

Data workup using MestreNova (or MNova)

This laboratory introduces the basic processing of one dimensional NMR data using the MestreNova software package. Throughout Chem 636 we will be using MNova to process NMR data. The primary goal of this HW is to gain familiarization with standard methods used to process NMR spectra. The MNova Quick Guide will be helpful initially in learning to use the software. Mestrelabs also has excellent documentation available at their website. The spectra needed for this assignment can be found in the **S:\Chem636-Fall2014\HW1\data** directory.

Install MNova onto your computer using the help guide on the NMR facility website. Connection to the Chemistry network via hardwire or the ChemVPN is required for the license authentication. See the links for both on the facility homepage: <http://www.chem.wisc.edu/~cic/nmr/main.html>.

I. Data directories used throughout the semester:

- On the Windows PCs in the facility, we have standardized server mounts:
 - **R:** is the *Apollo* ftp server, which backs up data from our older spectrometers, a Bruker AC-300 (Athena, introduced next week) and a Varian Mercury-300 (Hermes, later in the fall)
 - R:\ac300\athena** holds the data from Athena (300MHz – Bruker AC).
 - R:\hermes** holds the automation data collected on Hermes (300MHz – Varian Mercury) but for only 10 days. After that the data is moved to **R:\hf\group(chem636)\username\hermes**
 - **Q:** is the *Castor* server, which backs up data from our new Bruker Avance spectrometers, a 400 (Artemis, in 2 wks), and two 500s (Persephone and Callisto, wks 4 & 5).
 - Q:\username\public_html\av400** has data from Artemis (400MHz)
 - **S:\temp** may be used to temporarily store data (deleted on a monthly basis).
 - S:\Chem636-Fall2014\HW** holds data needed for specific homework assignments.
- The facility will do its best to archive your data. But for research, it is *your* work that is at risk. You should always back up your data to your own computer, flash drive, CD... regularly!!!
- From home: Apollo is an ftp server (**ftp:\\apollo.chem.wisc.edu**). See facility staff to get the username & password to access this server. Castor is accessed only via secured **sftp** or drive mounting. See the NMR facility homepage for information on how to connect to these servers.
- The MestreNova website is searchable and good at providing useful information. Manuals and tutorials are available at their website: mestrelab.com/resources.

Note: Other software products that tie into MNova are not separated or identified very well at the Mestrelab website, in particular those that do NMR spectrum predictions called NMRPredict and Verify. [This appears to be classic case of product teasers.] We do not have licenses to these products, so tutorials and guides directed toward them will not be useful.

II. Standard processing of 1D NMR spectra

Five steps are always involved in working up 1D data:

- i) **Zerofills:** The data points of the *free induction decay* (FID) have zeros added to end until filled out to the closest 2^n (if the FID is exactly at 2^n , nothing is done): 2^{14} , 2^{15} , or 2^{16} are typical values for 1D data sets. E.g., if the data are acquired to 16000 points, the *Fast Fourier Transform* (FFT) algorithm requires that zeros are added to $2^{14} = 16384$. This procedure is automatically performed by all FFT software.

One to two zerofills will usually be applied to improve the quality of the resulting spectrum. The initial fill as described in the previous paragraph is not counted as a *zerofill*; it is just part of the FFT requirement. The first zerofill would then, in the example above, add another 16384 zeros to the end of the FID. A 2^{nd} zerofill would add another 2×16384 zeros, resulting in a total number of points in the FID equaling 4×16384 or 2^{16} .

- ii) **Apodization:** The FID is usually multiplied by a mathematical function, and all have positive and negative attributes. A balance must be chosen between these attributes, as discussed in lecture. For now, we will use a standard method by applying an exponential *matched filter* to ^1H 1D spectra (although MNova's implementation of a Gaussian matched filter is preferred):

$$\text{matched filter: } lb = \frac{1}{AQ}$$

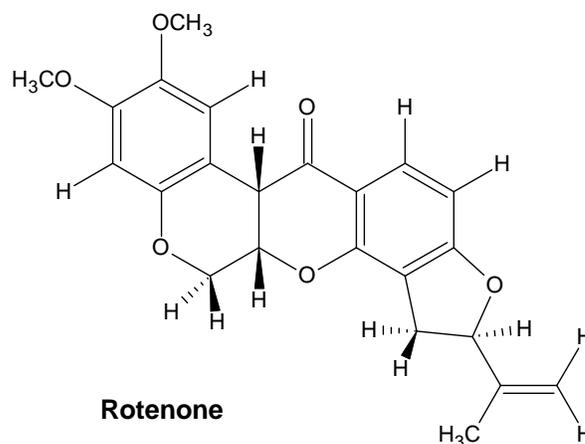
where lb = the constant in the $\exp(-lb \times t)$ function multiplied to the FID, and AQ is the acquisition time used to acquire the data. lb is perhaps best understood as a linebroadening: whatever the linewidth, $\Delta\nu$, of a peak would be in your spectrum without apodization, it will become $\Delta\nu + lb$ following application of the matched filter.

- iii) **FFT:** A Fourier transform is performed utilizing a fast Fourier transform algorithm. Most software performs this step using the command FT (as on all Bruker spectrometers); use the  icon in MNova. The spectrum will be composed of $\text{TDf} \times 2^{zf} / 2$ points, where TDf = number of points acquired filled to the next largest 2^n , and zf = number of zerofills. So in the example above with $zf = 1$ and $\text{TD} = 16\text{K}$, the spectrum size will be $16\text{K} \times 2 / 2 = 16\text{K}$.
- iv) **Phasing:** All NMR spectra must be phased to be positive absorptive. This topic will be discussed in lecture and labs. MNova performs an automatic phase correction, but for careful work, the automatic correction will rarely be sufficient and manual touch-up will be required.
- v) **Baseline correction:** To obtain accurate integrals, a baseline correction must be performed on the spectrum. MNova does not perform automatic baseline corrections, so these should be done manually (shortcut key B) on a routine, every-spectrum basis.

III. Exercises

1. Work up the FID named CGFROTH.001

- a) Open (ctrl-O or ) the data file named CGFROTH1.001. MNova automatically applies a fast Fourier Transform (FT or FFT), but no window function or baseline correction is applied. If you wish to see the FID, click on the arrow next to the  icon and choose 'original FID'.



- b) Zerofill the spectrum by clicking on the arrow by the  icon and choosing 'Zero Filling and LP' . The dialog box shows the number of acquired data points (Original size = 16384 or 16k for this data set) and it will automatically be zerofilled twice to 65536 or 64k. Zerofilling adds 'zeros' at the end of your FID and is used to increase digital resolution in the spectrum. (More than two zerofills will not help anything, and should not be used.)
- c) Phase correct the spectrum: MNova tries to phase correct for you, but doesn't do a perfect job. You will need to touch it up by *first* increasing the intensity using the middle mouse scroll button or  so that the TMS peak (right-most) goes to the top of the page. *Then* enter the phase correction mode by clicking on the arrow next to  and choosing 'Manual correction' (Shift-P). Change the 'pivot' to be either the left-most peak or the right-most peak (but not TMS) in the spectrum. Then with PH0 (zero-order phase) correct the peaks close to the pivot; ignore peaks far from the pivot point when applying a zero-order correction! Use PH1 (first-order phase) to correct the peaks on the opposite side of the spectrum.
- d) Zoom in on the TMS peak (the 'z' key will put your cursor in zoom mode, 'esc' to exit any mode) and click the TMS referencing icon  (L) to reference the TMS peak to 0.00 ppm.
- e) Peak pick the spectrum by choosing different peak picking options found under the  icon. The 'Manual Threshold'  option (K) is nice because it will let you set a different threshold on different regions. Use your best judgment here and don't pick the small contaminant peaks. (Note: this tool tends to over pick, i.e., it might pick 3 peaks where there is obviously only 1.)
- f) It is common to plot data to a standard spectral region. Display the spectral region exactly between 9.0 ppm and -1.0 ppm  and adjust the height so the tallest peak fits the page .
- g) One of the biggest problems we see with on-going research is poor documentation. We strongly recommend, therefore, that you get in the practice of properly documenting your data. Annotate this spectrum (T to enter text mode) with the following information:

the date

your name

homework number and plot number

the file name as saved (in this case CGFROTH1.001)

sample name and solvent (in this case Rotenone in CDCl₃)

how you referenced the spectrum. (TMS at 0ppm)

****For Chem 636, all plots of HW must contain this level of annotation. Failure to do so will result in reduction in the HW score.****

- h) Under 'file/page setup' make sure that the correct type of paper size is chosen (Letter) and that the orientation is 'landscape' position. You will submit this spectrum electronically. (**1st plot**)
- i) Select everything on this plot (ctrl-A) and then copy (ctrl-C). Create a new page by clicking on  and paste (ctrl-V) everything in here. Increase the intensity so that the TMS peak is halfway up the page. Reposition your annotation and leave in landscape orientation. [We can use this plot to better judge how well you phased and whether you did a baseline correction.] (**2nd plot**)

2. Work up the FID BIOTINH1.001 in a similar manner, but with more investigation.

- a) Open the spectrum and check that it has been zerofilled:



- b) Apply a 0.3 Hz exponential window function by clicking



on (W). You may see what each window function

does to your spectrum by checking the 'interactive' box in the lower right corner. We apply a mathematical function (or window function or apodization) to our NMR data to enhance sensitivity or resolution, or to remove artifacts. The 0.3 Hz exponential is called a '**matched filter**' because it equals the inverse of the acquisition time (2.7sec). The primary objective of apodization in ¹H spectra is to remove truncation artifacts (discussed later in lecture and lab).

- c) Touch up the phase correction as in 1c .

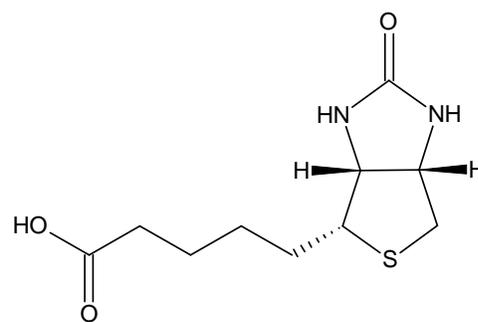
- d) Reference the spectrum  (L). This sample was dissolved in D₂O and has no TMS or DSS added for internal referencing. Therefore, we must reference to the residual HOD resonance at 4.7 ppm, which is the largest peak in the spectrum. Common internal reference standards are:

TMS = tetramethylsilane (CH₃)₄Si, typically used as an internal reference at 0.0 ppm

DSS = 2,2-dimethyl-2-silapentane-d₆-sulfonic acid sodium salt, a water soluble compound that is a common ¹H reference for D₂O/H₂O samples

- (Q1) Make two educated guesses as to problems that might arise due to referencing using the solvent peak (in this case HOD). Internal, substitution or external referencing is strongly recommended instead of referencing to solvent peaks like HOD. (So whenever there is a TMS or DSS peak in the spectrum, reference to it instead of the solvent.)

- e) Peak pick the spectrum , then change the peak picks to be in Hz (not ppm) and reposition the peak labels. To do this double click on the spectrum and in the GUI under the 'NMR spectrum' tab choose Peaks, change Units to 'Hz' and change Label Position to 'Peak Groups' (play with the other Label Position options to see what they do).



biotin

- f) Perform a baseline correction on the spectrum by clicking the arrow by  and choosing 'Baseline Correction...' (B). In the GUI that appears, define 'Bernstein Polynomial Fit', and you will notice a blue line appear on your spectrum. By changing the polynomial order, you will see the blue line change to fit your baseline. For this data, a polynomial order of 5 seems best. Click 'OK' to apply. The baseline correction is important to achieve accurate peak integrations!

Note: Be careful of the different baseline options. The Whittaker Smoother, for example, is useful when working with heterogeneous mixtures such as blood plasma. It works very hard to remove broad peaks that are present, but not useful, in such samples. But it can be disastrous when used on common small molecules, as it will remove broad –OH and >NH peaks. Use of the Bernstein Polynomial Fit and Polynomial Fit corrections are recommended.

- g) Manually integrate the spectrum by clicking on the arrow by  and choosing 'Manual correction' (I). MNova will automatically normalize your first integral to 1.0. You can change this by right-clicking on the integral and changing the normalized value. Make sure the total number of protons in the compound roughly equals that integrated [it is common that the sum of integrals will *not* equal the number of protons present]. The >NH and –OH peaks do not appear in the spectrum.

(Q2) Why are >NH and –OH peaks missing? Think about your solvent conditions. What could you do to make these protons observable?

- h) Display the spectral region between exactly 0.85 ppm and 5.25 ppm () and adjust the vertical scale so that the HOD peak goes off the scale. Fit the rest of the peaks to have the tallest go to the top of the page.
- i) Leave spectrum in 'landscape' and include annotations (1g). (3rd plot)
- j) Select everything on this plot (ctrl-A) and then copy (ctrl-C). Create a new page by clicking on  and paste (ctrl-V) everything in here.
- k) Zoom around the region of the HOD peak from 4.9ppm to 4.5ppm (). Then annotate the spectrum with arrows (found under 'annotate') and text (T) to show the following features. Artifacts can be observed about this HOD peak and the spectral region surrounding it. The most obvious artifact is the **large split** in the main peak, which is caused by poor magnetic field homogeneity, more commonly referred to as poor "shims". One of the most important adjustments prior to recording an NMR spectrum is to achieve a homogeneous magnetic field surrounding your sample. This process is known as "shimming" and in the next few weeks we will learn how to achieve a *good line shape* resulting from good "shims". Note also the **two smaller peaks** surrounding the HOD peak and equidistant from the HOD peak. These are known as 'spinning sidebands' resulting from errors in low order X/Y shims (known as non-spinning shims) and the need to spin the sample. On older spectrometers, such as the Bruker AC-300 (Athena) used to acquire this spectrum, the sample needs to be spinning in order to achieve a more homogeneous magnetic field by averaging out X/Y errors.
- l) Annotate the spectrum and use the  'Fit to Height' feature. (4th plot)

3. Work up the FID named CGFROTC.001

- Open up the spectrum and apply an exponential window function of (**lb=**) 2 Hz (). For ^{13}C spectra, sensitivity is usually much more important than resolution. Thus, this larger lb (remember for ^1H spectra lb typically = 0.3) window function trades off resolution by broadening the lines (by 2 Hz), but greatly reduces noise, improving the overall signal-to-noise (which is not technically “sensitivity”, but often described as such). ^{13}C 1D spectra are typically processed using an exponential filter with lb = 0.5 to 3 Hz (1 to 2 Hz being most common).
 - MNova automatically zerofills the spectrum once (check ). This should be fine.
 - The automatic phasing of ^{13}C spectra are often good enough; touch it up here by entering the ‘Manual correction’ mode  as done with the ^1H 1D spectra.
 - Reference the spectrum to the CDCl_3 peak (which appears as a 1:1:1 triplet just right of the center), and perform peak picking. Don’t forget your annotations! (**5th plot**)
- (Q3) Why does the CDCl_3 peak appear as a 1:1:1 triplet in the ^{13}C spectrum?

- ➔ Save your MNova document often and check it after the final Save.
- ➔ Convert your MestreNova plots to Adobe Acrobat *.pdf, by going to File → Export to PDF (ctrl-D). Name the file starting with your last name and ‘HWno1’ (*YourLastNameHWno1.pdf*) and select ‘Whole document’.
- ➔ View this *.pdf file to make sure it looks OK before submitting the homework.

Submit your HW assignment electronically. The TA will check this at the beginning of next week’s lab.

For this HW submit:

- A MestreNova file that contains 5 pages (one for each plot described above).
- A *.pdf file that is the converted MNova file.
- A hand written or typed sheet with the answers to 3 questions (Q1, Q2 and Q3). Please hand this to your TA at the beginning of your lab.

Move (or upload) your files into the S:\Chem636-Fall2014 folder located on the ‘temp’ drive. Put it into your lab section’s folder, so for example if you’re in the Tuesday 10am lab, the folder is called TuesdayAMlab, and so forth. Create a folder YourLastName-HW1 to put the above 2 files into. Let me or your TA know if you have questions about this. You will either need to put your files onto a USB device and then upload to the above folder in the routine NMR lab (2224), or mount **\\apollo.chem.wisc.edu\temp** as a hard drive on your computer. To mount the drive, your computer must be connected to the Chemistry network, either by hardwire, or by use of the ChemVPN.