Non-protein amino acids in peptide design[¶]

S ARAVINDA[a], N SHAMALA[a], RITUPARNA S ROY[b] and
P BALARAM[b,c]*
[a]Department of Physics, and [b]Molecular Biophysics Unit, Indian Institute of
Science, Bangalore 560 012, India
[c]Chemical Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific
Research, Jakkur Campus, Jakkur PO, Bangalore 560 064, India
e-mail: pb@mbu.iisc.ernet.in

Abstract. An overview of the use of non-protein amino acids in the design of
conformationally well-defined peptides, based on work from the author's laboratory,
is discussed. The crystal structures of several designed oligopeptides illustrate the use
a-aminoisobutyric acid (Aib) in the construction of helices, D-amino acids in the
design of helix termination segments and [D]Pro–Xxx segments for nucleating of
b-hairpin structures. *b*- and *g*-amino acid residues have been used to expand the range
of designed polypeptide structures.

Keywords. Peptide design; crystal structures; C–H···O hydrogen bond; hybrid
peptides; D-amino acids; *b*-amino acids.

1. Introduction

The 'first principles' approach to the design of polypeptide structures, which adopt well-
defined folded conformations, developed at Bangalore, relies on the use of stereo-
chemically constrained amino acids to direct peptide chain folding.[1,2] The *de novo* design
of polypeptide structures provides insights into the factors that govern the folding of
peptides and proteins. The approach is based on the ability of specific non-protein amino
acid residues to populate limited regions of conformational space, thereby serving as
local nuclei for the propagation of ordered secondary structures. This overview presents
the general background to the use of non-protein amino acids as stereochemical directors
of polypeptide chain folding and illustrates some recent examples of the design of
helices, hairpins and novel structures in sequences of mixed chirality. Recent work,
which addresses the issue of enhancing the repertoire of polypeptide structures by
incorporating *b*- and *g*-amino acid residues, is also highlighted.

2. Stereochemistry of polypeptide chains

Forty years have passed since the principles of polypeptide conformational analysis were
first enunciated by G N Ramachandran and his colleagues at the University of Madras.[3]
The Ramachandran plot provides a remarkably powerful and convenient means for
assigning the 'stereochemical allowedness' of the local conformations of individual

---

amino acid residues in polypeptide and protein structures. The variables used in the construction of the Ramachandran (ϕ, ψ) plot are the two torsion (dihedral) angles ϕ and ψ, which correspond to rotations about the two single bonds at each residue in the polypeptide chain, N–C^a (ϕ) and C^a–C (ψ). The two-dimensional ϕ, ψ plot simplifies the problem of considering three-dimensional chain folding in analytical terms. The regular structures of polypeptide α amino acid chains, the α helix and β sheet were first recognised by Linus Pauling, a little over half a century ago.[4] In the Ramachandran representation, an idealised secondary structure, generated by repetition of the ϕ, ψ dihedral angles along the chain, is simply a single point in ϕ, ψ space. The allowed regions of conformational space were generated by Ramachandran's group assuming the simple hard sphere contact criterion, which simply states that two proximal non-bonded atoms cannot approach one another at distances less than the sum of their van der Waals radii. Figure 1a shows a view of the classical Ramachandran plot for the L-alanine residue, with the regions of major regular structures indicated.[3,5,6] It is clear that almost two thirds of ϕ, ψ space is strictly disallowed for L-Ala, the prototype chiral amino acid. This map is broadly representative of the allowed regions for 18 of the 20 amino acids
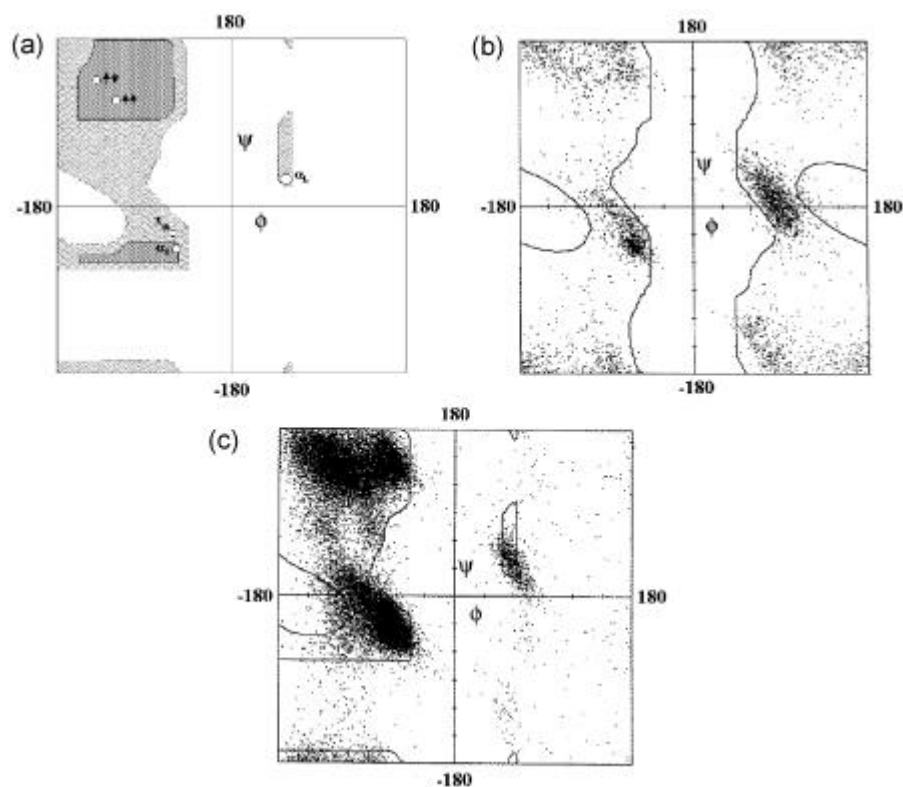

Figure 1. (**a**) Ramachandran map for N-acetyl-L-Ala-N′-methylamide. The shaded regions are stereochemically allowed. Regions corresponding to 3_{10}, α helical and parallel ($\uparrow\uparrow$) and antiparallel ($\uparrow\downarrow$) β-sheets are indicated. (**b**) Distribution of ϕ, ψ values of Gly residues from 250 protein structures ($\leq 2\cdot0$ Å resolution) (ref. 7b). (c) Distribution of non-Gly residues from 250 protein structures (ref. 7b).

that occur in proteins, the exceptions being glycine and proline. For Gly, the sole achiral residue in proteins, which lacks a substitutent at the C^a atom, the allowed regions of $\phi\psi$ space are considerably larger and symmetrically disposed with respect to the origin (figure 1b). The distribution of experimentally determined $\phi\psi$ values for individual residues in high resolution protein structures, 47612 residues from 250 proteins, is shown in figure 1c. Clearly, the experimental observations closely correspond to the allowed regions anticipated by Ramachandran. A similar distribution for Gly residues is illustrated in figure 1b. Notably, for this achiral residue the observed distribution is spread in all four quadrants of the $\phi\psi$ map.[7] Inspection of figures 1b and c reveals that alkylation at the C^a atom dramatically reduces the allowed regions of conformational space, on going from Gly to L-Ala. By extrapolation, further alkylation, as in the $C^{a,a}$ dialkylated residue a-aminoisobutyric acid (Aib) [H_2N–$C(CH_3)_2$–$COOH$], would result in a greater restriction of the allowed conformational space. In principle, the allowed regions for the Aib residue can be generated by overlapping the Ramachandran maps for L-Ala and D-Ala (figure 2a).[8] It is apparent that only small regions that correspond to the right- and left-handed helical structures are indeed allowed for this residue.[9,10]

3. *a*-Aminoisobutyric acid and helix nucleation

Aib is a naturally occurring amino acid, which is widely distributed in polypeptide antibiotics produced by diverse species of soil fungi. Interestingly, Aib and its homolog isovaline (Iva) [H_2N–$C(CH_3)(C_2H_5)$–$COOH$] are components of the amino acid mixtures found in meteorites and have been used as an indicator of the extraterrestrial origin of rock samples.[11] The peptaibol class of antibiotics produced by several fungal strains are characterised by a high proportion of Aib residues in the sequence and a C-terminal alcohol moiety. A very large number of peptaibol antibiotics have thus far been characterised[12] and the crystal structures of alamethicin,[13] zervamicin,[14] and antiamoebin[15,16] have been determined. The structures of the natural peptides are largely helical even
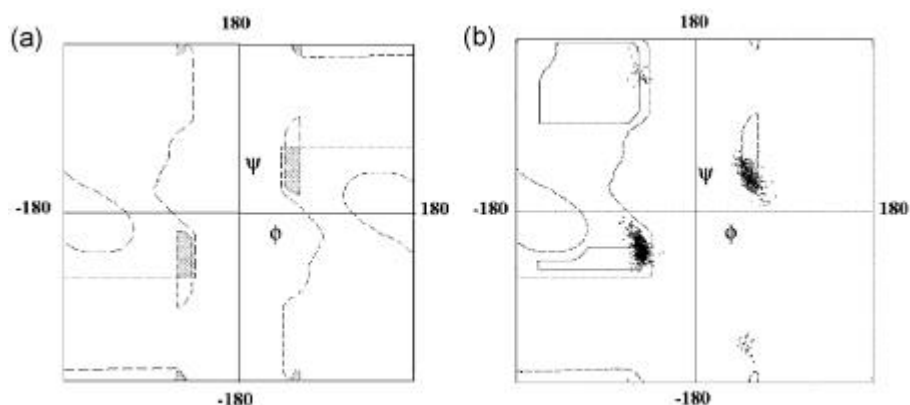

Figure 2. (**a**) Overlapped Ramachandran maps for N-acetyl-L-Ala-N′-methylamide and N-acetyl-D-Ala-N′-methyamide. The shaded regions are stereochemically allowed for both L- and D-Ala. (**b**) Distribution of the observed conformations of 1104 Aib residues in 367 independent crystal structures. (Coordinates for peptide structures have been obtained from the Cambridge Crystallographic Data Centre, ref. 72.)

when the sequences are interspersed with several Pro/Hyp residues; testimony to the helix promoting properties of the Aib residue. Interest in the structural chemistry of the peptaibol antibiotics has stemmed from the fact that they form voltage gated ion channels across artificial lipid bilayers.[17]

The stereochemical consequences of introducing Aib into peptide chains was first established at Bangalore, with the crystal structure determination and solution spectroscopic studies of the N-terminus tetrapeptide sequence of alamethicin.[18] This was followed by the establishment of a 3_{10}-helical conformation for the Aib oligopeptide Tosyl–(Aib)$_5$–OMe in crystals.[19] Almost concurrently, results of investigations of Aib containing sequences corresponding to fragments of peptaibol antibiotics appeared from the groups of Garland Marshall (St Louis)[20] and Gunther Jung (Tubingen),[21] providing strong support for the helix forming properties of Aib residues. It was quickly realised that the introduction of Aib residues into polypeptide chains limits the range of accessible conformations; nucleates of β-turns and helical structures and facilitates crystallisation presumably by restricting conformational flexibility in solution. Over the last 20 years a very large number of Aib containing peptides crystal structures have been determined, primarily by groups at Bangalore, Washington (Isabella L Karle) and Padova (Claudio Toniolo), which have provided valuable insights into the nature of helical conformations, helix packing in crystals, helix hydration, $3_{10}/\alpha$-helical transitions and the role of intramolecular hydrogen bonds in helix stabilisation. The extensive work on Aib peptides has resulted in the accumulation of a large body of information on polypeptide conformations.[1,2,22] Figure 2b shows the distribution of the observed ϕ, ψ values for Aib, representing 1104 residues from 367 independent crystal structures. A dramatic concentration of ϕ, ψ values in the right (α_R) and left handed (α_L) helical regions emphasizes the great propensity of Aib residues to occur in helical conformations. In the following section we briefly consider some specific issues related to helical structures in polypeptides.

4. 3_{10} and α-helical structures

Figure 3 illustrates the molecular conformation in crystals observed in three representative Aib containing decapeptides. The Aib oligomer pBrBz–(Aib)$_{10}$–OtBu adopts an almost perfect 3_{10} helical conformation stabilised by eight intramolecular $4 \rightarrow 1$ hydrogen bonds.[23] The decapeptide Boc–Aib–(Ala–Leu–Aib)$_3$–OMe adopts a mixed $3_{10}/\alpha$-helical structure with $4 \rightarrow 1$ hydrogen bonds (3_{10}) favored at the N-terminus and $5 \rightarrow 1$ hydrogen bonds (α) occurring at the C-terminus.[24] The decapeptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Ala–Leu–Aib–OMe adopts an almost perfect α-helical conformation stabilised by seven intramolecular $5 \rightarrow 1$ hydrogen bonds.[25] Table 1 summarises the potential $4 \rightarrow 1$ and $5 \rightarrow 1$ hydrogen bond parameters, which permit the choice of the interaction corresponding to either 3_{10} or α-helical structures. It must be emphasised that the distinction between 3_{10}, α and mixed helical structures is generally based only on the choice of the intramolecular hydrogen bond type. Relatively small variations in ϕ, ψ dihedral angles can result in switching of $4 \rightarrow 1$ and $5 \rightarrow 1$ hydrogen bonds. In helices the N$\cdots$O distances for both $4 \rightarrow 1$ and $5 \rightarrow 1$ hydrogen bonds can lie within acceptable limits (≤ 3.5 Å). It is therefore necessary that the complete characterisation of the donor acceptor geometries are carried out before choosing the type of hydrogen bond.[26] There has been considerable discussion in the literature on the relative stabilities of 3_{10} and α helical structures in Aib containing peptides.[27] The general consensus that has

emerged is that in Aib oligomers, 3_{10} helices are favoured. In sequences, which contain relatively few Aib residues, mixed $3_{10}/a$-helical structures and *a*-helical structures appear to be prominent.[22c] A survey of a large body of helical structures does not reveal any readily apparent correlation between the Aib content and positioning in the sequence, in the case of peptides having fewer than 50% Aib residues. In several crystal structures, multiple helical molecules have been observed in the crystallographic asymmetric unit, which adopt different hydrogen bonding patterns.[22c,e,f,28] It appears that the activation barriers required for the transformation of 3_{10} helical turns into *a*-helical turns is small, facilitating facile interconversion in solution. Attempts to establish diagnostic spectroscopic criteria, for example CD band shapes and positions[29] are likely to be complicated by dynamic interconversions between heterogeneous helical structures. The crystalline state may be viewed as providing a snapshot of specific of the molecular conformations that are present in solution, often entrapping a single distinct molecular species. The observation of multiple helical forms in crystals underscores the fact that the molecules probably exist in a broad potential well, with very small barriers separating various helical forms.
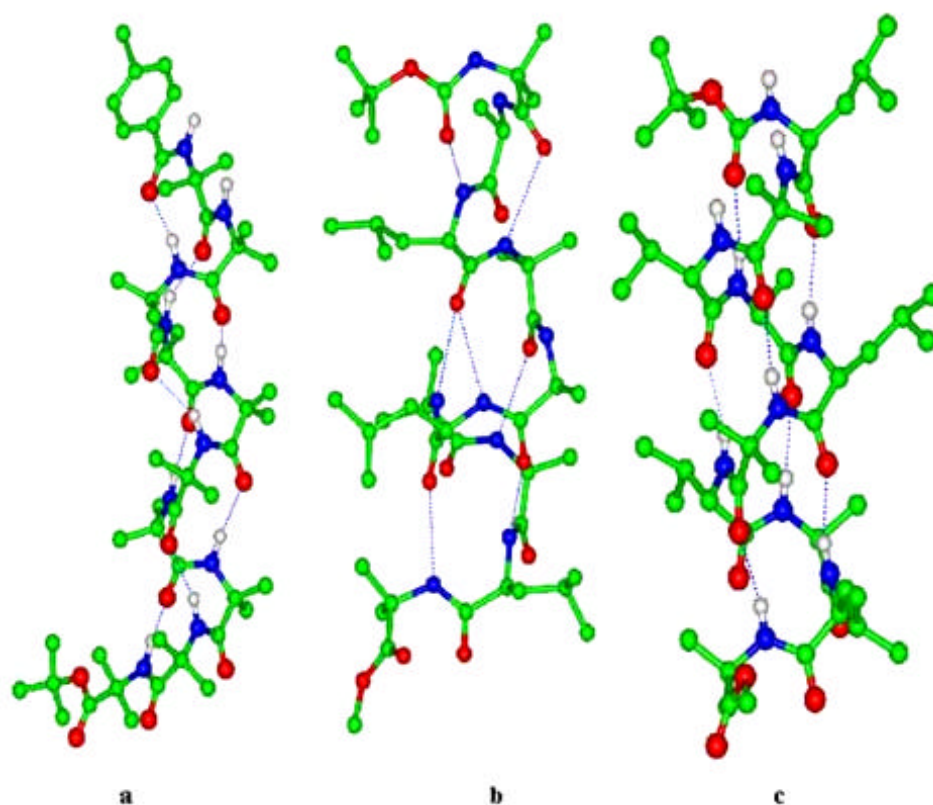

a   b   c

Figure 3. Molecular conformations observed in the crystals for three synthetic decapeptides (**a**) pBrBz–(Aib)$_{10}$–OtBu, 3_{10}-helix (ref. 23). (**b**) Boc–Aib–(Ala–Leu–Aib)$_3$–OMe, $3_{10}/a$ helix (ref. 24). (**c**) Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Ala–Leu–Aib–OMe, *a*-helix (LAL10, ref. 25). Dotted lines represent intramolecular hydrogen bonds.

Table 1. Potential hydrogen bond parameters[a] in three model helical decapeptides.

	Intramolecular		Ideal 3₁₀-helix			Mixed 3₁₀/a-helix			Ideal a-helix		
Type	Donor	Acceptor	N···O (Å)	H···O (Å)	N–H···O (deg)	N···O (Å)	H···O (Å)	N–H···O (deg)	N···O (Å)	H···O (Å)	N–H···O (deg)
4→1	N(3)	O(0)	2.945•	2.123	159.8	2.879•	2.092	151.8	3.559	3.104	115.4
4→1	N(4)	O(1)	2.975•	2.182	153.4	3.065•	2.240	160.6	3.200	2.779	111.8
4→1	N(5)	O(2)	2.979•	2.198	151.1	5.046	4.253	155.2	3.401	2.920	117.3
4→1	N(6)	O(3)	3.179•	2.382	154.4	2.966•	2.388	125.0	3.221	2.855	107.5
4→1	N(7)	O(4)	3.020•	2.183	164.2	3.377	3.001	108.8	3.393	2.923	116.3
4→1	N(8)	O(5)	3.211•	2.355	173.4	3.421	2.937	117.6	3.283	2.884	110.3
4→1	N(9)	O(6)	3.057•	2.225	163.0	3.227	2.632	127.4	3.390	2.932	115.3
4→1	N(10)	O(7)	2.907•	2.087	159.2	4.188	3.759	114.4	3.235	2.782	114.5
5→1	N(4)	O(0)	4.613	3.916	140.7	3.597	3.329	101.3	2.978•	2.165	157.7
5→1	N(5)	O(1)	4.337	3.665	137.3	5.200	4.633	127.4	3.113•	2.277	163.9
5→1	N(6)	O(2)	4.331	3.630	140.8	6.001	5.157	168.0	3.071•	2.237	163.3
5→1	N(7)	O(3)	4.600	4.012	128.8	3.209•	2.359	169.7	3.005•	2.174	162.1
5→1	N(8)	O(4)	4.791	4.181	131.2	3.089•	2.301	152.4	3.014•	2.183	162.3
5→1	N(9)	O(5)	5.200	4.547	136.0	3.145•	2.379	148.6	3.048•	2.209	165.2
5→1	N(10)	O(6)	4.600	3.970	133.0	2.925•	2.084	165.4	2.986•	2.194	153.0

[a]Average hydrogen bond parameters reported for polypeptide helices based on analysis of protein crystal structures are 3₁₀-helix (4→1), N···O = 3.05 Å, H···O = 2.17 Å, ∠N–H···O = 149°; a-helix (5→1), N···O = 2.99 Å, H···O = 2.06 Å, ∠N–H···O = 155° (ref. 26a)

•These are the acceptable hydrogen bonds

5. Helix-terminating motifs

The ability to synthetically construct defined helical peptide modules has permitted structural investigation of helix-terminating motifs. In proteins, right-handed α-helical segments often terminate with a residue adopting the left-handed helical (α_L) conformation (Schellman motif).[30] The achiral residue Gly has the greatest propensity to occur at the terminating (T) position. In helical peptides containing an achiral Aib residue at the penultimate position from C-terminus, formation of Schellman motifs, with Aib adopting an α_L conformation, has been frequently observed.[31] In these structures, pairs of hydrogen bonds $6 \rightarrow 1$ and $4 \rightarrow 1$ stabilise the helix terminating motif (figure 4).

As a part of a program to construct synthetic sequences containing multiple elements of secondary structure, we examined approaches to terminate helices at the C-terminus end. The introduction of D-amino acid residues, which favour α_L conformations should facilitate helix termination. Figure 5 shows the molecular conformation in crystals of the decapeptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe, (LAD10), which possesses two contiguous D-residues at positions 8 and 9. As anticipated, the right-handed helix formed by residues 1 to 7 terminated with ^{D}Ala(8) adopting an α_L conformation, generating a Schellman motif. However, this structure revealed an unanticipated feature with polypeptide chain reversal occurring over the C-terminus segment, residues 8 to 10, stabilised by an unusual C–H$\cdots$O hydrogen bond between Ala(4)C^{a}H and ^{D}Leu(9) CO groups.[32] An almost identical conformation is also established in crystals of the analog peptide LGD10, where Ala(4) is replaced by Gly. In this case, the intra-molecular C–H$\cdots$O hydrogen bond involved the *pro R* hydrogen of the Gly residue.[33]
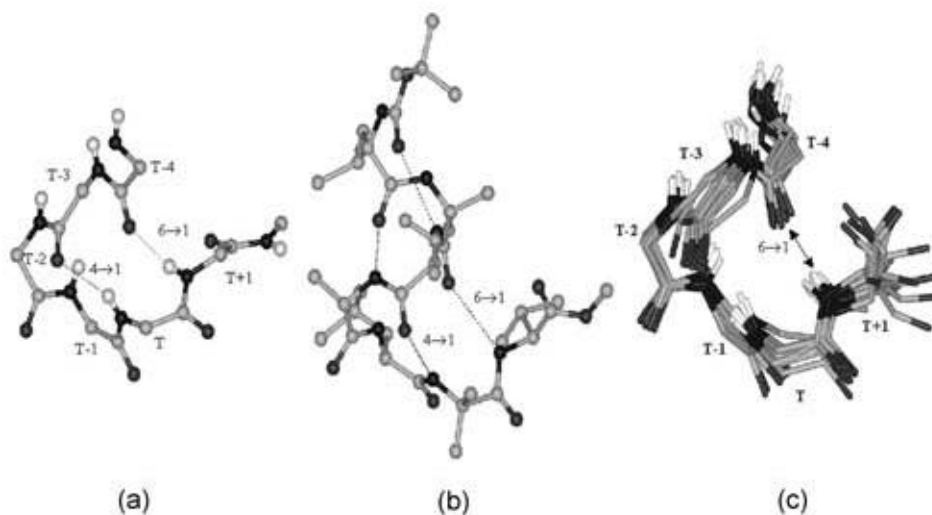

Figure 4. (**a**) Helix terminating Schellman motif stabilized by a pair of $4 \rightarrow 1$ and $6 \rightarrow 1$ hydrogen bonds. Residue T is the helix terminating residue. Residue T-1 to T-4 form the C-terminus of the helix. (**b**) Molecular conformation in crystals for the peptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–OMe (ref. 31c). Note the formation of the Schellman motif. Aib(6) adopts an α_L conformation and is the terminating residue T. (**c**) An overlay of helix terminating Schellman motifs observed in 18 crystal structures of helical peptides.
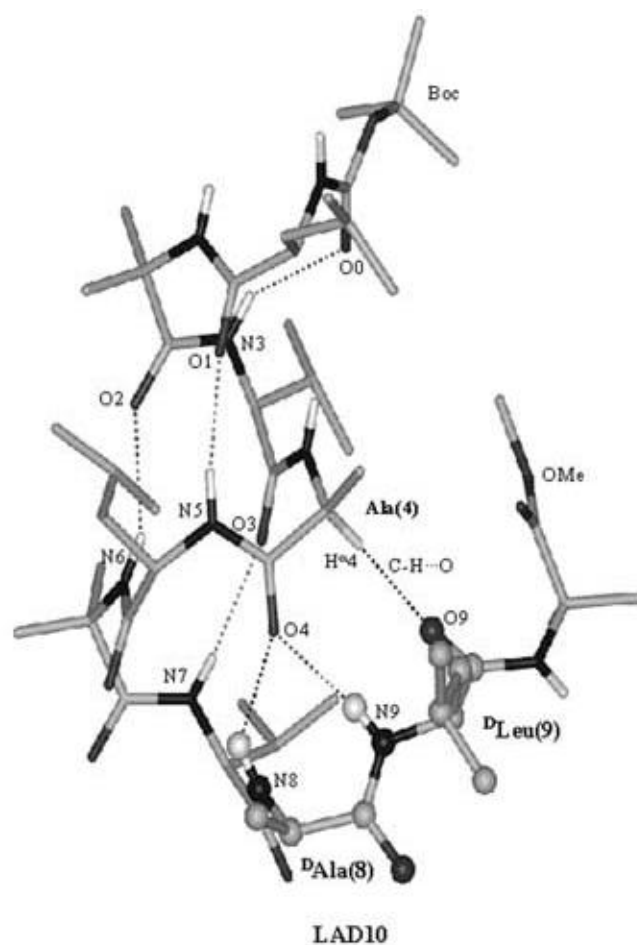
Figure 5. Molecular conformation of the decapeptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (LAD10, ref. 32) observed in crystals. Note the formation of the C–H···O hydrogen bond between Ala(4)C^{a}H and ^{D}Leu(9)CO groups.

Figure 6a shows the superposition of peptides LAD10 and LGD10 (RMSD = 0·54 Å for all the back bone atoms). A notable feature of this unusual motif is that it appears to facilitate registry of antiparallel helix and strand segments, stabilised by backbone C–H···O hydrogen bonds. A search of 634 high resolution protein structures revealed the occurrence of a similar motif in proteins. Figure 6b shows the superposition of 15 examples from protein structures on the structure of peptide LAD10.[34] The role of the C–H···O hydrogen bond in facilitating the novel chain reversal in peptides LAD10 and LGD10 has been established by determining the structures of two peptide analogs Boc–Leu–Aib–Val–^{D}Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (DA10) and Boc–Leu–Aib–Val–Aib–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (LUD10).[31] In these two cases, the *pro R* C^{a}H at T-4 has been replaced by a methyl group. Figure 7 shows a view of the molecular conformation of DA10 and LUD10 in crystals. Interestingly, both the peptides adopt conformations which are completely distinct from those observed for LAD10 and

LGD10, underscoring the importance of the interaction involving the T-4 C^{a}H group in determining the overall fold of the molecule. This example illustrates the role of a relatively long-range backbone interaction in determining peptide folding.

6. Residue and helix chirality

The availability of crystalline helical peptides generated by introducing Aib residues into sequences of L-amino acids has permitted the creation of novel backbone folds, which are not observed in protein structures. Figure 8 illustrates the crystal structures of two enantiomeric 14 residue peptides, generated by fusing helical sequences of opposite chirality. The peptides Boc–D–(Val–Ala–Leu–Aib–Val–Ala–Leu)–L–(Val–Ala–Leu–Aib–Val–Ala–Leu)–OMe (DL14)[35] and Boc–L–(Val–Ala–Leu–Aib–Val–Ala–Leu)–D–(Val–Ala–Leu–Aib–Val–Ala–Leu)–OMe (LD14)[36] were synthesised by condensation of the helical module Boc–Val–Ala–Leu–Aib–Val–Ala–Leu–OMe (UV7),[37] and its enantiomer. Notably, the two enantiomeric 14 residue sequences crystallise in polymorphic forms DL14 (space group = $P4_3$); LD14 (space group = $P2_1$, two molecules in the asymmetric unit); providing a view of three independent 'ambidextrous' structures. Figure 9 shows a schematic ribbon diagram of this unusual fold. The view down the helix
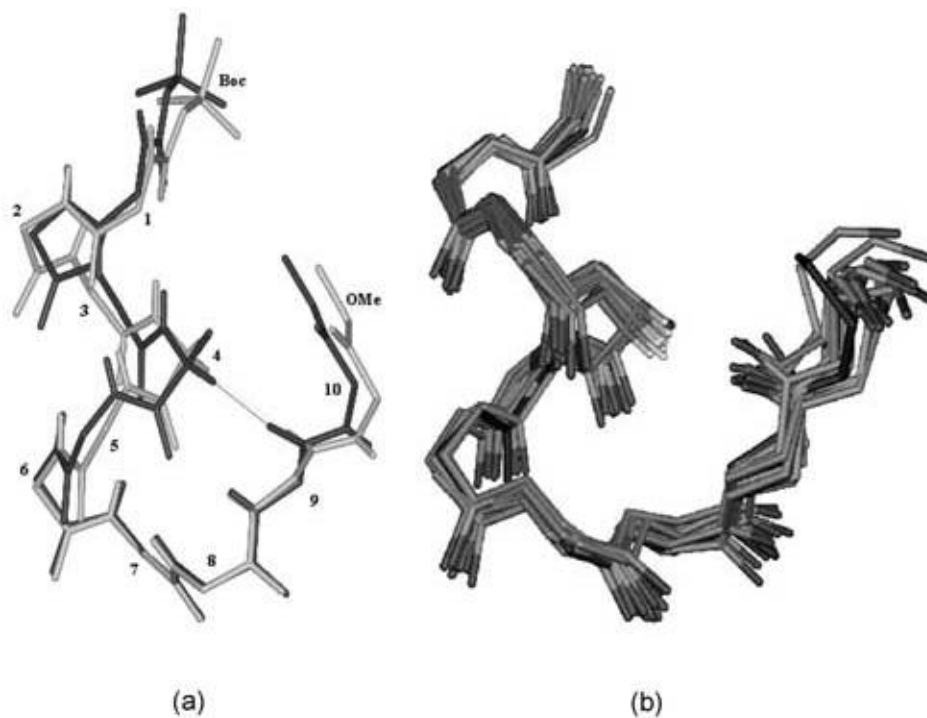

(a)   (b)

Figure 6. (a) Superposition of the crystal state conformations of the peptides Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (LAD10) and Boc–Leu–Aib–Val–Gly–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (LGD10), (ref. 33). (b) Overlay of 15 C–H⋯O hydrogen bond stabilised helix terminating motifs observed in protein structures with LAD10 (ref. 34a).
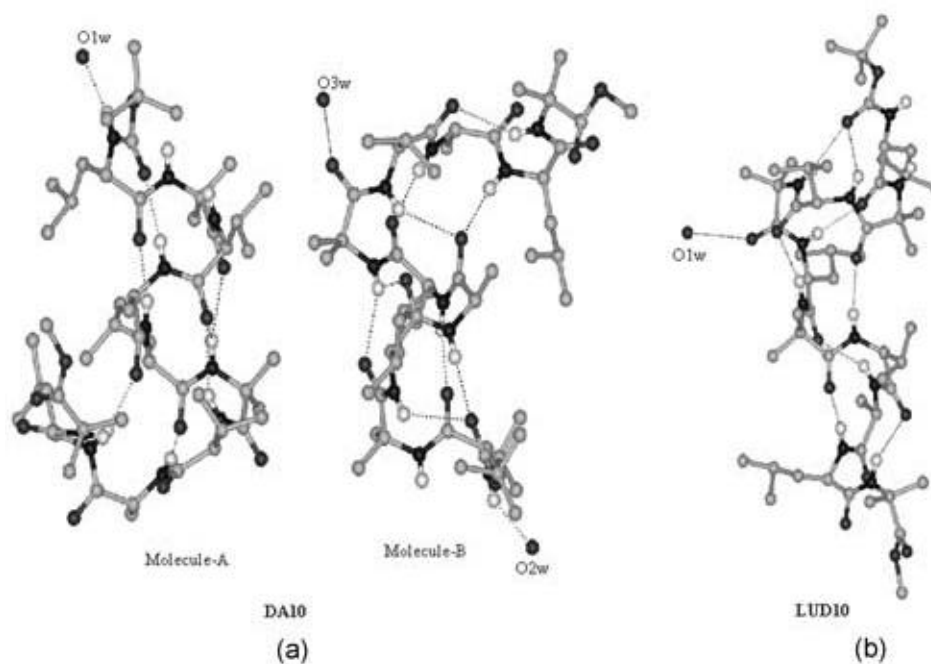
Figure 7. Molecular conformation in crystals for two synthetic decapeptides (**a**) Boc–Leu–Aib–Val–^{D}Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (DA10). Two molecules are observed in the crystallographic asymmetric unit. (**b**) Boc–Leu–Aib–Val–Aib–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–OMe (LUD10). Note the difference in the observed structures of these peptides as compared to the decapeptides illustrated in figure 6a.

axis illustrates the lateral displacement of the two helices. At the chiral junction, the fusion of N- and C-terminal helices of opposite hands is mediated through the formation of a Schellman motif. While polypeptides containing fused helical segments of opposite chirality have no natural analog at present, the possibility that such structures may find application is enhanced by recent studies of chiral conflict in synthetic polymers.[38]

The introduction of single D-amino acids or double D-segments into L-amino acid sequences has been anticipated to destabilise right handed helical structures. In the highly stable helical folds nucleated by Aib residues, D-amino acids can be accommodated into a_R conformations and conversely L-amino acids can be induced to adopt a_L conformations. The recent crystal structure determinations of predominately L-amino acid sequences containing guest D-residues illustrate this point. Figure 10 shows the molecular conformation of a 13-residue peptide Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–Leu–Aib–Val–OMe (LUV13). In this case the double D-segment ^{D}Ala–^{D}Leu is comfortably accommodated in a continuous *right handed* helical structure [^{D}Ala(8) $f= -49.3°$, $y= -48.9°$; ^{D}Leu(9) $f= -56.1°$, $y= -54.9°$].[39] In the structure of decapeptide DA10 (figure 7a) the ^{D}Ala(4) residue occurs in an a_R conformation [$f= -46.6°$, $y= -52.9°$ for molecule A and $f= -54.1°$, $y= -48.6°$ for molecule B] with the segment residues 1 to 7 forming a conventional right handed helix. In the decapeptide LUD10 (figure 7b), the N-terminal helix terminates at Aib(6) with the segment 6 to 9 residue

forming left handed helix. ^{L}Val(7) adopts an a_L conformation [f= 42·5°, y= 40·0°]. These examples point to the use of designed host helical sequences in investigating the effect of introduction of guest residues of opposite chirality.

7. D-Proline and the nucleation of *b*-hairpins

The recognition that the introduction of local conformational constrains can induce the formation of well defined structures in oligopeptides suggests that stereochemical control over polypeptide chain folding may be achieved by judicious introduction of amino acids

DL 14   LD14 (A)   LD14 (B)

(a)   (b)
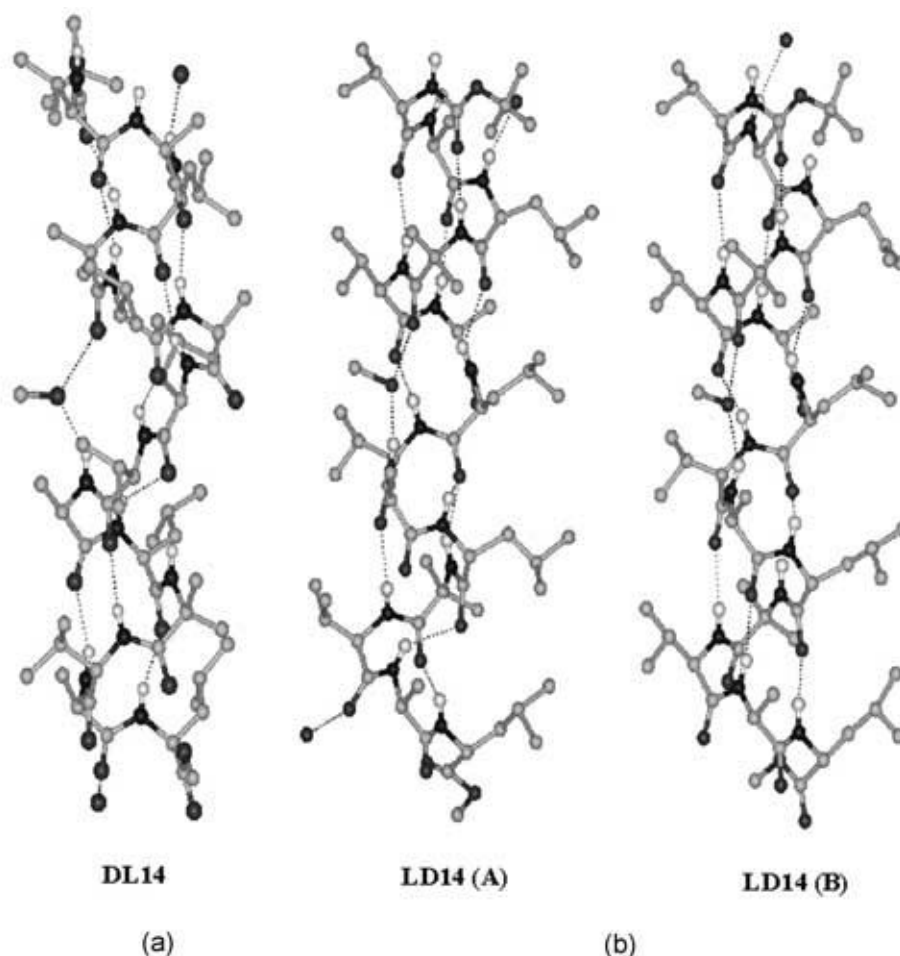
Figure 8. Molecular conformation in crystals observed for peptide sequences containing fused segments of opposite chirality. (**a**) Boc–D–(Val–Ala–Leu–Aib–Val–Ala–Leu)–L–(Val–Ala–Leu–Aib–Val–Ala–Leu)–OMe (DL14). (**b**) Boc–L–(Val–Ala–Leu–Aib–Val–Ala–Leu)–D–(Val–Ala–Leu–Aib–Val–Ala–Leu)–OMe (LD14). Two molecules observed in the asymmetric unit are shown. In all cases, hydration is observed at the centre of the molecule corresponding to the chiral junction (refs 35, 36).
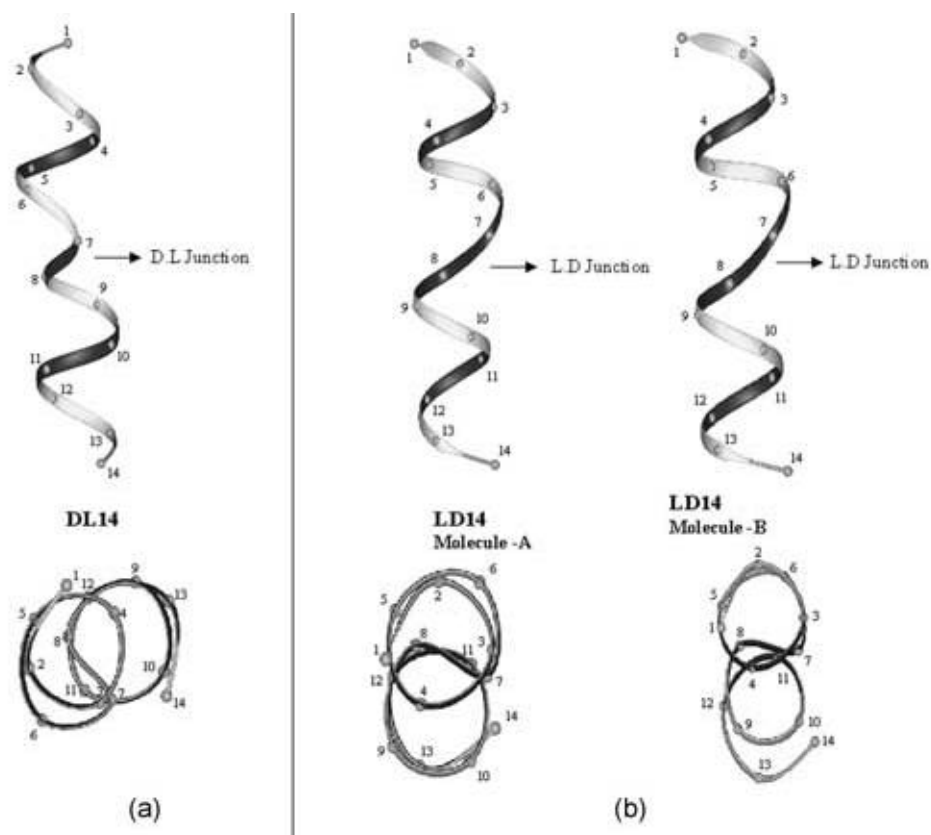
Figure 9. Ribbon representation of the ambidextrous helices shown in figure 8. (Top) View perpendicular to helix axis. (bottom) View down the helix axis showing the lateral displacement of the helical segments.

with definite conformational properties, into host L-amino acid sequences. This principle has been illustrated in the preceding section for the design of helical peptides. The β-hairpin constitutes a simple structural motif in which two antiparallel strands are stabilised by cross-strand hydrogen bonds. An analysis of protein structures by Janet Thornton and her colleagues suggested that hairpins in proteins are often nucleated by two residue β-turns (tight turns).[40] Most frequently, β-turns of type II′ or I′ form the nucleus for chain reversal. The 'prime' turns require a positive ϕ value ($\approx +60°$), for the $i + 1$ residue. In proteins, Gly often occupies this position. The other residue predominately found is Asn, which has a fairly high propensity for adopting conformations with positive ϕ values.[41] In synthetic design it is necessary to fix the stereochemistry of the turn segment in order to nucleate antiparallel hairpins. Figure 11a shows the allowed region of the Ramachandran map for ^{L}Pro and ^{D}Pro. In these residues, constraints of pyrrolidine ring formation restrict the value of ϕ to a narrow band, centred around $60°$. The preferred ϕ value for ^{L}Pro is $-60° \pm 20°$, while in ^{D}Pro the corresponding value is $+60° \pm 20°$. Figure 11b summaries the observed conformation for proline residues in a representative dataset of proteins.[7] Two strong clusters are observed corresponding to $\phi = -60°$, $\psi = -30°$ and $\phi = -60°$,

ψ = +120°, which are the values required at the $i + 1$ position of type I and II $\boldsymbol{b}$turns, respectively. We may therefore draw the inference that the enantiomeric residue ^{D}Pro will favour conformations which correspond to the $i + 1$ position in type I′ and II′ $\boldsymbol{b}$turns. The use of ^{D}Pro–Xxx segments to nucleate $\boldsymbol{b}$hairpin structures in peptides was developed at Bangalore[1] and independently in the group of Sam Gellman at Madison.[42] Spectroscopic investigation of the octapeptide Boc–Leu–Val–Val–^{D}Pro–Gly–Leu–Val–Val–OMe first
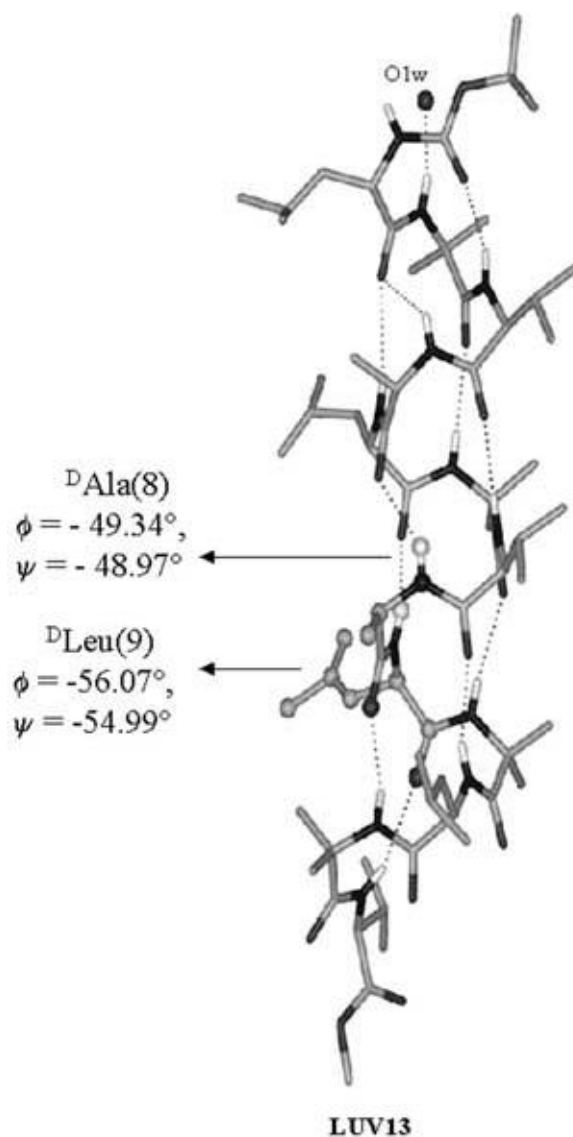
Figure 10. Molecular conformation in crystals of a 13-residue peptide helix Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–^{D}Ala–^{D}Leu–Aib–Leu–Aib–Val–OMe (LUV13, ref. 39) containing a centrally positioned ^{D}Ala(8)–^{D}Leu(9) double D-segment.
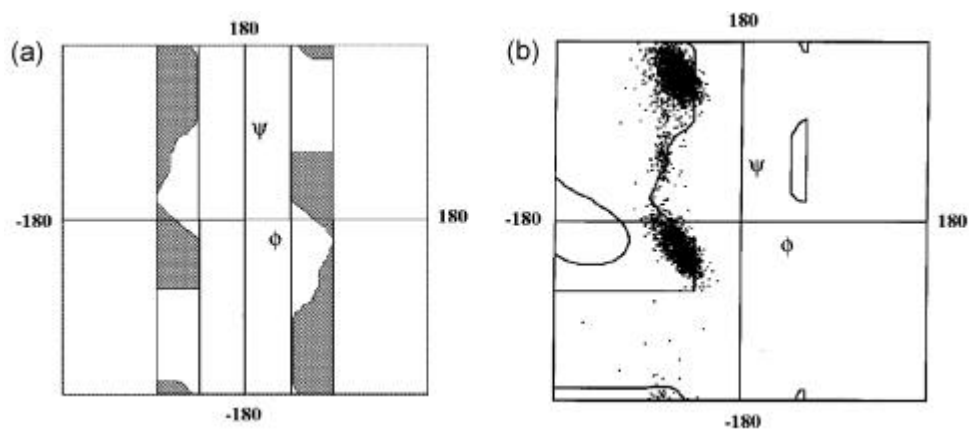
Figure 11. (**a**) The allowed regions in Ramachandran space for ^{L}Pro and ^{D}Pro residues (ref. 2). Note that the torsion angle f is restricted to a relatively narrow range of f values. ^{L}Pro = $-60 \pm 20°$; ^{D}Pro = $+60 \pm 20°$. (**b**) Observed distribution of conformational angles for 4995 Pro residues in 538 protein crystal structures (ref. 7b).

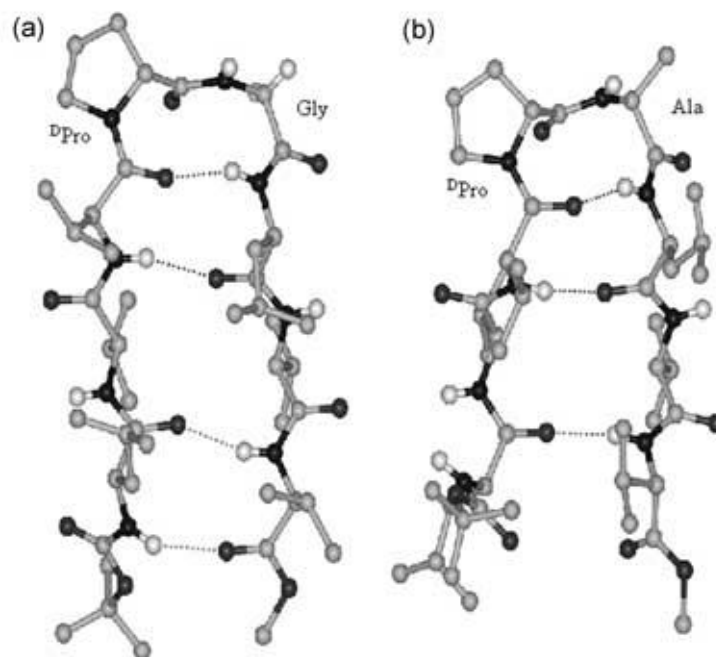**Figure 12.** Molecular conformation in crystals observed for two synthetic octapeptides (**a**) Boc–Leu–Val–Val–^{D}Pro–Gly–Leu–Val–Val–OMe (ref. 44). (**b**) Boc–Leu–Val–Val–^{D}Pro–Ala–Leu–Val–Val–OMe (ref. 45), Note there are two independent molecules in the asymmetric unit, having similar conformations; only one is shown.
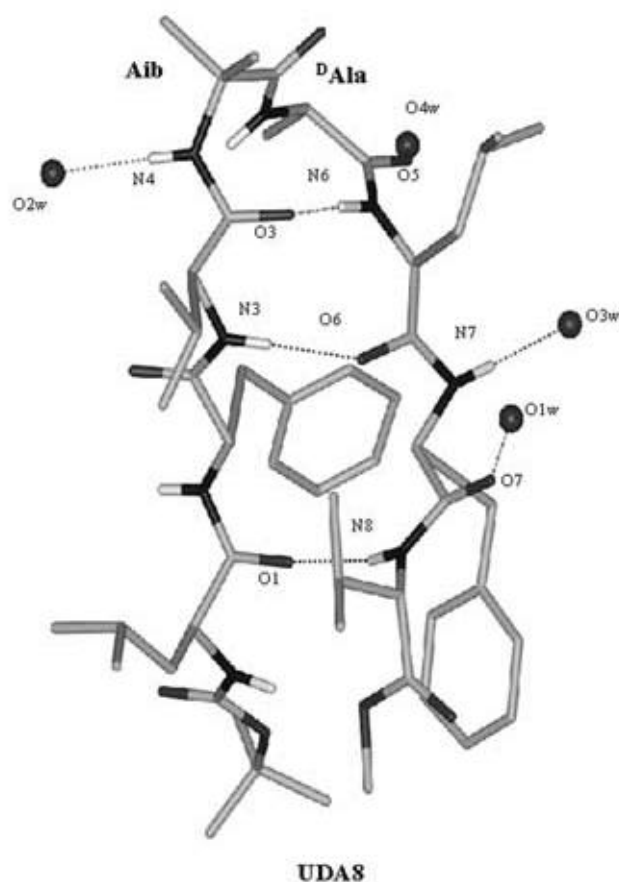
UDA8

Figure 13. Molecular conformation in crystals observed for the octapeptide Boc–Leu–Phe–Val–Aib–[D]Ala–Leu–Phe–Val–OMe (UDA8, ref. 49). The Aib-[D]Ala segment forms a type I′ *b*-turn.

revealed the formation of a stable hairpin in solution, as evidenced by the observation of all diagnostic interstrand nuclear Overhauser effects (NOEs).[43] The subsequent crystal structure determination revealed two independent molecules in the crystallographic asymmetric unit, both of which adopted a *b*hairpin conformation with the [D]Pro–Gly segment forming a type II′ *b*turn.[44] Figure 12 illustrates the molecular conformation in crystals, determined by X-ray diffraction, for the two octapeptides Boc–Leu–Val–Val–[D]Pro–Gly–Leu–Val–Val–OMe and Boc–Leu–Val–Val–[D]Pro–Ala–Leu–Val–Val–OMe, which contain centrally positioned, turn nucleating [D]Pro–Xxx segments.[45] *b*hairpins have also been demonstrated in crystals for peptides Boc–Leu–Val–Val–[D]Pro–Gly–Leu–Phe–Val–OMe, Boc–Leu–Phe–Val–[D]Pro–Ala–Leu–Phe–Val–OMe, Boc–Met–Leu–Phe–Val–[D]Pro–Ala–Leu–Val–Val–Phe–OMe, Boc–Leu–Val–Val–[D]Pro–Aib–Leu–Val–Val–OMe, Boc–Leu–Phe–Val–[D]Pro–Ac$_8$c–Leu–Phe–Val–OMe. In all cases, the central [D]Pro–Xxx segment adopts type II′ turn conformations (unpublished). These crystallographic investigations establish the simple principle of nucleating stable hairpins by inserting

^{D}Pro–Xxx segments into L-amino acid sequences. The extension of this approach to the design of three-, four- and five-stranded *b*sheet structures has been achieved by the insertion of multiple ^{D}Pro–Xxx segments into designed peptide chains. Thus far, the characterisation of multi stranded *b*sheets has been achieved in solution by two-dimensional NMR methods.[1,46] The largest sheet like structure achieved by synthetic design has been an eight stranded *b*sheet in a 70 residue C_2 symmetric polypeptide, stabilised by a single disulfide bond.[47] The search for alternative hairpin nucleating segments has encompassed both protein and non-protein amino acids. Asn–Gly sequences have been successfully employed in generating single *b*hairpins and three stranded *b*sheet structures characterised by NMR spectroscopy in solution.[48] We have explored the possibility of using Aib–^{D}Xxx sequences to nucleate type I′ *b*turns (3_{10} helical turns). Figure 13 shows the molecular conformations in crystals of a designed octapeptide Boc–Leu–Phe–Val–Aib–^{D}Ala–Leu–Phe–Val–OMe, which reveals a *b*hairpin conformation nucleated by an Aib–^{D}Ala type I′ *b*turn. In this structure, cross-strand aromatic interactions between the Phe residues at position 2 and 7 (centroid to centroid distance for aromatic rings is 5·52 Å) may lend further stability to the observed structure.[49] Several recent investigations address the issue of stabilising *b*hairpin conformations by specific cross-strand interactions between aromatic side chains. This approach is of particular relevance in the design of stable sheet like structures in water where hydrophobic clustering of side chains may lend additional stability to the design of backbone folds.[50]
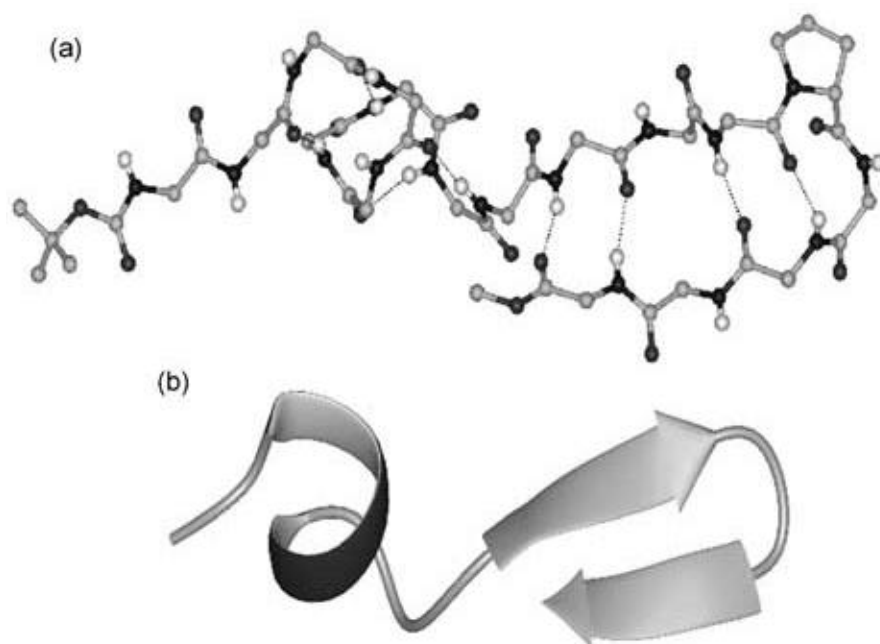

Figure 14. Molecular conformation in crystals for the synthetic 17-residue peptide Boc–Val–Ala–Leu–Aib–Val–Ala–Leu–Gly–Gly–Leu–Phe–Val–^{D}Pro–Gly–Leu–Phe–Val–OMe (ref. 52). (**a**) Note the two distinct secondary structure segments residues 1–7 form a helix, while residues 10–17 form a hairpin. (**b**) Ribbon representation illustrating the orientation of the two secondary structures.
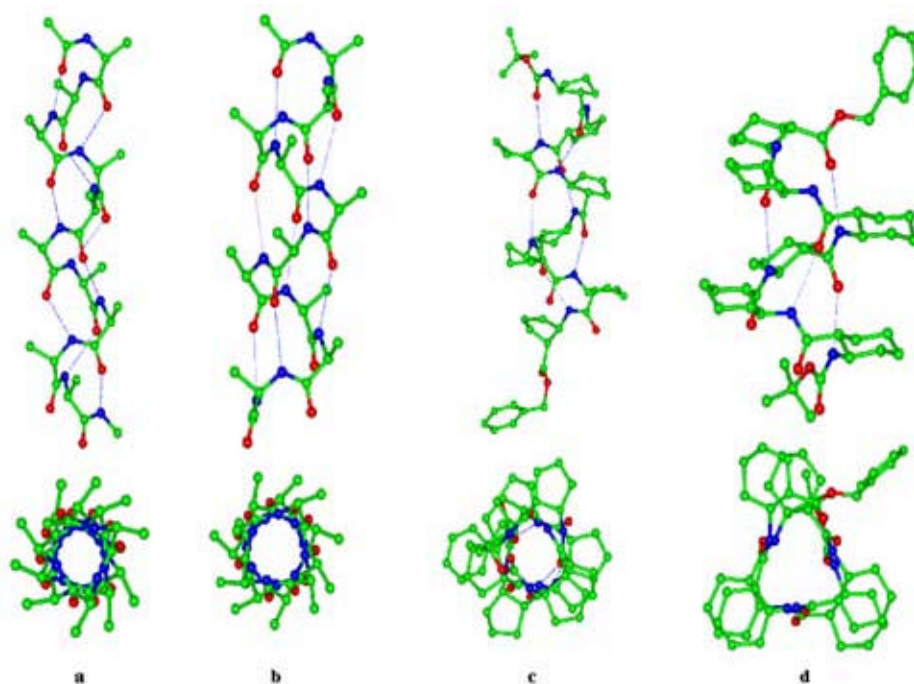
Figure 15. A comparison of the helical structures formed by poly *a*-peptides and poly *b*-peptides. (**a**) and (**b**) correspond to the 3_{10} and *a*-helical structures observed in polypeptides formed by *a*-amino acids. (**c**) 12-helix formed in the crystal structure of an oligomer of the *b*-amino acid ACPC (ref. 59). (**d**) 14-helix formed in the crystal structure of an oligomer of the *b*-amino acid ACHC (ref. 60). A view perpendicular to the helix axis is shown on top. The lower figures are a view down the helix axis.

8. Mixed *a*/*b* structures

Well-defined modules of secondary structure (*a* and *b*) have been combined in synthetic peptides to yield mixed *ab* structures. The existence of distinct helix and hairpin segments have been demonstrated in the 17 residue sequence, Boc–Leu–Aib–Val–Ala–Leu–Aib–Val–Gly–Gly–Leu–Val–Val–^DPro–Gly–Leu–Val–Val–OMe, although NMR studies were unable to clearly define the relative orientation of the two modules.[51] The related 17 residue peptide, Boc–Val–Ala–Leu–Aib–Val–Ala–Leu–Gly–Gly–Leu–Phe–Val–^DPro–Gly–Leu–Phe–Val–OMe, yielded single crystals permitting X-ray diffraction studies, which established a right-handed helical segment spanning residues 1 to 7, terminating in a Schellman motif with residue 8 at position T. The segment 10–17 forms a *b* hairpin nucleated by a type II′ *b* turn, with ^DPro–Gly as the corner residues (figure 14).[52] Building in greater *f*, *y* restrictions at the linking segments between secondary structures may permit greater control on the orientation of the helix and strand modules.

9. *b*- and *g*-amino acid residues in peptides

The stereochemistry of peptides and polypeptides containing omega amino acids has been reviewed, with specific attention to early studies on homopolymers.[53] In *b*-amino
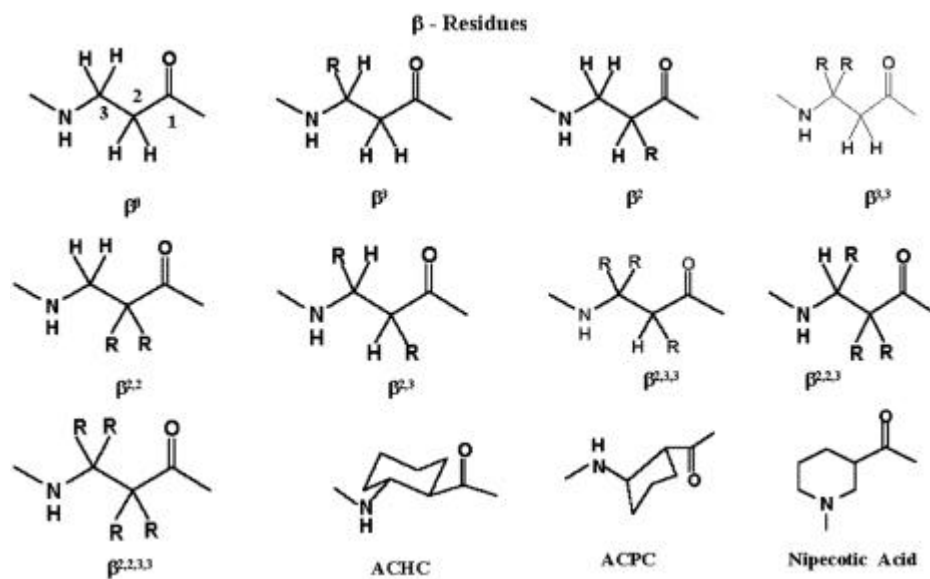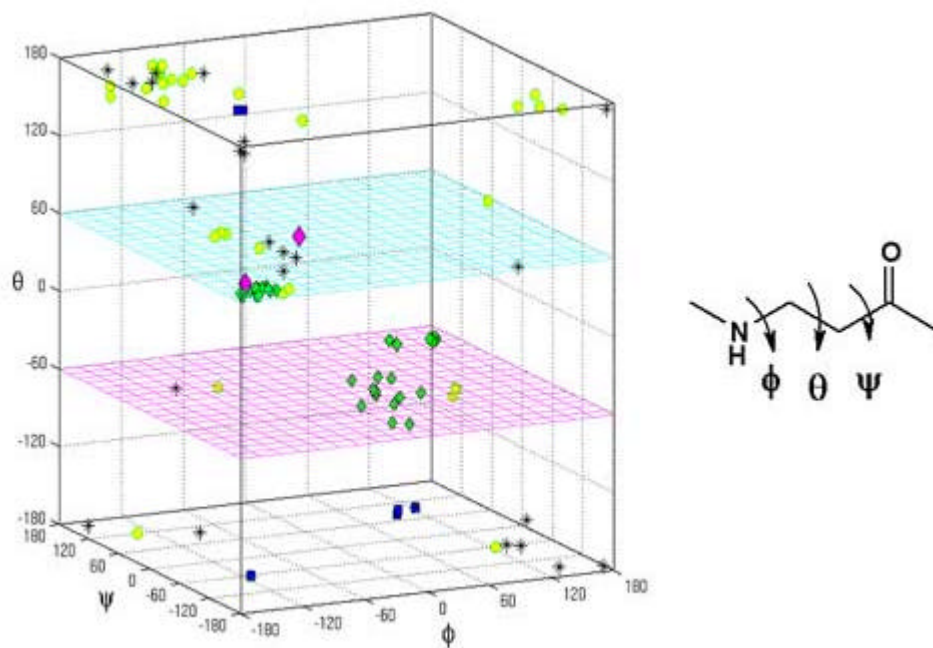
Figure 16. Nomenclature for *b*-residues.

Figure 17. Observed *b*-residue conformations in crystal structures of synthetic peptides representing on a three-dimensional f–q–y plot. The coloured planes correspond to the *gauche* conformation about C^a–C^b bonds. * (black): achiral acyclic unsubstituted *b*-amino acid, *b*-glycine (3-amino propionic acid); o (yellow): acyclic substituted *b*-amino acids; ◊ (green): chiral cyclic *b*-amino acids; (blue): nipecotic acid;(pink): idealised 12 - and 14-helices.
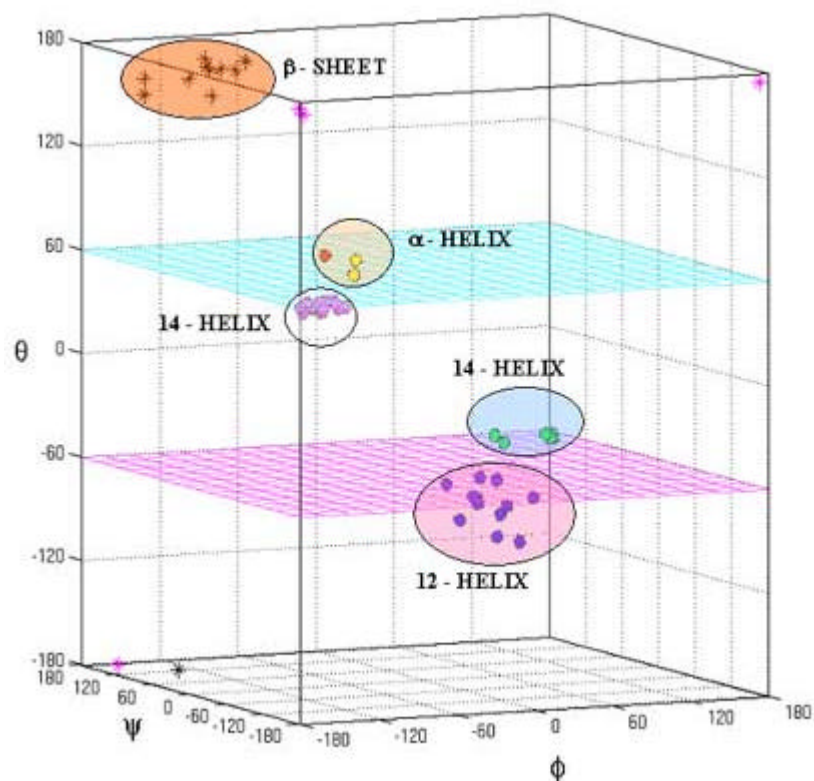
Figure 18. Location of the regions of ordered secondary structures for *b*-residues in *f*–*q*–*y* space. The *a*-helix and *b*-sheet are the classical structures for poly *a*-amino acids. *b*-residues occurring in the appropriate shaded region can be accommodated without disruption of secondary structures. The 12-helix and 14-helix are well characterised secondary structures for poly *b*-peptides.

acid residues the introduction of an additional degree of torsional freedom (*q*) into the backbone is expected to enhance conformational flexibility. Helical structures have been postulated for poly(*a*-alkyl-*b*-aspartates) on the bases of fibre diffraction, polarised IR spectroscopy and molecular mechanics and quantum chemical calculations by Munoz-Guerra, Subirana, Alemann and coworkers.[54] Interest in the conformations of *b*-peptides received a dramatic boost with the work of Dieter Seebach[55] and Sam Gellman[56] which established, unequivocally, the ability of oligopeptides containing *b*-residues to fold into helical conformations, which are completely distinct from those adopted by *a*-peptides. Figure 15 illustrates the well characterised helical forms observed in poly *a*- and poly *b*-peptides. The flood of recent research in the area of *b*- and *g*-peptides has been facilitated by the synthetic accessibility of many chiral, multiply substituted *b*- and *g*-residues.[57] Figure 16 defines the general formulae for *b*-amino acids and the nomenclature used for representing substituted residues. The structures of three cyclic *b*-amino acids in which rotation about the central C^a–C^b bond (*q*) is restricted are also shown. The formation of folded structures in *b*-peptides immediately suggests that these residues may be used to expand the range of designed peptide conformations, particularly when used in conjunction with stereochemically constrained residues.
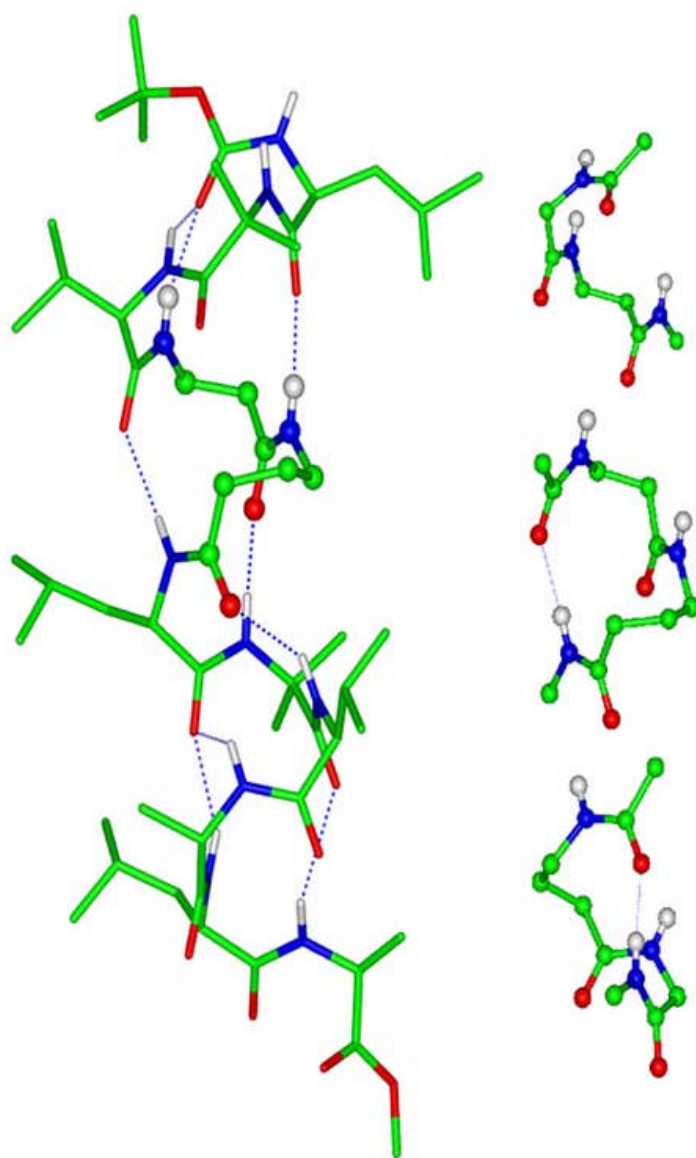
Figure 19. Molecular conformation in crystals for the 11 residue synthetic peptide Boc–Leu–Aib–Val–*b*-Gly–*g*-Abu–Leu–Aib–Val–Ala–Leu–Aib–OMe (ref. 61) accommodating a central *b*-Gly–*g*-Abu segment. The dipeptide segments are shown on the right. (top) Val–*b*–Gly, (middle) *b*-Gly–*g*-Abu and (bottom) *g*-Abu–Leu.

The rational use of *b* residues in peptide design requires an understanding of the nature of the local conformations, which are readily accessible. Conformational space for *b* residues can be conveniently represented in a three dimensional *f q-y* plot, in a simple extension of the Ramachandran analysis for *a* residues.[58] Figure 17 shows the observed distribution of *f, q, y* values for *b* residues in peptide crystal structures. Both *b*

oligopeptides and hybrid ***ab***-sequences are included. The conformation about the central C–C bond is overwhelmingly restricted to the anticipated *gauche*⁺ (g^+, $\boldsymbol{q}$ = +60°) *gauche*⁻ (g^-, $\boldsymbol{q}$ = –60°) and *trans* (*t*, $\boldsymbol{q}$ = 180°) conformations. The g^+ and g^- conformations induce helical folding. Figure 18 illustrates the observed clusters in conformational space for ***b***-residues incorporated into regular secondary structures. The 12-helix and 14-helix have been crystallographically characterised in oligomers of the constrained ***b***-amino acid ACPC[59] and ACHC[60] (see also figure 15). ***b***-residues can be incorporated into ***a***-helical and ***b***-sheet structures formed by predominately ***a***-amino acid sequences. We have undertaken a systematic characterisation of the conformational characteristics of hybrid peptides containing ***a***, ***b***, and ***g***-residues.

The crystal structures of the peptides Boc–Leu–Aib–Val–***b***-Gly–***g***-Abu–Leu–Aib–Val–OMe and Boc–Leu–Aib–Val–***b***-Gly–***g***-Abu–Leu–Aib–Val–Ala–Leu–Aib–OMe provided the first examples of the incorporation of the ***bg***-segment into helical ***a***-peptide structures.[61] (In the original report[61] the ***b***-Gly residue was called ***b***-Ala, which has been the trivial name used for 3-aminopropionic acid. Following the growth of the literature on ***b***-residues and ***b***-peptides, the designation ***b***-Ala is best reserved for the ***b***-residue obtained by backbone homologation of the residue Ala). Figure 19 shows the structure of
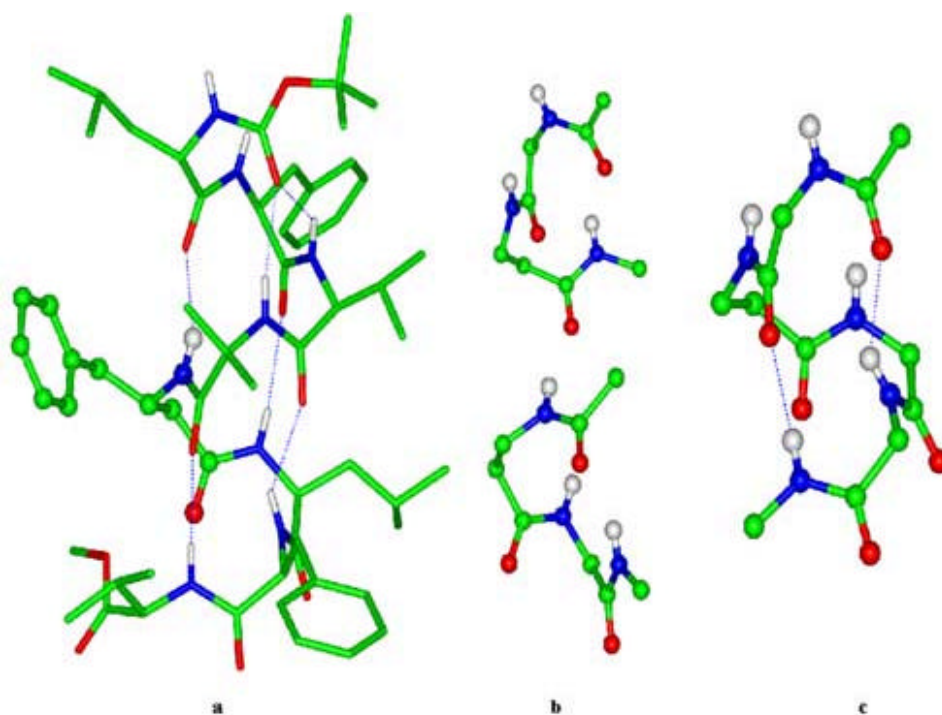

Figure 20. (**a**) Molecular conformation in crystals of the helical octapeptide Boc–Leu–Phe–Val–Aib–***b***–Phe–Leu–Phe–Val–OMe, which incorporates a single ***b***-Phe residue. (**b**) View of the dipeptide segments Aib–***b***-Phe (top) and ***b***-Phe–Leu (bottom). In both cases the N···O distance corresponding to a potential hydrogen bond are outside the normal limits. (**c**) An expanded view of Aib–***b***–Phe–Leu segment. Two C_{14} hydrogen bonds are observed, corresponding to the expansion of the ***a***-helical turn by insertion of an additional carbon atom into the peptide backbone.
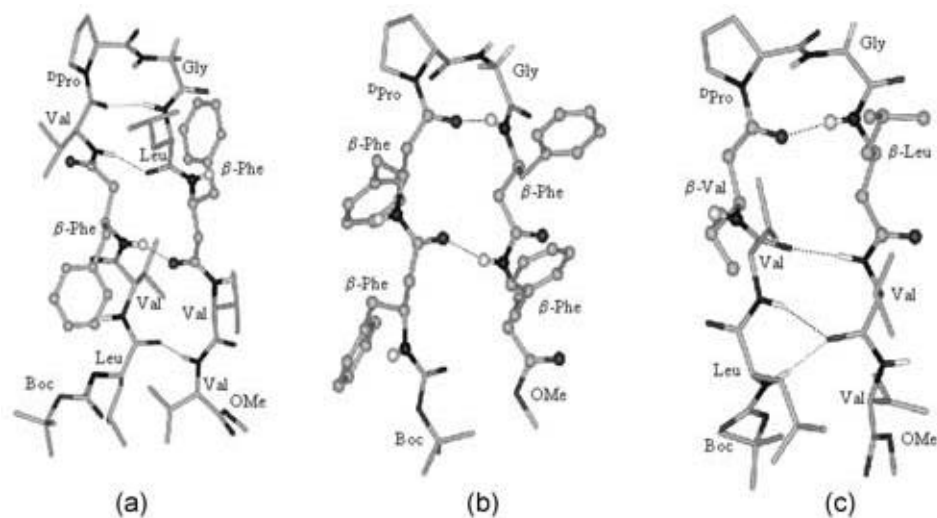
Figure 21. Molecular conformation observed in crystals for peptides containing *b*-residues in the strand segments of *b*-hairpins (**a**) Boc–Leu–Val–*b*–Phe–Val–^DPro–Gly–Leu–*b*–Phe–Val–Val–OMe, (^DPro–Gly: type I′ *b*-turn) (ref. 62), (**b**) Boc–*b*-Phe–*b*–Phe-^DPro–Gly–*b*–Phe–*b*-Phe-OMe, (^DPro–Gly: type II′ *b*-turn) (ref. 63), (**c**) Boc–Leu–Val–*b*–Val–^DPro–Gly–*b*–Leu–Val–Val–OMe (^DPro–Gly: type I′ *b*-turn) (ref. 58).

the 11 residue helix, incorporating a central *b*-Gly–*g*-Abu segment. Helical folding of the host sequence is facilitated by the introduction of Aib residues at positions 2, 7 and 11. The insertion of three additional CH_2 groups into the helix backbone does not have a major disrupting influence on the structure. Figure 19 also shows a view of the three dipeptide segments Val-*b*-Gly (*ab*), *b*-Gly-*g*-Abu (*bg*) and *g*-Abu–Val (*ga*). Internal $4 \rightarrow 1$ hydrogen bonds are observed in the *bg* (13 atom hydrogen bonded ring, C_{13}) and *ga* (12 atom hydrogen bonded ring, C_{12}) segments. The backbone torsion angles at the *bg* segments are *b*-Gly: $f = -103°$, $q_1 = 78°$ and $y = -107°$. *g*-Abu: $f = -121°$, $q_1 = 57°$, $q_2 = 62$ and $y = -121°$. In the eight residue peptide the corresponding values are *b*-Gly: $f = -130°$, $q_1 = 76°$ and $y = -162°$. *g*-Abu: $f = -108°$, $q_1 = 58°$, $q_2 = 66°$ and $y = -169°$. Accommodation of *b* and *g*-residues into helical folds is achieved by adoption of *gauche*$^+$ (g^+) conformations at the additional C–C bond. An example of the insertion of a single *b*-residue into an *a*-peptide helix is observed in the structure of the octapeptide Boc–Leu–Phe–Val–Aib–*b*-Phe–Leu–Phe–Val–OMe in crystals (unpublished). Figure 20 shows a view of the molecular conformation. An expanded view of the *aba* segment is also shown. The dipeptide segments Aib–*b*-Phe (*ab*) and *b*-Phe–Val (*ba*) do not form strong $4 \rightarrow 1$ hydrogen bonds, which would result in 11 atom rings. Instead hydrogen bonds of the $5 \rightarrow 1$ type, which result in a 14 atom hydrogen bonded ring are observed in the *aba* and *baa* segments. The observed conformational angles for the guest *b*-Phe residue in the host *a*-peptide helix are $f = -122·4°$, $q_1 = 1·3°$ and $y = -98·7°$.

While relatively few examples exist of the insertion of *b* and *g*-residues into *a*-peptide helices, incorporation into strand segments of hairpins is readily achieved. Several crystalline peptide hairpins, nucleated by ^DPro–Gly turning segments, containing

$\boldsymbol{b}$residue in the strands have been crystallographically characterized.[58,62,63] Figure 21 shows three representative $\boldsymbol{b}$hairpin structures containing $\boldsymbol{b}$residues. The insertion of $\boldsymbol{b}$ Phe residues in the strand segment results in a change of the polarity of the strand, as compared to the strands containing $\boldsymbol{a}$residues. In the case of $\boldsymbol{b}$peptide strands, adjacent carbonyl groups point in one direction, while the NH groups are oriented in the opposite direction, leading to the formation of *polar sheets* in crystals. In most of the observed structures, the $\boldsymbol{b}$residues used have been $\boldsymbol{b}$Phe, $\boldsymbol{b}$Val and $\boldsymbol{b}$Leu, with average torsion angles of $\boldsymbol{f} = -113 \pm 20°$, $\boldsymbol{q} = 163 \pm 7°$ and $\boldsymbol{y} = 127 \pm 19°$ (values averaged over 10 $\boldsymbol{b}$ residues). An interesting distortion is observed in the crystal structure of the octapeptide Boc–Leu–Val–$\boldsymbol{b}$-Val–^{D}Pro–Gly–$\boldsymbol{b}$-Leu–Val–Val–OMe (figure 21). While the hairpin nucleated by a type I′ ^{D}Pro–Gly $\boldsymbol{b}$turn is clearly observed, the $\boldsymbol{b}$Val(3) residue adopts a

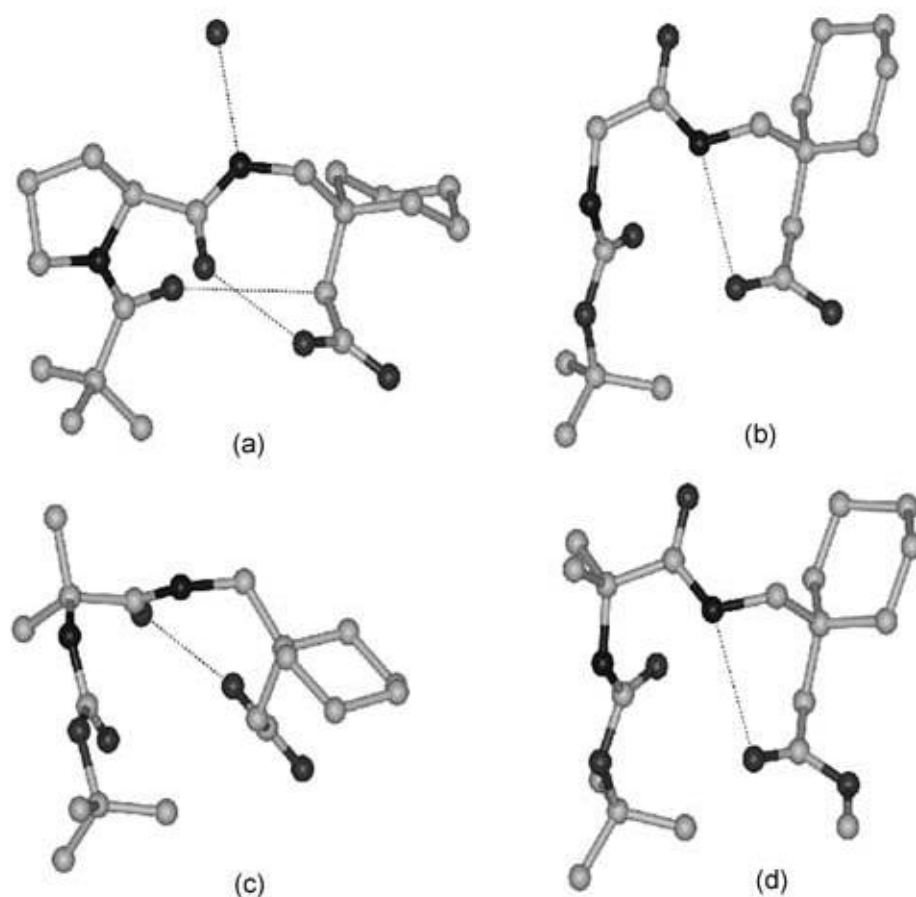**Figure 22.** Molecular conformations in crystals in four peptides containing the constrained $\boldsymbol{g}$amino acid residue gabapentin (Gpn). (**a**) Piv–Pro–Gpn–OH; Gpn $\boldsymbol{f} = 92.9°$, $\boldsymbol{q}_1 = -66.7°$, $\boldsymbol{q}_2 = -70.7°$ and $\boldsymbol{y} = 84.6°$ (**b**) Boc–Gly–Gpn–OH; Gpn $\boldsymbol{f} = -103.7°$, $\boldsymbol{q}_1 = -44.9°$, $\boldsymbol{q}_2 = -48.7°$ and $\boldsymbol{y} = -94.3°$ (**c**) Boc–Aib–Gpn–OH; Gpn $\boldsymbol{f} = 103.9°$, $\boldsymbol{q}_1 = -57.3°$, $\boldsymbol{q}_2 = -75.5°$ and $\boldsymbol{y} = -73.5°$ (**d**) Boc–Aib–Gpn–OMe; Gpn $\boldsymbol{f} = -102.1°$, $\boldsymbol{q}_1 = -48.2°$, $\boldsymbol{q}_2 = -50.3°$ and $\boldsymbol{y} = -90.0$ (ref. 65).

gauche$^+$ conformation $f = -131°$, $q = 65°$ and $y = -175°$. Despite the g^+ conformation about the C^a–C^b bond in bVal(3), cross hydrogen bonding is not disturbed with a compensating distortion at bVal resulting in an almost completely extended value ($-175°$) for y.[58]

$b$residues can also be accommodated in the turn segments of hairpins although no crystal structures have yet been determined for large hairpins. The dinipecotic acid segment has been shown to induce hairpin formation in short tetrapeptide sequences.[64] NMR studies in solution provide evidence for the accommodation of bPhe at the $i + 2$ position of the turn segment in the synthetic peptide Boc–Leu–Val–Val–^{D}Pro–b-Phe–Leu–Val–Val–OMe.[58] In recent work, we have began exploring the use of the stereo-chemically constrained, achiral b,bdisubstituted $g$amino acid, gabapentin (1-(amino-methyl) cyclohexaneacetic acid, Gpn) into short $a$amino acid containing peptides. The presence of the geminal substituents at the central atom restrict the torsion angles q_1 and q_2 to $\pm 60°$, resulting in obligatory folding of the chain upon insertion of the Gpn residue. Figure 22 shows the molecular conformation of four model peptides containing the Gpn residue.[65] In each case, the local folding of the chain is clearly evident with *gauche* conformations observed about the C^a–C^b (q_1) and C^b–C^g (q_2) bonds. Interestingly, the unsubstituted $d$amino acid, $d$aminovaleric acid (dAva) can be incorporated into designed $a$amino acid structures. The insertion of dAva at the $i + 2$ position of the turn segment of a $b$hairpin[66] and into the centre of a peptide helix[67] have been established by NMR studies of model peptides in solutions.

The incorporation of b- and $g$amino acids into hybrid peptide sequences can impart proteolytic stability to biologically active peptides.[68] It will be interest to design pharmacologically important peptides, to specially inhibit proteolysis at labile sites by backbone modification. In such cases the analog peptides must largely retain the confor-mation of the parent sequence in order to manifest biological activity. The exploration of the effect of b- and $g$residues on the conformation of host $a$peptides may provide a rational basis for the choice of sites for insertion.

10. Conclusion

The use of stereochemically constrained amino acid residues can limit the range of local conformations of polypeptide chains, thereby nucleating folding and formation of defined secondary structures. This review has focused primarily on the role of $a$aminoisobutyric acid (Aib) in promoting helix formation, D-amino acid residues in facilitating helix termination and ^{D}Pro–Xxx segments in nucleating $b$hairpin structures. The work reviewed has been largely based on the crystallographic characterisation of peptide conformations in the solid state, in the case of helices and hairpins. For multi-stranded $b$sheets, which have thus far not yielded single crystals, two-dimensional NMR spectroscopy has provided firm evidence for the nature of the designed structures in solution. The use of b- and $g$amino acids offers the possibility of extending the range of well defined peptide structures, beyond those normally observed in natural proteins. The availability of structurally well characterised peptide modules has facilitated investigation of side chain interactions, notably between aromatic groups[69] and permitted mechanistic investigations of proximity effects in catalysis of biologically relevant reactions, like the Amadori rearrangement of glycated peptides.[70] Furthermore, structurally rigid peptide scaffolds have provided the bases for the design of new catalytically active sequences.[71]

Acknowledgements

Reference

1. Kaul R and Balaram P 1999 *Bioorg. Med. Chem.* **7** 105
2. Venkatraman J, Shankaramma S C and Balaram P 2001 *Chem. Rev.* **101** 3131
3. Ramachandran G N, Ramakrishnan C and Sasisekharan V 1963 *J. Mol. Biol.* **7** 95
4. (a) Pauling L, Corey R B and Branson H R 1951 *Proc. Natl. Acad. Sci. USA* **37** 205; (b) Pauling L and Corey R B 1951 *Proc. Natl. Acad. Sci. USA* **37** 251
5. Ramakrishnan C and Ramachandran G N 1965 *Biophys. J.* **5** 909
6. Ramachandran G N and Sasisekharan V 1968 *Adv. Prot. Chem.* **23** 284
7. (a) Ramakrishnan C and Srinivasan N 1990 *Curr. Sci.* **59** 851; (b) Gunasekaran K 1997 *Stereochemical analysis of protein structures-lessons for design, engineering and prediction*, Ph D thesis, Indian Institute of Science, Bangalore
8. Ramachandran G N and Chandrasekaran R 1972 In *Progress in peptide research* (ed.) S Lande (New York: Gordon and Breach) vol II, p 195 (Proceedings of the second American peptide symposium, Cleveland, 1970)
9. Marshall G R and Bosshard H E 1972 *Circ. Res.* **30/31** (suppl. II) 143
10. Burgess A W and Leach S J 1973 *Biopolymers* **12** 2599
11. (a) Zhao M and Bada J L 1989 *Nature* (*London*) **339** 463; (b) Ehrenfreund P and Charnley S B 2000 *Annu. Rev. Astron. Astrophys.* **38** 427
12. Peptaibol Database: http://public-1.cryst.bbk.ac.uk/peptaibol/home.shtml; (b) Chugh J K and Wallace B A 2001 *Biochem. Soc. Trans.* **29** 565
13. (a) Pandey R C, Cook J C Jr and Rinehart K L Jr 1977 *J. Am. Chem. Soc.* **99** 8469; (b) Fox R O Jr and Richards F M 1982 *Nature* (*London*) **300** 325
14. (a) Rinehart K L, Gaudioso L A, Moore M L, Pandey R C and Cook J C Jr 1981 *J. Am. Chem. Soc.* **103** 6517; (b) Karle I L, Flippen-Anderson J L, Agarwalla S and Balaram P 1991 *Proc. Natl. Acad. Sci. USA* **88** 5307
15. (a) Pandey R C, Cook J C Jr and Rinehart K L Jr 1977 *J. Am. Chem. Soc.* **99** 5203; (b) Brückner H, Nicholson G J, Jung G, Kruse K and König W A 1980 *Chromatographia* **13** 209
16. (a) Karle I L, Perozzo M A, Mishra V K and Balaram P 1998 *Proc. Natl. Acad. Sci. USA* **95** 5501; (b) Snook C F, Woolley G A, Oliva G, Patthabi V, Wood S F, Blundell T L and Wallace B A 1998 *Structure* **6** 783; (c) Snook C F and Wallace B A 1999 *Acta Crystallogr.* **D55** 1539
17. (a) Sansom M S P 1991 *Prog. Biophys. Mol. Biol.* **55** 139; (b) Sansom M S P 1993 *Q. Rev. Biophys.* **26** 365; (c) Tieleman D P, Biggin P C, Smith G R and Sansom M S P 2001 *Q. Rev. Biophys.* **34** 473
18. (a) Shamala N, Nagaraj R and Balaram P 1977 *Biochem. Biophys. Res. Commun.* **79** 292; (b) Nagaraj R, Shamala N and Balaram P 1979 *J. Am. Chem. Soc.* **101** 16; (c) Nagaraj R and Balaram P 1981 *Acc. Chem. Res.* **14** 356
19. Shamala N, Nagaraj R and Balaram P 1978 *J. Chem. Soc. Chem. Commun.* 996
20. (a) Smith G D, Pletnev V Z, Duax W L, Balasubramanian T M, Bosshard H E, Czerwinski E W, Kendrick N E, Mathews F S and Marshall G R 1981 *J. Am. Chem. Soc.* **103** 1493; (b) Marshall G R, Hodgkin E E, Langs D A, Smith G D, Zabrocki J and Leplawy M T 1990 *Proc. Natl. Acad. Sci. USA* **87** 487
21. (a) Bruckner H, Konig W A, Greiner M and Jung G 1979 *Angew. Chem., Int. Ed. Engl.* **18** 476; (b) Butters T, Hutter P, Jung G, Pauls P, Schmitt H, Sheldrick G M and Winter W 1981

Angew. Chem., Int. Ed. Engl. **20** 889; (c) Jung G, Bosch R, Katz E, Schmitt H, Voges K P and Winter W 1983 *Biopolymers* **22** 241; (d) Jung G, Bruckner H, Bosch R, Winter W, Schaal H and Strahle J 1983 *Liebigs. Ann. Chem.* 1096; (e) Bosch R, Jung G and Winter W 1983 *Acta Crystallogr.* **C39** 776; (f) Bosch R, Schmitt H, Jung G and Winter W 1985 *Biopolymers* **24** 961 (g) Bosch R, Jung G, Schmitt H and Winter W 1985 *Biopolymers* **24** 979

22. (a) Prasad B V V and Balaram P 1984 *CRC Crit. Rev. Biochem.* **16** 307; (b) Toniolo C and Benedetti E 1988 *ISI atlas of science: Biochemistry* **1** 225; (c) Karle I L and Balaram P 1990 *Biochemistry* **29** 6747; (d) Toniolo C and Benedetti E 1991 *Trends Biochem. Sci.* **16** 350; (e) Karle I L 1992 *Acta Crystallogr.* **B48** 341; (f) Benedetti E 1996 *Biopolymers* (*Peptide Science*) **40** 3; (g) Karle I L 1996 *Biopolymers* (*Peptide Science*) **40** 157

23. Toniolo C, Crisma M, Bonora G M, Benedetti E, Di Blasio B, Pavone V, Pedine C and Santini 1991 *Biopolymers* **31** 129

24. Karle I L, Flippen-Anderson J L, Sukumar M, Uma K and Balaram P 1988 *Proc. Natl. Acad. Sci. USA* **85** 299

25. Datta S 1998 *Folding of the designed peptides: X-ray crystallographic studies on the structure, conformation, aggregation and interactions of oligopeptides containing conformationally constrained amino acids*, Ph D thesis, Indian Institute of Science, Bangalore

26. (a) Baker E N and Hubbard R E 1984 *Prog. Biophys. Mol. Biol.* **44** 97; (b) Datta S, Shamala N, Banerjee A and Balaram P 1997 *J. Peptide Res.* **49** 604

27. (a) Karle I L, Flippen-Anderson J L, Gurunath R and Balaram P 1994 *Protein Science* **3** 1547; (b) Millhauser G M 1995 *Biochemistry* **34** 3873; (c) Mammi S, Rainaldi M, Bellanda M, Schievano E, Peggion E, Broxterman Q B, Formaggio F, Crisma M and Toniolo C 2000 *J. Am. Chem. Soc.* **122** 11735; (d) Silva R A G, Yasui S C, Kubelka J, Formaggio F, Toniolo C and Keiderling T 2002 *Biopolymers* **65** 229

28. Otoda K, Kitagawa Y, Kimura S and Imanishi Y 1993 *Biopolymers* **33** 1337

29. (a) Sudha T S, Vijaykumar E K S and Balaram P 1983 *Int. J. Peptide Protein Res.* **22** 464; (b) Miick S M, Martinez G V, Fiori W R, Todd A P and Millhauser G L 1992 *Nature* (*London*) **359** 653; (c) Basu G and Kuki A 1993 *Biopolymers* **33** 995; (d) Toniolo C, Polese A, Formaggio F, Crisma M and Kamphius J 1996 *J. Am. Chem. Soc.* **118** 2744

30. (a) Schellman C 1980 In *Protein folding* (ed.) R Jaenicke (Amsterdam: Elsevier/North-Holland Biochemical Press) p. 53; (b) Milner-White E J 1988 *J. Mol. Biol.* **199** 915; (c) Nagarajaram H A, Sowdhamini R, Ramakrishnan C and Balaram P 1993 *FEBS Lett.* **321** 79; (d) Aurora R, Srinivasan R and Rose G D 1994 *Science* **264** 1126; (e) Gunasekaran K, Nagarajaram H A, Ramakrishnan C and Balaram P 1998 *J. Mol. Biol.* **275** 917

31. For examples in designed peptides: (a) Karle I L, Flippen-Anderson J L, Uma K and Balaram P 1993 *Int. J. Peptide Protein Res.* **42** 401; (b) Di Blasio B, Pavone V, Saviano R, Fattorusso C, Pedone E, Benedetti E, Crisma M and Toniolo C 1994 *Peptide Res.* **7** 55; (c) Banerjee A, Datta S, Pramanik A, Shamala N and Balaram P 1996 *J. Am. Chem. Soc.* **118** 9477; (d) Datta S, Shamala N, Banerjee A, Pramanik A, Bhattacharjya S and Balaram P 1997 *J. Am. Chem. Soc.* **119** 9246; (e) Datta S, Uma M V, Shamala N and Balaram P 1999 *Biopolymers* **50** 13

32. Aravinda S, Shamala N, Pramanik A, Das C and Balaram P 2000 *Biochem. Biophys. Res. Commun.* **273** 933.

33. Aravinda S, Shamala N, Bandyopadhyay A and Balaram P 2003 *J. Am. Chem. Soc.* (in press)

34. (a) Madan Babu M, Singh S K and Balaram P 2002 *J. Mol. Biol.* **322** 871 (b) Singh S K and Madan Babu M and Balaram P 2003 *Proteins: Struct. Funct. Genet.* **57** 167

35. Banerjee A, Raghothama S, Karle I L and Balaram P 1996 *Biopolymers* **39** 279

36. (a) Banerjee A 1996 *Non-standard amino acids in peptide design*, Ph D thesis, Indian Institute of Science, Bangalore; (b) Karle I L 2001 *Biopolymers* (*Peptide Science*) **60** 351

37. Karle I L, Flippen-Anderson J L, Uma K and Balaram P 1990 *Proteins: Struct. Funct. Genet.* **7** 62; Karle I L, Flippen-Anderson J L, Uma K and Balaram P 1993 *Biopolymers* **33** 827

38. Tang K, Green M M, Cheon K S, Selinger J V and Garetz B A 2003 *J. Am. Chem. Soc.* **125** 7313

39. Aravinda S, Shamala N, Desiraju S and Balaram P 2002 *Chem. Commun.* 2454

40. (a) Sibanda B L and Thornton J M 1985 *Nature* (*London*) **316** 170; (b) Wilmot C M and Thornton J M 1988 *J. Mol. Biol.* **203** 211; (c) Sibanda B L, Blundell T L and Thornton J M 1989 *J. Mol. Biol.* **206** 758

41. (a) Richardson J S 1981 *Adv. Prot. Chem.* **34** 167; (b) Srinivasan N, Anuradha V S, Ramakrishnan C, Sowdhamini R and Balaram P 1994 *Int. J. Pept. Prot. Res.* **44** 112
42. (a) Haque T S, Little J C and Gellman S H 1996 *J. Am. Chem. Soc.* **118** 6975; (b) Haque T S and Gellman S H 1997 *J. Am. Chem. Soc.* **119** 2303; (c) Gellman S H 1998 *Curr. Opin. Chem. Biol.* **2** 717; (d) Espinosa J F and Gellman S H 2000 *Angew. Chem., Int. Ed.* **39** 2330
43. Awasthi S K, Raghothama S and Balaram P 1995 *Biochem. Biophys. Res. Commun.* **216** 375
44. Karle I L, Awasthi S K and Balaram P 1996 *Proc. Natl. Acad. Sci. USA* **93** 8189
45. Das C, Naganagowda G A, Karle I L and Balaram P 2001 *Biopolymers* **58** 335
46. (a) Balaram P 1999 *J. Peptide. Res.* **54** 195; (b) Das C, Raghothama S and Balaram P 1998 *J. Am. Chem. Soc.* **120** 5812; (c) Das C and Balaram P 1999 *J. Chem. Soc., Chem. Commun.* 967; (d) Venkatraman J, Naganagowda G A, Sudha R and Balaram P 2001 *Chem. Commun.* 2660
47. Venkatraman J, Naganagowda G A and Balaram P 2002 *J. Am. Chem. Soc.* **124** 4987
48. (a) de Alba E, Jimenez M A and Rico M 1997 *J. Am. Chem. Soc.* **119** 175; (b) Ramirez-Alvarado M, Blanco F J and Serrano L 1996 *Natl. Struct. Biol.* **3** 604; (c) Maynard A J and Searle M S 1997 *J. Chem. Soc., Chem. Commun.* 1297
49. Aravinda S, Shamala N, Rajkishore R, Gopi H N and Balaram P 2002 *Angew. Chem., Int. Ed.* **41** 3863
50. (a) Tatko C D and Waters M L 2002 *J. Am. Chem. Soc.* **124** 9372; (b) Waters M L 2002 *Curr. Opin. Chem. Biol.* **6** 736
51. Das C, Shankaramma S C and Balaram P 2001 *Chem. Eur. J.* **7** 840
52. Karle I L, Das C and Balaram P 2000 *Proc. Natl. Acad. Sci. USA* **97** 3034
53. Banerjee A and Balaram P 1997 *Curr. Sci.* **73** 1067
54. (a) Fernandez-Santin J M, Aymami J, Rodriguez-Galan A, Munoz-Guerra S and Subirana J A 1984 *Nature* (*London*) **311** 53; (b) Bella J, Alemann C, Fernandez-Santin J M, Alegre C and Subirana J A 1992 *Macromolecules* **25** 5225; (c) Navas J J, Alemann C, Lopez-Carrasquero F and Munoz-Guerra S 1995 *Macromolecules* **28** 4487; (d) Lopez-Carrasquero F, Alemann C and Munoz-Guerra S 1995 *Biopolymers* **36** 263; (e) Aleman C, Navas J J and Munoz-Guerra S 1997 *Biopolymers* **41** 721
55. (a) Seebach D and Matthews J L 1997 *Chem. Commun.* 2015; (b) Seebach D, Abele S, Gademann, Guichard G, Hintermann T, Jaun B, Matthews J L and Schreiber J 1998 *Helv. Chim. Acta* **81** 932; (c) Seebach D, Abele S, Gademann K and Jaun B 1999 *Angew. Chem., Int. Ed.* **38** 1595
56. (a) Gellman S H 1998 *Acc. Chem. Res.* **31** 173; (b) Cheng R P, Gellman S H and DeGrado W F 2001 *Chem. Rev.* **101** 3219
57. Steer D L, Lew R A, Perlmutter P, Smith A I and Aguilar M I 2002 *Curr. Med. Chem.* **9** 811
58. Gopi H N, Roy R S, Raghothama S, Karle I L and Balaram P 2002 *Helv. Chim. Acta* **85** 3313
59. Appella D H, Christianson L A, Klein D A, Richards M R, Powell D R and Gellman S H 1999 *J. Am. Chem. Soc.* **121** 7574
60. Appella D H, Christianson L A, Karle I L, Powell D R and Gellman S H 1999 *J. Am. Chem. Soc.* **121** 6206
61. Karle I L, Pramanik A, Banerjee A, Bhattacharjya S and Balaram P 1997 *J. Am. Chem. Soc.* **119** 9087
62. Karle I L, Gopi H N and Balaram P 2001 *Proc. Natl. Acad. Sci. USA* **98** 3716
63. Karle I L, Gopi H N and Balaram P 2002 *Proc. Natl. Acad. Sci. USA* **99** 5160
64. (a) Chung Y J, Christianson L A, Stanger H E, Powell D R and Gellman S H 1998 *J. Am. Chem. Soc.* **120** 10555; (b) Chung Y J, Huck B R, Christianson L A, Stanger H E, Krauthäuser S, Powell D R and Gellman S H 2000 *J. Am. Chem. Soc.* **122** 3995
65. Aravinda S, Ananda K, Shamala N and Balaram P 2003 *Chem. Eur. J.* **9** 4789
66. Shankaramma S C, Singh S K, Sathyamurthy A and Balaram P 1999 *J. Am. Chem. Soc.* **121** 5360
67. Banerjee A, Pramanik A, Bhattacharjya S and Balaram P 1996 *Biopolymers* **39** 769
68. (a) Hintermann T and Seebach D 1997 *Chimia* **51** 244; (b) Frackenpohl J, Arvidsson P I, Schreiber J V and Seebach D 2002 *Chem. Biochem.* **2** 445; (c) Rueping M, Mahajan Y, Sauer

M and Seebach D 2002 *Chem. Biochem.* **3** 257; (d) Gopi H N, Ravindra G, Pal P P, Pattanaik P, Balaram H and Balaram P 2003 *FEBS Lett.* **535** 175

69. Aravinda S, Shamala N, Das C, Sriranjini A, Karle I L and Balaram P 2003 *J. Am. Chem. Soc.* **125** 5308
70. Venkatraman J, Aggarwal K and Balaram P 2001 *Chem. Biol.* **8** 611
71. (a) Jarvo E R, Copeland G T, Papaioannou N, Bonitatebus Jr P J and Miller S J 1999 *J. Am. Chem. Soc.* **121** 11638; (b) Copeland G T and Miller S J 2001 *J. Am. Chem. Soc.* **123** 6496; (c) Guerin J and Miller S J 2002 *J. Am. Chem. Soc.* **124** 2134
72. Allen F H and Kennard O 1993 *Chem. Des. Autom. News.* **8** 31