$\alpha-\gamma$ Hybrid Peptides that Contain the Conformationally Constrained Gabapentin Residue: Characterization of Mimetics of Chain Reversals

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Abstract: The crystal structures of four dipeptides that contain the stereochemically constrained γ-amino acid residue gabapentin (1-(aminomethyl)cyclohexaneacetic acid Gpn) are described. The molecular conformation of Piv-Pro-Gpn-OH (**1**), reveals a β-turn mimetic conformation, stabilized by a ten atom C–H$\cdots$O hydrogen bond between the Piv CO group and the *pro S* hydrogen of the Gpn CH_2–CO group. The peptides Boc-Gly-Gpn-OH (**2**), Boc-Aib-Gpn-OH (**3**), and Boc-Aib-Gpn-OMe (**4**) form compact, folded structures, in which a distinct reversal of polypeptide chain direction is observed. In all cases, the Gpn residue adopts a *gauche*,*gauche* (*g*,*g*) conformation about the C^{γ}–C^{β} (θ^1) and C^{β}–C^{α} (θ^2) bonds. Two distinct Gpn conformational families are observed. In peptides **1** and **3**, the average backbone torsion angle values for the Gpn residue are $\phi = 98^{\circ}$, $\theta^1 = -62^{\circ}$, $\theta^2 = -73^{\circ}$, and $\psi = 79^{\circ}$, while in peptide **2** and **4** the average values are $\phi = -103^{\circ}$, $\theta^1 = -46^{\circ}$, $\theta^2 = -49^{\circ}$, and $\psi = -92^{\circ}$. In the case of **1** and **3**, an intramolecular nine-membered O–H$\cdots$O hydrogen bond is formed between the C=O of the preceding residue and the terminal carboxylic acid OH group. All four $\alpha-\gamma$ dipeptide sequences yield compact folded backbone conformations; this suggests that the Gpn residue may be employed successfully in the design of novel folded structures.

Keywords: amino acids · beta-turn mimetics · hydrogen bonds · peptides · structure elucidation

Introduction

Polypeptide chain reversals nucleated by two contiguous residues, β-turns, are widely found in proteins,[1] and are commonly observed structural feature in biologically active peptides.[2] β-Turns were originally recognized in an attempt to stereochemically characterize the intramolecular hydrogen bonded conformations of "three linked peptide units".[3] Canonical β-turns in polypeptides, derived from α-amino acid residues, are stabilized by $4 \rightarrow 1$ (C_{10}) hydrogen bonds between CO_i and $N_{i+3}H$ groups. The residues $i+1$ and $i+2$ form the turning fulcrum of the polypeptide chain with the torsion angles ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, and ψ_{i+2}, each varying for different specific β-turn types.[4] The area of peptidomimetic design has seen considerable activity directed towards the synthesis of β-turn mimetics.[5] The impetus for these efforts derives from the role of β-turns as determinants of three-dimensional structure in a large number of pharmacologically important peptides.[6] During the investigations into the conformations of hybrid peptide sequences, that contain both α- and γ-amino acids, we observed an interesting C–H$\cdots$O hydrogen-bond-stabilized chain reversal in the peptide Piv-L-Pro-γ-Abu-NHMe (γ-Abu, γ-aminobutyric acid); this structure was determined ab initio from powder X-ray diffraction data.[7] In seeking to establish the generality of this conformational feature, and in order to explore the possibility of generating new β-turn mimetics, we investigated the structures of peptides that contain the conformationally constrained, achiral γ-amino acid residue, 1-(aminomethyl)cyclohexaneacetic acid (gabapentin (Gpn) Figure 1a). The amino acid gabapentin is a widely used anti-epileptic drug, which exists in solution as mixtures of two interconverting con-

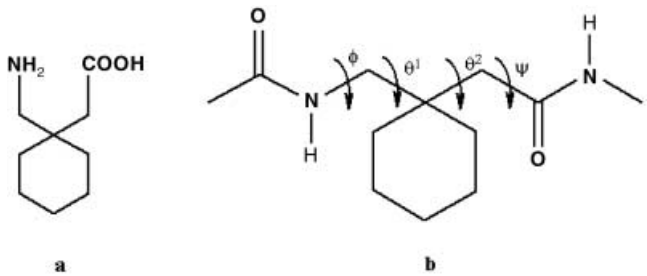

Figure 1. a) Schematic representation of gabapentin (Gpn) b) The parameters used to define the dihedral angles.[19e,f]

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formations that correspond to the two possible chair forms.[8] In crystals, the conformation with the aminomethyl group axial has been characterized.[9] Gpn is a β,β-dialkylated γ-amino acid residue with geminal substitutents at C^{β}; this restricts the range of accessible conformations about the torsion angles θ^1 and θ^2 (Figure 1b). Here, we describe the crystal structure of four peptides that contain the Gpn residues: Piv-L-Pro-Gpn-OH (**1**), Boc-Gly-Gpn-OH (**2**), Boc-Aib-Gpn-OH (**3**), and Boc-Aib-Gpn-OMe (**4**) (Aib, α-aminoisobutyric acid). We illustrate the conformational similarities of the chain reversals observed in specific $\alpha-\gamma$ sequences to the conventional β-turns observed in $\alpha-\alpha$ sequences.

Results and Discussion

Figure 2 shows the molecular conformation of peptides **1** to **4** in single crystals. The crystallographic data are given in Table 1. Table 2 summarizes the backbone dihedral angles, which serve as a descriptor of the fold for the polypeptide chain. Table 3 lists the observed intra- and intermolecular hydrogen bonds, while a view of the packing motif in the four structures is illustrated in Figure 3. In all four peptides, it is clear that the $\alpha-\gamma$ sequence results in a backbone chain reversal. This is clearly a consequence of the *gauche,gauche* (*g*,*g*) conformation adopted about the C^{γ}–C^{β} and C^{β}–C^{α} bonds of the Gpn residue. In all four peptides, the cyclohexane ring adopts an almost perfect chair conformation. In peptides **1** and **3** the carboxymethyl substitutent occupies an axial position, while in peptides **2** and **4** the aminomethyl group takes up the axial orientation. Clearly, both possible chair conformations are readily ac-

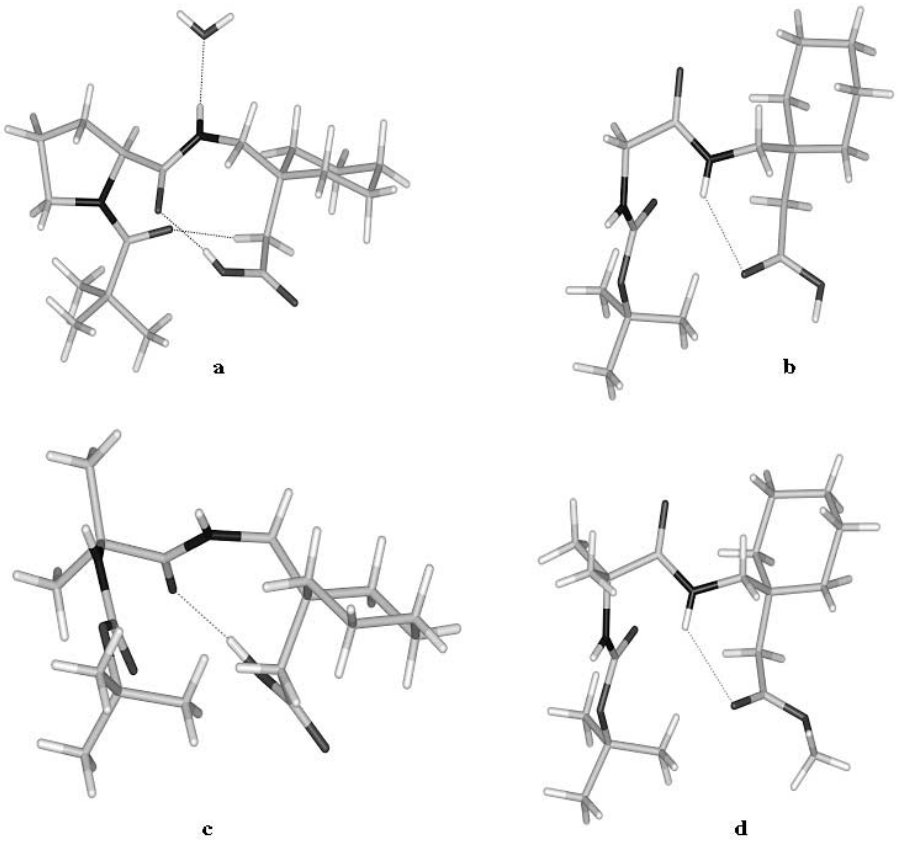

Figure 2. Molecular conformation of a) Piv-Pro-Gpn-OH (**1**), b) Boc-Gly-Gpn-OH (**2**), c) Boc-Aib-Gpn-OH (**3**), d) Boc-Aib-Gpn-OMe (**4**) in crystals.

Table 1. Crystal and diffraction parameters.

	1	**2**	**3**	**4**
formula	$C_{19}H_{32}N_2O_4 \cdot H_2O$	$C_{16}H_{28}N_2O_5$	$C_{18}H_{32}N_2O_5$	$C_{19}H_{34}N_2O_5$
crystal habit	clear	clear	clear	white
crystal size [mm]	$0.23 \times 0.19 \times 0.1$	$0.92 \times 0.76 \times 0.56$	$0.86 \times 0.5 \times 0.08$	$1.26 \times 0.58 \times 0.26$
crystallizing solvent	ethyl acetate	methanol/water	methanol/water	methanol
space group	$P2_12_12_1$	$P\bar{1}$	*Pbca*	$P2_1/c$
a [Å]	9.7899(9)	6.951(2)	9.718(2)	10.509(5)
b [Å]	23.854(2)	10.785(3)	12.379(3)	10.547(5)
c [Å]	8.9726(8)	12.927(4)	33.479(9)	20.240(9)
α [°]	90.0	78.556(5)	90.0	90.0
β [°]	90.0	79.095(5)	90.0	96.022(7)
γ [°]	90.0	84.330(5)	90.0	90.0
Volume [Å^3]	2095.3(3)	930.8(5)	4027.5(17)	2230.9(18)
Z	4	2	8	4
molecules/asymmetric unit	1	1	1	1
co-crystallized solvent	one water	none	none	none
M_r	370.48	328.40	356.46	370.48
ρcald [g cm^{3-}]	1.174	1.172	1.176	1.103
F (000)	808	356	1552	808
T [°C]	21	21	21	21
$2\theta_{max}$ [°]	54.08	54.36	53.8	53.72
measured reflections	15776	9862	28440	16121
R_{int}	0.0166	0.1104	0.1032	0.0369
independent reflections	4178	3730	4128	4381
observed reflections [$\|F\| > 4\sigma(F)$]	3756	3147	3012	3199
final *R* [%]	4.8	5.23	6.06	7.63
final w*R*2 [%]	13.32	14.17	11.78	21.45
goodness of fit	1.048	1.061	1.03	1.025
$\Delta\rho_{max}/\Delta\rho_{min}$[e Å^{-3}]	0.47/ – 0.20	0.26/ – 0.29	0.16/ – 0.14	0.65/ – 0.41
restraints/parameters	0/335	0/320	0/354	1/359
data to parameter ratio	11.2:1	9.8:1	8.5:1	8.9:1

Table 2. Backbone torsion angles [°][a,b] in $\alpha-\gamma$ peptides.

Peptide	ϕ_1	ψ_1	ω_1	ϕ_2	θ^1	θ^2	ψ_2	ω_2	Axial group
1	−56.9	145.7	180	92.9	−66.7	−70.7	84.6	–	carboxymethyl
2	−80.7	−7.1	−176.9	−103.7	−44.9	−48.7	−94.3	–	aminomethyl
3	55.4	46.2	174.0	103.9	−57.3	−75.5	73.5	–	carboxymethyl
4	−68.5	−25.3	−178.2	−102.1	−48.2	−50.3	−90.0	170.6	aminomethyl

[a] For α-residue nomenclature see ref. [4c], for ω-residue nomenclature see ref. [19ef]. [b] Peptides **2**–**4** are achiral and crystallize in centrosymmetric space groups that accommodate molecules with enantiomeric conformations. For convenience, the sign of the torsion angles listed has been chosen to correspond to the same signs for θ^1 and θ^2 as observed for the chiral peptide **1**. The estimated standard deviation is $\approx 0.2°$.

Table 3. Hydrogen bonds in peptides **1**–**4**.[a]

Donor	Acceptor	D···A [Å]	H···A [Å]	C=O···H [°]	C=O···D [°]	D–H···A [°]
peptide **1**						
intramolecular						
O3	O1	2.614	1.917	127.94	128.49	165.88
C2A	O0	3.554	2.571	127.98	130.76	169.63
intermolecular						
N2	O1w	2.865	1.972			166.11
O1w	O2[b]	2.908	2.078	155.60	158.87	163.08
O1w	O0[c]	2.876	1.980	144.60	142.01	167.29
peptide **2**						
intramolecular						
N2	O2	2.928	2.263	102.22	91.68	136.13
intermolecular						
N1	O2[d]	3.024	2.251	169.12	165.97	163.81
O3	O0[e]	2.643	1.734	140.30	143.24	170.93
peptide **3**						
intramolecular						
O3	O1	2.593	1.657	119.59	123.47	169.11
intermolecular						
N1	O1[f]	3.143	2.331	147.69	149.83	159.94
N2	O3[f]	2.933	2.078	127.82	124.06	163.22
peptide **4**						
intramolecular						
N2	O2	2.957	2.339	100.72	91.3	129.08
intermolecular						
N1	O1[g]	2.946	2.119	140.02	139.4	174.75

[a] The standard deviations in bond lengths are approximately 0.004 Å and those of bond angles are approximately 0.2°. [b] Symmetry related by $x+1, y, z$. [c] Symmetry related by $-x+1/2, -y, z+1/2$. [d] Symmetry related by $-x, -y+1, -z$. [e] Symmetry related by $x-1, y, z$. [f] Symmetry related by $-x+1/2, y+1/2, z$. [g] Symmetry related by $-x, y-1/2, -z+3/2$.

commodated in the observed peptide structures. Crystallographic and NMR investigations on the free amino acid Gpn and several of its derivatives suggests that the two possible chair forms differ only marginally in energy, and undergo rapid interconversion in solution.[8c].

Structural features in Gpn peptides

peptide 1: Piv-L-Pro-Gpn-OH adopts a folded conformation stabilized by two potential intramolecular hydrogen bonds: Piv C=O···H–C$^{\alpha}$ Gpn and Pro C=O···H–O Gpn. The relevant hydrogen-bond parameters are listed in Table 3. These values fall well within the range observed for potentially favorable C–H···O interactions.[10] The observed C–H···O interaction results in the formation of a ten-atom hydrogen-bonded ring, reminiscent of that observed in peptide β-turns, a feature previously detected in the peptide Piv-Pro-γ-Abu-NHMe by powder diffraction data. The second intramolecular nine-membered O–H···O hydrogen bond is formed between the Pro C=O group and O–H group of the terminal carboxylic acid moiety. This results in the adoption of the unusual *anti* conformation by the terminal carboxylic acid group. Theoretical calculations have estimated the energy difference between the *syn* and *anti* conformation of carboxylic acids to be about 4–8 kcal mol^{-1}; this results in the predominant population of *syn* forms in solution and in the solid state.[11] The L-Pro residue adopts a semi-extended conformation ($\phi = -56.9°$, $\psi = 145.7°$), which is similar to that observed for the $i+1$ position in type II β-turns in all α-amino acid structures.

In crystals, a lone water molecule bridges symmetry related molecules of the peptides, by forming hydrogen bonds to Gpn NH, Piv CO; and Gpn CO groups. The structure of peptide **1** may be considered as a formal analogue of a conventional β-turn, in which a ten-atom C–H···O hydrogen bond acts as a mimic for a ten-atom N–H···O (4→1) hydrogen bond. Notably, hydration of the central peptide unit, as seen in **1**, is also a feature commonly seen in β-turns in protein structures.[12]

Peptide 2: In Boc-Gly-Gpn-OH, a single intramolecular seven-membered hydrogen bond between the Gpn NH and CO groups is observed. The Gly residue adopts a conformation in the helical region of ϕ,ψ space, with appreciable distortion of both dihedral angles from ideal values. A view of the crystal packing is shown in Figure 3. Notably, the CO group of the Gly residue is not involved in any hydrogen bond.

Peptide 3: In Boc-Aib-Gpn-OH, a single intramolecular nine-membered O–H···O hydrogen bond is observed between the Aib CO moiety and the carboxylic acid OH group of Gpn. Inspection of the dihedral angles for the Gpn residue (Table 2) reveals a conformation almost identical to that observed in Piv-L-Pro-Gpn-OH (**1**). However, in contrast to peptide **1**, the Aib residue in **3** adopts ϕ,ψ values characteristic

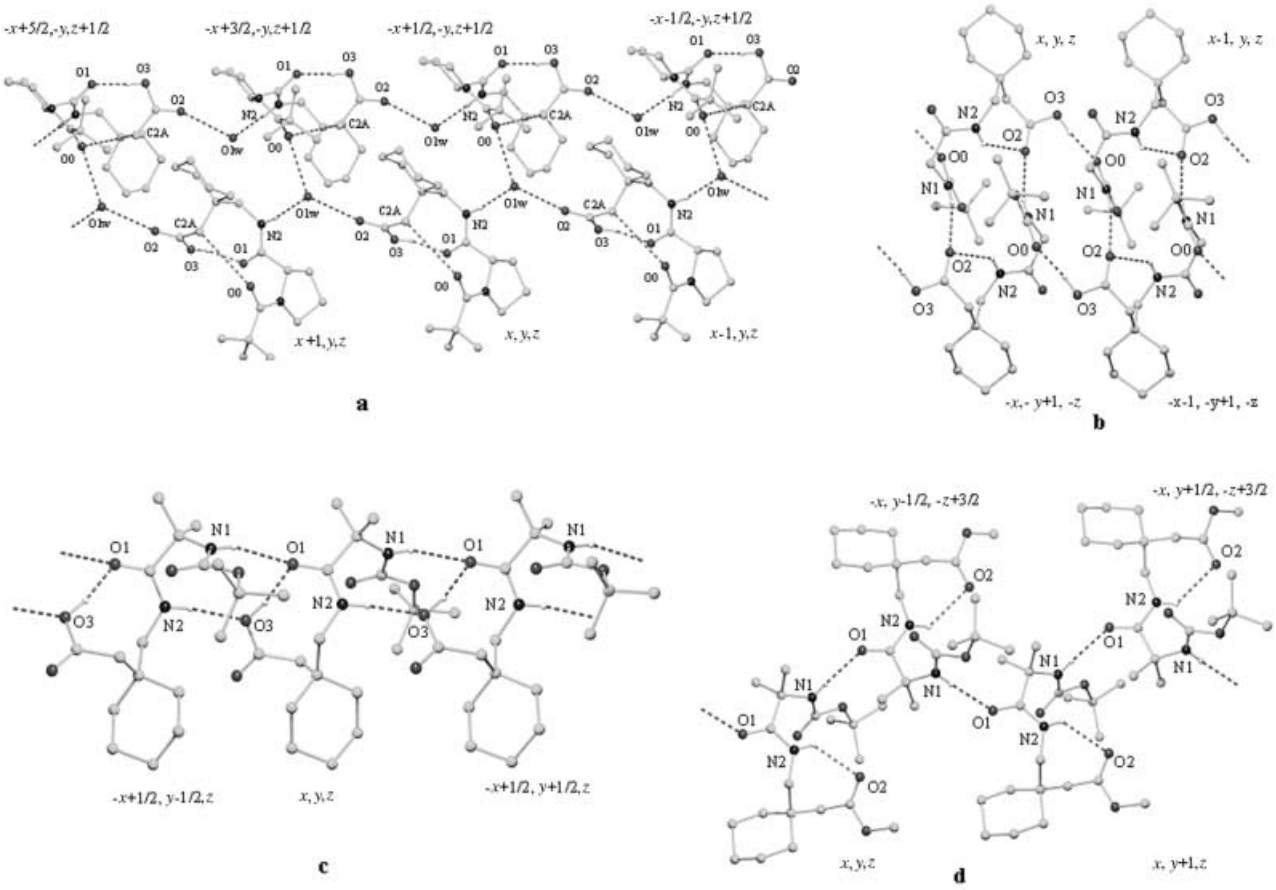

Figure 3. A view of crystal packing in a) Piv-Pro-Gpn-OH (**1**), b) Boc-Gly-Gpn-OH (**2**), c) Boc-Aib-Gpn-OH (**3**), d) Boc-Aib-Gpn-OMe (**4**). The intermolecular and intramolecular hydrogen bonds are shown as dotted lines.

of a helical conformation. This results in an altered orientation of the N-terminal urethane group, which leads to the absence of the C–H···O interaction noted in peptide **1**. In crystals, the carboxyl group of the terminal carboxylic acid is not involved in any strong hydrogen-bonding interaction. This may be in contrast to the situation in peptide **1**, in which both oxygen atoms of the terminal carboxylic acid group participate in hydrogen bond interactions.

Peptide 4: The observed molecular conformation in Boc-Aib-Gpn-OMe (**4**) is very similar to that observed in Boc-Gly-Gpn-OH (**2**). A single intramolecular seven-membered N–H···O hydrogen bond that involves the Gpn NH and CO groups is observed. The similarity between peptides **2** and **4** is also clearly evident upon comparison of observed backbone torsion angles (Table 2). In the packing motif shown in Figure 3, all the hydrogen bond donors and acceptors are paired in intermolecular hydrogen bonds. In this conformation, the distance between the O atom of the Boc group, and the C atom of the terminal ester methyl group is 6.618 Å; this is indicative of a sharp reversal of chain direction. In α-peptides, the $C^{\alpha}(i)$ to $C^{\alpha}(i+3)$ distance of $\leq$7.0 Å characterizes chain reversals nucleated by two residues, of which β-turns, with $4\rightarrow1$ intramolecular hydrogen bonds, are most abundant.[13]

Conformational characterization of $\alpha-\gamma$ chain reversals: Figure 4a shows a superposition of the conformations detected in peptides Piv-L-Pro-γ-Abu-NHMe[7] and Piv-L-Pro-Gpn-OH. It is clearly seen that the ten-atom C–H···O hydrogen-bonded rings superpose well, with the important difference that the central peptide unit linking the Pro and γ-amino acid residue adopts different orientations, which corresponds to simultaneous rotations about the torsion angles, ψ_{Pro} and $\phi_{\gamma\mathrm{Abu/Gpn}}$. Interconversion between different β-turn types, type I and type II, in $\alpha-\alpha$ sequences can occur by concerted motion involving flipping of the central peptide unit.[14] Thus, the two C–H···O stabilized turn conformations observed in the peptides Piv-L-Pro-γ-Abu-NHMe and Piv-L-Pro-Gpn-OH, appear to correspond to two distinct conformational families, which may be related to their counterparts in $\alpha-\alpha$ sequences. An important difference between the two $\alpha-\gamma$ turns is that in Piv-L-Pro-γ-Abu-NHMe, the *pro-R* hydrogen of γ-Abu CH_2–CO group is involved in the intermolecular interaction. In Piv-L-Pro-Gpn-OH, it is the *pro-S* hydrogen of the Gpn CH_2–CO group. Figure 4b shows a superposition of the structure of Piv-L-Pro-Gpn-OH with the type II β-turn conformation adopted by the peptide Piv-L-Pro-Aib-NHMe.[15] Figure 4c shows the superposition of the structure Piv-L-Pro-γ-Abu-NHMe and the type I β-turn conformation observed in Piv-Pro-Thr-NHMe.[16] The excellent superposition of the $\alpha-\alpha$ N–H···O hydrogen bond stabilized β-turn

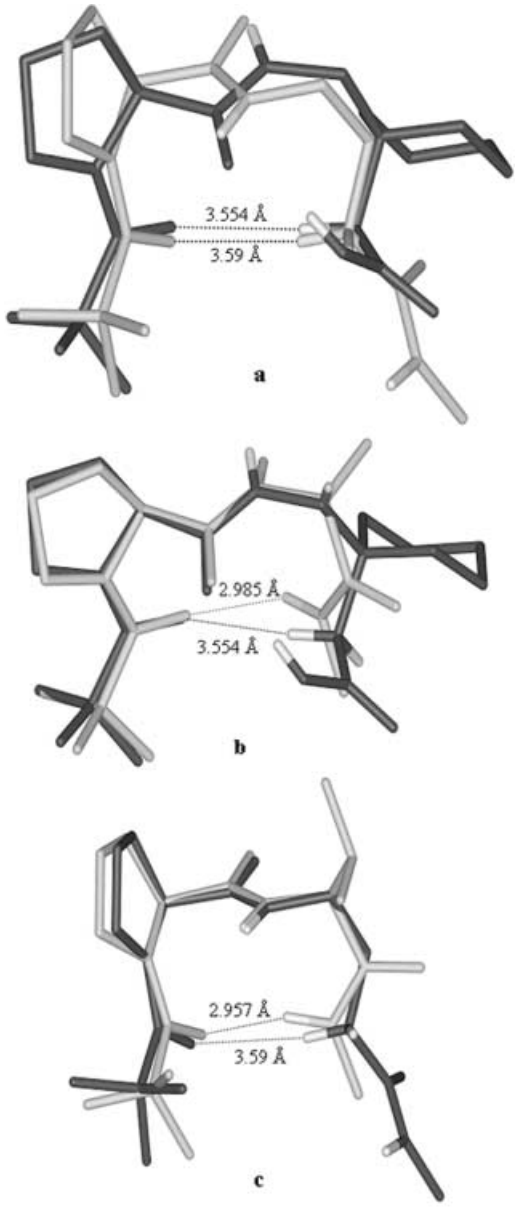

Figure 4. a) Superposition of the structures of Piv-Pro-Gpn-OH (black) and Piv-Pro-γ-Abu-NHMe (gray; $\phi_{Pro} = -71.0^\circ$, $\psi_{Pro} = -26.1^\circ$, $\phi_{\gamma Abu} = -77.2^\circ$, $\theta^1_{\gamma Abu} = -50.2$, $\theta^2_{\gamma Abu} = -172.2^\circ$ and $\psi_{\gamma Abu} = 140.0^\circ$).[7] b) Superposition of Piv-Pro-Gpn-OH (black) and Piv-Pro-Aib-NHMe[15] (gray; $\phi_{Pro} = -57.8^\circ$, $\psi_{Pro} = 139.2^\circ$, $\phi_{Aib} = 61.4^\circ$ and $\psi_{Aib} = 25.1^\circ$), RMSD = 0.36 Å. c) Superposition of Piv-Pro-γ-Abu-NHMe (black) and Piv-Pro-Thr-NHMe[16] (gray; $\phi_{Pro} = -65.8^\circ$, $\psi_{Pro} = -21.8^\circ$, $\phi_{Thr} = -102.8^\circ$ and $\psi_{Thr} = 6.5^\circ$), RMSD = 0.26 Å. Only the backbone atoms are used for the superposition. The C···O distances in the $\alpha-\gamma$ turn and the N···O distances in the $\alpha-\alpha$ turn are indicated. The representation was generated by using the program MolMol.[25]

segments, and $\alpha-\gamma$ C–H···O hydrogen bond stabilized chain reversals is evident. Thus it appears possible to generate mimetics of the common β-turns type I and type II in $\alpha-\gamma$ sequences. The oxy analogue of the β-turn with an intramolecular $4 \rightarrow 1$ O···H–O hydrogen bond has been observed in an N-protected tripepide acid Z-$(Aib)_3$-OH.[17]

In peptides Boc-Gly-Gpn-OH (**2**), Boc-Aib-Gpn-OH (**3**), and Boc-Aib-Gpn-OMe (**4**) there are no apparent hydrogen bond interactions; this restricts the torsional freedom at *both* residues and stabilizes the observed chain reversal. Nevertheless, in all these $\alpha-\gamma$ sequences, a compact folded structure is observed which brings the N- and the C-terminal groups into close proximity.

Gabapentin conformations: Gabapentin is unique among the γ-amino acid residues studied so far, in that, it is symmetrically substituted at the central β-carbon atom; this results in a restricted range of accessible values of θ^1 and θ^2. In all four structures, the observation of *gauche,gauche* (*g*,*g*) conformations with both the dihedral angles having the same sign, suggests that this is undoubtedly an energetically preferred structure for the Gpn residue. In all cases, the ϕ,ψ values in Gpn are semi-extended ($\phi \approx \pm 100^\circ$, $\psi \approx \pm 85^\circ$). If all four torsion angles are considered, the structures of peptides **1** to **4** reveal only two conformational families for the Gpn residue: 1) $\phi = 98^\circ$, $\theta^1 = -62^\circ$, $\theta^2 = -73^\circ$, $\psi = 79^\circ$ (mean values of **1** and **3**), and 2) $\phi = -103^\circ$, $\theta^1 = -46^\circ$, $\theta^2 = -49^\circ$, $\psi = -92^\circ$ (mean values of **2** and **4**). In case 1, an intramolecular nine-membered O–H···O hydrogen bond is formed between the C=O of the preceding residue and the terminal carboxylic acid OH group of the Gpn residue. In case 2, a short N···O distance is observed between the Gpn NH and Gpn CO groups (N···O = 2.928 Å in **2** and N···O = 2.957 Å in **4**). However, in both these cases the hydrogen bond angles (Table 3) appear to lie on the borderline of the limits; this is considered accepted for a stabilizing interaction.[18]

It is noteworthy that the two backbone conformational families observed for the Gpn residue appear to favor distinct orientation for the substitutents on the cyclohexane ring; in peptides **1** and **3** the carboxymethyl group adopts an axial position, while in peptides **2** and **4** the aminomethyl group is in the axial position.

Conclusion

This study provides an accurate conformational characterization of the stereochemically constrained β,β disubstituted γ-amino acid residue Gpn in four distinct peptide structures. The Gpn residue appears to contribute to the generation of a compact folded backbone conformation. This feature should be valuable in peptide design. The search for new peptidomimetics and foldamers, which provide access to a range of novel three- dimensional molecular structures, has been greatly facilitated by the findings of Seebach and Gellman; they found that peptides derived from β-amino acid residues provide an entry to conformational families, hitherto inaccessible for α-amino acids.[19] The rapidly developing field of β-peptide conformations has also stimulated investigations on γ- and δ-amino acid residues as novel elements in the design of folded structures.[20] The enhanced proteolytic stability of β- and γ-peptides is a particularly attractive feature for analogue design.[21] The use of hybrid sequences that incorporate both α- and ω-amino acids promises to greatly expand the repertoire of mimics of biologically active peptides.[22] The observation of a C–H···O hydrogen-bond-mediated chain reversal in Piv-Pro-Gpn-OH (**1**), which may serve as a β-turn surrogate, provides an entry to the design of a novel β-turn

mimetics in an $\alpha-\gamma$ peptide sequence. The possibility of extending such chain reversals to nucleate hairpin structures merits further investigation.[23] The structures of Boc-Gly-Gpn-OH (**2**), Boc-Aib-Gpn-OH (**3**), and Boc-Aib-Gpn-OMe (**4**) also suggest that Gpn residues may be used to generate chain reversals that do not require strong intramolecular hydrogen bonds for conformational stabilization.

Experimental Section

Gabapentin was the product of Hikal, Bangalore (India). Melting points were determined by using a Büchi melting point B-540 apparatus. ^{1}H NMR spectra were recorded on a Bruker AMX-400 MHz spectrometer by using tetramethylsilane as an internal standard. Mass spectra were recorded on a HP LCMSD 1100 electrospray mass spectrometer.

General procedure for the synthesis of peptides 1–3: A solution of H-Gpn-OH (6 mmol, 1.02 g, dissolved in 15 ml of 10% Na_2CO_3) was added to a stirred solution of N-protected *N*-hydroxy succinimide ester, Piv/Boc-AA-OSu (5 mmol), in THF, (20 mL). This was then stirred for 12 h at room temperature. After the reaction, THF was evaporated and the residue was dissolved in water. The aqueous layer was cooled, the pH was adjusted to $\approx$2 by the addition of 1N HCl, and then extracted with ethyl acetate. The pooled organic extracts were dried over Na_2SO_4, and after evaporation in vacuo, the peptides were present as white crystalline solids. The peptides were purified by medium-pressure liquid chromatography over a reverse-phase C_{18} column (40–60 μm). The identity of the peptides were confirmed by NMR spectroscopy (400 MHz) and electrospray mass spectrometry.

Piv-L-Pro-Gpn-OH (**1**): Yield: 82%; m.p. 109–110 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.26$ (s, 9H; Piv CH_3), 1.3–1.5 (m, 10H; cyclohexyl), 1.9–2.1 (m, 4H; Pro $C^\beta H_2$, $C^\gamma H_2$), 2.22, 2.29 (d, 2H; Gpn $C^\alpha H_2$), 3.17, 3.33 (dd, 2H; Gpn $C^\gamma H_2$), 3.71 (m, 2H; Pro $C^\delta H_2$), 4.63 (m, 1H; Pro $C^\alpha H$), 7.23 ppm (s, 1H; Gpn NH). MS: *m*/*z* cald: 352; found: 375.1 [M^++Na], 727.5 [$2M^+$+Na].

Boc-Gly-Gpn-OH (**2**): Yield: 78%; m.p. 119–120 °C; ^{1}H NMR ($CDCl_3$) $\delta = 1.2-1.6$ ((m, 10H; cyclohexyl) and (s, 9H; Boc CH_3)), 2.2 (s, 2H; Gpn $C^\alpha H_2$), 3.3 (d, 2H; Gpn $C^\gamma H_2$), 3.8 (d, 2H; Gly $C^\alpha H_2$), 5.5 (s, 1H; Gly NH), 7.0 ppm (s, 1H; Gpn NH); MS: *m*/*z* cald: 328; found: 351 [M^++Na], 679.3 [$2M^+$+Na].

Boc-Aib-Gpn-OH (**3**): Yield: 84%; m.p. 136–137 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.2-1.5$ ((m, 10H; cyclohexyl), (s, 6H; Aib CH_3) and (s, 9H; Boc CH_3)), 2.27 (s, 2H; Gpn $C^\alpha H_2$), 3.22 (d, 2H; Gpn $C^\gamma H_2$), 5.05 (s, 1H; Aib NH), 7.15 ppm (t, 1H; Gpn NH). MS: *m*/*z* cald: 356; found: 379 [M^++Na], 735.5 [$2M^+$+Na].

Boc-Aib-Gpn-OMe (**4**): Boc-Aib-OH (0.40 g, 2 mmol) was dissolved in THF (5 ml), 0.46 g (2 mmol) of Gpn-OMe obtained from its hydrochloride was added followed by DCC (0.40 g, 2 mmol) and HOBt (0.270 g). The mixture was stirred at room temperature for 12 h. The precipitated DCU was filtered and the THF was evaporated. It was redissolved in EtOAc and washed with HCl (1N), Na_2CO_3 (1M), and water. The solvent was then dried over anhydrous Na_2SO_4 and evaporated in vacuo, to yield the peptide as white crystalline solid. The peptide was purified by medium pressure liquid chromatography over a reverse-phase C_{18} column (40–60 μm). The identity of the peptide was confirmed by NMR spectroscopy (400 MHz) and electrospray mass spectrometry. Yield: 82%; m.p. 109–110 °C; ^{1}H NMR ($CDCl_3$) $\delta = 1.25-1.6$ ((m, 10H; cyclohexyl), (s, 6H; Aib CH_3) and (s, 9H; Boc CH_3)), 2.3 (s, 2H; Gpn $C^\alpha H_2$), 3.25 (d, 2H; Gpn $C^\gamma H_2$), 3.65 (s, 3H; OCH_3), 5.0 (s, 1H; Aib NH), 7.05 ppm (s, 1H; Gpn NH). MS: *m*/*z* cald: =370; found: 393.2 [M^++Na], 763.5 [$2M^+$+Na].

X-ray diffraction: Single crystals suitable for X-ray diffraction were obtained by slow evaporation of concentrated solution of the mixture of aqueous and organic solvents. Table 1 summarizes the crystallographic data and other details for compounds **1** to **4**. X-ray data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer, by using $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). ω-scan type was used. Structures were obtained by direct methods by using SHELXS-97.[24a] Refinement was carried out against F^2 with the full-matrix least-squares methods by using SHELXL-97.[24b] The hydrogen atoms were located from different Fourier maps, except for the Piv group in peptide **1**. The standard deviations in bond lengths were approximately 0.004 Å and those of bond angles were approximately 0.2°. CCDC-200611 **1**, CCDC-208874 **2**, CCDC-208872 **3**, and CCDC-208873 **4** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

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