Vanderbilt NMR Facilities

Instructions for Setup of 2D Heteronuclear Experiments Using TOPSPIN

INDEX

1. 2D Acquisition Prerequisites

The common procedures involved in the setup and processing of 2D heteronuclear experiments are described in this section. Specifics for each heteronuclear experiment have been provided in separate section.

1.1. Tuning the Probe

The tuning of both nuclei, ^{13}C and ^{1}H, are necessary for the 2D heteronuclear experiment (see Tuning Tutorial for details)

- Tune the lowest frequency first.
- Select the **TUNE** icon, type **atma,** or type **wobb f2** and **wobb f1** to tune the carbon and proton channels.

1.2. 1D ^{1}H Reference Spectrum

- Calibrate the 90° 1H pulse (see 90° Pulse Width Determination Tutorial for details)
- Acquire a 1D ^{1}H reference spectrum. (rpar AAA_proton.MV all, lock, tune, and shim – see separate Topspin manuals).
- Fourier Transform and reference the spectrum.
- Check for lineshape and spectral quality. This spectrum may be used for the 1H projection axis in the 2D heteronuclear plot.
- Note the spectrum reference, type **SR**.
- Optional: Adjustment of parameters **SW** and **O1**
    - Using the cursors, select the region of the 1D spectrum containing all your signals. Include a minimum of 0.5 ppm of baseline on either side of the spectrum.
    - Define **SW** and **O1** by selecting the icon  from the toolbar. (Fig. 1.1)

Figure 1 .1

    - Optional: Acquire a 1D ^{13}C reference spectrum.

1.3. The 2D Heteronuclear ^{1}H ^{13}C Experiment

- *Samples in organic solvents:* The default parameters in the "**AA_**..." experiments typically produce usable spectra without modification.
- The determination of **P1,** the 90° ^{1}H pulse, is required. (Section 1.2)
- *Aqueous samples* will require customization of the experimental parameters. All experiment specific parameters (ASED) must be double checked, and set correctly, for the experiment to yield a good data set.
- Check the **PulseProg** tab or Parameter Tables (page 18 ff) for information on specific parameter settings.

1.3.1. **ASED** (experiment specific acquisition parameters)

- Type **ased** or click on the custom button in the toolbar. (Fig. 1.2)

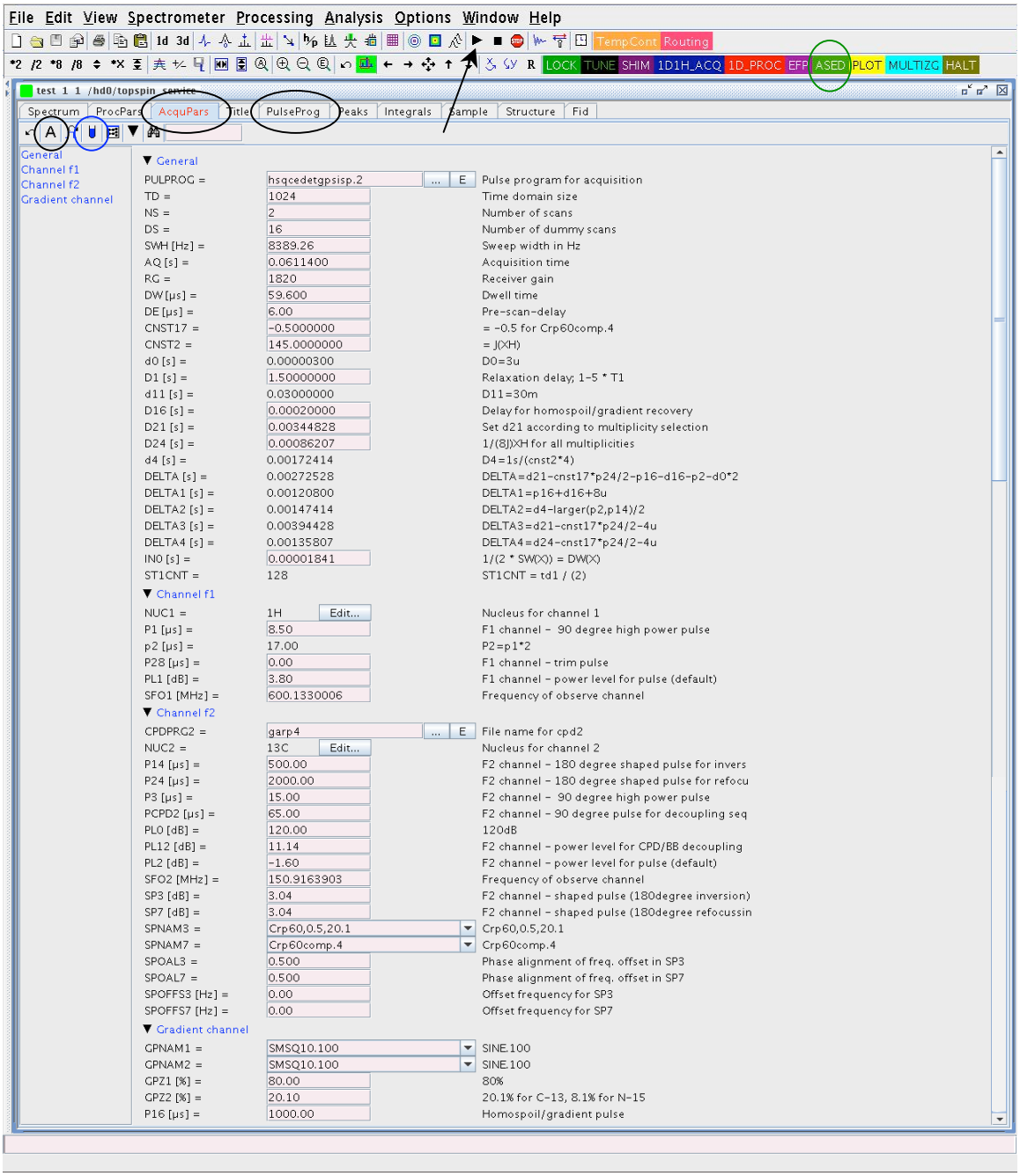

Figure 1.2

- Click on the **getprosol** icon.  (Fig. 1.2) This command loads appropriate pulses and gradient delays from a configuration table.
- For all solvents: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the calibrated proton pulse width <P1> and the corresponding pulse power <PL1> determined in Section 1.2. This command recalculates all the proton pulses according to the calibrated values entered.

- Verify the correctness of **all** parameters in the ASED window.

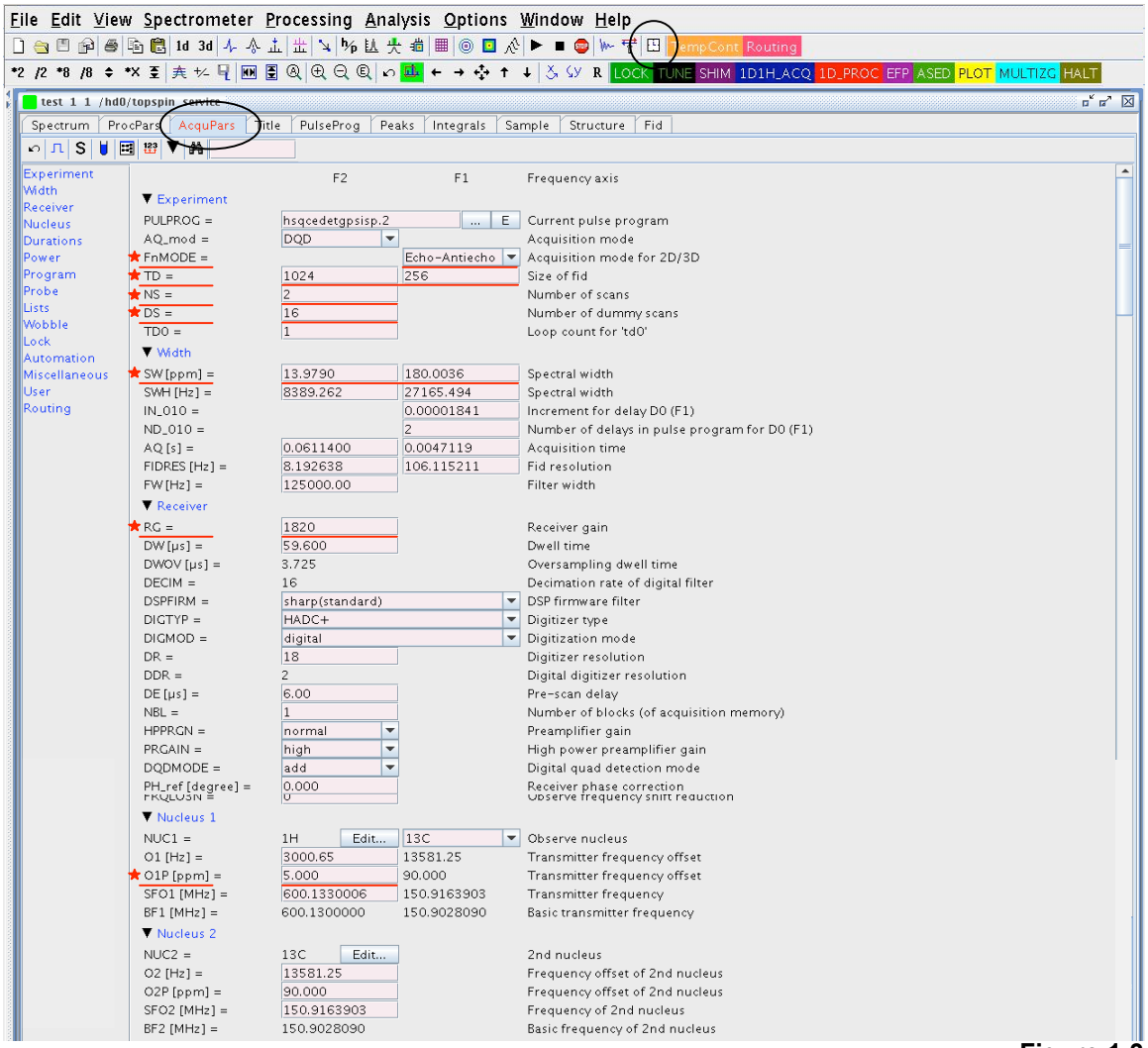

Figure 1.3

1.3.2. **EDA** (complete acquisition parameter list)

- Type **eda**, or click on **A** in the ASED window toolbar. (Fig. 1.2)
- Update **SW** and **O1**, as determined in Section 1.2.
- **RG** default values have been set according to the instrument specifications and normally do not need to be changed.
- Check the parameters for correctness, as outlined in the directions for the specific 2D experiment.
- Selection Tabs, listed in the left column of the window, are linked to associated parameters. (Fig. 1.3)

Time Estimate and Adjustment for 2D Experiment:

- To calculate the experiment time click on the clock in the toolbar  or type **expt**. (Fig. 1.3)
- Note: either changing **TD**(F1), or **NS**, will affect the experiment time the most.

- Changing **TD**(F1) directly affects the resolution in the indirect dimension; **NS** the signal intensity.
- Acquire the experiment with **zg** or select the ▶ button from the toolbar.
- 2D experiments can be stopped prematurely using the command, **stop** or **halt.** This affects the digital resolution in the indirect dimension.
- Multiple experiments may be queued by using the **multizg** command. The experiments must be sequentially numbered, and the first in the series displayed in the current window. If Topspin 2.0 or greater is used, experiments will be spooled, in the order executed, using the **zg** command.

1.4. Processing the 2D Spectrum

1.4.1. **RSER** (command line)

This command extracts single FID's out of a nD dataset. Without further argument, the 1D FID will be stored in the ~TEMP directory.
Example: **rser 1 99** extracts the first FID of an nD and saves the FID in experiment # 99 of the current project (any argument numbers are possible)

1.4.2. **EDP** (processing parameter list)

- Type **edp**, or select the "**ProcPars**" tab. (Fig. 1.4)
- Check the parameters for correctness, as outlined in the directions for the specific 2D experiments (chapters 2 -5).
- Use **xfb** to transform both dimensions of the spectrum.
- Phase the spectrum according to the directions for the specific 2D experiment.
- Use **abs1** and **abs2** to correct the baseline of the spectrum in either dimension.
- Using **xfb n** to transform the spectrum deletes the imaginary parts of the spectrum. The resulting data is only ¼ of the original size. The spectrum has to be phased properly prior to using the **xfb n** command.

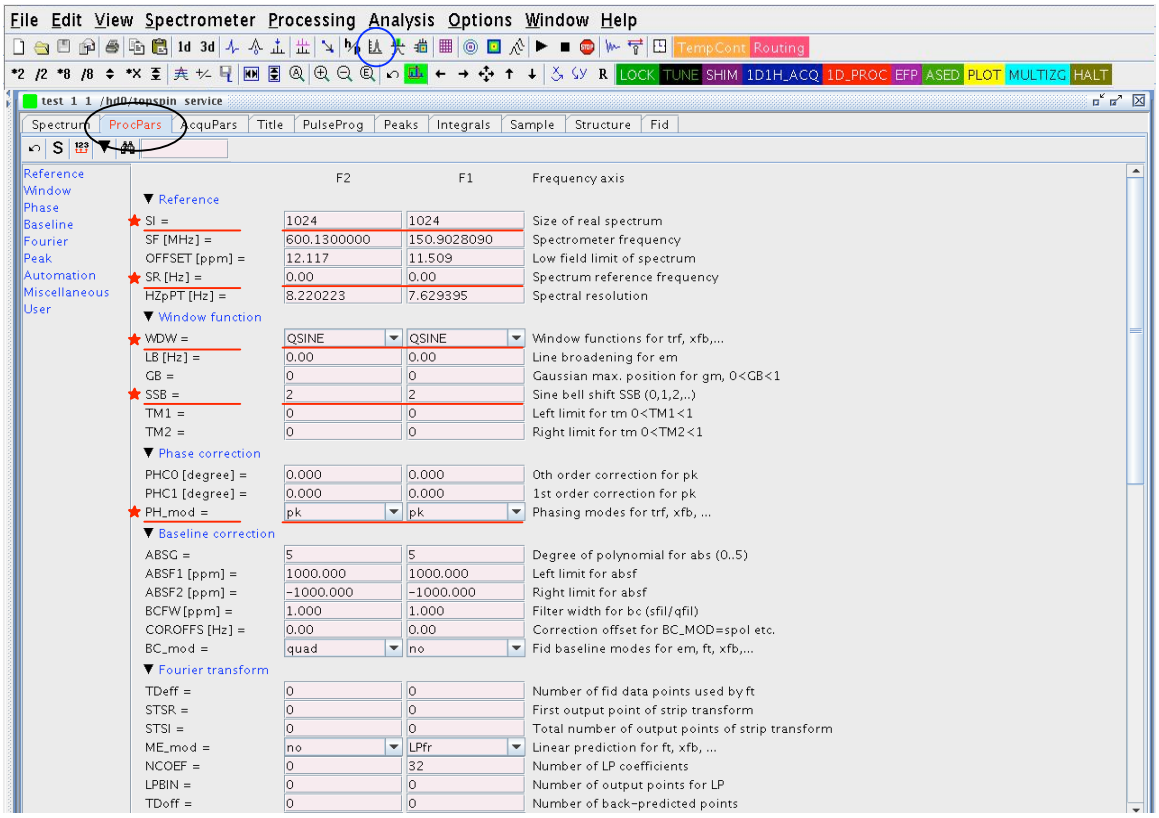

Figure 1.4

1.4.3. Contour Level Adjustment

Adjust the contour levels to improve visibility. Type the following command sequence:

nlev 21 (number of levels displayed)

levcalc (calculate contour level setting)

1.4.4. Projections

To set the 1D projections first select the icon.  (Fig. 1.4)

- Click the right mouse button inside the F2 projection and select "**External Projection**". (Fig. 1.5)
- Change the **EXPNO** to that of the reference spectrum collected in "**2D Acquisition Prerequisites**" (Section 1.2).
- Repeat the process for F1, if desired.

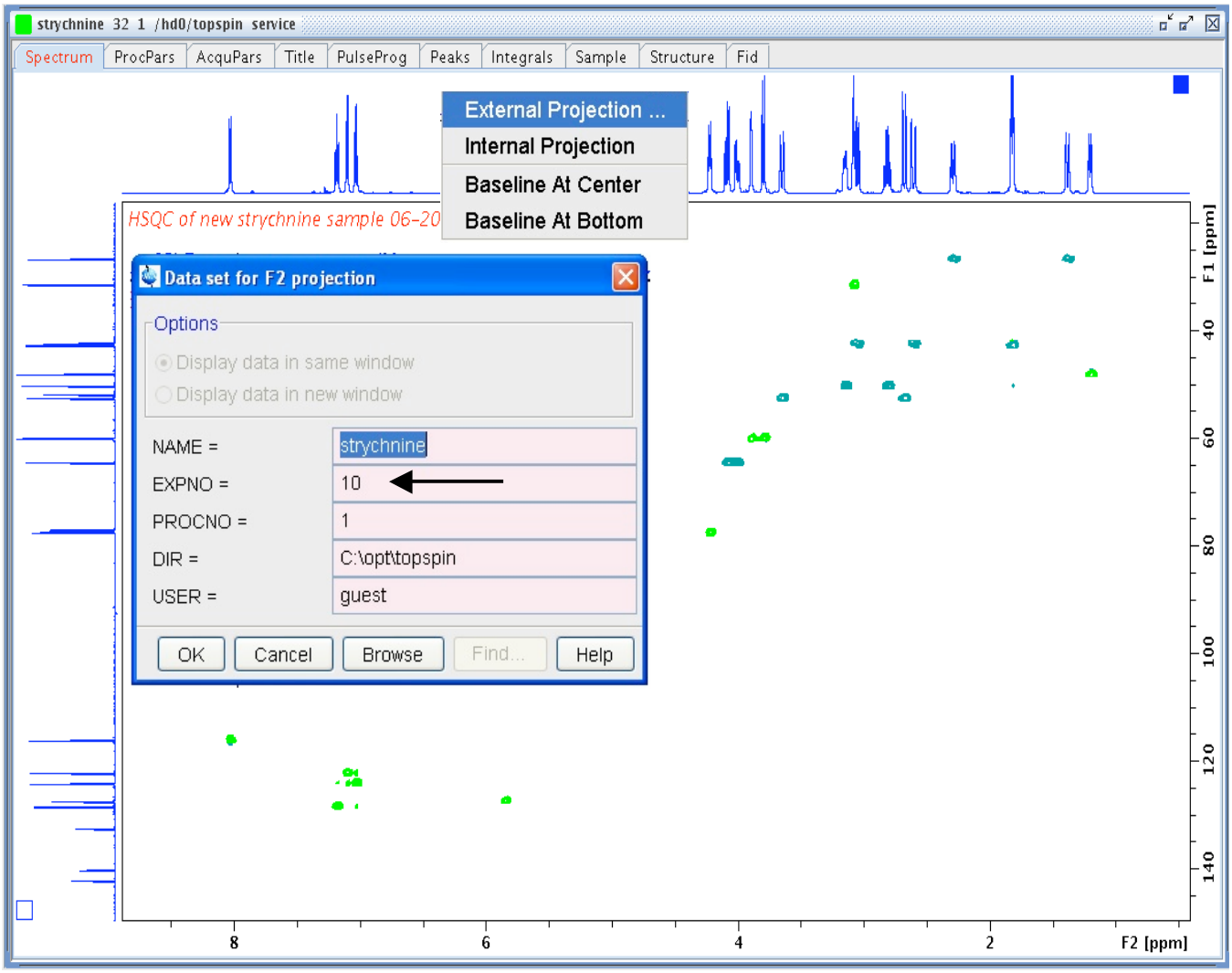
strychnine 32 1 /hd0/topspin service
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Fid
External Projection ...
Internal Projection
Baseline At Center
Baseline At Bottom
HSQC of new strychnine sample 06-20
Data set for F2 projection
Options
Display data in same window
Display data in new window
NAME =
strychnine
EXPNO =
10
PROCNO =
1
DIR =
C:\opt\topspin
USER =
guest
OK
Cancel
Browse
Find...
Help
F1 [ppm]
F2 [ppm]

Figure 1.5

2. HSQC

2.1. Experiment

- Run a 1D proton reference experiment. (Section 1.2)
- The precise determination of the 90° 1H pulse, **P1,** is required. (Section 1.2)
- In a new experiment, use default parameters "**AA_HSQC-multsp.MV**". (**rpar AA_ HSQC-multsp.MV all**)
- Users with aqueous samples will need to customize the experimental parameters.
- Check the **PulseProg** tab for directions indicating which parameter settings to use.
- Tune the ^{13}C and ^{1}H channels of the probe, respectively. (Section 1.1)

2.2. Acquisition

2.2.1. ASED (experiment specific acquisition parameters)

- Click on the **getprosol** icon 
- Verify the correctness of all parameters in this window.
- Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from Section 1.2.
- The J(XH) coupling constant, **CNST2 = 125 – 250Hz** (145 Hz ~ $^{1}J(C,H)$).
- The multiplicity selection, **D21 = 1 - 10ms;** = $(2J(XH))^{-1}$**.**

2.2.2. EDA (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = Echo-Antiecho**
- **TD = 1k** for F2, and **= 256** for F1
- **NS = 2**
- **DS >= 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the 1D reference spectrum.
- **ND010 = 2**

Receiver Tab:
- Do not adjust **RG.** Use the default value.

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with **zg** or select the ▶ button in the toolbar. **GS** may be used to further optimize the parameters after the start of the acquisition.

2.3. Processing

2.3.1. EDP (processing parameter list)

Reference Tab:
- **SI = 1k** for F1 and F2
- **SF = 13C** freq for F1 and **1H** freq F2 (MHz).
- **SR** = value copy and pasted from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 2** for F1 and F2.

Phase Tab:
- **PH_MOD = pk** for F2 and F1.

2.3.2. Process Spectrum

- **xfb** to transform both dimensions.
- Adjust the contour levels to improve visibility. (Section 1.4.2)
- Set the projections. (Section 1.4.3)
- **abs1** followed by **abs2** to subtract out the baseline noise.

2.3.3. Phase 2D Spectrum Interactively

- Enter the 2D phase mode by selecting the icon. 
- In the spectrum window, right click on a peak in the aromatic 1H region (7 – 8 ppm), and select "**Add**". Add a few more peaks from this region. (Fig. 2.1) If no peaks exist in the aromatic region, then pick the peak closest to 0 ppm, it has a high probability of being a -CH3 group.
- As with a DEPT 135, C-H and C-H_3 peaks are phased positive and CH_2 peaks negative.
- Click on the row icon, **R**, to phase the horizontal (F2) dimension. Select the "**0**" icon to apply zero order phasing, and the "**1**" icon to apply 1st order phasing. (Fig. 2.2) Insets in the figures 2.2 and 2.3, show the selected contour profiles before and after being phased "positive".
- Repeat this process for the "columns" as necessary, using the "**C**" icon to phase the vertical dimension.

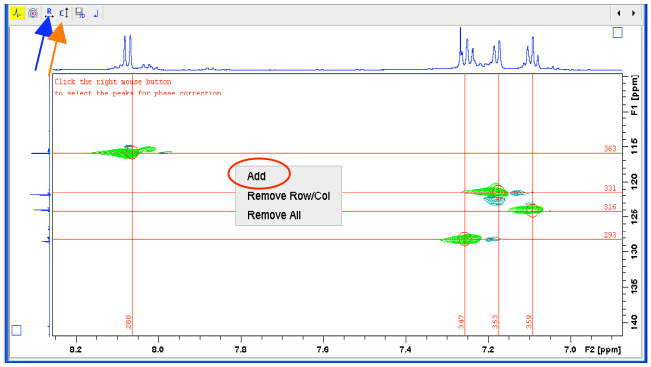

Figure 2.1

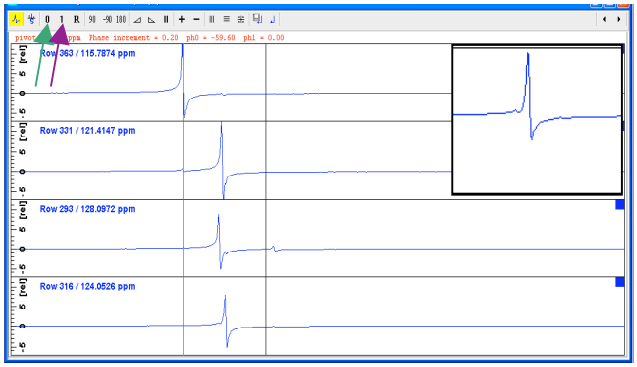

Figure 2.2

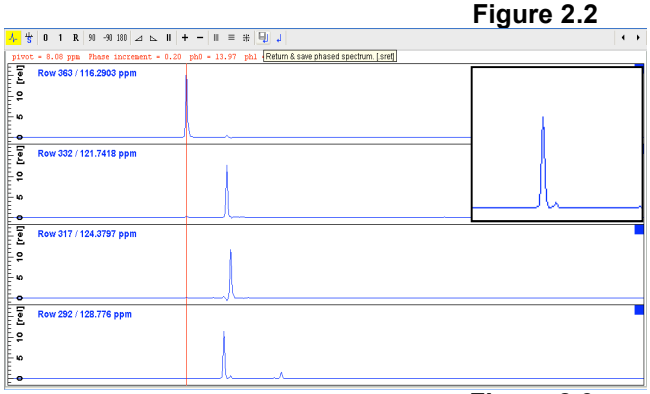

Figure 2.3

3. HMBC

3.1. Experiment

- Run a 1D proton reference experiment. (Section 1.2)
- Determination of the 90° 1H pulse, **P1,** is recommended. (Section 1.2)
- In a new experiment, use default parameters "**AA_HMBC-lpmag.MV**". (**rpar AA_HMBC-lpmag.MV all**)
- Users with aqueous samples will need to customize the experimental parameters.
- Check the **PulseProg** tab for directions indicating which parameter settings to use.
- Tune the ^{13}C and ^{1}H channels of the probe, respectively. (Section 1.1)

3.2. Acquisition

3.2.1. ASED (experiment specific acquisition parameters)

- Click on the **getprosol** icon. 
- Verify the correctness of all parameters in this window.
- Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from Section 1.2.
- The J(XH) coupling constant, **CNST2 = 125 - 250Hz** (145 Hz ~ ^{1}J(C,H)).
- Optimize J(XH) for long range couplings, **CNST13 = 5 - 15 Hz** (9 Hz ~ ^{1}J(C,H)).

3.2.2. EDA (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = QF**
- **TD = 2k** for F2, and **= 256** for F1
- **NS = 2**
- **DS >= 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the 1D reference spectrum.
- **ND010 = 2**

Receiver Tab:
- Do not adjust **RG.** Use the default value.

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with zg or select the ▶ button in the toolbar.

3.3. Processing

3.3.1. EDP (processing parameter list)

Reference Tab:
- **SI = = 1k** for F1 and F2.

- **SF = 13C** freq for F1 and **1H** freq F2 (MHz).
- **SR** = value copy and pasted from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 0** for F1 and F2.

Phase Tab:
- **PH_MOD = NO** for F2, and **= MC** for F1.

3.3.2. Process Spectrum

- **xfb** to transform both dimensions.
- Adjust the contour levels to improve visibility. (Section 1.4.2)
- Set the projections. (Section 1.4.3)
- **abs1** followed by **abs2** to subtract out the baseline noise.

4. H2BC

4.1. Experiment

- Run a 1D proton reference experiment. (Section 1.2)
- The precise determination of the 90° 1H pulse, **P1,** is required. (Section 1.2)
- In a new experiment, use default parameters "**AA_H2BCetgp.MV**". (**rpar AA_H2BCetgp.MV all**)
- Users with aqueous samples will need to customize the experimental parameters.
- Check the **PulseProg** tab for directions indicating which parameter settings to use.
- Tune the ^{13}C and ^{1}H channels of the probe, respectively. (Section 1.1)

4.2. Acquisition

4.2.1. **ASED** (experiment specific acquisition parameters)

- Click on the **getprosol** icon 
- Verify the correctness of all parameters in this window
- For aqueous solutions: Type: **getprosol 1H** <P1>**us** <PL1>**db**; inserting the values from Section 1.2.
- The MIN ^{1}J (XH) coupling constant, **CNST6 = 125 - 250Hz** (125 Hz ~ $^{1}J(C,H)$).
- The MAX ^{1}J (XH) coupling constant, **CNST7 = 125 - 250Hz** (170 Hz ~ $^{1}J(C,H)$).

4.2.2. **EDA** (complete acquisition parameter list)

Headings from the left column in the EDA window are linked to the parameters listed in **bold**. Check the following parameters in this window:

Experiment Tab:
- **AQ_mod = DQD**
- **FNMODE = Echo-Antiecho**
- **TD = 1k** for F2, and **= 256** for F1
- **NS = 2**
- **DS >= 16**

Width Tab:
- Update **SW**. If using non-default values, copy and paste from the 1D reference spectrum.
- **ND010 = 2**

Receiver Tab:
- Do not adjust **RG.** Use the default value.

Nucleus Tab:
- Update **O1**. If using a non-default value, copy and paste from the 1D reference spectrum.

Acquire the experiment with zg or select the ▶ button in the toolbar. GS may be used to further optimize the parameters after the start of the acquisition.

4.3. Processing

4.3.1. **EDP** (processing parameter list)

Reference Tab:
- **SI = 1k** for F1 and F2
- **SF = 13C** freq for F1 and **1H** freq F2 (MHz).
- **SR** = value copy and pasted from the 1D reference spectrum.

Window Tab:
- **WDW = QSINE** for F1 and F2
- **SSB = 2** for F1 and F2.

Phase Tab:
- **PH_MOD = pk** for both, F2 and F1

4.3.2. Processing Spectrum

- xfb to transform both dimensions.
- Adjust the contour levels to improve visibility (see section 1.4.2)
- Set the projections (see section 1.4.3)
- Use abs1 and abs2 to correct the baseline of the spectrum.

4.3.3. Phasing Spectrum

- Enter the 2D phase mode by selecting the icon 
- In the spectrum window, the peaks will appear in sets of 2 (anti-phase). Right click on one of these peaks, and select "**Add**". Select a few more peaks of the same color. (Fig. 4.1)
- Click on the row icon, **R**, to phase the horizontal (F2) dimension. Select the "**0**" icon to apply zero order phasing, and the "**1**" icon to apply 1st order phasing. (Fig. 4.3) Processing will be easiest if the peaks are phased positively. Insets in the figures 4.2 and 4.3, show the selected contour profiles before and after being phased "positive".
- Repeat this process for the "columns" as necessary, using the "**C**" icon to phase the vertical dimension.

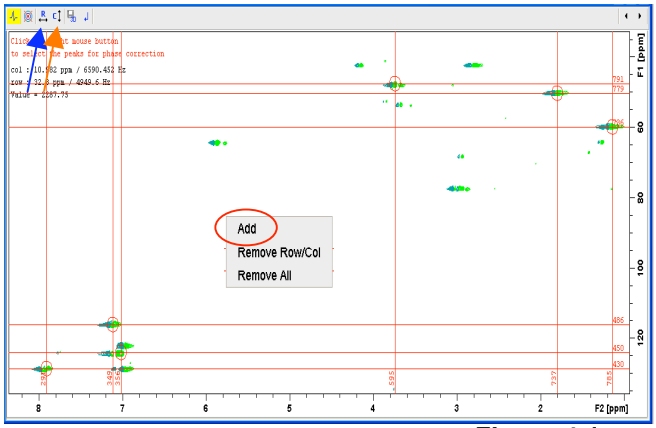

Figure 4.1

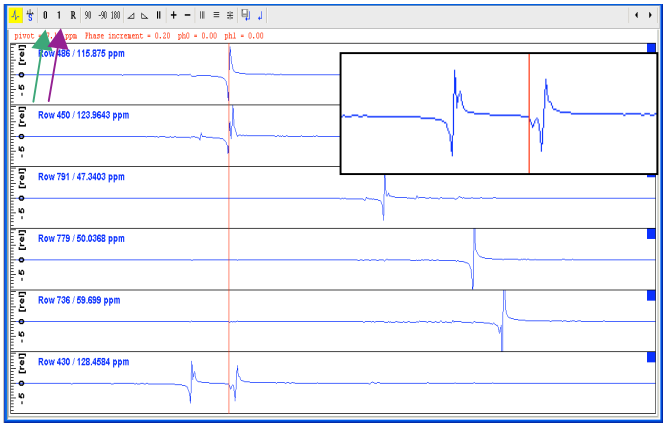

Figure 4.2

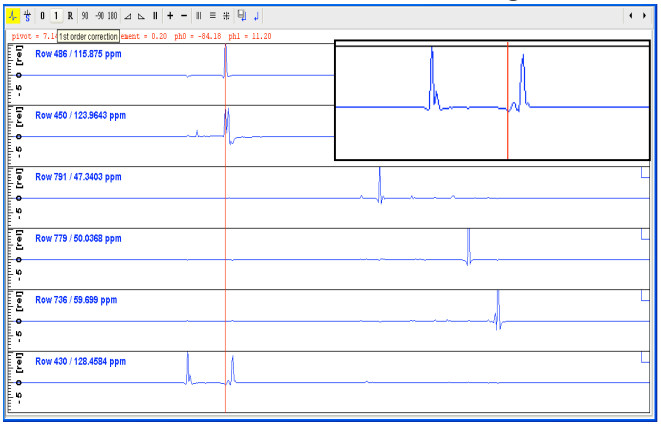

Figure 4.3

5. Parameter Tables

Summary of HMBC, H2BC, and HSQC parameters

NOTE: Changing one parameter might impact others, hence the integrity of all acquisition parameters must be verified. This can be done using the resources found in the pulse sequence description in the **PulseProg** tab, or by requesting help from a facility staff member.

ASED parameters

Experiment	Sensitivity Improved HSQC	Magnitude HMBC	Phase Sensitive H2BC
Parameter set	A_HSQC-multsp.MV,	A_HMBC-lpmag.MV	A_H2BCetgp.MV
Bruker PP name	hsqcedetgpsisp.2	hmbcgplpndqf	h2bcetgpl3
Description	Phased, decoupling, Sensitivity improved, 2D H-1/X correlation with double inept transfer (trim pulses), multiplicity editing	No phasing, 2D H-1/X correlation, long range coupling optimized	Phased, multiplicity edited, 2D H-1/X correlation, 3-fold low pass J-filter
CNST	CNST2 = J(XH)	CNST2 = J(XH) CNST13 = 5-15 Hz J(XH)	CNST6 = J(XH) min, CNST7 = J(XH) max
D21 (mult. sel.)	$(2J(XH))^{-1}$; XH, XH3 = +, XH2 = -	N/A	$(2J'(HH))^{-1}$
D24	$(8J(XH))^{-1}$; all multiplicities	N/A	N/A
P1	pw(^{1}H, 90°) @ PL1	P1(^{1}H, 90°) @ PL1	P1(^{1}H, 90°) @ PL1
P3	pw(^{13}C, 90°) @ PL2	P3(^{13}C, 90°) @ PL2	P3(^{13}C, 90°) @ PL2
PCPD2	^{1}H decoupling pulse	N/A	^{1}H decoupling pulse
PL1	^{1}H channel power level	^{1}H channel power level	^{1}H channel power level
PL2	^{13}C channel power level	^{13}C channel power level	^{13}C channel power level
GPNAM1 GPNAM2 GPNAM3 (1)	SMSQ10.100	SMSQ10.100	SMSQ10.100
GPZ1	80% for ^{13}C	50% for ^{13}C	80% for ^{13}C
GPZ2	20.1% for ^{13}C	30% for ^{13}C	N/A
GPZ3	N/A	40.1% for ^{13}C	N/A

(1) **SINE.100** may be used as well in these experiments, but is considered less efficient

EDA parameters

Bruker PP name	hsqcedetgpsisp.2	hmbcgplpndqf	h2bcetgpl3
FnMODE	Echo/Antiecho	QF	Echo/Antiecho
TD F2	512, **1024**, 2048	1024, **2048**, 4096	512, **1024**, 2048
TD F1	128, **256**, 512	128, **256**, 512, 1024	128, **256**, 512, 1024
NS (minimum)(2)	2	2	2
SW (F1, F2)	Compound specific	Compound specific	Compound specific
ND_010	2	2	2

(2) **NS** may be increased by multiples of n (integers)

EDP parameters

Bruker PP name	hsqcedetgpsisp.2	hmbcgplpndqf	h2bcetgpl3
SR (F1, F2)	values from ref. spectra	values from ref. spectra	values from ref. spectra
WDW (F1=F2)	QSINE	QSINE	QSINE
SSB (F1=F2)	2 - 3	0	2 - 3
PH_mod F2	pk	no	pk
PH_mod F1	**pk**, no	mc	**pk** , no
F2: PHC0, PHC1	determine	N/A	determine
F1: PHC0, PHC1	determine	N/A	determine