Week 4

2D NMR for Peak Assignments

4.1 HSQC, HMBC, and COSY

2/25:

- Questions.
  - Getting different answer consistently for PSet 2 SNR values between MNova methods?
    - That's fine; just be consistent.
  - JEOL 502 and Bruker 600 training?
    - The JEOL 502 can do simultaneous ^{1}H, ^{19}F, and ^{13}C, but no one uses it for that. It's just another autosampler machine with low queues.
    - The 600 also just has a low queue.
  - SampleJet caps?
    - Walt will show me the product ID next time I'm in the DCIF.
- Announcements.
  - PSet 1 answers posted, but grades not.
  - A note on PSet 1: Coupling constants have to be the same when we increase the quantity of ^{13}C.
  - Also, β-anomer coupling constant is almost exactly the same. Why doesn't the β-anomer couple to the anomeric carbon, but the α-anomer does?
    - It's coupling to a carbon 3-bonds away at the correct dihedral angle!
    - Dihedral angle wouldn't affect a 2-bond coupling.
- Today: Chemical shift assignment from scratch (adenosine).
  - We'll also start talking about the utility of 2D acquisition.
- Adenosine.
  - Nucleotide base in DNA, component of ATP, and occasionally used in chemical transformations.
  - Nice test system because soluble in DMSO and then will stay the same for a while.
  - 2 aromatic protons, 3 aromatic carbons that are not protonated, 5 non-aromatic carbons and associated protons, 3 hydroxyls, and amino group.
  - Dissolving it in D_2O removes the exchangeable protons; dissolving in DMSO-d6 shows us all protons.
  - As DMSO picks up H_2O, it will begin to broaden peaks for exchangeable protons.
- Assigning adenosine peaks (^{1}H spectrum).

  - Begin by integrating the spectrum.
  - 2 aromatic protons.
  - One 2H in the middle (that's probably aniline).
  - And 8 aliphatic protons (one of the 9 is hiding in the water signal).
  - Water in DMSO is 3.2-3.4 ppm depending on how many exchangeable protons there are in your solute.
  - Water in $CHCl_3$ can be between 1-5 ppm, depending on acidic protons in solution.

- Assigning adenosine peaks (^{13}C spectrum).
  - In general, you should start with the 1D carbon *before* going to the 1D proton!
    - Sensitivity is lower, but 1D chemical shift resolution is a big bonus!
    - 2 chemical shift overlaps in proton is an issue.
    - Carbon chemical shifts *can* overlap, but they do so much less often.
  - There are 10 carbons in the molecule, and we clearly see 10 carbon peaks.
  - Walt has integrated the carbons here. Quantitative carbon can be done at MIT, but this is not that??
  - Aside: Proton decoupling.
    - Before we apply the carbon pulse, we apply a moderately weak proton RF pulse that allows the proton-carbon NOE to build up. Thus, any proton will transfer its magnetization to the carbon. This enhances carbon signals by about threefold.
  - The two protonated carbons (because of NOE magnetization transfer) integrate a bit more.
  - For moderately accurate carbon integration, integrate close to the peak! It's not like proton where you can just integrate as far out as you want.
  - Aside: Quantitative carbon.
    - Have a longer t_1 relaxation delay so that the spins can all get back to equilibrium before the next pulse.
    - Use a 30° pulse instead of a 90° pulse so that it takes less time to relax back to equilibrium.
    - Don't use the NOE because you want quantitation, not maximal signal.
    - Long aquisition time as well, so you can digitize the signal as best as possible.

- **Heteronuclear single-bond quantum correlation**: An NMR experiment that shows you one-bond proton-carbon couplings in order to connect protons and carbons. *Also known as* **HSQC**.

- Assigning adenosine peaks (^{1}H-^{13}C HSQC).
  - 2 aromatic signals (proton around 8, carbon around 140).
  - 6 aliphatic signals.
    - 4 blue and 2 green.
    - This is a phase-sensitive and multiplicity-edited mode, allowing us to distinguish CH's, CH_2's, and CH_3's (analogous to DEPT experiments!).
    - DCIF has DEPT 90, 135, *and* 45!
  - Signals at the top and bottom are mirrored about the center.
    - Artifacts are pre-ordained by phase cycling, receiver gain, etc.
    - These are from the water in this case. The really strong signals can often be mirrored; this is because we didn't let the waters relax long enough between scans.
  - Gets us a labeling of the protons based on the carbon chemical shift.
    - To reiterate, carbon chemical shift is much more determining than proton chemical shifts.

- 1D vs. 2D experiments.
  - 1D is relaxation delay ($d1$), pulse, and aquisition.
  - 2D/fancy can be relaxation delay, a bunch of pulses, and *then* acquisition.
    - $d1$'s are on the order of seconds.
    - Pulses are on the order of milliseconds.
  - Two Fourier transforms.
- HMBC allows us to find 2- and 3-bond connections.
  - Proton-carbon has 120-170 Hz coupling for 2-bond, and 3-10 Hz couplings for 3-bond.
  - HMBC essentially optimizes for a different range of coupling constants, and filters out 1-bond couplings.
- **Homonuclear correlation spectroscopy**: A proton-proton 2D experiment. *Also known as* **COSY**.
  - A symmetric experiment that gives us the same cross-peaks on both sides of the diagonal.
- Assigning adenosine peaks (^{1}H-^{1}H COSY).
  - Connects nearby proton peaks.
  - The diagonal corresponds to correlation to between a proton and itself.
  - Off-diagonal elements give us what we want: 6-8, 8-9, 9-7, and 7-10.
  - 10 is the unique diastereotopic pair, so working backwards, we then get 7, 9, 8, and 6.
  - We can then assign the hydroxyl protons.
  - At this point, we've assigned the entire sugar but not the aromatic stuff.
- COSY is easier to interpret, but is there a reason we couldn't just measure coupling constants in the 1D ^{1}H NMR?
  - We could do that, but we are resolution-limited and there is much more overlap.
- Next time: Assigning the aromatic stuff with HMBC, nitrogen 2D experiments to figure out which nitrogen is which.