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The Crystal State Conformation of Aib-Rich Segments of Peptaibol Antibiotics

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Abstract: Ac-(Aib-Ala)$_3$-OH (a protected segment of the peptaibols gliodeliquescin and paracelsin), Z-Leu-Aib-Val-Aib-Gly-O*t*Bu (a segment of [Leu]^7-gliodeliquescin), Z-Val-Aib-Aib-Gln-O*t*Bu (a common segment of alamethicin, paracelsin, and hypelcin), and Ac-Aib-Pro-(Aib-Ala)$_2$-OMe and Z-Aib-Pro-(Aib-Ala)$_2$-OMe, which represent differently N$^\alpha$-protected 1–6 segments of alamethicin and hypelcin, have been synthesized by solution methods. The crystal-state conformations of these five Aib-containing peptides have been determined by X-ray diffraction analysis. We have confirmed that the 3_{10}-helical structure is preferentially adopted by Aib-rich short peptides. An experimentally unambiguous proof for the $3_{10} \rightarrow \alpha$-helix conversion has been provided by the two differently N-blocked -Aib-Pro-(Aib-Ala)$_2$-OMe hexapeptides. The β-bend ribbon conformation, commonly observed in the (Aib-Pro)$_n$ sequential oligopeptides, is not found in the -Aib-Pro-Aib-Ala-Aib-Ala- sequence. As expected on the basis of the L-configuration of the C$^\alpha$-monoalkylated residues, a right-handed helix screw sense was found in all peptides investigated. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: X-ray crystal structure; gliodeliquescin; alamethicin; hypelcin; paracelsin

INTRODUCTION

2-Aminoisobutyric acid, Aib, characterizes an important family of natural antibiotics, the peptaibols [1], which alter the ionic permeability of biological membranes by forming channels.

It is well established that the presence of two methyl groups on the C$^\alpha$-carbon in Aib residues imposes a marked restriction on the available conformational space [2–4]. As a consequence, folded and helical structures of the 3_{10}- and α-helical types [5,6] are significantly more stable than any other type of structure. On the other hand, the energy difference between the 3_{10}- and α-helical structures is rather small.

All Aib homo-peptides investigated so far adopt a regular 3_{10}-helical structure [5] (also described as a succession of type III or III′ β-bends) and no critical chain length for 3_{10}-helix formation was found.

The presence in an Aib peptide chain of other residues might switch the conformation from a 3_{10}-helix to an α-helix depending on the total length of the peptide, on its composition and Aib content [6]. The critical chain length for α-helix formation in an *p*BrBz-(Aib-Ala)$_n$-OMe sequential oligopeptide series was investigated both in the crystal state and in solution [7–9]. The octapeptide ($n = 4$) turned out to be the shortest peptide exhibiting the α-helical con-

Abbreviations: Ac, acetyl; *p*BrBz, *para*-bromobenzoyl; Me, methyl; Piv, pivaloyl; Z, benzyloxycarbonyl; *t*Bu, *tert* butyl; TFA, trifluoroacetic acid; EDCI, *N*-ethyl-*N*′-(3-dimethyl-aminopropyl)-carbodiimide HOBt, 1-hydroxybenzotriazole.

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formation. It is evident that the $3_{10} \rightarrow \alpha$-helical transition takes place as a consequence of the main-chain-length increase.

In addition, in a sequential peptide series with alternating Aib and Pro residues, partial disruption of the conventional H-bonding scheme typical of the 3_{10}-helix is observed because of the presence of Pro residues. The resulting structure is the so called β-bend ribbon conformation [10,11], which is extensively adopted by the longest oligomers in the terminally blocked -(Pro-Aib)$_n$- and -Aib-(Pro-Aib)$_n$- sequential peptide series [12]. This structure should be considered a subtype of the 3_{10}-helix, having nearly the same helical fold of the peptide chain and being stabilized by 50% of the usual intramolecular N–H···O=C H-bonds of type III β-bends.

Several peptaibol antibiotics have been isolated from different fungal sources. Our laboratories have been involved in the elucidation of the chemical structure and conformation of natural peptaibols and their segments [13–21]. To further investigate the conformation of these molecules and their segments and to establish the influence of amino acid composition and sequence on the type of helix adopted by the Aib-rich peptide chains we have analysed the crystal-state structures of the following peptides: Ac-(Aib-Ala)$_3$-OH and Z-Val-Aib-Aib-Gln-O*t*Bu, which are the protected segments 1–6 of gliodeliquescin [22] and paracelsin [23] and 15–18 of alamethicin [24], paracelsin and hypelcin [25], respectively, and Z-Leu-Aib-Val-Aib-Gly-O*t*Bu, the protected segment 7–11 of synthetic [Leu]^7-gliodeliquescin. We also report the crystal-state conformation of the two differently N$^{\alpha}$-blocked peptides Ac-Aib-Pro(Aib-Ala)$_2$-OMe and Z-Aib-Pro (Aib-Ala)$_2$-OMe, whose sequence represents the 1–6 segment of alamethicin and hypelcin. These two peptides, on the other hand, can also be considered analogues of the 1–6 fragment of gliodeliquescin A.

Our results show that the 3_{10}-helical structure is preferentially adopted by these short peptides, but they additionally demonstrate that a subtle balance between the 3_{10}- and α-helical structures is at hand, which in the crystal state is also influenced by crystal forces involving the terminal blocking groups.

RESULTS

The X-ray diffraction structures of Ac-(Aib-Ala)$_3$-OH, Z-Val-Aib-Aib-Gln-O*t*Bu and Z-Leu-Aib-Val-Aib-Gly-O*t*Bu are given in Figure 1A, B, and C, respectively. The structures of Ac-Aib-Pro-(Aib-Ala)$_2$-OMe and Z-Aib-Pro-(Aib-Ala)$_2$-OMe are given in Figure 2A and B, respectively. Ac-(Aib-Ala)$_3$-OH is found as a solvate: in the crystal a molecule of methanol bridges symmetry-related peptide molecules. The conformation of each molecule is analytically described by the ϕ, ψ and ω conformational angles [26] listed in Table 1 for all peptides. Intra- and intermolecular hydrogen-bonds are given in Table 2.

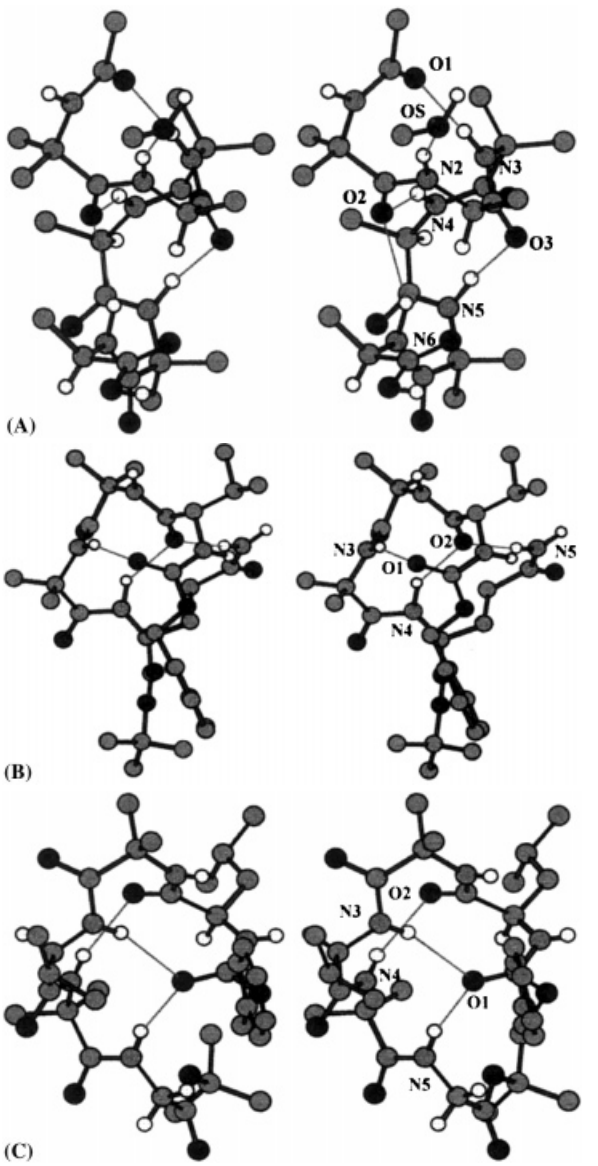

Figure 1 (A) Stereoview of the X-ray structure of Ac-(Aib-Ala)$_3$-OH. (B) Stereoview of the X-ray structure of Z-Val-Aib-Aib-Gln-O*t*Bu. (C) Stereoview of the X-ray structure of Z-Leu-Aib-Val-Aib-Gly-O*t*Bu. Intramolecular hydrogen-bonds are indicated as dashed lines.

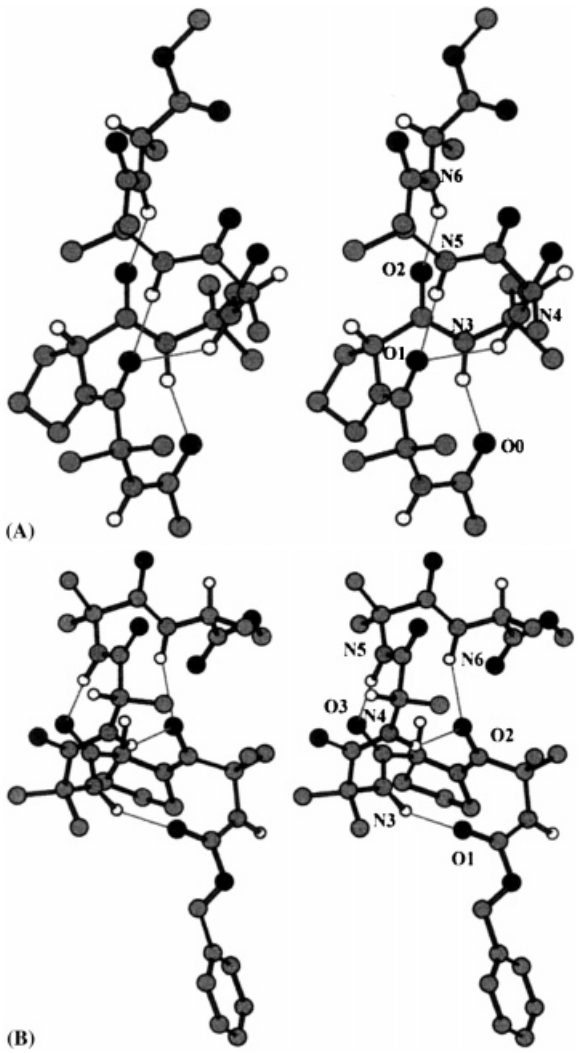

Figure 2 (A) Stereoview of the X-ray structure of Ac-Aib-Pro-Aib-Ala-Aib-Ala-OMe. (B) Stereoview of the X-ray structure of Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe. Intramolecular hydrogen-bonds are indicated as dashed lines.

As usually found in short or medium-sized Aib-rich peptides [5,6], the conformation of each peptide is either described as an (incipient) 3_{10}- or α-helix. In all molecules the helix screw sense is right-handed, as expected on the basis of the L-configuration of the C-monoalkylated residues.

The N-terminal blocked hexapeptide Ac-(Aib-Ala)$_3$-OH shows a right-handed 3_{10}-helical fold for the first three residues in the chain with average ϕ, ψ torsion angles $-55.1°$, $-30.9°$, very close to those typical of this type of helix [27], and the formation of two consecutive type III β-turns (C_{10} structures) stabilized by $1 \Leftarrow 4$ intramolecular C=O···H–N hydrogen-bonds. The right-handed screw sense is dictated by the presence in the peptide of the L-Ala residues. The incipient 3_{10}-helix is completed into one full turn of helix by an additional, distorted, type I β-turn in which the third (Aib^3) and fourth (Ala^4) residues are involved. The conformation of the C-terminal portion of the peptide is in part stabilized by the extremely weak intramolecular N6···O2 hydrogen-bond forming a C_{16} ring structure. This structural motif is typically found at the C-terminus of peptide helices [8,28]. The onset of this conformation, including a chiral reversal at the penultimate ($n-1$) residue, is facilitated by the achiral nature of Aib^5 and the intrinsic tendency of the residue at the $n-2$ position (Ala^4) to adopt a conformation in the bridge region of the ϕ, ψ space [29]. The O2 carbonyl oxygen is hydrogen-bonded as acceptor to two donor N–H groups (N4–H and N6–H). The Ala^6 residue falls in the extended region of the ϕ, ψ map. Interestingly, the C-terminal blocked peptide *p*BrBz-(Aib-Ala)$_3$-OMe, having the same peptide sequence but a different blocking group at the N-terminus and an ester C-terminal group, was found to be in a fully developed right-handed 3_{10}-helical conformation, with four consecutive $1 \Leftarrow 4$ intramolecular C=O···H–N hydrogen-bonds and average ϕ, ψ values $-62°$ and $-25°$ [7], without formation of the C_{16} ring structure.

The shorter pentapeptide Z-Leu-Aib-Val-Aib-Gly-O*t*Bu shows the same helical fold of the backbone as that found in Ac-(Aib-Ala)$_3$-OH for the first two residues, a type III β-turn involving the Aib^3 N–H group as donor and the urethane C=O group as acceptor. Then, a type I β-turn follows in which the Aib^4 N–H is H-bonded to the Leu^1 C=O. This folding motif is closed at the C-terminus by a C_{16} structure, analogous to that found in Ac-(Aib-Ala)$_3$-OH and stabilized by an intramolecular hydrogen-bond between the Gly^6 N–H group and the urethane C=O group. Thus, the urethane C=O group acts as acceptor of two hydrogen-bonds. Again, as in the structure of Ac-(Aib-Ala)$_3$-OH, the C_{16} structural motif is facilitated by the presence of an achiral residue (Aib^4) and a residue in the bridge region of the ϕ, ψ map (Val^3). This motif is a widely observed helix-terminating conformation in proteins and it is usually achieved by the adoption of a left-handed helical (α_L) conformation by a C-terminal residue and it could be readily mimicked in synthetic peptides by placing an achiral residue at the penultimate position of the helix. These conditions are both verified in Z-Leu-Aib-Val-Aib-Gly-O*t*Bu.

The protected tetrapeptide Z-Val-Aib-Aib-Gln-O*t*Bu is folded in two consecutive β-turns of type II

Table 1 Conformational Angles (in Degrees)

Peptides/Residues		1	2	3	4	5	6
Ac-(Aib-Ala)$_3$-OH	ϕ	−56.7(4)	−55.8(3)	−52.8(3)	−103.3(2)	81.0(2)	−75.5(3)
	ψ	−28.6(3)	−29.4(3)	−34.8(3)	17.9(2)	14.9(3)	160.5(2)
	ω	−178.1(2)	−179.7(2)	−170.9(2)	176.1(1)	169.5(2)	
Z-Val-Aib-Aib-Gln-O*t*Bu	ϕ	−64(1)	58(1)	58(1)	−77(1)		
	ψ	138.7(8)	38(1)	26(1)	136(1)		
	ω	168.8(9)	175(1)	173(1)	−169(1)		
Z-Leu-Aib-Val-Aib-Gly-O*t*Bu	ϕ	−54.0(3)	−54.0(3)	−123.0(2)	62.0(2)	−61.1(3)	
	ψ	−37.7(3)	−32.7(3)	25.0(3)	37.6(2)	−27.6(3)	
	ω	−174.5(2)	−174.0(2)	173.6(2)	163.1(2)	177.3(2)	
Ac-Aib-Pro-(Aib-Ala)$_2$-OMe	ϕ	−49.7(3)	−58.7(3)	−59.4(3)	−80.0(3)	−63.4(3)	−60.4(4)
	ψ	−43.4(3)	−33.6(3)	−39.8(3)	−36.3(3)	−35.9(3)	146.6(3)
	ω	−174.6(2)	−179.3(2)	179.9(2)	−177.3(2)	−175.8(2)	180.0(4)
Z-Aib-Pro-(Aib-Ala)$_2$-OMe	ϕ	−53.5(9)	−57.6(6)	−57.5(7)	100.4(7)	61.1(7)	123.8(8)
	ψ	−42.7(8)	−35.6(6)	−31.6(8)	19.1(7)	35.6(9)	165.9(7)
	ω	175.0(5)	177.3(5)	176.9(6)	175.5(5)	175.8(8)	179.9(8)

E.S.D.'s are given in parentheses.

and III′, stabilized by an extremely weak intramolecular interaction between the Aib^3 N–H group and the urethane C=O group, and by a stronger hydrogen-bond between the Gln^4 N–H group and the Val^1 C=O group, respectively. The Val^1 C=O group acts as acceptor of a second hydrogen-bond with the side-chain N–H group of the Gln^4 residue.

As usually observed in 3_{10}-helical folds [5,6] in the crystal structures of Ac(Aib-Ala)$_3$-OH, Z-Leu-Aib-Val-Aib-Gly-O*t*Bu and Z-Val-Aib-Aib-Gln-O*t*Bu the N–H groups of the first two residues along the sequence are not involved in the intramolecular hydrogen-bonding scheme, but rather they participate to intermolecular hydrogen-bonds with acceptor oxygen atoms of symmetry-related molecules or of the solvent. In fact, in the three structures discussed above, helical molecules pack with each other in a head-to-tail fashion, being stabilized by intermolecular hydrogen-bonds in which the N1–H and N2–H groups are involved. The head-to-tail interaction in Ac-(Aib-Ala)$_3$-OH is mediated by a solvent (MeOH) molecule located at the interface between two interacting peptide helical molecules.

The two hexapeptide analogues Ac-Aib-Pro-(Aib-Ala)$_2$-OMe and Z-Aib-Pro-(Aib-Ala)$_2$-OMe, which bear the same sequence but different N-terminal protecting groups, behave differently in the crystal state.

The acetyl derivative shows a right-handed helical fold made up of mixed 3_{10}- and α-helical turns (β- and α-turns [30]: C_{10} and C_{13} intramolecularly hydrogen-bonded structures). In fact, while the Aib^3 and Ala^4 N–H groups are involved in two consecutive type III β-turns, in which the N-terminal acetyl and the Aib^1 C=O groups, respectively, take part, the Aib^5 and Ala^6 N–H groups are involved in two consecutive α-turns with the Aib^1 and Pro^2 C=O groups, respectively. In this scheme the Aib^1 C=O group is hydrogen-bonded to the N–H groups of Ala^4 and Aib^5 residues. This mixed 3_{10}-/α-helical fold is similar to that observed previously in *p*BrBz-(Aib-Ala)$_4$-OMe [7], in which two intramolecular hydrogen-bonds of the C_{10}-type (3_{10}-helical fold), followed by four intramolecular hydrogen-bonds of the C_{13}-type (α-helical fold), were found.

On the contrary, the N-terminal benzyloxycarbonyl analogue Z-Aib-Pro-(Aib-Ala)$_2$-OMe shows an helical conformation quite similar to that observed for Ac-(Aib-Ala)$_3$-OH. Two consecutive type III β-turns and a type I β-turn are followed by a C_{16} hydrogen-bonded structure. The resulting right-handed structure is stabilized by four intramolecular hydrogen-bonds between the N–H groups of residues 3 to 6 and the urethane, Aib^1, Pro^2 and again Aib^1 C=O groups, respectively.

Head-to-tail packing of the helices in both Ac-Aib-Pro-(Aib-Ala)$_2$-OMe and Z-Aib-Pro-(Aib-Ala)$_2$-OMe hexapeptides is stabilized by N–H···O=C hydrogen-bonds with the involvement of only the N1–H group as donor. Other than those contacts between hydro-

Table 2 Intra- and Intermolecular Hydrogen-Bonds

Donor (D)	Acceptor (A)	D···A (Å)	D–H···A (°)	
Ac-(Aib-Ala)$_3$-OH				
A. Intramolecular hydrogen-bonds				
N3	O1	2.91(1)	159(4)	
N4	O2	2.98(1)	150(3)	
N5	O3	3.07(1)	155(3)	
N6	O2	3.38(1)	149(3)	
B. Intermolecular hydrogen-bonds				
N1	O4	2.93(1)	170(3)	$1-x,\ y,\ 1-z$
N2	OS[a]	2.92(1)	160(4)	$x,\ y,\ z$
O8	O5	2.67(1)	157(3)	$x,\ y,\ 1-z$
OS	O6	2.71(1)	176(4)	$-x,\ -1/2+y,\ -1-z$
Z-Val-Aib-Aib-Gln-O*t*Bu				
A. Intramolecular hydrogen-bonds				
N3	O1	3.54(1)	159(9)	
N4	O2	3.06(1)	141(9)	
N5	O2	2.88(1)	154(9)	
B. Intermolecular hydrogen-bonds				
N1	O3	3.15(1)	167(9)	$1/2-x,\ -1/2+y,\ 1/2-z$
N2	O5	2.80(1)	177(9)	$x,\ y,\ 1-z$
Z-Leu-Aib-Val-Aib-Gly-O*t*Bu				
A. Intramolecular hydrogen-bonds				
N3	O1	3.10(1)	160(2)	
N4	O2	3.13(1)	165(2)	
N5	O1	2.89(1)	160(2)	
B. Intermolecular hydrogen-bonds				
N1	O4	3.01(1)	160(2)	$-1-x,\ 1/2+y,\ 1/2-z$
N2	O5	3.10(1)	172(2)	$-1-x,\ 1/2+y,\ 1/2-z$
Ac-Aib-Pro-(Aib-Ala)$_2$-Ome				
A. Intramolecular hydrogen-bonds				
N3	O0	3.07(1)	158(3)	
N4	O1	3.07(1)	132(2)	
N5	O1	2.99(1)	163(3)	
N6	O2	3.13(1)	152(3)	
B. Intermolecular hydrogen-bonds				
N1	O4	2.94(1)	128(3)	$1/2-x,\ 2-y,\ -1/2+z$
N1	O6	3.14(1)	130(2)	$1/2-x,\ 2-y,\ -1/2+z$
Z-Aib-Pro-(Aib-Ala)$_2$-OMe				
A. Intramolecular hydrogen-bonds				
N3	O1	2.99(1)	137(5)	
N4	O2	3.00(1)	139(5)	
N5	O3	3.02(1)	160(6)	
N6	O2	3.18(1)	158(5)	
B. Intermolecular hydrogen-bond				
N1	O4	2.93(1)	131(6)	$1-x,\ y,\ z$

[a] OS, MeOH oxygen atom.

gen-bonded atoms no intramolecular contact less than 3.5 Å between atoms different from hydrogen-atoms are observed in both crystals. We can speculate that the presence of the electronically different urethane and amide blocking groups in the two peptides could result in slightly different overall dipole moments at the onset of the helix, which eventually, when a certain length is reached, achieves the energetically most stable conformation compatible with all other factors influencing the intra- and intermolecular interactions.

DISCUSSION

We have prepared a series of peptide segments of gliodeliquescin, alamethicin, paracelsin and hypelcin peptaibols. The crystal-state conformations of five Aib-containing peptides have been analysed by an X-ray diffraction study. The results have been compared to those reported in the literature for analogous peptides.

For the C^{α}-monosubstituted residues the 3_{10}-helix is energetically less favourable than the α-helix [31–33]. However, although the presence of 3_{10}-helical segments would now be well documented in protein crystal structures [34], the factors governing the transition between the 3_{10}- and α-helices appear to be quite subtle and not completely understood. This conformational transition is of great relevance to the problem of the relationship between three-dimensional structure (and helix self-association mode) and bioactivity of the membrane-active, channel-forming, ion-transporting peptaibol antibiotics.

In this study we have confirmed that N- and C-blocked peptides shorter than six residues, containing the helix-inducing Aib residue, have a very strong preference for the 3_{10}-helical conformation. We have also provided an experimentally unambiguous proof for the $3_{10} \rightarrow \alpha$-helix conversion in the terminally blocked hexapeptides Ac- and Z-Aib-Pro-$(Aib\text{-}Ala)_2$-OMe, i.e. in peptides with the same sequence but with different N^{α}-blocking groups. In the crystal state a subtle balance of factors, including main-chain length, Aib content, sequence, and polarity of crystallization solvents was known to take place between the two helical conformations [6]. All of these factors are heavily involved in the intra- and intermolecular interactions which are experimentally observed in the crystals. The disposition of the side-chains around the helix strongly influences the intra- and intermolecular contacts: minimization of short- and long-range non-bonded interactions is then better achieved in only one of the possible helical folds.

Finally, we have also shown that a β-bend ribbon conformation, proposed by Karle and coworkers [10,11] and observed in the $(Aib\text{-}Pro)_n$ sequential oligopeptides [12], cannot be induced by only one Pro residue in the Aib-Pro-Aib-Ala-Aib-Ala sequence.

MATERIALS AND METHODS

Synthesis and Characterization of Peptides

The protected amino acids Z-Ala-OH, Z-Val-OH, Z-Leu-OH, HCl·H-Pro-OMe, and HCl·H-Gly-O*t*Bu were purchased from Bachem (Bubendorf, Switzerland). Z-Aib-OH, H-Aib-O*t*Bu, Z-Aib-OPiv and Z-Aib-Aib-O*t*Bu were synthesized according to the literature [35].

All peptides were synthesized by conventional solution methods. For the synthesis of segments the Z/O*t*Bu or Z/OMe strategy was used, i.e. N-terminal amino acids were protected by the Z-group and C-terminal amino acids as O*t*Bu or OMe esters. The latter was employed in the course of the synthesis of Z-Aib-Pro-Aib-OH in order to avoid acidolytic cleavage of the acid labile Aib-Pro bond [24,36,37]. Coupling of intermediates was carried out in DMF as solvent with water-soluble EDCI and HOBt as activating agents. Z-groups were removed by hydrogenolysis in MeOH in the presence of 10% Pd on charcoal, O*t*Bu ester groups by TFA, and OMe ester groups by saponificatian with aqueous NaOH. As for acetylation of $H\text{-}(Ai\text{-}Ala)_3\text{-}O\textit{t}Bu$, Ac_2O in CH_2Cl_2 was used.

Analytical data of the peptides are compiled in Table 3 and include quantitative amino acid analysis (AAA) by HPLC [38], ionization mass spectrometry (ESI-MS) [21], melting points (uncorrected), and optical rotations.

X-ray Diffraction

Single crystals of all compounds were grown from a mixture of methanol, diethyl ether and *n*-hexane solutions by slow evaporation. The X-ray diffraction data were collected on a Nonius CAD-4 four circle diffractometer, using the graphite-monochromated CuK_{α} radiation ($\lambda = 1.54178$ Å). The independent reflections were measured in the $\omega/2\theta$-scan mode and in the θ range 1–70°. Unique cell parameters were determined by least-squares refinement of the

Table 3 Characterization of Peptides

Peptide	AAA, found (calc.)	ESI-MS $(M+Na)^+$, (m/z)	M.p. (°C)	$[\alpha]_D^{25}$ ($c = 1$, MeOH)
Ac-(Aib-Ala)$_3$-OH	Ala 3.00(3), Aib 3.08(3)	551	202	−4.7
Z-Val-Aib-Aib-Gln-O*t*Bu	Val 1.00(1), Aib 1.98(2), Glu 0.97(1)	628	164	−55.7
Z-Leu-Aib-Val-Aib-Gly-O*t*Bu	Leu 0.99(1), Aib 2.05(2), Val 1.00(1), Gly 1.00(1)	670	198	−0.2
Ac-Aib-Pro-(Aib-Ala)$_2$-OMe	Aib 3.07(3), Pro 0.93(1), Ala 2.00(1)	591	246–248	+5.5
Z-Aib-Pro-(Aib-Ala)$_2$-OMe	Aib 3.12(3), Pro 0.95(1), Ala 2.00(2)	683	182	+38.6

setting angles of 25 high-angle reflections ($20° < \theta < 30°$). For each data collection three standard reflections were measured periodically to monitor misalignments, crystal decay and electronic instability. Their intensities did not show variations greater than 5%, indicating stability in the data collection conditions. The main crystallographic data of the peptides investigated in the present study are given in Table 4. The crystal structures were solved by direct methods using SHELXS-90 [39]. The E-maps revealed the whole molecules except the hydrogen atoms. Refinements of the structures were performed using SHELX-76 [40] for all compounds except for Z-Leu-Aib-Val-Aib-Gly-O*t*Bu which was refined by SHELXL-93 [41]. Heavy atoms were refined with anisotropic thermal factors, while hydrogen atoms linked to nitrogens were located by difference Fourier maps and refined keeping the N–H distance to 1.03 Å. Other hydrogen atoms were placed in their stereochemically expected positions. An isotropic thermal factor was assigned to each hydrogen and kept fixed during refinement.

Table 4 Crystallographic Data

Compound	**1**	**2**	**3**	**4**	**5**
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
Unit cell dimensions					
a (Å)	9.296(1)	27.233(4)	9.449(1)	9.083(1)	0.190(1)
b (Å)	18.784(3)	14.026(2)	17.483(3)	16.139(2)	17.218(2)
c (Å)	9.373(2)	9.079(1)	22.116(4)	20.931(3)	10.400(1)
β (°)	112.49(2)	90.00	90.00	90.00	92.82(2)
Z	2	4	4	4	2
Density (calculated) (g cm^{-3})	1.230	1.159	1.178	1.230	1.165
Independent reflections	2867	3702	3887	3276	3793
Data [$1 > 3\sigma(I)$]	2794	1799	3805	3114	2427
No. of parameters	483	197	431	494	284
Goodness-of-fit	1.43	1.27	0.96	1.94	0.84
R indices [$1 > 3\sigma(I)$]					
R	0.034	0.091	0.037	0.035	0.053
$R_{\text{all data}}$	–	–	0.043	–	–
R_W	0.038	0.093	0.102	0.034	0.064
Largest difference peak (e Å^{-3})	0.13	0.29	0.12	0.13	0.15
Largest difference hole (e Å^{-3})	−0.16	−0.31	−0.24	−0.24	−0.19

(**1**) Ac-(Aib-Ala)$_3$-OH MeOH solvate; (**2**) Z-Val-Aib-Aib-Gln-O*t*Bu; (**3**) Z-Leu-Aib-Val-Aib-Gly-O*t*Bu; (**4**) Ac-Aib-Pro-(Aib-Ala)$_2$-OMe; (**5**) Z-Aib-Pro-(Aib-Ala)$_2$-OMe.
Compounds **1**, **2**, **4** and **5** were refined with SHELX-76 [40]; compound **3** was refined with SHELXL-93 [41] with a R_W and a goodness-of-fit on F^2. Crystals of **2** were of poor quality and the molecule was divided in two blocks for the refinement. Hydrogen-atoms were fixed for compounds **2**, **3** and **5**. Coordinates and anisotropic parameters were refined alternatively for compound **5**.

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REFERENCES

1. R.C. Pandey, H. Meng, J. Cook and K.L. Rinehart (1977). Structure of antiamoebin I from high resolution field desorption and gas chromatographic mass spectrometry studies. *J. Am. Chem. Soc. 99*, 5203–5205.
2. G.R. Marshall in: *Intra-Science Chemistry Reports*, N. Karaseh, Ed., p. 305–316, Gordon and Breach, New York, 1971.
3. B.V. Venkataram Prasad and V. Sasisekharan (1979). A case study of the conformation of poly (α-aminoisobutyric acid): α- or 3_{10}-helix. *Macromolecules 12*, 1107–1110.
4. Y. Paterson, S.M. Rumsey, E. Benedetti, G. Némethy and H.A. Scheraga (1981). Sensitivity of polypeptide conformation to geometry. Theoretical conformational analysis of oligomers of α-aminoisobutyric acid. *J. Am. Chem. Soc. 103*, 2947–2955.
5. E. Benedetti and C. Toniolo in: *Polymeric Materials Encyclopedia*, Vol. 8, J.C. Salamone, Ed., p. 6472–6481, CRC Press, Boca Raton, 1996.
6. I.L. Karle and P. Balaram (1990). Structural characteristics of α-helical peptide molecules containing Aib residues. *Biochemistry 29*, 6747–6756.
7. V. Pavone, E. Benedetti, B. Di Blasio, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma and L.Sartore (1990). Critical main-chain length for conformational conversion from 3_{10}-helix to α-helix in polypeptides. *J. Biomol. Struct. Dyn. 7*, 1321–1331.
8. E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma and L.Sartore (1990). Linear oligopeptides. Part 227. X-Ray crystal and molecular structures of two α-helix-forming (Aib-L-Ala)$_n$ sequential oligopeptides, *p*BrBz-(Aib-L-Ala)$_5$-OMe and *p*BrBz-(Aib-L-Ala)$_6$-OMe. *J. Chem. Soc. Perkin Trans. 2*, 1829–1837.
9. K. Otoda, Y. Kitagawa, S. Kimura and Y. Imanishi (1993). Chain length dependent transition of 3_{10}- to α-helix of Boc-(Ala-Aib)$_n$-OMe. *Biopolymers 33*, 1337–1345.
10. I.L. Karle, J. Flippen-Anderson, M. Sukumar and P. Balaram (1987). Conformation of a 16-residue zervamicin IIA analog peptide containing three different structural features: 3_{10}-helix, α-helix, and β-bend ribbon. *Proc. Natl. Acad. Sci. USA 84*, 5087–5091.
11. I.L. Karle, J. Flippen-Anderson, S. Agarwalla and P. Balaram (1991). Crystal structure of [Leu^1]zervamicin, a membrane ion-channel peptide. Implications for gating mechanism. *Proc. Natl. Acad. Sci. USA 88*, 5307–5311.
12. B. Di Blasio, V. Pavone, M. Saviano, A. Lombardi, F. Nastri, C. Pedone, E. Benedetti, M. Anzolin and C. Toniolo (1992). Structural characterization of the β-bend ribbon spiral. Crystallographic analysis of two long (L-Pro-Aib)$_n$ sequential peptides. *J. Am. Chem. Soc. 114*, 6273–6278.
13. C. Toniolo, G.M. Bonora, E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone and C. Pedone (1983). Linear oligopeptides. Peptaibol antibiotics: preferred conformation of the 2–9 segment of emerimicins III and IV and all related short sequences. *Int. J. Biol. Macromol. 7*, 357–362.
14. C. Toniolo, G.M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone (1985). Molecular structure of peptaibol antibiotics: solution conformation and crystal structure of the octapeptide corresponding to the 2–9 sequence of emerimicins III and IV. *J. Biomol. Struct. Dyn. 3*, 585–598.
15. M. Kokkinidis, D.W. Banner, D. Tsernoglou and H. Brückner (1986). Crystal structure of L-Pro-L-Leu-Aib-Aib-L-Glu-L-Valol, the C-terminal hexapeptide fragment of trichotoxin. *Biochem. Biophys. Res. Commun. 139*, 590–595.
16. M. Kokkinidis, D. Tsernoglou and H. Brückner (1986). Crystal structure of Z-Aib-Aib-Ala-Ala-Aib-O*t*Bu, a hexapeptide fragment of trichotoxin. *Biochem. Biophys. Res. Commun. 136*, 870–875.
17. R. Gessmann, H. Brückner and M. Kokkinidis (1991). Structural flexibility of Aib-containing peptides. The N-terminal tripeptide of trichotoxin. *Biochem. Biophys. Res. Commun. 174*, 878–884.
18. R. Gessmann, H. Brückner and M. Kokkinidis (1991). The crystal structure of Z-Aib-Gly-Aib-Leu-Aib-O*t*Bu, the synthetic, protected N-terminal pentapeptide of trichotoxin. *Peptide Res. 4*, 239–244.
19. R. Gessmann, H. Brückner and M. Kokkinidis (1994). The crystal structure of Z-Leu-Aib-Pro-Leuol, the synthetic, protected C-terminal tetrapeptide of trichovirin. *Zeit. Kristallogr. 209*, 597–603.
20. R. Gessmann, M. Currle, H. Brückner and M. Kokkinidis (1997). Unusual conformation adopted by standard amino-acids in Aib-containing oligopeptides. *Zeit. Kristallogr. 212*, 819–825.
21. D. Becker, M. Kiess and H. Brückner (1997). Structures of peptaibol antibiotics hypomurocin A and B from the ascomycetous fungus *Hypocrea muroiana* Hino et Katsumoto. *Liebigs Ann.*, 767–772.
22. H. Brückner and M. Przybylski (1984). Methods for the rapid detection, isolation and sequence determination of peptaibols and other Aib-containing peptides of fungal origin. I. Gliodeliquescin from *Gliocladium deliquescens*. *Chromatographia 19*, 188–199.
23. H. Brückner, H. Graf and M. Bokel (1984). Paracelsin; characterization by NMR spectroscopy and circular dichroism and hemolytic properties of a peptaibol antibiotic from the cellulolytically active mould *Trichoderma reesei*. Part B. *Experientia 40*, 1189–1197.

24. R.C. Pandey, J.C. Cook and K.L. Rinehart (1977). High resolution and field desorption mass spectrometry studies and revised structures of alamethicins I and II. *J. Am. Chem. Soc. 99*, 8429–8433.
25. T. Fujita, Y. Takaishi, K. Matsuura, Y. Takeda, Y. Yoshicka and H. Brückner (1984). Further investigation of the peptide antibiotic hypelcin A: isolation and structures of hypelcins A-I, A-II, A-III and A-IV. *Chem. Pharm. Bull. Jpn. 32*, 2879–2883.
26. IUPAC-IUB Commission on Biochemical Nomenclature. (1984). *Eur. J. Biochem. 138*, 9–37.
27. C. Toniolo and E. Benedetti (1991). The polypeptide 3_{10}-helix. *Trends Biochem. Sci. 16*, 350–353.
28. I.L. Karle, A. Banerjee, S. Bhattacharjya and P. Balaram (1996). Solid state and solution conformations of a helical peptide with a central Gly-Gly segment. *Biopolymers 38*, 515–526.
29. S.S. Zimmerman, M.S. Pottle, G. Némethy and H.A. Scheraga (1977). Conformational analysis of the 20 naturally occurring amino acid residues using ECEPP. *Macromolecules 10*, 1–9.
30. C. Toniolo (1980). Intramolecularly hydrogen-bonded peptide conformations. *CRC Crit. Rev. Biochem. 9*, 1–44.
31. G.N. Ramachandran, C.M. Venkatachalam and S. Krimm (1966). Stereochemical criteria for polypeptide and protein chain conformation. 3. Helical and hydrogen-bonded polypeptide chains. *Biophys. J. 6*, 849–872.
32. F.B. Sheinerman and C.L. Brooks (1995). Helices in peptides and proteins as studied by modified Zimm-Bragg theory. *J. Am. Chem. Soc. 117*, 10098–10103.
33. N. Gresh, G. Tiraboschi and D.R. Salahub (1998). Conformational properties of a model alanyl dipeptide and of alanine-derived oligopeptides: effects of solvation in water and in organic solvents. A combined SIBFA/continuum reaction field, ab initio self-consistent field, and density functional theory investigation. *Biopolymers 45*, 405–425.
34. D.J. Barlow and J.M. Thornton (1988). Helix geometry in proteins. *J. Mol. Biol. 201*, 601–619.
35. D.S. Jones, G.W. Kenner, J. Preston and R.C. Sheppard (1965). Synthesis of peptides and polymers of some sterically hindered amino acids via oxazolone intermediates. *J. Chem. Soc.*, 6227–6239.
36. H. Brückner and G. Jung (1980). Identification of *N*-acetyl-α-aminoisobutyric acid after selective trifluoroacetolysis of alamethicin and related peptide antibiotics. *Chromatographia 13*, 170–174.
37. H. Brückner, W.A. König, M Greiner and G. Jung (1979). The sequences of the membrane-modifying peptide antibiotic trichotoxin A-40. *Angew. Chem. Int. Ed. Engl. 18*, 476–477.
38. H. Brückner, T. Westhauser and H. Godel (1995). Liquid chromatographic determination of D- and L-amino acids by derivatization with o-phthaldialdehyde and *N*-isobutyryl-L-cysteine. Applications with reference to the analysis of peptidic antibiotics, toxins, drugs and pharmaceutically used amino acids. *J. Chromatogr. A 711*, 201–215.
39. G.M. Sheldrick (1990). Phase annealing in SHEL-90. Direct methods for larger structures. *Acta Crystallogr. A46*, 467–473.
40. G.M. Sheldrick (1976). *SHELX-76*, Program for crystal structure determination, University of Cambridge, UK.
41. G.M. Sheldrick (1993). *SHELXL-93*, Program for the refinement of crystal structures, University of Göttingen, Germany.

