Homework 11 Chem 636, Fall 2008

due at 5pm on the day of your lab the week of Dec 2-4

Automation on Hermes: ^{1}H-^{13}C HSQCs and HMBCs

In setting up these experiments, refer to the guide:

Use of Hermes, the Varian Mercury-300

Your unknown compound structure, and additional information about the unknown is now posted on the class webpage.

In this week's lab you will continue with acquiring 2D NMR data. Many different types of 2D NMR experiments exist in addition to COSY. This week's "indirectly detected" HSQC and HMBC experiments are useful in correlating ^{1}H resonances with ^{13}C resonances of a molecule. HSQC stands for Heteronuclear Single Quantum Coherence and gives 1-bond information, i.e., which ^{1}H is attached to which ^{13}C. HMBC stands for Heteronuclear Multiple Bond Correlation and gives 2-bond and 3-bond info, ie. which ^{1}H are 2-bonds or 3-bonds away from a particular ^{13}C. The combination of ^{1}H 1D, ^{1}H COSY, and ^{1}H-^{13}C HSQC spectra is very powerful, and will often allow complete assignment of all protons in a molecule. HMBC can be very useful in more difficult cases, especially in confirming bonding through heteroatoms, and will usually allow all carbons to be assigned.

Since HSQC and HMBC are ^{1}H-detected, significantly less amount of material is needed than for a ^{13}C 1D: 10mM concentrations are good at 300 MHz, with 5mM being more than sufficient at 500 or higher. But because both techniques rely on the sizes of J_{CH}-couplings, they are less reliable than ^{13}C 1D spectra in insuring that all ^{13}C nuclei are being observed.

Hsqc data from Hermes are of reasonably quality, if sufficient time is given to the experiment, and the resulting spectra can be quite valuable. Hermes, on the other hand, provides only an introduction to HMBC experiments. This experiment is complex to setup correctly and analyze; i.e., you will spend significant time studying the data generated, and typically you will only do this to solve a relatively complex assignment issue. The data quality for HMBC is very significantly improved by using our 500 or 600 MHz spectrometers, so a general recommendation is to ***not*** use Hermes for HMBC, but rather make the effort to get the data taken at 500 or 600 MHz. You will have to take Chem 637 to do this yourself; otherwise another student will have to take your data, or possibly Monika or I (cgf) will assist in obtaining the data.

For organic and inorganic chemists, two major categories of experiments have yet to be discussed: through-space NOE experiments, and dynamic NMR experiments. NOESY 2D and NOESY-1D (and their correlaries, ROESY and ROESY-1D) are very important for providing conformational, or even tertiary structural information. Dynamical NMR provides direct information about chemical or conformational exchange. NOESY1D can be run on hermes, and for some situations this data will be sufficient. But often higher field is needed, as once again the data quality is greatly improved when acquired at 500 or 600 MHz. Dynamic and kinetic NMR experiments require temperature control: the 360 or higher-field spectrometers are required to perform such experiments.

Setting Up and Submitting Experiments on Hermes

Use your **unknown in $CDCl_3$** to set up the following experiments to run in one night:

1. A **Hsqc** experiment with 4 scans per increment (**nt**) and 128 increments (**ni**). Change **d1** to 2 sec.

2. Note that, as with COSY requests, the spectrometer software automatically adds a ^{1}H 1D experiment if it has not already been requested when setting up either an **Hsqc** or **Ghmbc**.

3. A **Ghmbc** experiment with 8 scans per increment (**nt**) and 256 increments (**ni**). Change **d1** to 2 sec. Choose the coupling constant to be 8 Hz, since that is a good long range ^{1}H-^{13}C value.

4. A normal ^{13}C 1D, but with 256 scans. A ^{13}C 1D spectrum is not needed to analyze hsqc and hmbc spectra, but is useful when first working with such data. You acquired a ^{13}C 1D on Athena recently, and on Hermes in the 2nd week of class, but taking another spectrum now will be more convenient than trying to find and utilize that older data.

Submit these 4 experiments to the Night Queue in a single Study.

*Note the changes made above where the repetition time, **d1**=2, is set for each 2D experiment. Repetition time is an important parameter in these experiments: running too fast can generate significantly problematic artifacts, so setting **d1** to a value "long enough" is important. For most HSQC and HMBC experiment, the general rule-of-thumb is to set* $\boldsymbol{d1=1.5\times T_1(^1H)}$. *We used to have a lab in this course that involved measuring* $\boldsymbol{T_1(^1H)}$, *but it was removed due to limitations involved with the AC spectrometers. We are working to implement the* $\boldsymbol{T_1(^1H)}$ *experiment on Hermes. Until that time, the recommendation is that you ask senior students in your group what values of **d1** to use for your compound; similar compounds will have similar* $\boldsymbol{T_1(^1H)}$ *values. If they don't know, you can ask for assistance from the NMR staff. In general, **d1**=2 to 3 will suffice for moderately sized organic compounds. But if the sample is sealed under an inert atmosphere, as just one example, then d1>5 is often needed. In such cases, running a* $T_1(^1H)$ *experiment will be critical to optimizing the length of the 2D experiment.*

Data workup in NUTS:

1. Start with the proposed structure for your unknown compound.

PLOT1: Plot the structure page out and number the carbons in the compound by hand.

2. Workup the ^{13}C 1D spectrum, and see if the carbon count in the spectrum matches the structure.

PLOT2: Plot the ^{13}C spectrum out, and add an annotation that summarizes the carbon counts, e.g.:

21 carbons in structure
21 carbons in spectrum: 3 quat, 18 protonated [from intensities]

Otherwise, process the ^{1}H 1D and ^{13}C 1D as you normally would. Save both spectra as NUTS formatted spectra, as both will be used for Borders(projections) for your 2D data.

3. Workup the Hsqc data using the 'varian_hsqc.mac' macro. [Pay attention to whether or not the macro has a 'g' in its name, like 'varian_ghsqc.mac', which is only to be used with the Ghsqc pulse sequence!] This spectrum will most likely need to be phased, and then saved. The TA will demonstrate this procedure, which follows the NUTS Cheat Sheet. Add the ^{1}H 1D and the ^{13}C 1D spectra as Top and Left Borders on the 2D plot. There should be one to two crosspeaks in the HSQC for every protonated carbon in your ^{13}C spectrum. Quats will not show. −CH2− crosspeaks will show negative (red) in the HSQC, because of a flag in the spectrum setup that requested this type of multiplicity editing.

   If the borders/projections do not perfectly align, referencing the 2D spectrum to chosen peaks in the 1D spectra (using δ's from the 1D spectra) will fix the problem.

4. Draw lines through your HSQC spectrum using the instructions from the last page of the NUTS Cheat Sheet. ***Do this for 3 protons you have selected***, whose identity you can guess from the proposed structure of your unknown. Draw a line through each of these ^{1}H resonances (connecting into the 1H 1D border spectrum); then draw the orthogonal line connecting that ^{1}H to its attached ^{13}C. HSQC spectra will connect only protonated carbons, since it is a 1-bond ^{1}H–^{13}C connectivity experiment (j1xh=140 Hz).

PLOT3: Print the HSQC plot with the drawn lines and proper annotations. Draw in by hand the guessed-at assignments: if you guessed a proton was #1, then the crosspeak provides the assignment to carbon #1. You will not be scored on the accuracy of your assignments, but showing knowledge that an HSQC always connect the #*n* proton to the #*n* carbon is essential.

Never symmetrize HSQC or HMBC spectra; this would make no sense (ask if you're don't understand!).

5. Work up the Ghmbc data using the 'varian_ghmbc.mac' macro. This data will not need to be phased, since it is acquired in magnitude mode (like the Gcosy experiments). Again, use the ^{1}H 1D and ^{13}C 1D spectra for borders (projections) on the 2D plot. Draw lines through the same ^{13}C resonances, and now connect all protons that are now shown to have long-range (2- or 3-bond) connectivity to that ^{13}C. Choose one additional quaternary carbons, and drawn connectivity lines from it to all 2- to 3-bonded protons.

PLOT4: Turn in the HMBC plot with proper annotations. Again, draw in by hand the assignments. The protonated carbon assignments are given by the HSQC spectrum; do the long-range proton connections seem to make sense? Guess at the assignments for the connected protons and the quaternary carbon.

Turn in 4 plots