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NMR Structure in methanol of a β-hexapeptide with a disulfide clamp†

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A disulfide bridge between two cysteine side chains on amino acid residue 2 and 5 of a β-hexapeptide fixes the 3_{14}-helical structure, as shown by a 2D-NMR investigation.

Oligomers of β-amino acids (β-peptides) have made their debut as a promising class of peptide analogues: with short chain length they fold into well-ordered secondary structures such as helices, turns and sheets.[1] Their great structural diversity, together with the finding that β-peptides are resistant to degradation by peptidases,[2] renders them candidates for pharmaceutical applications.[3] The β-peptides built from homologated α-amino acids[4] form a left handed 3_{14}-helix with the side chains of amino acid residue *i* and *i* + 3 in juxtapositions.[5] Introduction of a conformational constraint by covalently linking HS–CH_2-groups in the aforementioned positions, forming a disulfide bridge, is expected to stabilise the helix, whereas a linkage between residue *i* and *i* + 4 will enforce new backbone conformations (Fig. 1). To demonstrate this, β-peptides with cysteine side chains in position i_α,i_β;[6] i_β, i_β + 3, i_β, i_β + 4 (Fig. 2) were synthesised and their CD spectra recorded (Fig. 3).[7]

It has been established by numerous CD measurements and corresponding NMR-structural investigations of β-peptides that a CD pattern exhibiting a trough at *ca*. 216 nm and a peak at *ca*. 198 nm is characteristic of a 3_{14}-helix with (*M*)-chirality.[8] The CD spectra of cysteine-containing β[3]-hexapeptides **1** and **2** as well as of heptapeptide **4** exhibit a pattern typical for a 3_{14}-helical structure in MeOH whereby compound **1**, the cyclic hexapeptide, shows a small blue shift of the *Cotton* effect at longer wavelength. β-Peptide **3**, which would be expected not to be able to fold into a 3_{14}-helix shows a different CD pattern with a trough at 200 nm.

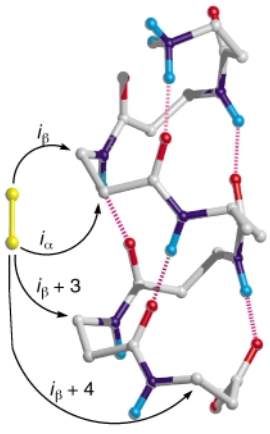

Fig. 1 Schematic presentation of a β-peptidic 3_{14}-helix. At each tetragonal carbon in the chain there is a *lateral* (perpendicular to the helix axis) and an *axial* position ('allowed' only for hydrogens).[5] A cystinic disulfide bridge (CH_2–S–S–CH_2) is possible between lateral positions i_α–i_β, i_β–i_β + 3, but not i_β–i_β + 4.

† Electronic supplementary information (ESI) available: NMR spectroscopy of hexapeptide **1** and heptapeptide **3**. See http://www.rsc.org/suppdata/cc/b0/b007503p/

These observations led us to examining β-peptides **1** and **3** by means of high-resolution NMR techniques. 2D-NMR Studies were carried out on a 500 MHz spectrometer with solutions in

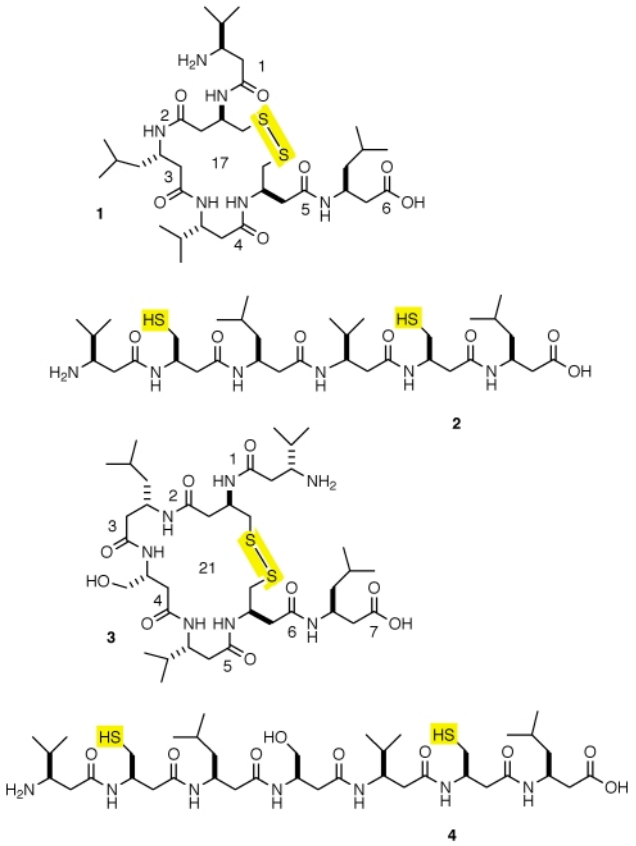

Fig. 2 Formulae **1**–**4** of the cyclic and linear β-hexa- and heptapeptides[7] included in the present NMR investigation.

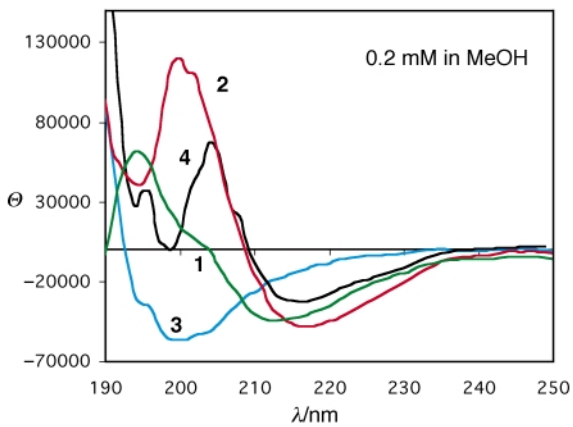

Fig. 3 CD Spectra of the four β-peptides in MeOH. A trough near 215 nm is considered characteristic of an (*M*) 3_{14}-helical structure (see CD of **1**, **2**, **4**). The macrocyclic β-heptapeptidic disulfide **3** with a linkage between residues 2 and 6 shows a completely different CD pattern.[7]

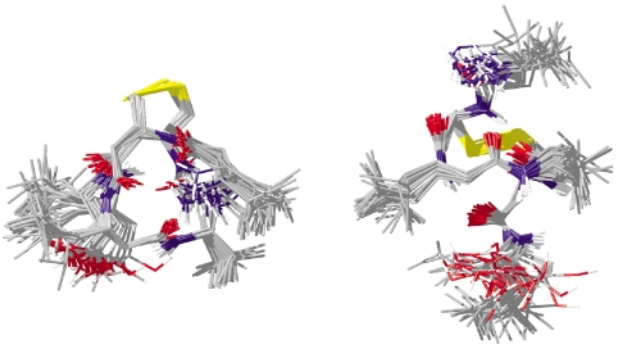

Fig. 4 Views along and perpendicular to the (*M*) 3_{14}-helix of an overlay of 20 lowest-energy structures obtained by simulated annealing, using NMR dihedral-angles and NOE-distance restraints with the XPLOR programme.

CD_3OH. DQF-COSY and TOCSY techniques were used to assign all ^1H resonances in their respective spin systems. HSQC and HMBC experiments led to the assignment of the sequence. From the large ^3J(NH; C(β)–H) coupling constants it can be concluded that the NH and C(β)–H protons are in an antiperiplanar arrangement. The diastereotopic $CH_2(\alpha)$ protons were assigned assuming that the axial protons (*cf.* Fig. 1) exhibit a large and the lateral a small coupling with C(β), which is in agreement with stronger NOEs from H–C(β) to the lateral H–C(α) protons than to the axial H–C(α) protons. ROESY spectra of **1** and **2** at different mixing times were acquired and NOEs were extracted from spectra with mixing time of 150 ms.

Qualitative analysis of the ROESY data obtained for β-hexapeptide **1** indicated that the 3_{14}-helical conformation is predominant, since the typical NOE correlations persisted, while data for **3** do not correspond to a 3_{14}-helix. A total of 62 NOEs of compound **1** were extracted and then classified according to their relative volume in the contour plot in three distance categories with the following upper bound distance limits: strong < 3.0 Å, medium < 3.5 Å and weak < 4.5 Å. These distance restraints were used together with 5 NH, C(β)–H dihedral angle restraints, derived from the coupling constants, in simulated annealing, following the XPLOR protocol. This calculation yielded a set of 25 structures with low restraint violation, of which 20 structures with minimum energy are depicted in Fig. 4. The structures show a left-handed helix with 14-membered hydrogen-bonded rings from NH of residue 2 to C=O of residue 4 and from NH of residue 3 to C=O of residue 5. The disulfide unit R–S–S–R in the 17-membered macrocyclic ring of **1** has (*P*)-chirality. Due to the conformational restraints imposed by the disulfide bond the helix formed by **1** is slightly twisted with the side chains being offset from one another, rather than on top of each other as in an idealised 3_{14}-helix and as in numerous *real* 3_{14}-helices published so far.[5,9]

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