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Introduction

This manual is intended for use with the AVANCE series of spectrometers, including the DMX, DRX, and DPX, and is written based on XWIN-NMR software version xwin-nmr1.1.1 and BSMS firmware version 940614. It covers the preparation, acquisition, and processing of several basic 1D and 2D experiments.

In general, later chapters of this manual assume information discussed in earlier chapters.

Unless otherwise specified, statements such as “click on calib” mean to move the cursor on top of the calib button on the screen, and click with the left-hand mouse button. Consider the left-hand mouse button the default; if another button is required, it will be explicitly stated.

Commands such as “Enter zg” mean to type zg followed by a return on the keyboard.

Words written in bold Helvetica font, e.g., acqu, refer to buttons or pulldown menus on the screen (items that can be selected with the mouse).

Words written in bold Courier font, e.g., edsp, refer to commands that can be typed in at the keyboard.

In several chapters, a list of references is included for those users who are interested in learning more about the particular experiment. These lists include only a few relevant references and are by no means meant to be complete.

We recommend to set the user interface for XWIN-NMR to the mode ‘extended’. This can be done with the XWIN-NMR command setres. Otherwise, some of the features described in this manual would not be available.

An Important Note on Power Levels

Several places throughout this manual, the user is asked to set the power levels pl1, pl3, etc. to the “high power” level for the corresponding channel (f1 or f2). In order to avoid damaging the probehead, the user is advised to use the power levels indicated below in Table 1. Note that these “power levels” are really attenuation levels, and so a higher value corresponds to a lower power. Also note that these power levels pertain only to the specific spectrometers and amplifiers listed below, which correspond to standard AVANCE instruments as of July 1994.
# Introduction

Table 1. Suggested “High Power” Levels for DMX, DRX, and DPX Spectrometers

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Spectrometer</th>
<th>Amplifier</th>
<th>Power Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>DMX</td>
<td>BLARH100</td>
<td>+3 dB</td>
</tr>
<tr>
<td></td>
<td>DRX</td>
<td>BLAXH40</td>
<td>-3 dB</td>
</tr>
<tr>
<td></td>
<td>DPX</td>
<td>BLAXH20</td>
<td>-6 dB</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>DMX</td>
<td>BLAX300</td>
<td>+3 dB</td>
</tr>
<tr>
<td></td>
<td>DRX</td>
<td>BLAXH40</td>
<td>-3 dB</td>
</tr>
<tr>
<td></td>
<td>DPX</td>
<td>BLAXH20</td>
<td>-6 dB</td>
</tr>
</tbody>
</table>
Preparing for Acquisition

Sample Preparation

The quality of the sample can have a significant impact on the quality of its NMR spectrum. The following is a brief list of suggestions to ensure high sample quality.

- Always use clean and dry sample tubes to avoid contaminating the sample.
- Always use good to high quality sample tubes to avoid unnecessary difficulties in shimming.
- Filter the sample solution.
- Always use the same sample volume or solution height. This minimizes the shimming that needs to be done between sample changes. Recommended values are for 5 mm tubes: 0.6 ml or 4 cm of solution, and for 10 mm tubes: 4.0 ml or 4 cm of solution.
- Use the depth gauge to position the sample tube correctly in the spinner. This is discussed further in Chapter 5 ‘Sample Positioning’ of the BSMS User’s Manual.
- Check that the sample tube is held tightly in the spinner so that it does not slip during an experiment.
- Wipe the sample tube clean before inserting it into the magnet.
- For experiments using sample spinning, be sure the spinner, especially the reflectors, is clean. This is important so that the correct spinning rate can be maintained.

Tuning and Matching the Probehead

Once the sample is inserted, the probehead should be tuned and matched. Notice that correct tuning and matching is especially important for higher frequencies. In general, the probehead should be tuned and matched each time a new sample is inserted, each time a new probehead is put in the magnet, and each time the observe or decouple nucleus is changed when using a broadband probe.

In a probehead there is a resonant circuit for each observe and decouple nucleus indicated on the probehead label (e.g., one for \(^1\text{H}\) and one for \(^13\text{C}\) in a dual \(^1\text{H}/^13\text{C}\) probehead; one for \(^1\text{H}\) and one for a wide range of nuclei in a BBO probehead). There is also a resonant circuit for the lock nucleus, but the standard user will never need to adjust this, so we will ignore it for now. Each of these circuits has a frequency at which it is most sensitive (the resonance frequency). Tuning is the process of adjusting this frequency until it coincides with the frequency of the pulses transmitted to the circuit. For example, the frequency at which the \(^1\text{H}\) resonant circuit is most sensitive must be set to the carrier frequency of the \(^1\text{H}\) pulses (which is \(sfo1\) if the \(^1\text{H}\) circuit is connected to the \(f1\) channel, \(sfo2\) if it is connected to the \(f2\) channel, etc.). A probehead is said to be tuned when all of its resonant circuits are tuned. Once a probehead has been tuned, it is not necessary to retune it after slight adjustments to the carrier frequency, since the probehead is...
Preparing for Acquisition

generally tuned over a range of a couple MHz. On the other hand, large adjustments to the carrier frequency, necessary when changing nuclei, do warrant retuning the probehead, so a broadband probe needs to be retuned each time the heteronucleus is changed.

Matching is the process of adjusting the impedance of the resonant circuit until it corresponds with the impedance of the transmission line connected to it. This impedance is 50Ω. Correct matching minimizes the power that is reflected by the probehead, and so is lost; or equivalently, maximizes the power that is transmitted to the coil, and so is available to do NMR. A probehead is said to be matched when all of its resonant circuits are matched. Again, once a probehead has been matched, it is not necessary to rematch it after slight adjustments to the carrier frequency. On the other hand, large adjustments to the carrier frequency, necessary when changing nuclei, do warrant rematching the probehead.

Tuning and matching are carried out simultaneously using the XWIN-NMR command \texttt{wobb} (wobble). During wobbling, a low power signal is transmitted to the probehead. This signal is swept over a frequency range determined by the parameter \texttt{wbsw} (the default value is 4 MHz) centered on the carrier frequency (i.e., \texttt{sfo1}, \texttt{sfo2}, etc., depending on which nucleus is being tuned/matched). Within the preamp (High Performance Preamplifier Assembly or HPPR), the impedance of the probe over this frequency range is compared to the impedance of a 50Ω resistor.

The results are shown both on the LED display of the HPPR and in the acquisition submenu in XWIN-NMR. Both displays show the reflected power of the probehead versus the frequency of the signal. The user observes either one or both of these displays while tuning and matching the probehead.

When the NMR experiments to be performed are \(^1\text{H}\) homonuclear experiments (e.g., \(^1\text{H}\) 1D spectroscopy, COSY, NOESY, or TOCSY), it is only necessary to tune and match the \(^1\text{H}\) circuit of the probehead.

Make sure that the sample is in the magnet, and the probehead is connected for standard \(^1\text{H}\) acquisition. Note that there is no special configuration for tuning and matching. Also, it is recommended to tune and match without sample spinning.

Set the parameters

In XWIN-NMR, enter \texttt{edsp} and set the following spectrometer parameters:

\begin{verbatim}
NUC1  1H
NUC2  OFF
NUC3  OFF.
\end{verbatim}

This automatically sets \texttt{sfo1} to a frequency appropriate for \(^1\text{H}\) tuning and matching. There is no need to adjust \texttt{sfo1} carefully now. Exit \texttt{edsp} by clicking \texttt{SAVE}.

Other \texttt{wobb} parameters are \texttt{wbsw}, which determines the wobble sweep width in MHz (the default value is 4 MHz), and \texttt{wbst}, which determines the number of wobble steps over the sweep width (the default value is 256). Both of these parameters may be found in the \texttt{eda} table. No other parameters are required.

Start wobbling
Tuning and Matching the Probehead

Before starting the wobbling procedure, ensure that no acquisition is in progress, e.g., enter `stop`.

Enter `acqu` to switch to the acquisition window of XWIN-NMR, if it is desired to use this to monitor the tuning and matching. Notice that being in the acquisition window slows down the wobbling procedure, so if the HPPR LED display will be used to monitor tuning and matching, it is best to remain in the main XWIN-NMR window and not switch to the acquisition window.

Start the frequency sweep by typing `wobb`. The curve that appears in the acquisition window is the reflected power as a function of frequency. Unless the probehead is quite far from the correct tuning and matching, there will be a noticeable dip in the curve. When the $^1$H circuit is properly tuned, the dip will be in the center of the window, denoted by the vertical marker; and when the circuit is properly matched, the dip will extend all the way down to the $x$ axis. Similar information is conveyed by the LED display on the HPPR. The horizontal row of LEDs indicates tuning and the vertical row matching. When the circuit is properly tuned and matched, the number of LED’s lit is minimized. This usually means that only green LED’s, not red, are lit both in the horizontal and vertical displays.

**Tune and match**

Adjust the tuning and matching screws (labeled T and M) at the base of the probehead with the special tool provided. Note that the screws are color coded and those for the $^1$H circuit are usually yellow. *Also note that the screws have a limited range and attempting to turn them beyond this range will damage the probehead.*

Since there is interplay between tuning and matching, it is generally useful to adjust the T and M screws in an iterative fashion. Turn the M screw until the dip is well matched at some frequency (the dip extends to the $x$ axis and the number of LED’s lit in the vertical HPPR display is minimized). Most likely this will **not** be the desired frequency. Adjust the T screw slightly to move the dip toward the center of the window, or equivalently, to reduce the number of LED’s lit in the horizontal HPPR display. Rematch the dip by adjusting the M screw. Again, adjust T to move the dip towards the center of the screen and rematch using M. In this manner, continue walking the dip towards the correct resonance frequency. Note that it is possible to run out of range on the M screw. If this happens, return M to the middle of its range, adjust T to get a well matched dip at some frequency, and walk the dip towards the correct frequency as described above.

As mentioned above, ideal tuning and matching is when the dip is centered in the window and extends to $y = 0$ (the $x$ axis) on the acquisition window, or equivalently, when the number of LED’s lit on the preamp is minimized in both the vertical and horizontal display.

When the $^1$H circuit is tuned and matched, exit the wobble routine by typing `stop`.

Click on `return` to exit the acquisition window and return to the main window.

---

**Tuning and Matching $^{13}$C**

Since most $^{13}$C experiments make use of $^1$H decoupling, when tuning and matching a probehead for $^{13}$C, it is generally a good idea also to tune and match for $^1$H.

When tuning and matching a probehead with multiple resonant circuits, it is best first to tune and match the lowest frequency circuit and then to proceed to higher
Preparing for Acquisition

frequency circuits. The larger capacitors and inductors found in lower frequency resonant circuits can be expected to have larger stray capacitance and inductance than the smaller elements in higher frequency circuits. Thus, one would expect tuning and matching lower frequency circuits to affect the tuning and matching of higher frequency circuits more so than vice versa. So when tuning and matching a probehead for both $^1H$ and $^{13}C$, it is best to make the $^{13}C$ adjustments first and the $^1H$ adjustments last.

Make sure that the sample is in the magnet, and the probehead is connected for the appropriate experiment. Also, it is recommended to tune and match without sample spinning.

**Set the parameters**

In XWIN-NMR, enter `edsp` and set the following spectrometer parameters:

```
NUC1  13C
NUC2  OFF
NUC3  OFF.
```

This automatically sets `sfoo` to a frequency appropriate for $^{13}C$ tuning and matching. Exit `edsp` by clicking `SAVE`.

Other wobb parameters are `wbaw` and `wbst`, as mentioned above. Both of these parameters may be found in the `eda` table. No other parameters are required.

**Start wobbling, tune and match**

Ensure that no acquisition is in progress, e.g., enter `stop`.

Enter `acqu` to switch to the acquisition window, if this will be used to monitor the tuning and matching.

Start the frequency sweep by typing `wobb`. The curve that appears in the acquisition window is for $^{13}C$. Adjust the tuning and matching following the guidelines given above for $^1H$. Notice that some probeheads (e.g., broadband probeheads) have sliding bars instead of screws, one set labeled tuning and another matching. These probeheads also have a menu of tuning and matching values for several nuclei. Set the tuning and matching sliding bars to the values indicated for $^{13}C$ on the menu. Adjust the tuning bar until the dip is well matched at some frequency, and then walk the dip towards the correct frequency as described above for $^1H$.

Once the $^{13}C$ circuit is tuned and matched, the $^{13}C$ wobbling must be stopped and $^1H$ wobbling begun. One straightforward way to do this is as follows: Exit the wobble routine by typing `stop`. Enter `edsp`, change to NUC1 to 1H, and exit by clicking `SAVE`. Then start the $^1H$ frequency sweep by typing `wobb`. After a few seconds the $^1H$ curve appears in the acquisition window and the $^1H$ circuit can be tuned and matched as described above.

Alternatively, if the user already has a data set in which NUC1 = $^1H$ and NUC2 = OFF, there is no need to redo `edsp` for the current data set. The user may simply read in the $^1H$ data set and then type `wobb`.

Once the probehead is tuned and matched for $^{13}C$ and $^1H$, exit the wobble routine by typing `stop`.

Click on `return` to exit the acquisition window and return to the main window.
Before running an NMR experiment, it is also necessary to lock and shim the magnetic field.

**Locking**

To display the lock signal enter `lockdisp`. This opens a new window in which the lock trace now appears.

The most convenient way for the standard user to lock is semi-automatically using the XWIN-NMR command `lock`. To start the lock-in procedure, enter `lock` and select the appropriate solvent from the menu that appears. Alternatively, enter the solvent name with the lock command, e.g., `lock cdcl3`. During lock-in, the lock power, field value, and frequency shift for the solvent are set according to the values in the 2H-Lock table (also known as the `edlock` table). These values can be edited with the command `edlock`. Note that the lock power listed in this table is the level used once lock-in has been achieved. The field-shift mode is then selected and autolock is activated. Once lock-in is achieved, the lock gain is set so that the lock signal is visible in the lock window. At this point the message “lock: finished” appears in the status line at the bottom of the window.

The lock-in procedure outlined above sets the frequency shift to the exact frequency shift value for the given solvent as listed in the `edlock` table. It also sets the field value to the value (which is the same for all solvents) listed in the `edlock` table and then adjusts this slightly to achieve lock-in. As a result, the absolute magnetic field is now nearly the same no matter what lock solvent is used. This has the advantage that offsets can now be defined in ppm, since the absolute frequency corresponding to a given ppm value no longer depends on the lock solvent.

Another advantage of following this lock-in procedure is that it automatically sets the parameter `solvent` correctly in the `eda` table. This is especially important if you wish to use the automatic calibration command `sref`, as described later (see “Spectrum Calibration and Optimization” on page 25).

It is recommended that each time the probehead is changed, the user adjust the phase of the lock signal while monitoring the sweep wiggles (i.e., while the field is not locked but is being swept). This is necessary if the original lock phase is very far wrong, in which case autolock may fail to achieve lock-in. If the original phase is reasonably close to correct, then lock-in can be achieved and the phase can be adjusted afterwards using autophase. Please note that the lock phase for each probehead is stored in the `edlock` table. To make shure that XWIN-NMR selects the `edlock` table assigned to the current probe, enter `edhead`, then click on Define Current and select your current probe.

The other lock parameter that may possibly be problematic when using the XWIN-NMR `lock` command is the lock power. In some instances, the power level listed in the `edlock` table is too high, meaning that the lock signal is always saturated. Usually, in this situation lock-in can be achieved, but since the signal is saturated, it oscillates. A quick fix is simply to reduce the lock power by hand once lock-in has been achieved. A better fix is to change the power level in the `edlock` table. Note that the appropriate lock power level depends on the lock solvent, the field value, and the probehead.

**Shimming**
Preparing for Acquisition

If the probehead has just been changed, the first step in shimming the magnetic field is to read in the shim file corresponding to the new probehead. Enter `rsh` and then select the appropriate file from the menu that appears.

Assuming that the shim file is a good one, or that a prior user has shimmed the field for the current probehead, the user need only adjust the Z and Z^2 shims (and possibly the X and Y). Generally, the shims are adjusted while viewing the lock signal and the best shim values correspond to the highest lock level (height of the lock signal in the window). For further discussion of shimming see Chapter 6 ‘Shim Operation’ of the BSMS User’s Manual.

Optimize lock settings (optional)

Once the magnetic field has been locked and shimmed, the user may wish to optimize the lock settings as described below. It is strongly recommended to follow this procedure before running any experiment requiring optimal stability (e.g., NOE difference experiments).

After the field is locked and shimmed, start the auto-power routine from the BSMS keyboard (see Chapter 2 ‘Key Description’ of the BSMS User’s Manual). For lock solvents with long T_1 relaxation times (e.g., CDCl_3), however, auto power may take an unacceptably long time and the lock power should be optimized manually. Simply increase the lock power level until the signal begins to oscillate (i.e., until saturation), and then reduce the power level slightly (approximately 3 dB). For example, if the lock signal begins to oscillate at a power of –15 dB, the optimal magnetic field stability can be expected when a level of approximately –18 dB (or even –20 dB) is used. The field stability will be significantly worse if a power level of, say, –35 dB is used instead.

When the lock power is optimized, start the auto-phase routine, and finally the auto-gain routine. Take note of the gain value determined by auto gain. Using this value, select the appropriate values for the loop filter, loop gain, and loop time as shown below in Table 2.
Table 2. Lock Parameters (BSMS Firmware Version 940614)

<table>
<thead>
<tr>
<th>Lock RX Gain (after auto gain) [dB]</th>
<th>Loop Filter [Hz]</th>
<th>Loop Gain [dB]</th>
<th>Loop Time [sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>20</td>
<td>−17.9</td>
<td>0.681</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>−14.3</td>
<td>0.589</td>
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<tr>
<td>110</td>
<td>50</td>
<td>−9.4</td>
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<td>1000</td>
<td>13.2</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>15.2</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>16.8</td>
<td>0.041</td>
</tr>
</tbody>
</table>

So, for example if auto gain determines a lock gain of 100dB, the user should set the loop filter to 160Hz, the loop gain to 0.3 dB, and the loop time to 0.220sec (see Chapter 4 ‘Menu Description’ of the BSMS User’s Manual for how to set these parameters from the BSMS keyboard).
Basic $^1$H Acquisition and Processing

Introduction

This chapter describes the acquisition and processing of a $^1$H spectrum acquired with the simple one-pulse sequence shown in Figure 1. The pulse sequence consists of the recycle delay $t_{rd}$ followed by an RF pulse. Data are collected following the RF pulse. In the figure the pulse angle is shown to be $\pi/2$, although, in practice it is often chosen to be somewhat less than this.

The two pulse sequence parameters shown in the figure, $d_1$ and $p_1$, correspond to the length of the recycle delay and the length of the RF pulse, respectively.

Note that the time intervals depicted in this pulse sequence diagram, as well as in the other pulse sequence diagrams in this manual, are not drawn to scale. For example $d_1$ is typically a few seconds while $p_1$ is typically a few microseconds in length.

Figure 1: $^1$H One-Pulse Sequence

Sample

The sample used to demonstrate the basic 1D $^1$H experiment in this chapter is 100 mg Cholesterylacetate in CDCl$_3$ with 0.5% TMS. In the procedure described below, however, the sample is treated as an unknown.

Preparation

Before proceeding make sure that you have done the following (see Chapter 2 ‘Preparing for Acquisition’):

- Inserted a suitable probehead (e.g., $^1$H selective or dual $^1$H/$^1$C) and read in the corresponding shim file.
- Inserted the sample.
- Locked the spectrometer.
- Optimized the Z and Z$^2$ shims.
- Tuned and matched the probehead for $^1$H.
Basic $^1$H Acquisition and Processing

Spectrometer and Acquisition Parameters

Before a spectrum is acquired it is necessary to create a new data set, and set the spectrometer and the acquisition parameters within the data set.

The spectrometer parameters are responsible for the hardware settings necessary for configuring the spectrometer for a particular experiment. They include which nucleus is to be observed, which nuclei are to be decoupled, and the basic frequencies of the observe and decouple nuclei. The command `edsp` calls up a window in which the spectrometer parameters may be set.

The acquisition parameters include all pulse sequence parameters, the number of data points, number of scans, receiver gain, and many others. These may be displayed and edited by entering `eda`. Notice that the spectrometer parameters are also listed in the `eda` table. It is important to set the spectrometer parameters before setting the acquisition parameters. This is because the values from `edsp` are automatically carried over to the `eda` table, overwriting whatever values were there previously.

Create a New File Directory for the Data Set

To create a new data set, type `edc` in the command line of the main XWIN-NMR window. This calls up a small window entitled “Current Data Parameters”. The parameters that can be set in this window are the data set name (NAME), experiment number (EXPNO), processed data number (PROCNO), disk unit (DU), user id (USER), and data type (TYPE). Change the parameters as follows:

```
NAME  proton
EXPNO 1
PROCNO 1.
```

Click on SAVE. This exits `edc` and creates the data set proton/1/1. The message “NO DATA AVAILABLE” should now appear on the screen.

Set Up the Spectrometer Parameters

Enter `edsp` and set the following spectrometer parameters:

```
NUC1  1H
NUC2  off
NUC3  off.
```

Since there is no decoupling, the only relevant spectrometer parameters are OFSH1, BF1, and SFO1. For the moment, let OFSH1 = 0 and note that BF1 = SFO1.

Click on SAVE to save the spectrometer parameters and return to the main window. The spectrometer is now prepared to pulse and detect at the base $^1$H frequency of the magnet.
Enter `eda` and set the acquisition parameters as shown below in Table 3. Note that those parameters which are set automatically or which are not immediately relevant have not been included below.

### Table 3. Basic $^1$H Spectrum Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 1 for pulse sequence diagram.</td>
</tr>
<tr>
<td>AQ_mod</td>
<td>qsim</td>
<td>the default setting.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td>not critical; 32 k is a fairly standard value for a high-resolution 1D spectrum.</td>
</tr>
<tr>
<td>PARMODE</td>
<td>1D</td>
<td>this is a 1D experiment.</td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td>do not bother to signal average until other parameters are optimized.</td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td>no need to collect dummy scans yet.</td>
</tr>
<tr>
<td>D<strong>Array</strong></td>
<td>D1 = 2 sec</td>
<td>the default unit for delays is seconds; simply entering “2” sets a delay of 2 seconds; $d_1$ is the only delay used in the pulse program zg.</td>
</tr>
<tr>
<td>P<strong>Array</strong></td>
<td>P1 = 3 μsec</td>
<td>the default unit for pulse lengths is microseconds; $p_1$ is the only pulse length used in the pulse program zg; this value will be optimized later.</td>
</tr>
<tr>
<td>SW</td>
<td>50 ppm</td>
<td>for the first spectrum of an unknown sample it is wise to use a large spectral width; also notice that when you enter “50” the value that is registered is slightly different.</td>
</tr>
<tr>
<td>RG</td>
<td>64</td>
<td>just a suggested value.</td>
</tr>
<tr>
<td>NUC1</td>
<td>1H</td>
<td>this parameter merely gives the user information; use edsp to change the nucleus.</td>
</tr>
<tr>
<td>SFO1</td>
<td></td>
<td>these parameters were set with edsp and should not be changed until something is known about the spectrum.</td>
</tr>
<tr>
<td>BF1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL<strong>Array</strong></td>
<td>PL1 =</td>
<td>select high power level (see “An Important Note on Power Levels” on page 7).</td>
</tr>
</tbody>
</table>

Click on **SAVE** to save the acquisition parameters and return to the main window. Everything is now prepared for acquisition.

Note that the parameters $d_1$, $p_1$, and $pl_1$ are included in the parameter arrays D, P and PL, respectively, in the `eda` table. To edit these parameters within `eda`, follow
Basic $^1$H Acquisition and Processing

this example for d1: In the eda table, find the button marked ‘***Array***’ next to the parameter ‘D’. Click on this **Array** button to call up the submenu of delays D0 to D31. In this submenu, set D1 to 2.0 and click on DONE to save the changes and return to the eda table (or click on SAVE to save all changes and exit eda). As with most acquisition parameters, however, d1, p1, and p2l can also be edited directly in the command line of the main XWIN-NMR window. For example, simply enter d1 and then 2.0 at the prompt.

Acquisition

Enter acqu to switch to the acquisition window. While it is possible to acquire a spectrum from the main window, the buildup of the FID can only be observed in the acquisition window.

Enter zg. This clears any previous data (‘zero’) and starts the experiment (‘go’).

Notice the message scan 1/l. This indicates that the spectrometer is performing the first scan and that only one scan will be performed.

Enter rga. The spectrometer automatically performs several acquisitions and sets a suitable value for the receiver gain (rg). Enter zg and the spectrometer now acquires a new FID with the adjusted value of rg.

If at some point here or in the following sections the message “DATA OUT OF WINDOW” appears, or if the scaling is unsuitably large or small, then one or more of the following steps may prove useful:

- Click on with the left mouse button. This resets the horizontal scaling to the full spectral width.
- Click on with the left mouse button. This resets the vertical scaling to full spectral height. The spectrum is expanded until a negative peak hits the bottom of the screen or a positive peak hits the top of the screen, whichever happens first.
- Click on with the left mouse button, hold down the mouse button and move the mouse up and down vertical for online scaling.

If, at any time, a submenu is entered accidentally, then clicking on return (located on the menu bar across the top of the window) always returns the display to the main window. From here, enter acqu to re-enter the acquisition window.

Processing

After the FID has been acquired the next step is to process the acquired data. The processing parameters may be displayed and edited by entering edp. It is also possible to change most processing parameters individually by typing the parameter name in the command line and then entering the desired value at the prompt.

Note that when using the digital filter, it is necessary to set PKNL = TRUE in edp in order to avoid artifacts due to the group delay.
Fourier Transformation

The most basic processing technique is the Fourier transformation, which is carried out by entering the command `ft`. The number of points used to form the resulting spectrum is determined by the processing parameter `si` (size). The spectrum consists of `si` real points and `si` imaginary points, so the default setting of `si` is `td/2`, where `td` is the acquisition parameter indicating the number of time domain data points collected. Both `td/2` and `si` are generally a power of 2. If `si < td/2` then not all time domain data are used in the Fourier transformation, and if `si > td/2` then the time domain data are filled out with `2(si) - td` zeroes before the Fourier transformation. In 1D spectroscopy, it is often recommended to zero fill one time, i.e., to set `si = td`.

Check the value of `si`. Enter `si` and when prompted enter a value of 32 k (appropriate since `td` is 32 k).

Enter `ft` and a spectrum now appears on the screen. The display automatically switches from the acquisition window to the main window. The FID can still be viewed by returning to the acquisition window. If the x axis of the Fourier transformed spectrum is displayed in Hz, click on `Hz/ppm` to convert to ppm. If necessary, use the , and buttons as described above to scale the spectrum appropriately.

Phase Correction

Once the spectrum is transformed, the next step is to phase correct it. For the simple experiment performed here, it should be possible to adjust the phase of the spectrum so that all peaks are positive.

Click on `phase` to enter the phase correction submenu.

Click on `biggest`. This selects the biggest peak of the spectrum as the reference peak for the 0th-order phase correction. Notice that the phase of the biggest peak is automatically adjusted. To adjust the 0th-order phase manually, place the cursor on `PH0`, hold down the left mouse button, and move the mouse until the reference peak is positive and the baseline on either side is as flat as possible.

Most likely, at this point, peaks to the left and right of the reference peak are not yet phased correctly. These require a 1st-order phase correction. To adjust the 1st-order phase correction, place the cursor on `PH1`, hold down the left mouse button, and move the mouse until the peaks far from the reference point are also positive.

Note that it is advisable to select the 0th-order phase correction reference peak to be near one end of the spectrum. For some samples, the biggest peak is towards the middle of the spectrum. When this is the case, click on `cursor` rather than `biggest`. This ties the cursor to the spectrum, and the user can then define the reference peak by moving the cursor to the desired peak and clicking the middle mouse button.

Once the spectrum is phased correctly, click on `return` to exit the submenu and save the phase corrections by selecting `Save & return`. The 0th- and 1st-order phase corrections are stored as processing parameters `phc0` and `phc1`, respectively. To quit the phase correction submenu without saving the corrections, simply click on `return` and select `return`. In either case, the display returns to the main menu and the spectrum appears on the screen.
Notice that once suitable values of \texttt{phc0} and \texttt{phc1} have been stored it is possible to use them to phase correct subsequent spectra by typing the command \texttt{pk}. In addition, it is possible to combine the Fourier transformation (\texttt{ft}) and phase correction (\texttt{pk}) into one step using the command \texttt{fp}.

### Windowing 3.4.3

Before Fourier transforming a spectrum, it is common to apply some sort of window or filter function to the time domain data. The two main reasons for doing this are either to improve the signal-to-noise ratio of the spectrum, or to improve the resolution of the spectrum. It is not possible to do both simultaneously. A filter function will either improve the signal-to-noise ratio at the cost of resolution, or vice versa. For a simple 1D spectrum as described in this chapter, it is most common to enhance the signal-to-noise ratio by multiplying the FID by a decaying exponential, achieved by the command \texttt{em}.

The rate of decay of the exponential determines the amount of line broadening that results from \texttt{em}. This rate is determined by the processing parameter \texttt{lb} (in Hz). Enter \texttt{lb} and set the value to 0.3 (0.3 Hz is an appropriate line broadening for high-resolution $^1$H spectra).

Enter \texttt{em} to perform the exponential multiply.

Enter \texttt{fp} to Fourier transform and phase correct the filtered data.

At this point it may be useful to define the macro \texttt{efp}, if it does not already exist, that combines the commands \texttt{em}, \texttt{ft}, and \texttt{pk}. Simply enter \texttt{edmac} to call up the menu of existing macros. If \texttt{efp} does not already exist, enter \texttt{efp} at the prompt “Type new name:”. The file “efp” is then opened by the vi editor. Using the vi editor, write a file that looks like the following:

```plaintext
  em
  ft
  pk
```

In the future, whenever you wish to combine the commands \texttt{em}, \texttt{ft}, and \texttt{pk}, you need only type \texttt{efp}.

The spectrum should resemble that of Figure 2 (the exact ppm values may not be the same, but the sw should be approximately 50 ppm).
Spectrum Calibration and Optimization

Figure 2: $^1$H Spectrum of 100 mg Cholesterylacetate in CDCl$_3$; No Signal Averaging

$^1$H NMR spectra are customarily calibrated by setting the TMS peak to 0 ppm.

First expand the spectrum about this peak. To do this, move the cursor so that it is anywhere within the data field and click the left mouse button to tie the cursor to the spectrum. Now moving the mouse causes the cursor to move along the spectrum and the precise frequency of its position to be displayed in the small window entitled “Data Set”. Move the cursor to the left of the TMS peak (the peak farthest to the right in the spectrum). Click the middle mouse button. Move the cursor to the right
of the TMS peak and click the middle mouse button again. The spectrum is now expanded about the TMS peak. Click the left mouse button again to release the cursor from the spectrum.

Now calibrate the TMS peak. Click on calib, tie the cursor to the spectrum as described above. Position the cursor on the top of the TMS peak. Click the middle mouse button and at the bottom of the window the prompt “Cursor frequency in ppm” appears. Enter 0. The TMS peak is now calibrated to 0 ppm. Click on return. Click on  to display the unexpanded spectrum.

With the new digital lock, provided parameters are set correctly in the edlock table and that lock-in was achieved using the UXNMR command lock, the magnetic field value is very nearly the same regardless of the lock solvent and so the spectra should be automatically calibrated. There may be an error of a few Hz, and this can be corrected by the automatic spectral referencing command sref. Notice that in order for the command sref to work properly, the parameter solvent must be set correctly in the eda table. This is taken care of automatically, however, when lock-in is achieved by the UXNMR command lock (recall that the solvent must be identified correctly here, see “Locking” on page 15).

Adjust the Spectral Width 3.5.1

Now it is evident that the $^1$H NMR spectrum of Cholesterylacetate lies in the region from 0 to 8 ppm, so there is no need for the spectral width to be 50 ppm. It makes much more sense to reduce the spectral width to a window of 10 or 11 ppm centered around the region of signal.

Before changing the spectral width, if you wish to preserve the original set of acquisition parameters as shown in the eda table, then a new data set must be created. Enter edc. Set the parameter EXPNO to 2 and click SAVE. This creates the data set proton/2/1. Notice that the acquisition parameters set for proton/1/1 are automatically carried over to proton/2/1.

Enter zg to acquire a new FID. Enter efp to add line broadening, Fourier transform and phase correct the data. Notice that this spectrum is calibrated like proton/1/1.

Click the left mouse button somewhere in the spectral window to tie the cursor to the spectrum. Position the cursor at approximately 10 ppm. Click the middle mouse button to set a marker at this frequency. Move the cursor to approximately –1 ppm and click the middle mouse button to expand the spectrum. The expanded region from –1 to 10 ppm now appears in the window. Click the left mouse button to release the cursor from the spectrum.

Click on sw-sfo1 while the expanded region is displayed. This adjusts sw so that it has the same value as the expanded region (here about 11 ppm), and also adjusts o1 (and thus sfo1) so that the carrier frequency lies in the center of the expanded region. (You can verify these changes by checking the eda table.) Notice that by reducing the spectral width, the acquisition time aq is increased while the parameter fidres is reduced. Finally, now that the acquisition parameters are optimized, it is a good idea to repeat the automatic receiver gain adjustment (rga).

Enter zg to acquire a new FID. Enter efp to apply line broadening, Fourier transform, and phase correct the spectrum. Notice that since the spectral width has been changed, it may be necessary to readjust the phase correction. The spectrum should now be similar to that of Figure 3.
Figure 3: $^1$H Spectrum of 100 mg Cholesterylacetate in CDCl$_3$; No Signal Averaging, SW and O1 Optimized

For future reference, the user may record the optimized values of $o_1$ and $sw$ for a $^1$H spectrum of 100 mg Cholesterylacetate in Table 54 in Appendix A 'Data Sets and Selected Parameters'.
Increase the Number of Scans

The signal-to-noise ratio of a spectrum may be improved by adding together the data acquired from a number of scans. The signal increases as the number of scans; however, the noise increases as the square root of the number of scans, so the overall signal-to-noise ratio increases as the square root of the number of scans.

To avoid overwriting previous data, first create a new data set. Enter edc, set EXPNO to 3, and click on SAVE to create the data set proton/3/1.

Enter eda, set NS to 64, and DS to 2. Equivalently, you may enter ns and then 64 when prompted for a value, then ds and 2 when prompted for a value. The parameters are ready now so that 64 spectra will be acquired and added together. The two dummy scans are to ensure that the system reaches steady state before any spectra are added together.

Click on acqu to enter the acquisition window. Enter zg to start the experiment. In the acquisition window it is possible to see the successive FID’s adding up. The residual experimental time, as well as the current scan and the total number of scans, are displayed in the small window entitled “Info”.

When the acquisition is complete, enter efp to add line broadening, Fourier transform, and phase correct the spectrum. The spectrum should resemble that shown in Figure 4. The signal-to-noise ratio of this spectrum should be about 8 times better than that of proton/2/1. The two spectra can be compared by using the dual function as follows: First enter edc2 to define the second data set. Within edc2, set EXPNO2 to 2, PROCNO to 1, and click SAVE. This defines proton/2/1 to be the second data set. Then with proton/3/1 displayed in the main window, click dual to call the dual subroutine. At this point, both proton/3/1 and proton/2/1 should appear in the window. The two data sets can then be manipulated as described in the “XWIN-NMR Manual: Chapter 13, The Display Menu”.
A straightforward way to plot 1D $^1$H spectra is by using most of the plotting parameters found in the plot parameter file standard1D. Read in the file standard1D by entering `rpar`, selecting `standard1D` from the menu of parameter file names, and then selecting `plot` from the menu of parameter file types that appears. Equivalently, simply enter `rpar standard1D plot`. This sets most of the plotting parameters to values which are appropriate for most 1D spectra, assuming
Basic $^1$H Acquisition and Processing

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 standard1D can be found in Appendix C ‘1D and 2D Plotting Parameters’.

For basic 1D $^1$H spectra no changes need to be made within the parameter menu edg itself; however, the spectral region and the integral range must be defined, and the spectrum title must be written.

To select the spectral region (full or expanded) to be plotted, first make sure the spectrum appears as desired on the screen, and then click DP1 and simply hit return in response to the following three (3) questions:

\[ F1 = \text{<return>} \]
\[ F2 = \text{<return>} \]
\[ \text{Change y-scaling on display according to PSCAL?<return>} \]

It is often useful to integrate $^1$H spectra, and so an integral range is required. The simplest way to define the integral range is by entering abs. The command abs performs an automatic baseline correction and also automatically defines the integral range.

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).
This chapter describes the acquisition and processing of a $^{13}$C spectrum acquired with a simple one-pulse sequence with and without $^1$H decoupling. Since NMR is much less sensitive to $^{13}$C nuclei than to $^1$H, it is advisable to replace the 100 mg sample of Cholesterylacetate used in Chapter 3 ‘Basic 1H Acquisition and Processing’ with a 1 g sample.

**Sample**

The sample used to demonstrate the basic 1D $^{13}$C experiments in this chapter is 1 g Cholesterylacetate in CDCl$_3$. In the procedure described below, however, the sample is treated as an unknown.

**Prepare the new data set**

Create a new data set starting from proton/3/1 created in the last chapter. Enter `edc` and change the following parameters:

- NAME: carbon
- EXPNO: 1
- PROCNO: 1

Click on `SAVE` to exit `edc` and create the data set carbon/1/1. The message “NO DATA AVAILABLE” should now appear on the screen.

Enter `edsp` and set the following spectrometer parameters:

- NUC1: 13C
- NUC2: 1H
- NUC3: off

The relevant spectrometer parameters are now OFSX1, BF1, and SFO1 for $^{13}$C, and OFSH1, BF2, and SFO2 for $^1$H. For the moment, let OFSX1 = 0 and OFSH1 = 0, and note that BF1 = SFO1 and BF2 = SFO2. The spectrometer is now ready to pulse and detect at the base $^{13}$C frequency of the magnet, and also to pulse at the base $^1$H frequency of the magnet.

Re-lock the spectrometer. Readjust the $Z$ and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^{13}$C and $^1$H.

**One-Pulse Experiment with No Decoupling**

The one-pulse sequence with no decoupling is identical to that used in Chapter 3 ‘Basic 1H Acquisition and Processing’ except that here the RF pulse is applied at the frequency of $^{13}$C. The pulse sequence diagram is shown in Figure 5.
**Basic $^{13}$C Acquisition and Processing**

*Figure 5: $^{13}$C One-Pulse Sequence with No $^1$H Decoupling*

![Figure 5: $^{13}$C One-Pulse Sequence with No $^1$H Decoupling](image)

Note that since there is no decoupling in this experiment, it is not necessary to have NUC2 defined to be 1H in `edsp`. Enter `edsp` and set NUC2 to off. It was suggested to set NUC2 to 1H in the introduction of this chapter merely so that this data set could be used for tuning the probehead for $^1$H as well as for $^{13}$C.

**Acquisition 4.2.1**

Enter `eda` and set the acquisition parameters values as shown in Table 4.

**Table 4. $^{13}$C Basic Acquisition Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 5 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td>not critical; 32k is a fairly standard value.</td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td>do not bother to signal average until other parameters are optimized.</td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td>no need for dummy scans yet.</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>3μsec</td>
<td>only a suggested value.</td>
</tr>
<tr>
<td>SW</td>
<td>350 ppm</td>
<td>$^{13}$C spectra cover a much broader spectral range than $^1$H spectra.</td>
</tr>
<tr>
<td>RG</td>
<td>8 k</td>
<td>or use rga.</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>for now leave this at 0; it will be optimized later.</td>
</tr>
</tbody>
</table>

Enter `rga` to start the automatic receiver gain adjustment.

Enter `zg` to acquire the FID. Notice that the X router display flashes.
Enter \texttt{si} and when prompted a value of 32\,k.

Enter \texttt{lb} to check the line broadening. Enter 3 when prompted (line broadening for $^{13}\text{C}$ spectra is typically 2 to 5\,Hz).

Enter \texttt{ef} to add line broadening and then Fourier transform the FID. Manually phase correct the spectrum and store the correction. Once this first $^{13}\text{C}$ spectrum has been phase corrected, the values of \texttt{phc0} and \texttt{phc1} are now correct for this experiment. Subsequent $^{13}\text{C}$ spectra may be processed with the command \texttt{efp}, which combines the exponential multiply, Fourier transformation, and phase correction.

The resulting spectrum is very noisy and most likely has only one visible peak, like the spectrum shown in Figure 6. This peak is the signal from the Chloroform solvent. Expand the spectrum about the Chloroform peak (notice that it is actually a triplet). Calibrate the spectrum so that the central peak of the triplet is 77\,ppm. This is equivalent to setting the TMS peak to 0\,ppm, but the TMS peak is still hidden in the noise. Again, with the digital lock, this calibration step may not be necessary. With the new digital lock, provided parameters are set correctly in the \texttt{edlock} table and that lock-in was achieved using the XWIN-NMR command \texttt{lock}, the magnetic field value is very nearly the same regardless of the lock solvent and so the spectra should be automatically calibrated. There may be an error of a few Hz, and this can be corrected by the automatic spectral referencing command \texttt{sref}. Notice that in order for the command \texttt{sref} to work properly, the parameter \texttt{solvent} must be set correctly in the \texttt{eda} table. This is taken care of automatically, however, when lock-in is achieved by the UXNMR command \texttt{lock} (recall that the solvent must be identified correctly here, see “Locking” on page 15).
Basic $^{13}\text{C}$ Acquisition and Processing

Figure 6: $^{13}\text{C}$ Spectrum of 1 g Cholesterylacetate in CDCl$_3$; No Signal Averaging, No Decoupling
One step that can be taken to improve the signal-to-noise ratio is to signal average. Enter `edc` and set `EXPNO` to 2. Click on `SAVE` to create the data set `carbon/2/1`.

Enter `ns` and change the current value to 64. Enter `ds` and change the current value to 4. The parameters are ready now so that 64 spectra will be acquired and added together. The four dummy scans are to ensure that the system reaches steady state before any spectra are added together. Enter `zg` to acquire the FID. Enter `efp` to add line broadening, Fourier transform, and phase correct the data. Because of the signal averaging, several more peaks are visible now; however, the signal-to-noise ratio is still unsatisfactory. A $^{13}$C spectrum of Cholesterylacetate after 64 scans is shown in Figure 7.

It is clear that the $^{13}$C signals are not centered in the current spectral width. To correct this, click `utilities` to enter the calibration submenu, and `o1` with the left mouse button to select `o1` calibration and tie the cursor to the spectrum. Move the mouse until the cursor is on top of the Chloroform peak and press the middle mouse button to set `o1` to this frequency. Click on `return` to exit the calibration submenu and return to the main window. Acquire and process another spectrum with this new value of `o1` (`zg`, `efp`).

The next step that can be taken to improve the signal-to-noise ratio is to apply $^1$H decoupling.
Basic $^{13}$C Acquisition and Processing

Figure 7: $^{13}$C Spectrum of 1g Cholesterylacetate in CDCl$_3$; Signal Averaging, No Decoupling
The one-pulse sequence with $^1$H decoupling is shown in Figure 8. Notice that the only difference between this sequence and the one shown in Figure 5 is that here $^1$H decoupling is applied for the duration of the pulse sequence.

**Figure 8: $^{13}$C One-Pulse Sequence with $^1$H Decoupling**

Before acquiring a $^1$H-decoupled $^{13}$C spectrum, the frequency of the Cholesterylacetate $^1$H signals must be determined.

Enter `re proton 3 1` to call up the data set proton/3/1. Ignoring the TMS and Chloroform peaks, all $^1$H signals lie in the range 0.5—5.5 ppm and most lie in the range 0.5—2.0 ppm. An appropriate frequency for $^1$H decoupling is, e.g., 1 ppm. (Note that as a general rule of thumb, when no optimized $^1$H spectrum is available, 5 ppm is a safe frequency to select for $^1$H decoupling.)

To set this frequency first click `utilities` to enter the calibration submenu. Then click `o1` with the left mouse button to select `o1` calibration and tie the cursor to the spectrum. Move the cursor to 1 ppm and press the middle mouse button to set `o1` to this frequency (this will be `o2` in the $^{13}$C spectrum). Click on `return` to exit the calibration submenu and return to the main window.

Return to the previous carbon spectrum by entering `re carbon 2 1`. Enter `edc` and set EXPNO to 3. Click on `SAVE` to create the data set carbon/3/1 for the $^1$H-decoupled $^{13}$C spectrum.

Enter `edsp` and set NUC2 to 1H. Set OFSH1 to the value of `o1` corresponding to 1 ppm in the $^1$H spectrum proton/3/1.

Enter `eda` and set the acquisition parameters as shown in Table 5.
Basic $^{13}$C Acquisition and Processing

Table 5. $^{13}$C Spectrum with $^1$H Decoupling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zgdc</td>
<td>see Figure 8 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td>not critical; 32k is a fairly standard value.</td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td>do not bother to signal average until other param-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ters are optimized.</td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td>no need to collect dummy scans yet.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL12</td>
<td></td>
<td>power level for cpd on f2 channel.</td>
</tr>
<tr>
<td>P1</td>
<td>$90^\circ$ $^{13}$C high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be $1-5\times T_1$($^{13}$C).</td>
</tr>
<tr>
<td>D11</td>
<td>30msec</td>
<td>delay for disk I/O; predefined.</td>
</tr>
<tr>
<td>SW</td>
<td>350 ppm</td>
<td>frequency of Chloroform peak in carbon/2/1.</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>frequency of 1ppm in the $^1$H spectrum proton/3/1.</td>
</tr>
<tr>
<td>O2</td>
<td></td>
<td>frequency of 1ppm in the $^1$H spectrum proton/3/1.</td>
</tr>
<tr>
<td>CPDPRG2</td>
<td>bb</td>
<td>broadband decoupling is not very efficient, but it</td>
</tr>
<tr>
<td></td>
<td></td>
<td>does not require calibrated pulses.</td>
</tr>
</tbody>
</table>

Enter zg to acquire an FID. Notice that both the $^1$H and the X router displays are active.

Processing 4.3.2

Enter efp to add line broadening, Fourier transform, and phase correct the data. A $^1$H decoupled $^{13}$C spectrum is shown in Figure 9. Notice that the TMS peak at 0 ppm is now visible and the signal-to-noise ratio is much improved. In general, the signal-to-noise ratio will depend on the decoupling frequency, which is set by sfo2, and on the power of the decoupling sequence pulses as set by pl12. (Avoid setting the power level too high, however, or the probehead may overheat.)

Since it is inefficient and can lead to sample and probehead heating, broadband decoupling is seldom used now. It is more common to use one of the so-called composite pulse decoupling (cpd) sequences, such as waltz16. Theoretically, cpd sequences can achieve the same decoupling as bb decoupling with about 50% less power. However, these cpd sequences require calibrated pulses. For example, waltz16 requires a correct $90^\circ$ pulse time pcpd2 with a correct pulse strength pl12. Pulse calibration is covered in Chapter 5 ‘Pulse Calibration’.
At this point the user may also wish to optimize $o_1$ and $sw$ so that the spectrum covers nearly the entire spectral width. For future reference, the optimized parameters $o_1$, $o_2$, and $sw$ for $^1H$-decoupled $^{13}C$ spectra of 1 g Cholesterylacetate may be recorded in Table 54 in Appendix A ‘Data Sets and Selected Parameters’.
A straightforward way to plot 1D $^{13}$C spectra is by using most of the plotting parameters found in the plot parameter file standard1D. Read in the file standard1D by entering `rpar`, selecting `standard1D` from the menu of parameter file names, and then selecting `plot` from the menu of parameter file types that appears. Equivalently, simply enter `rpar standard1D plot`. This sets most of the plotting parameters to values which are appropriate for these 1D spectra, 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 standard1D can be found in Appendix C ‘1D and 2D Plotting Parameters’.

To select the spectral region (full or expanded) to be plotted, first make sure the spectrum appears as desired on the screen, and then click `DP1` and simply hit return in response to the following three (3) questions:

- $F1 = \text{<return>}$
- $F2 = \text{<return>}$
- Change y-scaling on display according to PSCAL? $\text{<return>}$

For $^{13}$C spectra, it is a good idea to change the separation between tic marks on the $x$-axis. Enter `edg` to edit the plotting parameters. Click the `ed` next to the parameter EDAXIS to enter the X- and Y-axis parameters submenu. Change the value of the parameter XTICDIS from 0.1 to 5. This value is appropriate for a basic $^{13}$C spectrum with a large $sw$ as described in this chapter. For optimized spectra with narrower $sw$'s (e.g., less than 150 ppm), a value of 2.5 may be more appropriate. Click `SAVE` to save this change and return to the `edg` menu.

In addition, unless special precautions are taken to deal with the long $^{13}$C $T_1$ relaxation times and potential NOE build-up during $^1$H decoupling, the integrated intensities will not faithfully reflect the numbers of different types of $^{13}$C nuclei in a given molecule. Thus, it is best not to integrate standard $^{13}$C spectra. Within `edg`, click the `yes` next to the parameter INTEGR so that it toggles to `no`.

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`).
Pulse Calibration

Introduction 5.1

This chapter describes pulse calibration procedures for $^1$H and $^{13}$C as both the observe and the decouple nuclei. It is assumed that the user is already familiar with basic acquisition and processing. Note that while working through this chapter, 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 $o_1$, $o_2$, 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 this chapter.

$^1$H Observe 90° Pulse 5.2

To calibrate a $^1$H 90° pulse using the observe channel (f1), the one-pulse sequence described in Chapter 3 ‘Basic 1H Acquisition and Processing’ is used. The carrier frequency ($sfo_1$) is set to the resonance frequency of a peak in the $^1$H spectrum of an appropriate sample. That peak is monitored while the length ($p_1$) and/or strength ($p_{11}$) of the RF pulse is adjusted to determine the exact conditions for a 90° pulse.

Sample

A common sample to use for $^1$H pulse calibration is 0.1% Ethylbenzene in CDCl$_3$. Ethylbenzene has a simple $^1$H spectrum with well-separated signals, which makes it easy to select one signal for pulse calibration. The drawback of using this sample, however, is that due to the relatively long T$_1$ of Ethylbenzene, a long recycle delay time must be used.

Preparation 5.2.1

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z$^2$ shims until the lock level is optimized. Tune and match the probehead for $^1$H observation.

A few preparatory spectra need to be collected to determine the correct carrier frequency, spectral width, phase correction, and plotting region to be used in the actual calibration experiment.

First create a new data set. Since this will be a $^1$H observe experiment, it is helpful to create the new data set starting from a previous $^1$H data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter re proton 3 1 to call up proton/3/1, then enter edc and change the following parameters:

- NAME test1h
- EXPNO 1
- PROCNO 1

Click on SAVE to create the data set test1h/1/1.

Enter eda and set the acquisition parameter values as shown in Table 6.
Pulse Calibration

Table 6. $^1$H One-pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 1 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PI</td>
<td>3µsec</td>
<td>start with less than a 90° pulse.</td>
</tr>
<tr>
<td>DI</td>
<td>10sec</td>
<td>note long $T_1$ of ethylbenzene.</td>
</tr>
<tr>
<td>SW</td>
<td>20ppm</td>
<td>start with a large spectral width; this will be optimized later.</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>start with value from proton/3/1; this will be optimized later.</td>
</tr>
</tbody>
</table>

Enter *rga* to perform an automatic receiver gain adjustment.

Enter *zg* to acquire the FID.

Enter *edp* and set the processing parameters as shown in Table 7.

Table 7. $^1$H One-pulse Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>1Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td>this will be used by paropt below.</td>
</tr>
</tbody>
</table>

Add line broadening and then Fourier transform the spectrum with the command *ef*. Manually phase correct the spectrum and store the correction.

Type *sref* to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

**Set o1 and sw**

Now it is necessary to move *o1* to the signal that will be used to calibrate the 90° pulse, which in this case is the quartet of the Ethylbenzene $^1$H spectrum. Expand the spectrum so that only the quartet at 2.6ppm is displayed. Click on *utilities* to enter the calibration submenu. Click on *O1* with the left mouse button to select *o1* calibration. Move the cursor to the center of the quartet and click the middle mouse button.
button to assign $o_1$ to this frequency. Click on return to exit the calibration submenu and return to the main window.

Reduce the spectral width by entering $swh$ and changing the value to 1000Hz. Notice that with a digital filter, it is possible to reduce the spectral width this low and yet not have aliasing.

Enter $zg$ to acquire a new FID using the new values of $o_1$ and $swh$. Process the spectrum with the command ef.

**Define phase correction and plot region**

Now it is necessary to define the phase correction and spectral region that will be plotted in the output file of the automation program used to determine the 90° pulse time. Phase correct the spectrum so that the quartet is positive. Expand the spectrum so that the quartet covers approximately the central quarter of the screen. Click on DP1 with the left mouse button and hit return for the following 3 questions, or answer them as follows:

- F1 2.8 ppm
- F2 2.4 ppm
- change y-scaling on display according to PSCAL? y.

At this point, preparations are complete and we are ready to begin the actual calibration experiment.

### Calibration: High Power 5.2.2

A convenient way to calibrate a 90° pulse is with the automation program paropt. It is helpful to use paropt in the procedure outlined below when the user has no idea what the 90° time will be. Since it is somewhat time consuming, however, it is not the best procedure to follow if the user already has an idea of the correct pulse time and power level. (If this is the case, it is better to make educated guesses rather than to use paropt to check such a wide range of values).

To start the automation program, simply type xau paropt and answer the questions as follows:

- Enter parameter to modify: p1
- Enter initial parameter value: 2
- Enter parameter increment: 2
- Enter # of experiments: 16.

In this case, paropt acquires and processes 16 spectra while incrementing the parameter $p_1$ from 2µsec to 32µsec. For each value of $p_1$, only the spectral region defined above is plotted. All 16 spectra appear side-by-side in test1h/1/999, and the results should resemble those shown in Figure 10. The intensity of the quartet should vary sinusoidally over the 16 spectra. At the end of the experiment, the message “paropt finished” and a value for $p_1$ are displayed. This value is approximately the 90° pulse length of the $^1$H transmitter with the current power level $p_{ll}$. Write this value down and follow the procedure below to obtain a more accurate 90° time.

Note that the data set can be scaled horizontally with the button, and vertically with the and buttons.
Pulse Calibration

Return to the data set test1h/1/1 by entering `re 1 1`. Type `p1` and change the value to be approximately a 360° pulse (i.e., 4 times the 90° value determined by paropt).

Acquire and process another spectrum (`zg, efp`). Change `p1` by a small amount, acquire and process another spectrum, etc., until the quartet goes through a null, indicating a 360° pulse. Notice that when the phase correction is defined so that the quartet is positive for small pulse angles (as above), then the quartet will be negative when the pulse angle is slightly less than 360° and will be positive when the pulse angle is slightly more than 360°.

The 360° pulse time divided by 4 is the 90° pulse length for the $^1$H transmitter, with the current power level `pl1`, and using the current probehead.

*Figure 10: Paropt Results for $^1$H 90° Pulse Calibration*
The $^1\text{H}$ 90° pulses of the MLEV sequence used during the spinlock period of a TOCSY sequence should be 30 to 40 µsec, so it is necessary to find the corresponding power level. The procedure outlined below uses paropt to check a wide range of power levels. Alternatively, the user may make use of the rule of thumb that the pulse length should double, approximately, for every 6 dB decrease in power level. For example, say that it was determined that the 90° time ($p_1$) for $pl_1 = -6$ dB is 8 µsec. It could then be expected, roughly, that $p_1 = 16$ µsec for $pl_1 = 0$ dB and $p_1 = 32$ µsec for $pl_1 = 6$ dB. (Notice that $pl_1$ is an attenuation level, so higher numbers correspond to lower power).

Assuming the user has just finished calibrating the $^1\text{H}$ 90° pulse for high power level, as described in Section 5.2.2, return to test1h/1/1 (re: 1 1). Enter $p_1$ and change the value to 35 µsec. Use paropt to adjust the power level by typing xau paropt and answering the following questions (note that the appropriate initial power level will depend on the instrument):

- Enter parameter to modify: $pl_1$
- Enter initial parameter value: 0
- Enter parameter increment: 1
- Enter # of experiments: 16.

The results will be displayed in test1h/1/999. Notice that this time the result does not look like a simple decaying sinusoid. At the end of the experiment, the message “paropt finished” and a value for $pl_1$ are displayed. This value is the $^1\text{H}$ transmitter power level for a 90° pulse time of approximately 35 µsec. Write down this value and follow the steps below to obtain the exact 90° pulse time for this power level.

Return to test1h/1/1 (re: 1 1). Type $p_1$ and change the value to be approximately a 360° pulse (i.e., 4 times 35 µsec). Acquire and process a spectrum ($zg$, efp) using the power level $pl_1$ determined by paropt above. Change $p_1$ in small increments until the quartet goes through a null indicating a 360° pulse. Divide this 360° pulse time by 4 to get the 90° pulse time for this power level.

This is the power level and 90° pulse time for MLEV spinlocking with this probehead. Note that the parameters used by the TOCSY sequence are $p_6$ for the 90° pulse time and $pl_{10}$ for the power level, rather than $p_1$ and $pl_1$ as used here.

### Calibration: Low Power for ROESY Spinlock 5.2.4

The power level required for the cw spinlock used during ROESY corresponds to a 90° pulse length of 100 to 120 µsec. (Note, however, that no 90° pulse is actually used during a ROESY spinlock). The procedure outlined below uses paropt to check a wide range of power levels. Alternatively, the user may make use of the rule of thumb that the pulse length should double, approximately, for every 6 dB decrease in power level. For example, say that it was determined that the 90° time ($p_1$) for $pl_1 = -6$ dB is 8 µsec. It could then be expected, roughly, that $p_1 = 128$ µsec for $pl_1 = 18$ dB. (Notice that $pl_1$ is an attenuation level, so higher numbers correspond to lower power).

Return to test1h/1/1 (re: 1 1). Enter $p_1$ and change the value to 110 µsec. Use paropt to adjust the power level by typing xau paropt and answering the
following questions (note that the appropriate initial power level will depend on the instrument):

Enter parameter to modify: pl1
Enter initial parameter value: 10
Enter parameter increment: 1
Enter # of experiments: 16.

The results will be displayed in test1h/1/999. At the end of the experiment, the message “paropt finished” and a value for pl1 are displayed. This value is the $^1$H transmitter power level for a 90° pulse time of approximately 110µsec. Write down this value and follow the steps below to verify the 90° time for this power level.

Return to test1h/1/1 (re 1 1). Type pl and change the value to be approximately a 360° pulse (i.e., 4 times 110µsec). Acquire and process a spectrum (zg,efp) using the power level pl1 determined by paropt above. Change pl in small increments until the quartet goes though a null indicating a 360° pulse. Divide this 360° pulse time by 4 to get the 90° pulse time for this power level.

Notice that since ROESY uses cw spinlocking, only the power level, not the actual 90° time, is required. The above procedure is recommended simply to verify that the power level selected does in fact give a 90° time of 100 to 120µsec. Also, the parameter used by the ROESY sequence is pl11 for the cw power level, rather than pl1 as used here.

### 13C Observe 90° Pulse

#### Sample

13C observe pulse calibration experiments require a sample with a strong 13C signal. A good choice is 80% Benzene in Acetone-d6. If an appropriate sample is not available, it is possible to use the inverse mode 13C pulse calibration procedure described in Section 5.5 instead.

#### Preparation

Insert the sample in the magnet. Lock the spectrometer. Readjust the $Z$ and $Z_2$ shims until the lock level is optimized. Tune and match the probehead for 13C observation, $^1$H decoupling.

First create a new data set. Since this will be a 13C observe experiment, it is helpful to create the new data set starting from a previous 13C data set, for example carbon/2/1 (which was created in Section 4.2.3 on page 35). Enter re carbon 2 1 to call up carbon/2/1, then enter edc and change the following parameters:

- NAME = test13c
- EXPNO = 1
- PROCNO = 1.

Click on SAVE to create the data set test13c/1/1.

Enter eda and set the acquisition parameters as shown in Table 8.
Table 8. $^{13}$C One-pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 5 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PI</td>
<td>3µsec</td>
<td>start with less than a 90° pulse.</td>
</tr>
<tr>
<td>DI</td>
<td>20sec</td>
<td>note long $^{13}$C $T_1$.</td>
</tr>
<tr>
<td>SW</td>
<td>350ppm</td>
<td></td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>start with value from carbon/2/1; this will be optimized later.</td>
</tr>
</tbody>
</table>

Enter `rga` to perform an automatic receiver gain adjustment.

Enter `zg` to acquire the FID.

Enter `edp` and set the processing parameters as shown in Table 9.

Table 9. $^{13}$C One-pulse Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>3Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>

Add line broadening and then Fourier transform the spectrum with the command `ef`. Manually phase correct the spectrum and store the correction.

Type `sref` to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

**Set o1 and sw**

Now it is necessary to move o1 to the signal that will be used to calibrate the 90° pulse. Expand the spectrum so that only the doublet at 130 ppm is displayed. Click on `utilities` to enter the calibration submenu. Click on O1 with the left mouse button to select o1 calibration. Move the cursor to the center of the doublet and click the middle mouse button to assign o1 to this frequency. Click on `return` to exit the calibration submenu and return to the main window.
Reduce the spectral width by entering `swh` and changing the value to 1000 Hz. Notice that with a digital filter, it is possible to reduce the spectral width this low and yet not have aliasing. Acquire and Fourier transform another spectrum (`zg`, `ef`).

**Define phase correction and plot region**

Now it is necessary to define the phase correction and spectral region that will be plotted in the output file produced by paropt. Phase correct the spectrum so that the doublet is positive. Expand the spectrum so that the doublet covers approximately the central third of the screen. Click on `DP1` with the left mouse button and hit return for the following 3 questions, or answer them as follows:

- F1 133 ppm
- F2 127 ppm
- change y-scaling on display according to `PSCAL`?

At this point, preparations are complete and we are ready to begin the actual calibration experiment.

**Calibration: High Power 5.3.2**

Again, in the procedure outlined below, the automation program paropt is used to do the pulse calibration. This procedure is helpful when the user has no idea what the 90° time will be. Since it is somewhat time consuming, however, it is not the best procedure to follow if the user already has an idea of the correct pulse time. (If this is the case, it is better to make educated guesses rather than to use paropt to check such a wide range of pulse times).

To start the automation program, simply type `xau paropt` and answer the questions as follows:

- Enter parameter to modify: `p1`
- Enter initial parameter value: `2`
- Enter parameter increment: `2`
- Enter # of experiments: `16`.

Paropt acquires and processes 16 spectra, while incrementing the parameter `p1` from 2 µsec to 32 µsec, and displays the results side-by-side in `test13c/1/999`. The results should resemble those shown in Figure 11. The intensity of the doublet should vary sinusoidally over the 16 spectra. At the end of the experiment, the message “paropt finished” and a value for `p1` are displayed. This value is approximately the 90° pulse length of the $^{13}$C transmitter with the current power level `pl1`. Write this value down and follow the procedure below to obtain a more accurate 90° time.

Return to the data set `test13c/1/1` by entering `re 1 1`. Type `p1` and change the value to be approximately a 360° pulse (i.e., 4 times the 90° value determined by paropt).

Acquire and process another spectrum (`zg`, `efp`). Change `p1` by a small amount, acquire and process another spectrum, etc., until the doublet goes through a null, indicating a 360° pulse. The 360° pulse time divided by 4 is the 90° pulse length for the $^{13}$C transmitter, with the current power level `pl1`, and using the current probehead.
Note that if the 90° pulse length is less than 5µsec for 5 mm probes and less than 10µsec for 10 mm probes, there is a chance that the probe may arc. To prevent this from happening, if the 90° is too short change $p_{\text{ll}}$ to a higher value (i.e., increase the attenuation on the transmitter) and find the new 90° time.

**Figure 11:** Paropt Results for $^{13}$C 90° Pulse Calibration
Pulse Calibration

1H Decouple 90° Pulse

Sample
For 1H decoupling pulse calibrations, the sample must have a large 13C signal with detectable 1H coupling. A good choice is 80% Benzene in Acetone-d6.

Pulse sequence
The pulse sequence used in this procedure is the DECP90 sequence shown in Figure 12. This sequence consists of a recycle delay followed by a 90° 13C pulse, a delay 1/(2J_{XH}) for the creation of antiphase magnetization, a 1H pulse, and finally detection of the 13C signal. During calibration, the length and/or strength of the 1H pulse is adjusted. When the 1H pulse is exactly 90°, the 13C magnetization exists purely as multiple quantum coherence and so the signal disappears.

Figure 12: DECP90 Pulse Sequence

Preparation
Ideally, this procedure is carried out immediately following the 13C observe pulse calibration described above in Section 5.3, in which case the correct sample is already in the magnet, the magnet shimmed, the field locked, and the probehead tuned and matched for 13C and 1H.

Set o2
First create a new data set. Since this first spectrum will be a 1H observe experiment to determine the correct frequency for the DECP90 1H decoupling pulse, it is helpful to create the new data set starting from a previous 1H data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter re proton 3 1 to call up proton/3/1, then enter edc and change the following parameters:

NAME testdec
EXPNO 1
PROCNO 1

Click on SAVE to create the data set testdec/1/1.
Enter eda and set the acquisition parameters as shown in Table 6.

Table 10. ¹H One-pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 1 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>P1</td>
<td>3µsec</td>
<td>start with less than a 90° pulse.</td>
</tr>
<tr>
<td>D1</td>
<td>5sec</td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>20ppm</td>
<td>start with a large spectral width until the appropriate value is known.</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>start with value from proton/3/1.</td>
</tr>
</tbody>
</table>

Enter rga to perform an automatic receiver gain adjustment.

Enter zg to acquire an FID.

Enter edp and set the processing parameters as shown in Table 7.

Table 11. ¹H One-pulse Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>1Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>

Add line broadening and Fourier transform the data with the command ef.

Manually phase correct the spectrum and store the correction.

Type sref to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

With the cursor in the data field of the main window, click the left mouse button to tie the cursor to the spectrum. Move the cursor to the top of the Benzene peak at 7.3 ppm. Note the value of the cursor position in Hz shown in the small ‘Info’ window. This will be the value of o2 in the DECP90 experiment. Click the left mouse button to release the cursor from the spectrum.
**Pulse Calibration**

**Set o1 and sw**

This step was already carried out on test13c/1/1 in Section 5.3.1 on page 46. To transfer all the parameters from test13c/1/1 to the new data set, simply enter `re test13c 1 1`, then enter `edc` and change the following parameters:

- **NAME** testdec
- **EXPNO** 2
- **PROCNO** 1

Click on **SAVE** to create the data set testdec/2/1.

Here, however, 1H decoupling is required. Enter `edsp` and set NUC2 to 1H so that the spectrometer parameters are as follows:

- **NUC1** 13C
- **OFSX1** o1 from test13c/1/1
- **NUC2** 1H
- **OFSH1** o1 from testdec/1/1
- **NUC3** off

Enter `eda` and set the acquisition parameters values as shown in Table 12. Notice that o1 and swh should be set to the values used in test13c/1/1. Be sure to set o2 to the 1H offset frequency determined in the subsection “Set o2” on page 50. The DECP90 experiment will not work as described below unless both o1 and o2 are set correctly.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>decp90</td>
<td>see Figure 12 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel</td>
<td></td>
</tr>
<tr>
<td>PL2</td>
<td>high power level on f2 channel</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° 13C pulse determined in Section 5.3.2</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>2µsec</td>
<td>start with less than a 90° pulse; this will be optimized.</td>
</tr>
<tr>
<td>D1</td>
<td>5sec</td>
<td>note long 13C T1.</td>
</tr>
<tr>
<td>D2</td>
<td>3.125 msec</td>
<td>delay for creation of anti-phase magnetization (1/(2JxH)); calculated internally.</td>
</tr>
<tr>
<td>CNST2</td>
<td>160Hz</td>
<td>one-bond heteronuclear J-coupling (JxH); used to calculate d2.</td>
</tr>
<tr>
<td>SWH</td>
<td>1000Hz</td>
<td></td>
</tr>
</tbody>
</table>
Enter zg to acquire the FID. The receiver gain should already be set appropriately.

Enter edp and verify that the processing parameters are as shown in Table 13.

### Table 13. DECP90 Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>1Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>

Add line broadening and then Fourier transform the spectrum with the command ef. Manually phase correct the spectrum so that the left peak is positive and the right peak is negative. Store the correction.

The spectrum is already calibrated if the current data set was created from test13c/1/1.

Since paropt is not going to be used for this calibration procedure, it is not necessary to define the phase correction and plot region as described in the previous calibration procedures.

At this point the preparations are complete and we are ready to begin the actual calibration experiment.

### Calibration: High Power 5.4.2

The $^1$H decouple 90° pulse time should be close to the $^1$H observe 90° pulse time for the same power level, so it makes sense to do this calibration by hand rather than by using paropt.

Increase p3 to be the 90° time found in Section 5.2.2 on page 43. Acquire and process another spectrum (zg, ef, p). If the pulse angle is less than 90°, the left peak will remain positive and the right peak negative. If the pulse angle is greater than 90° but less than 270°, the left peak will be negative and the right peak positive. At 90°, the signals go through a null. This is shown in Figure 13.

Adjust p3 slightly until the signals go through a null. This is the 90° pulse length for high power.
The WALTZ-16 composite pulse decoupling (cpd) sequence requires a 90° decoupling pulse length of 80 to 100 µsec. Adjust $p_{12}$ and $p_3$ to determine the combination that gives a 90° pulse length in this range, keeping in mind that the 90° pulse time should approximately double for each 6 dB increase in $p_{12}$.

This is the power level and 90° pulse time for WALTZ-16 cpd with this probehead. Note that the parameters used by cpd sequences are $p_{cpd2}$ for the 90° pulse time and $p_{112}$ for the decoupler power level, rather than $p_3$ and $p_{12}$ as used here.
This calibration procedure should yield nearly the same results as the $^{13}$C observe 90° pulse calibration procedure outlined in Section 5.3 on page 46; however, it may be more convenient to implement because $^1$H signals are easier to detect than $^{13}$C signals and $^1$H $T_1$’s are shorter than $^{13}$C $T_1$’s.

**Sample**

For inverse experiment pulse calibrations, the detected nucleus is $^1$H, but $^{13}$C satellites must be visible. A sample with easily detected $^{13}$C satellites in the $^1$H spectrum is the $^1$H Lineshape Sample (i.e., 10% Chloroform in Acetone-d6 for frequencies ≤300 MHz, 3% Chloroform in Acetone-d6 for frequencies between 400 and 500 MHz, and 1% Chloroform in Acetone-d6 for frequencies ≥600 MHz).

![Figure 14: DECP90 Pulse Sequence](image)

The pulse sequence used in this calibration procedure is DECP90 and is shown in Figure 12. This is the same pulse sequence as was used for $^1$H decouple 90° pulse calibration in 5.4, except that now the roles of $^1$H and $^{13}$C have been exchanged.

**Preparation 5.5.1**

Insert the sample in the magnet. Lock the spectrometer. Readjust the $Z$ and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^1$H observation, $^{13}$C decoupling.

**Set $o_2$**

The first preliminary spectrum is a $^{13}$C observe experiment to determine the correct offset for $^{13}$C, which is $o_1$ here but will be $o_2$ in the inverse calibration experiment.

Create a new data set. Since this first spectrum will be a $^{13}$C observe experiment to determine the correct frequency for the DECP90 $^{13}$C decoupling pulse, it is helpful to create the new data set starting from a previous $^{13}$C data set. A good choice is
carbon/3/1 (which was created in Section 4.3.1 on page 37), since in this data set, the \( ^{13}\text{C} \) \( \omega_1 \) was set to the Chloroform peak. Enter `re carbon 3 1` to call up carbon/3/1, then enter `edc` and change the following parameters:

- NAME: `testinv`
- EXPNO: 1
- PROCNO: 1

Click on `SAVE` to create the data set testinv/1/1.

Enter `eda` and set the acquisition parameters values as shown in Table 14.

### Table 14. \( ^{13}\text{C} \) One-pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zgdc</td>
<td>see Figure 8 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL12</td>
<td></td>
<td>power level for cpd on f2 channel; use value determined in Section 5.4.3 on page 54.</td>
</tr>
<tr>
<td>P1</td>
<td>3( \mu )sec</td>
<td>start with less than a 90° pulse.</td>
</tr>
<tr>
<td>PCPD2</td>
<td></td>
<td>90° ( ^1\text{H} ) pulse for cpd sequence; use value determined in Section 5.4.3 on page 54.</td>
</tr>
<tr>
<td>D1</td>
<td>5 sec</td>
<td>note long ( ^{13}\text{C} ) ( T_1 ).</td>
</tr>
<tr>
<td>SWH</td>
<td>1000Hz</td>
<td></td>
</tr>
<tr>
<td>RG</td>
<td>8k</td>
<td>or use rga.</td>
</tr>
<tr>
<td>( \omega_1 )</td>
<td></td>
<td>start with value from carbon/3/1.</td>
</tr>
<tr>
<td>( \omega_2 )</td>
<td></td>
<td>start with value from carbon/3/1.</td>
</tr>
<tr>
<td>CPDPRG2</td>
<td>waltz16</td>
<td>a common cpd sequence for ( ^1\text{H} ) decoupling.</td>
</tr>
</tbody>
</table>

Notice that the \( \text{swh} \) value listed above is much smaller than the value customarily used for \( ^{13}\text{C} \) spectra. In this case it is ok to start with such a small value because we are only interested in the Chloroform signal and \( \omega_1 \) is already set to nearly the correct frequency.

In addition, for this first preliminary spectrum, we will just use an approximate value for \( \omega_2 \) taken from the data set carbon/3/1.

Enter `zg` to acquire the FID.

Enter `edp` and set the processing parameters as shown in Table 15.
Add line broadening and then Fourier transform the spectrum with the command `ef`. Manually phase correct the spectrum and store the correction.

Type `sref` to calibrate the spectrum and confirm the message “no peak found in ’sref’ default calibration done”.

Now it is necessary to move $o_1$ to the Chloroform peak at 77 ppm. Expand the spectrum to display the Chloroform peak only. Click on `utilities` to enter the calibration submenu. Click on $O_1$ with the left mouse button to select $o_1$ calibration. Move the cursor to the center of the signal and click the middle mouse button to assign $o_1$ to this frequency. Click on `return` to exit the calibration submenu and return to the main window. This $o_1$ value will be the $^{13}\text{C} o_2$ value for the DECP90 pulse sequence below.

**Set $o_1$ and $sw$**

The second preliminary spectrum is a $^1\text{H}$ observe experiment to determine the correct offset for $^1\text{H}$, which is $o_1$ both here and in the inverse calibration experiment.

First create a new data set. Since this will be a $^1\text{H}$ observe experiment, it is helpful to create the new data set starting from a previous $^1\text{H}$ data set, for example proton/3/1 (which was created in Section 3.5.2 on page 28). Enter `re proton 3 1` to call up proton/3/1, then enter `edc` and change the following parameters:

```plaintext
NAME testinv
EXPNO 2
PROCNO 1
```

Click on `SAVE` to create the data set `testinv/2/1`.

Enter `eda` and change the acquisition parameters as shown in Table 16.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>1 Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>
Pulse Calibration

Table 16. $^1$H One-pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>zg</td>
<td>see Figure 1 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>90° $^1$H pulse; use value determined in Section 5.2.2 on page 43.</td>
</tr>
<tr>
<td>D1</td>
<td>5 sec</td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>20 ppm</td>
<td>this will be reduced later.</td>
</tr>
<tr>
<td>RG</td>
<td></td>
<td>or use rga.</td>
</tr>
<tr>
<td>O1</td>
<td></td>
<td>start with value from proton/3/1; this will be optimized later.</td>
</tr>
</tbody>
</table>

Enter **rga** to perform an automatic receiver gain adjustment.

Enter **zg** to acquire the FID.

Enter **edp** and set the processing parameters as shown in Table 17.

Table 17. $^1$H One-pulse Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.3 Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>

Add line broadening and then Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref’ default calibration done”.

Now it is necessary to move **o1** to the Chloroform peak at 7.2 ppm. Expand the spectrum to display the Chloroform peak only. Click on **utilities** to enter the calibration submenu. Click on **O1** with the left mouse button to select **o1** calibration. Move the cursor to the center of the signal and click the middle mouse button to assign **o1** to this frequency. Click on **return** to exit the calibration submenu and return to the main window. This **o1** value will be the $^1$H **o1** value for the DECP90 pulse sequence below.
**13C Decouple 90° Pulse (Inverse Mode)**

It is now possible to reduce the spectral width. Enter `swh` and type in a value of 1000Hz at the prompt.

**Define phase correction**

The correct 1H and 13C frequencies have now been determined. The next preliminary spectrum required is a DECP90 spectrum to determine the appropriate phase correction.

Create a new data set. Enter `edc` and change EXPNO to 3. Click `SAVE` to create the data set testinv/3/1.

Next enable 13C decoupling. Enter `edsp` and set NUC2 to 13C. Also set OFSH1 to the value of `o1` determined in testinv/2/1 (the preliminary 1H spectrum) and OFSX1 to the value of `o1` determined in testinv/1/1 (the preliminary 13C spectrum).

Enter `eda` and set the acquisition parameters as shown in Table 16.

### Table 18. DECP90 Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>decp90</td>
<td>see Figure 12 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>high power level on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° 1H pulse; use value determined in Section 5.2.2 on page 43.</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>2µsec</td>
<td>start with less than a 90° pulse.</td>
</tr>
<tr>
<td>D1</td>
<td>5sec</td>
<td>relaxation delay; should be 1−5*T1(1H).</td>
</tr>
<tr>
<td>D2</td>
<td>2.34msec</td>
<td>delay for creation of anti-phase magnetization (1/(2JXH)); calculated internally.</td>
</tr>
<tr>
<td>CNST2</td>
<td>214Hz</td>
<td>one-bond heteronuclear J-coupling (JXH); used to calculate d2.</td>
</tr>
<tr>
<td>SWH</td>
<td>1000Hz</td>
<td></td>
</tr>
<tr>
<td>RG</td>
<td>use value from testinv/2/1.</td>
<td></td>
</tr>
<tr>
<td>O1</td>
<td>1H offset frequency of Chloroform peak; use o1 value from testinv/2/1.</td>
<td></td>
</tr>
<tr>
<td>O2</td>
<td>13C offset frequency of Chloroform peak; use o1 value from testinv/1/1.</td>
<td></td>
</tr>
</tbody>
</table>
Pulse Calibration

Enter zg to acquire the FID.

Enter edp and set the processing parameters as shown in Table 19.

Table 19. DECP90 Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.3 Hz</td>
<td></td>
</tr>
<tr>
<td>PSCAL</td>
<td>global</td>
<td></td>
</tr>
</tbody>
</table>

Apply line broadening and Fourier transform the spectrum (zg, ef). Expand the spectrum to display the region from about 8.5 to 7.5 ppm. This should include the Chloroform peak and its two 13C satellites. Correct the phase so that the left satellite is pointing up and the right satellite is pointing down.

Calibration: High Power 5.5.2

Increase p3 to be the 13C 90° time found in Section 5.3.2 on page 48. Acquire and process another spectrum (zg, efp). If the pulse angle is less than 90°, the left satellite will remain positive and the right satellite negative. If the pulse angle is greater than 90° but less than 270°, the left satellite will be negative and the right satellite positive. At 90°, the satellites go through a null. This is shown in Figure 15.

Adjust p3 slightly until the signals go through a null. This is the 90° pulse length for high power.
Figure 15: $^{13}$C Decouple 90° Pulse Calibration Results

- $\mathbf{p_3 > 90^\circ}$
- $\mathbf{p_3 = 90^\circ}$
- $\mathbf{p_3 < 90^\circ}$
Pulse Calibration

Calibration: Low Power for GARP CPD 5.5.3

The GARP composite pulse decoupling (cpd) sequence requires a 90° decoupling pulse length of 60 to 70 µsec. Adjust \( p_{12} \) and \( p_3 \) to determine the combination that gives a 90° pulse length in this range, keeping in mind that the 90° pulse time should approximately double for each 6 dB increase in \( p_{12} \).

This is the power level and 90° pulse time for GARP cpd with this probehead. Note that the parameters used by cpd sequences are \( p_{cpd2} \) for the 90° pulse time and \( p_{112} \) for the decoupler power level, rather than \( p_3 \) and \( p_{12} \) as used here.

1D Inverse Test Sequence 5.5.4

The 1D HMQC pulse sequence shown in Figure 16 may be used to determine how well parameters are set for inverse experiments. This is an inverse test experiment without \(^{13}\text{C}\) decoupling. The detected signal is from \(^1\text{H}\)'s bonded directly to \(^{13}\text{C}\)'s only. The signal from \(^1\text{H}\)'s bonded directly to \(^{12}\text{C}\)'s is canceled by phase cycling. Thus, the 1D HMQC spectrum of the current sample (10% Chloroform in Acetone-d6), should consist of the \(^{13}\text{C}\) satellites only, without the large central peak.

![Figure 16: 1D HMQC Pulse Sequence](image)

Ideally, this procedure is carried out immediately following the experiments described in Sections 5.5.2 and 5.5.3, in which case the correct sample is already in the magnet, the magnet shimmed, the field locked, and the probehead tuned and matched for \(^{13}\text{C}\) and \(^1\text{H}\).

Create a new data set starting from testinv/3/1, which was created in Section 5.5.1 on page 55. Enter \texttt{edc} and set EXPNO to 4. Click \\texttt{SAVE} to create the data set testinv/4/1.

Enter \texttt{eda} and set the acquisition parameters as shown in Table 20. The value of \( p_1 \) should be that for a high power \(^1\text{H}\) observe 90° pulse as determined in Section 5.2.2.
on page 43, and the value of p3 should be that for a high power 13C decouple 90° pulse as determined in Section 5.5.2 on page 60.

Table 20. 1D HMQC Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>inv4ndrd1d</td>
<td>see Figure 16 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>16</td>
<td>the number of scans should be 4*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL2</td>
<td></td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>90° 1H high power pulse on f1 channel.</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td>180° 1H high power pulse on f1 channel; calculated internally.</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td>90° 13C high power pulse on f2 channel.</td>
</tr>
<tr>
<td>D1</td>
<td>20sec</td>
<td>relaxation delay; should be 1–5*T1(1H).</td>
</tr>
<tr>
<td>D2</td>
<td>2.34msec</td>
<td>delay for creation of anti-phase magnetization (1/(2J_{XI})); calculated internally.</td>
</tr>
<tr>
<td>CNST2</td>
<td>214Hz</td>
<td>one-bond heteronuclear J-coupling (J_{XI}); used to calculate d2.</td>
</tr>
<tr>
<td>D13</td>
<td>3µsec</td>
<td>short delay.</td>
</tr>
</tbody>
</table>

Parameters such as zg, o1, and swh are already set correctly if this data set was created from testinv3/1.

Enter zg to acquire the FID.

Enter edp and set the processing parameters as shown in Table 21.

Table 21. 1D HMQC Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.30Hz</td>
<td></td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
</tbody>
</table>
Pulse Calibration

Add line broadening and Fourier transform the spectrum with the command \texttt{ef}. Manually correct the phase and store the correction.

A 1D HMQC spectrum of Chloroform is shown in Figure 17. Notice that, due to technical limitations of the spectrometer, phase cycling is never enough to completely eliminate the parent signal (the central peak arising from $^1$H’s bonded directly to $^{12}$C).

\textit{Figure 17: 1D HMQC Spectrum of 10\% Chloroform in Acetone-d6}
Distortionless Enhancement by Polarization Transfer is a polarization transfer technique and so is useful for the observation of low-γ nuclei (most commonly 13C) which are J-coupled to 1H. DEPT is a spectral editing sequence, that is, it can be used to generate separate 13C subspectra for methyl (CH₃), methylene (CH₂), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherences to differentiate between the different types of 13C signals. The pulse angle (θ) of the final 1H pulse (see Figure 18) is the basis of spectral editing with DEPT. CH₃ and CH₂ groups have maximum intensity when θ = π/4 and 0 intensity when θ = π/2; CH groups have maximum intensity when θ = π/2; and CH₂ groups have maximum negative intensity when θ = 3π/4. Quaternary carbons are missing from DEPT spectra because the large one-bond heteronuclear J-coupling (JXH) is used for polarization transfer. Quaternary carbons, by definition, are not directly bonded to any 1H’s, experience only small n-bond heteronuclear J-coupling (nJXH), and so undergo no polarization transfer.

DEPT may be run with or without 1H-decoupling. In the latter case, the familiar 1:2:1 triplets and 1:3:3:1 quartets are obtained for CH₂ and CH₃ groups, respectively. DEPT is relatively insensitive to the precise matching of delays with coupling constants, and so is much easier to use than the closely related INEPT sequence. DEPT, on the other hand, is more sensitive to pulse imperfections than INEPT.

In this chapter, DEPT-45, DEPT-90, and DEPT-135 experiments with 1H-decoupling will be described. These correspond to DEPT with θ = π/4, π/2, and 3π/4, respectively, and by appropriate adding and subtracting of the data, it is possible to obtain separate subspectra of CH, CH₂, and CH₃ signals.

Sample
The sample used to demonstrate DEPT in this chapter is 1 g Cholesterylacetate in CDCl₃.

Pulse Sequence Diagram

The DEPT pulse sequence is shown in Figure 18. The final 1H pulse is shown with pulse angle θ. This angle is set to 45° in the sequence DEPT-45, which yields spectra with positive CH, CH₂, and CH₃ signals; to 90° in DEPT-90, which yields spectra with only CH signals; and to 135° in DEPT-135, which yields spectra with positive CH and CH₃ signals and negative CH₂ signals.

Notice that the pulses p₁ and p₃ must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. Also, the cpd sequence used is WALTZ-16, which requires the calibrated 90° time pcpd₂. The 180° pulse lengths p₂ and p₄ are determined by the pulse program itself.
Figure 18: DEPT Pulse Sequence

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 \(^{13}\)C observation, \(^{1}\)H decoupling.

**Reference spectra**

Since DEPT is a \(^{13}\)C-observe, \(^{1}\)H-decouple experiment, the first step would be to obtain a reference \(^{1}\)H spectrum of the sample to determine the correct \(\delta_2\) for \(^{1}\)H decoupling. The second step would then be to obtain a \(^{1}\)H-decoupled \(^{13}\)C spectrum to determine the correct \(\sigma_1\) and \(\omega_2\) for the DEPT experiments. However, both of these steps were already carried out in Section 4.3 starting on page 37. So, a \(^{1}\)H-decoupled \(^{13}\)C reference spectrum of this sample can be found in carbon/3/1. (The one thing to be aware of is that broadband decoupling was used in carbon/3/1, but here the cpd sequence WALTZ-16 will be used).

**Create a new file directory for the data set**

Enter `re carbon 3 1` to call up the reference spectrum. Enter `edc` and change the following parameters:

- NAME: dept
- EXPNO: 1
- PROCNO: 1

Click `SAVE` to create the data set dept/1/1.

**Set up the acquisition parameters**

Enter `eda` and set the acquisition parameters as shown in Table 22. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters \(p_{11}\) and \(p_{1}\) (\(^{13}\)C observe high power level and 90° pulse time), \(p_{12}\) and \(p_{3}\) (\(^{1}\)H decouple high power level and 90° pulse time), and \(p_{112}\) and \(p_{cpd2}\) (\(^{1}\)H decouple low power level and JXH...
Acquisition and Processing

90° pulse time). Also be sure that CPDPRG2 is now set to "waltz16" and not "bb" as it was for carbon/3/1. Note that the dept, dept45, dept90, and dept135 pulse programs call an include file in which \textit{cnst2} (defined to be $J_{XH}$) is used to calculate $d_2$ ($d_2 = 1/(2*\textit{cnst2})$). Thus, it is only necessary for the user to set the value of \textit{cnst2}.

Table 22. DEPT Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>dept</td>
<td>(or dept45, dept90, or dept135) see Figure 18 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>4</td>
<td>the number of scans must be $4*n$ for the phase cycling to work correctly.</td>
</tr>
<tr>
<td>DS</td>
<td>8</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>PL2</td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>PL12</td>
<td>power level for cpd on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>P0</td>
<td>(\theta = ) high power pulse on f1 channel (only necessary for dept pulse program): 45°: all positive; 90°: XH only; 135°: XH and XH(_3) positive, XH(_2) negative.</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° (^{13}\text{C}) high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>180° (^{13}\text{C}) high power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>90° (^{1}\text{H}) high power pulse on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>180° (^{1}\text{H}) high power pulse on f2 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>PCD2</td>
<td>90° (^{1}\text{H}) pulse for cpd sequence.</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>2sec</td>
<td>relaxation delay; should be 1–5(\times T_1^{(13}\text{C})).</td>
</tr>
<tr>
<td>D2</td>
<td>3.45 msec</td>
<td>delay for creation of anti-phase magnetization ($1/(2J_{XH})$); calculated internally.</td>
</tr>
<tr>
<td>D12</td>
<td>20(\mu\text{sec})</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td>CNST2</td>
<td>145Hz</td>
<td>one-bond heteronuclear J-coupling ($J_{XH}$); 145Hz is a good intermediate value for (^{13}\text{C}).</td>
</tr>
<tr>
<td>CPDPRG2</td>
<td>waltz16</td>
<td>composite pulse decoupling sequence.</td>
</tr>
</tbody>
</table>
DEPT

**Acquire the spectrum**

First acquire a DEPT-45 spectrum. Either select the pulse program dept45 or set **p0** to the length of a 45° pulse (i.e., one half of **p1**). Enter **zg** to acquire the time domain data.

Notice that the receiver gain should already be set correctly if this data set was created from carbon/3/1. Also, this sample is quite concentrated; that is why an **ns** value of only 4 is sufficient. If the user wishes to try DEPT on another, less concentrated sample, an **ns** value of 64 may be more appropriate.

**Set up the processing parameters**

Enter **edp** and set the processing parameters as shown in Table 23.

### Table 23. DEPT Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>16 k</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td>exponential multiply.</td>
</tr>
<tr>
<td>LB</td>
<td>2</td>
<td>2 Hz line broadening.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
</tbody>
</table>

**Process the spectrum**

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum and store the correction. If all parameters have been set correctly, it will be possible to phase the spectrum so that all peaks are positive. The signals that appear in this spectrum are from the 13C’s in CH, CH₂, and CH₃ groups.

**Other spectra**

To obtain a DEPT-90 spectrum, create the data set dept/2/1, either select the pulse program dept90 or set **p0** to the length of a 90° pulse (i.e., equal to **p1**), acquire and process the data (**zg, ef**). This spectrum also has all positive peaks; however, only CH signals are visible here, so there should be fewer peaks in this spectrum than in the DEPT-45 spectrum.

To obtain a DEPT-135 spectrum, create the data set dept/3/1, either select the pulse program dept135 or set **p0** to the length of a 135° pulse (i.e., 1.5 times **p1**), acquire and process the data (**zg, ef**). This spectrum has both positive and negative peaks. The positive peaks arise from the 13C’s of CH and CH₃ groups and the negative peaks from the 13C’s of CH₂ groups.

**Plot the spectra**

A straightforward way to plot 1D 13C spectra, such as the DEPT spectra acquired here, is by using most of the plotting parameters found in the plot parameter file standard1D. Read in the file standard1D by entering **rpar**, selecting **standard1D** from the menu of parameter file names, and then selecting **plot** from the menu of parameter file types that appears. Equivalently, simply enter **rpar standard1D plot**. This sets most of the plotting parameters to values which are appropriate for
these 1D spectra, 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 standard1D can be found in Appendix C ‘1D and 2D Plotting Parameters’.

To select the spectral region (full or expanded) to be plotted, first make sure the spectrum appears as desired on the screen, and then click DP1 and simply hit return in response to the following three (3) questions:

F1 = <return>
F2 = <return>
Change y-scaling on display according to PSCAL?<return>

For $^{13}$C spectra, it is a good idea to change the separation between tic marks on the x-axis. Enter edg to edit the plotting parameters. Click the ed next to the parameter EDAXIS to enter the X- and Y-axis parameters submenu. Change the value of the parameter XTICDIS from 0.1 to 5. This value is appropriate for a basic $^{13}$C spectrum with a large sw as described in this chapter. For optimized spectra with narrower sw’s (e.g., less than 150 ppm), a value of 2.5 may be more appropriate. Click SAVE to save this change and return to the edg menu.

In addition, unless special precautions are taken to deal with the long $^{13}$C T$_1$ relaxation times and potential NOE build-up during $^1$H decoupling, the integrated intensities will not faithfully reflect the numbers of different types of $^{13}$C nuclei in a given molecule. Thus, it is best not to integrate standard $^{13}$C spectra. Within edg, click the yes next to the parameter INTEGR so that it toggles to no. 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).

DEPT-45, DEPT-90, and DEPT-135 spectra of 1 g Cholesterylacetate in CDCl$_3$ are shown in Figure 19.

The DEPT results can be compared with the standard $^1$H-decoupled $^{13}$C spectrum in carbon/3/1. Notice that some of the peaks that appear in carbon/3/1 do not appear in any of the DEPT spectra. These are the signals from the quaternary $^{13}$C’s (i.e., those not directly bonded to any $^1$H’s). From the combination of standard $^1$H-decoupled, and DEPT-45, -90, and -135 spectra, it is possible to determine which signals are from primary, secondary, tertiary, and quaternary $^{13}$C’s.
Figure 19: DEPT Spectra of 1 g Cholesterylacetate in CDCl₃
The spin-lattice relaxation time of the various $^1$H nuclei of a molecule may be determined by using the inversion recovery pulse sequence. The pulse sequence begins with a recycle delay ($t_{rd}$) that is sufficiently long to ensure that all magnetization returns to equilibrium (i.e., pure $z$-magnetization). A 180° pulse is applied which inverts the magnetization. The recovery delay follows to allow varying degrees of $T_1$ relaxation (depending on the value of the recovery delay time). The final 90° pulse then converts any $z$-magnetization into observable transverse magnetization, which is detected during the acquisition period immediately following the final pulse. Notice that if the recovery delay time is very short, the pulse sequence is equivalent to $t_{rd} - 270^\circ - \text{acq}$, and the detected signal has full, negative intensity. On the other hand, if the delay is very long, full $T_1$ relaxation occurs between the 180° and 90° pulses, and the detected signal has full positive intensity. $T_1$ can be determined by repeating the experiment with several different recovery delay values, processing the results identically, and plotting peak intensity with respect to recovery delay time. The resulting curve is an exponential with rate $1/T_1$. (Notice that for some intermediate value of the recovery delay, the peak intensity is zero and $T_1 = t_{null}/\ln 2$; however, the accuracy of this measure of $T_1$ is usually low.)

The procedure described in this chapter is for determining $^1$H $T_1$ values. A similar procedure may be used for measuring $^{13}$C $T_1$ values. However, for measuring $^{13}$C $T_1$'s, it is important to use inverse-gated $^1$H decoupling to improve the spectral signal-to-noise ratio without selectively enhancing peak intensities through NOE effects. It is also important to use a sufficiently long recycle delay (recall that $^{13}$C $T_1$'s can be much longer than $^1$H $T_1$'s).

**Sample**

The sample used to demonstrate a $T_1$ experiment in this chapter is 100 mM Pamoic Acid in DMSO-d6.

---

**Pulse Sequence Diagram**

The inversion recovery pulse sequence is shown in Figure 20. Notice that the pulse $p_1$ must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’. The 180° pulse length $p_2$ is determined by the pulse program itself.

The pulse sequence begins with the recycle delay $d_1$. The 180° pulse $p_2$ is followed by the recovery delay $v_d$. The value of $v_d$ is determined by the delays contained in the appropriate vdlst, and is varied over the course of the experiment. Observable magnetization is created by the final 90° pulse $p_1$, which is followed immediately by the acquisition period.
Measuring $T_1$

A 1D spectrum is obtained for each value of $vd$, and the results are stored in a 2D data set. The 2D data set is used by the $T_1$ calculation routine, which allows the user to determine $T_1$ for any number of peaks of the 1D spectrum.

*Figure 20: Inversion Recovery Pulse Sequence*

Acquisition and Processing 7.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the $Z$ and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^1H$ observation.

**Create a new file directory**

Enter `re proton 2` to call up the data set proton/2/1. Enter `edc` and change the following parameters:

- NAME: t1data
- EXPNO: 1
- PROCNO: 1

Click **SAVE** to create the data set t1data/1/1.

**$^1H$ reference spectrum**

Enter `rga` to perform an automatic receiver gain adjustment. Acquire and process a standard $^1H$ spectrum, as described in Chapter 3 ‘Basic $^1H$ Acquisition and Processing’ (notice that since the data set t1data/1/1 was created from proton/2/1, most acquisition parameters are already set). Calibrate the spectrum and optimize $sw$ and $ol$ so that the $^1H$ signals cover almost the entire spectral width. Acquire and process an optimized spectrum.

**Create a new file directory for the 2D data set**

From the data set t1data/1/1, enter `edc` and change EXPNO to 2. Click **SAVE** to create the data set t1data/2/1. This data set will be used for the inversion recovery experiment. Although inversion recovery is not technically a 2D experiment, it does generate an array of 1D spectra which are most easily handled as one 2D file. Thus, t1data/2/1 must be changed into a 2D data set as described below.

Enter `eda` and set PARMODE = 2D. Click **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.
Write the variable delay list

The inversion recovery experiment requires a variable delay list to provide all the values of the recovery time \( v_d \). To create the variable delay list, first enter `edlist`. A menu of list types appears. Select \( v_d \) from this menu. This calls up a menu of existing vdlst filenames and gives the user the option of creating a new file (`Type new name`). Simply type the name `t1delay`. This calls up the vi editor. Enter the delays desired, some appropriate values are listed below:

- 10s
- 5s
- 4s
- 3s
- 2s
- 1s
- 0.5s
- 0.25s
- 0.1s
- 0.01s

When the list is complete, save the file and exit the editor. (Note that for best results, it is recommended to begin and end the list with the longest \( v_d \) value (if all is working properly, then, the first and last spectra should be identical), and to scramble the order of the intermediate values.)

Set up the acquisition parameters

Enter `eda` and set the acquisition parameters as shown in Table 24. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters \( p_{l1} \) and \( p_{1} \) (\(^1\)H observe high power level and 90° pulse time). The relaxation delay time \( d_1 \) should be chosen to be longer than five times the longest \( T_1 \) value to be measured.

Be sure to set `vdlst` to the name of the appropriate variable delay list (here `t1delay`). This may be done in the `eda` menu either by typing `t1delay` in the box next to the parameter VDLIST, or by clicking with the right mouse button on this box to call up the menu of possible vdlsts and then selecting `t1delay` with the left mouse button. Also be sure to set `l4` equal to the number of entries in the vdlst (here 10).

The F2 parameters \( o_{1} \) and \( s_{w} \) (not shown in the table) should be identical to the values used in the optimized \(^1\)H reference spectrum (t1data/1/1). The only important F1 acquisition parameter is \( t_{d} \), and this should be set to the number of delays in the vdlst.

Table 24. Inversion Recovery Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>( t_{1ir} )</td>
<td>see Figure 20 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>16k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>the number of scans must be 8*n in order for the phase cycling to work properly.</td>
</tr>
</tbody>
</table>
# Measuring T<sub>1</sub>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>4</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>P1</td>
<td>90° 1H high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>180° 1H high power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>10s</td>
<td>relaxation delay; must be longer than five times the longest T&lt;sub&gt;1&lt;/sub&gt; to be measured.</td>
</tr>
<tr>
<td>D11</td>
<td>30msec</td>
<td>delay for disk I/O; predefined.</td>
</tr>
<tr>
<td>L4</td>
<td>10</td>
<td>loop counter; set to number of entries in vclist.</td>
</tr>
<tr>
<td>VDLIST</td>
<td>t1delay</td>
<td>name of vclist used to provide various recovery delays.</td>
</tr>
<tr>
<td>TD</td>
<td>10</td>
<td>number of experiments.</td>
</tr>
</tbody>
</table>

## Acquire the 2D data set

If this data set was created from the 1H reference spectrum t1data/1/1, the receiver gain is already set correctly.

Enter zg to acquire the time domain data. The approximate experiment time for the inversion recovery experiment with the acquisition parameters set as shown above is 30 minutes.

## Set up the processing parameters

Enter edp and set the processing parameters as shown in Table 25. Notice that the F1 parameter si must be set to a power of two greater than or equal to the number of delays in the vclist used.
Table 25. Inversion Recovery Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>spectrum reference frequency ($^1$H).</td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>1Hz</td>
<td></td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>

Process the spectra
The spectra will be processed by the automation program proc_t1. If desired, however, the spectra may be processed manually. Simply enter xf2 to multiply the time domain data by the window function and also perform the Fourier transform in F2 only. The 2D data set is displayed automatically.

Write the integral range file and baseline point file
The automation program proc_t1 which will be used to calculate $T_1$ for the defined peaks requires a predefined integral range file and baseline point file. These files must be written before running the automation program.

From the 2D data set, move to a 1D data set containing the row for which $vd$ is a maximum (here, row 1). This may be accomplished by entering rser 1. This copies the FID of the first row into the data set ~TEMP/1/1.

Enter ef to apply line broadening. Manually phase correct the spectrum and store the correction.

Click on integrate to enter the integration subroutine. Click the left mouse button to tie the cursor to the spectrum and then use the middle mouse button to select the integration regions. Click once at the beginning and once at the end of each region. A separate region must be selected for each peak for which $T_1$ will be calculated. When all the desired regions have been defined, click the left mouse button to release the cursor from the spectrum. Click on return and select Save as ‘intrng’ and return to store the regions and return to the main 1D window. Enter wmisc to call up the menu of miscellaneous list types. Select intrng to select the integral...
range file type. This calls up the list of possible files. Simply type the new name \textit{t1reg}. Now the integral regions selected above are written to the integral range file \textit{t1reg}.

From the main 1D window, enter \texttt{basl} to enter the baseline submenu and from here click on \texttt{def-pts} to enter the baseline point subroutine. In this subroutine, the cursor is tied to the spectrum. Use the middle mouse button to select the points for which $T_1$ will be calculated. One and only one point must be selected for each integral region defined above. Take care to select the point of maximum intensity for each peak (region). When finished, click the left-hand mouse button to release the cursor from the spectrum and store the baseline points. Next enter \texttt{wmisc} to call up the menu of miscellaneous list types. Select \texttt{baslpnts} to select the baseline point file type. This calls up the list of possible files. Simply type the new name \textit{t1bas}. Now the baseline points selected above are written to the baseline point file \textit{t1bas}.

Click on \texttt{return} to return to the main 1D window. From here, click on \texttt{2D} with the left mouse button to return to the full 2D data set.

\section*{\textbf{T}_1 \textbf{Calculation} \hspace{1cm} 7.4}

Once the $T_1$ data have been acquired and the integral range and baseline point files have been defined, the data may be processed and the $T_1$ calculation carried out using the automation program \texttt{proc\_t1}. This program first Fourier transforms and phase corrects the rows of the 2D $T_1$ data set. It then performs a $T_1$ calculation on all the peaks indicated by the integral range and baseline point files.

\textbf{The automation program proc\_t1}

Start the automation program from the 2D data set by entering \texttt{xau proc\_t1}. Answer the questions as follows:

\begin{itemize}
  \item Enter fid no. for phase determination: 1
  \item Enter left limit for baseline correction: 1000
  \item Enter right limit for baseline correction: -1000
  \item Enter no. of drift points: 20
  \item Enter name of baseline point file: t1bas
  \item Enter name of integral range file: t1reg
  \item Enter name of VD list to use: t1delay
  \item Enter calc. type ($T_1=1$, $T_2=2$): 1
\end{itemize}

The FID corresponding to the largest value of $v_d$ (i.e., full relaxation between the $180^\circ$ and $90^\circ$ pulses) should be used for phase determination. The values for left and right limit for baseline correction are in ppm. The values $\pm 1000$ are suggested merely to be sure that all spectra fall completely within these limits. The automation program applies a baseline correction in F2 (\texttt{abs2}) between these two limits, and it is important, obviously, to baseline correct the entire spectral width.

The number of drift points accounts for the fact that the maximum of a peak selected for a $T_1$ calculation is usually not at exactly the same position for each 1D spectrum. The number of drift points specifies how many digital points the peak maximum may vary. This parameter may need some optimization. It is important to select the number of drift points large enough so that you are always sure to find the peak maximum, yet small enough so that the maximum is always of the same peak. If the number of drift points is chosen incorrectly, peak picking will not work properly.
and the T₁ curves will not all be smooth exponential curves. (Peak picking can also fail if the integral regions and baseline points are not selected carefully.)

When proc_t1 is finished, the message “T1 result stored in t1r” appears. The full pathname of this file is /u/data/[user name]/nrm/t1data/2/pdata/1/t1r (i.e., it is in the same directory as the current processed data).

The peak intensity vs. vD time data are also gathered and plotted for each resonance. To view these results, select Analysis->Relaxation to enter the T₁/T₂ routine. The first T₁ curve should appear automatically in the window. Enter nxtp to view the T₁ curves for successive peaks.

**Check T₁ curves**

At this point it is a good idea to check the T₁ curve for each selected resonance to verify that the vD values were chosen so that all curves are clearly defined. For example, for a very short value of T₁, more short values of vD may be needed to define the beginning of the exponential curve, while for a very long value of T₁, more long values of vD will be required to define the “end” of the curve. If any T₁ curves are not well defined, it is necessary to edit the vdlst t1delay and rerun the experiment so that reliable T₁ measurements for those resonances may be obtained.

Also check all T₁ curves to be sure that they are smooth exponentials. If not, although it may not be necessary to rerun the experiment, it is necessary to redo the T₁ calculation. First, if there are only a few bad points per T₁ curve, it is possible to remove the bad points and redo the T₁ calculation by hand. Points may be removed from a curve one at a time by typing elim and then selecting a point with the middle mouse button (click the left mouse button to quit without choosing a peak). Eliminated points may be restored by entering rstp (this restores all eliminated points to all T₁ curves).

Once the bad points have been removed from a curve, enter ct1 to begin the T₁ calculation for that resonance. Enter nxtp to call up the next curve, remove the bad points, enter ct1 to calculate T₁ for that peak, and so on. Alternatively, remove the unwanted points from all curves and then enter dat1 to begin the T₁ calculation for all selected peaks. (Note that unless CURPRIN is changed before using ct1 or dat1 to recalculate T₁, the numerical results from proc_t1 will be overwritten as discussed below.)

If there are too many bad points for a given T₁ curve to be a reliable fit, proc_t1 should be rerun. It may be necessary to use a different number of drift points, or to redefine the integral range and baseline point files.

**Check numerical results**

The numerical results generated by the T₁ calculation routine may be stored in a file, displayed on the monitor, or sent directly to the printer. The automation program proc_t1 automatically stores the results in the file t1r in the processed data subdirectory. (After running proc_t1 enter edo to call up the plotter options menu and note that CURPRIN is set to t1r.) Each time a T₁ calculation is run with CURPRIN set to t1r, this file is overwritten. This means that it is overwritten each time proc_t1 is run, and may be overwritten when ct1 or dat1 is used. However, before using ct1 or dat1, the user also has the option to set CURPRIN to $screen or to the appropriate printer.

To display the numerical results on the screen, set CURPRIN to $screen as follows: enter edo, click the box next to CURPRIN with the left mouse button, and then
Measuring $T_1$

Enter $\text{screen}$. Click \textbf{SAVE} to exit the \textbf{edo} menu. To send the results directly to the printer, click the box next to CURPRIN with the right-hand mouse button to call up the menu of printer options, select one of these with the left-hand mouse button, and then click \textbf{SAVE} to exit the \textbf{edo} menu.

The numerical results consist of a table for each selected peak. These tables indicate TAU (i.e., $\text{vd}$ values), CURSOR, FREQ, PPM, INTEGRAL, and INTENSITY for each point. Below each table is the statement “[n] intensities fit” or “[n] areas fit”. This is an indication of how well the peak picking worked. For example, here 10 values of $\text{vd}$ were used, so if peak picking worked well, 10 intensities should have been fit for each peak selected. If 0 or very few intensities were fit for one or more peaks, it is a good idea to redefine the integral range and baseline point files and rerun \texttt{proc\_t1}.

Finally, for each peak selected, there is a table indicating the $T_1$ and standard deviation calculated.

**$T_1$ parameters**

If necessary, the user may edit a number of parameters used in the $T_1$ calculation routine. In the $T_1/T_2$ menu, select \texttt{edt1} from the \texttt{T1cmds} pulldown menu, or simply enter \texttt{edt1}. Some appropriate values are indicated in Table 26.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMPTS</td>
<td>10</td>
<td>number of delays in \text{vdlist}.</td>
</tr>
<tr>
<td>FITTYPE</td>
<td>intensity</td>
<td>$T_1$ will be calculated from peak intensity (rather than integrated area).</td>
</tr>
<tr>
<td>CURSOR</td>
<td>1</td>
<td>start with the first peak chosen.</td>
</tr>
<tr>
<td>CONV</td>
<td>$e^{-5}$</td>
<td>convergence criterion for the fit algorithm.</td>
</tr>
<tr>
<td>DRIFT</td>
<td>20</td>
<td>allowed peak drift for peak picking.</td>
</tr>
<tr>
<td>START</td>
<td>1</td>
<td>starting spectrum for peak picking.</td>
</tr>
<tr>
<td>INC</td>
<td>1</td>
<td>increment for next spectrum used in peak picking.</td>
</tr>
<tr>
<td>NUMTERM</td>
<td>3</td>
<td>number of variables used in fitting routine.</td>
</tr>
</tbody>
</table>

Create a Stacked Plot

This section describes a method for obtaining a stacked plot of the 2D data set. The plot is created by the \texttt{au} program \texttt{stack2d}, which uses the plot parameter set \texttt{stackplot}. Note that \texttt{stackplot} is a 1D plot parameter set.

To create the 1D parameter set, first return to the reference spectrum (enter \texttt{re11}). Use the mouse to select an appropriate region for plotting. Once the desired region is displayed, save it by clicking on \texttt{DP1} with the left mouse button and hitting return to answer the questions that follow.
Next create the plot parameter set to be used by the au program. Enter edg to call up the plot parameter menu. Make sure that SPECT is set to YES, but that XAXIS, YAXIS, TITLE, INTEGR, and PARAM are set to NO (see Appendix C, section C.2 ‘1D Plotting Parameters’). Click on the ED which appears next to the option ED-SPECT to call the submenu “Spectrum Plot Parameters”. The following selected parameter values are suggested for A4 (8.5” × 11”) paper.

Table 27. Spectrum Plot Parameters for Stacked Plot

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Value (A4)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXLLEFT</td>
<td>2.0 cm</td>
<td></td>
</tr>
<tr>
<td>SYLLEFT</td>
<td>1.0 cm</td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>20.0 cm</td>
<td></td>
</tr>
<tr>
<td>SHEI</td>
<td>20.0 cm</td>
<td></td>
</tr>
<tr>
<td>F1P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPMCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HZCM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHEI</td>
<td>19.0 cm</td>
<td></td>
</tr>
<tr>
<td>ZERO</td>
<td>4.0 cm</td>
<td></td>
</tr>
<tr>
<td>CY</td>
<td>6.0 cm</td>
<td></td>
</tr>
</tbody>
</table>

These parameters were set when DP1 was used to define the plot region.

Click SAVE to save these changes and return to the main edg menu. There, click SAVE to save all changes and exit edg.

Next, save these parameters as the plot parameter file stackpar. This is done by entering wpar stackpar plot.

Once the 1D parameters are set, return to the 2D processing menu (enter re 2 1).
Start the stacked plot automation program by entering `xau stack2d`. Answer the questions as follows:

- Enter NAME: `t1data`
- Enter EXPNO: `2`
- Enter PROCNO: `1`
- Enter USER: `[user name]`
- Enter DISK: `u`
- Repeat dialog (r) or continue (c): `c`
- Enter first row to plot: `10`
- Enter row increment: `-1`
- Enter number of rows: `10`
- Enter row for scaling: `1`
- Enter x increment [cm]: `0.2`
- Enter y increment [cm]: `1.2`

The resulting stacked plot is sent to the plotter specified by the parameter CURPLOT. To check or change this parameter, enter `edo` to call up the output device parameter menu. Using the right-hand mouse button, click on the box next to CURPLOT to open the menu of plotter options. Select one of these with the left-hand mouse button, and then click **SAVE** to exit the `edo` menu.

A stacked plot of the results of the inversion recovery sequence run on 100 mM Pamoic Acid in DMSO-d6 is shown in Figure 21. Notice that only a portion of the entire spectral width used is shown in the figure.
Figure 21: Inversion Recovery Spectra of 100 mM Pamoic Acid in DMSO-d6
Measuring $T_1$
Selective Excitation

Introduction

The hard pulses used in the other chapters of this manual are meant to excite the entire spectral width uniformly. In this chapter we discuss using a soft pulse to excite only one multiplet of a \(^1H\) spectrum selectively. Important characteristics of a soft pulse include its shape, its amplitude, and its length, where (although roughly) the pulse shape may be correlated with the shape of the excitation profile, the pulse amplitude with the flip angle, and the pulse length with the pulse selectivity (i.e., the width of the excited spectral region).

For example, the pulse shape used in this chapter is a Gaussian. The excitation profile of a Gaussian pulse falls off quickly and has no side lobes in the frequency domain (unlike the excitation profile of a square pulse). This means that a Gaussian pulse fulfills the condition of selectivity to a high degree of accuracy, i.e., it has a minimal effect on the rest of the spectrum.

The Gaussian pulse envelope must be truncated at some suitably low level so that the rf field is extinguished before the signal acquisition or application of other pulses begins. Truncation when the intensity falls below 1% of the maximum is acceptable, and is used here. The pulse width is then defined as the width between the 1% points.

In cases where the resonances of a given spin are well separated from all other resonances of the spin system, the effect of a selective 90° Gaussian pulse is (nearly) equivalent to the effect of a hypothetical nonselective 90° pulse applied selectively to that given spin, followed by a delay of suitable length.

The pulse length used in this chapter is 80 msec. The selectivity of a pulse is measured by its ability to excite a chosen resonance (or group of resonances) without appreciably affecting near neighbors. For a given net flip angle, the selectivity is determined by the duration of the soft pulse. Generally, the peaks to be excited should lie within ±1/2\(t_p\) Hz of the transmitter frequency, where \(t_p\) is the length of the selective pulse. The peaks to be left unperturbed should lie more than ±\(\pi/t_p\) Hz from the transmitter frequency.

Since the length of the selective pulse affects its selectivity, the length is selected based on the selectivity desired and then the pulse amplitude (i.e., power level) is adjusted to give a 90° (or 270°) flip angle.

Notice that it is necessary to set the transmitter offset frequency of the selective pulse to the frequency of the desired resonance. This transmitter frequency does not have to be the same as \(\omega_1\) (the offset frequency of the hard pulses), but for reasons of simplicity, they are often chosen to be identical.

Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments non-spinning.

Selective Excitation


Sample
The sample used to demonstrate selective pulse experiments in this chapter is 50 mM Gramicidin in DMSO-d6.

NOTE: Selective excitation experiments using shaped pulses are possible on DSX, DMX, and DRX spectrometers but not on DPX spectrometers since they have no rf-shape modulator.

Selective Pulse Calibration

8.2

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 recommended that selective experiments be run without sample spinning.

Before performing selective excitation experiments, it is first necessary to calibrate the selective pulse. The steps involved in this procedure are first, to obtain a 1H reference spectrum and determine the resonance frequency of the desired resonance; second, to define the shaped pulse; and third, to perform the pulse calibration experiment.

1H reference spectrum
The first step is to acquire and process a standard 1H spectrum. This 1H spectrum will be used to determine o1, sw, and the resonance for selective excitation.

Enter re proton 2 1 to call up the data set proton/2/1. Enter edc and change the following parameters:

NAME selex
EXPNO 1
PROCNO 1

Click SAVE to create the data set selex/1/1.

Enter rga to perform an automatic receiver gain adjustment. Acquire and process a standard 1H spectrum. Throughout this chapter, the ornithine peptide N-H resonance at 8.65ppm will be used for selective excitation. Change o1 to the frequency of this resonance as follows: Expand the spectrum about the peak at 8.65ppm. Click on utilities to enter the calibration submenu. Click on O1 with the left mouse button to select o1 calibration. Move the cursor to the center of the doublet and click the middle mouse button to assign o1 to this frequency. Click on return to exit the utilities submenu and return to the main window.

Make sure sw is large enough to include the entire 1H spectrum, even with this new value of o1. Acquire and process a second 1H spectrum with this new o1.

(Notice that it is possible to perform selective excitation experiments off-resonance, but for the sake of simplicity, only on-resonance experiments will be described here).
Selective one-pulse sequence

The pulse sequence used to calibrate the selective pulse is shown in Figure 22. Notice that this sequence is identical to the standard one-pulse sequence shown in Figure 1 on page 19, except that now the pulse is a low-power, shaped pulse. The pulse length \( p11 \) and the pulse strength \( sp1 \) must be adjusted so that the pulse is 90° or 270° (see below). In addition, the actual shape of the pulse must be defined.

Figure 22: Selective One-Pulse Sequence

Define the pulse shape

The shape of the selective pulse is defined by the program \texttt{xShape}, which is run outside of XWIN-NMR. Open a UNIX shell and enter \\texttt{/u/prog/\langleversion\rangle/xShape}, where \texttt{\langleversion\rangle} is the name of the current XWIN-NMR, e.g. \texttt{xwin-nmr1.1}, to start the program. This opens a window entitled “RF Pulse and Gradient Shapes.” The window includes a region in which the pulse shape will be plotted. Below this is a menu of options that the user can select. These options are selected by entering the appropriate number or letter at the command line at the bottom of the window.

The following describes how to set up a standard set of parameters for generating a Gaussian pulse shape:

At the prompt “Which function do you want>”, enter \texttt{I} to define the size of the shaped pulse. At the prompt “# of points to calculate (multiple of 2, real size)>” enter \texttt{1024}.

Again the prompt “Which function do you want>” appears. This time enter \texttt{2} to select a Gaussian shape. At the prompt “truncation level: (0.001% < level% < 100%)>” enter \texttt{1} to select a truncation level of 1%.

The selected shape is now plotted in the window. This shape should now be saved as follows: At the prompt “Which function do you want>”, enter \texttt{W} to write the file. The prompt “Storage as RF (=0), GRADIENT (=1) or GRADPROG (=2) shape (0/1/2)>” then appears. Enter \texttt{0} to indicate an RF shape. Next the prompt “Storage of whole file (=0) or fractional part of it (=1) (0/1)>” appears. Enter \texttt{0} to store the whole file, then the prompt “Apply attenuation Y/N” and enter \texttt{n}. Finally, at the prompt “Enter filename>” enter the filename \texttt{gaus1.1k}.

The message “going to write: /u/exp/stan/nmr/lists/wave/gaus1.1k” appears on the comment line.

Exit the shape program by typing \texttt{q} at the prompt “Which function do you want>”.

Return to the window running UXNMR.
Selective Excitation

**Acquire and process the selective one-pulse spectrum**

First, create a new data set for the 1D selective experiment. From the data set selex/1/1 enter `edc` and change EXPNO to 2. Click **SAVE** to create the data set selex/2/1. Then set up the acquisition parameters as shown in Table 28.

### Table 28. Selective One-Pulse Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>selzg</td>
<td>see Figure 22 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>1</td>
<td>no need for signal averaging yet.</td>
</tr>
<tr>
<td>DS</td>
<td>0</td>
<td>no need for dummy scans yet.</td>
</tr>
<tr>
<td>SP1</td>
<td>80dB</td>
<td>shaped pulse power level on f1 channel.</td>
</tr>
<tr>
<td>P11</td>
<td>80msec</td>
<td>90° shaped pulse on f1 channel.</td>
</tr>
<tr>
<td>D1</td>
<td>10sec</td>
<td>relaxation delay; select a long delay time to ensure correct pulse calibration results.</td>
</tr>
<tr>
<td>D12</td>
<td>20µsec</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td>SP</td>
<td>edit</td>
<td>enter this array to edit power level, offset, and filename for the shaped pulse.</td>
</tr>
</tbody>
</table>

To enter the power level, offset, and filename for the shaped pulse, click on the **edit** button next to the parameter **SP07** towards the bottom of the **eda** table. This calls up the table “Power for shaped pulses”, which has four columns: one for the shaped pulse index number (Index), one for the power level (Power[dB]), one for the offset frequency (Offset-Frequency), and one for the filename of the shaped pulse (Filename). The pulse program **selzg** makes use of shaped pulse 1 only, so here the user need only be concerned with entries in the row corresponding to index number 1.

In row 1, set the power level for the shaped pulse to 80dB. (This parameter is also known as **spl**.) Then, for on-resonance selective excitation, make sure that the offset frequency is set to 0Hz. Finally, click on the filename box with the right mouse button to call up the menu of possible shape files. From this list, select gauss1.1k with the left mouse button.

All other acquisition parameters should be the same as for the reference spectrum, in particular **td, ol, sw, and rg**.

Acquire and process a selective one-pulse spectrum. The spectrum should be processed with the command **efp** so that the **same phase correction as was used for the reference spectrum** is applied. The ornithine N-H resonance should appear in the middle of the window and no other peaks should be visible. Phase correct the ornithine N-H resonance using the 0th-order correction only. Note this value, but click **return** to return to the main menu **without** storing the phase correction. This additional phase correction might have to be applied to the shaped pulse only, not to the hard pulses (used in the pulse programs **selco** and **semlzfs** below).
Selective Pulse Calibration

To apply the additional phase correction to the shaped pulse it must be entered into the PHCOR(1) menu within eda. Enter eda and select the array PHCOR near the bottom of the table. This opens the table “correction angle for phase program,” which lists the correction in degrees for phases [0] to [31]. Since ph1 is the phase used for the shaped pulse, enter the phase correction beside “[1]”. (Rather than using the PHCOR table, it is also possible to type 2 phcor 1 and enter the phase correction directly.)

Now if the spectrum is reacquired and processed with efp, the ornithine peptide N-H should be phased properly.

Expand the spectrum so that the ornithine N-H doublet occupies approximately the center quarter of the window (e.g., so that the region from approximately 9.2 ppm to 8.1 ppm is displayed). Save this as a plotting region by clicking on DP1 with the left-hand mouse button and hitting return in response to the questions. This plotting region will be used by the au program paropt, below.

Perform the pulse calibration

As described in Chapter 5 ‘Pulse Calibration’, the au program paropt may be used to perform an automatic pulse calibration. Simply enter xau paropt and answer the questions as follows:

- Enter parameter to modify: sp1
- Enter initial parameter value: 90
- Enter parameter increment: –2
- Enter # of experiments: 20

At the end of the experiment, the message “paropt finished” and a value for sp1 are displayed. This value is the approximate ¹H transmitter power level for a 90° pulse time of 80 msec.

Note that the maximum intensity, at the 90° pulse, should occur at approximately 6 dB less attenuation than the null at the 180° pulse.

To obtain a more accurate 90° pulse, repeat paropt using a smaller increment for sp1. (At this point it may be useful to repeat the above procedure for a range of pl1 pulse lengths.)

Paropt results of selective excitation of the ornithine N-H resonance are shown in Figure 23. Notice that the peak corresponding approximately to a 270° pulse has a better lineshape than that corresponding approximately to a 90° pulse. This is because a 270° Gaussian pulse causes refocusing of evolution due to J-coupling that occurs during the pulse. The 270° pulse can be thought of as a 90° pulse followed by a 180° refocusing pulse. Since the 180° pulse is also selective, the evolution of the magnetization due to J-coupling is refocused in exactly the same way as if it were due to chemical shifts.

In fact, in many cases, a 270° Gaussian pulse is preferable to a 90° Gaussian pulse. For this reason, it is also suggested to use the above procedure to determine the correct sp1 value for a 270° pulse of 80 msec.

A selective one-pulse ¹H spectrum of the ornithine N-H resonance of Gramicidin, together with the reference spectrum, is shown in Figure 24. The selective spectrum was obtained using a 270° Gaussian pulse.
Selective Excitation

Figure 23: Selective One-pulse Paropt Results

~90°

~270°
Figure 24: Selective One-pulse Spectrum of 50mM Gramicidin in DMSO-d6

270° Gaussian Pulse

$^1$H Reference Spectrum
Selective Excitation

Selective COSY 8.3

Many 2D NMR experiments can be converted to analogous 1D experiments by using Gaussian pulses. A 1D sequence is advantageous when a limited amount of information is desired, which is often the case for medium-sized molecules. When this is the case, the total experiment and data manipulation times are shorter for the 1D experiment than for the 2D experiment, and the data storage capacity requirements are less.

The 2D COSY experiment is very effective at indicating coupling except in cases where the $^1$H chemical shifts are closely crowded together so that many cross-peaks overlap. Selective COSY gives the same $^1$H coupling information one site at a time, without involving a 2D Fourier transform. This is useful for probing regions of the spectrum where the $^1$H shifts are densely packed, provided that some $^1$H resonances are sufficiently well separated that they can be picked out for selective irradiation.

The selective COSY sequence begins with a 90° frequency selective excitation pulse. This is followed by a fixed delay (rather than the variable evolution period of the 2D COSY sequence) during which antiphase coherence is created by evolution due to J-coupling. The duration of this delay is measured from the middle of the Gaussian envelope. As with 2D COSY, the second (or coherence transfer) pulse is a hard 90° pulse. This pulse creates observable magnetization from the antiphase coherence present at the end of the fixed delay. The acquisition period follows immediately after the second pulse.

The frequency of the selective pulse is set to the chemical shift of a multiplet and the selectivity is chosen so that adjacent multiplets are unperturbed. The spectral width is set large enough to cover the entire chemical shift range whatever the transmitter offset. The intensity of the transferred signal depends on the magnitude of the appropriate coupling constant and on the length of the fixed delay, and varies in a sinusoidal fashion. There is a chance that a particular transfer falls accidentally at a null, in which case a coupling path would be overlooked. This risk can be minimized by selecting the precession interval short compared with the reciprocal of the largest expected coupling constant. The lower level of the delay is one half the Gaussian duration needed to get the required selectivity.

Since the final pulse gives coherence transfer to spins whose couplings are in antiphase to the selectively excited spin, 1D selective COSY gives rise to antiphase multiplets (which will unavoidably have adjacent positive and negative intensities). Thus, direct extraction of the coupling constants may be complicated due to annihilation of individual lines within the multiplet.

Notice that the final pulse also converts any longitudinal magnetization into transverse magnetization. The resulting signals are intense for all $^1$H sites other than the one excited by the selective pulse. These signals are eliminated by the same phase cycling as is used in 2D COSY; however, the corresponding signals in the 2D experiment are much weaker, and so are more easily eliminated by the phase cycling.
The selective COSY pulse sequence is shown in Figure 22. Notice that it is very similar to the standard COSY sequence shown in Figure 35 on page 134, except that here the first pulse is a low-power shaped pulse and the delay between the two pulses ($d_{14}$) is not incremented.

The high-power pulse $p_1$ must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’, and the shaped pulse $p_{11}$ to the appropriate 90° time found in Section 8.2 ‘Selective Pulse Calibration’. The delay $d_{14}$ should be set so that $(p_{11})/2 + d_{14} = 1/(2J_{HH})$ for the selected resonance. Notice that $p_{11}$ may also be chosen to be a 270° Gaussian pulse, in which case the delay $d_{14}$ should be set to $1/(2J_{HH})$.

**Figure 25: Selective COSY Pulse Sequence**

If this experiment is carried out directly after the pulse calibration described in Section 8.2, the correct sample is already in the magnet, the probehead tuned and matched, and the magnetic field shimmed and locked.

For best results, run selective COSY experiments non-spinning.

**Create a new file directory**

From the data set selex/2/1, enter edc and change the following parameters:

- NAME: selco
- EXPNO: 1
- PROCNO: 1

Click **SAVE** to create the data set selco/1/1.

**$^1$H reference spectrum**

The reference spectrum for the selective COSY experiment is the standard $^1$H spectrum of Gramicidin with $o_1$ set to the ornithine peptide N-H resonance. This may be found in selex/1/1.
Set up the acquisition parameters
Enter \texttt{eda} and set the acquisition parameters as shown in Table 29. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters \texttt{pl1} and \texttt{p1} (\textsuperscript{1}H observe high power level and 90° pulse time), and the values determined in Section 8.2 ‘Selective Pulse Calibration’ for the parameters \texttt{sp1} and \texttt{p11} (\textsuperscript{1}H selective low-power level and 90° pulse time).

Table 29. Selective COSY Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>selco</td>
<td>see Figure 22 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>64</td>
<td>number of scans must be 8*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>SP1</td>
<td>shaped pulse power level on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° \textsuperscript{1}H high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P11</td>
<td>80 msec</td>
<td>90° \textsuperscript{1}H shaped pulse on f1 channel.</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be 1–5*T\textsubscript{1}(\textsuperscript{1}H).</td>
</tr>
</tbody>
</table>
| D13       | 3 \textmu
sec | short delay; predefined.                                                 |
| D14       | 35 msec        | delay for evolution after shaped pulse ((p11)/2 + d14 = 1/(2J_{HH})).   |
| PHCOR(1)  |                | additional phase correction applied to shaped pulse; use value determined on page 86. |

Notice that in this pulse sequence, the delay \texttt{d14} is to ensure that the magnetization is antiphase when the second pulse is applied. There are two ways to accomplish this. The first, which is appropriate when the selective pulse is 90°, is to choose \texttt{d14} such that (\texttt{p11})/2 + \texttt{d14} = 1/2J_{HH} (i.e., \texttt{d14} = 31 msec, assuming \texttt{p11} is 80 msec and J_{HH} is 7 Hz). The second, which is appropriate when the selective pulse is 270°, is to choose \texttt{d14} to be 1/2J_{HH} (i.e., 71 msec). Here it is recommended to use a 90° selective pulse so that the shorter \texttt{d14} value may be used.

Acquire the data
Perform a routine acquisition with \texttt{zg}. The approximate experiment time for Selective COSY with the parameters set as shown above is 4 minutes.
Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 30.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>16k</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
</tbody>
</table>

Process the data

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum. The resulting spectrum should look like that in Figure 26.
Figure 26: Selective COSY Spectrum of 50 mM Gramicidin in DMSO-d6

selectively excited peak

correlation peak

$^1$H Reference Spectrum

8  6  4  2  ppm
Selective TOCSY

In practice it is often desirable to elucidate the entire spin system which is coupled to a given nucleus. In TOCSY a spin-locking pulse train distributes the magnetization of one nucleus over the whole spin system. There can be net magnetization transfer from one spin to another even without direct \( J \)-coupling. (In addition to connectivities via \( J \)-coupling, magnetization transfer via dipolar coupling and chemical exchange is observed during the spin-locking pulse train, but these will have a different sign in the phase-sensitive TOCSY spectrum.)

Selective TOCSY gives the same \( ^1H \) coupling information as 2D TOCSY one site at a time and without involving a 2D Fourier transform. The selective TOCSY sequence begins with a 90° frequency selective excitation pulse. This is followed by a fixed delay (rather than the variable evolution period of the 2D TOCSY sequence) during which in-phase coherence is created by evolution due to \( J \)-coupling. The duration of this delay is measured from the middle of the Gaussian envelope. Next, the coherence transfer occurs during the multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how “far” the spin coupling network will be probed. A general rule of thumb is that \( 1/(10J_{HH}) \) should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

Immediately following the spin-lock period is a z-filter, which is used to make the final 1D TOCSY spectrum easier to phase correct. Following this is the acquisition period. Since the TOCSY correlation peaks arise from magnetization that was in-phase during the fixed delay, they can be phase corrected to be positive and absorptive.

Pulse Sequence Diagram

The selective TOCSY pulse sequence is shown in Figure 27. Notice that it is very similar to the standard TOCSY sequence shown in Figure 44 on page 168, except that here the first pulse is a low-power shaped pulse, the following delay \( (d_{14}) \) is not incremented, and the spin-lock period is followed by a z-filter.

The high-power pulse \( p_1 \) and the low-power pulses \( p_6 \) and \( p_{17} \) must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. The shaped pulse \( p_{11} \) must be set to the appropriate 90° time found in Section 8.2 ‘Selective Pulse Calibration’. If \( p_{11} \) is chosen to be a 90° pulse, the delay \( d_{14} \) should be set so that \( (p_{11})/2 + d_{14} = 1/(J_{HH}) \) for the selected resonance. On the other hand if \( p_{11} \) is chosen to be a 270° pulse, \( d_{14} \) should be set to 20\( \mu \)sec (the time required for power switching).
Selective Excitation

Figure 27: Selective TOCSY Pulse Sequence

If this experiment is carried out directly after the pulse calibration described in Section 8.2, the correct sample is already in the magnet, the probehead tuned and matched, and the magnetic field shimmed and locked.

For best results, run selective TOCSY experiments non-spinning.

Create a new file directory
From the data set selco/1/1, enter edc and change the following parameters:

| NAME     | seltoc |
| EXPNO    | 1     |
| PROCNO   | 1     |

Click SAVE to create the data set seltoc/1/1.

$^1$H reference spectrum
The reference spectrum for the selective TOCSY experiment is the standard $^1$H spectrum of Gramicidin with o1 set to the ornithine peptide N-H resonance. This may be found in selex/1/1.
Write the variable delay list
The z-filter in the selective TOCSY experiment requires a variable delay list. To create the variable delay list, first enter \texttt{edlist}. A menu of list types appears. Select \texttt{vd} from this menu. This calls up a menu of existing vlist filenames and gives the user the option of creating a new file (‘Type new name’). Simply type the name \texttt{zf}. This calls up the vi editor. Enter the delays desired, some appropriate values are listed below:

\begin{verbatim}
0.004 s
0.016 s
0.010 s
0.006 s
0.004 s
0.010 s
0.017 s
0.011 s
0.018 s
0.012 s
\end{verbatim}

When the list is complete, save the file and exit the vi editor.

Set up the acquisition parameters
Enter \texttt{eda} and set the acquisition parameters as shown in Table 31. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters \texttt{pl1} and \texttt{p1} (\textsuperscript{1}H observe high power level and 90\degree pulse time), and \texttt{pl10} and \texttt{p6} (\textsuperscript{1}H low power level and 90\degree pulse time for MLEV spinlock). Use the values determined in Section 8.2 ‘Selective Pulse Calibration’ for the parameters \texttt{sp1} and \texttt{pl1} (\textsuperscript{1}H selective low-power level and 90\degree pulse time).

The parameter \texttt{l1} determines the number of cycles of the MLEV spinlock sequence, and thus determines the length of the “mixing period”. The mixing period typically lasts 20 to 100 msec, and so \texttt{l1} should be chosen so that the quantity \([\texttt{p6}*64 + \texttt{p5}]*\texttt{pl1} + (\texttt{p17}*2)]\) is 20 to 100 msec. The general rule of thumb is that \(1/10J_{HH}\) should be allowed for each coherence transfer step, and typically five transfer steps are desired, which means a mixing time of \(1/2J_{HH}\) or approximately 75 msec.

The parameter \texttt{pl7} determines the length of the trim pulses at the beginning and end of the mixing period. A good value for \texttt{pl7} is 2.5 msec. The trim pulses are used to ensure that the final 2D spectrum can be phased easily. Note, however, that for aqueous samples only the first trim pulse should be used, in which case \texttt{l1} should be adjusted so that \([(\texttt{p6}*64 + \texttt{p5})*\texttt{pl1} + \texttt{p17}]\) is 20 to 100 msec.

Be sure to set \texttt{vdlist} to the name of the appropriate variable delay list (here \texttt{zf}). This may be done in the \texttt{eda} menu either by typing \texttt{zf} in the box next to the parameter VDLIST, or by clicking with the right mouse button on this box to call up the menu of possible vdlists and then selecting \texttt{zf} with the left mouse button. Also be sure to set \texttt{l4} equal to the number of entries in the vdlist (here 10).
## Selective Excitation

Table 31. Selective TOCSY Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>selmlzf</td>
<td>see Figure 27 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>32k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>32</td>
<td>the number of scans must be 16*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL10</td>
<td></td>
<td>MLEV spin-lock power level on f1 channel.</td>
</tr>
<tr>
<td>SP1</td>
<td></td>
<td>shaped pulse power level on f1 channel.</td>
</tr>
<tr>
<td>P5</td>
<td>60° 1H low power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>90° 1H low power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>180° 1H low power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P11</td>
<td>80msec</td>
<td>90° 1H shaped pulse on f1 channel.</td>
</tr>
<tr>
<td>P17</td>
<td>2.5msec</td>
<td>trim pulse.</td>
</tr>
<tr>
<td>D1</td>
<td>2sec</td>
<td>relaxation delay; should be 1–5*1/1H.</td>
</tr>
<tr>
<td>D11</td>
<td>30msec</td>
<td>delay for disk I/O; predefined.</td>
</tr>
<tr>
<td>D12</td>
<td>20µsec</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td>D13</td>
<td>3µsec</td>
<td>short delay; predefined.</td>
</tr>
<tr>
<td>D14</td>
<td></td>
<td>delay for evolution after shaped pulse ((p11)/2 + d14 = 1/JHH).</td>
</tr>
<tr>
<td>L1</td>
<td>30</td>
<td>loop for MLEV cycle ((p6*64) + p5)<em>11 + (p17</em>2) = mixing time; this is generally between 15 and 55.</td>
</tr>
<tr>
<td>L4</td>
<td>10</td>
<td>number of delays in vdlst.</td>
</tr>
<tr>
<td>VDLIST</td>
<td>zf</td>
<td>name of vdlst used for z-filter.</td>
</tr>
<tr>
<td>PHCOR(1)</td>
<td></td>
<td>additional phase correction applied to shaped pulse; use value determined on page 86.</td>
</tr>
</tbody>
</table>

Notice that in this pulse sequence, the delay d14 is to ensure that the signals are in-phase at the beginning of the mixing period (not antiphase, as for the selective COSY sequence). There are two ways to accomplish this. The first, which is appropriate when the selective pulse is 90°, is to choose d14 such that (p11)/2 + d14 = 1/JHH (i.e., d14 = 103msec, assuming p11 is 80msec and JHH is 7Hz). The second, which is appropriate when the selective pulse is 270°, is to choose d14 to be 20µsec (in theory d14 should be 0, but in practice 20µsec are required for power...
switching). Here it is recommended to use a 270° selective pulse so that the shorter \texttt{d14} delay may be used.

**Acquire the data**

Perform a routine acquisition with \texttt{zg}. The approximate experiment time for selective TOCSY with the parameters set as shown above is 18 minutes.

**Set up the processing parameters**

Enter \texttt{edp} and set the processing parameters as shown in Table 32.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>16k</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
</tbody>
</table>

**Process the data**

Add line broadening and Fourier transform the time domain signal with the command \texttt{ef}. Manually phase correct the spectrum using the 0\textsuperscript{th}-order phase correction. The resulting spectrum should look like that in Figure 28.
Figure 28: Selective TOCSY Spectrum of 50 mM Gramicidin in DMSO-d6

Selective TOCSY Spectrum

$^1$H Reference Spectrum
The **Nuclear Overhauser Effect** is a net change of the signal intensity from one spin due to the relaxation of a saturated spin that is dipole-dipole coupled to the first spin. An NOE may be positive, meaning that there is a net increase in signal intensity, or it may be negative, meaning that there is a net decrease (this occurs for larger molecules). NOE’s develop due to through-space rather than through-bond interactions, and so contain information on the distances between spins.

The buildup of NOE’s depends on spin-lattice ($T_1$) relaxation, a process in which energy passes from the nuclear spins to the lattice (i.e., everything else) as heat. $T_1$ relaxation requires magnetic fields fluctuating at the appropriate frequency. For $^1$H’s and $^{13}$C’s in diamagnetic molecules, the dominant fields are due to the magnetic moments of $^1$H’s in the same molecule as it tumbles in solution (i.e., intramolecular dipole-dipole interactions).

The rate or efficiency of NOE buildup depends on the rate or efficiency of dipole-dipole relaxation. This depends on the strength and frequency of the fluctuating fields, which in turn depend on factors such as the distance between the nuclei involved, the tumbling rate of that portion of the molecule, and the nature of the nuclei themselves. Any other competing $T_1$ relaxation process will hinder the growth of NOE’s. In fact, the presence of paramagnetic molecules (e.g., metal ions, rust, or dissolved oxygen) can be deadly to an NOE experiment, since these may completely dominate $T_1$ relaxation processes. To get the largest possible useful NOE, it is necessary to maximize the contribution of the intramolecular dipole-dipole relaxation (e.g., eliminate paramagnetic species, use dilute solutions to eliminate problems from intermolecular interactions, also use a solvent without a high $^1$H concentration). It is also necessary to maximize the efficiency of the dipole-dipole relaxation. For example, dipole-dipole relaxation is most efficient when the molecular tumbling rate is intermediate. Small molecules tend to tumble too rapidly, and large molecules in viscous solvents too slowly, both yielding slow dipole-dipole relaxation. So, for a very small molecule with a very slow NOE growth, it is possible to speed up the experiment by using a solvent of higher viscosity (e.g.) to decrease the tumbling rate.

In an NOE difference experiment, a $^1$H resonance is selectively preirradiated until saturation is achieved. During the preirradiation period, NOE buildup occurs at another $^1$H resonance or at other $^1$H resonances. A $\pi/2$ pulse then creates observable magnetization, which is detected during the acquisition period that follows. The experiment is repeated using different preirradiation frequencies, including one which is off-resonance. The latter is used in obtaining a reference or control spectrum. Each final spectrum is displayed as the difference between a spectrum collected with on-resonance preirradiation and the control spectrum.

Very small phase or frequency shifts between two spectra will give rise to imperfect subtraction of signals that can ruin the NOE difference results. These subtraction artifacts look like dispersive responses (having no net integral) and care should be taken to minimize them. Some artifacts arise from essentially random, short time scale instabilities. To minimize these, it is best to use plenty of signal averaging and
the maximum line broadening acceptable, and to run the experiment when traffic around the magnet is at a minimum.

Other artifacts arise from longer time scale errors such as temperature or magnetic field drift. These may be minimized by acquiring the preirradiated and the control data in an interleaved manner (i.e., as nearly simultaneously as possible). Thus, each data set samples the same long term changes. Interleaved acquisition may be achieved as follows: Typically, a few dummy scans are acquired (to be sure that any influences of irradiation at the previous frequency in the cycle have completely decayed away) followed by 8 acquisitions with one preirradiation frequency. This is followed by a few dummy scans and 8 acquisitions with a second preirradiation frequency, and so on until the final preirradiation frequency. This entire cycle is repeated as many times as necessary to yield the desired number of signal averages for

The irradiation power level should be minimized to get the appropriate frequency selectivity, but should not be so low that the saturation is incomplete and the resulting NOE’s are very low, or that the frequency spread is so narrow and asymmetric that different lines in an irradiated multiplet are saturated to different extents. In this case it is possible to get selective population transfer (SPT) in spins J-coupled to the multiplet. The pulse sequence used in this chapter allows the user to loop through several frequencies of a multiplet during the preirradiation period of a given acquisition. Such cycling causes the NOE’s to co-add but the SPT effects to cancel, and enables one to use lower power, more selective preirradiation. When cycling through the frequencies of a multiplet, the irradiation time per line should be short so that there is little relaxation of previously irradiated lines during the final irradiation prior to the $\pi/2$ pulse, otherwise SPT effects reappear. On the other hand, the irradiation time per line should be long enough to avoid significant artifacts due to frequency modulation of the irradiation.


Sample
The sample used to demonstrate a 1D NOE difference experiment in this chapter is 100 mM Pamoic Acid in DMSO-d6. (Almost any sample can be used, as long as it has an observable nuclear Overhauser enhancement.)
The NOE difference pulse sequence is shown in Figure 29. The pulse sequence begins with the recycle delay time $d_1$. This is followed by the cw irradiation period of total time $14 \times d_{20}$. Here $d_{20}$ is the irradiation time for one particular frequency. The pulse program makes use of a frequency list ($fq2list$) to determine the frequency or frequencies for cw irradiation. Several frequencies are required only if the resonance to be irradiated is a multiplet. (Here it is necessary to define more than one frequency only if there is concern that a single irradiation point will not excite the full multiplet adequately.) For each of the $14$ time intervals $d_{20}$, the next frequency of the $fq2list$ is used. In this way, lines of a multiplet are irradiated in an interleaved manner so that those irradiated first have not relaxed by the time the last peaks are irradiated.

The final $90^\circ$ pulse $p_1$ creates the observable magnetization and is followed immediately by the acquisition period.

Several spectra are acquired during an NOE difference experiment, and for each spectrum a different $fq2list$ is used. In one of the spectra, the control or reference spectrum, the cw pulse is applied off-resonance, while for each of the other spectra, the cw pulse is applied on-resonances for one of the peaks, in order to saturate that peak. An NOE difference spectrum is then the difference between a spectrum with a saturated resonance, and the control spectrum.

The au program $noemult$ is used to acquire the spectra in an interleaved manner.

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the $Z$ and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^1H$ observation.

For best results it is recommended to optimize the lock parameters as described on page 16. Also note that NOE difference experiments should be run without sample spinning.

The parameters and spectra shown below are from a 300MHz spectrometer. The signal enhancements for Pamoic Acid in DMSO-d6 at other field strengths will be different than those shown here. If an NOE response is difficult to obtain, it may be necessary to change the sample temperature or solvent. In particular for this sample at 400 MHz, it is recommended to use a temperature of 40°C.
Create a new file directory
Enter re proton 2 1 to call up the data set proton/2/1. Enter edc and change the following parameters:

NAME      noe
EXPNO     1
PROCNO    1.

Click SAVE to create the data set noe/1/1.

Enter edasp and set both NUC1 and NUC2 to 1H. It is necessary to set NUC2 to 1H because the f2 channel will be used to provide the cw irradiation during the NOE experiment. By using edasp rather than edsp, the value of o1 is preserved rather than overwritten by the default value (i.e., OFSH1 remains equal to the o1 value of proton/2/1). For now o2 is unimportant, so let OFSH2 = 0.

1H reference spectrum
Enter rga to perform an automatic receiver gain adjustment. Acquire and process a standard 1H spectrum, as described in Chapter 3 ‘Basic 1H Acquisition and Processing’ (notice that since the data set noe/1/1 was created from proton/2/1, most acquisition parameters are already set). Calibrate the spectrum and optimize sw and o1. Keep in mind that the control spectrum should be irradiated well off-resonance (here –2 ppm is suggested), so do not choose sw to be too small. Acquire and process an optimized spectrum. This reference spectrum can be compared with the off-resonance control spectrum acquired during the NOE difference experiment.

Select the resonances for irradiation
The next step is to define the frequencies that will be used by the f2 channel during the preirradiation periods of the NOE experiment. These frequencies will be written to fq2lists. A separate list should be created for each resonance to be irradiated (be it a multiplet or singlet). A given list may have several frequencies if it is desired to irradiate a given resonance at several points. One of the lists must define a frequency well off-resonance to be used in generating the control spectrum.

For this example we will create lists with frequencies for the resonances at 4.8ppm and 8.5 ppm, and one with the off-resonance frequency –2 ppm. The frequency lists are defined in the frqlist routine, which is found in the utilities submenu. Note that if it is necessary to expand the spectrum in order to define the irradiation points accurately enough, this must be done before entering the frqlist routine. Also, within the calibration submenu it is not possible to expand the spectrum using the mouse.

Click utilities to enter the corresponding submenu. If desired, expand the spectrum and display the peak at 4.8 ppm using the buttons. Within the utilities submenu, click frqlist. Answer the questions as shown below:

Please enter type of list (f1, f2, f3): f1
Please enter name of f1 list: noedif.1
Write name of f1 list to acqu parameters? n .

If an f1 frequency list of the same name already exists, the following option also appears:

Frequency list exists, append (a), overwrite (o) or quit (q):.
Answer a if you wish to add new frequencies to the old list, o if you wish to overwrite the old list with the new list, or q if you wish to quit the frqlist routine and keep the old list.

(Point of clarification: Here, the “type of list (f1, f2, f3)” actually refers to the directory where the frequency list will be stored (not the spectrometer channel for which the list will be used). This must be set to f1. In any given acquisition parameter set, it is possible to define eight separate frequency lists (fq1list, fq2list, etc.). The pulse program noemul uses only one frequency list: fq2list. Therefore, within the eda menu, it is necessary to set the parameter fq2list to the appropriate list name (here, noedif.1). The automation program noemult redefines fq2list each time noemul is to be run with a different frequency list.)

Once the questions have been answered, move the mouse until the cursor is in the spectral window. The cursor is now tied to the spectrum. Click on the peak at 4.8 ppm with the middle mouse button. Finish the list by clicking the left mouse button. Remember that for a given list, multiple irradiation points should all be part of the same multiplet. A separate frequency list should be generated for each multiplet irradiated.

Repeat this procedure for the peak at 8.5 ppm and for the off-resonance frequency of –2 ppm. Write the lists to the files noedif.2 and noedif.3, respectively. Note that the automation program noemult used to run the NOE experiment requires that all frequency lists have the same base name and increasing extension numbers.

Click on return to leave the utilities submenu and return to the main menu.

Set up the acquisition parameters

Enter edc and change EXPNO to 2. Click SAVE to create the data set noe/2/1.

Enter eda and set the acquisition parameters as shown in Table 33. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters pl1 and p1 (1H observe high power level and 90° pulse time).

Be sure that NUC2 has been set to 1H in edasp.

Table 33. 1D NOE Difference Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>noemul</td>
<td>see Figure 29 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>16k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>the number of scans must be 8*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>4</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL14</td>
<td>70dB</td>
<td>power level for NOE buildup.</td>
</tr>
<tr>
<td>P1</td>
<td>90° 1H high power pulse on f1 channel.</td>
<td></td>
</tr>
</tbody>
</table>
The pulse program `noemul` operates such that `o2` is set to the first frequency of the `fq2list` and the selected multiplet is irradiated with this frequency for a time `d20`. Then `o2` is set to the next frequency (if there is one) of the `fq2list` and the selected multiplet is irradiated with this new frequency (or the first frequency if the `fq2list` has only one entry) for a time `d20`. This process continues until the multiplet is irradiated a total of `l4` times, using as many different frequencies as are in the `fq2list`.

### Optimize the Irradiation Power and Duration

The next step is to optimize the irradiation power and duration. An easy way to do this is, using the parameters shown above, to start the pulse program `noemul` with the command `zg`. For the full NOE difference experiment, the au program `noemult` will run the pulse program `noemul` with successive `fq2lists`. For optimizing the irradiation power and duration, however, it is only necessary to irradiate one resonance, and so the au program is not necessary.

Make sure that `PULPROG` is set to `noemul` and that `FQ2LIST` is set to `noedif.1` (or `noedif.2`, i.e., make sure that the cw irradiation will be applied on resonance for one of the multiplets). Start the acquisition with `zg`. Process the spectrum with `ef` (see the processing parameters listed below in Table 34). Manually phase correct the spectrum.

Compare this spectrum with the reference spectrum `noe/1/1`. It may be useful to use the dual routine. From the current data set (noe/2/1), enter `edc2` to define the second data set to be shown in the dual display. Set `EXPNO2` to 1 and `PROCNO2` to 1 and click `SAVE`. Click `dual` to enter the dual display. Both `noe/1/1` and `noe/2/1` should appear in the window. When finished comparing the spectra, click `return` to return to the main 1D processing window.

Ideally, the target resonance is completely saturated by the selective irradiation, while all other signals are completely unaffected by the irradiating field. In practice, the chemical shift difference between signals is often too small for this to be possible. For example, if a high enough power is used to saturate all lines of a multiplet, neighboring resonances may also be saturated.

It is almost always preferable to use low-power (and hence selective) irradiation rather than risk unwanted saturation of nearby resonances. However, uneven partial saturation of a multiplet leads to selective population transfer which may obscure NOE effects. To avoid this, as mentioned above, the lines (or several frequencies) of

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D1</strong></td>
<td>1sec</td>
<td>relaxation delay</td>
</tr>
<tr>
<td><strong>D12</strong></td>
<td>20µsec</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td><strong>D13</strong></td>
<td>3µsec</td>
<td>short delay; predefined.</td>
</tr>
<tr>
<td><strong>D20</strong></td>
<td>50msec</td>
<td>irradiation time.</td>
</tr>
<tr>
<td><strong>L4</strong></td>
<td>50</td>
<td>loop counter to determine overall irradiation time (l4*d20).</td>
</tr>
<tr>
<td><strong>FQ2LIST</strong></td>
<td>noedif.1</td>
<td>f2 frequency list for frequency of selective irradiation; this gets set by the automation program.</td>
</tr>
</tbody>
</table>
Acquisition and Processing

a target multiplet are irradiated in an interleaved manner during the preirradiation period before each scan.

If needed, adjust \texttt{pl14} to change the power level of the cw irradiation.

Note that the total cw irradiation time \((l4 \cdot d20)\) should be approximately equal to \(T_1\) of the irradiated peak, but with the au program \texttt{noemult}, it is necessary to use the same total irradiation time for each peak irradiated. Thus, the irradiation time should be chosen based on the longest \(T_1\). Here a total irradiation time of 2.5 sec is used, which is longer than the \(T_1\) of the peak at 8.5 ppm.

**Perform the multiple NOE experiment**

The automation sequence \texttt{noemult} is used to run the multiple NOE experiment. Simply type \texttt{xau noemult} and answer the questions as follows:

- base name of all frequency lists: \texttt{noedif}
- \# of frequency lists: 3
- \# of cycles through each list: 50
- \# of average cycles: 10 .

Here, the number of frequency lists is the number of fq2lists written above and will be the number of spectra acquired. The number of cycles through each list is the loop counter \texttt{l4}. The number of average cycles controls the total number of scans for each frequency list. For each frequency list (and hence, for each spectrum) the total number of scans is \(n_s \times \) (number of average cycles). So that each spectrum may reflect any long term drift equivalently (which is important in minimizing subtraction artifacts in the difference spectra), it is best to keep \(n_s\) small (e.g., 8) and then improve the signal-to-noise ratio by increasing the number of average cycles (to, e.g., 10).

Note that since this is a difference experiment, the observation of small NOE responses requires a high signal-to-noise ratio in the individual spectra, as well as careful temperature control. Be sure to run this experiment non-spinning, and it is also recommended to run it at night (or at some time when activity around the spectrometer is at a minimum).

The au program \texttt{noemult} automatically calls the pulse program \texttt{noemul}. Using the acquisition parameters defined in the current data set (here noe/2/1), and the \texttt{O2} frequency or frequencies defined in the first fq2list (here noedef1) for the preirradiation, \texttt{ds} dummy scans are run and \texttt{ns} signal averages are acquired and written to the current data set. Next, \texttt{ds} dummy scans and \texttt{ns} signal averages are performed using the \texttt{O2} frequency or frequencies defined in the second fq2list (here noedef2). The results are written to the next data set (here noe/3/1). This process is continued until the results obtained using the last frequency list (here noedef3) are written to the last data set (here noe/4/1). Notice that new data sets created by \texttt{noemult} have the same name as the original data set, but increasing EXPNO’s. Here the spectrum irradiated at 4.5 ppm is noe/2/1, that at 8.5 ppm is noe/3/1, and that at –2 ppm is noe/4/1.

The entire cycle is repeated until the experiment is finished. The number of times this cycle is performed is determined by the value entered for the number of average cycles.

The total experiment time with the parameters shown here is approximately 15 mins.
1D NOE Difference

Set up the processing parameters
Enter edp and set the processing parameters as shown in Table 34.

Table 34. NOE Difference Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>8k</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.30Hz</td>
<td></td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
</tbody>
</table>

Process the spectra
To process the spectra acquired by noemult it is first necessary to define the phase correction parameters.

Read in the first file (re 2 1). Apply the window function and Fourier transform with the command ef. Manually phase correct the spectrum and store the correction.

The remaining spectra must be processed identically. An easy way to accomplish this is by using the automation program multiefp. Simply enter xau multiefp and answer the questions as follows:

Enter first expno to process: 2
Enter number of expnos: 3.

Here, the “first expno to process” indicates the spectrum that is already Fourier transformed and phased correctly. The program multiefp reads all processing parameters (including 0th- and 1st-order phase corrections) from this data set and uses them to process the remaining spectra. Notice that this automation program assumes all spectra to be processed have the same name and increasing EXPNO’s.

At this point the data consists of a series of spectra with various saturated resonances (whether singlets or multiplets) and one reference spectrum. The procedure for creating the difference spectra is outlined below.

Create the NOE difference spectra
The NOE difference spectra are created by subtracting the control spectrum from each of the spectra preirradiated on-resonance. Within the data set of each preirradiated spectrum, then, it is necessary to use edc2 to define the second and third data sets. The second data set refers to the control spectrum and the third data set is where the difference spectrum will be stored.

To create the first difference spectrum, first make sure the first preirradiated spectrum is displayed (e.g., enter re 2 1). Enter edc2 to set up the second and third data sets. Within the edc2 menu set EXPNO2 and PROCNO2 to the values for the control spectrum, here EXPNO2 = 4 and PROCNO2 = 1.

Next, set EXPNO3 to the value of the current experiment number, and PROCNO3 to one greater than the current PROCNO, here EXPNO3 = 2 and PROCNO3 = 2. Click on SAVE to exit edc2 and return to the main menu.
Enter the dual submenu by clicking on dual. Both the current spectrum and the reference spectrum should now appear on the screen.

Click on Return and select Save & return to subtract the control spectrum from the current preirradiated spectrum. The difference spectrum appears automatically in the window.

Click store to save the results and return to the main menu. The message “result will be put into: DU = u, USER = <username>, NAME = noe, EXPNO = 2, PROCNO = 2, click OK if ok” appears. Click OK and notice that the current data set is now noe/2/2.

Move to the next preirradiated spectrum (e.g., enter re 3 1) and repeat the above procedure. Here EXPNO2 = 4, PROCNO2 = 1, EXPNO3 = 3 and PROCNO2 = 2, and the difference spectrum is stored in noe/3/2.

Two NOE difference spectra (with cw irradiation on-resonance at 8.5 ppm and 4.8 ppm) and the control spectrum (with cw irradiation off-resonance at –2 ppm) of the sample 100 mM Pamoic Acid in DMSO-d6 are shown in Figure 30. Notice that only the relevant portion of the spectral width is displayed (sw was actually much greater than what is shown).

For both the difference spectrum with cw irradiation at 8.5 ppm and that with cw irradiation at 4.8 ppm, the large negative peak is the irradiated resonance and the small positive doublet is the NOE. Note that these spectra were recorded on a DPX300 at 298 K. Similar spectra of this sample recorded at 500 MHz and 298 K will have negative NOE peaks, while those recorded at 400 MHz and 298 K may show no NOE peaks at all.
Figure 30: NOE Difference Spectra of 100 mg Pamoic Acid in DMSO-d6
Quantitate the NOE’s

To quantitate (roughly) an observed NOE, the integrated intensity of the NOE peak in the difference spectrum is compared with the integrated intensity of the peak that was irradiated to cause the NOE. However, this latter intensity should be measured in the control spectrum. Thus, it is necessary to integrate peaks in both the control and the difference spectrum, and to use the same normalization constant for the integrals in both spectra.

First integrate the appropriate peak in the control spectrum and set the normalization constant. Call up the reference spectrum (re 4 1). Click integrate to enter the integration submenu. Integrate the peak of interest (e.g., the peak at 8.5 ppm). To integrate the peak, first move the cursor into the spectral window and click the left mouse button to tie the cursor to the spectrum. Next, click the middle mouse button once at each end of the range of interest; the integral appears automatically. Click the left mouse button again to release the cursor from the spectrum. An asterisk should appear next to the integral (if not, select the integral with the left mouse button). Then, correct the baseline of the integral: with the cursor in the spectral window but not directly on the spectrum, move the mouse while holding down the middle mouse button to adjust the bias, and while holding down the right mouse button to adjust the slope. Again make sure the integral is selected (an asterisk appears next to it). Click on calib and enter 100 to calibrate this integral to 100%. Click on return and select Save & store ‘intrng’ to save the integral and normalization constant and return to the main 1D processing window.

Next call up the difference spectrum with the NOE you wish to quantitate (here the difference spectrum with cw irradiation frequency of 8.5 ppm, so enter re 2 2). Click on integrate to enter the integration subroutine. Integrate the NOE peak and adjust the slope and bias. Now click lastscal so that this integral is scaled using the same normalization constant that was defined for the previous data set. The number appearing below the NOE integral now indicates the percent integrated intensity (e.g., a value of 2.5 would mean that the NOE peak is 2.5% as intense as the irradiated peak). Note this value and then click on return to return to the main 1D processing window.

Repeat this procedure for other NOE peaks. For example, here read in the control spectrum (re 4 1), integrate the peak at 4.8 ppm, adjust the slope and bias, calibrate the integral to 100%, and click on return and select Save & store ‘intrng’ to save the integral and normalization constant and return to the main 1D processing window. Next read in the difference spectrum (re 3 2), integrate the NOE peak, adjust the slope and bias, and click lastscal to scale the integral.
Heteronuclear ($X$, $H$) shift CORRelation spectroscopy is a 2D technique that can be used to determine which $^1H$’s of a molecule are bonded to which $^{13}C$ nuclei (or other X nuclei). Like DEPT, XHCORR makes use of the large one-bond heteronuclear J-coupling ($J_{XH}$) for polarization transfer, and so only $^{13}C$’s bonded directly to $^1H$’s are detected. For $^{13}C$’s and directly attached $^1H$’s, $J_{XH} = 100$ to 200Hz, while for more distant $^1H$’s $J_{XH} = 5$ to 20Hz.

The XHCORR experiment yields a series of amplitude modulated $^{13}C$ spectra. The amplitude modulation contains information on $^1H$ chemical shifts and $J_{HH}$-couplings which are the source of $^1H$ evolution during $t_1$. The $^1H$ chemical shifts and coupling constants that appear in $t_1$ are those that have controlled the $^{13}C$ population distribution during that time period.

The short delay between the final $^{13}C$ pulse and the start of acquisition is a refocusing time added so that the $^{13}C$ lines no longer have opposite phase and thus do not cancel one another when $^1H$-decoupling is applied. The optimal refocusing time ($\Delta_2$) depends on whether the $^{13}C$ belongs to a CH, CH$_2$, or CH$_3$ group. Generally a compromise value of $\Delta_2 = 1/(3J_{XH})$ is chosen. $^{13}C$ couplings during $t_1$ are removed by adding a $^{13}C$ $\pi$ pulse in the middle of $t_1$, so that there is refocusing by the end of $t_1$. To enable maximum polarization transfer, a fixed delay $\Delta_1 = 1/(2J_{XH})$ is added after $t_1$. This delay allows anti-phase magnetization to be re-established.

Like DEPT, XHCORR requires phase cycling to eliminate signal from “natural magnetization,” i.e., $^{13}C$ z-magnetization during $t_1$ that is transformed into observable transverse magnetization by the final $^{13}C$ $\pi/2$ pulse. In a 2D experiment, such magnetization gives rise to an “axial” peak (large peak along $F1 = 0$). Extra phase cycling is required to obtain quadrature detection in $F1$.

The final 2D XHCORR spectrum has a projection onto the $F2$ axis which is the normal $^1H$-decoupled $^{13}C$ spectrum with all quaternary carbons missing, and a projection onto the $F1$ axis which is the normal $^1H$ spectrum with reduced signal to noise since only $^1H$’s directly attached to $^{13}C$ contribute to the signal. The XHCORR experiment is not phase-sensitive, and so the final 2D spectrum must be displayed in magnitude mode.


Sample

The sample used to demonstrate XHCORR in this chapter is 1 g Cholesterylacetate in CDCl$_3$. This is the same sample that was used to demonstrate DEPT.
The XHCORR pulse sequence is shown in Figure 31. Notice that the pulses $p_1$ and $p_3$ must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. Also, the cpd sequence used is WALTZ-16, which requires the calibrated 90° time $p_{31}$. The 180° pulse length $p_2$ is determined by the pulse program itself.

In this pulse sequence, the delay time $d_2$ determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1 = 1/(2J_{XH})$), and the time $d_3$ determines the length of the refocusing delay ($\Delta_2 = 1/(\alpha J_{XH})$, where $\alpha$ is usually chosen to be 3).

![Figure 31: XHCORR Pulse Sequence](image)

### Acquisition and Processing 10.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^{13}$C observation, $^{1}$H decoupling.

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

Note that while setting up to do an XHCORR 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 $o_1$, $o_2$, 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’.
**Acquisition and Processing**

\textbf{1H reference spectrum}

Since XHCORR is a $^{13}$C-observe, $^{1}$H-decouple experiment, the first step is to obtain a reference $^{1}$H spectrum of the sample. This reference spectrum will be used to determine the correct $o_2$ for $^{1}$H decoupling, the correct $sw$ for the F1 dimension, and can also be used as the F1 projection of the XHCORR spectrum.

Recall that in Chapter 3 ‘Basic 1H Acquisition and Processing’ we have already collected a few $^{1}$H spectra of 100mg Cholesterylacetate, so one of these can be used as the starting point for this reference spectrum. Enter \texttt{re proton 2 1} to call up the data set proton/2/1. Enter \texttt{edc} and change the EXPNO to 4. Click \texttt{SAVE} to create the data set proton/4/1.

Enter \texttt{rga} to perform an automatic receiver gain adjustment. Acquire and process a standard $^{1}$H spectrum. Calibrate the spectrum and optimize $sw$ and $o_1$ so that the $^{1}$H signals cover almost the entire spectral width.

Acquire an optimized spectrum to be used as the F1 projection of the XHCORR spectrum. (If desired, the number of scans may be increased for this spectrum.)

\textbf{13C reference spectrum}

The second step is to obtain a $^{1}$H-decoupled $^{13}$C reference spectrum to determine the correct $o_1$ and $sw$, and to be the F2 projection of the XHCORR spectrum. Since XHCORR detects only $^{13}$C’s bonded directly to $^{1}$H’s, a DEPT-45 spectrum is typically used as this reference spectrum.

Recall that in Chapter 6 ‘DEPT’ we already collected a DEPT-45 spectrum of this sample. Enter \texttt{re dept 1 1} to call up the data set dept/1/1. Enter \texttt{edc} and change EXPNO to 4. Click \texttt{SAVE} to create the data set dept/4/1.

Check $o_2$. This should be set to the value of $o_1$ in the $^{1}$H reference spectrum obtained above. Acquire and process a DEPT-45 spectrum. Optimize $sw$ and $o_1$ so that the $^{13}$C signals cover almost the entire spectral width.

\textbf{Create a new file directory for the 2D data set}

From the data set dept/4/1, enter \texttt{edc} and change the following parameters:

\begin{verbatim}
NAME xhcorr
EXPNO 1
PROCNO 1.
\end{verbatim}

Click \texttt{SAVE} to create the data set xhcorr/1/1. By creating the XHCORR data set from the data set of the DEPT reference spectrum, most of the F2 parameters for XHCORR are already set.

\textbf{Change to 2D parameter mode}

Enter \texttt{eda} and set PARMODE = 2D. Click on \texttt{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.

\textbf{Set up the acquisition parameters}

Enter \texttt{eda} and set the acquisition parameters as shown in Table 35. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters $pl1$ and $p1$ ($^{13}$C observe high power level and 90° pulse time), $pl2$ and $p3$ ($^{1}$H decouple high power level and 90° pulse time), and $pl12$ and $pcpd2$ ($^{1}$H decouple low power level and 90° pulse time). Note that the pulse program hxco calls an include file in which $cnst2$ (defined to be $J_{XH}$) and $cnst11$ (defined to be $\alpha$, which is usually chosen...
to be 3) are used to calculate $d_2$ ($d_2 = 1/(2 \times cnst2)$) and $d_3$ ($d_3 = 1/(cnst1 \times cnst2)$). Thus, it is only necessary for the user to set the values of $cnst2$ and $cnst1$. Similarly, the 180° pulse length $p_2$ is calculated from the corresponding 90° pulse length $p_1$, so the user need only set the value of $p_1$.

The F2 parameters $o_1$, $o_2$, and $sw$ (not shown in the table) should be identical to the values used in the optimized DEPT-45 reference spectrum (dept/4/1). The F1 parameters $sfo1$ and $sw$ should be identical to the values used in the optimized $^1H$ reference spectrum (proton/4/1).

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 35. XHCORR Acquisition Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>hxco</td>
<td>see Figure 31 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>1k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>the number of scans must be 4 * n for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL2</td>
<td></td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL12</td>
<td></td>
<td>power level for cpd on f2 channel.</td>
</tr>
<tr>
<td>P1</td>
<td>$90^\circ$ $^{13}$C high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>$180^\circ$ $^{13}$C high power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>$90^\circ$ $^1$H high power pulse on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>PCPD2</td>
<td></td>
<td>$90^\circ$ $^1$H pulse for cpd sequence.</td>
</tr>
<tr>
<td>D0</td>
<td>3$\mu$sec</td>
<td>incremented delay ($t_1/2$); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2sec</td>
<td>relaxation delay; should be about $1.25 \times T_1(^{13}$C).</td>
</tr>
<tr>
<td>D2</td>
<td>3.45msec</td>
<td>delay for creation of anti-phase magnetization ($1/(2J_{XH})$); calculated internally.</td>
</tr>
<tr>
<td>D3</td>
<td>2.30msec</td>
<td>refocusing delay; choose $1/(3J_{XH})$ for all multiplicities; calculated internally.</td>
</tr>
<tr>
<td>D11</td>
<td>30msec</td>
<td>delay for disk I/O; predefined.</td>
</tr>
<tr>
<td>CNST2</td>
<td>145Hz</td>
<td>one-bond heteronuclear J-coupling ($J_{XH}$); used to calculate $d_2$; 145Hz is a good intermediate value for $^{13}$C.</td>
</tr>
</tbody>
</table>
Acquire the 2D data set
If this data set was created from the DEPT-45 reference spectrum dept/4/1, the receiver gain is already set correctly.

Enter zg to acquire the time domain data. The approximate experiment time for XHCORR with the acquisition parameters set as shown above is 2.5 hours.

Set up the processing parameters
Enter edp and set the processing parameters as shown in Table 36.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there are two d0 periods per cycle and MC2 = QF.</td>
</tr>
<tr>
<td>IN0</td>
<td>$1/(2 \times SW_H) = DW_H$</td>
<td>$t_1$ increment.</td>
</tr>
<tr>
<td>SW</td>
<td>sw of the optimized $^1$H spectrum (proton/4/1).</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td>selects $^1$H frequency for F1.</td>
<td></td>
</tr>
</tbody>
</table>
### Table 36. XHCORR Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1 k</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>spectrum reference frequency ($^{13}\text{C}$).</td>
</tr>
<tr>
<td>WDW</td>
<td>EM</td>
<td>(for example).</td>
</tr>
<tr>
<td>LB</td>
<td>3 Hz</td>
<td>a value of 2—5 Hz is appropriate.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td>this is a magnitude spectrum.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>spectrum reference frequency ($^{1}\text{H}$).</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>mc</td>
<td>this is a magnitude spectrum.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>MC2</td>
<td>QF</td>
<td>determines type of FT in F1; QF results in a forward quadrature complex FT.</td>
</tr>
</tbody>
</table>

**Process the 2D data set**

Enter `xfb` to multiply the time domain data by the window functions and also perform the 2D Fourier transform. The 2D data set is displayed automatically.

**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. The button `#colors` is used to set the number of levels. This means the intensity range of the displayed peaks.

The user can choose to display positive peaks only, negative peaks only, or both positive and negative peaks by clicking on `+/-` with the left mouse button. Since this is a magnitude spectrum, only positive peaks need to be displayed.
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 by entering rpar, selecting standard2D from the menu of parameter file names, and then selecting plot from the menu of parameter file types that appears. Equivalently, simply 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.

Within the edg menu, it is a good idea to change the separation between tic marks on the F2 axis (i.e., the $^{13}$C axis). Click the ed next to the parameter EDAXIS to enter the F1- and F2-axis parameters submenu. Change the value of the parameter X2TICD from 0.1 to 2.5. Click SAVE to save this change and return to the edg menu.

Next it is necessary to define the files that will be used as the F1- and F2-projections of the XHCORR spectrum. 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/4/1)
PF1NAME proton
PF1EXP 4
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 dept/4/1)
PF2NAME dept
PF2EXP 4
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`).

An XHCORR spectrum of 1g Cholesteryl acetate in CDCl₃ is shown in Figure 32.
Figure 32: XHCORR Spectrum of 1 g Cholesterylacetate in CDCl$_3$
COLOC

Introduction 11.1

COrelation spectroscopy via LOng-range Coupling, like XHCORR, is a 2D heteronuclear correlation technique that can be used to determine which $^1$H’s of a molecule are bonded to which $^{13}$C nuclei (or other X nuclei). Unlike XHCORR, however, COLOC makes use of small long-range heteronuclear J-couplings ($^{n}J_X^H, n > 1$) for polarization transfer, and so all $^{13}$C’s are detected, even those which are not bonded directly to $^1$H’s.

In COLOC, the evolution time $t_1$ is incorporated in the polarization transfer period $\Delta_1 = 1/(2^{n}J_X^H)$. Since the long-range heteronuclear coupling constants are small ($^{3}J_{CH} = 5$ to $20\text{Hz}$), the time period $\Delta_1$ is long. By eliminating the need for an additional $t_1$ period, COLOC reduces the magnetization loss due to relaxation that is inevitable during such a long pulse sequence. The evolution time is included in $\Delta_1$ by shifting a pair of $\pi$ pulses throughout $\Delta_1$. Homo- and heteronuclear couplings evolve during $\Delta_1$ but $^1$H chemical shift evolves only in $\Delta_1 - t_1$. Notice that $\Delta_1 - t_1$ is negative when the $\pi$ pulses happen after $\Delta_1/2$.

The delays $\Delta_1$ and $\Delta_2$ can be optimized using the refocused INEPT sequence. Such an optimization holds for the entire experiment whereas for XHCORR applied to long-range correlations, similarly optimized parameters do not hold entirely because of evolution of homonuclear coupling in $t_1$.

The 2D spectrum generated by a 2D Fourier transform with respect to $t_1$ and $t_2$ must be displayed in magnitude mode.


Sample

The sample used to demonstrate COLOC in this chapter is 1 g Cholesteryl acetate in CDCl$_3$. This is the same sample that was used to demonstrate DEPT and XHCORR.

Pulse Sequence Diagram 11.2

The COLOC pulse sequence is shown in Figure 33. Notice that the pulses $p_1$ and $p_3$ must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. Also, the cpd sequence used is WALTZ-16, which requires the calibrated 90° time $p_{31}$. The 180° pulse lengths $p_2$ and $p_4$ are determined by the pulse program itself.

In this pulse sequence, the delay time $d_6$ determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1 = 1/(2^{n}J_X^H)$), and the time $d_{18}$ determines the length of the refocusing delay ($\Delta_2 = 1/(\alpha^{3}J_X^H)$, where $\alpha$ is generally chosen to be 3).

To ensure that the $\pi$ pulses occur during $\Delta_1$, the user must make sure that $d_6 \leq d_0 + (t_d(F1)^{*}\text{in}0) + (p_2 \text{ or } p_4)$; in other words, that $d_6$ is at least as long as the maximum evolution time ($t_1$) plus the length of the longest $\pi$ pulse ($p_2$ or $p_4$).
Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z\(^2\) shims until the lock level is optimized. Tune and match the probehead for \(^{13}\)C observation, \(^1\)H decoupling.

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

Note that while setting up to do a COLOC 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 \(o_1\), \(o_2\), 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’.

**\(^1\)H reference spectrum**

Since COLOC is a \(^{13}\)C-observe, \(^1\)H-decouple experiment, the first step is to obtain a reference \(^1\)H spectrum of the sample. This reference spectrum will be used to determine the correct \(o_2\) for \(^1\)H decoupling, the correct \(sw\) for the F1 dimension, and can also be used as the F1 projection of the COLOC spectrum.

A \(^1\)H reference spectrum of this sample was obtained in Chapter 10 ‘XHCORR’, and can be found in the data set proton/4/1.

**\(^{13}\)C reference spectrum**

The second step is to obtain a \(^1\)H-decoupled \(^{13}\)C reference spectrum to determine the correct \(o_1\) and \(sw\), and to be the F2 projection of the COLOC spectrum. Note
that unlike XHCORR, COLOC detects all types of $^{13}$C’s, not only those bonded directly to $^1$H’s. Thus, a standard $^1$H-decoupled $^{13}$C spectrum, not a DEPT-45 spectrum, should be used as the reference spectrum.

Recall that in Section 4.3 we already collected a $^1$H-decoupled $^{13}$C spectrum of this sample. Enter `carbon 3 1` to call up the data set carbon/3/1. Enter `edc` and change EXPNO to 4. Click `SAVE` to create the data set carbon/4/1.

Check `o2`. This should be set to the value of `o1` in the $^1$H reference spectrum above. Enter `eda` and set CPDPRG2 to waltz16. Also set `p1l2` and `pcpd2` to the appropriate power level and pulse time for a $^1$H low power 90° decouple pulse, as determined in Section 5.4.3 on page 54. The acquisition parameters are now correctly set for a $^{13}$C spectrum with WALTZ-16 composite pulse decoupling.

Acquire and process a spectrum. Optimize `sw` and `o1` so that the $^{13}$C signals cover almost the entire spectral width.

Create a new file directory for the 2D data set

From the data set carbon/4/1, enter `edc` and change the following parameters:

- NAME: coloc
- EXPNO: 1
- PROCNO: 1

Click `SAVE` to create the data set coloc/1/1. By creating the COLOC data set from data set of the $^{13}$C reference spectrum, many of the F2 parameters for COLOC 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 37. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters `pl1` and `p1` ($^{13}$C observe high power level and 90° pulse time), `p12` and `p3` ($^1$H decouple high power level and 90° pulse time), and `pl12` and `pcpd2` ($^1$H decouple low power level and 90° pulse time). Note that the pulse program coloc calls an include file in which `p1` and `p3` are used to calculate `p2` and `p4`, respectively. Thus, it is only necessary for the user to set the value of `p1` and `p3`. On the other hand, the delays `d6` and `d18` are not defined in the include file, and so must be set explicitly in `eda`.

The F2 parameters `o1`, `o2`, and `sw` (not shown in the table) should be identical to the values used in the optimized $^{13}$C reference spectrum (carbon/4/1). The F1 parameters `sfo1` and `sw` should be identical to the values used in the optimized $^1$H reference spectrum (proton/4/1).

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 37. COLOC Acquisition Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>coloc</td>
<td>see Figure 33 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>1 k</td>
<td>the number of scans should be 16*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>NS</td>
<td>64</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>PL2</td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>PL12</td>
<td>power level for cpd on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° 13C high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>180° 13C high power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>90° 1H high power pulse on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>180° 1H high power pulse on f2 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>PCPD2</td>
<td>90° 1H pulse for cpd sequence.</td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>3µsec</td>
<td>incremented delay (t₁/2); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be about 1.25*T₁(13C).</td>
</tr>
<tr>
<td>D6</td>
<td>~50 msec</td>
<td>delay for evolution of long-range couplings (1/(2<em>JₓH)); make sure that d6 ≥ d0 + (td1</em>inc0) + (p2 or p4).</td>
</tr>
<tr>
<td>D11</td>
<td>30 msec</td>
<td>delay for disk I/O; predefined.</td>
</tr>
<tr>
<td>D12</td>
<td>20 µsec</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td>D18</td>
<td>~33.3 msec</td>
<td>delay for evolution of long-range couplings (1/(α<em>JₓH)); generally a compromise value of 1/(3</em>JₓH) is chosen.</td>
</tr>
<tr>
<td>CPDPRG2</td>
<td>waltz16</td>
<td>composite pulse decoupling sequence.</td>
</tr>
</tbody>
</table>

**F1 Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>128</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there are two d0 periods per cycle and MC2 = QF.</td>
</tr>
<tr>
<td>IN0</td>
<td>1/(2*SWₓH) = DWₓH</td>
<td>t₁ increment.</td>
</tr>
</tbody>
</table>
Acquire the 2D data set
If this data set was created from the \(^{13}\text{C}\) reference spectrum carbon/4/1, the receiver gain is already set correctly.

Enter \texttt{zg} to acquire the time domain data. The approximate experiment time for COLOC with the acquisition parameters set as shown above is 5 hours.

Set up the processing parameters
Enter \texttt{edp} and set the processing parameters as shown in Table 38.

### Table 38. COLOC Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>sw of the optimized (^1\text{H}) spectrum (proton/4/1).</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td>select (^1\text{H}) frequency for F1.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F2 Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>SI</td>
<td>512</td>
</tr>
<tr>
<td>SF</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>2–5Hz</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F1 Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>SI</td>
<td>512</td>
</tr>
<tr>
<td>SF</td>
<td></td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
</tr>
<tr>
<td>PH_mod</td>
<td>mc</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
</tr>
<tr>
<td>MC2</td>
<td>QF</td>
</tr>
</tbody>
</table>
Process the 2D data set
Enter \texttt{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 magnitude spectrum, click on \texttt{+/=} 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 \texttt{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 \texttt{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 \texttt{DefPlot} and answer the following questions.

\begin{verbatim}
  Change levels? y
  Please enter number of positive levels? 6
  Display contours? n.
\end{verbatim}

Enter \texttt{edg} to edit the plotting parameters.

Click the \texttt{ed} next to the parameter EDAXIS to enter the axis parameters submenu.
Change the value of the parameter X2TICD from 0.1 to 2.5. Click \texttt{SAVE} to save this change and return to the \texttt{edg} menu.

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

\begin{verbatim}
PF1DU     u
PF1USER   (name of user for file proton/4/1)
PF1NAME   proton
PF1EXP    4
PF1PROC   1.
\end{verbatim}

Click \texttt{SAVE} to save these changes and return to the \texttt{edg} menu.

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

\begin{verbatim}
PF2DU     u
PF2USER   (name of user for file carbon/4/1)
PF2NAME   carbon
\end{verbatim}
PF2EXP 4
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 COLOC spectrum of 1 g Cholesterylaceitate in CDCl$_3$ is shown in Figure 34.
Figure 34: COLOC Spectrum of 1 g Cholesterylacetate in CDCl₃
COrrelation Spectroscopy is a homonuclear 2D technique that is used to correlate the chemical shifts of $^1$H 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 $^1$H nuclei are J-coupled.

Magnitude COSY

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.


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

### Pulse Sequence Diagram

The COSY-45 pulse sequence is shown in Figure 35. The pulse $p_1$ must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’.

*Figure 35: COSY-45 Pulse Sequence*

```
| $d_1$ | $d_0$ |
\hline
$1^H$ $t_{rd}$ $t_1$ $t_2$ acq
```

### Acquisition and Processing

Insert the sample in the magnet. Lock the spectrometer. Readjust the $Z$ and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $1^H$ 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 $o_1$, $o_2$, 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 $1^H$ spectrum will be used to determine $o_1$ 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 $1^H$ spectrum. Calibrate the spectrum, and optimize $sw$ and $o_1$ so that the $1^H$ 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 $^1$H 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 `p1` and `p1` ($^1$H 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 $^1$H 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 on **NUCLEI** for F1 parameters, $nd0$, and $sw$ correctly. This automatically sets $in0$ to the correct value.

### Table 39. COSY Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>F2 Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>cosy45</td>
<td>see Figure 35 for pulse sequence diagram.</td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>1k</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>the number of scans should be 4 *n in order for the phase cycling to work properly.</td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
<td></td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° $^1$H high power pulse on f1 channel.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>3µsec</td>
<td>incremented delay ($t_1$); predefined.</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>3sec</td>
<td>relaxation delay; should be about 1.25*$_{1.T_1}(^1H)$.</td>
<td></td>
</tr>
</tbody>
</table>
Acquire the 2D data set
If this data set was created from the $^1$H 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>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>1</td>
<td>there is one d0 period per cycle and MC2 = QF.</td>
</tr>
<tr>
<td>IN0</td>
<td>$1/(SW_{H}) = 2^*DW_{H}$</td>
<td>$t_1$ increment; should be $2^*dw$ used in F2 (which is in µs!).</td>
</tr>
<tr>
<td>SW</td>
<td>sw of the optimized $^1$H spectrum (proton/5/1); same as for F2.</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td>select $^1$H frequency for F1; same as for F2.</td>
<td></td>
</tr>
</tbody>
</table>

**F2 Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>spectrum reference frequency ($^1$H).</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>0</td>
<td>choose pure sine wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>yes</td>
<td>this is a magnitude spectrum.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>
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’.

### F1 Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>0</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>mc</td>
<td>this is a magnitude spectrum.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>MC2</td>
<td>QF</td>
<td>determines type of FT in F1; QF results in a forward quadrature complex FT.</td>
</tr>
</tbody>
</table>
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.
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 $F_1$ and $F_2$. 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 $^1$H’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.


Sample
The sample used to demonstrate DQF-COSY in this chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used for magnitude COSY.

The DQF-COSY pulse sequence is shown in Figure 37. The pulse $p_1$ 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 $d_1$ in order to avoid multiple-quantum artifacts in the spectrum. A suitable value for this sample is $d_1 = 3$ sec.
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 sf₀₁ and sw should be identical to the corresponding F2 values.
Finally, notice that $in_0$ and $sw(F1)$ are not independent. A convenient way to set $in_0$ is to set the F1 parameters $nucl$ by clicking NUCLEI for F1 parameters, $nd0$, and $sw$ correctly. This automatically sets $in_0$ to the correct value.

### Table 41. DQF-COSY Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PULPROG</td>
<td>cosydftp</td>
<td>see Figure 37 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>16</td>
<td>the number of scans should be 8*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>90° $^1$H high power pulse on f1 channel.</td>
</tr>
<tr>
<td>D0</td>
<td>3$\mu$s</td>
<td>incremented delay ($t_1$); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>3 sec</td>
<td>relaxation delay; should be about 1.25*$T_1$(H).</td>
</tr>
<tr>
<td>D13</td>
<td>3$\mu$s</td>
<td>short delay; predefined.</td>
</tr>
<tr>
<td><strong>F1 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>512</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there is one d0 period per cycle and MC2 = TPPI.</td>
</tr>
<tr>
<td>IN0</td>
<td>$1/(2*SW_H) = DW_H t_1$</td>
<td>increment.</td>
</tr>
<tr>
<td>SW</td>
<td></td>
<td>$sw$ of the optimized $^1$H spectrum (proton/5/1); same as for F2.</td>
</tr>
<tr>
<td>NUC1</td>
<td></td>
<td>select $^1$H frequency for F1; same as for F2.</td>
</tr>
</tbody>
</table>

**Acquire the 2D data set**

If this data set was created from the magnitude COSY spectrum cosy/l/l, 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td>spectrum reference frequency ((^1)H).</td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td></td>
<td>choose pure sine wave.</td>
</tr>
<tr>
<td>SSB</td>
<td>0</td>
<td>first determine 0- and 1st-order phase correction with phasing subroutine.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>quad</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1k</td>
<td>spectrum reference frequency ((^1)H).</td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td></td>
<td>choose pure sine wave.</td>
</tr>
<tr>
<td>SSB</td>
<td>0</td>
<td>first determine 0- and 1st-order phase correction with phasing subroutine.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
<td>TPPI determines type of FT in F1; TPPI results in a forward single real FT.</td>
</tr>
</tbody>
</table>

**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).
Double Quantum Filtered COSY

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 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) 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 50 mM Gramicidin in DMSO-d6 is shown in Figure 38. An expanded portion of the same spectrum is shown in Figure 39.
Figure 38: DQF-COSY Spectrum of 50 mM Gramicidin in DMSO-d6
Figure 39: Expanded DQF-COSY Spectrum of 50 mM Gramicidin in DMSO-d6
Introduction

Nuclear Overhauser Effect Spectroscopy is a 2D spectroscopy method whose aim is to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear $^1$H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicate which $^1$H’s are close to which other $^1$H’s in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and whose cross peaks indicate which $^1$H’s are close to which other $^1$H’s through the bonds of the molecule.

The basic NOESY sequence consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time $t_1$, which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period $\tau_m$ (the time during which cross relaxation occurs) where the longitudinal polarization of each spin is labelled by its resonance frequency. The longitudinal magnetization is allowed to relax during the mixing time $\tau_m$. Note that, for the basic NOESY experiment, $\tau_m$ is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time $t_2$. The NOESY spectrum is generated by a 2D Fourier transform with respect to $t_1$ and $t_2$.

Axial peaks, which originate from magnetization that has relaxed during $\tau_m$, can be removed by the appropriate phase cycling.

NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure.


Sample

The sample used to demonstrate NOESY in the chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY.

Pulse Sequence Diagram

The NOESY pulse sequence is shown in Figure 40. Notice that the pulse $p1$ must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’. The delay $d8$ determines the length of the mixing period, during which NOE buildup occurs.
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 NOESY, like all 2D experiments, be run without sample spinning.

¹H reference spectrum
Since NOESY is a homonuclear experiment only one reference spectrum is required. This ¹H spectrum will be used to determine $\omega_1$ and $sw$ for the NOESY experiment, and can also be used as both the F1 and the F2 projections of the NOESY 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 NOESY experiment is so similar to the COSY experiment, it makes sense to create the NOESY data set from the magnitude COSY data set. Enter `re cosy 1 1` to call up the data set cosy/1/1. Enter `edc` and change the following parameters:

```plaintext
NAME noesy
EXPNO 1
PROCNO 1.
```

Click `SAVE` to create the data set noesy/1/1.

If you do not wish to generate the NOESY data set from the COSY data set, it can be generated from the ¹H reference spectrum as follows: Enter `re proton 5 1` to call up the data set proton/5/1. Enter `edc` and change the following parameters:

```plaintext
NAME noesy
EXPNO 1
PROCNO 1.
```

Click `SAVE` to create the data set noesy/1/1.
**Change to 2D parameter mode**

If this data set was created from the magnitude COSY data set, it is already in 2D parameter mode. If not, 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 43. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters `pl1` 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 `nucl` by clicking on **NUCLEI** for F1 parameters, `nd0`, and `sw` correctly. This automatically sets `in0` to the correct value.

**Table 43. NOESY Acquisition Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PULPROG</td>
<td>noesytp</td>
<td>noesy using TPPI for quadrature detection in F1; see Figure 40 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>1k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>32</td>
<td>the number of scans must be 8*n for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° ¹H high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>3μsec</td>
<td>incremented delay (t1); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2sec</td>
<td>relaxation delay; should be about 1.25*T1(¹H).</td>
</tr>
<tr>
<td>D8</td>
<td>350msec</td>
<td>mixing time for NOE buildup; should be on the order of T1.</td>
</tr>
<tr>
<td><strong>F1 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there is one d0 period per cycle and MC2 = TPPI.</td>
</tr>
<tr>
<td>IN0</td>
<td>1/(2<em>SWH</em>DWH)</td>
<td>t1 increment.</td>
</tr>
</tbody>
</table>
Optimize d8

The parameter d8 determines the length of the mixing period during which NOE buildup occurs. This should be on the order of T1. The value listed in Table 43 is appropriate for this sample at 300 MHz and room temperature. In general, if the user is not sure of the appropriate value of d8, the following quick and easy procedure can be used.

First create a 1D data set from the NOESY 2D data set. Enter edc, set EXPNO to 2, and click SAVE to create the data set noesy/2/1. Enter eda, set PARMODE to 1D, click SAVE and ok the requests to delete acqu2, acqu2s and proc2, proc2s, and luta files.

In eda set PULPROG to zg (or equivalently, enter pulprog and then zg at the prompt). Set ns to 1 and ds to 0. Use zg and ef to acquire and process a 1D 1H spectrum. Manually phase correct the spectrum and store the correction. Notice that this phase correction is necessary for a correct interpretation of the t1ir1d data below.

In eda change PULPROG to the pulse program to t1ir1d (or equivalently, enter pulprog and then t1ir1d at the prompt). This is an inversion recovery sequence. Set d7 to approximately 500 msec (note that it is important to select a large enough starting value for d7 since the gs routine will only allow the user to vary d7 from 0 to twice the starting value).

Enter acqu to enter the acquisition window. Enter gs to start the go setup routine. Click the left mouse button to fix the “acquisition-gs” window somewhere on the screen, and then click on the box in the upper right hand corner of the window to send it away as an icon. Click on Display->Phasing->PK phasing to display the Fourier transformed data during gs and to apply the phase correction determined above to the Fourier transformed data. While monitoring the Fourier transformed data, adjust the value of d7 (simply enter d7 and then a new value at the prompt). Notice that for very short values of d7, all the signals are negative, while for very long values, all the signals are positive. Select the value of d7 for which most of the signals have just passed through a null. This length of time is sufficient for NOE buildup in small molecules (in order to avoid spin diffusion in macromolecules, it may be necessary to use a shorter length of time).

Return to the NOESY data set by typing re 1. Enter d8 and set this to the value of d7 determined above.

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 NOESY with the acquisition parameters set as shown above is 5.8 hours.
Set up the processing parameters

Enter `edp` and set the processing parameters as shown in Table 44.

Table 44. NOESY Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>1k</td>
<td>spectrum reference frequency ((^1)H).</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>apply 0- and 1(^{st})-order phase correction determined by phase correcting the first row.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>pk</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>quad</td>
</tr>
<tr>
<td><strong>F1 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>512</td>
<td>spectrum reference frequency ((^1)H).</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>first determine 0- and 1(^{st})-order phase correction with phasing subroutine.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td>TPPI results in a forward single real FT.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
<td></td>
</tr>
</tbody>
</table>

Process the 2D data set

Select window functions to optimize sensitivity or resolution. It is not possible with a window function, to suppress diagonal peaks relative to cross peaks.

Enter `xfb` to multiply the time domain data by the window functions and also perform the 2D Fourier transform. The 2D data set is displayed automatically.

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**

To simplify the phasing of the 2D NOESY spectrum, it helps first to phase correct the first row. Enter `rser 1` to transfer the first row to the 1D data set -TEMP/1/1. Enter `sinm` to apply the sine-bell windowing function, and enter `ft` to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum except that when you are finished, click `return` and select `save as 2D & return` to save the corrections `phc0` and `phc1` to the corresponding F2 parameters of the 2D data file noesy/1/1. Click 2D with the left mouse button to return to the 2D data set noesy/1/1.

Now enter `xfb` to Fourier transform the NOESY spectrum again, this time applying the appropriate phase correction to F2. The spectrum should now require additional phase correction only in F1, and this can be accomplished in the 2D phasing subroutine.

Click on phase to enter the phase correction submenu. To phase correct a 2D spectrum in the F1 dimension (i.e., the columns), first select three columns as described below.

In the phase correction submenu, click on `col` 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 vertical cross hair is aligned with a column towards one end of the spectrum. This column should contain a diagonal peak. Select the column by clicking the middle mouse button. If the selected column does not intersect the most intense portion of the diagonal peak, click on `+` or `-` with the left mouse button until it does. Once the desired column is selected, click on `mov 1` with the left mouse button to move the column to window 1, appearing in the upper right hand corner of the display.

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

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

Now that three columns have been selected, the 0th- and 1st-order phase corrections in F1 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 diagonal peak is not the biggest peak in the window, use `cur: 1` and the mouse to select the diagonal 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 diagonal 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 diagonal 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 xf1p option to apply this phase correction to the spectrum.

At this point, the spectrum should be phased correctly. If, however, the user wishes to make further adjustments, the above procedure can be repeated to adjust the F1 phasing. To further phase correct the spectrum in F2, repeat the above procedure using row rather than col to select three rows with diagonal peaks at one end of the spectrum, in the middle, and at the other end. Phase correct as described above and after selecting save & return after return, confirm the xf2p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by clicking on return. Select 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.

When the NOESY spectrum is properly phased, the diagonal peaks will be either all positive or all negative. The cross peaks may be positive or negative. Any anti-phase cross peaks are COSY artifacts.

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:
NOESY

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 NOESY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 41.
Figure 41: NOESY Spectrum of 50 mM Gramicidin in DMSO-d6
Rotating-frame Overhauser Effect Spectroscopy is an experiment in which homonuclear NOE effects are measured under spin-locked conditions. ROESY is especially suited for molecules with motional correlation times ($\tau_c$) such that $\omega \tau_c \sim 1$, where $\omega$ is the angular frequency $\omega = \gamma B$. In such cases the laboratory-frame NOE is nearly zero, but the rotating-frame NOE (or ROE) is always positive and increases monotonically for increasing values of $\tau_c$. In ROESY the mixing time is the spin-lock period. During this time spin exchange occurs among spin-locked magnetization components of different nuclei (recall that spin exchange in NOESY occurs while magnetization is aligned along the z axis). Different spectral density functions are relevant for ROESY than for NOESY and these cause the ROE’s to be positive for all values of $\tau_c$.

ROESY spectra can be obtained in 2D absorption mode. This is also useful for the identification of certain artifacts. Spurious cross peaks, both COSY-type and TOCSY-type, can be observed due to coherence transfer between scalar coupled spins. COSY-type artifacts (anti-phase) arise when the mixing pulse transfers anti-phase magnetization from one spin to another (the long spin-lock pulse acts like the mixing pulse in COSY). TOCSY-type artifacts (which have the same phase as the diagonal peaks, while ROESY cross peaks have the opposite phase) arise when the Hartmann-Hahn condition is met (e.g., when spins A and B have opposite but equal offsets from the transmitter frequency or when they have nearly identical chemical shifts). In general, to minimize these artifacts, it is suggested to limit the strength of the spin-locking field.


Sample
The sample used to demonstrate ROESY in this chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY and NOESY.

Pulse Sequence Diagram
The ROESY pulse sequence is shown in Figure 42. Notice that the pulse p1 must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’. The pulse p15 is the cw spinlock pulse, during which ROE buildup occurs.
Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and $Z^2$ shims until the lock level is optimized. Tune and match the probehead for $^1H$ observation.

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

$^1H$ reference spectrum
Since ROESY is a homonuclear experiment only one reference spectrum is required. This $^1H$ spectrum will be used to determine $\delta_1$ and $\sigma_2$ for the ROESY experiment, and can also be used as both the F1 and the F2 projections of the ROESY 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 ROESY experiment is so similar to the NOESY experiment, it makes sense to create the ROESY data set from the NOESY data set. From the data set noesy/1/1, enter edc and change the following parameters:

```
NAME     roesy
EXPNO    1
PROCNO   1
```

Click SAVE to create the data set roesy/1/1.

Change to 2D parameter mode
If this data set was created from the NOESY data set, it is already in 2D parameter mode.
Acquisition and Processing

Set up the acquisition parameters

Enter eda and set the acquisition parameters as shown in Table 45. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters pl and p1 (1H observe high power level and 90° pulse time), and pl1 (1H low power level for ROESY spinlock).

The parameter p15 sets the length of the cw spinlock pulse. The value listed in Table 45 is appropriate for this sample. For other samples with different relaxation properties, optimal results may be achieved with slightly different values. The typical range for p15 is from 50 to 300 msec. A good rule of thumb is that p15 for the ROESY experiment of a molecule should be about the same as d8 for the NOESY experiment of that molecule.

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

Table 45. ROESY Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 Parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PULPROG</td>
<td>roesyp</td>
<td>see Figure 42 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>1k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>32</td>
<td>the number of scans must 8*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL11</td>
<td></td>
<td>ROESY spin-lock power level on f1 channel.</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>90° 1H high power pulse on f1 channel.</td>
</tr>
<tr>
<td>P15</td>
<td>200msec</td>
<td>cw pulse for ROESY spin lock; should be approximately ½T1p.</td>
</tr>
<tr>
<td>D0</td>
<td>3µsec</td>
<td>incremented delay (t1); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2sec</td>
<td>relaxation delay; should be about 1.25*T1(1H).</td>
</tr>
<tr>
<td>D12</td>
<td>20µsec</td>
<td>delay for power switching; predefined.</td>
</tr>
<tr>
<td>F1 Parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Comments</td>
</tr>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
</tbody>
</table>
Acquire the 2D data set
If this data set was created from the NOESY spectrum noesy/1/1, the receiver gain is already set correctly.

Enter zg to acquire the time domain data. The approximate experiment time for ROESY 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 46.

Table 46. ROESY Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND0</td>
<td>2</td>
<td>there is one d0 period per cycle and MC2 = TPPI.</td>
</tr>
<tr>
<td>IN0</td>
<td>1/(2*SW_1) = Dw_1</td>
<td>t_1 increment.</td>
</tr>
<tr>
<td>SW</td>
<td>sw of the optimized 1H spectrum (proton/5/1); same as for F2.</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td>select 1H frequency for F1; same as for F2.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td>2 (3, 4)</td>
<td>choose pure cosine wave (or optimize the phase shift of the sine function).</td>
</tr>
<tr>
<td>SSB</td>
<td>pk</td>
<td>apply 0- and 1st-order phase correction determined by phase correcting the second row.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>PKNL</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td>2 (3, 4)</td>
<td>choose pure cosine wave (or optimize the phase shift of the sine function).</td>
</tr>
<tr>
<td>SSB</td>
<td>no</td>
<td>first determine 0- and 1st-order phase correction with phasing subroutine.</td>
</tr>
</tbody>
</table>
Process the 2D data set
Enter \texttt{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 \textbf{button}, holding down the middle or right mouse button, and moving the mouse back and forth.

Since this is a phase-sensitive spectrum, click on \texttt{+/-} 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 \texttt{DefPlot}.

**Phase correct the spectrum**
To simplify the phasing of the 2D ROESY spectrum, it helps first to phase correct the second row. Enter \texttt{rser 2} to transfer the second row to the 1D data set \texttt{~TEMP/1/1}. Enter \texttt{sinm} to apply the sine-bell windowing function, and enter \texttt{ft} to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum except that when you are finished, click \texttt{return} and select \texttt{save as 2D & return} to save the corrections \texttt{phc0} and \texttt{phc1} to the 2D data file \texttt{roesy/1/1}. Click \texttt{2D} to return to the 2D data set \texttt{roesy/1/1}.

Now enter \texttt{xfb} to Fourier transform the ROESY spectrum again, this time applying the appropriate phase correction to F2. The spectrum should now require additional phase correction only in F1, and this can be accomplished in the 2D phasing subroutine.

Click on \texttt{phase} to enter the phase correction submenu. To phase correct a 2D spectrum in the F1 dimension (i.e., the columns), first select three columns as described below.

In the phase correction submenu, click on \texttt{col} 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 vertical cross hair is aligned with a column towards one end of the spectrum. This column should contain a diagonal peak. Select the column by clicking the middle mouse button. If the selected column does not intersect the most intense portion of the diagonal peak, click on \texttt{+} or \texttt{-} with the left button until it does. Once the desired column is selected, click on \texttt{mov 1} with the left mouse button to move the column to window 1, appearing in the upper right hand corner of the display.

Click on \texttt{col} again and move the cross hair until it is aligned with a column containing a diagonal peak near the middle of the spectrum. Select the column by clicking the middle mouse button, adjust the selected column by clicking on \texttt{+} or \texttt{-} with the left mouse buttons, and finally move the desired column to window 2 by clicking on \texttt{mov 2} with the left mouse button.
Repeat the above procedure to select a column with a diagonal peak at the other end of the spectrum. Move this column to window 3 by clicking `mov 3` with the left mouse button.

Now that three columns have been selected, the 0th- and 1st-order phase corrections in F1 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 diagonal peak is not the biggest peak in the window, use `cur: 1` and the mouse to select the diagonal 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 diagonal peak in window 1). Next, move the cursor to the `ph1` button, hold down the left mouse 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 diagonal 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 xf1p option to apply this phase correction to the spectrum.

At this point, the spectrum should be phased correctly. If, however, the user wishes to make further adjustments, the above procedure can be repeated to adjust the F1 phasing. To further phase correct the spectrum in F2, repeat the above procedure using `row` rather than `col` to select three rows with diagonal peaks at one end of the spectrum, in the middle, and at the other end. Phase correct as described above and after selecting `return` followed by `save & return`, confirm the xf2p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by selecting `return` after clicking on `return`. Selecting `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.

When the ROESY spectrum is properly phased, the diagonal peaks will be all positive and the true ROESY cross peaks all negative (or vice versa).

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:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF1DU</td>
<td>u</td>
</tr>
<tr>
<td>PF1USER</td>
<td>(name of user for file proton/5/1)</td>
</tr>
<tr>
<td>PF1NAME</td>
<td>proton</td>
</tr>
<tr>
<td>PF1EXP</td>
<td>5</td>
</tr>
<tr>
<td>PF1PROC</td>
<td>1</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF2DU</td>
<td>u</td>
</tr>
<tr>
<td>PF2USER</td>
<td>(name of user for file proton/5/1)</td>
</tr>
<tr>
<td>PF2NAME</td>
<td>proton</td>
</tr>
<tr>
<td>PF2EXP</td>
<td>5</td>
</tr>
<tr>
<td>PF2PROC</td>
<td>1</td>
</tr>
</tbody>
</table>

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 ROESY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 43.
Figure 43: ROESY Spectrum of 50 mM Gramicidin in DMSO-d6
**TOCSY**

**Introduction**

**TOtal Correlation Spectroscopy** provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy in liquids. In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that the “net” coherence transfer produced can be arranged to create pure absorption mode spectra with positive intensity peaks (rather than “differential” coherence transfer which causes spectra with equal positive and negative intensities). In traditional COSY, cross peaks have zero integrated intensity and coherence transfer is restricted to directly spin-coupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as antiphase coherences.

The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how “far” the spin coupling network will be probed. A general rule of thumb is that $1/(10J_{HH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

The TOCSY spectrum can be phased so that all cross peaks and diagonal peaks have positive intensity.


**Sample**

The sample used to demonstrate TOCSY in this chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY, NOESY, and ROESY.

**Pulse Sequence Diagram**

The TOCSY pulse sequence is shown in Figure 44. Notice that the pulse $p_1$ must be set to the appropriate 90° time found in Chapter 5 ‘Pulse Calibration’. The MLEV-17 sequence used during the spinlock period requires the calibrated 90° time $p_6$. 
Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z\(^2\) shims until the lock level is optimized. Tune and match the probehead for \(^1\text{H}\) observation.

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

\(^1\text{H}\) reference spectrum
Since TOCSY is a homonuclear experiment only one reference spectrum is required. This \(^1\text{H}\) spectrum will be used to determine \(\omega_1\) and \(\omega_2\) for the TOCSY experiment, and can also be used as both the F1 and the F2 projections of the TOCSY spectrum.

A \(^1\text{H}\) 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
The TOCSY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter `re roesy 1` to call up the data set roesy/1/1. Enter `edc` and change the following parameters:

- `NAME` to `tocsy`
- `EXPNO` to `1`
- `PROCNO` to `1`.

Click `SAVE` to create the data set `tocsy/1/1`.

Change to 2D parameter mode
If this data set was created from a previous 2D 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 47. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters `pl1` and `p1` (\(^1\text{H}\) observe high power level and 90° pulse time), and `pl10` and `p6` (\(^1\text{H}\) low power level and 90° pulse time for MLEV spinlock).
The parameter \( l_1 \) determines the number of cycles of the MLEV spinlock sequence, and thus determines the length of the “mixing period”. The mixing period typically lasts 20 to 100 msec, and so \( l_1 \) should be chosen so that the quantity \([(p_6 \cdot 64) + p_5] \cdot 11 + (p_{17} \cdot 2)]\) is 20 to 100 msec. The general rule of thumb is that \( 1/\tau_{JHH} \) should be allowed for each transfer step, and typically five transfer steps are desired, which means a mixing time of \( 1/2 \tau_{JHH} \) or approximately 75 msec.

The parameter \( p_{17} \) determines the length of the trim pulses at the beginning and end of the mixing period. A good value for \( p_{17} \) is 2.5 msec. The trim pulses are used to ensure that the final 2D spectrum can be phased easily. Note, however, that for aqueous samples only the first trim pulse should be used, in which case \( l_1 \) should be adjusted so that \([(p_6 \cdot 64) + p_5] \cdot 11 + p_{17}]\) is 20 to 100 msec.

The F2 parameters \( o_1 \) 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 \( sf_{o1} \) and \( sw \) should be identical to the corresponding F2 values.

Finally, notice that \( in_0 \) and \( sw(F1) \) are not independent. A convenient way to set \( in_0 \) is to set the F1 parameters \( nuc_{1} \) by clicking on \textbf{NUCLEI} for F1 parameters, \( nd_{0} \), and \( sw \) correctly. This automatically sets \( in_0 \) to the correct value.

**Table 47. TOCSY Acquisition Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>mlevtp</td>
<td>see Figure 44 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>1k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>the number of scans should be 8 ( \times n ) in order for the phase cycling to properly.</td>
</tr>
<tr>
<td>DS</td>
<td>16</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL10</td>
<td></td>
<td>MLEV spin-lock power level on f1 channel.</td>
</tr>
<tr>
<td>P1</td>
<td>90° (^1H) high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>60° (^1H) low power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>90° (^1H) low power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>180° (^1H) low power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P17</td>
<td>2.5 msec</td>
<td>trim pulse to defocus non-spinlocked transverse magnetization.</td>
</tr>
<tr>
<td>D0</td>
<td>3(\mu)sec</td>
<td>incremented delay ( (t_1) ); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>2 sec</td>
<td>relaxation delay; should be about ( 1.25 \times T_1 ) (^1H).</td>
</tr>
<tr>
<td>D12</td>
<td>20(\mu)sec</td>
<td>delay for power switching; predefined.</td>
</tr>
</tbody>
</table>
Acquire the 2D data set

If this data set was created from roesy/1/1, or the data set of any of the other homonuclear 2D experiments run on this sample, the receiver gain is already set correctly.

Enter zg to acquire the time domain data. The approximate experiment time for TOCSY with the acquisition parameters set as shown above is 1.3 hours.

Set up the processing parameters

Enter edp and set the processing parameters as shown in Table 48.

Table 48. TOCSY Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>WDW</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>choose pure cosine wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>pk</td>
<td>apply 0- and 1st-order phase correction determined by phase correcting the second row.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>

F1 Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there is one d0 period per cycle and MC2 = TPPI.</td>
</tr>
<tr>
<td>IN0</td>
<td>1/(2*SWH) = DW_H</td>
<td>t1 increment.</td>
</tr>
<tr>
<td>SW</td>
<td>sw of optimized 1H spectrum (proton/5/1); same as for F2.</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td>select 1H frequency for F1; same as for F2.</td>
<td></td>
</tr>
</tbody>
</table>
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 `thres` button, holding down the middle or right mouse button, and moving the mouse back and forth.

Since this is a phase-sensitive spectrum, click on `p/n/a/u` 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
To simplify the phasing of the 2D TOCSY spectrum, it helps first to phase correct the second row. (Note that because of the phase-cycling routine used here, no spin-locking occurs during the acquisition of the first row, so the second row is the earliest row containing TOCSY signal which can be phase corrected.) Enter `rser 2` to transfer the second row to the 1D data set `~TEMP/1/1`. Enter `sinm` to apply the sine-bell windowing function, and enter `ft` to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum except that when you are finished, click `return` and select `save as 2D & return` to save the corrections `phc0` and `phc1` to the 2D data file `tocsy/1/1`. Click `to2D` to return to the 2D data set `tocsy/1/1`.

Now enter `xfb` to Fourier transform the TOCSY spectrum again, this time applying the appropriate phase correction to F2. The spectrum should now require additional phase correction only in F1, and this can be accomplished in the 2D phasing subroutine.

Click on `phase` to enter the phase correction submenu. To phase correct a 2D spectrum in the F1 dimension (i.e., the columns), first select three columns as described below.

In the phase correction submenu, click on `col` 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 vertical cross hair is aligned with a column towards one
end of the spectrum. This column should contain a diagonal peak. Select the column by clicking the middle mouse button. If the selected column does not intersect the most intense portion of the diagonal peak, click on + or - with the left and middle mouse buttons until it does. Once the desired column is selected, click on mov1 with the left mouse button to move the column to window 1, appearing in the upper right hand corner of the display.

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

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

Now that three columns have been selected, the 0th- and 1st-order phase corrections in F1 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 diagonal peak is not the biggest peak in the window, use cur: 1 and the mouse to select the diagonal 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 diagonal peak in window 1). Next, move the cursor to the ph1 button, hold down the left mouse 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 diagonal 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 xf1p option to apply this phase correction to the spectrum.

At this point, the spectrum should be phased correctly. If, however, the user wishes to make further adjustments, the above procedure can be repeated to adjust the F1 phasing. To further phase correct the spectrum in F2, repeat the above procedure using row rather than col to select three rows with diagonal peaks at one end of the spectrum, in the middle, and at the other end. Phase correct as described above and after return and selecting save & return, confirm the xf2p option.

Note that it is possible to exit the phase correction subroutine without saving the phase corrections by selecting return after clicking on return. Selecting 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.

It should be possible to phase correct the spectrum so that all TOCSY peaks are positive.

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 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 TOCSY spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 45.
Figure 45: TOCSY Spectrum of 50 mM Gramicidin in DMSO-d6
**Heteronuclear Multiple Quantum Correlation spectroscopy** is an inverse chemical shift correlation experiment that, like XHCORR, is used to determine which $^1$H’s of a molecule are bonded to which $^{13}$C nuclei (or other X nuclei). The advantage of HMQC over XHCORR is that in HMQC the nucleus with the highest $\gamma$ ($^1$H) is detected, and so it is possible to obtain the highest sensitivity. The challenge of an inverse chemical shift correlation experiment, however, is that the large signals from $^1$H’s not coupled directly to a $^{13}$C nucleus must be suppressed in a difference experiment. This poses a dynamic range problem: the signal of interest is that of $^1$H’s coupled directly to $^{13}$C nuclei; however, the signal detected is dominated by the contribution of $^1$H’s bonded directly to $^{12}$C nuclei. HMQC, which is based on multiple-quantum NMR, minimizes this dynamic range problem while optimizing the sensitivity of the experiment. The resonance frequency of low $\gamma$ spins can be detected with enhanced sensitivity by the creation and $^1$H detection of $^1$H-$^{13}$C (or other X nucleus) multiple-quantum coherence.

In the HMQC sequence, the first $^1$H pulse creates transverse magnetization, some of which evolves into anti-phase magnetization at the end of the first $1/(2J_{XH})$ delay. This anti-phase magnetization is converted into zero- and double-quantum coherence by the ($\pi/2$)$_X$ pulse. The zero- and double-quantum coherences evolve during $t_1$ and are exchanged by the $\pi_H$ pulse so that single-quantum $^{13}$C frequencies are observed in F1. The final ($\pi/2$)$_X$ pulse converts multiple-quantum coherence into observable $^1$H transverse magnetization. In analogy with XHCORR, if a delay $1/(2J_{XH})$ is inserted between the final ($\pi/2$)$_X$ pulse and the start of acquisition, then $^{13}$C decoupling can be used during acquisition. Without this delay, the $^1$H magnetization components would be anti-phase at the start of acquisition and so $^{13}$C decoupling would result in mutual cancellation of the $^1$H signals.

Note that since it is the longitudinal $^1$H magnetization present before the first ($\pi/2$)$_H$ pulse that is converted into heteronuclear multiple-quantum coherence, it is the $^1$H $T_1$ which determines the appropriate recycle delay. Thus, it is possible to use a shorter recycle delay for HMQC than for XHCORR.

For small molecules, it is useful to use a BIRD preparation period in conjunction with the HMQC experiment. The basic idea of this preparation period is to saturate all $^1$H’s not directly attached to a $^{13}$C nucleus, leaving $^1$H’s coupled to $^{13}$C unaffected or slightly intensified by homonuclear NOE (which is the case for molecules in the fast motion limit). Then at time $\tau$ ($d7$) when the inverted magnetization changes from negative to positive (i.e., when the $^1$H’s not coupled to $^{13}$C are nearly saturated) the first $\pi/2$ pulse of the HMQC experiment is applied. BIRD is not recommended for proteins and other macromolecules because the negative NOE effect during the delay time $\tau$ decreases the intensity of $^1$H’s coupled to $^{13}$C.

In practice, since the $T_1$’s of various $^1$H’s in the molecule vary, it is recommended to keep the delay time between experiments short: $T \approx 1.3 \times T_1$ of the fastest relaxing $^1$H of the molecule, where the delay time $T$ is defined to last from the start of data acquisition in one scan to the end of the preparation period ($3^{rd}$ $^1$H pulse) in the next
scan. Similarly, it is suggested that \( \tau \) be approximately \( \frac{T}{2.7} \). To fine tune \( \tau \), choose the value that minimizes the signal obtained from a single scan preceded by 2 dummy scans (i.e., that minimizes the signal from \( ^{1}\text{H} \)’s bonded to \( ^{12}\text{C} \) nuclei).

HMQC is a phase-sensitive experiment, and after a 2D Fourier transform with respect to \( t_1 \) and \( t_2 \), the 2D spectrum can be phased so that all peaks are purely absorptive.


**Sample**
The sample used to demonstrate HMQC in this chapter is 50 mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY, NOESY, ROESY, and TOCSY.

### Pulse Sequence Diagram 16.2

The HMQC pulse sequence is shown in Figure 46. This version of the sequence should be used on samples consisting of proteins and other macromolecules. The HMQC pulse sequence with BIRD is shown in Figure 47. This version of the sequence is useful for smaller molecules.

Notice that the pulses \( p_1 \) and \( p_3 \) must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. Also, the cpd sequence used is GARP, which requires the calibrated 90° time \( p_{cpd2} \). The 180° pulse lengths \( p_2 \) and \( p_4 \) are determined by the pulse program itself. In the HMQC sequence with BIRD, the delay \( d_7 \) should be optimized to minimize signal from \( ^{1}\text{H} \)’s bonded directly to \( ^{12}\text{C} \)’s.

**Figure 46: HMQC Pulse Sequence**

---

**Bruker**

AVANCE User’s Guide
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 ¹³C decoupling.

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

**¹H reference spectrum**

Since HMQC is a ¹H-observe, ¹³C-decouple experiment, the first step is to obtain a reference ¹H spectrum of the sample. This reference spectrum will be used to determine the correct ³o for ¹H, the correct ³sw for the F2 dimension, and can also be used as the F2 projection of the HMQC 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.

**¹³C reference spectrum**

It can be assumed that the sample used for an inverse experiment such as HMQC has too small a ¹³C signal to make it practical to obtain a ¹³C reference spectrum. Thus, the user will need to make an educated guess as to the appropriate values of ³o and ³sw for the F1 dimension. Actually, it is easier to use ³o₂p (in ppm) rather than ³o₂ (in Hz). This is because the XWIN-NMRlock routine was used to lock the magnetic field, and so 0ppm (for a given nucleus) is at the same absolute frequency regardless of the lock solvent.

In the HMQC experiment, no quaternary carbons lead to detected signal, so it is usually sufficient to select a ¹³C spectral width covering the range –10 ppm to 180 ppm. This corresponds to an ³o₂p value of 85 ppm and an ³sw value of 190 ppm.
**Create a new file directory for the 2D data set**

Enter `re proton 5 1` to return to the optimized $^1$H spectrum. From this data set, enter `edc` and change the following parameters:

- NAME: hmqc
- EXPNO: 1
- PROCNO: 1

Click `SAVE` to create the data set `hmqc/1/1`. By creating the HMQC data set from data set of the $^1$H reference spectrum, many of the F2 parameters for HMQC are already set.

Enter `edsp` and set NUC2 to 13C and OFSH1 to `o1` of the $^1$H reference spectrum `proton/5/1`. The parameter OFSX1 should have the value of `o2` corresponding to `o2p = 85 ppm`, but the best way to set this is simply to set `o2p` correctly in the main UXNMR window.

**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 49. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters `pl1` and `p1` ($^1$H observe high power level and 90° pulse time), `pl2` and `p3` ($^{13}$C decouple high power level and 90° pulse time), and `pl12` and `pcpd2` ($^{13}$C decouple low power level and 90° pulse time). Note that the pulse program invbtp calls an include file in which `cnst2` is used to calculate `d2` ($d2 = 1/(2*cnst2)$). Thus, it is only necessary for the user to set the value of `cnst2`. Similarly, the 180° pulse lengths `p2` and `p4` are calculated from the corresponding 90° pulse lengths `p1` and `p3`, so the user need only set the values of `p1` and `p3`.

The F2 parameters `o1` and `sw` (not shown in the table) should be identical to the values used in the optimized $^1$H reference spectrum (`proton/5/1`). Make sure to set `o2p` to 85 ppm as discussed above. The F1 parameter `sw` should also be set to 190 ppm as discussed above.

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 49. HMQC with BIRD Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PULPROG</td>
<td>invbtp</td>
<td>HMQC with BIRD; see Figure 47 for pulse sequence diagram; for HMQC without BIRD choose inv4tp.</td>
</tr>
<tr>
<td>TD</td>
<td>1 k</td>
<td>the number of scans should be 4 * n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>NS</td>
<td>8</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td></td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL2</td>
<td></td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
</tr>
<tr>
<td>PL12</td>
<td></td>
<td>power level for cpd on f2 channel.</td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td>90° 1H high power pulse on f1 channel.</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td>180° 1H high power pulse on f1 channel; calculated internally.</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td>90° 13C high power pulse on f2 channel.</td>
</tr>
<tr>
<td>P4</td>
<td></td>
<td>180° 13C high power pulse on f2 channel; calculated internally.</td>
</tr>
<tr>
<td>PCPD2</td>
<td></td>
<td>90° 13C pulse for cpd sequence.</td>
</tr>
<tr>
<td>D0</td>
<td>3 μsec</td>
<td>incremented delay (t1/2); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>1.5 sec</td>
<td>relaxation delay; should be about 1.25 * T1(1H).</td>
</tr>
<tr>
<td>D2</td>
<td>3.45 msec</td>
<td>delay for creation of anti-phase magnetization (1/(2JXH)); calculated internally.</td>
</tr>
<tr>
<td>D7</td>
<td></td>
<td>delay for inversion recovery (optimize).</td>
</tr>
<tr>
<td>D13</td>
<td>3 μsec</td>
<td>short delay; predefined.</td>
</tr>
<tr>
<td>CNST2</td>
<td>145 Hz</td>
<td>one-bond heteronuclear J-coupling (JXH); used to calculate d2; 145Hz is a good intermediate value for 13C.</td>
</tr>
<tr>
<td>CPDPRG2</td>
<td>garp</td>
<td>composite pulse decoupling sequence.</td>
</tr>
<tr>
<td><strong>F1 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>4</td>
<td>there are two d0 periods per cycle and MC2 = TPPL.</td>
</tr>
</tbody>
</table>
Optimize d7 (HMQC with BIRD only)

Set the acquisition parameters as shown above and choose a starting value of 400 msec for d7 (note that it is important to select a large enough starting value for d7 since the gs routine will only allow the user to vary d7 from 0 to twice the starting value). Enter acqu to enter the acquisition window. Enter gs to start the go setup routine. Click the left mouse button to fix the “acquisition-gs” window somewhere on the screen, and then click on the box in the upper right hand corner of the window to send it away as an icon. While monitoring the intensity of the time domain data, adjust the value of d7 (simply enter d7 and then a new value at the prompt). The optimum value of d7 corresponds to the minimum intensity. Once the optimum value of d7 is found and stored, enter rga to optimize the receiver gain for this minimum signal.

Acquire the 2D data set

Enter zg to start the HMQC experiment. With the acquisition parameters shown above, the approximate experiment time is 1.2 hours.

Set up the processing parameters

Enter edp and set the processing parameters as shown in Table 50.

Table 50. HMQC with BIRD Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>1k</td>
<td>spectrum reference frequency (1H).</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td>multiply data by phase-shifted sine-squared function.</td>
</tr>
<tr>
<td>WDW</td>
<td>QSINE</td>
<td>apply 0- and 1st-order phase correction determined by phase correcting the first row.</td>
</tr>
<tr>
<td>SSB</td>
<td>2</td>
<td>choose pure cosine-squared wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>pk</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td></td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>
Acquisition and Processing

**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**

To simplify the phasing of the 2D HMQC spectrum, it helps first to phase correct the first row. Enter `rser 1` to transfer the first row to the 1D data set ~TEMP/1/1. Enter `sinm` to apply the sine-bell windowing function, and enter `ft` to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum except that when you are finished, click `Return` and select `Save as 2D & return` to save the corrections `phc0` and `phc1` to the corresponding F2 parameters in the 2D data file `hmqc/1/1`. Then click `2D` with the left mouse button to return to the 2D data set `hmqc/1/1`.

Next, it is convenient to use the automation program `calcphinv` to determine the F1 phase correction. From the data set `hmqc/1/1`, simply enter `xau calcphinv`. Note that this automation program is designed specifically for HMQC-type experiments.

Now enter `xfb` to Fourier transform the HMQC spectrum again, this time applying the appropriate phase correction to F1 and F2. At this point, the spectrum should be phased correctly and all peaks should be positive. Further adjustments can be made in the 2D phase subroutine, as described in previous chapters.

---

**F1 Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>512</td>
<td>spectrum reference frequency ((^{13})C).</td>
</tr>
<tr>
<td>SF</td>
<td>SINE</td>
<td>multiply data by phase-shifted sine function.</td>
</tr>
<tr>
<td>WDW</td>
<td></td>
<td>choose pure cosine-squared wave.</td>
</tr>
<tr>
<td>PH_mod</td>
<td>pk</td>
<td>apply 0- and 1st-order phase correction determined by automation program <code>calcphinv</code>.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>no</td>
<td>determines type of FT in F1; TPPI results in a forward single real FT.</td>
</tr>
<tr>
<td>MC2</td>
<td>TPPI</td>
<td>determines type of FT in F1; TPPI results in a forward single real FT.</td>
</tr>
</tbody>
</table>
Plot the spectrum

Read in the plot parameter file standard2D, e.g., enter \texttt{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 \texttt{DefPlot} and answer the following questions.

- Change levels? \texttt{y}
- Please enter number of positive levels? 6
- Please enter number of negative levels? 3
- Display contours? \texttt{n}

Enter \texttt{edg} to edit the plotting parameters.

Click the \texttt{ed} next to the parameter EDAXIS to enter the axis parameters submenu. Change the value of the parameter X1TICD from 0.1 to 2.5. Click \texttt{SAVE} to save this change and return to the \texttt{edg} menu.

Since there is no $^{13}$C reference spectrum of this sample, the user may choose not to plot an F1 projection for the HMQC spectrum. To do this, simply click the YES adjacent to PROJ1 in the \texttt{edg} menu to toggle it to NO.

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

- PF2DU \texttt{u}
- PF2USER \texttt{(name of user for file proton/5/1)}
- PF2NAME \texttt{proton}
- PF2EXP \texttt{5}
- PF2PROC \texttt{1}

Click \texttt{SAVE} to save these changes and return to the \texttt{edg} menu.

Click \texttt{SAVE} to save all the above changes and exit the \texttt{edg} menu.

Next create a title for the spectrum. Enter \texttt{setti} to use the editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter \texttt{plot} (provided the correct plotter is selected in \texttt{edo}).

An HMQC spectrum of 50 mM Gramicidin in DMSO-d6 is shown in Figure 48.
Figure 48: HMQC Spectrum of 50 mM Gramicidin in DMSO-d6
Heteronuclear Multiple Bond Correlation spectroscopy is a modified version of HMQC suitable for determining long-range $^1$H-$^{13}$C connectivity. This is useful in determining the structure and $^1$H and $^{13}$C assignments of molecules. Since it is a long-range chemical shift correlation experiment, HMBC provides basically the same information as COLOC; however, since it is also an inverse experiment, HMBC has a higher sensitivity than COLOC.

The HMBC pulse sequence may be described simply as follows: The first $^{13}$C 90° pulse, which occurs $1/(2J_{\text{XH}})$ after the first $^1$H 90° pulse, serves as a low-pass J-filter to suppress one-bond correlations in the 2D spectrum. It does this by creating heteronuclear multiple quantum coherence for $^1$H’s directly coupled to a $^{13}$C nucleus. This unwanted coherence is removed from the 2D spectrum by phase cycling the first $^{13}$C 90° pulse with respect to the receiver. After the interval $\Delta_2$ (which is about 60 msec), the second $^{13}$C 90° pulse creates the desired heteronuclear multiple quantum coherence for $^1$H’s J-coupled to a $^{13}$C nucleus 2 or 3 bonds away. This is followed by the evolution time $t_1$. A $^1$H 180° pulse placed halfway through $t_1$ removes the effect of $^1$H chemical shift from the $t_1$ modulation frequency. The final $^{13}$C 90° pulse occurs directly after the evolution period, and is followed immediately by the detection period $t_2$. After the final $^{13}$C 90° pulse, the $^1$H signals originating from $^1$H-$^{13}$C multiple quantum coherence are modulated by $^{13}$C chemical shifts and homonuclear $^1$H J-couplings. Phase cycling of the second $^{13}$C 90° pulse removes signal from $^1$H’s that do not have a long-range coupling to $^{13}$C. The signal detected during $t_2$ is phase modulated by the homonuclear $^1$H J-couplings. The 2D spectrum is generated by a Fourier transform with respect to $t_1$ and $t_2$.

Because of phase modulation, the final spectrum has peaks which are a combination of absorption and dispersion lineshapes. It is not possible to phase correct the spectrum so that the peaks are purely absorptive, and so the spectrum must be presented in magnitude mode.

If more than one long-range $^1$H-$^{13}$C connectivity is detected for one particular proton, the relative intensities of the corresponding resonances are directly related to the magnitude of the coupling constant.


Sample

The sample used to demonstrate HMBC in this chapter is 50mM Gramicidin in DMSO-d6. This is the same sample that was used to demonstrate COSY, NOESY, ROESY, and TOCSY, and HMQC.
The HMBC pulse sequence is shown in Figure 49. Notice that the pulses $p_1$ and $p_3$ must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. The 180° pulse length $p_2$ is determined by the pulse program itself.

**Figure 49: HMBC Pulse Sequence**

Acquisition and Processing

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z$^2$ shims until the lock level is optimized. Tune and match the probehead for $^1$H observation $^{13}$C decoupling.

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

$^1$H reference spectrum

Since HMBC is a $^1$H-observe experiment, the first step is to obtain a reference $^1$H spectrum of the sample. This reference spectrum will be used to determine the correct $o_1$ for $^1$H, the correct $sw$ for the F2 dimension, and can also be used as the F2 projection of the HMBC spectrum.

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

$^{13}$C reference spectrum

It can be assumed that the sample used for an inverse experiment such as HMBC has too small a $^{13}$C signal to make it practical to obtain a $^{13}$C reference spectrum. Thus, the user will need to make an educated guess as to the appropriate values of $o_2$ and
sw for the F1 dimension. Actually, it is easier to use o2p (in ppm) rather than o2 (in Hz). This is because the UXNMR lock routine was used to lock the magnetic field, and so 0 ppm (for a given nucleus) is at the same absolute frequency regardless of the lock solvent.

Note that because HMBC is a multiple bond correlation experiment, we can expect to detect signals from 1H’s coupled to quaternary 13C’s, in addition to primary, secondary and tertiary 13C’s. Thus, the 13C spectral width should be larger than that used for HMQC. An appropriate spectral width would cover the range from –10 ppm to 250 ppm. This corresponds to an o2p value of 120 ppm and an sw value of 260 ppm.

Create a new file directory for the 2D data set
Enter re proton 5 1 to return to the optimized 1H spectrum. From this data set, enter edc and change the following parameters:

NAME          hmbc  
EXPNO         1  
PROCNO        1  

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

Enter edsp and set NUC2 to 13C and OFSH1 to o1 of the 1H reference spectrum proton/5/1. The parameter OFSX1 should have the value of o2 corresponding to o2p = 120 ppm, but the best way to set this is simply to set o2p correctly in the main UXNMR window.

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 51. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters pl1 and p1 (1H observe high power level and 90° pulse time), and pl2 and p3 (13C decouple high power level and 90° pulse time). Note that the pulse program inv4lpfrnd calls an include file in which cnst2 is used to calculate d2 (d2 = 1/(2 * cnst2)). Thus, it is only necessary for the user to set the value of cnst2. Similarly, the 180° pulse length p2 is calculated from the corresponding 90° pulse length p1, so the user need only set the value of p1. On the other hand, d6 is not defined in the include file, and so must be set explicitly in eda.

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). Make sure to set o2p to 120 ppm as discussed above. The F1 parameter sw should also be set to 260 ppm as discussed above.

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 51. HMBC Acquisition Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PULPROG</td>
<td>inv4plrnd</td>
<td>see Figure 49 for pulse sequence diagram.</td>
</tr>
<tr>
<td>TD</td>
<td>4k</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>64</td>
<td>the number of scans should be 16*n in order for the phase cycling to work properly.</td>
</tr>
<tr>
<td>DS</td>
<td>32</td>
<td>number of dummy scans.</td>
</tr>
<tr>
<td>PL1</td>
<td>high power level on f1 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>PL2</td>
<td>high power level on f2 channel (see “An Important Note on Power Levels” on page 7).</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>90° 1H high power pulse on f1 channel.</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>180° 1H high power pulse on f1 channel; calculated internally.</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>90° 13C high power pulse on f2 channel.</td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>3µsec</td>
<td>incremented delay (t1/2); predefined.</td>
</tr>
<tr>
<td>D1</td>
<td>1.5 sec</td>
<td>relaxation delay; should be about 1.25*T1(1H).</td>
</tr>
<tr>
<td>D2</td>
<td>3.45 msec</td>
<td>delay for creation of anti-phase magnetization (1/(2JXH)); calculated internally.</td>
</tr>
<tr>
<td>D6</td>
<td>~50 msec</td>
<td>delay for evolution of long range couplings (1/(nJXH)).</td>
</tr>
<tr>
<td>CNST2</td>
<td>145Hz</td>
<td>one-bond heteronuclear J-coupling (JXH).</td>
</tr>
<tr>
<td><strong>F1 Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>256</td>
<td>number of experiments.</td>
</tr>
<tr>
<td>ND0</td>
<td>2</td>
<td>there are two d0 periods per cycle and MC2 = QF.</td>
</tr>
<tr>
<td>IN0</td>
<td>1/(2*SWX) = DWX</td>
<td>t1 increment.</td>
</tr>
<tr>
<td>SW</td>
<td>sw of the 13C spectrum (here typically 260ppm).</td>
<td></td>
</tr>
<tr>
<td>NUC1</td>
<td></td>
<td>select 13C frequency for F1</td>
</tr>
</tbody>
</table>

**Acquire the 2D data set**

Enter zg to start the HMBC experiment. With the acquisition parameters shown above, the approximate experiment time is 13.5 hours.
Set up the processing parameters

Enter \textit{edp} and set the processing parameters as shown in Table 52.

Table 52. HMBC Processing Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2k</td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>QSINE</td>
<td>multiply data by phase-shifted sine-squared function.</td>
</tr>
<tr>
<td>WDW</td>
<td>0 (4)</td>
<td>choose pure sine wave (or optimize the phase shift of the sine-squared function).</td>
</tr>
<tr>
<td>PH_mod</td>
<td>no</td>
<td>this is a magnitude spectrum.</td>
</tr>
<tr>
<td>PKNL</td>
<td>TRUE</td>
<td>necessary when using the digital filter.</td>
</tr>
<tr>
<td>BC_mod</td>
<td>quad</td>
<td></td>
</tr>
</tbody>
</table>

Process the 2D data set

It is especially useful to do an automatic baseline correction in the F1 dimension of this 2D spectrum, in part because HMBC spectra usually have quite a bit of \( t_1 \) noise and also because they are magnitude mode.

Enter \texttt{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 \( \square \) button, holding down the left mouse button, and moving the mouse up and down.

Since this is a magnitude spectrum, click on \( \div/\times \) 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 EDAXIS to enter the axis parameters submenu. Change the value of the parameter X2TICD from 0.1 to 2.5. Click `SAVE` to save this change and return to the `edg` menu.

Since there is no $^{13}$C reference spectrum of this sample, the user may choose not to plot an F1 projection for the HMBC spectrum. To do this, simply click the YES adjacent to PROJ1 in the `edg` menu to toggle it to NO.

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`).

An HMBC spectrum of 50mM Gramicidin in DMSO-d6 is shown in Figure 50.
Figure 50: HMBC Spectrum of 50 mM Gramicidin in DMSO-d6
HMBC
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<td>wobble</td>
<td>12, 13, 14</td>
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<tr>
<td>XHCORR</td>
<td>115, 125, 175</td>
</tr>
<tr>
<td>zero fill</td>
<td>23</td>
</tr>
<tr>
<td>z-filter</td>
<td>95, 97</td>
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