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Review

Expanding the polypeptide backbone: hydrogen-bonded conformations in hybrid polypeptides containing the higher homologues of α-amino acids

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Half a century has passed since the hydrogen-bonded secondary structures of polypeptides and proteins were first recognized. An extraordinary wealth of conformational information is now available on peptides and proteins, which are formed of α-amino acid residues. More recently, the discovery of well-folded structures in oligopeptides containing β-amino acids has focused a great deal of current interest on the conformational properties of peptides constructed from higher homologues (ω) of α-amino acids. This review examines the nature of intramolecularly hydrogen-bonded conformations of hybrid peptides formed by amino acid residues, with a varying number of backbone atoms. The β-turn, a ubiquitous structural feature formed by two residue (αα) segments in proteins and peptides, is stabilized by a 10-atom (C_{10}) intramolecular $4\rightarrow1$ hydrogen bond. Hybrid turns may be classified by comparison with their αα counterparts. The available crystallographic information on hydrogen-bonded hybrid turns is surveyed in this review. Several recent examples demonstrate that individual ω-amino acid residues and hybrid dipeptide segments may be incorporated into the regular structures of α-peptides. Examples of both peptide helices and hairpins are presented. The present review explores the relationships between folded conformations in hybrid sequences and their counterparts in all α-residue sequences. The use of stereochemically constrained ω-residues promises to expand the range of peptide design strategies to include ω-amino acids. This approach is exemplified by well-folded structures like the C_{12} (αγ) and C_{14} (γγ) helices formed in short peptides containing multiply substituted γ-residues. The achiral γ-residue gabapentin is a readily accessible building block in the design of peptides containing γ-amino acids. The construction of globular polypeptide structures using diverse hybrid sequences appears to be a realistic possibility.

Keywords: hydrogen bonds; peptide conformation; hybrid peptides; ω-amino acids; backbone-homologated amino acids

1. INTRODUCTION

A little over 60 years ago, Maurice Huggins reviewed the hydrogen-bonded structures of polypeptide chains, at a time when no detailed structural information was available on oligopeptides and proteins (Huggins 1942, 1943). Attempts to rationalize limited X-ray diffraction data in terms of regular hydrogen-bonded polypeptide structures were summarized by Bragg *et al.* (1950) in a paper that concluded that 'there appears a real simplicity of chain structures in myoglobin, which will perhaps be shown by other favourably built proteins… There is hope that the study of such proteins may lead to a reliable determination of structure'. This expectation has been amply fulfilled in the last half century with the result that almost every feature of polypeptide chain folding is now firmly established by experimental studies. An interesting feature of the Bragg, Kendrew and Perutz paper is the consideration of intramolecularly hydrogen-bonded structures, which were later shown to be stereochemically and energetically unacceptable. Pauling's remarkable insights led to the correct formulation of the hydrogen-bonded helical structures of polypeptides (Pauling *et al.* 1951; Pauling & Corey 1952). One of the last attempts to propose alternative hydrogen-bonded structures was made by Huggins in 1952, when he suggested that a 11-membered hydrogen-bonded ring could be considered as an alternative to Pauling's α-helix, which is

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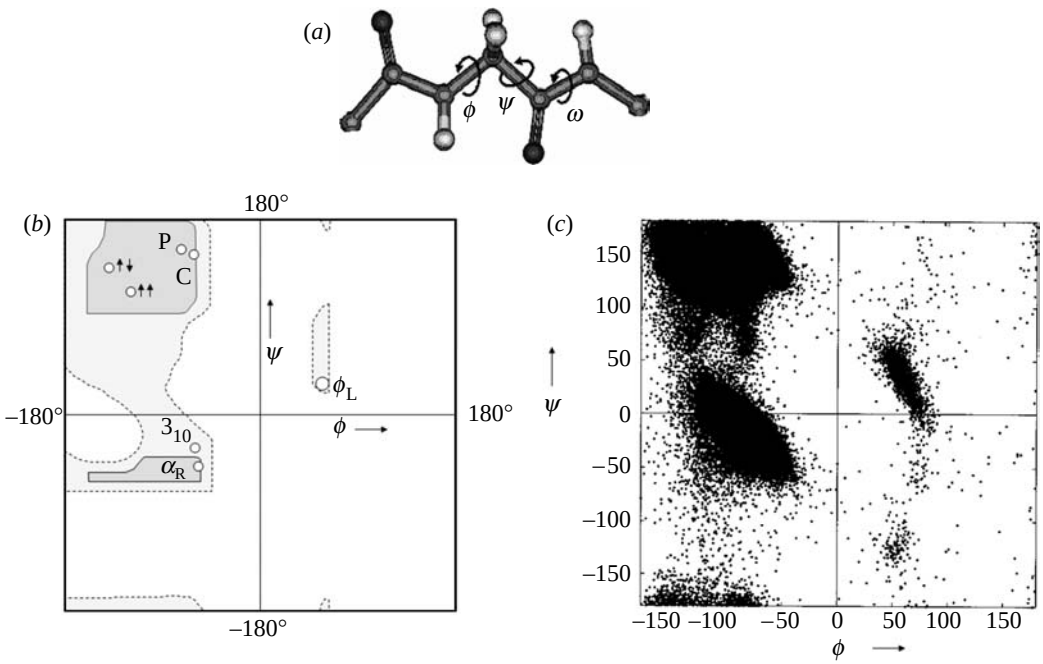

Figure 1. (*a*) A system of two linked peptide units defining the backbone torsion angles at an α-amino acid residue in a polypeptide chain. (*b*) Ramachandran map showing sterically allowed regions for L-alanine. The regions corresponding to the important secondary structures are marked α_R (right-handed α-helix), α_L (left-handed α-helix), 3_{10} (3_{10}-helix), two upward arrows (parallel β-sheet), left up, right down arrows (antiparallel β-sheet), P_{II} (polyproline II), C(collagen triple helix). (*c*) Scatter plot of 104 718 non-Gly residues in protein crystal structures (less than or equal to 2.0 Å) showing the distribution of the backbone dihedral angles ϕ and ψ (Singh 2001).

characterized by a 13-membered hydrogen bond (Huggins 1952*a*,*b*). This was quickly dismissed by Pauling, since the peptide units would have to deviate substantially from planarity to accommodate the 11-atom hydrogen-bonded ring (Pauling & Corey 1952). Current interest in the hydrogen-bonded structures of polypeptides chains stems from the realization that the repertoire of polypeptide chains can be vastly expanded when backbone-homologated amino acid residues are introduced (Gellman 1998; Cheng *et al.* 2001; Hill *et al.* 2001; Gademann *et al.* 2003; Lelias & Seebach 2004; Roy & Balaram 2004; Seebach *et al.* 2004, 2006; Kimmerlin & Seebach 2005). This overview considers recent research on hydrogen-bonded structures of peptides containing β-, γ- and δ-amino acids which have provided several examples of novel hydrogen-bonded patterns, hitherto unknown in all α-polypeptide structures.

Naturally occurring polypeptide chains found in proteins are heteropolymers of α-amino acid residues linked together by peptide bonds. The stereochemistry of the polypeptide chain is best described by analysing the various orientations of two planar peptide units linked together by a central tetrahedral carbon atom. The torsional degrees of freedom about the N–C^{α} and the C^{α}–C' bonds permit the chain to sample conformational space. Several stable conformations corresponding to energy minima are characterized by specific values of ϕ and ψ at each residue (Ramachandran & Sasisekharan 1968, figure 1). The nature of polypeptide chain folding is determined by short-range non-bonded interactions and intramolecular hydrogen bond formation between acceptor CO and donor NH groups. Over the course of a little more than half a century, the principles governing the folding of polypeptide chains and the nature of the stable secondary structures have been well established. In proteins, sequence variability generated by the incorporation of 20 different genetically coded α-amino acids, which differ in the substituent at C^{α}, is the key to structural and functional diversity.

β-Amino acids and higher backbone-homologated (ω) residues are found in nature as constituents of peptide metabolites produced by micro-organisms. Poly-γ-D-glutamate first reported from *Bacillus anthracis* (Hanby & Rydon 1946; Rydon 1964) has subsequently been shown to be produced by several species of bacillus (Ashiuchi & Misono 2002). Much of the early work on homopolypeptides of ω-amino acids was stimulated by the interest in the structures of nylons (Glickson & Applequist 1971; Lovinger 1978; Fernandez-Santin *et al.* 1984; Munoz-Guerra *et al.* 1985; Puiggali & Munoz Guerra 1986; Lopez-Carrasquero *et al.* 1995; Bella *et al.* 1992; Navas *et al.* 1995; Aleman *et al.* 1997). More recently, the characterization of well-defined helical conformations in homo-oligomeric β-peptide sequences (Appella *et al.* 1996, 1997; Seebach *et al.* 1996*a*,*b*, 1997; 1998*a*–*c*; Banerjee and Balaram 1997; Seebach & Matthews 1997*b*) has resulted in a sudden surge of interest in the conformational properties of peptides containing ω-amino acids. Hybrid polypeptides, heteropolymers composed of diverse ω-amino acids, are of special interest in attempts to mimic all α-peptide backbones, using sequences containing non-protein amino acids (Karle *et al.* 1997). This review surveys the conformational properties of hybrid peptides containing α-, β-, γ- and δ-amino acids as observed in crystal structures and advances the use of backbone torsion angles as convenient parameters for description of conformational variability.

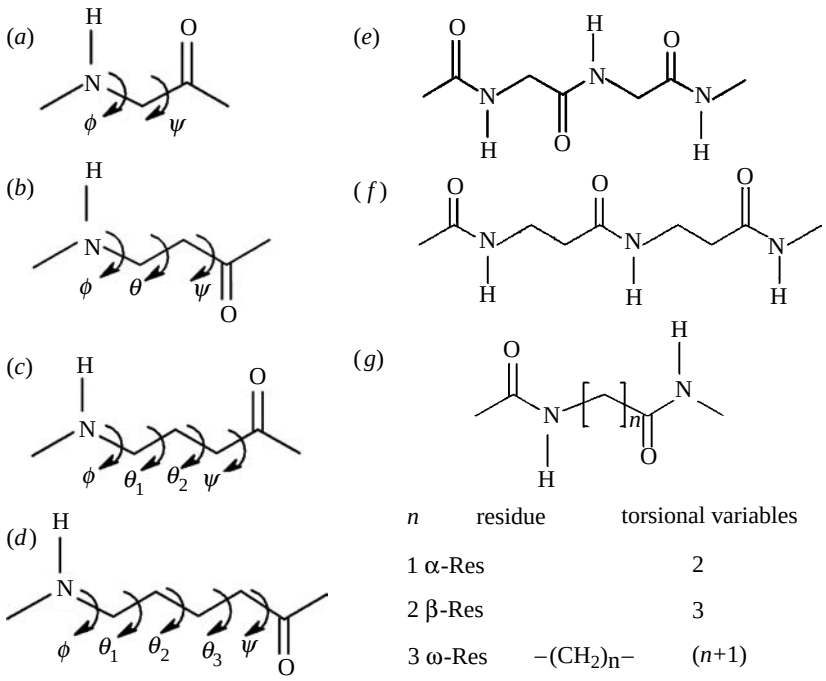

Figure 2. The nomenclature used for describing the backbone torsion angles in (*a*) α-residues, (*b*) β-residues, (*c*) γ-residues, (*d*) δ-residues, (*e*) all α-polypeptide chain, (*f*) all β-chain and (*g*) all ω-chain.

For unwary readers, a note of caution regarding nomenclature may be in order. For β and higher ω-amino acid residues, the backbone atoms are designated as C^{α}, C^{β}, C^{γ}, etc. beginning from the carboxyl end of the residue. For β-residues, the backbone torsion angles are ϕ, θ and ψ and for γ- and higher residues the backbone torsion angles (θ_n) follow the order θ_1, θ_2, …, θ_n in the reverse direction, i.e. θ_1 is towards the N-terminus while the θ_n is towards the C-terminus side of the residue. The residue designations α, β, γ must also be carefully distinguished from the use of the same Greek letters to denote well-established structural features in proteins and all α-polypeptides such as the α-helix, β-sheet, β-turns and γ-turns. Curiously, the α-turn is a feature involving three successive α-amino acids residues (ααα), while the β-turn involves two (αα) amino acid residues and the γ-turn involves one (α) amino acid residue. A convenient way out of this confusing situation does not appear to be feasible at this time, since usage of the various turns is well entrenched in the literature.

2. BACKGROUND TO CONFORMATIONAL ANALYSIS

Figure 1 shows the Ramachandran map for the L-alanine residue. The stereochemically allowed regions of conformational space are mapped on to the ϕ, ψ plane, with disallowed regions being characterized by unacceptably close approach of non-bonded atoms (Ramachandran *et al.* 1963). The validity of the Ramachandran map has been established by the observation that individual amino acid residue conformations, determined from protein crystal structures, cluster in the 'allowed regions'. The contact criterion, which simply states that non-bonded atoms cannot approach one another at distances less than the sum of their van der Waals' radii, has been found to be an excellent filter for distinguishing stereochemically allowed and disallowed conformations. The use of backbone torsion angles as variable parameters permits the analysis of a three-dimensional problem in two-dimensional, torsion angle space. Regular α-polypeptide structures, like the Pauling 3.6_{13} (α)-helix, 3_{10}-helix, parallel and antiparallel β-sheets and polyproline structures are ideally represented by a single point in ϕ, ψ space. The use of dihedral angles as descriptors of local conformations at individual residues has permitted systematic analysis of backbone structural features in a large body of experimentally determined protein structures.

For β- and the higher γ-residues, the number of torsional variables increases as a consequence of introduction of additional carbon atoms into the backbone. The conformation of β-residues can be described using three torsional variables ϕ, θ and ψ. Figure 2 summarizes the nomenclature used for describing backbone torsion angles in ω-residues. Three stable minima corresponding to θ values of $\pm 60°$ (*gauche*) and 180° (*trans*) are anticipated for β-residues. While homologated polypeptide backbones were examined structurally in the 1960s and 1970s (Banerjee & Balaram 1997), the observation of well-defined helical structures in oligopeptides by the groups of Seebach and Gellman in 1996, has led to a considerable resurgence of interest in the conformational properties of the higher homologues of α-amino acids. The characterization of the 12-helix [(M) 2.5_1 or (P) 2.5_1] (Appella *et al.* 1997, 1999*a*,*c*; Seebach & Matthews 1997*a*), which can be formally considered as an expanded version of the 3_{10}-helical structure of the α-polypeptides, the mixed 10/12-helix (Seebach & Matthews 1997*b*; Seebach *et al.* 1997, 1998*a*) and the C_{14}-helix [(M) 3_1 or (P) 3_1] (Appella *et al.* 1996, 1999*a*,*b*;

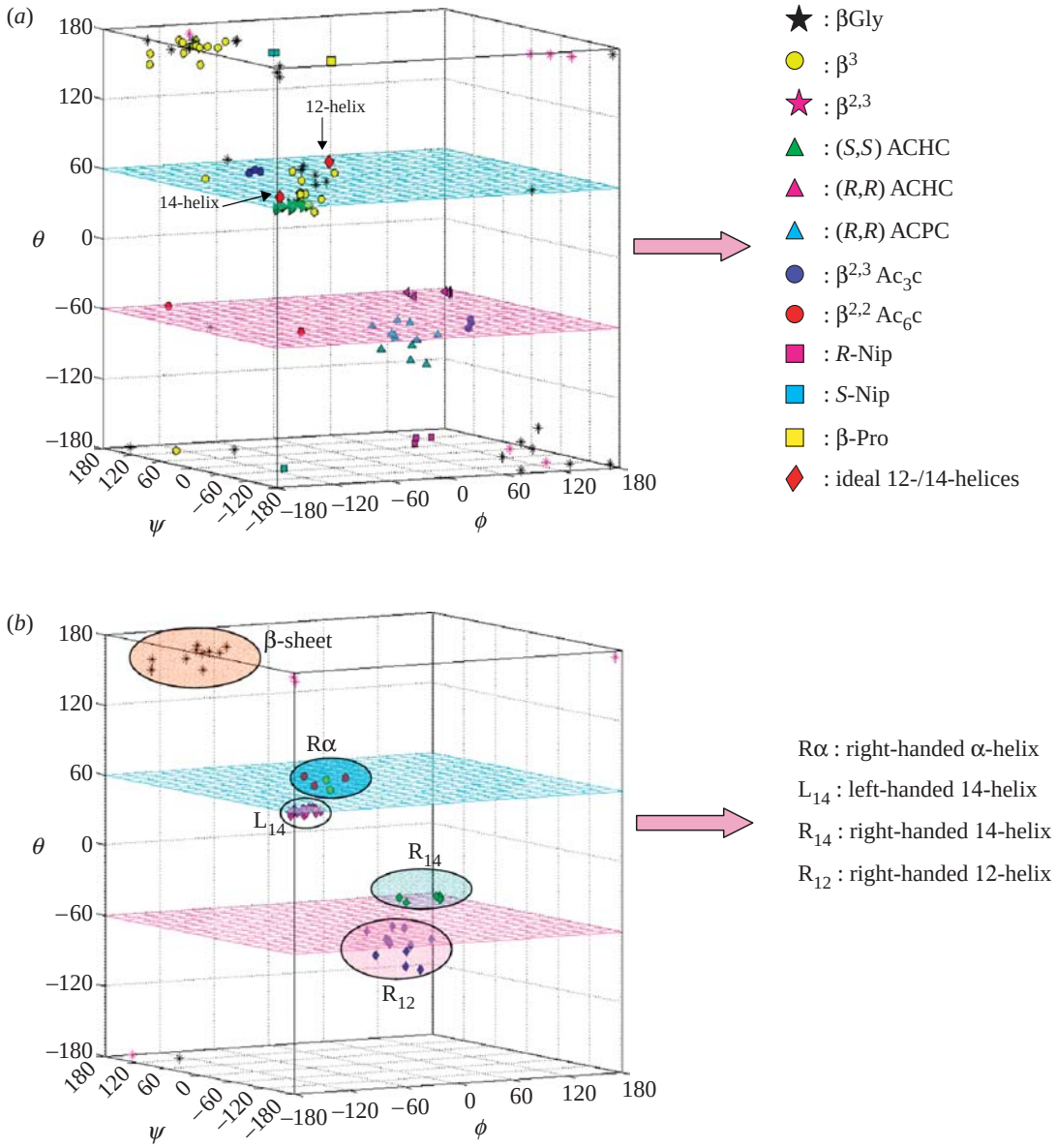

Figure 3. (*a*) Distribution of the observed β-residues, established from crystal structures of peptides, in ϕ–θ–ψ space. (*b*) Locations for ordered secondary structures for β-residues in ϕ–θ–ψ space (Roy 2005).

Seebach *et al.* 1996*a*,*b*, 1997*a*, 1998*a*), which has reversed hydrogen-bond directionality, provided the first evidence for the considerable potential of β- and higher ω-amino acid residues in expanding the repertoire of polypeptide chain conformations. For β- and higher ω-amino acids, torsion angle space becomes multidimensional as the number of rotatable backbone bonds increase, with the consequence that a simple graphical, Ramachandran-type representation of stereochemically allowed conformations is no longer possible. Figure 3 illustrates the distribution of the observed β-residue conformations, thus far established from the crystal structures of peptides, in three-dimensional ϕ, θ, ψ space. In considering the conformation of hybrid peptides, it is therefore convenient to use well-established, folded structures of all α-peptide backbones as the starting point. The conformational problem then reduces to questions on the effect of inserting additional backbone atoms into canonical α-peptide structures. This approach is illustrated below with specific examples.

3. REVERSE TURNS

Figure 4 schematically illustrates three common turn structures which facilitate polypeptide chain reversal in all α-residue sequences (Rose *et al.* 1985). The C_7 or γ-turn conformation is generated by the formation of a single seven-atom intramolecularly hydrogen-bonded ring and is characterized by the torsion angles $\phi \approx -70°$, $\psi \approx +70°$ or $\phi \approx +70°$, $\psi \approx -70°$ at residue $i+1$ (Mathews 1972; Milner-White & Poet 1986, 1987; Milner-White *et al.* 1988). The C_7 structure is effectively determined by the conformational preferences at a single residue. The C_{10} (β-turn) structure is a two-residue feature defined by torsion angles $\phi(i+1)$, $\psi(i+1)$, $\phi(i+2)$ and $\psi(i+2)$. β-Turns are structural elements widely found in proteins and peptides. They have been classified into distinct groups defined by the torsion angles at the two central residues. β-Turns are generally stabilized by a $4 \rightarrow 1$ hydrogen bond between $CO_{(i)}$ and $NH_{(i+3)}$ (Wilmot & Thornton 1988). The C_{13} or α-turn structure is determined by the conformational

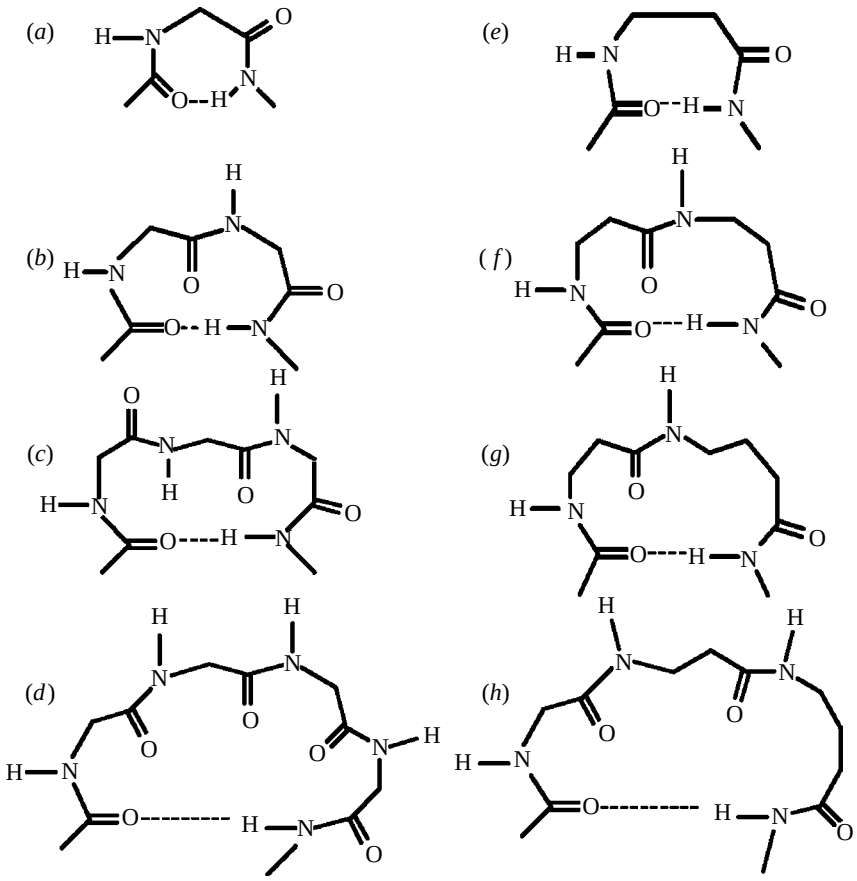

Figure 4. Schematic illustration of turns in all α-residue segments (*a*) γ-turn (C_7), (*b*) β-turn (C_{10}), (*c*) α-turn (C_{13}), (*d*) π-turn (C_{16}), (*e*) single-residue C_8 hydrogen-bonded ring (expanded γ-turn), (*f*) two-residue ββ C_{12} hydrogen-bonded ring (expansion of β-turns), (*g*) two-residue βγ C_{13} hydrogen-bonded ring (α-turn analogue) and (*h*) three-residue αβγ C_{16} hydrogen-bonded ring (π-turn analogue).

parameters at three successive α-residues and is characterized by a 5→1 hydrogen bond, between $CO_{(i)}$ and $NH_{(i+4)}$ (Nataraj *et al.* 1995; Pavone *et al.* 1996). Specific families in both C_{10}- and C_{13}-turn types can be successively repeated generating regular polypeptide helices. Notably, repetition of the type III β-turn motif results in a 3_{10}-helix (Donohue 1953; Toniolo & Benedetti 1991), while a repetitive set of α-turns results in the 3.6_{13} (α)-helix. In all α-residue polypeptides, the largest diameter helix that may be considered in which side chains protrude outwards is the 4.4_{16} (π) helix, in which the individual turn units are stabilized by a 6→1 hydrogen bond between the CO (i) and NH (i+5) (Ramachandran & Sasisekharan 1968). In proteins, the α-helix is the most widely observed secondary structure, its stability undoubtedly the consequence of optimum packing in the interior of the helical structure. For larger helices in this family, non-bonded interactions are far from optimal. The 3_{10}-helix is observed only in short stretches in proteins, but has been well characterized in synthetic peptides (Prasad & Balaram 1984; Karle & Balaram 1990). It may be noted that the β-helical structure observed in proteins is a large-diameter cylindrical structure. This is formed by winding an extended β-strand around a helix axis with parallel β-sheet hydrogen bonds between helical turns (Ienger *et al.* 2006).

Figure 4 also illustrates analogous structures generated by inserting one, two or three atoms into the polypeptide backbone. The C_8 conformation of a single β-residue may be considered as an expansion of the γ-turn. γ-turns are widely observed in the proteins but are generally not characterized in small peptides in the solid-state. A recent example of two consequtive C_7 hydrogen bonds is seen in the crystal structure of the dipeptide Piv-l–Pro-l-c_3–Dip–NHMe (c_3-Dip = 2, 2-diphenyl-1-aminocyclopropanecarboxylic acid) (Jimenez *et al.* 2004). A novel C_8 ribbon structure has been established in the tetrapeptide Boc-(1-(aminomethyl)cyclopropanecarboxylic acid)$_4$OMe (Abele *et al.* 1999). A ββ segment generated by two contiguous β-residues results in a C_{12}-turn, which is an expansion of the C_{10} (β-turn) structure of an αα segment. A βγ segment mimics the C_{13} (α)-turn formed by three contiguous α-residues. In all these cases, the hydrogen-bond directionality is maintained, with the acceptor CO group lying towards the N-terminus side, while the donor NH group is at the C-terminus end.

Reversal of hydrogen-bond directionality in isolated turns is generally not observed in all α-polypeptide structures, although the C_8-turn involving a *cis* peptide bond and a hydrogen bond between $NH_{(i)}$ and $CO_{(i+1)}$ has been considered in short peptides in order to

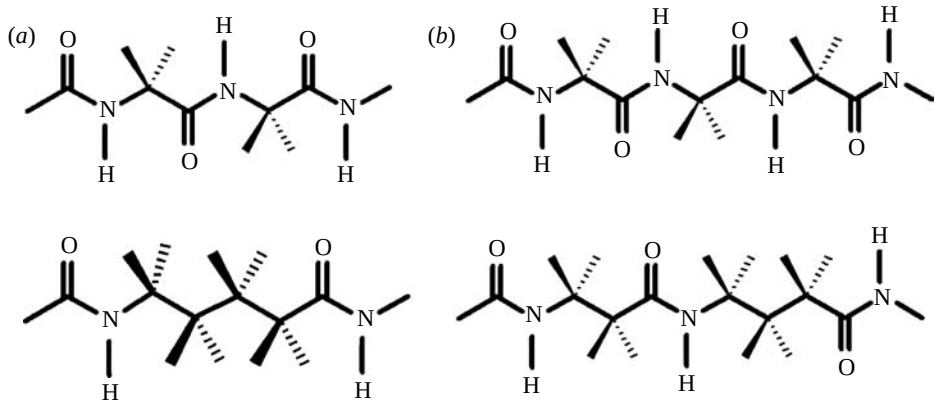

Figure 5. Comparison of the formally equivalent backbone segments (*a*) Gly–Gly and δ-Ava segment (number of backbone atoms is 6) and (*b*) Gly–Gly–Gly and β-Gly γ-Abu segment (number of backbone atoms is 9; Karle *et al.* 1997).

rationalize spectroscopic data (Pulla Rao *et al.* 1979). Structures with reversed hydrogen-bond directionality are stereochemically feasible in oligopeptides containing higher ω-amino acids, maintaining planarity of the peptide bond. A dramatic example is the C_{14}-helix formed in the all β-peptide Boc-(*trans*-ACHC)$_6$CO$_2$CH$_2$-Ph (Appella *et al.* 1996). A C_{10} hydrogen bond with a reversed hydrogen-bond directionality has also been characterized in the tripeptide Boc-(1-(aminomethyl) cyclohexanecarboxylic acid)$_3$OMe (Seebach *et al.* 1998*c*; Abele *et al.* 1999).

In considering reverse turns in hybrid αω sequences, it is useful to note that an α-residue contributes three atoms to the polypeptide backbone, while β-, γ- and δ-residues contribute 4, 5 and 6 atoms, respectively. Thus a δ-amino acid residue may be formally viewed as mimetic of a Gly–Gly segment in which the central peptide unit has been replaced by a CH_2–CH_2 group. Similarly, a βγ dipeptide segment which contains nine backbone atoms is formally related to an all α-tripeptide unit (figure 5, Banerjee *et al.* 1996). Figure 6 summarizes the hydrogen-bonded ring sizes that can be generated, in principle, from hybrid dipeptide sequences. In all cases, the directionality is assumed to be the same as that of the all α-polypeptide structures. Several recently determined peptide crystal structures provide examples of expanded hydrogen-bonded structures. Figure 7 shows examples of turn structures characterized by X-ray diffraction. In some cases, the hydrogen-bonded turns constitute part of a well-developed secondary structure in model peptides. Thus far, turn types containing up to 14 atoms in the hydrogen-bonded ring have been reported. C_{11}- and C_{12}-turns may be considered as backbone expanded analogues of αα C_{10}-turns (β-turns), while the C_{14}-turn may be considered as a one atom expanded analogue of the ααα C_{13}-turn (α-turn).

4. CONFORMATIONAL DIVERSITY OF PEPTIDE TURNS

In the case of α-peptides, the two-residue β-turn or β-bend is the most widely distributed and best characterized structure (Venkatachalam 1968; Chandrasekaran *et al.* 1973; Lewis *et al.* 1973; Richardson 1981; Wilmot & Thornton 1988). A 4→1 C_{10} hydrogen bond is a

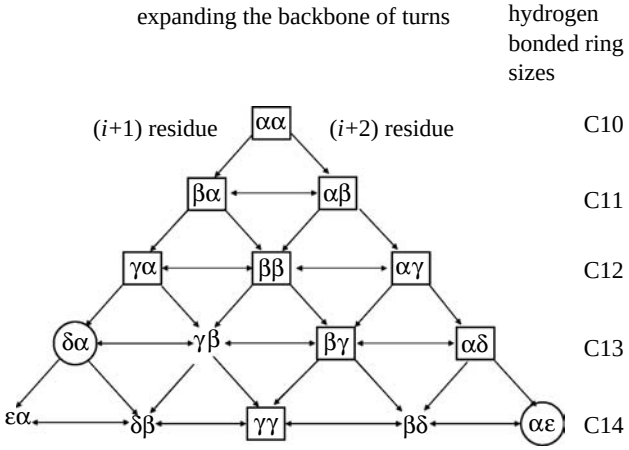

Figure 6. Hydrogen-bonded ring sizes that can be generated in principle from hybrid dipeptide sequences, the hydrogen-bond directionality being the same as in normal all α-polypeptide structures. Turns encircled with rectangles have been characterized in single crystals by X-ray diffraction. Turns established only by NMR are indicated with circles.

characteristic feature of this family of two-residue (αα) turns. Six distinct turn types I/I′, II/II′ and III/III′, which lead to sharp reversal of chain direction, have been extensively discussed in the literature. In these cases, all the three peptide bonds involved in the turn adopt *trans* geometry. Turn classification is based on the torsion angles ϕ, ψ at the corner residue, designated as the $(i+1)$ and $(i+2)$ positions of the turn (Type I: $\phi_{(i+1)} \sim -30°$, $\psi_{(i+1)} \sim -60°$, $\phi_{(i+2)} \sim -90°$, $\psi_{(i+2)} \sim 0°$; Type II: $\phi_{(i+1)} \sim -60°$, $\psi_{(i+1)} \sim 120°$, $\phi_{(i+2)} \sim 80°$, $\psi_{(i+2)} \sim 0°$; Type III: $\phi_{(i+1)} \sim -60°$, $\psi_{(i+1)} \sim -30°$, $\phi_{(i+2)} \sim -60°$, $\psi_{(i+2)} \sim -30°$). The prime turns I′, II′, III′ have the signs of all the torsion angles reversed. The type I and the type III turns are very closely related to each other and may be considered as a single family. Type II turns differ from type I turns in the orientation of the central peptide unit, which is flipped by about 180° resulting in a concerted change of $\psi_{(i+1)}$ and $\phi_{(i+2)}$ (Gunasekaran *et al.* 1998). A unique class of β-turns involving a central *cis* peptide bond, the type VI turn, is also encountered in proteins and peptides. Figure 8 shows a view of the type I, type II and type VI β-turns.

By analogy with the all α-peptide structures, conformational diversity is also anticipated in the

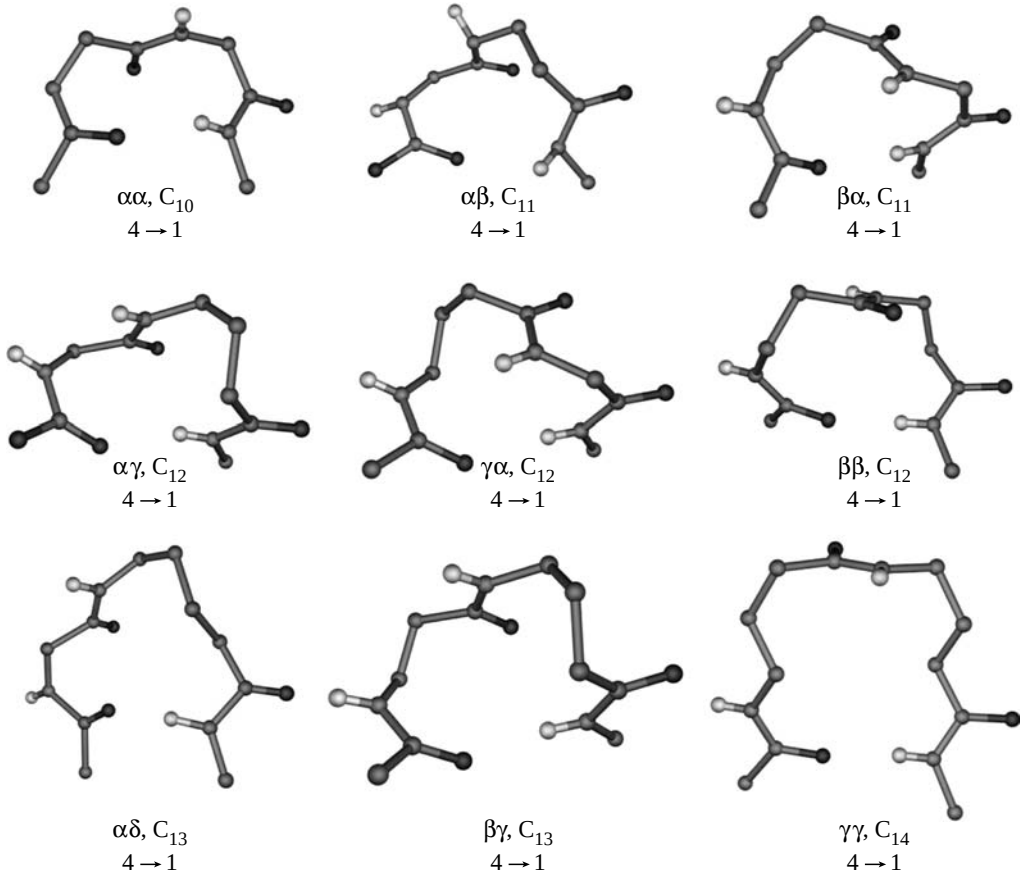

Figure 7. Examples of turn structures (normal hydrogen-bond direction) characterized by X-ray diffraction, αα, Boc–Leu–Phe–Val-^{D}**Pro**–**Gly** Leu–Phe–Val–OMe (Karle *et al.* 1996); αβ, Boc–Aib–**Aib**–**βGly** NHMe (Pavone *et al.* 1992); βα, Boc–Val–Ala–Phe–Aib–βVal–**βPhe**–**Aib**–Val–Ala–Phe–Aib–OMe (Roy *et al.* 2004); αγ, Boc–**Aib**–**Gpn**–Aib–Gpn–OMe (Ananda *et al.* 2005); γα, Boc–Aib–**Gpn**–**Aib**–Gpn–OMe (Ananda *et al.* 2005); ββ, Boc–(*trans* ACPC)$_8$-CO_2CH_2Ph (Appella *et al.* 1999*c*); αδ, Boc–Leu–Val–Val–^{D}**Pro**–**δAva**–Leu–Val–Val–OMe (Rai *et al.* in press); βγ, Boc–Leu–Aib–Val–**βGly** γ**Abu**–Leu–Aib–Val–OMe (Karle *et al.* 1997); γγ, Boc–**γ-ResA**–**γ-ResB**–NHMe (γ-ResA = 2 benzyl, 4 methyl-γ-aminoisobutyric acid, γ-ResB = 4 isopropyl, 2 methyl γ aminoisobutyric acid; Brenner & Seebach 2001).

case of the homologated turns obtained in hybrid sequences. Classification of the experimentally determined hydrogen-bonded hybrid structures may be readily achieved by comparison with their α-peptide counterparts. Figure 8 shows examples of homologated turns, which are formally analogous to the canonical β-turn structures in αα sequences. It is clear that although hybrid turns may contain the same number of atoms as their α-peptide counterparts, the precise turn type may vary. It must be emphasized that in the case of the αα, C_{10}-structures, type I/III turns nucleate helices, type II turns are generally isolated features, while type I′/II′ turns facilitate registered antiparallel hairpin formation (Venkatraman *et al.* 2001; Aravinda *et al.* 2003). As noted earlier, heterogeneity of turn types will undoubtedly also occur in the case of hybrid turns. In the history of the development of α-peptide stereochemistry, an initial theoretical analysis of all the possible intramolecularly hydrogen-bonded structures for a system of three linked peptide units by C. M. Venkatachalam in G. N. Ramachandran's laboratory in Madras (Venkatachalam 1968), played an important role in identifying the diverse nature of the β-turn types. A similar systematic investigation in the case of hybrid turns is merited.

Tables 1 and 2 list the torsion angles and hydrogen-bond parameters observed in the crystallographically determined turn structures involving ω-amino acid residues. In considering hybrid turns, we have chosen segments which may be a part of larger secondary structures. It should be emphasized that the repetition of specific turn types along a sequence results in the generation of helical polypeptide structures. In table 1, the analogous α-peptide turn type is indicated. This has been obtained by examining the superposition of the crystallographically characterized hybrid turns with the parent α-peptide turns.

Figure 9 illustrates examples of the superpositions of equivalent atoms of observed αβ/βα-turns with their α-peptide type I and type II turn counterparts. It is clearly evident that the overwhelming majority of C_{11}-turns in table 1 corresponds to a one-atom expansion of the backbone of a helix promoting α-peptide turn. The only example of a variant β-turn analogue is the case of the αβ C_{11}-turns observed in the tripeptide Boc–Aib–8-amino-cyclooct-4-enecarboxylic acid–Aib–OMe (Tanaka *et al.* 2001). Interestingly, this achiral peptide crystal contains two independent molecules in the asymmetric unit, with Aib adopting an unusual, semiextended conformation in both the cases, compatible with its occurrence at the

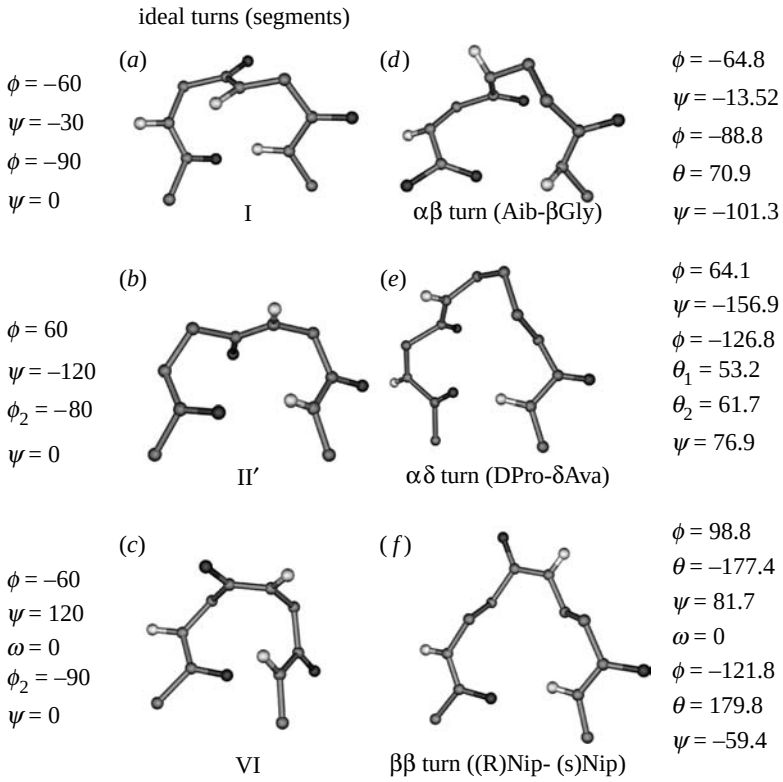

Figure 8. (*a*) Type I β-turns, (*b*) type II′ β-turns, (*c*) type VI β-turns and crystallographically characterized examples of homologated turns containing similar conformational features, (*d*) αβ-turn in Boc–Aib–Aib–β-Gly NHMe (Pavone *et al.* 1992), (*e*) αδ-turn in Boc–Leu–Val–Val–DPro–δAva–Leu–Val–Val–OMe (Rai *et al.* in press) and (*f*) ββ-turn Boc–β-Ala–(R)-Nip–(S)-Nip–β-Ala–NHMe (Chung *et al.* 2000). In the case of αβ- and αδ-turns, the ϕ, ψ values at residues $(i+1)$ are similar to those at $(i+1)$ position of type I and type II′ turns. The ββ-turn is an example of a structure with a *cis* central peptide bond which can be formally viewed as homologation of both $(i+1)$ and $(i+2)$ residues of a type VI β-turn.

$(i+1)$ position of a type II β-turn analogue. In comparing the αβ/βα-turns with their αα counterparts, the ϕ, ψ value of the α-residue appears to be indicative of the nature of the β-turn type.

An inspection of the results summarized in table 1 reveals that crystallographically characterized examples of αβ (C_{11}), αγ, ββ (C_{12}), βγ, αδ (C_{13}) and γγ (C_{14}) are available. In the cases of hydrogen-bonded ring sizes of 12 atoms or greater, direct analogies with corresponding all α-amino acid turns are not immediately apparent. Within each category, families of turns distinguished by specific values of backbone torsion angles may be identified. For example, two distinct groups are observed in the case of the ββ C_{12}-turn in which constrained β-residues are forced to adopt a θ value of approximately $+90°$ while in the other θ is approximately 180°. The hydrogen-bond parameters in table 2 do not reveal any significant difference between the various types of hydrogen-bonded turns. Hydrogen-bond optimization thus appears to have been achieved in all cases.

5. HYDROGEN-BONDED TURNS AS COMPONENTS OF SECONDARY STRUCTURES

The following features emerge from the extensive literature on protein and peptide structures. Two-residue αα-turns in proteins may be classified into three distinct types as follows.

— *Helix promoting turns* in which successive turn formation occurs and there is a shared residue between any pair of contiguous turns. For example, repetitive type III turns give rise to the 3_{10}-helix (note that the polypeptide 3_{10} and the α-helical structures lie very close to one another in conformational space and are separated by relatively low barriers to interconversion).

— *Hairpin promoting turns* in which a central two residue β-turn facilitates sharp chain reversal resulting in the formation of registered antiparallel strands which are held together by cross-strand hydrogen bonds. Analysis of protein structures in a large body of evidence accumulated from designed peptide structures establish that the prime turns I′/III′ and II′ promote hairpin formation when positioned centrally in sequences made up of L-amino acids.

— *Isolated turns* which do not lie within secondary structures but permit a change of polypeptide chain direction, a property essential for the formation of globular compact structures. While all turn types can, in principle, occur as isolated structural features, attention may be drawn to the type II β-turn structure which cannot promote conventional helix formation and also does not readily permit registry of antiparallel strands. Figure 10 illustrates the nucleation of helices and hairpins by local turn formation in the α-polypeptide structures.

Table 1. Torsion angle parameters observed in crystallographically determined turn structures involving ω-amino acid residues. βVal, β valine; βPhe, β phenylalanine; Aib, α-aminoisobutyric acid; ACPC, *trans*-2-aminocyclopentanecarboxylic acid; β-Gly, β glycine; Dec, (2S,4S,6S)-2-amino-6-hydroxy-4-methyl-8-odxe-ocanoic acid; MHA, (4S, 2E)-4-methylhex-2-enoic acid; DPDA, (2S)-*N*,*N*-dimethylpropane-l,2-diamine; Me Pro, Methyl proline; HyLeu, hydroxyleucine; DAP, diamino pimelic acid; β-Res[1], 8-aminocyclooct-4-enecarboxylic acid; Gpn, Gabapentin, (1-(aminomethyl) cyclohexaneacetic acid); γAbu, γ-aminoisobutyric acid; γ-Res Z, 2, 2 di-fluoro,3-oxo,4-methyl γ-aminoisobutyric acid; γ-ResX, 3-methoxy, 4-methyl γ-aminoisobutyric acid; γ-ResY, 4-methyl, 2–3 eneyl γ-aminoisobutyric acid; β-Res A, 2-benzyl, 3-(thio phenyl)methyl β-glycine; β-Res B, 3-ethyl,2-methyl β-glycine; Nip, nipecotic acid; δAva, δ aminovaleric acid; γ-Res A, 2-benzyl, 4-methyl γ-aminoisobutyric acid; γ-Res B, 4-isopropyl, 2-methyl γ-aminoisobutyric acid; γ- Res C, 2-, 3-, 4-trimethyl, γ-aminoisobutyric acid and γ-Res D, 2-isopropyl, 3-,4- dimethyl γ-aminoisobutyric acid.

peptide/reference	turn type	residue	torsion angles (deg.) ϕ	θ_1	θ_2	θ_3	ψ	analogous α-turn
αβ-turns (C_{11})								
Boc-Val-Alu-Phe-Aib-βVal-βPhe-Aib-Val-Alu-Phe-Aib-OMe/Roy *et al.* (2004)	βα	β-Phe 6	−88	80	—	—	−118	type III
		Aib 7	−55	—	—	—	−49	
Boc-(Aib-*trans* ACPC)$_4$-OBz/Schmitt *et al.* (2005)	βα[a]	*trans* ACPC	−95.8	94.5	—	—	−89.9	type III
		Aib	−53.5		—	—	−39.9	
Boc-β-Gly-Aib-Leu-Aib-OMe/ Banerjee *et al.* (2003)	βα	βGly1	−103.8	83.7	—	—	− 84.7	type III
		Aib 2	−56.1	—			−44.6	
Boc-(*trans* ACPC-*trans* ACPC-Phe)$_2$-OMe/Schmitt *et al.* (2006)	βα	*trans* ACPC 2	−67.5	105.8	—	—	−129.8	type III
		Phe 3	−69.9	—	—	—	−23.1	
Boc-(*trans* ACPC-Aib-Aib)$_2$-OMe/ Schmitt *et al.* (2006)	βα[b]	*trans* ACPC	−89.2	89.2	—	—	−94.3	type III
		Aib	−54.6		—	—	−32.3	
Boc-(*trans* ACPC- *trans*ACPC-Phe)$_2$-OMe/Schmitt *et al.* (2006)	αβ	Phe 3	−69.9	—	—	—	−23.1	type III
		trans ACPC 4	−112.5	74.9	—	—	163.0	
Boc-Aib-Aib-βGly-NHMe/Pavone *et al.* (1992)	αβ[c]	Aib 2	−64.8	—	—	—	−13.52	type III
		βGly 3	−88.8	70.9	—	—	−101.3	
Boc-(Aib-*trans* ACPC)$_4$-OBz/Schmitt *et al.* (2005)	αβ[a]	Aib	−53.9	—	—	—	−41.2	type III
		trans ACPC	−95.8	94.5	—	—	−89.9	
MHA-MePro-Dec-HyLeu-Leu-Leu-Aib-Aib-βGly-DPDA, Cerrini *et al.* (1989)	αβ	Aib 8	−66.8	—	—	—	−49.2	type III
		βGly 9	−103.3	79.7	—	—	−78.5	
Boc-(*trans* ACPC-Aib-Aib)$_2$-OMe/ Schmitt, *et al.* (2006)	αβ	Aib3	−52.0	—	—	—	−40.0	type III
		trans ACPC 4	−95.0	95.8	—	—	−85.1	
Boc-Aib-βRes[1]-Aib-OMe/Tanaka *et al.* (2001)	αβ	Mol. A						variant tupe
		Aib	46.26	—	—	—	−135.7	
		β-Res	−72.79	72.9	—	—	83.78	
		Mol.B						
		Aib	−47.38	—	—	—	130.2	
		β-Res	84.64	69.14	—	—	−193.71	
αγ-turns (C_{12})								
Boc-Aib-Gpn-Aib-Gpn-OMe/Ananda *et al.* (2005)	αγ	Aib 1	−59.8	—	—	—	−37.8	—
		Gpn 2	−126.8	52.1	63.8		−107.9	
Boc-Aib-Gpn-Aib-Gpn-OMe/Ananda *et al.* (2005)	γα	Gpn 2	−126.8	52.1	63.8	—	−107.9	—
		Aib 3	−51.5	—	—	—	−48.8	
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-OMe/Karle *et al.* (1997)	γα	γAbu5	−108	58	66	—	−169	—
		Leu 6	−86	—	—	—	−18	
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-Ala-Leu-Aib-OMe/Karle *et al.* (1997)	γα	γAbu5	−121	62	67	—	−169	—
		Leu 6	−57	—	—	—	−37	
Boc-Val-Ile-γResZ-Val-Ile-OMe/ Wolfe *et al.* (1998)	γα	γ-ResZ	−101.2	65.2	62.6	—	−140.5	
		Leu	90.0	—	—	—	−28.5	—
Boc-γ-Abu-Aib-Ala-Aib-OMe/Maji *et al.* (2002)	γα	γAbu1	92.7	−69.7	−65.8	—	155.7	—
		Leu 2	58.4	—	—	—	26.1	
Boc-γ-ResX-^{D}Pro-Gly-γ-ResY-OMe/ Hagihara *et al.* (1992)	γα	MolA						
		γ-ResX	−98.7	66.2	66.63	—	−149.3	
		Pro	−64.2	—	—	—	−14.2	—
		MolB						
		γ-ResX	−100.8	58.8	65.11	—	−160.1	—
		Pro	−69.9	—	—	—	−14.8	
Boc-γ-Abu-Aib-Ala-OMe/Maji *et al.* (2002)	γα	MolA						
		γAbu1	−109.2	65.2	62.6	—	−140.4	
		Aib2	−54.5	—	—	—	−37.0	—
		MolB						
		γAbu1	107.5	−62.3	−65.1	—	138.4	—

(*Continued.*)

Table 1. (*Continued.*)

peptide/reference	turn type	residue	torsion angles (deg.) ϕ	θ_1	θ_2	θ_3	ψ	analogous α-turn
		Aib2	58.35	—	—	—	36.36	
ββ-turns (C_{12})								
Boc-(*trans*-ACPC)$_8$-CO_2CH_2Ph/ Appella *et al.* (1999*c*)	ββ[d]	*trans* ACPC	97.9	96.4	—	—	106.3	—
		trans ACPC	97.9	96.4	—	—	106.3	
Boc-(*trans* ACPC-*trans* ACPC-Phe)$_2$ OMe/Schmitt *et al.* (2006)	ββ	*trans* ACPC1	−122.5	77.9	—	—	−112.0	—
		trans ACPC2	−67.5	105.8	—	—	−129.8	
Boc-(*trans* ACPC- *trans* ACPC-Phe)$_2$OMe/Schmitt *et al.* (2006)	ββ	*trans* ACPC4	−112.5	74.9	—	—	−78.8	—
		trans ACPC5	−92.2	90.1	—	—	−98.9	
Piv-β-Res A-Nip1-Nip2-β-Res B-NHMe/Chung *et al.* (1998)	ββ	Nip 1	115.7	−175.2	—	—	81.8	—
		Nip 2	−117.4	−176.6	—		−71.2	
Boc-B-Ala-(R)-Nip-(S)- Nip-β-Ala-NHMe/Chung *et al.* (2000)	ββ[e]	Nip 1	98.8	−177.4	—	—	81.7	—
		Nip 2	−121.8	179.3	—		−59.4	
βγ-turns (C_{13})								
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-OMe/Karle *et al.* (1997)	βγ	βGly4	−103	78	—	—	−107	—
		γAbu5	−121	63	57	—	−121	
αδ-turns (C_{13})								
Boc-Leu-Val-Val-^{D}Pro-δAva-Leu-Val-Val-OMe/Rai *et al.* (in press)	αδ	^{D}Pro 4	64.1	—	—	—	−156.9	—
		δAva5	−126.8	53.2	61.7	166.5	76.9	
γγ-turns (C_{14})								
Boc-γ-Res A-γ-Res B-NHMe/Brenner & Seebach (2001)	γγ	γ-Res1	157.9	−167	67.8	—	−113.6	—
		γ-Res2	−121.8	59.5	−177.8	—	149.6	
Ac-γ-Res A-γ-Res B-NHMe/Brenner & Seebach (2001)	γγ[e]	γ-Res1	148.1	−169.4	73.4	—	−112.6	—
		γ-Res2	−119.9	60.4	176.7	—	147.1	
Boc-(γ-ResC-γ-ResD-)$_2$OBz/Seebach *et al.* (2001*a*)	γγ[e]	γ-Res3	132.4	−69.9	−56.9	—	138.9	—
		γ-Res4	153.5	−70.4	−56.3	—	124.9	

[a] Torsion angle values for Aib residue in the αβ-turn is averaged over Aib residues 1, 3 and 5 and in the βα turn is averaged over the Aib residues 3, 5 and 7. Torsion angles of *trans* ACPC residue is averaged over residues 2, 4 and 6.
[b] Torsion angle values for *trans* ACPC is averaged over residues 1 and 4, while Aib is averaged over residues 2 and 5.
[c] Achiral peptide.
[d] Torsion angles averaged over the 7 *trans* ACPC residues of the peptide from the N-terminus. C-terminal residue has been excluded as the helix frays at the C-terminus.
[e] Torsion angles averaged over the residues in the two independent molecules in the crystal.

By analogy with the α-polypeptide structures, hybrid turns must also fall into distinct categories capable of generating hybrid helices or isolated chain reversals. Examination of available crystal structures of ω-amino acids containing peptides provides glimpses into the structural possibilities for creating new folded polypeptides. Hybrid turns have been characterized within the context of regular secondary structures. For example, insertion of two contiguous β-residues into the centre of a potentially helical host α-peptide in the sequence Boc–Val–Alu–Phe–Aib,βVal–βPhe–Aib–Val–Alu–Phe–Aib–OMe (Roy *et al.* 2004, figure 11), results in the formation of a C_{11} βα-turn between the CO of βVal 5 and NH of the Val 8 (table 1). Successive C_{11} H-bonded turns are observed in the alternating hybrid sequence Boc-(Aib- *trans* ACPC)$_4$OBz (ACPC=*trans*-2-aminocyclopentanecarboxylic acid; Schmitt *et al.* 2005, figure 11). This structure corresponds to a C_{11}-helix, which may be considered as an expanded version of the 3_{10}-helix formed by the insertion of one backbone atom at every alternate residue (table 1). The insertion of a βγ hybrid sequence into a host α-peptide helical sequence in the peptide, Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–OMe and Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–Alu–Leu–Aib–OMe (βGly=β-glycine, γAbu=γ-aminoisobutyric acid), results in the formation of C_{12}-turns within the context of a helical structure (table 1, Karle *et al.* 1997). The C_{12}-helix formed in the case of all β-peptide Boc-(*trans* ACPC)$_8$-CO_2CH_2Ph, is also a 3_{10}-helix analogue with homologation at each position along the chain (table 1, figure 11, Appella *et al.* 1999*c*). Regular C_{12}-helical structures can also be generated by $(\alpha\gamma)_n$ sequences as illustrated by the crystal structures of Boc–Aib–Gpn–Aib–Gpn–OMe (Gpn=1-(aminomethyl)cyclohexane acetic acid; Ananda *et al.* 2005, table 1, figure 11). A βγ segment forming a C_{13} hydrogen bond has been characterized in the crystals of Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–OMe and Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–Alu–Leu–Aib–OMe (Karle *et al.* 1997, table 1).This hydrogen-bonded turn is formally equivalent to a three-residue ααα C_{13}-turn. Seebach *et al.* (2001*a*) have established an incipient 2.6_{14} (14-helix) in a protected tetrapeptide formed from 2,3,4-trisubstituted γ-amino acids (figure 11, Seebach *et al.* 2001*a*).The formation of the two consequtive C_{14} hydrogen-bonded turn is undoubtedly promoted by the substitution pattern along the backbone (*R*, *R*, *R*) which promotes adoption of the *gauche* (+)–*gauche* (+) conformations about the C_β–C_γ (θ_1) and C_α–C_β(θ_2)

Table 2. Hydrogen-bond parameters observed in crystallographically determined turn structures involving ω-amino acid residues. (Abbreviations are the same as given in table legend 1.)

peptide sequence	turn type	turn residues	donor	acceptor	hydrogen bond parameters			
					O···H (Å)	N···O (Å)	C=O···H (deg.)	C=O···N (deg.)
11-membered hydrogen bonds								
Boc-(Aib-*trans* ACPC)$_3$-OBz/Schmitt *et al.* (2005)	βα	*trans* ACPC2 Aib3	*trans* ACPC4 NH	Aib 1 C=O	2.03	2.84	137.5	145.1
	βα	*trans* ACPC4 Aib5	*trans* ACPC6 NH	Aib 3 C=O	2.00	2.85	132.9	136.3
	βα	*trans* ACPC6 Aib7	*trans* ACPC8 NH	Aib 5 C=O	1.94	2.79	138.0	143.6
Boc-Val-Ala-Phe-Aib-βVal-βPhe-Aib-Val-Ala-Phe-Aib-OMe/Roy *et al.* (2004)	βα	β Phe6 Aib 7	Val 8 NH	β Val 5 C=O	2.57	3.05	122.5	137.0
Boc-β-Gly-Aib-Leu-Aib-OMe/Banerjee *et al.* (2003)	βα	βGly1 Aib2	Leu 3 NH	Boc 0 C=O	2.20	3.02	131.8	137.1
Boc-(*trans* ACPC-*trans* ACPC-Phe)$_2$-OMe/Schmitt *et al.* (2006)	βα	*trans* ACPC2 Phe 3	*trans* ACPC4 NH	*trans* ACPC1 C=O	2.17	2.93	150.7	160.8
Boc-(*trans* ACPC-Aib-Aib)2-OMe/ Schmitt *et al.* (2006)	βα	*trans* ACPC1 Aib2	Aib 3 NH	Boc 0 C=O	2.08	2.89	141.5	148.0
	βα	*trans* ACPC4 Aib5	Aib 6 NH	Aib 3 C=O	2.22	3.06	134.7	141.5
Boc-(Aib-*trans* ACPC)$_3$-OBz/Schmitt *et al.* (2005)	αβ	Aib1 *trans* ACPC2	Aib3 NH	Boc 0 C=O	2.10	2.93	141.0	145.0
	αβ	Aib3 *trans* ACPC4	Aib5 NH	*trans* ACPC2 C=O	2.00	2.88	144.3	146.8
	αβ	Aib5 *trans* ACPC6	Aib7 NH	*trans* ACPC4 C=O	2.03	2.91	150.4	149.7
Boc-(*trans* ACPC-Aib-Aib)2-OMe/ Schmitt *et al.* (2006)	αβ	Aib3 *trans* ACPC4	Aib2 NH	Aib5 C=O	2.07	2.95	138.8	139.9
Boc-Aib-Aib-βGly-NHMe/Pavone *et al.* (1992)	αβ	Aib 2 βGly 3	NHMe NH	Aib1 C=O	2.24	3.07	150.7	143.7
MHA-MePro-Dec-HyLeu-Leu-Leu-Aib-Aib-βGly-DPDA/Cerrini *et al.* (1989)	αβ	Aib 8 βGly 9	NHMe NH	Aib 7 C=O	2.03	3.01	96.5	114.2
Boc-Aib-βRes^1-Aib-OMe/Tanaka *et al.* (2001)	αβ	Aib 1 βRes2	Aib3 NH	Boc0 C=O	1.99	2.94	158.5	163.1
					2.07	3.08	165.8	164.4
12-membered hydrogen bonds								
Boc-Aib-Gpn-Aib-Gpn-OMe/Ananda *et al.* (2005)	αγ	Aib1 Gpn2	Aib3 NH	Boc0 C=O	2.11	2.93	140.9	144.6
	γα	Gpn2 Aib3	Gpn4 NH	Aib1 C=O	2.19	3.05	138.0	139.8
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-OMe/Karle *et al.* (1997)	γα	γAbu5 Leu6	Aib7 NH	βGly4 C=O	2.03	2.8	155.2	165.0
Boc-Leu-U-V-βG-γAbu-Leu-Aib-Val-Ala-Leu-Aib-OMe/Karle *et al.* (1997)	γα	γAbu5 Leu6	Aib7 NH	βGly4 C=O	1.88	2.8	144.8	148.0
Boc-γ-Abu-Aib-Ala-OMe/Maji *et al.* (2002)	γα	γ-Abu1	Ala 3	Boc 0	2.0	2.8	153.2	162.0
		Aib2	NH	C=O	2.1	2.9	155.1	163.0
Boc-γ-Abu-Aib-Ala-Aib-OMe/Maji *et al.* (2002)	γα	γ-Abu1 Aib2	Ala 3 NH	Boc 0 C=O	2.2	3.1	151.4	159.7
12-membered hydrogen bonds								
Boc-(*trans*-ACPC)$_8$-CO_2CH_2Ph/ Appella *et al.* (1999*c*)	ββ	*trans* ACPC1 *trans* ACPC2	*trans* ACPC 3 NH	Boc 0 C=O	2.16	2.93	170.3	175.1
	ββ	*trans* ACPC2 *trans* ACPC3	*trans* ACPC 4 NH	*trans* ACPC 1 C=O	2.03	2.83	155.7	148.3
	ββ	*trans* ACPC3 *trans* ACPC4	*trans* ACPC 5 NH	*trans* ACPC 2 C=O	2.02	2.83	152.8	149.7

(*Continued.*)

Table 2. (*Continued.*)

peptide sequence	turn type	turn residues	donor	acceptor	hydrogen bond parameters			
					O···H (Å)	N···O (Å)	C=O···H (deg.)	C=O···N (deg.)
	ββ	*trans* ACPC4 *trans* ACPC5	*trans* ACPC 6 NH	*trans* ACPC 3 C=O	2.03	2.88	148.3	147.3
	ββ	*trans* ACPC5 *trans* ACPC6	*trans* ACPC 7 NH	*trans* ACPC4 C=O	2.14	2.87	159.3	154.7
	ββ	*trans* ACPC6 *trans* ACPC7	*trans* ACPC 8 NH	*trans* ACPC5 C=O	2.02	2.87	151.0	145.9
Boc-(*trans* ACPC-*trans* ACPC-Phe)$_2$ OMe/Schmitt *et al.* (2006)	ββ	*trans* ACPC1 *trans* ACPC2	Phe3 NH	Boc 0 C=O	2.07	2.94	143.9	142.5
	ββ	*trans* ACPC4 *trans* ACPC5	Phe6 NH	Phe3 C=O	2.07	2.93	141.0	137.2
Boc-B-Ala-(R)-Nip-(S)- Nip-β-Ala-NHMe/Chung *et al.* (2000)	ββ	R-Nip2 S- Nip3	B-Ala4 NH	B-Ala1 C=O	2.11	2.92	153.6	161.5
13/14-membered hydrogen bonds								
Boc-Leu-Aib-Val-βGly-γAbu-Leu-Aib-Val-OMe/Karle *et al.* (1997)	βγ	βGly4 γAbu5	Leu (6) NH	Val (3) C=O	2.05	2.93	142.1	141.9
Boc-Leu-Val-Val-^{D}Pro-δAva-Leu-Val-Val-OMe/Rai *et al.* (in press)	αδ	DPro4 δAva5	Leu (6) NH	Val (3) C=O	2.06	3.03	151.8	147.3
Boc-(γ-Res C-γ-Res D)$_2$ OBz/Seebach *et al.* (2001*a*)	γγ-helix	γ-ResC γ-Res D	γ-Res C NH	Boc (0) C=O	2.09	2.89	154.4	170.4
Boc-γ-Res A-γ-Res B-NHMe/Brenner & Seebach (2001)	γγ-hairpin	γ-ResA γ-Res B	NHMe NH	Boc (0) C=O	2.04	3.24	150.6	166.2

bonds of the γ-residue. Recent modelling studies suggest that the construction of regular hybrid helices varying in the nature of the repeating hydrogen-bonded turn are indeed stereochemically and energetically favourable (Ananda *et al.* 2005; Baldauf *et al.* 2006*a*,*b*). While crystallographic characterization of hybrid helices has been restricted to relatively short peptides it is clear that crystal structures of longer hybrid sequences will undoubtedly be forthcoming.

In the creation of hybrid structures which mimic the regular α-polypeptide conformation, the additional atoms inserted into the backbone can sometimes be accommodated as protrusions. In such cases, the terminal backbone atoms involved in providing the scaffold for the hydrogen bond remain roughly at the same locations. A superposition of the all α-residue turn with a hybrid peptide analogue results in a good superposition of eight backbone atoms in the N- and the C-terminal ends of the turns. Figure 12 illustrates this point. It is noteworthy that the βγ-turn observed in peptide Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–Ala–Leu–Aib–OMe superposes very well (RMSD = 0.306 Å) with the α-helical ααα-turn established in the model peptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Ala–Leu–Aib–OMe (Aravinda *et al.* 2004). In contrast the αδ-turn observed in the hairpin peptide Boc–Leu–Val–Val–DPro–δAva–Leu–Val–Val–OMe, which contains the same number of backbone atoms as the α-helical turn superposes poorly (RMSD = 1.205 Å). Clearly of the two crystallographically characterized C_{13}-turns, the βγ example corresponds to an analogy of the α-peptide helical turn, while the αδ example mimics the hairpin forming prime turns observed in αα sequence.

Hybrid turns can also facilitate antiparallel β-hairpin formation. Solution NMR studies have established β-hairpin structures in the sequences Boc–Leu–Val–Val–DPro–βPhe–Leu–Val–Val–OMe (Gopi *et al.* 2002), Boc–Leu–Phe–Val–DPro–βAc_6c–Leu–Phe–Val–OMe and Boc–Leu–Phe–Val–DPro–Gpn–Leu–Phe–Val–OMe (Rai *et al.* in press). In these cases, the turns formed are of C_{11} (αβ)- or the C_{12} (αγ)-type with ^{D}Pro at (i+1) position. The only crystallographically characterized β-hairpin with a central hybrid turn is in Boc–Leu–Val–Val–DPro–δAva–Leu–Val–Val–OMe (figure 13, table 1, Rai *et al.* in press). The hairpin forming hybrid turns may be considered as Type II′ β-turn homologues.

6. STEREOCHEMICALLY CONSTRAINED ω-AMINO ACIDS IN PEPTIDE DESIGN

The design of well defined conformations in short α-peptide sequences has been greatly facilitated by the use of stereochemically constrained amino acid residues. Local restrictions on the backbone conformational angles ϕ and ψ serve to nucleate specific structural features which in turn promote secondary structure formation. While a large number of modified residues have been used, this approach is best exemplified by α-aminoisobutyryl residue (Aib) which promotes helix nucleation and ^{D}Pro residues which promotes prime

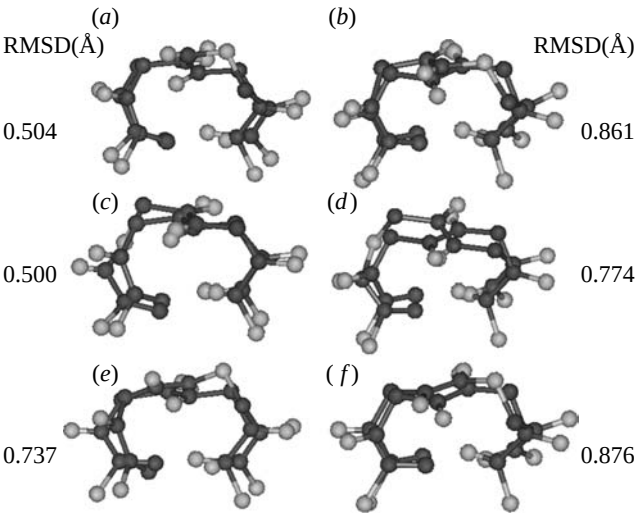

Figure 9. Superpositions of nine equivalent atoms (only C^{β} of the β-residue is omitted) of observed αβ/βα-turns with their α-peptide type I and type II turn counterparts (ideal structures). Superposition of (*a*) type I β-turn and αβ (1) turn of Boc-(Aib-*trans* ACPC)$_4$OBz, (*b*) type II β-turn and αβ (1) turn of Boc-(Aib-*trans* ACPC)$_4$OBz, (*c*) type I β-turn and βα (1) turn of Boc-(Aib-*trans* ACPC)$_4$OBz, (*d*) type II β-turn and βα (1) turn of Boc-(Aib-*trans* ACPC)$_4$OBz, (*e*) type I β-turn and αβ-turn of molecule B of Boc–Aib–β-Res–Aib–OMe and (*f*) type II β-turn and αβ-turn of molecule B of Boc–Aib–β-Res–Aib–OMe (β-Res in E and F = 8-aminocyclooct-4-ene carboxylic acid).

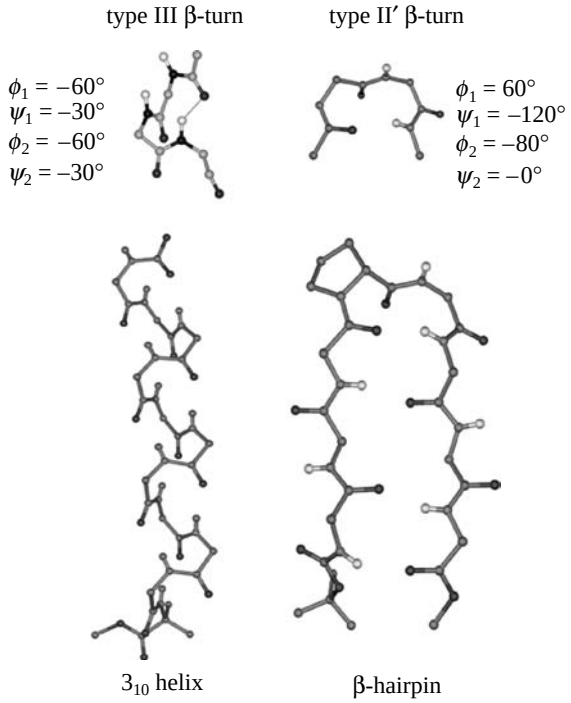

Figure 10. Nucleation of helices and hairpins by local turn formation in α-polypeptide structures. Turns shown are idealized structures. The helix is a view of pBrBz-$(Aib)_{11}$-O*t*Bu (Gesman *et al.* 2003). The average ϕ, ψ values for the 3_{10}-helix are −52.4 and −31.1°. The hairpin is from the structure of Boc–Leu–Val–Val–DPro–Gly–Leu–Val–Val–OMe (Karle *et al.* 1996). The ϕ, ψ values at the turn residues are: $\phi_{(i+1)} = 62.5°$, $\psi_{(i+1)} = -132.4°$, $\phi_{(i+2)} = -80.7°$ and $\psi_{(i+2)} = -3.4°$. Side-chain atoms of the residues and protecting groups in the helix and hairpin have been deleted for clarity.

turn formation resulting in the generation of hairpin structures (Venkatraman *et al.* 2001; Aravinda *et al.* 2003). Extension of this approach to the ω-amino acids is clearly warranted since the number of backbone torsional variables increase with the number of backbone atoms.

Figures 14 and 15 illustrate the structures of several β-amino acid residues in which rotation about the backbone torsion angles is restricted. For example, the Gellman group has successfully demonstrated structure formation in short β-peptides using the cyclic β-residues ACHC (*trans*-2-aminocyclohexanecarboxylic acid; Appella *et al.* 1996, 1999*b*) and ACPC (*trans*-2-aminocyclopentanecarboxylic acid; Appella *et al.* 1997, 1999*c*). In these cases, the constraints of cyclization restrict the values of θ to *gauche* conformations, which promote backbone folding. Nipecotic acid provides an example where the value of θ (approx. 180°) is restricted by cyclization to a value close to that required for an extended geometry (Chung *et al.* 1998, 2000). By analogy with the α-amino acids it is clear that substitution at backbone positions serves to restrict the range of accessible conformations. Substituent effects can be most readily understood in the case of α-amino acids by comparing the Ramachandran allowed regions for Gly, l-ala, Aib (Aravinda *et al.* 2003.). There is a progressive restriction in the accessible region of ϕ, ψ space upon backbone substitution. Similarly in β- and the higher ω-amino acid residues rotational restrictions about the flanking single bonds may be anticipated upon substitution at a specific position. The extensive literature on structure formation in peptides containing multiply substituted β- and γ-residues clearly illustrates the role of substituent effects (Hanessian *et al.* 1998; Rueping *et al.* 2002; Seebach *et al.* 2004). For example, Seebach and co-workers have established the effectiveness of α-, β-, γ-trisubstituted γ-amino acids in promoting folded structures in short sequences (Brenner & Seebach 2001; Seebach *et al.* 2001*a*). Figure 16 illustrates in interesting example of a hairpin conformation containing a δ-amino acid (sugar amino acid) at the $(i+2)$ position of the β-turn (Grotenberg *et al.* 2004).

Ongoing work in this laboratory has focused on two achiral geminally disubstituted β- and γ-amino acids, β-Ac_6c (1-aminocyclohexaneacetic acid) and Gpn (gabapentin, 1-(aminomethyl)cyclohexaneacetic acid; Ananda *et al.* 2003). These may be viewed as the homologues of the αα di-substituted residue Ac_6c (1-aminocyclohexane-1-carboxylic acid; figure 17, Paul *et al.* 1986). Gabapentin is a widely used anti-epileptic agent (Chadwick *et al.* 1998; Sill 2006) which has also been advanced for the treatment of deep neuropathic pain (Rosenberg *et al.* 1997; Gilron & Flatters 2006). The availability of gabapentin as a bulk drug makes it an attractive starting point in the synthesis of designed hybrid peptides containing γ-residues. The geminal substituents at the central C^{β}-atom restrict the values of θ_1, θ_2 to about $\pm 60°$. As a consequence, the gabapentin residue strongly promotes backbone folding with concomitant formation of intramolecular

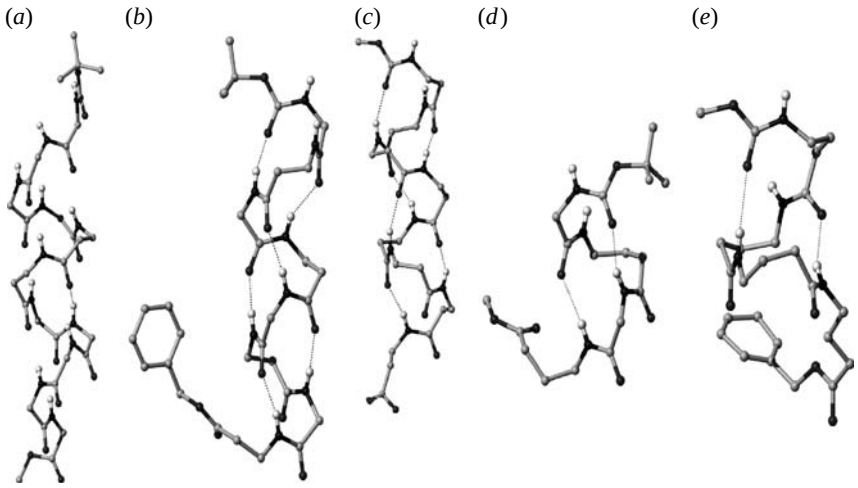

Figure 11. Helices containing ω-amino acids. (*a*) Contiguous ββ segment inserted into a potential helical peptide made up of all α-amino acids (Boc–Val–Ala–Phe–Aib–βVal–βPhe–Aib–Val–Ala–Phe–Aib–OMe, Roy *et al.* 2004), (*b*) C_{11}-helix formed in Boc-(Aib-*trans*-ACPC)$_4$OBz (Schmitt *et al.* 2005), (*c*) C_{12}-helix formed in Boc-(*trans* ACPC)$_8$CO$_2$CH$_2$Ph (Appella *et al.* 1999*c*), (*d*) C_{12}-helix in Boc–Aib–Gpn–Aib–Gpn–OMe (Ananda *et al.* 2005) and (*e*) C_{14}-helix in Boc-(γ-ResC-γ-ResD−)$_2$OBz. (γ-Res C=2,3,4-trimethyl, γ-aminoisobutyric acid; γ-Res D=2-isopropyl,3,4-dimethyl γ-aminoisobutyric acid; Seebach *et al.* 2001*a*). Side-chain atoms of the residues and protecting groups have been deleted for clarity.

hydrogen bonds. Figure 18 illustrates two distinct structural motifs that have been characterized in Gpn peptides. In Gpn oligomers, the C_9 ribbon (Vasudev *et al.* 2005) is formed which may be formally viewed as the expansion of the C_7 ribbon or the 2.2 helix in all α-polypeptides. In the alternating peptide Boc–Aib–Gpn–Aib–Gpn–OMe (Ananda *et al.* 2005), two consequtive C_{12}-turns are formed constituting an incipient C_{12}-helical $(\alpha\gamma)^n$ structure. A large body of crystallographic observations on synthetic peptides carried out in this laboratory reveal that the Gpn residue is almost exclusively restricted to the *gauche*, *gauche* conformation about the C^{α}–C^{β} and C^{β}–C^{γ} bonds (P. G. Vasudev, N. Shamala & P. Balaram, unpublished result).

7. CONCLUSION

The growing body of structural studies on hybrid peptides containing α-, β-, γ- and δ-amino acid residues suggests that backbone modification of the backbone by the homologation of the chain vastly expands the repertoire of polypeptide structures. Contrary to original expectations, higher homologues of the α amino acid residues can be readily accommodated into well-folded secondary structures. Thus even unsubstituted β-, γ- and δ-residues have been shown to adopt folded conformations when incorporated into peptide sequences (Karle *et al.* 1997; Rai *et al.* in press). Undoubtedly the energy differences between the *trans* and the *gauche* forms of the homologues of α-amino acid residues is not large and can be readily offset by the compensating contributions of intramolecular hydrogen bonds. Diversity in peptide structures has traditionally being generated by varying the nature of the side chain in the α-amino acid residues. In hybrid peptides, an additional mode of generating molecular diversity becomes apparent. The enhanced stability of peptide bonds formed by ω-amino acids to proteolysis is

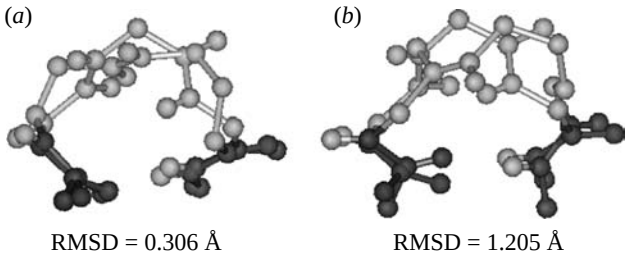

Figure 12. Superposition of an all α-residue turn with hybrid peptide analogue. Four backbone atoms in the N(C^{α}_i, C_i, O_i, $N_{(i+1)}$) and C ($C_{(i+2)}$, $O_{(i+2)}$, $N_{(i+3)}$, $C^{\alpha}_{(i+3)}$) terminal ends of the turns are superposed from crystal structures. Superposition of (*a*) βγ (C_{13})-turn observed in peptide Boc–Leu–Aib–Val–βGly γAbu–Leu–Aib–Val–Alu–Leu–Aib–OMe (Karle *et al.* 1997) with ααα-turn established in the model α-helical peptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Ala–Leu–Aib–OMe (Aravinda *et al.* 2004) and (*b*) αδ-turn observed in the peptide Boc–Leu–Val–Val–DPro–δAva–Leu–Val–Val–OMe (Rai *et al.* in press) with an ααα (C_{13})-turn established in a model α-helical peptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Ala–Leu–Aib–OMe (Aravinda *et al.* 2004).

an important consideration in the use of backbone-homologated amino acid residues in the design of peptide mimetics (Hintermann & Seebach 1997; Seebach *et al.* 1998*b*, 2001*b*; Frackenpohl *et al.* 2001; Porter *et al.* 2002, Gopi *et al.* 2003; Lelias & Seebach 2003; Hook *et al.* 2004; Schmitt *et al.* 2004). Conformational features in hybrid sequences can be established only in synthetic model peptides making the collection of structural data a slow process. This is in contrast to the case of α-polypeptides, where each structure determination of a naturally occurring protein provides an enormous wealth of conformational information. The propensity of higher homologues of α-amino acids to adopt folded conformations augurs well for the design of relatively long hybrid sequences,

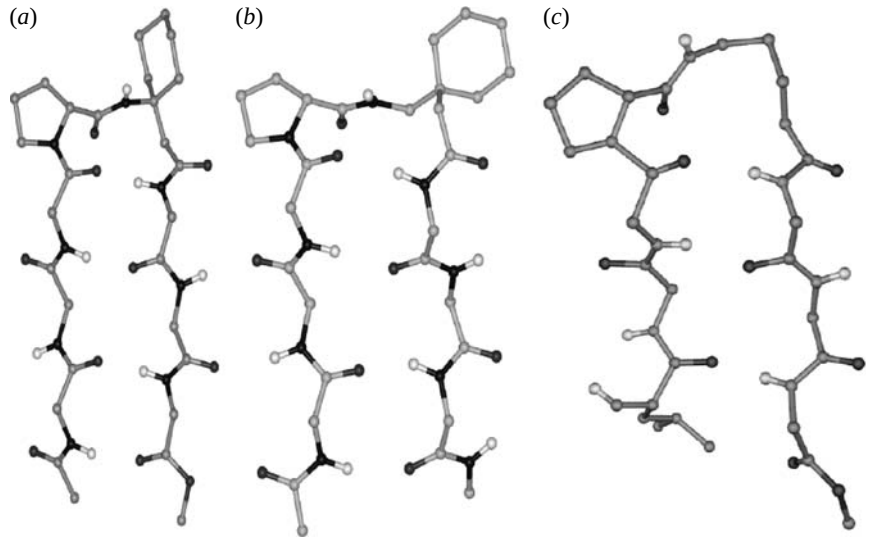

Figure 13. Hybrid turns facilitate antiparallel sheet formation. Hairpin containing central αω segments (*a*) αβ-turn in Boc–Leu–Phe–Val–^{D}Pro-βAc$_6$c-Leu–Phe–Val–OMe (Rai *et al.* in press), (*b*) αγ-turn in Boc–Leu–Phe–Val–^{D}Pro–Gpn–Leu–Phe–Val–OMe (Rai *et al.* in press), (*c*) αδ-turn in Boc–Leu–Val–Val–^{D}Pro–δAva–Leu–Val–Val–OMe (Rai *et al.* in press). (*a*) and (*b*) are NMR derived structures while (*c*) is determined by X-ray diffraction. Side-chains atoms of the residues in the hairpins and the protecting groups have been deleted for clarity.

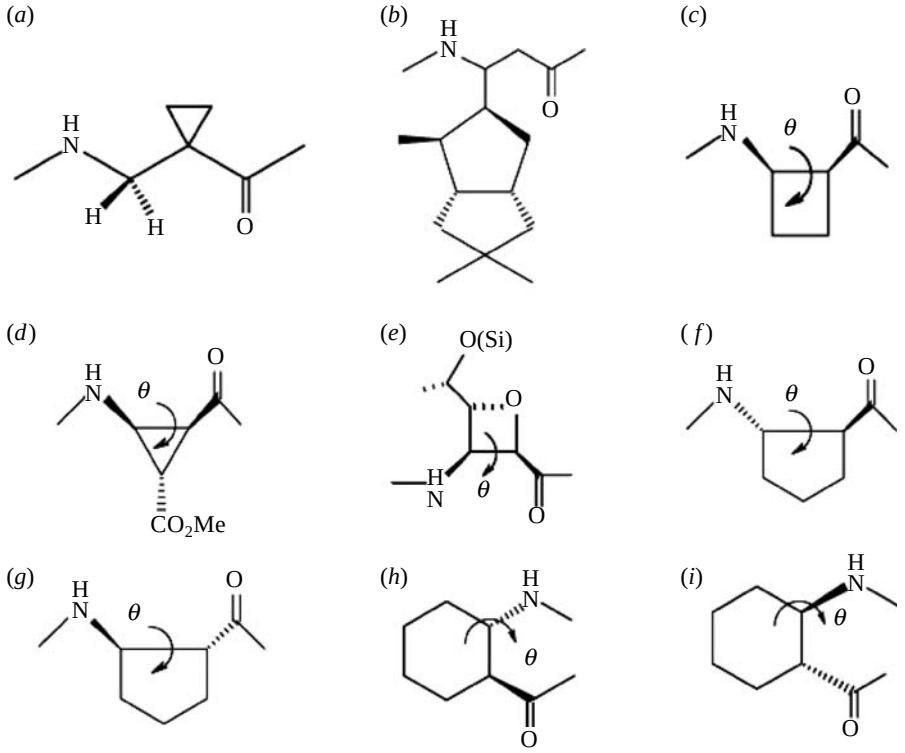

Figure 14. Helix promoting β-amino acid residues. (*a*) 1-(Aminomethyl) cyclopropane carboxylic acid (Abele *et al.* 1999); (*b*) C-linked carbo-β-amino acid (Sharma *et al.* 2006); (*c*) *cis*-β-amino cyclobutanecarboxylic acid (Izquierdo *et al.* 2004); (*d*) *cis*-β-aminocycloproanecarboxylic acid (De Pol *et al.* 2004); (*e*) (2R,3S)-oxetane 3-amino-2-carboxylic acid (Claridge *et al.* 2001); (*f*) (S, S)-*trans*-ACPC (ACPC=*trans*-2-aminocyclopentanecarboxylic acid, $\theta=90°$) (Appella *et al.* 1999*c*); (*g*) (R, R)-*trans*-ACPC ($\theta=-90°$) (Appella *et al.* 1999*c*); (*h*) (S, S)-*trans*-ACHC (ACHC=*trans*-2-aminocyclohexanecarboxylic acid, $\theta=60°$) (Appella *et al.* 1999*b*) and (*i*) (R, R)-*trans*-ACHC ($\theta=-60°$) (Appella *et al.* 1999*b*).

which may fold into globular structures, mimicking those formed in naturally occurring peptides/proteins. Clearly, the attributes of globularity and order can be achieved in polypeptides with non-α amino acid backbones. There is little doubt that many novel structural features remain to be established in hybrid peptide sequences. The availability of readily available, constrained ω-amino acids, of which gabapentin is an example, should greatly facilitate the task of rational design of predictably folded hybrid peptides.

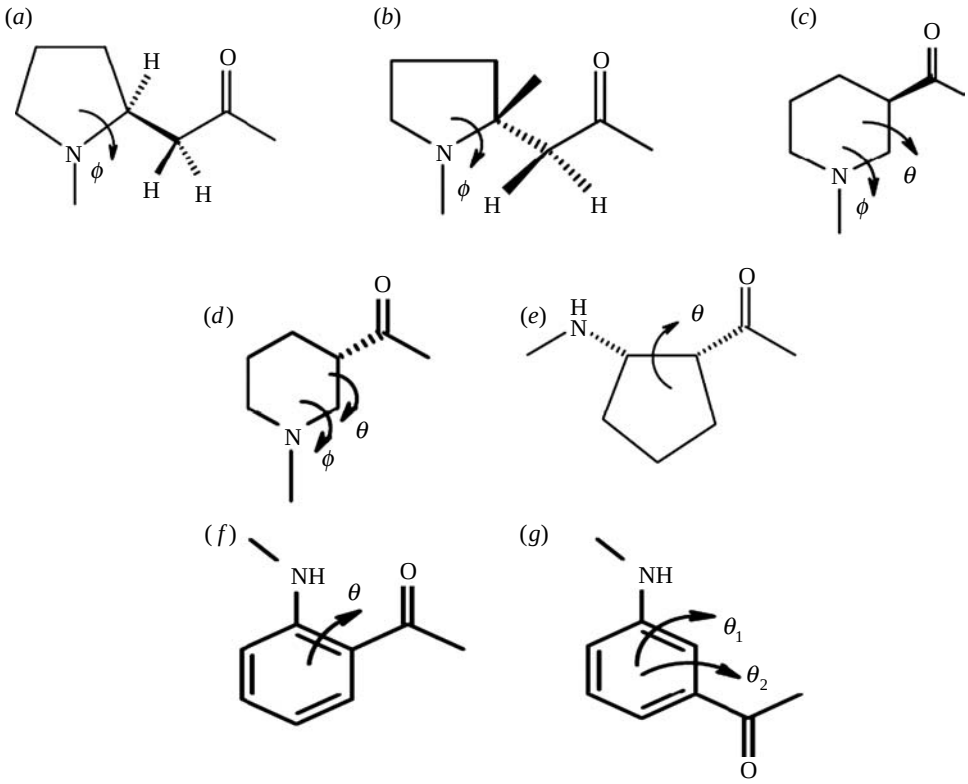

Figure 15. Constrained β-amino acids characterized in non-helical conformations. (*a*) (S)-(3-HProline ($\phi = -60°$) (Seebach *et al.* 2004); (*b*) (R)-(3-HProline ($\phi = 60°$) (Seebach *et al.* 2004); (*c*) (R)-nipecotic acid ($\phi = 100°$–$120°$, ($= 180°$) (Chung *et al.* 2001); (*d*) (S)-nipecotic acid ($\phi = -100°$ to $-120°$, $\theta = 180°$) (Chung *et al.* 2001); (*e*) (S, R)-*trans*-ACPC (Martinek *et al.* 2002); (*f*) 2-amino benzoic acid ($\theta = 0°$); (*g*) 3-amino benzoic acid ($\theta_1 = 180°$, $\theta_2 = 180°$) (Ramana Rao *et al.* 2003).

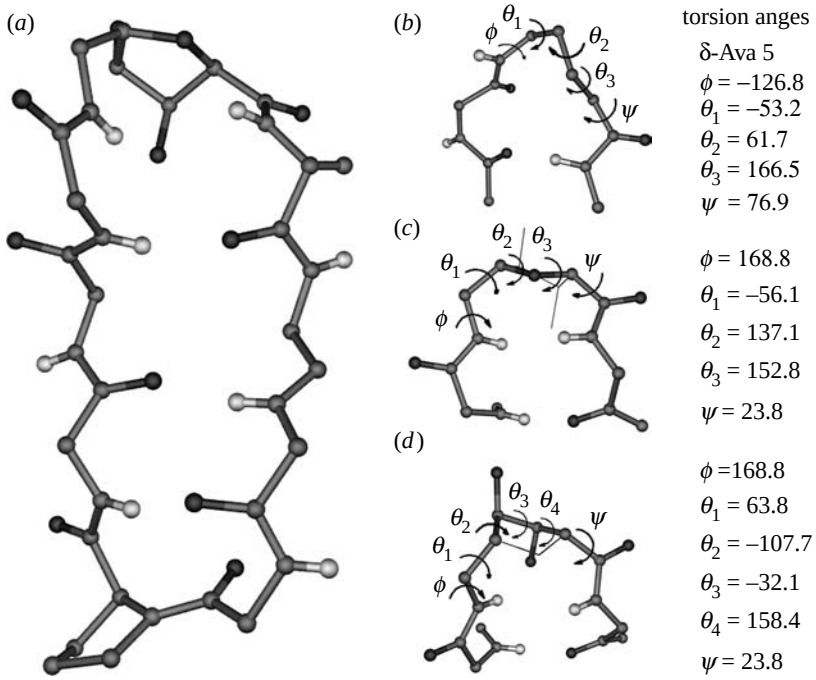

Figure 16. (*a*) View of cyclo-(SAA–Val–Orn–Leu–^{D}Phe–Pro–Val–Orn–Leu) (Grotenberg *et al.* 2004). (*b*) Central αδ-turn (C_{13}) segment in Boc–Leu–Val–Val–^{D}Pro–δAva–Leu–Val–Val–OMe (Rai *et al.* in press).The sugar amino acid residue (SAA) 2,5-anhydro-6-azido-6 deoxy-4-*o*-pivaloyl-D-gluconic acid may be viewed in two ways. (*c*) As a δ-residue in which the α and the δ carbon atoms are covalently bridged by CHR–CHR segment. The oxygen atom occupies the γ position and (*d*) as an ε-amino acid residue disubstituted at the γ and δ positions with β and ε atoms covalently bridged by the heteroatom in the sugar. The side-chain atoms have been deleted for clarity.

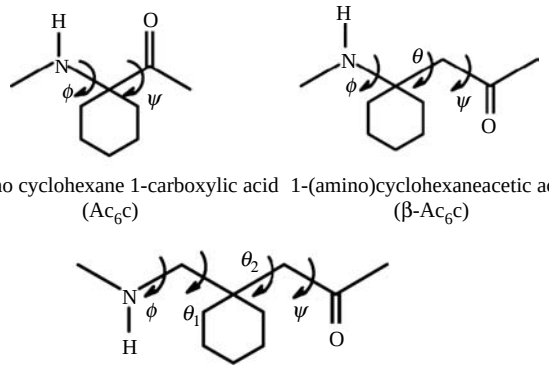

Figure 17. Structures of Ac_6c, β-Ac_6c and Gpn.

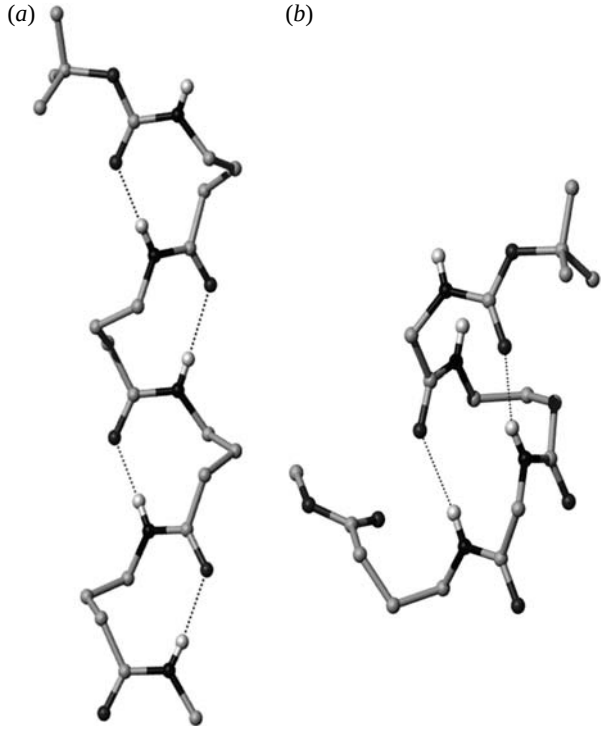

Figure 18. Two distinct structural motifs characterized in gabapentin peptides. (*a*) C_9 ribbon (expansion of 2.2_7-helix) in Boc–Gpn–Gpn–Gpn–Gpn–NHMe (Vasudev *et al.* 2005) and (*b*) C_{12} helix (expansion of 3_{10} (C_{10})-helix) in Boc–Aib–Gpn–Aib–Gpn–OMe (Ananda *et al.* 2005). Side-chain atoms of the residues have been deleted for clarity.

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