Spectral Assignment and Sequencing of Short Chain Peptides by ^{1}H and ^{13}C NMR

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Background

There is currently growing interest in the therapeutic benefits of short chain peptides. These molecules, like small molecule pharmaceuticals, require analysis and confirmation of structure, therefore procedures are required for their elucidation.

Presented is an example of the process used for assigning NMR spectroscopy data, required as part of the analysis of a short chain peptide.

Experimental

25 mg of a 7-mer cyclic peptide was dissolved in 0.75 ml of DMSO-d_6. Spectra were obtained using a Bruker Avance NMR spectrometer operating at 500.13 MHz running under TopSpin 2.1. The sample compartment temperature was set to 28.1°C. ^{1}H and ^{13}C spectra were assigned using DEPT-135[1], HSQC[2], HMBC[3], ROESY[4] and TOCSY[5] experiments. Mixing times for ROESY and TOCSY were both 200 ms. Total acquisition time was approximately 6 hours.

Assignment Procedure

The data was processed using TopSpin 3.1 and analysed using the following scheme.

Pick out the individual peaks in the HSQC (finger-printing) and determine their multiplicity with the aid of the DEPT-135 spectrum.

Confirm the correct number and multiplicity of carbons and protons.

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Use COSY and TOCSY data to assign the protons to individual amino acid side chains and confirm they agree with the expected residues.

Use HMBC data to link aliphatic and aromatic groups within these amino acid side chains

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Use ROESY (and/or HMBC data where possible) to determine the relative positions and sequence of amino acids within the molecule

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Confirm correct sequence of amino acids and report results

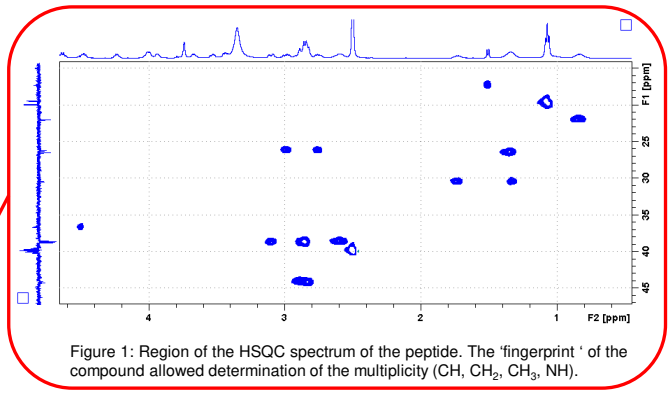

Figure 1: Region of the HSQC spectrum of the peptide. The 'fingerprint ' of the compound allowed determination of the multiplicity (CH, CH_2, CH_3, NH).

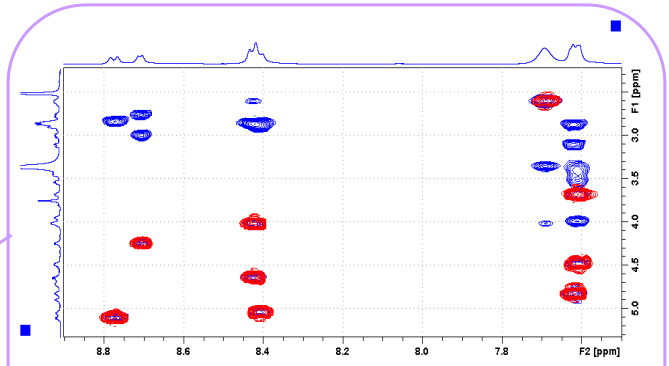

Figure 2: COSY correlations (red) between amide NH's and Cα-H. The TOCSY correlations in blue show the further correlations to the remaining protons in the amino acid side chain.

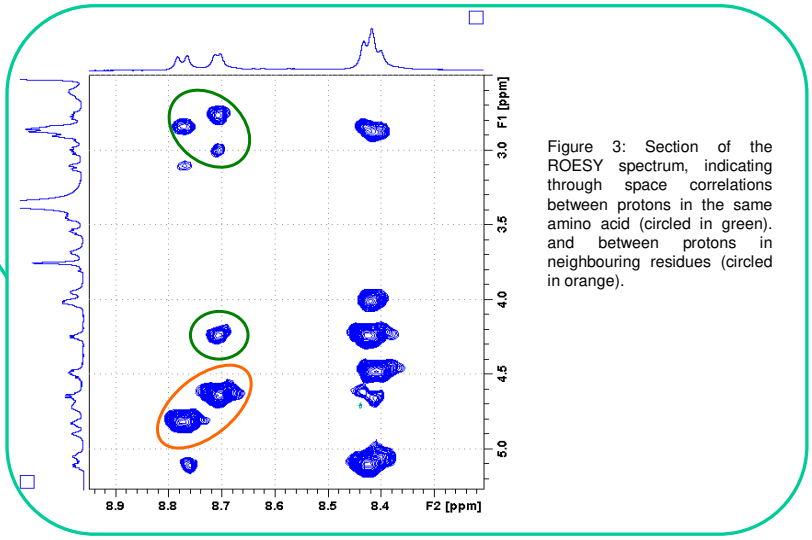

Figure 3: Section of the ROESY spectrum, indicating through space correlations between protons in the same amino acid (circled in green). and between protons in neighbouring residues (circled in orange).

Results

All ^{1}H and ^{13}C signals corresponding to the example peptide were assigned and indicated the molecules had the correct amino acid residues in the correct sequence. It was also determined that although the molecule was believed to be in a free base form an amine group in the molecule was protonated. The counterion was subsequently determined to be TFA. This assignment was used in further analyses of structurally related peptides, greatly reducing the analysis time for these molecules.

Conclusion

The 1H and 13C spectra of a peptide were fully assigned using a combination of 1D and 2D NMR techniques. The resolution was sufficient that an amine group present in one residue was identified as being protonated, further tests indicated the counterion was TFA.

The procedure outlined above demonstrates the value of analysing short peptide by NMR spectroscopy. The assignment procedure is now in place and can be used in future peptide analyses. Theoretically assignments should be feasible for peptides containing up to 50 amino acids.

References

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