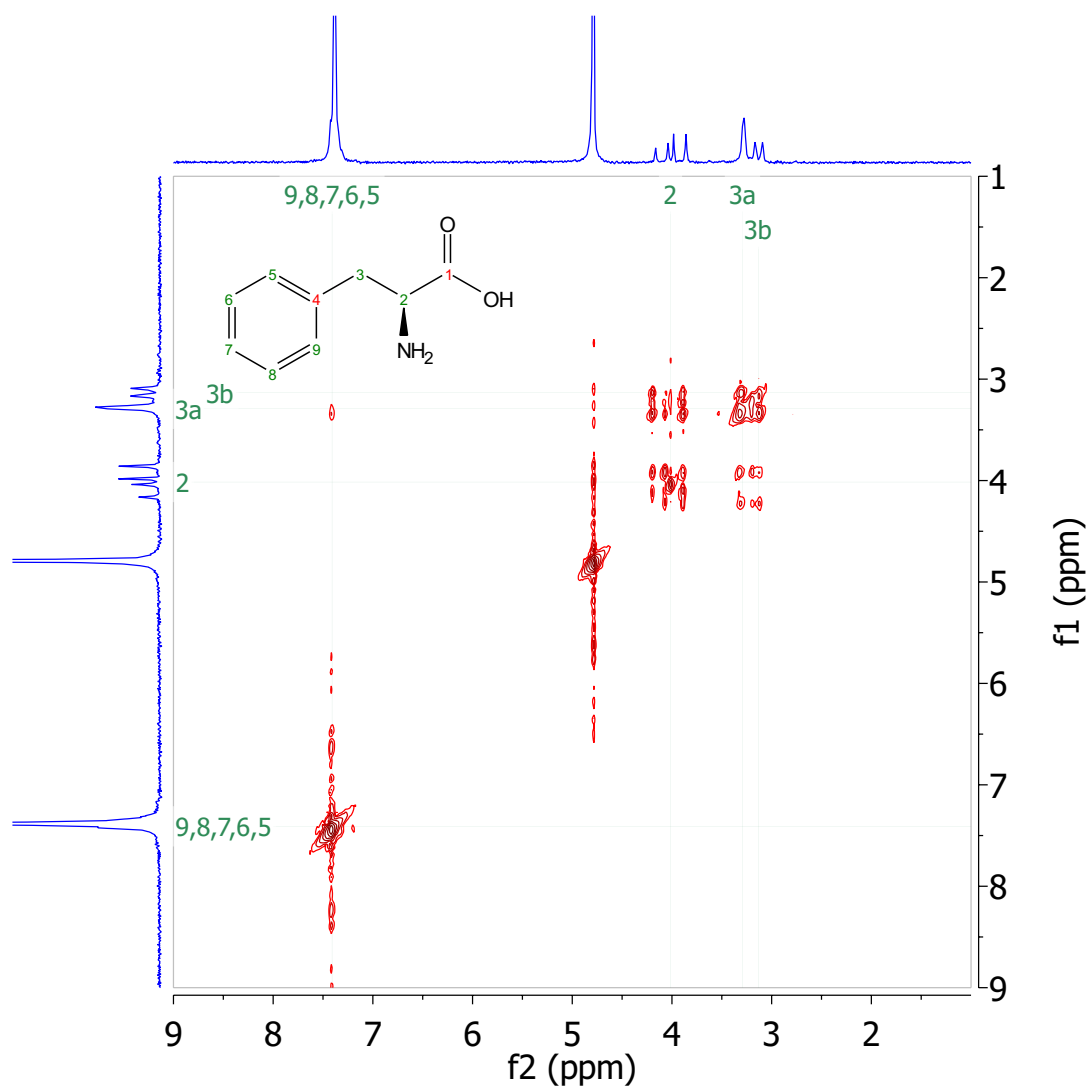


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Observing Spin Systems using COSY

^1H - ^1H COSY experiments produce 2D NMR spectra that identify proton coupling partners, which in many cases is directly related to the carbon skeleton connectivity. Thus COSY is one of the most informative and widely used 2D NMR experiments.

In this application note we demonstrate how COSY NMR spectra can be used to determine the composition of amino acids present in peptides using the Spinsolve benchtop NMR spectrometer.



'COSY' (**C**orrelated **S**pectroscopy) is a 2D homonuclear (^1H , ^1H)-correlated NMR experiment in which proton coupling partners can be identified. A COSY spectrum is shown as a contour plot in which the 1D proton NMR spectrum is plotted along the diagonal and protons that couple to each other result in a peak off the diagonal in line with each of the coupling protons (Figure 1).

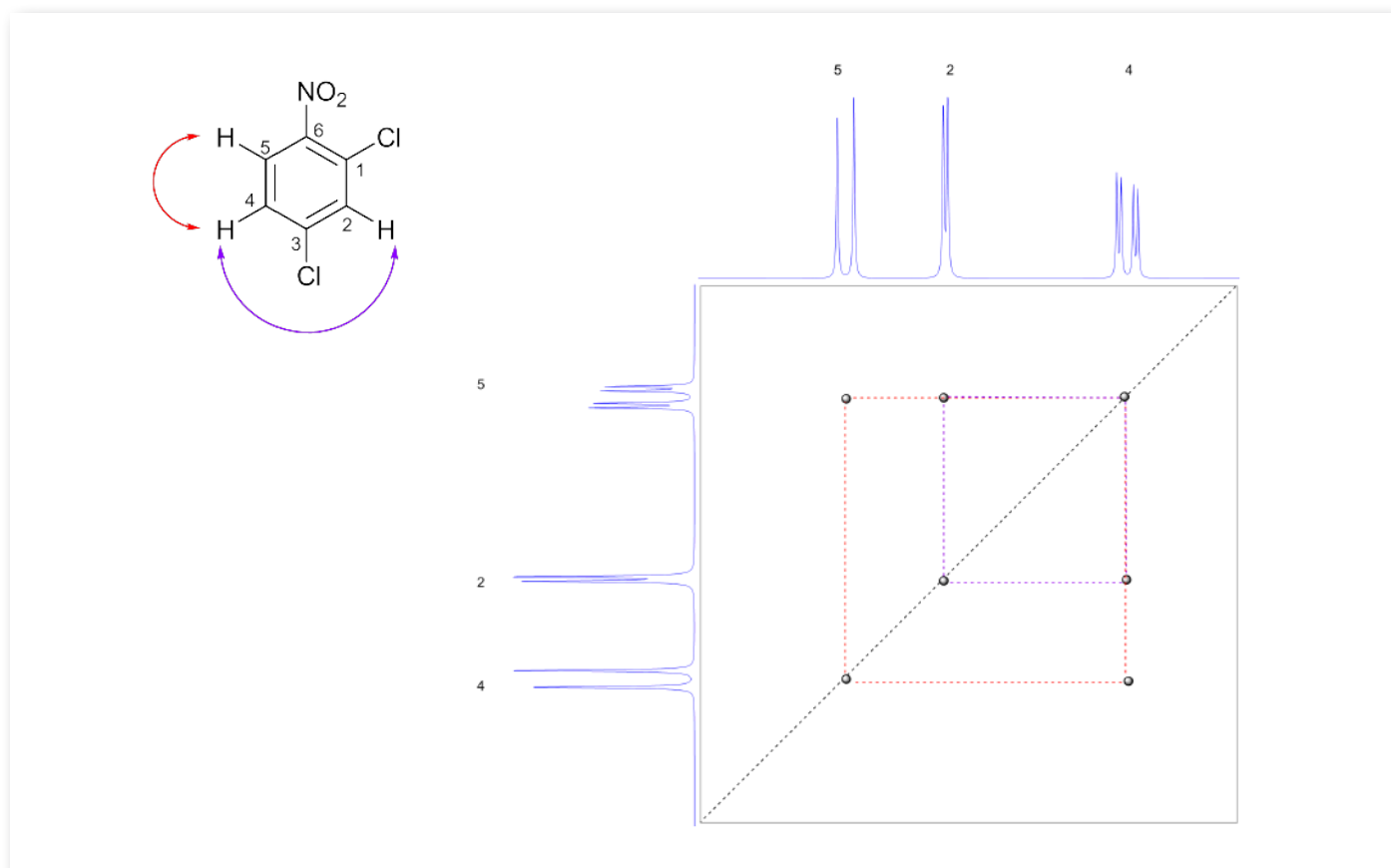


Figure 1: COSY Spectrum of 1,3-dichloro-6-nitro-benzene. Short range coupling is indicated on the spectrum with dashed lines and on the compound with double-headed arrows.

The analysis of COSY spectra often allows substantial piecing together of fragments in the molecule as coupling partners and therefore adjacent nuclei can be identified. In the process, the positions of functional groups or atoms that are not protonated can be identified as they provide a 'road-block' in the coupling for most molecules¹ (for example, a carbonyl). Fragments that are separated by these road blocks are known as *spin systems*. Spin systems are fragments of molecules that show short-range coupling within themselves, with no short-range coupling to the rest of the molecule. Often these form 'puzzle pieces' in structural elucidation that can be pieced together using other 2D NMR experiments.

¹ Longer range coupling (>3 bonds) may be observed in the COSY spectrum for some systems, particularly those with π -systems, e.g. benzene derivatives and alkenes.

Example: GLYCYL-L-PHENYLALANINE

One area where COSY spectra and the ability to identify spin systems is particularly useful is in the characterisation of peptides. Peptides are made up of amino acids (Figure 2) which each have a characteristic fingerprint in the COSY spectrum.

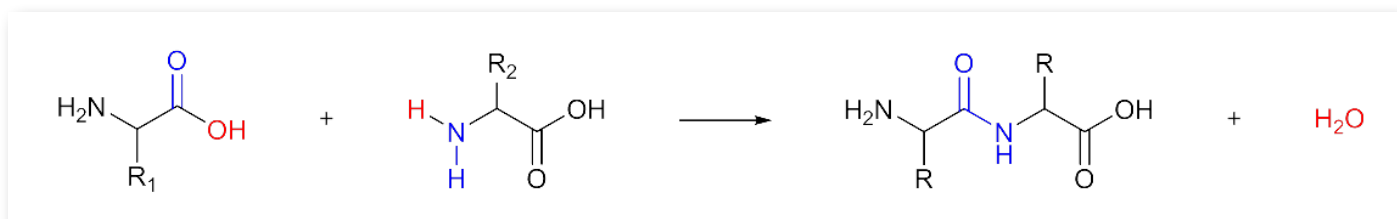


Figure 2: Formation of peptides through condensation of amino acids.

There is no coupling between different amino acid moieties as they are separated by peptide linkages (road-blocks) (Figure 3). Therefore COSY spectra can be used to identify the amino acids that make up simple peptide molecules.

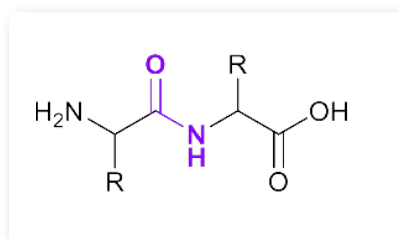
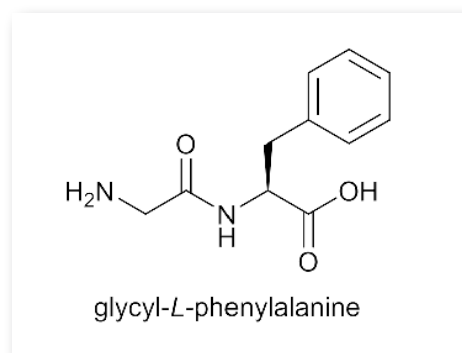


Figure 3: Peptide linkage.

Glycyl-L-Phenylalanine is produced in a third year synthesis laboratory experiment where students synthesise the dipeptide from the corresponding amino acids. Analysing the COSY NMR spectra of the amino acids shows that the dipeptide can be easily characterised.



In the COSY spectrum of phenylalanine, two different spin systems can be observed (Figure 4). The two spin systems are shown in different colours, and coupling is where the lines intersect on a peak off the diagonal (cross-peak). The protons in line with this cross-peak couple to each other.

Note that the methylene protons occur at different chemical shifts despite being attached to the same carbon atom, as the neighbouring carbon atom is chiral. This means that the two protons experience a different chemical environment (through space), resulting in a difference in chemical shift.

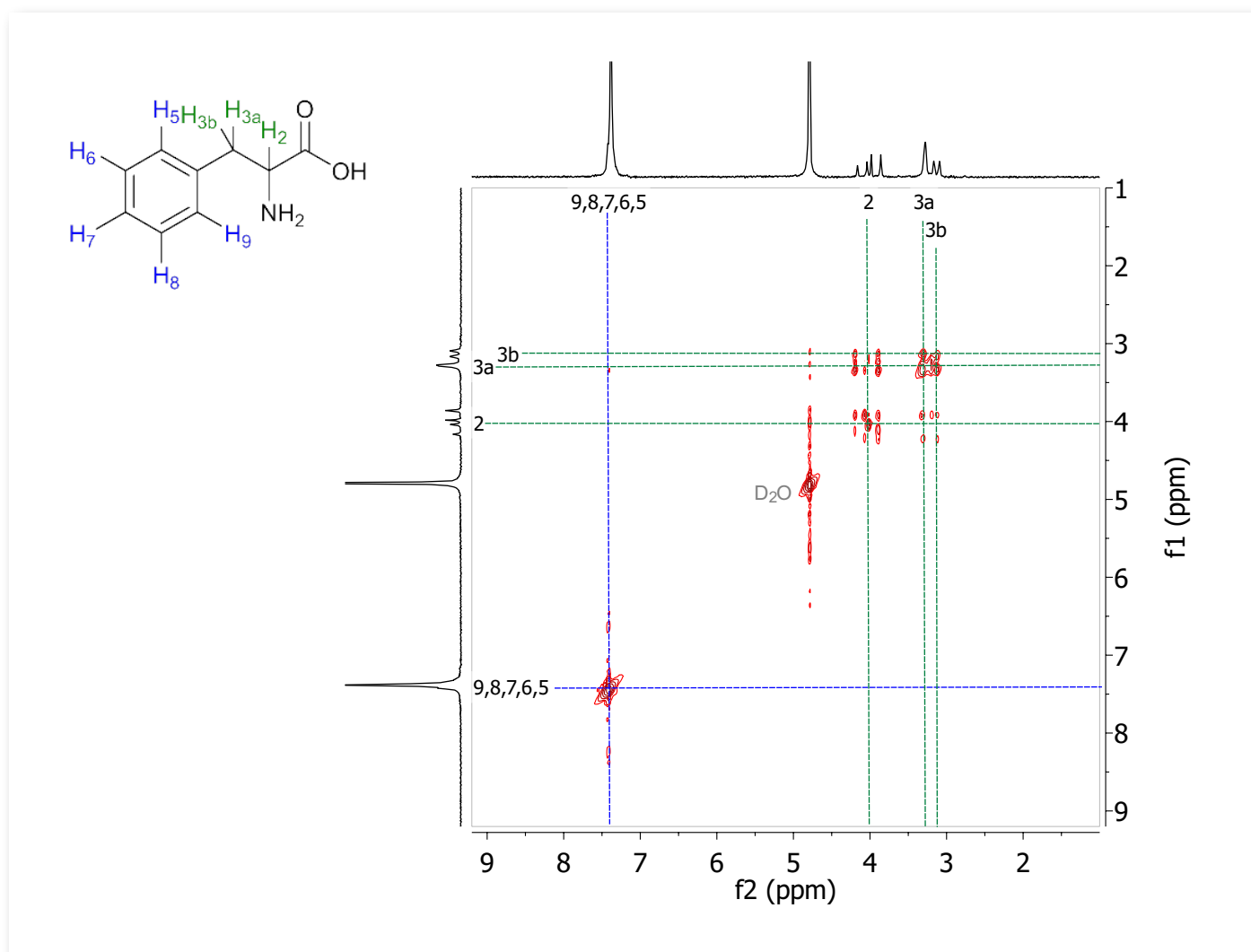


Figure 4: COSY spectrum of phenylalanine in D₂O (4.89 ppm).

The COSY spectrum of glycine (Figure 5) has only one peak along the diagonal and therefore no COSY correlations off the diagonal. This is because there is no chirality in the molecule (the only non-chiral amino acid) therefore the two hydrogen nuclei are equivalent.

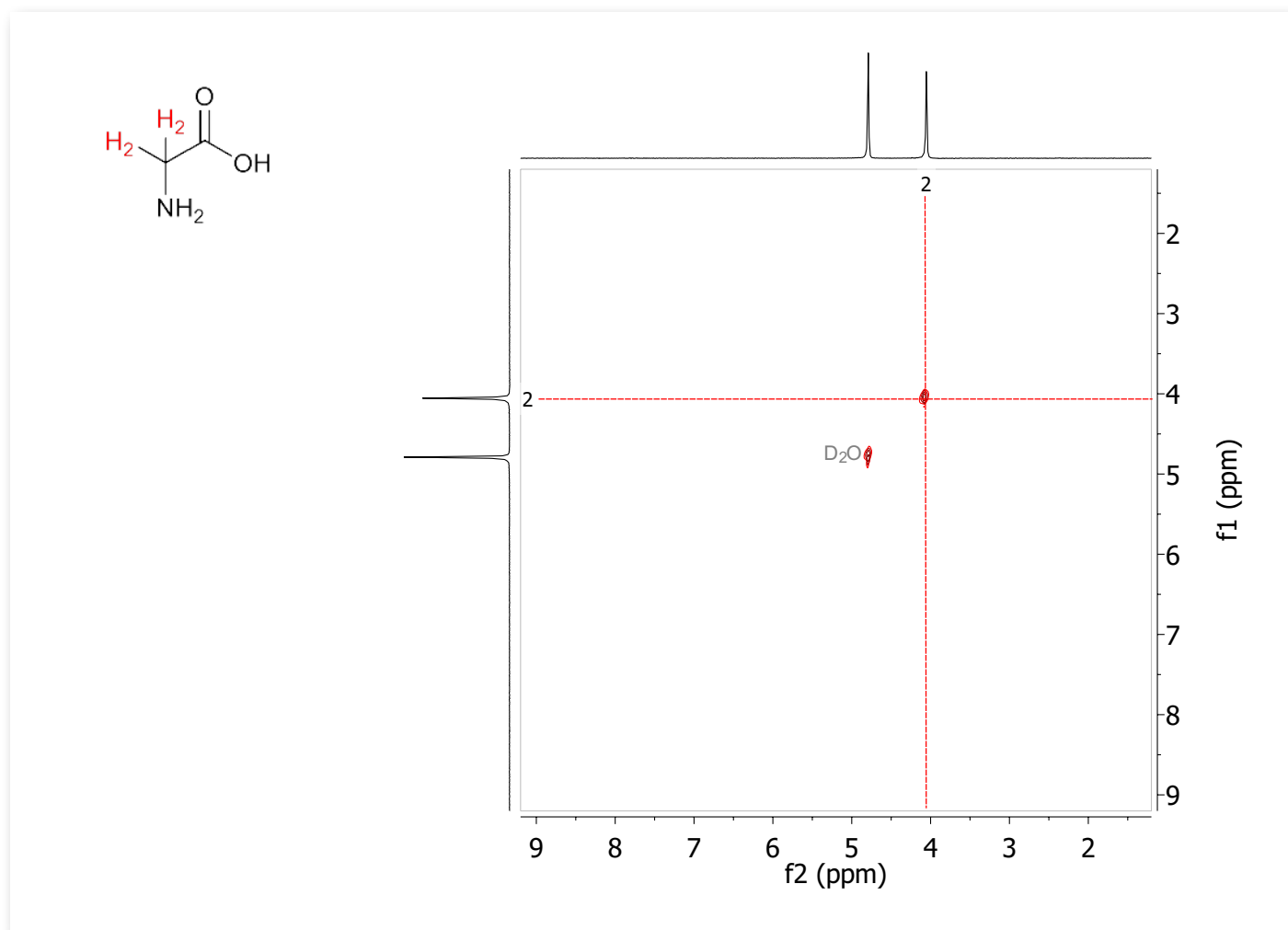


Figure 5: COSY spectrum of glycine in D_2O (4.89 ppm).

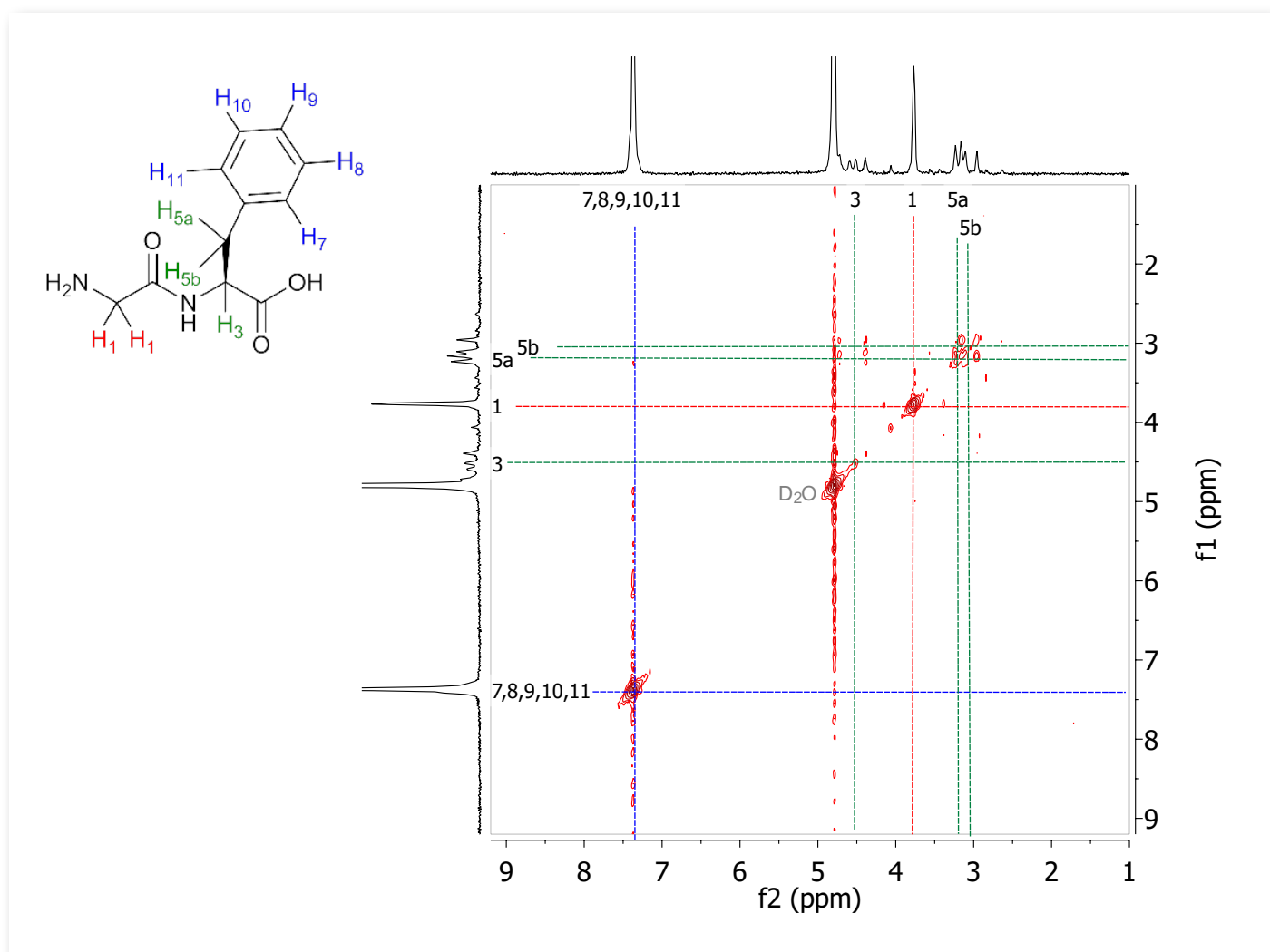


Figure 6: COSY spectrum of glycyl-L-phenylalanine in D₂O (4.89 ppm).

In the COSY spectrum of the dipeptide (Figure 6), the two amino acid moieties can easily be recognised due to their characteristic fingerprints that were observed in the COSY spectrum of the un-coupled amino acid. The same spin systems observed for the amino acids are translated into the COSY spectrum of the dipeptide.

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Conclusion

Spin systems are fragments of molecules that have short-range coupling within themselves. Correlated Spectroscopy (COSY) experiments are used to observe short-range coupling, and therefore spin systems can be identified. The 2D homonuclear COSY experiment was used to observe the spin systems in the amino acids L-phenylalanine and glycine, and the corresponding dipeptide glycyl-L-phenylalanine. It was observed that the spin systems in each amino acid moiety were preserved as peptide linkages prevent coupling between amino acids. This example was a simple demonstration of how spin

systems of amino acids have a characteristic fingerprint in the COSY spectrum of peptides which can be used to identify the amino acids. This analysis can be applied to larger peptides, which provides a non-destructive method for determining the amino acid composition.

References:

E. Gonzalez, D. Dolino, D. Schwartzburg, and M. A. Steiger, Dipeptide Structural Analysis Using Two-Dimensional NMR for the Undergraduate Advanced Laboratory, *Journal of Chemical Education*, 2014.

H. Friebolin, *Basic one- and two-dimensional NMR spectroscopy*, Wiley-VCH Verlag GmbH & Co. KGaA, 2011.

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