

Preparation and determination of X-ray-crystal and NMR-solution structures of $\gamma^{2,3,4}$ -peptides

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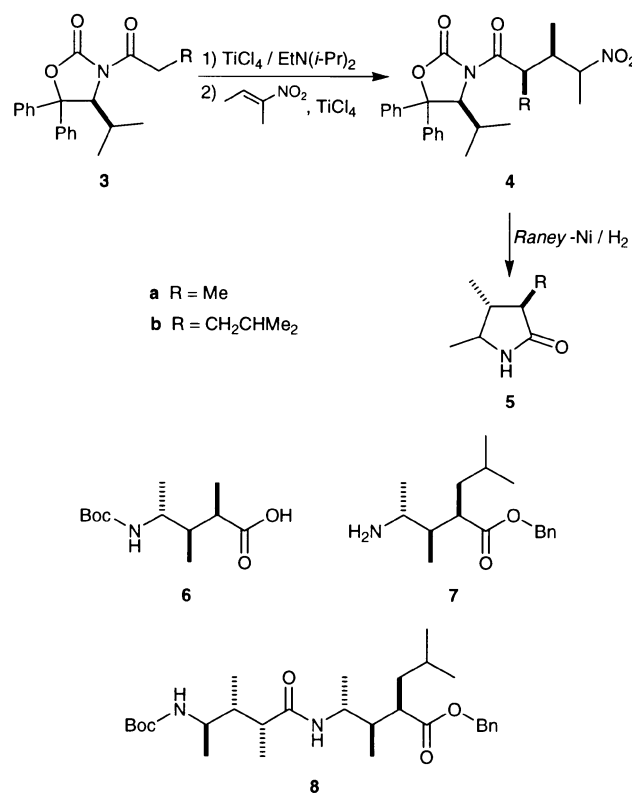
(*R,R,R*)- γ -Amino acids with side chains in the 2-, 3-, and 4-positions, prepared by addition of acyloxazolidinones to a nitroolefin and hydrogenation, have been coupled to γ -tetra-, and γ -hexapeptides which are shown to form (*M*)-2.6₁₄ helices in the crystal state and in MeOH solution.

While there is a lot of activity in the field of β -peptides, their homologs, the γ -peptides, have received much less attention, so far.^{1–3} It has been discovered that γ -peptides form helical secondary structures in solution, detectable by NMR spectroscopy, with chains as short as four residues, and that homologation of L- α -amino acids to L- β - and L- γ -amino acids leads to peptides, folding to helices of alternating polarity and helicity (α : N \leftrightarrow C (*P*), β : N \leftarrow C (*M*), γ : N \leftrightarrow C (*P*)), and of increasing stability. The γ -peptides studied hitherto consisted of γ^4 -residues (side chains at C(4))^{1,2} or of $\gamma^{2,4}$ -residues (two side chains, one at C(2) and one at C(4)),^{2,3} rel. configuration *l* or *u*.[†] Inspection of models leads to the conclusion that $\gamma^{2,3,4}$ -peptides of type **1/2** (Fig. 1), built of (*R,R,R*)-amino acid residues, should be able to form a 2.6₁₄-helix without steric interference of the side chains within the helical backbone.

The required γ -amino-acid building blocks were prepared stereoselectively by *Michael* addition of the modified *Evans* acyloxazolidinones **3** to nitrobutene (\rightarrow **4**),⁴ reductive cleavage (\rightarrow **5**), hydrolysis, and *N*-Boc or *C*-OBn protection (\rightarrow **6**, **7**) (Scheme 1). Coupling of amino acids **6** and **7** gave dipeptide **8**, which after appropriate deprotection yielded dipeptide building blocks which were coupled to tetra- and hexapeptides **1** and **2**, respectively.

Of the protected γ -tetrapeptide **1** we obtained crystals suitable for X-ray structure analysis.[‡] The quality and size of the samples allowed only for isotropic refinement and the determined structure is shown in Fig. 2a. The structure is characterized by two consecutive 14-membered H-bonded rings, one between the NH of residue 3 and the C=O of the Boc-protecting group and the other between the NH of residue 4 and the C=O of residue 1. Thus, both intramolecular H-bonds fit into the typical pattern of the 2.6₁₄-helix (H-bond between NH of

residue *i* and C=O of residue (*i* – 3)). The carbamate NH of residue 1 and the amide NH of residue 2 are involved in intermolecular H-bonding to the ester C=O and to the C=O of residue 3 respectively, resulting in a two-dimensional H-bonded network. Residues 1 to 3 show the typical backbone conformation found in the (*M*)-2.6₁₄ helix ((–)-*sc* for the C(2)–C(3) and the C(3)–C(4) ethane moieties). The side chains at C(2) and C(4) are in lateral positions, while the C(3)–Me bonds form angles of approximately 35° with the helix axis. The C-terminal residue has an extended backbone conformation ((±)-*ap* for the C(2)–C(3) and the C(3)–C(4) ethane moieties). Since the C-terminal ester group has no NH-group which could form an



Scheme 1 Synthesis of the building blocks used for the preparation of peptides **1** and **2**. Hydrolysis of lactam **5a** in 6 M HCl and subsequent Boc-protection yielded amino acid **6**. The same procedure was applied with **5b**, followed by benzylation and Boc-deprotection to obtain **7**. Compounds **4** and **5** were used as mixtures of diastereoisomers, and separation was performed at the stage of the corresponding γ -amino acid. Coupling of amino acids **6** and **7** with HATU [*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate] to dipeptide **8** proceeded smoothly. Appropriate deprotected derivatives of **8** were fragment coupled to tetrapeptide **1** and hexapeptide **2** using EDC (1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide-HCl)/DMAP as reagents.

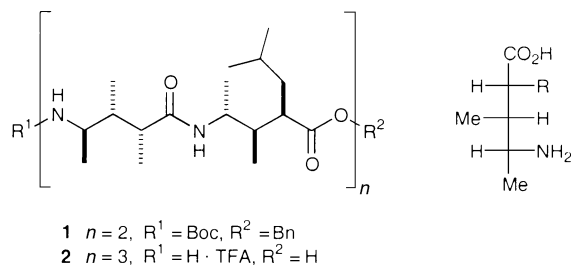


Fig. 1 $\gamma^{2,3,4}$ -Tetra- and hexapeptide derivatives **1** and **2** used for the structure determinations and Fischer representation of the required amino acid building blocks.

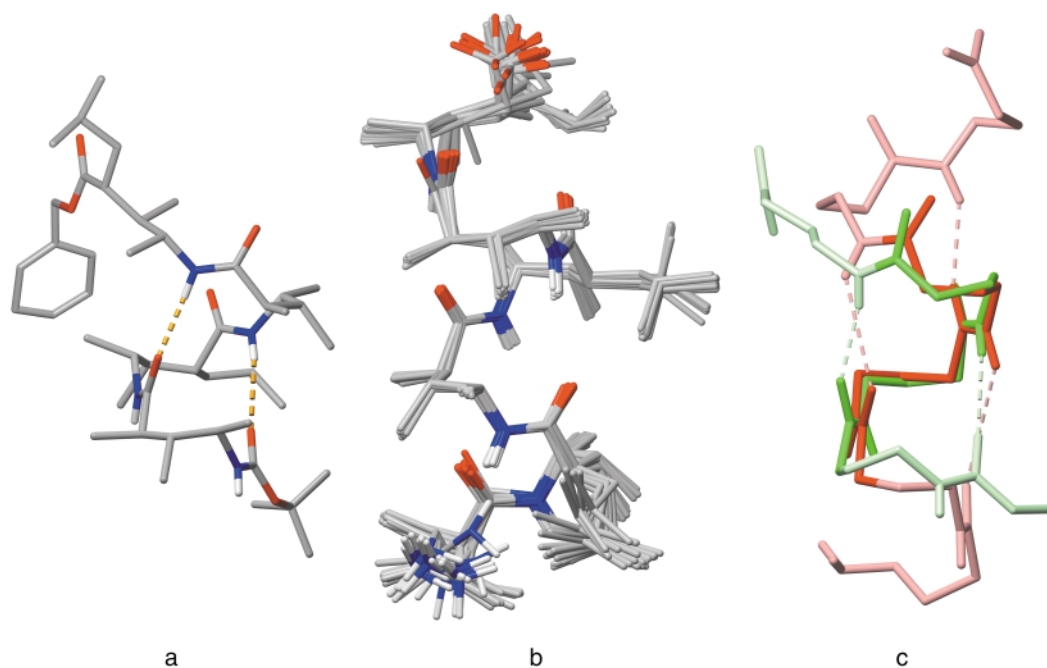


Fig. 2 (*M*)-2.6₁₄ Helical structures of γ -peptides **1** and **2**. a, Structure of γ -tetrapeptide **1** in the crystal state determined by X-ray structure analysis. b, Bundle of 20 conformers of hexapeptide **2** in MeOH obtained by simulated annealing calculations using restraints from NMR data. c, Superposition of the peptide backbones from the X-ray diffraction structure (blue) and NMR structure (red).

intramolecular H-bond the conformation of residue 4 may be determined by crystal packing factors.

The observation that γ -peptides with just four residues form a helical structure in the crystal state led us to examining γ -hexapeptide **2** by means of high-resolution NMR techniques. 2D-NMR Studies were carried out on a 500 MHz spectrometer with solutions in CD₃OH. We used DQF-COSY and TOCSY techniques to assign all ¹H resonances in their respective spin systems. HSQC and HMBC experiments led to the assignment of the amino acid sequence. ROESY spectra of **2** at different mixing times were acquired and NOEs were extracted from spectra with a mixing time of 300 ms.

The NOEs were classified according to their relative volume in three distance categories with the following upper bound distance limits: strong <3.0, medium <3.5 and weak <4.5 Å. A total of 83 NOEs were used as distance restraints in simulated annealing, following the XPLOR protocol. This calculation yielded a set of 20 structures with low restraint violation and minimum energy (Fig. 2b). The structures show a well-defined left-handed helix with three 14-membered hydrogen-bonded rings from C=O of residue *i* to NH of residue *i* + 3. The helix has a pitch of *ca.* 5.0 Å and has *ca.* 2.6 residues per turn. An overlay of the backbone of one of the NMR-derived structures of **2** and the backbone from the crystal-structure of tetrapeptide **1** is shown in Fig. 2c. This superposition shows a good agreement between the central residues of the two molecules.

This study shows that $\gamma^{2,3,4}$ -peptides constructed from (*R,R,R*)-trisubstituted γ -amino acid residues adopt well defined (*M*)-2.6₁₄-helices without steric interferences of the side chains, and allowed for the first time the characterization of this secondary structural motif by X-ray crystal structure analysis.

Notes and references

† Peptides built of $\gamma^{2,4}$ -amino acids with rel. configuration *l* form 2.6₁₄ helical structures, while a tetrapeptide consisting of the corresponding *u* residues was found to form a turn motif.

‡ Crystal data for C₄₆H₈₀N₄O₇ **1**: *M* = 801.14, monoclinic, space group *P*2₁, *a* = 9.462(2), *b* = 20.472(6), *c* = 13.866(4) Å, β = 106.14(2)°, *V* = 2580.1(12) Å³, *Z* = 2, *D*_c = 1.031 g cm⁻³, μ (Cu-K α) = 0.543 mm⁻¹, crystal size 0.30 × 0.20 × 0.02 mm. Data were collected on an Enraf Nonius CAD-4 diffractometer using graphite-monochromatized Cu-K α radiation. A total of 4479 unique reflections (3.32 < 2 θ < 66.23°) were processed of which 1140 were considered significant with *I*_{net} > 3 σ (*I*_{net}). Part of the structure was solved by direct method with SIR97,⁵ the remaining non-H-atoms were found from a difference Fourier map. The non-H atoms were refined isotropically with SHELXL97.⁶ The number of observed reflections did not allow anisotropic refinement. H-atoms were calculated at idealized positions and included in the structure factor calculation with fixed isotropic displacement parameters. Final residuals were *R* = 0.0898 and *R*_w = 0.1961 (GOF = 1.525) for 243 parameters. CCDC 182/1874.

The structure was determined by B. Schweizer of our X-ray service.

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