Structural studies of model peptides containing β-, γ- and δ-amino acids†

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The crystal structures of five model peptides Piv-Pro-Gly-NHMe (**1**), Piv-Pro-βGly-NHMe (**2**), Piv-Pro-βGly-OMe (**3**), Piv-Pro-δAva-OMe (**4**) and Boc-Pro-γAbu-OH (**5**) are described (Piv: pivaloyl; NHMe: *N*-methylamide; βGly: β-glycine; OMe: *O*-methyl ester; δAva: δ-aminovaleric acid; γAbu: γ-aminobutyric acid). A comparison of the structures of peptides **1** and **2** illustrates the dramatic consequences upon backbone homologation in short sequences. **1** adopts a type II β-turn conformation in the solid state, while in **2**, the molecule adopts an open conformation with the β-residue being fully extended. Piv-Pro-βGly-OMe (**3**), which differs from **2** by replacement of the C-terminal NH group by an O-atom, adopts an almost identical molecular conformation and packing arrangement in the solid state. In peptide **4**, the observed conformation resembles that determined for **2** and **3**, with the δAva residue being fully extended. In peptide **5**, the molecule undergoes a chain reversal, revealing a β-turn mimetic structure stabilized by a C–H $\cdots$ O hydrogen bond.

Introduction

Recent interest in the conformational properties of oligopeptides formed by β-amino acids and higher omega amino acid analogues has been stimulated by the recognition that new classes of folded structures can be formed by homooligomers of backbone homologated amino acids.[1–2] Hybrid sequences containing α- and ω-amino acids are of special interest in the rational design of secondary structures generated by insertion of additional atoms into polypeptide backbones.[3–7] As part of a systematic investigation, we have examined the effects of backbone homologation on the structures of simple proline containing peptides. β-turn formation is favorable in Pro–X sequences, because the two stable states for Pro residues, right handed helical, α_R ($\phi = -60°$, $\psi = -30°$) and polyproline II, P_{II} ($\phi = -60°$, $\psi = 120°$), are the conformations necessary at the $i + 1$ position of type I/III and type II β-turns, respectively.[8] In an earlier study, we have reported the characterization of the type II β-turn conformation in the model peptide Piv-Pro-Gly-NHMe (**1**) (Piv: pivaloyl; NHMe: *N*-methylamide), determined *ab initio* from powder diffraction data.[9] In this report, we describe the structures of **1** and its backbone homologue Piv-Pro-βGly-NHMe (**2**) (βGly: β-glycine) determined by single crystal X-ray diffraction. The choice of the pivaloyl blocking group for proline was based on earlier studies, which establish that the use of a bulky N-terminus protecting group restricts the amide bond preceding proline to the *trans* conformation.[10] A brief description of the crystal structure of Piv-Pro-βGly-NHMe appeared as early as 1989.[11] However, only backbone dihedral angles were reported and no coordinates are available in the Cambridge Structural Database (note that in ref. 11 the βGly residue is referred to as βAla, which was the originally used nomenclature. Subsequent to the rapid growth of the field of β-peptides, the term βHGly has been suggested,[1] simplified here as βGly). The structures of **1** and **2** are dramatically different. The structures of Piv-Pro-βGly-OMe (**3**) and Piv-Pro-δAva-OMe (**4**) (δAva: δ-aminovaleric acid; OMe: *O*-methyl ester) are shown to be remarkably similar to the extended conformation, characterized for Piv-Pro-βGly-NHMe (**2**). Clearly, β-turn disruption occurs upon insertion of the additional methylene group (–CH_2–) of βGly and the three –CH_2– groups of δAva into the polypeptide backbone. The structure of Boc-Pro-γAbu-OH (**5**) (γAbu: γ-aminobutyric acid) is also described. Here, the observed C–H $\cdots$ O hydrogen bond stabilized the β-turn domain, closely resembling that established earlier in Piv-Pro-γAbu-NHMe (**6**), determined by powder X-ray diffraction.[12]

Results and discussion

Peptide conformations in crystals

The conformations characterized for peptides **1–5** in crystals are shown in Fig. 1. The backbone torsion angles are summarized in Table 1. Table 2 lists the observed intra- and intermolecular hydrogen bond parameters. The structure observed for Piv-Pro-Gly-NHMe (**1**) is an almost ideal type II β-turn stabilized by a $4 \rightarrow 1$ hydrogen bond between the C=O of the Piv group and NH of the methylamide group (N3 $\cdots$ O0 = 2.962 Å; H $\cdots$ O0 = 2.266 Å; $\angle$NH $\cdots$ O = 154.9°). The RMSD obtained upon superposing the non-hydrogen atoms in the structures determined by powder diffraction[9] and single crystal (present work) methods is 0.09 Å. The near identity of the structures obtained using different datasets, powder and single crystal, is gratifying. The structure of peptide **2** provides insights into the effect of insertion of atoms into a folded peptide backbone, revealing disruption of

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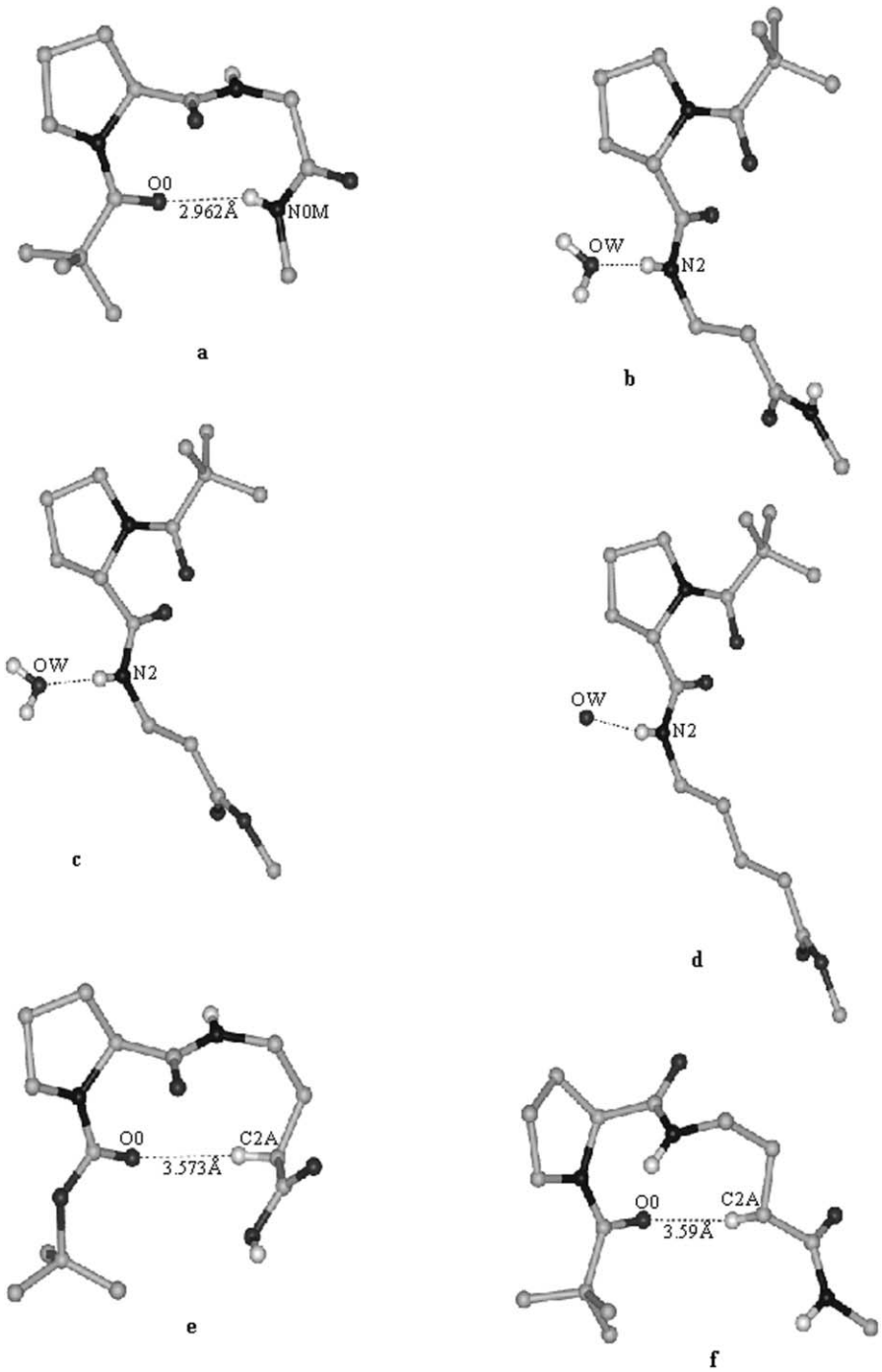

Fig. 1 Molecular conformations of a) Piv-Pro-Gly-NHMe (**1**), b) Piv-Pro-βGly-NHMe (**2**), c) Piv-Pro-βGly-OMe (**3**), d) Piv-Pro-δAva-OMe (**4**), e) Boc-Pro-γAbu-OH (**5**), f) Piv-Pro-γAbu-NHMe[12] (**6**) in crystals.

the β-turn conformation and loss of the intramolecular 4 → 1 hydrogen bond. The backbone torsion angles correspond closely to those determined in an earlier study.[11] While the Pro residue conformation is retained P_{II} (polyproline), the βGly residue adopts an extended geometry of $\theta \sim -179.4°$, very close to the ideal *trans* conformation of the C^{β}–C^{α} bond. The structure of Piv-Pro-βGly-OMe (**3**) is remarkably similar to that of (**2**), suggesting that the hydrogen bond involving the C-terminal NH group may not have a predominant influence in determining the molecular conformation and crystal packing. Interestingly, the Pro residue in Piv-Pro-δAva-OMe (**4**) also adopts the P_{II} conformation. Inspection of the structures shown in Fig. 1b, c and d reveals that there is a gross overall similarity between peptides **2**, **3** and **4**. The δAva residue adopts an all *trans* conformation about the C^{δ}–C^{γ}, C^{γ}–C^{β} and C^{β}–C^{α} bonds. The structure of peptide **5**, Boc-Pro-γAbu-OH reveals a folded conformation stabilized by a C–H · · · O hydrogen bond involving one of the α-methylene hydrogen atoms of the γAbu residue and the C=O group of Boc. A similar reverse turn has been observed in the structure of Piv-Pro-γAbu-NHMe determined from powder X-ray diffraction data (Fig. 1f).[12] Fig. 2

Table 1 Torsion angles (deg)[a] for peptides **1–5**

	Peptide	Residue	ϕ	θ_1	θ_2	θ_3	ψ	ω
	1	Pro	−59.5	—	—	—	133.5	−178.7
		Gly	72.3	—	—	—	13.4	−178.4
	2	Pro	−53.4	—	—	—	140.5	−175.8
		βGly	87.3	−179.4	—	—	−158.3	−179.0
	3	Pro	−54.1	—	—	—	144.9	178.3
		βGly	99.6	179.0			−164.1	−178.1
	4	Pro	−54.9	—	—	—	142.9	177.3
		δAva	94.2	−179.9	179.3	175.7	−147.4	−178.8
	5	Pro	−56.0	—	—	—	141.5	180.0
		γAbu	100.4	−60.8	−68.9	—	169.1	—

[a] For α-residue nomenclature see ref. 8c and for ω-residue nomenclature see ref. 7a.

Table 2 Hydrogen bond parameters in peptides **1–5**[a]

Peptide	Type	Donor (D)	Acceptor (A)	D···A (Å)	H···A (Å)	C=O···H (deg)	C=O···D (deg)	D–H···A (deg)
1	Intramolecular 4 → 1	N0M	O0	2.962	2.266	136.4	133.7	154.9
	Intermolecular	N2	O2[b]	2.938	2.081	127.6	128.9	174.6
2	Intermolecular	N2	OW	2.874	2.014			178.7
		N0M	O1[b]	3.088	2.185	126.7	126.9	171.0
		C1A	OW	3.472	2.641			140.5
		OW	O0[c]	2.841	1.854	144.0	143.3	177.1
		OW	O1[d]	2.816	1.971	130.6	132.5	172.9
3	Intermolecular	N2	OW	2.877	2.042			174.8
		C1A	OW	3.414	2.658			133.5
		OW	O1[e]	2.770	1.954	134.5	136.7	171.0
		OW	O0[f]	2.812	1.971	145.6	144.0	174.1
4	Intermolecular	N2	OW	2.890	2.041			169.0
		C1A	OW	3.301	2.499			138.9
		OW	O1[g]	2.775				
		OW	O0[h]	2.785				
5	Intramolecular	C2A	O0	3.573	2.632	124.3	125.3	174.4
	Intermolecular	N2	O1[i]	2.908	2.116	175.5	171.3	157.8
		O3	O0[j]	2.691	1.780	128.8	125.6	170.3
		C1D	O2[k]	3.613	2.648	152.3	152.2	173.5

[a] Estimated standard deviations in the hydrogen bond lengths and angles are approximately 0.004 Å and 0.5° respectively. [b] Symmetry related by ($x + 1$, y, z). [c] Symmetry related by ($x - 1$, y, z). [d] Symmetry related by ($-x - 1$, $y + 1/2$, $-z + 1/2$). [e] Symmetry related by ($-x$, $y + 1/2$, $-z + 1/2$). [f] Symmetry related by ($-x$, $y - 1/2$, $-z + 3/2$). [g] Symmetry related by ($-x - 1$, $y - 1/2$, $-z + 3/2$). [h] Symmetry related by ($x + 1/2$, $-y + 1/2$, $-z$). [i] Symmetry related by ($x + 1/2$, $-y + 1/2$, $-z + 1$). [j] Symmetry related by (y, $-x + y$, $z - 1/6$). [k] Symmetry related by ($x - y$, x, $z + 1/6$). [l] Symmetry related by ($x + 1$, y, z).

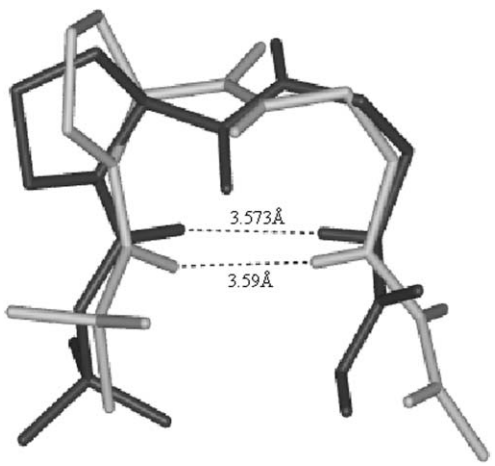

Fig. 2 Superposition of the structures Boc-Pro-γAbu-OH (black) and Piv-Pro-γAbu-NHMe (grey). The representation was generated by using the program MolMol.[45]

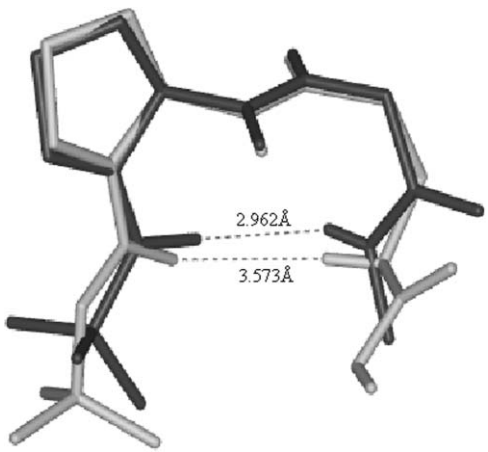

Fig. 3 Superposition of the peptides Boc-Pro-γAbu-OH (grey) and Piv-Pro-Gly-NHMe (black), RMSD = 0.32 Å. The representation was generated by using the program MolMol.[45]

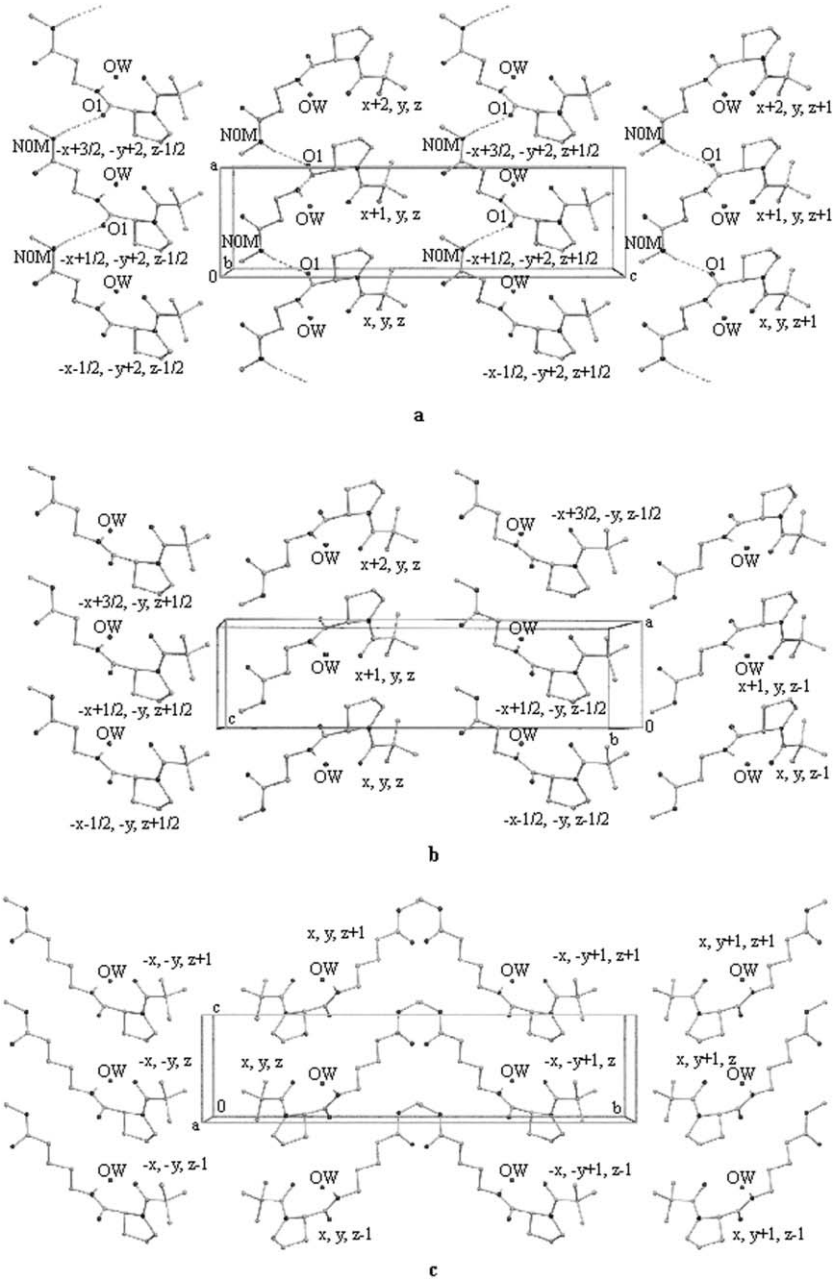

Fig. 4 A view of crystal packing in a) Piv-Pro-βGly-NHMe (**2**), b) Piv-Pro-βGly-OMe (**3**) and c) Piv-Pro-δAva-OMe (**4**). The intermolecular hydrogen bond for peptide **2** is shown as dotted lines.

shows a superposition of these two closely related structures. While the C–H···O hydrogen bond orientations in the two peptides superpose reasonably well, significant differences are observed in the orientation of the central peptide unit (Pro-γAbu). In **5**, Pro adopts a P_{II} conformation while the γAbu residue adopts the *gauche*, *gauche* (g^-g^-) conformation about the C^{γ}–C^{β} and C^{β}–C^{α} bonds (Table 1). In contrast, the γAbu residue in Piv-Pro-γAbu-NHMe, has the following torsion angles ($\phi_{Pro} = -71.0°$, $\psi_{Pro} = -26.1°$, $\phi_{\gamma Abu} = -77.2°$, $\theta^1{}_{\gamma Abu} = -50.2°$, $\theta^2{}_{\gamma Abu} = -172.2°$ and $\psi_{\gamma Abu} = 140.0°$).[12] The notable difference is that in this peptide, the γAbu residue adopts a g^-t conformation. The central peptide unit is flipped in peptide **5** as compared to Piv-Pro-γAbu-NHMe and the comparison of φ and θ_1 torsion angles reveals a compensating effect, which permits retention of the overall fold of the peptide chain. The C–H···O hydrogen bond mediated chain reversal in **5** mimics the N–H···O hydrogen bonded β-turn structure determined in **1**. Fig. 3 shows a superposition of the structures of peptides **1** and **5**, displaying the remarkable similarity of the overall fold of the backbone.

Molecular packing

A view of the packing motif in the three structures (**2–4**) is illustrated in Fig. 4. Peptides **2** and **3**, which differ only by

replacement of an NH group in **2** and an O-atom in **3** pack in an almost identical manner. Both peptides crystallized as monohydrate with water molecules linking symmetry related peptides. In addition, the single peptide hydrogen bond between the methylamide NH and the Pro C=O group of a symmetry related molecule is observed in the crystal structure of **2**. Replacement of the methylamide NH group by O in **3** does not disturb the packing arrangement, suggesting that this hydrogen bond may not be a major determinant of the solid state packing. Retention of the molecular conformation upon replacement of a hydrogen bonded NH by an O-atom has been earlier demonstrated in depsipeptide analogues in which an alanine residue is replaced by a lactic acid residue.[13] The co-crystallized water molecule is clearly an important determinant of the molecular packing in crystals of peptides **2** and **3**. An expanded view of the water environment in these two cases is shown schematically in Fig. 5a and 5b. In both cases, the water molecule forms three hydrogen bonds, acting as a hydrogen donor in two instances and as an acceptor in the third. The packing arrangement in peptide **4** is very similar with a single water molecule bridging three symmetry related peptides. A similarity of the water environment is also evident in Fig. 5c. There is no solvent molecule in the crystal structure of peptide **5**. Two independent hydrogen bonds Pro(1) C=O $\cdots$ H–N γAbu(2) and Boc(0) C=O $\cdots$ H–O γAbu(2) hold the peptide molecules in columns along the *c*-axis. The right-handed 6_1-screw axis results in generation of the peptide columns in crystals, shown in Fig. 6.

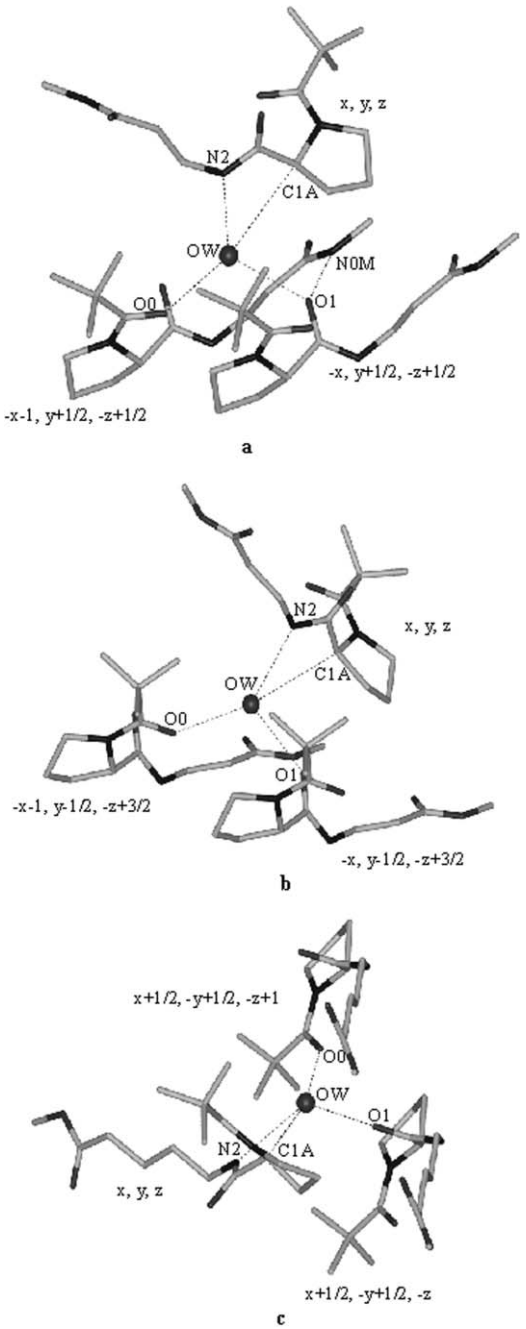

Fig. 5 A schematic view of the environment of water molecule in peptides a) Piv-Pro-βGly-NHMe (**2**), b) Piv-Pro-βGly-OMe (**3**) and c) Piv-Pro-δAva-OMe (**4**).

Potential C–H $\cdots$ O interaction

Considerable recent discussion has centered on the role of stabilizing C–H $\cdots$ O interactions in determining the packing of organic molecules in crystals[14] and in determining the folded structures of biological molecules.[15] In the structure of peptide **5**, attention has been drawn to an intramolecular C–H $\cdots$ O interaction, which appears to facilitate the formation of a β-turn mimetic conformation (Fig. 1e) as noted in earlier studies of αγ hybrid peptides.[12,16] This intra chain hydrogen bond mimics the 10-atom (C=$O_i \cdots$ H–N_{i+3}) hydrogen bond observed in a conventional β-turn structure.[17] In the structures described here, an additional C–H $\cdots$ O interaction involving the Pro C^{α}H and C^{δ}H groups may also be identified. In peptide **5**, there is a lateral C–H $\cdots$ O interaction involving the C^{δ} atom of Pro and the C=O group of γAbu with a symmetry related molecule (Table 2). This kind of weak C–H $\cdots$ O interaction is often observed in proteins.[15e] In peptides **2**, **3** and **4**, potential C–H $\cdots$ O interactions involving the C^{α}H Pro and the water molecule are observed (Table 2). Consideration of these possibilities would imply that the water molecule participates in two donor and two acceptor interactions.

Conformations of βGly residues

βGlycine, a glycine homolog (3-amino propanoic acid, referred to in the earlier literature as "β-alanine") is the simplest member of the omega amino acid series. Glycine occupies a special position in discussions of α-peptide conformations, since it is the only achiral residue in proteins as it lacks substituents at the C^{α}-atom. These features result in a symmetrical Ramachandran map for Gly residues encompassing a significantly larger degree of the conformational space as compared to the C^{α}-trisubstituted chiral residues.[18] By extension, βGly conformations serve as a starting point for a systematic understanding of β-peptide structures. The structure determination of peptides **2** and **3** prompted us to examine the conformational distribution of βGly residues in available peptide structures. Fig. 7 shows a distribution of observed conformations in ϕ, ψ space. Conformational families represented by three possible conformational states about the C^{β}–C^{α} bonds [$\theta \approx 180°$ (*t*), $\theta \approx -60°$ (g^-) and $\theta \approx 60°$ (g^+)] are marked by different

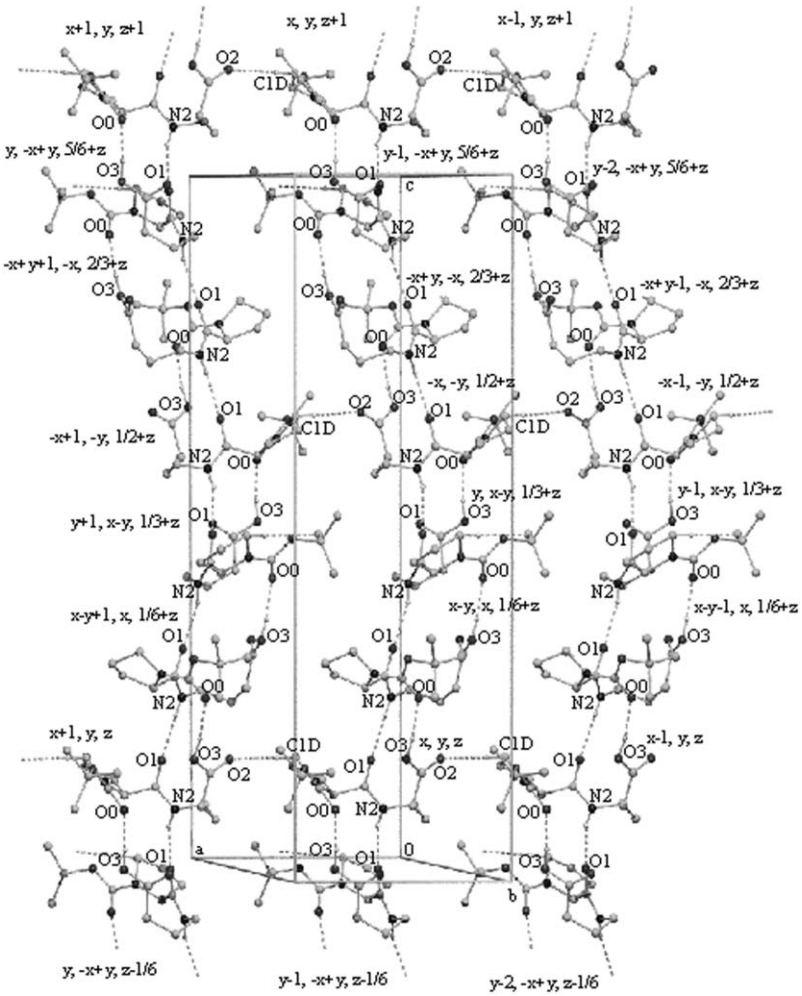

Fig. 6 Packing of peptide molecules Boc-Pro-γAbu-OH (**5**) in the unit cell.

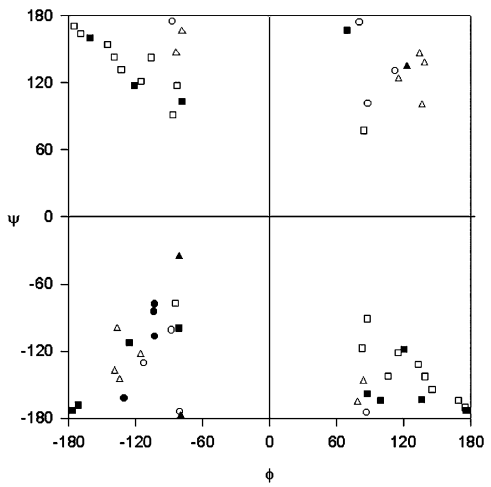

Fig. 7 Observed conformation of βglycine residues in the crystal structures of synthetic acyclic peptides represented on a two-dimensional ϕ–ψ plot. □: $\theta = 180°$ for achiral peptides; ■: $\theta = 180°$ for chiral peptides; △: $\theta = -60°$ for achiral peptides; ▲: $\theta = -60°$ for chiral peptides; ○: $\theta = +60°$ for achiral peptides; ●: $\theta = +60°$ for chiral peptides. Note the observed θ values for achiral peptides are listed twice in the figure ensuring both + and − values.

symbols. The observed clustering is skewed towards extended values of ϕ and ψ (Table 3).

Conclusions

A comparison of the structures of Piv-Pro-Gly-NHMe (**1**) and Piv-Pro-βGly-NHMe (**2**) reveals that insertion of a single sp^3 carbon atom into the backbone can result in a dramatic change in the molecular conformation. In αβ-hybrid peptide sequences an expanded β-turn of the C_{11}-type is possible,[4,19,21,38,39] but this has not been observed in the case of **2**. The near identity of the crystal structures of **2** and the analogue Piv-Pro-βGly-OMe (**3**), suggests that the hydrogen bond involving the terminal NH group in **2** is not a determinant of the molecular packing in crystals. A similar molecular conformation is observed in the homologous peptide Piv-Pro-δAva-OMe (**4**). In peptides **2**, **3** and **4**, all of the ω-amino acid residues adopt the *trans* conformation about the backbone C–C bonds (θ). In contrast, in Boc-Pro-γAbu-OH (**5**), the γAbu residue adopts a *gauche*, *gauche* (g^-g^-) conformation. The structures of Boc-Pro-γAbu-OH (**5**) and Piv-Pro-Gly-NHMe (**1**) show a striking resemblance, with a reversal of backbone direction. In the Pro-γAbu sequence, a C–H · · · O hydrogen bond

Table 3 Conformations of βGly residues in the crystal structure of acyclic peptides

Sequences		Residue	Torsion angles/deg ϕ	θ	ψ	References
		$\theta = 180°$				
Boc-βGly-mABA-OMe		βGly(1)	−139.2	173.1	142.8	20
Boc-Aib-βGly-NHMe		βGly(2)	−132.8	165.0	131.7	21
Boc-βGly-Aib-OMe	Mol B	βGly(1)	−106.4	161.2	142.7	22
Boc-βGly-Pda		βGly(1)	−115.3	173.1	121.6	23
Boc-βGly-^{D}Ala-NHMe		βGly(1)	−120.9	167.2	117.7	24
Boc-βGly-NHMe		βGly(1)	−145.5	171.6	154.5	25
Ac-Gly-βGly-Gly-βGly-NHpropyl		βGly(4)	175.0	−177.2	−170.5	26
Ac-Gly-βGly-Gly-βGly-NHpropyl		βGly(2)	169.0	180.0	−164.0	26
Boc-βGly-Aib-βGly-NHMe		βGly(3)	82.7	−177.4	−117.3	27
Boc-Aib-βGly-Aib-OMe		βGly(2)	−87.0	−177.2	91.1	28
Ac-βGly-(*R*)-Nip-(*S*)-Nip-βGly-NHMe	Mol A	βGly(1)	176.2	174.7	−173.4	29
		βGly(4)	−161.1	−178.4	160.5	29
Ac-βGly-(*R*)-Nip-(*S*)-Nip-βGly-NHMe	Mol B	βGly(1)	−171.5	171.4	−168.3	29
		βGly(4)	−176.9	175.6	−172.9	29
Boc-Ala-βGly-NHMe		βGly(2)	135.9	−175.8	−163.4	21
Piv-Pro-βGly-OMe		βGly(2)	99.5	178.9	−164.0	Present study
Piv-Pro-βGly-NHMe		βGly(2)	87.3	−179.3	−158.3	Present study
Boc-βGly-Ala-NHMe		βGly(1)	120.6	−167.1	−118.2	30
Boc-Leu-Aib-βGly-OMe	Mol A	βGly(3)	69.2	163.3	166.7	31
Boc-Leu-Aib-βGly-OMe	Mol B	βGly(3)	−125.5	−177.3	−112.4	31
Boc-Ala-Aib-βGly-OMe		βGly(3)	−81.4	−173.0	−99.3	32
Boc-βGly-Aib-βGly-OMe		βGly(3)	84.1	−175.6	77.5	33
Boc-βGly-Leu-Aib-Val-OMe		βGly(1)	−78.7	172.1	103.3	34
		$\theta = -60°$				
Boc-Aib-Val-Aib-βGly-OMe		βGly(4)	−81.0	−65.4	−35.7	35
Piv-βGly-OH		βGly(1)	−78.5	−68.2	166.1	Unpublished result
Boc-βGly-Aib-βGly-OMe		βGly(1)	−83.8	−77.6	146.8	33
Boc-βGly-Aib-βGly-NHMe		βGly(1)	136.3	−61.9	100.1	27
Boc-Ala-Gly-βGly-OMe		βGly(3)	−79.3	−60.4	−177.9	32
Boc-βGly-Ac$_6$c-OMe		βGly(1)	134.2	−64.8	145.8	36
Boc-βGly-Ac$_5$c-OMe	Mol B	βGly(1)	115.7	−61.2	123.4	42
Boc-βGly-Aib-OMe	Mol A	βGly(1)	138.8	−71.0	137.8	22
Boc-^{L}Pip-βGly-NHMe		βGly(2)	123.0	−60.2	134.7	37
		$\theta = 60°$				
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-Ala-Leu-Aib-OMe		βGly(4)	−102.6	78.5	−106.9	3*d*
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-OMe		βGly(4)	−130.3	75.9	−162.3	3*d*
Boc-βGly-Aib-Leu-Aib-OMe		βGly(1)	−103.8	83.7	−84.7	38
LeucinostatinA, acyclic nonapeptide from *Paecilomyces marquandii*		βGly(9)	−103.0	80.0	−78.0	39
Boc-βGly-Ac$_5$c-OMe	Mol A	βGly(1)	−112.6	67.8	−130.6	42
Boc-βGly-OH		βGly(1)	87.0	67.0	−175.0	40
N-Chloroacetyl-βGly		βGly(1)	80.5	73.1	174.2	41
Boc-Aib-Aib-βGly-NHMe		βGly(3)	−88.0	71.0	−101.3	21

acts as a mimetic of the N–H · · · O hydrogen bond in the classical peptide β-turn.

Experimental

Synthesis of peptides 1–5

Peptides **1–5** were synthesized by a conventional solution phase procedure, purified by reverse phase (C_{18}) medium pressure liquid chromatography and were characterized by electrospray ionization mass spectrometry.[43]

X-Ray diffraction

Crystals of peptides **1–5** were grown by slow evaporation from the solvents water (peptide **1**), dimethylsulfoxide–water (peptide **2**), methanol–water (peptide **3**) and ethyl acetate (peptides **4** and **5**). X-Ray intensity data for crystals **1–5** were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer with graphite monochromated MoK_α ($\lambda = 0.71073$ Å) radiation. The ω scan type was used. The structures of **1–5** were determined by direct phase determination using the program SHELXS-97.[44a] Refinements of all five structures were carried out against F^2, with a full matrix anisotropic least-squares method using the program SHELXL-97.[44b] The single water molecule was located from the difference Fourier maps in peptides **2**, **3** and **4**. Hydrogen atoms bonded to C1A(Pro); N2(Gly); N0M(NHMe) for peptide **1**, C1A(Pro); N0M(NHMe); OW for peptide **2**, C1A(Pro); N2(βGly); OW for peptide **3** and C1A(Pro); N2, C2A, C2B(γAbu); O3(OH) for peptide **5** were located from the difference Fourier maps. The remaining hydrogen atoms of peptides **1**, **2**, **3**, **5** and all the hydrogens of peptide **4**, 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 were bonded. In these all-light-atom structures with no significant anomalous

Table 4 Crystal and diffraction parameters

	Peptide **1**	Peptide **2**	Peptide **3**	Peptide **4**	Peptide **5**
Empirical formula	$C_{13}H_{23}N_3O_3$	$C_{14}H_{25}N_3O_3{\cdot}H_2O$	$C_{14}H_{24}N_2O_4{\cdot}H_2O$	$C_{16}H_{28}N_2O_4{\cdot}H_2O$	$C_{14}H_{24}N_2O_5$
Formula weight	269.0	301.0	302.0	330.0	300.0
Crystal habit	Rectangular	Rectangular	Rectangular	Plate	Rod
Crystal size (mm)	0.55 × 0.45 × 0.15	0.52 × 0.35 × 0.1	0.53 × 0.4 × 0.1	0.37 × 0.17 × 0.05	0.55 × 0.14 × 0.1
Crystallizing solvent	Water	Dimethylsulfoxide–water	Methanol–water	Ethyl acetate	Ethyl acetate
Space group	$P1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12$	$P6_1$
Cell parameters					
a/Å	5.8431(12)	6.297(3)	6.157(2)	11.330(10)	9.7591(16)
b/Å	7.9668(17)	11.589(5)	11.547(4)	25.56(2)	9.7591(16)
c/Å	9.1733(19)	22.503(9)	23.404(8)	6.243(6)	29.158(10)
α/deg	114.831(3)	90	90	90	90
β/deg	97.043(3)	90	90	90	90
γ/deg	99.449(3)	90	90	90	120
Volume/Å^3	373.43(13)	1642.2(11)	1663.9(10)	1808(3)	2405.0(10)
Z	1	4	4	4	6
Molecules/asym. unit	1	1	1	1	1
Co-crystallized solvent	None	One water	One water	One water	None
Molecular weight	269.34	301.39	302.37	328.40	300.35
Density/g cm^{-3} (calc)	1.198	1.219	1.207	1.206	1.244
F(000)/radiation	146/MoK$_\alpha$	656/MoK$_\alpha$	656/MoK$_\alpha$	712/MoK$_\alpha$	972/MoK$_\alpha$
Temperature/°C	20	20	20	20	20
2θ max (°)/R_{int}	54.38/0.0265	54.90/0.0409	55.0/0.0364	46.52/0.0368	54.8/0.1109
Measured reflections	3984	12803	13048	7348	19265
Independent reflections	2893	3455	3482	2578	3431
Unique reflections	1528	2054	2078	1533	1809
Observed reflections [$\lvert F_o\rvert > 4\sigma(\lvert F_o\rvert)$]	1496	1861	1874	1406	1537
Final R (%)/wR_2 (%)	3.65/9.79	4.39/12.11	5.00/13.79	9.19/23.44	7.73/12.43
Goodness-of-fit	1.069	1.076	1.124	1.143	1.263
$\Delta\rho_{max}$ (eÅ^{-3})/$\Delta\rho_{min}$ (eÅ^{-3})	0.180/−0.183	0.294/−0.151	0.325/−0.155	0.307/−0.285	0.187/−0.153
Restraints/parameters	3/184	0/206	0/206	1/208	1/218
Data-to-parameter ratio	8.1 : 1	9.0 : 1	9.1 : 1	6.8 : 1	7.1 : 1

scatterers the Friedel pairs were merged before the final refinement cycles. The relevant crystallographic data collection parameters and structures refinement details are summarized in Table 4. ‡

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