

Basic ^1H Acquisition and Processing

Introduction

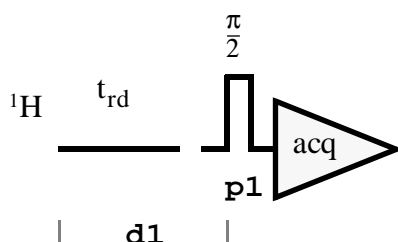
3.1

This chapter describes the acquisition and processing of a ^1H 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, **d1** and **p1**, 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 **d1** is typically a few seconds while **p1** is typically a few microseconds in length.

Figure 1: ^1H One-Pulse Sequence



Sample

The sample used to demonstrate the basic 1D ^1H experiment in this chapter is 100mg 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., ^1H selective or dual $^1\text{H}/^{13}\text{C}$) 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 ^1H .

Basic ^1H Acquisition and Processing

Spectrometer and Acquisition Parameters

3.2

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

3.2.1

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

3.2.2

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 ^1H frequency of the magnet.

Spectrometer and Acquisition Parameters

Set Up the Acquisition Parameters

3.2.3

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 ¹H Spectrum Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for pulse sequence diagram.
AQ_mod	qsim	the default setting.
TD	32k	not critical; 32 k is a fairly standard value for a high-resolution 1D spectrum.
PARMODE	1D	this is a 1D experiment.
NS	1	do not bother to signal average until other parameters are optimized.
DS	0	no need to collect dummy scans yet.
D**Array**	D1 = 2 sec	the default unit for delays is seconds; simply entering “2” sets a delay of 2 seconds; d1 is the only delay used in the pulse program zg.
P**Array**	P1 = 3 μsec	the default unit for pulse lengths is microseconds; p1 is the only pulse length used in the pulse program zg; this value will be optimized later.
SW	50 ppm	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.
RG	64	just a suggested value.
NUC1	1H	this parameter merely gives the user information; use edsp to change the nucleus.
SFO1		these parameters were set with edsp and should not be changed until something is known about the spectrum.
BF1		
O1		
PL**Array**	PL1 =	select high power level (see “An Important Note on Power Levels” on page 7).

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

Note that the parameters **d1**, **p1**, and **p11** are included in the parameter arrays D, P and PL, respectively, in the **eda** table. To edit these parameters within **eda**, follow

Basic ¹H 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 **p11** 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

3.3


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 1/1**. 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

3.4

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

3.4.1

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 $2(\mathbf{si}) - \mathbf{td}$ 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 32k (appropriate since **td** is 32k).

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

3.4.2

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.

Basic ¹H Acquisition and Processing

Notice that once suitable values of **phc0** and **phc1** have been stored it is possible to use them to phase correct subsequent spectra by typing the command **pk**. In addition, it is possible to combine the Fourier transformation (**ft**) and phase correction (**pk**) into one step using the command **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 **em**.

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

Enter **em** to perform the exponential multiply.

Enter **fp** to Fourier transform and phase correct the filtered data.

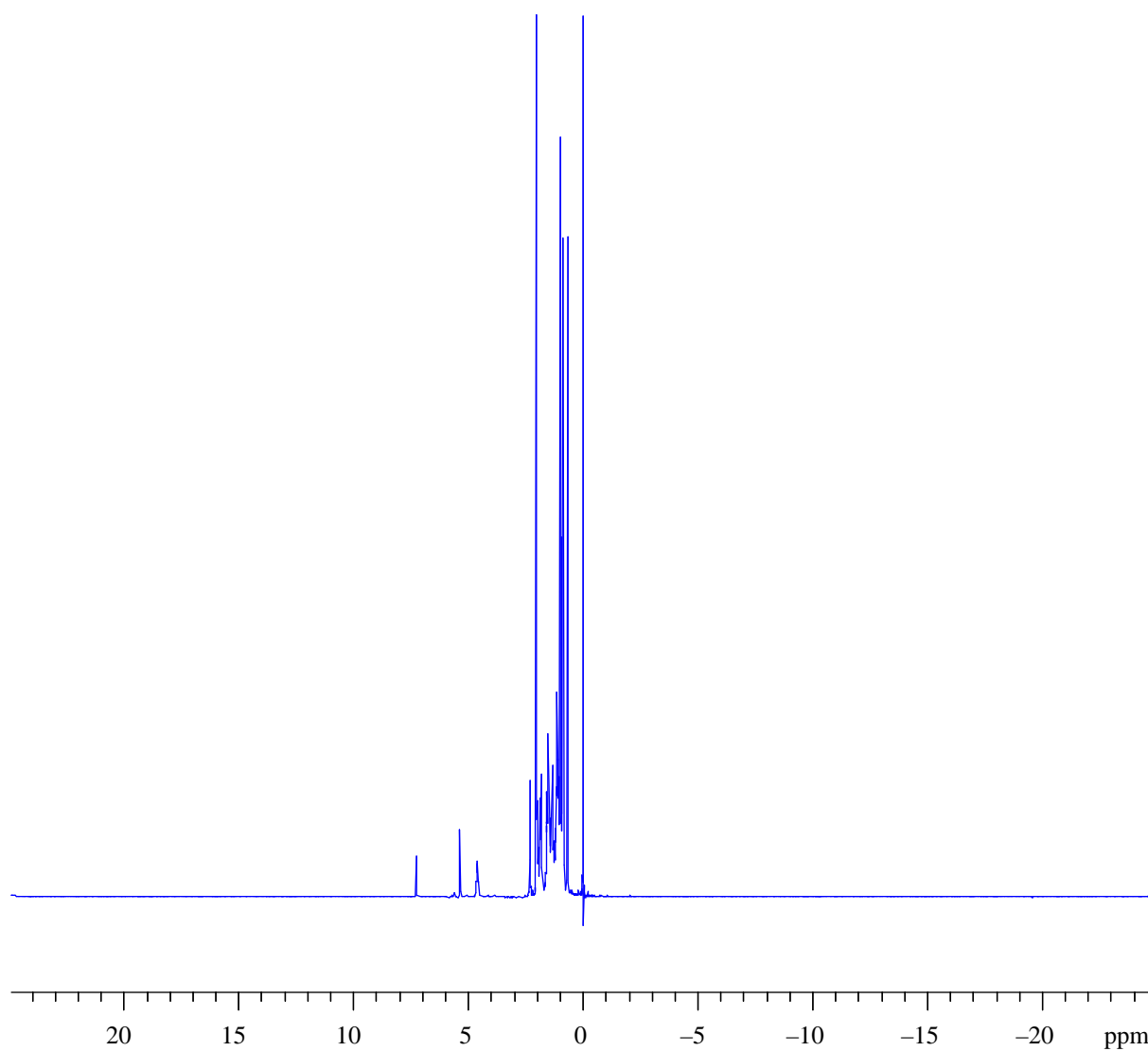
At this point it may be useful to define the macro **efp**, if it does not already exist, that combines the commands **em**, **ft**, and **pk**. Simply enter **edmac** to call up the menu of existing macros. If **efp** does not already exist, enter **efp** at the prompt "Type new name:". The file "efp"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

```
em  
ft  
pk .
```

In the future, whenever you wish to combine the commands **em**, **ft**, and **pk**, you need only type **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).

Figure 2: ^1H Spectrum of 100 mg Cholesterylacetate in CDCl_3 ;
No Signal Averaging



Spectrum Calibration and Optimization


3.5

^1H 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

Basic ¹H Acquisition and Processing

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 ¹H 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.

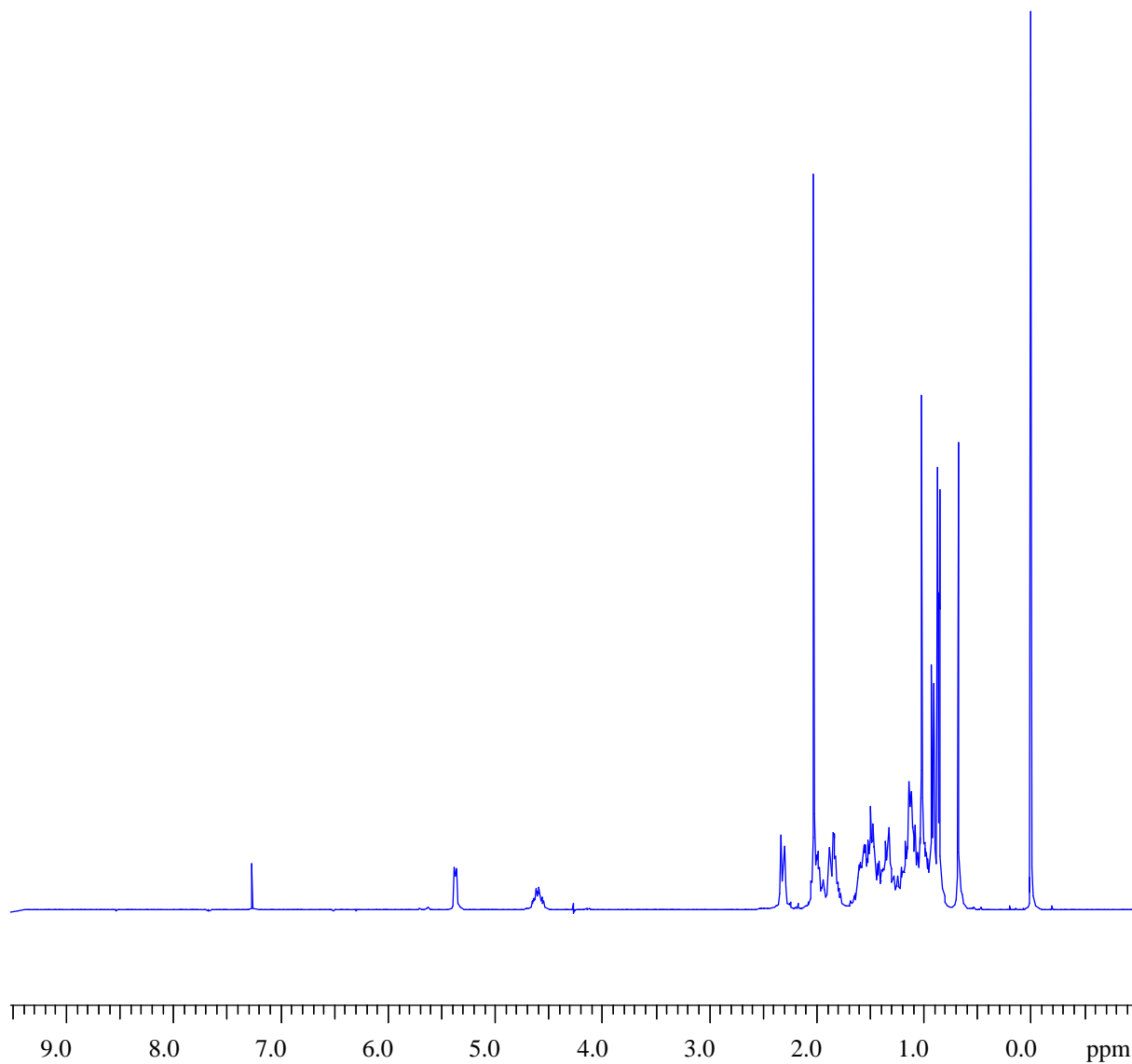
Click the left mouse button somewhere in the spectral window to tie the cursor to the spectrum. Position the cursor at approximately 10 ppm. Click the middle mouse button to set a marker at this frequency. Move the cursor to approximately -1 ppm and click the middle mouse button to expand the spectrum. 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.

Spectrum Calibration and Optimization

Figure 3: ^1H 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 **o1** and **sw** for a ^1H spectrum of 100mg Cholesterylacetate in Table 54 in Appendix A 'Data Sets and Selected Parameters'.

Basic ¹H Acquisition and Processing

Increase the Number of Scans

3.5.2

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.

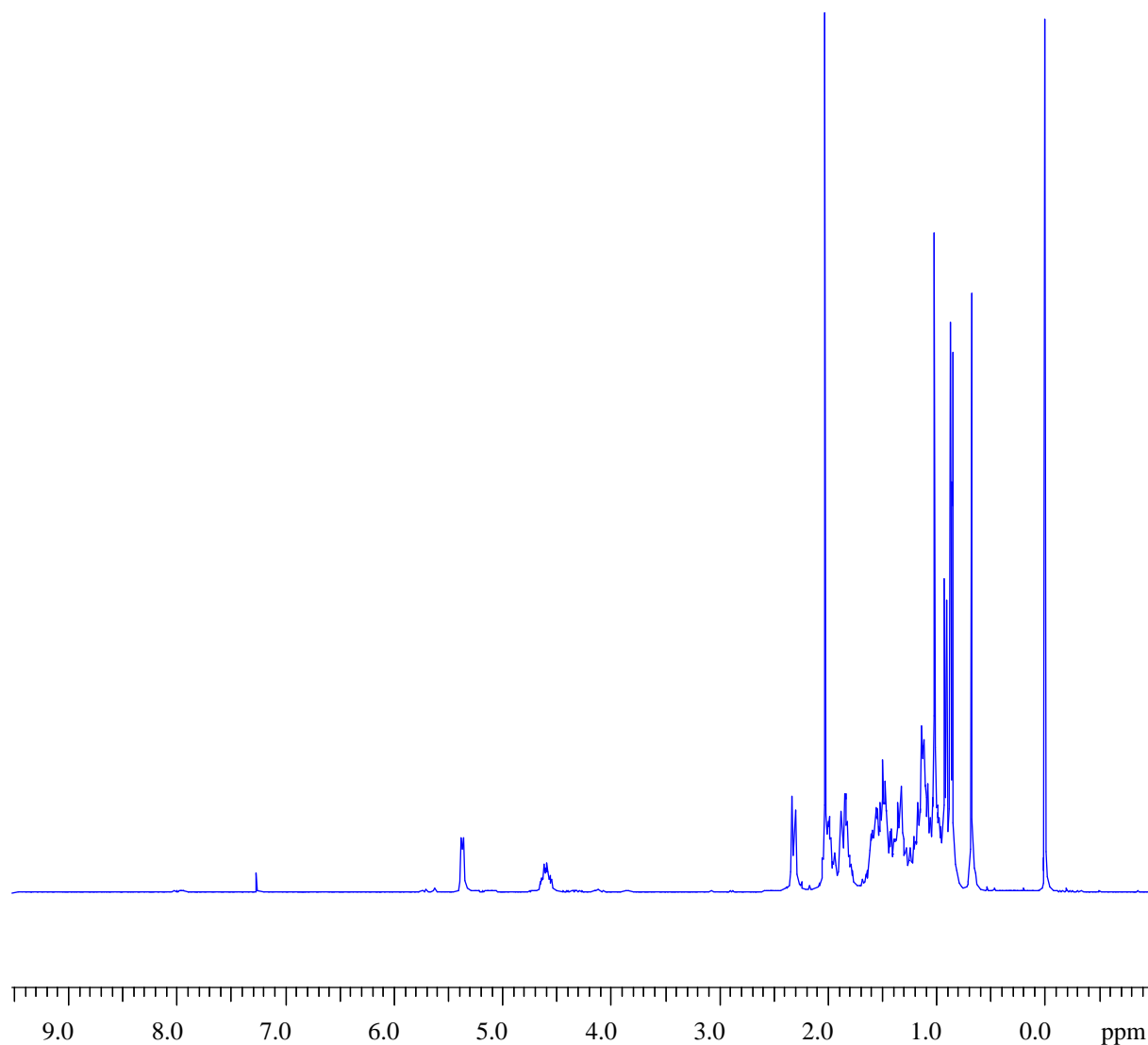
To avoid overwriting previous data, first create a new data set. Enter **edc**, set EXPNO to 3, and click on **SAVE** to create the data set proton/3/1.

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".

Figure 4: ^1H Spectrum of 100 mg Cholesterylacetate in CDCl_3 ;
Signal Averaging



Plotting 1D ^1H Spectra

3.6

A straightforward way to plot 1D ^1H 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 ¹H 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 ¹H 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 = <return>
F2 = <return>
Change y-scaling on display according to PSCAL?<return>
```

It is often useful to integrate ¹H 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**).