Homework 11 Chem 636, Spring 2014

due at the beginning of lab April 22-25 updated 14 Apr 2014 (cgf)

2D NMR: HMBC & Assignments in MNova

Use Artemis (Av-400) or Callisto (Av-500) for this week's HW.
Best to use the same sample as in HW#9+10.

Reading – **HMBC in Claridge section 6.4 (esp. 6.4.2)**
– **Assignments in MNova are best learned by doing it; there is a tutorial on their website at http://mestrelab.com/resources/assignments/. [The Automated Assignments tutorial is not useful since we don't have the Predict plug-in.]**

Goals – **Learn about common setup and use issues with HMBC.**
– **Combine 1D and 2D spectra to provide assignments, and in this session utilize the capabilities provided by MNova.**

I. HMBC: Heteronuclear multiple-bond correlation spectroscopy

HMBC is both similar and different from HSQC in setup and use. Key points are:

a) HMBC experiments are not as sensitive as HSQC: a common rule-of-thumb is HMBC takes 4× as long as HSQC. Note that often the minimum (good: **NS**=2) HSQC has more than sufficient signal-to-noise. In such cases, **NS**=8 may be more than needed for the HMBC.

b) Since quaternary carbons are observed in HMBC, the 13C spectral range is usually larger than with HSQC: 240ppm as default in HMBC vs 180ppm in HSQC.

c) Multiple-bond ^{1}H-^{13}C coupling have the ^{1}H then bound > 99% of the time to ^{12}C. Because ^{13}C assists in relaxing protons, the protons detected in HSQC relax faster than in HMBC. HMBC experiments therefore are less robust toward shorter **D1** values: resist pushing **D1** to too small of values for HMBC.

d) There are a large number of HMBC variants in the literature. For small molecules, the preferred experiments recommended on our Bruker spectrometers, HMBCETGPL3ND, is almost certainly the best for most situations. The number of variants begs the issues of problems with the data, however, and those do exist. HMBCETGPL3ND has a three-fold 1-bond filter that is very good at removing 1-bond artifacts. Important crosspeaks will still often be absent due to small J_{HC}; the most common work-around is to run a second experiment with a smaller value, e.g., CNST13 = 3 (default is 8 Hz).

Critical parameters for TOCSY, those that should always be checked, are:

D1 ≥ 1.5×T_1(longest)

NS ≥ 2 (often good to use 4×NS used for the HSQC)

TD1 = 256, 512 … up to perhaps 1024

CNST13 = 8 often run again at 3Hz to pick up small/missing crosspeaks

1☞ Acquire a good quality HMBC on the sample you've been using for the 2D labs. Make sure **NS** ≥ 2 (**NS**=8 is often best for research samples; but for facility-provided samples **NS**=4 will be ok), and set **TD** = 4096 and **TD1** ≥ 256.

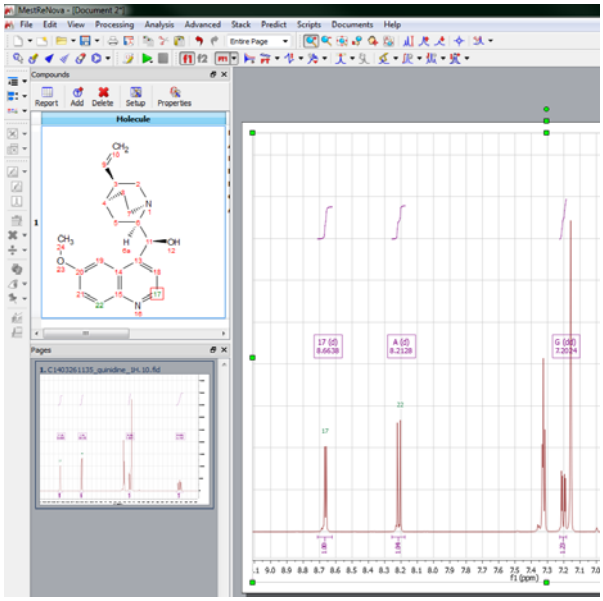
II. Data Assignments Using MNova

MetreNova provides useful tools to assist in making assignments based on sets of 1D and 2D NMR data. The first few tools we covered in HW #7, where multiplet analysis and datatables were introduced. The primary addition here involves adding a compound structure and making assignments directly to it.

a) Use ChemDraw or ChemSketch to copy-and-paste a compound structure into your ^{1}H 1D spectrum; CNTL-C and CNTL-V work fine for this.

Note: I (cgf) have been unable to get renumbering in ChemDraw to replicate in MNova, whereas the renumbering in ChemSketch is reproduced. In fact, pasting the ChemDraw compound into ChemSketch and then copy and paste into MNova will provide the correct numbering. Really annoying, especially when ChemSketch garps up the looks of the ChemDraw structure…. If any of you figures this out, let me know.

Recommended: It is difficult to not have the compound structure get in the way of spectral features when pasted into spectra. A way around this is to use **View → Tables → Compounds** to display the structure, as shown in the upper portion of the left panel in the figure. You can now delete the structure in the spectrum by clicking on the upper handlebar.



If you prefer having the compound in the spectrum, copy and paste it into each new spectrum as you add them. You do not need to do this when viewing the compound table.

b) It is best to use multiplet assignments in ^{1}H 1D spectra. [This is not required; one can make assignments based on peak picks; holding the SHIFT key down while doing this allows selection of a "peak" in the center of an overlapping doublet, as one example.]

→ Enter assignments mode by using the shortcut key **A**, or **Analysis → Assignments → Manual Assignment** . You will see a naphthalene ring attached to the mouse pointer. Click on an atom to be assigned, then on the multiplet box it is to be assigned to.

Note: If you have selected a compound that is pasted in a spectrum, typing A will replace the atom label with A. Undo, click on the background, then A again to get into assignment mode.

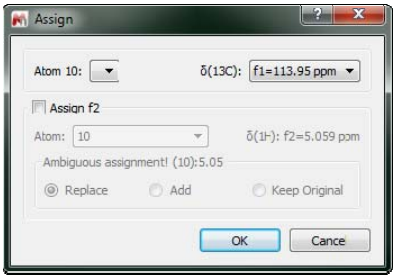
c) Assignments of carbons in a ^{13}C 1D spectrum are best done with simple peak picks. You don't have to do peak picks first; when in Assignment mode, the cursor will automatically choose peaks as you hover over them.

Note: ^{13}C chemical shift predictions can be quite useful for making (tentative) assignments. These should be confirmed with prior (literature) knowledge, but are best made using other data such as COSY and HSQC/HMBC.

d) COSY spectra are assigned by clicking the atom, then the center of diagonal peak to be assigned. Use only the top line for the assignment; i.e., uncheck the "Assign f2" box (the chemical shifts will not exactly match between the two).

Recommended: Always add the 1D traces that are available to your 2D spectra when making assignments. If you don't have a ^{13}C 1D (you should for this set of HW unless you're using a research compound), that's ok, and leave the f1 trace undisplayed.

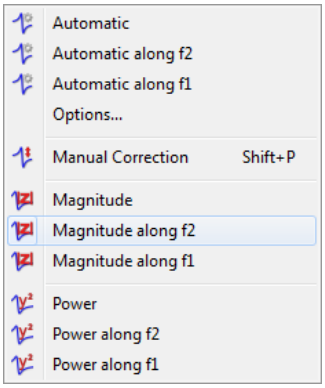
e) Assignments of HSQC crosspeaks require only clicking on the atom to be assigned, and then clicking in the center of the crosspeak. If the ^{1}H or ^{13}C is already assigned, remove it from the selection. In the example on the right, the ^{1}H assignment had been made already from the 1D spectrum, and is therefore "Assign f2" has been deselected. Only the ^{13}C chemical shift will be added.



Note: HSQC provides the simplest method for identifying non-equivalent CH_2 protons. In these cases choose the down-arrow box for Atom: above under Assign f2 and select 10', or 10". You will see other possibilities, e.g., ax or eq, and should make the choice that makes sense.

f) Assignments from HMBC crosspeaks will be similar to HSQC. It is a common methods for determining quaternary carbons chemical shifts, and for confirming or making other tentative or unknown assignments.

2☞ Work up the HMBC spectrum. These spectra should be magnitude mode in f2. If different colors are displayed, this has not be done by MNova: choose the dropdown for and "Magnitude along f2".



Make a reasonable number of assignments using all five of the data sets you have obtained: ^{1}H 1D, ^{13}C 1D, COSY, HSQC, HMBC [you can add in the TOCSY if you wish]. Save the assignments as a .mnova file and upload.

Plot HMBC spectra. Provide as many assignments as you can via compound structure. Upload 1 plot as .pdf and upload. Save a .mnova file containing five spectra, the compound structure, and your assignments.