Review

Design and Synthesis of Chiral α,α-Disubstituted Amino Acids and Conformational Study of Their Oligopeptides

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α,α-Disubstituted amino acids are α-amino acids in which the hydrogen atom at the α-position of the L-α-amino acid is replaced with an alkyl substituent. The introduction of an α-alkyl substituent changes the properties of amino acids, with the conformational freedom of the side chain in the amino acids and the secondary structure of their peptides being especially restricted. The author developed a synthetic route of optically active α-ethylated α,α-disubstituted amino acids using chiral cyclic 1,2-diol as a chiral auxiliary. It was found that the preferred secondary structure of peptides composed of chiral α-ethylated α,α-disubstituted amino acids is a fully extended C_5-conformation, whereas that of peptides composed of chiral α-methylated α,α-disubstituted amino acids is a 3_{10}-helical structure. Also, a new chiral cyclic amino acid; (3*S*,4*S*)-1-amino-3,4-di(methoxy)cyclopentanecarboxylic acid {(*S*,*S*)-Ac_5c^{dOM}}, and a bicyclic amino acid; (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 atom is not the chiral center but chiral centers exist at the side-chain cycloalkane skeleton, were designed and synthesized. The (*S*,*S*)-Ac_5c^{dOM} hexa- and octapeptides preferentially formed left-handed (*M*) helices, in which the helical-screw direction is exclusively controlled by the side-chain chiral centers. Contrary to the left-handed helices of (*S*,*S*)-Ac_5c^{dOM} peptides, the (*R*,*R*)-$Ab_{5,6=}c$ hexapeptide formed both diastereomeric right-handed (*P*) and left-handed (*M*) helices, and the twelve chiral centers at the side chain showed no preference for helical-screw direction. Thus, the chiral environment at the side chain is important for the control of helical-screw direction. Furthermore, the author designed a new class of chiral cyclic α,α-disubstituted amino acids that have pendant chiral centers at the substituent of the δ-nitrogen atom. The synthetic route would provide various optically-active cyclic α,α-disubstituted amino acids bearing a pendant chiral moiety.

Key words α,α-disubstituted amino acid; peptide; conformation; asymmetric synthesis; helix; secondary structure

1. Introduction

The design and synthesis of new molecules having various functions, which can not be attained by using natural products, have attracted much attention from organic, bioorganic, peptide, and medicinal chemists. The creation of such intelligently functionalized molecules is a new research area of chemistry in the 21st century. One category of such molecules might be 'foldamers', as named by Gellman.[1] The foldamers are any polymer with a strong tendency to adopt a specific compact conformation. Natural enzymes and receptors, which form defined three-dimensional shapes and have biological functions, might be the ultimate 'foldamers' designed and produced by evolution in nature. Unfortunately, oligopeptides composed of natural L-α-amino acids often form unordered or unstable secondary structures, particularly in the case of short peptides because of the flexibility of natural amino acids. The unordered or unstable secondary structure of natural oligopeptides is a drawback in the use of the oligopeptides as drug candidates, biological probes, and functionalized-device molecules. Thus, organic chemists are devoting great efforts to the design of new amino acid analogs and their oligomers with conformational restrictions.

2. α,α-Disubstituted Amino Acids

To restrict the conformational freedom of amino acids and their peptides, many designs and modifications have been developed so far. Among them, replacement of the α-hydrogen atom of L-α-amino acids with an alkyl substituent, which results in α,α-disubstituted amino acids (α-alkylated amino acid, α-alkyl amino acid, dialkyl glycine), has been reported.[2] The modification changes the properties of amino acids as follows: 1) increase of chemical stability, 2) increase of hydrophobicity, 3) restriction of conformational freedom of the side chains in amino acids, 4) restriction of conformational freedom of their peptides, and 5) metabolic stability of their peptides (Fig. 1).

α-Aminoisobutyric acid (Aib, dimethylglycine, α-methylalanine) is one of the most well-known α,α-disubstituted amino acids. The first report on Aib goes back to the synthesis of Aib by Urech in 1872.[3] Aib has also been isolated as a component of natural peptaibol antibiotics, such as antiamoebin, alamethicin, emerimicin, and zervamicin. Thus,

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α,α-disubstituted amino acids should be termed non-proteinogenic amino acids or non-coded amino acids instead of unnatural amino acids. Aib has been widely used for the construction of the helical secondary structure of peptides, as well as the design and synthesis of organo-catalysts and new drug candidates.[4—7] Retrieval using the key word '2-aminoisobutyric acid or Aib' in June 2006 hit more than 6000 articles, indicating that Aib is a very important amino acid for various kinds of research.

As other detailed α,α-disubstituted amino acids and the secondary structures of their peptides have already been reported in review articles,[2,4—7] I will only provide a minimal explanation of the secondary structures of oligopeptides, while focusing on the design, synthesis, and conformational studies of chiral α,α-disubstituted amino acids and their peptides in the author's group.

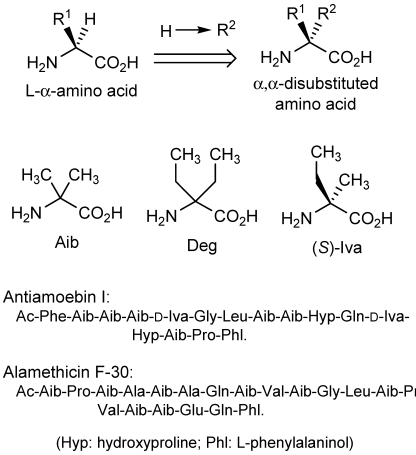

Fig. 1. Structure of α,α-Disubstituted Amino Acids and Peptaibol Antibiotics

3. Secondary Structures of Peptides Composed of α,α-Disubstituted Amino Acids

As for the secondary structures of peptides composed of α,α-disubstituted amino acids, Karle and co-workers reported the conformation of oligopeptides containing Aib, and Toniolo, Benedetti and co-workers reported those of achiral α,α-disubstituted amino acids and chiral α-methylated α,α-disubstituted amino acids.[5,8—11] Homopeptides composed of Aib assume a 3_{10}-helical structure both in solution and in the crystal state. There are both enantiomeric right-handed (*P*) and left-handed (*M*) 3_{10}-helices in the ratio of 1 to 1 because Aib is an achiral amino acid. In the 3_{10}-helix, an intramolecular hydrogen-bonded ($i \leftarrow i+3$) ring consisting of 10 atoms exists, and one 3_{10}-helical turn consists of 3.0 amino acid residues. On the other hand, in the α-helix (3.6_{13}-helix), which is often shown in proteins, an intramolecular hydrogen-bonded ($i \leftarrow i+4$) ring consisting of 13 atoms exists, and one α-helical turn consists of 3.6 amino acid residues, as shown in Fig. 2. Thus, the 3_{10}-helix is tighter than the α-helix; figuratively speaking, the 3_{10}-helix is a more strongly 'twisted towel' than the α-helix (Fig. 3). High-resolution X-ray crystallographic analysis has recently disclosed that the 3_{10}-helix often occurred as the secondary structure of proteins. The incorporation of Aib to peptides composed of L-α-amino acids induces right-handed (*P*) helices. It is known that the Aib peptides preferentially form the 3_{10}-helices in the case of shorter peptides containing a high percentage of Aib, and form the α-helices in the case of longer peptides having a low percentage of Aib. Aib is achiral and does not

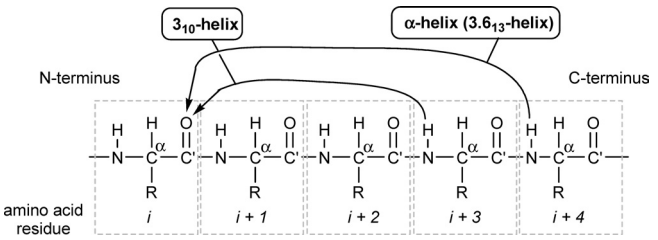

Fig. 2. Hydrogen Bonding Scheme of α-Helix and 3_{10}-Helix

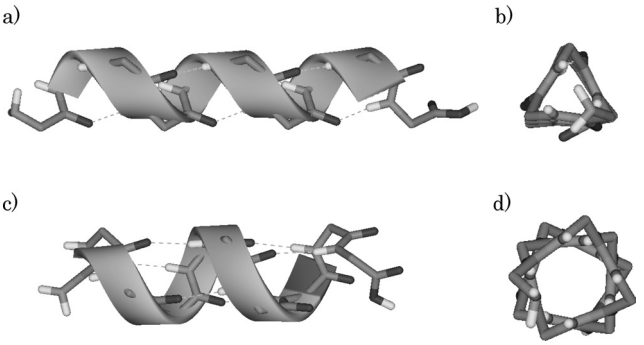

Fig. 3. Structure of 3_{10}-Helix and α-Helix

a) 3_{10}-Helix as viewed perpendicularly to the helical axis, b) 3_{10}-helix as viewed along the helical axis, c) α-helix as viewed perpendicularly to the helical axis, d) α-helix as viewed along the helical axis.

Masakazu Tanaka was born in Kagawa, Japan in 1963. He graduated with a BSc degree in Pharmaceutical Sciences from Kyushu University in 1986, and then received his MSc degree from the same university in 1988. He began work as a Research Fellow at the Osaka Branch of the National Institute of Health Sciences in 1988. In 1990, he was appointed as a Research Associate at Kyushu University. After receiving his PhD degree at Kyushu University under the supervision of Professor Kiyoshi Sakai, he performed postdoctoral work with Professor Dieter Seebach's group in 1994 at ETH in Switzerland. Since 1997, he has been an Associate Professor at the Graduate School of Pharmaceutical Sciences, Kyushu University (Professor Hiroshi Suemune's group). He received the Yamanouchi Seiyaku Award in Synthetic Organic Chemistry, Japan (1993), the Inoue Research Award for Young Scientists (1994), and The PSJ Award for Divisional Scientific Promotions (2006). His current research interests involve transition metal-catalyzed synthetic reactions, asymmetric catalysts, and medicinal chemistry.

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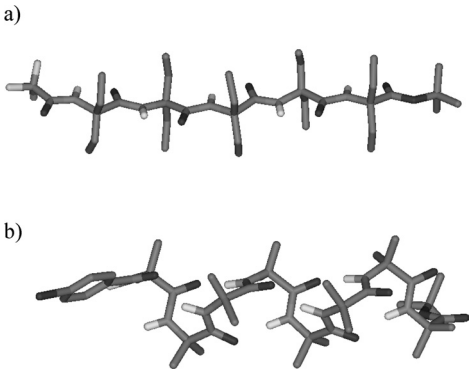

Fig. 4. 3_{10}-Helix and Planar Structures of Homopeptides Composed of α,α-Disubstituted Amino Acids

a) Fully planar C_5-conformation of Deg pentapeptide CF_3CO-$(Deg)_5$-OBu^t, b) 3_{10}-helix of Aib octapeptide *p*-BrBz-$(Aib)_8$-OBu^t.

have a helical-screw bias, and thus, the helical screw sense (right-handedness) of heteropeptides is controlled by the chiral center at the α-position of L-α-amino acids.

Contrary to the 3_{10}-helix of the Aib homopeptides, homopeptides composed of achiral diethylglycine (Deg) preferentially form a fully extended C_5-conformation (Fig. 4).[12—14] The extended C_5-conformation is an extended planar conformation of the peptide-backbone, showing two torsion angles of $\phi=180°$, $\varphi=180°$. Achiral homopeptides composed of dipropylglycine, dibutylglycine, or diphenylglycine also preferentially form the extended conformation. In his textbook of organic chemistry, Solomons mentions that the fully extended conformation is an ideal flat-sheet structure, having the repeat distance of 7.2 Å, and the structure does not exist as a secondary structure in proteins.[15] However, the achiral α,α-disubstituted amino acids have the same two side-chain substituents, and thus, their symmetrical homopeptides exceptionally assume the fully planar conformation. As an exception, the fully extended conformation is shown as a secondary structure of glycine tetrapeptide in tRNA synthetase by X-ray crystallographic analysis, because glycine has two hydrogen atoms at the α-position, and is symmetric.[11]

Concerning the conformation of chiral α,α-disubstituted amino acid peptides, the Toniolo[16—20] and Seebach groups[21] independently reported the preferred secondary structure of peptides composed of chiral α-methylated α,α-disubstituted amino acids. They focused on the conformation of isovaline (Iva) peptides. Isovaline, which is the simplest chiral α,α-disubstituted amino acid, has two different substituents consisting of methyl and ethyl groups. The Toniolo group prepared homopeptides composed of (*R*)-Iva; *p*BrBz-$\{(R)\text{-Iva}\}_n$-OBu^t ($n=3,4,5$), and studied their secondary structures by X-ray crystallographic analysis. They found that the homopeptides *p*BrBz-$\{(R)\text{-Iva}\}_n$-OBu^t assumed left-handed (*M*) 3_{10}-helices, and reported that the quaternary stereogenic center of (*R*)-Iva induces the left-handed (*M*) helical screw-sense of its homopeptides.[18] Contrary to the left-handed 3_{10}-helices of (*R*)-Iva peptides, the Seebach group reported that three homopeptides Boc-$\{(S)\text{-Iva}\}_n$-OMe ($n=3,4,6$) assumed both right-handed (*P*) and left-handed (*M*) 3_{10}-helices. This right-handedness and left-handedness are in a diastereomeric relationship. It is worthy of note that the hexapeptide assumed diastereomeric right-handed (*P*) and left-handed (*M*) 3_{10}-helices albeit the hexapeptide has six asymmetric centers on the peptide backbone α-carbon atom (Fig. 5). The ^{1}H-NMR measurement at low temperature ($-90\,°C$) showed two species of six peptide N–H protons in a ratio of 4 to 1, and the nuclear Overhauser and exchange spectroscopy (NOESY) ^{1}H-NMR spectrum at $-90\,°C$ revealed that the right-handed (*P*) 3_{10}-helix is a dominant conformation.[21] These results are different from those of the Toniolo group, and the difference is due to the fact that two homopeptides have different C- and N-terminal protecting groups. The ^{1}H-NMR spectra of (*R*)-Iva homopeptides at low temperature have not yet been reported.

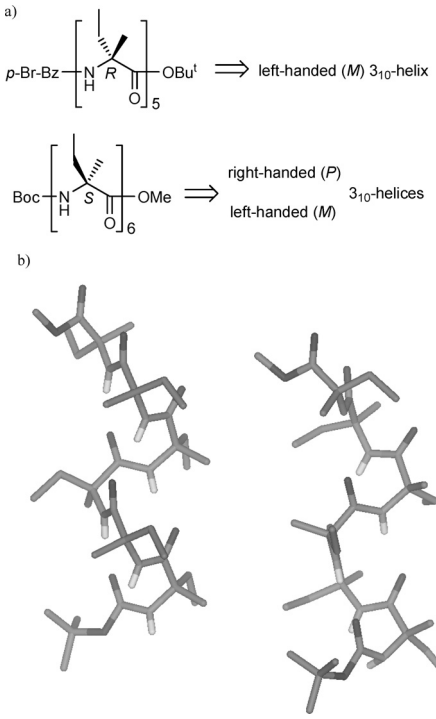

Fig. 5. Helical Secondary Structures of (*R*)- and (*S*)-Iva Homopeptides Reported by Toniolo,[18] and Seebach[21]

a) Summary of helical-screw sense of Iva homopeptides, b) both right-handed (*P*) and left-handed (*M*) 3_{10}-helices of Boc-$\{(S)\text{-Iva}\}_6$-OMe reported by Seebach.

Table 1. Reported Secondary Structures of Homopeptides Composed of Chiral α-Methylated α,α-Disubstituted Amino Acids[16—21]

Entry	Peptide	Secondary structure
1	*p*-BrBz-$\{(R)\text{-Iva}\}_5$-OBu^t	(*M*) 3_{10}-helix
2	Boc-$\{(S)\text{-Iva}\}_6$-OMe	(*P*) & (*M*) 3_{10}-helices
3	Cbz-$\{(S)$-αMeVal$\}_8$-OBu^t	(*P*) 3_{10}-helix
4	*p*-BrBz-$\{(R)$-αMeLeu$\}_4$-OH	(*P*) 3_{10}-helix
5	*p*-BrBz-$\{(R)$-αMePhe$\}_4$-OBu^t	(*P*) 3_{10}-helix
6	*p*-BrBz-$\{(R)$-αMeHph$\}_3$-OBu^t	(*M*) 3_{10}-helix

Iva: isovaline; αMeVal: α-methylvaline; αMeLeu: α-methylleucine; αMePhe: α-methylphenylalanine; αMeHph: α-methylhomophenylalanine.

Homo- and heteropeptides composed of α-methylated α,α-disubstituted amino acids such as (*S*)-α-methylvaline, (*R*)-α-methylleucine, and (*R*)-α-methylalanine were synthesized and their conformations have been studied by Toniolo (Table 1).[19,20] Their studies revealed that the α-quaternary stereogenic center affects the secondary structures of their peptides, and the helical-screw sense is dependent on the *R*

or *S* configuration at the α-carbon of α-methylated α,α-disubstituted amino acids.[16—21)]

4. Chiral α-Ethylated α,α-Disubstituted Amino Acids and Their Asymmetric Syntheses

Peptide chemists believed that peptides composed of chiral α-ethylated α,α-disubstituted amino acids would preferentially form a right-handed (*P*) or left-handed (*M*) 3_{10}-helical structure, since those of α-methylated amino acids prefer the 3_{10}-helix formation.[22,23)]

The author was interested in the preferred conformation of chiral α-ethylated amino acid peptides. This is because, in the case of a chiral α-ethylated α,α-disubstituted amino acid, for example, (*S*)-butylethylglycine {(*S*)-Beg}, the (*S*)-Beg has two different side chains, butyl and ethyl groups, but the properties of both the butyl and ethyl groups dictate the formation of the fully planar C_5-conformation. In other words, the homopeptides composed of dibutylglycine preferentially form the fully planar conformation, and those of diethylglycine tend to assume the same extended conformation. This raises the question as to how is the preferred conformation of homopeptides composed of (*S*)-Beg. That is, the fully planar conformation due to the fact that the properties of both side chains are the same planar C_5-conformation, or, the right-handed (*P*) or left-handed (*M*) 3_{10}-helix due to the fact that side chains are different substituents.

Although efficient synthetic procedures for optically active α-alkylated α,α-disubstituted amino acids[24,25)] have recently been reported by Kawabata,[26)] and Maruoka,[27)] the author developed an original synthetic route for optically active α-alkylated α,α-disubstituted amino acids. The author utilized diastereoselective alkylation using cyclohexane-1,2-diol as a chiral auxiliary to construct the quaternary stereogenic center (Chart 1).[28,29)] That is to say, the carbonyl function of ethyl 2-methylated or 2-ethylated acetylacetate was protected with (*S*,*S*)-cyclohexane-1,2-diol to give acetals (**1**). Then, the acetal **1** was alkylated, by treatment with lithium diisopropyl amide (LDA) and alkyl halide in THF-HMPA solution at $-78\,^{\circ}C$—$-40\,^{\circ}C$ in a highly diastereoselective manner to give enol ether products (**2**) (>95% de—92% de). After removal of the cyclohexane-1,2-diol moiety under acidic conditions, the resulting β-ketoesters (**3**) were converted to optically active α-alkylated α,α-disubstituted amino acids by the Schmidt rearrangement.[30)] The use of (*S*,*S*)-cyclohexane-1,2-diol afforded the *R* configuration of α,α-disubstituted amino acids, and the use of (*R*,*R*)-one gave the *S* configuration of amino acids. This synthetic strategy provides various optically active α,α-disubstituted amino acids in practice, albeit neither the synthetic steps nor chemical yields are satisfactory.[31)]

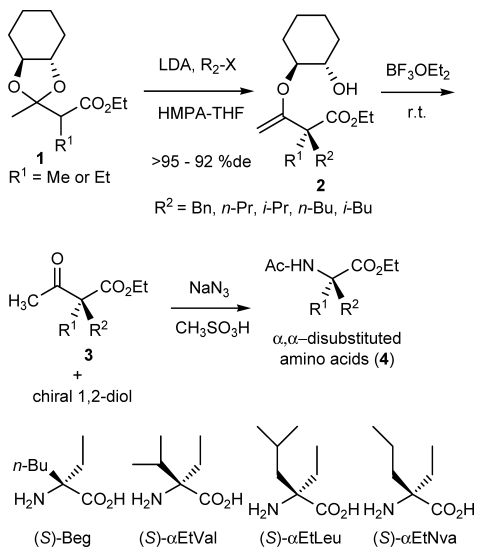

Chart 1. Diastereoselective Synthesis of Optically Active α,α-Disubstituted Amino Acids Using (*S*,*S*)-Cyclohexane-1,2-diol

5. Secondary Structure of Peptides Composed of Chiral α-Ethylated α,α-Disubstituted Amino Acids

The author prepared homopeptides composed of (*S*)-Beg; CF_3CO-{(*S*)-Beg}$_n$-OEt (n=1—6),[32)] and heteropeptides containing an (*S*)-Beg in Aib sequences; CF_3CO-{(*S*)-Beg}-$(Aib)_4$-OEt, and CF_3CO-$(Aib)_2$-{(*S*)-Beg}-$(Aib)_2$-OEt and studied the preferred secondary structures.[33,34)] The IR spectra of (*S*)-Beg homopeptides showed intramolecularly hydrogen-bonded weak N–H absorption [C–F$\cdots$H(N)$\cdots$O=C of the N-terminus] at 3380—3415 cm^{-1}, and also intramolecularly hydrogen-bonded strong peptide N–H absorption at 3335—3360 cm^{-1}. With an increase in the length of peptide, the peptide N–H absorption observed at 3335 cm^{-1} is shifted to 3360 cm^{-1}, with an increase in the relative intensity. These IR spectra are very similar to those of Deg homopeptides which preferentially form the fully planar conformation, and are different from those of Aib homopeptides which preferentially form the 3_{10}-helical structure. Also, the author studied the preferred secondary structure in $CDCl_3$ solution using ^{1}H-NMR experiments by the addition of a stable free radical, 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) or dimethyl sulfoxide (DMSO). These additives can become an acceptor of the hydrogen bond. The addition of TEMPO or DMSO had almost no influence on the peptide N–H protons of the (*S*)-Beg homopeptides in $CDCl_3$ solution. These results suggest that all of the peptide N–H protons are used for the intramolecular hydrogen bonds, meaning that the dominant secondary structure of the homopeptides in $CDCl_3$ solution is the fully planar C_5-conformation.

X-Ray crystallographic analysis revealed that the (*S*)-Beg tetrapeptide assumed the fully extended C_5-conformation in the crystal state. In the fully extended structure of the (*S*)-Beg tetrapeptide, the side-chain butyl and ethyl substituents alternate with respect to the peptide-backbone, and all of the peptide N–H amide protons are intramolecularly hydrogen-bonded to the carbonyl group of the same amino acid residues in the C_5-conformation. The fully planar structure shown in the crystal state is in good accordance with the preferred conformation in $CDCl_3$ solution (Fig. 6).[32)]

Contrary to the planar conformation of the (*S*)-Beg homopeptides, the IR and ^{1}H-NMR spectra indicated that the dominant conformation of the heteropeptides having an (*S*)-Beg in the Aib sequences seemed to be the 3_{10}-helical structure in $CDCl_3$ solution. X-Ray crystallographic analysis of CF_3CO-{(*S*)-Beg}-$(Aib)_4$-OEt showed both right-handed (*P*) and left-handed (*M*) 3_{10}-helices, while that of CF_3CO-$(Aib)_2$-{(*S*)-Beg}-$(Aib)_2$-OEt demonstrated a right-handed (*P*) 3_{10}-helix. These results mean that the heteropeptides have four Aib residues which have a strong propensity for 3_{10}-helix formation, and one (*S*)-Beg residue which might have a weak

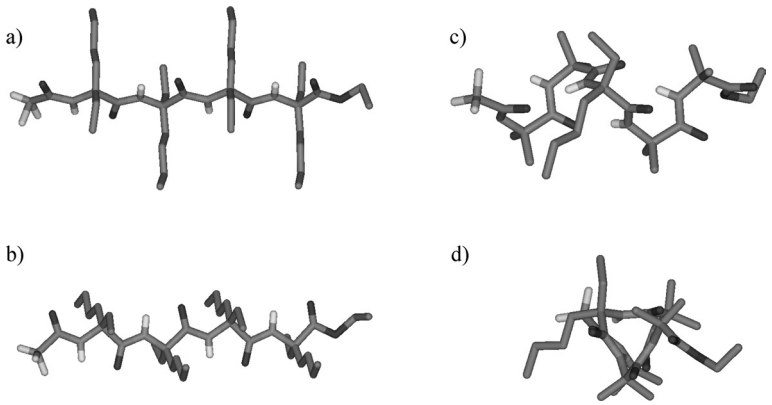

Fig. 6. Crystal Structures of (*S*)-Beg Homopeptide CF_3CO-{(*S*)-Beg}$_4$-OEt, and (*S*)-Beg Heteropeptide CF_3CO-$(Aib)_2$-{(*S*)-Beg}-$(Aib)_2$-OEt

a, b) CF_3CO-{(*S*)-Beg}$_4$-OEt: top view of planar conformation (a), side view (b), c, d) CF_3CO-$(Aib)_2$-{(*S*)-Beg}-$(Aib)_2$-OEt: 3_{10}-helix as viewed perpendicularly to the helical axis (c), as viewed along the helical axis (d).

propensity for planar structure formation and also a weak bias for the helical-screw handedness. As a result, both the diastereomeric right-handed (*P*) and left-handed (*M*) 3_{10}-helices exist in solution, and in the crystal state both the diastereomeric (*P*) and (*M*) 3_{10}-helices of CF_3CO-{(*S*)-Beg}-$(Aib)_4$-OEt exist, and (*P*) 3_{10}-helix of CF_3CO-$(Aib)_2$-{(*S*)-Beg}-$(Aib)_2$-OEt occurred either by chance or due to the effect of the crystal packing force.[33,34]

The author designed the pentapeptide CF_3CO-[{(*S*)-αEtVal}-{(*S*)-αEtLeu}-{(*S*)-αEtNva}-Deg-{(*S*)-Beg}]-OEt composed of diverse chiral α-ethylated α,α-disubstituted amino acids, and prepared the peptide starting from the C-terminal residue using 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDC) as a coupling reagent by solution-phase methods.[35] Because of steric hindrance, the yield of the pentapeptide by coupling N-terminal free tetrapeptide with α-ethylvaline was only 30% based on the recovered starting material. The IR spectra of heteropeptides (from dipeptide to pentapeptide) were very similar to those of (*S*)-Beg homopeptides, indicating the dominant conformation is the fully planar structure. The addition of radical TEMPO or DMSO to the $CDCl_3$ solution of the pentapeptide had only minimal influence on the peptide N–H protons, meaning all peptide N–H protons are used for the intramolecular hydrogen bond. These experiments also suggested that the preferred conformation in solution was the fully planar conformation.

Although the fully extended structure was preferred in $CDCl_3$ solution, the X-ray crystallographic analysis of the heteropeptides revealed different structures in the crystal state. The tripeptide CF_3CO-{(*S*)-αEtNva}-Deg-{(*S*)-Beg}-OEt formed a bent planar conformation, in which the N-terminal and C-terminal residues were planar C_5-conformation but the central Deg residue was bent. The tetrapeptide CF_3CO-{(*S*)-αEtLeu}-{(*S*)-αEtNva}-Deg-{(*S*)-Beg}-OEt assumed both diastereomeric right-handed (*P*) and left-handed (*M*) 3_{10}-helices in the ratio of 1 to 1 in the asymmetric unit of the crystal, while the pentapeptide CF_3CO-{(*S*)-αEtVal}-{(*S*)-αEtLeu}-{(*S*)-αEtNva}-Deg-{(*S*)-Beg}-OEt formed just the right-handed (*P*) 3_{10}-helix in the asymmetric unit (Fig. 7). These results might be attributed to the fact that in the 3_{10}-helical structure there are two intermolecular hydrogen bonds and the helices form one-dimensional chains,

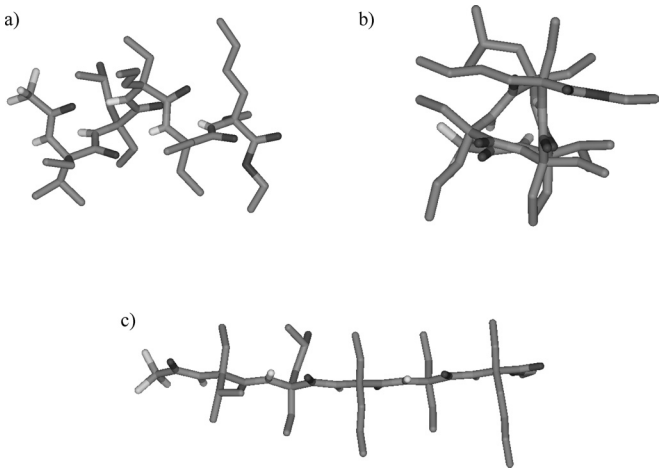

Fig. 7. Structures of α-Ethylated α,α-Disubstituted Amino Acid Heteropeptide; CF_3CO-[{(*S*)-αEtVal}-{(*S*)-αEtLeu}-{(*S*)-αEtNva}-Deg-{(*S*)-Beg}]-OEt

a, b) 3_{10}-Helix in the crystal state: as viewed perpendicularly to the helical axis (a), as viewed along the helical axis (b), c) fully extended conformation in solution by modeling.

while in the planar conformation there is no intermolecular hydrogen bond. Thus, the intermolecular hydrogen bonds affected the nucleation event, and the 3_{10}-helices that existed in a minute amount in solution occurred preferentially in the crystal state. That is to say, in solution there are both the 3_{10}-helix (minor conformation) and planar conformation (major conformation) in equilibrium, and the 3_{10}-helix as the minor conformation preferentially crystallized. The tetrapeptide formed both right-handed (*P*) and left-handed (*M*) 3_{10}-helices, and the pentapeptide assumed the right-handed (*P*) 3_{10}-helix. These results may be attributed to the fact that the pentapeptide has four chiral centers while the tetrapeptide has three chiral centers, and thus, one chiral center may be crucial for the control of helical-screw sense. On the other hand, it is possible that in solution both right-handed (*P*) and left-handed (*M*) 3_{10}-helices exist, and by chance or due to the influence of the crystal packing force,[35] the right-handed (*P*) 3_{10}-helix of the pentapeptide crystallized.

Although the fully planar secondary structure was believed to be formed in the case of achiral peptides such as Gly, Deg, and Dpg peptides, the author found that the formation of the

fully planar conformation does not require the symmetric element, and the oligopeptides composed of chiral α-ethylated α,α-disubstituted amino acids preferentially formed the fully planar conformation. It is noteworthy that the chiral α-methylated amino acid peptides prefer forming the 3_{10}-helices while the chiral α-ethylated peptides prefer forming the fully planar conformation, and thus only one methyl carbon atom at the side chain drastically changes the preferred secondary structures of their peptides.

6. Asymmetric Center at the Side Chain of α-Amino Acids

α-Helix shown as a secondary structure in proteins almost always forms a right-handed (*P*) helical-screw sense. The right-handedness of an α-helix is believed to result from the fact that L-α-amino acids, except for glycine, are chiral compounds that have an asymmetric center at the α-carbon atom. If oligopeptides composed of L-α-amino acids form left-handed α-helices, steric repulsion between the oxygen of the carbonyl function and the side-chain β-carbon atom would arise. Among the proteinogenic L-α-amino acids, isoleucine and threonine have another chiral center at the β-position in addition to the chiral carbon at the α-position. With regard to the side-chain chiral center of isoleucine and threonine, extensive research on the asymmetric syntheses of L-isoleucine, L-threonine, and their allo-amino acids has been reported. However, only scant attention has been paid to the effects of the side-chain chiral centers of amino acids on the secondary structures of their peptides. An exception is the Lorenzi group, who synthesized two kinds of homopeptides, up to hexapeptides, composed of L-isoleucine, and D-alloisoleucine, respectively, and studied their preferred conformation.[36] They reported that the spectroscopic data of two diastereomeric homopeptides showed almost no differences in the crystal state, and those of two homopeptides (up to pentapeptides) showed only small differences in solution. However, two diastereomeric hexapeptides showed different propensities for aggregation in trifluoroethanol (TFE) solution.[36] Also, the Toniolo group performed similar experiments and found that the side-chain chiral centers affected the stability of the β-sheet secondary structure.[37]

7. Design and Synthesis of Chiral Cyclic α,α-Disubstituted Amino Acid, and Its Homopeptides

The author envisaged the use of a chiral cyclic α,α-disubstituted amino acid to experimentally determine the effect of the side-chain chiral center on the secondary structure of its peptides. Chart 2 shows the structure of a chiral cyclic amino acid, (3*S*,4*S*)-1-amino-3,4-di(methoxy)cyclopentanecarboxylic acid {(*S*,*S*)-Ac_5c^{dOM}}, which was designed by the author. The cyclic amino acid (*S*,*S*)-Ac_5c^{dOM} has the following characteristics: 1) The amino acid is a new compound, which has not been designed or synthesized before; 2) The α-carbon atom is not a chiral center because both side-chain substituents are the same; 3) Asymmetric centers exist at the side-chain cyclopentane skeleton; 4) The coupling reaction of the amino acid would proceed smoothly because it has already been reported that the coupling yield of achiral cyclic amino acids was good; and 5) Hydrophilicity of the amino acid would be sufficiently high for it to be dissolved in water because the amino acid has ethereal functional groups at the side-chain cyclopentane.

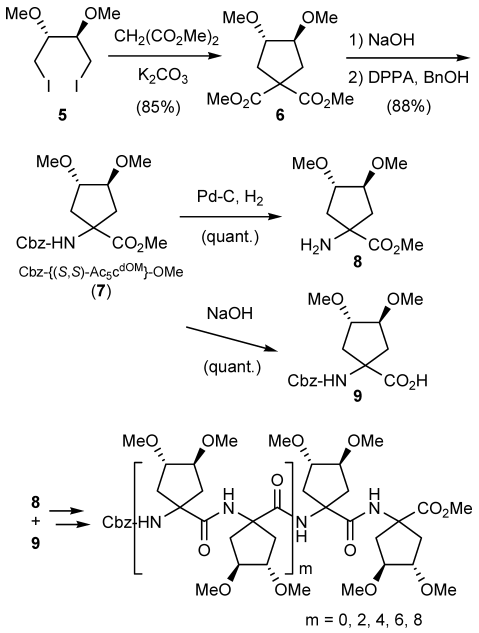

Chart 2. Synthesis of Chiral Cyclic α,α-Disubstituted Amino Acid (*S*,*S*)-Ac_5c^{dOM}, and Its Homopeptides Cbz-{(*S*,*S*)-Ac_5c^{dOM}}$_n$-OMe (n=6, 8, 10)

The chiral cyclic amino acid (*S*,*S*)-Ac_5c^{dOM} was synthesized starting from dimethyl L-(+)-tartrate as a chiral source. That is to say, diiodide (**5**) was prepared by a three-step sequence according to the reported methods,[38] and the dimethyl malonate was bisalkylated with the iodide **5** to give a 5-membered ring product (**6**) in 85% yield. Monohydrolysis of the diester **6** under basic conditions, followed by Curtius rearrangement[39] afforded both the C- and N-terminal protected amino acid Cbz-{(*S*,*S*)-Ac_5c^{dOM}}-OMe (**7**) in 88% yield. The C-terminal ester could be deprotected by hydrolysis under basic conditions, and the N-terminal Cbz-protecting group could be removed by hydrogenolysis using Pd–C as a catalyst.

The author prepared homopeptides, Cbz-{(*S*,*S*)-Ac_5c^{dOM}}$_n$-OMe (n=2, 4, 6, 8, 10), by coupling between the N-terminal free peptide H-{(*S*,*S*)-Ac_5c^{dOM}}$_n$-OMe and C-terminal free dipeptide acid Cbz-{(*S*,*S*)-Ac_5c^{dOM}}$_2$-OH using EDC as a coupling reagent. The coupling reaction proceeded without problem, and homopeptides, up to the decapeptide, were prepared. As expected, the decapeptide was readily dissolved in pure water (>5 mg/ml) due to the ethereal functional groups at the side chain.

8. Secondary Structure of Homopeptides Composed of Chiral Cyclic Amino Acid (*S*,*S*)-Ac_5c^{dOM}

At first, the preferred secondary structure of (*S*,*S*)-Ac_5c^{dOM} homopeptides in $CDCl_3$ solution was analyzed by using IR spectra. The IR spectra showed weak absorption of the hydrogen bond-free peptide N–H at 3420—3440 cm^{-1}, and strong absorption of intramolecularly hydrogen-bonded peptide N–H at 3320—3370 cm^{-1}. The latter absorption shifted from 3370 cm^{-1} to 3320 cm^{-1} with an increase in the peptide backbone length. These IR spectra are very similar to those of homopeptides composed of achiral cyclic α,α-disubstituted amino acids which form the 3_{10}-helical structures, but different from those of Deg or (*S*)-Beg homopeptides which

assume the fully planar conformation.

The rotating frame nuclear Overhauser and exchange spectroscopy (ROESY) ^{1}H-NMR spectrum of the (*S*,*S*)-Ac$_5$c^{dOM} hexapeptide showed successive d_{NN} correlations from the N-terminal N–H to the C-terminal N–H protons, suggesting the formation of the helical structure in $CDCl_3$ solution. Also, ^{1}H-NMR experiments by the addition of radical TEMPO or DMSO to the $CDCl_3$ solution indicated that the two N-terminal peptide N–H protons are easily accessible by the solvent, meaning the two peptide N–H protons are not forming the hydrogen bond. Thus, these results suggest the dominant conformation in solution is the helical structure.

The CD spectra of hexa-, octa- and decapeptides in TFE solution showed positive maxima at 208 nm and 222 nm. These results indicated that the homopeptides assumed the left-handed (*M*) helix conformation in solution. According to the R value, which is the ratio of two maxima at $\theta_{222}/\theta_{208}$, the hexapeptide preferentially formed a left-handed 3_{10}-helix, and the octa- and decapeptides formed left-handed α-helices. In water, the intensities of maxima in the CD spectra of octa- and decapeptides increased, and thus these homopeptides are more α-helical in water than in TFE solution.

The author succeeded in solving the X-ray crystallographic analysis of a hexapeptide and an octapeptide, respectively. The (*S*,*S*)-Ac$_5$c^{dOM} hexapeptide was solved in $P2_1$ space group, and there were three crystallographically independent molecules (*A*, *B*, and *C*) in the asymmetric unit. All three molecules formed left-handed 3_{10}-helices, and some conformational differences at the side chain were observed. Each molecule had four intramolecular hydrogen bonds, and there were two intermolecular hydrogen bonds among the molecules. Thus, the molecules form a head-to-tail alignment of $\cdots A \cdots B \cdots C \cdots A \cdots B \cdots C \cdots$ chains (Fig. 8). The (*S*,*S*)-Ac$_5$c^{dOM} octapeptide assumed one left-handed helix in the asymmetric unit of the crystal. Interestingly, the type of helix was not the 3_{10}-helix but the α-helix. In the α-helix of octapeptide, there were five intramolecular hydrogen bonds of type ($i \leftarrow i+4$), and intermolecularly the molecules were hydrogen-bonded by way of water molecules (Fig. 9).

Conformational search using a molecular mechanics calculation (AMBER*) produced the left-handed (*M*) α-helices as a global minimum energy conformation in the cases of both hexa- and octapeptides. The 3_{10}-helix observed in the crystal state of a hexapeptide was similar to a local minimum energy conformation (+3.22 kcal/mol) by calculation.

These results showed that the control of helical-screw handedness would be possible by the side-chain chiral centers of α-amino acids without a chiral center at the α-position. The left-handed helices of homopeptides composed of cyclic amino acid (*S*,*S*)-Ac$_5$c^{dOM} were stable even in pure water, and the 3_{10}-helix was changed to the α-helix by the elongation of peptide length from hexapeptide to octa- or decapeptides. In the case of the L-α-amino acids L-Ile and L-Thr, there is a chiral center at the α-position, and the influence of the chiral center at the α-position might be stronger than that of the side-chain chiral center, however, these experiments suggested that the side-chain chiral center might in addition affect the conformation of their peptides.[40)]

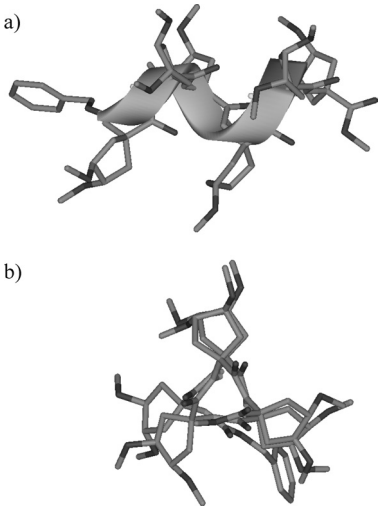

Fig. 8. Illustrative Structure of Hexapeptide Cbz-{(*S*,*S*)-Ac$_5$c^{dOM}}$_6$-OMe in the Crystal State (Molecule *A*)

a) 3_{10}-Helix as viewed perpendicularly to the 3_{10}-helical axis, b) as viewed along the 3_{10}-helical axis.

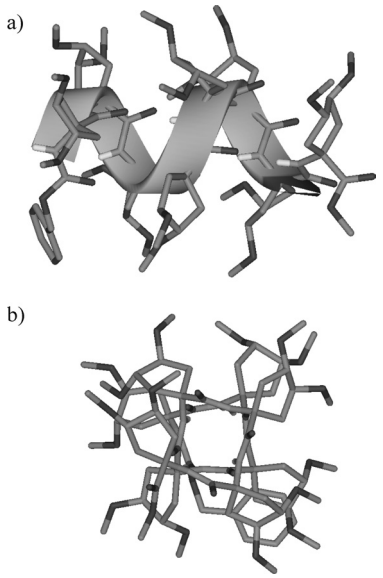

Fig. 9. Illustrative Structure of Octapeptide Cbz-{(*S*,*S*)-Ac$_5$c^{dOM}}$_8$-OMe in the Crystal State

a) α-Helix as viewed perpendicularly to the α-helical axis, b) as viewed along the α-helical axis.

9. Design and Synthesis of a Chiral Bicyclic α,α-Disubstituted Amino Acid, and Its Homopeptides

The side-chain chiral centers of (*S*,*S*)-Ac$_5$c^{dOM} controlled the helical-screw handedness of its homopeptides. However, it is not clear how strongly the side-chain chiral centers of amino acids affect the secondary structures of their peptides. To verify that the side-chain chiral centers have an effect on the secondary structures of their peptides, the author believed the synthesis of various chiral cyclic α,α-disubstituted amino acids would be needed. Thus, the author designed a chiral bicyclic α,α-disubstituted amino acid; (1*R*,6*R*)-8-aminobicyclo[4.3.0]non-3-ene-8-carboxylic acid {(*R*,*R*)-Ab$_{5,6=}$c}. It was expected that by modification of the olefin function, the bicyclic amino acid (*R*,*R*)-Ab$_{5,6=}$c and its peptides could be converted to various analogs of cyclic α,α-disubstituted

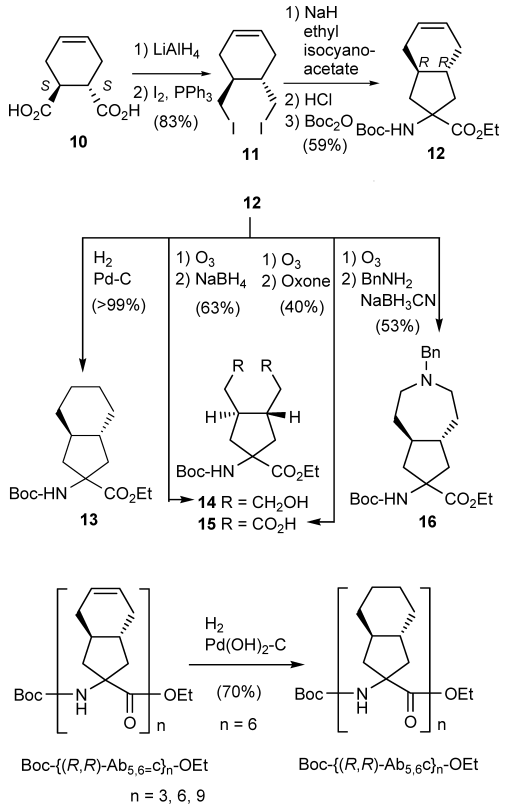

Chart 3. Synthesis of Chiral Bicyclic α,α-Disubstituted Amino Acid (R,R)-$Ab_{5,6=}c$ and Modification of Its Homopeptide Boc-{(R,R)-$Ab_{5,6=}c$}$_n$-OEt (n=6)

amino acids and their peptides (Chart 3).

The chiral bicyclic amino acid (R,R)-$Ab_{5,6=}c$ was synthesized from (*S*,*S*)-cyclohex-4-ene-1,2-dicarboxylic acid (**10**) prepared according to Bernardi's methods.[41] In other words, the acid **10** was converted to a diiodide (**11**) by reduction with $LiAlH_4$, and subsequent substitution with iodide. Then, ethyl isocyanoacetate was bisalkylated with **11**,[42] followed by acidic hydrolysis and protection of the N-terminus with Boc_2O to afford amino acid Boc-[(R,R)-$Ab_{5,6=}c$]-OEt in 59% yield. The N-terminal protecting group, and the C-terminal ester group could be quantitatively deprotected, respectively, and the N-terminal and C-terminal free amino acids were used for peptide synthesis. The olefin function in the amino acid (**12**) could be hydrogenated to afford saturated bicyclic amino acid (**13**), and also could be ozonolized to produce various cyclic amino acids. After the ozonolysis of olefin, reduction with $NaBH_4$ afforded a cyclic amino acid (**14**) having a hydroxyl function, oxidation gave an amino acid (**15**) with a dicarboxylic acid moiety, and reductive amination with $BnNH_2$ and $NaBH_3CN$ produced a bicyclic amino acid (**16**) having a 7-membered ring.

Homopeptides, Boc-{(R,R)-$Ab_{5,6=}c$}$_n$-OEt (n=3, 6, 9), were prepared by coupling between the N-terminal free peptide H-{(R,R)-$Ab_{5,6=}c$}$_n$-OEt and the C-terminal free tripeptide acid Boc-{(R,R)-$Ab_{5,6=}c$}$_3$-OH using *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as a coupling reagent. The six olefin functions in the hexapeptide Boc-{(R,R)-$Ab_{5,6=}c$}$_6$-OEt could be hydrogenated in one step to produce the saturated hexapeptide Boc-{(R,R)-$Ab_{5,6}c$}$_6$-OEt in 70% yield.

10. Secondary Structure of Homopeptides Composed of Chiral Bicyclic Amino Acid (R,R)-$Ab_{5,6=}c$

The IR spectra of the homopeptides Boc-{(R,R)-$Ab_{5,6=}c$}$_n$-OEt in $CDCl_3$ solution showed weak absorption of the hydrogen bond-free peptide N–H at 3420—3440 cm^{-1}, and strong absorption of intramolecularly hydrogen-bonded peptide N–H at 3320—3370 cm^{-1}. The latter absorption shifted from 3370 cm^{-1} (tripeptide) to 3320 cm^{-1} (nonapeptide) and the intensity increased with an increase in the peptide backbone length. These IR spectra are very similar to those of homopeptides composed of achiral cyclic α,α-disubstituted amino acids forming the helical structure.

In the ^{1}H-NMR experiments, the addition of radical TEMPO or DMSO to the $CDCl_3$ solution of hexa- and nonapeptides affected the two N-terminal peptide N–H protons, respectively. These results mean that the two N–H protons are easily accessible by the solvent, namely, the two N–H protons are free of the intramolecular hydrogen bond, suggesting the dominant conformation in $CDCl_3$ solution is the 3_{10}-helical structure. The ROESY ^{1}H-NMR spectrum of hexapeptide showed a complete series of d_{NN} correlation from the N-terminal N–H to the C-terminal N–H protons, suggesting the formation of a 3_{10}-helix, and that of nonapeptide showed partial d_{NN} correlation from the N-terminal peptide N(1)–H to the N(5)–H protons.

The CD spectra of (R,R)-$Ab_{5,6=}c$ homopeptides in TFE solution did not show characteristic maxima (208, 222 nm) for the helical structure. These results suggested that the homopeptides do not form the ordered secondary structure, or form a mixture of right-handed (*P*) and left-handed (*M*) helices in TFE solution. Taking the IR and ^{1}H-NMR spectra into consideration, both right-handed (*P*) and left-handed (*M*) helices might exist in solution. The hydrogenation of six olefins in the hexapeptide slightly changed the shape of the CD spectra, which suggests that the dominant secondary structure might be slightly changed by the hydrogenation.

X-Ray crystallographic analysis of (R,R)-$Ab_{5,6=}c$ hexapeptide showed four crystallographically independent molecules, *A*—*D*, along with two ethanol molecules. The molecules *A* and *D* showed right-handed (*P*) 3_{10}-helices, and the molecules *B* and *C* showed left-handed (*M*) 3_{10}-helices. The molecules *A* and *D*, and molecules *B* and *C*, respectively, are very similar with respect to the main-chain peptide backbone, but are different with respect to the cyclohexene structure as well as the C-terminal and N-terminal conformations. The relationship of right-handedness and left-handedness is diastereomeric because the bicyclic amino acid has chiral centers of *R*-configuration.[43] In the crystal packing, the molecules form two helical chains of $\cdots A(P)\cdots B(M)\cdots A(P)\cdots B(M)\cdots$ and $\cdots C(M)\cdots D(P)\cdots C(M)\cdots D(P)\cdots$ by intermolecular hydrogen bonds (Fig. 10).

Conformational calculation by AMBER* produced the right-handed (*P*) 3_{10}-helical conformation as a global minimum energy, and the left-handed (*M*) 3_{10}-helical conformation as a local minimum energy (+1.60 kcal/mol). Both conformations determined by calculation closely match the structures determined by the X-ray crystallographic analysis.

We succeeded in synthesizing the chiral bicyclic amino acid (R,R)-$Ab_{5,6=}c$, and its modification to various cyclic disubstituted amino acids. Also, we found that the (R,R)-$Ab_{5,6=}c$ hexapeptide having twelve chiral centers at the side-chain bi-

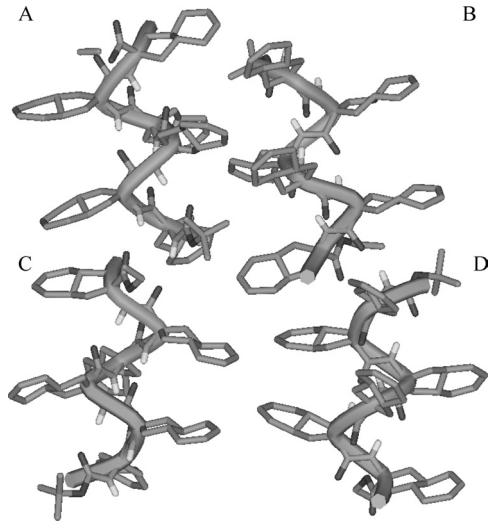

Fig. 10. Illustrative Structure of Hexapeptide Boc-{(*R*,*R*)-$Ab_{5,6=}c$}$_6$-OEt as Viewed Perpendicularly to the α-Helical Axis

Four crystallographically independent molecules, right-handed (*P*) 3_{10}-helices (*A* and *D*) and left-handed (*M*) 3_{10}-helices (*B* and *C*).

cyclic skeleton formed both diastereomeric right-handed (*P*) and left-handed (*M*) 3_{10}-helices. These results indicate that the chiral environment at the side chain is important for the control of the helical-screw direction of the peptide.[44)]

11. Design and Concise Synthetic Strategy toward Chiral Cyclic α,α-Disubstituted Amino Acids Bearing a δ-Nitrogen Atom

4-Aminopiperidine-4-carboxylic acid (Pip) is an achiral six-membered α,α-disubstituted amino acid bearing a δ-nitrogen atom, and has been focused upon because of the antimicrobial activity of its helical peptides.[45)] The author designed a new class of optically active Pip derivatives, in which asymmetric carbons exist in the appendant substituent at the δ-nitrogen atom, and believed that these chiral cyclic amino acids would be useful to study the effect of the pendant chiral moiety on the secondary structures of their peptides.

Although the Pip derivatives have already been synthesized from piperidone derivatives by the Strecker or Bücherer-Berg methods,[46)] the author developed a new synthetic route starting from dimethyl malonate (Chart 4). Briefly, bisalkylation of dimethyl malonate with 2-bromomethyl-1,3-dioxolane afforded a bis(dioxolanemethyl) diester (**17**) in 47% yield. The dioxolane group in **17** could be deprotected by 10% aqueous HCl to give a dialdehyde, which seemed to be unstable for purification. Thus, the crude dialdehyde was subjected to the next reaction without purification. After deprotection, the crude dialdehyde was condensed with chiral amines; (*S*)-phenylethylamine **a**, (*S*)-2-aminobutane **b**, and (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocamphenylamine **c** to give dienamines {**18a** (60%), **18b** (41%), and **18c** (56%)}, respectively. Hydrogenation of **18** produced the corresponding cyclic saturated diesters (**19a**—**19c**) in 67%, 86%, and 76% yields. Partial hydrolysis of the diester, followed by Curtius rearrangement, gave optically active 1-substituted Pip derivatives {**20a** (41%), **20b** (61%), and **20c** (30%)}, respectively.[47)]

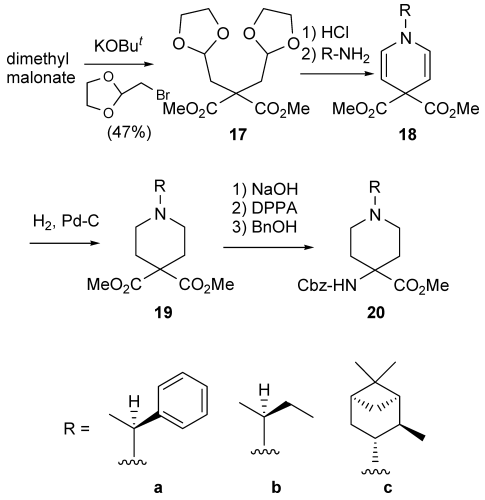

Chart 4. Synthesis of Various Optically Active Cyclic α,α-Disubstituted Amino Acids Bearing a Pendant Chiral Moiety.

This synthetic strategy can be applied to the preparation of various optically active cyclic α,α-disubstituted amino acids bearing a pendant chirality by using chiral amines, and may be applicable to combinatorial chemistry.

12. Conclusion

The author developed a practical synthetic route for optically active α-alkylated α,α-disubstituted amino acids using chiral cyclic 1,2-diol, and found that homooligopeptides composed of chiral α-ethylated α,α-disubstituted amino acid preferentially form the fully planar conformation, whereas the fully extended structure was believed to be the exception and was formed in the case of symmetrical peptides composed of achiral amino acids. The fully extended structures of peptides composed of chiral α-ethylated α,α-disubstituted amino acids contrast with the 3_{10}-helical structures of peptides composed of chiral α-methylated α,α-disubstituted amino acids.

Furthermore, the author designed and synthesized a chiral cyclic amino acid (*S*,*S*)-Ac_5c^{dOM} and a bicyclic amino acid (*R*,*R*)-$Ab_{5,6}c$, in which the α-carbon is not a chiral center but chiral centers exist at the side chain, respectively. Conformational analysis of their homopeptides revealed that the chiral centers at the side chain affect the secondary structure of their peptides, but the chiral environments such as flexibility and bulkiness, are important for the control of the helical-screw direction. Also, the author synthesized various chiral cyclic α,α-disubstituted amino acids bearing a pendant chirality, and they may be useful for the design of new peptide-foldamers.

With respect to the use of stable secondary structures of peptides, Hammer designed antimicrobial active peptides based on the helical secondary structure,[48)] and also synthesized an α,α-disubstituted amino acid peptide-inhibitor for aggregation and fibrillogenesis of amyloid β-protein based on the planar secondary structure.[49)] On the other hand, Toniolo and co-workers reported asymmetric epoxidation[50)] and oxidation reactions[51,52)] using the helical secondary structures of α,α-disubstituted amino acid peptides.[53)] The author has started to study the utilization of chiral cyclic α,α-disubstituted amino acids for drug design, and for the development

of asymmetric reactions.

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