136. On the Stacking of β-Rings: The Solution Self-Association Behavior of Two Partially *N*-Methylated Cyclo(hexaleucines)

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Cyclo(-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) (**1**) and cyclo(-L-Leu-D-Leu-L-MeLeu-D-Leu-L-Leu-D-Leu-) (**2**) were synthesized and used for a study of the interaction of β-rings in solution. In appropriate solvents, **1** and **2** formed dimers consisting of two β-rings connected through six interannular H-bonds and having the *N*-Me group(s) on the solvent-exposed face. The study also afforded indications that **2** formed different dimers of this kind, differing in the relative orientation of the two β-rings. These experimental observations provide strong support to the idea that unsubstituted D,L-alternating cyclooligopeptides, such as cyclo(hexaleucine), can self-assemble and give rise to long tubular stacks of β-rings.

Introduction. – In 1974, *De Santis* and coworkers [1] predicted that peptide rings formed by alternating D- and L-residues in equivalent β-like conformation (β-rings) should be able to pile up and form multi-ring aggregates with tubular structure. Such aggregates, of which two types are illustrated in *Fig. 1*, are more than a fascinating example of supramolecular architecture. Indeed, they appear to be uniquely suited for a number of special applications (*e.g.*, for mimicking biological channels, effecting separations of ions or molecules, ordering metal atoms in one-dimensional arrays), and they might even usher the development of novel optical and electronical devices. The first reports [2] [3] on synthetic D,L-alternating cyclopeptides do not provide any hint to such aggregates. In 1987, however, work from our laboratory [4] on some D,L-alternating cyclopeptides with 4, 6, or 8 valine, leucine, or phenylalanine residues showed that these cyclooligopeptides, in contrast to open-chain analogues, are remarkably insoluble[2]). This behavior is understandable, if these cyclopeptides have a tubular structure of the types shown in *Fig. 1*. IR Data [4] and the observation [5] of a β-ring-like molecular conformation in crystals of cyclo(hexavaline) and of cyclo(hexaphenylalanine) containing cocrystallized CF_3COOH provided some support for this possibility. Much stronger evidence for a tubular structure in a cyclopeptide, *i.e.*, in cyclo[-(D-Ala-L-Glu-D-Ala-L-Gln)$_2$-], was presented recently by *Ghadiri et al.* [6]. Despite these studies, however, an experimental demonstration of the stacking of β-rings is still lacking.

Aiming at studying this interaction, we considered the use of D,L-alternating cyclopeptides having some or all L(or D)-residues *N*-methylated. We expected that the *N*-Me group(s), which would protrude from one side of the β-rings, would allow a pairing of the

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[2]) The first observations [4] were taken to indicate that cyclo(hexavaline) is insoluble even in CF_3COOH. In fact, cyclo(hexavaline) is soluble in CF_3COOH, but at room temperature the dissolution in this solvent is slow.

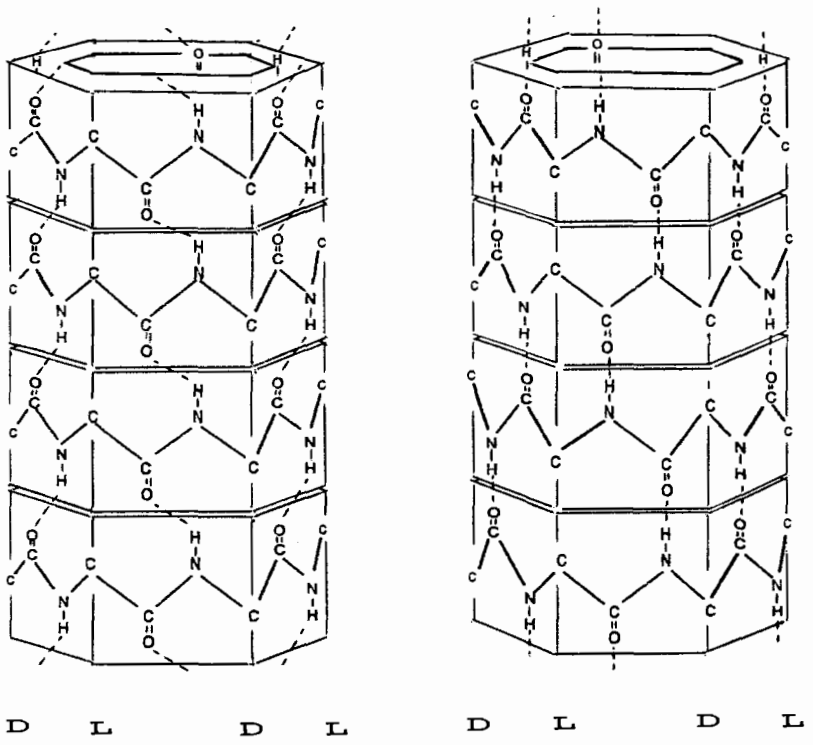

Fig. 1. *Stacks of β-rings with relative parallel* (left) *or antiparallel* (right) *orientation.* A cyclo(hexapeptide) is considered and the rings are represented by hexagonal, nut-like elements. For sake of clarity, only the atoms and bonds of the peptide backbone and the interannular H-bonds (---) are shown. The configurations of the C(α)'s (on the edges) are indicated at the bottom.

rings only through the other side, and we anticipated that the resulting two-ring stacks would be soluble enough to permit crystallization experiments and solution studies. In the present approach, we used two partially *N*-methylated analogs of D,L-alternating cyclo(hexaleucine), namely cyclo(-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-) (**1**) and cyclo(-L-Leu-D-Leu-L-MeLeu-D-Leu-L-Leu-D-Leu-) (**2**). In the case of **1**, we were able to determine the crystal structure [7] and to observe that the cyclopeptide forms indeed pairs of β-rings connected through their non-methylated face by six interannular NH···OC bonds. Here, we report and discuss results of a solution study of **1** and **2** that we carried out using IR and ^{1}H-NMR measurements. This work was presented already in preliminary form [8].

Experimental. – *General.* M.p.: *Mettler DSC 30.* IR Spectra: *Nicolet-5SXC Fourier*-transform IR spectrometer; in cm^{-1}. ^{1}H-NMR Spectra: *Bruker AMX 400* or *AMX 500* operating at 400.13 and 500.13 MHz, resp.; δ in ppm rel. to Me_4Si; individual assignment of the NH and *H*–C(α) signals of **2**, **6**, and **7** by COSY and NOESY; a β-type conformation and (*Z*)-configuration of the *N*-methylated peptide bond were assumed, and the NOE cross-peaks observed between *H*–C(α)'s and N*H*'s and between a *H*–C(α) and the *Me*N were attributed to protons of two

contiguous residues (*i* and *i* + 1) in the sequence, which yielded consistent results. 1D NOE Difference spectra of **1**: *Bruker AM-300-WB*; in CCl_4/C_6D_{12} at 25°: Molecular weight of **1**: vapor-pressure osmometry using a *Wescan-232-A* instrument; in $CHCl_3$ and CCl_4 at 25°; calibration with azobenzene. FAB-MS: *VG-ZAB2-SEQ* instrument.

*Cyclo(-*D*-Leu-*L*-MeLeu-*D*-Leu-*L*-MeLeu-*D*-Leu-*L*-MeLeu-)* (**1**). A soln. of 0.54 g (0.73 mmol) of H-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-OH (**3**; see below) in 200 ml of dimethylformamide (DMF; *Fluka, puriss.*, amine-free) was added dropwise in 2 h to a vigorously stirred, cold (0–4°) soln. of diphenylphosphoryl azide [9] (DPPA; 0.70 g, 2.5 mmol) and *N*-methylmorpholine (NMM; 0.25 g, 2.5 mmol) in 800 ml of DMF. After 2 days, the solvent was evaporated, the residue triturated in hexane, and the product collected by filtration, washed with hexane, and dried: 314 mg (60%) of **1**. The product was purified by recrystallization from MeOH. M.p. 289°. ^{1}H-NMR ($CDCl_3$, 20.3 mg/ml, 25°): 7.37 (*d*, 3 NH); 4.90 (*m*, 3 H–C(α) (L)); 4.85 (*m*, 3 H–C(α) (D)); 3.01 (*s*, 3 MeN); 1.70 (*m*, 3 $CH_2(\beta)$ (L)); 1.61 (*m*, 3 H–C(γ) (L)); 1.48 (*m*, 3 $CH_2(\beta)$ (D)); 1.39 (*m*, 3 H–C(γ) (D)); 1.00, 0.91 (2*m*, 6 Me(δ) (L)); 0.95, 0.89 (2*m*, 6 Me(δ) (D)). FAB-MS: 721.5 ($[M + H]^+$).

Peptide **3** was prepared by saponification of Boc-D-Leu-L-MeLeu-D-Leu-L-MeLeu-D-Leu-L-MeLeu-OMe (**4**; see below) and removal of the Boc group from the intermediate Boc-peptide acid: ^{1}H-NMR ($CDCl_3$, 20 mg/ml, 25°): 7.18, 6.83 (2*d*, 2 NH); 5.40–4.85 (3*m*, 5 H–C(α), 1 NH); 4.61 (*m*, 1 H–C(α)); 3.02 (*s*, 2 MeN); 2.95 (*s*, 1 MeN); 1.90–1.20 (*m*, 6 $CH_2(\beta)$, 6 H–C(γ)); 1.44 (*s*, Boc); 1.10–0.85 (*m*, 12 Me(δ)).

Peptide **4** (oil) was obtained starting from Boc-D-Leu-OH and from Boc-L-MeLeu-OMe [10]: First Boc-D-Leu-L-MeLeu-OMe (oil) was prepared, and from this, Boc-D-Leu-L-MeLeu-OH and H-D-Leu-L-MeLeu-OMe were obtained and coupled to yield Boc-D-Leu-L-MeLeu-D-Leu-L-MeLeu-OMe (oil). The latter was *N*-deprotected, and H-D-Leu-L-MeLeu-D-Leu-L-MeLeu-OMe was coupled with Boc-D-Leu-L-MeLeu-OH to give **4**. Boc-D-Leu-L-MeLeu-OH: M.p. 142° (from hexane). ^{1}H-NMR ($CDCl_3$, 12.0 mg/ml, 25°): 9.85 (br. *s*, COOH); 5.35 (*d*, NH); 5.13 (*m*, H–C(α.2)[3])); 4.70 (*m*, H–C(α.1)); 3.04 (*s*, MeN); 1.85–1.40 (2*m*, 2 $CH_2(\beta)$, 2 H–C(γ)); 1.42 (*s*, Boc); 0.98–0.88 (*m*, 4 Me(δ)).

*Cyclo(-*L*-Leu-*D*-Leu-*L*-MeLeu-*D*-Leu-*L*-Leu-*D*-Leu-)* (**2**). To a vigorously stirred, cold (0–4°) soln. of 0.71 g (1.1 mmol) of H-L-Leu-D-Leu-L-MeLeu-D-Leu-L-Leu-D-Leu-OH (**5**; see below) in 1000 ml of DMF, 0.82 g (2.9 mmol) of DPPA and 0.30 g (2.9 mmol) of NMM were added (→precipitation). After 2 days, the volume of the suspension was reduced to *ca.* 50 ml by evaporation and the product obtained collected by filtration and purified by washing with DMF and Et_2O. Drying gave 490 mg (71%) of **2**. M.p.: no melting up to 350°. ^{1}H-NMR ($CDCl_3$/CF_3COOH 45:1 (*v*/*v*), 11.0 mg/ml, 25°): 8.21 (*d*, NH(2)); 8.06 (*d*, NH(1)); 7.97 (*d*, NH(6)); 7.86 (*d*, NH(4)); 7.67 (*d*, NH(5)); 4.93 (*m*, H–C(α.2)); 4.92 (*m*, H–C(α.3)); 4.38 (*m*, H–C(α.1), H–C(α.4)); 4.31 (*m*, H–C(α.5)); 4.29 (*m*, H–C(α.6)); 3.14 (*s*, MeN); 1.75–1.30 (*m*, 6 $CH_2(\beta)$, 6 H–C(γ)); 0.96 (*m*, 12 Me(δ)).

Peptide **5** was prepared by saponification of Boc-L-Leu-D-Leu-L-MeLeu-D-Leu-L-Leu-D-Leu-OMe (**6**; see below) and removal of the Boc group from the intermediate Boc-peptide acid. Peptide **6** was obtained from Boc-D-Leu-L-MeLeu-D-Leu-L-Leu-D-Leu-OMe (**7**) by deprotection and subsequent coupling with Boc-L-Leu-OH. Analogously, **7** was synthesized from Boc-L-MeLeu-D-Leu-L-Leu-D-Leu-OMe [11] (a gift of *D. U. Römer*) and Boc-D-Leu-OH.

6: M.p. 176° (from $CHCl_3$/hexane). ^{1}H-NMR ($CDCl_3$, 14.2 mg/ml, 25°): 7.10 (*d*, NH(6)); 7.02 (*d*, NH(2)); 6.84 (*d*, NH(4), NH(5)); 5.32 (*d*, NH(1)); 5.09 (*m*, H–C(α.3)); 4.73 (*m*, H–C(α.2)); 4.58 (*m*, H–C(α.6)); 4.50 (*m*, H–C(α.5)); 4.28 (*m*, H–C(α.4)); 4.22 (*m*, H–C(α.1)); 3.71 (*s*, MeO); 2.94 (*s*, MeN); 1.46 (*s*, Boc); 1.58–1.50 (*m*, 6 $CH_2(\beta)$, 6 H–C(γ)); 0.96–0.87 (*m*, 12 Me(δ)).

7: M.p. 191° (from $CHCl_3$/hexane). ^{1}H-NMR ($CDCl_3$, 16.2 mg/ml, 25°): 7.13 (*d*, NH(5)); 6.82 (*d*, NH(3)); 6.45 (*d*, NH(4)); 5.32 (*d*, NH(1)); 5.21 (*m*, H–C(α.2)); 4.56 (*m*, H–C(α.5)); 4.49 (*m*, H–C(α.1), H–C(α.4)); 4.16 (*m*, H–C(α.3)); 3.70 (*s*, MeO); 2.97 (*s*, MeN); 1.42 (*s*, Boc); 1.58–1.50 (*m*, 5 $CH_2(\beta)$, 5 H–C(γ)); 0.98–0.88 (*m*, 10 Me(δ)).

The preparation and purification of the linear intermediates were carried out by standard methods [11] [12]. The purity of the products was checked by TLC.

Results and Discussion. – In contrast to cyclo(hexaleucine) which is soluble only in the presence of strong acids [4], **1** is soluble even in such solvents as $CHCl_3$ and CCl_4. At 25° in $CHCl_3$, **1** has a molecular weight (718 at 6.70 mg/ml) very close to that of the monomer

[3]) Here and in the following, the number in parenthesis indicates the residue to which the group belongs. The residues of the linear peptides are numbered consecutively starting from the N-terminal residue. The residues of **2** are numbered starting from the L-Leu residue which is at the N-terminus in the linear precursor **5**.

(721.05) and, under similar conditions, it yields ^{1}H-NMR spectra (see *Exper. Part*) showing no significant concentration dependence (investigated range: 2–24 mg/ml). At −100° in $CD_2Cl_2/CDCl_3$ 2:1 (*v/v*), **1** yields ^{1}H-NMR spectra with signals essentially at the same positions as at 25°. Some additional signals, possibly from an aggregate, are present, but the relative intensity of these signals is very weak (< 5%). Clearly, in these solvents, rings of **1** do not have much tendency to associate.

In CCl_4 and in CCl_4/C_6D_{12} mixtures, on the other hand, **1** displays properties which are markedly dependent on concentration. *E.g.*, upon increasing the concentration, the apparent molecular weight of **1** in CCl_4 increases (from 826 at 0.77 mg/ml to 1003 at 6.20 mg/ml), and the lower-frequency NH-stretching band (at 3309 cm^{-1}), which accompanies the band at 3360 cm^{-1} observable also with $CHCl_3$ solutions (*Fig. 2*), becomes more

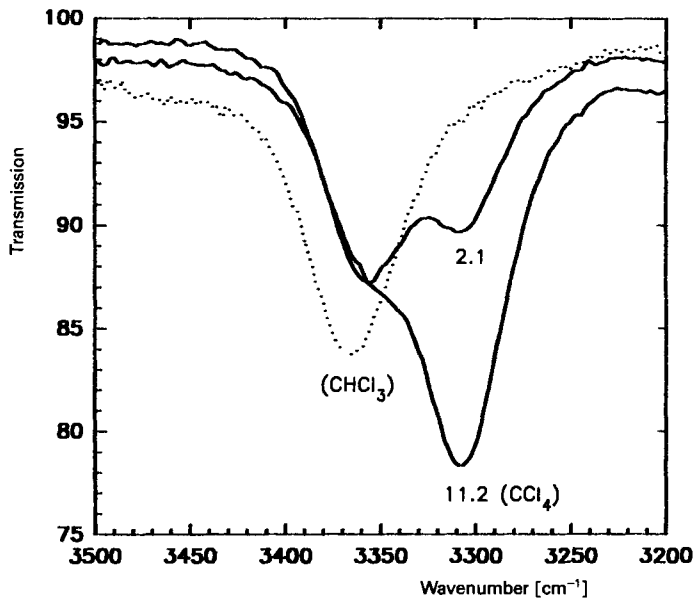

Fig. 2. *IR Spectra* (amide-A region) *of* **1** *in* $CHCl_3$ (···; 10.3 mg/ml) *and in* CCl_4 (–; concentrations indicated in mg/ml): $T = 25°$.

intensive. These indications of an association process through H-bonding are corroborated by NMR results showing (*Fig. 3*) a strong downfield shift of the signal of the 3 NH protons upon increasing the concentration. 1D NOE Difference spectra of **1** in CCl_4/C_6D_{12} (15.7 mg/ml) reveal a small NOE (3%) between the *NH* and upfield (5.35 ppm) *H*−C(α) resonances and another small NOE (2%) between the downfield (5.00 pm) *H*−C(α) and *Me*N resonances. These *Overhauser* effects and the values found for the coupling constants *J*(N*H*,*H*−C(α)) (6–8 Hz at 25°) suggest that there is a certain abundance of β-rings in the system. Recent X-ray work [7] showed that crystals of **1** (from MeOH) are made up of pairs of β-rings facing each other with their non-methylated face and connected by six interannular NH···OC bonds (*Fig. 4*). Thus, it seems likely that the

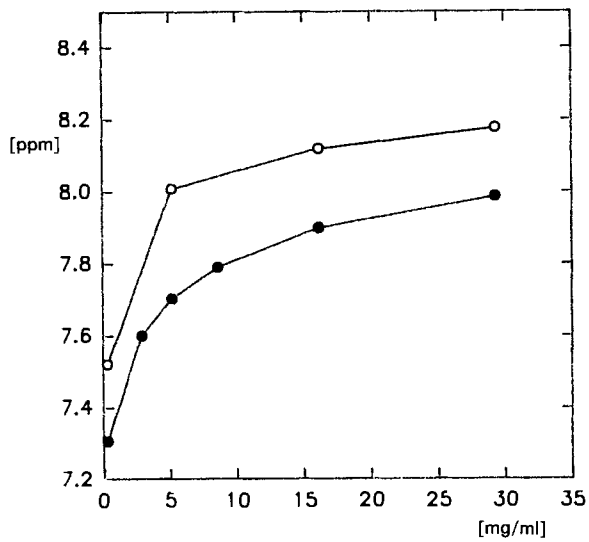

Fig. 3. *Concentration dependence of the chemical shifts of the NH protons of* **1** *in* CCl_4/C_6D_{12} *6:1 (v/v) at 5° (○) and at 25° (●)*

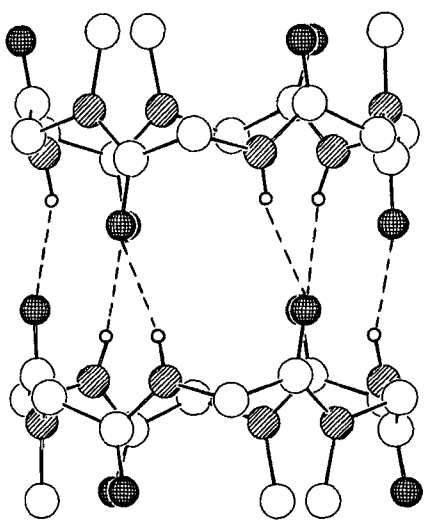

Fig. 4. *'Stick and ball' model of a dimer of β-rings of* **1** *as found by X-ray analysis* [7]. For the sake of clarity, the side chains are omitted; ○ = C-atoms, ○ = H-atoms, ◍ = N-atoms, and ◍ = O-atoms; --- = H-bonds between CO and NH of the D-residues.

behavior of **1** in the apolar solvent(s) used reflects a dimerization equilibrium involving dimers of β-rings connected to one another as in the crystalline state. Nonlinear curve-fitting to the lower set of data in *Fig. 3* would give for the equilibrium constant at 25° a value of *ca.* 80 M^{-1}.

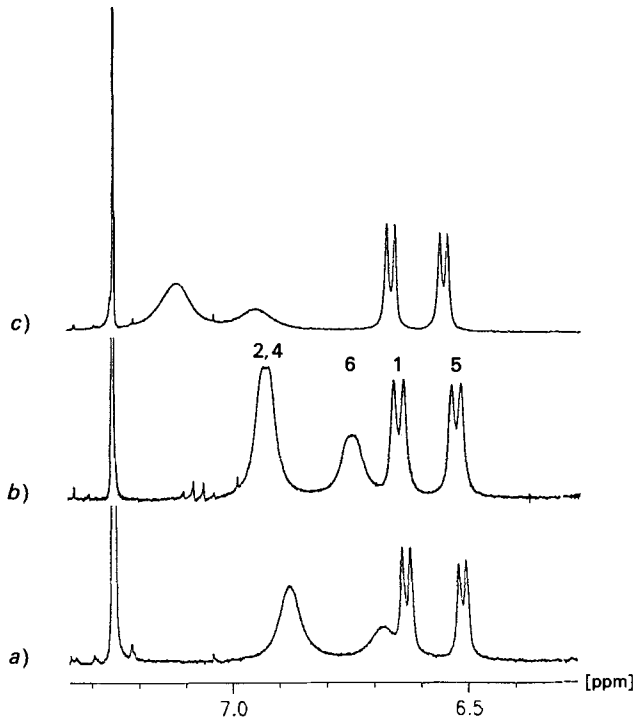

Fig. 5. *NH region of the ^{1}H-NMR spectrum of* **2** *in $CDCl_3/CF_3CH_2OH$ 13:1 (v/v) at different concentrations at 57°. a)* 3.0 mg/ml, *b)* 5.0 mg/ml and *c)* 10.0 mg/ml; the individual signals were assigned for *b*: 1 (NH(1)) and 5 (NH(5)) from L-residues, 2 (NH(2)), 4 (NH(4)); and 6 (NH(6)) from D-residues (for numbering, see *Footnote 3*).

In $CDCl_3$ containing some CF_3CH_2OH ($CDCl_3$ alone is not a good enough solvent for **2**) in the investigated temperature range (25°–57°), **2** exhibits for its 5 NH protons 2 sharp *d*'s ($J(NH,H-C(\alpha)) = 7.5$–8.5 Hz) and 2 broad signals of which one corresponding to 2 H (*Fig. 5*). The *d*'s are attributed to the L-leucine residues; they are the highest-field signals, and they move only slightly when changing the concentration. The broad signals, assigned to the NH's of the D-residues, are much more dependent on concentration: they are shifted downfield upon increasing the concentration, indicating that these NH's are involved in intermolecular H-bonds in some aggregation process. These are clearly features to be expected, if there is a dimerization equilibrium of the same type as suggested above for **1** in which only the NH protons of the D-residues contribute to the interannular H-bonding. The broadness of the signals of the NH's of the D-residues in *Fig. 5* seems to be another manifestation of this equilibrium. In fact, in the case of **2**, one can expect that there are 3 different dimers (*Fig. 6*) besides the monomer contributing to the NMR spectrum, and the interconversion among these species under the conditions used is possibly not very fast. Consistent with this interpretation, preliminary measurements reveal a progressive splitting of the broad NH signals of **2** between −20 and −40° upon lowering the solution temperature.

The structural features of the dimers which appear to be formed by **1** and **2** in solution reproduce well those of a two-ring element in a stack of antiparallel β-rings (*Fig. 1*). The

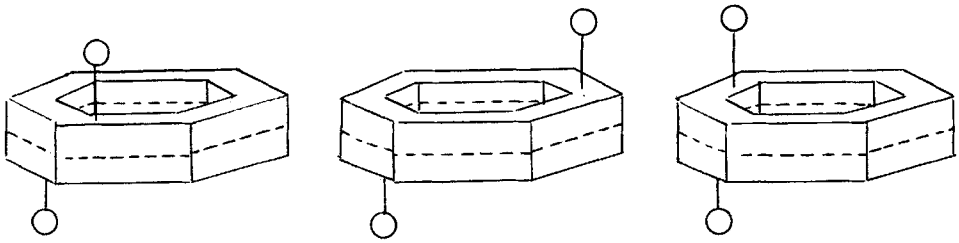

Fig. 6. *Schematic representation of the three different dimers of β-rings of* **2** *showing the relative position of the MeN groups* (○)

fact that these dimers can exist in solution provides strong support to our idea that the insolubility of the unsubstituted D,L-alternating cyclooligopeptides studied earlier [4] is the consequence of a much more extensive aggregation to long tubular structures.

The study of the interaction of **1** and **2** in solution with ions and with H_2O and other neutral molecules is planned.

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