139. Crystal Structure and Packing of Isocyclosporin A

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The crystal structure of isocyclosporin A (**1**), a rearrangement product of the immunosuppressant drug cyclosporin A, has been determined at 193 (2) K. Crystals are orthorhombic with cell dimensions $a = 26.684$ (7), $b = 26.936$ (3) Å, $c = 28.549$ (7) Å, space group $C222_1$. The structure was solved by direct methods and refined by full-matrix least-squares methods to a conventional R value of 0.110. In contrast to the structure of cyclosprin A in solution and in the crystal, isocyclosporin A (**1**) has no regular secondary structural elements. The backbone adopts an open, irregular conformation with *cis* amide bonds between residue 2 and 3, and 3 and 4, respectively. All the other amide bonds and the ester linkage are *trans*. Contrary to crystal structures of cyclosporin derivatives, this crystal structure is stabilized by two transannular and four intermolecular H-bonds.

Introduction. – The fungal metabolite cyclosporin A is widely used as an immunosuppressant drug in the prevention of allograft rejection in transplantation surgery [1]. The undecapeptide binds tightly to the intracellular receptor protein cyclophilin. The complex blocks the phosphatase calcineurin and inhibits subsequent steps in signal transduction pathways of T-cells [2] [3]. The molecular structure of free cyclosporin A in solution de-termined by NMR techniques [4] and in the crystal [5] was found to be very similar although in MeOH/H_2O several conformations were observed [6]. The structure comprises an antiparallel β-sheet with a β-II′-type turn around residue 3 and 4, and a *cis* amide bond between residue 9 and 10 held together by four intramolecular H-bonds. The structures of various cyclophilin-cyclosporin complexes have been investigated by NMR [7] and X-ray crystallography [8–10]. They show cyclosporin in a totally different (all-*trans*)-configuration with no regular secondary structural elements and only one intramolecular H-bond. This backbone conformation was also found for the water-soluble derivative [D-MeSer^3-D-Ser-(O-Gly)^8] cyclosporin in (D_6)DMSO and H_2O by NMR techniques [12].

Isocyclosporin A (**1**, *Fig. 1*) is formed from cyclosporin A by an intramolecular *N,O*-acyl migration from MeVal^{11} to the secondary alcohol function of the side chain of MeBmt^1 [13]. This reversible ring expansion is catalyzed by MsOH in aprotic solvents, *e.g.*, dioxane. The isomerization eliminates a peptide bond and adds an ester linkage (*Scheme 4*). The kinetics and mechanism of this rearrangement of cyclosporin and its analogues have been studied in detail by *Oliyai et al.* [14]. The free amino function allowed a modified *Edman* sequence analysis for determining the constitution of cyclosporin A [13]. Isocyclosporin A (**1**) is not able to bind cyclophilin and, therefore, has no immunosuppressive activity. In the cyclosporin-cyclophilin complex the β-OH group of MeBmt^1 forms a H-bond to the protein. This β-OH group is absent in **1**.

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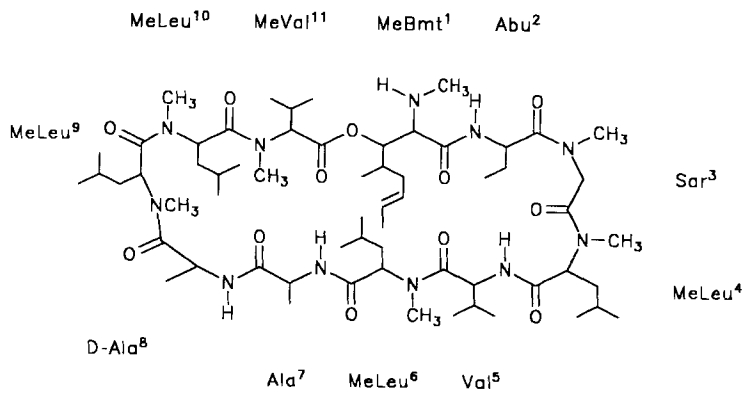
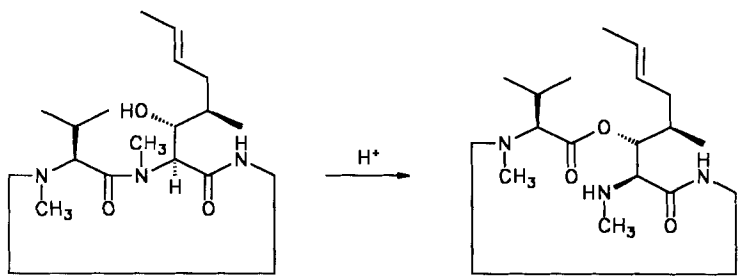


Fig. 1. *Chemical constitution of isocyclosporin A* (**1**)

Scheme



Cyclosporin A

1 Isocyclosporin A

Results and Discussion. – The amino-acid numbering is shown in *Fig. 1*, and the crystal structure and atom-numbering scheme of isocyclosporin A (**1**) are shown in *Fig. 2*. A stereoview of the molecule with 50% probability ellipsoids is given in *Fig. 3*. Selected bond lengths and angles are collected in *Table 1*, and the torsion angles of the peptide backbone in *Table 2*. All bond lengths, angles, and torsion angles are in the expected range found in peptide structures. The peptide linkages are essentially planar. The backbone conformation is substantially different from the conformations found for the various cyclosporin derivatives in the crystal [5] [15–18] and in hydrophobic solvents determined by NMR [5] [12] [17] and from the conformation of the bound peptide. The 34-membered ring adopts an irregular open conformation. No regular secondary structural elements can be identified, whereas cyclosporin A forms an antiparallel β-sheet structure with a β-II′-type turn. The peptide bonds between residues 2 and 3, and 3 and 4 have the *cis*-configuration; they are *trans* in cyclosporin A. All other amide bonds (including the amide bond between residue 9 and 10 which is *cis* in cyclosporin) and the ester bond between residue 1 and 11 are in *trans*-configuration. The relatively small chemical modification causes a dramatic change in the overall conformation of the peptide.

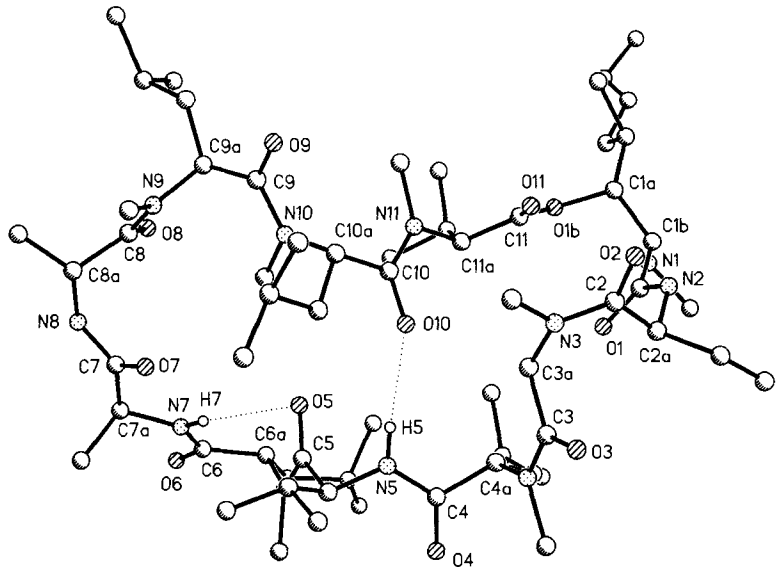


Fig. 2. *Crystal structure of* **1** (H-atoms except those involved in intramolecular H-bonds have been omitted for clarity)

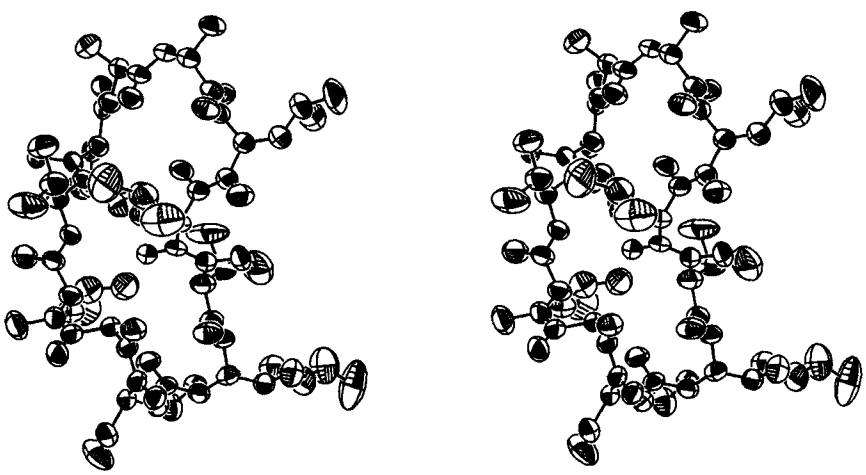


Fig. 3. *Stereoview of the molecule showing anisotropic displacement ellipsoids at 50% probability level*

This conformation is further stabilized by two transannular H-bonds N(5)–H(5)···O(10) (3.19 Å) and N(7)–H(7)···O(5) (3.07 Å). In contrast to the structure of cyclosporin, which is held together by four transannular H-bonds, this crystal structure is stabilized by intermolecular H-bonds to symmetry-related peptide molecules (distances 2.88 and 2.94 Å). These H-bonds are shown in *Figs. 4* and *5*. The geometries of the intra- and intermolecular H-bonds are given in *Table 3*.

Table 1. *Selected Bond Lengths* [Å] *and Angles* [°] *for* **1**

C(1)–O(1)	1.239(11)	C(1)–N(2)	1.327(12)	C(1)–C(1B)	1.515(13)
C(1B)–N(1)	1.392(13)	C(1B)–C(1A)	1.492(13)	C(1A)–O(1B)	1.462(10)
C(1A)–C(1C)	1.537(13)	O(1B)–C(11)	1.300(11)	C(2)–O(2)	1.242(11)
C(2)–N(3)	1.336(11)	C(2)–C(2A)	1.497(14)	C(2A)–N(2)	1.458(11)
C(3)–O(3)	1.212(11)	C(3)–N(4)	1.341(13)	C(3)–C(3A)	1.512(13)
C(3A)–N(3)	1.452(12)	C(4)–O(4)	1.224(10)	C(4)–N(5)	1.317(11)
C(4)–C(4A)	1.536(12)	C(4A)–N(4)	1.450(11)	C(5)–O(5)	1.224(10)
C(5)–N(6)	1.329(11)	C(5)–C(5A)	1.554(12)	C(5A)–N(5)	1.458(10)
C(6)–O(6)	1.206(10)	C(6)–N(7)	1.320(12)	C(6)–C(6A)	1.537(11)
C(6A)–N(6)	1.455(10)	C(7)–O(7)	1.221(10)	C(7)–N(8)	1.332(11)
C(7)–C(7A)	1.511(13)	C(7A)–N(7)	1.476(11)	C(8)–O(8)	1.238(10)
C(8)–N(9)	1.334(11)	C(8)–C(8A)	1.514(12)	C(8A)–N(8)	1.460(11)
C(9)–O(9)	1.207(11)	C(9)–N(10)	1.359(12)	C(9)–C(9A)	1.570(12)
C(9A)–N(9)	1.461(10)	C(10)–O(10)	1.230(10)	C(10)–N(11)	1.359(11)
C(10)–C(10A)	1.556(12)	C(10A)–N(10)	1.463(10)	C(11)–O(11)	1.227(11)
C(11)–C(11A)	1.527(13)	C(11A)–N(11)	1.471(12)		

O(1)–C(1)–N(2)	121.5(8)	O(1)–C(1)–C(1B)	119.2(9)
N(2)–C(1)–C(1B)	119.2(9)	N(1)–C(1B)–C(1A)	114.8(8)
N(1)–C(1B)–C(1)	112.7(9)	C(1A)–C(1B)–C(1)	108.3(8)
O(1B)–C(1A)–C(1B)	108.3(8)	O(1B)–C(1A)–C(1C)	107.6(7)
C(1B)–C(1A)–C(1C)	117.6(8)	C(11)–O(1B)–C(1A)	118.1(8)
O(2)–C(2)–N(3)	121.1(10)	O(2)–C(2)–C(2A)	120.0(9)
N(3)–C(2)–C(2A)	119.0(10)	N(2)–C(2A)–C(2)	109.0(7)
C(2)–C(2A)–C(2B)	110.2(9)	C(1)–N(2)–C(2A)	122.4(8)
O(3)–C(3)–N(4)	122.1(9)	O(3)–C(3)–C(3A)	120.8(10)
N(4)–C(3)–C(3A)	117.0(9)	N(3)–C(3A)–C(3)	111.3(8)
C(2)–N(3)–C(3A)	123.3(9)	C(3N)–N(33)–C(3A)	117.2(7)
O(4)–C(4)–N(5)	122.7(9)	O(4)–C(4)–C(4A)	120.8(8)
N(5)–C(4)–C(4A)	116.5(9)	N(4)–C(4A)–C(4)	109.6(8)
C(3)–N(4)–C(4A)	124.4(8)	O(5)–C(5)–N(6)	122.0(8)
O(5)–C(5)–C(5A)	117.5(8)	N(66)–C(55)–C(5A)	120.6(8)
N(5)–C(5A)–C(5)	105.2(7)	C(5B)–C(5A)–C(5)	111.9(8)
C(4)–N(5)–C(5A)	122.4(8)	O(6)–C(6)–N(7)	123.1(9)
O(6)–C(6)–C(6A)	123.3(9)	N(7)–C(6)–C(6A)	113.5(8)
N(6)–C(6A)–C(6B)	112.8(7)	N(6)–C(6A)–C(6)	111.0(7)
C(5)–N(6)–C(6A)	117.8(7)	C(5)–N(6)–C(6N)	122.2(8)
O(7)–C(7)–N(8)	122.6(9)	O(7)–C(7)–C(7A)	121.7(8)
N(8)–C(7)–C(7A)	115.7(9)	N(7)–C(7A)–C(7B)	112.1(8)
N(7)–C(7A)–C(7)	106.5(7)	C(7B)–C(7A)–C(7)	110.5(10)
C(6)–N(7)–C(7A)	124.2(8)	O(8)–C(8)–N(9)	123.2(8)
O(8)–C(8)–C(8A)	117.9(9)	N(9)–C(8)–C(8A)	118.8(8)
N(8)–C(8A)–C(8)	109.4(7)	N(8)–C(8A)–C(8B)	110.6(8)
C(8)–C(8A)–C(8B)	108.9(8)	C(7)–N(8)–C(8A)	119.3(8)
O(99)–C(99)–N(10)	123.6(9)	O(9)–C(9)–C(9A)	121.1(9)
N(10)–C(9)–C(9A)	115.2(9)	N(9)–C(9A)–C(9)	109.1(7)
C(8)–N(9)–C(9A)	119.3(8)	O(10)–C(10)–N(11)	122.5(9)
O(10)–C(10)–C(10A)	120.7(8)	N(11)–C(10)–C(10A)	116.8(8)
N(10)–C(10A)–C(10B)	112.1(7)	N(10)–C(10A)–C(10)	108.4(7)
C(9)–N(10)–C(10A)	117.7(8)	O(11)–C(11)–O(1B)	125.6(9)
O(11)–C(11)–C(11A)	122.5(9)	O(1B)–C(11)–C(11A)	111.9(9)
N(11)–C(11A)–C(11B)	115.0(8)	N(11)–C(11A)–C(11)	108.4(8)

Table 2. *Geometry of the Intra- and Intermolecular H-Bonds of* **1**

	D···A [Å]	D–H···A [°]
N(5)–H(5)···O(10)	3.19(1)	169.8(3)
N(7)–H(7)···O(5)	3.07(1)	142.5(3)
N(2)–H(2)···O(2)[a])	2.88(1)	161.7(3)
N(8)–H(8)···O(6)[b])	2.94(1)	151.8(3)

[a]) Symmetry operators $x, 1-y, 1-z$. [b]) Symmetry operators $1-x, y, 1.5-z$.

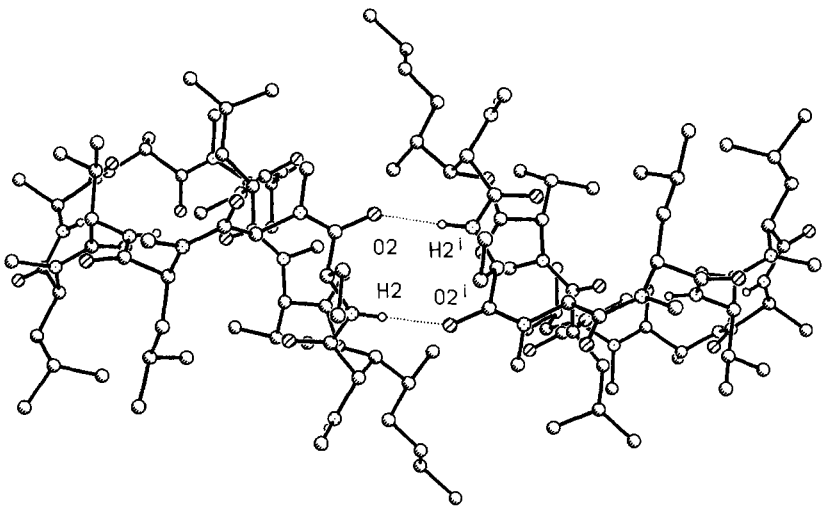


Fig. 4. *Intermolecular H-bonds of symmetry-related molecules*

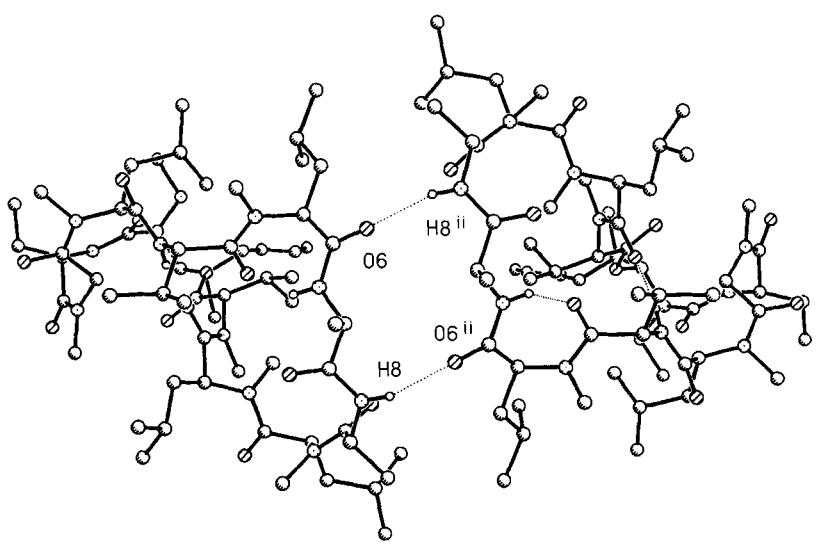


Fig. 5. *Intermolecular H-bonds of symmetry-related molecules*

Table 3. *Torsion Angles of the Peptide Backbone of* **1**

	ϕ	ψ	ω
MeBmt[1]	–	–	–
Abu[2]	−100.8(11)	111.8(9)	–
MeSar[3]	82.0(10)	−177.5(8)	−7.7(12)
MeLeu[4]	−124.1(10)	103.5(10)	3.5(14)
Val[5]	−115.3(10)	125.0(9)	177.9(8)
MeLeu[6]	−96.2(10)	54.2(10)	−176.7(7)
Ala[7]	−157.6(9)	143.1(8)	172.7(7)
D-Ala[8]	72.6(10)	−141.7(8)	−175.3(8)
MeLeu[9]	−131.8(9)	70.1(11)	−175.4(7)
MeLeu[10]	−130.4(9)	67.5(10)	−177.0(8)
MeVal[11]	−109.9(10)	–	−179.1(8)

The molecules form a helix around the crystallographic two-fold screw axis parallel to *c*. The four molecules per turn are linked alternatingly by the two types of intermolecular H-bonds. The packing diagram displayed in *Fig. 6*, shows a large channel along the crystallographic *c*-axis, which is filled with disordered solvent molecules. Two Me_3CO groups of Me_3COMe which was used for crystallization were visible as regular tetrahedra. These are disordered about crystallographic two-fold axes; one is shown in *Fig. 6*.

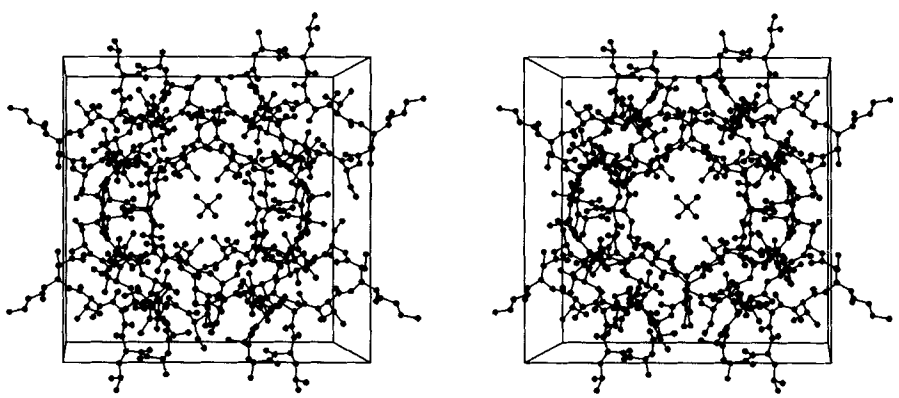


Fig. 6. *Stereoview of the crystal packing of* **1**, *perspective view along the z-axis*

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Experimental Part

Preparation and Crystallization of Isocyclosporin A (**1**): Isocyclosporin A (**1**; 10 mg) was dissolved in AcOEt/*t*-BuOMe 1:10 at 50°. The soln. was cooled down to 18° and tiny crystals formed overnight. To grow larger crystals, the soln. was heated to 50° until most of the crystals redissolved, then cooled again to 18° overnight. After repeating this procedure daily for one week, suitable crystals for the X-ray analysis were obtained.

Data Collection and Processing. Crystal data are summarized in *Table 4*. Data were collected on a *Stoe-Siemens-Huber* four-circle diffractometer with graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å). The

crystal was mounted on the tip of a glass fibre using the 'oil-drop' method [19]. The crystal was cooled to −80° by a cold N_2 stream with a locally built low-temp. device [20]. Intensities were obtained from ω-2θ scans, with a scan speed of one second per step, by a 'learnt profile' method [21]. To improve data quality, which proved absolutely necessary for successful structure solution, all *Friedel* opposites and a full set of symmetry-related reflections were measured. Three standard reflection were measured every 90 min to monitor crystal decay and apply linear decay correction. They showed a decrease of 10% over the 12 days of data collection.

Table 4. *Crystal and Refinement Data of* **1**

Empirical formula	$C_{65.75}H_{111}N_{11}O_{14.5}$	$F(000)$	5588
Formula weight [g/mol]	1287.66	2θ range	2–43
Crystal size [mm]	1.0 × 0.3 × 0.2	No. of reflections	26226
Crystal system	Orthorhombic	Independent	11917
Space group	$C222_1$	R_{int}	0.100
Temperature	−80	No. of data	11904
a [Å]	26.684(7)	No. of restraints	946
b [Å]	26.936(3)	No. of parameters	853
c [Å]	28.549(7)	$wR2$ (all data)	0.3589
V [Å^3]	20520(8)	$R1$ ($F > 4\sigma(F)$)	0.1097
Z	8	g_1, g_2	0.1960, 0
$\rho_{calc.}$ [Mgm^{-3}]	0.834	Max. [eÅ^{-3}]	0.459
μ [mm^{-1}]	0.059	Min. [eÅ^{-3}]	−0.310

$wR2 = [\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]]^{1/2}$.
$w^{-1} = \sigma^2(F_o)^2 + (g_1 \cdot P)^2 + g_2 \cdot P$, where $P = (F_o^2 + 2F_c^2)/3$.
$R1 = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$ for $F > 4\sigma(F)$.

Structure Solution and Refinement: The structure was solved by direct methods using SHELXS-90 [22]. The structure solution proved difficult although *ca.* 60% of the reflections are 'observed' [$I > 2\sigma(I)$] in the critical 1.1–1.2 Å range. This is probably due to the size of the unit-cell. The probability distribution for individual phase relations decreases with the factor $N^{-1/2}$, where N is the number of atoms per primitive unit-cell. For successful structure solution, it was necessary to increase the number of reflection used for tangent formula phase refinement. One correct solution starting from *ca.* 100000 random starting phase sets could be clearly identified by the combined figure of merit $CFOM = 0.101$ compared to $CFOM = 0.240$ for the second best solution. The usual *E-Fourier* recycling [23] led to the essentially complete structure. The data were further used to investigate the influence of high- and low-resolution data on structure solution. The weak (high-resolution) data have a considerable influence on the number and reliability of the negative quartets, whereas the very-low-resolution data enter into many triplet and quartet phase relations. The structure could not be solved when the very-low-resolution data (the eight reflections with $d > 5$ Å) were not used or when the original high-resolution data (the unique reflections plus *Friedel* pairs only) were employed. The measurements of equivalents in the 1.0–1.1 Å range, thus enabling more precise intensities to be obtained by merging four-fold redundant data rather than just two-fold, was apparently necessary before the structure could be solved.

All non-H-atoms were refined anisotropically against F^2 by full-matrix least-squares methods using SHELX-93 [24]. Rigid-bond restraints [25] (for 1,2 and 1,3 atom pairs) with e.s.d.'s of 0.01 Å^2 and 'similarity' restraints (for atoms closer than 1.7 Å) with e.s.d.'s of 0.05 Å^2 were applied to the anisotropic displacement parameters. The 1,2 and 1,3 distances of chemically equivalent residues were restrained to have similar values (within e.s.d.'s of 0.03 Å), but the torsion angles were free to vary. All carbonyl C-atoms were restrained to have a planar environment (e.s.d.'s 0.1 Å^2). These chemically reasonable restraints enabled a full anisotropic refinement despite rather weak data. In addition, they improve the convergence of the refinement significantly. The mean (Δ/σ) in the last refinement cycle is 0.0, the maximum (Δ/σ) is 0.012. H-Atoms were included in calculated positions and refined assuming a riding model. The isotropic displacement parameters of the H-atoms were set to 1.2 times (1.5 times for Me groups) the equivalent displacement parameters of the atom to which they were attached. Two disordered Me_3CO groups about crystallographic two-fold axis were refined with distance restraints and carbon scattering factors for all atoms. In addition, the five highest peaks in the difference electron density were refined as half occupied O-atoms of solvent molecules. The anisotropic displacement parameters of these atoms were restrained to be isotropic with e.s.d.'s of 0.1 Å^2. These molecules have no contacts to the peptide molecule. The peptide molecule

does not contain significant anomalous scatterers, so the absolute structure could not be determined experimentally; it was, therefore, fixed to be the same as that of cyclosporin A.

Lists of observed and calculated structure factors, crystal data, fractional atomic coordinates, anisotropic displacement parameters, full bond lengths and angles have been deposited at the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK.

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