Position of Side-chain Branching and Handedness of Turns and Helices of Homopeptides from Chiral C^{α}-Methylated Amino Acids. Crystal-state Structural Analysis of (αMe)Leu Trimer and Tetramer

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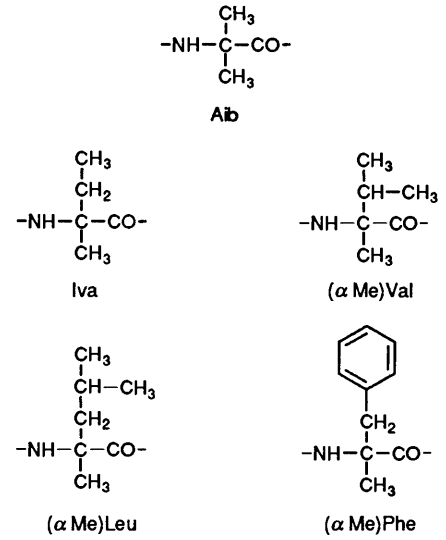
Terminally blocked homotri- and homotetra-peptides from (αMe)Leu, a chiral C_{α}-methylated, γ-branched α-amino acid, have been prepared by solution methods and fully characterized. The molecular and crystal structures of *p*BrBz-[D-(αMe)Leu]$_3$-OH monohydrate and *p*BrBz-[D-(αMe)-Leu]$_4$-OBu^t (where *p*BrBz indicates *p*-bromobenzoyl) were determined by X-ray diffraction. The tripeptide carboxylic acid adopts a type-III β-turn conformation followed by an uncommon oxy-analogue of a type-III β-turn, the latter being stabilized by a 1←4 C=O · · · H–O intramolecular H-bond. The three independent molecules in the asymmetric unit of the tetrapeptide ester are folded in a regular right-handed 3_{10}-helix. All (αMe)Leu residues exhibit φ, ψ torsion angles in the helical region of the conformational map. These results indicate that: (*i*) the (αMe)Leu residue is an effective β-turn and helix promoter and (*ii*) the relationship between (αMe)Leu chirality and turn and helix handedness is the same as that shown by the γ-branched (αMe)Phe residue, but it is opposite to that characteristic of isovaline (Iva), with a linear side chain, the β-branched (αMe)Val residue and protein amino acids (including Leu).

The stereochemistry of peptides containing C_{α}-methylated α-amino acids is rather unique as they possess significant constraints on their conformational freedom.[1] This property is of great relevance to the exploitation of these compounds as: (*i*) precise molecular rulers or as scaffolding units in the *de novo* design of protein and enzyme mimetics and in the investigation of molecular recognition processes,[2–4] and (*ii*) conformationally restricted, enzyme-resistant agonists and antagonists of bioactive peptides.[5–7]

Conformational energy computations, pioneered by Marshall in the early '70s,[1] showed that the presence of a *gem*-dimethyl group on the α-carbon of glycine imposes a marked restriction on the available conformational space of the resulting amino acid (Aib, α-aminoisobutyric acid or C_{α}-methylalanine) (Scheme 1). X-Ray diffraction analyses unequivocally established that only the type-III (III') β-turn[8–10] and the regular right- (and left-) handed 3_{10}-helix[11] are adopted by the achiral Aib homo-oligomers (to the decamer) in the crystal state.[3,12–15]

In our continuing investigation of the crystal-state preferred conformation of homopeptides from C_{α}-methylated α-amino acids, we have recently been able to solve the X-ray structures of a variety of homochiral homopeptides from the Iva (isovaline or C_{α}-methyl-α-aminobutyric acid), (αMe)Val (C_{α}-methylvaline) and (αMe)Phe (C_{α}-methylphenylalanine) residues.[16] The types of secondary structure observed are the same as those described above for Aib (β-turns and 3_{10}-helices). However, the relationship between α-carbon chirality and turn and helix screw sense strongly depends upon presence and position of side-chain branching. Specifically, Iva, with a linear side chain, and the β-branched, aliphatic (αMe)Val exhibit a normal behaviour (the same as that shown by protein amino acids), while for the γ-branched, aromatic (αMe)Phe the relationship is inverse.

With the aim of defining more accurately the role played by position of side-chain branching and aromaticity, we report here a crystal-state structural study by X-ray diffraction of the trimer and tetramer from (αMe)Leu, a C_{α}-methylated amino acid with a γ-branched, aliphatic side chain.



Scheme 1 α-Amino acids methylated at the α-carbon discussed in this work

Experimental

Materials.—The synthesis and characterization of the two (αMe)Leu homochiral homo-oligomers, the X-ray diffraction structures of which are discussed in this work, are reported below, while those of other members of the series (to the tetramer level) will be described elsewhere.[17]

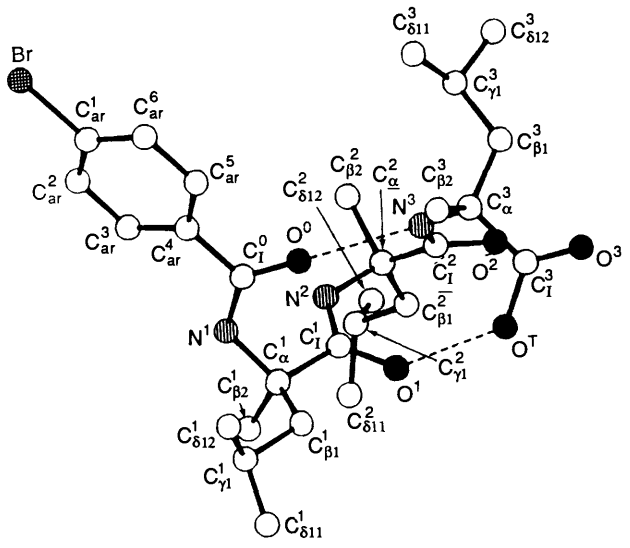


Fig. 1 X-Ray diffraction structure of *p*BrBz-[D-(αMe)Leu]$_3$-OH monohydrate with atom numbering. The two intramolecular H-bonds are indicated as dashed lines.

pBrBz-[D-(αMe)Leu]$_3$-OH (pBrBz, p-*bromobenzoyl*).—This compound was prepared by stirring *p*BrBz-[D-(αMe)Leu]$_3$-OBu^t in a 1:1 trifluoroacetic acid–dichloromethane mixture at room temperature for 2 h. The solvent was removed *in vacuo* and the residue evaporated several times from diethyl ether. Yield 80%. M.p. 219–220 °C (from ethyl acetate–light petroleum); $[\alpha]_D^{20}$ −5.8 (*c* 0.5 in methanol); TLC (silica gel plates 60F-254, Merck) R_{F1} (chloroform–ethanol 9:1) 0.40, R_{F2} (butan-1-ol–acetic acid–water 3:1:1) 0.95, R_{F3} (toluene–ethanol 7:1) 0.25; ν_{max}(1 mmol dm^{-3} $CDCl_3$)/cm^{-1} 3505, 3450, 3407, 3363, 1741, 1671 and 1649; δ_H(220 MHz; 10 mmol dm^{-3} $CDCl_3$; Me_4Si) 7.68 and 7.57 (2 m, 4 H, *p*BrBz CH), 7.53 (s, 1 H, NH), 7.25 (s, 1 H, NH), 7.10 (s, 1 H, NH), 2.50–2.23 (m, 3 H, 3 γ-CH), 1.90–1.50 (m, 6 H, 3β-CH_2), 1.70 (s, 3 H, β-CH_3), 1.64 (s, 3 H, β-CH_3), 1.60 (s, 3 H, β-CH_3) and 0.94–0.82 (m, 18 H, 6 δ-CH_3) (Found: C, 56.9; H, 7.6; N, 7.1. Calc. for $C_{28}H_{44}BrN_3O_5$: C, 57.7; H, 7.6; N, 7.2%).

pBrBz-[D-(αMe)Leu]$_4$-OBu^t.—This compound was synthesized from the oxazol-5(4*H*)-one from *p*BrBz-[D-(αMe)Leu]$_3$-OH and H-D-(αMe)Leu-OBu^t in acetonitrile under reflux for 43 h. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate and the organic solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, water, dried over Na_2SO_4, filtered and evaporated to dryness. The product was purified on a silica gel column eluted with a stepwise gradient of ethyl acetate in toluene. Yield 36%. M.p. 220–222 °C (from diethyl ether–light petroleum); $[\alpha]_D^{20}$ 31.4 (*c* 0.5 in methanol); TLC R_{F1} 0.95, R_{F2} 0.95, R_{F3} 0.70; ν_{max}(1 mmol dm^{-3} $CDCl_3$)/cm^{-1} 3389, 3364, 1716, 1676 and 1648; δ_H(10 mmol dm^{-3} $CDCl_3$) 7.97 (s, 1 H, NH), 7.71 and 7.55 (2 m, 4 H, *p*BrBz CH), 7.67 (s, 1 H, NH), 7.61 (s, 1 H, NH), 7.03 (s, 1 H, NH), 2.80, 2.63 and 2.41 (3 m, 4 H, 4 γ-CH), 1.75–1.45 (m, 8 H, 4 β-CH_2), 1.74 (s, 3 H, β-CH_3), 1.64 (s, 3 H, β-CH_3), 1.62 (s, 3 H, β-CH_3), 1.55 (s, 3 H, β-CH_3), 1.51 (s, 9 H, 3 OBu^tCH_3) and 0.89–0.81 (m, 24 H, 8 δ-CH_3) (Found: C, 61.1; H, 8.7; N, 7.2. Calc. for $C_{39}H_{65}BrN_4O_6$: C, 61.2; H, 8.6; N, 7.3%).

Crystallographic Data for pBrBz-[D-(αMe)Leu]$_3$-OH *monohydrate*.—$C_{28}H_{46}BrN_3O_6$, M = 600.6. Orthorhombic, a = 9.805(2), b = 17.453(3), c = 19.535(3) Å, V = 3343 Å^3, space group $P2_12_12_1$, Z = 4, D_c = 1.19 g cm^{-3}, F(000) = 1272, μ = 18.2 cm^{-1} (Cu-Kα), final R value 0.086.

Crystallographic Data for pBrBz-[D-(αMe)Leu]$_4$-OBu^t.—$C_{39}H_{65}BrN_4O_6$, M = 765.9. Trigonal, a = b = 19.583(3), c = 30.459(4) Å, V = 10 116 Å^3, space group $P3_2$, Z = 9, D_c = 1.03 g cm^{-3}, F(000) = 3690, μ = 14.66 cm^{-1} (Cu-Kα), final R value 0.111.

X-Ray Crystal Structure Determination of pBrBz-[D-(αMe)-Leu]$_3$-OH *monohydrate and* pBrBz-[D-(αMe)Leu]$_4$-OBu^t.—Colourless crystals of the trimer and tetramer were grown by slow evaporation of a methanol–water solvent mixture and a methanol solution, respectively. Enraf–Nonius CAD4 diffractometer, $\omega/2\theta$ scan mode up to θ = 60° for the trimer and θ = 70° for the tetramer; graphite-monochromated Cu-Kα radiation (λ = 1.5418 Å); 2813 and 12 390 unique reflections for the trimer and tetramer, respectively; 1397 and 5854 reflections with $I \geqslant 1\ \sigma(I)$ considered observed for the trimer and tetramer, respectively. The two structures were solved by SHELXS 86[18] and refined by blocked least squares with $w = 0.086/[\sigma^2(F) + 0.002\ F^2]$ for the trimer and $w = 2.219/[\sigma^2(F) + 0.0032\ F^2]$ for the tetramer. The thermal parameters were anisotropic for all non-hydrogen atoms of the trimer and most of the non-hydrogen atoms of the tetramer. The trimer co-crystallized with one water molecule and exhibited a remarkable disorder at the level of the three (αMe)Leu isobutyl side chains. The terminal parts of the three independent molecules in the asymmetric unit of the tetramer [(αMe)Leu side chains and *tert*-butyl ester group] undergo a significant thermal motion. Some side-chain C_α atoms were refined as staggered atoms with an occupancy factor of 2/3 and refined isotropically. Also the *tert*-butyl ester carbon atoms of molecule **C** were refined isotropically. Nevertheless, the peptide backbones are defined precisely. The hydrogen atoms of both trimer and tetramer were fixed by calculations and not refined. All calculations were performed using the SHELX 76 program.[19]

Fractional atomic coordinates, tables of hydrogen atoms coordinates, thermal parameters, bond lengths, bond angles, and torsion angles for the trimer and tetramer are available from the Cambridge Crystallographic Data Centre.*

Results and Discussion

We determined by X-ray diffraction the molecular and crystal structures of the following two (αMe)Leu homopeptides: *p*BrBz-[D-(αMe)Leu]$_3$-OH (in its monohydrate form) and *p*BrBz-[D-(αMe)Leu]$_4$-OBu^t. The *p*-bromobenzoyl group was incorporated at the *N*-terminus of the peptide chain to help solve the phase problem in the X-ray diffraction analyses, since it possesses a suitable heavy atom (Br). In the asymmetric unit of the tetramer three conformationally distinct, independent molecules (**A**, **B** and **C**) are observed. The molecular structures with the atomic numbering schemes are illustrated in Figs. 1 and 2. Relevant backbone and side-chain torsion angles[20] are given in Table 1. In Table 2 the intra- and inter-molecular H-bond parameters are listed.

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the *p*-bromobenzamido moiety,[21,22] the *tert*-butyl ester group[23] and the peptide unit.[24,25]

All 15 D-(αMe)Leu residues are found in the helical region *A* of the conformational map.[26] The average absolute φ and ψ values are 57 and 30°, matching perfectly those expected for a 3_{10}-helix.[27] In general, the signs of the φ and ψ values are negative, the only exceptions being represented by the *C*-terminal φ_4 and ψ_4 values of all three molecules of the tetramer.

* For details of the CCDC deposition scheme see 'Instructions for Authors (1994),' *J. Chem. Soc., Perkin Trans. 2*, 1994, issue 1.

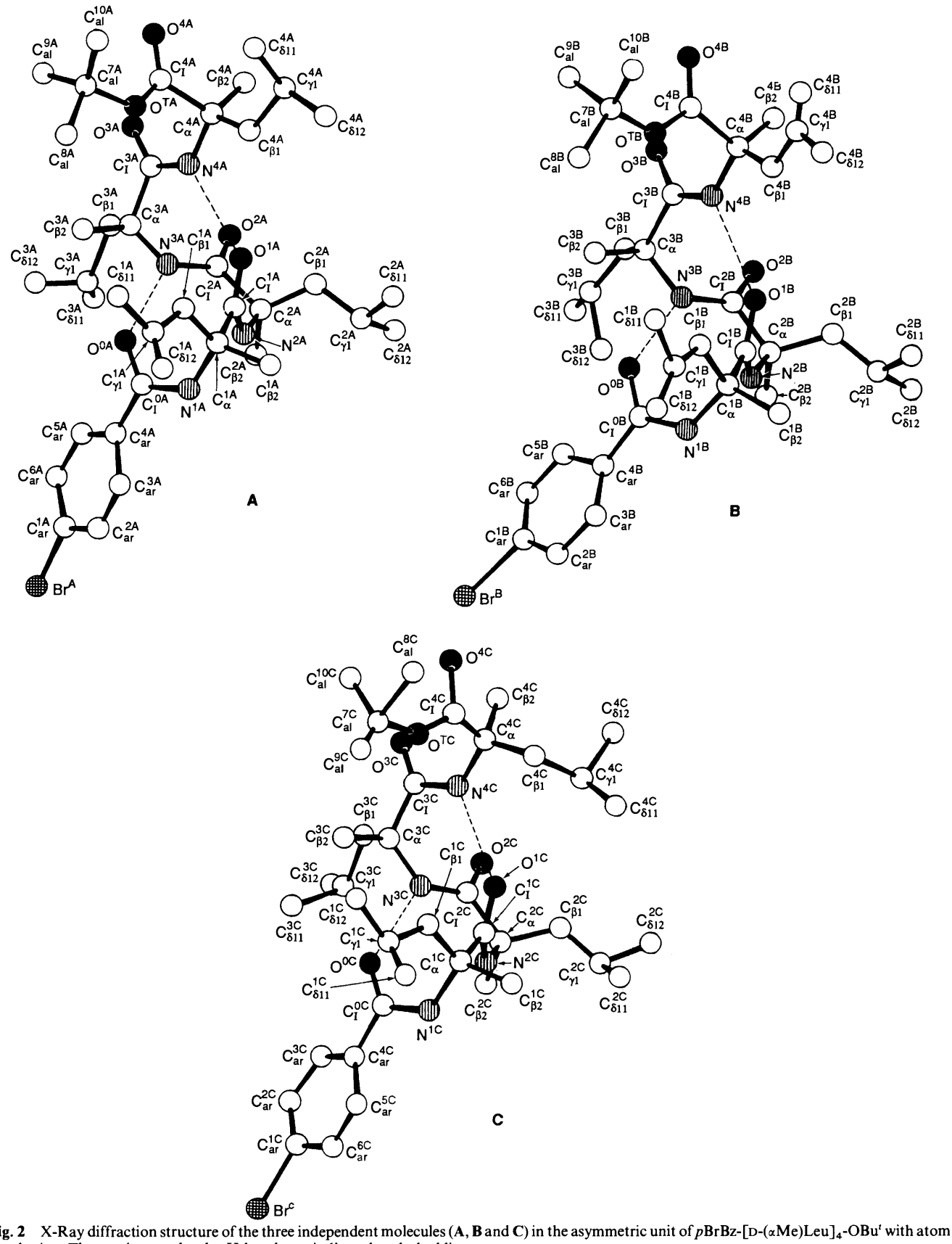


Fig. 2 X-Ray diffraction structure of the three independent molecules (**A**, **B** and **C**) in the asymmetric unit of *p*BrBz-[D-(αMe)Leu]$_4$-OBu^t with atom numbering. The two intramolecular H-bonds are indicated as dashed lines.

The N_α-blocked tripeptide carboxylic acid forms a regular type-III β-turn, followed by an unusual oxy-analogue of a type-III β-turn.[28] The intramolecular $N^3 \cdots O^0$ and $O^T \cdots O^1$ distances are 2.93(1) and 2.65(1) Å, respectively, within the range expected for such H-bonds.[29–33] The three molecules of the N^α-blocked tetrapeptide ester are folded in a regular, right-handed (incipient) 3_{10}-helical structure, characterized by two consecutive 1←4 C=O···H–N intramolecular H-bonds ($N^3 \cdots O^0$ and $N^4 \cdots O^1$). In general, the observed N···O separations are at the upper limit for such an interaction.[29–31]

The opposite handedness of the *C*-terminal residue of the tetrapeptide with respect to that of the preceding ones (see above) is a common observation for a 3_{10}-helix forming peptide ester.[34]

The distribution of the χ^1 torsion angle, the most relevant for characterizing side-chain conformations, for the 15 D-(αMe)Leu residues is 13 g^+ and 2 t, in agreement with the results of statistical analyses of the Leu residue in peptides and proteins.[25,35-37] The disposition of residue 4 of molecules **A** and **B** of the tetramer (t) compared with that of molecule **C** (g^+) represents the major conformational difference among these molecules.

All amide, peptide and ester groups (ω torsion angles) are *trans*, as expected, with only one peptide bond (ω_3 for molecule **C** of the tetramer) deviating more than 8° from planarity.[24,25] The θ torsion angle of the *p*BrBz group, giving the orientation of the aromatic ring relative to the amide plane, exhibits values in the range ±26–30°.[21,22] In particular, the sign for the three molecules of the tetramer is the same (positive).

The molecules of the N_α-blocked tripeptide carboxylic acid pack into the unit cell in rows parallel to the ***b*** direction with intermolecular H-bonds between the amide N^1–H and the peptide C_i^2=O^2 of a symmetry-related molecule. The water molecule plays the role of the acceptor of the H-bond from peptide N^2–H, while the donor of the H-bond to the acid C_i^3=O^3 of a symmetry-related molecule.[38,39] In the crystals of the N_α-blocked tetrapeptide ester molecules **A** form rows along the ***c*** direction with intermolecular H-bonds between the amide N^1–H and the peptide C_i^3=O^3 of a symmetry-related molecule **A**. The same observation applies to molecules **B** and **C**. No H-bonds between molecules of different types are seen.

Table 1 Selected torsion angles (degrees) for *p*BrBz-[D-(αMe)Leu]$_3$-OH monohydrate and *p*BrBz-[D-(αMe)Leu]$_4$-OBu^t

	Tripeptide	Tetrapeptide		
		Molecule **A**	Molecule **B**	Molecule **C**
θ	−20.3(19)	26.6(29)	29.6(30)	28.5(31)
ω_0	−177.5(11)	−179.7(18)	−177.0(17)	−175.5(17)
φ_1	−52.3(15)	−62.3(22)	−59.2(21)	−67.0(21)
ψ_1	−40.1(15)	−28.9(16)	−35.5(16)	−19.5(16)
ω_1	−176.6(11)	−178.4(10)	−177.5(10)	174.8(11)
φ_2	−46.7(16)	−61.1(15)	−56.0(16)	−52.9(16)
ψ_2	−35.5(19)	−10.9(21)	−17.4(22)	−17.0(17)
ω_2	177.9(14)	175.6(15)	178.0(14)	173.6(13)
φ_3	−45.5(19)	−59.6(22)	−60.7(22)	−56.0(23)
ψ_3	−40.6(18)[a]	−26.3(24)	−25.0(24)	−23.4(26)
ω_3	—	−172.8(17)	−173.5(17)	−166.5(19)
φ_4	—	51.9(26)	53.6(26)	65.1(37)
ψ_T	—	59.9(20)	48.9(20)	26.4(52)
ω_T	—	174.0(18)	173.9(20)	178.2(40)
χ_1^1	56.3(16)	51.1(26)	56.6(25)	42.1(21)
$\chi_1^{2,1}$	−106.6(18)	69.4(55)	−7.7(60)	57.9(38)
$\chi_1^{2,2}$	128.5(22)	−167.0(26)	178.5(23)	−175.9(18)
χ_2^1	57.6(17)	57.0(31)	55.8(29)	55.7(32)
$\chi_2^{2,1}$	118.3(20)	105.4(29)	106.9(27)	91.0(44)
$\chi_2^{2,2}$	−121.3(17)	−127.5(32)	−129.3(25)	−161.3(32)
χ_3^1	45.1(22)	58.2(25)	65.8(22)	38.6(25)
$\chi_3^{2,1}$	70.6(35)	97.0(31)	−55.4(41)	77.4(28)
$\chi_3^{2,2}$	−172.3(26)	−159.9(24)	−179.9(19)	−165.7(24)
χ_4^1	—	171.8(18)	−173.1(32)	70.4(26)
$\chi_4^{2,1}$	—	73.8(28)	0.9(88)	77.7(31)
$\chi_4^{2,2}$	—	−168.6(20)	160.8(45)	−155.2(25)

[a] N^3–C_α^3–C_i^3–O^T.

Conclusions

In this work we have described the crystal-state structural tendency of two homochiral homopeptides (the trimer and tetramer) of (αMe)Leu, as determined by X-ray diffraction. All (αMe)Leu residues examined are found in the helical region of the conformational map. A 1←4 C=O···H–N intramolecularly H-bonded β-turn conformation is adopted by the N^α-blocked tripeptide carboxylic acid. This folded conformation is additionally stabilized by an unusual 1←4 C=O···H–O intramolecular H-bond, giving rise to an oxy-analogue of a β-turn at the *C*-terminus. The tetrapeptide ester forms a regular (incipient) 3_{10}-helix with two 1←4 C=O···H–N intramolecular H-bonds. In addition, all D-(αMe)Leu residues (except those at the *C*-termini of the three independent molecules in the asymmetric unit of the tetramer) assume a right-handed helical conformation.

A comparison of the solid-state results described here for [(αMe)Leu]$_n$ homopeptides with the corresponding findings already reported for (Leu)$_n$ homo-oligopeptides[40-44] allows us to conclude that the (αMe)Leu residue is an effective β-turn and helix promoter, much stronger than its unmethylated parent compound Leu. Furthermore, in contrast to the Leu oligomers, there is no tendency for the (αMe)Leu oligomers to adopt the self-associated β-sheet conformation.

The crystal-state preferred conformation of the (αMe)Leu homo-oligomers, taken together with the corresponding tendency of the homopeptides derived from Aib (the prototype of C_α-methylated α-amino acids),[12-15] Iva, (αMe)Val, and

Table 2 Intra- and inter-molecular H-bond parameters for *p*BrBz-[D-(αMe)Leu]$_3$-OH monohydrate and *p*BrBz-[D-(αMe)Leu]$_4$-OBu^t

Peptide	Donor D–H	Acceptor A	Symmetry operations of A	Distance/Å D···A	Angle/degrees D···A=C
(*a*) *p*BrBz-[D-(αMe)Leu]$_3$-OH monohydrate	N^3–H	O^0	x, y, z	2.93(1)	135.3(8)
	O^T–H	O^1	x, y, z	2.65(1)	137.2(9)
	N^1–H	O^2	$1-x, -1/2+y, 3/2-z$	3.03(1)	164.5(9)
	N^2–H	O^w	$x, 1+y, z$	3.08(2)	—
	O^w–H	O^3	$1-x, -3/2+y, 3/2-z$	2.88(2)	115(1)
(*b*) *p*BrBz-[D-(αMe)Leu]$_4$-OBu^t	N^{3A}–H	O^{0A}	x, y, z	3.08(2)	131(1)
	N^{4A}–H	O^{1A}	x, y, z	3.33(2)[a]	132(1)
	N^{3B}–H	O^{0B}	x, y, z	2.85(2)	132(1)
	N^{4B}–H	O^{1B}	x, y, z	3.41(2)[a]	134(1)
	N^{3C}–H	O^{0C}	x, y, z	3.18(2)	135(1)
	N^{4C}–H	O^{1C}	x, y, z	3.18(2)	135(1)
	N^{1A}–H	O^{3A}	$1-x, 1+(x-y), -1/3+z$	3.21(2)	155(1)
	N^{1B}–H	O^{3B}	$1+(y-x), 1-x, 1/3+z$	3.16(2)	156(1)
	N^{1C}–H	O^{3C}	$2-x, 1+(x-y), -1/3+z$	3.04(2)	153(1)

[a] Very weak interaction.

(αMe)Phe residues[16] reinforces the conclusions that C_α-methylation induces a marked propensity for β-turn and 3_{10}-helix formation in the resulting homo-oligomers.

As for the relationship between chirality of C_α-methylated α-amino acids and turn and helix handedness, the available experimental data point to the normal behaviour of protein amino acids (an L-residue prefers φ, ψ backbone torsion angles typical of a right-handed helix) for the aliphatic Iva (with a linear side chain) and (αMe)Val (with a β-branched side chain) residues,[16] but to a strong preference for an inverse relationship for the γ-branched aliphatic (αMe)Leu (this work) and aromatic (αMe)Phe[16] residues. In other words, the inverse relationship appears to be dictated by the position of side-chain branching (at the γ-carbon), rather than by the electronic nature (whether aliphatic or aromatic) of the side chain.

Not unexpectedly, the energy difference between right- and left-handed helical structures for C_α-methylated chiral amino acids is much smaller than that of their protein counterparts.[45–47] A widely accepted view is that the left-handed helical handedness of protein amino acids (L-configuration) is energetically disfavoured with respect to the diastereoisomeric right-handed helix mainly because of the close contact between side-chain $C_{\beta L}$ atom and carbonyl oxygen of the same residue in the former conformation. Amino acids methylated at the α-carbon possess two side-chain C_β atoms (a substituted $C_{\beta L}$ atom and an unsubstituted $C_{\beta D}$ atom). Since the helix handedness usually adopted by L-Iva (carrying a $-CH_2R$ substituent in the L side chain) is the same as that of L-(αMe)Val [–CH(R)R] but opposite to that of L-(αMe)Leu and L-(αMe)Phe [both of the $-CH_2-CH(R)R$ type], it is reasonable to assume that not only the preferred rotamers about the $C_\alpha-C_\beta$ bond (χ^1 torsion angles) but even those about the $C_\beta-C_\gamma$ bond, more removed from the backbone (χ^2 torsion angles), may play a role in directing φ, ψ torsional preference of C_α-methylated amino acids. To clarify this point, a statistical analysis relating backbone and side-chain torsion angles of the known X-ray diffraction structures of peptides containing C_α-methylated amino acids[16] is currently in progress in our laboratory.

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