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Side-Chain Chiral Centers of Amino Acid and Helical-Screw Handedness of Its Peptides

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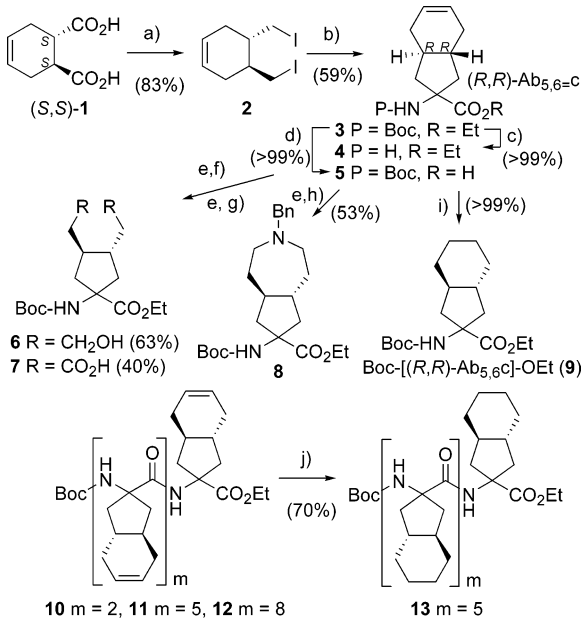
Helical structures[1] in peptides and proteins play a vital role in biological processes and, thus, attract much attention from bioorganic and peptide chemists and molecular biologists. The α- and 3_{10}-helices in proteins almost always form a right-handed (*P*) helical-screw sense, which is believed to result from the asymmetric center at the α-position of L-α-amino acids.[1a] Besides an asymmetric center at the α-position, isoleucine and threonine possess an additional chiral center at the side-chain *β*-position. However, so far, no attention has been paid as to how the asymmetric centers on the side chain affect the secondary structure.[2] We have previously reported that chiral cyclic α,α-disubstituted α-amino acids (dAA) bearing only side-chain chiral centers (Ac_5c^{dOM})[2a,3] control the helical-screw sense of its homopeptides.[2,4,5] Herein, we design chiral bicyclic dAA {(1*R*,6*R*)-8-aminobicyclo[4.3.0]non-3-ene-8-carboxylic acid; (*R*,*R*)-$Ab_{5,6=}c$}, in which the α-carbon is not the chiral center, but the asymmetric centers exist at the side-chain bicyclic skeleton. Furthermore, we describe modification of (*R*,*R*)-$Ab_{5,6=}c$ and its peptide and the effect of side-chain chiral centers on the secondary structure of their peptides.

The optically active (*R*,*R*)-$Ab_{5,6=}c$ was synthesized from (*S*,*S*)-cyclohex-4-ene-1,2-dicarboxylic acid **1**[6] as follows (Scheme 1). The acid (*S*,*S*)-**1** was converted into a diiodide **2** by reduction and subsequent substitution with iodide. Then, ethyl isocyanoacetate was bisalkylated with **2**,[7] followed by acidic hydrolysis and protection with Boc_2O to give amino acid Boc-[(*R*,*R*)-$Ab_{5,6=}c$]-OEt (**3**). Acidic hydrolysis of **3** afforded the N-free H-[(*R*,*R*)-$Ab_{5,6=}c$]-OEt (**4**), and alkaline hydrolysis gave the C-free Boc-[(*R*,*R*)-$Ab_{5,6=}c$]-OH (**5**). The olefin in the amino acid **3** could be easily converted into several functional groups. Ozonolysis of the olefin in **3**, followed by reduction with $NaBH_4$, afforded a dihydroxy amino acid **6** and by oxidation with Oxone gave a dicarboxylic amino acid **7**, and by reductive amination with $BnNH_2$ produced a bicyclic seven-membered ring amino acid **8**. Moreover, hydrogenation of the olefin in **3** afforded saturated amino acid Boc-[(*R*,*R*)-$Ab_{5,6}c$]-OEt (**9**). Homopeptides Boc-[(*R*,*R*)-$Ab_{5,6=}c]_n$-OEt (up to the nonapeptide; $n = 3, 6, 9$) were prepared by coupling the N-terminal-free peptide esters and the Boc-protected C-terminal-free tripeptide using HBTU[3] by solution-phase methods.[8] The six olefins in hexapeptide **11** were hydrogenated by H_2/20%Pd-$(OH)_2$−C in one step to produce the saturated peptide Boc-[(*R*,*R*)-$Ab_{5,6}c]_6$-OEt (**13**) in 70% yield.

At first, the preferred conformation of the hydrophobic homopeptides **10**−**12** in the $CDCl_3$ solution was studied by FT-IR absorption spectroscopy.[8] The IR spectra showed weak bands at the region 3420−3440 cm^{-1} [free (solvated) peptide NH groups]

Scheme 1. Synthesis of (*R*,*R*)-$Ab_{5,6=}c$, Modification, and Peptides[a]



[a] Reagents: (a) 1. $LiAlH_4$; 2. I_2, PPh_3; (b) 1. NaH, $CNCH_2CO_2Et$; 2. HCl; 3. Boc_2O; (c) H^+; (d) NaOH; (e) O_3; (f) $NaBH_4$; (g) Oxone; (h) $BnNH_2$, $NaBH_3CN$; (i) H_2, Pd−C; (j) H_2, $Pd(OH)_2$−C.

and strong bands at 3320−3370 cm^{-1} [intramolecularly H-bonded peptide NH groups]. The latter band observed at 3370 cm^{-1} in **10** shifts to lower wavenumbers (3320 cm^{-1} in **12**), and the relative intensity increases, as the length of the peptide chain becomes longer. These IR spectra are very similar to those of Ac_5c[8,9] and Ac_5c^{dOM} homopeptides, which form helical structures in solution.[2]

The ^1H NMR experiments, measured after addition of DMSO or the free-radical TEMPO (Figure 1), as well as at different peptide concentrations, indicated that the two NH signals [NH(1) and NH-(2)] of **11** and **12**, respectively, are very sensitive (solvent-exposed NH group), suggesting that these two NH groups are not intramolecularly H-bonded, and thus the peptide assumes a 3_{10}-helical structure in $CDCl_3$ solution. The ROESY ^1H NMR spectrum of **11** showed a complete series of sequential d_{NN} cross-peaks of NOEs, from the N-terminal NH(1) to the C-terminal NH(6), which are characteristic for a helical structure, albeit that of **12** just gives a partial series of sequential d_{NN} cross-peaks from NH(1) to NH(5) and from NH(6 or 7) to NH(8 or 9).[8] The ^1H NMR experiments of **13** showed the similar patterns.

The CD spectrum of hexapeptide **11** in TFE solution does not show characteristic maxima for a helical structure, assuming the secondary structure of **11** may be unstable in TFE solution, or both

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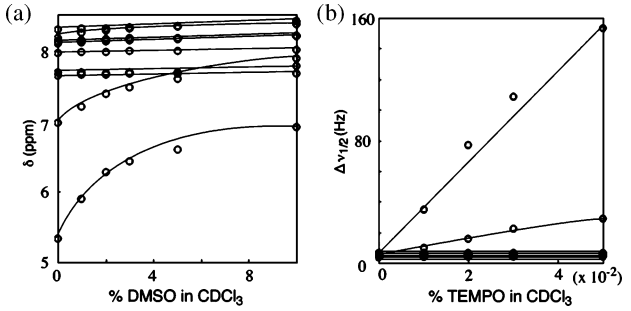

Figure 1. (a) Plots of NH chemical shifts in the ^{1}H NMR spectra of **12** (1.0 mM) as a function of increasing percentage of DMSO (v/v) added to the $CDCl_3$ solution. (b) Plots of bandwidth of the NH protons of **12** (1.0 mM) as a function of increasing percentage of TEMPO (w/v) added to the $CDCl_3$ solution.

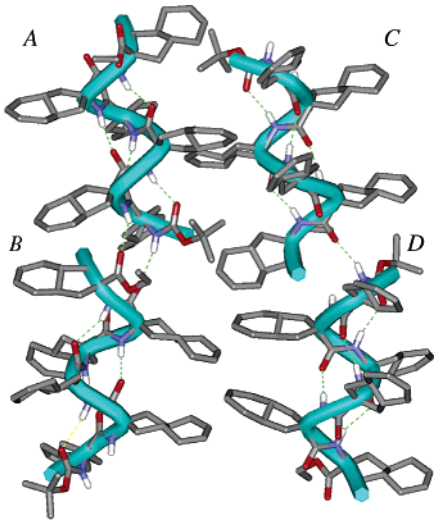

Figure 2. Four crystallographically independent molecules (*A*−*D*) of **11**, determined by X-ray crystallographic analysis.

the right-handed (*P*) and the left-handed (*M*) helices exist.[8] The CD spectrum of nonapeptide **12** shows weak negative maxima at 208 and 222 nm and a weak positive maximum at 192 nm, suggesting both the (*P*) and (*M*) helices, but the (*P*) helix would be slightly predominant. Interestingly, the conversion of **11** into the saturated **13** by hydrogenation changes the shape of the CD spectrum, which suggests the dominant conformation may slightly be changed.[8]

The crystal structure of hexapeptide **11** was solved in the *P*1 space group by a direct method using SHELXS-97,[10] by X-ray crystallographic analysis (Figure 2).[11] In the crystal state, four crystallographically independent molecules (*A*, *B*, *C* and *D*) along with two ethanol molecules, exist in the asymmetric unit, meaning the empirical molecular weight is 4594.0 [4($C_{67}H_{92}N_6O_9$)·2(C_2H_6O)]. Two molecules *A* and *D* are right-handed (*P*) 3_{10}-helices (mean value: $A\ \phi = -59.3°$, $\psi = -23.9°$; $D\ \phi = -59.2°$, $\psi = -25.5°$) and two molecules *B* and *C* are left-handed (*M*) 3_{10}-helices (mean value: $B\ \phi = 58.2°$, $\psi = 25.8°$; $C\ \phi = 57.6°$, $\psi = 24.8°$).[8,12] These two molecules, respectively, are very similar in the conformation of the peptide backbone, but small differences in the conformation, especially at the side-chain cyclohexene and at the C- and N-terminus, are observed.

Four intramolecular hydrogen bonds are found in each molecule. In the packing mode, the molecules *A* and *B* are connected by two intermolecular hydrogen bonds, and the molecules *C* and *D* are connected by one or two intermolecular hydrogen bonds. Thus, head-to-tail aligned chains of ···*A*(*P*)···*B*(*M*)···*A*(*P*)···*B*(*M*)··· and ···*C*(*M*)···*D*(*P*)···*C*(*M*)···*D*(*P*)··· are formed along the *c* direction in the crystal state.

Molecular-mechanics calculation of **11** with Macromodel (AMBER*) produced a (*P*) 3_{10}-helix as a global minimum-energy conformation (0 kcal/mol), and an (*M*) 3_{10}-helix as a local minimum-energy conformation (+1.60 kcal/mol). The (*P*) 3_{10}-helix and (*M*) 3_{10}-helix are similar to those in the crystal state.[8]

In summary, we synthesized a hydrophobic bicyclic dAA having chiral centers at the side-chain fused-ring junctions and described its modifications. The IR, ^{1}H NMR, CD spectra, and the X-ray analysis revealed that the (*R*,*R*)-$Ab_{5,6=}c$ hexapeptide **11** having twelve chiral centers forms both diastereomeric (*P*) and (*M*) 3_{10}-helices,[13] which is in contrast with the left-handed (*S*,*S*)-Ac_5c^{dOM} homopeptides controlled by side-chain chiral centers.[2a] These results indicate that the side-chain chiral environments (bulkiness or flexibility) might be important for control of the helical-screw sense of peptides.[2]

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Supporting Information Available: Experimental section, spectroscopic data of **1**−**13**, crystallographic details (CIF), IR, CD, ROESY ^{1}H NMR (PDF), and molecular mechanics calculation (PDB). This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) Abbreviation: $Ac_{(n)}c$: 1-aminocycloalkanecarboxylic acid (*n* = ring size); (*R*,*R*)-$Ab_{5,6}c$: (1*R*,6*R*)-8-aminobicyclo[4.3.0]nonane-8-carboxylic acid; HBTU:*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TEMPO: 2,2,6,6-tetramethyl-1-piperidinoxyl, free radical; TFE: 2,2,2-trifluoroethanol.

(4) For peptides composed of dAA with axial chirality, see: (a) Mazaleyrat, J. P.; Wright, K.; Gaucher, A.; Wakselman, M.; Oancea, S.; Formaggio, F.; Toniolo, C.; Setnicka, V.; Kapitan, J.; Keiderling, T. A. *Tetrahedron: Asymmetry* **2003**, *14*, 1879−1893. (b) Mazaleyrat, J.-P.; Wright, K.; Gaucher, A.; Toulemonde, N.; Wakselman, M.; Oancea, S.; Peggion, C.; Formaggio, F.; Sentnicka, V.; Keiderling, T. A.; Toniolo, C. *J. Am. Chem. Soc.* **2004**, *126*, 12874−12879.

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(11) Crystal data for **11**: 4($C_{67}H_{92}N_6O_9$)·2(C_2H_6O), M = 4594.0, space group *P*1, a = 15.765 Å, b = 16.535 Å, c = 26.90 Å, α = 74.22°, β = 82.32°, γ = 75.39°, V = 6514 Å^3, Z = 4, T = 123 K, μ (Mo Kα) = 0.78 cm^{-1}, 29 059 reflections measured, 14 531 unique reflections (R_{int} = 0.0530) R_1 ($I > 2\sigma$) = 0.0589, wR_2($I > 2\sigma$) = 0.1373, GOF = 0.885.

(12) The signs of ϕ, ψ torsion angles at the C-terminus are opposite to those of the preceding residues. Thus, the mean values refer to those of the amino acid residues 1−5, respectively.

(13) Concomitant occurrences of two diastereomeric (*P*) and (*M*) 3_{10}-helices of peptides having the main-chain asymmetric centers have already been reported. See: (a) Valle, G.; Crisma, M.; Toniolo, C.; Besswenger, R.; Rieker, A.; Jung, G. *J. Am. Chem. Soc.* **1989**, *111*, 6828−6833. (b) Jaun, B.; Tanaka, M.; Seiler, P.; Kühnle, F. N. M.; Braun, C.; Seebach, D. *Liebigs Ann. Recueil* **1997**, 1697−1710.

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