The 3_{10}-Helical Conformation of Aib-Containing Oligopeptide, Boc-(L-Leu-Aib_2)$_2$-OBzl

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The molecular and crystal structure of the Aib (α-aminoisobutyric acid)-containing oligopeptide, Boc-(L-Leu-Aib_2)$_2$-OBzl, are determined by X-ray diffraction analysis. The hexapeptide crystallizes in the space group $P2_1$, with a=19.912(2), b=16.098(1), c=14.710(2) Å, β=96.81(1)°, and Z=4. The unit cell contains two independent molecules (molecules A and B) in the asymmetric unit. The dihedral angles (ϕ and ψ) of the peptide back bone show that molecule A folds into a right-handed helix and molecule B folds into a left-handed helix. Both the molecules adopt a 3_{10}-helical conformation stabilized by four 4→1 intramolecular hydrogen bonds. The right- and left-handed helical molecules are arranged alternately, in head-to-tail fashion to make columns along the [101] direction. These columns are packed closely in the pseudo-hexagonal arrangement.

Due to the steric hindrance of the methyl groups and carbonyl oxygens, the dihedral angles ϕ and ψ of Aib residue are severely restricted to a small region including both the 3_{10}-helix (ϕ=±60°, ψ=±30°) and α-helix (ϕ=±57°, ψ=±47°).[1] Therefore, the Aib-containing oligopeptides form either 3_{10}- or α-helical conformation. In fact, the peptide fragments in membrane-channel-forming polypeptides, alamethicin and suzukacillin which contain a large proportion of Aib, are recognized to form 3_{10}- or α-helical conformation, especially 3_{10}-helical conformation in short peptides.[2-4] The above polypeptides in lipid membrane form potential-dependent, ion-conducting pores with discrete conductance values. Therefore, the conformational studies on Aib-containing peptides will contribute to a better understanding of excitation in nerve membranes. Further, one of the authors succeeded in improving the solubility of protected peptide fragments by utilizing the nature of Aib residue to promote helical folding in peptide. By using the same nature, the replacement of amino acid residues with Aib will generate novel proteins with improved physical properties.[5] Since the side chain distribution on the surface of helical core is very important for the peptide fragment to play the role in the protein structure, it is essential to know the helical type (α- or 3_{10}-helix) of peptide fragment after replacing by Aib residue. The recent structural study of the Aib-containing octapeptide consisted of (L-Leu_3-$Aib)_2$ showed that this peptide has an α-helical conformation.[6] In this paper, we will discuss the helical conformation and the crystal structure of Boc-(L-Leu-Aib_2)$_2$-OBzl.

Materials and Methods

The Boc-(L-Leu-Aib_2)$_2$-OBzl (Boc: *t*-butoxycarbonyl, Bzl: benzyl) was prepared by the fragment condensation reaction of Boc-L-Leu-Aib_2-OH with H-L-Leu-Aib_2-OBzl using dicyclohexylcarbodiimide and 1-hydroxy-1*H*-benzotriazole as coupling reagents. General procedures for the preparation of Boc-L-Leu-Aib_2-OH and H-L-Leu-Aib_2-OBzl were described in a previous paper.[5] Colorless, plate-like single crystals of Boc-(L-Leu-Aib_2)$_2$-OBzl were grown from a trichloromethane and *N*,*N*-dimethylformamide solution (5/3 by volume). The size of the crystal used for intensity measurement was about 0.5×0.3×0.1 mm^3. The lattice parameters and diffraction intensity were measured by a four-circle diffractometer (RASA-5RII, Rigaku Corporation) with graphite monochromatized Cu $K\alpha$ radiation (λ=1.5418 Å). The lattice parameters were refined by the least-squares fit using 20 reflections in the 2θ range of 40°—50°. The intensity measurements were carried out by the 2θ-ω scan mode with scan speed of 6 °min^{-1} and scan width ($\Delta\omega$) of (1.3+0.14 tan θ)°. A total of 3693 reflections with $|F_o|>3\sigma|F_o|$ was measured in the 2θ range of 3—100°. Three reflections ($1\bar{1}\bar{1}$, 202, and $\bar{7}6\bar{2}$) that were monitored after every 100 measurements showed no significant intensity deterioration during the data collection. The intensity was corrected for Lorentz and polarization factors but not for absorption. The density was measured by a flotation method using a solution of carbon tetrachloride and toluene. On the basis of the measured density and the unit cell volume, a unit cell contains four oligopeptide molecules.

Crystal data: $C_{40}H_{66}N_6O_9$, F.W.=775, monoclinic, space group $P2_1$, Z=4, a=19.912(2), b=16.098(1), c=14.710(2) Å, β=96.81(1)°, V=4682(1) Å^3, D_x=1.100, D_m=1.10 g cm^{-3}, $F(000)$=1680, μ(Cu$K\alpha$)=5.61 cm^{-1}.

The CD spectra were measured in the methanol solution by a JASCO J-40AS recording spectropolarimeter using cylindrical fused quartz cells of 0.2 mm path length at 1 mg ml^{-1} of sample concentration at room temperature. The band intensity is expressed as molar ellipticity [θ] (deg cm^2 dmol^{-1}).

Table 1. Fractional Coordinates and Isotropic Temperature Factors for Non-Hydrogen Atoms of Boc-(L-Leu-Aib$_2$)$_2$-OBzl, with Estimated Standard Deviations in Parentheses

	Atom	x	y	z	B_{iso}/Å^2
Molecule A					
Boc	C_1	0.8645(16)	−0.0349(0)	1.1211(21)	9.8(9)
	C_2	0.7957(14)	−0.0289(28)	0.9790(13)	8.0(7)
	C_3	0.7419(15)	−0.0764(29)	1.1314(22)	9.7(9)
	C_4	0.7909(16)	−0.0222(28)	1.0836(21)	7.5(7)
	O_1	0.7807(8)	0.0645(23)	1.0977(11)	5.9(4)
	C_5	0.7223(14)	0.1047(26)	1.0625(17)	5.5(6)
	O_2	0.6782(8)	0.0689(23)	1.0161(11)	5.8(4)
Leu$_1$	N	0.7264(9)	0.1851(23)	1.0875(12)	3.8(4)
	C_α	0.6643(12)	0.2310(26)	1.0609(16)	4.9(6)
	C′	0.6501(13)	0.2386(26)	0.9523(17)	4.9(6)
	O	0.5897(8)	0.2491(23)	0.9218(10)	5.0(4)
	C_β	0.6737(13)	0.3229(26)	1.0994(18)	6.5(7)
	C_γ	0.6735(15)	0.3233(29)	1.2075(21)	8.5(8)
	C_{δ_1}	0.6883(17)	0.4074(31)	1.2365(24)	11.6(10)
	C_{δ_2}	0.6103(18)	0.2963(31)	1.2272(24)	13.8(11)
Aib$_1$	N	0.7026(10)	0.2301(24)	0.9051(13)	4.4(4)
	C_α	0.6951(15)	0.2301(27)	0.8054(20)	6.7(7)
	C′	0.6362(13)	0.1729(25)	0.7626(18)	4.6(6)
	O	0.6029(9)	0.1912(23)	0.6872(13)	6.6(4)
	C_{β_1}	0.7603(12)	0.2057(26)	0.7700(17)	6.4(7)
	C_{β_2}	0.6738(14)	0.3243(27)	0.7680(19)	7.6(8)
Aib$_2$	N	0.6266(10)	0.1064(24)	0.8088(14)	4.7(5)
	C_α	0.5731(14)	0.0464(27)	0.7771(18)	6.3(7)
	C′	0.5044(12)	0.0876(25)	0.7569(15)	3.9(5)
	O	0.4598(8)	0.0536(23)	0.6991(10)	5.0(4)
	C_{β_1}	0.5702(12)	−0.0199(26)	0.8528(17)	5.5(6)
	C_{β_2}	0.5917(13)	0.0018(27)	0.6826(19)	7.2(7)
Leu$_2$	N	0.4930(9)	0.1518(23)	0.8056(13)	3.7(4)
	C_α	0.4233(10)	0.1903(24)	0.7882(14)	3.4(5)
	C′	0.4102(12)	0.2311(25)	0.6959(16)	4.1(5)
	O	0.3490(8)	0.2467(22)	0.6687(11)	5.1(4)
	C_β	0.4184(11)	0.2575(25)	0.8655(16)	5.3(6)
	C_γ	0.4085(14)	0.2130(28)	0.9580(20)	7.8(7)
	C_{δ_1}	0.4193(15)	0.2789(29)	1.0349(21)	9.4(9)
	C_{δ_2}	0.3384(17)	0.1781(29)	0.9575(23)	10.5(9)
Aib$_3$	N	0.4601(9)	0.2447(24)	0.6478(13)	4.1(4)
	C_α	0.4487(12)	0.2722(27)	0.5526(18)	5.8(6)
	C′	0.3951(13)	0.2059(28)	0.4974(19)	5.6(6)
	O	0.3485(9)	0.2396(23)	0.4408(12)	7.0(4)
	C_{β_1}	0.5145(14)	0.2587(28)	0.5067(21)	7.8(8)
	C_{β_2}	0.4213(13)	0.3629(27)	0.5485(19)	6.9(7)
Aib$_4$	N	0.4033(10)	0.1299(25)	0.5169(14)	5.1(5)
	C_α	0.3617(14)	0.0652(27)	0.4788(19)	7.4(7)
	C′	0.3524(21)	0.0760(31)	0.3596(24)	12.5(10)
	O	0.2841(12)	0.0708(25)	0.3441(15)	13.4(6)
	C_{β_1}	0.4045(15)	−0.0144(29)	0.4856(20)	8.7(8)
	C_{β_2}	0.2896(16)	0.0640(30)	0.5267(22)	10.8(9)
OBzl	O_1	0.3990(12)	0.1090(26)	0.3379(16)	14.0(7)
	C_1	0.3695(18)	0.1233(31)	0.2317(25)	14.1(10)
	C_2	0.4244(15)	0.0698(29)	0.1845(19)	7.8(7)
	C_3	0.4890(20)	0.0973(31)	0.1936(24)	12.9(10)
	C_4	0.5401(18)	0.0569(32)	0.1515(25)	12.9(10)
	C_5	0.5216(17)	−0.0102(31)	0.0949(22)	10.2(9)
	C_6	0.4555(19)	−0.0371(32)	0.0797(25)	12.6(11)
	C_7	0.4041(17)	0.0038(31)	0.1266(24)	11.9(9)

Table 1. (Continued)

	Atom	x	y	z	B_{iso}/Å^2
Molecule B					
Boc	C_1	0.3484(18)	0.5742(26)	0.6499(24)	12.8(10)
	C_2	0.2169(17)	0.5904(30)	0.6290(23)	11.6(9)
	C_3	0.2668(16)	0.5761(29)	0.4778(22)	10.3(9)
	C_4	0.2789(17)	0.5530(31)	0.5819(24)	11.3(9)
	O_1	0.2727(9)	0.4578(24)	0.6038(12)	7.3(5)
	C_5	0.2223(14)	0.4063(28)	0.5671(19)	6.1(6)
	O_2	0.1690(8)	0.4405(23)	0.5178(11)	6.1(4)
Leu_1	N	0.2368(10)	0.3301(25)	0.5739(15)	6.2(5)
	C_α	0.1877(11)	0.2633(25)	0.5410(15)	4.2(5)
	C′	0.1608(13)	0.2784(25)	0.4424(17)	4.6(6)
	O	0.1003(8)	0.2652(23)	0.4124(11)	5.3(4)
	C_β	0.1264(12)	0.2550(26)	0.6032(17)	6.2(6)
	C_γ	0.1570(16)	0.2298(29)	0.7026(23)	9.9(9)
	C_{δ_1}	0.1843(19)	0.1396(34)	0.7046(26)	15.1(12)
	C_{δ_2}	0.0863(16)	0.2302(30)	0.7521(22)	10.5(9)
Aib_1	N	0.2078(10)	0.2987(24)	0.3900(14)	5.5(5)
	C_α	0.1928(12)	0.2995(26)	0.2859(17)	5.1(6)
	C′	0.1320(13)	0.3651(26)	0.2617(18)	5.1(6)
	O	0.1000(8)	0.3533(23)	0.1831(12)	5.8(4)
	C_{β_1}	0.2558(12)	0.3508(26)	0.2565(17)	5.8(6)
	C_{β_2}	0.1808(13)	0.2189(26)	0.2442(18)	6.4(7)
Aib_2	N	0.1210(10)	0.4247(24)	0.3190(14)	4.8(5)
	C_α	0.0632(13)	0.4838(26)	0.2918(17)	5.5(6)
	C′	−0.0059(13)	0.4262(27)	0.2769(18)	5.6(6)
	O	−0.0513(8)	0.4623(24)	0.2191(11)	6.3(4)
	C_{β_1}	0.0522(13)	0.5288(26)	0.3846(18)	6.8(7)
	C_{β_2}	0.0717(12)	0.5402(26)	0.2084(17)	5.9(6)
Leu_2	N	−0.0076(10)	0.3558(23)	0.3105(13)	4.5(4)
	C_α	−0.0679(11)	0.3038(25)	0.2982(16)	4.4(5)
	C′	−0.0902(14)	0.2867(26)	0.1931(18)	5.5(6)
	O	−0.1508(9)	0.2721(23)	0.1729(11)	6.0(4)
	C_β	−0.1306(13)	0.3379(26)	0.3455(18)	7.0(7)
	C_γ	−0.1045(15)	0.3384(29)	0.4527(21)	9.5(8)
	C_{δ_1}	−0.0783(16)	0.2628(31)	0.5048(23)	11.3(9)
	C_{δ_2}	−0.1743(17)	0.3703(30)	0.4859(22)	11.2(9)
Aib_3	N	−0.0411(10)	0.2871(24)	0.1402(14)	4.8(5)
	C_α	−0.0624(13)	0.2712(27)	0.0398(18)	5.7(7)
	C′	−0.1111(15)	0.3352(28)	0.0080(20)	6.6(7)
	O	−0.1622(9)	0.3189(23)	−0.0517(12)	7.2(4)
	C_{β_1}	0.0061(14)	0.2902(27)	−0.0043(20)	6.6(7)
	C_{β_2}	−0.0879(14)	0.1844(28)	0.0192(20)	7.2(8)
Aib_4	N	−0.1021(10)	0.4133(25)	0.0358(15)	6.3(5)
	C_α	−0.1460(15)	0.4870(27)	0.0005(20)	8.0(7)
	C′	−0.1636(15)	0.4827(27)	−0.1005(20)	8.2(7)
	O	−0.2290(11)	0.4935(24)	−0.1257(14)	10.8(6)
	C_{β_1}	−0.1019(14)	0.5697(28)	0.0156(19)	7.7(7)
	C_{β_2}	−0.2066(14)	0.4928(27)	0.0589(19)	7.7(7)
OBzl	O_1	−0.1193(10)	0.4667(24)	−0.1516(14)	9.4(5)
	C_1	−0.1459(16)	0.4616(29)	−0.2477(22)	10.5(9)
	C_2	−0.0876(16)	0.4905(29)	−0.2966(21)	9.7(8)
	C_3	−0.0877(19)	0.5528(32)	−0.3602(26)	13.4(10)
	C_4	−0.0302(18)	0.5752(30)	−0.4047(22)	11.1(9)
	C_5	0.0233(18)	0.5185(33)	−0.3857(26)	13.7(11)
	C_6	0.0206(19)	0.4497(32)	−0.3326(25)	13.0(10)
	C_7	−0.0342(20)	0.4343(32)	−0.2827(26)	14.2(11)

Determination and Refinement of Structure

At first, the initial positions of 110 non-hydrogen atoms in the asymmetric unit were analyzed by the direct method. After several trials, however, it was recognized that obtaining atomic positions by this method was very difficult for the present crystal. Therefore, the pseudo-symmetry in the crystal was utilized. That is, in addition to the extinction of reflections with $k=2n+1$ in $(0k0)$, the intensity of the reflections with $h+1=2n+1$ in $(h01)$ reflection is fairly weak compared with that of $h+1=2n$ reflections. Therefore, at the first stage of the analysis, the space group of this crystal was assumed to be $P2_1/n$ instead of $P2_1$. The main chain structure was obtained by the direct method with SAPI-85[7] program for $P2_1/n$. After that, several Fourier syntheses and full-matrix least-squares refinement for the space group $P2_1$ were performed with isotropic thermal factors for all the non-hydrogen atoms. The obtained benzene ring was, however, fairly deviated from the standard benzene hexagon. Therefore, the benzene ring was fixed to the regular hexagon with C–C bond length of 1.395 Å in the major refinement cycles by SHELX-76[8] program. At the final refinement cycle, these constraining conditions were released. The final R value was 0.154 and R_w value was 0.143 for all the non-hydrogen atoms and for 3693 reflections with $|F_o|>3\sigma|F_o|$, where $w=1.0/\{\sigma|F_o|^2+(0.012|F_o|)^2\}$. The final atomic parameters for the non-hydrogen atoms are given in Table 1.[9]

The atomic scattering factors were taken from International Tables for X-Ray Crystallography, Vol. IV.[10] Computations were done on an A-70 minicomputer with the help of CRYSTAN program in RASA-5RII system (Rigaku Corporation) and on an ACOS 1000 computer at the Information Processing Center, Tokyo University of Agriculture and Technology.

Results and Discussion

For the convenience of atom specification, all the amino acid residues are numbered in series from N- to C-terminus. That is, the molecule of Boc-(L-Leu-Aib$_2$)$_2$-OBzl is denoted as Boc-Leu$_1$-Aib$_1$-Aib$_2$-Leu$_2$-Aib$_3$-Aib$_4$-OBzl. The names of each atom in the L-Leu and Aib residues are those given in the IUPAC-IUB recommendation,[11] while those in the protected groups at N- and C-termini are shown in Fig. 1.

Peptide-Backbone Conformation. A perspective view of the molecule is shown in Fig. 1. There are two independent molecules (A and B) in the asymmetric unit. The bond lengths, bond angles, and dihedral

Table 2. Bond Lengths, Bond Angles, and Dihedral Angles of the Peptide Main Chains in Molecules A and B, with Estimated Standard Deviations in Parentheses

	Leu$_1$	Aib$_1$	Aib$_2$	Leu$_2$	Aib$_3$	Aib$_4$
Molecule A						
Bond length/Å						
N_i–$C\alpha_i$	1.45(4)	1.46(3)	1.47(5)	1.51(3)	1.46(4)	1.40(5)
$C\alpha_i$–C'_i	1.59(3)	1.56(5)	1.52(4)	1.50(4)	1.65(5)	1.75(5)
C'_i–N_{i+1}	1.33(3)	1.29(5)	1.29(5)	1.31(3)	1.26(6)	—
Bond angle/°						
C'_{i-1}–N_i–$C\alpha_i$	113(2)	122(2)	121(2)	117(2)	122(2)	125(3)
N_i–$C\alpha_i$–C'_i	111(2)	113(3)	112(3)	113(2)	107(3)	108(3)
$C\alpha_i$–C'_i–N_{i+1}	117(2)	115(2)	117(2)	120(2)	117(3)	—
Dihedral angle/°						
ϕ (C'_{i-1}–N_i–$C\alpha_i$–C'_i)	−67(6)	−47(3)	−53(5)	−68(5)	−53(3)	50(5)
ψ (N_i–$C\alpha_i$–C'_i–N_{i+1})	−22(3)	−34(4)	−31(2)	−13(4)	−41(3)	28(4)
ω ($C\alpha_i$–C'_i–N_{i+1}–$C\alpha_{i+1}$)	176(6)	−180(4)	−177(3)	170(5)	178(4)	—
Molecule B						
Bond length/Å						
N_i–$C\alpha_i$	1.49(5)	1.52(3)	1.51(4)	1.46(4)	1.51(3)	1.53(5)
$C\alpha_i$–C'_i	1.51(3)	1.61(5)	1.65(5)	1.58(4)	1.45(5)	1.48(4)
C'_i–N_{i+1}	1.32(3)	1.31(5)	1.24(5)	1.32(3)	1.33(6)	—
Bond angle/°						
C'_{i-1}–N_i–$C\alpha_i$	123(2)	121(2)	118(2)	122(2)	116(2)	126(2)
N_i–$C\alpha_i$–C'_i	110(3)	106(2)	106(3)	111(2)	107(3)	111(3)
$C\alpha_i$–C'_i–N_{i+1}	114(2)	121(2)	121(3)	116(2)	121(3)	—
Dihedral angle/°						
ϕ (C'_{i-1}–N_i–$C\alpha_i$–C'_i)	54(5)	59(3)	60(4)	59(6)	59(3)	−42(4)
ψ (N_i–$C\alpha_i$–C'_i–N_{i+1})	45(3)	24(4)	20(5)	29(3)	41(3)	−46(3)
ω ($C\alpha_i$–C'_i–N_{i+1}–$C\alpha_{i+1}$)	168(5)	−180(4)	−180(4)	−180(6)	174(5)	—

angles (ϕ, ψ, and ω) of the peptide main chain for each molecule are given in Table 2. The dihedral angles (ϕ and ψ) show that the molecule A folds into a right-handed helix and the molecule B folds into a left-handed helix, with the exception of the Aib_4 residue at C-terminus, where ϕ and ψ values show the opposite helical sense for the rest of the molecule. The changing of the helical sense at the C-terminal Aib residue is usually observed in the oligopeptide helices with the protected moiety at C-terminus.[6,12-14] This may be attributed to the bulky protected moiety linked to the Aib residue and the restricted conformation of the Aib residue. Since ϕ and ψ values of an Aib residue are heavily restricted to two narrow regions, the helical sense of the C-terminus Aib residue has to be different from that of the preceding peptide region to avoid short atomic contacts between the protected moiety and the rest of the molecule.

The dihedral angles ω in the molecules A and B showed a very good planarity with the exception of those of Leu_2 residue in molecule A and the Leu_1 residue in molecule B (Table 2).

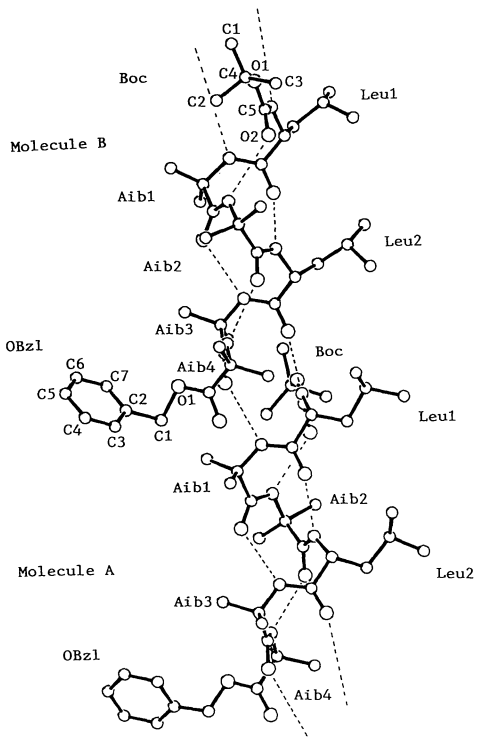

Fig. 1. Molecular structure of Boc-(L-Leu-Aib_2)$_2$-OBzl together with the atomic numbering in the terminal moieties (Boc and OBzl). Broken lines denote intra- and intermolecular hydrogen bonds.

Since the allowed regions of dihedral angles for α- and 3_{10}-helix almost overlap each other in the ϕ-ψ map, it is very difficult to decide the helical type on the basis of dihedral angles. Therefore, the helical parameters for each amino acid residue were calculated. This method revealed usefulness for the determination of the helical type in the case of Boc-(L-Leu_3-Aib_2)$_2$-OBzl molecule.[6] The helical parameters are the unit height (h) and the unit twist (θ). These are the height per residue along the helical axis and the rotational angle per residue about the helical axis, respectivlely. Assuming that the each amino acid residue forms its own helix, the helical parameters are derived from its bond lengths, bond angles, and dihedral angles by the method of Sugeta and Miyazawa.[15] These values for each residue except for the C-terminus Aib_4 are shown in Table 3. The average values in the molecule A are $<h>$=1.86 Å and $<\theta>$=113°, and those in B, $<h>$=1.85 Å and $<\theta>$=−110°. Since the helical parameters for α- and 3_{10}-helix are h=1.5 Å and θ=100°, and h=2.0 Å and θ=120°, respectively, it is obvious that both A and B molecules are close to a 3_{10}-helix rather than an α-helix.

In many cases, the helical conformation of Aib-containing oligopeptides have been determined by the intramolecular hydrogen bonding type. That is, the molecule with the hydrogen bonds between the C=O group of residue i and the N-H group of residue i+3 is classified as a 3_{10}-helix, and that with the bonds between residue i and i+4 classified as an α-helix. Hydrogen-bond lengths and angles of Boc-(L-Leu-Aib_2)$_2$-OBzl are given in Table 4. The hydrogen atoms of N-H group are located on their expected positions. Both A and B molecules are stabilized by four 4→1 intramolecular hydrogen bonds, which belong to the 3_{10}-helix. This result is agreed with the previous result from the helical parameters. Most hydrogen bonds are close to the standard value (2.9±0.1 Å)[16] with one exception of rather long bond length (N(Aib_2)···O2-(Boc)). On the other hand, the following four hydrogen bond angles are fairly deviated from the ideal angle (180°): N(Aib_4)···O(Aib_2) in molecules A and B, N(A:Aib_1)···O(B:Aib_3), and N(B:Aib_1)···O-(A:Aib_3).

Table 3. Helical Parameters for Each Amino Acid in Boc(L-Leu-Aib_2)$_2$OBzl

	Leu_1	Aib_1	Aib_2	Leu_2	Aib_3	Average
Molecule A						
h/Å	1.75	2.00	1.98	1.90	1.68	1.86
θ/°	110	115	113	116	109	113
Molecule B						
h/Å	1.87	1.92	1.98	1.88	1.62	1.85
θ/°	−101	−116	−118	−112	−102	−110

Table 4. Hydrogen-Bond Parameters, with Estimated Standard Deviations in Parentheses

Hydrogen bond	Bond length/Å (N···O)	Bond angle/° (N–H···O)
Intramolecular		
Molecule A		
$N(Aib_2)\cdots O2(Boc)$	3.16(3)	154
$N(Leu_2)\cdots O(Leu_1)$	2.88(3)	162
$N(Aib_3)\cdots O(Aib_1)$	2.96(3)	155
$N(Aib_4)\cdots O(Aib_2)$	3.04(3)	137
Molecule B		
$N(Aib_2)\cdots O2(Boc)$	2.98(3)	154
$N(Leu_2)\cdots O(Leu_1)$	2.87(3)	164
$N(Aib_3)\cdots O(Aib_1)$	3.00(3)	158
$N(Aib_4)\cdots O(Aib_2)$	2.88(3)	135
Intermolecular		
$N(A:Leu_1)\cdots O(B:Leu_2)$	2.97(3)	172
$N(A:Aib_1)\cdots O(B:Aib_3)$	3.05(3)	125
$N(B:Leu_1)\cdots O(A:Leu_2)$	2.83(3)	156
$N(B:Aib_1)\cdots O(A:Aib_3)$	2.97(3)	129

Table 5. Dihedral Angles of Side Chains in L-Leu Residues in Molecules A and B, with Estimated Standard Deviations in Parentheses

	χ_1	χ_2	χ'_2
Molecule A			
Leu_1	−72(5)	175(3)	−64(3)
Leu_2	−76(4)	168(4)	73(3)
Molecule B			
Leu_1	−63(6)	177(4)	−70(4)
Leu_2	−63(5)	−178(4)	−57(4)

Side Chain Conformations. The dihedral angles in the L-Leu side chains of molecules A and B are given in Table 5. All the side chains have the dihedral angles of $\chi_1(N-C_\alpha-C_\beta-C_\gamma)$ and $\chi'_2(C_\alpha-C_\beta-C_\gamma-C_{\delta_2})$ with gauche, and that of χ_2 $(C_\alpha-C_\beta-C_\gamma-C_{\delta_1})$ wih trans. These conformations are usually found in many other L-Leu-containing peptides.[12,17]

Crystal Structure of Boc-(L-Leu-Aib_2)$_2$-OBzl. The stereo view of the packing structure of Boc-(L-Leu-Aib_2)$_2$-OBzl is shown in Fig. 2. For making it clear, one of the molecules related by the crystallographic 2_1 symmetry are ignored in this figure. The independent molecules A and B are arranged in the head-to-tail fashion by use of four intermolecular hydrogen bonds between these molecules (Table 4) to form infinite helical columns along the [101] direction. All the side chains of the Leu residues are projected on one side of the column. On the other hand, all the benzyl moieties are projected on the opposite side of the column. These make a hydrophobic region between adjacent columns.

The helical axes of A and B molecules in the same column are almost parallel to the [101] direction and are separated along the crystallographic *b*-axis by about a diameter of these helices. Each column is surrounded by six crystallographically equivalent columns and these are packed closely in the pseudo-hexagonal arrangement. Among the six surrounding columns, two of them have the same direction as that of the center one, whereas the remaining four have the opposite direction. There are no hydrogen bonds between adjacent columns and only hydrophobic interaction stabilizes the crystal structure. The followings are fairly short atomic contacts between adjacent molecules: $C(A:Aib_1)\cdots O(B:Aib_3)$ 3.41(4) Å, $O(A:Aib_4)\cdots Cl(B:OBzl)$ 3.43(5) Å, $O(B:Leu_1)\cdots C4(B:OBzl)$ 3.36(6) Å and $O(B:Aib_1)\cdots Cl(A:Aib_1)$ 3.41(4)Å.

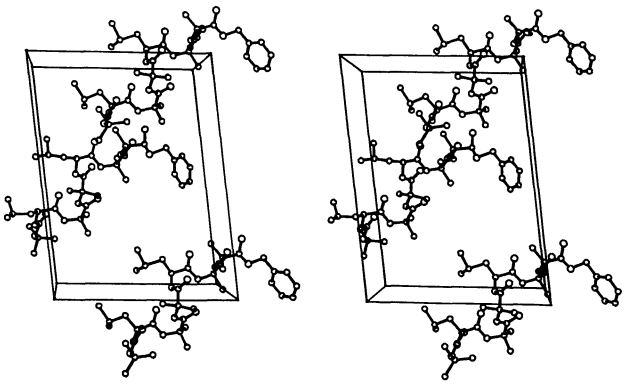

Fig. 2. Stereoscopic view of the packing structure of Boc-(L-Leu-Aib_2)$_2$-OBzl (ORTEP[19] drawing). The *a* axis points upwards, the *b* axis onto the plane of paper and the *c* axis from left to right. For the sake of clarity, the molecules related by the 2_1 symmetry are omitted.

Conformation in Solution. Figure 3 shows the CD spectrum of Boc-(L-Leu-Aib_2)$_2$-OBzl in a methanol solution, together with that of Boc-(L-Leu_3-Aib)$_2$-OBzl for the comparison. The latter peptide forms a right-handed α-helical conformation in the single crystal,[6] and it exhibits a CD spectrum for a right-handed helical conformation with two negative bands at 204($\pi-\pi^*$) and 220($n-\pi^*$) nm (Fig. 3). On the other hand, the Boc-(L-Leu-Aib_2)$_2$-OBzl exhibits two weak negative bands at 202 and 236 nm. Weakness of the bands of Boc-(L-Leu-Aib_2)$_2$-OBzl is attributed to shortness of the peptide chain compared with Boc-(L-Leu_3-Aib)$_2$-OBzl. The relationship between peptide length and band strength was reported for the series of Boc-X-(Aib-X)$_n$-OMe (n=1,2,3) and Boc-(Aib-X)$_5$-OMe where X=L-Ala and L-Val.[18] In these experiments, the similar weak negative bands were observed for short peptide compounds. The weak negative bands of Boc-(L-Leu-Aib_2)$_2$-OBzl, therefore, seem to suggest that a right-handed helix is likely in methanol solution. This means that the ratio of right-handed helix to left-handed helix in solution state is different

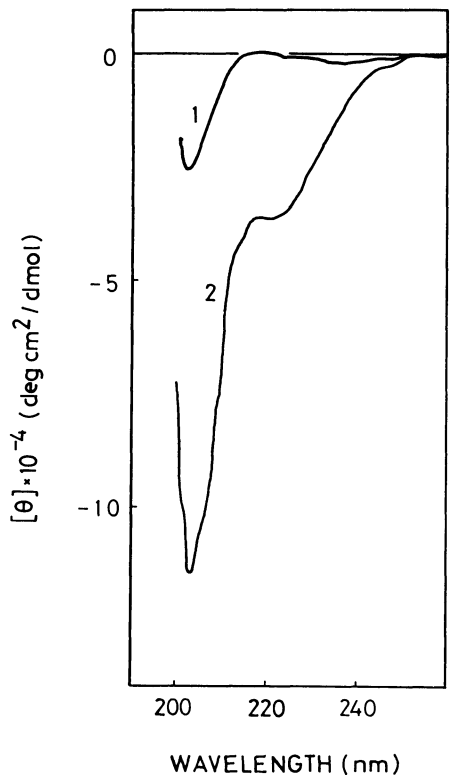

Fig. 3. CD spectra of Boc-(L-Leu-Aib2)2-OBzl (1) and Boc-(L-Leu3-Aib)2-OBzl (2).

from that in crystal state. The disadvantage for a left-handed helix of the peptide containing L-amino acid may be reduced by the intermolecular packing energy in solid state.

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