Automated Solid-Phase Synthesis and Structural Investigation of β-Peptidosulfonamides and β-Peptidosulfonamide/β-Peptide Hybrids: β-Peptidosulfonamide and β-Peptide Foldamers are Two of a Different Kind

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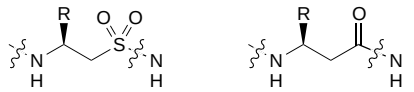
Dedicated to Professor *Dieter Seebach* on the occasion of his 65th birthday

Fmoc-protected β-aminoethane sulfonylchlorides can be employed for efficient automated solid phase synthesis of β-peptidosulfonamides and β-peptidosulfonamide/β-peptide hybrids containing one or more β-peptidosulfonamide residues. Thus, Fmoc-protected β-aminoethane sulfonylchlorides **5a**–**c** led to the hexa-β-peptidosulfonamide **9** and the nona-β-peptidosulfonamide **10**. In addition, the β-peptidosulfonamide/β-peptide hybrids **13** and **16**, consisting of six and nine β-residues, respectively, and containing a single β-peptidosulfonamide unit in the middle, as well as the peptidosulfonamide/β-peptide hybrid **15** with nine β-residues, including an N-terminal β-peptidosulfonamide residue, were synthesized by automated solid-phase synthesis. Both CD and NMR spectroscopic measurements did not indicate any helical secondary structure for **9** and **10**. As was shown by CD-measurements, the β-peptidosulfonamide residue in the hybrids **13**, **15**, and **16** acts as a 'helix breaker', especially when located in the middle of the hybrid chain (**13** and **16**), but, although to a lesser extent, also at the N-terminus.

Introduction. – The introduction of β-peptides was soon followed by commentaries such as 'β-peptides: Nature improved' [1] or statements referring to 'a brave new world' [2]. Although, at first glance, this may seem a little exaggerated and primary serving the purpose of attracting audience of the scientific community, the open mind will be clearly triggered and stimulated to investigate the elements of truth in these statements.

Likewise, when we introduced the sulfonamide moiety as a potential transition state isostere of the hydrolysis of the amide bond [3], we were interested in both comparing and evaluating the properties of the sulfonamide as compared to the amide moiety to find out how these peptidomimetics would behave as compared to their natural counterparts. This may even shed some light as why Nature 'chose' the amide moiety as a linkage to connect the building blocks of peptides and proteins. Indeed, one may wonder if additional branches to the evolution tree of biologically active macromolecules are possible in our world. From that perspective, we were interested to investigate the synthesis, structure, and properties of β-peptidosulfonamides. β-Peptides have been designated as a foldamer class [1][4], which indicates that its members have been shown to fold into defined three-dimensional structures similar to natural peptides. Clearly, the class of β-peptides, which has been thoroughly and extensively investigated by the groups of *Seebach* [5] and *Gellman* [6], represent the most important class of foldamers. We were, thus, interested if β-peptidosulfonamides could comprise another class. An especially attractive feature is the identical number of

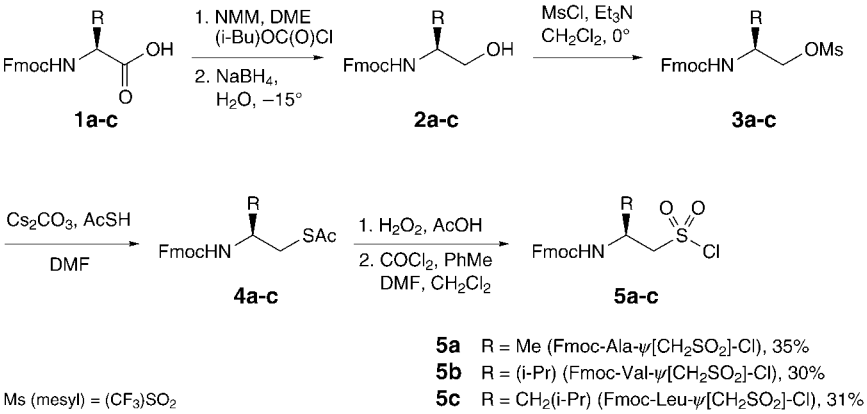
atoms in each backbone residue of β-peptidosulfonamides and β-peptides, which allows unequivocal conclusions regarding the influence of the sulfonamide moiety in both oligomers of β-peptidosulfonamides and β-peptidosulfonamide/β-peptide hybrids.



In order to achieve the synthesis of especially large and complex β-peptidosulfonamides or β-peptidosulfonamide/β-peptide hybrids, a solid-phase procedure is indispensable. An absolute prerequisite for its development is the easy availability of the necessary building blocks. Recently, we described an efficient synthesis of *N*-protected β-aminoethane sulfonylchlorides as versatile building blocks [7] for the convenient *manual* solid-phase synthesis of β-oligopeptidosulfonamides [8]. Here, we report in detail the *automated* solid-phase synthesis of oligopeptidosulfonamides and β-peptidosulfonamide/β-peptide hybrids and describe their structural characteristics.

Results and Discussion. – The required Fmoc[1])-protected β-aminoethane sulfonylchlorides **5a**–**c** were prepared in four steps according to *Scheme 1* [7]. In short, the Fmoc amino acids **1a**–**c** were reduced to the corresponding amino alcohols **2a**–**c** with sodium borohydride. This was followed by mesylation to **3a**–**c** and subsequent conversion to the thioacetates **4a**–**c**. Oxidation with H_2O_2 in AcOH gave the corresponding sulfonic acids, which were immediately transformed into the sulfonylchlorides **5a**–**c** in overall yields of 30–35%, corresponding to an average yield of 79% per step.

Scheme 1



For the automated solid-phase procedure, an *ABI 433A* peptide synthesizer was used. The Fmoc protecting group was cleaved from the *ArgoGel*-containing *Rink*-

[1]) For abbreviations, see *Exper. Part.*

linker with piperidine to give the free amine, which was treated with 4 equiv. of Fmoc-Leu-$\psi[CH_2SO_2]$-Cl and 6 equiv. of *N*-methylmorpholine (NMM) to give **6** (*Scheme 2*). Introduction of the second β-aminoethane sulfonamide residue, *i.e.*, Fmoc-Ala-$\psi[CH_2SO_2]$-Cl (**5a**), was performed in the same manner and led to **7**. Repetition of the deprotection and coupling cycles ultimately led to the hexa-β-peptidosulfonamide **8** attached to the solid support. NMM was used as a base in each coupling step. The appropriate Fmoc-protected β-aminoethane sulfonylchloride was weighed into the cartridge of the automated synthesizer and dissolved in CH_2Cl_2 before coupling[2]). The couplings proceeded smoothly within 3 h. This is comparable to the coupling time needed for the commonly used Fmoc-protected α-amino acids. In addition, the coupling efficiency was very high, as shown by the bromophenol blue (BPB) test, which was negative after each coupling, blue-colored beads were absent [9]. This observation was corroborated by on-line measurements of the total absorbance of the piperidine-dibenzofulvene adduct ($\lambda_{max} = 301$ nm) obtained after cleavage of the Fmoc group after each coupling step [10]. It was found that the loading of the growing oligopeptido-sulfonamide decreased only slightly with each coupling step.

After the removal of the last Fmoc group, the oligopeptidosulfonamide was cleaved from the resin with trifluoroacetic acid (TFA), H_2O, and triisopropylsilane (TIS) acting as scavengers. Mass spectral studies (EI-MS) unambiguously demonstrated the presence of the desired oligopeptidosulfonamide **9**, but the low absorbance of the sulfonamide moiety prevented a clear UV detection by HPLC. Originally, we had circumvented this problem by attachment of a UV-chromophore [8]. However, with an evaporative light-scattering detector (ELSD)[3]), this was no longer necessary, and, indeed, the peptidosulfonamide obtained after cleavage from the solid phase was very pure. *Fig. 1* shows the HPLC trace of the all-sulfonamide nonamer **10**.

In contrast to the corresponding β-hexapeptides (*vide infra*) [5a], circular dichroism (CD) studies of the β-hexapeptidosulfonamide did not indicate the presence of a defined helical secondary structure in solution (*Fig. 2*). This might be explained by the poor UV absorbance of the backbone sulfonamide moieties preventing the observation of a defined structure by this method. However, NMR spectroscopy also did *not* provide evidence for any defined secondary structure. Thus, oligopeptidosulfonamides of this length and/or with these residues may fail to assume a defined conformation. Therefore, the longer β-nonapeptidosulfonamide **10** was synthesized (*Scheme 2*), which, however, still did not show any indication of folding into a particular secondary structure either (*vide supra*). A likely explanation, therefore, is that the β-amino-sulfonamide residues have structural characteristics that interfere with or, at least, are not favorable for the formation of the helical structures described by *Seebach* and *Gellman* and their co-workers (*vide infra*).

To investigate this phenomenon, we decided to replace one β-amino acid residue in a known helical β-hexapeptide of *Seebach* and co-workers by a 2-aminosulfonamide

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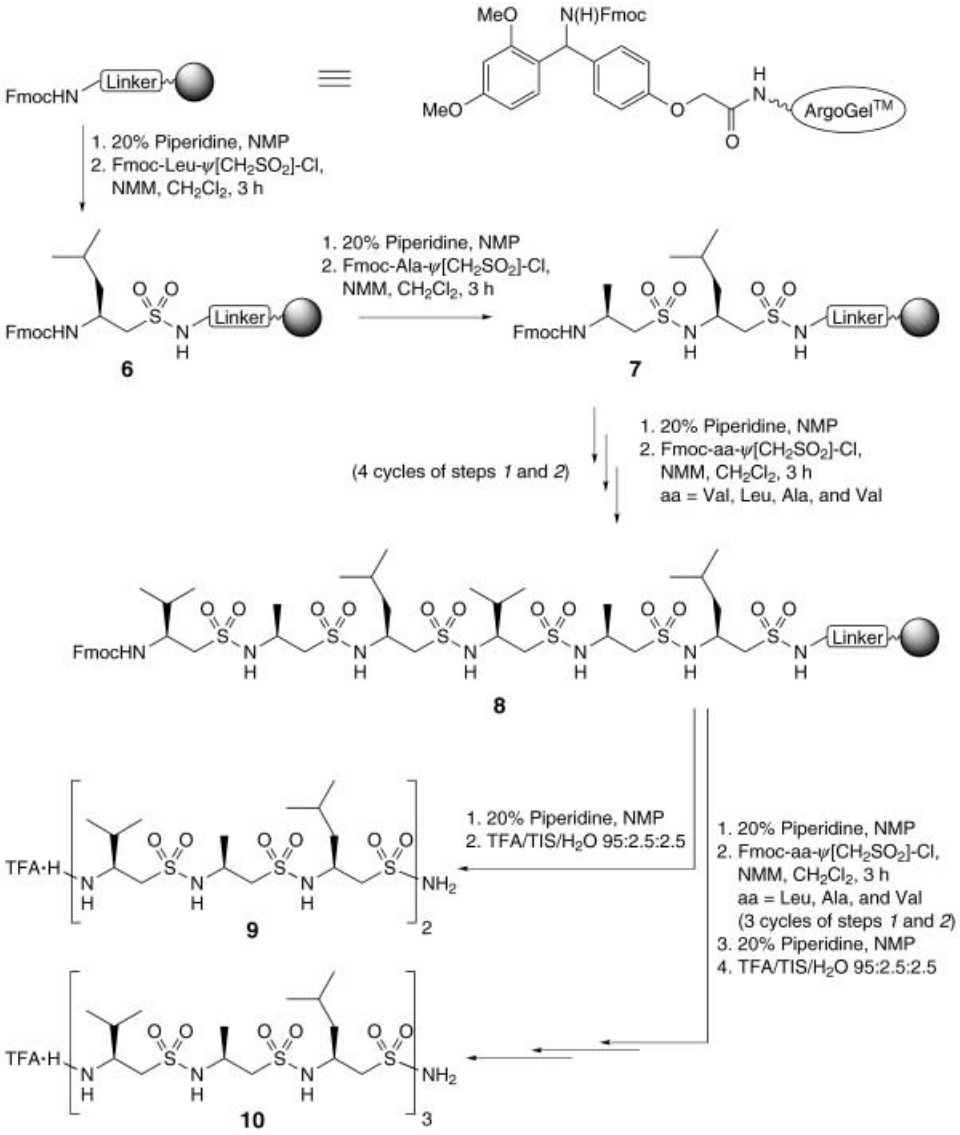
[2]) CH_2Cl_2 was the solvent of choice, since NMP led to decomposition of the sulfonyl chlorides.

[3]) ELS Detection is based on the ability of particles to cause photon scattering when they cross the path of a beam of light. The liquid effluent from an HPLC is first nebulized and the resultant vapor, containing the analyte particles, is directed through a light beam. A signal is generated that is proportional to the mass present and independent of the presence or absence of chromophores, fluorophores, or electroactive groups.

Scheme 2. *Automated Solid-Phase Synthesis of the Hexa- and Nonameric β-Peptidosulfonamides* **9** *and* **10**



residue. As a reference, the β-hexapeptide **12** was synthesized[4]). The synthesis of **12** and its analog **13** is shown in *Scheme 3*. Analogous to the procedures of *Seebach* and co-

[4]) At present, the 'S'-terminal amide (*e.g.*, in **9** or **10**) is best synthesized, therefore, for convenience and uniformity of all synthesized oligomers, S/C-terminal amides were synthesized. It is not expected that amide termination of the CO_2H or SO_3H groups will have a major impact on the observed secondary structure.

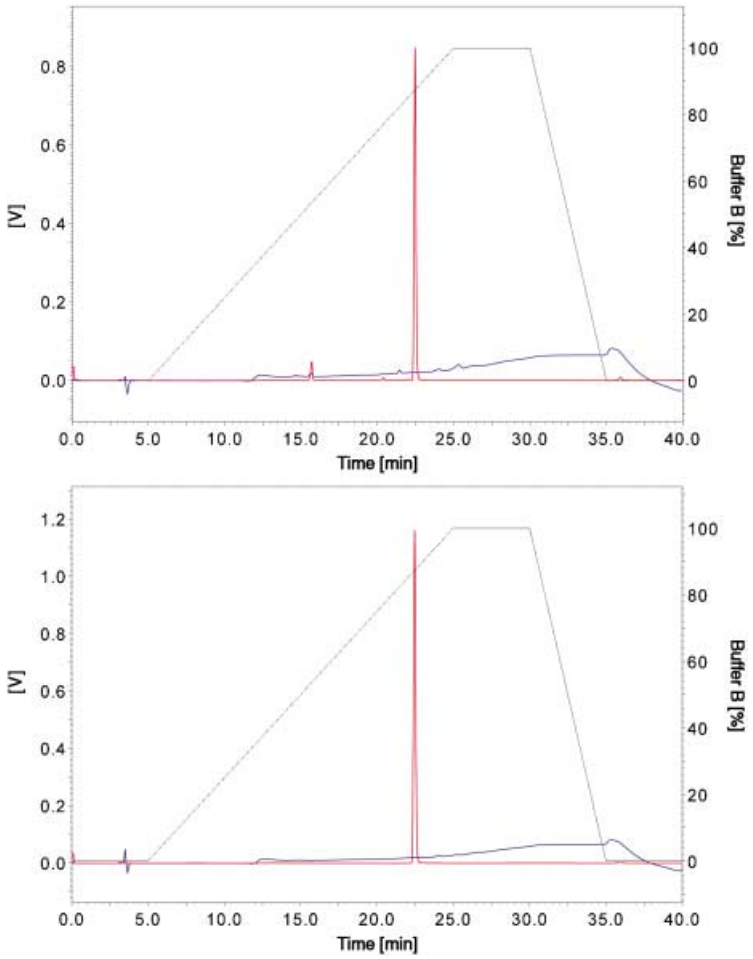


Fig. 1. *UV* (blue) *and ELSD* (red) *Detetection of HPLC traces of the nonameric β-peptidosulfonamide* **10**, *crude* (top) *and purified* (bottom)

workers, the β-hexapeptide amide and the β-peptide parts of the β-peptidosulfonamide/β-peptide hybrid **13** were prepared by solid phase synthesis. However, the β-amino sulfonamide residue was introduced using the required Fmoc-protected β-aminoethane sulfonylchloride **5c** dissolved in CH_2Cl_2 in the presence of NMM.

After cleavage from the solid phase and purification by HPLC, CD spectra of **12** and **13** were recorded (*Fig. 2*). As expected, the CD spectrum of the β-peptide-amide **12** was virtually identical with that published by *Seebach* and co-workers [5a]. In contrast, the CD-spectrum of the β-peptidosulfonamide/β-peptide hybrid **13** did not show the presence of a helical structure but rather a random coil structure. Thus, the presence of a single β-aminosulfonamide residue is sufficient to 'break' the 3_{14} helix of a β-hexapeptide! To investigate if the breaking capacity of this single residue could be overcome in a β-peptide part of sufficient length, the two nonameric β-peptidosulfon-

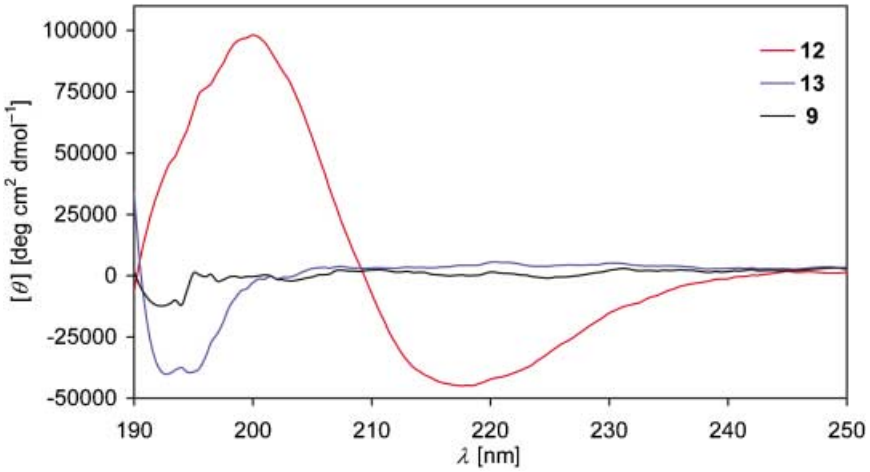


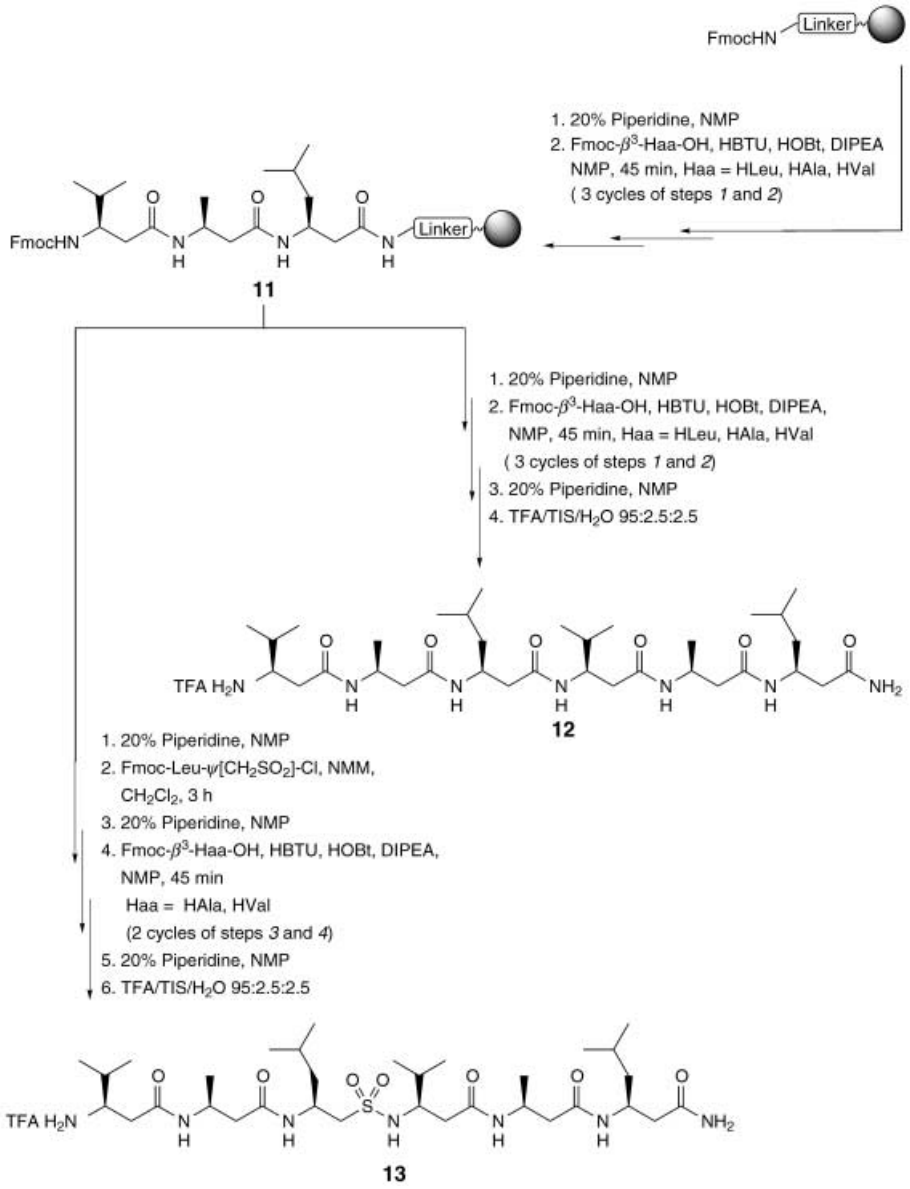
Fig. 2. *CD Spectra of the hexameric β-peptide* **12** *as compared to the corresponding β-peptidosulfonamide* **9** *and the β-peptidosulfonamide/β-peptide hybrid* **13**

amide/β-peptide hybrids **15** and **16** were synthesized (*Scheme 4*) and compared with the reference β-peptide **14**. As described by *Seebach* and co-workers, after coupling of the 5th residue, Fmoc removal of subsequently introduced β-amino acid residues becomes very sluggish, presumably because of secondary-structure formation on the resin [11]. However, when the 5th residue is a β-aminosulfonyl residue, as was the case in the preparation of **16**, Fmoc removal of the subsequently introduced β-amino acid residue proceeds smoothly (*Fig. 3*). This may be indicative of the absence of secondary structure formation on the resin. Finally, the β-peptidosulfonamide/β-peptide hybrid **15** was prepared (*Scheme 4*). Here, the synthesis of the β-peptide part was accompanied by the mentioned sluggish removal of the Fmoc group. Again, after introduction of the β-aminosulfonic acid residue as the N-terminus, Fmoc removal was quick, suggesting that, even at the very end of a β-peptide, a β-aminosulfonamide residue has a distinct influence on the compound's spatial structure.

After cleavage from the solid phase followed by reversed-phase HPLC, the pure β-peptide **14** and the pure β-peptidosulfonamide/β-peptide hybrids **15** and **16** were obtained. As an example, the HLPC traces of crude and purified **16** are shown in *Fig. 4*, indicating that the automated solid-phase synthesis proceeded very well and that the products were of high purity.

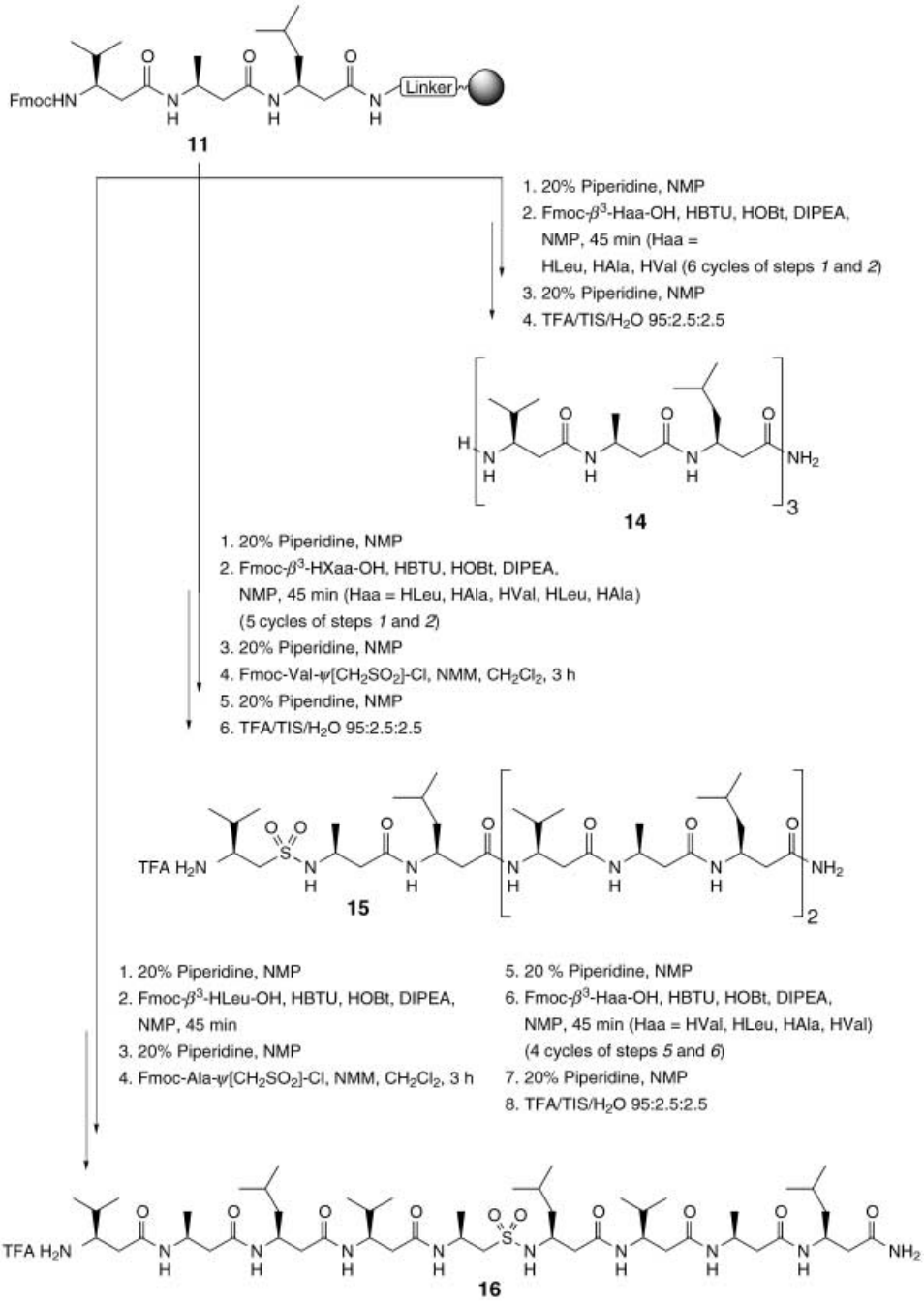
In contrast to the larger β-peptides of *Seebach* and co-workers [11a], DBU cannot be used for the removal of the Fmoc group in β-peptidosulfonamides, since it gives rise to side reactions. Fortunately, deprotection with piperidine can be easily monitored by UV as part of the automated solid-phase synthesis procedure [10]. Thus, the number and duration of piperidine treatments for completion of Fmoc-removal can be easily adjusted. Using this procedure, we found that it was not necessary to use DBU in the synthesis of β-peptides when repeated treatment with piperidine was carried out (*Fig. 3*). One additional Fmoc-deprotection cycle lasted 10 min. The CD spectra of **14** and the **10** as well as of the hybrids **15** and **16** are shown in *Fig. 5*.

Scheme 3. *Solid-Phase Synthesis of the Hexameric β-Peptide* **12** *and the β-Peptidosulfonamide/β-Peptide Hybrid* **13**



As mentioned above, the nonameric β-peptidosulfonamide **10** did not exhibit any CD signal whatsoever. In the nonameric β-peptidosulfonamide/β-peptide hybrid **15**, the β-peptide sequence part (eight residues) is sufficiently long to allow this compound to adopt a considerable helix character. However, the lower CD-amplitude of **15** as compared to the all-β-peptide **14** indicates that, even at the N-terminus, the

Scheme 4. *Automated Solid-Phase Synthesis of the Nonameric β-Peptide* **14** *and the Hybrids* **15** *and* **16**



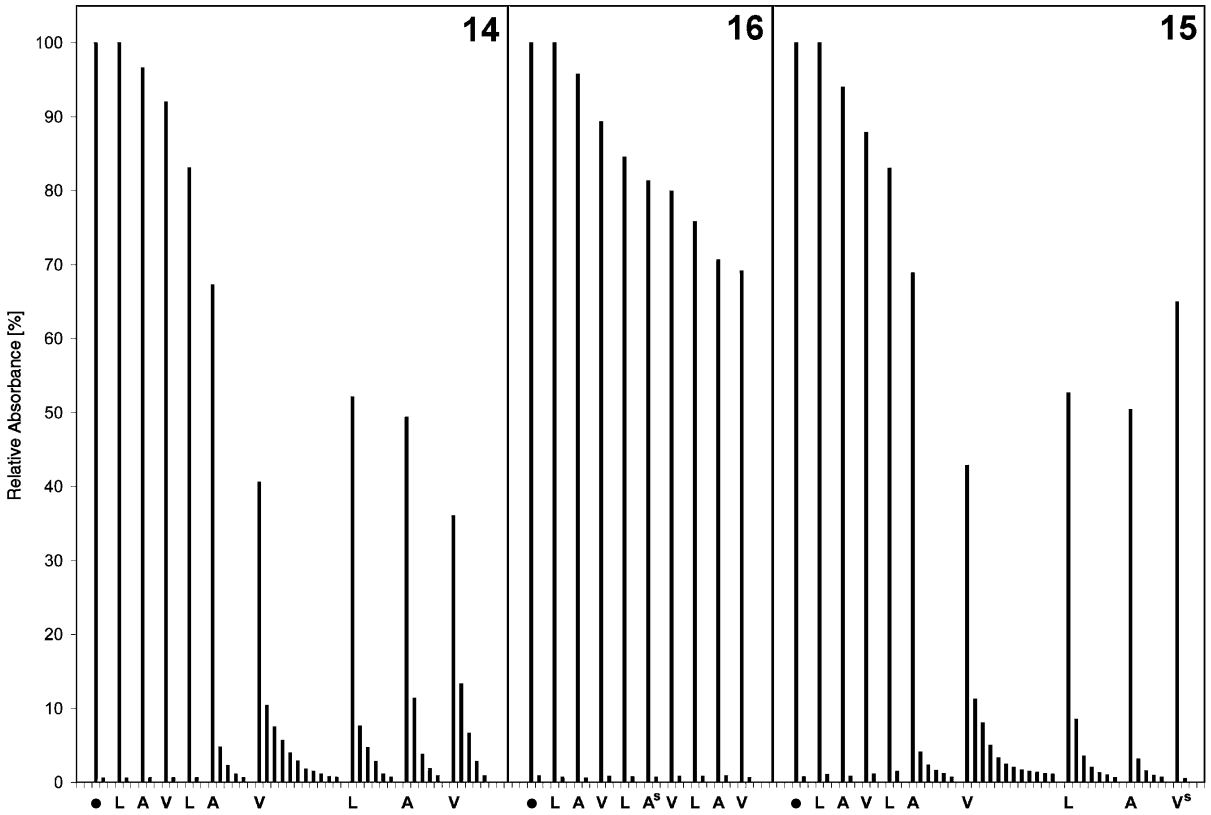


Fig. 3. *UV-Monitoring profile of the automated solid-phase synthesis of the nonameric β-peptide* **14** (left), *the hybrid* **16** (middle), *and the hybrid* **15** (right). The cleavage of the Fmoc group can be followed by the absorbance (arbitrary units) of the dibenzofulvene-piperidine adduct (single bars). When the second treatment with piperidine still gives rise to a significant absorbance, then more basic treatments were performed until a certain treshold was reached. The first and second piperidine treatment was carried out for 70 s, subsequent treatments lasted 10 min. The synthesis was carried out from the C- to the N-terminus. Designations: ●: dibenzofulvene-piperidine adduct resulting from Fmoc cleavage from the resin; L, A, V, A^s, V^s : dibenzofulvene-piperidine adducts resulting from Fmoc cleavage from coupled β-aminoethanesulfonic acid residues (*e.g.*, L = dibenzofulvene-piperidine adduct resulting from Fmoc cleavage from coupled Fmoc-β^3-Hleu-OH, A^s = dibenzofulvene-piperidine adduct resulting from Fmoc cleavage from coupled Fmoc-Ala-$\psi[CH_2SO_2]$-Cl).

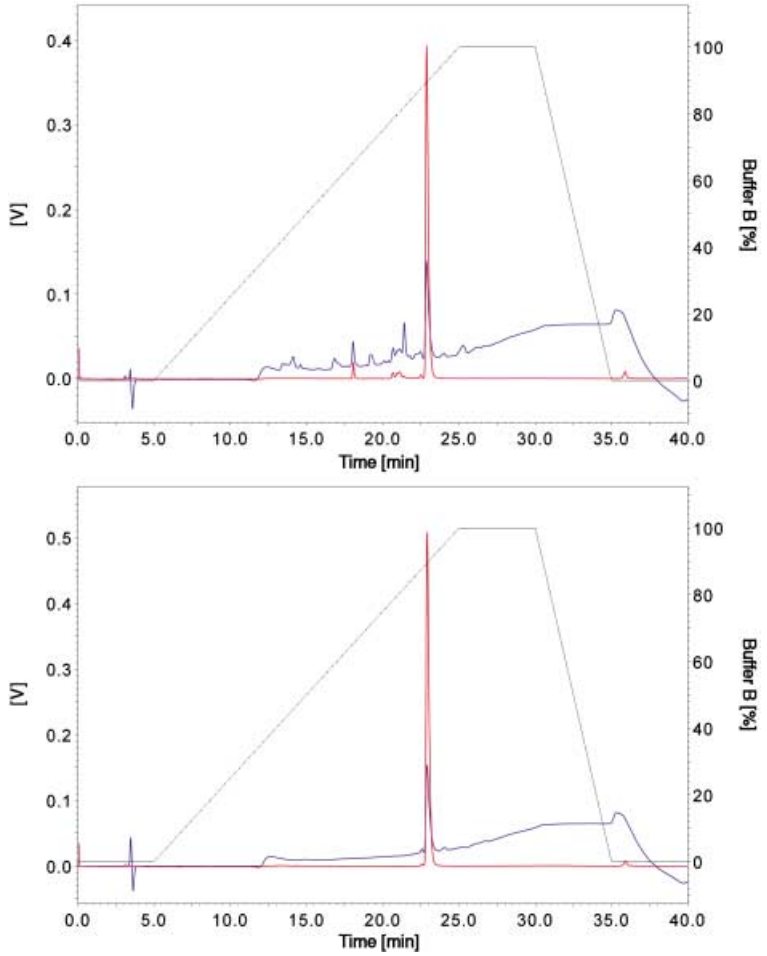


Fig. 4. *HPLC Traces of crude* versus *purified* **16** (upper *vs.* lower figure)

sulfonamide residue is capable of distorting the helix significantly. As expected from the findings with hybrid **13** with a sulfonamide residue in the middle, the helical character of **16**, also with a central sulfonamide residue, was even further reduced compared to **14**. This also indicated that the flanking β-peptide sequences at the amino end (four β-peptide residues) and at the sulfonamide end (five β-peptide residues) are not sufficiently long to overcome the helix-breaking effect of the sulfonamide residue.

In order to explain the helix-breaking character of sulfonamide residues in our hybrids, at least two structural features have to be taken into account. First, the sulfonamide moiety contains a strong H-bond donor (probably better than the peptide NH), but a poor H-bond acceptor[5]). The latter may be caused by a dichotomy of the acceptor since, in fact, it consists of two accepting sites, *i.e.*, both sulfonamide O-atoms.

[5]) A sulfonamide N–H is more acidic than an amide N–H.

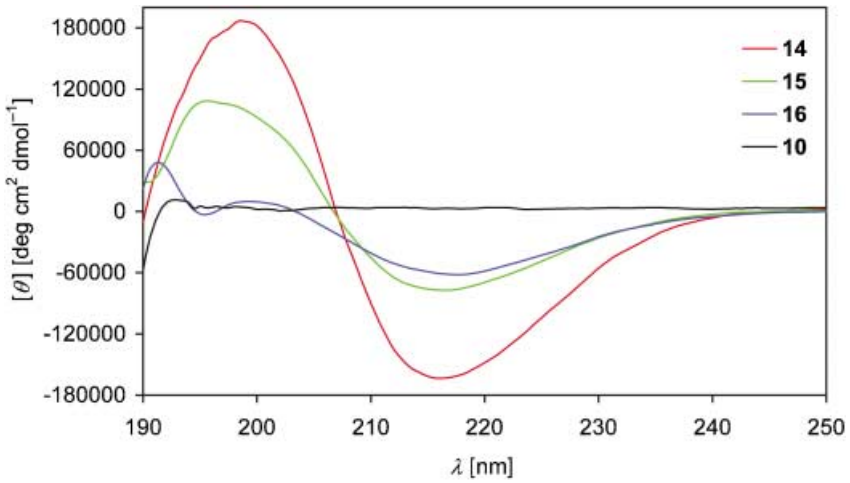


Fig. 5. *CD Spectra of the nonameric β-peptide* **14** *and the corresponding β-peptidosulfonamide* **10**, *as well as of the β-peptidosulfonamide/β-peptide hybrids* **15** *and* **16**, *resp.*

This may impair a helical H-bonding network. Second, in contrast to the peptide amide moiety, which is a relatively rigid, *trans*-configured structural element in a peptide, the sulfonamide bond is more freely rotatable, which might additionally impede the formation of a helix. As a result, the sulfonamide oxygens can assume varying positions, in which one O-atom occupies a *trans*- or *cis*-like position with respect to the amide N–H, while the other is neither *cis* nor *trans* [3d][12]. This could also prevent a proper alignment of the H-bonds to adopt a particular secondary structure.

Conclusions. – Orginally, we introduced β-peptidosulfonamides as peptidomimetics that contain the sulfonamide moiety as a potential transition-state isostere model for the hydrolysis of the amide bond [3a–c][3e]. Indeed, the peptidosulfonamide peptidomimetics are resistant towards degradation by proteases [3h]. The extension of the synthetic possibilities for the preparation of β-peptidosulfonamide building blocks [7] provided access to manual, and now also automated solid-phase synthesis of oligopeptidosulfonamides, opening up possibilities to investigate their behavior as a new class of foldamers [8]. This might even lead to the construction of proteinaceous sulfonamides. However, the β-peptidosulfonamide oligomer consisting of six or nine residues did not show any indications of secondary structure formation. Thus, although these compounds closely resemble β-peptides with respect to the number of atoms per residue as well as the presence of amide functionalities, replacement of the peptide amide carbonyl by a sulfonamide moiety has a profound influence on their ability to behave as foldamers. Moreover, a single β-aminoethane sulfonamide residue was capable of disordering the foldamer behavior of β-peptides. The presence of a β-aminoethane sulfonamide residue in the middle of a medium-sized β-peptide was practically detrimental to its 3_{14} helical structure and significantly reduced its helicity when present at the N-terminus.

Under present investigation is disturbance of the secondary structure in other peptides by β-aminoethane sulfonamide residues. The possibility to selectively

alkylate the sulfonamide NH group in hybrids derived from natural peptides [13] holds additional promises for influencing the spatial structures of β-peptidosulfonamides and mixed oligomers thereof.

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Experimental Part

General. Abbreviations: DIPCIDI: *N,N'*-diisopropylcarbodiimide, DIPEA: *N,N*-diisopropylethylamine, DMAP: 4-(*N,N*-dimethylamino)pyridine, Fmoc: [(fluoren-9-yl)methoxy]carbonyl, HBTU: *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*,tetramethyluronium hexafluorophosphate, HOBt: 1-hydroxybenzotriazole, MTBE: *tert*-butyl methyl ether, NMM: *N*-methylmorpholine, NMP: *N*-methylpyrrolidinone, TFA: trifluoroacetic acid, TIS: triisopropylsilane.

Liquid-chromatography electrospray-ionization mass spectrometry (LC-EI-MS) was carried out using a *Shimadzu LCMS QP-8000* single-quadrupole benchtop mass spectrometer coupled with a *QP-8000* data system. ^{1}H-NMR spectra were recorded on a *Varian Inova-500* (500 MHz) spectrometer and chemical shifts (δ) are given in ppm relative to CD_3OH (3.34 ppm). For the automated solid-phase peptide syntheses, an *Applied Biosystems 433A* peptide synthesizer coupled to a *759A* adsorbance detector was used. Fmoc deprotection was monitored by UV absorbance ($\lambda = 301$ nm) of the dibenzofulvene-piperidine adduct. All syntheses were carried out on *ArgoGel*™ Rink-NH-Fmoc resin functionalized with a 4-[(2',4'-dimethoxyphenyl)aminomethyl]phenoxyacetamido moiety (*Rink* amide linker). Anal. HPLC runs (unless stated otherwise) were performed on a *Shimadzu* automated HPLC system with an *Alltech Adsorbosphere C18* column (particle size: 5 μm, pore size: 300 Å , 250 × 4.6 mm) at a flow rate of 1.0 ml/min. The compounds were detected by UV light at 220 and 254 nm or by evaporative light scattering (ELSD) using a *Polymer Laboratories* ELS detector. Prep. HPLC runs (unless stated otherwise) were carried out with an *Applied Biosystems* HPLC workstation (UV detection at 214 nm) using an *Alltech Adsorbosphere C18* column (particle size: 10 μm, pore size: 300 Å, 250 × 22 mm) at a flow rate of 12 ml/min. Generally, a linear gradient of buffer B (0.085% TFA in MeCN/H_2O 95 : 5) (anal.: 100% in 20 min, prep.: 100% in 60 min) from 100% buffer A (0.1% TFA in H_2O). CD Spectra were recorded at 0.2 mM conc. in MeOH on an *OLIS RSM-1000* spectrometer in 0.5-nm intervals in the range of 190–250 nm at 20° (average of 5 baseline-corrected scans) using a 1 mm cylindrical cell. Optical rotations $[\alpha]_D$ were measured at 25° with a *Jasco P-1010* polarimeter.

All solvents were obtained as 'peptide grade' solvents and used without further purification.

Coupling of Fmoc-protected β-Amino Acids to the Solid Support (*Procedure A*). The Fmoc group of the *ArgoGel Rink*-amide linker resin was removed by treatment with 20% piperidine in NMP (25 ml/mmol, 3 × 10 min), and the resin was washed with NMP (25 ml/mmol, 5 × 2 min). Subsequently, the Fmoc-β-amino acid (4.0 equiv.) was dissolved in NMP (15 ml/mmol), activated with HBTU (3.8 equiv.), HOBt (4.0 equiv.), and DIPEA (7.8 equiv.), and added to the resin. The mixture was agitated for 45 min by bubbling anh. N_2 gas through it or by continuous shaking. Monitoring of the coupling reaction was performed by the bromophenol blue (BPB) test detecting free amino functions. In case of a positive BPB test, which indicates incomplete reaction, the coupling was prolonged for an additional 45 min. Subsequently, the resin was filtered and washed with NMP (25 ml/mmol, 5 × 2 min) prior to the next Fmoc deprotection step.

Coupling of Fmoc-protected β-Aminoethane Sulfonylchlorides to the Solid Support (*Procedure B*). The Fmoc group of the resin was removed by treatment with 20% piperidine in NMP (25 ml/mmol, 3 × 10 min) and the resin was washed with NMP (25 ml/mmol, 5 × 2 min) and CH_2Cl_2 (25 ml/mmol, 5 × 2 min). Subsequently, CH_2Cl_2 (15 ml/mmol) was added followed by the appropriate Fmoc-protected β-aminoethane sulfonylchloride (4 equiv.) and NMM (6 equiv.). The mixture was agitated for 3 h by bubbling anh. N_2 gas through it or by continuous shaking. Monitoring of the coupling reaction was performed with the BPB test. In case of incomplete coupling, the reaction time was prolonged for an additional 3 h[6]). Subsequently, the resin was filtered and washed with CH_2Cl_2 (25 ml/mmol, 5 × 2 min) and NMP (25 ml/mmol, 5 × 2 min) prior to the next deprotection step.

Coupling of Fmoc-protected β-Amino Acids to the Solid Support via *Peptide Synthesizer* (*Procedure C*). The resin was pre-swollen by washing with NMP (12 × 10 ml, 30 s) followed by Fmoc removal with 20% piperidine in

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[6]) Monitoring the reaction with the *Kaiser* test may be misleading due to the relatively acidic sulfonamide NH.

NMP (2×70 s). After washing the resin with NMP (6×10 ml, 30 s) to remove any residual piperidine, 0.4 mmol (4 equiv.) of the appropriate Fmoc-protected β-amino acid was dissolved in NMP (2 ml) and a soln. of HBTU/HOBt (0.143M, 2.8 ml) was added. Subsequently, a solution of DIPEA in NMP (0.8M, 0.5 ml) was added, and the activated Fmoc-protected β-amino acid was transferred to the reaction vessel containing the resin. After 45 min of intense agitation (*Vortex*) the vessel was drained and washed with NMP (6×10 ml, 30 s) prior to the following deprotection. After coupling of the final Fmoc-protected β-amino acid, the Fmoc group was removed and the resin was thoroughly washed with NMP (6×10 ml, 30 s) and CH_2Cl_2 (6×10 ml, 30 s).

Coupling of Fmoc-protected β-Aminoethane Sulfonylchlorides to the Solid Support via *Peptide Synthesizer* (*Procedure D*). The resin was pre-swollen by washing with NMP (12×10 ml, 30 s) and deprotected by treatment with 20% piperidine in NMP (2×70 s). After washing the resin with NMP (6×10 ml, 30 s) to remove any residual piperidine, the resin was thoroughly washed with CH_2Cl_2 (12×10 ml, 30 s) to remove residual NMP. The appropriate Fmoc-protected β-aminoethanesulfonyl chloride (4 equiv.) was dissolved in CH_2Cl_2 and treated with a soln. of NMM in CH_2Cl_2 (1.2M, 0.5 ml). This soln. was transferred to the reaction vessel containing the resin. After 3 h of intense agitation (*Vortex*), the vessel was drained and washed with CH_2Cl_2 (6×10 ml, 30 s) and NMP (6×10 ml, 30 s) prior to the next Fmoc deprotection step. After coupling of the final building block, the Fmoc group was removed by treatment with 20% piperidine in NMP, and the resin was washed with NMP (6×10 ml, 30 s) followed by CH_2Cl_2 (6×10 ml, 30 s).

Resin Cleavage (*Procedure E*). The peptide or peptidosulfonamide was cleaved from the resin by treatment with TFA/TIS/H_2O 95 : 2.5 : 2.5 (20 ml/mmol) at r.t. for 3 h, precipitated with MTBE/hexane 1 : 1 at $-20°$, and centrifuged. The supernatant was decanted, the pellet was washed with cold MTBE/hexane 1 : 1 and lyophilized from *t*-BuOH/H_2O 1 : 1.

H-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-NH₂ (**12**). According to *Procedure A*, six Fmoc-protected β-amino acids were subsequently coupled to the *Rink*-NH-Fmoc resin (103 mg, 0.33 mmol/g). Cleavage from the resin was accomplished according to *Procedure E* and afforded crude **12**. The product was purified by prep. HPLC and lyophilized to yield the TFA salt of **12** (14 mg, 53%) as a white solid. HPLC: t_R 15.3 min. $[\alpha]_D^{25} = +3.62$ ($c = 0.24$, MeOH). ^{1}H-NMR: 0.90 – 0.97 (*m*, 4 Me of Leu, 2 Me of Val)); 1.11 (*d*, $J = 6.9$, 2 Me of Val); 1.17 (*d*, $J = 6.6$, Me of Ala); 1.21 (*d*, $J = 6.6$, Me of Ala); 1.27 – 1.34 (*m*, 2 C*H*(H)(i-Pr) of Leu); 1.41 – 1.47 (*m*, 2 CH(*H*)(i-Pr) of Leu); 1.57 – 1.63 (*m*, 2 C*H*(Me_2) of Leu); 1.73 – 1.76 (*m*, C*H*(Me_2) of Val); 2.07 – 2.09 (*m*, C*H*(Me_2) of Val); 2.25 – 2.35 (*m*, 3 CH(*H*)C(O) of Ala, Leu, Val); 2.41 – 2.63 (*m*, 3 CH(*H*)C(O) of Ala, Leu, Val, 4 C*H*(H)C(O) of Ala, 2 Leu, Val); 2.69 – 2.75 (*m*, C*H*(H)C(O) of Ala); 2.78 – 2.84 (*m*, C*H*(H)C(O) of Val); 3.52 – 3.57 (*m*, NCH of Val); 4.20 – 4.27 (*m*, NCH of Val); 4.34 – 4.51 (*m*, 3 NCH of Ala and 2 Leu); 4.54 – 4.63 (*m*, NCH of Ala); 6.73 (*s*, 1 H, NH_2); 7.62 (*s*, 1 H, NH_2); 7.69 (*d*, $J = 8.2$, NH of Ala); 7.74 (*d*, $J = 9.3$, NH of Leu); 7.82 (br. *s*, NH_3^+ of Val); 8.02 (*d*, $J = 9.5$, NH of Val); 8.33 (*d*, $J = 9.0$, NH of Leu); 8.44 (*d*, $J = 9.2$, NH of Ala). EI-MS: 690.9 ($[M + Na]^+$), 668.7 ($[M + H]^+$).

H-(S)-β³-HVal-(S)-β³-HAla-(S)-Leu-ψ[CH₂SO₂]-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-NH₂ (**13**). According to *Procedure A*, three Fmoc-protected β-amino acids were subsequently coupled onto the *Rink*-NH-Fmoc resin (152 mg, 0.33 mmol/g) followed by coupling of Fmoc-Leu-$\psi[CH_2SO_2]$-Cl according to *Procedure B*. Then, two Fmoc protected β-amino acids were coupled according to *Procedure A*. Cleavage from the resin was accomplished following *Procedure E* and afforded crude **13**. The product was purified by prep. HPLC and lyophilized to obtain the TFA salt of **13** (20 mg, 49%) as a white solid. HPLC: t_R 14.8 min. $[\alpha]_D^{25} = -3.02$ ($c = 0.31$, MeOH). ^{1}H-NMR: 0.94 – 0.97 (*m*, 4 Me of Leu, 2 Me of Val); 1.03 – 1.06 (*m*, 2 Me of Val); 1.19 (*d*, $J = 6.6$, Me of Ala); 1.23 (*d*, $J = 6.7$, Me of Ala); 1.28 – 1.35 (*m*, C*H*(H)(i-Pr) of Leu); 1.47 – 1.53 (*m*, CH(*H*)(i-Pr) of Leu, CH_2(i-Pr) of Leu); 1.64 – 1.67 (*m*, 2 C*H*(Me_2) of Leu); 1.82 – 1.86 (*m*, C*H*(Me_2) of Val); 1.94 – 2.00 (*m*, C*H*(Me_2) of Val); 2.26 – 2.51 (*m*, 4 CH_2C(O) of 2 Ala, Leu, Val, C*H*(H)C(O) of Val); 2.59 – 2.63 (*m*, CH(*H*)C(O) of Val); 3.13 – 3.17 (*m*, C*H*(H)C(O) of Leu); 3.26 – 3.31(*m*, CH(*H*)C(O) of Leu); 3.38 – 3.43 (*m*, NCH of Val); 3.66 – 3.72 (*m*, NCH of Val); 4.23 – 4.29 (*m*, NCH of Ala); 4.34 – 4.37 (*m*, NCH of Ala, NCH of Leu); 4.48 – 4.50 (*m*, NCH of Leu); 6.86 (*s*, 1 H, NH_2); 7.73 (*s*, 1 H, NH_2); 7.88 (*d*, $J = 8.9$, NH of Leu); 7.96 – 7.99 (*m*, 2 NH of Ala); 8.12 (*d*, $J = 8.9$, NH of Leu); EI-MS: 726.8 ($[M + Na]^+$), 704.7 ($[M + H]^+$).

H-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-(S)-β³-HVal-(S)-β³-HAla-(S)-β³-HLeu-NH₂ (**14**). According to *Procedure C*, nine Fmoc-protected β-amino acids were subsequently coupled onto the *Rink*-NH-Fmoc resin (301 mg, 0.33 mmol/g). Cleavage from the resin according to *Procedure E* afforded crude **14**. The product was purified by prep. HPLC (*Phenomenex Jupiter-C4* column; particle size: 10 μm, pore size: 300 Å, 250×21.2 mm) and lyophilized to obtain the TFA salt of **14** (47 mg, 43%) as a white solid. HPLC (*Alltech Adsorbosphere C4*; particle size: 5 μm, pore size: 300 Å, 250×4.6 mm): t_R 15.6 min. $[\alpha]_D^{25} = -6.22$ ($c = 0.27$, MeOH). EI-MS: 1015.9 ($[M + Na]^+$), 994.0 ($[M + H]^+$), 497.9 ($[M + 2H]^{2+}$).

*H-(*S*)-Val-ψ[CH_2SO_2]-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*S*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*S*)-β³-H Val-(*S*)-β³-HAla-(*S*)-β³-HLeu-NH_2* (**15**). According to *Procedure C*, eight Fmoc-protected β-amino acids were subsequently coupled to the *Rink*-NH-Fmoc resin (302 mg, 0.33 mmol/g) followed by coupling of Fmoc-Val-ψ[CH_2SO_2]-Cl according to *Procedure D*. Cleavage from the resin following *Procedure E* afforded crude **15**. The product was purified by prep. HPLC and lyophilized to afford the TFA salt of **15** (62 mg, 55%) as a white solid. HPLC: t_R 23.9 min. $[\alpha]_D^{25} = +3.62$ ($c = 0.21$, MeOH). EI-MS: 1052.0 ($[M+Na]^+$), 1029.8 ($[M+H]^+$), 516.2 ($[M+2H]^{2+}$).

*H-(*S*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*S*)-β³-HVal-(*S*)-Ala-ψ[CH_2SO_2]-(*S*)-β³-HLeu-(*S*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-NH_2* (**16**). According to *Procedure C*, four Fmoc-protected β-amino acids were subsequently coupled to the Rink-NH-Fmoc resin (300 mg, loading 0.33 mmol/g) followed by coupling of Fmoc-Ala-ψ[CH_2SO_2]-Cl according to *Procedure D*. Four more Fmoc-protected β-amino acids were subsequently coupled following *Procedure C*. Cleavage from the resin (*Procedure E*) afforded crude **16**. The product was purified by prep. HPLC and lyophilized to afford the TFA salt of **16** (60 mg, 52%) as a white solid. HPLC: t_R 18.1 min. $[\alpha]_D^{25} = -12.06$ ($c = 0.35$, MeOH). EI-MS: 1051.7 ($[M+Na]^+$), 1029.7 ($[M+H]^+$), 515.7 ($[M+2H]^{2+}$).

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