ABSTRACT:
The design of folded structures in peptides containing the higher homologues of \(\alpha\)-amino acid residues requires the restriction of the range of local conformational choices. In \(\alpha\)-amino acids stereochemically constrained residues like \(\alpha,\alpha\)-dialkylated residue, aminoisobutyric acid (Aib), and \(\alpha\)-Proline (\(\alpha\)-Pro) have proved extremely useful in the design of helices and hairpins in short peptides. Extending this approach, backbone substitution and cyclization are anticipated to be useful in generating conformationally constrained \(\beta\)- and \(\gamma\)-residues. This brief review provides a survey of work on hybrid peptide sequences concerning the conformationally constrained \(\gamma\)-amino acid residue 1-aminomethyl cyclohexane acetic acid, gabapentin (Gpn). This achiral, \(\beta,\beta\)-disubstituted, \(\gamma\)-residue strongly favors gauche-gauche conformations about the \(C^\alpha-C^\beta\) (\(\theta_2\)) and \(C^\beta-C^\gamma\) (\(\theta_1\)) bonds, facilitating local folding. The Gpn residue can adopt both \(C_7\) (\(NH^{+3}\rightarrow CO_i\)) and \(C_9\) (\(CO_i\rightarrow NH^{+1}\)) hydrogen bonds which are analogous to the \(C_5\) and \(C_7\) (\(\gamma\)-turn) conformations at \(\alpha\)-residues. In conjunction with adjacent residues, Gpn may be used in \(\alpha\gamma\) and \(\gamma\alpha\) segments to generate \(C_{12}\) hydrogen bonded conformations which may be considered as expanded analogs of conventional \(\beta\)-turns. The structural characterization of \(C_{12}\) helices, \(C_{12}/C_{10}\) helices with mixed hydrogen bond directionalities and \(\beta\)-hairpins incorporating Gpn residues at the turn segment is illustrated. © 2010 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 94: 733–741, 2010.

Keywords: hybrid peptides; gabapentin; conformational constrained residues; helices and hairpins

INTRODUCTION

The importance of intermolecular hydrogen bonds in facilitating backbone folding in polypeptides was first recognized by Huggins \(^{1,2}\) and later, more famously, by Linus Pauling when he postulated the \(\alpha\)-helix \(^3\) and \(\beta\)-sheet \(^4\) structures for polymeric chains composed of \(\alpha\)-amino acids. Half a century of structural studies on peptides and proteins have established two major classes of helices, the \(3_{10}\)-helix stabilized by a repetitive \(4\rightarrow1\) (\(C_{10}\)) hydrogen bond between \(CO_i\cdot \cdot \cdot NH^{+3}\) and the \(\alpha\)-helix stabilized by a repetitive \(5\rightarrow1\) (\(C_{13}\)) hydrogen bond between \(CO_i\cdot \cdot \cdot NH^{+4}\) (see Figure 1). \(^{3,8–10}\) Helices with smaller hydrogen bonded rings like 2.2 \(\gamma\) (\(C_7\)) ribbon and the \(\pi\)-helix (\(C_{16}\)) are very rarely found in protein structures over any substantial length of polypeptide chain. \(^{11–13}\) Hydrogen bonding patterns in peptides and protein helices run, almost exclusively, in one direction with the acceptor CO group at the N-terminus of the chain while the donor NH group is toward the C-terminus. Alternative hydrogen bonded struc-
tures were considered in a paper published in 1950 by Bragg et al., although the important condition of the planarity of the peptide unit was not imposed.

Hydrogen bond directionalities opposite to those observed in \( \alpha \)-polypeptides were originally considered in studies of polypeptides containing \( \beta \)-peptide bonds. More recently, studies of peptides composed of backbone homologated amino acids established that helical structures with different hydrogen bond directionalities could be formed in oligo-\( \beta \)-peptides.

The work of the groups of Dieter Seebach in Zurich and Sam Gellman in Wisconsin conclusively demonstrated the occurrence of 12-helical (C\textsubscript{12}) and the 14-helical (C\textsubscript{14}) structures in \( \beta \)-amino acid peptides. In the C\textsubscript{12}-helix, the hydrogen bonding pattern is analogous to that of the \( \alpha \)-peptide 3\textsubscript{10}-helix, while the C\textsubscript{12} structure may be considered as a backbone expanded analog of the C\textsubscript{10}-helix in \( \alpha \)-amino acid sequences. The 14-helix is a novel structure in which the hydrogen bonds run in the opposite direction NH\(_i\) / CO\(_{i+2}\) (see Figure 1). Backbone homologation results in three degrees of torsional freedom for \( \beta \)-residues and four degrees for \( \gamma \)-residues, suggesting that conformational flexibility may impede the development of folded structures. In the case of \( \alpha \)-amino acids, the largest extent of conformationally accessible Ramachandran space is observed for the unsubstituted residue glycine (Gly). Introduction of a single substitution at the C\textsubscript{\alpha} atom, as in alanine (Ala), results in appreciable reduction in the extent of allowed conformational space.

In the case of unsubstituted \( \alpha \)-amino acid residues, backbone homologation can result in a substantial expansion of the degree of accessible conformational space. As illustrated in Figure 3, two torsional degrees of freedom, N-C\textsubscript{\alpha} (\( \phi \)) and C\textsubscript{\alpha}-C (\( \psi \)), have been used to define the conformational space for an individual residue. Backbone homologation results in three degrees of torsional freedom for \( \beta \)-residues and four degrees for \( \gamma \)-residues, suggesting that conformational flexibility may impede the development of folded structures. In the case of \( \alpha \)-amino acids, the largest extent of conformationally accessible Ramachandran space is observed for the unsubstituted residue glycine (Gly). Introduction of a single substitution at the C\textsubscript{\alpha} atom, as in alanine (Ala), results in appreciable reduction in the extent of allowed conformational space. Introduction of the second C\textsubscript{2} substituent, as in the case of the \( \alpha, \alpha \)-dialkylated residue, aminoisobutyric acid (Aib), results in a dramatic reduction of the allowed (\( \phi \), \( \psi \)) space, restricting the Aib residue to largely populate 3\textsubscript{10} or \( \alpha \)-helical conformations (see Figure 4). The fact

![FIGURE 1](image-url) 3\textsubscript{10} Helix and \( \alpha \)-helix observed \( \alpha \)-amino acid polypeptides. C\textsubscript{12} helix and C\textsubscript{14} helix in the crystal structures of Boc-(trans-ACPC)\textsubscript{5}-OBn\textsuperscript{5} and Boc-(trans-ACHC)\textsubscript{6}-OBn\textsuperscript{6} (Modified from Ref. 7).
that Aib residues strongly support helical folding in peptide sequences has been established by extensive structural studies beginning in the mid 1970s.\textsuperscript{34–39} Indeed, these properties have been used in the design of peptide conformational restraints for the construction of well-folded helices in oligopeptides about 7–9 residues in length.\textsuperscript{7,40–41} In the case of \(\alpha\)-polypeptides, the use of backbone conformational restraints has also been used in the design of peptide hairpins. A D-Proline (\(\delta\)-Pro) residue positioned in the center of the sequence is found to facilitate prime turn (Type I/Type II) formation.\textsuperscript{42–44} Side chain backbone cyclization in the case of Pro results in the formation of a pyrrolidine ring, restricting the range of accessible conformations for \(\phi\) to \(-60^\circ \pm 20^\circ\), in the case of \(\delta\)-Pro (see Figure 4). A large number of synthetic peptide hairpins with \(\delta\)-Pro-Xxx as the turn forming segment have been established by X-ray diffraction in crystals and NMR spectroscopy in solution.\textsuperscript{30,43–54} The introduction of backbone constraints may then be the method of choice in restricting local conformational choices in the higher homologues of \(\beta\)- and \(\gamma\)-residues. Indeed, the first successful crystallographic characterization of the 12-helix and 14-helix in oligo-\(\beta\)-peptides used sequences containing the cyclic \(\beta\)-amino acids, trans-2-aminocyclopentane carboxylic acid (ACPC) and trans-2-aminocyclohexane carboxylic acid (ACHC).\textsuperscript{5,25} In these residues, the torsional freedom about the \(C_\beta-C^\gamma\) bond (\(\theta\)) is restricted by covalent cyclization. The extensive work of Seebach and coworkers has also established the formation of folded structures in peptides containing \(\beta\)- and \(\gamma\)-residues substituted at backbone carbon atoms.\textsuperscript{16,19,20,22,26,55–57}

To develop a suitable stereochemically constrained residue for exploring the conformational properties of the peptides containing \(\gamma\)-amino acid residues, we turned to the readily available amino acid, 1-(aminomethyl)-cyclohexaneacetic acid, gabapentin (Gpn). Gpn is an achiral, \(\beta\),\(\beta\)-disubstituted backbone expanded analog of 1-aminocyclohexane-1-carboxylic acid (Ac6c) which has been extensively investigated.\textsuperscript{58}

Figure 5 illustrates the structures of 1-aminocyclohexane-1-
carboxylic acid (Ac6c), Gpn and two related isomers of the corresponding \( \beta \)-amino acids. The ready availability of Gpn, a consequence of its widespread use in the treatment of epileptic and neuropathic pain, makes it an attractive residue for use in synthetic peptide design studies.

**CONFORMATIONAL PROPERTIES OF GABAPENTIN PEPTIDES**

Extensive structural investigations of short model sequences containing Gpn residues carried out at Bangalore,59–62 have established that the geminal \( \beta \)-substituents restrict the torsional freedom about the \( C^\beta-C^\gamma(\theta_1) \) and \( C^\alpha-C^\beta(\theta_2) \) bonds as summarized in Figure 6.61 Both the \( \theta_1 \) and \( \theta_2 \) torsion angles, almost exclusively, adopt gauche conformations (\( \theta_1 \sim 60^\circ \) and \( \theta_2 \sim 60^\circ \)). The formation of both the \( C_7 \) and \( C_9 \) hydrogen bonded conformations at the Gpn residue has been demonstrated.61,62 For \( \gamma \)-residues, the \( C_7 \) structure is formed by a \( \text{NH} \rightarrow \text{CO} \) hydrogen bond, which is analogous to the \( C_5 \) structure, characteristic of the fully extended \( \alpha \)-polypeptide conformations in peptides containing \( \alpha,\alpha \)-diaminobutyric acid (Deg) and \( \alpha,\alpha \)-diproplylglycine (Dpg).63–66 In the \( C_9 \) structure, \( \text{CO}_{i+1} \rightarrow \text{NH}_{i+1} \) hydrogen bonds are formed at Gpn. This is analogous to the \( C_7 \) (\( \gamma \)-turn) structure observed for \( \alpha \)-amino acid residues (see Figure 7). The restriction of \( \theta_1 \), \( \theta_2 \) to gauche values facilitate local backbone folding in a manner analogous to that of Aib and Ac6c residues in \( \alpha \)-polypeptides. These conformational properties of Gpn residues may be used to advantage in the design of helical structures in hybrid peptides. Figure 8 illustrates the well-dispersed NMR spectrum observed for the octapeptide Boc-(Gpn-Aib)4-OMe obtained in chloroform solution. Figure 8 establishes a succession of \( \text{NH-H-CO-CO-H(N)} \) NOEs characteristic of a helical conformations. The formation of a continuous helical structure is confirmed by an X-ray diffraction study, which establishes the presence of four \( C_{12} \) hydrogen bonds in the segment Aib (2) to Aib (6). The N-terminal residues Gpn (1) and Gpn (7) adopt a local \( C_9 \) conformation. The \( d_{\text{NN}} \) distances for an \( \alpha \)-residue in a helical conformation is \( \sim 2.7 \) \( \text{Å} \), while that for a \( \gamma \)-residue is \( \sim 3.6 \) \( \text{Å} \). Inspection of the NOE data in Figure 8 reveals a pattern of strong (\( \alpha \)) and weak (\( \gamma \)) \( d_{\text{NN}} \) NOEs. The observed \( C_{12} \) helix in the \( (\alpha\gamma)_n \) sequence may be considered as a backbone expanded analog of the \( \alpha \)-polypeptide \( 3_{10} \) helix.67

The diversity of helical structures that may be generated in \( (\alpha\gamma)_n \) hybrid peptides is illustrated by the mixed \( C_{12}/C_{10} \) conformation established in crystals of the tetrapeptide, Boc-Leu-Gpn-Leu-Aib-OMe (see Figure 9).68 In this peptide, the
N-terminus $\alpha\gamma$ segment adopts the anticipated $C_{12}$ hydrogen bonded turn, while the $\gamma\alpha$ segment forms a $C_{10}$ hydrogen bonded structure. Inspection of the torsion angles at Leu (1), Gpn (2), and Leu (3) residues reveals that both the $\alpha$ residues adopt polyproline ($P_{12}$) conformations. The Gpn (2) residue adopts a conformation characterized by the formation of a $C_{12}/C_{10}$ hydrogen bonds. Comparison of the backbone dihedral angles with those determined for a regular $C_{12}$ helix (see Figure 8) reveals that a mixed hydrogen bonded structure is facilitated by a distinct conformation at the Gpn residue.

**FIGURE 5** Structures of $\text{Ac}_6\text{c}_c$, isomers of $\beta$-$\text{Ac}_6\text{c}_c$ and the backbone expanded analog gabapentin (Gpn).

**FIGURE 6** The observed distribution of the backbone torsions, $\theta_1$ and $\theta_2$, in the experimentally determined crystal structures of gabapentin containing peptides. The number of Gpn residues plotted is 81 (Modified from Ref. 61).

**FIGURE 7** Hydrogen bonded turns, $C_\alpha$, $C_\gamma$ ($\gamma$-turn) in $\alpha$-residues (left) and the corresponding analogs $C_7$, $C_9$ in $\gamma$-residues (right).
which has been termed as “nonhelical”.\textsuperscript{61} The mixed C\textsubscript{12}/C\textsubscript{10} hydrogen bonded pattern obtained in the \(\alpha\) segment may be propagated to obtain the mixed C\textsubscript{10}/C\textsubscript{12} helix in an \((\alpha\gamma)\)\textsubscript{n} sequence (see Figure 9). Figure 10 summarizes the backbone conformational requirements for the regular C\textsubscript{12} helix and the mixed C\textsubscript{12}/C\textsubscript{10} helix. In both cases, the Gpn residue adopts gauche-gauche conformations about the \(\text{C}^a-C^b\) (\(\phi_2\)) and \(\text{C}^b-C^c\) (\(\phi_1\)) bonds. In the regular \((\alpha\gamma)\)\textsubscript{n} C\textsubscript{12}-helix all the hydrogen bonds formed are of the type \(\text{CO}_i-\cdots\text{NH}_{i+3}\). In contrast, in the C\textsubscript{12}/C\textsubscript{10} helix the directionality of the C\textsubscript{12} (\(\text{CO}_i-\cdots\text{NH}_{i+3}\)) and C\textsubscript{10} (\(\text{NH}_i-\cdots\text{CO}_{i+1}\)) hydrogen bonds are opposite. This structural versatility of the Gpn residue may be used to design novel helices if the conformational choices at the \(\alpha\)-residue are restricted by covalent modification.

Despite the limited range of conformations that are sterically accessible at Gpn residues, it is possible to generate \(\beta\)-hairpins incorporating Gpn in the turn segment. This feature is illustrated in a structure of the octapeptide of Boc-Leu-Phe-Val-Gpn-Leu-Phe-Val-OMe (see Figure 11).\textsuperscript{67} The experimentally observed conformation for the Gpn residues in peptide structure cluster into limited regions of \((\phi, \psi)\) space, with \(\phi_1\) and \(\phi_2\) overwhelmingly clustering around values of \(\pm 60^\circ\).\textsuperscript{61}

**PROSPECTS FOR HYBRID PEPTIDE DESIGN**

The repertoire of hybrid peptide backbones which have been structurally characterized will rapidly expand with the use of \(\beta\)- and \(\gamma\)-residues, in which the range of accessible conformations is restricted. In hybrid sequences, variations are possible both along the backbone and in the side chains used. Figure 12 illustrates the structural characteristics of helices with varying sizes of hydrogen bonded rings in a 11-residue peptide sequence containing two centrally positioned \(\beta\)-amino
acids. This segment presents an opportunity to characterize $C_{14} (\alpha\beta\beta\alpha)$, $C_{15} (\alpha\beta)$, $C_{11} (\beta\alpha)$, $C_{14} (\beta\alpha\alpha)$ hydrogen bonds. The overall helical folding of the peptide bears a striking resemblance to $\alpha$-polypeptide helices. The design of biologically active peptides containing backbone homologated amino acids is anticipated to enhance the proteolytic

![Figure 9: Mixed $C_{12}/C_{10}$ helical conformation observed for the tetrapeptide, Boc-Leu-Gpn-Leu-Alb-OMe, as observed in the crystal structure. Middle: Reverse hydrogen bond directionalities observed in $C_{12}/C_{10}$ helix (Taken from Ref. 68). Right: Model for a mixed $C_{12}/C_{10}$ helix generated by extending the backbone conformation observed in the crystals (Modified from Ref. 60).](image)

![Figure 10: $(\Phi, \Psi)$ Representations for $\alpha$- and $\gamma$-residues. For $\gamma$-residues $\theta_1$ and $\theta_2$ are restricted to values of $\pm 60^\circ$ (gauche).](image)
stability of the peptide sequences. Retention of biological activity will demand the design of sequences ensuring placement of side chains in appropriate orientations on a folded backbone scaffold. This will become possible in a rational manner when further conformational data is obtained on a variety of hybrid sequences.

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FIGURE 11 left: β-hairpin conformation observed in the crystal structure of the octapeptide, Boc-Leu-Phe-Ala-Aib-Gpn-Leu-Phe-Val-OMe. The central Aib-Gpn segment adopts a novel C_{12} turn, with the Gpn adopting an unusual gauche-trans conformation (Modified from Ref. 61). Right: Sideview and the ribbon representation of the hairpin.

FIGURE 12 left: Consecutive β-residues incorporated in the middle of an all α-peptide sequence, Boc-Val-Ala-Phe-Alb-βVal-βPhe-Alb-Val-Ala-Phe-Alb-OMe. Right: The central αββααβαα segment highlighting the mixed C_{14}/C_{15}/C_{11}/C_{14} hydrogen bonds.


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