NAME _____

ID # _____

INTERPRETATION OF ORGANIC SPECTRA (4361/8361)

9:05 – 9:55 am, October 5, 2011

Exam 1

This exam is open book and open note. You are permitted to use any written materials you have brought as aids on this exam. You may also use a simple calculator. Other than this, please do not use any other electronic devices (cell phones, computers, recording devices, etc.) during the exam.

You may use pen or pencil. However, re-grades will be considered only for exams completed in pen.

Please write your answers in the boxes/spaces provided. If your answer is not in the appropriate space (say, for example, it's on the back of the page), draw us an arrow and/or note telling us where to look.

Feel free to remove the corner staple if this helps you analyze the spectra; you will have the opportunity to re-staple your exam at the end. The exam in this packet is designed to take 30 minutes to complete. You will be given 50 minutes total to finish the test. This exam contains one problem, which is split into parts. Many of these parts can be answered independently. *Do not get stuck* on one part and then assume that you will be unable to answer the rest of the question—move on. In addition, partial credit will be given for incorrect but still plausible answers, so *guess* on problems you cannot answer perfectly.

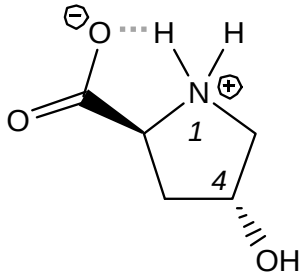
At the end of the 50 minute exam period you will be asked to return your exam to the proctor. Please do not take any part of the exam packet with you when you are done; everything will be returned to you after the exams are graded. This packet should contain 11 pages, including this one. Please check to make sure that your packet contains 11 pages before beginning your exam.

NAME _____

Scoring:	1. _____ / 46	5. _____ / 8
	2. _____ / 6	6. _____ / 15
	3. _____ / 10	7. _____ / 10
	4. _____ / 5	

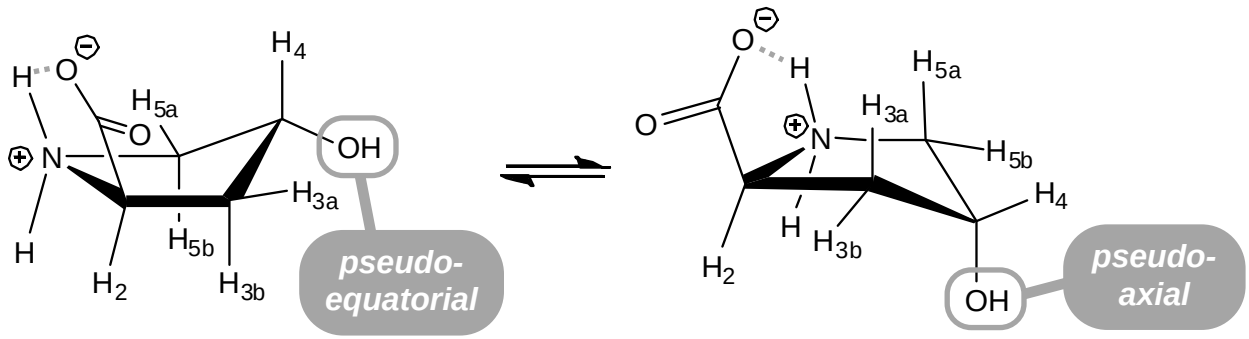
Total Score: _____ / 100

4-Hydroxyproline (4HP) is an uncommon, but important, amino acid found in post-translationally modified proteins. Like most amino acids, 4HP is a zwitterion in polar solvents, with a negatively charged carboxylate group and a positively charged ammonium nitrogen. Unlike linear amino acids, however, the ring in 4HP ensures that an intramolecular hydrogen bond is formed between one carboxylate oxygen and an ammonium hydrogen (even though the bent geometry of the H-bond isn't that great).



4-hydroxyproline
(4HP)

Computational studies have identified two stable, puckered conformations of 4HP: one with a pseudo-equatorial -OH group, and the other with a pseudo-axial -OH.



I've labeled inequivalent, geminal protons in this molecule according to whether they are on the same face as the carboxylate group (**a**) or on the opposite face (**b**). The main goals of this problem will be to assign chemical shifts to each of the nuclei in 4HP, and to determine which of the two conformations above predominates, using the ^{1}H (400 MHz, in D_2O) and ^{1}H-decoupled ^{13}C NMR (100 MHz, in D_2O) spectra attached to the end of this exam.

1. In the chart below (and continuing on the next page), assign each resonance in the ^{1}H spectrum to a proton in 4HP. If there are chemical shifts you feel you cannot assign definitively to one proton, but you can narrow it down to a few, name all possible protons in the corresponding box. List coupling constants to the nearest 0.5 Hz. If a coupling constant value appears more than once (say, $J_1 = J_2 = J_3$), then list it more than once. Then, for each coupling constant you list, propose a pair of protons that are responsible for that coupling constant.

   Do the best you can on this chart--we will give partial credit to assignments that are close to the correct answer, or that are indefinite when they could be definite (and vice versa). For some protons, I have given you more boxes than you need—you do not need to fill all the boxes. If a box is shaded, we won't be grading it, but feel free to fill it in for your own information.

δ (ppm)	**Name of proton** (H_n)	**Coupling constants *J* (Hz)**	**Assign coupling constants** $J(H_m,H_n)$
4.66			
4.33			
3.47			

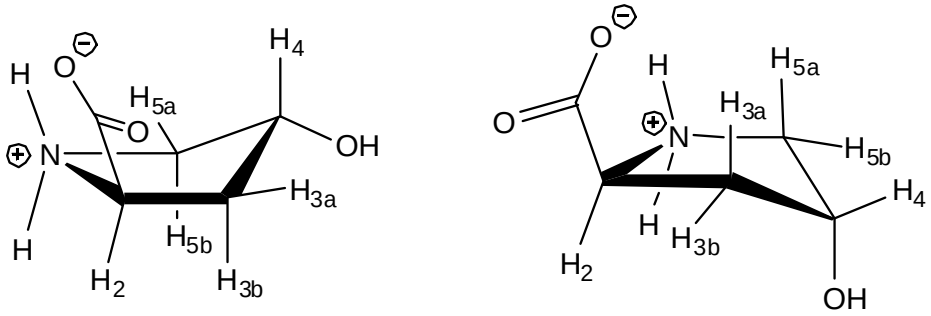
δ (ppm)	Name of proton (H_n)	Coupling constants *J* (Hz)	Assign coupling constants $J(H_m,H_n)$
3.35			
2.42			
2.15			

2. The -OH proton in 4HP can't be seen in the ^{1}H 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 ^{1}H NMR resonance, and assign it a chemical shift? Name two changes we might make.

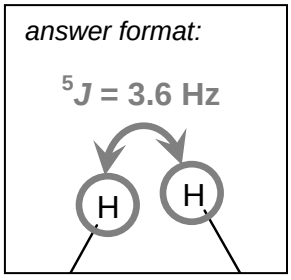
3. One of the multiplets in the ^{1}H NMR is labeled “resolution enhanced”. Briefly, explain what must be done to NMR data to achieve resolution enhancement. Why does resolution enhancement help?

4. Which conformation of 4HP predominates?

Circle one: **this one ?** *or* **this one ?**



5. Does 4HP exhibit any long-range (4-bond or 5-bond) ^{1}H-^{1}H 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.



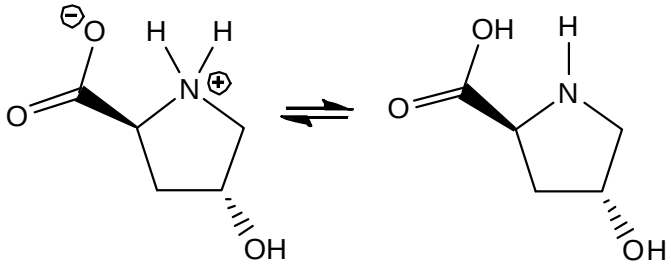
6. In the chart below, assign each resonance in the ^{13}C spectrum to a carbon in 4HP. If there are chemical shifts you feel you cannot assign definitively to one carbon, but you can narrow it down to a few, name all possible carbons in the corresponding box.

δ (ppm)	Name of carbon
177.0	
72.8	

δ (ppm)	Name of carbon
62.5	
55.7	

δ (ppm)	Name of carbon
40.2	

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

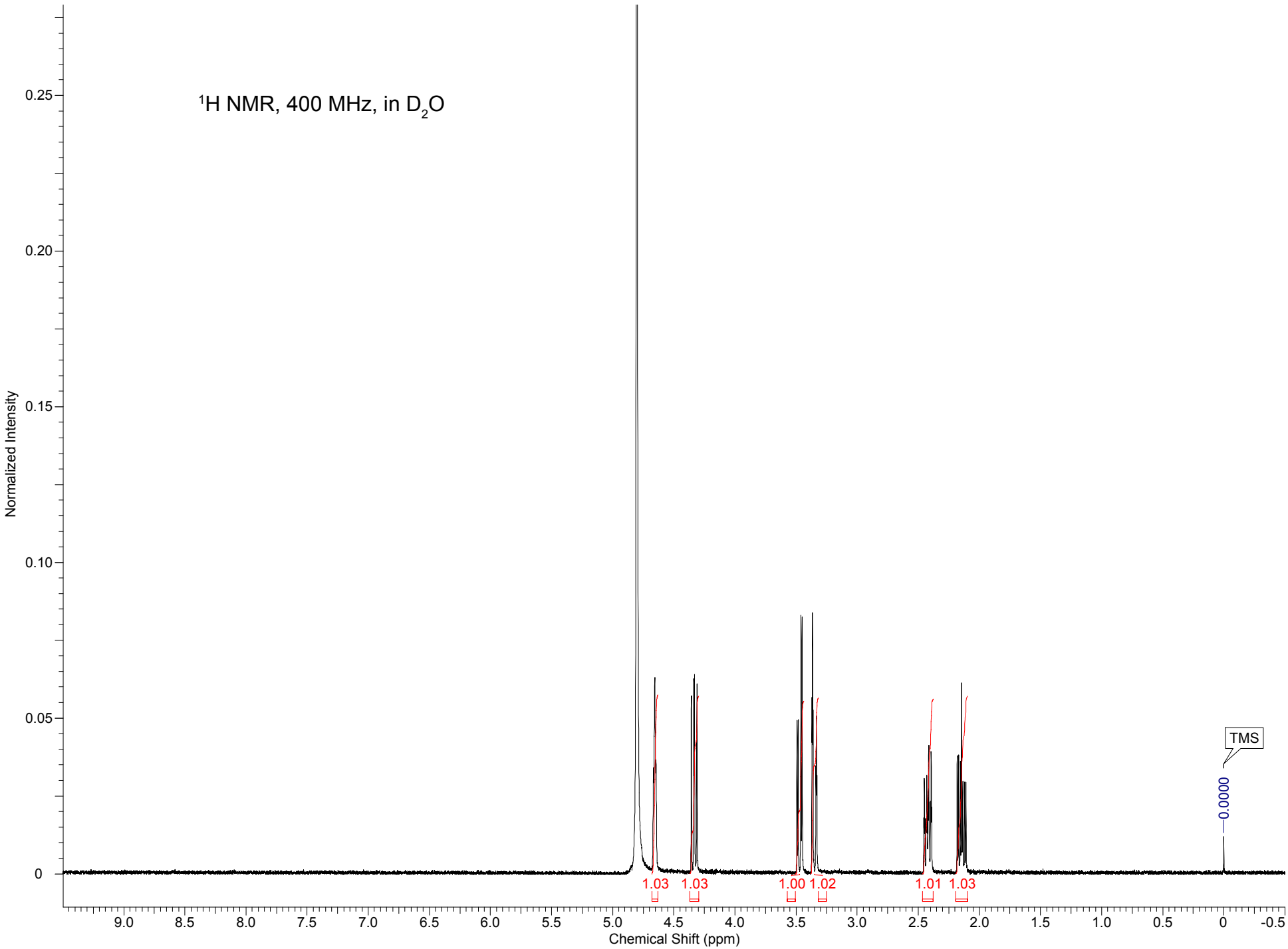
faster *or* **slower** (circle one)

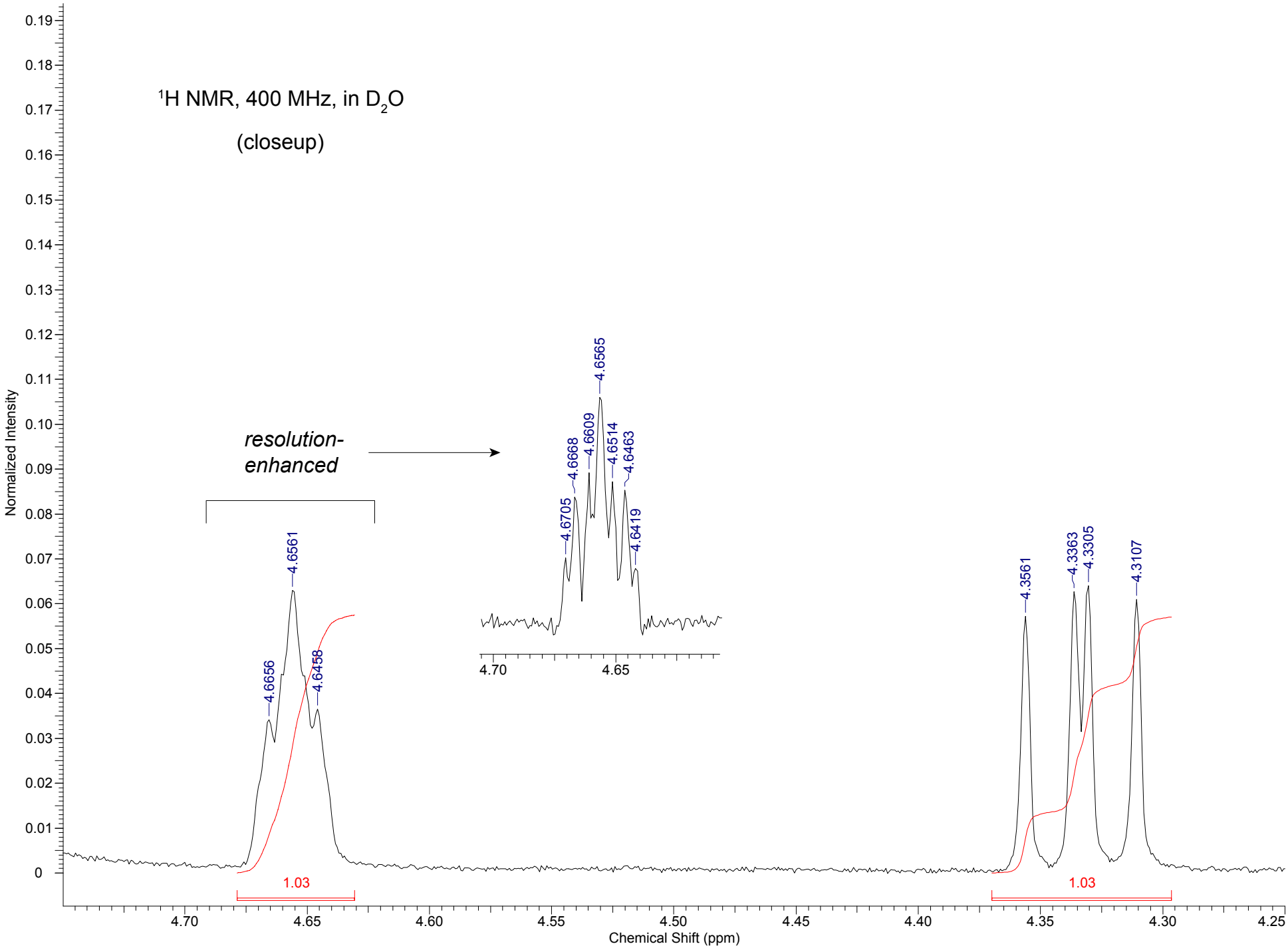
than the "NMR timescale"?

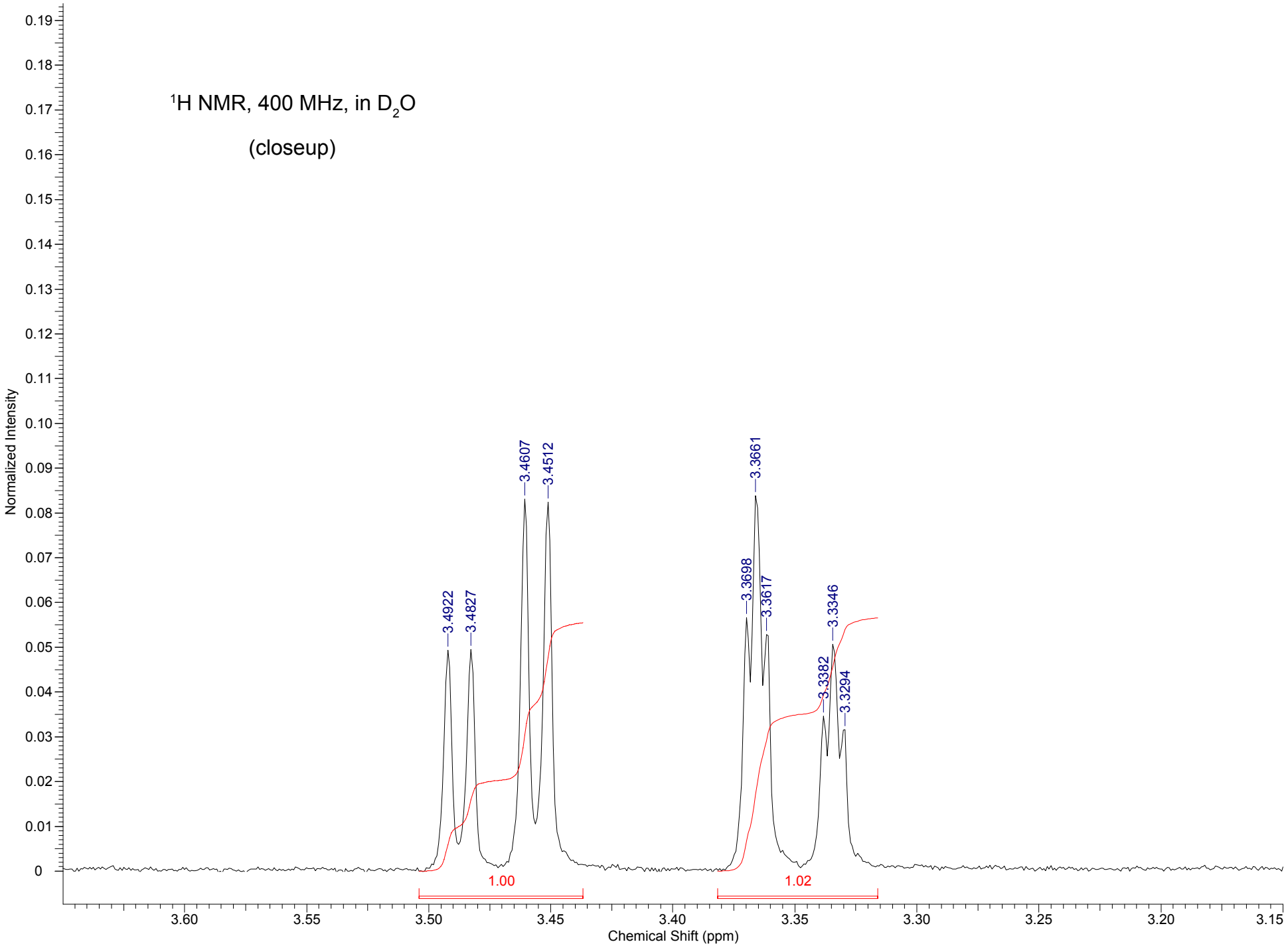
How would you expect the ^{13}C chemical shift of each of the following nuclei to change if the NMR were taken in acetone-d_6 instead of D_2O?

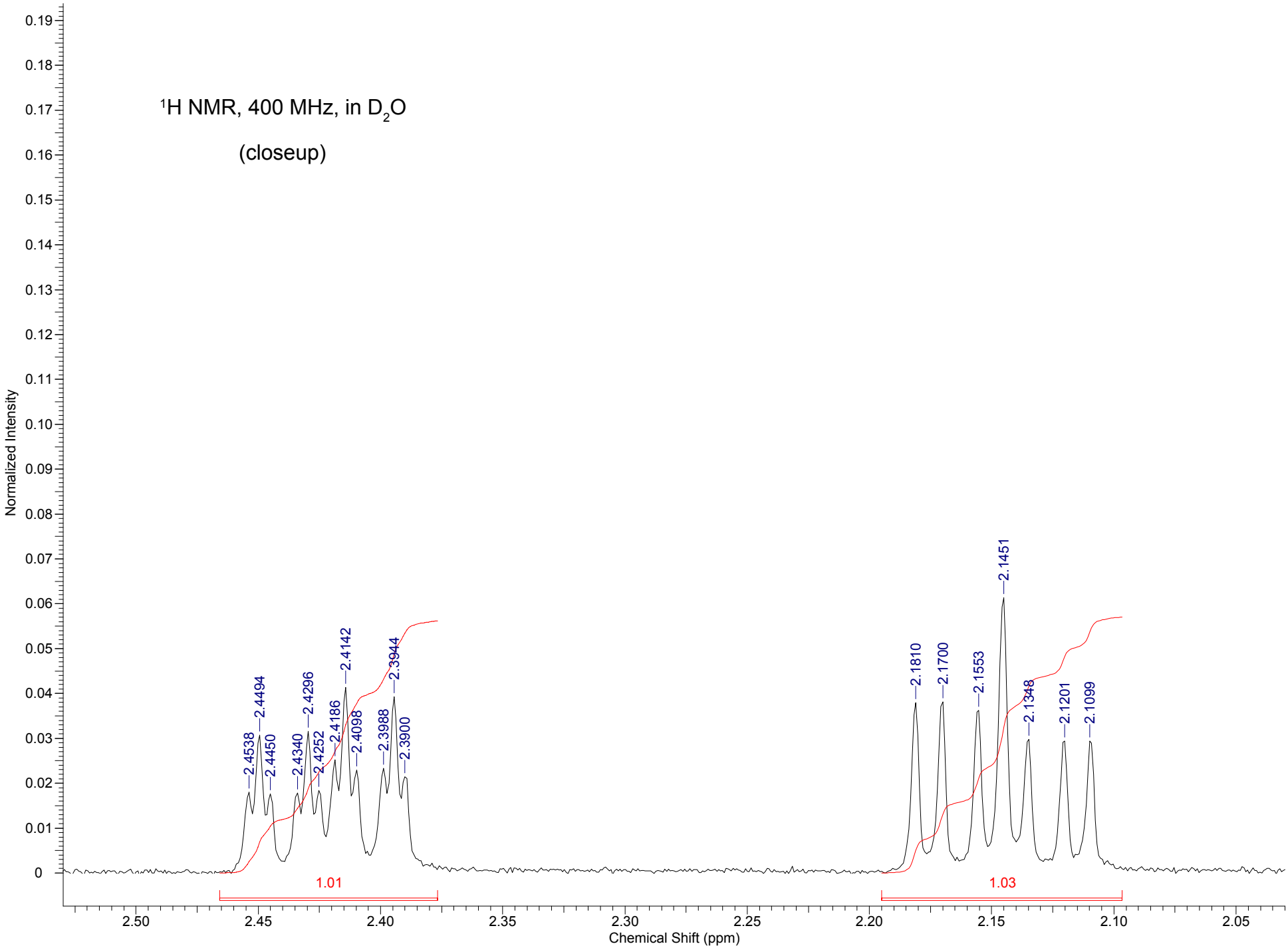
Would δ(**C**=O) **increase** , **decrease** , *or* **stay the same** ? (Circle one.)

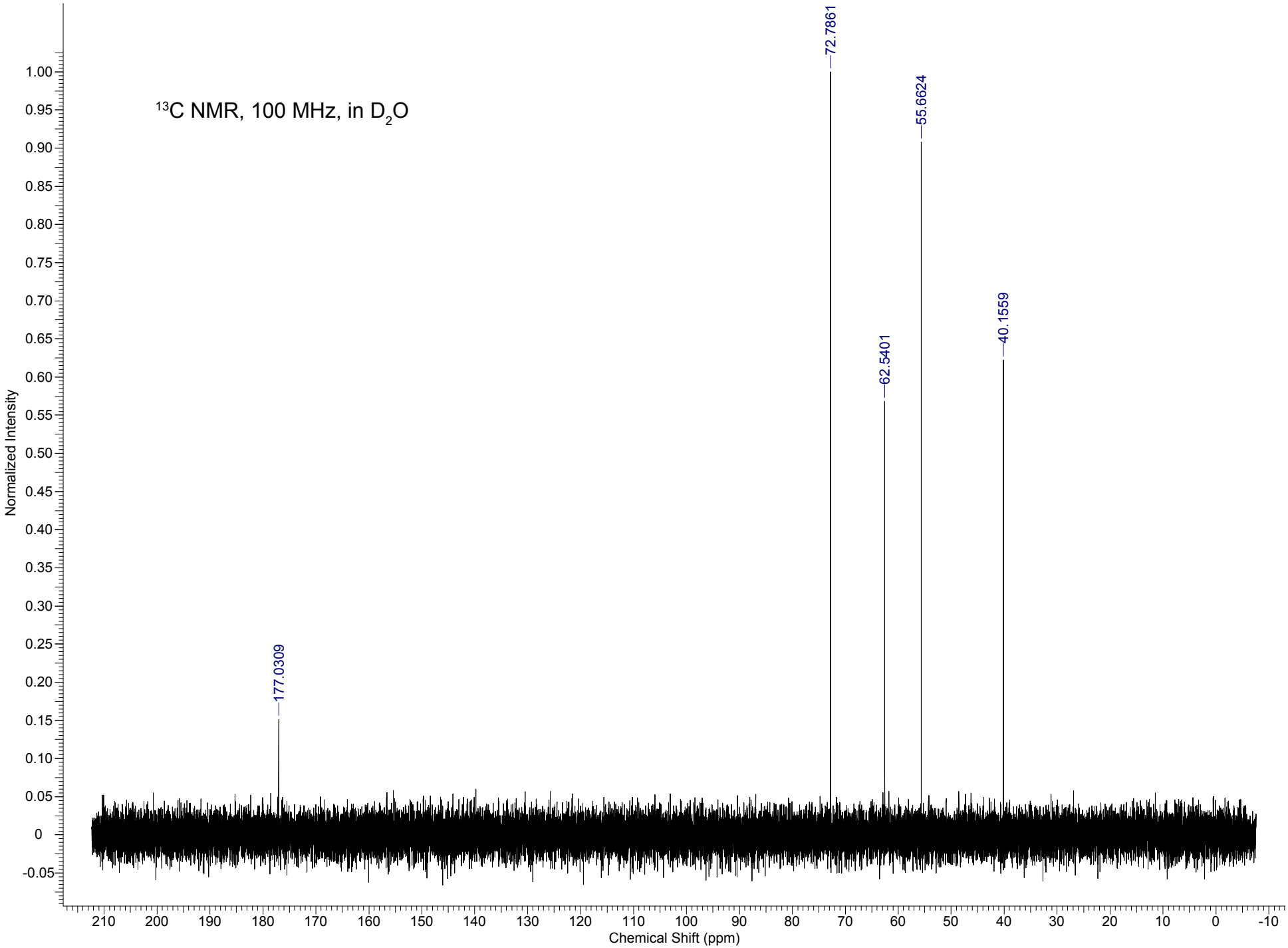
Would δ(**C**$_5$) **increase** , **decrease** , *or* **stay the same** ? (Circle one.)


^{1}H NMR, 400 MHz, in D_2O
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
0
1.03
1.03
1.00
1.02
1.01
1.03
TMS
0.0000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
Chemical Shift (ppm)


^{1}H NMR, 400 MHz, in D_2O
(closeup)
resolution-enhanced
4.6705
4.6668
4.6609
4.6565
4.6514
4.6463
4.6419
4.70
4.65
4.6656
4.6561
4.6458
1.03
4.3561
4.3363
4.3305
4.3107
1.03
Normalized Intensity
Chemical Shift (ppm)


^{1}H NMR, 400 MHz, in D_2O
(closeup)
Normalized Intensity
Chemical Shift (ppm)
3.4922
3.4827
3.4607
3.4512
3.3698
3.3661
3.3617
3.3382
3.3346
3.3294
1.00
1.02


^{1}H NMR, 400 MHz, in D_2O
(closeup)
Normalized Intensity
Chemical Shift (ppm)
2.4538
2.4494
2.4450
2.4340
2.4296
2.4252
2.4186
2.4142
2.4098
2.3988
2.3944
2.3900
2.1810
2.1700
2.1553
2.1451
2.1348
2.1201
2.1099
1.01
1.03


^{13}C NMR, 100 MHz, in D_2O
72.7861
55.6624
62.5401
40.1559
177.0309
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
Chemical Shift (ppm)