Helical Structures

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

Masakazu Tanaka, Yosuke Demizu, Mitsunobu Doi, Masaaki Kurihara, and Hiroshi Suemune**

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

[*] Prof. Dr. M. Tanaka, Y. Demizu, Prof. Dr. H. Suemune
Graduate School of Pharmaceutical Sciences
Kyushu University
Fukuoka 812-8582 (Japan)
Fax: (+81) 92-642-6545
E-mail: mtanaka@phar.kyushu-u.ac.jp
suemune@phar.kyushu-u.ac.jp
Prof. Dr. M. Doi
Osaka University of Pharmaceutical Sciences
Osaka 569-1094 (Japan)
Dr. M. Kurihara
Division of Organic Chemistry
National Institute of Health Sciences
Tokyo 158-8501 (Japan)

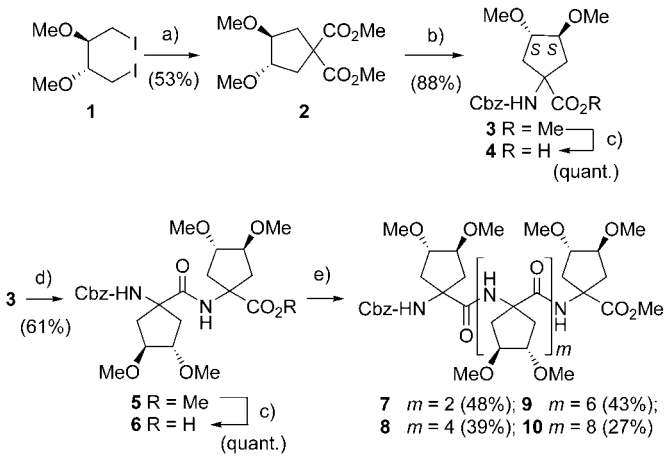
[**] This work was partly supported by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science and also by the Sasakawa Scientific Research Grant from the Japan Science Society.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/anie.200460420

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).

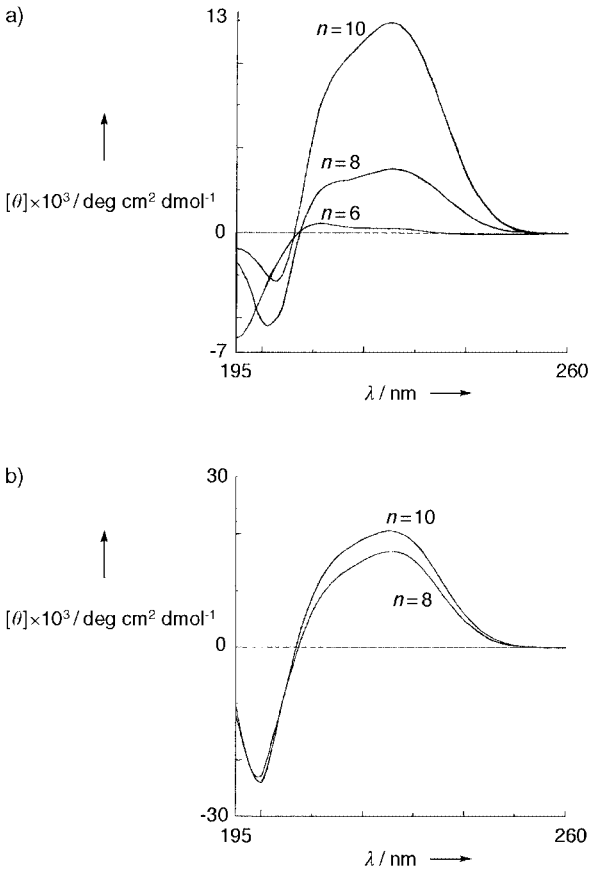


Figure 1. CD spectra of Cbz-[(*S*,*S*)-Ac_5c^{dOM}]$_n$-OMe **8**–**10** ($n = 6$, 8, 10) (0.5 mM) a) in TFE solution, b) in H_2O.

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

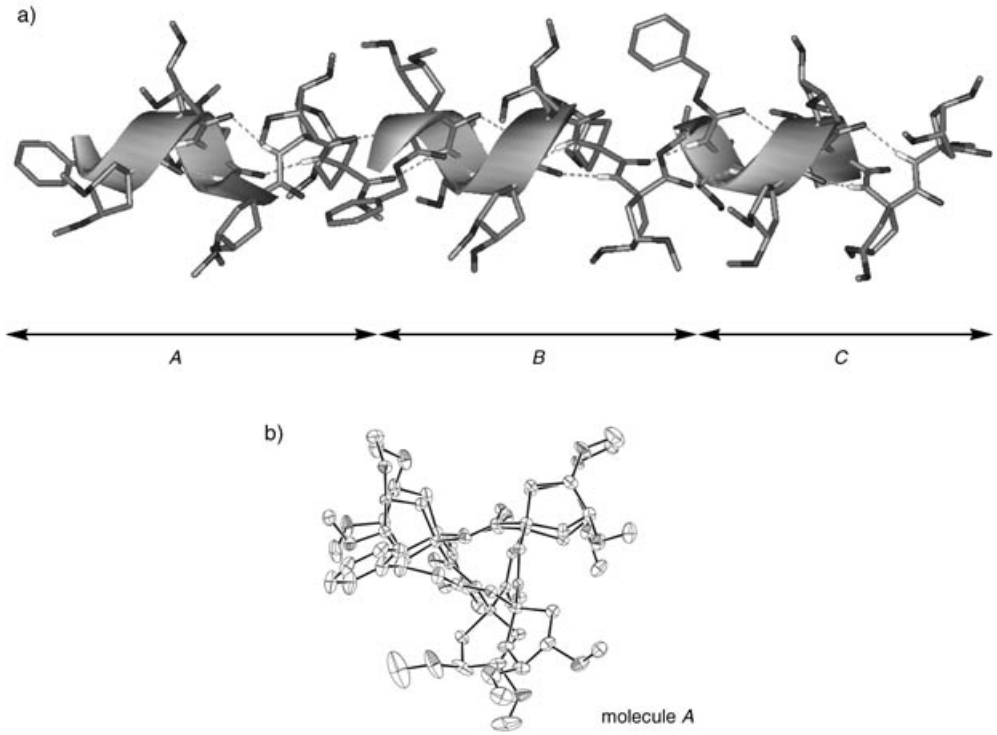


Figure 2. a) Illustrative structure of **8** (molecules *A*, *B* and *C*) as viewed perpendicular to the 3_{10}-helical axis; b) ORTEP drawing of molecule *A* as viewed along the 3_{10}-helical axis.

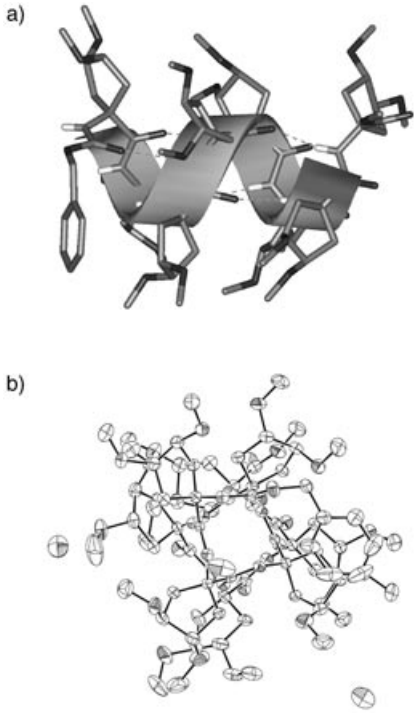


Figure 3. a) Illustrative structure of **9** as viewed perpendicular to the α-helical axis; b) ORTEP drawing as viewed along the α-helical axis (α-helical wheel).

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.

Received: April 23, 2004

Published Online: July 7, 2004

Keywords: amino acids · chirality · conformation analysis · helical structures · peptidomimetics

[1] a) C. Branden, J. Tooze, *Introduction to Protein Structure*, Garland, New York, **1991**, pp. 1–31; b) J. A. Robinson, *Synlett* **1999**, 429–441; c) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015–2022; d) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.

[2] Chiral N-alkylated glycine oligopeptides (peptoids) form a one-handed helix affected by the chiralities at the *N*-alkyl side-chains; however, the peptoids may have *cis* amides. See: C. W. Wu, K. Kirshenbaum, T. J. Sanborn, J. A. Patch, K. Huang, K. A. Dill, R. N. Zuckermann, A. E. Barron, *J. Am. Chem. Soc.* **2003**, *125*, 13525–13530.

[3] a) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; b) M. Tanaka, N. Imawaka, M. Kurihara, H. Suemune, *Helv. Chim. Acta* **1999**, *82*, 494–510; c) M. Gatos, F. Formaggio, M. Crisma, C. Toniolo, G. M. Bonora, Z. Benedetti, B. D. Blasio, R. Iacovino, A. Santini, M. Saviano, J. Kamphuis, *J. Pept. Sci.* **1997**, *3*, 110–122; d) B. Jaun, M. Tanaka, P. Seiler, F. N. M. Kühnle, C. Braun, D. Seebach, *Liebigs Ann./Recl.* **1997**, 1697–1710; e) M. Crisma, A. Moretto, M. Rainaldi, F. Formaggio, Q. B. Broxterman, B. Kaptein, C. Toniolo, *J. Pept. Sci.* **2003**, *9*, 620–637; f) N. Imawaka, M. Tanaka, H. Suemune, *Helv. Chim. Acta* **2000**, *83*, 2823–2835; g) M. Tanaka, S. Nishimura, M. Oba, Y. Demizu, M. Kurihara, H. Suemune, *Chem. Eur. J.* **2003**, *9*, 3082–3090.

[4] Toniolo's group reported that homopeptides of a C_2-symmetric binaphthyl dAA with only axial chirality form one-handed 3_{10}-helices in solution. See: J. P. Mazaleyrat, K. Wright, A. Gaucher, M. Wakselman, S. Oancea, F. Formaggio, C. Toniolo, V. Setnicka, J. Kapitan, T. A. Keiderling, *Tetrahedron: Asymmetry* **2003**, *14*, 1879–1893.

[5] I. Takahashi, K. Odashima, K. Koga, *Tetrahedron Lett.* **1984**, *25*, 973–976.

[6] See the Supporting Information for synthetic procedures, spectroscopic data of new compounds, IR spectra, ^{1}H NMR experiments (DMSO, TEMPO, concentration effects, and the ROESY spectrum), molecular mechanics calculations, torsion angles, and hydrogen-bond parameters.

[7] M. Tanaka, Y. Demizu, M. Doi, M. Kurihara, H. Suemune, *Pept. Sci. 2003 (Proceedings of the 40th JPS)* **2004**, 109–110.

[8] a) C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc.* **1996**, *118*, 2744–2745; b) P. Pengo, L. Pasquato, S. Moro, A. Brigo, F. Fogolari, Q. B. Broxterman, B. Kaptein, P. Scrimin, *Angew. Chem.* **2003**, *115*, 3510–3514; *Angew. Chem. Int. Ed.* **2003**, *42*, 3388–3392.

[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).

