

2D NMR Spectroscopy with VnmrJ

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Two Dimensional Experiments

- To select a 2D experiments, click on the experiment in the Experimental Protocols tab
- Proton spectra will be automatically run and the spectral window optimized
- Select save scout scans in proton experiment to save all spectra
- Run carbon spectra before “Hetero 2D” experiments to get automated plotting of the 1D spectra along the axes
- For **dilute** samples, increase **Scans per increment**
- For **overlapping or close ¹³C peaks**, increase the **Number of increments**

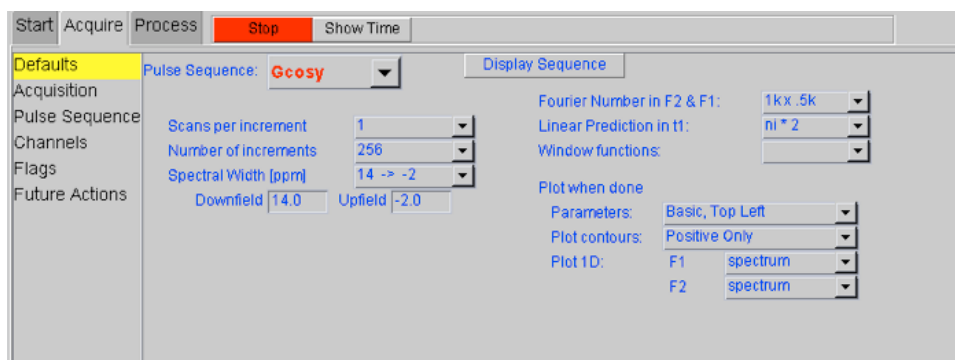
Homonuclear 2D



- Proton-proton correlation (scalar, through bond, or dipole through space)

Gcosy

- 1 scan per increment
- 128 or 256 increments (increase if overlapping proton spectrum)

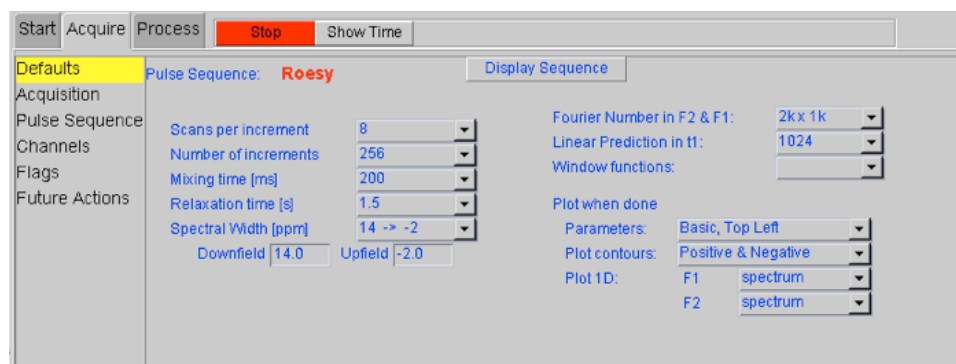


Cosy

- Gcosy, gradient version of Cosy, is preferred and faster
- 4 or 8 scans per increment (increase if very very dilute)

Roesy

- 8 or 16 scans per increments (increase if dilute)
- 128 or 256 increments (increase if overlapping proton spectrum)
- Mixing time 200 to 500 ms (increase for more interactions over longer distances)
- Use when molecular weight is 400 to 2000

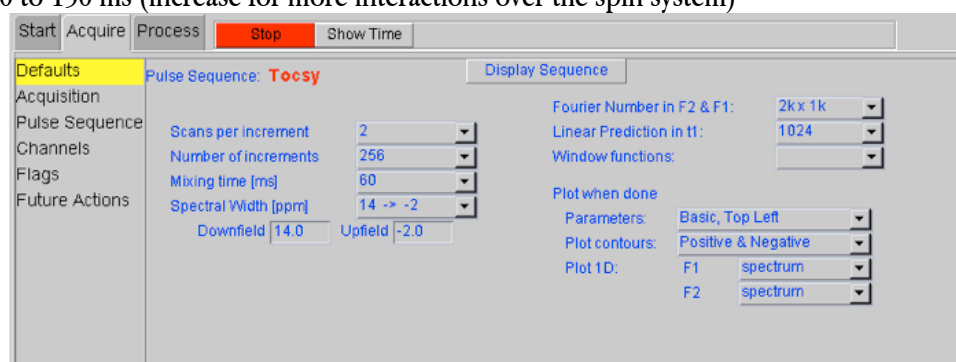


Noesy

- 8 or 16 scans per increment (increase if dilute)
- 128 or 256 increments (increase if overlapping proton spectrum)
- Mixing time 300 to 600 ms (increase for more interactions over longer distances)
- Use when molecular weight is less than 400 or greater than 2000

Tocsy

- 2 or 4 scans per increment (increase if dilute)
- 128 or 256 increments (increase if overlapping proton spectrum)
- Mixing time 40 to 150 ms (increase for more interactions over the spin system)



Ztocsy_zq

- Same as Tocsy but no out-of-phase multiplets (cleaner spectrum)—*preferred*
- Cannot change mix time

Noesy_zq

- Same as Noesy but no out-of-phase multiplets (cleaner spectrum)—*preferred*
- Mix time is in the *Pulse Sequence* page

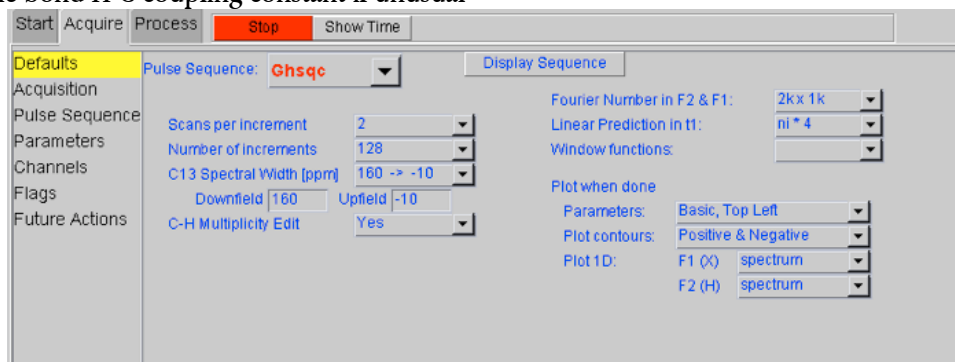
Heteronuclear 2D experiments

- Proton-carbon correlation

Std 1D	Homo 2D	
Hetero 2D	Sel 1D	Dosy 2D
Hmqc		Hmbc
Hsqc		Ghmbc
Ghmqc		Ghsqc
Hmctoxy		Hsqctoxy
Ghsqctoxy		Cigar2[3]

Ghsqcad or *Ghsqc*

- Ghsqcad uses adiabatic sweep pulses to give better carbon 180° pulses
 - Peaks near the edge of the F1 domain are bigger
 - The 1J value is linearly varied across the F1 domain
- Set carbon range if known to increase F1 resolution
- 2 or 4 scans per increment (increase if dilute)
- 128 or 256 increments (increase if carbon spectrum has peaks less than 1 ppm apart)
- Can specify one-bond H-C coupling constant if unusual



Hmqc or *Ghmqc*

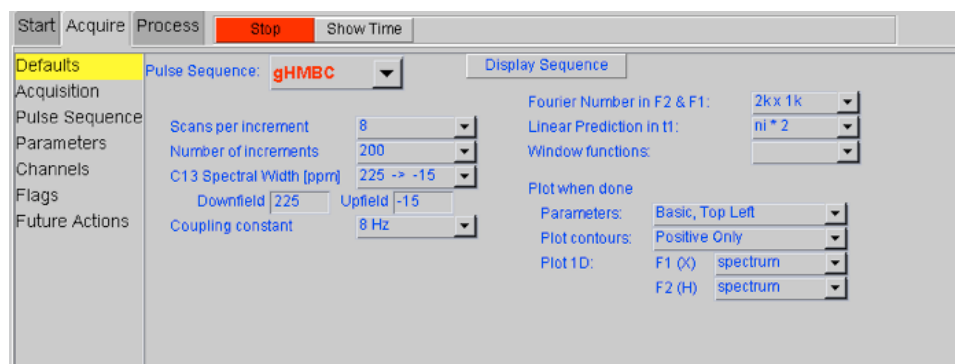
- Use Ghsqcad on Varian 400 or Mercury 400 (with autotuning probe)
- Use the *Ghmqc* on the Mercury 300 (see Ghsqcad for parameters)

Hsqc

- Use Ghsqcad unless very dilute sample
- 8 or 16 scans per increment (increase if dilute)

Ghmbc

- Use 8 or 16 scans per increment (increase if dilute)
- 200 or 400 increments (increase if carbon spectrum has peaks less than 1 ppm apart)
- Set C13 spectral width—important if no carbonyls in the compound
- Set multiple (2 or 3 bond) H-C coupling constant (8 Hz default)
- Can set one bond H-C coupling constant if unusual (130 Hz default)



Hmbc

- Use for dilute samples
- 16 or 32 scans per increment (increase if dilute)
- 256 increments (increase if carbon spectrum has peaks less than 1 ppm apart)
- Set C13 spectral width—important if no carbonyls in the compound
- Set multiple (2 or 3 bond) H-C coupling constant (8 Hz default)
- Can set one bond H-C coupling constant if unusual (130 Hz default)

Cigar2j3j

- Better version of Ghmhc
- Use same parameters as Ghmhc
- Can set a range of multiple-bond H-C coupling constant
- Can set a range of one-bond H-C coupling constant
- Use where H-C coupling constants vary (e.g. heterocycles, aziridines, epoxides)
- Some peaks are weaker or difficult to see

Ghsqctoxy

- Combined Hsqc and Tocsy
- Similar to Ghmhc but quaternary carbons not correlated
- Use instead of Hmqctoxy or Hsqctoxy

Two Dimensional Processing

Two dimensional spectra may be acquired in absolute value (magnitude) or phase sensitive modes. See Table 1 for a list of experiments and the usual acquisition mode. The processing will depend on the acquisition mode.

There are four different cases on processing; each will be discussed below. In each part, there will be common actions. These are:

- setting the linear prediction and zero-filling;
- applying the weighting function;
- applying the Fourier transform;
- symmetrising;
- using expansions, traces and projections;
- peak picking and listing peaks;
- plotting.

In the two dimensional spectra, the directly observed dimension is called f2 (F2, proton). Usually this is the proton dimension in the indirect experiments. The indirectly observed dimension is called f1 (F1, carbon or nitrogen or sili-

con etc) and, for heteronuclear experiments, is usually carbon and for homonuclear experiments, it is proton. The resolution of f_2 depends on the number of points while the resolution of f_1 depends on the number of increments. Increasing the number of points does not increase the length of the experiment significantly, but the length of the experiment is directly related to the number of increments. This usually means the resolution of f_1 is less than that of f_2 . We use linear prediction along f_1 to increase the resolution without increasing experiment time.

Table 1. Indirect 2D Experiments		
	Absolute Value	Phase Sensitive
Homonuclear	Gcosy, Gcosy45	Gdqcosy, Tocsy, Roesy
Heteronuclear	Hmbc [†] , Ghmbc, Cigar2j3j	Ghsqc, Ghsqcad

[†] May be mixed mode (phase sensitive in f_1)

Linear Prediction and Zero Filling

Linear prediction is an invaluable tool for increasing the resolution of 2D spectra by mathematical means. Since the exponential decay functions in the FID are well defined, using linear prediction produces the same result as running a 2D experiment 2 to 8 times longer. We will use linear prediction on f_1 only although it could also be used on f_2

Absolute value spectra are linear predicted only 2 fold because of the sinebell weighting function used. Larger amounts of linear prediction cause artifacts as the actual data used is reduced by the beginning of the sinebell.

Phase sensitive spectra can be linear predicted to larger values, up to 16 fold. If the linear prediction breaks down, large streaks appear in the spectra. Linear prediction requires good signal to noise.

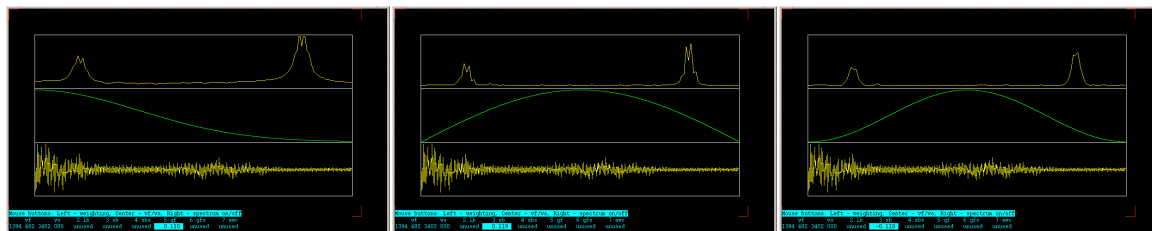
Weighting functions

Exponential (line broadening) weighting functions are used in 1D spectra. Having a weighting function falling to zero removes truncation wiggles—artifacts that look like wiggles at the bottom of the peaks. Since the acquisition time in 2D spectra is much shorter than 1D, this is important.

In the 2D case, different shapes of the weighting functions are used. For phase sensitive spectra, a Gaussian shape is used. The Gaussian function increases the resolution of the spectra while tailing off to zero to remove truncation wiggles.

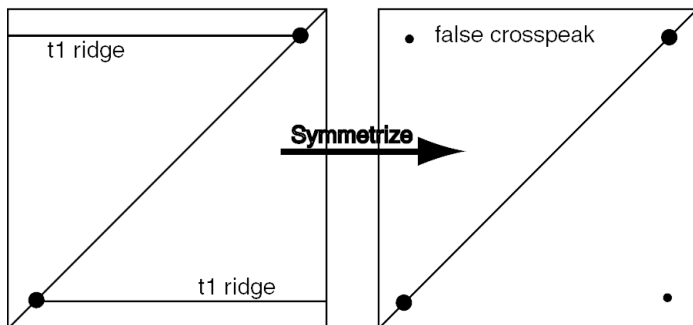
For absolute value mode experiments, a sinebell or squared sinebell is used. The squared sinebell is smoother near the edges but discards more of the FID information. Absolute value mode spectra have large tails around the peaks which may overlap from close peaks. Reducing the intensity at the beginning of the FID reduces these unwanted tails.

Pictures of these functions are shown below. (Left to right: Gaussian, sinebell and squared sinebell)



Symmetrization

Absolute value homonuclear experiments, like the Gcosy, are usually symmetrized (using **Foldt**). Symmetrization removed unwanted t1 noise (lines of noise that run parallel to the f1 axis). Watch for t1 ridges that appear as cross peaks after symmetrizing. It is a good idea to plot the spectrum before symmetrizing.



VnmrJ Processing Pages

Start Acquire Process **Cancel** Process Clear

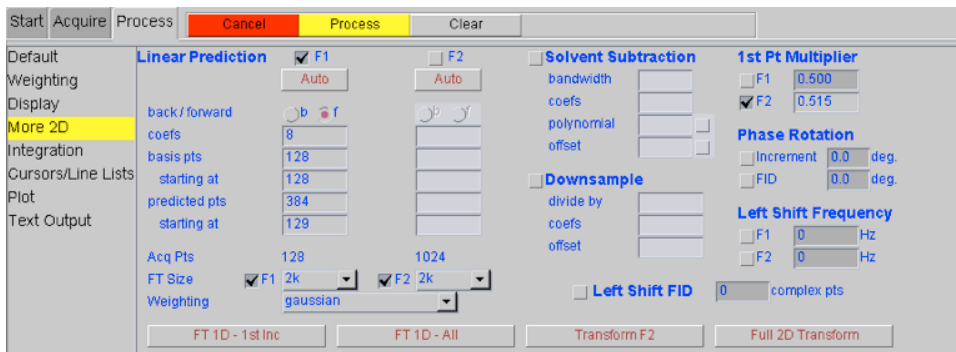
Default Weighting Display More 2D Integration Cursors/Line Lists Plot Text Output	Transform		Display		Display 1D # 1779
	FT Data Size	Acq Pts	Display 2D		Display Text
	<input checked="" type="checkbox"/> F1 1k	128	Display Trace		BC Correct (F1, F2)
	<input checked="" type="checkbox"/> F2 2k	712	Projections Full Screen		DC Correct (F1, F2)
	Transform Coefficients		AutoScale 2D		Referencing Reference F1 by Solvent Reference F2 by Solvent Set F1 cursor to: 0.00 Hz Set F2 cursor to: 0.00 Hz Plot
	1 0 1 0 0 1 0 -1		Trace <input checked="" type="radio"/> F1 <input type="radio"/> F2		
	Weighting: gaussian		Axis Display Mode		
	FT 1D - 1st Increment		F1 PPM Phased		
	FT 1D - All		F2 PPM Phased		
	Transform F2		Linear Prediction		
Full 2D Transform		<input checked="" type="checkbox"/> F1 Auto LP F1			
		<input type="checkbox"/> F2 Auto LP F2			

Start Acquire Process **Cancel** Process Clear

Default Weighting Display More 2D Integration Cursors/Line Lists Plot Text Output	AutoSelect Weighting		Weight Parameters		Transform		
	Both F1&F2 F2 only		F1 F2		FT Data Size	Acq Pts	
	exponential	exponential	line broadening	0.31831	1.5	<input checked="" type="checkbox"/> F1 1k	128
	gaussian	gaussian	sinebell	-0.05	-0.1	<input checked="" type="checkbox"/> F2 2k	712
	sine	sine	shift	0	0	Transform Coefficients	
	sq-sine	sq-sine	gaussian	0.01381	0.09210	1 0 1 0 0 1 0 -1	
	cosine	cosine	shift	0	0	FT 1D - 1st Increment	
	sq-cosine	sq-cosine	additive offset	0	0	FT 1D - All Increments	
	pseudo	pseudo	Interactive Weighting		FT 1D - All		
	res-enhance	res-enhance			Transform F2		
pi4ssbsq	pi4ssbsq			Full 2D Transform			
none	none						

Start Acquire Process **Cancel** Process Clear

Default Weighting Display More 2D Integration Cursors/Line Lists Plot Text Output	Display Mode		Axis		Screen Position		2D Contour Display		Referencing	
	F1 <input checked="" type="radio"/> Phased		F1 <input type="radio"/> Hertz		Full Center		Static + only - only both +/-		Reference F1	
	F2 <input type="radio"/> Absval		F2 <input checked="" type="radio"/> PPM		Left Right		Interactive + only - only both +/-		By Solvent	
	F2 <input type="radio"/> Power		F2 <input type="radio"/> kHz		Projections				Set F1 cursor to: 0.00 Hz	
	F2 <input type="radio"/> None				Display		Baseline Correct 2D		Reference F2	
	F2 <input checked="" type="radio"/> Phased		F2 <input type="radio"/> Hertz		Display 2D		BC F1 BC F2		By Solvent	
	F2 <input type="radio"/> Absval		F2 <input checked="" type="radio"/> PPM		Display Trace		DC F1 DC F2		By TMS	
	F2 <input type="radio"/> Power		F2 <input type="radio"/> kHz		AutoScale 2D				By Cursor	
	F2 <input type="radio"/> None				Message		1D Spectrum		Set F2 cursor to: 0.00 Hz	
	Processing Mode		<input checked="" type="radio"/> Full <input type="radio"/> Partial <input type="radio"/> Off		Foldt Foldj Foldcc Rotate		Display 1D # 155 Autoscale 1D Autophase 1D			
						1D Scaling Abs				



Processing quickly

- Click Auto LP F1
- Select weighting function in menu
- Click **Full 2D transform** to Fourier transform
- Click **Display 2D** to see 2D spectrum

Plotting 2D in Study Queue

- Plot button plots 2D with 1D spectra on sides
 - Only use plot buttons when processing from the study queue

Good values for processing

- ni is the number of increments
- The entry boxes calculate equations (4096-256 or 2*ni)

Gcosy

- Use 2*ni linear prediction in F1
- Use sinebell or sqsinebell weighting function
- Symmetrize (**Foldt**) after processing (*Display* page of *Processing* Tab)
- Absolute value—no phasing

Tocsy

- Use 4*ni linear prediction in F1
- Use gaussian weighting function
- Phase F1 and F2

Roesy

- Use 4*ni linear prediction in F1
- Use gaussian weighting function
- Phase F1 and F2

Noesy

- Use 4*ni linear prediction in F1
- Use gaussian weighting function
- Phase F1 and F2

Ghsqcad or Ghsqc

- Use 4*ni linear prediction in F1
- Use gaussian weighting function
- Phase F1 and F2

Gmbc

- Use 2*ni linear prediction in F1
- Use sinebell weighting function
- Absolute value—no phasing

Cigar2j3j

- Use 2*ni linear prediction in F1
- Use sinebell weighting function
- Absolute value—no phasing

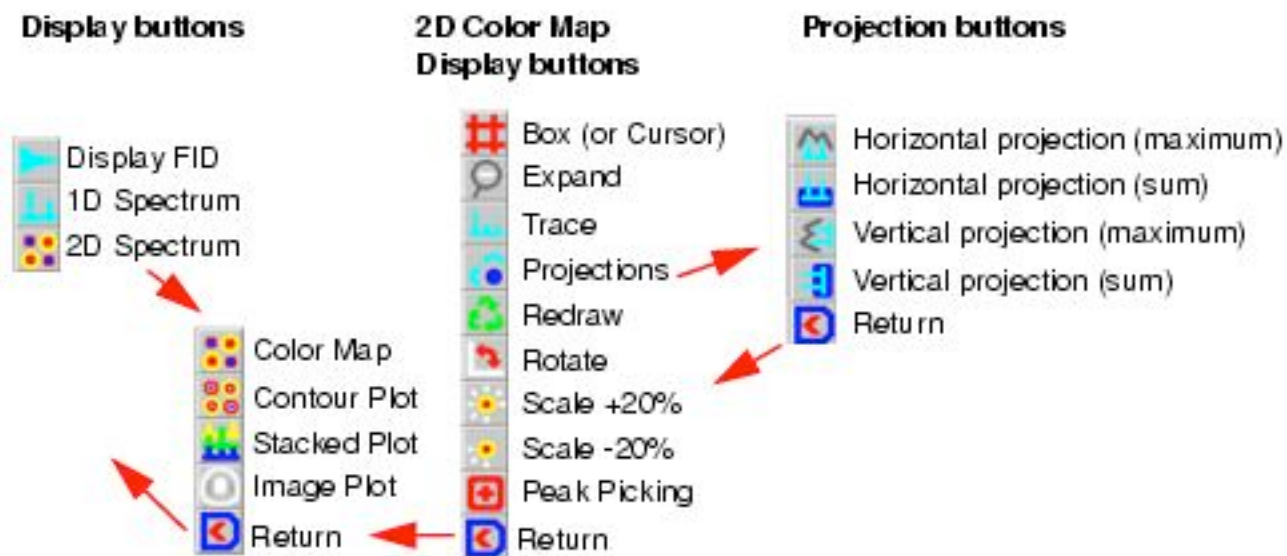
Hmbc

- Use 4*ni linear prediction in F1
- Use sinebell weighting function in F2
- Use gaussian weighting function in F2
- Under *Display mode* choose F1 Phased
- Under *Display mode* choose F2 Abs value
- Phase F1 only

Gbsqctoxy

- Use 4*ni linear prediction in F1
- Use gaussian weighting function
- Phase F1 and F2

2D spectral manipulation



- Use **Color Map** to manipulate the spectrum
- Use the left and right buttons on the mouse to move the two crosshairs
- The middle mouse button controls the vertical scale but using **Scale +20%** is easier
- The threshold is set through the colour bar on the right, middle clicking
- Use both the vertical scale and the threshold to show the contours
- Set the vertical scale so that there are no off-scale (white) peaks and use the threshold to hide noise or impurity peaks

Phasing 2D spectra

- Only phase phase-sensitive experiments
- Under the *Display* page, the processing mode should be **Full**
- Phasing a phase sensitive 2D spectrum requires phasing both dimensions
- Start with f1 (under the *Display* page select *Trace Axis F1*)
- Click the **Phase** button



- Select a trace with a peak in the top right of the spectrum
- The trace should be the maximum intensity for that peak and not the edge of the peak
- Click the **Spectrum 1** button
- Select a peak in the lower left of the spectrum
- Click the **Spectrum 2** button
- Click the **Phase** button; there should only be the **Spectrum 1**, **Spectrum 2** and **Return** buttons
- Click the **Spectrum 1** button
- Phase the right hand side of the trace, ignoring the left hand side
- Click the **Spectrum 2** button
- Phase the left hand side of the trace, ignoring the right hand side
- *IMPORTANT: After displaying the bottom trace, click on "phase" then click the right hand side of the spectrum, then phase on the left hand side. This selects the linear phase mode*
- Click the **Spectrum 1** button
- If necessary repeat until the phasing on f1 is correct.
- Phase F2 by rotating the spectrum (under the *Display* page select *Trace Axis F2*) and follow the procedure above.

Plotting using the command line

Homonuclear 2D experiments

The macro `plcosy` plots the contours with optionally the 1D spectra along the axes. The macro takes three arguments, for example `plcosy(10,2,1)`. The first is the number of contours (7 is the default). More contours give a darker plot. The second is the contour spacing factor, which must be greater than 1. A number closer to 1 gives closer spaced contours. The last argument is the location of the 1D spectrum, in this case experiment 1. Use a negative number if the 1D spectrum is not available. The plot size and scaling of the 1D spectra are automatically scaled. When the 1D has been specified by `plcosy`, it is copied into a sub experiment of the current experiment. Thereafter it is not necessary to specify the location and `plcosy` without arguments can be used.

When plotting homonuclear spectra, the diagonal of the chart and the diagonal of the spectrum can be aligned by typing `wp=wp1 sp=sp1`.

Heteronuclear 2D experiments

Use `plhxcor` to plot the CIGAR-MBC when the indirect carbon spectrum is available otherwise use `plhxcor2`. The macro `plhxcor` takes four arguments when first used: the first and second are the number of contours and the contour spacing factor, the third and fourth are the experiment numbers for the direct (proton) and indirect (carbon) experiments. Use negative numbers if the experiments are not available. For example, use `plhxcor(10,2,1,2)` if the proton is in experiment 1 and the carbon is in experiment 2. When `plhxcor` is run with four arguments, copies of the proton and carbon spectra are made in the 2D experiment. Calling `plhxcor` without arguments then plots those copies along the axes.