First Observation of a Helical Peptide containing Chiral α-Monosubstituted Residues without a Preferred Screw Sense

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We report the detailed X-ray structure of the fully blocked tetrapeptide Z-D-Val-$(Aib)_2$-L-Phe-OMe. The compound crystallizes in the space group $P2_1$ with four independent tetrapeptide molecules aligned in a parallel arrangement along the *c* axis. There is a regular alternation of right- and left-handed 3_{10}-helices hydrogen bonded head-to-tail along this axis. Pairs of molecules with the same handedness differ in the conformation of the side chains and of the *N*- and *C*-terminal blocking groups. This is the first observation, to the best of our knowledge, of a helical peptide containing chiral α-monosubstituted α-amino acids without a preferred screw sense. Conformational energy computations confirmed that those helices with different handedness have comparable stabilities. This work is a part of our studies on fully protected tetrapeptides containing homo and hetero chiral residues at *N*- and *C*-termini spaced by an achiral dipeptide segment, in order to understand the structural features responsible for the diastereoselective separation by reversed-phase HPLC.

Peptides containing α-aminoisobutyric acid (Aib) have been studied in our laboratories for several years. The interest in this class of compounds arises from (*i*) the overwhelming presence of the Aib residue in membrane-active, channel-forming peptaibol antibiotics,[1-13] and (*ii*) the unique conformational behaviour of this residue which can be used as a local structure determinant in designing simplified sequences for natural peptides and therefore providing a new type of conformational constraint in peptides.[14-28]

We have demonstrated that this residue has an high propensity to give folded structures and that in long homopeptides the 3_{10}-helix is observed.[29,32] Peptides containing α-monosubstituted amino acids can give rise to either α- or 3_{10}-helices depending on several factors.[8,31-33] We have also demonstrated that fully protected achiral homopeptides-$(Aib)_n$-(n = 3–10) adopt a 3_{10}-helical conformation of either right or left screw sense,[29,30,34-37] while peptides containing chiral α-monosubstituted residues have a handedness depending on the configuration of the incorporated optically active amino acids.[38-41] It has recently been reported[42] that the chiral peptide, containing only α,α-dialkylated residues, namely Ac-$(Aib)_2$-S-Iva-$(Aib)_2$-OMe (Ac: acetyl; Iva: isovaline) crystallizes as two crystallographically independent molecules differing essentially in the handedness of their 3_{10}-helical structure.

The solid state X-ray structure of the peptides Z-$(Aib)_3$-L-Val-OMe and Z-$(Aib)_3$-L-Val-Gly-OMe[41] (Z: benzyloxycarbonyl) seemed to indicate that the effect of chiral residues in determining the handedness of helices might also depend on the position of incorporation in Aib-containing fully protected linear peptides.

Therefore, in order to better understand and characterize the factors governing the handedness of helices in chiral peptides containing the Aib residue, we have characterized in the solid state the molecular conformation of the fully protected tetrapeptide Z-D-Val-$(Aib)_2$-L-Phe-OMe. Conformational energy calculations have also been performed in order to compare the relative stabilities of the right- and left-handed helices.

This work is a part of our studies on fully protected tetrapeptides containing homo and hetero chiral residues at *N*- and *C*-termini spaced by an achiral dipeptide segment, in order to understand the structural features responsible for the diastereoselective separation by reversed-phase HPLC. The driving force which determines the retention time in RP-HPLC is well known to be the hydrophobic interaction of a substrate with the octadecylated stationary phase. Therefore conformational differences[43,44] may determine the degree of separation of diastereoisomers in chromatography. From this viewpoint, Yamada *et al.*[45-53] have systematically studied the separation behaviour in reversed-phase HPLC of diastereoisomers of fully protected tetrapeptides containing either homo- or hetero-chiral amino acids at the N- and C-termini, spaced by an achiral dipeptide segment (X-Y), namely Z-(L/D)-Val-X-Y-L-Phe-OMe (X, Y = Sar, Gly, Ac_3c, Aib, Ac_5c, Ac_6c, Deg, Dpg, Dbu, Dbz, Dph) (Ac_3c: 1-aminocyclopropane-1-carboxylic acid; Ac_5c: 1-aminocyclopentane-1-carboxylic acid; Ac_6c: 1-aminocyclohexane-1-carboxylic acid; Deg: C^α,C^α-diethyl glycine; Dpg: C^α,C^α-dipropyl glycine; Dbu: C^α,C^α-dibutyl glycine; Dbz: C^α,C^α-dibenzyl glucine; Dph: C^α,C^α-diphenyl glycine).

The X-ray structure of a tetrapeptide from this class, namely Z-D-Val-Ac_6c-Gly-L-Phe-OMe, has already been reported.[54] The previous findings are in agreement with the results for the present structure and suggest possible structural features governing the overall hydrophobicity.

Experimental

Suitable, but low quality, crystals for X-ray diffraction studies were obtained by slow evaporation of an ethyl acetate solution.

Crystal Data.—$C_{32}H_{42}N_4O_8$, M = 582.7 amu. Monoclinic, a = 16.15(7), b = 13.11(8), c = 32.36(3) Å, β = 108.1(3)°, V = 6514 Å^3, λ = 1.541 78 Å, space group $P2_1$, Z = 8, D_m = 1.18 deg cm^{-3}, D_x = 1.188 deg cm^{-3}.

Data Collection and Processing.[55]—CAD4 Enraf-Nonius diffractometer (equipped with a MicroVax II and a Vax 750 Digital computers of the *Centro Interdipartimentale di Metodologie Chimico Fisiche* at the University of Napoli), $\omega/2\theta$ mode with ω scan width = (1.0 + 0.35 tan θ)°, ω scan speed

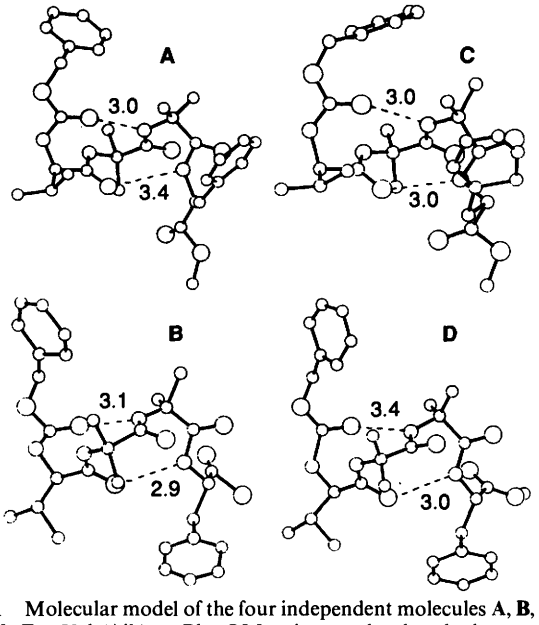

Fig. 1 Molecular model of the four independent molecules **A**, **B**, **C** and **D** of Z-D-Val-(Aib)$_2$-L-Phe-OMe; intramolecular hydrogen bond distances are indicated

1–5 deg min^{-1}, graphite-monochromated Cu-Kα radiation, 11 989 reflections measured ($1.0° \leq \Theta \leq 70°$), 3654 with $I > 1.5\sigma(I)$.

Structure Analysis and Refinement.—Straightforward applications of direct methods (SHELXS-86,[56] SIR,[57] PATSEE,[58] MULTAN-80,[59]) failed to give any stereochemical image of the molecules. The analysis of the observed structure factors revealed that few general reflections *hkl* with $l = 2n + 1$ were observed [189 reflections with $I \geq 1.5\sigma(I)$]. This was interpreted as a possible non-crystallographic symmetry along the *c* axis. Therefore we attempted to solve the structure in a unit cell with half *c* axis ($c' = c/2 = 16.18$ Å) and keeping the same space group $P2_1$. With this assumption, the structure was solved, in this reduced unit cell and containing only two independent molecules, using the Rantan procedure contained in SHELXS-86.[56] A total of 350 phase sets was developed. The E-map obtained from the phase set with the best figures of merit revealed 48 non-hydrogen atoms corresponding to two parts of two independent molecules. Using successive applications of the Dirdif package[60] it was possible to obtain the entire image of these two independent molecules, including double images of the *N*- and *C*-terminal blocking groups and of the phenylalanine side chains. The analysis of the packing in this reduced cell allowed us to properly position the four independent molecules in the real cell ($c = 32.36$ Å). Repeated geometry regularization, constrained refinement and slow relaxation of the structure, using a block procedure with the SHELXS-76 package,[61] converged to an *R* factor equal to 0.102 for 3654 independent reflections with $I \geq 1.5\sigma(I)$ ($R_w = 0.110$), with isotropic temperature factors. Hydrogen atoms were included in the structure factor calculation in their stereochemical expected position but not refined, with isotropic temperature factors equal to the equivalent *U* factor of the carrying atoms. Atomic scattering factors for all atomic species were calculated from Cromer and Waber.[62]

Conformational Energy Computations.—Right- and left-handed helices were built with standard residues directly drawn from the database of the Sybyl package.[63] Partial atomic charges were taken from Kollman's all atom force field[64] except those of the *N*-terminal Z group which were assigned as follows: the urethane COO charges were fixed equal to those of the same atoms in the urethane *t*-Boc (*tert*-butyloxycarbonyl), those of the benzyl group were taken from the same atoms in the phenylalanine residue with a slight modification on the CH_2 group in order to ensure the overall neutrality.[65–67] A dielectric distance-dependent constant has been used in the electrostatic term. A systematic search[68] of the energy minima was performed on the torsion angles χ_1^1, χ_4^2 and C_4^{α}-C′$_4$-O$_4$-CMe, χ_4^1, φ_4 and ψ_4. The backbone φ, ψ angles of the rest of the molecule were initially kept fixed in a standard either right or left 3_{10}-helical conformation. The Kollman united atoms force field[69] was used to evaluate single point energies of the conformational search. The united atoms representation was chosen in order to smooth the influence of short range interatomic contacts that might derive from keeping part of the molecule frozen. Few steps of full minimization on all the degrees of freedom were performed in each point of this search. An energy cut-off of 10 kcal mol^{-1} in the conformational search allowed us to obtain 12 conformers for the right handed structure and 21 for the structure of opposite handedness.* All these selected structures were fully minimized. The conformations corresponding to the absolute minima of these sets were successively minimized using the all-atoms Kollman force field.[64] The BFGS[70–73] minimization algorithm was used with a convergence criterion of 0.001 on the energy gradient. In addition, an energy minimization of the experimental structures *in vacuo* has been performed with the same criteria.

Results and Discussion

The structure of Z-D-Val-(Aib)$_2$-L-Phe-OMe in the solid state shows a geometry for all the residues that is in agreement with the literature data, within experimental error, except those of the *C*-terminal end. The atomic positions in this part of the molecules were poorly determined; the thermal motion of these atoms appears to be higher in relation to the rest of the molecule. Atomic co-ordinates for all four molecules are given in Table 1.†

Fig. 1 is a view of the four independent tetrapeptide molecules. The intramolecular hydrogen bond distances are indicated. The conformational parameters of the molecules are reported in Table 2.

Molecules **A** and **C** have a backbone conformation characterized by two consecutive type III β-turns and corresponding to incipient right-handed 3_{10}-helices stabilized by two intramolecular hydrogen bonds involving the carbonyl groups of the urethane moiety and of the D-Val^1 residue with the NH groups of the *C*-terminal residues Aib^3 and L-Phe.^{4} In molecule **A** the latter of these hydrogen bonds is weaker.

The *N*-terminal D-Val^1 and the two central Aib residues adopt the typical conformation in the right-handed helical region, while the *C*-terminal L-Phe^4 residue is in the F region of the Ramachandran plot (according to Zimmerman *et al.*[74]) for both molecules **A** and **C**. The side chain conformational parameters of the D-Val^1 residue are g^+, g^-, the χ^1 and χ^2 angles of the Phe^4 residue are also typical for this residue.[75]

As far as the benzyloxycarbonyl group is concerned, the C(1)–C(7)–O(1)–C(8) and the C(2)–C(1)–C(7)–O(1) torsion angles, giving the orientation of the phenyl ring relative to the urethane moiety, are both g^+. The urethane linkage is found in the usual *trans* conformation.[76]

Molecules **B** and **D** have a backbone conformation characterized by two consecutive slightly distorted type III′ β-

* 1 cal = 4.184 J.

† Thermal parameters, bond lengths and angles, and hydrogen atom co-ordinates have been deposited at the Cambridge Crystallographic Data Centre. See 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 2*, 1992, issue 1.

turns and corresponding to incipient left-handed 3_{10}-helices stabilized by two intramolecular hydrogen bonds involving the carbonyl groups of the urethane moiety and of the D-Val[1] residue and the NH groups of the *C*-terminal residues Aib[3] and L-Phe.[4] In molecule **D** the former of these hydrogen bonds is weaker.

The *N*-terminal D-Val[1] residue and the two central Aib residues adopt a typical conformation in the left-handed helical

Table 1 Atomic co-ordinates with esds in parentheses

	x/a	*y/b*	*z/c*		*x/a*	*y/b*	*z/c*
Molecule **A**							
C(7)	−0.0166(17)	0.2460(23)	0.5509(9)	C'_2	0.2321(12)	0.0266(17)	0.4769(6)
C(1)	−0.0373(12)	0.1608(17)	0.5118(6)	O_2	0.2638(10)	0.0068(14)	0.4486(5)
C(2)	−0.0421(16)	0.0674(22)	0.5211(8)	N_3	0.1863(12)	0.1122(16)	0.4681(6)
C(3)	−0.0672(18)	−0.0085(23)	0.4872(9)	$C^{\alpha}{}_3$	0.1687(11)	0.1752(15)	0.4296(5)
C(4)	−0.0928(18)	0.0240(24)	0.4467(9)	$C^{\beta 1}{}_3$	0.1311(23)	0.2773(31)	0.4354(12)
C(5)	−0.0921(14)	0.1228(19)	0.4381(7)	$C^{\beta 2}{}_3$	0.0917(19)	0.1096(25)	0.3958(9)
C(6)	−0.0686(15)	0.2010(21)	0.4675(8)	C'_3	0.2539(11)	0.1988(16)	0.4192(6)
O(1)	0.0610(8)	0.2160(11)	0.5825(4)	O_3	0.2231(10)	0.1982(14)	0.3732(5)
C(8)	0.1359(13)	0.2139(18)	0.5741(7)	N_4	0.3248(10)	0.2149(14)	0.4517(5)
O(2)	0.1371(9)	0.2364(12)	0.5375(5)	$C^{\alpha}{}_4$	0.3827(12)	0.2648(16)	0.4306(7)
N_1	0.2020(9)	0.1940(12)	0.6075(4)	$C^{\beta}{}_4$	0.4145(18)	0.3655(20)	0.4568(9)
$C^{\alpha}{}_1$	0.3007(13)	0.1823(18)	0.6104(7)	$C^{\gamma}{}_4$	0.3471(20)	0.4367(27)	0.4377(10)
$C^{\beta}{}_1$	0.3419(13)	0.2967(17)	0.6170(6)	$C^{\delta 1}{}_4$	0.3271(26)	0.4487(34)	0.3905(13)
$C^{\gamma 1}{}_1$	0.3512(16)	0.3461(21)	0.6636(8)	$C^{\varepsilon 1}{}_4$	0.2600(35)	0.5167(47)	0.3716(18)
$C^{\gamma 2}{}_1$	0.3025(21)	0.3794(27)	0.5788(10)	$C^{\zeta}{}_4$	0.2165(29)	0.5707(39)	0.3902(16)
C'_1	0.2979(14)	0.1299(19)	0.5663(7)	$C^{\varepsilon 2}{}_4$	0.2287(26)	0.5617(34)	0.4283(14)
O_1	0.3503(8)	0.1661(11)	0.5464(4)	$C^{\delta 2}{}_4$	0.2907(26)	0.4948(33)	0.4545(13)
N_2	0.2570(10)	0.0432(14)	0.5536(5)	C'_4	0.4503(12)	0.2169(16)	0.4590(7)
$C^{\alpha}{}_2$	0.2547(16)	−0.0239(21)	0.5200(8)	O_4	0.4786(12)	0.2072(16)	0.5014(7)
$C^{\beta 1}{}_2$	0.2029(14)	−0.1066(18)	0.5184(7)	O_4*	0.5044(12)	0.1870(16)	0.4334(7)
$C^{\beta 2}{}_2$	0.3453(16)	−0.0636(21)	0.5293(8)	C(9)	0.5899(12)	0.1640(16)	0.4615(7)
Molecule **B**							
C(7)	0.0345(23)	−0.1821(30)	0.3260(12)	C'_2	0.1820(17)	0.0959(22)	0.2226(8)
C(1)	−0.0238(14)	−0.1011(19)	0.3162(7)	O_2	0.2044(9)	0.1488(12)	0.1945(5)
C(2)	−0.1012(20)	−0.0749(26)	0.3303(10)	N_3	0.1513(10)	0.0045(14)	0.2195(5)
C(3)	−0.1607(19)	0.0030(26)	0.3167(10)	$C^{\alpha}{}_3$	0.1206(12)	−0.0467(17)	0.1796(6)
C(4)	−0.1511(22)	0.0787(29)	0.2916(11)	$C^{\beta 1}{}_3$	0.0296(14)	0.0068(19)	0.1479(7)
C(5)	−0.0747(23)	0.0792(30)	0.2780(11)	$C^{\beta 2}{}_3$	0.1018(17)	−0.1494(23)	0.1880(9)
C(6)	−0.0212(16)	−0.0132(21)	0.2835(8)	C'_3	0.1947(21)	−0.0220(27)	0.1562(11)
O(1)	0.1140(11)	−0.1478(14)	0.3541(5)	O_3	0.1684(10)	−0.0308(13)	0.1153(5)
C(8)	0.1779(18)	−0.1352(24)	0.3349(9)	N_4	0.2606(12)	−0.0747(16)	0.1810(6)
O(2)	0.1668(11)	−0.1520(15)	0.2960(6)	$C^{\alpha}{}_4$	0.3220(15)	−0.1163(16)	0.1603(8)
N_1	0.2259(10)	−0.0826(13)	0.3598(5)	$C^{\beta}{}_4$	0.4175(16)	−0.1168(21)	0.1937(8)
$C^{\alpha}{}_1$	0.3032(13)	−0.0889(18)	0.3459(7)	$C^{\gamma}{}_4$	0.4658(15)	−0.0113(20)	0.2005(8)
$C^{\beta}{}_1$	0.3794(13)	−0.0490(18)	0.3822(7)	$C^{\delta 1}{}_4$	0.4998(21)	0.0244(26)	0.2468(11)
$C^{\gamma 1}{}_1$	0.4015(17)	−0.1293(24)	0.4200(9)	$C^{\varepsilon 1}{}_4$	0.5509(23)	0.1272(33)	0.2496(12)
$C^{\gamma 2}{}_1$	0.4599(18)	−0.0257(23)	0.3692(9)	$C^{\zeta}{}_4$	0.5781(23)	0.1736(31)	0.2230(12)
C'_1	0.2839(14)	−0.0210(18)	0.3042(7)	$C^{\varepsilon 2}{}_4$	0.5544(22)	0.1334(30)	0.1837(12)
O_1	0.3211(8)	−0.0442(10)	0.2767(4)	$C^{\delta 2}{}_4$	0.4939(16)	0.0406(23)	0.1701(8)
N_2	0.2405(10)	0.0658(14)	0.3024(5)	C'_4	0.2906(12)	−0.2265(15)	0.1502(5)
$C^{\alpha}{}_2$	0.2274(13)	0.1402(17)	0.2703(7)	O_4	0.2460(12)	−0.2761(15)	0.1509(5)
$C^{\beta 1}{}_2$	0.3173(15)	0.1966(20)	0.2723(7)	O_4*	0.3542(12)	−0.2302(15)	0.1214(5)
$C^{\beta 2}{}_2$	0.1523(13)	0.2184(17)	0.2768(6)	C(9)	0.3494(48)	−0.3280(66)	0.1234(23)
Molecule **C**							
C(7)	−0.0079(15)	0.3451(20)	0.1751(7)	C'_2	−0.1979(11)	0.6090(15)	0.2736(6)
C(1)	0.0533(11)	0.4375(15)	0.1942(6)	O_2	−0.1881(11)	0.6728(15)	0.3076(6)
C(2)	0.0278(23)	0.5239(31)	0.2124(12)	N_3	−0.1546(10)	0.5306(13)	0.2797(5)
C(3)	0.0908(26)	0.5839(32)	0.2395(12)	$C^{\alpha}{}_3$	−0.1114(14)	0.4842(19)	0.3234(7)
C(4)	0.1720(24)	0.5995(32)	0.2410(12)	$C^{\beta 1}{}_3$	−0.0440(14)	0.5452(19)	0.3505(7)
C(5)	0.1919(32)	0.5189(43)	0.2184(16)	$C^{\beta 2}{}_3$	−0.0814(16)	0.3703(22)	0.3163(8)
C(6)	0.1408(23)	0.4452(32)	0.1947(12)	C'_3	−0.1836(9)	0.4451(13)	0.3447(5)
C(1)	−0.0891(10)	0.3885(13)	0.1478(5)	O_3	−0.1635(9)	0.4375(12)	0.3851(4)
C(8)	−0.1525(12)	0.3853(16)	0.1651(6)	N_4	−0.2739(10)	0.4539(14)	0.3193(5)
C(2)	−0.1416(10)	0.3556(13)	0.2047(5)	$C^{\alpha}{}_4$	−0.3388(14)	0.4129(18)	0.3365(7)
N_1	−0.2521(14)	0.4181(18)	0.1369(7)	$C^{\beta}{}_4$	−0.4300(15)	0.4164(20)	0.3029(8)
$C^{\alpha}{}_1$	−0.3224(14)	0.4391(19)	0.1546(7)	$C^{\gamma}{}_4$	−0.4790(17)	0.5121(23)	0.2950(9)
$C^{\beta}{}_1$	−0.3954(15)	0.4880(20)	0.1135(8)	$C^{\delta 1}{}_4$	−0.5015(23)	0.5519(34)	0.3356(12)
$C^{\gamma 1}{}_1$	−0.4328(17)	0.4120(22)	0.0788(9)	$C^{\varepsilon 1}{}_4$	−0.5379(20)	0.6257(28)	0.3304(10)
$C^{\gamma 2}{}_1$	−0.4660(18)	0.5273(25)	0.1333(9)	$C^{\zeta}{}_4$	−0.5725(20)	0.6821(27)	0.2927(10)
C'_1	−0.2988(11)	0.5060(15)	0.1937(6)	$C^{\varepsilon 2}{}_4$	−0.5496(20)	0.6426(29)	0.2522(11)
O_1	−0.3364(10)	0.4934(13)	0.2223(5)	$C^{\delta 2}{}_4$	−0.5018(17)	0.5634(22)	0.2587(8)
N_2	−0.2454(10)	0.5860(13)	0.1955(5)	C'_4	−0.3085(13)	0.2996(17)	0.3550(6)
$C^{\alpha}{}_2$	−0.2302(14)	0.6708(18)	0.2296(7)	O_4	−0.2624(10)	0.2382(13)	0.3389(5)
$C^{\beta 1}{}_2$	−0.3041(15)	0.7302(21)	0.2224(8)	O_4*	−0.3291(13)	0.2633(17)	0.3888(6)
$C^{\beta 2}{}_2$	−0.1661(21)	0.7343(29)	0.2276(11)	C(9)	−0.3226(21)	0.1507(28)	0.4012(10)

Table 1 (*continued*)

	x/a	*y/b*	*z/c*		*x/a*	*y/b*	*z/c*
Molecule **D**							
C(7)	−0.0106(17)	0.2990(23)	0.0509(9)	C'_2	0.2328(12)	0.0664(16)	−0.0225(6)
C(1)	−0.0461(21)	0.2097(29)	0.0320(11)	O_2	0.2471(9)	−0.0031(12)	−0.0534(5)
C(2)	−0.1145(29)	0.1695(40)	0.0552(15)	N_3	0.1889(9)	0.1403(13)	−0.0265(5)
C(3)	−0.1623(24)	0.0755(33)	0.0339(12)	$C^{\alpha}{}_3$	0.1538(16)	0.1911(22)	−0.0698(8)
C(4)	−0.1330(26)	0.0280(37)	0.0004(14)	$C^{\beta 1}{}_3$	0.1298(11)	0.2786(15)	−0.0628(6)
C(5)	−0.0892(26)	0.0749(35)	−0.0239(13)	$C^{\beta 2}{}_3$	0.0957(15)	0.1327(21)	−0.1056(8)
C(6)	−0.0530(19)	0.1754(28)	−0.0049(11)	C'_3	0.2279(16)	0.2061(23)	−0.0882(8)
O(1)	0.0693(10)	0.2718(14)	0.0888(5)	O_3	0.2552(8)	0.1948(12)	−0.1195(4)
C(8)	0.1423(12)	0.2583(16)	0.0767(6)	N_4	0.3046(11)	0.2517(15)	−0.0590(6)
O(2)	0.1480(8)	0.2732(11)	0.0399(4)	$C^{\alpha}{}_4$	0.3985(20)	0.2612(27)	−0.0604(10)
N_1	0.2067(11)	0.2265(15)	0.1108(6)	$C^{\beta}{}_4$	0.4024(30)	0.3962(38)	−0.0659(15)
$C^{\alpha}{}_1$	0.2918(12)	0.2106(16)	0.1092(6)	$C^{\gamma}{}_4$	0.3415(22)	0.4638(30)	−0.0948(11)
$C^{\beta}{}_1$	0.3539(19)	0.2956(26)	0.1245(10)	$C^{\delta 1}{}_4$	0.2890(36)	0.4389(46)	−0.1417(20)
$C^{\gamma 1}{}_1$	0.3623(18)	0.3110(23)	0.1709(9)	$C^{\varepsilon 1}{}_4$	0.2465(42)	0.5088(60)	−0.1651(22)
$C^{\gamma 2}{}_1$	0.3072(18)	0.3867(22)	0.0951(9)	$C^{\zeta}{}_4$	0.2205(28)	0.5894(37)	−0.1518(14)
C'_1	0.2987(12)	0.1568(16)	0.0690(6)	$C^{\varepsilon 2}{}_4$	0.2793(34)	0.6261(41)	−0.1178(17)
O_1	0.3473(11)	0.1958(16)	0.0487(6)	$C^{\delta 2}{}_4$	0.3357(39)	0.5553(56)	−0.0922(19)
N_2	0.2476(10)	0.0734(13)	0.0566(5)	C'_4	0.4705(17)	0.1926(24)	−0.0280(8)
$C^{\alpha}{}_2$	0.2632(12)	−0.0004(16)	0.0222(6)	O_4	0.4881(18)	0.1202(22)	−0.0047(9)
$C^{\beta 1}{}_2$	0.1838(16)	−0.0779(22)	0.0207(8)	O_4*	0.5431(17)	0.2257(22)	−0.0420(8)
$C^{\beta 2}{}_2$	0.3579(17)	−0.0430(22)	0.0365(8)	C(9)	0.6391(32)	0.1815(42)	−0.0141(16)

Table 2 Torsion angles

		Experimental				Theoretical	
Residue and angle		**A**	**B**	**C**	**D**	R	L
D-Val	φ	−45	77	−38	44	−39	50
	ψ	−45	37	−52	43	−41	32
	ω	−167	171	−171	168	178	−176
	$\chi_{1,1}$	62	72	69	67	62	63
	$\chi_{1,2}$	−57	−165	−62	−172	−61	−179
Aib	φ	−68	58	−57	55	−45	48
	ψ	−20	9	−20	32	−39	33
	ω	−170	−170	169	169	178	−176
Aib	φ	−54	54	−47	66	−52	51
	ψ	−44	53	−43	6	−38	37
	ω	162	168	−160	174	−177	179
L-Phe	φ	−105	−90	−145	−47	−132	54
N_4–$C^{\alpha}{}_4$–C'_4–O_4*	ψ	177	172	141	150	57	49
$C^{\alpha}{}_4$–C'_4–O_4*–C(9)	(ω)	171	165	162	166	−175	−171
	χ_1	−47	−90	−85	−78	−54	−52
	χ_2	−37	−62	−55	−66	−60	−73
C(2)–C(1)–C(7)–O(1)		87	76	58	45	53	122
C(1)–C(7)–O(1)–C(8)		85	−105	63	−104	70	−72
C(7)–O(1)–C(8)–N_1		−173	160	175	−172	−173	173
O(1)–C(8)–N_1–$C^{\alpha}{}_1$	(ω_0)	179	−163	−175	167	179	179
Conformational energy/kcal mol^{-1}						−35.1	−35.9

region, while the *C*-terminal L-Phe[4] residue is in the F region of the Ramachandran plot[74] for both molecules **B** and **D**. The side chain conformational parameters of the D-Val[1] and of the Phe[4] residue are also typical for these residues.[75]

As far as the benzyloxycarbonyl group is concerned, the C(1)–C(7)–O(1)–C(8) and the C(2)–C(1)–C(7)–O(1) torsion angles are g^+, s^- respectively. The urethane linkage is in the usual *trans* conformation.[76]

A comparison of the molecular conformation of the four independent tetrapeptide molecules shows the following features: (*i*) molecules **A** and **C** have the same handedness (right) as do molecules **B** and **D** (left); (*ii*) molecules **A** and **C** differ mainly in the conformation of the L-Phe[4] residue; (*iii*) molecules **B** and **D** differ slightly in the angles of the Aib residues and the conformation of the L-Phe[4] residue; (*iv*) molecules of opposite handedness differ also for the D-Val[1] side chain conformations (g^+, g^- and g^+, *t*); (*v*) right- and left-handed helices have the last residue Phe[4] in a conformation typical of L residues, independent of the handedness of the helix.

In Fig. 2 is reported the packing of the molecule as seen along the *b* axis. In Table 3 the 8 intermolecular hydrogen bond distances and the corresponding acceptors and donors are also reported. Molecules, translated along the *c* direction, have the N_1–H and N_2–H hydrogen bonded to the C'_2–O_2 and C'_3–O_3 groups, respectively. Rows of molecules along the *c* direction are formed by alternating molecules of right- and left-handedness, which are hydrogen bonded head-to-tail (see Fig. 3). These rows pack together as layers in an antiparallel fashion by hydrophobic interactions of the side chains and of the terminal blocking groups.

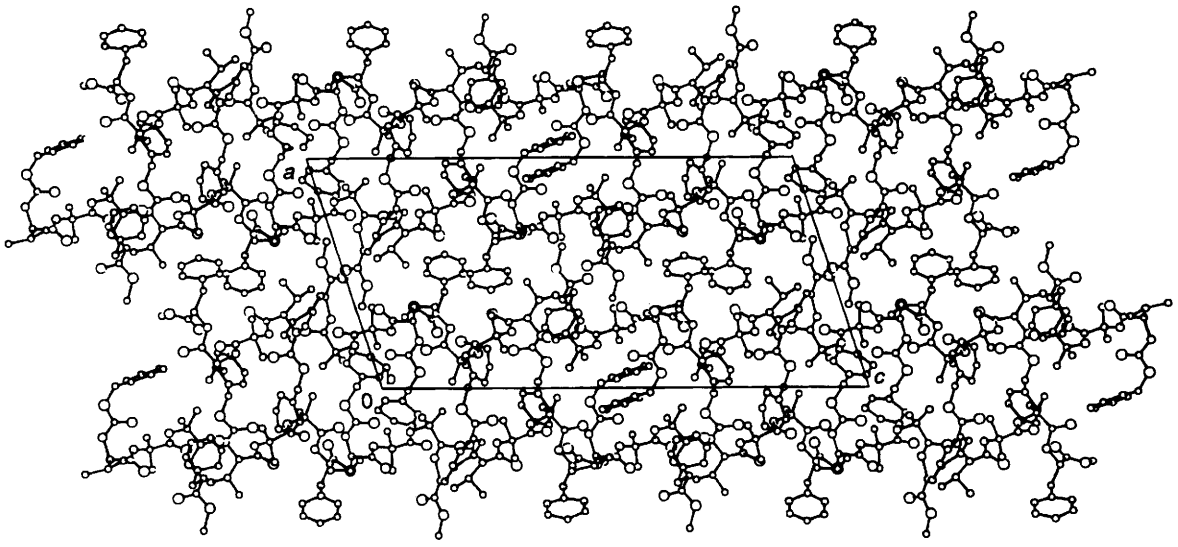

Fig. 2 Crystal packing viewed down the *b* axis

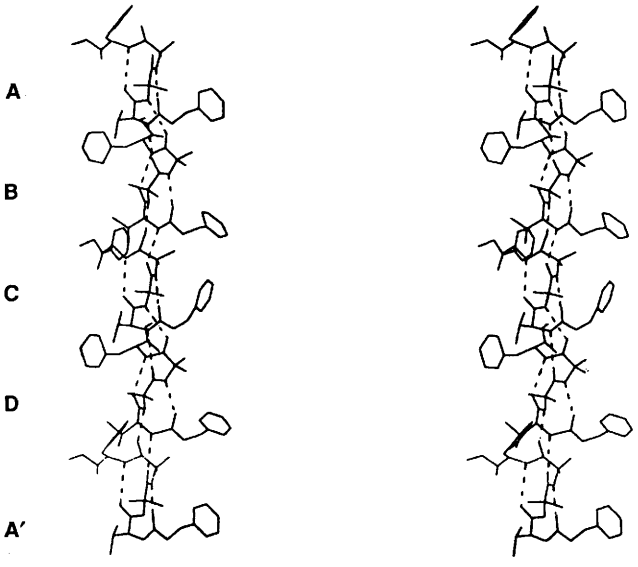

Fig. 3 Stereo view of the rows of molecules along the *c* direction which are formed by alternated molecules of opposite screw sense. The hydrogen bond pattern is indicated by dashed lines

Table 3 Intermolecular hydrogen bonds[a]

Acceptor	Molecule	Donor	Molecule	Bond distances/Å
O_2	**B**	N_1	**A**	2.9
O_3	**B**	N_2	**A**	2.9
O_2	**C**	N_1	**B**	3.0
O_3	**C**	N_2	**B**	3.0
O_2	**D**	N_1	**C**	2.8
O_3	**D**	N_2	**C**	3.2
O_2 (a)	**A**	N_1	**D**	2.9
O_3 (a)	**A**	N_2	**D**	2.8

[a] Symmetry operation (x, y, $z - 1$) to obtain the co-ordinates of the hydrogen-bonded atom.

The energy calculations indicate that several conformations of comparable energy could be obtained from a conformational search followed by partial energy minimization. Two searches were performed in both the right- and left-handed helical regions, varying the conformational parameters of the side chains, of the terminally blocking groups and of the φ, ψ angles of the L-phenylalanine residue. Several minima were then obtained in each region, and those having an energy difference of less than 10 kcal mol^{-1} from the absolute minima (12 conformers for the right-handed structure and 21 for the left-handed one), were subjected to a full minimization on all degrees of freedom. The absolute minima in both regions gave two structures, R and L, respectively. The corresponding conformational parameters and energies are reported in Table 1. These structures differ by 0.8 kcal mol^{-1}.

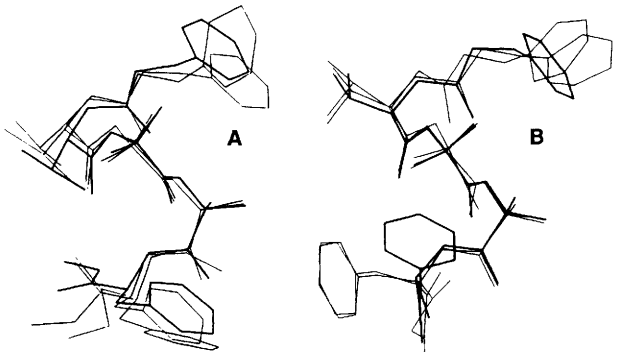

Fig. 4 Superimposition of right- (A) and left- (B) helices. Theoretical structures are indicated with thicker bonds.

The calculated right-handed structure, corresponding to the absolute minimum of the conformational energy in this region, is very similar to molecules **A** and **C** found in the crystal state except for the N_4–C^{α}_4–C'_4–O^*_4 torsion angle, while the calculated left-handed structure differs from those observed in the crystal structure in the D-Val^1 side chain, L-Phe^4 conformation and the Z group. In Fig. 4 is reported a backbone superposition of the observed and calculated right- and left-handed structures.

In order to analyse this behaviour better a comparison with minimized experimental structures has been performed. The energy minimum for the isolated molecules with a conformational behaviour similar to that of molecules **B** and **D** differs by 1 kcal mol^{-1} from the energy value corresponding to theoretical structure L. A similar comparison performed for right-handed structures shows that the experimental minimum is very similar to the theoretical structure R.

Conclusion

In conclusion the observed structure, when coupled with the results of the conformational energy computations *in vacuo* indicates that right- and left-handed 3_{10}-helices may have comparable stabilities. They lie in a wide region of the conformational space where several approximately isoenergetic minima are possible. Therefore it is likely that the crystallized conformations, in these helical regions, are further stabilized by favourable packing forces, like the head-to-tail hydrogen bonding or side-chain and end-groups hydrophobic interactions. This is the first example, to the best of our knowledge, of a helical peptide containing chiral α-monosubstituted α-amino acids without a preferred screw sense. These findings seem to indicate, together with previous observations,[41] that the screw sense of helical peptides containing Aib residues cannot be predicted from the configuration of the optically active α-monosubstituted α-amino acids when incorporated only at the terminal ends of a fully protected peptide.

As far as it regards the RP-HPLC behaviour of the L–L isomer in relation to the D–L isomer, presently investigated, the former has $k' = 10.41$ and the latter has $k' = 8.18$ with a separation factor $\alpha = 1.27$ as determined on a Cosmosil $5C_{18}$ [4.6 (i.d.) × 150 mm] using 65% methanol–water at a flow rate of 1.0 cm^3 min^{-1} at 30 °C. These results indicate that the molecular conformation of the L–L isomer imparts a greater hydrophobicity to the molecule.

Attempts to crystallize the L–L isomer have failed, so far, to give suitable crystals for X-ray analysis. The NMR studies in solution[77] are indicative of different conformational features that may account for the different RP-HPLC behaviour.

The studies are in agreement with our previous findings on Z–D–Val–Ac_6c–Gly–L–Phe–OMe and show that the differences in hydrophobicity, and therefore conformational differences, between diastereomers of the Aib–Aib derivatives are smaller than for the Ac_6c–Gly-containing tetrapeptides.

Further studies are presently in progress on other fully protected tetrapeptides containing either homo- or hetero-chiral sequences at the *N*- and *C*-termini to clarify and verify these findings.

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