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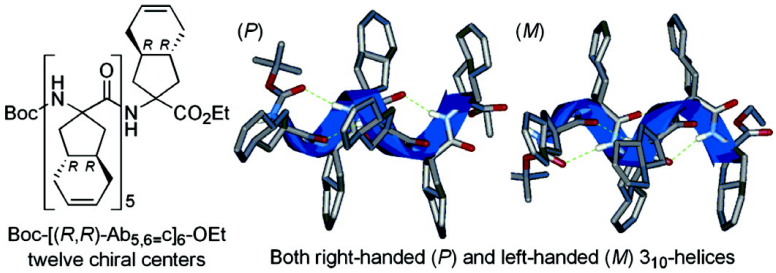
J. Am. Chem. Soc., **2005**, 127 (33), 11570-11571• DOI: 10.1021/ja053842c • Publication Date (Web): 30 July 2005



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Published on Web 07/30/2005

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

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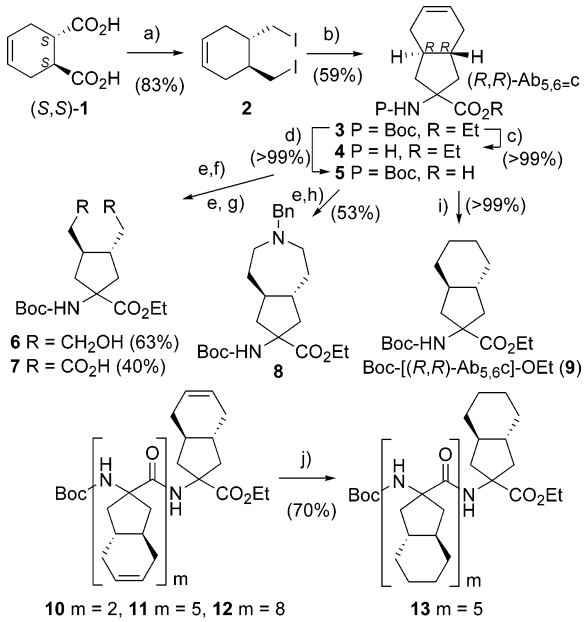
Helical structures[1] in peptides and proteins play a vital role in biological processes and, thus, attract much attention from bioorganic and peptide chemists and molecular biologists. The α- and 3_{10}-helices in proteins almost always form a right-handed (*P*) helical-screw sense, which is believed 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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10.1021/ja053842c CCC: $30.25

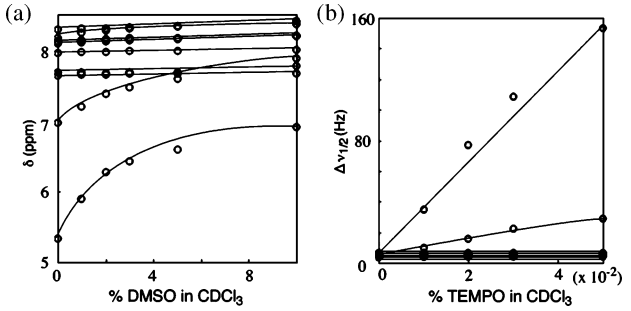

Figure 1. (a) Plots of NH chemical shifts in the ^{1}H NMR spectra of **12** (1.0 mM) as a function of increasing percentage of DMSO (v/v) added to the $CDCl_3$ solution. (b) Plots of bandwidth of the NH protons of **12** (1.0 mM) as a function of increasing percentage of TEMPO (w/v) added to the $CDCl_3$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

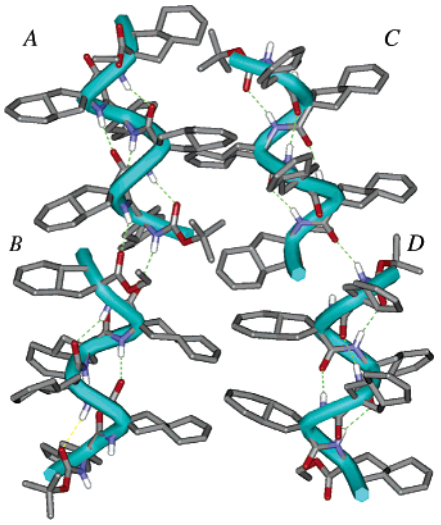

Figure 2. Four crystallographically independent molecules (*A*−*D*) of **11**, determined by X-ray crystallographic analysis.

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]

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research (B), and Young Scientists (B) from the Japan Society for the Promotion of Science, and also by the Sasakawa Scientific Research Grant from the Japan Science Society.

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.

JA053842C