

# Basic $^{13}\text{C}$ Acquisition and Processing

## Introduction

## 4.1

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

### Sample

The sample used to demonstrate the basic 1D  $^{13}\text{C}$  experiments in this chapter is 1g Cholesterylacetate in  $\text{CDCl}_3$ . 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	$^{13}\text{C}$
NUC2	$^1\text{H}$
NUC3	off .

The relevant spectrometer parameters are now OFSX1, BF1, and SFO1 for  $^{13}\text{C}$ , and OFSH1, BF2, and SFO2 for  $^1\text{H}$ . 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  $^{13}\text{C}$  frequency of the magnet, and also to pulse at the base  $^1\text{H}$  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  $^{13}\text{C}$  and  $^1\text{H}$ .

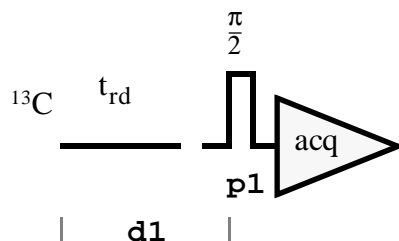
## One-Pulse Experiment with No Decoupling

## 4.2

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

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



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

### Acquisition

### 4.2.1

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

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

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

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

Enter **zg** to acquire the FID. Notice that the X router display flashes.

## One-Pulse Experiment with No Decoupling

## Processing

## 4.2.2

Enter **si** and when prompted a value of **32k**.

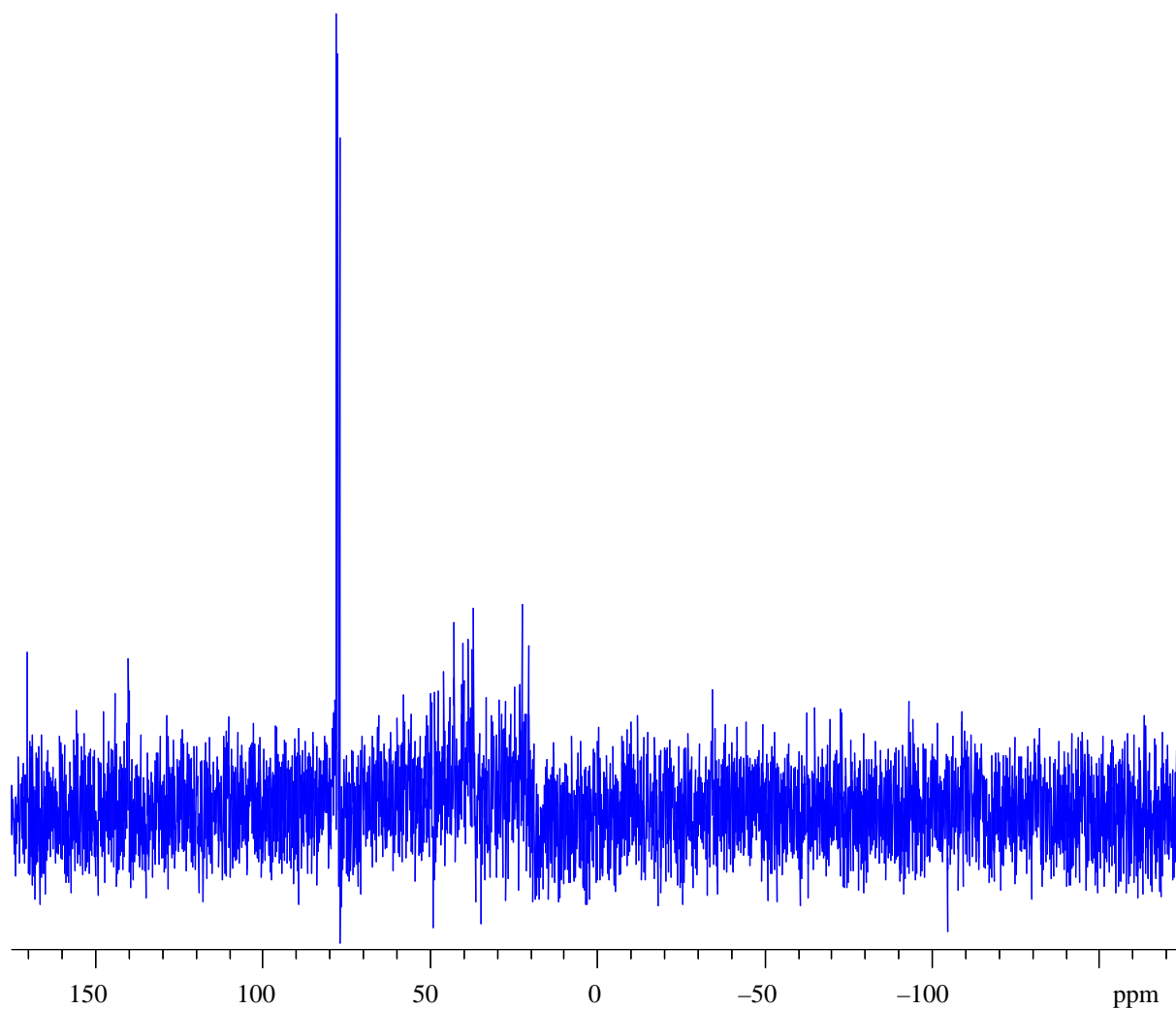
Enter **lb** to check the line broadening. Enter **3** when prompted (line broadening for  $^{13}\text{C}$  spectra is typically 2 to 5 Hz).

Enter **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 **phc0** and **phc1** are now correct for this experiment. Subsequent  $^{13}\text{C}$  spectra may be processed with the command **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 **edlock** table and that lock-in was achieved using the XWIN-NMR 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).

## Basic $^{13}\text{C}$ Acquisition and Processing

Figure 6:  $^{13}\text{C}$  Spectrum of 1 g Cholesterylacetate in  $\text{CDCl}_3$ ;  
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.

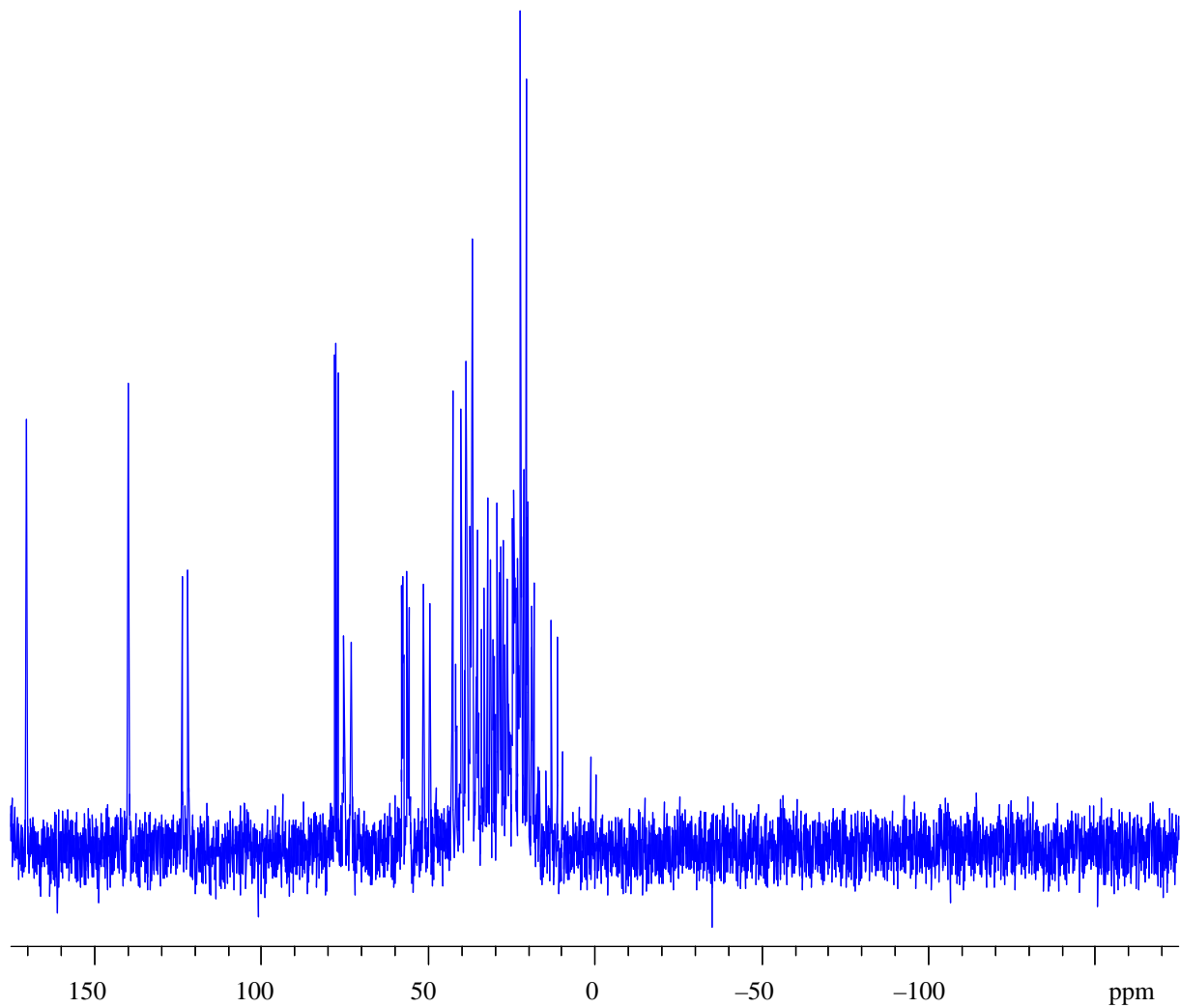
Enter **ns** and change the current value to 64. Enter **ds** and change the current value to 4. The parameters are ready now so that 64 spectra will be acquired and added together. The four dummy scans are to ensure that the system reaches steady state before any spectra are added together. Enter **zg** 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  $^{13}\text{C}$  spectrum of Cholesterylacetate after 64 scans is shown in Figure 7.

It is clear that the  $^{13}\text{C}$  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  $^1\text{H}$  decoupling.

## Basic $^{13}\text{C}$ Acquisition and Processing

Figure 7:  $^{13}\text{C}$  Spectrum of 1 g Cholesterylacetate in  $\text{CDCl}_3$ ;  
Signal Averaging, No Decoupling

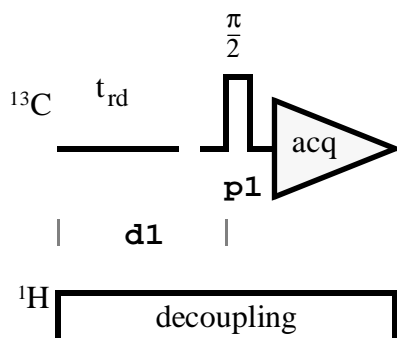


One-Pulse Experiment with  $^1\text{H}$  DecouplingOne-Pulse Experiment with  $^1\text{H}$  Decoupling

4.3

The one-pulse sequence with  $^1\text{H}$  decoupling is shown in Figure 8. Notice that the only difference between this sequence and the one shown in Figure 5 is that here  $^1\text{H}$  decoupling is applied for the duration of the pulse sequence.

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



## Acquisition

4.3.1

Before acquiring a  $^1\text{H}$ -decoupled  $^{13}\text{C}$  spectrum, the frequency of the Cholesterylacetate  $^1\text{H}$  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  $^1\text{H}$  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  $^1\text{H}$  decoupling is, e.g., 1 ppm. (Note that as a general rule of thumb, when no optimized  $^1\text{H}$  spectrum is available, 5 ppm is a safe frequency to select for  $^1\text{H}$  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  $^{13}\text{C}$  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  $^1\text{H}$ -decoupled  $^{13}\text{C}$  spectrum.

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

Enter **eda** and set the acquisition parameters as shown in Table 5.

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

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

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

### Processing

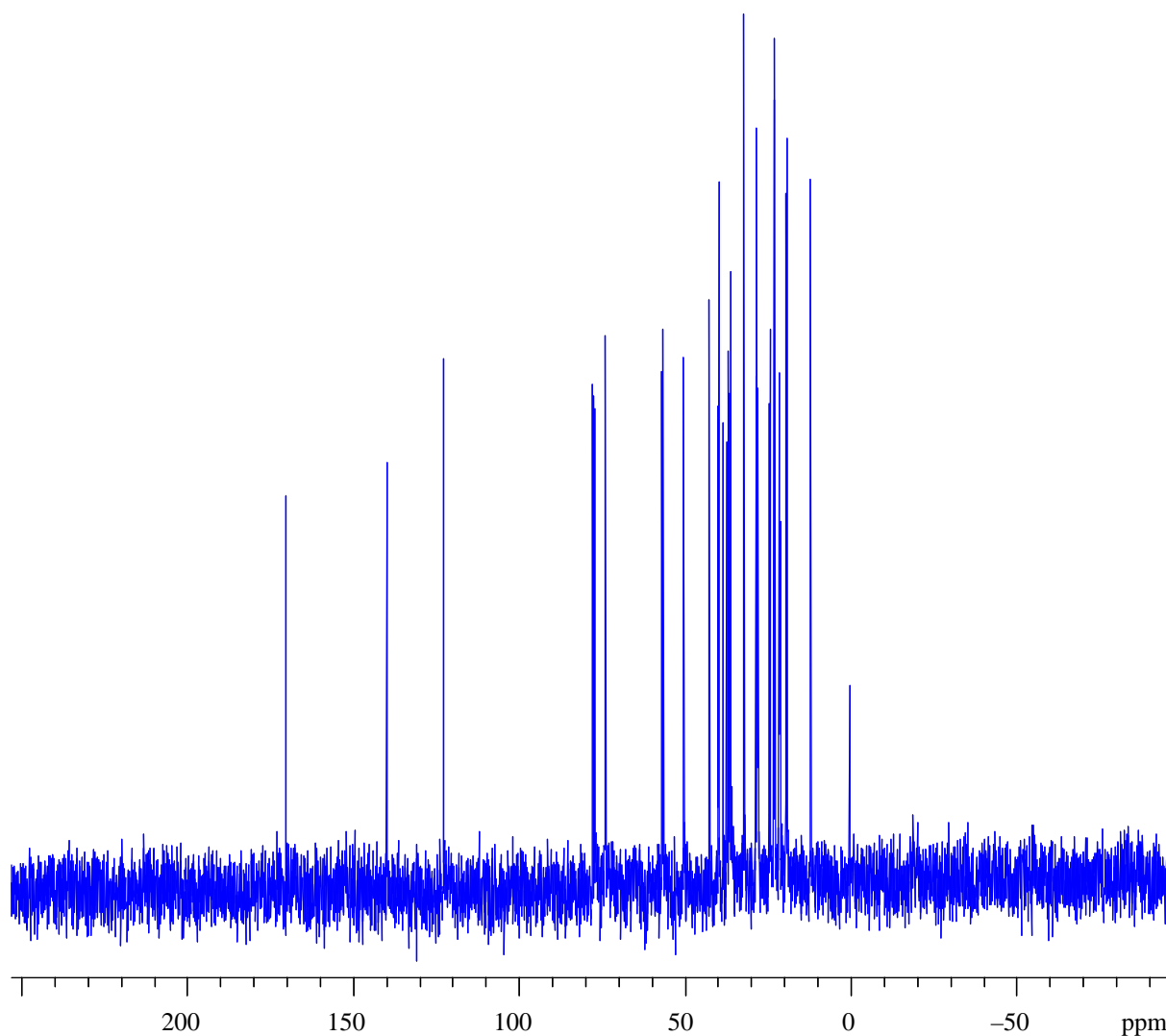
#### 4.3.2

Enter **efp** to add line broadening, Fourier transform, and phase correct the data. A  $^1\text{H}$  decoupled  $^{13}\text{C}$  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 **sf02**, and on the power of the decoupling sequence pulses as set by **p112**. (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 **p112**. Pulse calibration is covered in Chapter 5 ‘Pulse Calibration’.

One-Pulse Experiment with  $^1\text{H}$  Decoupling

Figure 9:  $^{13}\text{C}$  Spectrum of 1 g Cholesterylacetate in  $\text{CDCl}_3$ ;  
No Signal Averaging,  $^1\text{H}$  Decoupling



At this point the user may also wish to optimize **o1** and **sw** so that the spectrum covers nearly the entire spectral width. For future reference, the optimized parameters **o1**, **o2**, and **sw** for  $^1\text{H}$ -decoupled  $^{13}\text{C}$  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 <sup>13</sup>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 =                <return>
F2 =                <return>
Change y-scaling on display according to PSCAL?<return>
```

For <sup>13</sup>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 <sup>13</sup>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 <sup>13</sup>C T<sub>1</sub> relaxation times and potential NOE build-up during <sup>1</sup>H decoupling, the integrated intensities will not faithfully reflect the numbers of different types of <sup>13</sup>C nuclei in a given molecule. Thus, it is best not to integrate standard <sup>13</sup>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**).