15. Cyclo-β-peptides: Structure and Tubular Stacking of Cyclic Tetramers of 3-Aminobutanoic Acid as Determined from Powder Diffraction Data

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Dedicated to Professor *Hans Paulsen* on the occasion of his 75th birthday

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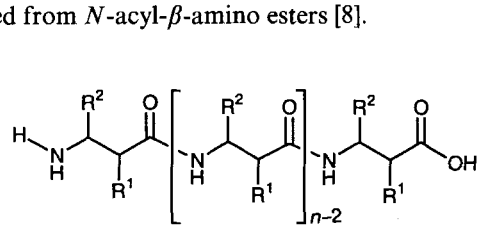
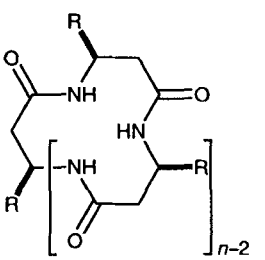
The solid-state structures of three stereoisomers, **1**–**3**, of the cyclic tetramer of 3-aminobutanoic acid are presented. These cyclo-β-peptides were found to be highly insoluble materials, and it proved to be impossible to grow crystals of sufficient quality for X-ray single-crystal analysis. The samples of **1**–**3** were, however, suitable candidates for structure determination from powder diffraction data (*Fig. 1*), and the application of this method is described. All three isomers have been found to adopt tubular structures (*Figs. 2–4*) in a fashion similar to those already observed for certain cyclo-α-peptides. The stacks of 16-membered rings are held together by four nonlinear C=O···H–N H-bonds between pairs of molecules (*Fig. 5*).

Introduction. – We have recently started investigations into the synthesis and structure of peptide analogues **A** that consist of β-amino acids exclusively (the so-called β-peptides) [1–3]. The β-substituted β-amino-acid building blocks (*cf.* $R^1 = H$, $R^2 \neq H$ in **A**) are readily available in an enantiomerically pure form by *Arndt-Eistert* homologation of α-amino acids [4–6]. The α-substituted β-amino acids (*cf.* $R^1 \neq H$, $R^2 = H$ in **A**) may be prepared by aminomethylation of *Evans*' enolates [3] [7], and we have prepared the α,β-disubstituted compounds (*cf.* $R^1 \neq H$, $R^2 \neq H$ in **A**) by alkylation of Li_2 derivatives generated from *N*-acyl-β-amino esters [8].



A



B

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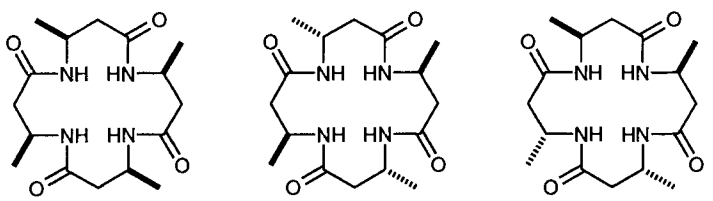
Much to the specialists' and our own surprise, even short-chain β-peptides form secondary structures such as turns [1], β-sheet-like arrangements [1] and helices [1–3]. These secondary structures have geometries, dimensions, functional-group relationships and overall shapes which are totally different from those adopted by α-peptides. In the course of our synthetic work, we have also prepared [1] [9] [10] cyclic β-peptides **B** which, in contrast to their α-peptide counterparts, are readily formed, and we wondered whether their structures also differ drastically from those of 'normal' cyclopeptides[3]). We were especially interested in cyclic oligomers of 3-aminobutanoic acid, as they can be considered to be the nitrogen analogues of the 3-hydroxybutanoic-acid-derived oligolides, and we have previously determined the crystal structures of many representatives of these and similar compounds [14–17]. Whilst their preparation[4]) and characterization is described in [9], we have now been able to determine the solid-state structure of three of the six possible stereoisomeric cyclo-β-tetrapeptides, derived from 3-aminobutanoic acid, by a combination of computer-aided model building and powder diffraction techniques.



1 **2** **3**

Cyclo-β-tetrapeptides. – The most remarkable property of the cyclo-β-peptides **1–3** is their poor solubility in both organic solvents and H_2O, and this is closely followed by their high melting/decomposition points. Thus, the C_4-symmetric (all-*S*) compound **1** is, to all intents and purposes, insoluble. (The best we could do was a 0.2 mM solution in CF_3CH_2OH, *i.e.*, *ca.* 0.2 g/l[5]).) The achiral S_4- and C_i-symmetric diastereoisomers (**2** and **3**, resp.) are somewhat more soluble, and pure samples for elemental analysis were obtained by sublimation (150°/10^{-5} Torr). All three compounds are thermally stable[6]) up to at least 300°. Due to these properties[7]), it has proved to be impossible to prepare single crystals suitable for X-ray structure analysis. We, therefore, tested the three compounds as candidates for structure determination using powder diffraction techniques. While laboratory single-crystal analysis requires crystals of *ca.* 50 μm on an edge, powder diffraction methods can be applied to crystallites of less than 1 μm. Recent advances in both

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[3]) For extensive discussions of the many possible conformations of cyclotetrapeptides, see [11–13] and ref. cit. therein.

[4]) The pentafluorophenyl esters of linear β-peptides are cyclized [1] (method of *U. Schmidt* [18]) in good yields: **1** (78%), **2** (55%), **3** (67%).

[5]) It is highly soluble in a saturated solution of LiCl in THF [19].

[6]) Note that the residues in **1–3** are constitutionally related to *Mannich* bases which are used for the preparation of α,β-unsaturated carbonyl compounds!

[7]) In contrast, the oligolide consisting of four 3-hydroxybutanoic-acid moieties is soluble in CH_2Cl_2/pentane and has a melting point of 178° [15].

instrumentation and computer algorithms in this area have made detailed structural investigations of both inorganic and organic materials using powder data possible[8]).

Data Collection and Refinement. – Powder diffraction data for each sample were collected in transmission mode (0.3-mm rotating capillary) on a high-resolution laboratory powder diffractometer (*Stoe STADI*) using CuK_α radiation (1.5406 Å) and 0.02° 2θ steps. Each pattern was indexed using standard methods [21]. Although the resulting unit cell dimensions and crystal systems proved to be remarkably different for the three materials (*Table*), each has one short dimension of *ca.* 5 Å, and the unit cell volumes are quite similar. The latter indicates that there are two molecules per unit cell in each case. The most probable space group was established by examining the data for systematically absent reflections. With powder data, this is not necessarily straightforward, because the affected reflections are often obscured by reflection overlap, but in these cases there were enough free standing reflections to allow a reasonable judgement to be made.

The short unit cell dimension dictates that the molecules lie approximately perpendicular to that cell edge, and the symmetry elements further require that the molecules lie in specific regions of the unit cell. Using these restrictions, a packing model was developed for each structure. Several models for each molecule were generated. These were consistent with the symmetry requirements of the space group and the chemical structural requirements (configuration of the stereogenic centres, the need for planarity of the peptide groups, bond distances and angles in similar compounds). These were then taken as starting models for *Rietveld* refinement using the program package XRS-82 [22].

Strong geometric restraints on the bond distances and angles were used during the initial stages of refinement [23]. As structure refinement progressed, the relative weight of these geometric restraints was reduced, but they were not removed completely. The model providing the best fit to the observed powder diffraction pattern was readily recognized in all cases, and a structure refinement converged satisfactorily. The number of non-H structural parameters refined remained quite low, with only 20 for **2** and 38 for both **1** and **3**, so the results can be viewed with confidence. Experimental and crystallographic data relating to the tetramers **1**–**3** are given in the *Table*; the observed, calculated and difference profiles for each refinement in *Fig. 1*. Further details regarding the data collection and/or the final structural parameters can be obtained from the authors.

Discussion and Conclusion. – The three 16-membered rings of **1**, **2**, and **3** adopt totally different conformations. The (all-*S*)-derivative looks like a parallelogram with the acute corners truncated (*Fig. 2*), the amide planes are within the sides, and the two tetrahedral C-atoms form the bridges between these sides, the four C=O and the four N–H bonds (mutually antiperiplanar) point, respectively, in the same direction, and the molecule is in a C_2-symmetrical arrangement, all four Me groups are in pseudo-equatorial positions. The backbone of the (*R*,*S*,*R*,*S*)-isomer **2** (*Fig. 3*) has the shape of a square, with the amide units as sides. Opposite pairs of C=O bonds point in the same direction, as do the N–H bonds. Again, the Me groups are in equatorial positions. The molecule has ideal S_4 symmetry. When viewed perpendicular to the average ring plane, the (*R*,*R*,*S*,*S*)-compound **3** has a rectangular appearance (*Fig. 4*); now the parallel C=O and N–H bonds

[8]) For a recent review containing information about structure determination of purely organic compounds from powder diffraction data, see [20].

Table. *Crystallographic Data for Cyclo-β-tetrapeptides* **1–3**

	1	**2**	**3**
Unit cell			
Crystal system	orthorhombic	tetragonal	monoclinic
a [Å]	11.3491(2)	13.6182(2)	12.6878(4)
b [Å]	16.4342(5)	a	4.9783(1)
c [Å]	4.7842(1)	4.8764(1)	18.6356(4)
β [°]	–	–	129.73(4)
Volume [Å^3]	892	904	906
Space group	$P2_12_12$	$I\bar{4}$	$P2_1/c$
Refinement			
2θ range (°2θ)	4–60	7.5–70	4–60
Standard peak (hkl, °2θ)	4–10°: 110, 9.46		
	10–60°: 120, 13.29	211, 23.34	002, 12.34
Peak range (No. of FWHM)	20	18	20
No. of observations	2777	3035	2628
No. of contributing reflections	159	108	226
No. of geometric restraints	75	39	75
No. of non-H struct. parameters	38	20	38
No. of H struct. parameters	42	21	42
No. of profile parameters	8	7	9
R_F	0.059	0.087	0.061
R_{wp}	0.097	0.123	0.114
R_{exp}	0.087	0.096	0.093

are on neighbouring sides, the symmetry is C_i. Thus, the necessity for the Me groups to be equatorial in all three stereoisomers (in order to both avoid a 1,5-repulsion ($A^{1,3}$ strain) [24–26] between a C=O O-atom and the Me group, and to permit intermolecular H-bonding) leads to different overall shapes of cyclo-β-tetrapeptides **1–3**.

Cyclic α-peptides have very complex conformational patterns. They only show a regular conformation leading to tubular crystal packing (with the C=O and N–H bonds alternately pointing up and down parallel to the stacking direction), when they are constructed from alternating (*R*)- and (*S*)-amino-acid residues. This behaviour was first predicted by *Sun* and *Lorenzi* [27] and recently confirmed by *Ghadiri* and coworkers [28]. *Karle et al.* have reported an X-ray crystal structure of a cyclic tetrapeptide containing two α- and two β-amino acids [cyclo(-Ser(O-*t*-Bu)-β-Ala-Gly-L-β-Asp(OMe)-)]. This cyclic peptide is a 14-membered ring and also adopts a conformation which leads to the formation of tubular stacks [29]. As is evident from *Figs. 2–4*, all three isomers of the cyclo-β-peptide studied exhibit tubular crystal packing, with nonlinear C=O···H–N H-bonding in all three cases. It is, therefore, clear that, in terms of their stacking ability, the cyclo-β-peptides are much more versatile (*Fig. 5*). It is the inclusion of the additional CH_2 units that allows the rings more freedom to adopt a conformation suitable for stacking (pseudo-equatorial Me groups), and hence, the cyclo-β-tetrapeptides do not suffer from the restrictions imposed on their α-counterparts.

The results described here demonstrate the power of powder diffraction methods for well-characterized systems. If enough chemical and symmetry information is available for reasonable starting models to be generated for a *Rietveld* structure refinement, the powder diffraction data will generally allow the correct model to be distinguished. In principle, the same structural information that is in a three-dimensional single-crystal data set (*i.e.*, the reflection intensities) is also in the one-dimensional powder diffraction

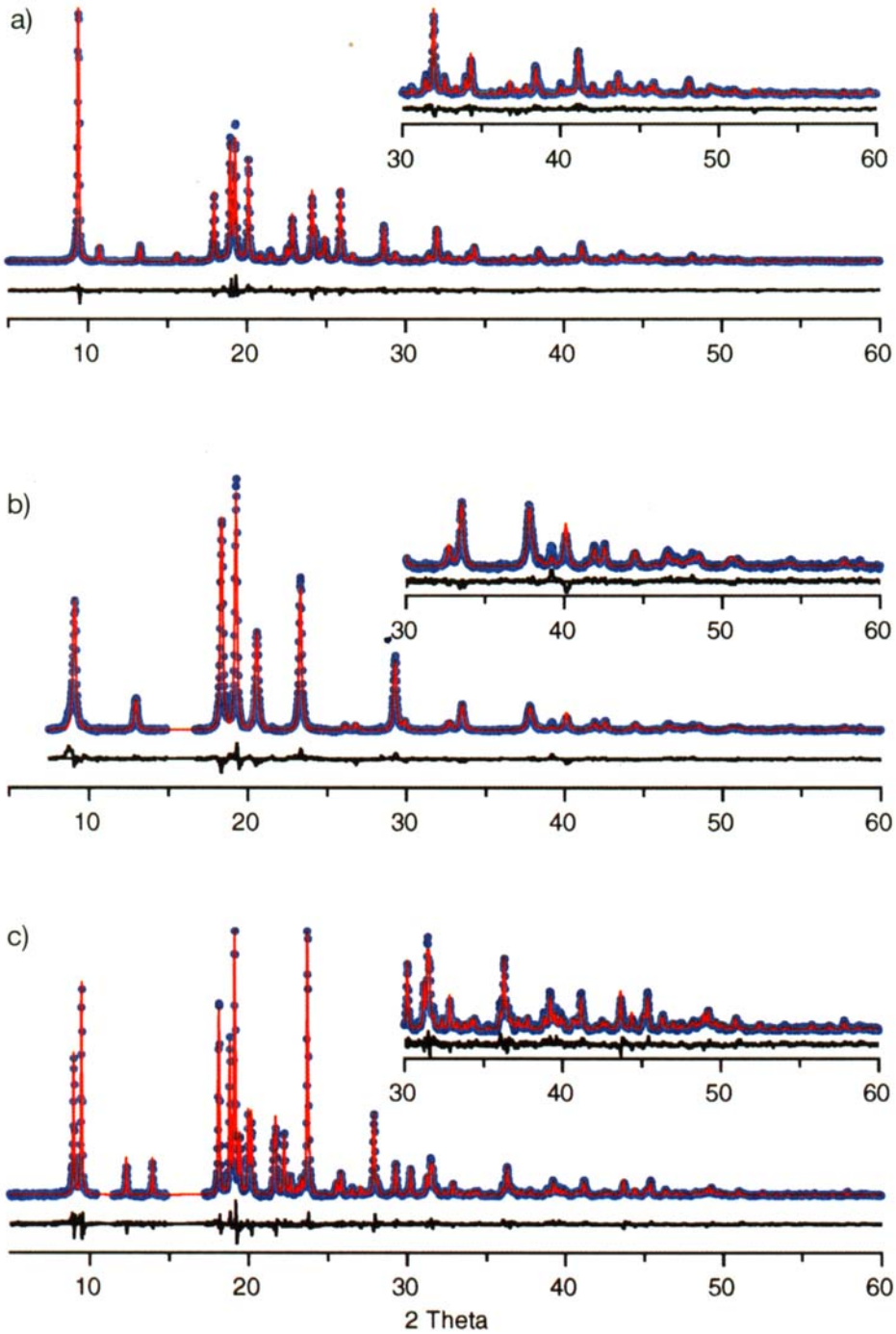

Fig. 1. *The* Rietveld *refinement profiles of* a) **1**, b) **2**, *and* c) **3**: *observed* (blue dots), *calculated* (red line), *and difference* (black line). The intensity scale for the insets has been increased by a factor of 10 to show more detail.

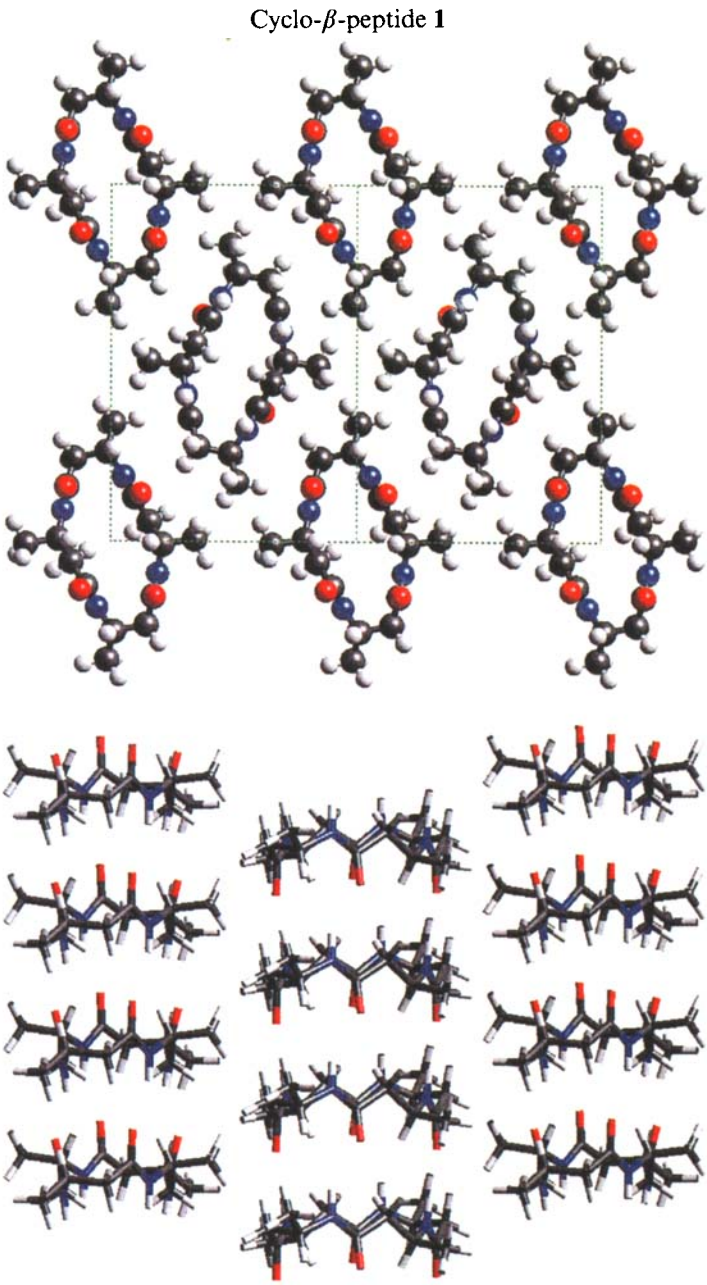

Fig. 2. *Structure of* **1** ((all-*S*)-configuration) *from powder diffraction data*: a) *view along [001]*, b) *view perpendicular to (a)*. O-Atoms in red, N-atoms in blue, C-atoms in black, H-atoms in grey. In two of the four CO–CH_2–CHMe–NH units of the 16-membered ring, the dihedral angle is (+)-*synclinal* (truncated acute corners), in the other two it is *anticlinal* (obtuse corners). In each tubular stack, all C=O bonds point in one direction and the N–H bonds in the opposite direction, and this results in polar tubes. Neighbouring tubes are arranged in opposite directions. The polar stacking may be responsible for the fact that the cyclo-β-peptide **1** is the least soluble of the three isomers studied.

Cyclo-β-peptide **2**

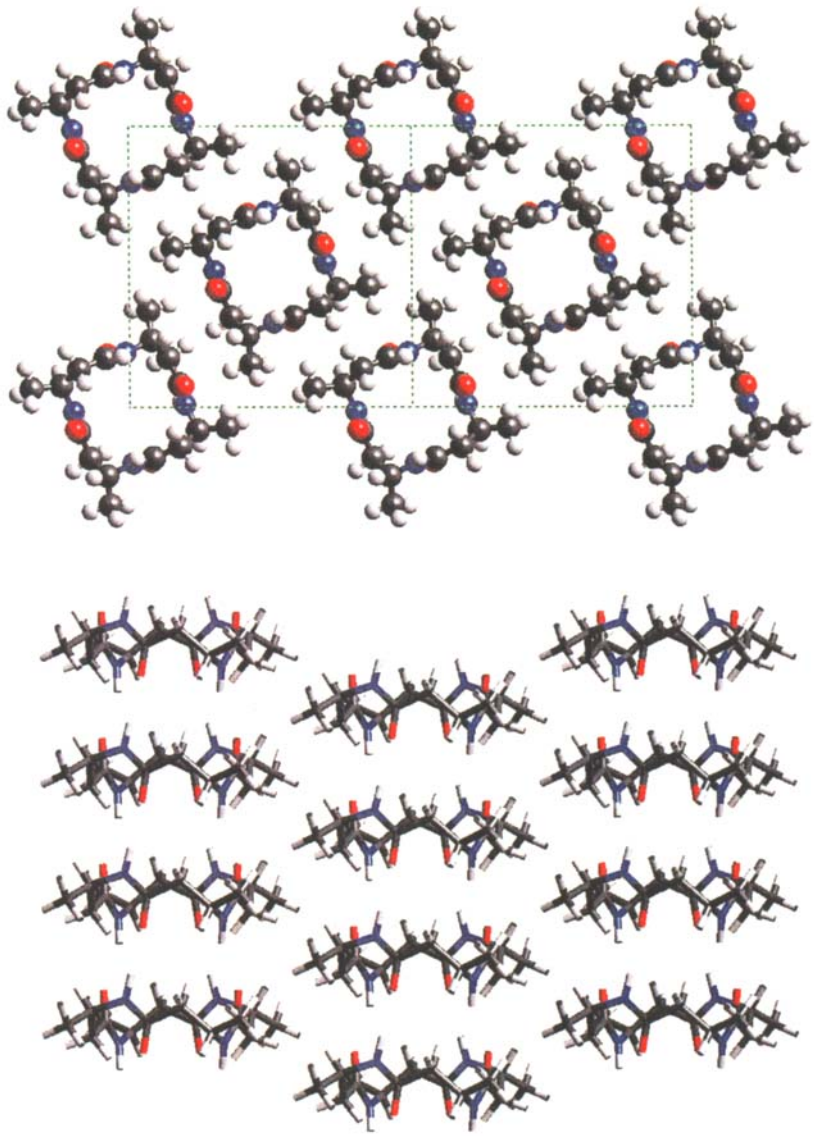

Fig. 3. *Structure of* **2** ((*R*,*S*,*R*,*S*)-configuration) *from powder diffraction data*: a) *view along [001]*, b) *view perpendicular to (* a*)*. Colours as in *Fig. 2*. All CO–CH_2–CHMe–NH dihedral angles are *synclinal* (*gauche*-conformation), with (+) and (–) signs on opposite corners of the square. The stacking occurs by H-bonding between unidirectional pairs of C=O and N–H bonds and is very reminiscent of that seen in the so-called nanotubes of cyclo-α-peptides with alternating (*R*)/(*S*)-configuration [27] [28].

Cyclo-β-peptide **3**

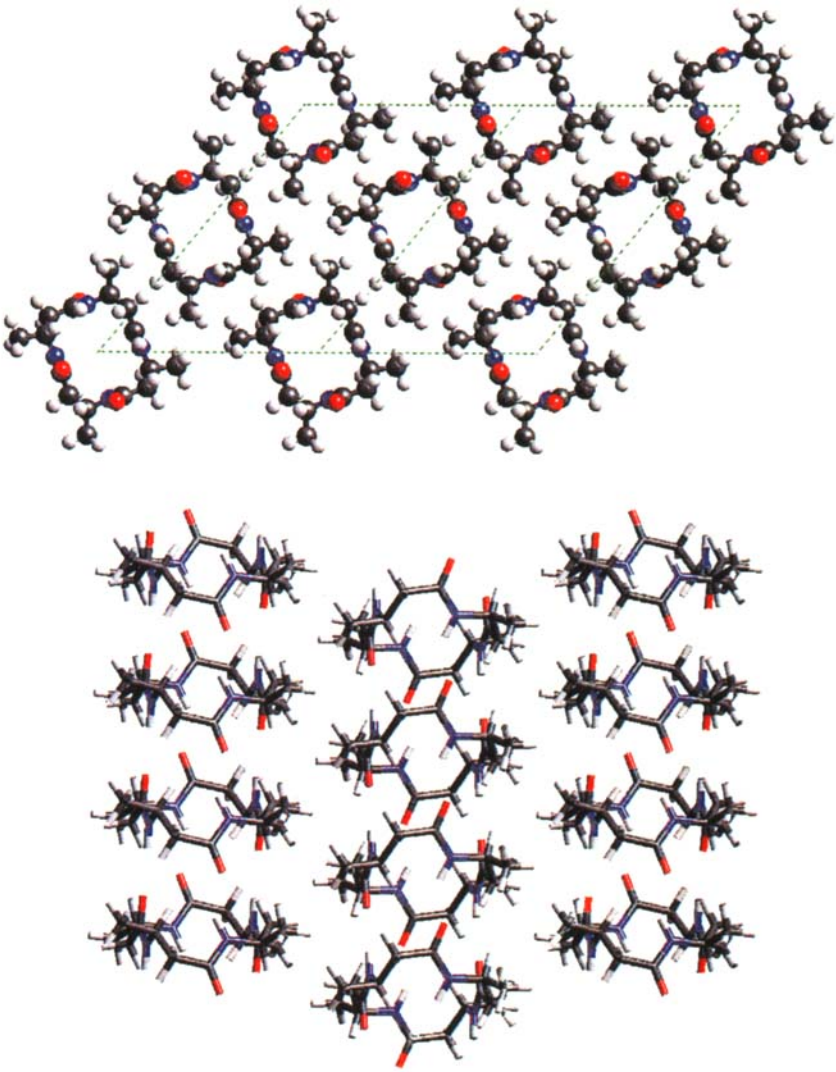

Fig. 4. *Structure of* **3** ((*R*,*R*,*S*,*S*)-configuration) *from powder diffraction data*: a) *view along [010]*, b) *view perpendicular to* (a). Colours as in *Fig. 2*. The CO–CH_2–CHMe–NH dihedral angles are (+)-, (+)-, (−)-, (−)-*synclinal* as each side of the ring is considered in turn. The molecules are held together by H-bonding between adjacent C=O and N–H atoms pointing pairwise up and down with respect to the average ring plane. In contrast to the stacking of **1** and **2** (*Figs. 2* and *3*), molecules **3** are tilted with respect to the stacking direction (*cf. Fig. 5*). The rectangular shape adopted by **3** is reminiscent of that seen in the structure of the cyclotetrapeptide [cyclo(-L-Ser(O-*t*-Bu)-β-Ala-Gly-L-β-Asp(OMe)-)] [29].

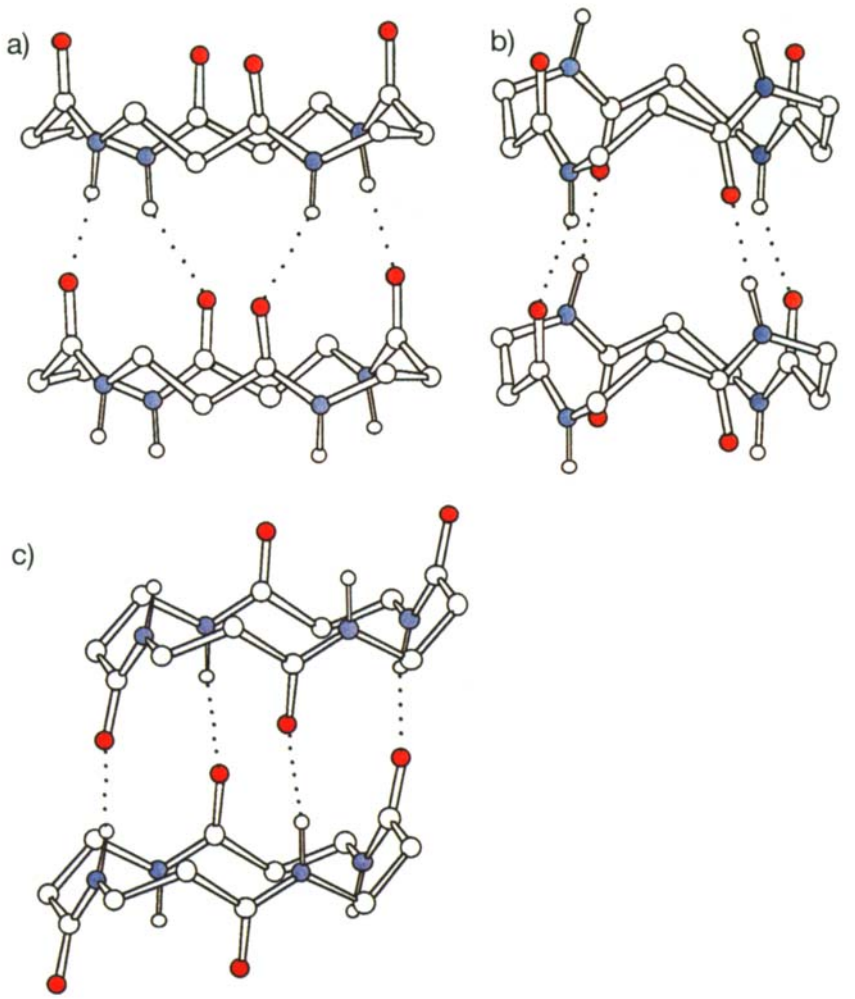

Fig. 5. *MacMoMo Presentation of the backbone and H-bonding of the cyclo-β-tetrapeptides* **1** *(*a*),* **2** *(*b*) and* **3** *(*c*) as found in the solid state.* (Me Substituents and backbone-bound H-atoms are omitted for clarity.) In all three cases, the C=O···H–N H-bonds are nonlinear. All rings containing two H-bonds between stacking molecules are 14-membered. In the (all-*S*)-compound **1**, all four 14-membered rings are built from two seven-atom components HNCCCNH and OCNCCCO. In the (*R*,*S*,*R*,*S*)-isomer **2**, four 14-membered rings result from H-bonding between a six-atom OCCCNH and an eight-atom component OCNCCCNH. Finally, in the stacking of the (*R*,*R*,*S*,*S*)-isomer **3**, two (7 + 7) and two (8 + 6) compositions of H-bonded rings occur. For comparison, 14-membered rings of (7 + 7) pattern containing two H-bonds occur in antiparallel β-sheets of α-peptides, and the tubular stacking of cyclo-α-peptides with alternating (*R*)- and (*S*)-configuration of the residues is made of ten- and 14-membered H-bonded rings [27] [28].

pattern; it is simply obscured by the overlap of reflections in 2θ. This makes it difficult, though not impossible [20], to solve a structure using conventional crystallographic methods, because many of the individual reflection intensities cannot be determined. However, the refinement of an approximate structure with powder data is almost routine.

Of course, the information content of a powder pattern is much lower than that of a single-crystal data set because of the reflection overlap, so the precision of the structural parameters is somewhat lower, and the number of parameters that can be refined is more limited. In these cases, for example, the standard deviations on the interatomic distances between non-H-atoms are *ca.* 0.02 Å. Nonetheless, powder diffraction techniques can often provide vital structural information that cannot be obtained in any other way.

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