The Miraculous CD Spectra (and Secondary Structures?) of β-Peptides as They Grow Longer

Preliminary Communication

by **Dieter Seebach***, **Jürg V. Schreiber**[1]), **Per I. Arvidsson**[2]), and **Jens Frackenpohl**[3])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

Dedicated to the memory of *Pascal A. Mathieu*, our dear friend and colleague

The recently improved conditions for solid-phase synthesis of β^3-peptides by the Fmoc strategy were used to synthesize a β-tetracosapeptide (**4**, *Scheme*) composed of eight different β-amino acid residues; 11 of the 24 residues carry functionalized proteinogenic side chains (namely those of Glu, Lys, Ser, and Tyr). The highly H_2O-soluble β-tetracosapeptide was identified by ^{1}H-NMR spectroscopy (in MeOH), analytical HPL chromatography, and ESI-mass spectrometry (*Fig. 1*). The expected 3_{14}-helical secondary structure of the new β-peptide was designed to have one hydrophobic and two hydrophilic faces, and to be compared with other β-peptides (**1**–**3**), two of which are also of amphipathic character in this secondary structure (*Fig. 2*). In the absence of NMR-structural proof, the CD spectra of the four β-peptides were compared (*Figs. 3* and *4*). The β-tetracosapeptide exhibits an unprecedented CD pattern (in MeOH and in H_2O solution) that may arise from a new type of secondary structure or from an unordered conformation.

β-Peptides built of homologated amino acids with proteinogenic side chains fold to secondary structures with as few as six residues [1], as detected by NMR measurements of their solutions in H_2O, MeOH, or pyridine, as well as by MD calculations [1f][4]). Thus, two different helices (designated 3_{14} and *12/10* helix) [1a][1b], a hairpin turn, and a parallel-sheet structure [1c][1d], as well as tubular stacking of cyclic β-peptides [1g–i] have been discovered.

We wondered up to what chain length these β-peptidic secondary structures would be stable in solution – α-peptidic helices, for instance, rarely exceed chain lengths of 25 residues in proteins[5]). Several problems had to be solved in order to produce larger β-peptides. all-β^3-Peptides (side chains in the 3-position of each residue) with non-

[1]) Part of the projected Ph.D. thesis of *J.V.S.*, ETH-Zürich; a Ph.D. scholarship from the *Novartis Stipendienfonds* is gratefully acknowledged.

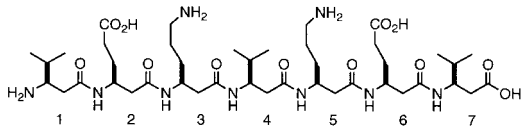
[2]) Postdoctoral fellow at ETH-Zürich (2000/2001), financed by the *Swedish Foundation for International Cooperation in Research and Higher Education* (*STINT*).

[3]) Postdoctoral fellow at ETH-Zürich (2000/2001), financed by the *Deutscher Akademischer Austauschdienst* (*Hochschulsonderprogramm III*).

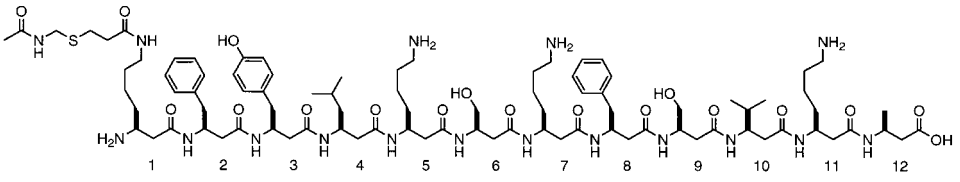
[4]) For helices and turns being entirely or partially constructed of cyclic β-amino acids with back-bone rotational restriction, see [2].

[5]) See the protein data bases [3] and the review article [4].

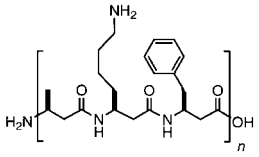
functionalized[6]) or protected functionalized side chains[7]) and with terminal protection become insoluble in common organic solvents (as well as in H_2O) with increasing chainlengths[8]) so that synthesis or fragment coupling[9]) in solution is prevented. Until recently, solid-phase synthesis of β-peptides according to the Fmoc strategy was complicated [1j] and failed with chain lengths of above *ca.* eight residues, an effect that we interpreted as a consequence of chain folding [1b][1j]. This problem has now been solved by use of a stronger base and longer reaction times for *N*-deprotection and coupling, a procedure that can also be applied to solid-phase fragment coupling, for instance of β-peptidic triades (see the series of compounds **3**) [1m][1n]. Thus, the method of choice now is the solid-phase synthesis of β^3-peptides with protected functionalized[7]) side chains and complete deprotection of all functional groups in the final step of detachment from the resin under acidic conditions. This leads to β-peptides of excellent solubility[10]) in H_2O and MeOH (see, *e.g.*, the β-heptapeptide **1** [7], the β-dodecapeptide **2** [8] and the β-oligopeptides **3** [1j][1k][1m][1n]). To test the improved synthetic methodology and to answer the question posed above (chain lengths *vs.*



1



2



3 $n = 1, 2, 3, 4, 5, 6$

---

6) … with the side chains of Ala, Val, Leu, Phe [1a][1b]. – We had obtained a β-pentadecapeptide on a solid support with such side chains after painstaking isolation and purification procedures; it was found to attain a 3_{14}-helical secondary structure (CD spectrum) and to undergo NH/ND exchange of certain central NH protons with a half life in the order of months (NMR analysis in CD_3OD) [1b].

7) … with O–^{t}Bu, S–Acm, S–Bn, NHZ, NHBoc, CO_2–^{t}Bu protection of the Ser, Tyr, Cys, Orn, Lys, Glu side chains [1j–n].

8) The opposite is true of β^2-/β^3-mixed β-peptides which are nonpolar and well-soluble in many organic solvents (chain lengths of up to twelve residues) [1b].

9) The cystein-mediated coupling of fragments in aqueous solution (the *Kemp* ligation [5]) has not yet been applied to β-peptides.

10) Even the single central Ser side chain in the β^3-heptapeptide H-β-HVal-β-HAla-β-HLeu-β-HSer-β-HVal-β-HAla-β-HLeu-OH suffices to make the compound well-soluble in H_2O (*ca.* 5 g/l) [1e][6].

secondary structure), we have now synthesized the all-β^3-tetracosapeptide **4** (*Scheme* and *Fig. 1*)[11]).

As outlined in the *Scheme*, the anchoring of the first amino acid to the *Wang* resin and the first six deprotection/coupling steps were carried out under standard conditions of the Fmoc methodology. For the next 17 steps, the stronger base DBU was employed for Fmoc cleavage, and the reaction times were increased (the total time required for assembly of the 24 β-amino acids, including cleavage from the resin with total deprotection, was *ca.* 240 h). The pentakis-trifluoroacetate salt of **4**, which contains eleven functionalized (hydrophilic) and 13 non-functionalized (hydrophobic) side chains, was formed in 28% purity (HPLC integration). Nevertheless, with the aid of the efficient purification offered by preparative reversed-phase (RP) HPLC, we were able to isolate 10 mg of the pure highly H_2O-soluble tetracosapeptide **4** (see *Fig. 1*, *a* and *b*), with the correct mass spectrum (*Fig. 1*, *c*). ^{1}H-NMR Measurement has been carried out with the CF_3COOH salt of **1** in CD_3OH (for the region of the NH and aromatic protons; see *Fig. 1*, *d*). It is thus demonstrated that we have the tools at hand to synthesize pure β-peptides of considerable chain length and complexity[12]).

What about the structure of the tetracosapeptide **4**? The primary structures (or sequences) of the β-peptides **1**–**4** may appear arbitrary to the untrained eye, but they have all been designed in view of the most common β-peptidic secondary structure, the left-handed or (*M*) 3_{14} helix, the idealized form of which (a 3_1 helix) is composed of three L-β-amino acid residues per turn, with the side chains in positions i and $i+3$ pointing perpendicularly to the helix axis and parallel to each other, in *ca.* 5-Å distance: the helix backbone circumscribes a virtual cylinder, which places three streaks of juxtaposed side chains, disposed from each other by 120°, on the surface of this cylinder (see **A** and the projections down along 3_1-helix axes in *Fig. 2*). If the β^3-peptides **1**–**4** would exist in such 3_1-helical forms, *i*) the side chains in **1** could form salt bridges [7] and experience hydrophobic interactions (between i-Pr groups) [1b], *ii*) the surface of the helix of **2** would be well-covered by polar (hydrophilic) side chains, *iii*) while two streaks of the 3_1-helical surface of **3** would be hydrophobic (Phe and Ala side chains) and one hydrophilic (charged Lys side chains)[13]), and *iv*) only one streak in the helix formed by **1** and **4** would be hydrophobic, the other two being predominantely hydrophilic (*cf.* amphipathic or amphiphilic 3.6_{13} helices of α-peptides and α-proteins [1k]).

Unfortunately, we have no *solid* evidence (from NMR measurements or by X-ray diffraction!) for the secondary structures[14]) of the β-peptides **2**–**4**, but we can discern from CD spectra[15]) that there must be dramatic differences (*Figs. 3* and *4*). There is ample evidence that the left-handed (*M*) 3_{14}-helical structure of β-peptides consisting of L-β-amino acids gives rise to a negative *Cotton* effect near 215 nm [1][2]. Thus, the

---

[11]) Comparison of the structures of **2** and **4** reveals that (apart from the special N-terminal linker group in **2**) the twelve C-terminal β-amino acids in **4** are identical to those in the dodecapeptide **2**. For the rationale regarding choice of the primary structures (sequences) of **1**–**4**, see *Fig. 2*.

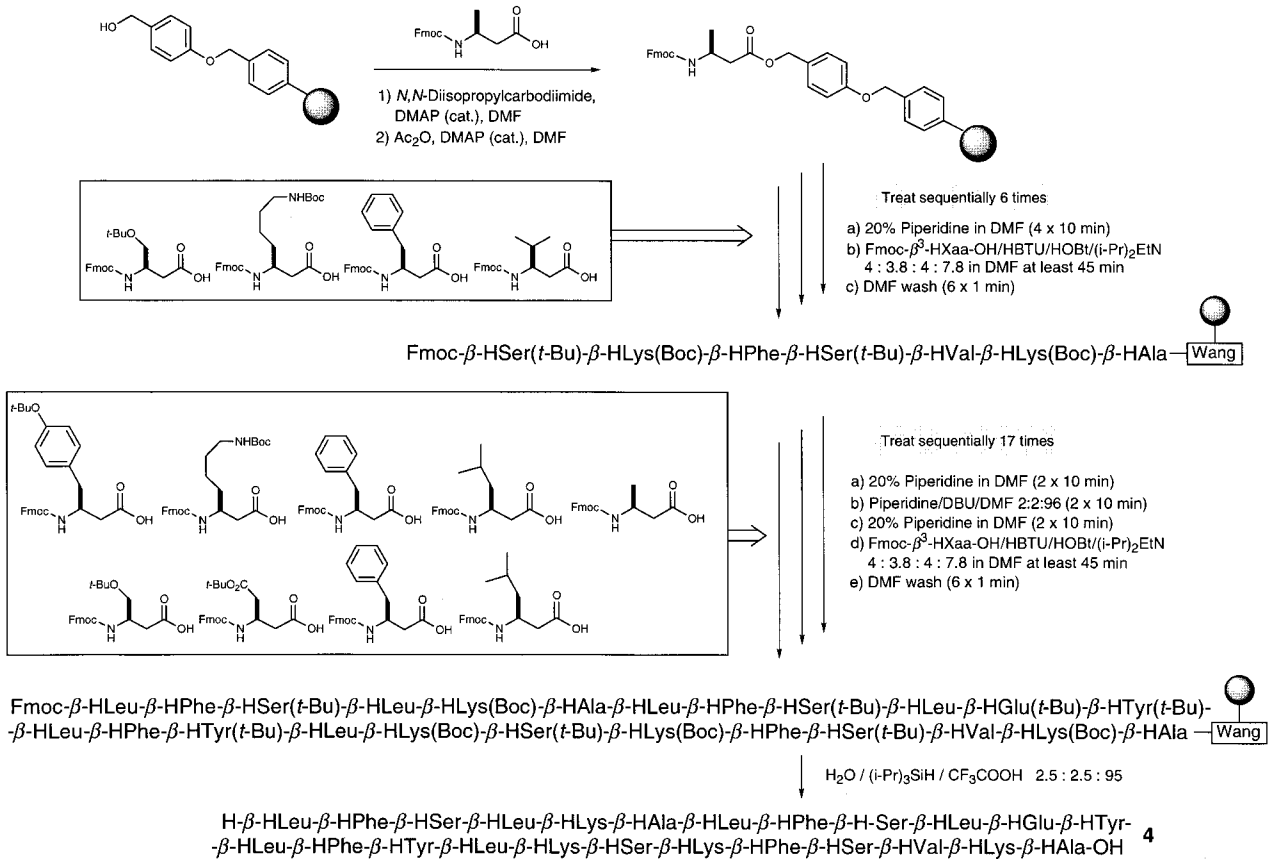
[12]) The required *N*-Fmoc-protected β-amino acids are either commercially available (*e.g.*, from *Fluka*), or have been prepared as previously described by us (*cf.* [1j][8][9] and refs. cit. therein).

[13]) Lysine side chains, although positively charged under physiological conditions in H_2O, are at the same time hydrophobic by their $(CH_2)_4$ linker!

[14]) For the NMR-solution structure of **1** in MeOH and H_2O, see [7].

[15]) For lists of characteristic CD patterns of β-peptides see Table 3 in [10] and the Table in [11].

Scheme. *Solid-Phase Synthesis of the β-Tetracosapeptide* **4** *on* Wang *Resin*. DMAP = 4-(Dimethylamino)pyridine, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.



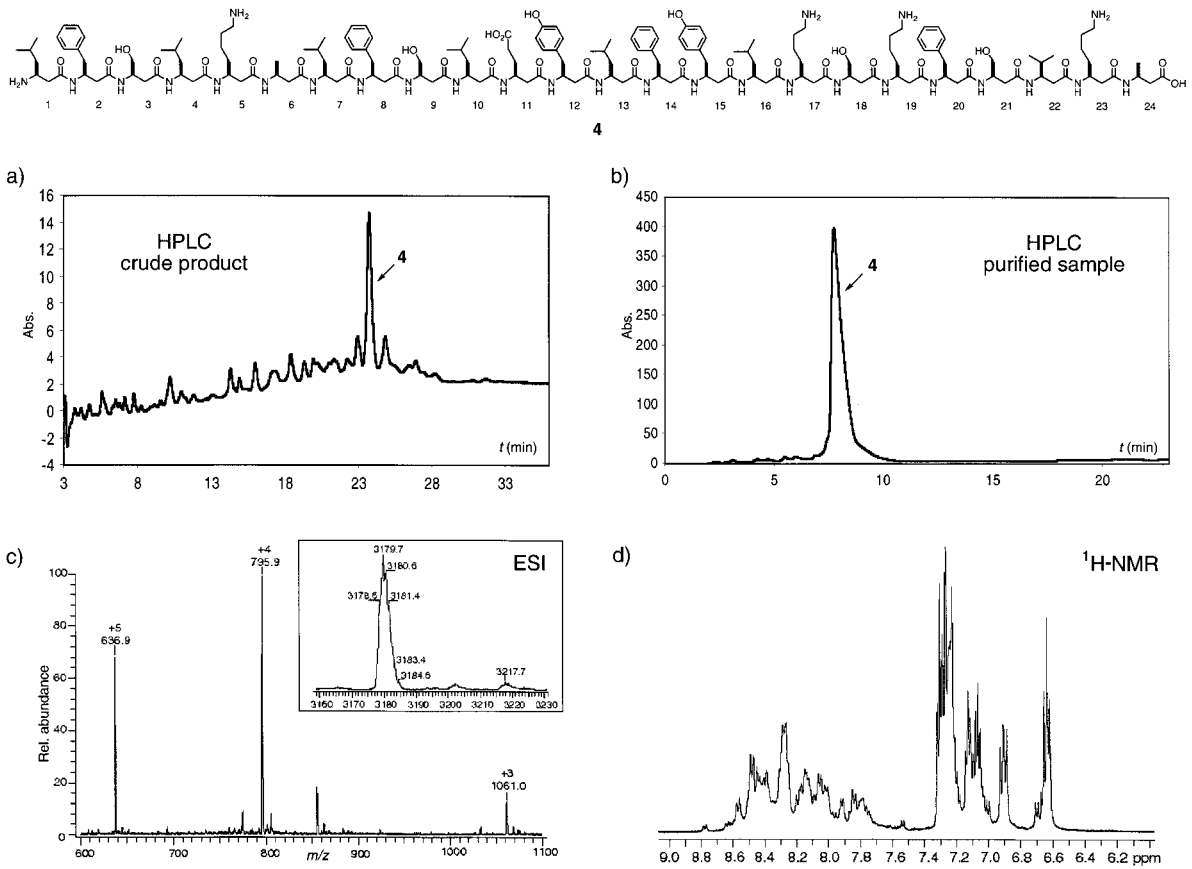


Fig. 1. *Formula, RP-HPLC traces, mass spectra, and 1D-^{1}H-NMR spectrum of the β-tetracosapeptide* **4**. *a*) Preparative HPLC elution profile of crude product as obtained after deprotective cleavage from the *Wang* resin (*RP-C18* column, linear gradient from H_2O containing 0.1% CF_3COOH to MeCN containing 0.1% CF_3COOH). *b*) Analytical RP-HPLC elution profile of **4** after preparative separation from the crude product mixture (*RP-C18* column, linear gradient of 60% to 40% H_2O, containing 0.1% CF_3COOH, in MeCN containing 0.1% CF_3COOH over 20 min). *c*) Electrospray mass spectra of **4** with multiply (positively) charged ion peaks and with the molecular-ion peak. *d*) Low-field region of the ^{1}H-NMR spectrum of **4** in CD_3OH showing the NH and aromatic protons. The β-peptide was employed as its CF_3COOH salt as obtained by lyophilization.

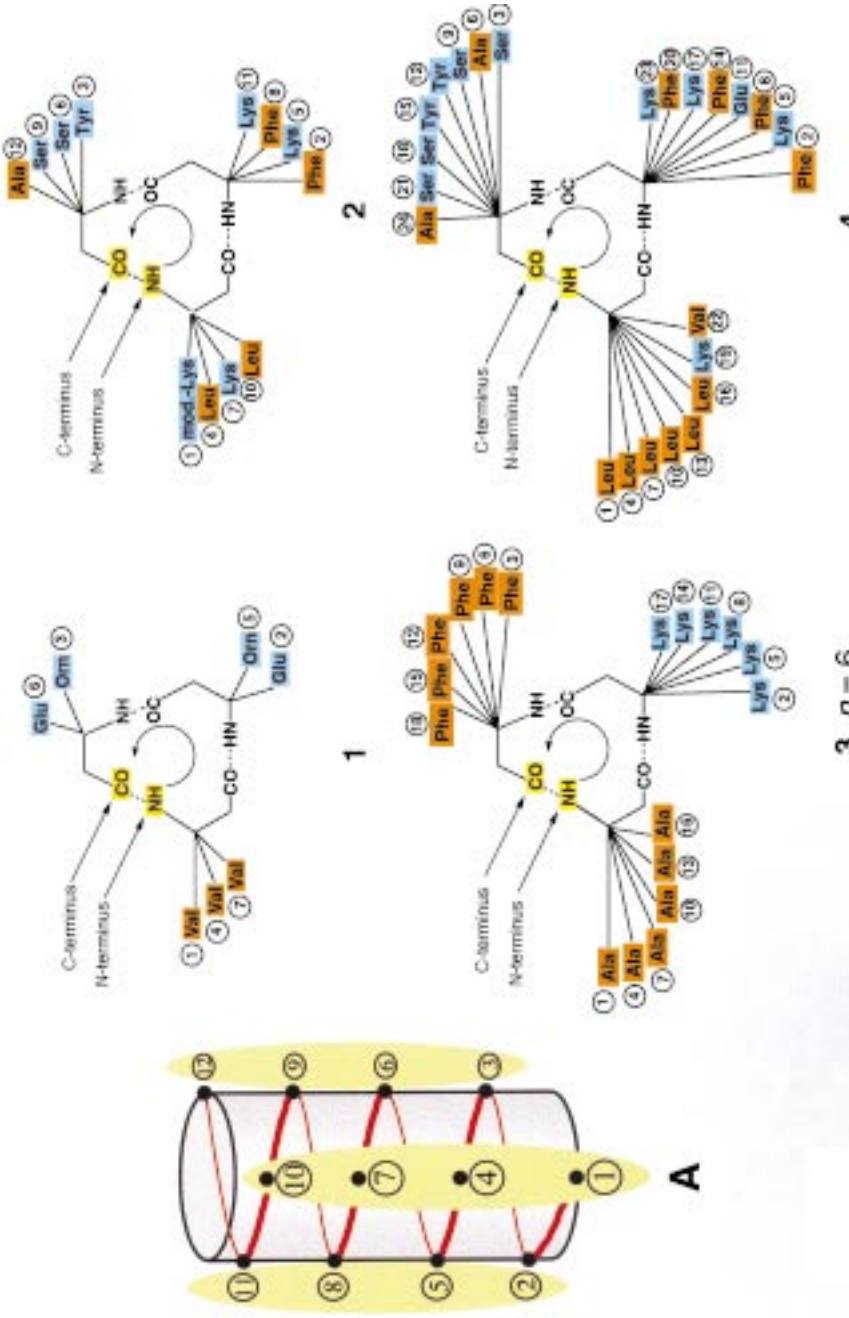


Fig. 2. *Schematic presentation* (**A**) *and views along the axes of possible idealized* 3_1*-helical secondary structures of the* β^3*-peptides* **1**–**4**. Color codes: *orange* for hydrophobic side chains, *blue* for functionalized (hydrophilic) side chains. The three letter-amino acid symbols stand here for the side chains on the β^3-amino acids.

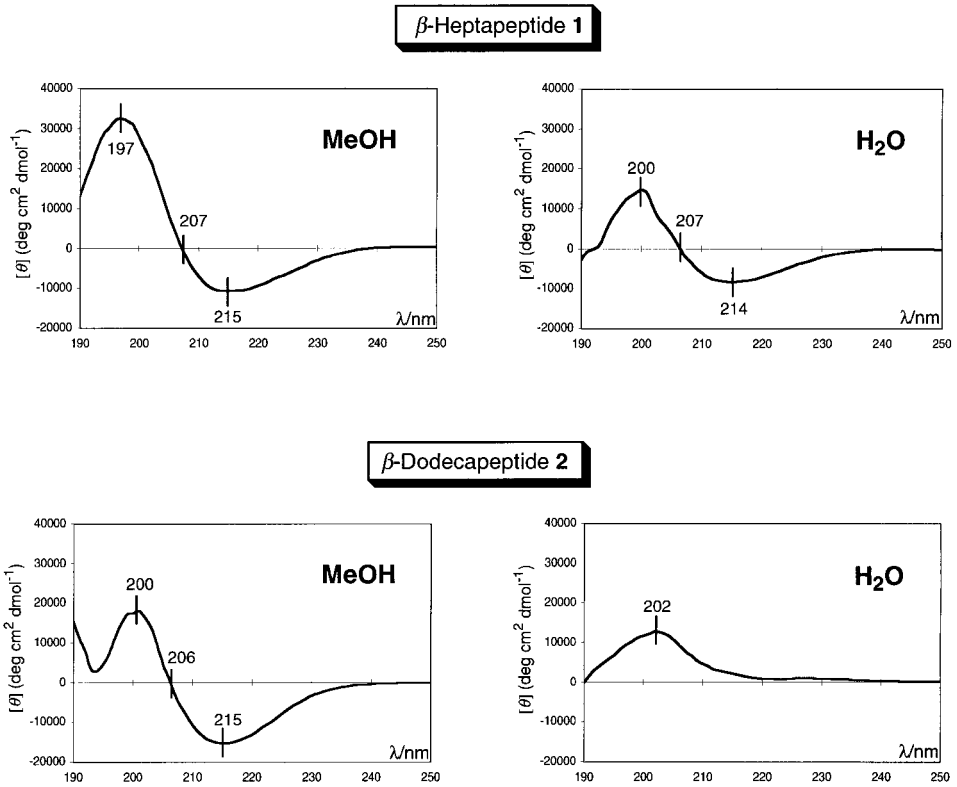


Fig. 3. *Normalized CD spectra in MeOH and in H_2O of the β-peptides* **1** *and* **2**. The spectra were recorded at 20.0° at a concentration of 0.2 mM. All of the peptides were measured as their TFA salts as obtained after lyophilization, and the aqueous solutions were buffered at pH 5.5 with 0.1M AcOK. The pattern of the spectra of **1** in both solvents and of **2** in MeOH is considered characteristic of β-peptidic 3_{14}-helical structures. The non-normalized spectra of **2** have been reported previously [8].

CD spectra of the β-peptides **1** (in MeOH and in H_2O) and **2** (in MeOH)[16]) are in accord with our experience and expectation (*Fig. 2*). Notably, only the β-heptapeptide **1** exhibits the characteristic pattern in aqueous solution[14]) at pH 5.5 (see *Fig. 3*, top). As pointed out before [8], the aqueous solution of the β-dodecapeptide **2** shows a positive-only CD curve[17]) with a maximum at 202 nm (*Fig. 3*, bottom right). In contrast, the β-octadecapeptide **3** ($n=6$) gives rise to a negative-only CD curve above 200 nm (in MeOH and H_2O), with an extremely intense[18]) *Cotton* effect at 208 nm in

[16]) … and also of the β-pentadecapeptide with purely aliphatic side chains (in MeOH), alluded to in footnote[6]) [1b][9b].

[17]) Such a single-positive CD pattern (with a strong (+)-*Cotton* effect at 200–210 nm) has been found for *12/10* helices [1b] and for hair-pin turns [1c], and it was proposed to arise from β-peptidic ten-membered H-bonded rings [1f].

[18]) The intensities of the *Cotton* effects *per* β-amino acid residue at wavelengths ≥200 nm are of the same magnitude in all the spectra shown ($\theta=5000-15000$), except for that of **3** ($n=6$), which has a molar ellipticity of 70000 per residue.

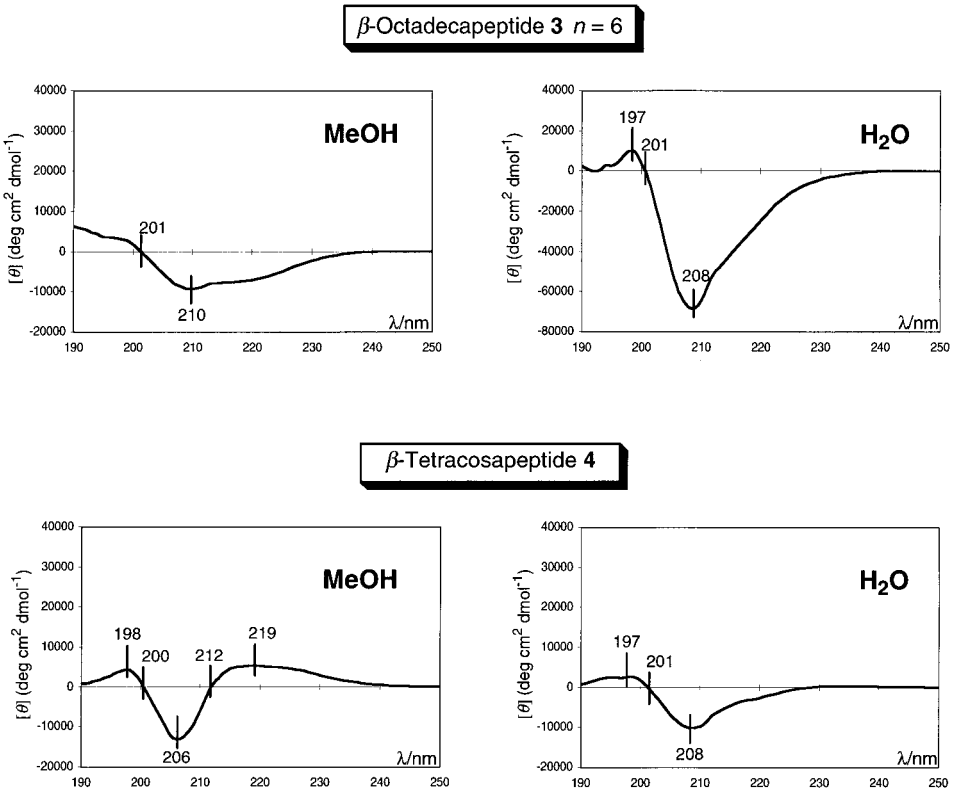


Fig. 4. *Normalized CD spectra in MeOH and in H_2O* of the *β-peptides* **3** ($n=6$) *and* **4**. The spectra were recorded at 20.0° at a concentration of 0.2 mM. The peptides were measured as their TFA salts as obtained after lyophilization, and the aqueous solutions were buffered at pH 5.5 with 0.1M AcOK. Note the different *y*-axis scale in the spectrum of **3** ($n=6$) in aqueous solution (upper right). For the CD spectrum of the similar octadecapeptide H-(β-HLeu-β-HLys-β-HLeu)$_6$-OH, see [1m].

H_2O at pH 5.5 (*cf.* the CD spectrum of H-(β-HLeu-β-HLys-β-HLeu)$_6$-OH at pH 7, reported by *DeGrado* and co-workers [1m]). Totally out of order is the CD spectrum of the β-tetracosapeptide **4** (*Fig. 4*, bottom): at first sight, both, the spectrum in MeOH and that in H_2O, look like mirror images of those obtained from the β-dodecapeptide **2** (*Fig. 3*, bottom), but a closer look shows that the extrema of opposite signs are shifted to longer wavelengths in the spectrum of the longer β-peptide[18]). Obviously, the β-peptide consisting of 24 residues has a different secondary structure, as compared to all other β-peptides considered here, at least in MeOH solution. It may be that the CD pattern is caused by a more complicated tertiary structure, and, hopefully, it is not the fingerprint of a random-coil or unfolded β-peptidic chain! The latter would not be completely surprising since the optimal length of an isolated β-peptidic helix may have been surpassed (*cf.* the natural α-peptidic helices [4]). Since the β-tetracosapeptide **4** contains only eight different residues[19]), it will be difficult to determine its NMR

[19]) 6 β-HLeu, 4 β-HLys, 4 β-HPhe, 4 β-HSer, 2 β-HAla, 2 β-HTyr, 1 β-HGlu, and 1 β-HVal.

solution structure[20]), and we are prepared to synthesize such large β-peptides with a greater variety of side chains in order to have a better chance of solving the riddle of the miraculous CD spectra of β-peptides, as they grow longer.

REFERENCES

[1] a) D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913; D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 2043; b) D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1998**, *81*, 932; c) D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem*. **1999**, *111*, 1700; *Angew. Chem.*, *Int. Ed*. **1999**, *38*, 1595; d) K. Gademann, T. Kimmerlin, D. Hoyer, D. Seebach, *J. Med. Chem.* **2001**, in press; e) D. Seebach, A. Jacobi, M. Rueping, K. Gademann, M. Ernst, B. Jaun, *Helv. Chim. Acta* **2000**, *83*, 2115; f) D. Seebach, J. V. Schreiber, S. Abele, X. Daura, W. F. van Gunsteren, *Helv. Chim. Acta* **2000**, *83*, 34; g) D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, *80*, 173; h) K. Gademann, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 957; i) H. C. Le, T. Hintermann, T. Wessels, Z. Gan, D. Seebach, R. R. Ernst, *Helv. Chim. Acta* **2001**, *84*, 208; j) S. Abele, G. Guichard, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2141; k) M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774; l) A. Jacobi, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1150; m) Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc*. **1999**, *121*, 12200; n) J. V. Schreiber, J. Frackenpohl, P. I. Arvidsson, D. Seebach, unpublished results.

[2] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; b) B. R. Huck, J. D. Fisk, S. H. Gellman, *Org. Lett*. **2000**, *2*, 2607.

[3] http://www.rcsb.org/pdb/.

[4] J. M. Scholtz, R. L. Baldwin, *Annu. Rev. Biomol. Struct.* **1992**, *21*, 95.

[5] D. S. Kemp, *Biopolymers* **1981**, *20*, 1793; J. P. Tam, Y.-A. Lu, C. F. Lium, J. Shao, *J. Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12485.

[6] J. V. Schreiber, unpublished results.

[7] P. I. Arvidsson, M. Rueping, D. Seebach, submitted.

[8] J. V. Schreiber, D. Seebach, *Helv. Chim. Acta* **2000**, *83*, 3139.

[9] a) G. Guichard, D. Seebach, *Chimia* **1997**, *51*, 315; b) G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 187.

[10] W. F. DeGrado, J. P. Schneider, Y. Hamuro, *J. Pept. Res.* **1999**, *54*, 206.

[11] D. Seebach, T. Sifferlen, P. A. Mathieu, A. M. Häne, C. M. Krell, D. J. Bierbaum, S. Abele, *Helv. Chim. Acta* **2000**, *83*, 2849.

Received January 12, 2000

---

[20]) As shown in *Fig. 1*, *d*, we cannot get a first hint of a possible secondary structure formed by **4** in CD_3OH; the signals in the NH region are neither well-dispersed nor collapsed.