Tetrahedron Vol. 49, No. 47, pp 10831-10842, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00
Pergamon Press Ltd

Parallel β-Sheet Conformation in Macrocycles

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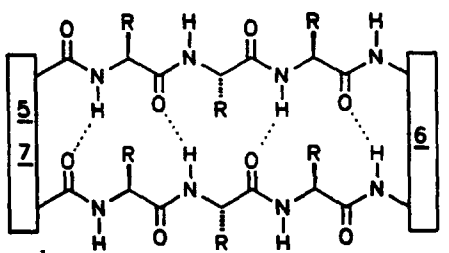
(*Received in Germany* 9 *August* 1993; *accepted* 24 *September* 1993)

Abstract: Amino acids (Val, Phe) are combined with two rigid spacers phenoxathiin-4,6-dicarboxylic acid (5) and 2,8-dimethyl-4,6-bis(aminomethyl)phenoxathiin-10,10-dioxide (6) to synthesize the cyclic structures 1 (5-Val/Val-6) and 2 (5-Phe/Phe-6). The diacid and diamino spacers 5 and 6 provide a distance between the attached short peptides which allows hydrogen bonding similar to that found in a parallel β-sheet. The β-sheet conformation of 1 and 2 is proved by NMR measurements at low temperatures. The derived dihedral angles and NOE distances are compared to the most stable conformations of 1 calculated with MM3 and AM1.

Introduction

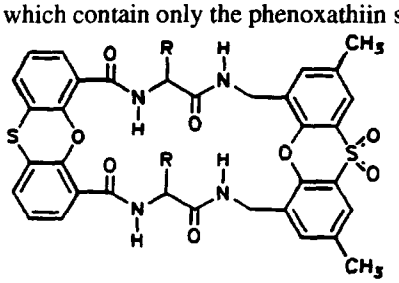
The structural motifs found in proteins are β-sheets, α-helices and β-turns. Several research groups have tried to induce such structures also in smaller units, as in cyclopeptides[1] or peptides containing additional conformationally rigid structures.[2-4] Surrogates for the amino acids are investigated in this context,[2] but also completly artificial spacer units were designed to induce helical[3] or β-sheet conformations.[4] The interest in this research is enhanced by the biological activity of structural parts of peptide hormones,[5] antibiotics[6] or models of active proteins.[7] It is surprising that in all of these studies no attempt has been made to induce the conformation of a *parallel* β-sheet. Here, we report on cyclic structures where two short peptide chains, fixed between a diacid- and a diamino-compound, are able to adopt the hydrogen bonding pattern of a parallel β-sheet.

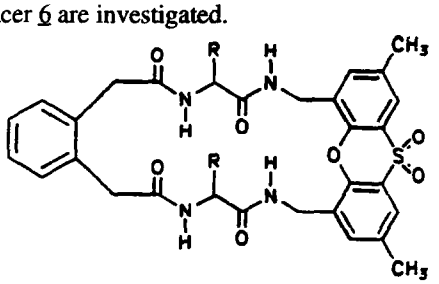


Scheme 1

A schematically drawn model of a parallel β-sheet is shown in scheme 1. Two peptide chains are held together by spacer units. Dihydroanthracene compounds substituted in 4,6-position with diamino- or dicarboxylate functions will provide in models the appropriate distance for hydrogen-bonding in the attached peptides

We report on the realization and conformational analysis of such structures, compounds 1 and 2 (scheme 2). Phenoxathiin derivatives 5 and 6 serve here as spacers. In addition the conformations of 3 and 4 which contain only the phenoxathiin spacer 6 are investigated.





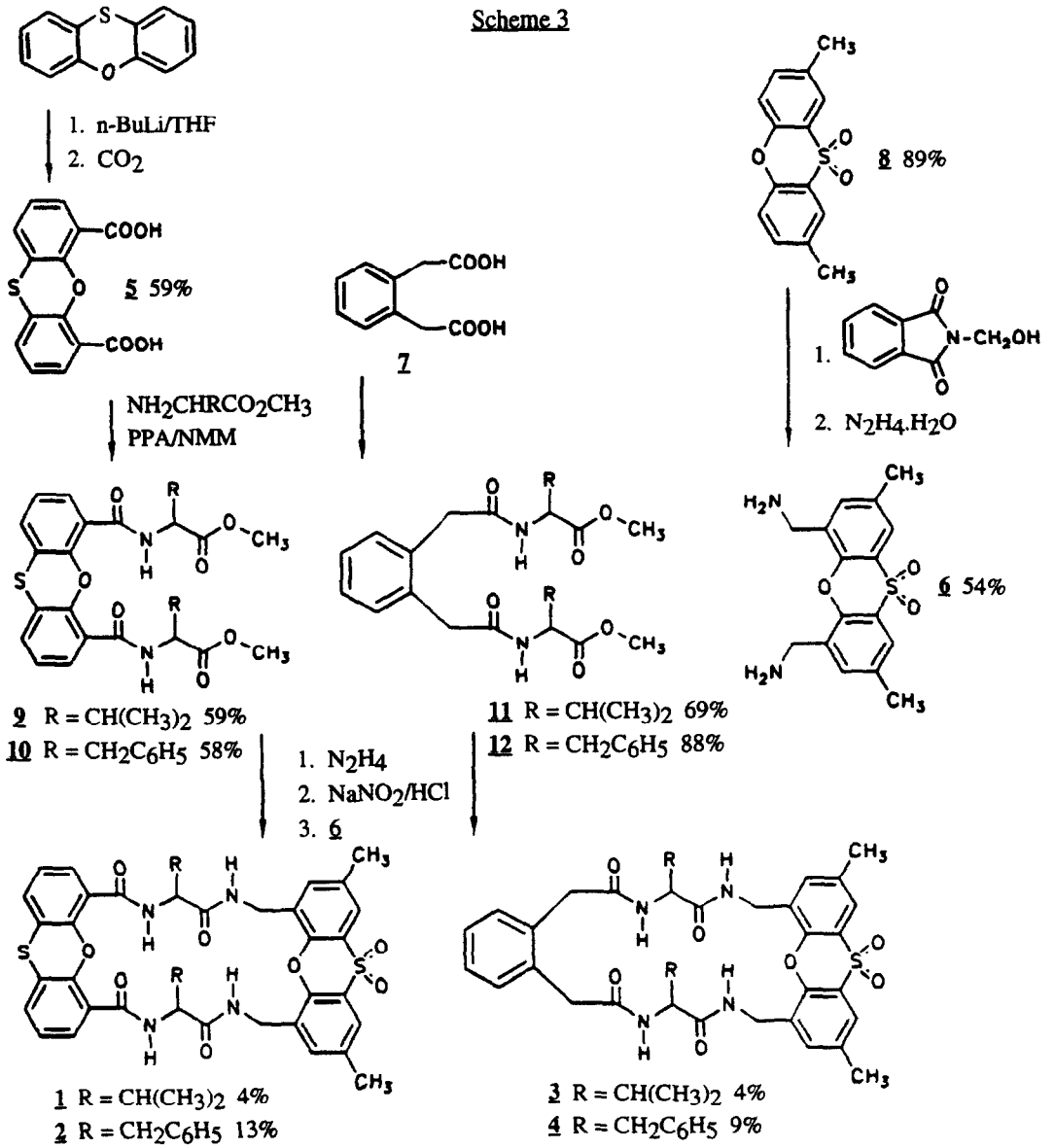
1: R = $CH(CH_3)_2$ 2: R = $CH_2C_6H_5$ 3: R = $CH(CH_3)_2$ 4: R = $CH_2C_6H_5$

Scheme 2

Synthesis

The spacer 5 is obtained from phenoxathiin[8] by deprotonation with n-butyllithium and quenching with solid CO_2[9] (scheme 3). The diamino compound 6 is prepared from 2,8-dimethylphenoxathiin-10,10-dioxide (8) by substitution with hydroxymethylphthalimide,[10] followed by reaction with hydrazine.[11] The methylesters of valine and phenylalanine[12] were attached to 5 and 7 with standard methods.[13] the cyclisation with 6 to 1, 2, 3 and 4 was successful with the azide method in low yields.[14] The macrocycles were purified by preparative TLC. Their molecular mass was proved by DCI mass spectrometry.

Scheme 3



Conformational analysis

Two identical peptide chains, held together by the described spacer molecules 5, 6 and 7, will be chemically different if they build a parallel β-sheet as shown in figure 1.

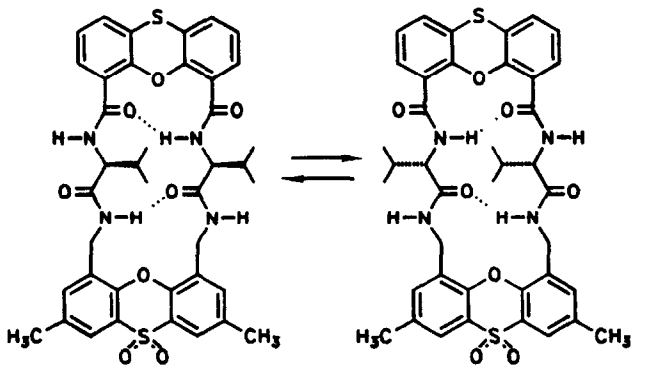


Figure 1: Degenerate exchange proposed for the parallel β-sheet conformation of 1.

However, the process shown in figure 1 is only observable by NMR techniques, if the interconversion between the two forms is not too fast. The NMR spectra of the macrocycles 1 and 2 show at temperatures below 0°C the distinct signals of two different peptide chains (figure 2, similar spectra are observed for compound 2). Coalescence of signals is seen at higher temperatures.

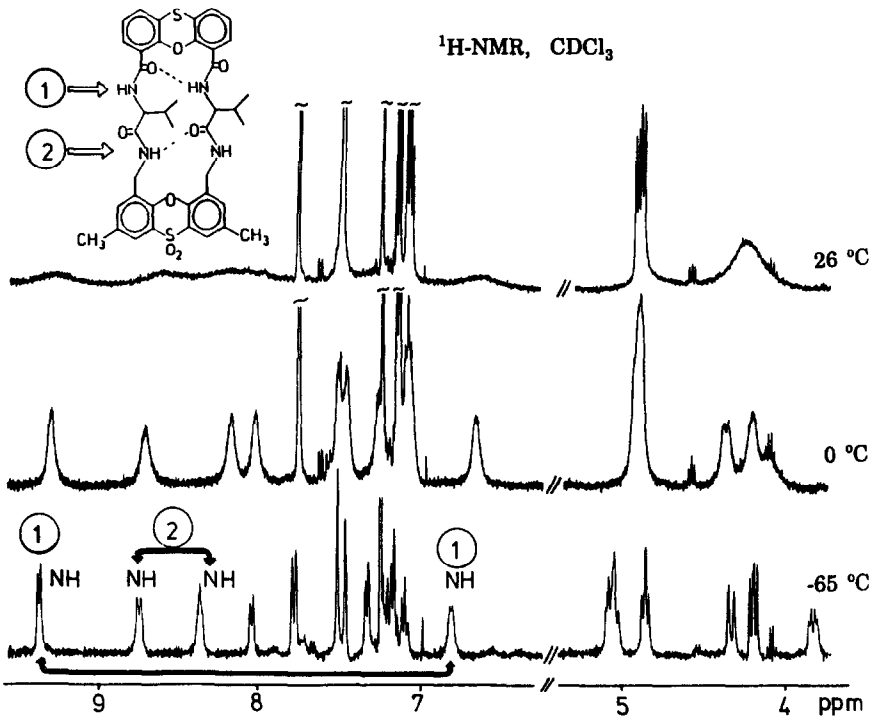


Figure 2: Parts of the 400 MHz ^{1}H-NMR spectrum of 1 in $CDCl_3$ at various temperatures. Two different peptide chains are observed below 0°C; the NH-signals are marked.

Standard NMR techniques (COSY, ROESY)[15] were used to analyze the signals of 1 and 2 confirming that two different peptide chains are present at low temperature.[16] In contrast, the macrocycles 3 and 4 exhibit sharp signals at room temperature which broaden at very low temperatures (-40°C and -60°C resp.) but never split into two sets.

Whereas the exchange phenomenon shown in figure 1 is in accordance with the NMR data, the conformation of the different peptide chains in 1 or 2 remains unknown. A few NMR data may serve as indicators. The coupling constants $J_{NH\text{-}\alpha H}$ are measured to 7.6 and 8.0 Hz indicating trans orientation at the dihedral angle ϕ. The ROESY spectrum of 1 (fig. 3) contains some conformational significant cross peaks. The connection I between an aromatic-H and one of the valine NH protons determines the local orientation between one of the peptide chains and the spacer 5. The NOE II from the α-H of one valine to the β-H of the corresponding residue in the other chain is only possible if a geometry as in figure 4 is maintained. There, one of the α-protons is directed to the inside of the ring, the other one to the outside. The connections III and IV indicate an U-type arrangement of NH- and α-proton within the peptide chains. All these informations support a β-sheet conformation as shown in figure 4. However, additional information is needed to support the existence or dominance of one specific conformation. Calculations may serve for this purpose.

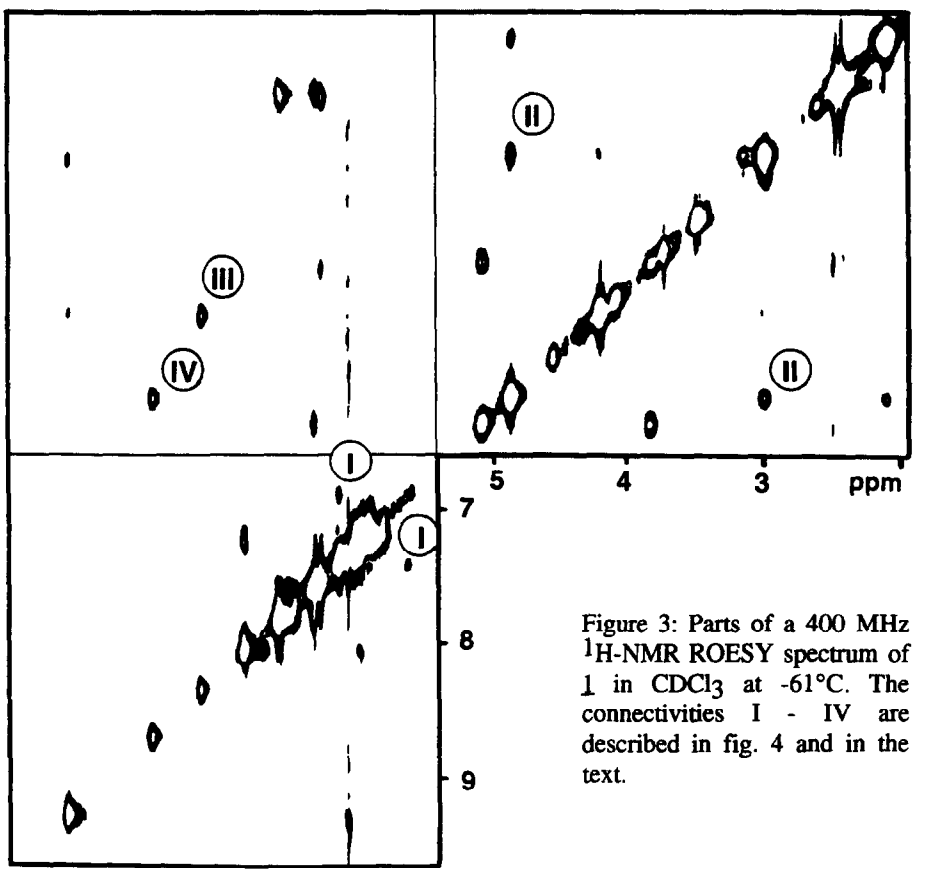


Figure 3: Parts of a 400 MHz ^{1}H-NMR ROESY spectrum of 1 in $CDCl_3$ at -61°C. The connectivities I - IV are described in fig. 4 and in the text.

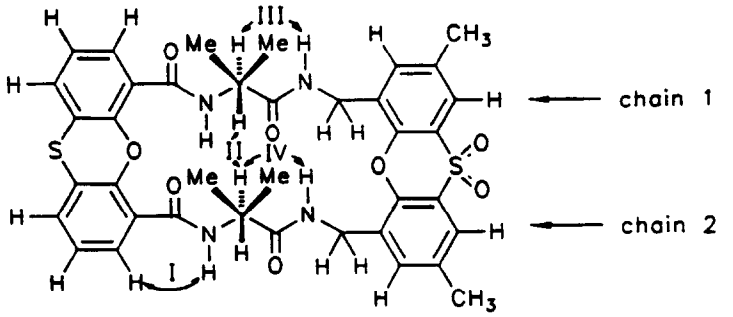


Figure 4: Conformational relevant NOE connectivities I - IV of 1.

A systematic search of all possible ring conformations of 1 was performed by Still's method.[17] For this purpose, the dihedral angles of six conformationally relevant backbone bonds in 1 were rotated systematically in increments of 60°. 27 ring structures were found and optimized with the MM3 force field[18] (a few new parameters were added to the force field to describe the phenoxathiin residue, see ref. 16). Only four of these structures were found in a narrow region of low energy (ΔΔE <6 kcal/mol). All other structures are associated with energies at least 20 kcal/mol higher than the global minimum found. The four structures were used as starting points for a systematic rotation of the valine side chains to get all possible staggered conformations (4 x 9). The subsequent force field optimizations yield 36 structures within a range of 12 kcal/mol. All geometries belong to only two different backbone conformations. The two structures of lowest energy of each class are shown in figure 5.

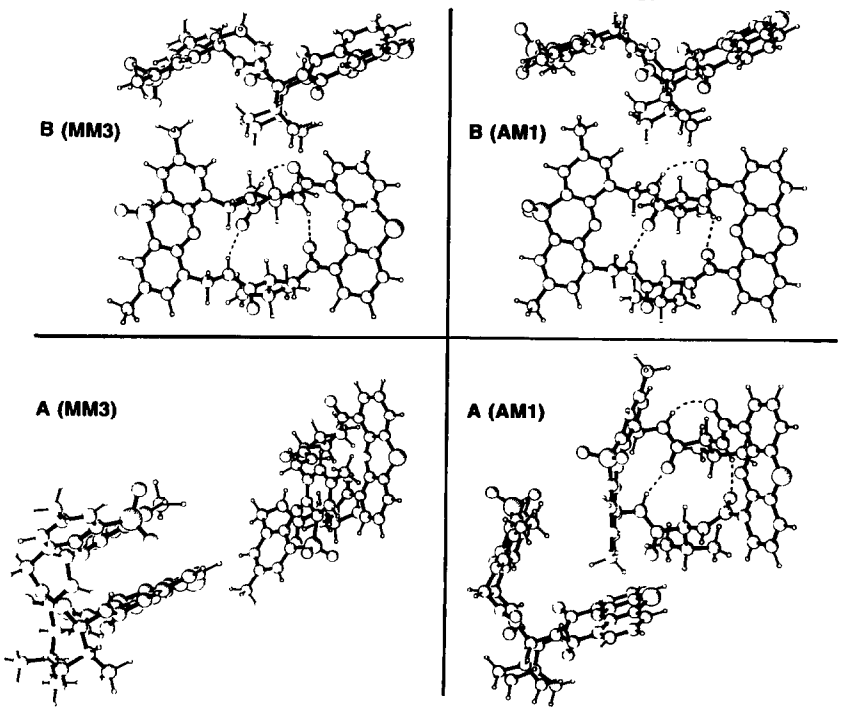


Figure 5: Low energy structures A and B of 1 calculated with MM3 and AM1.

Both forms, A and B in figure 5, contain the hydrogen bonding pattern of a parallel β-sheet. In addition, form B has developed one γ-loop in one of the chains. The form A is obviously stabilized by intramolecular Coulomb and van der Waals attractions which result in a very compact folded geometry. Especially the aromatic units are packed very tightly. Such globular structures are often seen if force field programs calculate flexible polar molecules in the gas phase. The energy difference between the forms A and B decreases when MM3 calculations are performed with increasing dielectric constant of the solvent (table 1).

Table 1: Relative MM3-energies (kcal/mol) of the forms A and B (fig. 5) calculated with different dielectric constants (ε).

geometry	ε	1.5	2.0	2.5	5	10	20	40	80	AM1
A	ΔHf(rel.)	0	0	0	0	0	0	0	0	0
B	ΔHf(rel.)	6.02	4.9	4.4	3.05	3.7	4.13	4.38	4.52	0.3

Structure B is nearly identical when calculated with MM3 or AM1. A(AM1) differs from A(MM3) (see text). The AM1-energies are given in the last column (gasphase).

The energy difference vanishes, if the semiempirical program AM1[19] is used to optimize the structures A and B (see table 1). However this comparison is not completely correct because the AM1 optimized structure of A differs from the original MM3 geometry. An additional H-bond is formed in one chain and the packing of the two aromatic spacers is somewhat released (see figure 5).

In summa, the calculations strongly support two basic structures (A and B) for the ring conformation of 1. The force field and semiempirical calculations are not sufficient to decide which form is energetically preferred in solution. However, the geometries of A and B may serve as the base to discuss the conformational conclusions of NMR experiments. The conformationally relevant dihedral angles in A and B are compared with the experimental values in table 2. The data of a NMR spectrum of 1 in $CDCl_3$ at -61°C are used in the Karplus analysis.[20]

Table 2: Dihedral angles ϕ (NH-CHα) of different geometries of 1 calculated with MM3 and AM1, corresponding coupling constants and experimental values $^3J_{NH\text{-}CH\alpha}$.

chain	dihedral angle	calculated angle / coupling constants (Hz)[a]				experiment[c]
	ϕ	A(MM3)	A(AM1)[b]	B(MM3)	B(AM1)	J(exp)
1	HN-CHα	143.4 / 7.0	160.9 / 9.0	147.1 / 7.5	158.8 / 8.6	8.0
1	HN-CH_2 (pro R)	2.1 / 9.5	30.0 / 7.4	161.8 / 8.8	176.6 / 9.6	<2
1	HN-CH_2 (pro S)	118.8 / 2.3	147.6 / 7.2	-81.0 / 0.5	-66.0 / 1.6	<2
2	2HN-CHα	-160.2 / 8.8	-147.2 / 7.2	-155.4 / 8.8	-149.7 / 8.2	7.6
2	HN-CH_2 (pro R)	94.6 / 0.6	80.2 / 0.6	-137.0 / 8.5	-144.8 / 7.4	6.8
2	HN-CH_2 (pro S)	-148.5 / 7.5	-163.0 / 9.2	-20.8 / 5.6	-27.7 / 7.5	6.8

a) The graphical Karplus equation[20] permits deviations of up to 0.8 Hz from the given values. b) AM1 geometries were obtained using the MM3 forms of A and B as starting point and optimizing all coordinates. Whereas B(AM1) is identical in shape to B(MM3) the geometries of A (MM3) and A(AM1) differ. c) Errors in ^3J(exp.) are approx. ±0.5 Hz.

The experimental coupling constants are completely reproduced only by structure B. The NH-CH_2 (pro-R) value (chain 2) is outside of the experimental range in the forms A(MM3) and A(AM1). This is supported by the NOE data in table 3.[21] The conformational characteristic short distances I - IV (see fig. 4) are reproduced by the structure B and by A(AM1). The U-type connection III does not exist in A(MM3). The NOE data compiled in table 3 are alone not conclusive. However, together with the dihedral information of table 2 the occurrence of the form B is manifested.

Table 3: Internuclear distances (Å) derived from NOE data[a)] and corresponding values in calculated structures of 1.

NOE[b)]	A(MM3)	A(AM1)	B(MM3)	B(AM1)	exp.[c)]
I	2.08	2.50	2.23	2.49	2.4
II	2.29	2.44	2.49	2.48	3.0
III	3.43	2.40	2.46	2.42	2.4
IV	2.27	2.31	2.24	2.31	2.5

a) Other observed NOE cross signals correspond also to short distances in A and B but are not conformational relevant. b) See figure 4. c) Error limits estimated to ±0.3Å.

Outlook

The experimental and theoretical data presented in this paper let us conclude that the spacer 5 induces in two attached parallel peptide chains a conformation which resembles the arrangement of a parallel β-sheet. This behaviour is observed in the compounds 1 and 2 in solution. Obviously, the hindered aryl-CO bond rotation in 5 contributes to the kinetic stability of the β-sheet. If the aryl-CO groups in 5 are replaced by aryl-CH_2-CO units, the two chains are not observed to be different anymore in the temperature region between 0 and -20°C.[16]

The question is open what geometry will be induced if the peptide chains are elongated. First attempts are made by the synthesis of the cycles 5-(ValPhe/ValPhe)-6 and 5-(PheVal/PheVal)-6. Their NMR spectra are characterized by a dynamical behavior similar to that found in 1.[16]

The way is open for the construction of artificial peptidic surfaces based on the spacer 5 or similar units. The side chains of the amino acids should be located here in defined chiral positions. The structures can be used in many fields of chemistry where preorganization of groups, functions and charges is desired.[22]

Experimental section

NMR spectra and distance calculations: ^{1}H-NMR spectra at 400 MHz (COSY and ROESY) were recorded on a Jeol-GX400 instrument. The 400MHz-ROESY spectrum in figure 3 serves as the basis of the distance evaluation. The spectrum was obtained on a degassed sample of 1 (6mg in 0.8 ml $CDCl_3$) at -61°C. The following measuring parameters were used: Pulssequency: 90°-Cw-spinlock $(32°\text{-}\tau)_x$-FID, 90°-puls = 20 µsec, 32°-puls = 7µsec, τ =-70 µsec, x = 4866 adding up to a total mixing time of 375 msec. The lock field of 1.25 kHz strength was centered at 5.2 ppm; spectral width 4.4 kHz; 2K points in f2, 128 f1 transients (16 scans each) zero filled to 512 data points in f1. The 2D matrix was transferred to an Iris-INDIGO workstation and processed with the FELIX 2.0 software.[23]

An exponential window function (lb = 0.73Hz) was used in f2; f1 was multiplied by a sine square function shifted by 90° (zero filling to 2K). The first 2 data points in f1 were constructed by linear prediction. The ratios sij (= Iij/Iii) of cross to diagonal peak volume integrals were divided by $\sin^2\alpha$ to correct for off resonance effects according to A. Bax[23] (α is the off resonance angle of spin j to the lock field). The corrected ratios sij were finally used to obtain distances rij by calibration with the distance ArHArMe (2.98Å) according to rij/rH-Me = (sH-Me/sij)$^{1/6}$. The experimental distances in table 3 are averaged over rij and rji if the volume integrals of both cross peaks are accessible. The correlation of the derived distances (NOE) with distances calculated by force field techniques is relatively good (see table 3) despite the fact that the method described above has severe limitations (isolated spin pair approximation, uniform correlation time, neglect of scalar coupling, referencing to only one "virtual" distance). ROESY spectra with shorter mixing times (100 msec) do not improve the correlation but noise and J-artefacts are significantly enhanced. Cross peaks due to chemical exchange were not observed at -61°C. DCI-mass spectra (direct chemical ionisation) were recorded on a MAT 8222 spectrometer. Melting points are uncorrected.

2,8-Dimethylphenoxathiin-10,10-dioxide (8)
70 ml of glacial acetic acid and 85 ml of hydrogen peroxide (30%) are added to 109 mmol (25 g) of dimethylphenoxathiin[8] giving a precipitate. The mixture is refluxed for 2 h and stirred overnight at room temperature. The white precipitate is separated and washed with water, through more product precipitates from the mother-lye. The combined crude product is dried under vaccuum and recrystallized from acetic acid or methanol giving colourless needles. Yield: 26.1 g (90%); m.p. 178°. ^{1}H-NMR (25°C, $CDCl_3$): 7.9ppm, d, 2H, aryl-H; 7.0ppm, AB, 4H, aryl-H; 2.27ppm, s, 6H, CH_3. Anal. ($C_{14}H_{12}O_3S$) calc. C, 64.59; H, 4.65; found C, 64.43; H, 4.76%.

2,8-Dimethyl-4,6-bis(phthalimidomethyl)-phenoxathiin-10,10-dioxide (8a)
10 mmol (2.6 g) of 8 and 30 mmol of N-hydroxymethylphthalimide[10] are suspended in 40 ml of sulfuric acid (cc), stirred overnight and let stand for 7 days at room temperature. The reaction mixture is poured on ice, the precipitate separated and treated with boiling acetone. Filtration and drying in vacuo gives a colourless powder. Yield: 5.14 g (89%). $C_{32}H_{22}N_2O_7S$. ^{1}H-NMR (DMSO-d_6): 7.9ppm, AA'BB', 8H, aryl-H; 7.8 and 7.5ppm, s, 2H, aryl-H; 5.2ppm, AB, 4H, CH_2; 2.35ppm, s,6H, CH_3. IR (cm^{-1}): 1670 (s), 1350 (s), 1120 (m).

2,8-Dimethyl-4,6-bis(aminomethyl)-phenoxathiin-10,10-dioxide (6)
To a stirred solution of 22.92 mmol (13.25 g) of 8a in 500 ml of ethanol and 350 ml of dioxane are added 250 mmol (12.15 ml) hydrazine hydrate (100%). The mixture is refluxed for 48 h and let stand at room temperature for 12 h, through phthalhydrazide precipitates quantitatively. The separated filtrate is concentrated and diluted with water giving a white precipitate. It is separated and dried under vacuo. Yield: 3.94 g (54%); m.p. 220 - 224°C. ^{1}H-NMR (25°C, DMSO-d_6): 7.5ppm, s, 4H, aryl-H; 4.0ppm, s, 4H, CH_2; 2.5ppm, s, 6H, CH_3; 3.0ppm, s, 4H, NH_2. Anal. ($C_{16}H_{18}N_2O_3S$) calc. C, 60.36; H, 5.7; N 8.8; found C, 59.7; H, 5.8; N, 8.8%. MS: EI (70 eV) m/z(%): 318 (6), 302 (19.4), 301 (100)

Phenoxathiin-4,6-dicarboxylic acid (5)
62.5 mmol (12.5 g) of phenoxathiin[8] are dissolved under nitrogen in 200 ml of tetrahydrofuran, 100 ml of n-butyllithium (1.6 m in hexane) are added and the mixture is stirred for 1 h at -40°. Cooling is removed, the mixture allowed to warm up at room temperature, stirred for 4 h and poured onto an excess of solid carbon dioxide. The residue obtained after evaporation of CO_2 is suspended in water. A yellow product precipitates when the solution is acidified with cc HCl to pH=1. The material is separated and suspended in 600 ml of water. Adding solid sodium hydroxide to pH=10 gives a yellow solution which is filtrated over Celite. By adding cc HCl the purified product precipitates again. It is recrystallized from butanone. Yield: 10.6 g (59%); m.p. 269 - 271°. ^{1}H-NMR (DMSO-d_6): 7.49 - 7.64ppm, m, 4H, aryl-H; 7.23 - 7.19ppm, t, 2H, aryl-H. ^{13}C-NMR (DMSO-d_6): 165.99, 149.32,

129.66, 128.63, 125.2, 124.28, 121.1. Anal. ($C_{14}H_8O_5S$) calc. C, 58.33; H, 2.8; found C, 58.65; H, 2.84%. MS: EI (70 eV) m/z(%): 288 (100).

Coupling phenoxathiin-4,6-dicarboxylic-acid (5) or phenylene-diacetic acid (7) with an amino acid methylester

10 mmol of 5 or 7 and 25 mmol of the amino acid methylester[12] are suspended in dry methylene chloride. The mixture is cooled with ice/NaCl. At -15° 55 mmol of N-methylmorpholine are added at once. The coupling reagent PPA[13] is added dropwise. The mixture is allowed to warm up to room temperature and stirred for 2 days. The solvent is removed under vacuo and the residue is dissolved in ethyl acetate. The solution is washed with aqueous sodium hydrogencarbonate, sodium chloride and sodium hydrogensulfate. The organic layer is separated, dried over $MgSO_4$, filtrated and concentrated. After adding petroleum ether the product precipitates at low temperature.

5-(ValOCH$_3$)$_2$ (9)

Yield: 3.044 g (59%); m.p. 138°C. ^{1}H-NMR (25°C, DMSO-d$_6$): 8.65ppm, d, 2H, -NH; 7.35ppm, d, 2H, aryl-H; 7.4ppm, d, 2H, aryl-H, 7.2ppm, t, 2H, aryl-H; 4.25ppm, m, 2H, α-H; 3.65ppm, s, 6H, -OCH$_3$; 2.2ppm, m, 2H, β-H; 0.9ppm, 2d, 12H, -CH$_3$. Anal. ($C_{26}H_{30}N_2O_7S$) calc. C, 60.68; H, 5.88; N, 5.44; found C, 60.49; H, 5.89; N 5.37%.

5-(PheOCH$_3$)$_2$ (10)

Yield: 3.55 g (58%); m.p. 90 - 92°. ^{1}H-NMR (25°, DMSO-d$_6$): 9.0ppm, d, 2H, -NH; 7.4ppm, m, 4H, aryl-H; 7.15 - 7.25ppm, m, 12H, aryl- and phenox.-H; 4.7ppm, m, 2H, α-H; 3.6ppm, s, 6H, -OCH$_3$; 3.2ppm, m, 4H, -CH$_2$. Anal. ($C_{34}H_{30}N_2O_7S$) calc. C, 66.87; H, 4.95; N, 4.59; found C, 66.48; H, 5.33; N, 4.59%.

7-(ValOCH$_3$)$_2$ (11)

Yield: 2.912 g (69%); m.p. 110°C. ^{1}H-NMR (25°C, DMSO-d$_6$): 8.4ppm, d, 2H, -NH; 7.25 - 7.1ppm, AA'BB', 4H, aryl-H; 4.15ppm, m, 2H, α-H; 3.6ppm, s, 6H, -OCH$_3$; 3.35ppm, s, 4H, -CH$_2$; 2.0ppm, m, 2H, β-H; 0.85ppm, 2d, 12H, -CH$_3$ Val. Anal. ($C_{22}H_{32}N_2O_6$) calc. C, 62.84; H, 7.67; N, 6.66; found C, 62.44; H, 7.67; N, 6.64%.

7-(PheOCH$_3$)$_2$ (12)

Yield: 4.54 g (88%); m.p.: 114°C. ^{1}H-NMR (25°C, DMSO-d$_6$): 8.5ppm, d, 2H, -NH; 7.2ppm, m, 10H, aryl-H Phe; 6.9-7.1ppm, AA'BB', 4H, aryl-H; 4.45ppm, m, 2H, α-H; 3.35ppm, d, 4H, -CH$_2$; 3.45ppm, s, 6H, -OCH$_3$; 2.9 and 3.1ppm, 2m, 4H, -CH$_2$ Phe. Anal. ($C_{30}H_{32}N_2O_6$) calc. C, 69.75; H, 6.24; N, 5.42; found C, 68.34; H, 6.23; N, 5.42%.

Hydrazinolysis of the methylesters 9, 10, 11 and 12

1 mmol of the corresponding methylester is suspended in 20 - 30 ml of methanol and heated in a water-bath. 10 mmol of hydrazine hydrate (100%) is added, the mixture is refluxed for a few hours and stirred overnight. The solvent is removed in vacuo. Methanol is added again and removed in vacuo. The procedure is repeated three times to remove excess hydrazinehydrate. The product is dried in vacuo and used in the next step without further purification.

7,10,20,23-Tetraaza-30,37-dioxa-2,15-dithia-28,32-dimethyl-2,21-bisisopropyl-heptacyclo-(32.3.1.3^{3,5}.3^{12,14}.3^{16,18}.1^{13,17}.1^{4,26})-tetraconta-1(26),3,5,12(34),13,16(28),17,25,28,31,35,39-dodecaen-2,8,11,19,22-hexoxid (cyclo-5-(Val/Val)-6) (1)

0.85 mmol 5-(ValNHNH$_2$)$_2$ are dissolved in 10 ml of dry DMF and cooled with ice/NaCl to -15°. 20.4 mmol cc HCl and 5.1 mmol of $NaNO_2$ (aqueous solution 14%) are added and the mixture is stirred for 30 - 45 min. 23.8 mmol of N-methylmorpholine are added. The diamine 6 is dissolved in 15 ml of dry DMF and dropped in very slowly to the reaction mixture at room temperature of 4°C.

The mixture is stirred for 5 - 7 days at 4°C. The solvent is removed in vacuo, the residue suspended in ethylacetate and washed carefully with saturated sodiumhydrogencarbonate (two times), saturated sodiumchloride, sodiumhydrogensulfate (5% aqueous solution) and saturated NaCl. The organic layer is separated, dried over $MgSO_4$ and concentrated. A crude product (171 mg, 26%) precipitates on adding petrolether. The material was purified in two steps by thin layer chromatography. A first chromatography with chloroform/methanol 95/5 gives a broad, intensive fraction of R_f=0.86 which contains **1** besides impurities (NMR). This material was again purified by TLC (chloroform). The fraction with R_f=0.12 yielded 25 mg (4%) of pure **1**. ^{1}H-NMR (-61.5°C, 400 MHz, $CDCl_3$): 9.35ppm, d, 1H, -NH Val; 8.75ppm, t, 1H, -NH'-6; 8.35ppm, t, 1H, -NH-6; 8.05ppm, d, 1H, H-5; 7.8ppm, d, 1H, H-5; 7.5ppm, d, 1H, H-6; 7.35ppm, d, 1H, H'-5; 7.2 - 7.1ppm, m, 4H, H-5; 6.8ppm, d, 1H, -NH' Val; 5.1ppm, m, 2H, -CH_2-phenox. and -CH_2'-phenox.; 4.85ppm, m, 1H, α-H'; 4.35ppm, m, 1H, -CH_2'-phenox.; 4.2ppm, m, 1H, α-H; 3.8ppm, m, 1H, -CH_2-phenox.; 2.95ppm, m, 1H, β-H'; 2.4ppm, 2s, 6H, -CH_3-phenox.; 2.05ppm, m, 1H, β-H; 1.0ppm, m, 3H, -CH_3-Val; 0.8ppm, m, 3H, -CH_3-Val. Anal. ($C_{40}H_{40}N_4O_8S_2$) calc. C, 62.48; H, 5.24; N, 7.29; S, 8.34; found C, 59.2; H, 5.43; N, 6.36; S, 7.35%. MS: DCI (NH_3, pos.), m/z(%): 769(100), 515(15), 358(20), 306(35), 177(25); DCI (NH_3, neg.):768(100); EI (70 eV): 768(100), 423(35), 395(30), 324(18).

7,10,20,23-Tetraaza-30,37-dioxa-2,15-dithia-2,21-dibenzyl-28,32-dimethyl-heptacyclo-(23.3.1.3^{3,5}.3^{12,14}.3^{16,18}.1^{13,17}.1^{4,26})-tetraconta-1(26),3,5,12(34),13,16(28),17,25,28,31,35,39-dodecaen-2,2,8,11,19,22-hexoxid (cyclo-5-(Phe/Phe)-6) (2)

The compound **2** is prepared in the same procedure as **1**. 1 mmol of 5-(PheNHNH$_2$)$_2$, 1 mmol of the diamine 6, 24 mmol of cc HCl, 6 mmol of $NaNO_2$ (14% aqueous solution), 28 mmol of N-methylmorpholine were used. The material is purified by thin layer chromatography with $CHCl_3$/methanol 95/5. Yields crude: 305 mg (35%), fine: 112 mg (13%), m.p. 230°. R_f-value: 0.84. ^{1}H-NMR (-20°C,400 MHz, $CDCl_3$): 9.2ppm, d, 1H, -NH Phe; 8.6ppm, t, 1H, -NH'-6; 8.25ppm, t, 1H, -NH-6; 7.85ppm, d, 2H, H-5; 7.75 and 7.25ppm, each 1s, 4H, H-6; 7.35 - 7.0ppm, m, 14H, C_6H_5-Phe and H-5; 6.85ppm, d, 1H, -NH'-Phe; 5.5ppm, m, 1H, α-H'; 4.9 and 3.9ppm, 2m, 2H, -CH_2-phenox.; 4.8ppm, m, 1H, α-H; 4.75 and 4.25ppm, 2d, 2H, -CH_2'-phenox.; 3.55ppm, m, 2H, -CH_2-Phe; 3.1ppm, m, 2H, -CH_2'-Phe; 2.4ppm, 2s, 6H, -CH_3-phenox.; Anal. ($C_{48}H_{40}N_4O_8S_2$) calc. C, 66.67; H, 4.63; N, 6.48; S, 7.41; found C, 64.46; H, 4.67; N, 6.25; S, 6.92%. MS: DCI (NH_3, pos.) m/z(%): 865 (100), 120(95); DCI (NH_3, neg.): 864(100); EI (70 eV): 864(100), 773(5), 520(8), 491(13), 417(21).

7,10,21,24-Tetraaza-31-oxa-2-thia-29,33-dimethyl-9,22-bisisopropyl-pentacyclo-(24.3.1.3^{3,5}.1^{4,27}.0^{13,18})-tetratrioconta-1(27),3,5(32),13,15,17,26,29,33-nonaen-8,11,20,23-tetroxid (cyclo-7-(Val/Val)-6) (3)

The material was prepared analoguous to **2** using 1 mmol of 7-(ValNHNH$_2$)$_2$. Yields crude: 137 mg (20%), fine: 26 mg (4%), R_f-value: 0.31; m.p. 160° (decomp.). $C_{36}H_{42}N_4O_7S$. ^{1}H-NMR (25°C, 400 MHz, $CDCl_3$): 7.8ppm, s, 2H, H-6; 7.65ppm, t, 2H, -NH-6; 7.45ppm, s, 2H, H-6; 7.25 and 7.15ppm, 2m, 4H, AB H-7; 7.1ppm, d, 2H, -NH Val; 4.85 and 4.25ppm, 2m, 4H, -CH_2-6; 4.1ppm, m, 2H, α-H Val; 3.65 and 3.5ppm, m, 4H, -CH_2-7; 2.4ppm, s, 6H, -CH_3-6; 2.05ppm, m, 2H, β-H Val; 0.85 and 0.75ppm, 2d, 12H, -CH_3-Val. MS: DCI (NH_3, pos.) m/z(%): 692(60), 675(100), 398(70), 361(50), 307(90), 132(95), 88(45), 72(55); DCI (NH_3, neg.): 674(100); EI (70 eV): 674(100), 631(5), 329(8%), 287(15), 230(19).

7,10,21,24-Tetraaza-31-oxa-2-thia-9,22-dibenzyl-29,33-dimethyl-pentacyclo-(24.3.1.3^{3,5}.1^{4,27}.0^{13,18})-tetratriaconta-1(27),3,5(32),13,15,17,26,29,33-nonaen-8,11,20,23-tetroxid (cyclo-7-(Phe/Phe)-6) (4)

The material was prepared in the same way as **2** using 1 mmol of 7-(PheNHNH$_2$)$_2$. Yields crude: 245 mg (32%), fine: 70 mg (9%); m.p. 195°. R_f-value: 0.47. ($C_{44}H_{42}N_4O_7S$). ^{1}H-NMR (27°C, 400 MHz, $CDCl_3$): 7.75ppm, s, 2H, H-6; 7.35ppm, t, 2H, -NH-6; 7.2ppm, s, 4H, aryl-H; 7.15ppm, s, 2H, H-6; 7.0ppm, m, 12H, H-Phe and NH-Phe; 4.75ppm, m, 2H, -CH_2-6; 4.65ppm, m, 2H, α-H; 4.0ppm, m, 2H, -CH_2-6; 3,65 - 3,5ppm, m, 4H, -CH_2-7; 3.0ppm, m, 4H, -CH_2-Phe; 2.4ppm, s, 6H, -CH_3-6.

MS: DCI (NH_3, pos.) m/z(%): 788(100), 771(55), 120(45); DCI (NH_3, neg.): 770(100); EI (70 eV): 770(100), 742(7), 679(22), 636(5), 504(6), 447(5), 357(16).

Acknowledgment: We thank Prof. Dr. D. Leibfritz, Dr. P. Schulze and I. Erxleben for the DCI-MS spectra and appreciate the help of Dipl. Chem. M. Stadelmann in preparing the manuscript. The financial support by the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie is gratefully acknowledged. G. Wagner thanks the DFG for a grant.

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