Junior Sophister
Organic and Inorganic Course

A introduction to Practical NMR

Introduction

- NMR is the **major** analytical technique used to identify and characterise materials today.
- NMR is used for :
  - Identification of nuclides and their environment
  - Conformational analysis
  - Interactions of molecules
  - Monitoring processes and reactions
- The main experimental methods are :
  - **FT NMR (superconducting magnets)**
  - Dynamic NMR, MRS, MRI

NMR of Liquids

- Most common method by which NMR is carried out.
- Materials and substances (liquids or solids) are dissolved in appropriate solvents – deuterium replaces hydrogen
- Many and varied experimental techniques in NMR, which are available to the user
- Usefulness is the fact that the spins under the influence of the magnetic field are dependent on the molecular environment and that peaks (resonances) are easily identifiable and reproducible from molecule to molecule

NMR of Liquids

- Applications :
  - Industrial (chemical, pharmaceutical, food)
  - Academic (research, teaching)

- In the fields of :
  - Chemistry, Biochemistry, Biology, Physics, Pharmaceutical Chemistry, Medicine, Genetics, Geology, Engineering, Forensic Science *etc*.

NMR Spectrometers

- Current equipment :
  - 600MHz (14.1T) solution instrument
  - 400MHz (9.4T) solution instruments

- Nuclides
  - ^{1}H, ^{13}C, ^{31}P, ^{19}F, ^{15}N
  - ^{2}D, ^{11}B, ^{17}O, ^{29}Si, ^{14}N
  - ^{111}Cd, ^{119}Sn, ^{195}Pt , ^{51}Vn

- Future direction
  - 800 MHz (18.8 T) instrument for large protein work and solid state MAS spectroscopy

In at the deep end !!!

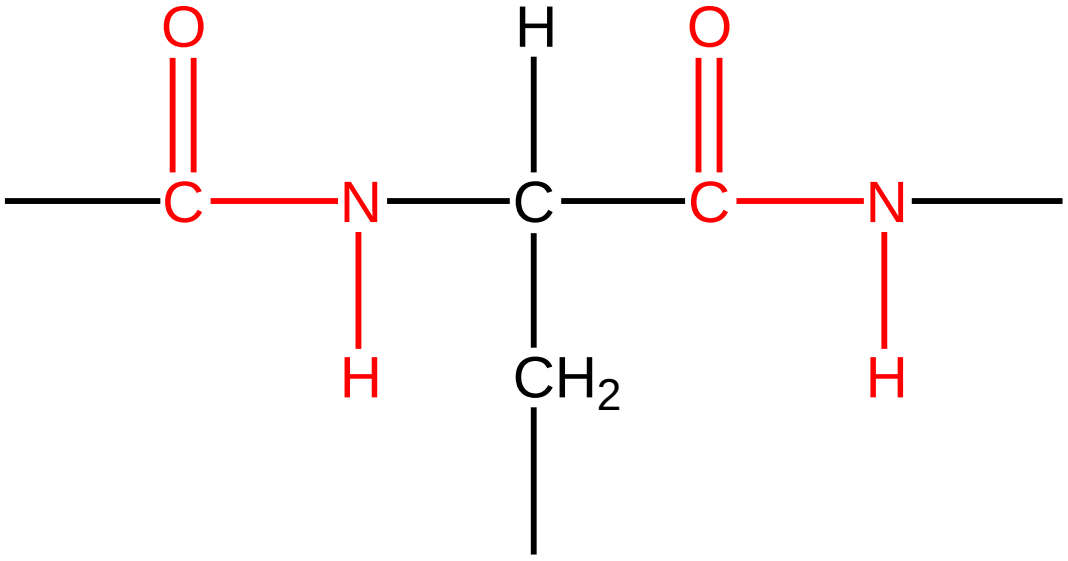
NMR on a Hormone

- Thyrotropin Releasing Hormone (TRH)

- TRH is a neuropeptide which is involved in some CNS disorders
- TRH has a function in the repair of brain and spinal disorders

A Case Study in NMR Methods

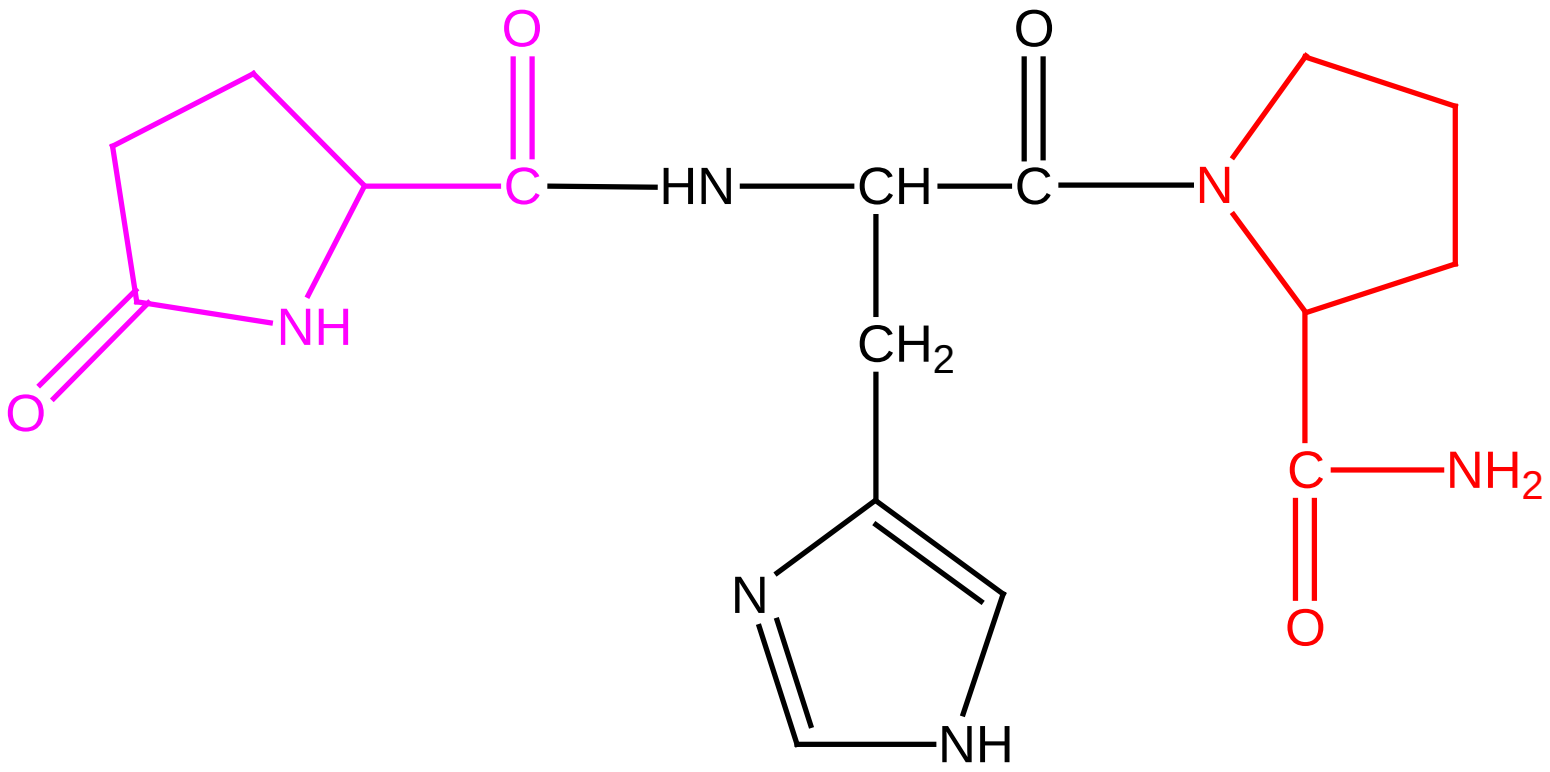
The TRH molecule is small peptide



- building block is an amide linkage

TRH

- a **Tripeptide** with an amino terminated proline
- p-Glutamic acid Histidine Proline



NMR of TRH

- The *aim* is to identify and assign *all* the positions of the atoms in the peptide
- Verify that the structure is correct

using

- Proton (Hydrogen) NMR
- Carbon-13 NMR
- Nitrogen-15 NMR

Getting Started

- The initial NMR experiment is a Proton NMR
- An analysis of the proton NMR determines which of other NMR techniques are required (or possible) to identify the compound
- Proton NMR is used because it is the most sensitive of the commonly active NMR nuclides

Proton (Hydrogen) NMR

- Position of the signal – resonance
- Hydrogen- hydrogen interaction
  - spin coupling
- Ratio of the amount of proton signals
  - integration

Carbon-13 NMR

- Carbon-13 is the active NMR nuclide

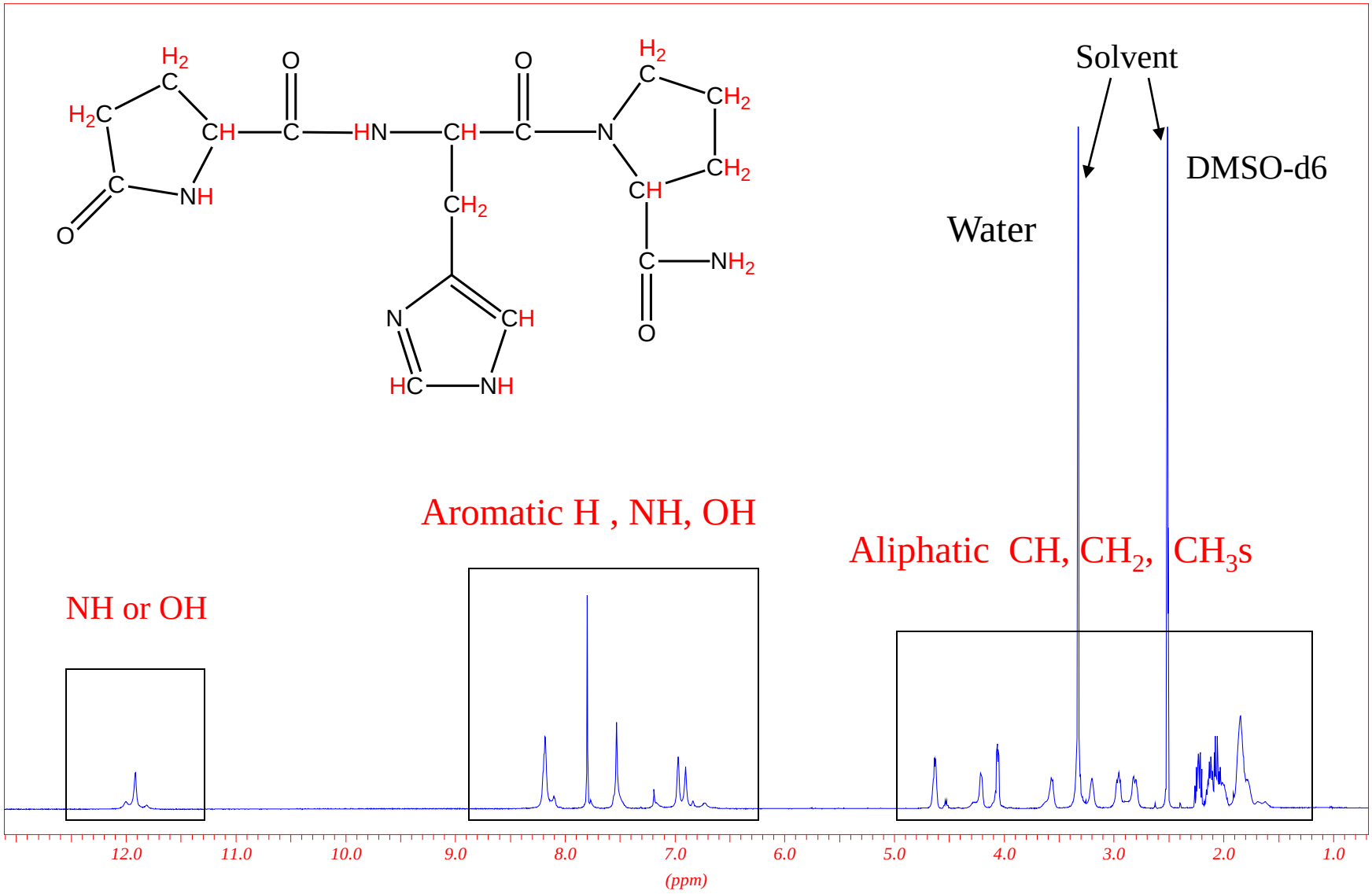
  - only 1% of all Carbon

- Position of the Carbon signals

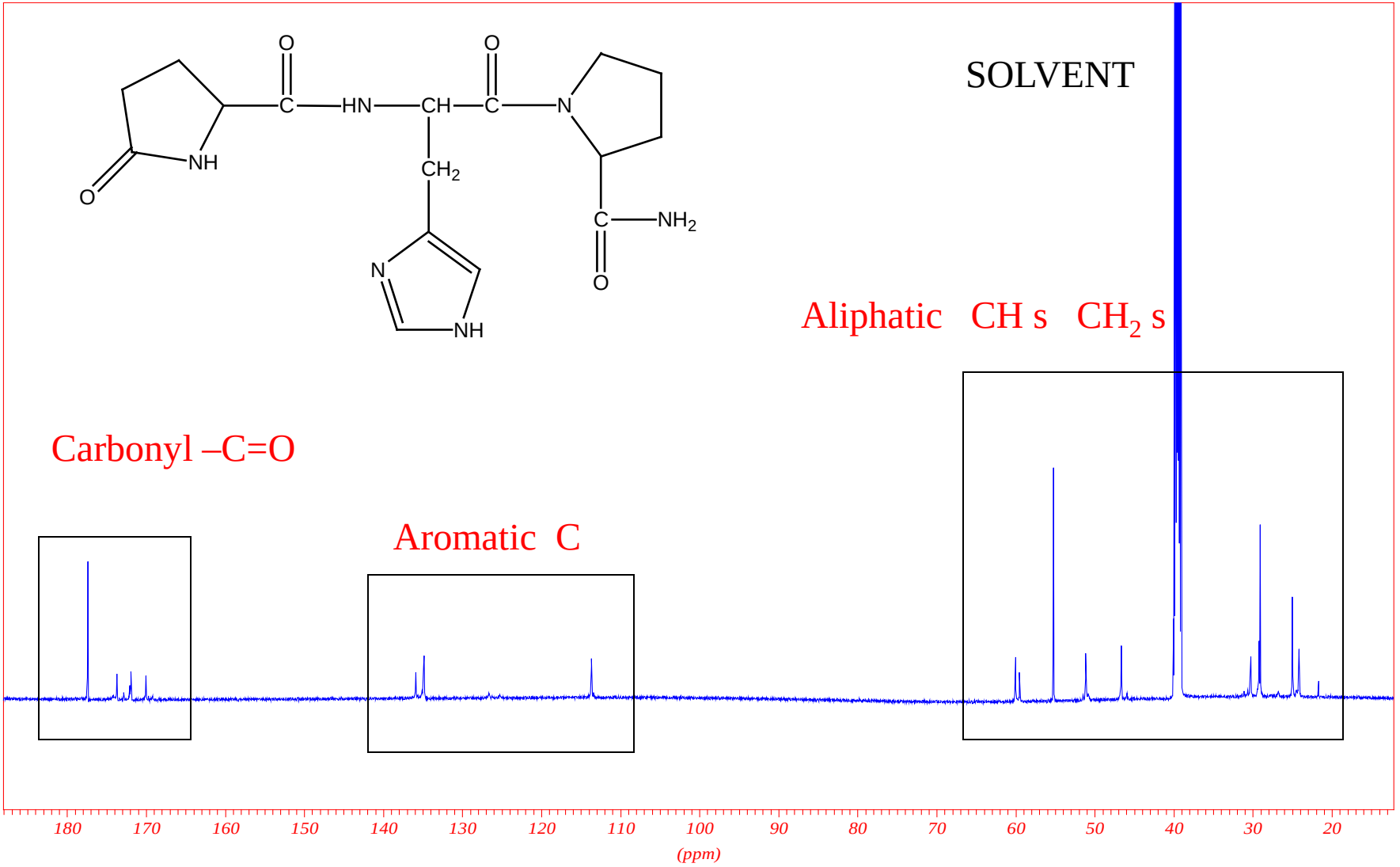
- Other carbon-13 experiments to determine the type number of hydrogens attached

  e.g. C, CH, CH_2, CH_3

Proton (HYDROGEN) NMR

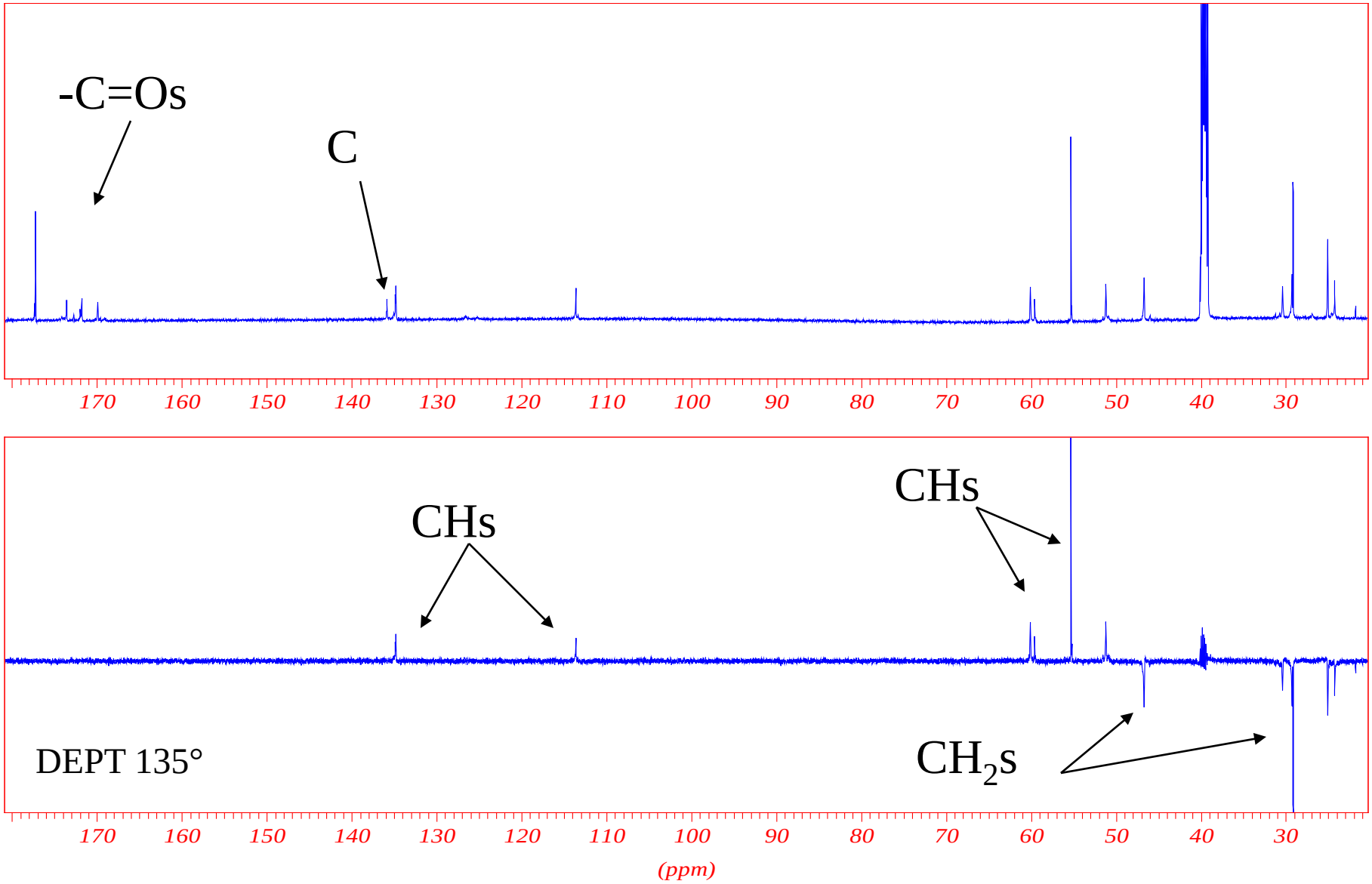


Carbon-13 NMR

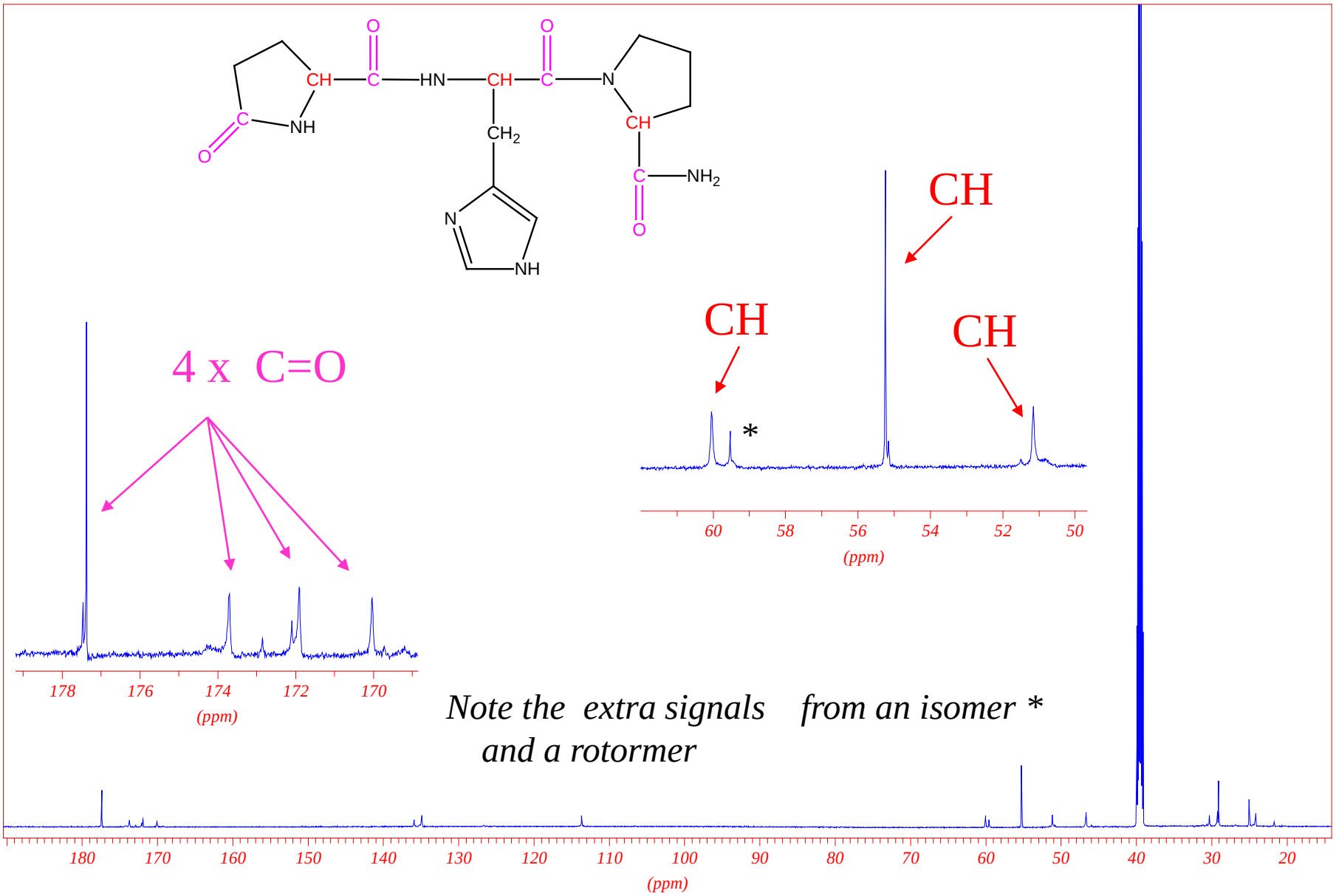


More Carbon-13 NMR

Carbon DEPT (distortionless enhancement by polarisation transfer)



^{13}C Carbonyl (C=O) and methine (CH) signals



More NMR tools - 2D

- CH and NH correlation – *through bonds*
  - direct (one) bond or further out
- HH correlation - *through bonds*, looks more complex
  - connections are the off diagonal signals
- *Through space* connections for HH - NOe
- Selective versions of the 2D experiments

NMR experiments

- The basic proton and carbon-13 NMR spectra yield a lot of general information

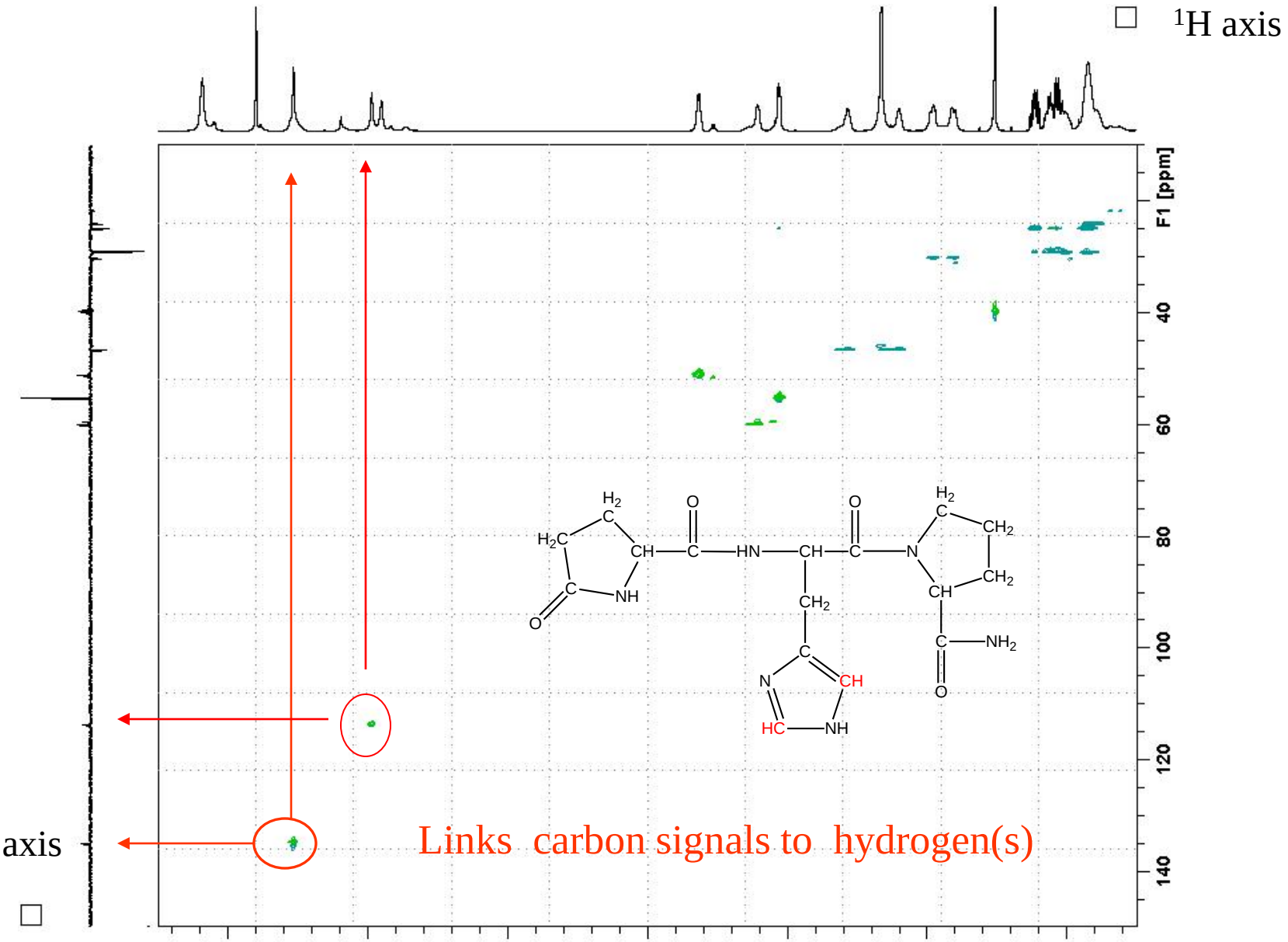
- The next stage is to link the information from the proton and carbon-13 data
- This will allow for the start of a full structural analysis
- The main methods are through the use 2-Dimensional (2D) NMR experiments and the related selective versions of 2D NMR

- The names of the experiments are :
  - COSY HX (direct H to C or N correlation) - HSQC-^{13}C and HSQC-^{15}N
  - Long range CH COSY (H to C links over 2 or 3 bonds) - HMBC

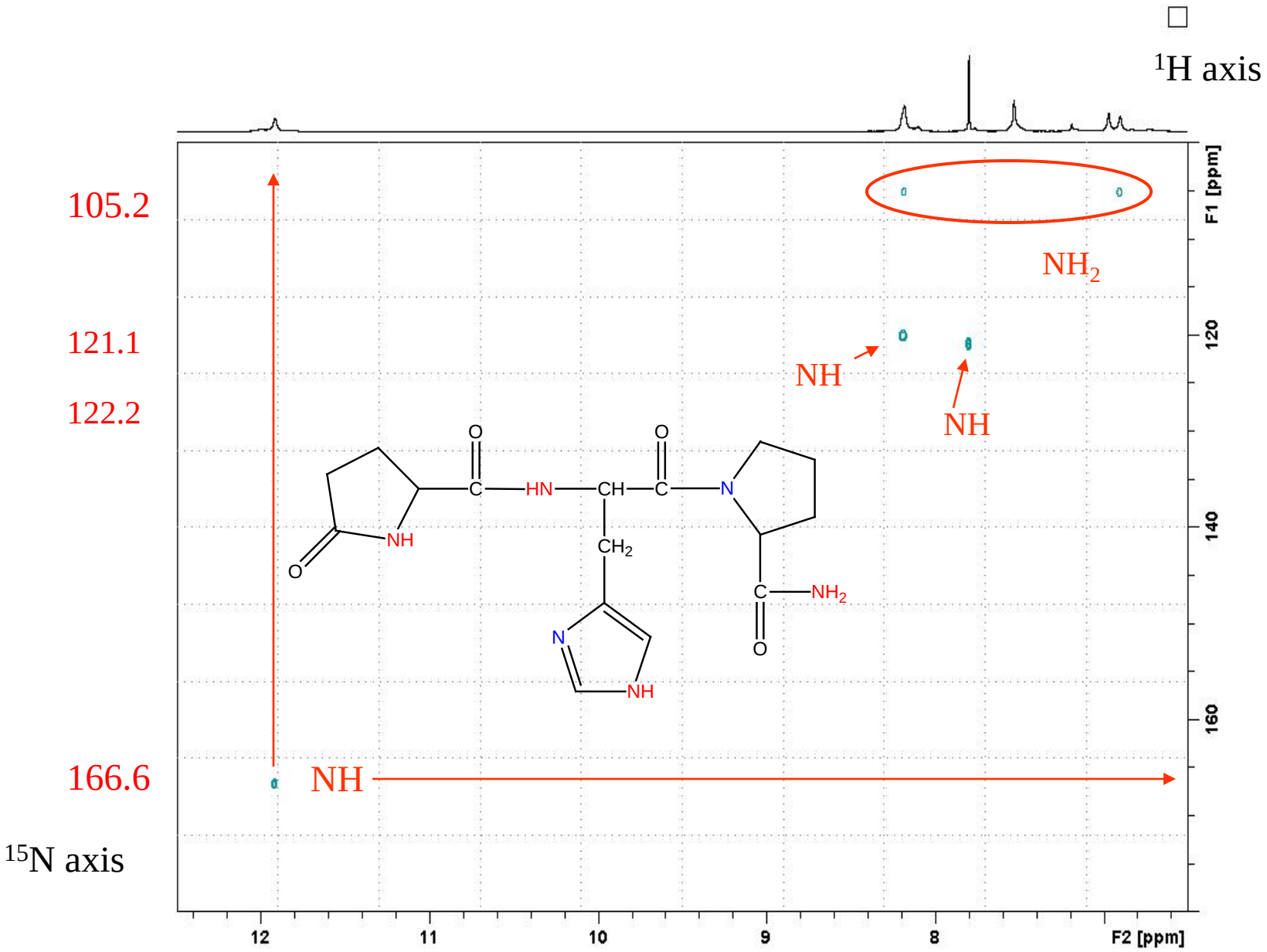
  - HH COSY or TOCSY - Hydrogen to Hydrogen correlation through **bonds**
  - NOESY or ROESY - Hydrogen to Hydrogen correlation through **space**
  - Selective TOCSY 1D version of the 2D experiment
  - Selective ROESY 1D version of the 2D experiment

NMR spectroscopists love fancy acronyms

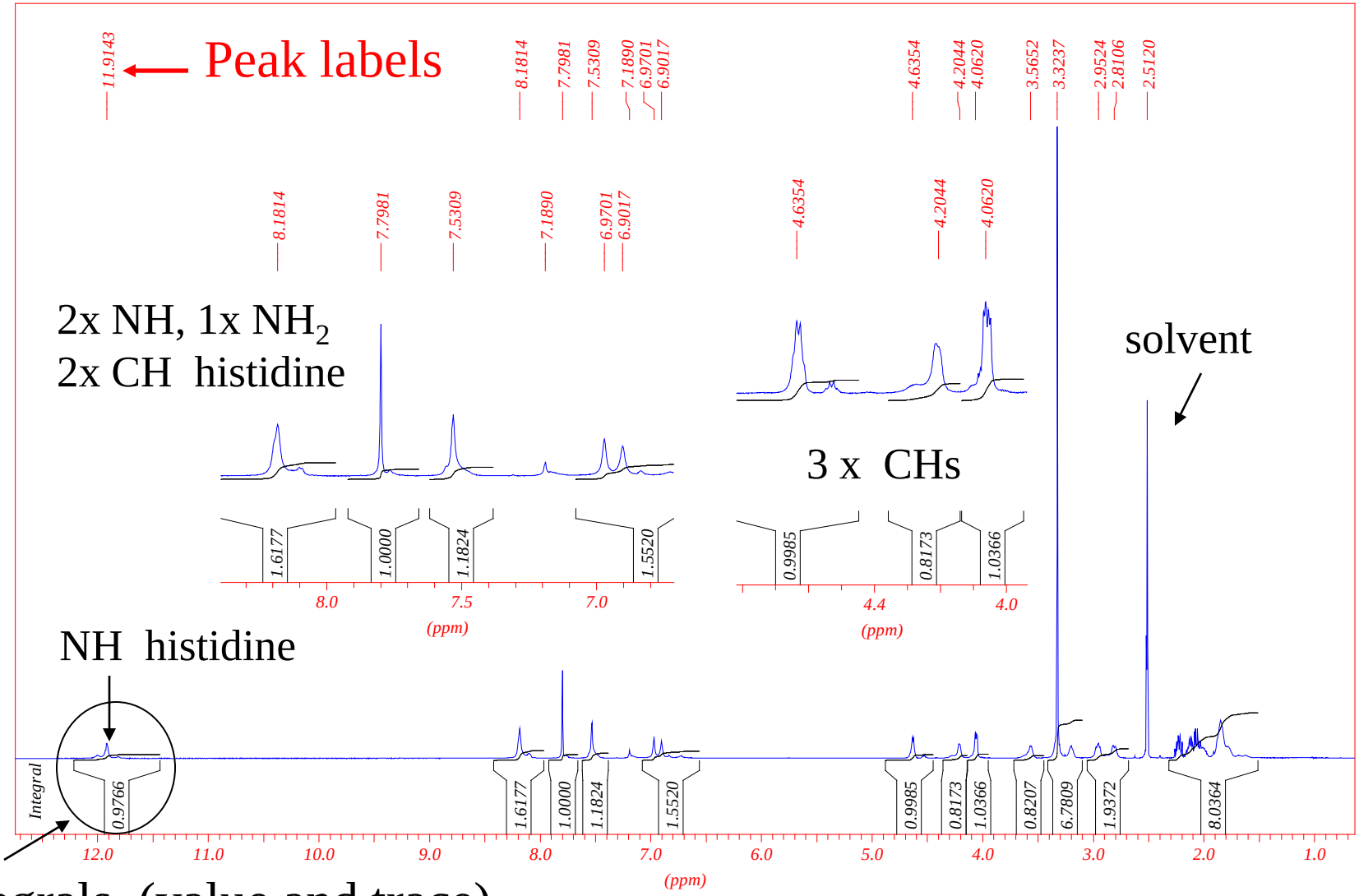
^{13}C-^{1}H Correlation



^{15}N-^{1}H Correlation



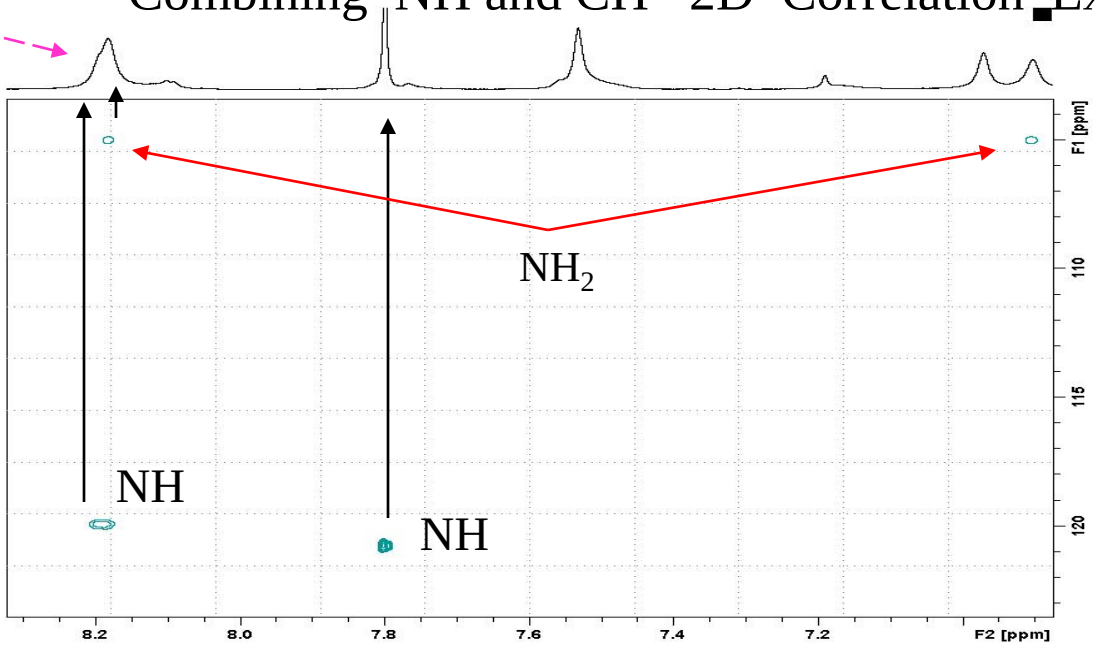
^{1}H NMR with peak *labels* and *integration* (ratio of signals with respect to one another)

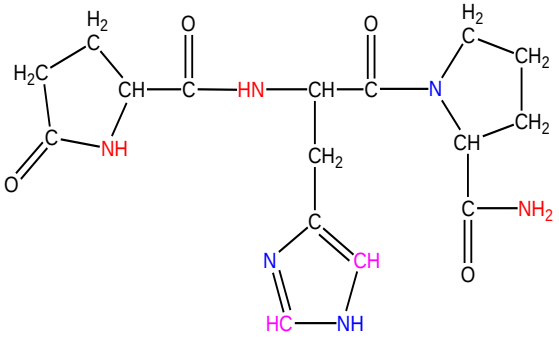


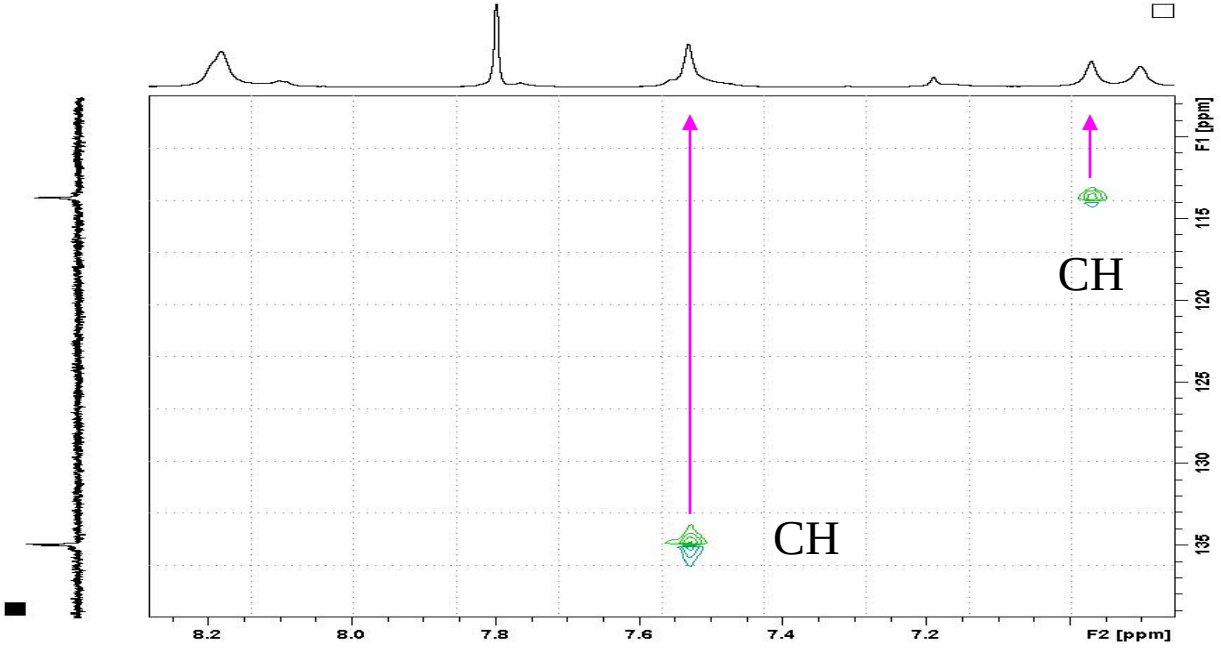
Integrals (value and trace)

Combining NH and CH 2D Correlation Experiments

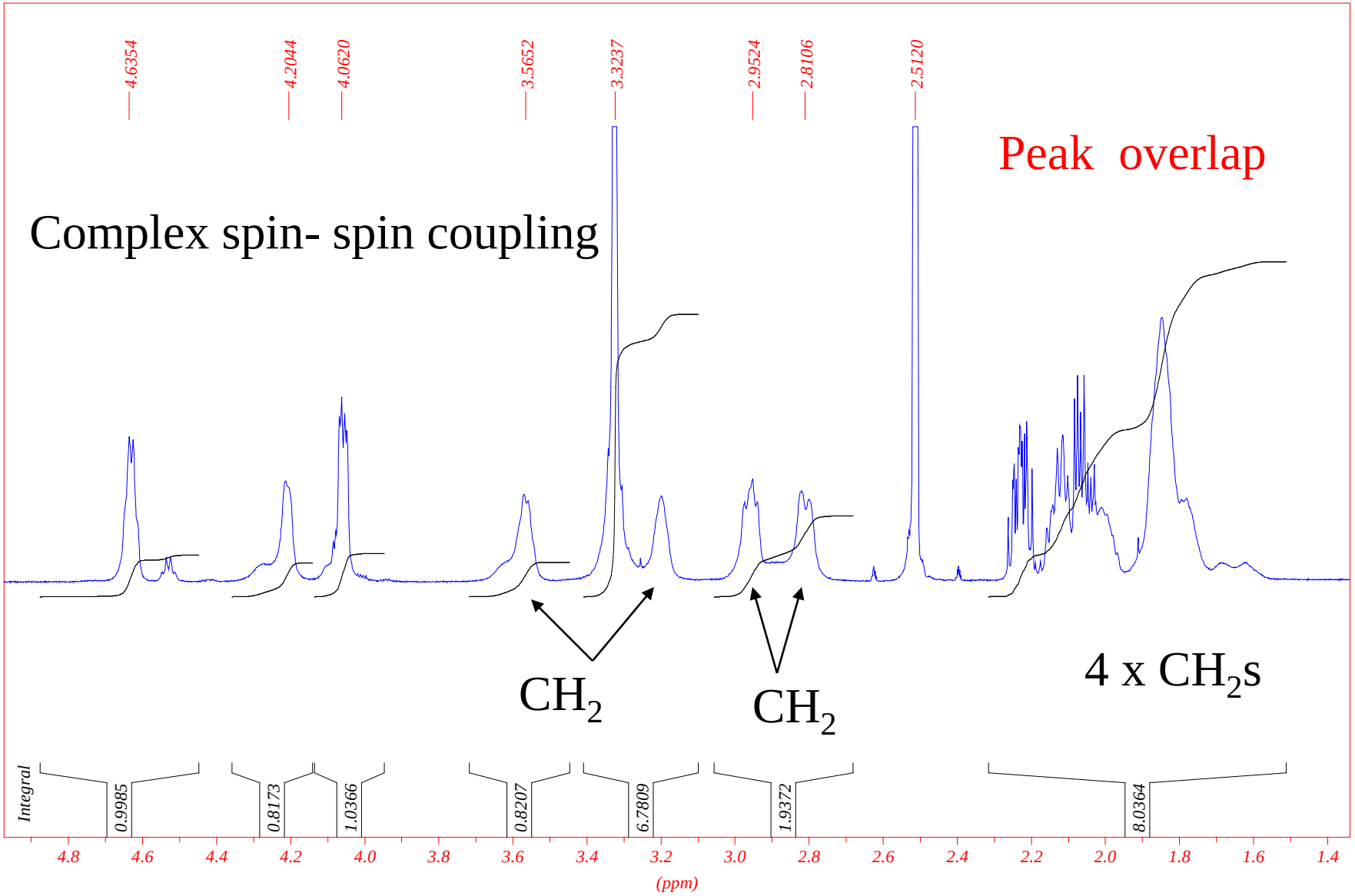
Peak overlap







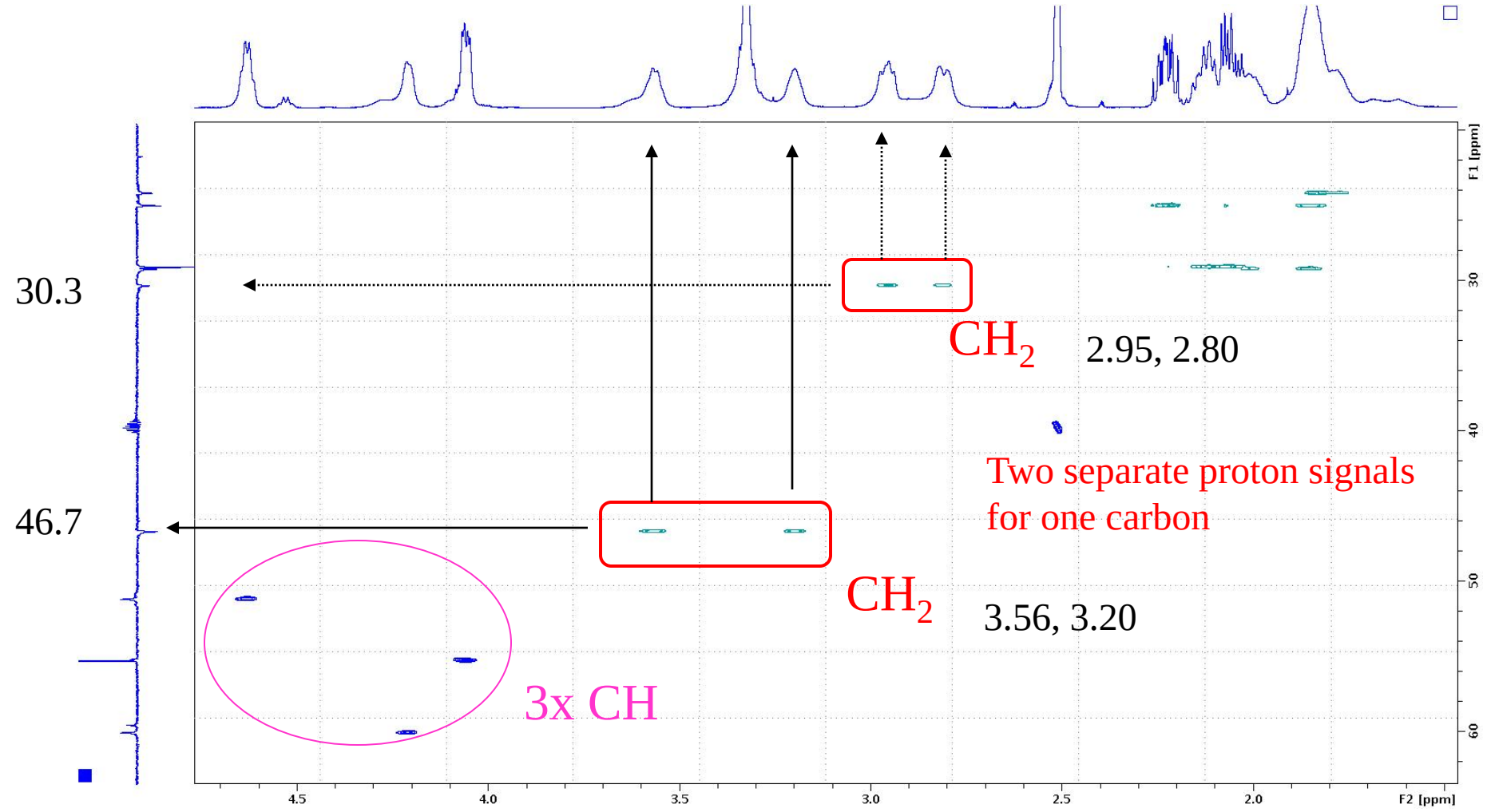
Problems in ^{1}H spectra



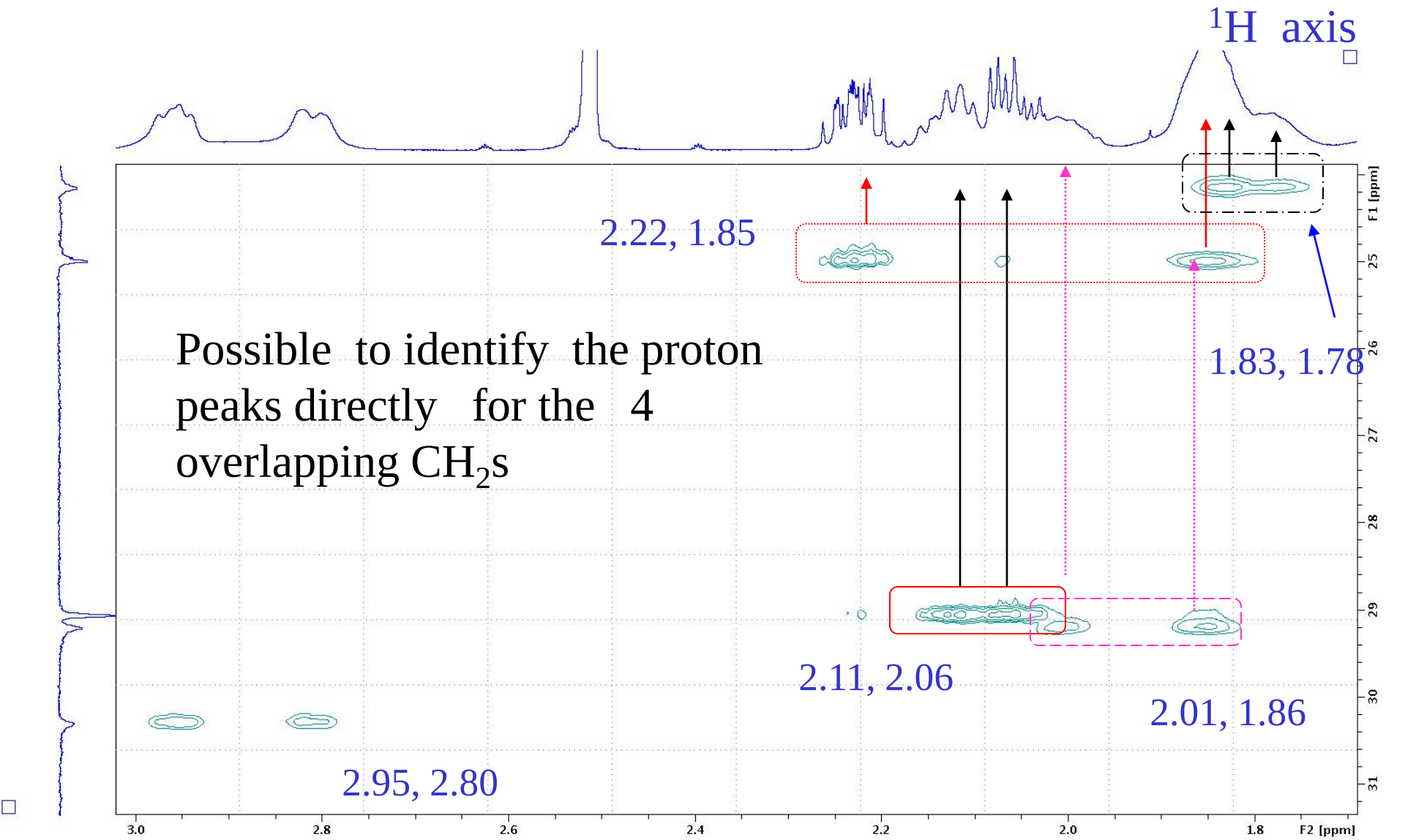
Review so far

- The two NH and CH COSY spectra begin to unlock the complexity of the proton spectra
- The aromatic histidine protons can be differentiated form the NH peaks by comparing the NH and CH COSY
- Overlap of proton signals is observed in methylene region (1.5-2.1ppm) and in the NH region at 8.0ppm
- The number of methylene signals in the 1.5 to 2.1ppm is obtained from the integral value of the entire region
- To solve this area , a closer look at the CH COSYs is required
- This is shown in the next two slides
- A useful tool is to tabulate the data in a table of peaks

CH correlation in aliphatic region



Identifying the remaining methylene peaks



^{13}C axis

Peak table

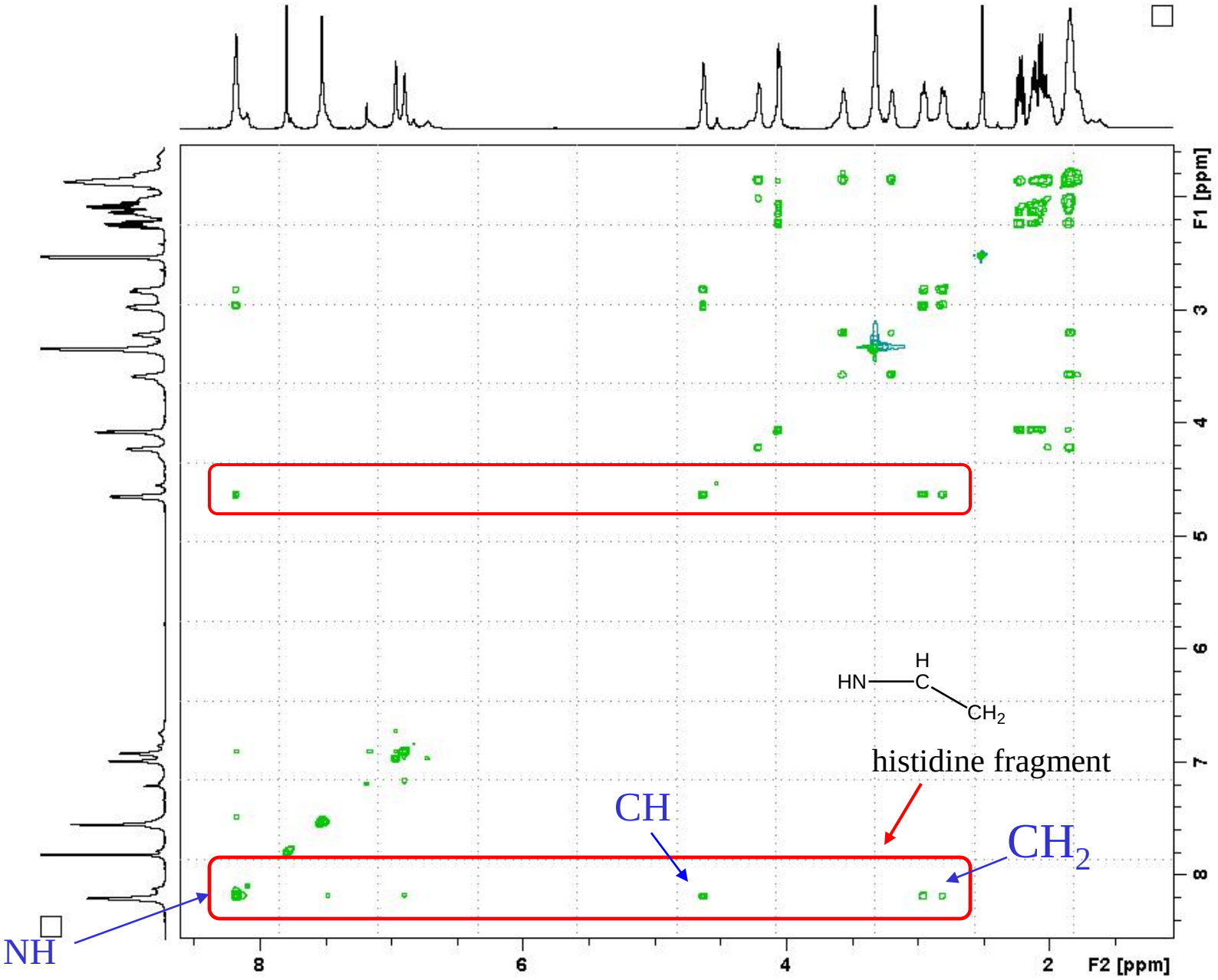
with assignments for carbon and nitrogen attached to protons

	δ ^{13}C ppm	δ ^{15}N ppm	δ ^{1}H ppm
CH aromatic	134.9	-	6.97
CH	113.7	-	7.53
CH aliphatic	60.1	-	4.21
CH	55.2	-	4.05
CH aliphatic	51.2		4.63
CH_2	46.7	-	3.56, 3.20
CH_2	30.3	-	2.95, 2.80
CH_2	29.2	-	2.01, 1.86
CH_2	29.0	-	2.11, 2.06
CH_2	25.0	-	2.22, 1.85
CH_2	24.2	-	1.83, 1.78
NH	-	166.6	11.92
NH	-	122.2	8.21
NH	-	121.1	7.80
NH_2	-	105.2	8.18, 6.90

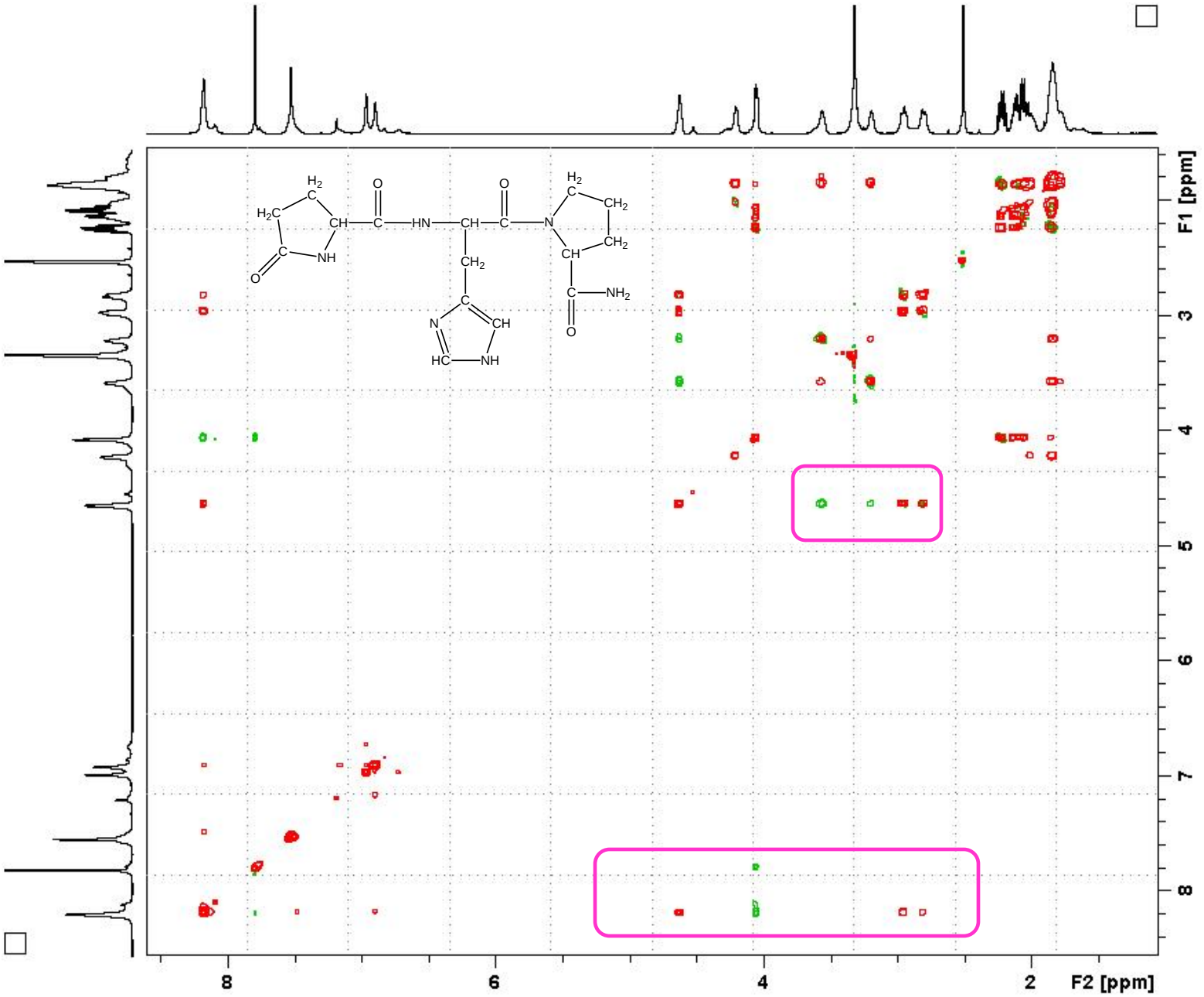
Assembling the structure

- Most of the peaks have now been identified and classified
- The next stage is to begin to assemble the peptide
- The HH COSY or its TOCSY cousin is the best way to start to link the various proton peaks with trough bond connections
- The HH COSY or TOCSY is read as follows :
  - The diagonal is the 1D spectrum
  - Off diagonal signal(s) display the connections of the spins
  - A true signal *must* have mirror image across the diagonal
- The spin splitting observed in the 1D proton spectrum is too complex or obscured to be of much use
- Through space HH links can be found using the ROESY experiment
- Combining this experiment with the HH COSY yields spatial and through bond links at the same time

HH correlation - links local protons



Combined trough bond (HH) and space (Noe) correlation



Selective versions of 2D NMR experiments

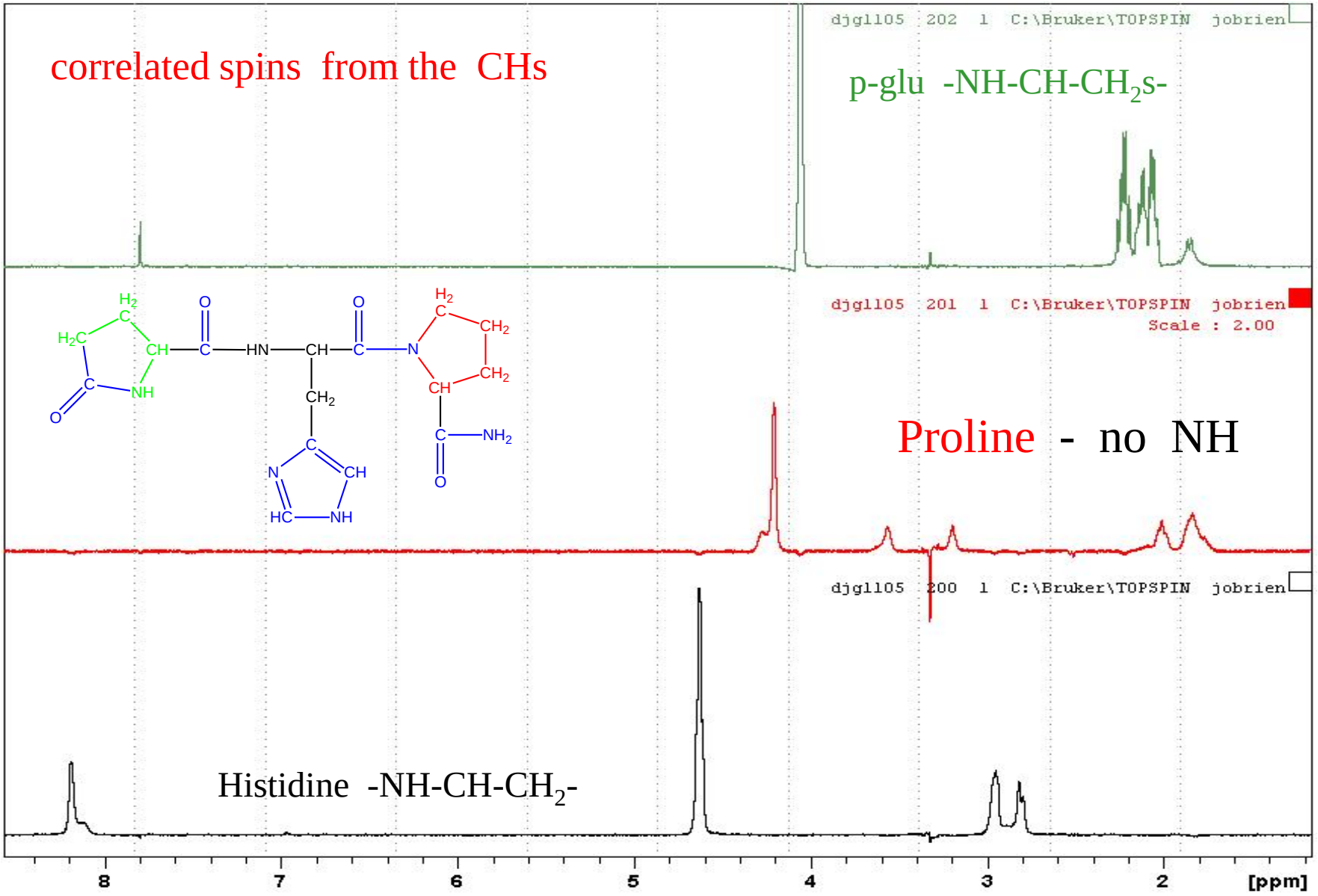
- Another set of tools available to the spectroscopist are the 1D versions of 2D NMR experiments

- The selective TOCSY focuses on the excitation of a single peak to give the through bond links with any other peak to which it is linked

- This can be tuned to locate a nearest coupled spin or an entire coupled spin system **Only** the spin coupled signals are observed which simplifies the spectrum

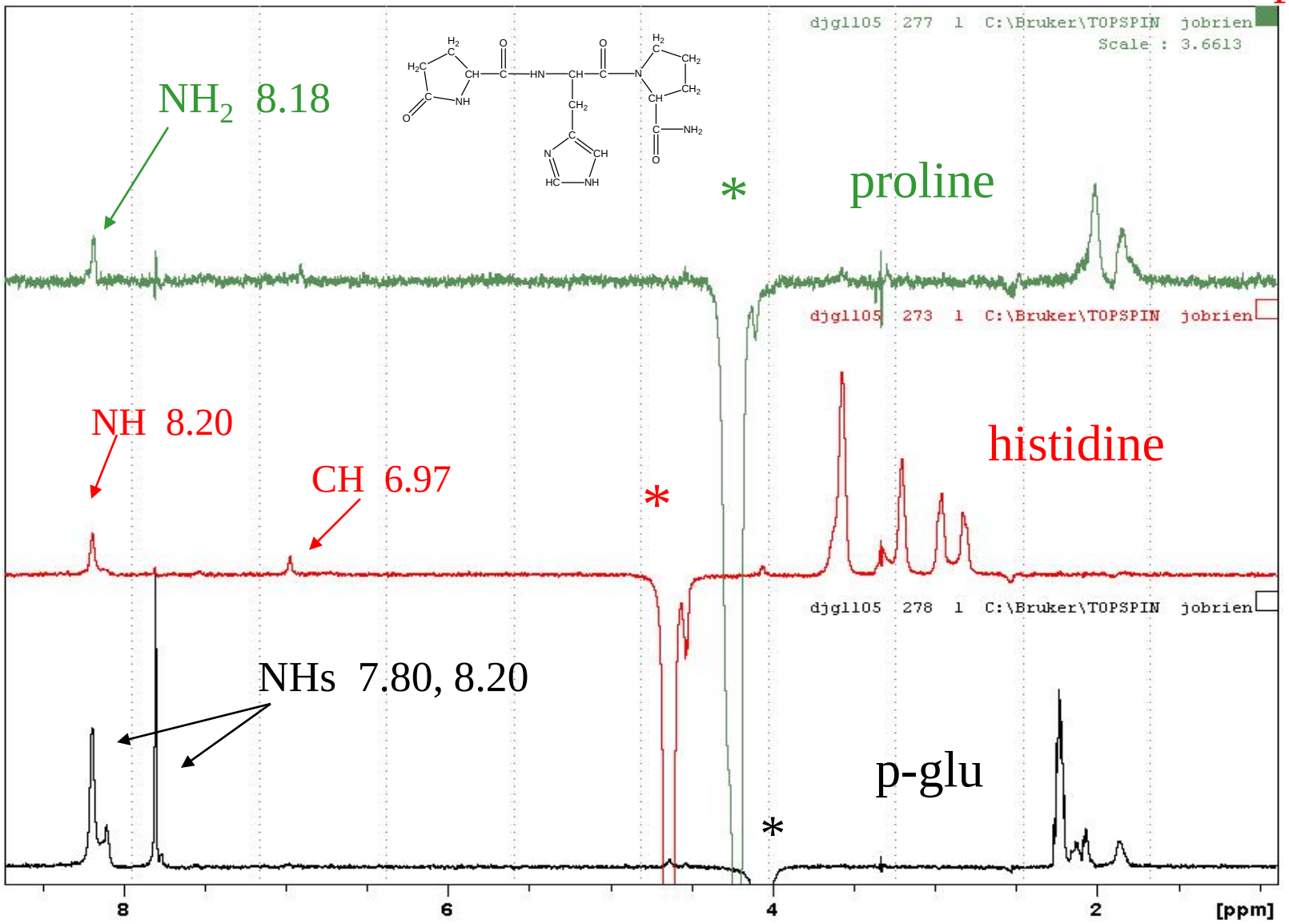
- The selective ROESY focuses on the excitation of a single peak to give the spatial links to other peaks in molecule (generally to a distance of 0.6nm)
- The peak which is excited goes fully antiphase (upside down) with spatial response(s) inphase (peak remains upwards). Only these signals are observed

Selective HH correlation experiments
same information as 2D TOCSY experiments



Selective Nuclear Overhauser (Noe) experiments - through space connections

* Irradiated CH peak

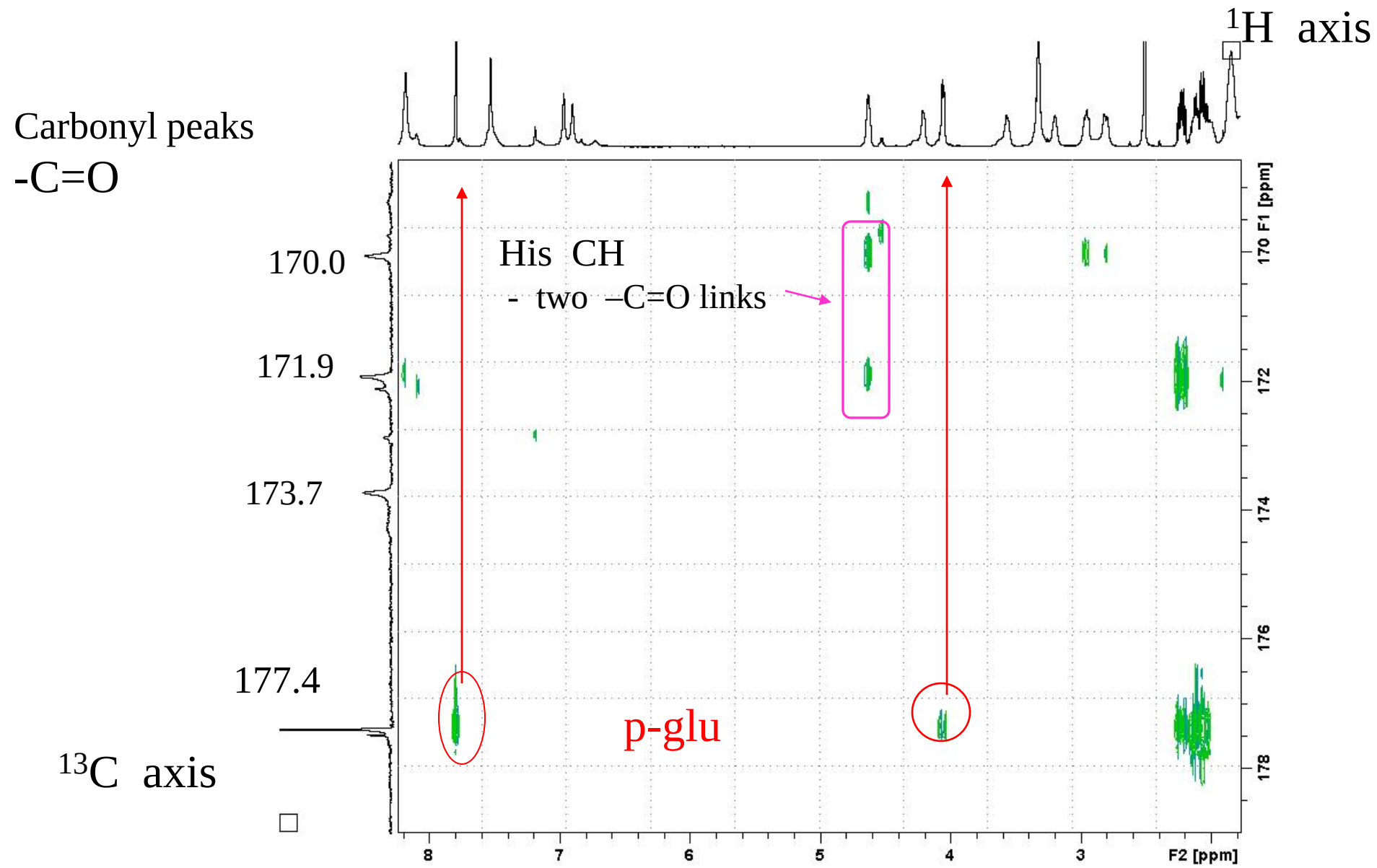


Fitting the jigsaw together with the Long Range CH COSY

- The long range CH COSY is a further variant on the CH COSY
- It is designed to locate hydrogens attached to carbons which are two or three bonds away (or *vice-versa*)
- It is often possible correlate several protons to a carbon
  (or *vice versa* - whichever is most appropriate)
- One of the most useful attributes is the links to carbon signals with NO protons directly attached
- It is now possible to complete an assignment from the information provided

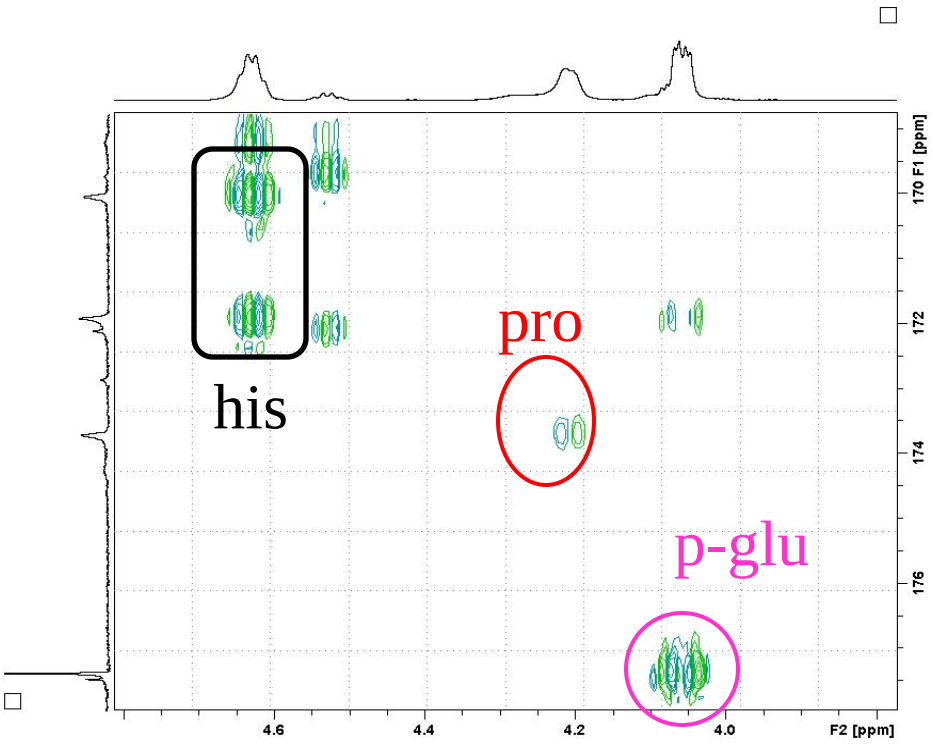
Long range CH correlation

Very useful - links proton to carbon with NO proton(s) attached

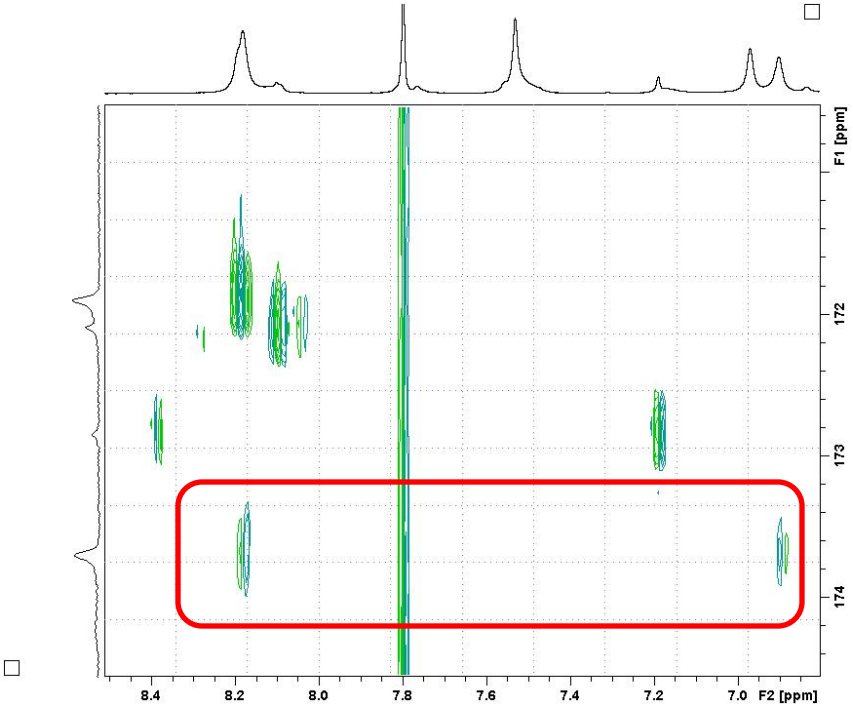


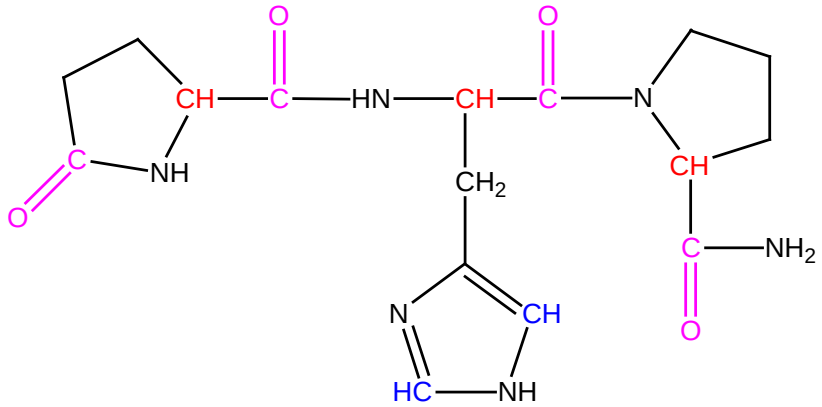
Long range CH correlation

-C=O to CH links

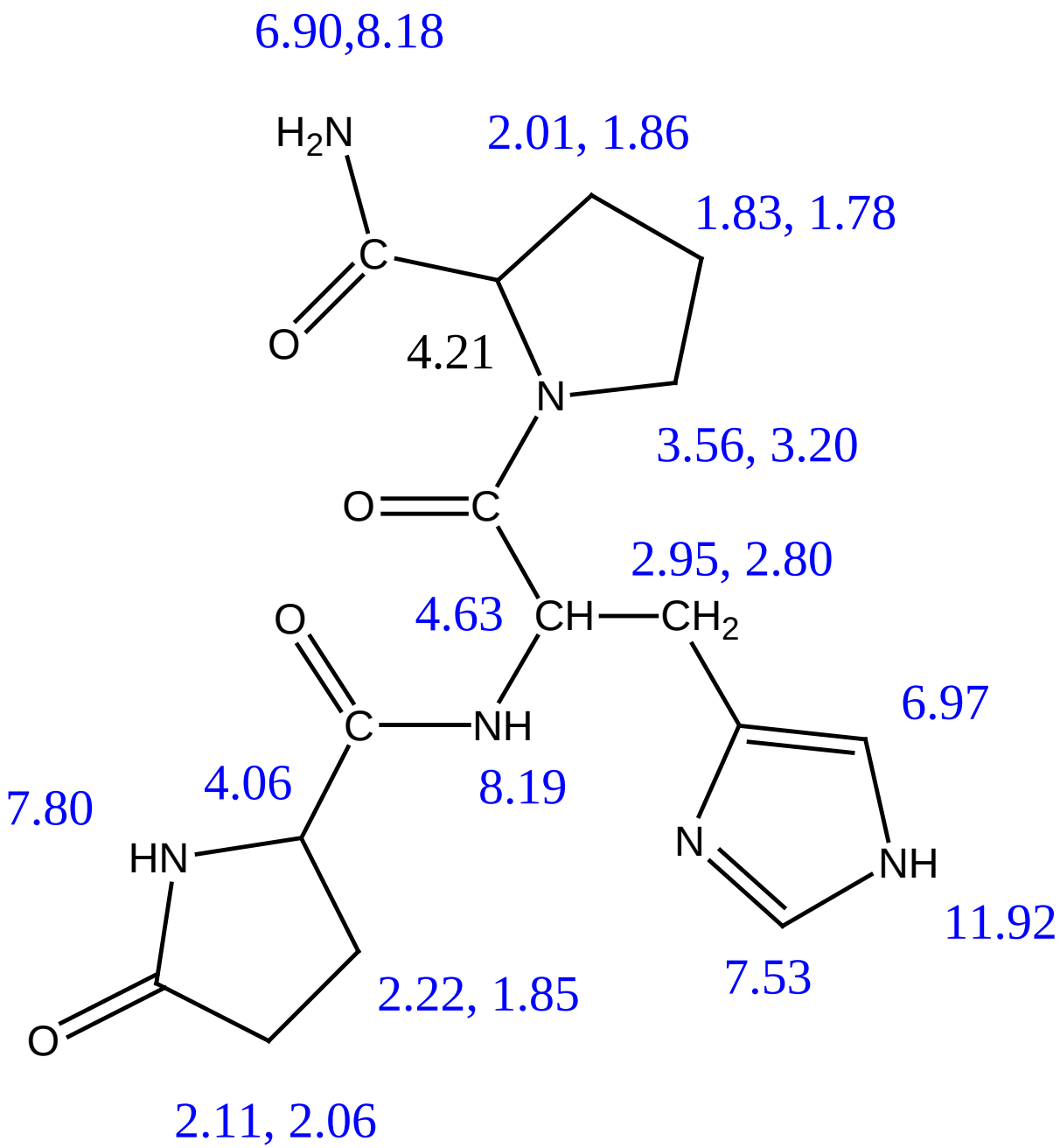


$-NH_2$ to -C=O





Proton NMR assignments



Carbon –13 NMR assignments