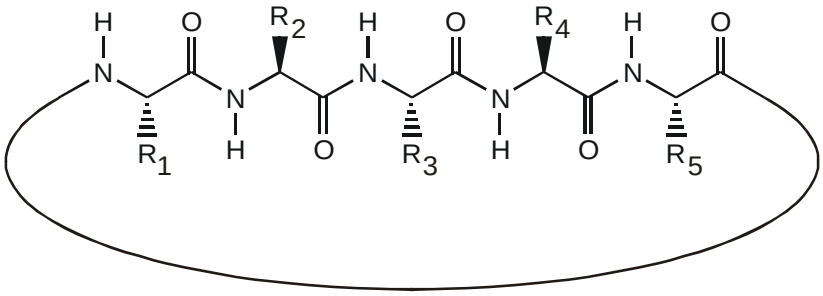
Chemistry 4361/8361 Fall 2012

Problem Set 6
TOCSY, NOE Correlation Spectroscopy
Due: Wednesday, November 7

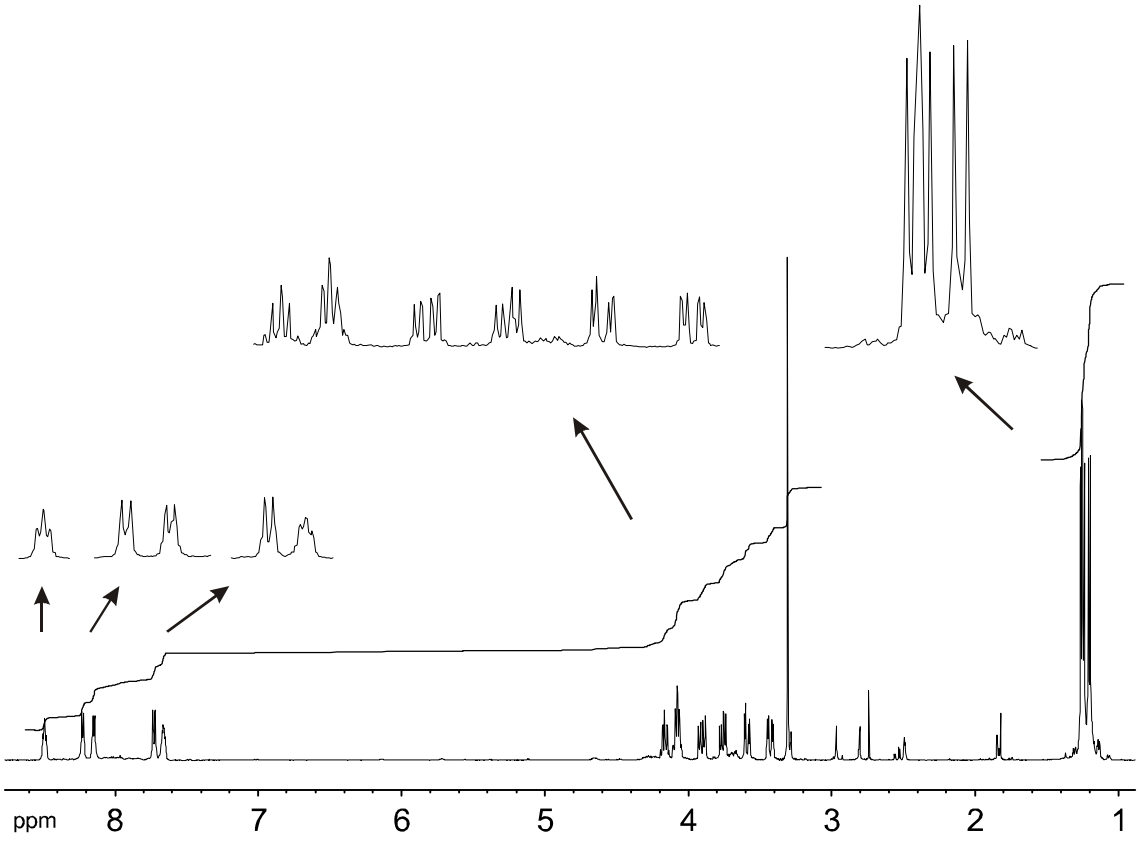
1. The spectra on the following pages refer to the cyclic pentapeptide shown below, which contains only glycine (R = H) and alanine (R = CH_3) residues.



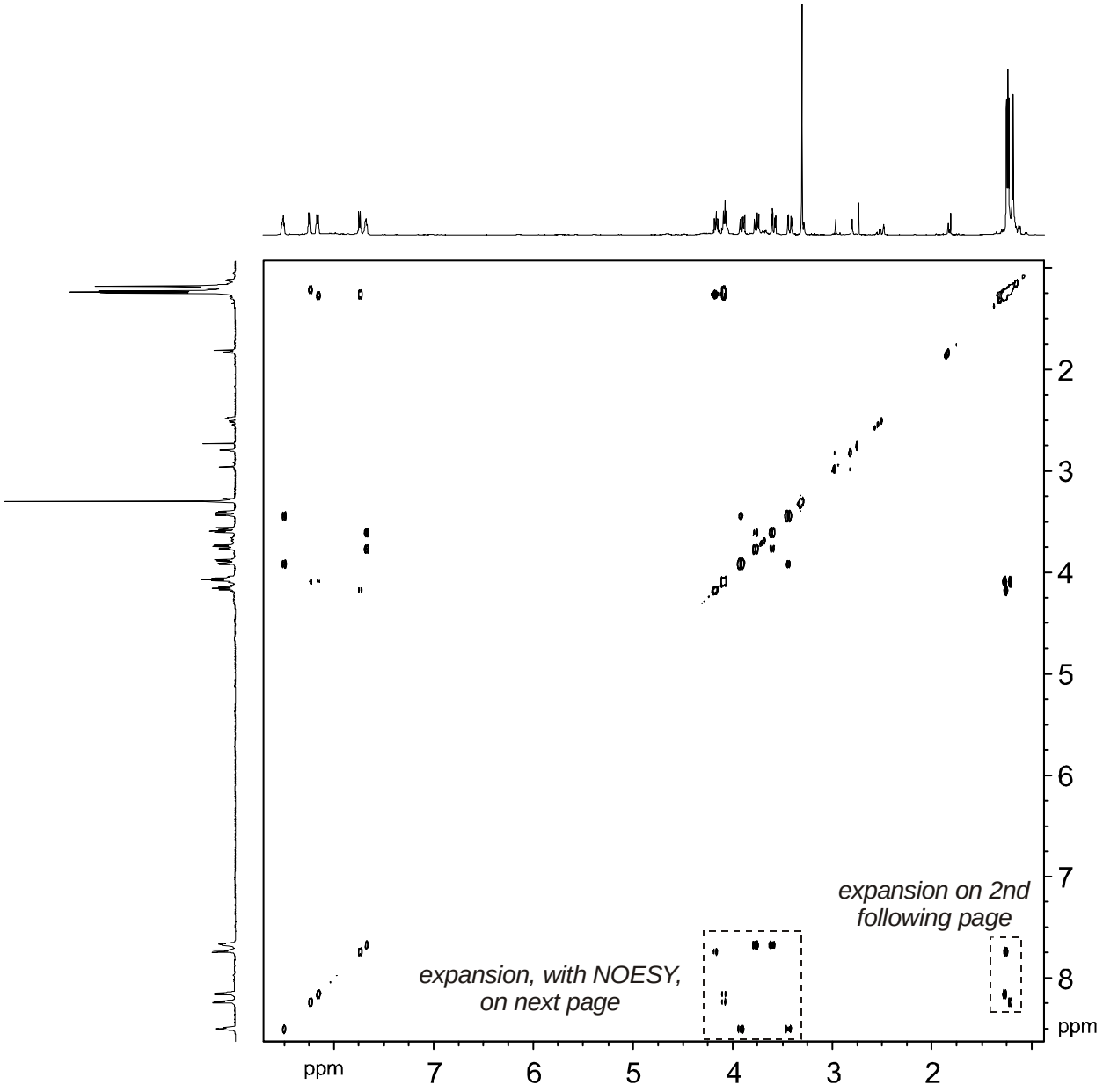
   a. How many glycine and how many alanine residues are there in the pentapeptide?

   b. Based on the 2D NMR data at your disposal, what is the most probable arrangement of glycines and alanines? (Hint: There are actually only two possible arrangements.)

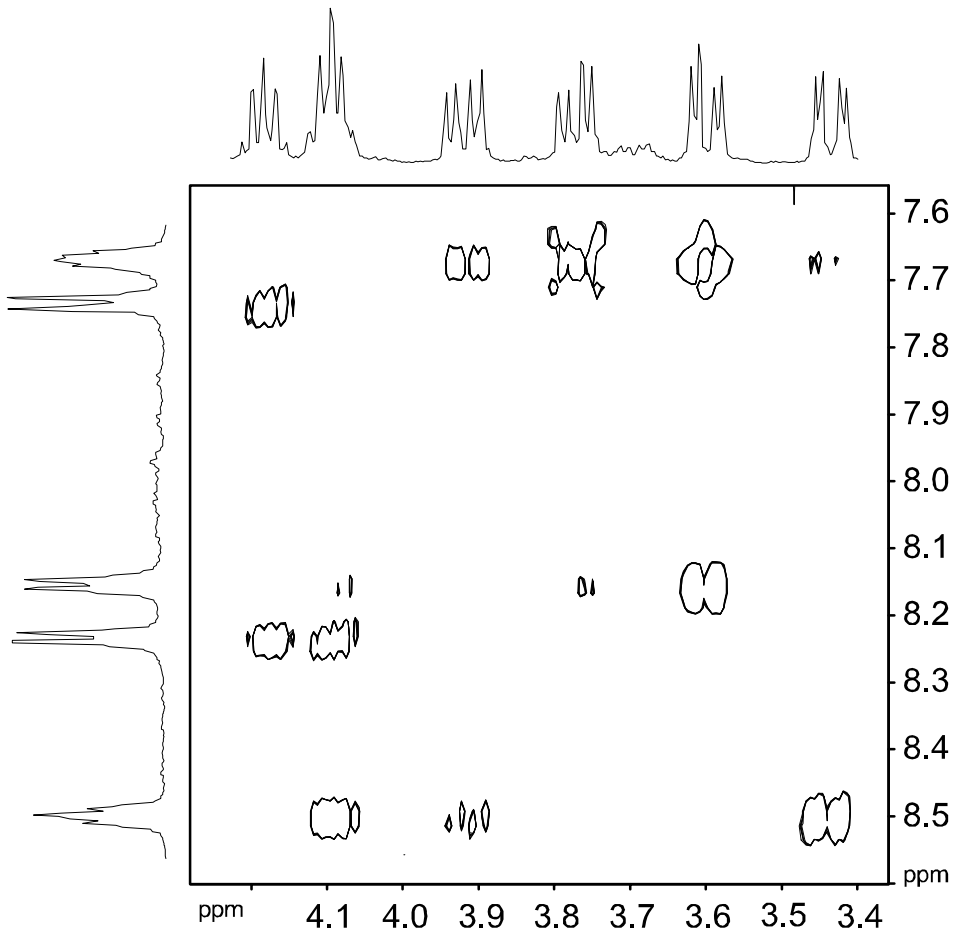
   c. Do the splittings and crosspeaks you observe all make sense in terms of your assignment?



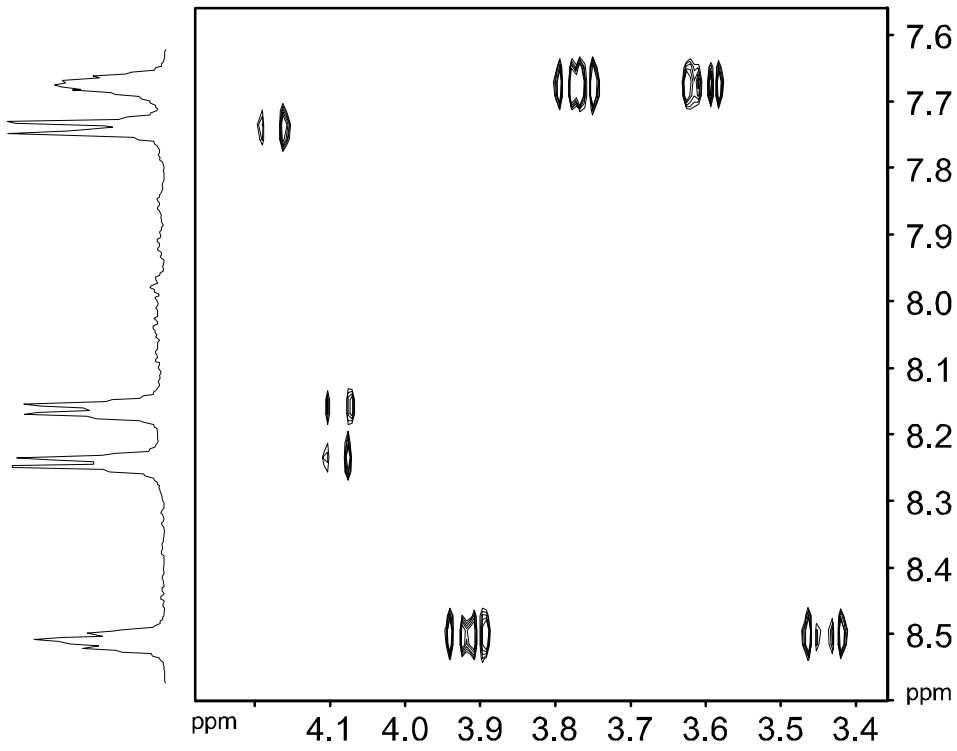
^{1}H NMR, 500 MHz, in $CDCl_3$



^{1}H-^{1}H TOCSY, 500 MHz, $CDCl_3$


7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.4
8.5
ppm
ppm
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4

NOESY


7.6
7.7
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ppm
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4.1
4.0
3.9
3.8
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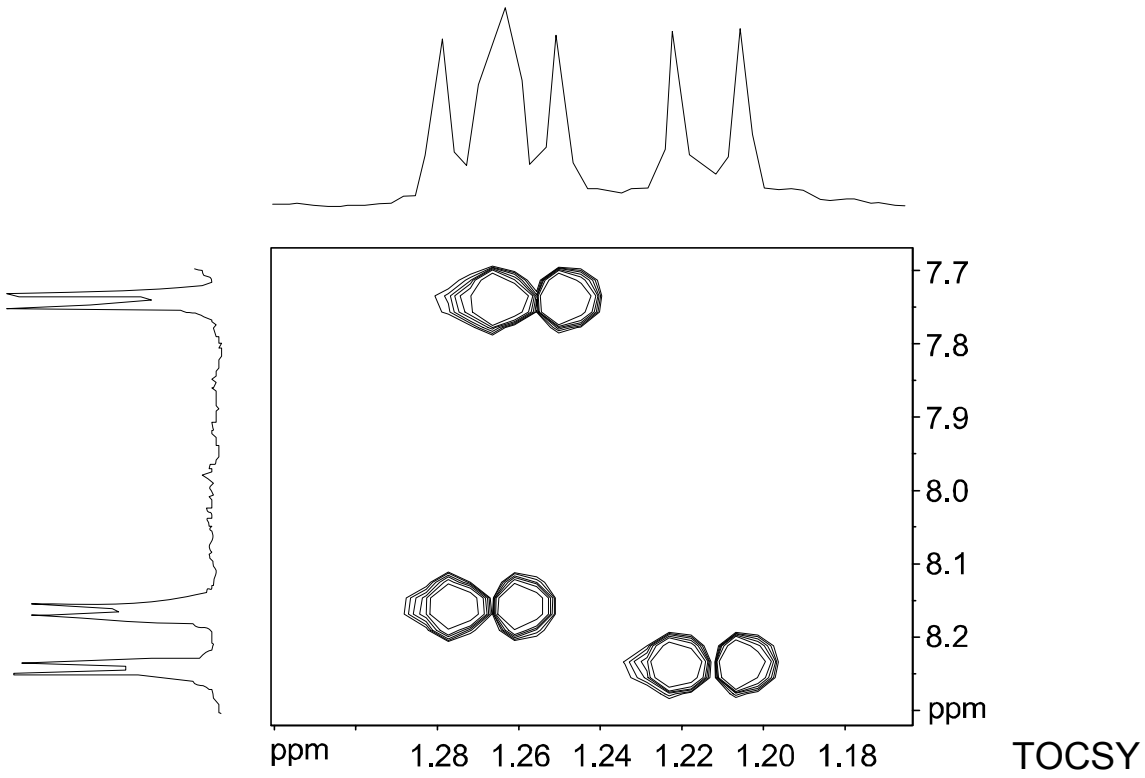
3.6
3.5
3.4

TOCSY


7.7
7.8
7.9
8.0
8.1
8.2
ppm
ppm
1.28
1.26
1.24
1.22
1.20
1.18
TOCSY

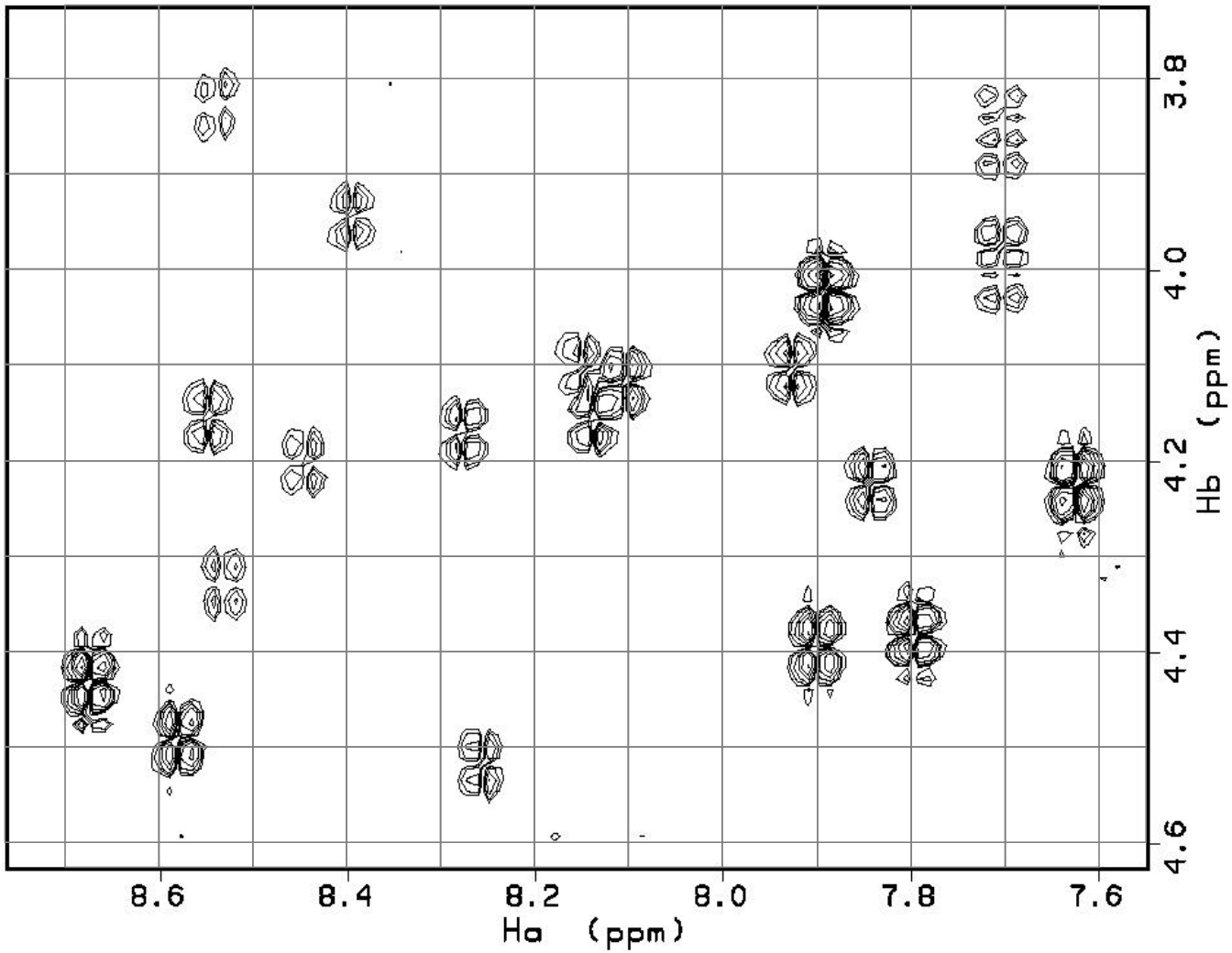
2. The COSY, TOCSY and NOESY spectra on the following pages were obtained from a twenty-amino acid peptide, with sequence **KTLTLEAALRNAWLREVGLK**, in a mixture of H_2O and D_2O. (A chart containing chemical structures and three- and one-letter abbreviations of all amino acids is attached to this problem set.)

   a. The COSY spectrum shows crosspeaks for direct ^3J couplings between NH protons and $CH_{\square}$ protons for all amino acids except proline. (Proline, **P**, has no NH proton, and shows no crosspeak in this region. Your peptide has no prolines in it though, so don't worry about this.) The first TOCSY spectrum also shows these crosspeaks, as well as additional peaks for serial correlations between NH protons and other protons in the coupled chain. Circle the two peaks that appear in the first TOCSY but not in the COSY. Based on the tables of amino acids chemical shifts and structures, which amino acids are these in your peptide?

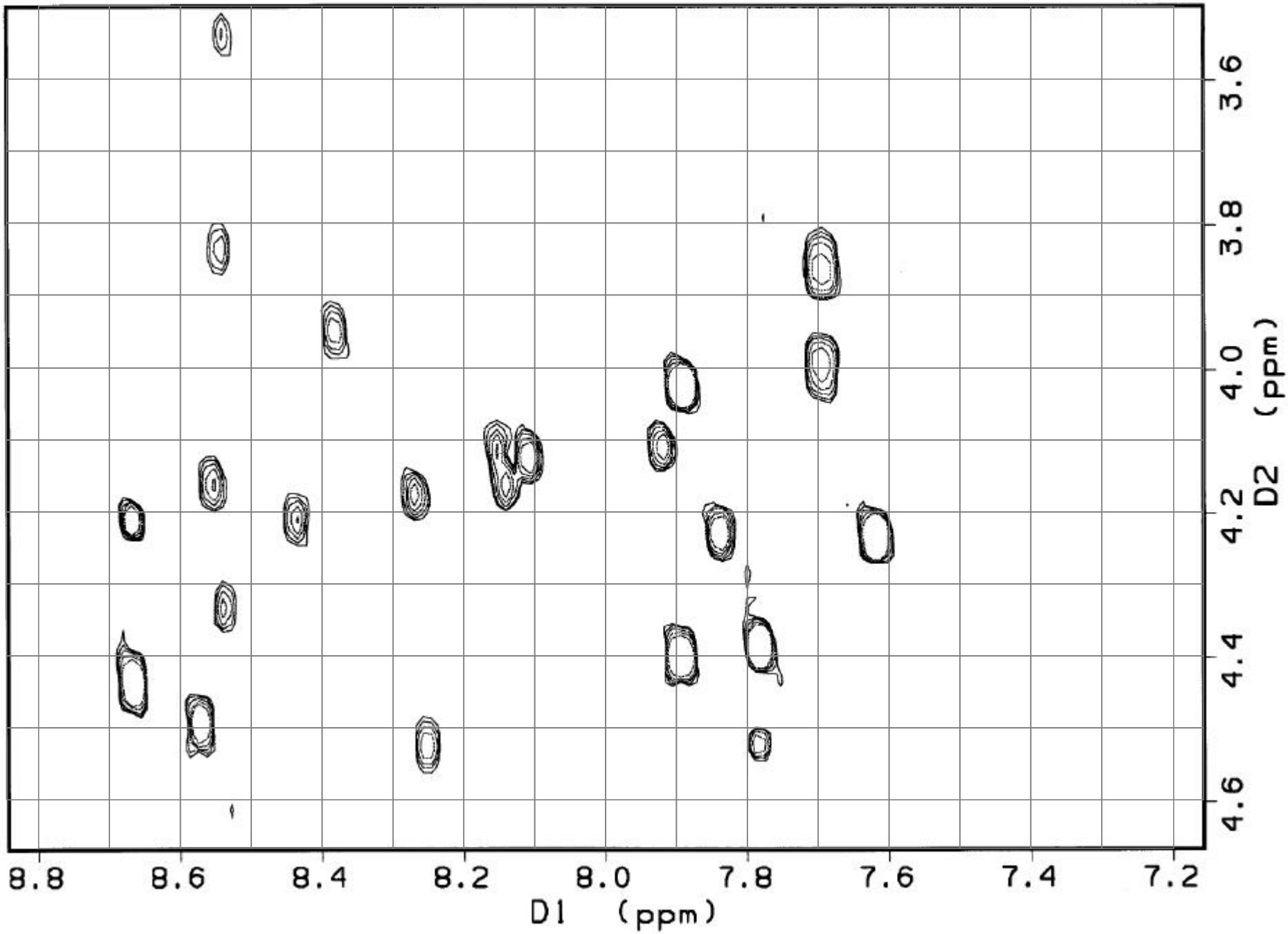
   b. The second TOCSY spectrum is complicated, but the presence of some amino acids can be confirmed by inspection. (Frequently TOCSY spectra are analyzed by computer to categorize amino acids present in a peptide.) In particular, **V**aline(17) is fairly easy to find. Give chemical shifts for the protons in **V**aline(17).

   c. The NOESY spectrum shows the same peaks present in the TOCSY, plus additional resonances for pairs of protons that are close in space. Can you identify a crosspeak corresponding to a **V**aline(17)-**G**lycine(18)? (What is a characteristic feature of glycine that makes it easy to spot?)

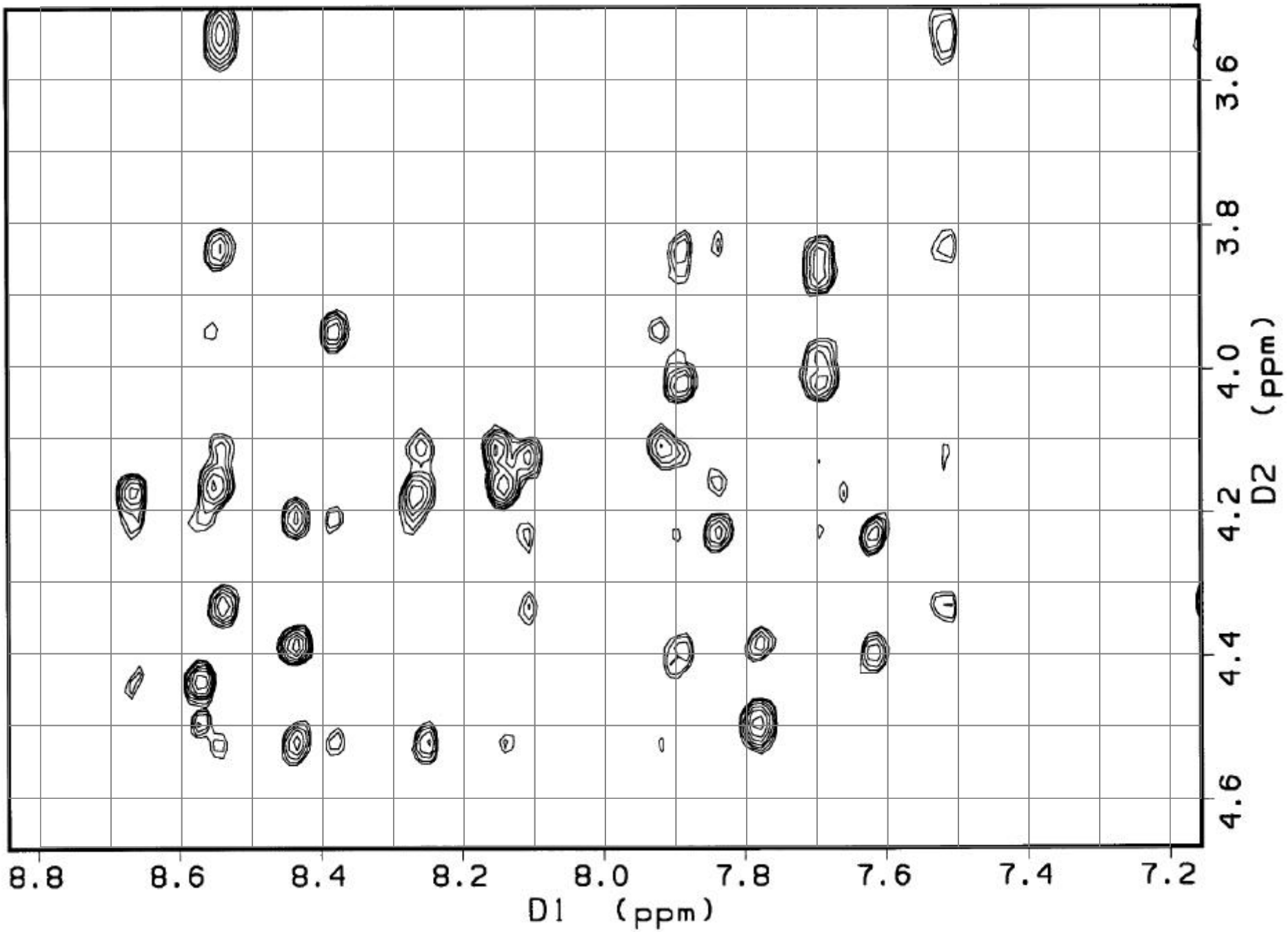
COSY



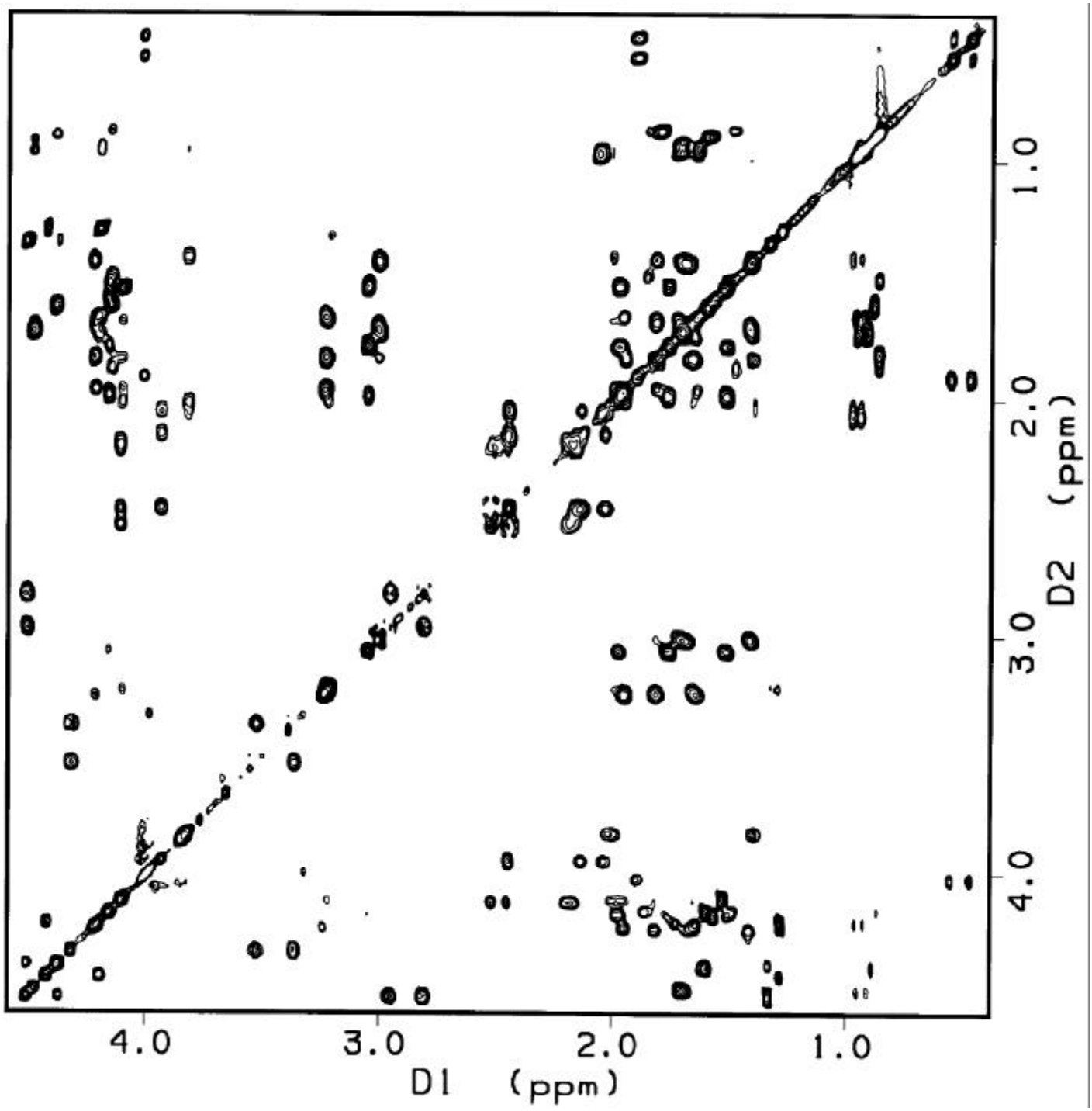
TOCSY



NOESY



TOCSY

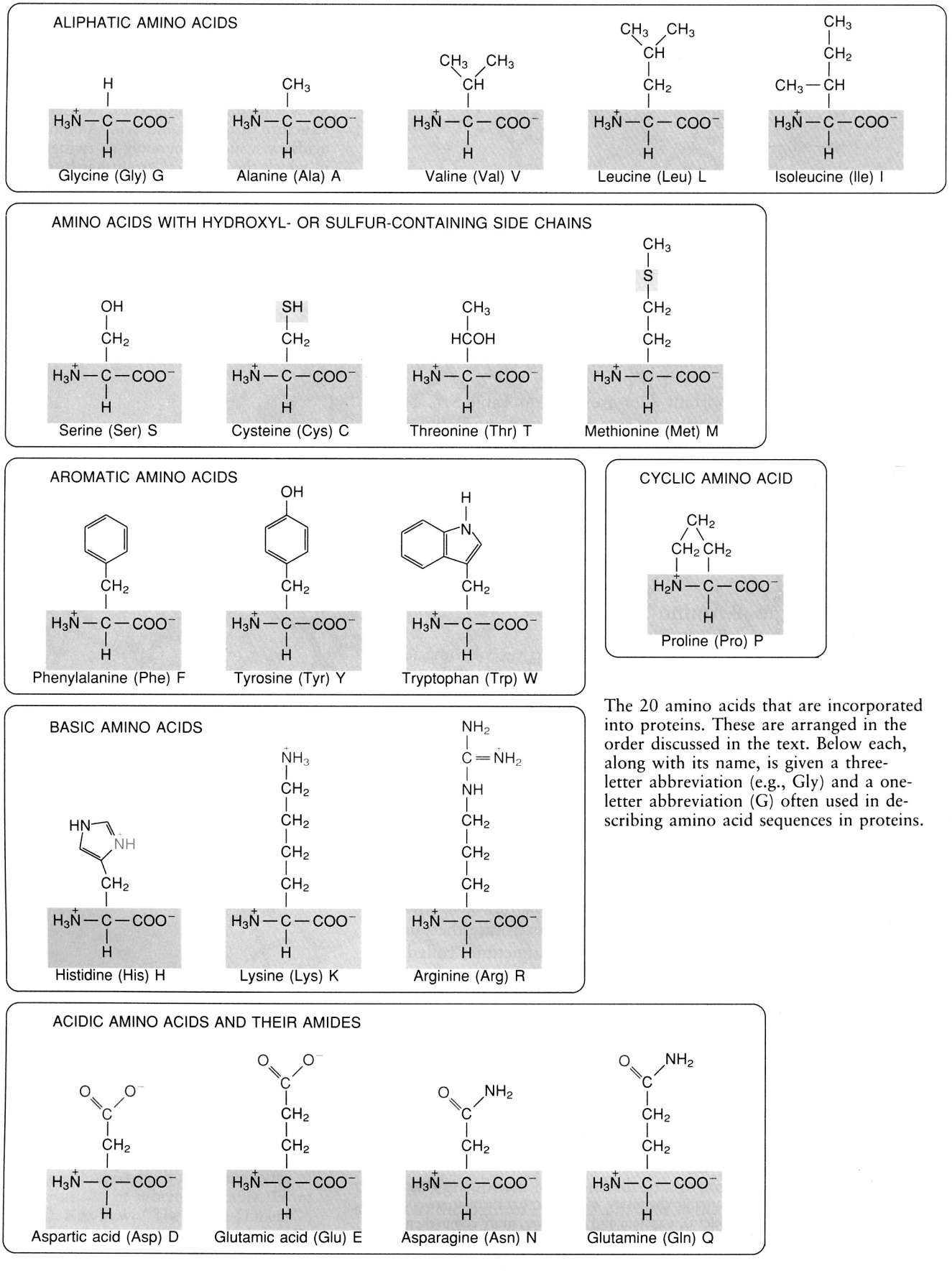


Typical proton chemical shifts for amino acids within a protein.

type	HN	HA	HB	other
gly	8.0	4.2,3.8		
ala	8.0	4.4	1.4β	
val	8.0	4.4	2.0β	1.0,0.9γ
ser	8.0	4.5	3.7,3.6β	
thr	8.0	4.5	4.4β	1.2γ
cys	8.0	4.5	3.3,3.1β	
asp	8.0	4.6	2.5,2.3β	
asn	8.0	4.6	2.5,2.3β	amine: 6.9,7.6
glu	8.0	4.5	2.2,2.1β	2.3,2.2γ
gln	8.0	4.5	2.2,2.1β	2.3,2.2γ amine: 6.9,7.6
ile	8.0	4.3	1.0β	0.9γ(methyl) 1.2,1.1γ 1.0δ(methyl)
leu	8.0	4.4	1.7,1.6β	1.6γ 1.0δ,0.9δ
lys	8.0	4.4	1.6,1.7β	1.5,1.4γ 1.7,1.6δ 3.0,3.1ε NH_3:6.9
arg	8.0	4.4	1.6,1.7β	1.5,1.4γ 1.7,1.6δ 3.3,3.2ε NH:7.1
met	8.0	4.5	2.0,2.1β	2.6,2.5γ methyl: 2.2
pro		4.5	2.2,2.0β	2.1,2.0γ 3.7,3.6δ
phe	8.0	4.5	3.0,2.8β	ring: H-C (3) 6.5 to 7.6
tyr	8.0	4.5	3.0,2.8β	ring: H-C (2) 6.6 to 7.4
trp	8.0	4.5	3.1,2.9β	ring: H-C (5) 6.5 to 7.9, NH:10.0
his	8.0	4.5	3.2,3.0β	ring: H-C (2) 6.5 to 8.5

Chemical shifts may vary by as much as approximately $\pm$ 1.5, and sometimes more, depending on local structure.

Sometimes OH proton of Thr and Ser may be observed near 5.6 ppm.



The 20 amino acids that are incorporated into proteins. These are arranged in the order discussed in the text. Below each, along with its name, is given a three-letter abbreviation (e.g., Gly) and a one-letter abbreviation (G) often used in describing amino acid sequences in proteins.