

Structure determination of racemic trichogin A IV using centrosymmetric crystals

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Direct methods of crystal structure solution are greatly facilitated in centrosymmetric space groups where the complexity of the phase-problem is reduced. For most peptides and proteins, crystallization in a centrosymmetric arrangement is precluded by an intrinsic dissymmetry due to the constituent chiral amino acid residues. The synthetic accessibility of peptide sequences containing amino acids of either chirality offers the possibility for co-crystallization of racemic crystals. We report here the first use of such an approach for the *de novo* structure determination of a medium-sized molecule, trichogin A IV, which is a constituent of a fungal lipopeptaibol mixture possessing membrane-modifying properties of biological interest.

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Simplifications of the X-ray crystallographic structure-determination process for medium-sized and macromolecules represent a research goal for many groups¹. Chief among the possibilities are direct-methods of structure solution, pioneered by Karle & Hauptman² and more recently amplified by other investigators³⁻⁵. Direct methods of structure solution are greatly facilitated in centrosymmetric space groups where the phase-problem is of reduced dimension compared to the non-centrosymmetric case since only two alternatives (0 or 180°) for the phase of each reflection need be considered. For most naturally occurring biomolecules, such as proteins and large peptides, crystallization in centrosymmetric arrangements is precluded by their intrinsic dissymmetry. Hence relatively few structures of intermediate (150-250 atoms) or large-sized biomolecules (>250 atoms) have been solved by direct methods.

For example the structure of Gramicidin A is the largest peptide to be solved *ab initio* by direct methods (317 nonhydrogen atoms)⁶; the structure of the 36 amino acid avian pancreatic polypeptide (which contains a zinc atom) originally solved using anomalous scattering information was subsequently solved by phase extension methods⁷; and a structure of (Leu⁵) enkephalin containing four crystallographically independent molecules (208 nonhydrogen atoms) was solved *de novo* by employing a combination of symbolic addition and tangent formula phase extension⁸.

A potential way around the noncentrosymmetric nature of biopolymer crystals by co-crystallization of equal numbers of enantiomeric molecules to form a racemic mixture was suggested by Mackay⁹. In the event that co-

crystallization leads to formation of a racemic compound the structure solution process should be greatly facilitated, however, co-crystallization neither guarantees formation of a solid phase corresponding to a racemic compound nor of a non-enantiomorphic crystal system. Crystallization of racemic molecules in space groups which do not possess inverse symmetry elements is a possibility, although only rarely observed¹⁰. A more likely, undesired outcome would entail crystallization as a conglomerate, wherein equal numbers of non-centrosymmetric crystals of separate D- and L-isomers are formed¹¹. Preferential occurrence of a crystalline racemate from solutions containing equal populations of two enantiomers is, however, predicted to be favoured based both on the additional packing interactions facilitated by centrosymmetric arrangements (165 for the centrosymmetric case as opposed to only 65 for homochiral crystals) and on statistics of known crystal structures¹¹, even for long, conformationally mobile peptides.

Background and rationale

The notion of producing centrosymmetric crystals of biomolecules was recently explored in the synthesis and co-crystallization of rubredoxin enantiomers¹², where centrosymmetric crystals of the protein were obtained after some considerable effort, and for a racemate sequence of the oligonucleotide d(CGCGCG)¹³. Solution of the protein structure by molecular replacement techniques starting from the coordinates of the all (L)-molecule was successful and the resulting electron density maps were reportedly of exceptional precision relative to those for other protein structures. Likewise, the oli-

gonucleotide structure was solved by the molecular replacement method using natural Z-DNA as the search probe. Attempts to solve those structures by direct methods have not been reported, nevertheless, the results were encouraging since racemic crystals were obtained in both instances. Recently the 'racemate method' was employed to solve another structure of Leu-enkephalin¹⁴. While crystallographic information about the naturally occurring all (L)-molecule was already available from several structures, the successful application of this approach to a highly flexible peptide system lends further credence to its general applicability for biomolecular crystallography.

We have been interested in the three-dimensional structures of peptaibols¹⁵, which are linear peptides of fungal origin containing large numbers of α -aminoisobutyric acid (Aib) residues¹⁶. These molecules, which include

some long sequences (19 amino acid residues) such as alamethicin^{17,18}, sequences of intermediate length (15 residues) such as zervamicins^{19,20} and antiameobins^{21,22}, and shorter sequences such as the trichogins²³ (10 residues) were shown to possess membrane-modifying properties of considerable biological importance including, in some cases, channel-forming properties. The trichogins are of special interest due to their incorporation of the octanoyl group (Oc) on the amino terminus (hence the term *lipopeptide*), their atypical amino acid composition as compared to longer sequences in this antibiotic family (in which the occurrence of glycine is rare), and their otherwise short lengths, which would seem to preclude the spanning of natural membranes by individual molecules as is proposed to be the case for longer, *N*-acetylated pore formers such as alamethicin.

In the course of our studies the all (L) leucinol (Leuol) to methoxy leucine (Leu-OME) analogue of trichogin A IV (sequence Oc-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Leuol) was synthesized and crystals of it were grown from a mixture of chloroform and petroleum ether. The unit cell size for these crystals $a=b=c=59.53$ Å, space group (I23) was consistent with the presence of four crystallographically independent molecules. For the noncentrosymmetric case this amounts to a difficult challenge for direct methods of structure solution, especially given the limited diffraction resolution (2 Å) for the dataset obtainable. Thus our attention was turned to the possibility of synthesizing an all (D) sequence of Trichogin A IV and attempts to form racemic crystals of this terminally blocked decapeptide. Here we demonstrate the straightforward, successful production of centrosymmetric crystals by traditional slow evaporation techniques. These crystals, which contain two unique formula units (159 total non-hydrogen atoms, none heavier than oxygen), were suitable for high resolution data collection and successful structure determination by direct methods without prior knowledge of a structure for the naturally occurring all (L) molecule.

Description of the structure

The molecular conformation of naturally occurring trichogin A IV has been extensively investigated in methanol by ¹H and ¹³C NMR and by circular dichroism²³. From these results it was proposed that the molecular conformation was predominantly, but not fully, right-handed α -helical. A hydrogen bonding pattern encompassing a few C₁₀ structures (β -bends and 3₁₀-helix) and several C₁₃ structures (α -helix) was proposed. In the trichogin A IV racemate crystal structure there are two crystallographically independent peptide molecules (A and B), with associated waters, which comprise the unique repeat. Predominantly helical molecules with right-handed screw sense for the L-molecules (and left-handed screw sense for the D-molecules) are observed (Fig. 1). The general conformational features of the two independent forms of the same chirality are virtually identical (Fig. 2 and Tables 1,2) but both molecules differ in detail from the structure proposed in the solution study. As with other deca- or undecapeptides containing several Aib residues which show mixed 3₁₀/ α -helix conformations^{24,25}, both trichogin A IV molecules com-

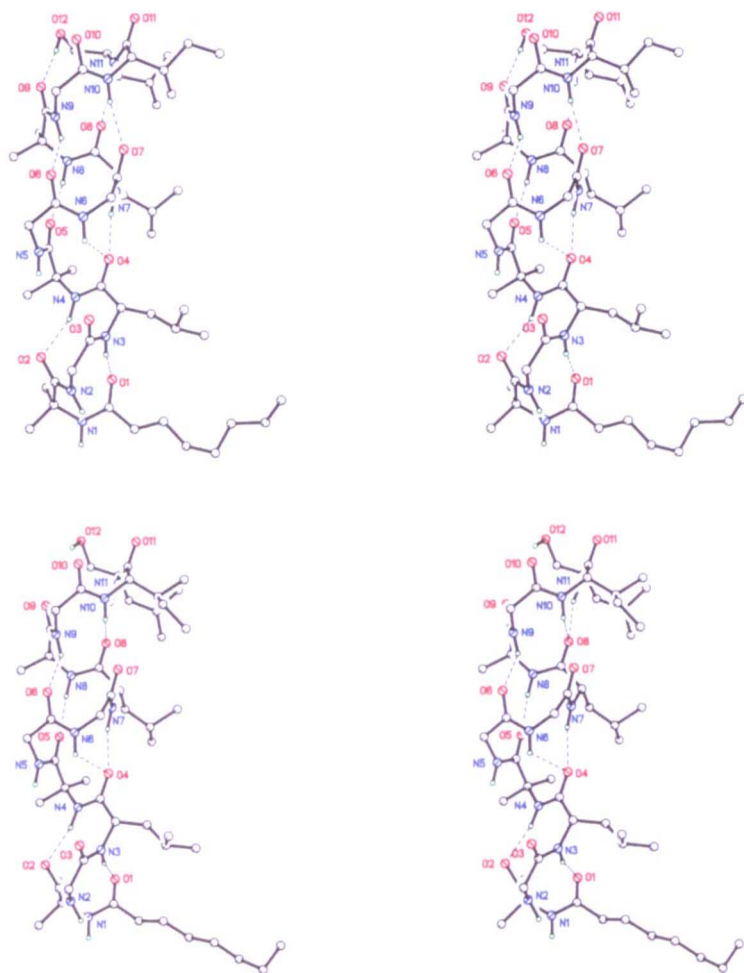


Fig. 1 Stereoviews of the two crystallographically independent, L-amino acid containing molecules from the structure of trichogin A IV. Molecule A is at the top and Molecule B at the bottom. Only heteroatoms are labelled and, for clarity, only amide hydrogens have been included. Intramolecular hydrogen bonds are illustrated with dashed lines.

bine an incipient, distorted 3_{10} -helix and a longer segment of irregular α -helix. At the N terminus the molecular conformation displays an extended octanoyl chain leading into a short, distorted 3_{10} -helix with two consecutive C_{10} structures, the first of which resembles a Type I β -bend. The C=O group [O 1] of the octanoyl moiety forms an intramolecular hydrogen bond with the nitrogen [N 3] of Leu 3 while the carbonyl oxygen of Aib 1 [O 2] is involved in a hydrogen bond with Aib 4 [N 4]. The helical hydrogen-bonding pattern is then interrupted at Gly 2 since its C=O [O 3] is captured

by hydrogen-bonding to a water molecule and is not involved in intramolecular hydrogen-bonding to other backbone atoms. Additional disruption of the helical hydrogen bonds through hydration occurs at the Gly 5 nitrogen [N 5] (Fig. 3). Then a weak interaction from the nitrogen of Gly 6, [N 6], to C=O of Leu 3 [O 4] forms a new C_{10} structure which is followed by five consecutive C_{13} structures involving carbonyl oxygens of Leu 3, Aib 4, Gly 5, Gly 6 and Leu 7. In molecule B the amide nitrogen of Gly 6 also interacts with a water molecule, thus participating in a bifurcated arrangement. At the carboxy terminus the C=O groups from Gly 9 [O 10] and Ile 10 [O 11] both interact with water molecules (Fig. 3) while the C=O of Aib 8 [O 9] is intramolecularly bonded (in molecule A) to the alcoholic OH [O 12] of Leuol. This arrangement in molecule A is illustrative of a C-terminal helical cap²⁶ involving hydrogen-bonding between a C-terminal blocking group and the peptide backbone. Though seen here for the unusual derivative of Leucine (Leuol), this example may well represent a model for the role of serine in other more common sequences²⁷. The only significant differences in conformational detail between the two molecules are found at either end, in the octanoyl chain, which is partially disordered in molecule A, in the Ile 10 side chain conformation and in the Leuol orientation.

Table 1 Principal torsion angles along the trichogin A IV backbone and sidechains

Residue		Mol A	Mol B
Oc-Aib	ω_0	-175.2	-171.6
Aib 1	ϕ	-53.2	-53.3
	ψ	-36.0	-35.8
	ω	-175.4	-178.8
Gly 2	ϕ	-70.3	-63.1
	ψ	-7.2	11.5
	ω	174.5	177.6
Leu 3	ϕ	-92.2	-99.4
	ψ	-13.9	-8.5
	ω	-179.6	-172.5
	χ^1	-68.0	-60.7
Aib 4	ϕ	-53.5	-56.0
	ψ	-45.7	-48.0
	ω	-177.0	-178.2
Gly 5	ϕ	-69.2	-67.2
	ψ	-32.9	-33.0
	ω	175.1	172.6
Gly 6	ϕ	-62.0	-57.1
	ψ	-46.5	-43.5
	ω	176.8	175.9
Leu 7	ϕ	-65.7	-65.1
	ψ	-46.1	-48.4
	ω	178.7	-179.9
	χ^1	-70.4	-69.9
Aib 8	ϕ	-54.3	-53.3
	ψ	-43.2	-40.5
	ω	-171.6	-174.5
Gly 9	ϕ	-76.0	-73.8
	ψ	-25.0	-17.9
	ω	173.6	175.3
Ile 10	ϕ	-90.6	-96.6
	ψ	-32.1	-39.6
	ω	176.5	-174.2
	χ^1	72.8	-67.3
Leuol	ϕ	-107.0	-100.3
	ψ	45.1	68.1
	χ^1	-56.3	-58.4

The torsion angles are defined in ref. 37. For Leuol ϕ is C'-N-C α -CH₂, while for ψ is N-C α -CH₂-O. Estimated standard deviations are approximately 1.0°. Values given are for the molecules containing L-residues.

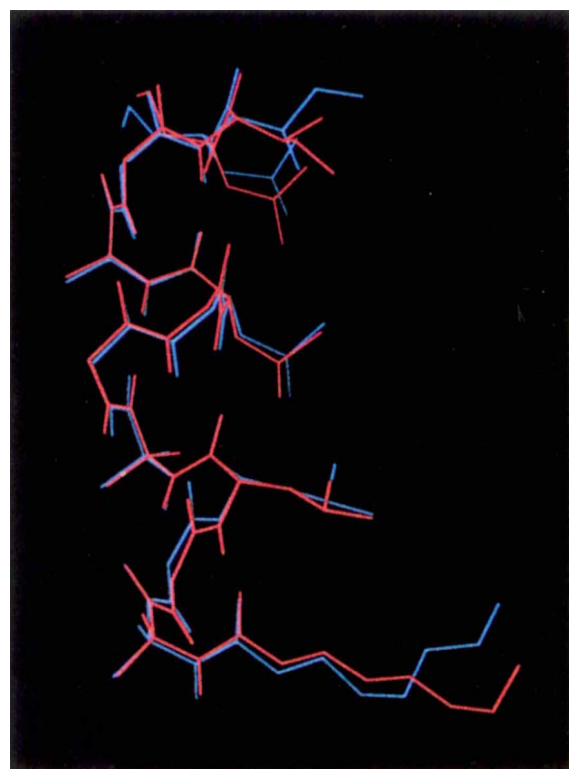


Fig. 2 An overlay of the two crystallographically independent molecules of trichogin A IV (Molecule A in cyan, Molecule B in red) illustrating the similarity in peptide backbone conformation. The molecules were fit by least-squares matching of all of the peptide backbone nitrogen atoms. The r.m.s. fit is 0.28Å.

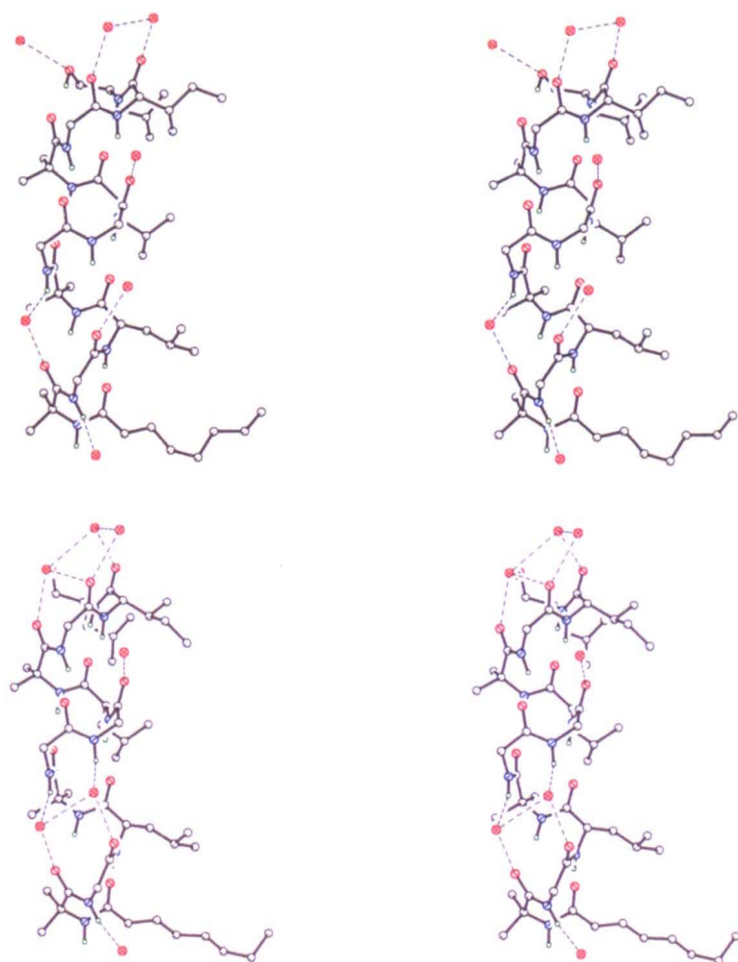


Fig. 3 Stereoviews of the trichogin A IV helices showing their hydration states. Water molecules are illustrated with cross-hatched spheres and hydrogen bonds as dashed lines. Molecule A is at the top and Molecule B at the bottom.

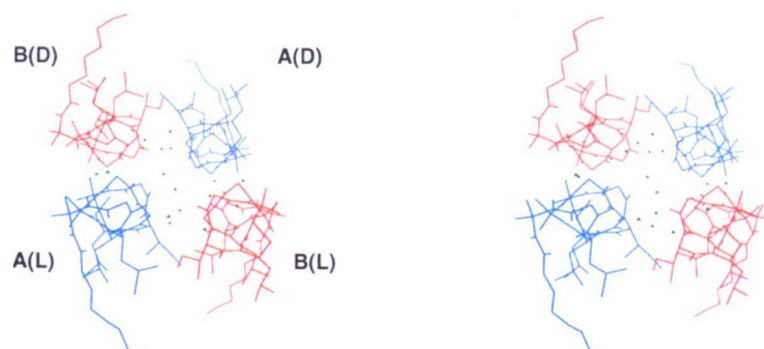


Fig. 4 A stereoview of the packing arrangement about a crystallographic inversion centre for molecules of trichogin A IV looking down the crystallographic *c*-axis. The two unique molecules of like-handedness (A in cyan, B in red) and their inversion related counterparts are illustrated and each molecule is labelled as (L) or (D). Water molecules, indicated by green triangles, principally occupy the hourglass-shaped hydrophilic channel between molecules of like-handedness.

The average values of the torsion angles through the helical regions are $\phi = -64.2^\circ$ and $\psi = -42.9^\circ$. The ψ value, generally thought to be correlated to α - versus 3_{10} -helical form, is identical to the -42° value expected for the former²⁸. All peptide bonds are within 8° of planar, *trans* geometry ($\omega = 180^\circ$) and the helical nature is amphipathic with all of the hydrophobic groups (octanoyl and Leu, Ile and Leuol aliphatic side chains) on one helix face and glycine residues comprising the hydrophilic face. Aligned on the border between those helical faces are the Aib methyl groups. In the molecular packing (Fig. 4) an hourglass-shaped hydrophilic channel defined by four molecules is observed. The two independent molecules of like-handedness are juxtaposed with their hydrophilic faces forming one half of the roughly rectangular channel which contains the majority of the crystallographically determined waters. This hydrophilic channel is completed by two all (D) molecules related to those other two molecules by a crystallographic inversion centre. Molecules of like-handedness, related by translation parallel to the crystallographic *c*-axis, are linked in head-to-tail chains through intermolecular hydrogen bonds involving the nitrogen of Aib 1 [N 1] and the C=O of Ile 10 [O 11] in molecule A, and the hydroxyl oxygen of Leuol of molecule B (Fig. 5) resulting in the formation of long rods of helical peptides. Enantiomeric molecules face each other with their hydrophobic faces intermeshed to form a network into which no water molecules intrude. There are no direct hydrogen bonds and only one water mediated interaction between peptide molecules of opposite handedness. The closest ($< 4\text{\AA}$) helix-helix contacts not involving heteroatoms are of two types, one of these involves meshing of hydrophobic side-chains (Ile with Ile, or Leu with Leu and Ile) and the other, close contacts between α -carbons of glycine residues.

Channel forming hypothesis

The peptaibol family of antibiotics constitute a class of molecules which are thought to act through pore or channel formation in cell membranes leading to leakage of the cytoplasmic material into surrounding areas and eventual cell death²⁹. A considerable body of evidence, including two crystal structures^{18,20}, has been accumulated to support the view that 15–19 amino acid peptaibols are helical and capable of forming channels in biological membranes through which water and ions may readily pass. Very different patterns of helical aggregation in crystal structures have been observed among them³⁰. For these sequences the helix length (32 Å for alamethicin and 29 Å for zervamicin) is sufficient to span the lipid bilayer (Fig. 6). In contrast, the helix length of trichogin A IV is approximately half that of alamethicin (16 Å from N 1 to N 11) and its sequence bears little resemblance to the longer peptaibols. It is reasonable to conclude that, if the mechanism of action for this class of lipopeptaibols involves channel formation in bilayers, the arrangement of molecules required to bring about such disruptions must be quite different from the longer sequences.

The crystal structure observed for the racemate may be revealing in light of this unique requirement. trichogin A IV, which lacks bulky sidechains on one face of the



Fig. 5 A stereoview of the packing arrangement perpendicular to the crystallographic *c*-axis in the crystal structure of racemic trichogin A IV. The head-to-tail hydrogen bonds between molecules of like-handedness are illustrated by the yellow dashed lines. Water molecules are illustrated as green triangles and the water channels between molecules of like-handedness run horizontally. α -Carbons of the glycine residues are highlighted in black. The network of hydrophobic sidechains and octanoyl groups between molecules of different handedness lie in the centre of the diagram.

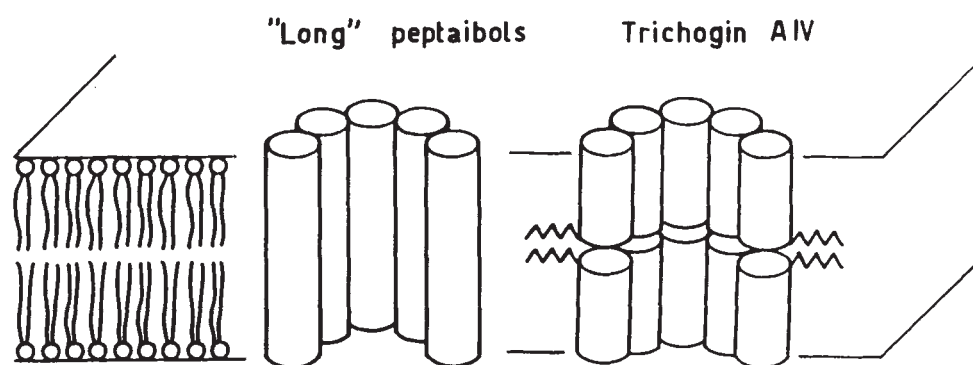


Fig. 6 A diagram of the proposed model for channel formation by peptaibols of varying length.

helix, is able to pack with other like molecules to form a channel in which water can clearly be accommodated. This channel extends infinitely beside pairs of molecules of like-handedness connected in a head-to-tail arrangement through hydrogen-bonding. Molecular aggregation is enhanced by the amphipathic helical nature which allows formation of a hydrophobic network of groups between molecules of opposite handedness. One layer of L (or D) molecules runs antiparallel to the layer of L (or D) molecules above or below it forming alternating hydrophobic networks and hydrophilic channels. Linkage of two trichogin molecules ('dimer' formation) at the membrane surface through either head-to-tail hydrogen-bonding (as observed in this structure) or, more likely, head-to-head interactions (Fig. 6), and insertion into the membrane, could lead to a molecular aggregate with glycine-rich faces at its interior to generate a water channel of sufficient length to span the bilayer. Experimental support for this hypothesis has been generated recently through a study of trichogin analogues with variable length $N\alpha$ -acyl chains³¹. Although this evidence does not confirm a 'channel-forming' role for the ten-residue trichogin A IV it is interesting to note that a model ten-residue helical sequence, Boc-(Ala-Aib-Ala-Aib-Ala)₂-OMe, has been shown to form channels in planar lipid bilayers³².

Functional and practical implications

The mode of molecular aggregation we hypothesize may represent a new mechanism of channel formation in membranes by short, helical peptide sequences and is similar to conclusions reached from studies of a ten-residue model sequence. The concept of glycine rich hydrophilic faces on helical peptides may be of more general significance. For example, an analogous structural motif has been proposed to occur in the putative α -helical domains of the class II major histocompatibility complex (MHC)³³.

Like Gramicidin A (ref. 6) and Leu-enkephalin (ref. 8) the trichogin A IV structure involves a large peptide system solved *de novo* by direct methods. Efforts to solve the Gramicidin structure were ongoing for more than ten years. For trichogin A IV only a few months expired between concept and structure solution. The success of the 'racemate method' as applied to solution of this and other structures bodes well for a broader applicability to molecules of increased complexity. Its applicability to the solution of biopolymer structures in which the molecular conformation is not severely limited by constraints imposed from the amino acid composition remains a fertile ground for investigation.

Table 2 Hydrogen-bonding interactions in the trichogin A IV structure

Type	Donor	Acceptor	Length (Å)	Angle (°) ¹		
Molecule A						
Intramolecular	N3	O1	2.982	164		
	N4	O2	3.102	157		
	N6	O4	3.030	110		
	N7	O4	2.994	168		
	N8	O5	2.911	169		
	N9	O6	2.922	145		
	N10	O7	3.163	144		
	N11	O8	2.885	162		
	O12	O9	2.823	156		
	Intermolecular Head-to-tail	N1	O11	2.935	158	
		Peptide Solvent	N2	O105	2.875	161
			N5	O101	2.817	148
O10			O108	2.75		
O101			O2	2.873	175	
O106			O3	2.738		
O109			O7	2.839	152	
O105			O11	2.890	180	
O103			O12	2.808	178	
Molecule B						
Intramolecular	N3		O1	2.871	166	
	N4	O2	3.083	166		
	N6	O4	3.023	110		
	N7	O4	3.048	160		
	N8	O5	2.940	169		
	N9	O6	2.892	147		
	N10	O8	3.080	140		
	N11	O8	2.894	163		
	Intermolecular Head-to-Tail	N1	O12	2.873	157	
		Peptide Solvent	N2	O104	2.824	158
	N5		O103	2.917	164	
N6	O107		2.892	143		
O12	O102		2.678	160		
O103	O2		2.847	179		
O107	O3		2.70			
O109	O7		2.806	179		
O102	O9		2.788	162		
O102	O10		3.102	172		
O106	O10		2.883	152		
O104	O11		2.798	168		
Solvent -Solvent						
O101	O109		2.79			
O102	O104		3.14			
O103	O107		3.11			
O104	O106	2.80				
O105	O107'	2.71				
O105	O108	2.93				
O107	O108	2.85				
O108	O108	2.82				

Methods

The synthesis of each enantiomer of trichogin A IV was performed separately in solution by stepwise elongation of the peptide chain starting from the C-terminal leucine methyl ester. α -benzyloxycarbonyl amino acid derivatives were incorporated in the peptide chain either by the mixed anhydride (in the case of protein amino acids) or by the symmetrical anhydride (in the case of Aib residues). The N-terminal octanoyl-Aib moiety was incorporated by the 5(4H)-oxazolone method. In the last step the C-terminal Leu-OMe was selectively reduced to Leuol with LiBH_4 . The purity and the identity of the final products were assessed by HPLC, FT-IR, ^1H NMR, mass spectrometry and chiral chromatography.

Crystals of the racemate were grown by slow evaporation of a methanol-diethyl ether solution containing equimolar amounts of both enantiomers. Crystallographic Details for Trichogin A IV: ($\text{C}_{52}\text{H}_{95}\text{N}_{11}\text{O}_{12} \cdot 4.5(\text{H}_2\text{O})$), $a = 17.711(3)\text{\AA}$, $b = 19.879(2)\text{\AA}$, $c = 20.712(4)\text{\AA}$, $\alpha = 109.04(3)^\circ$, $\beta = 101.86(2)^\circ$, $\gamma = 97.92(3)^\circ$; $T = 223(2)\text{ K}$; Space Group PT; $Z = 4$; density (calc) = 1.159 g cm^{-3} ; data resolution = 0.9\AA ; data collected 18,495; unique data 17,711; $R_{\text{int}} = 0.032$; observed data, $\geq 3\sigma(I)$ 10,318; variables: two blocks of 686 and 775 each; $R = 0.073$; $R_w = 0.089$; final difference density $+0.622, -0.445\text{ e \AA}^{-3}$. Data were collected from an irregularly shaped crystal, flash frozen in a nitrogen cold stream on an Enraf Nonius CAD-4 diffractometer with copper radiation from a rotating anode source ($\lambda = 1.54184\text{\AA}$) and a graphite monochromator. Intensity standards monitored at regular 3 hr. intervals indicated a 20% decay for which a correction was applied subsequent to corrections for measured backgrounds, Lorentz and polarization factors. A half-sphere of data was collected and symmetry equivalent reflections were averaged. Eventually, data were corrected for the effects of absorption using the DIFABS procedure³⁴. The structure was solved using SHELXS³⁵ which revealed positions for most of the peptide atoms for both of molecules A and B in the initial solution. Least-squares refinement (on F) using the SDP software³⁶ proceeded in two blocks straightforwardly. Weights, w , were applied to the data as $w = 4\text{Fo}^2/s(\text{Fo}^2)$ with $s(\text{Fo}^2) = (s^2/c) + (0.0016\text{ l}^2)$. Positions for the equivalent of nine water molecules (labelled O 101-O 109) were located from difference Fourier maps and were refined along with their anisotropic displacement parameters, with the exception that two positions which summed to full occupancy for each of O 107 and O 108 were refined isotropically. The carbonyl oxygen, O 10 was found to be disordered over two sites of equal occupancy. Atom C4 in the octanoyl group of molecule A was also disordered over two sites. Positions for hydrogen atoms attached to carbons were assigned based on geometric criteria and were held fixed along with isotropic temperature factors assigned as $1.3(B_{\text{eq}})$ of the attached nonhydrogen atom. Positions for all of the hydrogens attached to nitrogens and most of those attached to oxygens were suggested from difference Fourier maps. Others were calculated based on consideration of a plausible hydrogen-bonding scheme. The refinement converged (max $D/s = 0.01$) to the conventional crystallographic residuals listed above. Fractional atomic coordinates and full lists of bond lengths, bond angles and torsion angles have been deposited with the Cambridge Crystallographic Data Centre. Principal torsion angles for the peptide backbones are presented in Table 1 and metrical value for hydrogen-bonding interactions are listed in Table 2.

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Estimated standard deviations for distances are 0.008 \AA on average, but range to 0.02 \AA for distances involving disordered sites.

¹Angle as computed at the donor hydrogen-atom; those missing values are for interactions involving disordered atoms or where hydrogen atoms were not positioned.

1. Moffat, A.S. New methods make mid-sized molecules easier to solve. *Science* **256**, 309–310 (1992).
2. Karle, J. & Hauptman, H. A theory of phase determination for the four types of non-centrosymmetric space groups 1P22₂, 2P22₁, 3P12₁, 3P22. *Acta crystallogr.* **9**, 635–651 (1956).
3. Shiono, M. & Woolfson, M.M. Direct-space methods in phase extension and phase determination. I. Low-density elimination. *Acta crystallogr.* **A48**, 451–456 (1992).
4. DeTitta, G.T., Weeks, C.M., Thuman, P., Miller, R. & Hauptman, H. Structure solution by minimal-function phase refinement and Fourier filtering. I. Theoretical Basis. *Acta crystallogr.* **A50**, 203–210 (1994).
5. Weeks, C.M., DeTitta, G.T., Hauptman, H. A., Thuman, P. & Miller, R. Structure solution by minimal-function phase refinement and Fourier filtering. II. Implementation and Applications. *Acta crystallogr.* **A50**, 210–220 (1994).
6. Langs, D.A. Three-dimensional structure at 0.86 Å of the uncomplexed form of the transmembrane ion channel peptide Gramicidin A. *Science* **241**, 188–191 (1988).
7. Glover, I., Haneef, I., Pitts, J., Wood, S., Moss, T., Tickle, I. & Blundell, T. Conformational flexibility in a small globular hormone: X-ray analysis of avian pancreatic polypeptide at 0.98 Å resolution. *Biopolymers* **22**, 293–304 (1983).
8. Karle, I.L., Karle, J., Mastropaolo, D., Cameran, A. & Camerman, N. [Leu³] enkephalin: Four cocrystallizing conformers with extended backbones that form an antiparallel β-sheet. *Acta crystallogr.* **B39**, 625–637 (1983).
9. Mackay, A.L. Crystal enigma. *Nature* **342**, 133 (1989).
10. Jacques, J., Collet, A. & Wilen, S. H. in *Enantiomers, racemates and resolutions* (John Wiley & Sons, New York; 1981).
11. Brock, C.P., Schweizer, W.B. & Dunitz, J.C. On the validity of Wallach's rule: On the density and stability of racemic crystals compared with their chiral counterparts. *J. Am. chem. Soc.* **113**, 9811–9820 (1992).
12. Zawadke, L.E. & Berg, J.E. The structure of a centrosymmetric protein crystal. *Prot. Struct. Funct. Genet.* **16**, 301–305 (1993).
13. Doi, M. *et al.* Structural characteristics of enantiomeric DNA: Crystal analysis of racemates of the d(CGCGCG) duplex. *J. Am. chem. Soc.* **115**, 10432–10433 (1993).
14. Doi, M., Ishibe, A., Shinozaki, H., Urata, H., Inoue, M. & Ishida, T. Conserved and novel structural characteristics of enantiomeric Leu-enkephalin. *Int. J. Peptide Prot. Res.* **43**, 325–331 (1994).
15. Benedetti, E. *et al.* Peptaibol antibiotics: A study on the helical structure of the 2-9 sequence of Emerimicins III and IV. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7951–7954 (1982).
16. Bruckner, H., Cussin, C. & Kripp, T. Detection of new peptaibol antibiotics (mycotoxins) in species and strains of the fungal genus *Trichoderma*. in *Peptides: chemistry and biology*, 650–652 (ed. Marshall, G.R. ESCOM Science Publishers, Leiden, The Netherlands; 1988).
17. Pandey, R.C., Cook, J.C., Jr & Rinehart, K.L. High resolution and field desorption mass spectrometry studies and revised structures of Alamethicins I and II. *J. Am. chem. Soc.* **99**, 8469–8483 (1977).
18. Fox, R.O. & Richards, F.M. A voltage-gated ion channel model inferred from the crystal structure of Alamethicin at 1.5 Å resolution. *Nature*, **300**, 325–330 (1982).
19. Rinehart, K.L., *et al.* Structures of eleven zervamicin and two emerimicin peptide antibiotics studied by fast atom bombardment mass spectrometry. *J. Am. chem. Soc.* **103**, 6517–6520 (1981).
20. Karle, I.L., Flippen-Anderson, J.L., Agarwalla, S. & Balaran, P. Crystal structure of [Leu¹] Zervamicin, a membrane ion channel peptide: Implications for gating mechanisms. *Proc. natn. Acad. Sci. U.S.A.* **88**, 5307–5311 (1991).
21. Pandey, R.C., Meng, H., Cook, J.C., Jr & Rinehart, K.L., Jr. Structure of antiemoebic I from high resolution field desorption and gas chromatographic mass spectrometry studies. *J. Am. chem. Soc.* **99**, 5203–5205 (1977).
22. Das, K.M., Ragothama, S., Balaran, P. Membrane channel forming polypeptides. Molecular conformation and mitochondrial uncoupling activity of Antiemoebic, an α-aminoisobutyric acid containing peptide. *Biochemistry* **25**, 7110–7117 (1986).
23. Auvin-Guette, C., Rebuffat, S., Prigent, Y. & Bodo, B. Trichogin A IV, an 11-residue lipopeptaibol from *Trichoderma longibrachiatum*. *J. Am. chem. Soc.* **114**, 2170–2174 (1992).
24. Karle, I.L., Sukumar, M. & Balaran, P. Parallel packing of α-helices in crystals of the Zervamicin IIA analog Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe. 2H₂O. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9284–9288 (1986).
25. Bosch, R., Jung, G., Schmitt, H. & Winter, W. Crystal structure of the α-helical undecapeptide Boc-L-Ala-Aib-Ala-Aib-Ala-Glu(OBzl)-Ala-Aib-Ala-Aib-Ala-OMe. *Biopolymers* **26**, 961–978 (1985).
26. Presta, L.G. & Rose, G.D. Helix signals in proteins. *Science* **240**, 1632–1641 (1988).
27. Zhou, H.X., Lyu, P.C., Wemmer, D.E. & Kallenbach, N.R. Structure of a C-terminal α-helix cap in a synthetic peptide. *J. Am. chem. Soc.* **116**, 1139–1140 (1994).
28. Toniolo, C. & Benedetti, E. The polypeptide 3₁₀-helix. *Trends biochem. Sci.* **16**, 350–353 (1991).
29. Nagaraj, R. & Balaran, P. Alamethicin, a transmembrane channel. *Accs. chem. Res.* **14**, 356–362 (1981).
30. Karle, I.L. Folding, aggregation and molecular recognition in peptides. *Acta crystallogr.* **B48**, 341–356 (1992).
31. Bodo, B. *et al.* Synthesis and membrane-modifying properties of analogues of the lipopeptaibol Trichogin A IV with variable Nα-acyl chain length. in *Peptides 1994*, (ed. Maia, H.L.S.) (ESCOM Science Publisher, Leiden, The Netherlands, 1995).
32. Menestrina, G., Voges, K.-P., Jung, G. & Boheim, G. Voltage-dependent channel formation by rods of helical polypeptides. *J. memb. Biol.* **93**, 111–132 (1986).
33. Cosson, P. & Bonifacino, J.S. Role of transmembrane domain interactions in the assembly of class II MHC molecules. *Science* **258**, 659–662 (1992).
34. Walker, N. & Stuart, D. An empirical method for correcting diffractometer data for absorption effects. *Acta crystallogr.* **A39**, 158–166 (1983).
35. Sheldrick, G.M. in *Crystallographic Computing 3* eds Sheldrick, G.M., Kruger, C. & Goddard, R. 175–189 (Oxford University Press: England, 1985).
36. *Enraf Nonius Structure Determination Package*. (Enraf Nonius, Delft, The Netherlands, 1979).
37. *IUPAC-IUB Commission on Biochemical Nomenclature, Abbreviations and symbols for the description of the conformation of polypeptide chains*. *Biochemistry* **9**, 3471–3479 (1970).