

COSY

Introduction

12.1

CORrelation **S**pectroscop**Y** is a homonuclear 2D technique that is used to correlate the chemical shifts of ^1H nuclei which are J-coupled to one another. In this chapter, two types of COSY sequences will be discussed: magnitude COSY and double-quantum filtered (DQF-) COSY. Both pulse sequences are quite simple and can be explained generally as follows: The first pulse, the preparation pulse, creates transverse magnetization components for all allowed transitions. This is followed by the evolution period t_1 during which the various magnetization components are labeled with their characteristic precession frequencies (including chemical shift and homonuclear J-coupling). The mixing pulse then transfers magnetization components among all those transitions that belong to the same coupled spin systems. The final distribution of labeled magnetization components is detected by measuring their new precession frequencies during the detection period t_2 . The COSY spectrum is produced by a 2D Fourier transform with respect to t_1 and t_2 , and its cross peaks indicate which ^1H nuclei are J-coupled.

Magnitude COSY

12.2

There are several simple, two-pulse programs which can be used to obtain 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. Here it is recommended to use a pulse angle of 45° , because this will yield a spectrum with a better signal-to-noise ratio than a pulse angle of less than 45° , and with a simpler cross peak structure than a pulse angle of 90° .

The 2D spectrum acquired in one of these magnitude COSY experiments has lineshapes in both F1 and F2 that have both absorption and dispersion contributions. This means that it is not possible to phase the spectrum so that the peaks are purely absorptive, and the spectrum must be displayed in magnitude mode. Note that even though magnitude mode is used, it is important to ensure that the spectrum is acquired with a resolution that is sufficient to avoid mutual cancellation of nearby peaks of opposite amplitude (with the spectrum displayed in magnitude mode, it is not possible to discern the signs of the peak amplitudes but it is possible to suffer the adverse effects of mutual cancellation). A typical resolution is 3Hz/pt. This is sufficient for resolving large couplings, but not for resolving small ones. In order to resolve small J-couplings, it is necessary to use fine digital resolution and this requires a significant increase in the experiment time. In general, if higher resolution is desired, it is recommended to use DQF-COSY as described later in the chapter.

References: W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976); K. Nagayama, A. Kumar, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980).

Sample

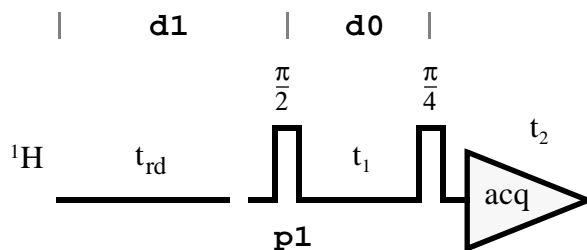
The sample used to demonstrate magnitude COSY in this chapter is 50 mM Gramicidin in DMSO-d₆.

Pulse Sequence Diagram

12.2.1

The COSY-45 pulse sequence is shown in Figure 35. The pulse **p1** must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’.

Figure 35: COSY-45 Pulse Sequence



Acquisition and Processing

12.2.2

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z² shims until the lock level is optimized. Tune and match the probehead for ¹H observation.

It is generally recommended that COSY, like all 2D experiments, be run without sample spinning.

Note that while setting up to do a COSY experiment, the user may find it helpful to refer to Appendix A ‘Data Sets and Selected Parameters’, and Appendix B ‘Pulse Calibration Results’. Appendix A lists data sets generated throughout the course of this manual and also provides a table in which the user can record the **o1**, **o2**, and **sw** values appropriate for the various samples used. Appendix B provides a table in which the user can record the pulse lengths and power levels determined during the pulse calibration procedures described in Chapter 5 ‘Pulse Calibration’.

¹H reference spectrum

Since COSY is a homonuclear experiment only one reference spectrum is required. This ¹H spectrum will be used to determine **o1** and **sw** for the COSY experiment, and can also be used as both the F1 and the F2 projections of the COSY spectrum.

Enter **re proton 2 1** to call up the data set proton/2/1. Enter **edc** and change the EXPNO to 5. Click **SAVE** to create the data set proton/5/1.

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ¹H spectrum. Calibrate the spectrum, and optimize **sw** and **o1** so that the ¹H signals cover almost the entire spectral width.

Acquire an optimized spectrum to be used as the F1 and F2 projections of the COSY spectrum. (If desired, the number of scans may be increased for this spectrum).

Create a new file directory for the 2D data set

From the data set proton/5/1, enter **edc** and change the following parameters:

```

NAME          cosy
EXPNO         1
PROCNO        1 .

```

Click **SAVE** to create the data set cosy/1/1. By creating the COSY data set from data set of the ^1H reference spectrum, most of the F2 parameters for COSY are already set.

Change to 2D parameter mode

Enter **eda** and set PARMODE = 2D. Click on **SAVE** and ok the message “Delete ‘meta.ext’ files?”. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 39. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **p11** and **p1** (^1H observe high power level and 90° pulse time). The correct pulse length for the 45° pulse is calculated from **p1** by the pulse program itself.

The F2 parameters **o1** and **sw** (not shown in the table) should be identical to the values used in the optimized ^1H reference spectrum (proton/5/1). The F1 parameters **sf01** and **sw** should be identical to the corresponding F2 values.

Finally, notice that **in0** and **sw(F1)** are not independent. A convenient way to set **in0** is to set the F1 parameters **nuc1** by clicking on **NUCLEI** for F1 parameters, **nd0**, and **sw** correctly. This automatically sets **in0** to the correct value.

Table 39. COSY Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	cosy45	see Figure 35 for pulse sequence diagram.
TD	1k	
NS	8	the number of scans should be $4*n$ in order for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
P1		90° ^1H high power pulse on f1 channel.
D0	3 μsec	incremented delay (t_1); predefined.
D1	3 sec	relaxation delay; should be about $1.25*T_1(^1\text{H})$.

COSY

F1 Parameters		
Parameter	Value	Comments
TD	256	number of experiments.
ND0	1	there is one d0 period per cycle and MC2 = QF.
IN0	$1/(SW_H) = 2 * DW_H$	t_1 increment; should be $2 * dw$ used in F2 (which is in $\mu s!$).
SW		sw of the optimized 1H spectrum (proton/5/1); same as for F2.
NUC1		select 1H frequency for F1; same as for F2.

Acquire the 2D data set

If this data set was created from the 1H reference spectrum proton/5/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for COSY with the acquisition parameters set as shown above is 1.4 hours.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 40.

Table 40. COSY Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	0	choose pure sine wave.
PH_mod	no	this is a magnitude spectrum.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	


F1 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (¹ H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	0	choose pure cosine wave.
PH_mod	mc	this is a magnitude spectrum.
BC_mod	no	
MC2	QF	determines type of FT in F1; QF results in a forward quadrature complex FT.

Process the 2D data set

Enter **xfb** to multiply the time domain data by the window functions and also perform the 2D Fourier transform.

For magnitude COSY, a sine-type window function is selected to suppress the diagonal peaks relative to the cross peaks. Such a window function is also resolution enhancing, which is appropriate for a magnitude mode 2D spectrum. By de-emphasizing the beginning of the time domain signal, the sine-type window function eliminates the dispersive tails of the magnitude signals and so enhances their resolution.

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the  button, holding down the left mouse button, and moving the mouse up and down.

Since this is a magnitude spectrum, click on **+/-** with the left mouse button until only the positive peaks are displayed.

The optimum display (both the threshold and which peaks are displayed) may be saved by clicking on **DefPlot**.

Phase correct the spectrum

Since this is a magnitude spectrum, no phase adjustment can be made.

Plot the spectrum

Read in the plot parameter file standard2D, e.g., enter **rpar standard2D plot**. This sets most of the plotting parameters to values which are appropriate for this 2D spectrum, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file standard2D can be found in Appendix C '1D and 2D Plotting Parameters'.

To set the region (full or expanded), threshold, and peak type (positive and/or negative), to be used in plotting the spectrum, first make sure the spectrum appears as desired on the screen, and then click **DefPlot** and answer the following questions.

```
Change levels?          y
Please enter number of positive levels?      6
Display contours?      n .
```

Enter **edg** to edit the plotting parameters.

Click the **ed** next to the parameter EDPROJ1 to enter the F1 projection parameters submenu. Edit the parameters from PF1DU to PF1PROC as follows:

```
PF1DU          u
PF1USER        (name of user for file proton/5/1)
PF1NAME        proton
PF1EXP         5
PF1PROC        1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters from PF2DU to PF2PROC as follows:

```
PF2DU          u
PF2USER        (name of user for file proton/5/1)
PF2NAME        proton
PF2EXP         5
PF2PROC        1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click **SAVE** to save all the above changes and exit the **edg** menu.

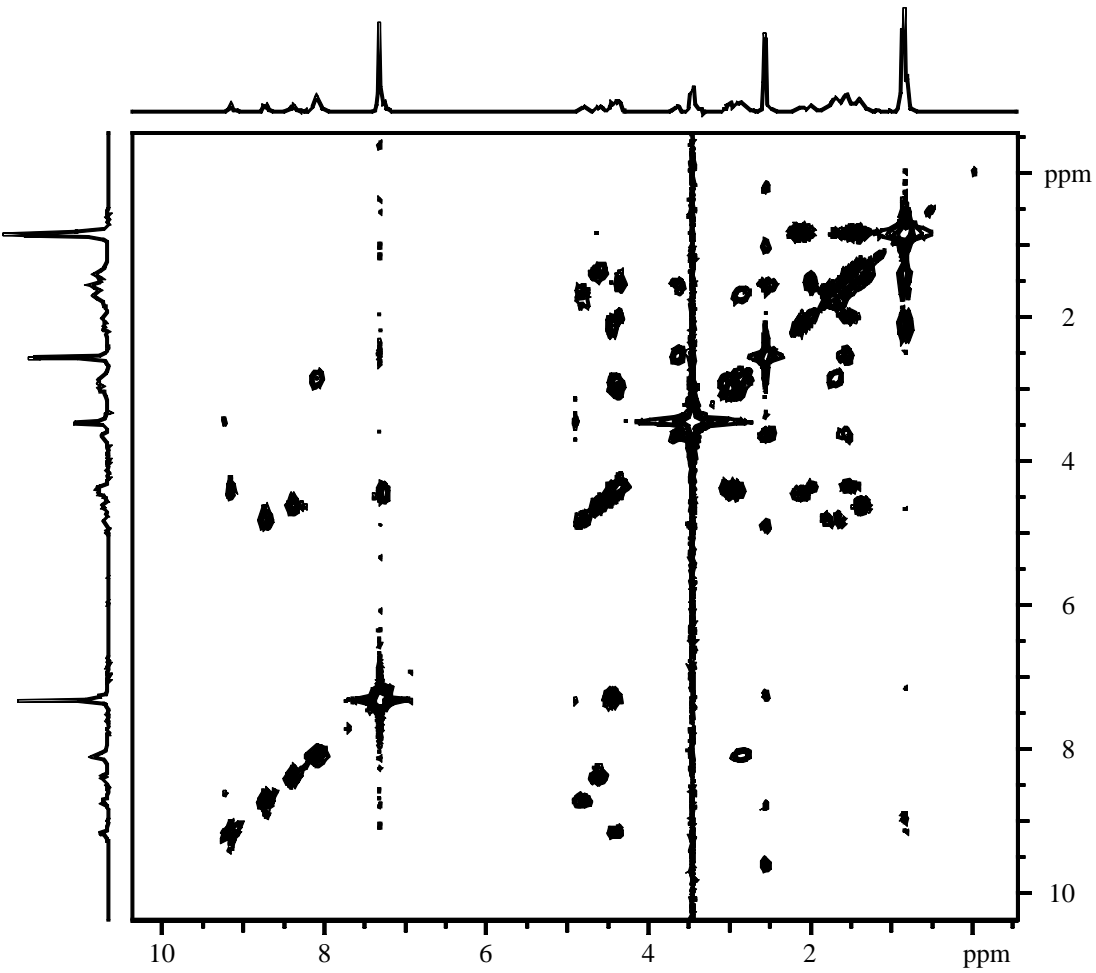
Next create a title for the spectrum. Enter **setti** to use the editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **plot** (provided the correct plotter is selected in **edo**).

A magnitude COSY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 36.

Magnitude COSY

Figure 36: COSY Spectrum of 50 mM Gramicidin in DMSO-d6



The DQF-COSY pulse sequence is a three-pulse sequence, with the third pulse occurring immediately after the second pulse. After the second pulse, the spin system exhibits multiple-quantum coherence. In a normal COSY experiment, the acquisition begins directly after the second pulse and only single-quantum coherence is detected. In a DQF-COSY experiment, the third pulse converts part of the multiple quantum coherence into observable single-quantum coherence. This is detected during the acquisition period which begins immediately following the third pulse. In DQF-COSY, then, only spins which exhibit multiple-quantum coherence (double or higher) after the second pulse are detected during t_2 . This means that only spins that are J-coupled to at least one other spin are detected.

One advantage of the DQF-COSY experiment is that it is phase-sensitive, i.e., the cross peaks of the DQF-COSY spectrum can be displayed with pure absorption lineshapes in both F1 and F2. A phase-sensitive spectrum has a higher resolution than an otherwise equivalent magnitude spectrum because the magnitude lineshape is broader than the pure absorption lineshape (the magnitude lineshape has a contribution from the slowly decaying wings of the dispersion lineshape).

With the appropriate phase cycling, the normal COSY sequence can also be used to obtain a phase-sensitive spectrum; however, the DQF-COSY sequence has two significant advantages. First, partial cancellation of the diagonal peaks in a DQF-COSY spectrum means that the diagonal ridge is much less pronounced in a DQF-COSY spectrum than it is in a normal COSY spectrum. This makes it easier to observe cross peaks between signals which are close together in chemical shift. Second, the double quantum filter eliminates the strong signals from, e.g., solvent ^1H 's that do not experience homonuclear J-coupling. These signals would otherwise dominate the spectrum and possibly be a source of troublesome t_1 noise.

References: M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, **117**, 479 (1984); A. Derome and M. Williamson, *J. Magn. Reson.*, **88**, 117 (1990).

Sample

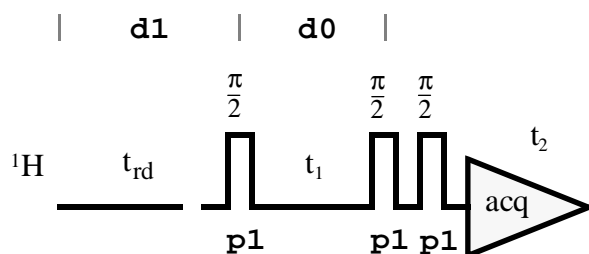
The sample used to demonstrate DQF-COSY in this chapter is 50 mM Gramicidin in DMSO- d_6 . This is the same sample that was used for magnitude COSY.

Pulse Sequence Diagram

12.3.1

The DQF-COSY pulse sequence is shown in Figure 37. The pulse **p1** must be set to the appropriate 90° time found in Chapter 5 'Pulse Calibration'. Notice that the DQF-COSY experiment is sensitive to too high a pulse-repetition rate, i.e., it is important to choose a long enough value of the recycle delay time **d1** in order to avoid multiple-quantum artifacts in the spectrum. A suitable value for this sample is **d1** = 3 sec.

Figure 37: DQF-COSY Pulse Sequence



Acquisition and Processing

12.3.2

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z² shims until the lock level is optimized. Tune and match the probehead for ¹H observation.

It is generally recommended that DQF-COSY, like all 2D experiments, be run without sample spinning.

¹H reference spectrum

Since COSY is a homonuclear experiment only one reference spectrum is required. This ¹H spectrum will be used to determine **o1** and **sw** for the COSY experiment, and can also be used as both the F1 and the F2 projections of the COSY spectrum.

A ¹H reference spectrum of this sample was already created for the magnitude COSY experiment. This spectrum is found in the data set proton/5/1.

Create a new file directory for the 2D data set

Since the DQF-COSY experiment is so similar to the magnitude COSY experiment, it makes sense to create the DQF-COSY data set from the magnitude COSY data set. From the data set cosy/1/1, enter **edc** and change EXPNO to 2. Click **SAVE** to create the data set cosy/2/1.

Change to 2D parameter mode

If this data set was created from the magnitude COSY data set, it is already in 2D parameter mode.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 41. Notice that it is generally recommended to use a larger data set (i.e., a larger value of **td** in both F1 and F2) and a larger number of scans (**ns**) for a DQF-COSY experiment than for a magnitude COSY experiment. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **p11** and **p1** (¹H observe high power level and 90° pulse time).

The F2 parameters **o1** and **sw** (not shown in the table) should be identical to the values used in the optimized ¹H reference spectrum (proton/5/1). The F1 parameters **sfo1** and **sw** should be identical to the corresponding F2 values.

Finally, notice that **in0** and **sw(F1)** are not independent. A convenient way to set **in0** is to set the F1 parameters **nuc1** by clicking **NUCLEI** for F1 parameters, **nd0**, and **sw** correctly. This automatically sets **in0** to the correct value.

Table 41. DQF-COSY Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	cosydfp	see Figure 37 for pulse sequence diagram.
TD	2k	
NS	16	the number of scans should be 8*n in order for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
P1		90° ¹ H high power pulse on f1 channel.
D0	3μsec	incremented delay (t ₁); predefined.
D1	3sec	relaxation delay; should be about 1.25*T ₁ (¹ H).
D13	3μsec	short delay; predefined.
F1 Parameters		
Parameter	Value	Comments
TD	512	number of experiments.
ND0	2	there is one d0 period per cycle and MC2 = TPPI.
IN0	$1/(2*SW_H) = DW_H$	t ₁ increment.
SW		sw of the optimized ¹ H spectrum (proton/5/1); same as for F2.
NUC1		select ¹ H frequency for F1; same as for F2.

Acquire the 2D data set

If this data set was created from the magnitude COSY spectrum cosy/1/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for DQF-COSY with the acquisition parameters set as shown above is 5.5 hours.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 42.


Table 42. DQF-COSY Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	2k	
SF		spectrum reference frequency (^1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	0	choose pure sine wave.
PH_mod	no	first determine 0- and 1 st -order phase correction with phasing subroutine.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	Value	Comments
SI	1k	
SF		spectrum reference frequency (^1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	0	choose pure sine wave.
PH_mod	no	first determine 0- and 1 st -order phase correction with phasing subroutine.
BC_mod	no	
MC2	TPPI	determines type of FT in F1; TPPI results in a forward single real FT.

Process the 2D data set

Enter **xfb** to multiply the time domain data by the window functions and also perform the 2D Fourier transform.

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the  button, holding down the left mouse button, and moving the mouse up and down.

Since this is a phase-sensitive spectrum, click on **+/-** with the left mouse button until both positive and negative peaks are displayed.

The optimum display (both the threshold and which peaks are displayed) may be saved by clicking on **DefPlot**.

Phase correct the spectrum

Click on **phase** to enter the phase correction submenu. Generally, a 2D spectrum is phase corrected first in the F2 dimension (i.e., rows), and then in the F1 dimension (i.e., columns). To phase correct the spectrum in F2, first select three rows as described below. Note that an important feature of a DQF-COSY spectrum is that the diagonal peaks have both an absorptive and a dispersive contribution, and so *no matter what the phase correction*, these peaks will have both absorptive and dispersive character. On the other hand, the cross peaks have only an absorptive contribution, and so when the phase correction is set properly, they are purely absorptive. This means that, unlike the case for many other types of 2D spectra, it is best to phase correct a DQF-COSY spectrum while examining the cross peaks rather than the diagonal peaks. When the spectrum is phased properly, the cross peaks will be purely absorptive (i.e., they will not have the slowly decaying wings characteristic of dispersion peaks). *However*, since DQF-COSY peaks are antiphase (i.e., each multiplet has adjacent positive and negative peaks), it is not possible to phase the spectrum so that all peaks are positive.

To phase correct the spectrum in F2, select three rows each with a cross peak, preferably on the same side of the diagonal (i.e., all three cross peaks above the diagonal, or all below the diagonal). One cross peak should be to the far left of the spectrum, one near the middle, and one to the far right of the spectrum.

In the phase correction submenu, click on **row** with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left hand corner of the display. Move the mouse until the horizontal cross hair is aligned with a row that has a cross peak towards one end of the spectrum. Select the row by clicking the middle mouse button. If the selected row does not intersect the most intense portion of the cross peak, click on **+** or **-** with the left mouse button until it does. Once the desired row is selected, click on **mov 1** with the left mouse button to move the row to window 1, appearing in the upper right hand corner of the display.

Click on **row** again and move the cross hair until it is aligned with a row containing a cross peak near the middle of the spectrum. Select the row by clicking the middle mouse button, adjust the selected row by clicking on **+** or **-** with the left mouse button, and finally move the desired row to window 2 by clicking on **mov 2** with the left mouse button.

Repeat the above procedure to select a row with a cross peak at the other end of the spectrum. Move this row to window 3 by clicking **mov 3** with the left mouse button.

Now that three rows have been selected, the 0th- and 1st-order phase corrections in F2 are determined by hand. Click on **big: 1** with the left mouse button to set the pivot point to the biggest peak in window 1. Note that if the desired cross peak is not the biggest peak in the window, use **cur: 1** and the mouse to select the cross peak by hand.

Move the cursor to the **ph0** button. Hold down the left mouse button and drag the mouse to adjust the 0th-order phase correction. Recall that the 0th-order phase correction should be adjusted so that the peak at the pivot point is phased correctly (i.e., here the cross peak in window 1). Next, move the cursor to the **ph1** button and drag the mouse to adjust the 1st-order phase correction. Recall that the 1st-order phase correction should be adjusted so that the peak farthest from the pivot point is phased correctly (i.e., here the cross peak in window 3).

When you are satisfied with the phase correction, click on **return** and select **save & return** to save the results and confirm the xf2p option to apply this phase correction to the spectrum.

To phase correct the spectrum in F1, repeat the above procedure using **col** rather than **row** to select three columns with cross peaks at one end of the spectrum, in the middle, and at the other end. Phase correct as described above and after selecting **return** and selecting **save & return**, confirm the xf1p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by clicking on **return** and selecting **return**. The selection **save & return** without confirming the xf2p or xf1p option means that the new phase correction is saved to the **edp** menu, but not applied to the spectrum.

While phase correcting the DQF-COSY spectrum, keep in mind that each multiplet is antiphase. Each multiplet has adjacent positive and negative portions. A phase-sensitive DQF-COSY spectrum *cannot* be phased so that all cross peaks are either positive or all are negative. Instead, as you phase correct the spectrum, you should attempt to eliminate any slowly decaying wings from the cross peaks.

If the data is to be retransformed with, e.g., different window functions, the phase correction determined above can be automatically applied by setting **PH_mod** to **pk** in both F1 and F2 of **edp**.

Plot the spectrum

Read in the plot parameter file standard2D, e.g., enter **rpar standard2D plot**. This sets most of the plotting parameters to values which are appropriate for this 2D spectrum, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file standard2D can be found in Appendix C '1D and 2D Plotting Parameters'.

To set the region (full or expanded), threshold, and peak type (positive and/or negative), to be used in plotting the spectrum, first make sure the spectrum appears as desired on the screen, and then click **DefPlot** and answer the following questions.

```
Change levels?          y
Please enter number of positive levels?    6
Please enter number of negative levels?    3
Display contours?      n .
```

Enter **edg** to edit the plotting parameters.

Click the **ed** next to the parameter EDPROJ1 to enter the F1 projection parameters submenu. Edit the parameters from PF1DU to PF1PROC as follows:

```
PF1DU          u
PF1USER        (name of user for file proton/5/1)
PF1NAME        proton
PF1EXP         5
PF1PROC        1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters from PF2DU to PF2PROC as follows:

PF2DU	u
PF2USER	(name of user for file proton/5/1)
PF2NAME	proton
PF2EXP	5
PF2PROC	1 .

Click **SAVE** to save these changes and return to the **edg** menu.

Click **SAVE** to save all the above changes and exit the **edg** menu.

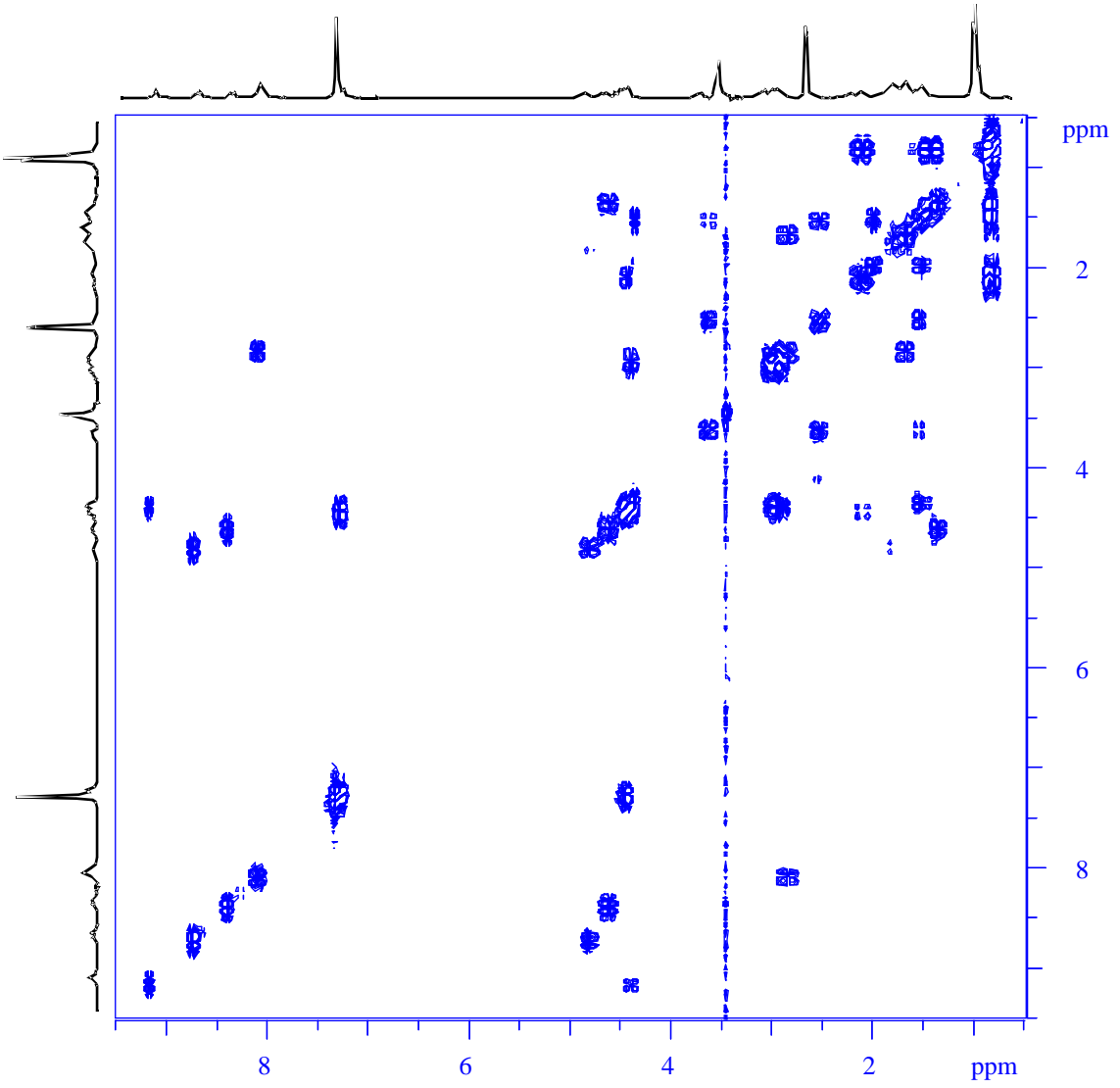
Next create a title for the spectrum. Enter **setti** to use the vi editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **plot** (provided the correct plotter is selected in **edo**).

A DQF-COSY spectrum of 50mM Gramicidin in DMSO-d6 is shown in Figure 38. An expanded portion of the same spectrum is shown in Figure 39.

Double Quantum Filtered COSY

Figure 38: DQF-COSY Spectrum of 50 mM Gramicidin in DMSO-d6



COSY

Figure 39: Expanded DQF-COSY Spectrum of 50 mM Gramicidin in DMSO-d6

