The Stereochemistry of Peptides Containing α-Aminoisobutyric Aci

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THE STEREOCHEMISTRY OF PEPTIDES CONTAINING α-AMINOISOBUTYRIC ACID

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I. INTRODUCTION

α-Aminoisobutyric acid (Aib),* a nonprotein amino acid first described synthetically,[1] has been found in diverse sources, ranging from peptides of microbial origin[2,3] to the Murchison meteorite.[4] Early studies of the chemistry of Aib were directed towards the synthesis of model peptides containing this "sterically hindered" amino acid.[5-7] There have been several reports on the synthesis of Aib containing analogs of biologically active peptides.[8] These include corticotropin,[9] angiotensin,[10,11] bradykinin,[12] and enkephalins.[13-16] Recent interest in Aib peptides has been stimulated primarily by the widespread occurrence of this residue in a large number of microbial peptide antibiotics several of which have been reported to form transmembrane ion channels (Figure 1).[17-29] The best studied member of this group of natural products is alamethicin,[30-32] a hydrophobic 20-residue peptide that forms voltage-dependent channels in lipid bilayer membranes.[33-35] There has been considerable uncertainty in establishing the precise sequences of these natural peptides, since the microbial products are frequently a complex mixture of closely related polypeptides.[26,36-38] Extensive mass spectrometric analysis using field desorption,[18] fast atom bombardment,[26] and ^{252}Cf-induced plasma desorption[39] methods, coupled with gas chromatographic studies[18,40,41] and HPLC separation of mixtures,[26,36] has permitted a resolution of the problems involved in primary structure determination of the naturally occurring Aib peptides. Attempts to establish conformation-function relationships for membrane channel-forming peptides containing Aib have focused attention on the structural influences exerted by Aib residues on the folding of the peptide backbone.[30-32] Aib residues (referred to in the earlier literature as 2-methylalanine, Mea) are structurally unique as compared to the normal amino acids, possessing a second methyl group at the C^{α} atom rendering the residue achiral. The presence of geminal methyl groups at C^{α} in Aib greatly restricts the range of accessible conformations for this residue. The relatively ready crystallizability of Aib peptides has permitted the accumulation of a considerable body of structural data, using single crystal X-ray diffraction techniques. This review summarizes available information on the solid-state conformations of Aib peptides.

II. THEORETICAL CONFORMATIONAL ANALYSIS

The convention for describing the peptide conformational angles used in this review follows the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature.[42]

* Abbreviations used include Ac, acetyl; Aib, α-aminoisobutyric acid; Boc, t-butyloxycarbonyl; Bz, benzyl; t-Bu, t-butyl; Me, methyl; Piv, Pivaloyl; Tosyl, *p*-toluenesulfonyl; Z, benzyloxycarbonyl. Configurations of all optically active amino acids are L, unless otherwise indicated.

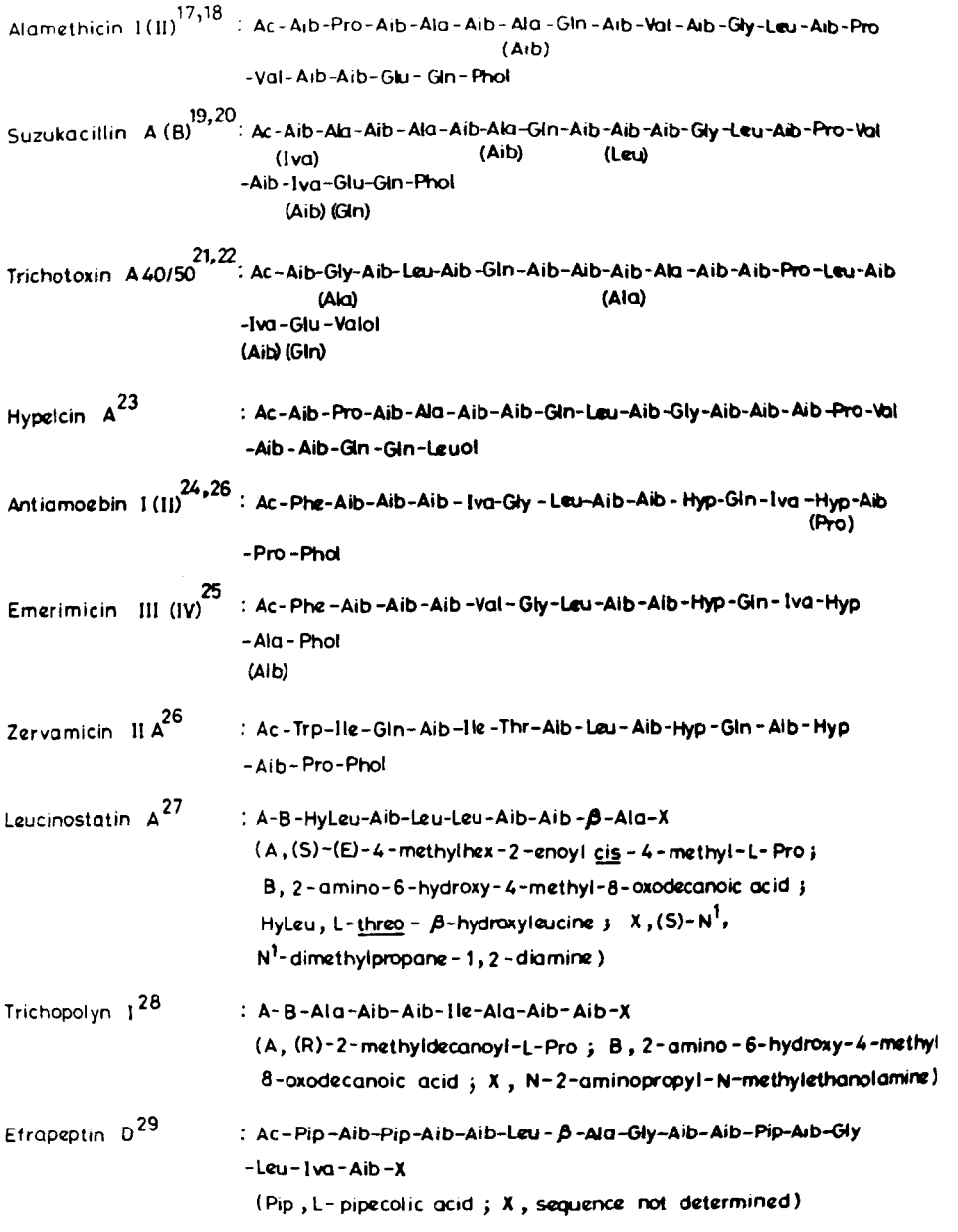
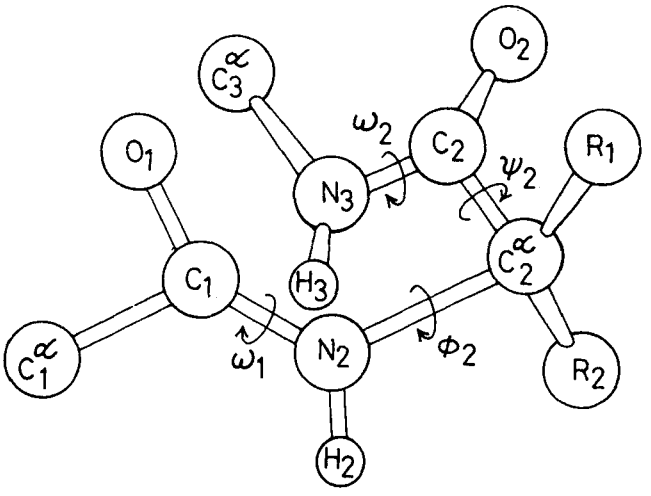


FIGURE 1. Sequences of representative acyclic peptide antibiotics containing Aib. Microheterogeneity of the natural products has only recently been resolved (see References 26, 36, and 38). Some specific replacements are indicated in parentheses. The sequence of suzukacillin has been revised and is taken from Figure 1 of Reference 20.

Several theoretical calculations on the conformational properties of Aib residues in peptides have been reported.[43-48] With two exceptions,[45,48] all theoretical analyses have used semi-empirical methods. These studies attempt to delineate the structural preferences of this amino acid. The basic structural unit used in these theoretical studies is a "dipeptide" unit. Figure 2 defines this basic unit and describes the various conformational variables.[49]

The presence of the geminal methyl groups at C^{α} in Aib implies that sterically allowed regions of conformational space must be restricted only to those regions which are allowed



$R_1 = CH_3$, $R_2 = H$ D-Ala
$R_1 = H$, $R_2 = CH_3$ L-Ala
$R_1 = H$, $R_2 = H$ Gly
$R_1 = CH_3$, $R_2 = CH_3$ Aib

FIGURE 2. Model dipeptide unit (Ac-X-NHMe) used in theoretical calculations. Conformational angles ϕ, ψ, and ω are defined.

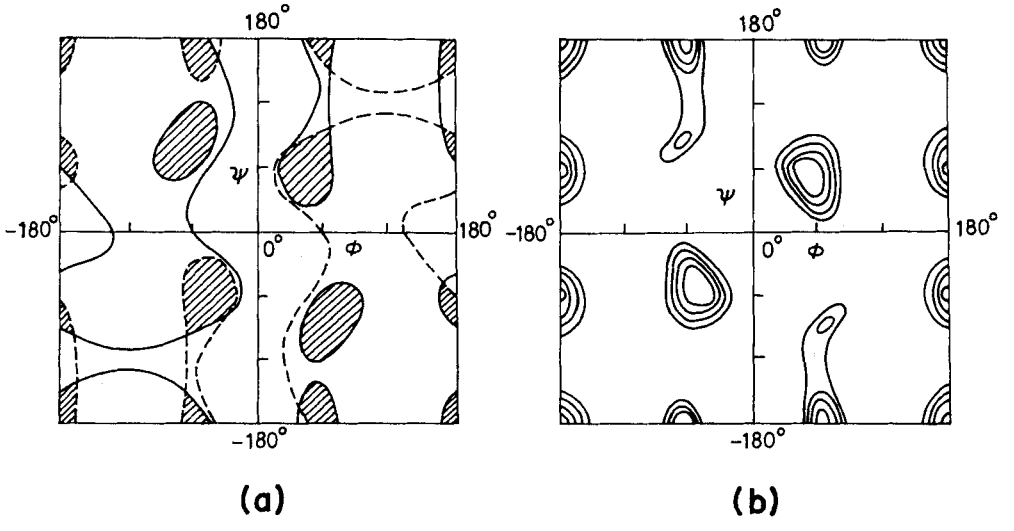


FIGURE 3. (a) Superposition of the conformational maps for L-Ala (continuous line) and D-Ala (broken line) residues. Only the contour for about 5 kcal/mol^{-1} above the minimum is represented. Shaded regions are allowed for both L and D residues. (b) Conformational energy map for the Aib residue. Contours are drawn at 1 kcal/mol^{-1} intervals with respect to the innermost contour enclosing the minimum.

for both L- and D-alanine. Figure 3a shows the superposition of the ϕ,ψ energy maps for L- and D-alanine. It can be seen that only limited regions of the conformational space indicated by shaded regions which are simultaneously accessible to both L- and D-Ala are sterically allowed for the Aib residue. The results of conformational energy calculations on the Aib

dipeptide, using the Kitaigorodsky potential function, were first described by Marshall and Bosshard in 1972.[43] This study indicated that right- and left-handed α-helical regions are energetically most favorable. Subsequent analyses[44-48] essentially corroborated these findings and also suggested that in repetitive Aib sequences, alternation of left- and right-handed conformations is not favorable.[45] Furthermore, specific hydrogen-bonded conformations like $3 \rightarrow 1$ (C_7) ($\phi = -80°$, $\psi = +70°$) have been shown to be energetically less favorable for an Aib residue even though the steric contacts are generally acceptable. Molecular orbital calculations, using the PCILO method, have shown that C_7^{eq} and C_7^{ax} hydrogen-bonded ring conformations are the most stable, although α-helical regions of the map have relatively low energy. It is believed that these C_7 conformations are of low energy because the repulsive parameters for this molecular orbital calculation are too soft and that the stabilization energy arising from the formation of an intramolecular hydrogen bond is too large.[45]

Theoretical calculations on Ac-Aib-NHMe have been carried out using both Kitaigorodsky and Buckingham "6-exp" potential functions.[46] Table 1 lists the total energy for the Aib residue in various regular conformations and also indicates the specific stereochemical contacts which are unfavorable. It is seen that the difference between α- and 3_{10}-conformations is small at the dipeptide level with only a single short contact being present in the latter. A particularly interesting feature is that the parallel and antiparallel β-pleated sheet conformations are extremely unfavorable, while the C_7 conformation is approximately 3.5 kcal/mol above the energy minimum. Apart from the α-helical region, other minimum energy regions in the (ϕ, ψ) map which have comparable energies are $\phi \sim 180°$, $\psi \sim 180°$ wherein intraresidue (C_5) hydrogen bond formation is facilitated; $\phi \sim |60°|$, $\psi \sim 180°$ and $\phi \sim 180°$, $\psi \sim |60°|$.

The ϕ, ψ energy map for the Aib dipeptide computed using the Buckingham "6-exp" function is shown in Figure 3b. This ϕ, ψ map indicates that the most probable regular structures for a homopolymer of Aib are α- and 3_{10}-helices. In early investigations, the similarity of the IR spectrum and the X-ray powder diffraction pattern of poly (Aib) with that of an α-helical polymer was noted and it was suggested that poly (Aib) adopts an α-helical structure.[50,51] However, the short distance between the methyl groups of the adjacent turns of an α-helix of poly(Aib) has led to the suggestion that a 3_{10}-helix should also be considered.[52]

Electron diffraction and IR studies on poly(Aib) have been reported by Malcolm.[53] A series of meridional reflections at spacings 5.98, 2.94, 1.99, 1.49, and 1.17 Å on the equator at 7.53 Å (strong) and 4.25 Å (weak) and a strong nonaxial reflection at 5.96 Å were observed. Indexing the meridional reflections on a cell with a *c* axis repeat of 5.96 Å and thereby assigning the only strong nonaxial reflection to the turn reflection of the helix with pitch 5.96 Å, the data have been interpreted in terms of the 3_{10}-helix. The regular α-helix has been eliminated inspite of the presence of a meridional reflection at 1.49 Å, for such a helix with pitch of 5.4 Å cannot account for the observed turn reflection.[53] A more recent electron diffraction investigation of poly(Aib) treated with dichloroacetic acid provides strong support for a 3_{10}-helical structure. Enantiomeric 3_{10}-helices are packed into a hexagonal cell forming a honeycomb structure containing dichloroacetic acid molecules in the holes.[158]

A novel fourfold helical structure which is both energetically favorable and compatible with electron diffraction data has been suggested from systematic helical energy calculations,[46] which incorporate nonplanar distortions of the peptide unit ($\Delta\omega = \omega - 180°$) and deviations in $NC^{\alpha}C$ and $C^{\beta L}C^{\alpha}C^{\beta D}$ angles. In the range of $\Delta\omega \sim -10$ to 10° studied, α-helical conformations are preferred in the region $-3 < \Delta\omega < 10°$ and 3_{10}-helical conformations are preferred in the region $-3 > \Delta\omega > -10°$. Minimum energy conformations for right-handed structures are found in the positive region of $\Delta\omega$, and correspondingly for left-handed structures in the negative region of $\Delta\omega$. For $\Delta\omega \sim 6°$, α-helical structures have four or near fourfold symmetry with $h \sim 1.5$ Å. Such a helix with $n = 4$ and $h = 1.5$ Å

Table 1
RELATIVE STABILITIES OF THE AIB RESIDUE IN VARIOUS REGULAR CONFORMATIONS

Conformation	Conformational angles (°) ϕ	ψ	Total energy* (kcal/mol^{-1})	Short contacts
Lowest energy	−50	−50	−2.63	—
α-Helix	−60	−50	−2.37	—
3_{10}-Helix	−60	−30	−1.69	$N_2 \ldots (N_3, H_3)$
2.2_7-Helix (γ-turn)	−80	80	0.96	$C^{\beta L} \ldots O_2$; $C^{\beta D} \ldots (N_3, H_3)$; $O_1 \ldots C^{\beta D}$
Collagen	−80	150	3.19	$c^{\beta D} \ldots (N_3, H_3)$; $(C_1, O_1) \ldots C^{\beta D}$
Parallel β-structure	−120	120	8.24	$C^{\beta D} \ldots (N_3, H_3)$; $C^{\beta D} \ldots (C_1, O_1)$
Antiparallel β-structure	−140	140	8.00	$C^{\beta D} \ldots (N_3, H_3)$; $C^{\beta D} \ldots (C_1, O_1)$
Poly(glycine)I	−150	150	5.57	$C^{\beta D} \ldots (N_3, H_3)$; $C^{\beta D} \ldots (C_1, O_1)$

* Total energy is the sum of nonbonded, electrostatic, and torsional contributions, estimated as described in Reference 49. Energies have been calculated for the "dipeptide" Ac-Aib-NHMe.

is termed an α′-helix.[46] The energetically best 3_{10}- and α′-helical structures suggested for poly (Aib) are illustrated in Figure 4. Further experimental investigations are necessary to establish the precise helical conformation of poly (Aib).

During the past 5 years a remarkably large number of crystal structures of Aib peptides have been determined. With very few exceptions, ϕ, ψ values for Aib residues have generally been clustered in the right- and left-handed 3_{10}-/α-helical regions of the (ϕ, ψ) map, as shown in Figure 5. These structures are examined in greater detail in the subsequent sections.

Figure 6 shows the average geometry of the Aib residue as determined from crystal structure analyses, which may be employed in further refinement of theoretical calculations. A general feature regarding the bond lengths in all these structures is that they are quite unexceptional and conform to average bond lengths reported for similar systems.[54] One interesting feature that emerges is that bond angles around the C^α atom of Aib are intimately correlated to the conformation.[47] For a residue having right-handed helical conformation, the angles N–C^α–$C^{\beta D}$ and C–C^α–$C^{\beta D}$ tend to become larger than the tetrahedral value, whereas the N–C^α–$C^{\beta L}$ and C–C^α–$C^{\beta L}$ become smaller. Exactly the reverse holds true for an Aib residue in a left-handed helical conformation. This asymmetric geometry around C^α atom of the Aib residue has been implicated in rationalizing the preference for the 3_{10}-helical conformation in Aib peptides.[47]

Nonplanar distortion of the peptide unit may be important in relieving unfavorable steric contacts. The survey of Aib peptide crystal structures does not reveal any specific tendency of peptide bonds involving Aib as N- or C-terminal residue to be distorted significantly from the value of 180°. The generally observed values of $\Delta\omega$ are no different from those observed in the crystal structures of peptides in general.[54] There are, however, two examples of Aib-containing peptides having large deviations. These are the cyclic peptide dihydrochlamydocin[55] where the Aib-Phe peptide bond has $\Delta\omega = -18°$ and X-Aib has $\Delta\omega = 13°$, and the acyclic peptide Z-(Aib-Pro)$_2$-OMe, where the Pro-Aib peptide bond has $\Delta\omega = 14°$.[56] Another feature meriting comment is that the directionality of the nonplanar distortion in the peptide unit

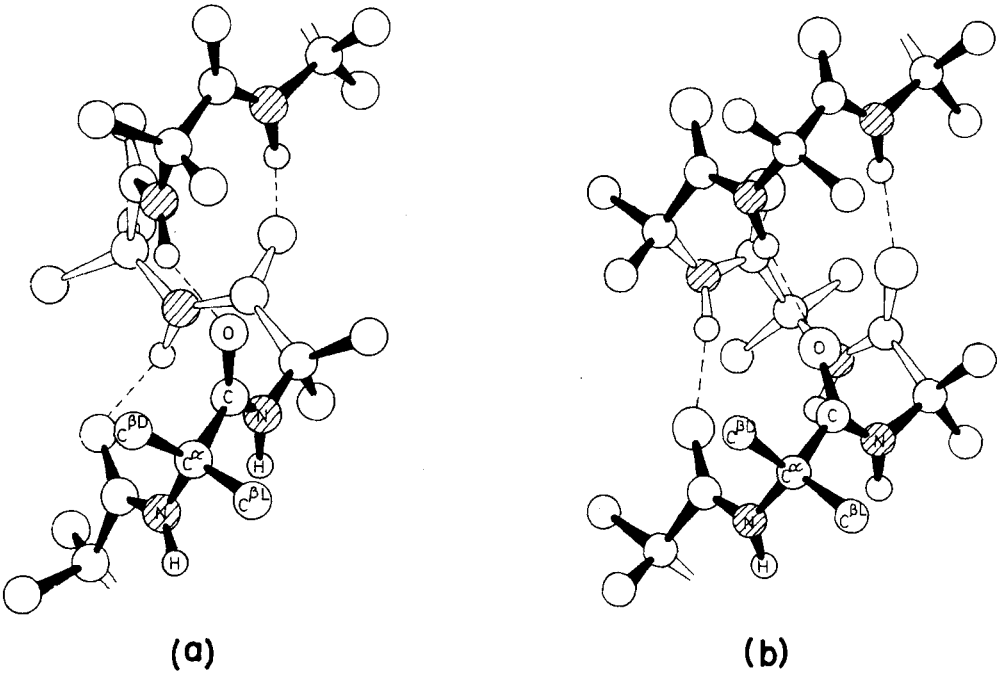


FIGURE 4. Helical conformations proposed for poly (Aib). (a) 3_{10}-helix [n = 3.19, h = 1.89 Å; $\phi = -50°$, $\psi = -35°$, $\Delta\omega = 0°$, τ (NĈ$^{\alpha}$ C) = 112°]. The intramolecular hydrogen bonds are indicated by dashed lines (N... O, 2.88 Å; HN̂O = 17.5°). (b) α'-helix[46] [n = 4.00, h = 1.48 Å; $\phi = -55°$, $\psi = -60°$, $\Delta\omega = 7°$, τ (NĤ$^{\alpha}$ C) = 110°; N...O = 3.07 Å, HN̂O = 9.3°].

connecting two Aib residues is related to conformation. In Aib-Aib sequences adopting right-handed helical conformations, the peptide unit has positive values of $\Delta\omega$ and in those adopting left-handed helical conformations, it has negative values of $\Delta\omega$. Specific ω values are listed in Tables 2 to 5.

III. β-TURN CONFORMATIONS

β-turns (Figure 7), formed by structures involving three linked peptide units, are responsible for chain reversal in polypeptide structures.[57,58] The stereochemistry of β-turns is determined by the backbone conformational angles of the two corner residues (denoted as i + 1 and i + 2). These structures were orginally classified into three major categories:[57,58] type I — $\phi_{i+1} = -60°$, $\psi_{i+1} = -30°$, $\phi_{i+2} = -80°$, $\psi_{i+2} = 0°$; type II — $\phi_{i+1} = -60°$, $\psi_{i+1} = 120°$; $\phi_{i+2} = 80°$, $\psi_{i+2} = 0°$; type III — $\phi_{i+1} = \phi_{i+2} = -60°$, $\psi_{i+1} = \psi_{i+2} = -30°$ and the corresponding enantiomeric structures (types I′, II′, and III′), where the signs of all the ϕ, ψ angles are reversed. In all these structures an intramolecular 4 → 1 hydrogen bond between the NH of residue i + 3 and the CO of residue i stabilizes the folded conformation. The peptide bonds are *trans* in all three types of β-turns. Type II and II′ structures have also been referred to as LD or DL bends, since the presence of amino acids of opposite chirality at the corners favors these structures.[59] The Type I and III β-turns differ very slightly in the ϕ, ψ values for residue i + 2 and should really be considered as only small variants of the same general conformation. Subsequent analyses of protein crystal structure data have led to more elaborate classifications involving a central *cis* peptide unit (type VI) and structures not involving 4 → 1 hydrogen bonds.[60,61] In these cases, $C_i^{\alpha} - C_{i+3}^{\alpha}$ distances of 4 to 7 Å have been used as criteria for establishing

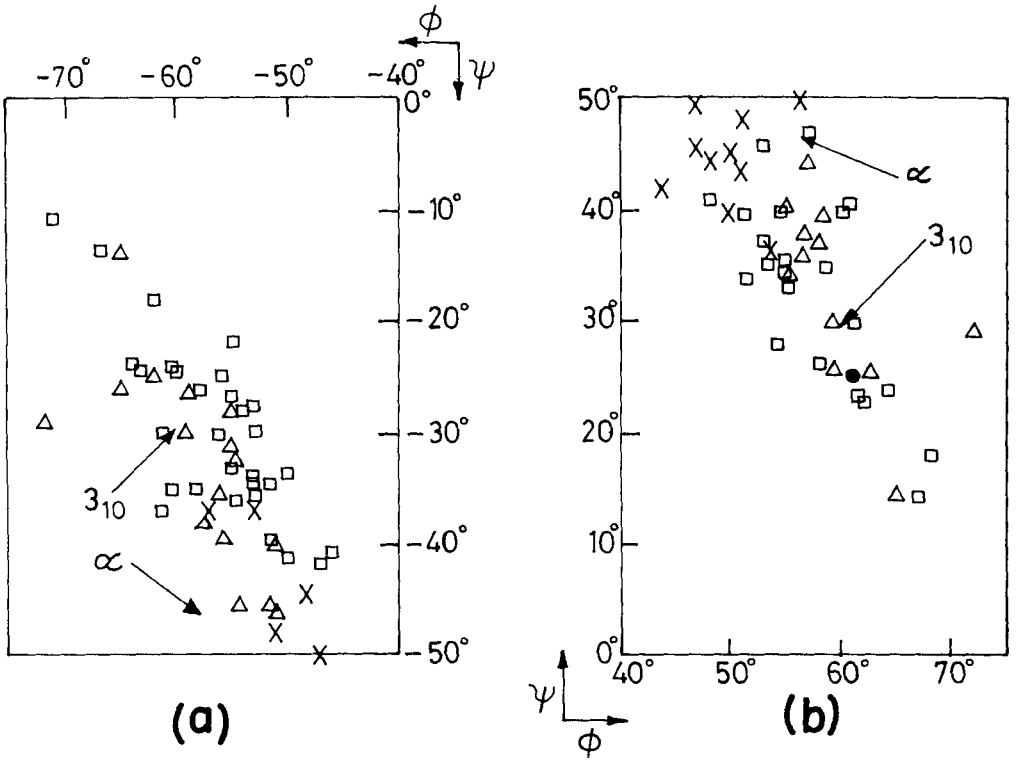


FIGURE 5. Crystallographic observations for Aib residues in peptides represented on the ϕ, ψ map. (a) Right-handed helical region. (b) Left-handed helical region. Tips of arrows indicate ideal right- and left-handed α- and 3_{10}-helixes. Δ, N-terminal residues in amino-protected peptides. □, Nonterminal residues. C-terminal Aib, protected as amide. X, C-terminal Aib present as ester or free acid. Definition of ψ is taken as N–C^{α}–C–O, where O is not the carbonyl oxygen. A general feature is that in helical Aib peptides, the C-terminal Aib adopts conformations of opposite chirality to the rest of the molecule.

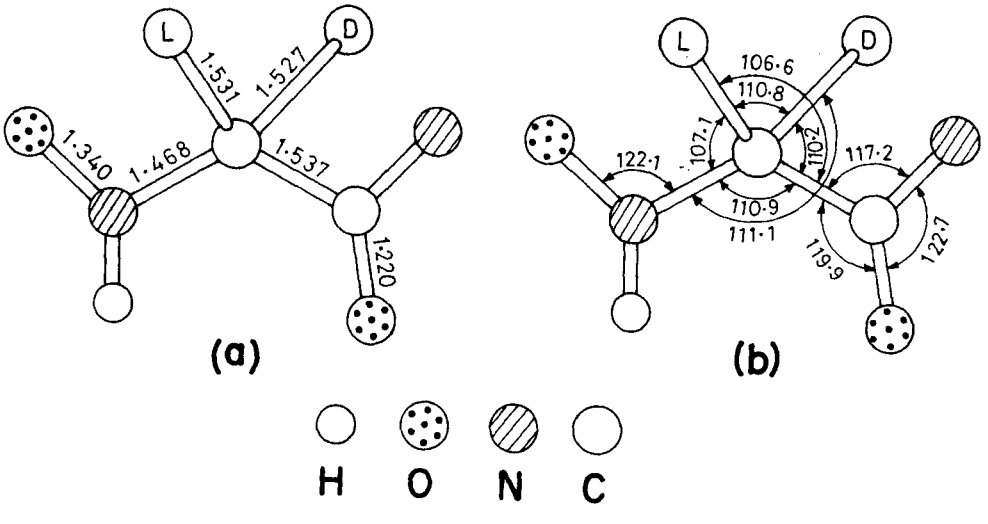


FIGURE 6. Average geometry for the Aib residue derived from crystal structure data for residues in peptides. (a) Bond lengths. (b) Bond angles.

Table 2
CONFORMATIONAL PARAMETERS FOR AIB CONTAINING β-TURN STRUCTURES

			Conformational angles (°)			Hydrogen bond parameters			
Peptide	R value	Residue	φ	ψ	ω	N . . . O (Å)	(H–N̂ . . . O) (°)	Type of β-turn	Ref.
Z-Aib-Pro-NHMe	0.054	Z	—	—	−175				63
		Aib	−51	−40	−174				
		Pro	−65	−25	180	3.12	(28.6)	III	
Z-Aib-Pro-Aib-Pro-OMe	0.051	Z	—	—	179				56
		Aib	−55	−31	180				
		Pro	−72	−4	166				
		Aib	57	47	−177	3.10	(16.0)	I	
		Pro	−77	158[a]					
Z-Aib-Pro-Aib-OMe	—	Z	—	—	—				64
		Aib	−52	−39	—				
		Pro	−73	−15	—				
		Aib	−50	−45[a]	—			III	
Piv-Pro-Aib-NHMe	0.061	Piv	—	—	175				
		Pro	−58	139	−179				68
		Aib	61	25	177	2.98	(30.5)	II	
Z-Aib-Aib-Ala-OMe	0.053	Z	—	—	167				72
		Aib	58	37	176				
		Aib	68	18	−178	3.10	(16.0)		
		Ala	−136	56[a]	—				
Z-Aib-Aib-Aib-O-t-Bu	0.053	Z	—	—	173				71
		Aib	−65	−15	170				
		Aib	−47	−41	179				
		Aib	57	37[a]	—	3.03		III or III′	
Z-Aib-Aib-Aib-OH.H_2O	—	Z	—	—	—				64
		Aib	−59	−27	—				
		Aib	−67	−14	—				
		Aib	−47	−53[a]	—			III or III′	

[a] Definition of ψ is taken as N–C^{α}–C–O, where O is not the carbonyl oxygen.

Table 3
CONFORMATIONAL PARAMETERS OF CONSECUTIVE β-TURN STRUCTURES

Peptide	R value	Residue	Conformational angles (°)			Hydrogen bond parameters		Type of β-turn	Ref.
			ϕ	ψ	ω	N . . . O (Å)	(H–N̂ . . . O) (°)		
Boc-Pro-Aib-Ala-Aib-OBz	0.087	Boc	—	—	−174				77
		Pro	−53	−38	−177				
		Aib	−50	−41	−174				
		Ala	−91	−11	−176	3.01		III	
		Aib	44	42[a]	—	3.10		I	
Z-Aib-Pro-Aib-Ala-OMe	0.031	Z	—	—	−176				73, 74
		Aib	−51	−45	−171				
		Pro	−55	−36	170				
		Aib	−72	−11	−173	3.16		III	
		Ala	−68	156[a]	—	3.06		I	
Z-Aib-Aib-Aib-Aib-OH	0.075	Z	—	—	−165				71
		Aib	−56	−36	−178				
		Aib	−54	−28	174				
		Aib	−58	−35	179	2.95		III or III′	
		Aib	48	44[a]	—	2.88		III or III′	
Boc-Aib-Aib-Phe-Met-NH_2	0.103	Boc	—	—	—				80
		Aib	−54	−46	−169				
		Aib	−60	−35	−173				
		Phe	−84	−7	170	3.05	(28.6)	III	
		Met	−82	−8	—	3.06	(12.2)	I	
Boc-Cys-Pro-Aib-Cys-NHMe (S–S)	0.069	Boc	—	—	175				82, 83
		Cys	−121	66	175				
		Pro	−59	−34	179				
		Aib	−62	−18	176	2.94	(28.1)	III	
		Cys	−71	−19	−179	2.98	(13.3)	III	

Table 3 (continued)
CONFORMATIONAL PARAMETERS OF CONSECUTIVE β-TURN STRUCTURES

Peptide	R value	Residue	Conformational angles (°)			Hydrogen bond parameters		Type of β-turn	Ref.
			ϕ	ψ	ω	N . . . O (Å)	(H–N̂ . . . O) (°)		
Boc-Hyp-Aib-Aib-Phol	0.056	Boc	—	—	−173				79
		Hyp	−60	−30	180				
		Aib	−56	−27	180				
		Aib	−56	−25	173	3.00		III	
		Phol	−74	−49	—	2.97		III	
Z-Aib-Aib-Aib-Val-OMe	0.052	Z	—	—	175				81, 161
		Aib	56	38	174				
		Aib	61	23	180				
		Aib	54	40	172			III′	
		Val	−65	−30[a]	—			III′	
p-Chlorobenzoyl-Pro-Aib-Ala-Aib-Ala-OMe	0.036	Pcb[b]	—	—	−178				84
		Pro	−56	145	175				
		Aib	55	34	178				
		Ala	56	38	179	3.05		II	
		Aib	61	40	168	3.00		III′	
		Ala	−53	144[a]	—	3.10		III′	

[a] Definition of ψ is taken as N–C^{α}–C–O, where O is not the carbonyl oxygen.
[b] Pcb = p-chlorobenzoyl.

Table 4
CONFORMATIONAL PARAMETERS OF 3_{10}-HELICAL AIB PEPTIDES[a]

Peptide	R value	Residue	Conformational angles (°) φ	ψ	ω	Hydrogen bond parameters N . . . O (Å)	(H–N̂ . . . O) (°)	Ref.
Tosyl-Aib-Aib-Aib-Aib-Aib-OMe (Pbca)	0.08	Aib	−62	−25	−179			90
		Aib	−51	−39	−173			
		Aib	−54	−36	−174	3.01		
		Aib	−64	−24	−172	3.06		
		Aib	53	38[b]	—	3.07		
Tosyl-Aib-Aib-Aib-Aib-Aib-OMe ($P\bar{1}$)	0.124	Aib	−72	−29	−173			91
		Aib	−53	−35	−172			
		Aib	−58	−26	−176	2.89	(20.3)	
		Aib	−57	−35	−178	3.02	(13.1)	
		Aib	−51	48[b]	−	3.10	(24.0)	
Boc-Leu-Aib-Pro-Val-Aib-OMe	0.069	Boc	—	—	180			92, 94
		Leu	−104	−30	178			
		Aib	−46	−41	−171			
		Pro	−65	−15	173			
		Val	−59	−38	174	2.92	(9.9)	
		Aib	51	43[b]	—	3.27	(25.4)	
Z-Aib-Aib-Aib-Aib-Aib-O-*t*-Bu	0.062	Z	—	—	−173			71
		Aib	−59	−29	180			
		Aib	−52	−34	−174			
		Aib	−55	−33	−172	3.01		
		Aib	−61	−30	−174	3.05		
		Aib	51	145[b]	—	3.17		
Boc-Ala-Aib-Ala-Aib-Aib-OMe	0.065	Boc	—	—	−177			92, 93
		Ala	−58	−37	−175			
		Aib	−56	−30	171			
		Ala	−64	−13	170	3.04	(11.0)	
		Aib	−55	−22	177	3.09	(13.0)	
		Aib	56	51[b]	—	3.01	(8.0)	

Table 4 (continued)
CONFORMATIONAL PARAMETERS OF 3_{10}-HELICAL AIB PEPTIDES[a]

Peptide	R value	Residue	Conformational angles (°)			Hydrogen bond parameters		Ref.
			φ	ψ	ω	N . . . O (Å)	(H–N̂ . . . O) (°)	
Boc-Aib-Pro-Val-Aib-Val-OMe·H_2O	0.101	Boc	—	—	−171			91
		Aib	−51	−46	180			
		Pro	−74	−11	−176			
		Val	−106	−52	−176	3.08	(14.0)	
		Aib	−61	−37	179	3.01	(20.0)	
		Val	−104	−56[b]	—	3.27	(21.0)	
Z-Aib-Aib-Aib-Val-Gly-OMe	0.049	Z	—	—	−179			81, 161
		Aib	−55	−33	−178			
		Aib	−53	−30	−174			
		Aib	−60	−24	−179			
		Val	−109	15	−178			
		Gly	−66	164[b]	—			

[a] The crystal structure of Ac-Aib-Pro-Aib-Ala-Aib-OBz has been reported in Reference 156. However, no φ, ψ values are available. The peptide has three intramolecular hydrogen bonds and may represent a 3_{10}-helical conformation.

[b] Definition of ψ is taken as N–C^{α}–C–O, where O is not the carbonyl oxygen.

Table 5
CONFORMATIONAL PARAMETERS OF Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe[102]

	Conformational angles			Hydrogen bond parameters	
Residue	ϕ	ψ	ω	N . . . O (Å)	HN̂ . . . O(°)
Boc	—	—	−174	—	—
Aib	−53	−35	−176	—	—
Pro	−59	−27	−179	—	—
Val	−70	−16	−178	3.19	9
Aib	−53	−34	−176	3.00	15
Val	−64	−12	175	3.46	6
Ala	−61	−16	171	3.02	4
Aib	−50	−33	−177	3.13	0
Ala	−65	−20	176	2.94	9
Aib	−63	−24	−179	3.00	19
Aib	50	40[a]	—	3.11	12

[a] Definition of ψ is taken as N–C$^{\alpha}$–C–O, where O is not the carbonyl oxygen.

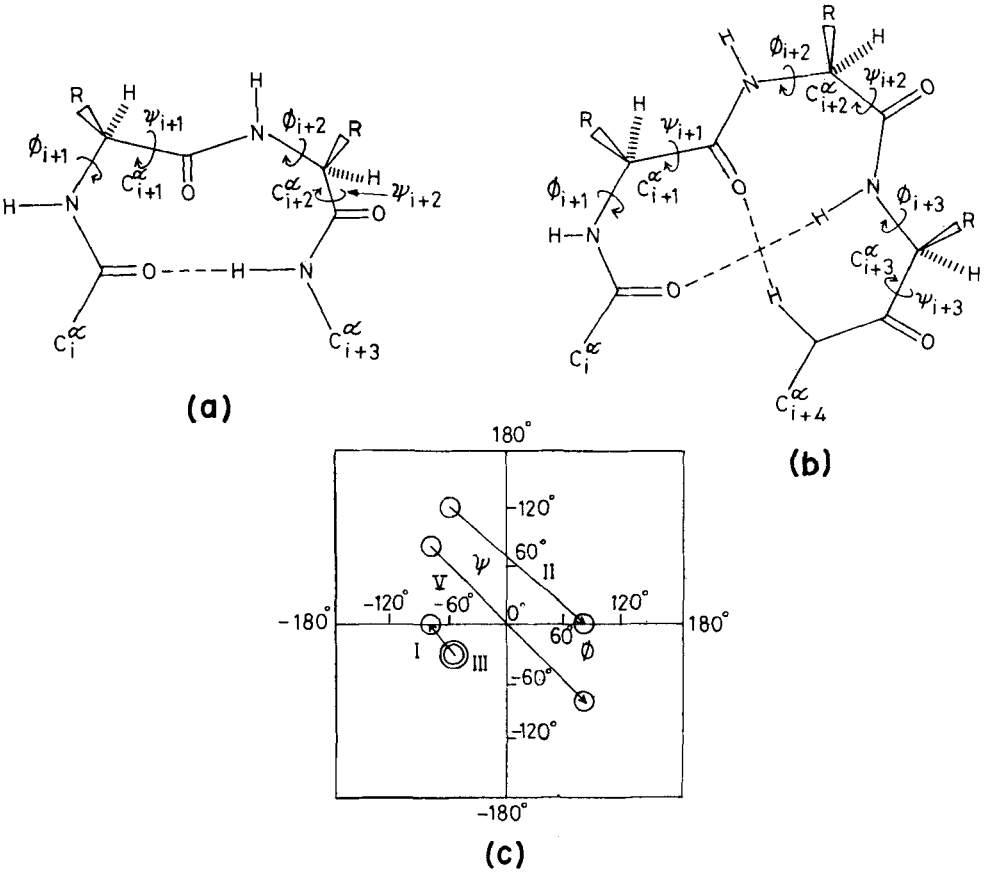


FIGURE 7. (a) Schematic representation of the β-turn structure. (b) Consecutive β-turn conformation. (c) Specific β-turn conformations represented on a ϕ, ψ map. Arrows point from residue i + 1 to residue i + 2.

chain reversals. In the present context of surveying β-turns in small Aib peptides, only the type I, II, and III β-turns (and their enantiomers) are relevant.

Aib residues in acyclic peptides largely favour ϕ, ψ values of $\sim \pm 60° \pm 20°$ and $\pm 30° \pm 20°$. The positive ϕ, ψ values correspond to the left-handed helical regions of the conformational map, while negative ϕ, ψ values lie in the right-handed helical region (α-helix $\phi \sim \pm 55°$, $\psi \sim \pm 45°$; 3_{10}-helix $\phi \sim \pm 60°$, $\psi \sim \pm 30°$). The presence of Aib results in a stabilization of β-turn conformations.[32] Aib residues can be readily accommodated at both corners of type I, I′, III, and III′ β-turns. Further, Aib can also occupy the i + 2 position (right-hand corner) of type II and II′ β-turns. Crystallographic studies of Aib containing protected di and tripeptides have provided several examples of specific β-turn structures. Table 2 lists the conformational parameters for various Aib peptides adopting β-turn structures in the solid state. Figure 8 illustrates the backbone conformation in selected examples of type I, II, III, and III′ β-turns. In all cases there is an intramolecular 4 → 1 hydrogen bond, with N—O distances ranging from 2.90 to 3.12 Å. These values are similar to N—O distances reported in other β-turn peptides.[54,62]

The structures of Z-Aib-Pro-Aib-OMe[64] and Z-$(Aib\text{-}Pro)_2$-OMe[56] are interesting examples of L-Pro residues occupying the i + 2 position of type I(III) β-turns. Theoretical calculations have led to the conclusion that L-X-Pro β-turns are energetically unfavorable due to nonbonded contacts between the C^δ atom of the pyrrolidine ring and the C^β group of the X residue.[65] This conclusion has been reinforced by an analysis of the Pro-containing β-turn structures observed in proteins. Of the 459 β-turn structures examined from 29 protein crystal structures, 58 occur with Pro at the i + 1 position and 12 with Pro at the i + 2 position. Of the latter, 8 have *cis* X-Pro bonds (type VI β-turn).[61] However, in the case of the Aib peptides, the presence of the Aib residue appears to dictate peptide chain folding, forcing the L-Pro residue into the less favored i + 2 position of a type I(III) β-turn. In Aib-Pro sequences, *cis* conformations about the Aib-Pro bond are sterically highly unfavorable, precluding the formation of type VI β-turns. ^{13}C NMR studies have established an almost exclusive preference for *trans* Aib-Pro geometries in solution for a very large number of peptides.[66,67]

The Pro-Aib sequence can, in principle, adopt either type II or III conformations. In Piv-Pro-Aib-NHMe an almost ideal type II β-turn is observed in the solid state.[68] Intriguingly, both Pro-L-Ala and Pro-D-Ala sequences, in protected dipeptide alkylamides, adopt type II β-turn conformations in the crystalline state.[69,70] Conformational energy calculations for Ac-Pro-Aib-NHMe suggest that there is only a small energy difference of 1.5 kcal/mol^{-1} in favor of the type II conformation.[68] Examples of Pro-Aib sequences adopting type III structures are considered later.

In the achiral peptides Z-$(Aib)_3$-O-t-Bu and Z-$(Aib)_3$-OH, the centrosymmetric crystals consist of both the enantiomeric type III and III′ β-turn conformations.[71] In Z-Aib-Aib-Ala-OMe, the presence of a single chiral center leads to a preference for a Type III′ structure.[72] So far the only classical β-turn conformation which has not been characterized in an Aib peptide is the type II′ structure. An analysis of a D-Pro-Aib sequence should undoubtedly fill this gap.

IV. CONSECUTIVE β-TURNS

The marked tendency of Aib residues to occupy either position i + 1 or i + 2 in β-turns results in the possibility of generating consecutive β-turn structures[73,74] in -X-Aib-Y sequences. These conformations are characterized by three sets of conformational angles: (1) ϕ_{i+1}, ψ_{i+1}; (2) ϕ_{i+2}, ψ_{i+2}; and (3) ϕ_{i+3}, ψ_{i+3} (Figure 7b). In such structures the central Aib residue occupies the right-hand corner position of the first β-turn and the left-hand corner position of the second β-turn when the chain is viewed as progressing from the

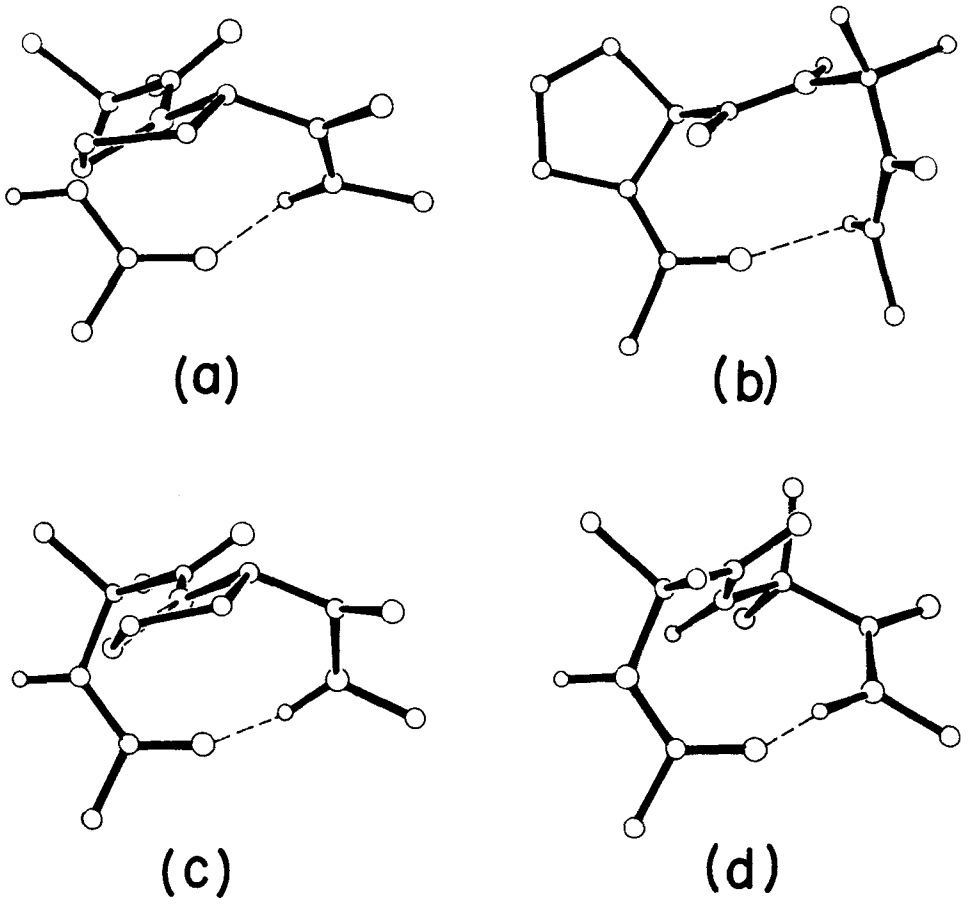


FIGURE 8. β-turn conformations in Aib peptides. (a) Z-Aib-Pro-Aib-Pro-OMe (Aib-Pro, type I).[56] (b) Piv-Pro-Aib-NHMe (Pro-Aib, type II).[68] (c) Z-Aib-Pro-NHMe (Aib-Pro, type III).[63] (d) Z-Aib-Aib-Ala-OMe (Aib-Aib, type III').[72] Only the β-turn portion of the structures is shown. C = O...H–N 4 → 1 hydrogen bonds are indicated.

N to C terminal. The presence of a common residue limits the number of consecutive β-turns that are stereochemically possible. These are

1. Type III-III (or III'-III'), where $\phi_{i+1} = \phi_{i+2} = \phi_{i+3} \sim -60°$ (+60°) and $\psi_{i+1} = \psi_{i+2} = \psi_{i+3} \sim -30°$ (+30°). These structures form incipient right- and left-handed 3_{10}-helices.
2. Type II-III', where $\phi_{i+1} \sim -60°$, $\psi_{i+1} \sim 120°$; $\phi_{i+2} \sim 80°$, $\psi_{i+2} \sim 0°$; $\phi_{i+3} \sim 80°$, $\psi_{i+3} \sim 0°$, and its enantiomer type II'-III, where the signs of all the ϕ, ψ angles are reversed. In considering these structures the types I and III (or I' and III') β-turns have not been separately classified. For sequences with Aib as the central (i + 2) residue, all these conformations are possible, in principle, and the precise structure obtained may depend on the structural preference of the X and Y residues. Repetitive β-turns may also be favored when the amino acid at position i + 2 is a strongly β-turn-favoring residue like L-Pro.[75] The existence of multiple bend structures in proteins has in fact been analyzed.[76]

Table 3 lists the conformational parameters for consecutive β-turns observed in Aib peptide crystal structures. Three illustrative examples are shown in Figure 9. There are six examples

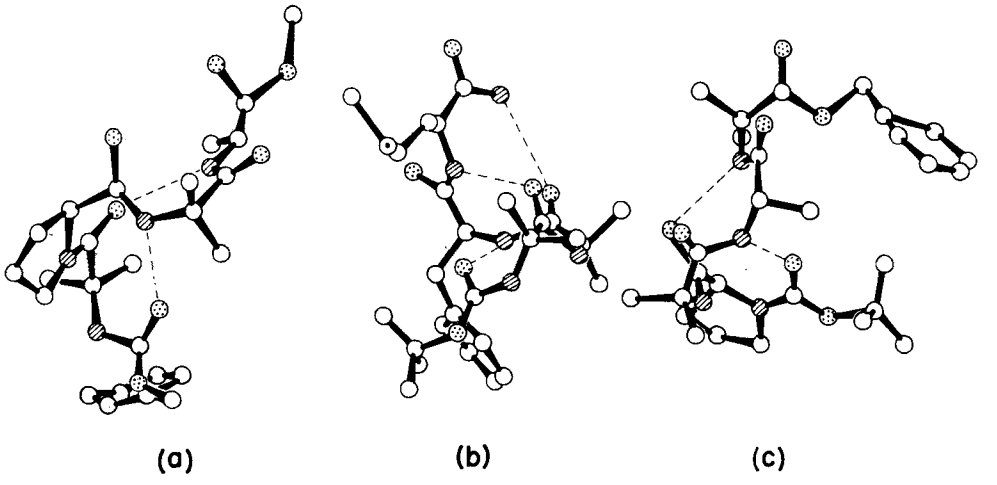


FIGURE 9. Consecutive β-turns in Aib peptides. (a) Z-Aib-Pro-Aib-Ala-OMe.[73] (b) Boc-Aib-Aib-Phe-Met-NH_2.[80] (c) Boc-Pro-Aib-Ala-Aib-OBz. Coding scheme for C, N, O, S atoms is shown. This is followed in all subsequent figures. (FIGURE 9c redrawn from Smith, G. D. et al., Crystal structures and conformational calculations of fragments of alamethicin containing aminoisobutyric acid, *J. Am. Chem. Soc.*, 103, 1493, 1981. With permission.)

of structures which can be considered as incipient 3_{10}-helices as they are composed of repetitive type III-III or type III-I β-turns. In all cases two intramolecular 4 → 1 hydrogen bonds stabilize the structure with N-O distances ranging from 2.94 to 3.16 Å. The structure of Boc-Pro-Aib-Ala-Aib-OBz (Figure 9c)[77] provides a rare example of a *trans* conformation for the urethane protecting group, where Pro is the amino-terminal residue. This presumably is a consequence of the additional stabilization resulting from formation of the Pro-Aib β-turn. In general, urethane groups in crystal structures of peptides with L-terminal Pro have been observed to favor the *cis* geometry.[78,159] A *trans* conformation is also observed for the urethane moiety in Boc-Hyp-Aib-Aib-Phol.[79]

In Z-Aib-Pro-Aib-Ala-OMe (Figure 9a) the central residue (i + 1) is L-Pro, constraining ϕ_{i+1} to a value of ~ −60°, thus leading to a type III-I structure.[73,74] Z-Aib-Pro-Aib-Ala-OMe and Boc-Pro-Aib-Ala-Aib-OBz provide clear examples of the accommodation of Pro residues at the N-terminal end of helical peptides. This is relevant in view of the proposal that helical folding may be nucleated by initial formation of consecutive β-turns in sequences which are rich in β-turn-promoting residues.[75]

In the achiral peptide Z-$(Aib)_4$-OH, incipient right- and left-handed 3_{10}-helical conformations (type III-III and type III′-III′) are observed in the centrosymmetric crystal.[71] The crystal structure of Boc-Aib-Aib-Phe-Met-NH_2La a fragment of a biologically active enkephalin analog (Figure 9b), reveals a consecutive type III-III structure.[80] There is also evidence for further folding of this peptide chain by formation of the type III(I) Phe-Met- β-turn, though the distance between Aib(2)CO and the C-terminal carboxamide nitrogen is 3.35 Å, which is rather long for a good hydrogen bond. Z-$(Aib)_3$-Val-OMe adopts a consecutive type III′-III′ structure which corresponds to an incipient left-handed 3_{10}-helix.[81] The effect of lengthening the peptide chain in this sequence upon helix handedness is considered later. A novel disulfide-bridged consecutive β-turn structure has been established in the peptide Boc-Cys-Pro-Aib-Cys-NHMe (Figure 22a).[82,83] Here the -Pro-Aib-Cys- residues generate

S S

the type III-III conformation and the S-S bridge lies approximately parallel to the axis of the incipient helix. This also constitutes an example of an Aib residue in a cyclic structure, and this aspect is considered in the section on cyclic peptides.

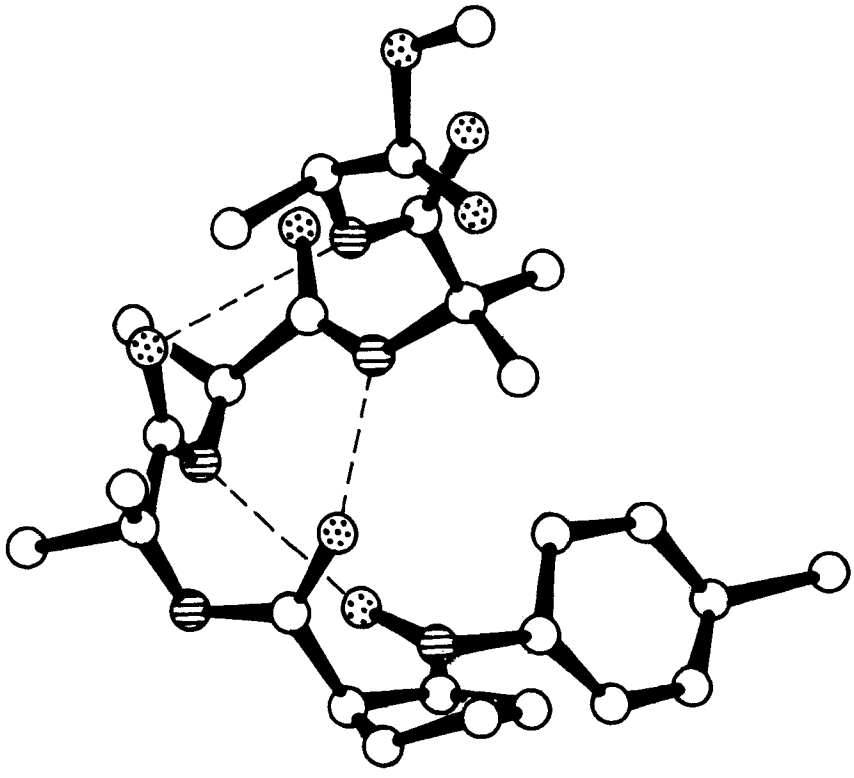


FIGURE 10. Molecular conformation of *p*-chlorobenzoyl-Pro-Aib-Ala-Aib-Ala-OMe.[84] (Redrawn from published coordinates.)

The crystal structure of *p*-chlorobenzoyl-Pro-Aib-Ala-Aib-Ala-OMe (Figure 10) exemplifies a type II-III′ consecutive β-turn.[84] Here Aib(2) is at position i + 2 with $\phi = 55°$, $\psi = 34°$. The repetitive β-turn conformation is further continued to generate a third type III′ β-turn with Ala(3) and Aib(4) as the corner residues. This leads to positive ϕ, ψ values for Ala(3) ($\phi = 56°$, $\psi = 38°$), a situation rarely observed for an L residue in peptide crystal structures.[69,85-87] The only example of a consecutive type III′-I structure so far is in the peptide Piv-D-Pro-L-Pro-L-Ala-NHMe.[88,89]

V. HELICAL PEPTIDES

The overwhelming preference of Aib residues for ϕ, ψ values lying in the left- or right-handed helical regions results in the marked stabilization of helical structures in oligopeptides, with Aib-rich sequences. The crystal structures of Aib containing peptides with five or more residues provide clear examples of helical conformations in the solid state and have allowed the detailed characterization of 3_{10}- and α-helical structures. A 3_{10}-helical conformation was first observed in the crystal structure of Tosyl-$(Aib)_5$-OMe (Figure 11a), which is composed of three consecutive type III β-turns with three intramolecular 4 → 1 hydrogen bonds.[90] The centrosymmetric crystal (Pbca) contains both right- and left-handed helical peptides. A second crystal form (P1) has also been studied. The molecular conformation is largely unchanged, the incorporation of solvent leading to significant changes in the crystal packing arrangement.[91] Subsequent investigations of several oligopeptides established 3_{10}-helical structures in many cases. The conformational parameters determined in helical Aib oligopeptides are summarized in Table 4.

Z-$(Aib)_5$-O-*t*-Bu (Figure 11b)[71] adopts a conformation very similar to that for Tosyl-$(Aib)_5$-OMe. The crystal structures of two suzukacillin A fragments, Boc-Ala-Aib-Ala-Aib-

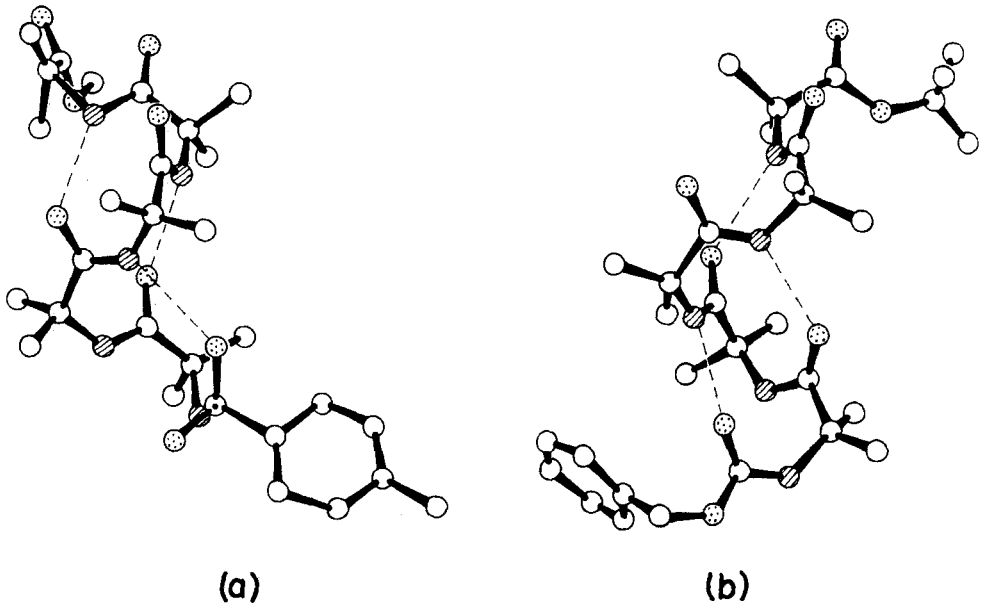


FIGURE 11. Molecular conformation of 3_{10}-helical pentapeptides. (a) Tosyl-(Aib)$_5$-OMe.[90] (b) Z-(Aib)$_5$-O-*t*-Bu.[71]

Aib-OMe[92,93] (residues 2 to 6 in the revised sequence) and Boc-Leu-Aib-Pro-Val-Aib-OMe (residues 11 to 16)[92,94] (Figure 12) establish 3_{10}-helical conformations in these peptides. The former is folded into an almost ideal right-handed 3_{10}-helix. The presence of L-amino acids results in a preference for the right-handed structure (negative ϕ, ψ values). A similar structure has also been reported for Boc-Aib-Ala-Aib-Ala-Aib-OMe.[20,165] The peptide Boc-Leu-Aib-Pro-Val-Aib-OMe is an interesting example of a 3_{10}-helical structure with a central Pro residue.[94] This sequence which occurs in both alamethicin and suzukacillin is of particular interest since the Pro residue precludes formation of a Leu-Aib β-turn by preventing 4 → 1 hydrogen bonding. Therefore, the possibility of structures with mixed 4 → 1 and 5 → 1 hydrogen bonding patterns have been considered from spectroscopic studies.[95,96] The solid-state conformation consists of two consecutive type III β-turns with Aib(2)-Pro(3) and Pro(3)-Val(4) as the corner residues. Two intramolecular 4 → 1 hydrogen bonds stabilize this structure.

The pentapeptide Boc-Aib-Pro-Val-Aib-Val-OMe adopts a distorted helical structure in the solid state (Figure 12a).[97] The folded conformation is stabilized by two 5 → 1 hydrogen bonds [Boc CO—HN Aib(4) and Aib(1)CO—HN Val(5)] and one 4 → 1 hydrogen bond [Boc CO—HN Val(3)]. The Boc CO group simultaneously hydrogen bonds to two NH groups. The structure is therefore different from either purely 3_{10}- or α-helical conformations. The conformation has been described as a "4-fold helix" with an average pitch of 5.58 Å, since a projection of the C^{α} atoms down the helix axis shows an approximate 4-fold symmetry (Figure 15).[97] An examination of the ϕ, ψ values in this structure suggests that the two Aib and Pro residues adopt conformations close to right-handed 3_{10}- or α-helical structures. Large deviations are, however, apparent for the central Val(3) residue. This crystal structure also incorporates one molecule of water, which is hydrogen bonded to the CO oxygens of the Pro(2) residue of one molecule and the Val(3) residue of a symmetry-related molecule. It is possible that interaction of water with the Pro(2)-Val(3) segment may contribute to distortion of the structure from a classical helical conformation.

The pentapeptide Z-(Aib-)$_3$-Val-Gly-OMe is also an example of a right-handed 3_{10}-helical structure.[81,161] Here the three Aib residues adopt ϕ, ψ values close to those for an ideal 3_{10}-

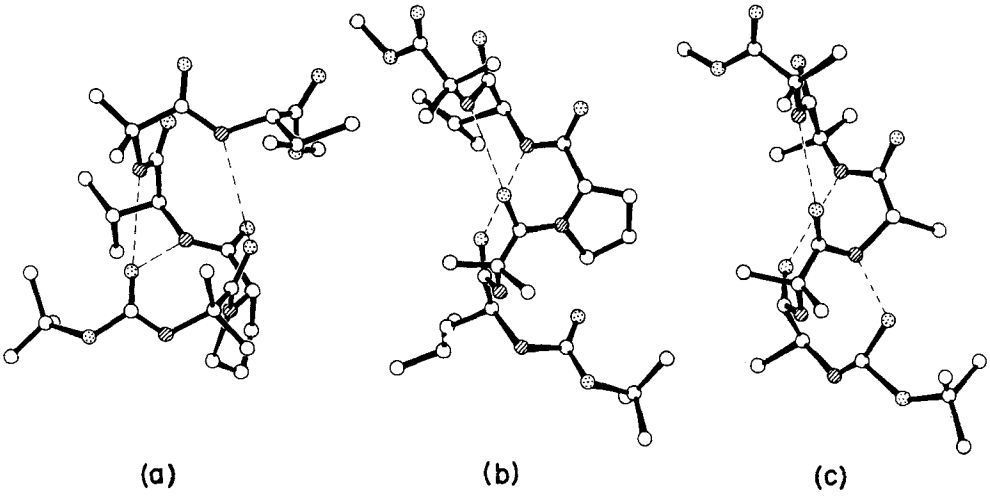


FIGURE 12. Helical pentapeptide structures. (a) Boc-Aib-Pro-Val-Aib-Val-OMe.[92,97] (b) Boc-Leu-Aib-Pro-Val-Aib-OMe.[92,94] (c) Boc-Ala-Aib-Ala-Aib-Aib-OMe.[92,93]

helix. A large deviation is observed for the Val residue which terminates the helix. As noted earlier, the related tetrapeptide Z-$(Aib)_3$-Val-OMe adopts an incipient *left-handed* 3_{10}-helical conformation.[81,161] Addition of an achiral Gly residue in this sequence thus results in a dramatic reversal of helix sense. In the tetrapeptide the Val residue is not part of a β-turn and merely provides an NH group for the 4 → 1 hydrogen bond stabilizing the Aib(2)-Aib(3) Type III′ β-turn. In conrast, in the pentapeptide, there is a further lengthening of the peptide helix requiring a type III β-turn, with Aib(3)-Val(4) as the corner residues. In this situation, the L-Val residue forces a reversal of helix sense, since negative ϕ, ψ values, corresponding to a right-handed twist, are generally favored for the L residue. The distribution of ω values for the helical peptides in Table 4 suggests that the direction of nonplanar distortions of the peptide units may be related to the handedness of the helix.

A noteworthy feature of the examples in Table 4 is the effect of Val residues on the backbone conformation of these helical peptides. Val has a relatively low probability of occurring in helical segments in proteins.[98] In the peptides considered here, the backbone folding is largely dominated by Aib residues. However, in the structures of Boc-Aib-Pro-Val-Aib-Val-OMe[97] and Z-$(Aib)_3$-Val-Gly-OMe,[81,161] the ϕ, ψ values for the Val residue deviate substantially from ideal helical conformations. Boc-Leu-Aib-Pro-Val-Aib-OMe[94] is an exception in that the Val residue occurs in an almost ideal helical conformation.

Two recent crystal structure determinations of 10- and 11-residue peptides serve to illustrate long segments of both 3_{10}- and α-helical conformations. The solid-state conformation of Boc-Ala-$(Aib\text{-}Ala)_2$-Glu(OBz)-Ala-$(Aib\text{-}Ala)_2$-OMe is shown in Figure 13b.[99,100] The 11-residue peptide folds into an α-helix with 9 of the 11 residues forming 2.5 turns of the helix. The C-terminal residues (10 and 11) are not part of the helix. Six intramolecular –C=O.... HN– 5 → 1 hydrogen bonds and one 4 → 1 hydrogen bond stabilize the structure. Details of the intramolecular hydrogen bonds have not been specifically discussed in References 99 and 100. However, the figures in References 99 and 100 also indicate a 6 → 1 hydrogen bond between Glu(6)CO and Ala(11)NH. The helix is slightly more tightly wound near the C-terminal end. The ϕ, ψ values for residues 1 to 9 fall into the right-handed helical region of the Ramachandran map.[49,101] This structure has been used to argue strongly that α-helical conformations are favored in longer peptides, while 3_{10}-helixes may occur only in shorter peptides.[20,99,100] This conclusion does not appear to be justified, since the decapeptide Boc-

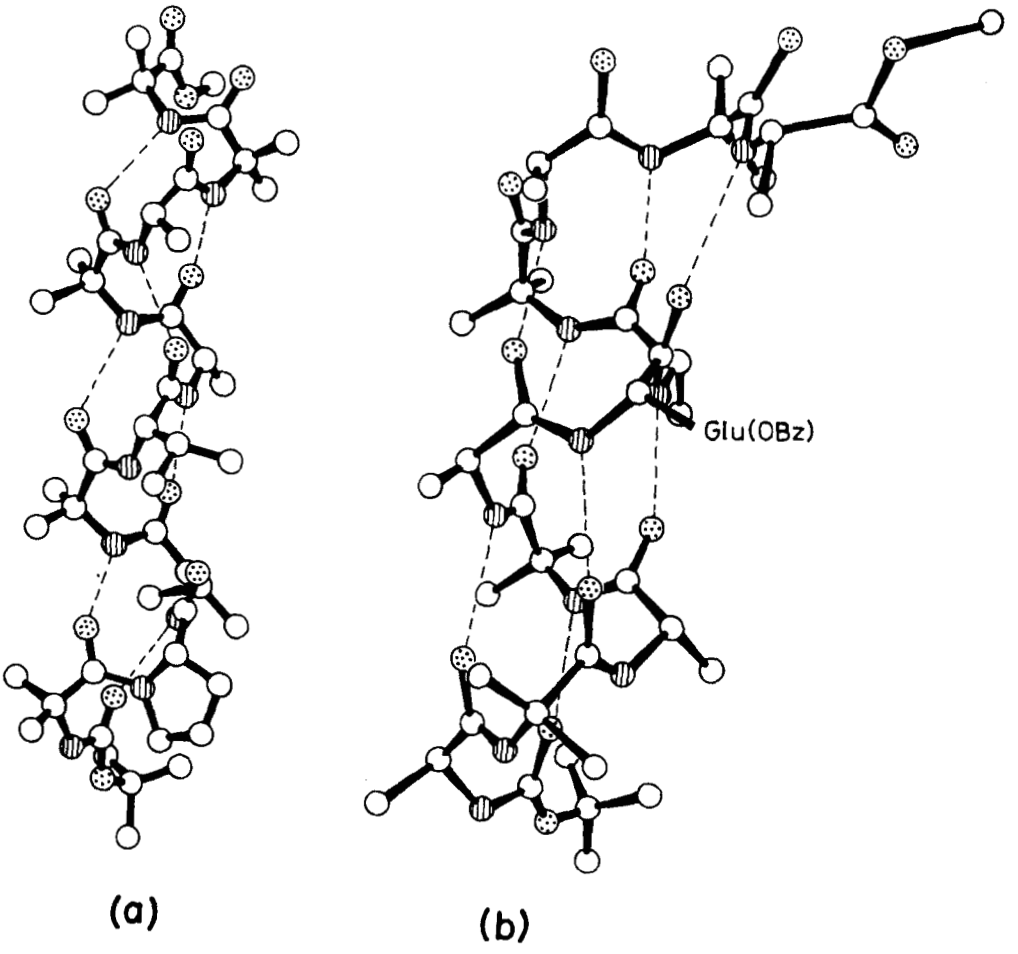


FIGURE 13. Molecular conformation of helical oligopeptides. (a) Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (3_{10}-helix).[102] (b) Boc-Ala-Aib-Ala-Aib-Ala-Glu(OBz)-Ala-Aib-Ala-Aib-Ala-OMe. (FIGURE 13b redrawn from Butters, T. et al., Zur struktur des Helikalen N-Terminus in Alamethicin', α-Helix order 3_{10}-Helix?, *Agnew. Chem.*, 93, 904, 1981; *Agnew. Chem. Int. Ed. Engl.*, 20, 889, 1981. With permission.)

Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe adopts a 3_{10}-helical conformation in the solid state.[102] Figure 13a shows a view of the molecular conformation of this 10-residue peptide. The backbone folds into an almost ideal 3_{10}-helix, stabilized by 7 intramolecular 4 → 1 hydrogen bonds. The conformational parameters are listed in Table 5. The interaction between the Val(5)NH and Pro(2)CO appears to be rather weak and the N—O distance of 3.46 Å is too large to be indicative of a hydrogen bond.[102] The solid-state conformation of the two pentapeptide fragments — Boc-Aib-Pro-Val-Aib-Val-OMe[92,97] and Boc-Ala-Aib-Ala-Aib-Aib-OMe[92,93] — of this decapeptide have already been described. While residues 6 to 10 retain the 3_{10}-helical folding pattern in the decapeptide, the 1 to 5 segment is distorted in the pentapeptide structure, but folded as a 3_{10}-helix in the longer peptide. Both Val residues in the decapeptide have ϕ, ψ values close to an ideal 3_{10}-helix. The absence of solvent in the decapeptide crystals may account for the difference in the conformation of the amino-terminal segment, as compared to Boc-Aib-Pro-Val-Aib-Val-OMe, which crystallized as a monohydrate.[97]

An important difference between the 10- and 11-residue peptides is that the latter contains only 4 Aib residues out of a total of 11 amino acids, whereas the former has 5 Aib residues.

The 10-peptide also has a L-Pro residue in the amino-terminal segment, which may promote 3_{10}-helical folding. Further, the 11-peptide contains a central triplet of L-amino acids (-Ala-Glu(OBz)-Ala-). The effect of this segment of non-Aib residues on backbone folding remains unclear.[64,103] Further investigations of carefully chosen model oligopeptides are necessary to establish the role of chain length, sequence, and solvent effects in modulating the precise nature of the helical conformation adopted by Aib-rich peptide sequences.

The crystal structure of alamethicin (Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol), crystallized from CH_3CN/CH_3OH, has been determined at 1.5 Å resolution using isomorphous heavy atom derivatives prepared by diffusion of dimethyl mercury into the crystals.[104] The three independent molecules in the crystallographic asymmetric unit adopt largely α-helical conformations, stabilized by $5 \rightarrow 1$ hydrogen bonds. A short 3_{10}-helical turn, involving a $4 \rightarrow 1$ hydrogen bond between Leu(12)CO and Val(15)NH, is also observed. The CO groups of Aib(10) and Gly(11) do not form intramolecular hydrogen bonds and are relatively accessible to solvent. The availability of refined coordinates for this structure would help in the analysis of the stereochemistry of oligopeptide helices.

VI. PACKING OF HELICAL PEPTIDES IN CRYSTALS

The evidence for the propensity of Aib-containing sequences to fold into 3_{10}- or α-helical conformations is overwhelming. There is little doubt that the Aib containing membrane channel-forming polypeptides (Figure 1) are constrained to adopt helical conformations, with diameters too narrow to permit passage of cations through the helix interior.[30,32,105] In this respect the Aib peptide channels differ from poly-LD peptides like gramicidin A, which adopt π_{LD} helical or antiparallel $\pi\pi_{LD}$ double-helical structures, with a large channel through the helix interior.[106-108] There is considerable evidence available that functional membrane channels of Aib peptides are composed of peptide aggregates.[30,109-111] Several recent models envisage association of helical monomers in the membrane phase, resulting in formation of a central pore.[30,32,104,105,112] Detailed models[30,112] also invoke interactions between helix macrodipoles[113,114] to explain fluctuations between different conductance states and the observed voltage dependence of ion conduction. In this context the crystal structures of helical peptides afford a means of visualizing helix-helix association.

Table 6 lists crystal data for helical Aib peptides. Figures 14 to 16 illustrate the packing modes in some representative cases, as viewed down the helix axis. In all cases, the helix axis is approximately aligned with one of the crystallographic axes. Peptide helices in these structures are arranged in head-to-tail fashion in columns which are stabilized, by intermolecular hydrogen bonds involving free NH groups at the N terminal of one helix with free CO groups at the C terminal of a translated molecule. Each column may then be viewed as an infinitely long helical structure. Adjacent columns are aligned in parallel or antiparallel fashion as illustrated in the packing motifs exemplified in Figures 14 to 16. For the space groups $P2_12_12_1$, Pbca, and P1, no net crystal polarity is obtained.[115] On the other hand, in the space group $P2_1$, crystals with a polar axis are possible, in principle.[115] The sole example of a "polar crystal" is in the case of Boc-Leu-Aib-Pro-Val-Aib-OMe. This arrangement corresponds to a high dipole state of the peptide aggregates. The significance of the polar axis in organic crystals has been recently considered.[115] The generation of structures in which vacancies are created by removal of a peptide column may be valuable in stereochemical modeling of transmembrane channels.

The sequences of the naturally occurring channel forming Aib peptides necessarily require that helical aggregates lead to the formation of pores with largely hydrophobic cores.[30,105] These will be distinct from the commonly accepted notion of *hydrophilic* channels across membranes.[116] The crystal structure of the triclinic form of Tosyl-$(Aib)_5$-OMe is an interesting

Table 6
CRYSTAL PARAMETERS IN HELICAL OLIGOPEPTIDES

Peptide	Space group	Cell constants			Heix axis[a] (Polar)[b]	Ref.
Tosyl-$(Aib)_5$-OMe	Pbca	a = 18.172				90
		b = 16.562			b (−)	
		c = 22.553				
Tosyl-$(Aib)_5$-OMe	$P\bar{1}$	a = 8.312	α =	92.51		91
		b = 12.122	β =	105.57	b (−)	
		c = 17.912	γ =	91.17		
Z-$(Aib)_5$-O-*t*-Bu	$P\bar{1}$	a = 9.185	α =	105.79		71
		b = 11.540	β =	93.92	b (−)	
		c = 18.737	γ =	102.98		
Boc-Leu-Aib-Pro-Val-Aib-OMe	$P2_1$	a = 11.034				92,
		b = 10.894	β =	104.79	b (+)	94
		c = 15.483				
Boc-Ala-Aib-Ala-Aib-Aib-OMe	$P2_12_12_1$	a = 11.671				92,
		b = 14.534			a (−)	93
		c = 17.906				
Boc-Aib-Pro-Val-Aib-Val-OMe·H_2O	$P2_12_12_1$	a = 10.192				97
		b = 10.440			b (−)	
		c = 32.959				

[a] Indicates the cell axis which is approximately parallel to the helix axis.
[b] Indicates whether crystal possesses a polar axis as defined in Reference 115.

example of a peptide generating hydrophobic channels by virtue of a unique packing mode (Figure 17).[91] A hydrophobic channel with a diameter of 5.2 Å runs through the crystal, which accommodates grossly disordered solvent. The helix axes of the peptides lie approximately perpendicular to the channel axis. This structure is therefore not strictly relevant in building channel models for alamethicin and related peptides.

VII. NON-HYDROGEN-BONDED CONFORMATIONS

Table 7 lists the peptides lacking intramolecular C=O—H–N hydrogen bonds in the solid state. These structures are of protected amino acid derivatives, dipeptide acids, dipeptide esters, acids, esters, and methylamides of tripeptides. In all of these cases the peptides are either too short to form β-turns or 4 → 1 hydrogen bonding is prevented by an appropriately positioned Pro residue.

The simplest protected derivative whose structure in the solid state has been established is Boc-Aib,[64,117] which crystallizes in the centric space group $P2_1/c$. The φ value of ±54° falls into the energetically favored region of the Ramachandran map. The ψ value defined as N–C^{α}–C–OH for C-terminal amino acids is ±44°. Ac-Aib-NHMe which serves as the theoretician's model "dipeptide" crystallizes rather intriguingly in the noncentric space group Cc and adopts φ, ψ values characteristic of an ideal helical residue.[118]

The solid-state conformations of three dipeptide acids — Z-Aib-Aib-OH,[64,119] Boc-Ala-Aib-OH,[119,120] and Boc-Gly-Aib-OH[77] — are shown in Figure 18. There has been some interest in establishing the occurrence of "oxy analogs" of the 4 → 1 hydrogen-bonded structure in peptides.[121,122] In such a conformation the CO group of residue i is hydrogen bonded to the terminal carboxylic acid group of residue i + 2, resulting in a 10-atom hydrogen-bonded ring. Z-Aib-Aib-OH would have been expected to adopt such a structure if both residues had φ, ψ values in the helical region of the conformational map. Such a structure would be analogous to the β-turn conformation observed in Z-Aib-Aib-Ala-OMe[72]

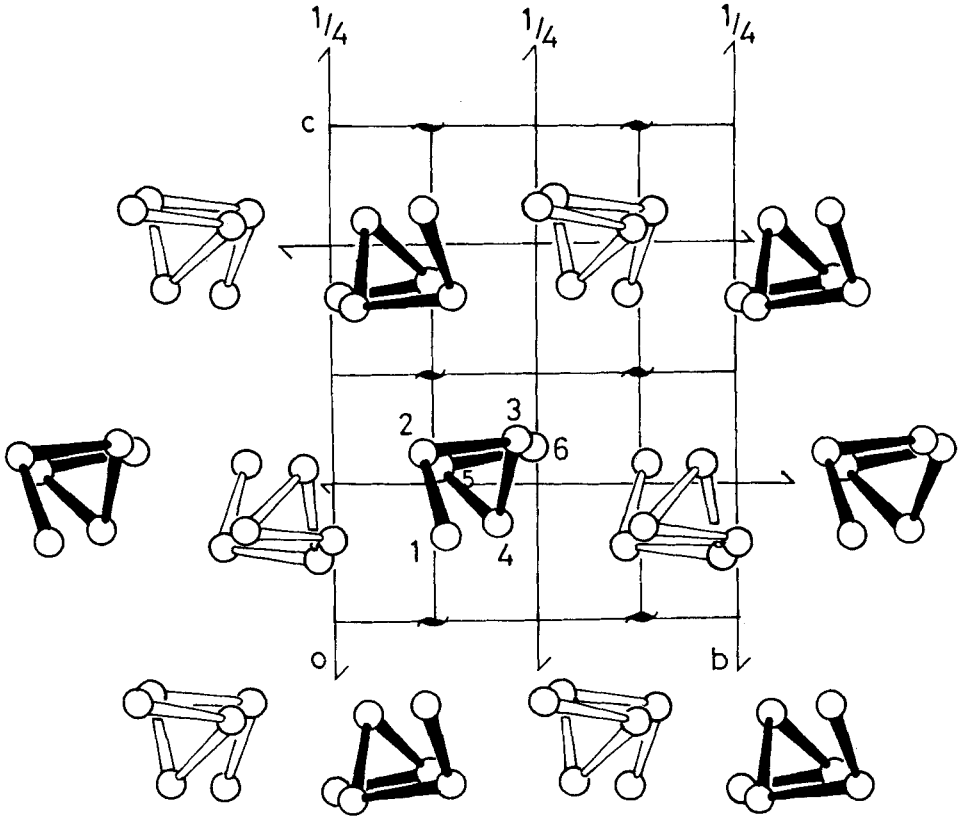


FIGURE 14. Helix packing in crystals of Boc-Ala-Aib-Ala-Aib-Aib-OMe viewed down *a* axis. Only the Boc O atom (marked as 1) and the backbone C^{α} atoms are shown. The shaded and light molecules correspond to helices which are antiparallel in the crystal structure.

or Z-$(Aib)_3$-OH.[64] However, the molecule does not crystallize in this conformation. Interestingly, the two Aib residues have ϕ, ψ values close to those for 3_{10}-helices (type III β-turn) but have opposite *senses of twist*. Consequently, the β-turn analog conformation is not observed. The carboxylic acid groups in this structure interact strongly with one another via a pair of O–H....O hydrogen bonds. Presumably, packing considerations then favor the observed structure. Replacement of the N-terminal Aib by Gly or L-Ala also leads to peptides which favor nonhydrogen-bonded structures. In Gly-Aib-OH the urethane group appears to be appreciably nonplanar ($\omega_o = -159°$).[77] An unusual feature of the Boc-Ala-Aib-OH structure is the conformation of the C-terminal Aib residue. The ϕ, ψ values of -175 and $-170°$, respectively, result in an extended structure which is only about 0.5 kcal/mol^{-1} above the global minimum (Figure 3b) (unpublished results). In this extended structure there is the possibility of an intramolecular C_5 hydrogen bond between the NH and CO groups of the Aib residue. The N....O distance of 2.59 Å and H–N....O angle of 46° provide evidence for such an interaction. C_5 hydrogen bonds have only been infrequently inferred from crystal structure data.[121,122] Examples include Boc-Gly-Ala-OH[123] and Boc-Gly-Pro-OH.[124] The structure of Boc-Gly-Ala-Aib-OMe incorporates a Gly-Ala type III β-turn with the terminal Aib in the left-handed helical region.[125]

Crystal structures of small peptides containing Aib also illustrate the possibility of Aib residues occurring in nonhelical regions of the conformation map. In Boc-Aib-Aib-OBz[126] the Aib(2) residue has $\phi = 57°$ and $\psi = -138°$. Two other examples are observed in the structures of peptide oxazolones derived from di- and tripeptide acids containing Aib (Figure

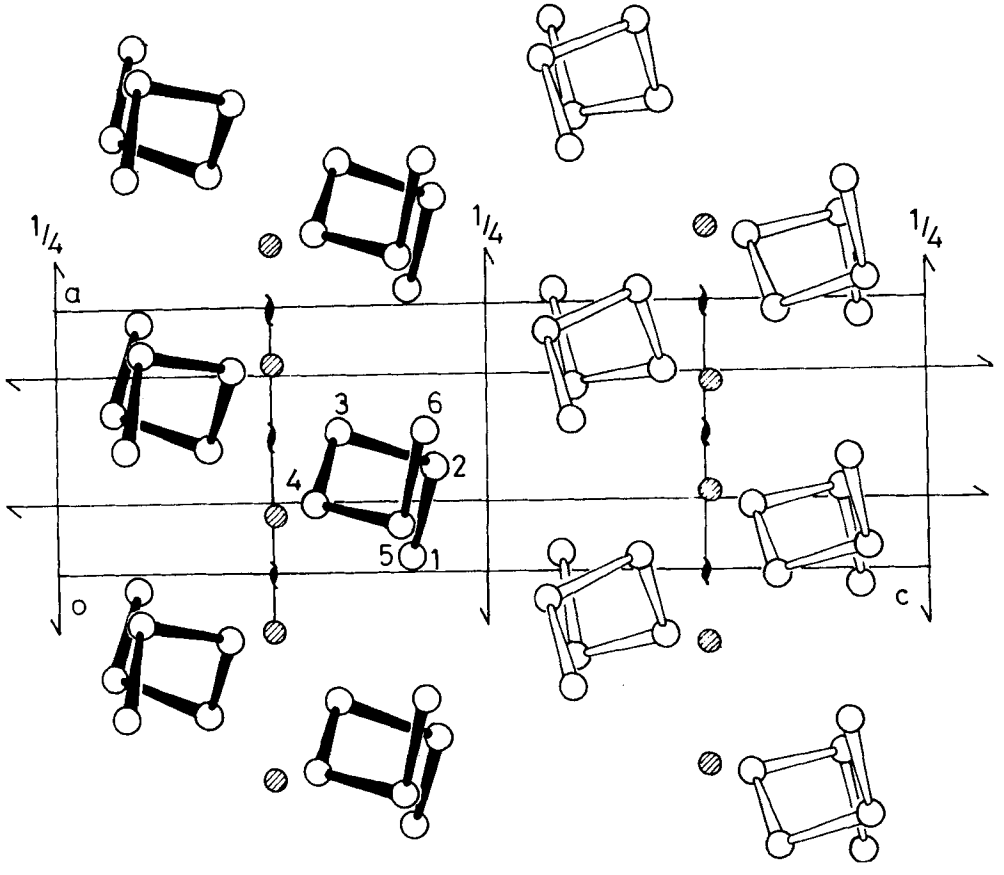


FIGURE 15. Helix packing in crystals of Boc-Aib-Pro-Val-Aib-Val-OMe·H_2O viewed down *b* axis. See legend to Figure 14.

19).[127,128] While the C-terminal Aib in these peptides is forced into an unusual geometry by covalent constraints, the residues Aib(1) in the dipeptide ($\phi = 43.7°$, $\psi = -142.5°$)[126] and Aib(2) in the tripeptide ($\phi = 51°$, $\psi = -145°$)[127] lie well outside the $3_{10}/\alpha$-helical region of conformational space. It is pertinent to note that there is a small sterically allowed region with $\phi \sim 50°$, $\psi \sim -140°$, which lies ~3.5 kcal/mol^{-1} above the global minimum (Figure 3b).

The crystal structure of Boc-Leu-Aib-Pro-OH is a novel example of a chain reversal in a small peptide, which is not stabilized by a 4 → 1 hydrogen bond (Figure 20a).[77] The Leu ($\phi = -84°$, $\psi = +163°$) and Aib ($\phi = 53°$, $\psi = 37°$) residues adopt conformations very similar to those required for the corner residues of a type II β-turn. The related peptide Boc-Leu-Aib-Pro-OBz adopts a dramatically different overall conformation (Figure 20b).[77] The ϕ, ψ values for the central Aib residue are almost unchanged; the large difference arising almost exclusively from a complete reorientation about the C^{α}-CO (ψ) bond of the Leu residue.

Two peptides of the type Boc-Aib-X-Pro-NHMe (X = Pro, Leu) have been examined as possible models for 5 → 1 hydrogen-bonded conformations in acyclic peptides. In these sequences the 4 → 1 hydrogen-bonded β-turn would be expected to be destabilized by the presence of the Pro residue. In principle, a 5 → 1 hydrogen-bonded structure corresponding to an ideal α-helical turn could occur in this sequence. The molecular conformations, in the solid state, of the peptides Boc-Aib-Pro-Pro-NHMe[129,157] and Boc-Aib-Leu-Pro-NHMe[162]

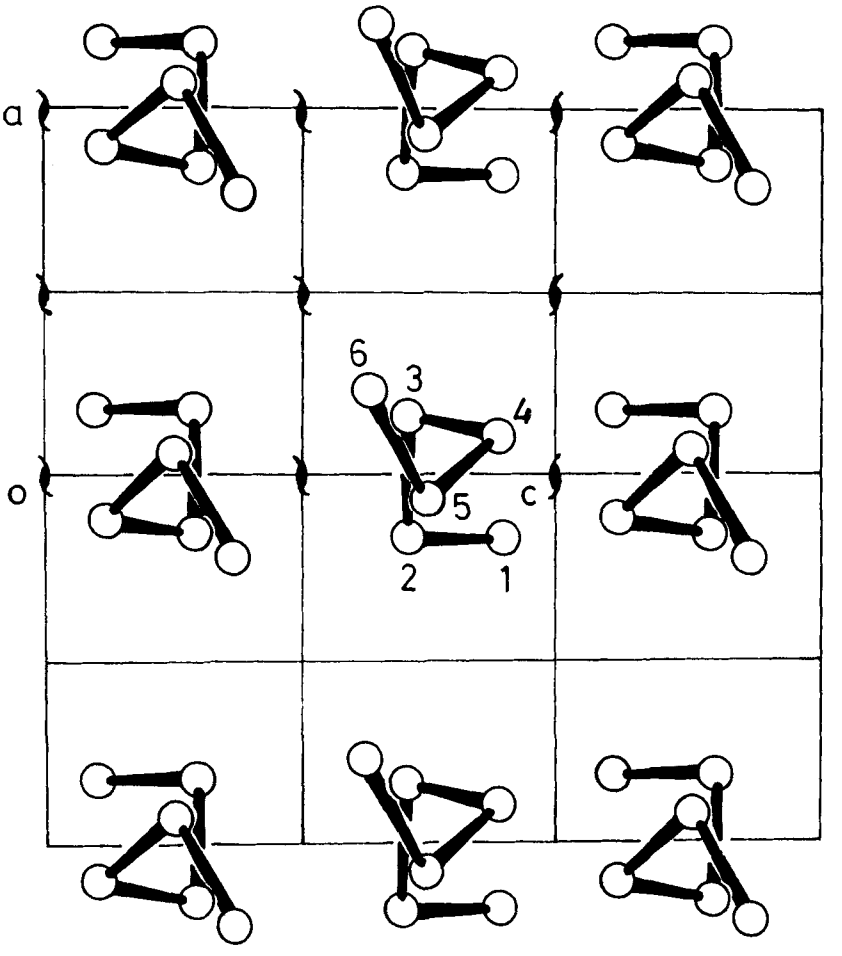


FIGURE 16. Helix packing in crystals of Boc-Leu-Aib-Pro-Val-Aib-OMe viewed down *b* axis. See legend to Figure 14. Note that this is the sole example of parallel packing of helixes throughout the crystal (See Table 6).

are illustrated in Figure 21. Both peptides adopt extended structures with the Aib residues falling into the left-handed helical region of ϕ, ψ space. The crystals of these peptides incorporate water molecules; three per asymmetric unit in the Pro-Pro peptide and two in the Leu-Pro peptide. It is difficult to evaluate the influence of interactions with water on the intrinsic conformational preferences of the peptide backbone. In general,where specific folding of the peptide backbone into β-turns or helices is not strongly favored, as in longer sequences, it is likely that crystal packing forces may influence the observed conformations of these peptides.

VIII. AIB-PRO AND PRO-AIB SEQUENCES: PYRROLIDINE RING GEOMETRY

Proline is the most sterically restricted of the amino acids commonly found in proteins. The pyrrolidine ring restricts ϕ_{Pro} to $\sim -60° \pm 20°$ in L-Pro. Only a limited region of ϕ, ψ space in the vicinity of ψ $-50°$ (α-helix), $\psi \sim +70°$ (C_7 or γ-turn) and $\psi \sim +120°$ (poly Pro) are energetically favored for L-Pro residues.[130] The use of Pro residues in conjunction with Aib in synthesizing model peptides of well-defined geometries is an attractive possibility.[131] The pyrrolidine ring invariably occurs in puckered conformations with the C^{γ}

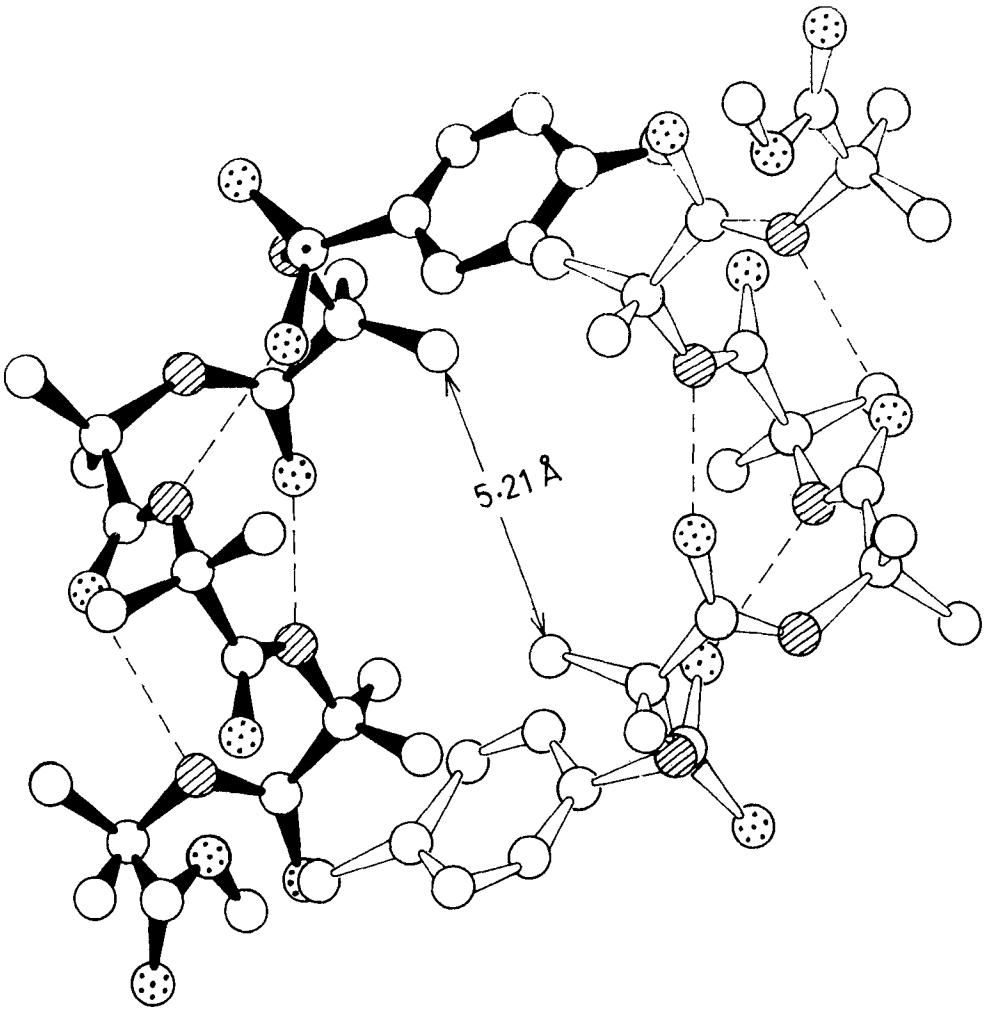


FIGURE 17. Packing of helical peptide molecules of Tosyl-$(Aib)_5$-OMe in the space group P1 leads to formation of a hydrophobic channel.[91] Note that the channel of diameter ~5.2 Å accommodates grossly disordered solvent molecules.

or C^{β} atoms displaced from the mean plane of the ring. Considerable attention has been focused on the stereochemical preferences and dynamic interconversions of the Pro ring in peptides.[130] The availability of several crystal structures, with Aib-Pro or Pro-Aib sequences in β-turn or 3_{10}-helical conformations, permits an evaluation of the effects of the amino acid preceding or succeeding the Pro residue and backbone conformation on ring geometry.

Table 8 summarizes the torsion angles obtained for the Pro ring in peptides with well-defined backbone conformations. The overall ring conformation is defined as C^{γ}-*exo* or C^{γ}-*endo*.[132,133] Structures with C^{γ} on the same side as the Pro C = O group are termed as C^{γ}-*endo* while in C^{γ}-*exo* structures they lie on opposite sides of the mean plane of the ring. In the C^{γ}-*exo* conformations, χ_1 values are negative and χ_2 values are positive. In C^{γ}-*endo* structures, χ_1 is positive and χ_2 negative. There are five examples of Aib-Pro sequences, all of which adopt type III β-turns or the closely related type I conformation. Of these, in four cases the pyrrolidine geometry is C^{γ}-*exo*. The sole example of a C^{γ}-*endo* structure is observed in Boc-Leu-Aib-Pro-Val-Aib-OMe.[94] In this peptide the Pro ring is flattened, with extremely low values of the angles χ_1, χ_2, χ_3, and χ_4. This, presumably, is a consequence

Table 7
AIB PEPTIDES LACKING INTRAMOLECULAR HYDROGEN BONDS

Peptide	R value	Residue	Conformational angles (°) φ	ψ	ω	Ref.
Boc-Aib-OH	0.037	Boc	—	—	—	64, 117
		Aib	−54	−43[a]	—	
Ac-Aib-NHMe	0.037	Ac	—	—	−173	118
		Aib	−55	−39	−175	
Boc-Ala-Aib-OH	0.053	Boc	—	—	177	119, 120
		Ala	−66	−24	172	
		Aib	−175	−170[a]	—	
Boc-Gly-Aib-OH	0.065	Boc	—	—	−159	77
		Gly	89	165	−176	
		Aib	47	45[a]	—	
Z-Aib-Aib-OH	0.037	Z	—	—	−175	64, 119
		Aib	−66	−26	−172	
		Aib	51	44[a]	—	
Boc-Aib-Aib-OBz	—	Boc	—	—	—	126
		Aib	—	—	—	
		Aib	51	−138[a]	—	
Boc-Gly-L-Ala-Aib-OMe	0.037	Boc	—	—	180	125
		Gly	−62	−18	175	
		Ala	−61	−30	−174	
		Aib	50	39[a]	—	
Boc-Leu-Aib-Pro-OH	0.054	Boc	—	—	171	77
		Leu	−84	163	−178	
		Aib	53	37	177	
		Pro	−71	161[a]	—	
Boc-Leu-Aib-Pro-OBz	0.058	Boc	—	—	−174	77
		Leu	−63	−42	170	
		Aib	53	46	169	
		Pro	−74	162[a]	—	
Boc-Aib-Pro-Pro-NHMe	0.069	Boc A	—	—	16	129, 157
		B	—	—	4	
		Aib A	57	44	177	
		B	58	39	175	
		Pro A	−71	158	179	
		B	−67	157	173	
		Pro A	−72	149	179	
		B	−62	154	172	
Boc-Aib-Leu-Pro-NHMe	0.099	Boc	—	—	176	162
		Aib	68	23	178	
		Leu	−134	143	177	
		Pro	−70	152	175	
Z-$(Aib)_3$-OH oxazolone	0.055	Z	—	—	−178	128
		Aib	−57	−38	−178	
		Aib	51	−145	−178	
Z-$(Aib)_2$-OH oxazolone	0.041	Z	—	—	−178	127
		Aib	44	−142	−175	

[a] Definition of ψ is taken as N–C^{α}–C–O, where O is not the carbonyl oxygen.
[b] Unpublished results.

of the position of the Pro residue within the 3_{10}-helical turn. In Z-Aib-Pro-Aib-Ala-OMe[73] and Z-Aib-Pro-Aib-Pro-OMe[56] the Pro residue within the 3_{10}-helical turn is flanked by Aib on both sides and adopts C^{γ}-*exo* geometries.

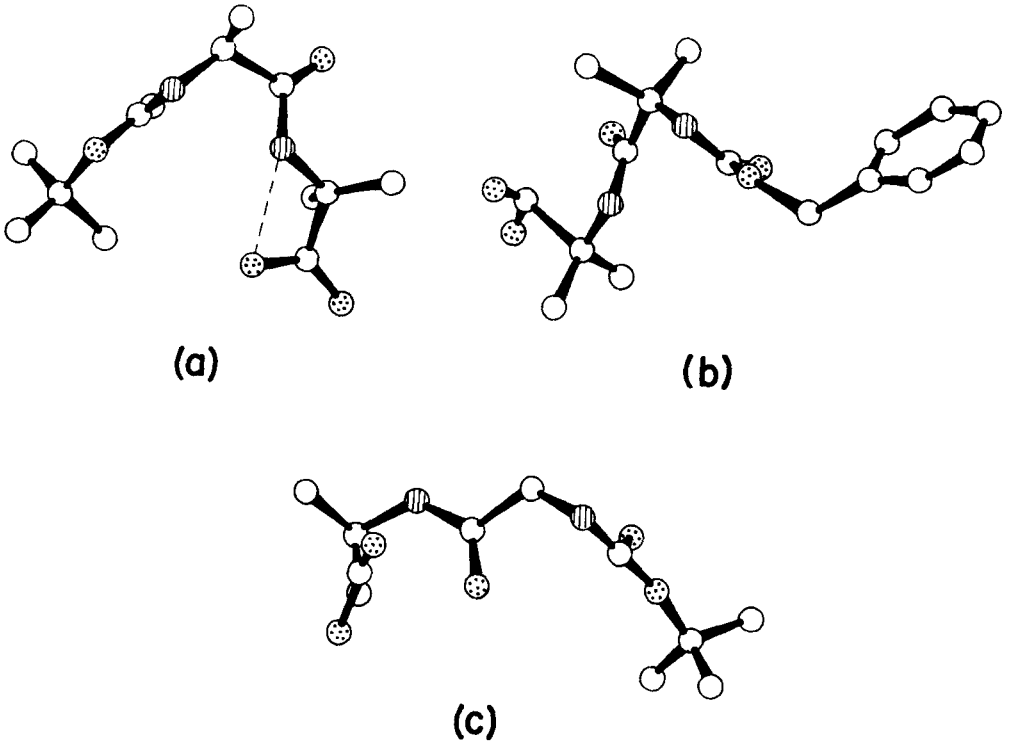


FIGURE 18. Solid-state conformations of (a) Boc-Ala-Aib-OH,[119] (b) Z-Aib-Aib-OH,[119] and (c) Boc-Gly-Aib-OH. (FIGURE 18c redrawn from Smith, G. D. et al., Crystal structures and conformational calculations of fragments of almethicin containing aminoisobutyric acid, *J. Am. Chem. Soc.*, 103, 1493, 1981. With permission.)

The Pro-Aib sequence occurs in four peptides. Both type II and type III conformations are stereochemically possible. Two peptides adopt the former structure, while the other two fall into the latter category. In the type II Pro-Aib β-turns observed in Piv-Pro-Aib-NHMe[68] and *p*-chlorobenzoyl-Pro-Aib-Ala-Aib-Ala-OMe,[84] the Pro residue in the former adopts a C^{γ}-*exo* conformation, while it is C^{γ}-*endo* in the latter. In the two examples of Pro-Aib type III β-turns, the pyrrolidine ring is C^{γ}-*endo* in Boc-Cys-Pro-Aib-Cys-NHMe,[82,83] but C^{γ}-*exo*

in Boc-Pro-Aib-Ala-Aib-OBz.[77] These results suggest that in Aib-Pro peptides the pyrrolidine ring favors a C^{γ}-*exo* conformation. However, in Pro-Aib sequences both C^{γ}-*exo* and C^{γ}-*endo* structures are readily accommodated, irrespective of the precise backbone conformation. This observation is compatible with the results of theoretical calculations for Ac-Pro-Aib-NHMe, which suggest that changes in ring puckering do not appreciably affect the energy of the type II and type III β-turn conformations.[68]

There are four examples of Aib-Pro sequences in non-β-turn structures. Of these, one is the C-terminal Aib-Pro sequence in Z-Aib-Pro-Aib-Pro-OMe,[56] in which the amino terminal is in a β-turn conformation. In all cases the pyrrolidine ring adopts a C^{γ}-*endo* geometry. However, the stereochemical significance of this observation is obscure.

IX. CYCLIC PEPTIDES

There have been relatively few reports of crystallographic studies of Aib residues incorporated into cyclic peptides. Table 9 lists the conformational parameters for one cyclic tetrapeptide and three cyclic dipeptides (diketopiperazines) containing Aib. An example of an Aib residue in a 14-membered cyclic peptide disulfide has already been illustrated in the

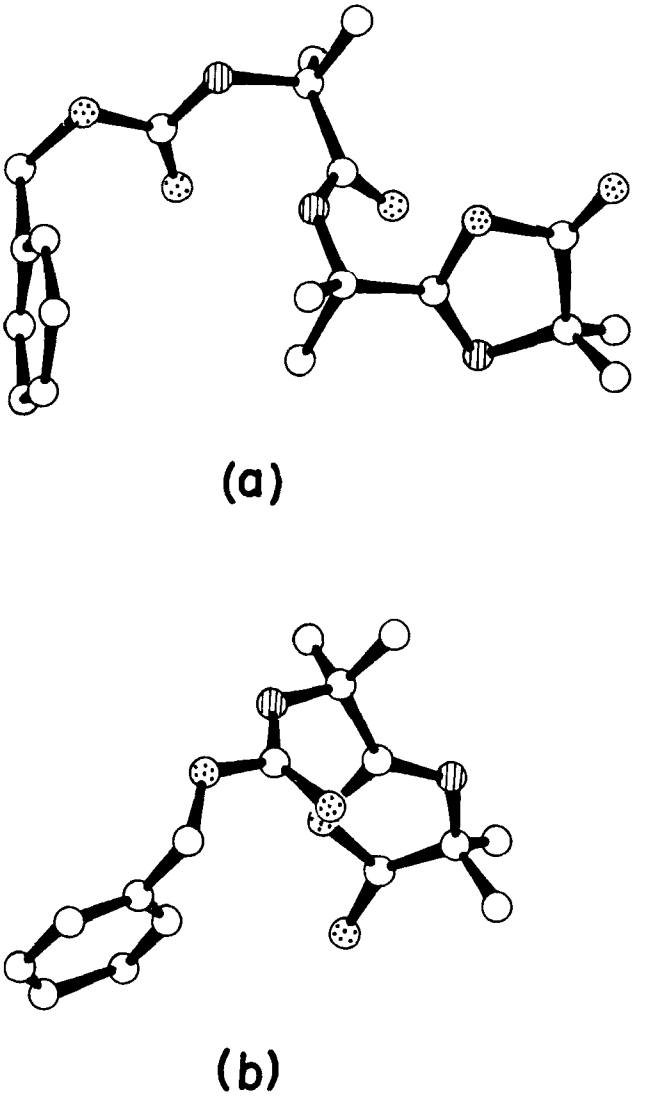


FIGURE 19. Molecular conformations of two-peptide oxazolones containing Aib. (a) Oxazolone derived from Z-$(Aib)_3$-OH.[128] (b) Oxazolone derived from Z-$(Aib)_2$-OH.[127]

peptide Boc-Cys-Pro-Aib-Cys-NHMe [82,83] The solid-state conformation of this molecule is shown in Figure 22a and the conformational parameters are listed in Table 3. As noted earlier, this peptide is formally a disulfide-bridged 3_{10}-helix and the Aib residue adopts a conformation similar to that observed in 3_{10}-helical oligopeptides (Table 3). The solid-state conformation of dihydrochlamydocin,[55] a derivative of the naturally occurring cytotoxic peptide chlamydocin, is shown in Figure 22b. The 12-membered ring accommodates an all *trans* peptide backbone, with very large deviations from planarity for all peptide units ($\Delta\omega$ ranges from 14 to 24°). Two intramolecular 3 → 1 hydrogen bonds are formed within the ring. The N—O distance characterizing the 3 → 1 hydrogen bond spanning the Aib residue is 2.82 Å, while that centered at D-Pro is 2.94 Å. This is the only example of a C_7- or γ-turn conformation[58] at an Aib residue ($\phi = 72°$, $\psi = -65°$). An examination of Table 1 (Figure 3b) suggests that the C_7 conformation is ~3 kcal/mol^{-1} less stable than the lowest

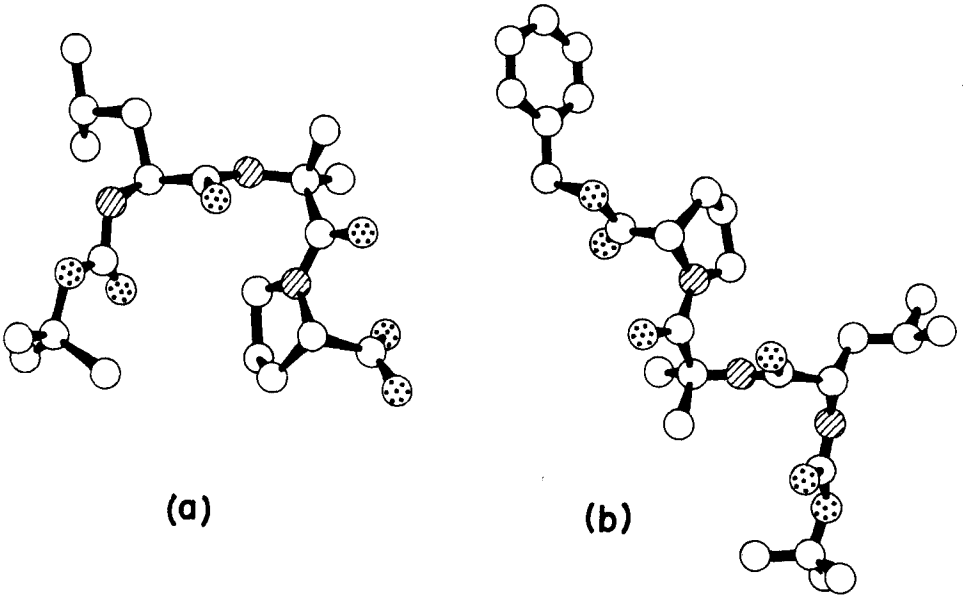


FIGURE 20. Molecular conformations of (a) Boc-Leu-Aib-Pro-OH and (b) Boc-Leu-Aib-Pro-OBz. (Reproduced with permission from Smith, G. D., Pletnev, V. Z., Duax, W. L., Balasubramanian, T. M., Bosshard, H. E., Czerwinski, E. W., Kendrick, N. E., Mathews, F. S., and Marshall, G. R., *J. Am. Chem. Soc.*, 103, 1483, 1981. Copyright American Chemical Society.)

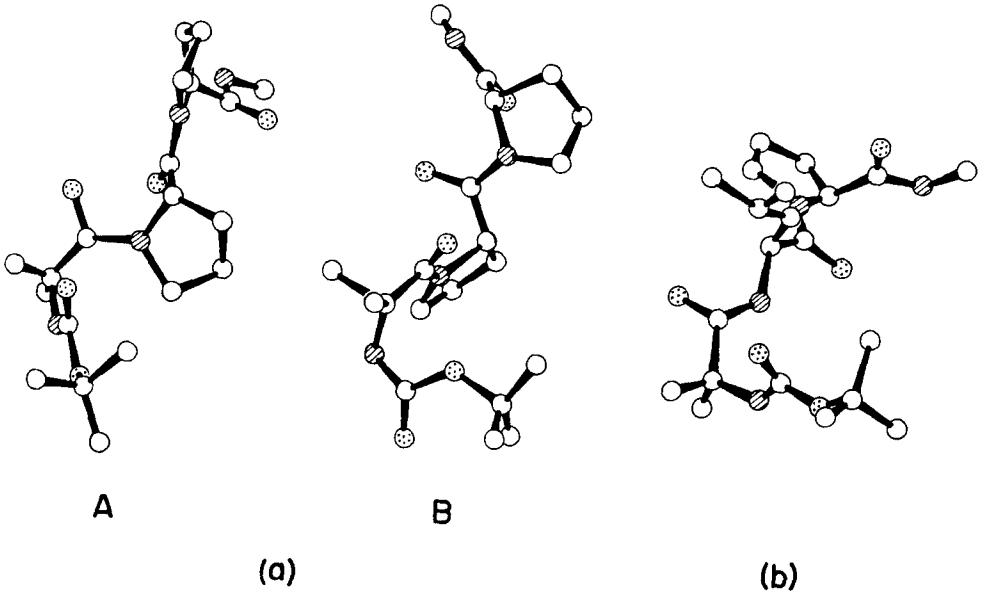


FIGURE 21. Solid-state conformations of Boc-Aib-X-Pro-NHMe sequences. (a) X = Pro. A, B are the two independent molecules in the asymmetric unit.[129] (b) X = Leu[161].

energy of helical region for Aib. The constraint of cyclization presumably forces the Aib residue into the C_7 structure in this case. The C_7 conformation should probably be considered for Aib residues in small peptides under conditions where 4 → 1 hydrogen-bonded β-turns are precluded. IR data supporting the formation of C_7 structures in Aib peptides in apolar solvents have been reported.[95,118]

Table 8
PYRROLIDINE RING CONFORMATIONS IN AIB-PRO AND PRO-AIB SEQUENCES

Peptide	β-turn type	Ring conformational angles[a] (°) χ^1	χ^2	χ^3	χ^4	θ	Puckering	Ref.
Aib-Pro β-turn								
Z-*Aib-Pro*-NHMe	III	−18	29	−27	16	0	C^γ-*exo*	63
Z-*Aib-Pro*-Aib-Ala-OMe	III	−27	37	−31	14	8	C^γ-*exo*	73
Z-*Aib-Pro*-Aib-Pro-OMe	I	−21	32	−29	17	3	C^γ-*exo*	56
Boc-Leu-*Aib-Pro*-Val-Aib-OMe	III	9	−13	12	−6	−1	C^γ-*endo*	92, 94
Boc-*Aib-Pro*-Val-Aib-Val-OMe	III	−15	27	−27	18	−3	C^γ-*exo*	97
Pro-Aib β-turn								
Piv-*Pro-Aib*-NHMe	II	−26	40	−40	25	1	C^γ-*exo*	68
Boc-Cys-*Pro-Aib*-Cys-NHMe (S–S)	III	23	−37	35	−22	0	C^γ-*endo*	82, 83
Boc-*Pro-Aib*-Ala-Aib-OBzl	III	−30	36	−27	6	15	C^γ-*exo*	77
Boc-*Hyp-Aib*-Aib-Phol	III	−32	41	−33	14	11	C^γ-*exo*	79
p-Chlorobenzoyl-*Pro-Aib*-Ala-Aib-Ala-OMe	II	29	−40	34	−15	−9	C^γ-*endo*	84
Non-β-turn structures								
Boc-Leu-Aib-*Pro*-OH	—	34	−40	30	−7	—	C^γ-*endo*	77
Boc-Leu-Aib-*Pro*-OBzl	—	31	−38	31	−11	−12	C^γ-*endo*	77
Boc-Aib-*Pro*-Pro-NHMe								129,
A	—	14	−15	10	0	−8	C^γ-*endo*	157
B	—	27	−35	29	−12	−10	C^γ-*endo*	
Z-Aib-Pro-Aib-*Pro*-OMe	—	28	−41	37	−21	−7	C^γ-*endo*	56

[a] Ring conformational angles are defined as follows: χ^1: N–C^α–C^β–C^γ; χ^2: C^α–C^β–C^γ–C^δ; χ^3: C^β–C^γ–C^δ–N; χ^4: C^γ–Cγ–N–C^α θ:C^δ–N–C^α–C^β.

Table 9
CONFORMATIONAL PARAMETERS IN CYCLIC PEPTIDES

Conformational angles (°)	Cyclic peptides			
	c(Aib-Aib)[a]	c(Aib-Ile)[a]	c(Aib-Phe)[b]	c(Aib-Phe-Pro-X)[c] (dihydrochlamydocin)
ϕ	−6, 6	10, 5	7, 4	72, −106, 83, −106
ψ	5, −5	−8, −3	−4, −1	−64, 94, −73, 105
ω	6, −6	−4, 1	−2, −4	162, −166, 157, −164

[a] From Suguna et al.[135]
[b] Unpublished results.
[c] From Flippen and Karle.[55]

Diketopiperazines have been widely investigated as models for *cis* peptide conformations and for examining side chain interactions with peptide units.[134] The crystal structures of three Aib containing diketopiperazines — c(Aib-Aib),[135] c(Aib-Ile),[135] and c(Aib-Phe) (unpublished) — are illustrated in Figure 23. All three cyclic dipeptides adopt almost planar conformations, with slight distortions. c(Aib-Aib) has been described as a "very flat chair", in which the Aib C^{α} atoms are displaced by 0.07 Å on either side of the mean plane of the ring.[135] c(Aib-Ile) is best described as a flattened boat, with the Aib and Ile C^{α} atoms displaced from the mean plane on the same side, by 0.11 and 0.05 Å, respectively.[135] A similar conformation is obtained in c(Aib-Phe), with the Aib and Phe C^{α} atoms displaced on the same side of the ring by 0.063 and 0.034 Å, respectively. In this case the extent of flattening is more than that for c(Aib-Ile). The Phe ring folds over the six-membered ring in a manner analogous to that observed in other diketopiperazines containing aromatic residues.[134] The bond angles C^{α}–N–C′ (τ_N) for the Aib residue in these systems range from 127.9 to 130.1°, which is significantly larger than the average value of 122.1° reported for acyclic Aib peptides.[47] Theoretical energy calculations may be helpful in analyzing the stereochemical features of these systems.

X. COMPARISONS OF SOLUTION AND SOLID-STATE CONFORMATIONS

The presence of Aib residues greatly restricts backbone conformational freedom of acyclic peptides. Consequently Aib peptides generally adopt a restricted range of conformations in solution, and comparisons between solid-state and solution structures become feasible. NMR studies of flexible acyclic peptides are complicated by problems of dynamic averaging over widely different conformational states. Interpretations based on "average conformations" lead to doubtful conclusions.[136] Optical spectroscopic techniques like CD and vibrational (IR/Raman) methods yield a molecular view on a time scale much shorter than in NMR, giving rise to spectra which are a composite of those derived from several different conformations. The recognition of spectral features corresponding to specific conformations is generally difficult. The development of spectroscopic techniques in peptide conformational analysis has therefore been based on cyclic peptide model systems, of which gramicidin-S is the preeminent example.[134,137]

The conformational conclusions derived from spectroscopic studies in solution of Aib containing oligopeptides have been extensively compared with the results of crystallographic studies in the solid state. The consecutive β-turn conformation in Z-Aib-Pro-Aib-Ala-OMe was initially inferred from NMR studies, which established the inaccessibility of two NH groups to the solvent. This incipient 3_{10}-helical structure was then established to exist in the solid state.[74] Subsequent to this study, several reports of ^{1}H NMR studies in model Aib

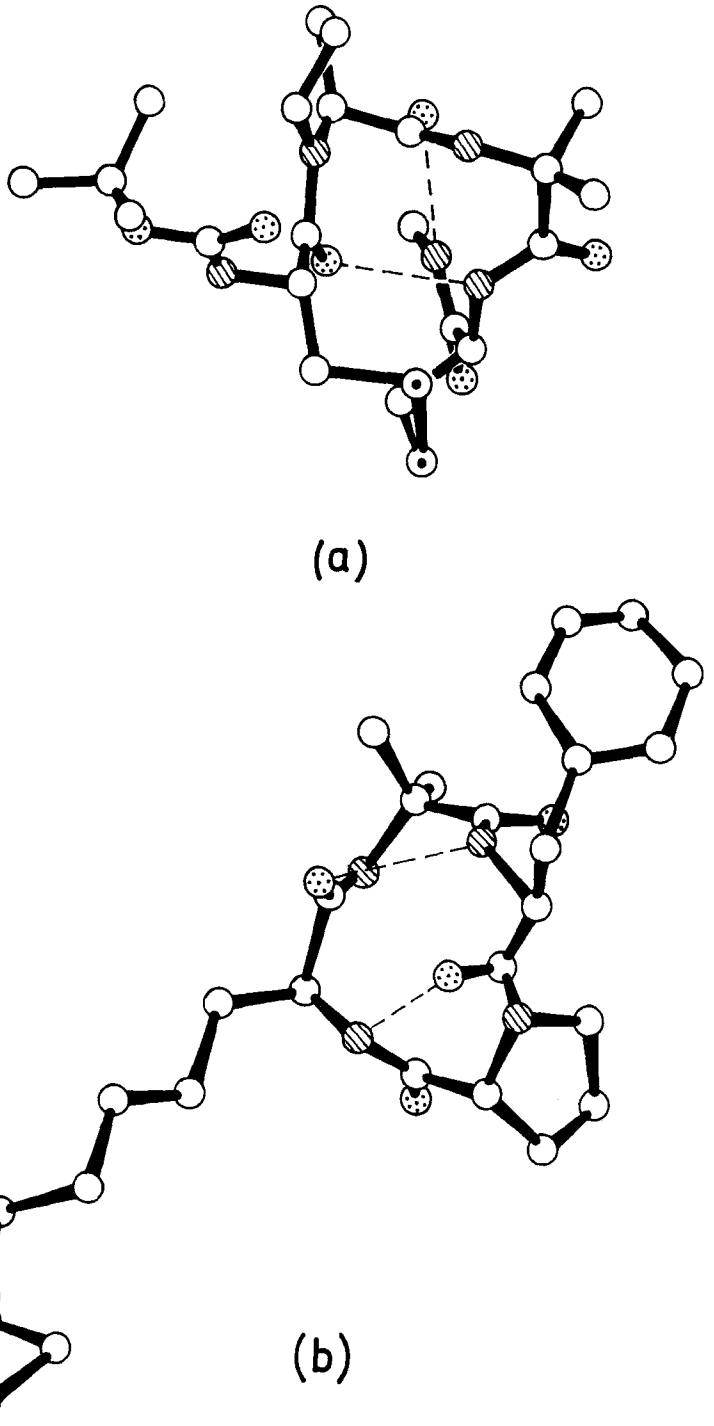


FIGURE 22. Cyclic peptides containing Aib. (a) Boc-Cys-Pro-Aib-Cys-NHMe [82] (b) Dihydrochlamydocin.[52,62]

(FIGURE 22b redrawn from Elliott, A. et al., conformation of the polypeptide chain in the solid state, in *Poly* α-*Amino Acids, Polypeptides and Proteins*, Stahmann, M. A., Ed., University of Wisconsin Press, Madison, 1962, 255. With permission.)

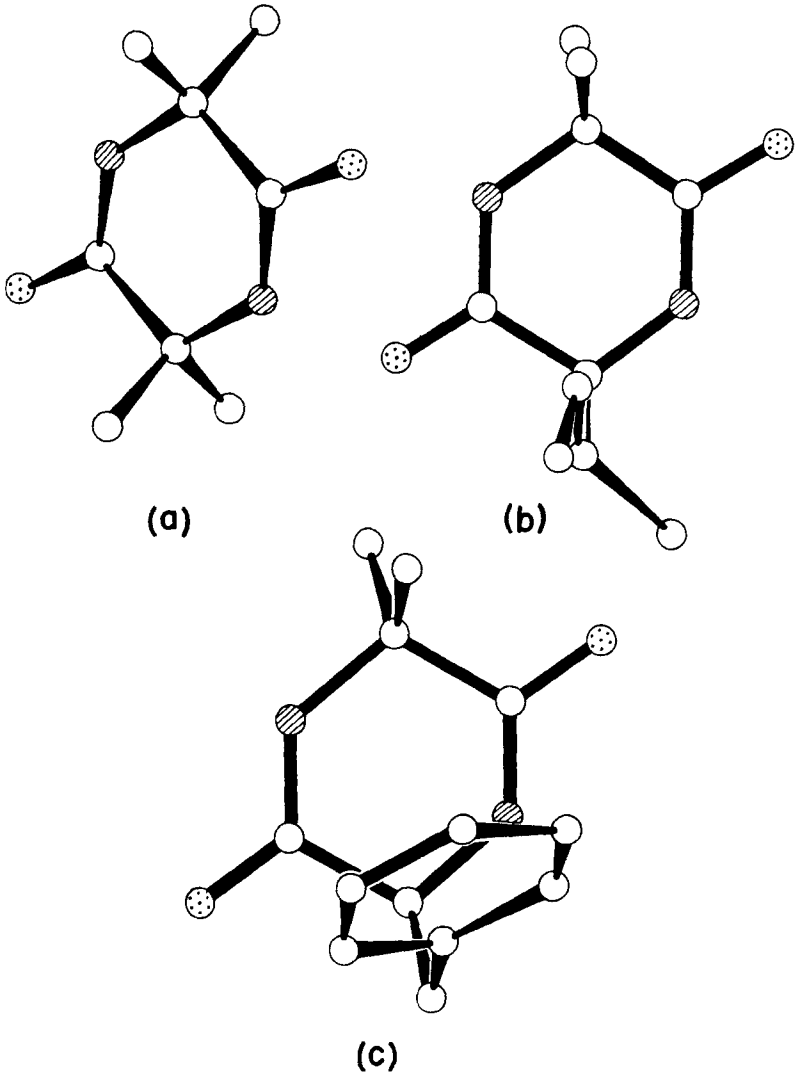


FIGURE 23. Structures of Aib containing diketopiperazines. (a) Cyclo(Aib-Aib).[135] (b) Cyclo(Aib-Ile).[135] (c) Cyclo(Aib-Phe) (unpublished).

peptides[103,138-142] and synthetic fragments of membrane channel peptides[96,105,143-145] have appeared. From analyses of the number of solvent-shielded (presumably hydrogen-bonded) NH groups, 3_{10}-helical conformations have been proposed even for peptides as long as 16 residues.[146] The structural restraints imposed by Aib result in conformations which are largely solvent independent, permitting comparisons between studies in polar and apolar solvents. Further, very good correlations have been obtained between the number of hydrogen bonds determined in the solid state for folded alamethicin fragments and model peptides and IR intensities for the hydrogen-bonded NH stretching band in dilute chloroform solutions.[147] These peptides therefore appear to be conformationally homogeneous in solution and their solid-state backbone conformations are not significantly influenced by crystal packing forces.

Circular dichroism studies of Aib containing oligopeptides of moderate length ($\geq$10 residues) have generally been interpreted in terms of α-helical structures. This follows from the broad resemblance of the observed CD spectra to classical helical patterns.[148-150] The determination of an α-helical conformation for the 11-residue peptide Boc-Ala(Aib-Ala)$_2$-

Glu(OBz)-Ala(Aib-Ala)$_2$-OMe in the solid state has been used to support such interpretations of CD spectra. However, recent studies in this laboratory[160] suggest that it may be difficult to establish a ready chiroptical distinction between 3_{10}- and α-helical conformations. Further, helical peptide association frequently results in dramatic changes in CD spectra, rendering conformational interpretations suspect.

XI. PROSPECTS

The possibility of using Aib residues to synthesize model peptides with well-defined conformations has been realized in studies of β-turn conformations and 3_{10}-helices.[131] Aib-containing sequences have been used in characterizing CD (unpublished) and Raman spectral features associated with these conformations.[151] Helix development in Aib peptides has been probed using the geminal nonequivalence of Aib C^{β} atoms in ^{13}C NMR studies.[152,153] Further research in this area will undoubtedly focus on defining the sequence requirements for generating 3_{10}- or α-helical structures. It may also be possible to generate antiparallel β-sheet conformations, using Aib and Pro residues for stabilizing a chain reversal in the center of a peptide sequence rich in β-sheet-promoting residues. The judicious use of Aib residues should permit the design of peptide backbone structures, which allow specific positioning of reactive amino acid side chains. Such an approach may be of value in the design of enzyme models[154,155] and in constructing structures to mimic many functional aspects of biologically active molecules. The recognition of the role of α-alkylation in restricting backbone conformations has led to the synthesis of Aib analogs of several biologically active peptides in an attempt to delineate the nature of the conformations recognized at receptor sites. The full promise of this approach has yet to be exploited.[8-16] While in the past few years the impetus for studies of Aib peptide stereochemistry has been to develop structure-function relationships for alamethicin and related membrane channels, there is little doubt that in the future Aib and similar α-alkyl amino acids will be used to engineer model peptides for application in many areas of bioorganic chemistry.

NOTE ADDED IN PROOF

Structural details for the following peptides have been reported since submission of this review: Ac-Aib-Ala-Aib-OMe (very distorted β-bend, with Aib in a Type III′ conformation);[163] Z-Aib-Pro-Aib-OMe (Type I (III) β-turn);[164] Boc-Aib-Ala-Aib-Ala-Aib-OMe.$2H_2O$ (3_{10}-helix, three Type III β-turns)[165] and Boc-Pro-Aib-Ala-Aib-OMe (two consecutive Type III β-turns).[166] Structures solved in this laboratory include Boc-Aib-Phe-Leu.H_2O (Type I β-turn) (Velumurugan, D. and Prasad, B.V.V. unpublished) and Boc-Val-(Aib-Val)$_3$-OMe (3_{10}-helix, five Type III β-turns) (Francis, A. K. and Vijayan, M., unpublished). Preliminary crystallographic data on the Aib containing 19 residue natural peptide trichorzianine A-1 has been reported.[167]

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