Structure determination of a 20 amino acid peptide by NMR

The twenty amino acid peptide contains over 100 unique protons that are potentially observable by NMR. When placed in a magnetic field, each proton within the peptide can absorb or emit radiofrequency (RF) energy at a specific frequency, depending on its local chemical environment within the peptide structure. The frequency of the RF energy emited by each proton in the peptide is reported in units of "chemical shift". "Chemical shift" is actually proportional to Hz (a more familiar unit of frequence), and the proportionality constant depends on the magnetic field strength of the particular NMR instrument used in the experiment.

The structure determination proceeds in two stages. The first stage is the solving of the "assignment problem". Solving the assignment problem means identifying the unique NMR resonance frequency (or chemical shift) of each chemically unique proton within the peptide.

To solve the assignment problem for the peptide, the following information is helpful:

a) A table of typical proton NMR chemical shifts for protons within amino acids will be useful. This table is available on the web page.

b) It will be helpful to know the primary sequence of the 20 amino acid peptide. It is:

K1 T2 L3 T4 L5 E6 A7 A8 L9 R10 N11 A12 W13 L14 R15 E16 V17 G18 L19 K20

c) The 2-D TOCSY spectrum of the peptide will be needed. TOCSY is an abbreviation for "total correlated spectroscopy". The 2-D TOCSY spectrum identifies the chemical shifts of pairs of protons that are within the same amino acid. The TOCSY spectum is available on the web page. A view of the full TOCSY spectrum is shown, plus expanded views of six sections of the TOCSY spectrum.

d) The 2-D NOE spectrum of the peptide will be needed. NOE is an abbreviation for "nuclear Overhauser effect". The 2-D NOE spectrum identifies identifies the chemical shifts of pairs of protons that are close together within the peptide structure (specifically, within about 6 Å of each other). These protons may or may not be within the same amino acid. The NOE spectum is available on the web page. A view of the full NOE spectrum is shown, plus expanded views of 9 sections of the NOE spectrum. [Note: two versions of the NOE spectrum are shown on the web page, an "80 msec mixing time" spectrum and a "400 msec mixing time" spectrum. I suggest starting with the "80 msec mixing time NOE spectrum". The difference between the spectra will be discussed later.]

Getting started on the assignment problem:

A good place to start is with the valine. The peptide contains only one valine, V17. Try to assign the chemical shifts of each of the protons in the valine.

To do this, first look up the chemical shifts of typical protons in a valine in the table. Chemical shift values for a typical valine are:

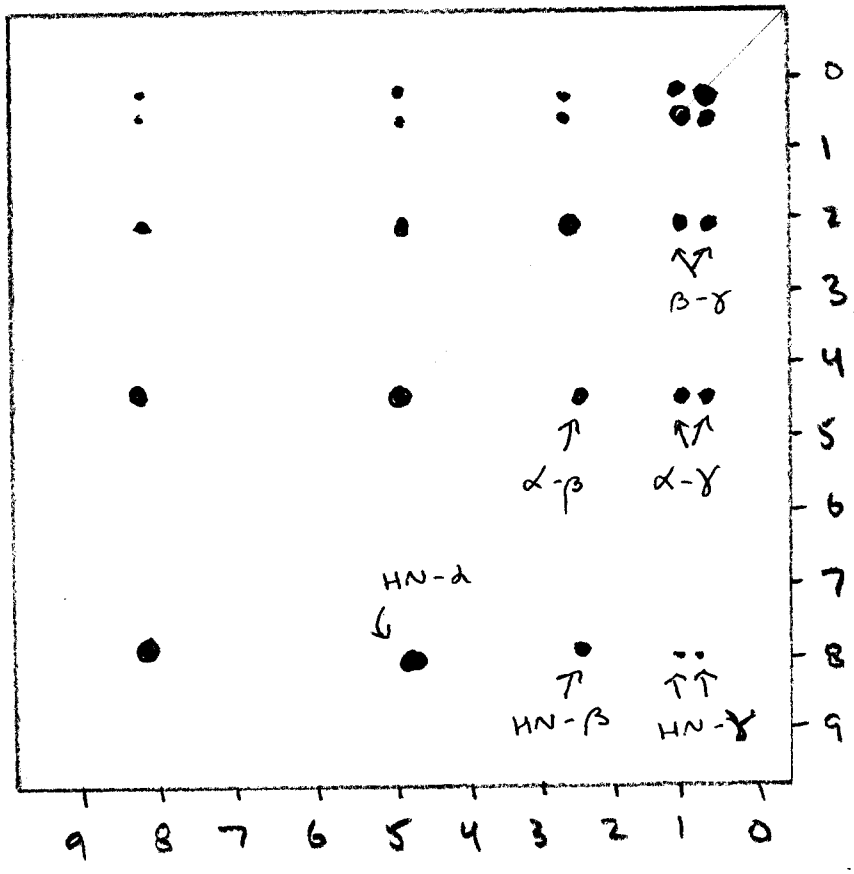
8.0 for the amide proton (HN)
4.4 for the alpha proton (αH)
2.0 for the beta proton (βH)
1.0 and 0.9 for the two methyl groups (γH)

Remember, the chemical shift values for Val17 in the peptide will not have these exact values, but will probably be similar.

To assign the chemical shifts of the valine in the peptide, first look at the TOCSY spectrum.

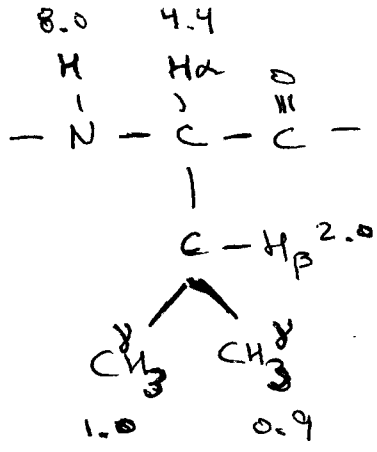
The pattern of peaks in the TOCSY spectrum from protons in the valine should look something like that shown below. Notice that the strongest peaks are for those protons separated by 3 bonds. Protons separated by 4 or 5 bonds give weaker peaks in the TOCSY spectrum.

Pattern of peaks in a TOCSY spectrum from a typical valine →



Note: Sometimes the HN-γ peaks are very weak and not observed.

A typical valine, with typical chemical shifts:



The chemical shifts of Val17 in the peptide are:

HN = 7.90
Hα = 4.03
Hβ = 1.91
Hγ = 0.48, 0.58