NMR Data workup using NUTS

This laboratory and homework introduces the basic processing of one dimensional NMR data using the NUTS software package. This is the primary software used for the class and for research in the Chemistry Department. *Use the "NUTS Cheat Sheet" to guide you while you work with NUTS*; this document is accessed through the NMR Facility website at:

http://www.chem.wisc.edu/~cic/nmr/main.html

The Acorn website (www.acornnmr.com) also has excellent help indexes and tutorials on NUTS.

I. Data locations and backups

The spectra needed for this assignment can be found on the NMR facility's ftp server, **ftp:\\apollo.chem.wisc.edu** in the **ac300\Chem636\HW1** folder. The apollo server is mounted as the **R:** drive on the facility PCs. This semester you will be collecting data on the 300 MHz instrument called Homer, so your saved data will be on **R:\ac300\homer**. Automation data collected on Hermes (300MHz) will be saved on **R:\hermes*username***.

Data older than 10 days are archived (moved) to two other locations: hermes data to **R:\hf*group**username*\hermes**, and data from Homer to **R:\ac300\dataarchives\homer**. This archiving makes new data easier to find, but regular personal backups of data are still very important and expected from users. Backups typically would be made through the ftp server to your personal or group computer; CD writing and USB memory key backups can also be done using the facility PCs. An **S:\temp** folder is available within the facility to temporarily store data (the others folders are read-only), but this data is deleted on a monthly basis.

II. Routine workup of 1H 1D NMR data

A. Work up the rotenone proton FID named CGFROTH.001

a) Use the common sequence of commands for working up this data, starting with:

   GA BC LB EM ZF FT

b) Set the line broadening parameter [**LB**] to 0.3 Hz. This is a '**matched filter**' setting— **LB** = 1/**AQ**, where **AQ**=2.7 s—that reduces "truncation artifacts" for moderate resolution ^1H spectra (where AQ is set < 4 s). The line broadening is applied using the **EM** (exponential multiplication) command.

c) Following Fourier transformation [**FT**], the spectrum needs to be phase corrected. There are two methods for doing this: **PH** is often used for phasing proton spectra, whereas **PE** is recommended for ^{13}C spectra. Use the **PH** method here.

   Expand the spectrum vertically and use the baseline as the primary guide to getting a good phase adjustment: the baseline should connect when extended virtually beneath the peaks. If you are having trouble, or something is not clear, make sure you ask for help.

d) Reference the spectrum, and then perform a peak pick. Display the peak pick results in units of Hertz [using **DP H**]; coupling constants are typically derived from peak picks of ^1H spectra. The frequency axis is left in units of ppm.

e) This is a reasonably well-shimmed proton spectrum. The shim (resolution and line shape) quality is best inspected using the TMS peak. Expand the region horizontally and vertically about the TMS peak so the ^{29}Si satellites are clearly displayed, and include this expansion as an inset [**IS A**] in your plot.

f) Annotate [**NO**] the spectrum with the following information:
   - your name
   - the date
   - homework number and plot number
   - filename as saved on homer or hermes
   - sample name and solvent (in this case, Rotenone in $CDCl_3$)
   - how you referenced the spectrum

   Plots turned in for Chem 636 should always include this information.

PLOT-1: Plot the spectrum [**PL**] with peak picks and the annotation in the 'landscape' orientation.

Rotenone
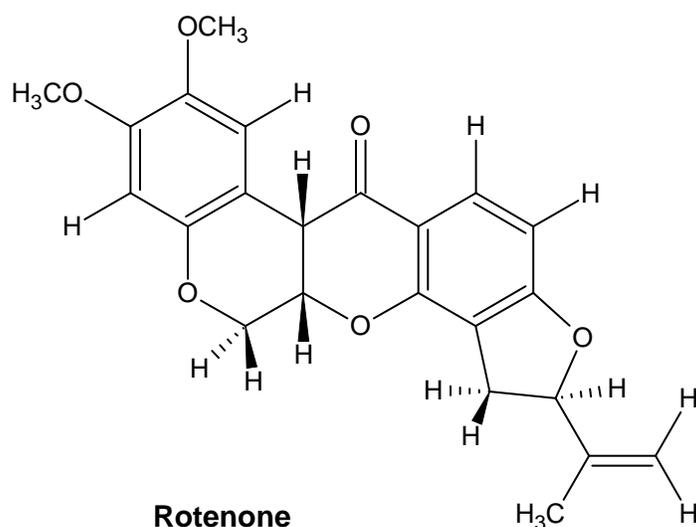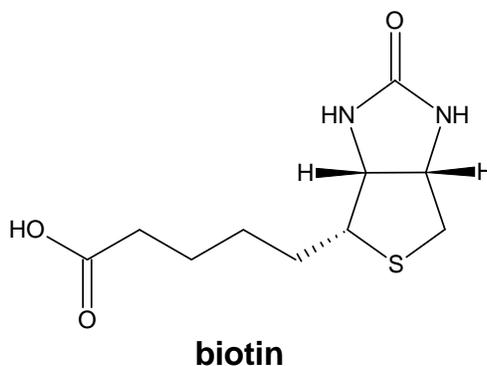
biotin

B. Work up the biotin proton FID named BIOTINH1.001

a) Perform the normal processing steps for a proton spectrum.

b) For samples having no TMS or DSS (a silyl sodium salt often used in D_2O/H_2O samples), as with this sample, referencing is not straightforward. A solvent peak is often used, even when it is not a very good reference (as is usually the case for HOD). Reference this spectrum to the HOD resonance at 4.7 ppm (which is the largest peak in the spectrum).

c) This sample was not shimmed very well. Inspect the line shape by zooming around the region of the HOD peak. Note the **large split** (called a Z2-split) in the main peak. One of the most important procedures needed to obtain a good-quality NMR spectrum is "shim" in a homogeneous magnetic field surrounding your sample. In the next few weeks, you will learn how to shim in a *good line shape*. Note **two smaller peaks** surrounding the HOD peak and equidistant from the HOD peak. These are known as 'spinning sidebands' resulting from errors in non-axial (X/Y) shims. You can measure their distance from the main HOD peak as 18 Hz; spinning sidebands will always show up at 1× (and sometimes also at 2×) the spinning speed of the sample.

Line shape imperfections arising from poor shimming are reproduced identically throughout a spectrum. Very poor line shape, and especially Z2-splits, can confuse spectral interpretations by making each line appear to be small doublets. Shim/line shape quality is easiest to inspect by looking at solvent peaks that are known to be singlets (as is the case with the central ^{28}Si peak of TMS, and with $CHCl_3$ and HOD).

Measure the size of the Z2-split of the main HOD peak by using *left-mouse-hold* on one side, and then *right-mouse-drag* to the other; both mouse buttons are held down simultaneously while reading the value from the lower right corner.

Q1→ Inspect the left "dd" portion of the ABX multiplet at 4.48 ppm. State whether and why you believe the minor splitting observed there is due to shimming errors, or rather is a small J-coupling.

Q2→ Also inspect the "dd" portion of a different ABX multiplet at 2.88 ppm (running from ~2.92 to 2.84 ppm). List the three "apparent" J-coupling values displayed in this multiplet. State whether you believe the smallest is a real J-coupling, or is due to the poor shim quality?

d) Perform a baseline correction on the full spectrum [**FB L**]. This procedure should always be done prior to setting up integrations; good baseline corrections are crucial to achieving reproducibly accurate integrals.

e) Manually integrate the spectrum [**ID**]. Normalize the integral values by choosing a multiplet as representing 1, 2, or 3 protons [*left-mouse-hold*+**V**]. The total number of protons from the integrals should roughly equal the number in the compound, but impurities and relaxation can alter this substantially. Note that the NH and OH peaks do not appear due to D exchange with the solvent.

PLOT2: Display the spectral region running from exactly 5.00 and 1.00 ppm [**ZO F**]. Plot the properly annotated spectrum with the integration results [*cntl*-**I**].

C. Work up the rotenone carbon FID named CGFROTC.001

a) Carbon data are processed similar to proton, with an important difference being the **LB** parameter, now typically set to 2 Hz (0.5 and 1 Hz are also commonly used for ^{13}C spectra). Although the same processing algorithm is used [**EM**], the intent now is to reduce noise in the ^{13}C spectrum (rather than reducing truncation artifacts in ^{1}H spectra): larger values of **LB** provide more noise reduction. The compromise is loss in resolution: the peak line widths are increased by **LB** Hz, so larger **LB** reduces resolution. Use **LB**=2 for this spectrum.

b) Reference the spectrum to the $CDCl_3$ peak, the 1:1:1 triplet to the right of center-spectrum; use 77.25 ppm for the center peak of the 1:1:1 triplet.

c) Perform a peak pick (note: chemical shifts are now the desired information, so display the peak picks in ppm). Count the number of carbons. Make sure the minimum height is set properly. The number of carbon peaks in a relatively clean sample should always equal the number of carbons in the proposed compound; this is not as useful as a mass spec, but is an important check of the chemical composition. The number should not match the rotenone structure for this spectrum.

D. Work up the rotenone carbon FID named "rot204mg_I500_080317_13C1d.fid"

a) The data you have processed until now were acquired on the Bruker AC spectrometers (which you will use in two weeks). This new dataset is from our Varian INOVA-500, and is typical of data from newer spectrometers. The raw data are stored in a set of files (in this case four of them: *fid* containing the raw data points; *procpar* containing the acquisition parameters; *log* with status information; *text* containing optional user-entered information) all stored within a folder having the dataset name.

Open a 2nd occurrence of NUTS (this will allow you compare this new data to the CGFROTC.001 spectrum). To open Varian data, use **GA** and click into the folder named

above, then double click (or select then OPEN) on the *fid* file. Data from our automation spectrometer, named hermes (a Varian Mercury-300 spectrometer that you will use next week), is opened in a similar manner. NUTS shows the data as a "Varian UNIX type" prior to displaying the FID.

b) Process normally, again using **LB**=2. After the **FT**, phase-correct this spectrum using **PE** (the previous dataset, CGFROTC.001, was unusual in not needing a phase correction).

c) Perform a peak pick, similar to that done on the CGFROTC.001 dataset. Count the number of carbons in this spectrum. Find the region where the peaks are different, and annotate this in the plot of this spectrum.

Carbon spectra are normally not quantitative in their intensities. Those with close-by (bonded) protons will be most intense; quaternary carbons will be less intense. In rotenone, one protonated and one quaternary carbon have very similar chemical shifts. Depending on the solvent conditions, these two peaks can overlap. The spectrum taken on the INOVA-500 resolves the last (23rd) carbon simply because the CDCl3 had "aged" (becomes somewhat more acidic). We will discuss later in the semester various methods for resolving such issues with ^{13}C spectra.

PLOT-3: Plot the full spectrum with peak picking. Include the normal annotation, and another pointing out which carbons, now resolved, that were overlapped in the CGFROTC.001 spectrum.

d) If you wish, go back to the CGFROTC.001 spectrum and process it with **LB**=0.5. The two overlapped carbons are now resolved, but note that they are opposite in relative shift compared to the INOVA-500 data: the quat is slightly upfield, rather than downfield, of the protonated carbon.

Hand in 3 plots and 2 answers to questions stapled together.