Solid-Phase Synthesis of a β-Dodecapeptide with Seven Functionalized Side Chains and CD-Spectroscopic Evidence for a Dramatic Structural Switch When Going from Water to Methanol Solution

by **Jürg V. Schreiber**[1]) and **Dieter Seebach***

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

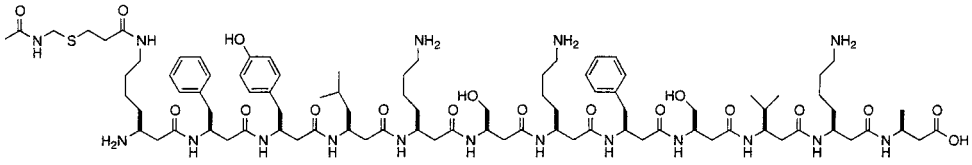
An all-β^3-dodecapeptide with a protected N-terminal thiol-anchoring group and with seven side chains has been synthesized in multi-mg amounts by the manual solid-phase technique, applying Fmoc methodology and the *Wang* resin. The sequence is β-HLys-β-HPhe-β-HTyr-β-HLeu-β-HLys-β-HSer-β-HLys-β-HPhe-β-HSer-β-HVal-β-HLys-β-HAla-OH (from N- to C-terminus; see **1**). The functional groups in the side chains of the building blocks were Boc (β-HLys) or *t*-Bu ether (β-HSer, β-HTyr) protected to allow for simultaneous deprotection and detachment from the resin with trifluoroacetic acid. All coupling steps were achieved with HBTU (= *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate)/HOBt (= 1-hydroxy-1*H*-benzotriazole) in DMF. For Fmoc (= (9*H*-fluoren-9-yl)methoxycarbonyl) deprotection, a protocol was developed to surmount the previously reported problems arising in solid-phase synthesis of β-peptides when the chain length exceeds seven or eight amino-acid moieties: for up to seven amino acids, a 20% solution of piperidine in DMF was used for removal of Fmoc; for the subsequent five amino acids, DBU and piperidine were employed for complete deprotection. The crude product was purified by preparative reversed-phase HPLC, and the yield of pure β-dodecapeptide derivative (**1**) was 23%. As the compound is well-soluble in H_2O, it was characterized by ^{1}H-NMR (in MeOH and H_2O), ^{13}C-NMR (in MeOH), and CD spectroscopy (in MeOH and in H_2O at pH values ranging from 3.5 to 11), and its molecular weight and composition were confirmed by high-resolution mass spectrometry (*Figs. 1–4*). In MeOH solution, the β-dodecapeptide exhibits the expected CD pattern typical of an (*M*)-3_{14}-helical secondary structure. In H_2O, however, the characteristic trough near 215 nm is missing in the CD spectrum, only a strong positive *Cotton* effect at 202 nm was observed, indicating the presence of β-peptidic secondary structures, containing ten-membered H-bonded rings, such as the *12/10* helix (*Fig. 4*, right) or the hairpin. Only a detailed NMR solution-structure analysis will provide the clues necessary for understanding the effects leading to the observed dramatic structural change of the highly functionalized β-dodecapeptide described.

1. Introduction. – For biological investigations of β^3-peptides consisting entirely of homologated proteinogenic α-amino acid residues, we needed to have compounds with hydrophobic, water-solubilizing side chains and with a protected thiol functionality for N-terminal attachments. Thus, we prepared the β-dodecapeptide derivative **1** by manual solid-phase synthesis; **1** is an all-L[2]) β^3-peptide H-β-HLys($N^{\varepsilon}CO(CH_2)_3$-S-Acm)-β-HPhe-β-HTyr-β-HLeu-β-HLys-β-HSer-β-HLys-β-HPhe-β-HSer-β-HVal-β-HLys-β-HAla-OH, with a protected ω-*N*-mercaptopropionyl group on the *N*-terminal β-HLys residue. So far, all β^3-oligopeptides of six or more residues with proteinogenic

[1]) Part of the projected Ph.D. thesis of *J.V.S.*, ETH Zürich.

[2]) The *Fischer* convention is more convenient, here, than the *CIP* system: the twelve β-amino acid residues all belong to the same chirality family (they are homochiral in *Lord Kelvin*'s definition), still the *CIP* descriptors are (from N- to C-terminus): *S*, *S*, *S*, *S*, *S*, *R*, *S*, *S*, *R*, *R*, *S*, *S*.

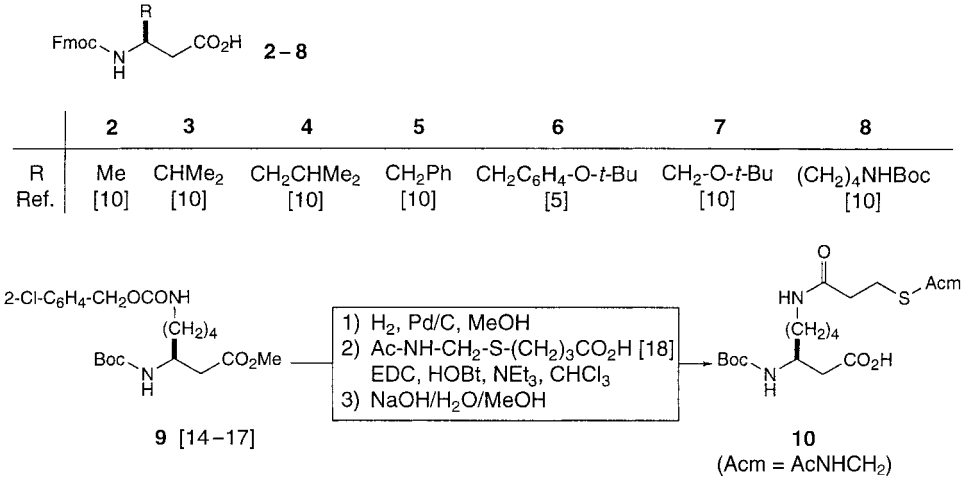
side chains investigated by us have been shown by NMR and CD analysis to fold to 3_{14} helices in methanol solution [1–8]. The β-dodecapeptide was devised such that, in an (M)-3_{14}-helical secondary structure, *Coulomb* repulsions between positively charged Lys side chains in (i) and ($i+3$) positions would be avoided (in the ideal 3_{14} helix, there is a juxtaposition at *ca.* 4.5 Å distance of side chains on residues in these positions!)[3]). Thus, we expected to see what we consider a 3_{14}-helix-typical CD spectrum of **1**. This turned out to be the case in MeOH, but not in H_2O solution.



1

2. Results and Discussion. – 2.1. *Synthesis.* We chose the Fmoc (=(9*H*-fluoren-9-yl)methoxycarbonyl) strategy for the synthesis of **1** on a solid support. The preparation of the required *N*-Fmoc-protected amino acids with hydrophobic or orthogonally protected side chains, **2**–**8**, has been described by us previously (*Scheme 1*), in full experimental detail [5][10][4]). Catalytic hydrogenation of **9**, followed by subsequent

Scheme 1. *Fmoc-Protected β-Amino Acids* **2**–**8** *and Preparation of the N-Terminal Building Block* **10** *for the β-Dodecapeptide* **1**



[3]) For β-peptides with juxtaposition of Lys side chains, see [5][9–13]. The may mimic amphipathic α-peptidic helices [12][13].

[4]) In the present work, **5** was prepared by catalytic hydrogenation of Boc-β^3-HPhe-OBn (prepared by decomposition of the corresponding phenylalanine-derived diazo ketone [16] in THF/$PhCH_2OH$ with catalytic amounts of silver benzoate), subsequent Boc deprotection, and treatment with *N*-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}oxy)succinimide (Fmoc-OSu) in H_2O/acetone in the presence of Na_2CO_3 (see *Exper. Part*).

conventional peptide coupling to *S*-acetamidomethyl-protected mercaptopropionic acid [18] with *N*-[3-(dimethylamino)propyl]propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC)/1-hydroxy-1*H*-benzotriazole (HOBt), and saponification under standard conditions furnished the *N*-Boc-protected modified β^3-HLys derivative **10** (see *Scheme 1*).

With the appropriate protected β-amino-acid building blocks at hand, we attached the first β-amino acid to the 4-(benzyloxy)benzyl-alcohol resin (*Wang* resin [19]) *via* classical 4-(dimethylamino)pyridine (DMAP)-catalyzed esterification with the symmetrical anhydride generated *in situ*, as previously decribed for α-peptides [20]. Thus, treatment of **2** (10 equiv. relative to resin loading) with diisopropylcarbodiimide (DIPCDI, 10 equiv.) in CH_2Cl_2/DMF afforded the intermediate that was added successively to preswelled *Wang* resin (initial loading: 0.99 mmol/g). DMAP (1 equiv. relative to resin loading) was added, and, after 1 h, the resin was filtered, washed, and dried overnight. The resin loading was determined, after treatment with 20% piperidine in DMF, by measuring the absorbance of the dibenzofulvene-piperidine adduct at 290 nm, using a calibration curve (see *Exper. Part*). A satisfactory esterification yield (75%) was monitored. The unreacted OH groups were 'neutralized' by acetylation using Ac_2O (10 equiv. relative to resin loading) in the presence of DMAP (*Scheme 2*).

We previously discussed the difficulties encountered in the syntheses and purification steps of longer β-peptides [5][10][17]. It is well-known in α-peptide chemistry that peptides with a chain length exceeding seven amino acids may be hard to separate from mismatched peptides by HPLC. *De Grado* and co-workers overcame this problem in syntheses of longer β-peptides (up to a β-octadecapeptide) by coupling fully protected β-tripeptide acids to the *N*-deprotected peptide on a solid support [13]. However, this method is not really suitable for the synthesis of long-chain β-peptides without repetitive sequences[5]). Furthermore, difficulties arose due to insufficient coupling and/or incomplete Fmoc removal. In the present work, we performed the peptide-bond formation under standard conditions (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU)/HOBt hydrate with $(i\text{-}Pr)_2EtN$); but, we used longer coupling time (at least 45 min), and, if necessary, we repeated the coupling step[6]).

Incomplete Fmoc deprotection has previously been observed in the course of solid-phase syntheses of long-chain α- [22] and β-peptides [10][17]. We reported that the yield was not improved by higher concentrations of piperidine in DMF or by longer deprotection times. However, replacement of piperidine by the stronger base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) provided more satisfactory results [10][7]). Further investigations have shown that the Fmoc group of the β-amino acid introduced at position 7 was removed incompletely during an automated synthesis of a β^3-

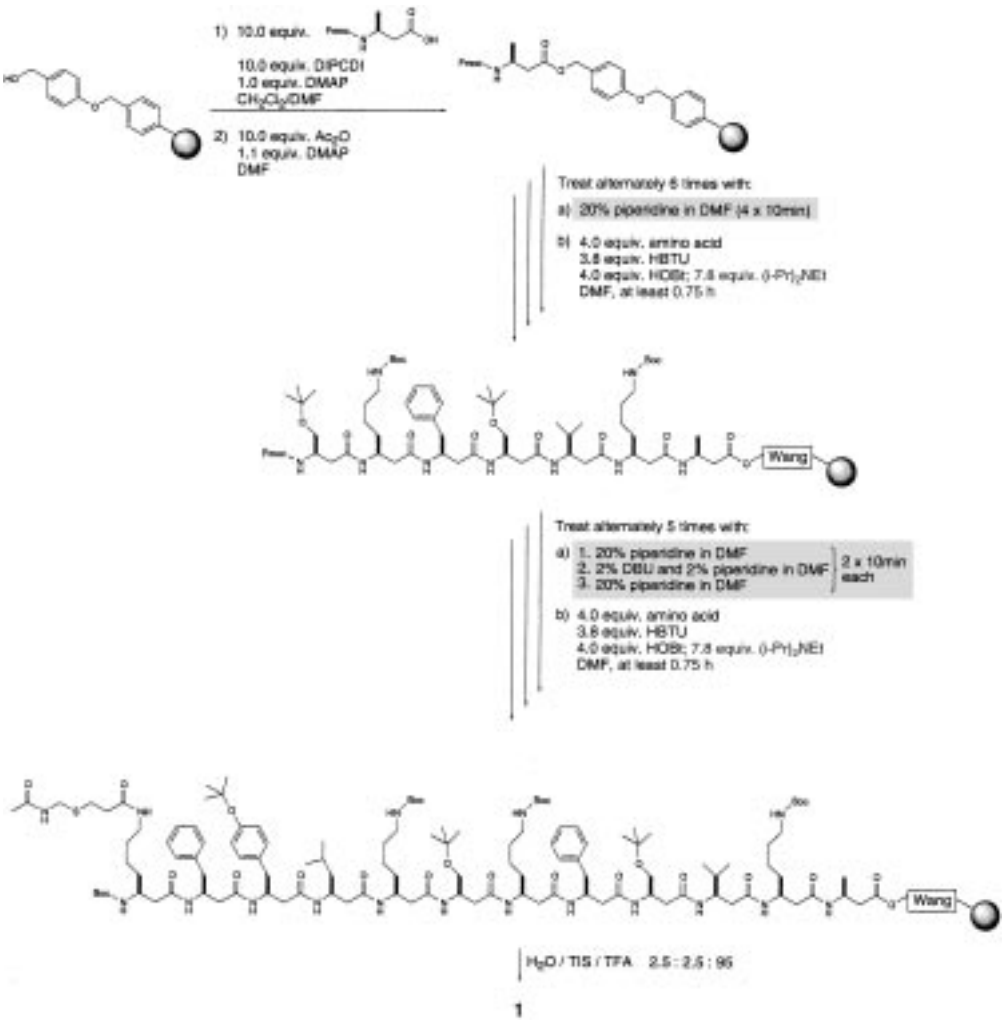
---

5) Of course, each peptide can, in principle, be synthesized by this strategy.

6) Coupling reactions were monitored with 2,4,6-trinitrobenzenesulfonic acid (TNBS) [21]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was either allowed to react for a longer time, or another 1–2 equiv. of the corresponding amino acid, together with coupling reagents, were added. Generally, longer coupling times do not, of course, lead to racemization or epimerization in the case of β^3-peptides.

7) A stronger base such as DBU was employed for the first time in the solid-phase synthesis of α-peptides [23].

Scheme 2. *Solid-Phase Synthesis of the β-Dodecapeptide* **1** *on* Wang *Resin*. TIS = $(i\text{-}Pr)_3SiH$, TFA = CF_3COOH. For other abbreviations, see accompanying text.



heptapeptide[8]) [24]. We attribute the incomplete Fmoc deprotection to secondary structures formed by the β-peptide[9]). Based on this knowledge, we have used a conventional protocol for the Fmoc removal (4×10 min piperidine/DMF 1:4) to introduce the first seven amino acids of **1**. The other amino acids (8th to 12th amino acid) were attached after deprotection of the Fmoc group according to a modified

---

[8]) The synthesis was performed on an *ABI 433* peptide synthesizer by established machine-assisted solid-phase methods with HBTU/HOBt/NMP activation protocols for Fmoc chemistry (*FastMoc*® protocol, *Applied Biosystems*).

[9]) A fully protected β-heptapeptide already adopts a 3_{14} helix in MeOH, according to CD investigation [8].

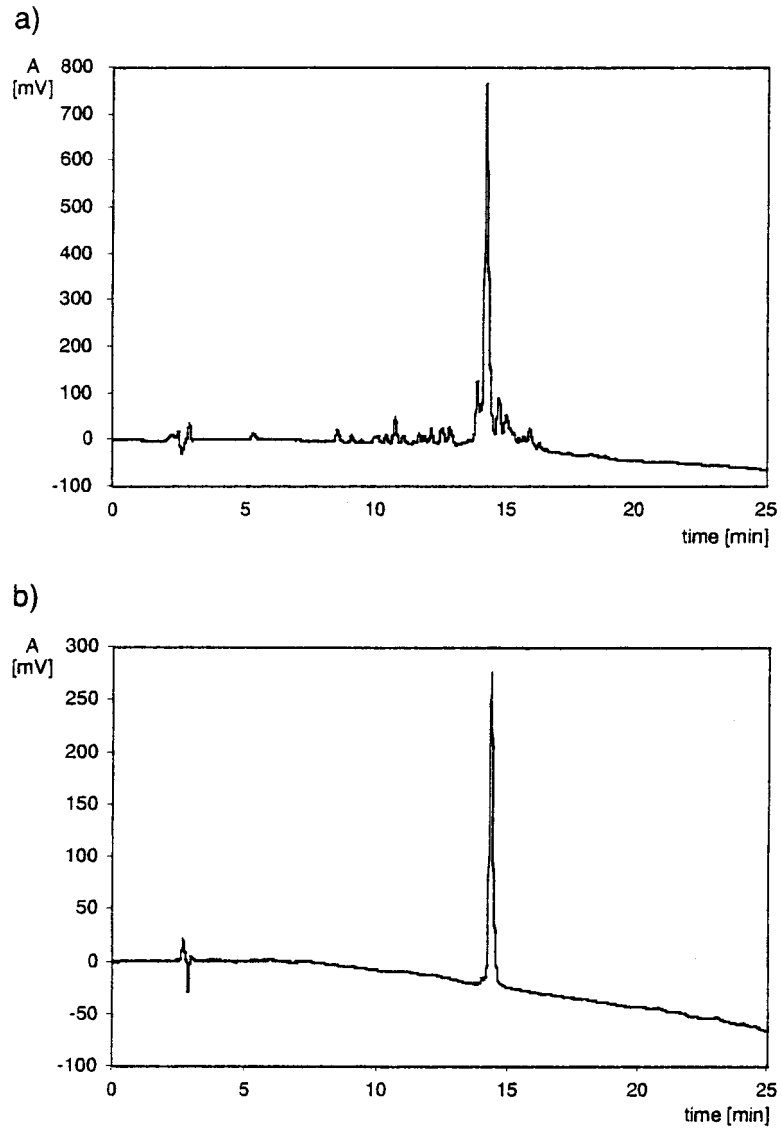


Fig. 1. *HPLC Elution profiles of crude* (a) *and purified* (b) **1** *(RP-C18* column, linear gradient of 0.1% TFA in H_2O and MeCN; see *Exper. Part*)

protocol with the stronger base DBU: 2×10 min piperidine/DMF, 2×10 min DBU/piperidine/DMF 1:1:48, 2×10 min piperidine/DMF 1:4 (*Scheme 2*). Treatment of the peptide resin with CF_3COOH(TFA)/$(i\text{-}Pr)_3SiH/H_2O$ 95:2.5:2.5 and precipitation with Et_2O afforded the crude thiol-protected peptide **1** (*Scheme 2*). The crude product had a purity of 59%, as determined by reversed-phase (RP) HPLC (*Fig. 1,a*). HPLC Purification on a preparative *C18* column afforded the pure β-dodecamer **1** in an

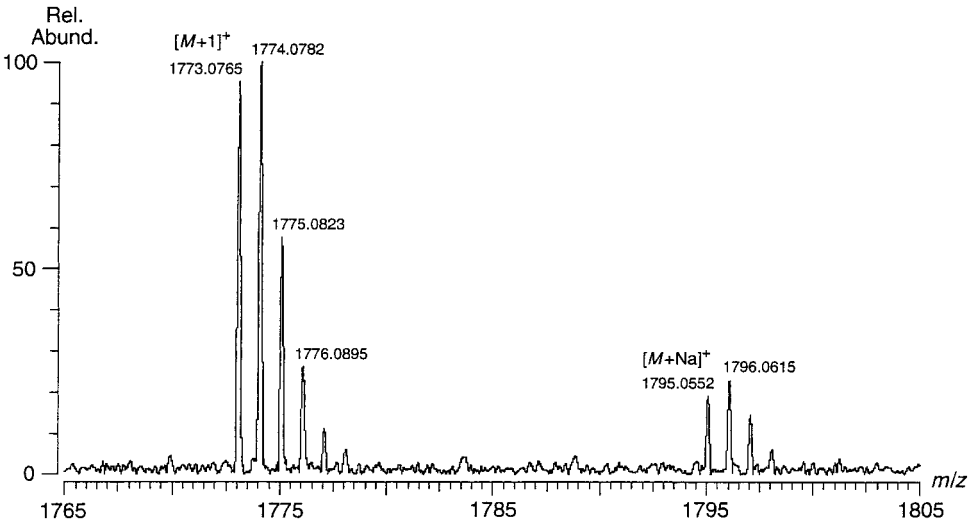


Fig. 2. *HR-MALDI Mass spectrum of* **1** (see *Exper. Part*)

overall yield of 23%[10]) with a purity of more than 99%, as determined by RP-HPLC (*Fig. 1,b*).

2.2. *Spectroscopic Identification.* The characterization of β^3-dodecapeptide **1** was performed by well-established methods of analytical spectroscopy. The composition of **1** was confirmed by high-resolution matrix-assisted laser-desorption ionization (HR-MALDI) mass spectrometry (*Fig. 2*). The spectrum of **1** shows mainly the peak of the monocation (MH^+) with its isotopic patern. Peaks at higher *m*/*z* can be assigned to Na complexes of the corresponding molecular ions. The difference between the experimentally determined accurate mass (*m*/*z* 1773.0765) and the calculated molecular mass of the monocation (*m*/*z* 1773.0747) is lower than the tolerance of the instrument (5 ppm). To judge the synthetic procedure by spectroscopic product characterization, the primary structure of **1** was verified by ^{1}H- and ^{13}C-NMR spectroscopy (*Fig. 3*). NMR Measurements have been carried out with the TFA salt of **1** in CD_3OH and in H_2O/D_2O 9:1. For a first hint of the secondary structure, we measured CD spectra of **1** in 0.2 mM MeOH and H_2O solutions. We expected **1**, which carries all substituents in the β^3-position, to exhibit the CD pattern typical of a 3_{14} helix with a trough between 215 and 220 nm, a zero-crossing between 205 and 210 nm, and a positive peak near 200 nm. This was the case in MeOH ($\theta = -16.8 \cdot 10^4$ [10 deg · cm^2 · mol^{-1}] at 215 nm, $\theta = 0$ at 207 nm, $\theta = 19.8 \cdot 10^4$ at 201 nm); however, in H_2O solution, the CD pattern with a single maximum at 202 nm ($\theta = 11.4 \cdot 10^4$) was more typical of what we assign to a *12*/*10*-helical structure (*Fig. 4,a* and *b*) [8]. We also investigated the pH-value dependence of the *Cotton* effect: the CD curves of **1** in H_2O at pH 3.5 and 7 (the amino groups are protonated and thus positively charged!) do not differ substantially ($\theta = 13.7 \cdot 10^4$ at 202 nm and $\theta = 14.6 \cdot 10^4$ at 201 nm, resp.), whereas the

[10]) The TNBS test has been performed several times before removing the next Fmoc group. This led to a non-quantified loss of resin.

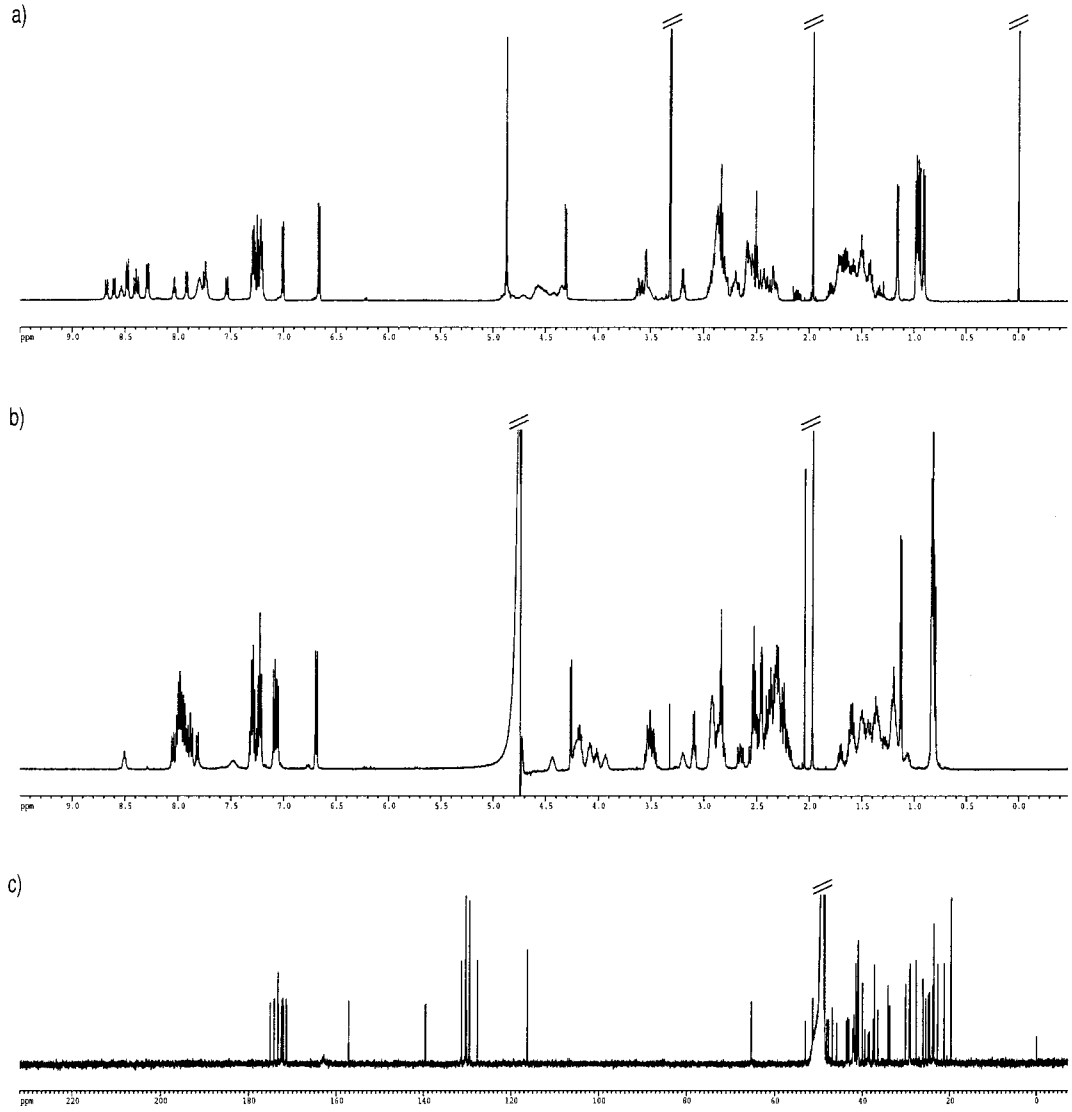


Fig. 3. *NMR Spectra of* **1**. ^{1}H-NMR Spectra measured in CD_3OH (*a*) and in H_2O/D_2O 9:1 (*b*). ^{13}C-NMR Spectrum measured in CD_3OH (*c*). The β-peptide was employed as its TFA salt as obtained by lyophilization.

CD pattern of **1** at pH 11 exhibits a significantly lower intensity at around 202 nm (*Fig. 5*).

3. Conclusion. – We have for the first time synthesized on solid phase a larger β^3-peptide with many functionalized side chains. As compared to previous attempts [10], the yield could be greatly improved with DBU as base for Fmoc deprotection, as the chain length exceeded seven residues. This simple modification will, no doubt, be

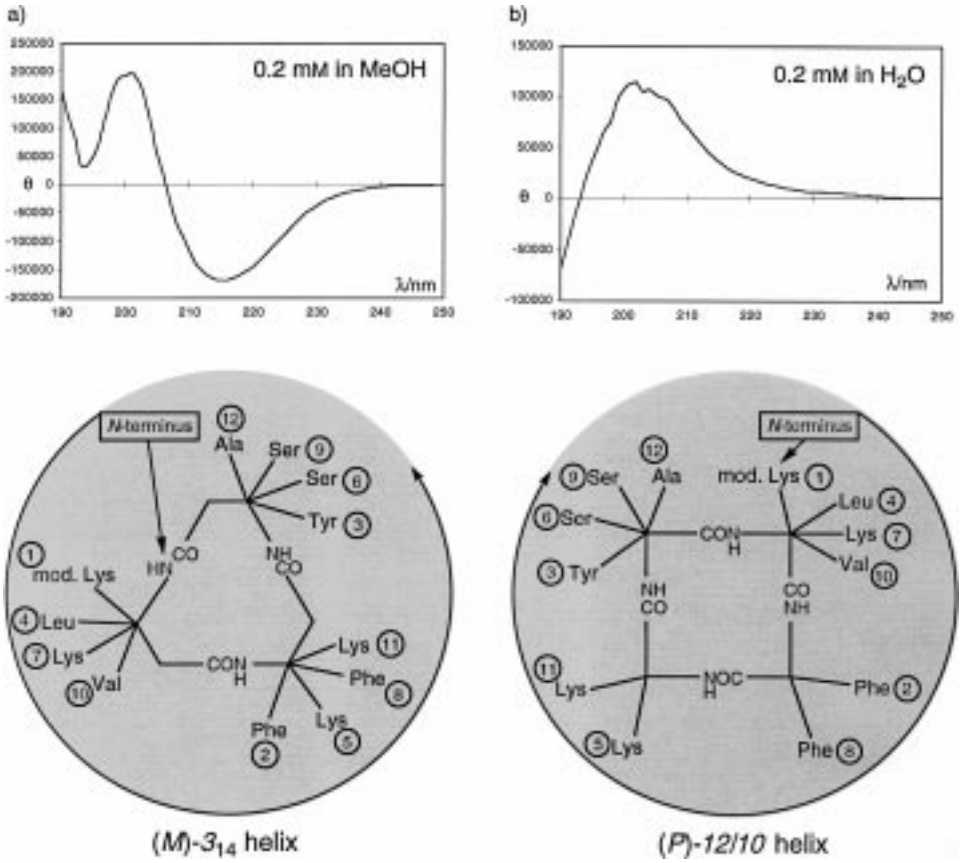


Fig. 4. *CD Spectra of β-dodecapeptide* **1** *in methanol and water solution. a*) CD Curve of **1** in MeOH. The negative *Cotton* effect between 210 and 220 nm is considered characteristic of the 3_{14}-helical structure. In such a structure, the side chains would be in juxtapositions as indicated in a schematic top view of an idealized 3_{14} helix. Direct destabilization of positively charged HLys residues is prevented by the intermitting hydrophobic side chains of HPhe. *b*) CD-Curve of **1** in H_2O. In the more polar solution, **1** exhibits the single maximum pattern that we assign to *12*/*10*-helical structures. In this case, other side chains could be in juxtaposition, as presented in a schematic top view of an idealized *12*/*10* helix: here, the polar side chains of Lys are 'on the one side' and the hydrophobic side chains of Phe 'on the other side'! The spectra were recorded at room temperature. The concentration was 0.2 mM. Molar ellipticity $[\theta]$ in 10 deg · cm^2 · mol^{-1}. Compound **1** was employed as its TFA salt obtained after lyophilization. The three letter code of the amino acids used here are meant to indicate the side chains on the corresponding β^3-amino acid residues.

applicable for the SPS of much larger β-oligopeptides. The protected thiol anchoring group chosen will allow for attachment of our β-dodecapeptide to proteins or to other biomacromolecules, and to solid support (*cf.* affinity chromatography). The tentative assignment for the secondary structure in H_2O to a (*P*)-*12*/*10*-helix (*Fig. 4*, right side) will have to be confirmed – or disproved – by a detailed NMR solution analysis. From previous CD investigations, we must also consider a hairpin structure with antiparallel β-peptidic pleated sheet(s) [8][17][25]. It will be especially exciting to find out which subtle interactions are governing the interconversion between the two secondary

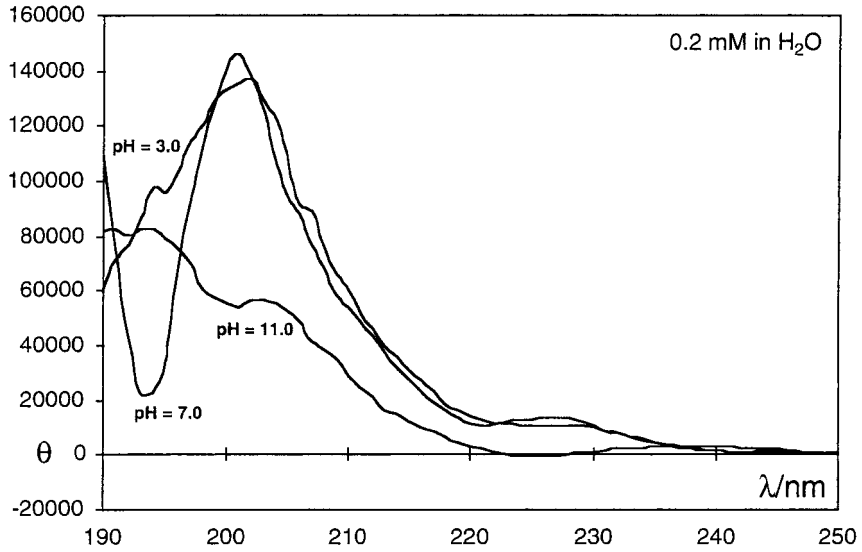


Fig. 5. *CD Spectra of β-dodecapeptide* **1** *in aqueous solution at different pH values.* The spectra were recorded at room temperature, at a concentration of 0.2 mM. Molar ellipticity [θ] in 10 deg · cm^2 · mol^{-1}; **1** was employed as its TFA salt as obtained by lyophilization.

structures of **1** upon solvent change. The two structures are probably quite close in energy (*cf.* the 3_{14}-helical 'impurity' present in the MeOH solution of a *12/10*-helical β^2/β^3-peptide, as detected by *GROMOS 96* molecular-modelling calculation [26], confirming some 'disturbing' NOEs in the NMR structure analysis [3][8]). In the world of α-peptides, solvents such as MeOH, CF_3CH_2OH, and $(CF_3)_2CHOH$ are well-known to have a stabilizing effect on 3.6_{13}- or 3_{10}-helical structures *vs.* random-coil arrangements [27]. Equilibria between two fundamentally different helical structures do, however, not occur with α-peptides and proteins.

A generous gift of amino acid derivatives by *Degussa AG*, Wolfgang, is gratefully acknowledged. We thank the *Novartis Stipendienfonds* for a scholarship granted to *J.V.S. Novartis Pharma AG* (Basel) has also supported this work. Dr. *S. Abele* and Dr. *G. Guichard* are acknowledged for supplying us with samples of the β-amino acids **3**, **4**, and **6**–**9**.

Experimental Part

1. *General. Abbreviations:* FC (flash chromatography), h.v. (high vacuum, 0.01–0.1 Torr), β-HXxx (β-homoamino acid) [1–3][28], RV (rotatory evaporator). Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). Et_3N was distilled from CaH_2 and stored over KOH. $(i\text{-}Pr)_2NH$ was freshly distilled over CaH_2. Amino acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. All other reagents were used as received from *Fluka*. The β-amino acids were prepared according to literature procedures [5][10]. ***Caution:*** The generation and handling of CH_2N_2 requires special precautions [29]. Reactions carried out with the exclusion of light were performed in flasks completely wrapped in aluminium foil. TLC: *Merck* silica gel *60 F254* plates; detection with UV or dipping into a soln. of ninhydrin (300 mg), HOAc (3 ml), and BuOH (100 ml), followed by heating. FC: *Fluka* silica gel *60* (40–63 mm); at *ca.* 0.3 bar. Anal. HPLC: *Knauer* HPLC system (pump type *WellChrom K-1000 Maxi-Star*, *EuroChrom 2000* integration package, degasser, UV detector (variable-wavelength monitor)), *Macherey-Nagel* C_{18} column (*Nucleosil 100-5* C_{18}

(250 × 4 mm)). Prep. HPLC: *Knauer* HPLC system (pump type *64*, programmer *50*, UV detector (variable-wavelength monitor)), *Merck/Hitachi* HPLC system (pump type *L-6250*, UV detector *L-4000*), and *Waters* HPLC system (HPLC pump type *515*, dual wavelength-absorbance detector *2487*, fraction collector); *Macherey-Nagel* C_{18} column (*Nucleosil 100-7* C_{18} (250 × 21 mm)). M.p.: *Büchi-510* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t. The used $CHCl_3$ (*Fluka*) was filtered over Alumina N, Akt. I (*ICN Biomedicals GmbH*, Germany). CD Spectra: *Jasco J-710* spectropolarimeter, from 190 to 250 nm at r.t. in 1-mm rectangular cells. The optical system was flushed with N_2 at a flow rate of *ca.* 10 l/min; parameters: band width 1.0 nm, resolution 0.2–1 nm, sensitivity 100 mdeg, response 0.5 s, speed 50 nm/min, 5 accumulations. All spectra were corrected for the corresponding solvent spectrum. Peptide concentration 0.2 mM. The molar ellipticity $[\theta]$ in 10 deg · cm^2 · mol^{-1} (λ in nm) is calculated for the corresponding peptide (not normalized), taking into account the mass of TFA for each free amino group. Smoothing was done by *Jasco* software. Solvents: MeOH (HPLC grade), aq. buffers: pH 3.5: 0.1M AcOK/AcOH, pH 7.0: 0.1M KH_2PO_4/K_2HPO_4, prepared according to [30]; pH 11.0: 0.05M $NaHCO_3$/NaOH, prepared according to [31]. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz) or *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (= 0 ppm); *J* values in Hz; compound **1** shows the presence of a rotamer (see the comment in the individual procedure). MS: *IonSpec Ultima 4.7 T FT* Ion Cyclotron Resonance (ICR) mass spectrometer (*Hi Res MALDI*, in a 2,5-dihydroxybenzoic-acid matrix); in *m/z* (% of basis peak). Elemental analyses were performed by the Mikroanalytisches Laboratorium, Laboratorium für Organische Chemie, ETH-Zürich.

2. *Reversed-Phase (RP) HPLC Analysis and Purification*. RP-HPLC Analysis was performed on a *Macherey-Nagel* C_{18} column/*Nucleosil 100-5* C_{18} (250 × 4 mm) with a linear gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN) at a flow rate of 1 ml/min; UV detection at 220 nm; t_R in min. RP-HPLC Purification was performed on a *Macherey-Nagel* C_{18} column/*Nucleosil 100-7* C_{18} (250 × 21 mm) with a linear gradient of *A* and *B* at a flow rate of 20 ml/min (*Knauer* and *Waters* system) or 15 ml/min (*Merck/Hitachi* system), UV detection at 220 nm.

3. *Preparation of the β-Amino Acids and Synthesis of β^3-Dodecapeptide* **1**. *Benzyl (3S)-3-{[(tert-butoxy)carbonyl]amino}-4-phenylbutanoate* (Boc-β^3-HPhe-OBn). According to the reported procedure [32], Boc-Phe-CHN_2 (prepared as described in [33]; 13.4 g, 46.3 mmol) was dissolved in THF/BnOH 8.5 : 1.5 (*v/v*) (0.25M). Under the exclusion of light, the soln. was cooled to −25°, and a soln. of AgO_2CPh (1.16 g, 5.07 mmol) in Et_3N (18.7 ml, 134.17 mmol) was added. The resulting mixture was allowed to warm to r.t. and stirred for 3 h in the dark. The solvent was removed under reduced pressure and the residue dissolved in AcOEt. The org. phase was washed with sat. aq. $NaHCO_3$, NH_4Cl, and NaCl solns. and dried ($MgSO_4$). The solvent and the excess BnOH were removed by evaporation on RV under h.v. Recrystallization (AcOEt/hexane) yielded Boc-β^3-HPhe-OBn (11.97 g, 70%). Colorless needles. M.p. 77.5–78.0°. $[\alpha]_D^{rt} = -4.9$ (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3438*w*, 3008*w*, 2980*w*, 1707*s*, 1498*s*, 1455*m*, 1391*m*, 1367*m*, 1163*s*, 1050*w*, 1029*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.40 (*s*, *t*-Bu); 2.49 (*dd*, *J* = 15.9, 5.9, 1 H, CH_2CO); 2.54 (*dd*, *J* = 15.9, 5.5, 1 H, CH_2CO); 2.80 (*dd*, *J* = 13.5, 7.7, 1 H, PhCH_2); 2.89–2.95 (*m*, 1 H, PhCH_2); 4.11–4.21 (*m*, CHN); 5.03–5.08 (br. *d*, NH); $\nu_A = 5.10$, $\nu_B = 5.15$ (*AB*, $J_{AB} = 12.3$, CH_2O); 7.12–7.14 (*m*, 2 arom. H); 7.18–7.28 (*m*, 3 arom. H); 7.30–7.39 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 28.4 (Me); 37.7, 40.3 (CH_2); 48.9 (CH); 66.4 (CH_2); 79.4 (C); 126.6, 126.8, 128.3, 128.4, 128.5, 128.6, 129.4 (CH); 135.7, 137.7, 155.1, 171.5 (C). HR-MALDI-MS: 392.1830 (41, $[M + Na]^+$, $C_{22}H_{27}NNaO_4^+$; calc. 392.1838), 292.1312 (100, $[M - Boc + Na]^+$, $C_{17}H_{19}NNaO_2^+$; calc. 292.1313). Anal. calc. for $C_{22}H_{27}NO_4$ (369.46): C 71.52, H 7.37, N 3.79; found: C 71.37, H 7.43, N 3.79.

(S)-3-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-4-phenylbutanoic acid (Fmoc-(*S*)-β^3-HPhe-OH; **5**): Boc-β^3-HPhe-OBn (4.26 g, 11.52 mmol) was dissolved in MeOH (115 ml), and Pd/C (10%, 0.43 g) was added. The apparatus was evacuated and flushed with H_2 (3×), and the mixture was stirred under H_2 (1 bar) for 10 h. The mixture was filtered through *Celite* and concentrated under reduced pressure. The residue was dried under h.v. The crude carboxylic acid was Boc-deprotected according to the procedure described in [1]. The residue was dissolved in CH_2Cl_2 (25 ml) and cooled to 0° (ice-bath). TFA (25 ml) was added, and the mixture was allowed to warm to r.t. and then stirred for 1.5 h. Concentration under reduced pressure and drying under h.v. yielded the crude TFA salt, which was Fmoc-protected according to [8]. A stirred soln. of the TFA salt in 0.6M aq. Na_2CO_3 (57.6 ml) was treated with a soln. of Fmoc-OSu (4.28 g, 12.69 mmol) in acetone (57.6 ml). The pH was readjusted to 9–10 with additional Na_2CO_3. After 6 h, the mixture was diluted with H_2O and extracted with Et_2O. The aq. phase was carefully adjusted to pH 1–2 at 0° (ice-bath) with 1N HCl and extracted with AcOEt (3×). The org. layer was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. Recrystallization (CH_2Cl_2/hexane) afforded Fmoc-(*S*)-β^3-HPhe-OH (4.26 g, 92%). ^{1}H-NMR Data are in agreement with those in [10].

Methyl (S)-7-({3-[(Acetylamino)methylsulfanyl]propionyl}amino)-3-{[(tert-butoxy)carbonyl]amino}heptanoate (Boc-(*S*)-β^3-HLys(N$^\omega$CO(CH$_2$)$_3$-S-Acm)-OMe). Compound **9** (prepared as described [17]; 2.24 g, 5.06 mmol) was dissolved in MeOH (100 ml), and Pd/C (10%, 0.22 g) was added. The apparatus was evacuated and flushed with H_2 (3×), and the mixture was stirred under H_2 (1 bar) for 14 h. The mixture was filtered through *Celite* and concentrated under reduced pressure. The residue was dried under h.v. According to [34], the crude N^ω-deprotected amino acid was coupled to 3-{[(acetylamino)methyl]sulfanyl}-propionic acid (prepared according to [18]). A soln. of the amine fragment in $CHCl_3$ (22 ml) at 0° (ice-bath) under Ar was treated successively with Et_3N (0.90 ml, 6.46 mmol), a soln. of 3-{[(acetylamino)methyl]sulfanyl]propionic acid (1.15 g, 6.49 mmol) in $CHCl_3$ (10 ml), HOBt (0.98 g, 6.49 mmol), and EDC (1.25 g, 6.51 mmol). The mixture was allowed to warm to r.t., and stirring was continued for 23 h. The mixture was diluted with $CHCl_3$ and washed with 1N HCl, aq. sat. $NaHCO_3$, and NaCl solns. The org. phase was dried ($MgSO_4$) and evaporated. Recrystallization ($CHCl_3$/hexane) yielded Boc-(*S*)-β^3-HLys(N$^\omega$CO(CH$_2$)$_3$-S-Acm)-OMe (2.01 g, 92%). White microcrystalline solid. M.p. 114.5–116.0°. $[\alpha]_D^{r.t.} = -17.7$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3441*m*, 3008*m*, 2940*m*, 1667*s*, 1506*s*, 1439*m*, 1368*s*, 1166*s*, 1090*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.27–1.59 (*m*, 3 CH$_2$); 1.44 (*s*, *t*-Bu); 2.03 (*s*, Ac); 2.51–2.56 (*m*, 2 CH$_2$); 2.84–2.87 (*m*, CH$_2$); 3.24–3.25 (*m*, CH$_2$); 3.68 (*s*, MeO); 3.86–3.89 (*m*, NCH); 4.36 (*dd*, $J = 14.0$, 6.3, 1 H, NCH$_2$S); 4.38 (*dd*, $J = 14.0$, 6.4, 1 H, NCH$_2$S); 5.08 (br. *d*, $J = 8.7$, NH); 6.30–6.38 (*m*, NH); 6.88–6.95 (*m*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.2 (CH$_2$); 23.3 (Me); 26.8 (CH$_2$); 28.4 (Me), 29.0, 34.1, 37.1, 39.1, 39.4, 40.7 (CH$_2$); 47.4 (CH); 51.7 (Me); 79.4, 155.6, 170.5, 171.7, 172.1 (C). HR-MALDI-MS: 456.2134 (16, $[M + Na]^+$, $C_{19}H_{35}N_3NaO_6S^+$; calc. 456.2144), 356.1619 (44, $[M - Boc + Na]^+$, $C_{14}H_{27}N_3NaO_4S^+$; calc. 356.1620), 263.1431 (100, $[M - Boc - Acm + H]^+$, $C_{11}H_{23}N_2O_3S^+$; calc. 263.1429). Anal. calc. for $C_{19}H_{35}N_3O_6S$ (433.57): C 52.64, H 8.14, N 9.69; found: C 52.75, H 8.17, N 9.64.

(S)-7-({[3-(Acetylamino)methylsulfanyl]propionyl}amino)-3-{[(tert-butoxy)carbonyl]amino}heptanoic acid (Boc-(*S*)-β^3-HLys(N$^\omega$CO(CH$_2$)$_3$-S-Acm)-OH; **10**). According to [35], a soln. of Boc-(*S*)-β^3-HLys(N$^\omega$CO(CH$_2$)$_3$-S-Acm)-OMe (0.44 g, 1.02 mmol) in MeOH (2.55 ml) was treated with aq. 1N NaOH (3.1 ml). Aq. 1N NaOH was added in portions (1.5 ml after 10 h and 1.5 ml after 15.5 h), and, after 21.5 h, the soln. was diluted with H_2O and extracted with pentane (2×). The soln. was adjusted to pH 2 with 1N HCl and extracted with AcOEt (5×). The org. phase was washed with H_2O, dried ($MgSO_4$), concentrated under reduced pressure, and the residue was dried under h.v. to yield **10** (0.32 g, 74%). Slightly yellow oil. For anal. purposes, a soln. of **10** in sat. aq. $NaHCO_3$ was extracted with AcOEt (1×), adjusted to pH 2 with 1N HCl, and extracted with AcOEt. The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure and the residue was dried under h.v. This purification sequence was performed twice. $[\alpha]_D^{r.t.} = -10.7$ ($c = 0.8$, $CHCl_3$). IR ($CHCl_3$): 3670*w*, 3439*m*, 3340*m*, 3007*m*, 2936*m*, 1706*s*, 1662*s*, 1506*s*, 1393*m*, 1368*s*, 1167*s*, 1088*w*, 1010*w*, 865*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.38–1.60 (*m*, 3 CH$_2$); 1.44 (*s*, *t*-Bu); 2.05 (*s*, Ac); 2.49–2.64 (*m*, 2 CH$_2$); 2.83–2.87 (*m*, CH$_2$); 3.22–3.30 (*m*, CH$_2$); 3.85–3.96 (*m*, NCH); 4.37 (*dd*, $J = 14.0$, 6.4, 1 H, NCH$_2$S); 4.40 (*dd*, $J = 14.0$, 6.4, 1 H, NCH$_2$S); 5.12 (br. *d*, $J = 7.5$, NH); 6.29–6.35 (*m*, NH); 6.65–6.75 (*m*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.9 (CH$_2$); 23.2 (Me); 26.7 (CH$_2$); 28.4 (Me, CH$_2$); 33.8, 37.0, 38.9, 39.2, 40.6 (CH$_2$); 47.2 (CH); 79.6, 155.7, 171.2, 171.9, 174.1 (C). HR-MALDI-MS: 442.1985 (22, $[M + Na]^+$, $C_{18}H_{33}N_3NaO_6S^+$; calc. 442.1988), 342.1467 (76, $[M - Boc + Na]^+$, $C_{13}H_{25}N_3NaO_4S^+$; calc. 342.1463), 249.1276 (100, $[M - Boc - Acm + H]^+$, $C_{10}H_{21}N_2O_3S^+$; calc. 249.1273).

H-β³-HLys(N$^\omega$CO(CH$_2$)$_3$-S-Acm)-β-³-HPhe-β³-HTyr-β³-HLeu-β³-HLys-β³-HSer-β³-HLys-β³-HPhe-β³-HSer-β³-HVal-β³-HLys-β³-HAla-OH (**1**). Esterification of the Fmoc-β^3-HAla-OH with *Wang* resin was performed according to [20]. A soln. of Fmoc -β^3-HAla-OH (prepared according to [10]; 0.61 g, 1.86 mmol) in CH_2Cl_2 (9.6 ml) and DMF (1 ml) was cooled (ice-bath). DIPCDI (0.29 ml, 1.87 mmol) was then added successively. After stirring for 30 min at 0° (ice-bath), CH_2Cl_2 was removed (RV). DMF (6 ml) was added to the residue, and the milky soln. obtained was filtered over a microfilter (*CHROMAFIL*, 'Einmalfilter', *Macherey-Nagel*, *Typ PET-45/25*, pore ∅ = 0.45 μm, filter ∅ = 25 mm) and added to the resin (189 mg; initial loading: 0.99 mmol/g) preswelled in DMF (1 ml) for 50 min. The suspension was then treated with DMAP (23.6 mg, 0.19 mmol) and mixed by Ar bubbling for 1 h at r.t. Subsequently, the resin was filtered off, washed with DMF (3 ml, 5 × 1 min) and CH_2Cl_2 (3 ml, 5 × 1 min), and dried under h.v. overnight. The loading was determined by measuring the absorbance of the benzofulvene-piperidine adduct: two aliquots of the Fmoc-amino acid resin were weighed exactly (m_1(resin) = 5.125 mg, m_2(resin) = 2.230 mg) and dissolved in graduated cylinders with piperidine (20%) in DMF to a total volume of 25 ml (V^1, [ml]) and 10 ml (V^2, [ml]), resp. After 30 min, the mixtures were suspended in an UV cell, and piperidine (20%) in DMF in another UV cell (blank), and the absorbance (A) was measured at 290 nm. The concentrations (c_1 and c_2 in [mM]) of the benzofulvene piperidine

adduct in soln. was determined using a calibration curve [24]. The loading (Subst) was then calculated according to *Eqn. 1*.

$$\text{Subst}_n\ [\text{mmol/g resin}] = c_n \cdot V_n/(m_n(\text{resin}) - [c_n \cdot V_n \cdot \{\text{MW} - 18.0\}/1000]) \quad (1)$$

MW = molecular weight of the Fmoc-protected β-amino acid

The yield for the attachment the resin (loading yield) was determined by *Eqn. 2*.

$$\text{Loading yield} = [(\text{Subst}_1 + \text{Subst}_2)/2]/\text{Subst}_{\text{theor.}} \quad (2)$$

Loading (as an average of the two determined substitutions): 0.75 mmol/g (75%); 136 μmol of anchored Fmoc-β^3-HAla-OH. The peptide resin was covered with DMF (2 ml), and the unreacted OH groups were capped using Ac_2O (176 ml; 18.6 mmol) and DMAP (24.9 mg, 0.20 mmol; added as a soln. in 1 ml DMF) for 70 min under Ar bubbling. The resin was then filtered and washed with DMF (2 ml, 8 × 1 min). The Fmoc group of the first amino acid attached to the *Wang* resin was removed using 20% piperidine in DMF (2 ml, 4 × 10 min) under Ar bubbling. After filtration, the resin was washed with DMF (2 ml, 3 × 1 min). Solid-phase synthesis was continued by sequential incorporation of **8** (prepared as described in [10]), **3** (prepared as described in [10]), **7** (prepared as described in [10]), **5**, and **8**. For each coupling step, the resin was treated with a soln. of the Fmoc-β^3-amino acid (4 equiv.), HBTU (3.8 equiv.), and HOBt (4 equiv.) in DMF (1.5 ml). After mixing by Ar bubbling for 5 min, $(i\text{-Pr})_2$EtN (7.8 equiv.) in DMF (0.5 ml) was added, and the suspension was further mixed by Ar bubbling for 45–60 min. Monitoring of the coupling reaction was performed with TNBS [21]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 1–12 h, or after filtration, the peptide resin was treated again with the same Fmoc-amino acid (1–2 equiv.) and coupling reagents. The resin was then filtered off and washed with DMF (2 ml, 3 × 1 min) prior to the following Fmoc-deprotection step with 20% piperidine in DMF (2 ml, 4 × 10 min). The Fmoc group of the 6th amino acid was removed with 20% piperidine in DMF (2 ml, 2 × 10 min), DBU/piperidine/DMF 1:1:48 (2 ml, 2 × 10 min), and 20% piperidine in DMF (2 ml, 2 × 10 min) under Ar bubbling. After filtration, the resin was washed with DMF (2 ml, 3 × 1 min), and solid-phase synthesis was continued by sequential incorporation of **7**, **8**, **4** (prepared as described in [10]), **6** (prepared as described in [5]), **5**, and **10**. For each coupling step, the resin was treated as described above[11]). The resin was then filtered off, washed with DMF (2 ml, 5 × 1 min), AcOH (2 ml, 5 × 1 min), CH_2Cl_2 (2 ml, 5 × 1 min), and MeOH (2 ml, 5 × 1 min), and dried over KOH under h.v. for 24 h. The dry peptide-resin was then treated for 2 h with 10 ml of TFA/H_2O/$(i\text{-Pr})_3$SiH 95:2.5:2.5. The resin was removed by filtration, washed with TFA, and the org. phase containing the peptide was concentrated under reduced pressure. The precipitate, which formed upon addition of cold Et_2O to the oily residue, was collected by centrifugation: crude **1** as TFA salt (192 mg), purity 59% (RP-HPLC). Purification by RP-HPLC (*Knauer* and *Waters* system; 5–50% *B* in 40 min; *Merck*/*Hitachi* system: 5–20% *B* in 10 min, then 20–35% *B* in 25 min) yielded the TFA salt of **1** (56 mg, 23%). White solid. RP-HPLC (10–60% *B* in 20 min, then 60–99% *B* in 5 min): t_R 14.3 min; purity >99%. M.p. 133–134° (dec.). CD (0.2 mM in MeOH): $-16.8 \cdot 10^4$ (215 nm), 0 (207 nm), $19.8 \cdot 10^4$ (201 nm). CD (0.2 mM in H_2O): $11.4 \cdot 10^4$ (202 nm). CD (0.2 mM pH 3.5): $+13.7 \cdot 10^4$ (202 nm). CD (0.2 mM pH 7.0): $+14.6 \cdot 10^4$ (201 nm). ^{1}H-NMR (500 MHz, CD_3OH; solvent suppression by presaturation): 0.91 (*d*, *J* = 6.6, Me); 0.95 (*d*, *J* = 6.5, Me); 0.97 (*d*, *J* = 6.8, Me); 0.98 (*d*, *J* = 6.7, Me); 1.16 (*d*, *J* = 6.7, Me); 1.29–1.83 (*m*, 28 CH); 1.96 (*s*, Ac); 2.30–2.96 (*m*, 38 H); 3.17–3.22 (*m*, CH_2); 3.46–3.66 (*m*, 6 H); 4.30 (*d*, *J* = 6.3, SCH_2N); 4.32–4.92 (*m*, ≥7 H, partially saturated); 6.66 (*d*, *J* = 8.6, 2 arom. H); 6.66 (*d*, *J* = 4.6, NH); 7.01 (*d*, *J* = 8.6, 2 arom. H); 7.20–7.31 (*m*, 10 arom. H); 7.54 (*d*, *J* = 9.1, NH); 7.74–7.79 (*m*, NH, NH_3^+); 7.92 (*d*, *J* = 8.7, NH); 8.03 (*t*, *J* = 5.5, CH_2NH); 8.29 (*d*, *J* = 8.5, 2 NH); 8.38 (*d*, *J* = 11.4, NH); 8.40 (*d*, *J* = 9.9, NH); 8.48 (*d*, *J* = 9.1, NH); 8.53 (*t*, CH_2NH); 8.61 (*d*, *J* = 8.9, NH); 8.68 (*d*, *J* = 8.9, NH). ^{1}H-NMR (500 MHz, H_2O/D_2O 9:1, solvent suppression by presaturation): 0.80–0.84 (*m*, 4 Me); 1.06–1.73 (*m*, 25 CH); 1.13 (*d*, *J* = 6.7, Me); 1.97 (*s*, Ac); 2.05 (*s*, Ac, rotamer); 2.18–2.57 (*m*, 28 H); 2.63–2.68 (*m*, CH); 2.80–2.93 (*m*, 10 H); 3.08–3.12 (*m*, CH_2); 3.15–3.22 (*m*, 1 H); 3.45–3.56 (*m*, 4 H); 3.93–4.27 (*m*, 10 H); 4.39–4.48 (*m*, 1 H); 6.69 (*d*, *J* = 8.5, 2 arom. H); 7.05–7.10 (*m*, 4 arom. H); 7.21–7.33 (*m*, 9 H); 7.42–7.52 (*m*, NH); 7.82 (*d*, *J* = 9.0, NH); 7.87–8.02 (*m*, 8 NH); 8.05 (*d*, *J* = 9.5, NH); 8.51 (*t*, NH). ^{13}C-NMR (125 MHz, CD_3OH, solvent suppression by presaturation): 19.7, 21.3, 22.8, 23.6 (Me); 23.7, 23.9, 24.6, 24.9, 25.4 (CH_2); 26.1 (CH); 27.7, 29.1, 29.2, 29.3, 30.1,

---

11) After addition of the 8th amino acid, **8**, for a second time, the TNBS test was still positive. Thus, the unreacted peptide resin was capped with Ac_2O (130 μl, 1.38 mmol) and $(i\text{-Pr})_2$EtN (120 μl, 0.61 mmol) in 1 ml of DMF (2 ml) for 12 h under Ar bubbling.

33.8 (CH_2); 34.1 (CH); 36.5, 37.2, 37.3, 37.6, 38.4, 38.6, 38.8, 39.4, 40.0, 40.8, 40.9, 41.0, 41.1, 41.5, 41.6, 41.9, 42.0, 42.1, 42.2, 42.2, 43.1 (CH_2); 43.3 (CH); 43.7 (CH_2); 45.9 (CH); 46.9 (CH_2); 47.7, 47.8, 48.2, 51.4, 53.0 (CH); 65.3 (CH_2); 116.3, 127.7, 129.5, 129.6 (CH); 130.2 (C); 130.4, 130.4, 131.4 (CH); 139.5, 139.7, 157.3, 171.4, 171.5, 171.5, 172.2, 172.2, 172.3, 172.4, 172.5, 172.5, 172.7, 173.4, 174.2, 175.1 (C). HR-MALDI-MS: 1795.0552 (19, $[M+Na]^+$, $C_{89}H_{145}N_{17}NaO_{18}S^+$; calc. 1795.0572), 1773.0765 (95, $[M+H]^+$, $C_{89}H_{146}N_{17}O_{18}S^+$; calc. 1773.0752), 1702.0383 (34, $[M-Acm+H]^+$, $C_{86}H_{141}N_{16}O_{17}S^+$; calc. 1702.0381).

REFERENCES

[1] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.

[2] 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.

[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.

[4] J. L. Matthews, K. Gademann, B. Jaun, D. Seebach, *J. Chem. Soc., Perkin Trans. 1* **1998**, 3331.

[5] S. Abele, G. Guichard, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2141.

[6] A. Jacobi, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1150.

[7] T. Sifferlen, M. Rueping, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 2067.

[8] D. Seebach, J. V. Schreiber, S. Abele, S. Daura, W. F. van Gunsteren, *Helv. Chim. Acta* **2000**, *83*, 34.

[9] T. Hintermann, D. Seebach, *Chimia* **1997**, *51*, 244.

[10] G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 187.

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

[12] M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774.

[13] Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, *121*, 12200.

[14] K. Gademann, M. Ernst, D. Hoyer, D. Seebach, *Angew. Chem.* **1999**, *111*, 1302; *Angew. Chem., Int. Ed.* **1999**, *38*, 1223.

[15] K. Gademann, Dissertation No. 13556, ETH-Zürich, 1999.

[16] K. Gademann, M. Ernst, D. Seebach, *Helv. Chim. Acta* **2000**, *83*, 16.

[17] S. Abele, Dissertation No. 13203, ETH-Zürich, 1999.

[18] P. Marbach, J. Rudinger, *Helv. Chim. Acta* **1974**, *57*, 403.

[19] S.-W. Wang, *J. Am. Chem. Soc.* **1973**, *95*, 1328.

[20] *Novabiochem* Catalog 2000, S32, method 11S, and ref. cited therein.

[21] W. S. Hancock, J. E. Battersby, *Anal. Biochem.* **1976**, *71*, 260.

[22] J. D. Fontenot, J. M. Ball, M. A. Miller, C. M. David, R. C. Montelaro, *Pept. Res.* **1991**, *4*, 19.

[23] J. D. Wade, J. Bedford, R. C. Sheppard, G. W. Tregear, *Pept. Res.* **1991**, *4*, 194.

[24] P. I. Arvidsson, Postdoctoral research fellow, ETH-Zürich, 2000, unpublished results.

[25] D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, *111*, 1700; *Angew. Chem., Int. Ed.* **1999**, *38*, 1595.

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

[27] M. Goodman, I. Listowsky, Y. Masuda, F. Boardman, *Biopolymers* **1963**, *1*, 33; M. Goodman, I. G. Rosen, *Biopolymers* **1964**, *2*, 537; M. Goodman, A. S. Verdini, C. Toniolo, W. D. Phillips, F. A. Bovey, *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *64*, 444; G. Conio, E. Patrone, S. Brighetti, *J. Biol. Chem.* **1970**, *245*, 3335; C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphius, *J. Am. Chem. Soc.* **1996**, *118*, 2744; N. Hirota, K. Mizuno, Y. Goto, *Protein Sci.* **1997**, *6*, 416.

[28] T. Hintermann, D. Seebach, *Synlett* **1997**, 437.

[29] P. Lombardi, *Chem. Ind. (London)* **1990**, Nov. 5, 708; S. Moss, *Chem. Ind. (London)* **1994**, Feb. 21, 122.

[30] F. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl, 'Short Protocols in Molecular Biology', 3rd edn., John Wiley & Sons, New York, 1995.

[31] R. C. Weast, ' Handbook of Chemistry and Physics', 1st Student Edition, CRC Press, Cleveland, 1988, p. D-87.

[32] J. L. Matthews, M. Overhand, F. N. M. Kühnle, P. E. Ciceri, D. Seebach, *Liebigs Ann. Chem.* **1997**, 1371.

[33] J. Podlech, D. Seebach, *Liebigs Ann. Chem.* **1995**, 1217.
[34] J. A. Sheehan, P. A. Cruickshank, J. L. Boshart, *J. Org. Chem*. **1961**, *26*, 2525. N. L. Benoiton, K. Kuroda, F. M. F. Chen, *Int. J. Pept. Protein Res.* **1979**, *13*, 403.
[35] M. Bodanszky, B. Bodanszky, 'The Practice of Peptide Synthesis', Springer-Verlag, New York, 1984, 177.

Received August 14, 2000