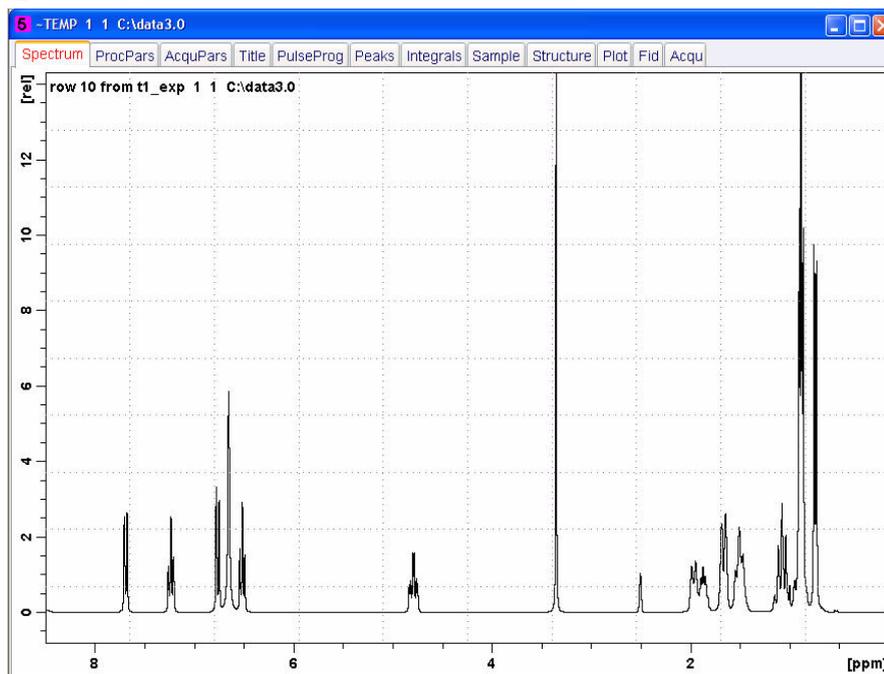
Figure 8.195



5. Select Slice Number **10**

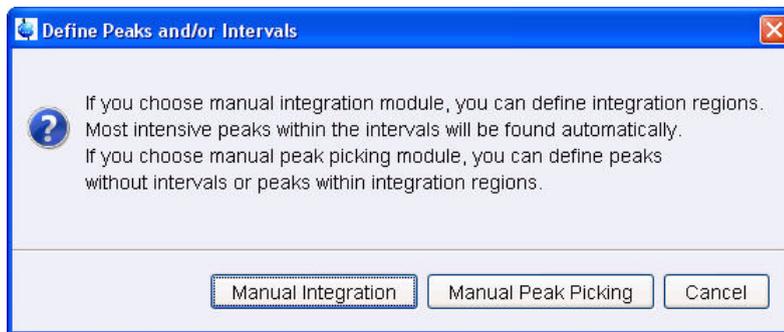
6. Click on

Figure 8.20



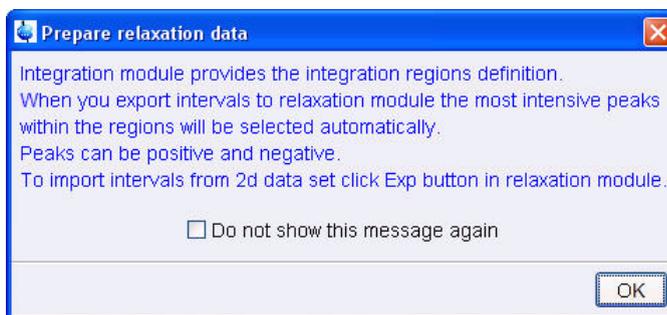
7. Click on 'Define Peaks/Ranges'

Figure 8.21



8. Click on **Manual Integration**

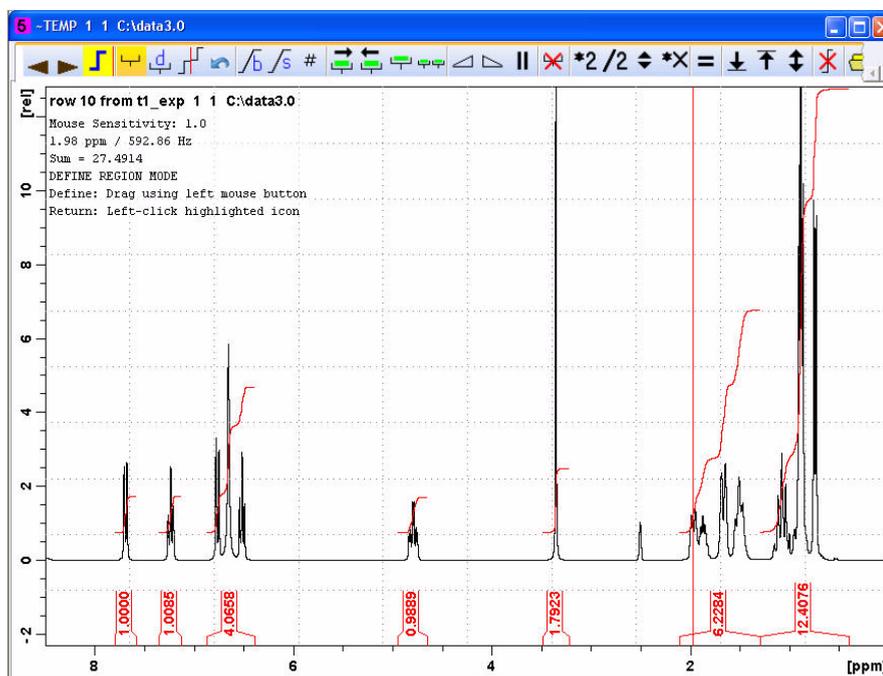
Figure 8.22



9. Click on **OK**

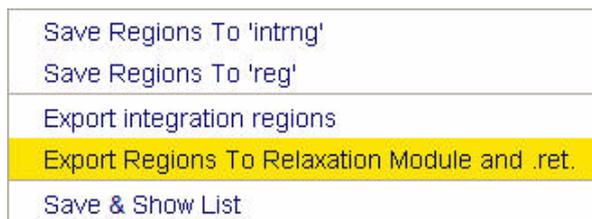
10. Define the regions by clicking the left mouse button and the use of the cursor lines

Figure 8.23



11. Click on 

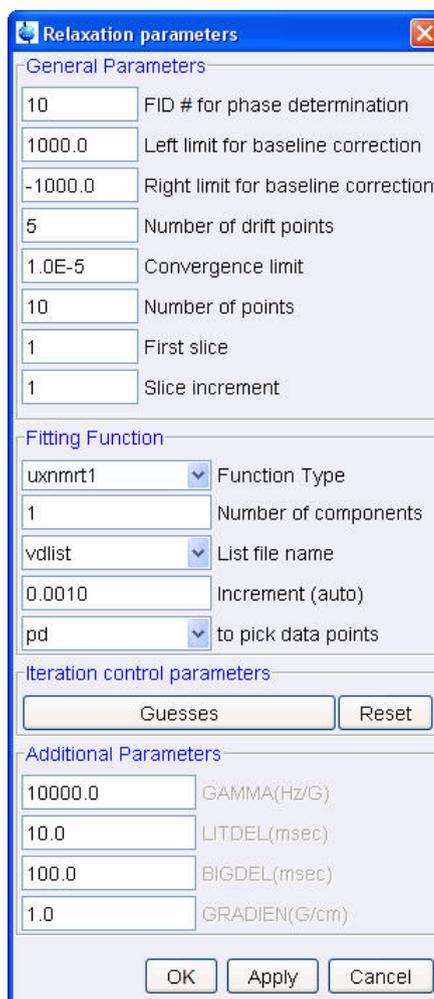
Figure 8.24



12. Select **'Export Region To Relaxation Module'** by clicking on it

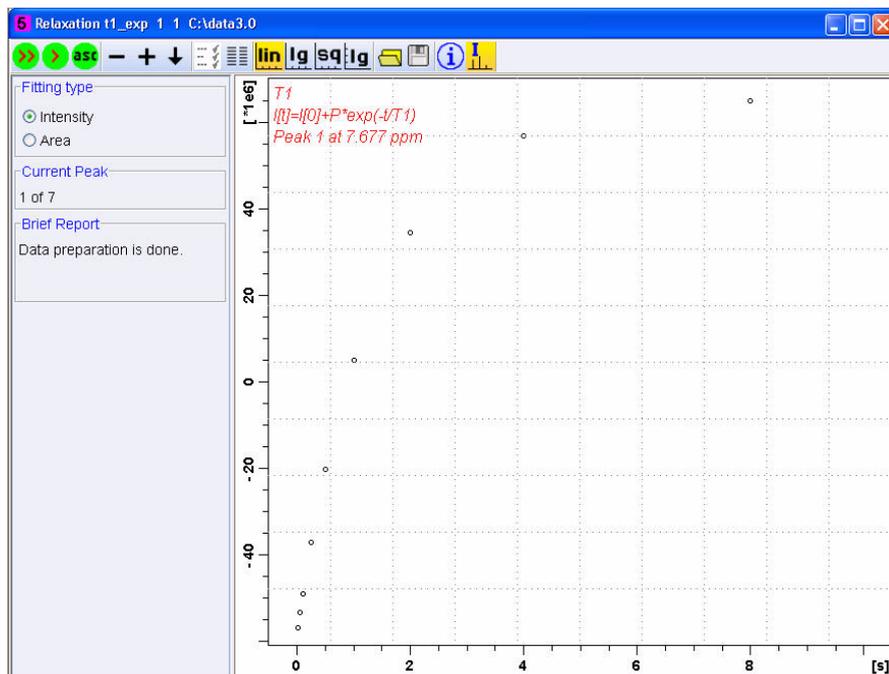
13. Click on  **Relaxation** **'Relaxation Window'**

Figure 8.25



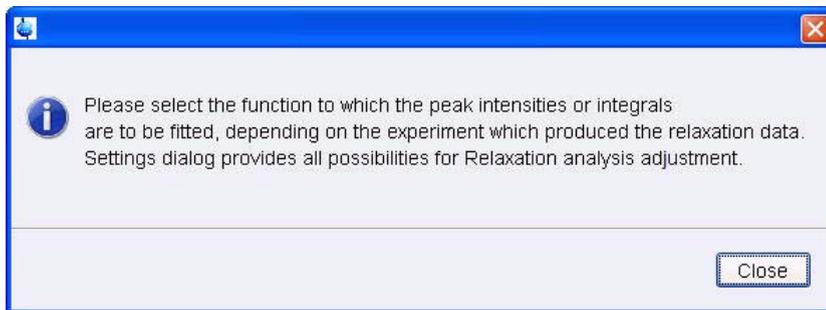
14. Click on 

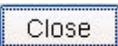
Figure 8.26



15. Click on  **Fitting** 'Fitting Functions'

Figure 8.27



16. Read the message and then click on 

17. Click on 

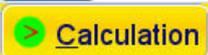
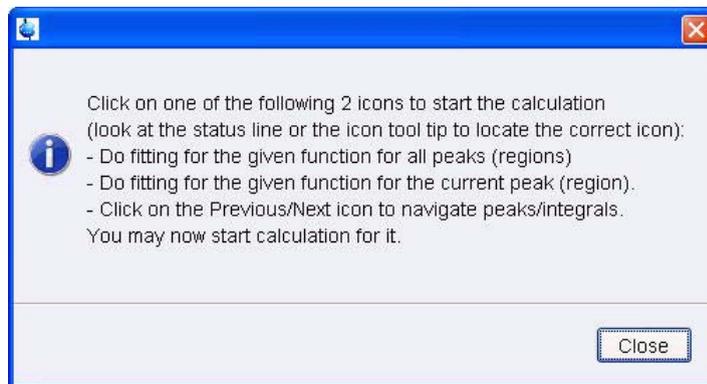
18. Click on  **Calculation** 'Start Calculating'

Figure 8.28



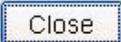
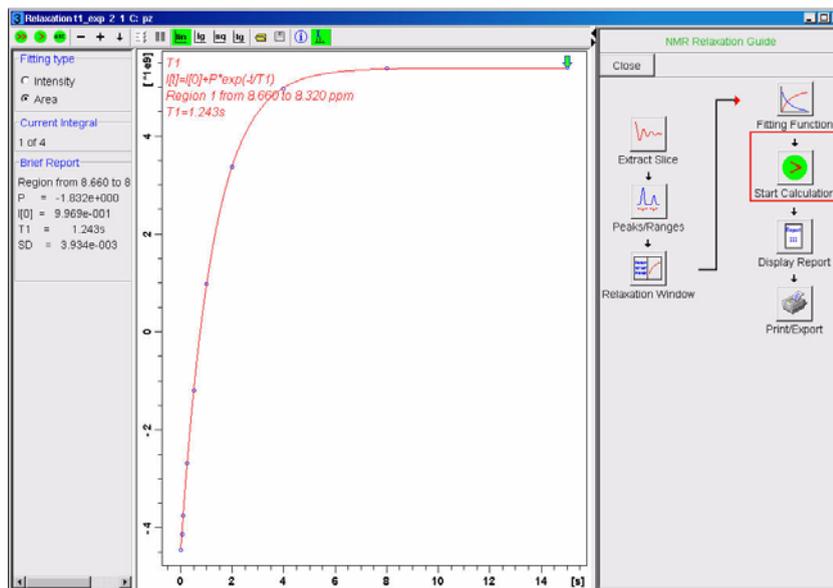
19. Click on 
20. Select Area for 'Fitting Type'

Figure 8.29



21. In the T1 data display window click on  to calculate all regions

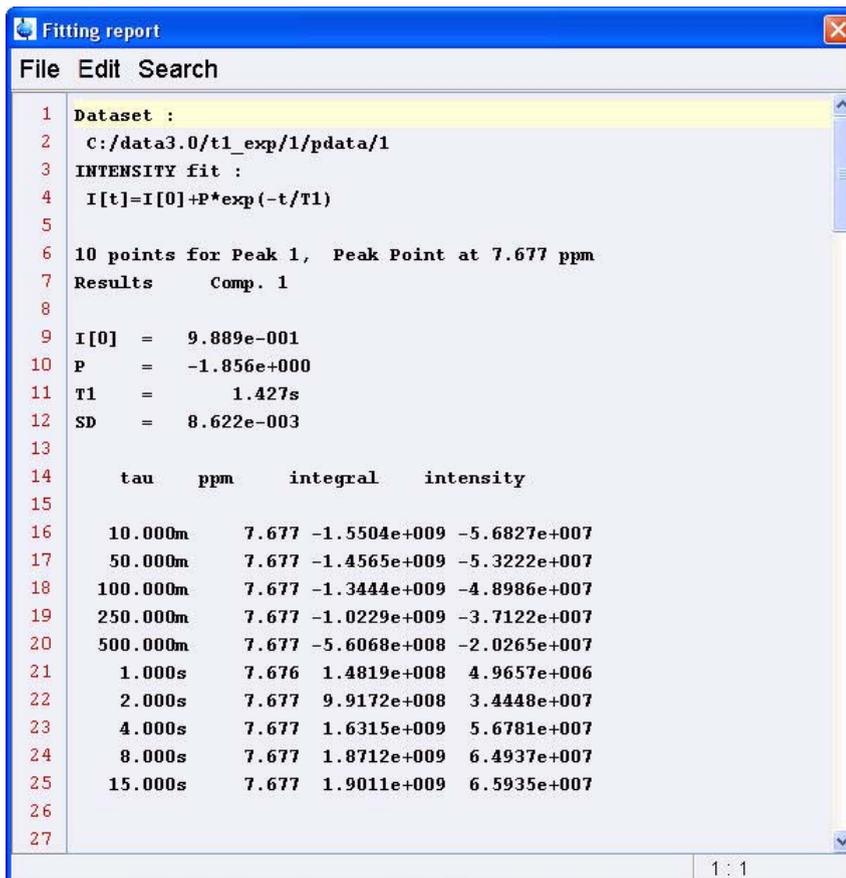
Figure 8.30

```

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

22. Click on  'Display Report'

Figure 8.31



8.3 Observations



Bruker BioSpin, your solution partner

Bruker BioSpin provides a world class, market-leading range of analysis solutions for your life and materials science needs.

Our solution-oriented approach enables us to work closely with you to further establish your specific needs and determine the relevant solution package from our comprehensive range, or even collaborate with you on new developments.

Our ongoing efforts and considerable investment in research and development illustrates our long-term commitment to technological innovation on behalf of our customers. With more than 40 years of experience meeting the professional scientific sector's needs across a range of disciplines, Bruker BioSpin has built an enviable rapport with the scientific community and various specialist fields through understanding specific demand, and providing attentive and responsive service.

● **Bruker BioSpin Group**

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