Design, synthesis, and structural characterization of helix-forming aliphatic homo-δ-peptides based on conformational restriction due to the structural characteristics of cyclopropane

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ABSTRACT: Considerable effort has been directed toward developing artificial peptide-based oligomers that fold into a specific secondary structure, i.e., peptide foldamers. To date, however, detailed structural analysis of crystals of δ-peptide foldamers consisting of aliphatic δ-amino acids, which have a more extended carbon backbone compared with well-studied β- and γ-amino acids, have not been reported. We rationally designed aliphatic homo-δ-peptide foldamers forming a stable helical structure utilizing a chiral cyclopropane δ-amino acid as a monomer unit whose conformation was tightly restricted by the structural characteristics of cyclopropane depending on its stereochemistry. We stereoselectively synthesized the cyclopropane δ-amino acid monomer and prepared its various homo-oligomers. Structural analysis of the homo-δ-peptides using nuclear magnetic resonance, circular dichroism, and infrared spectroscopy revealed that they form a stable 14-helical structure in solution. Furthermore, the effective conformational regulation of the backbone due to the characteristics of cyclopropane allowed us to achieve X-ray crystallographic analysis of the homo-δ-peptides, showing their common right-handed 14-helical structures. The helical structures were consistent with both those predicted by theoretical calculations and those obtained based on nuclear magnetic resonance spectroscopy in solution. A critical point is that the helical structures of these δ-peptides are theoretically predictable by calculations. To our knowledge, this is the first example of aliphatic homo-δ-peptide foldamers forming a stable helical structure both in solution and in crystal.

INTRODUCTION

Homo-oligomers of α-amino acids, i.e., α-peptides, which constitute proteins, that form into ordered secondary structures, such as α-helixes, β-sheets, and β-turns, have essential biologic functions. Many artificial oligomers, called foldamers,[1-2] that self-organize into a specific secondary structure usually mimicking or expanding natural secondary structures of α-peptides, have been developed over the last several decades.[3-8] Peptide foldamers have a wide range of applications.[9-13] Significant studies of foldamers comprising homo-oligomers of β- and γ-amino acids (β- and γ-peptides) with a structurally restricted backbone triggered the recent intense interest in foldamer research.[14-19]

Most of β- and γ-peptide foldamers have a non-aromatic, i.e., aliphatic, backbone. In contrast, representative foldamers based on homo-oligomers of δ-amino acids (δ-peptides) are aromatic oligoamides as shown in Figure 1a. Quinoline oligomers (**1**)[20] adopting a stable helical structure have been extensively investigated and applied to various studies,[21-22] including ribosomal peptide synthesis and B-DNA mimics.[23-24] Pentamers of 2-aminophenoxyacetic acid (**2**)[25] forming a helical structure in crystal and tris-pyridine-based oligoamides (**3**)[26-28] fixed in a plane structure mimicking an α-helix have also been reported as other aromatic δ-peptide foldamers.

Although reported examples of aliphatic δ-peptide foldamers (Figure 1b) are rather limited, homo-oligomers of oxetane- (**4**) and furanose-based δ-sugar amino acids (δ-SAAs, **5**) have been extensively studied by Fleet's and Chakraborty's groups: nuclear magnetic resonance (NMR) spectroscopy analysis suggested that these δ-peptides adopt a helix or β-turn-like structure in solution.[29-32] To the best of our knowledge, however, other than δ-SAA oligomers, only two foldamer studies based on aliphatic homo-δ-peptides have been reported. Cyclohexylether-δ-peptides (**6**) were predicted to form a secondary structure in solution based on their circular dichroism (CD) spectra, although the detailed structure was not confirmed.[33] Oligomers of L-ornithine with an aromatic ring as a side chain (**7**) were suggested by their NMR analysis to be a stable zipper-featured structure in solution due to the charge-transfer interaction between the alternately-positioned electron-deficient and electron-rich aromatic side chains on the α-amino group.[34]

The backbone length of δ-amino acids (NH–C–C–C–C–CO) is analogous to that of a dipeptide unit of natural α-amino acids (NH–C–CO–NH–C–CO), and thus δ-peptides with a stable secondary structure are potentially effective mimetics of functional α-peptides. Significantly fewer aliphatic δ-peptide foldamers have been reported to date, however, compared with aliphatic β- and γ-peptide foldamers, and no crystal structures of aliphatic δ-peptide foldamers are described. Compared with β- and γ-amino acids, aliphatic δ-amino acids have more

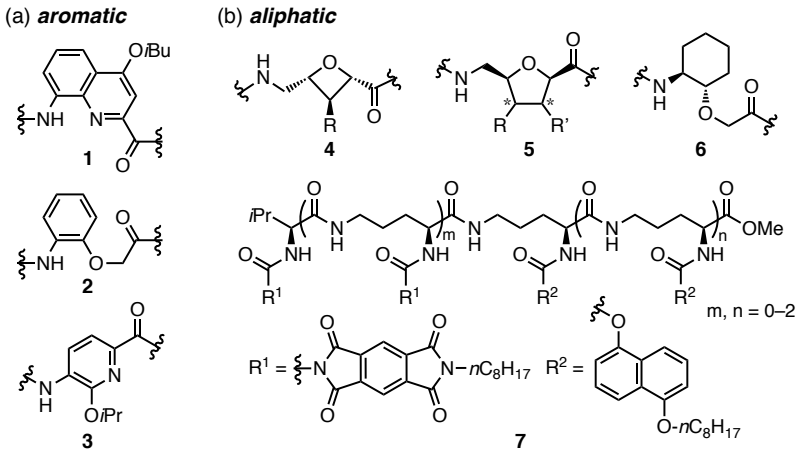


Figure 1. Structures of the units of representative (a) aromatic and (b) aliphatic homo-δ-peptide foldamers **1**–**7**.

rotatable bonds. Thus, its backbone structures are relatively flexible, making it challenging to obtain aliphatic δ-peptides with a stable secondary structure, such as foldamers.

In the present study, we have rationally designed aliphatic homo-δ-peptide foldamers that form into a stable helical structure utilizing cyclopropane δ-amino acid as a monomer unit that was conformationally restricted due to the structural characteristics of cyclopropane. We stereoselectively synthesized a chiral cyclopropane δ-amino acid containing four asymmetric carbon centers and prepared its tetramers, hexamers, and octamers. Structural analysis based on CD, infrared (IR), and NMR spectra revealed that the homo-oligomers have a stable 14-helical structure in solution. The effective conformational regulation of the backbone due to cyclopropane allowed us to achieve X-ray crystallographic analysis of the oligomers, showing their right-handed 14-helical structures. The 14-helix secondary structures were analogous in solution and in crystal, and consistent with that obtained by rational design using calculations. This is the first example of aliphatic δ-peptide foldamers forming a stable helical structure both in solution and in crystal.

RESULTS AND DISCUSSION

Rational Design. We have been developing small bioactive molecules whose conformation is strictly regulated by the structural characteristics of cyclopropane.[35-42] Cyclopropane can restrict the conformation of compounds to a *cis*- or *trans*-form (*cis*/*trans* restriction) and is less likely to cause steric hindrance when the compound binds to the target protein due to its minimal ring structure. Furthermore, unlike four- or more-membered cycloalkanes, cyclopropane has no ring flip. The *cis*-oriented substituents on cyclopropane are fixed in an eclipsed conformation, and a robust steric repulsion — "cyclopropylic strain"— occurs between the substituents (Figure 2a).[43] This steric effect limits the C–C bond rotation between the cyclopropane (C1) and adjacent carbon (C1') so that the smallest substituent on the C1' orients toward the cyclopropane side. Cyclopropylic strain is analogous to 1,3-allylic strain, but it can more effectively restrict C1–C1' bond rotation than 1,3-allylic strain due to the steric repulsion by the two substituents (or protons) on the C2 and C3 of cyclopropane. We previously confirmed by NMR and X-ray crystallographic analysis that the cyclopropylic strain actually restricts the conformation of various cyclopropane compounds.[37,44-45] By tak-

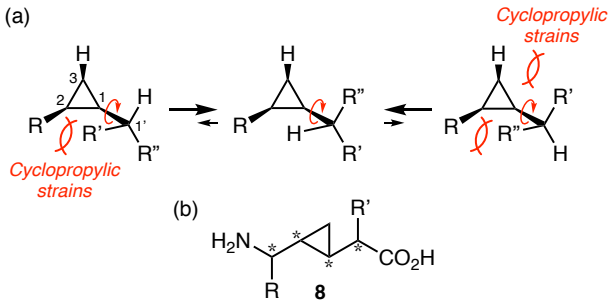


Figure 2. (a) Conformational restriction by steric effects due to the structural characteristics of cyclopropane, i.e. cyclopropylic strain. (b) General structure of the disubstituted cyclopropane δ-amino acid (**8**).

ing advantage of these structural characteristics of cyclopropane, we designed cyclopropane δ-amino acids (**8**, Figure 2b) having two asymmetric carbons adjacent to cyclopropane, i.e., 5-amino-3,4-methano-2,5-disubstituted pentanoic acids, which comprise 16 stereoisomers (8 diastereomers and their corresponding 8 enantiomers) due to the 4 asymmetric centers, as conformation restricting units for regulating the three-dimensional (3D) structure of peptides. Because the backbone (N–Cδ–Cγ–Cβ–Cα–CO) conformation of the cyclopropane δ-amino acids is tightly restricted due to the structural characteristics of cyclopropane, their most stable conformations differ from each other depending on the stereochemistry, as shown in Figure 3: the orientation of the Cδ–Cγ–Cβ–Cα backbone is regulated to a *cis*- or *trans*-conformation depending on the Cβ- and Cγ-configuration on the cyclopropane ring, and the orientations of the N–Cδ and Cα–CO bonds relative to the Cδ–Cγ–Cβ–Cα backbone are regulated to a "folded" or "extended" conformation by the cyclopropylic strain due to the Cδ- and Cα-configurations, respectively. We thus considered that incorporating each of the cyclopropane δ-amino acids into a peptide would regulate the 3D structure of the peptide differentially depending on the rigid conformation of the cyclopropane δ-amino acid. We previously synthesized cyclic hexapeptides comprising five natural α-amino acids and one of the conformationally restricted cyclopropane δ-amino acids, which regulated the overall 3D structures of the peptides depending on the conformation of the incorporated δ-amino acid, and not

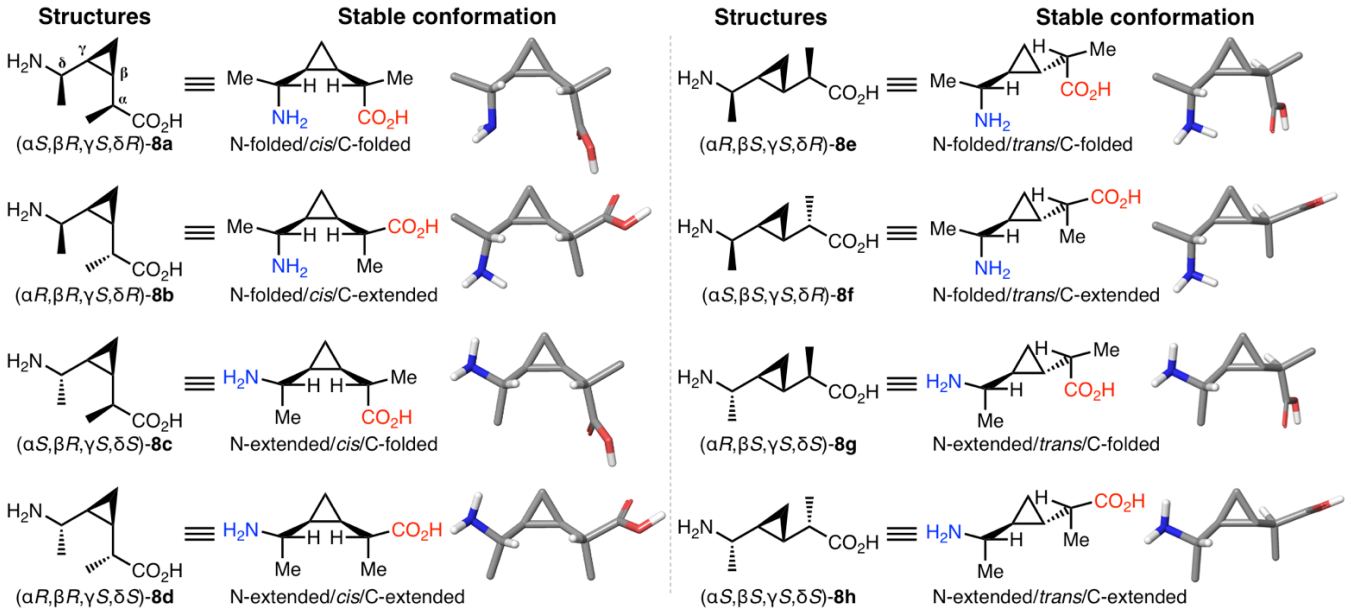


Figure 3. Chemical structures of the eight diastereomers (**8a**–**h**) of cyclopropane δ-amino acid 8 with methyl substituents at both the α- and δ-positions and their most stable conformations, calculated by a Monte Carlo conformational search using MacroModel 10.9 (Schrödinger, Inc.) with MMFFs as a force field and H_2O as a solvent condition. Hydrogens except for NH, CO_2H, and the α- and δ-positions were omitted for clarity in the calculated models. 'N-folded' and 'N-extended' mean that the N–Cδ bond adopts the "folded" and "extended" conformation, respectively. 'C-folded' and 'C-extended' mean that the Cα–CO bond adopts the "folded" and "extended" conformation, respectively. In the calculations of each diastereomer, all obtained conformations within 2.7 kcal/mol from the global minimum were similar to the most stable conformation, in which the N- and C-terminal backbone orientations (folded or extended) were restricted by the cyclopropylic strain depending on the configurations at the α- and δ-positions, respectively, as shown in this figure.

on the sequence.[46] Because passive membrane transportation of cyclic peptides is related to the 3D structure of peptides,[47] the membrane permeability of the cyclic peptides was effectively tuned depending on the cyclopropane δ-amino acid type. As a result, a dramatically highly membrane-permeable cyclic peptide with a particular restricted3D structure was successfully identified. On the basis of these results, we considered that regulation of the 3D peptide-structure by utilizing the cyclopropane δ-amino acids as irreplaceable key units might allow us to develop new aliphatic homo-δ-peptide foldamers.

Hofmann and co-workers investigated the secondary structures of a hexamer of unsubstituted linear δ-amino acids **9** (Figure 4a) by theoretical calculations in detail and predicted that the δ-hexapeptide potentially forms six different types of helixes (8-, 10-, 14-, 16-, 20-, and 22-helix) stabilized by intramolecular hydrogen bonds.[48] They also showed backbone torsion angles of the peptides for each type of the hydrogen-bonded pseudocycles in these six potential helixes, which provides beneficial information for rationally designing stable helixes of the δ-hexapeptide. Thus, we calculated the most stable conformation for each of the eight diastereomers **8a–h** of cyclopropane δ-amino acids, as shown in Figure 3, and obtained the backbone torsion angles in each calculated stable conformation. Among the diastereomers, (α*R*,β*S*,γ*S*,δ*R*)-isomer **8e** with an N-folded/trans/C-folded-stable conformation has backbone torsion angles θ (–86.0°), ζ (139.7°), and ρ (–89.0°), which correspond to *gauche*$^-$, *anticlinal*$^+$, and *gauche*$^-$, respectively (Figure 4 and Table 1). The ζ fixes at 139.7° (*anticlinal*$^+$) due to the *trans*-configuration in the rigid cyclopropane ring. Both θ and ρ are very stable at around –88° (*gauche*$^-$) because the cyclopropylic strain restricts rotation around the Cγ–Cδ and Cα–Cβ bonds, which are clearly shown in the Ramachandran plot of these angles (Figure S1). We noticed that these angles of **8e** are similar to angles θ (*gauche*$^-$), ζ (*trans*), and ρ (*gauche*$^-$), respectively, of the 14-helical structure of δ-hexapeptide **9** calculated by Hofmann (Table 1).[48]

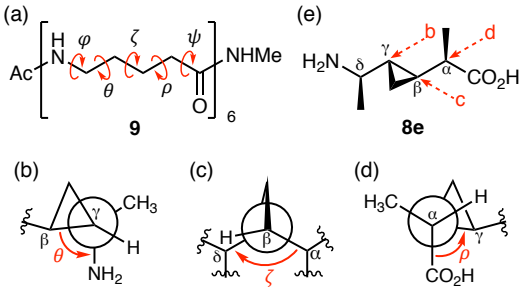


Figure 4. (a) Structure of an unsubstituted linear δ-hexapeptide **9** and its backbone torsion angles defined according to the literature.[48] (b–d) Newman projections showing the backbone torsion angles (b) θ, (c) ζ, and (d) ρ, respectively, in (α*R*,β*S*,γ*S*,δ*R*)-**8e**. (e) Red dotted arrows b–d on **8e** indicate the directions of looking at the bond in Newman projections b–d, respectively.

These findings regarding the torsion angles suggested that using this preorganized δ-amino acid monomer **8e** as a key component would allow us to rationally design δ-peptide foldamers that spontaneously fold into a 14-helical structure. Thus, we designed homo-oligomers using δ-amino acid **8e** as the constituent unit (Figure 5a) and calculated their most stable structures, revealing that the homo-δ-peptides longer than a tetramer adopt a common right-handed 14-helix, similar to that predicted by Hofmann (Figure 5b). The torsion angles ζ, θ, and ρ of the δ-amino acid units in *N*-Ac-tetramer **11a** were unchanged from those of the monomer **8e** (Table 1). Furthermore,

Table 1. The torsion angles[a] of the backbone in cyclopropane δ-amino acid 8e obtained by calculation, tetramer 11a obtained by calculation or experimentally (average and range for the four residues), and the potential 14-helical structure of δ-hexapeptide 9 (average and range for the six residues).

Structures	φ	θ	ζ	ρ	ψ
Monomer **8e**, calcd[b]	–	–86.0	139.7	–89.0	–
N-Ac-tetramer **11a**, calcd[b]	140.8 (132.0–147.8)	–84.4 (–82.7 to –86.3)	138.9 (138.4–139.3)	–89.8 (–88.9 to –91.2)	136.8 (121.8–156.2)
N-Ac-tetramer **11a**, NMR[c]	149.5 (141.2–160.1)	–99.4 (–73.1 to –126.2)	142.1 (141.7–142.4)	–73.4 (–48.7 to –117.8)	120.3[f] (93.2–135.2)[f]
N-Ac-tetramer **11a**, crystal[d]	125.7 (101.7–150.0)	–83.4 (–81.2 to –89.2)	143.9 (141.2–146.7)	–82.7 (–79.0 to –85.4)	129.1 (107.8–152.8)
14-helical δ-hexapeptide **9**, calcd[e]	106.5 (100.3–117.5) *anticlinal*$^+$	–72.8 (–71.1 to –74.1) *gauche*$^-$	171.6 (169.9–175.8) *trans*	–76.9 (–75.0 to –79.9) *gauche*$^-$	113.7 (108.8–128.9) *anticlinal*$^+$

[a]Angles in degrees. Ranges are indicated in parentheses. [b]Most stable conformation calculated by a Monte Carlo conformational search using MacroModel 10.9 (force field, MMFFs; solvent, H_2O). [c]Lowest energy 3D-structure in CD_3OH from the NMR-based calculation. [d]X-ray crystallographic structure. [e]HF/6-31G* backbone torsion angles for the most stable 14-helix in an unsubstituted δ-hexapeptide described in the literature.[48] [f]Average and range of the angles for the three residues except for the C-terminal residue (residue *4*).

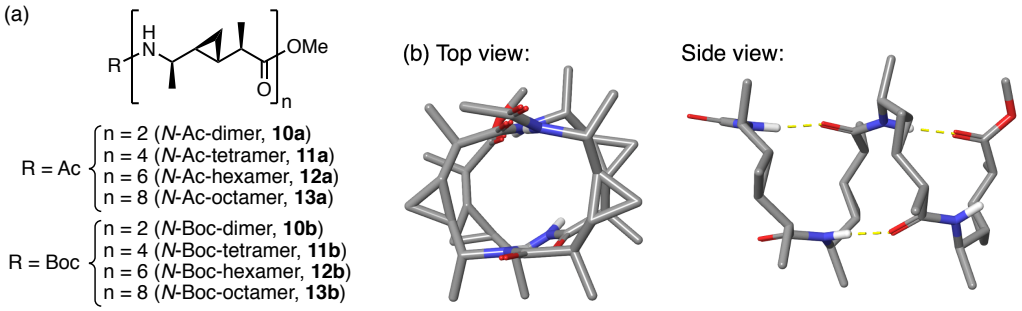


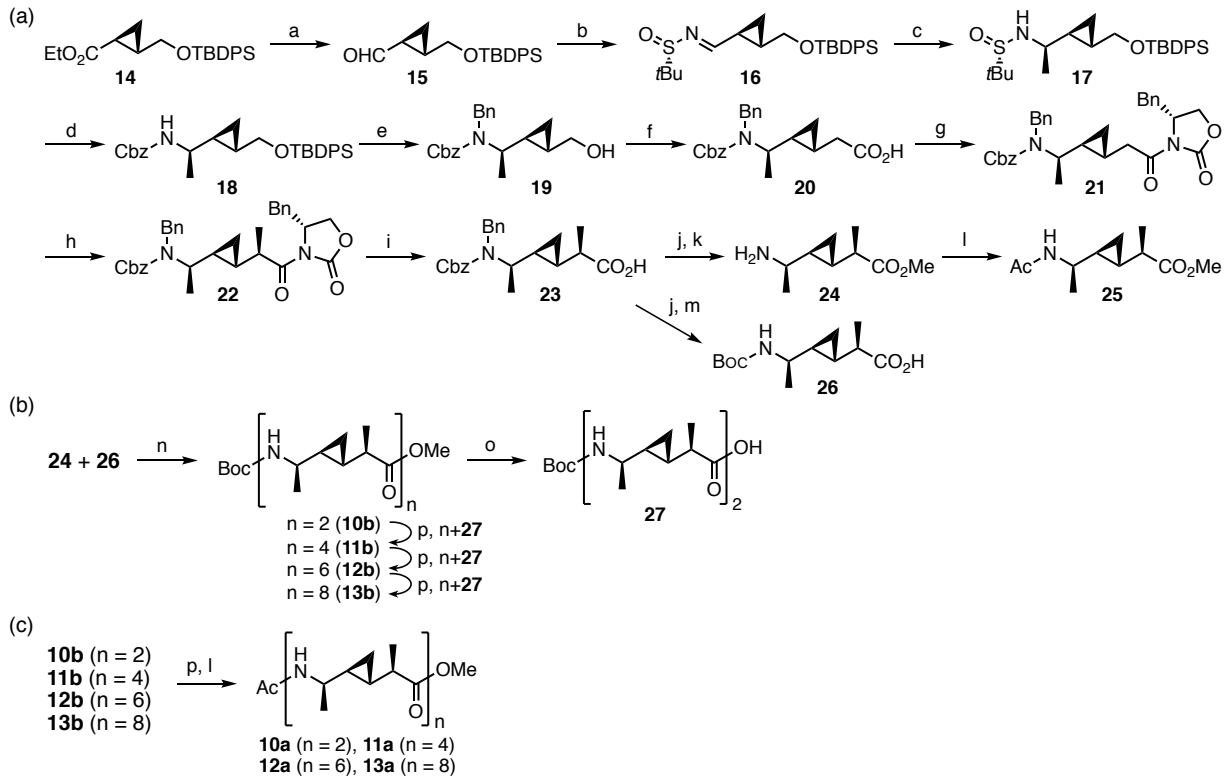
Figure 5. (a) Structures of the synthesized homo-oligomers of **8e**. The N-terminus structure is an Ac or Boc group and the C-terminus structure is a methyl ester group. (b) Top and side views of the most stable 3D structure of *N*-Ac-tetramer **11a** predicted by calculations (MacroModel 10.9; force field, MMFFs; solvent, H_2O). Hydrogens except for NH are omitted for clarity. Yellow dots indicate intramolecular H-bonds.

all the torsion angles, including the outside angles φ and ψ, were close to the values calculated by Hofmann.

Synthesis. First, the δ-amino acid monomer unit with four asymmetric centers was stereoselectively synthesized (Scheme 1a). The optically active *trans*-cyclopropane **14**, obtained according to the previously reported method,[49] was converted into cyclopropane aldehyde **15**[50] by a $LiAlH_4$-reduction of the ester group and subsequent Swern oxidation. After converting aldehyde **15** to Ellman imine **16**,[38] a methyl group was introduced by a stereoselective Grignard reaction to form **17**. The configuration of the constructed asymmetric carbon was confirmed using modified Mosher's method[51] (Scheme S1 and Figure S2). After removing the sulfinyl group under acidic conditions, the resulting primary amino group was protected by the Cbz group to yield **18**. Further protection of the amino group by benzyl group and subsequent removal of the TBDPS group gave alcohol **19**. The hydroxy group of **19** was converted to a cyano group, which was hydrolyzed to a carboxyl group under basic conditions to afford **20**. Condensation of **20** and an oxazolidinone chiral auxiliary by the mixed anhydride method was conducted to provide **21**. Methylation at the α-position of the carbonyl group in a stereoselective manner and subsequent hydrolysis of the oxazolidinone with lithium hydroxide and hydrogen peroxide[52] afforded N-protected δ-amino acid **23**. After simultaneously removing the Cbz and Bn groups of **23** by catalytic hydrogenation, methyl esterification of the carboxyl group or Boc-protection of the amino group of the obtained free δ-amino acid was conducted to yield δ-amino acid methyl ester **24** or *N*-Boc-protected δ-amino acid **26**, respectively. N-Acetylation of **24** gave the cyclopropane δ-amino acid derivative **25**, which was used for conformational analysis. The configuration at the α-position was confirmed by the PGME method[53] (Scheme S2 and Figure S3).

The cyclopropane δ-amino acid was oligomerized in liquid-phase synthesis (Scheme 1b). δ-Amino acid methyl ester **24** and *N*-Boc-protected δ-amino acid **26** were condensed by treatment with HATU and DIPEA in DMF to form a dimeric δ-peptide, *N*-Boc-dimer **10b**. Dimer **10b** was converted to a C-terminal carboxylic acid **27** by hydrolysis under basic conditions. After removing the *N*-Boc group of **10b** by TFA treatment, condensation with **27** gave a desired homo-δ-tetrapeptide, *N*-Boc-tetramer **11b**. Similarly, *N*-Boc-hexamer

Scheme 1. Synthetic route of homo-oligomers 10a,b–13a,b[a]



[a]Reagents and conditions: (a) (1) $LiAlH_4$, THF, 0 °C to rt; (2) $(COCl)_2$, DMSO, CH_2Cl_2, –78 °C, then Et_3N, –78 °C to rt; (b) (*S*)-*t*BuSONH$_2$, $CuSO_4$, CH_2Cl_2; (c) MeMgBr, toluene; (d) (1) HCl, AcOEt, 0 °C; (2) CbzCl, $NaHCO_3$, aq. THF; (e) (1) BnBr, NaH, DMF, 0 °C; (2) TBAF, THF; (f) (1) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt; (2) NaCN, NaI, DMSO; (3) KOH, aq. EtOH, reflux; (g) (1) PivCl, Et_3N, CH_2Cl_2; (2) (*R*)-4-benzyl-2-oxazolidinone, BuLi, THF, –78 °C; (h) MeI, NaHMDS, THF, –78 °C; (i) LiOH, H_2O_2, aq. THF, 0 °C to rt; (j) H_2, $Pd(OH)_2$, MeOH; (k) $SOCl_2$, MeOH, –40 °C; (l) Ac_2O, Et_3N, CH_2Cl_2; (m) $(Boc)_2O$, Et_3N, aq. dioxane; (n) HATU, *i*Pr$_2$NEt, DMF; (o) LiOH, aq. THF; (p) TFA, CH_2Cl_2.

12b and -octamer **13b** were also synthesized. Acidic removal of the *N*-Boc group of these oligomers **10b–13b** and subsequent N-acetylation afforded the corresponding *N*-Ac-dimer **10a**, -tetramer **11a**, -hexamer **12a**, and -octamer **13a**, respectively (Scheme 1c).

Conformational analysis of the monomer in solution. The conformation of the *trans*-δ-amino acid monomer unit **25** in $CDCl_3$ was confirmed by nuclear Overhauser effect (NOE) experiments (Figure 6). Strong NOEs were observed between the α-proton (CαH) and the two protons on the different carbons in the cyclopropane ring (CγH and Cγ′Ha) as well as between the δ-proton (CδH) and the other two protons on the cyclopropane ring (CβH and Cγ′Hb). Furthermore, weak NOEs were observed between the protons at the top of the cyclopropane ring and the Me group protons at the α- and δ-positions (between Cγ′Ha and Cα-CH_3; Cδ-CH_3 and Cγ′Hb, respectively). These results showed that both the minimum substituents (H) at the α- and δ-positions orient toward the cyclopropane side, consistent with the most stable conformation obtained by the calculations (Figure 3). These findings indicate that the cyclopropylic strain in **25** functions effectively as expected to restrict the orientations of the N–Cδ and Cα–CO bonds to an N-folded/C-folded form in solution, in which both torsional angles θ and ρ of the backbone of **25** would be regulated to the *gauche*$^-$ conformation. On the basis of these results, homo-oligomers of this highly conformationally restricted *trans*-cyclopropane δ-amino acid monomer would yield a 14-helix foldamer, as predicted by the calculations described above.

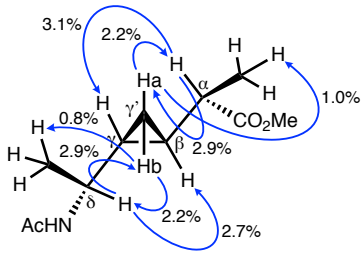


Figure 6. The observed NOEs in *trans*-cyclopropane δ-amino acid monomer **25** in $CDCl_3$ (400 MHz).

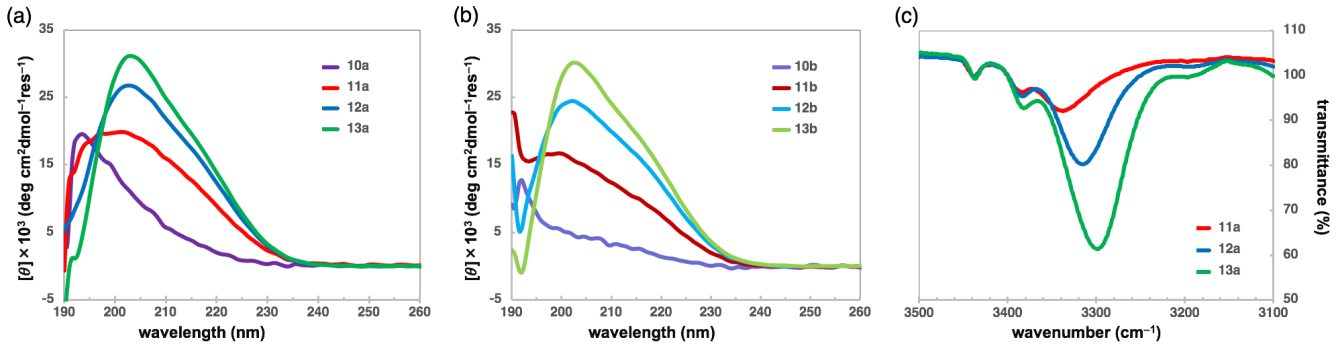


Figure 7. (a, b) Overlay of normalized CD spectra of (a) *N*-Ac-oligomers **10a**–**13a** and (b) *N*-Boc-oligomers **10b**–**13b** in MeOH (0.1 mM) at 20 °C. The y-axis indicates molar ellipticity per one δ-amino acid. The spectra are the averages of three independent measurements. (c) Overlay of expanded IR spectra of *N*-Ac-oligomers **11a**–**13a** in $CDCl_3$ (10 mM) at 20 °C. The spectra are the averages of three independent measurements.

CD and IR analysis of the oligomers in solution. Far-ultraviolet CD spectroscopy is useful for obtaining secondary structural information not only on natural α-peptides but also on unnatural oligopeptides in solution.[54] We measured the CD of *N*-Ac-dimer **10a**, -tetramer **11a**, -hexamer **12a**, and -octamer **13a** in MeOH (0.1 mM) at 20 °C. As shown in Figure 7a, all of the oligomers showed a single positive Cotton effect, and the spectra changed depending on the oligomer length. When the oligomers lengthened from a dimer to a tetramer, the maximum wavelength was red shifted from 194 nm to approximately 203 nm. The maximum positive wavelength of the tetramer, hexamer, and octamer was unchanged at approximately 203 nm, and the intensity of the positive Cotton effect per residue increased as the oligomers became longer. These results suggest that the tetramer and longer oligomers adopt an analogous secondary structure in MeOH, and the longer the oligomer, the more stable the secondary structure. The CD spectra of oligomers **10b**, **11b**, **12b**, and **13b** with a Boc group at the N-terminus instead of an Ac group in MeOH were almost the same as those of *N*-Ac-oligomers **10a**, **11a**, **12a**, and **13a**, respectively (Figure 7b), indicating that their secondary structures in solution were unaffected by the N-terminal group of the oligomers. Further, the CD spectra of *N*-Ac-octamer **13a** in a different concentration range of 0.01–0.1 mM and also a different temperature range of 0–60 °C showed no significant change (Figures S4 and S5), indicating that the secondary structure of the oligomer is remarkably stable, insensitive to both the oligomer concentration and temperature. Thus, the structural characteristics of the cyclopropane δ-amino acid unit would effectively regulate the backbone of the oligomers to form a common stable secondary structure.

IR spectra of peptides are useful for detecting intramolecular hydrogen-bond formation[55] and thus we investigated the absorption for N–H stretch vibrations of *N*-Ac-tetramer **11a**, -hexamer **12a**, and -octamer **13a** in $CDCl_3$ (Figure 7c). As the oligomer lengthened, the absorption for hydrogen-bonded N–H markedly increased at approximately 3300 cm^{-1}, whereas the weak absorption for free N–H stretch was unchanged at approximately 3440 cm^{-1}. These findings indicate that only the number of NH-forming intramolecular hydrogen bonds increases as the number of δ-amino acid residues of oligomers increases. The δ-peptides, regardless of the length, are likely to adopt a repeating secondary structure with the same hydrogen-bonding pattern in solution.

NMR analysis of the oligomers in solution. In the ^1H NMR spectrum of the *N*-Ac-tetramer **11a** in CD_3OH, the H_N-

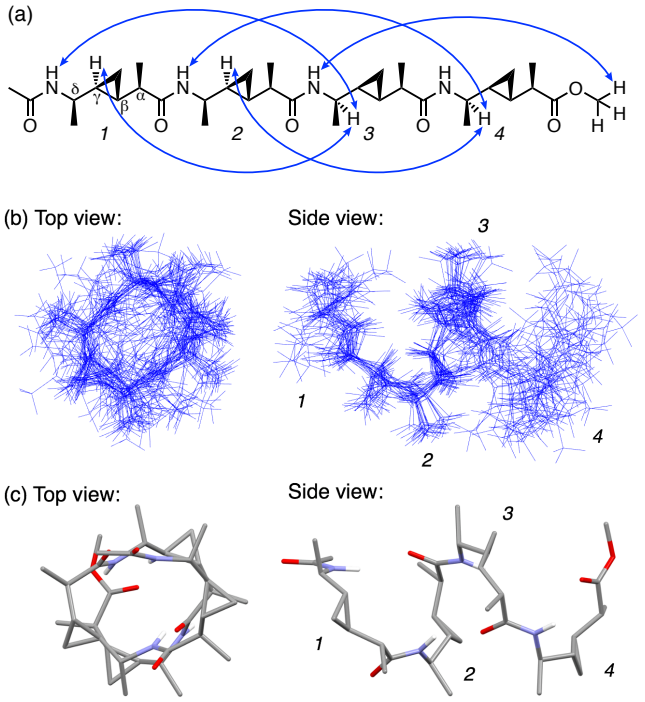


Figure 8. (a) The observed long-range NOEs of the *N*-Ac-tetramer **11a** in CD_3OH supporting the helical structure; (b) Overlay of the 20 lowest-energy structures of **11a** obtained from the NMR-based calculations; (c) Top and side views of the lowest energy 3D structure of **11a** in CD_3OH from the NMR-based calculation. Hydrogens except for NH are omitted for clarity.

proton signals from the two N-terminal residues (residues *1* and *2*) were observed in lower magnetic field around δ 8.5–8.35 ppm than those of the two C-terminal residues (residues *3* and *4*; δ 7.85–7.60 ppm) (Figure S6). These downfield shifts of the H_N-protons of residues *1* and *2* indicate the formation of intramolecular hydrogen bonds in solution. The 3D structure of tetramer **11a** in CD_3OH was then investigated on the basis of 2D NOESY spectra. For residues *1* and *2*, long-range NOEs between $H_N(i)$ and $H_\delta(i + 2)$, and $H_\gamma(i)$ and $H_\delta(i + 2)$ were observed (Figures 8a and S7). Additionally, a long-range NOE was also observed between the H_N of residue *3* and the OCH_3 moiety of residue *4* (Figure S7). These long-range NOEs support the notion that the oligomer folded into a helical structure. Furthermore, based on the 117 NOE-derived distance restraints and four backbone dihedral angles restraints obtained from ^3J

couplings between H_N and H_δ, a simulated annealing calculation using XPLOR was performed for the tetramer **11a**. Figure 8b shows that overlay of the 20 lowest-energy structures obtained from the 100 calculations. As shown in Figure 8b, the three N-terminal residues (residues *1*–*3*) converged well with the mean pairwise RMSD of the backbone, 0.76 ± 0.25 Å, and for all the heavy atoms, 1.48 ± 0.35 Å. The C-terminal residue (residue *4*) was less structurally converged and the NOE intensity of the residue was weaker than that of the other residues. In the lowest energy 3D structure shown in Figure 8c, the NH(*i*)···O=C(*i* + 1) pairs from residues *1* and *2* form intramolecular 14-atom-ring hydrogen bonds; the geometric criteria for the H-bond assignments are N···O distance <4.0 Å and N–H···O angle >130°.[56] These analyses indicate that even in the short length tetramer **11a**, in which average values of each torsion angle (φ, θ, ζ, ρ, and ψ) were similar to not only those in the calculated stable form of **11a** but also those in the potential 14-helical structure of unsubstituted δ-hexapeptide **9** presented by Hofmann as summarized in Table 1, a stable 14-helical structure was formed in solution.

The longer *N*-Ac-oligomers, i.e., hexamer **12a** and octamer **13a**, were also analyzed by ^{1}H NMR in CD_3OH. The chemical shifts of H_N signals from two C-terminal residues in **12a** were almost identical to those of **11a** (Figure S6). Although complete assignment of the spectrum of octamer **13a** was unsuccessful due to severe overlapping of the resonances, the chemical shifts of the H_N proton resonances seem to be consistent with those of tetramer **11a** and hexamer **12a**, and as the number of residues increased, the number of H_N signals increased in the same region as the two N-terminal residues of tetramer **11a**. These results suggest that the increased residues form new intramolecular hydrogen bonds, and these *N*-Ac-oligomers share a 14-helical structure regardless of the peptide length. NMR spectra of *N*-Ac-oligomers **11a**, **12a**, and **13a** at different concentrations (0.2–7.4 mM) showed no change in the chemical shift of the signals (Figure S6), suggesting that these secondary structures were independent of the concentration. The *N*-Boc-oligomers **11b**, **12b**, and **13b** were also analyzed. The chemical shifts of the H_N-protons in the *N*-Boc-oligomers were almost identical to those of the *N*-Ac-oligomers except for the chemical shift difference due to the different N-terminal chemical structure (amide or carbamate) (Figure S8). These spectra suggest that all of the homo-oligomers **11a,b**–**13a,b** share an ordered 14-helical secondary structure in CD_3OH, regardless of the difference in their length and N-terminal structure. Further, the conformational restriction of the δ-peptide backbone by the structural characteristics of cyclopropane makes the 14-helical structure stable in these oligomers, as predicted by the calculations in the molecular design (Figure 5b), even in protic polar solvent.

Crystal-structures of the oligomers. The stable secondary structure of the cyclopropane δ-peptides in solution encouraged us to tackle their crystallization. A solvent diffusion method with MeOH successfully gave a single crystal of the *N*-Ac-tetramer **11a**. The X-ray crystallographic analysis showed that the tetramer has a right-handed helical structure, in which all the possible 14-membered-ring hydrogen bonds are observed as anticipated from the molecular design, and the helical structure is similar to that in solution obtained by the NMR-based calculations (Figure 9). The average values of each torsion angle (φ, θ, ζ, ρ, and ψ) adopted the *anticlinal*$^+$, *gauche*$^-$, *anticlinal*$^+$, *gauche*$^-$, and *anticlinal*$^+$ conformations, respectively, and these angles, except for ζ, were in good agreement with the torsion angles of the hexamer of the linear unsubstituted δ-amino acids forming a 14-helix structure predicted by Hofmann by calculations as described above (Table 1). In all of the residues *1*–*4*, each torsion angle θ, ζ, and ρ was homogeneous (θ, –81.2° to –89.2°; ζ, 141.2°–146.7°; ρ, –79.0° to –85.4°, respectively), indicating that these angles were precisely controlled by the conformation restricting effect of cyclopropane in the oligomer.

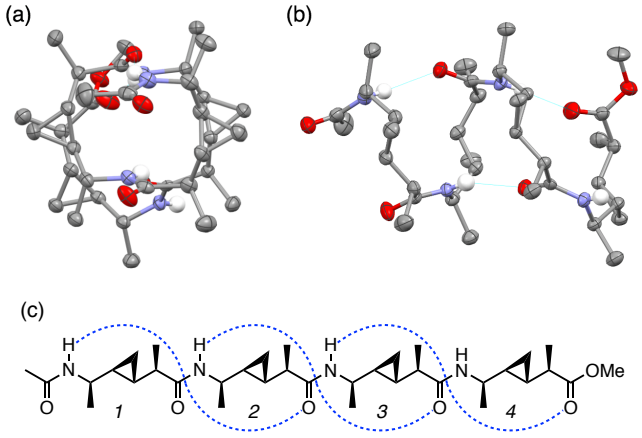


Figure 9. (a, b) The X-ray crystal structure of *N*-Ac-tetramer **11a**: (a) top view and (b) side view. Solvated molecules and hydrogens except for NH are omitted for clarity. Probability level: 50%. CCDC 2049402; (c) Blue dots lines indicate the H-bonding patterns in the crystal structure of **11a**.

The other oligomers, *N*-Boc-tetramer **11b** and *N*-Boc-hexamer **12b**, were also successfully crystalized by the solvent diffusion method using MeOH and the counter diffusion method using $CHCl_3$ and MeCN, respectively. The X-ray crystallography revealed that these oligomers also adopted a 14-helical structure like tetramer **11a** (Figures 10 and 11). The average values of each backbone torsion angle (φ, θ, ζ, ρ, and ψ) were almost the same in the three δ-peptides **11a**, **11b**, and **12b** (Tables 1 and S1). These results indicate that the secondary structure of the cyclopropane δ-amino acid oligomers was unaffected by the differences in the N-terminal group (Ac or Boc) and oligomer length (4 or 6). Regardless of the oligomer length, the strong conformational regulation of the *trans*-cyclopropane δ-amino acid monomer unit was effective in the oligomer, and the entire oligomer folded stably to a helical structure as expected.

Effects of the cyclopropylic strain on the helix folding. As described above, effective formation of the stable 14-helical structure of homo-oligomers of cyclopropane δ-amino acids having a methyl group at both the α- and δ-positions was experimentally demonstrated. We speculated that the spontaneous folding of these homo-δ-peptides into definite secondary structures was caused by the conformational restriction of the backbone due to the characteristic steric effect of cyclopropane, i.e., *cis*/*trans*-restriction and cyclopropylic strain. The cyclopropylic strain is caused by steric effects due to the substituents at the α- and δ-positions adjacent to the cyclopropane ring, as described under **Rational Design**. To confirm the actual contribution of the steric effect on the helix formation due to the substituents, we synthesized homo-hexamers **12c**–**e** of cyclopropane δ-amino acid derivatives without one or both of the methyl groups at the α- and/or δ-positions (Figure 12,

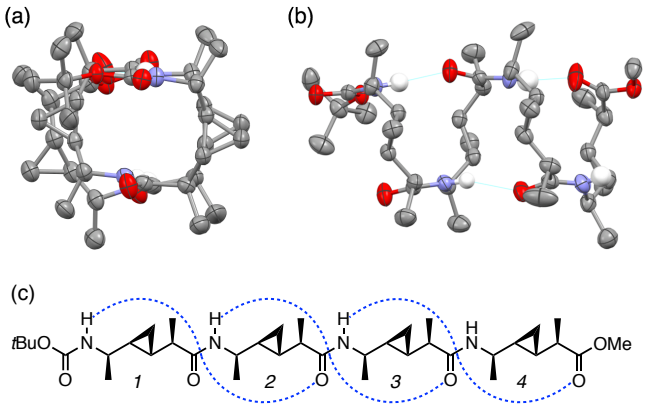


Figure 10. (a, b) The X-ray crystal structure of *N*-Boc-tetramer **11b**: (a) top view and (b) side view. Solvated molecules and hydrogens except for NH are omitted for clarity. Probability level: 50%. CCDC 2049403; (c) Blue dots lines indicate the H-bonding patterns in the crystal structure of **11b**.

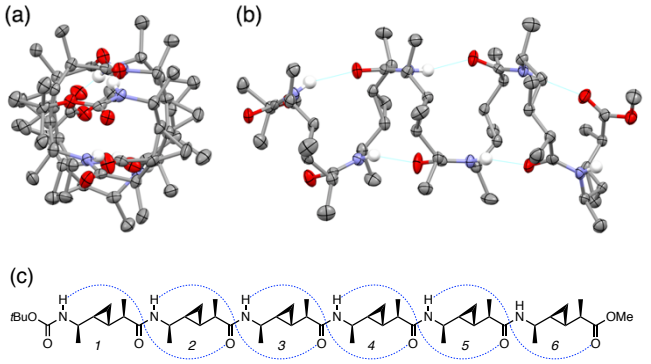


Figure 11. (a, b) The X-ray crystal structure of *N*-Boc-hexamer **12b**: (a) top view and (b) side view. Solvated molecules and hydrogens except for NH are omitted for clarity. Probability level: 50%. CCDC 2049404; (c) Blue dots lines indicate the H-bonding patterns in the crystal structure of **12b.**

Schemes S3 and S4). In the CD spectra of these hexamers **12c–e** in MeOH (0.1 mM), no distinct Cotton effect was observed at a wavelength range longer than 200 nm, in contrast to the demethylated hexamer **12b** (Figure 12). The results suggest that these homo-oligomers **12c–e** with a decreased number of methyl groups in the monomer unit did not form a helical structure in solution, unlike the homo-oligomers of **8e** with methyl groups at the both α- and δ-positions. Further, the NMR spectra of these hexamers **12c–e** exhibited no NOE signals supporting the formation of the folded structure (data not shown). These results confirm that the substituents at both the α- and δ-positions inducing the cyclopropylic strain are essential for the formation of a stable helical structure in homo-oligomers consisting of cyclopropane δ-amino acids.

In this study, even short δ-peptides, such as tetramers, formed a helical structure stable enough to be crystallized. This result indicates that cyclopropane in the backbone of peptides has high ability to regulate their 3D structure. The backbone torsion angles of the oligomers in both the NMR-based and crystal structures well-agreed with those of the most stable structure obtained by theoretical calculations in the molecular design, indicating that the 3D structures of the oligomers in the crystal, solution, and calculations were almost identical. This good agreement among the secondary structures obtained by calculated prediction and experimental analysis proves that the use of the cyclopropane δ-amino acids with its precisely conformational-regulating ability as the monomer unit enables the rational design of new aliphatic δ-peptide foldamers.

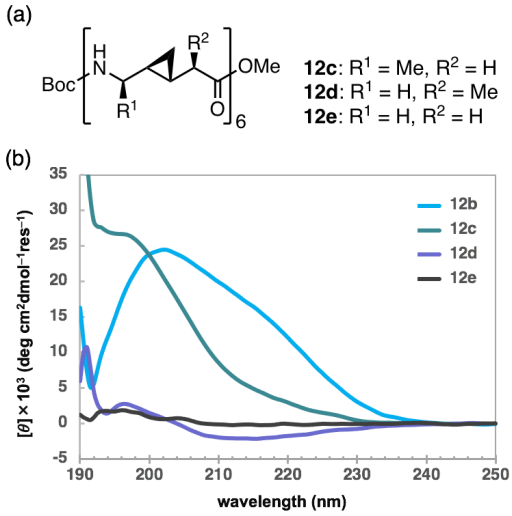


Figure 12. (a) Structures of *N*-Boc-hexamers **12c–e**, which were without one or both of the methyl groups at the α- and/or δ-positions in the cyclopropane δ-amino acid unit; (b) Overlay of normalized CD spectra of **12b–e** in MeOH (0.1 mM) at 20 °C. The y-axis indicates molar ellipticity per one δ-amino acid. The spectra are the averages of three independent measurements.

We should emphasize that the 14-helical foldamers confirmed in the present study were predicted by employing the 14-helical structure potentially formed by an unsubstituted homo-δ-hexapeptide **9**, previously presented by Hofmann and co-workers' theoretical calculations,[48] as a prototype. Thus, the present study experimentally demonstrated the validity of Hofmann's work, and provides valuable clues for developing various foldamers of aliphatic δ-peptides other than the 14-helical foldamers.

Aliphatic δ-amino acids have the same backbone length as α-dipeptides, and therefore the α- and δ-positions correspond to the positions of the two side chains of α-dipeptides. Thus, these cyclopropane δ-peptide foldamers, in which various functional groups instead of the methyl groups are synthetically introducible at the α- and δ-positions, would effectively mimic the structures and functions of natural α-peptides of biologic importance.

CONCLUSIONS

We developed the first aliphatic homo-δ-peptide helical foldamer based on a rational molecular design with a conformationally restricted cyclopropane δ-amino acid that has backbone torsion angles tightly regulated by the structural characteristics of cyclopropane. Oligomerization of the cyclopropane δ-amino acids monomer was predicted by calculations to form into a stable helical structure. The synthesized cyclopropane δ-peptides, even those with shorter length like tetramers, definitely adopted a stable right-handed 14-helical structure, as expected, both in crystals and in solution. Critical points are that the 3D structures of the aliphatic homo-δ-peptide folda-

mers in this strategy are rationally predictable by theoretical calculations. Thus, this work may open a new class of non-natural peptides forming a secondary structure that will contribute to improving and expanding the structures and functions of foldamers.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Figures S1–S8, Schemes S1–S4, Table S1, Summary of crystallographic data (Table S2), Synthetic procedures and characterization of compounds, Protocol of NMR structural calculation, and NMR spectra (PDF)

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Author Contributions

M.W. and S.S. conceived this work, designed and calculated the compounds, analyzed the whole data, and wrote the paper; M.N., M.U., N.O, W.I, and K.F. performed the synthesis and the CD measurements. R.D. and Y.S. performed the X-ray crystallography. T.K. conducted the calculations. K.T. performed the NMR analysis of the oligomers and wrote the paper. All authors have read and agreed to the final version of the manuscript.

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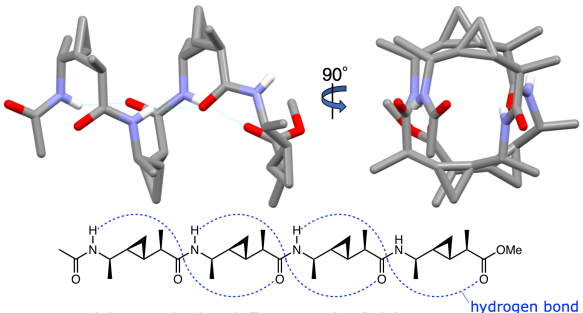

Stable 14-helical δ-peptide foldamer