J|A|C|S ARTICLES

Published on Web 09/30/2010

Water-Stable Helical Structure of Tertiary Amides of Bicyclic β-Amino Acid Bearing 7-Azabicyclo[2.2.1]heptane. Full Control of Amide Cis−Trans Equilibrium by Bridgehead Substitution

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Abstract: Helical structures of oligomers of non-natural β-amino acids are significantly stabilized by intramolecular hydrogen bonding between main-chain amide moieties in many cases, but the structures are generally susceptible to the environment; that is, helices may unfold in protic solvents such as water. For the generation of non-hydrogen-bonded ordered structures of amides (tertiary amides in most cases), control of cis−trans isomerization is crucial, even though there is only a small sterical difference with respect to cis and trans orientations. We have established methods for synthesis of conformationally constrained β-proline mimics, that is, bridgehead-substituted 7-azabicyclo[2.2.1]heptane-2-*endo*-carboxylic acids. Our crystallographic, 1D- and 2D-NMR, and CD spectroscopic studies in solution revealed that a bridgehead methoxymethyl substituent completely biased the cis−trans equilibrium to the *cis*-amide structure along the main chain, and helical structures based on the *cis*-amide linkage were generated independently of the number of residues, from the minimalist dimer through the tetramer, hexamer, and up to the octamer, and irrespective of the solvent (e.g., water, alcohol, halogenated solvents, and cyclohexane). Generality of the control of the amide equilibrium by bridgehead substitution was also examined.

Introduction

Among peptides composed of non-natural amino acids, β-peptides have been extensively studied due to their ability to form ordered structures (especially helices), their structural resemblance to natural α-peptides, and their enhanced stability to protease digestion.[1] In many cases, helical structures of oligomers of non-natural β-amino acids are significantly stabilized by intramolecular hydrogen bonding between main-chain amide moieties,[2] but such structures are generally susceptible to the environment; that is, helices are often unfolded (destabilized) in protic solvents such as water.[3] Thus, development of β-peptides with robust organized structure without utilizing hydrogen bonding is an important goal in the area of peptide engineering and medicinal chemistry.[1b,4]

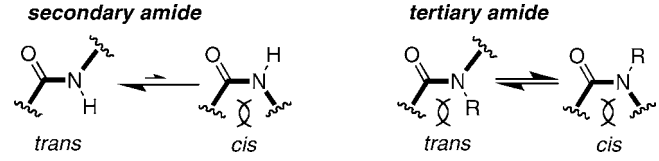

Figure 1. The cis−trans isomerization of amides.

Control of cis−trans (*E*−*Z*) isomerization of the amide linkage is crucial for the robustness of ordered structures. Nevertheless, tailoring of cis−trans isomerization of non-hydrogen-bonding amides, that is, tertiary amides in most cases, is intrinsically difficult due to the small sterical difference with respect to cis and trans orientations (Figure 1).[5] This is significantly different from the situation in the case of secondary amides, which show strong preference for trans-conformation along the main chain, together with hydrogen-bond formation. Changing the equilibrium of cis−trans isomerization of tertiary amides in the case of α-amino acids, particularly α-proline and its mimetics, has been accomplished sterically (by introduction

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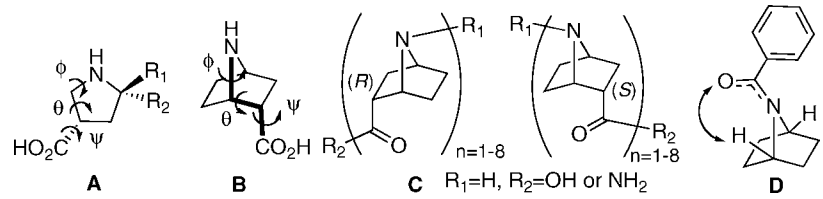

Figure 2. (A) 2,2-Disubstituted-pyrrolidine-4-carboxylic acid. (B) β-Amino acid bearing 7-azabicyclo[2.2.1]heptane and (C) homooligomers of (B) described in the previous study.[11] (D) 1,3-Allylic strain of bicyclic amides.

of alkyl group(s) at the α-position of the nitrogen atom,[6,7] etc.) or stereoelectronically (by use of the gauche effect, which influences puckering of the pyrrolidine ring, leading to direct interaction of the amide linkage and the carboxylic functionality,[8] etc.). In most cases, the *trans*-amide structure was favored over the *cis*-amide, although 5,5-dimethyl-α-proline[6c,d] and its oxazolidine and thiazolidine analogues[7] drove the equilibrium toward the *cis*-amide. Thus, steric and stereoelectronic factors can be used to modify the cis−trans amide isomerization to various extents, but complete control of cis−trans amide isomerization to obtain a single conformer is very difficult, even in the case of α-proline derivatives.

While control of cis−trans equilibrium of α-proline derivatives has been studied intensively, that of tertiary amide-type β-amino acids has been little explored,[9] probably because the *N*-amide linkage in a β-amino acid is distal from the intraresidual carboxylic acid functionality and direct interaction is ineffective. As a precedent of non-hydrogen-bonding β-peptides, 2,2-disubstituted-β-proline (2,2-disubstituted-pyrrolidine-4-carboxylic acid, **A**, Figure 2) oligomers have been studied,[10] and length-dependent induction of helical structures has been demonstrated. If full control of cis−trans isomerization of β-tertiary amides, that is, suppression of the contributions of interconverting rotamers, and that of torsion angles (ϕ, θ, and ψ) along the main chain (in **A**, Figure 2) could be achieved, conformationally homogeneous secondary structures would be obtained, leading to length-independent induction of ordered structures.

Recently, the synthesis and crystallographic and CD/UV spectral analyses of β-amino acid homooligomers based on bicyclic 7-azabicyclo[2.2.1]heptane have been studied (**B** and **C**, Figure 2).[11] The bicyclic skeleton was found to constrain the torsion angles (ϕ, θ, and ψ) along the main chain (**B**).[12] This rigid bicyclic system was proposed to drive the formation of CD-active ordered structures of the long oligomers. Furthermore, the present bicyclic structure and the presence of two bridgehead hydrogen atoms of the 7-azabicyclo[2.2.1]heptane amide have a great influence on amide planarity: they promote nitrogen pyramidalization and twisting of the amide linkage, while monocyclic amides such as the pyrrolidine amides take planar structures.[13] This nonplanarity stems from angle strain of the bicyclic structure and 1,3-allylic-type strain between the amide moiety and the bridgehead hydrogen atoms (**D**, Figure 2). Therefore, we expected that bridgehead substitution of the relevant bicylic skeleton could influence cis−trans isomerization of the amide group to a great extent. The 7-azabicyclo[2.2.1]heptane structure is also contained in epibatidine, a biologically active alkaloid, as the basic skeleton, and thus synthetic access to this ring system has been well investigated in the past.[14−16]

In this study, we established synthetic methods leading to conformationally constrained β-proline mimics, that is, bridgehead-substituted 7-azabicyclo[2.2.1]heptane-*2-endo*-carboxylic acid (e.g., **3** in Figure 4). We studied the robustness of secondary structures of the homooligomers and found that bridgehead substitution completely biased the cis−trans equilibrium to the *cis*-amide structure (along the main chain). Helical structures based on the *cis*-amide linkage were generated independently of the number of the residues, from the minimalist dimer through the tetramer, hexamer, and up to the octamer, and irrespective of the solvent, including water, alcohol, halogenated solvents,

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(11) Otani, Y.; Futaki, S.; Kiwada, T.; Sugiura, Y.; Muranaka, A.; Kobayashi, N.; Uchiyama, M.; Yamaguchi, K.; Ohwada, T. *Tetrahedron* **2006**, *62*, 11635–11644.

(12) Ab initio calculations of the cis- and trans-dimers of the bicyclic β-amino acid **B** ((*S*)-enantiomer) suggested that ϕ, θ, and ψ converged to−86°,−162°, and−75°, respectively, while ψ had one additional minimum at−95° within 0.1 kcal/mol difference.

(13) Otani, Y.; Nagae, O.; Naruse, Y.; Inagaki, S.; Ohno, M.; Yamaguchi, K.; Yamamoto, G.; Uchiyama, M.; Ohwada, T. *J. Am. Chem. Soc.* **2003**, *125*, 15191–15199.

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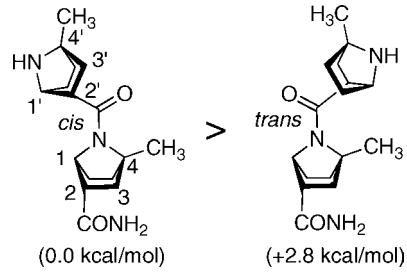


Figure 3. Energy differences between cis and trans rotamers of the methyl-substituted dimer at the MP2/6-31G*//B3LYP/6-31G* level.

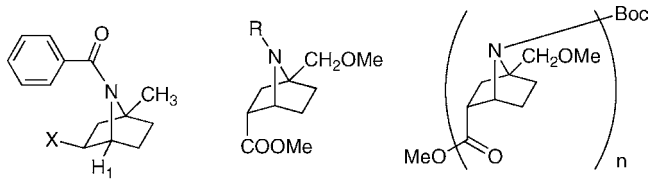


Figure 4. 7-Azabicyclo[2.2.1]heptane derivatives in this study.

and cyclohexane. We also studied the effect of transformation of the bridgehead substituents to various functionalities.

Results and Discussion

Bridgehead Methyl-Substituted Bicyclic Amide. Molecular Design and Synthesis. To evaluate the effect of the bridgehead substituent on the amide cis−trans isomerization, we carried out quantum chemical calculations for the dimer of bicyclic β-amino acid substituted with a methyl group at the bridgehead (C4) position (Figure 3). The calculations showed that the *cis*-conformer is more stable than the *trans*-conformer by 2.8 kcal/mol (MP2/6-31G*//B3LYP/6-31G*). This result suggested that the combination of the present bicyclic skeleton and the bridgehead substitution would completely tip the equilibrium toward the *cis*-amide.

To examine this predicted effect, we synthesized the nitrogen-amides of 4-methyl-7-azabicyclo[2.2.1]heptane (**1** and **2**, Figure 4) by means of a modification of the procedure employed in epibatidine synthesis by Corey et al.[15] and Fletcher et al.[16] (Scheme 1). First, a methyl group was introduced at the α position of the carboxylic acid moiety of **10** using lithium diisopropylamide (LDA) and hexamethylphosphoramide (HMPA), and then Curtius rearrangement followed by methanol treatment gave the carbamate (**12**).[17] Because a trifluoroacetyl group is reported to be the best auxiliary for diastereo-selective bromination in the next step,[18,19] **12** was treated with trimethylsilyl iodide to give the primary amine **13** quantitatively, and **13** was acylated with trifluoroacetic anhydride to give **14**. Bromination of **14** with 2 equiv of Me_3PhNBr_3 proceeded stereoselectively in a manner similar to that of the nonmethylated precursors[18] to give a single *trans*-dibromo compound **15**. Its structure was confirmed by X-ray crystal structure analysis (Figure S1). Deprotection of the trifluoroacetyl group followed by transannular nucleophilic cyclization both proceeded smoothly under weakly basic conditions (K_2CO_3, 35 °C), in a manner similar to the epibatidine case. The resultant amine (**16**) was subjected to benzoylation with benzoyl chloride to give bridgehead-substituted bicyclic amide **2**. The bromoamide **2** was also converted to the simple *N*-benzoyl amide **1** via ^{t}BuOK-mediated elimination of HBr, followed by hydrogenation of the resultant alkene **17**.

Evaluation of the Effect of the Bridgehead Methyl Substituent on Amide Cis−Trans Equilibrium. X-ray crystallographic analyses showed that benzoyl amides **1** and **2** both take *cis*-amide structures; that is, the phenyl group and the bridgehead H are on the same side with respect to the amide bond (Scheme 1, Figure 5a, and Figure S2; the torsion angle $\omega(C_1-C_2-N-C_3)$ values for **1** and **2** were −32.0° and −41.3°, respectively). The $\omega(C_1-C_2-N-C_3)$ values are close to 0°, consistent with the *cis*-conformation of the amides. However, these values apparently deviated from 0° because of nitrogen pyramidalization.

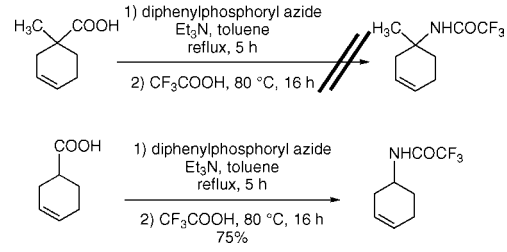
Solution structures of **1** and **2** were also elucidated by 1D-^{1}H and 2D NMR spectroscopy (Supporting Information). The 1D-^{1}H and ^{13}C NMR spectra in chloroform-d_1 and methanol-d_4 showed the presence of a single conformer, and NOESY experiments detected NOE between the phenyl protons of the benzoyl group and the bridgehead proton in both cases (Figures 5b and Figure S3 (2D NOESY spectrum in CD_3OD)). Only a *cis* conformer was detected for compound **1** in the ^{1}H NMR spectra in the range of temperature from −20 to 100 °C in 1,1,2,2-tetrachloroethane-d_2 (data not shown). These data indicated that both **1** and **2** take a single conformation with the *cis*-amide linkage in solution.

Bridgehead-Substituted Bicyclic β-Amino Acid Derivatives and Their Homooligomers. Synthesis of Bridgehead Methoxymethyl-Substituted Bicyclic β-Amino Acid. The above strategy can be applied to bridgehead-substituted bicyclic β-amino acid derivatives. First, we chose an alkoxymethyl group as a bridgehead substituent because of its synthetic compatibility with the additional carboxylic acid functionality of the β-amino acids. It turned out that this substituent increased the water solubility of the oligomers, and this feature makes it possible to conduct various spectroscopic measurements in water. Thus, compounds **3**−**9** (Figure 4) were chosen as synthetic targets.

For the preparation of the N- and C-protected β-amino acid derivative **3** (see Scheme 3), it was necessary to change the strategy used for the methyl-substituted amides **1** and **2**.[20] After several attempts, we decide to utilize the bridgehead ester-substituted bicyclic alcohol **18** (Scheme 2) as a synthetic precursor; it was synthesized according to Avenoza et al.[21] Next, the Wittig reaction (leading to **20**) was used as a key step in the introduction of a one-carbon unit at the β-position, and this

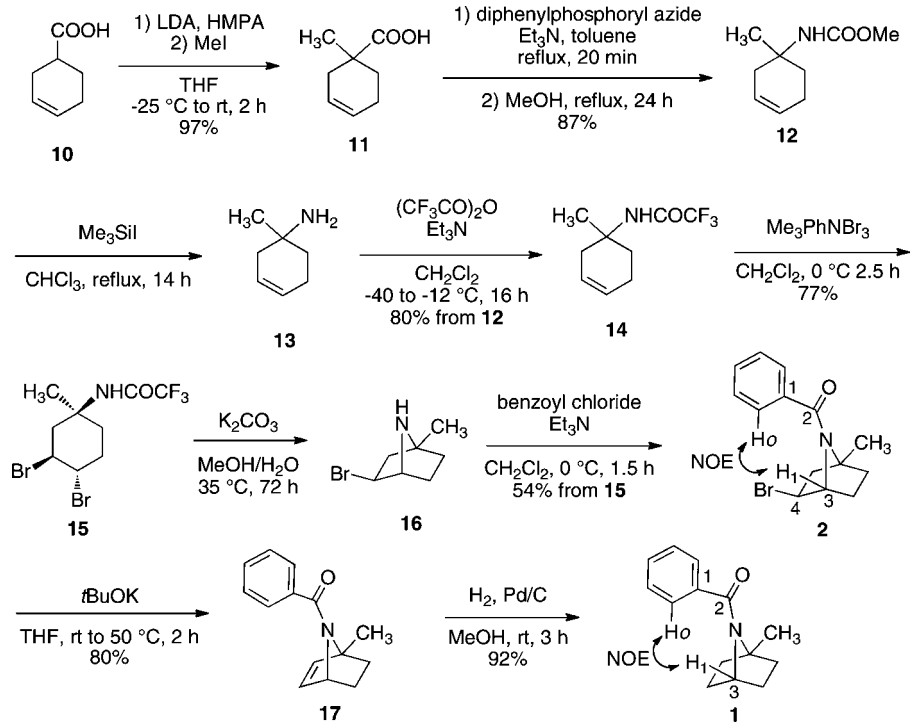
(17) Pfister, J. R.; Wymann, W. E. *Synthesis* **1983**, *1*, 38–40.
(18) Kapferer, P.; Vasella, A. *Helv. Chim. Acta* **2004**, *87*, 2764–2789.

(19) Direct trapping of the acyl azide intermediate with trifluoroacetic acid instead of methanol during the Curtius rearrangement was attempted, but was unsuccessful with substrate **9** because of steric hindrance of the methyl group (see below and ref 17).



(20) Attempts at nucleophilic or electrophilic substitution of the bromo group of **2** with carboxylic acid equivalents were unsuccessful.

Scheme 1. Synthesis of Methyl-Substituted 7-Azabicyclo[2.2.1]heptane Amide[a]



[a] THF = tetrahydrofuran.

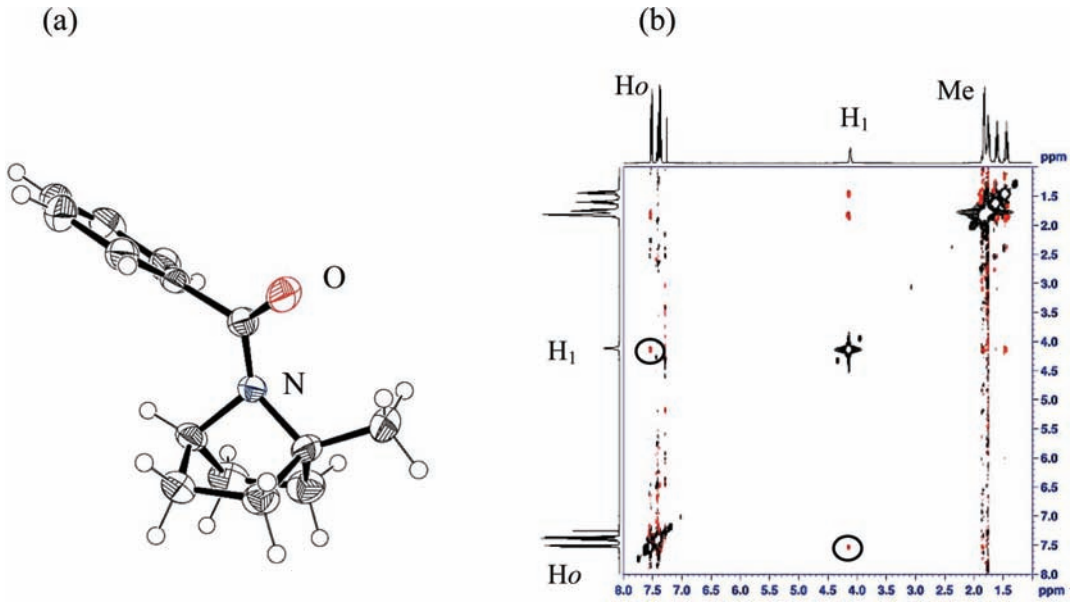


Figure 5. (a) X-ray crystallographic structure of **1**. (b) Diagnostic NOESY peaks of **1** measured in chloroform-*d*.

in turn was converted to a carboxylic acid functionality (Scheme 2 and see below).

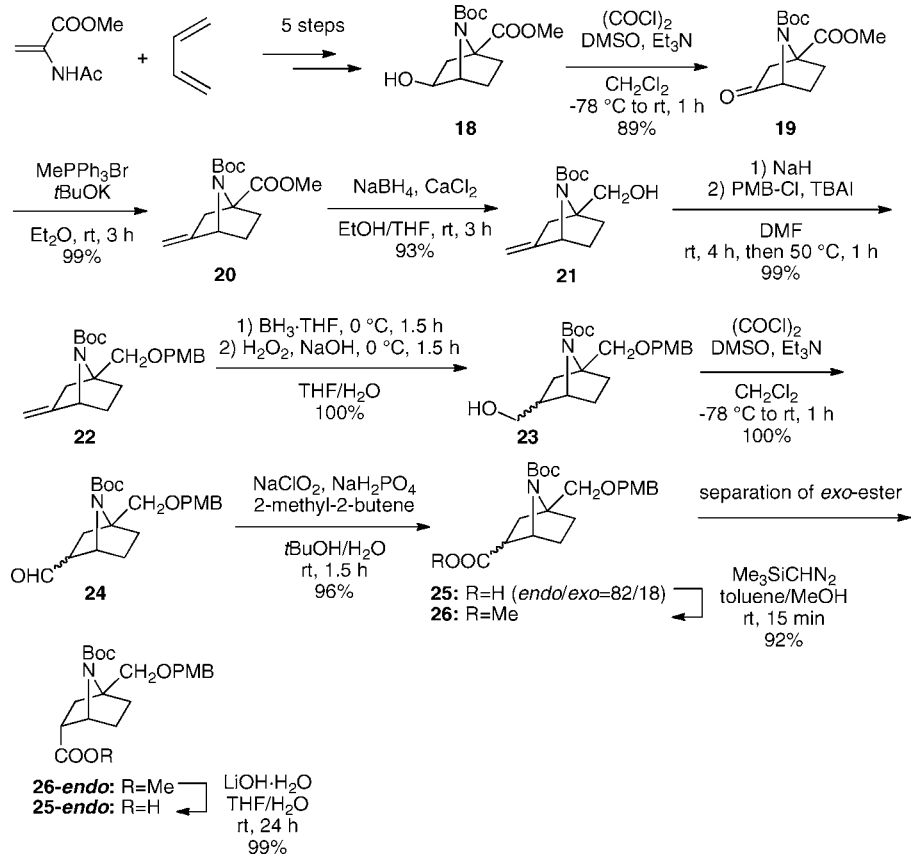
Ketone **19** was obtained from alcohol **18** by Swern oxidation, and then **19** was converted to olefin **20** via the Wittig reaction. The bridgehead ester was reduced to alcohol **21** by using a combination of $NaBH_4$ and $CaCl_2$,[22] and the alcohol group was protected with a *p*-methoxybenzyl (PMB) group to give **22**.[23] The exocyclic methylene group of **22** was converted to alcohol **23** quantitatively by hydroboration with BH_3·THF (as a mixture of endo/exo isomers (82:18)), and then **23** was subjected to Swern oxidation and subsequent Pinnick oxidation to give the β-carboxylic acid **25**. The ratio of the endo/exo isomers with

(21) Avenoza, A.; Busto, J. H.; Peregrina, J. M. *Org. Lett.* **2007**, *9*, 1235–1238.

(22) Avenoza, A.; Barriobero, J. I.; Busto, J. H.; Cativiela, C.; Peregrina, J. M. *Tetrahedron: Asymmetry* **2002**, *13*, 625–632.

(23) When we tried other protecting groups such as a MOM and a silyl ether group, the yields of the coupling reactions with camphorsultam were rather low because of the formation of undefined byproducts. In the case of benzyl protection, the coupling yield was moderate, but the subsequent deprotection of the benzyl group by catalytic hydrogenation proceeded only slowly.

Scheme 2. Synthesis of Bridgehead-Substituted Bicyclic β-Amino Acid **25**-*endo*[a]



[a] DMSO = dimethylsulfoxide, TBAI = tetrabutylammonium iodide, DMF = *N*,*N*-dimethylformamide.

respect to the β-substituent remained constant during these oxidation processes. Conversion of the resultant carboxylic acid **25** to methyl ester **26** with (trimethylsilyl)diazomethane enabled separation of the endo/exo isomers by column chromatography on silica gel. The isolated *endo*-ester **26**-*endo* was hydrolyzed to give the racemic β-amino acid **25**-*endo*. All the steps proceeded in high yields, and the present procedure is applicable at a 10 g scale. The structure of the resultant β-amino acid was confirmed by X-ray crystallographic analysis of the methoxymethyl-substituted *N*-benzoyl derivative *rac*-**4a** (Figure 6a and also Scheme 3), which was synthesized in the course of a preliminary study (see Supporting Information, Scheme S1).

To separate the *endo*-ester enantiomers, an optically active camphorsultam group was introduced into the β-amino acid **25**-*endo* as a chiral auxiliary to give **27** (Scheme 3). During optimization to identify suitable conditions for large-scale and reproducible separation, we found that the enantiomers of the deprotected hydroxymethyl-substituted derivative **28** could be separated by silica-gel column chromatography (eluent: *n*-hexane:Et_2O = 2:1).[24] Other protected hydroxymethyl-substituted derivatives were difficult to separate by column chromatography on a preparative scale.

The chiral alcohol **28** was converted to the methyl ether **29** with (trimethylsilyl)diazomethane in the presence of aqueous fluoroboric acid,[25] and hydrolysis gave the chiral β-amino acid **30**. The stereochemistry was confirmed by X-ray crystallographic analysis of the homodimer (*S*)-**5** (vide post, see Figure 6b). The values of optical rotation $[\alpha]^{24}_D$ of the *N*-benzoylmethyl ester were $-34.7°$ for (*R*)-**4a** (c = 1.56, $CHCl_3$) and $+35.3°$ for (*S*)-**4a** (c = 1.48, $CHCl_3$), respectively.[26]

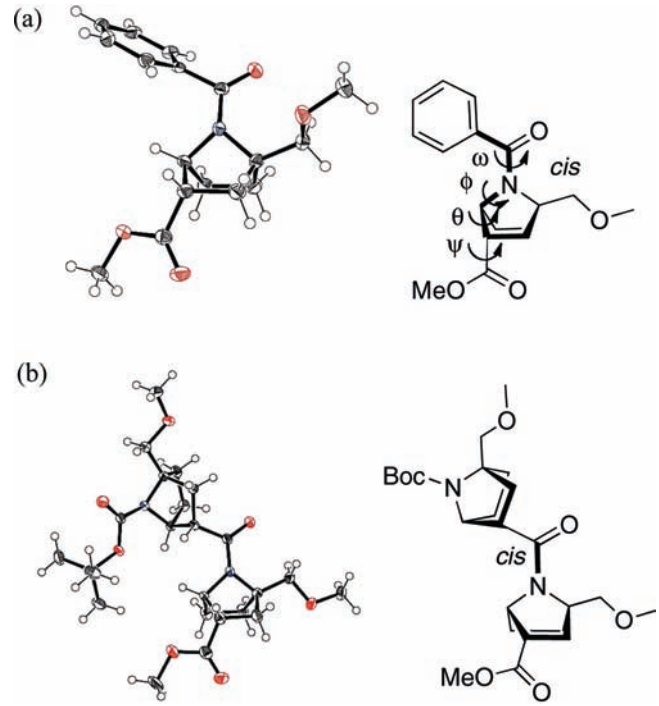
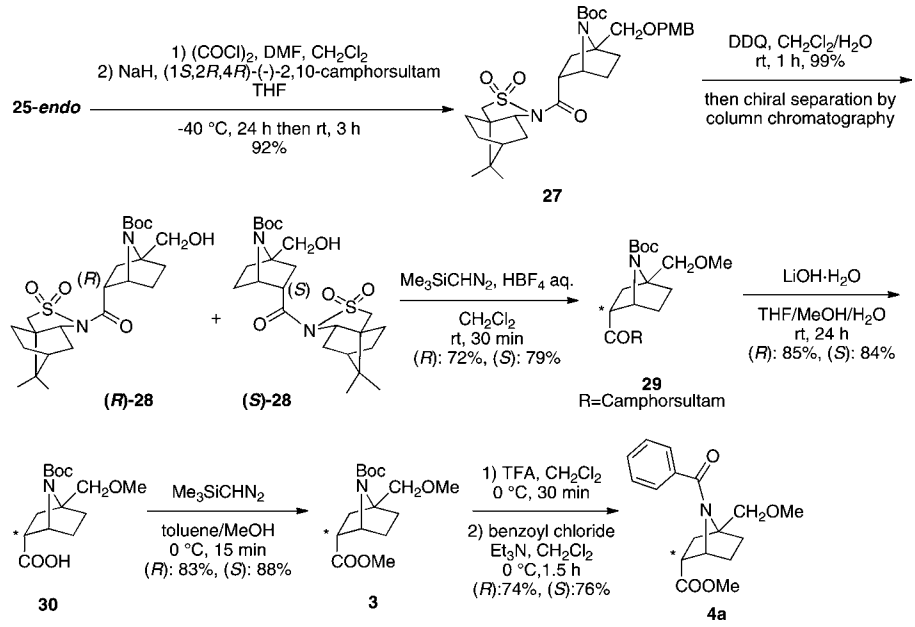


Figure 6. ORTEP drawings of the crystal structures of (a) *rac*-**4a** and (b) (*S*)-**5**.

(24) 7 g of **28** can be separated at one time by repeated column chromatography.
(25) Aoyama, T.; Shioiri, T. *Tetrahedron Lett.* **1990**, *31*, 5507–5508.

Scheme 3. Optical Resolution and Synthesis of Chiral β-Amino Acids[a]



[a] DDQ = 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, TFA = trifluoroacetic acid.

In the course of homocoupling of the β-amino acid unit (*R*)-**3** (vide infra), we unexpectedly obtained *N*-acetyl amide (*R*)-**4b** (Figure 4) during deprotection of the Boc group of (*R*)-**3** in the presence of HCl in ethyl acetate (acetic acid was present as a contaminant). The *N*-acetyl amide (*R*)-**4b** was an oily material, which showed NOE effects between the acetyl methyl protons and the bridgehead proton in the 2D-NOESY spectrum in methanol-d_4, indicating the presence of *cis*-amide structure in solution (Figure S4). Only a single conformer was detected in methanol-d_4, and the spectrum did not change even at 50 °C. These findings show that even the sterically small *N*-acetyl group on the nitrogen atom generates the *cis*-amide structure in the presence of the bridgehead substituent.

Next, homooligomers, the dimer (*R*)-**5**, the trimer (*R*)-**6**, the tetramer (*R*)-**7**, the hexamer (*R*)-**8**, and the octamer (*R*)-**9** were synthesized by means of normal solution-phase peptide coupling procedures using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and (4-dimethylamino)pyridine (DMAP) (Scheme 4). The hexamer (*R*)-**8** was synthesized by amide coupling of the amine of the dimer (*R*)-**5** and the carboxylic acid of the tetramer (*R*)-**7**. The octamer (*R*)-**9** was synthesized by the coupling of the amine of the hexamer (*R*)-**8** and the carboxylic acid of the dimer (*R*)-**5**. The dimer composed of the (*S*)-enantiomer of the β-amino acid ((*S*)-**5**) was synthesized in a manner similar to that of (*R*)-**5** (see Supporting Information).

Structural Analysis of Monomeric and Oligomeric β-Amino Acid. Crystallographic Analyses and NMR Spectroscopy. Single-crystal structure analyses of the racemic *N*-benzoyl monomer (*rac*-**4a**) and the dimeric β-amino acid ((*S*)-**5**) were successfully conducted (Figure 6). The observed main-chain torsional angles of these molecules are summarized in Table 1. Both *rac*-**4a** ($\omega = -38.2°$) and (*S*)-**5** ($\omega = -24.8°$ and 13.4°) take *cis*-amide structures. The torsion angle θ was well converged to constant absolute value, because of the rigid bicyclic skeleton, while the angles ω and ϕ vary to some extent depending upon the degree of nitrogen pyramidalization.[27] The free-rotating torsion angle ψ was −87.5° in the case of (*S*)-**5**, which was consistent with the calculated value of the dimer of the bridgehead-unsubstituted case (**C**).[12]

1D-^{1}H, 1D-^{13}C, and 2D-NMR (COSY, HOHAHA, and NOESY) analyses were conducted in chloroform-d_1 and methanol-d_4 for the dimer (*R*)-**5**, the trimer (*R*)-**6**, and the tetramer (*R*)-**7** of the unit β-amino acid (see Supporting Information). Diagnostic interresidual NOE effects between the β_i proton and the bridgehead (α_{i+1}) proton were observed in the 2D-NOESY spectra (Figure 7 and Supporting Information). All the data indicated the presence of a single conformer having *cis*-amide linkages. The NMR spectra of the trimer (*R*)-**6** were also measured in various solvents: methylene chloride-d_2, methanol-d_4, acetone-d_6, and D_2O (Figure S5). Only a single conformer was present in all of these solvents. In the range of the temperature from 20 to 50 °C, there was no significant change of the shapes and chemical shifts of the peaks, in either chloroform-d_1 or methanol-d_4 (Supporting Information).

The 2D-NOESY NMR spectra of the trimer (*R*)-**6** measured in D_2O showed correlation patterns similar to those observed in methanol-d_4 (Figure S6).

The present results clearly indicated that the short β-peptide oligomers ((*R*)-**5**, (*R*)-**6**, and (*R*)-**7**) based on the bridgehead-substituted bicyclic structure adopted distinct folding structures and showed highly homogeneous conformations in a range of solvents from water to chloroform. The combination of the present bicyclic skeleton and unsymmetrical bridgehead sub-

(26) The chiralities of the monomers (*R*)- and (*S*)-**3** or their homooligomers were consistent with those of the bridgehead unsubstitued compounds (ref 11) in terms of the sign of the optical rotations and CD absorptions. The characteristic deshielding ^{1}H-NMR chemical shift of the bridgehead (H_1) proton of the camphorsultam derivative, (*R*)-**28** (multiplet, 4.77−4.48 ppm), as compared to that of (*S*)-**28** (multiplet, 4.72−4.70 ppm), was also consistent with the case of the corresponding bridgehead unsubstituted compounds ((*R*)-camphorsultam amide, multiplet, 4.71−4.69 ppm; and (*S*)-camphorsultam amide, multiplet, 4.66−4.64 ppm, respectively (see ref 11)).

(27) The tilt angles of nitrogen pyramidalization of amide bonds for **1**, **2**, *rac*-**4a**, (*S*)-**5**, **45**, and **47** are 27.4°, 29.4°, 28.5°, 10.3°, 23.2°, and 40.7°, respectively.

Scheme 4. Synthesis of the Homooligomers (*R*)-**5**, (*R*)-**6**, (*R*)-**7**, (*R*)-**8**, and (*R*)-**9**

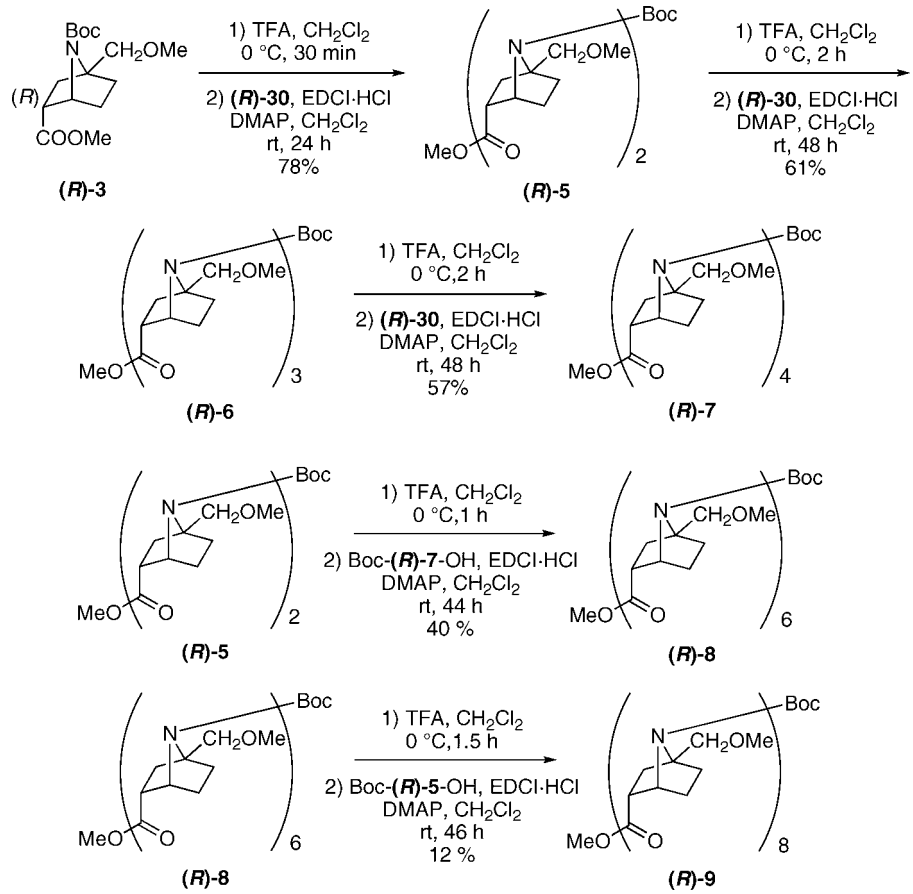


Table 1. Main-Chain Torsional Angles in the Crystal Structure[a]

compound	residue[b]	ω (deg)[c]	ϕ (deg)	θ (deg)	ψ (deg)
1	1	−32.0			
2[d]	1	−41.3			
rac-**4a**[e]	1	−38.2	157.0	162.0	(49.1)[f]
(*S*)-**5**	1	−24.8	(−80.3)[g]	−169.5	−87.5
	2	13.4	−136.5	−158.4	(−55.2)[f]
45[e]	1	24.9	99.7	152.9	(65.3)[f]
47[e]	1	−51.8	167.9	165.9	(38.8)[f]

[a] See Figure 6a for the definition of the torsion angles. [b] Numbering from the N-terminal. [c] In the case of compounds **1** and **2**, ω means the torsion angle ($C_1-C_2-N-C_3$) (Scheme 1). [d] The values of the enantiomer of (*S*)-configuration with respect to the C_4 atom are shown. [e] The values of the (*R*)-enantiomer are shown. [f] The torsion angle concerns the C-terminal ester. [g] The torsion angle concerns the N-terminal *N*-Boc bond.

stitution thus permits unprecedented full control of selectivity in favor of the *cis*-amide.

Circular Dichroism. The CD spectra of the dimer (*R*)-**5**, the trimer (*R*)-**6**, and the tetramer (*R*)-**7** at 100 μM, the hexamer (*R*)-**8** at 45 μM, and the octamer (*R*)-**9** at 32 μM in water at 20 °C are shown in Figure 8a. Intensities of the spectra were normalized in terms of concentration and the number of residues. The spectra showed strong signals at 190−195 nm and at around 215−223 nm, and, surprisingly, the intensities of the signals per residue ([θ] (deg cm^2 dmol^{-1} residue^{-1})) were almost the same throughout the range of oligomers: $[\theta]_{214} = -2.9 \times 10^4$ for (*R*)-**5**, $[\theta]_{215} = -2.9 \times 10^4$ for (*R*)-**6**, $[\theta]_{218} = -2.7 \times 10^4$ for (*R*)-**7**, $[\theta]_{222} = -2.4 \times 10^4$ for (*R*)-**8**, and $[\theta]_{223} = -2.4 \times 10^4$ for (*R*)-**9**. This is in sharp contrast to the oligomers of bridgehead-unsubstituted bicyclic amino acid (**C**, Figure 2)[11] or β-proline derivatives (**A** in Figure 2),[10] which showed length-dependent increase of the CD intensities (per residue). Because a mixture of *cis*- and *trans*-conformers was observed in the NMR spectra of the bridgehead-unsubstituted dimer, it was suggested that the ratio of *cis*-amide conformer increased as the chain length increased in the case of the bridgehead-unsubstituted oligomers.[11] The dimer of the *S*-enantiomer ((*S*)-**5**) showed a symmetric spectrum with respect to that of (*R*)-**5** in terms of the Cotton effects (Figure S7). Furthermore, the CD spectrum of the tetramer (*R*)-**7** in water did not change significantly as the temperature was raised to 80 °C (Figure S8).

Further, the CD spectra of (*R*)-**7** in water in the concentration range of 25 μM to 1 mM were almost the same (Figure S9a), and the CD spectra of (*R*)-**9** in water in the concentration range of 8−32 μM were also almost the same (Figure S9b), which indicates that no aggregation occurred. The methoxymethyl groups, which protrude outside the helix (see Figure 9), probably contributed to the increased solubility in water. We also measured the CD spectra of the tetramer (*R*)-**7** in other solvents: methanol, 2,2,2-trifluoroethanol (TFE), and cyclohexane (c-Hex) (Figure 8b). While (*R*)-**7** in water solution showed about 20% enhanced CD intensities as compared to the methanol solution, the CD spectra in all the solvents had similar peak shapes and intensities, indicating that the structure of (*R*)-**7** in these solvents is essentially conserved.

These results of the CD spectral studies clearly suggest that the oligomers **5**, **6**, **7**, **8**, and **9** take highly homogeneous and robust ordered structures, probably *cis*-helical structures based on the *cis*-amide linkage. The calculated CD spectrum of the dimer (*R*)-**5** at the TD-DFT level (TDDFT/B3LYP/aug-cc-

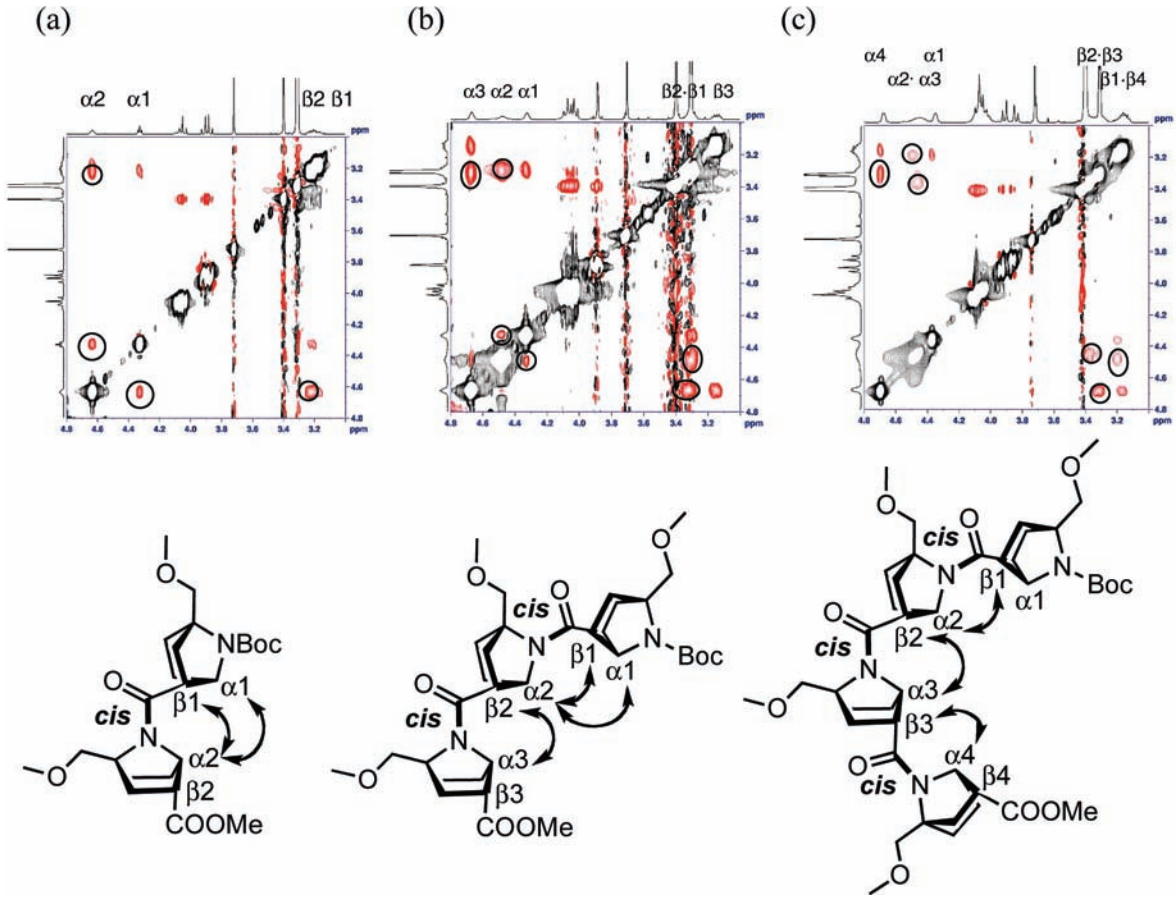


Figure 7. Diagnostic interresidue NOE signals (open circles) of (a) (*R*)-**5**, (b) (*R*)-**6**, and (c) (*R*)-**7** in CD_3OD at 25 °C.

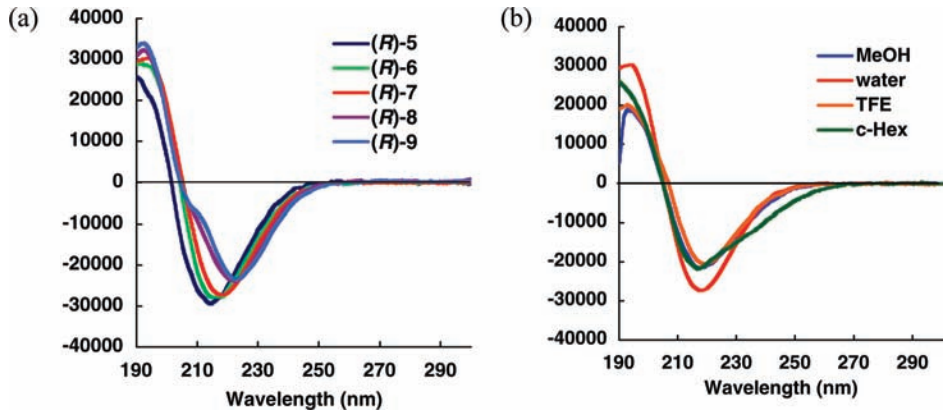


Figure 8. CD spectra of (a) (*R*)-**5**, (*R*)-**6**, and (*R*)-**7** at 100 μM, (*R*)-**8** at 45 μM, and (*R*)-**9** at 32 μM in water at 20 °C and (b) CD spectra of (*R*)-**7** at 100 μM in methanol, water, TFE, and cyclohexane (c-Hex) at 20 °C. The molar ellipticity [θ] values were normalized in terms of the concentration and the number of residues.

pVDZ//B3LYP/6-31G*)[28] showed that the *cis*-amide structure of (*R*)-**5**, rather than *trans*-amide structure, is consistent with the experimental CD spectra (Figure S10). This and the aforementioned X-ray and NMR results support the above interpretation of the CD spectra.

The computational conformational search of the *N*-formyl-(*S*)-octamer revealed that the *cis*-helical structure is the lowest-energy structure (Figure 9), and the torsion angles of the eight residues ($\omega = -23.6°$ to $-16.9°$, $\phi = -103.3°$ to $-97.5°$, $\theta = -160.6°$ to $-158.6°$, and $\psi = -83.6°$ to $-79.4°$) are similar in magnitude to those found in the crystal structures of **4a** and (*S*)-**5** (Table 1). It is anticipated from this model structure that the bridgehead substituents protrude outside the helix, and they are expected to be useful for functionalization of the helix surface.

Transformation of Bridgehead Substituents. Bicyclic ester derivative **18** could be transformed to compounds substituted with ethyl (**35**) and *i*-propyl (**39**) groups at the bridgehead position (Scheme 5, (1) and (2)). The hydroxyl group of **18** was protected with a *tert*-butyldimethylsilyl (TBS) group, and then the bridgehead ester of **31** was reduced with $NaBH_4$ and $CaCl_2$[22] to give **32**. The bridgehead hydroxymethyl group of

(28) Frisch, M. J.; et al. *Gaussian 03*; Gaussian, Inc.: Pittsburgh, PA, 2003.

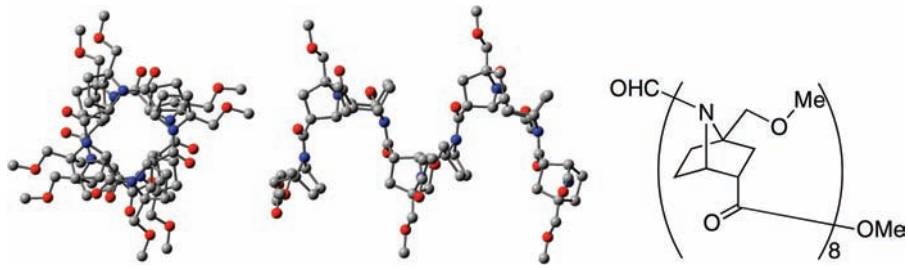
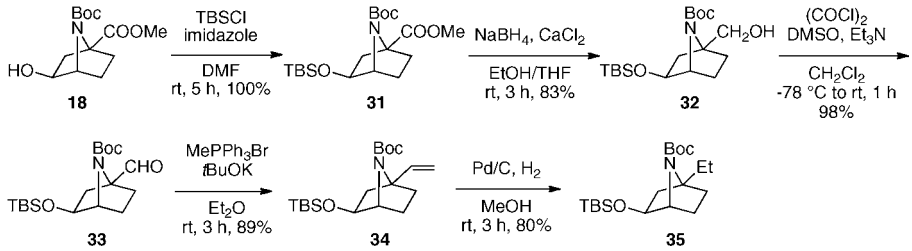


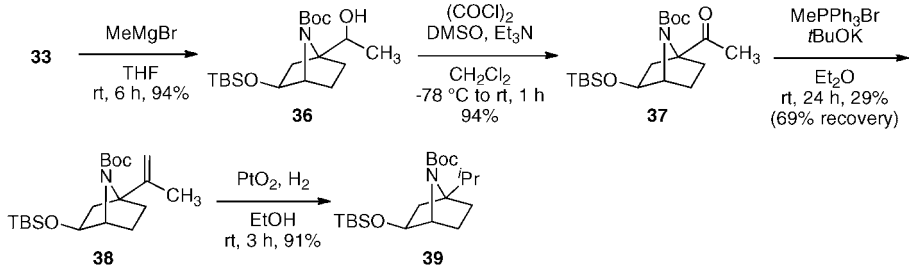
Figure 9. Top view (left) and side view (middle) of the energy-minimum structure of the octamer (right) obtained by Monte Carlo conformation search followed by DFT-optimization (B3LYP/6-31G*). Hydrogen atoms are omitted for clarity.

Scheme 5. Synthesis of Various Bridgehead-Substituted Bicyclic Amine Derivatives

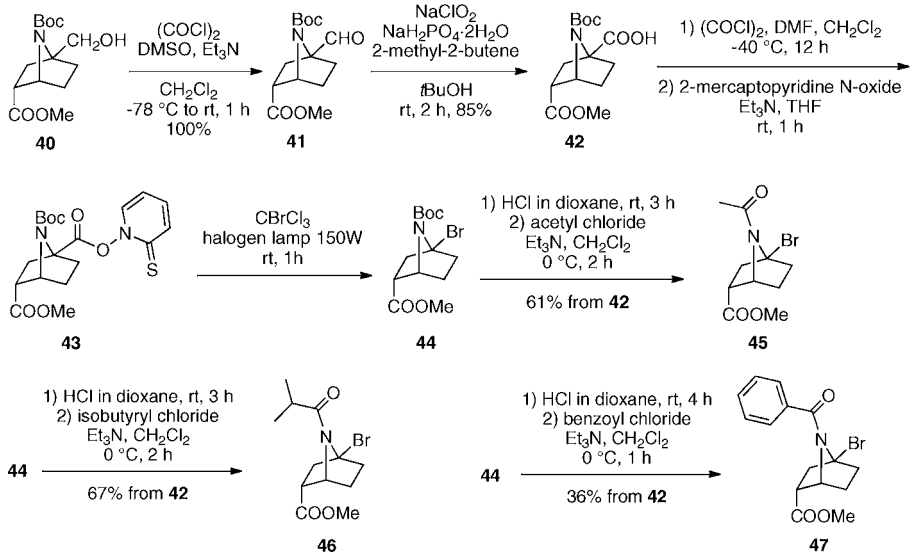
(1) Bridgehead Ethyl Substitution



(2) Bridgehead Isopropyl Substitution



(3) Bridgehead Bromo Substitution



32 was oxidized by Swern oxidation to give aldehyde **33**, whose aldehyde group was converted to alkene via a Wittig reaction to give **34**. The ethyl-substituted bicyclic compound **35** was obtained by hydrogenation of **34**. For the preparation of the isopropyl-substituted compound, an additional methyl group was introduced into aldehyde **33** with a methyl Grignard reagent to give the secondary alcohol **36**, which was oxidized to give ketone **37**. While the following Wittig reaction proceeded slowly, probably due to steric hindrance near the bridgehead position, the resultant alkene **38** was hydrogenated over PtO_2

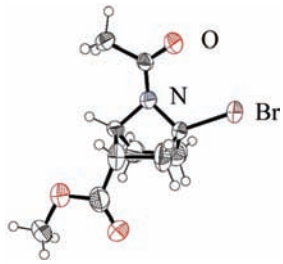


Figure 10. ORTEP drawing of the crystal structure of bridgehead Br-substituted *N*-acetylamide **45**.

to give the isopropyl-substituted bicyclic compound **39** in high yield. These *N*-Boc-protected alkyl-substituted bicyclic compounds (**35** and **39**) can be easily converted to β-amino acid derivatives by means of the procedure described in this work.

The bromo-substituted compounds **45**, **46**, and **47** (all racemic) were synthesized by a modification of the procedure of Avenoza et al. via bridgehead radical reaction (Scheme 5, (3)):[29] the bridgehead alcohol of **40** was oxidized to the carboxylic acid **42**, and then a mixture of the Barton ester **43** and bromotrichloromethane was irradiated with a 150 W halogen lamp to give the bromo compound **44** in a good yield, and this was used for the next step without further purification. Compound **44** can be derived to *N*-acetamide **45**, *N*-isopropyl amide **46**, and *N*-benzoyl amide **47** in good yields. ^{1}H NMR studies, including the NOE experiments, showed that all of these bromo-substituted amides (**45**−**47**) were present as a single conformer and took *cis*-amide conformations (Supporting Information). In the range of temperature from 20 to 50 °C in methanol-d_4, no new peaks appeared in the ^{1}H NMR spectrum of *N*-isopropyl amide **46**, excluding apparent equilibrium between the amide rotamers. The *cis*-amide structures of the *N*-acetyl amide **45** and the *N*-benzoyl amide **47** were also supported by crystallographic analysis (Figure 10 and Figure S11). Therefore, these bridgehead substituents also strongly bias the amide cis−trans isomerization of the present bicyclic β-amino acid derivatives to the *cis*-amide structure.

(29) Avenoza, A.; Busto, J. H.; Cativiela, C.; Peregrina, J. M. *Tetrahedron* **2002**, *58*, 1193–1197.

Conclusion

Herein, we described the synthesis of β-amino acids bearing the 7-azabicyclo[2.2.1]heptane structure substituted with a functional group at the bridgehead position. We showed that homooligomers of β-amino acid bearing a bridgehead methoxymethyl group generated robust secondary structures without hydrogen bonds. Crystallographic and NMR-NOE analysis showed that these homooligomers had strong predominance of the *cis*-amide structure, and they adopted helix-type folded structures in the solid state and in solution. Furthermore, the CD and NMR spectra indicated that these secondary structures were retained in water solution even in the case of homooligomer as short as the dimer. These results indicate that the oligomers can be utilized as robust minimalist elements of ordered secondary structures, both in hydrophobic and in hydrophilic environments. Furthermore, on the basis of the shape and sign of the CD spectra, the CD-active structures of the homooligomers of bridghead-unsubstituted 7-azabicyclo[2.2.1]heptane-*2*-*endo*-carboxylic acid (**C** in Figure 2)[11] can be concluded to be helix structures involving the *cis*-amide linkage. The present robust helical oligomers should therefore be available for the creation of novel helix-mimicking compounds with a range of potential biochemical applications.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science. We thank Mr. Hiroaki Kurouchi and Ms. Fumika Karaki of our group for help in the synthesis of (*R*)-**8** and (*R*)-**9**. Some of the calculations were carried out using Research Center for Computational Science, Okazaki, Japan. We thank the computational facility for generous allotments of computer time.

Supporting Information Available: Supporting scheme and figures. Experimental section including the details of synthesis, NMR spectral data, CD and UV spectra, X-ray crystallographic data, and the details of calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

JA1017877