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The Crystal Structures of the Synthetic C-Terminal Octa- and Dodecapeptides of Trichovirin

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Abstract: The structures of two synthetic peptides with sequences corresponding to the C-terminal region of the naturally occurring 14-residue peptaibol trichovirin have been determined. The crystal structures of 8- and 12-residue segments are presented and are compared with the structures of the tetrapeptide and of the 9-residue segment, which have been reported earlier. A comparison between these segments leads to the hypothesis that the three-dimensional structure of trichovirin is to a large extent determined by the properties of a periodically repeating -Aib-Pro- pattern in the sequence of the peptide. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptaibols; helix formation; X-ray diffraction

INTRODUCTION

Peptaibols [1,2] are naturally occurring peptides of fungal origin consisting of 11–20 residues. They are acetylated at the N-terminus and possess a C-terminal 1,2-aminoalcohol. Peptaibols, alamethicin in particular, have been extensively studied in connection with their property to associate, forming weakly selective, ion conducting pores that penetrate lipid bilayer membranes [3]. Conductance through the pores is a voltage-dependent [4,5] or a voltage-independent [6] process. The properties of the pores are largely determined by the helical character of the peptides. All peptaibols exhibit a high content of α-aminoisobutyric acid (Aib). Due to conformational constraints imposed on the peptide backbone by the gem-dimethyl group, Aib occurs overwhelmingly in helical conformations; the only exceptions are observed when Aib is placed at the C-terminus of a peptide [7,8]. Furthermore, insertion of a small number of Aib residues in sequences with low helical propensities promotes the formation of helical structures [9]; with respect to peptide folding, Aib residues override the effects of helix-breaking residues [10]. The conformational space available to Aib comprises the regions of left- and right-handed α- and 3_{10}-helices [11]. At the level of their sequences, most petaibols are microheterogeneous, a typical example being the decatetrapeptide trichovirin from the mold *Trichoderma viride* NRRL 5243 [12]. One of the major components, trichovirin I-4A has the sequence Ac-Aib^1-Asn^2-Leu^3-Aib^4-Pro^5-Ala^6-Val^7-Aib^8-Pro^9-Aib^{10}-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14}. The authors report here the crystal structures of the 8- and 12-residue C-terminal segments. These could help develop a structural model for trichovirin, since no crystals suitable for X-ray diffrac-

Abbreviations: Z, benzyloxycarbonyl; OMe, methoxy; O*t*Bu, *tert* butoxy; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide; ESI–MS, electron spray–ionization mass spectrometry.

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Table 1 Characterization of Key Tripeptides Z-Leu$^{3(11)}$-Aib-Pro$^{5(13)}$-OMe (**A**) and Z-Val^{7}-Aib-Pro^{9}-OMe (**B**), and of title compound **I** (Z-Val^{7}---Leuol^{14}) and **II** (Z-Leu3---Leuol^{14})

	Peptide m.p.[a] (°C)	$[\alpha]_D^{20}$ [b] ($c = 1$, MeOH)	ESI–MS[c] (m/z)	Racemization[d] (%)	Elemental analysis[e]
A	132	−86.6	484.3 $[M+Na]^+$	D-Leu (1.9) D-Pro (2.5)	For $C_{24}H_{35}N_3O_6$ (461.56): calc. C 62.45, H 7.64, N 9.10; found C 62.67, H 7.65, N 9.14
B	76–80	−89.8	470.2 $[M+Na]^+$	D-Val (0.5) D-Pro (1.2)	For $C_{23}H_{33}N_3O_6$ (447.53): calc. C 61.73, H 7.43, N 9.39; found C 61.53, H 7.46, N 9.29
I	217	−7.8	913.6 $[M+H]^+$	D-Val (0.6) D-Leu (4.2) D-Pro (1.8)	For $C_{47}H_{74}N_8O_{10}$ (911.15): calc. C 61.96, H 8.19, N 12.03; found C 61.59, H 8.44, N 12.15
II	211	−6.5	1302.2 $[M+Na]^+$	D-Val (0.7) D-Leu (4.2) D-Pro (2.3) D-Ala (2.2)	For $C_{65}H_{104}N_{12}O_{14}\cdot H_2O$ (1295.64): calc. C 60.26, H 8.24, N 12.97; found C 60.51, H 8.50, N 12.95

[a] m.p., Melting point, open capillaries, not corrected (Model B 520 apparatus, Büchi, Switzerland).
[b] Optical rotation, model 241 polarimeter (Perkin-Elmer, Überlingen, Germany).
[c] Electron spray–ionization mass spectrometry (ESI–MS), TSQ-700 (Finnigan, Bremen, Germany).
[d] Racemization (uncorrected) determined after total hydrolysis by gas chromatography and flame ionization detector on chiral columns Lipodex E (Macherey-Nagel, Düren, Germany) and Chirasil-Val (Chrompack, Middelburg, The Netherlands).
[e] Elemental analysis on a CHN elemental analyser (Carlo Erba, Milan, Italy).

tion structure analysis have been obtained from this peptaibol.

MATERIALS AND METHODS

Peptide Synthesis

For the coupling of trichovirin segments by conventional solution-phase procedures, water-soluble carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) were used without exception. Z-Leu$^{3(11)}$-Aib$^{4(12)}$-O*t*Bu was synthesized from Z-Leu-OH and H-Aib-O*t*Bu. After C-terminal deprotection with TFA, the dipeptide was coupled with H-Pro-OMe resulting in Z-Leu$^{3(11)}$-Aib$^{4(12)}$-Pro$^{5(13)}$-OMe, occurring twice in trichovirin I-4A. Racemization of residues and characterization of this peptide, the peptide Z-Val^{7}-Aib^{8}-Pro^{9}-OMe and both final products are listed in Table 1. The methyl esters were selected in order to circumvent any acidic treatment of the acid-labile Aib-Pro bond. After saponification of the methyl ester with NaOH, the resulting tripeptide acid was coupled with H-Ala-OMe yielding the tetrapeptide Z-Leu^{3}-Aib^{4}-Pro^{5}-Ala^{6}-OMe. Saponification and coupling of the tripeptide mentioned above with L-Leuol as amino component yielded the tetrapeptide Z-Leul^{11}-Aib^{12}-Prol^{13}-Leuol^{14} and the following hydrogenolysis furnished H-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14}.

Coupling Z-Val-OH with H-Aib-O*t*Bu provided the dipeptide Z-Val^{7}-Aib^{8}-O*t*Bu. After C-terminal deprotection with TFA, the dipeptide was coupled with H-Pro-OMe resulting in the tripeptide Z-Val^{7}-Aib^{8}-Pro^{9}-OMe. After saponification the resulting Z-Val^{7}-Aib^{8}-Pro^{9}-OH was coupled with H-Aib-OMe yielding Z-Val^{7}-Aib^{8}-Pro^{9}-Aib^{10}-OMe. After saponification the resulting tetrapeptide acid was coupled with H-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14} and the octapeptide Z-Val^{7}-Aib^{8}-Pro^{9}-Aib^{10}-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14} (title compound **I**) was obtained. Saponification of Z-Leu^{3}-Aib^{4}-Pro^{5}-Ala^{6}-OMe, hydrogenolysis of Z-Val^{7}-Aib^{8}-Pro^{9}-Aib^{10}-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14} and coupling of these resulting segments provided Z-Leu^{3}-Aib^{4}-Pro^{5}-Ala^{6}-Val^{7}-Aib^{8}-Pro^{9}-Aib^{10}-Leu^{11}-Aib^{12}-Pro^{13}-Leuol^{14} (title compound **II**) [13].

X-ray Diffraction

Crystals suitable for X-ray diffraction from both compounds **I** and **II** were obtained from a methanol/water mixture by slow evaporation. X-ray diffraction data were collected using an Enraf-Nonius CAD4 diffractometer with graphite monochromated, Ni-fil-

Table 2 Crystallographic Data for Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (**I**) and Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (**II**)

	I	**II**
Molecular formula	$C_{47}H_{74}N_8O_{10}$	$C_{65}H_{104}N_{12}O_{14}$
Co-crystallized solvent	$2H_2O$, $0.5{\cdot}CH_3OH$	$4H_2O$
Formula unit (amu)	$911.15+2{\cdot}18.015+0.5{\cdot}32.04=963.2$	$1277.61+4{\cdot}18.015=1349.67$
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	9.588(6)	9.523(5)
b (Å)	19.082(5)	19.261(15)
c (Å)	30.415(14)	41.977(26)
Volume (Å^3)	5565 (8)	7699 (15)
Z (molecules/cell)	4	4
Density (calc.) (g/cm^3)	1.150	1.164
Crystal size (mm)	$1.2\times0.6\times0.4$	$0.8\times0.5\times0.3$
Absorption coeff. (mm^{-1})	0.691	0.708
Absorption correction	Semi-empirical	Semi-empirical
max/min Transmission	0.99/0.63	0.99/0.6
Total number of collected reflections (including systematic absences)	4662	5454
Independent reflections	4629	5416
$\sigma(I)$ omitted	2.5	1.5
Data/restraints/parameters	2392/116/606	3400/185/857
R/wR_2	0.085/0.217	0.106/0.262
GooF/restr. GooF	1.122/1.103	1.11/1.092
Max. shift/estimated S.D.	0.04	0.007
Max./min. final electron density (e/Å^3)	0.25/−0.25	0.33/−0.34

tered CuK$_\alpha$ radiation ($\lambda = 1.54184$ Å). The crystals were sealed in capillaries. Unit cell parameters were obtained by a least-squares procedure using the angular parameters of 25 reflections with $12° < \Theta < 29°$ (**I**) and $11 < \Theta < 20°$ (**II**). Data sets consisting of unique reflections were collected up to 60° in θ, at 295 K, ω–θ scan mode for **I** and ω–2θ scan mode for **II**. Five standard reflections were monitored periodically (every half hour for **I** and every hour for **II**) and the total loss of intensity was 2.4% for **I** and 7.0% for **II**. For a 'semi-empirical' absorption correction of reflection intensities, curves obtained from ψ scans of reflections with χ close to 90° were used (five for the data of **I** and six for the data of **II**). Crystal data are summarized in Table 2. The poor quality of the weakly diffracting crystals is the main reason for the relatively high R values obtained after structure refinement.

The structures of both title compounds were solved by Patterson fragment search techniques, using the PATSEE program [14], whereby the structure of **II** was determined first; for title compound **I**, a 25-atom fragment of the structure of title compound **II**, comprising the C=O group of Val1; N, C$_\alpha$, C$_{\beta L}$, C$_{\beta R}$, C, O of Aib2 and Aib4; N, C$_\alpha$, C$_\beta$, C, O of Pro3 and Leu5; and N of Aib6, was used; this fragment forms three intramolecular $1\leftarrow4$ hydrogen bonds. The solution shows the values TPRSUM = 0.707, TFOM = 0.236 and $Re = 0.236$. All other 40 non-hydrogen atom positions, plus the position of two water molecules, could be determined by assigning phases to the 250 strongest normalized structure factors and expanding the structure by using the TEXP option in SHELXS-86 [15]. A methanol molecule with a disordered oxygen, the C-terminal hydrogen and two hydrogens belonging to one water molecule were located in difference Fourier syntheses, all other hydrogen atom positions were calculated and refined using the riding model approach. The structure was refined isotropically with the SDP package [16] using least-squares on F. Anisotropic, full-matrix least-squares refinement for non-hydrogen atoms was carried out with SHELX-96 [17]; thermal parameters for hydrogens

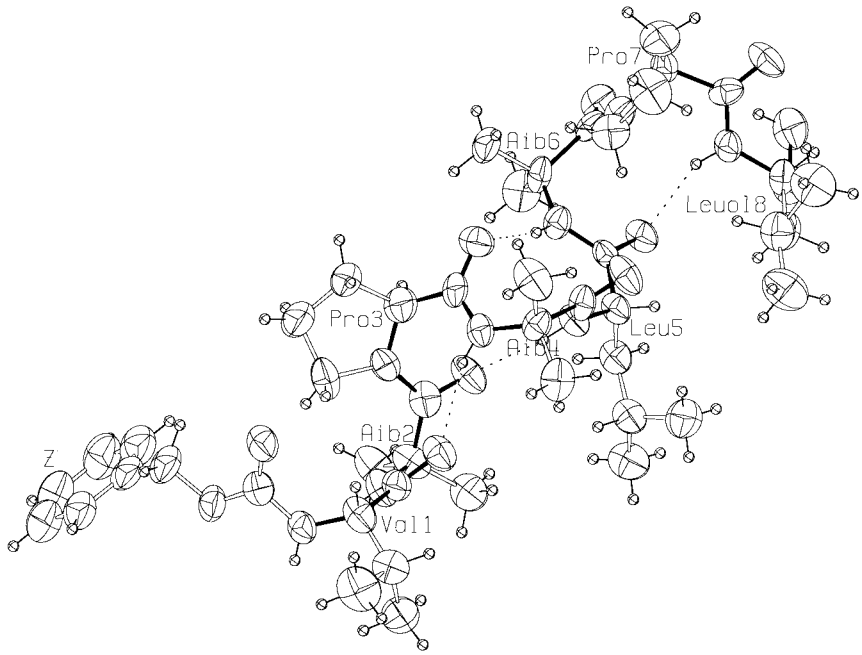

Figure 1 Perspective ORTEP-drawing of the structure of Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol. The backbone is highlighted and intramolecular hydrogen bonds are indicated as broken lines. The probability is contoured at 35%.

were fixed to 1.5 times (C-terminal and methyl group hydrogens) and 1.2 times (all other) the equivalent isotropic thermal parameter of the atom they are riding on. One hundred and sixteen restraints were introduced concerning thermal parameters and the position of the two hydrogens bonded to one water oxygen. The methanol molecule was given 50% occupancy due to high temperature factors and was refined isotropically.

The structure of **II** was solved with a 17-atom fragment of the structure of Boc-Leu-Aib-Pro-Val-Aib-OMe [18], comprising two $1 \leftarrow 4$ intramolecular hydrogen bonds, built by the C=O group of Leu1; N, C_α, C, O of Aib2; N, C_α, C_β, C, O of Pro3; N, C_α, C_β, C, O of Val4; and N of Aib5. This resulted in the fragment C=O (Val5) to N (Leu9). The values of this solution are TPRSUM = 0.731, TFOM = 0.219 and $Re = 0.27$. An additional 65 non-hydrogen atom positions were found by assigning phases to the 250 strongest normalized structure factors and expanding the structure by using the TEXP option in SHELXS-86. The remaining nine positions of non-hydrogen atoms and the position of four water molecules were located in difference Fourier syntheses, as well as the positions of four hydrogen atoms bound to water molecules and the position of the C-terminal hydrogen. All other hydrogen atom positions were calculated and refined using the riding model approach. The structure was refined isotropically with the SDP package. For anisotropical, full-matrix least-squares refinement, SHELX-96 was used. Thermal parameters for hydrogens were fixed to 1.5 times (C-terminal and methyl group hydrogens) and 1.2 times (all other) the equivalent isotropic thermal parameter of the atom they are riding on. One hundred and eighty-five restraints were introduced concerning thermal parameters and the position of the four hydrogens bonded to two water oxygen.

RESULTS AND DISCUSSION

Molecular Structure of Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol

A perspective view of the molecule, based on the experimentally determined coordinates and thermal parameters is shown in Figure 1, using the ORTEP [19] computer program. The backbone and side-chain torsion angles [20] are listed in Table 3. The overall folding of the octapeptide fits approximately the pattern of a right-handed 3_{10}-helix. The helix consists of two consecutive β-turns of type III [21,22], formed by Val1, Aib2, Pro3, Aib4 and by Aib2, Pro3, Aib4, Leu5, respectively, both stabilized by a $1 \leftarrow 4$ hydrogen bond (Table 4) and two β-turns

Table 3 Backbone and Side chain Torsion Angles (°) for Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (a) and for Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (b)

	φ	ψ	ω	χ^1	χ^2	χ^3	χ^4	θ^4
(a)								
Val1	−90(1)	−19(2)	179(1)	−63(2)/63(2)				
Aib2	−49(2)	−39(2)	178(1)					
Pro3	−53(2)	−20(2)	174(1)	−26(2)	40(2)	−38(2)	21(2)	3(2)
Aib4	−51(2)	−33(1)	−174(1)					
Leu5	−89(2)	−7(2)	−171(1)	−58(2)	−52(2)/−179(1)			
Aib6	−56(2)	−44(1)	−177(1)					
Pro7	−98(1)	17(2)	179(1)	40(2)	−39(2)	23(2)	3(1)	−27(1)
Leuol8	−109(1)			−54(2)	−49(2)/−178(1)			
(b)								
Leu1	−57(2)	−51(2)	−170(2)	−71(2)	−63(3)/171(2)			
Aib2	−56(2)	−55(2)	−177(2)					
Pro3	−69(2)	−24(3)	−178(1)	29(3)	−38(3)	32(2)	−13(2)	−9(2)
Ala4	−75(2)	−38(2)	−177(1)					
Val5	−91(2)	−23(2)	−175(1)	−61(2)/173(2)				
Aib6	−52(2)	−38(2)	−177(1)					
Pro7	−58(2)	−17(2)	174(1)	−29(2)	44(2)	−41(2)	23(2)	4(2)
Aib8	−52(2)	−30(2)	−175(1)					
Leu9	−83(2)	−3(2)	179(1)	−58(2)	−31(5)/180(2)			
Aib10	−42(2)	−53(2)	−173(1)					
Pro11	−95(2)	10(2)	−177(1)	40(2)	−39(2)	25(2)	2(2)	−27(2)
Leuol12	−107(2)			−56(2)	−51(2)/−177(1)			

of type I (formed by Pro3, Aib4, Leu5, Aib6 and by Leu5, Aib6, Pro7, Leuol8), also both stabilized by a $1 \leftarrow 4$ hydrogen bond. The parameters of the hydrogen bonds are within the expected range for hydrogen bonds of organic structures [23] and of amino acids and peptides [24]. The φ, ψ values of Val1, Pro7, Leu5 and Leuol8 residues differ considerably from the $\alpha/3_{10}$-helical region [25–27]. This deviation of the φ, ψ values of Val and Leu residues from the average α- or 3_{10} values in proteins has been also observed in other Aib-containing peptides [28–30] and seems to be a characteristic feature for these residues in Aib-containing peptides.

The peptide units adopt the usual *trans* planar conformation with an average deviation of 3.4° from the ideal geometry ($\omega = 180°$). The side chain of Val1 (Table 3) is in the rather rare g^+g^- conformation [31,32], and the side chains of Leu5 and Leuol8 adopt the most common conformation, which belongs to the type g^+ for χ_1 and g^+t for χ_2 if the definitions of reference [31] are used, or $g^-(tg^-)$ according to reference [32]. The pyrrolidine ring of Pro3 adopts the C_γ-*exo* [33] conformation with puckering parameters [34] $Q = 0.4$ Å and $\Phi = 282°$, and the pyrrolidine ring of Pro7 adopts the C_γ-*endo* conformation with puckering parameters $Q = 0.4$ Å and $\Phi = 69°$.

Crystal Packing of Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Lenol

In the crystal, the peptides are head-to-tail hydrogen bonded along the [0 0 1]-direction via two hydrogen bonds (Table 4), and an additional potential hydrogen bond between the N–H group of Val1 and the C=O group of Pro7, which is however, considerably weaker and less linear than the two other hydrogen bonds. Due to an angle of about 110° between the helical axes of two molecules bonded in that way, the head-to-tail hydrogen bonded columns are formed in a zigzag fashion (Figure 3). The carbonyl groups of Z and Aib2 are hydrogen bonded to a disordered methanol oxygen, building a connection between parallel packed zigzag columns in the [1 0 0]-direction. Furthermore, these columns are hydrogen bonded via a water molecule (OW). A hydrogen bond exists between the C=O group of Aib6 and the second water molecule (OV), between the two water molecules, and between the second water molecule (OV) and the second conformation of the methanol oxygen, forming an only

Table 4 Hydrogen-Bond Parameters (Å, °) for Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (a) and for Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol (b)

Don N	Acc O	N···O	H···O	N–H···O	H···O=C		
(a)							
Intramolecular							
Aib4	Val1	3.004(14)	2.17	164	137		
Leu5	Aib2	2.928(12)	2.09	166	133		
Aib6	Pro3	3.028(13)	2.18	169	115		
Leuol8	Leu5	2.976(13)	2.17	156	117		
Intermolecular							
Val1[a]	Pro7[(1)]	3.469(14)	2.91	125	113		
Val1	Leuol8[(1)]	3.030(14)	2.26	149	120		
Aib2	Pro7 [(1)]	2.862(14)	2.24	129	164		
Solvent–peptide							
Don O	Acc O	O···O				C–O(H)···O[c]	O(H)···O=C[c]
OM2[b]	Z[(2)b]	2.940(61)				149(3)	156(2)
OM2	Aib2	3.042(64)				76(3)	112(1)
Ov[b]	Aib6[(3)]	2.816(15)					134.5(8)
				O–H···O			
Ow[b]	Pro3	2.851(14)	2.20	125	131		115.9(8)
OW	Leu5[(3)]	2.887(13)	1.96	159	141		139.1(8)
Peptide–solvent							
Leuol8	OV[(2)]	2.825(16)	2.18	136			
Solvent–solvent							
OV	OW	2.764(16)					
OM1[b]	OV[(4)]	2.96(10)				67(3)	
Symmetry operation:							
(1) $3/2-x$, $1-y$, $-1/2+z$							
(2) $1+x$, y, z							
(3) $-1+x$, y, z							
(4) $1/2+x$, $3/2-y$, $1-z$							
(b)							
Intramolecular							
Ala4[a]	Z[b]	3.403(21)	2.66	145	139		
Ala4	Leu1	3.065(15)	2.50	124	105		
Val5	Leu1	2.965(12)	2.15	158	170		
Aib6	Aib2	3.045(16)	2.32	142	147		
Aib8	Val5	3.000(14)	2.16	164	141		
Leu9	Aib6	2.992(14)	2.14	171	133		
Aib10	Pro7	3.066(16)	2.22	167	120		
Leuol12	Leu9	3.035(15)	2.21	161	119		
Intermolecular							
Leu11	Leuol12[(1)]	2.914(17)	2.06	174	112		
Aib2	Pro11[(1)]	2.850(16)	2.09	147	162		
Don O	Acc O	O···O					O(H)···O=C[c]
OZ[b]	Pro3[(2)]	3.088(35)					129(2)
OZ	Ala4[(2)]						101(1)
OY[b]	Ala4[(2)]	2.772(33)					154(2)
OY	Aib6[(3)]	3.017(29)					124(1)
				O–H···O			
OW[b]	Pro7[(4)]	2.921(18)	2.17	135	119		121(1)
OW	Leu9	2.941(16)	2.13	142	134		139(1)

Table 4 (continued)

Don N	Acc O	N···O	H···O	N–H···O	H···O=C	
OX[b]	Aib10	2.897(21)	2.22	127	140	131(1)
Peptide–solvent						
Leuol12	OX	2.742(19)	1.99	153		
Solvent–solvent						
OX	OW	2.837(22)	2.22	122		
OY	OZ	2.754(51)				

Symmetry operation:
(1) $3/2-x, -y, -1/2+z$
(2) $-1/2+x, -1/2+y, -z$
(3) $1/2+x, -1/2-y, -z$
(4) $-1+x, y, z$

[a] Very weak hydrogen bond.
[b] Z, benzyloxycarbonyl; OX, OW, water each with two hydrogens; OY, OV, OZ, water were only the oxygen could be located.
[c] Designates the angles between non-hydrogen atoms.

statistically existent hydrogen bond between antiparallel packed columns in the [010]-direction.

Molecular Structure of Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Lenol

A perspective ORTEP-drawing of the molecule is shown in Figure 2, the backbone and side chain torsion angles are listed in Table 3. A pattern of bifurcated hydrogen bonds (Table 4) exists at the N-terminus indicating a mixed $\alpha/3_{10}$-helical character for this part of the peptide. The N–H group of Ala4 is involved in a weak hydrogen bond of type $1\leftarrow5$ with the C=O group of the Z protecting group and a shorter but less linear one of type $1\leftarrow4$ with the C=O group of Leu1. In addition, the C=O group of Leu1 is involved in a further hydrogen bond of type $1\leftarrow5$ to the N–H group of Val5. These parameters of hydrogen-bonding geometries are in good agreement with the results obtained from a compilation of hydrogen bond geometries of type $1\leftarrow4$ and $1\leftarrow5$ in globular proteins [35]. Another hydrogen bond of type $1\leftarrow5$ is formed between the C=O group of Aib2 and the N–H group of Aib6. Near the C-terminus, the backbone folding consists of two consecutive β-turns of type III built by Val5, Aib6, Pro7, Aib8 and Aib6, Pro7, Aib8, Leu9 and two consecutive β-turns of type I formed by Pro7, Aib8, Leu9, Aib10 and Leu9, Aib10, Pro11, Leu12; each of these four β-turns are stabilized by a $1\leftarrow4$ hydrogen bond. The φ, ψ values of Val5, Leu9, Pro11 and Leu12 differ (as observed also in the crystal structure of the C-terminal octapeptide) considerably from the average α- or 3_{10}-helical values in peptides and proteins [25–27]. The peptide units adopt the usual *trans* planar conformation with an average deviation of 4.4° from the ideal geometry. All three Leu/Leuol side chains and the Val side chain adopt the usual conformation, g^+t according to [31] or tg^- according to [32]. The pyrrolidine ring of Pro3 adopts the C_γ-*endo* conformation with puckering parameters $Q = 0.35$ Å and $\Phi = 94°$, the pyrrolidine ring of Pro7 adopts the C_γ-*exo* conformation with puckering parameters $Q = 0.43$ Å and $\Phi = 282°$, and the pyrrolidine ring of Pro11 adopts C_γ-*endo* conformation with puckering parameters $Q = 0.41$ Å and $\Phi = 70°$.

The folding of the dodecapeptide (mixed $\alpha/3_{10}$-helical at the N-terminus, 3_{10}-helical at the C-terminus) is in good agreement with a model derived from NMR and CD experiments for the peptaibol trichosporin B–IVa [36] and the crystal structures of alamethicin [37] and [Leu[1]]zervamicin [38,39].

Crystal Packing of Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol

The arrangement of the 12-residue peptide resembles that of the 8-residue peptide (compare Figure 3 with Figure 4). This can already be seen from the unit cell parameters of the two title compounds: in both cases the *a* and *b* parameters are similar, while *c* is longer for **II** and coincidences with the direction of the helical axis. The peptides here are head-to-tail hydrogen bonded along the [0 0 1]-direction via two hydrogen bonds; one is formed between the N–H group of Leu1 and the C=O group of Leu12 and one between the N–H group of Aib2 and

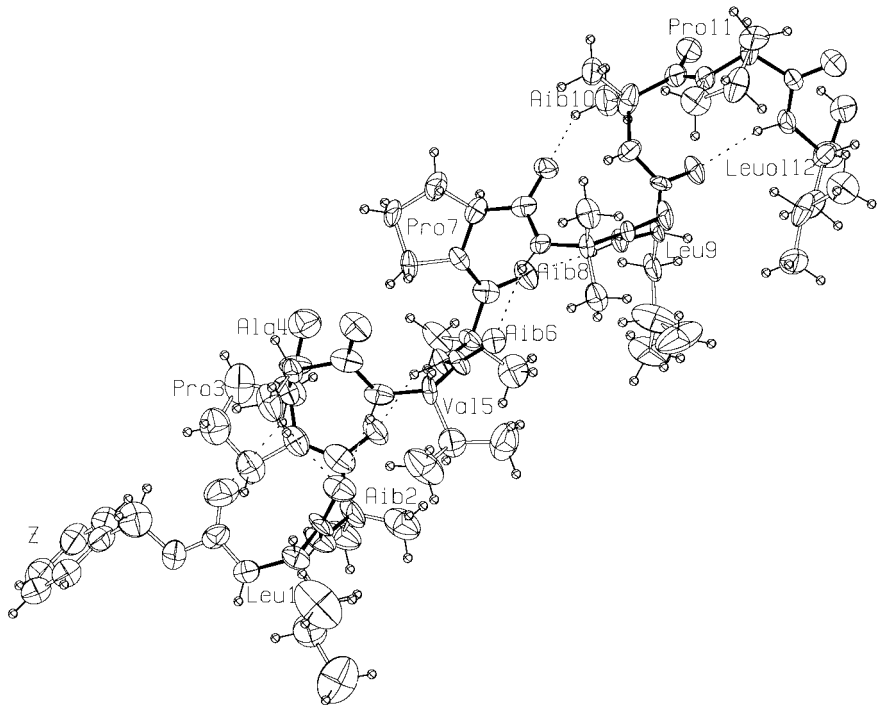

Figure 2 Perspective ORTEP-drawing of the structure of Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol. The backbone is highlighted and intramolecular hydrogen bonds are indicated as broken lines. The probability is contoured at 35%.

the C=O group of Pro11 (Table 4). Due to an angle of about 130° between the helical axes of two molecules bonded in that way, the head-to-tail hydrogen bonded columns are formed in a zigzag fashion (Figure 4). These zigzag columns are packed parallel to the [100]-direction. Two of the four co-crystallized water molecules interact via hydrogen bonds with the parallel columns: one water (OY) is involved in hydrogen bonds with the C=O group of Ala4 and with the C=O group of Aib6 in the *x*-translated molecule, and one water (OW) interacts via hydrogen bonds with the C=O group of Leu9 and the C=O group of Pro7 of the *x*-translated molecule. In the [0 1 0]-direction, the antiparallel columns interact via van der Waals crystal contacts. The two other water molecules saturate the remaining capacity of the polar groups of the peptide, and only the C=O group of Aib8 has no hydrogen bond partner. All waters exist in the crystal in hydrogen-bonded pairs and in total, the four water molecules are involved in ten hydrogen bonds. The overall arrangement of the two title compounds is in excellent agreement with observations of other Aib-containing peptides [40,41]. Theoretical calculations [42] indicate that the alignment of the peptide dipole columns in an antiparallel manner is energetically more favorable than the parallel arrangement. In almost all cases surveyed to date by the authors, parallel columns are hydrogen-bonded; these hydrogen bonds may provide stabilization energy [43] necessary for the parallel arrangement of helices.

Valence Geometry Around the C_α-atom in Aib Residues

Experimental and theoretical studies have shown that the valency geometry around the C_α-atom for Aib residues in 3_{10}-helical structures is asymmetric [11]. If one designates as $C_{\beta L}$ and $C_{\beta R}$ the atoms that occupy the same position as C_β- and the C_α-bonded hydrogen in L-amino acids, respectively, the angles $N–C_\alpha–C_{\beta L}$ and $C–C_\alpha–C_{\beta L}$ in right-handed 3_{10}-helices are usually significantly smaller than the tetrahedral value (109.45°); the opposite behavior is observed for the angles $N–C_\alpha–C_{\beta R}$ and $C–C_\alpha–C_{\beta R}$. No such correlation could be found for the title compounds (Table 5) or for the two independent molecules in the asymmetric unit of the four residues comprising the C-terminal part of the trichovirin. The origin for this atypical behavior of Aib residues when they are incorporated in the trichovirin molecules is presently under investigation.

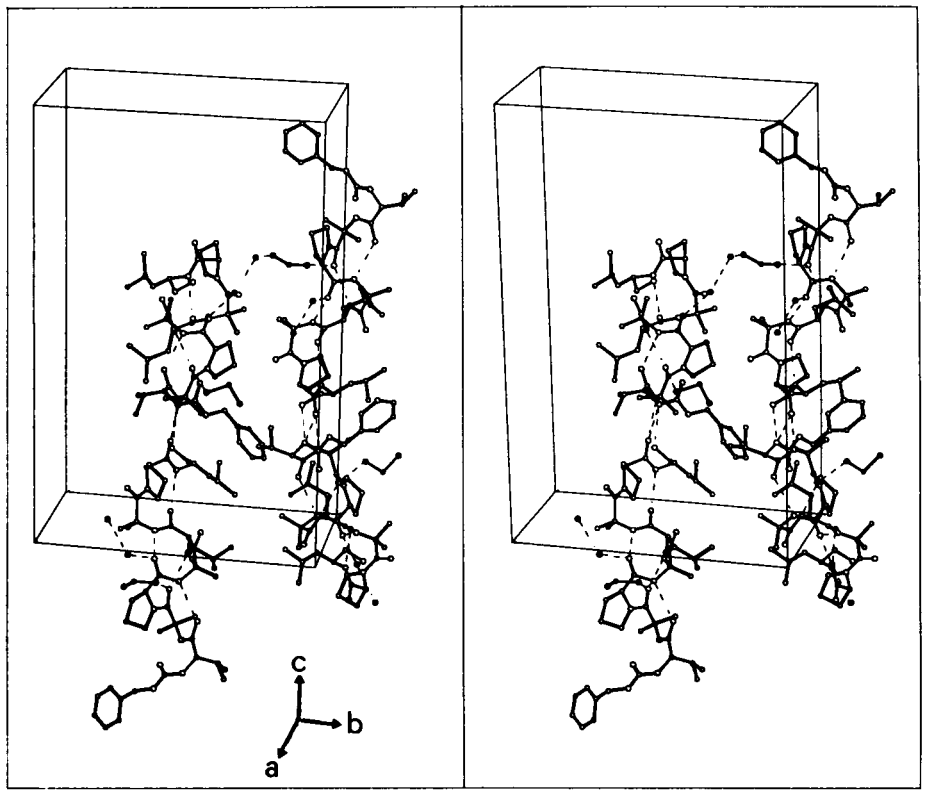

Figure 3 Crystal packing of Z-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol viewed approximately down the *a*-axis. Solvent atoms are shown as full circles and hydrogen bonds are indicated as broken lines. Hydrogens are omitted for clarity.

CONCLUSIONS

Least-squares superpositions of the main-chain atoms (comprising the C=O group of the N-terminal protecting moiety and the atoms N, C_α, C and O of each residue) were performed between the 4-, 8-, 9-residue peptides and the 12-residue peptide using the program LSQ [44] (Figure 5 and Table 6). Since the first residue of the 9-residue peptide [45] is a bulky Ser(*t*Bu), which is likely to adopt a different backbone conformation compared with the conformation of the naturally occurring Ala or Ser at this position of the trichovirin sequences, it was not included in the least-square superposition. A second least-squares superposition was performed between the same atoms of all peptides without the C=O group of the Leuol and the results are listed in Table 6. The latter was considered necessary, as the C=O group of Leuol in the 9-residue peptide and in one of the tetrapeptides adopts a different orientation [conformation angle N–C_α–C–O of Leuol $-73°$ (9-residue peptide), 58° (**II**), 65° (**I**), $-171°$ and 56° (4-residue peptides A and B], probably due to different intermolecular interactions.

Interestingly, the r.m.s. deviations obtained from these superpositions are very small, and the structures differ mostly at the N- and at the C-termini. The overall folding is identical. In the case of trichovirin, it is therefore possible to 'predict' the structure of longer segments stepwise based on the shorter segments. As the structures of the peptides studied appear to be insensitive to crystalline environment, the authors conclude that these structures are determined by the properties of their amino acid sequences. In this context, one could speculate that the structure of trichovirin is to a large extent determined by the characteristic sequence pattern resulting from the periodic occurrence of the highly constrained -Aib-Pro- dipeptide, which imposes locally a rigid three-dimensional structure. The shorter peptides, however, differ in the conformation of the side chains probably due to different interactions with the crystal environment. Differences in the conformations of the C-terminal group in these structures are associated with different orientations of the hydrogen bonds in which this group is involved.

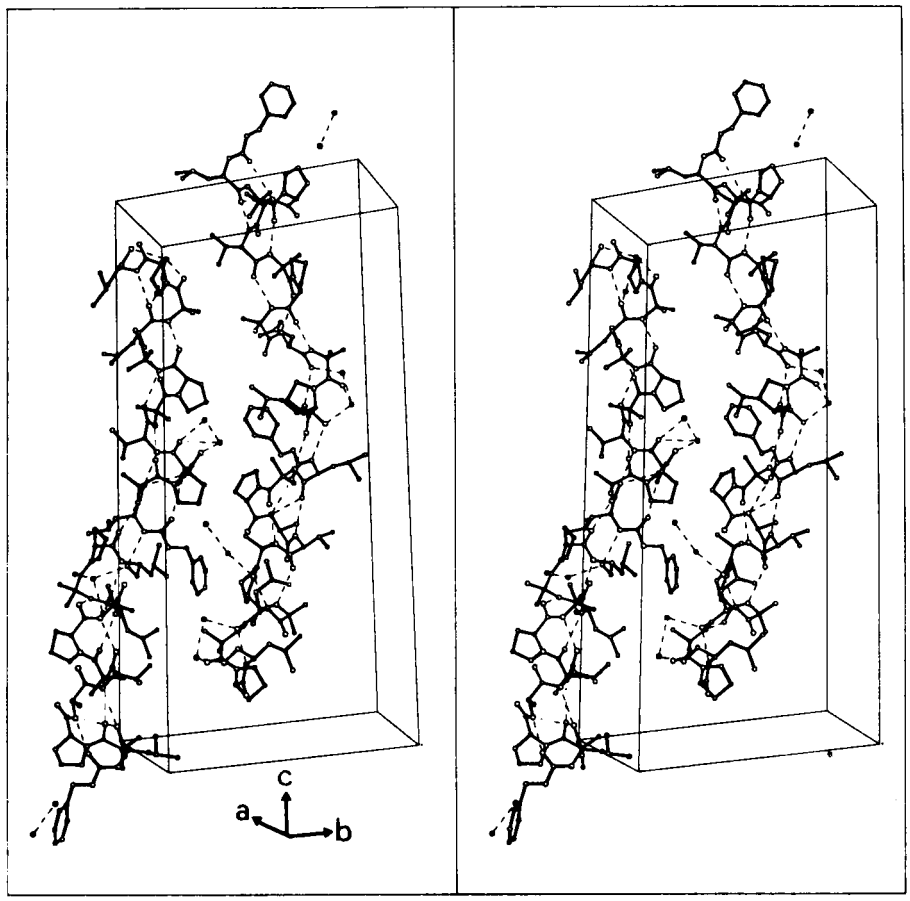

Figure 4 Crystal packing of Z-Leu-Aib-Pro-Ala-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leuol viewed approximately down the *a*-axis. Solvent atoms are shown as full circles and hydrogen bonds are indicated as broken lines. Hydrogens are omitted for clarity.

Trichovirin has one of the shortest peptaibol sequences found so far. The total length of the 12-residue segment (**II**) spans 21.5 Å. An extension of this segment by modeling the N-terminus of trichovirin (Ac-Aib-Asn-) in a helical conformation prolongs the helical structure to a total length of about 25 Å. This is not long enough to span a undistorted lipid bilayer (30 Å). Pore formation might either take place in a local distortion of the lipid bilayer to accommodate the length mix-match or the peptide molecules might undergo some degree of head-to-tail aggregation. Both models have been discussed [3] for the amphiphilic peptides mastoparan [46] and bombolitin I–V [47], and for some hydrophobic 'designed' peptaibol analogs of short length (5-, 10- and 15-residues) that perform channel formation [48].

Table 5 Bond Angles Defining the Valence Geometry around the Aib C_α-Atoms

Residue	N–C_α–$C_{\beta L}$	C–C_α–$C_{\beta L}$	N–C_α–$C_{\beta R}$	C–C_α–$C_{\beta R}$
Aib2 (**I**)	108.7(0.9)	108.7(1.0)	109.5(1.0)	109.0(1.0)
Aib4 (**I**)	109.9(0.9)	107.6(0.9)	110.1(0.9)	108.7(0.8)
Aib6 (**I**)	110.5(0.9)	107.2(0.9)	111.6(1.0)	108.8(0.9)
Aib2 (**II**)	106.0(1.2)	110.8(1.9)	108.6(1.9)	115.0(1.6)
Aib6 (**II**)	107.6(1.1)	108.9(1.3)	107.7(1.3)	110.1(1.4)
Aib8 (**II**)	109.1(1.1)	106.6(1.2)	110.3(1.1)	109.3(1.0)
Aib10 (**II**)	112.3(1.4)	113.0(1.6)	104.7(1.5)	107.4(1.4)

Due to the periodicity of the trichovirin sequence, hydrophobic side chains [Leu1, Val5, Aib8, Leu9 and Leu12 (of **II**), see Figure 4; same for **I**] occupy the same side of the helix. All polar groups in the structure, which are exclusively hydrogen-bonded to water molecules (these are the C=O groups of Pro3, Ala4 and Aib10 in **II**), occupy the other side of the helix, which exhibits a pronounced amphiphilic

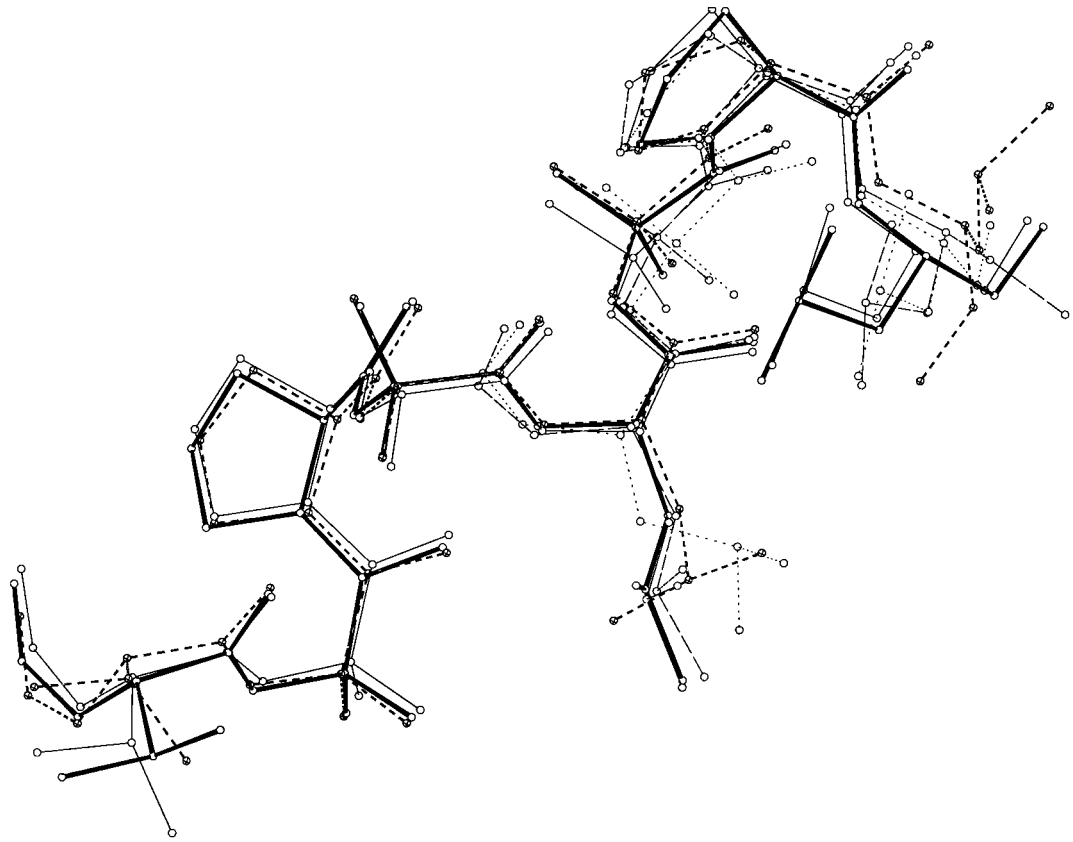

Figure 5 Least-square superposition of the molecular structures of the 12- (**—**), 9 (**– – –**), 8- (—) and 4- (– –, molecule **A**; ·····, molecule **B**) residue C-terminus segments of trichovirin.

Table 6 r.m.s Deviation of the Least-Square Superposition[a]

Fit	r.m.s. deviation			r.m.s deviation without C=O group of Leuol		
	Average	Maximum	At atom	Average	Maximum	At atom
9 → 12	0.429	3.001	O (Leuol)	0.325	1.319	O (Pro8, 11)
8 → 12	0.293	0.522	C (Z, Ala)	0.295	0.498	C (Z, Ala4)
4 mol**A** → 12	0.445	1.364	O (Leuol)	0.317	0.716	C_α (Leuol)
4 mol**B** → 12	0.438	1.200	O (Leuol)	0.322	0.681	C_α (Leuol)

[a] The numbers 4, 8, 9 and 12 indicate the peptide lengths.

character. One could therefore speculate, that the interaction with the lipid bilayer is performed by the hydrophobic side of the helix, while the side of the helix involved in hydrogen bonding with solvent molecules forms the inside of the pore. This is confirmed by the modeled position of the polar Asn side chain, which points to the hydrophilic side of the helix.

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