Inversion of 3₁₀-Helix Screw Sense in a (D-αMe)Leu Homo-tetrapeptide Induced by a Guest D-(αMe)Val Residue

FERNANDO FORMAGGIO[1], MARCO CRISMA[1], CLAUDIO TONIOLO[1], ETTORE BENEDETTI[2], BENEDETTO DI BLASIO[2], MICHELE SAVIANO[2], STEFANIA GALDIERO[2], JOHAN KAMPHUIS[3] and ANTONELLO SANTINI[4]

[1] Biopolymer Research Centre, CNR, Department of Organic Chemistry, University of Padova, Italy
[2] Biocrystallography Centre, CNR, Department of Chemistry, University of Naples 'Federico II', Naples, Italy
[3] DSM Research, Bio-organic Chemistry Section, Geleen, The Netherlands
[4] Department of Food Science, University of Naples 'Federico II', Naples, Italy

Abstract: The terminally blocked tetrapeptide pBrBz-[D-(αMe)Leu]₂-D-(αMe)Val-D-(αMe)Leu-OtBu is folded in the crystal state in a left-handed 3₁₀-helical structure stabilized by two consecutive $1 \leftarrow 4$ C=O···H—N intramolecular H-bonds, as determined by X-ray diffraction analysis. A CD study strongly supports the view that this conformation is also that largely prevailing in MeOH solution. A comparison with the published conformation of pBrBz-[D-(αMe)Leu]₄-OtBu indicates that incorporation of a single internal β-branched (αMe)Val guest residue into the host homo-tetrapeptide from the γ-branched (αMe)Leu residue is responsible for a dramatic structural perturbation, i.e. an inversion of the 3₁₀ screw sense from right to left-handed.

Keywords: (αMe) amino acids; CD spectroscopy; 3₁₀-helix; peptide 3D-structure; X-ray structure

Abbreviations

pBrBz para-bromobenzoyl; (αMe)Leu, Cα-methyl leucine; (αMe)Val, Cα-methyl valine; Abu, α-aminobutyric acid; Deg, Cα,α-diethylglycine; OtBu, tert-butoxy; EDC, N-ethyl-N'-[3-dimethylamino) propyl] carbodiimide.

INTRODUCTION

In the late 1970s Goodman and co-workers [1, 2] pioneered the approach of synthetically inserting a single guest protein amino acid into selected positions of a well-characterized, monodispersed homo-oligopeptide chain with the aim of gaining useful information into the factors influencing the conformational stability of peptides. We have recently extended this methodology to the field of Cα,α-disubstituted glycines which, in addition to the classical α-helix, are known to prefer less common secondary structures, such as the 3₁₀-helix and the fully extended conformation (or 2.0₅ helix) [3, 4]. More specifically, we have been able to demonstrate that a modest point defect, represented by the incorporation of an Abu guest residue interrupting the side-chain uniformity of the host (Deg)₅ homo-peptide, while altering only marginally the conformation in a solvent of low polarity, is responsible for a dramatic perturbation of the crystal state structure, from the flat fully extended conformation of the host pentapeptide to the 3₁₀-helix of the host/guest analogue [5].

Recent works from our laboratories have shown that the 3₁₀-helix is preferentially adopted by peptides rich in Cα-methylated chiral amino acids [4]. Intriguing experimental findings on the impact of Cα

Address for correspondence: Prof. Claudio Toniolo, Department of Organic Chemistry, University of Padova, via Marzolo 1, 35131 Padova, Italy.

chirality on helix screw sense have been reported, which demonstrate that C^α-methylated amino acids with a linear or a β-branched side chain exhibit a normal relationship (i.e. the same as that shown by protein amino acids: L-amino acids give right-handed helices), whereas the relationship characterizing C^α-methylated amino acids with a γ-branched side chain is opposite.

As a part of our ongoing study aiming at establishing the implications of point defects (e.g. side-chain irregularities) on the secondary structure of $C^{\alpha,\alpha}$-disubstituted glycine-rich homo-peptides, we describe in this paper the experimental results obtained using X-ray diffraction and CD spectroscopy on the terminally blocked tetrapeptide pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-D-(αMe)Leu-OtBu. In comparison with the (αMe)Leu residue of the host homo-tetrapeptide pBrBz-[D-(αMe)Leu]$_4$-OtBu, the single (αMe)Val residue of the host/guest peptide has one side-chain carbon atom less, i.e. it is a β-branched, instead of a γ-branched, amino acid.

MATERIALS AND METHODS

Synthesis and Characterization of Peptides

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin-Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel 60F$_{254}$ precoated plates using the following solvent systems: 1 (CHCl$_3$–EtOH, 9:1), 2 (nBuOH–AcOH–H$_2$O, 3:1:1); 3 (toluene–EtOH, 7:1). The chromatograms were examined by UV fluorescence or developed by chlorine–starch–potassium iodide or ninhydrin chromatic reaction as appropriate. All the compounds were obtained in a chromatographically homogeneous state. The solid-state IR absorption spectra (KBr disk technique) were recorded with a Perkin-Elmer model 580B spectrophotometer equipped with a Perkin-Elmer model 3600 data station. The ^{1}H-NMR spectra were recorded with a Bruker model AC 250 spectrometer. Measurements were carried out in deuterochloroform (99.96% d, Merck) with tetramethylsilane as the internal standard.

pBrBz-[D-(αMe)Leu]$_2$-D(-αMe)Val-OtBu. The 5(4H)-oxazolone from pBrBz-[D-(αMe)Leu]$_2$-OH [6] (0.14 g, 0.32 mmol) and H-D-(αMe)Val-OtBu (obtained from

Pd–C catalysed hydrogenolysis of the corresponding Z-protected amino acid ester [7] (0.40 g, 1.24 mmol)) were refluxed in CH$_3$CN for 6 h. The solvent was removed under reduced pressure, the organic phase washed with 10% KHSO$_4$, water, 5% NaHCO$_3$ and water, dried over Na$_2$SO$_4$, and evaporated to dryness. The product was purified by flash-chromatography by eluting the column with a 1:5 isocratic mixture of AcOEt:petroleum ether. Yield 78%; m.p. 194–195 °C (from AcOEt/petroleum ether); $R_{F1} = 0.95$, $R_{F2} = 0.95$, $R_{F3} = 0.60$; $[\alpha]_D^{20} = 6.4°$ ($c = 0.5$, MeOH), $[\alpha]_{436}^{20} = 15.9°$ ($c = 0.5$, MeOH); IR (KBr): 3403, 3363, 1715, 1672, 1651 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 50 mM): δ 7.92 (s, 1H, NH), 7.31 (m, 5H, 4 pBrBz-phenyl CH and 1 NH), 6.84 (s, 1H, NH), 2.70 (m, 2H, Leu βCH$_2$), 2.45 (m, 1H, Val βCH), 1.72 (s, 3H, Val βCH$_3$), 1.62 and 1.61 (2s, 6H, 2Leu βCH$_3$), 1.56 (m, 4H, Leu βCH$_2$, 2Leu γCH), 1.50 (s, 9H, OtBu CH$_3$), 1.01 (d, 3H, Val γCH$_3$), 0.86 (m, 15H, Val γCH$_3$ and 4Leu δCH$_3$).

pBrBz-[D-(αMe)Leu]$_2$-D(-αMe)Val-OH. This compound was prepared by treatment of pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-OtBu (0.13 g, 0.21 mmol) with a 2:1 TFA:CH$_2$Cl$_2$ mixture for 90 min at room temperature. The solvent mixture was removed in vacuo. The resulting oil, treated several times with diethyl ether, afforded the solid title compound. Yield 90%; m.p. 196–197 °C; $R_{F1} = 0.45$, $R_{F2} = 0.95$; $R_{F3} = 0.25$; $[\alpha]_D^{20} = -10.4°$ ($c = 0.5$, MeOH), $[\alpha]_{436}^{20} = -20.9°$ ($c = 0.5$, MeOH); IR (KBr): 3321, 1735, 1647 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 50 mM): δ 7.62 (m, 5H, 4 pBrBz-phenyl CH and 1 NH), 7.28 (s, 1H, NH), 6.84 (s, 1H, NH), 6.67 (s, 1H, NH), 2.54 (m, 1H, Val βCH$_2$), 1.85 (m, 6H, 2Leu βCH$_2$ and 2Leu γCH), 1.64, 1.58 and 1.54 (3s, 9H, 2Leu βCH$_3$ and Val βCH$_3$), 1.08 (d, 3H, Val γCH$_3$), 0.99 (m, 9H, Val γCH$_3$ and 2Leu δCH$_3$), 0.83 (2d, 6H, 2Leu δCH$_3$).

5(4H)-Oxazolone from pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-OH. This compound was prepared from pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-OH (0.094 g, 0.165 mmol) and EDC·HCl (0.035 g, 0.182 mmol) in a 1:1 CH$_3$CN:AcOEt mixture. After stirring for 1 h at room temperature, the solvent was removed under reduced pressure, the residue dissolved in AcOEt and the organic phase washed with 10% KHSO$_4$, water, 5% NaHCO$_3$ and water, dried over Na$_2$SO$_4$, and evaporated to dryness. Oil. Yield 93%. $R_{F1} = 0.95$, $R_{F2} = 0.95$, $R_{F3} = 0.75$; $[\alpha]_D^{20} = -13.2°$ ($c = 0.5$, AcOEt), $[\alpha]_{436}^{20} = -28.2°$ ($c = 0.5$, AcOEt); IR (KBr): 3375, 1827, 1655 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 10 mM): δ 7.84 (s, 1H, NH), 7.70 and 7.56 (m, 4H, pBrBz-phenyl CH), 7.29 (s, 1H, NH), 2.84 (2d, 1H, Leu βCH$_2$), 2.49 (2d, 1H, Leu βCH$_2$), 2.05 (m, 1H, Val

βCH), 1.75 and 1.72 (2s, 6H, 2Leu βCH₃), 1.61 (m, 4H, Leu βCH₂ and 2Leu γCH), 1.41 (s, 3H, Val βCH₃), 0.90 (m, 18H, 4Leu δCH₃ and 2Val γCH₃).

pBrBz-(D-(αMe)Leu)₂-D-(αMe)Val-D-(αMe)Leu-OtBu.

This compound was prepared from the 5(4H)-oxazolone from pBrBz-[D-(αMe)Leu]₂-D-(αMe)Val-OH (0.075 g, 0.14 mmol) and H-D-(αMe)Leu-OtBu [obtained from Pd-ccatalysed hydrogenolysis of the corresponding Z-protected amino acid ester [6] (0.30 g, 0.90 mmol)] according to the procedure described above for pBrBz-[D-(αMe)Leu]₂-D-(αMe)Val-OtBu (refluxed in CH_3CN for 24 h). The product was purified by flash-chromatography by eluting the column with a 1:6 to 1:4 step-gradient mixture of AcOEt:petroleum ether. Yield 66%; m.p. 192–193 °C; $R_{F1} = 0.95$, $R_{F2} = 0.95$, $R_{F3} = 0.60$; $[\alpha]_D^{20} = -16.2°$ (c = 0.5, MeOH); IR (KBr): 3412, 1727, 1659 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 10 mM): δ 7.89 (s, 1H, NH), 7.70 and 7.55 (2m, 5H, 4 pBrBz-phenyl CH and 1 NH), 7.46 (s, 1H, NH), 7.06 (s, 1H, NH), 2.60 (m, 3H, Leu βCH₂ and Val γCH), 1.65 (m, 7H, 2Leu βCH₂ and 3Leu γCH), 1.74, 1.72, 1.60 and 1.55 (4s, 12H, 3Leu βCH₃ and Val βCH₃), 1.50 (s, 9H, OtBu CH₃), 1.04 (d, 3H, Val γCH₃), 0.92 (m, 21H, 6Leu δCH₃ and Val γCH₃).

X-ray Diffraction

Colourless single crystals were obtained by slow evaporation from a methanol solution. A crystal with dimensions 0.2 × 0.3 × 0.4 mm was used for unit cell determination and data collection on a CAD4-Turbo Enraf Nonius automated diffractometer using graphite monochromated $CuK\alpha$ radiation. The crystals are tetragonal, space group P4₃ with $a = b = 19.0574(2)$ Å, $c = 11.3009(3)$ Å and $Z = 4$. A total of 4325 unique reflections were measured at room temperature in the range $0 < 2\theta < 140°$, 3669 of which were classified as observed having $I > 3\sigma(I)$ and used for structure determination and refinement. The structure was solved using the SIR 92 package [8]. The best E map revealed most of the non-H atoms. Remaining ones were recovered from subsequent difference Fourier maps. H atoms were in part located on successive difference Fourier maps, and in part calculated in their stereochemically expected positions. Refinement of the structure was performed by a full matrix least-squares procedure minimizing the quantity $\Sigma w(F_o^2 - F_c^2)^2$, with $w = 1/\sigma(F_o^2)$. All non-H atoms were refined anisotropically. H atoms were introduced in the calculations with isotropic thermal factors equal to the Beq of the

Table 1 Positional Parameters and Their Estimated Standard Deviations for pBrBz-[D-(αMe)Leu]₂-D-(αMe)Val-D-(αMe)Leu-OtBu

Atom	x	y	z	B (Å²)
Br	0.96037(3)	0.83814(5)	0.293	7.32(2)
C(1)	0.7547(2)	0.8293(3)	0.5129(4)	3.7(1)
C(2)	0.8099(3)	0.7861(3)	0.5397(5)	4.4(1)
C(3)	0.8711(3)	0.7869(3)	0.4725(5)	5.1(1)
C(4)	0.8768(3)	0.8347(3)	0.3819(5)	4.6(1)
C(5)	0.8217(3)	0.8790(3)	0.3530(5)	5.1(1)
C(6)	0.7604(3)	0.8753(3)	0.4186(5)	4.8(1)
C_0'	0.6879(3)	0.8306(3)	0.5811(4)	3.8(1)
O_0	0.6337(2)	0.8556(2)	0.5386(4)	4.74(8)
N_1	0.6889(2)	0.8042(2)	0.6935(4)	3.73(8)
C_1^α	0.6299(2)	0.8156(2)	0.7743(4)	3.43(9)
$C_1^{\beta1}$	0.6216(3)	0.8931(3)	0.7992(6)	4.7(1)
C_1^β	0.6458(3)	0.7726(3)	0.8868(5)	3.9(1)
C_1^γ	0.5922(3)	0.7796(3)	0.9883(5)	4.4(1)
$C_1^{\delta1}$	0.5754(4)	0.7100(4)	1.0384(7)	8.1(2)
$C_1^{\delta2}$	0.6173(4)	0.8283(4)	1.0848(6)	7.9(2)
C_1'	0.5620(2)	0.7860(3)	0.7198(4)	3.6(1)
O_1	0.5049(2)	0.8136(2)	0.7398(3)	4.43(8)
N_2	0.5691(2)	0.7279(2)	0.6540(4)	3.88(9)
C_2^α	0.5106(3)	0.6861(3)	0.6090(5)	3.9(1)
$C_2^{\beta1}$	0.4716(3)	0.6537(3)	0.7126(6)	5.5(1)
C_2^β	0.5401(3)	0.6261(3)	0.5309(6)	4.9(1)
C_2^γ	0.5660(3)	0.6408(3)	0.4063(6)	5.0(1)
$C_1^{\gamma1}$	0.5817(4)	0.5711(4)	0.3448(7)	7.9(2)
$C_2^{\gamma2}$	0.6300(3)	0.6884(4)	0.4010(6)	6.3(2)
C_2'	0.4589(2)	0.7284(2)	0.5332(5)	3.9(1)
O_2	0.4000(2)	0.7041(2)	0.5142(4)	5.24(9)
N_3	0.4817(2)	0.7896(2)	0.4893(4)	3.66(8)
C_3^α	0.4415(3)	0.8358(3)	0.41104(4)	3.8(1)
$C_3^{\beta1}$	0.4165(3)	0.7955(3)	0.3031(5)	5.8(1)
C_3^β	0.4919(3)	0.8970(3)	0.3763(5)	4.5(1)
$C_3^{\gamma1}$	0.5051(3)	0.9840(3)	0.4745(6)	5.1(1)
$C_3^{\gamma2}$	0.4685(4)	0.9367(4)	0.2660(6)	7.1(2)
C_3'	0.3773(3)	0.8677(3)	0.4745(5)	3.7(1)
O_3	0.3301(2)	0.8957(2)	0.4154(3)	4.52(8)
N_4	0.3773(2)	0.8680(2)	0.5928(4)	3.71(8)
C_4^α	0.3225(2)	0.9041(3)	0.6610(5)	3.8(1)
$C_4^{\beta1}$	0.3216(3)	0.9827(3)	0.6315(7)	5.6(1)
C_4^β	0.3397(3)	0.8990(3)	0.7918(6)	5.4(1)
C_4^γ	0.3420(4)	0.8287(4)	0.8487(6)	7.9(2)
$C_4^{\delta1}$	0.2775(5)	0.7943(5)	0.8784(9)	10.2(3)
$C_4^{\delta2}$	0.3806(7)	0.8412(9)	0.9729(8)	19.6(5)
C_4'	0.2512(2)	0.8711(2)	0.6322(4)	3.32(9)
O_4	0.2429(2)	0.8147(2)	0.5863(3)	4.00(7)
OT	0.1994(2)	0.9125(2)	0.6684(3)	3.76(7)
C(7)	0.1247(2)	0.8947(3)	0.6512(5)	3.9(1)
C(8)	0.0881(3)	0.9602(3)	0.6999(5)	4.7(1)
C(9)	0.1091(3)	0.8867(3)	0.5215(6)	6.2(1)
C(10)	0.1060(3)	0.8301(3)	0.7226(7)	6.0(2)

Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $(4/3)^*[a^{2*}B(1,1) + b^{2*}B(2,2) + c^{2*}B(3,3) + ab(\cos \gamma)^*B(1,2) + ac(\cos \beta)^*B(1,3) + bc(\cos \alpha)^*B(2,3)]$.

carrier atom and their parameters were not refined. Final R and R_w values were 0.042 and 0.040, respectively. In the final difference Fourier synthesis the maximum and minimum electronic densities were 0.32 and -0.24 e Å^{-3}, respectively. Positional atomic parameters and equivalent thermal factors for non-hydrogen atoms with their standard deviations are reported in Table 1. Tables of anisotropic thermal factors, hydrogen positional and thermal parameters, bond lengths, bond angles, torsion angles and observed vs. calculated structure factors have been deposited as Supplementary Material.

Circular Dichroism

The CD spectra were recorded on a JASCO (Tokyo, Japan) model J-600 spectropolarimeter equipped with a Haake thermostat. Cylindrical fused quartz cells of 10 and 1 mm path lengths were employed. The values are expressed in terms of $[\theta]_M$, the total molar ellipticity (deg cm^2 dmol^{-1}). MeOH (C. Erba, Rodano, Italy) was used as solvent.

RESULTS

Crystal-state Conformation

The X-ray diffraction structure of pBrBz-[D-(αMe)-Leu]$_2$-D-(αMe)Val-D-(αMe)Leu-OtBu is illustrated in Figure 1. Bond lengths and bond angles are in accordance with previously reported values for the geometry of para-bromobenzamido [9] and tert-butyl ester [10] groups, (αMe)Leu [11] and (αMe)Val [12] residues, and the peptide unit [13].

The molecules of the N^α-blocked tetrapeptide ester are folded in an (incipient) left-handed 3_{10}-helical structure with $\phi_1 = 58.4°$, $\psi_1 = 34.4°$, $\phi_2 = 56.8°$, $\psi_2 = 18.6°$, $\phi_3 = 66.6°$ and $\psi_3 = 19.5°$ [14]. The helix is stabilized by two consecutive $1 \leftarrow 4$ C=O$\cdots$H—N intramolecular H-bonds (N$_3\cdots$O$_0$ = 3.21 Å and N$_4\cdots$O$_1$ = 3.12 Å). The observed N$\cdots$O separations are at the upper limit for such an interaction [15]. The C-terminal D-(αMe)Leu residue is semi-extended ($\phi_4 = 61.4°$, $\psi_4 = -164.7°$). All amide, peptide and ester groups (ω torsion angles) are trans, as expected, with only the pBrBz-NH ω_0 angle deviating more than 7° from planarity.

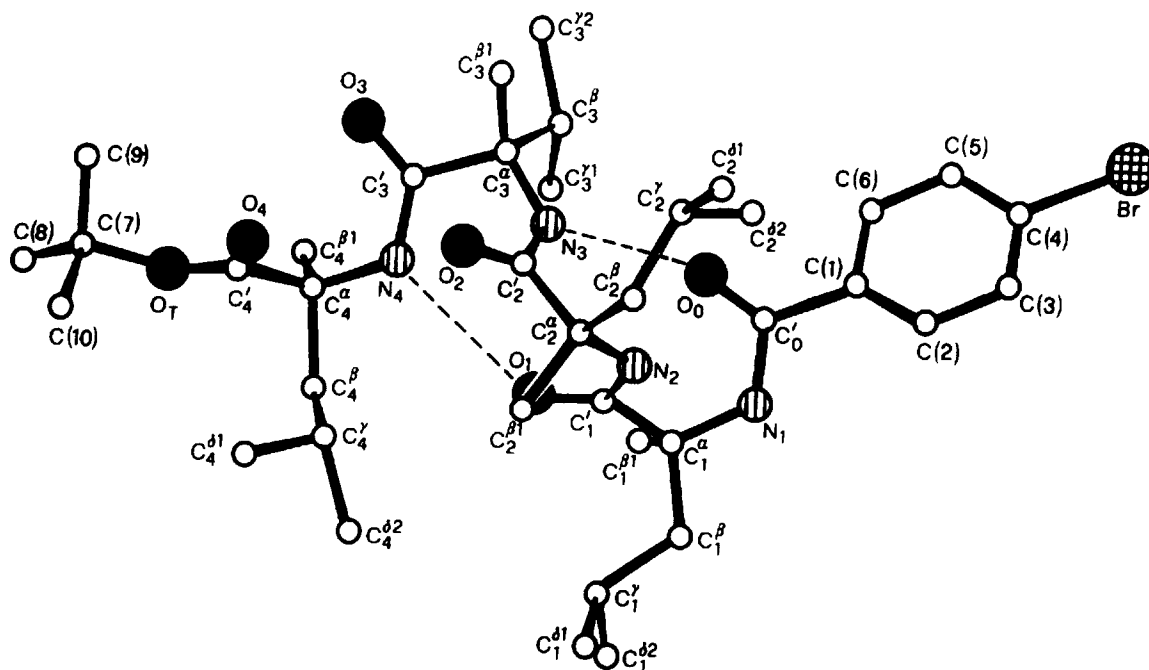Figure 1 X-ray diffraction structure of pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-D-(αMe)Leu-OtBu with numbering of the atoms. The two intramolecular H-bonds are represented by dashed lines.

The value of the θ torsion angle of the pBrBz group, giving the orientation of the aromatic ring relative to the amide plane, is in the usual range (20°) [9]. The distribution of the χ^1 and χ^2 torsion angles characterizing the side-chain conformations is: D-(αMe)Leu^1 $t(s^+, s^-)$, D-(αMe)Leu^2 $g^-(g^+, t)$, D-(αMe)Val^3 t, g^-, and D-(αMe)Leu^4 $g^-(t, g^-)$ [16].

In the mode of packing of the tetrapeptide along the c-axis a helical arrangement of the molecules around the fourfold screw axis is observed. The molecules in the crystal are held together by one rather weak intermolecular hydrogen bond of the N—H···O=C type [15] [the N—H group of D-(αMe)Leu^1 in one molecule is H-bonded to the C=O group of D-(αMe)Leu^4 of a symmetry-related molecule (x, $1 - y$, $z - 1/4$) around the fourfold screw axis, with an N···O distance of 3.12 Å], and by a number of van der Waals interactions between the hydrophobic tert-butyl, isopropyl, isobutyl and phenyl groups.

Solution Conformation

We have previously shown that the para-bromo-benzamido chromophore at the N-terminus of a peptide chain is an excellent CD probe for the assignment of the screw sense of 3_{10}-helical peptides, irrespective of the C$^\alpha$-configuration of the constituent α-amino acids [17]. Two intense, oppositely signed bands, positive at higher wavelengths, indicate the onset of a significant population of right-handed helical structure, while a left-handed helical structure is characterized by two oppositely signed bands, negative at higher wavelengths. The cross-over point between the two components of the exciton split curve is seen in the vicinity of 240 nm, the region where the absorption maximum of the para-bromobenzamido chromophore is found [18].

In Figure 2, the CD spectrum in the 210–300 nm region in MeOH solution of pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-D-(αMe)Leu-OtBu is compared with that of pBrBz-[D-(αMe)Leu]$_4$-OtBu [17]. It is evident that the signs of the exciton split CD curve of the host/guest tetrapeptide associated with the para-bromobenza-mido chromophore are opposite to those of the host homo-tetrapeptide. In particular, the long wavelength CD band is negative for the former, whereas it is positive for the latter. These findings are strongly in favour of the conclusion that in MeOH solutions the host/guest tetrapeptide is preferentially folded in a left-handed helix, whereas the host homo-tetrapeptide helix prefers the opposite screw sense.
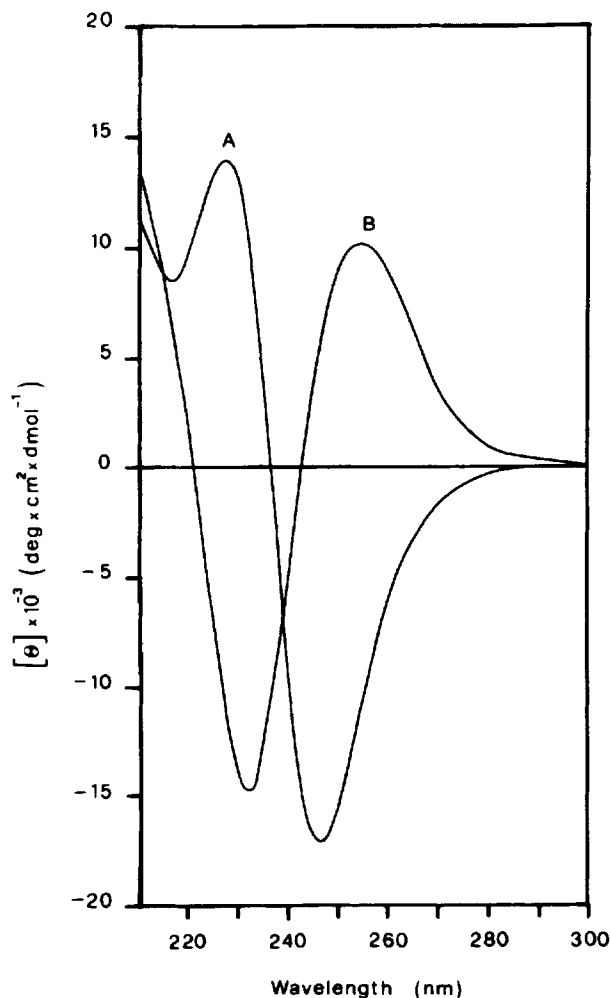

Figure 2 Circular dichroism spectra in the 210–300 nm region of pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)Val-D-(αMe)Leu-OtBu (A) and pBrBz-[D-(αMe)Leu]$_4$-OtBu (B) in MeOH solution (peptide concentration 1 mM). Curve (B) was adapted from [17].

DISCUSSION

In this article we have shown that a helix screw sense reversal is undergone by peptides formed by C$^\alpha$-methylated, chiral amino acids. In the crystal state, as well as in methanol solution, the terminally blocked tetrapeptide pBrBz-[D-(αMe)Leu]$_2$-D-(αMe)-Val-D-(αMe)Leu-OtBu is folded in a left-handed 3_{10}-helical structure. These findings contrast dramatically with those of the host homo-tetrapeptide pBrBz-[D-(αMe)Leu]$_4$-OtBu which is known to prefer the right-handed 3_{10}-helical handedness [11, 17], despite the two compounds possessing identical N-and

C-blocking groups. These results clearly indicate that the conformational tendency of a single, internal, β-branched D-(αMe)Val guest residue (left-handed helix) [4] is strong enough to overtake that of two internal γ-branched D-(αMe)Leu residues (right-handed helix) [4]. Solution and crystal-state evidence for the co-existence of opposite screw senses in 3_{10}-helical oligopeptides built-up of C^{α}-methylated amino acids has been reported [19, 20].

Acknowledgements

REFERENCES

1. F. Naider, J. M. Becker, A. Ribeiro and M. Goodman (1978). The effect of homologous amino acid replacement on the conformation of oligopeptides. III. CD studies on co-oligopeptides of methionine and valine or methionine and glycine in organic solutions. *Biopolymers 17*, 2213–2224.

2. A. Ribeiro, M. Goodman and F. Naider (1978). Use of a guest amino acid for the complete proton nuclear magnetic resonance assignments of blocked linear methionine homo-oligopeptides. *J. Am. Chem. Soc. 100*, 3903–3907.

3. C. Toniolo and E. Benedetti (1991). Structures of polypeptides from α-amino acids disubstituted at the α-carbon. *Macromolecules 24*, 4004–4009.

4. C. Toniolo, M. Crisma, F. Formaggio, G. Valle, G. Cavicchioni, G. Précigoux, A. Aubry and J. Kamphuis (1993). Structures of peptides from α-amino acids methylated at the α-carbon. *Biopolymers 33*, 1061–1072.

5. E. Benedetti, C. Pedone, V. Pavone, B. Di Blasio, M. Saviano, R. Fattorusso, M. Crisma, F. Formaggio, G. M. Bonora, C. Toniolo, K. Kaczmarek, A. S. Redlinski and M. T. Leplawy (1994). Defect peptide chemistry: perturbations in the structure of a homopentapeptide induced by a guest residue interrupting side-chain regularity. *Biopolymers 34*, 1409–1418.

6. C. Toniolo, M. Pantano, F. Formaggio, M. Crisma, G. M. Bonora, A. Aubry, D. Bayeul, A. Dautant, W. H. J. Boesten, H. E. Schoemaker and J. Kamphuis (1994). Onset of the fully extended conformation in (αMe)Leu derivatives and short peptides. *Int. J. Biol. Macromol. 16*, 7–14.

7. C. Toniolo, M. Crisma, G. M. Bonora, B. Klajc, F. Lelj, P. Grimaldi, A. Rosa, S. Polinelli, W. H. J. Boesten, E. M. Meijer, H. E. Schoemaker and J. Kamphuis (1991). Peptides from chiral $C^{\alpha,\alpha}$-disubstituted glycines. Synthesis and characterization, conformational energy com-

putations and solution conformational analysis of C^{α}-methyl, C^{α}-isopropylglycine [(αMe)Val] derivatives and model peptides. *Int. J. Peptide Protein Res. 38*, 242–252.

8. A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori and M. Camalli (1994). SIR 92. A program for automatic solution of crystal structures by direct methods. *J. Appl. Crystallogr. 27*, 435.

9. E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma and L. Sartore (1990). Linear oligopeptides. Part 227. X-ray crystal and molecular structures of two α-helix forming (Aib-L-Ala) sequential oligopeptides, pBrBz-(Aib-L-Ala)$_5$-OMe and pBrBz-(Aib-L-Ala)$_6$-OMe. *J. Chem. Soc., Perkin Trans. 2*, 1829–1837.

10. W. B. Schweizer and J. D. Dunitz (1982). Structural characteristics of the carboxylic ester group. *Helv. Chim. Acta 65*, 1547–1554.

11. A. Aubry, D. Bayeul, G. Précigoux, M. Pantano, F. Formaggio, M. Crisma, C. Toniolo, W. H. J. Boesten, H. E. Schoemaker and J. Kamphuis (1994). 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. *J. Chem. Soc., Perkin Trans. 2*, 525–529.

12. F. Formaggio, M. Pantano, G. Valle, M. Crisma, G. M. Bonora, S. Mammi, E. Peggion, C. Toniolo, W. H. J. Boesten, H. E. Schoemaker and J. Kamphuis (1993). Structural versatility of peptides from $C^{\alpha,\alpha}$-disubstituted glycines. Synthesis, characterization, and solution and crystal-state conformational analysis of homopeptides from C^{α}-methyl-C^{α}-isopropylglycine, [(αMe)Val)]. *Macromolecules 26*, 1848–1852.

13. E. Benedetti in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein (Ed.), p. 105–184, Dekker, New York 1982.

14. IUPAC-IUB Commission on Biochemical Nomenclature (1970). Abbreviations and symbols for the description of the conformation of polypeptide chains. *Biochemistry 9*, 3471–3479.

15. C. H. Görbitz (1989). Hydrogen-bond distances and angles in the structures of amino acids and peptides. *Acta Crystallogr. B45*, 390–395.

16. E. Benedetti, G. Morelli, G. Némethy and H. A. Scheraga (1983). Statistical and energetic analysis of side-chain conformations in oligopeptides. *Int. J. Peptide Protein Res. 22*, 1–15.

17. C. Toniolo, F. Formaggio, M. Crisma, H. E. Schoemaker and J. Kamphuis (1994). The p-bromobenzamido chromophore as a circular dichroic probe for the assignment of the screw sense of helical peptides. *Tetrahedron: Asymmetry 5*, 507–510.

18. N. Harada and K. Nakahishi in: *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA 1983.

19. R. P. Hummel, C. Toniolo and G. Jung (1987). Conformational transitions between enantiomeric 3_{10}-helices. *Angew. Chem. Int. Ed. Engl.* **26**, 1150–1152.

20. G. Valle, M. Crisma, C. Toniolo, R. Beisswenger, A. Rieker and G. Jung (1989). First observation of a helical peptide containing a chiral residue without a preferred screw sense. *J. Am. Chem. Soc.* **111**, 6828–6833.