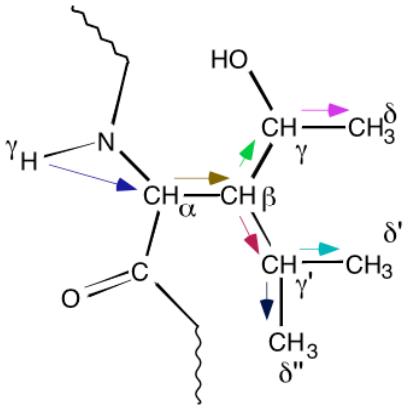
** This examination is open book, but is to be worked on ***independently***. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is ***very important***.

Due in class, 9:30am, Thursday, April 10

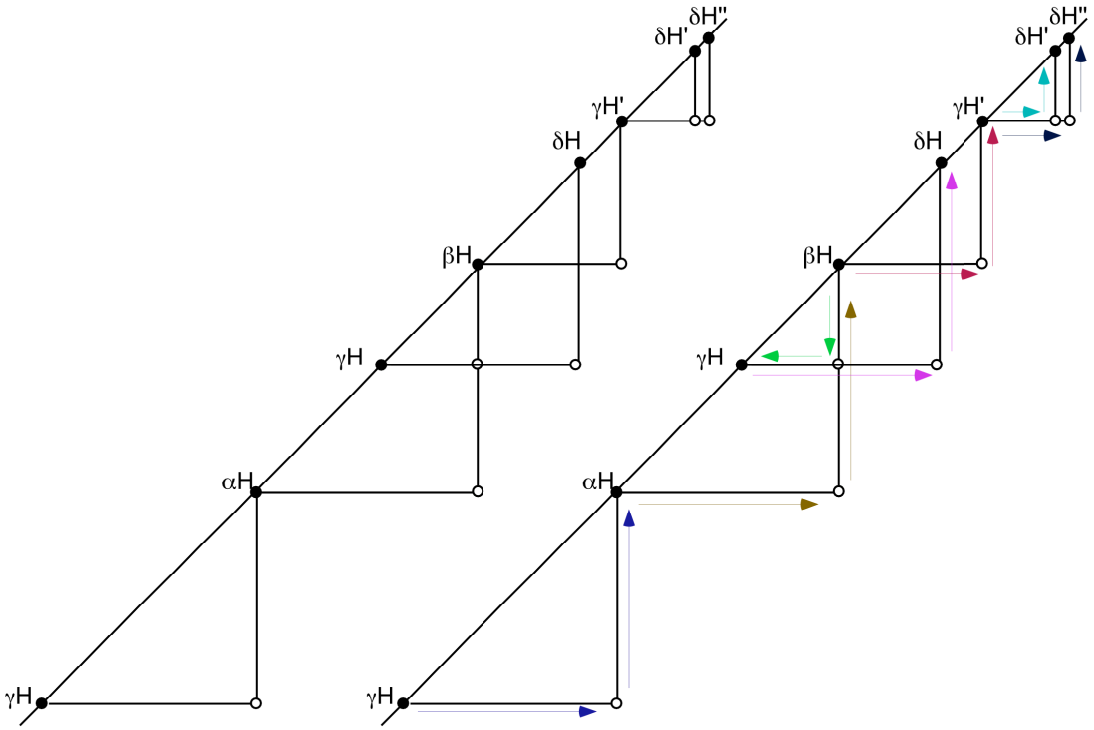
Show your work for full credit. Be concise, but complete.
Avoid long rambling answers which indicate that you don't really understand the question.

1. (20 points) You've just designed a cell that can express proteins with a new amino acid (this technology does exist). The amino acid is shown at right. Create a spin connectivity diagram like the kind you've seen in the hand-out (see "Amino acid spin connectivity diagrams" on the course WEB page). Try to be neat…

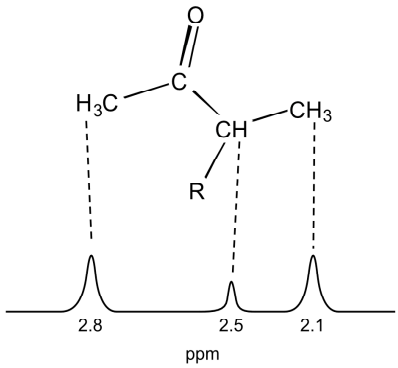


The answer is given below left. To its right is a reprint, with arrows indicating the connections, for clarity. The arrows are color-coded to match similar arrows at right.

Connectivity is most important here, but reasonable guesses at chemical shifts should also be made.



2. Consider the system at right.

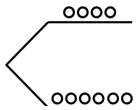


a) (10 points) Consider first, only the spin at 2.5 ppm and the following pulse sequence:

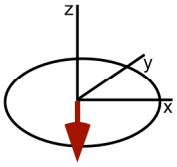
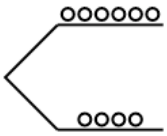
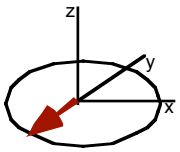
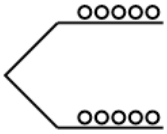
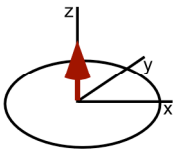
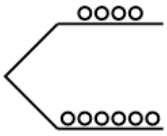
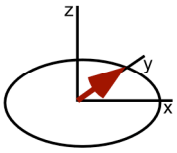
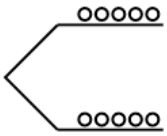
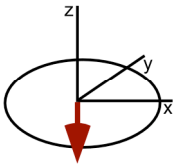
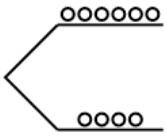
$$- 90°_y - \Delta_D - 90°_y -$$

Show, on the laboratory frame spin vector diagrams below, what will happen for each value of Δ_D below (ie. what is the state of the ***single*** magnetization vector precessing at Ω_o (corresponding to 2.5 ppm) immediately after the pulse sequence?). The applied field lies along the z-axis, as usual.

b) (5 points) As before, use the kind of diagram at right, to show the resulting spin population at the end of the sequence for each value of Δ_D



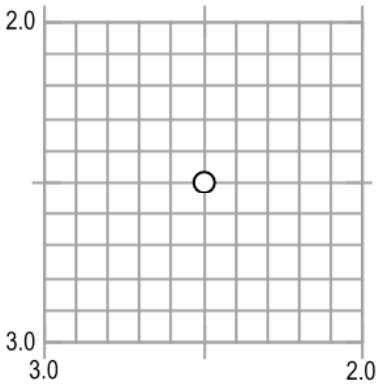
c) (5 points) Finally, show the signal (i.e., an NMR peak) resulting from an FID following the pulse sequence

Δ_D	Laboratory Frame	Spin Population	NMR Signal
$\Delta_D = 0$			
$\Delta_D = \frac{\pi}{2\Omega_\Omega}$			
$\Delta_D = \frac{\pi}{\Omega_\Omega}$			
$\Delta_D = \frac{3\pi}{2\Omega_\Omega}$			
$\Delta_D = \frac{2\pi}{\Omega_\Omega}$			

2 (continued)

d) (5 points) Draw a "bird's eye" view of the resulting 2D plot one would get by Fourier transforming a set of experiments like those above with respect to first the FID time and then by the delay time τ_D. What are the axes? Put values on the axes and place the "peaks" accordingly. Consider only the 2.5 ppm peak.

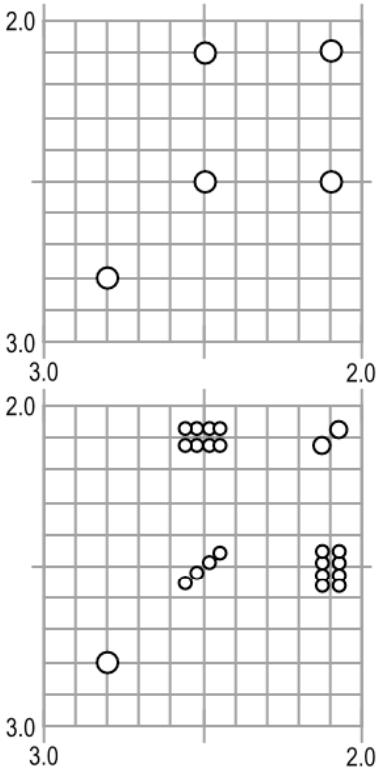
From above, we see that the signal will oscillate sinusoidally according to its own frequency. So for a peak at 2.5 ppm along one axis (its chemical shift), Fourier transform along the second axes will reveal the oscillatory frequency – its own resonant frequency! Thus if we plot both scales at right as ppm, there will be a (diagonal) peak at 2.5/2.5



e) (5 points) Finally, now imagine all 3 resonances and assume that all 3 undergo the same 90° pulses (but each has its own γB_1). What might the above plot look like now? Think about inter-peak interactions. Explain.

Think about the proton corresponding to chemical shift 2.5 ppm. We will of course have the peak mentioned above, but now we can see that the populations of the neighboring alpha and beta spins at 2.1 ppm are fluctuating according to the resonant frequency of the 2.1 ppm spins. The signal intensity of each of the 2.5 ppm peaks will therefore fluctuate (not invert, but fluctuate) at the frequency of the 2.1 ppm protons. The Fourier transform with respect to delay time will thus yield two peaks for the 2.5 ppm protons – the one above, plus this new "cross peak." The plot at upper right ignores the splitting of each peak and is an acceptable answer. The plot at lower right is more correct, including the J-coupled splitting in the 1D spectrum.

This kind of behavior is the basis for the COSY experiment…



3. On the accompanying page, you will find COSY and NOESY NMR spectra of a pentapeptide. The composition of the peptide is one each of Gly, Phe, Ser, Thr, and Val. Assign each of the peaks in the spectra and then answer the following questions.

a) (25 points) On the answer sheet that follows the spectra, indicate the approximate chemical shift for each proton. If a class of protons is inequivalent, indicate the multiple chemical shifts values as follows "3.52/3.64." If you cannot determine the chemical shift of a proton, indicate so and explain why.

See next page

b) (10 points) Place the sequence of the peptide on the answer sheet that follows the spectra

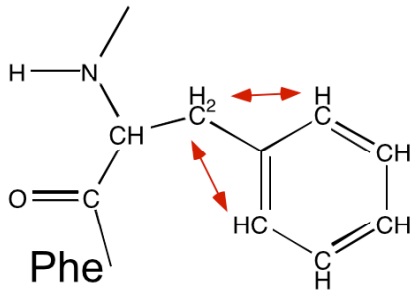
See next page

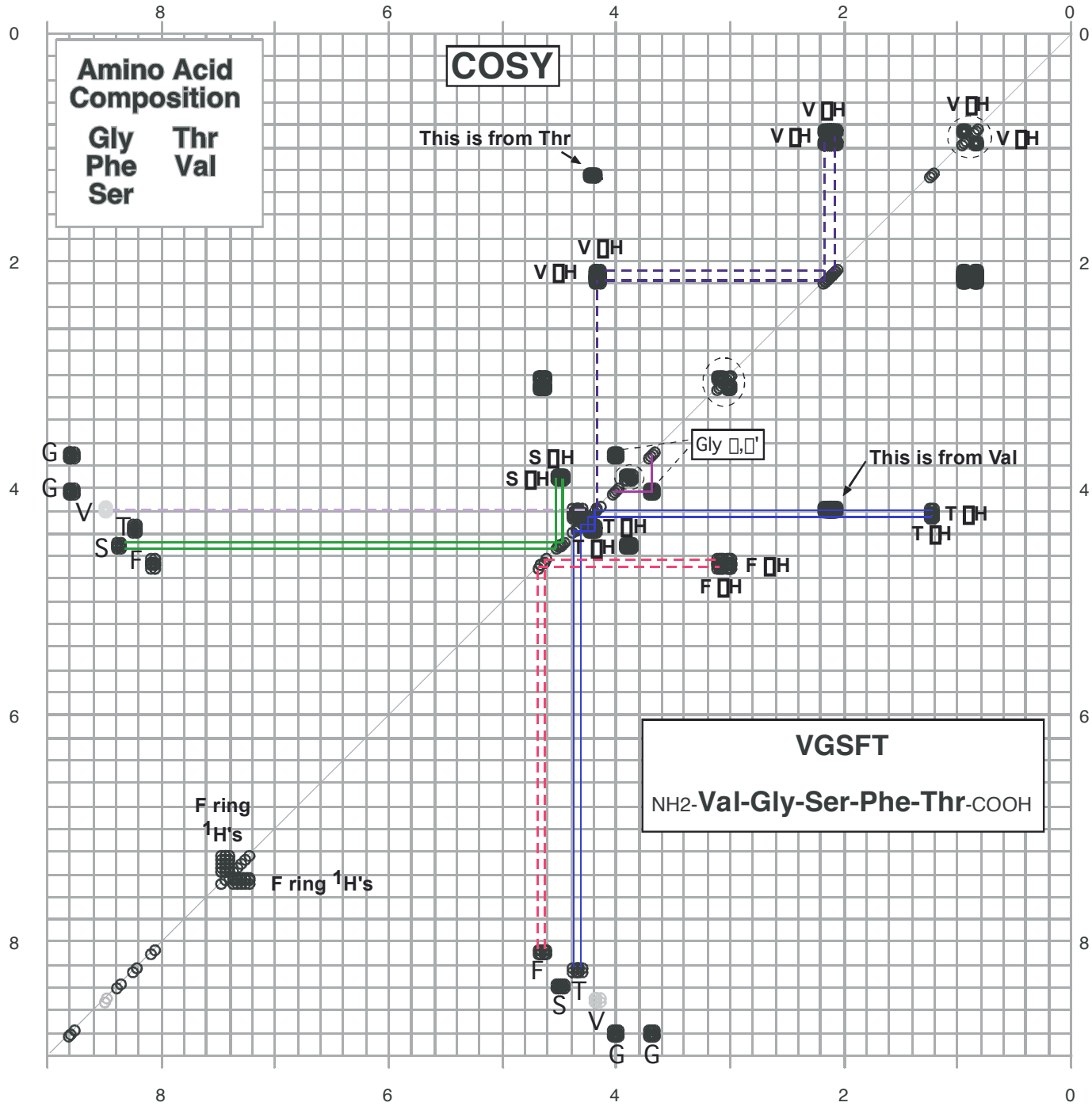
c) (10 points) Near the diagonal, there is a cluster of barely off-diagonal peaks near 3.0-3.1 ppm. What are these off-diagonal peaks?

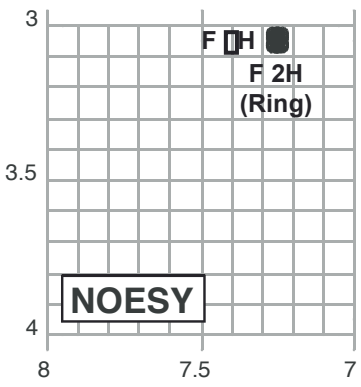
These arise because the two Phe beta protons are slightly inequivalent. As a result, they split each other and give rise to these off-diagonal peaks, indicating the J-coupling. Similar cross peaks are seen for the Val β and Gly α protons.

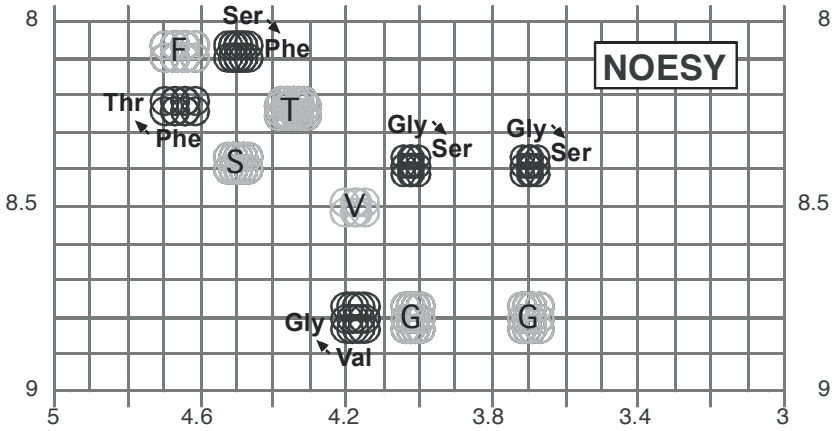
d) (5 points) Explain the peak in the NOESY spectrum blow up at lower left (near the intersection of 3.05 and 7.25 ppm.

These are the Phe β protons coupling to the protons closest on the Phe ring. With the proper assignment of the Phe β protons, this is easy (conversely, this result can help you with the assignment of the Phe spin system). 2D spectra are full of these mutually useful bits of information.









Val-Gly-Ser-Phe-Thr

Name: _____

Peptide Sequence:

NH2 - Val - Gly - Ser - Phe - Thr **- COOH**

Chemical Shift Assignments