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Twisted Structure of a Cyclic Hexapeptide Containing a Combination of Alternating L-Leu-D-Leu-Aib Segments

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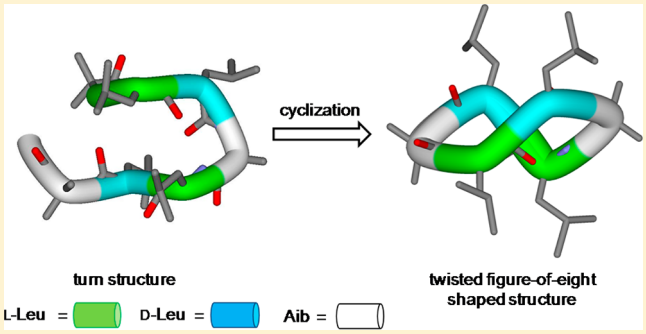
Supporting Information

ABSTRACT: We designed and synthesized a C_2-symmetric cyclic hexapeptide, *cyclo*(L-Leu-D-Leu-Aib)$_2$ (**2**), which contains L- and D-amino acids and achiral Aib residues. The conformation of **2** was analyzed in the crystalline state and in solution, which was a unique figure-eight-shaped conformation.



The de novo design of peptides is important in various fields of organic chemistry, nanotechnology, and medicinal chemistry. It is often necessary to fold such peptides into well-defined structures, and so, a variety of approaches to controlling peptide conformations involving the use of β-amino acids,[1] α,α-disubstituted α-amino acids,[2] and cross-linked side chains[3] have been investigated. Cyclic peptides are also used to control their conformations, and in particular, small-size cyclic peptides have been developed because they are able to form stable structures.[4] As templates for peptide cyclization, combinations of an L-amino acid and a D-amino acid (L-AA-D-AA) are frequently used, and cyclic peptides with alternating L-AA-D-AA segments are known to form antiparallel β-sheet-like cylindrical structures.[4] Furthermore, 2-aminoisobutyric acid (Aib) residues have also been incorporated into cyclic peptides to induce a β-turn structure.[5] However, to date, the conformations of cyclic peptides containing a combination of L-AA-D-AA and Aib residues have not been investigated.

Recently, we reported that the linear hexapeptide Boc-(L-Leu-D-Leu-Aib)$_2$-OMe (**1**) (Boc, *tert*-butoxycarbonyl; L-Leu, L-leucine; D-Leu, D-leucine; OMe, methyl ester), which contains alternating L-Leu-D-Leu segments and Aib residues, forms a turn structure in the crystalline state (Figure 1).[6] In the crystal structure of **1**, the N-terminal L-Leu(1) residue is close to the C-terminal Aib(6) residue, and therefore, we speculated that the cyclic peptide **2** could be easily built from the linear peptide **1** (Figure 1).

Herein, we designed and synthesized the C_2-symmetric cyclic hexapeptide *cyclo*(L-Leu-D-Leu-Aib)$_2$ (**2**) as a model cyclic peptide containing a combination of L-AA-D-AA and Aib residues and studied its preferred conformation in the crystalline state and in solution.

The cyclic hexapeptide **2** was synthesized as follows (Scheme 1). First, the N- and C-terminal free hexapeptide was prepared via the deprotection of the C-terminal methyl ester and N-terminal Boc group. Then, intramolecular cyclization of the linear peptide using EDC/HOBt gave the cyclic peptide **2** in 63% yield.

The cyclic hexapeptide **2** formed good crystals for X-ray crystallographic analysis after slow evaporation of MeOH/H_2O at room temperature. The structure of **2** was solved using the SHELXS 97 direct methods[7] and expanded by the Fourier technique.[8] All non-H-atoms were given anisotropic thermal parameters, some H-atoms were refined isotropically, and the remaining H-atoms were placed at the calculated positions.[9] The relevant backbone and side-chain torsion angles and the intra- and intermolecular hydrogen-bond parameters are listed in Tables 1 and 2.

The structure of *cyclo*(L-Leu-D-Leu-Aib)$_2$ (**2**) was solved in space group *P*1. Two crystallographically independent molecules, **a** and **b**, were present in the asymmetric unit together with two methanol molecules (Figure 2). Both molecules **a** and **b** formed near C_2-symmetric twisted figure-of-eight conformations.[11] They contained β-turn structures at the Aib(3) and Aib(6) residues and rippled-sheet structures between opposing L-Leu and D-Leu residues [for example; L-Leu(1) and D-Leu(5), Figure 3a,b],[10,12] and filled much the void compared with other

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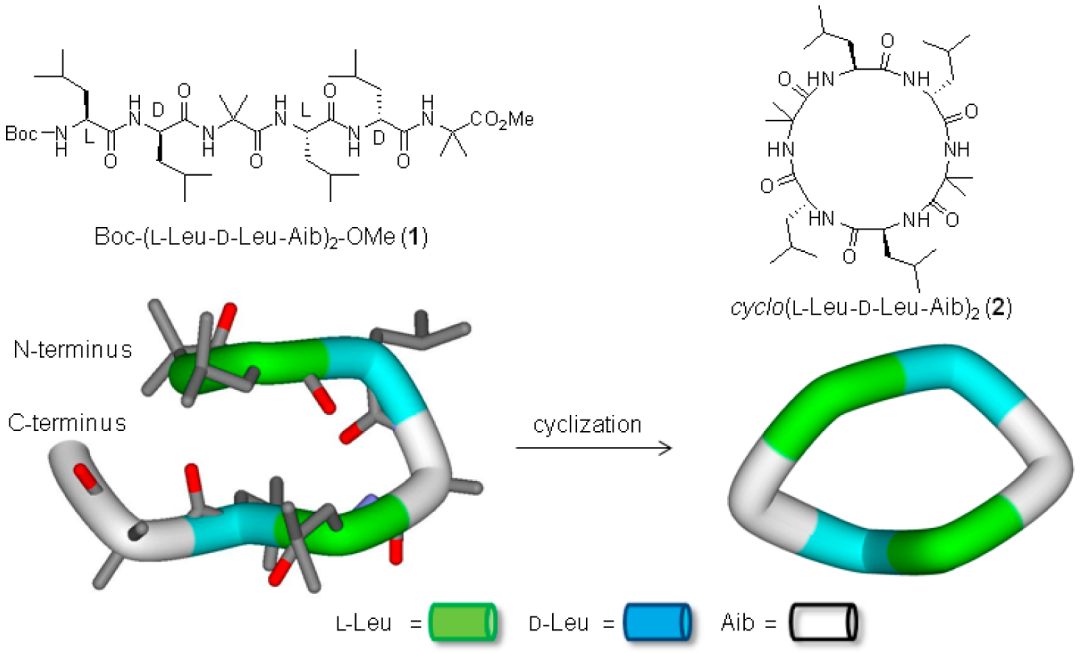

Figure 1. Chemical structures and tube models of linear hexapeptide **1** and cyclic hexapeptide **2**.

Scheme 1. Synthesis of Cyclic Hexapeptide 2

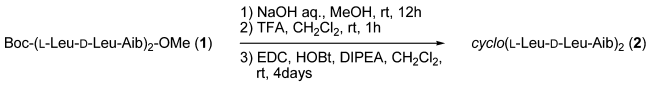

Table 1. Selected Torsion Angles (ω, ϕ, ψ, and χ) (deg) for Peptide 2[10]

	torsion angle			
residue	ϕ	ψ	ω	χ
molecule **a**				
L-Leu(1)	−140.9	108.6	178.7	−164.9
D-Leu(2)	47.8	−121.6	−177.1	174.3
Aib(3)	−57.9	−15.3	−179.2	
L-Leu(4)	−100.8	6.6	174.2	−57.5
D-Leu(5)	88.3	−74.3	171.8	169.0
Aib (6)	−52.1	−40.1	178.3	
molecule **b**				
L-Leu(1)	−139.1	113.7	176.5	−69.0
D-Leu(2)	52.08	−126.4	−175.5	−175.5
Aib(3)	−60.2	−20.6	178.5	
L-Leu(4)	−82.8	−8.6	−175.2	−62.2
D-Leu(5)	93.4	−72.8	173.9	69.3
Aib (6)	−47.4	−45.7	−178.9	

cyclic hexapeptides having L-AA-D-AA segments (Figure 3c,d).[4] The conformations of molecules **a** and **b** were well matched except for small differences in the conformations of their side chains (Figure 4). In molecule **a**, two hydrogen bonds of the $i \leftarrow i+3$ type were observed between H−N(4a) and C(1a)═O(1a) [N(4a)···O(4a) = 2.88 Å; N−H···O 156.6°] and between H−N(5a) and C(2a)═O(2a) [N(5a)···O(2a) = 2.92 Å; N−H···O 163.5°]. Furthermore, one intramolecular hydrogen bond of the $i \leftarrow i+2$ type was observed between H−N(6a) and C(4a)═O(4a) [N(6a)···O(4a) = 2.95 Å; N−H···O 132.9°]. Molecule **b** similarly contained two intramolecular hydrogen bonds of the $i \leftarrow i+3$ type between H−N(4b) and C(1b)═O(1b) [N(4b)···O(1b) 2.89 Å; N−H···O 150.3°], and between H−N(5b) and C(2b)═O(2b) [N(5b)···O(2b) 2.94 Å; N−H···O 166.2°], and one weak intramolecular hydrogen bond of the $i \leftarrow i+2$ type between H−N(6b) and C(4b)═O(4b) [N(6b)···O(4b) = 3.18 Å; N−H···O 134.9°]. In packing mode, molecules **a** and **b** were alternately connected by intermolecular hydrogen bonds (Figure 5). Peptide **2** formed an antiparallel sheet-like structure between conformers **a** and **b**, but did not form cylindrical structures due to the influence of the twisted conformations of L-Leu(1)-D-Leu(2) and L-Leu(4)-D-Leu(5).

Table 2. Intra- and Intermolecular H-Bond Parameters for Peptide 2[a]

donor D−H	acceptor A	distance D···A	angle (deg) D−H···A	symmetry operations
molecule **a**				
N_{4a}-H	O_{1a}	2.88	156.6	*x*, *y*, *z*
N_{5a}-H	O_{2a}	2.92	163.5	*x*, *y*, *z*
N_{6a}-H	O_{4a}	2.95	132.9	*x*, *y*, *z*
N_{3a}-H	O_{Ma}[b]	2.93	168.9	*x*, *y*, *z*
N_{2a}-H	$O_{4b'}$	2.86	167.8	*x*, *y*, 1 + *z*
molecule **b**				
N_{4b}-H	O_{1b}	2.89	150.3	*x*, *y*, *z*
N_{5b}-H	O_{2b}	2.94	166.2	*x*, *y*, *z*
N_{6b}-H	O_{4b}	3.18[c]	134.9	*x*, *y*, *z*
N_{2b}-H	O_{Mb}	2.90	175.6	*x*, *y*, *z*
N_{3b}-H	$O_{3a'}$	2.97	176.2	*x*, *y*, 1 + *z*
N_{6b}-H	$O_{6a'}$	3.15[c]	129.7	*x*, *y*, 1 + *z*
O_{Ma}-H	O_{5b}	2.89	122.9	*x*, *y*, *z*
O_{Mb}-H	O_{Ma}	2.86	136.8	1 + *x*, *y*, *z*

[a]The numbering of the amino acid residues begins at the N-terminus of the peptide. [b]O_M: MeOH molecule. [c]The distance is a bit long for a hydrogen bond.

On the basis of the 1D ^{1}H NMR spectrum, peptide **2** was found to have *C*2-symmetric conformation in solution (equivalent sets of protons in the NMR spectrum).[11] Figure 6 shows the 4.0—8.0 ppm region of 2D NOESY spectrum of **2**

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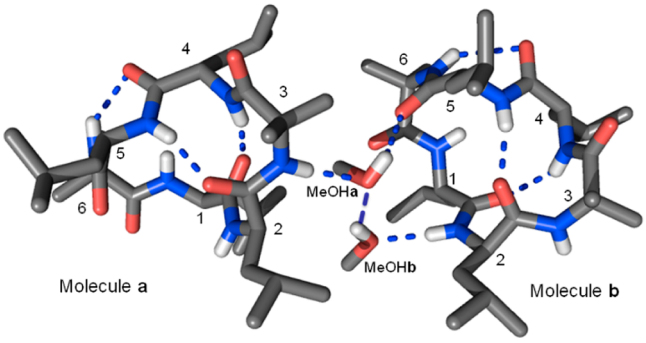

Figure 2. X-ray diffraction structure of cyclic hexapeptide **2**. Hydrogen bonds are indicated as blue dashed lines. The numbering refers to amino acid residues.[10]

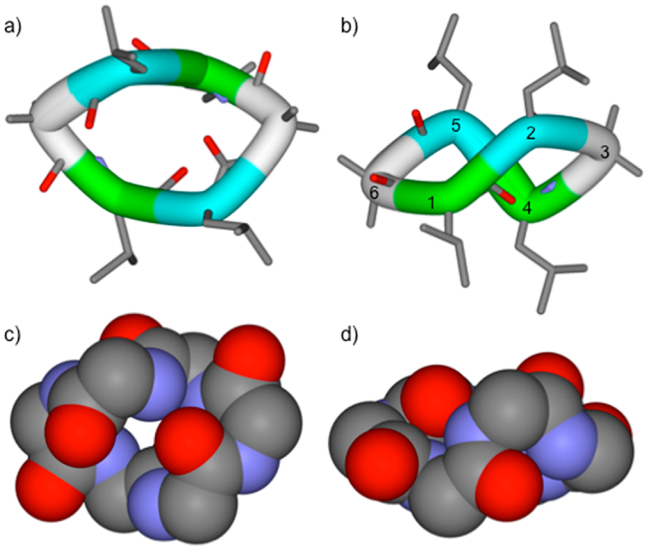

Figure 3. Structure of molecule **a** from (a) the overhead view and (b) the side view. The numbering refers to amino acid residues.[10] The space-filling structure of molecule **a** from (c) the overhead view and (d) the side view. (The side chains composed of amino acids have been omitted.)

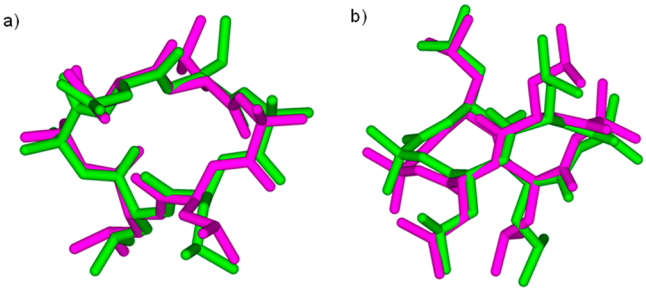

Figure 4. Overlaid structures of molecules **a** (green) and **b** (purple) from (a) the overhead view and (b) the side view.

in $CDCl_3$. The spectrum shows four dipolar interactions dαN(1→2), dNN(1→2), dαN(2→3), and dNN(3→4). These interactions might support that peptide **2** forms a twisted structure in solution, which is in agreement with the crystal structure of **2**.

The conformational search calculation of peptide **2** was performed using MacroModel with OPLS_2005 force field to obtain the global-minimum energy conformation. The minimum energy conformation is similar to those in the crystal with some differences at the side chains of Leu residues.[11]

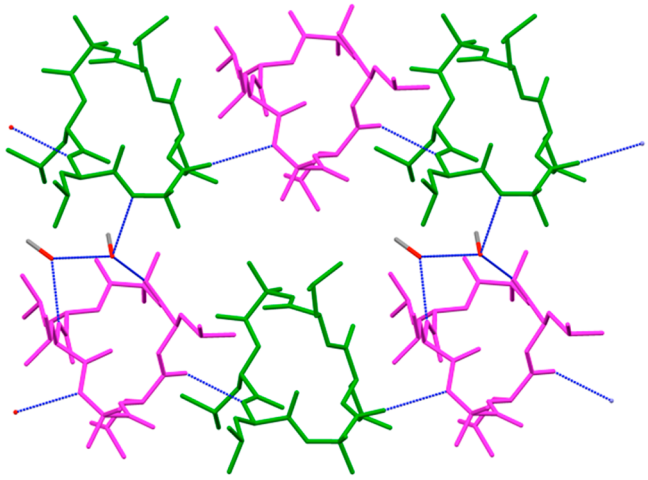

Figure 5. Packing of molecules **a** (green) and **b** (purple) in the crystalline state. Intermolecular hydrogen bonds are indicated as dashed lines.

We synthesized the cyclic hexapeptide *cyclo*(L-Leu-D-Leu-Aib)$_2$ (**2**) as a model cyclic peptide containing a combination of L-AA-D-AA and Aib residues. The conformation of **2** in the crystal state was determined by X-ray crystallographic analysis, which revealed that **2** formed a twisted figure-of-eight structure. The cyclic hexapeptide produced a new secondary structure that has not previously been identified. Compared with the conformation of *cyclo*(Gly-D-Leu-L-Leu)$_2$,[4a,13] two Aib residues of peptide **2** played a very important role to form the twisted structure, because Aib acts as a strong bend inducer. Furthermore, a rippled-sheet structure, which was constructed of opposing L-Leu and D-Leu residues, was also important for the formation of the twisted structure. Peptides composed of alternating L-AA-D-AA-Aib segments would be good models for rippled-sheet peptides and would represent new peptide materials.

EXPERIMENTAL SECTION

Synthesis of Peptide 2. A solution of linear hexapeptide **1** (377 mg, 0.5 mmol) and 1 N aqueous NaOH (1.0 mL, 1.0 mmol) in MeOH (5 mL) was stirred at room temperature for 12 h. Then, the solution was neutralized with 1 N aqueous HCl, and MeOH was evaporated. The aqueous solution was extracted with AcOEt and dried over Na_2SO_4. Removal of the solvent afforded hexapeptide-carboxylic acid (370 mg, >99%) as colorless crystals, which were used for the next reaction without further purification. Trifluoroacetic acid (1 mL) was added to a solution of the above acid in CH_2Cl_2 (5 mL) at 0 °C, and the whole was stirred at room temperature for 1 h. Removal of the solvent afforded a crude N- and C-terminal free hexapeptide, which was used without further purification. A mixture of EDC (115 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol), DIPEA (209 μL, 1.2 mmol), and the above N- and C-terminal free hexapeptide in CH_2Cl_2 (50 mL) was stirred at rt for 4 days, and the solution was washed with 3% aqueous HCl, 5% aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel (*n*-hexane/AcOEt = 1:1) to give cyclic hexapeptide **2** (196 mg, 63%): colorless crystals; mp 254—256 °C (recryst from MeOH/H_2O); $[\alpha]^{24}_D$ = +117.8 (*c* 1.0, $CHCl_3$); IR (ATR, cm^{-1}) 3426, 2960, 1677, 1376, 1228, 908; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 6.8 Hz, 2H), 6.87 (br s, 2H), 6.35 (d, *J* = 8.0 Hz, 2H), 4.43 (m, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 1.88 (m, 2H),

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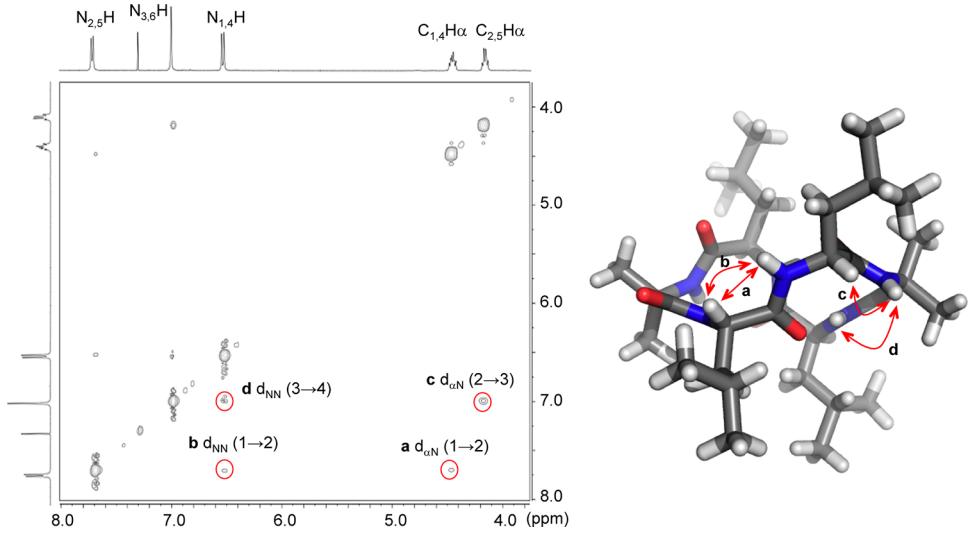

Figure 6. Nuclear Overhauser effect spectroscopy (NOESY) ^{1}H NMR spectrum of peptide **2** in the 4.0—8.0 ppm region. $C_nH\alpha$ (n = residue number) refers the α-proton of amino acids, and N_nH refers the amide proton.

1.39—1.73 (m, 22H), 0.89—0.95 (m, 24H); ^{13}C NMR(100 MHz, $CDCl_3$) δ 173.9, 173.7, 172.2, 56.9, 52.1, 51.5, 40.0, 37.4, 27.6, 25.0, 24.5, 23.1, 23.0, 22.7, 22.2, 21.6; [HR-ESI(+)] m/z calcd for $C_{32}H_{59}N_6O_6$ $[M + H]^+$ 623.4491, found 623.4479.

■ ASSOCIATED CONTENT

Supporting Information

Crystallographic data, molecular mechanics calculations, and copies of the ^{1}H NMR and ^{13}C NMR spectra of peptide **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(9) Crystal data for **2**: $C_{32}H_{58}O_6N_6,CH_3OH$; M_r = 654.89; triclinic; *P*1, *a* = 11.759 Å, *b* = 13.661 Å, *c* = 13.815 Å; α = 73.33°, β = 67.47°, γ = 83.35°; *V* = 1963.7 Å^3; *Z* = 2; D_{calc} = 1.108 g/cm^3; μ (Mo Kα) = 0.78 cm^{-1}; no. of observations ($I > 2\sigma(I)$) = 7947; no. of variables = 831; R_1 = 0.0774, and R_w = 0.2288. CCDC-879292 for **2** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(10) The L-Leu residues in which ϕ = −140.9 and ψ = 108.6 for molecule **a**, and in which ϕ = −139.1 and ψ = 113.7 for molecule **b**, are both numbered as residue 1.

(11) See the Supporting Information.

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