Homework 2 Chem 636, Fall 2008

due at the beginning of the 3rd week lab (week of Sept 15)

Automation on Hermes: ^{1}H 1D, ^{13}C 1D, DEPT-90 and DEPT-135

In this week's lab you will learn how to use our automated spectrometer, a Varian Mercury 300, named Hermes. Experiment setup on this instrument is simple, as long as you follow the instructions. You will each be given a login name, and temporary password. Please change your password the first time you use Hermes. The TAs will demonstrate how to access your account and how to change your password, as well as how to set up experiments.

You will setup a standard set of ^{1}H and ^{13}C 1D experiments. We will ask you to change the most commonly adjusted parameters, to assist you in becoming familiar with the procedure for changing them. The defaults parameters will often work fine.

In setting up these experiments, refer to the guide located at:

http://www.chem.wisc.edu/~cic/nmr/Guides/VUG/Use_of_Hermes.pdf

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Remember while setting up your sample for automation:

- Insert the sample into the spinner and check the depth using the gold depth gage at the top of the automation table; if the solvent height < 4.5cm, center it on the detect region.
- Check the sample height by using the 9" scale on the front of the sample table (the NMR tube should not be longer than 9"; there's no limit on shortness, as long as it fits the spinner).
- Make sure slot 0 is empty, and make sure the slot the robot arm is above is empty.
- Do not use slots 99 or 100.

Acquire data on your unknown (in $CDCl_3$) for the following 4 experiments. Submit all 4 experiments in a single Study (all in one night, within a single data folder). You don't need to have Hermes plot data (you will work it up yourself in NUTS):

1. Acquire a **^{1}H 1D** spectrum: use 32 transients [**nt**], and an acquisition time [**at**] of 3.5 sec. Set the sweep width [**sw**] to go from 17 ppm to –1 ppm (in pulldown menu or use the provided boxes).

   Aside: **nt** for ^{1}H spectra only need be increased if the concentration is fairly low (< 10mM). **at** is the fundamental limitation on resolution ($\Delta\nu \geq 1/$**at**), beyond the quality of the shims (which are automatically performed on hermes). Large **sw** improves baseline (and thus integral) quality of ^{1}H spectra. **sw** may also need to be increased to observe protons shifted outside the normal range for organic compounds, e.g., ^{1}H close to metal nuclei.

   A few important parameters used in Varian and Bruker spectrometers are:

Varian	Bruker	description
nt	NS	number of transients or scans
ss	DS	number of steady-state or dummy scans
d1	RD or D1	relaxation or recycle delay
at	AQ	acquisition time
np	TD	number of data points

2. Acquire a standard **^{13}C 1D** spectrum (with normal ^{1}H decoupling): use 256 transients [**nt**], and a relaxation delay [**d1**] of 2 sec. Set the sweep width [**sw**] to go from 220 ppm to –5 ppm (use provided boxes). Note that the decoupling mode is set to 'Decoupled + NOE'.

   Aside: **nt** for ^{13}C spectra will often need to be adjusted to match the concentration of your solution. 200mM is easy (64 scans), 50mM harder (512 scans). ^{13}C data should not be attempted on Hermes for concentrations at or below 20mM. Adjustment of **d1** to larger values (up to 5s) can be more optimal for observing quaternary carbons (varies considerably depending on the chemistry). The standard decoupler mode greatly improves sensitivity (through NOE build-up), and simplifies measurement of ^{13}C chemical shifts (by removing ^{1}H–^{13}C couplings). We will learn more about the different decoupling modes later in the semester.

3. Acquire a **Dept-90** spectrum: use 256 transcients [**nt**], and a relaxation delay [**d1**] of 2 sec. Set the sweep width [**sw**] to go from 220 ppm to –5 ppm, as in the ^{13}C 1D. For the CH multiplicity selection, leave on "CH only", which ensures the data will be that of a DEPT-90 (CH carbons appear positive while the rest are nulled).

4. Acquire a **Dept-135** spectrum: use 256 transcients [**nt**], and a relaxation delay [**d1**] of 2 sec. Set the sweep width [**sw**] to go from 220 ppm to –5 ppm, as in the ^{13}C 1D. For the CH multiplicity selection, leave on "CH, CH3 Up and CH2 Down", which ensures the data will be that of a DEPT-135 (CH and CH_3 carbons are positively phased while CH_2 carbons are negatively phased).

***Once you have set up all 4 experiments, click on the "Submit Night Queue" button, as instructed in the 'Use of Hermes' guide. If you have set up all the above experiments correctly the total time (in lower left corner) will be 52 minutes.

Retrieving the data on the routine lab PCs:

Your data will appear under the R:\\apollo…\hermes (or use ftp:\\apollo…) drive. Your *study* directory will exist in this folder (starting with your operator/login name). When opening the data files in NUTS, go into the *study* folder, then the data folder, then the folder of the specific experiment type, and open the 'fid' file, e.g.,: ***studyname* → data → Proton_01.fid → fid**

Data workup in NUTS:

PLOT1: Use standard processing for the ^{1}H 1D spectrum. This includes using a "matched filter" (**LB** = 1/**at**, where at=3.5 sec here), and one full zero fill (**ZF**) to improve the digital resolution. Integrate the peaks (don't forget to use **FB** prior to integrations). Make sure to include the annotation as instructed in HW#1, with the original file name being the name of this data set on Hermes (the experiment name).

PLOT2: Use standard processing for the ^{13}C 1D spectrum, which includes setting **LB** to 2 Hz. Peak pick the spectrum in ppm and include your annotation. Save the worked up spectrum to the S:\temp drive to use in part 3.

5. Process the DEPT-90 the same as for the ^{13}C spectrum. This spectrum should show only CH peaks (there may be small intensity artifacts from some of the other carbons, especially from intense methyl peaks).

   Save the worked up data onto the S:\temp drive (temporarily). Then open the DEPT-135 data in the same NUTS session, use the same **LB** value and use the **PS** command (phase same) to apply the same phase correction that was used for the DEPT-90. The DEPT-135 should now be phased correctly (or at least close to). For a DEPT-135, CH_2 peaks will be negative and all other protonated carbon peaks (CH and CH_3) will be positive. Quaternary carbons do not show in DEPT spectra. Save this spectrum to the S:\temp drive as well.

6. a) Now use the NUTS **BU** subroutine (buffers mode) to transfer the DEPT-135 spectrum to a buffer (with the command **A**). Choose **View → View buffers outside of BU mode** and exit **BU** mode. You should see 2 spectra in the Base Level of NUTS, both the same (the upper in the buffer, the lower in the current working area).

   b) Open the worked-up DEPT-90 spectrum, then go back into **BU** and transfer this to a buffer (another **A**). Exit **BU**; you'll now see three spectra: top is D135, middle is D90 in buffer, bottom D90 in active area.

   c) Finally, open the ^{13}C 1D spectrum. This final spectrum will replace the bottom DEPT-90 spectrum. In **BU** mode you may adjust the position and intensity of each buffer (see the NUTS Cheat Sheet for details).

   d) Label which spectrum is which using **Edit → Edit Current Buffer** and enter the info in the comment line. For the ^{13}C 1D spectrum at the bottom, you can do this from **View → Spectral Parameters** and change the comment there.

PLOT3: Annotate this stacked spectrum and print it out.

7. From the stack plot, determine which carbons are 1º, 2º, 3º and 4º (1º ≡ CH_3, 2º ≡ CH_2, 3º ≡ CH, 4º ≡ C). Tabulate the numbers of each type of carbon:
   for example, there are _____ 1º = CH_3 carbons
   _____ 2º = CH_2 carbons
   _____ 3º = CH carbons
   _____ 4º = C carbons
   Indicate these *multiplicity* assignments on the ^{13}C spectrum (PLOT2). Compare to the FW provided for your unknown (will be more useful when we provide the structure later on).

→ Hand in the 3 PLOTS that you have processed in NUTS.