

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 ^1H , ^{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- d_6 shows us all protons.
 - As DMSO picks up H_2O , it will begin to broaden peaks for exchangeable protons.
- Assigning adenosine peaks (^1H 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 acquisition 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 (^1H - ^{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 acquisition.
 - 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 (^1H - ^1H 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 ^1H 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.

4.2 Heteroatom Correlation Experiments

2/27:

- Today: Finish the complete assignment of adenosine.
- More on COSY.
 - **t_1 stripes**.
 - The spectrum can also be displayed in 3D, instead of as a contour/topological plot.
- **t_1 stripe**: A stripe in a COSY spectrum.
 - These are the product of not waiting long enough between scans or between the increments in the indirect dimension.
 - They are just noise, and we have to go down to a point where the spectrum looks rational.
- More on HSQC.
 - Even if a mirror reflection lines up with a carbon peak, we have to be able to spot that it's not.
 - If we're still unsure, we can change the spectral width or center of the spectrum. If the point moves with the spectrum, it's a reflection; if the point has the same apparent chemical shift, it's real.

- Assigning the hydroxyl peaks.
 - Three-bond ^1H - ^1H couplings will be slightly different magnitudes through C–C vs. C–O bonds.
 - COSY (in DMSO-d6) does the trick.
 - Spiking DMSO-d6 with a bit of D₂O and running two consecutive experiments (with and without spiking) can also be helpful.
- A pitch for DMSO in NMR.
 - We don't like to use it because it's hard to get rid of.
 - But if you use a gentle stream of dry nitrogen on your 0.5 mL sample, it will get rid of the DMSO in about half a day!
- ^1H - ^{13}C HMBC.
 - Can't routinely detect signal up vs. signal down (as in DEPT), so all of the signals are just magnitude (blue).
 - Carbon 6 should be correlated to proton 8, proton 9, and the hydroxyl on 8.
 - HMBC's are very rich in information, but too rich to make an absolute assignment. That's why you need COSY, too!
 - What's up with the signals that don't line up with a proton?
 - The center of the pair of signals line up with a proton; that's the breakthrough of the one-bond coupling.
 - Three-bond connections are bigger than two-bond connections in every case except 5-membered nitrogenous heterocycles.
 - Account for the big signals first, and then go after the little ones if you want.
- Don't try to do direct observe ^{15}N unless it's fully labeled; only do correlation spectroscopy.
 - Only do direct observe if we're doing ^{14}N spectroscopy.
- ^1H - ^{15}N HSQC.
 - Only one peak, corresponding to the NH₂ protons.
 - The nitrogen peak is measured indirectly, by projecting the nitrogen correlation over. This is as good as a direct result, though!
 - If a reviewer asks for a direct ^{15}N spectrum, argue with them!
- ^1H - ^{15}N HMBC.
 - One nitrogen sees one aromatic proton, and two sugar protons. This is the bridging nitrogen!
- Walt pitches MestReNova's multiplet tool and automatic peak reporting.
- Next week: NMR tools for stereochemical assignments, gradient tools, final project.