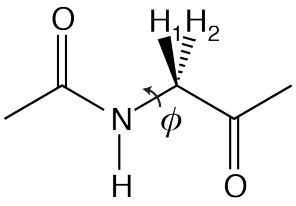
BCMB/CHEM 8190
ANSWERS TO PROBLEM SET 6

1) For short mixing times, NOE intensities depend approximately on distance (r) as $1/r^6$. Here a standard (known) distance of 1.78 Å results in an NOE with intensity 28.0. The distances then for the NOEs with intensities of 1.6 and 0.5 are:

$$\frac{28}{\left(1/(1.78)^6\right)} = \frac{1.6}{\left(1/(r)^6\right)} \quad r = 2.87 \qquad \frac{28}{\left(1/(1.78)^6\right)} = \frac{0.5}{\left(1/(r)^6\right)} \quad r = 3.48$$

So, the dihedral angle ϕ should be such that the $H\alpha_2$- H_N distance is 2.87 Å and the $H\alpha_1$- H_N distance is 3.48 Å.



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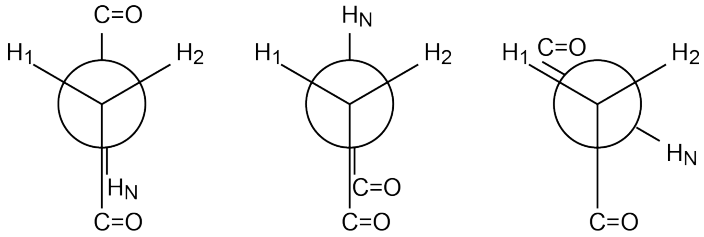
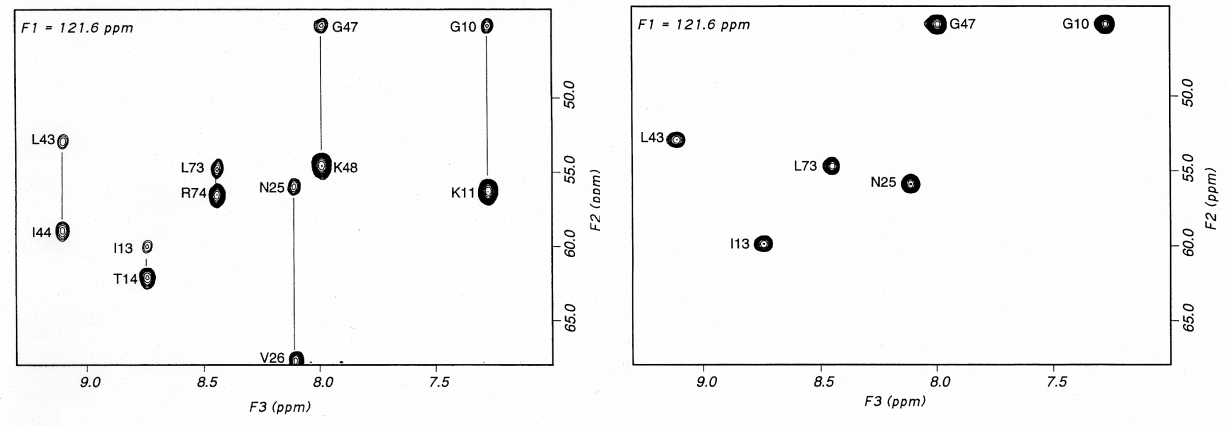


In the diagrams shown, conformation 1 results in distances between H_N and the $H\alpha$ hydrogens of ~2.95 Å, and conformation 2 results in distances of ~2.65 Å. Conformation 3 gives a distance of 2.65 Å for the HN-$H\alpha_2$ distance, and about 3.1 Å for the HN- $H\alpha_1$ distance, which is the only result that gives distances close to those predicted by the NOEs. So, the dihedral angle ϕ would be ±120°.

2)



a) The assignments are shown
b) In the HNCA spectrum, the amide ^1H and ^{15}N chemical shifts are correlated with the alpha carbon ^{13}C chemical shift of the same amino acid and also with the alpha carbon

^{13}C chemical shift of the preceding amino acid in the sequence. Typically, the experiment is performed such that the signal from the preceding amino acid is less intense that that of the same amino acid. However, this is not always the case, and is the reason for performing the HN(CO)CA experiment (to differentiate between the intra- and inter-residue correlation). In any case, for this protein, for each of the signals shown, it happens that the signals for the preceding amino acid are weaker.
c) No. In this example, coincidentally the upfield signals were all from inter-residue correlations that, coincidentally, all happened to be less intense than the intra-residue signals.

3)

	(LEU)	GLU	ILE	VAL	THR	ALA	ILE	PHE	(LYS)
	(123)	124	125	126	127	128	129	130	(131)
^{1}HN	8.41	7.78	7.74	8.59	8.76	7.77	7.55	8.40	7.60
^{15}N	119.6	124.0	120.6	116.5	119.1	123.8	112.9	121.8	116.3
$^{1}H\alpha$	3.91	4.02	3.85	3.77	3.73	4.08	3.61	3.91	3.99
$^{13}C\alpha$	ND*	60.5	65.7	65.8	68.1	55.4	63.9	60.5	57.8
$^{1}H\beta$	ND*	2.30	2.15	2.30	4.42	1.84	1.90	3.28	1.85
$^{13}C\beta$	ND*	28.8	38.8	31.8	68.7	18.7	37.9	40.1	31.9
$^{13}C=O$	179.2	178.8	177.2	178.5	174.4	178.2	179.1	177.6	176.6

*ND, could not be determined

Residues 130-126 show $^{1}H_N(i)$-$^{1}H\alpha(i-3)$ NOEs, which are diagnostic of alpha helical structure, as well as strong sequential $^{1}H_N$-$^{1}H_N$ NOEs that also are consistent with alpha helical structure.