[5] M. Böltau, S. Walheim, J. Mlynek, G. Krausch, U. Steiner, *Nature* **1998**, *361*, 877 – 879.
[6] S. B. Carter, *Nature* **1965**, *208*, 1183 – 1187.
[7] N. B. Maroudas, *Nature* **1973**, *244*, 353 – 354.
[8] G. P. López, M. W. Albers, S. L. Schreiber, R. Carroll, E. Peralta, G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, *115*, 5877 – 5878.
[9] R. Singhvi, A. Kumar, G. P. López, G. N. Stephanopoulos, D. I. C. Wang, G. M. Whitesides, D. E. Ingber, *Science* **1994**, *264*, 696 – 698.
[10] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425 – 1428.
[11] D. W. Branch, J. M. Corey, J. A. Weyhenmeyer, G. J. Brewer, B. C. Wheeler, *Med. Bio. Eng. Comput.* **1998**, *36*, 135 – 141.
[12] W. M. Lackowski, P. Ghosh, R. M. Crooks, *J. Am. Chem. Soc.* **1999**, *121*, 1419 – 1420.
[13] Y. Xai, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568 – 594; *Angew. Chem. Int. Ed.* **1998**, *37*, 550 – 575.
[14] Y. Zhou, M. L. Bruening, D. E. Bergbreiter, R. M. Crooks, M. Wells, *J. Am. Chem. Soc.* **1996**, *118*, 3773 – 3774.
[15] A. S. G. Curtis, J. V. Forrester, F. Lawrie, *J. Cell Biol.* **1983**, *97*, 1500 – 1506.
[16] S. R. Sheth, D. Leckband, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 8399 – 8404.
[17] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Biotechnol. Prog.* **1998**, *14*, 356 – 363.
[18] J. G. Franchina, W. M. Lackowski, D. L. Dermody, R. M. Crooks, D. E. Bergbreiter, K. Sirkar, R. J. Russell, M. V. Pishko, *Anal. Chem.*, submitted.
[19] M. Zhao, Y. Zhou, M. L. Bruening, D. E. Bergbreiter, R. M. Crooks, *Langmuir* **1997**, *13*, 1388 – 1391.
[20] M. L. Bruening, Y. Zhou, G. Aguilar, R. Agee, D. E. Bergbreiter, R. M. Crooks, *Langmuir* **1997**, *13*, 770 – 778.

Pleated Sheets and Turns of β-Peptides with Proteinogenic Side Chains

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The properties of peptides and proteins depend on their three-dimensional structure, which itself is determined by the sequence of the amino acids, that is, the primary structure. The mechanisms of formation and the parameters determining the stability of secondary structures of proteins—including not only the helix, the pleated sheet, and the turn, but also the random-coiled region—are not yet fully understood.[1] In contrast, β-peptides (oligomers of β-amino acids[2]) adopt predictable[3] secondary structures that can also be identified by calculations.[4] This also holds for β-peptides whose backbones are not conformationally restricted by cyclic residues. Thus, β-peptides composed of more than five homochiral β^2-, β^3-, or *like*-$\beta^{2,3}$-amino acids[5] with proteinogenic side chains form a 3_{14} helix in methanol with all substituents in lateral positions;[3] on the other hand, chains consisting of (*R*)-β^2/(*S*)-β^3 or *unlike*-$\beta^{2,3}$ residues were expected to adopt an extended conformation, with formation of pleated sheets (Figure 1a); in β-peptide sections with (*S*)-β^2/β^3 or with *geminally* disubstituted $\beta^{2,2}$-amino acid moieties we have observed the formation of ten-membered hydrogen-bonded rings (Figure 1b, c). These results and conclusions have provided guidance in our search for β-peptidic parallel and antiparallel pleated sheets and turns, and we have prepared (by known methods[3, 9]) the β-peptides **1** (in solution) and **2** (on solid phase), containing *unlike*-$\beta^{2,3}$-amino acids. Herein, we report their structures.

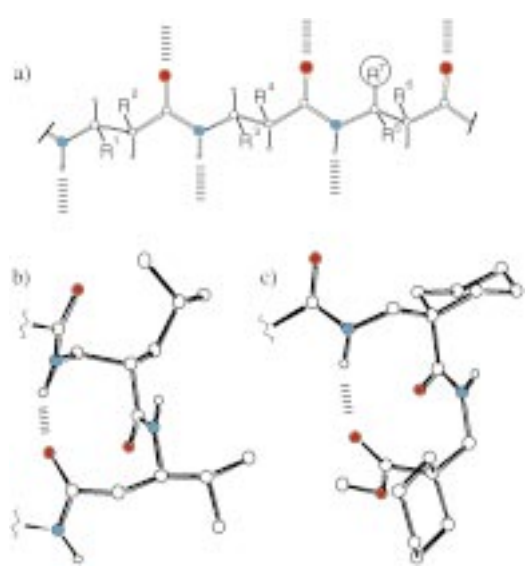
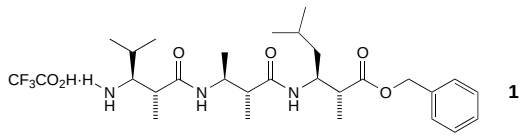
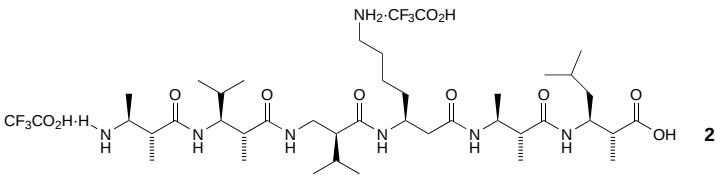


Figure 1. a) Model of a fully extended β-peptide chain. β-Amino acid sequences with R^1 to $R^6 \neq H$, $R^7 = H$ (*unlike*-$\beta^{2,3}$, type I) or, for example, R^1, R^4, $R^5 \neq H$, R^2, R^3, R^6, $R^7 = H$ ((*S*)-β^3/(*R*)-β^2, type II) preclude a β-peptide from adopting the 3_{14}-helical secondary structure; the linear arrangement is enforced.[2, 3, 6] Geminal disubstitution, for example R^5 *and* $R^7 \neq H$, prevents both the formation of the helix and the aggregation to a pleated sheet (see 14-membered hydrogen-bonded turns in Figure 2 and the discussion in reference [3]). b), c) Ten-membered hydrogen-bonded turns found in the β^2-β^3-segments of the 12/10/12 helix[3, 7] or formed by geminally disubstituted β-peptides.[8]





Unsurmountable solubility problems arise upon chain elongation with formation of β-peptides such as **1** (type I in Figure 1a), so that attempted dimerizing coupling of **1** to give a hexapeptide was unsuccessful.[10] The crystal structure and crystal packing of **1**, which indeed forms sheets, are shown in Figure 2.[11] The parallel amide planes in the individual strands

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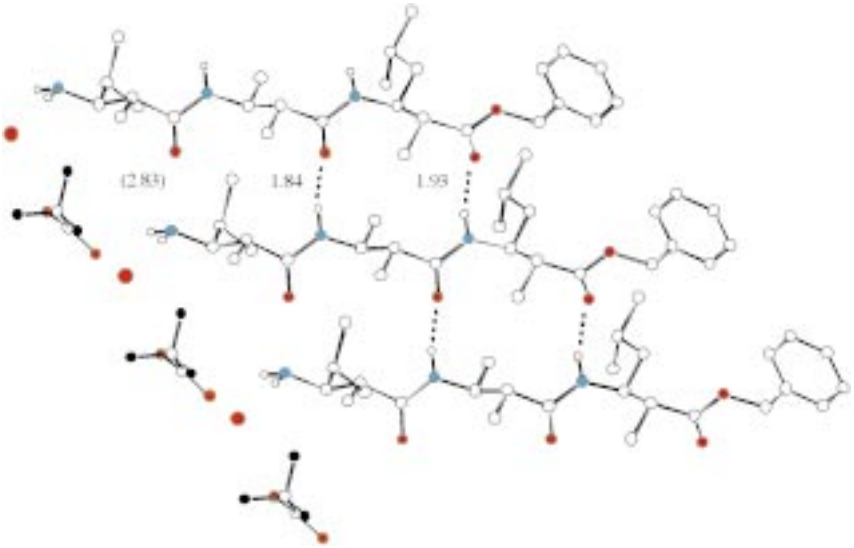

Figure 2. Parallel pleated sheet structure of β-tripeptide ester **1**·H_2O. The angles N–H···O are 159.7 and 165.6°; the distance N···O is shown for the N-terminal hydrogen bond. The X-ray structure analysis was performed by P. Seiler.

are connected through CHR–CHMe ethane moieties, in which R and Me are antiperiplanar as are HN and CO; 14-membered hydrogen-bonded rings connect the strands in the *parallel* pleated sheet structure. In contrast to α-peptidic pleated sheets, where neighboring O=C bonds point in opposite directions, these bonds are unidirectional in the β-peptide structure, leading to a polar packing that might be an additional reason for the low solubility of compounds of this type (cf. cyclo-β-tetrapeptides[12]).

We chose the turn (Figure 1b) composed of a β^2/β^3-dipeptide sequence (with the side chains of valine and lysine) for the construction of an *antiparallel* pleated sheet arrangement. This turn has the same substitution pattern as the central ten-membered hydrogen-bonded ring of the 12/10/12 helix.[3, 7] Two dipeptide segments of *unlike*-$\beta^{2,3}$-amino acids, which enforce the extended conformation, were attached on each end of this unit and were supposed to form intra- rather than intermolecular hydrogen bonds. In fact, the resulting β-peptide **2** is well soluble, even in water (due to the ω-aminobutyl group[13]).

The structure of hexapeptide **2** in CD_3OH was determined by 2D NMR spectroscopy. All resonances in the ^1H NMR spectrum were assigned unequivocally by the evaluation of DQF-COSY and TOCSY measurements. The coupling constants between the protons of the peptide backbone are collected in Table 1. The *J* values of the backbone protons of amino acids 2 and 5 are large, implying an antiperiplanar arrangement of the corresponding NH and C(β)-H as well as C(β)-H and C(α)-H protons; that there is more than one preferred conformation of the terminal amino acids 1 and 6 is suggested by their smaller *J* values. However, the values observed for the central amino acids 3 and 4 indicate a completely different, nonextended conformation, and they resemble those found for the ten-membered hydrogen-bonded ring of the 12/10/12 helix.[3, 7] ROESY measurements were used to obtain information about the distances between the protons, and the volumina of 20 NOE cross-peaks were classified in three distance categories (Table 2). Long-range NOEs between amino acids 1 and 6 as well as between 2 and 5 are especially significant. An extended section of the structure is suggested by NOEs between NH(3), C(α)-H(2) and NH(2), as well as between NH(6), C(α)-H(5), and NH(5). The 20 NOEs and 12 *J* values were used as distance and torsion-angle restraints in molecular dynamics simulations according to the simulated annealing method (programme X-PLOR). The resulting conformations converged to a unique structure of peptide **1** (Figure 3): β-peptide **2** adopts a hairpin arrange-

Table 1. Coupling constants for β-hexapeptide **2** (CD_3OH, 500 MHz).[a]

Amino acid	*J*(NH, C(β)-H)	*J*(C(α)-H, C(β)-H)
1	–	7
2	10.1	9.7
3	*J*(NH, C(β)-H^{Si}) 7.4	*J*(C(β)-H^{Si}, C(α)-H) 3.8
	J(NH, C(β)-H^{Re}) 3.8	*J*(C(β)-H^{Re}, C(α)-H) 10.7
4	9.0	*J*(C(β)-H, C(α)-H^{Si}) 4.1
		J(C(β)-H, C(α)-H^{Re}) 9.6
5	10.6	9.2
6	7	7

[a] The backbone CH_2 protons of amino acids 3 and 4 were assigned stereospecifically. The large values for the amino acids 2 and 5 suggest an extended conformation, whereas the *J* values for 1 and 6 indicate several preferred conformations. The coupling constants in residues 3 and 4 are in agreement with a well-defined, nonextended conformation.

Table 2. NOEs of β-hexapeptide **2** extracted from the 150ms-ROESY spectrum (CD_3OH, 500 MHz).[a]

H atom		H atom		NOE	H atom		H atom		NOE
NH	2	C*H*Me_2	2	m	NH	5	α-Me	5	w
NH	2	C(α)-H	2	m	NH	5	β-Me	5	m
NH	2	C(β)-H	1	w	NH	5	C(α)-H^{Si}	4	m
NH	2	C(α)-H	1	s	NH	5	C(α)-H	5	m
β-H^{Si}	3	C*H*Me_2	3	m	NH	5	C(β)-H	4	m
β-H^{Si}	3	C(α)-H	3	m	NH	6	C(α)-H	6	m
NH	3	C(β)-H	2	m	NH	6	C*H*Me_2	6	m
NH	3	C(α)-H	2	s	C(α)-H	1	C(β)-H	6	w
NH	4	α-H^{Si}	4	w	C(α)-H	2	C(β)-H	5	m
NH	4	α-H^{Re}	4	s	δ-Me	2	α-Me	5	w

[a] The NOEs were classified in three distance categories: s (strong, < 3 Å), m (medium, < 3.5 Å), and w (weak, < 4.5 Å).

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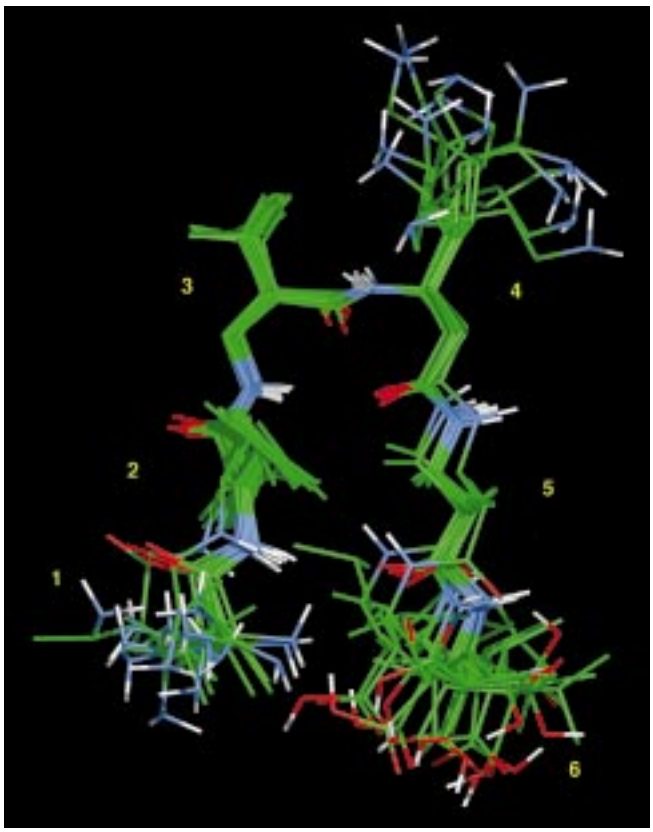

Figure 3. Structure of β-peptide **2** as determined by NMR spectroscopy (CD_3OH). Bundles of the 15 lowest energy conformers showing no significant violation of the experimental restraints (NOE and J values). According to this analysis, the C- and N-terminal amino acids 1 and 6 are rather flexible, and two conformations are observed for 6 that are in perfect agreement with the experimental data. The figure was generated by MOLMOL.[14]

ment,[15] a secondary structural element of α-peptides which is frequently responsible for interactions with receptors.[16]

The results described herein now complete the demonstration that oligomers (β-peptides) consisting of the homologues of simple proteinogenic amino acids form helices, pleated sheets, and turns, just like the α-peptidic proteins. However, the β-peptidic secondary structures are not only of larger variety (three different helices have already been discovered[2b, 3]) and of much more pronounced stability (even with short chain lengths[3, 17]), but they are also predictable and amenable to rational planning and theoretical calculations. Furthermore, β-peptides were found to be stable to the most aggressive peptidases.[18] Thus, the door is wide open to large structures (β-proteins, β-enzymes) and to β-peptidic drugs.

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[1] C. Branden, J. Tooze, *Introduction to Protein Structure*, 2nd ed., Garland, New York, **1998**; Y. Duan, P. A. Kollman, *Science* **1998**, *282*, 740 – 744; H. J. C. Berendsen, *Science* **1998**, *282*, 642 – 643.

[2] a) D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913 – 941; b) review: D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, *21*, 2015 – 2022; S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173 – 180.

[3] 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 – 982.

[4] X. Daura, K. Gademann, B. Jaun, D. Seebach, W. F. van Gunsteren, A. E. Mark, *Angew. Chem.* **1999**, *111*, 249 – 253; *Angew. Chem. Int. Ed.* **1999**, *38*, 236 – 240.

[5] The superscripted number after β specifies the position of the side chain on the corresponding β-amino acid; see T. Hintermann, D. Seebach, *Synlett* **1997**, 437 – 438.

[6] T. Hintermann, D. Seebach, *Chimia* **1997**, *51*, 244 – 247.

[7] D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1997**, *80*, 2033 – 2038.

[8] D. Seebach, S. Abele, T. Sifferlen, M. Hänggi, S. Gruner, P. Seiler, *Helv. Chim. Acta* **1998**, *81*, 2218 – 2243.

[9] G. Guichard, D. Seebach, *Chimia* **1997**, *51*, 315 – 318; G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 187 – 206; S. Abele, G. Guichard, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2141 – 2156.

[10] Peptide **1** is only soluble in hot MeOH; the β-heptapeptide H-(*S*)-β^2-HVal-(*R*)-β^3-HAla-(*S*)-β^2-HPhe-(*R*)-β^3-HPhe-(*S*)-β^2-HVal-(*R*)-β^3-HAla-(*S*)-β^2-HLeu-OH of type II (Figure 1a) also shows very poor solubility.

[11] Crystal structure data of **1**: $C_{29}H_{64}N_3O_6F_3 \cdot H_2O$, $M_r = 607.72$. Crystals were grown by slow evaporation of a solution of **1** in methanol. Crystal size: $0.20 \times 0.10 \times 0.10$ mm^3, orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 6.0636(7)$, $b = 20.191(4)$, $c = 27.139(4)$ Å, $V = 3322.7(9)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.22$ g cm^{-3}, $\mu = 0.816$ mm^{-1}, $2.73 < \theta < 59.94^\circ$; of 3074 reflections observed, 2658 were unique; Nonius CAD4 diffractometer (193 K, $\lambda(Cu_{K\alpha}) = 1.5418$ Å). The structure was solved by direct methods (SHELXS86; G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467 – 473) and refined by full-matrix least-squares analysis (G. M. Sheldrick, *SHELXL93*, *Program for the Refinement of Crystal Structures*, Universität Göttingen, **1993**); $w = 1/[\sigma^2(F_o^2) + (0.0710P)^2 + 0.83P]$, where $P = (F_o^2 + 2F_c^2)/3$ (heavy atoms anisotropic, hydrogen atoms isotropic, where hydrogen positions are based on stereochemical considerations). $R(F) = 0.039$, $wR(F^2) = 0.105$ for 392 variables and 2409 reflections with $I > 2\sigma(I)$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-113798. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[12] D. Seebach, J. L. Matthews, A. Meden, T. Wessels, C. Baerlocher, L. B. McCusker, *Helv. Chim. Acta* **1997**, *80*, 173 – 182.

[13] In H_2O, MeOH, and CF_3CH_2OH **2** shows an intensive positive Cotton effect at about 205 nm (molar ellipticity $[\Phi]$ of up to 1.2×10^5 (2×10^{-4} M **1**)), similar to the β-peptides that fold into the 12/10/12-helical structure,[3] an indication that this Cotton effect may be produced mainly by the ten-membered hydrogen-bonded ring.

[14] R. Koradi, M. Billeter, K. Wüthrich, *J. Mol. Graphics* **1996**, *14*, 51 – 54.

[15] Hitherto, turns of β-peptides were realized only by incorporating "unnatural" building blocks with cyclic α-and β-amino acids, as for example D-Pro-Xaa- or (*R*)-β^2-HPro-(*S*)-β^2-HPro-: S. Krauthäuser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719 – 11720; Y. J. Chung, L. A. Christianson, H. E. Stanger, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 10555 – 10556.

[16] First example of a pharmacologically active β-peptide with affinity for a human receptor: K. Gademann, M. Ernst, D. Hoyer, D. Seebach, *Angew. Chem.* **1999**, *111*, 1302 – 1304; *Angew. Chem. Int. Ed.* **1999**, *38*, 1223 – 1226.

[17] K. Gademann, B. Jaun, D. Seebach, R. Perozzo, L. Scapozza, G. Folkers, *Helv. Chim. Acta* **1999**, *82*, 1.

[18] D. Seebach, S. Abele, J. V. Schreiber, B. Martinoni, A. K. Nussbaum, H. Schild, H. Schulz, H. Hennecke, R. Wössner, F. Bitsch, *Chimia* **1998**, *52*, 734 – 739.

  1433-7851/99/3811-1597 $ 17.50+.50/0