Crystal-state Conformation of $C^{\alpha,\alpha}$-Dialkylated Peptides Containing Chiral β-*homo*-Residues

ALESSANDRA ROMANELLI[a], **ISIDORO GARELLA**[b], **VALERIA MENCHISE**, **ROSA IACOVINO**[b], **MICHELE SAVIANO**[a], **DANIELA MONTESARCHIO**[c], **CLAUDE DIDIERJEAN**[d], **PAOLA DI LELLO**[b], **FILOMENA ROSSI**[a] and **ETTORE BENEDETTI**[a,*]

[a] Centro di Studio di Biocristallografia, C.N.R. and Dipartimento di Chimica,
Università degli Studi di Napoli 'Federico II', Napoli, Italy
[b] Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Caserta, Italy
[c] Dipartimento di Chimica Organica e Biologica, Università degli Studi di Napoli 'Federico II', Napoli, Italy
[d] Université Henri Poincaré, Nancy 1, Faculté des Sciences, Vandoeuvres-lès-Nancy Cedex, France

Abstract: Secondary structure formation and stability are essential features in the knowledge of complex folding topology of biomolecules. To better understand the relationships between preferred conformations and functional properties of β-*homo*-amino acids, the synthesis and conformational characterization by X-ray diffraction analysis of peptides containing conformationally constrained $C^{\alpha,\alpha}$-dialkylated amino acid residues, such as α-aminoisobutyric acid or 1-aminocyclohexane-1-carboxylic acid and a single β-*homo*-amino acid, differently displaced along the peptide sequence have been carried out. The peptides investigated are: Boc-βHLeu-(Ac₆c)₂-OMe, Boc-Ac₆c-βHLeu-(Ac₆c)₂-OMe and Boc-βHVal-(Aib)₅-O*t*Bu, together with the *C*-protected β-*homo*-residue HCl·H-βHVal-OMe. The results indicate that the insertion of a βH-residue at position 1 or 2 of peptides containing strong helix-inducing, bulky $C^{\alpha,\alpha}$-disubstituted amino acid residues does not induce any specific conformational preferences. In the crystal state, most of the NH groups of β-*homo* residues of tri- and tetrapeptides are not involved in intramolecular hydrogen bonds, thus failing to achieve helical structures similar to those of peptides exclusively constituted of $C^{\alpha,\alpha}$-disubstituted amino acid residues. However, by repeating the structural motifs observed in the molecules investigated, a β-pleated sheet secondary structure, and a new helical structure, named (14/15)-helix, were generated, corresponding to calculated minimum-energy conformations. Our findings, as well as literature data, strongly indicate that conformations of βH-residues, with the μ torsion angle equal to $-60°$, are very unlikely. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: α,α-dialkylated amino acids; β-*homo* amino acids; peptide synthesis; X-ray structure; β-pleated sheet

INTRODUCTION

The assembly of medium-sized peptide blocks, adopting predictable and defined secondary structures in solution, aiming at a more complex folding topology, represents a common feature in all strategies for protein design. Consequently, a detailed knowledge of the process of secondary structure formation and stability is essential. Numerous studies using *homo*-oligopeptides [1] or the host-guest approach [2] contributed to our knowledge of the critical main-chain length, solvent and sequence dependencies of $\alpha/3_{10}$-helices and β-pleated sheet formation. With regard to their use in protein design, the stabilization of specific secondary structures by the introduction of molecular blocks in the sequence is a way of achieving, by a specific molecular 'tool', a given conformation. In this connection,

* Correspondence to: Centro di Studio di Biocristallografia, C.N.R. and Dipartimento di Chimica, Università degli Studi di Napoli 'Federico II', Via Mezzocannone 4, I-80134 Napoli, Italy. E-mail: benedetti@chemna.dichi.unina.it

the incorporation of a $C^{\alpha,\alpha}$-dialkylated amino acid residue into an oligopeptide chain is a commonly used tool for stabilizing helical conformations; in particular, systematic studies on the different structural propensity of individual C^{α}-methylated amino acids have given valuable insight for stabilizing right-handed helical structures [3,4].

β-Amino acid oligomers, the so-called β-peptides, provide an interesting alternative to conventional peptides for design purposes, as the β-peptide backbone offers greater opportunity to modulate the conformational rigidity [5]. Short β-peptides have recently been shown to adopt, in organic solvents [6–8], each of the three types of regular secondary structures (helix, sheet and turn) which are observed in proteins.

In particular, Appella *et al.* [5] found that six-residue oligomers of the optically pure *trans*-2-aminocyclohexane-carboxylic acid (ACHC) adopt a new type of helical structure stabilized by intramolecular, 14-membered ring hydrogen bonds (14-helix), both in organic solvents and in the crystal state. These authors have proposed that the backbone constraint provided by the cyclohexane ring should confer a very high conformational stability to the ACHC oligomers.

Recently, research in β-peptides, built exclusively with β-amino acids, has gained interest because of their supposed unique tendency to form stable secondary structures in solution [9–11], and because of their supposed resistance to common proteases [12].

On the other hand, in previous papers [13,14], we have not been able to observe any preferred new secondary structure or preference in the folding pattern, both in solution (NMR) and/or crystal state (X-ray) in short linear peptide sequences built with new synthetic β-*homo* amino acids. The extra methylene group addition brought in by the β-*homo* amino acid insertion in short linear peptides does not appear to alter the overall conformation of the backbone. In addition, our theoretical results suggest that the use of β-*homo*-residues is to be preferred when a larger conformational freedom for the ϕ angle, with respect to the ψ angle, is required. As only few data are available to date on the crystal-state behaviour of this new class of peptides, it seems wise not to draw any 'a priori' general conclusion about the conformational preferences of the various β-*homo* amino acid residues.

In view of the close relationship between preferred conformation and functional properties of β-*homo* amino acids, we here report on the synthesis and structural characterization of conformationally constrained peptides containing bulky amino acids such as the $C^{\alpha,\alpha}$-dialkylated amino acids Aib (α-aminoisobutyric acid) and Ac_6c (1-aminocyclohexane-1-carboxylic acid), and a single β-*homo* amino acid residue differently positioned along the peptide sequence.

The peptides, the conformation of which was investigated in the crystal state by X-ray diffraction, are: Boc-βHLeu-Ac_6c-Ac_6c-OMe (Boc, *tert*-butyloxycarbonyl; OMe, methoxy) (**1**), Boc-Ac_6c-βHLeu-Ac_6c-Ac_6c-OMe (**2**) and Boc-βHVal-(Aib)$_5$-OtBu (OtBu, *tert*-butoxy) (**3**), together with the C-protected β-*homo*-residue HCl·H-βHVal-OMe (all β-*homo*-amino acids discussed in this work have the L-configuration).

MATERIALS AND METHODS

Peptide Synthesis and Characterization

α-Amino acids and the activating agent benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBop) were purchased from Novabiochem (Switzerland). Solvents were reagent grade; the other organic reagents, commercially available, were used without further purification. Optical rotations were measured using a Perkin–Elmer model 141 polarimeter. Silica gel plates 60F-254 (Merck) were visualized by ultraviolet (UV) lamp and/or ninhydrin chromatic reaction. High-performance liquid chromatography (HPLC) analyses were performed on a Beckman System Gold instrument equipped with UV 166 module detector and a Chromotopac R6A recorder. Purifications were carried out on a Millipore–Waters Delta Prep 3000 HPLC system. NMR measurements were carried out on Varian Gemini 200 and Varian Unity 400 MHz spectrometers. All ^{1}H NMR spectra were recorded at 298 K; measurements were carried out in CDCl$_3$ (99.96% ^{2}H isotope, Aldrich, Milwaukee, WI, USA). MALDI-TOF mass measurements were performed on a Shimadzu Kratos Maldi 4 instrument.

The synthesis of *N*-protected, optically pure βHLeu and βHVal was carried out starting from Boc-L-Leu-OH and Boc-L-Val-OH, respectively, following literature procedures [15]. We converted the starting amino acid into the corresponding *N*-protected β-amino acid *via* the β-amino alcohol, the β-amino iodide and the β-amino nitrile, followed by hydrolysis of the latter compound under strong acidic conditions. Then, the resulting terminally

deprotected, enantiomerically pure βHLeu and βHVal [16] were quantitatively converted to their *N*-protected form using di-*tert*-butyl-dicarbonate (Boc)$_2$O by conventional synthetic procedures.

Boc-βHLeu-OH and Boc-βHVal-OH were then coupled by solution methods to the Ac$_6$c and Aib residues for the preparation of the peptides discussed below.

Boc-βHLeu-Ac$_6$c-Ac$_6$c-OMe (1)

To a solution of 165 mg of Boc-βHLeu-OH (0.76 mmol) and 395 mg of PyBop (0.76 mmol) dissolved in 1 mL of CH$_2$Cl$_2$, 300 mg (0.76 mmol) of the dipeptide TFA$^-$ $^+$H$_2$-(Ac$_6$c)$_2$-OMe (TFA, trifluoroacetate), prepared according to a standard procedure in 1 mL of CH$_2$Cl$_2$ and 0.78 mL of DIEA (diisopropylethylamine) (4.56 mmol), were added under stirring at room temperature. The reaction mixture was kept under stirring for 2 days, then was dried under reduced pressure, and the residue taken up in ethyl acetate (EtOAc). After washings with 20 mL of a 10% aqueous citric acid solution, 20 mL of a saturated NaHCO$_3$ solution and 20 mL of H$_2$O, the organic phase was dried over anhydrous Na$_2$SO$_4$ and taken to dryness. Tripeptide **1** was purified by flash column (FC) chromatography, eluted with a mixture of EtOAc and petroleum ether (1:1, v/v) to give 360 mg (yield 93%) of the pure title compound. $R_f = 0.50$, in EtOAc:petroleum ether (1:1, v/v); $[\alpha]_D^{20}$ $-7.0°$ ($c = 0.15$, CH$_3$CN); m/z (MALDI-TOF) 510 for (M−H)$^+$, calcd. 509.

^{1}H NMR (200 MHz; CDCl$_3$), δ (ppm): 7.80 (s, 1H, NH Ac$_6$c); 6.07 (s, 1H, NH Ac$_6$c); 5.10 (d, 1H, NH βHLeu); 3.88 (m, 1H, αCH βHLeu); 3.63 (s, 3H, CH$_3$ OMe); 2.43 (d, 2H, βCH$_2$ βHLeu); 2.20–1.20 (complex signals, 23H, β'CH$_2$ and, γCH βHLeu and cyclohexyl Ac$_6$c protons); 1.40 (s, 9H, CH$_3$ Boc); 0.88 (d, 6H, δCH$_3$ βHLeu).

Boc-Ac$_6$c-βHLeu-Ac$_6$c-Ac$_6$c-OMe (2)

50 mg of tripeptide **1** (0.1 mmol) were treated with TFA for 1 h at room temperature under stirring. The solution was dried under reduced pressure and coevaporated several times with Et$_2$O (diethyl ether). The deprotected fragment was redissolved in the minimum amount of CH$_3$CN, and added to a 1 mL CH$_3$CN solution containing 49 mg of Boc-Ac$_6$c-OPfp (OPfp, pentafluorophenoxy) (0.12 mmol), previously prepared according to the literature [17]. The mixture was kept under stirring at 50°C for 1 day. The solvent was evaporated and the residue dissolved in CH$_3$CN. After the usual work-up, the organic phase

was dried on anhydrous Na$_2$SO$_4$ and taken to dryness. The crude product was purified by HPLC on a RP-Vydak C$_{18}$ column (2.2 × 25 cm) eluted with a linear gradient of H$_2$O with 0.1% TFA (solvent A) and CH$_3$CN with 0.1% TFA (solvent B) from 20% B to 80% B in 30 min; $\lambda = 210$ nm; flow = 12 mL/min. 14.0 mg of the pure title compound were obtained (yield 22%). $R_f = 0.40$, EtOAc:petroleum ether (1:1, v/v). $[\alpha]_D^{20}$ $-8.3°$ ($c = 0.13$, CH$_3$CN); m/z (MALDI-TOF) 635 for (M−H)$^+$, calcd. 634.

^{1}H NMR and 2D (400 MHz; CDCl$_3$), δ (ppm): 7.95 (s, 1H, NH Ac$_6$c); 6.95 (d, 1H, NH βHLeu); 6.76 (s, 1H, NH Ac$_6$c); 4.94 (s, 1H, NH Ac$_6$c^1); 4.27 (m, 1H, αCH βHLeu); 3.69 (s, 3H, CH$_3$ OMe); 2.48 (m, 1H, βCH$_2$ βHLeu); 2.31 (m, 1H, βCH$_2$ βHLeu); 2.36, 2.18 and 2.09–1.20 (complex signals, 30H, cyclohexyl Ac$_6$c protons); 1.66 (m, 1H, γCH βHLeu); 1.53 (m, 1H, β'CH$_2$ βHLeu); 1.42 (m, 1H, β'CH$_2$ βHLeu); 1.42 (s, 9H, CH$_3$ Boc); 0.88 (d, 3H, δCH$_3$ βHLeu); 0.87 (d, 3H, δCH$_3$ βHLeu).

Boc-βHVal-(Aib)$_5$-O*t*Bu (3)

39 mg of Boc-βHVal-OH (0.17 mmol) and 88 mg of PyBop (0.17 mmol) dissoved in 1 mL of CH$_2$Cl$_2$ were stirred for 10 min, then 86 mg of H-(Aib)$_5$-O*t*Bu (0.17 mmol), prepared according to a described procedure [18], dissolved in 2 mL of CH$_2$Cl$_2$ with 0.175 mL DIEA (1.02 mmol), were added. The reaction mixture was stirred for 2 days at room temperature. The crude mixture was dried under reduced pressure, redissolved in CH$_3$CN, and purified by HPLC on a Vydac C$_{18}$ column (2.2 × 25 cm), with the linear gradient from 20% B to 80% B in 35 min ($\lambda = 210$ nm, flow = 12 mL/min) to give 20 mg of pure title compound (yield 16%). $[\alpha]_D^{20}$ $-15.5°$ ($c = 0.12$, CH$_3$CN; m/z (MALDI-TOF) 715 for (M−H)$^+$, calcd. 713.

^{1}H NMR (200 MHz; CDCl$_3$), δ (ppm): 7.72; 7.67; 7.49; 7.35; 7.05; 6.23 (6s, 6H, 6 NH); 4.54 (m, 1H, αCH βHVal); 2.56 (m, 1H, βCH$_2$ βHVal); 2.35 (m, 1H, βCH$_2$ βHVal); 1.44 (overlapped signals, 19H, CH$_3$ Boc, CH$_3$ O*t*Bu and β'CH βHVal); 0.91 (overlapped signals, 36H, βCH$_3$ Aib and γCH$_3$ βHVal).

X-Ray Diffraction Analysis

Colourless single crystals of HCl·H-βHVal-OMe, Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe, Boc-βHLeu-(Ac$_6$c)$_2$-OMe, and Boc-βHVal-(Aib)$_5$-O*t*Bu were grown by slow evaporation at room temperature from the solvents reported in Table 1.

A poor quality crystal of HCl·H-βHVal-OMe was mounted on a four-circle Nonius Kappa CCD

Table 1   Crystallographic Data for $^+H_2$-(βHVal)-OMe Cl$^-$, Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe, Boc-βHLeu-(Ac$_6$c)$_2$-OMe, Boc-βHVal-(Aib)$_5$-OtBu

	$^+H_2$-βHVal-OMe Cl$^-$	Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe	Boc-βHLeu-(Ac$_6$c)$_2$-OMe	Boc-βHVal-(Aib)$_5$-OtBu
Empirical formula	C$_7$H$_{16}$N$_1$O$_2$Cl	C$_{34}$H$_{61}$N$_4$O$_7$	C$_{27}$H$_{47}$N$_3$O$_6$	C$_{35}$H$_{69}$N$_6$O$_9$
Formula weight (a.m.u.)	171.33	637.87	509.68	717.96
Crystallization solvent	Methanol	Acetonitrile/methanol	Acetonitrile/methanol	Methanol
Crystal system	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic
Space group	P2$_1$2$_1$2$_1$	P2$_1$2$_1$2$_1$	P2$_1$	C2
a (Å)	5.426 (1)	6.128 (1)	6.187 (1)	18.048 (5)
b (Å)	10.765 (1)	15.169 (3)	21.057 (3)	11.451 (3)
c (Å)	17.242 (1)	40.564 (5)	11.863 (2)	21.555 (9)
β (°)	90.000	90.000	102.61 (1)	97.92 (3)
V (Å^3)	1007.1 (2)	3771 (1)	1508.2 (2)	4412 (2)
Z (molecules/unit cell)	4	4	2	4
Density (calc.) (g/cm^3)	1.198	1.124	1.122	1.081
Independent reflections	631	4115	2956	4389
Observed reflections	563	2015	1531	1902
S	1.924	1.159	1.077	1.359
Final R indexes [$I > 2\sigma(I)$]	$R1 = 0.071$, $wR2 = 0.172$	$R1 = 0.088$, $wR2 = 0.216$	$R1 = 0.069$, $wR2 = 0.169$	$R1 = 0.108$, $wR2 = 0.258$
R indexes (all data)	$R1 = 0.096$, $wR2 = 0.240$	$R1 = 0.154$, $wR2 = 0.261$	$R1 = 0.128$, $wR2 = 0.214$	$R1 = 0.184$, $wR2 = 0.297$
Temperature	293	293	293	293
Radiation (λ, Å)	AgKα (0.56090)	CuKα (1.54178)	CuKα (1.54178)	CuKα (1.54178)

diffractometer using a graphite-monochromated AgKα ($\lambda = 0.56087$ Å) X-ray radiation. The diffraction experiments were carried out with the COLLECT software [19]: the 'INDEX' procedure was used to determine the cell parameters and the data collection parameters, given by the STRATEGY procedure, were followed. The data were integrated and scaled using the HKL suite [20]. The redundancy and the R_{sym} are 5.8 and 8.8%, respectively. The completeness of the 1 Å data set is 98%.

The X-ray data for the other peptides were collected on an Enraf–Nonius CAD4 diffractometer (Delft, The Netherlands), using graphite-monochromated CuKα radiation. During all data collections, three reflections were measured every 120 min, in order to check the stability of the crystals and the electronics. The observed intensity decreases were within 3%. The intensities of all reflections were corrected for Lorentz and polarization factors, but no absorption correction was applied. Unit cell determinations were carried out for all crystals by least-square refinement of the setting angles of at least 25 high angle reflections accurately centred.

All structures were solved by direct methods using the SIR97 program [21]. In all cases, the E maps corresponding to the best figures of merit revealed all the non-H-atoms.

The structures were refined using the program SHELXL93 [22] by the full-matrix least-square procedure on F^2 (all data). All non-H atoms were refined anisotropically. The positions of the H-atoms were calculated, and during the refinement, the H-atoms were allowed to ride on their carrying atom, with U$_{iso}$ set equal to 1.2 times the U$_{eq}$ of the attached atom.

The scattering factors for all atomic species were calculated from Cromer and Waber [23]. Details of the crystallographic data and diffraction parameters for the four structures are given in Table 1. Further details of the crystal structures, including final atomic parameters for the non-H-atoms, have been deposited with, and are available, on request, from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (UK), on quoting the full journal citation. The C$^\alpha$, C$^\beta$ symbology for backbone atoms does not follow the recommendations of the IUPAC-IUB Commission, but the Seebach nomenclature [10].
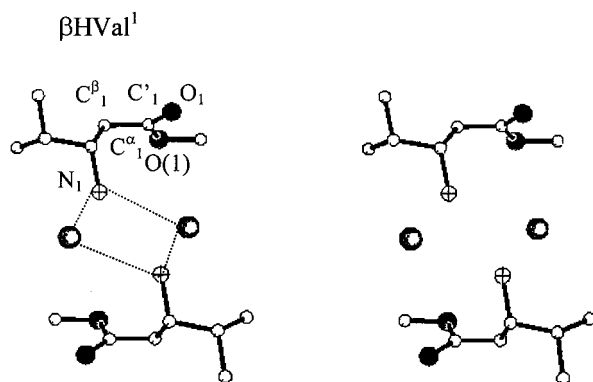
βHVal[1]

Figure 1 Stereo drawing of the X-ray diffraction structure of HCl·H-βHVal-OMe, with numbering of the backbone atoms. The intermolecular interactions existing between the charged amino groups and the chlorine ions in the crystals are indicated as dotted lines.

RESULTS AND DISCUSSION

The conformations of peptides **1**-**3**, together with that of the hydrochloride of the *C*-protected βHVal amino acid, were investigated in the crystal state by single-crystal X-ray diffraction analysis. In Figures 1–4, the stereo drawings of the molecular structures are illustrated. For all structures, bond lengths and bond angles show values in agreement with literature data [24,25]. In particular, the average C^α-$C^\beta H_2$-C' bond angle of the βH-residues

(113.0°), as determined from the structures investigated in the present work and from previous structures, the coordinates of which have been reported in the literature, does not show any substantial difference with respect to the average value (113.2°) determined for the βHGly residue (usually termed as β-Ala), using the structures available in the Cambridge Crystallographic Data Bank [26]. Relevant backbone and side-chain torsion angles are listed in Table 2, while in Table 3, the intra- and intermolecular H-bond parameters are reported.

The molecular structures of the Boc-βHLeu-(Ac₆c)₂-OMe (**1**) (Figure 2) and Boc-Ac₆c-βHLeu-(Ac₆c)₂-OMe (**2**) (Figure 3) do not show any intramolecular H-bond. In the folded conformation observed, the C=O carbonyl groups and the N–H amino groups are grossly all positioned on opposite directions with respect to the mean plane of the molecular backbone. In this conformation, the N–H···O=C H-bond intermolecular interactions are optimized (see below). The urethane, peptide and ester groups are all found in the *trans* conformation.

The values of the φ, μ and ψ torsion angles (where φ is C'-N-C^α-C^β, μ is N-C^α-C^β-C', and ψ is C^α-C^β-C'-N) for the βHLeu residue are −87.1°, 179.8° and 119.4° for **1**, and −82.2°, −177.7° and 116.3° for **2**, respectively, in agreement with one of the minimum-energy conformation regions theoretically predicted for the fully protected Ac-βHAla-NHMe (Ac,
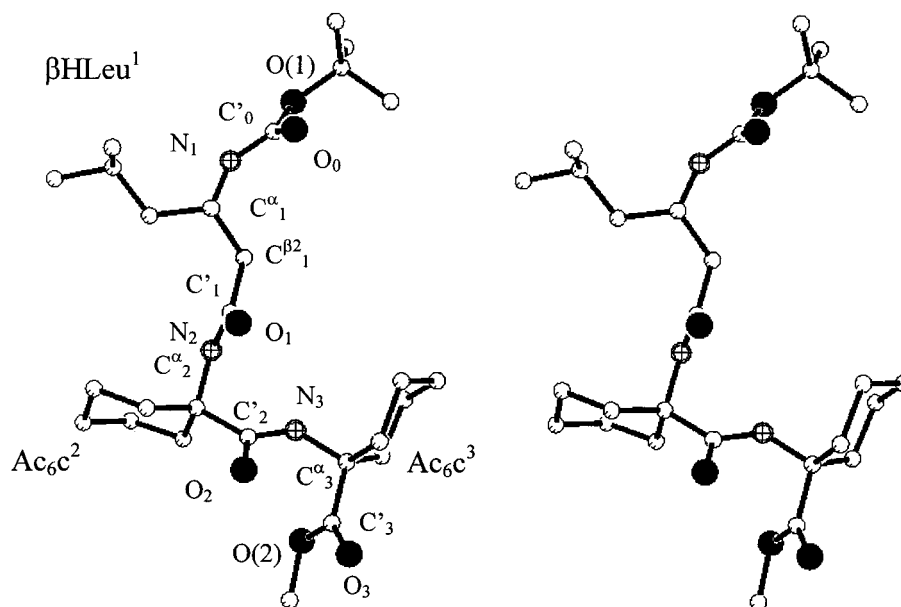

Figure 2 Stereo drawing of the X-ray diffraction structure of the tripeptide Boc-βHLeu-(Ac₆c)₂-OMe with numbering of the backbone atoms.
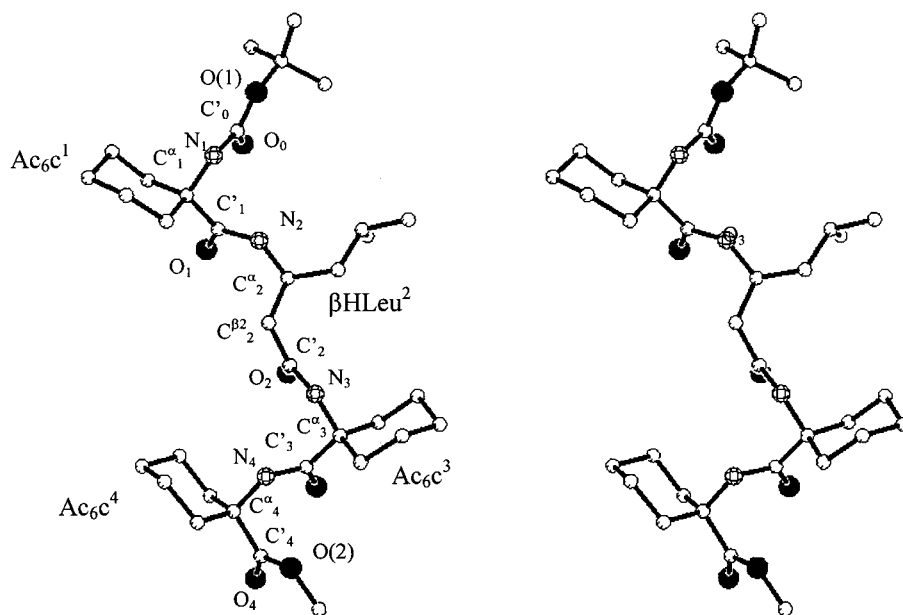
Figure 3   Stereo drawing of the X-ray diffraction structure of the tetrapeptide Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe with numbering of the backbone atoms.
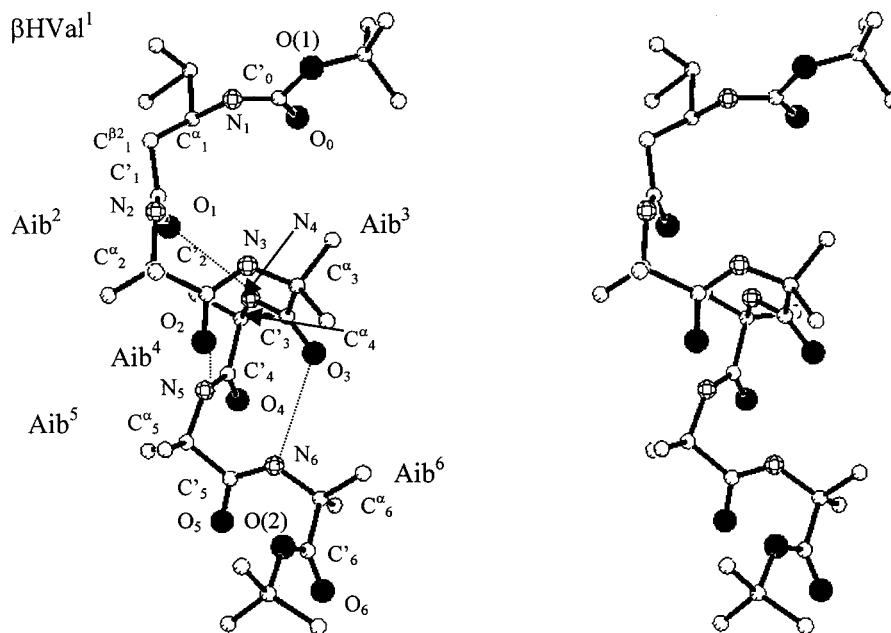

Figure 4   Stereo drawing of the X-ray diffraction structure of the hexapeptide Boc-βHVal-(Aib)$_5$-OtBu with numbering of the backbone atoms. The three intramolecular H-bonds are represented by dotted lines.

acetyl; NHMe, methylamino) model peptide [13], namely region B of the φ, ψ map ($\varphi = -90°$, $\psi = 120°$), with $\mu = 180°$.

All of the Ac$_6$c residues are found in the helical region A (or A*) of the conformational map [27] for α-residues with average values for the φ, ψ backbone torsion angles of $\pm 52.4°$, $\pm 48.3°$, close to

those expected for right- and left-handed $3_{10}/\alpha$-helical conformations [28]. It is worth noting that, in both structures, the *C*-terminal Ac$_6$c residue assumes a helical conformation with opposite handedness with respect to that of the preceding residue, as commonly found in consecutive *C*-terminally positioned C$^{\alpha,\alpha}$-dialkylated aminoacid residues [29].

Table 2 Selected Backbone Torsion Angles (°) for $^+$H$_2$-βHVal-OMe Cl$^-$, Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe, Boc-βHLeu-(Ac$_6$c)$_2$-OMe and Boc-βHVal-(Aib)$_5$-OtBu

Torsion angle	Cl$^-$ $^+$H$_2$-βHVal-OMe	Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe	Boc-βHLeu-(Ac$_6$c)$_2$-OMe	Boc-βHVal-(Aib)$_5$-OtBu
ϕ_1		58.0(9)	$-87.1(8)$	$-149(1)$
μ_1	71(1)	–	179.8(6)	87(1)
ψ_1	$-127(1)$	48.3(9)	119.4(7)	$-129(1)$
ω_1		168.5(6)	178.9(6)	$-169(1)$
ϕ_2		$-82.2(8)$	$-59.2(8)$	$-61(1)$
μ_2		170.8(6)	–	–
ψ_2		116.3(8)	$-45.6(8)$	$-28(1)$
ω_2		$-177.7(6)$	$-169.9(6)$	$-178.3(8)$
ϕ_3		$-58.4(9)$	42.5(9)	$-50(1)$
ψ_3		$-46.2(9)$	54.6(8)	$-36(1)$
ω_3		$-169.7(7)$	173.7(8)	$-174.8(9)$
ϕ_4		46.9(9)		$-58(1)$
ψ_4		45.1(8)		$-31(1)$
ω_4		$-179.3(7)$		$-174.1(9)$
ϕ_5			$-55(1)$	
ψ_5			$-39(1)$	
ω_5			$-173.3(9)$	
ϕ_6			54(1)	
ψ_6			45(1)	
ω_6			176.9(8)	

The bond angles for the Ac$_6$c residues indicate an asymmetric geometry for the C$^\alpha$ atom. The average value for the conformationally sensitive N-C$^\alpha$-C' (τ) bond angle, external to the cyclic system, for the Ac$_6$c residues is 109°, similar to that exhibited by the C$^{\alpha,\alpha}$-dialkylated glycines forming regular helices (110–111°) [30–34]. The cyclic side chains of the Ac$_6$c residues are found in the typical chair conformation, with an average total puckering amplitude Q value of 0.56 ± 0.01 Å, and an average amplitude of distortion θ value of $176.0° \pm 1.5°$ [35]. It is very interesting to note that the conformation of the sequence -βHLeu-(Ac$_6$c)$_2$-OMe common to **1** and **2** are almost superimposable (Figure 5), with a root mean square deviation of 0.088 Å.

By repeating the motif represented by the -βHLeu-(Ac$_6$c)$_2$- moiety one can generate a β-pleated sheet structure, stabilized by intermolecular H-bonds of the N–H···O=C type involving all carbonyl and amino groups which point to opposite direction in each peptide chain, as represented in Figure 6 in a parallel fashion. Our preliminary calculations show that the isolated β-pleated sheet structure is among the minimum-energy conformations available to a [-βHAla-(Aib)$_2$-]$_n$ chain.

In both compounds the N-terminal *tert*-butyloxycarbonyl blocking group is found in the usual con-

formation, in which the three methyl substituents of the quaternary C(1) atom are staggered with respect to the mean plane of the C(1)-O(1)-C$_0'$(=O$_0$)-N$_1$ moiety [36].

At variance with the structures found for the peptides discussed above, the structure of the hexapeptide Boc-βHVal-(Aib)$_5$-OtBu (**3**) (Figure 4) is mainly helical. The βHVal residue at position 1 presents values of the φ, μ, and ψ conformational angles of $-149°$, $87°$, and $-129°$, respectively. These values correspond to a conformation quite different from that observed for the βHLeu residues in the tri- and tetrapeptides discussed above, but they are, however, in agreement with the other calculated minimum-energy conformation region determined for Ac-βHAla-NHMe [13], with $\mu = 60°$, [region A of the φ, ψ map with $\varphi = -150°$, $\psi = -110°$]. Similarly, the βHVal residue in HCl·H-βHVal-OMe (Figure 1) shows a value for the torsion angle μ of 71° (g^+ conformation), while the ψ angle shows a value of $-127°$, close to those observed for the βHVal residue in the hexapeptide.

The sequence 2–5 of the hexapeptide **1** is folded in a right-handed 3_{10}-helical structure (mean values of the φ, ψ torsion angles for Aib^2-Aib^5 residues are $-56°$ and $-34°$, respectively), being stabilized by three $i \leftarrow i + 3$ C=O···H–N intramolecular H-bonds

Table 3   Intra- and Intermolecular H-bond Parameters for the Amino Acid Derivative and Peptides

Compound	Type	Donor	Acceptor	Length(Å) $(N\cdots O)/(N\cdots O)$	Angle(°) $(C'{=}O\cdots N)/$ $(C'{=}O\cdots O)$	Symmetry operation
$^+H_2$-βHVal-OMe Cl$^-$						
	Intermolecular	N	Cl	3.19(1)		x, y, z
		N	Cl	3.160(9)		$x-1, y, z$
Boc-Ac$_6$c-βHLeu-(Ac$_6$c)$_2$-OMe						
	Intermolecular	N_1	O_1	2.990(7)	146.8(3)	$x-1, y, z$
		N_2	O_2	2.931(6)	171.6(3)	$x-1, y, z$
		N_3	O_3	3.339(7)	148.2(3)	$x-1, y, z$
Boc-βHLeu-(Ac$_6$c)$_2$-OMe						
	Intermolecular	N_1	O_1	2.851(8)	158.5(3)	$x-1, y, z$
		N_2	O_2	3.200(8)	144.9(3)	$x-1, y, z$
		N_3	O_3	2.964(8)	111.7(3)	$x-1, y, z$
Boc-βHVal-(Aib)$_5$-OtBu						
	Intramolecular $(1 \leftarrow 4)$	N_4	O_1	2.99(1)	162(1)	x, y, z
		N_5	O_2	2.96(1)	156(1)	x, y, z
		N_6	O_3	3.15(1)	149(1)	x, y, z
Boc-βHval-(AIB)$_5$-OtBu						
	Intermolecular	N_1	O_4	3.06(1)	146(1)	$x, y+1, z$
		N_2	O_5	2.91(1)	164(1)	$x, y+1, z$

(Table 3). In this structure, the range of observed H-bonded $N\cdots O$ distances is 2.98–3.16 Å, while that of the $N\cdots O{=}C$ angles is 150–163° [37–39]. The C-terminal Aib residue shows again a conformation with opposite helical handedness (left-handed, $\varphi = 54°$, $\psi = 45°$) with respect to that shown by the preceding residues. The urethane N-terminal group, the peptide units and the ester group at the C-terminu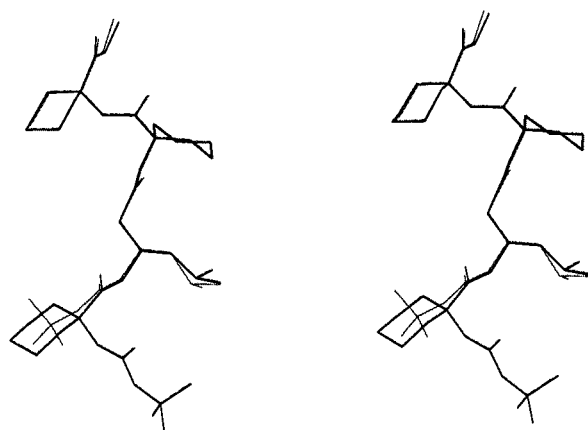s of the hexapeptide molecule, as well as the ester group of HCl·H-βHVal-OMe are all found in the *trans* planar conformation. The N-terminal *tert*-butyloxycarbonyl blocking group is found in the usual conformation (see above) and, analogously, the C-terminal *tert*-butyl ester blocking group
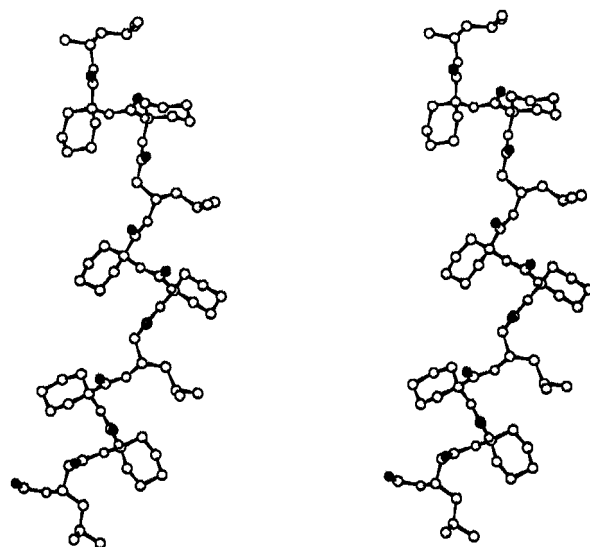
Figure 5   Stereo drawing of the superposition of the -βHLeu-(Ac$_6$c)$_2$- sequences of the tri- and the tetrapeptide molecules. The calculated r.m.s. deviation is 0.088 Å.

Figure 6   Stereo drawing of the β-pleated sheets structure generated by the repetition of the -βHLeu-(Ac$_6$c)$_2$- sequence observed in the structures of the tri- and tetrapeptide.

adopts a conformation in which the three methyl substituents of the quaternary C(5) atom are staggered with respect to the mean plane of the C_6^α-$C_6'(=O_6)$-O(2) moiety [36].

It is also interesting to note that, by repeating of the -βHVal-Aib- dipeptide sequence, a helix is obtained, which is stabilized by intramolecular H-bonds (the alternating C_{14} and C_{15} ring structures, as represented in Figure 7). We name this helix a 14/15-helix. Preliminary calculations show that this structure is among the minimum-energy conformations available to sequential peptides constituted by a βH-residue and a $C^{\alpha,\alpha}$-dialkylated residue with torsion angles similar to those observed for the first two residues in peptide **3**.

In the tri- (**1**) and tetrapeptide (**2**) structures the βHLeu side-chain χ^1 (N-C^α-C^β-C^γ) torsion angle is close to $-60°$ (g^- conformation), while the $\chi^{2,1}$ (C^α-$C^{\beta'}$-C^γ-$C^{\delta1}$) and $\chi^{2,2}$ (C^α-C^β-C^γ-$C^{\delta2}$) torsion angles are close to $-60°$ and $180°$, respectively (g^-, t conformation). The overall conformation of the βHLeu side chain is similar to the most populated conformer found for the proteinogenic Leu residue [25], thereby demonstrating that the short-range interactions responsible for this conformation are similar in α- and βH-residues. Similarly, the βHVal side-chain conformation in both HCl·H-βHVal-OMe and Boc-βHVal-(Aib)$_5$-OtBu (**3**) is of the g^- t, type, in agreement with the results of statistical analyses of the Val residue in α-peptides [25], again showing that the short-range interactions responsible for this conformation are similar in the α- and βH-residues.

The Boc-βHLeu-(Ac$_6$c)$_2$-OMe (**1**) molecules pack one on top of the other along the a direction. Indeed,
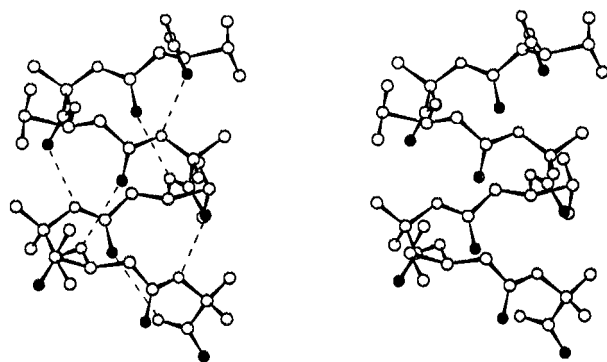

Figure 7 Stereo drawing of the 14/15-helical structure obtained by repeating the -βHVal-Aib- motif found in the hexapeptide structure. Dashed lines represent N–H···O=C H-bonds which generate alternating C_{14} and C_{15} ring structures along the helix.

the observed conformation with the C=O groups pointing to the opposite direction with respect to that of the N–H groups, relative to the mean plane of the backbone atoms, allows the formation of a β-pleated sheet structure (Figure 8), in which molecules are H-bonded to symmetry-translated molecules along the a crystallographic direction by means of three intermolecular H-bonds of the N–H···O=C type (Table 3). Further stabilization of the sheets is achieved by van der Waals interactions between the hydrophobic groups protruding from the average plane of the β-pleated sheets. In particular, along the b direction layers of β-pleated sheets (packed in the ac plane in a parallel fashion) alternate with similar layers of β-pleated sheets running in opposite direction (antiparallel with respect to adjacent layers).

Analogously, in the tetrapeptide structure (**2**), we observe the formation of a β-pleated sheet structure, in which again molecules are held together in the crystal by three intermolecular H-bonds of the N–H···O=C type between molecules translated along the a axis (Table 3).

The packing mode of Boc-βHVal-(Aib)$_5$-OtBu (**3**) is characterized by two N–H···O=C' intermolecular H-bonds (N_1-H···O_4 and N_2-H···O_5). Both intermolecular H-bonds link symmetry related molecules (translated along b) giving rise to columns of helical molecules aligned in a head-to-tail fashion along the b direction. The hexagonal packing of the resulting rows of molecules is stabilized by van der Waals interactions between hydrophobic groups of adjacent rows, which pack in a parallel fashion, forming layers parallel to the ab plane. These layers are packed in an antiparallel fashion with similar layers along the c direction.

In the crystal packing of HCl·H-βHVal-OMe, the Cl$^-$ ion acts as a bridge, connecting symmetry-related amino ester molecules by two strong N–H···Cl intermolecular electrostatic interactions (Table 3). The values of the N···Cl distances are 3.17 and 3.18 Å. These interactions give rise to rows of molecules linked through the N–H$^+$···Cl$^-$···$^+$H–N motif. Hydrophobic interactions hold together rows of molecules in the other directions.

CONCLUSIONS

Seebach *et al.* [40] have reported general rules for the allowed conformations of β-*homo* amino acid residues, a relatively new class of residues found in several natural peptides [41–43]. In addition, other
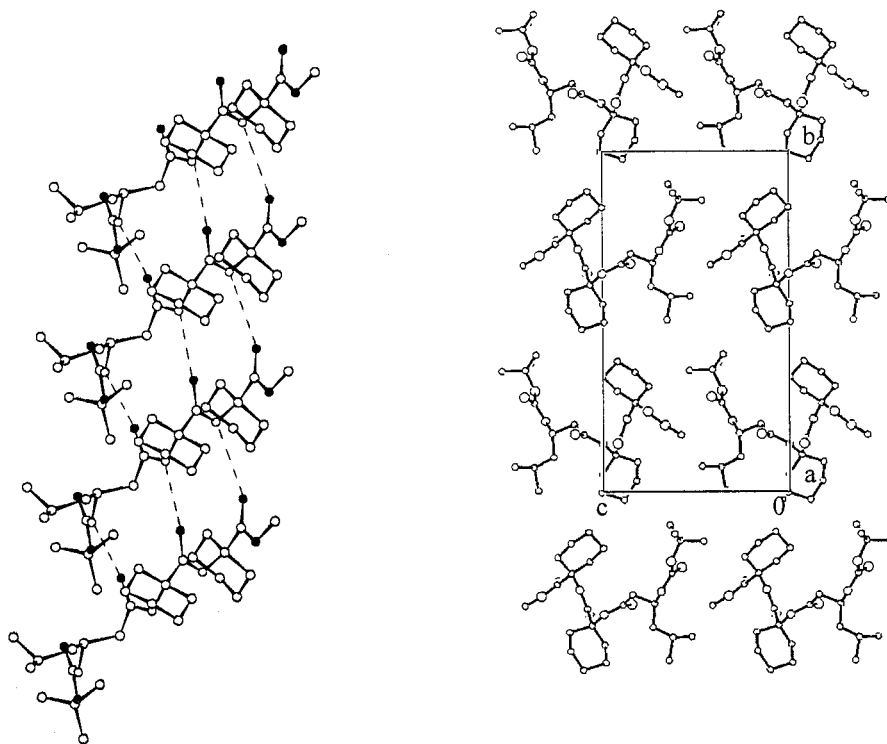
Figure 8 Left: the β-pleated sheets structure of the tripeptide Boc-βHLeu-(Ac$_6$c)$_2$-OMe molecules, obtained by translation along the a direction of the molecules. Right: the packing of the β-pleated sheets as seen in the bc plane (each molecule represents the projection of a β-pleated sheet).

authors have shown the onset of new stable secondary structures for particular β-*homo* amino acid residues. We have prepared few new β-*homo* amino acids, which have been variously included in peptides containing α-residues, or have served for the synthesis of homo-oligopeptides composed exclusively by these new β-*homo* residues [13,14,16].

A theoretical analysis of the conformations allowed to peptides containing β-*homo* amino acids by minimum-energy calculations of Ac-βHAla-NHMe clearly indicates that no general preferred conformational behaviour can be predicted 'a priori' or ruled out. In particular, for each of the possible staggered backbone conformations (g^-, g^+, and t, corresponding to $-60°$, $+60°$, and $180°$, respectively) around the central C$^\alpha$-C$^\beta$ bond (μ angle), the calculated φ and ψ maps show two broad flat regions of minimum energy, in which the φ and ψ angles are both negative (region A), or present negative φ values, and positive ψ values (region B), which should correspond to possible preferred secondary structures in solution, and in the crystal state. All of the β-*homo* residues studied by us to

date show conformational angles which fall within regions A or B of these φ and ψ maps.

However, while the results of the present work, as well as those of our previous papers [13,14,16], still fail to indicate strongly preferred conformations for peptides containing a low percentage of β-*homo* amino acid residues, a rather strong evidence indicate that conformations of β-*homo* residues, with μ angle equal to $-60°$, are very unlikely, as in no case has this conformation ever been observed among the crystal-state structures reported to date.

Furthermore, the insertion of a β-*homo* residue at position 1 or 2 of peptides containing strong helix-inducing, bulky C$^{\alpha,\alpha}$-disubstituted amino acid residues does not produce any conformational preference. Actually, at variance with tri- or longer homo-peptides constituted exclusively of C$^{\alpha,\alpha}$-dialkylated residues, which assume in the crystal state a helical structure, either a 3$_{10}$- or α-helix, in the case of tri- or longer peptides, in which only one β-*homo* residue is present in the sequence, they fail to show a preferred secondary structure. In the crystal state, the NH groups of β-*homo* residues of

the tri- and the tetrapeptides investigated here are not involved in any intramolecular hydrogen bond, thus failing to achieve helical structures similar to those of peptides exclusively constituted of $C^{\alpha,\alpha}$-disubstituted amino acid residues. However, by repeating the structural motifs observed in these molecules, new secondary structures, a β-pleated sheets structure and a new helical structure, a 14/15-helix, are generated, corresponding to calculated minimum-energy conformations. We are currently involved in the preparation and conformational analysis, both in solution and in the crystal state, of the appropriate sequential peptides in order to confirm this prediction.

Further systematic studies on peptides containing β-*homo* residues should led to a clearer definition of the conformational behaviour of these residues.

Acknowledgements

REFERENCES

1. Abd El Rahman S, Auzinger H, Mutter M. Relationship between conformation and physicochemical properties of polypeptides. Synthesis of homo- and co-oligopeptides by the liquid-phase method. *Biopolymers* 1980; **19**: 173–187.
2. Toniolo C, Bonora GM, Mutter M, Pillai VNR. Linear oligopeptides. 78. Effect of the insertion of a proline residue on the solution conformation of host peptides. *Makromol. Chem.* 1981; **182**: 2007–2014.
3. Toniolo C, Crisma M, Formaggio F, Valle G, Cavicchioni G, Précigoux G, Aubry A, Kamphuis J. Structure of peptides from α-aminoacids methylated at the C^{α} atom. *Biopolymers* 1993; **33**: 1061–1071.
4. Altmann RH, Altmann E, Mutter M. Conformational studies on peptides containig enantiomeric α-methyl α-aminoacids. Differential conformational properties of (R)- and (S)-2-methyl aspartic acid. *Helv. Chim. Acta* 1992; **75**: 1198–1210.
5. Appella DH, Christianson LA, Karle IL, Powell DR, Gellman SH. Peptide foldamers: robust helix formation in a new family of α-amino acid oligomers. *J. Am. Chem. Soc.* 1996; **118**: 13071–13072.
6. Appella DH, Christianson LA, Klein DA, Powell DR, Huang X, Barchi Jr JJ, Gellman SH. Residue-based control helix shape in β-peptide oligomers. *Nature* 1997; **387**: 381–384.
7. Seebach D, Matthews JLJ. Peptides: a surprise at every turn. *J. Chem. Soc. Chem. Commun.* 1997; 2015–2022.
8. Chung YJ, Christianson LA, Stanger HE, Powell DR, Gellman SH. A β-peptide reverse turn that promotes hairpin formation. *J. Am. Chem. Soc.* 1998; **120**: 10555–10556.
9. Koert U. Peptides: novel secondary structures take shape. *Angew Chem. Int. Ed. Engl.* 1997; **36**: 1836–1837.
10. Guichard G, Abele S, Seebach D. Preparation of N-Fmoc-protected β^2- and β^3- amino acids and their use as building blocks for the solid-phase synthesis of β-peptides. *Helv. Chim. Acta* 1998; **81**: 187–206.
11. Iverson BL. Betas are brought into the fold. *Nature* 1997; **385**: 113–115.
12. Hintermann T, Seebach D. The biological stability of β-peptides. No interactions between α and β peptidic structures. *Chimia* 1997; **51**: 244–247.
13. Rossi F, Bucci E, Isernia C, Saviano M, Iacovino R, Romanelli A, Di Lello P, Grimaldi M, Montesarchio D, De Napoli L, Piccialli G, Benedetti E. Effect of lengthening of peptide backbone by insertion of chiral β-*homo* amino acid residues. Conformational behavior of linear peptides containing L-leucine and β-*homo*-L-leucine residues. *Biopolymers* 2000; **53**: 140–149.
14. Isernia C, Bucci E, De Napoli L, Di Lello P, Iacovino R, Montesarchio D, Piccialli G, Rossi F, Saviano M, Benedetti E. Synthesis and conformation of dipeptide taste ligands containing *homo*-β-aminoacid residues. *J. Phys. Org. Chem.* 1999; **12**: 577–587.
15. Caputo R, Cassano E, Longobardo L, Palumbo G. Synthesis of enantiopure N- and C-proteted *homo*-β-amino acid by direct homologation of α-amino acids. *Tetrahedron* 1995; **51**: 12337–12350.
16. Benedetti E, Iacovino R, Pedone C, Rossi F, Saviano M, Isernia C, Montesarchio D, De Napoli L, Piccialli G. Aminoacid residues: conformational characterization of an N- and C-protected *homo*-β-(S)-leucine. *Lett. Pept. Sci.* 1997; **4**: 129–134.
17. Schmidt U, Griesser H, Lieberknecht A, Tolbiersky J. A novel method for the preparation of ansapeptides; synthesis of model peptide alkaloids. *Angew Chem. Int. Ed. Engl.* 1981; **20**: 280–281.
18. Toniolo C, Bonora GM, Barone V, Bavoso A, Benedetti E, Di Blasio B, Grimaldi P, Lelj F, Pavone V, Pedone C. Conformation of pleionomers of α-aminoisobutyric acid. *Macromolecules* 1985; **18**: 895–902.
19. Nonius. *COLLECT. Kappa CCD Program Package*. Nonius BV: Delft, The Netherlands, 1998.
20. Otwinoski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* 1997; **276**: 307–326.

21. Altomare A, Burla MC, Camalli M, Cascarano GM, Giacovazzo C, Guagliardi GA, Moliterni GG, Polidori G, Spagna R. *A Program for Automatic Solution and Refinement of Crystal Structures*. University of Bari: Bari, Italy, 1997.

22. Sheldrick GM. *SHELXL 93. Program for Crystal Structure Refinement*. University of Göttingen: Göttingen, Germany, 1993.

23. Cromer DT, Waber JT. *International Tables for X-Ray Crystallography*, Table 2.2 B, vol. IV. Kynoch Press: Birmingham, England, 1974 (now distributed by D. Reidel Publishing Co.).

24. Benedetti E. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, vol. 6, Weinstein B (ed.). Dekker: New York, 1982; 105–184.

25. Benedetti E, Morelli G, Némethy G, Scheraga HA. Statistical and energetic analysis of side-chain conformations in oligopeptides. *Int. J. Pept. Protein Res.* 1983; **22**: 1–15.

26. Cambridge Structural Database, version 5.18, October 1999.

27. Zimmerman SS, Pottle MS, Némethy G, Scheraga HA. Conformational analysis of the 20 naturally occurring aminoacids residues using ECEPP. *Macromolecules* 1977; **10**: 1–9.

28. Toniolo C, Benedetti E. The polypeptide 310-helix. *Trends Biochem. Sci.* 1991; **16**: 350–353.

29. Pavone V, Benedetti E, Barone V, Di Blasio B, Lely F, Pedone C, Santini A, Crisma M, Bonora GM, Toniolo C. A conformational energy computation and x-ray diffraction study of homopeptides from 1-aminocyclohexane-1-carboxylic acid. *Macromolecules* 1998; **21**: 2064–2070.

30. Toniolo C, Benedetti E. Structures of polypeptides from α-amino acids disubstituted at the α-carbon. *Macromolecules* 1991; **24**: 4004–4009.

31. Benedetti E, Toniolo C. In *Polymeric Materials Encyclopedia*, vol. 8P, Salamone JC (ed.). CRC Press: Boca Raton, FL, 1996; 6472–6481.

32. Moretto V, Formaggio F, Crisma M, Bonora GM, Toniolo C, Benedetti E, Santini A, Saviano M, Di Blasio B, Pedone C. Preferred conformation of peptides rich in Ac8c, a medium-ring alicyclic $C^{\alpha,\alpha}$-disubstituted glycine. *J. Pept. Sci.* 1996; **2**: 14–27.

33. Toniolo C, Bonora GM, Bavoso A, Benedetti E, Di Blasio B, Pavone V, Pedone C. Preferred conformations of peptides containing α,α-disubstituted α-amino acids. *Biopolymers* 1983; **22**: 205–215.

34. Paterson Y, Rumsey SM, Benedetti E, Némethy G, Scheraga HA. Sensitivity of polypeptide conformation to geometry. Theoretical conformational analysis of oligomers of α-aminoisobutyric acid. *J. Am. Chem. Soc.* 1981; **103**: 2947–2955.

35. Cremer D, Pople JA. Definition of ring puckering coordinates. *J. Am. Chem. Soc.* 1975; **97**: 1354–1358.

36. Schweizer WB, Dunitz JD. Structural characteristics of the carboxylic ester group. *Helv. Chim. Acta* 1982; **65**: 1547–1554.

37. Ramakrishnan C, Prasad N. Study of hydrogen bonds in amino acids and peptides. *Int. J. Protein Res.* 1971; **3**: 209–231.

38. Taylor R, Kennard O, Versichel W. The geometry of the N–H⋯O=C hydrogen bond. 3. Hydrogen-bond distances and angles. *Acta Crystallogr.* 1984; **B40**: 280–288.

39. Görbitz CH. Hydrogen-bond distances and angles in the structures of amino acids and peptides. *Acta Crystallogr.* 1989; **B45**: 390–395.

40. Gademann K, Jaun B, Seebach D, Perozzo R, Scapozza L, Folkers G. Temperature-dependent NMR and CD spectra of β-peptides: on the thermal stability of β-peptide helices. Is the folding process of β-peptides non-cooperative? *Helv. Chim. Acta* 1999; **82**: 1–11.

41. Sone H, Nemoto T, Ishiwata H, Ojika M, Yamada K. Isolation, structrure and synthesis of dolastin D, a cytotoxic cyclic depsipeptide from the sea hare *Dolabella auricularia*. *Tetrahedron Lett.* 1993; **34**: 8449–8452.

42. Crews P, Manes LV, Boehler M. Jasplakinolide, a cyclodepsipeptide from the marine sponge, *japis sp.* *Tetrahedron Lett.* 1986; **27**: 2797–2800.

43. Morita H, Nagashima S, Takeya K, Itokawa H, Iitaka Y. Structure and conformation of antitumor cyclic pentapeptides astins A, B and C, from *Aster tataricus*. *Tetrahedron* 1995; **51**: 1121–1132.