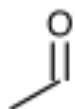


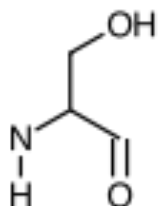
In-class NMR Problem

- The challenge here was to determine the sequence of a peptide with an N-acetyl group containing one serine and one glutamate residue.
- A 2D COSY spectrum will be used to assign resonances to each of the “residues” including the acetyl group.
- A 2D NOESY spectrum will indicate the sequence.

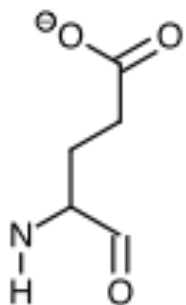
1. Assign chemical shifts to all expected protons



1



3

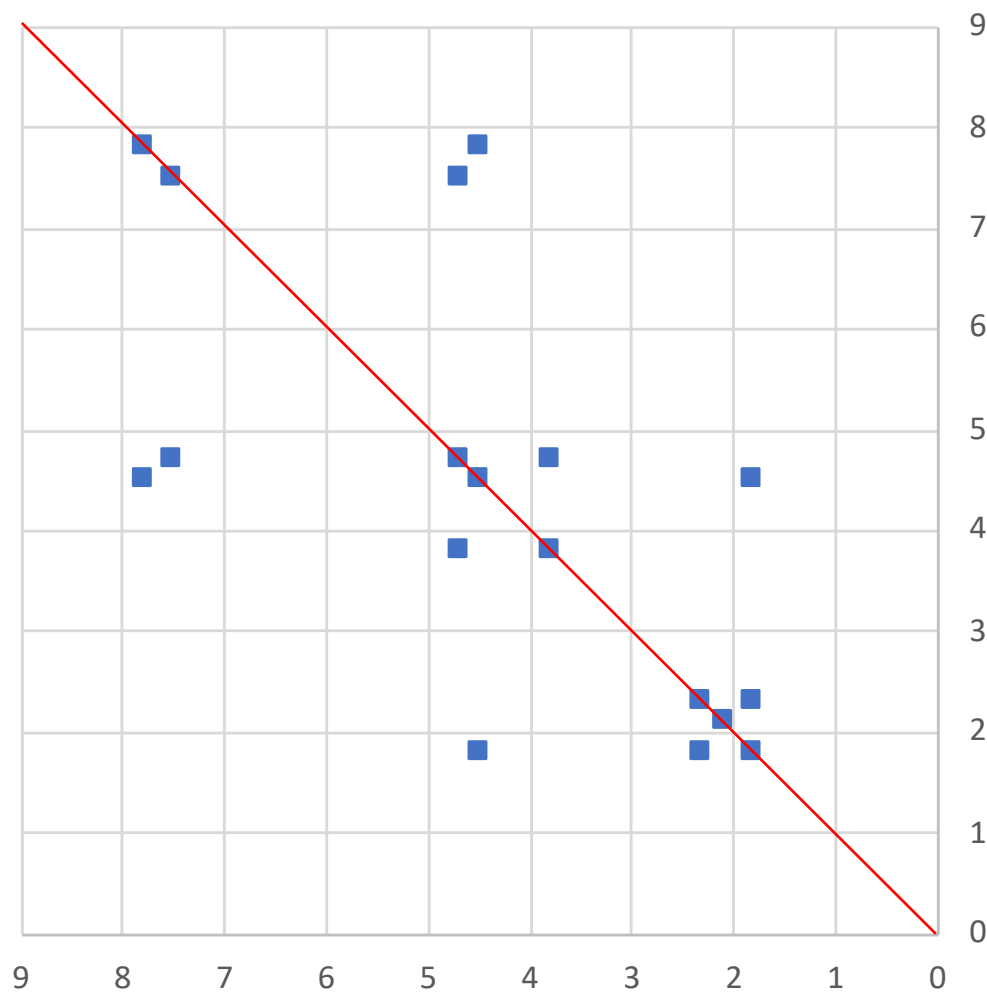


4

Group	Shift
Acetyl	2.1
Ser-NH	7.5
Ser-CA	4.8
Ser-CB	3.8
Glu-NH	7.8
Glu-CA	4.6
Glu-CB	1.8
Glu-CG	2.3

0. Are the required number of resonances present? Check diagonal.

Note that there are 8 self-cross peaks on the diagonal indicating 8 distinct resonances, as expected. Two are between 7-8 (NH protons) three are below 2.5 ppm (aliphatic protons) and three are around 4 ppm (mixture of aliphatic and C alpha protons).



COSY Spectrum

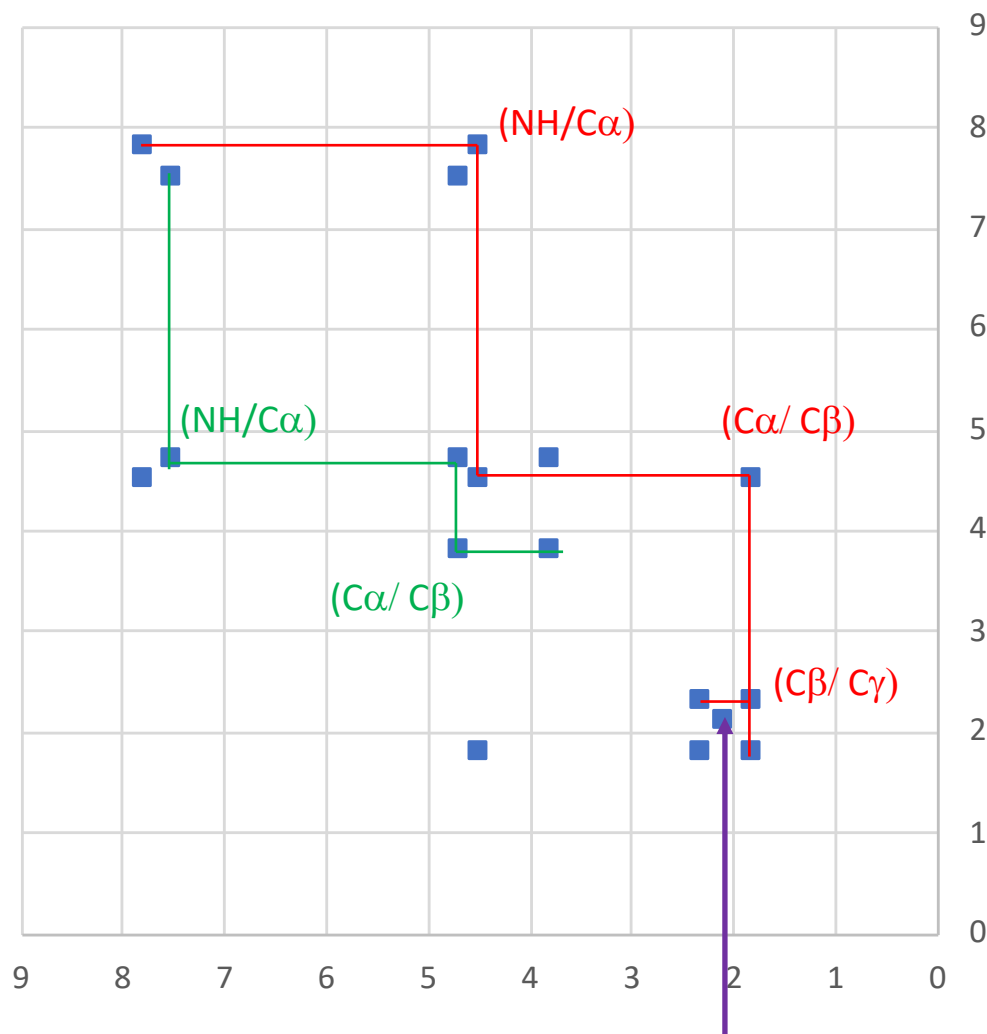
2. Group resonances to individual residues.

Starting with the NH at 7.8 ppm I find a x-peak to a $C\alpha$ resonance (NH/ $C\alpha$).

The $C\alpha$ then has a x-peak to a resonance at 1.8 ppm ($C\alpha$ / $C\beta$).

The $C\beta$ then has a x-peak to a resonance at 2.3 ppm ($C\beta$ / $C\gamma$).
Must be Glu

Similar logic gets me x-peaks for the second residue, serine.



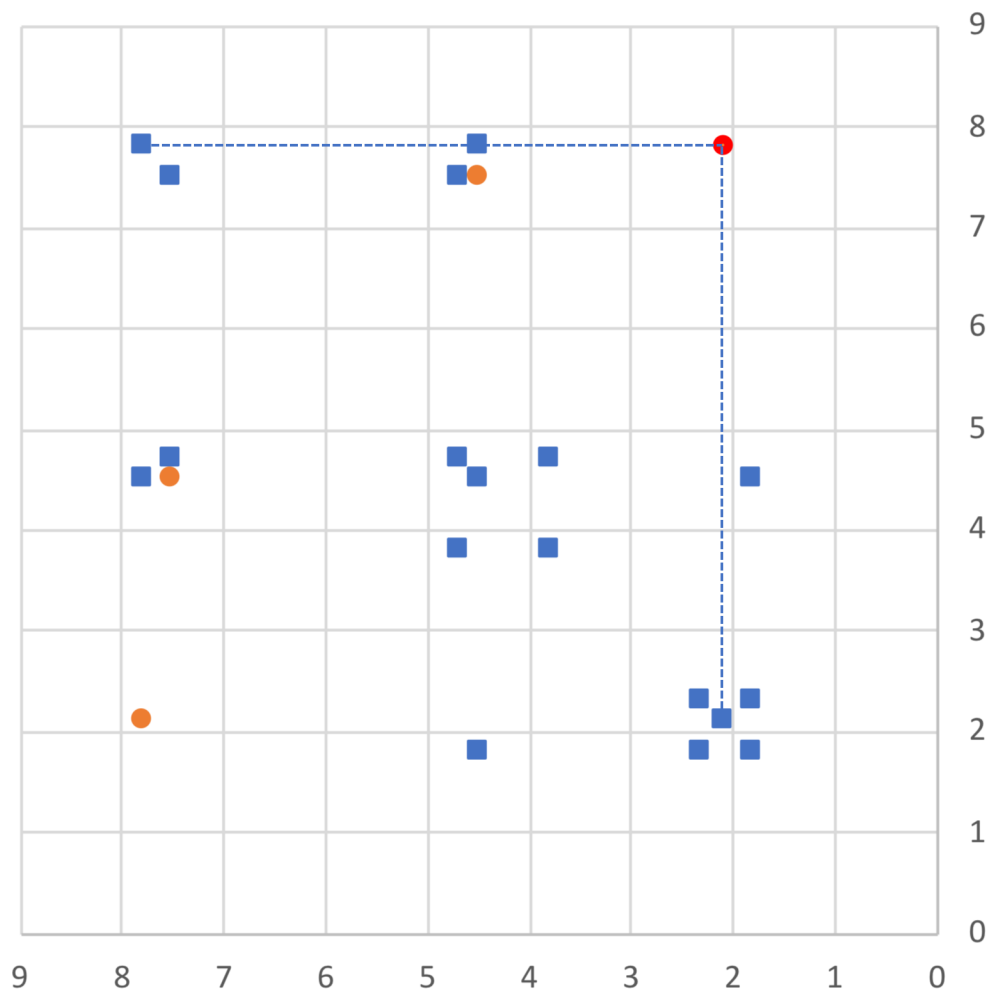
No crosspeaks to the resonance at 2.1 ppm
This must be Acetyl group.

COSY Spectrum

3. Use NOESY to determine sequence

Now the question is sequence – which aa is first – glutamate or serine. What we see in the NOESY is 4 additional x-peaks.

The one that matters is one formed from acetyl group to an NH. We see that it goes the the NH of Glu – that is the first residue in the sequence.



NOESY Spectrum