139. 'Mixed' β-Peptides: A Unique Helical Secondary Structure in Solution

Preliminary Communication

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β-Hexapeptides **1**–**5** and a β-dodecapeptide **6** with sequences containing two different types of β-amino acids (aliphatic proteinageous side chains in the 2- or in the 3-position) have been prepared. CD (*Fig. 1*) and NMR measurements indicate that, with one exception, the secondary structures formed by these new β-peptides differ from those of isomers studied previously. Detailed NMR analysis of the β-hexapeptide **5** (with alternating β^2,β^3-building blocks) and molecular-dynamics simulations have produced a minimum energy conformation (*Fig. 2,b*) which might be described as a novel irregular helix containing ten- and twelve-membered H-bonded rings. This demonstrates the great structural variability of β-peptides, since three different helical secondary structures have been discovered to date.

It has come as a surprise to specialists that short-chain β-peptides **A** (n = 6, 7) form distinct secondary structures in solution [1–6]. The β-peptides studied so far contain either β^2-amino acids (R^3 = H in **A**) [3], or β^3-amino acids (R^2 = H in **A**) [1] [2], or cyclic β-amino acids (R^2–R^3 = $(CH_2)_n$, n = 3 or 4, in **A**) [4] [5]. The most remarkable type of secondary structure is helical, and two entirely different helices have hitherto been identified: a 3_1 [1] [2] [4] and a $2,5_1$ [5] helix. The former contains 14-membered H-bonded rings (3_{14} helix), and has a dipole negative at the N- and positive at the C-terminus, the latter contains 12-membered rings ($2,5_{12}$ helix), with the dipole in the opposite direction. With substituents R^2 and R^3 in the (*Si*) half-space of the stereogenic centers in **A**, the 3_1 helix is left-handed (*M*) and the $2,5_1$ helix is right-handed (*P*) [6]. So far, only the 3_1 helix has been observed by NMR spectroscopy of β-peptides containing rotationally unrestricted β-amino-acid residues, and a distinct CD pattern (trough at 215 nm, peak at 200 nm) has been assigned to the (*M*) helix [2].

We have now synthesized 'mixed' β-peptides **1**–**6** containing both β^2- and β^3-amino acids (which were prepared and coupled by the methods described previously [1–3]). The

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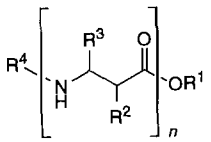
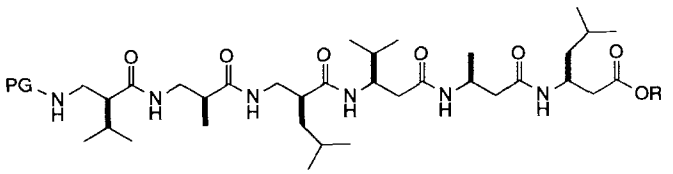
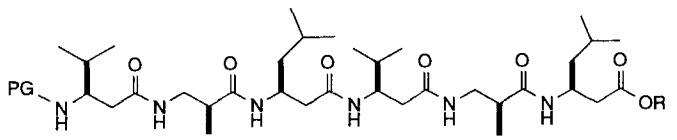


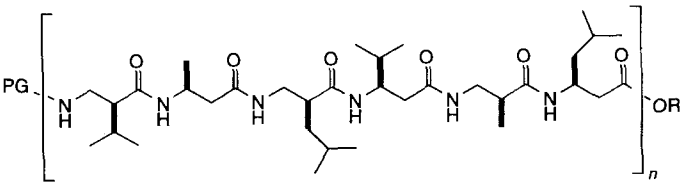
A



1 PG = Boc, R = Bn
2 PG = R = H, trifluoroacetate



3 PG = Boc, R = Bn



4 *n* = 1, PG = Boc, R = Bn
5 *n* = 1, PG = R = H, hydrochloride
6 *n* = 2, PG = Boc, R = Bn

β-hexapeptides **1** and **2** are constructed from a triad of β^2- and a triad of β^3-amino-acid residues, while **3** results from fragment coupling of two β^3,β^2,β^3-tripeptides. The third type (**4**–**6**) contains sequences of alternating β^2- and β^3-amino acids. To stay as close as possible to natural α-peptides and to the previously reported β-peptides, we chose the side chains of valine, alanine, and leucine, and the configuration of all stereogenic centers such that the now well-established (*M*) 3_1 helix could have been adopted by all β-peptides **1**–**6**.

We were stunned by the fact that only the deprotected β-hexapeptide **2** shows the familiar 215/200-nm CD pattern in MeOH solution, while all other new β-peptides **1** and **3**–**5**, including the β-dodecapeptide **6** show a new type of CD spectrum with a single peak at *ca.* 205 nm (*Fig. 1*), and a record intensity of molar ellipticity of $6.5 \cdot 10^5$ for **6**.

An NMR investigation of a C_5D_5N solution of β-hexapeptide **5**, using TOCSY and ROESY experiments [7], resulted in full assignment of all resonances in the ^1H spectrum and in the determination of the sequence. Inspection of the ROESY cross-peaks showed

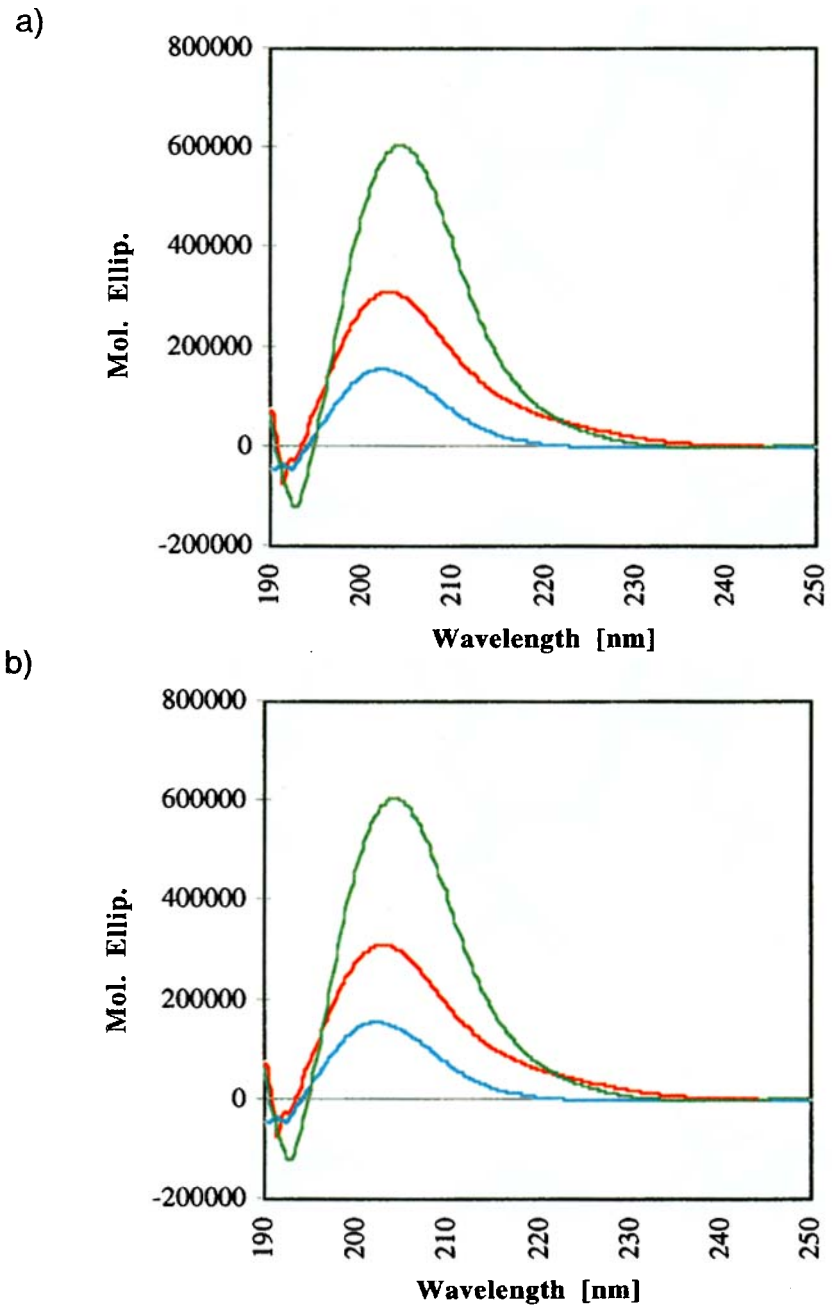


Fig. 1. *CD Spectra of β-peptides* **1**–**6** (*ca.* 0.2 mM in MeOH; mol. ellipticity in deg · cm^2 · $dmol^{-1}$). Only the deprotected hexapeptide **2** shows the characteristic pattern (blue curve in a) assigned to a β-peptide 3_1 helix [2]. All other samples (of **1** and **3**–**6**) give rise to peaks at *ca.* 205 nm. *a*) CD Spectra of β-hexapeptides consisting of trimer blocks; *red*: **1**, *blue*: **2**; *green*: **3**. b) CD Spectra of β-peptides with alternating β^2,β^3 sequences; *red*: **4**; *blue*: **5**; *green*: **6**.

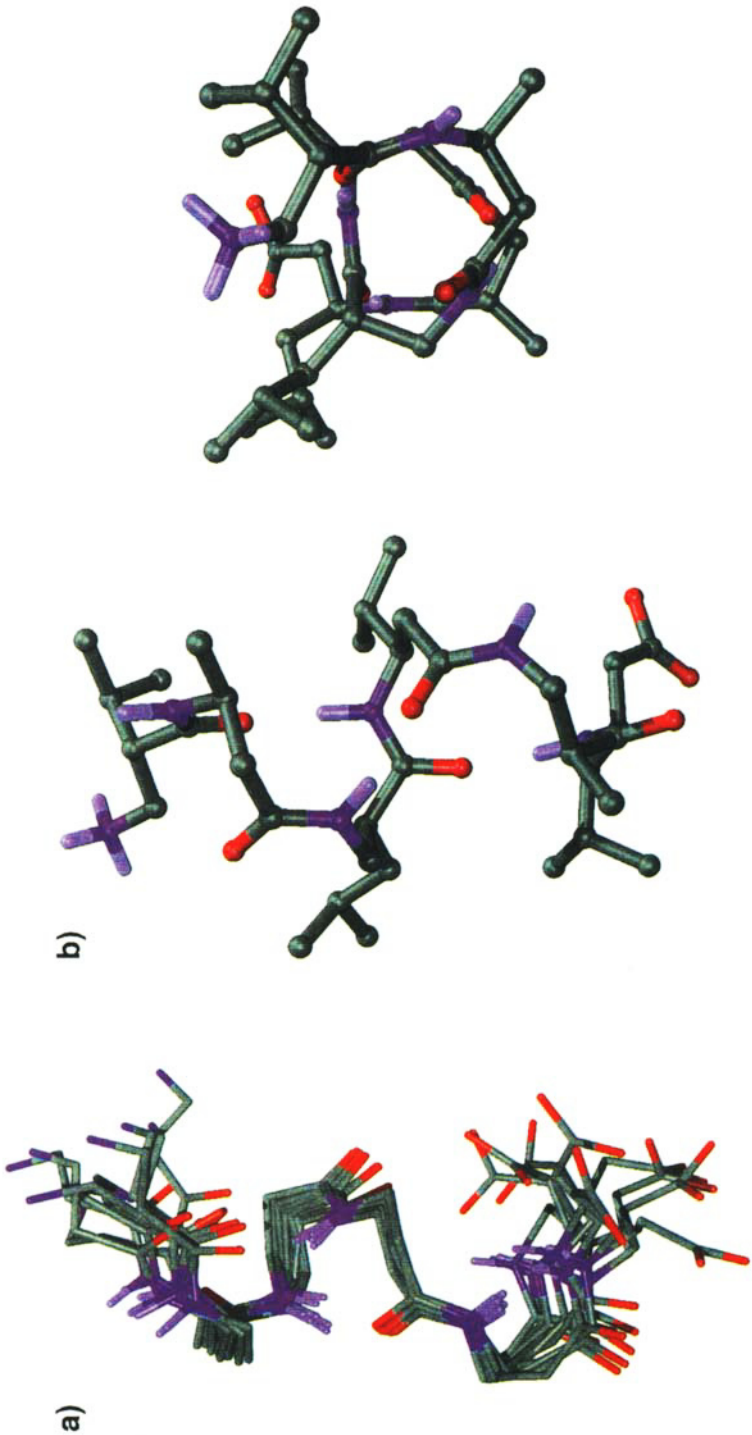


Fig. 2. *Structures of the β-hexapeptide H-β²-HVal-β³-HAla-β²-HLeu-β³-HVal-β²-HAla-β³-HLeu-OH hydrochloride* (**5**) *as determined from NMR measurements* (C_5D_5N solution). *a*) Side view of the structure obtained by NOE-restrained annealing MD simulation. *b*) Side view and top view of a low-energy conformer of **5** obtained by an unrestrained MD simulation *in vacuo* at 50 K, resulting from one of the structures shown in *a*. Figures were generated using the Raster3D program [11] [12].

significant NOEs between residues 2 and 4, and residues 4 and 6. This is in contrast to NOEs observed for a typical 3_1 helix, where additional i to $i + 3$ relations are found. Forty ROESY cross-peaks were ordered in three distance categories (strong < 2.8 Å, medium < 3.5 Å and weak < 4.5 Å) which served as upper-bound distance restraints for standard annealing molecular-dynamics simulations using the X-PLOR package [8]. The calculation converged to a bundle of structures with a central ten-membered H-bonded turn shown in *Fig. 2,a*, while the H-bonding between residues 1 and 4, as well as 3 and 6 is very weak. Since the force field used in this calculation does not account for H-bonding interactions, we decided to perform an unrestrained 50-ps molecular-dynamics simulation using AMBER* (MacroModel) [9] [10]. A conformation taken from the NOE-restrained modeling was used as starting structure for a molecular-dynamics simulation at 50 K *in vacuo*. The ten lowest-energy conformations of the trajectory were each minimized in energy and all converged to a single conformer depicted in *Fig. 2,b*. This minimized structure is still consistent with the NMR-derived structural family, but, in addition to the central ten-membered turn, twelve-membered H-bonding rings are present in both the C- and N-terminal regions.

This structure might be considered as a very unusual kind of helix, consisting of a wide, a narrow, and another wide turn. The sense of helicity is right-handed (*P*), and the helix has a strongly reduced dipole, since its amide C=O bonds point alternately up and down the helix axis. From the top view in *Fig. 2,b*, it is evident that the hydrophobic valine and leucine side chains are in juxtaposition on one side of the structure. If the β-peptide were to adopt a 3_1 helical conformation, the alignment of substituents described above would not occur and, thus, may be the reason why a 3_1 helical structure is not observed in this case (a detailed disussion will be given in a forthcoming full paper).

The so far unknown NMR structure of the β-peptide **5** in MeOH solution will tell us whether the characteristic CD spectra shown in *Fig. 1* are actually indicative of the helical structure described herein. It will also be interesting to learn whether the 12/10/12 ring pattern in this structure is repetitive in longer-chain analogs of **5** (such as **6**). Furthermore, the strikingly high solubility of the protected β-hexa- and β-dodecapeptide **4** and **6** in organic solvents (such as AcOEt) and the fact that the dodecamer **6** moves much faster on a thin-layer chromatography plate than the hexamer **4** will hopefully be understood as more structural information about the 'mixed' β-peptides with alternating β^2,β^3 residues becomes available.

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