Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 1, pp 75–83, 1997

PII: S0968-0896(96)00213-1
0968-0896/97 $17.00 + 0.00

Synthesis of Characteristic Lipopeptides of the Human *N-Ras* Protein and their Evaluation as Possible Inhibitors of Protein Farnesyl Transferase

Paul Stöber, Michael Schelhaas, Edgar Nägele, Patrizia Hagenbuch, János Rétey* and Herbert Waldmann*

Universität Karlsruhe, Institut für Organische Chemie, Richard-Willstätter-Allee 2, D-76128 Karlsruhe, Germany

Abstract—Lipopeptides carrying a farnesyl thioether or a palmitic acid thioester and a farnesyl thioether were prepared from *S*-farnesyl cysteine methyl ester by *N*-terminal extension of the peptide chain employing the base labile Fmoc blocking group or the palladium(0) sensitive Aloc urethane. By means of this technique a lipohexapeptide representing the completely functionalized, i.e. palmitoylated and farnesylated *C*-terminus of the human N-*Ras* protein, was prepared. If acid labile blocking functions like the Boc group were used, upon deprotection an undesired addition of the acid to the double bonds of the farnesyl residue occurred. Therefore, acid labile blocking groups should not be employed in the synthesis of farnesylated lipopeptides. The lipopeptide methyl esters which carry only a farnesyl group do not inhibit protein farnesyl transferase, whereas palmitoylated peptides are weak inhibitors of this enzyme. Copyright © 1997 Elsevier Science Ltd

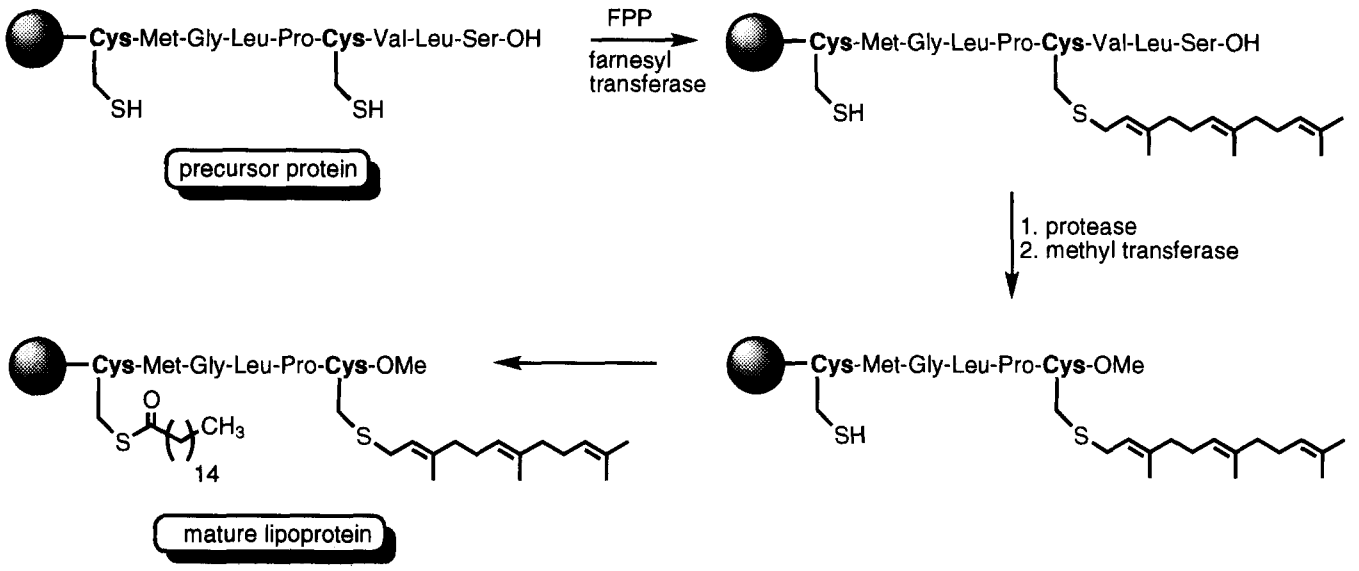
Introduction

The *Ras* proteins are a class of plasma-membrane associated lipoproteins which serve as molecular switches in mitogenic signalling across the cell membrane. Their ability to transfer growth signals from transmembrane receptors to intracellular kinases puts the *Ras* proteins in the heart of the central signalling pathways that control normal proliferation of cells.[1] If the regulation of cell growth is, however, disturbed or interrupted an uncontrolled proliferation may occur, which ultimately may result in the establishment of cancer. Indeed, point mutations in the *ras* oncogenes which lock *Ras* in its active GTP-bound state lead to malignant transformation and are among the most frequently identified mutations in human cancers.[2]

In order to perform both their normal and oncogenic functions, the *Ras* proteins must be membrane associated[3] and the covalent attachment of lipid residues to their peptide chains is critical to this localization. In these post-translational processing events a conserved CAAX amino acid sequence (C is cysteine, A is an aliphatic amino acid, X is serine or methionine) is recognized and farnesylated at the cysteine by the enzyme protein farnesyl transferase (PFT; Scheme 1). After subsequent proteolytic removal of the three *C*-terminal amino acids and conversion of the resulting farnesylated *C*-terminal cysteine to the respective methyl ester the peptide chain is further modified by introduction of palmitic acid thioesters. The initial farnesylation is a prerequisite for all subsequent modifications.

The discovery that the presence of covalently bound lipids is crucial to oncogenic *Ras* signalling has led to the idea that preventing the attachment of these lipids might disrupt the aberrant growth signals of oncogenic *Ras* proteins. In particular, the inhibition of protein farnesyl transferase was identified as a promising target and intense efforts of several research groups resulted in the development of numerous highly potent peptidic and nonpeptidic inhibitors of this enzyme.[4,5] Some of these inhibitors prevented the growth of malignant human lung cells[6] or human pancreatic adenocarcinoma cells[7] transplanted into nude mice and reduced established mammary and salivary tumors in transgenic mice.[8]

In general, inhibitors of *Ras* farnesylation were designed exclusively on the basis of the substrate specificity of PFT, i.e. analogues of the lipid or of the peptide sequence to which the lipid is transferred were employed. In a few cases also bisubstrate inhibitors were investigated.[9–12] However, in enzyme-mediated reaction cascades the phenomenon of 'feedback-inhibition' is common,[13] i.e. the inhibition of a given biocatalyst by the product of an enzymatic transformation which occurs several steps downstream in the entire biosynthetic pathway. For the development of possible inhibitors of *Ras* processing this opens up the possibility that farnesyl transferase might be inhibited by lipopeptide methyl esters carrying a farnesyl thioether or both an *S*-farnesyl thioether and an *S*-palmitoyl thioester, but this question has not been addressed so far. To investigate the relevance of this notion peptide conjugates are needed which represent the characteristic and correctly lipidated *C*-terminal amino acid sequences of the *Ras* proteins. However, due to the acid-sensitivity of the farnesyl group and the base lability of the palmitic acid thioester, their



FPP = farnesyl pyrophosphate

Scheme 1.

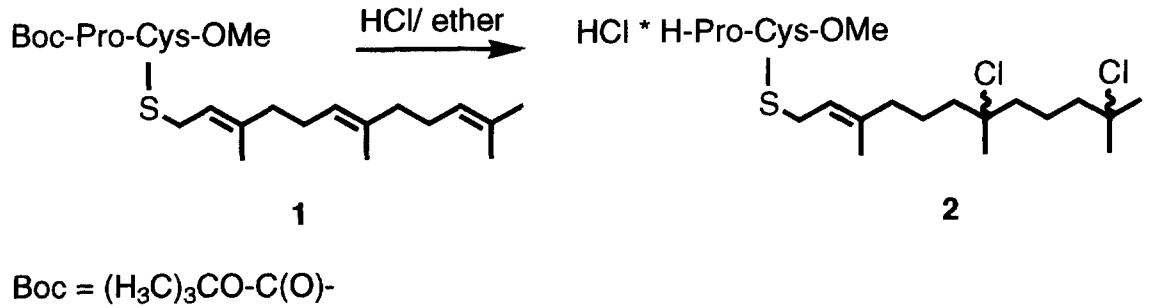
construction poses a formidable challenge to the organic chemist.

Recently, we have reported on the development of enzymatic protecting group techniques for the synthesis of such sensitive peptide conjugates which allowed for the first construction of the acid and base labile *S*-palmitoylated and *S*-farnesylated *C*-terminal lipohexapeptide of the human *N-Ras* protein and analogues thereof.[14,15] In this paper we describe an alternative nonenzymatic access to such lipopeptides and their investigation as possible inhibitors of protein farnesyl transferase.

Synthesis of the lipidated peptides

Our initial attempts to build up the characteristic *C*-terminal lipohexapeptide sequence of the human *N-Ras* protein[16] focused an a stepwise *N*-terminal extension of the peptide chain from *S*-farnesylated cysteine methyl ester. In explorating experiments it was found that the classical acid labile Boc protecting group could be removed from *S*-allylated cysteine peptides without any attack of the acid on the double bond of the allyl group. Therefore, the Boc protected *S*-farnesylated dipeptide **1** was built up and treated with an ethereal solution of HCl to remove the *N*-terminal blocking group (Scheme 2). However, under these conditions the acid also added to the central and the terminal olefin present in the farnesyl ether to give **2**. This undesired side reaction could not be overcome by switching to different Brönstedt or Lewis acids. These findings demonstrate that acid labile protecting groups like the Boc urethane cannot be employed in the construction of farnesylated lipopeptides.

This problem can be overcome by switching to the enzyme-labile *p*-acetoxybenzyloxycarbonyl group which is removed by means of an enzyme-initiated fragmentation process under almost neutral conditions.[14] However, since the farnesyl thioethers are not base sensitive, the desired selective *N*-terminal deprotection of farnesylated peptides can also be achieved by removal of the base labile Fmoc group[17] or by palladium(0) mediated cleavage of the allyloxycarbonyl urethane.[18] It should be noted, however, that basic deprotection conditions cannot be applied if lipopep-



Boc = $(H_3C)_3CO$-C(O)-

Scheme 2.

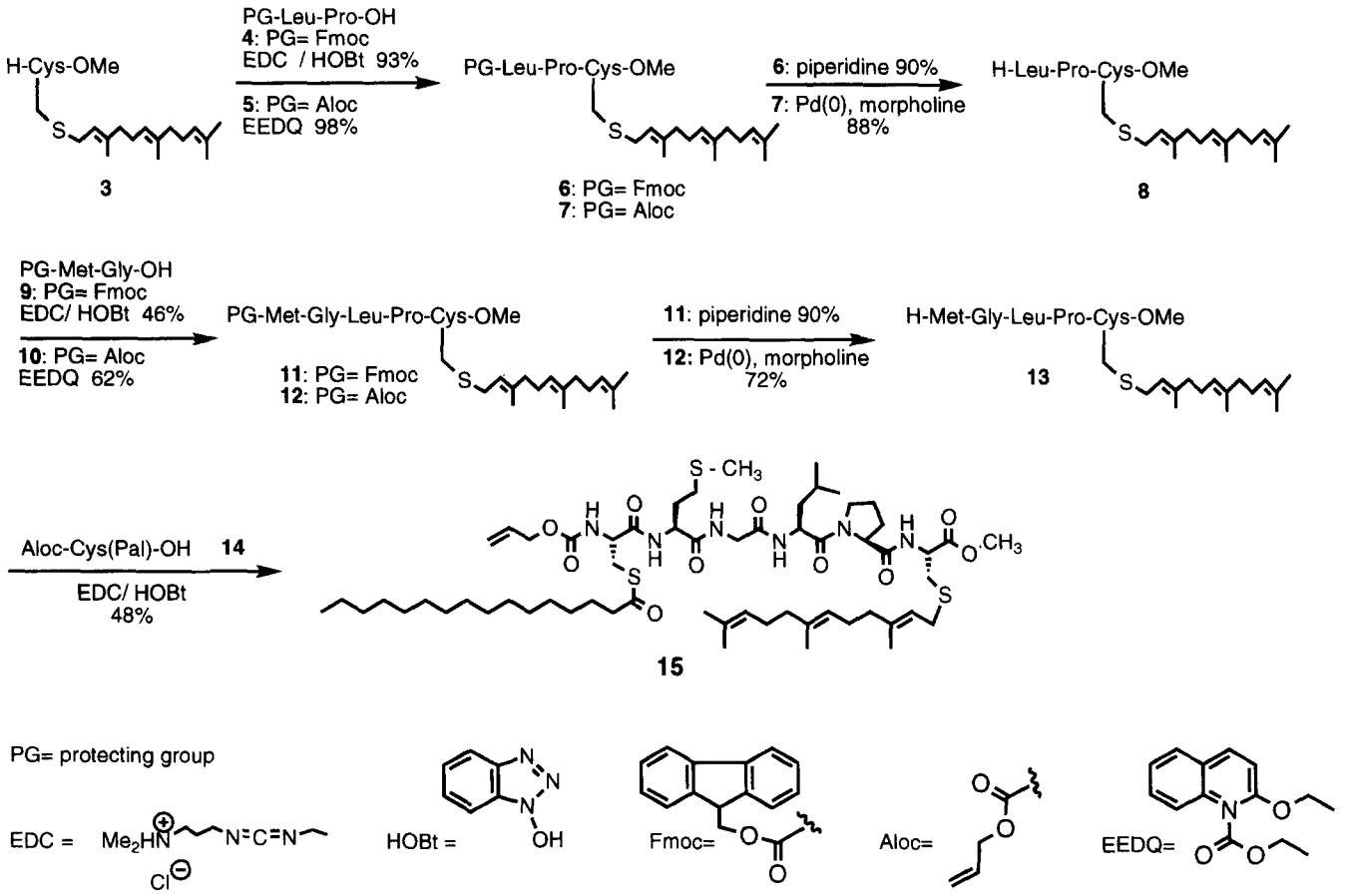
tides have to be unmasked which carry a sensitive palmitic acid thioester.[15]

To build up different farnesylated lipopeptides and the characteristic farnesylated and palmitoylated *C*-terminal hexapeptide fragment **15** of the human *N-Ras* protein, *S*-farnesyl cysteine methyl ester **3**[19] was coupled with the Fmoc- or Aloc protected dipeptides **4** or **5** (Scheme 3) by using a carbodiimide and *N*-hydroxybenzotriazole as activating reagents. The dipeptides **4** and **5** were readily obtained by selective removal of the *C*-terminal blocking function from the respective *tert*-butyl esters (see the Experimental). From the Fmoc-masked peptide **6** the *N*-terminal urethane group was cleaved off by treatment with piperidine in high yield. Similarly, from the Aloc-protected analogue **7** the blocking function could be removed by allyl transfer to morpholine as accepting nucleophile in the presence of catalytic amounts of a palladium(0) catalyst. The selectively unmasked lipotripeptide **8** obtained thereby was then condensed with the dipeptide **9** or **10** to give the fully protected lipopentapeptides **11** and **12** carrying once more an *N*-terminal Fmoc or an Aloc group, respectively. Dipeptide **9** was also obtained from the respective *tert*-butyl ester by acidic cleavage of the ester group, **10** was synthesized by lipase-mediated hydrolysis of the dipeptide heptyl ester (see Experimental). From the acid- but not base-sensitive peptide conjugates **11** and **12** the urethane blocking functions again were removed by treatment with base to eliminate the fluorenylmethyl moiety and by means of a palladium(0)-catalysed allyl transfer, respectively. In both cases the desired *N*-terminally unmasked lipopentapeptide **13** was isolated in high yield. Finally, the synthesis was completed by coupling of **13** with the *S*-palmitoylated cysteine derivative **14** to give the palmitoylated and farnesylated lipohexapeptide **15**, which represents the correctly functionalized *C*-terminus of the human *N-Ras* protein.

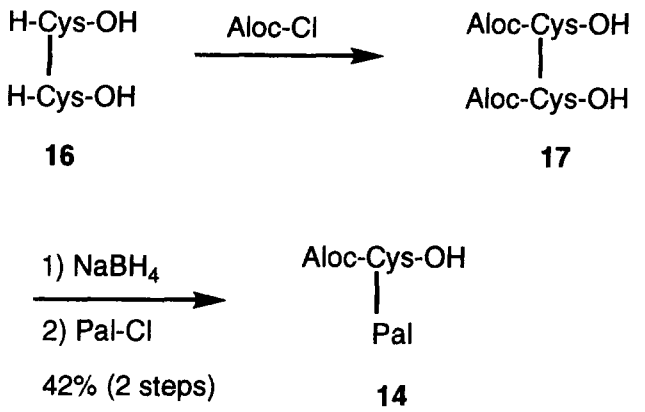
The protected cysteinyl thioester **14** required for the final elongation of the peptide chain was built up from cystine (Scheme 4). To this end the amino acid **16** was first masked as the bis-Aloc urethane **17**. After reductive opening of the disulfide bridge the liberated mercapto groups were acylated by means of palmitoyl chloride to give the thioester **14** in moderate yield.

Farnesyl transferase inhibition

The possible inhibitory effect of the above mentioned lipopeptides and the related compound **18**[15] on protein farnesyl transferase was determined by essentially following the procedure described by F. Tamanoi et al.[20] employing yeast farnesyl transferase which is structurally and functionally very similar to the mammalian enzymes.



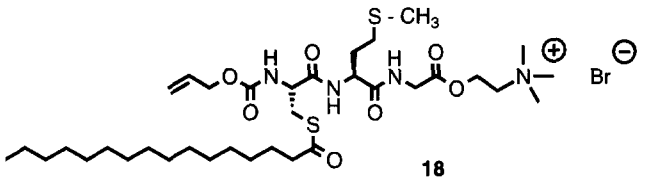
Scheme 3.



Scheme 4.

In brief, the farnesyl transferase was obtained by simultaneous overexpression of the DPR 1 and the RAM 2 genes in *S. cerevisiae* and used as crude extract. As substrate a glutathione *S*-transferase fused to the *C*-terminal 12 amino acids of yeast *Ras 2* protein was employed (GST-CIIS).[20] In the assays the transfer of ^{3}H farnesyl from ^{3}H farnesylpyrophosphate to the cysteine of the *C*-terminal CIIS sequence was measured in the presence of varying amounts of synthetic lipopeptide (for details see the Experimental).

Table 1 summarizes the results of the inhibition assays. The lipopeptides which carry only a farnesyl group do not inhibit protein farnesyl transferase up to millimolar concentrations (Table 1, entries 1–4). However, if a palmitoyl residue is present IC_{50} values in the low millimolar range were determined (Table 1, entries 5 and 6). These results are in agreement with the finding that farnesyltransferase modifies a *C*-terminal CAAX sequence of its natural substrates[16] and that efficient inhibitors must mimic this motif.



Scheme 5.

Table 1. Inhibition of protein farnesyl transferase by synthetic *Ras* lipopeptides

Entry	No.	Substrate	IC_{50} (mM)
1	**6**	Fmoc-Leu-Pro-Cys(Far)OMe	no inhibition
2	**8**	H-Leu-Pro-Cys(Far)-OMe	no inhibition
3	**11**	Fmoc-Met-Gly-Leu-Pro-Cys(Far)-OMe	no inhibition
4	**13**	H-Met-Gly-Leu-Pro-Cys-(Far)-OMe	no inhibition
5	**18**	Aloc-Cys(Pal)-Met-Gly-OCho	4.0
6	**15**	Aloc-Cys(Pal)-Met-Gly-Leu-Pro-Cys(Far)-OMe	5.5

On the other hand, the observation that upon incorporation of a *S*-palmitoyl residue at the *N*-terminus of the model peptides inhibition (albeit only weak[21]) of the enzyme occurs is new and might open novel ways to intercept this biocatalyst. In addition it raises the question whether palmitoylation of the native *Ras* proteins only serves as additional anchoring of these lipoproteins in the cell membrane or whether it also is involved in the regulation of the activity of protein farnesyl transferase.

Experimental

^{1}H NMR spectra were recorded on a Bruker AM 400 spectrometer, high-resolution MS and FABMS were recorded on a Finnigan MAT 90 machine. Specific rotations were measured with a Perkin–Elmer polarimeter 241. Flash column chromatography was carried out on columns packed with Baker silica gel (30–60 μm). TLC on Kieselgel $60F_{254}$ aluminium sheets (Merck, Darmstadt, Germany) was used to monitor the reactions and to ascertain the purity of the products.

***S*-Farnesylcysteine methyl ester 3 (H-Cys(Far)OMe)[19].** To a solution of 0.5 g (2.9 mmol) of HCysOMe, HCl and 1.0 mL (7.25 mmol) of NEt_3 in DMF at 0 °C was added 0.84 g (0.80 mL, 2.9 mmol) of farnesylbromide. After 30 min the precipitate was filtered off, the solution was poured into 50 mL water and the pH was adjusted to 9 with Na_2CO_3. The aqueous phase was extracted eight times with 30 mL of hexane. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **1** was isolated as a light yellow oil from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (50:50 [v:v]) as eluent. Yield: 0.74 g (75%); $[\alpha]^{20}_D$ −12.8° (*c* 2.5, methanol); R_f=0.25 (hexane:ethyl acetate 50:50 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 1.6 (s, 6H, 2 × Far CH_3), 1.7 (s, 6H, 2 × Far CH_3), 1.8 (s, 2H, NH_2), 1.9–2.1 (m, 8H, 4 × Far CH_2), 2.7 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2a}), 2.9 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys CH_{2b}), 3.2 (m, 2H, Far α-CH_2), 3.65 (m, 1H, Cys α-CH), 3.75 (s, 3H, OCH_3), 5.1 (m, 2H, Far CH), 5.2 (t, *J*=7 Hz, 1H, Far CH). MS: *m/z* (%) 339 (17) [M^+], 270 (5), 202 (8), 185 (5), 135 (15), 103 (15), 69 (100). $C_{19}H_{33}NO_2S$: calcd 339.2232, found 339.2242 [M^+] (MS).

Fluorenylmethoxycarbonylleucyl-proline *tert*-butyl ester (FmocLeuProOBut). To a solution of 2.25 g (6.3 mmol) of FmocLeuOH, 1.3 g (6,3 mmol) of HProOt-Bu·HCl, and 0.89 mL (6.3 mmol) of NEt_3 in 50 mL THF at 0 °C were added 1.7 g (12.6 mmol) of HOBt and 1.6 g (7.56 mmol) of DCC in 50 mL THF. The solution was stirred for 12 h at rt, the precipitate was filtered off and the solvent was removed in vacuo. The residue was dissolved in 100 mL CH_2Cl_2 and the solution was extracted with 25 mL 1 M HCl, 25 mL of 1 M $NaHCO_3$ and 25 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product was isolated as a white amorphous

material from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (60:40 [v:v]) as eluent. Yield: 2.14 g (67%); $[\alpha]^{20}_D$ −52° (*c* 0.4, $CHCl_3$); R_f=0.4 (hexane:ethyl acetate (60:40 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 1.0 (2×d, *J*=7 Hz, 6H, 2×Leu CH_3), 1.4 (s, 9H, Bu^t), 1.5 (m, 2H, Leu β-CH_2), 1.75 (m, 1H, Leu γ-CH), 1.9–2.0 (m, 3H, Pro CH_2), 2.15 (m, 1H, Pro CH_2), 3.5 (m, 1H, Pro CH_2), 3.65 (m, 1H, Pro CH_2), 4.2 (t, *J*=7 Hz, 1H, Fmoc CH), 4.3 (d, *J*=7 Hz, 2H, Fmoc CH_2), 4.4 (m, 1H, Leu α-CH), 4.5 (m, 1H, Pro α-CH), 5.5 (d, *J*=10 Hz, 1H, urethane NH), 7.25–7.4 (m, 4H, Fmoc), 7.5 (d, *J*=7 Hz, 2H, Fmoc), 7.7 (d, *J*=7 Hz, 2H, Fmoc). MS: *m/z* (%) 506 (0.2) [M$^+$], 284 (4.3), 178 (63), 86 (100), 70 (24), 57 (1). $C_{30}H_{38}N_2O_5$ calcd: 506.2780, found: 506.2769.

Fluorenylmethoxycarbonylleucyl proline, 4 (FmocLeu-ProOH). To cleave the *tert*-butyl protecting group 2 g (3.9 mmol) of FmocLeu-ProOBut was added to 5 mL trifluoroacetic acid containing 0.5 mL thioanisole and the solution was stirred for 1 h at rt. The solution was evaporated to dryness and the product was isolated as a white amorphous material from the remaining residue by flash chromatography on silica gel using ethyl acetate:methanol (90:10 [v:v]). Yield: 1.67 g (95%); $[\alpha]^{20}_D$ −46° (*c* 1, methanol); R_f=0.3 (ethyl acetate:methanol 90:10 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 1.0 (2×d, *J*=7 Hz, 6H, 2×Leu CH_3), 1.5 (m, 2H, Leu β-CH_2), 1.75 (m, 1H, Leu γ-CH), 1.9–2.0 (m, 3H, Pro CH_2), 2.15 (m, 1H, Pro CH_2), 3.5 (m, 1H, Pro CH_2), 3.65 (m, 1H, Pro CH_2), 4.2 (m, 2H, Fmoc CH, α-CH), 4.3 (d, *J*=7 Hz, 2H, Fmoc CH_2), 4.4 (m, 1H, α-CH), 5.5 (d, *J*=10 Hz, 1H, urethane NH), 7.25–7.4 (m, 4H, Fmoc), 7.5 (d, *J*=7 Hz, 2H, Fmoc), 7.7 (d, *J*=7 Hz, 2H, Fmoc). MS: *m/z* (%) 450 (0.8) [M$^+$], 308 (3), 178 (100), 70 (10). $C_{26}H_{30}N_2O_5$ calcd: 450.2155, found: 450.2165.

Allyloxycarbonylleucyl-proline *tert*-butyl ester (AlocLeu-ProOBut). To a solution of 0.86 g (5 mmol) of AlocLeuOH, 1.22 g (5 mmol) of HProOtBu in 50 mL THF at 0 °C were added 0.123 g (1 mmol) of DMAP and 0.76 g (6 mmol) of DIC in 50 mL THF. The solution was stirred for 12 h at rt, the precipitate was filtered off and the solvent was removed in vacuo. The residue was dissolved in 100 mL of CH_2Cl_2 and the solution was extracted with 25 mL 1 M HCl, 25 mL 1M $NaHCO_3$ and 25 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product was isolated as a white amorphous material from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (60:30 [v:v]) as eluent. Yield: 0.855 g (47%); $[\alpha]^{20}_D$ −95° (*c* 1, MeOH); R_f=0.35 (hexane:ethyl acetate (60:30 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.95 (d, *J*=7 Hz, 3H, Leu CH_3), 1.0 (d, *J*=7 Hz, 3H, Leu CH_3), 1.45 (s, 9H, Bu^t), 1.55 (m, 2H, Leu β-CH_2), 1.75 (m, 1H, Leu γ-CH), 1.9–2.05 (m, 3H, Pro CH_2), 2.15 (m, 1H, Pro CH_2), 3.5 (m, 1H, Pro CH_2), 3.65 (m, 1H, Pro CH_2), 4.4 (m, 1H, Leu α-CH), 4.5 (m, 3H, Pro α-CH, Aloc OCH_2), 5.2–5.45 (m, 3H, urethane NH, Aloc H_2C=CH), 5.9 (m, 1H, Aloc CH_2=CH). MS: *m/z* (%) 368.3 (5) [M$^+$], 312 (8), 170 (92), 70 (100). $C_{19}H_{32}N_2O_5$ calcd: 368.2311, found: 368.2323.

Allyloxycarbonylleucyl-proline 5 (AlocLeuProOH). To cleave the *tert*-butyl protecting group 0.780 g (2.12 mmol) of AlocLeuProOtBu was added to 5 mL trifluoroacetic acid containing 0.5 mL thioanisole and the solution was stirred for 1 h at rt. The solution was evaporated to dryness, the remaining residue was dissolved in water and extracted at pH 1 with dichloromethane. The organic layers were dried with $MgSO_4$, the solvent was removed in vacuo and the product **5** was isolated as a colourless oil. Yield: 0.704 g (quant); $[\alpha]^{20}_D$ −80° (*c* 1, methanol); 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.95 (d, *J*=7 Hz, 3H, Leu CH_3), 1.0 (d, *J*=7 Hz, 3H, Leu CH_3), 1.55 (m, 2H, Leu β-CH_2), 1.75 (m, 1H, Leu g-CH), 1.9–2.05 (m, 3H, Pro CH_2), 2.15 (m, 1H, Pro CH_2), 3.5 (m, 1H, Pro CH_2), 3.65 (m, 1H, Pro CH_2), 4.5 (m, 3H, Pro α-CH, Aloc OCH_2), 4.6 (m, 1H, Leu α-CH), 5.2–5.45 (m, 3H, urethane NH, Aloc H_2C=CH), 5.9 (m, 1H, Aloc H_2=CH). MS: *m/z* (%) 312 (2.2) [M$^+$], 281 (5), 212 (6), 170 (100), 70 (50). $C_{15}H_{24}N_2O_5$ calcd: 312.1685, found: 312.1670.

Fluorenylmethoxycarbonylleucyl-prolyl-*S*-farnesyl-cysteine methyl ester, 6 (FmocLeuProCys(Far)OMe). To a solution of 233 mg (0.68 mmol) of the farnesylated cysteine methyl ester **1** and 310 mg (0.68 mmol) of the dipeptide **2** in 25 mL CH_2Cl_2 at 0 °C was added 185 mg (1.36 mmol) of 1-hydroxybenzotriazole (HOBt) and 158 mg (0.816 mmol) of ethyl-(dimethylamino)propyl-carbodiimide (EDC) in 10 mL CH_2Cl_2 and the solution was stirred for 12 h at rt. The solution was extracted with 10 mL 1 M HCl, 10 mL 1 M $NaHCO_3$ and 10 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **3** was isolated from the residue by flash chromatography on silica gel using hexane:ethyl acetate (70:30 [v:v]) as eluent as a light yellow oil. Yield 0.488 g (93%); $[\alpha]^{20}_D$ −63° (*c* 1, methanol); R_f=0.25 (hexane:ethyl acetate 70:30 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.9 (m, 6H, Leu CH_3), 1.5 (m, 2H, Leu β-CH_2), 1.6 (s, 6H, Far CH_3), 1.7 (s, 6H, Far CH_3), 1.75 (m, 1H, Leu γ-CH), 1.9–2.2 [m, 11H, 4×Far CH_2, Pro CH_2(3H)], 2.35 (m, 1H, Pro CH_2), 2,7 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2a}), 2.9 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2b}), 3.0–3.2 (m, 2H, Far α-CH_2), 3.55 (m, 1H, Pro CH_2), 3.7 (m, 1H, Pro CH_2), 3.75 (s, 3H, OCH_3), 4.2 (t, 1H, Fmoc CH), 4.3 (m, 2H, Fmoc CH_2), 4.5–4.7 (m, 2H, 2×α-CH), 4.8 (m, 1H, α-CH), 5.1 (m, 2H, Far CH), 5.2 (t, *J*=7 Hz, 1H, Far CH), 5.45 (d, *J*=10 Hz, 1H, urethane NH), 7.2–7.4 (m, 5H, amide NH, Fmoc), 7.4 (d, *J*=7 Hz, 2H, Fmoc), 7.5 (d, *J*=7 Hz, 2H, Fmoc). MS: *m/z* (%) 771 (0.002) [M$^+$], 549 (1), 178 (100). FABMS (3-NBA:TFA 5:1): *m/z* (%) 794 (20) [M+Na$^+$], 772 (22) [M+H$^+$], 179 (100).

Allyloxycarbonylleucyl-prolyl-S-farnesyl-cysteine methyl ester 7 (AlocLeuProCys(Far)OMe). To a solution of 640 mg (1.89 mmol) of the farnesylated cysteine methyl ester **3** and 500 mg (1.60 mmol) of the dipeptide **5** in 25 mL CH_2Cl_2 at 0 °C was added 793 mg (3.03

mmol) of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in 10 mL CH_2Cl_2 and the solution was stirred for 12 h at rt. The solution was extracted with 10 mL 1 M HCl, 10 mL 1 M $NaHCO_3$ and 10 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **7** was isolated from the residue by flash chromatography on silica gel using hexane:ethyl acetate (60:30 [v:v]) as eluent as a light yellow oil. Yield 1.00 g (98%); $[\alpha]^{20}_D$ −84° (*c* 1; methanol); R_f=0.15 (hexane:ethyl acetate 60:30 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.9 (m, 6H, Leu CH_3), 1.5 (m, 2H, Leu β-CH_2), 1.6 (s, 6H, Far CH_3), 1.7 (s, 6H, Far CH_3), 1.75 (m, 1H, Leu γ-CH), 1.9–2.2 [m, 11H, 4×Far CH_2, Pro CH_2(3H)], 2.35 (m, 1H, Pro CH_2), 2,7 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2a}), 2.9 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2b}), 3.0–3.2 (m, 2H, Far α-CH_2), 3.55 (m, 1H, Pro CH_2), 3.7 (m, 1H, Pro CH_2), 3.75 (s, 3H, OCH_3), 4.5–4.7 (m, 5H, 3×α-CH, Aloc OCH_2), 5.1–5.3 (m, 5H, Aloc H_2C=CH, 3×Far CH), 5.45 (d, *J*=10 Hz, 1H, urethane NH), 5.9 (m, 1H, Aloc H_2C—C*H*), 7.35 (d, *J*=10 Hz, 1H, amide NH). MS: *m/z* (%) 633 (40) [M^+], 428 (35), 170 (50), 70 (100). $C_{34}H_{55}N_3O_6S$ calcd: 633.3812, found: 633.3792.

Leucyl-prolyl-(*S*-farnesyl)cysteine methyl ester, 8 (HLeuProCys(Far)OMe). (a) To a solution of 490 mg (0.63 mmol) of **6** in 10 mL CH_2Cl_2 was added 2 mL piperidine and the solution was stirred for 1 h at rt. The solution was evaporated to dryness and the product **8** was isolated from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (20:80 [v:v]) as eluent as a light yellow oil. Yield: 0.311 g (90%). (b) To a solution of 45.0 mg (0.071 mmol) of **7** in 10 mL THF was added 10 mg (0.115 mmol) of morpholine and 25 mg (0.02 mmol) of tetrakis(triphenylphosphine)palladium(0) and the solution was stirred for 1 h at rt. The solvent was removed in vacuo and the product **8** was isolated from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (20:80 [v:v]) as eluent as a light yellow oil. Yield: 34.3 g (88%); $[\alpha]^{20}_D$ −50° (*c* 1.7, $CHCl_3$); R_f=0.16 (hexane:ethyl acetate 20:80 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ=0.9 (m, 6H, Leu CH_3), 1.6 (s, 6H, Far CH_3), 1,7 (s, 6H, Far CH_3), 1.5 (m, 2H, Leu β-CH_2), 1.75 (m, 1H, Leu γ-CH), 1.9–2.2 [m, 13H, 4×Far CH_2, Pro CH_2(3H), NH_2], 2.3 (m, 1H, Pro CH_2), 2,7 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2a}), 2.9 (dd, J_1=7 Hz, J_2=14 Hz, 1H, Cys β-CH_{2b}), 3.0–3.2 (m, 2H, Far α-CH_2), 3.55 (m, 1H, Pro CH_2), 3.65 (m, 1H, α-CH), 3.7 (m, 1H, Pro CH_2), 3.75 (s, 3H, OCH_3), 4.5 (m, 2H, α-CH), 5.1 (m, 2H, Far CH), 5.2 (t, *J*=7 Hz, 1H, Far CH), 7.3 (d, *J*=10 Hz, 1H, amide NH). MS: *m/z* (%) 549 (2) [M^+], 339 (19), 154 (100), 69 (66), 68 (15). $C_{30}H_{51}N_3O_4S$: calcd: 549.3600, found: 549.3577.

Fluorenylmethoxycarbonylmethionyl-glycine *tert*-butyl ester (FmocMetGlyOt Bu). To a solution of 2.33 g (6.3 mmol) of FmocMetOH and 1.05 g (6,3 mmol) of HGlyOtBu·HCl and 0.87 mL NEt_3 (6.3 mmol) in 50 mL THF at 0 °C were added 1.7 g (12.6 mmol) of 1-hydroxybenzotriazole (HOBt) and 1.55 g (7.56 mmol) of dicyclohexylcarbodiimide (DCC) in 50 ml THF. The solution was stirred for 12 h at rt. The precipitate was filtered off and the solvent was removed in vacuo. The residue was dissolved in 100 mL CH_2Cl_2 and extracted with 25 mL 1 M HCl, 25 mL 1 M $NaHCO_3$ and 25 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product was isolated from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (60:40 [v:v]) as eluent as a white amorphous material. Yield: 2.20 g (72%); $[\alpha]^{20}_D$ −4.5° (*c* 0.6, $CHCl_3$); R_f=0.37 (hexane: ethyl acetate (60:40 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 9H, OtBu), 1.8–2.0 (m, 1H, Met γ-CH_2), 2.10 (s, 3H, Met CH_3), 2.1 (m, 1H, Met γ-CH_2), 2.6 (t, *J*=7 Hz, 2H, Met β-CH_2), 4.0 (m, 2H, Gly CH_2), 4.3 (t, *J*=7 Hz, 1H, Fmoc CH), 4.5 (m, 3H, Met α-CH, Fmoc CH_2), 6.0 (d, *J*=10 Hz, 1H, urethane NH), 7.1 (br, 1H, amide), 7.2–7.4 (m, 4H, Fmoc), 7.5 (d, *J*=7 Hz, 2H, Fmoc), 7.7 (d, *J*=7 Hz, 2H, Fmoc). MS: *m/z* (%) 484 (0.37), 411 (0.66), 354 (1.4), 178 (100), 104 (32.9), 57 (11). $C_{26}H_{32}N_2O_5S$: calcd: 484.2031, found: 484.2045.

Fluorenylmethoxycarbonylmethionyl-glycine, 9 (FmocMetGlyOH). To cleave the *tert*-butyl-protecting group 1.88 g (3.9 mmol) of FmocMet-GlyOtBu was added to 5 mL trifluoroacetic acid (TFA) containing 0.5 mL thioanisole and was stirred for 1 h at rt. The solvent was evaporated in vacuo. The product **9** was isolated as a white amorphous material from the remaining residue by recrystallization from ethyl acetate. Yield: 1.59 g (95%); $[\alpha]^{20}_D$ −2.5° (*c* 0.3, $CHCl_3$) after recrystallization from ethyl acetate. 400 MHz ^{1}H NMR ($CDCl_3$): δ=1.8–2.0 (m, 1H, Met γ-CH_{2a}), 2.10 (s, 3H, Met CH_3), 2.1 (m, 1H, Met γ-CH_{2b}), 2.6 (t, *J*=7 Hz, 2H, Met β-CH_2), 3.8–4.0 (m, 2H, Gly CH_2), 4.3 (t, *J*=7 Hz, 1H, Fmoc CH), 4.5 (m, 3H, Met α-CH, Fmoc CH_2), 6.0 (d, *J*=10 Hz, 1H, urethane NH), 7.1 (br, 1H, amide), 7.2–7.4 (m, 4H, Fmoc), 7.5 (d, *J*=7 Hz, 2H, Fmoc), 7.7 (d, *J*=7 Hz, 2H, Fmoc). MS: *m/z* (%) 428 (0.2) [M^+], 354 (0.6), 178 (100), 165 (35), 61 (3). $C_{22}H_{24}N_2O_5S$: calcd: 428.1406, found: 428.1414.

Fluorenylmethoxycarbonylmethionyl-glycyl-leucyl-prolyl (*S*-farnesyl)cysteine methyl ester, 11 (FmocMetGlyLeuProCys(Far)OMe). To a solution of 460 mg (1.1 mmol) of **9** and 600 mg (1.1 mmol) of **8** in 25 mL CH_2Cl_2 at 0 °C was added 300 mg (2.2 mmol) of 1-hydroxybenzotriazole (HOBt) and 250 mg (1.3 mmol) of ethyl-(dimethylamino)propyl-carbodiimide (EDC) in 10 mL CH_2Cl_2 and the mixture was stirred for 12 h. The solution was extracted with 10 ml 1M HCl, 10 mL 1 M $NaHCO_3$ and 10 mL water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **11** was isolated from the residue by flash chromatography on silica gel using ethyl acetate:methanol (95:5 [v:v]) as eluent as a light yellow oil. Yield: 460 mg (46%); $[\alpha]^{20}_D$ −46° (*c* 0.25; $CHCl_3$); R_f=0.45 (ethyl acetate:methanol 95:5 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): 0.9 (2×d, *J*=7 Hz, 6H, Leu CH_3), 1.4 (m, 2H, Leu β-CH_2), 1.5 (s, 6H, Far CH_3), 1.65 (2×s, 6H, Far CH_3), 1.7 (m, 1H, Leu CH),

1.8 (m, 3H, Pro CH_2), 1.9–2.2 [m, 14H, 4x Far CH_2, Pro CH_2(1H), Met γ-CH_2, Met CH_3], 2.55 (m, 2H, Met β-CH_2), 2.7 (dd, $J_1=7$ Hz, $J_2=14$ Hz, 1H, Cys β-CH_{2a}), 2.9 (m, 1H, Cys β-CH_{2b}), 3.0–3.25 (m, 2H, Far α-CH_2), 3.5–3.7 (m, 2H, Pro CH_2), 3.7 (s, 3H, OCH_3), 4.0 (m, 2H, Gly CH_2), 4.2 (t, $J=7$ Hz, 1H, Fmoc), 4.3–4.5 (m, 3H, Fmoc CH_2, α-CH), 4.65 (m, 2H, 2 × α-CH), 4.8 (m, 1H, α-CH), 5.1 (t, $J=7$ Hz, 2H, Far CH), 5.15 (t, $J=7$ Hz, 1H, Far CH), 5.6 (d, $J=10$ Hz, 1H, urethane NH), 7.0 (m, 1H, amide NH), 7.2–7.4 (m, 5H, Fmoc, amide NH), 7.45 (d, $J=10$ Hz, 1H, amide NH), 7.5 (d, $J=7$ Hz, 2H, Fmoc), 7.7 (d, $J=7$ Hz, 2H, Fmoc). MS: *m/z* (%) 178 (10), 154 (50), 86 (60), 70 (100), 69 (70), 61 (8).

Allyloxycarbonylmethionyl-glycine, 10 (AlocMetGlyOH). Allyloxycarbonylmethionyl-glycine **10** was synthesized via the corresponding dipeptide heptyl ester and a lipase mediated hydrolysis of the ester as described previously.[21]

Allyloxycarbonylmethionyl- glycyl- leucyl- prolyl-(S-farnesyl)cysteine methyl ester, 12 (AlocMetGlyLeuProCys(Far)OMe). To a solution of 460 mg (0.45 mmol) of **10** and 131 mg (0.45 mmol) of **8** in 25 mL CH_2Cl_2 at 0 °C was added 300 mg (0.6 mmol) of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in 10 mL CH_2Cl_2 and the mixture was stirred for 12 h at rt. The solution was extracted with 10 ml 1M HCl, 10 mL 1 M $NaHCO_3$ and 10 mL of water. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **12** was isolated from the residue as a light yellow oil by flash chromatography on silica gel using ethyl acetate:acetone 80:20 [v:v]) as eluent. Yield: 230 mg (62%); $[\alpha]^{20}_D$ −53° (*c* 1, MeOH); $R_f=0.3$ (ethyl acetate:acetone 80:20 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): 0.9 (2 × d, $J=7$ Hz, 6H, Leu CH_3), 1.4 (m, 2H, Leu β-CH_2), 1.5 (s, 6H, Far CH_3), 1.65 (2 × s, 6H, Far CH_3), 1.7,(m, 1H, Leu γ-CH), 1.8 (m, 3H, Pro CH_2), 1.9–2.2 (m, 14H, 4x Far CH_2, Pro CH_2 (1H), Met γ-CH_2, Met CH_3), 2.55 (m, 2H, Met β-CH_2), 2.7 (dd, $J_1=7$ Hz, $J_2=14$ Hz, 1H, Cys β-CH_{2a}), 2.9 (m, 1H, Cys β-CH_{2b}), 3.0–3.25 (m, 2H, Far α-CH_2), 3.5–3.7 (m, 2H, Pro CH_2), 3.7 (s, 3H, OCH_3), 4.1 (m, 2H, Gly CH_2), 4.3 (m, 2H, 2 × α-CH), 4.55 (d, $J=5.5$ Hz, 2H, Aloc OCH_2), 4.65(m, 1H, α-CH), 4.8 (m, 1H, α-CH), 5.1 (t, $J=7$ Hz, 2H, Far CH), 5.15 (t, $J=7$ Hz, 1H, Far CH), 5.2–5.3 (m, 2H, Aloc CH=C$\underline{H}_2$), 5.7 (d, $J=10$ Hz, 1H, urethane NH), 5.9 (m, 1H, Aloc C$\underline{H}$=CH_2), 7.0 (m, 1H, amide NH), 7.6 (m, 1H, amide NH), 7.7 (m, 1H, amide NH).

Methionyl-glycyl-leucyl-prolyl-(S-farnesyl) cysteine methyl ester 13 (HMetGlyLeuProCys(Far)OMe). (a) To a solution of 180 mg (0.18 mmol) of **11** in 5 mL THF was added 1 mL piperidine and the solution was stirred for 1 h at rt. The solvent was removed in vacuo and the product **13** was isolated from the remaining residue by flash chromatography on silica gel using ethyl acetate:methanol (90:10 [v:v]) as eluent as a light yellow oil. Yield: 0.120 g (90%) (b) To a solution of 200 mg (0.245 mmol) of **12** in 5 mL CH_2Cl_2 was added 380 mg (2.45 mmol) of dimethyl barbituric acid and 25 mg of tetrakis(triphenylphosphine)palladium(0) and the solution was stirred for 1 h at rt. The solvent was removed in vacuo and the product **13** was isolated from the remaining residue by flash chromatography on silica gel using ethyl acetate:acetone (60:30 [v:v]) as eluent as a light yellow oil. Yield: 0.130 g (72%); $[\alpha]^{20}_D$ −78.5°, (*c* 0.5; $CHCl_3$); $R_f=0.25$ (ethyl acetate/methanol 80:20 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): 0.9 (2 × d, 6H, Leu CH_3), 1.4 (m, 2H, Leu β-CH_2), 1.5 (s, 6H, Far CH_3), 1.65 (2 × s, 6H, Far CH_3), 1.7(m, 1H, Leu γ-CH), 1.8 (m, 3H, Pro CH_2), 1.9–2.2 (m, 14H, 4 × Far CH_2, Pro CH_2(1H), Met CH_2, Met CH_3), 2.55 (m, 2H, Met β-CH_2), 2.7 (dd, $J_1=7$ Hz, $J_2=14$ Hz, 1H, Cys β-CH_{2a}), 2.9 (m, 1H, Cys β-CH_{2b}), 3.0–3.25 (m, 2H, Far α-CH_2), 3.5–3.7 (m, 2H, Pro CH_2), 3.7 (s, 3H, OCH_3), 4.0 (m, 2H, Gly CH_2), 4.4–4.6 (m, 3H, 3 × α-CH), 4.8 (m, 1H, α-CH), 5.1 (t, $J=7$ Hz, 2H, Far CH), 5.15 (t, $J=7$ Hz, 1H, Far CH), 7.0 (m, 1H, amide NH), 7.3(d, $J=10$ Hz, 1H, amide NH), 7.45 (d, $J=10$ Hz, 1H, amide NH). FABMS (glycerine TFA 5:1): 738 (8.4) [M+H]. $C_{37}H_{64}N_5O_6S_2$: calcd: 738.4298, found: 738.4290.

***N*-Allyloxycarbonyl-(*S*-palmitoyl)cysteine, 14 (AlocCys(Pal)OH).** To a solution of 3 g (7.35 mmol) of $(AlocCysOH)_2$ in 14 mL 1 M NaOH at 0 °C was added 1.1 g (29.4 mmol) of $NaBH_4$ and the mixture was stirred at rt for 24 h. The pH of the solution was adjusted to 2 by addition of 5 M HCl and the solution was extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and concentrated in vacuo. To a solution of 2.8 g (13.65 mmol) of crude AlocCysOH in 25 mL THF was added at 0 °C 1.9 mL (13.65 mmol) NEt_3, 0.32 g (2.73 mmol) dimethylaminopyridine (DMAP) and 5.53 g (20.5 mmol) palmitoyl chloride. The solution was stirred for 2 h at 0 °C and then concentrated in vacuo. The product **8** was isolated from the remaining residue by flash chromatography on silica gel using hexane:ethyl acetate (30:70 [v:v]) as eluent as a white wax. Yield: 2.74 g (42%); $[\alpha]^{20}_D$ −28° (*c* 1, methanol); $R_f=0.6$ (hexane:ethyl acetate 30:70 [v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): 0.9 (t, $J=7$ Hz, 3H, ω-Pal), 1.2–1.4 (m, 24H, Pal CH_2), 1.65 (m, 2H, Pal β-CH_2), 2.6 (t, $J=7$ Hz, 2H, Pal α-CH_2), 3.4 (m, 2H, Cys β-CH_2), 4.5 (m, 1H, Cys α-CH), 4.6 (d, $J=7$ Hz, 2H, Aloc OCH_2), 5.3 (m, 2H, Aloc CH=C$\underline{H}_2$), 5.5 (d, $J=10$ Hz, 1H, urethane NH), 5.9 (m, 1H Aloc C$\underline{H}$=CH_2). MS: m/z (%): 443 (0.16), 398 (0.75), 340 (3.25) 239 (100). $C_{23}H_{41}NO_5S$: calcd: 443.2705, found: 443.2729.

***N*-Allyloxycarbonyl-(*S*-palmitoyl)cysteinyl-methionyl-glycyl-leucyl-prolyl-(S-farnesyl)cysteine methyl ester, 15 (AlocCys(Pal)MetGlyLeuProCys(Far)OMe).** To a solution of 17 mg (0.023 mmol) of **13** and 30 mg (0.069 mmol) of **14** in 10 mL CH_2Cl_2 at 0 °C was added 16 mg (0.115 mmol) of 1-hydroxybenzotriazole (HOBt) and 14 mg (0.069 mmol) of ethyl-(dimethylamino)propyl-carbodiimide (EDC) in 5 mL CH_2Cl_2 and the mixture was stirred for 24 h. The solution was extracted with 5 ml of 1M HCl, 5 mL 1 M $NaHCO_3$ and 5 mL water.

The organic layers were dried with $MgSO_4$ and concentrated in vacuo. The product **15** was isolated from the residue by flash chromatography on silica gel using hexane:ethyl acetate:methanol (50:50:10 [v:v:v]) as a light yellow oil. Yield: 12.8 mg (48%); $[\alpha]^{20}_D$ −33° (*c* 0.66, $CHCl_3$); R_f=0.7 (hexane:ethyl acetate:methanol 50:50:10 [v:v:v]). 400 MHz ^{1}H NMR ($CDCl_3$): 0.88 (t, *J*=7 Hz, 3H, ω-Pal CH_3), 0.94 (d, 3H, *J*=7 Hz, Leu CH_{3a}), 0.98 (d, 3H, *J*=7 Hz, Leu CH_{3b}), 1.20–1.35 (m, 26H, 2H Leu β-CH_2, 24H Pal CH_2), 1.55 (s, 6H, Far CH_3), 1.6 (m, 2H, Pal β-CH_2), 1.65 (2×s, 6H, Far CH_3), 1.75 (m, 1H, Leu γ-CH), 1.9–2.20 (m, 16H, 4×Far CH_2, Pro CH_2(3H), Met γ-CH_2, Met CH_3), 2.2 (m, 1H, Pro CH_2), 2. 55 (m, 2H, Pal α-CH_2), 2.60 (m, 2H, Met β-CH_2), 2.8 (m, 1H, Cys β-CH_{2a}), 2.90 (m, 1H, Cys β-CH_{2b}), 3.0–3.2 (m, 2H, Far α-CH_2), 3.4 (m, 2H, Cys β-CH_2), 3.5 (m, 2H, Pro CH_2), 3.7 (s, 3H, OCH_3), 4.1 (d, 2H, Gly CH_2), 4.5–4.7 (m, 4H, 4×α-CH), 4.6 (d, *J*=7 Hz, 2H, Aloc OCH_2), 4.8 (m, 1H, α-CH), 5.1 (m, 2H, Far CH), 5.2 (t, *J*=7 Hz, 1H, Far CH), 5.3 (m, 2H, Aloc CH=CH_2), 5.9 (m, 1H, Aloc CH=CH_2), 6.0 (m, 1H, urethane NH), 7.2–7.8 (4×d, 4H, 4×amide NH). FABMS: 1186 [M+Na^+].

***N*-Allyloxycarbonyl-(*S*-palmitoyl)cysteinyl-methionyl-glycine choline ester bromide, 18 (AlocCys(Pal)Met-GlyOCho).** Compound **18** was synthesized as described previously.[15]

Expression of protein farnesyl transferase and the GST-CIIS fusion protein

The plasmids YEp13/RAM2 and YEP24/DPR1 were supplied by F.Tamanoi.[23] For expression the *Saccharomyces cerevisiae* strain W303–1B (*MAT*α leu2 his3 trp1 ura 3 ade2–1 can1 cyr$^+$) was used. Cells were grown in YNB medium lacking uracil and leucine.

The pGEX-Vector for producing the GST-CIIS protein (glutathione *S*-transferase fused to the *C*-terminal 12 amino acids of yeast RAS2 protein) was supplied by M. H. Gelb.[24] *Escherichia coli* XL1-B cells were used for expression and the fusion protein purified with glutathione-agarose beads, essentially as described.[25]

^{3}H Farnesylpyrophosphate (Fpp) was purchased from Amersham (17,2 Ci/mmol) or Dupont (22.3 Ci/mmol).

Farnesyltransferase assay

To examine the inhibition of PFTase by lipopeptides a PFT filter-binding assay was used in a similar way as described.[23,26]

The reaction mixture (25 µL) contained 50 mM Tris/HCl, pH 8, 10 mM $MgCl_2$, 5mM DTT, 5 µM $ZnCl_2$, 0.93–1.76 µM ^{3}H Fpp, 19 µg GST-CIIS protein, variable concentrations of lipopeptides (dissolved in methanol) and enzyme. The assay mixture was incubated at 37 °C for 10 min and the reaction was stopped by the addition of EDTA. The reaction mixture was spotted on a filter paper, treated with 10% trichloroacetic acid and washed with ethanol:75 mM phosphoric acid (1/1; v/v).[27] Scintillation fluid (Quicksafe A, Zinsser) was added and the samples were counted.

Preparation of the crude enzyme extracts was performed as previously described.[23] W303–1B cells carrying YEp13/RAM2 and YEP24/DPR1 were grown to late-exponential phase, suspended in a buffer with 0.1 M Mes, pH 6.5, 0.1mM $MgCl_2$, 0.1 mM EDTA, 1mM DTT containing 2 mM Pefabloc®SC, 5 mM benzamidine, 3.1 µg/mL bestatin, 6.7 µg/mL pepstatin and broken by glass beads. The resulting lysate was centrifuged at 100,000 g for 1 h after low speed centrifugation. Protein concentrations were measured by the method of Warburg[28] and a modified method of Lowry[29,30] and were in the range 1.6–2.5 mg/mL.

Acknowledgments

We thank Professor F. Tamanoi for the generous gift of the DPR 1 and RAM 2 genes from *S. cerevisiae*. This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Deutschen Chemischen Industrie. P.H. and M.S. thank the Land Baden-Württemberg for a graduate scholarship.

References

1. Reviews: (a) Egan, S. E.; Weinberg, R. A. *Nature* **1993**, *365*, 781; (b) Boguski, M. S.; McCormick, F. **1993**, *366*, 643; (c) Bos, J. L. *Mutat. Res.* **1988**, *195*, 255.

2. Levitzki, A. *Eur. J. Biochem.* **1994**, *226*, 1.

3. Hall, A. *Science* **1994**, *264*, 1413, and ref therein.

4. Buss, J. E.; Marsters, J. C. Jr. *Chem. Biol.* **1995**, *2*, 787.

5. (a) Leftheris, K.; Kline, T.; Vite, G. D.; Cho, Y. H.; Bhide, R. S.; Patel, D. V.; Patel, M. M.; Schmidt, R. J.; Weller, H. N.; Andahazy, M. L.; Carboni, J. M.; Gullo-Brown, J. L.; Lee, F. Y. F.; Ricca, C.; Rose, W. C.; Yan, N.; Barbacid, M.; Hunt, J. T.; Meyers, C. A.; Seizinger, B. R.; Zahler, R.; Manne, V. *J. Med. Chem.* **1996**, *39*, 224; (b) Hunt, J. T.; Lee, V. G.; Leftheris, K.; Seizinger, B.; Carboni, J.; Mabus, J.; Ricca, C.; Yan, N.; Manne, V. *J. Med. Chem.* **1996**, *39*, 353.

6. Sun, J; Qian, Y.; Hamilton, A. D.; Sebti, S. M. *Cancer Res.* **1995**, *55*, 4243.

7. Kohl, N. E.; Wilson, F. R; Mosser, S. D.; Guiliani, E.; de Solms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; Lee, T.-J.; Smith, R. L.; Graham, S. L.; Hartman, G. D.; Gibbs, J. B.; Oliff, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9141.

8. Kohl, N. E.; Omer, C. A.; Conner, M. W.;Anthony, N. J.; Davide, J. P.; deSolms, S. J.; Giuliani, E. A.; Gomez, R. P.; Graham, S. L.; Hamilton, K.; Handt, L. K.; Hartman, G. D.; Koblan, K. S.; Kral, A. M.; Miller, P. J.; Mosser, S. D.; O'Neill, T. J., Rands, E., Schaber, M. D., Gibbs, J. B., Oliff, A. *Nat. Med.* **1995**, *1*, 792.

9. Manne, V.; Yan, N.; Carboni, J. M.; Tuomari, A. V.; Ricca, C. S.; Brown, J. G.; Andahazy, M. L.; Schmidt, R. J.; Patel, D.; Zahler, R.; Weinmann, R.; Der, C. J.; Cox, A. D.; Hunt, J. T.; Gordon, E. M.; Barbacid, M.; Seizinger, B. R. *Oncogene* **1995**, *10*, 1763.

10. Schaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. D.; Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Friedman, P. A.; Dixon, R. A. F.; Gibbs, J. B. *J. Biol. Chem.* **1990**, *265*, 14701.

11. Moores, S. L.; Schaber, M. D.; Mosser, S. D.; Rands, E.; O'Hara, M. B.; Garky, V. M.; Marshall, M. S.; Pompliano, D. L.; Gibbs, J. B. *J. Biol. Chem.* **1991**, *266*, 14603.

12. Pompliano, D. L.; Rands, E.; Schaber, M. D.; Mosser, S. D.; Anthony, N. J.; Gibbs, J. B. *Biochem.* **1992**, *31*, 3800.

13. see e. g. Stryer, L. *Biochemistry*, W. H. Freeman: New York, **1995**; 4th edn.

14. Waldmann, H; Nägele, E. *Angew. Chem.* **1995**, *107*, 2425; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2259.

15. Schelhaas, M.; Glomsda, S.; Hänsler, M.; Jakubke, H.-D.; Waldmann, H. *Angew. Chem.* **1996**, *108*, 82; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 106.

16. Hancock, J. F.; Magee, A. I.; Childs, J. E.; Marshall, C. J. *Cell* **1989**, *57*, 1167.

17. Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748.

18. Kunz, H.; Unverzagt. K. *Angew. Chem.* **1984**, *96*, 426; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 436.

19. Liakopoulos-Kyriakides, M. *Phytochem.* **1985**, *24*, 1593.

20. Tamanoi, F. *TIBS* **1993**, *18*, 349.

(*Received in U.S.A.* 1 *April* 1996; *accepted* 23 *April* 1996)

21. We note however, that at pH 7.0–7.5 the hydrolysis of the thioester present in **15** and **18** is slow (see also: Shahinian, S.; Silvius, J. R. Biochemistry **1995**, *34*, 3813), i.e. the concentration of free palmitic acid in the assay is at most very low. At 1 mmol concentration of added palmitic acid we did not observe any inhibition of farnesyl transferase.

22. Gomez, R.; Goodman, L. E.; Tripathy, S. K.; O'Rourke, E.; Manne, V.; Tamanoi, F. *Biochem. J.* **1993**, 25.

23. Finegold, A. A.; Johnson, D. I.; Farnsworth, C. C.; Gelb, M. H.; Judd, S. R.; Glomset, J. A.; Tamanoi, F, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4448.

24. Smith, D. B.; Johnson, K. S. *Gene* **1988**, *67*, 31.

25. Goldman, L. E.; Judd, S. R.; Farnsworth, C. C.; Powers, S.; Gelb, M. H.; Glomset, J. A.; Tamanoi, F. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9665.

26. Roskowski, R. Jr; Ritchie, P.; Gahn, L. G. *Anal. Biochem.* **1994**, *222*, 275.

27. Warburg, O.; Christian, W. *Biochem. Z.* **1942**, *310* , 384.

28. Bensadoun, A.; Weinstein, D. *Anal. Biochem.* **1976**, *70*, 241.

29. Bach, T. J.; Rogers, D. H.; Rodney H. *Eur. J. Biochem.* **1986**, *154*, 103.

30. Braun, P.; Waldmann, H.; Vogt, W.; Kunz, H. *Liebigs Ann. Chem.* **1991**, 165.