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Synthesis of the Palmitoylated and Prenylated C-terminal Lipopeptides of the Human R- and N-*Ras* Proteins

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Abstract—For the study of biological phenomena influenced by the R- and N-*Ras* proteins, characteristic peptides which embody the correct lipid modifications of their parent proteins (palmitoyl thioesters, geranylgeranyl thioethers, and farnesyl thioethers), as well as analogues thereof, may serve as efficient tools. For the construction of such acid- and base labile peptide conjugates the allyl ester was developed as C-terminal protecting group. Allyl esters are cleaved selectively and in high yields from lipidated peptides by Pd(0)-mediated allyl transfer to accepting N- or C-nucleophiles like morpholine and *N*,*N*′-dimethylbarbituric acid. This protecting group technique formed the key step in the synthesis of the characteristic *S*-palmitoylated and *S*-isoprenylated C-terminus of human R-*Ras* and human N-*Ras* proteins, as well as several analogues thereof. Deprotections are so mild that no undesired side reactions of the lipid conjugates are observed.

Introduction

Lipid modified proteins are critically involved in the transduction of stimuli from the extracellular space across the plasma membrane into the cell, and ultimately to the cell nucleus.[1,2] The biological significance of lipidated proteins is highlighted by the crucial roles of the so-called *Ras* proteins in maintaining the regular life cycle of cells. H-, N-, and K-*Ras* proteins are plasma-membrane bound lipoproteins which are found in organisms as diverse as mammals, flies, worms and yeast. They serve as central molecular switches,[3,4] and translate the signals given by growth factors via a well-balanced series of non-covalent protein/protein interactions into a cascade of highly specific protein phosphorylations, resulting in the activation of transcription factors (Scheme 1). Thus, *Ras* regulates cell growth and proliferation. If this regulation is disturbed or interrupted, uncontrolled proliferation may occur which may result in transformation of the cell. Thus, a point mutation in the *ras* oncogenes coding for the *Ras* proteins is found in ca. 30% of all human cancers, a figure which rises up to 80% for some of the major malignancies like pancreas cancer.[5] Furthermore, the R-*Ras* protein may have a key regulatory role in a signal transduction pathway involved in the regulation of programmed cell death[6] (apoptosis), i.e. the physiological mechanism by which cells can actively regulate their own and/or each other's death, thereby preventing damaged cells from harming the organism.

All *Ras* proteins are post transitionally modified.[7] In this process precursor proteins are S-prenylated, then the three C-terminal amino acids are proteolized and the C-terminal amino acid is converted into the methyl ester. Finally, further cysteine residues may be *S*-palmitoylated. Thus, H-, N- and K-*Ras* are *S*-farnesylated, and H-, and N-*Ras* carry additional palmitic acid thioesters (Scheme 2). The R-*Ras* protein is *S*-geranylgeranylated and *S*-palmitoylated (Scheme 2). H-, N- and K-*Ras* must be lipid-modified and plasma membrane associated to perform both their normal and oncogenic biological functions. In the non lipidated forms the *Ras* proteins are cytosolic and are not able to transduce signals,[3,8] and it is believed that the interaction between *Ras* and its downstream effector *Raf* (Scheme 1) is mediated at least in part via the farnesyl group present in *Ras*.[9,10] The lipidated C-terminus of R-*Ras* was shown to associate with the apoptosis-suppressing proto-oncogene product Bcl-2,[11] suggesting a role for R-*Ras* in the regulation of programmed cell death (see above).

The finding that lipidation can be crucial to guarantee correct cellular signaling may have far reaching consequences for biological and pharmaceutical research. It may provide important clues for a better understanding of biological signal transduction, as well as improve the efficiency of drugs which influence signal transduction processes.[5,12]

Key words: *Ras* proteins; signal transduction; lipidated peptides; protecting groups; allyl esters.

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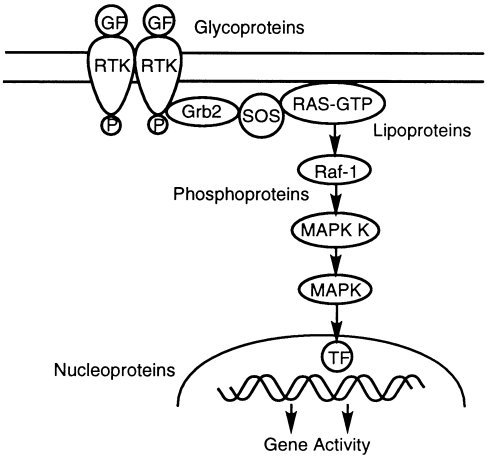
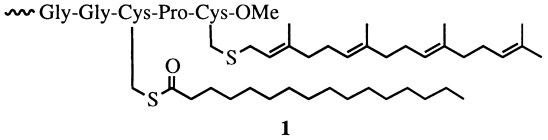
For the study of biological phenomena which are influenced by lipid-modified proteins the combination of techniques of cell biology, organic synthesis and biophysics opens up new and alternative opportunities.[13–19] For such studies characteristic lipidated peptides embodying the correct lipid groups and amino acid sequences of their parent lipid-modified proteins are useful reagents.[15–18] Such compounds can for instance shed light on cellular mechanisms by which these covalently modified polypeptides may be targeted to their subcellular destination, i.e. the plasma membrane.[17,18,20] Unfortunately, the synthesis of such peptide conjugates as for instance the characteristic C-terminal lipidated peptides **1** and **2** of human R-*Ras* and N-*Ras* is severely complicated by their pronounced acid- and base lability (Scheme 2).[13,21–25] Thus, during acid-mediated removal of the Boc group from *S*-farnesylated cysteinyl peptides, an attack of the acid on the double bonds of the farnesyl residue always occurs,[21] whereas the thioesters present in *S*-palmitoylated lipidated peptides hydrolyze spontaneously even at pH 6–7 in aqueous solution.[22–24] Due to this pronounced acid and base lability, different orthogonally stable blocking functions have to be employed that can be selectively removed under the mildest conditions.[26]

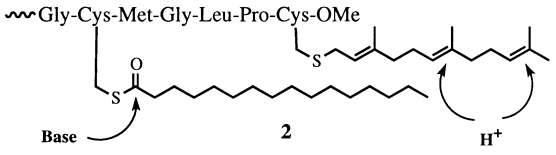


Scheme 1. The *Ras* signal transduction pathway.



1

C-terminus of the post-translationally modified human R-*Ras* protein



C-terminus of the post-translationally modified human N-*Ras* protein

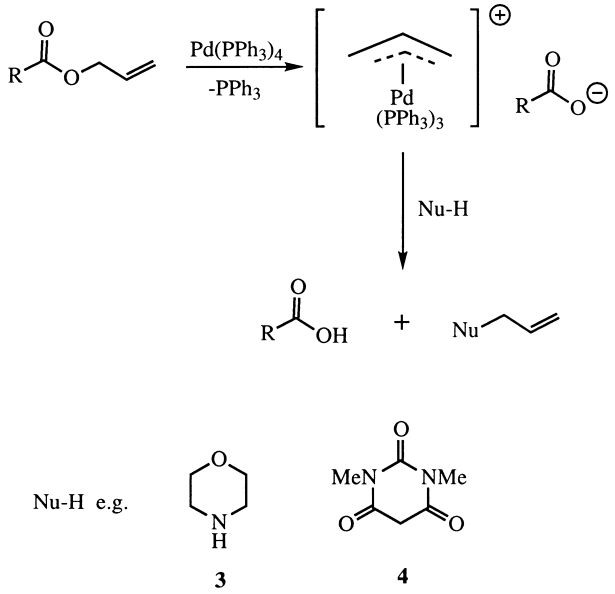
Scheme 2. Structures of the characteristic acid and base labile C-terminal lipopeptides of human N- and R-*Ras* proteins.

Allyl-based protecting groups have proven to be versatile tools for the synthesis of complex, multifunctional and sensitive compounds.[27] In general, they can be removed by means of a Pd(0)-mediated allyl transfer to accepting nucleophiles like morpholine **3** and *N*,*N*′-dimethylbarbituric acid **4** under very mild conditions (see Scheme 3 for a description of the general process as exemplified for allyl esters). In particular, the use of allyl ester type protecting groups[28] has allowed the synthesis of complex, sensitive glycopeptides in solution and on the solid phase.[27,29] These advantageous properties of allyl-type blocking functions have prompted us to investigate their application in the synthesis of *S*-acylated and *S*-prenylated peptides. We now report that characteristic lipidated peptides which represent lipid modified substructures of the human R- and N-*Ras* proteins as well as analogues thereof can be built up efficiently by employing the allyl ester as protecting group.[30]

Results and Discussion

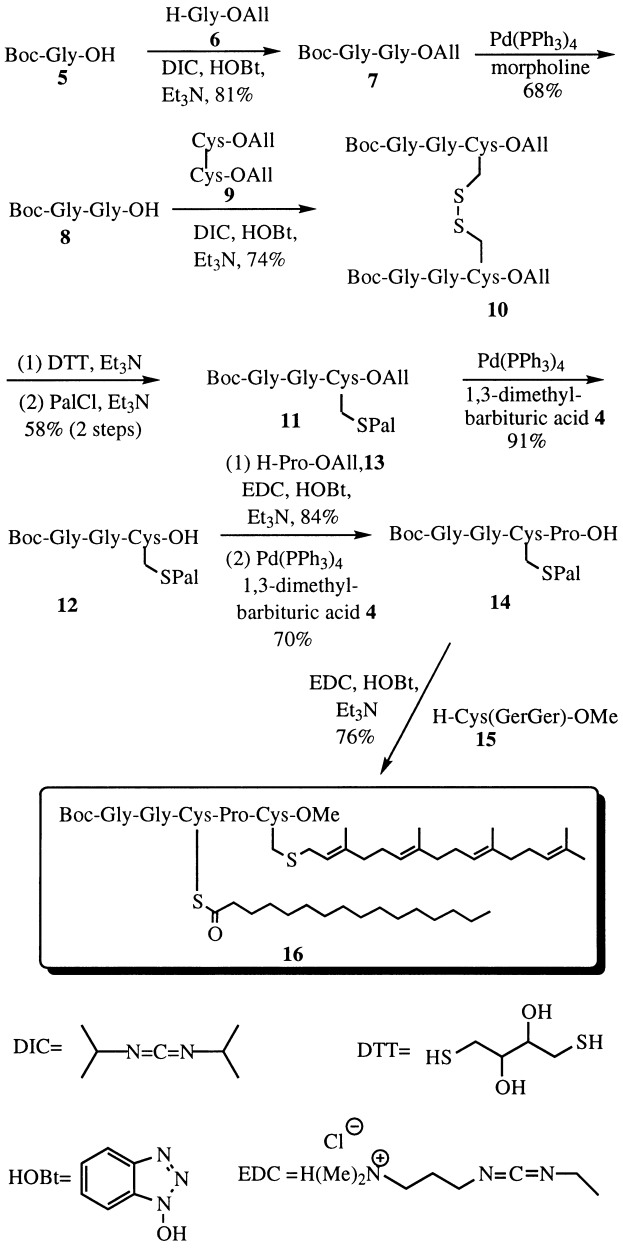
Synthesis of the *S*-palmitoylated and *S*-geranyl geranylated C-terminus of human R-*Ras* protein

For the construction of an S-palmitoylated and S-geranylgeranylated R-*Ras* peptide[7] and analogues to **1**, the cystinyl tripeptide allyl ester **10** was built up as the N-terminal building block (Scheme 4). To this end, Boc-glycine **5** was coupled with glycine allyl ester **6**. From the resulting fully protected dipeptide **7** the allyl ester was removed selectively by Pd(0)-mediated allyl transfer to morpholine **3**. Dipeptide acid **8** was then condensed with cystine bis(allyl ester) **9** to yield the desired intermediate **10**. Reduction of the disulfide **10** with dithiothreitol and subsequent treatment of the resulting cysteine peptide with palmitoyl chloride gave *S*-palmitoylated tripeptide allyl ester **11**. The thioester



3 **4**

Scheme 3. Removal of allyl protecting groups by Pd(0) mediated allyl transfer to accepting nucleophiles.



Scheme 4. Synthesis of *S*-palmitoylated and *S*-geranylgeranylated C-terminal pentapeptide **16** of human R-*Ras* protein.

present in tripeptide **11** is very sensitive to nucleophilic attack.[22,24] Therefore, the choice of the allyl-accepting nucleophile in the projected Pd(0)-catalyzed removal of the allyl ester protecting group is crucial to the successful realization of the entire synthetic strategy. The allyl-trapping reagent must be nucleophilic enough to attack the intermediary formed π-allylpalladium complex (Scheme 3). It should not induce a β-elimination of the entire palmitic acid or attack the activated thioester itself. For the selective removal of allyl protecting groups a variety of nucleophiles is available that may fulfill these criteria.[27] To solve the challenging problem posed by the selective C-terminal deprotection of **11**, we used *N*,*N*′-dimethylbarbituric acid **4** as nucleophile. This compound has already proven to be an efficient allyl-trapping reagent in glycopeptide chemistry.[27,31] It is weakly C−H acidic, thus basic conditions are prevented during the deprotection step.

Upon treatment of tripeptide allyl ester **11** with $Pd(PPh_3)_4$ in the presence of C-nucleophile **4** the C-terminal carboxylic acid was smoothly deprotected without any harm to the base-sensitive thioester bond to yield tripeptide carboxylic acid **12** in excellent yield. Thus *N*,*N*′-dimethylbarbituric acid promises to be a generally useful allyl accepting nucleophile for the removal of allyl-type blocking groups from lipidated peptides. Elongation of the peptide chain by proline allyl ester **13** and a further Pd(0)-mediated allyl ester cleavage in the presence of C-nucleophile **4** delivered the selectively unmasked *S*-palmitoylated tetrapeptide carboxylic acid **14** in high yield. Finally, the synthesis was completed by coupling of acid **14** with *S*-geranylgeranylated cysteine methyl ester **15**[32] to give the desired R-*Ras* peptide **16**.

The allyl ester cleavages performed in the sequence shown in Scheme 4 proceeded with complete selectivity and without any undesired side reactions. The conditions of the noble-metal complex mediated allyl transfer are so mild that neither an attack on the base-sensitive thioester nor a base-induced β-elimination of the palmitoyl group occurred.

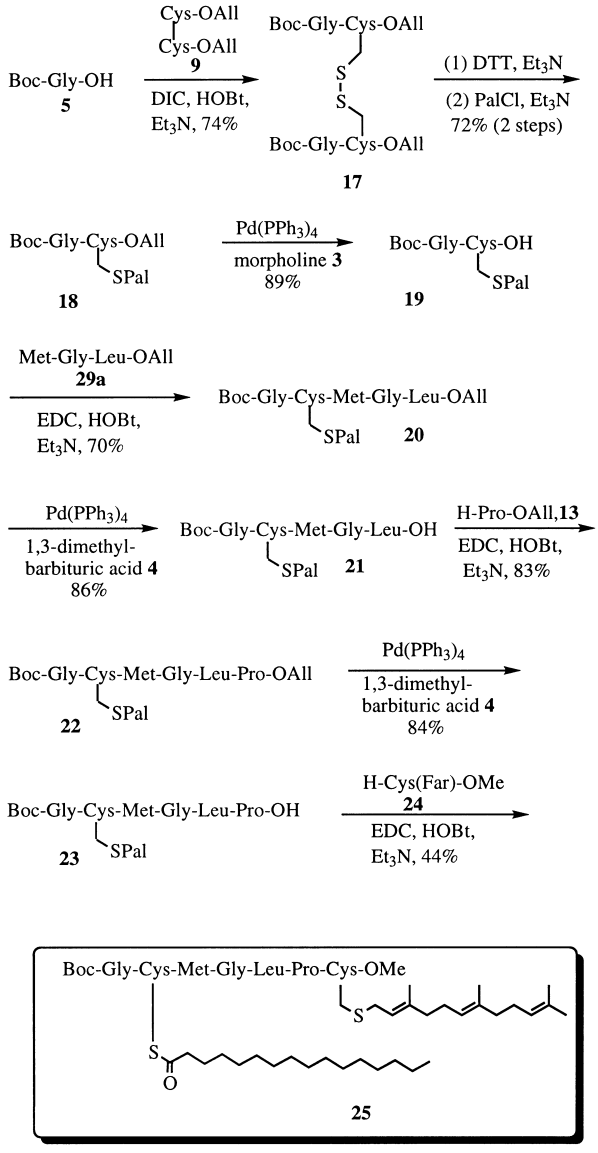
Synthesis of the *S*-palmitoylated and *S*-farnesylated C-terminus of human N-*Ras* protein

The strategy underlying the synthesis of the R-*Ras* peptide described above proved also very fruitful for the synthesis of N-*Ras* peptides and analogues thereof. Thus, first *S*-palmitoylated and C-terminally unmasked dipeptide **19** was built up (Scheme 5). To this end, Boc-glycine **5** was coupled with cystine bis(allyl ester) **9** to yield disulfide **17**. Reductive cleavage of the disulfide and subsequent *S*-palmitoylation as described above delivered *S*-acylated dipeptide allyl ester **18**. Encouraged by the efficiency of the allyl ester deprotection under weakly acidic conditions (see above) we investigated whether the noble-metal complex mediated selective removal of this blocking group from sensitive *S*-palmitoylated peptides might also be feasible under weakly basic conditions. Morpholine **3** is an N-nucleophile with a relatively low pK_A value (8.4). It was advantageously applied in the removal of allyl-type protecting groups in glycopeptide chemistry.[29,30] Therefore, the use of this nitrogen base as allyl-trapping reagent in the unmasking of *S*-allylated peptide **18** was investigated. Upon treatment of allyl ester **18** with $Pd(PPh_3)_4$ in the presence of morpholine the allyl ester protecting group was split off without any undesired side reaction and dipetide building block **19** was obtained in high yield (Scheme 5). No β-elimination or attack on the thioester was detected. Thus morpholine also promises to be a generally useful allyl-accepting nucleophile for the construction of base labile *S*-acylated lipopeptides. The peptide chain was then elongated by condensation of carboxylic acid **19** with N-terminally unmasked tripeptide allyl ester **29a** (for synthesis of **29a** see Scheme 6). Upon treatment of *S*-palmitoylated pentapeptide allyl ester **20** obtained

thereby with a Pd(0) catalyst in the presence of C-nucleophile **4** the C-terminal allyl ester protecting group was cleaved off smoothly to give rise to pentapeptide carboxylic acid **21** in high yield. Further chain elongation by proline allyl ester **13** and subsequent allyl ester cleavage yielded selectively deprotected hexapeptide carboxylic acid **23**. In order to complete the synthesis of N-*Ras*- peptide **25**, the *S*-palmitoylated and C-terminally deblocked hexapeptide **23** was condensed with *S*-farnesylated cysteine methyl ester **24**.[21,33] As in the synthesis of the R-*Ras* peptide detailed above, all allyl ester cleavage reactions proceeded without β-elimination of palmitic acid or attack on the thioester bond.

Synthesis of *S*-palmitoylated and *S*-farnesylated analogues of the C-terminus of N-*Ras*

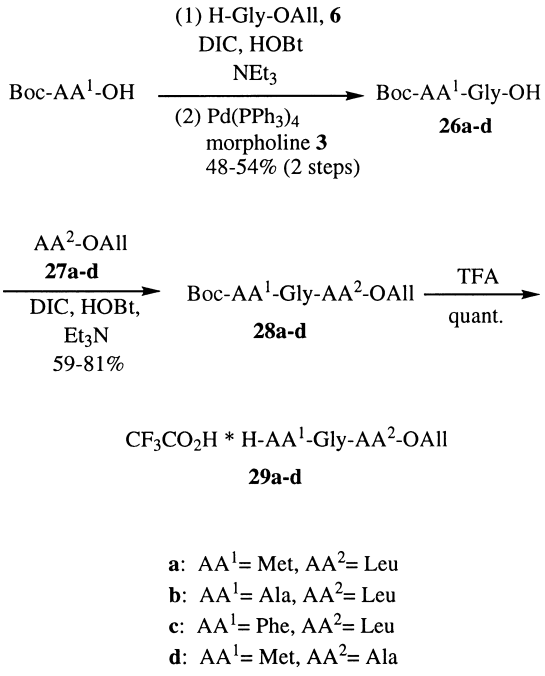
Analogues of N-*Ras* peptide **25** may be useful tools for exploring the precise role of the N-*Ras* C-terminus for instance in membrane binding or in processing and post-translational modification of the *Ras* proteins. As described above, N-*Ras* peptide **25** essentially was built up from four building blocks: a) an S-palmitoylated dipeptide b) a central tripeptide c) a proline and d) *S*-farnesylated cysteine methyl ester (see Scheme 5). By varying the structure of either one of these building blocks, but keeping the sequence of coupling and deprotection steps unchanged, analogues of *Ras* peptide **25** should be readily accessible.



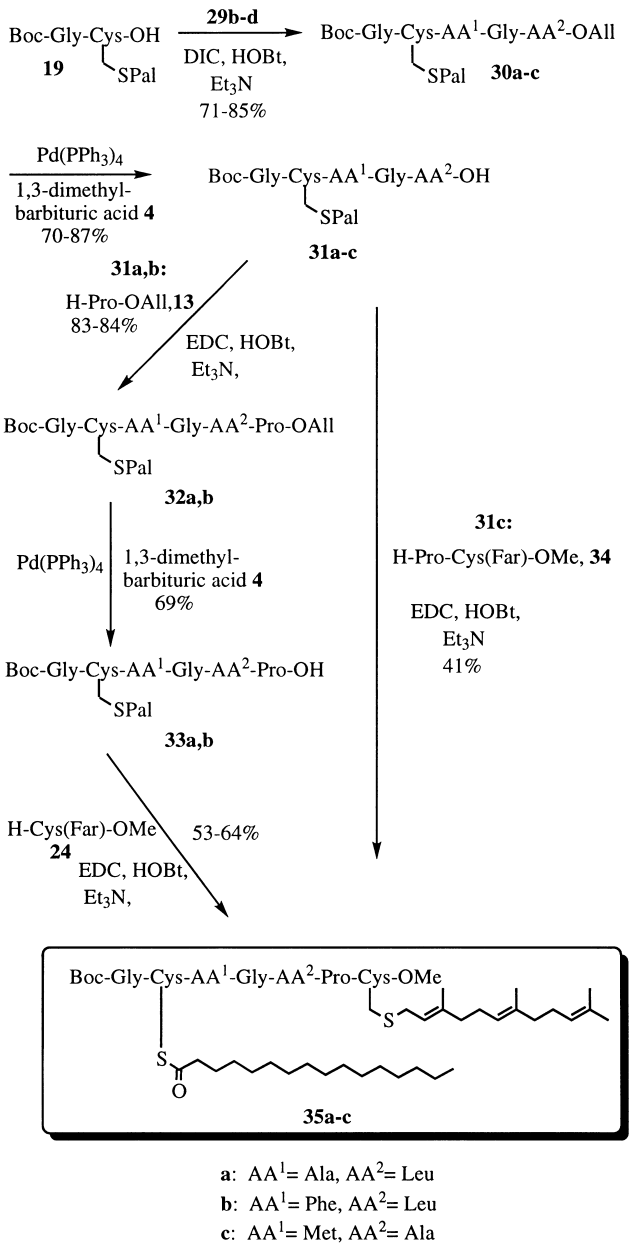
Scheme 5. Synthesis of *S*-palmitoylated and *S*-farnesylated C-terminal heptapeptide **25** of human N-*Ras* protein.

To this end, the amino acid sequence of the central tripeptide unit was varied. Thus, tripeptide allyl esters **29a–d** were built up by condensation of amino acid allyl esters **27** with Boc-protected glycine dipeptide carboxylic acids **26** and subsequent removal of the Boc group from the intermediary formed tripeptides **28** (Scheme 6). Boc protected, C-terminally unmasked dipeptides **26**, were synthesized by condensation of the corresponding Boc amino acid with glycine allyl ester **6** and subsequent removal of the allyl group (see the Experimental).

Coupling of *S*-palmitoylated dipeptide building block **19** with the N-terminally deprotected tripeptide allyl esters **29b–d** yielded lipopeptides **30a–c** in high yields (Scheme 7). The allyl ester protecting group could be removed from esters **30a–c** employing the Pd(0) mediated deprotection technique described in detail above. To give pentapeptide carboxylic acids **31a–c** in high yields. In the case of **31a** and **31b** the peptide chain was then elongated by proline allyl ester and the C-terminus of the resulting palmitoylated hexapeptides **32** was once more deprotected by Pd(0) catalyzed allyl transfer to *N*,*N*′-dimethylbarbituric acid. Finally, the carboxylic acids **33a**,**b** obtained thereby were condensed with *S*-farnesylated cysteine methyl ester **24** to yield the desired analogues **35a** and **35b** of the N-*Ras* peptide. In



Scheme 6. Synthesis of tripeptide allyl esters **29**.



Scheme 7. Synthesis of lipidated peptides **35** that are analogues to N-*Ras* peptide **25**.

addition, selectively deprotected pentapeptide **31c** was directly condensed with N-terminally deprotected farnesylated dipeptide ester **34**[21] to give analogue **35c**. All Pd(0) catalyzed allyl ester cleavage reactions performed in these reaction sequences proceeded smoothly and without undesired side reactions. In no case could a β-elimination or a nucleophilic attack on the activated thioesters be observed.

Conclusion

The use of the allyl ester as C-terminal protecting group makes sensitive *S*-palmitoylated and *S*-isoprenylated peptides corresponding to the C-terminus of human R- and N-*Ras* protein available in an efficient manner. The conditions of the noble-metal complex-mediated transfer of the allyl group to accepting N- or C-nucleophiles like morpholine and *N*,*N*′-dimethylbarbituric acid are so gentle that no undesired side reactions occur. In addition to the results described above for allyl esters we have recently obtained evidence that also the Pd(0)-mediated selective removal of *N*-allyloxycarbonyl urethane protecting groups from lipidated peptides can be achieved successfully.[24] Taken together these findings demonstrate that allyl-type protecting groups advantageously complement the recently developed enzymatic techniques for synthesis of *S*-acylated and *S*-farnesylated peptides[18,22,23] that proceed under extremely mild conditions. By means of these protecting group strategies[26] further functionalized lipidated peptides carrying e.g. fluorescent labels or biotin units by which they can be traced in biological systems may be synthesized efficiently.[17,18]

Experimental

General. ^{1}H NMR spectra were recorded on Bruker AC 250 (250 MHz), Bruker AM 400 (400 MHz), and Bruker DRX 500 (500 MHz) NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker AC 250 (62.8 MHz), Bruker AM 400 (100.6 MHz), and Bruker DRX 500 (125.6 MHz) NMR spectrometers. Optical rotations were recorded on Perkin–Elmer 241 digital polarimeter. Melting points were measured on Heraeus CHN-rapid melting point apparatus without correction.

***N-tert*-Butyloxycarbonyl-glycyl-glycine allyl ester (7).** To a solution of Boc-protected glycine **5** (1.75 g, 10 mmol) and glycine allyl ester hydro-*p*-toluenesulfonate **6** (2.96 g, 10 mmol) in CH_2Cl_2 (30 mL) was added 1-hydroxybenzotriazole (2.70 g, 20 mmol) and triethylamine (1.38 mL, 10 mmol). After stirring for 5 min at room temperature, diisopropylcarbodiimide (1.70 mL, 11 mmol) was added. The mixture was allowed to stir for 12 h at room temperature, and then was washed with 1 N hydrochloric acid (20 mL), with aqueous (5%) sodium bicarbonate (20 mL) and finally with water (2×20 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent was removed in vacuo. The resulting crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, 2/3 v/v) to afford **7** (2.21 g, 81%) as a colorless oil: R_f = 0.55 (hexane/ethyl acetate, 2/3 v/v); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.48 (s, 9H, CH_3), 3,85 (d, 2H, J = 6.2 Hz, CH_2-NH), 4.12 (d, 2H, J = 4.5 Hz, CH_2-NH), 4.65 (d, 2H, J = 5.1 Hz, CH_2-C-O), 5.10–5.23 (m, 3H, HN urethane + H_2C=C), 5.82–6.01 (m, 1H, HC=C), 6.72 (m br, 1H, HN); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 28.3 (CH_3), 41.2 (CH_2-NH), 44.1 (CH_2-NH), 66.1 (CH_2-C-O), 80.2 (Cq), 119.0 (H_2C=), 131.4 (HC=), 156.2 (C=O urethane), 169.5 (C=O), 170.1 (C=O).

N*-tert-*Butyloxycarbonyl-glycyl-glycine (8). Dipeptide allyl ester **7** (1.60 g, 5.9 mmol) was dissolved in THF (50 mL) at room temperature under argon. Tetrakis(triphenylphosphine) palladium (10 mg) and 5 min

later morpholine (0.56 mL, 6.4 mmol) were added. The mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the resulting oil was dissolved in aqueous (2.5%) sodium bicarbonate (30 mL). The aqueous layer was extracted with Et_2O (2×20 mL). The pH was adjusted to 2 with 1 N hydrochloric acid and extracted with ethyl acetate (3×20 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent was evaporated in vacuo. The dipeptide **8** was obtained as a white solid (930 mg, 68%): R_f = 0.80 (ethyl acetate/MeOH, 4/1 v/v); mp 125–126 °C (lit.[34] 125–127 °C); ^{1}H NMR (MeOD, 400 MHz) δ 1.40 (s, 9H, CH_3), 3.65 (s, 2H, CH_2-NH), 3,80 (s, 2H, CH_2-NH); ^{13}C NMR (MeOD, 100.5 MHz) δ 29.2 (CH_3), 42.9 (CH_2-NH), 44.4 (CH_2-NH), 79.7 (Cq), 157.4 (C=O urethane), 171.3 (C=O), 171.6 (C=O).

Bis-(*N*-*tert*-butyloxycarbonyl-glycyl-glycyl)-L-cystine bis-(allyl ester) (10). 1-Hydroxybenzotriazole (827 mg, 6.12 mmol) and triethylamine (0.43 mL, 3.06 mmol) were added to a suspension of **8** (710.0 mg, 3.06 mmol) and cystine bis(allyl ester) hydro-*p*-toluenesulfonate **9** (917 mg, 1.53 mmol) in CH_2Cl_2/DMF (10/1, 15 mL). After stirring for 5 min at room temperature, diisopropylcarbodiimide (0.52 mL. 3.36 mmol) was added. The mixture was allowed to stir for 12 h at room temperature, and then was washed with 1 N hydrochloric acid (10 mL), with aqueous (5%) sodium bicarbonate (10 mL), and finally with water (2×10 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent was removed in vacuo. The resulting crude product was purified by column chromatography on silica gel (MeOH/ethyl acetate, 1/10 v/v) to afford **10** (780 mg, 81%) as a colorless viscous oil: R_f = 0.35 (MeOH/ethyl acetate, 1/10 v/v); $[\alpha]_D^{22}$ −52° (*c* 1.20, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.47 (s, 18H, CH_3), 2.95–3.25 (m, 4H, CH_2-S), 3.80–4.15 (m, 8H, 2×CH_2-NH), 4.63 (d, 4H, *J* = 5,7 Hz, CH_2-O), 4.80–4.92 (m, 2H, HC-NH), 5.22–5.40 (m, 4H, H_2C=C), 5.65 (m br, 2H, NH urethane), 5.80–5.97 (m, 2H, HC=C), 7.35 (m br, 2H, NH), 7.52 (m br, 2H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 28.1 (CH_3), 41.4 (CH_2-NH), 44.2 (CH_2-NH), 47.8 (CH_2-S), 51.7 (HC-NH), 66.6 (CH_2-O), 80.9 (Cq), 119.1 (H_2C=C), 131.4 (HC=C), 156.3 (C=O urethane), 169.8 (C=O), 170.2 (C=O), 170.7 (C=O).

***N*-*tert*-Butyloxycarbonyl-glycyl-glycyl-(*S*-palmitoyl)-L-cysteine allyl ester (11).** Triethylamine (0.32 mL, 2.28 mmol) and 1,4-DL-dithiothreitol (527 mg, 3.42 mmol) were added under argon atmosphere to a solution of **10** (780 mg, 1.04 mmol) in CH_2Cl_2 (30 mL). After stirring for 2 h at room temperature, the solution was washed with 1 N hydrochloric acid (20 mL), with water (2×20 mL), and dried over sodium sulfate. The solution was filtered and cooled to 0 °C. Triethylamine (0.32 mL, 2.28 mmol) and palmitoyl chloride (1.38 mL, 4.56 mmol) were added. The mixture was allowed to stir for 2 h at room temperature. After filtration and evaporation of CH_2Cl_2 in vacuo, a crude viscous oil was obtained, which was purified by column chromatography on silica gel (ethyl acetate). Compound **11** was isolated as a white solid (827 mg, 65%): R_f = 0.20 (hexane/ethyl acetate, 1/1 v/v); $[\alpha]_D^{22}$ −13 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, *J* = 7.0 Hz, CH_3 Pal), 1.25 (s br, 24H, CH_2 Pal), 1.45 (s, 9H, CH_3 Boc), 1.65 (m, 2H, CH_2-Me Pal), 2.55 (t, 2H, *J* = 7.5 Hz, CH_2-C=O Pal), 3.35 (dd, 1H, *J* = 14.0 Hz, *J* = 6.0 Hz, HC(H)-S), 3.43 (dd, 1H, *J* = 14.0 Hz, *J* = 4.5 Hz, HC(H)-S), 3.85 (m, 2H, CH_2-NH), 3.90–3.95 (dd, 1H, *J* = 17 Hz, *J* = 5.5 Hz, HC(H)-NH), 4.00–4.10 (dd, 1H, *J* = 17 Hz, *J* = 5.5 Hz, HC(H)-NH), 4.60 (m, 2H, 2H, CH_2-O), 4.75 (m, 1H, CH-NH), 5.25–5.30 (dd, 1H, *J* = 10.5 Hz, *J* = 1.5 Hz, H(H)C=C), 5.30–5.35 (dd, 1H, *J* = 17.0 Hz, *J* = 1.5 Hz, H(H)C=C), 5.45 (t br, 1H, NH urethane), 5.80–6.00 (m, 1H, HC=), 7.00 (s br, 1H, NH), 7.10 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 14.0 (CH_3 Pal), 22.7 (CH_2 Pal), 25.5 (CH_2 Pal), 28.3 (CH_3 Boc), 29.0–30.0 (12 CH_2 Pal), 31.9 (CH_2-S), 42.7 (CH_2-NH), 44.0 (CH_2-NH), 52.7 (CH-NH), 66.5 (CH_2-O), 80.3 (Cq), 119.1 (H_2C=C), 131.3 (HC=C), 156.0 (C=O urethane), 168.8 (C=O), 169.5 (C=O), 170.0 (C=O), 199.7 (C=O thio ester); Anal. calcd for $C_{31}H_{55}N_3O_7S$ (613.80): C, 60.66; H, 9.03; N, 6.85. Found: C, 60.47; H, 8.75; N, 7.02.

***N*-*tert*-Butyloxycarbonyl-glycyl-glycyl-(*S*-palmitoyl)-L-cysteine (12).** Tetrakis(triphenylphosphine) palladium (5 mg) and 1,3-dimethyl-pyrimidone-2,4,5-trione **4** (85.4 mg, 0.55 mmol) were added under argon atmosphere to a solution of **11** (560 g, 0.91 mmol) in THF (10 mL) at room temperature. The resulting mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the resulting oil was dissolved in CH_2Cl_2 (5 mL) and hexane was added until all the product precipitated. **12** was isolated as a yellow solid (474 mg, 91%) and used without further purification: R_f = 0.15 (ethyl acetate/MeOH, 10/1 v/v); $[\alpha]_D^{22}$ −16° (*c* 1.54, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.91 (t, 3H, *J* = 6.9 Hz, CH_3 Pal), 1.25 (s br, 24H, 12 CH_2 Pal), 1.47 (s, 9H, CH_3 Boc), 1.61–1.73 (m, 2H, CH_2-Me Pal), 2.62 (t, 2H, *J* = 7.5 Hz, CH_2-C=O Pal), 3.29 (dd, 1H, *J* = 14.0 Hz, *J* = 6.1 Hz, HC(H)-S), 3.44 (dd, 1H, *J* = 14.0 Hz, *J* = 4.5 Hz, HC(H)-S), 3.81–3.96 (m, 3H, CH_2-NH + HC(H)-NH), 4.05–4.20 (m, 1H, HC(H)-NH), 4.68 (m, 1H, CH-NH), 5.60 (m br, 1H, NH urethane), 7.35 (m br, 2H, 2×NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 22.2 (CH_2 Pal), 25.5 (CH_2 Pal), 28.3 (CH_3 Boc), 28.8–30.3 (12 CH_2 Pal), 31.9 (CH_2-S), 42.8 (CH_2-NH), 44.0 (CH_2-NH), 52.6 (CH-NH), 66.5 (CH_2-O), 80.3 (Cq), 157.2 (C=O urethane), 169.4 (C=O), 170.0 (C=O), 173.2 (C=O), 200.1 (C=O thio ester).

***N*-*tert*-Butyloxycarbonyl-glycyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-proline (14).** 1-Hydroxybenzotriazole (80.3 mg, 0.592 mmol) and triethylamine (41.3 μL, 0.296 mmol) were added to a suspension of tripeptide **12** (170 mg, 0.296 mmol) and proline allyl ester hydro-*p*-toluenesulfonate **13** (100 mg, 0.296 mmol) in CH_2Cl_2 (10 mL). After stirring for 5 min at room temperature, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (85.2 mg, 0.444 mmol) was added. The mixture was allowed to stir for 12 h at room temperature. Then it was washed with 1 N hydrochloric acid (5 mL), with aqueous (5%) sodium bicarbonate (5 mL), and finally

with water (2×5 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated in vacuo. The resulting oil was dissolved in anhydrous THF (5 mL) at room temperature under argon atmosphere, tetrakis(triphenyl-phosphine) palladium (2 mg) and 5 min later 1,3-dimethyl-pyrimidone-2,4,5-trione **4** (6.6 mg, 42 μmol) were added. The mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the resulting oil was dissolved in CH_2Cl_2 (2 mL) and hexane was added until all the product had precipitated. **14** was isolated as a light yellow viscous oil (104.7 mg, 59% for 2 steps): R_f = 0.35 (ethyl acetate); $[\alpha]_D^{22}$ −30° (*c* 1.57, MeOH); ^{1}H NMR (MeOD, 400 MHz) δ 0.83 (t, 3H, *J* = 7.0 Hz, CH_3 Pal), 1.21 (s br, 24H, CH_2 Pal), 1.42 (s, 9H, CH_3 Boc), 1.51–1.63 (m, 2H, CH_2-Me Pal), 1.85–2.21 (m, 4H, 2 CH_2 Pro), 2.48 (t, 2H, *J* = 7.4 Hz, CH_2-C=O Pal), 2.72–2.80 (dd, 1H, *J* = 14.0 Hz, *J* = 6.2 Hz, H(H)C-S), 3.34–3.42 (dd, 1H, *J* = 14.0 Hz, *J* = 4.5 Hz, H(H)C-S), 3.64–3.75 (m, 2H, CH_2-N Pro), 3.77–3.93 (m, 4H, 2 CH_2-NH), 4.28 (m, 1H, CH-N Pro), 4.76 (m, 1H, CH-NH); ^{13}C NMR (MeOD, 100.5 MHz) δ 14.2 (CH_3 Pal), 22.7 (CH_2 Pal), 25.5 (CH_2 Pal), 28.3 (CH_3 Boc), 29.0–31.5 (12 CH_2 Pal + 2 CH_2 Pro), 32.3 (CH_2-S), 42.6 (CH_2-NH), 44.0 (CH_2-NH), 48.3 (CH_2-N), 52.6 (CH-NH), 61.4 (CH-N), 80.1 (Cq), 158.8 (C=O urethane), 168.8 (C=O), 170.0 (C=O), 170.1 (C=O), 170.7 (C=O), 200.6 (C=O thio ester).

***N*-*tert*-Butyloxycarbonyl-glycyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-prolyl-(*S*-geranylgeranyl)-L-cysteine methyl ester (16).** Carboxylic acid **14** (33 mg, 49 μmol) and HOBt (13.3 mg, 98 μmol) were dissolved in CH_2Cl_2 (5 mL). (*S*-geranylgeranyl)-L-cysteine methyl ester **15** (19.3 mg, 49 μmol) and 5 min later *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (14 mg, 73.5 μmol) were added. The mixture was allowed to stir for 24 h at room temperature. The solvent was evaporated in vacuo, and the residue was purified by column chromatography on silica gel (ethyl acetate). **16** was obtained as colorless oil (38 mg, 76%): R_f = 0.25 (ethyl acetate); $[\alpha]_D^{22}$ −78° (*c* 1.90, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.84 (t, 3H, *J* = 7.0 Hz, CH_3 Pal), 1.28 (s br, 24H, CH_2 Pal), 1.42 (s, 9H, CH_3 Boc), 1.55 (m, 2H, CH_2-Me Pal), 1.58 (s, 9H, 3 CH_3 GerGer), 1.67 (s, 3H, CH_3 GerGer), 1.69 (s, 3H, CH_3 GerGer), 1.82–2.13 (m, 15H, 6 CH_2 GerGer, H(H)C Pro + CH_2 Pro), 2.29–2.34 (m, 1H, H(H)C Pro), 2.55 (t, 2H, *J* = 7.3 Hz, CH_2-C=O Pal), 2.72–2.80 (dd, 1H, *J* = 14.0 Hz, *J* = 6.1 Hz, H(H)C-S), 2.91–3.22 (m, 4H, CH_2-S Cys, CH_2-S GerGer), 3.27–3.34 (dd, 1H, *J* = 14.0 Hz, *J* = 4.3 Hz, H(H)C-S), 3.64–3.77 (m, 2H, CH_2 Pro), 3.72 (s, 3H, CH_3-OC=O), 3.81–3.93 (m, 4H, 2 CH_2-NH), 4.05–4.13 (m, 1H, CH-N Pro), 4.68 (m, 1H, CH-NH), 4.86 (m, 1H, CH-NH), 5.08 (m, 3H, 3 HC=); 5.17 (t, 1H, *J* = 7.0 Hz, HC=), 5.24 (s br, 1H, NH urethane), 7.04 (m, 1H, NH), 7.32 (m, 1H, NH), 7.41 (m, 1H, NH); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 14.2 (CH_3 Pal), 16.0 (CH_3 GerGer), 16.1 (CH_3 GerGer), 16.2 (CH_3 GerGer), 17.7 (CH_3 GerGer), 22.7 (CH_2 Pal), 25.5 (CH_2 Pal), 25.7 (CH_3 GerGer), 26.6 (CH_2 GerGer), 26.7 (CH_2 GerGer), 26.8 (CH_2 GerGer), 28.4 (CH_3 Boc), 29.0–33.2 (12 CH_2 Pal, 2 CH_2 Pro, 3 CH_2 GerGer + CH_2-S Pal), 39.8 (2 CH_2-S GerGer), 42.7 (CH_2-NH), 44.1 (CH_2-NH), 47.7 (CH_2-N), 51.0 (CH-NH), 51.8 (CH-NH), 52.6 (CH_3-0C=O), 60.2 (CH-N), 80.3 (Cq), 119.5 (HC=), 123.8 (HC=), 124.2 (HC=), 124.4 (HC=), 131.3 (Cq=), 135.0 (Cq=), 135.5 (Cq=), 140.1 (Cq=), 156.2 (C=O urethane), 168.8 (C=O), 170.0 (C=O), 170.1 (C=O), 170.7 (C=O), 171.2 (C=O), 200.8 (C=O thio ester); Anal. calcd for $C_{57}H_{97}N_5O_9S_2$ (710.96): C, 64.55; H, 9.12; N, 6.60. Found: C, 64.53; H, 8.93; N, 6.94.

Bis-(*N*-*tert*-butyloxycarbonyl-glycyl)-L-cystine bis(allyl ester) (17). 1-Hydroxybenzotriazole (6.17 g, 45.72 mmol) and triethylamine (1.59 mL, 11.43 mmol) were added to a solution of *N*-*tert*-butyloxycarbonyl glycine **5** (2 g, 11.43 mmol) and cystine allyl ester hydro-*p*-toluenesulfonate **9** (3.8 g, 5.71 mmol) in CH_2Cl_2 (100 mL). After stirring for 5 min at room temperature diisopropylcarbodiimide (1.95 mL, 12.57 mmol) was added. The mixture was allowed to stir for 12 h at room temperature. The solution was washed with 1 N hydrochloric acid (100 mL), with aqueous (5%) sodium bicarbonate (100 mL), and with water (2×100 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, 1/3 v/v) to afford **17** (2.68 g, 74%) as a colorless viscous oil: R_f = 0.5 (hexane/ethyl acetate, 1/2 v/v); $[\alpha]_D^{22}$ −44° (*c* 1.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 18H, CH_3 Boc), 3.20 (d, 4H, *J* = 5.0 Hz, CH_2-NH), 3.80–4.00 (m, 4H, CH_2-S), 4.60 (d, 4H, *J* = 6.0 Hz, CH_2-O), 4.90 (m, 2H, CH-NH), 5.20–5.40 (m, 4H, H_2C=C), 5.70 (m br, 2H, NH urethane), 5.80–6.00 (m, 2H, HC=C), 7.30 (m br, 2H, NH); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 28.0 (CH_3 Boc), 41.0(CH_2-S), 45.3 (CH_2-NH), 51.8 (CH-NH), 67.1 (CH_2-O), 81.2 (Cq), 119.0 (H_2C=C), 132.4 (HC=C), 157.1 (C=O urethane), 169.7 (C=O), 170.0 (C=O); Anal. calcd for $C_{26}H_{42}N_4O_{10}S_2$ (634.23): C, 49.19; H, 6.67; N, 8.82. Found: C, 49.16; H, 6.69; N, 8.77.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteine allyl ester (18).** Triethylamine (0.77 mL, 5.51 mmol) and 1,4-DL-dithiothreitol (2.12 g, 13.79 mmol) were added under argon atmosphere to a solution of **17** (1.75 g, 2.75 mmol) in CH_2Cl_2 (120 mL). After stirring for 2 h at room temperature, the solution was washed with 1 N hydrochloric acid (100 mL), with water (2×100 mL), and dried over sodium sulfate. The solution was filtered and cooled to 0 °C before adding triethylamine (0.77 mL, 5.51 mmol) and palmitoyl chloride (4.18 mL, 13.79 mmol). The mixture was stirred for 2 h at room temperature. After filtration and evaporation of CH_2Cl_2 in vacuo, a crude oil was obtained, which was purified by column chromatography (hexane/ethyl acetate, 1/1 v/v). Compound **18** was isolated as a white solid (2.15 g, 72%): R_f = 0.25 (hexane/ethyl acetate, 5/2 v/v); $[\alpha]_D^{22}$ −14° (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, *J* = 7.0 Hz, CH_3 Pal), 1.25 (s br, 24H, CH_2 Pal), 1.47 (s, 9H, CH_3 Boc), 1.65 (m, 2H, CH_2-Me Pal), 2.55 (t, 2H, *J* = 7.5 Hz, CH_2-C=O Pal), 3.35 (dd, 1H, *J* = 14.0 Hz, *J* = 6.0 Hz, HC(H)-S), 3.43 (dd, 1H, *J* = 14.0 Hz, *J* = 4.5 Hz, HC(H)-S), 3.75–3.85 (m, 2H, CH_2-NH), 4.65 (m, 2H, CH_2-O), 4.80 (m, 1H, CH-NH),

5.20 (m br, 1H, NH urethane) 5.25–5.35 (m, 2H, H_2C=C), 5.85–5.95 (m, 1H, HC=C), 6.95 (m br, 1H, NH); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 14.1 (CH_3 Pal), 22.7 (CH_2 Pal), 25.5 (CH_2 Pal), 28.3 (CH_3 Boc), 28.9–29.6 (CH_2 Pal), 30.4 (CH_2 Pal), 31.9 (CH_2-S), 44.0 (CH_2-NH), 52.1 (CH-NH), 66.5 (CH_2-O), 80.1 (Cq), 119.0 (H_2C=C), 131.3 (HC=C), 155.9 (C=O urethane), 169.5 (C=O), 169.6 (C=O), 198.8 (C=O thio ester); Anal. calcd for $C_{29}H_{52}N_2O_6S$ (556.81): C, 62.56; H, 9.41; N, 5.03. Found: C, 62.52; H, 9.18; N, 5.18.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteine (19).** Tetrakis(triphenylphosphine) palladium (2 mg) was added under argon atmosphere to a solution of **18** (170 mg, 0.305 mmol) in THF (5 mL). 5 min later morpholine **3** (30 μL, 0.340 mmol) was added. The resulting mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, the resulting oil was dissolved in CH_2Cl_2 (2 mL) and hexane was added until all the product had precipitated. **19** was isolated as a yellow semi-cristalline product (140 mg, 89%) and used without further purification: R_f=0.15 (ethyl acetate/MeOH, 10/1 v/v); $[\alpha]_D^{22}$ −5° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.90 (t, 3H, *J*=6.9 Hz, CH_3 Pal), 1.26 (s br, 24H, CH_2 Pal), 1.49 (s, 9H, CH_3 Boc), 1.60–1.73 (m, 2H, CH_2-Me Pal), 2.58 (t, 2H, *J*=7.5 Hz, CH_2-C=O Pal), 3.22–3.38 (m, 1H, HC(H)-S), 3.42–3.58 (m, 1H, HC(H)-S), 3.72–3.84 (m, 1H, HC(H)-NH), 3.90–4.03 (m, 1H, HC(H)-NH), 4.70–4.88 (m, 1H, CH-NH), 5.45 (NH urethane), 7.10 (m br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.2 (CH_3 Pal), 22.2 (CH_2 Pal), 25.5 (CH_2 Pal), 28.1 (CH_3 Boc), 28.5–30.2 (CH_2 Pal), 30.4 (CH_2 Pal), 32.1 (CH_2-S), 44.8 (CH_2-NH), 53.1 (CH-NH), 80.0 (Cq), 157.0 (C=O urethane), 169.2 (C=O), 169.8 (C=O), 199.1 (C=O thio ester); Anal. calcd for $C_{26}H_{48}N_2O_6S$ (516.72): C, 60.43; H, 9.36; N, 5.42. Found: C, 60.22; H, 9.21; N, 5.18.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-leucine allyl ester (20).** 1-Hydroxybenzotriazole (52.1 mg, 0.386 mol) and triethylamine (40 μL, 0.283 mmol) were added to a solution of tripeptide **29a** (91.6 mg, 0.193 mmol) and dipeptide **19** (100 mg, 0.193 mmol) in CH_2Cl_2 (5 mL) After stirring for 5 min at room temperature, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (55 mg, 0.290 mmol) was added. The mixture was allowed to stir for 12 h at room temperature, and then was washed with 1 N hydrochloric acid (5 mL), with aqueous (5%) sodium bicarbonate (5 mL), and finally with water (2×5 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvent was evaporated in vacuo. The resulting oil was purified by column chromatography on silica gel (ethyl acetate) to afford **20** (116.2 mg, 70%) as a colorless oil: R_f=0.15 (ethyl acetate); $[\alpha]_D^{22}$ −5.2° (*c* 0.64, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.86 (t, 3H, *J*=7.1 Hz, CH_3 Pal), 0.93 (d, 6H, *J*=4.9 Hz, CH_3 Leu), 1.23 (s br, 24H, CH_2 Pal), 1.44 (s, 9H, CH_3 Boc), 1.51–1.72 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.97–2.25 (m, 2H, CH_2 Met), 2.08 (s, 3H, CH_3 Met), 2.50–2.58 (m, 4H, H_2C-S Met, H_2C-C=O), 3.22–3.31 (m, 2H, H_2C-S Cys), 3.90–4.26 (m, 4H, 2×H_2C-NH), 4.66 (s br, 2H, H_2C-O), 4.50–4.72 (m, 3H, 3×CH-NH), 4.91 (s br, 1H, NH urethane), 5.13–5.21 (dd, 1H, *J*=10.3 Hz, *J*=1.5 Hz, H(H)C=C), 5.27–5.35 (dd, 1H, *J*=17.0 Hz, *J*=1.5 Hz, H(H)C=C), 5.80–5.97 (m, 1H, HC=H), 7.41 (s br, 1H, NH), 7.95 (s br, 2H, 2×NH), 8.26 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 15.3 (SCH_3), 21.9 (CH_3 Leu), 22.7 (CH_2 Pal), 22.9 (CH_3 Leu), 23.5 ($CH(Me)_2$), 25.6 (CH_2 Pal), 28.4 (CH_3 Boc), 29.0–30.2 (CH_2 Pal), 30.4 (CH_2 Met), 31.9 (H_2C-S Met), 41.2 (CH_2 Leu), 43.1 (H_2C-NH), 44.0 (H_2C-NH), 50.7 (HC-NH), 52.7 (HC-NH), 53.2 (HC-NH), 65.8 (H_2C-O), 80.0 (Cq), 118.6 (H_2C=C), 131.7 (HC=C), 156.4 (C=O urethane), 168.8 (C=O), 169.7 (C=O), 170.4 (C=O), 171.2 (C=O), 172.7 (C=O), 199.6 (C=O thio ester); Anal. calcd for $C_{42}H_{75}N_5O_9S_2$ (858.21): C, 58.78; H, 8.81; N, 8.16; Found: C, 58.79; H, 8.92; N, 8.03.

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-leucine (21).** Pentapeptide ester **20** (70 mg, 81.1 μmol) was dissolved, under argon atmosphere, in THF (2 mL). Tetrakis(triphenylphosphine) palladium (1 mg) and 5 min later morpholine **3** (10.7 μL, 121 μmol) were added. The mixture was allowed to stir for 3 h at room temperature. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (ethyl acetate/MeOH, 7/3 + 1% acetic acid v/v). **21** was isolated as a light yellow viscous oil (56.7 mg, 86%): $[\alpha]_D^{22}$ −33° (*c* 1.20, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 0.89 (t, 3H, *J*=7.1 Hz, CH_3 Pal), 0.91 (d, 6H, *J*=4.9 Hz, CH_3 Leu), 1.21 (s br, 24H, CH_2 Pal), 1.44 (s, 9H, CH_3 Boc), 1.48–1.73 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.97–2.25 (m, 2H, CH_2 Met), 2.08 (s, 3H, CH_3 Met), 2.50–2.58 (m, 4H, H_2C-S Met, H2C-C=O), 3.22–3.31 (m, 2H, H_2C-S Cys), 3.85–4.23 (m, 4H, 2×H_2C-NH), 4.66 (s br, 2H, H_2C-O), 4.50–4.72 (m, 3H, 3×CH-NH).

***N*-*tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-leucyl-L-proline allyl ester (22).** 1-Hydroxybenzotriazole (19.8 mg, 14.61 μmol) and triethylamine (10.2 μL, 7.34 μmol) were added to a suspension of **21** (60 mg, 7.34 μmol) and proline allyl esterhydro-*p*-toluenesulfonate **13** (24.1 mg, 7.34 μmol) in CH_2Cl_2 (3 mL). After stirring for 5 min at room temperature, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (21 mg, 10.95 μmol) was added. The mixture was allowed to stir for 12 h at room temperature. Then it was washed with 1 N hydrochloric acid (2 mL), with aqueous (5%) sodium bicarbonate (2 mL), and finally with water (2×2 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvent was evaporated in vacuo. The resulting oil was purified by column chromatography on silica gel (ethyl acetate) to afford **22** (58.4 mg; 83%) as a colorless oil: R_f=0.70 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −44° (*c* 1.16, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.89 (t, 3H, *J*=7.0 Hz, CH_3 Pal), 0.92 (d, 3H, *J*=7.0 Hz, CH_3 Leu), 0.97 (d, 3H, *J*=7.0 Hz, CH_3 Leu), 1.22 (s br, 24H, CH_2 Pal), 1.41 (s, 9H, CH_3Boc), 1.49–1.64 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.99–2.22 (m, 6H, 2×CH_2 Pro, CH_2 Met), 2.08 (s, 3H, CH_3 Met), 2.51–2.58 (m, 4H, H_2C-S Met, H_2C-C=O Pal), 3.17–3.29 (m, 2H, H_2C-S Cys), 3.90–4.26 (m, 6H, CH_2 Pro + 2×H_2C-NH), 4.64 (s

br, 2H, H_2C-O), 4.47–4.63 (m, 4H, 3×CH-NH, CH-N), 4.82 (s br, 1H, NH urethane), 5.10–5.22 (dd, 1H, J = 10.3 Hz, J = 1.5 Hz, H(H)C=C), 5.26–5.35 (dd, 1H, J = 17.0 Hz, J = 1.5 Hz, H(H)C=C), 5.76–5.95 (m, 1H, HC=H), 7.41 (s br, 1H, NH), 7.95 (s br, 1H, NH), 8.26 (s br, 2H, 2×NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.0 (CH_3 Pal), 15.4 (S-CH_3), 21.8 (CH_3 Leu), 22.7 (CH_2 Pal), 23.1 (CH_3 Leu), 23.5 ($CH(Me)_2$), 25.8 (CH_2 Pal), 28.4 (CH_3 Boc), 29.0–30.2 (CH_2 Pal), 30.4–31.0 (CH_2 Met, 2×CH_2 Pro), 31.9 (H_2C-S Met), 41.0 (CH_2 Leu), 43.6 (H_2C-NH), 44.8 (H_2C-NH), 48.0 (CH_2 Pro), 51.1 (HC-NH), 52.5 (HC-NH), 53.8 (HC-NH), 54.1 (HC-N), 66.2 (H_2C-O), 80.9 (Cq), 118.3 ((H_2C=C), 132.0 (HC=C), 157.1 (C=O urethane), 169.4 (C=O), 169.9 (C=O), 170.3 (C=O), 170.7 (C=O), 171.5 (C=O), 172.1 (C=O), 199.6 (C=O thio ester); Anal. calcd for $C_{47}H_{82}N_6O_{10}S_2$ (955.33): C, 59.09; H, 8.65; N, 8.79; Found: C, 59.31; H, 8.81; N, 9.02.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-leucyl-L-proline (23).** Tetrakis(triphenylphosphine) palladium (1 mg) and 1,3-dimethyl barbituric acid **4** (5 mg, 31.5 μmol) were added under argon atmosphere to a solution of hexapeptide ester **22** (50 mg, 52.4 μmol) in THF (2 mL). The mixture was allowed to stir for 2 h at room temperature. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (ethyl acetate/MeOH, 7/3 + 1% acetic acid v/v). **23** was isolated as a light yellow viscous oil (40 mg, 84%): R_f = 0.60 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −37° (*c* 2, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 0.87 (t, 3H, J = 7.0 Hz, CH_3 Pal), 0.95 (d, 6H, J = 7.1 Hz, 2×CH_3 Leu), 1.24 (s br, 24H, CH_2 Pal), 1.44 (s, 9H, CH_3Boc), 1.46–1.68 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 2.01–2.23 (m, 6H, 2×CH_2 Pro, CH_2 Met), 2.09 (s, 3H, CH_3 Met), 2.51–2.58 (m, 4H, H_2C-S Met, H_2C-C=O Pal), 3.10–3.21 (m, 2H, H_2C-S Cys), 3.99–4.37 (m, 6H, CH_2 Pro, 2×H_2C-NH), 4.54–4.71 (m, 4H, 3×CH-NH, CH-N); ^{13}C NMR (MeOD, 62.8 MHz) δ 14.1 (CH_3 Pal), 15.4 (S-CH_3), 21.9 (CH_3 Leu), 22.7 (CH_2 Pal), 23.0 (CH_3 Leu), 23.4 ($CH(Me)_2$), 25.8 (CH_2 Pal), 28.9 (CH_3 Boc), 29.0–30.2 (CH_2 Pal), 30.6–31.1 (CH_2 Met + 2×CH_2 Pro), 32.2 (H_2C-S Met), 41.1 (CH_2 Leu), 43.9 (H_2C-NH), 45.1 (H_2C-NH), 48.2 (CH_2 Pro), 51.4 (HC-NH), 52.5 (HC-NH), 54.0 (HC-NH), 54.7 (HC-N), 80.0 (Cq), 157.6 (C=O urethane), 169.4 (C=O), 169.7 (C=O), 170.0 (C=O), 170.7 (C=O), 171.9 (C=O), 172.6 (C=O), 200.2 (C=O thio ester).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-leucyl-L-prolyl-(*S*-farnesyl)-L-cysteine methyl ester (25).** Carboxylic acid **23** (40 mg, 43.7 μmol) and HOBt (11.9 mg, 87 μmol) were dissolved in CH_2Cl_2 (2 mL). (S-farnesyl)-L-cysteine methyl ester **24** (14.8 mg, 43.8 mmol) and 5 min later *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (12.6 mg, 65.7 μmol) were added. The mixture was allowed to stir for 24 h at room temperature. The solvent was evaporated in vacuo, and the residue was directly purified by column chromatography on silica gel (ethyl acetate). **25** was isolated as colorless viscous oil (23.1 mg, 36% for 2 steps): R_f = 0.60 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −52° (*c* 0.46, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3H, J = 7.0 Hz, CH_3 Pal), 0.94 (d, 6H, J = 6.6 Hz, 2×CH_3 Leu), 1.25 (s br, 24H, CH_2 Pal), 1.43 (s, 9H, CH_3 Boc), 1.47–1.63 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.59 (s, 6H, 2×CH_3 Far), 1.66 (s, 3H, CH_3 Far), 1.68 (s, 3H, CH_3 Far), 1.99–2.22 (m, 14H, 4×CH_2 Far, 2×CH_2 Pro, CH_2 Met), 2.09 (s, 3H, CH_3 Met), 2.48–2.61 (m, 4H, H_2C-S Met, H_2C-C=O), 2.68–3.20 (m, 6H, 2×H_2C-S Far, H_2C-S Pal), 3.70–3.90 (m, 4H, 2×H_2C-NH), 3.74 (s, 3H, H_3C-OC=O), 4.27–4.70 (m, 7H, CH_2 Pro, 5×CH-NH + CH-N), 5.05 (m, 2H, 2×HC=C), 5.16 (m, 1H, HC=C), 5.41 (s br, 1H, NH urethane), 7.00 (s br, 1H, NH), 7.38 (s br, 2H, 2×NH), 7.61 (s br, 1H, NH), 8.00 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3 Pal), 15.6 (S-CH_3), 16.2 (2×CH_3 Far), 18.0 (CH_3 Far), 22.0 (CH_3 Leu), 22.7 (CH_2 Pal), 23.3 (CH_3 Leu), 23.5 ($CH(Me)_2$), 25.8 (CH_2 Pal), 28.4 (CH_3 Boc), 29.0–30.2 (14 CH_2 Pal, CH_3 Far, 4×CH_3 Far), 30.4–31.0 (CH_2 Met; 2×CH_2 Pro), 31.9 (H_2C-S Met), 40.5 (CH_2 Far), 41.2 (CH_2 Leu), 43.6 (H_2C-NH), 45.0 (H_2C-NH), 48.0 (CH_2 Pro), 51.1 (HC-NH), 51.8 (CH-NH), 52.5 (HC-NH), 53.8 (HC-NH), 54.1 (HC-N), 80.7 (Cq), 118.6 ((HC=C), 121.6 (HC=C), 125.8 (HC=C), 132.8 (Cq Far), 136.1 (Cq Far), 141.0 (Cq Far), 155.9 (C=O urethane), 169.7 (C=O), 170.0 (C=O), 170.3 (C=O), 170.7 (C=O), 171.1 (C=O), 171.5 (C=O), 172.1 (C=O), 199.6 (C=O thio ester); Anal. calcd for $C_{63}H_{109}N_7O_{11}S_2$ (1204.73): C, 62.81; H, 9.11; N, 8.13. Found: C, 63.08; H, 8.99; N, 9.27.

Preparation of the tripeptides 29a–d

Synthesis of dipeptide carboxylic acids 26: General procedure. 1-Hydroxybenzotriazole (4.33 g, 32.10 mmol) and triethylamine (2.23 mL, 16.05 mmol) were added to a suspension of glycine allyl ester hydro-*p*-toluenesulfonate **6** (16.05 mmol) and of a Boc-amino acid (16.05 mmol) in CH_2Cl_2 (80 mL). Diisopropylcarbodiimide (2.73 mL, 17.66 mmol) was added after stirring for 5 min. The mixture was allowed to stir for 12 h at room temperature, and then washed with 1 N hydrochloric acid (50 mL), with aqueous (5%) sodium bicarbonate (50 mL), and with water (2×50 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, 2/3 v/v). The resulting dipeptide (8.66 mmol) was dissolved in THF (60 mL). Tetrakis(triphenylphosphine) palladium (5 mg) and 5 min later morpholine **3** (7.54 mL, 86.60 mmol) were added. The resulting mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo and the resulting oil was purified by column chromatography on silica gel (MeOH/ethyl acetate, 1/4 v/v).

***N-tert*-Butyloxycarbonyl-L-methionyl-glycine (26a). 26a** was isolated as a yellow oil (48% for 2 steps). $[\alpha]_D^{22}$ −8° (*c* 1, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 1.44 (s, 9H, CH_3 Boc), 1.81–2.18 (m, 2H, CH_2 Met), 2.11 (s, 3H, CH_3 Met), 2.57 (m, 2H, H_2C-S), 3.82–4.08 (m, 2H, H_2C-NH), 4.21–4.33 (m, 1H, HC-NH); ^{13}C NMR (MeOD, 100 MHz) δ 15.4 (S-CH_3), 24.8 (CH-$(Me)_2$), 28.9 (CH_3 Boc), 30.5 (CH_2 Met), 31.7 (CH_2 Met), 42.0

(H_2C-NH), 53.6 (HC-NH), 80.6 (Cq), 155.6 (C=O urethane), 168.6 (C=O).

***N-tert*-Butyloxycarbonyl-L-alanyl-glycine (26b). 26b** was isolated as a light yellow oil (54% for 2 steps): $[\alpha]_D^{22}$ +8° (*c* 4.1, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 1.39 (d, 3H, *J* = 7.2 Hz, CH_3 Ala), 1.44 (s, 9H, CH_3 Boc), 3.84–4.24 (m, 3H, CH-NH + CH_2-NH); ^{13}C NMR (MeOD, 62.8 MHz) δ 18.5 (CH_3 Ala), 28.2 (CH_3 Boc), 42.7 (CH_2-NH), 50.8 (CH-NH), 80.2 (Cq), 155.1 (C=O urethane), 169.3 (C=O).

***N-tert*-Butyloxycarbonyl-L-phenylalanyl-glycine (26c). 26c** was isolated as a light yellow oil (51% for 2 steps): $[\alpha]_D^{22}$ −10° (*c* 3.24, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 1.41 (s, 9H, CH_3 Boc), 2.88–3.12 (m, 2H, H_2C-Ph), 3.83–4.07 (m, 2H, H_2C-NH), 4.44–4.56 (m, 2H, CH Phe), 7.18–7.34 (m, 5H, HC=C ar.); ^{13}C NMR (MeOD, 62.8 MHz) δ 39.9 (CH_2 Phe), 42.8 (CH_2-NH), 56.1 (CH-NH), 128.6, 129.9, 130.1 (HC=C ar.), 135.0 (HC=C ar.), 170.2 (C=O), 170.7 (C=O).

Synthesis of tripeptide allyl esters 28: general procedure. 1-Hydroxybenzotriazole (686.2 mg, 5.08 mmol) and triethylamine (0.35 mL, 2.54 mmol) were added to a suspension of amino acid allyl ester hydro-*p*-toluenesulfonate **27** (2.54 mmol) and of Boc-dipeptide **26** (2.54 mmol) in CH_2Cl_2/DMF (10/1, 20 mL). Diisopropylcarbodiimide (0.43 mL, 2.79 mmol) was added 5 min later. The mixture was allowed to stir for 12 h at room temperature, washed with 1 N hydrochloric acid (20 mL), with aqueous (5%) sodium bicarbonate (20 mL), and with water (2×20 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated in vacuo. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, 1/3 v/v).

***N-tert*-Butyloxycarbonyl-L-methionyl-glycyl-L-leucine allyl ester (28a). 28a** was isolated as a colorless viscous oil in 81% yield: R_f = 0.30 (hexane/ethyl acetate, 1/3 v/v); $[\alpha]_D^{22}$ −18° (*c* 1.80, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.93 (d, 6H, *J* = 7.0 Hz, CH_3 Leu), 1.42 (s, 9H, CH_3 Boc), 1.54–1.71 (m, 3H, CH Leu, CH_2 Leu), 1.81–2.18 (m, 2H, CH_2 Met), 2.11 (s, 3H, CH_3 Met), 2.57 (m, 2H, H_2C-S), 3.82–4.08 (m, 2H, H_2C-NH), 4.21–4.33 (m, 1H, HC-NH), 4.55–4.64 (m, 3H, HC-NH; H_2C-O), 5.22–5.28 (dd, 1H, *J* = 10.4 Hz, *J* = 1.5 Hz, H(H)C=C), 5.30–5.39 (dd, 1H, *J* = 17.0 Hz, *J* = 1.5 Hz, H(H)C=C), 5.52 (m, 1H, NH urethane), 5.81–6.00 (m, 1H, HC=C), 7.12 (m, 1H, NH), 7.23 (m, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.2 (S-CH_3), 21.7 (CH_3 Leu), 22.7 (CH_3 Leu), 24.7 (CH-$(Me)_2$), 28.2 (CH_3 Boc), 30.1 (CH_2 Met), 31.5 (CH_2 Met), 41.0 (CH_2 Leu), 42.0 (H_2C-NH), 50.8 (HC-NH), 53.6 (HC-NH), 65.8 (H_2C-O), 80.1 (Cq), 118.6 (H_2C=C), 131.5 (HC=C), 155.6 (C=O urethane), 168.6 (C=O), 172.2 (C=O), 172.4 (C=O); Anal. calcd for $C_{21}H_{37}N_3O_6S$ (459.60): C, 54.88; H, 8.11; N, 9.14. Found: C, 54.96; H, 7.89; N, 9.10.

***N-tert*-Butyloxycarbonyl-L-alanyl-glycyl-L-leucine allyl ester (28b). 28b** was obtained as a colorless viscous oil in 77% yield: R_f = 0.30 (hexane/ethyl acetate, 1/3 v/v); $[\alpha]_D^{22}$ −15° (*c* 5.85, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.92 (d, 6H, *J* = 7.1 Hz, CH_3 Leu), 1.38 (d, 3H, *J* = 7.3 Hz, CH_3 Ala), 1.43 (s, 9H, CH_3 Boc), 1.53–1.71 (m, 3H, CH Leu; CH_2 Leu), 3.84–4.24 (m, 3H, CH-NH + CH_2-NH), 4.51–4.63 (m, 3H, CH-NH; H_2C-O), 5.20–5.35 (m, 3H, H_2C=C; NH urethane), 5.79–5.98 (m, 1H, HC=C), 7.15 (s br, 1H, NH), 7.31 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 18.5 (CH_3 Ala), 21.6 (CH_2 Leu), 22.5 (CH_3 Leu), 25.0 ($CH(Me)_2$), 28.2 (CH_2 Boc), 41.1 (CH_2 Leu), 42.0 (CH_2-NH), 50.4 (CH-NH), 50.9 (CH-NH), 66.0 (H_2C-O), 80.2 (Cq), 119.1 (H_2C=C), 131.3 (HC=C), 155.7 (C=O urethane), 169.3 (C=O), 171.8 (C=O); Anal. calcd for $C_{19}H_{33}N_3O_6$ (399.49): C, 57.12; H, 8.32; N, 10.51. Found: C, 57.18; H, 8.34; N, 10.50.

***N-tert*-Butyloxycarbonyl-L-phenylalanyl-glycyl-L-leucine allyl ester (28c). 28c** was obtained as a white solid in 65% yield: R_f = 0.25 (hexane/ethyl acetate, 1/3 v/v); $[\alpha]_D^{22}$ −5° (*c* 2.60, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.92 (m, 6H, CH_3 Leu), 1.39 (s, 9H, CH_3 Boc), 1.58–1.75 (m, 3H, CH Leu, CH_2 Leu), 2.91–3.02 (m, 1H, HC(H)-Ph), 3.10–3.20 (m, 1H, HC(H)-Ph), 3.73–3.88 (m, 1H, HC(H)-NH), 3.99–4.11 (m, 1H, HC(H)-NH), 4.30–4.39 (m, 1H CH-NH Leu), 4.51–4.62 (m, 3H, CH Phe; H_2C-O), 5.04 (m, 1H, NH urethane), 5.18–5.32 (m, 2H, H_2C=C), 5.80–5.95 (m, 1H, HC=C), 6.75 (s br, 1H, NH), 6.95 (s br, 1H, NH), 7.18–7.34 (m, 5H, HC=C ar.); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 21.6 (CH_3 Leu), 23.2 (CH_3 Leu), 25.2 (HC-$(Me)_2$), 39.1 (CH_2 Phe), 41.6 (CH_2 Leu), 42.4 (CH_2-NH), 53.1 (CH-NH Leu), 56.0, (CH-NH Phe), 66.3 (H_2C-O), 118.4 (H_2C=C), 128.8, 130.1, 130.5 (HC=C ar.), 133.3 (HC=C), 135.6 (HC=C ar.), 170.2 (C=O), 170.7 (C=O), 173.7 (C=O); Anal. calcd for $C_{25}H_{37}N_3O_6$ (475.59): C, 63.14; H, 7.84; N, 8.83. Found: C, 63.22; H, 8.01; N, 8.91.

***N-tert*-Butyloxycarbonyl-L-methionyl-glycyl-L-alanine allyl ester (28d). 28d** was isolated as a viscous oil in 59% yield: R_f = 0.30 (hexane/ethyl acetate, 1/3 v/v); $[\alpha]_D^{22}$ −4° (*c* 5.30, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.42 (s br, 12H, CH_3 Ala, CH_3 Boc), 1.84–2.13 (m, 2H, CH_2 Met), 2.11 (s, 3H, CH_3-S), 2.58 (m, 2H, CH_2-S), 3.82–4.08 (m, 2H, H_2C-NH), 4.21–4.32 (m, 1H, CH Ala), 4.52–4.66 (m, 3H, CH Met, H_2C-O), 5.20–5.39 (m, 3H, H_2C=C, NH urethane), 5.80–5.95 (m, 1H, HC=C), 6.92 (s br, 1H, NH), 7.01 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 15.0 (S-CH_3), 17.7 (CH_3 Ala), 28.9 (CH_3 Boc), 29.9 (CH_2 Met), 32.0 (CH_2-S), 42.9 (H_2C-NH), 51.1 (HC-NH), 53.0 (HC-NH), 66.4 (H_2C-O), 80.4 (Cq), 118.8 (H_2C=C), 131.9 (HC=C), 155.6 (C=O urethane), 169.4 (C=O), 170.1 (C=O), 170.8 (C=O); Anal. calcd for $C_{18}H_{31}N_3O_6S$ (417.52): C, 51.78; H, 7.48; N, 10.06. Found: C, 51.99; H, 7.76; N, 9.77.

Synthesis of N-terminally deprotected tripeptides 29: general procedure. Trifluoroacetic acid (2.46 mL, 32.2 mmol) was added to a solution of **28** (1.61 mmol) in CH_2Cl_2 (10 mL). The mixture was allowed to stir for 1 h at room temperature, and then toluene (30 mL) was added. After the evaporation of the solvents in vacuo, the desired products **29** were obtained as viscous oils.

L-Methionyl-glycyl-L-leucine allyl ester hydrotrifluoroacetate (29a). 29a was isolated as a viscous colorless oil in quantitative yield: $[\alpha]_D^{22}$ +14° (*c* 0.78, CH_2Cl_2); ^1H NMR ($CDCl_3$, 250 MHz): δ 0.91 (m, 6H, CH_3 Leu), 1.52–1.72 (m, 3H, CH Leu, CH_2 Leu), 2.03 (s, 3H, CH_3 Met), 2.11–2.24 (m, 2H, CH_2 Met), 2.61 (m, 2H, H_2C-S), 3.62–3.78 (m, 1H, H(H)C-NH), 4.20–4.58 (m, 3H, H(H)C-NH + HC-NH + H_2C-O), 5.15–5.35 (m, 2H, H_2C=C), 5.73–5.94 (m, 1H, HC=C), 7.14 (m, 1H, NH), 7.23 (s br, 3H, NH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.1 (S-CH_3), 21.9 (CH_3 Leu), 23.3 (CH_3 Leu), 25.9 (CH-$(Me)_2$), 29.7 (CH_2 Met), 31.9 (CH_2 Met), 41.5 (CH_2 Leu), 42.8 (H_2C-NH), 52.3 (HC-NH), 53.6 (HC-NH3), 66.7 (H_2C-O), 97.2 (CF_3), 118.7 (H_2C=C), 133.2 (HC=C), 170.2 (C=O), 170.8 (C=O), 173.6 (C=O), 175.8 (C=O); Anal. calcd for $C_{18}H_{30}F_3N_3O_6S$ (473.51): C, 45.66; H, 6.38; N, 8.87. Found: C, 45.29; H, 6.17; N, 8.97.

L-Alanyl-glycyl-L-leucine allyl ester hydrotrifluoroacetate (29b). 29b was isolated as a viscous colorless oil in quantitative yield: $[\alpha]_D^{22}$ +12° (*c* 1.80, MeOH); ^1H NMR (MeOD, 250 MHz) δ 0.92 (d, 3H, *J* = 7.0 Hz, CH_3 Leu), 0.96 (d, 3H, *J* = 7.0 Hz, CH_3 Leu), 1.52 (d, 3H, *J* = 7.6 Hz, CH_3 Ala), 1.59–1.77 (m, 3H, CH Leu, CH_2 Leu), 3.91–4.08 (m, 3H, CH-NH, CH_2-NH), 4.40–4.50 (m, 1H, HC-NH), 4.60 (d, 2H, *J* = 5.6 Hz, H_2C-O), 5.22 (d, 1H, *J* = 10.2 Hz, H_2C=C), 5.34 (d, 1H, *J* = 17.1 Hz, H_2C=C), 5.85–6.01 (m, 1H, HC=C).

L-Phenylalanyl-glycyl-L-leucine allyl ester hydrotrifluoroacetate (29c). The peptide **29c** was obtained as a viscous (light yellow) oil with quantitative yield: $[\alpha]_D^{22}$ −10° (*c* 1.12, MeOH); ^1H NMR (MeOD, 250 MHz) δ 0.91 (d, 6H, *J* = 7.2 Hz, CH_3 Leu), 1.55–1.72 (m, 3H, CH Leu, CH_2 Leu), 2.88–3.15 (m, 2H, H_2C-Ph), 3.75–3.82 (m, 1H, H_2C-NH), 4.33–4.40 (m, 1H, HC-NH Leu), 4.25–4.54 (m, 1H HC-NH Phe), 4.63 (d, 2H, *J* = 5.6 Hz, H_2C-O), 5.18 (d, 1H, *J* = 10.2 Hz, H(H)C=C), 5.31 (d, 1H, *J* = 17.0 Hz, H(H)C=C), 5.74–5.9 (m, 1H, HC=C), 7.12–7.29 (m, 5H, HC=C ar.)

L-Methionyl-glycyl-L-alanyl allyl ester hydrotrifluoroacetate (29d). 29d was isolated as a viscous (colorless) oil in quantitative yield: $[\alpha]_D^{22}$ −15° (*c* 1.76, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40 (d, 3H, *J* = 7.3 Hz, CH_3 Ala), 2.04–2.17 (m, 2H, CH_2 Met), 2.11 (s, 3H, CH_3-S), 2.61 (t, 2H, *J* = 7.5 Hz, CH_2-S), 3.30 (m, 2H, H_2C-NH), 4.01 (m, 1H, CH Ala), 4.43 (m, 1H, CH Met), 4.61 (d, 2H, *J* = 5.5 Hz, H_2C-O), 5.22 (d, 1H, *J* = 10.3 Hz, H(H)C=C), 5.32 (d, 1H, *J* = 17.2 Hz, H(H)C=C), 5.88–5.97 (m, 1H, HC=C); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.1 (S-CH_3), 17.5 (CH_3 Ala), 29.7 (CH_2 Met), 31.8 (CH_2-S), 42.8 (H_2C-NH), 50.3 (HC-NH), 53.5 (HC-NH), 66.7 (H_2C-O), 97.9 (CF_3), 118.6 (H_2C=C), 133.1 (HC=C), 170.0 (C=O), 170.4 (C=O), 173.6 (C=O).

Preparation of the pentapeptides 30a–c

Peptides **30** were prepared according to the procedure given for the synthesis of peptides **28**.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-alanyl-glycyl-L-leucine allyl ester (30a).** The pentapeptide **30a** was obtained as a colorless viscous oil in 85% yield: R_f = 0.15 (ethyl acetate); $[\alpha]_D^{22}$ −2.5° (*c* 4.10, CH_2Cl_2); ^1H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, 3H, *J* = 6.8 Hz, CH_3 Pal), 0.95 (d, 6H, *J* = 5.2 Hz, CH_3 Leu), 1.23 (s br, 24H, 12×CH_2 Pal), 1.44 (s br, 12H, CH_3 Boc, CH_3 Ala), 1.53–1.78 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 2.49 (t, 2H, *J* = 7.7 Hz, H_2C-C=O Pal), 3.24–3.42 (m, 2H, H_2C-S Cys), 3.91–4.18 (m, 4H, 2×H_2C-NH), 4.24–4.35 (m, 1H, CH-NH), 4.53–4.90 (m, 4H, 2 CH-NH, H_2C-O), 5.21 (dd, 1H, *J* = 10.1 Hz, *J* = 1.2 Hz, H(H)C=C), 5.32 (dd, 1H, *J* = 17.3 Hz, *J* = 1.2 Hz, H(H)C=C), 5.79–5.98 (m, 1H, HC=H), 6.05 (s br, 1H, NH urethane), 7.54 (s br, 1H, NH), 8.21 (s br, 1H, NH), 8.34 (s br, 1H, NH), 8.42 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 22.0 (CH_3 Ala), 22.7 (2×CH_3 Leu), 22.8 (CH_2 Pal), 24.8 (CH-$(Me)_2$), 25.6 (CH_2 Pal), 28.4 (CH_3 Boc), 29.0–29.9 (12×CH_2 Pal), 31.9 (H_2C-S), 41.2 (CH_2 Leu), 43.2 (H_2C-NH), 44.0 (H_2C-NH), 49.3 (HC-NH), 50.7 (HC-NH), 52.8 (HC-NH), 65.7 (H_2C-O), 79.7 (Cq), 118.5 ((H_2C=C), 131.7 (HC=C), 156.4 (C=O urethane), 168.9 (C=O), 169.4 (C=O), 170.5 (C=O), 172.5 (C=O), 172.7 (C=O), 199.8 (C=O thio ester); Anal. calcd for $C_{40}H_{71}N_5O_9S$ (798.09): C, 60.20; H, 8.96; N, 8.77. Found: C, 60.18; H, 8.76; N, 8.66.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-phenylalanyl-glycyl-L-leucine allyl ester (30b).** The pentapeptide **30b** was obtained as light yellow viscous oil (yield 78%): R_f = 0.15 (ethyl acetate); $[\alpha]_D^{22}$ +1.8° (*c* 2.10, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, 3H, *J* = 6.9 Hz, CH_3 Pal), 0.97 (d, 6H, *J* = 5.2 Hz, CH_3 Leu), 1.22 (s br, 24H, 12×CH_2 Pal), 1.44 (s , 9H, CH_3 Boc), 1.49–.72 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 2.50 (t, 2H, *J* = 7.5 Hz, H_2C-C=O Pal), 2.88–2.99 (m, 1H, H(H)C-Ph), 3.09–3.20 (m, 1H, H(H)C-Ph), 3.20–3.41 (m, 2H, H_2C-S Cys), 3.88–4.12 (m, 4H, 2×H_2C-NH), 4.29–4.35 (m, 1H, CH-NH), 4.50–4.95 (m, 4H, 2 CH-NH + H_2C-O), 5.09 (s br, 1H, NH urethane), 5.19 (dd, 1H, *J* = 10.4 Hz, *J* = 1.3 Hz, H(H)C=C), 5.31 (dd, 1H, *J* = 17.3 Hz, *J* = 1.3 Hz, H(H)C=C), 5.80–5.96 (m, 1H, HC=H), 7.11–7.28 (m, 5H, HC ar.), 7.35 (s br, 1H, NH), 7.98 (s br, 1H, NH), 8.14 (s br, 1H, NH), 8.28 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.2 (CH_3 Pal), 22.7 (2×CH_3 Leu), 22.8 (CH_2Pal), 24.9 (CH-(Me)2), 25.6 (CH_2 Pal), 29.0 (CH_3 Boc), 29.0–30.1 (12×CH_2 Pal), 32.0 (H_2C-S), 39.4 (CH_2 Phe), 41.4 (CH_2 Leu), 43.7 (H_2C-NH), 44.2 (H_2C-NH), 50.9 (HC-NH), 53.2 (HC-NH), 56.3 (CH-NH Phe), 65.7 (H_2C-O), 80.3 (Cq), 118.8 (H_2C=C), 128.8, 130.4, 130.8 (HC=C ar.), 132.1 (HC=C), 136.2 (HC=C ar.), 156.9 (C=O urethane), 168.1 (C=O), 169.9 (C=O), 171.8 (C=O), 172.0 (C=O), 172.2 (C=O), 200.3 (C=O thio ester); Anal. calcd for $C_{46}H_{75}N_5O_9S$ (873.19): C, 63.20; H, 8.64; N, 8.01. Found: C, 63.48; H, 8.79; N, 7.84.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-alanine allyl ester (30c). 30c** was isolated as a viscous oil and in 71% yield: R_f = 0.15 (ethyl acetate); $[\alpha]_D^{22}$ +2.2° (*c* 4.25, CH_2Cl_2); ^1H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, 3H, *J* = 7.1 Hz, CH_3 Pal),

1.22 (s br, 24H, 12×CH_2 Pal), 1.45 (s, 9H, CH_3 Boc), 1.48 (d, 3H, J = 7.2 Hz, CH_3 Ala), 1.52–1.58 (m, 2H, CH_2-Me Pal), 2.02–2.24 (m, 2H, CH_2 Met), 2.09 (s, 3H, CH_3 Met), 2.49–2.62 (m, 4H, H_2C-S Met, H2C-C=O), 3.21–3.42 (m, 2H, H_2C-S Cys), 3.90–4.20 (m, 4H, 2×H_2C-NH), 4.24–4.41 (m, 1H, CH-NH) 4.63 (s br, 2H, H_2C-O), 4.65–4.81 (m, 2H, 2×CH-NH), 5.02 (s br, 1H, NH urethane), 5.22 (d, 1H, J = 10.3 Hz, H(H)C=C), 5.34 (d, 1H, J = 17.0 Hz, H(H)C=C), 5.83–6.02 (m, 1H, HC=H), 6.23 (s br, 1H, NH), 7.83 (s br, 1H, NH), 8.38 (s br, 1H, NH), 8.60 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 15.3 (S-CH_3), 18.4 (CH_3 Ala), 22.7 (CH_2 Pal), 25.6 (CH_2 Pal), 29.0 (CH_3 Boc), 29.3–29.8 (CH_2 Pal), 30.6 (CH_2 Met), 31.9 (H_2C-S Met), 43.2 (H_2C-NH), 44.0 (H_2C-NH), 48.1 (HC-NH), 48.2 (HC-NH), 52.4 (HC-NH), 66.0 (H_2C-O), 79.5 (Cq), 118.6 ((H_2C=C), 131.6 (HC=C), 156.2 (C=O urethane), 168.4 (C=O), 169.6 (C=O), 169.9 (C=O), 171.1 (C=O), 172.6 (C=O), 198.7 (C=O thio ester); Anal. calcd for $C_{39}H_{69}N_5O_9S_2$ (816.13): C, 57.39; H, 8.52; N, 8.58. Found: C, 57.29; H, 8.75; N, 8.54.

Preparation of C-terminally deprotected pentapeptides 31a–c

Peptide carboxylic acids **31** were prepared according to the general procedure given for the synthesis of peptide **21**.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-alanyl-glycyl-L-leucine (31a). 31a** was isolated as a yellow viscous oil (yield 70%): $[\alpha]_D^{22}$ −30° (*c* 1.36, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 0.88 (t, 3H, J = 6.8 Hz, CH_3 Pal), 0.97 (d, 6H, J = 5.4 Hz, CH_3 Leu), 1.21 (s br, 24H, 12(CH_2 Pal), 1.44 (s br, 12H, CH_3 Boc, CH_3 Ala), 1.50–1.72 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 2.47 (t, 2H, J = 7.7 Hz, H_2C-C=O Pal), 3.26–3.41 (m, 2H, H_2C-S Cys), 3.97–4.23 (m, 4H, 2×H_2C-NH), 4.20–4.29 (m, 1H, CH-NH), 4.71–4.90 (m, 2H, 2 CH-NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 21.3 (CH_3 Leu), 22.4 (CH_3 Ala), 22.7 (CH_3Leu), 22.9 (CH_2 Pal), 25.0 (CH-$(Me)_2$), 25.8 (CH_2 Pal), 28.8 (CH_3 Boc), 28.9–30.3 (12×CH_2 Pal), 32.2 (H_2C-S), 41.4 (CH_2Leu), 43.9 (H_2C-NH), 44.4 (H_2C-NH), 50.6 (HC-NH), 50.9 (HC-NH), 53.2 (HC-NH), 80.9 (Cq), 156.0 (C=O urethane), 168.9 (C=O), 169.4 (C=O), 171.5 (C=O), 172.8 (C=O), 173.3 (C=O), 200.1 (C=O thio ester).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-phenylalanyl-glycyl-L-leucine (31b). 31b** was isolated as a viscous oil (yield 87%): $[\alpha]_D^{22}$ −12° (*c* 1.40, MeOH); ^{1}H NMR (MeOD, 250 MHz) δ 0.89 (t, 3H, J = 6. Hz, CH_3 Pal), 0.93 (d, 3H, J = 5.1 Hz, CH_3 Leu), 0.97 (d, 3H, J = 5.4 Hz, CH_3 Leu), 1.23 (s br, 24H, 12×CH_2 Pal), 1.46 (s , 9H, CH_3 Boc), 1.51–1.70 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 2.50 (t, 2H, J = 7.4 Hz, H_2C-C=O Pal), 2.93–3.14 (m, 2H, H_2C-Ph), 3.16–3.39 (m, 2H, H_2C-S Cys), 3.91–4.14 (m, 4H, 2×H_2C-NH), 4.27–4.36 (m, 1H, CH-NH), 4.57–4.78 (m, 2H, 2×CH-NH), 7.09–7.27 (m, 5H, HC ar.); ^{13}C NMR (MeOD, 62.8 MHz) δ 14.3 (CH_3 Pal), 22.2 (CH_3 Leu), 22.7 (CH_3 Leu), 22.9 (CH_2 Pal), 25.0 (CH-$(Me)_2$), 25.8 (CH_2 Pal), 29.1 (CH_3 Boc), 29.2–30.3 (12 CH_2 Pal), 32.1 (H_2C-S), 39.6 (CH_2 Phe), 41.9 (CH_2 Leu), 43.9 (H_2C-NH), 44.3 (H_2C-NH), 51.0 (HC-NH), 53.9 (HC-NH), 56.3 (CH-NH Phe), 79.8 (Cq), 128.6, 130.8, 130.9 (HC=C ar.), 135.9 (HC=C ar.), 157.1 (C=O urethane), 168.9 (C=O), 170.0 (C=O), 170.3 (C=O), 170.7 (C=O), 171.7 (C=O), 199.9 (C=O thio ester).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-alanine (31c). 31c** was isolated as a light yellow solid (yield: 82%): $[\alpha]_D^{22}$ −15° (*c* 0.60, MeOH); mp 170–172 °C; ^{1}H NMR (MeOD, 250 MHz) δ 0.89 (t, 3H, J = 7.1 Hz, CH_3 Pal), 1.22 (s br, 24H, CH_2 Pal), 1.43 (s, 9H, CH_3 Boc), 1.47 (d, 3H, J = 7.2 Hz, CH_3 Ala), 1.50–1.57 (m, 2H, CH_2-Me Pal), 1.99–2.17 (m, 2H, CH_2 Met), 2.07 (s, 3H, CH_3 Met), 2.49–2.58 (m, 4H, H_2C-S Met, H_2C-C=O), 3.20–3.42 (m, 2H, H_2C-S Cys), 3.88–4.15 (m, 4H, 2×H_2C-NH), 4.20–4.32 (m, 1H, CH-NH), 4.69–4.84 (m, 2H, 2×CH-NH); ^{13}C NMR (MeOD, 62.8 MHz) δ 14.4 (CH_3 Pal), 15.3 (S-CH_3), 18.2 (CH_3 Ala), 23.5 (CH_2 Pal), 26.4 (CH_2 Pal), 28.7 (CH_3 Boc), 29.8–30.8 (CH_2 Pal), 31.0 (CH_2 Met), 32.7 (H_2C-S Met), 43.3 (H_2C-NH), 44.6 (H_2C-NH), 50.0 (HC-NH), 54.1 (HC-NH), 54.7 (HC-NH), 80.8 (Cq), 158.1 (C=O urethane), 169.2 (C=O), 170.4 (C=O), 172.1 (C=O), 172.9 (C=O), 173.5 (C=O), 200.8 (C=O thio ester).

Preparation of the hexapeptides (32a,b)

Peptides **32a**,**b** were prepared according to the general procedure given for the synthesis of peptide **22**.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-alanyl-glycyl-L-leucine-L-proline allyl ester (32a). 32a** was isolated as a colorless oil (yield 84%): R_f = 0.65 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −38° (*c* 0.84, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.87 (t, 3H, J = 7.1 Hz, CH_3 Pal), 0.93 (m, 6H, CH_3 Leu), 1.22 (s br, 24H, 12×CH_2 Pal), 1.42 (s, 9H, CH_3 Boc), 1.47 (d, 3H, J = 6.8 Hz, CH_3Ala), 1.58–1.76 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.96–2.21 (m, 4H, 2×CH_2 Pro), 2.54 (t, 2H, J = 7.4 Hz, CH_2-C=O Pal), 3.11–3.32 (m, 2H, CH_2-S Cys), 3.58–3.72 (m, 4H, 2×CH_2-NH), 3.82–4.11 (m, 2H, CH_2 Pro), 4.23–4.32 (m, 1H, CH-NH), 4.43–4.52 (m, 2H, CH-NH, CH-N), 4.61 (d, 2H, J = 5.5 Hz, CH_2-O), 4.72–4.83 (m, 1H, CH-NH), 5.21–5.33 (m, 3H, H_2C=C, NH urethane), 5.80–5.97 (m, 1H, HC=C), 7.03 (s br, 1H, NH), 7.48 (s br, 2H, 2×NH), 7.62 (s br, 1 H, NH), 8.19 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.1 (CH_3 Pal), 21.6 (CH_3 Leu), 22.1 (CH_3 Ala), 22.6 (CH_2 Pal), 23.0 (CH_3 Leu), 23.5 ($CH(Me)_2$), 25.7 (CH_2 Pal), 28.8 (CH_3 Boc), 29.2–30.5 (CH_2 Pal), 30.8 (2×CH_2 Pro), 33.2 (H_2C-S), 40.8 (CH_2 Leu), 43.8 (CH_2-NH), 44.4 (CH_2-NH), 48.2 (CH_2 Pro), 50.8 (HC-NH), 51.9 (HC-NH), 52.7 (HC-NH), 53.4 (HC-N), 54.0 (HC-NH), 66.6 (H_2C-O), 80.9 (Cq), 118.5 (H_2C=C), 131.7 (HC=C), 158.0 (C=O urethane), 169.9 (C=O), 170.1 (C=O), 170.8 (C=O), 171.2 (C=O), 172.1 (C=O), 172.8 (C=O), 200.9 (C=O thio ester).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-phenylalanyl-glycyl-L-leucine-L-proline allyl ester (32b). 32b** was isolated as a colorless oil (yield 83%): R_f = 0.65 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −24 (*c* 3.85, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.91 (t,

3H, CH_3 Pal), 0.93 (d, 3H, $J=5.2$ Hz, CH_3 Leu), 0.99 (d, 3H, $J=5.4$ Hz, CH_3 Leu), 1.22 (s br, 24H, CH_2 Pal), 1.39 (s, 3H, CH_3 Boc), 1.52–1.89 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.93–2.20 (m, 4H 2×CH_2 Pro), 2.53 (t, 2H, $J=7.2$ Hz, H_2C-C=O Pal), 3.02–3.24 (m, 4H, H_2C-Ph, H_2C-S Cys), 3.40–3.72 (m, 4H 2×CH_2-NH), 3.82–4.04 (m, 2H, CH_2 Pro), 4.31–4.50 (m, 3H, 2×CH-NH, CH-N), 4.58 (d, 2H, $J=5.4$ Hz, H_2C-O), 4.70–4.80 (m, 1H, CH-NH), 5.21–5.36 (m, 3H, $H_2C=C$, NH urethane), 5.72–5.98 (m, 1H, HC=C), 7.07–7.32 (m, 8H, HC ar. + 3×NH), 7.40 (s br, 1H NH), 8.31 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.0 (CH_3 Pal), 21.9 (CH_3 Pal), 22.5 (CH_2 Pal), 22.8 (CH_3 Leu), 24.7 ($CH(Me)_2$), 25.8 (CH_2 Pal), 29.0 (CH_3 Boc), 29.1–30.4 (12 CH_2 Pal), 31.1 (CH_2 Pro), 31.3 (CH_2 Pro), 32.9 (H_2C-S), 39.1 (CH_3 Phe), 41.2 (CH_2Leu), 43.2 (CH_2-NH), 44.6 (CH_2-NH), 48.8 (CH_2 Pro), 50.2 (HC-NH), 51.5 (HC-NH), 52.7 (HC-NH), 53.6 (HC-NH), 54.8 (HC-NH), 80.4 (Cq), 118.2 (H_2C=C), 128.0, 130.1, 130.5 (HC ar.), 132.0 (HC=C), 136.2 (HC ar.), 158.2 (C=O urethane), 170.0 (C=O), 170.3 (C=O), 170.9 (C=O), 171.4 (C=O), 172.0 (C=O), 200.6 (C=O thio ester).

Preparation of C-terminally deprotected hexapeptides (33a,b)

Peptide carboxylic acids **33a**,**b** were prepared according to the general procedure given for the synthesis of peptide **23**.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-alanyl-glycyl-L-leucyl-L-proline (33a). 33a** was isolated as a colorless viscous oil (yield 69%): $R_f=0.15$ (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −30° (*c* 0.61, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, 3H, $J=7.1$ Hz, CH_3 Pal), 0.95 (m, 6H, CH_3 Leu), 1.23 (s br, 24H, CH_2 Pal), 1.44 (s, 9H, CH_3 Boc), 1.49 (d, 3H, $J=6.9$ Hz, CH_3 Ala), 1.58–1.76 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.92–2.20 (m, 4H, 2×CH_2 Pro), 2.53 (t, 2H, $J=7.3$ Hz, CH_2-C=O Pal), 3.06–3.26 (m, 2H, CH_2-S Cys), 3.47–3.65 (m, 4H, 2×CH_2-NH), 3.84–4.10 (m, 2H, CH_2 Pro), 4.20–4.30 (m, 1H, CH-NH), 4.4–4.59 (m, 2H, CH-NH + CH-N), 4.69–4.80 (m, 1H, CH-NH), 5.45 (s br, 1H, NH urethane), 7.09 (s br, 1H, NH), 7.65 (s br, 3H, 3×NH), 7.72 (s br, 1 H, NH).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-phenylalanyl-glycyl-L-leucyl-L-proline (33b). 33c** was isolated as a colorless viscous oil (yield 69%): $R_f=0.10$ (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −21 (*c* 1.36, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.90 (t, 3H, CH_3 Pal), 0.97 (m, 6H, CH_3 Leu), 1.23 (s br, 24H, CH_2 Pal), 1.42 (s, 3H, CH_3 Boc), 1.50–1.81 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.90–2.11 (m, 4H 2×CH_2 Pro), 2.51 (t, 2H, $J=7.2$ Hz, H_2C-C=O Pal), 2.99–3.25 (m, 4H, H_2C-Ph, H_2C-S Cys), 3.43–3.70 (m, 4H, 2×CH_2-NH), 3.87–4.08 (m, 2H, CH_2 Pro), 4.22–4.56 (m, 3H, 2×CH-NH + CH-N), 4.73–4.85 (m, 1H, CH-NH), 5.21–5.36 (m, 1H, NH urethane), 7.07–7.32 (m, 8H, HC ar. + 3×NH), 7.40 (s br, 1H NH), 8.31 (s br, 1H, NH).

Preparation of the heptapeptides (35a–c)

Peptide methyl esters **35a**–**b** were prepared according to the general procedure given for the synthesis of peptide **25**.

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-alanyl-glycyl-L-leucine-L-prolyl-(*S*-farnesyl)-L-cysteine methyl ester (35a). 35a** was obtained as a colorless oil (yield 64%): $R_f=0.60$ (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −46° (*c* = 0.43, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.88 (t, 3H, $J=7.0$ Hz, CH_3 Pal), 0.94 (d, 6H, $J=6.5$ Hz, 2×CH_3 Leu), 1.24 (s br, 24H, CH_2 Pal), 1.40–1.45 (s br , 12H, CH_3 Boc, CH_3 Ala), 1.47–1.63 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.61 (s, 6H, 2×CH_3 Far), 1.66 (s, 3H, CH_3 Far), 1.69 (s, 3H, CH_3 Far), 1.96–2.23 (m, 12H, 4×CH_2 Far, 2×CH_2 Pro), 2.52 (t, 2H, $J=7.3$ Hz, H_2C-C=O Pal), 2.55–3.22 (m, 4H, 6H, 2×H_2C-S Far, H_2C-S Pal), 3.61–3.75 (m, 4H, 2×H_2C-NH), 3.73 (s, 3H, H_3C-OC=O), 4.11–4.70 (m, 7H, CH_2 Pro, 5×CH-NH + CH-N), 5.05 (m, 2H, 2×HC=C), 5.16 (m, 1H, HC=C), 5.43 (s br, 1H, NH urethane), 6.98 (s br, 1H, NH), 7.48 (s br, 2H, 2×NH), 7.64 (s br, 1H, NH), 7.78 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.8 MHz) δ 14.2 (CH_3 Pal), 16.5 (2×CH_3 Far), 18.2 (CH_3 Far), 22.0 (CH_3 Leu), 22.2 (CH_3 Ala), 22.7 (CH_2 Pal), 23.5 (CH_3 Leu), 23.8 ($CH(Me)_2$), 25.8 (CH_2 Pal), 28.8 (CH_3 Boc), 29.1–30.2 (14 CH_2 Pal, CH_3 Far, 4×CH_2 Far), 30.9 (2×CH_2 Pro), 40.2 (CH_2 Far), 41.6 (CH_2 Leu), 43.3 (H_2C-NH), 44.8 (H_2C-NH), 48.1 (CH_2 Pro), 51.6 (HC-NH), 51.9 (CH-NH), 52.7 (HC-NH), 53.2 (HC-NH), 54.0 (HC-N), 79.6 (Cq), 118.9 ((HC=C), 121.0 (HC=C), 127.2 (HC=C), 132.8 (Cq Far), 136.0 (Cq Far), 141.1 (Cq Far), 154.1 (C=O urethane), 169.9 (C=O), 170.5 (C=O), 170.8 (C=O), 170.9 (C=O), 171.4 (C=O), 171.8 (C=O), 172.8 (C=O), 200.9 (C=O thio ester).

***N-tert*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-phenylalanyl-glycyl-L-leucine-L-prolyl-(*S*-farnesyl)-L-cysteine methyl ester (35b). 35b** was isolated as a light-yellow oil (yield 53%): $R_f=0.55$ (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −44° (*c* 1.41, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, 3H, $J=7.0$ Hz, CH_3 Pal), 0.96 (m, 6H, 2×CH_3 Leu), 1.26 (s br, 24H, CH_2 Pal), 1.42 (s, 9H, CH_3 Boc), 1.49–1.60 (m, 5H, CH Leu, CH_2 Leu, CH_2-Me Pal), 1.64 (s, 6H, 2×CH_3 Far), 1.67 (s, 3H, CH_3 Far), 1.70 (s, 3H, CH_3 Far), 1.90–2.18 (m, 12H, 4×CH_2 Far, 2×CH_2 Pro), 2.54 (t, 2H, $J=7.5$ Hz, H_2C-C=O Pal), 2.59–3.27 (m, 8H, 2×H_2C-S Far, H_2C-S Pal, CH_2 Phe), 3.74–3.91 (m, 4H, 2×H_2C-NH), 3.79 (s, 3H, H_3C-OC=O), 4.15–4.80 (m, 7H, CH_2 Pro, 5×CH-NH, CH-N), 5.04 (m, 2H, 2×HC=C), 5.18 (m, 1H, HC=C), 5.29 (s br, 1H, NH urethane), 6.98 (s br, 1H, NH), 7.03 (s br, 1H, NH), 7.14–7.31 (m, 6H, HC ar. + NH), 7.44 (s br, 1H, NH), 8.05 (s br, 2H, 2×NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3 Pal), 16.6 (2×CH_3 Far), 18.0 (CH_3 Far), 22.3 (CH_3 Leu), 22.6 (CH_2 Pal), 23.7 (CH_3 Leu), 23.8 ($CH(Me)_2$), 26.0 (CH_2 Pal), 29.1 (CH_3 Boc), 29.0–30.0 (14 CH_2 Pal, CH_3 Far, 4×CH_2 Far), 30.7 (2×CH_2 Pro), 39.2 (CH_2 Phe), 40.4 (CH_2 Far), 41.9 (CH_2 Leu), 43.3 (H_2C-NH), 44.0 (H_2C-NH), 48.1 (CH_2 Pro), 51.8 (HC-NH), 52.1 (CH-NH), 52.3 (HC-NH), 52.9 (HC-NH), 54.6 (HC-N), 81.0 (Cq), 118.9 ((HC=C), 121.0 (HC=C), 127.2 (HC=C), 128.1, 130.2, 131.4 (HC=C ar.), 133.4 (Cq Far), 136.2 (HC=C ar.), 137.1 (Cq Far), 142.6 (Cq Far), 154.0 (C=O urethane), 169.2 (C=O), 169.9 (C=O), 170.2

(C=O), 170.5 (C=O), 171.7 (C=O), 172.1 (C=O), 173.2 (C=O), 201.0 (C=O thio ester).

***N*-*t*-Butyloxycarbonyl-glycyl-(*S*-palmitoyl)-L-cysteyl-L-methionyl-glycyl-L-alanyl-L-prolyl-(*S*-farnesyl)-L-cysteine methyl ester (35c).** 1-Hydroxybenzotriazole (8.7 mg, 6.44 μmol) was added to a suspension of **31c** (25 mg, 3.22 μmol) and L-prolyl-(S-farnesyl)-L-cysteine methyl ester 34[21] (14 mg, 3.22 μmol) in CH_2Cl_2/DMF (10/1, 2 mL). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (9.26 mg, 4.83 μmol) was added after stirring for 5 min at room temperature. The mixture was allowed to stir for 12 h at room temperature, and then was washed with 0.5 N hydrochloric acid (2 mL), with aqueous (5%) sodium bicarbonate (5 mL), and finally with water (2×5 mL). The organic layer was dried over magnesium sulfate, filtered, and the solvents were removed in vacuo. The resulting oil was purified by column chromatography on silica gel (ethyl acetate/MeOH, 9/1 v/v) to afford **35c** (15.3 mg, 41%) as a colorless oil: R_f=0.60 (ethyl acetate/MeOH, 8/1 v/v); $[\alpha]_D^{22}$ −58° (*c* 0.77, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, 3H, *J*=7.0 Hz, CH_3 Pal), 1.22 (s br, 24H, 12×CH_2 Pal), 1.45 (s, 9H, CH_3 Boc), 1.48 (d, 6H, *J*=7.1 Hz, CH_3 Ala), 1.52–1.59 (m, 2H, CH_2-Me Pal), 1.61 (s, 6H, 2×CH_3 Far), 1.66 (s, 3H, CH_3 Far), 1.71 (s, 3H, CH_3 Far), 2.05–2.31 (m, 14H, 4×CH_2 Far, 2×CH_2 Pro, CH_2 Met), 2.11 (s, 3H, CH_3 Met), 2.37–2.51 (m, 4H, H_2C-S Met, H_2C-C=O), 2.84–3.18 (m, 6H, 2 (H_2C-S Far, H_2C-S Pal), 3.71–3.98 (m, 4H, 2×H_2C-NH), 3.77 (s, 3H, H_3C-OC=O), 4.30–4.69 (m, 7H, CH_2 Pro, 5×CH-NH, CH-N), 5.06 (m, 2H, 2×HC=C), 5.14 (m, 1H, HC=C), 5.55 (s br, 1H, NH urethane), 7.09 (s br, 1H, NH), 7.43 (s br, 1H, NH), 7.84 (s br, 2H, 2×NH), 8.18 (s br, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9 (CH_3 Pal), 15.6 (S-CH_3), 16.6 (2×CH_3 Far), 18.1 (CH_3 Far), 18.7 (CH_3 Ala), 22.7 (CH_2 Pal), 23.6 ($CH(Me)_2$), 25.8 (CH_2 Pal), 28.7 (CH_3 Boc), 29.1–30.2 (14 CH_2 Pal, CH_3 Far, 4×CH_3 Far), 30.4–31.0 (CH_2 Met, 2×CH_2 Pro), 32.3 (H_2C-S Met), 40.8 (CH_2 Far), 44.1 (H_2C-NH), 44.9 (H_2C-NH), 48.5 (CH_2 Pro), 50.8 (HC-NH), 52.0 (CH-NH), 52.9 (HC-NH), 53.8 (HC-NH), 54.4 (HC-N), 79.6 (Cq), 118.0 ((HC=C), 121.6 (HC=C), 125.8 (HC=C), 132.1 (Cq Far), 136.5 (Cq Far), 141.2 (Cq Far), 156.3 (C=O urethane), 170.2 (C=O), 170.4 (C=O), 171.3 (C=O), 171.7 (C=O), 171.9 (C=O), 172.5 (C=O), 173.1 (C=O), 201.0 (C=O thio ester).

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