5.46 (NMR Spectroscopy and Organic Structure Determination) Problem Sets

Steven Labalme

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# Contents

# 1   Working with NMR Spectra

2/19:   1. One of the directories in the 5.46_NMR_data folder is called 'glucose2_320mM'; inside this directory
          are a number of datasets which were acquired at different times or using different NMR experiments.

      a) This dataset was, surprisingly, generated from a 320 mM sample of glucose. Glucose exists as a
          mixture of α- and β-anomers. Draw the structures of each in chair form, and label each anomer.

          Answer.
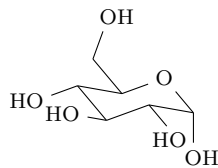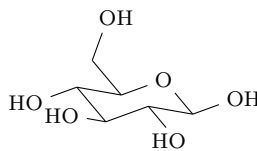
          (a) α-D-glucose.   (b) β-D-glucose.
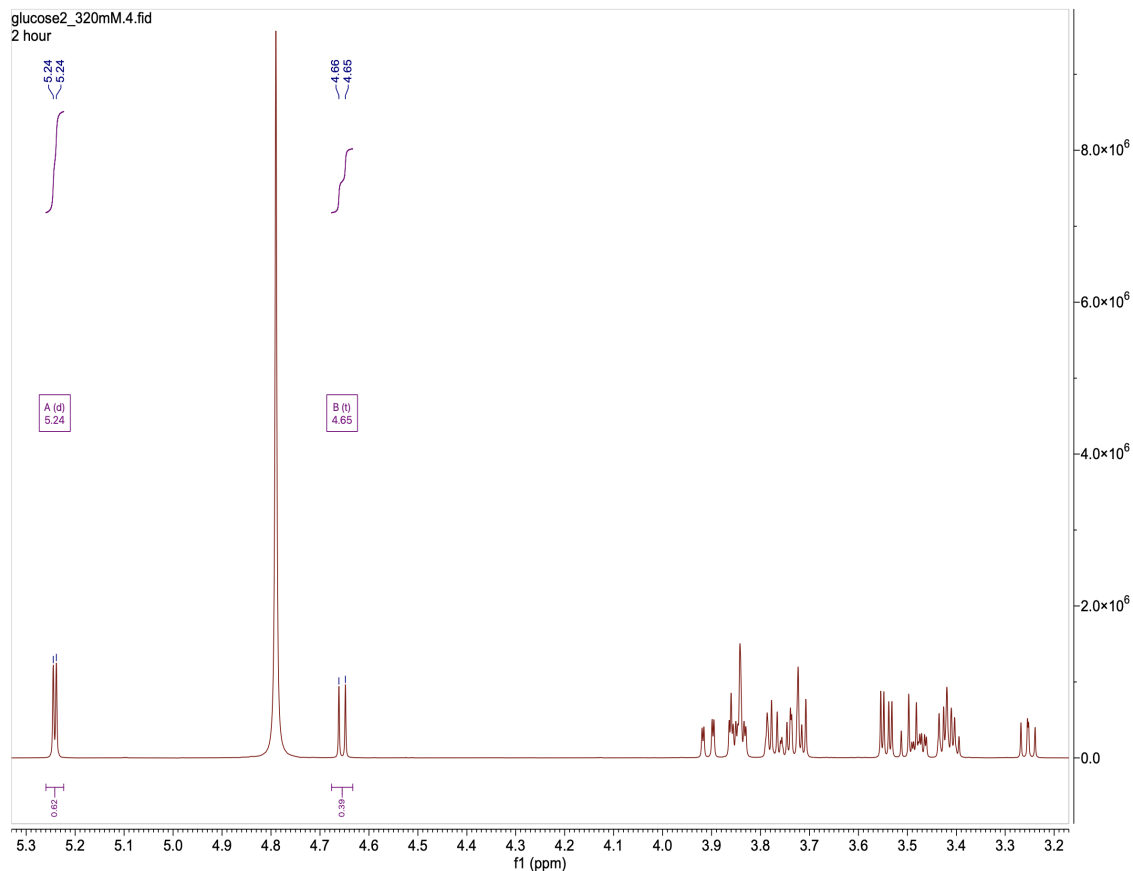
          □

      b) Find experiment #4, load it into MestReNova or TopSpin, and generate a properly phased spec-
          trum. Use the software to integrate the peaks or regions, and expand the spectrum to include
          only the region around water to include the anomeric protons.

          Answer.

          □

c) Measure the $^3J_{HH}$ coupling constant and the $^1J_{CH}$ coupling constant for each anomeric proton. Make a table showing the chemical shift for each anomeric proton, the relative integrals (which should total 100%), and the two coupling constants.

Answer.

Pyranose	Chemical Shift	Integral	$^3J_{HH}$	$^1J_{CH}$
α-D-glucose	5.24 ppm	61%	3.81 Hz	169.50 Hz
β-D-glucose	4.65 ppm	39%	7.96 Hz	164.62 Hz

□

d) Using the fact that the β-anomer is expected to be about 60% of the total, rationalize the variation in chemical shift and coupling constants for each.

Answer. Contrary to the expected integrals, the peak characteristic of the α-anomer integrates to approximately 61% of the total in the provided data file. Perhaps the anomers were taken out of equilibrium before the spectrum was taken and are in the process of reequilibrating.

Regardless, the β-anomer's anomeric proton should be more shielded because of the anomeric effect's no-bond resonance placing a δ^- on that proton; such resonance does not occur in the α-anomer. This effect is clearly observed, as the β-anomer's doublet is 0.59 ppm upfield from the α-anomer's doublet. Note that the downfield doublets were originally identified as the anomeric peaks because the anomeric protons should be the most deshielded due to their position on the glucose hemiacetal (the position nearest to the most electron-withdrawing hydroxyl groups).

Additionally, the β-anomer's $^3J_{HH}$ coupling constant should be larger than the α-anomer's due to the Karplus correlation. Indeed, the β-anomer's anomeric proton is oriented antiperiplanar to the C2 proton, while the α-anomer's anomeric proton is oriented gauche. This effect is also clearly observed, as the β-anomer has a $^3J_{HH}$ value 4.15 Hz greater than the α-anomer's $^3J_{HH}$ value.

Lastly, the β-anomer's $^1J_{CH}$ coupling constant should be smaller than the α-anomer's because the relevant C–H bond is longer in the β-anomer. This bond elongation is again a result of the anomeric effect, specifically $n_O \rightarrow \sigma^*_{CH}$ donation. This effect is again observed, as the β anomer has a $^1J_{CH}$ value 4.88 Hz smaller than the α-anomer's $^1J_{CH}$ value.   □

2. A second directory in the data folder is called 'glucose1_320mM'; this sample is also glucose at 320 mM with one difference – this glucose is fully ^{13}C labeled. Analyze the anomeric region of spectrum #4 in the same way as you did for the first sample. Explain the difference in apparent anomeric ratio as best you can.

Answer.

Pyranose	Chemical Shift	Integral	$^3J_{HH}$	$^1J_{CH}$
α-D-glucose	5.24 ppm	62%	??	169.92 Hz
β-D-glucose	4.65 ppm	39%	??	165.08 Hz

Of note is that the central chemical shift values do not change (as expected), and the coupling constants only increase marginally (perhaps insignificantly). The $^1J_{CH}$ values are also difficult to measure — especially for the α-anomer — because of the appearance of apparently new couplings that split the peaks into pseudo-quartets.

Additionally, there does not appear to be a significant difference in the anomeric ratio. Rather, the $\alpha : \beta$ ratio appears to be approximately 62 : 38 instead of 61 : 39.   □

3. For the spectra you have been working with, measure the amount of water relative to the glucose. Estimate the amount of water, and think about where this water signal comes from. Which glucose sample has more water in it?

Answer. The residual solvent peak at $4.79\,\text{ppm}$ in glucose2_320mM.4.fid integrates to 3.82 times the height of the sum of the anomeric peaks. This means that for each glucose anomeric proton in solution, there are 3.82 water protons. Since there is one glucose anomeric proton per glucose molecule but two water protons per molecule, it follows that there are 1.91 water molecules for each glucose molecule in solution. Thus, the concentration of water in this sample is

$$1.91 \times 320\,\text{mM} = 611\,\text{mM}$$

By an analogous calculation, the concentration of water in glucose1_320mM.4.fid is

$$2.01 \times 320\,\text{mM} = 643\,\text{mM}$$

Thus, $\boxed{\text{glucose1\_320mM.4.fid has more water in it.}}$ The water signal most likely comes partially from leftover H_2O contamination in the isotopically enriched D_2O solvent, and partially from proton-deuterium exchange between D_2O and undeuterated glucose.   □

2   Signal-To-Noise Ratio

2/25:   We often measure the sensitivity of a system using a standard sample and a standard experimental design; this allows us to compare one NMR system to another, or one probe to another in the same NMR system. It also allows us to estimate the result we can expect when we run a real sample (one that we care about).

For this exercise we will use data collected for adenosine in d6-DMSO to compare some of the systems in the DCIF with respect to both proton and carbon acquisition. The method is as follows — choose the largest adenosine signal in the spectrum (do not use the water or DMSO peaks) and use either MestReNova (the non-interactive approach is fine) or Topspin to measure SNR (the signal-to-noise ratio) using the software. For the proton spectrum one of the aromatic singlets will be largest; for the carbon spectrum, it will be one of the sugar carbons.

1. For the 20 mM adenosine data acquired at 500 MHz, experiments 1 and 2 are proton acquisitions using 16 and 128 scans, respectively. Measure the SNR for each and confirm that the increase in SNR with scans matches what you expect.

    Answer. For both experiments 1 and 2, I first applied Auto Phase Correction and Auto Baseline Correction, and referenced the central DMSO peak to 2.50 ppm. Then, I used MestReNova's Tools > NMR Tools > SNR Calculation Non-Interactive. . . with Noise Region 8.70-8.65 ppm and Signal Region 8.38-8.31 ppm. The results are as follows.

Experiment	SNR
1	8388.99
2	25 299.58

    Since experiment 2 has eight times as many scans, its SNR should be $\sqrt{8}$ times as high as experiment 1. In reality, $8388.99 \cdot \sqrt{8} = 23727.6$, so we have about 6% error.   □

2. Compare experiment 1 of the 20 mM adenosine sample from the 500 with experiment 1 of the 10 mM sample from the 500 (labeled `5.46_2025_adenosine_10mM_500`), and again confirm that the difference in SNR is what you expect.

    Answer. Using the same settings as in Q1, the SNR is 3886.10. Halving the concentration should halve the sensitivity. In reality, $8388.99/2 = 4194.50$, so we have about 7% error.   □

3. Now compare the SNR for the 10 mM adenosine samples for both proton (experiment 1) and carbon (experiment 2) across all three NMR spectrometers (400, 500, and 600). For both proton and carbon, how many scans would you need at 400 MHz and 500 MHz to give you the same SNR as you see at 600 MHz?

    Answer. For each proton experiment, I used the settings described in my answer to Q1. For each carbon experiment, I used Noise Region 72-71 ppm and Signal Region 70.8-70.5 ppm.[1] The results are as follows.

Spectrometer	Experiment	SNR
400	1	1536.43
	2	28.86
500	1	3886.10
	2	43.00
600	1	17 821.86
	2	54.98

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[1]This is after once again applying Auto Phase Correction, applying Auto Baseline Correction, and referencing the central DMSO peak to 39.52 ppm.

To achieve the same SNR on a $400\,\text{MHz}$ ^1H spectrum as on a $600\,\text{MHz}$ ^1H spectrum, you would need

$$\left\lceil 16 \cdot \left(\frac{17821.86}{1536.43}\right)^2\right\rceil = \lceil 2153\rceil = \boxed{4096\,\text{scans}}$$

To achieve the same SNR on a $500\,\text{MHz}$ ^1H spectrum as on a $600\,\text{MHz}$ ^1H spectrum, you would need

$$\left\lceil 16 \cdot \left(\frac{17821.86}{3886.10}\right)^2\right\rceil = \lceil 337\rceil = \boxed{512\,\text{scans}}$$

To achieve the same SNR on a $400\,\text{MHz}$ ^{13}C spectrum as on a $600\,\text{MHz}$ ^{13}C spectrum, you would need

$$\left\lceil 4096 \cdot \left(\frac{54.98}{28.86}\right)^2\right\rceil = \lceil 14866\rceil = \boxed{16384\,\text{scans}}$$

To achieve the same SNR on a $500\,\text{MHz}$ ^{13}C spectrum as on a $600\,\text{MHz}$ ^{13}C spectrum, you would need

$$\left\lceil 4096 \cdot \left(\frac{54.98}{43.00}\right)^2\right\rceil = \lceil 6697\rceil = \boxed{8192\,\text{scans}}$$

□

4. From the results in Q3, use the DCIF fee schedule to calculate the cost of achieving the same SNR for the AVIII 400, Neo500, and Neo600. Assume the proton spectra take 6 min and the carbon spectra take 72 min.

Answer. We will assume that multiplying the number of scans by n makes the experiment take n times as long and hence cost n times as much. Let's begin.

Since Q3 tells us that it takes $4096/16 = 256$ times as many scans on the 400 to reach the SNR of a 600 proton experiment, the cost will be

$$256 \times \frac{6\,\text{min}}{1} \times \frac{1\,\text{h}}{60\,\text{min}} \times \frac{\$28}{1\,\text{h}} = \boxed{\$716.80}$$

Since Q3 tells us that it takes $512/16 = 32$ times as many scans on the 500 to reach the SNR of a 600 proton experiment, the cost will be

$$32 \times \frac{6\,\text{min}}{1} \times \frac{1\,\text{h}}{60\,\text{min}} \times \frac{\$35}{1\,\text{h}} = \boxed{\$112.00}$$

Assuming it only takes 6 min to run a 600 proton experiment, the cost will be

$$6\,\text{min} \times \frac{1\,\text{h}}{60\,\text{min}} \times \frac{\$38}{1\,\text{h}} = \boxed{\$3.80}$$

Running analogous calculations for ^{13}C gives $\boxed{\$134.40, \$84.00, \text{ and } \$45.60, \text{ respectively.}}$ □

5. Finally, using the $10\,\text{mM}$ $400\,\text{MHz}$ proton and carbon spectra, measure the SNR with different line broadening (apodization) values — $0\,\text{Hz}$, $1\,\text{Hz}$, $5\,\text{Hz}$, $10\,\text{Hz}$, and $20\,\text{Hz}$. Which line broadening gives the highest SNR for each nucleus? Why?

Answer. For the proton and carbon experiments, I used the settings described in my answers to Q1 and Q3, respectively. The results are as follows.

Experiment	Apodization (Hz)	SNR
1	0	516.91
	1	1783.98
	5	1643.11
	10	1058.36
	20	486.38
2	0	21.16
	1	27.24
	5	34.14
	10	33.98
	20	22.75

1 Hz gives the highest SNR for ^{1}H, and 5 Hz gives the highest SNR for ^{13}C.

Aquisition times for nuclei other than ^{1}H (e.g., ^{13}C) are typically cut short, so a more extreme window function helps to smooth out the FID before Fourier transforming it.   □

3 Peak Assignments

3/6: Darolutamide is an anti-androgen compound used in the treatment of prostate cancer in older men.
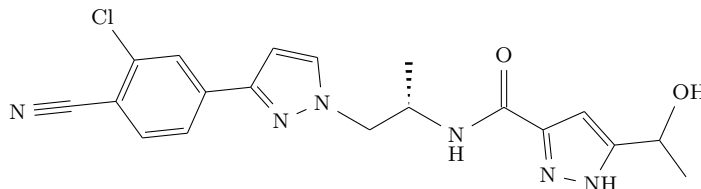
A single 300 mg dose tablet weighs approximately 610 mg. A sample of darolutamide was produced by crushing a single tablet, adding approximately 1.5 mL of d6-DMSO, mixing thoroughly, and then spinning the mixture in a microfuge at $10\,000 \times$ g for 10 min to separate the liquid phase. This solution was used to generate the data in the `unknown-D_5.46_2025` dataset. The experiments in the dataset are as follows.

1. ^{1}H 1D.

2. ^{13}C 1D.

4. DEPT 135 (^{13}C).

5. ^{1}H COSY.

6. ^{1}H-^{13}C HSQC.

7. ^{1}H-^{13}C HMBC.

8. ^{1}H-^{15}N HSQC.

The sample is clearly a mixture of the known compound (darolutamide) and excipients/fillers from the tablet. Use everything you know about NMR so far to identify the proton and carbon signals that correspond to the drug to the best of your ability. Your final result should be a structure with the carbons labeled according to the ^{13}C signals in the spectrum, where possible from the data, starting with the carbon at 147.8 ppm labeled as carbon #1, and a table of proton/carbon correlations with proton chemical shifts and multiplicities, similar to what we did in class with adenosine. Indicate where you used the COSY and HMBC data to support your conclusions.

Answer. To the best of my ability, here are the assignments for the darolutamide portion of the provided data.
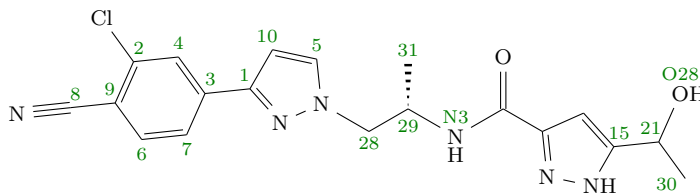
Here is the desired table.

MNova #	Carbon #	^{13}C (ppm)	DEPT	^{1}H (ppm)	^{1}H Multiplicity	COSY	HMBC
11	1	147.46	Quat				4,5,6(w),7,10
15	2	139.63	Quat				4,6,7
13	3	135.98	Quat				4,6,7
14	4	135.05		7.93	s		
9	5	133.24		7.78	d	10	10,28
17	6	125.77		8.03	dt	7	
18	7	124.20		7.93	s	6	6
20	8	116.36	Quat				4,6(w),7(w)
16	9	110.00	Quat				4,6,7
10	10	104.25		6.88	d	5	5
24	15	81.44					O28
26	21	73.36		4.79	q	30,O28	
1	28	55.81	CH$_2$	4.27,4.35	dd,dd	29	5,29,31,N3
2	29	44.92		4.45	dq	N3,28,31	28,31,N3
27	30	23.87		1.38	d	21	
8	31	18.07		1.12	d	29	28,29,N3
3	N3			8.23	d	29	
28	O28			3.35	s	21	

And here are the numbered ^{1}H and ^{13}C NMR spectra, still with the original MNova numbers.
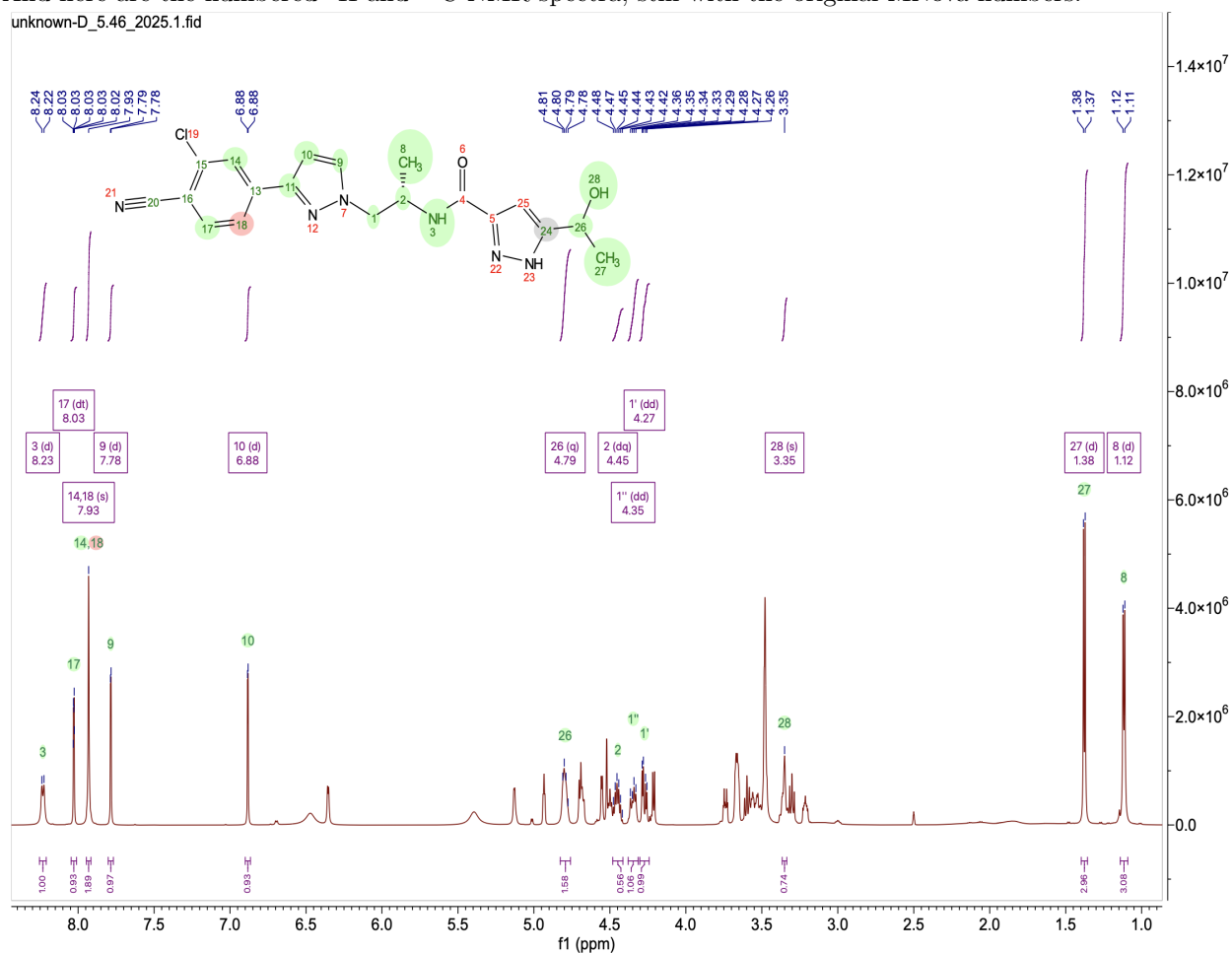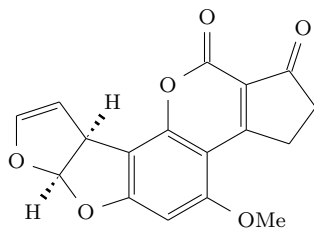
unknown-D_5.46_2025.2.fid
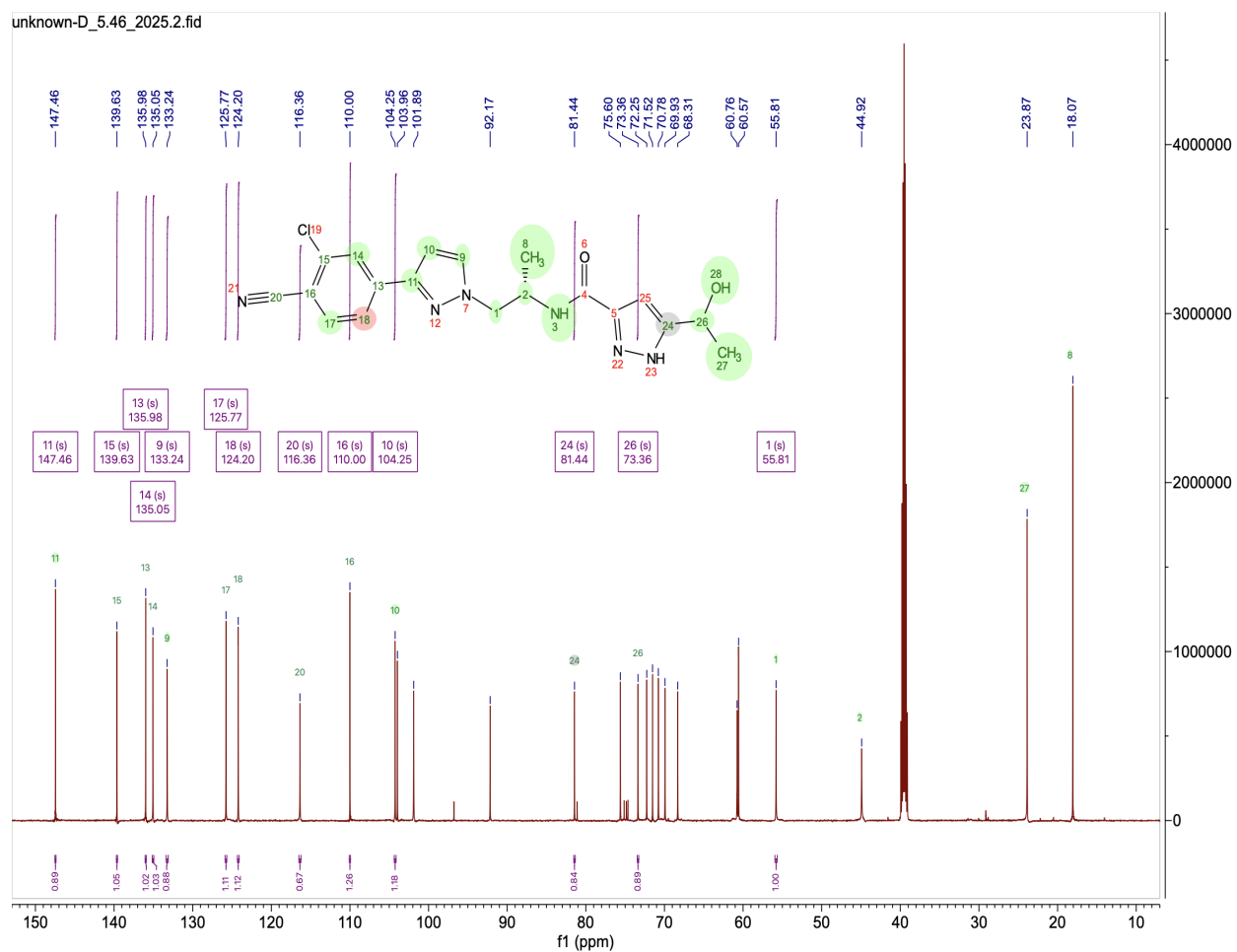
# 4   Determining Stereochemistry

3/18: Aflatoxin B1 is a toxin produced by a fungus which grows on a number of plant species, but is best known for causing liver carcinogenicity from contaminated peanuts.
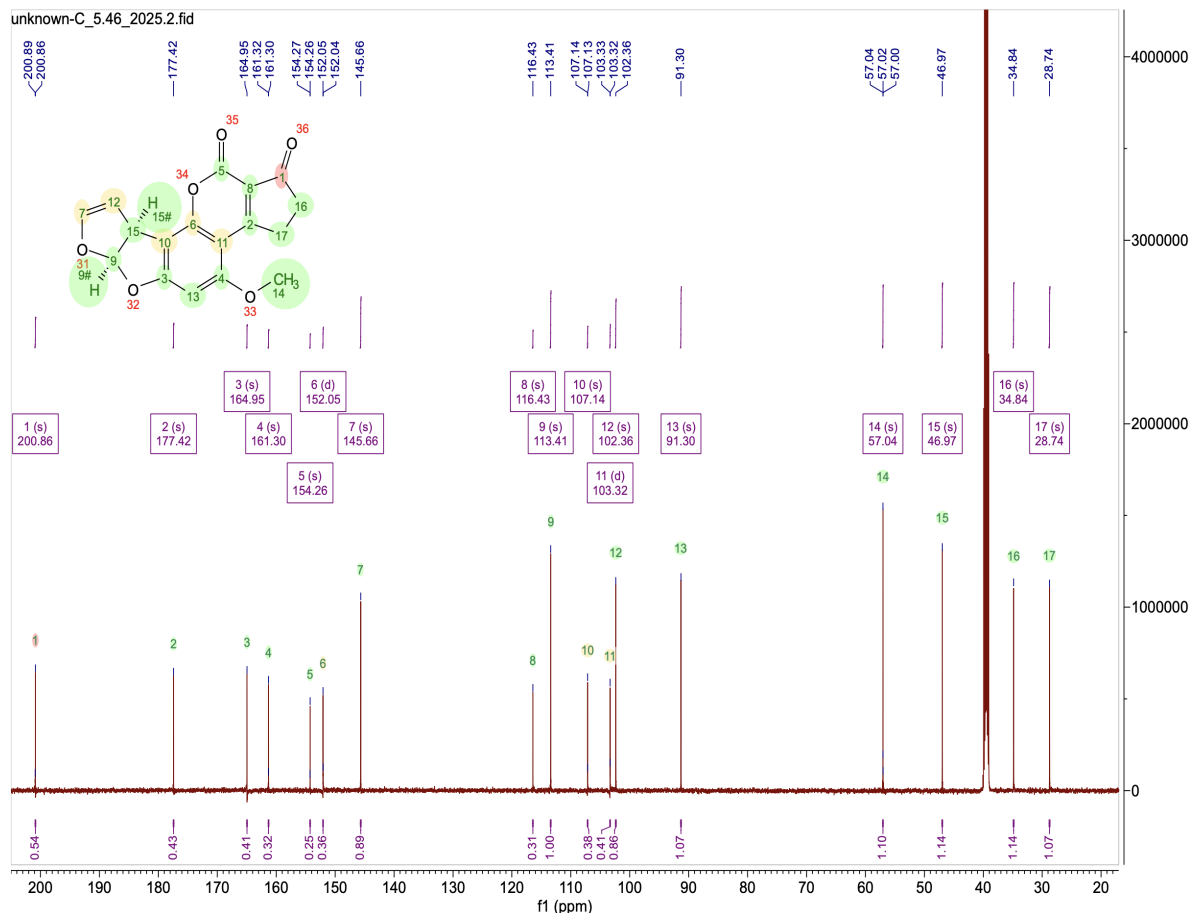

The `unknown-C_5.46_2025` dataset contains a series of spectra which you should be able to identify (if this is untrue, let me know and I'll send out a list). For this exercise, I'd like you to identify the ROESY crosspeaks in experiment #9 and the NOESY crosspeaks in #37 as follows.

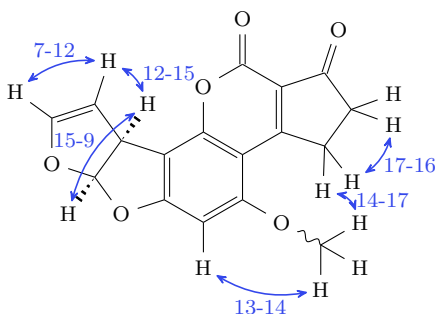1. Assign all the protons in the molecule using the standard ^{13}C-directed approach.

Answer.
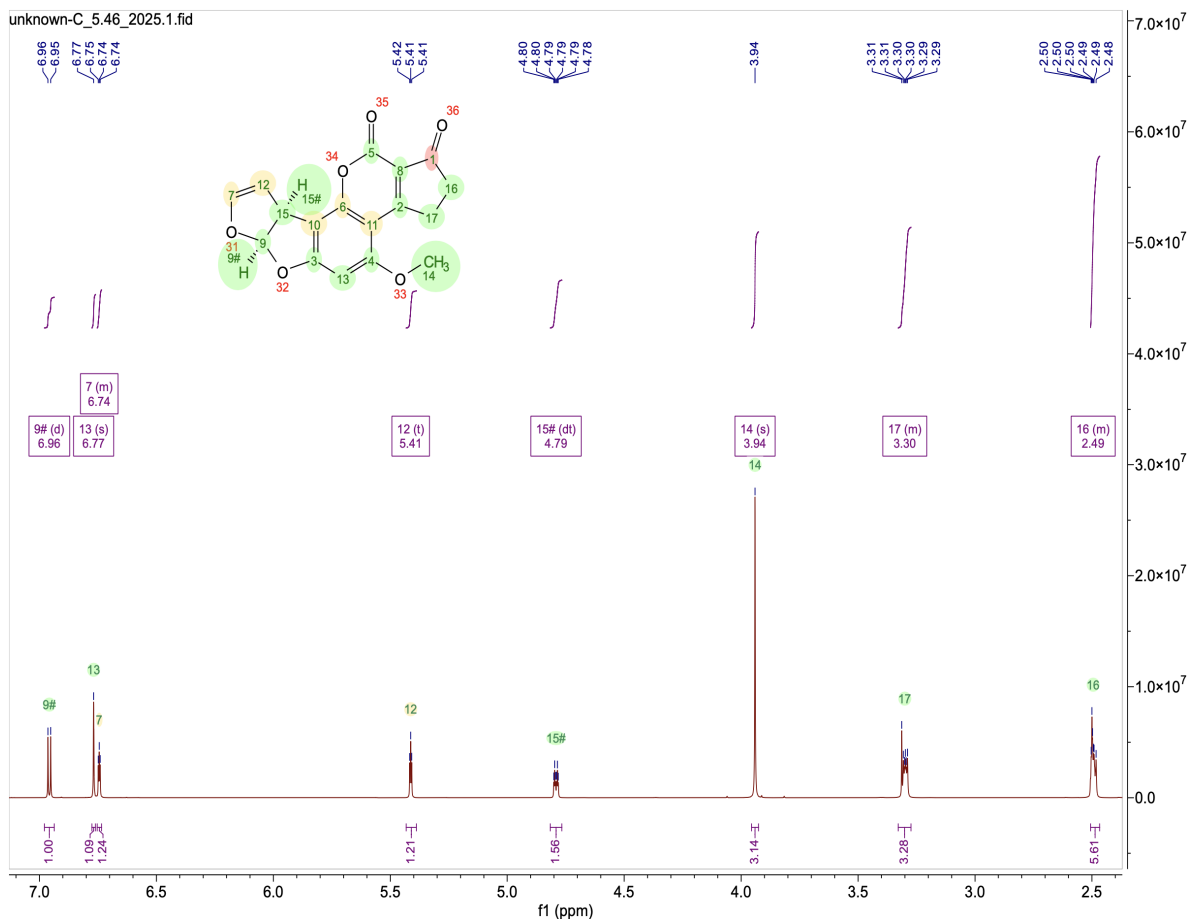
2. Show the ROE/NOE crosspeaks on the structure of Aflatoxin B1 and explain whether any are missing that you would expect.

Answer.

Some cross peaks are small, but all the ones I expect to see are distinguishable from noise at some level of zoom on both spectra. The 14-17 crosspeak is the hardest to distinguish on both spectra.   □

3. Confirm the stereochemistry of the bridged ring.

Answer. Since we can observe a significant 15-9 NOESY, the saturated bridgehead carbons on the western fragment of Aflatoxin B1 must bear two *cis* protons. Without a chiral resolving reagent, it is impossible to determine the absolute stereochemistry of the molecule.   □

4. Experiment #13 shows a ^{1}H-^{13}C HSQC in which the decoupling is turned off during acquisition; indicate on the structure which proton-carbon couplings are larger than usual and explain why you think this is.

Answer. To begin, here is an accounting of all $^1J_{\mathrm{CH}}$ coupling constants in the visible in the coupled HSQC spectrum.

Proton #	Shift (ppm)	$^1J_{\mathrm{CH}}$ (Hz)
9	6.96	188.41
13	6.77	167.62
7	6.74	202.33
12	5.41	181.30
15	4.79	146.88
14	3.94	146.80
17	3.30	139.69
16	2.49	132.41

The largest proton-carbon coupling constant corresponds to an sp^2-hybridized carbon-proton bond, as we might expect since increasing s-character means shorter bonds and more coupling. However, with all of the strain in this molecule, it is very difficult to qualitatively predict the magnitude of the $^1J_{\mathrm{CH}}$ couplings.   □