

**Exam 1
Answer Key**

Exam 1 Mean: 64
Exam 1 Median: 67
Exam 1 St. Dev.: 18

1. 4-Hydroxyproline (4HP) has some protons that will exchange with the deuterium in D₂O—the ammonium protons, and the -OH proton—and that, as a result, won't show up in the ¹H NMR spectrum. The other 6 protons in 4HP are inequivalent. The cationic nitrogen, the -OH group, and the acid carbonyl group are all deshielding, but not by the same amounts; Pretsch's summary table for alkanes (p. 160 in the 4th edition) says that the downfield shifting effect (Z_{α}) of an -OH group is the largest, followed by cationic nitrogen, and then the carbonyl group. That means I would expect that H₄ would be the most downfield, followed by H₂ (which is adjacent to both cationic nitrogen and a carbonyl group), then H_{5a} and H_{5b}, then last H_{3a} and H_{3b}. So our initial assignments would be:

δ (ppm)	Name of proton (H _n)	Coupling constants J (Hz)	Assign coupling constants $J(H_m, H_n)$
4.66	H ₄		
4.33	H ₂		
3.47	H _{5a/b}		
3.35	H _{5a/b}		
2.42	H _{3a/b}		
2.15	H _{3a/b}		

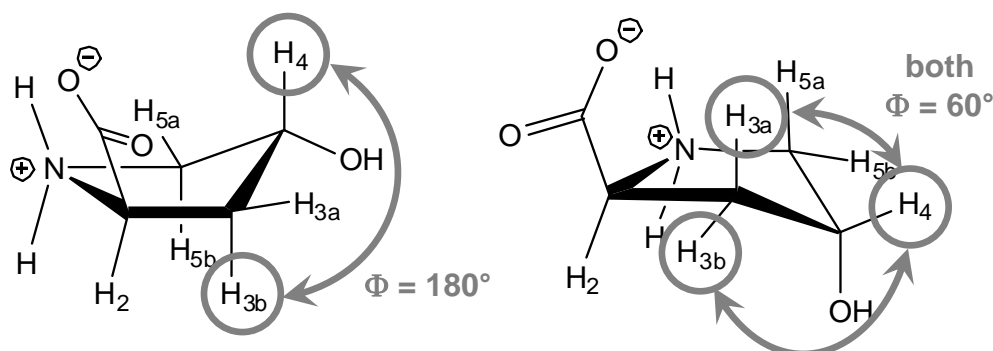
These assignments are consistent with the splitting observed for some of the multiplets, but at first glance, maybe not all of them. The resonance at $\delta = 4.33$ ppm (H₂) is a doublet of doublets, with two coupling partners, just like H₂ should have (to both H₃ protons); $\delta = 3.47$ ppm (H_{5a/b}) is also a doublet of doublets, with two coupling partners (the other H₅ and H₄); and $\delta = 2.15$ ppm (H_{3a/b}) is a doublet of doublet of

doublets, with three coupling partners, just like H₃ should have (to the other H₃, to H₂, and to H₄). The resonance at $\delta = 4.66$ ppm is difficult to interpret, even with resolution enhancement, so we can't say much there; we do know, however, that none of the four coupling constants we'd expect for H₄ could be very big. And, most puzzling, it looks like the resonances at $\delta = 3.55$ and 2.42 ppm have an *extra* coupling constant each, beyond what we'd expect for simple geminal and vicinal coupling alone. We'll see what that's about later.

We can start assigning coupling constants from the H₂ side:

δ (ppm)	Name of proton (H _n)	Coupling constants <i>J</i> (Hz)	Assign coupling constants <i>J</i> (H _m ,H _n)
4.33	H ₂	10.2 7.9	<i>J</i> (H ₂ ,H _{3a/b}) <i>J</i> (H ₂ ,H _{3a/b})
2.42	H _{3a/b}	14.1 7.9 1.8 1.8	<i>J</i> (H _{3a/b} ,H _{3a/b}) <i>J</i> (H ₂ ,H _{3a/b})
2.15	H _{3a/b}	14.4 10.3 4.4	<i>J</i> (H _{3a/b} ,H _{3a/b}) <i>J</i> (H ₂ ,H _{3a/b})

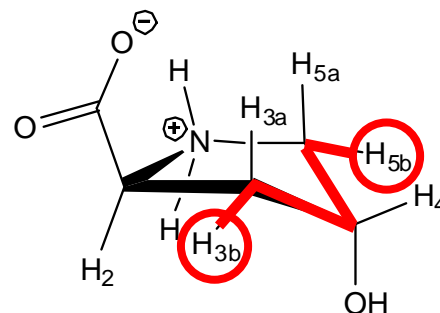
At this point in the analysis, I'm not sure we can choose which H₃ proton is 3a or 3b, but we can assign all the coupling partners between H₂ and the two H₃'s. In addition, we can predict that each H₃ should couple to H₄, that one constant in each box should be *J*(H₃,H₄). Both of the proposed *J*(H₃,H₄) values are small, suggesting that the dihedral angle between each H₃/H₄ pair should be close to 90°. Out of our two 4HP conformers, that is only true for the pseudo-axial OH; in the pseudo-equatorial OH conformer, there is a 180° dihedral angle that you'd expect to give a large *J*:



I think that means we can make the rest of our assignments under the assumption that the conformer on the right is the most representative (though, as you'll see later, you still got credit if you picked the wrong conformer). If that's true, both $J(H_4, H_{5a/b})$ coupling constants should also be small—because both H_4 - H_5 dihedral angles are also 60° —and the big coupling constant shared by the H_5 protons must be due to geminal coupling.

δ (ppm)	Name of proton (H_n)	Coupling constants J (Hz)	Assign coupling constants $J(H_m, H_n)$
4.66	H_4		
4.33	H_2	10.2 7.9	$J(H_2, H_{3a/b})$ $J(H_2, H_{3a/b})$
3.47	$H_{5a/b}$	12.6 3.8	$J(H_{5a/b}, H_{5a/b})$ $J(H_4, H_{5a/b})$
3.35	$H_{5a/b}$	12.6 1.5 1.5	$J(H_{5a/b}, H_{5a/b})$ $J(H_4, H_{5a/b})$??
2.42	$H_{3a/b}$	14.1 7.9 1.8 1.8	$J(H_{3a/b}, H_{3a/b})$ $J(H_2, H_{3a/b})$ $J(H_{3a/b}, H_4)$??
2.15	$H_{3a/b}$	14.4 10.3 4.4	$J(H_{3a/b}, H_{3a/b})$ $J(H_2, H_{3a/b})$ $J(H_{3a/b}, H_4)$

The only loose J assignment we haven't made is the one marked ?? above, which must correspond to a long-range 4J between one H_3 proton and one H_5 proton. Four-bond coupling in alkyl systems is sometimes called "W-coupling", because the optimal arrangement of atoms is a W shape. In our preferred conformer, this could only happen between H_{3b} and H_{5b} .



This allows us to complete the table.

δ (ppm)	Name of proton (H_n)	Coupling constants J (Hz)	Assign coupling constants $J(H_m, H_n)$
4.66	H_4		
4.33	H_2	10.2 7.9	$J(H_2, H_{3a})$ $J(H_2, H_{3b})$
3.47	H_{5a}	12.6 3.8	$J(H_{5a}, H_{5b})$ $J(H_4, H_{5a})$
3.35	H_{5b}	12.6 1.5 1.5	$J(H_{5a}, H_{5b})$ $J(H_4, H_{5b})$ $J(H_{3b}, H_{5b})$
2.42	H_{3b}	14.1 7.9 1.8 1.8	$J(H_{3a}, H_{3b})$ $J(H_2, H_{3b})$ $J(H_{3b}, H_4)$ $J(H_{3b}, H_{5b})$
2.15	H_{3a}	14.4 10.3 4.4	$J(H_{3a}, H_{3b})$ $J(H_2, H_{3a})$ $J(H_{3a}, H_4)$

Rubric: (46 points total this part.)

We did not grade your a/b assignments in this problem—we only graded the number on the H. (I think you needed to get the letter correct for stuff later on in the exam, but we didn't take off points for that here.)

If you put incorrect entries in the “name of proton” column, the graders did their best to use this information to give you credit in the “assign coupling constants” column. In other words, you didn't have to get the first column right to receive points for the third, but the two columns did have to match.

3 points each proton number. Switching a and b is fine.

1 point each coupling constant value, to within 0.5 Hz.

1 point each coupling assignment.

Assignment must match number in column 2; no points for just having a correct pair name in column 3.

2. The -OH proton in 4HP can't be seen in the ^1H NMR spectrum, so we don't know what its chemical shift might be. How might we change the experiment so that we could see this proton's ^1H NMR resonance, and assign it a chemical shift? Name two changes we might make.

Use an aprotic NMR solvent in place of D_2O , to avoid chemical H/D exchange.

3

Make the solution dilute to avoid H-bonding. (Though, this wouldn't work in D_2O —you'd still need to run in aprotic solvent.)

3

If using an aprotic solvent, make sure it's rigorously dried to avoid H-bonding.

3

Some answers that we didn't accept:

- Changing delay times. Exchange and H-bonding in the -OH group isn't affected by the delay time τ —the problem here isn't T_1 .
 - Lowering the sample temperature. This won't change the exchange of H for D, and even if the experiment were performed in an aprotic solvent, changes in H-bond geometry and proton exchange are so rapid that it would be difficult to freeze them out.
3. One of the multiplets in the ^1H NMR is labeled "resolution enhanced". Briefly, explain what must be done to NMR data to achieve resolution enhancement. Why does resolution enhancement help?

In resolution enhancement, the FID is multiplied by a "window function"—usually a function that de-emphasizes the very beginning and the very end of the FID trace. (The most common functions are a combination of an exponential decay and a Gaussian function.)

Resolution enhancement helps because it addresses two problems in FID quality: detector overload at early times, and poor signal-to-noise at long times. It isn't really correct to say it helps because it sharpens peaks that are broadened; it doesn't help at all if the source of the broadening is chemical exchange or rotational restriction, only if the problem is with the FID.

Rubric: (10 points total this part.)

5 points for defining resolution enhancement.

2 points for stating that method operates on "FID". (Just writing "FID" is enough, really.)

3 points for stating that method applies a window function to the FID. (Just "function" is enough.)

5 points for explaining how resolution enhancement helps with detector overload & signal-to-noise.

3 points partial for just one of these two justifications.

No partial credit for just saying it makes the peaks look nicer.

4. Which conformation of 4HP predominates?

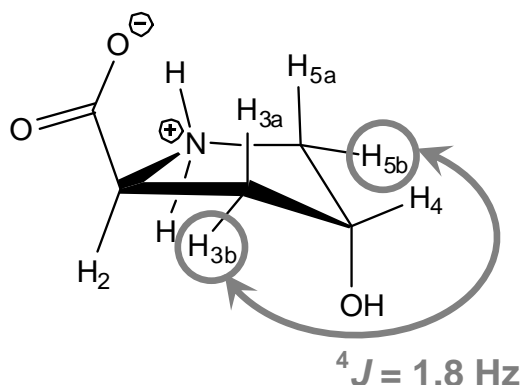
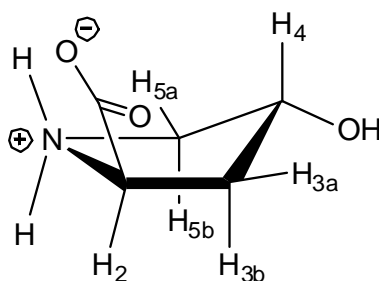
Circle one:

this one ?

or

this one ?

5

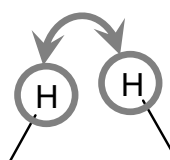


5. Does 4HP exhibit any long-range (4-bond or 5-bond) ^1H - ^1H coupling? If so, circle the two coupled protons on your chosen structure above, and connect them with a double headed arrow. Then, label the arrow with the appropriate coupling constant and J type.

(Answer explained in problem 1.)

answer format:

$^5J = 3.6 \text{ Hz}$



Rubric: (8 points total this part.)

No need to get problem 4 correct to get these points.

4 points for drawing a W-coupling.

Could also draw H_{3a} - H_{5a} coupling on structure on the left and get these points.

2 points partial for drawing a non-W 4J between an H_3 and an H_5 .

No partial credit for drawing a different 4-bond coupling, or a 5-bond coupling.

2 points for accurately naming it a 4J .

2 points for coupling constant (within 0.5 Hz).

6. In the chart below, assign each resonance in the ^{13}C spectrum to a carbon in 4HP. If there are chemical shifts you feel you cannot assign definitively to one carbon, but you can narrow it down to a few, name all possible carbons in the corresponding box.

Out of the five peaks in the ^{13}C spectrum, I think only one is obvious: the carbonyl carbon at $\delta = 177$ ppm. Looking at our molecule, we have four other carbons to assign:

- One that's α to an alcohol group (C_4);
- One that's α to both a carbonyl and a cationic nitrogen atom (C_2);
- One that's α to just a cationic nitrogen atom (C_5);
- And one that's not α to anything (C_3).

Pretsch has a number of additivity tables that predict the effect of substituents on ^{13}C chemical shifts. In general, those tables say that the $-\text{OH}$ group has the most influence, but that the sum of effects of an $\alpha\text{-C=O}$ and an $\alpha\text{-N}^+$ are almost as large. So for me, that meant it wasn't obvious which carbon of these two should be farther downfield, but together they should be the $\delta = 72.8$ and 62.5 ppm peaks.

The carbon that has just the nitrogen should be upfield of the one that has the nitrogen and the carbonyl, and then the carbon with nothing should be upfield of that. Putting it all together:

δ (ppm)	Name of carbon
177.0	C=O
72.8	C₄ (or C₂)

δ (ppm)	Name of carbon
62.5	C₂ (or C₄)
55.7	C₅

δ (ppm)	Name of carbon
40.2	C₃

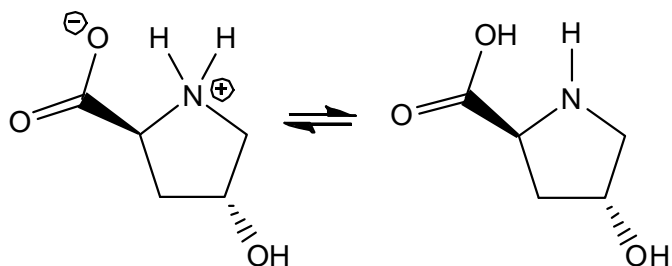
3 points each.

Rubric: (15 points total this part.)

3 points for each correct answer..

-1 point for each incorrect answer included with the correct answer.

7. Amino acid zwitterions always equilibrate with a non-ionic tautomer. In less-polar solvents, this equilibrium lies more towards the non-ionic form.



Would you expect this equilibrium to be

2 **faster** or **slower** (circle one)

than the "NMR timescale"?

Proton transfer is super fast.

How would you expect the ^{13}C chemical shift of each of the following nuclei to change if the NMR were taken in acetone- d_6 instead of D_2O ?

Would $\delta(\text{C}=\text{O})$ 4 **increase** decrease, or **stay the same**? (Circle one.)

Switching to acetone- d_6 would make the carboxylate group less negative (by protonating the charge), and would thus decrease the electron density around the carbon atom. This would de-shield the carbon, sending its resonance downfield, to a higher δ value.

Would $\delta(\text{C}_5)$ increase, **decrease**, or **stay the same**? (Circle one.)

Switching to acetone would make the nitrogen atom less positively charged (by deprotonating it), and thus less electron-withdrawing. There would be more electron density around C_5 to shield it, and this would shift the C_5 resonance upfield (to lower δ).