Helical Structures

Chiral Centers in the Side Chains of α-Amino Acids Control the Helical Screw Sense of Peptides**

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Understanding the secondary structures of peptides and proteins, for example, the α-helix, β-sheet, and reversed-turn, is important as they play a vital role in molecular biology, the life sciences, and drug discovery.[1] The α-helices and the 3_{10}-helices in proteins almost always form a right-handed (*P*) helical screw, which is believed to result from the asymmetry of the α-carbon (*S* enantiomer) in terrestrial L-α-amino acids.[1a] Among proteinogenic L-α-amino acids, only isoleucine and threonine possess an additional chiral center at the side-chain β-carbon besides the α-carbon. However, so far it has not been clear how chiral centers in the side chain affect the secondary structure of peptides.[2] Here we describe how the asymmetric centers of the α-amino acid side chain alone control the screw sense of the helices formed by oligopeptides made up of amino acids without a chiral center at the α-carbon.

Replacement of the α-hydrogen atom of an α-amino acid with an alkyl substituent results in an α,α-disubstituted α-amino acid (dAA), such as 2-aminoisobutyric acid (Aib), diethylglycine (Deg), 1-aminocycloalkanecarboxylic acid (Ac_nc, n = ring size), and isovaline.[3] Oligopeptides containing dAAs show stable secondary structural preferences, such as β-turns, 3_{10}-helices, and extended planar C_5 conformations. We designed a chiral cyclic dAA [(*S*,*S*)-Ac_5c^{dOM}], in which the α-carbon does not have an asymmetric center but has two side chain γ-carbons that are asymmetric centers. In the case of Ac_5c^{dOM} homopeptides, the asymmetric centers do not lie along the main-chain backbone of the peptides but rather in the side chain cyclopentane ring. Thus, the secondary

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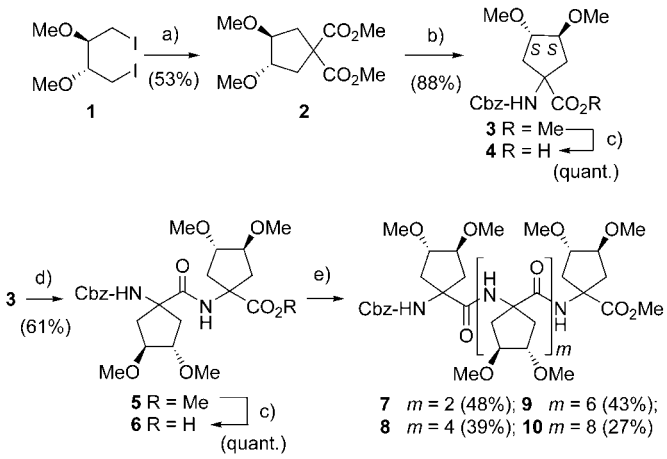
structure is affected by the side chain chiral centers of (*S*,*S*)-Ac_5c^{dOM} peptides but not by the α-carbons.[4]

The optically active (*S*,*S*)-Ac_5c^{dOM} was synthesized from dimethyl-L-(+)-tartrate as follows (Scheme 1): Dimethyl-L-(+)-tartrate was converted into a diiodide **1** by conventional procedures;[5] then dimethyl malonate was alkylated with **1** to give the cyclic diester **2**. Monohydrolysis of **2** followed by Curtius rearrangement with diphenylphosphoryl azide (DPPA) afforded the C- and N-terminal protected Cbz-(*S*,*S*)-Ac_5c^{dOM}-OMe **3**. Hydrolysis with an alkaline solution gave the N-protected Cbz-(*S*,*S*)-Ac_5c^{dOM}-OH **4**. Homopeptides Cbz-[(*S*,*S*)-Ac_5c^{dOM}]$_n$-OMe (up to the decamer; $n = 2$, 4, 6, and 10) were prepared by coupling the N-terminal free peptides and C-terminal free dipeptide **6** by solution-phase methods.[6] Octapeptide **9** and decapeptide **10** can be dissolved in water (**10**: $> 5\ mg\,cm^{-1}$) because of the hydrophilic ethereal groups at the cyclopentane.[7]



Scheme 1. Synthesis of (*S*,*S*)-Ac_5c^{dOM} and its homopeptides. Reagents and conditions: a) dimethyl malonate, KO*t*Bu; b) 1. NaOH, 2. DPPA, 3. BnOH; c) NaOH; d) 1. Pd/C, H_2, 2. EDC, HOBt, **4**, MeCN, RT; e) 1. Pd/C, H_2, 2. EDC, HOBt, **6**, MeCN, RT. Cbz = benzyloxycarbonyl, EDC = 2-(3-dimethylaminopropyl)-1-ethylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole.

The preferred secondary structure of the homopeptides in the $CDCl_3$ solution was first studied by FT-IR and ^{1}H NMR spectroscopies. In the IR spectra, the weak bands in the region 3420–3440 cm^{-1} are assigned to free (solvated) peptide NH groups, and the strong bands at 3320–3370 cm^{-1} to peptide NH groups with N–H···O=C intramolecular hydrogen bonds of differing strengths. As the length of the peptide chain increases, the strong band observed at 3370 cm^{-1} in **7** shifts to slightly lower wave numbers (3320 cm^{-1} in **10**), and the relative intensity of this band gradually increases.[6] These IR spectra are very similar to those of achiral Ac_4c peptides, which form 3_{10}-helices in solution,[3c] but very different from those of Deg peptides, which form extended planar conformations.[3b]

In the ^{1}H NMR spectra measured after addition of DMSO or the free radical 2,2,6,6-tetramethyl-1-piperidinoxyl (TEMPO), as well as at different peptide concentrations, the two NH signals [NH1 and NH2] of the hexapeptide **8** and octapeptide **9**, respectively, are very sensitive (solvent-exposed NH group). This suggests the absence of two intramolecular hydrogen bonds at these NH groups and thus indicates that the peptides assume a helical structure in $CDCl_3$ solution.[6] Also, the ROESY ^{1}H NMR spectrum of **8** shows a complete series of sequential d_{NN} cross peaks of NOEs, from the N-terminal NH1 to the C-terminal NH6, characteristic of a helical secondary structure.[6]

The CD spectra of **8**–**10** in 2,2,2-trifluoroethanol (TFE) show positive maxima and intensity for two bands at 222 nm and 208 nm, indicating that the screw sense of the helix is left-handed (*M*) (Figure 1 a). The ratio of R [$\theta_{222}/\theta_{208}$] suggests that the secondary structure of **8** is a 3_{10}-helix, and that those of **9** and **10** are α-helices.[8] Interestingly, the intensity of the CD spectra of **9** and **10** in water become stronger, indicating that these peptides are more helical when dissolved in water than in TFE (Figure 1 b).

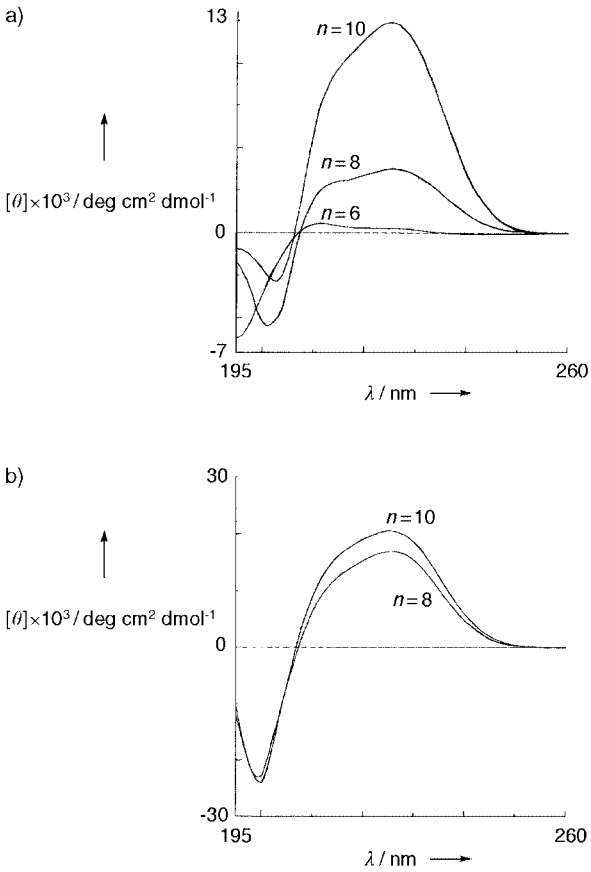


Figure 1. CD spectra of Cbz-[(*S*,*S*)-Ac_5c^{dOM}]$_n$-OMe **8**–**10** ($n = 6$, 8, 10) (0.5 mM) a) in TFE solution, b) in H_2O.

The molecular and crystal structures of the terminally protected hexapeptide **8** (Figure 2) and octapeptide **9** (Figure 3) were determined by X-ray crystallographic analysis. In the crystal structure of **8** three crystallographically independent molecules *A*, *B*, and *C* exist in the asymmetric unit. All three molecules are left-handed (*M*) 3_{10}-helices (mean value: $\phi = 58.5°$, $\psi = 30.5°$), showing small differences in the conformation of the side chains, and four intramolecular hydrogen bonds are found in each molecule. The molecules *A*, *B*, and *C* are connected by two intermolecular

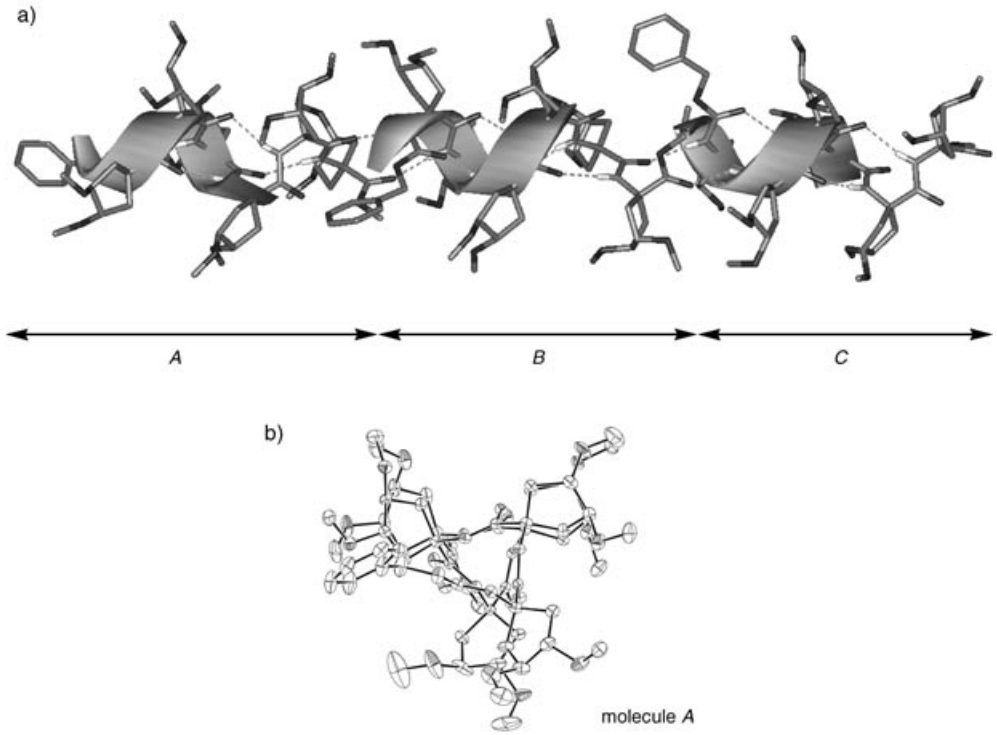


Figure 2. a) Illustrative structure of **8** (molecules *A*, *B* and *C*) as viewed perpendicular to the 3_{10}-helical axis; b) ORTEP drawing of molecule *A* as viewed along the 3_{10}-helical axis.

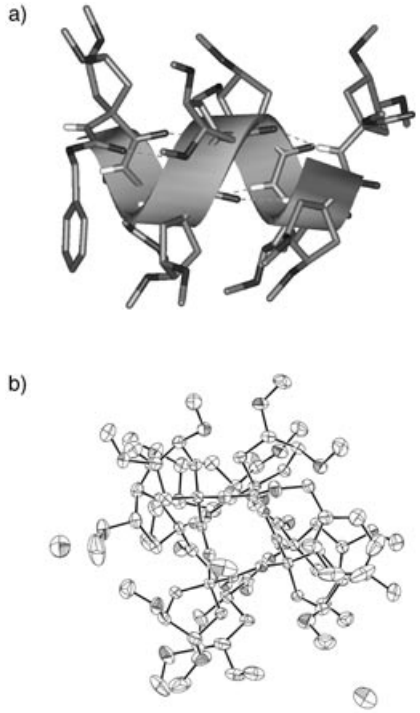


Figure 3. a) Illustrative structure of **9** as viewed perpendicular to the α-helical axis; b) ORTEP drawing as viewed along the α-helical axis (α-helical wheel).

hydrogen bonds, forming a head-to-tail alignment of (··A···B···C···A···B···C··) chains.[9]

In the asymmetric unit of **9** one left-handed (*M*) helical structure (mean value: $\phi = 60.9°$, $\psi = 46.8°$),[10] which is not a 3_{10}-helix but an α-helix, exists along with three water molecules. Five intramolecular hydrogen bonds exist in the α-helical molecule, and in the packing mode the chains of intermolecularly hydrogen-bonded (*M*) α-helices are formed by means of the water.

The conformational search calculation with Macromodel (AMBER*) produced left-handed (*M*) α-helices as a global minimum-energy conformation for both **8** and **9**. The (*M*) 3_{10}-helix of **8**, which is similar to those in the crystal, was obtained as a local minimum-energy conformation (+3.22 kcal mol^{-1}).[6]

We have efficiently synthesized a new chiral cyclic dAA and studied the secondary structure of (*S*,*S*)-Ac_5c^{dOM} homopeptides. It is notable that: 1) Chiral centers in the side chain of the α-amino acid strongly control the helical screw sense of its peptides; 2) The (*M*) α-helix of the (*S*,*S*)-Ac_5c^{dOM} peptides is stable even in water; 3) The transition from the 3_{10}-helix into the α-helix occurs for longer (*S*,*S*)-Ac_5c^{dOM} peptides; and 4) The α-helix is formed in dAA homopeptides without a natural α-amino acid in the solid state.

These results strongly imply that the side chain chiral centers of isoleucine and threonine would affect the secondary structure of their oligopeptides; albeit, these residues also exhibit a strong screw-sense bias due to their chiral α-carbon atom, and they are poorly helicogenic residues. Preparation of the enantiomer and incorporation of (*S*,*S*)-Ac_5c^{dOM} into natural α-amino acid sequences are currently underway.

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[9] CCDC-236749 and CCDC-236750 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223–336–033 or deposit@ccdc.cam.ac.uk). Crystal data: **8**: $3(C_{57}H_{88}N_6O_{21})$, M_r = 3579.99 (1193.33), space group $P2_1$, a = 22.889, b = 11.9061, c = 33.711 Å, β = 91.887°, V = 9182.0 Å^3, Z = 6, T = 90 K, $\mu(Mo_{K\alpha})$ = 0.99 cm^{-1}, 31196 reflections measured, 29355 unique reflections (R_{int} = 0.0326) R_1 ($I > 2\sigma$) = 0.0775, wR_2 ($I > 2\sigma$) = 0.1972, GOF = 1.095. **9**: $C_{73}H_{114}N_8O_{27}\cdot 3H_2O$, M_r = 1589.77, space group $P2_1$, a = 15.639, b = 16.431, c = 15.989 Å, β = 95.85°, V = 4087 Å^3, Z = 2, T = 123 K, $\mu(Mo_{K\alpha})$ = 1.0 cm^{-1}, 16850 reflections measured, 12478 unique reflections (R_{int} = 0.0609) R_1 ($I > 2\sigma$) = 0.0579, wR_2 ($I > 2\sigma$) = 0.1401 ($I > 2\sigma$), GOF = 1.031.

[10] The signs of ϕ, ψ torsion angles at the C-terminus are opposite to those of the preceding residues. The average is amino acid residues (1–7).

