The Synthesis of Acid- and Base-Labile Lipopeptides on Solid Support

Björn Ludolph and Herbert Waldmann*[a]

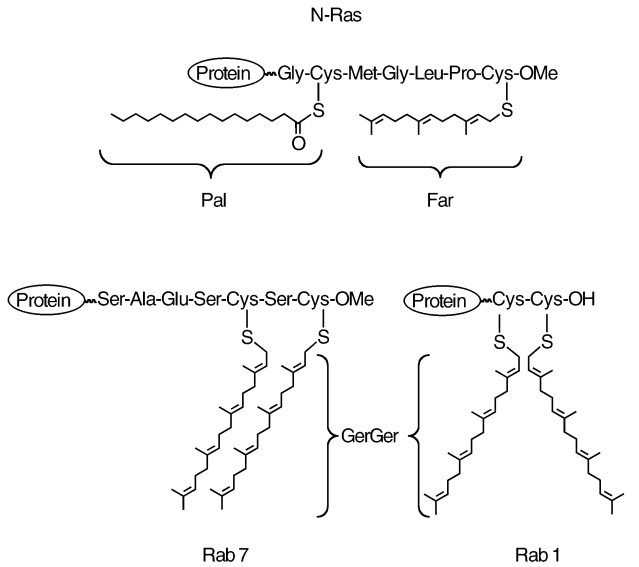
Abstract: Lipidated peptides, including characteristic partial structures of human Ras proteins, were synthesized by means of a new solid-phase technique in 22–68% yield. This technique gives access to farnesylated, palmitoylated, and doubly lipidated peptides as methyl esters or carboxylic acids carrying a fluorescent tag or a maleimide moiety for coupling to proteins. The peptide backbones were built up on the resin by using 9-fluorenylmethoxycarbonyl chemistry together with the oxidatively cleavable hydrazide linker. As a key step, the acid-labile farnesyl and basic-labile palmitoyl lipid groups were introduced onto the resin after the cleavage of appropriate acid- or reduction-sensitive protecting groups from the cysteine residues. Optional introduction of different fluorescent tags or a maleimide group into the peptide was followed by release of the resin-bound target peptide as the methyl ester or carboxylic acid by very mild copper(II)-mediated oxidation in slightly acidic or basic media. This new methodology should substantially facilitate the access to lipidated peptides for the study of important biological phenomena like biological signal transduction, localization, and vesicular transport.

Keywords: lipopeptides · lipoproteins · protecting groups · Ras proteins · solid-phase synthesis

Introduction

Lipidated proteins play important roles in the transduction of extracellular signals across the cell membrane and in numerous intracellular processes like organization of the cytoskeleton, vesicle formation, and targeting.[1] Lipidation of these proteins is a prerequisite for correct biological function. The lipid groups are believed to be involved in protein–protein and protein–lipid interactions and to serve as anchors of the proteins to different membranes. Several different lipid modifications are known. For instance, the Ras proteins embody both farnesyl thioethers and palmitic acid thioesters and terminate in a cysteine methyl ester, whereas the Rab proteins are *S*-geranylgeranylated and carry either a carboxylic acid or a methyl ester at the C terminus (Scheme 1).[1a, 2] Tailormade lipidated peptides representing the characteristic lipid-modified partial structures of their parent proteins are efficient tools for the investigation of these biological processes in molecular detail.[2c, 3]

The synthesis of lipidated peptides is severely complicated by the pronounced acid- and base-sensitivity of the isoprenyl thioethers and the palmitoyl thioesters, respectively (Scheme 2), and requires the application of blocking groups that are cleavable under the mildest conditions.[2a, 3c] Currently differently functionalized and differently lipidated peptides are only available through multistep solution-phase methods employing, for instance, enzyme- or noble-metal-catalyzed transformations as key steps.[3a–c, 4] The solid-phase synthesis of exclusively *S*-palmitoylated[5] or *S*-farnesylated[6] peptides



Scheme 1. Structures of the lipidated C terminus of N-Ras and of the Rab proteins. Far = farnesyl, GerGer = geranylgeranyl, Pal = palmitoyl.

[a] Prof. Dr. H. Waldmann, Dipl.-Chem. B. Ludolph
Max-Planck-Institut für molekulare Physiologie
Abteilung Chemische Biologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
and
Universität Dortmund
Fachbereich 3, Organische Chemie
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de

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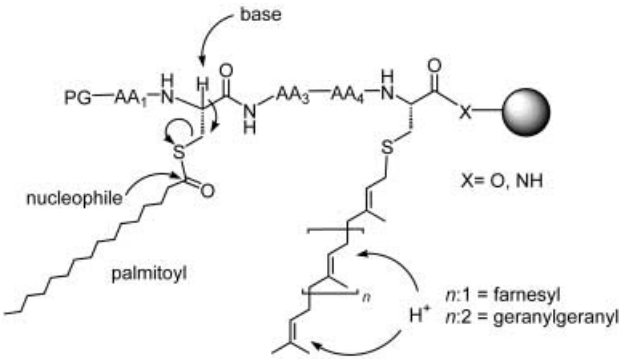


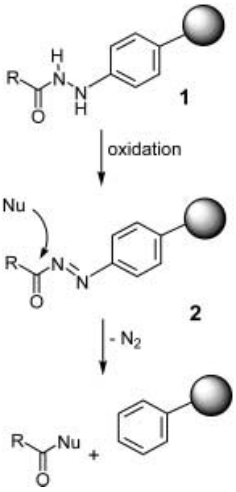
Scheme 2. Acid-labile isoprenyl thioethers and base- and nucleophile-labile palmitoyl thioesters in lipopeptides. AA = amino acid, PG = protecting group.

has been described in a few cases but no solid-phase method that gives access to lipopeptides incorporating isoprenoid groups together with thioesters is available. The major challenge to be met by such a flexible solid-phase technique lies in the development of a set of suitable orthogonally stable protecting groups and a linker to the solid support that allows for selective introduction of the different lipid groups as well as additional functional groups for tracing such peptides in biological systems (for example, a fluorescent group) or for coupling them to expressed proteins (such as a maleimide moiety). Also, the linker should allow for selective elongation of the peptide chain and, finally, release of the desired products into solution as the ester or acid under the mildest conditions and without any harm to the acid- and base-sensitive lipid groups. Herein we report on the development of a solid-phase technique that fulfils the demands raised above. The technique relies on the combined use of the base-labile 9-fluorenylmethoxycarbonyl (Fmoc) group, the Pd^0-sensitive allyloxycarbonyl (Aloc) group, and the very acid-sensitive trityl (Trt) group for protection of the amino groups, the application of acid- and reduction-labile protecting groups for the cysteine side chain, *S*-farnesylation and *S*-palmitoylation of the growing peptide chain on the solid support, and the use of the oxidation-sensitive hydrazide linker. Part of this work was published in a preliminary communication.[7]

Results

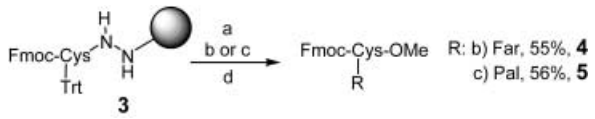
To develop the principle set-up of the method, we first focused on the oxidation-sensitive hydrazide linker **1** for attachment to the solid support. It can be cleaved by oxidation with Cu^{II} or *N*-bromosuccinimide to form an intermediate acyldiazene **2** that is then trapped by an added nucleophile to form an ester or amide bond, thereby releasing the molecule from the solid support (Scheme 3). This linker has been employed before in the synthesis of small peptide esters and amides[8] and cyclic peptides.[9]

Farnesylated and palmitoylated cysteine methyl esters: In an initial series of experiments farnesylated and palmitoylated cysteine methyl esters were synthesized to investigate the compatibility of the hydrazide linker with the demands



Scheme 3. Cleavage of the hydrazide linker. Nu = nucleophile.

imposed by the sensitivity of the lipids. To this end, the Fmoc group of commercially available Fmoc-4-hydrazinobenzoyl resin (NovaGel resin, Novabiochem) was cleaved and *S*-trityl-protected Fmoc-cysteine was coupled to the hydrazide resin to yield immobilized amino acid **3** with a loading of 0.35 mmol g^{-1}. The *S*-trityl group was removed by treatment with 50% TFA in dichloromethane and then *S*-farnesylation was carried out by treatment of the liberated thiol with farnesyl bromide in the presence of a tertiary amine, that is, under basic conditions (Scheme 4). The farnesylated intermediate was cleaved from the resin by treatment with



Scheme 4. Solid-phase synthesis of lipidated cysteine methyl esters **4** and **5**. a) 50% TFA, TES, CH_2Cl_2; b) Far-Br, DIEA, DMF; c) Pal-Cl, HOBt, Et_3N, CH_2Cl_2, DMF; d) $Cu(OAc)_2$, pyridine, O_2, CH_2Cl_2, MeOH, and in the case of (c) additional acetic acid. TFA = trifluoroacetic acid, TES = triethylsilane, Far-Br = *trans,trans*-farnesyl bromide, DIEA = *N,N'*-diisopropylethylamine, DMF = *N,N*-dimethylformamide, Pal-Cl = palmitoyl chloride, HOBt = 1-hydroxybenzotriazole.

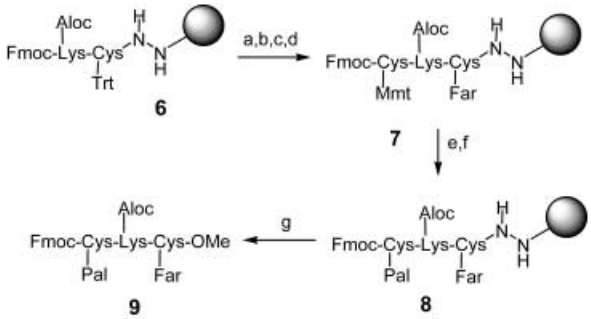
$Cu(OAc)_2$ and oxygen in dichloromethane and in the presence of methanol and pyridine to give ester **4** in 55% yield. This is the first example of peptide farnesylation on a solid support.

In a similar series of experiments, the on-resin palmitoylation and release of an *S*-palmitoylated model compound were investigated. To this end, the trityl group was cleaved from polymer-bound *S*-trityl-protected cysteine **3** and the liberated thiol was converted into the corresponding palmitic acid thioester by treatment with palmitoyl chloride in the presence of HOBt and triethylamine (Scheme 4). The best results were observed when 10 equivalents of both reagents were used for 15 h; application of only four equivalents for 2 h resulted in lower yields and impure product. *S*-palmitoylated cysteine methyl ester **5** was released in a yield of 56% from the solid support by oxidation with $Cu(OAc)_2$ in dichloromethane and in the presence of pyridine and acetic acid. Under these

conditions undesired hydrolysis of the thioester was not observed. Cleavage from the resin with an excess of $Fe(NO_3)_3$ as the oxidant instead of $Cu(OAc)_2$ resulted in lower yield (34%).

These results clearly demonstrate that the hydrazide linker is compatible with the demands posed by acid- and base-labile lipid residues and is therefore, in principle, suitable for lipopeptide synthesis on the solid support. It is, however, intriguing that the overall yield for the reaction sequence is only moderate, a fact that raises the question of whether undesired side reactions occur in the lipidation or cleavage procedures. To investigate the possibility of such processes, a model peptide incorporating a double linker composed of the hydrazide linker and the Wang linker was built up. After releasing the product from the resin by oxidation of the hydrazide, the Wang linker was cleaved under acidic conditions. However a major side product could not be identified by LC-MS. Rather, several minor side products were formed which could not be identified unambiguously.

Lipopeptide synthesis: Based on these results the synthesis of a variety of mono- and double-lipidated peptides was carried out. Commercially available Fmoc-4-hydrazinobenzoyl NovaGel resin (Novabiochem) was employed for Fmoc-protected solid-phase peptide synthesis methods. Typically resins with a loading of 0.35 – 0.43 mmol g^{-1} of the first amino acid were used, as determined by quantification of Fmoc groups on the resin by UV analysis. Peptide **9** was prepared as the first example for the solid-phase synthesis of an acid-labile *S*-isoprenylated and base-labile *S*-palmitoylated peptide (Scheme 5). After assembling dipeptide **6** on the resin the

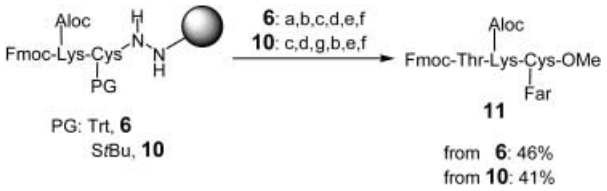


Scheme 5. Synthesis of the farnesylated and palmitoylated tripeptide **9**. a) 50% TFA, TES, CH_2Cl_2; b) Far-Br, DIEA, DMF; c) piperidine, DMF; d) DIC, HOBt, Fmoc-Cys(Mmt)-OH, DMF; e) 1% TFA, TES, CH_2Cl_2; f) Pal-Cl, HOBt, Et_3N, CH_2Cl_2, DMF; g) $Cu(OAc)_2$, acetic acid, pyridine, O_2, CH_2Cl_2, MeOH. DIC = *N*,*N*′-diisopropylcarbodiimide.

trityl group was removed with 50% TFA and the cysteine thiol group farnesylated under basic conditions. The Fmoc group was removed and the peptide elongated to tripeptide **7**. The mercapto group of the second cysteine in the sequence had been protected as a monomethoxytrityl (Mmt) thioether. Cleavage of the methoxytrityl group was achieved with 1% TFA in dichloromethane and in the presence of triethylsilane. The farnesyl group remained unattacked under these mild, weakly acidic conditions.[10] *S*-palmitoylation was then carried out as described above to yield doubly lipidated polymer-bound peptide **8**. Cleavage with Cu^{II}/O_2 under acidic conditions yielded doubly lipidated peptide methyl ester **9** in 25% overall yield. (Overall yields are given with respect to the amount of the first amino acid bound to the resin.) Purification of the final product was readily achieved by simple column chromatography during which the copper was completely removed. An undesired attack on the acid-sensitive farnesyl thioether or the base-labile palmitoyl thioester was not recorded.

However, a loss of peptide from resin could be observed during peptide synthesis. Determination of the Fmoc groups remaining on the resin by the established UV method[11] showed a lower loading of the resin than expected, based on the expected increase of weight of the resin-bound peptide during the course of the synthesis. We assume that under basic conditions oxidation of the hydrazide linker can take place and subsequent cleavage reduces the amount of peptide on resin. This view was supported by double linker experiments similar to the one mentioned above. Also, in a related case it has been shown that a hydrazide-based linker system was not stable against oxygen in the presence of amines and this resulted in hydrazide cleavage as well.[12] To avoid these side reactions, in subsequent experiments all steps were carried out under argon and piperidine was freshly distilled prior to use.

Farnesylated tripeptide **11** was prepared as shown in Scheme 6. Either the acid-sensitive trityl or the orthogonally stable reduction-sensitive *tert*-butyl disulfide (S*t*Bu) was

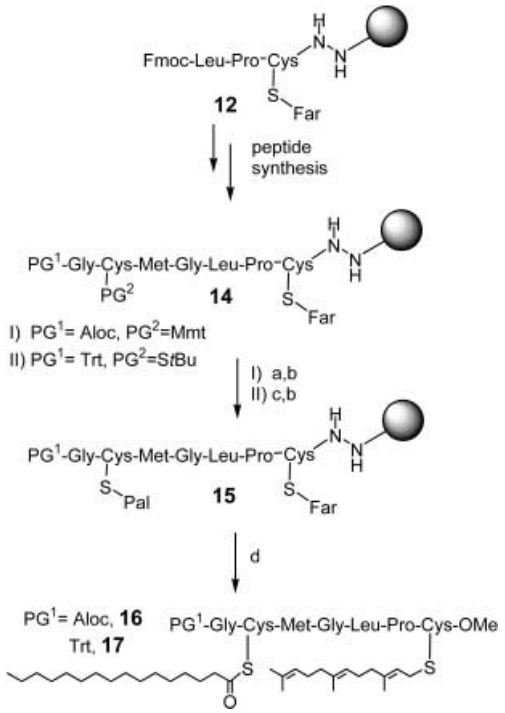


Scheme 6. Synthesis of farnesylated tripeptide **11**. a) 50% TFA, TES, CH_2Cl_2; b) Far-Br, DIEA, DMF; c) piperidine, DMF; d) HBTU, HOBt, DIEA, Fmoc-Thr(Trt)-OH, DMF; e) 1% TFA, TES, CH_2Cl_2; f) $Cu(OAc)_2$, acetic acid, pyridine, O_2, CH_2Cl_2, MeOH; g) PBu_3, H_2O, DMF, CH_2Cl_2. HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide.

employed as the protecting group for the cysteine residue. In the case with the trityl-protected cysteine, **6**, the trityl group was removed at the stage of the dipeptide. The thiol function was farnesylated and this was followed by subsequent elongation with protected threonine. The trityl protecting group of the threonine hydroxy function was cleaved from the readily assembled tripeptide with 1% TFA and the N-terminally Fmoc-protected peptide was liberated with copper acetate in an acetic acid buffer to give target peptide **11** in 46% overall yield. Alternatively, farnesylation at the stage of the tripeptide was achieved after cleaving the *tert*-butyl disulfide used as a masking group for the cysteine thiol function from **10** with PBu_3 and H_2O. Again, as the last step on the resin, the trityl group of the threonine was removed with 1% TFA and the product was obtained after copper-mediated

cleavage in 41% overall yield. Thus, both protecting group strategies were successfully established for lipopeptide synthesis on the solid support.

Having established all the necessary protecting-group and lipidation procedures, we next turned to the synthesis of doubly lipidated heptapeptides **16** and **17**, which mimic the C terminus of N-Ras (Scheme 7). Individual steps were performed as described above for the synthesis of peptide **9**.



Scheme 7. Synthesis of farnesylated and palmitoylated peptides **16** and **17** which resemble the C terminus of N-Ras. a) 1% TFA, TES, CH_2Cl_2; b) PalCl, HOBt, Et_3N, CH_2Cl_2, DMF; c) PBu_3, H_2O, DMF, CH_2Cl_2; d) $Cu(OAc)_2$, acetic acid, pyridine, O_2, CH_2Cl_2, MeOH.

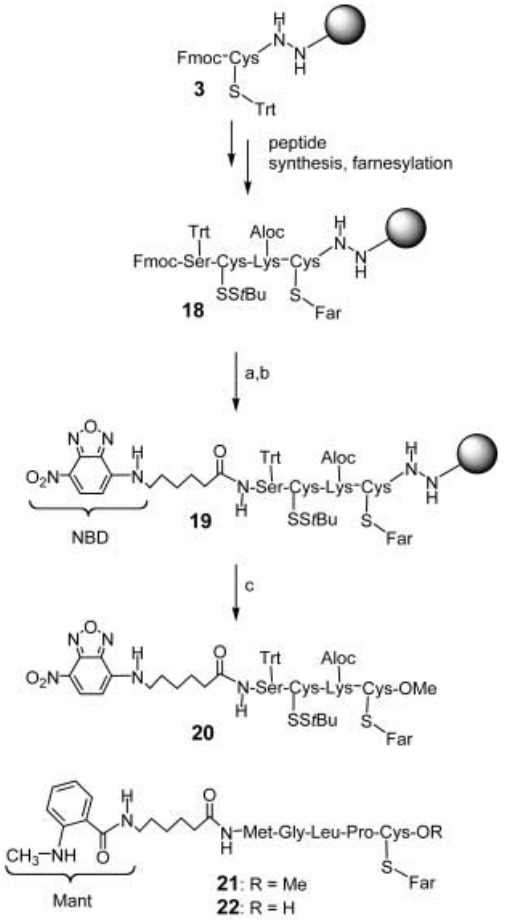
Basic conditions were applied under argon, and according to quantification of Fmoc groups by the UV method after the addition of the second, third, and sixth amino acids no major loss of peptide was observed. Part of the resin was subjected to cleavage at the stage of the farnesylated tripeptide **12** to give Fmoc-Leu-Pro-Cys(Far)-OMe (**13**) in an overall yield of 50%. After elongation to the heptapeptide **14** and deprotection of the second cysteine residue, palmitoylation in dichloromethane, that is, under conditions which had worked very well in case of the tripeptide **9**, was not successful. This might be due to the occurrence of peptide aggregation or secondary structure formation on the solid support. To overcome this problem, DMF was added to the palmitoylation mixture and the reaction proceeded smoothly to yield intermediate **15**. Finally, the desired N-Ras peptide methyl ester **16** was released from the solid support in 42% overall yield. By means of this procedure multimilligram amounts of pure lipopeptide are readily obtained within days, whereas the synthesis of the same compound by means of solution-phase methods[4c, 13, 14] requires weeks.

A different set of protection groups had to be used for the synthesis of the N-terminally trityl-protected N-Ras heptapeptide **17**. As described for **16**, peptide **17** was assembled on the solid support, but the mercapto group of the second cysteine residue in the sequence was protected with *tert*-butyl disulfide as a masking group, which was removed with PBu_3 and H_2O to give the free thiol. After palmitoylation, cleavage was carried out under very weakly acidic conditions with only a slight excess of acetic acid to give **17** in an overall yield of 68%. However, under these conditions partial cleavage (approximately 10%) of the palmitoyl thioester was observed as well. During all steps the very acid-labile N-terminal trityl protecting group present was not affected. We would like to point out that the N-terminal Aloc urethane or trityl protecting group can be removed selectively without any harm to the *S*-palmitoyl thioester or the *S*-farnesyl thioether from lipidated peptides including **16** and **17**;[3d, 14] this method should thereby give access to intermediates which can be equipped with, for example, fluorescent groups or a biotin tag to be used in further biological experiments.

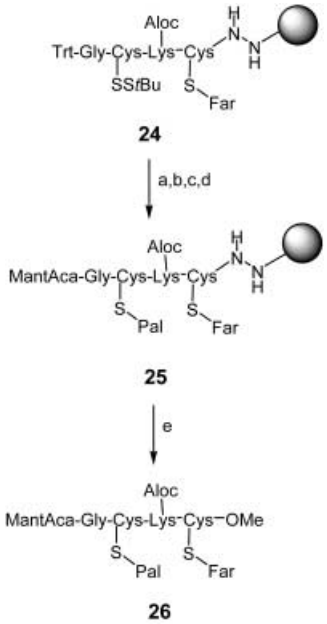
Synthesis of fluorescently labeled and maleimidocaproyl (MIC) labeled lipidated peptides: To investigate whether such differently lipidated and additionally tagged peptides are directly accessible by means of the solid-phase method described above, the synthesis of fluorescently labeled lipopeptides and of a lipopeptide carrying a maleimido group (for coupling to proteins through conjugate addition of cysteine thiol groups[3a,b]) was investigated.

By employing the methodology delineated above *S*-farnesylated tetrapeptide **20** incorporating the fluorescent 4-nitrobenz-2-oxa-1,3-diazole (NBD) group was synthesized (Scheme 8). After assembly of the peptide chain to give intermediate **18** and subsequent N-terminal deprotection, the NBD fluorescent label was coupled to the N terminus. The resulting labeled compound **20** was released from the solid support and was obtained in 47% overall yield and with high purity after simple flash column chromatography. In the course of this synthesis neither the acid-labile trityl protecting group nor the reduction-sensitive NBD label and *tert*-butyl disulfide masking group were attacked. By using the same methodology N-Ras peptide **21** incorporating the fluorescent *N*-methylanthraniloyl (Mant) label was prepared in 49% overall yield. In order to demonstrate that not only can peptide esters be obtained by this solid-phase technique but that lipidated peptides with an unmasked carboxylic acid at the C terminus (as, for instance, are required for Rab-derived peptides; see Scheme 1) are also available, the activated intermediate formed in the oxidative release of the Mant-labeled peptide from the solid support was alternatively trapped with water instead of methanol to give lipopeptide carboxylic acid **22**.

In addition, doubly lipidated peptides carrying a tag for subsequent biological experiments were prepared. To this end, farnesylated tetrapeptide **24** was assembled on the solid support (Scheme 9). It incorporates a reduction-sensitive *tert*-butyl disulfide as a masking group for the cysteine thiol, an acid-labile trityl blocking function, and a lysine with an *N*-Aloc-protected side-chain amino group. This set of protecting groups and the hydrazide linker are orthogonally stable. The *tert*-butyl disulfide group was cleaved by treatment with



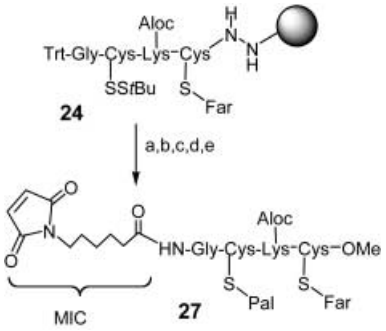
Scheme 8. Solid-phase synthesis of fluorescently labeled and *S*-farnesylated peptides **20**–**22**. a) piperidine, DMF; b) HBTU, HOBt, DIEA, NBDAca-OH (**23**), DMF; c) $Cu(OAc)_2$, pyridine, O_2, CH_2Cl_2, MeOH. NBDAca = *N*-(4-nitrobenz-2-oxa-1,3-diazol-7-yl)aminocaproyl.



Scheme 9. Solid-phase synthesis of Mant-labeled and doubly lipidated peptide **26**. a) PBu_3, H_2O, DMF, CH_2Cl_2; b) Pal-Cl, HOBt, Et_3N, CH_2Cl_2, DMF; c) 1% TFA, TES, CH_2Cl_2; d) HBTU, HOBt, DIEA, MantAca-OH, DMF; e) $Cu(OAc)_2$, acetic acid, pyridine, O_2, CH_2Cl_2, MeOH. MantAca = *N*-methylanthraniloylaminocaproyl.

PBu_3/H_2O and this was followed by palmitoylation of the liberated mercapto group. The N-terminal trityl group was removed from the formed intermediate under weakly acidic conditions and then the Mant label was introduced by coupling with Mant-aminocaproic acid, DIC, and HOBt in the presence of triethylamine to yield immobilized fluorescently labeled lipopeptide **25**. Finally, desired lipopeptide methyl ester **26** was released from the solid support by oxidation with Cu^{II} in methanol and in the presence of pyridine and acetic acid. After flash column chromatography it was obtained in pure form in 29% overall yield for 13 steps on the polymeric carrier.

Finally, we investigated the possibility of introducing a maleinimido group, which is an important linker group for the coupling of chemically synthesized lipopeptides to expressed proteins. For instance, it has been shown that lipopeptides equipped with a maleimido group can react with the mercapto groups of proteins to yield biologically fully functional Ras proteins.[3a, b] With tetrapeptide **24** as the starting material, removal of the *tert*-butyl disulfide group, subsequent palmitoylation of the free thiol, and N-terminal deprotection (removal of the trityl group) were carried out as described above. Commercially available maleimidocaproic acid was coupled to the immobilized peptide with DIC, HOBt, and triethylamine to yield the resin-bound target peptide. Cleavage from the resin by oxidation with Cu^{II} in methanol and in the presence of pyridine and acetic acid furnished the desired MIC-equipped lipopeptide **27** in 22% overall yield after column chromatography (Scheme 10). Figure 1 displays the



Scheme 10. Solid-phase synthesis of MIC-tagged, doubly lipidated peptide **27**. a) PBu_3, H_2O, DMF, CH_2Cl_2; b) Pal-Cl, HOBt, Et_3N, CH_2Cl_2, DMF; c) 1% TFA, TES, CH_2Cl_2; d) HBTU, HOBt, DIEA, MIC-OH, DMF; e) $Cu(OAc)_2$, acetic acid, pyridine, O_2, CH_2Cl_2, MeOH. MIC = maleinimidocaproyl.

HPLC trace of the product. For the peptides discussed above similar traces were obtained after release from the solid support. These findings demonstrate that the method detailed in this paper yields very pure lipidated peptides that require hardly any further purification.

Conclusion

We have developed the first solid-phase method for the synthesis of differently lipidated and additionally labeled peptides. It gives access to farnesylated, palmitoylated, and

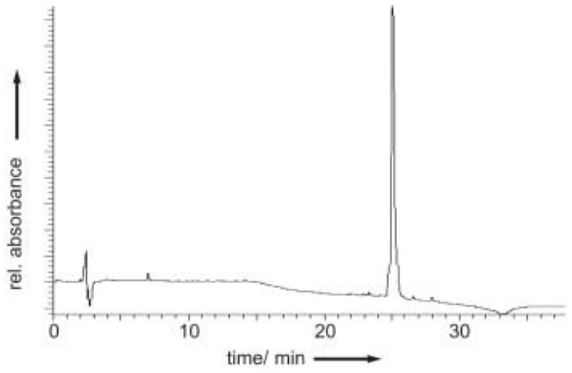


Figure 1. HPLC trace of compound **27**. An RP-C4 column with a water/acetonitrile gradient and detection at 210 nm was employed.

doubly lipidated peptides as methyl esters or carboxylic acids carrying an additional fluorescent group or a maleimido moiety for further biological studies. This new methodology should substantially facilitate the use of lipidated peptides, and the lipidated proteins accessible from them by combination of organic synthesis and molecular biology techniques,[3a, b] in the study of important biological phenomena like biological signal transduction, localization, and vesicular transport.

Experimental Section

General: Unless otherwise noted, reagents and chemicals were obtained from Acros, Chimica, Advanced Chemtech, Aldrich, AppliChem, Avocado, Biosolve, Fluka, Novabiochem, or Senn Chemicals and used without further purification. Dichloromethane and piperidine were refluxed under argon over CaH_2 and freshly distilled prior to use. ^1H and ^{13}C NMR spectroscopic data were recorded on a Varian Mercury 400 or a Bruker DRX500 spectrometer at room temperature. ^1H and ^{13}C NMR spectra were afterwards calibrated to the solvent signals of $CDCl_3$ ($\delta = 7.26$ ppm and 77.16 ppm, respectively). ESI-MS was carried out by using a Agilent 1100 series binary pump together with a reversed-phase HPLC column (Macherey-Nagel) and a Finnigan Thermoquest LCQ. FAB MS measurements were taken with a Jeol SX102A apparatus by using a 3-nitrobenzyl alcohol (3-NBA) matrix. Optical rotations were measured with a Perkin–Elmer Polarimeter 341. Flash chromatography was performed with Merck silica gel 60. TLC was performed with aluminium-backed silica gel 60 F_{254} plates (Merck). MALDI MS was carried out with a Voyager-DE Pro BioSpectrometer from PerSeptive Biosystems by using a 2,5-dihydroxybenzoic acid (DHB) matrix. The yield and scale of the solid-phase reactions are given with respect to the amount of the first amino acid coupled onto the resin and the resin loading was determined by measuring the Fmoc groups remaining on the resin by the established UV method. After cleavage from the resin no major byproducts could be detected by HPLC. However, column chromatography was carried out to remove copper salts.

General conditions for the synthesis of lipopeptides with the phenylhydrazide linker: For all reactions commercially available Fmoc-4-hydrazinobenzoyl NovaGel resin from Novabiochem was used. All reactions were carried out under an argon atmosphere in a 50-mL solid-phase peptide synthesis reactor. Agitation was achieved by bubbling argon gas through the glass sinter or by using an orbital shaker. After liberation of the thiol group of cysteine the subsequent reaction was carried out without any delay. Loading of the resin was determined by the amount of Fmoc groups on the resin. To this end, a small amount of dried resin (5–9 mg) was treated with freshly prepared piperidine/DMF (1:4; 18 mL) for 10 min and then the UV absorption of the solution at 301 nm ($\varepsilon = 7800\ M^{-1}cm^{-1}$) was determined.

Fmoc cleavage: Fmoc cleavage was achieved by using a solution of 50% piperidine in DMF two times for 7 min.

Peptide coupling: Unless stated otherwise all amino acids were coupled by using HBTU/HOBt chemistry. Typically, amino acid (4 equiv) was treated for 2–3 min with HBTU (3.6 equiv), HOBt (4 equiv) and DIEA (8 equiv) in DMF. The solution was added to the resin and agitated for 2 h at room temperature. Cysteine derivatives were coupled by using DIC/HOBt chemistry in order to avoid extensive racemization.[15] Typically amino acid (4 equiv) was treated with DIC (4 equiv) and HOBt (6 equiv) in DMF for 2–3 min and then added to the resin. Reaction times varied from 3–4 h at room temperature.

Cleavage of the Trt group from cysteine: The Trt group was cleaved with 3% TES and 50% TFA in dichloromethane for 1 h. The resin was washed several times with dichloromethane and then with DMF.

Removal of the Trt group from nitrogen atoms/removal of the Mmt group from cysteine: Cleavage was achieved with 1% TFA and 2% TES in dichloromethane. In the case of deprotection of a nitrogen atom the procedure was repeated twice. The resin was washed six times with dichloromethane and then with DMF.

Removal of the *tert*-butyl disulfide group from cysteine: The *tert*-butyl disulfide group was cleaved with PBu_3 (100 equiv) and H_2O (400 equiv) in DMF/dichloromethane (1:1) for 12 h. Typically 6.4 mL of PBu_3 together with 2 mL of H_2O were used in 40 mL of solvent. The resin was washed six times with dichloromethane and then with DMF.

Farnesylation: The farnesyl group was introduced onto the freshly deprotected thiol with farnesyl bromide (5 equiv) and DIEA (12 equiv) in DMF for 4 h. The resin was washed six times with DMF.

Palmitoylation: The palmitoyl group was introduced with palmitoyl chloride (20 equiv), HOBt (20 equiv), and Et_3N (22 equiv) in DMF/dichloromethane (1:3) for 15 h. The resin was washed six times with dichloromethane and then with DMF.

Fmoc-Cys(Far)-OMe (4): Fmoc-4-hydrazinobenzoyl NovaGel resin was deprotected with piperidine, and Fmoc-Cys(Trt)-OH was coupled to the resin by using DIC/HOBt chemistry to give cysteine-bound resin (137 mg) with a loading of 0.35 mmol g^{-1} (0.047 mmol). The Trt group was cleaved and the thiol was farnesylated. The resin was treated with a solution of $Cu(OAc)_2$ (18 mg, 0.1 mmol), pyridine (280 μL, 3.46 mmol), and methanol (1 mL) in dichloromethane (10 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with ethyl acetate/cyclohexane (1:4) as the eluent furnished **4** (14.5 mg, 0.026 mmol, 55%). $R_f = 0.35$ (cyclohexane/ethyl acetate (4:1)); $[\alpha]_D^{20} = -48.4$ ($c = 0.5$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $^3J = 7.4$ Hz, 2H; Fmoc), 7.62–7.60 (m, 2H; Fmoc), 7.40 (t, $^3J = 7.2$ Hz, 2H; Fmoc), 7.32 (t, $^3J = 7.4$ Hz, 2H; Fmoc), 5.59 (d, $^3J = 8.2$ Hz, 1H; NH), 5.21 (t, $^3J = 7.7$ Hz, 1H; C=C*H*–CH_2S), 5.10–5.07 (m, 2H; 2 × C=CH Far), 4.62–4.58 (m, 1H; αCH Cys), 4.35–4.45 (m, 2H; CH_2 Fmoc), 4.22–4.26 (m, 1H; CH Fmoc), 3.78 (s, 3H; OCH_3), 3.21–3.12 (m, 2H; CH_2 Far), 2.85–3.00 (m, 2H; βCH_2 Cys), 2.12–1.95 (m, 8H; CH_2 Far), 1.68 (s, 3H; CH_3 Far), 1.66 (s, 3H; CH_3 Far), 1.60 (s, 6H; CH_3 Far) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 171.4$, 155.8, 143.9, 141.3, 140.2, 135.4, 131.4, 127.8, 127.2, 125.2, 124.4, 123.7, 120.0, 119.6, 67.4, 53.8, 52.8, 47.3, 40.0, 38.9, 33.8, 30.3, 26.9, 26.7, 25.9, 17.9, 16.4, 16.3 ppm; MS (ESI +): *m*/*z* calcd for $C_{34}H_{44}NO_4S$ $[M+H]^+$: 562.3; found: 562.2; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 562.2991; found: 562.3004.

Fmoc-Cys(Pal)-OMe (5): The Trt group was cleaved from resin-bound Fmoc-Cys(Trt) (210 mg, with a loading of 0.38 mmol g^{-1}, 0.080 mmol), and the thiol function was palmitoylated. The resin was treated with a solution of $Cu(OAc)_2$ (6 mg, 0.033 mmol), pyridine (123 μL, 1.52 mmol), acetic acid (175 μL, 3.06 mmol), and MeOH (400 μL, 9.88 mmol) in dry dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with ethyl acetate/cyclohexane (1:2) as the eluent furnished **5** (26.7 mg, 0.045 mmol, 56%). $R_f = 0.56$ (ethyl acetate/cyclohexane (1:2)); $[\alpha]_D^{20} = +18.9$ ($c = 1.04$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $^3J = 7.3$ Hz, 2H; Fmoc), 7.60 (d, $^3J = 7.2$ Hz, 2H; Fmoc), 7.40 (t, $^3J = 7.6$ Hz, 2H; Fmoc), 7.31 (t, $^3J = 7.5$ Hz, 2H; Fmoc), 5.56 (s, 1H; NH), 4.61 (m, 1H; αCH Cys), 4.38 (d, $^3J = 7.0$ Hz, 2H; CH_2O Fmoc), 4.24 (t, $^3J = 7.0$ Hz, 1H; CH Fmoc), 3.77 (s, 3H; OMe), 3.39 (m, 2H; βCH_2 Cys), 2.57 (t, $^3J = 7.4$ Hz, 2H; αCH_2 Pal), 1.65 (m, 2H; βCH_2 Pal), 1.25 (m, 24H; Pal), 0.88 (t, $^3J = 6.8$ Hz, 3H; ωCH_3 Pal) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 199.0$, 170.9, 155.9, 144.0,

141.6, 127.9, 127.3, 125.4, 120.2, 67.5, 53.9, 53.0, 47.3, 44.3, 32.2–29.1 (several signals), 25.8, 22.9, 14.2 ppm; MS (ESI +): *m/z* calcd for $C_{35}H_{50}NO_5S$ $[M+H]^+$: 596.3; found: 596.1; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 596.3410; found: 596.3419.

Fmoc-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe (9): Fmoc-Lys(Aloc)-OH was coupled to resin-bound Fmoc-Cys(Trt) (488 mg, with a loading of 0.38 mmol g^{-1}, 0.185 mmol) by using HBTU/HOBt chemistry. The Trt group was cleaved and the free thiol was farnesylated. The Fmoc group was removed and Fmoc-Cys(Mmt)-OH was coupled onto the dipeptide by using DIC/HOBt chemistry. The Mmt group was cleaved and the free thiol was palmitoylated. The resin was treated with a solution of $Cu(OAc)_2$ (7.9 mg, 0.043 mmol), pyridine (140 μL, 1.74 mmol), acetic acid (200 μL, 3.50 mmol), and MeOH (441 μL, 10.9 mmol) in dry dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with ethyl acetate/cyclohexane (1:1) as the eluent furnished **9** (51.8 mg, 0.047 mmol, 25%). R_f = 0.45 (cyclohexane/ethyl acetate (1:1)); $[\alpha]_D^{20} = +46.8$ ($c = 2.06$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.68$ (d, $^3J = 7.6$ Hz, 2H; Fmoc), 7.52 (d, $^3J = 9.6$ Hz, 2H; Fmoc), 7.32 (t, $^3J = 7.4$ Hz, 2H; Fmoc), 7.23 (t, $^3J = 7.4$ Hz, 2H; Fmoc), 6.94 (s, 1H; NH), 6.82 (s, 1H; NH), 5.83–5.75 (m, 2H; NH, C*H*=CH$_2$), 5.19 (d, $^3J = 18$ Hz, 1H; CH=C*H*$_{2a}$), 5.15–4.97 (m, 5H; CH=C*H*$_{2b}$,C=C*H*−CH$_2$S, 2 × C=CH Far, NH), 4.66 (m, 1H; αCH Cys), 4.50–4.24 (m, 6H; Fmoc, αCH Lys, O−CH$_2$ allyl), 4.14 (m, 1H; αCH Cys), 3.67 (s, 3H; OMe), 3.30–2.75 (m, 8H; 2 × βCH$_2$ Cys, βCH$_2$ Lys, αCH$_2$ Far), 2.51 (t, $^3J = 7.4$ Hz, 2H; αCH$_2$ Pal), 2.1–1.81 (m, 8H; CH$_2$ Far), 1.68–1.59 (m, 2H; βCH$_2$ Pal), 1.60 (s, 3H; CH$_3$ Far), 1.58 (s, 3H; CH$_3$ Far), 1.52 (s, 6H; 2 × CH$_3$ Far), 1.46–1.43 (m, 2H; δCH$_2$ Lys), 1.36–1.33 (m, 2H; γCH$_2$ Lys), 1.18 (s, 24H; Pal), 0.81 (t, $^3J = 7.0$ Hz, 3H; ωCH$_3$ Pal) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 200.6$, 171.3, 171.2, 170.3, 156.7, 144.0, 141.5, 140.4, 135.6, 133.3, 131.5, 128.0, 127.3, 125.4, 124.5, 124.0, 120.2, 119.7, 117.7, 67.8, 65.7, 55.8, 53.3, 52.9, 52.0, 47.3, 44.3, 40.5, 39.9, 33.1–22.3 (several signals), 17.9, 16.4, 16.2, 14.3 ppm; MS (ESI +): *m/z* calcd for $C_{63}H_{94}N_4O_9S_2$ $[M+H]^+$: 1115.7; found: 1115.3; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 1115.7; $[M+Na]$: 1137.6; found: 1115.4, 1137.4.

Fmoc-Thr-Lys(Aloc)-Cys(Far)-OMe (11): Fmoc-Lys(Aloc)-OH was coupled to resin-bound Fmoc-Cys(Trt) (332 mg, with a loading of 0.37 mmol g^{-1}, 0.123 mmol) by using HBTU/HOBt chemistry. The Trt group was cleaved and the free thiol was farnesylated. The Fmoc protection group was removed and Fmoc-Thr(Trt)-OH was attached; this was followed by cleavage of the Trt protecting group of the hydroxy moiety. The resin was treated with a solution of $Cu(OAc)_2$ (10 mg, 0.055 mmol), pyridine (280 μL, 3.47 mmol), acetic acid (409 μL, 7.15 mmol), and MeOH (1 mL, 24.7 mmol) in dry dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (20:1) furnished **11** (49.6 mg, 0.057 mmol, 46%). The route with the *tert*-butyl disulfide protecting group was analogous except that farnesylation was carried out at the stage of the tripeptide. R_f = 0.4 (dichloromethane/methanol (20:1)). $[\alpha]_D^{20} = -20.7$ ($c = 0.99$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $^3J = 7.3$ Hz, 2H; Fmoc), 7.60 (d, $^3J = 7.2$ Hz, 2H; Fmoc), 7.40 (t, $^3J = 7.6$ Hz, 2H; Fmoc), 7.31 (t, $^3J = 7.5$ Hz, 2H; Fmoc), 7.05 (s, 1H; NH), 6.99 (s, 1H; NH), 5.89–5.87 (m, NH, 2H; C*H*=CH$_2$ allyl), 5.27 (d, $^3J = 18$ Hz, 1H; CH=C*H*$_{2a}$ allyl), 5.20–5.17 (m, 2H; CH=C*H*$_{2b}$ allyl, C=C*H*−CH$_2$S), 5.10–5.07 (m, 2H; C=CH Far), 4.98 (s, 1H; NH), 4.73–4.72 (m, 1H; αCH Cys), 4.54–4.21 (m, 8H; Fmoc, 2 × αCH, βCH Thr, O−CH$_2$ allyl), 3.75 (s, 3H; OMe), 3.19–3.10 (m, 4H; αCH$_2$ Far, CH$_2$ εLys), 2.97–2.92 (m, 1H; βCH$_2$ Cys), 2.85–2.80 (m, 1H; βCH$_2$ Cys), 2.10–1.91 (m, 8H; 4 × CH$_2$ Far), 1.68 (s, 3H; CH$_3$ Far), 1.66 (s, 3H; CH$_3$ Far), 1.59 (s, 6H; 2 × CH$_3$ Far), 1.51–1.36 (m, 4H; δCH$_2$, γCH$_2$ Lys), 1.19 (d, $^3J = 6.3$ Hz, 3H; CH$_3$ Thr) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.6$, 171.4, 171.1, 156.9, 143.9, 141.5, 140.4, 135.6, 133.2, 131.6, 128.0, 127.3, 125.3, 124.5, 123.9, 120.2, 119.6, 117.9, 67.5, 65.8, 59.1, 53.6, 53.4, 52.9, 52.0, 47.3, 40.4, 40.0, 33.2, 31.4, 30.0, 29.5, 27.0, 26.7, 25.9, 22.4, 18.7, 17.9, 16.4, 16.2 ppm; MS (ESI +): *m/z* calcd for $C_{48}H_{67}N_4O_9S$ $[M+H]^+$: 875.5; $[M+Na]^+$: 897.5; found: 875.2, 897.4; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 875.4629; found: 875.4617.

Fmoc-Leu-Pro-Cys(Far)-OMe (13): The Fmoc group was cleaved from resin-bound Fmoc-Cys(Trt) (119 mg, with a loading of 0.35 mmol g^{-1}, 0.0042 mmol) and Fmoc-Pro-OH was coupled. The Trt group was removed and the dipeptide was farnesylated; this was followed by deprotection and coupling of Fmoc-Leu-OH to give the resin-bound tripeptide **12**. The resin was treated with a solution of $Cu(OAc)_2$ (6.7 mg, 0.037 mmol), pyridine (119 μL, 1.47 mmol), acetic acid (170 μL, 2.97 mmol), and MeOH (374 μL, 9.2 mmol) in dichloromethane (15 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with ethyl acetate/cyclohexane (1:1) as the eluent furnished **13** (15.8 mg, 0.021 mmol, 50%). R_f = 0.4 (cyclohexane/ethyl acetate (1:1)); $[\alpha]_D^{20} = -102.4$ ($c = 0.41$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (d, $^3J = 7.6$ Hz, 2H; Fmoc), 7.59 (m, 2H; Fmoc), 7.39 (t, $^3J = 7.4$ Hz, 2H; Fmoc), 7.31 (t, $^3J = 7.4$ Hz, 2H; Fmoc), 5.48 (d, $^3J = 9.0$ Hz, 1H; NH), 5.19, (t, $^3J = 7.6$ Hz, 1H; C=C*H*−CH$_2$S Far), 5.08 (m, 2H; 2 × C=CH Far), 4.70–4.65 (m, 1H; αCH), 4.60–4.56 (m, 1H; αCH), 4.37–4.34 (m, 2H; CH$_2$ Fmoc), 4.22–4.19 (m, 1H; CH Fmoc), 3.50–3.80 (m, 5H; OMe, CH$_2$ Pro), 3.18–3.08 (m, 2H; CH$_2$ Far), 2.94–2.97 (m, 1H; βCH$_{2a}$ Cys), 2.80–2.75 (m, 1H; βCH$_{2b}$ Cys), 2.20–1.10 (m, 27H; γCH Leu, βCH$_2$ Leu, 4 × CH$_2$ Far, 2 × CH$_2$ Pro, 4 × CH$_3$ Far), 1.01–0.94 (m, 6H; 2 × δCH$_3$ Leu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 173.2$, 171.3, 171.0, 156.5, 144.1, 141.5, 140.2, 135.6, 131.5, 129.7, 127.9, 127.3, 125.4, 124.5, 124.0, 120.2, 119.8, 67.3, 60.0, 52.7, 52.0, 51.1, 47.4, 42.7, 42.4, 39.9, 33.5, 30.0, 27.4, 27.0, 26.7, 25.9, 25.2, 24.9, 23.7, 21.8, 17.9, 16.4, 16.2 ppm; MS (ESI +): *m/z* calcd for $C_{45}H_{62}N_3O_6S$ $[M+H]^+$: 772.4; found: 772.3; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 772.4359; found: 772.4387.

Aloc-Gly-Cys(Pal)-Met-Gly-Leu-Pro-Cys(Far)-OMe (16): Resin-bound tripeptide Fmoc-Leu-Pro-Cys(Far) **12** was synthesized from resin-bound Fmoc-Cys(Trt) (302 mg, with a loading of 0.35 mmol g^{-1}, 0.106 mol) as described above. The Fmoc group was cleaved and this was followed by subsequent HBTU/HOBt-mediated coupling with Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Cys(Mmt)-OH, and Aloc-Gly-OH. The Mmt group was removed and the thiol was palmitoylated. The resin was treated with a solution of $Cu(OAc)_2$ (20 mg, 0.11 mmol), pyridine (280 μL, 3.47 mmol), acetic acid (400 μL, 6.99 mmol), and MeOH (882 μL, 21.7 mmol) in dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (20:1) as the eluent furnished **16** (53.3 mg, 0.044 mmol, 42%). R_f = 0.2 (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -52.8$ ($c = 0.74$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.96$ (s, 1H; NH), 7.60 (s, 1H; NH), 7.51 (s, 1H; NH), 7.34 (s, 1H; NH), 7.05 (s, 1H; NH), 5.88–5.84 (s, 1H; C*H*=CH$_2$ allyl), 5.31 (d, $^3J = 17.4$ Hz, 1H; CH=CH$_{2a}$ allyl), 5.21 (d, $^3J = 10.4$ Hz, 1H; CH=CH$_{2b}$ allyl), 5.19–5.16 (m, 1H; C=C*H*−CH$_2$S), 5.08 (m, 2H; 2 × C = CH Far), 4.8–4.36 (m, 11H; 5 × αCH, O−CH$_2$ allyl, 2 × CH$_2$ Gly), 3.5–4.0 (m, 7H; CH$_2$ Pro, OMe, βCH$_2$ Cys), 3.36–2.76 (m, 4H; CH$_2$ Far, βCH$_2$ Cys), 2.54 (m, 4H; γCH$_2$ Met, αCH$_2$ Pal), 2.3–1.8 (m, 17H; 4 × CH$_2$ Far, 2 × CH$_2$ Pro, γCH$_2$ Met, SCH$_3$), 1.70–1.30 (m, 17H; γCH Leu, βCH Leu, 4 × CH$_3$ Far, βCH$_2$ Pal), 0.90–1.20 (m, 24H; Pal), 0.88–0.70 (m, 9H; ωCH$_3$ Pal, 2 × δCH$_3$ Leu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 200.5$, 173.2, 172.6, 171.6, 171.2, 170.5, 169.6, 196.0, 157.4, 140.3, 135.6, 132.8, 131.5, 124.5, 123.9, 119.8, 118.1, 66.3, 60.1, 53.9, 53.0, 52.7, 52.0, 49.3, 47.6, 45.0, 44.3, 43.4, 42.0, 41.2, 33.3–24.0 (several signals), 17.9, 16.4, 16.2, 16.0, 14.3 ppm; MS (ESI +): *m/z* calcd for $C_{62}H_{106}N_7O_{11}S_3$ $[M+H]^+$: 1220.7; found: 1220.5; MS (MALDI, DHB): calcd for $[M+Na]^+$: 1242.7; $[M+K]^+$: 1258.8; found: 1243.1, 1259.0.

Trt-Gly-Cys(Pal)-Met-Gly-Leu-Pro-Cys(Far)-OMe (17): Fmoc-Pro-OH and Fmoc-Leu-OH were coupled to resin-bound Fmoc-Cys(Trt) (127 mg, with a loading of 0.34 mmol g^{-1}, 0.043 mmol). The Trt group was cleaved and the thiol farnesylated to give **12**. The peptide was elongated with Fmoc-Gly-OH, Fmoc-Met-OH, Fmoc-Cys(S*t*Bu)-OH, and Trt-Gly-OH by using Fmoc chemistry. The *tert*-butyl disulfide group was cleaved and the thiol was palmitoylated. The resin was treated with a solution of $Cu(OAc)_2$ (18 mg, 0.10 mmol), pyridine (280 μL, 3.47 mmol), acetic acid (228 μL, 3.99 mmol), and MeOH (1 mL, 24.7 mmol) in dry dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (20:1) as the eluent furnished **17** (40.6 mg, 0.029 mmol, 68%). R_f = 0.35 (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -36.1$ ($c = 0.76$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.4–7.1$ (m, 15H; Trt), 5.10–4.95 (m, 3H; 3 × C=CH Far), 4.8–4.1 (m, 8H; 4 × αCH, 2 × αCH$_2$ Gly), 3.6–3.3 (m, 7H; CH$_2$ Pro, OMe, βCH$_2$ Cys), 3.3–2.7 (m, 4H; CH$_2$ Far, βCH$_2$ Cys), 2.4–2.6 (m, 4H; γCH$_2$ Met, αCH$_2$ Pal), 2.2–1.6 (m, 17H; 4 ×

CH_2 Far, $2\times CH_2$ Pro, γCH_2 Met, SCH_3), 1.6–1.3 (m, 17H; γCH Leu, βCH Leu, $4\times CH_3$ Far, βCH_2 Pal), 1.2–0.95 (m, 24H; Pal), 0.9–0.6 (m, 9H; ωCH_3 Pal, $2\times\delta CH_3$ Leu) ppm; MS (ESI +): *m/z* calcd for $C_{77}H_{116}N_7O_9S_3$ $[M+H]^+$: 1378.8; $[M+Na]^+$: 1400.8; found: 1378.8, 1400.7; MS (FAB, 3-NBA): calcd for $[M+Na]^+$: 1400.8; found: 1400.1.

***N*-(4-Nitrobenz-2-oxa-1,3-diazol-7-yl)aminocaproic acid (23)**: DIEA (850 µl, 5.00 mmol) and slowly, over an hour, solid aminocaproic acid (315 mg, 2.40 mmol) were added to a solution of NBD-Cl (401 mg, 2.01 mol) in methanol (20 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the remaining material was purified by chromatography on silica with ethyl acetate/MeOH (10:1) as the eluent. The substance was recrystallized from methanol/water to give **23** (339 mg, 1.15 mmol, 57 %). ^{1}H NMR (400 MHz, DMSO): $\delta = 9.4$ (s, 1H; COOH), 8.41 (d, $^3J = 8.8$ Hz, 1H; CH NBD), 6.32 (d, $^3J = 8.8$ Hz, 1H; CH NBD), 3.41 (m, 2H; CH_2 Aca), 2.18 (t, $^3J = 7.4$ Hz, 2H; CH_2 Aca), 1.65 (m, 2H; CH_2 Aca), 1.53 (m, 2H; CH_2 Aca), 1.35 (m, 2H; CH_2 Aca) ppm; ^{13}C NMR (100 MHz, DMSO): $\delta = 175.0$, 145.7, 145.0, 144.7, 138.5, 121.1, 99.6, 49.2, 34.2 28.0, 26.6, 24.8; MS (ESI +): *m/z* calcd for $C_{12}H_{15}N_4O_5$ $[M+H]^+$: 295.10; found: 295.2; MS (ESI −): *m/z* calcd for $[M-H]^-$: 293.10; found: 293.2; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 295.1042; found: 295.1042.

NBDAca-Ser(Trt)-Cys(S*t*Bu)-Lys(Aloc)-Cys(Far)-OMe (20): The Fmoc group was cleaved from resin-bound Fmoc-Cys(Trt) (269 mg, with a loading of 0.38 mmol g^{-1}, 0.102 mmol) and Fmoc-Lys(Aloc)-OH was coupled. The Trt group was removed and the liberated thiol group was farnesylated. Subsequently, Fmoc-Cys(S*t*Bu)-OH, Fmoc-Ser(Trt)-OH, and NBDAca-OH were coupled. The resin was treated with a solution of $Cu(OAc)_2$ (7.8 mg, 0.043 mmol), pyridine (140 µL, 1.74 mmol), and MeOH (441 µL, 10.9 mmol) in dichloromethane (20 mL) for 80 min under oxygen. The resin was filtered and the crude mixture was directly subjected to flash column chromatography with ethyl acetate/cyclohexane (1:5) and 2 % Et_3N as the eluent to give **20** (64.5 mg, 0.048 mmol, 47 %). $R_f = 0.5$ (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -55.1$ ($c = 0.118$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.36$ (d, $^3J = 8.6$ Hz, 1H; CH NBD), 7.45–7.06 (m, 19H; Trt, $4\times$ NH), 6.28 (m, 1H; CH NBD), 6.07 (s, 1H; NH), 5.79 (m, 1H; C*H*=CH_2 allyl), 5.34 (s, 1H; NH), 5.19–5.00 (m, 7H; CH=CH_2 allyl, C=C*H*−CH_2S, $2\times$C=CH Far, $2\times\alpha$CH), 4.72–4.59 (m, 2H; $2\times\alpha$CH), 4.45–4.35 (m, 4H; $2\times\alpha$CH, O−CH_2 allyl), 3.80–3.55 (m, 4H; OMe, βCH_{2a} Ser), 3.4–3.6 (m, 2H; αCH_2 Aca), 3.35–3.04 (m, 7H; βCH_{2b} Ser, αCH_2 Far, βCH_2 Cys, εCH_2 Lys), 2.85–2.84 (m, 1H; βCH_2 Cys), 2.76–2.71 (m, 1H; βCH_2 Cys), 2.18–2.16 (m, 2H; εCH_2 Aca), 1.99–1.86 (m, 8H; $4\times CH_2$ Far), 1.71–1.30 (m, 24H; βCH_2 Lys, γCH_2 Lys, δCH_2 Lys, $4\times CH_3$ Far, βCH_2 Aca, γCH_2 Aca, δCH_2 Aca), 1.21 (s, 9H; S*t*Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.0$, 171.5, 171.4, 170.6, 170.0, 156.8, 144.5, 144.3, 143.6, 143.4, 140.2, 136.8, 135.6, 133.2, 131.5, 128.7, 128.4, 127.7, 124.5, 123.9, 119.8, 117.6, 98.7, 87.7, 65.7, 63.0, 54.9, 54.9, 53.9, 52.8, 52.1, 49.1, 41.6, 40.7, 39.9, 36.0, 33.1, 31.2, 30.0, 29.8, 29.6, 28.2, 27.0, 26.7, 26.6, 25.9, 24.9, 24.8, 23.0, 17.9, 16.4, 16.2 ppm; MS (ESI −): *m/z* calcd for $C_{70}H_{92}N_9O_{12}S_3$ $[M-H]^-$: 1346.6; $[M+Cl]^-$: 1382.6; found: 1346.5, 1382.5.

MantAca-Met-Gly-Leu-Pro-Cys(Far)-OMe (21): The Fmoc group was cleaved from resin-bound Fmoc-Cys(Trt) (517 mg, with a loading of 0.38 mmol g^{-1}, 0.196 mmol) and Fmoc-Pro-OH was coupled. The Trt group was removed and the liberated thiol group was farnesylated. Subsequently Fmoc-Leu-OH, Fmoc-Gly-OH, Fmoc-Met-OH, and MantAca-OH were coupled by using standard HBTU/HOBt chemistry. The resin was treated with a solution of $Cu(OAc)_2$ (7.8 mg, 0.043 mmol), pyridine (140 µL, 1.74 mmol), and MeOH (441 µL, 10.9 mmol) in dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (20:1) as the eluent furnished **21** (95 mg, 0.097 mmol, 49 %). $R_f = 0.3$ (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -37.6$ ($c = 2.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.81$ (m, 1H; NH), 7.53 (m, 1H; NH), 7.43–7.39 (m, 2H; NH), 7.34 (d, $^3J = 7.8$ Hz, 1H; Mant), 7.22 (t, $^3J = 7.8$ Hz, 1H; Mant), 6.87–6.85 (m, 1H; NH), 6.73 (m, 1H; NH), 6.59 (d, $^3J = 8.4$ Hz, 1H; Mant), 6.50 (t, $^3J = 7.0$ Hz, 1H; Mant), 5.12 (t, $^3J = 7.4$ Hz, 1H; C=C*H*−CH_2S), 5.06–5.03 (m, 2H; $2\times$CH=C Far), 4.79–4.76 (m, 1H; αCH), 4.65–4.58 (m, 4H; $4\times\alpha$CH), 4.00 (m, 2H; αCH Gly), 3.76–3.73 (m, 1H; CH_{2a} Pro), 3.67 (s, 3H; OMe), 3.61–3.57 (m, 1H; CH_{2b} Pro), 3.33–3.29 (m, 2H; αCH_2 Aca), 3.16–3.11 (m, 1H; αCH_{2a} Far), 3.04–2.98 (m, 1H; αCH_{2b} Far), 2.85–2.89 (m, 1H; βCH_{2a} Cys), 2.79 (d, $^3J = 4.9$ Hz, 3H; NCH_3), 2.67–2.64 (m, 3H; βCH_{2b} Cys, γCH_2 Met), 2.48–2.45 (m, 2H; εCH_2 Aca), 2.20–2.16 (m, 3H; CH_2 Pro, CH_{2a} Pro), 2.10–1.90 (m, 15H; βCH_2 Met, CH_3S Met, γCH, Leu, $4\times CH_2$ Far, CH_{2b} Pro), 1.63–1.30 (m, 20H; βCH_2 Aca, γCH_2 Aca, δCH_2 Aca, $4\times CH_3$ Far, βCH_2 Leu), 0.90–0.87 (m, 6H; $2\times CH_3$ Leu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 173.5$, 172.4, 172.0, 171.5, 171.3, 170.1, 168.6, 150.6, 140.1, 135.5, 132.7, 131.4, 127.6, 124.4, 123.8, 119.7, 115.6, 114.5, 111.0, 60.0, 52.5, 52.3, 52.1, 49.1, 47.5, 43.1, 41.7, 39.8, 39.5, 36.1, 32.9, 31.8, 30.1, 29.8, 29.7, 29.2, 28.4, 26.8, 26.6, 26.5, 25.8, 25.2, 24.9, 24.7, 23.4, 22.0, 17.8, 16.2, 16.1, 15.4 ppm; MS (ESI +): *m/z* calcd for $C_{51}H_{82}N_7O_8S_2$ $[M+H]^+$: 984.6; found: 984.6; MS (FAB, 3-NBA): calcd for $[M+H]^+$: 984.5666; found: 984.5728.

MantAca-Met-Gly-Leu-Pro-Cys(Far)-OH (22): Synthesis was carried out as described above for compound **21** with resin-bound Fmoc-Cys(Trt) (239 mg,with a loading of 0.39 mmol g^{-1}, 0.093 mmol). MantAca-OH was coupled by using DIC/HOBt chemistry. The resin was treated with a solution of $Cu(OAc)_2$ (10 mg, 0.055 mmol), pyridine (280 µL, 3.47 mmol), and acetic acid (400 µL, 6.99 mmol) in THF (10 mL) containing H_2O (350 µL, 19.4 mmol) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. A solution of the crude product in dichloromethane was absorbed onto trisaminoethyl HL resin (200 mg, Novabiochem) and shaken for 2 h. The resin was washed with dichloromethane/H_2O then the product was carefully eluted with THF/H_2O (20:1) followed by THF/H_2O/AcOH (20:1:0.5) and dried in vacuo to give **22** (28 mg, 0.029 mmol, 31 %). $[\alpha]_D^{20} = -31.5$ ($c = 1.3$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$ (d, $^3J = 7.8$ Hz, 1H; Mant), 7.29–7.26 (m, 1H; Mant), 7.06 (m, 1H; NH), 6.75 (m, 1H; NH), 6.62 (d, $^3J = 8.4$ Hz, 1H; Mant), 6.54 (t, $^3J = 7.2$ Hz, 1H; Mant), 5.15 (m, 1H; C=C*H*−CH_2S), 5.07 (m, 2H; $2\times$ CH=C, Far), 4.76–4.31 (m, 5H; $5\times\alpha$CH), 4.0–4.38 (m, 2H; $4\times\alpha$CH Gly), 3.72 (m, 1H; CH_{2a} Pro), 3.56 (m, 1H; CH_{2b} Pro), 3.33 (m, 2H; αCH_2 Aca), 3.31–2.91 (m, 4H; αCH_2 Far, βCH_2 Cys), 2.86–2.66 (m, 5H; NCH_3, γCH_2 Met), 2.50 (m, 2H; εCH_2 Aca), 2.26–1.86 (m, 15H; $2\times CH_2$ Pro, CH_3S Met, $4\times CH_2$ Far), 1.66–1.57 (m, 21H; βCH_2 Aca, γCH_2 Aca, δCH_2 Aca, $4\times CH_3$ Far, βCH_2 Leu, γCH Leu), 0.87 (m, 6H; $2\times CH_3$ Leu) ppm; MS (ESI +): *m/z* calcd for $C_{50}H_{80}N_7O_8S_2$ $[M+H]^+$: 970.5; found: 970.5; MS (ESI −): *m/z* calcd for $[M-H]^-$: 968.5; found: 968.5; MS (FAB, 3-NBA): calcd for $[M+Na]^+$: 992.5329; found: 992.5376.

MantAca-Gly-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe (26): The Fmoc group was cleaved from resin-bound Fmoc-Cys(Trt) (249 mg, with a loading of 0.43 mmol g^{-1}, 0.107 mmol) and Fmoc-Lys(Aloc)-OH was coupled. The Trt group was removed and the liberated thiol group was farnesylated. Subsequently Fmoc-Cys(S*t*Bu)-OH and Trt-Gly were coupled by using standard HBTU/HOBt chemistry. The *tert*-butyl disulfide group was cleaved and the liberated thiol was palmitoylated. The Trt group was cleaved from the glycine residue and MantAca-OH was coupled (111 mg, 0.42 mmol) by treatment with DIC (61.9 µL, 0.40 mmol), HOBt (97 mg, 0.64 mmol), and Et_3N (15 µL, 0.11 mmol) for 4 h. The resin was treated with a solution of $Cu(OAc)_2$ (18 mg, 0.10 mmol), pyridine (280 µL, 3.47 mmol), acetic acid (400 µL, 6.99 mmol), and MeOH (882 µL, 21.7 mmol) in dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (30:1) furnished **26** (36.7 mg, 0.031 mmol, 29 %). $R_f = 0.3$ (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -19.3$ ($c = 1.6$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.35$ (d, $^3J = 6.6$ Hz, 1H; Mant), 7.28 (d, $^3J = 8.6$ Hz, 1H; Mant), 6.62 (m, 1H; Mant), 6.56–6.52 (m, 1H; Mant), 5.88–5.81 (m, 1H; C*H*=CH_2 allyl), 5.25–5.07 (m, 5H; CH=CH_2 allyl, C=C*H*−CH_2S, $2\times$C=CH Far), 4.80–4.39 (m, 5H; $3\times\alpha$CH, OCH_2 allyl), 4.08–3.96 (m, 2H; αCH_2 Gly), 3.72 (s, 3H; OMe), 3.37–3.09 (m, 8H; βCH_2 Cys, εCH_2 Lys, αCH_2 Far, αCH_2 Aca), 2.92–2.80 (m, 2H; βCH_2 Cys), 2.82 (s, 3H; CH_3 Mant), 2.54 (t, $^3J = 7.1$ Hz, 2H; αCH_2 Pal), 2.31–2.27 (m, 2H; εCH_2 Aca), 2.07–1.95 (m, 8H; $4\times CH_2$ Far), 1.90–1.37 (m, 24H; βCH_2 Aca, γCH_2 Aca, δCH_2 Aca, $4\times CH_3$ Far, βCH_2 Lys, δCH_2 Lys, γCH_2 Lys), 1.23 (s, 24H; Pal), 0.87 (t, $^3J = 6.6$ Hz, 3H; ωCH_3 Pal) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 200.4$, 174.2, 171.8, 171.4, 170.2, 169.8, 169.6, 156.7, 150.7, 140.3, 135.6, 133.3, 132.8, 131.5, 127.6, 124.7, 123.9, 119.7, 117.6, 115.7, 114.6, 111.2, 65.6, 53.2, 52.7, 52.5, 44.3, 43.9, 40.8, 39.9, 39.6, 35.9, 32.8–22.5 (several signals), 17.9, 16.4, 16.2, 14.3 ppm; MS (ESI +): *m/z* calcd for $C_{64}H_{106}N_7O_{10}S_2$ $[M+H]^+$: 1196.7; found: 1196.7.

MIC-Gly-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe (27): The synthesis was carried out as described above for compound **26** with resin-bound Fmoc-Cys(Trt) (128 mg, 0.055 mmol). After cleavage of the Trt group MIC-OH was coupled by using MIC-OH (73.9 mg, 0.35 mmol), DIC (31 μL, 0.20 mmol), HOBt (46 mg, 0.30 mmol), and Et_3N (34 μL, 0.20 mmol) for 3 h. The resin was treated with a solution of $Cu(OAc)_2$ (19 mg, 0.10 mmol), pyridine (140 μL, 1.74 mmol), acetic acid (200 μL, 3.50 mmol), and MeOH (441 μL, 10.9 mmol) in dichloromethane (20 mL) for 2 h under oxygen. The resin was filtered off, the solvent was evaporated under reduced pressure, and the crude reaction mixture was dried in vacuo. Flash column chromatography with dichloromethane/methanol (20:1) furnished **27** (13.9 mg, 0.012 mmol, 22 %). $R_f = 0.3$ (dichloromethane/MeOH (20:1)); $[\alpha]_D^{20} = -12.2$ ($c = 0.85$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$ (s, 1H; NH), 7.18 (s, 1H; NH), 6.67 (s, 2H; CH=CH MIC), 6.52 (s, 1H; NH), 5.90 (m, 1H; C*H*=CH_2 allyl), 5.29–5.08 (m, 5H; CH=CH_2, C=C*H*–CH_2S, 2 × C=CH Far), 4.68–4.52 (m, 5H; 3 × αCH, OCH_2 allyl), 4.00–3.85 (m, 2H; αCH_2 Gly), 3.73 (s, 3H; OMe), 3.52–3.48 (m, 2H; NCH_2 MIC), 3.27–3.09 (m, 6H; βCH_2 Cys, εCH_2 Lys, αCH_2 Far), 2.95–2.78 (m, 2H; βCH_2 Cys), 2.57 (t, $^3J = 6.0$ Hz, 2H; αCH_2 Pal), 2.28–2.24 (m, 2H; CH_2 MIC), 2.1–1.90 (m, 8H; 4 × CH_2 Far), 1.8–1.1 (m, 48H; βCH_2 Aca, γCH_2 Aca, δCH_2 Aca, βCH_2 Lys, δCH_2 Lys, γCH_2 Lys, 4 × CH_3 Far, Pal), 0.87–0.85 (m, 3H; ωCH_3 Pal) ppm; MS (ESI +): *m*/*z* calcd for $C_{60}H_{99}N_6O_{11}S_2$ $[M+H]^+$: 1143.7; found: 1143.6; MS (MALDI, DHB): calcd for $[M+Na]^+$: 1165.7; found: 1165.9.

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