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Conformation of di-n-propylglycine residues (Dpg) in peptides: crystal structures of a type I′ β-turn forming tetrapeptide and an α-helical tetradecapeptide

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Abstract: The crystal structures of two oligopeptides containing di-n-propylglycine (Dpg) residues, Boc-Gly-Dpg-Gly-Leu-OMe (**1**) and Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe (**2**) are presented. Peptide **1** adopts a type I′ β-turn conformation with Dpg(2)–Gly(3) at the corner positions. The 14-residue peptide **2** crystallizes with two molecules in the asymmetric unit, both of which adopt α-helical conformations stabilized by 11 successive $5 \rightarrow 1$ hydrogen bonds. In addition, a single $4 \rightarrow 1$ hydrogen bond is also observed at the *N*-terminus. All five Dpg residues adopt backbone torsion angles (ϕ, ψ) in the helical region of conformational space. Evaluation of the available structural data on Dpg peptides confirm the correlation between backbone bond angle N–C^{α}–C′ (τ) and the observed backbone ϕ,ψ values. For $\tau > 106^{\circ}$, helices are observed, while fully extended structures are characterized by $\tau < 106^{\circ}$. The mean τ values for extended and folded conformations for the Dpg residue are $103.6^{\circ} \pm 1.7^{\circ}$ and $109.9^{\circ} \pm 2.6^{\circ}$, respectively. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Di-n-propylglycine; peptide helices; helical conformations; crystal structures

INTRODUCTION

The conformational characteristics of α,α-dialkylated amino acid residues when incorporated into peptide chains have been extensively investigated [1–21]. The prototype residue α-aminoisobutyric acid (Aib), which contains a pair of methyl substitutants at the C^{α} carbon atom has been shown to strongly favor local helical conformations, characterized by Ramachandran angles $\phi \approx 60^{\circ} \pm 20^{\circ}$, $\psi \approx 30^{\circ} \pm 20^{\circ}$ [22–24]. Earlier studies of the higher homologues of Aib, di-n-propylglycine (Dpg) and di-n-butylglycine (Dbg) suggested that these residues tend to favor fully extended conformations $\phi \approx 180^{\circ}$, $\psi \approx 180^{\circ}$ [11,25–30]. These conclusions were based on the crystal structures of homo-oligopeptides containing Dpg residues [25]. Subsequent crystallographic studies, however, demonstrated that Dpg and Dbg residues could also adopt helical conformations and were readily incorporated into helical polypeptide structures [31–40]. Several crystallographic results together with theoretical calculations suggested a correlation between the backbone N–C^{α}–C′ bond angle (τ) and the torsion angles about N–C^{α} (ϕ), C^{α}–C′ (ψ) bonds [25,27,38,40–43]. The demonstration of the coexistence of both helical and extended conformation of Dpg residues in the crystal structure of the tripeptide Boc-Leu-Dpg-Val-OMe [43] underscores the fact that the two distinct conformations may not differ appreciably in energy, in contrast to the case of Aib residues. Limited structural information is available on the conformations of Dpg residues when placed in different sequence contexts. We present in this article crystal structures of a tetrapeptide Boc-Gly-Dpg-Gly-Leu-OMe (**1**) and a 14-residue peptide Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe (**2**). Peptide **2** crystallizes with two molecules in the asymmetric unit, thereby providing conformational information on four independent Dpg residues. These results establish that all five Dpg residues adopt helical conformations with τ values lying between 106.6° and 111.3°.

MATERIALS AND METHODS

Synthesis of Peptides 1 and 2

Dpg was synthesized from the di-n-propyl ketone in good yield and purified by reported procedures [44]. The peptides **1** and **2** were synthesized by conventional solution phase procedures using a fragment condensation strategy as described elsewhere for related sequences [45]. The Boc group was used for the *N*-terminal protection and the *C*-terminus was protected as a methyl ester. The esterification of Dpg was effected

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Table 1 Crystal and diffraction parameters of peptides **1** and **2**

	Peptide **1**	Peptide **2**
Empirical formula	$C_{24}H_{44}N_4O_7$	$C_{78}H_{142}N_{14}O_{17}$
Crystal habit	Clear plate	Clear needle
Crystal size (mm)	1.1 × 0.2 × 0.05	0.57 × 0.17 × 0.17
Crystallizing solvent	Methanol-water	Methanol-water
Space group	$P2_12_12_1$	$P2_1$
Cell parameters		
a (Å)	8.309(2)	12.851(2)
b (Å)	12.970(3)	40.384(7)
c (Å)	28.434(5)	19.367(3)
β (deg)		93.283(3)
Volume (Å^3)	3 064(1)	10 035(3)
Z	4	4
Molecules/asymmetric unit	1	2
Molecular weight	500.63	1548.1
Density (g/cm^3)(calc.)	1.085	1.025
F(000)	1 088	3 376
Radiation (Å)	MoK$_\alpha$ ($\lambda = 0.7107$)	MoK$_\alpha$ ($\lambda = 0.7107$)
Temperature (°C)	20	20
2θ max (deg)	53.74	46.5
Scan type	ω	ω
Measured reflections	23 293	105 809
Independent reflections	6 125	28 464
Unique reflections	3 558	14 652
Observed reflections ($\|F_o\| > 4\sigma(\|F_o\|)$)	2 746	11 753
Final R (%)	6.99	12.16
w$R2$ (%)	17.09	22.95
Goodness of fit (S)	1.083	1.105
$\Delta\rho_{max}$(e Å^{-3})	0.277	0.235
$\Delta\rho_{min}$(e Å^{-3})	−0.211	−0.168
No. of restraints/parameters	0/340	73/2033
Data to parameter ratio	8.1 : 1	5.8 : 1

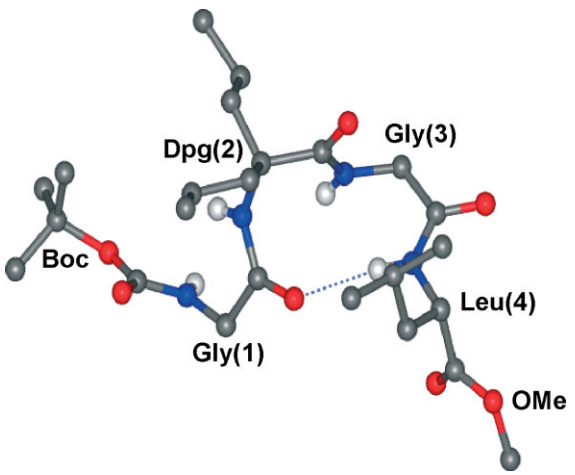

Figure 1 Molecular conformation of peptide **1** in crystals. The type I′ β-turn is stabilized by an intramolecular 4 → 1 hydrogen bond, shown as broken dotted line.

by passing dry HCl gas (until saturation) into solutions of amino acids in dry methanol, followed by storage at −10 °C for 3 days and then refluxing for 6 h. Deprotections were performed using 98% formic acid and saponification for the *N*- and *C*-terminus, respectively. Coupling reactions in the preparation of dipeptides were mediated by DCC in DCM and all other couplings were carried out in DMF in the presence of DCC and HOBt. The final step in the synthesis of peptide **1** was achieved by the fragment condensation of Boc-Gly-Dpg-OH and H_2N-Gly-Leu-OMe. Peptide **2** was synthesized by fragment condensation of Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-OH and H_2N-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe using DCC/HOBt as coupling agents. Fragment Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe was synthesized by extending the fragment Leu-Dpg-Val in both *N*- and *C*- terminal direction to overcome the problem of low coupling yields. All the intermediate peptides were characterized by ^{1}H-NMR (80 MHz) and TLC, and used without further purification. The final peptides were purified by medium-pressure liquid chromatography (MPLC) on a C_{18} column (40–60 μ) followed by HPLC (C_{18}, 5–10 μ), employing methanol–water gradients. The homogeneity of the purified peptides was ascertained by analytical HPLC. The purified peptides were characterized by ESI-MS, and 500 MHz NMR spectra. Peptide **1**: $MNa^+_{obs} = 523$ Da, Mcal = 500 Da and Peptide **2**: $MNa^+_{obs} = 1571$ Da, Mcal = 1548 Da.

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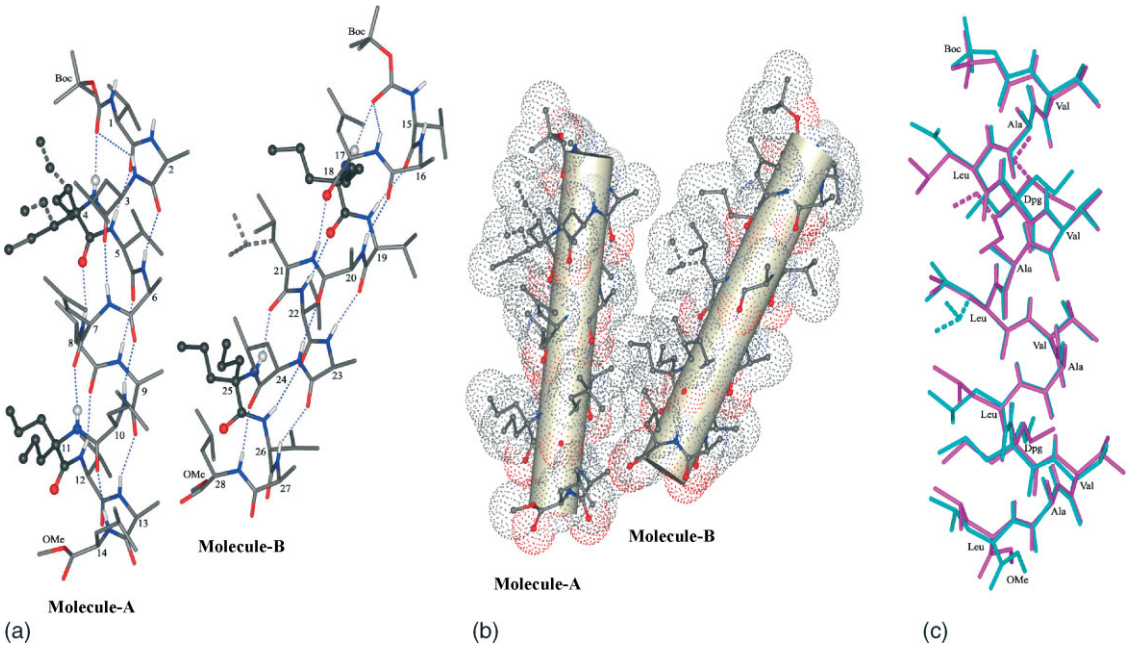

Figure 2 (a) The molecular conformation of peptide **2** in crystals showing molecules A and B in the asymmetric unit. The alternate positions of Dpg(4) in Molecule-A and Leu(7) in Molecule-B are indicated by thick broken lines. Hydrogen bonds are shown as broken lines. (b) Schematic representation of the asymmetric unit of peptide **2**. (c) Backbone superposition of the two molecules in the asymmetric unit of peptide **2** (RMSD = 0.18 Å). Molecule-A is shown in magenta while Molecule-B is shown in cyan. The figure was generated using the program MOLMOL [51].

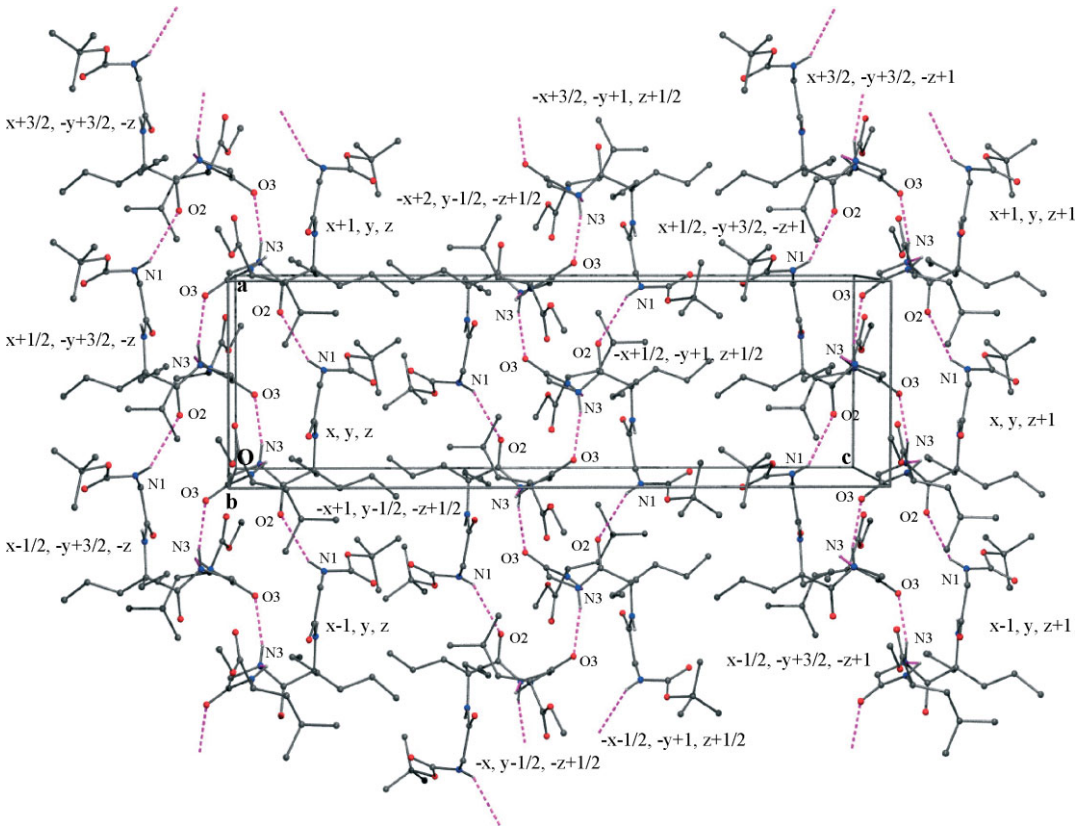

Figure 3 Packing of molecules in peptide**1** projected down the crystallographic *b*-axis. The intermolecular hydrogen bonds are indicated by dotted lines.

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Table 2 Torsion angles [a] (deg) of peptides **1** and **2**

Residue	ϕ	ψ	ω	χ^1	χ^2
Peptide **1**					
Gly(1)	−84.7[b]	5.3	172.0	—	—
Dpg(2)	62.5	20.6	−178.0	−56.9, −62.5	−178.5, 173.1
Gly(3)	74.6	13.8	−178.0	—	—
Leu(4)	−96.5	161.8[c]	179.1[d]	−62.7	−64.6, 169.3
Peptide **2**					
Molecule A					
Val(1)	−60[f]	−42	−178	−62, 171	—
Ala(2)	−61	−40	178	—	—
Leu(3)	−66	−49	−176	−175	64, −168
Dpg(4)[e]	−54	−52	−178	53 (171), 163 (−75)	−171(−174), −76(−180)
Val(5)	−65	−43	173	−62, 167	—
Ala(6)	−50	−45	180	—	—
Leu(7)	−71	−36	172	−69	−24, −174
Val(8)	−60	−44	177	−64, 171	—
Ala(9)	−58	−43	175	—	—
Leu(10)	−61	−48	−179	−84	−46, 172
Dpg(11)	−53	−45	−172	52, 179	−177, −139
Val(12)	−73	−43	−175	−66, 171	—
Ala(13)	−76	−32	−179	—	—
Leu(14)	−108	1[g]	−176[h]	−54	−61, 178
Molecule-B					
Val(1)	−70[i]	−35	−175	−71, 179	—
Ala(2)	−68	−28	179	—	—
Leu(3)	−77	−39	175	−70	6, −176
Dpg(4)	−54	−53	−176	64, 177	−176, 179
Val(5)	−64	−41	−180	−68, 171	—
Ala(6)	−62	−38	179	—	—
Leu(7)[e]	−72	−38	176	−69(179)	−65(79), 170(−175)
Val(8)	−67	−41	172	−66, 178	—
Ala(9)	−53	−50	−180	—	—
Leu(10)	−57	−47	−174	−170	60, −174
Dpg(11)	−59	−38	−178	58, 179	−166, 178
Val(12)	−83	−28	178	−68, 84	—
Ala(13)	−73	−43	−177	—	—
Leu(14)	−84	−28[j]	−176[k]	−70	−56, −180

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ and ω) and about bonds of the amino acid side chains (χ^1, χ^2) as suggested by the IUPAC-IUB Commission on Biochemical Nomenclature [50]. Estimated standard deviations ~0.5° for peptide **1** and 1.0° for peptide **2**.
[b] C0′–N1–C1A–C1′.
[c] N4–C4A–C4′–O0M.
[d] C4A–C4′–O0M–C0M.
[e] Side chain is disordered. The values in the parenthesis are for the corresponding disordered position.
[f] C02–N1–C1A–C1′.
[g] N14–C14A–C14′–O1M.
[h] C14A–C14′–O1M–C1M.
[i] C04-N15-C15A-C15′.
[j] N28–C28A–C28′–O2M.
[k] C28A–C28′–O2M–C2M.

X-ray Diffraction

Single crystals suitable for X-ray diffraction were grown from methanol–water mixture by slow evaporation for peptides **1** and **2**. X-ray diffraction data for the peptide crystals were collected at room temperature, from a dry crystal mounted on a Bruker AXS SMART APEX CCD diffractometer using MoK_α radiation. The ω scan type was used.

Structure Solution and Refinement

Peptide **1** crystallized in the orthorhombic space group $P2_12_12_1$ with one molecule in the asymmetric unit. The

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Table 3 Hydrogen-bond parameters in peptides **1** and **2**

Type	Donor (D)	Acceptor (A)	N···O (Å)	H···O (Å)	C=O···H (deg)	C=O···N (deg)	O···HN (deg)
			Peptide **1**				
Intramolecular							
4 → 1	N4	O1	3.016	2.159	124.9	128.3	167.6
Intermolecular	N1	O2[a]	3.137	2.615	129.2	130.3	120.8
Intermolecular	N3	O3[b]	2.917	2.103	121.4	117.5	164.5
			Peptide **2**				
Intramolecular							
Molecule A							
4 → 1	N3	O02	3.119	2.576	117.6	128.3	122.1
5 → 1	N4	O02	3.106	2.249	159.2	160.4	174.6
5 → 1	N5	O1	2.902	2.048	157.2	159.3	172.3
5 → 1	N6	O2	3.076	2.233	139.8	143.5	166.7
5 → 1	N7	O3	3.012	2.182	155.4	159.3	162.0
5 → 1	N8	O4	2.999	2.164	155.8	160.1	163.6
5 → 1	N9	O5	3.002	2.157	157.3	159.8	167.6
5 → 1	N10	O6	3.052	2.229	147.5	152.4	160.2
5 → 1	N11	O7	2.979	2.138	148.4	151.9	165.8
5 → 1	N12	O8	3.070	2.281	150.6	156.7	152.7
5 → 1	N13	O9	3.004	2.190	148.9	154.7	157.8
5 → 1	N14	O10	2.871	2.071	153.8	159.3	154.4
Molecule-B							
4 → 1	N17	O04	3.254	2.603	110.2	119.0	133.4
5 → 1	N18	O04	3.317	2.469	150.5	153.3	169.0
5 → 1	N19	O15	2.971	2.117	158.7	160.6	172.0
5 → 1	N20	O16	3.067	2.257	138.6	144.6	157.2
5 → 1	N21	O17	3.042	2.227	151.3	156.7	158.2
5 → 1	N22	O18	3.132	2.302	151.4	156.0	162.1
5 → 1	N23	O19	2.924	2.072	156.3	158.8	170.8
5 → 1	N24	O20	2.981	2.152	145.4	150.3	161.9
5 → 1	N25	O21	3.031	2.221	144.7	150.6	156.8
5 → 1	N26	O22	3.284	2.497	146.8	151.7	152.4
5 → 1	N27	O23	3.085	2.307	149.2	156.9	150.5
5 → 1	N28	O24	3.010	2.168	157.4	160.0	166.0
Intermolecular							
Molecule-A	N1	O13[c]	2.880	2.027	108.0	107.4	171.2
Molecule-A	N2	O12[c]	2.867	2.101	141.0	146.6	148.0
Molecule-B	N15	O27[d]	2.908	2.049	120.9	120.5	177.0
Molecule-B	N16	O26[d]	2.922	2.106	166.3	165.9	158.0

[a] Symmetry related by (x + 1, y, z).
[b] Symmetry related by (x + 1/2, −y + 3/2, −z).
[c] Symmetry related by (−x + 1, y + 1/2, −z + 1).
[d] Symmetry related by (−x, y + 1/2, −z). Estimated standard deviations in the hydrogen- bond lengths and angles are ~0.005 Å and 0.3° for peptide **1** and 0.024 Å and 1.0° for peptide **2**.

structure was determined by direct phase determination using the program SHELXS-97 [46]. Refinement was carried out against F^2 with full-matrix least squares methods by using SHELXL-97 [47]. At the end of isotropic refinement, the *R*-factor was 19.72% and dropped to 14.90% after the anisotropic refinement. Hydrogen atoms bonded to N1 (Gly); N3, C3A (Gly); N4, C4A (Leu) were located from the difference Fourier maps. The remaining hydrogen atoms, which could not be located, were fixed geometrically in the idealized positions and refined in the final cycle as riding over the heavier atom to which they are bonded. The final *R*-factor was 6.99% (wR_2 = 17.09%) for 2746 observed reflections. Peptide **2** crystalized in the monoclinic space group $P2_1$ with two molecules in the asymmetric unit. The asymmetric unit in the crystal structure of **2** contains 218 nonhydrogen atoms. Attempts to solve the structure by conventional direct method technique using phase annealing [48] failed to yield any fragments. The structure was solved by iterative dual space direct methods using SHELXD [49]. Two fragments were obtained containing 84 and 89 atoms. The remaining

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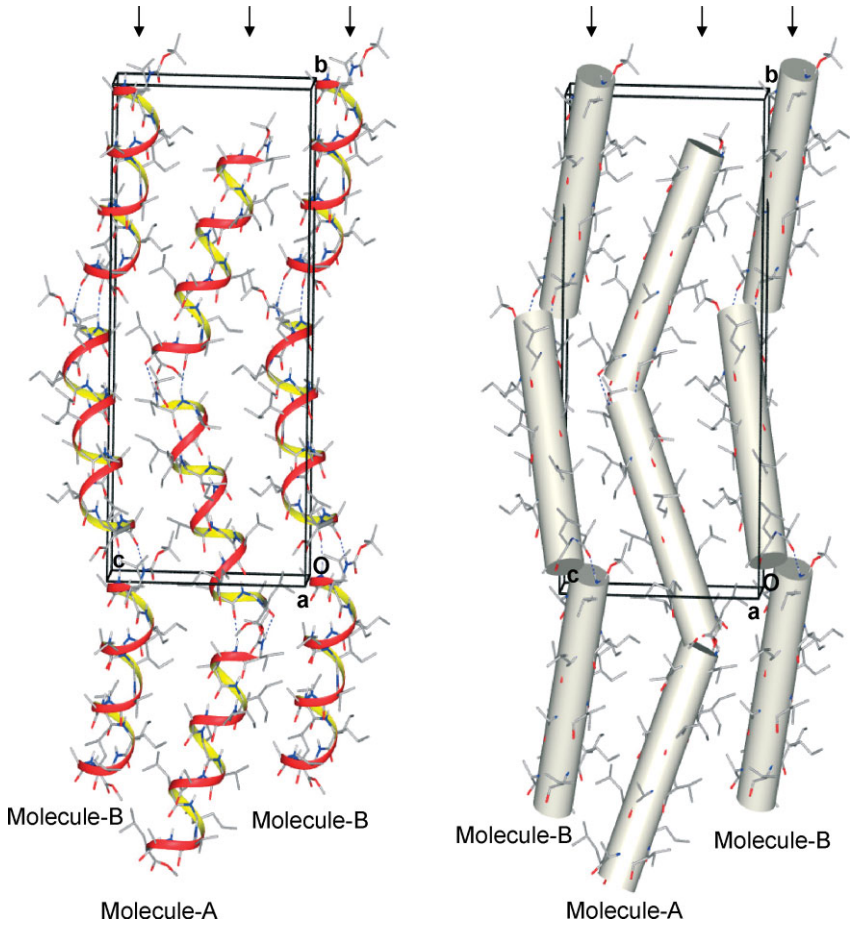

Figure 4 Packing of molecules (peptide **2**) in crystals. Head-to-tail NH···O hydrogen bonds stabilize the columns of helices, which are shown by dotted lines. The arrows indicate the direction of the helix axes and mode of aggregation.

atoms were located from difference Fourier maps after several cycles of refinement. Refinement was carried out with a blocked full-matrix least squares method against F^2 by using SHELXL-97 [47]. The entire asymmetric unit was divided into two blocks with one molecule forming each block. All nonhydrogen atoms were initially refined isotropically. Positional disorder was observed in the CG and CD atoms of Dpg(4) in molecule-A and Leu(7) of molecule-B in the asymmetric unit. The thermal parameters of these atoms were refined isotropically till the final cycle of refinement and were refined anisotropically in the final cycle. Seven atoms showed 'nonpositive definite' temperature factor during the course of refinement. Hence these atoms were refined anisotropically with the U_{ij} components restrained to approximate isotropic behavior (ISOR) [48] in the final cycle of refinement. The hydrogen atoms were fixed geometrically in the idealized positions and refined in the final cycle as riding over the heavier atom to which they are bonded. The final *R*-factor was 12.16% ($wR_2 = 22.95\%$) for 11 753 observed reflections. The relevant crystallographic data collection parameters and structure refinement details for peptides **1** and **2** are summarized in Table 1. Crystallographic data (excluding structure factors) for peptides **1** and **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-656859 and CCDC-656860, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or E-mail: deposit@ccdc.cam.ac.uk).

RESULTS

Molecular conformations determined in crystals of the tetrapeptide **1** and the 14-residue peptide **2** are shown in Figures 1 and 2, respectively. The backbone and side- chain torsion angles are listed in Table 2, and the intra and intermolecular hydrogen-bond parameters are listed in Table 3.

Boc-Gly-Dpg-Gly-Leu-OMe (1)

The tetrapeptide adopts a type I′ β-turn conformation with Dpg(2) and Gly(3) occupying the i + 1 and i + 2 positions. The reverse turn structure is stabilized by an intramolecular 4 → 1 hydrogen bond involving the Gly(1) CO and Leu(4) NH groups. The Dpg(2) residue adopts a left-handed helical (α_L) conformation.

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Table 4 Conformations of Gly-Dpg-Gly segments in peptides

No	Sequence	Gly		Dpg		Gly		References
		ϕ	ψ	ϕ	ψ	ϕ	ψ	
1.	Boc-Gly-Dpg-Gly-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe							
	Molecule-A	−94	−162	−53	−50	−64	−36	35
	Molecule-B	−96	−153	−56	−47	−65	−40	
2.	Boc-Gly-Dpg-Gly-OH	−72	−32	178	171	−63	—	37
3.	Boc-Val-Ala-Leu-Gly-Dpg-Gly-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe	−66	−51	−52	−44	−63	−34	37
4.	Boc-Gly-Dpg-Gly-Gly-Dpg-Gly-NHMe	72	−166	−54	−46	−78	−9	38
		−80	−18	56	32	85	−3	—
5.	Boc-Gly-Dpg-Gly-Leu-OMe	−84.7	5.3	62.5	20.6	74.6	13.8	Present study

Peptide molecules in the crystal associate via two intermolecular hydrogen bonds between symmetry-related molecules (Figure 3). The Dpg(2) NH does not participate in any intermolecular hydrogen bond formation. Table 4 compares the conformations of Gly-Dpg-Gly segments observed in crystal structures of diverse model peptides. With the exception of tripeptide acid Boc-Gly-Dpg-Gly-OH, in all other cases the Dpg residue adopts an α_R or α_L conformation, with Dpg-Gly unit adopting type I or I′ β-turn structures. The conformation of the glycine residue preceding the Dpg is somewhat more variable. Interestingly, in the case of the tetrapeptide Boc-Gly-Dpg-Gly-Leu-OMe (**1**) established in the present study, Gly(1) adopts an α_R conformation while Gly(3) adopts an α_L conformation. Switching the Gly(1) conformation to the α_L region would have resulted in a structure with two intramolecular hydrogen bonds. In crystals, the absence of this additional intramolecular interaction is undoubtedly compensated by the formation of an intermolecular hydrogen bond.

Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe (2)

The 14-residue peptide exhibits a right-handed helical conformation over the entire length of the peptide in both the molecules in the asymmetric unit (Figure 2). Inspection of the hydrogen bond pattern reveals an α-helix stabilized by 11 successive $5 \rightarrow 1$ hydrogen bonds in both the molecules. At the *N*-terminus an additional $4 \rightarrow 1$ interaction between the Boc CO and Leu(3) NH groups is also observed. The Boc CO group participates in a bifurcated hydrogen-bond interaction of the $4 \rightarrow 1$ and $5 \rightarrow 1$ types in both the molecules. The Dpg(4) and Dpg(11) residues adopt a right-handed helical (α_R) conformations in all the cases. In the asymmetric unit the two peptide helices lie at an angle of 20.4° (Figure 2(b)). Figure 2(c) shows a superposition of the two molecules in the asymmetric unit of peptide **2** (RMSD = 0.18 Å), establishing that both the molecules have very similar backbone conformations. A large deviation is observed at the side chains of Leu(3) and Leu(10). The molecules pack into the crystals as columns of helices, approximately parallel to each other, held together in each column by two intermolecular head-to-tail N–H···O hydrogen bonds (Figure 4, Table 3). Helical conformations observed in peptide **2** are remarkably similar to those established in the related 15-residue peptide sequence Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe, which contains Aib residues at position 4, 11 and 15 [52]. Figure 5 shows the superposition of the structures of the Aib analog and the Dpg containing peptide helices determined in the present study. Clearly, the observed structures are remarkably similar.

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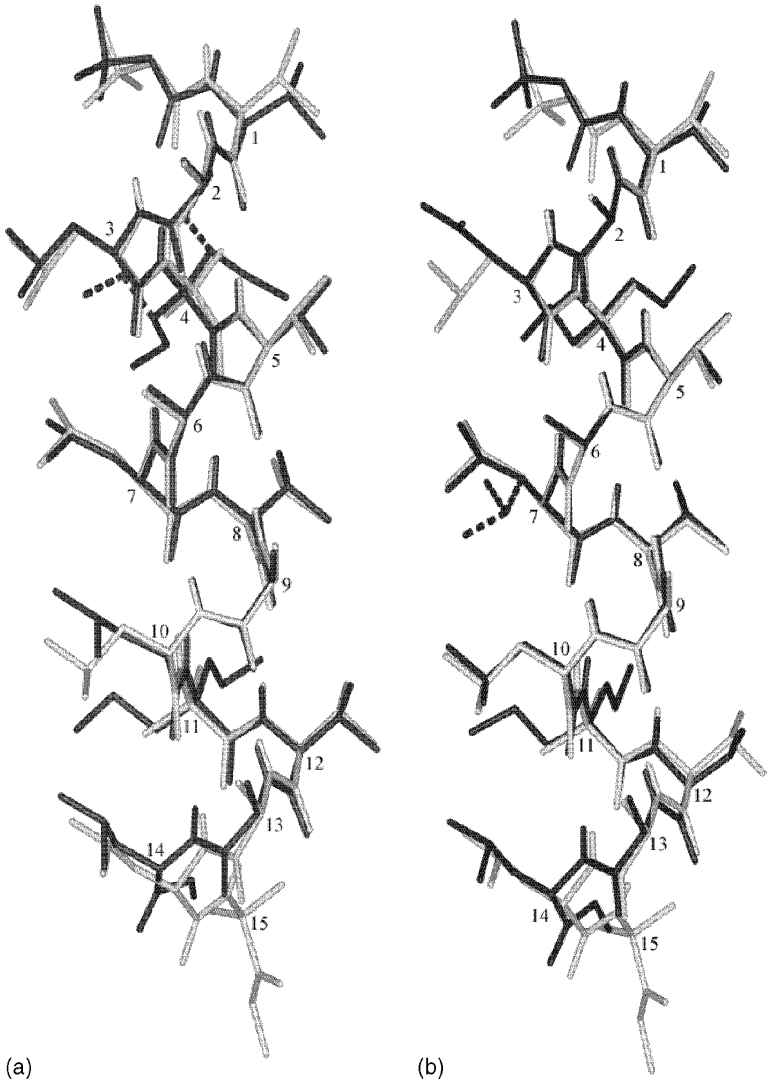

Figure 5 (a) Superposition of peptide **2** of Molecule-A (black) with VALU15 (gray) (Backbone atom of residues 2–13 are used, RMSD = 0.21 Å) (b) Superposition of peptide **2** of Molecule-B (black) with VALU15 (gray) (Backbone atom of residues 2–13 are used, RMSD = 0.19 Å). VALU15: Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe.

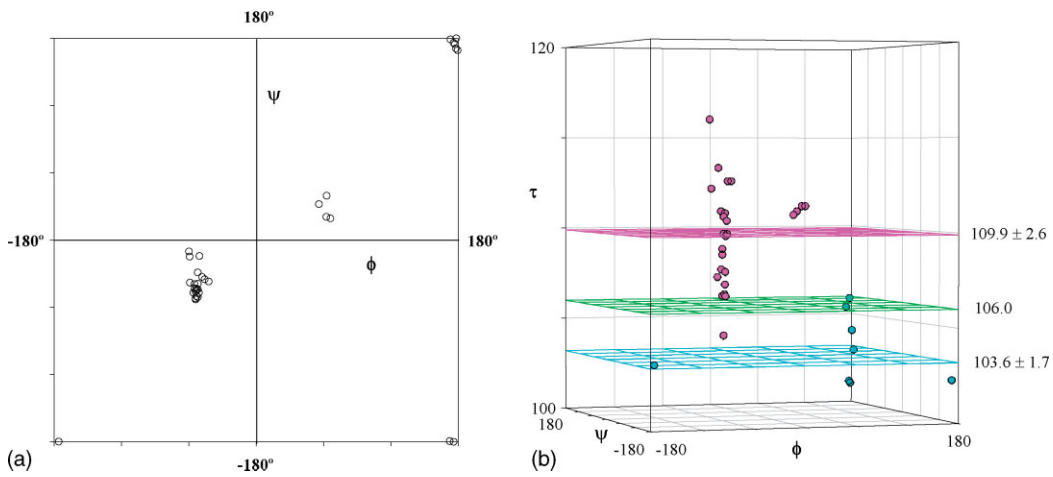

Figure 6 (a) Distribution of torsion angles for Dpg residues in peptides. 35 Dpg residues from 22 peptide sequences. (b) Three-dimensional ϕ-ψ-τ plot shows the correlation between bond angle N–C^{α}—C' (τ) and backbone torsion angles ϕ,ψ in extended and folded conformations. The τ value 106° may be taken as the limiting value for extended and folded conformations.

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Table 5 Dpg residue conformation in peptides

No	Peptides/CCDC code	ϕ	ψ	τ	$\chi^{1,1}$	$\chi^{2,1}$	$\chi^{1,2}$	$\chi^{2,2}$	References
1.	Tfa-Dpg-DBH. (cughob)								
	Molecule-A	176.0	175.9	101.2	58.1	−167.0	−55.9	−173.1	25
	Molecule-B	177.0	174.8	101.1	51.3	−173.8	−53.5	−174.5	
2.	Tfa-$(Dpg)_2$-DBH. (cughiv)	179.5	169.4	103.0	59.3	179.5	−31.6	156.4	25
		172.5	−179.4	102.3	55.0	−165.6	−58.0	173.7	
3.	mClAc-Dpg-OH. (dovxan)	178.3	180.0	105.9	57.7	173.4	−59.0	180.0	26
4.	N-formyl-Met-Dpg-Phe-OMe.	173.1	179.0	105.4	−62.3	176.8	53.2	175.4	29
5.	Tfa-$(Dpg)_3$-DBH. (vovzip)	−54.9	−39.3	108.0	57.6	−172.8	−177.9	−173.9	31
		−59.9	−10.2	116.0	47.7	−166.1	71.4	−176.9	
		−48.3	−32.9	111.0	61.4	−174.5	173.6	−171.8	
6.[a]	Boc-Leu-Dpg-Val-OMe. (cahpad)								
	Molecule-A	176.0	−180.0	102.0	59.8	−179.4	−56.7	−173.3	43
	Molecule-B	62.8	39.6	111.0	−60.4	−175.4	179.5	−174.6	
7.	Boc-Aib-Ala-Leu-Ala-Leu-Dpg-Leu-Ala-Leu-Aib-OMe. (yetpao)	−56.0	−46.9	108.8	60.5	−163.0	177.5	−169.8	32
8.	Boc-Leu-Dpg-Leu-Ala-Leu-Aib-OMe.								
	Molecule-A (triclinic) (yetpes)	−54.8	−43.2	106.6	66.7	−177.5	−178.5	−172.0	32
	Molecule-B (triclinic)	−53.4	−43.4	104.5	65.8	−171.4	176.3	−172.8	
	Molecule-A (orthorhombic) (yetpiw)	−42.4	−37.0	112.7	54.9	−173.2	69.3	160.5	
9.	Boc-Gly-Dpg-Leu-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe. (zevbad)	−51.0	−47.0	110.6	62.2	−158.2	−179.4	−173.8	33
10.	Boc-Gly-Dpg-Pro-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe. (zevbeh)	−52.0	−51.0	109.9	62.9	−155.3	177.8	−172.7	33
11.	Boc-Gly-Dpg-Ala-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe. (zevbil)	−46.0	−35.0	112.7	53.6	−178.8	−178.9	−172.3	33
12.[a]	Boc-Ala-Dpg-Ala-OMe.	66.2	19.3	111.3	−69.8	169.1	−59.5	179.2	34
13.	Boc-Ala-Dpg-Ala-NHMe.								
	Molecule-A	−50.6	−14.2	113.4	−67.9	−165.4	58.9	−175.3	34
	Molecule-B	−59.3	−14.8	112.3	42.2	173.9	61.2	−179.4	
14.	Boc-Gly-Dpg-Gly-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe.								
	Molecule-A (nicpaq)	−52.9	−49.5	109.8	63.1	−153.3	177.6	−175.6	35
	Molecule-B	−56.2	−47.1	109.1	57.4	−142.3	176.2	−179.4	
15.	Boc-Gly-Dpg-Gly-OH. (neznov)	178.0	171.0	104.1	−57.0	−170.0	56.3	−170.4	37
16.	Boc-Val-Val-Ala-Leu-Gly-Dpg-Gly-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe. (neznub)	−52.0	−44.0	107.2	−69.3	177.7	169.6	−178.3	37
17.[a]	Boc-Gly-Dpg-Gly-Gly-Dpg-Gly-NHMe. (nuwrec)	−54.3	−46.1	109.9	62.9	−170.3	176.9	177.8	38
		56.0	32.3	110.8	−55.7	176.6	178.9	−179.2	
18.	Ac-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe. (maqwoq)	−52.0	−39.0	110.8	61.4	−172.8	173.0	163.7	39
19.[b]	Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe. (maqwuw)	−52.0	−29.0	111.1	—	—	—	—	39

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Table 5 (*Continued*)

No	Peptides/CCDC code	ϕ	ψ	τ	$\chi^{1.1}$	$\chi^{2.1}$	$\chi^{1.2}$	$\chi^{2.2}$	References
20.	Boc-Val-Dpg-Val-OMe. (woqzil)	−176.0	−179.9	103.4	−55.1	−179.0	63.3	−166.2	40
21.[a]	Boc-Gly-Dpg-Gly-Leu-OMe	62.5	20.6	111.3	−56.9	−178.5	−62.5	173.1	Present study
22.[b]	Boc-Val-Ala-Leu-Dpg-Val-Ala-Leu-Val-Ala-Leu-Dpg-Val-Ala-Leu-OMe.								
	Molecule-A	−54.0	−52.0	107.9	—	—	—	—	Present study
		−53.0	−45.0	106.7	52	−177	179	−139	
	Molecule-B	−54.0	−53.0	106.6	64	−176	177	179	
		−59.0	−38.0	107.6	58	−166	179	178	

τ is the bond angle N–C^{α}—C′.
[a] Dpg residue adopts positive helical ϕ,ψ values.
[b] Disordered Dpg side chain values are not included.

DISCUSSION

All five Dpg residues crystalographically characterized in this study adopt helical conformations (α_R or α_L). Table 5 list the backbone conformational angles and the N–C^{α}—C′ bond angle (τ) observed at the Dpg residues in crystal structures deposited in Cambridge Structural Database [53]. Figure 6 provides a cluster plot relating the distribution in ϕ,ψ space with the bond angle (τ) at the C^{α} carbon atom. There are significantly more example of Dpg residues lying in the helical region of ϕ,ψ space. This observation may, of course, reflect the influence of the nature of the sequences studied thus far, where isolated Dpg residues often have been placed in the context of strongly helix-promoting sequences. There appears to be a clear transition in backbone conformation (ϕ,ψ) as a function of the bond angle (τ). Helices require a bond angle $\tau > 106^{\circ}$, while the fully extended conformations are characterized by bond angles between 101° and 106°. The mean τ values for extended and folded conformations for the Dpg residue are $103.6^{\circ} \pm 1.7^{\circ}$ and $109.9^{\circ} \pm 2.6^{\circ}$, respectively. These results have been rationalized by theoretical studies of Dpg residues [27,41,42].

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