β-Thiopeptides: Synthesis, NMR Solution Structure, CD Spectra, and Photochemistry

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To test the effect of NH–C=S groups (*Scheme 1*) on the stability of β-peptide secondary structures, we have synthesized three β-thiohexapeptide analogues of H-(β-HVal-β-HAla-β-HLeu)$_2$-OH (**1**) with one, two, and three C=S groups in the N-terminal positions (*cf.* **2**–**4** and model in *Fig. 1*). The first C=S group was introduced selectively by treatment with *Lawesson* reagent of Boc-β-dipeptide esters (**6** and **8**). A series of fragment-coupling steps (with reagents as for the corresponding sulfur-free building blocks) and another thionation reaction led to the title compounds with a C=S group in residues 1, 1, and 3, as well as 1, 2, and 3 of the β-hexapeptide (*Schemes 2* and *3*). The sulfur derivatives, especially those with three C=S groups, were much more soluble in organic media than the sulfur-free analogues (> 1000-fold in $CHCl_3$; *Table 1*). The UV and CD spectra (in $CHCl_3$, MeOH, and H_2O) of the new compounds were recorded and compared with those of the parent β-hexapeptide **1** (*Figs. 2–4*); they indicate the presence of more than one secondary structure under the various conditions. Most striking is a pronounced exciton splitting ($\Delta\lambda$ *ca.* 20 nm, amplitude up to + 121000) of the $\pi\pi^*_{C=S}$ band near 270 nm with the β-trithiohexapeptide (with and without terminal protecting groups), and strong, so-called 'primary solvent effects', in the CD spectra. The CD spectrum of the β-dithiohexapeptide **3** undergoes drastic changes upon irradiation with 266-nm laser light of a MeOH solution (*Fig. 5*). The NMR structure in CD_3OH of the unprotected β-trithiohexapeptide **4** was determined to be an (*M*)-3_{14}-helix (*Fig. 7*), very similar to that of the non-thionated analogue (*cf.* **1**). NMR and mass spectra of the β-hexapeptides with C=S and with C=O groups are compared (*Figs. 6* and *8*).

1. Introduction and Aim. – In recent years, unnatural amino-acid oligomers, which are able to form stable and well-defined secondary structures, have received much attention. We and others have demonstrated that oligomers consisting exclusively of β-amino acids (with as few as six or seven residues) can adopt a large variety of secondary structures in solution as well as in the solid state[3]). Thus, the main structural elements of proteins (helices [2–6], parallel and antiparallel pleated sheets [2][7][8], turns [7][8], and tubular stacks [9–11]) have been identified in the realm of β-peptides by X-ray crystal-structure, NMR, and CD analyses. Moreover, negative *Ames*' tests [12] and stability towards mammalian proteases [2][12][13] are properties which make β-peptides candidates for medicinal applications [14][15].

Backbone modification is of great importance in the field of α-peptides[4]). It may afford analogues possessing an enhanced stability to enzymic hydrolysis, as well as a greater affinity and specificity towards biological receptors. Thus, the thioamide

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[2]) Part of the projected dissertations of *M.R.* and *K.G.*, ETH-Zürich.

[3]) For recent reviews on β-peptides, see [1].

[4]) For our own activities in this field, see the reviews [16–18].

replacement Ψ[CSNH][5])[6]), in which the amide O-atom has been substituted by an S-atom, constitutes one example of seemingly minimal and subtle backbone modification. The interest in thionated α-peptides was boosted, thanks to the introduction of convenient thionating reagents [21]. Previous studies [22–29] have shown that the introduction of one C=S function constitutes a nearly isosteric substitution, and that this group, like the amide group itself, preferentially adopts a (*Z*) planar configuration[7]). Still, these two types of functional groups possess different physical and chemical properties (*Scheme 1*) that may lead to surprising conformational changes [20][30], to enhanced stability towards proteases [31], and to greater biological activity [32–34] in α-peptides[8]).

Of particular importance is the H-bonding ability of the thioamide group. Its NH is more acidic [38], and thus a stronger H-bond donor [45], while the S-atom is generally considered a weaker acceptor [28][9]). Together with the higher barrier to rotation around the thioamide bond, this may enhance the conformational stability of peptides thionated in key positions of the backbone. On the other hand, the larger C=S bond length may cause 'widening' of secondary structures, and thus increases solvent accessibility to the more polar thioamide group (see the data collected in *Scheme 1*).

To study the effect of thioamide groups on the known secondary structure of a β-peptide, we chose to introduce Ψ[CSNH] replacements in the β-hexapeptide H-(β^3-HVal-β^3-HAla-β^3-HLeu)$_2$-OH (**1**), which has been shown to form a left-handed or (*M*)-3_{14}-helix in CD_3OH [3] and in C_5D_5N [2], with H-bonds between the NH group of amino acid *i* to the C=O group of amino acid *i* + 2. To ensure maximum increase of H-bonding (from thioamide NH to amide C=O!)[10]), we decided to position C=S groups in the three N-terminal positions of the β-hexapeptide **1** (see *Fig. 1*). Thus, our synthetic target molecules are the β-mono-, β-di-, and β-trithio-hexapeptides **2**–**4**.

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[5]) For this nomenclature, see the recommendations of the *IUPAC-IUB Joint Commission on Biochemical Nomenclature* [19]. The thioamide replacement is most frequently called thionation, and the term endothiopeptide is used for peptide derivatives containing one ore more –C(S)NH– function(s) in the peptide backbone.

[6]) *Cf.* cyclosporin thionations by our group [20] and by others [18].

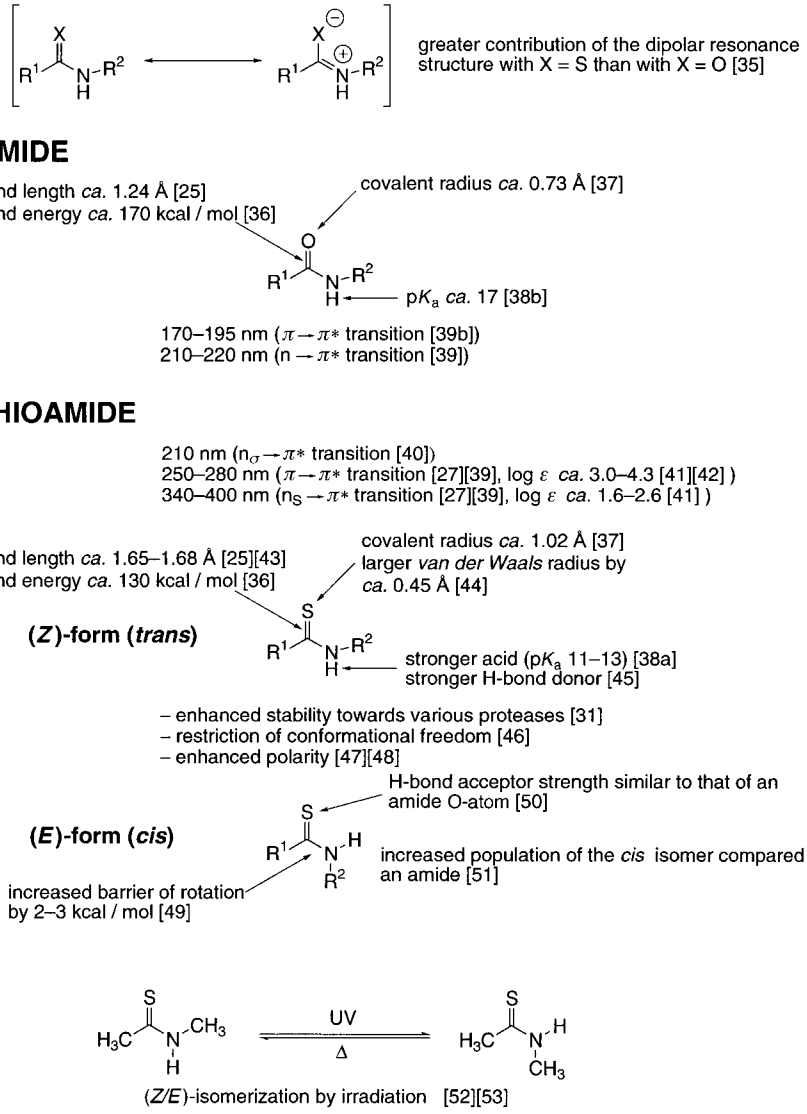
[7]) We use the terms *form* or *configuration*, and the (*E*/*Z*) *convention* for specification of the isomers arising from hindered rotation around the RC(X)–NHR partial double bond (rather than the terms *conformation*, *cis*/*trans* or *rotamers*) throughout this paper.

[8]) Biological studies have shown that the effects of the C=O/C=S exchange is difficult to predict: introduction of thioamide groups may give rise to analogues with increased (leucine enkephalin [32a], dermorphin [33a]), decreased (leucine enkephalin [32a,b], dermorphin [33a], growth hormone releasing hexapeptide [33b]), or nearly equivalent activities (thyrotropin-releasing hormone [34]).

[9]) Although there is also evidence that the H-bonding-acceptor capacity of the thioamide S-atom may equal that of the amide O-atom [50][54].

[10]) Recently β-(sulfonamido)peptides, another type of sulfur-containing peptide analogues, were studied as potential unnatural oligomers possessing defined secondary structures [55]. The ability of the sulfonamide group to form an intramolecular H-bond was investigated, and it was concluded that the amido group is a stronger H-bond acceptor than the sulfonamido group.

Scheme 1. *Comparison of Bonding, Structure, and Spectroscopy of Amide and Thioamide Groups*



2. Synthesis of the Thio-β-hexapeptides 2 – 4. – Since the first successful synthesis of thiopeptides with P_2S_5 in 1926 [56], important developments in the field of thionation have been realized. A major and notable breakthrough was, in 1978, the introduction of the *Lawesson* reagent, a convenient racemization-free thionating agent [21][57][11]). For the preparation of endothiopeptides several methods are now available, but the main

[11]) Later, more potent and more selective derivatives were developed [58].

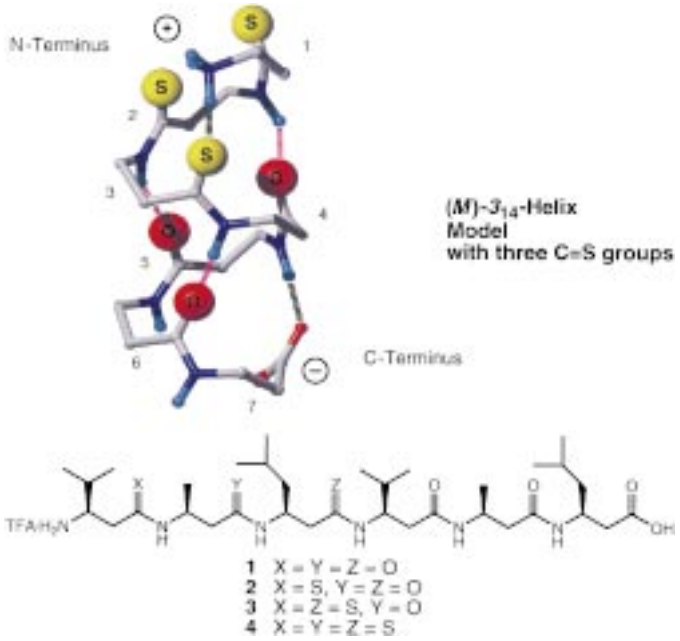


Fig. 1. *β-Thiopeptides.* The model of a helical β-trithioheptapeptide (without side chains) was constructed with the data from the NMR analysis of **1** [2][3]. The position of three S-atoms in residues 1, 2, and 3 (*cf.* **4**) is such that three H-bonds of increased strength (violet dotted lines) between CS–NH and O=C should result. The β-monothio- and β-dithiopeptides **2** and **3**, respectively, have only one and two such H-bonds, respectively.

strategy is still thionation of a preformed dipeptide and subsequent incorporation into large peptides by fragment coupling[12]).

For the synthesis of the β^3-thiohexapeptides, we selected this last-mentioned approach, which allows for regioselective reaction of the internal peptide bond [23][57][60][13]). For the preparation of the fully protected dipeptides[14]) **5** and **7**, we first synthesized (in enantiomerically pure form) the *N*-Boc-protected β^3-amino acids, as well as the corresponding methyl esters[15]).Then, the β^3-dipeptides **5** and **7** were

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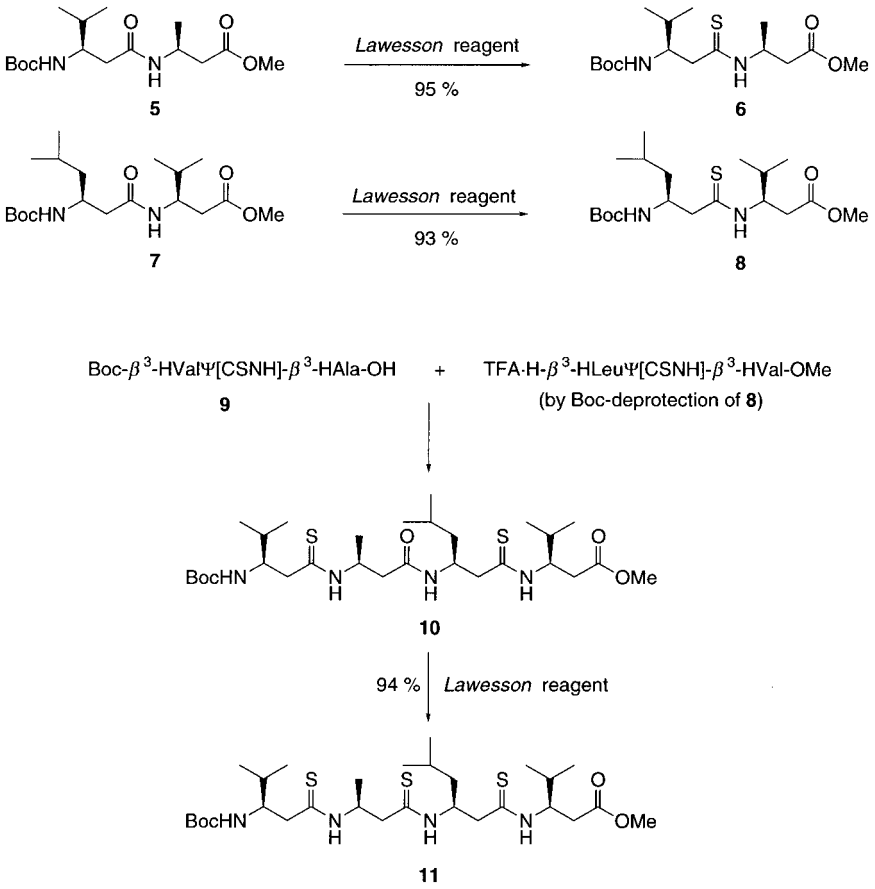
[12]) Thioacylation is an alternative possible route to endothiopeptides, although this method gives variable results. The following thioacylating reagents have been reported: thioesters [59a], dithioesters [59b][59c], amino thioacids [59d][59e], thiacyl-*N*-phthalimides [59f], thioacyl-benzimidazolines [59g], and nitro-benzotriazoles [59h].

[13]) Ordinary protected dipeptides contain three C=O groups: in the carbamate (*N*-terminal protection), in the peptide, and in the ester moiety (*C*-terminal protection). The *Lawesson* reagent replaces selectively the O-atom of the internal amide group [23][57][60]. Previous work showed that, for a successful thionation, the size of peptide is generally limited to dipeptides because of the loss of regioselectivity and poor solubility often encountered with larger peptides. Regioselective *mono*thionations of tripeptides or tetrapeptides are sometimes feasible with sufficiently large differences of steric hindrance between the internal amide groups [18][20][23][58b].

[14]) It was shown that both, the free amino- and the free carboxylic-acid function react with *Lawesson* reagent [21b]. Therefore, *N*-protected dipeptide esters are used.

[15]) These chiral building blocks, with the proteinogenic side-chains of valine, alanine, and leucine, were obtained by *Arndt-Eistert* homologation of the commercial *N*-Boc-protected α-amino acids, as described previously [2][61].

Scheme 2. *Selective Thionation of Fully Protected Dipeptides* **5** *and* **7**, *and of Tetrapeptide* **10** *to the* N-*Boc-protected* β*-Thiopeptides* **6**, **8**, *and* **11**



obtained by conventional peptide solution synthesis with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC)/(HOBt) [2][3] (yield *ca.* 85%). The subsequent thionations were carried out in THF[16]), according to a previously described procedure, in which a molar ratio of 1:2 between the *Lawesson* reagent and the substrates **5** and **7** was used [58b][58d][17]). Under these conditions, the thionations

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16) Thionation can be realized in anhydrous toluene [21][29][57][60c], xylene [21c], 1,2-dimethoxyethane [58d], HMPA [21b], or DMPU [20]. In peptide chemistry, THF shows a pronounced solvent effect on the course of the thionation reactions [58b] [58d]: most amides and lactames are thionated at room temperature or below, with short reaction times. At room temperature, and depending on the substrate, the required time for completion of the reaction can range from a few minutes to several hours or days (when relatively large side chains flank the amide group, or when proline is involved [58b]). The *Lawesson* reagent has low solubility in THF, but the heterogeneous reaction mixture becomes clear as the thionation proceeds.

17) Best yields were obtained with the molar ratio 1:2 [58d]. That a molar ratio of 1:4 still afforded yields above 75% confirms that 1 equiv. of *Lawesson* reagent is able to achieve up to four thionations.

proceeded smoothly, regioselectively, and with high yields of the β-thiodipeptide derivatives **6** and **8** (95% after 2.5 h, and 93% after 4 h, respectively; see *Scheme 2*)[18]).

A particularly efficient control of thionation rests upon a characteristic chemical shift displayed by C=S groups in the ^{13}C-NMR spectra: the C=S C-atoms resonate *ca.* 30 ppm downfield relative to the corresponding C=O C-atoms. Thus, in the β-dipeptide esters **5** and **7** the C=O C-atoms of the urethane, of the methyl ester, and of the amide groups have chemical shifts between 155.6 and 172.4 ppm, and, after thionation, only one downfield ^{13}C-resonance is observed at 200.6 ppm for **6** and at 201.1 ppm for **8**. Another evidence for the monothionation is obtained from the IR spectra (KBr): the dipeptides **5** and **7** show three well separated and identified bands, corresponding to the three types of C=O groups; after thionation the amide band has disappeared[19])[20]).

After having prepared the first two β-thiopeptides, and after having established the analytical tools to identify the presence and the position of a C=S group in a β-peptide, we were ready to perform fragment couplings and an additional thionation step (*Schemes 2* and *3*). It is known from syntheses of α-thiopeptides that the necessary *N*-Boc and Me-ester deprotections are not trivial: the thioamide group can undergo both acid- and base-catalyzed hydrolysis[21]). Still, successful Boc cleavages with CF_3CO_2H (TFA) [29], HCl [54], or, preferably $SnCl_4$ [64] have been reported. Also, reaction conditions appropriate for ester saponification, avoiding S/O exchange on a thioamide group in the same molecule, have been described [23][42][54]. Furthermore, activation of the CO_2H group in an α-thiodipeptide is known to cause cyclization to a thioazlactone[22]) (*cf.* the *Edman* degradation!), with concomitant racemization [42]; we assumed that this complication would not arise with our β-peptide analogues, as long as we are dealing with β^3-amino-acid residues[22]). Finally, the S-atom of the C=S groups has been found to be able, under certain conditions, to hop between positions [67], and we had to be aware of this possibility in our β-thiopeptides as well.

In spite of so many possibilities for failure, the feasibility of our synthetic strategy was proved by experiment (*Scheme 2*): the β-thiodipeptide ester **6** was saponified with

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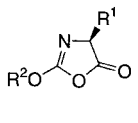
[18]) The reported yields for monothionations of fully protected α-dipeptides range from 70 to >99%.

[19]) In the IR spectra (KBr) of **5**–**8** the ester, the urethane and the amide carbonyl absorptions are at 1718–1747, 1679–1690 and 1642–1654 cm^{-1}, respectively. Similar IR absorptions for these three kinds of carbonyl groups were reported for protected α-dipeptides [23][60b][60c].

[20]) Unlike C=O groups, C=S groups do not give rise to characteristic IR stretch bands; their vibration occurs at much lower frequencies (in the C–O or C–N region near 1000 cm^{-1} [62]). Therefore thioamide C=S groups are difficult to assign.

[21]) For a recent review describing the conversion of thiocarbonyl groups into carbonyl groups see [63].

[22]) The thia analogue **ii** of the well-known racemization-causing oxazolinone **i** [65] can be formed from activated RO_2CNH-protected amino acids. No racemization is likely to occur with the corresponding β-peptide derivative (*cf.* **iii** and [66]).



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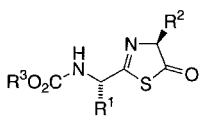

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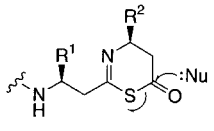

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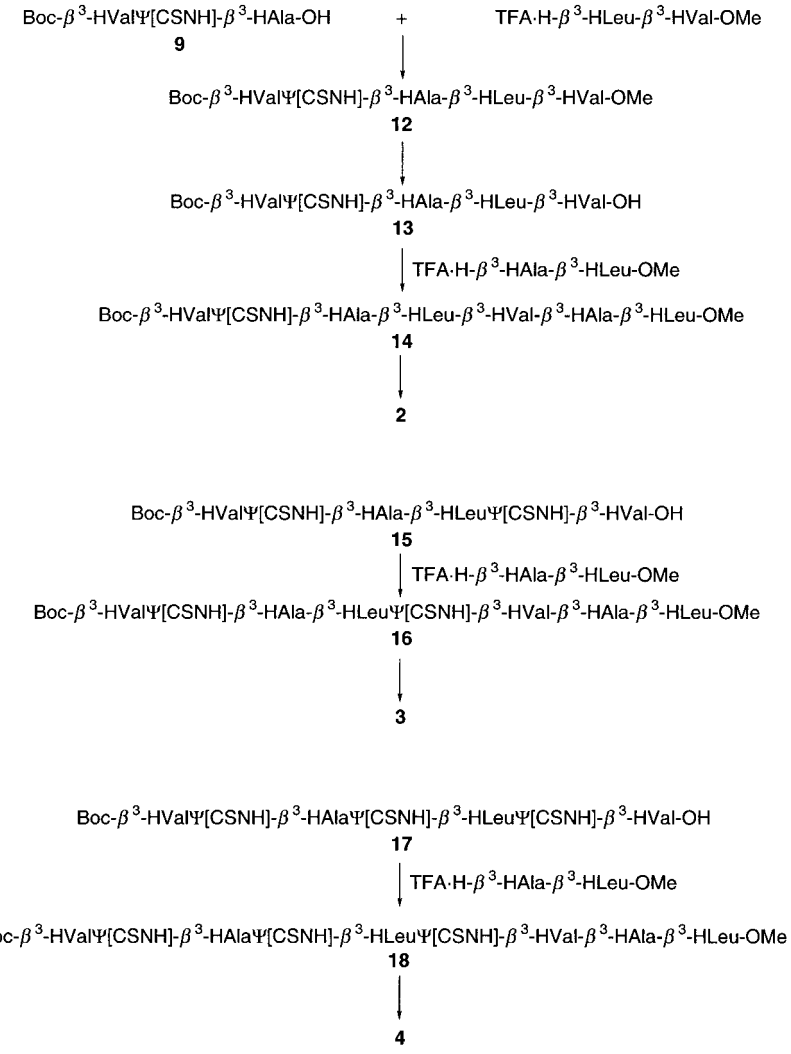
Scheme 3. *Fragment Coupling towards the β-Hexapeptides* **2**, **3**, *and* ***4***. The TFA salts of the β^3-dipeptide esters H-β-HLeu-β-HVal-OMe and H-β-HAla-β-HLeu-OMe, used for the preparation of **12**, **14**, **16**, and **18**, have been described before [2] and are used as formed by Boc-deprotection without further purification.



NaOH[23]) to give the acid **9** quantitatively, the Boc deprotection of the β-thiodipeptide ester **8** with TFA also proceeded smoothly, and coupling of the two resulting fragments (EDC, HOBt, Et_3N) provided the fully protected β-thiotetrapeptide **10** in 80% yield

[23]) For successful ester hydrolysis, a small excess of aqueous NaOH (1.1 – 1.4 equiv.) was used, and these saponifications were performed in MeOH between 20 and 40°.

(after purification), which, in turn, was thionated on the central amide group to give the β-trithiotetrapeptide **11** in almost quantitative yield after chromatography[24])[25]).

For the synthesis of the target compound **2**, dipeptide **7** was *N*-deprotected with TFA and coupled with **9** to give the β-monothiotetrapeptide **12** (82%; see *Scheme 3*). Saponification of **12** in CF_3CH_2OH yielded the acid **13** (99%), which, in turn, was coupled with H-β^3-HAla-β^3-HLeu-OMe [2] to give the fully protected β-thiohexapeptide **14**.

Due to poor solubility in organic solvents, flash-chromatographic purification of **14** was not possible, thus it was recrystallized from MeOH (yield 48%). Methyl-ester hydrolysis of **14** required somewhat harsh conditions (100 equiv. of NaOH, 120 h at 40°),which proved to be compatible with the other functional groups of this thiopeptide, and the expected acid was obtained in 85% yield[26]). Subsequent Boc deprotection with TFA and preparative RP-HPLC purification gave the free β-monothiohexapeptide **2** (62%). For the preparation of the target compounds **3** and **4**, the protected β-thiotetrapeptide esters **10** and **11** were quantitatively saponified (room temperature, 1.2 equiv. of NaOH) to the acids **15** and **17**. Subsequent fragment coupling with H-β^3-HAla-β^3-HLeu-OMe [2] led to the fully protected hexapeptides **16** and **18**, which, in contrast to **14**, could be readily purified by flash chromatography (60 and 56% yield, respectively)[27]). Due to their remarkably good solubility in MeOH, the fully protected β-thiohexapeptides **16** and **18** could be readily and quantitatively saponified (room temperature, 6 equiv. of NaOH) to the corresponding acids. Without further purification, a Boc deprotection and a subsequent preparative RP-HPLC purification provided the desired β-dithio- and β-trithiohexapeptides **3** and **4** (*ca.* 60%), respectively. The free β-hexapeptides **2**–**4** were isolated as TFA salts and were fully characterized by analytical HPLC, electrospray mass spectrometry, and ^{1}H-, ^{13}C-NMR, IR, and CD spectroscopy. The NMR structure of the trithio derivative **4** is described in *Sect. 4*. Electrospray mass spectroscopy (ESI-MS) proved to be the method of choice to further corroborate the structure of these compounds (see *Fig. 8* in the *Exper. Part*).

A notable difference of our synthesized C=S peptides, compared to their C=O counterparts is the much higher solubility in organic solvents. The general tendency is an increase of solubility with the number of C=S groups. Thus, the solubility of thiopeptides with two or three thioamide groups is greatly improved, while no big change is observed for monothiopeptides (see *Table 1*). Thus, the difference between the mono- and the trithiotetrapeptides **12** and **11**, respectively, is 5 *vs.* 720 mg/ml $CHCl_3$. The increased solubility leads to a significant improvement in the *C*-terminal deprotection step (less basic, shorter reaction time; *vide supra*).

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[24]) Conditions as for the reactions **5** → **6** and **7** → **8**, except that the temperature was raised somewhat (to 110–140° in order to thionate the urethane or ester groups with the *Lawesson* reagent [21c][23]).

[25]) Three C=S C-atoms (at 199.5, 200.0, and 200.7 ppm) and two C=O C-atoms (156.7 and 171.9 ppm) are detected by ^{13}C-NMR (*cf. Fig. 6*).

[26]) The ^{1}H- and ^{13}C-NMR spectroscopy showed that no S/O exchange had taken place during the saponification of **14.**

[27]) Unlike the β-monothiohexapeptide **14**, the di- and trithio derivatives contained by-products (*ca.* 5% of the theoretical yield), formed by partial loss of sulfur (in **18**) and, perhaps, also by a migration of sulfur (in **16**; *cf.* [67]) under the coupling conditions. These 'defects' were carried through to the target compounds **3** and **4**, where they were 'eliminated' by HPLC purification.

Table 1. *Influence of Thioamide Groups on the Solubility in $CHCl_3$ of β-Peptides*

Compound	Solubility [mg/ml]
Boc-β-HValΨ[CSNH]-β-HAla-β-HLeu-β-HVal-OMe (**12**)	5
Boc-β-HValΨ[CSNH]-β-HAla-β-HLeu-β-HVal-OH (**13**)	3
Boc-β-HValΨ[CSNH]-β-HAla-β-HleuΨ[CSNH]-β-HVal-OH (**15**)	210
Boc-β-HValΨ[CSNH]-β-HAlaΨ[CSNH]-β-HLeuΨ[CSNH]-β-HVal-OMe (**11**)	720
Boc-β-HVal-β-HAla-β-HLeu-β-HVal-β-HAla-β-HLeu-OMe (protected **1**)	< 0.5
Boc-β-HValΨ[CSNH]-β-HAla-β-HLeu-β-HVal-β-HAla-β-HLeu-OMe (**14**)	0.5 – 1
Boc-β-HValΨ[CSNH]-β-HAla-β-HLeuΨ[CSNH]-β-HVal-β-HAla-β-HLeu-OMe (**16**)	90
Boc-β-HValΨ[CSNH]-β-HAlaΨ[CSNH]-β-HLeuΨ[CSNH]-β-HVal-β-HAla-β-HLeu-OMe (**18**)	120

3. CD Spectroscopy and Photochemistry of β-Thiopeptides. – Circular dichroism (CD) has special advantages for exploration of the conformational environment of a thioamide group which may be considered as a chromophoric derivative of an amide group[28]). The CD spectra of 'endothiopeptides' present three well-separated bands,

	1	**2**		**3**		**4**	
λ_{max} [nm]	205	206	268	207	268	207	266
$\varepsilon \cdot 10^{-3}$ [$mol^{-1} \cdot l \cdot cm^{-1}$]	5.1	10.1	12.5	7.6	20.2	6.7	28.7

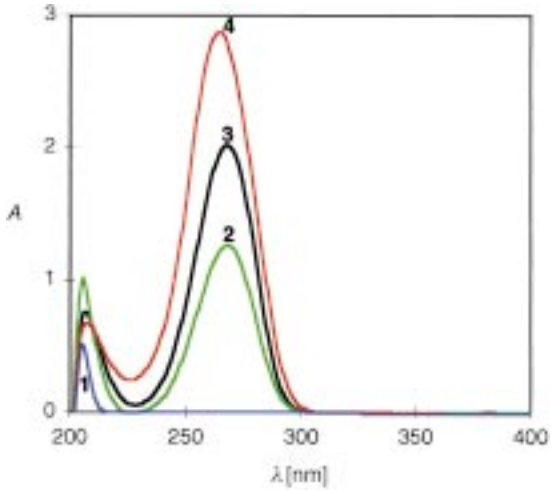

Fig. 2. *Overlay of the UV spectra of the β-hexapeptide* **1** (blue) *and of the β-monothio β-dithio-, and β-trithiohexapeptides* **2** (green), **3** (black), *and* **4** (red), *respectively, in MeOH* (*ca.* 10^{-4}M). The exact extinction coefficients ε [$mol^{-1} \cdot l \cdot cm^{-1}$] and λ_{max} values [nm] are collected in the accompanying table.

[28]) All CD bands are sensitive to the geometry of the molecule around the chromophore, and, therefore, CD spectroscopy is a valuable tool in the study of the stereochemistry of thioamides [23]. *Hollosi et al.* showed that, in α-endothiopeptides, the optical activity of the thioamide chromophore is dominated by the chiral contribution of perturbing groups attached to the C(α)-atom, at the NH side of the chromophore [68]. Thus, the positions, magnitudes, and sign pattern of the nπ* and ππ* bands mainly reflect the local conformation of the amino-acid residue following the thioamide group in the chain.

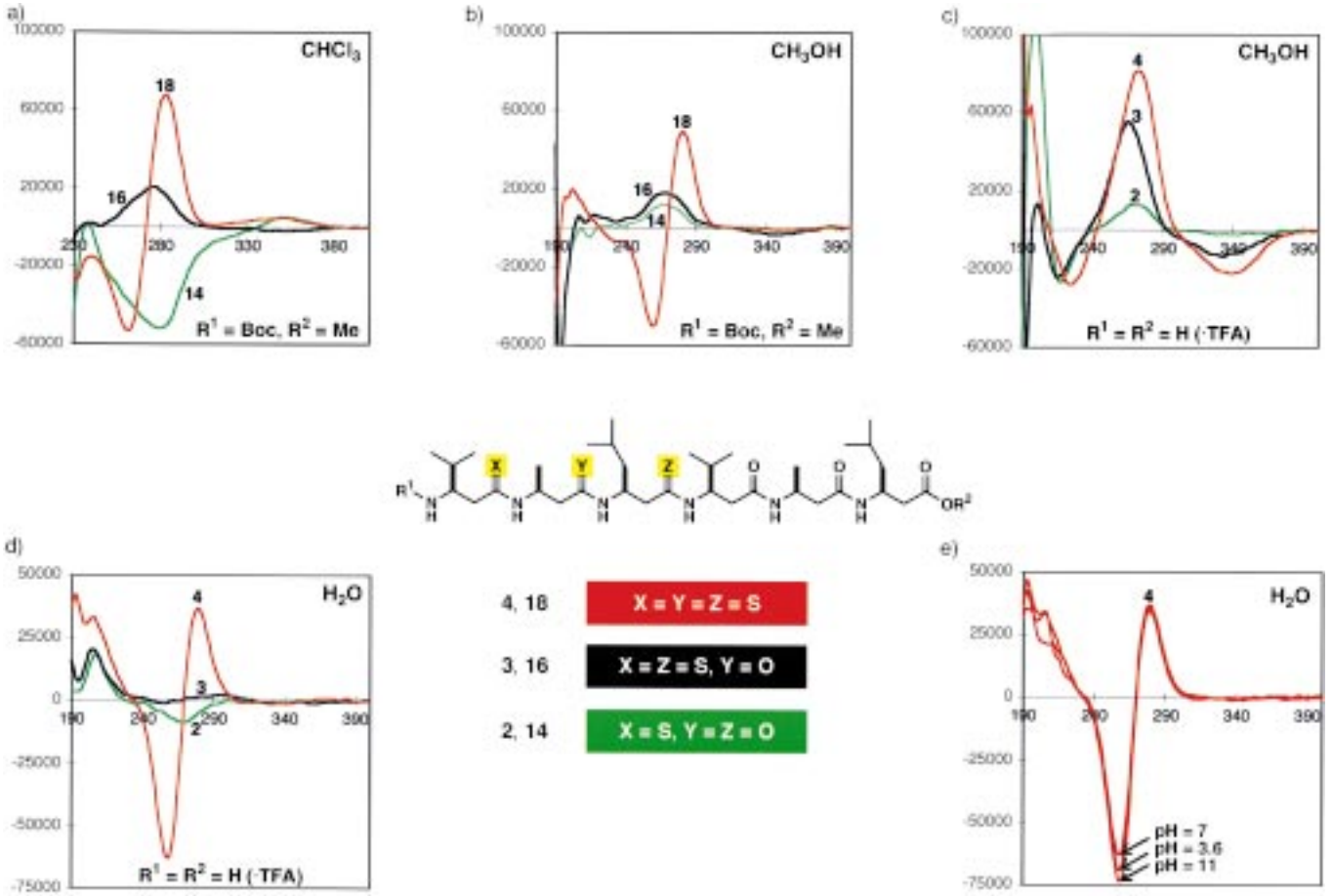

Fig. 3. *CD Spectra of β-thiohexapeptide derivatives* **2**–**4**, **14**, **16**, *and* **18**. Fully protected thiopeptides **14**, **16**, **18** *a*) in $CHCl_3$, *b*) in MeOH. Free thiopeptides **2**–**4** *c*) in MeOH, and *d*) in H_2O. *e*) pH Dependence of the CD spectrum of the *β-trithiohexapeptide* **4** in H_2O. All β-peptides were measured as 0.2 mM solutions. Molar ellipticity [ϕ] in 10 deg·cm^2·mol^{-1}. The *Cotton* effect near 340 nm in *a*), *b*), and *c*) is due to the n$\pi^*_{C=S}$, and that near 270 nm is due to the $\pi\pi^*_{C=S}$ transition. The Boc-*β*-trithiohexapeptide ester **18** shows an exciton splitting of the $\pi\pi^*_{C=S}$ transition in $CHCl_3$ (*a*; *Davydov* splitting 22 nm, amplitude 121000) and MeOH (*b*; *Davydov* splitting 21 nm, amplitude 99000), the corresponding unprotected *β*-trithiohexapeptide **4** only in H_2O (*d*; *e*; *Davydov* splitting 21 nm, amplitude 100000–110000, depending on pH). In MeOH, there is only a gradual difference of the *Cotton* effects at 270 and 340 nm in the spectra of the free *β*-monothio-, *β*-dithio-, and *β*-trithiohexapeptides **2**–**4**, respectively.

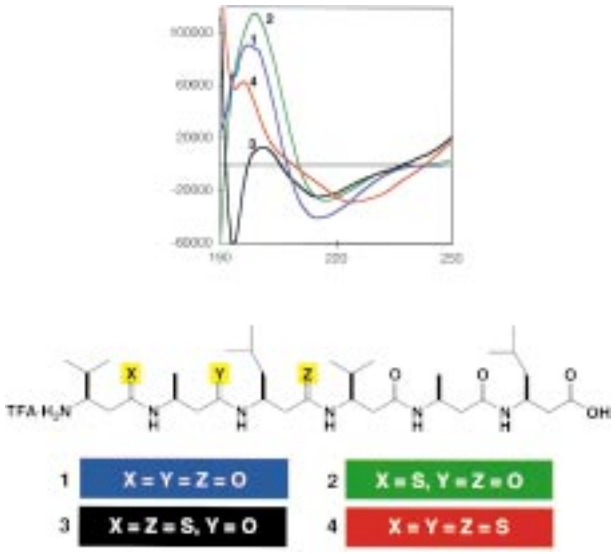

Fig. 4. *Short-wavelength part of the CD spectra of β-hexapeptides* **1**–**4**. All β-peptides were measured as 0.2 mM solutions in MeOH. Molar ellipticity $[\phi]$ in 10 deg · cm^2 · mol^{-1}.

with spectral positions corresponding to the maxima or shoulders found in the UV spectra (see *Scheme 1*). The thioamide $n\pi^*_{(C=S)}$ band is by 130–180 nm red-shifted relative to the amide $n\pi^*_{(C=O)}$ band, and the $\pi\pi^*_{(C=S)}$ band appears in the spectral range of 250–280 nm and is, therefore, well-separated from that of amides (*Scheme 1*)[29]. The UV spectra (in MeOH) and the CD spectra (in different solvents) of the β-thiopeptides **2**–**4** and of the non-thionated parent β-peptide **1** are shown in *Figs. 2–4*. The most striking feature in the CD spectra are non-continuous changes going from the mono- and di- to the trithio derivatives (green and black *vs.* red curves in *Fig. 3*).

Only for the non-protected β-thiohexapeptides **2**–**4** in MeOH, there is a simple, gradual change of the CD spectra (*Fig. 2,c*): troughs near 340 ($n\pi^*_{(C=S)}$) and 220 ($n\pi^*_{(C=O)}$), and peaks near 270 ($\pi\pi^*_{(C=S)}$) and 200 nm ($\pi\pi^*_{(C=O)}$), with increasing intensity of the longer-wavelength *Cotton* effects with increasing number of C=S groups[30]).

Inspection of the short-wavelength part of these spectra, as shown in *Fig. 4*, reveals a similarity in the CD pattern associated with the (*M*)-3_{14}-helix (trough at 216, peak at 198 nm) [2][3], but caution is advised, because a thioamide chromophore is known to have a so-called $n_o\pi^*$ band in this very same region of the spectrum [40][31]).

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[29]) CD Measurements in the 250–400 nm range can be performed in a greater variety of solvents than at shorter wavelengths.

[30]) It is known that, for thioamides, the wavelength of a given maximum changes as much for different compounds in a given solvent as for the same compound in different solvents [27].

[31]) For the fully protected thiohexapeptides **14**, **16**, and **18**, the short-wavelength parts of the CD spectra in MeOH show rather no pattern at all. Thus, like the parent β-hexapeptide Boc-(β-HVal-β-HAla-β-HLeu)$_2$-OMe, they may not form the 3_{14}-helix in MeOH. In contrast to our original report (Fig. 4,a, in [2]) and according to CD measurements by *J. V. Schreiber* (hitherto unpublished results, ETH-Zürich, 1998), the fully protected β^3-hexapeptide Boc-(β-HVal-β-HAla-β-HLeu)$_2$-OMe does not have a 3_{14}-helical secondary structure in MeOH.

In all other cases, the CD spectra of the β-trithiohexapeptide[32]) show a remarkable exciton splitting [69] of the $\pi\pi^*_{C=S}$ band at *ca.* 270 nm (see the red curves in *Fig. 3*,*a*,*b*,*d*, and *e*)[33]). Especially surprising are the changes occurring in the CD spectra going from the β-thiohexapeptides **2**, **3**, and **4** themselves (*Fig. 3*,*c*) to their fully protected derivatives **14**, **16**, and **18**, respectively, in MeOH (*cf. Fig. 3*,*c* with *b*), and, likewise, going from MeOH to H_2O with the unprotected β-thiohexapeptides (*cf. Fig. 3*,*c* with *d*). While the Boc-protected β-monothio- and β-dithiohexapeptide esters **14** and **16**, respectively, show a positive *Cotton* effect at 270 nm, albeit of lower intensity than the unprotected compounds, the trithio derivative **18** exhibits a *Davydov* splitting (21 nm) of the 270-nm band with a positive amplitude. In H_2O, the unprotected β-monothiohexapeptide **2** gives a negative, rather than a positive *Cotton* effect at *ca.* 270 nm; the dithio analogue **3** shows essentially no CD effect above 230 nm in H_2O; the trithio compound **4**, however, gives rise to an intensive splitting in H_2O. Yet another type of change occurs between MeOH and the non-H-bonding solvent $CHCl_3$ in the CD spectra of the fully protected derivatives **14**, **16**, and **18** (*cf. Fig. 3*,*b* with *a*): whereas the spectra of the trithio and dithio derivatives **18** and **16**, respectively, are almost unchanged in the range of 240–300 nm, the *Cotton* effect from the monothio compound **14** is reversed.

Before knowing the solution structures of the β-thiohexapeptides, it is not possible to interpret the surprising effects exhibited in the CD spectra. Thus, we should make a few general remarks as to what these effects *may* mean. *i*) The splitting of CD bands is caused by exciton interaction between dipoles of chromophores, which are arranged in a non-coplanar fashion in a chiral environment [68][69][34]). *ii*) The relative position in space of the C=S groups in β-trithiopeptide **4** must change when the solvent MeOH (no couplet of the $\pi\pi^*_{C=S}$ band) is replaced by H_2O (unsymmetrical couplet; *Fig. 3*,*c*,*d*, and *e*). *iii*) The protected and the unprotected β-hexapeptides, **18** and **4**, respectively, must have different secondary structures in MeOH (*Fig. 3*,*b* and *c*); such an effect of protecting groups on the structures has been previously observed and interpreted [3][4][31]). *iv*) The most unusual 'chameleon-like' behavior of the $\pi\pi^*_{C=S}$ band from the β-monothiohexapeptide (−8700 unprotected in H_2O, +12000 unprotected in MeOH, +12000 protected in MeOH, and −52000 unprotected in $CHCl_3$ in the range of 270–280 nm; see green curves in *Fig. 3*) indicates that the C=S group within the secondary structure of this compound has different orientations, depending on solvent and protection status[35]).

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[32]) For the tetrapeptide **11**, in $CHCl_3$, we also observed a splitting (positive amplitude of *ca.* 99000), and in all the CD spectra showing such splittings (of **4** and **18**; see *Fig. 3*,*a*,*b*,*d, and e*) the contribution of the $n\pi^*_{C=S}$ transition is negligible, an effect which had been previously observed with α-thiopeptides [68].

[33]) *Hollosi et al.* described a splitting of the $\pi\pi^*$ band for the α-dipeptide AcΨ[CSNH]-Pro-GlyΨ[CS]-$NHCH_3$ which adopts a type-II β-turn in $CHCl_3$ [68].

[34]) To give such splitting of the $\pi\pi^*_{C=S}$ band, it is not necessary that the thioamide groups are located near each other in two adjacent amino acids, as shown with AcΨ[CSNH]-Pro-GlyΨ[CS]-$NHCH_3$ [68].

[35]) Intramolecular H-bonds are better stabilized in $CHCl_3$ than in MeOH (*cf.* **14** in MeOH and $CHCl_3$). A strong shift of the equilibrium between different conformations is called a 'primary solvent effect' [40a]. In this case, the geometry of the solute molecules responsible for the chiroptical properties is strongly dependent on the nature of the solvent. Consequently, large differences, even sign changes of some of the bands, can be found in CD spectra when measured in different solvents [69].

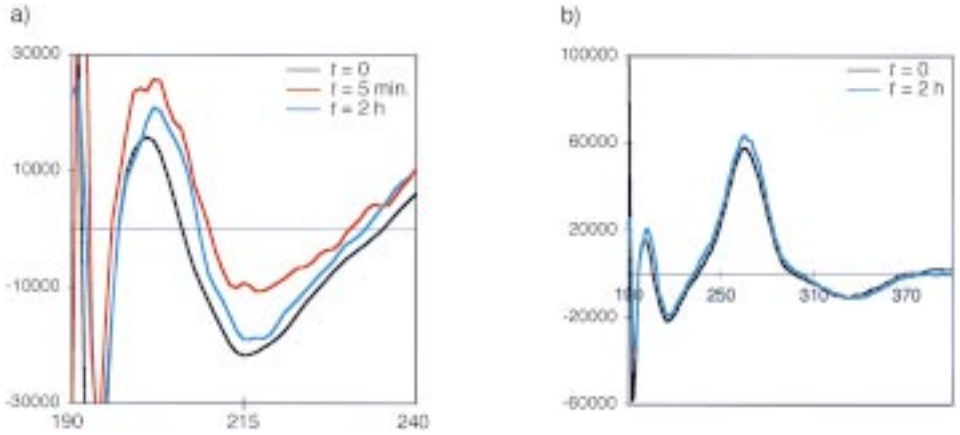

Fig. 5. *CD Spectra measured after UV irradiation of β-dithiohexapeptide* **3** (0.2 mM in MeOH). *a*) Short-wavelength range of the CD spectrum before irradiation ($t = 0$; black curve, *cf. Fig. 3,c*). The solution was irradiated in a quarz cuvette for 30 s (266-nm laser light). Samples were withdrawn from this solution (kept in the dark) after 5 min and 2 h, and the short-wavelength CD spectra were measured again (red and blue curves in *a*). *b*) Entire CD spectrum of **3** before and 2 h after end of irradiation; obviously, no photodecomposition of the thiopeptide has occurred. The wavy CD traces observed after irradiation could be due to small particles (of an (*E*)-isomer?) separating from and floating in the solution (invisible to the eye). At the end of the dark period, they must have redissolved, because the original spectrum is restored (*cf.* [52][53]). Molar ellipticity [ϕ] in 10 $deg \cdot cm^2 \cdot mol^{-1}$.

One of the NMR-solution structures of a β-thiopeptide is described in the following section, the other structures are currently under investigation.

To determine whether the photochemical (*Z*)/(*E*) switching, as observed for simple thioacetamides [52] and for an α-monothiotetrapeptide [53], is also applicable to β-thiopeptides, we have conducted some experiments with the β-dithiopeptide **3**. A solution of **3** in MeOH (typical CD concentration of 0.2 mM) was irradiated with 266-nm laser light for up to 30 s. As can be seen in *Fig. 5*, the CD spectrum of **3** (190–240 nm range) undergoes a drastic change of intensities upon irradiation, but reverses to the original pattern in the dark. There is no sign of photodecomposition under the conditions chosen. This result demonstrates that β-peptides, just like α-peptides [53], into which C=S groups have been incorporated, can be reversibly forced into unusual conformations by irradiation.

4. NMR Solution Structure of the β-Trithiohexapeptide 4. – In a previous work, we have established that the structure of hexapeptide **1** in (D_5)pyridine and CD_3OH is a 3_{14} helix [2][3]. A number of other β-peptides have been shown to have this same secondary structure in MeOH solution [4][11]. Hence, it was of interest to determine, first of all, the secondary structure of the β-trithiopeptide **4** in this solvent[36]) to learn whether our original plan (see *Introduction* and *Fig. 1*) had been realized.

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[36]) The NMR structures of other β-thiopeptides, described here, and of **4** in other solvents are currently under investigation in our laboratory.

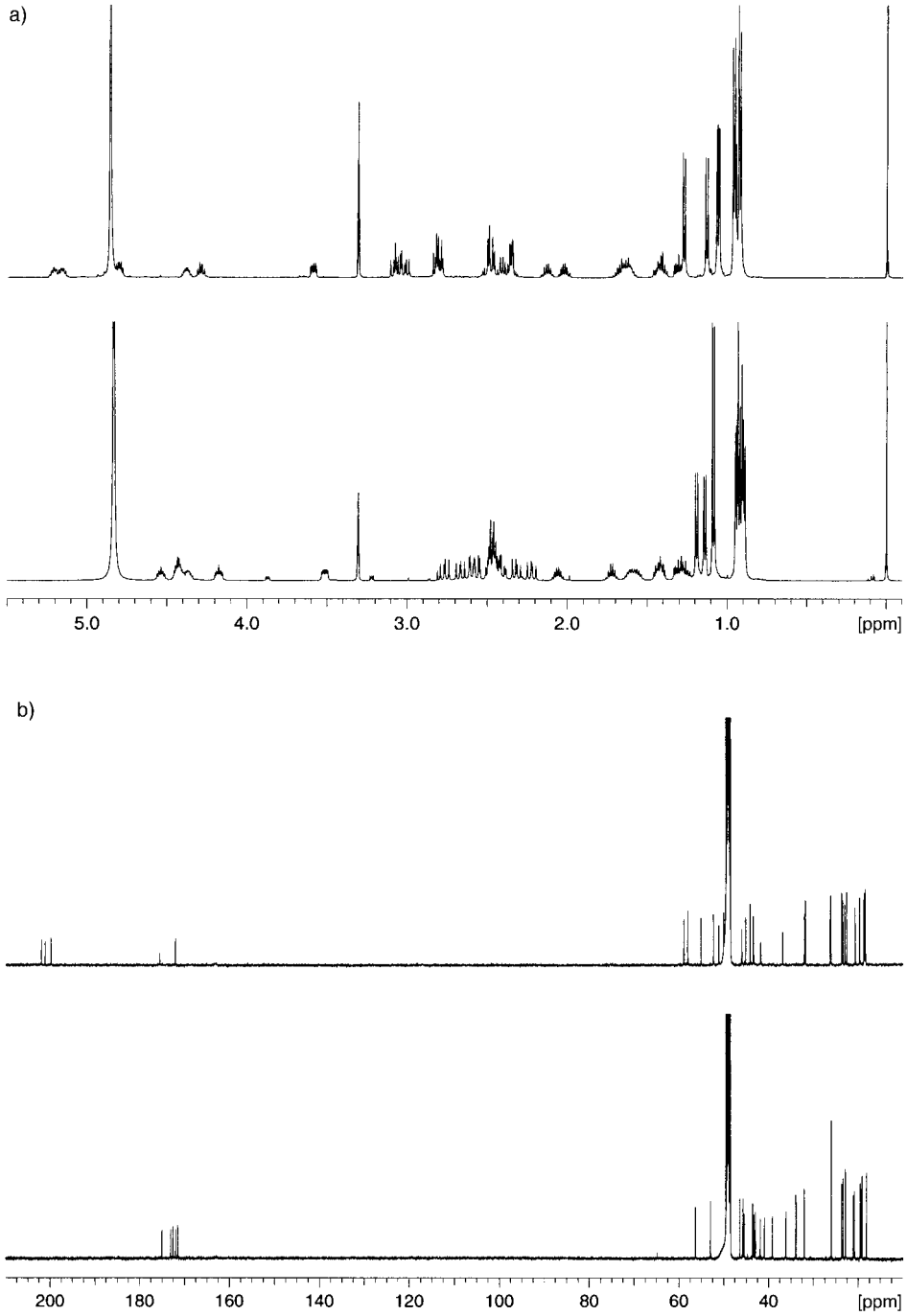

Fig. 6. *^{1}H- and ^{13}C-NMR Spectra of the parent β-hexapeptide* **1** *and of the β-trithiohexapeptide* **4** *in CD_3OD* (500 MHz for ^{1}H, 125 MHz for ^{13}C). *a*) Upper trace ^{1}H-NMR spectrum of **4**, lower trace of **1**; in the sulfur derivative the C*H*(R)N and (to a lower degree) the CH_2CO proton signals are spred over a much wider shift range than the corresponding signals of the sulfur-free compound. *b*) Upper trace ^{13}C-NMR spectrum of **4**, lower trace of **1**; while the high-field signals show a similar pattern in the two spectra, the ^{13}CS and the ^{13}CO signals are clearly separated, with the C=S C-atoms resonating at *ca.* 200 ppm, 25–30 ppm lower than the normal amide C=O C-atoms.

Table 2. *^{1}H-NMR Chemical Shifts of the β-Trithiopeptide* **4** in MeOH

β-Amino acid	NH	2 H–C(α) H_{ax}/H_{la}[a])	H–C(*B*)	H–C(γ), 2 H–C(γ) or Me–C(γ)	Me–C(δ) or H–C(δ)	Me–C(ε)
Val[1]		2.80/3.04	3.57	2.01	1.04	
Ala[2]		3.07/2.79	5.15	1.27		
Leu[3]	9.80	3.01/2.80	5.20	1.25/1.40	1.64	0.95/0.90
Val[4]	9.66	2.48/2.48	4.78	2.11	0.94/0.92	
Ala[5]	7.81	2.34/2.39	4.27	1.12		
Leu[6]	7.78	2.40/2.46	4.37	1.42/1.32	1.61	0.91/0.90

[a]) The C–H bonds of H_{ax} and H_{la}, the diastereotopic back-bone CH_2 H-atoms are parallel and approximately perpendicular, respectively, to the helix axis.

At first sight, the ^{1}H- and ^{13}C-NMR spectra of the parent β-hexapeptide **1** and of the trithio derivative **4** are quite different (*Fig. 6*). However, detailed 2D-NMR analysis of **4** has revealed the presence of a left-handed 3_{14}-helical conformation in CD_3OH solution.

The sequence-specific assignment of all resonances in the ^{1}H-NMR spectrum was achieved by DQF-COSY, TOCSY, HSQC, and HMBC experiments, as previously reported for other β-peptides. The chemical shifts are collected in *Table 2*.

The signals of the N-terminal NH_3^+ protons and the NH of β-HAla[2] are broadened beyond determination. All other NH protons show coupling constants J(NH,H–C(β)) in the range of 9–10 Hz, which correspond to an *anti*-periplanar arrangement of NH and H–C(β). For all residues, the H_{ax}–C(α) exhibits a large and the H_{la}–C(α) a small coupling with H–C(β), as evident from the ^{1}H-NMR and DQF-COSY spectra. Together with the observation that the H–C(β) consistently shows a stronger NOE to the H–C(α) with the smaller vicinal coupling (see below), this indicates that H_{ax}–C(α) is *anti*-periplanar and H_{la}–C(α) *syn*-clinal in relation to H–C(β). To collect additional information regarding the three-dimensional structure of the trithio peptide, ROESY spectra at two different mixing times were acquired. NOEs obtained from the spectrum measured with a mixing time of 150 ms were classified according to their cross peak volume in three categories: strong, medium, and weak (*Table 3*). A total of 58 NOEs were extracted, of which 38 are intraresidual, 10 sequential, 2 from residue *i* to residue $(i+2)$, and 8 from residue *i* to residue $(i+3)$.

Qualitative inspection reveals that the NOE pattern typical for 3_{14}-helical β-peptides is displayed in the ROESY spectra of **4**. Thus, the NH protons of residues 3 (fully), 4 (partially), and 5 (partially) show the set of NOEs to H_{ax}–C(α_{i-1}, H_{ax}–C($\alpha)_i$, H–C($\beta)_{i+2}$, and H–C($\beta)_{i+3}$. Unfortunately, the rapid exchange of NH–Ala_2 prevents observation of a corresponding pattern at the N-terminus.

Next to this characteristic pattern, however, a second set of NOEs from NH_i to H–C($\beta)_{i-1}$ ($i=3, 4, 5$), which is clearly not compatible with a 3_{14} helix, is observed. A short distance between NH_i and H–C($\beta)_{i-1}$ is possible only if the dihedral backbone angle around the 2 H–C(α)/C=O bond is in the *syn*-clinal range as opposed to the *anti*-clinal conformation in the 3_{14} helix. This type of NOEs, although at lower relative intensity, have been observed in β-peptides before [2][3][6c]. In particular, hexapeptide **1**, the oxygen analogue of trithiopeptide **4**, exhibited such NOEs for residues 2, 4, and 5 in CD_3OH and (D_5)pyridine. Interestingly, for the β-heptapeptide H-β-HVal-β-

Table 3. *Weak* (4.5 Å), *Medium* (3.5 Å), *and Strong* (3.0 Å) *NOEs Observed in the ROESY NMR Spectrum of Compound* **4** *in MeOH*

Residue	H-Atom(s)	Residue	H-Atom(s)	NOE	Residue	H-Atom(s)	Residue	H-Atom(s)	NOE
1	β	1	α^{la}	weak	4	β	4	α^{la}	medium
1	β	1	α^{ax}	medium	4	β	4	γ	strong
1	β	1	γ	strong	4	β	4	α^{la}	weak
2	β	3	NH	medium	4	β	1	γ	weak
2	β	5	γ	strong	4	β	1	δ	weak
2	β	2	α^{la}	medium	4	α^{la}	1	δ	weak
2	β	2	γ	strong	5	NH	4	β	weak
3	NH	5	β	weak	5	NH	5	β	medium
3	NH	6	β	weak	5	NH	5	α^{ax}	strong
3	NH	2	β	medium	5	NH	4	α^{ax}	medium
3	NH	2	α^{ax}	strong	5	NH	5	γ	medium
3	NH	3	α^{la}	medium	5	β	5	α^{la}	strong
3	NH	3	α^{ax}	medium	5	β	2	α^{ax}	strong
3	NH	3	δ	strong	5	β	3	γ	weak
3	NH	3	γ	weak	5	α^{ax}	2	α^{ax}	strong
3	NH	3	γ	weak	6	NH	6	β	medium
3	NH	3	ε	weak	6	NH	5	α^{ax}	strong
3	β	3	α^{la}	strong	6	NH	6	δ	weak
3	β	3	α^{ax}	medium	6	NH	6	γ	medium
3	β	3	γ	medium	6	NH	6	γ	weak
3	β	3	γ	medium	6	NH	6	ε	weak
3	β	3	ε	strong	6	β	6	α^{ax}	weak
4	NH	3	β	weak	6	β	6	α^{la}	medium
4	NH	4	β	weak	6	β	6	δ	weak
4	NH	3	α^{ax}	strong	6	β	6	γ	medium
4	NH	3	α^{la}	medium	6	β	6	γ	weak
4	NH	4	α^{ax}	medium	6	β	6	ε	strong
4	NH	4	α^{la}	weak	6	β	3	α^{ax}	weak
4	NH	4	γ	weak					

HAla-β-HLeu-β-HAla(αMe)-β-HVal-β-HAla-β-HLeu-OH, presumably forming the most stable 3_{14} helix of those β^3-oligopeptides studied in our laboratory, as yet, this type of NOE was not observed. Since model considerations and MD calculations (see below) indicate that no single conformer of **4** is consistent with all observed NOEs, other conformations besides the regular 3_{14} helix must be populated at 300 K in CD_3OH.

The NOE data were used as distance restraints in the slow-cooling simulated annealing protocol of X-PLOR [70]. In addition, 9 angle restraints were derived from the large coupling constants J(NH,H–C(β)) and J(H_{ax}–C(α),H–C(β)) mentioned above. From calculations with the program X-PLOR six conformations were selected, which fulfill the restrains without the NOEs data NH_i to H–C(β) (see above). A bundle of six structures with a RMSD of less than 0.3 Å is shown in *Fig. 7*.

5. Conclusion and Outlook. – Our investigation has shown that β-thiopeptides can be readily prepared by methods previously employed in the field of α-peptides. No

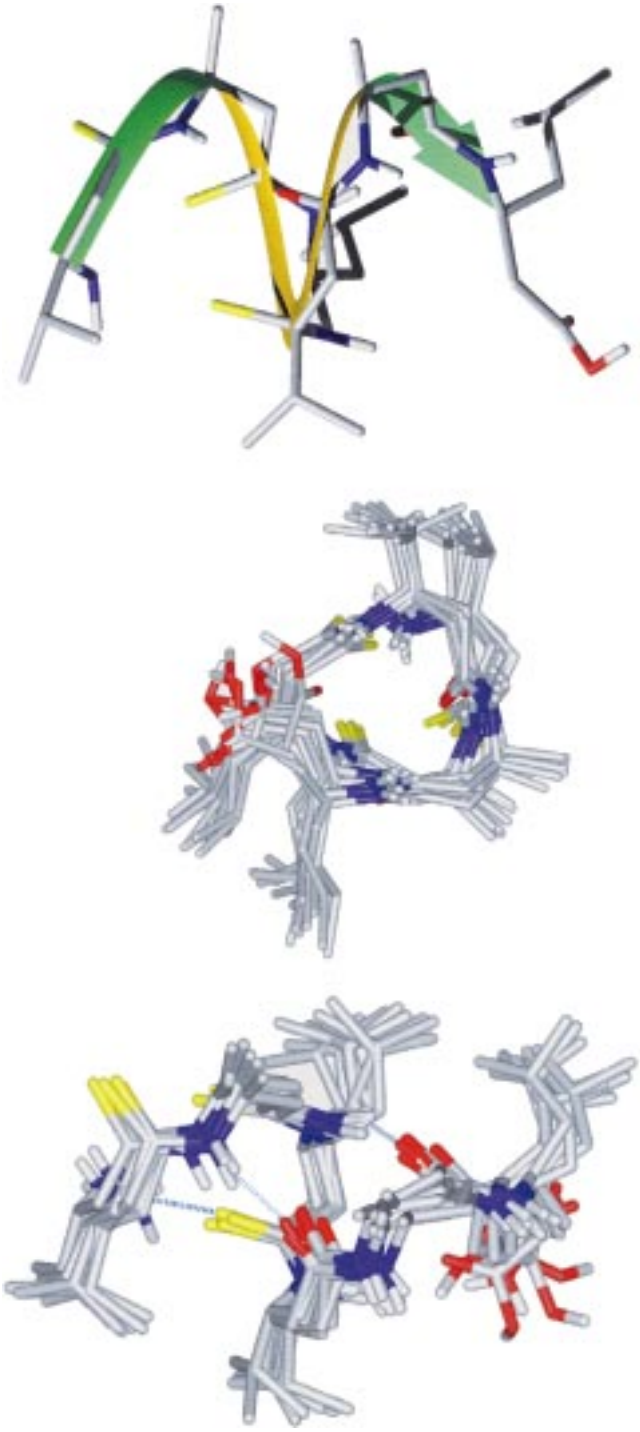

Fig. 7. *(M)-3$_{14}$-Helical NMR structure of the β-trithiopeptide* **4** *in CD_3OH*. Bundle of six structures with root-mean-square deviation (RMSD) ≤ 0.3 Å in side view (*left*) and top view (*middle*). The H-bonds between C=S and HNCO, and between C=S and the N-terminal $NH_3^{\oplus}$ group are indicated by blue dashed lines. CH H-Atoms have been omitted for clarity. C-Backbone in grey, S in yellow, O in red, N in blue. The best-fit structure of **4** with ribbon is shown on the right side. The figures were generated by MolMol [71].

special precautions had to be taken due to the increased acidity of the α-CH_2 groups[37]), and no instabilities of the thioamide moieties, other than those known from α-peptide work, were encountered. While the incorporation of N-terminal C=S groups leads to the presence of a 3_{14}-helical structure (see NMR structure in CD_3OH), there must be other secondary structures to discover with the synthesized β-thiopeptides (see CD spectra; *Fig. 3*) in other solvents, with protected *termini*, and after irradiation (*Fig. 5*). We will, hopefully, be able to actually *read* the structural information signalled by the exciton splitting in the CD spectra of the β-trithiopeptide **4** when we compare the NMR structures of the β-monothio-, β-dithio-, and β-trithiopeptides determined under various conditions, a task which is being undertaken in our laboratory. Finally, the switching between conformations of peptides by incorporating a single C=S group and carrying out photochemical (Z)/(E) isomerization, with slow equilibration is an intriguing process [52][53], and has now been shown to be possible also with β-peptides.

The simple replacement of C=O groups by C=S groups in β-peptides, as any good research project, has opened new possibilities, and the results pose more questions than they provide answers.

We thank Prof. Dr. *P. Chen* and Dr. *K. Redmond* for helping us with the irradiation experiment. The services of the NMR, MS, and elemental analysis divisions of our laboratory are gratefully acknowledged. We thank the *Swiss National Science Foundation*[1]) and *Novartis Pharma AG*, Basel, for generous financial support.

Experimental Part

1. *General*. Abbreviations: EDC: 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, FC: flash chromatography, HOBt: 1-hydroxy-1*H*-benzotriazole, β-HXxx: β-homoamino acid [2][3], LR: *Lawesson* reagent, TFA: CF_3CO_2H. THF was freshly distilled over K/benzophenone under Ar before use. Et_3N was distilled from CaH_2 and stored over KOH. $(i\text{-}Pr)_2NH$ was freshly distilled over CaH_2. $CHCl_3$ employed for the coupling reactions was filtered over Al_2O_3 (Alumina *Woelm* N, activity I) to remove EtOH. Solvents for chromatography and workup procedures were distilled from *Sikkon* (anh. $CaSO_4$; *Fluka*). *Lawesson* reagent was purchased from *Fluka* (*purum* quality). Amino-acid derivatives were purchased from *Bachem*, *Senn*, or *Degussa*. All other reagents were used as received from *Fluka*. The diazo ketones Boc-(R)-Val-CHN_2, Boc-(S)-Ala-CHN_2, and Boc-(S)-Leu-CHN_2, as well as the β-amino-acid derivatives Boc-(R)-β^3-HVal-OMe, Boc-(R)-β^3-HVal-OH, Boc-(S)-β^3-HAla-OMe, Boc-(S)-β^3-HAla-OH, Boc-(S)-β^3-HLeu-OMe, Boc-(S)-β^3-HLeu-OH, and Boc-(S)-β^3-HAla-(S)-β^3-HLeu-OMe were prepared according to literature procedures [2][3][61]. ***Caution:*** The generation and handling of CH_2N_2 require special precautions [72]. Flasks and stirring bars for the thionations were dried for *ca.* 12 h at 120° and allowed to cool in a desiccator over silica gel. Reactions carried out with the exclusion of light were performed in flasks completely wrapped in aluminium foil. All indicated temp. were monitored with an internal thermometer (*Ebro-TTX-690* digital thermometer). TLC: *Merck* silica gel *60* F_{254} plates; detection with UV and anisaldehyde (9.2 ml of anisaldehyde, 3.75 ml of AcOH, 12.5 ml of conc. H_2SO_4, 350 ml of EtOH). FC: *Fluka* silica gel *60* (40–63 μm). Anal. HPLC: *Knauer* HPLC system *K 1000*, pump type *64*, *EuroChrom 2000* integration package, degaser, 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 400 nm at 20°; 1-mm rectangular cell; average of five scans, corrected for the baseline; peptide concentration 0.2 mM in MeOH, H_2O, or $CHCl_3$; molar ellipticity [ϕ] in deg · cm^2 · mol^{-1} (λ in nm); smoothing by

[37]) Possible eliminations did not hamper reactions, which had to be carried out under basic conditions (see *Sect. 2*; saponification of the methyl ester **14**) during our syntheses.

Jasco software. IR Spectra: *Perkin-Elmer-782* spectrophotometer. UV Spectra: *Kontron Uvikon 860* spectrophotometer, recording from 200 to 400 nm at r.t.; 1-cm rectangular cell; concentration 0.1 mM in MeOH (see *Fig. 2*). NMR Spectra: *Bruker AMX-II 500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *AMX 400* (^{1}H: 400 MHz, ^{13}C: 100 MHz); chemical shifts (δ) in ppm downfield from internal TMS ($\delta = 0.0$ ppm); *J* values in Hz. MS: *Hitachi Perkin-Elmer RHU-6M* (FAB, in a 3-nitrobenzyl alcohol matrix) and *Finnigan-MAT-TSQ 7000* (ESI, sprayed from a 10^{-5}M MeOH soln.; 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 1a* (*GP 1a*). According to the reported procedure [2][3], the *N*-Boc-protected amino-acid or oligopeptide was dissolved in CH_2Cl_2 (0.8M) and cooled to 0°. An equal volume of TFA was added, and the mixture was allowed to warm to r.t. and then stirred for further 1 h. Concentration under reduced pressure and drying of the residue under h.v. (2 h) yielded the crude TFA salt, which was identified by NMR and used without further purification.

General Procedure 1b (*GP 1b*). The S-containing oligopeptide was dissolved in CH_2Cl_2 (0.8M) and cooled to 0°. A half volume of TFA was added, and the mixture was then stirred for 1 h at 0°. Concentration under reduced pressure and drying of the residue under h.v. (2 h) yielded the crude TFA 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 (0.05–0.4M according to solubility) and treated with 1N NaOH (1.2–6 equiv.) at r.t. After hydrolysis, 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 dried ($MgSO_4$), concentrated under reduced pressure, and dried under h.v.

General Procedure 2b (*GP 2b*). The fully protected oligopeptide was dissolved in CF_3CH_2OH (0.05–0.4M according to solubility), treated with aq. NaOH (5–100 equiv.), and heated at 40°. 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. aq. 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 TFA salt was dissolved in $CHCl_3$ (0.5M) and cooled to 0°. This soln. was treated successively with Et_3N (4 equiv.), HOBt (1.2 equiv.), a soln. of the *N*-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 15 h with the exclusion of light. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N HCl (3×), sat. aq. $NaHCO_3$ soln. (2×), and sat. aq. NaCl soln. (1×). 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 TFA salt was dissolved in $CHCl_3$ (0.15M) and cooled to 0°. This soln. was treated successively with Et_3N (4 equiv.), HOBt (1.2 equiv.), a soln. of the *N*-Boc-protected fragment (1 equiv.) in DMF (0.15M), and EDC (1.2 equiv.). The mixture was allowed to warm to r.t. and then stirred for 15 h with the exclusion of light. The resulting mixture was dispersed in $CHCl_3$ (0.01M), and the suspension was washed with 1M HCl (3×), sat. aq. $NaHCO_3$ soln. (2×), and sat. aq. NaCl soln. (1×). The org. phase was concentrated under reduced pressure, and recrystallization yielded the pure peptide.

5. *Thionation of Oligopeptides. General Procedure 4* (*GP 4*): According to the reported procedure [58d], the fully protected oligopeptide was dissolved in dry THF (10 ml per mmol), and, after addition of the LR (0.5 equiv.), the resulting heterogeneous mixture was stirred at r.t. (thionation of dipeptides) or at 50° (thionation of tetrapeptides). After completion of the reaction (TLC), the mixture was concentrated under reduced pressure. FC yielded the pure thiopeptide.

6. *Reversed-Phase (RP) HPLC Analysis and Purification of β-Peptides. General Procedure 5* (*GP 5*). RP-HPLC Analysis was performed with a linear gradient of *A* (0.1% TFA 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 using a gradient of *A* and *B* at a flow rate of 20 ml/min with UV detection at 220 nm. The pure peptide was then lyophilized.

*Boc-(*R*)-β^3-HVal-(*S*)-β^3-HAla-OMe* (**5**). Boc-(*S*)-β^3-HAla-OMe (2.32 g, 10.67 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (21 ml), and treated with Et_3N (5.2 ml, 37.30 mmol), HOBt (1.73 g, 12.81 mmol), Boc-(*R*)-β^3-HVal-OH (2.47 g, 10.67 mmol) in $CHCl_3$ (21 ml), and EDC (2.45 g, 12.81 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 18:1) yielded **5** (2.99 g, 9.05 mmol, 85%). Colorless solid. M.p. 145–146° ((i-Pr)$_2$O). R_f (CH_2Cl_2/MeOH 18:1) 0.38. $[\alpha]_D = -31.2$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3350*s*, 2964*m*, 1745*s*, 1690*s*, 1642*s*, 1529*s*, 1431*w*, 1368*m*, 1333*w*, 1307*m*, 1273*m*, 1251*m*, 1179*m*, 1150*m*, 1114*w*, 1052*w*, 1024*w*, 970*w*, 860*w*, 655*w*, 607*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.91 (*d*, $J = 6.8$, 2 Me); 1.21 (*d*, $J = 6.8$, Me); 1.43 (*s*, *t*-Bu);

1.74–1.87 (*m*, CH); 2.28–2.59 (*m*, 2 CH_2); 3.60–3.71 (*m*, CHN); 3.69 (*s*, MeO); 4.29–4.41 (*m*, CHN); 5.11 (*d*, *J* = 9.3, NH); 6.43 (*d*, *J* = 7.4, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.4, 19.4, 19.9, 28.4 (Me); 32.2 (CH); 39.5, 40.1 (CH_2); 42.1 (CH); 51.7 (MeO); 53.3 (CH); 79.3, 156.1, 170.4, 172.0 (C). FAB-MS: 683 (16, $[2M + Na]^+$), 661 (31, $[2M + 1]^+$), 353 (20, $[M + Na]^+$), 332 (22, $[M + 2]^+$), 331 (100, $[M + 1]^+$), 330 (6, M^+), 275 (17), 232 (14, $[M - Boc + 2]^+$), 231 (89, $[M - Boc + 1]^+$). Anal. calc. for $C_{16}H_{30}N_2O_5$ (330.42): C 58.16, H 9.15, N 8.48; found: C 58.34, H 9.17, N 8.53.

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-OMe* (**6**). Compound **5** (2.99 g, 9.05 mmol) was dissolved in THF (90 ml) and was treated with LR (1.83 g, 4.52 mmol) according to *GP 4* (reaction time: 2.5 h). 2 × FC (CH_2Cl_2/MeOH 72:1) yielded **6** (2.99 g, 8.63 mmol, 95%). Colorless solid. M.p. 128–130°. R_f (CH_2Cl_2/MeOH 72:1) 0.22. $[\alpha]_D = -20.7$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3390*m*, 3317*m*, 2975*m*, 1747*s*, 1679*s*, 1514*s*, 1456*m*, 1406*m*, 1366*m*, 1304*m*, 1245*m*, 1169*s*, 1105*m*, 1078*m*, 1048*w*, 1010*m*, 891*w*, 846*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90–0.97 (*m*, 2 Me); 1.32 (*d*, *J* = 6.8, Me); 1.43 (*s*, *t*-Bu); 1.78–1.91 (*m*, CH); 2.63–3.02 (*m*, 2 CH_2); 3.71 (*s*, MeO); 3.68–3.77 (*m*, CHN); 4.71–4.81 (br., NH); 4.89–5.00 (*m*, CHN); 8.53–8.62 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.8, 18.3, 19.4, 28.4 (Me); 32.1 (CH); 38.4 (CH_2); 48.1 (CH); 50.6 (CH_2); 51.8 (MeO); 54.7 (CH); 79.8, 156.3, 171.8, 200.6 (C). FAB-MS: 693 (8, $[2M + 1]^+$), 692 (10, $2M^+$), 349 (11, $[M + 3]^+$), 348 (30, $[M + 2]^+$), 347 (100, $[M + 1]^+$), 346 (62, M^+), 345 (13, $[M - 1]^+$), 292 (13), 291 (72), 247 (17, $[M - Boc + 1]^+$), 245 (13, $[M - Boc - 1]^+$). Anal. calc. for $C_{16}H_{30}N_2O_4S$ (346.49): C 55.46, H 8.73, N 8.08, S 9.25; found: C 55.52, H 8.61, N 8.04, S 9.14.

*Boc-(*S*)-β³-HLeu-(*R*)-β³-HVal-OMe* (**7**). Boc-(*R*)-β³-HVal-OMe (2.70 g, 11.03 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (20 ml) and treated with Et_3N (5.4 ml, 38.74 mmol), HOBt (1.79 g, 13.24 mmol), Boc-(*S*)-β³-HLeu-OH (2.70 g, 11.03 mmol) in $CHCl_3$ (20 ml), and EDC (2.53 g, 13.24 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 36:1) yielded **7** (3.48 g, 9.36 mmol, 85%). Colorless solid. M.p. 129–130°. R_f (CH_2Cl_2/MeOH 36:1) 0.16. $[\alpha]_D = -36.2$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3316*m*, 2955*m*, 1738*s*, 1686*s*, 1654*s*, 1538*m*, 1439*w*, 1387*w*, 1364*m*, 1302*w*, 1272*m*, 1244*w*, 1175*m*, 1124*w*, 1044*w*, 997*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89–0.94 (*m*, 4 Me); 1.25–1.34 (*m*, CH); 1.37–1.53 (*m*, CH); 1.43 (*s*, *t*-Bu); 1.57–1.69 (*m*, CH); 1.76–1.89 (*m*, CH); 2.29–2.55 (*m*, 2 CH_2); 3.68 (*s*, MeO); 3.85–3.96 (*m*, CHN); 4.02–4.12 (*m*, CHN); 5.13–5.21 (br., NH); 6.18–6.26 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.9, 19.3, 22.2, 22.9 (Me); 25.0 (CH); 28.4 (Me); 31.3 (CH); 36.5, 41.5, 43.8 (CH_2); 46.4, 51.5 (CH); 51.8 (MeO); 79.1, 155.6, 170.5, 172.4 (C). FAB-MS: 395 (13, $[M + Na]^+$), 374 (17, $[M + 2]^+$), 373 (70, $[M + 1]^+$), 372 (8, M^+), 371 (6, $[M - 1]^+$), 317 (20), 274 (19, $[M - Boc + 2]^+$), 273 (100, $[M - Boc + 1]^+$). Anal. calc. for $C_{19}H_{36}N_2O_5$ (372.50): C 61.26, H 9.74, N 7.52; found: C 61.38, H 9.64, N 7.54.

*Boc-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-OMe* (**8**). Compound **7** (2.28 g, 6.14 mmol) was dissolved in THF (60 ml) and was treated with LR (1.24 g, 3.07 mmol) according to *GP 4* (reaction time: 4 h). 2 × FC (CH_2Cl_2/MeOH 72:1) yielded **8** (2.23 g, 5.75 mmol, 93%). Colorless solid. M.p. 76–77°. R_f (CH_2Cl_2/MeOH 72:1) 0.28. $[\alpha]_D = -47.3$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3367*m*, 2954*m*, 2871*w*, 1725*s*, 1689*s*, 1523*s*, 1444*m*, 1413*m*, 1397*m*, 1367*m*, 1328*w*, 1306*w*, 1274*m*, 1248*m*, 1230*m*, 1208*m*, 1173*s*, 1105*m*, 1015*w*, 755*w*, 620*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.90 (*d*, *J* = 6.5, Me); 0.91 (*d*, *J* = 6.6, Me); 0.96 (*d*, *J* = 6.8, Me); 0.97 (*d*, *J* = 6.8, Me); 1.43 (*s*, *t*-Bu); 1.34–1.48 (*m*, CH_2); 1.62–1.74 (*m*, CH); 1.99–2.12 (*m*, CH); 2.65 (*dd*, *J* = 5.2, 16.1, CH); 2.69 (*dd*, *J* = 5.0, 16.1, CH); 2.86–2.96 (*m*, CH_2); 3.69 (*s*, MeO); 3.85–4.00 (*m*, CHN); 4.67–4.76 (*m*, CHN); 4.76–4.88 (br., NH); 8.45–8.58 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.2, 19.3, 22.0, 23.0 (Me); 24.8 (CH); 28.4 (Me); 30.6 (CH); 34.6, 44.0 (CH_2); 48.7 (CH); 51.9 (MeO); 53.3 (CH_2); 57.6 (CH); 79.7, 155.8, 172.2, 201.1 (C). FAB-MS: 391 (15, $[M + 2]^+$), 390 (68, $[M + 1]^+$), 389 (50, M^+), 388 (16, $[M - 1]^+$), 333 (20), 299 (19), 289 (37, $[M - Boc + 1]^+$), 287 (22, $[M - Boc - 1]^+$). Anal. calc. for $C_{19}H_{36}N_2O_4S$ (388.57): C 58.73, H 9.34, N 7.21, S 8.25; found: C 58.81, H 9.51, N 7.12, S 8.11.

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-OH* (**9**). Compound **6** (1.79 g, 5.16 mmol) was dissolved in MeOH (16 ml) and treated with 1N NaOH (6.2 ml, 6.2 mmol, 1.2 equiv.) according to *GP 2a* (reaction time: 5 h), yielding **9** (1.69 g, 5.10 mmol, 98%). Colorless solid. M.p. 149–152°. R_f (CH_2Cl_2/MeOH 18:1) 0.08. $[\alpha]_D = +49.5$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3386*w*, 3313*m*, 2971*m*, 1712*s*, 1682*s*, 1516*s*, 1446*m*, 1402*s*, 1366*m*, 1309*m*, 1246*m*, 1167*s*, 1079*m*, 1050*w*, 1012*w*, 742*w*, 572*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88 (*d*, *J* = 6.8, Me); 0.94 (*d*, *J* = 6.8, Me); 1.33 (*d*, *J* = 6.7, Me); 1.44 (*s*, *t*-Bu); 1.73–1.83 (*m*, CH); 2.39 (*t*, *J* = 12.0, CH); 2.58 (*dd*, *J* = 3.2, 17.7, CH); 2.65 (*dd*, *J* = 4.0, 17.7, CH); 2.93 (*dd*, *J* = 3.0, 12.1, CH); 4.17–4.27 (*m*, CHN); 4.79–4.90 (*m*, CHN); 6.75 (*d*, *J* = 10.2, NH); 8.67–8.78 (br., *J* = 8.1, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.0, 17.2, 19.4, 28.0 (Me); 32.8 (CH); 37.2 (CH_2); 47.7 (CH); 51.9 (CH_2); 57.6 (CH); 81.8, 159.4, 177.6, 200.2 (C). FAB-MS: 703 (17, $[2M + K - 1]^+$), 687 (13, $[2M + Na]^+$), 665 (16, $[2M + 1]^+$), 664 (26, $2M^+$), 439 (13), 355 (35, $[M + Na]^+$), 334 (25, $[M + 2]^+$), 333 (100, $[M + 1]^+$), 332 (61, M^+), 331 (15, $[M - 1]^+$), 278 (13), 277 (77), 233 (13, $[M - Boc + 1]^+$), 231 (9, $[M - Boc - 1]^+$).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-OMe* (**10**). Compound **8** (1.31 g, 3.38 mmol) was deprotected according to *GP 1b*, dissolved in $CHCl_3$ (7.5 ml), and treated with Et_3N (1.5 ml, 10.76 mmol), HOBt (549 mg, 4.06 mmol), **9** (1.12 g, 3.38 mmol) in $CHCl_3$ (7.5 ml), and EDC (779 mg, 4.06 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 36:1) yielded **10** (1.63 g, 2.71 mmol, 80%). Colorless solid. M.p. 73–76°. R_f (CH_2Cl_2/MeOH 36:1) 0.12. $[\alpha]_D = -30.1$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3291*m*, 2962*s*, 2872*m*, 1718*s*, 1686*s*, 1647*s*, 1527*s*, 1438*s*, 1412*s*, 1366*s*, 1308*m*, 1248*m*, 1171*s*, 1100*m*, 735*w*, 668*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.87–1.00 (*m*, 6 Me); 1.30 (*d*, $J = 6.7$, Me); 1.43 (*s*, *t*-Bu); 1.37–1.54 (*m*, 2 CH); 1.57–1.68 (*m*, CH); 1.79–1.90 (*m*, CH); 2.01–2.14 (*m*, CH); 2.43–2.55 (*m*, CH_2); 2.61–2.68 (*dd*, $J = 5.0$, 16.0, CH); 2.71 (*dd*, $J = 4.6$, 16.0, CH); 2.81–3.00 (*m*, 2 CH_2); 3.68 (*s*, MeO); 3.70–3.82 (*m*, CHN); 4.06–4.24 (*m*, CHN); 4.60–4.74 (*m*, CHN); 4.75–4.92 (*m*, CHN); 4.84 (*d*, $J = 9.5$, NH); 6.79–6.88 (br., NH); 8.60–8.69 (br., NH); 8.91–8.98 (br., $J = 8.0$, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.8, 18.5, 19.0, 19.3, 19.4, 22.0, 22.9 (Me); 25.0 (CH); 28.4 (Me); 30.6, 32.3 (CH); 34.5, 41.3, 43.0 (CH_2); 47.8, 49.3 (CH); 50.3 (CH_2); 51.9 (MeO); 52.6 (CH_2); 54.7, 57.6 (CH); 79.7, 156.3, 170.8, 172.3, 200.3, 200.8 (C). FAB-MS: 619 (19), 605 (17, $[M+3]^+$), 604 (40, $[M+2]^+$), 603 (100, $[M+1]^+$), 602 (70, M^+), 601 (33, $[M-1]^+$), 571 (13), 569 (23), 503 (49, $[M-\text{Boc}+1]^+$), 432 (24), 272 (56), 259 (30). Anal. calc. for $C_{29}H_{54}N_4O_5S_2$ (602.90): C 57.77, H 9.03, N 9.29, S 10.64; found: C 57.85, H 8.89, N 9.08, S 10.39.

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAlaΨ[CSNH]-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-OMe* (**11**). Compound **10** (823 mg, 1.36 mmol) was dissolved in THF (14 ml) and was treated with LR (276 mg, 0.68 mmol) according to *GP 4* (reaction time: 5 h). 2 × FC (CH_2Cl_2/MeOH 36:1) yielded **11** (799 mg, 1.29 mmol, 94%). Colorless solid. M.p. 61–63°. R_f (CH_2Cl_2/MeOH 36:1) 0.16. $[\alpha]_D = +1.3$ ($c = 1.0$, $CHCl_3$). IR (KBr): 3267*s*, 2967*s*, 1722*s*, 1683*s*, 1522*s*, 1439*s*, 1411*s*, 1367*s*, 1244*m*, 1172*s*, 1100*m*, 756*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.86–1.00 (*m*, 6 Me); 1.38 (*d*, $J = 6.7$, Me); 1.44 (*s*, *t*-Bu); 1.59–1.72 (*m*, 3 CH); 1.79–1.90 (*m*, CH); 2.03–2.13 (*m*, CH); 2.67 (*dd*, $J = 5.7$, 16.0, CH); 2.70 (*dd*, $J = 5.0$, 16.0, CH); 2.82–3.21 (*m*, 3 CH_2); 3.68 (*s*, MeO); 3.63–3.75 (*m*, CHN); 4.49–4.62 (*m*, CHN); 4.64–4.73 (*m*, 2 CHN); 4.77 (*d*, $J = 9.0$, NH); 8.77–8.86 (br., NH); 8.90 (*d*, $J = 6.8$, NH); 9.14–9.22 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.7, 18.8, 19.2, 19.3, 21.9, 23.0 (Me); 25.2 (CH); 28.4 (Me); 30.6, 32.5 (CH); 34.7, 42.0, 50.6, 51.1 (CH_2); 51.9 (MeO); 52.2 (CH_2); 54.3, 54.8, 57.6 (CH); 80.3, 156.7, 171.9, 199.5, 200.0, 200.7 (C). FAB-MS: 621 (16, $[M+3]^+$), 620 (39, $[M+2]^+$), 619 (77, $[M+1]^+$), 618 (100, M^+), 617 (40, $[M-1]^+$), 585 (80), 519 (19, $[M-\text{Boc}+1]^+$), 272 (99, $[M-346]^+$). Anal. calc. for $C_{29}H_{54}N_4O_4S_3$ (618.96): C 56.27, H 9.05, S 15.54; found: C 56.25, H 8.97, N 9.25, S 15.54.

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-OMe* (**12**). Compound **7** (830 mg, 2.22 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (5 ml), and treated with Et_3N (1.25 ml, 8.96 mmol), HOBt (362 mg, 2.67 mmol), **9** (741 mg, 2.22 mmol) in $CHCl_3$ (5 ml), and EDC (513 mg, 2.67 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 18:1) yielded **12** (1.07 g, 1.82 mmol, 82%). Colorless solid. M.p. 205–206°. R_f (CH_2Cl_2/MeOH 18:1) 0.20. $[\alpha]_D = -44.0$ ($c = 0.5$, $CHCl_3$). IR (KBr): 3295*m*, 2961*m*, 2873*w*, 1719*m*, 1690*m*, 1646*s*, 1540*s*, 1438*m*, 1367*m*, 1309*w*, 1246*m*, 1173*s*, 1047*w*, 1011*w*, 723*w*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 2:1): 0.86–0.98 (*m*, 6 Me); 1.27 (*d*, $J = 6.6$, Me); 1.25–1.34 (*m*, CH); 1.39–1.52 (*m*, *t*-Bu, CH); 1.52–1.66 (*m*, CH); 1.74–1.88 (*m*, 2 CH); 2.25–2.90 (*m*, 4 CH_2); 3.68 (*s*, MeO); 3.80–3.89 (*m*, CHN); 4.05–4.21 (*m*, 2 CHN); 4.75–4.86 (*m*, CHN). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD 2:1): 17.8, 18.3, 18.6, 19.1, 19.4, 21.8, 23.3 (Me); 25.2 (CH); 28.5 (Me); 32.0, 32.7 (CH); 36.9, 41.5, 41.9, 43.1 (CH_2); 45.9 (CH); 52.0 (MeO); 55.8 (CH); 79.6, 156.9, 171.4, 171.6, 172.8, 201.1 (C). FAB-MS: 693 (25), 588 (21, $[M+2]^+$), 587 (67, $[M+1]^+$), 586 (14, M^+), 585 (26, $[M-1]^+$), 571 (49), 488 (26, $[M-\text{Boc}+2]^+$), 487 (100, $[M-\text{Boc}+1]^+$), 485 (17, $[M-\text{Boc}-1]^+$), 415 (40). Anal. calc. for $C_{29}H_{54}N_4O_6S$ (586.83): C 59.36, H 9.27, N 9.55, S 5.46; found: C 59.36, H 9.20, N 9.48, S 5.54.

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-OH* (**13**). Compound **12** (756 mg, 1.28 mmol) was dissolved in 2,2,2-trifluoroethanol (3.5 ml) and treated with 1N NaOH (6.5 ml, 6.5 mmol, 5 equiv.) according to *GP 2b* (reaction time: 54 h), yielding **13** (733 mg, 1.28 mmol, 99%). Colorless solid. M.p. 179–180°. R_f (CH_2Cl_2/MeOH 9:1) 0.22. $[\alpha]_D = -5.9$ ($c = 0.25$, CH_3OH). IR (KBr): 3311*w*, 2967*m*, 2422*w*, 1717*m*, 1689*m*, 1639*s*, 1522*w*, 1456*s*, 1389*m*, 1367*m*, 1283*w*, 1250*w*, 1172*m*, 1106*w*, 756*w*. ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 2:1): 0.86–0.99 (*m*, 6 Me); 1.26 (*d*, $J = 6.5$, Me); 1.25–1.34 (*m*, CH); 1.42 (*s*, *t*-Bu); 1.40–1.53 (*m*, CH); 1.52–1.68 (*m*, CH); 1.72–1.91 (*m*, 2 CH); 2.27–2.91 (*m*, 4 CH_2); 3.78–3.88 (*m*, CHN); 3.97–4.11 (*m*, CHN); 4.11–4.21 (*m*, CHN); 4.73–4.84 (*m*, CHN). ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD 2:1): 17.7, 18.2, 18.6, 19.1, 19.3, 21.8, 23.1 (Me); 25.1 (CH); 28.5 (Me); 31.8, 32.6 (CH); 36.7, 41.3, 41.7, 43.1 (CH_2); 45.8, 49.4 (CH); 49.4 (CH_2); 51.9, 55.6 (CH); 79.6, 156.8, 171.3, 171.5, 174.3, 200.9 (C). FAB-MS: 1187 (13, $[2M+2\text{Na}-4]^+$), 613 (21, $[M+\text{K}+2]^+$), 612 (23, $[M+\text{K}+1]^+$), 611 (13, $[M+\text{K}]^+$), 599 (11, $[M+\text{Na}+4]^+$), 598 (31, $[M+\text{Na}+3]^+$), 597 (62, $[M+\text{Na}+2]^+$), 596 (64, $[M+\text{Na}+1]^+$), 595 (34, $[M+\text{Na}]^+$), 575 (14, $[M+$

3]$^+$), 574 (18, [M+2]$^+$), 573 (16, [M+1]$^+$), 572 (10, M^+), 475 (34, [M – Boc + 3]$^+$), 474 (37, [M – Boc + 2]$^+$), 473 (22, [M – Boc + 1]$^+$), 260 (100, [M – 312]$^+$).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe* (**14**). Boc-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (413 mg, 1.19 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (9 ml), and treated with Et_3N (0.6 ml, 4.30 mmol), HOBt (195 mg, 1.43 mmol), **13** (687 mg, 1.19 mmol) in DMF (9 ml), and EDC (276 mg, 1.43 mmol) according to *GP 3b*. Recrystallization (MeOH) yielded **14** (456 mg, 0.57 mmol, 48%). Colorless solid. M.p. 231° (dec.). R_f (CH_2Cl_2/MeOH 9:1) 0.36. $[\alpha]_D = -26.8$ (c = 0.25, CF_3CH_2OH). CD (0.2 mM in $CHCl_3$): $-5.2\cdot10^4$ (279 nm), $+5.0\cdot10^3$ (351 nm). CD (0.2 mM in CH_3OH): $+1.2\cdot10^4$ (267 nm), $-2.3\cdot10^3$ (340 nm). IR (KBr): 3296*m*, 3075*w*, 2961*m*, 2872*w*, 1719*m*, 1647*s*, 1544*s*, 1438*m*, 1367*m*, 1311*w*, 1243*w*, 1174*m*, 994*w*, 724*w*, 600*w*. ^{1}H-NMR (500 MHz, $CDCl_3$/CD_3OD 2:1): 0.87–0.97 (*m*, 8 Me); 1.16 (*d*, *J* = 6.7, Me); 1.26 (*d*, *J* = 6.4, Me); 1.24–1.35 (*m*, 3 CH); 1.42 (*s*, *t*-Bu); 1.40–1.52 (*m*, CH); 1.52–1.68 (*m*, 2 CH); 1.73–1.88 (*m*, 2 CH); 2.18–2.89 (*m*, 6 CH_2); 3.68 (*s*, MeO); 3.81–3.88 (*m*, CHN); 3.98–4.06 (*m*, CHN); 4.09–4.17 (*m*, CHN); 4.17–4.35 (*m*,2 CHN); 4.77–4.84 (*m*, CHN). ESI-MS (neg.): 798 (100, [M – 1]$^-$). ESI-MS (pos.): 838 (8, [M + K]$^+$), 822 (100, [M + Na]$^+$).

*H-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeu-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH·CF₃CO₂H* (**2**). Compound **14** (162 mg, 0.20 mmol) was dissolved in 2,2,2-trifluoroethanol (3.5 ml) and treated with 5N NaOH (4 ml, 20 mmol, 100 equiv.) according to *GP 2b* (reaction time: 120 h), yielding the corresponding acid (133 mg, 0.17 mmol, 85%). Without further purification, the Boc-protected hexapeptide (96 mg, 0.12 mmol) was treated with TFA according to *GP 1b*. The crude peptide was purified by prep. RP-HPLC (30 → 70% *B* in 20 min) according to *GP 5*, yielding **2** (60 mg, 0.075 mmol, 62%). Colorless solid. RP-HPLC (30 → 70% *B* in 20 min). t_R 16.6 min, purity > 98%. M.p. 184° (dec.). CD (0.2 mM in CH_3OH): $+1.1\cdot10^5$ (199 nm), $-2.6\cdot10^4$ (217 nm), $+1.2\cdot10^4$ (267 nm), $-2.4\cdot10^3$ (340 nm). CD (0.2 mM in H_2O, pH = 7): $-8.7\cdot10^3$ (269 nm). IR (KBr): 3293*m*, 3061*m*, 2962*s*, 1658*s*, 1544*s*, 1448*m*, 1375*m*, 1202*s*, 1139*m*, 835*w*, 800*w*, 721*m*, 599*w*. ^{1}H-NMR (500 MHz, CD_3OD): 0.83–0.98 (*m*, 6 Me); 1.06–1.18 (*m*, 3 Me); 1.27 (*d*, *J* = 6.6, Me); 1.22–1.35 (*m*, 2 CH); 1.38–1.47 (*m*, 2 CH); 1.51–1.68 (*m*, 2 CH); 1.68–1.77 (*m*, CH); 2.08–2.18 (*m*, CH); 2.23 (*dd*, *J* = 11.9, 14.9, CH); 2.32 (*dd*, *J* = 10.9, 15.3, CH); 2.41 (*dd*, *J* = 4.4, 15.1, CH); 2.43–2.53 (*m*, 4 CH); 2.54 (*dd*, *J* = 4.1, 17.3, CH); 2.61 (*dd*, *J* = 4.6, 15.2, CH); 2.92 (*dd*, *J* = 3.2, 14.1, CH); 2.96 (*dd*, *J* = 11.7, 15.4, CH); 3.09 (*dd*, *J* = 11.4, 14.1, CH); 3.84 (*ddd*, *J* = 3.1, 6.1, 11.2, CHN); 4.18 (*ddd*, *J* = 4.2, 7.3, 15.9, CHN); 4.36–4.53 (*m*, 3 CHN); 5.06–5.15 (*m*, CHN); 7.59 (*d*, *J* = 9.8, NH); 7.74 (*d*, *J* = 8.9, NH); 8.25 (*d*, *J* = 9.2, NH). ^{13}C-NMR (125 MHz, CD_3OD): 18.7, 18.8, 19.4, 19.5, 19.7, 20.9, 22.8, 22.9, 23.4, 23.7 (Me); 26.1, 32.2, 33.9 (CH); 39.2, 41.0, 42.0, 42.3, 43.0 (CH_2); 43.5, 45.4, 45.6 (CH); 45.7, 45.9, 46.4 (CH_2); 50.0, 52.8, 59.7 (CH); 171.5, 172.0, 172.7, 172.9, 175.1, 200.5 (C). ESI-MS (neg.): 683 (100, [M – 1]$^-$) (see *Fig. 8*). ESI-MS (pos.): 723 (20, [M + K]$^+$), 707 (100, [M + Na]$^+$), 685 (74, [M + 1]$^+$).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-OH* (**15**). Compound **10** (333 mg, 0.55 mmol) was dissolved in MeOH (1.5 ml) and treated with 1N NaOH (0.67 ml, 0.67 mmol, 1.2 equiv.) according to *GP 2a* (reaction time: 6 h), yielding **15** (324 mg, 0.55 mmol, 99%). Colorless solid. M.p. 85–87°. R_f (CH_2Cl_2/MeOH 9:1) 0.32. $[\alpha]_D = -1.7$ (c = 1.0, $CHCl_3$). IR (KBr): 3283*s*, 2962*s*, 2873*m*, 1716*s*, 1681*s*, 1654*s*, 1522*s*, 1410*s*, 1367*s*, 1249*m*, 1169*s*, 1100*m*, 738*w*, 668*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88–1.01 (*m*, 6 Me); 1.28 (*d*, *J* = 6.7, Me); 1.44 (*s*, *t*-Bu); 1.40–1.50 (*m*, CH); 1.57–1.70 (*m*, CH); 1.72–1.83 (*m*, CH); 1.86–1.97 (*m*, CH); 2.14 (*dd*, *J* = 3.0, 15.5, CH); 2.16–2.27 (*m*, CH); 2.35–2.42 (*m*, CH); 2.43 (*dd*, *J* = 4.0, 15.5, CH); 2.68 (*dd*, *J* = 4.0, 17.0, CH); 2.71 (*dd*, *J* = 4.0, 17.0, CH); 2.79 (*dd*, *J* = 4.5, 13.0, CH); 2.86 (*dd*, *J* = 3.8, 13.0, CH); 3.61 (*dd*, *J* = 10.9, 12.5, CH); 3.77–3.89 (*m*, CHN); 4.21–4.32 (*m*, CHN); 4.48–4.58 (*m*, CHN); 4.61–4.72 (*m*, CHN); 5.99 (*d*, *J* = 6.1, NH); 6.19 (*d*, *J* = 10.0, NH); 8.44 (*d*, *J* = 8.8, NH); 9.78 (*d*, *J* = 7.0, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.8, 17.9, 19.2, 19.4, 20.2, 21.9, 23.0 (Me); 25.3 (CH); 28.2 (Me); 29.7, 33.1 (CH); 34.4, 38.5, 42.3 (CH_2); 48.6 (CH); 50.1, 50.6 (CH_2); 53.5, 56.9, 58.2 (CH); 81.5, 158.9, 173.4, 175.8, 199.1, 201.7 (C). FAB-MS: 695 (44), 612 (13, [M + Na + 1]$^+$), 611 (40, [M + Na]$^+$), 590 (36, [M + 2]$^+$), 589 (100, [M + 1]$^+$), 588 (59, M^+), 587 (43, [M – 1]$^+$), 555 (28), 490 (15, [M – Boc + 2]$^+$), 489 (51, [M – Boc + 1]$^+$), 258 (61).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe* (**16**). Boc-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (190 mg, 0.55 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (4 ml), and treated with Et_3N (0.2 ml, 1.43 mmol), HOBt (90 mg, 0.66 mmol), **15** (324 mg, 0.55 mmol) in $CHCl_3$ (4 ml), and EDC (127 mg, 0.66 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 18:1) yielded **16** (271 mg, 0.33 mmol, 60%). Colorless solid. M.p. 105–107°. R_f (CH_2Cl_2/MeOH 18:1) 0.17. $[\alpha]_D = -45.4$ (c = 1.0, $CHCl_3$). CD (0.2 mM in $CHCl_3$): $+2.0\cdot10^4$ (276 nm), $-2.3\cdot10^3$ (352 nm). CD (0.2 mM in CH_3OH): $+1.7\cdot10^4$ (268 nm), $-3.6\cdot10^3$ (344 nm). IR (KBr): 3300*m*, 3063*w*, 2960*m*, 2872*w*, 1719*m*, 1652*s*,

1534*s*, 1438*m*, 1367*m*, 1309*w*, 1248*w*, 1172*m*, 1102*w*, 730*w*. ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$ 2 : 1): 0.88 – 0.99 (*m*, 8 Me); 1.17 (*d*, *J* = 6.7, Me); 1.26 (*d*, *J* = 6.6, Me); 1.42 (*s*, *t*-Bu); 1.26 – 1.51 (*m*, 5 CH); 1.52 – 1.68 (*m*, 2 CH); 1.73 – 1.89 (*m*, CH); 1.97 – 2.92 (*m*, 6 CH_2); 3.68 (*s*, MeO); 3.78 – 3.90 (*m*, CHN); 4.09 – 4.19 (*m*, CHN); 4.25 – 4.41 (*m*, 2 CHN); 4.67 – 4.85 (*m*, 2 CHN); 5.67 (*d*, *J* = 9.5, NH); 7.54 – 7.63 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$ 2 : 1): 17.7, 18.4, 18.8, 19.2, 19.4, 19.7, 21.9, 22.0, 23.1, 23.3 (Me); 25.1, 25.2 (CH); 28.5 (Me); 31.4, 32.7 (CH); 37.2, 40.0, 41.5, 42.7, 43.6, 43.7 (CH_2); 43.7, 44.9, 47.9 (CH); 49.1 (CH_2); 49.3 (CH); 51.8 (CH_2); 52.0 (MeO); 55.8, 58.5 (CH); 79.6, 156.9, 171.3, 172.6, 200.9, 201.0 (C). ESI-MS (neg.): 814 (100, $[M-1]^-$). ESI-MS (pos.): 854 (10, $[M+K]^+$), 838 (100, $[M+Na]^+$), 816 (10, $[M+1]^+$).

*H-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAla-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**3**). Compound **16** (204 mg, 0.25 mmol) was dissolved in MeOH (5 ml) and treated with 1N NaOH (1.5 ml, 1.5 mmol, 6 equiv.) according to *GP 2a* (reaction time: 5 h), yielding the corresponding acid (195 mg, 0.24 mmol, 97%). Without further purification, the Boc-protected hexapeptide (48 mg, 0.059 mmol) was treated with TFA according to *GP 1b*. The crude peptide was purified by prep. RP-HPLC (45 → 65% *B* in 25 min) according to *GP 5* yielding **3** (28 mg, 0.034 mmol, 57%). Colorless solid. RP-HPLC (45 → 65% *B* in 25 min). t_R 9.0 min, purity > 98%. CD (0.2 mM in MeOH): $-6.0\cdot10^4$ (193 nm), $-2.4\cdot10^4$ (215 nm), $+5.5\cdot10^4$ (265 nm), $-1.3\cdot10^4$ (333 nm). CD (0.2 mM in H_2O, pH = 7): $+2.3\cdot10^3$ (294 nm). IR (KBr): 3254*m*, 3070*m*, 2964*s*, 1654*s*, 1542*m*, 1458*m*, 1203*s*, 1138*s*, 837*w*, 801*w*, 722*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.89 – 0.98 (*m*, 6 Me); 1.06 (*d*, *J* = 6.9, Me); 1.07 (*d*, *J* = 6.9, Me); 1.13 (*d*, *J* = 6.7, Me); 1.27 (*d*, *J* = 6.7, Me); 1.27 – 1.34 (*m*, 2 CH); 1.39 – 1.48 (*m*, 2 CH); 1.56 – 1.68 (*m*, 2 CH); 1.98 – 2.07 (*m*, CH); 2.07 – 2.15 (*m*, CH); 2.30 – 2.55 (*m*, 7 CH); 2.62 – 2.71 (*m*, 2 CH); 2.78 (*dd*, *J* = 8.9, 13.5, CH); 2.88 (*dd*, *J* = 10.4, 15.6, CH); 3.00 (*dd*, *J* = 3.0, 15.6, CH); 3.61 – 3.68 (*m*, CHN); 4.25 – 4.32 (*m*, CHN); 4.32 – 4.41 (*m*, CHN); 4.58 – 4.67 (*m*, CHN); 4.76 – 4.83 (*m*, CHN); 4.89 – 4.95 (*m*, CHN). ^{13}C-NMR (125 MHz, CD_3OD): 18.3, 18.7, 18.8, 19.1, 19.7, 20.6, 22.5, 22.7, 23.6, 23.7 (Me); 26.1, 32.0, 32.1 (CH); 37.0, 41.4, 41.9, 43.3 (CH_2); 44.2 (CH); 44.6, 45.1, 45.4 (CH_2); 45.9, 50.1 (CH); 51.9 (CH_2); 58.4, 58.9 (CH); 171.6, 172.1, 172.4, 175.3, 199.9, 202.6 (C). ESI-MS (neg.): 699 (100, $[M-1]^-$) (see *Fig. 8*). ESI-MS (pos.): 739 (14, $[M+K]^+$), 723 (100, $[M+Na]^+$), 702 (91, $[M+1]^+$).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAlaΨ[CSNH]-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-OH* (**17**). Compound **11** (890 mg, 1.43 mmol) was dissolved in MeOH (6 ml) and treated with 1N NaOH (1.75 ml, 1.75 mmol, 1.2 equiv.) according to *GP 2a* (reaction time: 24 h), yielding **17** (853 mg, 1.41 mmol, 98%). Colorless solid. M.p. 75 – 77°. R_f (CH_2Cl_2/MeOH 18 : 1) 0.12. $[\alpha]_D = -3.9$ (*c* = 1.0, $CHCl_3$). IR (KBr): 3258*s*, 2962*s*, 1713*s*, 1683*s*, 1522*s*, 1412*s*, 1367*m*, 1249*m*, 1169*s*, 1100*m*, 755*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.78 – 1.07 (*m*, 6 Me); 1.37 (*d*, *J* = 6.6, Me); 1.44 (*s*, *t*-Bu); 1.53 – 1.90 (*m*, 4 CH); 2.06 – 2.31 (*m*, CH); 2.48 – 3.40 (*m*, 4 CH_2); 3.65 – 3.78 (*m*, CHN); 4.08 – 4.78 (*m*, 3 CHN); 4.77 – 4.86 (br., NH); 6.00 – 6.09 (br., NH); 8.57 – 8.68 (br., NH); 8.78 – 8.89 (br., NH); 8.95 – 9.06 (br., NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 17.6, 18.8, 19.2, 19.3, 21.9, 23.0 (Me); 25.2 (CH); 28.4 (Me); 30.7, 32.4 (CH); 34.6, 42.1, 50.4, 51.1 (CH_2); 51.9 (CH); 52.1 (CH_2); 54.4, 54.7, 57.6 (CH); 80.5, 156.8, 175.4, 199.8, 200.3, 200.7 (C). FAB-MS: 627 (16, $[M+Na]^+$), 606 (12, $[M+2]^+$), 605 (27, $[M+1]^+$), 604 (43, M^+), 603 (17, $[M-1]^+$), 571 (38), 505 (10, $[M-Boc+1]^+$), 259 (21), 258 (100).

*Boc-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAlaΨ[CSNH]-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe* (**18**). Boc-(*S*)-β³-HAla-(*S*)-β³-HLeu-OMe (476 mg, 1.38 mmol) was deprotected according to *GP 1a*, dissolved in $CHCl_3$ (3 ml), and treated with Et_3N (0.67 ml, 4.80 mmol), HOBt (224 mg, 1.65 mmol), **17** (835 mg, 1.38 mmol) in $CHCl_3$ (3 ml), and EDC (318 mg, 1.65 mmol) according to *GP 3a*. FC (CH_2Cl_2/MeOH 18 : 1) yielded **18** (644 mg, 0.77 mmol, 56%). Colorless solid. M.p. 210 – 211° (dec.). R_f (CH_2Cl_2/MeOH 18 : 1) 0.16. $[\alpha]_D = -17.1$ (*c* = 1.0, $CHCl_3$). CD (0.2 mM in $CHCl_3$): $-5.3\cdot10^4$ (261 nm), $+6.7\cdot10^4$ (283 nm), $+4.7\cdot10^3$ (351 nm). CD (0.2 mM in CH_3OH): $-5.0\cdot10^4$ (259 nm), $+4.9\cdot10^4$ (280 nm). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.85 – 1.04 (*m*, 8 Me); 1.26 (*d*, *J* = 5.6, Me); 1.33 (*d*, *J* = 6.2, Me); 1.20 – 1.37 (*m*, CH); 1.45 (*s*, *t*-Bu); 1.39 – 1.79 (*m*, 5 CH); 1.79 – 1.91 (*m*, CH); 2.16 – 2.28 (*m*, CH); 2.32 – 3.31 (*m*, 6 CH_2); 3.69 (*s*, MeO); 4.00 – 4.15 (*m*, CHN); 4.36 – 4.49 (*m*, 2 CHN); 4.72 – 4.83 (*m*, CHN); 4.95 – 5.28 (*m*, 2 CHN, NH); 6.62 – 6.78 (br., NH); 7.31 – 7.47 (br., NH); 8.91 – 9.03 (br., NH); 9.32 – 9.52 (br., 2 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 18.0, 18.4, 19.3, 19.4, 20.2, 22.0, 22.4, 22.9, 23.2 (Me); 24.9, 25.3 (CH); 28.5 (Me); 30.3, 32.7 (CH); 35.8, 38.8, 41.6, 42.1 (CH_2); 42.9, 44.0 (CH); 48.7, 49.2, 51.0 (CH_2); 51.4 (CH), 52.0 (MeO); 53.7, 55.2, 57.8 (CH); 79.1, 156.5, 169.9, 170.1, 172.6, 199.0, 200.7 (C). ESI-MS (neg.): 829 (100, $[M-1]^-$). ESI-MS (pos.): 853 (100, $[M+Na]^+$), 731 (20, $[M-Boc+1]^+$).

*H-(*R*)-β³-HValΨ[CSNH]-(*S*)-β³-HAlaΨ[CSNH]-(*S*)-β³-HLeuΨ[CSNH]-(*R*)-β³-HVal-(*S*)-β³-HAla-(*S*)-β³-HLeu-OH · CF_3CO_2H* (**4**). Compound **18** (205 mg, 0.24 mmol) was dissolved in MeOH (5 ml) and treated with 1N NaOH (1.5 ml, 1.5 mmol, 6 equiv.) according to *GP 2a* (reaction time: 10 h), yielding the corresponding acid (196 mg, 0.23 mmol, 96%). Without further purification, the Boc-protected hexapeptide (65 mg, 0.079 mmol) was treated with TFA according to *GP 1b*. The crude peptide was purified by prep. RP-HPLC (30 → 70% *B* in

20 min) according to *GP 5*, yielding **4** (39 mg, 0.046 mmol, 58%). Colorless solid. RP-HPLC (30 → 70% *B* in 20 min). t_R 16.0 min, purity > 98%. M.p. 136–138°. CD (0.2 mM in CH_3OH): $+6.3\cdot10^4$ (196 nm), $-2.8\cdot10^4$ (224 nm), $+8.1\cdot10^4$ (272 nm), $-2.2\cdot10^4$ (338 nm). CD (0.2 mM in H_2O, pH = 3.6): $-6.9\cdot10^4$ (258 nm), $+3.4\cdot10^4$ (279 nm). CD (0.2 mM in H_2O, pH = 7): $-6.3\cdot10^4$ (258 nm), $+3.6\cdot10^4$ (279 nm). CD (0.2 mM in H_2O, pH = 11): $-7.3\cdot10^4$ (258 nm), $+3.5\cdot10^4$ (279 nm). IR (KBr): 3242*s*, 3055*s*, 2962*s*, 1654*s*, 1540*s*, 1448*s*, 1202*s*, 1138*s*, 836*w*, 800*w*, 722*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.89–0.98 (*m*, 6 Me); 1.05 (*d*, *J* = 6.9, Me); 1.06 (*d*, *J* = 6.9, Me); 1.13 (*d*, *J* = 6.7, Me); 1.27 (*d*, *J* = 6.6, Me); 1.27–1.34 (*m*, CH); 1.38–1.47 (*m*, 2 CH); 1.57–1.73 (*m*, 3 CH); 1.98–2.06 (*m*, CH); 2.08–2.17 (*m*, CH); 2.31–2.55 (*m*, 6 CH); 2.76–2.85 (*m*, 3 CH); 2.98–3.11 (*m*, 3 CH); 3.59 (*ddd*, *J* = 3.2, 5.4, 9.9, CHN); 4.25–4.33 (*m*, CHN); 4.34–4.42 (*m*, CHN); 4.77–4.84 (*m*, CHN); 5.12–5.24 (*m*, 2 CHN). ^{13}C-NMR (125 MHz, CD_3OD): 18.4, 18.5, 18.6, 18.8, 19.7, 20.7, 22.6, 23.0, 23.4, 23.7 (Me); 26.1, 26.3, 31.8, 32.0 (CH); 36.9, 41.8, 43.4, 43.5, 44.2 (CH_2); 44.2 (CH); 45.2 (CH_2); 46.0 (CH); 50.1, 51.2 (CH_2); 52.4, 55.1, 58.1, 58.9 (CH); 172.1, 172.1, 175.6, 199.9, 201.2, 202.0 (C). ESI-MS (neg.): 715 (100, $[M-1]^-$) (see *Fig. 8*). ESI-MS (pos.): 755 (16, $[M+K]^+$), 739 (100, $[M+Na]^+$), 717 (96, $[M+1]^+$).

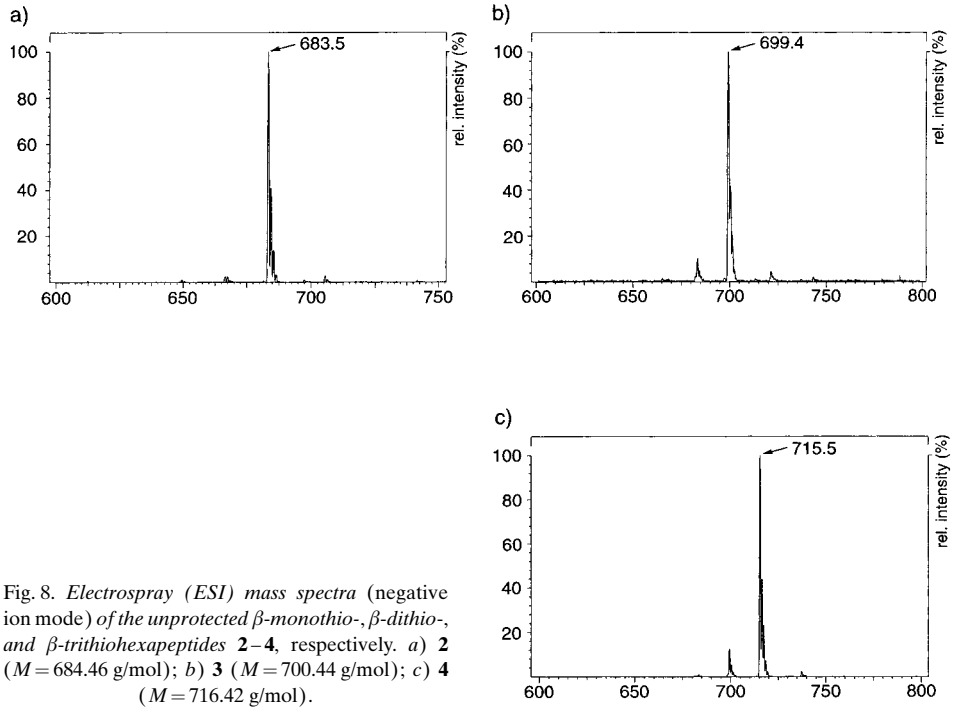

Fig. 8. *Electrospray (ESI) mass spectra* (negative ion mode) *of the unprotected β-monothio-, β-dithio-, and β-trithiohexapeptides* **2**–**4**, respectively. *a*) **2** (*M* = 684.46 g/mol); *b*) **3** (*M* = 700.44 g/mol); *c*) **4** (*M* = 716.42 g/mol).

7. *NMR Spectroscopy of β-Trithio-hexapeptide* **4**. Sample: 9 mg of **4** dissolved in 0.6 ml of CD_3OH. 1D-NMR (*AMX500*): ^{1}H-NMR (500 MHz): Suppression of the CD_3OH signal by presaturation; 90-K data points, 64 scans, 5.6-s acquisition time. {^{1}H}-BB-decoupled-^{13}C-NMR (125 MHz): 80-K data points, 27-K scans, 1.3-s acquisition time, 1-s relax. delay 45° excitation pulse. Processed with 1.0-Hz exponential line broadening. 2D-NMR (see *Table 2*). All with solvent suppression by presat. DQF.COSY (500 MHz, CD_3OH) with pulsed-field gradients (PFG) for coherence pathway selection [73]: *Acquisition:* 2K(t_2) × 512 (t_1) data points. 8 scans per t_1 increment, 0.17-s acquisition time in t_2; relaxation delay 2.0 s. TPPI Quadrature detection in ω_1. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/3$ in ω_2 and $\pi/2$ in ω_1. HSQC with PFG [74] (500, 125 MHz, CD_3OH): *Acquisition:* 2K(t_2) × 512 (t_1) data points, 48 scans per t_1 increment. ^{13}C-GARP Decoupling during t_2. 0.17-s acq. time in t_2. *Processing:* Zero filling and FT to 1K × 1K real/real data points after multiplication with sin^2 filter shifted by $\pi/2$ in ω_2 and sin filter shifted by $\pi/2$ in ω_1. HMBC with PFG [75] (500, 125 MHz, CD_3OH): *Acquisition:* Delay for evolution of long-range antiphase

magn. 50 ms. No ^{13}C-decoupling, otherwise identical to parameters for HSQC. *Processing:* Zero filling and FT to $1K \times 1K$ after multiplication with $\cos^2$ filter in ω_2 and gaussian filter in ω_1; power spectrum in both dimensions. ROESY [76] (500 MHz, CD_3OH): see *Table 3*. *Acquisition:* 2 ROESY spectra with mixing times of 80 and 150 ms were acquired. CW Spin lock (3.8 kHz) between trim pulses, $2K(t_2) \times 480$ (t_1) data points, 64 scans per t_1 increment. 0.17-s acquisition time in t_2, other parameters identical to DQF.COSY. *Processing:* Zero filling and FT to $1K \times 512K$ real/real data points after multiplication by $\cos^2$ filter in ω_2 and ω_1. Baseline correction with 3rd degree polynomial in both dimensions.

8. *NMR Structure Determination*. Calculations were performed using X-PLOR 3.581 [70] on a *Silicon Graphics* O_2 (*R 10000*) workstation under Irix 6.3. Visualisation was carried out using MolMol [71] (see *Fig. 7*).

The simulated annealing protocol of Quanta 98, X-PLOR 3.851 was used to generate the structures starting from a given helical conformation. Initial time: 800 K, 4000 high steps, 4000 cooling steps, 2-fs time step, NOE scaling 30, all other parameters were left unchanged. The resulting structures converged to a left-handed helical structure. The obtained structures were analyzed using the analyze protocol with the following acceptance criteria: No NOE violations >0.4 Å, rms difference for bond deviations from ideality <0.01 Å, rms difference for angle deviations from ideality $<2°$. All structures fulfilled this test. We chose this bundle of structures (*Fig. 7*) as representative for the structure in solution.

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