Synthesis of the N-Terminal Lipohexapeptide of Human $G_{\alpha O}$-Protein and Fluorescent-Labeled Analogues for Biological Studies

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Abstract: For the study of biological signal transduction via heterotrimeric *N*-myristoylated and *S*-palmitoylated G proteins, useful reagents may be lipidated peptides that contain the lipid groups and amino acid sequences of their parent lipoproteins. The synthesis of *S*-palmitoylated peptides like Myr-Gly-Cys(Pal)-Thr-Leu-Ser-Ala-OH (**1**), which represents the characteristic *N*-terminus of the α-subunit of human $G_{\alpha O}$ protein, is complicated by the pronounced base-lability of the thioester. Lipidated G-protein peptide **1** and various fluorescent-labeled analogues thereof were built up efficiently by employing either the Pd^0-mediated removal of the allyl ester or the butyryl choline esterase-catalysed cleavage of the choline ester as key step. The removal of both blocking functions proceeds under very mild conditions and without undesired side reactions. In the cases studied the allyl ester proved to be superior to the enzyme-labile choline ester. The fluorescent-labeled lipopeptides were subjected to microinjection experiments in NIH-3T3 cells, which revealed that the compounds meet basic requirements for application in biology.

Keywords: cell signaling · enzyme reactions · lipopeptides · protecting groups · proteins

Introduction

Heterotrimeric G proteins (guanine nucleotide-binding regulatory proteins) composed of a guanine nucleotide-binding α subunit and a tight complex of β and γ subunits function as central molecular switches in diverse signaling pathways.[1] In response to extracellular signals (such as hormones, neurotransmitters, odorants, and light), receptors coupled to the G proteins activate them and mediate their dissociation into a guanosine triphosphate GTP–α complex and a β,γ dimer. They then trigger a variety of biological responses to the incoming signal. To fulfill their key functions, G proteins must be membrane-associated. Covalent lipid modifications that anchor the G proteins in the plasma membrane are found on both the α and γ subunits. The γ subunits are *S*-prenylated at their carboxyl termini, and α subunits (α_o, α_i, α_z) often are *S*-palmitoylated at a cysteine residue close to an amino-terminal myristoylated glycine.[1d] *S*-Palmitoylation is also found on α subunits (α_s, α_q, α_{12}), which are not *N*-myristoylated.[2, 9] A significant difference between these three types of lipid modification is that, whereas myristoylation and prenylation are permanent, palmitoylation is rapidly reversible.[3] Palmitoylation and depalmitoylation may be important mechanisms for regulating the interactions of G proteins with upstream receptors and downstream effectors.[4] For instance, desensitization of the G-protein-coupled β_2 adrenoceptor following binding of an agonist (i.e., switching the signal off) is accompanied by depalmitoylation of the receptor.[5] Furthermore, addition of the agonist brings about an increase in the degree of palmitoylation of the $G_{s\alpha}$ protein of the β_2 adrenoceptor, also causing the signal to be switched off.[6]

For the study of biological processes in which G proteins are involved, useful reagents may be lipidated peptides that contain the lipid groups and amino acid sequences of their parent lipoproteins and also carry labels by which they can be traced in biological systems.[7] Here we report on the enzymatic and non-enzymatic synthesis of lipid-modified hexapeptide **1**[8] and fluorescent-labeled analogues thereof. This peptide conjugate represents the *N*-terminus of the α-subunit of human G_o protein (Figure 1),[9] which is expressed in olfactory neuroepithelium. Peptide **1** embodies the characteristic Myr-Gly-Cys(Pal)-AA-AA-Ser/Thr-AA (AA = amino acid) sequence motif found in many G proteins.[9]

Results and Discussion

The synthesis of *S*-palmitoylated peptides is severely complicated by the pronounced base lability of the palmitic acid

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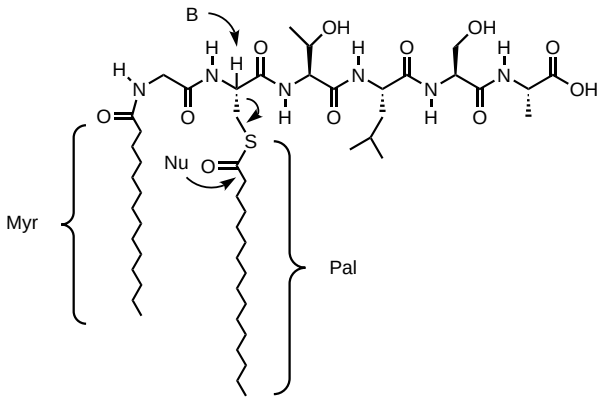


(Myr)Gly-Cys(Pal)-Thr-Leu-Ser-Ala-OH

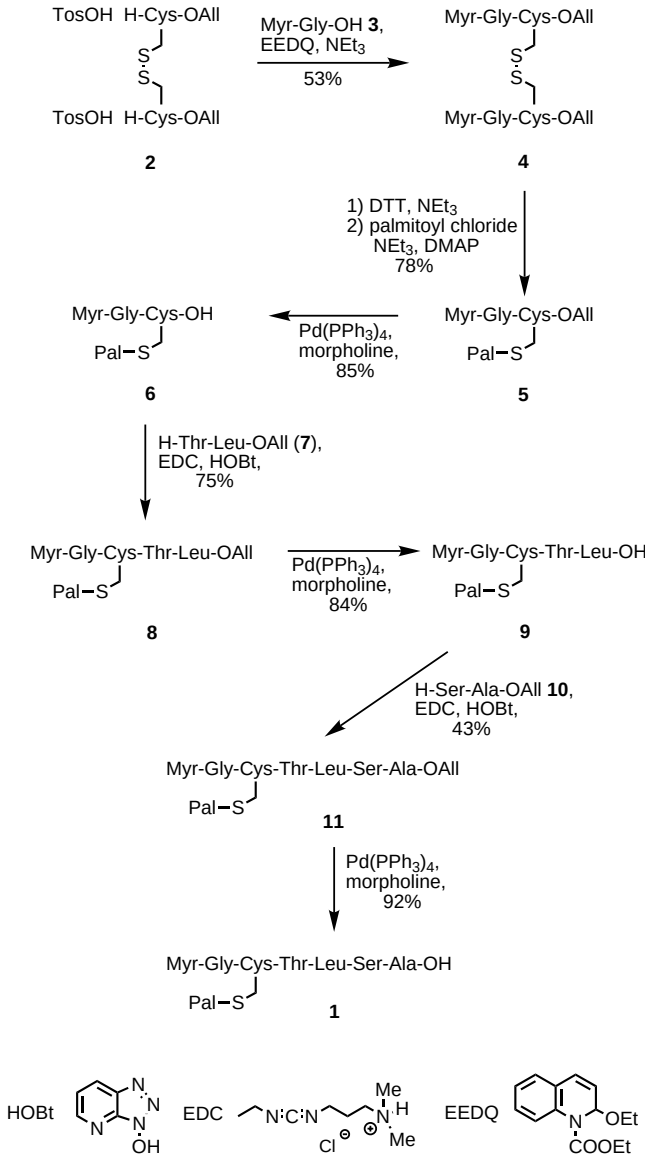
1

Figure 1. Structure and base lability of the *N*-myristoylated and *S*-palmitoylated N-terminal hexapeptide of human $G_{\alpha O}$ protein

thioester, which is already cleaved at pH 7 in aqueous solution,[10, 11] excluding the use of base-labile blocking functions. In addition, the palmitoyl group may be lost by means of a base-induced β-elimination resulting in α,β-dehydroalanine formation (Figure 1).[12] Thus, protecting groups must be employed that can be removed selectively under mild, preferably neutral, conditions. To solve this problem two protecting-group strategies were developed. In the synthesis strategy explored first, the allyl ester was used as carboxy-protecting group. This blocking function can be removed under gentle conditions by Pd^0-mediated allyl transfer to nucleophiles like morpholine or *N*,*N*′-dimethylbarbituric acid[13] and has already been applied with great success in the construction of glycopeptides[14] and further complex natural products.[15]

Since enzymatic protecting-group techniques[15, 16] have offered powerful alternatives to classical chemical methods, in the second strategy the enzyme-labile choline ester was used as C-terminal blocking function. The conditions for the selective hydrolysis of choline esters by means of the enzyme butyrylcholine esterase are very mild and this method has already been profitably employed in the synthesis of an *S*-palmitoylated and *S*-farnesylated lipohexapeptide, which represents the characteristic C-terminus of the human *N*-Ras protein.[11]

For the synthesis of the *N*-myristoylated and *S*-palmitoylated peptide **1** by means of the allyl ester route, cystine (bis)allyl ester **2**[11] was coupled with *N*-myristoylated glycine **3** in the presence of 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihyroquinoline (EEDQ) as condensing reagent (Scheme 1). The fully masked disulfide **4** thus obtained was then reductively cleaved by treatment with dithiothreitol (DTT) and the liberated mercapto groups were *S*-palmitoylated to give the protected thioester **5** in high yield. From this very base-labile intermediate, the C-terminal allyl ester protecting group was removed with complete selectivity and in high yield by Pd^0-mediated allyl transfer to morpholine as accepting nucleophile. The amino acid chain of the *S*-palmitoylated and selectively unmasked peptide **6** was then elongated by



Scheme 1. Synthesis of $G_{\alpha O}$ peptide **1** with an allyl ester C-terminal protecting group.

coupling with N-terminally deprotected dipeptide **7** in the presence of a carbodiimide and *N*-hydroxybenzotriazole (HOBt) as condensing reagents (Scheme 1). Treatment of lipidated and fully masked tetrapeptide allyl ester **8** with $(PPh_3)_4Pd^0$ and morpholine once more resulted in a smooth and completely selective deprotection of the C-terminus to give carboxylic acid **9** in high yield. Further extension of the peptide chain by the dipeptide allyl ester **10** and final Pd^0-mediated removal of the allyl group delivered the desired *N*-myristoylated and *S*-palmitoylated *N*-terminal fragment **1** of the $G_{\alpha O}$ protein (Scheme 1).

The three allyl ester cleavages performed in this sequence proceeded with complete selectivity and without any undesired side reaction. The conditions of the noble metal complex mediated allyl transfer are so mild that neither attack on the base-sensitive thioester nor base-induced β-elimination of the palmitoyl group occurred. These results clearly demonstrate that the allyl ester can be used very advantageously for the construction of *S*-palmitoylated base-sensitive peptides.

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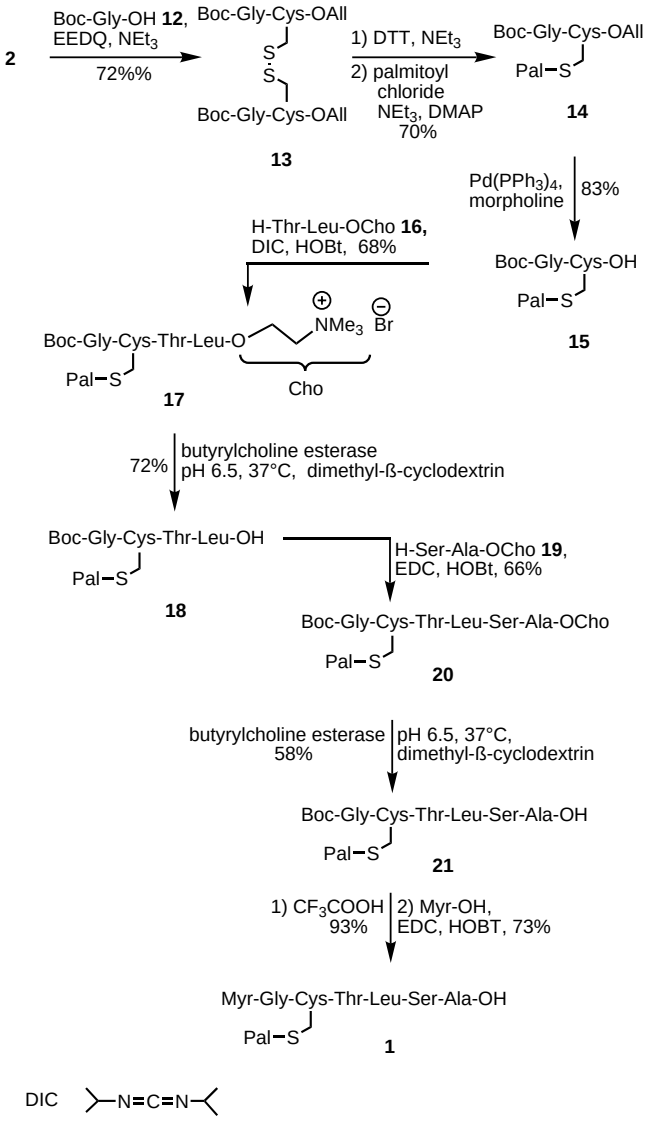
The N-terminally unmasked dipeptide allyl esters used in the synthesis detailed above were obtained by acid-mediated removal of the N-terminal Boc group from the analogous Boc-protected dipeptide allyl esters according to published procedures[11, 17] (see the Experimental Section).

In order to investigate whether an enzymatic blocking group technique may offer an advantageous alternative to the allyl ester, in the second strategy we employed the enzyme-labile choline ester as C-terminal protecting group. To this end, first the C-terminally deprotected *S*-palmitoylated dipeptide **15** was built up (Scheme 2) and condensed with



Scheme 2. Synthesis of $G_{\alpha O}$ peptide **1** with a choline ester C-terminal protecting group.

dipeptide choline ester **16**. The resulting *S*-palmitoylated peptide choline ester **17** was then subjected to enzymatic hydrolysis by butyrylcholine esterase in aqueous buffer at pH 6.5. The optimization of this biocatalyzed transformation turned out to be a formidable challenge. Peptide choline esters usually are highly soluble in water. Thus, the substrates become readily accessible to the biocatalyst and the use of additional solubilizing organic cosolvents that might denature the enzyme may be reduced or is even rendered unnecessary. However, the *S*-palmitoylated ester **17** is only sparingly soluble in a purely aqueous solution. Therefore, in initial experiments 5 vol% of dioxane, DMF, or methanol were employed as solubilizing cosolvents. Under these conditions the enzymatic removal of the protecting group proceeded smoothly and without undesired side reaction. Unfortunately, the choline esterase is sensitive to organic cosolvents and is rapildy denatured in their presence. Therefore, under these conditions the desired selectively unmasked tetrapeptide **18** was obtained only in 40–47% yield. Addition of cyclodextrins[18] instead of organic cosolvents, however, led to a significant rise in yield. Deprotected tetrapeptide **18** was obtained in 58% yield if 15 equivs of α-cyclodextrin were employed and in 72% yield if the same amount of dimethyl-β-cyclodextrin was added to the buffer solution. These results can be interpreted by the assumption that the hydrophobic cavity of the cyclodextrins serves as host for the palmitoyl chain. The formation of such an inclusion complex increases the solubility of the lipopeptide and the substrate is better accessible to the biocatalyst. It should be noted that cyclodextrins did not act as a mimic of an esterase in which the hydroxyl group of cyclodextrin reacts with the thioester bond and hydrolyses the palmitoyl group.[19] If instead of the Boc-protected *S*-palmitoylated choline ester **17** the analogous *N*-myristoylated peptide was used as substrate, the enzymatic deprotection did not proceed at all. The *N*-myristoylated and *S*-palmitoylated choline ester obviously is no longer soluble in the aqueous solution, and the addition of the cyclodextrin did not improve this situation. These solubility problems also explain why in the synthesis shown in Scheme 2 the Boc group was used from the start instead of the required myristic acid amide. In addition, the synthesis of the choline ester analogous to the allyl ester **14** is problematic. Choline esters are usually generated from 2-bromoethyl esters by treatment with NMe_3 in acetone.[17] Under these conditions, the base-sensitive palmitoylated cysteine might be attacked. Therefore, the allyl ester was used in the initial steps shown in Scheme 2.

After suitable conditions for the selective enzymatic unmasking of the palmitoylated choline ester **17** had been found, the amino acid chain was elongated by condensing carboxylic acid **18** and dipeptide choline ester **19** to yield peptide **20**. From this peptide choline ester once more the C-terminal blocking group was split off with complete selectivity under very mild conditions and without any side reaction in the presence of dimethyl-β-cyclodextrin. Finally, the synthesis was completed by removal of the Boc group from hexapeptide **21** and myristoylation of the liberated N-terminal glycine residue.

These results demonstrate that in principle the enzyme-labile choline ester also is an advantageous function for the synthesis of sensitive *S*-palmitoylated peptide conjugates like **1**. However, a direct comparison of the two syntheses shown in Schemes 1 and 2 demonstrates that for the construction of *N*-myristoylated and *S*-palmitoylated lipopeptides the allyl ester is clearly superior to the choline ester.

The N-terminally deprotected dipeptide choline esters **16** and **19** employed in the reaction sequence shown in Scheme 2

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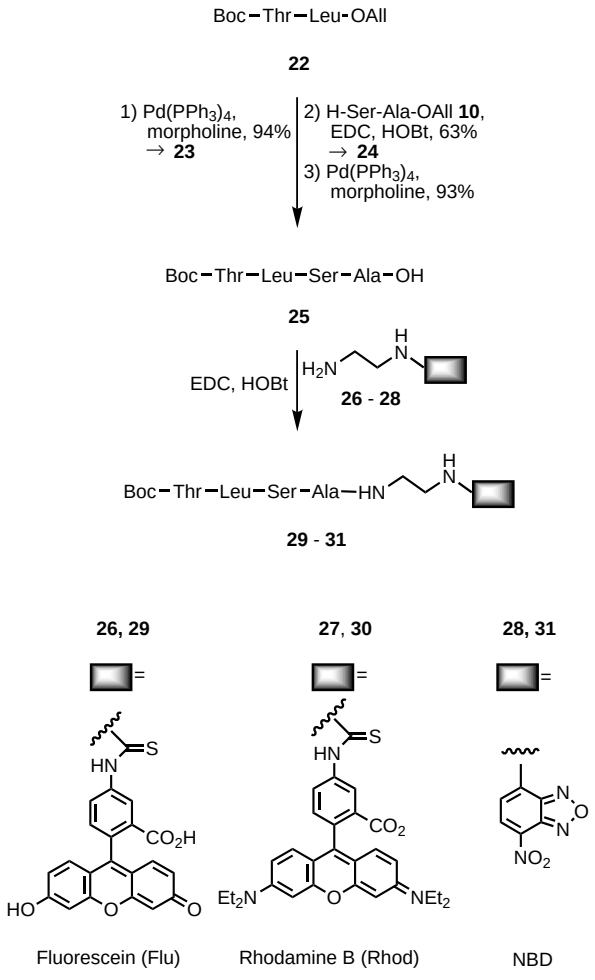
were synthesized from the analogous Boc-protected dipeptide choline esters by treatment with HBr/CH_3COOH according to established procedures (see the Experimental Section).[11, 17]

Lipidated peptides that embody the characteristic structural elements of their parent lipoproteins as well as functional groups that can be traced by appropriate analytical techniques may serve as efficient molecular probes in biological studies.[7] For instance, the application of fluorescent-labeled lipidated peptides in cell-biological and biophysical experiments[11, 20–22] has yielded insight into the molecular details of specific lipoprotein localization to subcellular membranes. For the study of biological phenomena that may be influenced by the *N*-terminus of the $G_{\alpha O}$ protein and related G proteins several lipid-modified and fluorescent labeled peptides were built up.

To this end, the *C*-terminal tetrapeptide unit of peptide **1** was synthesized and coupled to different fluorescent dyes (Scheme 3). The resulting labeled tetrapeptides were then

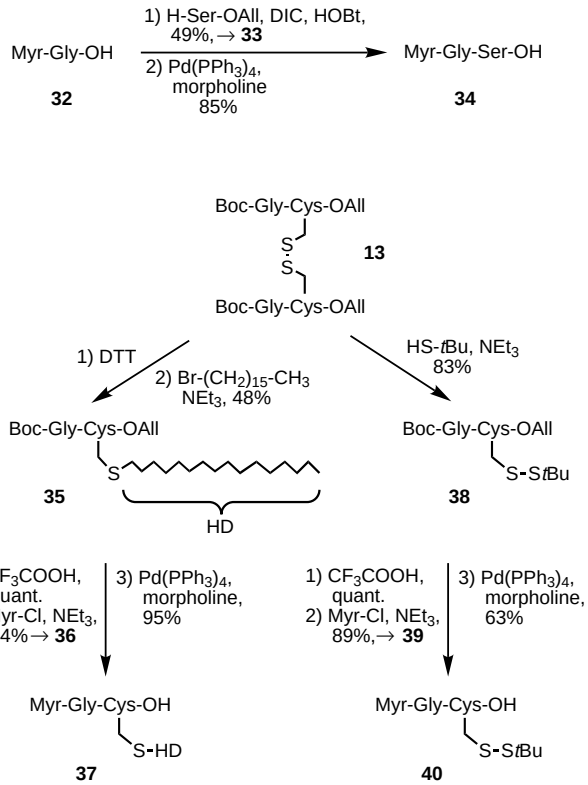


Scheme 3. Synthesis of fluorescent-labeled tetrapeptides **29**–**31**.

equipped with various lipid-modified N-terminal dipeptides to give various fluorescent analogues of the $G_{\alpha O}$-peptide **1** (see Scheme 5).

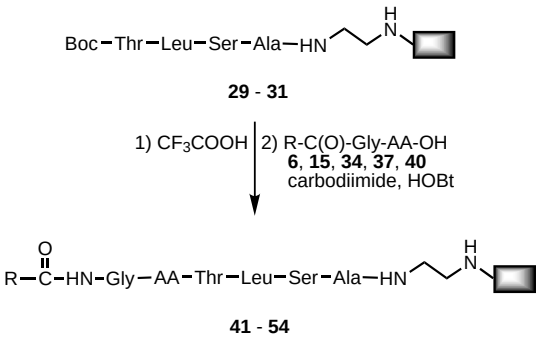
The synthesis of the fluorescent-labeled analogues of the C-terminal tetrapeptide is shown in Scheme 3. Dipeptide allyl ester **22** was *C*-terminally deprotected by Pd^0-mediated allyl ester cleavage. After elongation of the peptide chain a second allyl ester removal yielded Boc-tetrapeptide **25**. This compound was condensed with ethylenediamine derivatives **26**–**28** of fluorescein, rhodamine B, and 7-nitrobenz-2-oxadiazole (NBD) to yield fluorescent-labeled Boc-protected tetrapeptides **29**–**31** (Scheme 3). Fluorescein and rhodamine B derivatives **26** and **27** were obtained by reaction of the corresponding isothiocyanate with ethylenediamine, and NBD derivative **28** was synthesized by treatment of 4-chloro-7-nitro benzofurazane with the diamine (see the Experimental Section).[23]

As dipeptide units to be coupled to the labeled tetrapeptide, the various lipidated peptides **6**, **15**, **34**, **37**, and **40** were chosen. Peptide **6** (Scheme 1) is myristoylated and palmitoylated and thus resembles the correct lipid modification of $G_{\alpha O}$ protein. In peptide **15** (Scheme 2) the *N*-terminal myristic acid is replaced by an acid-labile Boc group. This opens up the opportunity to synthesize an *S*-palmitoylated but N-terminally unmasked analogue. In peptide **34** (Scheme 4) a serine



Scheme 4. Synthesis of lipid-modified dipeptides **34**, **37**, and **40**.

instead of a cysteine is close to the N-terminus. This compound should no longer be palmitoylated in cells.[11, 20] It was synthesized from *N*-myristoyl glycine by coupling with serine allyl ester and selective C-terminal deprotection. Cysteinyl peptides **37** and **40** embody an *S*-hexadecyl-modified cysteine, that is, a thioether instead of a thioester, and an S-protected cysteine that can readily be unmasked, for example, by treatment with a thiol. They were built up from cystine dipeptide **13** as shown in Scheme 4. Thus, on the one



	R-C(O)-	AA		yield [%]
41	Boc	Cys(Pal)	Flu	55
42	Myr	Cys(Pal)	Flu	50
43	Myr	Cys(HD)	Flu	53
44	Myr	Cys(S-*t*Bu)	Flu	57
45	Boc	Cys(Pal)	Rhod	51
46	Myr	Cys(Pal)	Rhod	37
47	Myr	Cys(HD)	Rhod	32
48	Myr	Cys(S-*t*Bu)	Rhod	53
49	Myr	Ser	Rhod	27
50	Boc	Cys(Pal)	NBD	54
51	Myr	Cys(Pal)	NBD	41
52	Myr	Cys(HD)	NBD	33
53	Myr	Cys(S-*t*Bu)	NBD	78
54	Myr	Ser	NBD	28

Scheme 5. Synthesis of various fluorescent-labeled and lipid-modified $G_{\alpha O}$ hexapeptides **41**–**54**.

hand the disulfide in **13** was cleaved reductively by treatment with dithiothreitol (DTT) and the liberated mercapto group was alkylated with hexadecyl bromide to give the protected dipeptide **35**. Exchange of the Boc group for myristic acid and subsequent allyl ester cleavage yielded *S*-hexadecylated peptide **37**. On the other hand, treatment of cystine derivative **13** with *tert*-butyl thiol resulted in the formation of peptide **38** in which the mercapto group of cysteine is masked as *tert*-butyl disulfide. This compound was readily converted into the selectively unmasked dipeptide **40** by protecting group manipulation (Scheme 4).

Finally, Boc-protected fluorescent-labeled tetrapeptides **29**–**31** were selectively unmasked at the N-terminus by treatment with trifluoroacetic acid and the resulting amines were immediately coupled with lipid-modified dipeptides **6**, **15**, **34**, **37**, and **40** (Scheme 5). By this convenient procedure various fluorescent-labeled lipidated peptides **41**–**54** are accessible in a straightforward and efficient manner.

In order to determine whether fluorescent labeled lipid-modified peptides **41**–**54** fulfill the basic requirements for application in biological experiments in a first series of orientating experiments peptides **51** (*N*-myristoylated and *S*-palmitoylated), **52** (*N*-myristoylated and *S*-hexadecyl-modified), **53** (*N*-myristoylated and *S*-*tert*-butyl protected), and **54** (*N*-myristoylated and serine instead of a cysteine) were subjected to microinjection into NIH-3T3 cells (mouse fibroblasts). The distribution of the peptides within the cells was monitored with a fluorescence microscope. In all cases the fluorescence intensity was high enough to give clear pictures. Figure 2 shows results obtained two minutes after injection for *N*-myristoylated and *S*-palmitoylated NBD-labeled peptide **51** and the analogous *S*-hexadecyl-modified peptide **52**. The figures indicate that the fluorescent peptides appear to be distributed evenly in the cytosol. The nucleus, however, is not labeled.

These initial experiments indicate that lipid-modified peptides such as **41**–**54** and analogues with, for example, varied peptide sequence and lipid modification fulfill the basic requirements for use in biological experiments.

Experimental Section

General procedures: ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 250, AM 400, and DRX-500 spectrometers. Mass spectra were measured on a Finnigan MAT MS 70 spectrometer. Analytical chromatography was performed on E. Merck silica gel 60 F_{254} plates. Specific rotations were measured with a Perkin–Elmer polarimeter 241. Flash chromatography was performed on Baker silica gel. Butyrylcholine esterase was obtained from Sigma (Deisenhofen, Germany) or isolated from horse serum as described in the literature.[26] All solvents were dried and distilled using standard procedures.[27]

***N*,*N*-Bis-(myristoyl-glycyl)-L-cystine bis(allyl) ester** (**4**): To an ice-cold solution of MyrGlyOH (810 mg, 2.84 mmol) and $(HCysOAll)_2 \cdot 2p$TosOH[11] (858 mg, 1.29 mmol) in CH_2Cl_2 (40 mL) and isopropyl alcohol (20 mL) was added NEt_3 (260 mg, 360 μL, 2.58 mmol) and EEDQ (1.27 mg, 5.16 mmol). The mixture was stirred at 40 °C for 24 h, the precipitated urea was filtered off, and the solvent was washed with 1M HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **4** was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 50/50 [*v*/*v*] as eluent. Yield: 582 mg (53%); m.p. 109–111 °C; $R_f = 0.48$ (*n*-hexane/ethyl acetate 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +7.4$ ($c = 0.1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 6H, 2 CH_3 Myr), 1.27 (s, 40H, 20 CH_2 Myr), 1.50–1.68 (m, 4H, 2 β-CH_2 Myr), 2.25 (t, $J = 7$ Hz, 4H, 2 α-CH_2 Myr), 3.18 (d, $J = 5.7$ Hz, 4H, 2 CH_2 Gly), 3.85 (dd, $J = 16.2$ Hz, $J = 5.5$ Hz, 2H, 2 CH_a Cys), 3.90 (dd, $J = 16.2$ Hz, $J = 5.3$ Hz, 2H, 2 CH_b Cys), 4.66 (dt, $J = 5.8$ Hz, $J = 1.1$ Hz, 4H, 2 CH_2 allyl), 4.89 (dt, 2H, $J = 11.3$ Hz, $J = 5.7$ Hz, 2 α-CH Cys), 5.27 (dt, $J = 11$ Hz, $J = 1.1$ Hz, 2H, 2 CH_a=), 5.35 (dt, $J = 17.2$ Hz, $J = 1.1$ Hz, 2H, 2 CH_b=), 5.76 (s, 2H, 2NH urethane), 5.91 (ddt, $J = 17.2$ Hz, $J = 11$ Hz, $J = 5.8$ Hz, 2H, 2 CH=), 7.41 (d, $J = 7.4$ Hz, 2H, 2NH); anal. calcd for $C_{44}H_{78}N_4O_8S_2$: C 61.79, H 9.19, N 6.55; found: C 61.52, H 9.11, N 6.18.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteine allyl ester, MyrGlyCys(Pal)-OAll** (**5**): To a solution of $(MyrGlyCysOAll)_2$ (**4**) (807 mg, 0.95 mmol) in CH_2Cl_2 (50 mL) was added DTT (727 mg, 4.71 mmol) and NEt_3 (192 mg, 265 μL, 1.90 mmol). The mixture was stirred at 20 °C for 1 h. The solution was washed twice with distilled water (50 mL) and dried over $MgSO_4$. To the crude product BocGlyCysOAll in CH_2Cl_2 were added NEt_3 (192 mg, 265 μL, 1.90 mmol) and a catalytic amount of DMAP and palmitoyl choride (1.30 g, 4.75 mmol). After stirring the mixture at 20 °C for 2 h, the precipitated white solid was filtered off to give product **5** (679 mg). Additional product **5** (303 mg) was isolated from the residue by flash chromatography on silica gel using *n*-hexane/ethyl acetate 70/30 [*v*/*v*] as eluent. Yield: 980 mg (78%); m.p. 72–73 °C; $R_f = 0.56$ (*n*-hexane/ethyl acetate 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +10$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 6H, CH_3 Pal, CH_3 Myr), 1.27 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.50–1.68 (m, 4H, β-CH_2 Pal, β-CH_2 Myr), 2.25 (t, $J = 7$ Hz, 2H, α-CH_2 Myr), 2.55 (t, $J = 7$ Hz, 2H, α-CH_2 Pal), 3.22 (dd, $J = 13.5$ Hz, $J = 6$ Hz, 1H, CH_a Cys), 3.44 (dd, $J = 13.5$ Hz, $J = 4$ Hz, 1H, CH_b Cys), 3.97 (dd, $J = 15$ Hz, $J = 5.2$ Hz, 1H, CH_a Gly), 4.02 (dd, $J = 15$ Hz, $J = 5.2$ Hz, 1H, CH_b Gly), 4.66 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 4.82 (td, $J = 6$ Hz, $J = 4$ Hz, 1H, α-CH Cys), 5.26 (dd, $J = 12$ Hz, $J = 1.2$ Hz, 1H, =CH_a), 5.33 (dd, $J = 16$ Hz, $J = 1.2$ Hz, 1H, =CH_b), 5.92 (ddt, $J = 16$ Hz, $J = 12$ Hz, $J = 5.7$ Hz, 1H, =CH), 6.20 (t, $J = 5.2$ Hz, 1H, NH), 6.90 (d, $J = 6$ Hz, 1H, NH); $C_{38}H_{70}N_2O_5S$; FAB MS (glycerol/3-NBA); *m*/*z*: 667.5 $[M+H]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteine, MyrGlyCys(Pal)OH** (**6**): To a solution of MyrGlyCys(Pal)OAll (**5**, 500 mg, 0.75 mmol) in CH_2Cl_2

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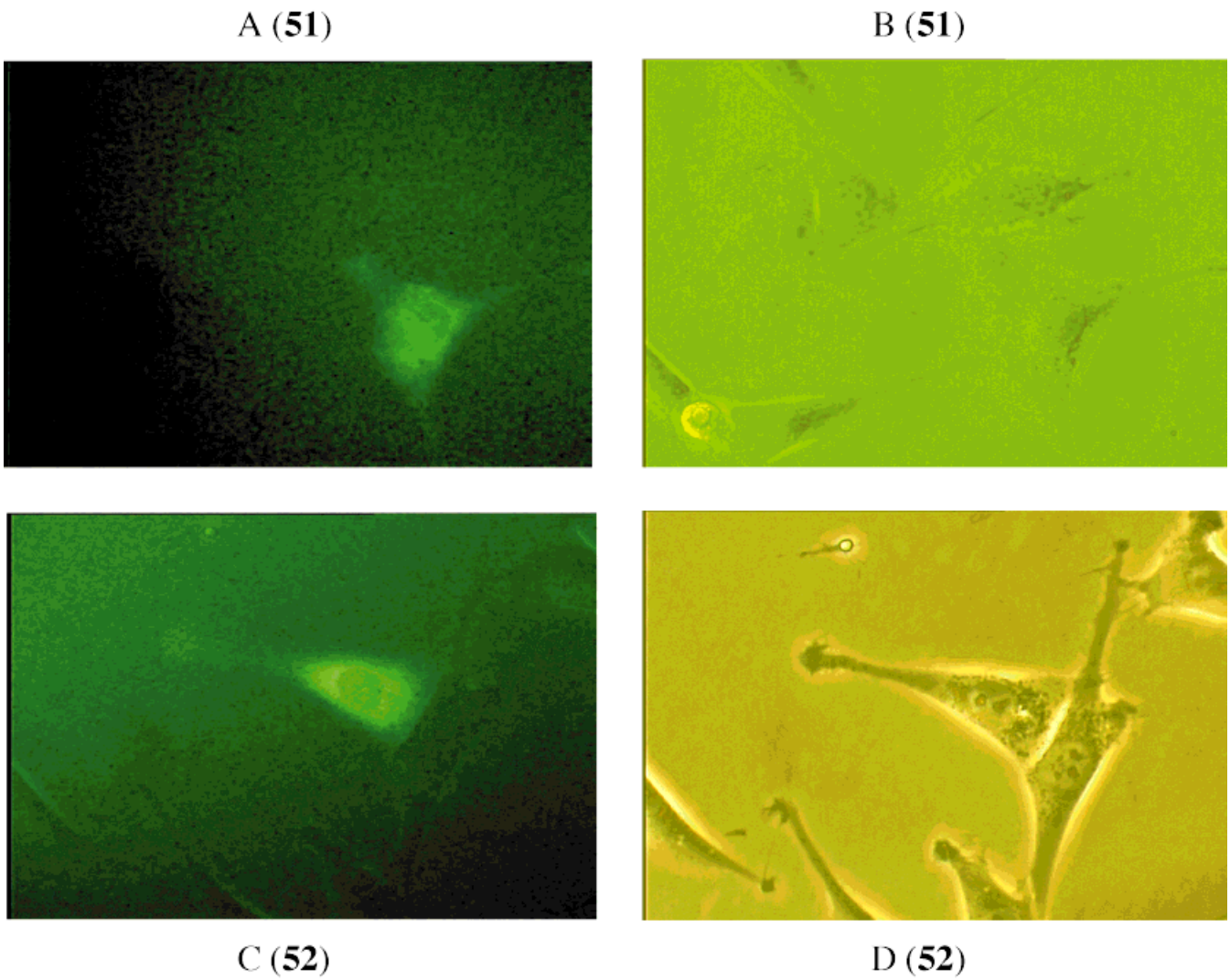

Figure 2. Microinjection experiments with NBD-labeled lipopeptides. The NBD-labeled *N*-myristoylated peptides with additional *S*-palmitoylation (**51**) or *S*-hexadecyl modification (**52**) were injected into NIH-3T3 fibroblast cells as described in the Experimental Section. A) Fluorescence image of a fibroblast cell injected with the palmitoylated lipopeptide **51**; B) corresponding phase contrast micrograph; C) fluorescence image of an NIH-3T3 cell after injection of hexadecyl-modified lipopeptide **52**; D) corresponding phase-contrast micrograph. Stock solution of peptide **51** was 53 μM (peptide **52**: 330 μM). Dilution of the peptide solution after injection approx. 1:10. Owing to bleaching the NBD signal was observed only for about 15 min after injection.

(50 mL) was added under argon morpholine (87 mg, 85 μL, 0.97 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(0), and the mixture was stirred at 20 °C for 1 h. The precipitated dipeptide morpholinium salt was filtered off and recrystallized from methanol by addition of HCl (1M) (50 μL) to the hot methanol after the salt had dissolved. The precipitated carboxylic acid **6** was filtered off and isolated as a white solid. Yield: 399 mg (85 %); m.p. 90–92 °C; $R_f = 0.66$ (ethyl acetate/methanol/acetic acid 90/10/1 [*v*/*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD 55 °C): $\delta = 0.89$ (t, $J = 7$ Hz, 6H, CH_3 Pal, CH_3 Myr), 1.29 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.53–1.71 (m, 4H, β-CH_2 Pal, β-CH_2 Myr), 2.27 (t, $J = 8.2$ Hz, 2H, α-CH_2 Myr), 2.56 (t, $J = 7.6$ Hz, 2H, α-CH_2 Pal), 3.24 (dd, $J = 13.8$ Hz, $J = 7.3$ Hz, 1H, CH_a Cys), 3.50 (dd, $J = 13.7$ Hz, $J = 4.3$ Hz, 1H, CH_b Cys), 3.76 (d, $J = 16.6$ Hz, 1H, CH_a Gly), 3.92 (d, $J = 16.6$ Hz, 1H, CH_b Gly), 4.42 (dd, $J = 7.3$ Hz, $J = 4.3$ Hz, 1H, α-CH Cys); anal. calcd for $C_{35}H_{66}N_2O_5S$: C 67.05, H 10.61, N 4.46; found: C 67.03, H 10.42, N 4.16; EI MS (70 eV); *m*/*z*: 626.5 $[M]^+$.

Synthesis of H-Thr-Leu-OAll · CF_3COOH (7), *N-tert*-Butyloxycarbonyl-L-threonyl-L-leucine allyl ester, BocThrLeuOAll: To a solution of BocThrOH (2.50 g, 11.4 mmol) and HLeuOAll · *p*TsOH (3.92 g, 11.4 mmol) CH_2Cl_2 (200 mL) was added NEt_3 (1.15 g, 1.58 mL, 11.4 mmol), HOBt (1.54 g, 11.4 mmol), and, finally, diisopropylcarbodiimide (DIC) (3.17 g, 3.90 mL, 22.8 mmol). The mixture was stirred at 20 °C for 16 h, the solvent was washed with 1M HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product was isolated as a colorless oil from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 60/40 [*v*/*v*] as eluent. Yield: 3.36 g (79 %); $R_f = 0.42$ (ethyl acetate/*n*-hexane 50/50 [*v*/*v*]); $[\alpha]_D^{22} = -62.8$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.93$ (d, $J = 5$ Hz, 6H, 2 CH_3 Leu), 1.19 (d, $J = 7.5$ Hz, 3H, CH_3 Thr), 1.44 (s, 9H, 3 CH_3 Boc), 1.55–1.75 (m, 3H, CH_2 Leu, γ-CH Leu), 4.22–4.35 (m, 2H, α-CH Thr, β-CH Thr), 4.50–4.61 (m, 1H, α-CH Leu), 4.63 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 5.26 (dd, $J = 10.4$ Hz, $J = 1.2$ Hz, 1H, =CH_a), 5.34 (dd, $J = 13.6$ Hz, $J = 1.2$ Hz, 1H, =CH_b), 5.62 (d, $J = 7.8$ Hz, 1H, NH urethane), 5.91 (ddt, $J = 13.6$ Hz, $J = 10.4$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.15 (d, $J = 8$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 17.78$ (CH_3 Thr), 21.56 (CH_3 Leu), 22.77 (CH_3 Leu), 24.67 (γ-CH Leu), 28.26 (3 CH_3 Boc), 40.82 (CH_2 Leu), 50.83 (α-CH Leu), 57.88 (α-CH Thr), 65.85 (CH_2 allyl), 66.93 (β-CH Thr), 80.35 (Cq Boc), 118.69 (=CH_2), 131.50 (=CH), 156.26 (C=O urethane), 171.16 (C=O), 172.31 (C=O); anal. calcd for $C_{18}H_{32}N_2O_6$: C 58.05, H 8.66, N 7.52; found: C 58.16.07, H 8.70, N 7.09.

L-Threonyl-L-leucine allyl ester hydrotrifluoroacetate (7): To a solution of BocThrLeuOAll (500 mg, 1.34 mmol) at 0 °C was added trifluoroacetic acid (4 mL) and the solution was stirred for 1 h. Ether was added (40 mL) and the precipitated oil was washed several times with ether. After drying in vacuo the deprotected dipeptide **7** was isolated as a yellowish oil. Yield: 495 mg (96 %); $[\alpha]_D^{22} = -23.6$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz,

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CD_3OD): $\delta = 0.94$ (d, $J = 6.3$ Hz, 3H, CH_3 Leu), 0.97 (d, $J = 6.3$ Hz, 3H, CH_3 Leu), 1.36 (d, $J = 7.5$ Hz, 3H, CH_3 Thr), 1.48–1.70 (m, 3H, CH_2 Leu, γ-CH Leu), 3.65 (d, $J = 6.4$ Hz, 1H, α-CH Thr), 3.95 (q, $J = 6.4$ Hz, 1H, β-CH Thr), 4.51 (t, $J = 7.3$ Hz, 1H, α-CH Leu), 4.61 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 5.23 (dd, $J = 10.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.33 (dd, $J = 14$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.93 (ddt, $J = 14$ Hz, $J = 10.5$ Hz, $J = 5.7$ Hz, 1H, =CH); $C_{15}H_{25}F_3N_2O_6$; EI MS (70 eV); m/z: 273.2 $[M - CF_3CO_2]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucine allyl ester, MyrGlyCys(Pal)ThrLeuOAll (8)**: To a solution of MyrGlyCys(Pal)OH (**6**, 43 mg, 0.068 mmol) and HThrLeuOAll · CF_3CO_2H **7** (26 mg, 0.068 mmol) in CH_2Cl_2 (10 mL) and DMF (3 mL) was added NEt_3 (7 mg, 9.5 μL, 0.068 mmol), HOBt (9 mg, 0.068 mmol) and then EDC (27 mg, 0.14 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure, and the residue was dissolved in a small volume of CH_2Cl_2 and precipitated with diethyl ether (50 mL). The ether layer was dried over $MgSO_4$ and concentrated in vacuo. The product **8** was purified from the residue by flash chromatography on silica gel using ethyl acetate/methanol 95/5 [*v*/*v*] as eluent. Yield: 45 mg (75%); white solid; m.p. 81–83 °C; $R_f = 0.29$ (ethyl acetate/methanol 90/10 [*v*/*v*]); $[\alpha]_D^{22} = -5.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 7$ Hz, 6H, CH_3 Pal, CH_3 Myr), 0.93 (d, $J = 6$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 6$ Hz, 3H, CH_3 Leu), 1.17 (d, $J = 6.5$ Hz, 3H, CH_3 Thr), 1.25 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.50–1.72 (m, 7H, β-CH_2 Pal, β-CH_2 Myr, β-CH_2 Leu, γ-CH Leu), 2.26 (t, $J = 7.8$ Hz, 2H, α-CH_2 Myr), 2.55 (t, $J = 7.8$ Hz, 2H, α-CH_2 Pal), 3.15 (dd, $J = 14.3$ Hz, $J = 5$ Hz, 1H, CH_a Cys), 3.32 (dd, $J = 14.3$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 4.01 (d, $J = 4.6$ Hz, 2H, CH_2 Gly), 4.32 (dd, $J = 6.3$ Hz, $J = 3.6$ Hz, 1H, α-CH Thr), 4.52–4.65 (m, 4H, α-CH Cys, β-CH Thr, CH_2 allyl), 4.77 (d, $J = 7$ Hz, 1H, α-CH Leu), 5.24 (dd, $J = 10.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.33 (dd, $J = 13.8$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.91 (ddt, $J = 13.8$ Hz, $J = 10.5$ Hz, $J = 5.7$ Hz, 1H, =CH), 6.62 (s, 1H, NH), 7.43 (d, $J = 7.3$ Hz, 2H, NH), 7.87 (d, $J = 6.3$ Hz, 1H, NH); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 14.13$ (CH_3 Pal, CH_3 Myr), 17.99 (CH_3 Thr), 21.76 (CH_3 Leu), 22.70 (CH_2 Pal, CH_2 Myr), 22.89 (CH_3 Leu), 24.85 (γ-CH Leu), 25.51 (CH_2 Cys), 29.00–30.00 (12 CH_2 Pal, 10 CH_2 Myr), 31.93 (α-CH_2 Pal), 36.23 (α-CH_2 Myr), 40.65 (CH_2 Leu), 44.06 (CH_2 Gly), 51.22 (α-CH Leu), 53.78 (α-CH Cys), 57.99 (α-CH Thr), 65.88 (CH_2 allyl), 66.90 (β-CH Thr), 118.66 ($=CH_2$), 131.73 (=CH), 169.24 (C=O), 169.80 (C=O), 169.95 (C=O), 172.25 (C=O), 174.48 (C=O), 200.35 (C=O thioester); anal. calcd for $C_{48}H_{88}N_4O_8S$: C 65.42, H 10.06, N 6.35; found: C 64.93, H 9.79, N 6.52; MALDI-TOF MS (MeOH/TFA 9/1); m/z: 882.2 $[M+H]^+$, 1005.3 $[M+Na]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucine, MyrGlyCys(Pal)ThrLeuOH (9)**: To a solution of MyrGlyCys(Pal)ThrLeuOAll (**8**, 35 mg, 0.039 mmol) in CH_2Cl_2 (10 mL) was added under argon morpholine (5 mg, 5 μL, 0.05 mmol), a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**), and the mixture was stirred at 20 °C for 1 h. The solvent was washed with 1M HCl (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **9** was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent. Yield: 28 mg (84%); m.p. 127–131 °C; $R_f = 0.56$ (CH_2Cl_2/methanol/acetic acid) 90/10/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = -6.4$ ($c = 0.5$ in DMF); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.86$ (t, $J = 7$ Hz, 6H, CH_3 Pal, CH_3 Myr), 0.90 (d, $J = 5.5$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 5.5$ Hz, 3H, CH_3 Leu), 1.17 (d, $J = 6.3$ Hz, 3H, CH_3 Thr), 1.23 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.40–1.51 (m, 7H, β-CH_2 Pal, β-CH_2 Myr, β-CH_2 Leu, γ-CH Leu), 2.27 (t, $J = 8$ Hz, 2H, α-CH_2 Myr), 2.55 (t, $J = 8$ Hz, 2H, α-CH_2 Pal), 3.13 (dd, $J = 14$ Hz, $J = 8$ Hz, 1H, CH_a Cys), 3.23 (dd, $J = 14$ Hz, $J = 4$ Hz, 1H, CH_b Cys), 3.75 (d, $J = 17$ Hz, 1H, CH_a Gly), 3.95 (d, $J = 17$ Hz, 1H, CH_b Gly), 4.21 (t, $J = 5.8$ Hz, 1H, α-CH Leu), 4.33 (d, $J = 3.5$ Hz, α-CH Thr), 4.40 (dd, $J = 8$ Hz, $J = 4$ Hz, 1H, α-CH Cys), 4.40–4.47 (m, 1H, β-CH Thr); ^{13}C NMR (100.6 MHz, CD_3OD): $\delta = 14.15$ (CH_3 Pal, CH_3 Myr), 18.79 (CH_3 Thr), 21.65 (CH_3 Leu), 22.79 (CH_2 Pal, CH_2 Myr), 23.00 (CH_3 Leu), 24.97 (γ-CH Leu), 25.61 (CH_2 Cys), 29.00–30.00 (12 CH_2 Pal, 10 CH_2 Myr), 32.04 (α-CH_2 Pal), 36.22 (α-CH_2 Myr), 40.71 (CH_2 Leu), 44.10 (CH_2 Gly), 51.67 (α-CH Leu), 54.45 (α-CH Cys), 58.53 (α-CH Thr), 67.25 (β-CH Thr), 170.53 (C=O), 170.79 (C=O), 175.41 (3 C=O), 201.22 (C=O thioester); $C_{45}H_{84}N_4O_8S$; FAB MS (glycerol); m/z: 842.1 $[M+H]^+$.

Synthesis of Ser-Ala-OAll · CF_3COOH (10), *N*-*tert*-butyloxycarbonyl-L-seryl-L-alanine allyl ester: To a solution of BocSerOH (470 mg, 2.29 mmol) and HAlaOAll · *p*TsOH (680 mg, 2.29 mmol) in CH_2Cl_2 (30 mL) was added NEt_3 (231 mg, 320 μL, 2.29 mmol), and finally EEDQ (1.10 g, 4.58 mmol). The mixture was stirred at 20 °C for 16 h, the solvent was washed with 1M HCl (3 × 50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 50/50 [*v*/*v*] as eluent. Yield: 638 mg (88%); m.p. 50–53 °C; $R_f = 0.36$ (ethyl acetate/*n*-hexane 50/50 [*v*/*v*]); $[\alpha]_D^{22} = -37.8$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.44$ (d, $J = 6.5$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3 CH_3 Boc), 3.65 (dd, $J = 11.5$ Hz, $J = 5.5$ Hz, 1H, β-CH_a Ser), 4.09 (dd, $J = 11.5$ Hz, $J = 2$ Hz, 1H, β-CH_b Ser), 4.18 (dd, $J = 5.5$ Hz, $J = 2$ Hz, 1H, α-CH Ser), 4.59 (quintet, $J = 7.3$ Hz, 1H, α-CH Ala), 4.64 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 5.26 (dd, $J = 10.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.33 (dd, $J = 13.8$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.59 (d, $J = 8$ Hz, 1H, NH urethane), 5.91 (ddt, $J = 13.8$ Hz, $J = 10.5$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.10 (d, $J = 8$ Hz, 1H, NH); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 17.70$ (CH_3 Ala), 28.30 (3 CH_3 Boc), 48.32 (α-CH Ala), 55.52 (α-CH Ser), 63.03 (CH_2 Ser), 66.04 (CH_2 allyl), 80.16 (Cq Boc), 118.73 ($=CH_2$), 131.55 (=CH), 156.02 (C=O urethane), 171.07 (C=O), 172.59 (C=O); anal. calcd for $C_{14}H_{24}N_2O_6$: C 53.15, H 7.65, N 8.86; found: C 53.08, H 7.56, N 9.08; EI MS (70 eV); m/z: 317.2 $[M+H]^+$.

L-Seryl-L-alanine allyl ester hydrotrifluoroacetate, HSerAlaOAll · CF_3CO_2H (10): To a solution of BocSerAlaOAll (50 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added trifluoroacetic acid (2 mL) and the solution was stirred for 30 min. Ether was added (30 mL) and the precipitated product was washed several times with ether. After drying in vacuo the deprotected dipeptide **10** was isolated as a white solid. Yield: 49 mg (95%); m.p. 124–125 °C; $[\alpha]_D^{22} = -15.6$ ($c = 1$ in DMF); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 1.43$ (d, $J = 7.3$ Hz, 3H, CH_3 Ala), 3.78 (dd, $J = 17$ Hz, $J = 5.3$ Hz, 1H, β-CH_a Ser), 3.96 (dd, $J = 17$ Hz, $J = 4$ Hz, 1H, β-CH_b Ser), 4.02 (dd, $J = 5.3$ Hz, $J = 4$ Hz, 1H, α-CH Ser), 4.51 (quintet, $J = 7.3$ Hz, 1H, α-CH Ala), 4.63 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 5.24 (dd, $J = 10.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.31 (dd, $J = 13.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.95 (ddt, $J = 13.5$ Hz, 10.5 Hz, $J = 5.7$ Hz, 1H, =CH); anal. calcd for $C_{11}H_{17}F_3N_2O_6$: C 40.00, H 5.19, N 8.48; found: C 40.10, H 5.28, N 8.58; EI MS (70 eV); m/z: 217.2 $[M - CF_3CO_2]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine allyl ester, MyrGlyCys(Pal)ThrLeuSerAlaOAll (11)**: To a solution of MyrGlyCys(Pal)ThrLeuOH **9** (20 mg, 0.023 mmol) and HSerAlaOAll · CF_3CO_2H **10** (8 mg, 0.023 mmol) in CH_2Cl_2 (10 mL) and DMF (2 mL) was added NEt_3 (2.3 mg, 3.2 μL, 0.023 mmol), HOBt (3.1 mg, 0.023 mmol), and finally EDC (8.8 mg, 0.046 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL), and washed with 1M HCl (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from CH_2Cl_2/ethyl acetate gave a white solid. Yield: 10.2 mg (43%); $R_f = 0.38$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); $[\alpha]_D^{22} = -3.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.90$ (t, $J = 6.8$ Hz, 6H, CH_3 Pal, CH_3 Myr), 0.93 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.22 (d, $J = 6.5$ Hz, 3H, CH_3 Thr), 1.27 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.45 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 1.53–1.78 (m, 7H, β-CH_2 Pal, β-CH_2 Myr, β-CH_2 Leu, γ-CH Leu), 2.30 (t, $J = 7$ Hz, 2H, α-CH_2 Myr), 2.59 (t, $J = 7$ Hz, 2H, α-CH_2 Pal), 3.24 (dd, $J = 16$ Hz, $J = 5$ Hz, 1H, CH_a Cys), 3.35 (dd, $J = 16$ Hz, $J = 3$ Hz, 1H, CH_b Cys), 3.70 (m, 4H, CH_2 Ser, α-CH Ser, α-CH Thr), 4.25 (d, $J = 12$ Hz, CH_2 Gly), 4.31–4.56 (m, 4H, α-CH Cys, β-CH Thr, α-CH Ala, α-CH Leu), 4.64 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 5.24 (dd, $J = 11$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.32 (dd, $J = 14$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.90 (ddt, $J = 14$ Hz, $J = 11$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.50–7.95 (m, 6H, NH); $C_{54}H_{98}N_6O_{11}S$; FAB MS (glycerol); m/z: 1040.2 $[M+H]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine, MyrGlyCys(Pal)ThrLeuSerAlaOH (1)**: To a solution of MyrGlyCys(Pal)ThrLeuSerAlaOAll (**11**, 30 mg, 0.029 mmol) in CH_2Cl_2 (10 mL) was added under argon morpholine (3.3 mg, 3.3 μL, 0.037 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**), and the mixture was stirred at 20 °C for 1 h. The solvent was washed with 1M HCl (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate/methanol 95/5 [*v*/*v*] as eluent. Yield: 27 mg (92%). The product is identical to the lipopeptide obtained by the choline ester route from the Boc protected intermediate **21** (see below for detailed analytical data).

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***N*,*N*-Bis-(*tert*-Butyloxycarbonyl-glycyl)-L-cystine bis-allyl ester, (BocGlyCysOAll)$_2$ (13)**: To an ice-cold solution of BocGlyOH (1.73 g, 9.92 mmol) and (HCysOAll)$_2 \cdot 2p$TosOH[11] (3.00 g, 4.51 mmol) in CH_2Cl_2 (50 mL) was added NEt_3 (0.91 mg, 1.25 mL, 9.02 mmol), and EEDQ (4.90 g, 19.8 mmol). The mixture was stirred at 20 °C for 12 h, the precipitated urea was filtered off, and the solvent was washed with 1M HCl (25 mL), saturated $NaHCO_3$ (25 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **2** was isolated as a viscous clear oil from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 50/50 [*v*/*v*] as eluent. Yield: 2.07 g (72%); $R_f = 0.32$ (ethyl acetate/*n*-hexane 50/50 [*v*/*v*]); $[\alpha]_D^{22} = -44$ ($c = 0.5$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (s, 18H, 6 CH_3 Boc), 3.18 (d, $J = 5.7$ Hz, 4H, 2 CH_2 Gly), 3.85 (dd, $J = 16.2$ Hz, $J = 5.5$ Hz, 2H, 2 CH_a Cys), 3.90 (dd, $J = 16.2$ Hz, $J = 5.3$ Hz, 2H, 2 CH_b Cys), 4.66 (dt, $J = 5.8$ Hz, $J = 1.1$ Hz, 4H, 2 CH_2 allyl), 4.89 (dt, 2H, $J = 11.3$ Hz, $J = 5.7$ Hz, 2 α-CH Cys), 5.27 (dt, $J = 11$ Hz, $J = 1.1$ Hz, 2H, 2 Ch_a=), 5.35 (dt, $J = 17.2$ Hz, $J = 1.1$ Hz, 2H, 2 CH_b=), 5.76 (s, 2H, 2NH urethane), 5.91 (ddt, $J = 17.2$ Hz, $J = 11$ Hz, $J = 5.8$ Hz, 2H, 2CH=), 7.41 (d, $J = 7.4$ Hz, 2H, 2NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta =$ 28.22 (6 CH_3 Boc), 40.55 (2 CH_2 Cys), 44.03 (2 CH_2 Gly), 51.88 (2 α-CH Cys), 66.41 (2 CH_2 allyl), 80.04 (2 Cq Boc), 119.09 (2 CH_2=), 131.15 (2 CH=), 156.14 (2 C=O urethane), 169.75 (2 C=O), 169.99 (2 C=O); anal. calcd for $C_{26}H_{42}N_4O_{10}S_2$: C 49.19, H 6.67, N 8.83; found: C 49.00, H 6.52, N 8.82.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteine allyl ester, BocGlyCys(Pal)OAll (14)**: To a solution of (BocGlyCysOAll)$_2$ (**13**, 1.00 g, 1.57 mmol) in CH_2Cl_2 (50 mL) was added dithiothreitol (DTT) (1.21 g, 7.85 mmol) and NEt_3 (320 mg, 0.44 mL, 3.14 mmol). The mixture was stirred at 20 °C for 1 h. The solution was washed twice with distilled water (25 mL) and dried over $MgSO_4$. To the crude product BocGlyCysOAll in CH_2Cl_2 was added NEt_3 (320 mg, 0.44 mL, 3.14 mmol) and a catalytic amount of DMAP and palmitoyl choride (2.15 g, 7.85 mmol). After stirring the mixture at 20 °C for 1 h, the solution was concentrated in vacuo and the product **14** was isolated as a white solid from the residue by flash chromatography on silica gel using *n*-hexane/ethyl acetate 70/30 [*v*/*v*] as eluent. Yield: 1.22 g (70%); m.p. 39–40 °C; $R_f = 0.68$ (ethyl acetate/*n*-hexane 50/50 [*v*/*v*]); $[\alpha]_D^{22} = +15.7$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 7$ Hz, 3H, CH_3 Pal), 1.25 (s, 24H, 12 CH_2 Pal), 1.46 (s, 9H, 3 CH_3 Boc), 1.63 (t, $J = 6.9$ Hz, 2H, β-CH_2 Pal), 2.57 (t, $J = 7.7$ Hz, 2H, α-CH_2 Pal), 3.33 (dd, $J = 16.6$ Hz, $J = 5.7$ Hz, 1H, CH_a Cys), 3.42 (dd, $J = 16.6$ Hz, $J = 4.7$ Hz, 1H, CH_b Cys), 3.78 (d, $J = 5.7$ Hz, 1H, CH_a Gly), 3.86 (d, $J = 4.7$ Hz, 1H, CH_b Gly), 4.63 (d, $J = 5.8$ Hz, 2H, CH_2 allyl), 4.82 (ddd, 1H, $J = 7.6$ Hz, $J = 6.3$ Hz, $J = 4.7$ Hz, α-CH Cys), 5.26 (d, $J = 11.6$ Hz, 1H, =CH_a), 5.35 (d, $J = 17.2$ Hz, 1H, =CH_b), 5.37 (brs, 1H, NH urethane), 5.91 (ddt, $J = 17.2$ Hz, $J = 11.6$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.04 (d, $J = 7.6$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 14.01$ (CH_3 Pal), 22.57 (CH_2 Pal), 25.43 (CH_2 Cys), 28.19 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 31.81 (α-CH_2 Pal), 43.89 (CH_2 Gly), 52.07 (α-CH Cys), 66.39 (CH_2 allyl), 80.02 (Cq Boc), 118.98 (=CH_2), 131.22 (=CH), 155.87 (C=O urethane), 169.53 (C=O), 169.59 (C=O), 198.74 (C=O thioester); anal. calcd for $C_{29}H_{52}N_2O_6S$: C 62.55, H 9.41, N 5.03; found: C 62.54, H 9.52, N 5.95; EI MS (70 eV): m/z: 556.4 $[M]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteine, BocGlyCys(Pal)-OH (15)**: To a solution of BocGlyCys(Pal)OAll (**14**, 1.10 g, 1.97 mmol) in CH_2Cl_2 (30 mL) was added under argon morpholine (228 mg, 223 µL, 2.57 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) and the mixture was stirred at 20 °C for 2 h. The solvent was washed with 1M HCl (25 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **15** was isolated as a white solid from the residue by flash chromatography on silica gel eluting with a gradient ethyl acetate/*n*-hexane 50/50 [*v*/*v*] to ethyl acetate. Yield: 842 mg (83%); m.p. 94–95 °C; $R_f = 0.40$ (ethyl acetate/*n*-hexane/acetic acid) 50/50/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = +1.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 3H, CH_3 Pal), 1.25 (s, 24H, 12 CH_2 Pal), 1.45 (s, 9H, 3 CH_3 Boc), 1.63 (t, $J = 7.2$ Hz, 2H, β-CH_2 Pal), 2.56 (t, $J = 7.2$ Hz, 2H, α-CH_2 Pal), 3.40 (d, $J = 14$ Hz, 2H, CH_2 Cys), 3.77 (dd, $J = 11.6$ Hz, $J = 5.7$ Hz, 1H, CH_a Gly), 3.96 (dd, $J = 11.6$ Hz, $J = 5.2$ Hz, 1H, CH_b Gly), 4.77 (brs, 1H, α-CH Cys), 5.53 (s, 1H, NH urethane), 7.18 (d, $J = 6.20$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 14.16$ (CH_3 Pal), 22.75 (CH_2 Pal), 25.60 (CH_2 Cys), 28.33 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 31.96 (α-CH_2 Pal), 44.08 (CH_2 Gly), 52.50 (α-CH Cys), 80.70 (Cq Boc), 156.42 (C=O urethane), 170.52 (C=O), 172.21 (C=O), 199.68 (C=O thioester); anal. calcd for $C_{26}H_{48}N_2O_6S$: C 60.43, H 9.36, N 5.42; found: C 60.33, H 9.28, N 5.65.

Synthesis of H-Thr-LeuOCho · HBr (16), *N*-*tert*-butyloxycarbonyl-L-threonyl-L-leucine 2-bromoethyl ester, BocThrLeuOEtBr: To a solution of BocThrOH (522 mg, 2.38 mmol) and HLeuOEtBr · HCl (652 mg, 2.38 mmol) in CH_2Cl_2 (50 mL) was added NEt_3 (241 mg, 330 µL, 2.38 mmol), HOBt (322 mg, 2.38 mmol), and DIC (660 mg, 810 µL, 5.24 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was washed with 1M HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **5** was isolated as a white solid from the residue by flash chromatography on silica gel using *n*-hexane/ethyl acetate 80/20 [*v*/*v*] as eluent. Yield: 985 mg (94%); m.p. 44–45 °C; $R_f = 0.64$ (ethyl acetate/*n*-hexane 70/30 [*v*/*v*]); $[\alpha]_D^{22} = -29.8$ ($c = 1$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.93$ (d, $J = 5.5$ Hz, 3H, CH_3 Leu), 0.95 (d, $J = 5.5$ Hz, 3H, CH_3 Leu), 1.20 (d, $J = 6$ Hz, 3H, CH_3 Thr), 1.46 (s, 9H, 3 CH_3 Boc), 1.54–1.77 (m, 3H, CH_2 Leu, γ-CH Leu), 3.36 (brs, 1H, OH), 3.52 (t, $J = 6.1$ Hz, 2H, CH_2Br), 4.08 (dd, $J = 8$ Hz, $J = 2$ Hz, 1H, α-CH Thr), 4.34 (qd, $J = 6$ Hz, $J = 2$ Hz, 1H, β-CH Thr), 4.40 (dd, $J = 12$ Hz, $J = 6$ Hz, 1H, OCH_a), 4.48 (dd, $J = 12$ Hz, $J = 6$ Hz, 1H, OCH_b), 4.59 (t, $J = 9$ Hz, 1H, α-CH Leu), 5.52 (d, $J = 7.6$ Hz, 1H, NH urethane), 6.97 (d, $J = 7.6$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 17.89$ (CH_3 Thr), 21.63 (CH_3 Leu), 22.82 (CH_3 Leu), 24.77 (γ-CH Leu), 28.26 (3 CH_3 Boc), 28.55 (CH_2Br), 40.78 (CH_2 Leu), 50.89 (α-CH Leu), 57.69 (α-CH Thr), 64.49 (OCH_2), 66.81 (β-CH Thr), 80.35 (Cq Boc), 156.43 (C=O urethane), 171.44 (C=O), 172.23 (=O); anal. calcd for $C_{17}H_{31}BrN_2O_6$: C 46.47, H 7.11, N 6.37; found: C 46.34, H 7.09, N 6.28; EI MS (70 eV); m/z: 439.2 $[M+H]^+$.

***N*-*tert*-Butyloxycarbonyl-L-threonyl-L-leucine choline ester bromide, BocThrLeuCho**: To a solution of BocThrLeuOEtBr (4.15 g, 9.44 mmol) in acetone (40 mL) at −78 °C was added NMe_3 (1.00 g, 1.70 mL, 19 mmol) and the mixture was stirred for 48 h. The product BocThrLeuCho was precipitated with diethyl ether (50 mL) and was obtained as a white hygroscopic solid. Yield: 4.33 g (92%); m.p. 66–68 °C; $[\alpha]_D^{22} = -37.3$ ($c = 1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 0.90$ (d, $J = 6$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 6$ Hz, 3H, CH_3 Leu), 1.18 (d, $J = 5.2$ Hz, 3H, CH_3 Thr), 1.45 (s, 9H, 3 CH_3 Boc), 1.48–1.70 (m, 3H, CH_2 Leu, γ-CH Leu), 3.25 (s, 9H, 3 CH_3 Cho), 3.72 (t, $J = 4.6$ Hz, 2H, CH_2N), 4.04 (d, $J = 4$ Hz, 1H, α-CH Thr), 4.15 (qd, $J = 5.2$ Hz, $J = 4$ Hz, 1H, β-CH Thr), 4.55 (dd, $J = 9.2$ Hz, $J = 5.2$ Hz, 1H, α-CH Leu), 4.60–4.72 (m, 2H, OCH_2); ^{13}C NMR (62.8 MHz, CD_3OD): $\delta = 18.97$ (CH_3 Thr), 21.58 (CH_3 Leu), 22.92 (CH_3 Leu), 24.65 (γ-CH Leu), 28.34 (3 CH_3 Boc), 39.86 (CH_2 Leu), 51.21 (CH Leu), 54.38 (3 CH_3 Cho), 58.47 (α-CH Thr), 58.87 (CH_2N), 64.72 (OCH_2), 67.41 (β-CH Thr), 79.84 (Cq Boc), 156.04 (C=O urethane), 171.26 (C=O), 171.86 (C=O); $C_{20}H_{40}BrN_3O_6$; FAB MS (glycerol); m/z: 418.2826 $[M-Br]^+$.

L-Threonyl-L-leucine choline ester bromide hydrobromide, HThrLeuCho · HBr (16): To a solution of BocThrLeuCho (210 mg, 0.40 mmol) in CH_2Cl_2 (5 mL) −50 °C was added HBr/CH_3CO_2H (5 mL). After stirring the mixture for 15 min, the product **16** was precipitated and washed several times with ether. The dipeptide was isolated as a white solid. Yield: 1.32 g (95%); m.p. 206–208 °C; $[\alpha]_D^{22} = -14.1$ ($c = 1$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.95$ (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.99 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.39 (d, $J = 6.4$ Hz, 3H, CH_3 Thr), 1.66–1.78 (m, 3H, CH_2 Leu, γ-CH Leu), 3.27 (s, 9H, 3 CH_3 Cho), 3.79–3.83 (m, 3H, CH_2N, α-CH Thr), 4.06 (qd, $J = 6.4$ Hz, $J = 6.2$ Hz, 1H, β-CH Thr), 4.50 (dd, $J = 9.8$ Hz, $J = 5$ Hz, 1H, α-CH Leu), 4.55–4.69 (m, 2H, OCH_2); ^{13}C NMR (125.6 MHz, CD_3OD): $\delta = 20.32$ (CH_3 Thr), 21.73 (CH_3 Leu), 23.26 (CH_3 Leu), 25.79 (CH Leu), 40.61 (γ-CH_2 Leu), 52.52 (α-CH Leu), 54.55 (3 CH_3 Cho), 59.73 (α-CH Thr), 59.79 (CH_2N), 65.81 (OCH_2), 67.50 (β-CH Thr), 168.88 (C=O), 172.69 (C=O); $C_{15}H_{32}Br_2N_3O_4$; FAB MS (glycerol); m/z: 318.2346 $[M-2Br]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucine choline ester bromide, BocGlyCys(Pal)ThrLeuCho (17)**: To a solution of BocGlyCys(Pal)OH **15** (210 mg, 0.40 mmol) and HThrLeuCho · HBr (**16**, 194 mg, 0.40 mmol) in CH_2Cl_2 (20 mL) and DMF (5 mL) was added NEt_3 (41 mg, 56 µL, 0.40 mmol), HOBt (55 mg, 0.40 mmol), and EDC (155 mg, 0.80 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure, the residue was dissolved in a small volume of CH_2Cl_2 and precipitated with diethyl ether (50 mL). The crude product was then dissolved in water (50 mL) and extracted with CH_2Cl_2 (4 × 25 mL). The organic layers were dried over $MgSO_4$ and concentrated in vacuo to give a white hygroscopic solid. Yield: 250 mg

(68%); $R_f = 0.5$ (CH_2Cl_2/methanol/acetic acid) 80/20/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = -30$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 7$ Hz, 3H, CH_3 Pal), 0.88 (d, $J = 6$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 6$ Hz, 3H, CH_3 Leu), 1.25 (s, 24H, 12 CH_2 Pal), 1.35 (d, $J = 6$ Hz, 3H, CH_3 Thr), 1.47 (s, 9H, 3 CH_3 Boc), 1.60–1.82 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.60 (t, $J = 7$ Hz, 2H, α-CH_2 Pal), 3.27 (s, 9H, 3 CH_3 Cho), 3.20–3.50 (m, 2H, CH_2 Cys), 3.60–3.80 (m, 4H, CH_a Gly, CH_2N, α-CH Thr), 4.10–4.22 (m, 3H, CH_b Gly, β-CH Thr, α-CH Leu), 4.51–4.59 (m, 3H, α-CH Cys, OCH_2), 6.30 (br s, 1H, NH urethane), 7.62 (d, $J = 6$ Hz, 1H, NH), 7.82 (d, $J = 5.3$ Hz, 1H, NH), 8.48 (d, $J = 7.7$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 13.98$ (CH_3 Pal), 19.75 (CH_3 Thr), 21.11 (CH_3 Leu), 22.57 (CH_2 Pal), 22.80 (CH_3 Leu), 24.65 (γ-CH Leu), 25.41 (CH_2 Cys), 28.22 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 31.80 (α-CH_2 Pal), 39.02 (CH_2 Leu), 43.97 (CH_2 Gly), 51.31 (α-CH Leu), 54.08 (3 CH_3 Cho), 55.82 (α-CH Cys), 58.40 (CH_2N), 59.97 (α-CH Thr), 64.68 (OCH_2), 66.85 (β-CH Thr), 80.14 (Cq Boc), 156.56 (C=O, urethane), 170.93 (C=O), 171.31 (C=O), 171.60 (C=O), 172.51 (C=O), 200.69 (C=O thioester); $C_{41}H_{78}BrN_5O_9S$; FAB MS (glycerol); *m*/*z*: 816.5 $[M - Br]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucine, BocGlyCys(Pal)ThrLeuOH (18)**: To a solution of dimethyl-β-cyclodextrin (800 mg, 0.68 mmol) in phosphate buffer (10 mL, 0.6 mM, pH = 6.5) was added BocGlyCys(Pal)ThrLeuOCho (**17**, 40 mg, 0.045 mmol) and butyrylcholine esterase (100 U). The mixture was stirred at 37 °C for 48 h, the solution was diluted with 1M HCl (50 mL) and benzyltriethylammonium bromide (5 g, 17.7 mmol) was added. The precipitated product **18** was filtered off, washed several times with distilled water, and then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 90/10 [*v*/*v*] as eluent. Yield: 25 mg (77%); colorless solid; m.p. 115–118 °C; $R_f = 0.5$ (CH_2Cl_2/methanol/acetic acid) 90/10/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = -22.8$ ($c = 0.5$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 0.88$ (t, $J = 7$ Hz, 3H, CH_3 Pal), 0.92 (d, $J = 5.8$ Hz, 3H, CH_3 Leu), 0.96 (d, $J = 5.8$ Hz, 3H, CH_3 Leu), 1.15 (d, $J = 6.4$ Hz, 3H, CH_3 Thr), 1.25 (s, 24H, 12 CH_2 Pal), 1.46 (s, 9H, 3 CH_3 Boc), 1.55–1.80 (m, 5H, β-CH_2 Pal, β-CH_2 Leu, γ-CH Leu), 2.56 (t, $J = 7.3$ Hz, 2H, α-CH_2 Pal), 3.24 (dd, $J = 14.3$ Hz, $J = 5.2$, 1H, CH_a Cys), 3.34 (dd, $J = 14.3$ Hz, $J = 7.3$, 1H, CH_b Cys), 3.81 (d, $J = 17.4$ Hz, 2H, CH_2 Gly), 4.18 (qd, $J = 6.4$ Hz, $J = 4$ Hz, 1H, β-CH Thr), 4.45 (d, $J = 4$ Hz, 1H, α-CH Thr), 4.51 (dd, $J = 7.3$ Hz, $J = 5.2$ Hz, 1H, α-CH Cys), 4.61 (dd, $J = 7.3$ Hz, $J = 4.8$ Hz, 1H, α-CH Leu); ^{13}C NMR (100.6 MHz, CD_3OD): $\delta = 14.09$ (CH_3 Pal), 18.62 (CH_3 Thr), 21.95 (CH_3 Leu), 22.66 (CH_2 Pal), 22.86 (CH_3 Leu), 24.87 (γ-CH Leu), 25.51 (CH_2 Cys), 28.22 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 31.50 (α-CH_2 Pal), 41.11 (CH_2 Leu), 44.00 (CH_2 Gly), 51.00 (α-CH Leu), 52.50 (α-CH Cys), 58.85 (α-CH Thr), 68.02 (β-CH Thr), 80.23 (Cq Boc), 157.08 (C=O urethane), 170.11 (3 C=O), 175.28 (C=O), 200.00 (C=O thioester); anal. calcd for $C_{36}H_{66}N_4O_9S$: C 59.15, H 9.10, N 7.66; found: C 58.84, H 9.10, N 7.26.

Synthesis of H-Ser-Ala-OCho · HBr (19), *N*-*tert*-butyloxycarbonyl-L-seryl-L-alanine choline ester bromide, Boc-Ser-Ala-Cho: To a solution of BocSerOH (500 mg,2.43 mmol) and HAlaOCho · HBr (816 mg, 2.43 mmol) in CH_2Cl_2 (30 mL) and DMF (30 mL) was added NEt_3 (246 mg, 340 µL, 2.43 mmol), HOBt (328 mg, 2.43 mmol) and then DIC (613 mg, 750 µL, 4.86 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure, the residue was dissolved in a small volume of methanol, and the product was precipitated with 50 mL diethyl ether. Purification by flash chromatography on silica gel using CH_2Cl_2/methanol 70/30 [*v*/*v*] as eluent gave a white hygroscopic solid. Yield: 432 mg (40%); $[\alpha]_D^{22} = -26$ ($c = 1$ in MeOH); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 1.44$ (s, 9H, 3 CH_3 Boc), 1.45 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 3.31 (s, 3 CH_3 Cho), 3.71 (dd, $J = 12$ Hz, $J = 6$ Hz, 1H, β-CH_a Ser), 3.89 (dd, $J = 12$ Hz, $J = 4$ Hz, 1H, β-CH_b Ser), 3.93 (t, $J = 4.5$ Hz, 2H, CH_2N), 4.18 (dd, $J = 6$ Hz, $J = 4$ Hz, 1H, α-CH Ser), 4.48 (quintet, $J = 7.2$ Hz, 1H, α-CH Ala), 4.63 (br s, 2H, OCH_2); ^{13}C NMR (100.6 MHz, CD_3OD): $\delta = 17.55$ (CH_3 Ala), 28.69 (3 CH_3 Boc), 49.34 (α-CH Ala), 54.60 (3 CH_3 CHO), 57.69 (α-CH Ser), 59.79 (CH_2N), 63.61 (CH_2 Ser), 65.83 (OCH_2), 80.65 (Cq Boc), 157.49 (C=O urethane), 164.75 (C=O ester), 174.42 (C=O); $C_{16}H_{32}BrN_3O_6$; FAB MS (glycerol); *m*/*z*: 362.4 $[M - Br]^+$.

L-Seryl-L-alanine choline ester bromide hydrobromide, HSerAlaCho · HBr (19): To a solution of BocThrLeuCho (1.46 g, 2.93 mmol) in CH_2Cl_2 (5 mL) at −50 °C was added HBr/CH_3CO_2H (5 mL). After the mixture had been stirred for 15 min, the product **19** was precipitated and washed several times with ether. The dipeptide was isolated as a white hygroscopic solid. Yield: 1.32 g (95%); $[\alpha]_D^{22} = -10.2$ ($c = 1$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.49$ (d, $J = 7.3$ Hz, 3H, CH_3 Ala), 3.29 (s, 3 CH_3 Cho), 3.85 (t, $J = 4.7$ Hz, 2H, CH_2N), 3.93 (dd, $J = 11.7$ Hz, $J = 6$ Hz, 1H, β-CH_a Ser), 4.03 (dd, $J = 12$ Hz, $J = 4$ Hz, 1H, β-CH_b Ser), 4.09 (dd, $J = 6$ Hz, $J = 4$ Hz, 1H, α-CH Ser), 4.55 (quintet, $J = 7.3$ Hz, 1H, α-CH Ala), 4.64 (br s, 2H, OCH_2); ^{13}C NMR (100.6 MHz, CD_3OD): $\delta = 17.03$ (CH_3 Ala), 49.79 (α-CH Ala), 54.66 (3 CH_3 Cho), 55.91 (α-CH Ser), 59.94 (CH_2N), 61.64 (CH_2 Ser), 65.91 (OCH_2), 168.31 (C=O, ester), 172.81 (C=O); $C_{11}H_{25}Br_2N_3O_4$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine choline ester bromide, BocGlyCys(Pal)ThrLeuSerAlaCho (20)**: To a solution of BocGlyCys(Pal)ThrLeuOH (**18**, 100 mg, 0.14 mmol) and HSerAlaCho · HBr (**19**, 58 mg, 0.14 mmol) in CH_2Cl_2 (20 mL) and DMF (5 mL) was added NEt_3 (14 mg, 19 µL, 0.14 mmol), HOBt (19 mg, 0.14 mmol), and then EDC (53 mg, 0.28 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure, and the residue was dissolved in a small volume of CH_2Cl_2 and precipitated with diethyl ether (50 mL). The crude product was then dissolved in water (50 mL) and extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give a white hygroscopic solid. Yield: 95 mg (66%); $R_f = 0.26$ (CH_2Cl_2/methanol/acetic acid) 80/20/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = -3.6$ ($c = 0.5$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.84$ (t, $J = 6.7$ Hz, 3H, CH_3 Pal), 0.92 (d, $J = 6$ Hz, 3H, CH_3 Leu), 0.98 (d, $J = 6$ Hz, 3H, CH_3 Leu), 1.23 (d, $J = 7.3$ Hz, 3H, CH_3 Thr), 1.28 (s, 24H, 12 CH_2 Pal), 1.44 (d, $J = 6$ Hz, 3H, CH_3 Ala), 1.46 (s, 9H, 3 CH_3 Boc), 1.58–1.85 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.60 (t, $J = 6.5$ Hz, 2H, α-CH_2 Pal), 3.20 (dd, $J = 14.3$ Hz, $J = 4$ Hz, 1H, CH_a Cys), 3.44 (dd, $J = 14.3$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 3.27 (s, 9H, 3 CH_3 Cho), 3.60–3.85 (m, 7H, CH_2 Gly, CH_2 Ser, CH_2N, α-CH Thr), 4.22–4.26 (m, 2H, β-CH Thr, α-CH Leu), 4.21 (dd, $J = 8$ Hz, $J = 4$ Hz, 1H, α-CH Cys), 4.36 (t, $J = 4.8$ Hz, 1H, α-CH Ser), 4.46 (quintet, $J = 7.3$ Hz, 1H, α-CH Ala), 4.47–4.58 (m, 2H, OCH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 14.46$ (CH_3 Pal), 16.92 (CH_3 Ala), 20.31 (CH_3 Thr), 21.84 (CH_3 Leu), 23.53 (CH_2 Pal), 23.64 (CH_3 Leu), 25.76 (γ-CH Leu), 26.53 (CH_2 Cys), 28.22 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 32.96 (α-CH_2 Pal), 40.97 (CH_2 Leu), 44.74 (CH_2 Gly), 49.84 (α-CH Ala), 54.38 (α-CH Leu), 54.52 (3 CH_3 Cho), 55.07 (α-CH Cys), 57.05 (α-CH Ser), 59.83 (CH_2N), 61.04 (α-CH Thr), 62.71 (OCH_2), 65.71 (CH_2 Ser), 68.20 (β-CH Thr), 80.78 (Cq Boc), 158.30 (C=O urethane), 172.15 (C=O), 172.77 (2 C=O), 172.94 (C=O), 173.35 (C=O), 174.89 (C=O), 200.75 (C=O thioester); $C_{47}H_{88}BrN_7O_{12}S$; FAB MS (glycerol); *m*/*z*: 974.8 $[M - Br]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine, Boc-Gly-Cys(Pal)-Thr-Leu-Ser-Ala-OH (21)**: To a solution of dimethyl-β-cyclodextrin (100 mg, 0.068 mmol) in phosphate buffer (10 mL, 0.6 mM, pH = 6.5) was added BocGlyCys(Pal)ThrLeuSerAlaOCho (**20**, 20 mg, 0.019 mmol) and butyrylcholine esterase (50 U). The mixture was stirred at 37 °C for 48 h, the solution was diluted with 1M HCl (30 mL), and benzyltriethylammonium bromide (2 g, 7.10 mmol) was added. The precipitated product **21** was filtered off, washed several times with distilled water, and then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 90/10 [*v*/*v*] as eluent. Yield: 9.7 mg (58%); yellowish solid; $R_f = 0.45$ (CH_2Cl_2/methanol/acetic acid) 80/20/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = -7.4$ ($c = 0.5$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.90$ (t, $J = 6.8$ Hz, 3H, CH_3 Pal), 0.93 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.20 (d, $J = 6.3$ Hz, 3H, CH_3 Thr), 1.28 (s, 24H, 12 CH_2 Pal), 1.41 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 1.46 (s, 9H, 3 CH_3 Boc), 1.58–1.76 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.59 (t, $J = 6.5$ Hz, 2H, α-CH_2 Pal), 3.27 (dd, $J = 14$ Hz, $J = 4$ Hz, 1H, CH_a Cys), 3.41 (dd, $J = 14$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 3.73 (t, $J = 7$ Hz, 2H, CH_2 Ser), 3.81 (d, $J = 7$ Hz, 1H, CH_a Gly), 3.83 (d, $J = 7$ Hz, 1H, CH_b Gly), 4.19 (qd, $J = 6.3$ Hz, $J = 4.4$ Hz, 1H, β-CH Thr), 4.32 (d, $J = 4.4$ Hz, 1H, α-CH Thr), 4.33-4.49 (m, 3H, α-CH Cys, α-CH Ser, α-CH Ala), 4.62 (dd, $J = 7.6$ Hz, $J = 5.5$ Hz, 1H, α-CH Leu); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 14.44$ (CH_3 Pal), 17.87 (CH_3 Ala), 20.12 (CH_3 Thr), 21.87 (CH_3 Leu), 23.59 (CH_2 Pal), 23.71 (CH_3 Leu), 25.82 (γ-CH Leu), 26.63 (CH_2 Cys), 28.75 (3 CH_3 Boc), 29.00–30.00 (12 CH_2 Pal), 33.05 (α-CH_2 Pal), 41.36 (CH_2 Leu), 44.78 (CH_2 Gly), 47.92 (α-CH Ala), 53.60 (α-CH Leu), 54.76 (α-CH Cys), 56.73 (α-CH Ser), 60.40 (α-CH Thr), 63.05 (β-CH_2 Ser), 68.35 (β-CH Thr), 80.87 (Cq Boc), 158.30 (C=O, urethane), 171.72 (C=O), 172.44 (C=O), 172.94 (3 C=O), 172.99 (C=O), 174.75 (C=O), 200.75 (C=O thioester); $C_{42}H_{76}N_6O_{12}S$; FAB MS (glycerol/NMP); *m*/*z*: 889.6 $[M+H]^+$.

***N*-myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine, MyrGlyCys(Pal)ThrLeuSerAlaOH (1)**: To a solution of MyrOH

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(8.5 mg, 0.037 mmol), HOBt (5 mg, 0.037 mmol), and EDC (7 mg, 0.037 mmol) in CH_2Cl_2 (2 mL) was added after 15 min, HGlyCys(Pal)ThrLeuSerAlaOH · CF_3CO_2H (43 mg, 0.037 mmol) (this product was obtained by Boc deprotection of BocGlyCys(Pal)ThrLeuSerAlaOH **21** using CF_3CO_2H. Yield: 93%) dissolved in DMF (2 mL) containing NEt_3 (3.7 mg, 5 μL, 0.037 mmol). The mixture was stirred at 20 °C for 2 h, the solvents were evaporated under reduced pressure. The product **1** was isolated as a white solid from the residue by flash chromatography on silica gel eluting with a gradient of CH_2Cl_2/methanol 95/5 [*v*/*v*] to CH_2Cl_2/methanol 80/20 [*v*/*v*]. Yield: 27 mg (73%); $R_f = 0.27$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); $[\alpha]_D^{22} = +4$ (*c* = 1 in DMF); ^{1}H NMR (400 MHz, CD_3OD, 50 °C): $\delta = 0.88$ (t, *J* = 7 Hz, 6H, CH_3 Pal, CH_3 Myr), 0.91 (d, *J* = 6.5 Hz, 3H, CH_3 Leu), 0.95 (d, *J* = 6.5 Hz, 3H, CH_3 Leu), 1.22 (d, *J* = 7.2 Hz, 3H, CH_3 Thr), 1.26 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.43 (d, *J* = 7.2 Hz, 3H, CH_3 Ala), 1.55–1.76 (m, 7H, β-CH_2 Pal, β-CH_2 Myr, β-CH_2 Leu, γ-CH Leu), 2.28 (t, *J* = 7.8 Hz, 2H, α-CH_2 Myr), 2.58 (t, *J* = 7.8 Hz, 2H, α-CH_2 Pal), 3.15–3.45 (m, 2H, CH_2 Cys), 3.72–3.98 (m, 4H, CH_2 Ser, α-CH_2 Gly), 4.18–4.55 (m, 6H, α-CH Cys, α-CH Thr, α-CH Leu, α-CH Ser, α-CH Ala, β-CH Thr); anal. calcd for $C_{51}H_{97}N_6O_{11}S$: C 61.11, H 9.75, N 8.38; found: C 61.07, H 9.05, N 6.62; MALDI-TOF MS (MeOH/TFA 9/1); *m*/*z*: 1002.6 $[M+H]^+$, 1025.7 $[M+Na]^+$, 1042 $[M+K]^+$.

***N-tert*-Butoxycarbonyl-L-threonyl-L-leucine, BocThrLeuOH (23)**: To a solution of BocThrLeuOAll (**22**, 1.49 g, 4.00 mmol) in CH_2Cl_2 (50 mL) was added under argon morpholine (453 mg, 450 μL, 5.20 mmol), a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**), and the mixture was stirred at 20 °C for 2 h. The solvent was washed with 1M HCl (50 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the product **23** was isolated as a white solid. Yield: 1.25 g (94%); m.p. 126–127 °C; $R_f = 0.45$ (ethyl acetate); $[\alpha]_D^{22} = -27$ (*c* = 0.5 in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.92$ (d, *J* = 5.3 Hz, 3H, CH_3 Leu), 0.94 (d, *J* = 5.3 Hz, 3H, CH_3 Leu), 1.18 (d, *J* = 6.3 Hz, 3H, CH_3 Thr), 1.46 (s, 9H, 3 CH_3 Boc), 1.52–1.75 (m, 3H, CH_2 Leu, γ-CH Leu), 4.17 (dd, *J* = 8 Hz, *J* = 2.5 Hz, 1H, α-CH Thr), 4.28 (qd, *J* = 6.4 Hz, *J* = 2.5 Hz, 1H, β-CH Thr), 4.57 (dt, *J* = 8.2 Hz, *J* = 6 Hz, 1H, α-CH Leu), 5.72 (d, *J* = 7.6 Hz, 1H, NH urethane), 7.23 (d, *J* = 8.1 Hz, 1H, NH); anal. calcd for $C_{15}H_{28}N_2O_6$: C 54.20, H 8.49, N 8.43; found: C 54.14, H 8.40, N 8.34.

***N-tert*-Butoxycarbonyl-L-threonyl-L-leucyl-L-seryl-L-alanine allyl ester, BocThrLeuSerAlaOAll (24)**: To a solution of BocThrLeuOH (**23**, 268 mg, 0.81 mmol) and HSerAlaOAll · CF_3CO_2H **10** (266 mg, 0.81 mmol) in CH_2Cl_2 (20 mL) and DMF (2 mL) was added NEt_3 (82 mg, 112 μL, 0.81 mmol), HOBt (109 mg, 0.81 mmol), and finally EDC (309 mg, 1.62 mmol). The mixture was stirred at 20 °C for 16 h, and the solvent was washed with 1M HCl (3 × 20 mL), saturated $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **24** was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 272 mg (63%); m.p. 126–127 °C; $R_f = 0.33$ (ethyl acetate); $[\alpha]_D^{22} = -55$ (*c* = 1 in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.91$ (d, *J* = 6.5 Hz, 6H, 2 CH_3 Leu), 1.13 (d, *J* = 6.2 Hz, 3H, CH_3 Thr), 1.44 (d, *J* = 6.9 Hz, 3H, CH_3 Ala), 1.46 (s, 9H, 3 CH_3 Boc), 1.55–1.70 (m, 3H, CH_2 Leu, γ-CH Leu), 3.72 (dd, *J* = 11.4 Hz, *J* = 5 Hz, 1H, CH_a Ser), 3.79 (dd, *J* = 11.4 Hz, *J* = 6 Hz, 1H, CH_b Ser), 4.09 (dt, *J* = 8 Hz, *J* = 6 Hz, 1H, α-CH Ser), 4.41 (d, *J* = 4.3 Hz, *J* = 3 Hz, 1H, α-CH Thr), 4.32 (qd, *J* = 6.2 Hz, *J* = 4.4 Hz, 1H, β-CH Thr), 4.59 (quintet, *J* = 7.3 Hz, 1H, α-CH Ala), 4.60 (dd, *J* = 14 Hz, *J* = 5.7 Hz, 1H, CH_a allyl), 4.66 (dd, *J* = 14 Hz, *J* = 5.7 Hz, 1H, CH_b allyl), 4.86 (q, *J* = 5.6 Hz, 1H, α-CH Leu), 5.26 (dd, *J* = 11 Hz, *J* = 1.2 Hz, 1H, =CH_a), 5.33 (dd, *J* = 14 Hz, *J* = 1.2 Hz, 1H, =CH_b), 5.79 (d, *J* = 8.4 Hz, 1H, NH urethane), 5.91 (ddt, *J* = 14 Hz, *J* = 11 Hz, *J* = 5.7 Hz, 1H, =CH), 7.74 (d, *J* = 8.4 Hz, 1H, NH), 7.83 (d, *J* = 7.3 Hz, 1H, NH), 7.91 (d, *J* = 8 Hz, 1H, NH); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 17.81$ (CH_3 Ala), 18.13 (CH_3 Thr), 22.00 (CH_3 Leu), 22.95 (CH_3 Leu), 24.73 (γ-CH Leu), 28.35 (3 CH_3 Boc), 41.37 (CH_2 Leu), 48.30 (α-CH Ala), 51.80 (α-CH Leu), 54.67 (α-CH Ser), 58.34 (α-CH Thr), 63.02 (β-CH_2 Ser), 66.08 (CH_2 allyl), 68.46 (β-CH Thr), 79.99 (Cq Boc), 118.77 (=CH_2), 131.54 (=CH), 156.13 (C=O urethane), 170.27 (C=O), 170.49 (C=O), 172.44 (C=O), 173.06 (C=O); anal. calcd for $C_{24}H_{42}N_4O_9$: C 54.33, H 7.98, N 10.56; found: C 53.93, H 8.01, N 10.41.

***N-tert*-Butoxycarbonyl-L-threonyl-L-leucyl-L-seryl-L-alanine, BocThrLeuSerAlaOH (25)**: To a solution of BocThrLeuSerAlaOAll (**24**, 170 mg, 0.32 mmol) in abs. CH_2Cl_2 (40 mL) was added under argon morpholine (37 mg, 37 μL, 0.42 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**), and the mixture was stirred at 20 °C for 30 min. The precipitated tetrapeptide morpholinium salt was filtered off and dissolved in 1M HCl (30 mL). The product was extracted with ethyl acetate (3 × 40 mL). The organic layers were washed with brine (50 mL), dried over $MgSO_4$, and concentrated in vacuo. The product **25** was isolated as a white solid. Yield: 145 mg (93%); m.p. 72–75 °C; $R_f = 0.20$ (ethyl acetate/acetic acid 100/1 [*v*/*v*]); $[\alpha]_D^{22} = -42.4$ (*c* = 0.5 in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.93$ (d, *J* = 6.4 Hz, 3H, CH_3 Leu), 0.96 (d, *J* = 6.4 Hz, 3H, CH_3 Leu), 1.19 (d, *J* = 6.2 Hz, 3H, CH_3 Thr), 1.41 (d, *J* = 7.3 Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3 CH_3 Boc), 1.61–1.72 (m, 3H, CH_2 Leu, γ-CH Leu), 3.79 (d, *J* = 5.5 Hz, 1H, CH_2 Ser), 4.07 (d, *J* = 3.7 Hz, 1H, α-CH Thr), 4.12 (quintet, *J* = 6.9 Hz, 1H, α-CH Ala), 4.38–4.50 (m, 3H, β-CH Thr, α-CH Leu, α-CH Ser); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 17.72$ (CH_3 Ala), 19.95 (CH_3 Thr), 21.90 (CH_3 Leu), 23.51 (CH_3 Leu), 25.74 (γ-CH Leu), 28.63 (3 CH_3 Boc), 41.52 (CH_2 Leu), 48.31 (α-CH Ala), 53.35 (α-CH Leu), 56.57 (α-CH Ser), 61.05 (α-CH Thr), 63.05 (β-CH_2 Ser), 68.64 (β-CH Thr), 80.83 (Cq Boc), 157.99 (C=O urethane), 171.74 (C=O), 173.45 (C=O), 174.70 (C=O), 175.75 (C=O); anal. calcd for $C_{21}H_{38}N_4O_9$: C 51.41, H 7.81, N 11.41; found: C 51.25, H 7.58, N 10.68.

Thiocarbamoyl ethylenediamine fluorescein, H_2NEtFlu (26): To a solution of ethylenediamine (10 mL, 0.15 mol) was added fluorescein isothiocyanate (250 mg, 0.64 mmol); the mixture was stirred at 20 °C for 16 h. The solvent was evaporated under reduced pressure and the product **26** was isolated as a red solid from the residue by flash chromatography on silica gel using methanol as eluent. Yield: 122 mg (42%); m.p. 216–222 °C; $R_f =$ 0.38 (methanol); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 3.35$ (t, *J* = 7.5 Hz, 2H, CH_2 Et), 4.09 (t, *J* = 7.5 Hz, CH_2 Et), 6.80–7.49 (m, 8H), 7.85–8.05 (m, 1H); $C_{23}H_{19}N_3O_5S$.

Thiocarbamoyl ethylenediamine rhodamine, H_2NEtRhod (27): To a solution of ethylenediamine (175 mg, 190 μL, 2.90 mmol) in DMF (10 mL) was added rhodamine isothiocyanate (78 mg, 0.15 mmol). The mixture was stirred at 20 °C for 16 h. The solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of CH_2Cl_2 and precipitated with diethyl ether (20 mL), the product **27** was isolated as a green solid. Yield: 76 mg (87%); m.p. 135 °C (decomp); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 1.20$ (t, *J* = 7 Hz, 12H, 4 CH_3 Et), 3.25 (q, *J* = 7 Hz, 8H, 3 CH_2 Et), 2.78–3.00 (m, 2H, CH_2 Et), 3.50–3.62 (m, 2H, CH_2 Et), 6.20–6.90 (m, 8H), 8.10 (br s, 1H); $C_{31}H_{37}N_5O_3S$.

***N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino ethylamine, H_2NEtNBD (28)**: To a solution of ethylenediamine (1.20 g, 1.30 mL, 20 mmol) in MeOH (20 mL) was added 4-chloro-7-nitro benzofurazane (200 mg, 1 mmol). The precipitated product **28** was filtered off and washed several times with ether. Yield: 217 mg (97%); brown solid; ^{1}H NMR (250 MHz, [D_6]DMSO): $\delta = 3.37$ (t, *J* = 7 Hz, 2H, CH_2 Et), 3.82–4.08 (m, 2H, CH_2 Et), 6.65 (t, *J* = 8.5 Hz, 1H), 8.67 (t, *J* = 8.5 Hz, 1H); $C_8H_9N_5O_3$.

***N-tert*-Butyloxycarbonyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethylthioureido fluorescein, BocThrLeuSerAlaHNEtFlu (29)**: To a solution of BocThrLeuSerAlaOH (**25**, 75 mg, 0.15 mmol), HOBt (20 mg, 0.15 mmol), and of EDC (30 mg, 0.15 mmol) in DMF (20 mL) was added after 10 min at 0 °C H_2NEtFlu (**29**, 83 mg, 0.18 mmol) dissolved in DMF (10 mL) containing NEt_3 (18 mg, 25 μL, 0.18 mmol). The mixture was stirred at 20 °C for 16 h and the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with 50 mL diethyl ether. The crude product was then purified by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent to give a yellow solid. Yield: 98 mg (69%); $R_f = 0.52$ (ethyl acetate/methanol 90/10 [*v*/*v*]); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 0.88$ (d, *J* = 6 Hz, 3H, CH_3 Leu), 0.92 (d, *J* = 6 Hz, 3H, CH_3 Leu), 1.13 (d, *J* = 6.3 Hz, 3H, CH_3 Thr), 1.43 (d, *J* = 6.5 Hz, 3H, CH_3 Ala), 1.46 (s, 9H, 3 CH_3 Boc), 1.55–1.68 (m, 3H, CH_2 Leu, γ-CH Leu), 3.45–3.60 (m, 2H, CH_2 Et), 3.64–3.82 (m, 4H, CH_2 Ser, CH_2 Et), 4.11 (d, *J* = 3.2 Hz, 1H, α-CH Thr), 4.14–4.18 (m, 1H, α-CH Ser), 4.38–4.53 (m, 3H, α-CH Ala, β-CH Thr, α-CH Leu), 6.42–6.60 (m, 6H), 7.10 (d, *J* = 11 Hz, 1H), 7.62 (t, *J* = 11 Hz, 1H), 8.12 (s, 1H); $C_{44}H_{55}N_7O_{13}S$; MALDI-TOF MS (MeOH); *m*/*z*: 923.1 $[M+H]^+$, 946.2 $[M+Na]^+$.

***N-tert*-Butyloxycarbonyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido rhodamine, BocThrLeuSerAlaHNEtRhod (30)**: To a solution of BocThrLeuSerAlaOH **25** (44 mg, 0.09 mmol), HOBt (12 mg, 0.09 mmol), and DIC (11.2 mg, 14 μL, 0.09 mmol) in DMF (4 mL) and CH_2Cl_2 (6 mL) was added after 10 min at 0 °C, H_2NEtRhod (**27**, 50 mg, 0.09 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under

reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with 50 mL diethyl ether. The crude product was then purified by flash chromatography on silica gel eluting with a gradient CH_2Cl_2/methanol 95/5 [*v*/*v*] to CH_2Cl_2/methanol 80/20 [*v*/*v*]. Yield: 60 mg (65%); red solid; $R_f = 0.62$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.93$ (d, $J = 6.4$ Hz, 3H, CH_3 Leu), 0.97 (d, $J = 6.4$ Hz, 3H, CH_3 Leu), 1.13–1.25 (m, 12H, 4CH_3 Et), 1.29 (d, $J = 7$ Hz, 3H, CH_3 Thr), 1.38 (d, $J = 7.3$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3CH_3 Boc), 1.62–1.74 (m, 3H, CH_2 Leu, γ-CH Leu), 3.30–3.38 (m, 10H, 4CH_2 Et, CH_2 Et), 3.60–3.78 (m, 2H, CH_2 Et), 3.76 (dd, $J = 11$ Hz, $J = 5.8$ Hz, 1H, CH_a Ser), 3.84 (dd, $J = 11$ Hz, $J = 5.6$ Hz, 1H, CH_b Ser), 4.07 (d, $J = 3.8$ Hz, 1H, α-CH Thr), 4.13–4.20 (m, 1H, α-CH Ser), 4.28–4.42 (m, 3H, α-CH Ala, β-CH Thr, α-CH Leu), 6.08–6.50 (m, 4H), 7.35–7.40 (m, 2H), 7.69 (d, $J = 7$ Hz, 1H), 7.77 (d, $J = 7$ Hz, 1H), 8.05 (s, 1H); $C_{52}H_{73}N_9O_{11}S$.

***N-tert*-Butyloxycarbonyl-L-threonyl-L-leucyl-L-seryl-L-alanine ethylenediamine NBD, BocThrLeuSerAlaHNEtNBD (31)**: To a solution of BocThrLeuSerAlaOH (**25**, 55 mg, 0.11 mmol), HOBt (15 mg, 0.11 mmol) and EDC (21 mg, 0.22 mmol) in DMF (10 mL) was added after 10 min at 0 °C, H_2NEtNBD (**28**, 30 mg, 0.13 mmol) dissolved in NMP (4 mL). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure. The crude product was then purified by flash chromatography on silica gel eluting with CH_2Cl_2/methanol 95/5 [*v*/*v*] to give a yellow solid. Yield: 35 mg (45%); $R_f = 0.58$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.91$ (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 6.4$ Hz, 3H, CH_3 Leu), 1.22 (d, $J = 7$ Hz, 3H, CH_3 Thr), 1.40 (d, $J = 7.3$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3CH_3 Boc), 1.55–1.78 (m, 3H, CH_2 Leu, γ-CH Leu), 3.57–3.86 (m, 4H, 2CH_2 Et), 3.78 (dd, $J = 11.7$ Hz, $J = 3.4$ Hz, 1H, CH_a Ser), 3.94 (dd, $J = 11.7$ Hz, $J = 4$ Hz, 1H, CH_b Ser), 4.08–4.29 (m, 4H, β-CH Thr, α-CH Thr, α-CH Ser, α-CH Leu), 4.38 (quintet, $J = 7.3$ Hz, 1H, α-CH Ala), 5.82 (d, $J = 7.2$ Hz, 1H, NH urethane), 6.21 (d, $J = 8.5$ Hz, 1H), 7.36 (d, $J = 8$ Hz, 1H, NH), 7.42–7.58 (m, 2H, 2NH), 7.77 (d, $J = 7$ Hz, 1H, NH), 8.18 (t, $J = 6.5$ Hz, 1H, NH), 8.48 (d, $J = 8.5$ Hz, 1H); $C_{29}H_{45}N_9O_{11}$.

***N*-Myristoyl-glycyl-L-serine allyl ester, MyrGlySerOAll (33)**: To a solution of MyrGlyOH (200 mg, 0.70 mmol) and HSerOAll·*p*TsOH (222 mg, 0.70 mmol) in CH_2Cl_2 (20 mL) and DMF (5 mL) at 0 °C was added NEt_3 (71 mg, 98 µL, 0.70 mmol), HOBt (95 mg, 0.70 mmol) and then DIC (176 mg, 216 µL, 1.40 mmol). The mixture was stirred at 20 °C for 16 h, the solvents were evaporated under reduced pressure. The product **33** was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 50/50 [*v*/*v*] as eluent. Yield: 136 mg (49%); m.p. 85–86 °C; $R_f = 0.35$ (ethyl acetate/*n*-hexane 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +15.7$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.90$ (t, $J = 7$ Hz, 3H, CH_3 Myr), 1.27 (s, 20H, 10CH_2 Myr), 1.52–1.60 (m, 2H, β-CH_2 Myr), 2.27 (t, $J = 7.5$ Hz, 2H, α-CH_2 Myr), 3.82 (dd, $J = 12$ Hz, $J = 4$ Hz, 1H, CH_a Ser), 3.85 (dd, $J = 17$ Hz, $J = 5.8$ Hz, 1H, CH_a Gly), 3.91 (dd, $J = 12$ Hz, $J = 4.5$ Hz, 1H, CH_b Ser), 3.95 (dd, $J = 17$ Hz, $J = 5.8$ Hz, 1H, CH_b Gly), 4.42–4.47 (m, 1H, α-CH Ser), 4.60 (dd, $J = 14$ Hz, $J = 5.7$ Hz, 1H, CH_a allyl), 4.66 (dd, $J = 14$ Hz, $J = 5.7$ Hz, 1H, CH_b allyl), 5.26 (dd, $J = 11$ Hz, $J = 1.2$ Hz, 1H, =CH_a), 5.33 (dd, $J = 14$ Hz, $J = 1.2$ Hz, 1H, =CH_b), 5.82 (t, $J = 5.8$ Hz, 1H, NH urethane), 5.91 (ddt, $J = 14$ Hz, $J = 11$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.74 (d, $J = 8.4$ Hz, 1H, NH); $C_{22}H_{40}N_2O_5$; EI MS (70 eV); *m*/*z*: 413.3 $[M+H]^+$.

***N*-Myristoyl-glycyl-L-serine, MyrGlySerOH (34)**: To a solution of MyrGlySerOAll **33** (50 mg, 0.13 mmol) in abs. CH_2Cl_2 (20 mL) was added under argon morpholine (15 mg, 15 µL, 0.17 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**) and the mixture was stirred at 20 °C for 1 h. To the solution was added 1M HCl (20 mL), the precipitated dipeptide **34** was filtered off and isolated as a white solid. Yield: 38 mg (85%); m.p. 97–99 °C; $R_f = 0.42$ (CH_2Cl_2/methanol/acetic acid) 90/10/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = +6$ ($c = 0.5$ in DMF); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.89$ (t, $J = 7.1$ Hz, 3H, CH_3 Myr), 1.29 (s, 20H, 10CH_2 Myr), 1.63 (t, $J = 7.3$ Hz, 2H, β-CH_2 Myr), 2.27 (t, $J = 7.5$ Hz, 2H, α-CH_2 Myr), 3.83 (dd, $J = 11.3$ Hz, $J = 3.8$ Hz, 1H, CH_a Ser), 3.90 (dd , $J = 16.7$ Hz, 1H, CH_a Gly), 3.91 (dd, $J = 11.3$ Hz, $J = 4.5$ Hz, 1H, CH_b Ser), 3.94 (dd, $J = 16.7$ Hz, 1H, CH_b Gly), 4.49 (t, $J = 4.1$ Hz, 1H, α-CH Ser); ^{13}C NMR (125.6 MHz, CD_3OD): $\delta =$ 14.44 (CH_3 Myr), 23.73 (CH_2 Myr), 26.82 (CH_2 Myr), 30.00–30.76 (9CH_2 Myr), 36.89 (α-CH_2 Myr), 43.42 (CH_2 Gly), 56.06 (α-CH Ser), 62.83 (CH_2 Ser), 171.59 (C=O), 173.17 (C=O), 176.89 (C=O); anal. calcd for $C_{19}H_{36}N_2O_5$: C 61.26, H 9.74, N 7.52; found: C 61.84, H 9.35, N 7.16.

***N-tert*-Butoxycarbonyl-glycyl-(*S*-hexadecyl)-L-cysteine allyl ester, BocGlyCys(HD)OAll (35)**: To a solution of $(BocGlyCysOAll)_2$ (**13**, 1.40 g, 2.21 mmol) was added DTT (1.70 g, 11.05 mmol) and NEt_3 (0.45 g, 0.61 mL, 4.42 mmol). The crude BocGlyCysOAll was dissolved in abs. THF (50 mL) and to the solution was added NEt_3 (1.10 g, 0.61 mL, 11.05 mmol), hexadecyl bromide (3.9 g, 3.9 mL, 12.8 mmol), and a catalytic amount of NaI. After stirring for 5 d the precipitated solid was filtered off, the solution was concentrated in vacuo and the product BocGlyCys(HD)OAll (**35**) was isolated from the residue as a white amorphous solid by flash chromatography on silica gel using *n*-hexane/ethyl acetate 70/30 [*v*/*v*] as eluent. Yield: 1.14 g (48%); $R_f = 0.48$ (*n*-hexane/ethyl acetate 3:2 [*v*/*v*]); $[\alpha]_D^{22} = -10.2$ (c = 1, methanol); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 3H, CH_3 HD), 1.25 (s, 26H, 13CH_2 HD), 1.45 (s, 9H, CH_3 Boc), 1.51–1.64 (m, 2H, β-CH_2 HD), 2.49 (t, $J = 7.5$ Hz, 2H, α-CH_2 HD), 2.97 (dd, $J = 14$ Hz, $J = 7.6$ Hz, 1H, CH_a Cys), 3.01 (dd, $J = 14$ Hz, $J = 5.3$ Hz, 1H, CH_b Cys), 3.85 (dd, $J = 16.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Gly), 4.04 (dd, $J = 16.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Gly), 4.65 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 4.80 (dt, $J = 7.6$ Hz, $J = 5.3$ Hz, 1H, α-CH Cys), 5.26 (dd, $J = 11.6$ Hz, $J = 1.2$ Hz, 1H, =CH_a), 5.29 (d, $J = 5$ Hz, 1H, NH urethane), 5.33 (dd, $J = 17$ Hz, $J = 1.2$ Hz, 1H, =CH_b), 5.92 (ddt, $J = 17$ Hz, $J = 11.6$ Hz, $J = 5.7$ Hz, 1H, =CH), 6.92 (d, $J = 7.5$ Hz, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 14.13$ (CH_3 HD), 25.70 (CH_2 Cys), 28.35 (CH_3 Boc), 29.00–30.00 (13CH_2 HD), 34.03 (α-CH_2 HD), 44.04 (CH_2 Gly), 51.93 (α-CH Cys), 66.39 (CH_2 allyl), 80.31 (Cq Boc), 119.25 (=CH_2), 131.30 (=CH), 155.90 (C=O urethane), 168.30 (C=O), 170.21 (C=O); anal. calcd for $C_{29}H_{54}N_2O_5S$: C 64.17, H 10.03, N 5.16; found: C 64.19, H 9.95, N 5.24; EI MS (70 eV); *m*/*z*: 542.4 $[M+H]^+$.

***N*-Myristoyl-glycyl-(*S*-hexadecyl)-L-cysteine allyl ester, MyrGlyCys(HD)OAll (36)**: To a solution of HGlyCys(HD)OAll·CF_3CO_2H (90 mg, 0.16 mmol) (this product was obtained by Boc deprotection of BocGlyCys(HD)OAll (**35**) using CF_3CO_2H. Yield: quant.) in CH_2Cl_2 (20 mL) was added NEt_3 (32 mg, 44 µL, 0.32 mmol) and myristoyl chloride (42 mg, 50 µL, 0.17 mmol). The mixture was stirred at 20 °C for 16 h, the solvent was washed with brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from CH_2Cl_2/ether gave a white solid. Yield: 78 mg (74%); m.p. 68 °C; $R_f = 0.41$ (*n*-hexane/ethyl acetate 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +5.1$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 6H, CH_3 Myr, CH_3 HD), 1.25 (s, 46H, 13CH_2 HD, 10CH_2 Myr), 1.51–1.67 (m, 4H, β-CH_2 HD, β-CH_2 Myr), 2.24 (t, $J = 7.5$ Hz, 2H, α-CH_2 Myr), 2.49 (t, $J = 7.5$ Hz, 2H, α-CH_2 HD), 2.97 (dd, $J = 14$ Hz, $J = 7.6$ Hz, 1H, CH_a Cys), 3.01 (dd, $J = 14$ Hz, $J = 5.3$ Hz, 1H, CH_b Cys), 3.98 (dd, $J = 16.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Gly), 4.04 (dd, $J = 16.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Gly), 4.65 (d, $J = 5.7$ Hz, 2H, CH_2 allyl), 4.80 (dt, $J = 7.6$ Hz, $J = 5.3$ Hz, 1H, α-CH Cys), 5.26 (dd, $J = 11.6$ Hz, $J = 1.2$ Hz, 1H, =CH_a), 5.33 (dd, $J = 17$ Hz, $J = 1.2$ Hz, 1H, =CH_b), 5.92 (ddt, $J = 17$ Hz, $J = 11.6$ Hz, $J = 5.7$ Hz, 1H, =CH), 6.30 (d, $J = 5$ Hz, 1H, NH), 6.92 (d, $J = 7.5$ Hz, 1H, NH); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 14.13$ (CH_3 HD, CH_3 Myr), 22.70 (CH_2 Myr), 25.63 (CH_2 Cys), 29.00–30.00 (13CH_2 HD, 10CH_2 Myr), 34.03 (α-CH_2 HD), 36.43 (α-CH_2 Myr), 44.04 (CH_2 Gly), 52.13 (α-CH Cys), 66.39 (CH_2 allyl), 119.65 (=CH_2), 131.30 (=CH), 168.92 (C=O), 170.21 (C=O); $C_{38}H_{72}N_2O_4S$.

***N*-Myristoyl-glycyl-(*S*-hexadecyl)-L-cysteine, MyrGlyCys(HD)OH (37)**: To a solution of MyrGlyCys(HD)OAll (**36**, 64 mg, 0.10 mmol) in abs. CH_2Cl_2 (20 mL) was added under argon morpholine (12 mg, 12 µL, 0.13 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**), and the mixture was stirred at 20 °C for 1 h. To the solution was added 1M HCl (20 mL); the precipitated dipeptide **37** was filtered off and isolated as a white solid. Yield: 58 mg (95%); m.p. 98–102 °C; $R_f = 0.22$ (CH_2Cl_2/methanol/acetic acid 90/10/1 [*v*/*v*/*v*]); ^{1}H NMR (250 MHz, $CDCl_3$+CD_3OD): $\delta = 0.88$ (t, $J = 7$ Hz, 6H, CH_3 HD, CH_3 Myr), 1.25 (s, 46H, 13CH_2 HD, 10CH_2 Myr), 1.50–1.71 (m, 4H, β-CH_2 HD, β-CH_2 Myr), 2.24 (t, $J = 7.1$ Hz, 2H, α-CH_2 Myr), 2.52 (t, $J = 7.3$ Hz, 2H, α-CH_2 HD), 2.96 (dd, $J = 11$ Hz, $J = 5$ Hz, 1H, CH_a Cys), 3.02 (dd, $J = 11$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 3.84 (d, $J = 16.6$ Hz, 1H, CH_b Gly), 3.99 (d, $J = 16.6$ Hz, 1H, CH_b Gly), 4.54 (t, $J = 5$ Hz, 1H, α-CH Cys); $C_{35}H_{68}N_2O_4S$; EI MS (70 eV); *m*/*z*: 612.9 $[M]^+$.

***N-tert*-Butoxycarbonyl-glycyl-(*S-tert*-Butyl)-L-cysteine allyl ester, BocGlyCys(*S-t*Bu)OAll (38)**: To a solution of $(BocGlyCysOAll)_2$ (**13**, 797 mg, 1.25 mmol) in (50 mL) dioxane (797 mg, 1.25 mmol) was added NEt_3 (303 mg, 0.4 mL, 3 mmol) and *tert*-butyl thiol (303 mg, 0.4 mL, 3 mmol). The mixture was stirred under air for 5 d at room temperature. The solvent

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was distilled under reduced pressure and the product **38** was isolated as a clear oil from the residue by flash chromatography on silica gel using ethyl acetate/*n*-hexane 60/40 [*v*/*v*] as eluent. Yield: 838 mg (83%); $R_f = 0.35$ (*n*-hexane/ethyl acetate 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +23$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.31$ (s, 9H, *S*-*t*-Bu), 1.45 (s, 9H, CH_3 Boc), 3.16 (dd, $J = 14$ Hz, $J = 5.8$ Hz, 1H, CH_a Cys), 3.21 (dd, $J = 14$ Hz, $J = 4.8$ Hz, 1H, CH_b Cys), 4.02 (d, $J = 6$ Hz, 2H, CH_2 Gly), 4.60 (d, $J = 6$ Hz, 2H, CH_2 allyl), 4.90 (dt, $J = 5.6$ Hz, $J = 4.8$ Hz, 1H, α-CH Cys), 5.26 (dd, $J = 13.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.33 (dd, $J = 16$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.40 (m, 1H, NH urethane); 5.92 (ddt, $J = 16$ Hz, $J = 13.5$ Hz, $J = 5.7$ Hz, 1H, =CH), 7.10 (d, $J = 7$ Hz, 1H, NH); anal. calcd for $C_{17}H_{30}N_2O_5S_2$: C 62.56, H 9.41, N 5.03; found: C 62.52, H 9.18, N 5.18; EI MS (70 eV); *m*/*z*: 406.1 $[M]^+$.

***N*-Myristoyl-glycyl-(*S*-*tert*-butyl)-L-cysteine allyl ester, MyrGlyCys(*S*-*t*Bu)OAll (39)**: To a solution of HGlyCys(*S*-*t*-Bu)OAll · CF_3CO_2H (78 mg, 0.18 mmol) (this product was obtained by Boc deprotection of **38** using CF_3CO_2H. Yield: quant. in CH_2Cl_2 (20 mL)) was added NEt_3 (36 mg, 50 µL, 0.36 mmol) and myristoyl chloride (44 mg, 50 µL, 0.18 mmol). The mixture was stirred at 20°C for 16 h, the solvent was washed with brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Recrystallization from CH_2Cl_2/ether gave a white solid. Yield: 83 mg (89%); m.p. 48–50°C; $R_f = 0.33$ (*n*-hexane/ethyl acetate 70/30 [*v*/*v*]); $[\alpha]_D^{22} = +17.2$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 6.8$ Hz, 3H, CH_3 Myr), 1.25 (s, 20H, 10CH_2 Myr), 1.31 (s, 9H, *S*-*t*-Bu), 1.55–1.72 (m, 2H, β-CH_2 Myr), 2.25 (t, $J = 7.1$ Hz, 2H, α-CH_2 Myr), 3.16 (dd, $J = 14$ Hz, $J = 5.8$ Hz, 1H, CH_a Cys), 3.21 (dd, $J = 14$ Hz, $J = 4.8$ Hz, 1H, CH_b Cys), 4.02 (d, $J = 6$ Hz, 2H, CH_2 Gly), 4.66 (d, $J = 5.8$ Hz, 2H, CH_2 allyl), 4.88 (dt, $J = 5.6$ Hz, $J = 4.8$ Hz, 1H, α-CH Cys), 5.26 (dd, $J = 13.5$ Hz, $J = 1.2$ Hz, 1H, $=CH_a$), 5.33 (dd, $J = 16$ Hz, $J = 1.2$ Hz, 1H, $=CH_b$), 5.92 (ddt, $J = 16$ Hz, $J = 13.5$ Hz, $J = 5.7$ Hz, 1H, =CH), 6.38 (br s, 1H, NH), 7.07 (d, $J = 7.6$ Hz, 1H, NH); $C_{26}H_{48}N_2O_4S_2$; EI MS (70 eV); *m*/*z*: 516.3 $[M]^+$.

***N*-myristoyl-glycyl-(*S*-*tert*-Butyl)-L-cysteine, MyrGlyCys(*S*-*t*Bu)OH (40)**: To a solution of MyrGlyCys(*S*-*t*-Bu)OAll (**39**, 74 mg, 0.14 mmol) in CH_2Cl_2 (20 mL) was added under argon morpholine (16 mg, 16 µL, 0.18 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium(**0**) and the mixture was stirred at 20°C for 1 h. The solvent was washed with 1M HCl (20 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product **40** was isolated as a white solid from the residue by flash chromatography on silica gel using ethyl acetate as eluent. Yield: 42 mg (63%); m.p. 71–72°C; $R_f = 0.20$ (CH_2Cl_2/methanol/acetic acid 90/10/1 [*v*/*v*/*v*]); $[\alpha]_D^{22} = +13.9$ ($c = 0.5$ in DMF); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, $J = 6.8$ Hz, 3H, CH_3 Myr), 1.25 (s, 20H, 10CH_2 Myr), 1.31 (s, 9H, *S*-*t*Bu), 1.62 (t, $J = 7.3$ Hz, 2H, β-CH_2 Myr), 2.25 (t, $J = 7.9$ Hz, 2H, α-CH_2 Myr), 3.13 (dd, $J = 16.6$ Hz, $J = 7$ Hz, 1H, CH_a Cys), 3.25 (dd, $J = 16.6$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 3.97 (dd, $J = 16.7$ Hz, $J = 5$ Hz, 1H, CH_a Gly), 4.11 (dd, $J = 16.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Gly), 4.81 (td, $J = 7$ Hz, $J = 5$ Hz, 1H, α-CH Cys), 6.95 (s, 1H, NH), 7.35 (d, $J = 7$ Hz, 1H, NH); ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 14.12$ (CH_3 Myr), 22.70 (CH_2 Myr), 25.67 (CH_2 Cys), 29.00–30.00 (10CH_2 Myr), 29.69 (CH_3 *S*-*t*Bu), 31.92 (α-CH_2 Myr), 36.36 (CH_2 Gly), 48.16 (Cq *S*-*tBu*), 52.61 (α-CH Cys), 172.29 (C=O), 174.62 (C=O); anal. calcd for $C_{23}H_{44}N_2O_4S_2$: C 57.95, H 9.30, N 5.87; found: C 57.57, H 9.68, N 5.33.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido fluorescein, BocGlyCys(Pal)ThrLeuSerAlaHNEtFlu (41)**: To a solution of BocGlyCys(Pal)OH (**15**, 10 mg, 0.019 mmol), HOBt (2.5 mg, 0.019 mmol), and EDC (3.6 mg, 0.019 mmol) in DMF (3 mL) was added after 10 min at 0°C, HThrLeuSerAlaHNEtFlu · CF_3CO_2H (17 mg, 0.019 mmol) (this product was obtained by Boc deprotection of BocThrLeuSerAlaHNEtFlu (**26**) using CF_3CO_2H. Yield: 93%) dissolved in DMF (2 mL) containing NEt_3 (1.9 mg, 2.6 µL, 0.019 mmol). The mixture was stirred at 20°C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent to give a yellow solid. Yield: 13.7 mg (55%); m.p. 171–174°C; $R_f = 0.21$ (ethyl acetate/methanol 90/10 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.89$ (t, $J = 6.6$ Hz, 3H, CH_3 Pal), 0.90 (d, $J = 6.8$ Hz, 3H, CH_3 Leu), 0.92 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.20 (d, $J = 6.3$ Hz, 3H, CH_3 Thr), 1.28 (s, 24H, 12CH_2 Pal), 1.40 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3CH_3 Boc), 1.56–1.74 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.57 (t, $J = 7.2$ Hz, 2H, α-CH_2 Pal), 3.17–3.46 (m, 4H, CH_2 Cys, CH_2 Et), 3.58–3.95 (m, 6H, CH_2 Ser, CH_2 Gly, CH_2 Et), 4.15–4.40 (m, 5H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala), 4.58-4.62 (m, 1H, α-CH Leu), 6.48–6.70 (m, 6H), 7.12 (d, $J = 11$ Hz, 1H), 7.81 (dd, $J = 11$ Hz, $J = 1.5$ Hz, 1H), 8.12 (s, 1H); $C_{65}H_{93}N_9O_{16}S$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido fluorescein, MyrGlyCys(Pal)ThrLeuSerAlaHNEtFlu (42)**: To a solution of MyrGlyCys(Pal)OH (**6**, 11 mg, 0.017 mmol), HOBt (2.4 mg, 0.017 mmol), and EDC (3.3 mg, 0.017 mmol) in DMF (3 mL) was added after 10 min at 0°C, HThrLeuSerAlaHNEtFlu · CF_3CO_2H (16 mg, 0.015 mmol) (this product was obtained by Boc deprotection of **26** using CF_3CO_2H. Yield: 93%) dissolved in DMF (2 mL) containing NEt_3 (1.7 mg, 2.3 µL, 0.017 mmol). The mixture was stirred at 20°C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent to give a yellow solid. Yield: 12 mg (50%); m.p. 179–184°C; $R_f = 0.25$ (ethyl acetate/methanol 90/10 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.87$–0.98 (m, 12H, CH_3 Pal, CH_3 Myr, 2CH_3 Leu), 1.20 (d, $J = 6.3$ Hz, 3H, CH_3 Thr), 1.25 (s, 44H, 12CH_2 Pal, 10CH_2 Myr), 1.40 (d, $J = 7$ Hz, 3H, CH_3 Ala), 1.55–1.74 (m, 7H, β-CH_2 Myr, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.23 (t, $J = 6$ Hz, 2H, α-CH_2 Myr), 2.58 (t, $J = 6$ Hz, 2H, α-CH_2 Pal), 3.12–3.55 (m, 6H, CH_2 Cys, CH_2 Ser, CH_2 Et), 3.60–3.95 (m, 4H, CH_2 Gly, CH_2 Et), 4.10–4.40 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, CH Leu), 6.48–6.70 (m, 6H), 7.12 (d, $J = 11$ Hz, 1H), 7.78–7.84 (m, 1H), 8.12 (s, 1H); $C_{74}H_{111}N_9O_{15}S$.

***N*-Myristoyl-glycyl-(*S*-hexadecyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido fluorescein, MyrGlyCys(HD)ThrLeuSerAlaHNEtFlu (43)**: To a solution of MyrGlyCys(HD)OH (**37**, 9 mg, 0.015 mmol), HOBt (2 mg, 0.015 mmol), and EDC (2.8 mg, 0.015 mmol) in DMF (3 mL) was added after 10 min at 0°C, HThrLeuSerAlaHNEtFlu · CF_3CO_2H (14 mg, 0.015 mmol) (this product was obtained by Boc deprotection of **26** using CF_3CO_2H. Yield: 93%) dissolved in DMF (2 mL) containing NEt_3 (1.5 mg, 2 µL, 0.015 mmol). The mixture was stirred at 20°C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent to give a yellow solid. Yield: 11 mg (53%); m.p. 180–185°C; $R_f = 0.25$ (ethyl acetate/methanol 90/10 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.88$ (t, $J = 7$ Hz, 6H, CH_3 HD, CH_3 Myr), 0.90 (d, $J = 7.3$ Hz, 3H, CH_3 Leu), 0.94 (d, $J = 7.3$ Hz, 3H, CH_3 Leu), 1.20 (d, $J = 6.5$ Hz, 3H, CH_3 Thr), 1.25 (s, 46H, 13CH_2 HD, 10CH_2 Myr), 1.41 (d, $J = 7$ Hz, 3H, CH_3 Ala), 1.55–1.74 (m, 7H, β-CH_2 Myr, β-CH_2 HD, CH_2 Leu, γ-CH Leu), 2.21–2.27 (m, 2H, α-CH_2 Myr), 2.55–2.59 (m, 2H, α-CH_2 HD), 3.12–3.55 (m, 6H, CH_2 Cys, CH_2 Ser, CH_2 Et), 3.60–3.95 (m, 4H, CH_2 Gly, CH_2 Et), 4.10–4.40 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, CH Leu), 6.55–6.70 (m, 6H), 7.12 (d, $J = 11$ Hz, 1H), 7.75 (d, $J = 11$ Hz, 1H), 7.99 (s, 1H); $C_{74}H_{113}N_9O_{14}S$; MALDI-TOF MS (MeOH); *m*/*z*: 1407.5 $[M+Na]^+$, 1423.7 $[M+K]^+$.

***N*-Myristoyl-glycyl-(*S*-*tert*-butyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido fluorescein, MyrGlyCys(*S*-*t*Bu)ThrLeuSerAlaHNEtFlu (44)**: To a solution of MyrGlyCys(*S*-*t*Bu)OH (**40**, 11 mg, 0.023 mmol), HOBt (3.1 mg, 0.023 mmol), and EDC (4.4 mg, 0.023 mmol) in DMF (3 mL) was added after 10 min at 0°C, HThrLeuSerAlaHNEtFlu · CF_3CO_2H (21 mg, 0.023 mmol) (this product was obtained by Boc deprotection of **26** using CF_3CO_2H. Yield: 93%) dissolved in DMF (2 mL) containing NEt_3 (2.3 mg, 3.2 µL, 0.023 mmol). The mixture was stirred at 20°C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using ethyl acetate/methanol 90/10 [*v*/*v*] as eluent to give a yellow solid. Yield: 17 mg (57%); m.p. 161–165C; $R_f = 0.22$ (ethyl acetate/methanol 90/10 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 0.85$ (d, $J = 6.2$ Hz, 3H, CH_3 Leu), 0.89 (t, $J = 7.1$ Hz, 3H, CH_3 Myr), 0.92 (d, $J = 6.2$ Hz, 3H, CH_3 Leu), 1.28 (d, $J = 7.5$ Hz, 3H, CH_3 Thr), 1.32 (s, 20H, 10CH_2 Myr), 1.36 (s, 9H, *S*-*t*Bu), 1.40 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 1.52–1.73 (m, 5H, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.26 (m, 2H, α-CH_2 Myr), 3.06 (dd, $J = 13.7$ Hz, $J = 8.2$ Hz, 1H, CH_a Cys), 3.21 (dd, $J = 13.7$ Hz, $J = 5.1$ Hz, 1H, CH_b Cys), 3.35–3.95 (m, 8H, CH_2 Ser, CH_2 Gly, 2CH_2 Et), 4.20–4.40 (m, 5H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala), 4.68 (dd, $J = 5$ Hz, $J = 4$ Hz, 1H, α-CH Leu), 6.50–6.80 (m,

6H), 7.15 (d, $J=11$ Hz, 1H), 7.80 (d, $J=11$ Hz, 1H), 8.12 (s, 1H); $C_{62}H_{89}N_9O_{14}S_3$; MALDI-TOF MS (MeOH); m/z: 1280.3 $[M+H]^+$, 1303.3 $[M+Na]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-SERYL-L-alanine aminoethyl thioureido rhodamine, BocGlyCys(Pal)ThrLeuSerAlaHNEtRhod (45)**: To a solution of BocGlyCys(Pal)OH (**15**, 2.6 mg, 5.11 μmol), HOBt (0.7 mg, 5.11 μmol), and DIC (0.6 mg, 0.80 μL, 5.11 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtRhod · CF_3CO_2H (6 mg, 5.11 μmol) (this product was obtained by Boc deprotection of BocThrLeuSerAlaHNEtRhod (**27**) using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1 mg, 1.40 μL, 10.20 μmol). The mixture was stirred at 20 °C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with 20 mL diethyl ether. The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a red solid. Yield: 3.7 mg (51 %); m.p. 120 °C (decomp); $R_f=0.63$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta=0.90$ (t, $J=7$ Hz, 3H, CH_3 Pal), 0.92 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 1.16 – 1.19 (m, 15H, 4 CH_3 Et, CH_3 Thr), 1.29 (s, 24H, 12 CH_2 Pal), 1.39 (d, $J=7.2$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3 CH_3 Boc), 1.63 – 1.82 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.55 (t, $J=7$ Hz, 2H, α-CH_2 Pal), 3.20 – 3.92 (m, 18H, 6 CH_2 Et, CH_2 Cys, CH_2 Ser, CH_2 Gly), 4.03 – 4.32 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, α-CH Leu), 6.20 – 6.50 (m, 4H), 7.26 – 7.30 (m, 2H), 7.68 (d, $J=8$ Hz, 2H), 8.05 (s, 1H); $C_{73}H_{111}N_{11}O_{14}S_2$; MALDI-TOF MS (MeOH); m/z: 1452.7 $[M+Na]^+$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido rhodamine, MyrGlyCys(Pal)ThrLeuSerAlaHNEtRhod (46)**: To a solution of MyrGlyCys(Pal)OH **6** (3.2 mg, 5.11 μmol), HOBt (0.7 mg, 5.11 μmol), and DIC (0.6 mg, 0.80 μL, 5.11 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtRhod · CF_3CO_2H (6 mg, 5.11 μmol) (this product was obtained by Boc deprotection of **27** using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1 mg, 1.40 μL, 10.20 μmol). The mixture was stirred at 20 °C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a red solid. Yield: 2.9 mg (37 %); m.p. 95 °C (decomp); $R_f=0.63$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.87-0.98$ (m, 12H, CH_3 Pal, CH_3 Myr, 2 CH_3 Leu), 1.16 – 1.20 (m, 15H, 4 CH_3 Et, CH_3 Thr), 1.25 (s, 44H, 12 CH_2 Pal, 10 CH_2 Myr), 1.40 (d, $J=7$ Hz, 3H, CH_3 Ala), 1.55 – 1.74 (m, 7H, β-CH_2 Myr, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.22 – 2.29 (m, 2H, α-CH_2 Myr), 2.56 – 2.60 (m, 2H, α-CH_2 Pal), 3.12 – 3.55 (m, 14H, 5 CH_2 Et, CH_2 Cys, CH_2 Ser), 3.60 – 3.95 (m, 4H, CH_2 Gly, CH_2 Et), 4.10 – 4.40 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, CH Leu), 6.20 – 6.50 (m, 4H), 7.26 – 7.30 (m, 2H), 7.68 (d, $J=8$ Hz, 2H), 8.05 (s, 1H); $C_{82}H_{129}N_{11}O_{13}S$.

***N*-Myristoyl-glycyl-(*S*-hexadecyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethylthioureido rhodamine, MyrGlyCys(HD)ThrLeuSerAlaHNEtRhod (47)**: To a solution of MyrGlyCys(HD)OH (**37**, 3.1 mg, 5.11 μmol), HOBt (0.7 mg, 5.11 μmol), and DIC (0.6 mg, 0.80 μL, 5.11 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtRhod · CF_3CO_2H (6 mg, 5.11 μmol) (this product was obtained by Boc deprotection of **27** using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1 mg, 1.40 μL, 10.20 μmol). The mixture was stirred at 20 °C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a red solid. Yield: 2.5 mg (32 %); m.p. 101 °C (decomp); $R_f=0.63$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta=0.90$ (t, $J=7$ Hz, 6H, CH_3 HD, CH_3 Myr), 0.92 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 1.18 (t, $J=7$ Hz, 12H, 4 CH_3 Et), 1.16 – 1.19 (m, 3H, CH_3 Thr), 1.29 (s, 46H, 13 CH_2 HD, 10 CH_2 Myr), 1.39 (d, $J=7.2$ Hz, 3H, CH_3 Ala), 1.63 – 1.85 (m, 7H, β-CH_2 HD, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.15 (t, $J=7$ Hz, 2H, α-CH_2 Myr), 2.54 (t, $J=7$ Hz, 2H, α-CH_2 HD), 3.48 (q, $J=7$ Hz, 8H, 4 CH_2 Et), 3.20 – 3.90 (m, 10H, CH_2 Cys, CH_2 Ser, 2 CH_2 Et, CH_2 Gly), 4.01 – 4.25 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, CH Leu), 6.25 – 6.50 (m, 4H), 7.21 – 7.28 (m, 2H), 7.62 – 7.75 (m, 2H), 8.05 (s, 1H); $C_{82}H_{131}N_{11}O_{12}S_2$; MALDI-TOF MS (MeOH); m/z: 1551.8 $[M+Na]^+$.

***N*-Myristoyl-glycyl-(*S*-*tert*-butyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethylthioureido rhodamine, MyrGlyCys(*S*-*t*Bu)ThrLeuSerAlaHNEtRhod (48)**: To a solution of MyrGlyCys(*S*-*t*Bu)OH (**40**, 2.4 mg, 5.11 μmol), HOBt (0.7 mg, 5.11 μmol), and DIC (0.6 mg, 0.80 μL, 5.11 μmol) in DMF (2 mL) was added after 10 min at 0 °C, CHThrLeuSerAlaHNEtRhod · CF_3CO_2H (6 mg, 5.11 μmol) (this product was obtained by Boc deprotection of **27** using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1 mg, 1.40 μL, 10.20 μmol). The mixture was stirred at 20 °C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a red solid. Yield: 3.8 mg (53 %); m.p. 95 °C (decomp); $R_f=0.63$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.90$ (t, $J=7$ Hz, 3H, CH_3 Myr), 0.92 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 1.16 (t, $J=6.7$ Hz, 12H, 4 CH_3 Et), 1.28 (s, 20H, 10 CH_2 Myr), 1.25 – 1.30 (m, 3H, CH_3 Thr), 1.32 (s, 9H, *S*-*t*Bu), 1.37 (d, $J=7$ Hz, 3H, CH_3 Ala), 1.55 – 1.80 (m, 5H, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.28 (t, $J=7$ Hz, 2H, α-CH_2 Myr), 3.02 – 3.95 (m, 18H, 6 CH_2 Et, CH_2 Cys, CH_2 Ser, CH_2 Gly), 4.03 – 4.89 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, α-CH Leu), 6.20 – 6.50 (m, 4H), 7.26 – 7.28 (m, 2H), 7.65 – 7.80 (m, 2H), 8.03 (s, 1H); $C_{70}H_{107}N_{11}O_{12}S_3$; MALDI-TOF MS (MeOH); m/z: 1414.8 $[M+Na]^+$.

***N*-Myristoyl-glycyl-L-seryl-L-threonyl-L-leucyl-L-seryl-L-alanine aminoethyl thioureido rhodamine, MyrGlySerThrLeuSerAlaHNEtRhod (49)**: To a solution of MyrGlySerOH (**34**, 1.9 mg, 5.11 μmol), HOBt (0.7 mg, 5.11 μmol), and DIC (0.6 mg, 0.80 μL, 5.11 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtRhod · CF_3CO_2H (6 mg, 5.11 μmol) (this product was obtained by Boc deprotection of **27** using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1 mg, 1.40 μL, 10.20 μmol). The mixture was stirred at 20 °C for 16 h, the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a red solid. Yield: 1.8 mg (27 %); m.p. 118 °C (decomp); $R_f=0.63$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (500 MHz, CD_3OD): $\delta=0.90$ (t, $J=7$ Hz, 3H, CH_3 Myr), 0.92 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 1.12 – 1.20 (m, 15H, 4 CH_3 Et, CH_3 Thr), 1.28 (s, 20H, 10 CH_2 Myr), 1.40 (d, $J=6$ Hz, 3H, CH_3 Ala), 1.59 – 1.73 (m, 5H, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.27 (t, $J=7$ Hz, 2H, α-CH_2 Myr), 3.20 – 3.95 (m, 18H, 6 CH_2 Et, 2 CH_2 Ser, CH_2 Gly), 4.02 – 4.60 (m, 6H, β-CH Thr, α-CH Thr, α-2 CH Ser, α-CH Ala, α-CH Leu), 6.15 – 6.50 (m, 4H), 7.20 – 7.35 (m, 2H), 7.67 (d, $J=6.6$ Hz, 1H), 7.71 (d, $J=8$ Hz, 1H), 8.03 (s, 1H); $C_{66}H_{99}N_{11}O_{13}S$; MALDI-TOF (MeOH); m/z: 1308.5 $[M+Na]^+$.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine NBD-aminoethyl amide, BocGlyCys(Pal)ThrLeuSerAlaHNEtNBD (50)**: To a solution of BocGlyCys(Pal)OH (**15**, 3.5 mg, 6.75 μmol), HOBt (0.9 mg, 6.75 μmol), and DIC (1.7 mg, 1.95 μL, 13.50 μmol) in DMF (2 mL) was added after 10 min at 0 C, HThrLeuSerAlaHNEtNBD · CF_3CO_2H (6 mg, 8.45 μmol) (this product was obtained by Boc deprotection of BocThrLeuSerAlaHNEtNBD (**28**) using CF_3CO_2H. Yield: 95 %) dissolved in DMF (1 mL) containing NEt_3 (1.3 mg, 1.90 μL, 13.50 μmol). The mixture was stirred at 20 °C for 16 h; the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a yellow solid. Yield: 4 mg (54 %); m.p. 190 °C (decomp); $R_f=0.55$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta=0.83$ (t, $J=7$ Hz, 3H, CH_3 Pal), 0.86 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 0.90 (d, $J=6.5$ Hz, 3H, CH_3 Leu), 1.18 (d, $J=7.2$ Hz, CH_3 Thr), 1.29 (s, 24H, 12 CH_2 Pal), 1.34 (d, $J=7.3$ Hz, 3H, CH_3 Ala), 1.45 (s, 9H, 3 CH_3 Boc), 1.56 – 1.72 (m, 5H, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.59 (t, $J=7.4$ Hz, 2H, α-CH_2 Pal), 3.18 – 4.20 (m, 15H, 2 CH_2 Et, CH_2 Cys, CH_2 Ser, CH_2 Gly, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Leu), 4.38 (q, $J=7.3$ Hz, 1H, α-CH Ala), 6.19 (d, $J=8.2$ Hz, 1H), 8.44 (d, $J=8.2$ Hz, 1H); $C_{50}H_{83}N_{11}O_{14}S$.

***N*-Myristoyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine NBD-aminoethyl amide, MyrGlyCys(Pal)ThrLeuSerAlaHNEtNBD**

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(**51**): To a solution of MyrGlyCys(Pal)OH (**6**, 4.2 mg, 6.75 μmol), HOBt (0.9 mg, 6.75 μmol), and DIC (1.7 mg, 1.95 μL, 13.50 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtNBD · CF_3CO_2H (6 mg, 8.45 μmol) (this product was obtained by Boc deprotection of **28** using CF_3CO_2H. Yield: 95 %) dissolved in DMF (1 mL) containing NEt_3 (1.3 mg, 1.90 μL, 13.50 μmol). The mixture was stirred at 20 °C for 16 h; the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a yellow solid. Yield: 3.4 mg (41 %); m.p. 150 °C (decomp); $R_f = 0.55$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.82$ (t, $J = 7$ Hz, 6H, CH_3 Pal, CH_3 Myr), 0.86 (d, $J = 7.2$ Hz, 3H, CH_3 Leu), 0.89 (d, $J = 7.2$ Hz, 3H, CH_3 Leu), 1.18 (d, $J = 6.3$ Hz, 3H, CH_3 Thr), 1.20 (s, 44H, 12CH_2 Pal, 10CH_2 Myr), 1.38 (d, $J = 7.4$ Hz, 3H, CH_3 Ala), 1.44–1.72 (m, 7H, β-CH_2 Myr, β-CH_2 Pal, CH_2 Leu, γ-CH Leu), 2.22–2.29 (m, 2H, α-CH_2 Myr), 2.57 (t, $J =$ 7 Hz, 2H, α-CH_2 Pal), 3.19 (dd, $J = 11$ Hz, $J = 4$ Hz, 1H, CH_a Cys), 3.26 (dd, $J = 11$ Hz, $J = 3$ Hz, 1H, CH_b Cys), 3.45–4.40 (m, 14H, 2CH_2 Et, CH_2 Ser, CH_2 Gly, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, α-CH Leu), 6.18 (d, $J = 8.8$ Hz, 1H), 8.43 (d, $J = 8.8$ Hz, 1H); $C_{59}H_{101}N_{11}O_{13}S$.

***N*-myristoyl-glycyl-(*S*-hexadecyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine NBD-aminoethyl amide, MyrGlyCys(HD)ThrLeuSerAlaHNEtNBD** (**52**): To a solution of MyrGlyCys(HD)OH (**37**, 4.1 mg, 6.75 μmol), HOBt (0.9 mg, 6.75 mol), and DIC (1.7 mg, 1.95 μL, 13.50 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtNBD · CF_3CO_2H (6 mg, 8.45 μmol) (this product was obtained by Boc deprotection of **28** using CF_3CO_2H. Yield: 85 %) dissolved in DMF (1 mL) containing NEt_3 (1.35 mg, 1.90 μL, 13.50 μmol). The mixture was stirred at 20 °C for 16 h; the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a yellow solid. Yield: 4 mg (33 %); m.p. 140 °C (decomp); $R_f = 0.55$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.90$ (t, $J = 7$ Hz, 6H, CH_3 HD, CH_3 Myr), 0.92 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.97 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.16–1.19 (m, 3H, CH_3 Thr), 1.29 (s, 46H, 13CH_2 HD, 10CH_2 Myr), 1.39 (d, $J = 7.2$ Hz, 3H, CH_3 Ala), 1.63–1.85 (m, 7H, β-CH_2 Myr, β-CH_2 HD, CH_2 Leu, γ-CH Leu), 2.15 (t, $J = 7$ Hz, 2H, α-CH_2 Myr), 2.54 (t, $J = 7$ Hz, 2H, α-CH_2 HD), 3.20–3.90 (m, 10H, CH_2 Cys, CH_2 Ser, 2CH_2 Et, CH_2 Gly), 4.01–4.25 (m, 6H, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser, α-CH Ala, α-CH Leu), 6.18 (d, $J =$ 8.8 Hz, 1H), 8.43 (d, $J = 8.8$ Hz, 1H); $C_{59}H_{103}N_{11}O_{12}S$; MALDI-TOF MS (MeOH); *m*/*z*: 1214.2 $[M+Na]^+$, 1231.7 $[M+K]^+$.

***N*-Myristoyl-glycyl-(*S*-*tert*-butyl)-L-cysteyl-L-threonyl-L-leucyl-L-seryl-L-alanine NBD-aminoethyl amide, MyrGlyCys(*S*-*t*Bu)ThrLeuSerAlaHNEtNBD** (**53**): To a solution of MyrGlyCys(*S*-*t*Bu) OH (**40**, 3.2 mg, 6.75 μmol), HOBt (0.9 mg, 6.75 μmol), and DIC (1.7 mg, 1.95 μL, 13.50 μmol) in DMF (2 mL) was added after 10 min at 0 °C, HThrLeuSerAlaHNEtNBD · CF_3CO_2H (6 mg, 8.45 μmol) (this product was obtained by Boc deprotection of **28** using CF_3CO_2H. Yield: 95 %) dissolved in DMF (1 mL) containing NEt_3 (1.3 mg, 1.90 μL, 13.50 μmol). The mixture was stirred at 20 °C for 16 h; the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a yellow solid. Yield: 5.6 mg (78 %); m.p. 180 °C (decomp); $R_f = 0.55$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.88$ (t, $J = 6.3$ Hz, 3H, CH_3 Myr), 0.92 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.95 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.22 (t, $J = 7$ Hz, 3H, CH_3 Thr), 1.25 (s, 20H, 10CH_2 Myr), 1.34 (s, 9H, *S*-*t*Bu), 1.41 (d, $J = 7$ Hz, 3H, CH_3 Ala), 1.43–1.62 (m, 5H, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.29 (t, $J =$ 7 Hz, 2H, α-CH_2 Myr), 3.09 (dd, $J = 14$ Hz, $J = 7.3$ Hz, 1H, CH_a Cys), 3.16 (dd, $J = 14$ Hz, $J = 5$ Hz, 1H, CH_b Cys), 3.61 (d, $J = 5$ Hz, 2H, CH_2 Gly), 3.64–3.80 (m, 4H, 2CH_2 Et), 3.87 (dd, $J = 12$ Hz, $J = 3.5$ Hz, 1H, CH_a Ser), 3.97 (dd, $J = 12$ Hz, $J = 5.3$ Hz, 1H, CH_b Ser), 3.95–4.15 (m, 6H, CH_2 Gly, β-CH Thr, α-CH Thr, α-CH Cys, α-CH Ser), 4.38 (quartet, $J = 7.3$ Hz, 1H, α-CH Ala), 4.59 (t, $J = 5$ Hz, 1H, α-CH Leu), 6.30 (d, $J = 8.6$ Hz, 1H), 8.51 (d, $J = 8.6$ Hz, 1H); $C_{47}H_{79}N_{11}O_{12}S_2$.

***N*-Myristoyl-glycyl-L-seryl-L-threonyl-L-leucyl-L-seryl-L-alanine NBD-aminoethyl amide, MyrGlySerThrLeuSerAlaHNEtNBD** (**54**): To a solution of MyrGlySerOH (**34**, 2.4 mg, 6.75 μmol), HOBt (0.9 mg, 6.75 μmol), and DIC (1.7 mg, 1.95 μL, 13.50 μmol) in DMF (2 mL) was added after 10 min at 0 °C HThrLeuSerAlaHNEtNBD · CF_3CO_2H (6 mg, 8.45 μmol) (this product was obtained by Boc deprotection of **28** using CF_3CO_2H. Yield: 95 %) dissolved in DMF (1 mL) containing NEt_3 (1.3 mg, 1.90 μL, 13.50 μmol). The mixture was stirred at 20 °C for 16 h; the solvent was evaporated under reduced pressure. The residue was dissolved in a small volume of methanol and precipitated with diethyl ether (20 mL). The crude product was then purified by flash chromatography on silica gel using CH_2Cl_2/methanol 80/20 [*v*/*v*] as eluent to give a yellow solid. Yield: 1.8 mg (28 %); m.p. 145 °C (decomp); $R_f = 0.50$ (CH_2Cl_2/methanol 80/20 [*v*/*v*]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.87$ (t, $J = 7$ Hz, 3H, CH_3 Myr), 0.88 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 0.93 (d, $J = 6.5$ Hz, 3H, CH_3 Leu), 1.25 (s, 20H, 10CH_2 Myr), 1.29 (d, $J = 6.5$ Hz, 3H, CH_3 Thr), 1.40 (d, $J = 7$ Hz, 3H, CH_3 Ala), 1.59–1.73 (m, 5H, β-CH_2 Myr, CH_2 Leu, γ-CH Leu), 2.27 (t, $J = 7$ Hz, 2H, α-CH_2 Myr), 3.40–3.95 (m, 14H, 2CH_2 Ser, CH_2 Gly, 2CH_2, β-CH Thr, α-CH Thr, α-2CH Ser), 4.10 (q, $J = 7.3$ Hz, 1H, α-CH Ala), 4.38 (t, $J =$ 4 Hz, 1H, α-CH Leu), 6.25 (d, $J = 8.8$ Hz, 1H), 8.50 (d, $J = 8.8$ Hz, 1H); $C_{43}H_{71}N_{11}O_{13}$; MALDI-TOF MS (MeOH); *m*/*z*: 973.7 $[M+Na]^+$.

Microinjection experiments: NIH-3T3 cells were grown in Dulbeccos modified eagles medium (DMEM) supplemented with 10 % fetal bovine serum (Gibco) in a humidified CO_2 (7.5 %) incubator at 37 °C in Falcon polystyrene tissue culture dishes. NBD-labelled lipohexapeptides were dissolved in methanol (1 % [w/v]) and mixed with a tenfold volume of 10 mM HEPES, 140 mM KCl, 8 mM NaCl, and 1 mM $MgCl_2$. The pH of these buffer solutions was adjusted prior to mixing to pH 6.5 for the palmitoylated lipopeptides and to pH 7.4 for the hexadecylated peptides. Methanol was removed from the suspensions in a speed-vac centrifuge, the supernatants were homogenized by filtration through extruder equipment[24] with polycarbonate filters (pore size 100 nm). Concentrations of the lipopeptid solutions were determined in a Kontron Uvikon 332 UV/Vis spectrometer.[25] The resulting stock solutions were ca. 0.1 mM in peptide concentration and were applied without further dilution in microinjection experiments.

For injections the Zeiss Microinjection Workstation (AIS) and thin borosilicate glass capillaries with filament (Hilgenberg) with a tip diameter < 0.5 μm were used.

Phase contrast and fluorescence microscopy was performed in a Zeiss Axiovert microscope supplemented with a Zeiss long-distance Achrostigmat 32× lens and a filter system for fluorescein dyes (filter block I: excitation 450–490 nm, FT 510, long pass 520 nm). Images were recorded with a Sony 3CCD color video camera, digitized with a Matrox Meteor RGB frame grabber and processed with a Kontron KS300 imaging system.

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