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# One-Pot Synthesis of Orthogonally Protected Enantiopure *S*-(Aminoalkyl)-cysteine Derivatives[‡]

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*Dedicated to the memory of Arno F. Spatola[‡‡]*

**Keywords:** Thia diamino acids / *S*-Alkylation / Diversity / Cysteine / β-Iodoamines

The general synthesis of a new class of non-natural diamino acids, 2-amino-3-[(2′-aminoalkyl)thio]propanoic acids or *S*-(aminoalkyl)cysteines, is reported. Under the conditions devised, enantiopure *N*-Boc-protected β-iodoamines, readily generated from proteinogenic α-amino acids, are treated with L-cysteine ethyl ester hydrochloride, using $Cs_2CO_3$ as a base. The *S*-alkylation products, obtained in high yields (96–98 %) and without any detectable traces of accompanying byproducts, are hydrolysed to yield the free carboxyl group. An orthogonal protection is then introduced on the free amino group by treatment with Fmoc-OSu under standard conditions. The inclusion of one of these orthogonally protected diamino acids in a solid-phase growing pentapeptide is also reported.


## Introduction

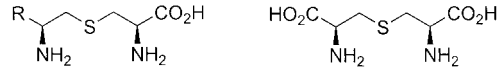
Nowadays it is thoroughly recognized that natural peptides, despite the potential interest to exploit them as pharmaceutical lead compounds, are indeed poor drug candidates because of their low oral bioavailability, potential immunogenicity and insufficient metabolic stability in vivo.[1]

Recent efforts to ameliorate disadvantageous peptide characteristics and thus generate viable pharmaceutical therapies have focused on the creation of non-natural peptide mimics. These "peptidomimetics" can be based on any oligomer that mimics peptide primary structure through the use of amide bond isosteres and/or modification of the native peptide backbone, including chain extension or heteroatom incorporation.[2] Peptidomimetic oligomers are often protease-resistant and may have reduced immunogenicity and improved bioavailability relative to peptide analogues.

In addition to primary structural mimicry, a select subset of the sequence-specific peptidomimetic oligomers, the so-called "foldamers",[3] exhibits well-defined secondary structural elements such as helices, turns and small sheetlike structures. When peptide bioactivity is contingent upon a precise 3-D structure, the capacity of a biomimetic oligomer to fold can be indispensable. Examples of simple peptidomimetics include azapeptides, oligocarbamates and oligoureas, and common examples of foldamers include β-peptides, γ-peptides, oligo(phenylene ethynylene)s, vinylogous sulfonopeptides and poly-*N*-substituted glycines (peptoids).[2–5]

However, the main road to generate peptide diversity still resides on the preparation of non-natural amino acids and their incorporation in specific (bioactive) peptide sequences.

In this paper, we report the enantioselective synthesis of new thia diamino acids **1** that represent a new class of non-natural amino acids resembling natural lanthionine (**2**)[6] one of whose carboxyl groups has been replaced by the side chain (R in **1**) of a proteinogenic α-amino acid.




**1** **2**

To the best of our knowledge, the sole literature precedent for these compounds is the so-called "thialysine" ("Tlys") (R = H in **1**), known for its interesting cytotoxic properties towards human acute leukaemia Jurkat T cells.[7] Thialysine, whose preparation dates back to nearly half a century ago,[8,9] should formally represent the parent compound of this class of thia diamino acids.

In view of the biological properties that could be expected for such compounds and also their usefulness in in-

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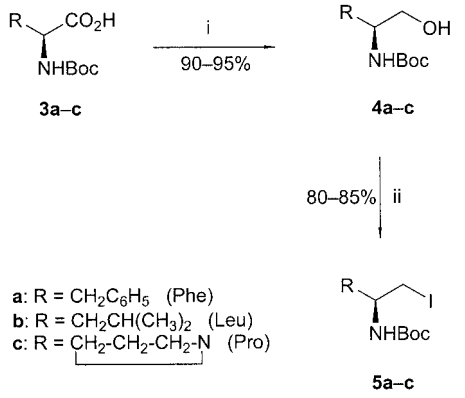
troducing elements of diversity if incorporated in synthetic peptide molecules, we have realized a simple and efficient synthesis that leads to orthogonally protected thia diamino acids **1**, by *S*-alkylation of *C*-protected L-cysteine with different *N*-Boc-β-iodoamines **5**, in their turn prepared as depicted in Scheme 1.



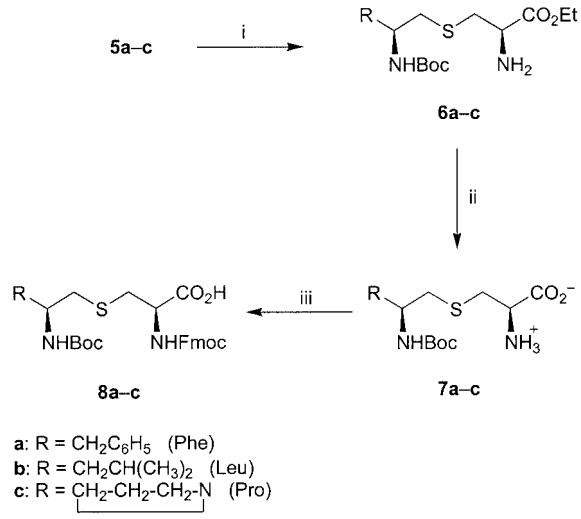

Scheme 1. Conversion of *N*-Boc-*α*-amino acids into *N*-Boc-β-iodoamines. i) THF, NMM, MeOCOCl, then $NaBH_4$ in $H_2O$; ii) TPP-$I_2$, ImH (imidazole), DMC, reflux, 1 h.

In fact, the reaction is known, and several reports of *S*-alkylation of *N*-Boc-β-iodoamines by miscellaneous mercaptans can be found in the current literature.[10–14] The rationale of their use in the synthesis of thia diamino acids **1** lies in the opportunity to access a series of synthetic building blocks, chiral aminoalkyl cation equivalents that carry through the chirality of their parent proteinogenic α-amino acids.

## Results and Discussion

The enantiopure *N*-Boc-β-iodoamines **5a**–**c** were readily prepared in excellent yields from the corresponding natural α-amino acids **3a**–**c**, as depicted in Scheme 1. The *N*-Boc-protected β-amino alcohols **4a**–**c**, coming from the reduction of the α-amino acids, were converted into the corresponding iodoamines by using a polymer-bound triarylphosphane/$I_2$ complex in dry dichloromethane (DCM) in the presence of imidazole.[15] In consideration of the somewhat high cost of such a triarylphosphane, we also carried out the conversion by using the inexpensive triphenylphosphane, with quite comparable results (see Exp. Sect.).

Under our experimental conditions, the *N*-Boc-β-iodoamines **5a**–**c**, generated from Phe, Leu and Pro,[16] respectively, were treated with L-cysteine ethyl ester hydrochloride and $Cs_2CO_3$, in dry DMF under argon (Scheme 2). The *S*-alkylations proceeded in high yields (96–98%); neither alkylation of the cysteinyl amino group nor traces of elimination products and/or aziridines from **5a**–**c** could be detected by TLC or $^1H$ NMR of the crude alkylation products **6a**–**c**.




Scheme 2. Synthesis of orthogonally protected thia diamino acids. i) HCl·L-Cys-OEt, $Cs_2CO_3$, DMF, room temp., 3 h; ii) aq. LiOH, MeOH, room temp., 2 h; iii) Fmoc-OSu, THF, $Na_2CO_3$, 0 °C, 1 h.

The *S*-alkylation procedure may appear as a slight modification of a method already reported for the synthesis of lanthionine derivatives.[17–19] Indeed, the resemblance is only formal, since in our case the *S*-alkylation occurs on an iodide β to a carbamate function (*N*-Boc-β-iodoamines **5**) whereas the cysteine alkylation leading to lanthionines occurs on the β-iodide of *N*-trityl-protected serine alkyl ester that is naturally prone to undergo either hydrogen iodide elimination or aziridine ring closure.

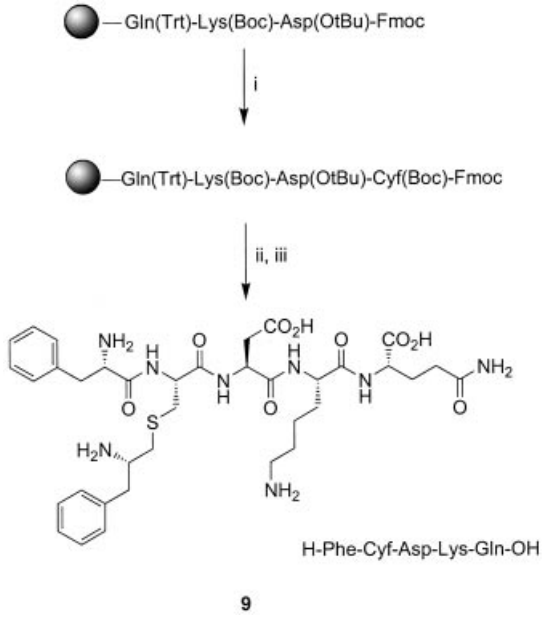
Following alkylation, the products **6a**–**c** were hydrolysed by LiOH in MeOH to the corresponding monoprotected thia diamino acids **7a**–**c** that, without being isolated, were treated with Fmoc-OSu under standard conditions. The final orthogonally protected thia diamino acids **8a**–**c** were obtained in excellent (79–82%) overall yields (based on the starting *N*-Boc-β-iodoamines) after purification by flash chromatography. Their regio- and stereochemical integrity was ascertained by TLC, RP-HPLC, $^1H$ and $^{13}C$ NMR spectroscopy, and MS analyses. DEPT and COSY $^1H$ NMR experiments also showed the absence of traces of other isomers.

In order to avoid complicated systematic names in current laboratory practice, we propose for these new thia diamino acids a nomenclature system in which each compound is given an acronym composed of "Cy" (from "Cys") and the one-letter code denoting the α-amino acid whose side chain (R in formula **1**) is present in it. A referee suggested using the one-letter code in lower case, to be more in connection with the three-letter symbolism of amino acids. Accordingly, the three compounds we have synthesised and reported in this paper should be indicated as Fmoc-L-Cyf(Boc)-OH (**8a**), Fmoc-L-Cyl(Boc)-OH (**8b**) and Fmoc-L-Cyp(Boc)-OH (**8c**).

As an illustrative example of the versatility of our orthogonally protected thia diamino acids, compound **8a** was included in a growing pentapeptide on a PAC-PEG-PS (4-hydroxymethylphenoxyacetic acid polyethyleneglycol poly-

styrene) support with a classical peptide coupling in DMF. HATU [2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] and NMM (*N*-methylmorpholine) were used as activating agents (Scheme 3).




**9**

Scheme 3. Solid-phase synthesis of pentapeptide foldamer **9**. i) 2% DBU in DMF, Fmoc-Cyf(Boc)-OH, HATU, NMM, 1 h; ii) 2% DBU in DMF, Boc-Phe-OH, HATU, NMM, 30 min; iii) TFA, TES, TA, room temp., 2 h.

In the final step, the product was cleaved from the polymeric support, and the expected pentapeptide **9** was obtained. One single compound with the expected molecular weight could be detected by analytical RP-HPLC and MALDI-TOF MS analysis of the crude product.

## Conclusions

We have proposed a synthesis of orthogonally protected thia diamino acids that are ready to be inserted into solid-phase growing peptides to obtain foldamers.

It is noteworthy that the same synthetic approach can even be applied to the modification of native proteins (bioconjugation),[20,21] considering that the most widely used bioconjugation strategy exploits the latent nucleophilicity of the thiol side chain of cysteine. It might be interesting to use chiral β-iodoamines as aminoalkyl cation equivalents for the *S*-alkylation of cysteines and the introduction of proteinogenic α-amino acid moieties in native proteins.

However, successful work is already in progress in our laboratory in preparing new orthogonally protected non-natural diamino acids such as **8a**–**c** with various heteroatoms other than sulfur.

## Experimental Section

**General:** Melting points were measured with a Kofler apparatus and are uncorrected. Optical rotations were measured with a Jasco 1010 polarimeter. RP-HPLC was carried out with a Shimadzu SCL-10 Avp system with a photodiode-array detector, using a binary solvent system consisting of 0.01% TFA in $H_2O$ (solvent A) and 0.01% TFA in MeCN (solvent B); an analytical Vydac $C_{18}$ column was used (4.6 mm diameter with a flow rate of 1 mL/ min). $^1H$ and $^{13}C$ NMR spectra were recorded with Varian Inova 500 and Bruker DRX-400 spectrometers: chemical shifts are in ppm ($\delta$) and $J$ coupling constants in Hz. Low-resolution MALDI-TOF mass spectra were obtained with a Voyager DE-PRO (PE-Biosystem), using 2,3-dihydroxybenzoic acid (DHB) as matrix. High-resolution ES mass spectra were obtained with a Micromass Q-TOF Ultima™ API using Leu-Enk as standard and NaI for calibration. TLC was carried out on silica gel Merck 60 F254 plates (0.2 mm layer thickness) and developed with ninhydrin (0.25% in MeOH) or visualized by UV. Column chromatography was performed on Merck Kieselgel 60 (70–230 mesh). Dry solvents were distilled immediately before use.

**Preparation of *N*-Boc-β-Iodoamines 5a–c. General Procedure:** To a stirred suspension of polystyryl-diphenylphosphane (ca. 3 mmol/g, 4 g, ca. 12 mmol) in dry DMC (70 mL), under argon, $I_2$ (3.62 g, 12.7 mmol) and imidazole (1.70 g, 25 mmol) were added in sequence. After 15 min, one of the *N*-Boc-β-aminols **4a**–**c** (5 mmol), dissolved in the same solvent (10 mL), was added in one portion to the heterogeneous reaction mixture, that was then refluxed for 1 h (TLC monitoring, EtOAc/hexane, 15:85). After cooling, it was diluted with DMC (100 mL) and filtered through a glass septum funnel to remove the polymeric material. The filtrate was washed with aq. 10% $Na_2S_2O_3$ (2 × 50 mL) and finally with pure water, then concentrated in vacuo. The resulting *N*-Boc-β-iodoamines **5a**–**c**, obtained in high yields (95–98%), were used directly in the next step. When, under the same experimental conditions, polystyryl-diphenylphosphane was replaced by soluble TPP (triphenylphosphane) (1.64 g, 6.25 mmol), the final *N*-Boc-β-iodoamines needed further purification by flash chromatography (EtOAc/hexane, 7:3) and were obtained in somewhat lower yields (82–86%).

***tert*-Butyl [(1*S*)-1-Benzyl-2-iodoethyl)carbamate (5a):** 96%; m.p. 110–111 °C (from $Et_2O$/hexane). $[\alpha]_D^{20}$ = +18.3 ($c$ = 1.3, $CHCl_3$) {ref.[22] m.p. 118 °C; $[\alpha]_D^{20}$ = +18.9 ($c$ = 2.9)}; $R_f$ (EtOAc/hexane, 85:15) = 0.51. $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 1.42 (s, 9 H), 2.76 (dd, $J$ = 6.7 and 13.4 Hz, 1 H), 2.90 (dd, $J$ = 5.0 and 13.4 Hz, 1 H), 3.16 (dd, $J$ = 3.3 and 10.0 Hz, 1 H), 3.38 (br. dd, $J$ = 4.0 and 10.0 Hz, 1 H), 3.45–3.70 (m, 1 H), 4.57–4.72 (m, 1 H), 7.20–7.40 (m, 5 H) ppm.

***tert*-Butyl [(1*S*)-1-(Iodomethyl)-3-methylbutyl]carbamate (5b):** 98%; m.p. 58 °C (from $Et_2O$/hexane). $[\alpha]_D^{20}$ = –31.6 ($c$ = 1.5, $CHCl_3$) {refs.[15,23] m.p. 55–57 °C; $[\alpha]_D^{20}$ = –29.9 ($c$ = 1.3)}; $R_f$ (EtOAc/hexane, 85:15) = 0.56. $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 0.92 (d, $J$ = 6.7, 3 H), 0.93 (d, $J$ = 6.7, 3 H), 1.30–1.37 (m, 2 H), 1.45 (s, 9 H), 1.53–1.68 (m, 1 H), 3.28 (dd, $J$ = 2.7 and 9.2 Hz, 1 H), 3.35–3.45 (m, 1 H), 3.47 (dd, $J$ = 3.7 and 9.2 Hz, 1 H), 4.52 (br. d, $J$ = 6.0, 1 H) ppm.

***tert*-Butyl [(1*S*)-2-Iodo-1-(pyrrolidin-1-yl)ethyl]carbamate (5c):** 95%; m.p. 38–40 °C (from EtOAc/hexane). $[\alpha]_D^{20}$ = –34.7 ($c$ = 1.3, $CHCl_3$) {ref.[24] m.p. 102–104 °C; $[\alpha]_D^{20}$ = –32.8 ($c$ = 1.5)}; $R_f$ (EtOAc/hexane, 85:15) = 0.48. $^1H$ NMR ($CDCl_3$, 500 MHz): $\delta$ = 1.46 (s, 9 H), 1.81–1.83 (m, 1 H), 1.86–1.89 (m, 2 H), 2.03–2.05 (m, 1 H), 3.11–3.15 (t, $J$ = 9.5, 1 H), 3.34–3.37 (m, 2 H), 3.43–3.48 (t, 1 H), 3.82–3.85 (m, 1 H) ppm.

***S*-Alkylations of Cysteine Ethyl Ester. General Procedure:** To a stirred solution of one of the β-iodoamines **5a**–**c** (0.33 mmol) and L-cysteine ethyl ester hydrochloride (62 mg, 0.33 mmol) in dry DMF (5 mL) and under argon, solid $Cs_2CO_3$ (216 mg, 0. 66 mmol)

was added in one portion. Stirring was continued in the dark at room temperature for 3 h. Most of the solvent was carefully (< 45 °C) evaporated in vacuo and the residue was suspended in EtOAc (50 mL) and shaken with $H_2O$ (3 × 20 mL). The organic layer was then dried ($Na_2SO_4$) and the solvents were evaporated in vacuo.

**Ethyl (2*R*)-2-Amino-3-{(2′*S*)-2′-[(*tert*-butyloxycarbonyl)amino]-3′-phenylpropylthio}propanoate, H-Cyf(Boc)-OEt (6a):** Oil, ca. 97%; $R_f$ (DCM/MeOH, 95:5) = 0.23. Chromatographically pure sample: $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 1.24 (t, 3 H), 1.39 (s, 9 H), 2.62 (d, $J$ = 5.5, 2 H), 2.76–2.93 (m, 3 H), 2.9 (dd, $J$ = 4.6 and 13.4 Hz, 1 H), 3.60 (dd, $J$ = 4.6 and 7.0 Hz, 1 H), 3.92–4.01 (m, 1 H), 4.15 (q, 2 H), 5.03 (d, $J$ = 8.4, 1 H), 7.16–7.28 (m, 5 H) ppm. $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ = 13.9, 28.1, 36.6, 37.7, 39.3, 51.2, 54.1, 61.0, 79.0, 126.3, 128.2, 129.2, 137.4, 155.1, 173.7 ppm. $C_{19}H_{30}N_2O_4S$ (382.5): calcd. C 59.66, H 7.91; found C 59.61, H 7.87 (specially prepared analytical sample).

**Ethyl (2*R*)-2-Amino-3-{(2′*S*)-2′-[(*tert*-butoxycarbonyl)amino]-4′-methylpentylthio}propanoate, H-Cyl(Boc)-OEt (6b):** Oil, ca. 98%; $R_f$ (DCM/MeOH, 95:5) = 0.21. Chromatographically pure sample: $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 0.82 (d, 6 H), 1.21 (t, 3 H), 1.31 (t, 2 H), 1.41 (s, 9 H), 1.6 (m, 1 H), 2.61 (d, $J$ = 5.5, 2 H), 2.75 (dd, $J$ = 7.0 and 13.4 Hz, 1 H), 2.79 (dd, $J$ = 4.6 and 13.4 Hz, 1 H), 3.51 (dd, $J$ = 4.6 and 7.0 Hz, 1 H), 3.9 (m, 1 H), 4.18 (q, 2 H), 4.9 (br. d, 1 H) ppm. $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ = 14.3, 22.3, 23.2, 25.1, 28.6, 38.4, 39.1, 43.2, 48.6, 54.5, 61.3, 79.7, 155.6, 174.1 ppm. $C_{16}H_{32}N_2O_4S$ (348.5): calcd. C 55.14, H 9.26; found C 55.20, H 9.27 (specially prepared analytical sample).

**Ethyl (2*R*)-2-Amino-3-{[(2′*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2′-yl]methylthio}propanoate, H-Cyp(Boc)-OEt (6c):** Oil, ca. 96%; $R_f$ (DCM/MeOH, 95:5) = 0.29. Chromatographically pure sample: $^1$H NMR ($CD_3OD$, 400 MHz): $\delta$ = 1.28 (t, 3 H), 1.45 (s, 9 H), 1.85–1.97 (m, 4 H), 2.55–2.58 (m, 1 H), 2.84–2.88 (m, 3 H), 3.36–3.38 (m, 2 H), 3.60–3.63 (m, 1 H), 3.87–3.89 (m, 1 H), 4.16–4.21 (q, 2 H) ppm. $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ = 14.4, 22.9, 28.7, 30.2, 36.5, 37.8, 47.2, 54.5, 57.1, 61.3, 79.8, 154.4, 174.1 ppm. $C_{15}H_{28}N_2O_4S$ (332.5), calcd. C 54.19, H 8.49; found C 54.24, H 8.43 (specially prepared analytical sample).

**Alkaline Hydrolysis of Crude 6a–c and Fmoc Protection of the Crude Diamino Acids 7a–c. General Procedure:** To a stirred solution of one of the *S*-alkylated compounds **6a–c** (0.3 mmol) in MeOH (5 mL) aq. 1 M LiOH (1 mL) was added in one portion. After 2 h of stirring at room temperature, MeOH was evaporated in vacuo. The residue was redissolved in THF (10 mL) and the solution cooled to 0 °C. Fmoc-OSu (112 mg, 0.33 mmol) was then added portionwise over 1 h, maintaining an alkaline pH (ca. 10) by dropwise addition of 10% aq. $Na_2CO_3$, then by addition of excess solid $Na_2CO_3$. THF was evaporated in vacuo and the crude reaction product was dissolved in EtOAc (75 mL) and worked up as usual, under acidic conditions (aq. 0.1 M HCl). The final *N*-Fmoc,*N*′-Boc thia diamino acids **8a–c** were purified by flash chromatography, using a 0–5% MeOH gradient in DCM.

**(2*R*)-3-[(2′S)-2′-(*tert*-Butyloxycarbonyl)amino-3′-phenylpropylthio]-2-[(fluorenylmethoxycarbonyl)amino]propanoic Acid, Fmoc-Cyf(Boc)-OH (8a):** 80% (overall yield from **5a**); $R_f$ (DCM/MeOH, 9:1) = 0.47. Analytical sample, crystallized from hot absolute EtOH: M.p. 135–136 °C. $[\alpha]_D^{20}$ = –20.0 ($c$ = 1.0, MeOH). $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ = 1.40 (s, 9 H), 2.62–2.67 (m, 3 H), 2.84–2.90 (m, 2 H), 3.04–3.06 (dd, $J$ = 4.5 Hz, 1 H), 3.86–3.87 (m, 1 H), 4.22–4.27 (t, $J$ = 7.7, 1 H), 4.31–4.37 (m, 3 H), 7.13–7.20 (m, 5 H), 7.27–7.31 (t, $J$ = 7.5 Hz, 2 H), 7.35–7.39 (t, 2 H), 7.67–7.69 (d, $J$ = 7.4 Hz, 2 H), 7.77–7.79 (d, 2 H) ppm. $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ = 29.9, 36.8, 39.5, 42.1, 54.6, 56.6, 59.5, 69.3, 81.2, 122.0, 127.5, 128.4, 129.3, 129.9, 130.4, 131.5, 140.9, 143.7, 146.4, 170.2 ppm. MS-MALDI-TOF ($C_{32}H_{36}N_2O_6S$): $m/z$ = 599.14 [M + $Na^+$], 615.18 [M + $K^+$], 477.30 [M – Boc]. HR ES-MS (EI): calcd. 577.2765 [$MH^+$], found 577.2789.

**(2*R*)-3-[(2′*S*)-2′-(*tert*-Butyloxycarbonyl)amino-4′-methylpentylthio]-2-[(fluorenylmethoxycarbonyl)amino]propanoic Acid, Fmoc-Cyl-(Boc)-OH (8b):** 82% (overall yield from **5b**); $R_f$ (DCM/MeOH, 9:1) = 0.37. Analytical sample, crystallized from hot absolute EtOH: M.p. 130–133 °C. $[\alpha]_D^{20}$ = –29.2 ($c$ = 0.6, MeOH). $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ = 0.88 (d, $J$ = 5.8 Hz, 6 H), 1.28–1.36 (m, 2 H), 1.42 (s, 9 H), 1.62–1.65 (m, 1 H), 2.61–2.64 (m, 2 H), 2.91–2.94 (m, 1 H), 3.05–3.08 (m, 1 H), 3.66–3.70 (m, 1 H), 4.23–4.33 (m, 4 H), 7.29–7.32 (t, $J$ = 7.5 Hz, 2 H), 7.36–7.40 (t, 2 H), 7.69–7.70 (d, $J$ = 7.4 Hz, 2 H), 7.78–7.80 (d, 2 H) ppm. $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ = 19.5, 29.9, 36.8, 39.5, 42.1, 54.6, 56.6, 59.5, 69.3, 81.1, 122.0, 127.5, 129.3, 129.9, 130.4, 131.5 140.9, 143.7, 146.4, 159.0, 159.6, 160.5, 171.4 ppm. MS-MALDI-TOF ($C_{29}H_{38}N_2O_6S$): $m/z$ = 565.32 [M + $Na^+$], 581.33 [M + $K^+$], 443.25 [M – Boc]. HR ES-MS (EI): calcd. 543.2940 [$MH^+$], found 543.2965.

**(2*R*)-3-{[(2′*S*)-1-(*tert*-Butyloxycarbonyl)pyrrolidin-2′-ylmethyl]-thio}-2-[(fluorenylmethoxycarbonyl)amino]propanoic Acid, Fmoc-Cyp(Boc)-OH (8c):** 79% (overall yield from **5c**); $R_f$ (DCM/MeOH, 9:1) = 0.45. Analytical sample, crystallized from hot absolute EtOH: M.p. 130–133 °C. $[\alpha]_D^{20}$ = –25.2 ($c$ = 0.6, MeOH). $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ = 1.36 (s, 9 H), 1.67–1.69 (m, 1 H), 1.80–1.85 (m, 3 H), 2.49–2.57 (m, 1 H), 2.76–2.81 (m, 3 H), 2.99–3.01 (m, 2 H), 3.07–3.10 (m, 1 H), 3.80–3.82 (m, 2 H), 4.13–4.16 (t, $J$ = 7.7 Hz, 1 H), 4.20–4.26 (m, 3 H), 7.20–7.22 (t, $J$ = 7.5 Hz, 2 H), 7.26–7.28 (t, 2 H), 7.57–7.59 (d, $J$ = 7.4 Hz, 2 H), 7.68–7.70 (d, $J$ = 7.4 Hz, 2 H) ppm. $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ = 22.1, 28.1, 29.1, 33.6, 35.6, 46.3, 52.4, 57.1, 65.7, 79.8, 120.1 125.3, 127.1, 127.6, 140.7, 143.8, 153.9, 157.2, 172.3 ppm. MS-MALDI-TOF ($C_{28}H_{34}N_2O_6S$): $m/z$ = 549.6 [M + $Na^+$], 565.7 [M + $K^+$], 426.5 [M – Boc]. HR ES-MS (EI): calcd. 527.2731 [$MH^+$], found 527.2704.

**Solid-Phase Synthesis of H-Phe-Cyf-Asp-Lys-Gln-OH (9):** In a manual peptide synthesizer Fmoc-Asp(O*t*Bu)-Lys(Boc)-Gln(Trt)-PAC-PEG-PS was grown up starting from Fmoc-Gln(Trt)-PAC-PEG-PS (118 mg, 0.16 mmol/g, 0.019 mmol). The Fmoc group was removed using 2% DBU (1,8-diazobicyclo[5.4.0]undec-7-ene) in DMF (3 mL) for 30 min. The orthogonally protected thia diamino acid **8a** (43 mg, 0.076 mmol) was then added together with HATU (29 mg, 0.076 mmol) and NMM (8 μL) in DMF (3 mL). The coupling proceeded for 1 h. The resin was washed with DMF (3 × 5 mL) and DCM (3 × 5 mL) and the protecting Fmoc group was again removed with 2% DBU in DMF (3 mL) for 30 min. The last coupling was performed by using Boc-Phe-OH (20 mg, 0.076 mmol), HATU (29 mg, 0.076 mmol) and NMM (8 μL) in DMF (3 mL). The product was eventually washed with DMF, DCM, and $Et_2O$, dried and cleaved from the polymeric support using a mixture of TFA (1.9 mL), TES (triethylsilane) (60 μL) and TA (thioanisole) (60 μL) at room temperature for 2 h. After precipitation in dry $Et_2O$, it was washed 3 times with the same solvent. Under analytical RP-HPLC conditions (solvents A/B, 95:5 over 5 min, then from 95:5 to 8:2 over 10 min, finally from 8:2 to 1:1 over 10 min) a single peak could be detected at $t$ = 12.6 min (220 nm). MS-MALDI-TOF ($C_{36}H_{52}N_8O_9S$): $m/z$ (%) = 773.24 (100) [M], 795.21 (50) [M + $Na^+$], 811.21 (10) [M + $K^+$], 755.23 (30) [M – $H_2O$]. HR ES-MS (EI): calcd. 773.3746 [$MH^+$], found 773.3759.

**Supporting Information:** Supporting information for this article, including full characterisation ($^1$H NMR, $^{13}$C NMR, MALDI-TOF and HR MS) of compounds **6a**–**c** throughout **9** is available (see the footnote on the first page of this paper).

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