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Conformational Behaviour of $C^{\alpha,\alpha}$-diphenylglycine: Folded *vs.* Extended Structures in DϕG-containing Tripeptides

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Abstract: The crystal structures of three fully protected tripeptides containing the Dϕg residue ($C^{\alpha,\alpha}$-diphenylglycine) in the central position are reported, namely Z-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**). The molecular conformations are quite unusual because the Dϕg residue adopts a folded conformation in the 3_{10}-helical region when the following residue adopts a folded conformation of opposite handedness (peptides **b** and **c**). In contrast, the Dϕg residue adopts the more frequently observed fully extended conformation when the following residue adopts a semi-extended conformation (peptide **a**). These findings are in agreement with the theoretical calculations on Ac-Dϕg-Aib-$NHCH_3$and Ac-Aib-Dϕg-$NHCH_3$ also reported in this work. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

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INTRODUCTION

The development of peptides as potential therapeutic agents represents the main goal of many scientists, working in the fields of pharmacology, medicinal chemistry and biology. With the aim of obtaining highly potent biologically active molecules, a continuous and growing interest is currently devoted to the rational design of peptide sequences capable of adopting a well-determined, three-dimensional structure [1, 2]. It has been recognized that conformational rigidity is an essential requirement (i) to increase potency and selectivity; (ii) to improve bioavailability; and (iii) to enhance the resistance to peptidases [3, 4]. Local backbone modifications and short-, medium- or long-range cyclization have been shown to restrict the conformational flexibility of peptides [1–5]. In particular, the incorporation of $C^{\alpha,\alpha}$-disubstituted amino acids into peptide sequences provides an excellent tool to constrain the backbone conformation and to induce well-defined secondary structures [6–8].

The prototypical *achiral* amino acid Aib is a strong helix-promoting residue [6–8]. The pioneering conformational energy computations of the monopeptide Ac-Aib-NHMe, performed by Marshall [9] and Burgess and Leach [10] in the early 1970s, showed that only the helical regions (of 3_{10}- or α-type) of the Ramachandran map are available to the Aib residue. The 3_{10}- ($\phi = \pm 60°$; $\psi = \pm 30°$) and the α- ($\phi = \pm 55°$; $\psi = \pm 45°$) helices are both low-energy conforma-

Abbreviations: Aib, α-aminoisobutyric acid; NHMe, methylamino; Deg, $C^{\alpha,\alpha}$-diethylglycine; Dpg, $C^{\alpha,\alpha}$-di-*n*-propylglycine; Dϕg, $C^{\alpha,\alpha}$-diphenylglycine; NHBzl, benzylamino; Z, benzyloxycarbonyl; EtOAc, ethyl acetate; ESD, estimated standard deviations.

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tions and they are significantly more stable than extended structures ($\phi \approx \psi \approx 180°$). These theoretical results were confirmed by numerous crystal structures of homo- and hetero-peptides containing Aib residues [11–15].

These studies were subsequently extended to peptides containing different $C^{\alpha,\alpha}$-disubstituted glycines, with both linear or cyclic alkyl side chains. Peptides containing 1-aminocycloalkane-1-carboxylic acids are characterized by a conformational behaviour similar to the Aib residue, because folded backbone conformations are preferred [6–8]. Residues disubstituted at the C^{α} atom with linear alkyl side chains show a different behaviour. Theoretical calculations for Deg and Dpg suggested that fully extended (C_5) conformations are energetically favoured over helical conformations [16, 17]. This theoretical finding was confirmed by early crystal structure analyses on homopeptides containing Deg and Dpg [17–21]. HCO-Met-Dpg-Phe-OMe also showed a fully extended backbone conformation at the Dpg residue [22]. More recently, Deg and Dpg residues have been found in folded conformations in both homo- and hetero-peptides [23–26].

In order to clarify the stereochemical preferences of $C^{\alpha,\alpha}$-dialkyl amino acids and to evaluate the role of the side chain bulkiness in inducing a well-defined conformation, we have undertaken a systematic structural analysis of homo- and hetero-peptides incorporating the Dϕg residue. This amino acid is the constituent of the most widely used anticonvulsant, 5,5-diphenyl-hydantoin [27–29].

Conformational analyses both in solution and in the solid state have been performed in our and in other laboratories on homo- and hetero-peptides containing the Dϕg residue [30–34]. These studies, in agreement with conformational energy computations [31–33], indicate that Dϕg prefers a fully extended conformation even in peptides containing the highly helix-inducing Aib residue or the more flexible Gly [30].

The crystal structure determination of Ac-Dϕg-NHBzl, recently reported by Toniolo *et al.* [33], provides an example of a Dϕg residue adopting a folded conformation, with ϕ,ψ torsion angles intermediate between those of the 3_{10}- and α-helices. In contrast, in $CDCl_3$ solution the fully extended conformation seems to be preferred.

We have synthesized and characterized by single crystal X-ray analysis fully protected tripeptides containing Dϕg in combination with amino acids of different conformational tendencies, namely Aib and Gly [30]. The X-ray crystal structure of Z-Aib-Dϕg-Gly-OMe, in which Dϕg adopts a fully ext ended conformation, has already been reported [30]. In this paper the crystal structures of the three peptides Z-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**) are described. The first peptide shows an extended conformation, while peptides(**b**) and (**c**) fold into type III/III′ β-turn. The results from conformational energy computations of two terminally blocked dipeptides, Ac-Dϕg-Aib-NHMe and Ac-Aib-Dϕg-NHMe, are also reported, in order to understand the forces that may stabilize folded or extended structures in Dϕg-containing peptides.

MATERIALS AND METHODS

Synthesis

The three peptides (**a**), (**b**) and (**c**) were synthesized as previously reported [30, 35]. A solution of Z-Gly-OH or Z-Aib-OH (11 mmol), diphenylmethani mine (11 mmol) and methyl isocyanoacetate or methyl-2-isocyano-2-methylpropionate (10 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 14 days. The solvent was removed under vacuum, the residue was dissolved in EtOAc and the solution was washed with 1 M HCl, 1 M $NaHCO_3$ and H_2O, and dried over Na_2SO_4. The organic solvent was evaporated and the crude product was purified by flash chromatography or open column chromatography and/or by recrystallization from EtOAc/hexane.

Z-Gly-Dϕg-Gly-OMe **(a).** Yield 31%; m.p. 148–149 °C. Found: C, 66.27%; H, 5.45%; N, 8.60%. Calcd for $C_{27}H_{27}N_3O_6$: C, 66.25%; H, 5.56%; N, 8.58%.

^{1}H-NMR ($CDCl_3$). δ (p.p.m.) = 3.69 (s, 3H, OCH_3), 3.85 (d, 2H, J = 5.4 Hz, Gly^1-CH_2), 3.99 (d, 2H, J = 5.4 Hz, Gly^3-CH_2), 5.10 (s, 2H, Z-CH_2), 5.43 (br, 1H, Gly^1-NH), 6.41 (br, 1H, Gly^3-NH), 7.27–7.44 (m, 15H, phenyl-CH), 7.89 (s, 1H, Dϕg-NH).

^{13}C-NMR ($CDCl_3$). δ (p.p.m.) = 41.9, 45.0 (Gly-αC), 52.3 (OCH_3), 67.0 (Z-CH_2), 70.5 (Dϕg-αC), 127.8, 128.0, 128.1, 128.17, 128.20, 128.23, 128.3, 128.4, 136.2, 139.1 (phenyl-C), 156.5 (Z–C=O), 167.3, 169.5, 171.8 (C′=O).

Z-Gly-Dϕg-Aib-OMe **(b).** Yield 16%; m.p. 141–142 °C. Found: C, 67.46%; H, 6.27%; N, 8.07%.

Calcd for $C_{29}H_{31}N_3O_6$: C, 67.30%; H, 6.04%; N, 8.12%.

^{1}H-NMR ($CDCl_3$): δ (p.p.m.) = 1.45 (s, 6H, Aib-βCH_3), 3.68 (s, 3H, OCH_3), 3.88 (d, 2H, J = 5.4 Hz, Gly-CH_2), 5.11 (s, 2H, Z-CH_2), 5.41 (br, 1H, Gly-NH), 6.42 (s, 1H, Aib-NH), 7.28–7.46 (m, 15H, phenyl-CH), 7.90 (s, 1H, Dϕg-NH).

^{13}C-NMR ($CDCl_3$): δ (p.p.m.) = 24.1 (Aib-βC), 44.9 (Gly-αC), 52.7 (OCH_3), 57.2 (Aib-αC), 67.0 (Z-CH_2), 70.2 (Dϕg-αC), 128.0, 128.08, 128.14, 128.3, 128.4, 128.47, 128.53, 136.2, 139.3 (phenyl-C), 156.4 (Z–C=O), 167.0, 170.5, 174.3 (C′=O).

Z-Aib-Dϕg-Aib-OMe **(c).** Yield 35%; m.p. 172–173 °C. Found: C, 68.25%; H, 6.53%; N, 7.68%. Calcd for $C_{31}H_{35}N_3O_6$: C, 68.24%; H, 6.47%; N, 7.70%.

^{1}H-NMR ($CDCl_3$): δ (p.p.m.) = 1.47 (s, 6H, Aib-βCH_3), 1.49 (s, 6H, Aib-βCH_3), 3.65 (s, 3H, OCH_3), 5.08 (s, 2H, Z-CH_2), 5.42 (s, 1H, Aib^1-NH), 6.85 (br, 1H, Aib^3-NH), 7.2–7.4 (m, 15H, phenyl-CH), 7.87 (s, 1H, Dϕg-NH).

^{13}C-NMR ($CDCl_3$): δ (p.p.m.) = 24.3, 25.1 (Aib-βC), 52.4 (OCH_3), 56.9 (Aib-αC), 57.2 (Aib-αC), 66.7 (Z-CH_2), 69.8 (Dϕg-αC), 127.8, 128.0, 128.1, 128.2, 128.5, 136.2, 140.1 (phenyl-C), 155.1 (Z–C=O), 170.3, 172.2, 174.5 (C′=O).

X-ray Diffraction

Suitable crystals for X-ray diffraction analysis were grown by slow evaporation of ethyl acetate solutions at room temperature for all the peptides. Preliminary oscillation and Weissemberg photographs taken with CuKα radiation indicated a monoclinic system, space group $P2_1/n$ for the three molecules.

Data collections were performed using a graphite monochromated CuKα radiation and a pulse-height discrimination on a CAD4 Enraf-Nonius diffractometer equipped with a MicroVax 3100 Server computer of the 'Centro di Studio di Biocristallografia del C.N.R.' at the University of Napoli. The analysis of the peak profiles suggested an ω–2θ scan mode with a scan angle $\triangle\omega = (1.0 + 0.35 \tan \theta)°$; background counts were taken on an additional area of $\triangle\omega/4°$ on both sides of the main scan for each reflection. A crystal-to-counter distance of 368 mm was used with horizontal and vertical counter entrance apertures of 4 mm and $(3.0 + 1.0 \tan \theta)$ mm, respectively. The tube placed between the goniometer head and the detector was evacuated using a vacuum pump. Pre-scan runs were made at a speed of 5°/min. Reflections with a net intensity $I \leq 0.5\ \sigma(I)$ were flagged as 'weak'; those having $I > 0.5\ \sigma(I)$ were measured at a lower speed (in the range $1 \div 5°$/min) depending on the value of $\sigma(I)/I$. Two intensity control reflections were measured every 60 min of X-ray exposure in order to monitor the crystal decay and the electronic stability of the apparatus; no significant change in their intensities was observed during the entire data collection.

4761, 5196 and 5760 total independent reflections in the range 1–70° of θ were collected forZ-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**), respectively. Reflections having a net intensity greater than $3.0\sigma(I)$ (3166, 3060 and 3531 for peptides (**a**), (**b**) and (**c**), respectively) were considered as 'observed' and were used in the subsequent calculations after correction for Lorentz and polarization effects. A summary of the crystal data of the three peptides is given in Table 1.

Structure Determination and Refinement

The three structures were solved with straightforward applications of direct methods using the SIR92 program [36]. The E-map obtained from the phase set with the best figures of merit revealed all non-hydrogen atoms. Only a few hydrogen atoms were clearly visible in the subsequent difference Fourier analysis. Thus, all the hydrogen atoms were introduced in their stereochemically expected positions, but not refined, with isotropic temperature factors equal to the equivalent B factor of the carrying atoms. The structures were refined by a full-matrix least square procedure, minimizing the quantity $\sum w[F_o - F_c]^2$, with weights w equal to $1/\sigma(F_0)^2$. All heavy atoms were refined with anisotropic temperature factors; the SDP package [37] of crystallographic programs was used. Refinements were ended when the shifts in the atomic coordinates and anisotropic temperature factors for the N, C and O atoms were less than a fifth and a third of the corresponding standard deviations, respectively. Atomic scattering factors for all atomic species were calculated according to Cromer and Waber [38]. The final conventional and weighted R factors were 0.050 and 0.048, 0.055 and 0.047, 0.051 and 0.054, for Z-Gly-Dϕg-Gly-OMe, Z-Gly-Dϕg-Aib-OMe and Z-Aib-

Table 1 Crystal Data for Z-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**)

	a	**b**	**c**
Molecular formula	$C_{27}H_{27}N_3O_6$	$C_{29}H_{31}N_3O_6$	$C_{31}H_{35}N_3O_6$
Molecular weight (a.m.u.)	489.5	517.6	545.6
Space group	$P2_1/n$	$P2_1/n$	$P2_1/n$
Z (molecules/unit cell)	4	4	4
a (Å)	12.012(1)	10.900(1)	11.081(1)
b (Å)	13.289(6)	17.446(1)	17.690(1)
c (Å)	16.464(2)	15.041(1)	16.218(1)
β (°)	107.43(9)	106.52(3)	107.06(3)
V (Å^3)	2507	2742	3039
$D_{calc.}$ (g/cm^3)	1.297	1.254	1.192
F (000)	1032	1096	1160
μ (cm^{-1})	7.252	6.881	6.433
Collected reflections	5133	5588	6168
Independent reflections	4761	5196	5760
Observed reflections	3166 ($I > 3.0\sigma(I)$)	3060 ($I > 3.0\sigma(I)$)	3531 ($I > 3.0\sigma(I)$)
Solved by	SIR92 [36]	SIR92	SIR92
Refined by	SDP [37]	SDP	SDP
S	1.008	1.001	1.172
Refined parameters	325	344	362
R (unweighted)	0.050	0.055	0.051
R (weighted)	0.048	0.047	0.054
w	$1/\sigma(Fo)^2$	$1/\sigma(Fo)^2$	$1/\sigma(Fo)^2$
Temperature	Ambient	Ambient	Ambient
Radiation (λ, Å)	CuKα 1.54178	CuKα 1.54178	CuKα 1.54178
Scan method	ω–2θ	ω–2θ	ω–2θ
θ range (°)	1–70	1–70	1–70
Crystallization solvent	Ethyl acetate	Ethyl acetate	Ethyl acetate
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.167/−0.114	0.225/−0.168	0.269/−0.181

Dϕg-Aib-OMe, respectively. Final positional parameters, equivalent thermal factors for non-hydrogen atoms, anisotropic temperature factors, bond lengths and bond angles for the three structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as a supplementary publication. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44-1223-336-033; e-mail teched@chemcryst.cam.ac.uk).

Conformational Energy Computations

The geometrical parameters of the Dϕg residue in folded and extended conformations were derived from the X-ray structures of the peptides reported in this paper and in reference [30], respectively; those of Aib were taken from literature data [16]. The standard geometries of Scheraga and co-workers [39, 40] were used for the acetamido and methylmido N- and C-blocking groups.

Conformational energy calculations were performed using the INSIGHT/DISCOVER package, with the consistent valence force field (CVFF) [41–43]. A dielectric constant of 1 was assumed in all calculations. The conformational space was mapped by calculating the conformational energy at 5° intervals for the ϕ,ψ angles. The ω angles were fixed at 180°. The terminal methyl groups were frozen into staggered conformations [44] and the ϕ and ψ angles of Aib residue fixed at 60° and 30°, respectively. Minimum-energy conformations were obtained in the low-energy regions located in the above search, minimizing the energy with respect to bond distances, bond angles and dihedral angles, using the Newton–Raphson algorithms [45]. Conformational energies are expressed as $\Delta E = E - E_0$, where E_0 is the energy of the most stable conformation. All computations were performed on a Silicon Graphics Personal Iris 4D35 GT Turbo of the Centro Interuniversitario di Ricerca su Peptidi Bioattivi at the University of Napoli.

RESULTS AND DISCUSSION

X-ray Analysis

The crystal structures of three, fully protected Dϕg-containing tripeptides, namely Z-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**) are reported here. Stereoviews of the molecular conformation of peptides (**a**), (**b**) and (**c**) are shown in Figures 1–3, respectively.

All three peptides crystallize in monoclinic space group $P2_1/n$. They lack any chiral amino acid and therefore molecules of opposite conformational parameters are both present in the centrosymmetric space group. All bond geometries are in agreement with literature data, and all the peptide bonds are *trans*. The significant backbone torsion angles are listed in Table 2 (the conformation of only those molecules with a negative ϕ_1 angle will be discussed for a consistent comparison); the torsion angles of Z-Aib-Dϕg-Gly-OMe (**a′**) are also reported for comparison.

In detail, the overall conformation of peptide (**a**) can be described, like our previous findings on Z-Aib-Dϕg-Gly-OMe [30], as a succession of three different local conformation for each amino acid residue (folded–extended–semi-extended). The Gly^1 residue adopts a folded conformation with ϕ,ψ torsion angles corresponding to the B region of the Ramachandran plot ($\phi = -83.1(4)°$, $\psi = -8.0(4)°$) [44]. The bond angle τ (N_1–C^{α}_1–C'_1) has a value of 117.5(4)°, much wider than the ideal tetrahedral value. The Dϕg residue is in the expected fully extended C_5 conformation ($\phi = -174.1(4)°$, $\psi = +173.3(4)°$) [30–33]. The $N_2 \cdots O_2$ intra-residue distance is 2.549(3) Å. The bond angle τ (N_2–C^{α}_2–C'_2) has a value of 102.8(3)°, remarkably compressed with respect to the tetrahedral value. The Gly^3 residue is in a *semi*-extended conformation, falling in the F* region of the Ramachandran map ($\phi = 82.2(4)°$, $\psi = -162.4(4)°$) [44]. The bond angle τ (N_3–C^{α}_3–C'_3) has a value of 112.0(4)°, slightly wider

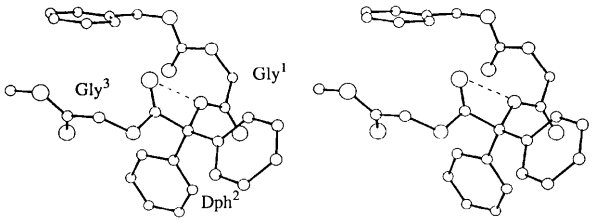

Figure 1 Stereoview of the molecular model of Z-Gly-Dϕg-Gly-OMe. Intramolecular hydrogen bonds are indicated as dashed lines.

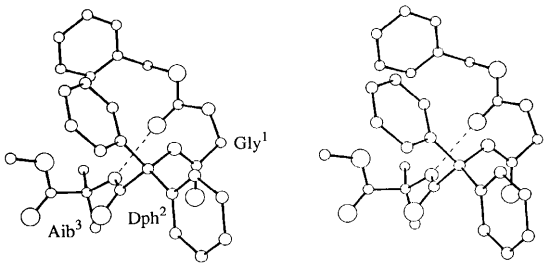

Figure 2 Stereoview of the molecular model of Z-Gly-Dϕg-Aib-OMe. Intramolecular hydrogen bonds are indicated as dashed lines.

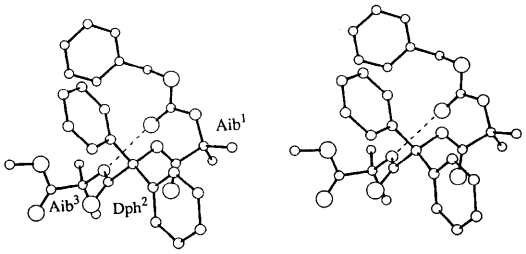

Figure 3 Stereoview of the molecular model of Z-Aib-Dϕg-Aib-OMe. Intramolecular hydrogen bonds are indicated as dashed lines.

Table 2 Torsion angles for Z-Gly-Dϕg-Gly-OMe (**a**), Z-Aib-Dϕg-Gly-OMe (**a′**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**) (ESDs are in parentheses)

	ϕ (°)	ψ (°)	ω (°)		ϕ (°)	ψ (°)	ω (°)
(a)				**(a′)**			
Gly	−83.1(4)	−8.0(4)	178.9(5)	Aib	−62.4(5)	−38.4(4)	−173.4(6)
Dϕg	−174.1(4)	173.3(4)	−177.1(5)	Dϕg	−160.9(5)	157.3(6)	−178.4(7)
Gly	82.2(4)	−162.4(4)	−177.7(5)	Gly	110.7(6)	−171.7(7)	−177.4(7)
(b)				**(c)**			
Gly	−59.1(5)	−30.4(4)	177.2(5)	Aib	−58.6(4)	−34.7(4)	−164.9(5)
Dϕg	−60.0(4)	−24.6(4)	−169.6(5)	Dϕg	−61.4(4)	−25.4(4)	−178.9(5)
Aib	48.9(4)	49.3(4)	−177.4(5)	Aib	50.4(4)	50.5(4)	−179.2(6)

than the ideal tetrahedral value. The values of the τ angles for the two Gly residues agree well with the theoretical results reported by Barone *et al.* [16]: values of the bond angle τ larger than the ideal tetrahedral value are usually found for residues that adopt folded conformations, whereas extended conformations present smaller values of the τ angle. This observation also holds true for the $C^{\alpha,\alpha}$-dialkylated glycines Aib, Deg and Dϕg [16]. The stabilization of the extended C_5 structure on decreasing the τ angle can be ascribed to a reduction in the steric repulsion between non-bonded atoms. The large deviation of the Dϕg τ angle from the ideal tetrahedral value is consistent with the observed C_5 structure and is comparable to those previously reported for the Dϕg residue in the extended conformation [30–33].

The two phenyl rings of the Dϕg residue are twisted towards each other. The angle between normals to the average planes of the two phenyl rings is 56.7(1)°. The bond angles between the two side chain phenyl ring $C_2^{\beta 1}$–C_2^{α}–$C_2^{\beta 2}$ (σ) has a value of 116.1(3)°. The side-chain $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles of the Dϕg residue, defined as reported in the literature [31], are 18.7(4)° and 73.2(4)°. Peptide (**a**) shows a *trans* N-terminal urethane bond with the Z-group conformation of type B [46].

The crystal packing viewed down the **b** axis is reported in Figure 4. The packing is stabilized by two intermolecular hydrogen bonds between the Gly^1NH and the Gly^3C′O, and between the Gly^3NH and the urethane CO group. Hydrogen bond parameters are listed in Table 3(**a**). Furthermore, several hydrophobic interactions occur between the phenyl rings that are facing each other in alternate orthogonal and parallel arrangements.

The molecular structures of peptides (**b**) and (**c**) are quite similar. They are characterized by a succession of ϕ,ψ torsion angles in the 3_{10}-helical region. It is noteworthy that the folded conformation is quite unusual for Dϕg-containing peptides, even though it was recently observed in the crystal structure of the monopeptide Ac-Dϕg-NHBzl [33].

Compound (**b**) adopts an almost ideal type III(III′) β-turn conformation with Gly^1 and Dϕg^2 as corner residues ($\phi_1 = -59.1(5)°$, $\psi_1 = -30.4(4)°$; $\phi_2 = -60.0(4)°$, $\psi_2 = -24.6(4)°$). The conformation is stabilized by a $i \leftarrow i+3$ intramolecular hydrogen bond (Aib^3 NH $\rightarrow$ urethane CO) with a N$\cdots$O distance of 3.118(3) Å. Aib^3 also adopts a conformation in the helical region but with opposite handedness ($\phi = 48.9(4)°$, $\psi = 49.3(4)°$). This reversed peptide chain folding has often been observed in crystal structures of Aib-containing oligopeptides [11–15].

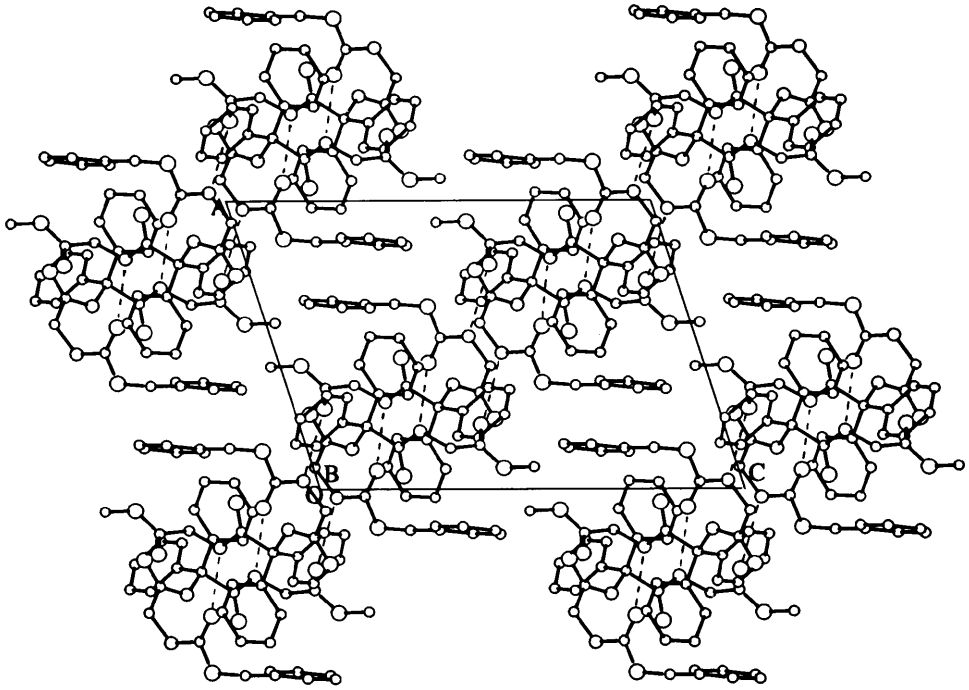

Figure 4 Molecular packing of the Z-Gly-Dϕg-Gly-OMe molecules viewed along the *b* axis. Intermolecular hydrogen bonds are indicated as dashed lines.

Table 3 Intra- and intermolecular hydrogen bonds for Z-Gly-Dϕg-Gly-OMe (**a**), Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**) (ESDs are in parentheses)

Compound	Donor D	Acceptor A	Distance (Å) D···A	Angle (°) D···A–X	Symmetry equiv.
Intramolecular					
(**a**)	N_2	O_2	2.549(3)	67.5(2)	–
(**b**)	N_3	O_0	3.118(3)	133.4(2)	–
(**c**)	N_3	O_0	3.311(3)	129.2(2)	–
Intermolecular					
(**a**)	N_3	O_0	2.945(3)	163.2(2)	$\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}-z$
(**a**)	N_1	O_3	2.999(2)	126.6(2)	$x-\frac{1}{2}, -y-\frac{1}{2}, z-\frac{1}{2}$
(**b**)	N_1	O_2	2.756(3)	161.8(2)	$x+\frac{1}{2}, \frac{3}{2}-y, z+\frac{1}{2}$
(**b**)	N_2	O_3	3.134(3)	145.7(2)	$x+\frac{1}{2}, \frac{3}{2}-y, z+\frac{1}{2}$
(**c**)	N_1	O_2	2.834(3)	173.3(2)	$\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}-z$
(**c**)	N_2	O_3	3.362(3)	149.7(2)	$\frac{1}{2}-x, \frac{1}{2}+y, \frac{1}{2}-z$

The bond angle τ has values of 116.8(5)° for Gly[1], wider than the ideal tetrahedral value and of 110.0(4)° and 108.7(4)° for Dϕg[2] and Aib[3], respectively. These latter values are close to the regular tetrahedral value, but the τ angle of the Dϕg[2] residue is remarkably different from that found in all the other structures, in which Dϕg adopts the fully extended conformation [30–33]. The two phenyl rings are oriented similarly to other Dϕg peptides and are twisted to each other, with the angle between normals to the average planes of the two phenyl rings having a value of 73.1(1)°. The bond angle between the $C_2^{\beta 1}$–C_2^{α}–$C_2^{\beta 2}$ (σ) has a value of 112.5(4)°. The side-chain $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles of the Dϕg residue are 27.8(5)° and 69.6(5)°. Peptide (**b**) shows a N-terminal *trans* urethane bond with a Z group conformation of type B [46].

Two intermolecular hydrogen bonds, listed in Table 3(**b**) and involving the Gly[1] and Dϕg[2] NHs and the Dϕg[3] and Aib[3]C′O groups of symmetry-related molecules, stabilize the crystal packing. This feature gives rise to rows of molecules, along the ***c*** direction, linked by the intermolecular hydrogen bonds. These rows pack together by several hydrophobic interactions between the phenyl rings. It is worth mentioning that the Dϕg residue in the folded conformation is able to form intermolecular hydrogen bonds, while in the fully extended conformation only intra-residues hydrogen bonds have been observed. The packing of the molecule, viewed along the [100] direction, is reported in Figure 5.

The structure of peptide (**c**) is also characterized by a type III(III′) β-turn with corner residues Aib[1] ($\phi = -58.6(4)°$, $\psi = -34.7(4)°$) and Dϕg[2] ($\phi = -61.4(4)°$, $\psi = -25.4(4)°$). The conformation is stabilized by a $i \leftarrow i+3$ intramolecular hydrogen bond, with an Aib[3] NH → urethane CO distance of 3.311(3) Å. The Aib[3] residue also adopts a helical conformation ($\phi = 50.4(4)°$, $\psi = 50.5(4)°$) with a handedness that is opposite to that of the preceding residue. The bond angle τ has values of 110.8(4)°, 109.8(4)° and 108.9(4)° for Aib[1], Dϕg[2] and Aib[3], respectively. The value of the τ angle of the Dϕg residue is similar to that found for compound (**b**),

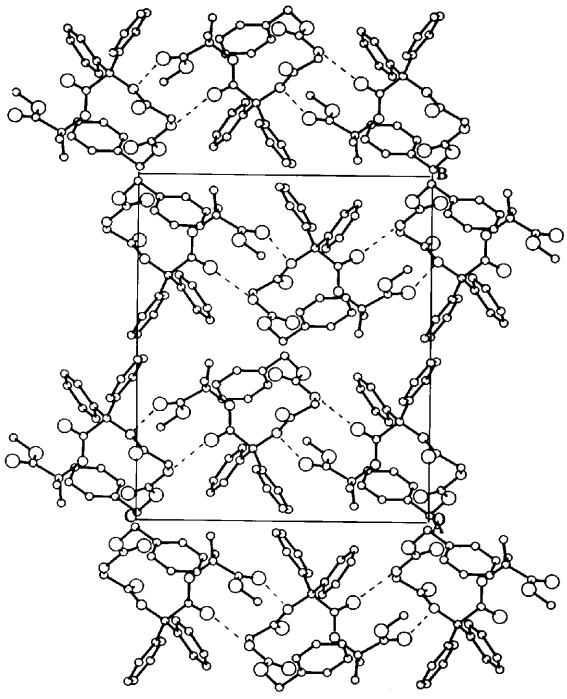

Figure 5 Molecular packing of the Z-Gly-Dϕg-Aib-OMe molecules viewed along the [100] direction. Intermolecular hydrogen bonds are indicated as dashed lines.

and very close to the ideal tetrahedral value. The angle between normals to the average planes of the two phenyl rings is 74.0(1)°. The bond angles between the $C_2^{\beta 1}$–C_2^{α}–$C_2^{\beta 2}$ (σ) has a value of 112.6(4)°. The side-chain $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles of the Dϕg residue are 27.4(5)° and 68.7(5)°.

The crystal structure is stabilized by the same pattern of intermolecular hydrogen bonds found in peptide (**b**) (see Table 3(**c**)), involving the exposed Aib[1] NH, Dϕg[2] NH and Dϕg[2] C′O, Aib[3] C′O groups. Similarly to peptide (**b**), rows of molecules along the *c* direction are formed. The crystal packing of peptide (**c**), viewed along the [100] direction, is reported in Figure 6.

Conformational Energy Computations

In order to better understand the effect on the conformational behaviour of the Dϕg residue when a bulky amino acid (Aib) is preceding or following, theoretical calculations on the two dipeptides Ac-Aib-Dϕg-NHMe and Ac-Dϕg-Aib-NHMe were carried out.

The average geometries for the energy computations were derived for the Dϕg residue in the fully extended C_5 ring structure and in the folded 3_{10}-helical conformation from an analysis of the available crystal structures (Figure 7). The bond angle at the C^{α} atom (τ) of the Dϕg residue in the folded structure was initially set to be similar to that found for the Aib residue [11–16]. In contrast, a smaller τ angle for the fully extended conformation was selected, similar to the τ angle observed for Deg and Dpg residues [17–21].

Conformational energy maps using the average geometries have been computed for the Ac-Aib-Dϕg-NHMe and Ac-Dϕg-Aib-NHMe dipeptides. Rigid rotor (ϕ,ψ) maps of these dipeptides are shown in Figures 8 and 9, respectively, when the average bond geometry in the 3_{10}-helical conformation for Dϕg residue is used. The ϕ,ψ maps do not differ considerably from the previous calculations when bond geometries of the fully extended Dϕg residue were used. For both dipeptides the conformational space is sterically restricted. In fact, when the Aib residue precedes the Dϕg, the inspection of the map shows restricted values for the ϕ angle in the range 60° and around 180°, and a larger conformational freedom for the ψ angle. In contrast, when the Aib residue follows the Dϕg, the ψ angle is sterically restricted in the range 60° and around 180°, while the ϕ angle shows a large conformational freedom.

The minimum energy conformations from the rigid rotor maps after the full minimization procedure are given in Table 4. For the Ac-Aib-Dϕg-NHMe dipeptide, the analysis reveals that, keeping the Aib

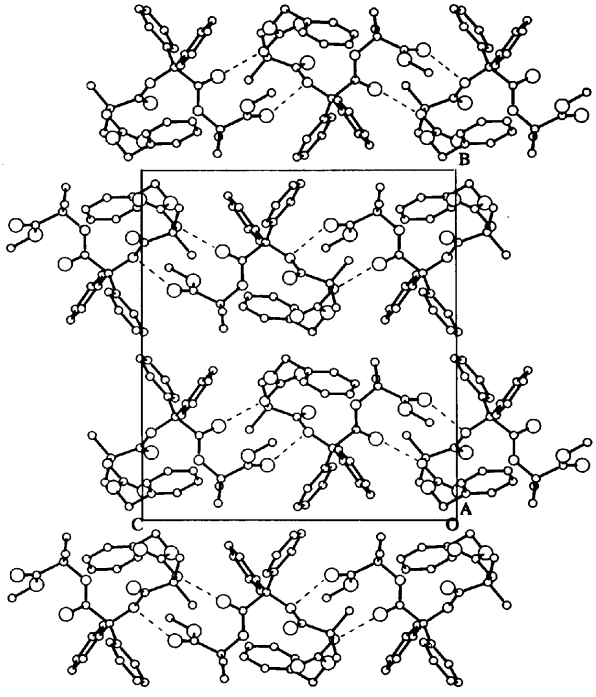

Figure 6 Molecular packing of the Z-Aib-Dϕg-Aib-OMe molecules viewed along the [100] direction. Intermolecular hydrogen bonds are indicated as dashed lines.

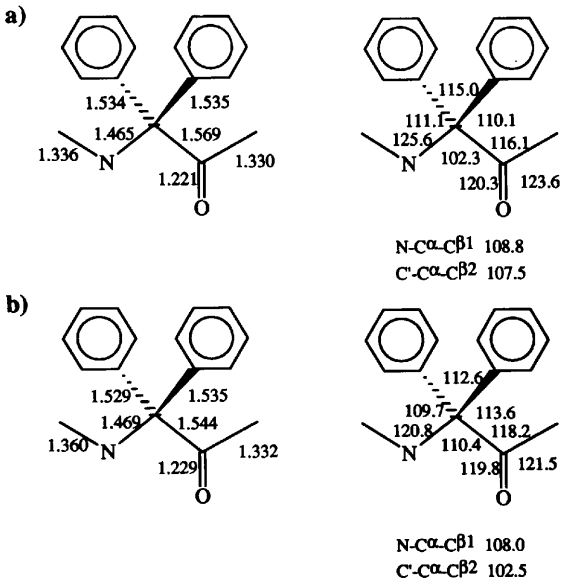

Figure 7 Average geometries of the Dϕg residue derived from crystal state analyses: (a) folded conformation and (b) fully extended conformation.

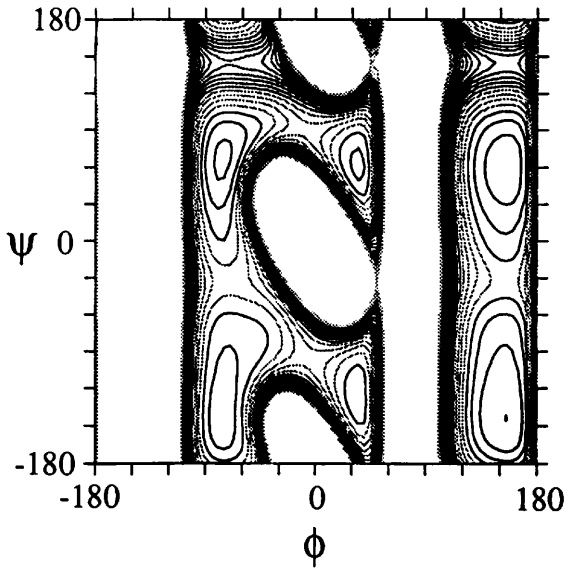

Figure 8 Rigid rotor (ϕ,ψ) maps of Ac-Aib-Dϕg-NHMe with the Aib conformation sets to $+60°$, $+30°$. Contour lines are drawn every 41.86 kJ/mol (10 kcal/mol).

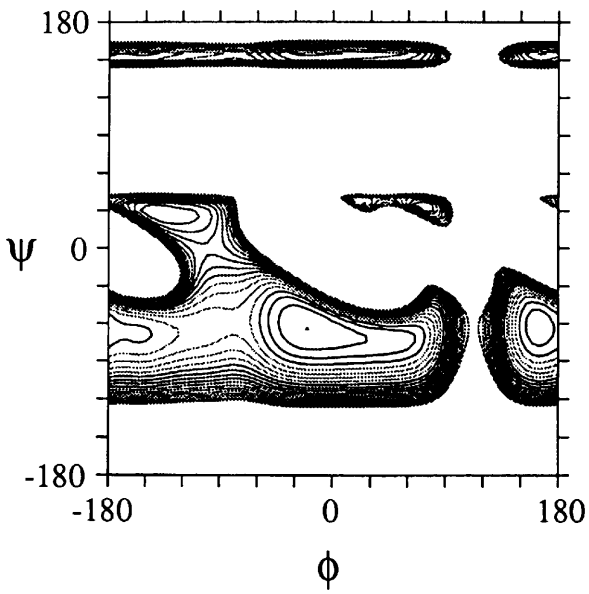

Figure 9 Rigid rotor (ϕ,ψ) maps of Ac-Dϕg-Aib-NHMe with the Aib conformation sets to $+60°$, $+30°$. Contour lines are drawn every 41.86 kJ/mol (10 kcal/mol).

residue in the left-handed helical region, the lowest minimum energy conformation corresponds to a fully extended C_5-ring structure for the Dϕg residue. However, helical and C_7 structures are not much higher in energy, so that these conformations might also be observed for the Dϕg residue. The analysis of the reversed sequence dipeptide Ac-Dϕg-Aib-NHMe reveals a different behaviour. In fact, the minimum-energy conformation corresponds to a right-handed helical conformation for the Dϕg residue. In this dipeptide, the extended conformation also results in higher energy compared with the 3_{10}-helical conformation. The analysis of minimum energy conformations also shows that in all cases the structures have similar mutual orientation of the phenyl rings, in agreement with that found in the crystal state.

The two dipeptides under investigation, Ac-Aib-Dϕg-NHMe and Ac-Dϕg-Aib-NHMe, both contain the minimum sequence able to form a β-turn stabilized by an intramolecular hydrogen bond between the acetyl CO and methylamide NH. However, the theoretical calculations on Ac-Aib-Dϕg-NHMe revealed that Aib residue in a folded conformation ($\phi = \pm 60$, $\psi = \pm 30$) is unable to force the Dϕg to a folded conformation. These findings are in agreement with the crystal structure of Z-Aib-Dϕg-Gly-OMe previously reported [30[. An unexpected result was instead obtained from the theoretical calculations on Ac-Dϕg-Aib-NHMe. The Aib residue in its

Table 4 Minimum-energy conformations for Ac-Aib-Dϕg-NHMe and Ac-Dϕg-Aib-NHMe

	Aib		Dϕg				
Compound	ϕ (°)	ψ (°)	ϕ (°)	ψ (°)	$\chi^{1,1}$ (°)	$\chi^{1,2}$ (°)	$\triangle E$ (kcal/mol)
Ac-Aib-Dϕg-NHMe	55	34	154	−160	35	51	0.0
	56	33	42	23	−23	−77	1.5
	50	24	−81	60	20	78	1.5
Ac-Dϕg-Aib-NHMe	54	32	−51	−31	23	77	0.0
	52	30	164	−44	18	−85	0.8
	50	33	50	15	−23	−77	1.8
	40	35	167	162	22	−83	2.2
	45	34	−55	146	21	81	3.1

typical folded conformation is also able to force the preceding Dϕg residue to adopt a folded conforation. More surprisingly, folded conformations of alternated handedness are the lowest energy structures. This alternated right- and left-handed conformation corresponds to the rarely observed α-pleated sheet [47, 48]. The X-ray analyses of Z-Gly-Dϕg-Aib-OMe (**b**) and Z-Aib-Dϕg-Aib-OMe (**c**) confirmed the theoretical results, because the Dϕg-Aib segment adopts an α-pleated sheet conformation. In this particular case the comparison between methyl amide and methyl ester derivatives is quite feasible. In fact, the lowest energy conformation of Ac-Dϕg-Aib-NHMe is not characterized by any intramolecular H-bond involving the NHMe group. It is reasonable to predict that the presence of the OMe group, as in peptides (**b**) and (**c**), instead of the NHMe group, as in the theoretically studied peptide Ac-Dϕg-Aib-NHMe, would further increase the energy difference between the α-pleated conformation and the β-turn structure for the lack of the H-bonding donor in the OMe derivatives.

The observation that the sequence –C(=O)-Aib-Dϕg-NH- is helical in peptide (**c**), but not in peptide (**a′**), should therefore be attributed to the nature of the residue following this particular sequence. In the former peptide an Aib residue is present and it adopts a folded conformation of opposite handedness. In the latter a Gly residue is present and it adopts a semi-extended conformation.

These observations may help to further the understanding of the general occurrence of opposite handedness at the C-terminal end of 3_{10}- and incipient 3_{10}-helices.

CONCLUSIONS

The results described demonstrate that despite the previously reported propensity of the Dϕg residue to assume the fully extended conformation, it can be incorporated into turn structures. The crystal structure of the four tripeptides containing the sequences (**a**)-Gly^1-Dϕg^2-Gly^3-, (**a′**)-Aib^1-Dϕg^2-Gly^3-, (**b**)-Gly^1-Dϕg^2-Aib^3 and (**c**)-Aib^1-Dϕg^2-Aib^3- have shown that: (i) peptides (**a**) and (**a′**) have an almost identical conformation, the superimposition of all the common atoms giving a root mean square displacement of 0.27 Å; (ii) residue 1 in peptides (**a**) and (**a′**) despite being in a folded conformation is not incorporated in a type III(III′) β-turn; (iii) residue 2 in peptides (**a**) and (**a′**) is in an extended conformation; (iv) residue 3 in peptides (**a**) and (**a′**) is in a semi-extended conformation; (v) peptides (**b**) and (**c**) have an almost identical conformation, the superimposition of all the common atoms giving a root mean square displacement of 0.23 Å; (vi) residue 1 in peptides (**b**) and (**c**) is in a folded conformation and occupies the $i+1$ position of a type III(III′) β-turn; (vii) residue 2 in peptides (**b**) and (**c**) is in a folded conformation and occupies the $i+2$ position of a type III(III′) β-turn; (viii) residue 3 in peptides (**b**) and (**c**) is also in a folded conformation, but with handedness opposite to that of the preceding residue.

Theoretical calculations on the Ac-Aib-Dϕg-NHMe and Ac-Dϕg-Aib-NHMe dipeptides have clarified the different behaviour of the Dϕg residue. In the first case the fully extended conformation is energetically more stable over the helical structure; in contrast, when the strong helix-promoting residue Aib follows Dϕg, the folded conformation becomes more favourable. The crystal structures of Dϕg containing peptides, in agreement with our theoretical calculations, indicate that both conformations are experimentally observable.

The structure versatility of this bulky amino acid represents an attractive feature in developing new peptide molecules. The Dϕg residue can be conveniently used in the *de novo* design of conformational restrained peptides and in the design of agonists or antagonists of bioactive peptides.

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