

Conformational properties of hybrid peptides containing α - and ω -amino acids*

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Abstract: This review briefly surveys the conformational properties of guest ω -amino acid residues when incorporated into host α -peptide sequences. The results presented focus primarily on the use of β - and γ -residues in $\alpha\omega$ sequences. The insertion of additional methylene groups into peptide backbones enhances the range of accessible conformations, introducing additional torsional variables. A nomenclature system, which permits ready comparisons between α -peptides and hybrid sequences, is defined. Crystal structure determination of hybrid peptides, which adopt helical and β -hairpin conformations permits the characterization of backbone conformational parameters for β - and γ -residues inserted into regular α -polypeptide structures. Substituted β - and γ -residues are more limited in the range of accessible conformation than their unsubstituted counterparts. The achiral β,β -disubstituted γ -amino acid, gabapentin, is an example of a stereochemically constrained residue in which the torsion angles about the $C^\beta-C^\gamma$ (θ_1) and $C^\alpha-C^\beta$ (θ_2) bonds are restricted to the *gauche* conformation. Hybrid sequences permit the design of novel hydrogen bonded rings in peptide structures.

The conformational properties of peptides derived from α -amino acid residues are well established. The Ramachandran (ϕ,ψ) dihedral angles and intramolecular hydrogen bonding patterns serve as descriptors of polypeptide backbone structures. These parameters provide a convenient means of identifying and classifying secondary structural features in peptides and proteins (1,2). Limited attention has been focused on the stereochemistry of the polypeptides containing higher ω -amino acid (3), until the finding of the groups of Seebach and Gellman that the oligo- β -peptides adopt novel folded structures, with hydrogen bonding

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patterns distinct from those observed in poly- α -peptides (4–11). The term ω -amino acid is used to refer to the entire family of residues generated by homologation of the backbone. The growing body of work on peptides containing β -amino acid residues points to a tendency of oligomeric sequences to adopt well defined structures, despite the possibility of enhanced conformational freedom, because of the presence of an additional torsional variable (θ) about the C^α – C^β bond. More recent studies have expanded the range of the β -amino acids used in the design of synthetic peptides and have extended the exploration of conformational space to studies of oligomers of γ - and δ -amino acid residues (12,13). The use of ω -amino acid residues in conjunction with α -residues permits systematic exploration of the effects of introducing additional backbone atoms into well characterized α -peptide structures. Modification of the backbone of α -peptides can result in proteolytically stable sequences, a property of some importance in the design of analogs of biologically active sequences. Arno Spatola reviewed this area two decades ago, introducing the concept of peptide bond surrogates and the psi-bracket nomenclature for backbone modified structures (14). We present here a brief overview of ongoing studies on $\alpha\omega$ hybrid sequences in our laboratory.

Conformational Properties of Hybrid $\alpha\omega$ -Sequences

Torsion angle and hydrogen bond nomenclature

The nomenclature of the canonical hydrogen bonded structures found in poly- α -peptides is summarized in Table 1. All

widely observed hydrogen bonded structures have N-terminus acceptor $C=O$ groups ($C=O_i$) interacting with C-terminus donor NH -groups ($N_{i+n}-H$). For $n = 2$ to 4 we get the well known γ -turn (C_7), β -turn(C_{10}) and α -turn (C_{13}) structures. Repetition of these hydrogen bonded structures can lead to regular helices notably 2.2_7 , 3_{10} and α -helical structures. Propagation of a helical structure is necessarily contingent upon adoption of specific backbone (ϕ, ψ) angles (1). The $n = 1$ structure results in the formation of an eight-membered ring (hydrogen bond directionality reversed, $N \rightarrow C$) which is feasible only if the central peptide unit adopts a *cis*-geometry (17). The $n = 5$ structure, the (π -turn) which is stabilized by $6 \rightarrow 1$ hydrogen bonding is less common and is often observed at the C-terminus end of helices, resulting in the formation of Schellman motifs (18). The π -helix which results from repetition of C_{16} hydrogen bonded turns is intrinsically unstable; its larger diameter resulting in poor packing in the helix interior, with attendant loss of favorable nonbonded interactions. In all cases, the directionality of the hydrogen bond with respect to the chain direction ($C \leftarrow N$) is the same.

The most striking finding of the early work on oligomeric β -peptides is the observation of regular structures with hydrogen bonding patterns, which run in both directions, with respect to the polypeptide chain. Figure 1 illustrates five regular structures which have been experimentally realized or theoretically postulated for oligomeric β -peptides. The 8- and 12-helices have a hydrogen bond direction ($C \leftarrow N$), which is the same as that observed in α -peptides, whereas in the C_{10} and C_{14} structures the hydrogen bond directions ($N \rightarrow C$) are reversed. Figure 2 provides a summary of the potential hydrogen bonds in two residue $\alpha\omega$ sequences. In hybrid sequences, the insertion of additional atoms may perturb the formation of intramolecularly

Table 1. Canonical α - and β -polypeptide structures

Hydrogen-bonded ring size and directionality	Turn/helix ^a	Backbone torsion angles (helices)			References
		ϕ (°)	θ (°)	ψ (°)	
C_7 , $C \leftarrow N$	γ -Turn, 2.2_7 -helix	–78		59	(1)
C_{10} , $C \leftarrow N$	β -Turn, 3_{10} -helix	–49		–26	(1,15)
C_{13} , $C \leftarrow N$	α -Turn, α -helix	–57		–47	(1,15)
C_{16} , $C \leftarrow N$	π -Turn, π -helix	–57		–70	(1,15)
C_8 , $C \leftarrow N$	8-Helix	120	–72	0	(6)
C_{10} , $N \rightarrow C$	10-Helix	64	59	75	(16)
C_{12} , $C \leftarrow N$	12-Helix	95	–94	103	(11)
C_{14} , $N \rightarrow C$	14-Helix	–134	60	–140	(11)

a. Note that multiple turn types are possible which are characterized by different sets of ϕ , ψ angles. Only specific turn types give rise to the repetitive helical structures.

Figure 1. Novel helices formed by oligo- β -peptides. The structures have been generated using insight II program using theoretically or experimentally determined backbone torsion angles. Views obtained perpendicular to the helix axis and down the helix axis are shown. The values used for models are: 8-helix: $\phi = 120^\circ$, $\theta = -72^\circ$, $\psi = 0^\circ$; 10-helix: $\phi = 64^\circ$, $\theta = 59^\circ$, $\psi = 75^\circ$; 12-helix: $\phi = 100.2^\circ$, $\theta = -95.5^\circ$, $\psi = 103.8^\circ$; 14-helix: $\phi = -134.4^\circ$, $\theta = 60^\circ$, $\psi = -139.9^\circ$. For the 10/12-helix see ref. [25].

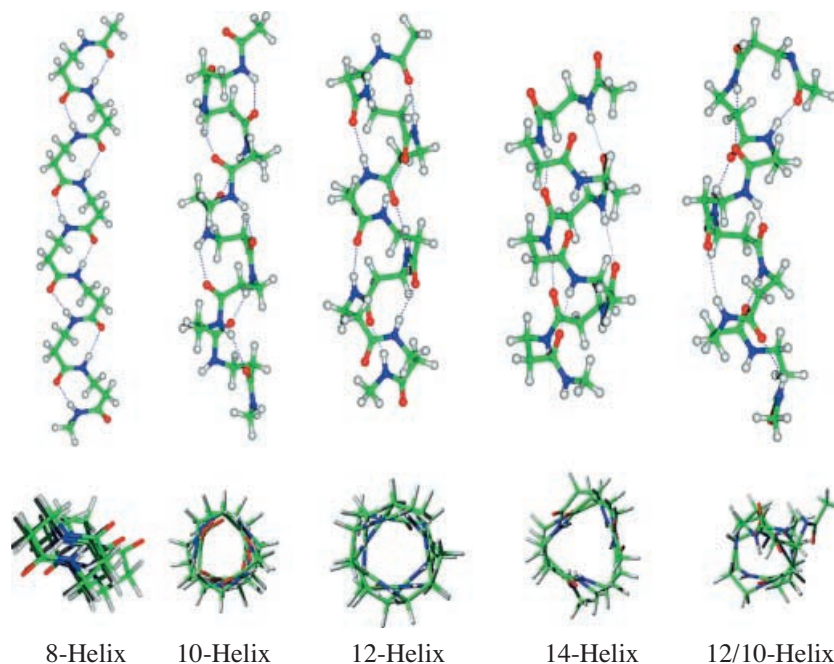


Figure 2. Potential hydrogen bonded rings in $\alpha\omega$ -hybrid sequences. Dipeptide segments which have been observed in crystal structures are circled. Segments characterized by nuclear magnetic resonance are enclosed in squares.

	$C_i=O \quad H \quad N_{i+3}$			
	α	β	γ	δ
α	(10)	(11)	12	(13)
β	(11)	(12)	(13)	14
γ	12	(13)	(14)	15
δ	(13)	14	15	16

	$N_i \quad H \quad O \quad =C_{i+1}$			
	α	β	γ	δ
α	8	9	10	11
β	9	(10)	11	12
γ	10	11	12	13
δ	11	12	13	14

$\alpha\alpha, C_{10}^{C \leftarrow N}$

$\beta\beta, C_{10}^{N \rightarrow C}$

hydrogen-bonded structures. It should be noted that if the number of backbone atoms are considered, correspondence may be obtained between $\alpha\omega$ sequences and a stretch of α -residues. For example, $\alpha\delta$ or $\beta\gamma$ segments result in insertion of nine atoms into a peptide backbone and may be formally viewed as similar to a stretch of three contiguous α -residues (Fig. 3).

Inspection of the sizes of the hydrogen bonded rings for various combinations of residues in dipeptide segments

reveal that the 10- and 13-membered hydrogen bonded rings commonly observed in α -peptide structures may be mimicked by specific hybrid combinations. For example, the conventional α -peptide β -turn may be mimicked by a $\beta\beta$ dipeptide, with a reversal of the direction of the intramolecular hydrogen bond. Figure 2 also highlights the hydrogen bonded rings, which have thus far been definitively characterized by X-ray diffraction in crystals or inferred from nuclear magnetic resonance (NMR) data in solution.

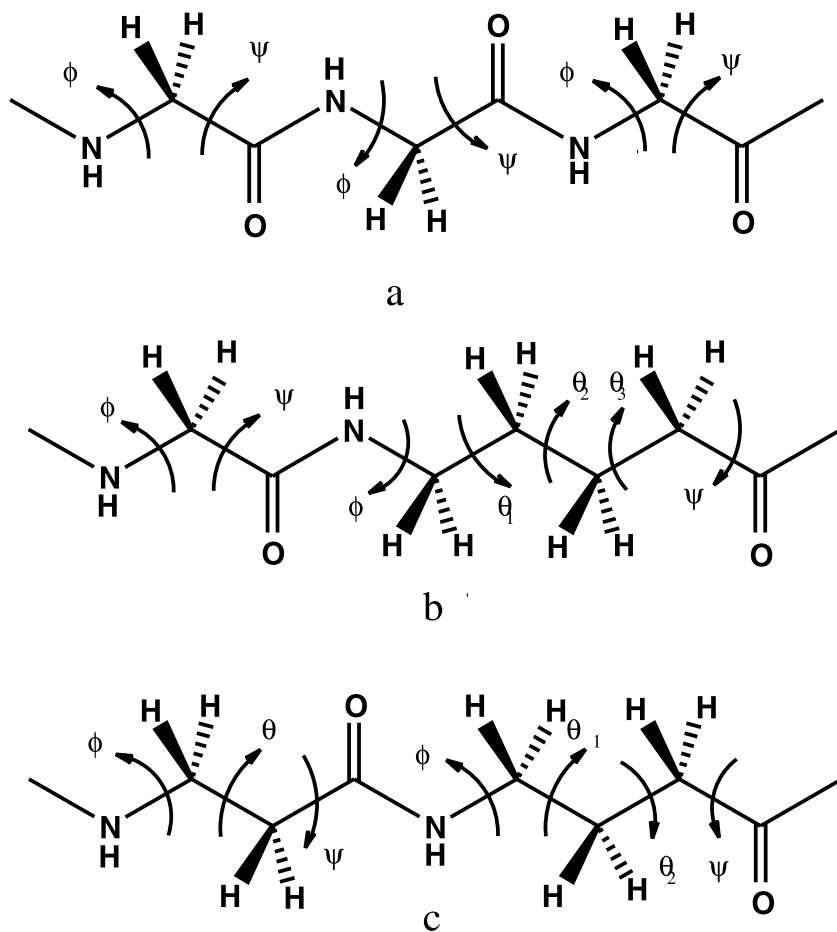


Figure 3. Definition of backbone torsion angles. (A) Gly-Gly-Gly (α, α, α) segment, (B) Gly- δ Ava ($\alpha\delta$) segment, (C) β -HGly- γ Abu ($\beta\gamma$) segment.

In describing conformations of ω -amino acid residues and relating observed stereochemical characteristics to the vast data available on α -peptides, it is convenient to follow a backbone torsion angle nomenclature, which readily permits direct comparison. In this description, ϕ and ψ continue to refer to the torsion angles about the N-C ω and C ω -CO bonds. Torsions about polymethylene chains are denoted by the angles $\theta_1, \theta_2, \dots, \theta_n$, with increasing subscript numbers referring to the progression of the chain from the N-terminus end to the C-terminus. Residue numbers may be indicated as superscripts, e.g. θ_n^i for the i th residue. Note that in the conventional nomenclature for β and higher ω amino acids the C $^\beta$ /C $^\omega$ atom precedes the C $^\alpha$ atom, when the chain is read from the N-terminus.

Extended structures: β -sheets and β -hairpins

α -Residues can be incorporated into extended strand structures which assemble into sheets by backbone hydrogen bonding involving proximal strands. The backbone dihedral angles for the β -sheet structure lie in the region $\phi = -120 \pm 30^\circ$ and $\psi = 120 \pm 30^\circ$. In the case of ω -amino

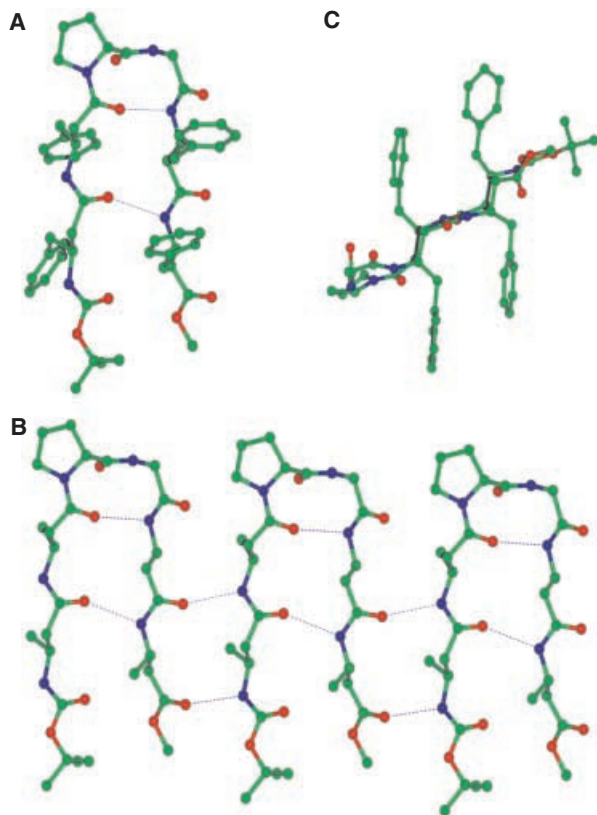
acids, comfortable accommodation into extended strand structures require the dihedral angles (θ_n) about the central C-C bonds to adopt a largely *trans*(180°) conformation. This requirement may be anticipated to be particularly stringent for β -residues, while in the case of the higher ω -amino acids the possibility of compensating torsion angles about contiguous C-C bonds may permit greater variation. Earlier studies from this laboratory have explored the use of D Pro-Xxx sequences in nucleating β -hairpin structures (19). We have systematically investigated the effect of inserting ω -residues at different positions of the strands. Table 2 illustrates observed torsion angles of five peptide hairpins containing facing ω -residues. In all cases, good interstrand hydrogen bonds between facing antiparallel strands have been observed. Figure 4 illustrates representative β -hairpin structures in a hybrid α/β -sequences. Notably, in the structure of octapeptide Boc-Leu-Val- β^3 -HVal- D Pro-Gly- β^3 -HLeu-Val-Val-OMe, the β^3 -HVal₃ residue adopts a *gauche* conformation about the C $^\alpha$ -C $^\beta$ bond ($\theta = +65^\circ$), which is the only example, thus far reported, where such a large deviation from a generally observed *trans* value is established (22). In this case, an almost fully extended ψ -value ($\psi = -175^\circ$) is observed for β^3 -HVal₃, presumably to restore

Table 2. Torsion angles (ϕ , θ , ψ) of β,γ -residues in crystals of hybrid peptide hairpins

Peptide sequences ^a	β,γ -Residues	Torsion angles ($^{\circ}$)				References
		ϕ	θ_1	θ_2	ψ	
Boc-L-V- β F-V- ^D P-G-L- β F-V-V-OMe	β F3	-116	168	-	106	(20)
	β F8	-109	172	-	128	
Boc - β F- β F- ^D P-G- β F- β F-OMe	β F1	-128	163	-	115	(21)
	β F2	-113	153	-	110	
	β F5	-84	171	-	115	
	β F6	-99	166	-	147	
	β V3	-131	65	-	-175	(22)
Boc-L-V- β V- ^D P-G- β L-V-V-OMe	β L6	-136	-178	-	115	
Boc-L- β F-V- ^D P-G-L- β F-V-OMe	β F2(A)	-141.1	150.2	-	158.8	^b
	β F2(B)	-145.4	160.5	-	151.1	
	β F7(A)	-101	166.7	-	118.5	
	β F7(B)	-91.9	166.3	-	115.5	
Boc L-V- γ Abu-V- ^D Pro-Gly-L- γ Abu-V-V-OMe	γ Abu3(A)	-118.7	107.2	172.9	-158	^b
	γ Abu3(B)	-116.2	-172.8	176.3	105.9	
	γ Abu8(A)	94.1	-174.8	-65.1	143.4	
	γ Abu8(B)	-178.8	-171.3	-135.3	131.5	

a. For simplicity, β -HPhe, β -HVal and β -HLeu are abbreviated as β F, β V and β L respectively.

b. I.L. Karle personal communication.

**Figure 4.** (A) A crystalline β -hairpin with β -amino acids in the strands, Boc- β^3 -HPhe- β^3 -HPhe-^DPro-Gly- β^3 -HPhe- β^3 -HPhe-OMe. (B) Infinite β -sheets formed by the peptide. (C) A sideview illustrating the sidechain orientation ref. (21).

the hydrogen bonding interaction. Insertion of β - residues into β -sheet structures changes the nature of hydrogen bonding faces of the strands. In the case of α -residues, CO and NH groups alternately point inward and outward, whereas in β -residues the CO groups are directed towards one face and the NH groups face the opposite direction, resulting in the formation of 'polar sheets' (Fig. 4) (21,23). Furthermore, the insertion of additional atoms into the strand backbone place the sidechains of successive β -residues on the same face of the sheets as opposed to α -peptide sheets in which they lie in opposite directions (Fig. 4). Thus, β -amino acid residues may be used to generate peptide assemblies with a distinctly different hydrogen bonding pattern when compared with α -peptides. Furthermore, scaffolds with a unique disposition of sidechains may be generated.

β -Residues may also be inserted into turn segments of hairpins. In the peptide Boc-Leu-Val-Val-^DPro- β^3 -HPhe-Leu-Val-Val-OMe, β^3 -HPhe is placed at the $i + 2$ position of the β -turn in order to assess the effect of expansion of the nucleating turn to yield a 11-membered hydrogen bonded ring (22). NMR studies reveal the observation of several critical nuclear Overhauser effects (NOEs), characteristic of a β -hairpin with a nucleating hybrid $\alpha\beta$ turn. The higher ω -amino acids may also be inserted into the strand

segments of hairpins as exemplified by the structure of the decapeptide sequence Boc-Leu-Val- γ Abu-Val- D Pro-Gly-Leu- γ Abu-Val-Val-OMe, in which two facing γ Abu residues are observed in antiparallel strands of a β -hairpin structure in the crystal (I.L. Karle, personal communication). Attempts have also been made to examine the role of ω -amino acid insertion into the turn segment of peptide hairpins, specifically at the $i + 2$ position of D Pro-Xxx sequences. NMR analysis provides evidence of a hairpin structure for the peptide Boc-Leu-Val-Val- D Pro- δ Ava-Leu-Val-Val-OMe, with a nucleating $\alpha\delta$ turn (24). Further evidence is desirable for the definitive delineation of the effect of inserting higher ω -amino acids into β -hairpin structures. The rate of accumulation of crystal structure data for β -hairpin peptides has been slow, because of the low tendency of β -sheets to form single crystals, suitable for X-ray diffraction. In our hands,

β -hairpins containing ω -amino acids in the strand segments have yielded crystals, only infrequently. We have thus far been unable to crystallize hairpins with expanded turn segments. Interpretation of NMR data is sometimes hampered by resonance overlap, which obscures critical inter-strand NOEs between facing ω -amino acids in synthetic model peptides.

The effect of insertion of ω -amino acids into the strand segments of β -hairpins on the thermodynamic stability of the fold have not been assessed thus far. Preliminary studies with the designed peptides suggest that the β^3 -residues strongly favor conformations accommodated into extended strands, with several examples of stable hairpins having been (2) demonstrated in solution. It is likely that the stability of registered anti-parallel hairpins may be compromised by the insertion of higher ω -amino acids into strands, as conform-

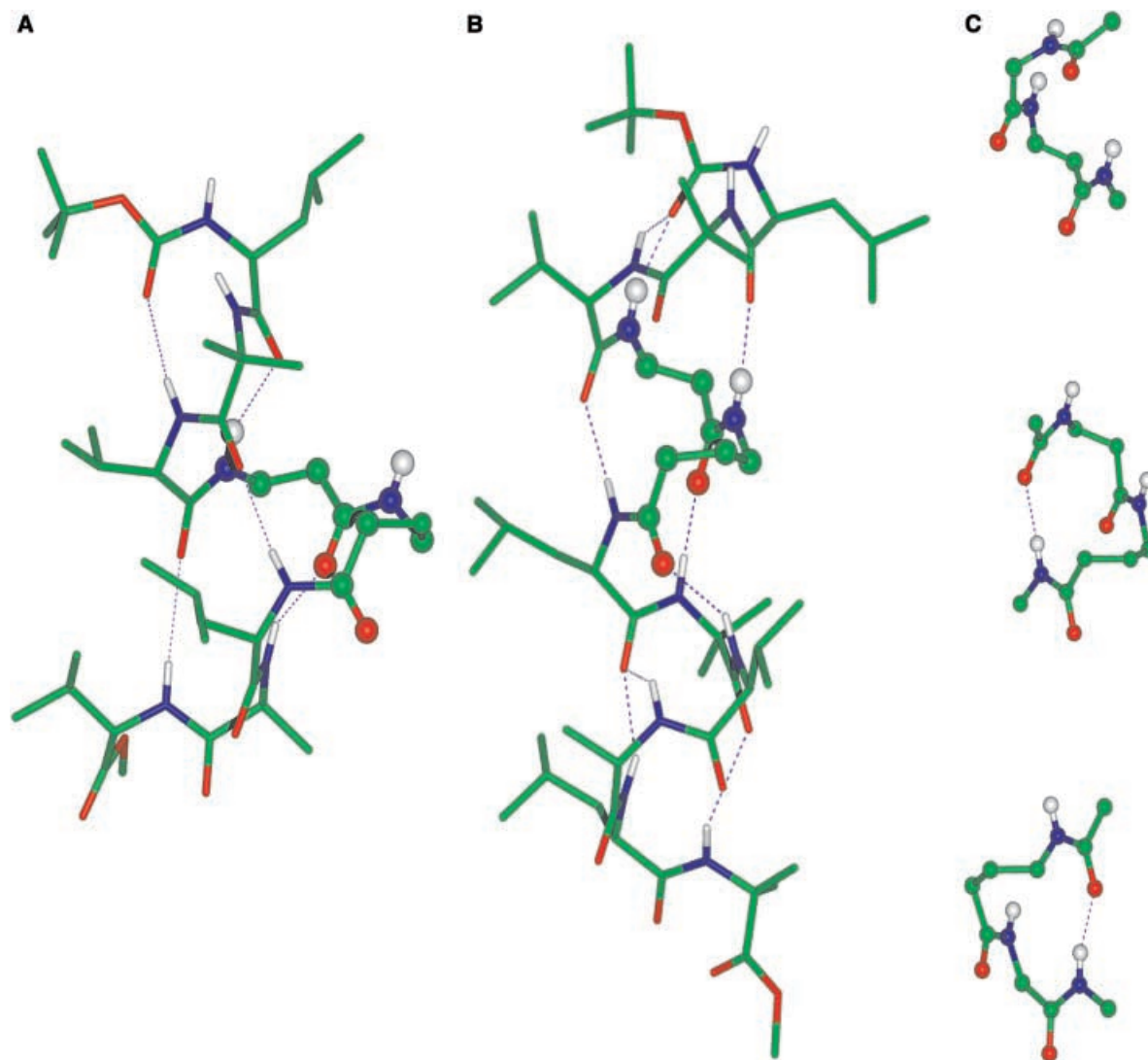


Figure 5. Molecular conformation of hybrid helices in crystals. (a) Eight-residue peptide (Boc-Leu-Aib-Val- β -HGly- γ Abu-Leu-Aib-Val-OMe). (B) Eleven-residue peptide, (Boc-Leu-Aib-Val- β -HGly- γ Abu-Leu-Aib-Val-Ala-Leu-Aib-OMe). (C) The hybrid dipeptide segments are shown (top) Val- β -HGly, (middle) β -HGly- γ Abu and (bottom) γ Abu- Leu [ref. (26)].

Table 4. Conformation Sensitive Interproton Distances (Å)^a

Distances (Å)	β -Sheet (X-ray)	Helix (X-ray)
α -Residues		
d_{NN}^{α}	4.2	2.8
$d_{\alpha N}^{\alpha}$	2.2	3.4
$d_{N\alpha}^{\alpha}$	2.7	2.6
β -Residues ^b		
d_{NN}^{β}	4.8	3.5
$d_{\beta N}^{\beta}$	4.1	3.9
$d_{\alpha N}^{\beta}$	2.2, 3.1	2.2, 3.2
$d_{N\beta}^{\beta}$	2.8	2.7
$d_{N\alpha}^{\beta}$	2.6, 3.0	2.5, 3.5
$d_{\beta\alpha}^{\beta}$	2.4, 2.8	2.5, 2.8,
γ -Residues ^c		
d_{NN}^{γ}	6.1	4.4
$d_{\gamma N}^{\gamma}$	4.1, 4.7	3.7, 4.9
$d_{\beta N}^{\gamma}$	3.7, 4.2	4.3, 4.4
$d_{\alpha N}^{\gamma}$	2.2, 3.0	2.2, 3.0
$d_{N\gamma}^{\gamma}$	2.4, 2.7	2.3, 2.7
$d_{N\beta}^{\gamma}$	2.5, 3.0	2.7, 3.6
$d_{N\alpha}^{\gamma}$	4.2, 4.5	3.0, 4.1
$d_{\gamma\beta}^{\gamma}$	2.1–2.8	2.3–2.8
$d_{\beta\alpha}^{\gamma}$	2.2–2.8	2.3–2.8
$d_{\gamma\alpha}^{\gamma}$	2.5–3.3	3.0–4.1

a. Distances calculated for ϕ , θ , ψ values observed in specific secondary structures using crystallographically determined coordinates. Values are averaged over several residues. The superscripts indicate the residue type while the subscripts indicate the atom type.

b. Distances crystallographically determined for β^3 -residues.

c. $d_{\gamma\beta}^{\gamma}$, $d_{\beta\alpha}^{\gamma}$ and $d_{\gamma\alpha}^{\gamma}$ distances are given as ranges which represent the upper and lower limits in the γ -Abu residue.

and ACHC (trans 2-aminocyclohexanecarboxylic acid), the 10 helix and the mixed 10/12 helix have thus far not been observed in crystals. The mixed 10/12 helix has been postulated in the structure of the synthetic peptide (H₂N- β^3 -HVal- β^2 -HAla- β^3 -HLeu- β^2 -HVal- β^3 -HAla- β^2 -HLeu- β^3 -HVal- β^2 -HAla- β^3 -HLeu-OH) from NMR data (25). In hybrid sequences, an important question to be addressed is whether the ω -amino acid can be accommodated into the helical fold generated in the host α -peptide sequences. Two early crystal structures of 8- and 11-residue peptide helices demonstrated that the β Gly- γ Abu segment could be incorporated into a helical fold, without significant perturbation of the secondary structure (26). Figure 5 shows a view of the helices formed and also illustrates the expanded hydrogen bonded rings of the $\alpha\beta$, $\beta\gamma$, and $\gamma\alpha$ dipeptide segments. Table 3 lists the conformational parameters for the β - and γ -residues incorporated in host α -peptide structures. In all the cases, the

torsion angles about the C $^{\alpha}$ -C $^{\beta}$ and C $^{\beta}$ -C $^{\gamma}$ lie close to an ideal *gauche* conformation. Interestingly, the ϕ , ψ values for the β - and γ -residues are both somewhat extended, but have the same sign (negative for a right-handed helical twist).

Recent work in our laboratory suggests that the incorporation of contiguous β -residues into helical α -peptide structures can be accomplished without any dramatic structural perturbation. The structure of the peptide Boc-Val-Ala-Phe-Aib- β^3 -HVal- β^3 -HPhe-Aib-Val-Ala-Phe-Aib-OMe determined by X-ray diffraction, reveals a continuous helix stabilized by intramolecular hydrogen bonds (I.L. Karle personal communication).

The solution conformational analysis in solution of β - and γ -residues in peptides depend critically on the observation of specific NOEs. Figure 6 shows a perspective overview of α , β and γ -residues when incorporated into host α -peptide sequences that closely resembles α -helical and β -sheet structures. Relevant interproton distances are defined. Table 4 lists the interproton distances for β - and γ -residues accommodated into extended strand and helical conformation in host regular structures.

Constrained ω -amino acids

The use of ω -amino acids in which torsions about rotatable bonds are restricted by freezing into cyclic structures are useful in probing the promotion of specific conformations in oligopeptides. Figure 7 shows some representative structures of amino acids with restricted torsional freedom. The successful characterization of the 12 and 14 helical structures of oligo- β -peptides by X-ray diffraction are striking examples of the utility of introducing conformational constraints (8–11). Nipecotic acid, a β -amino acid which incorporates both ϕ and θ constraints, has been introduced into a reverse turn motif, where a heterochiral dinipecotic acid segment occupies the $i + 1$ and $i + 2$ positions of a C₁₂ turn (27).

(S)-H- β^3 -HPro-OH, derived by backbone homologation of L-proline, is a readily available amino acid in which the torsion angle $\phi = -60 \pm 20^\circ$. Ordered structures of homooligomeric sequences of β^3 -homoproline and β^2 -homoproline (nipecotic acid) have been inferred from circular dichorism (CD) data (28). In principle, (S)-H- β^3 -HPro-OH residue can be readily accommodated in the $i + 2$ position in expanded β -turns in $\alpha\beta$ sequences.

Trans-3-ACPC (*trans*-3-aminocyclopentanecarboxylic acid) is a constrained γ -amino acid. *Trans*-3-ACPC has been incorporated into the strand segment of a parallel sheet structure nucleated intramolecularly using a D-prolyl-(1,1-

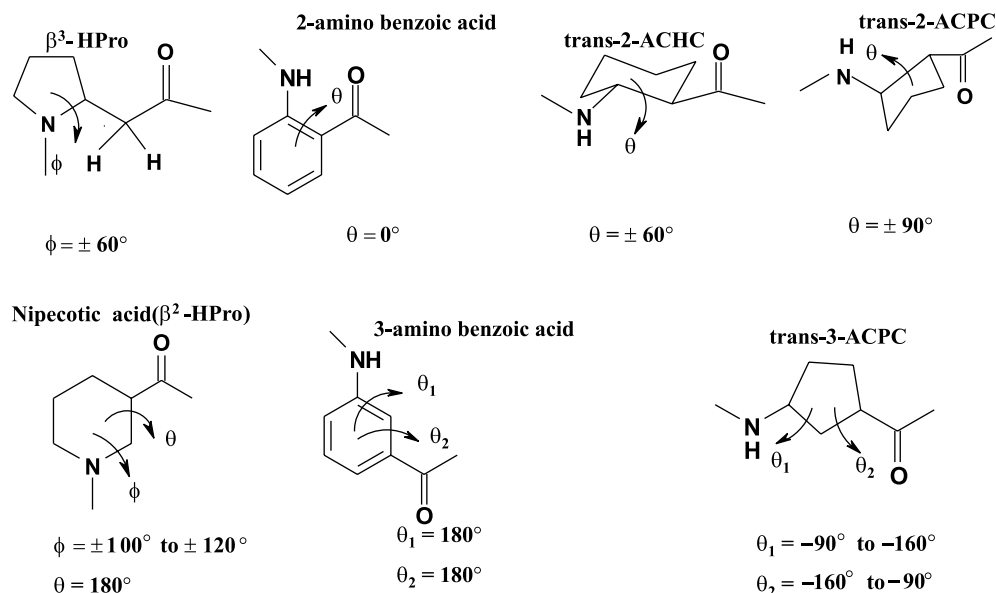


Figure 7. The structures of constrained ω -amino acid residues. Relevant torsion angles which are restricted are indicated.

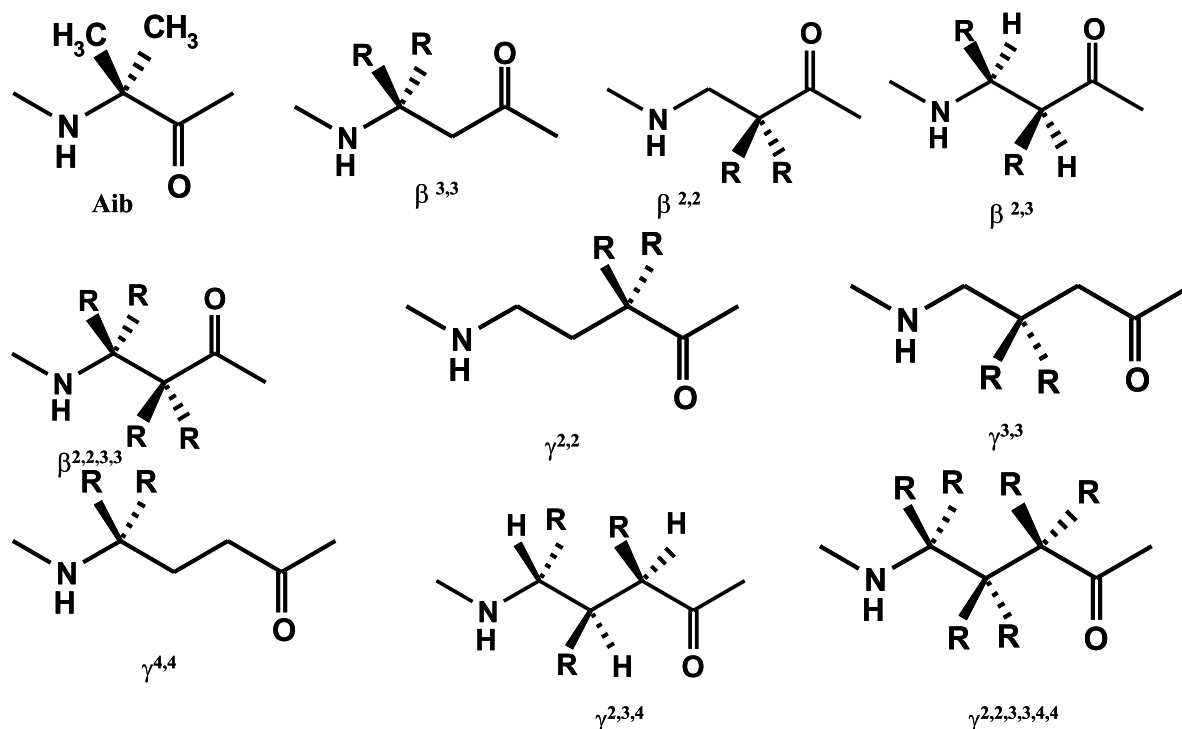


Figure 8. The structures and nomenclature of acyclic, substituted ω -amino acid residues. The prototype residue Aib is also shown.

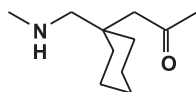
dimethyl)-1,2-diaminoethyl unit as the turn segment (29). 3-Aminobenzoic acid which is a γ -amino acid with $\theta_1 = \theta_2 = 180^\circ$, is comfortably accommodated into the strand segment of model hairpin structures as established by NMR studies (30).

The addition of substituents at the C^α -carbon atom in α -amino acids restricts the range of sterically allowed conformations about the torsional angles ϕ and ψ . The $C^{\alpha,\alpha}$

dialkylated amino acid, α -aminoisobutyric acid (Aib) the prototype residue in this class, has been shown to adopt an extremely limited range of (ϕ, ψ) values, resulting in the stabilization of ordered structures in short sequences (19,31). By extension, substitution along the carbon backbone of acyclic ω -amino acid residues should restrict torsional freedom. In the case of β and γ -amino acids multiple possibilities of substitution exist permitting selective con-

formational restrictions to be imposed about the ϕ , θ , ψ torsional angles (see Fig. 8 for the nomenclature for substituted acyclic β and γ -residues).

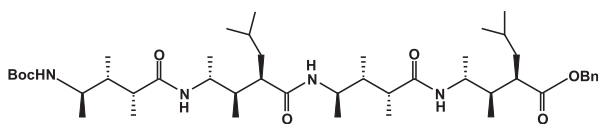
The widely used anti-epileptic drug gabapentin [1-(aminomethyl) cyclohexanecarboxylic acid, Gpn] is an achiral β,β -disubstituted γ -amino acid ($\gamma^{3,3}$) Fig. 8. The pair of geminal substituents at the central C^β -carbon atom restrict the range of conformations available about the torsion angles θ_1 and θ_2 . The determination of several X-ray structures of gabapentin derivatives reveals the almost exclusive adoption of *gauche-gauche* conformations about the $C^\gamma-C^\beta(\theta_1)$ and $C^\beta-C^\alpha(\theta_2)$ bonds (32). Preliminary studies in our laboratory suggest that Gpn residues in hybrid sequences promote obligatory reversed of chain direction, even in the absence of stabilizing intramolecular hydrogen bonds as exemplified in the crystal structure of Boc-Gpn- β^3 -HPhe-Leu-OMe (Ananda, K., Aravinda, S., Shamala, N. and Balaram, P., Unpubl. data).



Gabapentin (Gpn)

Crystal structure determination of N-protected dipeptide acids and esters like Piv- L Pro-Gpn-OH, Boc-Aib-Gpn-OH, Boc-Gly-Gpn-OH, Boc-Aib-Gpn-OMe establishes the folding of the peptide chain at the Gpn residue (33).

Novel helical incipient structures have been crystallographically demonstrated in short sequences containing γ -amino acids. Seebach *et al.* have established a 2_614 -helical structure in a tetrapeptide containing $\gamma^{2,3,4}$ -amino acids (34).



In short γ -peptide sequences evidence of nine-membered hydrogen bonded rings have also been obtained (33,34).

The use of hybrid sequences permits characterization of novel hydrogen bonded rings in short peptides. The struc-

ture of the tripeptide Boc- β^3 -HPhe-Gpn-Phe-OMe reveals a compact folded conformation stabilized by two intramolecular hydrogen bonds; C_{13} ($C \leftarrow N$), encompassing the $\beta\gamma$ dipeptide segment and C_{10} ($N \rightarrow C$) encompassing the $\beta\alpha$ dipeptide segment (Vasudev, P.G., Ananda, K., Shamala, N. and Balaram, P., Unpubl. data).

Conclusions

Systematic exploration of the conformational characteristics of hybrid dipeptide segments enumerated in Fig. 2 may prove valuable in formulating approaches for the insertion of such segments into host α -peptide sequences in the design of analogs of biologically active peptides. The insertion of β , γ and δ residues provides a convenient means for replacing amide bonds by 'surrogate ethylene units' and for perturbing the local structure in helices and β -sheets by insertion of additional atoms into peptide backbones. Analogs, which confer site-directed proteolytic stability (35), without perturbation of the overall fold in biologically active sequences, may be of special importance. The growing body of literature on naturally occurring, biologically active peptides containing diverse β -amino acid residues adds relevance to attempts to explore the conformational characteristics of peptides containing the higher homologs of α -amino acids (36).

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