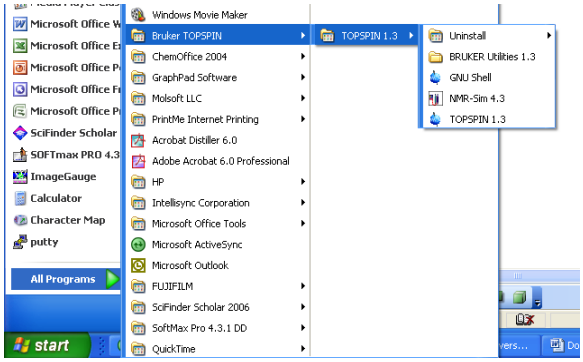
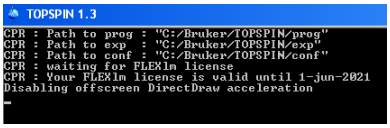
Processing 1D NMR spectra with TOPSPIN



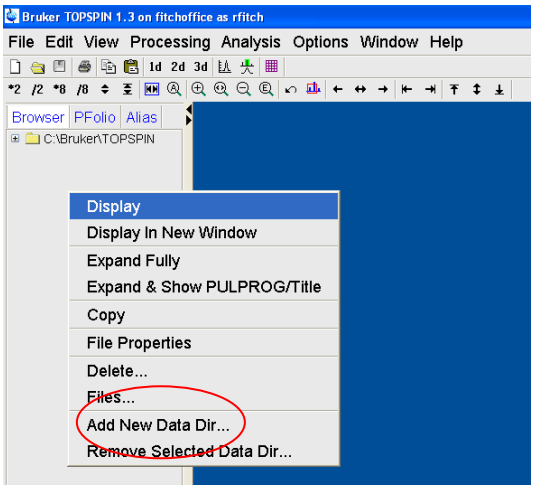
Processing of NMR spectra may be done at the spectrometer workstation at the time of acquisition or, more commonly, offline at one of the departmental computers. Either way, you will process from the Topspin program. On the NMR workstation, Topspin is open because it is running the spectrometer. On the departmental computers, you will need to start it by clicking on the Topspin icon on the desktop after you have logged into the computer or from the Start menu as shown. A black DOS box will open and some commands will be issued. This box stays open so do not try to close it. However, you will not have any interaction with this box unless there is an error, such as the machine is unable to find its license. Once Topspin is up, you will see the standard window that you use for processing and acquisition. (NOTE: Do not attempt to acquire from the department computers. They are set up as data stations only.)

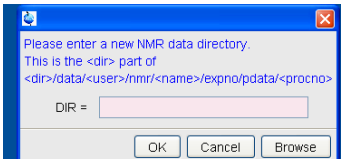


Opening an NMR data file

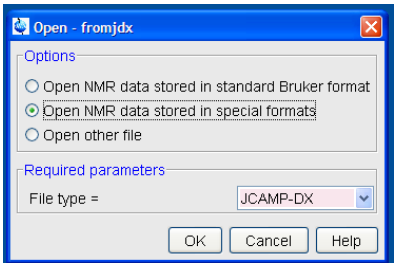
A. From the NMR Workstation. You can open a file either through the Open dialog from the File menu or through the data browser at the left of the screen. If you use the open dialog, you will need to browse to the file as you normally would for opening any file. The data are stored in C:\Bruker\Topspin\Data\<your username>\nmr\<filename>. If you have multiple experiments, a dialog box will open asking you which dataset you wish to open. If you use the browser, simply expand the directory tree to the location where your data are stored. Then select the dataset you wish to work with and click and drag into the main window.

B. From a Departmental Computer. First, you will need to access your data directory. To do this, you will need to specify the directory in the data browser as shown. If you do not see the data browser, there will be a pair of arrows on the top left corner of the main screen. Click on the arrow to show the data browser or go to View – Browser Panel On/Off. You can also use <ctrl> + D to toggle the display. Either way, you will see the directory list at the left. The browser is a directory list that first specifies the local directory and then any other areas where data is stored. To include your data directory, right click in the browser area and select Add New Data Dir… A window will open and you will be able to type the path or (preferably) select Browse. This will open a window as shown. Select your home directory (the one with your username) from the drop-down menu at the top and then select the directory NMR400 in your folder and hit OK (Do not open the directory). You should now see your NMR400 directory in the data browser. To open a spectrum for processing, simply open the NMR400 directory and select the subdirectory containing your spectrum. Once you find the experiment you are looking for, simply double click on the folder, or drag and drop into the main window. *If you are trying to open emailed datasets, this will not work.* This is because the datasets are in JCAMP-DX format.

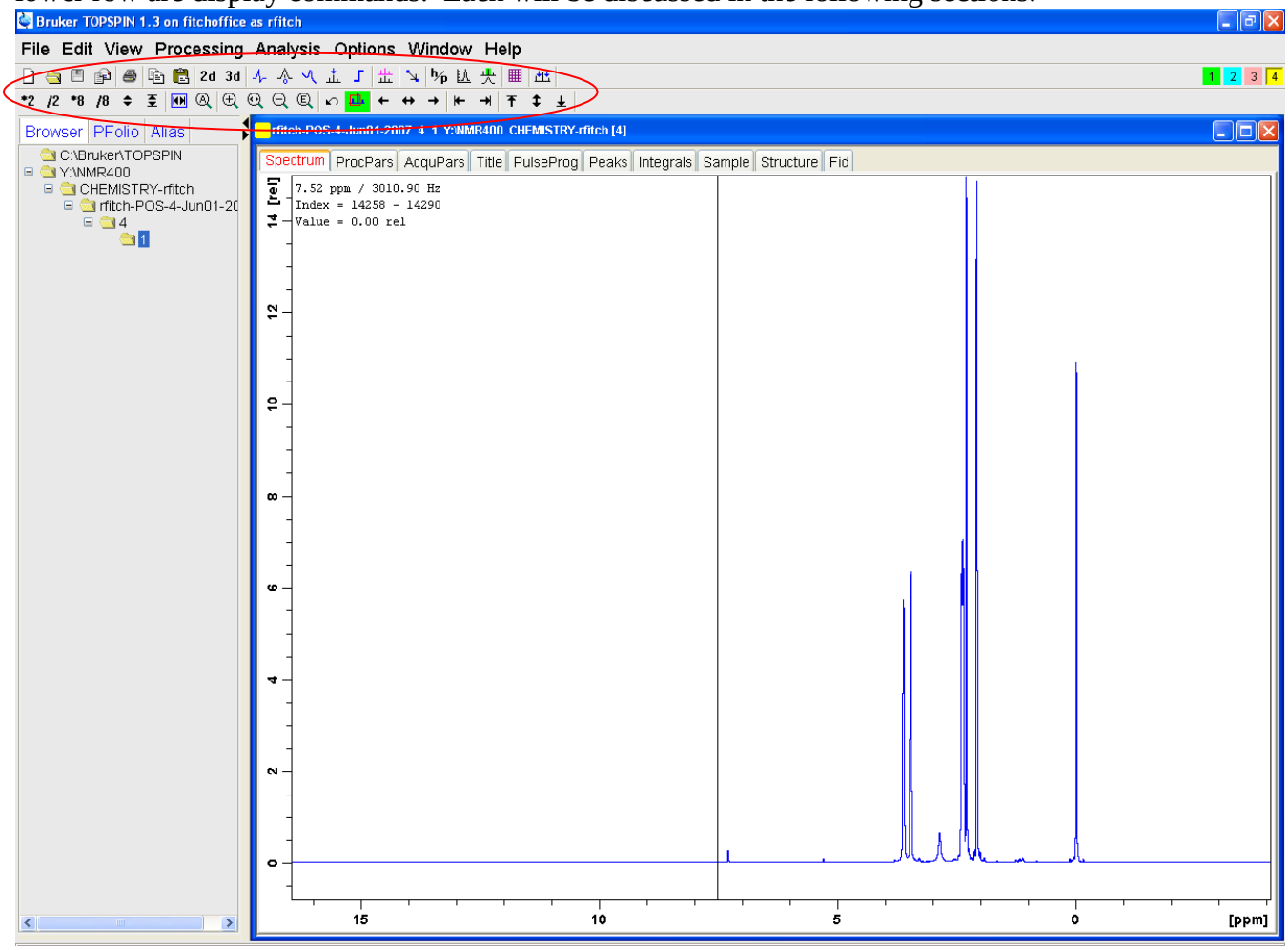




C. Opening emailed datasets. To open an emailed dataset with Topspin, select Open, and when the dialog box comes up, select "Open NMR data stored in special formats". The default file type is JCAMP-DX, so select OK. Then select Browse in the next dialog box and browse to the file.



In any of these scenarios, your dataset will open and the window will look something like that below (Note: You may or may not have a spectrum to display. Don't worry about this just yet.). The area in the red oval is the main toolbar and contains the commands used most often in processing spectra. The upper row of buttons are subroutines for integration, peak picking, calibration and other functions. The lower row are display commands. Each will be discussed in the following sections.



General notes on commands

There are often several paths to get to the same command in Topspin. For those who like the menu approach, most of the commands we will discuss can be found in the appropriate drop-down menus from the menu bar. For those preferring the point-and-click approach, there are interactive buttons on the main toolbars and in the toolbars in the subroutines we will discuss below. For those preferring to type commands at the command line, there are commands available for most, if not all of the functions we will discuss. The typed commands can be found next to the selections from the menu bar or in the popup information balloons when you rest the mouse over a button. The typed command is found in brackets and you may find this to be faster as you become more proficient with the software. All paths work equally well, so use what you like.

Transforming the FID to a spectrum

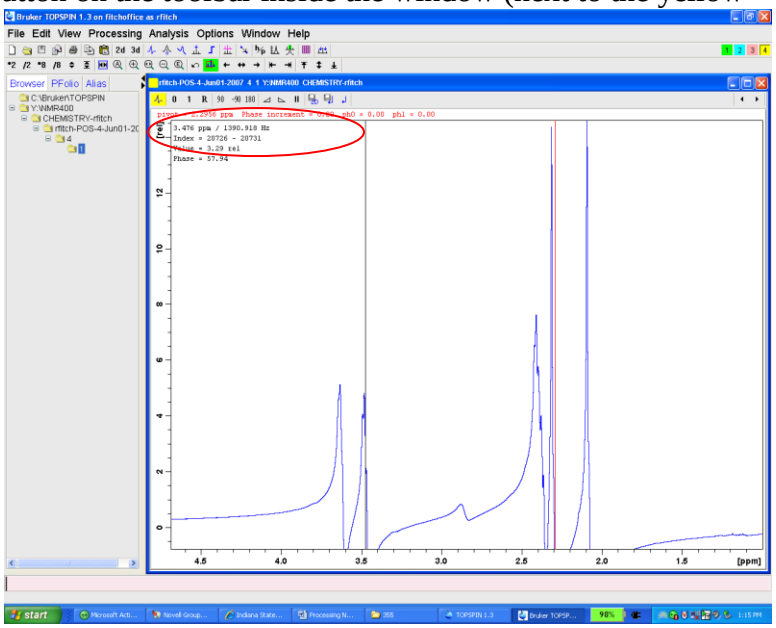
Most often, you will have a spectrum stored, but occasionally you will have only an FID. If this is the case, then the spectrum window will say "No processed data available. Raw 1D data available." In this case, you need to fourier transform the spectrum. There are several ways to do this, but the most straightforward method is to type **efp** at the command line. Efp is a combination of three commands (**e**xponential multiplication [em], **f**ourier transform [ft], and **p**hase correction [pk]). Often the spectrum will be out of phase (positive and negative peaks) after efp. This is because the computer takes whatever values are in memory for the phase and applies them. If this is the case then type **apk** (**a**utomatic **p**hase **c**orrection) to fix the problem.

Manipulating the spectrum.

The spectrum view may be manipulated with any of the buttons in the bottom row (above, circled).The best way to get used to using them is simpy to try them out. All of the toolbar buttons have balloons that tell you what they are. Also as you pass over them, the button definition will show up at the bottom of the window below the command line. However, a few buttons are of note. The vertical double arrow fifth from left is a continuous scaling. All of the double arrow buttons behave this way. The horizontal scale (magnifying glass with double horizontal arrow) zooms in and out by clicking and dragging side-to-side. Likewise with the horizontal shift button (seventh from right). If you make a mistake on scaling you can hit the magnifying glass with the A inside to show the entire spectrum and rezoom, or simply hit the undo button (curved arrow) to back up by one expansion.

Phasing your spectrum

Most of the time, apk does a pretty good job at phasing. However, sometimes you will want to manually phase the spectrum because you need a very accurate integration or because apk failed to give an acceptable spectrum for some reason (This often happens with biphasic spectra like APT and DEPT135). To phase the spectrum, type **.ph** or select the interactive phase correction button (looks like an out of phase peak and is next to the 3d button on the upper row of the toolbar). This will open a window that looks like that below.The red line indicates the pivot point and is generally set on the tallest peak in the spectrum. Click and drag the **0** button on the toolbar inside the window (next to the yellow highlighted phase button) to change the zero order phase (phases all peaks equally). Next click and drag the **1** button and adjust the phase of the peaks according to their distance from the pivot point. At this point you should have a reasonable looking spectrum. If you go too far, you can develop a rolling baseline that does not behave correctly. If this happens, simply click on the **R** button to reset the phase values and try again. Also, if you retry apk, this will sometimes clean things up to where you can work with them again. When you are finished, leave the routine by clicking on save and return (the button with the enter symbol and the little floppy disk, second from right).

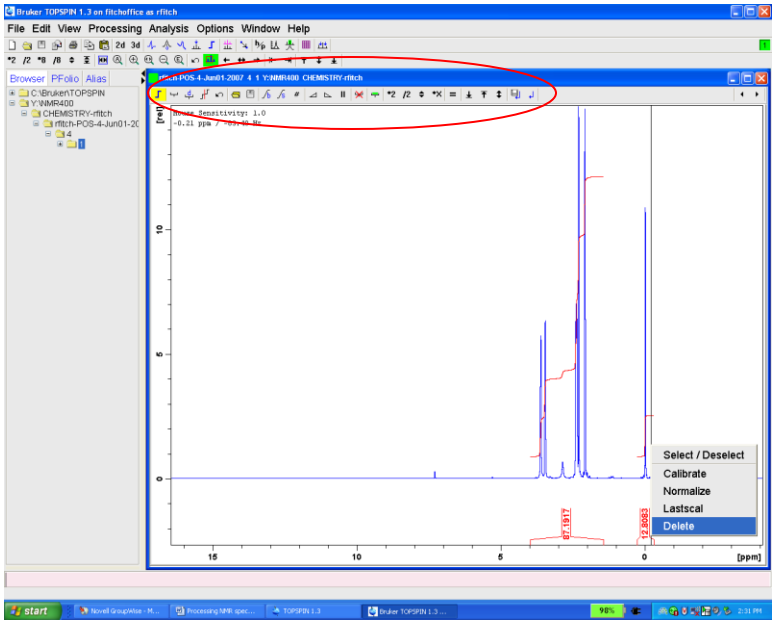


Setting the reference

If you have TMS in your sample, you can simply type sref (set reference) at the command line. This will calibrate the peak to 0 ppm (Caution: If you have other peaks close to zero, you can inadvertently pick the wrong one. This commonly happens with other silicon derivatives such as silyl ether protecting groups.). If you wish to calibrate the chemical shift to another peak, say $CDCl_3$, simply expand and select the peak of interest. Then click on the calibrate button (next to the phase button, looks like a peak with a zero underneath it). Then click on the center of the peak you wish to reference and type the chemical shift into the window that appears.

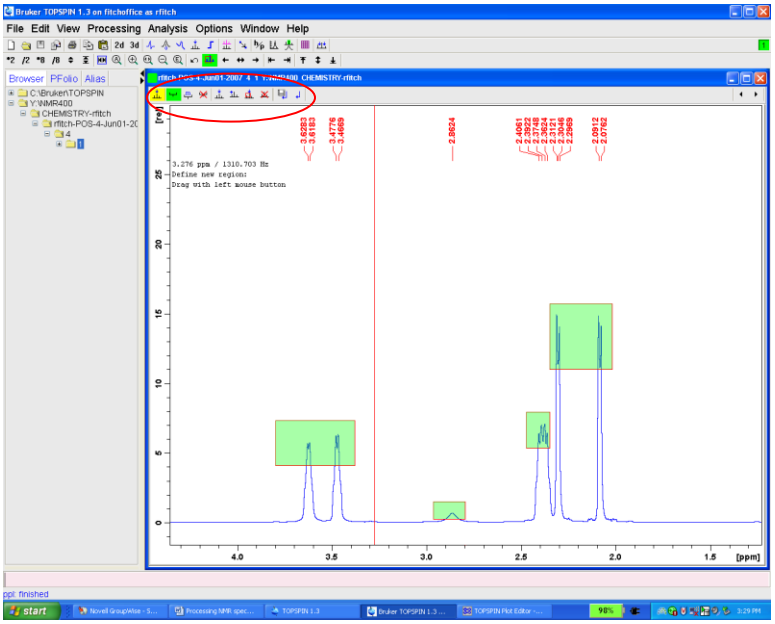
Integration

To integrate your spectrum, there are two options. The one which I prefer is to type **abs** (auto baseline) at the command line. This both corrects the baseline to flatness and integrates the spectrum and is the routine that generates the integrals in automation. However, this often does not separate peaks the way we like. Nonetheless, it is a good first step. Next type **.int** on the command line or click on the button that looks like an integral on the top row of the toolbar to enter the interactive integration routine. You will see a window that looks much like below. The routine opens an interactive window similar to the phase routine. There are more buttons on the toolbar and again there are popup balloons to show the functions of the buttons. You will notice there are similar display buttons on the right side of the bar to those on the main toolbar. The buttons on the main toolbar manipulate the specrum while those in the integration window manipulate the integrals. You will notice that the abs routine has integrated the TMS peak at 0 ppm. Since we do not need to know the integration of this peak, we can delete the integral simply by right clicking on the integral as shown and selecting Delete from the menu. You will also notice that the rest of the significant peaks have been integrated as a whole. While this is acceptable and the individual integrations could be sorted out with a ruler, it would be more convenient if we could integrate them separately. This can be done by clicking on the split integral button on the toolbar in the window (third to the right of the yellow integration symbol with the vertical red line through the integral). The button will turn green to indicate it is active. With this button selected, we simply move the mouse between the integrals we want to split and click. The integral will be split at that point. In order to turn the function off, simply click on the button again. If you make an error, you can click on the undo button in the window. If you wish to add a new integral, you first click on the define region button (button with a bracket next to the yellow button). Then click and drag left to right across the multiplet you wish to integrate and it will appear (the button must be active or you will simply expand the spectrum about your multiplet). To define an area for an integral, simply right click and select Calibrate from the menu that appears and enter the area you desire. When you have finished, click the save and exit button. If you have made errors so severe that you feel you cannot recover from them, simply hit the exit without saving button and then go back into the routine and start over.



Peak Picking

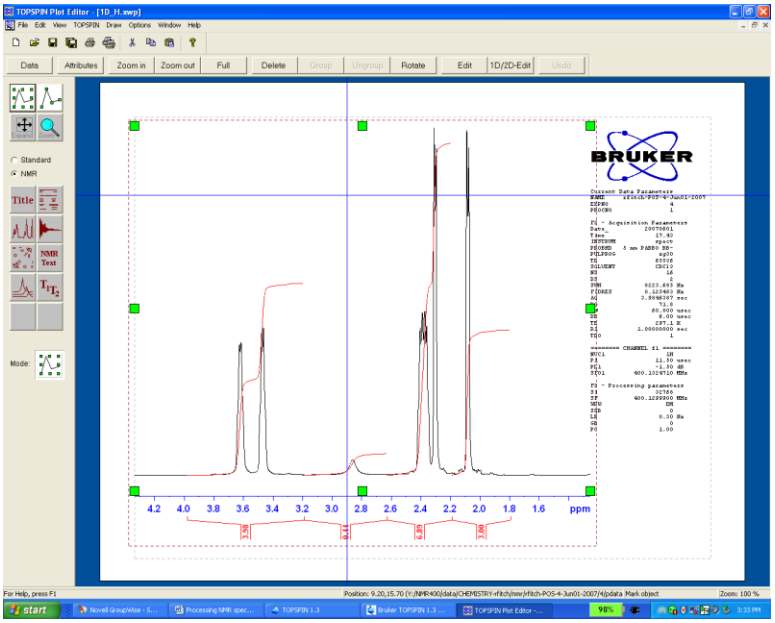
Peaks are identified in the abs routine as described above under Integration. However, often one wants more or fewer peaks than are identified with this routine. There are two ways of changing the number of peaks. One is with the **mi** command which establishes the minimum intensity that can be read from the y-axis on the spectrum. Thus for the example spectrum above, specifying mi above 1 will exclude the singlet at 2.75 ppm and above 6 will exclude the two signals at 3.6 ppm. This is followed by the **ppf** command for peak picking the full spectrum. However, often one wants to be more precise and this is done in the interactive mode by selecting interactive peak picking by clicking the button with the triplet and the arrow from the top toolbar (next to the integration button) or by typing **.pp** at the command line. This will give the window shown.

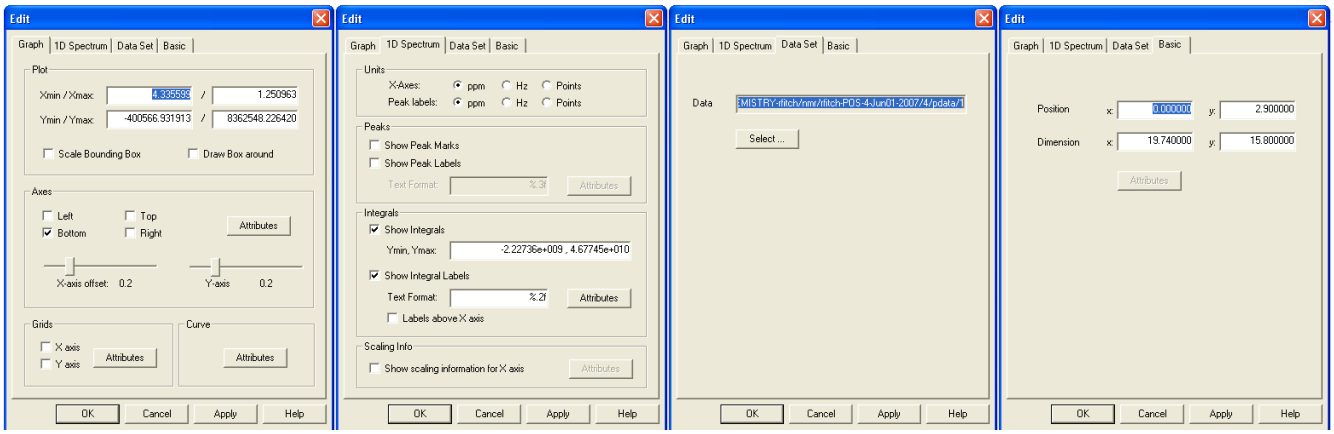


You clear all peaks by right clicking inside the spectrum and selecting Delete All Regions, or by clicking on the Delete all peaks button on the toolbar. From there you can select peaks by clicking on the select button on the toolbar (shown in green next to the yellow peak picking button). You can then drag a window across peaks to peak pick them. It is helpful to expand your regions of interest with the display buttons on the main toolbar and to reduce the scale to enable peak picking for the tallest peaks in the spectrum as shown. When you are satisfied with the results click on the save and exit button.

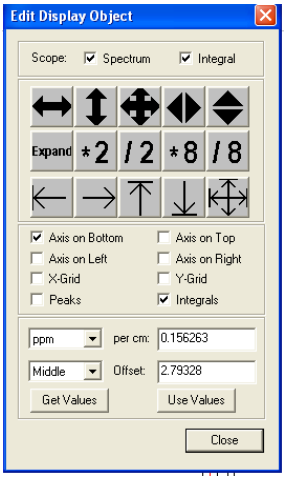
Plotting 1D spectra

Plotting your spectrum can be done a number of ways. Automation uses the **autoplot** command. However, this automatically reprocesses your spectrum and will not generally give the result you want. A more useful approach is to use the plot editor. When you click on the print button on the main menu, a dialog box will open giving you a set of options. Select Print with layout – start plot editor, or simply type **plot** at the command line. This opens up the plot editor as shown. Clicking on the spectrum highlights it and activates several buttons across the top. The two of greatest interest are the Edit and 1D/2D-Edit buttons toward the right. Either double-clicking on the spectrum or clicking the Edit button open a routine that allow manual setting of the plot region. The routine has several tabbed screens that will allow you to select the axis units, change the file or resize the plot as shown below.





The Edit routine is excellent for setting the x scaling and the items described above, but not particularly good for rescaling in the y-direction. This is much better done using the 1D/2D-Edit routine. Clicking on the button brings up a different window as shown at right. This routine has most of the buttons you are used to on the main toolbar. However it is not easily adapted to handling fixed ppm regions, say for comparison purposes. However, the ability to smoothly scale spectrum and integrals together or separately makes up for this shortcoming.



Insets. It is sometimes desirable to do an inset of a multiplet when the spectrum is overly congested. In this case, one can highlight the window by clicking on it and copy and paste either using <ctrl>+c and <ctrl>+v or right clicking the mouse and selecting copy and paste. This will paste the existing window over itself, so it will appear that nothing has happened. However if you click on one of the green border boxes and drag them over, you can resize the window to create an inset. The inset window can be manipulate in the same way the original window was with the Edit and 1D/2D-Edit routines to expand the area in which you are interested. Below is a spectrum which is shown in the usual proton range of 0-10 ppm with insets showing the peak picking for the individual multiplets and was done by simply making an inset, followed by turning on the peaks in the 1D/2D-Edit and resizing the peak region to fit the window. Much of this requires simply playing around with the routines until you get what you want, but between Edit, 1D/2D-Edit, and resizing the windows by dragging borders, you can be as simple or as elaborate as you wish. When you are satisfied, simply plot using the print button (not the multiple print). When finished simply close the window and choose No when it asks to save changes.