Vanderbilt NMR Facilities

Instructions for Setup of 2D Heteronuclear Experiments Using TOPSPIN

INDEX

1. 20	D Acquisition Prerequisites	2
1.1.	Tuning the Probe	2
1.2.	1D ¹ H Reference Spectrum	2
1.3.	The 2D Heteronuclear ¹ H ¹³ C Experiment	2
1.4.	Processing the 2D Spectrum	5
2. H	ISQC	
2.1.	Experiment	8
2.2.	Acquisition	8
2.3.	Processing	8
3. H	IMBC	
3.1.	Experiment	10
3.2.	Acquisition	10
3.3.	Processing	10
4. H	2BC	
4.1.	Experiment	12
4.2.	Acquisition	12
4.3.	Processing	12
5. Pa	arameter Tables	14
Sum	nmary of HMBC, H2BC, and HSQC parameters	14

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, ¹³C and ¹H, are necessary for the 2D heteronuclear experiment (see Tuning Tutorial for details)

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

1.2. 1D ¹H Reference Spectrum

- Calibrate the 90° 1H pulse (see 90° Pulse Width Determination Tutorial for details)
- Acquire a 1D ¹H 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 **b** from the toolbar. (Fig. 1.1)

<u>F</u> ile	<u>E</u> dit	⊻ie	v <u>S</u> pe	ctror	neter	Proc	essing	<u>A</u> naly	rsis	<u>O</u> pt	ions	<u>W</u> indow	i <u>H</u> elp				
	3 🗏 é	2 3	Ð 🖪	2d 3d	1. ~	. く 並	」出	` ₄ ʰ∕p	赵 ;			= 👓 🕪 *	7 🖽 🗸	्र 🕹 🗖	empCont R	outing	1 🔁 3 🕨
*2 /	2 *8 /	8 🗢	E HH	& €	ତ୍ପ୍	Q N	→ 🛄	↔ →	⊬ -	+ ₹	‡ 4	LOCK TU	JNE SHIM	1D1H_A	CQ 1D_PRO	C EFP ASEC	<mark>PLOT MULTIZG</mark> H
-																	Figure 1 .1

• Optional: Acquire a 1D ¹³C reference spectrum.

1.3. The 2D Heteronuclear ¹H ¹³C 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)

File Edit View	Spectrometer Pro	cessing Analysis	Options W	indow Help	
n 🔿 🖪 🏟 👼 🖻	🖺 1d 3d 👍 🙏 📩	出 い か は チ 道 [∎ ⊚ ⊡ ∧ ∎	► ■ 📾 🔤 🖶 TempCont Routing	
*2 /2 *8 /8 ≜ *X					ALT
		4 444 •• <mark>•</mark> •	/	O 37 X LOCK TONE SHIM IDITIACY IDITION OF A SUBJECT MORTED T	
test 1 1 /hd0/to	pspin service		/	- Ya Ya-1	
Spectrum ProcPa	ars AcquPars itle	PulseProg Peaks II	ntegrals Samp	ble Structure Fid	
		\smile	,		
General Chappel 51	▼ General				^
Channel f2	PULPROG =	hsqcedetgpsisp.2	E	Pulse program for acquisition	
Gradient channel	TD =	1024		Time domain size	
	NS =	2		Number of scans	
	DS = SWH [H=1 -	16		Number of dummy scans Sweep width in Hz	
	AQ [s] =	0.0611400		Acquisition time	
	RG =	1820		Receiver gain	
	DW [µs] =	59.600		Dwell time	
	DE [µs] =	6.00		Pre-scan-delay	
	CNST17 =	-0.5000000		= -0.5 for Crp60comp.4	
	CNST2 =	145.0000000		= J(XH)	
	dU[s] =	1 50000000		DU=30 Relaxation delay: 1-5 * T1	
	d11[s] =	0.03000000		D11=30m	
	D16[s] =	0.00020000		Delay for homospoil/gradient recovery	
	D21[s] =	0.00344828		Set d21 according to multiplicity selection	
	D24 [s] =	0.00086207		1/(8J)XH for all multiplicities	
	d4 [s] =	0.00172414		D4=1s/(cnst2*4)	
	DELTA [s] =	0.00272528		DELTA = d21-cnst17*p24/2-p16-d16-p2-d0*2	
	DELTAI [s] =	0.00120800		DELTA1=p16+d16+8u DELTA2=d4-larger(p2.p14)/2	
	DELTA2 [5] =	0.00394428		DELTA2=04 (arger(p2,p14))2	
	DELTA4 [s] =	0.00135807		DELTA4=d24-cnst17*p24/2-4u	
	INO [s] =	0.00001841		1/(2 * SW(X)) = DW(X)	
	ST1CNT =	128		ST1CNT = td1 / (2)	
	▼ Channel f1				
	NUC1 =	1H Edit		Nucleus for channel 1	
	P1 [µs] =	8.50		F1 channel – 90 degree high power pulse	
	p2 [µs] =	17.00		P2=p1*2	
	P20 [µs] =	3.80		F1 channel - unimpulse F1 channel - nower level for pulse (default)	
	SFO1 [MHz] =	600.1330006		Frequency of observe channel	
	▼ Channel f2				
	CPDPRG2 =	garp4	E	File name for cpd2	
	NUC2 =	13C Edit		Nucleus for channel 2	
	P14 [µs] =	500.00		F2 channel – 180 degree shaped pulse for invers	
	P24 [µs] =	2000.00		F2 channel – 180 degree shaped pulse for refocu	
	P3 [µs] =	15.00		F2 channel – 90 degree high power pulse	
	PLO [dB] =	120.00		120dB	
	PL12 [dB] =	11.14		F2 channel – power level for CPD/BB decoupling	
	PL2 [dB] =	-1.60		F2 channel – power level for pulse (default)	
	SFO2 [MHz] =	150.9163903		Frequency of observe channel	
	SP3 [dB] =	3.04		F2 channel – shaped pulse (180degree inversion)	
	SP7 [dB] =	3.04		F2 channel – shaped pulse (180degree refocussin	
	SPNAM3 = SPNAM7 -	Crp60;0.5;20.1		Crp60;0.5,20.1	
	SPOAL3 =	0.500		Phase alignment of freq. offset in SP3	
	SPOAL7 =	0.500		Phase alignment of freq. offset in SP7	
	SPOFFS3 [Hz] =	0.00		Offset frequency for SP3	
	SPOFFS7 [Hz] =	0.00		Offset frequency for SP7	
	Gradient channel				
	GPNAM1 =	SMSQ10.100	-	SINE.100	
	GPNAM2 =	SMSQ10.100	-	SINE.100	
	GP21 [%] = CP72 [%] -	20.10		00% 20.1% for C=13, 8.1% for N=15	
	P16 [µs] =	1000.00		Homospoil/gradient pulse	
Ľ					

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 <u>all</u> parameters in the ASED window.



- 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 is 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 **b** 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.

Image: Set	<u>File</u> <u>E</u> dit ⊻ie	w <u>S</u> pectrometer <u>I</u>	Processing Ana	alysis <u>O</u> ptions	<u>W</u> indow <u>H</u> elp
*2 /2 *8 /8 ** × ≦ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ ★ <td< th=""><th>0 🔄 🖱 🖗 🛎</th><th>🗈 🔀 1d 3d 🎶 🚸 .</th><th>山出、外区</th><th>🕂 🏥 🔘 🛄 🔥</th><th>· ▶ ■ 🐵 🐆 향 🖽 TempCont Routing</th></td<>	0 🔄 🖱 🖗 🛎	🗈 🔀 1d 3d 🎶 🚸 .	山出、外区	🕂 🏥 🔘 🛄 🔥	· ▶ ■ 🐵 🐆 향 🖽 TempCont Routing
test 1 1 Indifference F2 F1 Frequency axis Reference F2 F1 Frequency axis Window Reference F2 F1 Frequency axis Pase Baseline Size of real spectrum Spectrometer frequency Pointer SF [MHz] = 1024 1024 Size of real spectrum Power SF [MHz] = 600.1300000 150.9028090 Spectrometer frequency Power SF [MHz] = 0.000 0.000 Spectrum reference frequency Power SF [Hz] = 0.000 0.000 Spectrum reference frequency Miscellaneous WDW = 0.000 0.000 Spectrum reference frequency WDW = QSINE Window functions for trf, xfb, Uise broadening for em WDW = QSINE Window functions for trf, xfb, Uise broadening for em WDW = 0.000 0.000 Left limit for trm 0 <tm1<1< td=""> TM2 = 0 0 Register for trm 0<tm1<1< td=""> TM2 = 0 Register for trd 0 PHC0 [degree] = 0.000 0.000 Dooooo Dooooooooooooooooooooooooooooooooo</tm1<1<></tm1<1<>	*2 /2 *8 /8 \$	*X 至 夫 ½ 및 M		o <mark>, 💷 ← → 💠 ↑</mark>	↓ Š SY R LOCK TUNE SHIM 1D1H_ACQ 1D_PROC EFP ASED PLOT MULTIZC HALT
SpectruProcParscquParsTitlePlaseSectronF2F1Frequency axisMindowF2F1PreferenceF2F1Frequency axisMindowPreferencePaseSolutionAutomationMiscellaneousUserQSINEQSINEWindow functionWord word functionWord for functionPreferencePreferencePreferenceQSINEWind for functionWord for function </th <th>test 1 1 /hd</th> <th>0/topspin service</th> <th></th> <th></th> <th></th>	test 1 1 /hd	0/topspin service			
SF2F1Frequency axisReferenceF2F1Frequency axisPhaseSI=10241024SPInseSF[600.1300000150.9028090Spectrometer frequencyOFFSET [ppm] =12.117PeakAutomationSR [Hz] =0.00AutomationSR [Hz] =0.000.00MiscellaneousSpectrum reference frequencyUserWindow functionWindow functions for trf, xfb,UserWindow functionUne broadening for emCB =00Caussian max, position for gm, 0 <cb<1< td="">SSB =22Sine bell shift SSB (0,1,2,)TM 1 =00Left hill for tm 0<tm141< td="">TM 2 =00Right limit for tm 0<tm2<1< td="">V Phase correctionPHC0 [degree] =0.0000.000PHC1 [degree] =0.0000.000V braseline correctionpkpkV Basiline correctionABSG =5ABSC =55ABSC =55ABSC =55ABSC =55ABSC =1000.0001000.000ABSC =55ABSC =55ABSC =1000.0001000.000ABSC =55ABSC =5ABSC =1000.0001000.000ABSC =5ABSC =5ABSC =5ABSC =5ABSC =5ABSC</tm2<1<></tm141<></cb<1<>	Spectrum		le PulseProg Pe	aks Integrals Sa	imple Structure Fid
ReferenceWindow Phase Baseline FourierSI =10241024SF [MH2] =10241024Size of real spectrum Spectrum terfrequency Spectrum for spectrumPeak Automation MiscellaneousSR [H2] =0.000.00SR [H2] =0.000.00Spectrum reference frequency Spectral resolutionWMW =QSINE VMW =QSINE QSINE QSINE VMOW function for gm, 0 <cb<1< td="">WMW =00.000.00User00WDW =00QSINE VBW =00QSINE VBW =00QSINE VBW =00QSINE VBW =00VBW =00VBW =00QSINE VBW =00VBW =0VBW =0<!--</th--><th>n S 🔛 🔻</th><th>m</th><th></th><th></th><th></th></cb<1<>	n S 🔛 🔻	m			
WindowPriceProduction yaxisPhase BaselineSile10241024Fourier PeakSF [MHz] =600.1300000150.9028090Peak Automation MiscellaneousSR [Hz] =0.000.00WDW = LB [Hz] =0.000.00Spectrum reference frequencyWDW = LB [Hz] =0.000.00Casisan max. position for gm, 0 < CB < 1	Reference		52	F1	For many states and the states of the states
Phase Neterine Baseline SI = 1024 1024 Size of real spectrum Fourier SF [MH2] = 600.1300000 150.9028090 Spectrometer frequency Peak OFFSET [ppm] = 12.117 11.509 Low field limit of spectrum Automation SR [H2] = 0.00 Spectrum reference frequency Miscellaneous HZPT [H2] = 8.22023 7.629395 Spectrum reference frequency VBW = QSINE Window functions for trf, xfp, Line broadening for em Line broadening for em User WW = QSINE QSINE Window function for gm, 0 <gb<1< td=""> SSB = 2 2 Sine bell shift SSB (0,1,2,) TM1 = D 0 Q QSINE Window function for gm, 0<gb<1< td=""> TM2 = 0 0 Line broadening for em Line broadening for em UB (H2) = 0.000 Line broadening for em Line broadening for em Line broadening for em UB (H2) = 0.000 Dool Caussian max. position for gn, 0<gb<1< td=""> String for tro o<tm1< th=""><th>Window</th><th>• D.6</th><th>F2</th><th>F1</th><th>Frequency axis</th></tm1<></gb<1<></gb<1<></gb<1<>	Window	• D.6	F2	F1	Frequency axis
Baseline SI = 1024 1024 Size of real spectrum Fourier SF [MHz] = 600.1300000 150.9022090 Spectrometer frequency Peak OFFSET [ppm] = 12.117 11.509 Low field limit of spectrum Automation Miscellaneous SR [Hz] = 0.00 0.00 Spectrometer frequency Miscellaneous VMdew function Vindow function Vindow function of the broadening for em Set of a spectrum reference frequency VBW = QSINE QSINE Window function for gm, 0 <cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Window function for gm, 0<cb<1< td=""> VBW = QSINE QSINE Nine for the 0 VBW = QSINE QSINE Nine for the 0 VB = QSINE QS</cb<1<></cb<1<></cb<1<></cb<1<></cb<1<></cb<1<></cb<1<>	Phase	Keterence			
FourierSr [MHz] =600.1300000150.3020305Spectrometer trequencyPeakOFFSET [ppm] =12.11711.509Low field limit of spectrumAutomationSR [Hz] =0.000.00Spectrum reference frequencyMiscellaneousHZpPT [Hz] =8.2202237.629395Spectral resolutionV Mdow functionVMdow functionVMdow functions for trf, xfb,UserQSINEQSINEWindow functions for gm, 0 <gb<1< td="">SS =00.00Gaussian max, position for gm, 0<gb<1< td="">CB =00Left limit for tm 0<tm1<1< td="">TM1 =00Left limit for tm 0<tm2<1< td="">TM2 =0.0000.000Oth order correction for pkPHC0 [degree] =0.0000.0001st order correction for pkPHC1 [degree] =0.0000.0001st order correction for pkPH_mod =pkphasPhasing modes for trf, xfb,V Baseline correctionABSG =5Degree of polynomial for abs (0.5)ABSG =55Degree of polynomial for abs (0.5)ABSF1 [ppm] =1000.0001000.000Left limit for absfBCFW [ppm] =1.0001.000Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<></gb<1<></gb<1<>	Baseline	SI =	1024	1024	Size of real spectrum
Peak OF-SE1 [ppm] = 12.117 11.509 Low Held limit of spectrum Automation SR [H2] = 0.00 0.00 Spectrum reference frequency Miscellaneous HZpPT [H2] = 8.220223 7.629395 Spectrum reference frequency User WDW = QSINE ▼ QSINE ▼ QSINE ▼ Window functions for trf, xfb, Image: Comparison of the provided in the provid	Fourier	SF [MHz] =	600.1300000	150.9028090	Spectrometer frequency
Automation SR [Hz] = 0.00 0.00 Spectrum reference frequency Miscellaneous HZpPT [Hz] = 8.220223 7.629395 Spectral resolution VBW 0.00 0.00 Line broadening for em B GB = 0 0 Caussian max. position for gm, 0 <gb<1< td=""> SSB = 2 2 Sine bell shift SSB (0,1,2,) TM1 = 0 0 Left limit for tm 0<tm1<1< td=""> TM2 = 0 0 Right limit for tm 0<tm2<1< td=""> V Phase correction PHC0 [degree] = 0.000 Oth order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk PH_mod = pk pk Phasing modes for trf, xfb, V Baseline correction ABSC = 5 Degree of polynomial for abs(05) ABSC [ppm] = 1000.000 1000.000 Left limit for absf ABSC [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<></gb<1<>	Peak	OFFSET [ppm] =	12.117	11.509	Low field limit of spectrum
HzpPI [Hz] =Szectral resolutionUserSpectral resolution \forall Whdow functionWhdow functions for trf, xfb, \forall WDW =QSINE \checkmark QSINE \checkmark Whdow functions for trf, xfb, B [Hz] =0.00Line broadening for em $GB =$ 0Gaussian max, position for gm, 0 <gb<1< th="">B [Hz] =0Caussian max, position for gm, 0<gb<1< th="">$CB =$0Caussian max, position for gm, 0<gb<1< th="">$GB =$0Caussian max, position for gm, 0<gb<1< th="">$TM =$0Caussian max, position for mo$TM =$0Caussian max, position for TM1<1</gb<1<></gb<1<></gb<1<></gb<1<></gb<1<></gb<1<></gb<1<></gb<1<></gb<1<></gb<1<>	Miccelleneous	SR [Hz] =	0.00	0.00	Spectrum reference frequency
▼ Wndow function WDW = QSINE ▼ QSINE ▼ Wndow functions for trf, xfb, LB [H2] = 0.00 0.00 Line broadening for em GB = 0 0 Gaussian max. position for gm, 0 <gb<1< td=""> SSB = 2 2 Sine bell shift SSB (0, 1, 2,) TM1 = 0 0 Left limit for tm 0<tm1<1< td=""> TM2 = 0 0 Right limit for tm 0<tm2<1< td=""> ▼ Phase correction PHC0 [degree] = 0.000 PHC1 [degree] = 0.000 0.000 1st order correction for pk PHC1 [degree] = pk ▼ pk ♥ Phasing modes for trf, xfb, ▼ Baseline correction ABSC = 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 1000.000 Left limit for absf ABSF1 ABSF2 [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<></gb<1<>	User	HZpP1[Hz] =	8.220223	7.629395	Spectral resolution
WDW= QSINE QSINE Window functions for trf, xtb, LB [H2] = 0.00 0.00 Line broadening for em GB = 0 0 Gaussian max. position for gm, 0 <ge<1< td=""> SSB = 2 2 Sine bell shift SSB (0,1,2,) TM1 = 0 0 Left limit for tm 0<tm1<1< td=""> TM2 = 0 0 Right limit for tm 0<tm2<1< td=""> V Phase correction 0.000 Oth order correction for pk PHC0 [degree] = 0.000 0.000 VB1 [degree] = 0.000 O.000 VB2 0 0 VB3 PHC [degree] = 0.000 VB4 pk pk V PHand = pk pk VB3 D00.000 Left limit for abs for tf, xfb, V Baseline correction ABSC = 5 ABSC 1 [ppm] = 1000.000 Left limit for abs f ABSC 2 [ppm] = 1000.000 Right limit for abs f ABSF1 [ppm] = 1000.000 Hord constructions for tf, rdp abs f BCFW [ppm] = 1.000 1000.000 Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<></ge<1<>		Window function			
LB [Hz] = 0.00 0.00 Line broadening for em CB = 0 0 Gaussian max. position for gm, 0 < CB < 1 SSB = 2 2 Sine bell shift SSB (0, 1, 2,) TM1 = 0 0 Left limit for tm 0 < TM1 < 1 TM2 = 0 0 Right limit for tm 0 < TM2 < 1 ♥ Phase correction PHC0 [degree] = 0.000 0.000 PHC1 [degree] = 0.000 0.000 1st order correction for pk PH_mod = pk ♥ Phasing modes for trf, xfb, ♥ ABSG = 5 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 (ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)		🛨 WDW =	QSINE	QSINE 💌	Window functions for trf, xfb,
GB = 0 0 Gaussian max. position for gm, 0 <gb<1< td=""> SSB = 2 2 Sine bell shift SSB (0,1,2,) TM1 = 0 0 Left limit for tm 0<tm1<1< td=""> TM2 = 0 0 Right limit for tm 0<tm2<1< td=""> ▼ Phase correction PHC0 [degree] = 0.000 0.000 PHC1 [degree] = 0.000 0.000 1st order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk PHC3 pk< pk Phasing modes for trf, xfb, ▼ Baseline correction Degree of polynomial for abs (0.5) ABSG = 5 5 Degree of polynomial for abs (0.5) ABSF1 [ppm] = 1000.000 1000.000 Left limit for absf ABSF2 [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<></gb<1<>		LB [Hz] =	0.00	0.00	Line broadening for em
\$ SSB = 2 2 Sine bell shift SSB (0,1,2,) TM1 = 0 0 Left limit for tm 0 <tm1<1< td=""> TM2 = 0 0 Right limit for tm 0<tm2<1< td=""> V Phase correction 0 0.000 Oth order correction for pk PHC0 [degree] = 0.000 0.000 Ist order correction for pk PHC1 [degree] = 0.000 0.000 Ist order correction for pk V PH.mod = pk< v pk V Phasing modes for trf, xfb, V Baseline correction ABSC = 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 -1000.000 Right limit for abs f ABSF2 [ppm] = -1000.000 Filter width for bc (sfil/qfil)</tm2<1<></tm1<1<>		GB =	0	0	Gaussian max. position for gm, 0 <gb<1< th=""></gb<1<>
TM1 = 0 0 Left limit for tm 0 < TM1 <1 TM2 = 0 0 Right limit for tm 0 < TM2 <1 V Phase correction PHC0 [degree] = 0.000 0.000 Oth order correction for pk PH2 [degree] = 0.000 0.000 1st order correction for pk PH_mod = pk Phasing modes for trf, xfb, V Baseline correction ABSC = 5 5 ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 (ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 1.000		🗙 SSB =	2	2	Sine bell shift SSB (0,1,2,)
TM2 = 0 0 Right limit for tm 0 <tm2<1< td=""> ▼ Phase correction PHC0 [degree] = 0.000 0.000 Oth order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk </tm2<1<>		TM1 =	0	0	Left limit for tm 0 <tm1<1< th=""></tm1<1<>
▼ Phase correction PHC0 [degree] = 0.000 0.000 0th order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk PHC1 [degree] = pk ▼ [pk ♥ Phasing modes for trf, xfb, ▼ Baseline correction ABSC = 5 Degree of polynomial for abs (0.5) ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 [ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 Filter width for bc (sfil/qfil)		TM2 =	0	0	Right limit for tm 0 <tm2<1< td=""></tm2<1<>
PHC0 [degree] = 0.000 0.000 Oth order correction for pk PHC1 [degree] = 0.000 0.000 1st order correction for pk PH_mod = pk pk Phasing modes for trf, xfb, V Baseline correction ABSC = 5 Degree of polynomial for abs (05) ABSC 1 [ppm] = 1000.000 Left limit for absf ABSC 2 [ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 Filter width for bc (sfil/qfil)		Phase correction			
PHC1 [degree] = 0.000 1st order correction for pk ★ PH_mod = pk pk Phasing modes for trf, xfb, ▼ Baseline correction ABSC = 5 ABSC = 5 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 [ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 1.000		PHC0 [degree] =	0.000	0.000	Oth order correction for pk
★ PH_mod = pk ▼ Phasing modes for trf, xfb, ▼ Baseline correction ABSC = 5 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 [ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 1.000		PHC1 [degree] =	0.000	0.000	1st order correction for pk
✓ Baseline correction ABSC = 5 5 Degree of polynomial for abs (0.5) ABSF1 [ppm] = 1000.000 1000.000 Left limit for absf ABSF2 [ppm] = -1000.000 -1000.000 Right limit for absf BCFW [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)		📌 PH_mod =	pk 🗖	🖌 pk 🔍 💌	Phasing modes for trf, xfb,
ABSC = 5 5 Degree of polynomial for abs (05) ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 (ppm] = -1000.000 Right limit for absf BCFW [ppm] = 1.000 Filter width for bc (sfil/qfil)		Baseline correction	1		
ABSF1 [ppm] = 1000.000 Left limit for absf ABSF2 [ppm] = -1000.000 -1000.000 BCFW [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)		ABSG =	5	5	Degree of polynomial for abs (05)
ABSF2 [ppm] = -1000.000 -1000.000 Right limit for absf BCFW [ppm] = 1.000 1.000 Filter width for bc (sfil/qfil)		ABSF1 [ppm] =	1000.000	1000.000	Left limit for absf
BCFW[ppm] = 1.000 Filter width for bc (sfil/qfil)		ABSF2 [ppm] =	-1000.000	-1000.000	Right limit for absf
		BCFW [ppm] =	1.000	1.000	Filter width for bc (sfil/qfil)
COROFFS [Hz] = 0.00 0.00 Correction offset for BC_MOD=spol etc.		COROFFS [Hz] =	0.00	0.00	Correction offset for BC_MOD=spol etc.
BC_mod = quad 💌 no 💌 Fid baseline modes for em, ft, xfb,		BC_mod =	quad	no 💌	Fid baseline modes for em, ft, xfb,
▼ Fourier transform		▼ Fourier transform			
TDeff = 0 0 Number of fid data points used by ft		TDeff =	0	0	Number of fid data points used by ft
STSR = 0 0 First output point of strip transform		STSR =	0	0	First output point of strip transform
STSI = 0 0 Total number of output points of strip transform		STSI =	0	0	Total number of output points of strip transform
ME_mod = no V LPfr V Linear prediction for ft, xfb,		ME_mod =	no	LPfr 💌	Linear prediction for ft, xfb,
NCOEF = 0 32 Number of LP coefficients		NCOEF =	0	32	Number of LP coefficients
LPBIN = 0 0 Number of output points for LP		LPBIN =	0	0	Number of output points for LP
TDoff = 0 0 Number of back-predicted points		TDoff =	0	0	Number of back-predicted points
Figure 1.4					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.



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 ¹³C and ¹H 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 ~ ¹J(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 expe	riment with za or select the \blacktriangleright button in the toolbar GS may be used to further optimize the				

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.

A

- As with a DEPT 135, C-H and C-H₃ peaks are phased positive and CH₂ 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.



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 ¹³C and ¹H 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 ~ ¹J(C,H)).

U

• 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 ¹³C and ¹H 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 ¹J (XH) coupling constant, **CNST6 = 125 250Hz** (125 Hz \sim ¹J(C,H)).
 - The MAX ¹J (XH) coupling constant, CNST7 = 125 250Hz (170 Hz \sim ¹J(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.



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	ASED	parameters
-----------------	------	------------

Sensitivity Improved HSQC	Magnitude HMBC	Phase Sensitive H2BC
A_HSQC-multsp.MV,	A_HMBC-lpmag.MV	A_H2BCetgp.MV
hsqcedetgpsisp.2	hmbcgplpndqf	h2bcetgpl3
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
CNST2 = J(XH)	CNST2 = J(XH) CNST13 = 5-15 Hz J(XH)	CNST6 = J(XH) min, CNST7 = J(XH) max
(2J(XH)) ⁻¹ ; XH, XH3 = +, XH2 = -	N/A	(2J'(HH)) ⁻¹
(8J(XH)) ⁻¹ ; all multiplicities	N/A	N/A
pw(¹ H, 90 [°]) @ PL1	P1(¹ H, 90 [°]) @ PL1	P1(¹ H, 90 [°]) @ PL1
pw(¹³ C, 90 [°]) @ PL2	P3(¹³ C, 90 [°]) @ PL2	P3(¹³ C, 90 [°]) @ PL2
¹ H decoupling pulse	N/A	¹ H decoupling pulse
¹ H channel power level	¹ H channel power level	¹ H channel power level
¹³ C channel power level	¹³ C channel power level	¹³ C channel power level
SMSQ10.100	SMSQ10.100	SMSQ10.100
80% for ¹³ C	50% for ¹³ C	80% for ¹³ C
20.1% for ¹³ C	30% for ¹³ C	N/A
N/A	40.1% for ¹³ C	N/A
	Sensitivity Improved HSQC A_HSQC-multsp.MV, hsqcedetgpsisp.2 Phased, decoupling, Sensitivity improved, 2D H-1/X correlation with double inept transfer (trim pulses), multiplicity editing CNST2 = J(XH) (2J(XH)) ⁻¹ ; XH, XH3 = +, XH2 = - (8J(XH)) ⁻¹ ; all multiplicities pw(¹ H, 90°) @ PL1 pw(¹³ C, 90°) @ PL2 ¹ H decoupling pulse ¹ H channel power level ¹³ C channel power level SMSQ10.100 80% for ¹³ C 20.1% for ¹³ C N/A	Sensitivity Improved HSQCMagnitude HMBCA_HSQC-multsp.MV,A_HMBC-Ipmag.MVhsqcedetgpsisp.2hmbcgplpndqfPhased, decoupling, Sensitivity improved, 2D H-1/X correlation with double inept transfer (trim pulses), multiplicity editingNo phasing, 2D H-1/X correlation, long range coupling optimizedCNST2 = J(XH)CNST2 = J(XH) CNST13 = 5-15 Hz J(XH) $(2J(XH))^{-1}$; XH, XH3 = +, XH2 = -N/A $(8J(XH))^{-1}$; all multiplicitiesN/A $pw(^{14}, 90^{\circ})$ @ PL1P1(14 , 90°) @ PL2 14 decoupling pulseN/A 14 channel power level 14 channel power level 13 C channel power levelSMSQ10.100 80% for 13 C 50% for 13 C 80% for 13 C 30% for 13 C N/A 40.1% for 13 C

(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, <u>1024</u> , 2048	1024, <u>2048</u> , 4096	512, <u>1024</u> , 2048
TD F1	128, <u>256</u> , 512	128, <u>256</u> , 512, 1024	128, <u>256</u> , 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	<u>pk</u> , no	mc	<u>pk</u> , no
F2: PHC0, PHC1	determine	N/A	determine
F1: PHC0, PHC1	determine	N/A	determine