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

Dieter Seebach,* Meinrad Brenner, Magnus Rueping, Bernd Schweizer and Bernhard Jaun

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich. E-mail: seebach@org.chem.ethz.ch; Fax: +41 1632 1144; Tel: +41 1632 2990

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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*)-2.6_{14} 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 ↦ C (*P*), β: N ↤ C (*M*), γ: N ↦ 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 2.6_{14}-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 (→ **4**),[4] reductive cleavage (→ **5**), hydrolysis, and *N*-Boc or *C*-OBn protection (→ **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 2.6_{14}-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*)-2.6_{14} 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

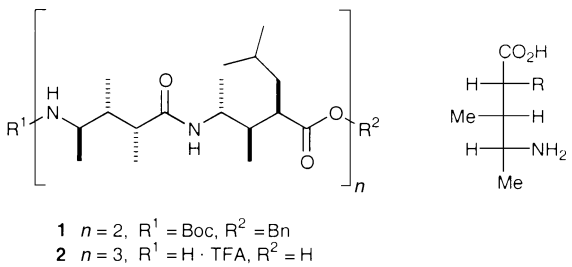
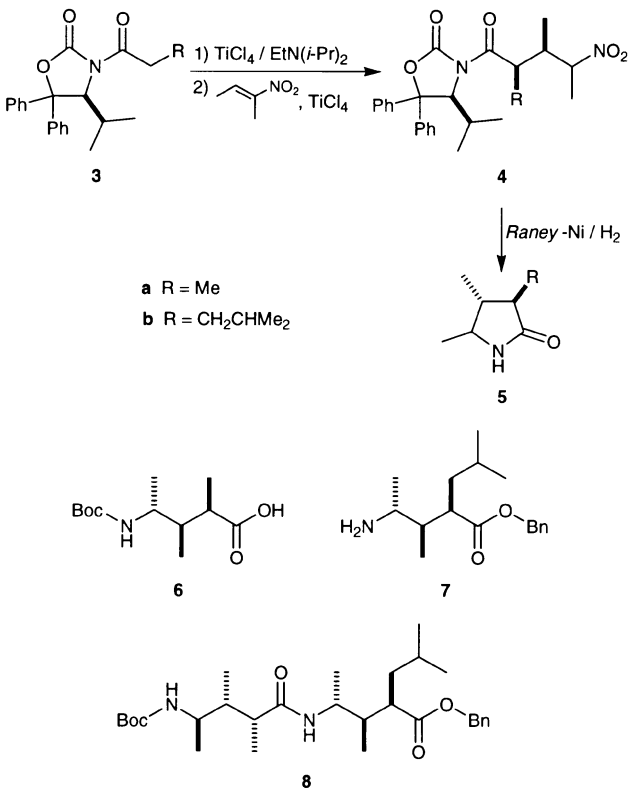


Fig. 1 $\gamma^{2,3,4}$-Tetra- and hexapeptide derivatives **1** and **2** used for the structure determinations and *Fischer* representation of the required amino acid building blocks.



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.

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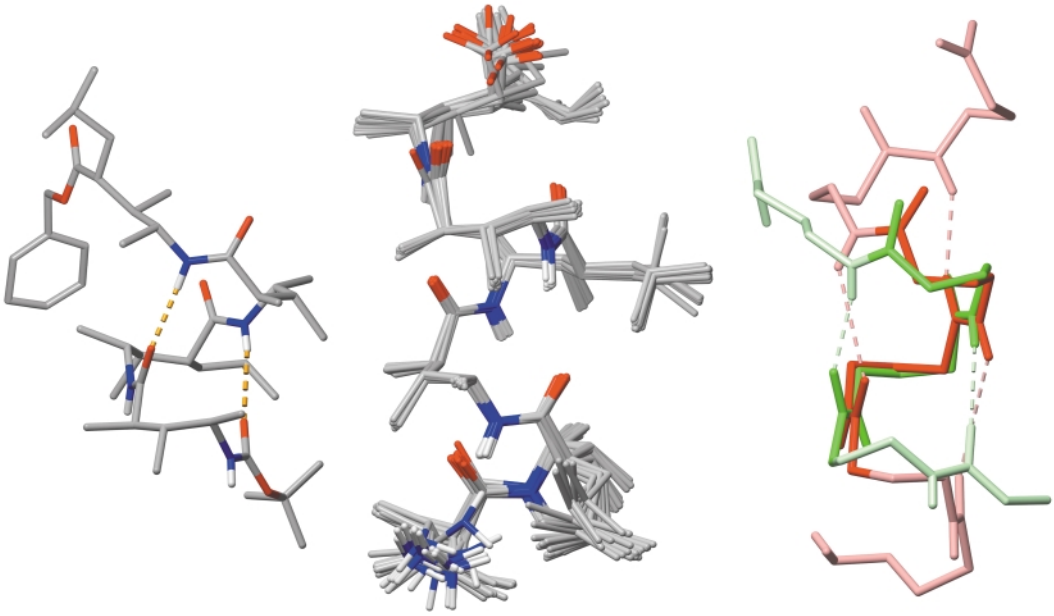

Fig. 2 (*M*)-2.6$_{14}$ Helical structures of γ-peptides **1** and **2**. a, Structure of γ-tetrapeptide **1** in the crystal state determined by X-ray structure analysis. b, Bundle of 20 conformers of hexapeptide **2** in MeOH obtained by simulated annealing calculations using restraints from NMR data. 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 by 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 CD_3OH. 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 γ^{2,3,4}-peptides constructed from (*R*,*R*,*R*)-trisubstituted γ-amino acid residues adopt well defined (*M*)-2.6$_{14}$-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 γ^{2,4}-amino acids with rel. configuration *l* form 2.6$_{14}$ helical structures, while a tetrapeptide consisting of the corresponding *u* residues was found to form a turn motif.

‡ *Crystal data* for $C_{46}H_{80}N_4O_7$ **1**: M = 801.14, monoclinic, space group $P2_1$, a = 9.462(2), b = 20.472(6), c = 13.866(4) Å, β = 106.14(2)°, V = 2580.1(12) Å^3, Z = 2, D_c = 1.031 g cm^{-3}, μ(Cu-Kα) = 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-Kα radiation. A total of 4479 unique reflections (3.32 < 2Θ < 66.23°) were processed of which 1140 were considered significant with $I_{net} > 3\sigma(I_{net})$. 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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