MestReNova Manual for Chem 201/202. January, 2020.

1. Introduction to 1-D NMR Data Processing with MestReNova

The MestReNova program can do all of the routine NMR data processing needed for Chem 201 and 202 and will be available through the Reed downloads site or on the computers in Chem 203. This manual covers basic ("1-D") data processing for ^{1}H NMR and guides you through a typical operating session.

2. What does an NMR spectrum look like?

The following spectrum is typical (Fig. 1).

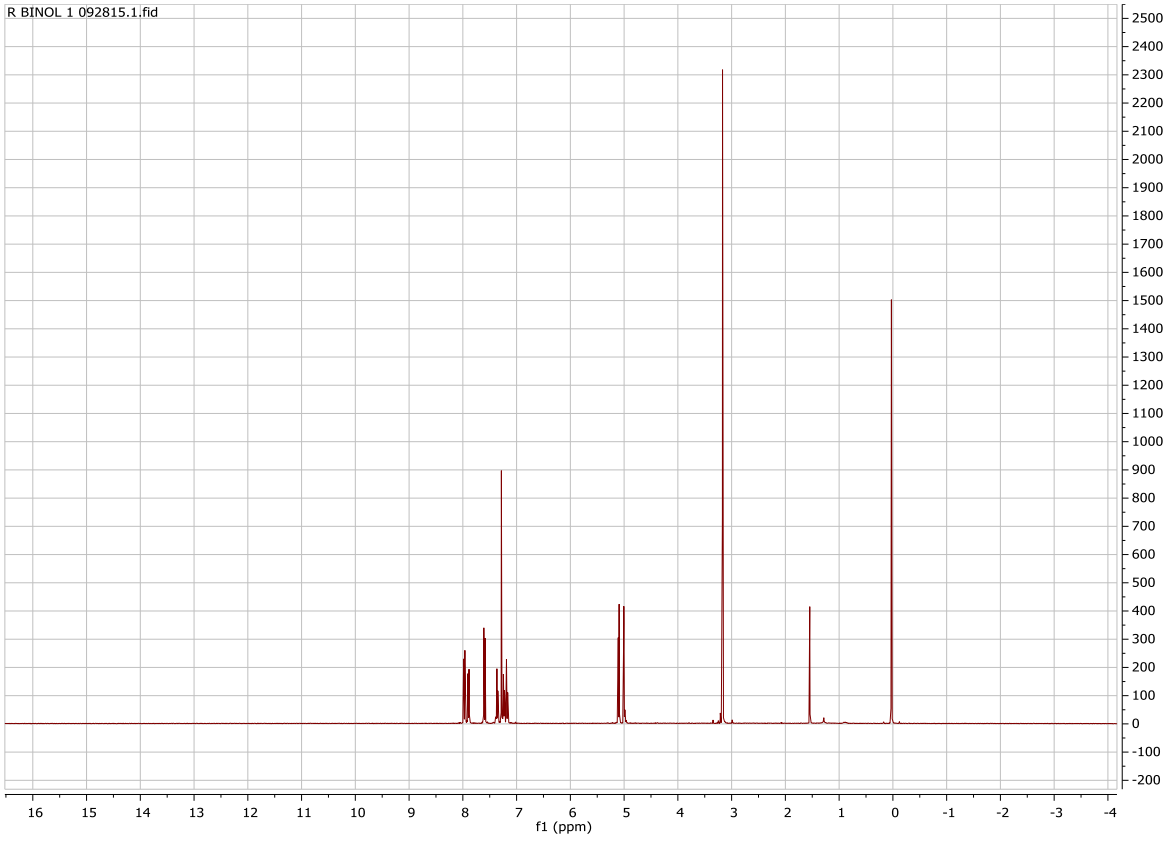


Figure 1

It contains several groups of signals separated by stretches of horizontal baseline. A chemist can extract information about molecular structure by noticing where signals appear, and also by noticing where they don't. Because 'absence of signal' is just as important as 'signal', it is essential to record and print a "full" spectrum, one that runs from -0.5 to about 14 ppm.

Another typical thing: the spectrum is extremely hard to read and not useful in its present format. We could correct this by (Fig. 2):

- printing the spectrum on a larger piece of paper
- zooming in on, or “expanding” selected regions of the spectrum.

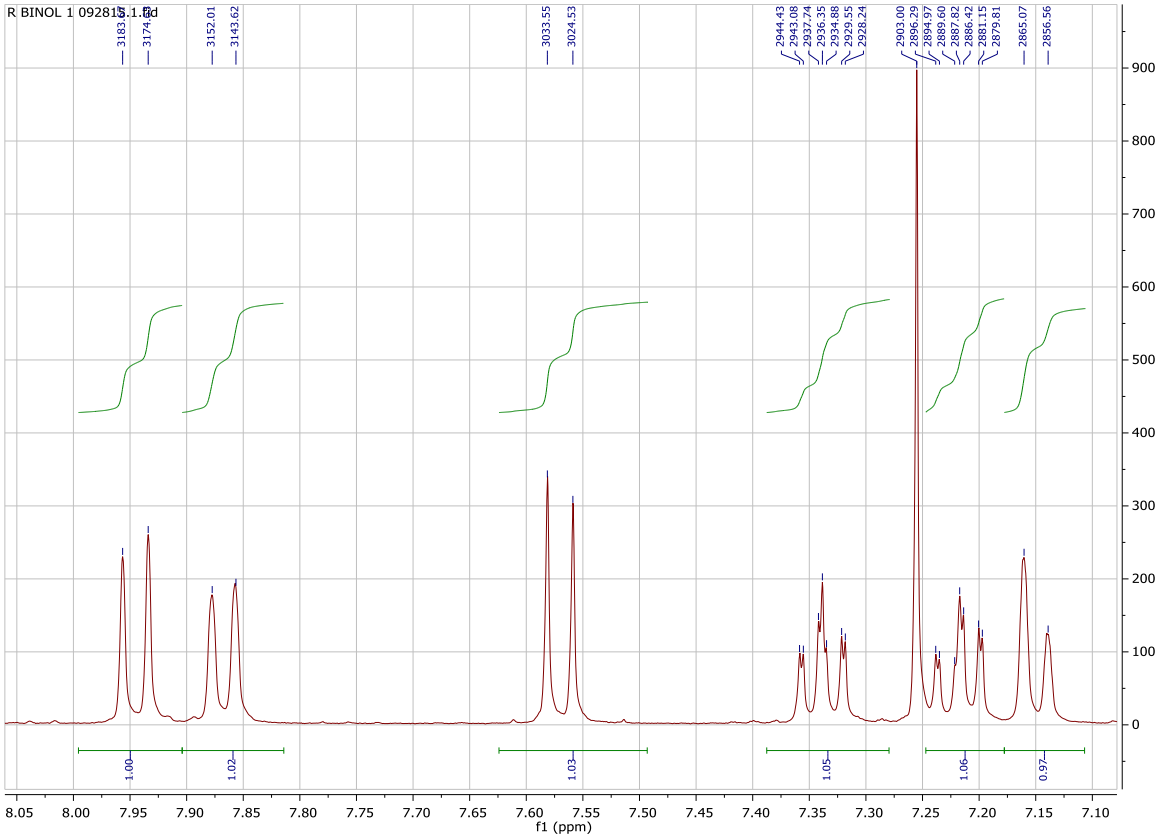


Figure 2

A complete “expansion” might show the shape of the signals, their chemical shifts (in ppm, scale shown on x-axis), integrals (rising green curves, blue numerical values below each set of peaks), and the exact frequency of each peak (in Hz, blue values above peaks). This manual shows you how to print a “full” spectrum and then how to create expansions like this one. To do this, you will need to:

- download and process your data to make a spectrum
- control which part of the spectrum is displayed (full vs. expansion)
- analyze the spectrum by integrating and “peak picking” (getting frequencies of individual peaks)
- print the spectrum

The description of each set of operations starts on a separate page so you can quickly find a desired set of instructions by flipping pages. The first time, however, you should go through the full menu without skipping any pages.

3. Downloading and processing your data

Your data is stored on a computer attached to the NMR spectrophotometer. It is stored in a read-only folder and you can download this folder as many times as you like. In the unlikely event that data processing makes a mess of your data, simply download a fresh copy of this folder.

1. Click on the NMR server icon

on the desktop.

2. Navigate to the folder containing your NMR data (usually Chem201labday).

3. Plow down into this folder until you find a folder labeled EXP.DAY.INI (or whatever file format you used) and drag this entire folder to the Desktop or to My Documents to save a copy of the data.

4. Close the NMR/data explorer window.

5. Double-click the desktop icon for MestReNova

or open from the programs in the start menu.

6. Drag your entire NMR data folder into the MestReNova window to load your data (your spectrum will be set up similar to Figure 1). An alternative way to open your data:

   a. Under File, choose Open (Or Ctrl+O)
   b. Select the fid file from your saved NMR data folder (NMR folder → 1 → fid)

Note: FID refers to “free induction decay” which is a signal vs. time spectrum and this is how your raw data looks when it is transferred from the NMR (Fig. 3). MNova automatically processes your data and applies Fourier transformation (FT), which changes your data to a signal vs. frequency (chemical shift) spectrum.

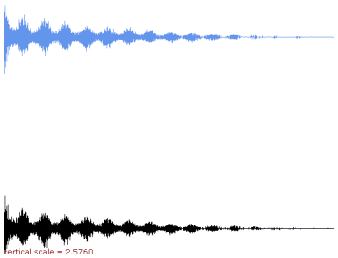


Figure 3

To obtain a copy of MestReNova for your own computer. In any web browser, go to **downloads.reed.edu.**

   a. Click on Mnova
   c. Download the Mnova license
   d. Open MestReNova via the start menu under “programs”
   e. When prompted for the license file, select the license file
   f. Restart Mnova

4. Controlling the display

What you see in your display can be controlled by a variety of vertical and horizontal adjustments. Try each of the adjustment tools listed below. Once you have tried them, restore the full spectrum.

Full Spectrum (Shortcut: Type F)
Clicking on this button will restore your full spectrum, so will typing F

Pan (Shortcut: Type P)
Left click on your spectra, hold, and drag. Allows you to access different parts of your spectrum by dragging.

Fit to Highest Intensity/Fit To Highest Compound
Will adjust the relative height of all of your peaks at once. Useful when printing.

Zoom In (Shortcut: Type Z)
Either left click once near where you want to zoom OR hold the left mouse button and select a region. Usful when integrating, peak picking, printing.

Peak Height Adjustment
Increase/Decrease peak height respectively (Can also do this by scrolling the wheel on your mouse)

Zoom Out (Shortcut: Hold Shift + Z)
Left click (as many times as you need to) near where you want to zoom out

Manual Zoom
Allows you to set the horizontal display range of your spectrum.

Expansion (Shortcut: Type E)
Allows you to create an expansion of one particular region of interest in your spectra. Left click on the left side of the region of interest, hold, then select. A new window will appear with the selected region which you can adjust independently of the original spectrum

5. Phasing (& Baseline correction? Do we want them to do this?)

The phasing adjustment affects the rise and fall of the baseline on each side of your signal. MestReNova attempts to make this adjustment for you *automatically* when you initially process your data, but you may need to make additional adjustments. The following diagram (Fig. 4) shows the same set of signals with "bad" and "good" phasing. Notice the difference in the rise and fall of the baselines.

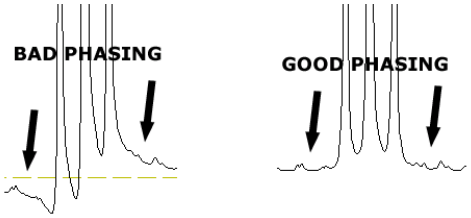


Figure 4

Inspect the baseline around each of the signals in your spectrum. If the baseline around *all* of the peaks looks acceptable, proceed to **6. Calibrating** on the next page. Otherwise, additional phasing is required.

1. Click the Phasing button to adjust your spectrum
2. Select "Automatic Phasing" from the drop down menu
3. If your spectrum is still out of phase following this, select the "Manual Phasing" option from the menu
4. Follow the window instructions to adjust **ph0** and **ph1** ("zeroth-order" and "first-order" phase controls, respectively) and then close the window. These sliders have very different effects.
   a. **ph0** – These sliders affect the entire spectrum in the same way. Adjust them so that the baseline around the *tallest* signal looks acceptable (a thick colored vertical bar underneath the spectrum marks the location of the *tallest* signal).
   b. **ph1** – These sliders have little or no effect near the *tallest* signal, but their effect grows with increasing distance from this signal. Adjust them so that the baseline looks acceptable around signals that are *far* from the tallest signal.

6. Calibrating

Chemists use the signal provided by TMS (tetramethylsilane) to calibrate the chemical shift (horizontal) scale of an NMR spectrum. The TMS signal usually appears further right (upfield) than any other signal. The chemical shift of the TMS signal is arbitrarily set to 0.0 ppm (parts per million). If the solvent does not contain TMS, use the residual solvent peak to reference.

1. Zoom in on the TMS signal. You may need to do this a couple of times so that the signal looks quite broad (Fig. 5). If the solvent does not contain TMS, zoom into the residual solvent peak for the NMR solvent. This is approximately 7.26 ppm for Chloroform-*d*.
2. Click on the calibration tool button in the MestReNova menu (**Shortcut: Type L**).
3. Move your mouse cursor over the peak, and you should see a red line appear at the center of the peak, left click.
4. A box will appear titled “Reference along f1” (Fig. 6)
5. For TMS, manually enter 0 or alternatively click on the “Solvents>>” button in the lower right of the box and select TMS from the list. For Chloroform-*d*, manually enter 7.26 or select Chloroform-*d* from the solvent list.
6. Restore the full spectrum display (**Type F**).

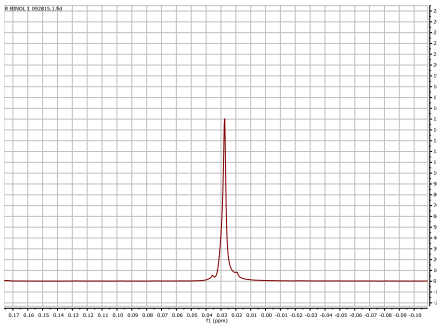


Figure 5

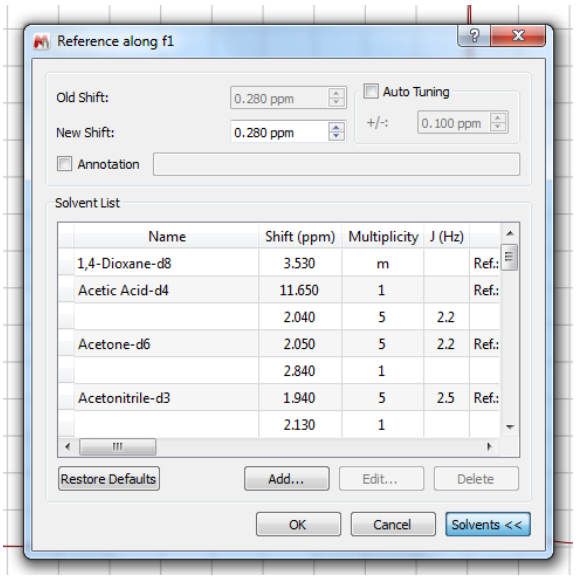


Figure 6

7. Integrating

Integrating the spectrum means finding the *area* underneath the peaks that interest you. This peak area, or *integral*, is proportional to the number of nuclei that create these signals. Therefore, an integral is useful only in comparison to another integral. Furthermore, only the *relative* integral size is meaningful; two integrals with values of 1 and 4 mean exactly the same thing as two integrals with values of 0.2 and 0.8. Remember, only integrate the signals that interest you. *Do not integrate contaminants* like TMS or the signals produced by solvent(s).

Reed College NMR Spectrometer disclaimer: The NMR spectra for Chem 201 & 202 are gathered in a way that can easily distort the values of integrals, making them artificially too large or too small (peak size distortion is an artifact of our "fast scan" procedure). These distortions need not affect all of the integrals in the same way and distortions of 10-20% are routine.

Integration involves three steps:

1. Proper phasing. We will assume this has been done already.
2. Selecting integration regions. Generally, a region should contain all of the signals produced by *one* type of hydrogen.
3. Calibrating the value of one integral.

The detailed instructions, beginning with “selecting integration regions,” go like this:

1. Try to parse your spectrum by identifying signals that look like they come from a single type of hydrogen. Pay attention only to those signals that might be produced by your compound. Ignore contaminants like TMS, $CHCl_3$, and H_2O.
2. Zoom into the peak of interest
3. Click on the triangle symbol to the right of the integration tool button in the MestReNova menu to access the dropdown menu. Select Manual (**Shortcut: Type I**).
4. Click the baseline on the left of the peak, and drag the pink highlighting box to the right side of the signal pattern to select it (Fig. 7). This will produce an integration curve and the value of the integral underneath this curve will appear below the signal.
5. Move the spectrum horizontally to the left until you find the next pattern and repeat step #4.
6. When all of the integrals have been marked, calibrate one integral by selecting it, then right clicking, and selecting “Edit Integral” from the menu that appears.
7. A box will appear titled “Integral Manager” (Fig. 8)

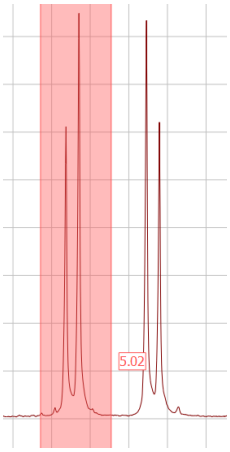


Figure 7

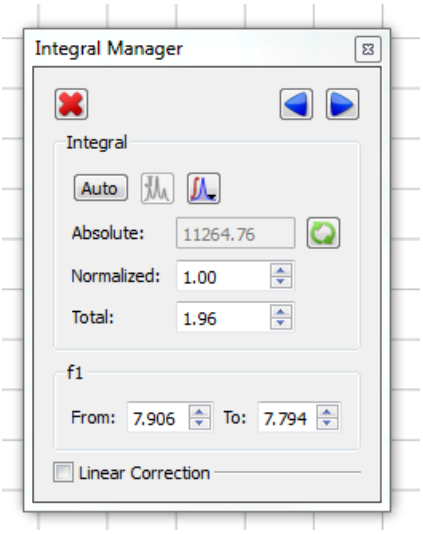


Figure 8

8. Type the value you would like to assign to the integral in the “Normalized” slot, close the Integral Manager window. You should notice that the values of all the other integrals are reset, not just the integral you selected. Remember, all that counts with integrals are their relative values. Values of 0.634 and 1.902 have exactly the same meaning as values of 1.000 and 3.000. That said, the latter are easier to use.
9. To adjust the green integration curve (optional), hover your mouse cursor over the curve, a red box should appear selecting it, then use the green boxes to adjust vertical height of the curve. To adjust positioning above the peak, left click, hold and drag the box (Fig. 9).
10. Restore the full spectrum display (**Type F**).

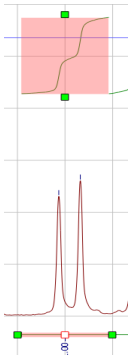
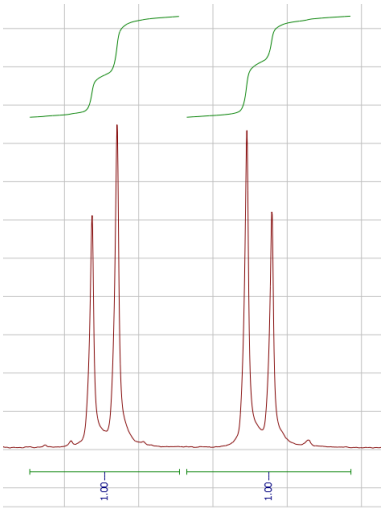


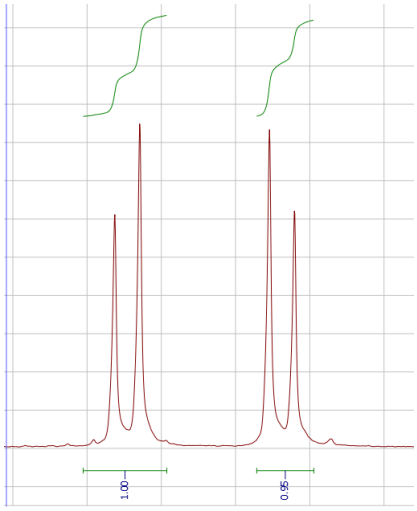
Figure 9

Note: The most common student mistake is to set the edges of the integral on the sloping curve of the signal instead of the flat baseline (Fig. 10). Notice that the bad integral starts and stops on the signal, not on the baseline. The good integral runs from baseline to baseline. Not only that, it encompasses enough baseline on each side of the signal so that the “flat” character of the baseline can be verified. If your integral is too narrow, either select one or both of the green boxes at the edge of the integral to widen it, or delete it by moving your cursor over either the green integration curve above the peak or over the integration value (it will become highlighted), then right click and select ‘Delete Integral’ from the menu. If you want to delete all the integrals select ‘Delete All’.

Figure 10



Good Integrals –
Regions run baseline to baseline



Bad Integrals –
Integral regions are too narrow

8. Peak picking

The most reliable way to obtain coupling constants (*J* values) is to measure the distance between signals ("peaks") in Hz. Peak picking prints the frequencies of the selected peaks above those peaks. The tops of the peaks are marked with small vertical lines. Peak picking is applied only to the peaks that 1) are currently visible in the window, and 2) rise above a threshold value so peak picking requires the following steps:

1. On your spectrum zoom into the signals of interest (and *only* these signals) into the window.
2. Click on the triangle to the right of the peak picking tool button in the MestReNova menu to access the dropdown menu. Select Manual Threshold (**Shortcut: Type K**).
3. Left click to the side of the peaks you want to pick, and then drag the pink box over the tops of all the peaks (the box you create is the "threshold" and peaks below this box will not be selected).
4. Repeat steps 6-8 for different signals of interest.
5. To delete a single peak, move your cursor over the peak label to select it (it will turn red), then right click, and select "Delete Peak" from the menu that appears. To delete a group of peaks, click on the peak picking dropdown menu, and select "Delete Manually", then left click and drag the purple highlighting tool over the peaks you would like to delete. To delete all the peaks, select "Delete All" from the dropdown menu the peak picking dropdown menu.

Final spectra in chemical journals or lab reports are typically displayed with peak labels in ppm. This is the default setting for peak labels in MNova. However, if you are calculating *J* values it is much easier to temporarily convert them into Hertz.

6. To display the peak labels in Hertz, under "Edit" select "Properties…" or right click on the spectrum.
7. A box will appear (Fig. 11).
8. On the left hand side under the "NMR Spectrum" tab select "Peaks".
9. Then on the right hand side, where it says "Units:" select Hz from the dropdown menu.
10. Click "Apply" then "Ok" to close the window.

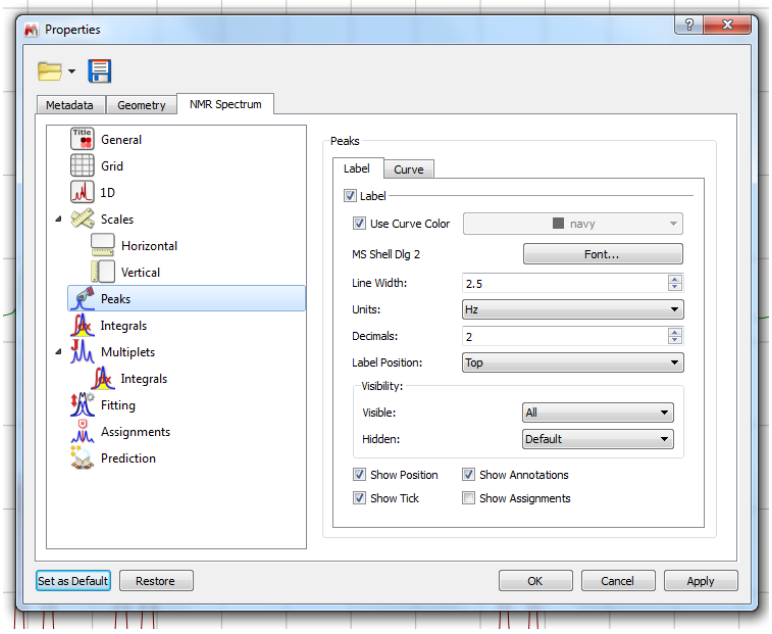


Figure 11

9. Saving your data

Once you have loaded your spectrum and fully annotated it, you will want to save your data. I recommend saving two versions.

1. To save a copy of your data that you can reopen in MestReNova and edit further without losing any changes you've already made annotating (e.g. integrals, peak picking, etc.) save as a .mnova file extension. To reopen this document, the computer you are using will have to have MestReNova installed on it. Remove the .fid part of your file name before creating the file. To do this, Select "File" then "Save As...".

   Tip: Spectra can also be directly copied and pasted from the MestReNova program into a word document for example by selecting, then copying and pasting.

2. To save a copy that you can open on many devices save as a .pdf file extension. To do this, Select "File" the "Save as…". Select pdf from the available file extensions before you save.

10. Printing a full spectrum

A proton NMR spectrum will contain signals from every hydrogen-containing compound in your sample. A full spectrum, one that displays chemical shifts (horizontal scale) from -0.5 to approximately 14 ppm, will display all of these signals, both the signals that you think your compound has produced and also the signals produced by contaminants.

Organic chemists normally expect to "interpret" every signal in an NMR spectrum. Conversely, they expect to find a signal for every hydrogen nucleus in their sample. The assignment of signals to nuclei, and of nuclei to signals, means finding a satisfactory interpretation for every signal's chemical shift, integration, and coupling pattern. A full spectrum reveals all of the signals and allows an unbiased observer to consider alternative interpretations of the NMR data.

Printing note: Although there is a printer in the Chemistry computer lab, it is strongly recommended that you print your spectra as PDF files, save these files in a secure location, and then print these files on another printer or copy the image directly into your lab report.

3. Click the Manual Zoom button to adjust horizontal display. Set the range from: -0.5 to 14 ppm.
4. Adjust the vertical scale so that tallest signal fits on screen using the mouse wheel or by clicking on the Fit To Highest Intensity button .
5. Select "Edit" then "Edit Page Title…" and enter a descriptive title. Useful title components include: sample, NMR solvent, your name, date, and **EXP.INI.DAY** folder label. Click "OK" to close window.
6. Create a .pdf file. Select "File" and then "Export to PDF…" or "Save as…" a pdf. If necessary, select "File" then "Print" to find the printer named **Microsft Print to PDF**, and print your spectrum. You will be asked to provide a name for your PDF file and a location where you want it saved.
7. Find your PDF file and open it. Inspect the *horizontal* scale (-0.5 to 14 ppm?). Inspect the *vertical* scale (Peaks large enough? Not too large?). Inspect the *paper alignment* (Landscape mode?). If everything seems satisfactory, go to the next page. Otherwise, make corrections and print your spectrum again.

Printing in the Chemistry computer lab. It is, in fact, possible to print on the printer in the computer lab and this could save you time in an emergency. That said, your instructors are not responsible for maintaining this printer and it seems to work wonderfully for long stretches and then behave very poorly for equally long stretches. Caveat printer. The time and money you waste on this printer is *your own*. If you want to try it, here's what you must do:

1. Log in to the printer (printer name: **Clab**).
2. Open your PDF file and print it using the **Clab** printer (keep your fingers crossed).
3. Don't forget to *log out* when you are done.

11. Printing expansions

Once you load up a spectrum with integrals and peak picks, you will want to print your data. Unfortunately, a full spectrum will cram all of this information into a small space rendering it unreadable in most cases (see pages 2-3 above). A better alternative is to expand regions of interest so that the integrals and peak picks in these regions can be read clearly.

1. Zoom in on the chemical shift region of interest (**Type Z, then click and drag to highlight the region**).
2. Adjust the vertical scale so that tallest signal fits on screen using the mouse wheel or by clicking on the Fit To Highest Intensity button .
3. Select “Edit” then “Edit Page Title…” and enter a descriptive title. Useful title components include: sample, NMR solvent, your name, date, and **EXP.INI.DAY** folder label. Click “OK” to close window.
4. Select “File” then “Export to PDF…” or alternatively “Save As…”. Create a .pdf file. Remove the .fid part of the filename before saving. If necessary, select “File” then “Print” to find the printer named **Microsoft Print to PDF**, and print your spectrum. You will be asked to provide a name for your PDF file and a location where you want it saved.
5. Find your PDF file and open it. Inspect the *horizontal* scale (The region you intended?). Inspect the *vertical* scale (Peaks large enough? Integral and peak pick data easy to read?). Inspect the *paper alignment* (Landscape mode?). If everything seems satisfactory, expand and print another region by repeating steps 1-5.
6. Another way of including an expansion is to create an expansion window and place it in the full spectrum. To do this click on the Expansion tool (**Shortcut: Type E**), then select the region of interest. A secondary window of that region will appear, which you can select and independently edit from the rest of your spectrum (Fig. 12). Avoid having more than one expansion window in your spectrum or it will look crowded.

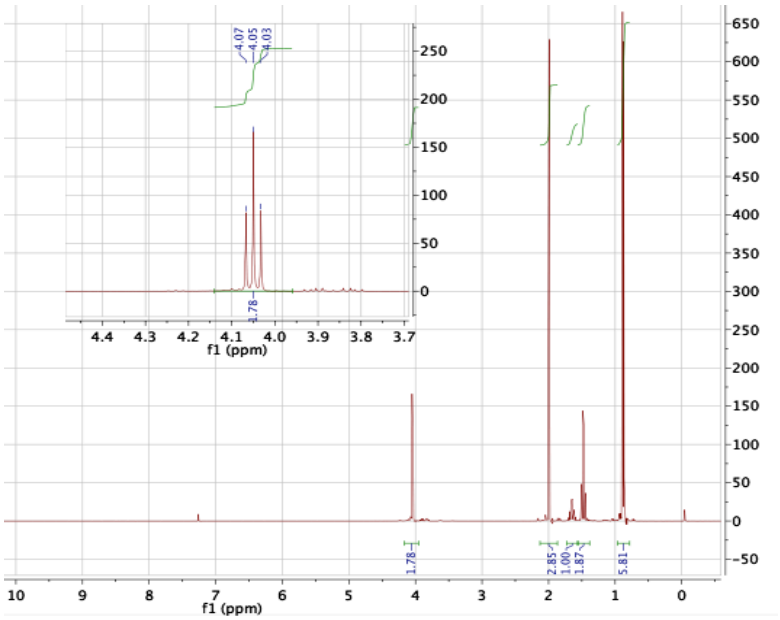


Figure 12