5.46 (NMR Spectroscopy and Organic Structure Determination) Notes

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Weeks

List of Figures

Week 1

1D NMR Principles and Practices

1.1 Underlying Principles of NMR

1/4:

- Philosophy: NMR is a complicated and useful set of tool for chemists.
- Background on Walt.
  - Training: Masters, PhD, Postdoc, and 5 jobs in NMR.
  - 20 years industry experience, 10 years academia experience, some small company experience.
  - Considers himself somewhere in the middle between knowing nothing and everything about NMR.
  - Experience in natural products discovery, drug discovery, biological NMR, etc.
- Goal of the class: Distill what's most important for us to know.
- Announcements.
  - Syllabus now posted on Canvas!
  - Everything important will be posted on Canvas.
  - MestReNova is what we should use; TopSpin is what Walt is more comfortable with.
  - Slides posted to Canvas at the end of the day.
- This week: The fundamentals.
  - Chemical shift, coupling constants, correlations (new), NMR relaxation
- The NMR periodic table.
  - ^{1}H has almost 100% spin 1/2.
  - ^{12}C is 99% abundant and has spin 0.
    - Thus, we can only do NMR with ^{13}C.
- The resonant frequency depends on an absolutely fundamental physical property called the **gyromagnetic ratio** (γ).
  - Highest gyromagnetic ratio is tritium (^{3}H), then fluorine, then a whole bunch, then phosphorus, carbon, nitrogen (with a bunch in between these three as well).
- Higher magnetic field gives more signal.
  - But 1/4 as much for ^{13}C as for ^{1}H, because γ for ^{13}C is 1/4 what it is for ^{1}H.
- The highest field NMR systems commercially available are at 1.2 GHz.
  - Walt will typically only go up to 600 MHz in this class, corresponding to a 14.1 T magnet.

- Range of chemical shifts.

Figure 1.1: Chemical shift ranges of common nuclei.

  - The range of chemical signals we'll see is tiny, though; only about 6000 Hz if we're talking about a 10 ppm window.
  - Different nuclei appear in different windows and with different ranges (think of how carbon is 0-200 ppm vs. proton -5-15!!).
  - Note that the ranges in Figure 1.1 are to scale relative to each other, but have been scaled up absolutely by 10 times.

- All atoms' spins are active as soon as we magnetize the sample in the magnet bore. Differentiating between them is now an electronics problem.

- α- and β-D-glucose's anomeric protons have significantly different chemical shifts (4.6 ppm vs. 5.1 ppm, roughly).

- Oxygen is virtually all spin 0 ^{16}O, but protons will couple to each other and to ^{13}C (giving carbon satellites).

  - 1% of the time, the proton is coupled to ^{13}C, and gets massively split.
  - All couplings exist; it's just a question of whether we can see them!
    - 170 Hz coupling for the 1-bond carbon-to-proton coupling.
    - 2-bond connection is then expected to be much smaller, maybe 25-30 Hz.
  - Protons are present in much higher concentration, though, so we see their splitting much more (but it's also smaller because they're farther away!). This is why vicinal protons couple in 4-8 Hz instead of 200 Hz.

- Sergei: Why no coupling to the alcohol protons?

  - Because the sample is in D_2O, we get exchange everywhere to OD.
  - Since deuterium is spin 1, it should split the spin 1/2 nucleus into a triplet. But it also has 1/6 the gyromagnetic ratio. Additionally, fast exchange prevents any meaningful coupling from developing.
  - Dissolving the sample in (very dry) DMSO-d_6 will *not* lead to proton exchange, and we *can* observe the couplings to the hydroxyl protons!

- Equations.

  - 5/2 exponent for gyromagnetic ratio means it *really* matters for sensitivity.

- 600 MHz denotes the resonance for protons at the set magnetic field.

  - Carbon would be at 150 MHz in this case (because 1/4 gyromagnetic ratio)!
  - 140-150 A of current in the magnetic.

- A 4 Hz coupling is on the order of parts per billion, so to discern it, we need parts per billion homogeneity in the magnetic field. This is why we need shimming.

  - Shimming is done with additional coils that impart additional magnetism to parts of the sample.

  - Shimming is done for all of the few dozen coils every once in a while, and then with some of the coils for each particular sample.
  - To shim, you measure the deuterium lock signal and how broad or narrow/high the peak is and then you fiddle with the coils!
  - All you really have to look at is the height because the area is the same, so the height of the lock signal correlates to how good the shimming is.
  - Today, we do **radian shimming**, which tells us how to change the current in the coils to make the shimming better.
  - Shim coils can only be adjusted so far; if there's no sample below the coil, the shimming likely can't compensate enough to get a good spectrum.

- Administrivia.
  - There will be some kind of group project.
  - Final project is for us to use our skills to do something useful.
  - Write up our PSets independently, but we can work together on them.

1.2 Recording NMR Data

2/6:

- Deuterated solvents are used both to remove the proton background *and* for lock.
- If you run a sample automatically, everything we do in the next 20 minutes is gonna be automated.
- Locking.
  - If your sample doesn't lock in automation, the sample will fail.
  - If you've got 50:50 $CDCl_3$ to MeOD, the system may or may not lock on the chloroform signal, specifically.
  - The spectrometer is sweeping resonant frequencies across a relatively small range for deuterium.
  - x-axis is frequency, y-axis is intensity; perhaps an FID??
  - When lock is on, we're picking a deuterium frequency. If DMSO-d6 resonates at 2.49 ppm, we relate everything else back to that.
- Shimming.
  - We don't use R_f pulses, but rather magnetic gradient pulses.
  - A constant gradient across the measured window will give a broad line because samples at one part will go at one frequency, and samples at another part will go at a different frequency.
  - Various currents for various gradients that you add together properly can be added together, like Fourier analysis! Creating a straight line as the sum of nonstraight lines!
  - There's error in the machine picking the deuterium sample exactly right.
    - This is what makes the machine say $CHCl_3$ is at 7.19-7.28 ppm.
    - There's a difference between robustness and precision; the machine probably loses some precision for the sake of robustness.
- Tuning.
  - Looking at the response of the entire R_f system.
  - Is that response maximum at the frequency at which I'm looking?
  - You need to tune the system to your sample, because otherwise, your sample's response will be much weaker.

- Phasing.
  - Maximizing the real and imaginary components.
- The FID.
  - The FID goes down due to a **relaxation effect** (t_2) that we'll discuss more later.
  - Exponential multiplication of 0.5 Hz, i.e., (reciprocal) 2 s.
  - Hertz/seconds conversions are good math to practice on.
  - Getting rid of the signal after 2 seconds gives less noise, but you lose signal intensity.
  - Losing 0.5 Hz couplings is fine if you're mainly looking for 3-5 Hz couplings.
  - Zero-filling gives an increase in resolution, but it has limited advantages.
  - The further out you go in time, the more frequency discrimination you get. But lose S/N as well.
- t_2 is the relaxation to equilibrium perpendicular to the magnetic field.
- t_1 is the relaxation to equilibrium parallel to the magnetic field.
  - This determines if spins actually get back to equilibrium after you do something with them (e.g., pulses).
- Pulse length.
  - 5 µs by default.
  - What if we lengthen it to 500 µs?
    - Things get out of phase. Manual phasing allows you to see it, but you get a broad background.
  - At 5 ms, you don't get anything really interpretable, although the peaks are in roughly the same space.
  - 1/5 µs is 20 kHz, which is parts per million on a 600 MHz spectrum.
- Project #1.
  - Task: Prepare a presentation for the class, and a report for the class.
  - Purpose: Share as much information about a range of useful nuclei as we can with each other.
  - We're not gonna touch the stuff in this class for a long time, so it will be good for our future selves to have resources.
  - List of references that people can go to is really important (online, published articles, etc.).
  - Stay within the allotted time no matter what.
  - Report and slides can be the same, but just make sure that all of your references go in the slides, too.

Week 2

Project 1 Presentations

2.1 Introduction to Proton, Carbon, Nitrogen, and Phosphorus

1/11:

- Our presentation.
- ^{13}C NMR presentation (Angel, Nate).
  - Broadband decoupled ^{13}C NMR gives no coupling with protons, so the number of peaks is the number of distinct carbons.
  - Low abundance of ^{13}C gives 100 times weaker signal than ^{1}H.
  - Gyromagnetic ratio γ is 1/4 that of ^{1}H.
    - The signal intensity is proportional to γ^3, so overall, proton signal is about 6400 times stronger than ^{13}C.
  - Solution: Increase sample concentration, longer relaxation delay (d1), higher field strength NMR (600 MHz), DEPT, 2D NMR.
  - Chemical shifts: 0-220 ppm.
    - Two regions: Above and below 100 ppm.
    - Aliphatic: 0-50 ppm.
    - EWG-substituted aliphatic: 50-100 ppm.
    - Aromatic: 100-150 ppm.
    - Since carbon is more electronegative than hydrogen, adding carbon substituents shifts signals downfield.
    - Resonance structures and partial charges can help predict shifts.
    - Steric effects: Up to 10 ppm shifts from van der Waals interactions of atoms being near each other, especially in rigid molecules.
  - Impurities.
    - $CDCl_3$ has an equally heighted triplet at 77 ppm due to the spin 1 deuteron splitting the carbon peak into 3 peaks of equal height.
  - Functional groups (shifts and couplings).
    - Alkenes: 100-150 ppm.
      - ➢ One-bond coupling of about 150 Hz.
    - Alkynes: 70-90 ppm.
      - ➢ Results from differences in electronic configuration around the carbon nuclei.
      - ➢ One-bond coupling to proton in acetylene of about 249 Hz (*sp*-hybridized carbons have huge couplings; shorter bonds!).
      - ➢ Two-bond coupling to other proton of about 49 Hz.

        - Aldehydes.
        - Halides.
            - Big bulky electron density on iodine pushes shift for alkyl iodides to −20 to −40 ppm.
    - Why isn't ^{13}C NMR quantitative?
        - We'll talk about it, but it might have something to do with NOESY.
        - Polarization transfer can amplify signals *and* decouple.
        - Turning off NOE, very long relaxation delay, and can make ^{13}C NMR quantitative!
    - For ^{1}H, we don't have an issue with chemical shift anisotropy. For almost any heteroatom (and carbon), we will have this issue. And it increases with the square of the field strength, so there's an ideal field strength range for carbon NMR whereas for proton, you can go as high as they make them.
- ^{15}N and ^{31}P NMR (Natalie, Rosalind).
    - For both nuclei: Typical chemical shifts, proton-heteroatom coupling constants, and what this can look like in biomolecular NMR.
    - ^{15}N.
        - Spin 1/2.
        - 0.37% abundant.
        - Low γ.
        - $>$ 1000 ppm range of chemical shifts.
        - Most groups fall within 0-500 ppm. Metal nitrosyl (M−NO) complexes are roughly 300-1200 ppm, but this is helpful for identifying metal complexes (such as iron sulfur complexes, i.e., metalloproteins which store or transport NO)!
        - Proton-nitrogen couplings are difficult to detect. Magnitude affected by solvent used as well as intermolecular interactions (e.g., hydrogen bonding).
        - A variety of techniques be used to study biomolecules (e.g., at MIT in Mei Hong's lab). HSQC experiments, solid-state, isotopic labeling, and many more.
        - DNA is only made of four simple nitrogen-containing heterocycles, so looking at isolated nucleotides can be very helpful.
        - Shifts affected by post-translational modifications, DNA shape, protonation, etc.
        - 100-130 ppm for backbone nitrogens in proteins, varies drastistically for side chains (30-220 ppm).
        - HSQC is a protein fingerprint, as well as the gateway into deuterium exchange experiments. Can be used to study the folding of proteins.
    - ^{31}P.
        - Spin 1/2 and 100% isotopic abundance. Thus, very easy to measure!
        - 2000 ppm shift range.
            - Upfield defined by P_4 at −527 ppm.
            - Downfield defined by...
        - Proton-phosphorus J-coupling allows us to tell how far part phosphorus and hydrogen atoms are. Very useful tool!
            - Great examples in the slides.
        - Chirality determination with a chiral phosphorus reagent and ^{31}P NMR.
        - ^{31}P NMR in DNA.
            - Gives information about backbone conformation (e.g., A vs. B vs. Z).
            - Dickerson dodecamer backbone; researchers were able to correlate ^{31}P NMR shift with the percent of a certain conformation in the sample.
        - Cummins and Radosevich labs will have a lot to say on ^{31}P-^{31}P couplings!
    - These nuclei are also not often studied at higher fields; you lose stuff even at 600 MHz.