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**Conformational studies on peptides having chiral five-membered ring amino acid with two azido or triazole functional groups within the sequence of Aib residues**

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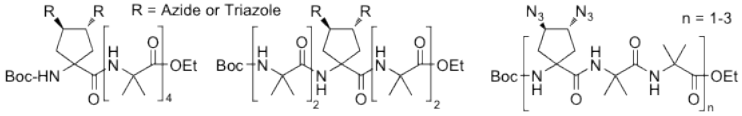

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# Conformational studies on peptides having chiral five-membered ring amino acid with two azido or triazole functional groups within the sequence of Aib residues

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ABSTRACT

The chiral cyclic α,α-disubstituted α-amino acid, (3*R*,4*R*)-1-amino-3,4-diazido-1-cyclopentanecarboxylic acid [(*R*,*R*)-Ac$_5$c$^{dN3}$], was introduced into achiral α-aminoisobutyric acid (Aib) peptides. The azido groups of (*R*,*R*)-Ac$_5$c$^{dN3}$ in the peptides were efficiently converted into 1,2,3-triazole functional groups. FT-IR, $^1$H NMR, and CD spectra revealed that the dominant conformations of all peptides in solution were 3$_{10}$-helical structures without controlling the helical-screw sense. X-ray crystallographic analyses of peptides containing (*R*,*R*)-Ac$_5$c$^{dN3}$ showed that both the right-handed (*P*) and left-handed (*M*) 3$_{10}$-helical structures were present in the crystal state.



## 1. Introduction

α-Aminoisobutyric acid (Aib)[1] and cyclic α,α-disubstituted α-amino acid (Ac$_n$c)[2] have been shown to stabilize the helical secondary structures of peptides and are widely used as helical inducers for peptide foldamers. Aib and Ac$_n$c are achiral amino acids; and thus, their peptides have no helical-screw sense bias, both right-handedness (*P*) and left-handedness (*M*). Many studies have attempted to regulate the helical-screw sense of Aib peptides by introducing chiral amino acids into the peptide sequence.[3] Controlling the direction of the helical-screw sense is essential when designing peptide foldamers for biologically active molecules[4] and asymmetric organocatalysts[5]. We recently demonstrated that the chiral cyclic α,α-disubstituted α-amino acid bearing only side-chain chiral centers, (3*R*,4*R*)-1-amino-3,4-diazido-1-cyclopentanecarboxylic acid [(*R*,*R*)-Ac$_5$c$^{dN3}$] controlled the right-handed (*P*) helical-screw sense of its homopeptide in the crystal state.[6] A feature of Ac$_5$c$^{dN3}$ was that the azido groups in the side chain could be converted into various 1,2,3-triazole functional groups by the Huisgen 1,3-dipolar cycloaddition reaction (click reaction). In the present study, we investigated whether the attachment of one, two, or three chiral amino acids bearing azido groups [(*R*,*R*)-Ac$_5$c$^{dN3}$] or 1,2,3-triazole functional groups [(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$] was capable of controlling the helical-screw sense of Aib peptides. We have designed and synthesized Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**), Boc-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_4$-OEt (**2**), Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**), Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_2$-OEt (**4**), and Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_n$-OEt [n = 1 (**5**), n = 2 (**6**), n = 3 (**7**)], and determined their preferred conformations in solution and also in the crystal state (Fig. 1).

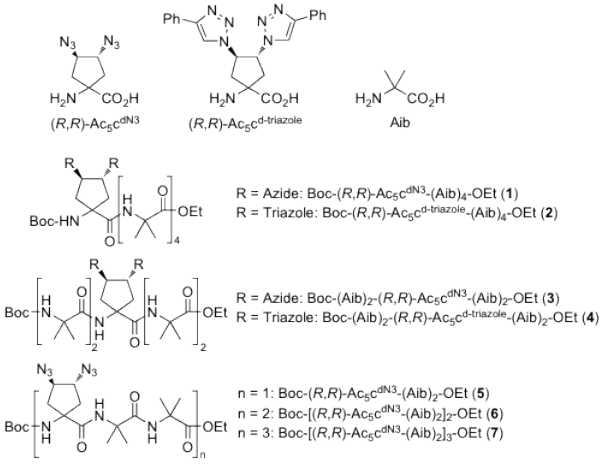


**Fig. 1.** α,α-Disubstituted α-amino acids and their peptides in the present study.

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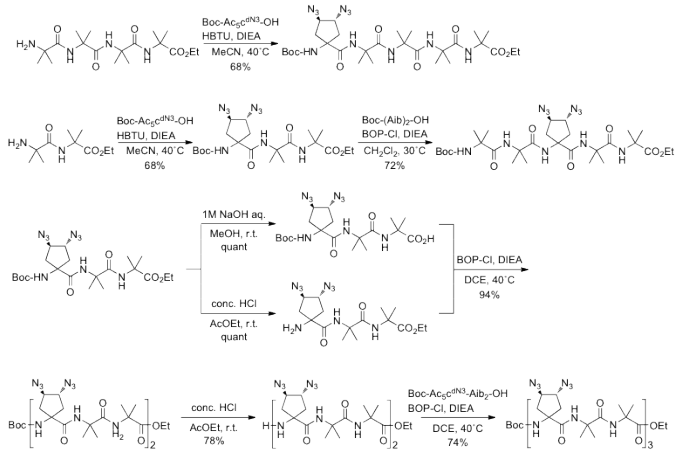
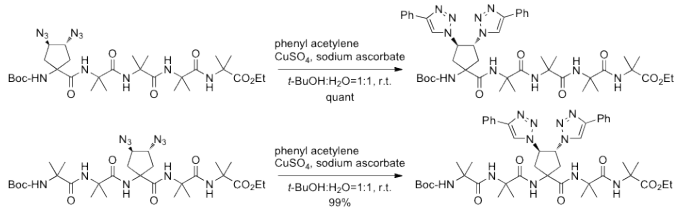
## 2. Results

### 2.1. Synthesis of amino acids and peptides

The chiral five-membered ring amino acids Boc-(*R*,*R*)-$Ac_5c^{dN3}$-OMe (**8**) and Boc-(*R*,*R*)-$Ac_5c^{d\text{-triazole}}$-OMe (**9**) were synthesized according to previously reported methods.[6] The synthesis of peptides **1**, **3**, and **5**–**7** was achieved using fragment condensation solution-phase methods with *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluoro-phosphate (HBTU) or bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) as the coupling reagent (Scheme 1).[7] The azido functional groups in peptides **1** and **3** were converted into triazole functional groups by a treatment with phenylacetylene in the presence of $CuSO_4$ and sodium ascorbate, and products **2** (quantitatively) and **4** (99%) were subsequently isolated, respectively (Scheme 2).




**Scheme 1.** Synthesis of peptides composed of (*R*,*R*)-$Ac_5c^{dN3}$ and Aib.




**Scheme 2.** Click conversion of the side-chain azido function of (*R*,*R*)-$Ac_5c^{dN3}$.

### 2.2. Conformational analysis in solution

The dominant conformations of the peptides in $CDCl_3$ solution were initially examined using by FT-IR absorption spectroscopy. Fig. 2 shows the IR absorption spectra of Boc-(*R*,*R*)-$Ac_5c^{dN3}$-$(Aib)_4$-OEt (**1**) and Boc-(*R*,*R*)-$Ac_5c^{d\text{-triazole}}$-$(Aib)_4$-OEt (**2**) (Fig. 2a), Boc-$(Aib)_2$-(*R*,*R*)-$Ac_5c^{dN3}$-$(Aib)_2$-OEt (**3**) and Boc-$(Aib)_2$-(*R*,*R*)-$Ac_5c^{d\text{-triazole}}$-$(Aib)_2$-OEt (**4**) (Fig. 2b), and Boc-[(*R*,*R*)-$Ac_5c^{dN3}$-$(Aib)_2]_n$-OEt [n = 1 (**5**), n = 2 (**6**), n = 3 (**7**)] (Fig. 2c) in the 3250–3500 $cm^{-1}$ region. The weak bands in the 3420–3440 $cm^{-1}$ region were assigned to free (solvated) peptide NH groups, and the strong bands in the 3310–3370 $cm^{-1}$ region were assigned to the intramolecularly hydrogen-bonded peptide NH groups. The IR spectra of peptides **1**–**7** were very similar to those of the helical peptides in solution,[1b,2b,3b,3c] but differed markedly from those of peptides that formed the extended planar $C_5$ conformation.[8] The intensity of the band around the 3350 $cm^{-1}$ and 3335 $cm^{-1}$ of (*R*,*R*)-$Ac_5c^{dN3}$ peptides **1** and **3**, respectively, decreased after the click reaction in (*R*,*R*)-$Ac_5c^{d\text{-triazole}}$ peptides **2** and **4**, which was consistent with the findings of a previous study.[6] Furthermore, the low-frequency band observed at 3370 $cm^{-1}$ in tripeptide **5** shifted to a lower wavelength of 3310 $cm^{-1}$ in nonapeptide **7**, and its intensity steadily increased with the elongation of the peptide length. These results were very similar to those for the $Ac_nc$ homopeptides, which adopted a helical secondary structure in solution.[2]

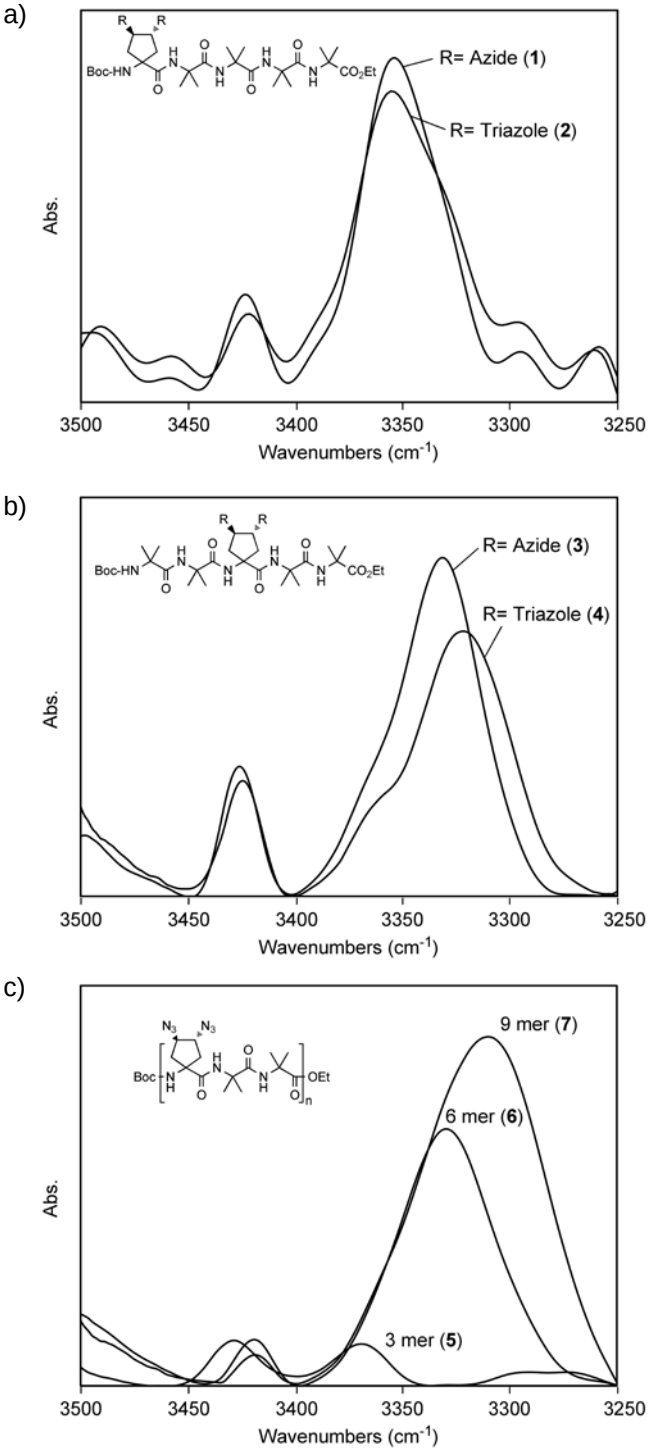



**Fig. 2.** FT-IR spectra (3250–3500 $cm^{-1}$ region) of a) pentapeptides (R = azide: **1**; R = triazole: **2**), b) pentapeptides (R = azide: **3**; R = triazole: **4**), and c) Boc-[(*R*,*R*)-$Ac_5c^{dN3}$-$(Aib)_2]_n$-OEt (n = 1, 3 mer: **5**; n = 2, 6 mer: **6**; n = 3, 9 mer: **7**) in $CDCl_3$ solution. Peptide concentrations: 1 mM (a), 10 mM (b), and 5 mM (c).

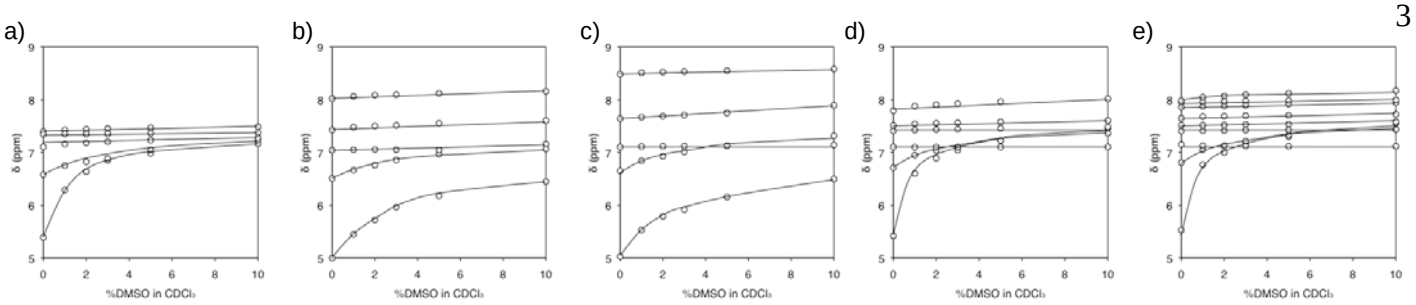



**Fig. 3.** Plots of N–H chemical shifts in the $^1$H NMR spectra of the peptides a) Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**), b) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**), c) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_2$-OEt (**4**), d) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**), and e) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_3$-OEt (**7**) as a function of an increase in the percentage of DMSO (*v*/*v*) added to the $CDCl_3$ solution. Peptide concentration: 1.0 mM.

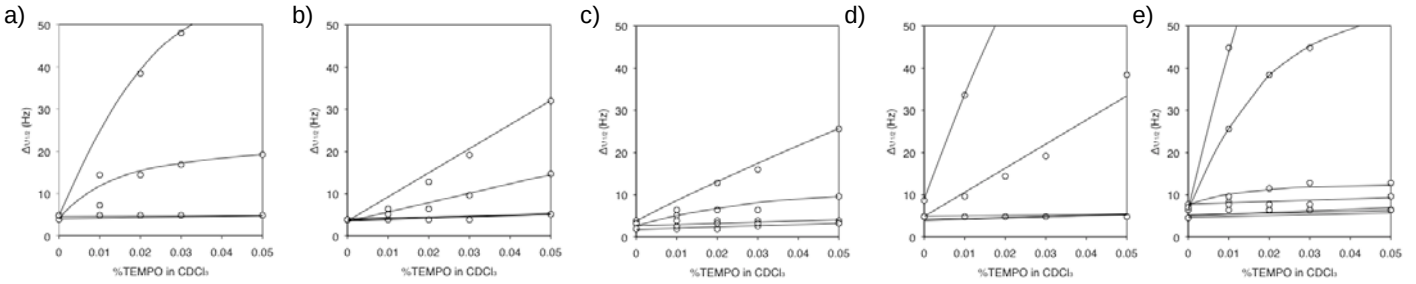
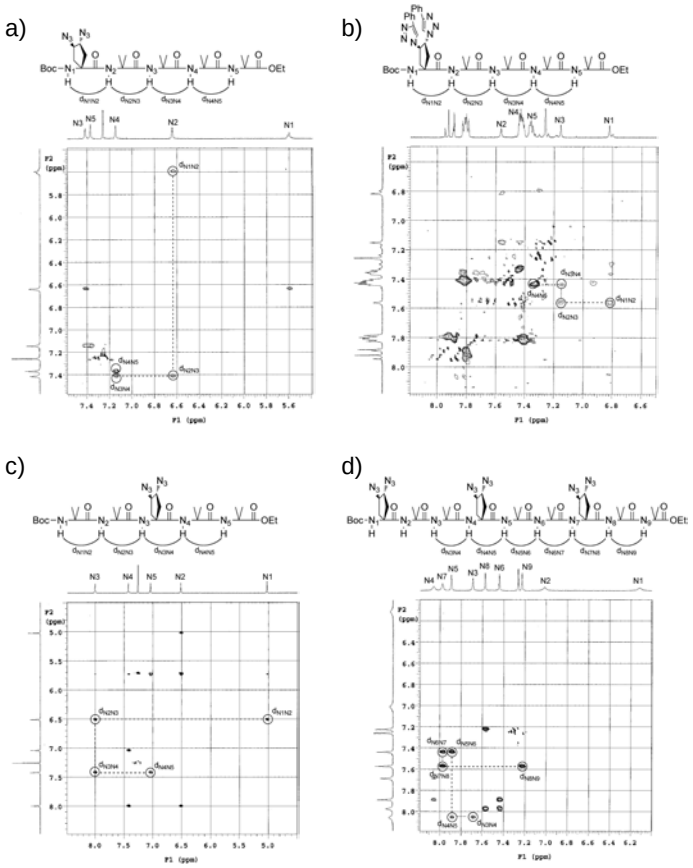



**Fig. 4**. Plots of the bandwidths of the N–H protons in the peptides a) Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**), b) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**), c) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_2$-OEt (**4**), d) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**), and e) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_3$-OEt (**7**) as a function of an increase in the percentage of TEMPO (*w*/*v*) added to the $CDCl_3$ solution. Peptide concentration: 1.0 mM.

$^1$H NMR experiments were performed for peptides **1**, **3**, **4**, **6**, and **7** in $CDCl_3$ solution with the addition of dimethyl sulfoxide (DMSO; 0–10% (*v*/*v*)) (Fig. 3) or the free radical 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; 0–0.05% (*w*/*v*)) (Fig. 4). Peptide **2** could not be analyzed because of the overlapping of several NH protons with the signals of triazole functional groups. Free NH groups without intramolecular hydrogen bonds were affected by the addition of DMSO or TEMPO. In the $^1$H NMR spectra of peptides **1**, **3**, **4**, **6**, and **7**, the N(1)H signals at the *N*-terminus could be unambiguously determined by their high-field positions at δ = 5.39 ppm in **1**, δ = 5.00 ppm in **3**, δ = 5.03 ppm in **4**, δ = 5.42 ppm in **6**, and δ = 5.53 ppm in **7**, due to their urethane structure, whereas the remaining four, five, or eight NH protons could not be assigned at this stage. Two NH chemical shifts in peptides **1**, **3**, **4**, **6**, and **7** were sensitive to the addition of the perturbing reagent DMSO, and the bandwidths of two NH proton signals were broadened by the addition of the TEMPO radical. These results demonstrated that the two NH protons were solvent-exposed, which suggested that they may not have been intramolecularly hydrogen-bonded. Therefore, peptides **1**, **3**, **4**, **6**, and **7** may have adopted a $3_{10}$-helical structure, in which two NH groups [N(1)H and N(2)H] were free (solvated) of the intramolecular hydrogen bond.

The nuclear Overhauser effect spectroscopy (NOESY) and/or rotating-frame nuclear Overhauser effect spectroscopy (ROESY) $^1$H NMR spectra of peptides **1**–**4**, **6**, and **7** were measured in $CDCl_3$ solution (Fig. 5). The NOESY $^1$H NMR spectra of peptides **1**–**4** and **6** revealed a complete series of sequential NH (*i*→*i*+1) dipolar interactions, from the *N*-terminal N(1)H to the *C*-terminal N(5)H or N(6)H, respectively. Sequential NH (*i*→*i*+1) dipolar interactions were used to identify the helical structures;[9] however, these interactions alone did not enable an assessment of regarding whether a $3_{10}$- or α-helical conformation was present. The ROESY $^1$H NMR spectrum of nonapeptide **7** showed a series of sequential NH (*i*→*i*+1) dipolar interactions from the N(3)H to the *C*-terminal N(9)H, respectively. Although the interactions $d_{NN}$ (*1*→*2*) and $d_{NN}$ (*2*→*3*) are not shown, the series of sequential NOE cross peaks suggested the helical structure of **7**. In L-α-amino acid peptides and proteins, two NOE constraints, [$d_{\alpha N}$ (*i*→*i*+2)] and [$d_{\alpha N}$ (*i*→*i*+4)] were useful for the assignment of a $3_{10}$- or α-helical structure. No hydrogen at the α-carbon atom in the peptides **1**–**4**, **6**, and **7**; thus, we could not discriminate a $3_{10}$- or α-helical conformation.



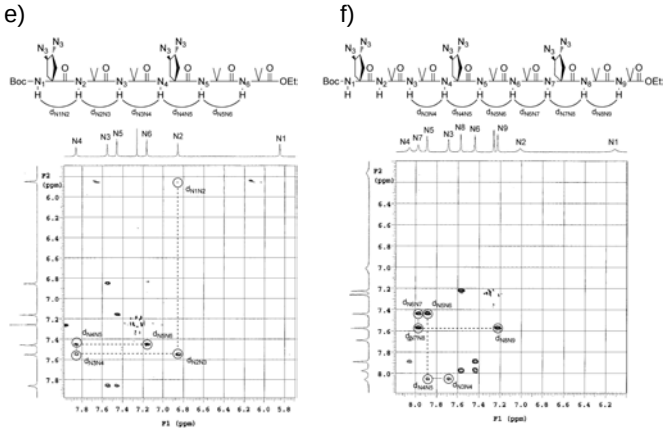



**Fig. 5.** The NOESY $^1$H NMR spectra of a) Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**), b) Boc-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_4$-OEt (**2**), c) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**), d) Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{d\text{-triazole}}$-(Aib)$_2$-OEt (**4**), and e) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**), and the ROESY $^1$H NMR spectrum of f) Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_3$-OEt (**7**).

The CD spectra of peptides **1**–**4**, **6**, and **7**, and amino acids **8** and **9** were measured in 2,2,2-trifluoroethanol solution to obtain information on the helical-screw sense of the peptides (Fig. S1 in the Supplementary Data). However, the spectra of peptides **1**–**4**, **6**, and **7** did not show the maximum characteristic of a helical structure (208 nm and 222 nm), which suggested the existence of roughly equivalent amounts of both right-handed (*P*) and left-handed (*M*) helices. The conversion of azido functional groups into triazole groups enhanced the intensity of the spectra (Figs. S1a and S1b); however, the chromophore of azido and triazole functional groups may have directly affected the CD spectra of their peptides (Figs. S1d and S1e).

### 2.3. Conformational analysis in the crystal state

**Table 1.** Crystal and diffraction parameters of pentapeptide **3** and hexapeptide **6**.

| | Pentapeptide **3** | Hexapeptide **6** |
|---|---|---|
| Empirical formula | $C_{29}H_{49}N_{11}O_8$ | $C_{35}H_{46}N_{18}O_9$ |
| *Mr* | 679.79 | 872.98 |
| Crystal diameters [mm] | 0.50 × 0.25 × 0.20 | 0.40 × 0.30 × 0.25 |
| Crystal system | monoclinic | monoclinic |
| Lattice parameters: | | |
| *a*, *b*, *c* [Å] | 16.761, 11.651, 20.192 | 15.472, 17.458, 17.110 |
| *α*, *β*, *γ* [°] | 90, 112.95, 90 | 90, 94.081, 90 |
| *V* [Å$^3$] | 3631.0 | 4610.0 |
| Space group | *P2$_1$* | *P2$_1$* |
| *Z* value | 4 | 4 |
| *D* calc [g/cm$^3$] | 1.244 | 1.258 |
| *μ* (Mo*Kα*) [cm$^{-1}$] | 0.93 | 0.94 |
| No. of observations | 8386 [$I > 2\sigma(I)$] | 9044 [$I > 2\sigma(I)$] |
| No. of variables | 884 | 1178 |
| $R_1$, $R_w$ | 0.0387, 0.1030 | 0.0947, 0.2571 |
| Solvent | MeOH/H$_2$O | MeOH/H$_2$O |

Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**) and Boc-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**) formed good crystals for X-ray crystallographic analysis due to slow evaporation of the solvent (MeOH/H$_2$O) at room temperature.[10] The crystal and diffraction parameters of **3** and **6** are summarized in Table 1. The molecular structures of **3** and **6** are given in Figs. 6 and 7, respectively. Relevant backbone and side-chain torsion angles as well as intra- and intermolecular hydrogen-bond parameters are listed in Tables 2 and 3, respectively.

**Table 2.** Selected torsion angles *ω*, *ϕ*, and *ψ* [°] for **3** and **6**, as determined by X-ray crystallographic analysis.

| Torsion | Pentapeptide **3** | | Hexapeptide **6** | |
|---|---|---|---|---|
| Angle | *A* | *B* | *C* | *D* |
| *θ*0 | –171.4 | 175.6 | –176.2 | 178.4 |
| *ω*0 | –166.9 | 163.9 | –170.0 | 171.8 |
| *ϕ*1 | –57.6 | 58.2 | –62.2 | 59.8 |
| *ψ*1 | –38.8 | 40.6 | –36.9 | 37.3 |
| *ω*1 | –176.1 | 175.4 | –172.3 | 171.9 |
| *ϕ*2 | –51.8 | 52.7 | –52.6 | 52.5 |
| *ψ*2 | –36.0 | 35.3 | –37.6 | 36.9 |
| *ω*2 | –173.5 | 173.1 | –176.1 | 176.4 |
| *ϕ*3 | –59.9 | 59.9 | –55.4 | 56.3 |
| *ψ*3 | –28.1 | 28.0 | –28.3 | 28.9 |
| *ω*3 | –179.7 | 179.6 | 179.9 | –179.3 |
| *ϕ*4 | –57.7 | 59.5 | –59.8 | 56.6 |
| *ψ*4 | –38.0 | 36.2 | –20.2 | 24.7 |
| *ω*4 | –175.2 | 174.8 | 178.0 | –179.0 |
| *ϕ*5 | –55.0 | 55.0 | –50.9 | 48.2 |
| *ψ*5 | –49.0 | 45.8 | –44.4 | 42.1 |
| *ω*5 | 178.8 | –179.5 | –175.9 | 166.4 |
| *ϕ*6 | — | — | 28.7 | –56.0 |
| *ψ*6 | — | — | 63.7 | 153.1 |
| *ω*6 | — | — | –166.3 | 171.1 |
| *χ*1 | — | — | 130.4 | 101.8 |
| *χ*1 | — | — | –103.7 | –80.5 |
| *χ*3 | 138.1 | 149.0 | — | — |
| *χ*3 | –111.0 | –122.7 | — | — |
| *χ*4 | — | — | 155.2 | 153.1 |
| *χ*4 | — | — | –82.8 | –127.2 |

**Table 3.** Intra- and intermolecular H-bond parameters for **3** and **6**.

| Peptide | Donor | Acceptor | Distance [Å] | Angle [°] | Symmetry operation |
|---|---|---|---|---|---|
| Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**) | | | | | |
| *A* (*P*) | $N_{3a}$-H | $O_{0a}$ | 2.95 | 150 | x, y, z |

| | | | | | |
|---|---|---|---|---|---|
| | $N_{4a}$-H | $O_{1a}$ | 2.97 | 151 | x, y, z |
| | $N_{5a}$-H | $O_{2a}$ | 3.07 | 144 | x, y, z |
| *B* (*M*) | $N_{3b}$-H | $O_{0b}$ | 2.95 | 149 | x, y, z |
| | $N_{4b}$-H | $O_{1b}$ | 2.98 | 152 | x, y, z |
| | $N_{5b}$-H | $O_{2b}$ | 3.05 | 145 | x, y, z |
| | $N_{1a}$-H | $O_{4a'}$ | 2.90 | 170 | x, 1+y, z |
| | $N_{2a}$-H | $O_{5a'}$ | 3.00 | 141 | x, 1+y, z |
| | $N_{1b}$-H | $O_{4b'}$ | 2.91 | 171 | x, –1+y, z |
| | $N_{2b}$-H | $O_{5b'}$ | 3.00 | 142 | x, –1+y, z |
| Boc-[(*R*,*R*)-$Ac_5c^{dN3}$-Aib-Aib]$_2$-OEt (**6**) | | | | | |
| *C* (*P*) | $N_{3c}$-H | $O_{0c}$ | 3.37[a] | 147 | x, y, z |
| | $N_{4c}$-H | $O_{1c}$ | 2.88 | 155 | x, y, z |
| | $N_{5c}$-H | $O_{2c}$ | 2.94 | 163 | x, y, z |
| | $N_{6c}$-H | $O_{3c}$ | 3.10 | 145 | x, y, z |
| *D* (*M*) | $N_{3d}$-H | $O_{0d}$ | 3.27[a] | 146 | x, y, z |
| | $N_{4d}$-H | $O_{1d}$ | 2.87 | 150 | x, y, z |
| | $N_{5d}$-H | $O_{2d}$ | 2.95 | 163 | x, y, z |
| | $N_{6d}$-H | $O_{3d}$ | 3.12 | 163 | x, y, z |
| | $N_{1c}$-H | $O_{4d'}$ | 2.85 | 168 | 1–x, 0.5+y, 1–z |
| | $N_{2c}$-H | $O_{5d'}$ | 2.99 | 124 | 1–x, 0.5+y, 1–z |
| | $N_{1d}$-H | $O_{4c'}$ | 2.86 | 167 | –x, 0.5+y, –z |
| | $N_{2d}$-H | $O_{5c'}$ | 2.94 | 124 | –x, 0.5+y, –z |

[a]The D···A distance is a bit long for a hydrogen bond.

Two crystallographically independent conformers *A* and *B* existed in the asymmetric unit of Boc-(Aib)$_2$-(*R*,*R*)-$Ac_5c^{dN3}$-(Aib)$_2$-OEt (**3**) (Fig. 6). One azido group in conformer *B* was disordered. Conformer *A* was a right-handed (*P*) $3_{10}$-helical structure while conformer *B* was a left-handed (*M*) $3_{10}$-helical structure; thus, the relationship between the two conformers was diastereomeric (*P*) and (*M*) helices. The average values of the torsion angles $\phi$ and $\psi$ of amino acid residues (1–5) were –56.4°, –38.0° in conformer *A*, and 57.1°, 37.2° in conformer *B*. The three consecutive intramolecular hydrogen bonds of $i \leftarrow i$+3 type ($i$ = 0–2), that correspond to the $3_{10}$-helical structure were detcted in each conformer. Conformer *A* had three intramolecular hydrogen bonds between the H-N(3a) and C(0a)=O(0a) [N(3a)···O(0a) = 2.95 Å; N–H···O 150°], the H-N(4a) and C(1a)=O(1a) [N(4a)···O(1a) = 2.97 Å; N–H···O 151°], and the H-N(5a) and C(2a)=O(2a) [N(5a)···O(2a) = 3.07 Å; N–H···O 144°]. Conformer *B* shows three intramolecular hydrogen bonds between the H-N(3b) and C(0b)=O(0b) [N(3b)···O(0b) = 2.95 Å; N–H···O 149°], the H-N(4b) and C(1b)=O(1b) [N(4b)···O(1b) = 2.98 Å; N–H···O 152°], and the H-N(5a) and C(2a)=O(2a) [N(5a)···O(2a) = 3.05 Å; N–H···O 145°]. In the packing mode, conformer *A* was connected by intermolecular hydrogen bonds between the H-N(1a) and C(4a')=O(4a') [N(1a)···O(4a') = 2.90 Å; N–H···O 170°] and the H-N(2a) and C(5a')=O(5a') [N(2a)···O(5a') = 3.00 Å; N–H···O 141°] of a symmetry-related molecule (x, 1+y, z), while conformer *B* was connected by intermolecular hydrogen bonds between the H-N(1b) and C(4b')=O(4b') [N(1b)···O(4b') = 2.91 Å; N–H···O 171°] and the H-N(2b) and C(5b')=O(5b') [N(2b)···O(5b') = 3.00 Å; N–H···O 142°] of a symmetry-related molecule (x, –1+y, z), thereby forming a head-to-tail alignment of $3_{10}$-helical chains, *i.e.*, ···*A*···*A*···*A*··· and ···*B*···*B*···*B*···.

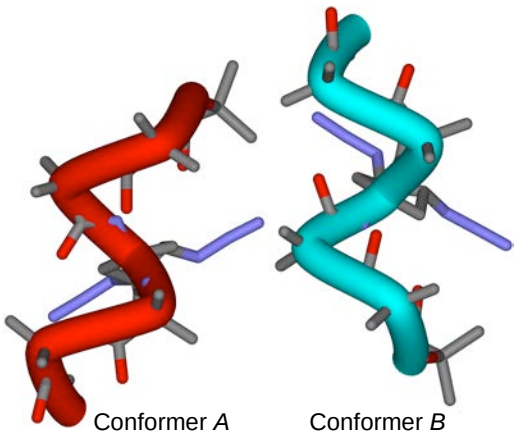



**Fig. 6.** $3_{10}$-Helical secondary structures of Boc-(Aib)$_2$-(*R*,*R*)-$Ac_5c^{dN3}$-(Aib)$_2$-OEt (**3**), determined by X-ray crystallographic analysis.

The structure of Boc-[(*R*,*R*)-$Ac_5c^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**) was solved in the space group $P2_1$. Two crystallographically independent molecules *C* and *D* were found in the asymmetric unit. A five-membered ring at residue 1 in conformer *C* was disordered by puckering. Conformers *C* and *D* were folded into (*P*) and (*M*) $3_{10}$-helical structures, respectively (Fig. 7). The mean values of the torsion angles $\phi$ and $\psi$ of the amino acid residues (1–5) were –56.2° and –33.5° in conformer *C*. Reversal of the torsion angles occurred at the *C*-terminus residues, that is, the values of the $\phi$ and $\psi$ torsion angles of Aib(6) were 28.7°, 63.7° in conformer *C*. The average torsion angles $\phi$ and $\psi$ of the amino acid residues (1–5) in conformer *D* (54.7° and 34.0°) were closed to those for an ideal left-handed (*M*) $3_{10}$-helical structure (60° and 30°), while the values of the $\phi$ and $\psi$ torsion angles of Aib(6) were –56.0°, 153.1°, which appeared to be a half extended conformation. In conformers *C* and *D*, two hydrogen bonds of the $i \leftarrow i$+3 type were observed between the H-N(4c, d) and C(1c, d)=O(1c, d) [N(4c, d)···O(1c, d) = 2.88 Å (c), 2.87 Å (d); N–H···O 155° (c), 150° (d)], and the H-N(5c, d) and C(2c, d)=O(2c, d) [N(5c, d)···O(2c, d) = 2.94 Å (c), 2.95 Å (d); N–H···O 163° (c), 163° (d)]. Furthermore, one weak hydrogen bond was observed between the H-N(6c, d) and C(3c, d)=O(3c, d) [N(6c, d)···O(3c, d) = 3.10 Å (c), 3.12 Å (d); N–H···O 145° (c), 163° (d)]. The distance between the H-N(3c, d) and C(0c, d)=O(0c, d) [N(3c, d)···O(0c, d) = 3.37 Å (c), 3.27 Å (d); N–H···O 147° (c), 146° (d)] was a bit long for an intramolecular hydrogen bond. In the packing mode, four intermolecular hydrogen bonds were observed between the H-N(1c) and C(4d')=O(4d') [N(1c)···O(4d') = 2.85 Å; N–H···O 168°] and the H-N(2c) and C(5d')=O(5d') [N(2c)···O(5d') = 2.99 Å; N–H···O 124°] of a symmetry-related molecule (1–x, 0.5+y, 1–z), and the H-N(1d) and C(4c')=O(4c') [N(1d)···O(4c') = 2.86 Å; N–H···O 167°] and between the H-N(2d) and C(5c')=O(5c') [N(2d)···O(5c') = 2.94 Å; N–H···O 124°] of a symmetry-related molecule (–x, 0.5+y, –z). The $3_{10}$-helical chains of *C* and *D* were packed to form a head-to-tail alignment of chain ···*A*···*B*···*A*···*B*···*A*···*B*···.

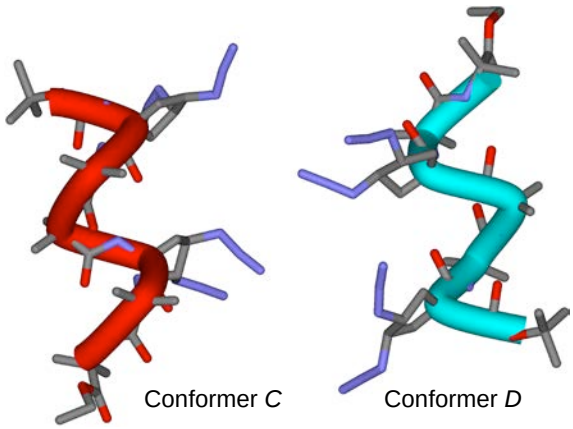



**Fig. 7.** $3_{10}$-Helical secondary structures of Boc-[(*R*,*R*)-$Ac_5c^{dN3}$-$(Aib)_2$]$_2$-OEt (**6**), determined by X-ray crystallographic analysis.

## 3. Discussion

An optically active cyclic amino acid (*R*,*R*)-$Ac_5c^{dN3}$ having two azido functional groups was synthesized from L-tartaric acid.[6] The synthesis of peptides **1**, **3**, and **5**–**7**, which were composed of (*R*,*R*)-$Ac_5c^{dN3}$ and Aib, was achieved using the fragment condensation solution-phase method with HBTU or BOP-Cl as the coupling reagent with moderate yields (68–94%) (Scheme 1). In the click reaction of the azido groups in peptides **1** and **3** with phenylacetylene, the isolated products were di(1,2,3-triazole) products with excellent yields (99–100%) (Scheme 2), but not mono(1,2,3-triazole) products, which is consistent with the findings of a previous study.[6]

The preferred conformation of peptides **1**–**7** was determined in solution. The FT-IR spectra of the peptides were very similar to those of the Aib and $Ac_nc$ peptides,[1b,2b] which indicated the existence of helices (Fig. 2). The NOESY (ROESY) $^1$H NMR spectra of the peptides showed a complete (peptides **1**–**4** and **6**) or partial (peptide **7**) series of sequential NH dipolar interactions [$d_{NN}$ ($i \rightarrow i$+1)] from the *N*-terminal NH to the *C*-terminal NH, which suggested the formation of helical structures (Fig. 5). The $^1$H NMR measurements obtained for peptides **1**, **3**, **4**, **6**, and **7** in the presence of DMSO (Fig. 3) or the TEMPO radical (Fig. 4) suggested the existence of $3_{10}$-helical conformations in solution because two NH proton signals were affected by both additives. However, the CD spectra of peptides **1**–**4**, **6**, and **7** did not show characteristic maxima for a helical structure (Fig. S1). The chromophore of the azido and triazole functional groups appeared to directly affect the CD spectra (Figs. S1d and S1e). Taken together, all the peptides formed $3_{10}$-helical structures in solution without controlling their helical-screw sense. These results were consistent with the findings of previous studies, in which one chiral α,α-disubstituted α-amino acid could not control the $3_{10}$-helical-screw sense of the Aib tetrapeptides.[3b,3c,3e] Although peptides **6** and **7** possessed two and three (*R*,*R*)-$Ac_5c^{dN3}$ in four and six Aib residues, respectively, they did not immobilize the helical structures in one direction. More (*R*,*R*)-$Ac_5c^{dN3}$ or (*R*,*R*)-$Ac_5c^{d\text{-triazole}}$ amino acids should be introduced to control the helical-screw sense of Aib peptides. Furthermore, conversion of the azido groups of peptides into triazole groups did not cause a marked conformational change. A bulkier substituent than the phenyl group may have affected the secondary strucutre. X-ray crystallographic analyses revealed that the conformations of pentapeptide **3** and hexapeptide **6** were both right-handed (*P*) and left-handed (*M*) $3_{10}$-helical structures in the crystal state. The helical-screw sense of the peptides designed in this study was not controllable not only in solution, but also in the crystal state.

## 4. Conclusion

The chiral cyclic α,α-disubstituted α-amino acids (*R*,*R*)-$Ac_5c^{dN3}$ and (*R*,*R*)-$Ac_5c^{d\text{-triazole}}$ with azido and triazole functional groups in the side chain, respectively, were introduced into Aib peptides. The dominant conformations of the peptides in solution were found to be $3_{10}$-helical structures by FT-IR, $^1$H NMR, and 2D NOESY and/or ROESY spectra. Furthermore, the CD spectra suggested the existence of roughly equivalent amounts of both right-handed (*P*) and left-handed (*M*) helices. The preferred conformations of peptides **3** and **6** were studied by X-ray crystallographic analyses, and the results obtained indicated the existence of both right-handed (*P*) and left-handed (*M*) $3_{10}$-helices in the crystal state. The side-chain chiral centers in (*R*,*R*)-$Ac_5c^{dN3}$ and (*R*,*R*)-$Ac_5c^{d\text{-triazole}}$ could not control the helical-screw sense to one-handedness in these Aib-based peptides.

## 5. Experimental section

### 5.1. General

(3*R*,4*R*)-3,4-Diazido-1-(*tert*-butoxycarbonylamino)cyclopentanecarboxylic acid methyl ester [Boc-(*R*,*R*)-$Ac_5c^{dN3}$-OMe: **8**], Boc-(*R*,*R*)-$Ac_5c^{d\text{-triazole}}$-OMe (**9**), Boc-(*R*,*R*)-$Ac_5c^{dN3}$-OH, H-$(Aib)_4$-OEt, H-$(Aib)_2$-OEt, and Boc-$(Aib)_2$-OH were prepared according to the previously reported methods.[1,2] Optical rotations $[\alpha]^{rt}_D$ were measured with a Jasco DIP-316 polarimeter using a 0.5 or 1.0 dm cell. Circular dichroism (CD) spectra were measured with a Jasco J-720W spectropolarimeter using a 1.0 mm path length cell. Infrared (IR) spectra were recorded on a Nicolet Avatar-320 spectrometer, Shimazu IRAffinity-1, or Jasco FT/IR-420 spectrometer for conventional measurement (KBr), and the solution ($CDCl_3$) method using 0.1 mm path length of NaCl cell. $^1$H NMR spectra were determined at 400 or 500 MHz (Varian Unity). FAB-MS spectra were taken on a Jeol JMS-D300 or Jeol JMS-SX 102 spectrometer.

### 5.2. Synthesis of peptides

*5.2.1. Boc-(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**).* A solution of acid Boc-(*R*,*R*)-$Ac_5c^{dN3}$-OH (125 mg, 0.40 mmol), amine H-$(Aib)_4$-OEt (147 mg, 0.38 mmol), *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluoro-phosphate (HBTU, 151 mg, 0.40 mmol), and $^i$Pr$_2$EtN (130 μL, 0.76 mmol) in MeCN (2 mL) was stirred at 40˚C for 4 days. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% MeOH in $CHCl_3$ gave pentapeptide **1** (177 mg, 68%) as colorless crystals: m.p. 243˚C (decomp.); $[\alpha]_D^{23}$ = –13.2 (*c* 1.00, $CHCl_3$); IR ($CDCl_3$) *ν* 3348, 2985, 2360, 2111, 1730, 1676, 1527, 1256 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) *δ* 7.42 (br s, 1H), 7.37 (br s, 1H), 7.14 (br s, 1H), 6.62 (br s, 1H), 5.54 (br s, 1H), 4.14 (q, *J* = 6.8 Hz, 2H), 3.98—3.99 (m, 2H), 2.79 (m, 1H), 2.51 (m, 1H), 2.28 (m, 1H), 1.80 (m, 1H), 1.55 (s, 4H), 1.52 (s, 10H), 1.46—1.48 (m, 15H), 1.44 (s, 4H), 1.25 (t, *J* = 6.8 Hz, 3H); FAB(+)HRMS calcd for $C_{29}H_{50}N_{11}O_8$ [$M^+$ + H]: 680.3844; found: 680.3812.

*5.2.2. Boc-(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**5**).* A solution of acid Boc-(*R*,*R*)-$Ac_5c^{dN3}$-OH (196 mg, 0.63 mmol), amine H-$(Aib)_2$-OEt (136 mg, 0.63 mmol), HBTU (239 mg, 0.63 mmol), and $^i$Pr$_2$EtN (220 μL, 1.26 mmol) in MeCN (10 mL) was stirred at 40˚C for 2

days. After evaporation of the solvent, the residue was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 30% EtOAc in *n*-hexane gave tripeptide **5** (218 mg, 68%) as colorless crystals: m.p. 158—160˚C; $[\alpha]_D^{23}$ = –15.8 (*c* 1.05, $CHCl_3$); IR ($CDCl_3$) *ν* 3293, 2983, 2106, 1727, 1694, 1660, 1530, 1254, 1172 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 7.07 (br s, 1H), 6.62 (br s, 1H), 5.07 (br s, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.93 (m, 1H), 3.85 (m, 1H), 2.77 (m, 1H), 2.44 (m, 1H), 2.20 (m, 1H), 1.71 (m, 1H), 1.49 (s, 7H), 1.45—1.47 (m, 14H), 1.19 (t, *J* = 7.4 Hz, 3H); FAB(+)HRMS calcd for $C_{21}H_{36}N_9O_6$ $[M^+ + H]$: 510.2789; found: 510.2792.

*5.2.3. Boc-(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OH.* A solution of tripeptide **5** (47 mg, 0.098 mmol) in MeOH (3 mL) and 0.1 M aqueous NaOH (8.9 mL, 0.89 mmol) was stirred at room temperature for 24 h. After acidification with 1M aqueous HCl, MeOH was evaporated. The aqueous solution was then extracted with EtOAc, and dried over $Na_2SO_4$. Removal of the solvent produced a crude carboxylic acid Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OH (47 mg, quant), which was used for next reaction without purification: $^1H$ NMR (400 MHz, $CD_3OD$) *δ* 7.87 (br s, 1H), 7.57 (br s, 1H), 3.81—3.95 (m, 2H), 2.84 (m, 1H), 2.25 (m, 1H), 2.12 (m, 1H), 1.75 (m, 1H), 1.47 (s, 21H).

*5.2.4. H-(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt.* A mixture of tripeptide **5** (218 mg, 0.43 mmol) in concentrated aqueous HCl (3 mL) and EtOAc (10 mL) was stirred at room temperature for 12 h. The solution was alkalized with saturated aqueous $NaHCO_3$, extracted with EtOAc, dried over $MgSO_4$. Removal of the solvent produced a crude amine H-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (176 mg, quant), which was used for next reaction without purification: $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 8.09 (br s, 1H), 7.29 (br s, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 4.03 (m, 1H), 3.95 (m, 1H), 2.73 (m, 1H), 2.43 (m, 1H), 2.03 (m, 1H), 1.76 (br s, 1H), 1.55 (s, 12H), 1.26 (t, *J* = 7.2 Hz, 3H).

*5.2.5. Boc-(Aib)$_2$-(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**).* A solution of acid Boc-(Aib)$_2$-OH (62 mg, 0.215 mmol), amine H-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (73 mg, 0.18 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl, 55 mg, 0.215 mmol), and $^i$Pr$_2$EtN (63 μL, 0.36 mmol) in $CH_2Cl_2$ (3 mL) was stirred at 30˚C for 7 days. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 25% EtOAc in *n*-hexane gave pentapeptide **3** (63 mg, 72% based on recovered material) as colorless crystals: m.p. 211—213˚C; $[\alpha]_D^{23}$ = +12.0 (*c* 1.05, $CHCl_3$); IR ($CDCl_3$) *ν* 3291, 2986, 2111, 1731, 1681, 1652, 1538, 1300, 1169 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 8.09 (br s, 1H), 7.52 (br s, 1H), 7.09 (br s, 1H), 6.65 (br s, 1H), 5.50 (br s, 1H), 4.14 (q, *J* = 6.4 Hz, 2H), 3.92 (m, 1H), 3.79 (m, 1H), 3.13 (m, 1H), 2.53 (m, 1H), 2.17 (m, 1H), 1.85 (m, 1H), 1.53 (s, 6H), 1.51 (s, 11H), 1.48 (s, 2H), 1.43 (s, 4H), 1.23 (t, *J* = 7.2 Hz, 3H); FAB(+)HRMS calcd for $C_{29}H_{50}N_{11}O_8$ $[M^+ + H]$: 680.3844; found: 680.3834.

*5.2.6. Boc-[(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (**6**).* A solution of crude acid Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OH (239 mg, 0.496 mmol), crude amine H-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (234 mg, 0.48 mmol), BOP-Cl (126 mg, 0.496 mmol), and $^i$Pr$_2$EtN (173 μL, 0.992 mmol) in $ClCH_2CH_2Cl$ (8 mL) was stirred at 45˚C for 17 days. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% MeOH in $CHCl_3$ gave hexapeptide **6** (406 mg, 94%) as colorless crystals: m.p. 221˚C (decomp.); $[\alpha]_D^{27}$ = +7.25 (*c* 1.11, $CHCl_3$); IR ($CDCl_3$) *ν* 3406, 3326, 2987, 2938, 2111, 1670, 1528, 1256, 1156 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 7.86 (br s, 1H), 7.55 (br s, 1H), 7.46 (br s, 1H), 7.16 (br s, 1H), 6.85 (br s, 1H), 5.85 (br s, 1H), 4.14 (q, *J* = 6.4 Hz, 2H), 3.99—4.04 (m, 2H), 3.95 (m, 1H), 3.87 (m, 1H), 3.06 (br s, 1H), 2.92 (br s, 1H), 2.58 (m, 1H), 2.26—2.35 (m, 3H), 1.99 (m, 1H), 1.90 (m, 1H), 1.45—1.56 (m, 33H), 1.24 (t, *J* = 7.1 Hz, 3H); FAB(+)HRMS calcd for $C_{35}H_{57}N_{18}O_9$ $[M^+ + H]$: 873.4556; found: 873.4551.

*5.2.7. H-[(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt.* A mixture of hexapeptide **6** (75 mg, 0.086 mmol) in concentrated aqueous HCl (1 mL) and EtOAc (3 mL) was stirred at room temperature for 12 h. The solution was alkalized with saturated aqueous $NaHCO_3$, extracted with EtOAc, dried over $MgSO_4$. Removal of the solvent produced a crude amine H-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (59 mg, 78%), which was used for next reaction without purification: $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 8.29 (br s, 1H), 7.99 (br s, 1H), 7.44 (br s, 1H), 7.14 (br s, 1H), 6.33 (br s, 1H), 4.09—4.14 (m, 2H), 3.87—3.95 (m, 2H), 2.96 (br s, 1H), 2.55—2.63 (m, 2H), 2.29—2.34 (m, 2H), 2.04—2.09 (m, 2H), 1.83 (br s, 2H), 1.70 (m, 1H), 1.50 (s, 24H), 1.43 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 3H).

*5.2.8. Boc-[(R,R)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_3$-OEt (**7**).* A solution of crude acid Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OH (76 mg, 0.157 mmol), crude amine H-[(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$]$_2$-OEt (94 mg, 0.122 mmol), BOP-Cl (40 mg, 0.157 mmol), and $^i$Pr$_2$EtN (43 μL, 0.244 mmol) in $ClCH_2CH_2Cl$ (3 mL) was stirred at 45˚C for 19 days. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$, washed with saturated aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% MeOH in $CHCl_3$ gave nonapeptide **7** (43 mg, 74% based on recovered material) as colorless crystals: m.p. 238˚C (decomp.); $[\alpha]_D^{27}$ = +25.0 (*c* 0.89, $CHCl_3$); IR ($CDCl_3$) *ν* 3308, 2982, 2933, 2357, 2110, 1661, 1533, 1255, 1169 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 7.98 (br s, 1H), 7.94 (br s, 1H), 7.86 (br s, 1H), 7.65 (br s, 1H), 7.52 (br s, 1H), 7.41 (br s, 1H), 7.15 (br s, 1H), 6.81 (br s, 1H), 5.53 (br s, 1H), 4.14 (q, *J* = 6.9 Hz, 2H), 4.00 (m, 4H), 3.92 (m, 2H), 3.12—3.65 (m, 4H), 2.68 (m, 2H), 2.67—2.69 (m, 2H), 2.25—2.27 (m, 2H), 1.84—2.17 (m, 4H), 1.45—1.54 (m, 45H), 1.22—1.24 (m, 3H); FAB(+)HRMS calcd for $C_{49}H_{78}N_{27}O_{12}$ $[M^+ + H]$: 1236.6323; found: 1236.6295.

*5.2.9. Boc-(R,R)-Ac$_5$c$^{d\text{-}triasole}$-(Aib)$_4$-OEt (**2**).* A mixture of Boc-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_4$-OEt (**1**) (50 mg, 0.074 mmol), phenylacetylene (19.5 μL, 0.18 mmol), sodium ascorbate (7 mg, 0.037 mmol), and $CuSO_4$ (6 mg, 0.037 mmol) in *t*-BuOH (2 mL) and $H_2O$ (1 mL) was stirred at room temperature for 4 days. After evaporation of the solvent, the residue was diluted with $H_2O$, extracted with $CH_2Cl_2$, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% MeOH in $CHCl_3$ gave peptide **2** (42 mg, quant) as colorless crystals: m.p. 228—230˚C; $[\alpha]_D^{23}$ = –43.5 (*c* 1.00, $CHCl_3$); IR ($CDCl_3$) *ν* 3349, 2986, 2361, 1730, 1677, 1525 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ* 7.93 (br s, 1H), 7.87 (br s, 1H), 7.79—7.83 (m, 4H), 7.56 (br s, 1H), 7.41—7.45 (m, 5H), 7.35—7.38 (m, 3H), 7.14 (br s, 1H), 6.79 (br s, 1H), 5.79 (m, 1H), 5.43 (m, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 3.27 (m, 1H), 3.14 (m, 1H), 3.03 (m, 1H), 2.77 (m, 1H), 1.54 (s, 12H), 1.53 (s, 9H), 1.51 (s, 6H), 1.48 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H); FAB (+)HRMS calcd for $C_{45}H_{62}N_{11}O_8$ $[M^+ + H]$: 884.4783; found: 884.4812.

*5.2.10. Boc-(Aib)$_2$-(R,R)-Ac$_5$c$^{d\text{-}triasole}$-(Aib)$_2$-OEt (**4**).* A mixture of Boc-(Aib)$_2$-(*R*,*R*)-Ac$_5$c$^{dN3}$-(Aib)$_2$-OEt (**3**) (48 mg, 0.071 mmol), phenylacetylene (37.2 μL, 0.34 mmol), sodium ascorbate (14 mg, 0.072 mmol), and $CuSO_4$ (11 mg, 0.072 mmol) in *t*-BuOH (2 mL) and $H_2O$ (1 mL) was stirred at room temperature for 7 days. After evaporation of the solvent, the residue was diluted with $H_2O$, extracted with $CH_2Cl_2$, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% MeOH in $CHCl_3$ gave peptide **4** (62 mg, 99%) as colorless crystals: m.p. 145—147˚C; $[\alpha]_D^{23}$ = –49.1 (*c* 0.86, $CHCl_3$); IR ($CDCl_3$) *ν* 3299, 2986, 2937, 2351, 1731, 1681, 1538, 1165 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) *δ* 8.46 (br s, 1H), 8.12 (br s, 1H), 7.79 (d, *J* = 7.7 Hz, 2H), 7.73 (d, *J* = 7.1 Hz, 2H), 7.72 (br s, 1H), 7.65 (br s, 1H), 7.37—7.42 (m, 4H), 7.29—7.33 (m, 2H), 7.10 (br s, 1H), 6.66 (br s, 1H), 5.78 (m, 1H), 5.48 (m, 1H), 5.05 (s, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.96 (m, 1H), 3.43 (br s, 1H), 3.18 (m, 1H), 2.84 (m, 1H), 1.46—1.59 (m, 33H), 1.24 (t, *J* = 7.1 Hz, 3H); FAB (+)HRMS calcd for $C_{45}H_{62}N_{11}O_8$ [$M^+$ + H]: 884.4783; found: 884.4764.

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10. CCDC-997384 (**3**) and 997385 (**6**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via: www.ccdc.cam.ac.uk/data_request/cif.

## Supplementary Data

Supplementary data associated with this article can be found at http://XXXX.