How To Stabilize or Break β-Peptidic Helices by Disulfide Bridges: Synthesis and CD Investigation of β-Peptides with Cysteine and Homocysteine Side Chains

by **Albrecht Jacobi**[1]) and **Dieter Seebach***

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

all-L-β^3-Penta-, hexa-, and heptapeptides with the proteinogenic side chains of valine, leucine, serine, cysteine, and methionine have been prepared by previously described procedures (**12**, **13**, **14**, **15**; *Schemes 2–5*). Thioether cleavage with Na/NH_3 in β-HMet residues has also provided a β^3-hexapeptide with homocysteine (CH_2CH_2S) side chains (**13e**). The $HS-(CH_2)_n$ groups were positioned on the β-peptidic backbone in such a way that, upon disulfide-bridge formation, the corresponding β-peptide was expected to maintain either a 3_1-helical secondary structure (**1**, **2**) (*Fig. 1*) or to be forced to adopt another conformation (**3**, **4**). The 13-, 17-, 19-, and 21-membered-ring macrocyclic disulfide derivatives and their open-chain precursors, as well as all synthetic intermediates, were purified (crystallization, flash or preparative HPL chromatography; *Fig. 5*) and fully characterized (m.p., $[\alpha]_D$, CD, IR, NMR, FAB or ESI mass spectroscopy, and elemental analysis, whenever possible; *Fig. 2* and *Exper. Part*). The structures in MeOH and H_2O of the new β-peptides were studied by CD spectroscopy (*Figs. 3* and *4*), where the characteristic 215-nm-trough/200-nm-peak pattern was used as an indicator for the presence or absence of (*M*)-3_1-helical conformations. A $CH_2-S_2-CH_2$ and, somewhat less so, a $(CH_2)_2-S_2-(CH_2)_2$ bracket between residues *i* and *i*+3 (**1** *vs.* **12d**, and **2** *vs.* **13e** in *Fig. 3*) give rise to CD spectra which are compatible with the presence of 3_1-helical structures, while $CH_2-S_2-CH_2$ brackets between residues *i* and *i*+2 (**3** *vs.* **14c**) or *i* and *i*+4 (**4** *vs.* **15c** in *Fig. 4*) do not.

1. Introduction. – β-Peptides consisting of as few as six residues and carrying proteinogenic side chains have been shown to form stable secondary structures in MeOH solution. Thus, the NMR structure of a simple β^3-hexapeptide[2]) H-(β^3-HVal-β^3-HAla-β^3-HLeu)$_2$-OH, built from homologated α-amino acids, is a left-handed 3_1 or 3_{14} helix[2]) [1], that of the isomer H-(β^2-HVal-β^3-HAla-β^2-HLeu-β^3-HVal-β^2-HAla-β^3-HLeu)-OH is a right-handed 12/10/12 helix [3], and that of H-(*u*-$\beta^{2,3}$-HAla(α-Me)-*u*-$\beta^{2,3}$-HVal(α-Me)-β^2-HVal-β^3-HLys-*u*-$\beta^{2,3}$-HAla(α-Me)-*u*-$\beta^{2,3}$-HLeu(α-Me))-OH is a hairpin with an antiparallel pleated sheet [4][3]). Two schematic representations of the β^3-peptidic 3_1 helix are shown in *Fig. 1*, a_1 (side view) and a_2 (top view), with the lateral substituents indicated as simple spheres. The helical-wheel-type top view reveals the juxtaposition (in 5-Å distance) of the side chain of amino acids *i* and *i*+3. A proper covalent link between two such neighboring positions should stabilize the helix and a short clamp between *i* and *i*+2, or *i* and *i*+4 positions will enforce new backbone

[1]) Postdoctoral Fellow 1998/99, partially funded by the *Deutsche Forschungsgemeinschaft* (*DFG*).

[2]) In contrast to our original report (Fig. 4, a, in [2]) and in agreement with a recently described effect of protecting groups on the stability of the 3_{14} helix [3], the fully protected derivative Boc-(β-HVal-β-HAla-β-HLeu)$_2$-OMe does not form the 3_1 helix in MeOH, according to CD measurements by *J. V. Schreiber* (hitherto unpublished results, ETH-Zürich, 1998).

[3]) For reviews on β-peptides, see [5–7].

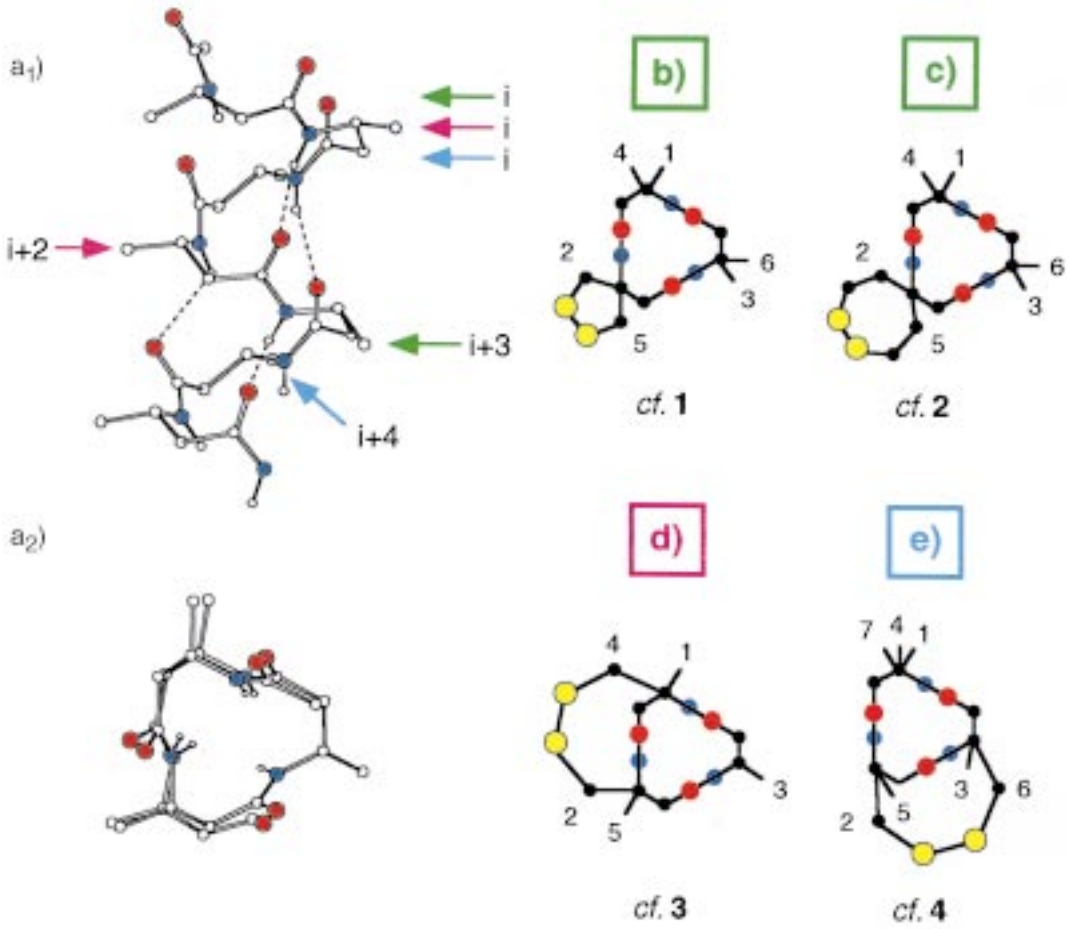


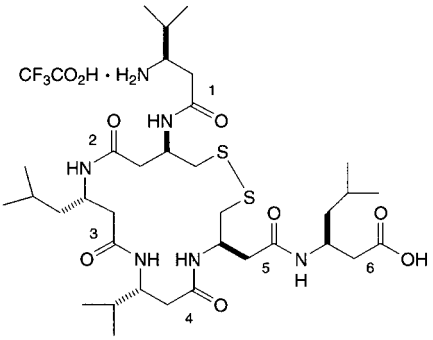
Fig. 1. *Schematical representation of some possible disulfide-bridging modes in short β-oligopeptides.* a_1) Side view of a β-peptidic left-handed or (*M*)-3_1-helix; top: *N*-terminus, bottom: *C*-terminus (colored arrows indicate sulfur side chain positions resulting in varying disulfide-ring sizes as shown in *b*)–*d*)). a_2) Top view of a β-peptidic left-handed or (*M*)-3_1-helix. *b*)–*d*) Cross-sectional helical wheel-type representations of the top views of β^3-peptide with bridging disulfide bonds.

conformations of the β-peptide. We have now synthesized β^3-peptides with cysteine (CH_2SH) and homocysteine (CH_2CH_2SH) side chains to be able to test the structural modifications caused by disulfide-bond formation[4]). Thus, β^3-hexapeptides with $(CH_2)_nSH$ groups in the 2- and 5-position should be able to form 17- and 19-membered disulfide rings that prevent the helix from unwinding (*Fig. 1*, *b* and *c*; see the *Formulae* **1** and **2**). On the other hand, disulfide bridges formed by oxidative coupling of β^3-HCys side chains in the 2- and 4-positions of a β^3-pentapeptide (*Fig. 1*, *d*) or in the 2- and 6-

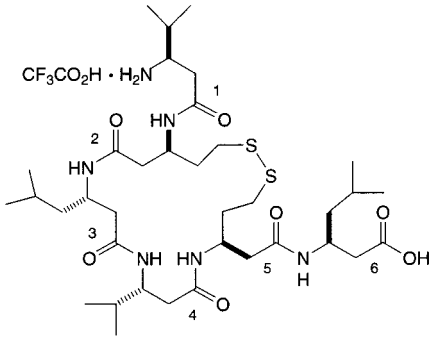
[4]) Various strategies for the introduction of conformational constraint by covalent linkages have been employed in the case of α-peptidic helices (*e.g.*, side-chain lactamization [8], disulfide bridging [9], and ring-closing metathesis [10]).

positions of a β^3-heptapeptide (*Fig. 1*, *e*), with 13- and 21-membered rings, are not compatible with the 3_1 helical structure (see the *Formulae* **3** and **4**). We chose CD spectroscopy as tool for structural analysis in solution. The CD spectra of numerous β-peptides in CF_3CH_2OH, MeOH [11] and in H_2O [12] have led to assignment of a 216-nm trough followed by a 198-nm peak as pattern characteristic of the 3_1 helix of (*M*)-configuration. With the serine side chain in the 4-position of β-heptapeptide **4**, we expected to be able to test the helix-breaking effect of the disulfide bridge in H_2O (*cf.* [12a]).

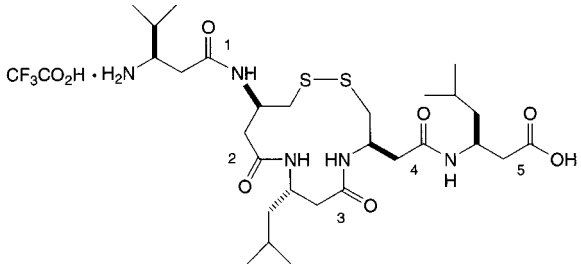
Besides the possibility of disulfide-bond formation, the sulfanyl group in cysteine is of great interest with respect to its capacity as a metal-ligating functional group [13][5]). Furthermore, it is intriguing to note that the α-amino-acid sequence Cys–X–X–Cys occurs frequently at the active sites of various metal-containing [15] and metal-free proteins[6]).



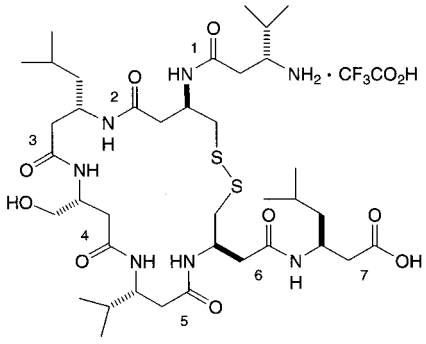
1



2



3



4

---

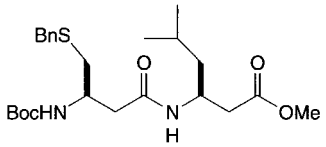
[5]) SH-Group-mediated fixation of β-peptides to a gold surface is of particular interest, as it should thus be possible to obtain structural data about β-peptide monolayers from STM methods [14].

[6]) Examples for the occurrence of the Cys–X–X–Cys sequence in proteins are the zinc-finger motif [16], iron-sulfur clusters [17], oxidoreductases like thioredoxins and glutaredoxins [18], and cytochrome *c*, where the cysteine residues form a thioether linkage between the apoprotein and the prosthetic group [19].

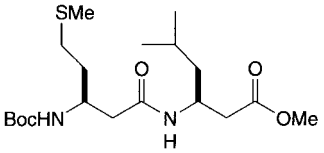
2. Synthesis of the β-Peptides 1–4. – We employed solution synthesis with *N*-terminal Boc and *C*-terminal MeO protection[7]). Starting from the commercial *N*-Boc-protected α-amino acids *S*-benzylcysteine and methionine[8]), and by the *Arndt-Eistert* homologation procedure as described previously [1][20] for the other β-amino-acid building blocks, we prepared Boc-(*R*)-β^3-HCys(Bn)-OH (**5**) and Boc-(*R*)-β^3-HMet-OH (**6**)[9]) and coupled them to give the β-di-, tri-, and tetrapeptide derivatives **7**–**11** in good yields (79–86%; see *Scheme 1*). *C*-Terminal deprotection of the β-tripeptides **9a** and **10a** by methyl-ester hydrolysis gave **9b** (91%) and **10b** (92%), respectively.

5

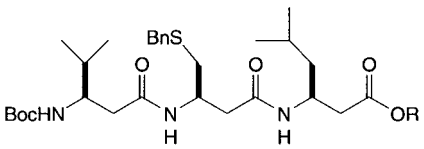
6



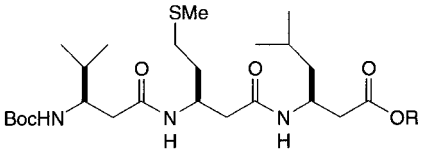
7



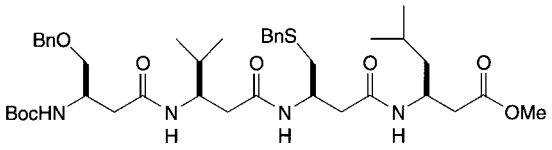
8



9a (R = Me)
b (R = H)



10a (R = Me)
b (R = H)



11

The fully protected hexapeptide **12a** was obtained by fragment coupling of Boc-deprotected **9a** with **9b** (yield: 71%) (*Scheme 2*). Due to poor solubility in organic

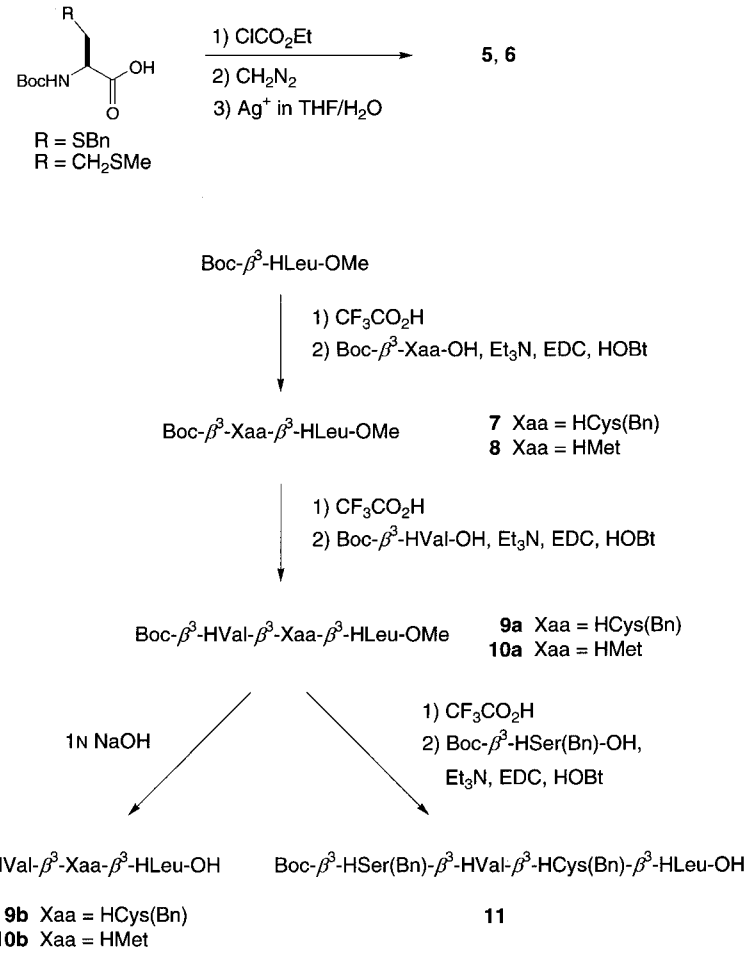
---

[7]) This particular set of protecting groups was chosen for being orthogonal to alkyl protection of the sulfanyl group. Protecting groups susceptible to removal by hydrogenolysis could not be employed because of the catalyst-poisoning effect of thioether and thiol functions.

[8]) We were confident that the methionine side chain in β-peptides would be amenable to demethylation by Na/NH_3, a reaction which had been performed successfully on α-methionine [21].

[9]) As pointed out before for the homologative conversion of (*S*)- or L-valine to (*R*)- or L-β^3-homovaline [1], the retentive homologation of (*S*)- or L-methionine takes place with change of the chirality sense, caused by reversal of the *CIP* priority sequence.

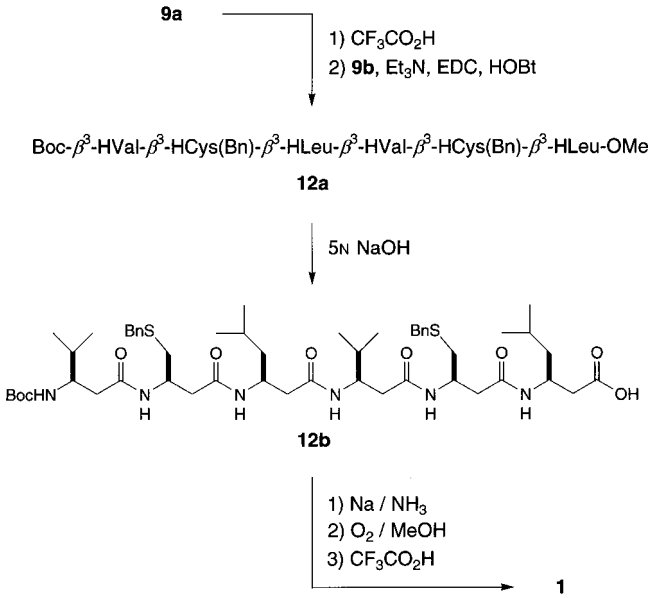
Scheme 1. *Preparation of* (R)-β^3-*Amino-Acid Derivatives* **5** *and* **6** *by* Arndt-Eistert *Homologation and Preparation of Peptide Derivatives* **7**–**11**. Xaa = β-Amino acid with various substitution patterns. For other abbreviations, see *General* in *Exper. Part.*



solvents (as encountered before with other β^3-hexa- and β^3-heptapeptides [1][2]), flash chromatography (FC) was not possible with **12a**, which was instead purified by recrystallization from MeOH. The conditions employed for the methyl-ester hydrolysis of hexapeptide derivative **12a** were more drastic than for the smaller peptides; the yield of **12b** was nevertheless high (94%) and no side products were observed.

The elaboration of the subsequent deprotection and cyclization protocol yielding the cyclic disulfide-bridged peptide **1** required considerable experimental effort. While the cleavage of the *S*-benzyl groups in **12b** was found to proceed smoothly with fivefold molar excess of Na in liquid NH_3, the resulting crude linear dithiol peptide was found to be very sensitive to air oxidation. Reversed-phase HP liquid chromatography (RP-HPLC) of Boc-deprotected samples revealed the formation of several less and more

Scheme 2



polar species only minutes after exposure to air[10]). Most of these species (not identified, probably disulfide oligomers of different molecular weight and connectivity) were, in turn, susceptible to reductive cleavage. Treatment of the mixture with excess dithiothreitol resulted in only two major products (by RP-HPLC analysis). As a consequence of these findings, the *S*-benzyl deprotection and cyclization of **12b** was conducted in a one-pot procedure: upon completion of the reductive cleavage, the Na/NH_3 solution was quenched and immediately diluted with MeOH to an 80 μM peptide concentration, in order to prevent aggregation and intermolecular side reactions (see *GP 4* in *Exper. Part*). Air oxidation yielded a single cyclic peptide derivative, which was Boc-deprotected to give **1** in a rather good yield (53% after purification by prep. RP-HLPC)[11]).

The hexapeptide derivative **13b** with methionine side-chains was also prepared in two steps starting from Boc-deprotected **10a** and **10b** (*Scheme 3*). Cleavage of the sulfanylmethyl groups in **13b** required forcing *Birch* conditions (large excess of Na, prolonged reaction times of up to 24 h) but proceeded without considerable side reactions (*e.g.*, degradation of the main chain, desulfuration[12])) to yield the dithiol **13d**. Boc-Deprotection followed by preparative RP-HPLC purification gave the free dithiol

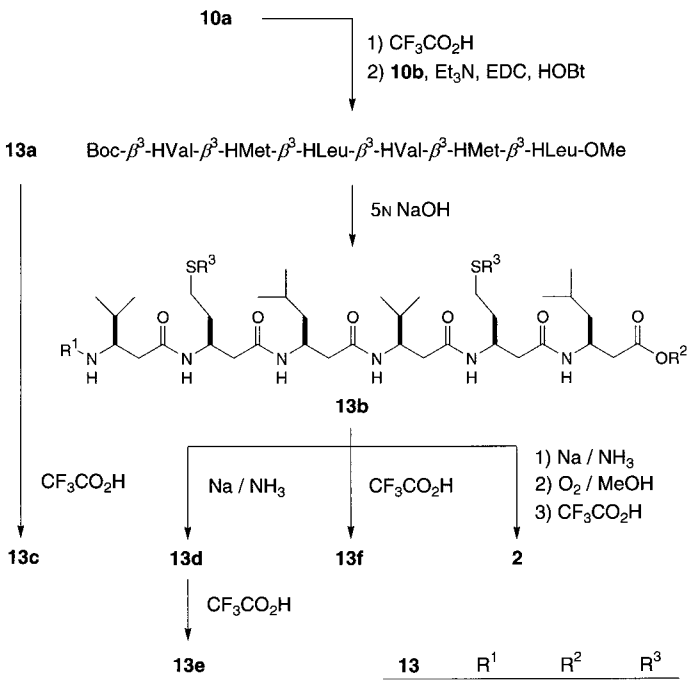
---

[10]) Addition of catalytic amounts dithiothreitol (0.1 equiv.) to these mixtures of fully deprotected peptide species in MeOH (degassed, 1% Et_3N) and stirring for 5 days under Ar did not result in a reduced number of compounds (as judged by the RP-HPLC analysis) (*cf.* [22]).

[11]) For comparison of thiol oxidation methods generating cyclic disulfides in peptides, see [23].

[12]) A side reaction, which had been observed during *S*-debenzylation of the A chain of porcine insuline under *Birch* conditions [24].

Scheme 3



13	R^1	R^2	R^3
b	Boc	H	Me
c	TFA·H	Me	Me
d	Boc	H	H
e	TFA·H	H	H
f	TFA·H	H	Me

peptide **13e** (positive *Ellman*'s test, see *Exper. Part*), which could be cyclized in MeOH/NH_3 to give the macrocycle **2**[13]).

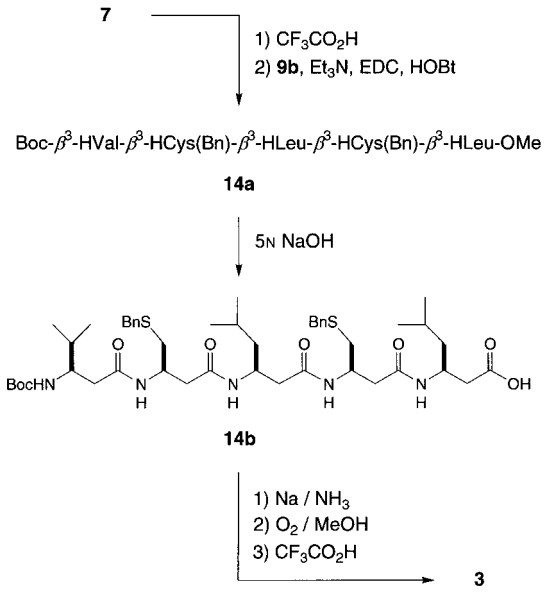
The fully protected pentapeptide **14a** was prepared by coupling of Boc-deprotected **7** with **9b** (*Scheme 4*). Deprotection and cyclization steps were performed as described above, while this particular cyclization to a 13-membered ring in **3** proceeded in slightly lower yield (38% after prep. RP-HPLC purification) than with the larger cyclic disulfides.

The fully protected heptapeptide **15a** was prepared in the same way, from the Boc-deprotected tetrapeptide **11** and the tripeptide acid **9b** (*Scheme 5*). Compound **15a** is well soluble in organic solvents ($CHCl_3$, MeOH) and was purified by FC (yield: 42%); subsequent methyl-ester saponification gave the *N*-Boc-peptide acid **15b** (yield: 96%).

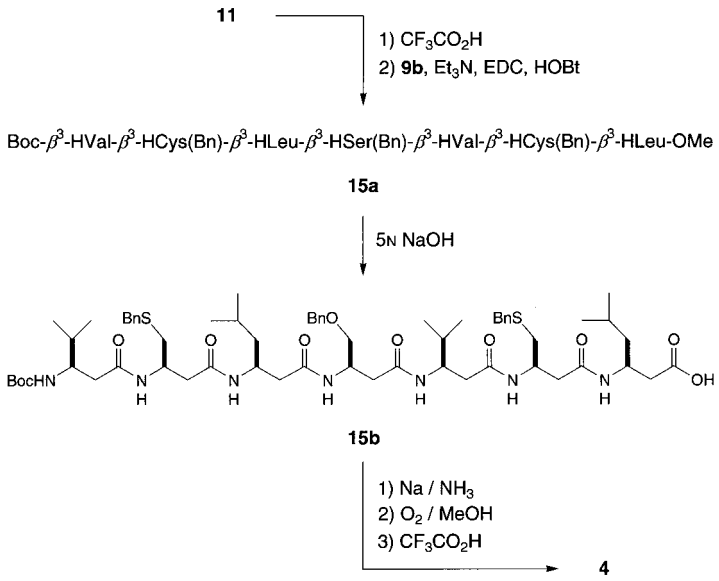
---

[13]) Compound **2** could also be prepared from **13b** by the one-pot deprotection/cyclization procedure (described for **12b**), followed by prep. RP-HPLC purification (yield: 51%). Earlier attempts to cyclize **13d** or **13e** by oxidation with H_2O_2 [23] had failed. DMSO-Mediated oxidation of **13e** [25] had given **2** in low and unreproducible yields, and additional difficulties were encountered in the removal of DMSO from the product.

Scheme 4



Scheme 5



Simultaneous debenzylation of the ether and thioether moieties in the acid **15b** under *Birch* conditions (see above) provided no difficulties, and the macrocycle **4** was obtained after oxidative cyclization and prep. RP-HPLC purification in 65% overall yield.

While the purity of the cyclic disulfide-bridged peptides **1**–**4** was established by analytical RP-HLPC (see *Fig. 5* in *Exper. Part*)[14]), electrospray-ionization mass spectroscopy (ESI-MS) proved to be the method of choice to further corroborate the structures of these compounds (see *Fig. 2*). The cyclic compound **2** and its reduction product **13e** could easily be distinguished by a mass difference of two Da in the positive- as well as the negative-ion mode. Correct ESI mass spectra were also obtained for peptides **1**, **3**, and **4** (see *Exper. Part*).

3. CD Spectroscopy. – As concluded from numerous CD measurements and corresponding NMR structural investigations of β-peptides [1][2][8][9], the CD pattern of a trough at *ca.* 216 and a peak at *ca.* 198 nm is indicative of a 3_1 helix of (*M*)-configuration. While the chromophoric groups in the linear peptides studied here are essentially the same as in the earlier reports, the macrocyclic peptides **1**–**4** have an additional disulfide chromophore [26]. Considering a possible CD contribution of this unit (which results from axial chirality of the S–S bond), we recorded CD spectra of the disulfide-bridged peptides **1**–**4** in the range from 190 to 350 nm[15]). None of them shows any *Cotton* effect above 230 nm[16]).

The CD spectra of cysteine-containing β^3-hexapeptide derivatives **1**, **12c**, and **12d** are shown in *Fig. 3*, *a*. Compound **1** exhibits a pattern typical for a 3_1-helical structure in MeOH, but with a small blue shift. A stronger shift in this direction, accompanied by a decreased intensity of the *Cotton* effect, is detected for **1** in H_2O. Nevertheless, a considerable amount of helical structure must still be present for **1** in aqueous solution, as judged from its CD spectrum[17]).

Reductive opening of the 17-membered ring of **1** mediated by excess dithiothreitol (see *GP 6* in *Exper. Part*) yields **12d**, the CD spectrum of which shows a strong 200-nm maximum followed by a 216-nm minimum indicating an undisturbed 3_1 helix (see above). Interestingly, the *S*-benzyl side chains in **12c** appear to break the secondary structure: a complete loss of the 216-nm minimum is observed.

The oxidative cyclization of **13e** yields peptide **2** with a 19-membered disulfide-bonded ring, and thus with a ring size increased by two atoms as compared to **1**. Apart from decreased intensities, the CD spectra of **2** and **13e** are similar (*Fig. 3*,*b*). If at all, the helical conformation of **2** is only marginally maintained in aqueous solution. This means that, while the 17-membered ring in **1** allows for preservation of certain features of the helical secondary structure in H_2O, the 19-membered ring in **2** is not able to

---

[14]) Ellman's tests, which were performed on samples of **1**–**4** after prep. RP-HPLC purification, showed that no free thiol groups were present.

[15]) According to calculations, the *Cotton* effect of a disulfide group in the region of the amide-backbone transitions (below 230 nm) should be negligible [27]. For further theoretical work on disulfide stereochemistry and chiroptical properties, see [28].

[16]) A possible explanation for these findings is that the energy difference between the two diastereoisomeric species resulting from (*P*)- or (*M*)-configuration of the S–S unit is very small and, as the barrier for the rotation about the S–S bond is low, their respective *Cotton* effects cancel each other [27][28].

[17]) One must keep in mind that linking, and thus conformationally locking, the central residues of a helix as done in peptide **1** (*cf. Fig. 1*,*b*) cannot prevent the helix from unwinding at its termini, which could account for the decreased CD intensities observed upon switching from a less protic (MeOH) to a more competitive solvent such as H_2O.

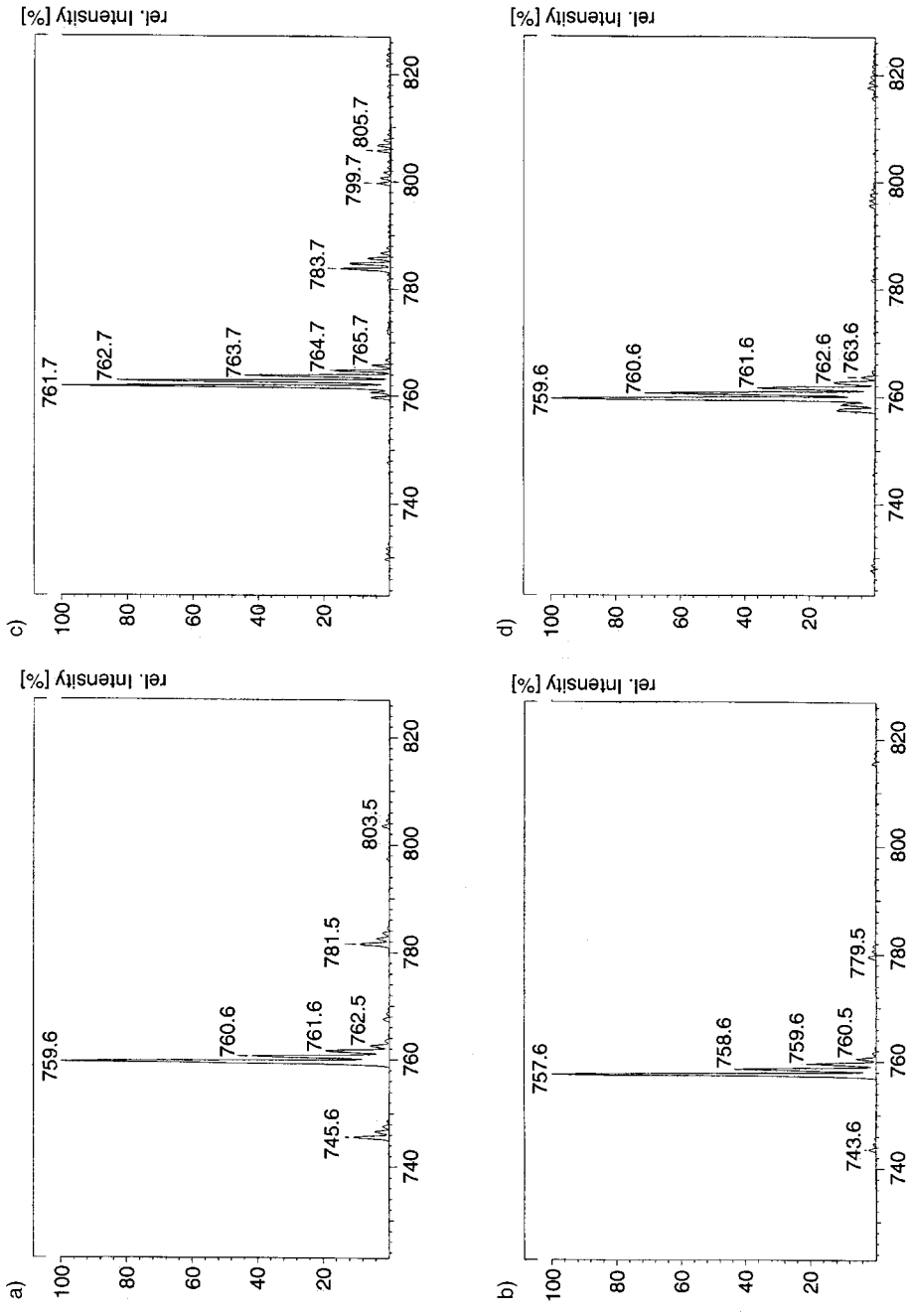


Fig. 2. *ESI Mass spectra of cyclic* (**2**) *and linear β-peptides* (**13e**) (for experimental conditions, see *General* in *Exper. Part*). *a*) **2** (*m/z* 758.44; positive-ion mode). *b*) **2** (*m/z* 758.44; negative-ion mode). *c*) **13e** (*m/z* 760.46; positive-ion mode). *d*) **13e** (*m/z* 760.46; negative-ion mode)

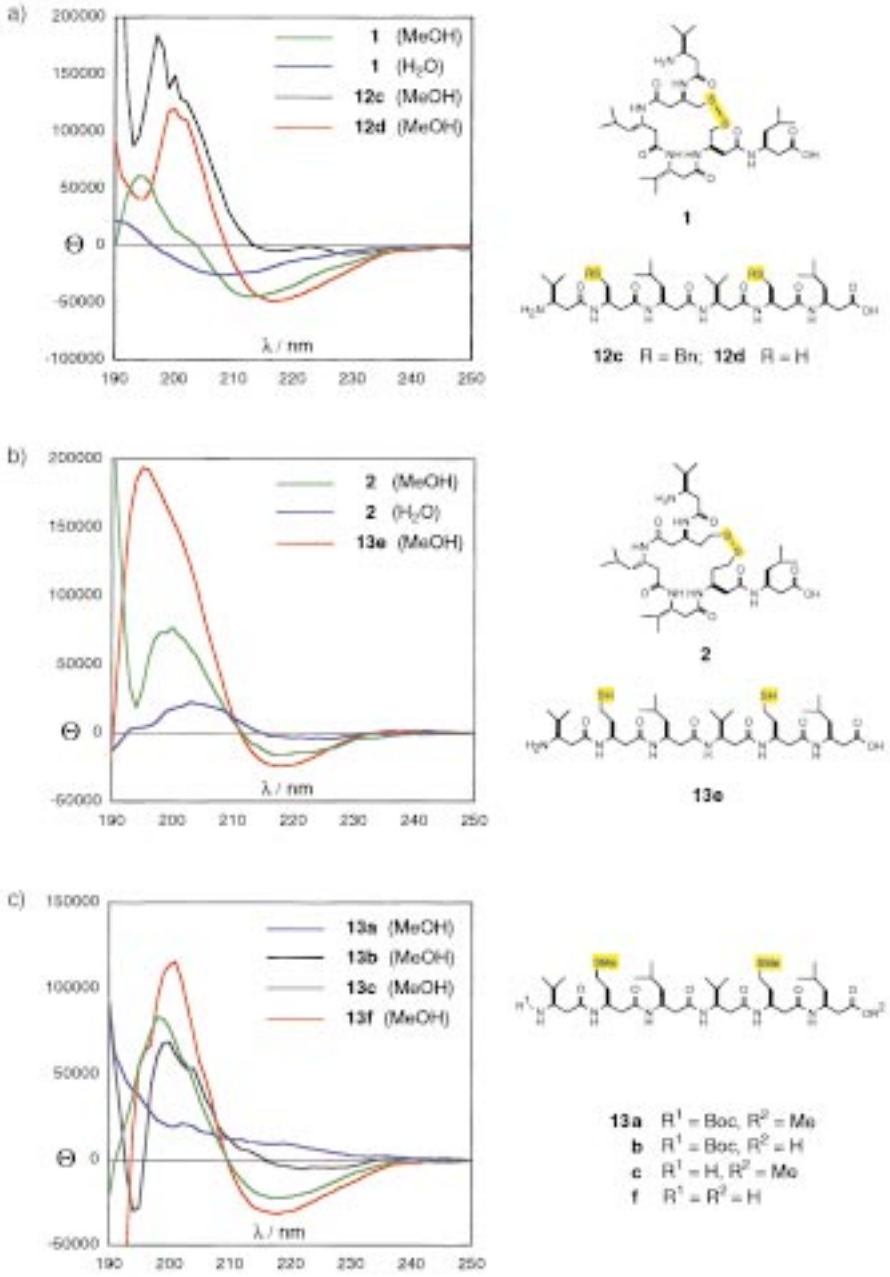


Fig. 3. *Overlay of CD spectra of linear and cyclic β-peptides* **1**, **2**, **12c**, **12d**, **13a**–**c**, **13e**, *and* **13f** *in MeOH and* H_2O. *a*) β-Peptides **1**, **12c**, and **12d**. *b*) β-Peptides **2** and **13e**. *c*) β-Peptides **13a**–**c** and **13f**. Molar ellipticity $[\Theta]$ in 10 deg · cm^2 · mol^{-1}. All *N*-deprotected β-peptides were measured as their TFA salts (see *Exper. Part*).

enforce the helical structure to the extent required for survival of the competitive H-bonding in aqueous media.

The effect of the main-chain protecting groups on the formation and stability of the 3_1 helix[2]) was investigated with the linear methionine peptide derivatives **13a–c** and **13f** in MeOH (*Fig. 3,c*): whereas the fully protected hexapeptide **13a** shows rather no pattern at all, *N*-Boc-peptide acid **13b** exhibits a 'distorted' 3_1 helical pattern, which is nervertheless clearly present, with a pronounced maximum at 200 nm but only weak minimum at 222 nm. Boc-Deprotected methyl ester **13c** gives the familiar pattern, which is almost as strong as for the fully deprotected peptide **13f** (molar ellipticity: $+1.15 \cdot 10^5$ (201 nm); $-3.2 \cdot 10^4$ (218 nm)[18]). While the influence of protecting groups on the stability of the 3_1 helix can not be rationalized simply in terms of intramolecular H-bonding[19]), the interaction between the charged terminal groups and the helix macrodipole has been proposed to cause this effect[20]). Thus, the positive charge at the H_3N^+ terminus (negative end of the macrodipole) should have a stabilizing effect on the β-peptidic 3_1 helix, and the same is true for the negative charge at the CO_2^- terminus (positive end of the macrodipole)[21]). Clearly, a careful investigation of pH and solvent dependence, as well as of salt effects [30][31], will be necessary for better understanding.

The 13-membered cyclic β-pentapeptide **3**, which can not possibly form a 3_1 helix (*Fig. 4*, *a*), gives rise to a CD curve with a single minimum below 200 nm. Upon reductive cleavage of the disulfide bond in **3** with excess dithiothreitol, the pattern is almost reversed (**14c**: molar ellipticity $+1.05 \cdot 10^5$ at 198 nm; $-9.0 \cdot 10^3$ at 220 nm), and a gross pattern results similar to that associated with the 3_1 helix. That a pronounced trough is missing supports the proposed minimum number of six residues necessary to form a stable β-peptidic 3_1 helix [1]. In contrast to all other compounds described herein, the CD spectrum of **3** in H_2O does not differ much from its spectrum in MeOH. This weak solvent dependence could be due to a comparatively rigid backbone induced by the 13-membered ring in **3**.

Finally, the β-heptapeptide **4** contains a 21-membered ring, which should not fit with a 3_1-helical structure (*cf. Fig. 1*). Indeed, **4** shows a novel CD pattern in MeOH with a 200 nm trough, which collapses in aqueous solution[22]). Upon reductive ring cleavage in **4**, the characteristic 3_1-helical CD pattern is fully restored (see the spectrum of **15c**).

A particular feature of the CD pattern exhibited by the helical β-peptides described herein is a variation of the relative peak and trough intensities (*cf. Figs. 3* and *4*). The

---

[18]) The protecting-group effect was found to be independent of the peptide concentration in the range from 0.02 to 0.4 mM in MeOH.

[19]) While in the case of the α-peptidic α and 3_{10} helices [29], and also in the β-peptidic 12/10/12 helix [3] an *N*-terminal Boc group can account for an additional H-bond with a main-chain amide group, the β-peptidic 3_1 helix lacks this possibility for additional stabilization, as all NH groups are pointing towards the C-terminus (*cf. Fig. 1*, a_1).

[20]) As proposed in the helix dipole model for α-peptides [30], charges on side chains which are opposite to the resulting helix macrodipole charge should also stabilize the helix structure of β-peptides.

[21]) As pointed out before, the α-peptidic α helix and the β-peptidic 3_1 helix have opposite polarity with respect to their C- and N-termini [5].

[22]) The H_2O solubility of **4** (0.7 mM), containing one serine side chain, is moderately increased in comparison to **1** (0.3 mM).

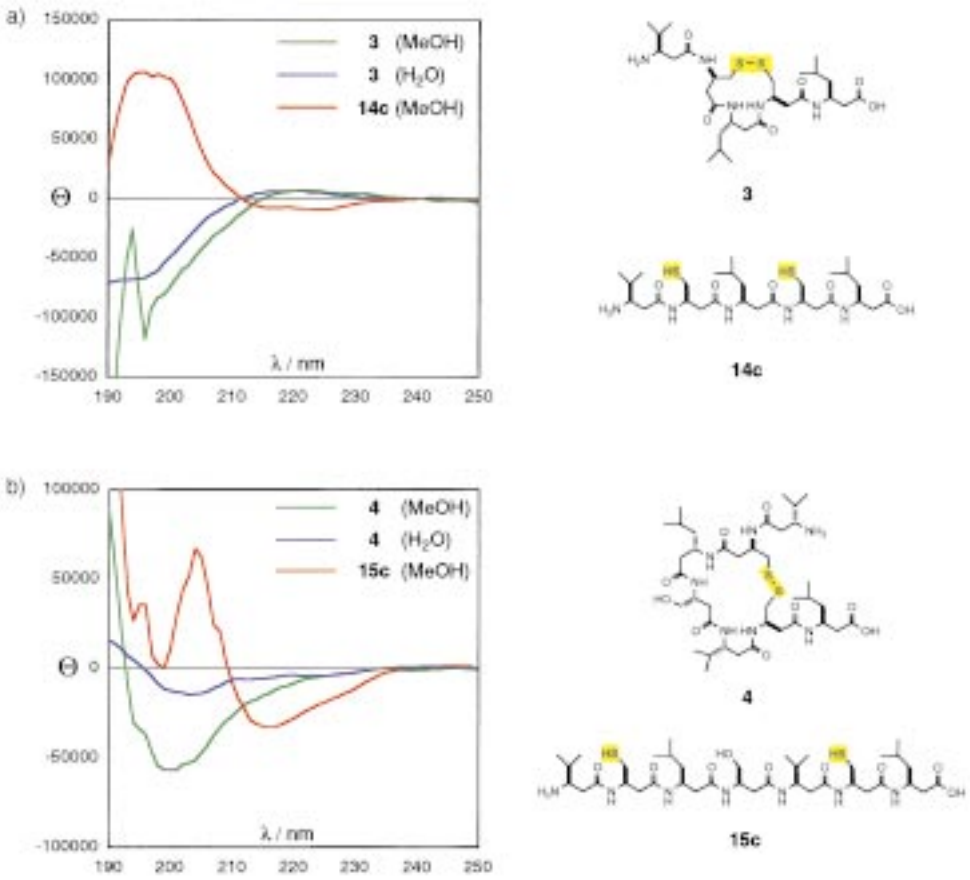


Fig. 4. *Overlay of CD spectra of linear and cyclic β-peptides* **3**, **4**, **14c**, *and* **15c** *in MeOH and* H_2O. *a*) β-Peptides **3** and **14c**. *b*) β-Peptides **4** and **15c**. Molar ellipticity $[\Theta]$ in 10 deg · cm^2 · mol^{-1}. All *N*-deprotected β-peptides were measured as their TFA salts (see *Exper. Part*).

intensity ratios $R = [\Theta]_{217}/[\Theta]_{200}$ in MeOH of the two extremes associated with the 3_1-helical peptide structure range from $R = 0.1$ in **13e** to $R = 0.8$ in H-(β^3-HVal-β^3-HAla-β^3-HLeu)$_2$-OH [1]. It is possible that these changes of *R* correspond to only small structural changes within the 3_1 helix[23]).

Structural determinations by NMR spectroscopy are currently under way in order to establish a better correlation between 'known' CD and structural pattern; in addition, the NMR structures of compounds exhibiting a novel CD pattern remain to be determined.

---

[23]) The ratio $R = [\Theta]_{222}/[\Theta]_{207}$ (corresponding to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ backbone amide transitions, respectively) was taken as an indicator to distinguish α-helical from 3_{10} helical conformations in α-peptides [32]. CD Spectra of poly(β-amino acid) helices were recently calculated and compared with experimental data [33].

4. Conclusion. – We have prepared and studied β^3-peptides with cysteine and homocysteine side chains in order to demonstrate the general feasibility of disulfide bridging in β-peptide chemistry. Thus, the cyclic peptides **1**–**4** were obtained in reasonable yields by air oxidation from their corresponding open-chain precursors.

Structural features of the new peptides were elucidated by CD spectroscopy: Whereas the cyclic peptides **1** and **2** show weaker *Cotton* effects than their respective linear analogues in MeOH (which could mean that the geometry imposed on the 3_1 helix by cyclization is not optimal in either case), a net stabilization was found for **1** in H_2O: The *Cotton* effect detected for **1** was stronger than for the linear 3_1-helical peptides with proteinogenic side chains known to date [1][12].

On the other hand, the helix-disrupting effect of disulfide linkages attached to positons that do not stack above each other in the 3_1 helix was clearly demonstrated with the cyclic peptides **3** and **4**.

In summary, it may be said that disulfide linkages in β-peptides are – as they are in α-peptides – a valuable tool for introducing conformational restrictions. This tool can be used advantageously in the design of novel secondary and possibly tertiary structures, and thus will allow us to rationally proceed with investigations of β-peptides.

We gratefully acknowledge the financial support of the *Deutsche Forschungsgemeinschaft* (*DFG*) (fellowship No. JA 918/1-1 to *A. J.*). *D. Rigo* is thanked for the preparation and characterization of Boc-(*R*)-β^3-HCys(Bn)-OH, *K. Gademann* for a donation of Boc-(*R*)-β^3-HSer(Bn)-OH, which was used for the preparation of the β^3-heptapeptide **4**. We gratefully acknowledge the assistance by the staff in our analytical division: *B. Brandenberg* and *P. Zumbrunnen* (NMR), Dr. *W. Amrein*, *O. Greter*, and *R. Häfliger* (MS) and *D. Manser* (elemental analysis). Continuing support of *Novartis Pharma AG*, Basel, is greatly appreciated.

Experimental Part

1. *General*. Abbreviations: Boc_2O: di(*tert*-butyl) dicarbonate, DTT: dithiothreitol, EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: flash chromatography, HOBt: 1-hydroxy-1*H*-benzotriazole, h.v.: high vacuum, 0.01–0.1 Torr, β-HXaa: β-homoamino acid. 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. $ClCO_2Et$ was distilled and stored at $+4°$ (refrigerator) under Ar. All indicated temp. were monitored with an internal thermometer (*Ebro-TTX-690* digital thermometer). Amino-acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. All other reagents were used as received from *Fluka*. The diazo ketones Boc-(*S*)-Cys(Bn)-CHN_2 and Boc-(*S*)-Met-CHN_2 [34], as well as the β-amino-acid derivatives Boc-(*R*)-β^3-HVal-OH [1], Boc-(*R*)-β^3-HSer(Bn)-OH [35], Boc-(*R*)-β^3-HMet-OH (**6**) [35] and, Boc-(*S*)-β^3-HLeu-OMe [1], were prepared according to literature procedures. *Caution:* The generation and handling of CH_2N_2 requires special precautions [36]. Reactions carried out with the exclusion of light were performed in flasks completely wrapped in aluminum foil. TLC: *Merck* silica gel *60* F_{254} plates; detection with UV, and anisaldehyde or I_2. FC: *Fluka* silica gel *60* (40–63 μm); at *ca.* 0.2 bar. Anal. HPLC: *Knauer* HPLC System *K 1000*, *EuroChrom 2000* Integration Package, degasser, UV detector *K 2000* (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); *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. Circular dichroism (CD) spectra: *Jasco J-710* recording from 190 to 250 nm at r.t.; 1-mm rectangular cell; average of five scans, corrected for the baseline; peptide concentration 0.2 mM in MeOH or H_2O; molar ellipticity Θ in deg · cm^2 · $dmol^{-1}$ (λ in nm); smoothing by *Jasco* software. IR Spectra: *Perkin-Elmer-782* spectrophotometer. NMR Spectra: *Bruker AMX 500* (^1H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^1H: 400 MHz, ^{13}C: 100 MHz), *ARX 300* (^1H: 300 MHz), *Varian Gemini 300* (^1H: 300 MHz, ^{13}C: 75 MHz); chemical shifts δ in ppm downfield from internal Me_4Si (= 0 ppm); *J* values in Hz. MS: *VG Tribrid* (EJ), *VG ZAB2-SEQ* (FAB, in a 3-nitrobenzyl-alcohol matrix), and *Finnigan TSQ 7000* (ESI, sprayed from a 10^{-5} M methanolic solution; volumetric flow 20 μl/min) spectrometer; in *m/z* (% of basis peak). Elemental

analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

2. *Boc Deprotection: General Procedure 1* (*GP 1*). Similarly to the reported procedure [1][2], the Boc-protected amino acid was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. An equal volume of CF_3CO_2H was added, and the mixture was allowed to slowly warm to r.t. and then stirred for further 1.5 h. Concentration under reduced pressure and drying of the residue under h.v. (12 h) yielded the crude CF_3CO_2H salt, which was identified by NMR and used without further purification.

3. *Methyl-Ester Hydrolysis: General Procedure 2a* (*GP 2a*). The fully protected oligopeptide was dissolved in MeOH (0.1M) and treated with 1N NaOH (1.2 equiv.). After 24 h, the mixture was diluted with H_2O, and the pH was adjusted to 2–3 with 1N HCl. The soln. was extracted with AcOEt, and the combined org. phases were washed successively with sat. aq. NaCl soln. and H_2O. The org. phase was evaporated and dried under h.v.

General Procedure 2b (*GP 2b*). The fully protected oligopeptide was dissolved in CF_3CH_2OH (0.1M), treated with 5N NaOH (100 equiv.), and heated to 45°. After completion of the reaction (TLC), the mixture was diluted with H_2O, and the pH was adjusted to 2–3 with 5N HCl (0°). The soln. was extracted with AcOEt, and the combined org. phases were washed successively with sat. NaCl soln. and H_2O. The org. phase was evaporated and dried under h.v.

4. *Peptide Coupling with EDC: General Procedure 3a* (*GP 3a*). The appropriate CF_3CO_2H salt was dissolved in $CHCl_3$ (0.5M) and cooled to 0°. The soln. was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in $CHCl_3$ (0.5M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and then stirred for 18 h. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N HCl, sat. aq. $NaHCO_3$ soln., and sat. aq. NaCl soln. The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure. FC yielded the pure peptide.

General Procedure 3b (*GP 3b*). The appropriate CF_3CO_2H salt was dissolved in $CHCl_3$ (0.5M) and cooled to 0°. The soln. was treated successively with Et_3N (5 equiv.), HOBt (1.2 equiv.), a soln. of the Boc-protected fragment (1 equiv.) in DMF (0.1M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and then stirred for 18 h. The solvents were removed under reduced pressure, and the residue was dispersed in $CHCl_3$ (0.02M). The resulting suspension was washed with 1M HCl, sat. aq. $NaHCO_3$ soln., sat. aq. NaCl soln., and H_2O. The org. phase was concentrated under reduced pressure. FC or recrystallization yielded the pure peptide.

5. *Cleavage of* S-*Benzyl Groups/*O-*Benzyl Groups*[24]) *and Cyclization to the Disulfide. General Procedure 4* (*GP 4*). The Boc-protected oligopeptide was dissolved in NH_3 (2 mM) at −33°; 5 equiv. of Na per Bn-group was added. The dark blue soln. was stirred for 1 h and subsequently quenched by addition of MeOH. The resulting methanolic solution of NH_3 was immediately diluted to a peptide concentration of 0.08 mM. Air oxidation was allowed to proceed while air was bubbled through the soln. with slow stirring. Aliquots were removed at different times in order to monitor reaction progress by RP-HPLC (after workup and Boc-deprotection of these samples). When oxidation was judged to be complete (*Ellman*'s test [38] was negative), MeOH was removed under reduced pressure and the resulting crude cyclic peptide was Boc-deprotected according to *GP 1*.

6. *Reversed Phase (RP) HPLC Analysis and Purification of β-Peptides: General Procedure 5* (*GP 5*). RP-HPLC Analysis was performed on a *Macherey-Nagel* C_{18} column/*Nucleosil 100-5* C_{18} (250 × 4 mm) by using a linear gradient of *A* (0.1% CF_3CO_2H in H_2O) and *B* (MeCN) at a flow rate of 1 ml/min with UV detection at 220 nm. t_R in min. Crude products were purified by prep. RP-HPLC (*Macherey-Nagel* C_{18} column/*Nucleosil 100-7* C_{18} (250 × 21 mm) using a gradient of *A* and *B* at a flow rate of 20 ml/min with UV detection at 220 nm) and then lyophilized.

7. *S−S Reduction of Cyclic Disulfide-Bridged Oligo-β-peptides: General Procedure 6* (*GP 6*). A 0.2 mM methanolic solution of the cyclic peptide was purged with Ar and treated with 50 equiv. KOH and 25 equiv. DTT under Ar. The reaction was monitored by RP-HPLC (*Fig. 5*). After 1 h, the soln. was acidified by addition of CF_3CO_2H and stored under Ar.

*Boc-(*R*)-β*^{3}*-HCys(Bn)-OH* (**5**). According to [1], a soln. of Boc-(*S*)-Cys(Bn)-CHN_2 (6.71 g, 20 mmol) in THF (50 ml) containing 10% H_2O at −25° (bath temp.) was treated with a soln. of CF_3COOAg (486 mg, 2.2 mmol) in Et_3N (5.86 g, 58 mmol) under Ar with the exclusion of light. The mixture was allowed to warm to r.t. within 4 h in the dark. After 12 h, the soln. was reduced to half of its initial volume, diluted with Et_2O (35 ml) and extracted with sat. aq. $NaHCO_3$ soln. The aq. phase was carefully adjusted to pH 2–3 at 0° with 5N HCl and extracted with Et_2O. The org. phase was dried ($MgSO_4$) and then concentrated under reduced pressure.

---

[24]) For a detailed description of a *Birch* reduction (experimental setup and procedure), see [37].

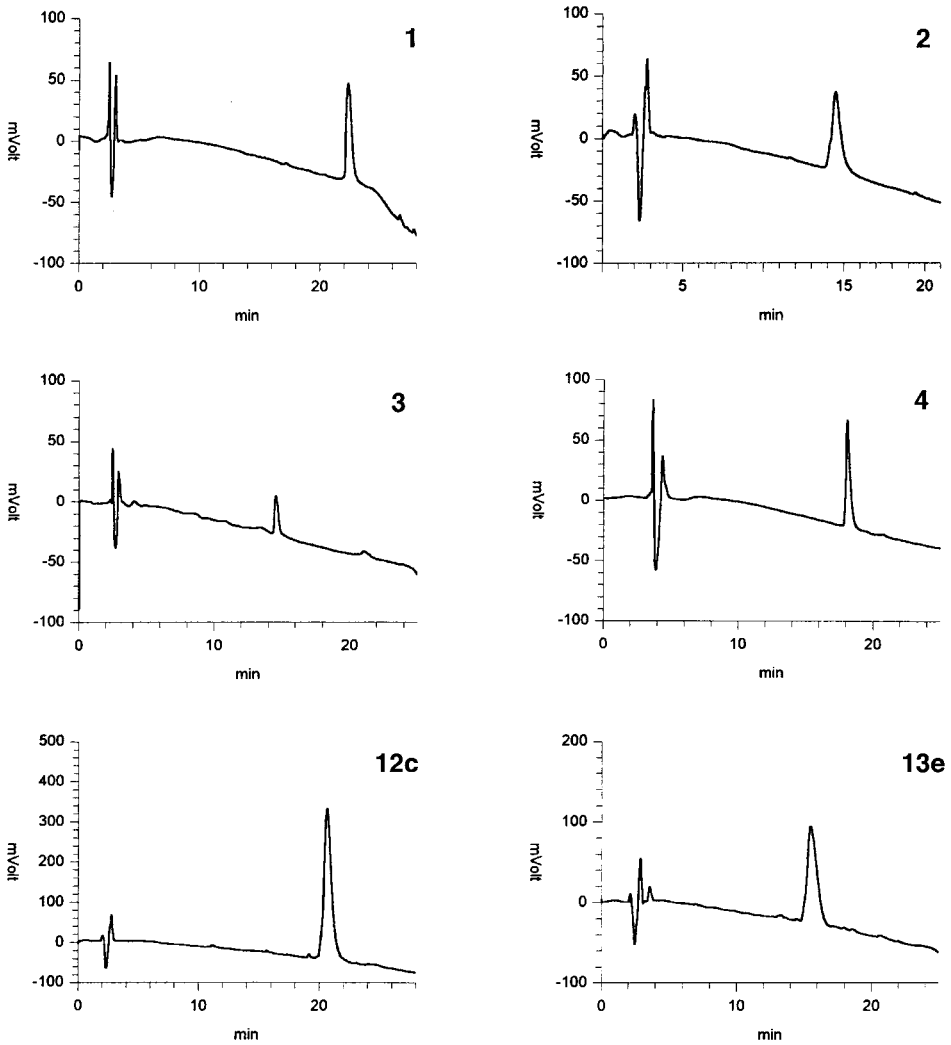


Fig. 5. *HPLC Profiles of purified* β-peptides **1**–**4**, **12c**, *and* **13e** (*RP-*C_{18} column, linear gradient of *A* (0.1% CF_3COOH in H_2O and *B* (MeCN); see *GP 7* in *Exper. Part*)

Recrystallization from Et_2O/pentane yielded **5** (3.97 g, 61%). White solid. M.p. 112–113°. R_f (CH_2Cl_2/MeOH 15:1) 0.45. $[\alpha]_D^{rt.} = +4.3$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3365*m*, 2979*m*, 1682*s*, 1517*s*, 1365*m*, 1299*m*, 1250*m*, 1165*s*, 1047*w*, 1018*w*, 768*w*, 695*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.45 (*s*, *t*-Bu); 2.57–2.71 (*m*, CH_2CO, CH_2S); 3.73 (*s*, SCH_2Ph); 4.05–4.16 (*m*, CHN); 5.10 (br., NH); 7.21–7.34 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 28.4 (Me); 35.2, 36.3, 37.3 (CH_2); 46.6 (CH); 79.9 (C); 127.2, 128.6, 129.0 (arom. C); 155.3, 176.6 (C). FAB-MS: 326 (44, $[M+1]^+$), 307 (100), 289 (46), 270 (48), 226 (16, $[M-\text{Boc}+1]^+$). Anal. calc. for $C_{16}H_{23}NO_4S$ (325.42): C 59.05, H 7.12, N 4.30; found: C 59.10, H 7.07, N 4.23.

*Boc-(*R*)-*β^3*-HMet-OH* (**6**). Treatment of a soln. of Boc-(*S*)-Met-CHN_2 (10.90 g, 40 mmol) according to [1] followed by recrystallization of the crude product from Et_2O/pentane yielded **6** (7.57 g, 73%). White solid. M.p. 83–84°. R_f (Et_2O/pentane 2:1) 0.15. Spectroscopical data in agreement with [35]. $[\alpha]_D^{rt.} = -20.2$ ($c = 1.0$, $CHCl_3$). ([35]: $[\alpha]_D^{rt.} = -19.9$ ($c = 1.0$, $CHCl_3$)).

*Boc-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**7**). Boc-(*S*)-β³-HLeu-OMe (3.89 g, 15 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (30 ml), and treated with Et_3N (7.58 g, 75 mmol), HOBt (2.43 g, 18 mmol), **5** (5.03 g, 15 mmol) in $CHCl_3$ (30 ml), and EDC (3.44 g, 18 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 20:1) yielded **7** (5.87 g, 83%). White solid. M.p. 105–106°. R_f (CH_2Cl_2/MeOH 20:1) 0.6. $[\alpha]_D^{rt} = -8.4$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3340*s*, 2959*s*, 1738*s*, 1685*s*, 1643*s*, 1521*s*, 1436*m*, 1367*m*, 1303*m*, 1277*m*, 1251*m*, 1161*s*, 1050*m*, 1023*w*, 764*w*, 704*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, $J = 6.6$, 2 Me); 1.24–1.31 (*m*, CH); 1.44 (*s*, *t*-Bu); 1.38–1.50 (*m*, 1 H, CH_2); 1.52–1.62 (*m*, 1 H, CH_2); 2.41–2.75 (*m*, 2 CH_2CO, CH_2S); 3.67 (*s*, MeO); 3.74 (*s*, SCH_2Ph); 3.98–4.05 (*m*, CHN); 4.26–4.35 (*m*, CHN); 5.63 (br., NH); 6.05 (br., NH); 7.21–7.35 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.1, 22.9 (Me); 25.0 (CH); 28.4 (Me); 35.3, 36.4, 38.6, 38.9, 43.1 (CH_2); 44.2, 47.5 (CH); 51.7 (Me); 79.4 (C); 127.1, 128.5, 129.0 (arom. C); 138.2, 155.3, 170.0, 172.1 (C). FAB-MS: 489 (16, $[M+Na]^+$), 467 (52, $[M+1]^+$), 367 (100, $[M-Boc+1]^+$). Anal. calc. for $C_{24}H_{38}N_2O_5S$ (466.64): C 61.77, H 8.21, N 6.00, S 6.87; found: C 61.88, H 8.15, N 5.98, S 6.87.

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**9a**). Compound **7** (4.87 g, 10.5 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (21 ml), and treated with Et_3N (5.35 g, 53 mmol), HOBt (1.70 g, 12.6 mmol), Boc-(*R*)-β³-HVal-OH (2.42 g, 10.5 mmol) in $CHCl_3$ (21 ml), and EDC (2.41 g, 12.6 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 15:1) yielded **9a** (4.76 g, 79%). White solid. M.p. 154–155°. R_f (CH_2Cl_2/MeOH 15:1) 0.6. $[\alpha]_D^{rt} = -19.5$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3304*s*, 2960*s*, 1741*s*, 1687*s*, 1647*s*, 1540*s*, 1437*m*, 1367*m*, 1310*m*, 1248*m*, 1174*s*, 1047*m*, 1021*w*, 770*w*, 705*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89–0.92 (*m*, 4 Me); 1.24–1.32 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.40–1.50 (*m*, 1 H, CH_2); 1.54–1.63 (*m*, 1 H, CH_2); 1.77–1.82 (*m*, CH); 2.24–2.81 (*m*, 3 CH_2CO, CH_2S); 3.67 (*s*, MeO); 3.67–3.74 (*m*, CHN); 3.74 (*s*, SCH_2Ph); 4.22–4.83 (*m*, 2 CHN); 5.15 (br., NH); 6.33 (br., NH); 6.96 (br., NH); 7.21–7.35 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.5, 19.5, 22.1, 22.9 (Me); 25.0 (CH); 28.4 (Me); 32.2 (CH); 34.7, 36.3, 38.4, 39.2, 39.3, 43.2 (CH_2); 44.5, 46.5 (CH); 51.8 (Me); 53.5 (CH); 79.1 (C); 127.1, 128.6, 129.0 (arom. C); 138.2, 156.0, 170.3, 170.8, 172.2 (C). FAB-MS: 602 (28, $[M+Na]^+$), 580 (79, $[M+1]^+$), 480 (100, $[M-Boc+1]^+$). Anal. calc. for $C_{30}H_{49}N_3O_6S$ (579.79): C 62.15, H 8.52, N 7.25, S 5.53; found: C 62.00, H 8.42, N 7.18, S 5.52.

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OH* (**9b**). Compound **5a** (1.16 g, 2 mmol) was dissolved in CF_3CH_2OH (20 ml) and treated with 40 ml of 5N NaOH (200 mmol), according to *GP 2b* (reaction time: 12 h) yielding **9b** (1.03 g, 91%). White solid. M.p. 171–172°. R_f (CH_2Cl_2/MeOH 15:1) 0.36. $[\alpha]_D^{rt} = -8.6$ ($c = 0.5$, MeOH). IR (KBr): 3335*s*, 2960*s*, 1740*s*, 1685*s*, 1646*s*, 1540*s*, 1367*m*, 1308*m*, 1248*m*, 1172*s*, 1017*w*, 775*w*, 698*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88–0.92 (*m*, 4 Me); 1.26–1.33 (*m*, CH); 1.40 (*s*, *t*-Bu); 1.40–1.50 (*m*, 1 H, CH_2); 1.53-1.64 (*m*, 1 H, CH_2); 1.73–1.81 (*m*, CH); 2.19–2.58 (*m*, 3 CH_2CO, CH_2S); 3.70–3.78 (*m*, CHN); 3.74 (*s*, SCH_2Ph); 4.26–4.35 (*m*, 2 CHN); 6.35 (br., NH); 7.18–7.34 (*m*, 5 arom. H); 7.79 (br., NH); 7.87 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.3, 19.8, 22.2, 23.7 (Me); 26.1 (CH); 28.9 (Me); 33.5 (CH); 36.2, 37.0, 40.2, 41.0, 44.5 (CH_2); 46.0, 47.8, 54.8 (CH); 80.0 (C); 128.0, 129.5, 130.2 (arom. C); 139.9, 158.1, 172.2, 173.5, 174.9 (C). FAB-MS: 588 (71, $[M+Na]^+$), 566 (60, $[M+1]^+$), 466 (100, $[M-Boc+1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**12a**). Compound **9a** (495 mg, 0.85 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (1.7 ml), and treated with Et_3N (431 mg, 4.3 mmol), HOBt (136 mg, 1 mmol), **9b** (483 mg, 0.85 mmol) in DMF (8.5 ml), and EDC (196 mg, 1 mmol) according to *GP 3b*. Recrystallization from MeOH yielded **12a** (623 mg, 71%). White solid. M.p. 225–227°. R_f (CH_2Cl_2/MeOH 10:1) 0.56. $[\alpha]_D^{rt} = -0.6$ ($c = 0.5$, MeOH). IR (KBr): 3302*s*, 2958*s*, 1739*s*, 1686*s*, 1648*s*, 1540*s*, 1437*m*, 1367*m*, 1311*m*, 1248*m*, 1173*s*, 1028*w*, 772*w*, 706*m*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 3:1): 0.89–0.93 (*m*, 8 Me); 1.24–1.31 (*m*, 2 CH); 1.42 (*s*, *t*-Bu); 1.40–1.47 (*m*, CH_2); 1.54–1.62 (*m*, CH_2); 1.79–1.83 (*m*, 2 CH); 2.23–2.68 (*m*, 6 CH_2CO, 2 CH_2S); 3.67 (*s*, MeO); 3.72, 3.73 (2*s*, 2 SCH_2Ph); 3.74–3.85 (*m*, CHN); 4.05–4.11 (*m*, CHN); 4.25–4.40 (*m*, 4 CHN); 5.83 (br., NH); 7.20–7.36 (*m*, 10 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD 3:1): 18.3, 18.6, 19.1, 19.3, 22.2, 23.1, 23.2 (Me); 25.1 (CH); 28.5 (Me); 32.0, 32.4 (CH); 35.5, 35.7, 36.4, 36.6, 38.4, 38.9, 39.5, 39.7, 39.8, 41.7, 43.8, 43.9 (CH_2); 44.6, 45.5, 46.5, 46.6 (CH); 52.0 (Me); 52.2, 53.7 (CH); 79.4 (C); 127.2, 127.3, 128.7, 128.7, 129.1, 129.2 (arom. C); 138.3, 138.5, 156.8, 170.7, 170.9, 171.4, 171.5, 172.1, 172.6 (C). FAB-MS: 1050 (100, $[M+Na]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OH* (**12b**). Compound **12a** (425 mg, 0.41 mmol) was dissolved in CF_3CH_2OH (4.1 ml) and treated with 8.2 ml of 5N NaOH (41 mmol), according to *GP 2b* (reaction time: 48 h) yielding **12b** (394 mg, 94%). White solid. M.p. 233–235° (dec.). R_f (CH_2Cl_2/MeOH 10:1) 0.37. $[\alpha]_D^{rt} = +1.3$ ($c = 0.5$, MeOH). IR (KBr): 3299*s*, 2958*s*, 1687*s*, 1647*s*, 1541*s*, 1453*m*, 1367*m*, 1311*m*, 1247*m*, 1173*s*, 1029*w*, 773*w*, 707*m*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 3:1): 0.89–0.92 (*m*, 8 Me); 1.21–1.35 (*m*, 2 CH); 1.42 (*s*, *t*-Bu); 1.42–1.50 (*m*, CH_2); 1.52–1.63 (*m*, CH_2); 1.80–1.94 (*m*, 2 CH); 2.28–2.67 (*m*, 6 CH_2CO, 2 CH_2S); 3.71, 3.73 (2*s*, 2 SCH_2Ph); 3.73–3.80 (*m*, CHN); 4.02–4.07 (*m*, CHN); 4.22–4.41 (*m*, 4 CHN); 7.20-7.38 (*m*, 10 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD

3:1): 18.2, 18.6, 19.1, 19.3, 22.3, 23.1, 23.2 (Me); 25.1 (CH); 28.5 (Me); 32.0, 32.4 (CH); 35.8, 35.9, 36.5, 36.8, 38.2, 38.8, 39.6, 39.9, 40.1, 41.6, 44.0, 44.2 (CH_2); 44.9, 45.3, 46.4, 46.6 (CH); 52.1, 53.6 (CH); 79.4 (C); 127.2, 127.3, 128.7, 128.7, 129.1, 129.2 (arom. C); 138.3, 138.5, 156.8, 170.5, 171.0, 171.4, 171.6, 172.3, 174.1 (C). FAB-MS: 1036 (100, $[M+Na]^+$).

*H-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OH · CF₃-CO₂H* (**12c**). Compound **12b** (33 mg, 0.033 mmol) was deprotected according to *GP 1*. The crude peptide was purified by prep. RP-HPLC (50 → 90% *B* in 25 min) according to *GP 5* yielding **12c** (19 mg, 58%). White solid. RP-HPLC (40 → 90% B in 25 min) t_R 20.6 min, purity > 98%. M.p. 198–199° (dec.). CD (0.2 mM in MeOH): $+1.48 \cdot 10^5$ (200 nm). IR (KBr): 3289*m*, 3084*m*, 2961*m*, 1654*s*, 1550*s*, 1420*w*, 1387*w*, 1308*w*, 1201*m*, 1138*m*, 800*w*, 701*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.89–0.96 (*m*, 6 Me); 1.08 (*d*, *J* = 6.9, 2 Me); 1.22–1.33 (*m*, 2 CH); 1.37–1.46 (*m*, CH_2); 1.51–1.64 (*m*, CH_2); 1.67–1.75 (*m*, CH); 2.02–2.10 (*m*, CH); 2.18–2.85 (*m*, 6 CH_2CO, 2 CH_2S); 3.50–3.55 (*m*, CHN); 3.65, 3.70 (2*d*, *J* = 13.1, SCH_2Ph); 3.77 (*s*, SCH_2Ph); 3.73–3.80 (*m*, CHN); 4.14–4.20 (*m*, CHN); 4.35–4.44 (*m*, 2 CHN); 4.48–4.55 (*m*, CHN); 4.65–4.72 (*m*, CHN); 7.16–7.34 (*m*, 10 arom. H); 7.48 (br., NH); 7.74 (br., NH); 7.77 (br., NH); 8.19 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.2, 19.2, 19.5, 19.5, 22.9, 23.0, 23.3, 23.8 (Me); 26.0, 26.1, 32.1, 34.0 (CH); 36.1, 36.9, 37.1, 37.7, 38.1, 38.9, 40.6, 40.7, 40.9, 41.7 (CH_2); 45.4, 45.8 (CH); 46.6 (CH_2); 47.1, 52.7, 56.2 (CH); 128.0, 128.1, 129.5, 129.5, 130.1, 130.2 (arom. C); 139.7, 139.8, 171.0, 172.0, 172.1, 172.5, 173.1, 175.0 (C). ESI-MS (pos.): 958 (14, $[M+2\,Na-H]^+$), 936 (45, $[M+Na]^+$), 914 (100, $[M+1]^+$). ESI-MS (neg.): 912 (100, $[M-1]^-$).

*cyclo-H-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF₃CO₂H* (**1**). Compound **12b** (80 mg, 0.079 mmol) was deprotected and cyclized according to *GP 4*. The crude peptide was purified by prep. RP-HPLC (20 → 50% *B* in 30 min) according to *GP 5* yielding **1** (35 mg, 53%), which gave a negative *Ellman*'s test [38]. White solid. RP-HPLC (20 → 50% *B* in 20 min, then 50 → 100% *B* in 5 min) t_R 22.3 min, purity > 95%. M.p. 145–147°. CD (0.2 mM in MeOH): $+1.3 \cdot 10^4$ (200 nm); $-4.5 \cdot 10^4$ (213 nm). CD (0.2 mM in H_2O): $-2.6 \cdot 10^4$ (209 nm). IR (KBr): 3284*m*, 3081*m*, 2961*m*, 1654*s*, 1544*s*, 1466*w*, 1388*w*, 1309*w*, 1203*s*, 1138*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.88–0.96 (*m*, 6 Me); 1.08 (2*d*, *J* = 6.9, 2 Me); 1.24–1.71 (*m*, 2 CH_2, 3 CH); 2.03–2.10 (*m*, CH); 2.31–3.25 (*m*, 6 CH_2CO, 2 CH_2S); 3.49–3.53 (*m*, CHN); 4.12–4.16 (*m*, CHN); 4.35–4.40 (*m*, CHN); 4.47–4.53 (*m*, CHN); 4.57–4.64 (*m*, CHN). ^{13}C-NMR (125 MHz, CD_3OD): 18.1, 18.8, 19.0, 19.6, 22.4, 23.0, 23.3, 23.6 (Me); 26.1, 26.1, 32.1, 34.0 (CH); 36.1, 37.5, 37.6, 39.5, 40.6, 40.9, 41.8, 44.9 (CH_2); 45.8, 46.0 (CH); 46.5 (CH_2); 47.3, 47.6 (CH); 48.1 (CH_2); 52.2, 56.5 (CH); 172.1, 172.4, 172.4, 172.5, 173.3, 175.0 (C). ESI-MS (pos.): 776 (12, $[M+2\,Na-H]^+$), 754 (38, $[M+Na]^+$), 732 (100, $[M+1]^+$). ESI-MS (neg.): 730 (100, $[M-1]^-$).

*H-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF₃CO₂H* (**12d**). Compound **12d** was prepared from **1** according to *GP 6*. CD (0.2 mM in MeOH): $+1.2 \cdot 10^5$ (200 nm); $-4.8 \cdot 10^4$ (216 nm).

*Boc-(*R*)-β³-HMet-(*S*)-β³-HLeu-OMe* (**8**). Boc-(*S*)-β³-HLeu-OMe (1.297 g, 5 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (10 ml), and treated with Et_3N (2.525 g, 25 mmol), HOBt (0.811 g, 6 mmol), **6** (1.317 g, 5 mmol) in $CHCl_3$ (10 ml), and EDC (1.150 g, 6 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 15:1) yielded **8** (1.738 g, 86%). White solid. M.p. 112–113°. R_f (CH_2Cl_2/MeOH 15:1) 0.45. $[\alpha]_D^{r.t.} = -38.2$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3302*s*, 2955*s*, 1742*s*, 1687*s*, 1656*s*, 1537*s*, 1441*m*, 1365*m*, 1274*m*, 1172*s*, 1053*m*, 1026*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.90 (*d*, *J* = 6.8, Me); 0.90 (*d*, *J* = 6.5, 2 Me); 1.24–1.33 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.42–1.51 (*m*, 1 H, CH_2); 1.54–1.61 (*m*, 1 H, CH_2); 1.75–1.85 (*m*, CH_2); 2.08 (*s*, MeS); 2.32–2.59 (*m*, 2 CH_2CO, CH_2S); 3.68 (*s*, MeO); 3.91–3.95 (*m*, CHN); 4.29–4.34 (*m*, CHN); 5.40 (br., NH); 6.12 (br., NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.5, 22.1, 22.8 (Me); 25.0 (CH); 28.4 (Me); 30.9, 34.0, 38.9, 40.7, 43.1 (CH_2); 44.2, 47.7 (CH); 51.7 (Me); 79.2, 155.6, 170.1, 172.2 (C). FAB-MS: 427 (6, $[M+Na]^+$), 405 (100, $[M+1]^+$). Anal. calc. for $C_{19}H_{36}N_2O_5S$ (404.57): C 56.41, H 8.97, N 6.92, S 7.93; found: C 56.60, H 8.76, N 6.94, S 7.73.

*Boc-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OMe* (**10a**). Compound **8** (4.04 g, 10 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (20 ml), and treated with Et_3N (5.05 g, 50 mmol), HOBt (1.62 g, 12 mmol), Boc-(*R*)-β³-HVal-OH (2.31 g, 10 mmol) in $CHCl_3$ (20 ml), and EDC (2.30 g, 12 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 15:1) yielded **10a** (4.23 g, 82%). White solid. M.p. 176–177°. R_f (CH_2Cl_2/MeOH 15:1) 0.30. $[\alpha]_D^{r.t.} = -31.1$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3304*s*, 2961*m*, 1741*m*, 1687*s*, 1645*s*, 1540*s*, 1437*w*, 1367*w*, 1249*w*, 1174*m*, 1050*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.89–0.92 (*m*, 4 Me); 1.25–1.34 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.43–1.62 (*m*, CH_2); 1.74–1.85 (*m*, CH_2); 1.97–2.02 (*m*, CH); 2.08 (*s*, MeS); 2.23–2.60 (*m*, 3 CH_2CO, CH_2S); 3.69 (*s*, MeO); 3.61–3.74 (*m*, CHN); 4.06–4.19 (*m*, CHN); 4.25–4.37 (*m*, CHN); 5.11 (br., NH); 6.50 (br., NH); 6.90 (br., NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 15.5, 18.4, 19.4, 22.1, 22.9 (Me); 25.0 (CH); 28.4 (Me); 30.9 (CH_2); 32.3 (CH); 33.2, 39.3, 39.5, 40.4, 43.2 (CH_2); 44.6, 47.2 (CH); 51.8 (Me); 53.6 (CH); 79.2, 156.1,

170.5, 171.0, 172.4 (C). FAB-MS: 540 (38, $[M+Na]^+$), 518 (54, $[M+1]^+$), 418 (54, $[M-Boc+1]^+$). Anal. calc. for $C_{25}H_{47}N_3O_6S$ (517.72): C 58.00, H 9.15, N 8.12, S 6.19; found: C 57.87, H 9.36, N 8.27, S 6.10.

*Boc-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OH* (**10b**). Compound **10a** (2.07 g, 4 mmol) was dissolved in MeOH (10 ml) and treated with 10 ml of 1N NaOH (10 mmol), according to *GP 2a*. Recrystallization from MeOH yielded **10b** (1.85 g, 92%). White solid. M.p. 198–199°. R_f (CH_2Cl_2/MeOH 15:1) 0.3. $[\alpha]_D^{r.t.} = -11.0$ ($c = 0.5$, MeOH). IR (KBr): 3348*s*, 3315*s*, 2956*m*, 1728*m*, 1682*s*, 1646*s*, 1593*m*, 1549*s*, 1442*w*, 1365*w*, 1228*w*, 1172*m*, 1057*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.85–0.88 (*m*, 4 Me); 1.21–1.32 (*m*, CH); 1.38 (*s*, *t*-Bu); 1.40–1.48 (*m*, 1 H, CH_2); 1.51–1.61 (*m*, 1 H, CH_2); 1.69–1.81 (*m*, CH_2, CH); 2.04 (*s*, MeS); 2.15–2.50 (*m*, 3 CH_2CO, CH_2S); 3.63–3.67 (*m*, CHN); 4.06–4.11 (*m*, CHN); 4.21–4.27 (*m*, CHN). ^{13}C-NMR (100 MHz, CD_3OD): 15.3, 18.1, 19.2, 22.0, 23.2 (Me); 25.2 (CH); 28.5 (Me); 30.8 (CH_2); 32.7 (CH); 33.4, 39.3, 40.1, 41.4, 43.5 (CH_2); 45.0, 47.3, 54.0 (CH); 79.6, 156.8, 171.3, 172.6, 174.1 (C). FAB-MS: 504 (99, $[M+1]^+$), 404 (100, $[M-Boc+1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OMe* (**13a**). Compound **10a** (518 mg, 1 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (2 ml), and treated with Et_3N (505 mg, 5 mmol), HOBt (162 mg, 1.2 mmol), **10b** (503 mg, 1 mmol) in DMF (10 ml), and EDC (230 mg, 1.2 mmol) according to *GP 3b*. Recrystallization from MeOH yielded **13a** (687 mg, 76%). White solid. M.p. 255° (dec.). R_f (CH_2Cl_2/MeOH 15:1) 0.45. $[\alpha]_D^{r.t.} = -3.6$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3298*m*, 3079*w*, 2959*m*, 1740*m*, 1686*m*, 1647*s*, 1541*s*, 1438*m*, 1367*m*, 1248*w*, 1174*m*, 1051*w*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 3:1): 0.90–0.94 (*m*, 8 Me); 1.26–1.33 (*m*, 2 CH); 1.44 (*s*, *t*-Bu); 1.45–1.50 (*m*, CH_2); 1.57–1.65 (*m*, CH_2); 1.77–1.84 (*m*, 6 H, 2 CH_2, 2 CH); 2.09, 2.10 (2*s*, 2 MeS); 2.24–2.56 (*m*, 6 CH_2CO, 2 CH_2S); 3.70 (*s*, MeO); 3.70–3.74 (*m*, CHN); 4.04–4.12 (*m*, CHN); 4.18–4.28 (*m*, 3 CHN); 4.29–4.35 (*m*, CHN); 5.96 (br., NH); 7.49 (br., NH); 7.59 (br., NH); 7.64 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 3:1): 15.4, 15.5, 18.3, 18.5, 19.2, 19.3, 22.1, 23.2, 23.3 (Me); 25.3 (CH); 28.6 (Me); 30.9 (CH_2); 32.2, 32.9 (CH); 33.9, 34.2, 38.8, 39.2, 40.2, 41.2, 41.6, 42.1, 43.9, 44.0 (CH_2); 45.0, 45.8, 47.0, 47.1 (CH); 52.1 (Me); 52.5, 54.0 (CH); 79.6, 157.0, 171.3, 171.4, 171.8, 172.1, 172.6, 172.7 (C). FAB-MS: 926 (65, $[M+Na]^+$), 904 (28, $[M+1]^+$), 804 (76, $[M-Boc+1]^+$), 559 (100).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OH* (**13b**). Compound **13a** (226 mg, 0.20 mmol) was dissolved in CF_3CH_2OH (2 ml) and treated with 4 ml of 5N NaOH (20 mmol) according to *GP 2b* (reaction time: 24 h) yielding **13b** (213 mg, 96%). White solid. M.p. 245–246° (dec.). R_f (CH_2Cl_2/MeOH 10:1) 0.18. CD (0.2 mM in MeOH): $+6.9 \cdot 10^4$ (200 nm); $-6.0 \cdot 10^3$ (222 nm). IR (KBr): 3296*m*, 3079*w*, 2960*m*, 1689*m*, 1648*s*, 1542*s*, 1438*m*, 1367*m*, 1248*w*, 1173*m*, 1048*w*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 3:1): 0.89–0.94 (*m*, 8 Me); 1.22–1.34 (*m*, 2 CH); 1.39–1.45 (*m*, CH_2); 1.45 (*s*, *t*-Bu); 1.56–1.66 (*m*, CH_2); 1.75–1.81 (*m*, 2 CH_2, 2 CH); 2.09, 2.10 (2*s*, 2 MeS); 2.26–2.57 (*m*, 6 CH_2CO, 2 CH_2S); 3.76–3.85 (*m*, CHN); 4.08–4.15 (*m*, CHN); 4.30–4.44 (*m*, 4 CHN). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 3:1): 15.5, 15.6, 18.1, 19.0, 19.1, 19.2, 22.5, 22.6, 23.3 (Me); 25.4, 25.4 (CH); 28.6 (Me); 30.9, 31.0 (CH_2); 32.8 (CH); 35.2, 38.0, 39.0, 40.9, 41.5, 41.8, 42.7 (CH_2); 45.1, 46.0, 46.2, 46.4 (CH); 52.0, 53.6 (CH); 79.6, 157.4, 170.5, 171.4, 171.7, 172.1, 173.2, 176.5 (C). FAB-MS: 912 (100, $[M+Na]^+$), 790 (22, $[M-Boc+1]^+$).

*H-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OMe·CF₃CO₂H* (**13c**). Compound **13a** (50 mg, 0.055 mmol) was deprotected according to *GP 1*, yielding **13c** (49 mg, 98%). White solid. M.p. 218–219° (dec.). CD (0.2 mM in MeOH): $+8.3 \cdot 10^4$ (198 nm); $-2.3 \cdot 10^4$ (218 nm). IR (KBr): 3294*m*, 3105*w*, 2962*m*, 1646*s*, 1545*s*, 1439*m*, 1370*w*, 1311*w*, 1262*w*, 1204*m*, 1133*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88–0.96 (*m*, 6 Me); 1.09 (*d*, $J = 6.9$, 2 Me); 1.22–1.45 (*m*, 2 CH, CH_2); 1.54–2.10 (*m*, 3 CH_2, 2 CH); 2.08, 2.08 (2*s*, 2 MeS); 2.18–2.92 (*m*, 6 CH_2CO, 2 CH_2S); 3.53–3.57 (*m*, CHN); 3.71 (*s*, MeO); 4.21–4.33 (*m*, CHN); 4.35–4.60 (*m*, 4 CHN); 7.43 (br., NH); 7.71 (br., NH); 7.73 (br., NH); 8.23 (br., NH). ESI-MS (pos.): 826 (11, $[M+Na]^+$), 804 (100, $[M+1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-OH* (**13d**). Compound **13b** (400 mg, 0.45 mmol) was dissolved in 135 ml of NH_3 at −33° [37]. Na (1.04 g, 45 mmol) was added. The dark-blue soln. was stirred for 24 h and subsequently quenched by adding 3 g (56 mmol) of NH_4Cl. NH_3 was allowed to evaporate completely, and the residue was taken up in 100 ml of H_2O. The pH was adjusted to 2–3 with 1N HCl, resulting in the precipitation of **13d** (337 mg, 87%), which was not further purified. White solid. M.p. 212–213° (dec.). R_f (CH_2Cl_2/MeOH 10:1) 0.18. $[\alpha]_D^{r.t.} = -0.1$ ($c = 0.25$, MeOH). IR (KBr): 3276*m*, 3081*w*, 2960*m*, 1689*m*, 1641*s*, 1544*s*, 1444*m*, 1368*m*, 1250*w*, 1171*m*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 3:1): 0.90–0.94 (*m*, 8 Me); 1.24–1.36 (*m*, 2 CH); 1.45 (*s*, *t*-Bu); 1.45–1.49 (*m*, CH_2); 1.57–1.63 (*m*, CH_2); 1.77–1.89 (*m*, 2 CH_2, 2 CH); 2.27–2.55 (*m*, 6 CH_2CO, 2 CH_2S); 3.74–3.81 (*m*, CHN); 4.06–4.14 (*m*, CHN); 4.27–4.42 (*m*, 4 CHN). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 3:1): 18.2, 18.7, 19.1, 19.2 (Me); 21.3, 21.3 (CH_2); 22.4, 23.3 (Me); 25.3 (CH); 28.6 (Me); 32.5, 33.0 (CH); 38.4, 39.0, 39.3, 39.6, 40.5, 41.1, 41.5, 41.9, 44.4, 44.6 (CH_2); 44.9,

45.1, 45.5, 46.0, 46.1 (CH); 52.3, 53.9 (CH); 79.6, 157.2, 170.9, 171.3, 171.6, 172.0, 173.0, 174.4 (C). ESI-MS (neg.): 860 (100, $[M-1]^-$).

*H-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-OH·CF₃CO₂H* (**13e**). Compound **13d** (51 mg, 0.059 mmol) was deprotected according to *GP 1*. The crude peptide was purified by prep. RP-HPLC (45 → 65% *B* in 30 min) according to *GP 5* yielding **13e** (36 mg, 71%). White solid. RP-HPLC (45 → 65% B in 20 min) t_R 15.5 min, purity > 98%. M.p. 202–203° (dec.). CD (0.2 mM in MeOH): $+1.92\cdot10^5$ (197 nm); $-2.3\cdot10^4$ (218 nm). IR (KBr): 3281*m*, 3081*w*, 2961*m*, 1654*s*, 1547*s*, 1466*w*, 1438*w*, 1387*w*, 1309*w*, 1202*m*, 1137*m*, 1045*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.88–0.96 (*m*, 6 Me); 1.09 (*d*, *J* = 6.9, 2 Me); 1.21–1.33 (*m*, 2 CH); 1.37–1.46 (*m*, CH_2); 1.51–2.11 (*m*, 3 CH_2, 2 CH); 2.20–2.89 (*m*, 6 CH_2CO, 2 CH_2S); 3.51–3.56 (*m*, CHN); 4.17–4.23 (*m*, CHN); 4.34–4.44 (*m*, 2 CHN); 4.46–4.54 (*m*, CHN); 4.59–4.66 (*m*, CHN); 7.50 (br., NH); 7.64 (br., NH); 7.72 (br., NH); 8.22 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 18.3, 19.2, 19.3, 19.5, 19.5 (Me); 21.7, 21.9 (CH_2); 23.0, 23.3, 23.8 (Me); 26.1, 32.1, 34.0 (CH); 36.1, 38.8, 40.8, 41.2, 41.4, 41.6, 41.7, 41.8 (CH_2); 45.3, 45.7 (CH); 45.8, 46.2, 46.6, 46.7 (CH_2); 52.7, 56.3 (CH); 171.1, 172.3, 172.4, 172.5, 173.1, 175.0 (C). ESI-MS (pos.): 784 (17, $[M+Na]^+$), 762 (100, $[M+1]^+$). ESI-MS (neg.): 760 (100, $[M-1]^-$].

*H-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HMet-(*S*)-β³-HLeu-OH·CF₃CO₂H* (**13f**). Compound **13b** (59 mg, 0.066 mmol) was deprotected according to *GP 1*. The crude peptide was purified by prep. RP-HPLC (35 → 70% *B* in 35 min) according to *GP 5*, yielding **13f** (35 mg, 59%). White solid. RP-HPLC (35 → 70% B in 20 min) t_R 17.1 min, purity > 95%. M.p. 214–215° (dec.). CD (0.2 mM in MeOH): $+1.15\cdot10^5$ (201 nm); $-3.2\cdot10^4$ (218 nm). IR (KBr): 3308*m*, 3083*w*, 2964*m*, 1684*s*, 1644*s*, 1558*m*, 1446*m*, 1210*s*, 1142*s*, 844*m*, 803*m*, 725*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.89–0.96 (*m*, 6 Me); 1.09 (*d*, *J* = 6.9, 2 Me); 1.21–1.34 (*m*, 2 CH); 1.37–1.46 (*m*, CH_2); 1.51–2.11 (*m*, 3 CH_2, 2 CH); 2.08, 2.09 (2*s*, 2 MeS); 2.26–2.87 (*m*, 6 CH_2CO, 2 CH_2S); 3.51–3.56 (*m*, CHN); 4.16–4.23 (*m*, CHN); 4.38–4.49 (*m*, 3 CHN); 4.52–4.58 (*m*, CHN); 7.52 (br., NH); 7.69 (br., NH); 7.74 (br., NH); 8.22 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 15.6, 18.3, 19.2, 19.5, 19.5, 23.0, 23.0, 23.3, 23.8 (Me); 26.0, 26.1 (CH); 31.5, 31.8 (CH_2); 32.1, 34.0 (CH); 36.2, 36.5, 36.7, 38.9, 40.8, 41.5, 41.6, 41.8 (CH_2); 45.3, 45.7 (CH); 45.8, 46.6 (CH_2); 46.7, 47.4, 52.8, 56.3 (CH); 171.2, 172.2, 172.6, 173.2, 174.9 (C). ESI-MS (pos.): 834 (15, $[M+2\,Na-H]^+$), 828 (14, $[M+K]^+$), 812 (71, $[M+Na]^+$), 790 (100, $[M+1]^+$). ESI-MS (neg.): 788 (100, $[M-1]^-$).

*cyclo-H-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*R*)-β³-HHcy-(*S*)-β³-HLeu-OH·CF₃CO₂H* (**2**). Compound **13b** (35 mg, 0.041 mmol) was cyclized and deprotected according to *GP 4*. The crude peptide was purified by prep. RP-HPLC (20 → 40% *B* in 30 min) according to *GP 5*, yielding **2** (18 mg, 51%), which gave a negative *Ellman*'s test [38]. White solid. RP-HPLC (20 → 50% in 20 min) t_R 14.5 min, purity > 95%. M.p. 128–130° (dec.). CD (0.2 mM in MeOH): $+7.7\cdot10^4$ (200 nm); $-2.4\cdot10^4$ (218 nm). CD (0.2 mM in H_2O): $+2.2\cdot10^4$ (203 nm); $-0.6\cdot10^4$ (226 nm). IR (KBr): 3293*m*, 3098*m*, 2962*m*, 1654*s*, 1550*s*, 1472*w*, 1399*m*, 1203*s*, 1137*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.89–0.96 (*m*, 6 Me); 1.08 (*d*, *J* = 6.9, 2 Me); 1.24–2.21 (*m*, 4 CH_2, 4 CH); 2.24–3.17 (*m*, 6 CH_2CO, 2 CH_2S); 3.52–3.56 (*m*, CHN); 4.14–4.24 (*m*, CHN); 4.38–4.45 (*m*, 2 CHN); 4.59–4.64 (*m*, CHN); 4.71–4.78 (*m*, CHN); 7.62 (br., NH); 7.71 (br., NH); 7.74 (br., NH); 8.22 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.1, 19.2, 19.6, 19.6, 23.0, 23.2, 23.3, 23.7 (Me); 26.1, 32.1 (CH); 33.7 (CH_2); 34.0 (CH); 35.6, 36.0, 36.4, 36.6, 38.8, 40.0, 41.2, 41.9 (CH_2); 45.5, 45.7 (CH); 45.7 (CH_2); 46.0 (CH); 46.4, 46.5 (CH_2); 52.9, 56.5 (CH); 171.3, 171.8, 172.7, 173.0, 175.3 (C). ESI-MS (pos.): 782 (10, $[M+Na]^+$), 760 (100, $[M+1]^+$). ESI-MS (neg.): 758 (100, $[M-1]^-$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**14a**). Compound **7** (128 mg, 0.275 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.6 ml), and treated with Et_3N (140 mg, 1.4 mmol), HOBt (45 mg, 0.35 mmol), **9b** (156 mg, 0.275 mmol) in DMF (2.8 ml), and EDC (64 mg, 0.35 mmol) according to *GP 3b*. FC (CH_2Cl_2/MeOH 13 : 1) yielded **14a** (225 mg, 89%). White solid. M.p. 201–202°. R_f (CH_2Cl_2/MeOH 13 : 1) 0.33. $[\alpha]_D^{rt} = -1.4$ (*c* = 0.5, MeOH). IR (KBr): 3302*s*, 2957*s*, 1684*s*, 1636*s*, 1541*w*, 1451*s*, 1366*m*, 1310*w*, 1260*m*, 1173*m*, 1019*m*, 804*w*, 699*m*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 3 : 1): 0.89–0.94 (*m*, 6 Me); 1.24–1.33 (*m*, 2 CH); 1.41 (*s*, *t*-Bu); 1.41–1.47 (*m*, CH_2); 1.53–1.61 (*m*, CH_2); 1.80–1.84 (*m*, CH); 2.23–2.69 (*m*, 5 CH_2CO, 2 CH_2S); 3.66 (*s*, MeO); 3.66–3.70 (*m*, CHN); 3.74, 3.74 (2*s*, 2 SCH_2Ph); 4.20–4.31 (*m*, 4 CHN); 7.11–7.34 (*m*, 10 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 3 : 1): 18.3, 19.4, 22.0, 23.1, 23.4 (Me); 25.1 (CH); 28.5 (Me); 32.4 (CH); 35.2, 35.4, 36.5, 39.1, 39.5, 39.6, 39.8, 42.0, 43.4, 43.5 (CH_2); 44.8, 45.4, 46.8 (CH); 51.9 (Me); 53.8 (CH); 79.5 (C), 127.3, 127.3, 128.8, 129.2, 129.2 (arom. C); 138.4, 138.4, 156.7, 170.8, 170.9, 171.0, 171.4, 172.1, 172.5 (C). FAB-MS: 937 (7, $[M+Na]^+$), 915 (17, $[M+1]^+$), 815 (100, $[M-Boc+1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OH* (**14b**). Compound **14a** (197 mg, 0.22 mmol) was dissolved in CF_3CH_2OH (2.2 ml) and treated with 4.4 ml of 5N NaOH (22 mmol), according to *GP 2b* (reaction time: 12 h) yielding **14b** (185 mg, 94%). White solid. M.p. 211–212°. R_f (CH_2Cl_2/

MeOH 15:1) 0.13. $[\alpha]_D^{rt} = -0.7$ ($c = 0.25$, MeOH). IR (KBr): 3297*s*, 2957*s*, 1686*s*, 1647*s*, 1541*s*, 1451*s*, 1366*m*, 1313*w*, 1248*m*, 1172*m*, 1029*m*, 770*w*, 699*m*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 3:1): 0.87–0.96 (*m*, 6 Me); 1.21–1.33 (*m*, 2 CH); 1.39 (*s*, *t*-Bu); 1.39–1.62 (*m*, 2 CH_2); 1.73–1.81 (*m*, CH); 2.25–2.80 (*m*, 5 CH_2CO, 2 CH_2S); 3.68–3.80 (*m*, CHN); 3.74 (br. *s*, 2 SCH_2Ph); 4.39–4.56 (*m*, 4 CHN); 7.23–7.38 (*m*, 10 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 3:1): 18.0, 19.6, 22.1, 23.1, 23.4 (Me); 25.0, 25.0 (CH); 28.5 (Me); 31.8 (CH); 35.7, 36.2, 36.5, 40.1, 40.6, 41.6, 43.6, 44.4 (CH_2); 44.9, 45.9, 46.6, 53.4 (CH); 80.1 (C); 127.4, 127.8, 128.8, 129.0, 129.1 (arom. C); 138.0, 138.1, 157.2, 172.5, 173.8 (C). FAB-MS: 923 (100, $[M + Na]^+$), 800 (12, $[M - Boc + 1]^+$).

*cyclo-H-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**3**). Compound **14b** (60 mg, 0.067 mmol) was deprotected and cyclized according to *GP 4*. The crude peptide was purified by prep. RP-HPLC (20 → 40% *B* in 30 min) according to *GP 5*, yielding **3** (21 mg, 38%), which gave a negative *Ellman*'s test [38]. White solid. RP-HPLC (20 → 50% *B* in 20 min) t_R 14.5 min, purity > 95%. M.p. 170–172° (dec.). CD (0.2 mM in MeOH): $-8.3 \cdot 10^4$ (198 nm). CD (0.2 mM in H_2O): $-6.1 \cdot 10^4$ (198 nm). IR (KBr): 3314*m*, 3112*m*, 2962*m*, 1671*s*, 1558*s*, 1400*m*, 1307*w*, 1204*s*, 1136*s*. ^{1}H-NMR (500 MHz, CD_3OD): 0.90–0.95 (*m*, 4 Me); 1.01–1.04 (*m*, 2 Me); 1.28–1.62 (*m*, 2 CH_2, 2 CH); 1.93–2.01 (*m*, CH); 2.19–3.25 (*m*, 5 CH_2CO, 2 CH_2S); 3.44–3.50 (*m*, CHN); 3.83–3.94 (*m*, CHN); 4.25–4.30 (*m*, CHN); 4.34–4.39 (*m*, 2 CHN); 7.89 (br., NH); 8.03 (br., NH). ^{13}C-NMR (125 MHz, CD_3OD): 18.2, 18.6, 22.2, 22.6, 23.4, 23.6 (Me); 26.1, 26.2, 31.7 (CH); 35.3, 40.2, 41.0, 41.3, 41.7, 42.1, 44.5, 44.6, 45.3 (CH_2); 46.1, 46.1, 55.6 (CH); 172.0, 172.0, 172.2, 173.1, 174.9 (C). ESI-MS (pos.): 663 (8, $[M + 2\,Na - H]^+$), 656 (10, $[M + K]^+$), 640 (45, $[M + Na]^+$), 618 (100, $[M + 1]^+$). ESI-MS (neg.): 616 (100, $[M - 1]^-$).

*H-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**14c**). Compound **14c** was prepared from **3** according to *GP 6*. CD (0.2 mM in MeOH): $+1.05 \cdot 10^5$ (198 nm); $-9.0 \cdot 10^3$ (220 nm).

*Boc-(*R*)-β³-HSer(Bn)-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**11**). Compound **9a** (232 mg, 0.4 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.8 ml), and treated with Et_3N (202 mg, 2 mmol), HOBt (65 mg, 0.48 mmol), Boc-(*R*)-β³-HSer(Bn)-OH (124 mg, 0.4 mmol) in $CHCl_3$ (0.8 ml), and EDC (95 mg, 0.48 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 13:1) yielded **11** (257 mg, 83%). White solid. M.p. 159–160°. R_f (CH_2Cl_2/MeOH 15:1) 0.48. $[\alpha]_D^{rt} = -6.1$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–0.92 (*m*, 3 Me); 1.24–1.31 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.42–1.49 (*m*, 1 H, CH_2); 1.51–1.62 (*m*, 1 H, CH_2); 1.74–1.83 (*m*, CH); 2.25–2.77 (*m*, 4 CH_2CO, CH_2S); 3.47–3.58 (*m*, CH_2O); 3.66 (*s*, MeO); 3.71 (*s*, SCH_2Ph); 3.94–4.02 (*m*, CHN); 4.07–4.17 (*m*, CHN); 4.18–4.26 (*m*, CHN); 4.26–4.36 (*m*, CHN); 4.51 (*s*, CH_2O); 5.51 (br., NH); 6.35 (br., NH); 6.69 (br., NH); 7.01 (br., NH); 7.20–7.36 (*m*, 10 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.0, 19.6, 22.1, 22.9 (Me); 25.0 (CH); 28.4 (Me); 31.5 (CH); 34.8, 36.2, 38.3, 38.5, 38.7, 39.1, 43.2 (CH_2); 44.5, 46.5, 47.9 (CH); 51.8 (Me); 52.1 (CH); 71.5, 73.3 (CH_2); 79.1 (C); 127.1, 127.7, 127.8, 128.4, 128.6, 129.0 (arom. C); 138.0, 138.1, 155.6, 170.2, 170.5, 170.8, 172.2 (C). FAB-MS: 793 (14, $[M + Na]^+$), 771 (47, $[M + 1]^+$), 671 (100, $[M - Boc + 1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HSer(Bn)-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OMe* (**15a**). Compound **11** (212 mg, 0.275 mmol) was deprotected according to *GP 1*, dissolved in $CHCl_3$ (0.6 ml), and treated with Et_3N (140 mg, 1.4 mmol), HOBt (45 mg, 0.35 mmol), **9b** (156 mg, 0.275 mmol) in DMF (2.8 ml), and EDC (64 mg, 0.35 mmol) according to *GP 3b*. FC (CH_2Cl_2/MeOH 7:1) yielded **15a** (140 mg, 42%). White solid. M.p. 220–222°. R_f (CH_2Cl_2/MeOH 16:1) 0.29. $[\alpha]_D^{rt} = -2.6$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3291*s*, 3063*m*, 2958*s*, 1648*s*, 1542*s*, 1453*s*, 1367*m*, 1311*w*, 1248*m*, 1174*m*, 1028*m*, 743*w*, 699*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85–0.92 (*m*, 8 Me); 1.15–1.70 (*m*, 4 CH, 2 CH_2, *t*-Bu); 2.38–3.31 (*m*, 7 CH_2CO, 2 CH_2S); 3.49–3.79 (*m*, 2 SCH_2Ph, MeO); 4.05–4.16 (*m*, 2 CHN); 4.27–4.38 (*m*, CHN); 4.41–4.55 (*m*, 3 CHN, 2 CH_2O); 4.74–4.86 (*m*, CHN); 5.49 (br., NH); 7.19–7.34 (*m*, 15 arom. H); 7.51 (br., NH); 7.61 (br., NH); 7.76 (br., NH); 8.31 (br., NH); 8.57 (br., NH); 8.83 (br., NH); 8.93 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.1, 19.3, 19.4, 22.8, 23.1 (Me); 24.7, 25.0 (CH); 28.5 (Me); 32.5, 32.8 (CH); 35.5, 36.8, 37.7, 37.9, 39.6, 39.8, 40.1, 41.4 (CH_2); 44.0 (CH); 44.8 (CH_2); 45.0 (CH); 45.1 (CH_2); 46.7, 47.2 (CH); 52.2 (Me); 52.4 (CH); 71.6, 73.0 (CH_2); 80.2 (C); 111.1, 118.4, 123.7, 124.7, 126.9, 127.1, 127.3, 127.8, 128.1, 128.4, 128.4, 128.6, 128.9 (arom. C); 137.7, 138.1, 157.5, 170.6, 170.8, 170.90, 171.4, 171.6, 173.0, 174.5 (C). FAB-MS: 1119 (100, $[M - Boc + 1]^+$).

*Boc-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-(*R*)-β³-HSer(Bn)-(*R*)-β³-HVal-(*R*)-β³-HCys(Bn)-(*S*)-β³-HLeu-OH* (**15b**). Compound **15a** (91 mg, 0.075 mmol) was dissolved in CF_3CH_2OH (1 ml) and treated with 1.5 ml of 5N NaOH (7.5 mmol), according to *GP 2b* (reaction time: 48 h) yielding **15b** (87 mg, 96%). White solid. M.p. 241° (dec.). R_f (CH_2Cl_2/MeOH 10:1) 0.22. $[\alpha]_D^{rt} = -0.4$ ($c = 1.0$, CF_3CH_2OH). IR (KBr): 3308*m*, 2961*m*, 1637*s*, 1542*w*, 1451*s*, 1366*m*, 1261*m*, 1172*m*, 1098*m*, 1028*w*, 802*w*, 698*w*. ^{1}H-NMR (400 MHz, CD_3OD): 0.85–0.92 (*m*, 8 Me); 1.14–1.83 (*m*, 4 CH, 2 CH_2, *t*-Bu); 2.33–2.78 (*m*, 7 CH_2CO, 2 CH_2S); 3.31 (*m*, 1 H); 3.48–3.53 (*m*, CHN); 3.71 (*d*, $J = 4.4$, SCH_2Ph); 3.76 (*d*, $J = 2.4$, SCH_2Ph); 3.84–3.88 (*m*, CHN); 4.27–4.35

(*m*, 3 CHN); 4.43–4.64 (*m*, 2 CHN, 2 CH_2O); 7.17–7.34 (*m*, 15 arom. H); 7.49 (br., NH); 7.82 (br., NH); 8.30 (br., NH). ^{13}C-NMR (100 MHz, CD_3OD): 19.0, 19.1, 19.3, 22.3, 22.8, 23.2, 23.3 (Me); 25.0, 25.2 (CH); 28.5 (Me), 32.5, 32.7 (CH); 36.5, 36.6, 36.7, 37.5, 37.7, 37.9, 38.7, 39.7, 40.3, 41.8 (CH_2); 44.7 (CH); 44.9 (CH_2); 45.5 (CH); 45.7 (CH_2); 46.2, 47.5, 51.8, 53.2 (CH); 71.9, 73.3 (CH_2); 79.2 (C); 127.2, 127.3, 127.8, 127.9, 128.0, 128.6, 128.7, 129.1, 129.2, 131.3 (arom. C); 138.3, 138.3, 138.4, 157.4, 170.0, 170.7, 171.1, 171.4, 172.7, 173.9 (C). FAB-MS: 1233 (100, $[M+29]^+$).

*cyclo-H-(*R*)-β³-HVal(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HSer-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**4**). Compound **15b** (60 mg, 0.050 mmol) was deprotected and cyclized according to *GP 4*. The crude peptide was purified by prep. RP-HPLC (20 → 40% *B* in 30 min) according to *GP 5*, yielding **4** (27 mg, 65%), which gave a negative *Ellman*'s test [38]. White solid. RP-HPLC (20 → 50% *B* in 20 min) t_R 18.1 min, purity > 98%. M.p. 190–191° (dec.). CD (0.2 mM in MeOH): $-5.7 \cdot 10^4$ (200 nm). CD (0.2 mM in H_2O): $-1.5 \cdot 10^4$ (203 nm). IR (KBr): 3295*m*, 3110*m*, 2975*m*, 1676*s*, 1545*s*, 1437*m*, 1400*m*, 1315*w*, 1207*s*, 1136*s*, 1052*w*. ^{1}H-NMR (500 MHz, CD_3OD): 0.91–0.98 (*m*, 6 Me); 1.03–1.06 (*m*, 2 Me); 1.24–1.84 (*m*, 2 CH_2, 3 CH); 1.97–2.03 (*m*, CH); 2.24–3.06 (*m*, 7 CH_2CO, 2 CH_2S); 3.41–3.44 (*m*, CHN); 3.46–3.57 (*m*, CH_2CO); 4.05–4.09 (*m*, CHN); 4.31–4.38 (*m*, 4 CHN); 4.42–4.48 (*m*, CHN); 7.89 (br., NH). ^{13}C-NMR (125 MHz, CD_3OD): 18.2, 18.8, 19.0, 19.3, 22.4, 22.4, 23.6, 23.7 (Me); 26.1, 26.1, 32.0, 34.0 (CH); 36.2, 38.5, 38.7, 40.0, 40.1, 41.4, 43.2, 44.7, 44.9, 45.2, 45.7 (CH_2); 46.1, 46.6, 48.2, 53.4, 56.1 (CH); 64.5 (CH_2); 171.7, 172.0, 172.1, 172.5, 173.2, 173.5, 175.4 (C). ESI-MS (pos.): 877 (20, $[M+2\,Na-H]^+$), 855 (24, $[M+Na]^+$), 833 (100, $[M+1]^+$). ESI-MS (neg.): 831 (100, $[M-1]^-$).

*H-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-(*R*)-β³-HSer-(*R*)-β³-HVal-(*R*)-β³-HCys-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**15c**). Compound **15c** was prepared from **4** according to *GP 6*. CD (0.2 mM in MeOH): $+6.6 \cdot 10^4$ (204 nm); $-3.3 \cdot 10^4$ (216 nm).

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] D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, submitted.

[5] D. Seebach, J. L. Matthews, *J. Chem. Soc., Chem. Commun.*, **1997**, *21*, 2015.

[6] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173.

[7] K. Gademann, T. Hintermann, J. V. Schreiber, *Curr. Med. Chem.* **1999**, *111*, 1700; *ibid., Int. Ed.* **1999**, *38*, 1595.

[8] D. Y. Jackson, D. S. King, J. Chmielewski, S. Singh, P. G. Schultz, *J. Am. Chem. Soc.* **1991**, *113*, 9391.

[9] M. Chorev, E. Roubini, R. L. McKee, S. W. Gibbons, M. E. Goldman, M. P. Caulfield, M. Rosenblatt, *Biochemistry* **1991**, *30*, 5968.

[10] H. E. Blackwell, R. H. Grubbs, *Angew. Chem.* **1998**, *110*, 3469; *ibid. Int. Ed.* **1998**, *37*, 3281.

[11] a) T. Hintermann, D. Seebach, *Synlett* **1997**, 437; b) G. Guichard, D. Seebach, *Chimia* **1997**, *51*, 315; c) G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* **1998**, *52*, 187.

[12] a) S. Abele, G. Guichard, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2141; b) B. W. Gung, D. Zou, A. M. Stalcup, C. E. Cottrell, *J. Org. Chem.* **1999**, *64*, 2176; c) D. A. Appella, J. J. Barchi, S. R. Durell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 2309.

[13] E. Block, J. Zubieta, in 'Advances in Sulfur Chemistry', Ed. E. Block, Jai Press Inc., Greenwich, CT, 1994, p. 133.

[14] E. P. Fries, J. E. T. Andersen, L. L. Madsen, P. Moller, R. J. Nichols, K. G. Olesen, J. Ulstrup, *Electrochimica Acta* **1998**, *43*, 2889.

[15] T. V. O' Halloran, *Science* **1993**, *261*, 715.

[16] S. J. Lippard, J. M. Berg, 'Principles of Bioinorganic Chemistry', University Science Books, Mill Valley, CA, 1994, p. 178.

[17] K. T. Yasunobu, M. Tanaka, in 'Iron Sulfur Proteins'. Ed. E. Lovenberg, Academic Press Inc., London 1973, Vol. 2, p. 27.

[18] H. J. Dyson, *Method. Enzymol.* **1995**, *252*, 293.

[19] H. Tuppy, in 'Sulfur in Proteins' Eds. R. Benesch, R. E. Benesch, P. D. Boyer, I. M. Klotz, W. R. Middlebrook, A. G. Uzent-Györgi, Academic Press Inc., London 1959, p. 141.
[20] C. Guibourdenche, J. Podlech, D. Seebach, *Liebigs Ann. Chem.* **1996**, 1121.
[21] G. Grue-Sørensen, E. Kelstrup, A. Kjær, J. Øgaard Madsen, *J. Chem. Soc., Perkin Trans. I* **1984**, 1091.
[22] T. D. Clark, K. Kobayashi, M. R. Ghadiri, *Chem. Eur. J.* **1999**, *5*, 782.
[23] a) D. Andreu, F. Albericio, N. A. Solé, M. C. Munson, M. Ferrer, G. Barany in 'Peptide Synthesis Protocols', Eds. M. W. Pennington, B. M. Dunn, Humana Press Inc., Totowa, NJ, 1994, Vol. 35, p. 91; b) J. Eichler, R. A. Houghten, *Protein Pep. Lett*. **1997**, *4*, 157; c) E. V. Kudryavtseva, M. V. Sidorova, R. P. Evstigneeva, *Russ. Chem. Rev*. **1998**, *67*, 545.
[24] Y. Shimonishi, H. Zahn, W. Puls, *Z. Naturforschg*. **1969**, *24b*, 422.
[25] J. P. Tam, C.-R. Wu, W. Liu, J.-W. Zhang, *J. Am. Chem. Soc*. **1991**, *113*, 6657.
[26] R. W. Woody, A. K. Dunker in 'Circular Dichroism and the Conformational Analysis of Biomolecules', Ed. G. D. Fasman, Plenum Press, New York, NY, 1996, p. 109.
[27] A. Rauk, *J. Am. Chem. Soc*. **1984**, *106*, 6517.
[28] G. Siligardi, M. M. Campbell, W. A. Gibbons, A. F. Drake, *Eur. J. Biochem.* **1992**, *207*, 25.
[29] a) I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Proteins: Struct. Funct. Genet*. **1990**, *7*, 62; b) I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Biopolymers* **1993**, *33*, 827; c) C. Toniolo, E. Benedetti, *Trends Biochem. Sci*. **1991**, *16*, 350.
[30] K. R. Shoemaker, P. S. Kim, E. J. York, J. M. Stewart, R. L. Baldwin, *Nature* **1987**, *326*, 563.
[31] D. J. Lockhart, P. S. Kim, *Science* **1993**, *260*, 198.
[32] C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc*. **1996**, *118*, 2744.
[33] K. A. Bode, J. Applequist, *Macromolecules* **1997**, *30*, 2144.
[34] J. Podlech, D. Seebach, *Liebigs Ann. Chem.* **1995**, 1217.
[35] K. Gademann, hitherto unpublished results, ETH-Zürich 1998.
[36] P. Lombardi, *Chem. Ind. (London)* **1990**, 708; S. Moss, *ibid.* **1994**, 122.
[37] M. Bodanszky, A. Bodanszky, 'Practice of Peptide Synthesis', Springer Verlag, Berlin, Heidelberg, 1994, p. 135.
[38] G. L. Ellman, *Arch. Biochem. Biophys.* **1959**, *82*, 70.

Received May 3, 1999