Electrophilic *S*-Trifluoromethylation of Cysteine Side Chains in α- and β-Peptides: Isolation of Trifluoromethylated *Sandostatin*® (Octreotide) Derivatives

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Dedicated to *Duilio Arigoni* on the occasion of his 80th birthday

The new electrophilic trifluoromethylating 1-(trifluoromethyl)-benziodoxole reagents **A** and **B** (*Scheme 1*) have been used to selectively attach CF_3 groups to the S-atom of cysteine side chains of α- and β-peptides (up to 13-residues-long; products **7**–**14**). Other functional groups in the substrates (amino, amido, carbamate, carboxylate, hydroxy, phenyl) are not attacked by these *soft* reagents. Depending on the conditions, the indole ring of a Trp residue may also be trifluoromethylated (in the 2-position). The products are purified by chromatography, and identified by ^{1}H-, ^{13}C-, and ^{19}F-NMR spectroscopy, by CD spectroscopy, and by high-resolution mass spectrometry. The CF_3 groups, thus introduced, may be replaced by H (Na/NH_3), an overall Cys/Ala conversion. The importance of trifluoromethylations in medicinal chemistry and possible applications of the method (spin-labelling, imaging, PET) are discussed.

1. Introduction. – In one of our groups, there is a long-standing interest in the modification of peptides: naphthylselenation [1], dehydration of serine moieties [2], C=O → C=S thionylation [3], electrochemical decarboxylation [4], Li-enolate formation and reactions with electrophiles [5], and homologation of amino-acid residues [6][7]. In continuation of this tradition, we have now investigated the conversion of CH_2-S-H groups of cysteine side chains in peptides to CH_2-S-CF_3 groups, by using a method [8][9] that has been recently introduced by another group of co-authors of the present article[5]). The method uses the hypervalent iodine reagents **A**

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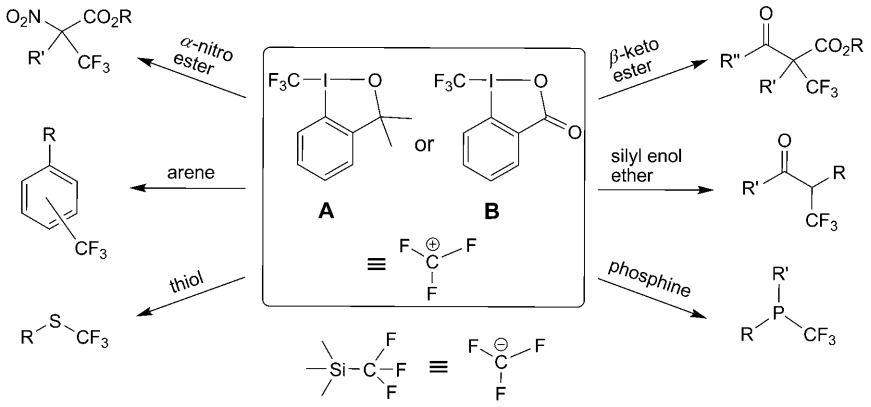
[3]) Postdoctoral Research Fellow at ETH-Zürich, 2004–2006, financed by the *Deutsche Forschungsgemeinschaft* (DFG), by the *Swiss National Science Foundation* (Project No. 200020-100182), and by *Novartis Pharma AG*, Basel.

[4]) Part of the Ph.D. Thesis of *G. L.*, ETH-Zürich, 2000–2004, Dissertation No. 15429.

[5]) Both our groups have a history of preparing and investigating organo-fluoro compounds [8–11].

and **B** with a CF_3 group attached to the I-atom. They are capable of transferring CF_3 to nucleophilic centers of substrate molecules. This electrophilic trifluoromethylation reaction is remarkably mild and selective. Examples are shown in *Scheme 1:* s*oft* nucleophiles (with high-lying HOMO) such as the anions of β-keto esters, or of α-nitro esters, silyl enol ethers, electron-rich aromatic systems (phenol, pyrrol, indole), phosphanes, and thiols are preferred over *harder* nucleophiles such as amino or OH groups to an extent that the reactions can be carried out in $MeOH/H_2O$ (at low temperature) [8][9]. The CF_3-I σ^* orbital (LUMO) in these molecules must be low-lying. The new method provides an *umpolung* of the nucleophilic trifluoromethylation with Me_3SiCF_3/F^- (*Ruppert–Prakash* reagent) [12].

Scheme 1. *Molecular Formulae of the CF_3-Substituted Benziodoxoles* **A** *and* **B**, *and of the Products Obtained with Various Nucleophiles.* **A** and **B** correspond to CF_3^+ synthons, while Me_3SiCF_3 corresponds to a CF_3^- synthon.



1.1 *Preparation of CF_3-S Derivatives.* Reagents **A** and **B** are readily prepared from inexpensive starting materials, and the I-containing reduced species can be recovered after delivery of the CF_3 fragment [8][9]. It has already been shown that these hypervalent iodine(III)$-CF_3$ reagents can be used for *S*-trifluoromethylation of cysteine esters (with or without *N*-protection) in high yields; the reaction is carried out in MeOH at dry-ice temperature in the absence of base [9]. Before invention of the reagents **A** and **B**, SCF_3 substituents were introduced into organic molecules by functional-group interconversions under rather drastic conditions [13], by direct transfer of the SCF_3 moiety [14], or by the delivery of CF_3 to a S-atom by a nucleophilic, or, more often, a radical reaction [15][16]. The hitherto less common electrophilic *S*-trifluoromethylation with (trifluoromethyl)dibenzothio-, -seleno-, or -tellurophenium salts (*Umemoto* and *Ishihara* [17]) requires preformed sodium thiolates, and the product is accompanied by a considerable amount of disulfide. In particular, for the direct preparation of *S*-(trifluoromethyl)-cysteine derivatives, reaction conditions involving *t*-BuO_2H [15][18] or liquid NH_3 [19] had to be employed. In contrast, with reagent **A** essentially no disulfide by-product is formed. As outlined above, the $SH \rightarrow SCF_3$ transformation exhibits a remarkably high functional-group

selectivity: amino, hydroxy, carboxy, amido, thioacetal, and alkinyl groups do not interfere.

Here, we present applications of the electrophilic trifluoromethylation of cysteine side chains in α- and β-peptides consisting of up to 13 amino-acid residues with a variety of other side-chain functional groups.

1.2. *Why Trifluoromethylation of Cysteine Side Chains?* The introduction of F-atoms, in particular CF_3 groups, into drug candidates constitutes an important tool of the lead-finding process of medicinal chemists [20]. The reduced polarity and the increased metabolic stability brought about by the CF_3 moiety can lead to higher biological activity and improved bioavailability of a potential drug. Very often, when CH_3 is replaced by CF_3, there is no more P_{450}-dependent hydroxylation of neighboring sites, and the lipophilicity rises considerably (*Hansch* parameter π of SCH_3 0.62, of SCF_3 1.58 in the '3-substituted phenoxy acetic acid' system) [21]. Thus, trifluoromethylation of a cysteine residue, not involved in a disulfide bridge, of a peptide or a protein may greatly alter its properties. Furthermore, conversion of a cystine group, $CH_2-S-S-CH_2$, to a $[CH_2-S-CF_3/CF_3-S-CH_2]$ moiety, while removing a structure-stabilizing covalent bond, may create a hydrophobic pocket within the peptide or protein. As a first example of this kind of structural change, we chose the cyclic disulfide derivative *Sandostatin*® (octreotide) [22][6]).

2. Preparation of the Peptides for Trifluoromethylation. – As simple peptidic substrates for this reaction, we chose the α-dipeptides R-Phe-Cys-OMe and R-Cys-Ala-OMe (R = Boc or H), which we generated *in situ* from the corresponding disulfides (cystine derivatives) **1** and **2** (*Scheme 2*) by reduction with Et_3P in aqueous MeOH. As analogous β-dipeptide substrates Boc-β^3hPhe-β^3hCys-OH (**3**) and Boc-β^3hCys-β^3hAla-OH (**4**) were chosen (*Scheme 3*). All six dipeptides were prepared by coupling appropriately protected amino acids with HATU in CH_2Cl_2 solution as outlined in *Schemes 2* and *3*, and as described in detail in the *Exper. Part*.

Two more complex peptides **5** and **6** with the functional groups in the side chains of Lys, Asp, Trp, and Thr, and without terminal protection were also prepared for trifluoromethylation reactions. The β-tridecapeptide **5** was prepared by solid-phase peptide synthesis (SPPS) on *Wang* resin, using the Fmoc strategy [26]. For the last coupling step, Boc-protected β^3hCys(Trt)-OH was used. After cleavage from the resin and removal of the protecting groups [27], the linear peptide was purified by preparative reversed-phase HPLC to provide the CF_3COOH (TFA) salt of **5**. The α-octapeptide derivative **6** is the ring-opened form of the drug *Sandostatin*® (octreotide) marketed by *Novartis*[6]). A sample of the cyclic disulfide octreotide acetate[7]) was dissolved in degassed phosphate buffer and treated with 1,4-dithiothreitol (DTT) to cleave the disulfide bond. The resulting solution was diluted in aqueous TFA and directly subjected to reversed-phase HPLC purification, to afford the dithiol **6** as TFA salt.

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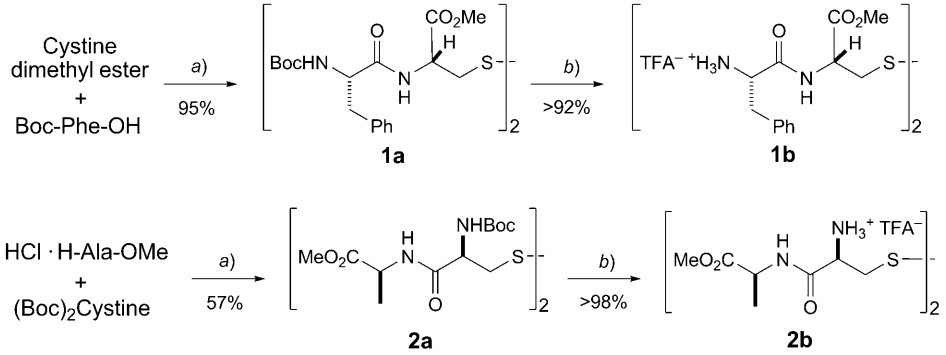
[6]) For recent discussions of the pharmacological properties of this drug, see [23–25].

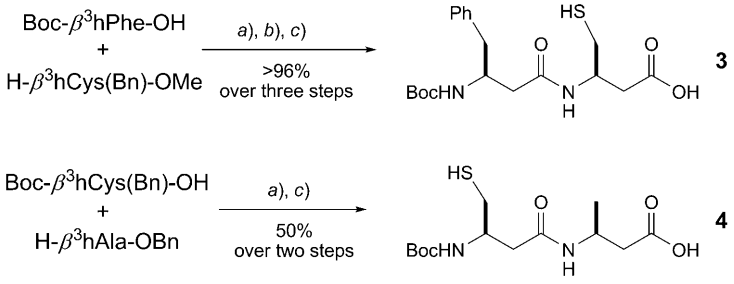
[7]) As provided by *Novartis Pharma AG*, Basel.

Scheme 2. *Preparation of the Disulfanediyl-Bridged Dipeptide Diesters* **1** *and* **2**. All starting materials and reagents were used as purchased.



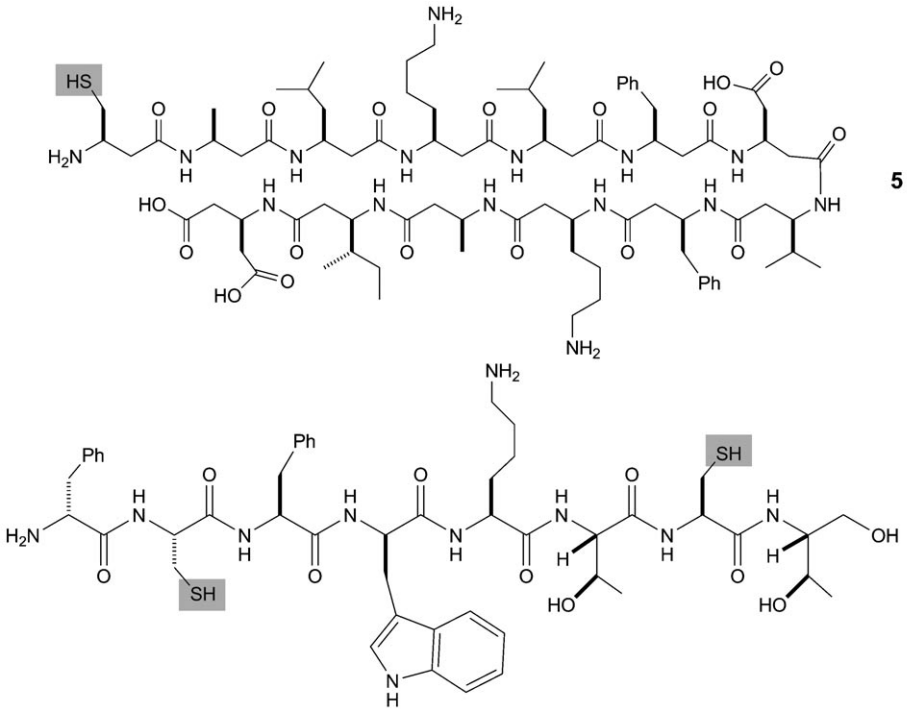
a) 2-(7-Aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 2,6-dimethylpyridine (DMP), CH_2Cl_2/$OCHNMe_2$ (DMF), 12 h. *b*) CF_3CO_2H (TFA), CH_2Cl_2, 3 h.

Scheme 3. *Preparation of the Boc-β-Dipeptides* **3** *and* **4**. The starting materials were purchased or prepared according to known procedures (see references in the *Exper. Part*). For abbreviations, see *Scheme 2*.



a) HATU, Et_3N, CH_2Cl_2, 12 h. *b*) NaOH, MeOH, 50°, 12 h. *c*) Na/NH_3, *t*-BuOH, THF, −80°, 10 min.

3. Trifluoromethylations of Peptide Cysteine Side Chains to Give Derivatives 7–11. – First, the cysteine derivatives **1** and **2** were cleaved to the corresponding peptide thiols by treating solutions in MeOH/H_2O 9:1 with 2 equiv. of Et_3P at room temperature. Subsequent addition at −78° of 2.3 equiv. of reagent **A** (dissolved in MeOH), warming to room temperature, and removing the solvent *in vacuo* gave the crude products **7a** and **7b**, and **8a** and **8b**, which were purified by chromatography and isolated in yields ranging between 50 and 60%. Likewise, the reactions (in MeOH, −78° → +25°) of the β-dipeptides **3** and **4** with 1.1 equiv. of reagent **A** provided the trifluoromethylated products **9** and **10**, respectively, in essentially quantitative yields. The β-tridecapeptide **5** (containing, besides the SH group, six unprotected functional groups, plus the twelve amide groups) was subjected to a slight excess of trifluoromethylating reagent **A** under the same conditions (degassed MeOH solution, inert atmosphere, −78° to +25°), to give a 40% yield of its CF_3 derivative **11** (after preparative RP-HPLC purification) on a 5-μM scale (for details and characterization of **11** see the *Exper. Part*).



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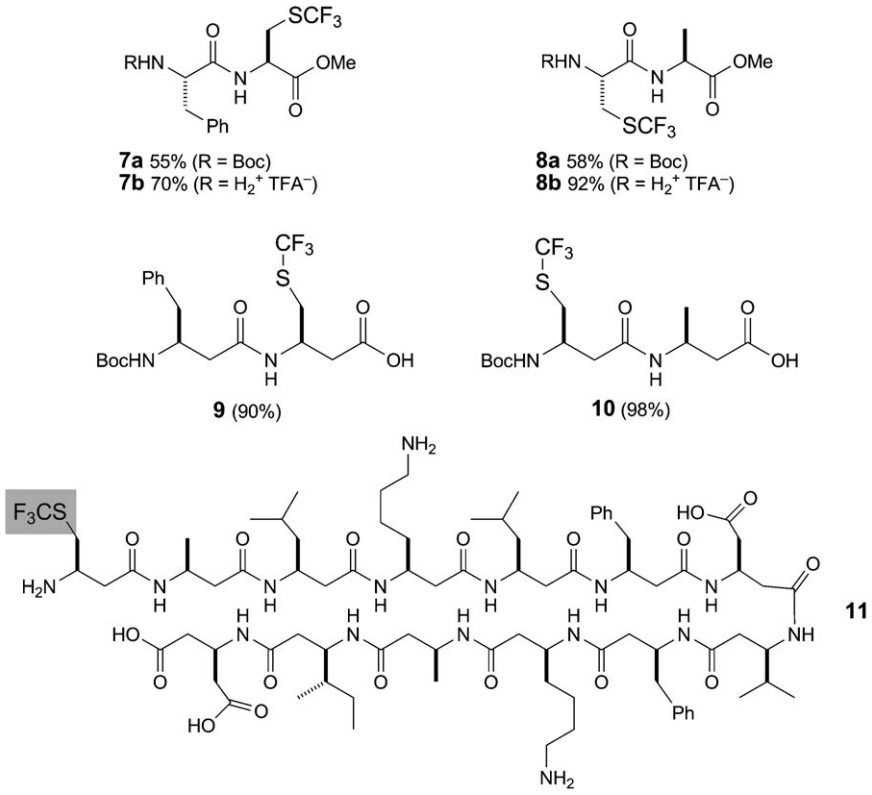
4. Trifluoromethylation of the Octreotide Derivative 6. – From the reaction (under various condition) of disulfanyl derivative **6** with reagent **A** or **B**, we were able to isolate three products: the octreotide **12** with a single CF_3 group in position 2 of the D-Trp residue, the open-chain peptide derivatives **13** with two $S-CF_3$ groups, and **14** with trifluoromethylated indole ring and two $S-CF_3$ groups. The three compounds were separated by preparative RP-HPLC, and characterized by NMR and CD spectroscopy, and by mass spectrometry (LC/MS). Under the standard conditions, reagent **A** (2.9 equiv.) produced **12**, **13**, and **14** in a ratio of *ca.* 1:6:6 in a total yield (after purification) of 34% (+3% octreotide). As can be seen from the analytical HPLC trace of the crude-product mixture in *Fig. 1*, *a*, a number of minor by-products is also formed. Notably, octreotide itself is not trifluoromethylated by reagent **A**. With 2.1 equiv. of reagent **B** and disulfanyl derivative **6**, the mono-trifluoromethylated octreotide **12** and octreotide, the product of disulfide coupling, are formed (ratio 1:3, combined yield 50% after RP-HPLC separation/purification). The reaction with reagent **B** (the 'harder' one, as compared to **A**) is much cleaner (see *Fig. 1*, *b*). If the SH groups of **6** are deprotonated by addition of 3 equiv. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), the only product detectable by NMR analysis in the reaction with the benziodoxole derivative **B** is the bis(trifluoromethyl) derivative **13** – a remarkable switch from attack on the indole ring to reaction at the S-atom (CF_3 transfer from the *hard* reagent **B** is charge-controlled).



The CD spectra of octreotide, and of its derivatives **6**, **12**, **13**, and **14** are shown in *Fig. 2*. As it had to be expected, the [F_3C-Trp]-octreotide **12** gives rise to a pattern similar to that of octreotide itself, with a weak positive *Cotton* effect between 250 and 220 nm, and a stronger negative one between 205 and 200 nm. Why the depth of the short-wavelength peak observed for the trifluoromethylated compound is so much smaller is not clear at this point. The open-chain starting material, *i.e.*, disulfanyl derivative **6**, and the tris(trifluoromethylated), likewise open-chain product **14** give rise to negative-only spectra between 250 and 195 nm with very similar shapes. Again, the spectrum of lower intensity belongs to the CF_3 derivative. There is a resemblance of the CD spectra of these two compounds (minima near 220 and 205 nm) with those considered characteristic of α-helices (minima at 220 and 210 nm)[8]. Interestingly, it has been shown by X-ray and NMR investigations of octreotide that the molecule can adopt both, antiparallel β-sheet and 3_{10}-helix-like secondary structures, in which the C-terminal residues fold to a 'helical ensemble' [29].

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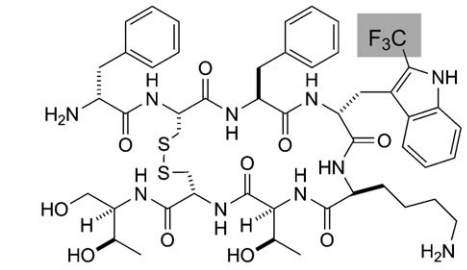
[8] For typical CD curves of α-helices, β-sheets, and random coils, see textbooks of peptide and protein chemistry, for instance, Fig. 5.12 in [28].

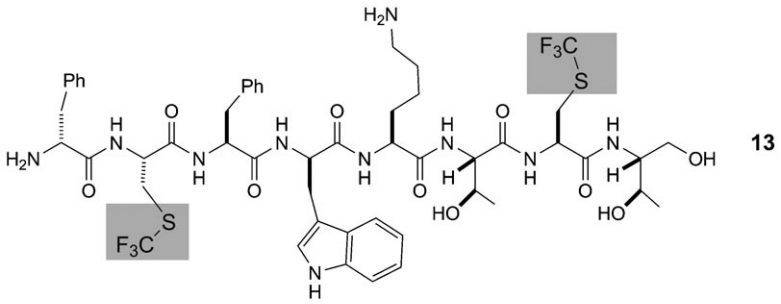
Detailed structural analysis of the new octreotide derivatives and their affinities for somatostatin receptors are under investigation and will be published in a forthcoming article.

The electrophilic trifluoromethylation described here may be used for an overall reductive removal of the SH group in a cysteine side chain: we have treated the trifluoromethylated compound **10** under *Birch*-reduction conditions (Na in liq. NH_3), which led to a quantitative yield of diastereoisomerically pure *N*-Boc-β^3hAla-β^3hAla-OH, as established by NMR comparison with an authentic sample (*Scheme 4* and *Fig. 3*).

5. Conclusions and Outlook. – We have shown that the new electrophilic trifluoromethylating reagents **A** and **B** can be used for remarkably selective transformations of peptides. The reactions can be carried out in the protic solvents MeOH and H_2O, and the primary goal of attack is the SH group of cysteine. Amino groups of lysine residues, free N-terminal amino groups, Boc-carbamoyl groups, amide groups in



[F_3C-Trp]-Octreotide (**12**)



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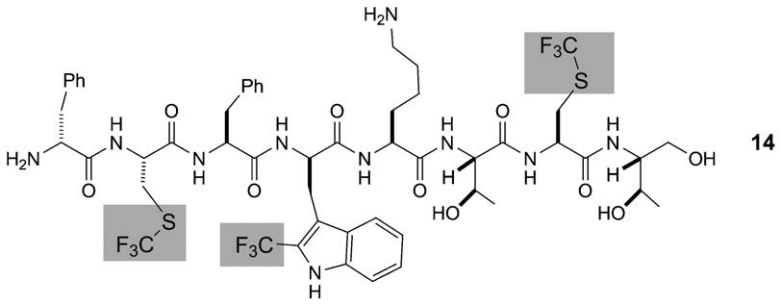

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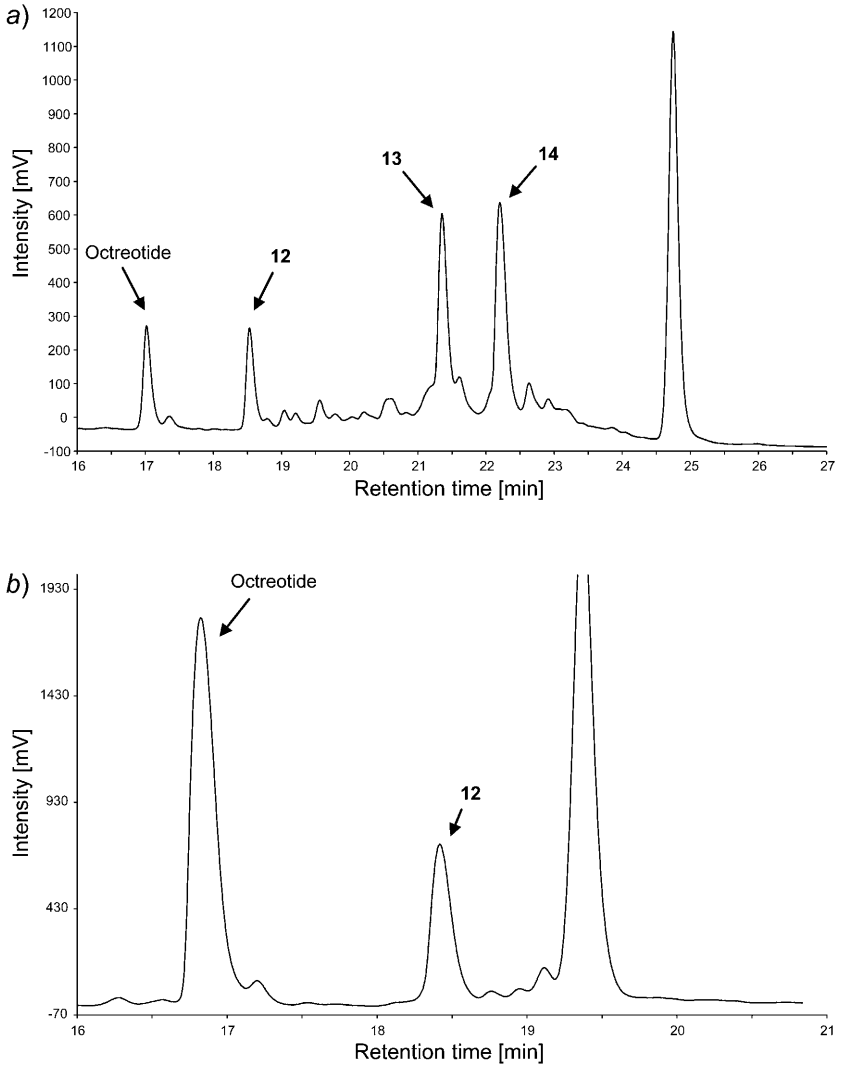


Fig. 1. *HPLC Traces of the crude product from reaction of the dithiol* **6** *with* a) *reagent* **A** (2.9 equiv. of **A** in MeOH at −78°, 12 h; 34%) *and* b) *reagent* **B** (2.1 equiv. of **B** in MeOH at −78°, 12 h; 50%). Chromatographic conditions: *C18* column (*Macherey-Nagel* 250 mm × 4.6 mm × 300 Å, 5 μ; 5–95% MeCN in H_2O/0.1% TFA (1 ml/min) in 45 min: linear gradient). The intensive peaks at 24.6 min (*a*) and 19.4 min (*b*) correspond to 2-(2-iodophenyl)propan-2-ol and 2-iodobenzoic acid, the products formed from **A** and **B**, resp., after the electrophilic CF_3 group has been 'delivered'.

the chain, carboxylate groups of aspartic acid, OH groups of threonine, and benzene rings of phenylalanine side chains do not appear to be able to compete for the electrophilic CF_3 group of the reagents **A** and **B**. By preliminary optimization experiments, it was demonstrated that, depending on the conditions and the reagent used, only the indole ring of tryptophan competes with the SH groups, but at higher pH

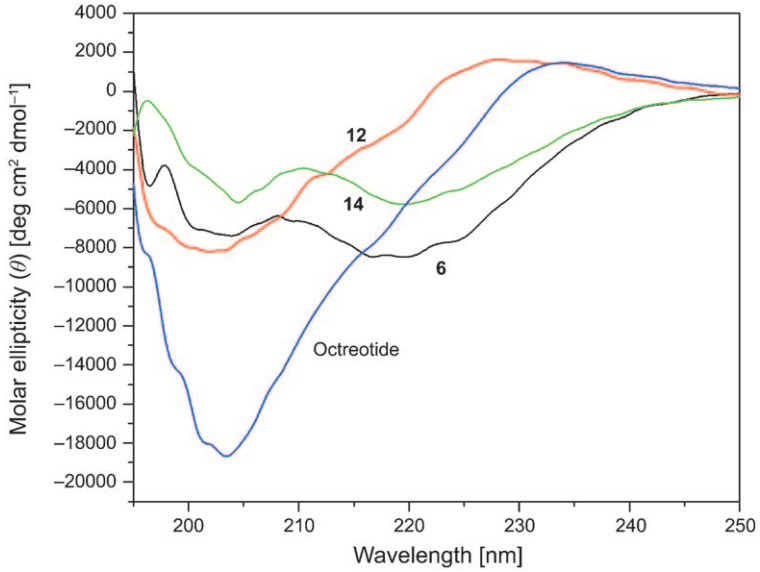
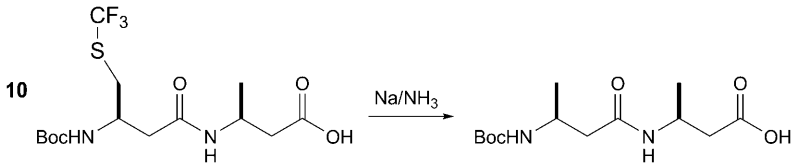


Fig. 2. *Normalized CD spectra in MeOH* (0.2 mM) *of Octreotide* (blue), *and of its derivatives* **6** (black), **12** (red), *and* **14** (green). Solutions were prepared by weighing the peptide on a μg-precision balance and dissolving it in a volumetric flask.

Scheme 4



(*cf.* addition of DBU) the generated thiolate wins. Independent work by one of our groups has shown that *ortho*-/*para*-trifluoromethylation of phenol groups (*cf.* tyrosine) occurs more slowly than electrophilic attack at an indole ring, an effect, which should be pH-dependent.

To the peptide chemist the transformations described herein may look like standard reactions: very small scales, LC/MS detection, preparative HPLC separation, and purification, lyophilization, HR-MS characterization, and CD and NMR spectra. For the preparative chemist, the reported experiments must be considered preliminary and mainly analytical. Thus, future experiments will have to address upscaling and further optimization of selectivity of the trifluoromethylation of peptides.

What could this reaction be useful for? One aspect has been discussed in the introduction: CF_3-modified compounds are interesting drug candidates. Another application could be the overall replacement of the cysteine SH group by other groups X, (*cf. Scheme 4*), for instance through the nucleophilic or radical processes involving dehydro-alanine moieties, as outlined in *Scheme 5* [30]. The CF_3S group should be an

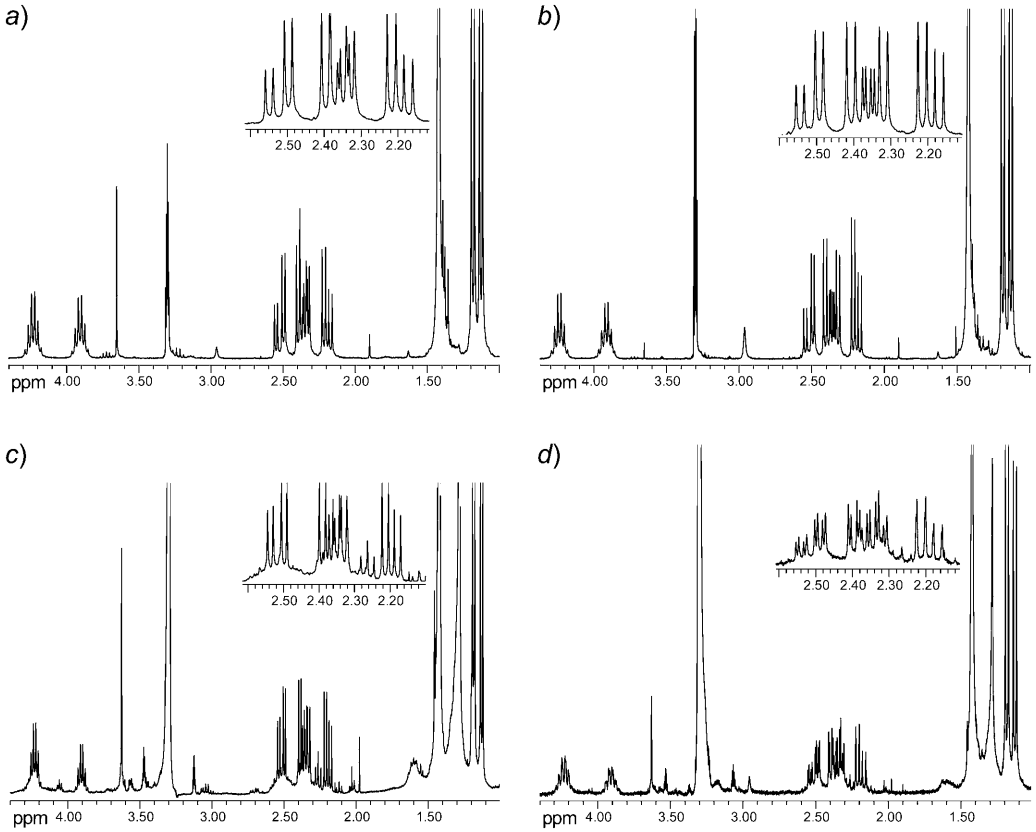


Fig. 3. *Analysis of the reductive cleavage of* **10**. NMR Spectra of *a*) Boc-β^3hAla-β^3hAla-OH, *b*) of the epimer Boc-(R)-β^3hAla-β^3hAla-OH, *c*) crude product of the reaction of **10** with Na/NH_3, *d*) mixture obtained by adding Boc-(R)-β^3hAla-β^3hAla-OH to the crude reaction mixture from **10** and Na/NH_3.

excellent leaving group, if we consider the increased stability of CF_3S^- as compared to alkyl–S^- (the pK_a value of an alkyl-thiol is *ca.* 7, the pK_a value of F_3CS–H could well be 5 to 10 units lower)[9]). Indeed, in a preliminary experiment we have found that, by treating compound **8a** with *t*-BuOK in MeOD, CF_3SH is eliminated from the cysteine residue, giving rise to a dehydroalanine residue. Of course, the SeH group of selenocysteine (Sec) is expected to be an even 'better target' for the trifluoromethylating reagents **A** and **B**, and in corresponding CF_3Se derivatives CF_3Se^- will be an even better leaving group than CF_3S^-.

The most attractive use of the trifluoromethylation reaction is in the fields of biochemistry, biology, and medicine. It is a well-known technology to use *surface* cysteines in proteins for attaching all kinds of groups [32], either through disulfide

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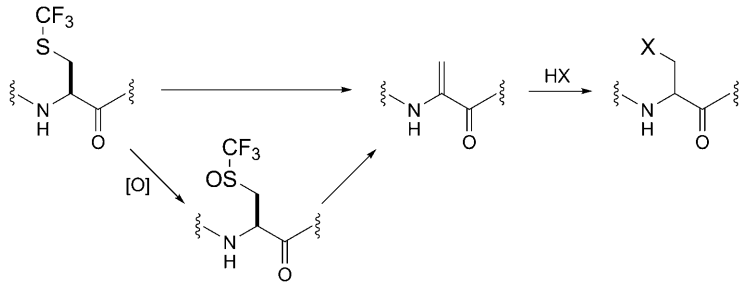
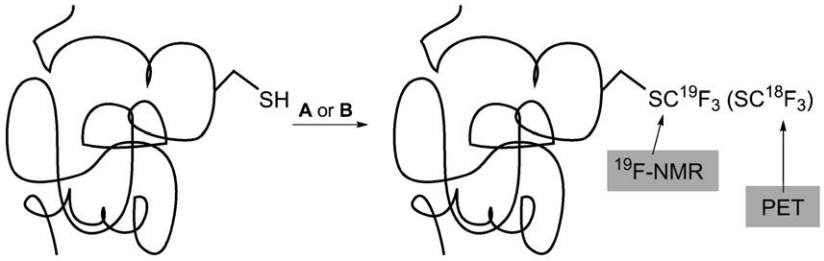
[9]) See pK_a tables in [31] or *Evans* pK_a Table. Examples are CH_3/CF_3CH_2OH (ΔpK_a 4), CH_3/CF_3CO_2H (ΔpK_a 5), CH_3/CF_3SO_3H (ΔpK_a 11).

Scheme 5



formation or by elimination and addition (*cf. Scheme 5*). This has been applied for glycoconjugation, for introduction of N_3 or HC≡C groups (*cf.* 'click' chemistry [33]), for attachment of fluorophores, and for many other transformations ('post-translational mimicking' [34]). If the trifluoromethylation of such surface SH groups in proteins and enzymes were possible (*Scheme 6*), without drastically altering their physiological properties, three important applications could be envisioned. *i*) The CF_3 group will provide a unique identification of the species by ^{19}F-NMR spectroscopy; there is no interference of ^{19}F-resonance with any other magnetic nucleus in the molecule, which would allow facile NMR imaging in cells, in tissues, and in plants or animals. *ii*) The CF_3 group could be used as a spin label, exploiting nuclear *Overhauser* effects for probing its neighborhood within a complex molecule. *iii*) Carried out with a ^{18}F-labelled CF_3 group, the reaction would allow for a quick, one-step introduction of the positron-emitting *isotope*, and thus for positron-emission tomography (PET)[10]). For these purposes [36], the phenol ring of tyrosine or the indole ring of tryptophan at the surface of a protein are also possible targets of trifluoromethylation by reagents of type **A** or **B**[11]). Work along the lines discussed in the previous paragraphs is under-way in our laboratories or is being pursued in collaboration with specialists in the corresponding fields.

Scheme 6. *Possible Trifluoromethylation of Cysteine Side Chains at the 'Surface' of Proteins*



[10]) For an example and for leading references, see an article entitled: *'First ^{18}F-Labeled Tracer Suitable for Routine Clinical Imaging of sst Receptor-Expressing Tumors Using PET'* [35].

[11]) The reactivity, and thus the selectivity, of these reagents could be modified by putting electron-donating or -withdrawing substituents in various positions of their benzene rings.

Experimental Part

1. *General. Abbreviations:* Bn: benzyl; Boc: (*tert*-butoxy)carbonyl; DTT: dithiothreitol; HATU: 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TFA: CF_3COOH; FC: flash chromatography; h.v.: high vacuum (0.01 – 0.1 Torr); HR-MALDI: high-resolution matrix-assisted laser desorption ionization; RP: reversed-phase; TLC: thin layer chromatography; SPPS: solid-phase peptide synthesis.

Materials and Methods. Solvents for reactions, workup procedures, and/or chromatography were distilled (AcOEt, MeOH (*Sikkon*, anh. $CaSO_4$), CH_2Cl_2 (P_2O_5), Et_2O (KOH and filtered through Al_2O_3 (Alumina *Woelm* N, activity I)), THF (Na)), or used in *p.a.* quality (*Fluka*, *Aldrich*). All other reagents were used as received from *Fluka* or *Aldrich*. *Wang* resins were purchased from *Novabiochem*. TLC: silica gel *Merck 60* F_{254} plates (0.2-mm layer) and FC on *Merck Kieselgel 60* (230 – 400 mesh). Anal. RP-HPLC: *Merck/Hitachi* HPLC system (*LaChrom*, *L-7150* pump, UV detector *L-7400*, interface *D-7000*); column: *Nucleosil 100-5 C18* (250 × 4 mm, *Macherey-Nagel*). HPLC Analyses: 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. Retention time (t_R) in min. Prep. RP-HPLC: *Merck/Hitachi* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC manager *D-7000*); column: *Nucleosil 100-7 C18* (250 × 21 mm, *Macherey-Nagel*). Crude products were purified with a gradient of *A* (0.1% TFA in H_2O) and *B* (MeCN) at a flow rate of 10 ml/min with UV detection at 220 nm, and then lyophilized. UV-Grade TFA (> 99% GC) was used for RP-HPLC. Lyophilization: *Hetosicc* cooling condenser with a h.v. pump. CD Spectra: *Jasco J-710* spectropolarimeter from 190 – 250 nm at r.t. in 1-mm pathlength cells. The optical system was flushed with N_2 at a flow rate of ca. 10 l/min. Parameters: band width 1.0 nm, resolution 0.2 ± 1 nm, sensitivity 100 mdeg, response 0.5 s, speed 50 nm/min, 5 accumulations. All spectra were corrected for the corresponding solvent spectrum. The molar ellipticity $[\theta]$ in deg cm^2 mol^{-1} (λ in nm) is calculated for the corresponding peptide and normalized, taking into account the mass of TFA for each free amino group. Smoothing was done by *Jasco* software. Solvents: MeOH (HPLC grade). M.p.: in open-end glass cap. tubes on a *Büchi 510* apparatus, uncorrected. $[\alpha]_D^{20}$: *Jasco PIP-370* polarimeter (1.0-dm cell); solvent MeOH, unless otherwise specified. IR Spectra: *Perkin-Elmer Spectrum-100* spectrometer; in cm^{-1}. NMR Spectra: *Bruker DPX-500* (^{1}H: 500 MHz, ^{13}C: 125 MHz), *DRX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz, ^{19}F: 376 MHz), *DPX-400* (^{1}H: 400 MHz, ^{13}C: 100 MHz, ^{19}F: 376 MHz), *ARX-300* (^{1}H: 300 MHz, ^{13}C: 75 MHz), or *Varian Mercury-300BB* (^{1}H: 300 MHz, ^{13}C: 75 MHz, ^{19}F: 282 MHz); chemical shifts δ in ppm; *J* values in Hz; all spectra recorded in CD_3OD, unless otherwise specified; $CFCl_3$ external standard for ^{19}F-NMR. MS: *IonSpecUltima 4.7-T-FT Ion Cyclotron Resonance* (ICR; HR-MALDI, in 2,5-dihydroxybenzoic acid matrix) spectrometer; in *m*/*z*. LC/MS was performed on a *Thermo Separation Products* (TSP) HPLC system connected to a PDA detector (*UV6000LB*, *TSP*) and to an ion-trap mass-spectrometry detector (*LCQdeca, Finnigan*). Linear gradients of 5 – 60% MeCN in H_2O/0.1% HCOOH (0.2 ml/min) on a *Waters Atlantis dC18-3* 3 × 100 mm column were used.

Preparation and characterization of Boc-(*R*)-β^3hCys(Bn)-OMe [37][38], Boc-(*R*)-β^3h-Cys(Bn)-OH [38][39], and Boc-(*S*)-β^3hAla-OBn [7][40] has been previously reported.

2. *Boc Deprotection: General Procedure 1* (*GP 1*). *GP 1a:* The Boc-protected α-peptide was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. An equal volume of TFA was added, and the mixture was allowed to warm slowly to r.t. and stirred for further 3 h. Concentration under reduced pressure and co-evaporation with hexane of the residue under h.v. yielded the crude TFA salt, which was lyophilized from dioxane or MeCN/H_2O 3:7 (containing 0.1% TFA), identified by NMR, and used without further purification.

GP 1b: The Boc-protected β-amino acid was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. An equal volume of TFA was added, and the mixture was allowed to warm slowly to r.t. and stirred for further 1.5 h. Concentration under reduced pressure, co-evaporation with CH_2Cl_2, and drying of the residue under h.v. yielded the crude TFA salt, which was identified by NMR and used without further purification.

3. *Peptide Coupling: General Procedure 2* (*GP 2*). *GP 2a:* The HCl salt of the α-amino acid was dissolved in CH_2Cl_2/DMF 1:1 (*v*/*v*, 0.1M) and cooled to 0°. This soln. was treated successively with collidine (2.7 equiv.), a soln. of the Boc-protected α-amino acid (1.1 equiv.) in CH_2Cl_2 (0.25M), and HATU (1.1 equiv.). The mixture was allowed to warm to r.t. and stirred for 12 h. Subsequent dilution

with CH_2Cl_2 was followed by thorough washing with 0.5M HCl, sat. aq. $NaHCO_3$ (3×) and NaCl solns. (1×). The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. FC or recrystallization yielded the pure peptide.

GP 2b: The TFA salt of the β-amino acid was dissolved in CH_2Cl_2 (0.5M) and cooled to 0°. This soln. was treated successively with Et_3N (5 equiv.), a soln. of the Boc-protected β-amino acid (1 equiv.) in CH_2Cl_2 (0.25M), and HATU (1.05 equiv.). The mixture was allowed to warm to r.t. and stirred for 12 h. Subsequent dilution with CH_2Cl_2 was followed by thorough washing with 0.5M HCl, sat. aq. $NaHCO_3$ (3×), and NaCl solns. (1×). The org. phase was dried ($MgSO_4$) and concentrated under reduced pressure. FC or crystallization yielded the pure peptide.

4. *Deprotection of Cysteine Residue of β-Peptides: General Procedure 3* (*GP 3*). *GP 3:* A three-necked round-bottomed flask was fitted with a dry ice condenser and two septum cups and flamed-dried under N_2 flow. The flask was then cooled to −78° (acetone/CO_2) and NH_3 gas (25 ml) was condensed into the flask *via* needle. The liq. NH_3 was kept under N_2 at −78°. Na (3.2 mmol, 74 mg) was added and, once the metal had dissolved, the appropriate β-peptide (320 mg, 0.64 mmol) dissolved in THF (6 ml) and *t*-BuOH (4% (*v*/*v*)) was added slowly by syringe. The temp. was kept at −78°, and the mixture was stirred for 10 min at this temp. MeOH (2 ml) was slowly added, and after the blue color disappeared, the mixture was allowed to warm to r.t., and the NH_3 was evaporated under a stream of N_2. H_2O (5 ml) was finally added, followed by AcOEt (5 ml), and the org. layer was washed with 1M HCl and brine. The aq. layers were extracted with AcOEt (2 × 5 ml), the combined org. layers were dried (Na_2SO_4), filtered, and the solvent was removed under reduced pressure to yield a white solid, which was used without further purifications.

5. *Trifluoromethylation of Peptide Fragments: General Procedure 4* (*GP 4*). *GP 4a:* Et_3P (2 equiv., 1M in THF) was added to a stirred soln. of α-peptide disulfide in degassed MeOH/H_2O 9:1 (*v*/*v*; 33 mM) at r.t. After 2 h, the mixture was cooled to −78°, and a soln. of 1,3-dihydro-3,3-dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (2.3 equiv.) in degassed MeOH (46 mM) was added dropwise. The slight yellow mixture was allowed to warm to r.t. and stirred for 12 h. The soln. was evaporated to dryness under vacuum, and the remaining oil was subjected to FC or HPLC purification.

GP 4b: The appropriate β-peptide was dissolved in degassed MeOH (0.15M) and cooled to −78°. 1,3-Dihydro-3,3-dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (1.1 equiv.) dissolved in degassed MeOH (0.3M) and cooled to −78° was added, and the reaction was kept at −78° under N_2 atm for 2 h, then allowed to warm to r.t., and stirred for 12 h. The solvent was removed under vacuum, and the crude product was purified by RP-HPLC.

6. *Preparation of [Boc-(*S*)-Phe-(*R*)-Cys-OMe]$_2$* (**1a**) *and [H-(*S*)-Phe-(*R*)-Cys-OMe]$_2$* (**1b**). *(6*S*,9*R*,14*R*,17*S*)-Dimethyl 6,17-Dibenzyl-2,2,21,21-tetramethyl-4,7,16,19-tetraoxo-3,20-dioxa-11,12-dithia-5,8,15,18-tetraazadocosane-9,14-dicarboxylate* ([Boc-(*S*)-Phe-(*R*)-Cys-OMe]$_2$; **1a**). [H-(*R*)-Cys-OMe]$_2$ (1.45 mmol, 389 mg) was coupled with Boc-(*S*)-Phe-OH (3.63 mmol, 965 mg) according to *GP 2a*. Recrystallization from AcOEt/hexane yielded **1a** (1.050 g, yield 95%). White solid $[\alpha]_D^{20} = -42.3$ (*c* = 0.13, MeOH). IR: 3318*w*, 2975*w*, 1738*m*, 1686*m*, 1649*s*, 1521*s*, 1495*m*, 1454*m*, 1436*m*, 1390*m*, 1365*m*, 1293*m*, 1245*s*, 1164*s*, 1045*m*, 1022*m*, 927*w*, 865*m*, 753*m*, 699*s*, 642*m*. ^{1}H-NMR (300 MHz): 1.38 (*s*, Me_3C); 2.74–2.94 (*m*, CH_2); 3.02 (*dd*, *J* = 7.9, 14.0, 1 H, CH_2); 3.12 (*dd*, *J* = 5.2, 14.0, 1 H, CH_2); 3.72 (*s*, MeO); 4.32–4.44 (*m*, CH); 4.70–4.80 (*m*, CH); 7.20–7.40 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz): 29.3 (Me_3C); 39.7, 40.5 (CH_2); 53.2 (CH); 53.3 (MeO); 57.3 (CH); 80.8 (Me_3C); 128.0, 129.5, 130.5 (arom. CH); 138.6 (arom. C); 157.7, 172.2, 174.7 (CO). HR-MS (MALDI): 785.285 ($[M + Na]^+$, $C_{36}H_{50}N_4NaO_{10}S_2^+$; calc. 785.286). The molecular peak ($C_{36}H_{50}N_4O_{10}S_2$) was not observed.

*(2*S*,5*R*,10*R*,13*S*)-Dimethyl 2,13-Diamino-3,12-dioxo-1,14-diphenyl-7,8-dithia-4,11-diazatetradecane-5,10-dicarboxylate* ([H-(*S*)-Phe-(*R*)-Cys-OMe]$_2$; **1b**). Dipeptide **1a** (0.69 mmol, 525 mg) was Boc-deprotected according to *GP 1a*. The residue was lyophilized from MeCN/H_2O (30%, containing 0.1% TFA) to give the TFA salt of **1b**, which was used without further purifications (504 mg, yield 92%). Part of this product was purified by RP-HPLC (*C18*; 5% of *B* for 5 min, then from 5 to 57% in 40 min; t_R 27 min, 40% *B*). $[\alpha]_D^{20} = -58.5$ (c = 0.82, MeOH). IR: 2955*m*, 1740*m*, 1665*s*, 1522*m*, 1456*m*, 1437*m*, 1305*m*, 1176*s*, 1129*s*, 1023*m*, 835*m*, 798*s*, 747*m*, 721*s*, 700*s*. ^{1}H-NMR (300 MHz): 3.00–3.13 (*m*, CH_2); 3.19–3.28 (*m*, CH_2); 3.74 (*s*, MeO); 4.21 (*dd*, *J* = 5.9, 8.1, CH); 4.79 (*dd*, *J* = 5.3, 8.2, CH). ^{13}C-NMR (75 MHz): 38.6, 40.3 (CH_2); 53.2 (CH); 53.4 (MeO); 55.6 (CH); 128.9, 130.2, 130.7 (arom. CH); 135.5

(arom. C); 170.0, 171.8 (CO). HR-MS (MALDI): 563.199 (M^+, $C_{26}H_{35}N_4O_6S_2^+$; calc. 563.199), 585.181 ($[M+Na]^+$).

7. *Preparation of [Boc-(*R*)-Cys-(*S*)-Ala-OMe]$_2$* (**2a**) *and [H-(*R*)-Cys-(*S*)-Ala-OMe]$_2$* (**2b**). *(2*R*,5*S*,10*S*,13*R*)-Dimethyl 5,10-Bis[(*tert*-butoxycarbonyl)amino]-2,13-dimethyl-7,8-dithia-3,12-diazatetradecanedioate* ([Boc-(*R*)-Cys-(*S*)-Ala-OMe]$_2$; **2a**). H-(*S*)-Ala-OMe (5.0 mmol, 516 mg) was coupled with [Boc-(*R*)-Cys-OH]$_2$ (2.28 mmol, 1.0 g) according to *GP 2a*. Recrystallization from AcOEt/hexan yielded **2a** (795 mg, 57%). White solid. M.p. 151.7–154.6. $[\alpha]_D^{20} = -91.0$ ($c = 0.12$, MeOH). ^{1}H-NMR (300 MHz): 1.40 (*d*, $J = 7.5$, Me); 1.45 (*s*, Me_3C); 2.90 (*dd*, $J = 9.6$, 12.3, 1 H, CH_2); 3.12–3.21 (*m*, 1 H, CH_2); 3.71 (*s*, MeO); 4.38–4.52 (*m*, 2 CH). ^{13}C-NMR (75 MHz): 18.1 (Me); 29.3 (Me_3C); 43.2 (CH_2)[12]; 53.4 (MeO); 55.8 (CH); 81.5 (Me_3C); 158.3, 173.5, 175.0 (CO). HR-MS (MALDI): 649.199 ($[M+K]^+$), 633.224 ($[M+Na]^+$, $C_{24}H_{42}N_4NaO_{10}S_2^+$; calc. 633.223), 611.241 (M^+).

*(2*R*,5*S*,10*S*,13*R*)-Dimethyl 5,10-Diamino-2,3-dimethyl-7,8-dithia-3,12-diazatetradecanedioate* ([H-(*R*)-Cys-(*S*)-Ala-OMe]$_2$; **2b**). Compound **2a** (0.82 mmol, 500 mg) was Boc-deprotected according *GP 1a*. Lyophilization from dioxane afforded **2b** (337 mg, >98%), used as such without further purifications. White solid. $[\alpha]_D^{20} = -121.9$ (c = 0.31, MeOH). IR: 2961*w*, 1720*m*, 1663*s*, 1556*m*, 1431*m*, 1383*m*, 1293*m*, 1178*s*, 1130*s*, 1058*m*, 974*m*, 833*m*, 800*m*, 719*s*, 637*m*. ^{1}H-NMR (300 MHz): 1.43 (*d*, $J = 7.1$, Me); 3.10 (*dd*, $J = 8.8$, 14.7, 1 H, CH_2); 3.46 (*dd*, $J = 4.1$, 14.7, 1 H, CH_2); 3.72 (*s*, MeO); 4.26–4.37 (*m*, CH); 4.41–4.56 (*m*, CH). ^{13}C-NMR (75 MHz): 16.8 (Me); 38.8 (CH_2); 49.26 (CH); 52.3 (CH); 52.5 (MeO); 168.1, 173.6 (CO). HR-MS (MALDI): 433.118 ($[M+Na]^+$), 411.136 (M^+, $C_{14}H_{27}N_4O_6S_2^+$; calc. 411.137).

8. *Preparation of Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys-OH* (**3**). Boc-(*R*)-β^{3}hCys(Bn)-OMe (2.18 mmol, 742 mg) was Boc-deprotected according to *GP 1b* and coupled with Boc-(*S*)-β^{3}hPhe-OH (2.18 mmol, 611 mg) according to *GP 2b*. Filtration on silica gel (CH_2Cl_2/Et_2O/hexan 0.5:1:1) afforded Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys(Bn)-OMe (1.068 g, 98%). White solid. M.p. 146.9–147.7. $[\alpha]_D^{20} = -18.7$ (c = 1.12, MeOH). IR: 3346*m*, 3027*w*, 1733*m*, 1685*s*, 1641*m*, 1524*s*, 1436*m*, 1367*m*, 1345*m*, 1328*m*, 1310*m*, 1288*m*, 1272*m*, 1249*m*, 1213*m*, 1159*s*, 1139*m*, 1131*m*, 1098*m*, 1082*m*, 1072*m*, 1042*m*, 1032*m*, 1020*s*, 997*m*, 762*m*, 735*m*, 696*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.40 (*s*, Me_3C); 2.21 (*dd*, $J = 5.8$, 15.1, 1 H, CH_2); 2.36 (*dd*, $J =$ 4.9, 15.1, 1 H, CH_2); 2.50–2.86 (*m*, 2 CH_2, 1 H of CH_2); 2.88–3.02 (*m*, 1 H, CH_2); 3.67 (*s*, MeO); 3.73 (*s*, PhCH_2S); 3.98–4.13 (*m*, CH); 4.35–4.50 (*m*, CH); 5.40–5.55 (*m*, NH); 6.14 (*d*, $J = 8.8$, NH); 7.15–7.40 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 28.3 (Me_3C); 34.7, 36.1, 36.8, 38.8, 40.1 (CH_2); 44.8, 49.4 (CH); 51.7 (MeO); 79.1 (Me_3C); 126.3, 127.1, 128.4, 128.5, 128.8, 129.2 (arom. CH); 137.8, 138.0 (arom. C); 155.3, 170.3, 171.6 (CO). HR-MS (MALDI): 539. 1970 ($[M+K]^+$), 523.223 ($[M+Na]^+$, $C_{27}H_{36}N_2NaO_5S^+$; calc. 523.224), 501.242 (M^+).

*Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys(Bn)-OH.* Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys(Bn)-OMe (134 mg, 0.267 mmol) was dissolved in MeOH (4 ml) and 5N NaOH (4 ml) and the soln. was warmed to 50° and kept at this temp. for 12 h. The solvent was removed under vacuum, and the residue was diluted with AcOEt and washed with 5N HCl. The org. phase was washed with brine, dried (Na_2SO_4), and the solvent was removed under vacuum. The residue was dissolved in dioxane and lyophilized to afford Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys(Bn)-OH (130 mg, >99%) and used without further purifications. White solid. $[\alpha]_D^{20} = -18.0$ (c = 0.2, MeOH). IR: 3307*w*, 2922*w*, 1717*m*, 1686*m*, 1666*m*, 1625*m*, 1516*m*, 1453*m*, 1408*m*, 1391*m*, 1366*m*, 1307*m*, 1274*m*, 1248*m*, 1210*m*, 1158*s*, 1081*m*, 1023*m*, 914*m*, 856*m*, 778*m*, 756*m*, 701*s*, 664*m*, 622*m*. ^{1}H-NMR (300 MHz): 1.34 (*s*, Me_3C); 2.34 (*d*, $J = 6.5$, CH_2); 2.45–2.78 (*m*, 2 CH_2, 1 H of CH_2); 2.86 (*dd*, $J = 5.9$, 13.5, 1 H, CH_2); 3.75 (*s*, PhCH_2S); 4.00–4.16 (*m*, CH); 4.35–4.48 (*m*, CH); 7.11–7.41 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz): 28.8 (Me_3C); 36.2, 36.9, 38.9, 41.6, 41.9 (CH_2); 47.0, 51.3 (CH); 80.0 (Me_3C); 127.4, 128.0, 129.4 129.5 130.2, 130.6 (arom. CH); 139.8 (2 overlapped arom. C); 157.6, 173.0, 174.5 (CO). HR-MS (MALDI): 525.182 ($[M+K]^+$), 509.207 ($[M+Na]^+$, $C_{26}H_{34}N_2NaO_5S^+$); calc. 509.208).

*(*R*)-3-[(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-phenylbutanamido]-4-(sulfanyl)butanoic acid* (Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys-OH; **3**). Boc-(*S*)-β^{3}hPhe-(*R*)-β^{3}hCys(Bn)-OH was deprotected at the cysteine side chain according to *GP 3* to afford **3** (47 mg, >99%). White solid, which was used without further purifications. M.p. 143.2 (dec.). $[\alpha]_D^{20} = -26.4$ (c = 0.16, MeOH). IR: 3287*w*, 2929*w*, 1680*m*, 1645*m*, 1555*s*,

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[12]) The missing CH signal is overlapped by the signal of the solvent.

1410*m*, 1367*m*, 1249*m*, 1156*s*, 1114*s*, 1046*m*, 1024*m*, 756*m*, 736*m*, 699*s*, 661*m*. ^{1}H-NMR (300 MHz): 1.34 (*s*, *Me*$_3$C); 2.20–2.47 (*m*, CH_2); 2.50–3.05 (*m*, 3 CH_2); 3.98–4.18 (*m*, CH); 4.20–4.40 (*m*, CH); 7.10–7.30 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz): 28.8 (*Me*$_3$C); 30.8, 38.0, 41.9, 42.0 (CH_2); 50.2, 51.4 (CH); 80.1 (Me_3C); 127.4, 129.4, 130.6 (arom. CH); 139.8 (arom. C); 157.6, 173.2, 174.5 (CO). HR-MS (MALDI): 419.161 ($[M+Na]^+$, $C_{19}H_{28}N_2NaO_5S^+$; calc. 419.161), 397.179 (M^+).

9. *Preparation of Boc-(*R*)-β*^{3}*hCys-(*S*)-β*^{3}*hAla-OH* (**4**). Boc-(*R*)-β^{3}hCys(Bn)-(*S*)-β^{3}hAla-OBn. Boc-(*S*)-β^{3}hAla-OBn (3.69 mmol, 1.08 g) was Boc-deprotected according to *GP 1b* and coupled with Boc-(*R*)-β^{3}hCys(Bn)-OH (3.69 mmol, 1.20 g) according to *GP 2b*. FC (Et_2O/hexan 1:1.5) afforded Boc-(*R*)-β^{3}hCys(Bn)-(*S*)-β^{3}hAla-OBn (918 mg, yield 50%). White solid. M.p. 123.0–124.3. $[\alpha]_D^{20} = -15.7$ (*c* = 0.42, MeOH). IR: 3353*w*, 3316*w*, 2980*w*, 1729*s*, 1720*s*, 1683*s*, 1635*s*, 1525*s*, 1495*m*, 1454*m*, 1433*w*, 1411*w*, 1391*w*, 1370*w*, 1362*w*, 1347*m*, 1301*s*, 1272*m*, 1242*m*, 1215*m*, 1197*m*, 1171*s*, 1159*s*, 1137*s*, 1065*m*, 1041*m*, 1022*s*, 996*m*, 970*m*, 911*w*, 875*w*, 777*w*, 755*m*, 745*m*, 696*s*, 689*s*. ^{1}H-NMR (300 MHz): 1.14 (*d*, *J* = 6.8, Me); 1.43 (*s*, *Me*$_3$C); 2.24–2.62 (*m*, 3 CH_2); 3.73 (*s*, PhC*H*$_2$S); 3.97–4.10 (*m*, CH); 4.20–4.33 (*m*, CH); 5.07 (*d*, *J* = 12.1, 1 H, PhC*H*$_2$O); 5.12 (*d*, *J* = 12.1, 1 H, PhC*H*$_2$O); 7.18–7.40 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz): 20.1 (Me); 28.5 (*Me*$_3$C); 36.3, 36.6, 41.1, 41.4 (CH_2); 43.4 (CH)[12]; 67.0 (CH_2); 79.8 (Me_3C); 127.5, 128.7, 128.8, 128.9, 129.1, 129.7 (arom. CH); 137.0, 139.3 (arom. C); 157.0 (Me_3CO); 171.7, 171.9 (CO). HR-MS (MALDI): 501.241 (M^+), 523.223 ($[M+Na]^+$, $C_{27}H_{36}N_2NaO_5S^+$; calc. 523.224), 539.197 ($[M+K]^+$).

*(*S*)-3-{(*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-sulfanylbutanamido}butanoic acid* (Boc-(*R*)-β^{3}hCys-(*S*)-β^{3}hAla-OH; **4**). Boc-(*R*)-β^{3}hCys(Bn)-(*S*)-β^{3}hAla-OBn (320 mg, 0.64 mmol) was deprotected at the cysteine side chain according to *GP 3* to yield **4** (206 mg, >99%), which was used without further purifications. White solid. M.p. 135 (dec.). $[\alpha]_D^{20} = -8.2$ (c = 1.2, MeOH). IR: 3334*w*, 2976*w*, 1686*s*, 1688*m*, 1603*w*, 1524*s*, 1445*w*, 1423*w*, 1391*w*, 1367*m*, 1351*w*, 1275*m*, 1248*m*, 1164*s*, 1108*w*, 1060*m*, 1029*m*, 984*w*, 935*w*, 858*w*, 778*w*, 751*w*, 648*m*. ^{1}H-NMR (300 MHz): 1.19 (*d*, *J* = 6.5, Me); 1.43 (*s*, *Me*$_3$C); 2.34–2.44 (*m*, CH_2, 1 H of CH_2); 2.53 (*dd*, *J* = 15.6, *J* = 6.2, 1 H, CH_2); 2.60–2.65 (*m*, CH_2); 3.87–4.00 (*m*, CH); 4.17–4.30 (*m*, CH). ^{13}C-NMR (75 MHz): 20.0 (Me); 28.4 (*Me*$_3$C); 29.0, 40.3, 41.1 (CH_2); 43.4, 51.8 (CH); 80.0 (Me_3C); 157.4, 171.9, 174.4 (CO). HR-MS (MALDI): 343.130 ($[M+Na]^+$, $C_{13}H_{24}N_2NaO_5S^+$; calc. 543.130), 321.148 (M^+).

10. *Preparation of β-tridecapeptide* **5**. *$CF_3CO_2H \cdot H_2N$-β^{3}hCys-β^{3}hAla-β^{3}hLeu-β^{3}hLys-β^{3}hLeu-β^{3}hPhe-β^{3}hAsp-β^{3}hVal-β^{3}hPhe-β^{3}hLys-β^{3}hAla-β^{3}hIle-β^{3}hAsp-OH* (**5**). Esterification of the Fmoc-β^{3}hAsp(*t*-Bu)-OH with *Wang* resin was performed according to [26] by the MSNT/MeIm method. The resin was placed into a dried manual SPPS reactor, swelled in CH_2Cl_2 (20 ml/g resin) for 1 h, and washed with CH_2Cl_2. In a separate dry round-bottomed flask equipped with magnetic stirrer, Fmoc-β^{3}hAsp(*t*-Bu)-OH (1,25 mmol, 532 mg, 5 equiv.) was dissolved in CH_2Cl_2 (4 ml), then MeIm (3.75 equiv.) and MSNT (5 equiv.) were added under Ar. Stirring was continued until MSNT was dissolved. Thereafter, the soln. was transferred *via* syringe to the SPPS reactor containing the resin (269 mg, 0.93 mmol/g, 100–200 mesh) and mixed by Ar bubbling for 2 h. Subsequently, the resin was filtered, washed with DMF (5 ml, 5 × 1 min) and CH_2Cl_2 (5 ml, 5 × 1 min), and dried under h.v. for 24 h. The resin substitution was determined by measuring the absorbance of the dibenzofulvene–piperidine adduct: two aliquots of the Fmoc-amino acid resin were weighed exactly and suspended in piperidine (20%) in DMF, in volumetric flasks (10 ml). After 30–40 min, the mixtures were transferred to a UV cell and piperidine (20%) to another UV cell (blank), and the absorbance was measured at 290 nm. The concentrations (c_1 and c_2, [mM]) of the benzofulvene–piperidine adduct in soln. were determined using a calibration curve [26]. The loading (*Subst*) was then calculated according to *Eqn. 1:*

$$Subst\ [\text{mmol/g resin}] = c \cdot V / \{m(\text{resin}) \cdot [c \cdot V \cdot (\text{MW} \cdot 18)/1000]\} \quad (1)$$

(MW = molecular weight of the Fmoc-protected amino acid)

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

$$\text{Loading yield} = [(Subst_1 + Subst_2)/2]/Subst_{\text{theor.}} \quad (2)$$

The loading was determined to be 0.67 mmol/g (72%), corresponding to 0.18 mmol of Fmoc-(*S*)-β^3hAsp-OH. The peptide resin was covered with DMF (5 ml), and unreacted OH groups were capped using Ac_2O (1 ml, 10 equiv.) and 4-(dimethylamino)pyridine (DMAP; 50 mg, 0.1 equiv.) for 2 h under Ar bubbling. The resin was then washed with DMF (5 ml, 5 × 1 min) and CH_2Cl_2 (5 ml, 5 × 1 min). The Fmoc group was removed by treating the resin with 20% piperidine in DMF (4 ml, 4 × 10 min) under Ar bubbling. After filtration, the resin was washed with DMF (5 ml, 4 × 1 min). Solid-phase synthesis was continued by sequential incorporation of Fmoc-protected amino acids. For each coupling step, the resin was treated with a soln. of the Fmoc-protected amino acid (4 equiv.), HATU (3.7 equiv.), and $EtN(i\text{-}Pr)_2$ (DIPEA; 8 equiv.) in DMF (5 ml) for 1 h. Monitoring of the coupling reaction was performed with the TNBS test [41]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react further for 0.5–4 h, or, after filtration, the peptide–resin was treated again with the same Fmoc-protected amino acid (1–3 equiv.) and with the coupling reagents. The resin was then filtered and washed with DMF (5 ml, 5 × 1 min) prior to the following deprotection step. For the last coupling, Boc-protected β^3hCys(trityl)-OH [42] was used. The cleavage from the resin and the peptide deprotection were performed according to [27]. The dry peptide–resin was suspended in a soln. of TFA/H_2O/ethane-1,2-dithiol (EDT)/triisopropylsilane (TIS) 94:2.5:2.5:1 (10 ml) for 2.5 h. The resin was removed by filtration, washed with TFA (2 ×), and the org. phase was concentrated under reduced pressure. The resulting oily residue was treated with cold Et_2O, and the formed precipitate was separated. Part of the crude peptide (60 mg) was purified by prep. RP-HPLC (*C-8*, 50% *B* for 5 min then from 50 to 63% *B* in 20 min, flow rate: 17 ml/min, t_R 15 min, 56% B) to afford 20 mg (33%) of **5** as TFA salt. White solid. Anal. RP-HPLC (*C-4*, 2–95% *B* in 40 min, t_R 23.4 min, 55% B): purity >97%. ^{1}H-NMR (400 MHz): 0.80–1.10 (*m*, 9 Me); 1.11–1.23 (*m*, Me, 2 CH_2); 1.24–1.80 (*m*, 7 CH_2, 4 CH); 2.19–3.10 (*m*, 13 α-CH_2, 7 CH_2); 4.26–4.80 (*m*, 13 β-CH); 7.10–7.39 (*m*, 10 arom. H); 8.16 (*d*, J = 8.7, NH); 8.29 (*d*, J = 9.6, NH); 8.33–8.50 (m, 2 NH); 8.59 (d, J = 8.6, NH). HR-MS (MALDI): 1665.009 (M^+, $C_{83}H_{137}N_{15}O_{18}S^+$; calc. 1665.006). For the CD spectrum, see *Fig. 4*.

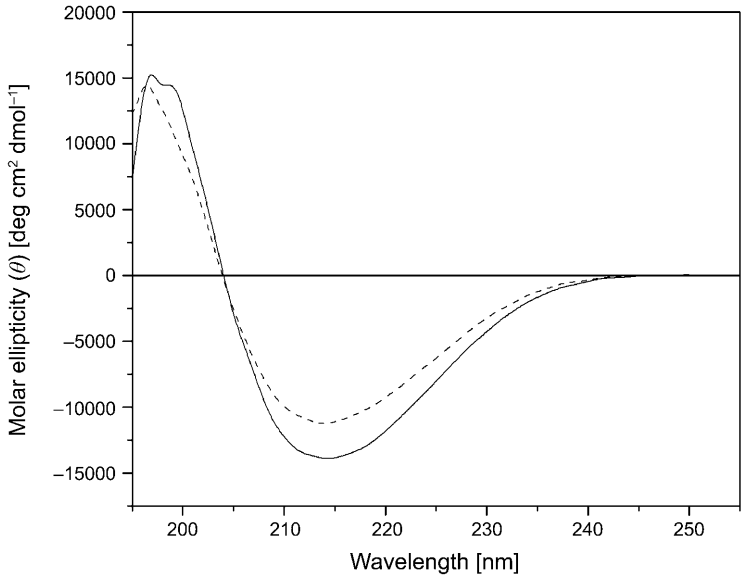

Fig. 4. *Normalized CD spectra of compounds* **5** *and* **11** *in MeOH* (0.2 mM). Solutions were prepared by weighing the peptides on a μg-precision balance and dissolving them in volumetric flasks.

11. *Preparation of α-Octapeptide* **6**. *$CF_3CO_2H \cdot H_2N$-(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr-ol* (**6**). Octreotide acetate (130 mg, 0.12 mmol) was dissolved in 10 ml of degassed phosphate buffer

(100 mM, pH 7.5), and 1,4-dithio-DL-threitol (DTT; 278 mg, 1.8 mmol) was added, and the mixture was stirred for 1 h. Then the soln. was diluted with H_2O (containing 0.1% TFA) and purified by RP-HPLC. Prep. RP-HPLC (*C-8*, from 5% to 70% *B* in 50 min, t_R 32 min, 47% B) afforded 105 mg (70%) of **6** as a TFA salt. White solid. Anal. RP-HPLC (*C-8*, 5–95% *B* in 40 min, t_R 18 min, 46% B): purity > 98%. LC/MS (*C-18*, 5% *B* for 2 min, then from 5 to 95% *B* in 20 min, t_R 15 min, $[M+H]^+$: 1022.3; ($[M+2H]^+/2$): 512.8). ^{1}H-NMR (300 MHz): 0.70–0.80 (*m*, 2 H); 1.16 (*d*, *J* = 6.6, Me); 1.22 (*d*, *J* = 6.3, Me); 1.27–1.47 (*m*, 4 H); 1.67–1.82 (*m*, 1 H); 2.60–2.75 (*m*, 2 H); 2.80–3.05 (*m*, 5 H); 3.07–3.27 (*m*, 3 H); 3.55–3.70 (*m*, 2 H); 3.80–3.90 (*m*, 1 H); 4.01–4.30 (*m*, 4 H); 4.33–4.46 (*m*, 2 H); 4.58–4.68 (*m*, 1 H); 4.70–4.83 (*m*, 2 H); 7.00–7.43 (*m*, 14 arom. H); 7.50 (*d*, *J* = 7.7, arom. H); 7.67 (*d*, *J* = 9.1, NH); 8.12 (*d*, *J* = 8.5, NH); 8.23 (*d*, *J* = 7.8, NH); 8.32 (*d*, *J* = 8.0, NH); 8.39 (*d*, *J* = 8.0, NH); 8.51 (*d*, *J* = 5.8, NH); 10.35 (br. *d*, NH). HR-MS (ESI): 1021.468 ($[M+H]^+$), 1043.446 ($[M+Na]^+$, $C_{49}H_{68}N_{10}NaO_{10}S_2^+$; calc. for 1043.445).

12. *Trifluoromethylation of Peptide Cysteine Side Chain in α-Peptides. (*S*)-Methyl 2-[(*S*)-2-[(*tert-*Butoxycarbonyl)amino]-3-phenylpropanamido]-3-[(trifluoromethyl)sulfanyl]propanoate* (Boc-(*S*)-Phe-(*R*)-Cys(CF_3)-OMe; **7a**). Compound **1a** (76 mg, 0.1 mmol) was trifluoromethylated according to *GP 4a*. FC (cyclohexanes/AcOEt 2:1) afforded **7a** (49.3 mg, 55%). White solid. Part of the peptide was further purified by RP-HPLC (*C-18*, from 5% to 80% of *B* in 60 min; t_R 55 min, 70% B). Anal. RP-HPLC (*C-18*, from 5% to 95% of *B* in 40 min, flow rate 1 ml/min; t_R 29 min, 70% B). M.p. 122.3–122.8. $[\alpha]_D^{20} = -18.7$ (c = 0.12, MeOH). IR: 3324*w*, 2936*w*, 1742*m*, 1682*m*, 1665*s*, 1518*s*, 1438*m*, 1392*m*, 1368*m*, 1321*m*, 1297*m*, 1250*m*, 1218*m*, 1148*s*, 1106*s*, 1046*m*, 1027*m*, 934*w*, 863*m*, 796*m*, 756*m*, 698*m*, 642*m*. ^{1}H-NMR (300 MHz): 1.40 (*s*, Me_3C); 2.82 (*dd*, *J* = 9.0, 13.8, 1 H, CH_2); 3.09 (*dd*, *J* = 5.8, 13.8, 1 H, CH_2); 3.24 (*dd*, *J* = 7.6, 14.3, 1 H, CH_2); 3.45 (*dd*, *J* = 5.6, 14.3, 1 H, CH_2); 3.72 (*s*, MeO); 4.31 (*dd*, *J* = 5.8, 9.0, CH); 4.66 (*dd*, *J* = 5.6, 7.6, CH); 7.18–7.35 (*m*, 5 arom. H). ^{13}C-NMR (63 MHz): 27.2 (Me_3C); 30.2, 37.7 (CH_2); 51.8 (MeO); 52.1, 55.9 (CH); 79.3 (Me_3C); 126.3, 128.0, 129.0 (arom. CH); 131.0 (*q*, *J* = 305.1, CF_3); 137.0 (arom. C); 155.8, 169.7, 173.1 (CO). ^{19}F-NMR (188 MHz): −41.42 (*s*, CF_3), −75.19 (*s*, TFA). HR-MS (EI): 450.144 (M^+, $C_{19}H_{25}F_3N_2O_5S^+$; calc. 450.143).

*(*S*)-Methyl 2-[(*S*)-2-amino-3-phenylpropanamido]-3-[(trifluoromethyl)sulfanyl]propanoate* (H-(*S*)-Phe-(*R*)-Cys(CF_3)-OMe; **7b**). Compound **1b** (55 mg, 0.07 mmol) was trifluoromethylated according to *GP 4a*. FC (cyclohexanes/AcOEt 2:1) afforded **7b** (42 mg, 70%). White solid. Part of the product was further purified by prep RP-HPLC (*C-18*, from 5% to 20% of *B* in 5 min, than from 20% to 70% of *B* in 45 min, t_R 26 min, 43% B). Anal. RP-HPLC (*C-18*, from 5% to 40% of *B* in 30min, t_R 28 min, 40% B). $[\alpha]_D^{20} = -15.2$ (c = 0.56, MeOH). IR: 2960*w*, 1744*m*, 1664*s*, 1530*m*, 1499*m*, 1456*m*, 1439*m*, 1319*m*, 1179*s*, 1104*s*, 837*s*, 798*s*, 756*s*, 721*s*, 700*s*. ^{1}H-NMR (300 MHz): 3.05 (*dd*, *J* = 8.2, 14.3, 1 H, CH_2); 3.22–3.33 (*m*, CH_2); 3.48 (*dd*, *J* = 5.5, 14.3, 1 H, CH_2); 3.76 (*s*, MeO); 4.15 (*dd*, *J* = 6.0, 8.2, CH); 4.75 (*dd*, *J* = 7.7, 5.5, CH); 7.26–7.42 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz): 30.1, 37.0 (CH_2); 51.9 (Me); 52.2, 54.0 (CH); 127.4, 128.7, 129.1 (arom. CH); 130.8 (*q*, *J* = 302.7, CF_3); 133.8 (arom. C); 168.3, 169.3 (CO). ^{19}F-NMR (282 MHz): −41.37 (*s*, SCF_3), −75.19 (*s*, TFA). HR-MS (ESI): 351.099 ($[M+H]^+$, $C_{14}H_{18}F_3N_2O_3S^+$; calc. 351.098).

*(*S*)-Methyl 2-[(*R*)-2-[(*tert-*butoxycarbonyl)amino]-3-[(trifluoromethyl)sulfanyl]propanamido]propanoate* (Boc-(*R*)-Cys(CF_3)-(*S*)-Ala-OMe; **8a**). Compound **2a** (60.9 mg, 0.1 mmol) was trifluoromethylated according *GP 4a*. FC (cyclohexanes/AcOEt 2:1) afforded **8a** (0.116 mmol, 58%). White solid. $[\alpha]_D^{20} = -110.0$ (c = 0.05, MeOH). IR: 3315*m*, 1747*m*, 1693*m*, 1657*m*, 1526*m*, 1447*w*, 1389*w*, 1369*w*, 1341*m*, 1293*m*, 1279*m*, 1251*m*, 1213*m*, 1146*m*, 1105*s*, 1050*m*, 1026*m*, 981*m*, 871*m*, 809*w*, 780*w*, 757*m*, 666*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.43 (*d*, *J* = 7.2, Me); 1.48 (*s*, Me_3C); 3.23 (*dd*, *J* = 5.7, 14.1, 1 H, CH_2); 3.32 (*dd*, *J* = 5.7, 14.1, 1 H, CH_2); 3.78 (*s*, MeO); 4.46 (*m*, CH); 4.57 (*m*, CH); 5.29 (*d*, *J* = 8.4, NH); 6.87 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 18.2 (CH_3); 28.2 (Me_3C); 31.3 (CH_2); 48.3 (CH); 52.6 (MeO); 53.5 (CH); 81.1 (Me_3C); 130.7 (*q*, *J* = 306.4, CF_3); 155.3, 169.1, 172.8 (CO). ^{19}F-NMR (188 MHz, $CDCl_3$): −41.2 (*s*, CF_3). HR-MS (EI): 318.049 ($[M-C_4H_9]^+$, $C_{13}H_{21}F_3N_2O_5S^+$; calc. 318.049).

*(*S*)-Methyl 2-[(*R*)-2-amino-3-[(trifluoromethyl)sulfanyl]propanamido]propanoate* ((*R*)-Cys(CF_3)-(*S*)-Ala-OMe; **8b**). Compound **2b** (66 mg, 0.1 mmol) was trifluoromethylated according *GP 4a* to afford **8b** (135 mg crude product, yield 92%, as estimated by ^{1}H-NMR). Part of the product was purified by prep. RP-HPLC (*C-18*, 5% of *B* for 5 min then from 5% to 70% of *B* in 55 min; t_R 24 min, 28% B). Anal. RP-HPLC (*C-18*, 5% of *B* for 5 min then from 5% to 40% of *B* in 30 min; t_R 14 min, 21% B). White solid. $[\alpha]_D^{20} = -10.8$ (c = 0.41, MeOH). IR: 3334*w*, 2962*m*, 1732*s*, 1663*s*, 1599*w*, 1554*w*, 1479*w*, 1452*w*, 1375*w*,

1334*w*, 1231*w*, 1155*w*, 1107*s*, 1050*w*, 980*w*, 929*w*, 843*w*, 756*w*, 618*w*. ^{1}H-NMR (400 MHz): 1.34 (*d*, *J* = 7.3, Me); 3.20–3.23 (*m*, CH_2); 3.27 (*dd*, *J* = 7.9, 14.8, 1 H, CH_2); 3.49 (*dd*, *J* = 5.3, 14.8, 1 H, CH_2); 3.64 (*s*, MeO); 4.08 (*dd*, *J* = 5.3, 8.0, CH); 4.39 (*dd*, *J* = 7.3, 14.4, CH). ^{13}C-NMR (100 MHz): 17.2 (Me); 31.3 (CH_2); 49.8 (CH); 52.9 (MeO); 53.4 (CH); 131.8 (*q*, *J* = 306.0, CF_3); 167.5, 173.9 (CO). ^{19}F-NMR (376 MHz): −42.95 (*s*, CF_3), −75.20 (*s*, TFA). HR-MS (EI): 318.049 ($M^+ - C_4H_9$, $C_{13}H_{21}F_3N_2O_5S$; calc. 318.049).

13. *Trifluoromethylation of the Peptide Cysteine Side Chain in β-Peptides. (*S*)-3-{(*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-phenylbutanamido}-4-[(trifluoromethyl)sulfanyl]butanoic Acid* (Boc-(*S*)-β^3hPhe-(*R*)-β^3hCys(CF_3)-OH; **9**). Compound **3** (31.8 mg, 0.08 mmol) was trifluoromethylated according to *GP 4b* to give **9** (57 mg crude product, yield 90%, as estimated by ^{1}H-NMR). The crude peptide was dissolved in 30% MeCN in H_2O (containing 0.1% TFA) and purified by prep. RP-HPLC (*C-18*, 30% of *B* for 5 min, then from 30% to 70% of *B* in 40 min, t_R 38 min, 62% B). Anal. RP-HPLC (*C-18*, 5% to 95% of *B* in 40 min, t_R 25 min, 60% B). $[\alpha]_D^{20} = -2$ (c = 0.05, MeOH). IR: 3400*w*, 3293*w*, 2981*w*, 1721*m*, 1666*s*, 1632*s*, 1568*m*, 1512*s*, 1452*w*, 1411*w*, 1393*w*, 1367*m*, 1307*m*, 1271*w*, 1254*w*, 1208*m*, 1191*w*, 1147*s*, 1122*s*, 1104*s*, 1083*s*, 1025*m*, 854*w*, 779*w*, 757*w*, 703*s*, 656*w*. ^{1}H-NMR (300 MHz): 1.34 (*s*, Me_3C); 2.30–2.60 (*m*, 2 CH_2); 2.63–2.90 (*m*, CH_2); 3.05–3.18 (*m*, 1 H, CH_2); 3.22 (*dd*, *J* = 5.9, 13.5, 1 H, CH_2); 4.02–4.20 (*m*, $PhCH_2CH$); 4.40–4.55 (*m*, H–C(3)); 7.15–7.35 (*m*, 5 arom. H). ^{13}C-NMR (DMSO, 100 MHz): 28.1 (Me_3C); 37.6, 37.7, 40.5 (CH_2)[13]; 45.4, 49.2 (CH); 77.3 (Me_3C); 125.7, 127.8 (arom. CH); 129.8 (*q*, *J* = 275, CF_3); 131.2 (arom. CH); 138.7 (arom. C); 154.6 (CO); 169.9, 171.5 (CO). ^{19}F-NMR (282 MHz): −40.89 (*s*, CF_3); −75.22 (*s*, TFA). HR-MS (MALDI): 487.149 ($[M + Na]^+$, $C_{20}H_{27}F_3N_2NaO_5S^+$; calc. 487.148). The molecular peak ($C_{36}H_{50}N_4O_{10}S_2^+$) was not observed.

*(*S*)-3-{(*R*)-3-[(*tert*-Butoxycarbonyl)amino]-4-[(trifluoromethyl)sulfanyl]butanamido}butanoic acid* (Boc-(*R*)-β^3hCys(CF_3)-(*S*)-β^3hAla-OH; **10**). Compound **4** (100 mg, 0.31 mmol) was trifluoromethylated according to *GP 4b* to yield **4** (197 mg crude product, yield > 98%, as estimated by ^{1}H-NMR). The crude peptide was dissolved in 30% MeCN in H_2O (containing 0.1% TFA) and purified by prep. RP-HPLC (*C-18*, 30% for 5 min, then from 30% to 70% of *B* in 45 min; t_R 29 min, 53% B). Anal. RP-HPLC (*C-18*, 5% to 95% of *B* in 40 min; t_R 21.3 min, 53% B). M.p. 165.6–166.5. $[\alpha]_D^{20} = -18.2$ (c = 0.48, MeOH). IR: 3388*w*, 2983*w*, 2938*w*, 1720*m*, 1669*s*, 1621*s*, 1551*m*, 1513*s*, 1445*w*, 1419*m*, 1392*w*, 1369*m*, 1320*w*, 1295*w*, 1275*m*, 1251*w*, 1193*m*, 1156*s*, 1106*s*, 1069*m*, 1046*m*, 1032*m*, 952*w*, 877*m*, 845*w*, 781*w*, 756*m*, 664*w*. ^{1}H-NMR (400 MHz): 1.19 (*d*, *J* = 6.7, Me); 1.43 (*s*, Me_3C); 2.38 (*dd*, *J* = 7.1, 15.6, 1 H, CH_2); 2.45 (*d*, *J* = 6.6, CH_2); 2.53 (*dd*, *J* = 6.2, 15.6, 1 H, CH_2); 3.07–3.21 (*m*, CH_2); 4.08–4.20 (*m*, CH); 4.21–4.28 (*m*, CH). ^{13}C-NMR (100 MHz): 20.2 (Me); 28.7 (Me_3C); 34.8, 40.8, 41.3 (CH_2); 43.7 (CH)[12]; 80.0 (Me_3C); 132.6 (*q*, *J* = 378.7, CF_3); 157.4, 171.4, 174.6 (CO). ^{19}F-NMR (282 MHz): −40.95 (*s*, CF_3); −75.22 (*s*, TFA). HR-MS (MALDI): 411.116 ($[M + Na]^+$, $C_{14}H_{23}F_3N_2NaO_5S^+$; calc. 411.117).

$CF_3CO_2H \cdot H_2N$-β^3hCys(CF_3)-β^3hAla-β^3hLeu-β^3hLys-β^3hLeu-β^3hPhe-β^3hAsp-β^3hVal-β^3hPhe-β^3hLys-β^3hAla-β^3hIle-β^3hAsp-OH (**11**). Compound **5** (10 mg, 5 μmol) was trifluoromethylated according to *GP 4b*. The crude peptide was purified by prep. RP-HPLC (*C-8*, 50% *B* for 10 min, then from 50 to 95% *B* in 45 min; t_R 25 min, 64% B) to afford **11** as TFA salt (4.2 mg, 40%). White solid. Anal. RP-HPLC (*C-8*, 5–95% *B* in 40 min; t_R 30 min, 64% B): purity > 98%. LC/MS (*C-18*, 5% *B* for 2 min, then from 5 to 95% *B* in 20 min; t_R 19.9 min; $[M + H]^+$: 1733.7; ($[M + 2\,H]^+/2$): 868.9; ($[M + 3\,H]^+/3$): 580.1; for $[C_{50}H_{65}F_3N_{10}O_{10}S_2]$). ^{1}H-NMR (400 MHz): 0.80–1.10 (*m*, 8 Me); 1.11–1.23 (*m*, 2 Me, CH); 1.24–1.80 (*m*, 9 CH_2, 3 CH); 2.19–3.10 (*m*, 20 CH_2); 4.26–4.80 (*m*, 13 CH); 7.10–7.39 (*m*, 10 arom. H); 8.16 (*d*, *J* = 8.7, NH); 8.29 (*d*, *J* = 9.6, NH); 8.33–8.50 (*m*, 2 NH); 8.59 (*d*, *J* = 8.6, NH). ^{19}F-NMR (282 MHz): −41.21 (*s*, CF_3); −75.22 (*s*, TFA). HR-MS (MALDI): 1732.998 ($[M + H]^+$, $C_{83}H_{138}N_{15}O_{18}S^+$; calc. 1732.993). For the CD spectrum, see *Fig. 4*.

14. *Trifluoromethylation of* **6**. *$CF_3CO_2H \cdot H_2N$-(*D*)Phe-[Cys-Phe-(*D*)-(2-CF_3)Trp-Lys-Thr-Cys]-Thr-ol* (**12**), *$CF_3CO_2H \cdot H_2N$-(*D*)Phe-Cys(CF_3)-Phe-(*D*)-Trp-Lys-Thr-Cys(CF_3)-Thr-ol* (**13**), *and $CF_3CO_2H \cdot H_2N$-(*D*)Phe-Cys(CF_3)-Phe-(*D*)-(2-CF_3)Trp-Lys-Thr-Cys(CF_3)-Thr-ol* (**14**). Compound **6** (17.6 mg, 14.1 μmol) was dissolved in degassed MeOH (7.05 mM) and cooled to −78°. 1,3-Dihydro-3,3-dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (2.9 equiv.) dissolved in degassed MeOH (1 ml) and cooled to −78° was added to this soln., and the mixture was kept at −78° under N_2 for 2 h, and was then

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[13]) The missing CH_2 signals are overlapped by the signal of the solvent.

allowed to warm to r.t. and stirred for 12 h. The solvent was removed under vacuum, and the crude products were purified by prep. RP-HPLC (*C-8*, 5–70% *B* in 50 min) to afford 0.4 mg of **12** (t_R 35 min, 50% B), 3 mg of **13** (t_R 40 min, 57% B), and 3 mg of **14** (t_R 41.5 min, 59% B) as TFA salts. White solids.

Data of **12**. Anal. RP-HPLC (*C-8*, 5–95% *B* in 40 min, t_R 18.3 min): purity >98%. LC/MS (*C-18*, 5% *B* for 2 min, then from 5 to 95% *B* in 20 min; t_R 15.5 min, $[M+H]^+$: 1087.4; $([M+2H]^+/2)$: 544.7; for $[C_{50}H_{65}F_3N_{10}O_{10}S_2]$). ^{1}H-NMR (300 MHz): 0.62–0.90 (*m*, 1 H); 1.00–1.40 (*m*, 7 H); 1.42–1.62 (*m*, 2 H); 1.83–2.22 (*m*, 3 H); 2.42–2.63 (*m*, 2 H); 2.65–3.05 (*m*, 6 H); 3.33–3.42 (*m*, 1 H); 3.50–3.63 (*m*, 3 H); 3.64–3.87 (*m*, 3 H); 3.90–4.07 (*m*, 2 H); 4.09–4.40 (*m*, 3 H); 4.50–4.60 (*m*, 1 H); 5.02–5.17 (*m*, 2 H); 6.95–7.50 (*m*, 14 arom. H). ^{19}F-NMR (282 MHz): −58.32 (s, CF3); −75.21 (s, TFA).

Data of **13**. Anal. RP-HPLC (*C-8*, 5–95% *B* in 40 min; t_R 21.3 min): purity >95%. LC/MS (*C-18*, 5% *B* for 2 min, then from 5 to 95% *B* in 20 min; t_R 16.3 min; $[M+H]^+$: 1157.8; $([M+2H]^+/2)$: 580.3; for $[C_{51}H_{66}F_6N_{10}O_{10}S_2]$). ^{1}H-NMR (300 MHz): 0.70–0.80 (*m*, 1 H); 1.14 (*d*, *J* = 6.6, Me); 1.23 (*d*, *J* = 6.3, Me); 1.27–1.45 (*m*, 4 H); 1.67–1.82 (*m*, 1 H); 2.58–2.78 (*m*, 2 H); 2.78–3.25 (*m*, 8 H); 3.40–3.70 (*m*, 5 H); 3.80–3.90 (*m*, 1 H); 4.01–4.10 (*m*, 1 H); 4.11–4.28 (*m*, 2 H); 4.35–4.45 (*m*, 2 H); 4.55–4.65 (*m*, 2 H); 4.61–4.78 (*m*, 1 H); 7.00–7.43 (*m*, 14 arom. H); 7.52 (*d*, *J* = 7.5, 1 arom. H). ^{19}F-NMR (282 MHz): −41.41 (*s*, SCF3); −41.51 (*s*, SCF3); −75.21 (*s*, TFA).

Data of **14**. Anal. RP-HPLC (*C-8*, 5–95% *B* in 40 min, t_R 22.2 min): purity >98%. LC/MS (*C-18*, 5% *B* for 2 min, then from 5 to 95% *B* in 20 min; t_R 16.3 min; $[M+H]^+$: 1225.8; $([M+2H]^+/2)$: 615.5; for $[C_{52}H_{65}F_9N_{10}O_{10}S_2]$). ^{1}H-NMR (500 MHz): 0.68 (*quint.*, *J* = 7.5, 1 H, CH_2); 1.16 (*d*, *J* = 6.6, Me); 1.18 (*d*, *J* = 6.4, Me); 1.20–1.43 (*m*, 2 CH_2); 1.62–1.72 (*m*, 1 H, CH_2); 2.69–2.73 (*m*, CH_2); 2.95 (*d*, *J* = 7.5, CH_2); 3.01–3.11 (*m*, 3 H, 3 CH_2); 3.23–3.33 (*m*, 3 H, 3 CH_2); 3.39 (*dd*, *J* = 13.9, *J* = 10.3, 1 H, CH_2); 3.49 (*dd*, *J* = 13.5, 6.1, 1 H, CH_2); 3.62–3.72 (*m*, CH_2); 3.87 (*td*, *J* = 6.3, 3.1, CH); 4.08 (*qd*, *J* = 6.6, 3.1, CH); 4.13–4.18 (*m*, 2 CH); 4.26 (*qd*, *J* = 6.4, 5.1, CH); 4.39 (*dd*, *J* = 10.3, 5.7, CH); 4.44 (*d*, *J* = 5.1, CH); 4.60 (*t*, *J* = 7.5, CH); 4.8–4.9 (*m*, CH); 4.92 (*dd*, *J* = 7.5, 6.0, CH); 7.1–7.5 (*m*, 13 arom. H); 7.66 (*d*, *J* = 8.1, arom. H). ^{19}F-NMR (376 MHz): −42.90 (*s*, CF_3); −42.96 (*s*, CF_3); −59.08 (*s*, CF_3).

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