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REVIEW ARTICLE

Stereochemistry of peptides and polypeptides containing omega amino acids

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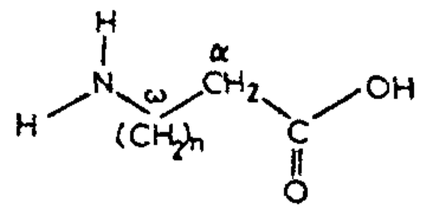
The omega amino acids have a larger degree of conformational variability than the alpha amino acids, leading to a greater diversity of backbone structures in peptides and polypeptides. The synthetic accessibility of chiral β-amino acids and the recent observation of novel helical folds in oligomers of cyclic β-amino acids has led to renewed interest in the stereochemistry of ω-amino acid containing peptides. This review focuses on the conformational characteristics of the polymethylene chain in ω-amino acid segments and surveys structural features in peptides established by X-ray diffraction. The literature on polymers of achiral ω-amino acids (nylon derivatives) and chiral, substituted derivatives derived from trifunctional α-amino acids, reveals that while sheet-like, intermolecular hydrogen bonded structures are formed by the former, folded helices appear favoured by the latter. ω-Amino acids promise to expand the repertoire of peptide folds.

THE genetic code determines the translation of nucleic acid sequences in genes into amino acid sequences in proteins. The alphabet of amino acids specified by the genetic code is generally limited to twenty. The genetically coded amino acids are all α-amino acids, structures in which the carboxyl and the amino groups are linked to a common tetrahedral carbon centre, the α-carbon atom. Variants of the α-amino acids are found in nature in which the amino and the carboxyl groups are placed on different carbon atoms. In ω-amino acids, the two functional groups are separated by polymethylene units of variable length (Figure 1). The introduction of additional C–C bonds into the polyamide (polypeptide)* backbone introduces additional degrees of conformational freedom, which can, in principle, have profound effects on structural and functional properties of peptides containing ω-amino acids. Recent studies have established convenient synthetic routes to chiral β-amino acids[1–3]. The observation of novel helical folds in peptide oligomers of acyclic[1] and cyclic β-amino acids[4,5], the characterization of α-helical structures in peptides incorporating β-Ala–γ-Abu segments[6] and the demonstration of proteolytic stability of a model β-hexapeptide[7] have provided a dramatic new impetus for the use of ω-amino acids in peptide and protein design. This report presents a brief overview of the structural features established in peptides containing ω-amino acids.

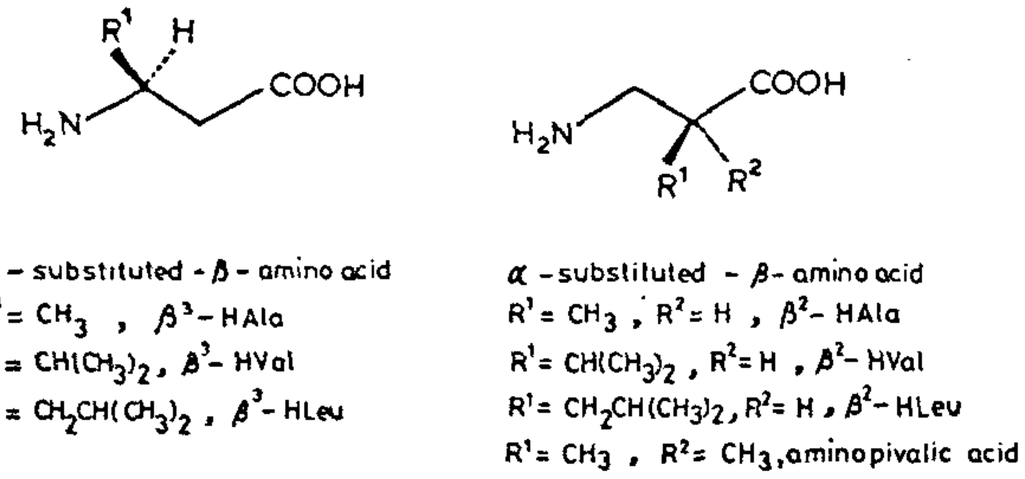
*We shall use the term peptide to describe amide linkages involving ω- amino acids in oligomeric sequences. We use the broad definition of a peptide bond as the linkage between amino acids, not necessarily restricted to α-amino acids[8]. The term polyamide is confined to the descriptions of polymeric sequences involving ω-amino acids encompassing the entire range of nylons[9]. The term isopeptide has been used in the literature to describe peptide bonds formed by side chain carboxyl or amino group in trifunctional amino acids like Asp, Glu and Lys. In the present review, the term β-peptide is adopted for homooligomeric sequences containing β-amino acids. The nomenclature used follows the current literature[1].

Achiral, acyclic, unsubstituted ω-amino acids



n = 1, β-Ala ; n = 2, γ- Abu
n = 3, δ-Ava ; n = 4, ε - Acp

Chiral, acyclic substituted ω-amino acids



β - substituted - β - amino acid
$R^1 = CH_3$, β^3 - HAla
$R^1 = CH(CH_3)_2$, β^3 - HVal
$R^1 = CH_2CH(CH_3)_2$, β^3 - HLeu

α - substituted - β - amino acid
$R^1 = CH_3$, $R^2 = H$, β^2 - HAla
$R^1 = CH(CH_3)_2$, $R^2 = H$, β^2 - HVal
$R^1 = CH_2CH(CH_3)_2$, $R^2 = H$, β^2 - HLeu
$R^1 = CH_3$, $R^2 = CH_3$, aminopivalic acid

Chiral, cyclic ω-amino acids

trans - 2 - amino cyclopentanecarboxylic acid

trans - 2 - amino cyclohexanecarboxylic acid

trans - 4 - amino cyclohexanecarboxylic acid

Figure 1. Structures of achiral unsubstituted ω-amino acids, chiral, acyclic, substituted β-amino acids and cyclic ω-amino acids.

ω-Amino acids are widespread in nature and are metabolically derived in diverse organisms. β-Alanine (β-Ala) occurs widely in the animal and plant kingdoms. Representative β-Ala containing natural peptides[10] include carnosine[11], efrapeptin[12], roseotoxin[13], and leucinostatin[14]. β-Ala is also a constituent of the vitamin pantothenic acid. γ-Aminobutyric acid (γ-Abu) is found in the mammalian brain[15], where it is enzymatically produced and functions as a neurotransmitter[16]. Isolation of δ-aminovaleric acid derived from rumen ciliate protozoa has been reported[17]. The occurrence of γ-Abu and higher homologs in natural peptides have not yet been documented. The formation of peptide bonds using side chain amino/carboxyl functions of some trifunctional amino acids like Asp, Glu, Lys and α,γ-diamino butyric acid (Dab) leads to peptides containing substituted ω-amino acids. Some important examples of peptide bond (isopeptide) formation, involving side chains include glutathione[18], calbindin[19], fibrinogen[20] and polymyxin B[21]. A particularly intriguing example is the case of the antigenic, capsular polypeptide, poly(γ-D-Glu), produced by *Bacillus subtilis* and *Bacillus anthracis*[22]. Isopeptide bond formation is also a feature in clavicepamines, lysine rich basic proteins isolated from saprophytic culture of ergot, which show anti-cell proliferative activity[23]. In recent years, considerable effort has been expended in research directed to enhance stability and/or potency of physiologically-active peptides. Increased stability towards enzymatic degradation may be achieved by incorporating ω-amino acids that are not normally substrates for proteolytic enzymes[24]. A number of reports describe the incorporation of ω-amino acids into bioactive peptides[25]. A particular interesting example is the replacement of Gly16–Gly17 by δ-aminovaleric acid (δ-Ava) in bovine pancreatic trypsin inhibitor (BPTI) using semisynthesis, resulting in diminished inhibition of trypsin[26].

There have also been many reports of synthetic incorporation of ω-amino acids into structurally-interesting peptides and polypeptides. Considerable attention has been focused on polymers of ω-amino acids. Polyamides derived from ω-amino acids assume special importance because of the extensive interest in nylons[9]. While β-Ala and its achiral higher homologs are readily available and can be easily incorporated into peptides, chiral substituted β-amino acids pose a greater problem, requiring efficient synthesis of chiral starting materials, an area of great current activity[1–3,27]. A convenient synthetic strategy involves Arndt–Eistert homologation of diazo ketones derived from the chiral α-amino acids and subsequent incorporation into peptides (Figure 2). Synthetic routes to cyclic β-amino acids containing five and six-membered rings of defined stereochemistry have been reported[28,29]. The use of readily available chiral trifunctional amino acids like Dab, Glu, Orn and Lys provides an entry to backbone substituted derivatives of peptides containing higher ω-amino acids.

Scheme 1

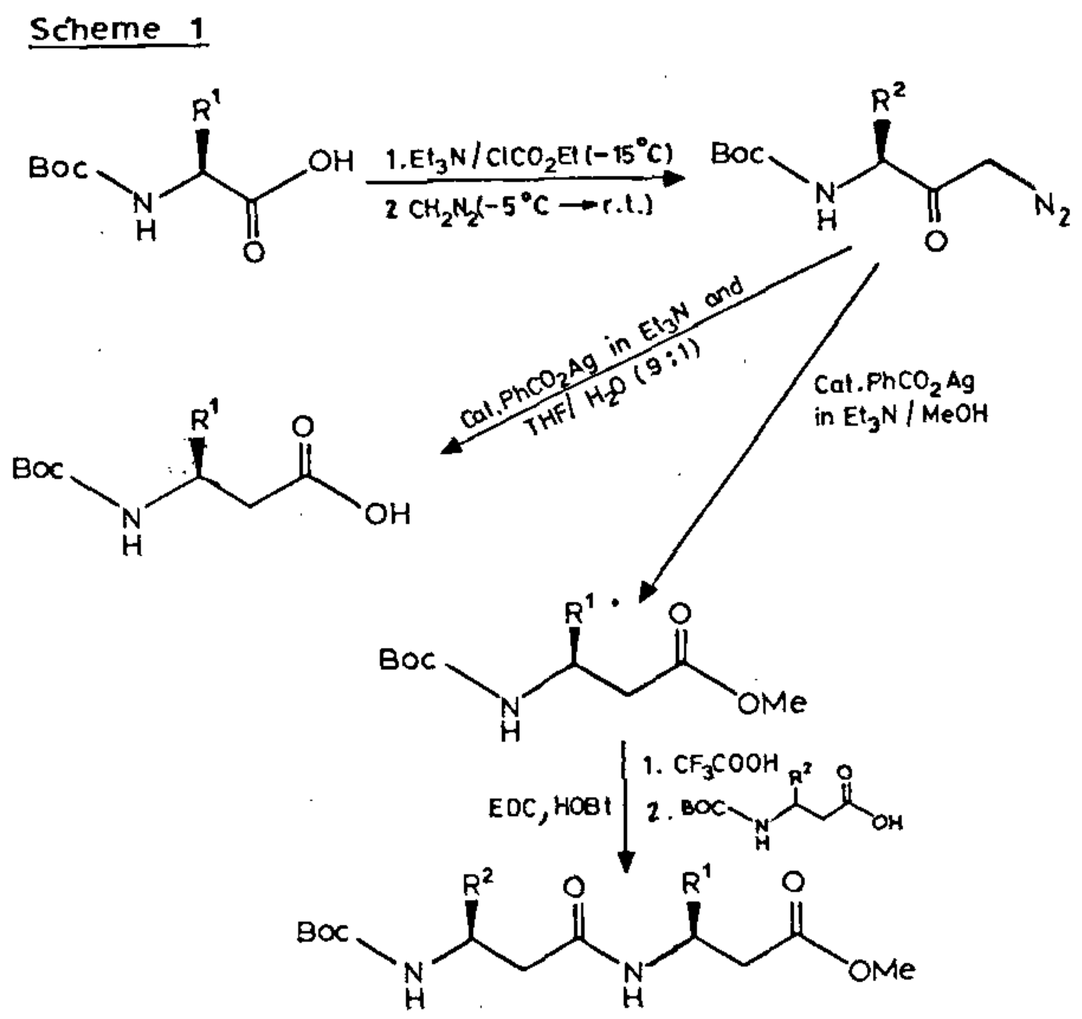


Figure 2. Synthetic scheme for preparation of chiral β-amino acids (from ref. 1).

The stereochemistry of ω-amino acid-containing peptides

Conformations of a polypeptide backbone made up of only α-amino acids are defined by three sets of torsion angles ϕ, ψ and ω (ref. 30). The torsion angle about the peptide bond is generally limited to a *trans* geometry ($\omega = 180°$), with a *cis* ($\omega = 0°$) conformation being rarely found in peptides and proteins. Non-bonded interactions dictate polypeptide backbone folding, resulting in the restriction that the ϕ, ψ values lie within the sterically-allowed regions of the Ramachandran map[31]. Due to the insertion of one or more methylene groups between the N and C^{α}-atoms of glycine, the accessible conformational space for ω-amino acid residues is greater than that for the α-amino acids, when incorporated into peptide chains. The nomenclature for the backbone torsion angles in ω-amino acids is introduced in Figure 3. The C^{α}–CO bond is designated as ψ, while N–C^{ω} bond is designated as ϕ in the ω-amino acids. The C–C torsion angles along the polymethylene chains are defined as θ_n, with numbering beginning from the N-terminus*. Figure 3 also illustrates the structural similarity between $(Gly)_n$ segments (where $n = 2, 3$) and corresponding ω-amino acids. It also depicts the substitution of a $(Gly)_3$ segment in a polypeptide chain with a β-alanyl-γ-aminobutyryl unit. This kind of replacement can be termed as homomorphous, with the number of atoms in the ω-amino acid containing moiety being exactly the same as the number of atoms in the peptide segment that has been replaced[32]. In ω-amino acids, the torsion angles about C–C bonds of the polymethylene chain can lie close to the *gauche* ($\theta = \pm 60°$) and *trans* ($\theta = 180°$) conformations. Folded structures can be readily obtained by populating *gauche* conformations.

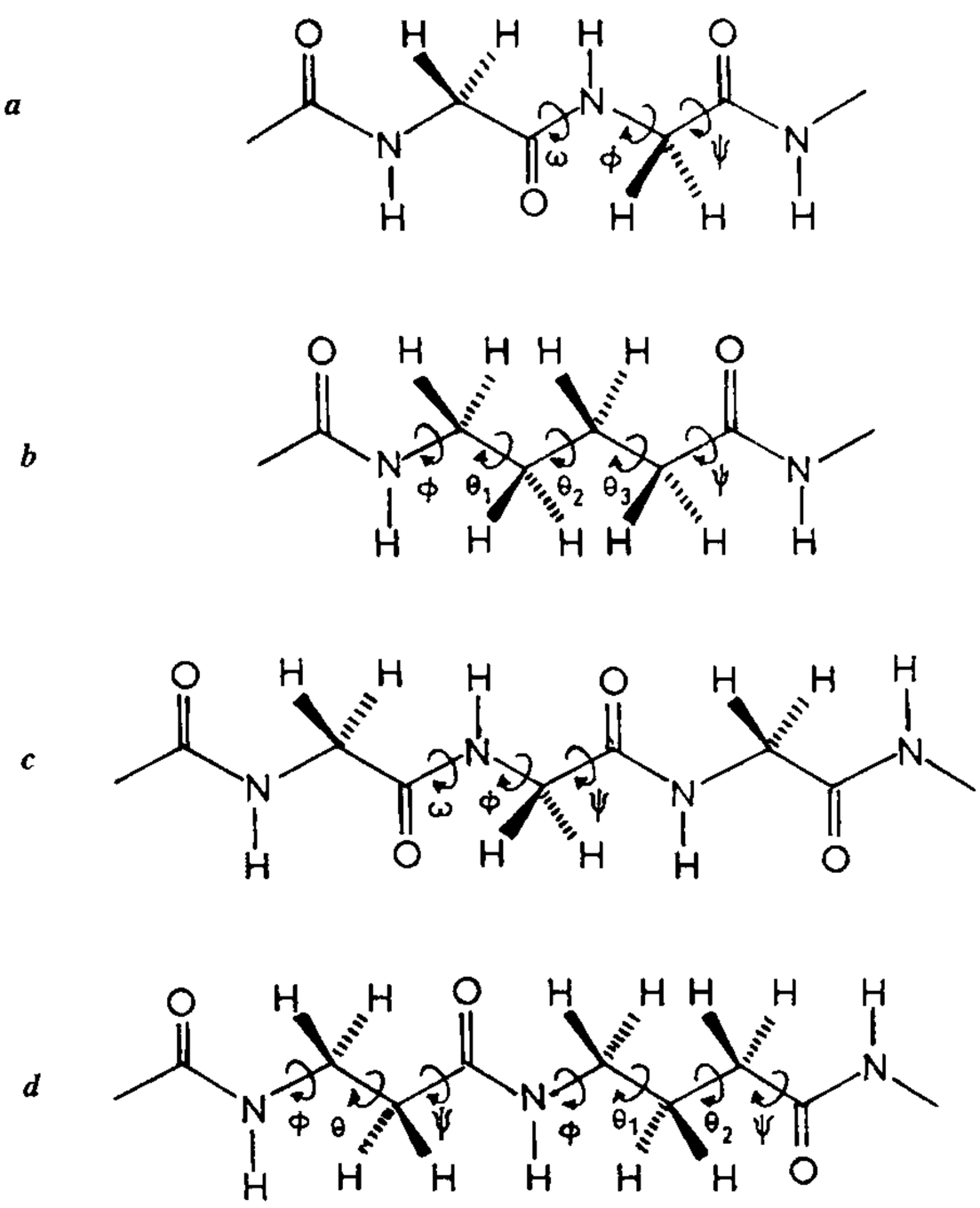


Figure 3. Definition of backbone torsion angles. *a*, Gly residue in a Gly–Gly segment. *b*, δ-amino valeryl (δ-Ava) residue. Note that the number of atoms bridging the two flanking peptide units is the same in (*a*) and (*b*). *c*, $(Gly)_3$ segment. *d*, β-Ala–γ-Abu segment. *e*, 7-amino-heptanoyl residue. Note that (*d*) and (*e*) are formally homomorphous with (*c*).

Peptide crystal structures

There are relatively few reports of crystal structure determinations of peptides containing ω-amino acids, with β-Ala being most widely investigated. Table 1 lists backbone conformational parameters observed in crystal structures. In the case of β-Ala, there are fourteen and nine examples for *gauche* and *trans* conformations, respectively. There are only a limited number of examples of structure determinations of γ-Abu and δ-Ava, ε-Acp in peptides. Table 1 reveals occurrence of both *gauche* and *trans* conformations in the higher ω-amino acids also. While the observation of *gauche* forms in cyclic peptides is unsurprising because of the constraints of ring closure, the adoption of θ values near $\pm 60°$ in acyclic peptides is a clear indicator of the intrinsic tendency of the polymethylene units to fold into compact conformations. Indeed theoretical calculations on succinamide[33,34], glutaramide[35] and adipamide[34] suggest that *gauche* conformations are predominantly favoured. Evidence for folded conformations in solution for simple amides derived from β-Ala and γ-Abu has been reported[36]. Figure 4 illustrates two helical peptide structures which contain a centrally positioned β-Ala–γ-Abu segment, that is formally homomorphous to a $(Gly)_4$

*This nomenclature has the advantage that the backbone conformation is defined by the values of θ_n, read sequentially from the N-terminus to the C-terminus, following the same order as the ϕ, ψ angles. Alternative definitions, involving sequential labelling of the C–C torsion angles from the C-terminus C^{α}-carbon (for example, the C^{α}–C^{β} torsion angle is denoted in the early literature as μ_1 or χ_1) are less convenient, because the numbering of the polymethylene dihedral angles runs opposite to the numbering of the ϕ, ψ dihedral angles in peptide chains, where the residues are sequentially numbered from N- to C-terminus.

Table 1. Torsional angles ϕ, θ_n, and ψ of ω-amino acids in derivatives and peptides

Residue(s)	Compounds	Torsion angles (deg.)	Referen
β-Ala	LeucinostatinA, acyclic nonapeptide	$\phi = -103$, $\theta = 80$, $\psi = -78$	69
β-Ala	Boc–Aib–β-Ala–Aib–OMe	$\phi = -87$, $\theta = -177.2$, $\psi = 91.1$	70
β-Ala	Carnosine(β-Ala–L-His)	$\theta = -177$, $\psi = -38$	71
β-Ala	Carnosine(β-Ala–L-His)	$\theta = -177.4$, $\psi = 141.2$	72
β-Ala	β-Alanine (Zwitterionic)	$\theta = -154.8$	73
β-Ala	N -Boc–β-Ala–(L)-Ala–$NHCH_3$	$\phi = 130.57$, $\theta = -150.2$, $\psi = -109.45$	74
β-Ala	Cyclo (Pro–Phe–Phe–β-Ala–β-Ala)	β-Ala4: $\phi = 71$, $\theta = 79$, $\psi = -106$, β-Ala5: $\phi = 171$, $\theta = -70$, $\psi = 157$	75
β-Ala	Acetyl-(Gly–β-Ala)$_2$ NH Propyl	β-Ala2: $\phi = 169$,. $\theta = 180$, $\psi = -164$ β-Ala4: $\phi = 175$,. $\theta = -177$, $\psi = -171$	76
β-Ala	3-ammonium propionic acid monohydrogen phosphite ($H_3NCH_2H_4CO_2H$)(HPO_3H)	$\theta = -48.5$	77
β-Ala	Cyclo[L-Ser(O-tBu)–β-Ala–Gly–L-βAsp(OMe)]	$\phi = -103.4$, $\theta = -57.6$, $\psi = 69.1$	78
β-Ala	β-Alanylciliatine	$\theta = 66.1$, $\psi = -157$	79
β-Ala	Cyclo(L-Pro–L-Phe–β-Ala–β-Ala)	Mol.A; β-Ala3:$\phi = -133.9$, $\theta = 63.6$, $\psi = -170$; β-Ala4: $\phi = 133$, $\theta = 70.4$, $\psi = -103.5$ Mol.B; β-Ala3: $\phi = -140.4$, $\theta = 61.9$, $\psi = -167.2$; β-Ala4: $\phi = 104.2$, $\theta = 72.4$, $\psi = -98.1$	80
β-Ala	(a) Boc–L-Ala–β-Ala–NHMe (b) Boc–Aib–β-Ala–NHMe (c)Boc–Aib–Aib–β-Ala–NHMe	(a) $\phi = 136$, $\theta = -175.8$, $\psi = -163.4$ (b) $\phi = -132.8$, $\theta = 165$, $\psi = 131.7$ (c) $\phi = -88$, $\theta = 71$, $\psi = -101.3$	81
β-Ala	cyclo(Pro– β-Ala–Pro– β-Ala)	β Ala2: $\phi = 105$, $\theta = 57.8$, $\psi = -157.4$ β Ala4: $\phi = 81.6$, $\theta = 64.4$, $\psi = -158$	82
β-Ala	Boc–β-Ala–Leu–Aib–Val–OMe	β Ala: $\phi = -78$, $\theta = 173$, $\psi = 103$	83
β-Ala	cyclo(Pro–Pro–β-Ala–β-Ala)	β Ala3: $\phi = 76.2$, $\theta = 62.5$, $\psi = -143.5$ β Ala4: $\phi = -101.6$, $\theta = 52.2$, $\psi = 89.3$	84
γ-Abu	γ-Aminobutyric acid (zwitterionic)	$\theta_1 = 175.9$, $\theta_2 = -67.6$	85
γ-Abu	γ-Aminobutyric acid hydrochloride	$\theta_1 = -177.6$, $\theta_2 = -169.3$	86
γ-Abu	(a) Copper(II)-di-γ-amino butyrate (b) Copper(II)-di-γ-aminobutyrate dihydrate	(a) $\theta_1 = -178.7$, $\theta_2 = 58.9$ (b) $\theta_1 = -175.6$, $\theta_2 = -173.7$	87
(a) β-Ala (b) γ-Abu	Boc–Leu–Aib–Val–β-Ala–γ-Abu–Leu–Aib–Val–OMe	(a) $\phi = -130$, $\theta = 76$, $\psi = -162$ (b) $\phi = -108$, $\theta_1 = 58$, $\theta_2 = 66$ $\psi = -169$	37
(a)β-Ala (b) γ-Abu	Boc–Leu–Aib–Val–β-Ala–γ-Abu–Leu–Aib–Val–Ala–Leu–Aib–OMe	(a) $\phi = -103$, $\theta = 78$, $\psi = -107$ (b) $\phi = -121$, $\theta_1 = 57$, $\theta_2 = 62$ $\psi = -121$	37
γ-Abu	(a) γ-Aminobutyric acid choline ester diiodide (b) γ-Aminobutyric acid choline ester (±)tartarate	(a) $\theta_1 = -179.7$, $\theta_2 = 71.8$, $\psi = 178.5$ (b) $\theta_1 = -70$, $\theta_2 = 177$, $\psi = 176$	88
δ-Ava	Cyclo(L-Leu1–L-Tyr2–δ-Ava3–δ-Ava4)	Crystal A (acetone/DMSO) δ-Ava3: $\phi = 138$, $\theta_1 = 178$, $\theta_2 = 177$, $\theta_3 = 59$, $\psi = -117$, δ-Ava4: $\phi = -118$, $\theta_1 = 66$, $\theta_2 = -177$, $\theta_3 = 177$, $\psi = -150$ Crystal A (acetone/H_2O): δ-Ava 3: $\phi = 175$, $\theta_1 = 94$ $\theta_2 = 178$, $\theta_3 = 64$, $\psi = -132$ δ-Ava 4: $\phi = -108$, $\theta_1 = 61$, $\theta_2 = -176$, $\theta_3 = 177$, $\psi = -140$	89
δ-Ava	Cyclo[Gly–Pro–Ser(O^{t}Bu)–Gly–δ–Ava]	$\phi = 113$, $\theta_1 = -49.5$, $\theta_2 = -82.78$ $\theta_2 = 168.86$, $\psi = -88.47$	90
Acp	ε-Aminocaproic acid (zwitterionic)	$\theta_1 = 176$, $\theta_2 = -106.83$ $\theta_3 = -171.44$, $\theta_4 = -41.58$	43
Acp	Boc–Tyr–Ala–Leu–Aib–Val–Ala–Leu–Acp–OMe	$\phi = -94$, $\theta_1 = -70$, $\theta_2 = 173$, $\theta_3 = -164$, $\theta_4 = -21$, $\psi = 94$	40
Acp	Boc–L-Val–L-Ala–L-Leu–Aib–L-Val–L-Ala–L-Leu–Acp–D-Val–D-Ala–D-Leu–Aib–D-Val–D-Ala–D-Leu–OMe	$\phi = -96$, $\theta_1 = -65$, $\theta_2 = -51$, $\theta_3 = -53$, $\theta_4 = -63$, $\psi = -102$	40
Acp	Boc–L-Val–L-Ala–L-Leu–Aib–L-Val–L-Ala–L-Leu–Acp–L-Val–L-Ala–L-Leu–Aib-L-Val–L-Ala–L-Leu–OMe	$\phi = 117$, $\theta_1 = -62$, $\theta_2 = -164$, $\theta_3 = -162$, $\theta_4 = -63$, $\psi = 124$	39

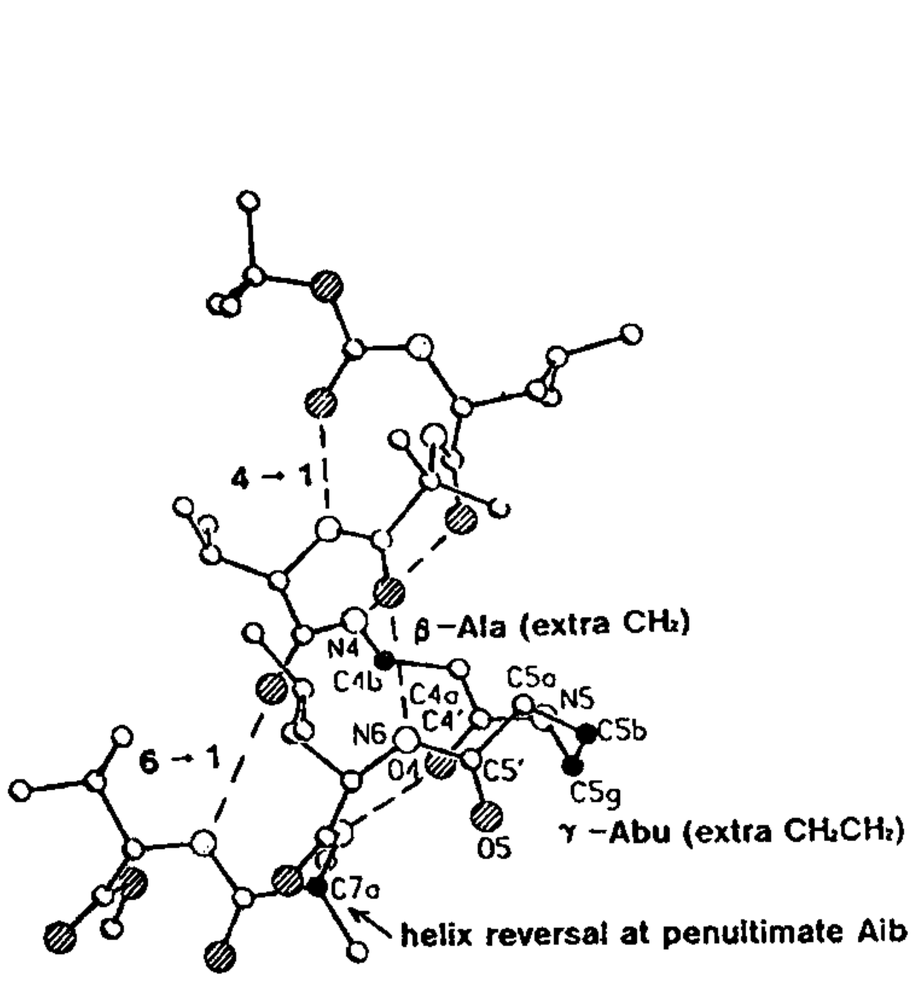


Figure 4. *a*, Molecular structure of the octapeptide Boc–Leu–Aib–Val–β-Ala–γ-Abu–Leu–Aib–Val–OMe determined in crystals[6]. There is a helix reversal at Aib(7), resulting in a Schellman motif, with a 6→1 hydrogen bond between N(8) and O(3). The darkened back-bone atoms represent the carbon atoms of the extra CH_2 group in β-Ala (C4b) and γ-Abu (C5b and C5g) and C7a of Aib7, the site of helix sense reversal. *b*, Molecular structure of a undecapeptide Boc–Leu–Aib–Val–β-Ala–γ-Abu–Leu–Aib–Val–Ala–Leu–Aib–OMe determined in crystals[6]. The structure is a mixed $3_{10}/\alpha$-helix without a helix reversal as seen in (*a*). The darkened atoms are the same as in (*a*).

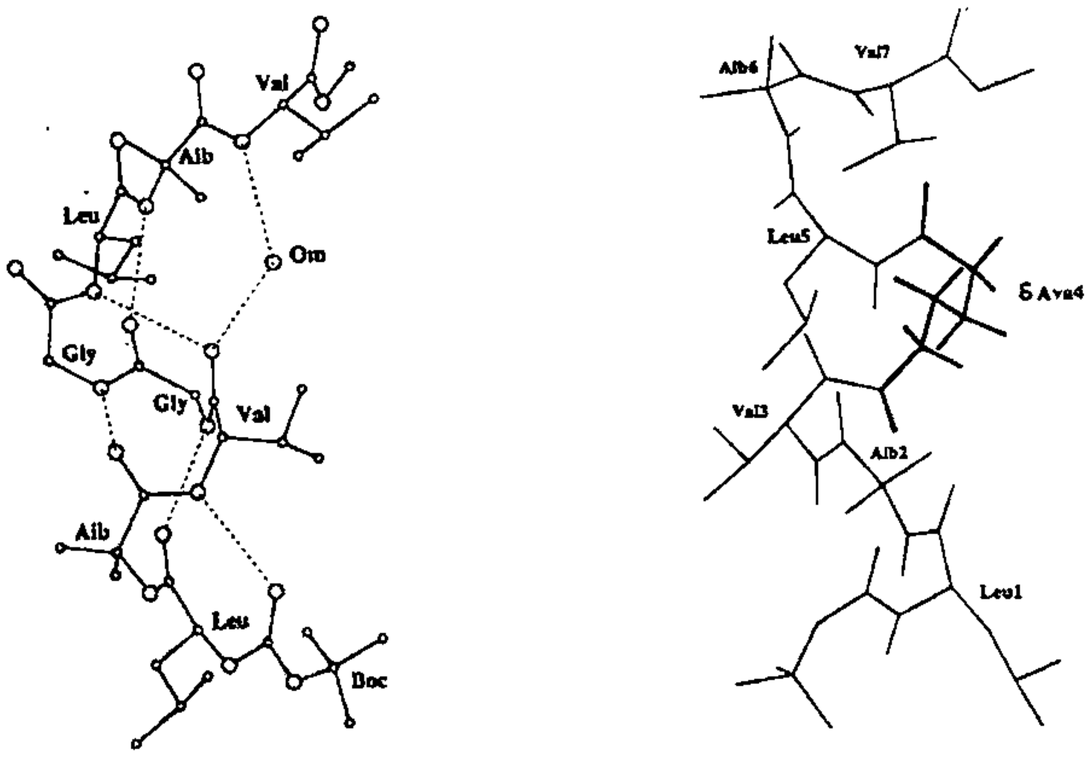
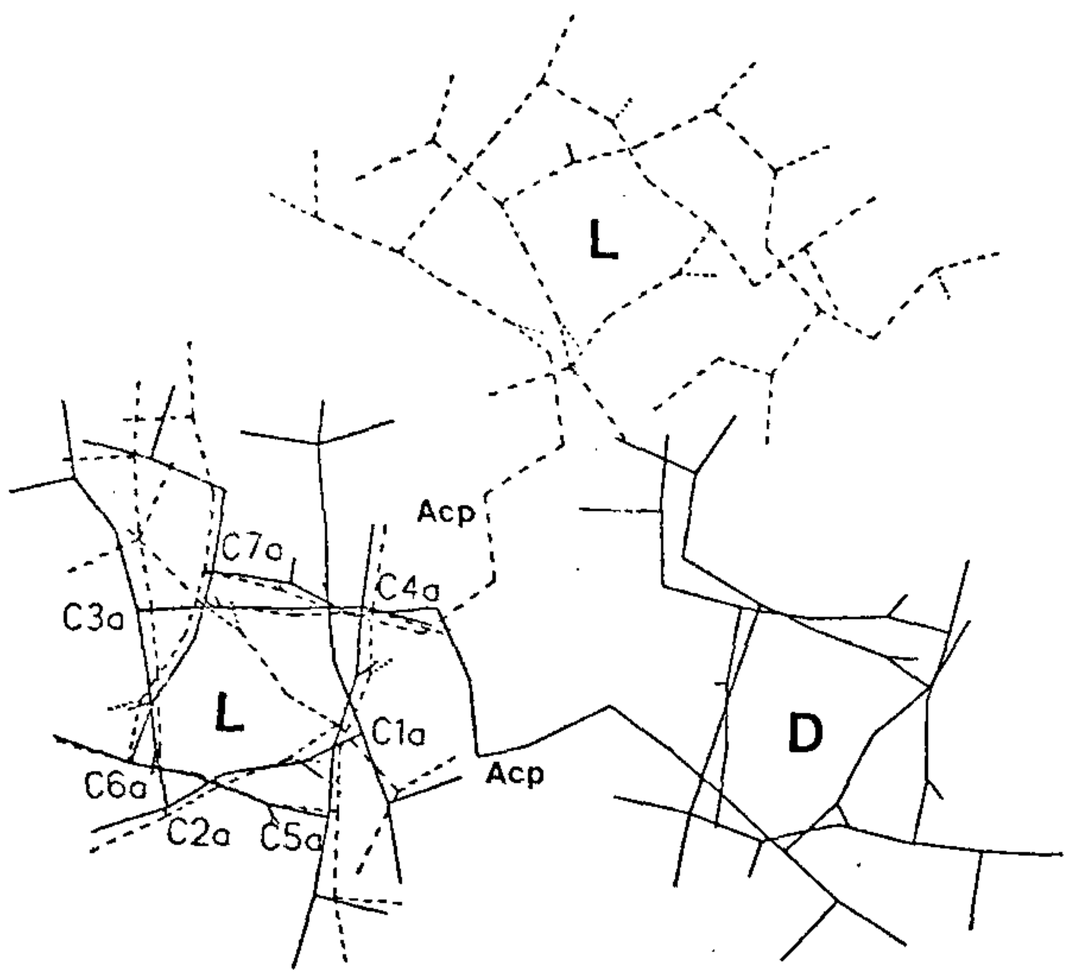


Figure 5. A comparison of peptide structures containing central Gly–Gly and δ-Ava segments (left). Conformation in crystals of Boc–Leu–Aib–Val–Gly–Gly–Leu–Aib–Val–OMe[38] (right). An NMR-derived structure for the analog Boc–Leu–Aib–Val–δ-Ava–Leu–Aib–Val–OMe[32]. The central δ-Ava residue is darkened and methylene hydrogens are indicated.

unit[6]. In both sequences, helical folding is driven by the presence of α-aminoisobutyryl (Aib) residues[37]. The additional methylene groups are comfortably accommodated within the helical fold with a corresponding change in the number of atoms in the hydrogen bonded rings (11, 12, 16 and 19-membered hydrogen bonded rings are observed). The retention of helical conformations in solution for the peptide Boc–Leu–Aib–Val–β-Ala–γ-Abu–Leu–Aib–Val–Ala–Leu–Aib–OMe[6] is evident from NMR studies in $CDCl_3$. The adoption of *gauche* conformations about C–C bonds is a key feature permitting incorporation of polymethylene units into a helical fold in both solid states and in solution[6]. While no crystal structure determinations are available for acyclic δ-Ava containing peptides, an NMR study of a model heptapeptide with a centrally-located δ-Ava residue reveals a 3_{10} helical conformation[32] (Figure 5). Figure 5 also shows the crystal structure of an octapeptide with a centrally positioned Gly–Gly segment (left panel)[38]. In this case δ-Ava, which is formally homomorphous with a Gly–Gly segment is accommodated into a helical fold with modelled dihedral angles of $\phi = -67°$, $\theta_1 = -64°$, $\theta_2 = 176°$, $\theta_3 = -59°$, $\psi = -45°$. ε-Acp has been used as a flexible linker in the synthetic construction of helix–helix motifs in peptides[39,40].



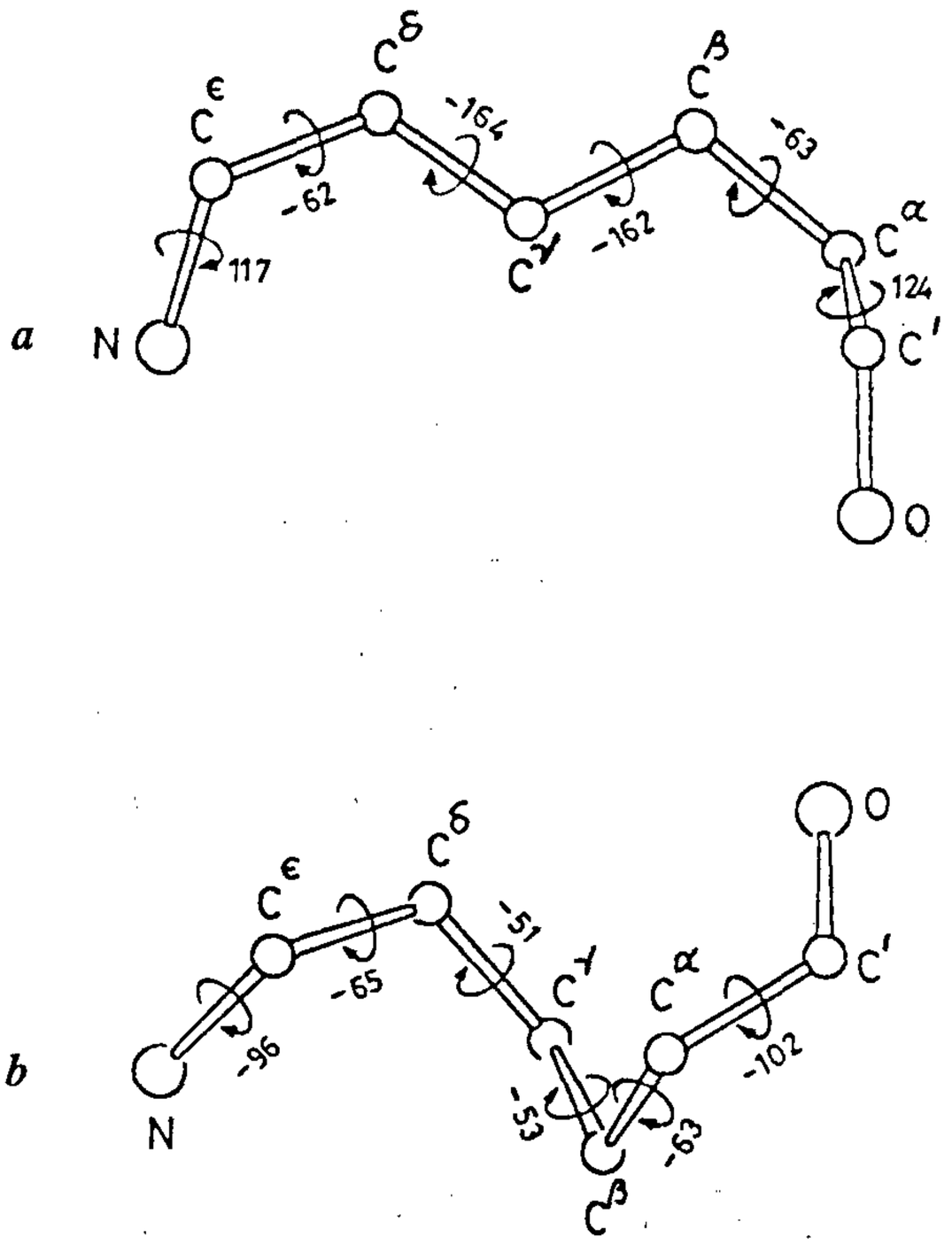


Figure 6. Two examples of crystalline peptides containing a central Acp residue[40]. A superposition of the 15-residue peptide structures Boc–Val–Ala–Leu–Aib–Val–Ala–Leu–Acp–Val–Ala–Leu–Aib–Val–Ala–Leu-(L,L-peptide, all chiral residues have the same L-configuration) and Boc–L-Val–L-Ala–L-Leu–Aib–L-Val–L-Ala–L-Leu–Acp–D-Val–D-Ala–D-Leu–Aib–D-Val–D-Ala–D-Leu–OMe (L, D peptide). The ε-Acp conformations in the two peptides are different (Table 1).

Figure 6 illustrates a comparison of structure determinations of two 15-residue peptides containing two independent helical modules, separated by an ε-Acp residue. In one case, both modules are right-handed helices formed by L-amino acids (designated as L,L-peptide) while in the other the two helical modules have opposite screw senses (designated as L,D-peptide). A superposition of these structures, viewed down the helix axis, is shown in Figure 5. In both cases the Acp linker is partially extended breaking the polypeptide chain into two segments. The conformational variability of the ε-Acp segment is clearly seen in a comparison of three different crystal structure determinations shown in Figure 7.

The examples described thus far have ω-amino acids incorporated into heteromeric sequences. The nature of the structures formed by homooligopeptides of ω-amino acids is of special interest, since new hydrogen bonding patterns unobserved in poly α-amino acids are possible. Figure 8 shows possible hydrogen bonding patterns that may be considered for helical structures formed by β-amino acid oligomers. In β-Ala itself, the central C–C bond is unconstrained and can adopt θ values of approximately ± 60° (*gauche*) and ± 180° (*trans*). Severe constraints on the value of θ may be imposed in the cyclic β-amino acids. Spectacular success in generating novel helical folds has been achieved by Gellman and coworkers as revealed by the crystal structures of

Figure 7. Observed ε-Acp conformations in three independent peptide crystal structures. *a*, 15-residue L,L peptide; *b*, 15-residue L,D peptide. Sequences in Figure 5 legend. *c*, Boc–Tyr–Ala–Leu–Aib–Val–Ala–Leu–Acp–OMe[40].

homooligomeric β-peptides of *trans* 2-aminocyclohexanecarboxylic acid, and *trans* 2-aminocyclopentanecarboxylic acid respectively[4,5]. The former peptide forms a '14-helix' with 14-membered hydrogen bonded rings (Figure 9), whereas the latter peptide adopts a '12-helix' structure, in which 12-membered hydrogen bonded rings are formed. The preservation of '12-helix' structures in solution for the oligomers of *trans* 2-aminocyclopentanecarboxylic acid is confirmed by the

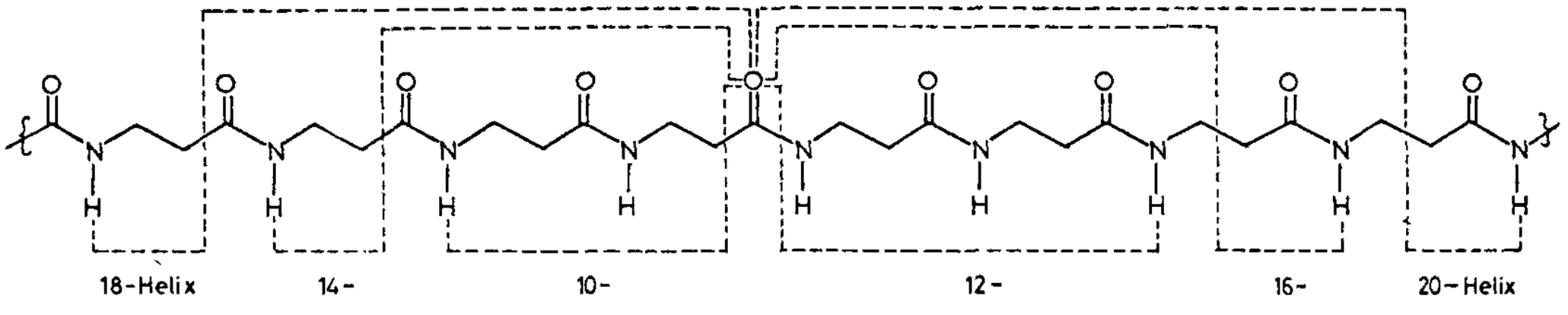


Figure 8. Schematic representation of putative intramolecular hydrogen bonding schemes in oligomeric β-alanine peptides. The numbers represent the number of atoms in the hydrogen bond ring.

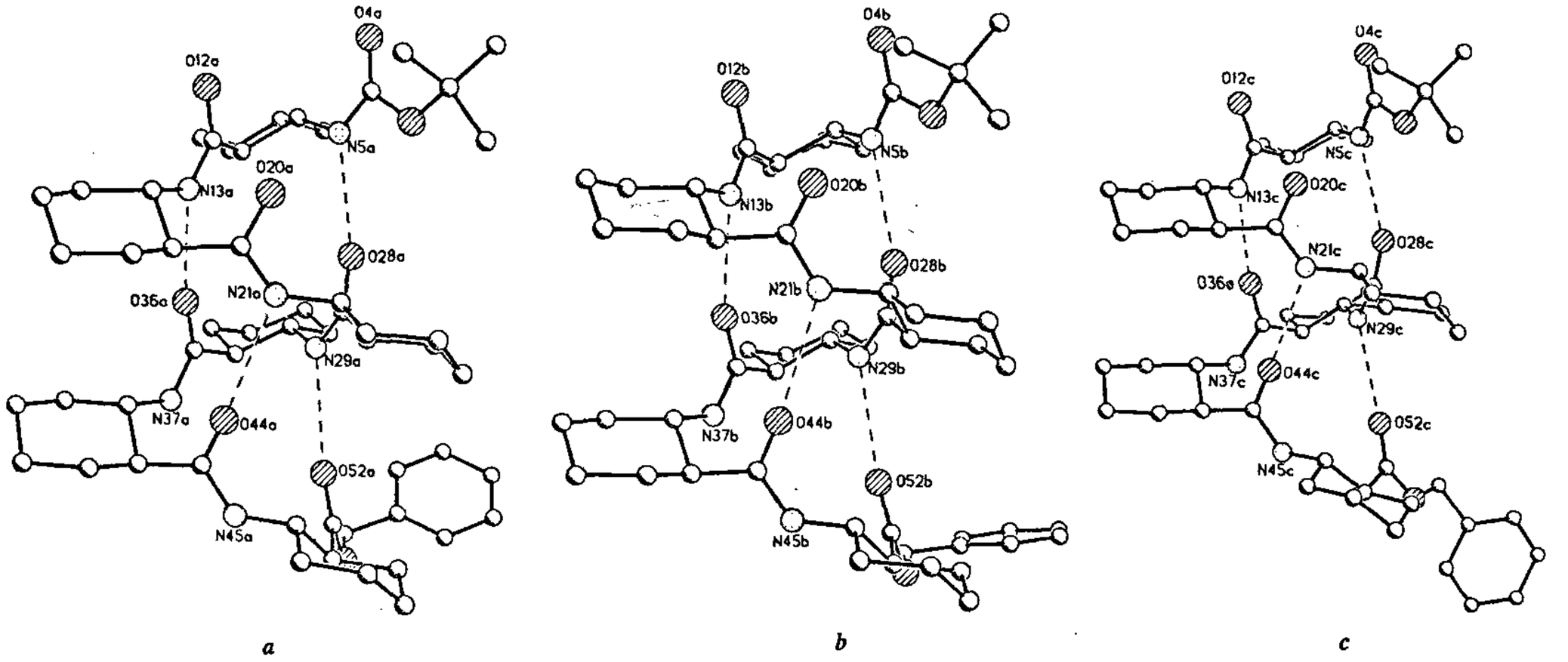


Figure 9. Three independent helical molecules observed in the crystal structure of a hexapeptide derived from *trans* 1-aminocyclohexane-2-carboxylic acid. The hydrogen bonding scheme corresponds to a '14-helix' (ref. 4).

observation of characteristic NOE data[5]. Restriction of conformational freedom about the C^{α}–C^{β} bond in acyclic β-amino acids is also imposed by substituents at the C^{α} and C^{β} atoms. The elegant work of Seebach and coworkers has provided an entry to oligomeric peptides containing chiral, substituted β-amino acids[1,41]; NMR and model-building studies clearly demonstrate helical structures. The amino acid *trans*-4-aminocyclohexane carboxylic acid, a conformationally restricted analog of δ-Ava has been used to replace the Gly(2)–Gly(3) segment in dynorphin A[42]. Crystal structures are as yet unavailable for peptides containing this residue. These recent developments suggest that ω-amino acids are of great promise in peptide design and may permit construction of novel folding units, 'foldamers'[4], in which backbone stereochemistry can be modulated in a rational manner.

Polymers of ω-amino acids

Table 2 summarizes representative studies on achiral polyamides related to nylons. The parent poly α-amino acid, poly(Gly), has been shown to exist in two structural forms. Form I is an extended β-sheet structure[43] while form II is a semi-extended collagen type helix[44,45]. Poly β-Ala, the simplest member of the nylon family, has also been shown to exist in at least two forms. X-ray powder diffraction analysis suggests that in both forms interchain hydrogen bonded sheet type structures are formed[46]. The absence of high quality fibre diffraction data limits the structural characterization of these polymeric materials. A preliminary NMR study of poly(β-Ala) was interpreted in terms of absence of ordered secondary structure in solution[47]. Munoz-Guerra and coworkers have carried out extensive analysis of the heteromeric polypeptide, poly(Gly–β-Ala)[48,49], characterizing multiple forms, all of which adopt structures stabilized by interchain hydrogen bonds. Sheet-like hydrogen bonded arrays have also been favoured for poly(ε-Acp) (nylon-6)[50]. An important point to note is that in the case of the unsubstituted poly-ω-amino acids there is no compelling evidence for *intramolecularly* hydrogen bonded helical structures, requiring folded conformations about C–C bonds, as described in the preceding section for small peptides.

Polymers of chiral, substituted ω-amino acids do provide evidence for the formation of helical folding pat-

Table 2. Polyamides related to nylons

Compounds	Methods	Comments
Poly (Gly) I	X-ray powder diffraction and IR spectroscopy	Common β-sheet type structure[91]
Poly (Gly) II	X-ray diffraction	Collagen like helix with interchain hydrogen bonds, $\phi = -78°$, $\psi = +146°$ (ref. 44); $\phi = -76°$, $\psi = +150°$ (ref. 45) for a left handed helical with a screw sense.
Poly (β-Ala) I (Nylon-3)	X-ray powder diffraction and electron microscopy	Interchain hydrogen bonded sheet type structure with all carbonyls pointing in the same direction[46]
Poly (β-Ala) II (Nylon-3)	X-ray powder diffraction and electron microscopy	Interchain hydrogen bonded sheet type structure with carbonyls pointing in the opposite directions in alternating planes of the structure[46]
Poly (β-Ala) (Nylon-3)	Proton magnetic resonance and deuterium exchange studies	Lack of any ordered secondary structure in aqueous solution[47].
Poly (Gly–β-Ala) I	X-ray (powder) and electron diffraction.	Antiparallel interchain hydrogen bonded sheet type structure[48]
Poly (Gly–β-Ala) I	Electron microscopy and X-ray (powder) diffraction	Antiparallel interchain hydrogen bonded sheet type structure[49]
Poly (Gly–β-Ala) II	X-ray (powder) and electron diffraction.	Poly glycine II/collagen like helix with interchain hydrogen bonds[48]
Poly (Gly–β-Ala) II	Electron microscopy and X-ray (powder) diffraction	Poly glycine II/collagen like helix with interchain hydrogen bonds[48]
Poly (ε-Acp) (Nylon 6)	X-ray powder diffraction	Interchain hydrogen bonded sheets[50]

terns (Table 3). The naturally occurring poly(γ-D-Glu), produced by *Bacillus subtilis* and *Bacillus anthracis* was the subject of early investigations. Based on ORD data Rydon suggested that poly(γ-D-Glu) adopts ordered helical, forms at acidic pH, when the α-carboxyl group is unionized and noted the absence of structure in the ionized form. The precise nature of the helical form has not been established although Rydon proposes several intramolecularly hydrogen bonded structures[51]. Subsequent CD studies of poly(γ-D-Glu) and the enantiomeric polypeptide poly(γ-L-Glu) have been interpreted in terms of 'hypercoiled non-regular structure' at low pH[52]. In an attempt to investigate the role of the carboxyl substituent, Kovacs *et al.* studied the co-polymer, poly-(γ-D-Glu–γ-Abu)[53]. In this case the carboxyl substituent is absent in every alternate residue. No evidence for structure formation was obtained[53]. CD studies of high molecular weight optically active poly[(S)-β-aminobutyric acid] in fluoroalcohol solvents yield spectra which resembles those obtained from β-sheet structures of poly α-amino acids[54]. CD spectra of films cast from hexafluoroacetone hydrate or hexafluoroisopropanol show an intense negative CD band at 212 nm with a positive band near 190 nm. Balasubramanian and coworkers have reported the synthesis and CD characterization of polypeptides derived from chiral ω-amino acids (e.g. L-Orn and L-Lys)[55,56]. Structure formation has been demonstrated at high pH when the α-amino groups are uncharged. CD spectra with negative bands at about 215–218 nm are observed, a feature that has been interpreted in terms of β-sheet formation. The interpretation of CD spectral features observed in poly-ω-amino acids in a manner similar to that for poly-α-amino acids is fraught with uncertainty. Differences in the arrangements of amide chromophores in these two types of systems may complicate detailed interpretation. The poly(α-alkyl-β-aspartates) are the best characterized polymers of β-amino acids. In a series of detailed investigation Munoz-Guerra, Subirana, Alleman and coworkers have used fibre diffraction data, polarized IR spectroscopy, molecular mechanics and quantum chemical calculations to characterize the nature of helical structures that are formed in these derivatives of nylon-3 (ref. 57–63). While poly (β-Ala), nylon-3, forms extended structures, the α-substituent in poly(β-L-Asp) promotes helical folding. The extensive studies of the Spanish group demonstrate that the poly(α-alkyl-β-L-aspartates) can adopt helical structures which are topologically similar to the α-helix formed by α-amino acids. The most stable structure is the 13/4 arrangement, which contains 3.25 residues per turn. Intramolecular hydrogen bonds are formed between NH_i and CO_{i+2}, yielding 14 atoms within the hydrogen-bonded ring. Although both right- and left-handed structures are stereochemically possible, the right handed-screw sense appears to be favoured[63]. The '13/4-helix' has the conformational parameters $\phi = -146.2°$, $\xi\ (\theta) = -59.8°$ and $\psi = 128.8°$ and is practically identical to the '14-helix' observed in the crystal structure of the β-hexapeptide[4] derived from *trans*-2-aminocyclohexanecarboxylic acid (Figures 9 and 10)[4]. An important feature of this structure is that N-terminus NH groups interact with C-terminus CO groups, yielding a hydrogen bond and macroscopic dipole moment directionality opposite to that in poly-α-amino acid helices. Poly-β-amino acids are structurally more versatile, being capable of adopting energetically favourable structures with both types of hydrogen bonding schemes. Two alternate helical forms, a 17/4 helix and 4/1-helix (an ideal integral helix with 4 residues per turn) are also energetically feasible structures.

Table 3. Chiral ω-polypeptides derived from trifunctional amino acids

Polymers (sequences)	Methods used for structure determinations	Comments
Poly(γ–D-Glu)	ORD (a) Un-ionized form: $a_o = 295°$, $b_o = -226°$ $\lambda_o = 197$ mμ (b) ionized form: $a_o = -111°$, $\lambda_c = 222$ mμ	(a) helical in un-ionized state and two types of structures are proposed, (b) unordered structure in ionized form[51]
Poly(γ–L-Glu)	CD: (a) at pH 7: $\lambda_{max} = 217$nm, $[\theta] = 2.7 \times 10^3$ at cross over point $\lambda = 207$ nm, at $\lambda_{max} = 190$ nm, $[\theta] = -10 \times 10^3$ (b) at pH 2: $\lambda_{max} = 201$ nm, $[\theta] = 12 \times 10^3$ at cross over point $\lambda = 195$ nm, at $\lambda_{max} = 190$ nm, $[\theta] = -25 \times 10^3$	(a) unordered structure at high pH and (b) hypercoiling of nonregular random coil structure are proposed at low pH[52]
Poly(γ–D-glutamyl-γ-aminobutyric acid)	CD at pH 2: $\lambda_{max} = 212$ nm, $[\theta]_M = \approx -14 \times 10^3$	unordered structure[53]
Poly [(S)-β-aminobutyric acid]	CD at 70% aqueous HFA: $\lambda_{max} = 212$ nm, $[\theta]_M = \approx -48 \times 10^3$ and $\lambda_{max} = 191$ nm $[\theta]_M = \approx 42 \times 10^3$	β-sheet type structure[54]
Poly(δ–L-ornithine)	CD at pH 12, $\lambda_{max} = 215$ nm, residue molar ellipticity $= -1.5 \times 10^3$ cross over point $\lambda = 205$ nm	β-pleated sheet conformation; the decrease in intensity of the negative band at 215 nm with the lowering of pH indicates pH dependent conformational transition[55]
Poly(N^{α}-Boc–δ–L-ornithine)	CD in methanol: $\lambda_{max} = 217$ nm, residue molar ellipticity $= -5 \times 10^3$ and at $\lambda_{max} = 203$ nm, residue molar ellipticity $= -8.6 \times 10^3$	Partially (40%) β-pleated sheet type folding is proposed[55]
Poly(ε-L-lysine)	CD (a) at high pH there is a negative band centered at 215 nm and the cross over point is 205 nm. (b) at pH <4 only one positive band centered around 192 nm and $[\theta]_M = 11 \times 10^3$	(a) Antiparallel β-sheet type conformation is preferred at high pH. (b) At low pH the structure is not typical random coil, but, there may be an electrostatically expanded chain due to the repulsion between the charged α-NH_3^+ side chain groups[56]
Poly(β-L-Asp)	CD at pH 12 doubly dichroic spectrum was observed with a small positive band at 218 nm ($[\theta]_M = 2.5 \times 10^3$) and a large negative band near 200 nm ($[\theta]_M = -15 \times 10^3$)	Un-ordered structure[57]
Poly(α-isobutyl-L-Asp)	Fibre X-ray diffraction and model-building studies	Left-handed helical structure with 3.25 units per turn (13/4-helix)[58]
Poly(α-isobutyl-L-Asp)	Fibre X-ray diffraction analysis and model-building studies + CD (in TFE: a negative peak at 189 nm and a positive band at 205 nm	Two types of left handed helical structures were postulated: in one case 3.25 residues per turn and the other helix has 4 residues per turn[59]
Poly(α–isobutyl-L-Asp)	Model-building studies based on the fibre diffraction data previously obtained	Right-handed helical structures were proposed[60]
Poly(α-n-butyl-β-L-aspartate)	Molecular mechanics calculations and linked-atom-least-squares (LALS) refinements using X-ray fibre diffraction data	Two crystalline forms were found depending upon the preparation of the sample. For the hexagonal form a right-handed 13/4-helix (13 residues in 4 turns) composed of 14-membered hydrogen bonded rings was favoured while for the tetragonal form a right-handed 4/1-helix (4 residues per turn) was preferred[61]
Poly(α-alkyl-β-L-aspartate)s	Polarized IR spectroscopic analysis (in polyethylene oxide)+ model building based on fibre X-ray diffraction data	Right-handed 13/4-helical structure most favoured over the other possible helical structures[62]
Poly(α-alkyl-β-L-aspartate)s	Quantum chemical calculations	A right-handed helical conformation involving hydrogen bonds between NH groups of ith residues and C=O groups of $i + 2$ residues is the most stable[63]

Polyamides derived from poly(β-L-Asp) bearing long paraffinic sidechains are of special interest, because they give rise to 'comb like' polymers exhibiting novel physical properties. The fusion of rigid cylindrical backbones, with potentially fluid hydrocarbon sidechains, can give rise to interesting thermotropic properties[64].

Prospects

Insertion of ω-amino acids selectively into peptides composed of α-amino acids permits the design of useful analogs, in which potentially scissile peptide bonds are replaced by C–C linkages. The ability of polymethylene chains to be accommodated into classical helical peptide

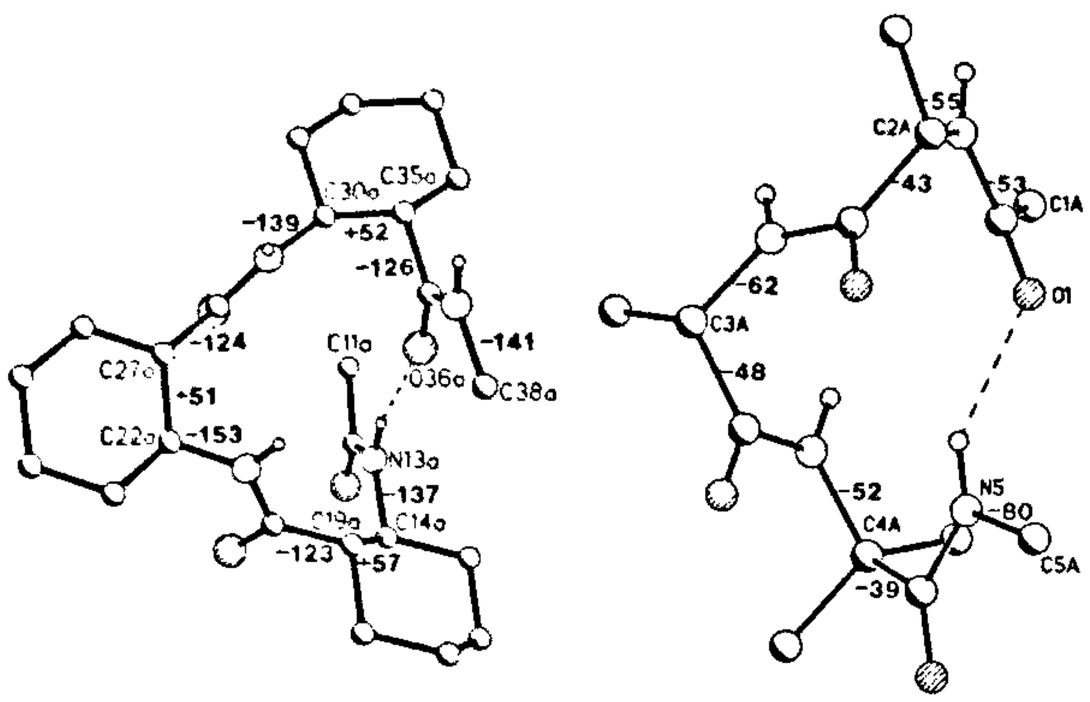


Figure 10. A comparison of the hydrogen bonding schemes and backbone dihedral angles in a single hydrogen bonded helical turn in (left) a 'β-foldamer' 14-helix and (right) α-helix. (I. L. Karle, personal communication).

folding patterns augurs well for design of proteolytically stable analogs of important peptides in which helical conformations are critical for biological activity. Peptide structures in which peptide units are replaced by CH_2–CH_2 linkages also allow investigations of the effect of intramolecular hydrogen bonds on secondary structure stability. The use of ω-amino acids of variable chain length and the use of such residues in sequential positions, e.g. β-Ala–γ-Abu or γ-Abu–β-Ala, will permit the alteration of registry of hydrogen bonds in secondary structures. Oligomeric sequence of chiral, substituted ω-amino acids are likely to yield important new information on polypeptide folding patterns. The recent success in generation of novel folds using oligomers of chiral substituted β-amino acids is a pointer in this direction. Very convenient stereoselective synthetic routes for all four stereoisomers of α- and β-substituted acyclic β-amino acids have recently been reported[65]. Considerable recent activity on the synthesis of chiral β-peptides, including procedures for Arndt–Eistert homologation in the solid phase[66] promises to quickly provide entry to several classes of new structures. Stereoselective synthesis of γ-substituted γ-amino acids from the corresponding α-amino acids[67] holds future promise for the construction of peptides containing substituted, chiral γ-amino acids. Schreiber and coworkers reported '10-helix', '12-helix' and antiparallel β-sheet type structures in peptides containing α,β-unsaturated γ-amino acids[68]. The flurry of recent activity in structure determination of peptides containing ω-amino acids, together with advances in synthetic methodology, suggests that several new folding patterns will soon be characterized for these systems. While initial studies on unsubstituted polyamides provided evidence only for extended polypeptide chains, aggregating into sheet-like structures, introduction of backbone substituents appears to facilitate folding into new types of helical structures. Although most recent work has focused on β-amino acids, it is clear that much uncharted territory remains to be explored in the case of the higher ω-amino acids.

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