γ-Peptide Structures

DOI: 10.1002/ange.200501055

C_9 Helices and Ribbons in γ-Peptides: Crystal Structures of Gabapentin Oligomers**

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The insertion of additional atoms into α-polypeptide backbones by the introduction of β-, γ-, and higher ω-amino acid residues expands the range of polypeptide secondary structures.[1] Two new helices, the 2.5_{12} ((*P*)-2.5_1) helix and the 3_{14} ((*M*)-3_1) helix, were first identified in studies of oligopeptides containing β-amino residues.[2] Several recent studies have focused on the conformations of peptides containing β- and γ-amino acids.[3] The symmetrically β,β-disubstituted γ-amino acid gabapentin (Gpn, Figure 1a) is a readily available

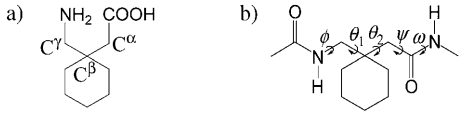

Figure 1. a) The amino acid gabapentin (Gpn); b) parameters used to define the backbone dihedral angles of the Gpn residue.

stereochemically constrained γ-amino acid residue.[4] Four degrees of torsional freedom, ϕ, θ_1, θ_2, and ψ (Figure 1b), are available for the Gpn residue. The presence of geminal substituents at the central C^β atom limits the range of conformations about the C^γ–C^β (θ_1) and C^β–C^α (θ_2) bonds to the two possible *gauche* conformations ($\theta_1 \approx \theta_2 \approx \pm 60°$).[4a,5] Herein, we describe the crystal structures of the peptides Boc-Gpn-Gpn-NHMe and Boc-Gpn-Gpn-Gpn-Gpn-NHMe (Boc = *tert*-butoxycarbonyl), which provide examples of C_9-hydrogen-bond-stabilized, folded conformations (in which the hydrogen bonds enclose a ring of nine assorted atoms). Repetition of this hydrogen-bonded motif reveals new families of C_9 ribbons and C_9 helices.

Figure 2 shows the molecular conformations determined in crystals by X-ray diffraction for Boc-Gpn-Gpn-NHMe and

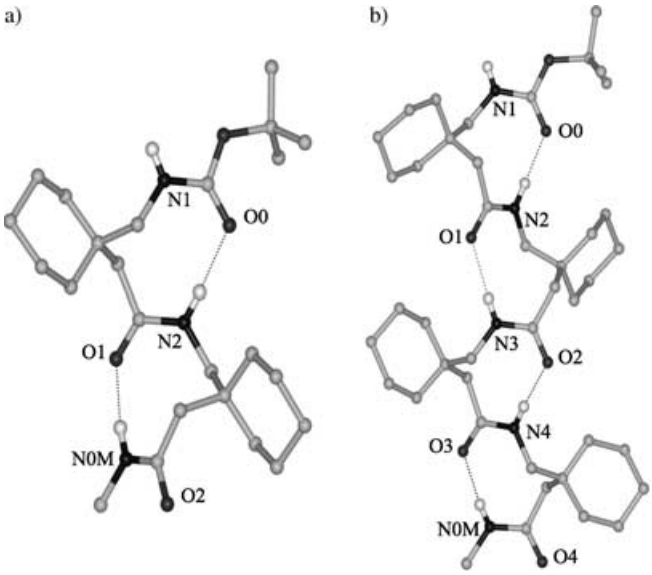

Figure 2. Molecular conformation of a) Boc-Gpn-Gpn-NHMe and b) Boc-Gpn-Gpn-Gpn-Gpn-NHMe in crystals; the intramolecular hydrogen bonds are represented as dotted lines.

Boc-Gpn-Gpn-Gpn-Gpn-NHMe. The observed backbone torsion angles and hydrogen-bond parameters are summarized in Table 1 and Table 2. In all six independent Gpn residues in these two structures, a C_9 hydrogen bond between the C=O moiety of the preceding residue (i–1) and the N–H group of the succeeding residue (i+1) is observed. The

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[**] This research was supported by a grant from the Council of Scientific and Industrial Research (CSIR) and Program Support in the area of Molecular Diversity and Design, Department of Biotechnology, India. We thank Dr. K. Nagarajan of Hikal R&D Centre, Bangalore (India), for the sample of gabapentin. P.G.V. and K.A. thank the CSIR for a senior research fellowship and research associateship, respectively. X-ray diffraction data were collected on the CCD facility funded under the IRHPA program of the Department of Science and Technology, India.

Table 1: Backbone torsion angles [°] for Boc-Gpn-Gpn-NHMe and Boc-Gpn-Gpn-Gpn-NHMe.[a]

	ϕ	θ_1	θ_2	ψ	ω	Orientation of aminomethyl group
Boc-Gpn-Gpn-NHMe						
Gpn (1)	108.9	−63.2	−76.6	87.2	−174.9	axial
Gpn (2)	108.2	−61.9	−78.2	88.0	−178.1	equatorial
Boc-Gpn-Gpn-Gpn-NHMe						
Gpn (1)	100.5	−69.6	−72.8	84.5	−171.6	equatorial
Gpn (2)	−103.8	69.9	73.4	−90.5	−174.5	axial
Gpn (3)	−112.2	66.8	72.6	−88.4	174.8	axial
Gpn (4)	104.6	−70.7	−71.5	97.2	179.0	equatorial

[a] Estimated standard deviation for the dihedral angles is approximately 0.4°.

Table 2: Intramolecular hydrogen bonds in Boc-Gpn-Gpn-NHMe and Boc-Gpn-Gpn-Gpn-NHMe.[a]

Donor	Acceptor	N···O [Å]	H···O [Å]	∢ N–H···O [°]
Boc-Gpn-Gpn-NHMe				
N2	O0	2.90	2.00	172.5
N0M	O1	2.86	2.00	170.8
Boc-Gpn-Gpn-Gpn-NHMe				
N2	O0	2.82	1.90	176.3
N3	O1	2.89	2.05	173.6
N4	O2	2.87	2.03	169.9
N0M	O3	2.90	2.05	168.4

[a] Estimated standard deviations in the hydrogen-bond lengths N···O and H···O and angles are approximately 0.01 Å, 0.02 Å, and 2.5°, respectively.

backbone torsion angles for all six residues reveal that they adopt very similar *gauche*,*gauche* conformations. Interestingly, three of the observed cyclohexane rings have the amino methyl group in an axial orientation, whereas in the other three this group adopts an equatorial orientation. A comparison of the di- and tetrapeptide structures, however, reveals an important difference. In the protected dipeptide the two Gpn residues adopt the same combination of signs for the four backbone torsion angles. In sharp contrast, in the protected tetrapeptide the combination of signs for the torsion angles for residues 1 and 4 is opposite to that of residues 2 and 3. Notably, as both structures are of achiral peptides crystallizing in centrosymmetric space groups, the choice of sign reported in Table 1 is arbitrary. However, differences in the handedness of conformations between residues in the same structure can be observed.

The repetitive C_9-hydrogen-bonded structures determined in the two peptides can be viewed as analogues of the corresponding C_7-hydrogen-bonded structures described for polypeptides composed of α-amino acids. The isolated γ turn stabilized by a 3→1 hydrogen bond between C=O ($i-1$) and N–H ($i+1$) has been observed in the structure of proteins and peptides.[6] The repetitive C_7 structure, the 2.2_7 helix, was first proposed for α polypeptides by Donohue.[7] Subsequently, two structures were theoretically considered, the 2_7 ribbon ($\phi = -70°$, $\psi = 70°$) and the 2.2_7 helix ($\phi = -78.1°$, $\psi = 59.2°$).[8] However, these structures have not been experimentally observed in the crystal structures of polypeptides and proteins. A recent report describes two consecutive γ-turn conformations in a synthetic dipeptide.[9] Ab initio molecular orbital calculations have been used to examine all possible periodic structures of γ-peptides. These studies suggest that helical structures with hydrogen bonds enclosing 14- and 9-membered rings are most stable.[10] An isolated C_9 hydrogen bond has been described in the crystal structure of a γ-dipeptide.[11] A C_9-ribbon structure has also been postulated on the basis of NMR spectroscopy data for γ-peptide oligomers of *cis*-γ-amino-L-proline.[12] The C_9 structures described above constitute an expansion of the C_7 structures by the insertion of two additional backbone atoms.

Figure 3 shows views of three possible repetitive polypeptide structures generated by using the C_9-repeat motif. Figure 3b shows the C_9 helix generated by repeating the structure determined for the dipeptide Boc-Gpn-Gpn-NHMe. In this case, the monomeric unit is a single Gpn residue and the combination of signs for the torsion angles is the same for all residues in the helical structure. The number of residues/turn (n) value in this C_9 helix is 2.67 and the height/residue (h) value is 3.40 Å. The view down the axis of this 2.7_9 helix is shown in Figure 3c. A projection of the analogous C_7-helical structure of α polypeptides (2.2_7 helix) is also shown for comparison (Figure 3a).

Inspection of the torsion angles in Table 1 for the tetrapeptide Boc-Gpn-Gpn-Gpn-Gpn-NHMe reveals that the signs of the four torsion angles between adjacent Gpn residues can also be inverted. It is thus possible to consider a range of repetitive C_9 structures employing different handedness at adjacent residues. The helical structure pictured in Figure 3b may be represented by the notation ++++, which indicates that the handedness of all residues along the polymeric chain is identical. Two alternative structures, +−+− and ++−−++−−, are illustrated in Figure 3d–g. The regular alternation of sign in the +−+− structure leads to a C_9 ribbon with a significant degree of curvature, which can result in cyclization after 79 residues (Figure 4). Figure 3f and g illustrates two views of the ++−−++−− structure, where the repeating unit consists of four Gpn residues. This structure can be obtained by repeating the backbone torsion angles determined in the structure of Boc-Gpn-Gpn-Gpn-Gpn-NHMe. Once again, the structure can be viewed as a ribbon but one with a much smaller degree of curvature than the C_9 ribbon obtained from the regular +−+− combination (Figure 3d).

The structures described herein for Gpn oligomers reveal a new family of polypeptide structures which are C_9 ribbons and helices. The C_9-hydrogen-bond structure is determined by a specific combination of the four degrees of torsional freedom at the Gpn residue and is independent of the flanking residues. In Gpn homooligomers, C_9 helices and ribbons will undoubtedly be the energetically favored structures. In mixed sequences, where Gpn is inserted into polypeptides containing α- and β-amino acids, alternative hydrogen-bonding patterns may be anticipated.[1b]

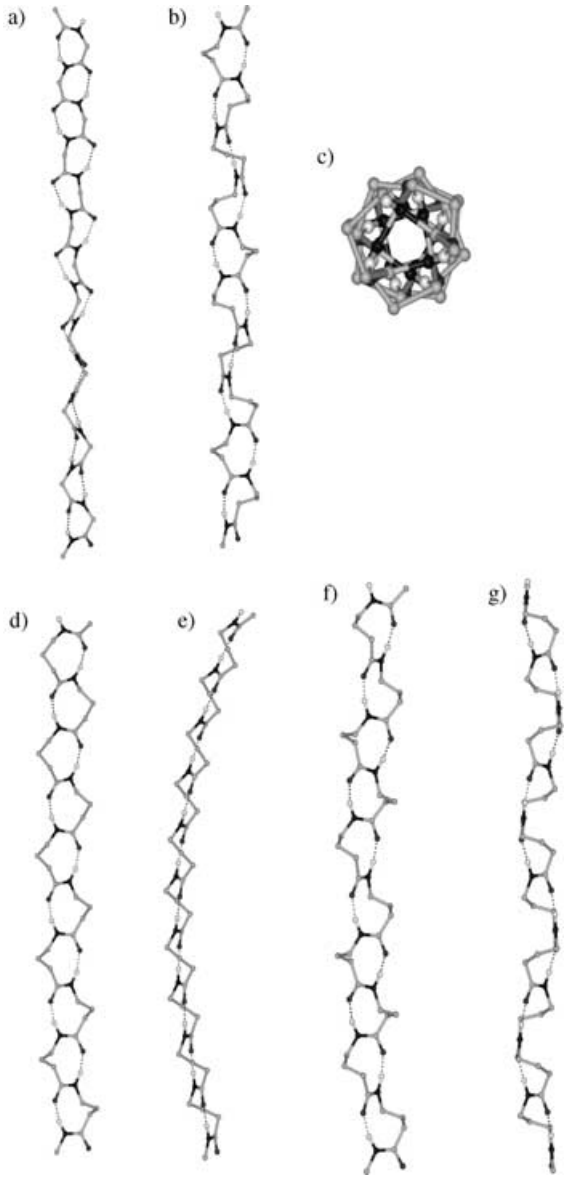

Figure 3. a) 2.2_7 helix formed by α-amino acids; b) 2.7_9 (C_9) helix formed by the Gpn ++++ repeat motif; c) view down the helix axis of the 2.7_9 (C_9) helix; d) C_9 ribbon formed by the Gpn +−+− repeat motif; e) side view of the C_9 ribbon formed by the Gpn +−+− repeat motif; f) C_9 ribbon formed by the Gpn ++−−++−− repeat motif; g) side view of the ribbon formed by the Gpn ++−−++−− repeat motif. (Signs + and − denote the handedness of the Gpn residues.)

Figure 4. Cyclic structure generated by the repetition of the Gpn +−+− motif.

Experimental Section

Peptides Boc-Gpn-Gpn-NHMe (peptide 1) and Boc-Gpn-Gpn-Gpn-Gpn-NHMe (peptide 2) were synthesized by conventional solution-phase procedures. Coupling was mediated by *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) in tetrahydrofuran (THF). Boc-Gpn-NHMe was synthesized from Boc-Gpn-OH by using a mixed anhydride strategy and employing methylamine as the amino compound. Subsequent elongation of the chain was carried out by formic acid mediated deprotection of the Boc group, followed by coupling to Boc-Gpn-OH. Stepwise elongation was used up to the tetrapeptide structure. The peptides, which were obtained as crystalline solids, were further purified by medium-pressure liquid chromatography over a reversed-phase C_{18} column (40–60 μm) and characterized by 500 MHz ^{1}H NMR spectroscopy; m.p.: 96–97 °C for peptide 1; 162–163 °C for peptide 2.

Single crystals suitable for X-ray diffraction were grown by slow evaporation from mixtures of ethyl acetate/petroleum ether (peptide 1) and methanol/chloroform (peptide 2). X-ray intensity data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer, by using $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The ω scan type was used. In total, 37219 reflections were measured (5232 independent reflections) with $2\theta_{max} = 53.6°$ and $R_{int} = 0.06$ for peptide 1 ($C_{24}H_{43}N_3O_4$, $M_w = 437.6$). The data were collected from a crystal of dimensions $0.14 \times 0.10 \times 0.03$ mm. The space group is *Pbca* with unit cell dimensions and parameters of $a = 11.771(6)$, $b = 20.724(11)$, and $c = 22.206(12)$ Å, $V = 5233(5)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.11$ g cm^{-3}, $\mu = 0.07$ mm^{-1}, and $F(000) = 1920$. For peptide 2 ($C_{42}H_{73}N_5O_6$, $M_w = 744.0$) 31521 reflections were measured (8500 independent reflections) with $2\theta_{max} = 53.4°$ and $R_{int} = 0.03$. The crystal dimensions are $0.22 \times 0.14 \times 0.10$ mm. The space group is $P2_1/c$ with unit cell dimensions and parameters of $a = 10.004(7)$, $b = 12.648(8)$, and $c = 35.240(2)$ Å, $\beta = 92.632°$ (12), $V = 4454.0(5)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.11$ g cm^{-3}, $\mu = 0.07$ mm^{-1}, and $F(000) = 1632$. Both the structures were solved by direct methods by using SHELXS-97[13a] and refined against F^2, with full-matrix least squares methods, by using SHELXL-97.[13b] All the hydrogen atoms were located from the difference Fourier map. The final R value for peptide 1 was $R_1 = 0.0616$ ($wR_2 = 0.1461$) for 3134 observed reflections with $F_o \geq 4\sigma|F_o|$ and for 452 parameters. The data to parameter ratio is 6.9:1.0 and the goodness of fit $S = 0.99$. The largest difference peak was 0.18 e Å^{-3} and the largest difference hole was −0.15 e Å^{-3}. The final R value for peptide 2 was $R_1 = 0.0655$ ($wR_2 = 0.1782$) for 5474 observed reflections with $F_o \geq 4\sigma|F_o|$ and for 758 parameters. The data to parameter ratio is 7.2:1.0 and the goodness of fit $S = 1.16$. The largest difference peak was 0.27 e Å^{-3} and the largest difference hole was −0.16 e Å^{-3}. CCDC 266651 (Boc-Gpn-Gpn-NHMe) and CCDC 266652 (Boc-Gpn-Gpn-Gpn-Gpn-NHMe) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Received: March 23, 2005
Published online: July 8, 2005

Keywords: helical structures · hydrogen bonds · peptide conformation · ribbon structures

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