REVIEW

Aib Residues in Peptaibiotics and Synthetic Sequences: Analysis of Nonhelical Conformations

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The α-aminoisobutyric (Aib) residue has generally been considered to be a strongly helicogenic residue as evidenced by its ability to promote helical folding in synthetic and natural sequences. Crystal structures of several peptide natural products, peptaibols, have revealed predominantly helical conformations, despite the presence of multiple helix-breaking Pro or Hyp residues. Survey of synthetic Aib-containing peptides shows a preponderance of 3_{10}-, α-, and mixed $3_{10}/\alpha$-helical structures. This review highlights the examples of Aib residues observed in *nonhelical* conformations, which fall 'primarily' into the polyproline II (P_{II}) and fully extended regions of conformational space. The achiral Aib residue can adopt both left (α_L)- and right (α_R)-handed helical conformations. In sequences containing chiral amino acids, helix termination can occur by means of chiral reversal at an Aib residue, resulting in formation of a *Schellman* motif. Examples of Aib residues in unusual conformations are illustrated by surveying a database of Aib-containing crystal structures.

1. Introduction. – The achiral residue α-aminoisobutyric acid (Aib) has been found as a major constituent of nonribosomally synthesized fungal peptides [1–6]. The term 'peptaibiotics' has been introduced to describe this class of peptide natural products, which contains the Aib residue and possess antibiotic activities [7]. The term 'peptaibol' has been reserved as a descriptor of linear Aib-containing peptides of natural origin, which contain a C-terminal alcohol group [8]. Alamethicin [9] and antiamoebin [10] were among the earliest group of natural peptides in this family to be chemically characterized and widely studied. The advent of fast-atom-bombardment mass spectrometry (FAB-MS) in the 1970s resulted in the determination of the sequences of many families of microheterogeneous, Aib-containing acyclic peptides [11]. The more recent methods of liquid chromatography coupled with electrospray mass spectrometry (LC/ESI-MS) have resulted in an explosive growth in the number of sequences of Aib-containing peptides of natural origin [12].

The unusual conformational properties of Aib residues were first anticipated by theoretical analysis in the early 1970s [13]. The presence of geminal dialkyl substituents at the tetrahedral C(α)-atom imposes major steric restrictions on the energetically accessible conformational space, mostly limiting the Aib residue to the helical region of the *Ramachandran* map ($\phi = \pm 60^\circ \pm 20^\circ$; $\psi = \pm 30^\circ \pm 20^\circ$) [14]. *Fig. 1* illustrates a

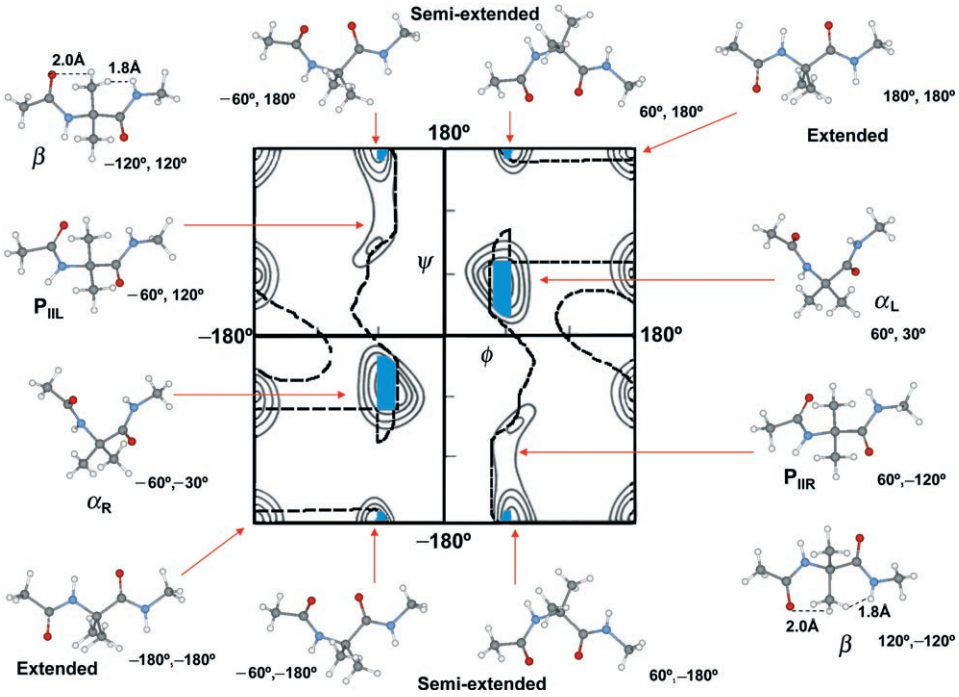

Fig. 1. *Superposition of the allowed region of the ϕ,ψ space determined by hard sphere criteria Ac-Aib-NHMe and conformational energy map for an Aib residue.* The allowed conformations for Aib residues are illustrated.

conformational energy map for Aib, computed using classical potential-energy functions [15]. The deepest minima lie in the right- and left-handed $3_{10}/\alpha$-helical regions of the ϕ,ψ space. These minima correspond to the region of overlap obtained by superposition of the *Ramachandran* maps for L-Ala and D-Ala residues [13]. In addition to the helical regions, minima are also observed in the fully extended and semi-extended regions of the ϕ,ψ space. Conformations corresponding to β-strand structures ($\phi \approx -120^\circ$, $\psi \approx 120^\circ$) are strongly disallowed because of short contacts between one of the Me groups at C(β) and the C=O group of the preceding residue, and the NH group of succeeding residue. These expectations were first realized in the structure determination of small synthetic Aib-containing peptides [16–18], which provided definitive experimental evidence for the ability of this unusual residue to stabilize helical conformations [19]. Over the last three decades, a large body of experimental evidence has accumulated establishing the prominent role of Aib residue in stabilizing helical folding in peptides. Although there has been considerable discussion in the literature on the distinction between 3_{10}- and α-helical conformation of Aib-containing peptides [20], in this review we consider both these helical subtypes as closely related structures. There is, however, a limited number of examples in which Aib residues adopt semi-extended polyproline II (P_{II}) conformations. The achiral nature of Aib also permits this residue to adopt ϕ,ψ values corresponding to both right (α_R)- and left (α_L)-

handed conformations. The choice of the handedness of the helix is generally determined by the chirality of the residues in peptide sequences which contain the protein amino acids and by the positioning of the Aib residue. In a large number of peptide helices, the C-terminal Aib often adopts a helix handedness, which mirrors that of the body of the peptide. This review summarizes structural studies on natural peptaibiotics and focuses attention on unusual conformations observed in synthetic peptides.

2. Conformational Distributions in Peptide Crystal Structures. – The experimentally determined peptaibiotics structures are listed in *Table 1* [4][21–43] and the molecular conformations are illustrated in *Figs. 2–4*. *Fig. 5*, *a*, provides a scatter plot for Aib residue conformations in the peptaibiotic structures. It is seen that 112 out of 113 Aib residues cluster in the right-handed-helical region of the ϕ,ψ space establishing that nonhelical conformations of Aib are highly improbable in this group of peptide natural products. A single case has been reported for the structure of antiamoebin I by *Snook et al.*, with Aib(2) having conformational angles of $\phi = -137.1^\circ$, $\psi = -53.9^\circ$ [25]. Interestingly, disorder of the first five residues in one of the two molecules in the crystallographic asymmetric unit has been noted, with the data collected up to 1.4 Å. In another structure of antiamoebin I reported in the literature, at a resolution of 1.0 Å, Aib(2) has a torsion angle of $\phi = -56.5^\circ$, $\psi = -43.4^\circ$ [26]. Curiously, the first example of the crystal structure of an Aib-containing natural product was the cyclic tetrapeptide dihydrochlamydocin in which the Aib residue has $\phi = 71.9^\circ$, $\psi = -63.7^\circ$, corresponding to the C_7/γ-turn region of the *Ramachandran* map [46].

In synthetic peptides, Aib residues can be placed in achiral sequences, or in sequences containing amino acids of one hand, or sequences containing residues of mixed chirality. *Fig. 5*, *b*, shows a scatter plot of Aib residues in both natural and synthetic, acyclic (linear) peptide sequences. For cases of achiral sequences crystallizing in centric space groups, the right-handed sense (α_R) has been arbitrarily chosen. An interesting feature of this plot is the observation of the clusters in the regions corresponding to P_{IIL} ($\phi \approx -60^\circ$, $\psi \approx 120^\circ$) and P_{IIR} ($\phi \approx 60^\circ$, $\psi \approx -120^\circ$) conformations. In addition, several of the examples in the left-handed helical (α_L) region correspond to Aib residues occurring in a helix terminating position. *Fig. 5*, *c*, shows a representation of all Aib residues observed in a nonhelical context. C-Terminal Aib residues, which do not participate in determining the fold of the peptide have been excluded. Of the 40 examples, 16 correspond to α_L conformations occurring in the helix-terminating *Schellman* motif [47]. *Fig. 5*, *d*, shows the distribution of Aib residues in cyclic peptides. The covalent constraint of cyclization can force Aib residues into conformations, which have not been observed in acyclic sequences.

3. Polyproline II (P_{II}) Conformations. – The semi-extended polyproline II (P_{II}) conformation is often observed in protected dipeptides and tripeptides. *Fig. 6* provides a view of the molecular conformations established in crystals for Aib residues in the P_{II} region of the ϕ,ψ space [48–59]. In the case of peptides **1**–**8**, the Aib residue occupies the $i+1$ position of a type II/II′ β-turn conformation. In Ac-Aib-Gly-Aib-OH (**3**), a type-III β-turn conformation corresponding to a single 3_{10}-helical turn might have been anticipated with Aib(1) adopting an α_R conformation. However, the observed structure

Table 1. *Peptaibiotic Structures*

Entry	Sequence (PDB or CCDC code) space group[a])	No. of residues	Method	Comment	Reference
1	Alamethicin I (1AMT) $P2_1$ Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe-OH	20 3 molecules in the asymmetric unit	X-Ray 1.5 Å	Bend angle ~33° Alamethicin F 30 is a mixture of two components, F 30 I and II	[4]
2	Leucinostatin (JATZIN) $P2_12_12_1$ MHA-MePro-Dec-HyLeu-Aib-Leu-Leu-Aib-Aib-β-Ala-DPDA	11 1 molecule in the asymmetric unit	X-Ray	α-Helix	[21]
3	Leu1-Zervamicin (KIYPUD) $P2_1$ Ac-Leu-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH·10 H_2O	16 1 molecule in the asymmetric unit	X-Ray 0.93 Å	Bend angle ~36°	[22]
4	Leu1-Zervamicin (–) $P2_1$ Ac-Leu-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH·6.5 H_2O	16 1 molecule in the asymmetric unit	X-Ray 1.09 Å	Bend angle ~26°	[23]
5	Leu1-Zervamicin (–) $P2_12_12_1$ Ac-Leu-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH·5.5 $H_2O \cdot C_2H_5OH$	16 1 molecule in the asymmetric unit	X-Ray 1.2 Å	Bend angle ~47°	[24]
6	Antiamoebin I (1JOH) $P1$ Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phe-OH	16 2 molecules in the asymmetric unit	X-Ray 1.4 Å	Bend angle ~56° for Chain A and 48° for Chain B	[25]
7	Antiamoebin I (FEJQOA) $P2_12_12_1$ Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phe-OH·3-octan-1-ol	16 1 molecule in the asymmetric unit	X-Ray 1.0 Å	Bend angle ~39–48° [b])	[26]
8	Peptaibolin (SUWKEA) $P2_1$ Ac-Leu-Aib-Leu-Aib-Phe-OH	5 1 molecule in the asymmetric unit	X-Ray	–	[27]
9	Trichotoxin A50E (1M24) $P1$ Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Gln-Val-OH	18 2 molecules in the asymmetric unit	X-Ray 0.9 Å	Bend angle ~8° for Chain A and 10° for Chain B	[28]
10	Ampullosporin A (BEKZIB) $P2_1$ Ac-Trp-Ala-Aib-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Gln-Leu-Aib-Gln-Leu-OH	15 1 molecule in the asymmetric unit	X-Ray 0.77 Å	Bend angle ~53–64° [b])	[29]
11	Cephaibol A (1OB4/ETUYOH) $P2_12_12$ Ac-Phe-Aib-Aib-Aib-Aib-Gly-Leu-Iva-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phe-OH	16 1 molecule in the asymmetric unit	X-Ray 0.91 Å	Bend angle ~55°	[30]

Table 1 (cont.)

Entry	Sequence (PDB or CCDC code) space group[a])	No. of residues	Method	Comment	Reference
12	Cephaibol B (1OB6/ETUYUN) $P2_1$ Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Iva-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phe-OH	16 2 molecules in the asymmetric unit	X-Ray 0.89 Å	Bend angle ~55°	[30]
13	Cephaibol C (1OB7/ETUZAU) $P2_12_12_1$ Ac-Phe-Aib-Aib-Aib-Aib-Gly-Leu-Div-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH	16 1 molecule in the asymmetric unit	X-Ray 0.89 Å	Bend angle ~55°	[30]
14	Chrysopermin C (1EE7) Ac-Phe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Aib-Aib-Aib-Gln-Trp-OH	19 (average structure)	NMR	Bend angle ~38°	[31]
15	Zervamicin IIB (1DLZ) Ac-Trp-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH	16 20 models (isotropic solvent)	NMR	Bend angle ~47°	[32]
16	Zervamicin IIB (1IH9) Ac-Trp-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH	16 20 models (bound to DCP micelles)	NMR	Bend angle ~23°	[33]
17	Antiamoebin I (1GQ0) Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phe-OH	16 20 models (MeOH)	NMR	–	[34]
18	Zervamicin IIB (1R9U) Ac-Trp-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phe-OH	16 24 models (MeOH)	NMR	Bend angle ~20°	[35]
19	Trichorzianine A III C (−) $C222_1$ Ac-Aib-Ala-Ala-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Ile-Gln-Gln-Trp-OH	19 1 molecule in the asymmetric unit	X-Ray 0.9 Å	Coordinates not available	[36]
20	Trichogin A IV (MORHUW) $P\bar{1}$ Oc-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OH	11 2 molecules in the asymmetric unit	X-Ray 0.9 Å	Coordinates not available	[37]
21	Tricholongins BI Ac-Aib-Gly-Phe-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Leu-OH	19	NMR	–	[38]
22	Tricholongins BII Ac-Aib-Gly-Phe-Aib-Aib-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Iva-Gln-Gln-Leu-OH	19	NMR	–	[38]

Table 1 (cont.)

Entry	Sequence (PDB or CCDC code) space group[a])	No. of residues	Method	Comment	Reference
23	Trichorzianin TA VII Ac-Aib-Ala-Ala-Aib-Iva-Gln-Aib-Aib-Aib-Ser-Leu-Aib-Pro-Val-Aib-Ile-Gln-Gln-Phe-OH	19	NMR	Bend angle ~90–100°	[39]
24	Alamethicin analogue (CCDC-604861) *C2* Ac-Aib-Pro-Aib-Ala-Aib-Ala-Glu(OMe)-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-TOAC-Aib-Glu(OMe)-Glu(OMe)-Phe-OH	20 2 molecules in the asymmetric unit	X-Ray 0.95 Å	–	[40]
25	Trichodecenin I (NUPLIT) $P2_1$ Z-Gly-Gly-D-Leu-Aib-Gly-D-Ile-D-Leu-OMe	7 2 molecules in the asymmetric unit	X-Ray 0.87 Å	–	[41]
26	Trichogin GA IV analogue (SASKON) $P2_12_12_1$ Boc-Bpa-Aib-Gly-Leu-Aib-Gly-Gly-Leu-TOAC-Gly-Ile-Leu-OMe	12 2 molecules in the asymmetric unit	X-Ray 0.82 Å	For analogues of trichogin GA IV synthesized and studied, see [42]	[43]

[a]) All Iva residues are in D-configuration. [b]) Taken from http://www.imb-jena.de/peptaibols/BENDING_TABLE.html.

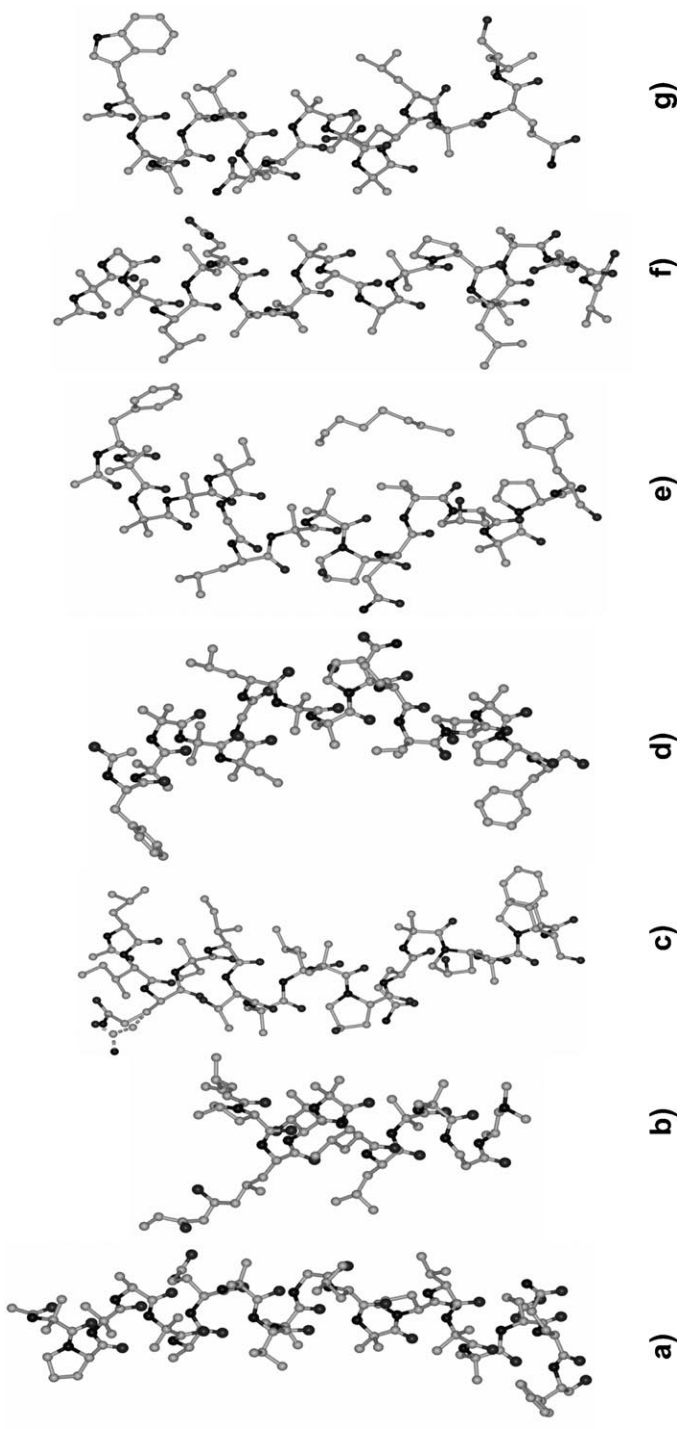

Fig. 2. *Crystal structures of peptaibiotics. a*) Alamethicin [4]. *b*) Leucinostatin [21]. *c*) Leu-Zervamicin [22]. *d*) Antiamoebin [25]. *e*) Antiamoebin [26]. *f*) Trichotoxin A50E [28]. *g*) Ampullosporin A [29]. Wherever multiple molecules are present in the asymmetric unit, only one molecular structure is shown. The figures are drawn form the coordinates obtained from the *Protein Data Bank* (*PDB*) [44] or *Cambridge Crystallographic Data Centre* (*CCDC*) [45].

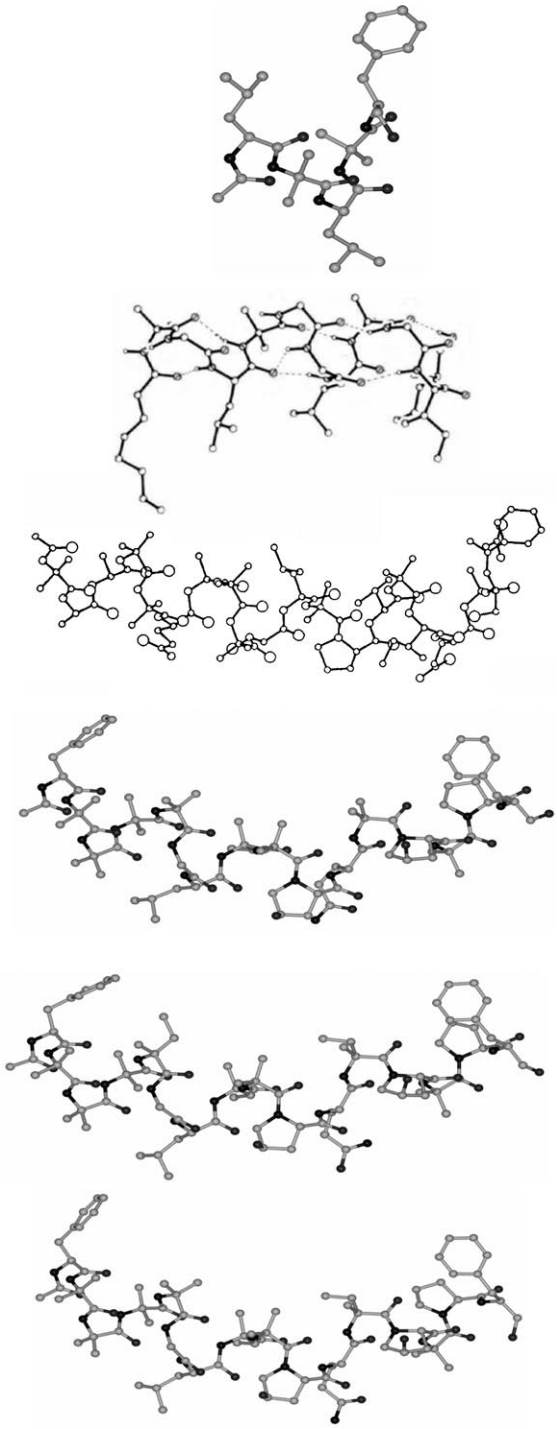

Fig. 3. *Crystal structures of peptaibiotics. a*) Cephaibol A [30]. *b*) Cephaibol B [30]. *c*) Cephaibol C [30]. *d*) Trichorzianine A III C [36] (reproduced from [19b]). *e*) Trichogin A IV [37]. *f*) Peptaibolin [27]. *a*–*c* and *f* are drawn form the coordinates, while *d* and *e* are reproduced from the articles.

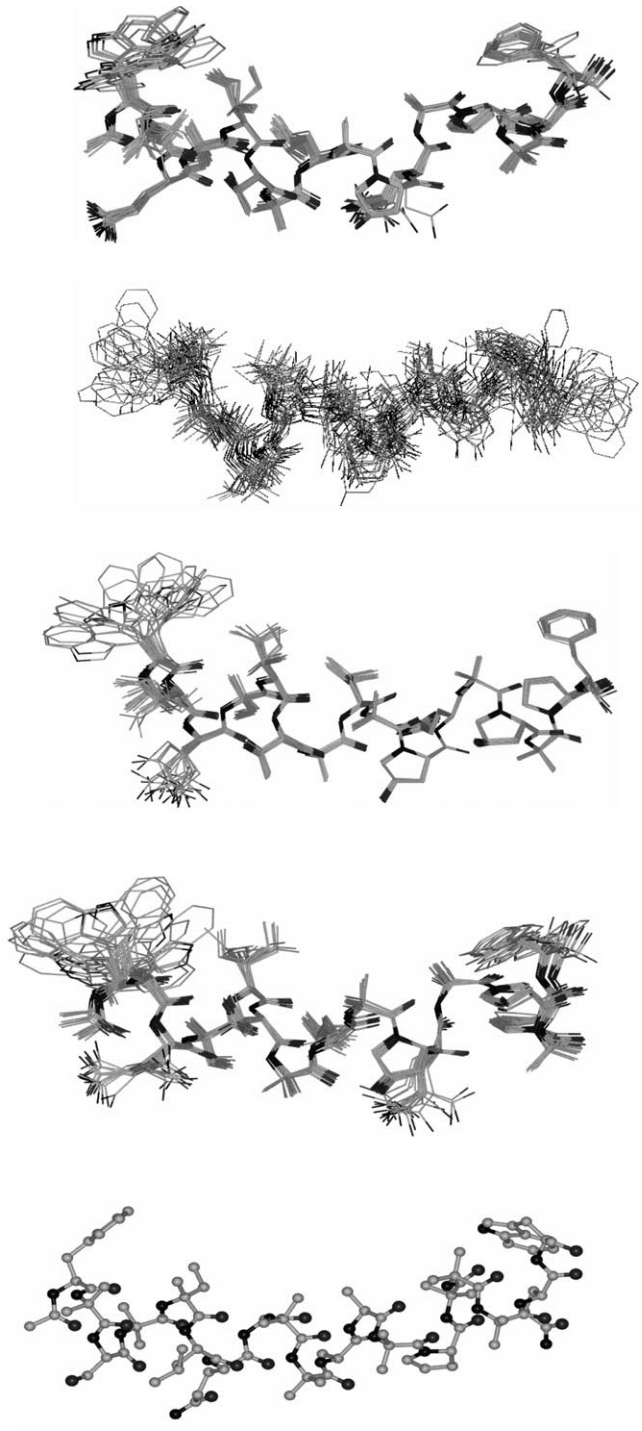

Fig. 4. *NMR structures of peptaibols. a*) Chrysospermin [31]. *b*) Zervamicin IIB [32]. *c*) Zervamicin IIB [33]. *d*) Antiamoebin I [34]. *e*) Zervamicin IIB [35]. The figures are drawn from the coordinates obtained from PDB. In *a*, the average structure is shown, while in *b*–*e* the structure consistence with the NMR data is shown.

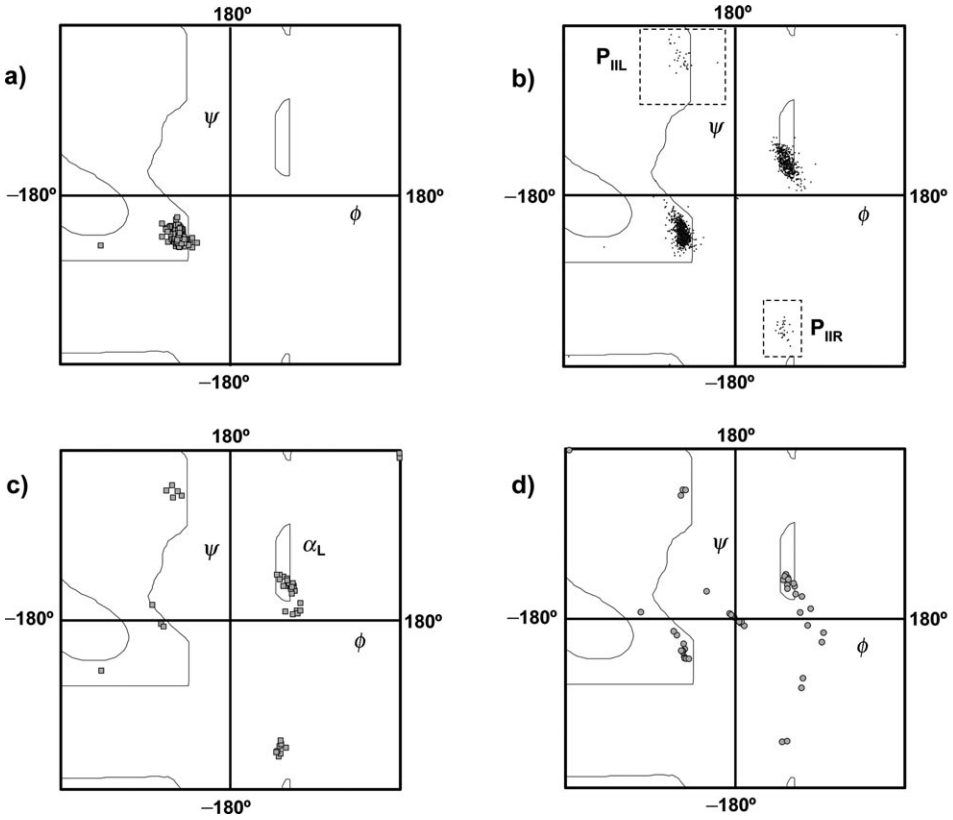

Fig. 5. *Distribution of Aib residues in the conformational space. a*) Peptaibiotics. *b*) Natural and synthetic acyclic (linear) peptides. *c*) Nonhelical and left-handed peptides. *d*) Cyclic peptides.

reveals Aib(1) in a P_{II} conformation [50]. A similar situation is observed in the case of peptides **4** [51] and **5** [52]. Interestingly, in the case of dipeptide esters, Boc-Aib-Ac_6c-OMe (**1**) [48] and Tfa-Aib-Aib-O^{t}Bu (**2**) [49], which cannot form a $4\rightarrow1$ H-bond because of the absence of the NH group at the C-terminus, the molecule adopts a quasi type-II′ β-turn with the ester O-atom occupying the position of the NH group. The observed O···O distances for the $4\rightarrow1$ interaction are 3.46 and 3.58 Å, respectively. In the other examples illustrated, Aib residues adopt the P_{II} conformation despite the absence of any significant intramolecular interactions. These examples suggest that the energy difference between α_R and P_{II} minima, which are estimated to be *ca*. 3.5 kcal/mol from theoretical calculations, can be compensated by intermolecular interactions in crystals.

4. Distorted Conformations in the Helical Region. – Conformations of Aib residues which lie in the bridge region of the *Ramachandran* map close to the right (α_R)- and left (α_L)-handed helical conformations ($\psi\approx0^\circ$) are not uncommon. Some examples are illustrated in *Fig. 7* [60–64]. In all these cases, the observed ψ angle lies between -7° and 16°.

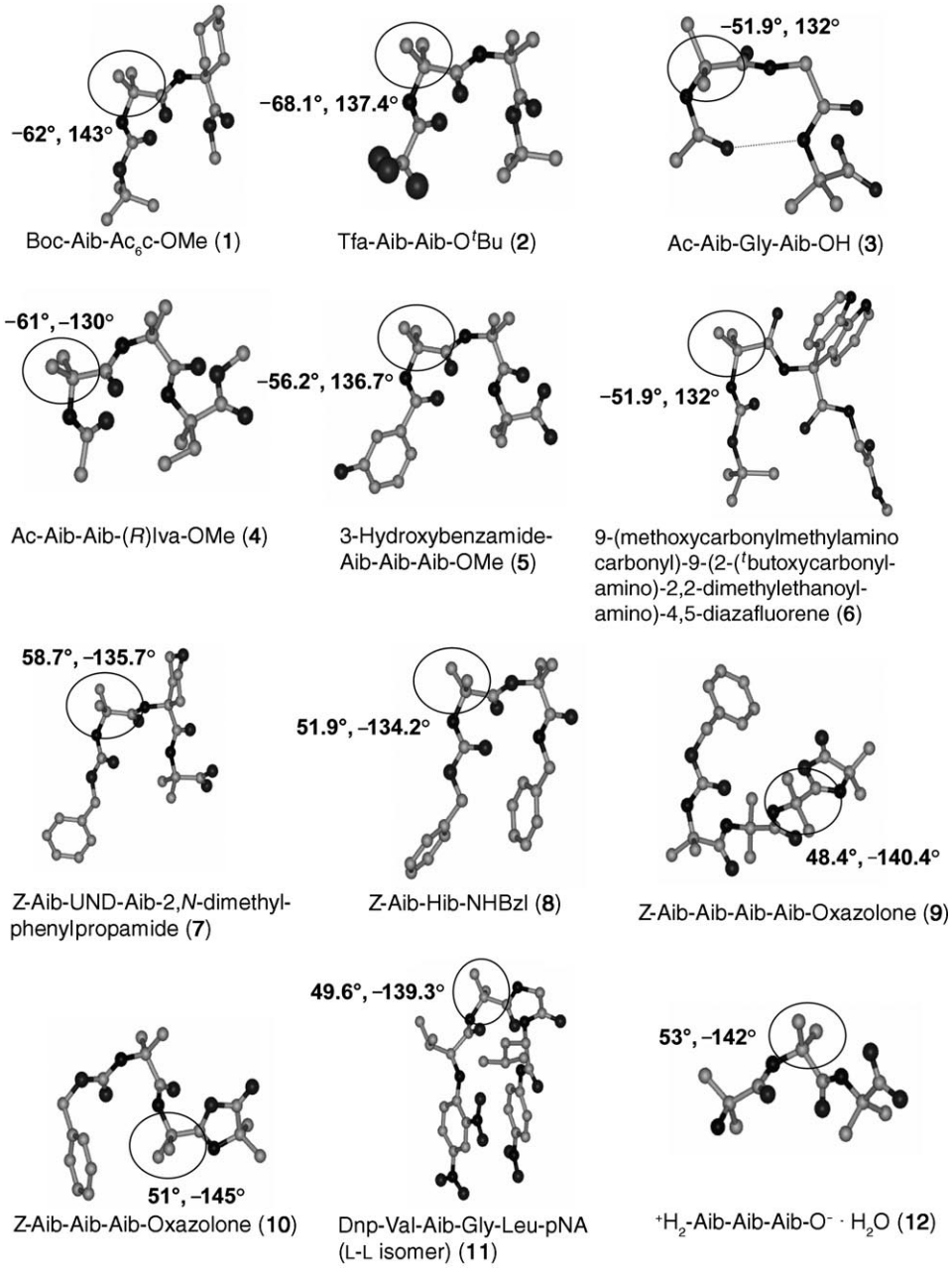

Fig. 6. *Examples of Aib residues which adopt polyproline II* (P_{II}) *conformations.* The ϕ, ψ values are indicated.

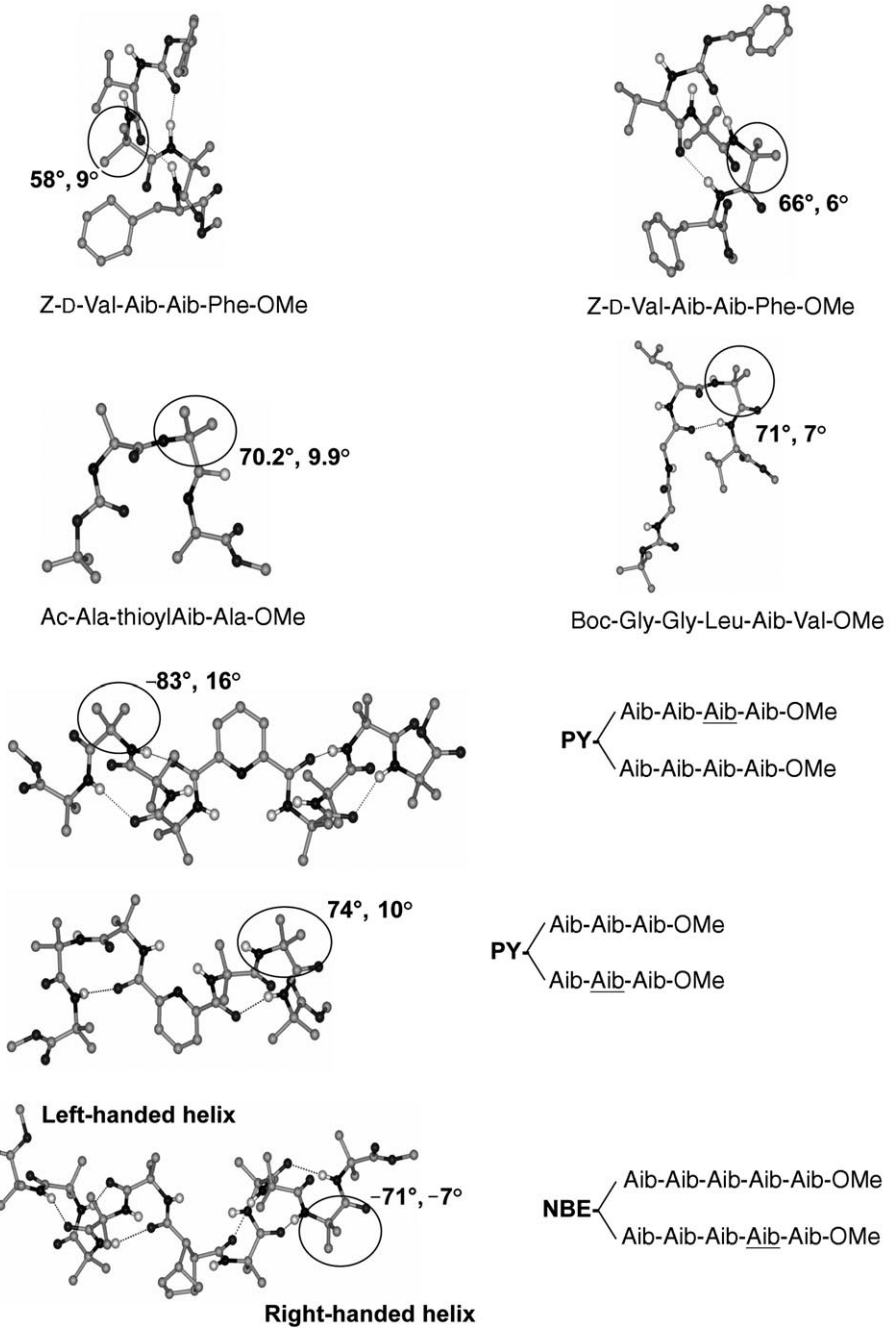

Fig. 7. *Crystal structures of Aib containing peptides, where Aib-residue* ϕ,ψ *values fall in the bridge region* ($\psi \approx 0^{\circ}$) *of the* Ramachandran *map*

5. Extended Conformations. – Two examples of fully extended Aib conformations have been observed so far (*Fig. 8*). In both these cases, $\phi \approx 180°$, $\psi \approx 180°$ [63][65]. Although, the fully extended regions are sterically and energetically permissible for Aib residues, these conformations are rarely observed. In contrast, several examples of fully extended conformation have been characterized for higher homologues of Aib, containing symmetrical dialkyl substituents, like diethylglycine (Deg), dipropylglycine (Dpg) and dibutylglycine (Dbg) [66]. In the cases of Deg, Dpg, and Dbg, both α_R and extended conformations have been crystallographically observed [67]. In the case of α_R conformations the observed tetrahedral bond angle $\tau(N-C(\alpha)-C')$ is *ca.* 110°, while in the fully extended conformation τ is *ca.* 104°. In the case of the Aib peptides shown in *Fig. 8*, the observed bond angle τ is *ca.* 104°. This may be compared to the standard bond angle observed for Aib residue in helical conformations ($\tau \approx 111°$) [17]. Interestingly, fully extended conformations have recently been reported even for chiral *C(α)*-ethylated dialkyl amino acid residues [68].

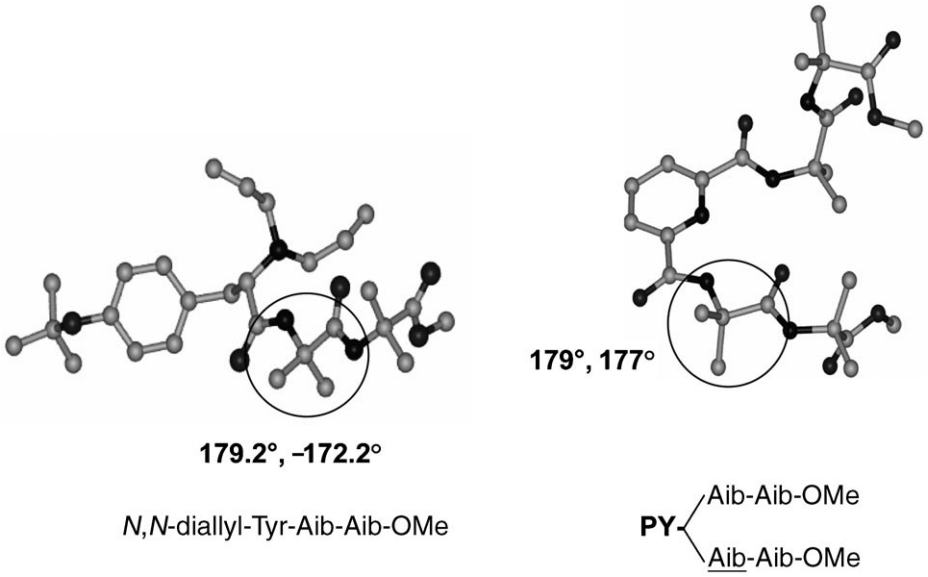

Fig. 8. *Molecular conformations of peptides, where the Aib residue adopts an extended conformation* [63][65]

6. Helical Sense of Aib Residue. – Since the Aib residue is achiral, both the α_R and α_L conformations are equally possible in achiral peptide sequences. In sequences containing chiral residues, the handedness of Aib within intramolecularly H-bonded peptide conformations is determined by the handedness of the chiral residue. Thus, Aib residues placed within a sequence containing L-amino acids invariably adopt the α_R conformation ($\phi \approx -60°$, $\psi \approx -30°$). Aib Residues at the C-terminal end of right-handed helices often adopt the α_L conformations ($\phi \approx +60°$,$\psi \approx +30°$) [69][70] or polyproline II (P_{IIL}) conformation ($\phi \approx -60°$, $\psi \approx +120°$) [69][71] as shown in *Fig. 9*. We have attempted to rationalize this observation by surveying 143 crystal structures of

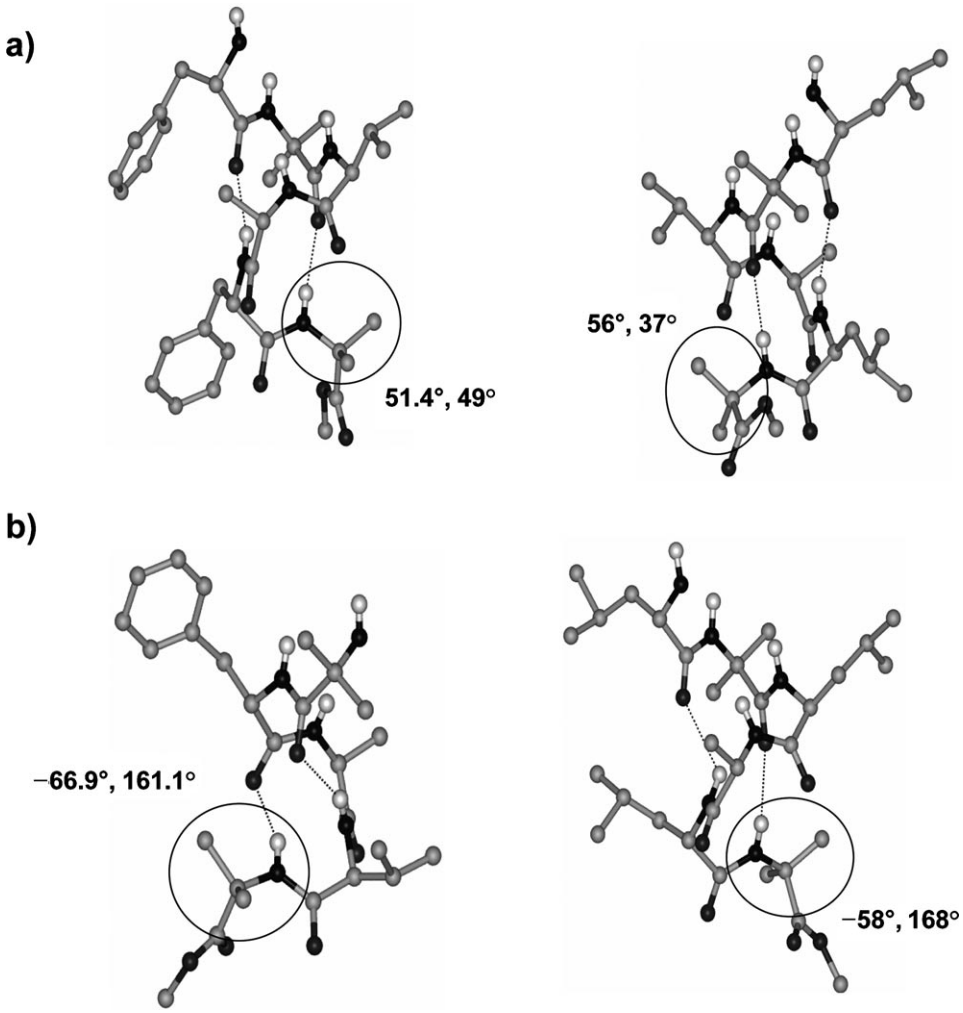

Fig. 9. *Representative examples of Aib residues at the C-terminus. a*) Left-handed (α_L) conformation [69][70]. *b*) Polyproline II (P_{II}) conformation [69][71]. Only the last six residues are shown. The ϕ,ψ values are indicated.

Aib-containing peptide helices of length ≥ 4 residues, which contain a C-terminal Aib residue. Out of 192 examples examined, only 13.5% adopt the same helix sense as the body of the peptide. 66.2% of the residues adopt the opposite sense, while 20.3% lie in the less favored polyproline P_{II} region. *Fig. 10*, *a*, summarizes the observed distribution. In the structure of peptide helices, helical molecules are arranged in columns by head-to-tail H-bonding between exposed back-bone CO and NH groups. Packing considerations which optimize interaction between adjacent residues in a column are likely to be responsible for this unusual conformational feature. *Fig. 10*, *b*, shows an example of head-to-tail H-bonding between the N- and C-termini of adjacent molecules in a

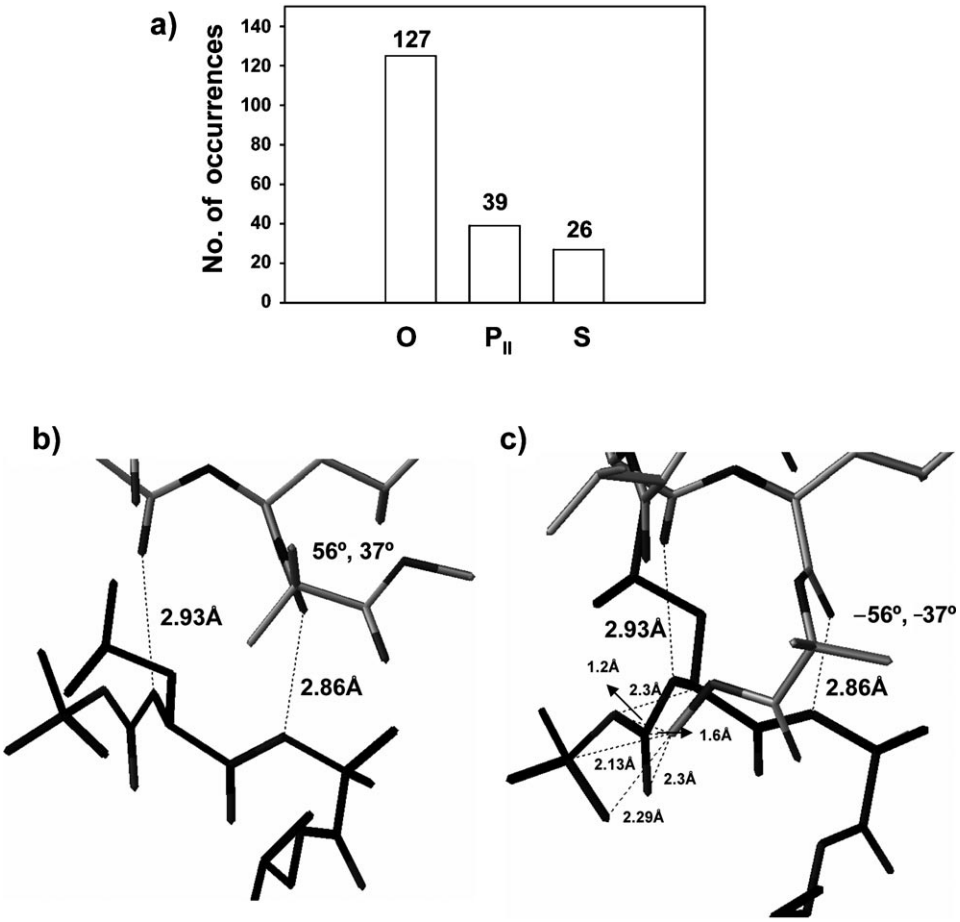

Fig. 10. a) *Histogram showing the distribution of conformations for the C-terminal Aib residues in helical peptides of length* $\geq$*4 residues.* 192 examples are distributed into three classes. O, Aib residues have ϕ,ψ values of *opposite* sign as the body of the helix; P_{II}, polyproline II conformation; S, Aib residues have the *same* sign of ϕ,ψ values as the body of the helix. Values shown in the figure correspond to the number of occurrences. b) *Head-to-tail region of a helical column in the peptide Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe* [70]. The conformational angles of the C-terminus Aib residue are marked. The intermolecular N−H···O H-bonds are also marked. c) *Model generated by reversing the signs of the* ϕ,ψ *angles for the C-terminal Aib residue.* Short contacts between atoms in adjacent molecules are marked.

peptide, which has been protected with the Boc group at the amino terminus and a methyl ester function at the C-terminus. In this case, the body of the peptide forms a right-handed helix, while the C-terminal Aib residue adopts a left-handed helical conformation. *Fig. 10,c*, illustrates the consequence of altering the handedness of the terminal Aib residue. It is immediately apparent that several short contacts are observed between the methyl ester and the Boc groups of adjacent molecules in the

column. It thus appears that registering intermolecular H-bonds appropriately may be the driving force for the terminal Aib residue to adopt the *opposite* helical sense. In the database surveyed, there are 26 examples (13.5%) of C-terminal Aib residues, which adopt the *same* helix sense. In many of the cases, hydration/solvation and the nature of the protecting groups appears to be responsible for this conformational feature.

There are a few examples in which the handedness of the Aib residue in the body of the peptide and the helical sense of the peptide merits comment. In the peptides pClBz-L-Pro-Aib-L-Ala-Aib-L-Ala-OMe [72] and Boc-L-Pro-Aib-L-Ala-Aib-L-Ala-OH [73], Pro(1) adopts a polyproline II (P_{II}) conformation, while the segment Aib(2)-Ala(3)-Aib(4) forms a left-handed 3_{10}-helix, with both Aib residues in α_L conformations (*Fig. 11*). Interestingly, the L-alanine residue in both cases adopts the unusual α_L conformation. In this case, the driving force for the formation of the structure is undoubtedly the N-terminus type-II′ β-turn, which results in the Pro-Aib segment adopting a P_{II}-α_L structure. The hexapeptide Boc-Leu-Aib-Aib-Leu-Aib-Aib-OBzl presents an unusual example of the coexistence of helices of opposite hand in the crystallographic asymmetric unit (*Fig. 12*,*a*) [74]. In the left-handed helix, both the L-leucine residues adopt the less frequently observed α_L conformation. The sequence Ac-Aib-Aib-(*S*)-Iva-Aib-Aib-OMe presents another interesting example of coexistence of molecules of opposite helix sense in the asymmetric unit of the crystal, in the chiral space group *P*1 (*Fig. 12*,*b*) [75]. A similar feature was also observed in the following peptide sequences: Ac-$(Aib)_3$-(*S*)-Iva-Aib-OMe [76], Bz-$(Aib)_4$-L-(αMe)Val-O^{t}Bu [77], pBrBz-$(Aib)_4$-L-(αMe)Val-O^{t}Bu [77], pNBz-$(Aib)_4$-L-(αMe)Val-O^{t}Bu [77], pMeBz-$(Aib)_4$-L-(αMe)Val-O^{t}Bu [77], pBrBz-L-Val-$(Aib)_4$-O^{t}Bu [78], Tfa-$(Aib)_2$-[(*S*)-αEtLeu]-$(Aib)_2$-OEt [79], and Tfa-[(*S*)-αEtLeu]-$(Deg)_4$-OEt [79]. Surprisingly, a chiral reversal at the N-terminal Aib residue is observed in one of the molecules in the peptide sequence Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe (*Fig. 13*) [80].

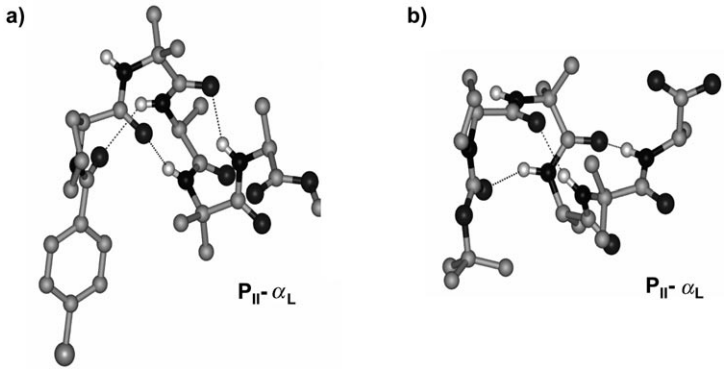

Fig. 11. *Crystal structure of* a) *pClBz-L-Pro-Aib-L-Ala-Aib-L-Ala-OMe* [72] *and* b) *Boc-L-Pro-Aib-L-Ala-Aib-L-Ala-OH* [73]

Particularly interesting examples reported by *Karle et al.* are the structures of [Boc-Cys-$(Aib)_n$-OMe]$_2$, ($n=3, 4$) [81]. *Fig. 14* shows a view of the molecular conformation observed in the peptides along with a scatter plot of the observed ϕ,ψ values. In the case of the peptide with $n=3$, the molecule has perfect twofold symmetry; the molecular

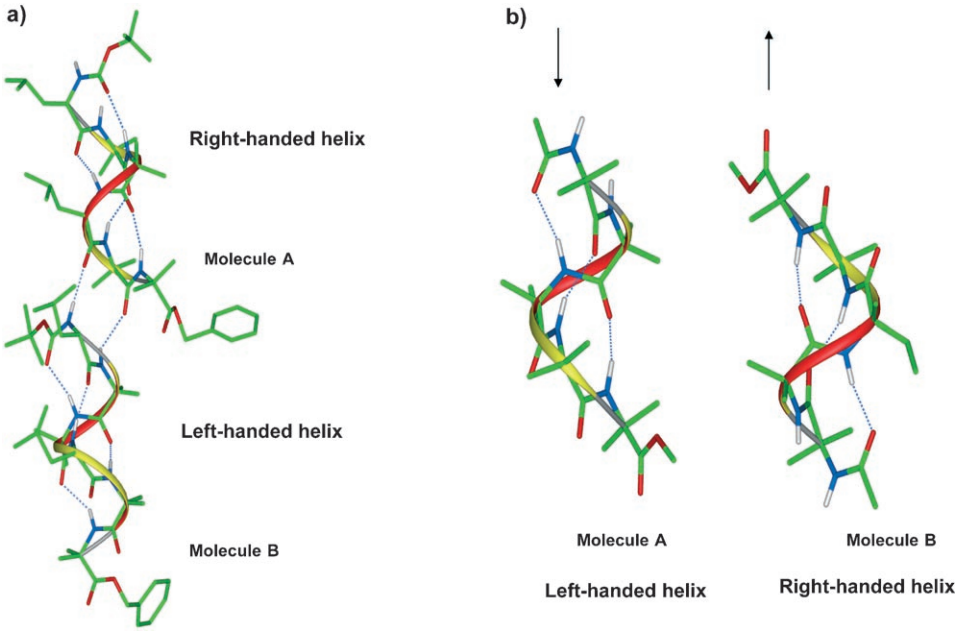

Fig. 12. *Molecular conformation of* a) *Boc-Leu-Aib-Aib-Leu-Aib-Aib-OBzl* [74] *and* b) *Ac-Aib-Aib-(*S*)-Iva-Aib-Aib-OMe* [75], *where both right* (α_R) *and left* (α_L)*-handed helix are present in the asymmetric unit*

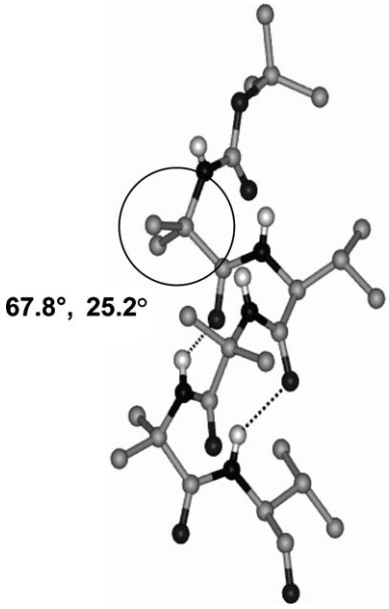

Fig. 13. *Molecular conformation of Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe* [80]. The N-terminal Aib(1) adopts positive ϕ,ψ values. This is an example of a chiral reversal at the N-terminus. Only the first five residues are shown.

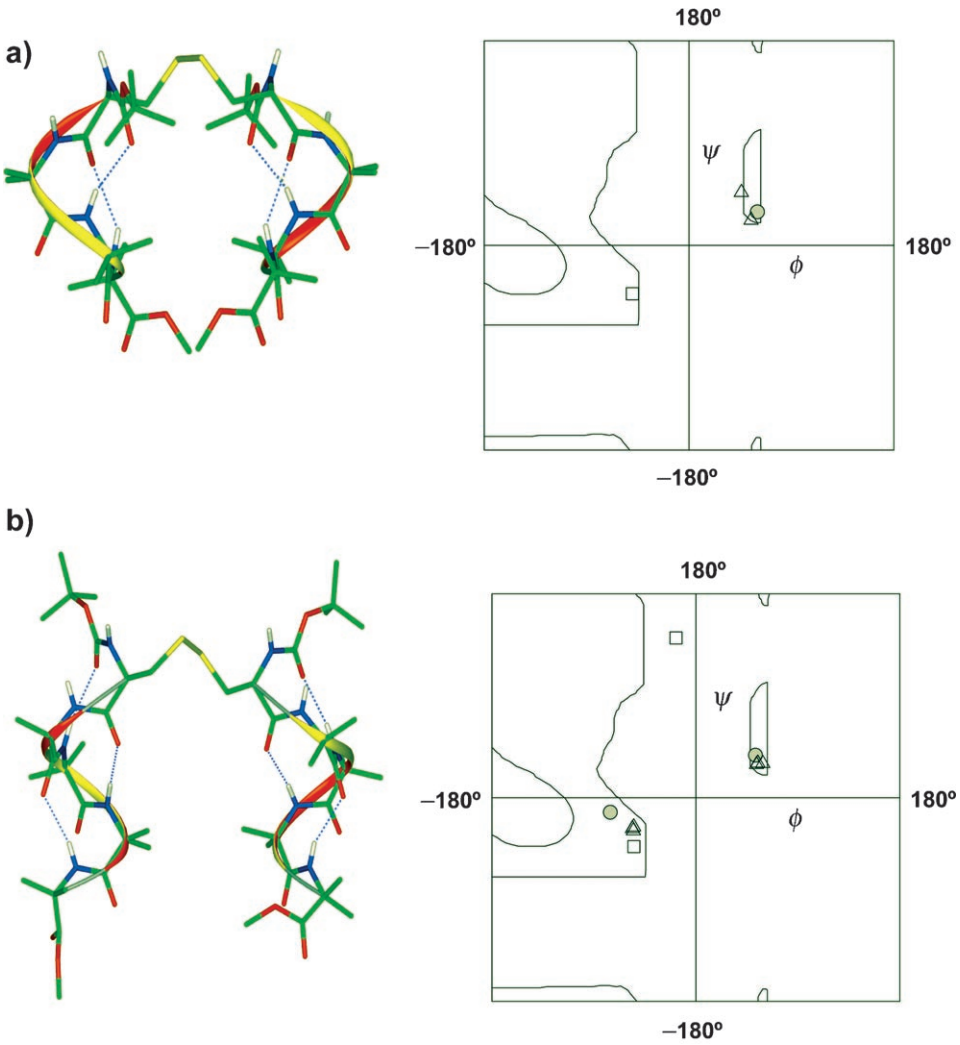

Fig. 14. *The molecular conformation of [Boc-Cys-(Aib)$_n$-OMe]$_2$ in crystals* [81]. *a*) $n=3$, *b*) $n=4$. The observed ϕ,ψ values are plotted in a *Ramachandran* map. The symbols ●, △, and □ correspond to Cys, Aib, and the terminal Aib residues, resp.

axis coincides with the crystallographic twofold axis (space group $C2$). In both cases, the helical fold of the peptide backbone is left-handed, with Cys(1) adopting an α_L conformation ($\phi=60.1^\circ$, $\psi=29.4^\circ$). Chiral reversal is observed at Aib(4) ($\phi=-49.8^\circ$, $\psi=-42.6^\circ$). In contrast, the two helical arms in the peptide with $n=4$ adopt the opposite senses of helical twist. In one arm, the Cys residues adopt an α_R conformation, while, in the other, the Cys residue adopts an α_L conformation. In the case of both peptides, the unusual α_L conformation of Cys(1) is observed. Normally, the lone chiral residue in a sequence might have been expected to impose the sense of helical twist on

an homo-oligo $(Aib)_n$ segment. Surprisingly, in this case despite the L-configuration of the Cys residue, the energetically unfavorable left-handed twist has been selected. *DeGrado* and co-workers have estimated energy differences of *ca.* 0.95 kcal/mol between α_L and α_R conformations of D-alanine [82].

7. *Schellman* Motif. – In synthetically designed peptides, the tendency of the Aib residue to adopt α_L conformations can be exploited to generate the helix-terminating *Schellman* motif [47]. In this structure, the last turn of a right-handed helix is capped by a residue adopting an α_L conformation. In proteins, the achiral Gly residue invariably serves as the terminating residue [83]. Synthetic sequences containing Aib as the penultimate residue from the C-terminus usually yield structures exhibiting the helix-terminating *Schellman* motif [84]. *Fig. 15,a*, illustrates the definition of residues in the *Schellman* motif and provides an example for the case of Aib as the terminating residue [85]. An interesting example of chiral reversal in the center of a synthetic sequence is observed in the peptide sequence Boc-Leu-Aib-Val-Aib-Leu-Aib-Val-D-Ala-D-Leu-Aib-OMe [86]. The segment Leu(1) to Leu(5) adopts right-handed helical conformation. Aib(6) adopts α_L conformation resulting in a short sequence of left-handed helix at the C-terminus. The two helical segments of opposite sense of twist are fused seamlessly (*Fig. 15,b*).

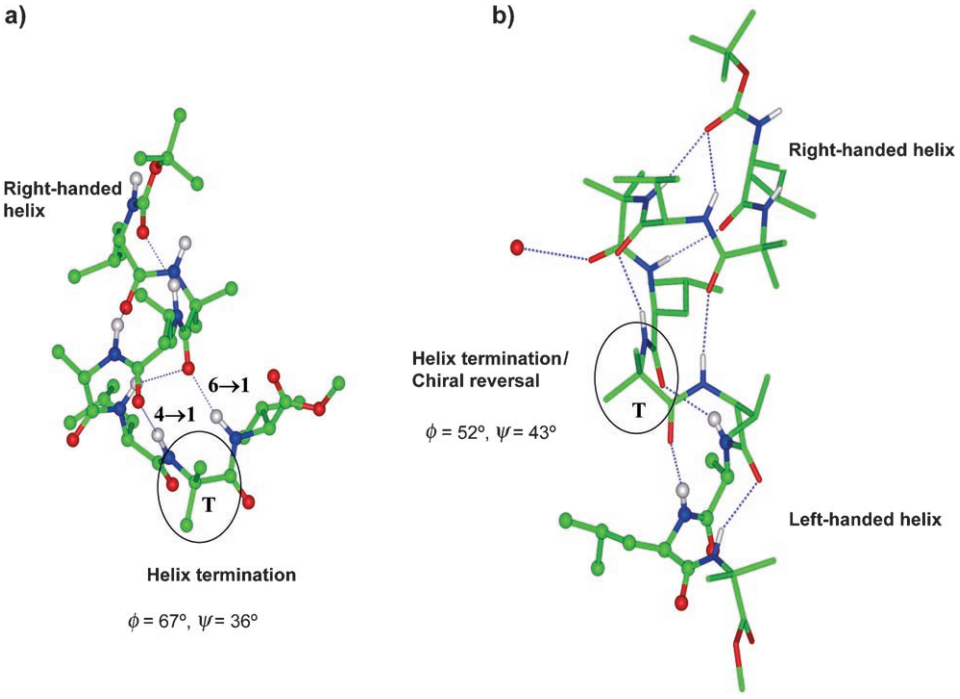

Fig. 15. *Molecular conformation of* a) *Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-OMe* [85] *and* b) *Boc-Leu-Aib-Val-Aib-Leu-Aib-Val-*D*-Ala-*D*-Leu-Aib-OMe* [86]. T is the helix-terminating residue, which adopts positive ϕ,ψ values.

8. Cyclic Peptides. – A survey of the crystal structure database revealed 22 examples of cyclic peptides containing Aib residues (*Table 2*) [46][87–104]. In several cases, the Aib residues adopted anticipated ϕ,ψ values lying in the α_R and α_L region of the *Ramachandran* map (*Fig. 5,d*). Two examples of Aib residue adopting nonhelical conformations are shown in *Fig. 16*. In the natural product dihydrochlamydocin, the lone Aib residue adopts a C_7 (γ-turn) conformation [46]. In the synthetic cyclic cystine peptide Boc-[Cys-Val-Aib-Ala-Leu-Cys]-NHMe, the Aib residue occupies the $i+1$ position of the type-II′ β-turn conformation, with $\phi = 49.9°$, $\psi = -131.2°$ (P_{IIR}) [91]. In piperazine-2,5-diones, for example, the imperative of a nearly flat six-membered ring locks Aib into the normally disallowed conformations near $\phi \approx 0°$, $\psi \approx 0°$ [88][90].

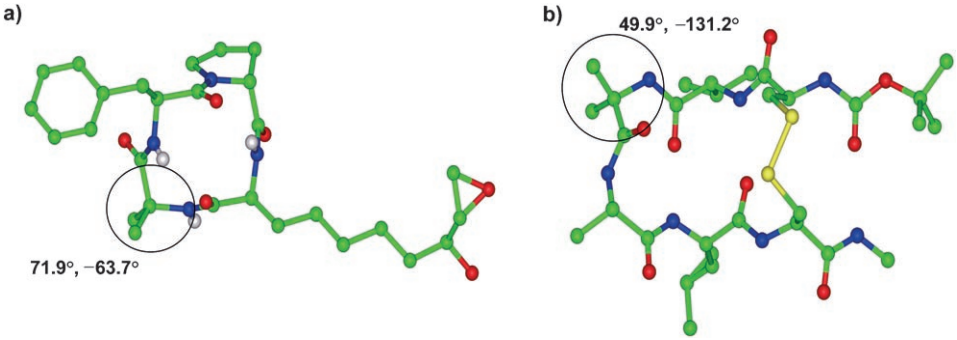

Fig. 16. *Molecular conformation of* a) *dihydrochlamydocin* [46] *and* b) *Boc-[Cys-Val-Aib-Ala-Leu-Cys]-NHMe* [91]

9. Conclusions. – Aib Residues are strongly constrained to the helical region of the ϕ,ψ space and thus limit the range of accessible backbone conformations when incorporated into peptide sequences. The ability of Aib-containing sequences to adopt well-defined folds and their high degree of conformational homogeneity in solution has resulted in the crystallization of a large number of Aib-containing peptides. X-Ray diffraction studies have permitted detailed structural characterization, with the result that Aib-containing peptides are the best-studied group of oligopeptides in the crystal state. There are, however, unusual nonhelical conformations that can be populated by Aib residues, albeit, much less frequently, which need attention for designing appropriately folded synthetic peptides and in the conformational analysis of Aib containing-peptides of natural origin. This overview has attempted to highlight the circumstances in which Aib residues have been observed in unusual conformations.

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Table 2. *ϕ,ψ Values* [°] *of Aib Residues in Cyclic Peptides* ('cyclic' abbreviated as c)

Entry	Sequence [CCDC code]	ϕ	ψ	Space group	Reference
1	c(Aib-Phe-Pro-X)·H_2O dihydrochlamydocin [DHCMYD]	71.9	−63.7	$P2_1$	[46]
2	c(Pro-Aib) [BUYGUX]			$P2_1$	[87]
	Molecule A	−30.3	28.6		
	Molecule B	−1.0	0.9		
3	c(Aib-Aib) [BOCSIV]	5.8	−5	$P\bar{1}$	[88]
		−5.8	5		
4	c(Aib-Ile) [BOCSOB]	9.7	−8.0	$P2_12_12_1$	[88]
5	Boc-Cys-Pro-Aib-Cys-NHMe·DMSO [BEWDIQ]	−61.8	−17.9	$P2_12_12_1$	[89]
6	c(Aib-Phe) [COYRIR]	6.7	−3.6	$P2_1$	[90]
7	Boc-[Cys-Val-Aib-Ala-Leu-Cys]-NHMe·DMSO + H_2O [GAGDIB]	49.9	−131.2	$P2_12_12_1$	[91]
8	c(Pro-Pro-Phe-Phe-Aib-Aib-Ile-Ala-Val) [JUJHUR]	−53.8	−41.0	$P2_12_12_1$	[92]
		−54.6	−27.1		
9	c(Pro-Pro-Phe-Phe-Aib-Aib-Ile-Ala-Val)·CH_3CN + 2 H_2O [LINCOA]	−53.6	−42.4	$P2_12_12_1$	[93]
		−53.4	−32.7		
10	c(Gly-Aib-Gly)$_2$·Formic acid [RAQVOU]	55.3	35.2	$P2_1/c$	[94]
		−55.3	−35.2		
11	c(Pro-Phe-Phe-Aib-Leu)$_2$	63	34	$P2_1$	[95]
		62	37		
12	c(Phe-Phe-Aib-Leu-Pro) [FEGJAD]	70.4	−73.9	$P6_5$	[96]
13	c(Phe-Ser-Ser-Phe-Aib) [XACJAM] (Ser = *O*-benzylseryl; Aib = *N*-methyl-Aib)	−57.5	130.5	*P*1	[97]
14	Boc-Val-[Hse-Leu-Aib-Val-Hse]-Leu-OMe·CH_2Cl_2 + H_2O [GOGFAJ] (Hse = Homoserine)	−55.5	−35.9	$P2_12_12_1$	[98]
15	c(Aib-Aib-Aib-Aib-Tro) [QENREG]			$P2_1/n$	[99]
	Molecule A	−55.4	136.4		
		55.5	31.4		
		92.0	−25.4		
		−51.9	−42.9		
	Molecule B	−52.5	136.1		
		51.1	40.7		
		70.9	23.1		
		−100	6.5		
16	c(Pro-Thr-Aib-β-Phe-ethylgly)·H_2O [AWONOP]	56.0	43.0	$P3_1$	[100]
17	c(Aib-Aib) [BOCSIV01]	4.2	−3.7	$P\bar{1}$	[101]
		−4.2	3.7		
18	c(Pro-Aib-Gly-Aib-Aib-Phe)·Hexafluorophosphate monohydrate [FAZPIG]	−65.1	−13.8	$P2_12_12_1$	[102]
		53.8	46.7		
		80.2	10.1		
19	c(Gly-Aib-Aib-Gly-Aib-Phe)·3 H_2O [DALPOW]	52.6	44.7	$P2_1$	[103]
		64.2	25.7		
		−55.8	−34.2		
20	c(Gly-α(Me)Phe-Aib-Gly-Aib-Phe) [DALPUC]	69.0	6.1	$P2_12_12_1$	[103]
		−49.1	−43.1		
21	c(Gly-Aib-Leu-Aib-Phe-Aib) [FINPEY]	76.9	−7.6	$P2_12_12_1$	[104]
		55.2	−130.4		
		−175.9	178.6		
22	c(Leu-Aib-Phe-Gly-Aib-Aib)·ethanol isopropanol solvate dehydrate [FINPIC]	−57.1	−34.4	$P2_1$	[104]
		56.4	41.2		
		93.8	−15.3		

REFERENCES

[1] G. W. Kenner, R. C. Sheppard, *Nature* **1958**, *181*, 48; H. Brückner, H. Graf, *Experientia* **1983**, *39*, 528.

[2] M. J. Thirumalachur, *Hindustan Antibiot. Bull.* **1968**, *10*, 287; M. G. Vaidya, P. V. Deshmukh, S. N. Chari, *Hindustan Antibiot. Bull.* **1968**, *11*, 81.

[3] R. C. Pandey, H. Meng, J. C. Cook Jr., K. L. Rinehart Jr., *J. Antibiot.* **1978**, *31*, 241.

[4] R. J. Fox, F. M. Richards, *Nature* **1982**, *300*, 325.

[5] L. Whitmore, J. K. Chugh, C. F. Snook, B. A.Wallace, *J. Pept. Sci*. **2003**, *9*, 663; L. Whitmore, B. A. Wallace, *Nucleic Acids Res.* **2004**, *32*, 593.

[6] J. F. Daniel, E. R. Filho, *Nat. Prod. Rep.* **2007**, *24*, 1128.

[7] T. Degenkolb, A. Berg, W. Gams, B. Schlegel, U. Grafe, *J. Pept. Sci.* **2003**, *9*, 666; A. Szekeres, B. Leitgeb, L. Kredics, Z. Antal, L. Hatvani, L. Manczinger, C. Vágvölgyi, *Acta Microbiol. Immunol. Hung.* **2005**, *52*, 137; H. Duclohier, *Chem. Biodivers.* **2007**, *4*, 1023; T. Degenkolb, J. Kirschbaum, H. Brückner, *Chem. Biodivers.* **2007**, *4*, 1052.

[8] E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, G. M. Bonora, *Proc. Natl. Acad. Sci. U.S.A*. **1982**, *79*, 7951.

[9] D. R. Martin, R. J. P. Williams, *Biochem. J.* **1976**, *153*, 181; R. C. Pandey, J. C. Cook Jr., K. L. Rinehart Jr., *J. Am. Chem. Soc.* **1977**, *99*, 8469; R. Nagaraj, P. Balaram, *Acc. Chem. Res.* **1981**, *14*, 356; M. K. Mathew, P. Balaram, *Mol. Cell. Biochem*. **1983**, *50*, 47; M. S. P. Sansom, *Prog. Biophys. Mol. Biol*. **1991**, *55*, 139; D. S. Cafiso, *Annu. Rev. Biophys. Biomol. Struct*. **1994**, *23*, 141; B. Leitgeb, A. Szekeres, L. Manczinger, C. Vágvölgyi, L. Kredics, *Chem. Biodivers.* **2007**, *4*, 1027.

[10] R. C. Pandey, H. Meng, J. C. Cook Jr., K. L. Rinehart Jr., *J. Am. Chem. Soc.* **1977**, *99*, 5203; H. Brückner, G. J. Nicholson, G. Jung, K. Kruse, W. A. König, *Chromatographia* **1980**, *13*, 209; M. K. Das, S. Raghothama, P. Balaram, *Biochemistry* **1986**, *25*, 7110; E. N. Lyukmanova, Z. O. Shenkarev, A. S. Paramonov, A. G. Sobol, T. V. Ovchinnikova, V. V. Chupin, M. P. Kirpichnikov, M. J. J. Blommers, A. S. Arseniev, *J. Am. Chem. Soc*. **2008**, *130*, 2140.

[11] K. L. Rinehart Jr., L. A. Gaudioso, M. L. Moore, R. C. Pandey, J. C. Cook Jr., M. Barber, R. D. Sedgwick, R. S. Bordoli, A. N. Tyler, B. N. Green, *J. Am. Chem. Soc.* **1981**, *103*, 6517; B. Bodo, S. Rebuffat, M. E. Hajji, D. Davoust, *J. Am. Chem. Soc.* **1985**, *107*, 6011; C. Auvin-Guette, S. Rebuffat, Y. Prigent, B. Bodo, *J. Am. Chem. Soc.* **1992**, *114*, 2170; C. Auvin-Guette, S. Rebuffat, I. Vuidepot, M. Massias, B. Bodo, *J. Chem. Soc., Perkin Trans. 1* **1993**, 249; S. Rebuffat, L. Conraux, M. Massias, C. Auvin-Guette, B. Bodo, *Int. J. Pept. Protein Res.* **1993**, *41*, 74; A. Iida, M. Sanekata, T. Fujita, H. Tanaka, A. Enoki, G. Fuse, M. Kanai, P. J. Rudewicz, E. Tachikawa, *Chem. Pharm. Bull.* **1994**, *42*, 1070.

[12] A. Jaworski, H. Brückner, *J. Chromatogr., A* **1999**, *862*, 179; A. Jaworski, H. Brückner, *J. Pept. Sci.* **2000**, *6*, 149; A. Jaworski, H. Brückner, *J. Pept. Sci.* **2001**, *7*, 433; J. Kirschbaum, C. Krause, R. K. Winzheimer, H. Brückner, *J. Pept. Sci.* **2003**, *9*, 799; C. Krause, J. Kirschbaum, H. Brückner, *Amino Acids* **2006**, *30*, 435; C. Krause, J. Kirschbaum, G. Jung, H. Brückner, *J. Pept. Sci.* **2006**, *12*, 321; T. Degenkolb, T. Gräfenhan, A. Berg, H. I. Nirenberg, W. Gams, H. Brückner, *J. Agric. Food Chem.* **2006**, *54*, 7047; T. Degenkolb, T. Gräfenhan, H. I. Nirenberg, W. Gams, H. Brückner, *Chem. Biodivers.* **2006**, *3*, 593.

[13] G. R. Marshall, H. E. Bosshard, *Circ. Res.* **1972**, *30–31 (suppl. II)*, 143; G. N. Ramachandran, R. Chandrasekaran, in 'Progress in Peptide Research Volume II: Proceedings of the Second American Peptide Symposium, Cleveland, 1970', Ed. S. Lande, Gordon and Breach, New York, 1972, p. 195.

[14] G. N. Ramachandran, V. Sasisekharan, *Adv. Protein Chem*. **1968**, *23*, 283.

[15] A. W. Burgess, S. J. Leach, *Biopolymers* **1973**, *12*, 2599; B. V. V. Prasad, V. Sasisekharan, *Macromolecules* **1979**, *12*, 1107; B. V. V. Prasad, V. Sasisekharan, *Curr. Sci.* **1979**, *48*, 517; T. T. Tran, H. Treutlein, A. W. Burgess, *Protein Eng., Des. Sel*. **2006**, *19*, 401.

[16] N. Shamala, R. Nagaraj, P. Balaram, *Biochem. Biophys. Res. Commun*. **1977**, *79*, 292; N. Shamala, R. Nagaraj, P. Balaram, *J. Chem. Soc., Chem. Commun*. **1978**, 996; E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, M. Crisma, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc*. **1982**, *104*, 2437; C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, *Biopolymers* **1983**, *22*, 205.

[17] B. V. V. Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984**, *16*, 307.
[18] I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747.
[19] a) C. Toniolo, E. Benedetti, *ISI Atlas of Science: Biochemistry* **1988**, *1*, 225; C. Toniolo, E. Benedetti, *Trends Biochem. Sci*. **1991**, *16*, 350; C. Toniolo, E. Benedetti, *Macromolecules* **1991**, *24*, 4004; I. L. Karle, *Acta Crystallogr., Sect. B* **1992**, *48*, 341; b) E. Benedetti, *Biopolymers* **1996**, *40*, 3; c) I. L. Karle, *Biopolymers* **1996**, *40*, 157; R. Kaul, P. Balaram, *Bioorg. Med. Chem*. **1999**, *7*, 105; J. Venkatraman, S. C. Shankaramma, P. Balaram, *Chem. Rev*. **2001**, *101*, 3131; C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396; S. Aravinda, N. Shamala, R. S. Roy, P. Balaram. *Proc. Indian Acad. Sci. Chem. Sci.* **2003**, *115*, 373.
[20] I. L. Karle, J. L. Flippen-Anderson, R. Gurunath, P. Balaram, *Protein Sci*. **1994**, *3*, 1547; G. L. Millhauser, *Biochemistry* **1995**, *34*, 3873; G. Yoder, A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo, T. A. Keiderling, *J. Am. Chem. Soc*. **1997**, *119*, 10278; S. Mammi, M. Rainaldi, M. Bellanda, E. Schievano, E. Peggion, Q. B. Broxterman, F. Formaggio, M. Crisma, C. Toniolo, *J. Am. Chem. Soc*. **2000**, *122*, 11735; R. A. G. Silva, S. C. Yasui, J. Kubelka, F. Formaggio, C. Toniolo, T. Keiderling, *Biopolymers* **2002**, *65*, 229.
[21] S. Cerrini, D. Lamba, A. Scatturin, C. Rossi, G. Ughetto, *Biopolymers* **1989**, *28*, 409
[22] I. L. Karle, J. L. Flippen-Anderson, S. Agarwalla, P. Balaram, *Proc. Natl. Acad. Sci. U.S.A*. **1991**, *88*, 5307.
[23] I. L. Karle, J. L. Flippen-Anderson, S. Agarwalla, P. Balaram, in 'Structure and Function: Volume 2: Proteins. Proceedings of the Seventh Conversation in Biomolecular Stereodynamics', Eds. R. H. Sarma, M. H. Sarma, Adenine Press, New York, 1992, p. 97.
[24] I. L. Karle, J. L. Flippen-Anderson, S. Agarwalla, P. Balaram, *Biopolymers* **1994**, *34*, 721.
[25] C. F. Snook, G. A. Woolley, G. Oliva, V. Pattabhi, S. P. Wood, T. L. Blundell, B. A. Wallace, *Structure* **1998**, *6*, 783.
[26] I. L. Karle, M. A. Perozzo, V. K. Mishra, P. Balaram, *Proc. Natl. Acad. Sci. U.S.A*. **1998**, *95*, 5501.
[27] M. Crisma, A. Barazza, F. Formaggio, B. Kaptein, Q. B. Broxterman, J. Kamphuis, C. Toniolo, *Tetrahedron* **2001**, *57*, 2813.
[28] J. K. Chugh, H. Brückner, B. A. Wallace, *Biochemistry* **2002**, *41*, 12934.
[29] M. Kronen, H. Görls, H. Nguyen, S. Reißmann, M. Bohl, J. Sühnel, U. Gräfe, *J. Pept. Sci*. **2003**, *9*, 729.
[30] G. Bunkóczi, M. Schiell, L. Vértesy, G. M. Sheldrick, *J. Pept. Sci.* **2003**, *9*, 745.
[31] R. Anders, O. Ohlenschläger, V. Soskic, H. Wenschuh, B. Heisel, L. R. Brown, *Eur. J. Biochem.* **2000**, *267*, 1784.
[32] T. A. Balashova, Z. O. Shenkarev, A. A. Tagaev, T. V. Ovchinnikova, J. Raap, A. S. Arseniev, *FEBS Lett*. **2000**, *466*, 333.
[33] Z. O. Shenkarev, T. A. Balashova, R. G. Efremov, Z. A. Yakimenko, T. V. Ovchinnikova, J. Raap, *Biophys. J*. **2002**, *82*, 762.
[34] T. P. Galbraith, R. Harris, C. P. Driscoll, B. A. Wallace, *Biophys. J.* **2003**, *84*, 185.
[35] Z. O. Shenkarev, T. A. Balashova, Z. A. Yakimenko, T. V. Ovchinnikova, A. S. Arseniev, *Biophys. J.* **2004**, *86*, 3687.
[36] B. Bachet, C. Brassy, I. Morize, E. Surcouf, J. P. Mornon, B. Bodo, S. Rebuffat, *J. Mol. Biol.* **1983**, *170*, 795; M. LeBars, B. Bachet, J. P. Mornon, *Z. Kristallogr.* **1988**, *185*, 588.
[37] C. Toniolo, C. Peggion, M. Crisma, F. Formaggio, X. Shui, D. S. Eggleston, *Nat. Struct. Biol.* **1994**, *1*, 908.
[38] S. Rebuffat, Y. Prigent, C. Auvin-Guette, B. Bodo, *Eur. J. Biochem*. **1991**, *201*, 661.
[39] E. Condamine, S. Rebuffat, Y. Prigent, I. Ségalas, B. Bodo, D. Davoust, *Biopolymers* **1998**, *46*, 75.
[40] M. Crisma, C. Peggion, C. Baldini, E. J. MacLean, N. Vedovato, G. Rispoli, C. Toniolo, *Angew. Chem., Int. Ed*. **2007**, *46*, 2047.
[41] V. Monaco, F. Formaggio, M. Crisma, C. Toniolo, X. Shui, D. S. Eggleston, *Biopolymers* **1996**, *39*, 31.
[42] C. Peggion, F. Formaggio, M. Crisma, R. F. Epand, R. M. Epand, C. Toniolo, *J. Pept. Sci.* **2003**, *9*, 679.
[43] M. Saviano, R. Improta, E. Benedetti, B. Carrozzini, G. L. Cascarano, C. Didierjean, C. Toniolo, M. Crisma, *ChemBioChem* **2004**, *5*, 541.

[44] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **2000**, *28*, 235.
[45] F. H. Allen, *Acta Crystallogr., Sect. B* **2002**, *58*, 380.
[46] J. L. Flippen, I. L. Karle, *Biopolymers* **1976**, *15*, 1081.
[47] C. Schellman, in 'Protein Folding' Ed. R. Jaenicke, Amsterdam, Elsevier/North-Holland Biochemical Press 1980, p. 53; E. J. Milner-White, *J. Mol. Biol*. **1988**, *199*, 915; H. A. Nagarajaram, R. Sowdhamini, C. Ramakrishnan, P. Balaram, *FEBS Lett*. **1993**, *321*, 79; R. Aurora, R. Srinivasan, G. D. Rose, *Science* **1994**, *264*, 1126.
[48] R. Bardi, A. M. Piazzesi, C. Toniolo, M. Sukumar, P. A. Raj, P. Balaram, *Int. J. Pept. Protein Res.* **1985**, *25*, 628.
[49] G. Valle, C. Toniolo, G. Jung, *Gazz. Chim. Ital*. **1987**, *117*, 549.
[50] R. Gessmann, H. Brueckner, M. Kokkinidis, *Biochem. Biophys. Res. Commun*. **1991**, *174*, 878.
[51] G. Valle, M. Crisma, C. Toniolo, R. Beisswenger, A. Rieker, G. Jung, *Liebigs Ann. Chem*. **1989**, 337.
[52] J. M. Villalgordo, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 748.
[53] C. Peggion, M. Crisma, F. Formaggio, C. Toniolo, K. Wright, M. Wakselman, J. P. Mazaleyrat, *Biopolymers* **2002**, *63*, 314.
[54] C. Strässler, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **1997**, *80*, 1528.
[55] G. Valle, R. Bardi, A. M. Piazzesi, M. Crisma, C. Toniolo, G. Cavicchioni, K. Uma, P. Balaram, *Biopolymers* **1991**, *31*, 1669.
[56] M. Crisma, F. Formaggio, C. Toniolo, *Acta Crystallogr., Sect. C* **2000**, *56*, 695.
[57] C. Toniolo, G. M. Bonora, M. Crisma, E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, *Int. J. Pept. Protein Res.* **1983**, *22*, 603.
[58] T. Yamada, M. Nakao, T. Miyazawa, S. Kuwata, M. Sugiura, Y. In, T. Ishida, *Biopolymers* **1993**, *33*, 813.
[59] A. N. Jayashree, C. G. Suresh, M. Vijayan, C. Toniolo, G. M. Bonora, *Acta Crystallogr., Sect. C* **1987**, *43*, 1618.
[60] V. Pavone, A. Lombardi, F. Nastri, M. Saviano, B. Di Blasio, F. Fraternali, C. Pedone, T. Yamada, *J. Chem. Soc., Perkin Trans. 2* **1992**, *6*, 971.
[61] R. Bardi, A. M. Piazzesi, C. Toniolo, O. E. Jensen, R. S. Omar, A. Senning, *Biopolymers* **1988**, *27*, 747.
[62] S. Datta, N. Shamala, A. Banerjee, P. Balaram, *Biopolymers* **1997**, *41*, 331.
[63] D. Ranganathan, S. Kurur, A. C. Kunwar, A. V. S. Sarma, M. Vairamani, I. L. Karle, *J. Pept. Res.* **2000**, *56*, 416.
[64] D. Ranganathan, S. Kurur, I. L. Karle, *Biopolymers* **2000**, *54*, 249.
[65] J. L. Flippen-Anderson, C. George, J. R. Deschamps, *Acta Crystallogr., Sect. C* **1994**, *50*, 1095.
[66] E. Benedetti, C. Toniolo, P. Hardy, V. Barone, A. Bavoso, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, I. Lingham, *J. Am. Chem. Soc.* **1984**, *106*, 8146; G. Valle, G. M. Bonora, C. Toniolo, P. M. Hardy, M. T. Leplawy, A. Redlinski, *J. Chem. Soc., Perkin Trans. 2* **1986**, *6*, 885; E. Benedetti, V. Barone, A. Bavoso, B. Di Blasio, F. Lelj, V. Pavone, C. Pedone, G. M. Bonora, C. Toniolo, M. T. Leplawy, K. Kaczmarek, A. Redlinski, *Biopolymers* **1988**, *27*, 357; A. R. Dentino, P. A. Raj, K. K. Bhandary, M. E. Wilson, M. J. Levine, *J. Biol. Chem.* **1991**, *266*, 18460; R. Bardi, A. M. Piazzesi, M. Crisma, C. Toniolo, Sudhanand, R. B. Rao, P. Balaram, *Z. Kristallogr.* **1992**, *202*, 302.
[67] B. Di Blasio, V. Pavone, C. Isernia, C. Pedone, E. Benedetti, C. Toniolo, P. M. Hardy, I. N. Lingham, *J. Chem. Soc., Perkin Trans.* 2 **1992**, 523; I. L. Karle, R. B. Rao, S. Prasad, R. Kaul, P. Balaram, *J. Am. Chem. Soc.* **1994**, *116*, 10355; M. Crisma, G. Valle, C. Toniolo, S. Prasad, R. B. Rao, P. Balaram, *Biopolymers* **1995**, *35*, 1; S. Datta, R. Kaul, R. B. Rao, N. Shamala, P. Balaram, *J. Chem. Soc., Perkin Trans. 2* **1997**, 1659; R. Kaul, S. Banumathi, D. Velmurugan, R. B. Rao, P. Balaram, *Biopolymers* **2000**, *54*, 159; R. P. Hegde, S. Aravinda, R. Rai, R. Kaul, S. Vijayalakshmi, R. B. Rao, N. Shamala, P. Balaram, *J. Pept. Sci*. **2008**, *14*, 648.
[68] N. Imawaka, M. Tanaka, H. Suemune, *Helv. Chim. Acta* **2000**, *83*, 2823; M. Tanaka, *Chem. Pharm. Bull.* **2007**, *55*, 349.

[69] S. Aravinda, N. Shamala, C. Das, A. Sriranjini, I. L. Karle, P. Balaram, *J. Am. Chem. Soc*. **2003**, *125*, 5308.
[70] S. Aravinda, S. Datta, N. Shamala, P. Balaram, *Angew. Chem., Int. Ed*. **2004**, *43*, 6728.
[71] I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Biopolymers* **1990**, *29*, 1835.
[72] T. S. Cameron, A. W. Hanson, A. Taylor, *Cryst. Struct. Commun*. **1982**, *11*, 321.
[73] R. Bosch, G. Jung, H. Schmitt, W. Winter, *Acta Crystallogr., Sect. C* **1985**, *41*, 1821.
[74] K. Okuyama, S. Nakanishi, Y. Saga, H. Umeyama, M. Narita, *Bull. Chem. Soc. Jpn*. **1990**, *63*, 3149.
[75] G. Valle, M. Crisma, C. Toniolo, R. Beisswenger, A. Rieker, G. Jung, *J. Am. Chem. Soc*. **1989**, *111*, 6828.
[76] M. Crisma, G. Valle, M. Pantano, F. Formaggio, G. M. Bonora, C. Toniolo, J. Kamphuis, *Recl. Trav. Chim. Pays-Bas* **1995**, *114*, 325.
[77] E. Benedetti, M. Saviano, R. Iacovino, C. Pedone, A. Santini, M. Crisma, F. Formaggio, C. Toniolo, Q. B. Broxterman, J. Kamphuis, *Biopolymers* **1998**, *46*, 433.
[78] S. Benedetti, M. Saviano, R. Iacovino, M. Crisma, F. Formaggio, C. Toniolo, *Z. Kristallogr*. **1999**, *214*, 160.
[79] M. Oba, M. Tanaka, M. Kurihara, H. Suemune, *Helv. Chim. Acta* **2002**, *85*, 3197.
[80] I. L. Karle, J. L. Flippen-Anderson, K. Uma, H. Balaram, P. Balaram, *Proc. Natl. Acad. Sci. U.S.A*. **1989**, *86*, 765.
[81] I. L. Karle, D. Ranganathan, C. Lakshmi, *Biopolymers* **2001**, *59*, 301.
[82] R. Fairman, S. J. Anthony-Cahill, W. F. DeGrado, *J. Am. Chem. Soc.* **1992**, *114*, 5458.
[83] K. Gunasekaran, H. A. Nagarajaram, C. Ramakrishnan, P. Balaram, *J. Mol. Biol*. **1998**, *275*, 917.
[84] E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma, L. Sartore, *J. Chem. Soc., Perkin Trans. 2* **1990**, *11*, 1829; I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Int. J. Pept. Protein Res.* **1993**, *42*, 401; S. Datta, M. V. Uma, N. Shamala, P. Balaram, *Biopolymers* **1999**, *50*, 13; I. L. Karle, *Biopolymers* **2001**, *65*, 351.
[85] A. Banerjee, S. Datta, A. Pramanik, N. Shamala, P. Balaram, *J. Am. Chem. Soc*. **1996**, *118*, 9477.
[86] S. Aravinda, N. Shamala, A. Bandyopadhyay, P. Balaram, *J. Am. Chem. Soc*. **2003**, *125*, 15065.
[87] M. Gdaniec, *Pol. J. Chem*. **1981**, *55*, 1795.
[88] K. Suguna, S. Ramakumar, N. Shamala, B. V. V. Prasad, P. Balaram, *Biopolymers* **1982**, *21*, 1847.
[89] B. V. V. Prasad, A. Ravi, P. Balaram, *Biochem. Biophys. Res. Commun*. **1981**, *103*, 1138; A. Ravi, B. V. V. Prasad, P. Balaram, *J. Am. Chem. Soc*. **1983**, *105*, 105.
[90] K. Suguna, S. Ramakumar, R. Nagaraj, P. Balaram, *Acta Crystallogr., Sect. C* **1985**, *41*, 284.
[91] I. L. Karle, R. Kishore, S. Raghothama, P. Balaram, *J. Am. Chem. Soc*. **1988**, *110*, 1958.
[92] B. Di Blasio, F. Rossi, E. Benedetti, V. Pavone, M. Saviano, C. Pedone, G. Zanotti, T. Tancredi, *J. Am. Chem. Soc*. **1992**, *114*, 8277.
[93] M. Saviano, F. Rossi, M. Filizola, B. Di Blasio, C. Pedone, *Acta Crystallogr., Sect. C* **1995**, *51*, 663.
[94] E. Escudero, X. Vidal, X. Solans, E. Peggion, J. A. Subirana, *J. Pept. Sci*. **1996**, *2*, 59.
[95] F. Rossi, M. Saviano, P. Di Talia, B. Di Blasio, C. Pedone, G. Zanotti, M. Mosca, G. Saviano, T. Tancredi, K. Ziegler, E. Benedetti, *Biopolymers* **1996**, *40*, 465.
[96] G. Zanotti, M. Saviano, G. Saviano, T. Tancredi, F. Rossi, C. Pedone, E. Benedetti, *J. Pept. Res.* **1998**, *51*, 460.
[97] C. J. Creighton, T. T. Romoff, J. H. Bu, M. Goodman, *J. Am. Chem. Soc*. **1999**, *121*, 6786.
[98] H. E. Blackwell, R. H. Grubbs, *Angew. Chem., Int. Ed*. **1998**, *37*, 3281; H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary, R. H. Grubbs, *J. Org. Chem*. **2001**, *66*, 5291.
[99] K. N. Koch, G. Hopp, A. Linden, K. Moehle, H. Heimgartner, *Helv. Chim. Acta* **2001**, *84*, 502.
[100] F. Rossi, G. Zanotti, M. Saviano, R. Iacovino, P. Palladino, G. Saviano, P. Amodeo, T. Tancredi, P. Laccetti, C. Corbier, E. Benedetti, *J. Pept. Sci*. **2004**, *10*, 92.
[101] H. Koshima, H. Miyamoto, I. Yagi, K. Uosaki, *Cryst. Growth Des.* **2004**, *4*, 789.
[102] T. Jeremic, A. Linden, H. Heimgartner, *Helv. Chim. Acta* **2004**, *87*, 3056.
[103] T. Jeremic, A. Linden, H. Heimgartner, *Chem. Biodivers.* **2004**, *1*, 1730.
[104] T. Jeremic, A. Linden, K. Moehle, H. Heimgartner, *Tetrahedron* **2005**, *61*, 1871.

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