Phys. Lett. **1999**, *302*, 347–353; c) C. C. Chiang, J. Y. Cheng, T.-C. Chang, *Proc. Natl. Sci. Counc. Repub. China Part A* **1999**, *23*, 679–694.
[12] R. Chattopadhyaya, S. Ikuta, K. Grzeskowiak, R. E. Dickerson, *Nature* **1988**, *334*, 175–179.
[13] a) Q. Wu, M. Tsiang, J. E. Sadler, *J. Biol. Chem.* **1992**, *267*, 24408–24412; b) L. R. Paborsky, S. N. McCurdy, L. C. Griffin, J. J. Toole, L. L. K. Leung, *J. Biol. Chem.* **1993**, *268*, 20808–20811; c) K. Y. Wang, S. H. Krawczyk, N. Bischofberger, S. Swaminathan, P. H. Bolton, *Biochemistry* **1993**, *32*, 11285–11292.
[14] J. A. Kelly, J. Feigon, T. O. Yeates, *J. Mol. Biol.* **1996**, *256*, 417–422.
[15] T.-C. Chang, C. C. Chiang, G. J. Small, S. H. Chou, *Chem. Phys. Lett.* **1994**, *223*, 190–196.

Received: March 30, 2000 [Z 28]

Synthesis of Characteristic H-Ras Lipopeptides by Employing Noble-Metal-, Acid-, and Reduction-Labile Blocking Groups

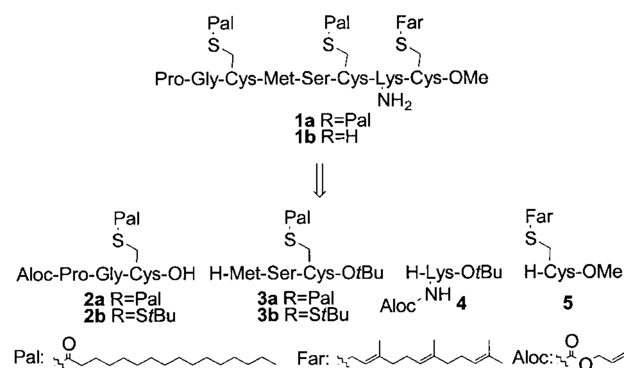
Dieter Kadereit[b] and Herbert Waldmann*[a]

KEYWORDS:

peptides · protecting groups · protein lipidation · Ras proteins · signal transduction

Numerous proteins covalently modified by fatty acid thioesters or prenyl thioethers are involved in biological events of paramount importance, such as signal transduction, organization of the cytoskeleton, and vesicular transport.[1, 2] Lipidation of these proteins is a prerequisite to correct biological function, and the lipid groups are believed to participate in protein–protein and protein–membrane interactions which, for instance, may determine the selective intracellular localization of lipid-modified proteins.[2–5] By combining techniques of organic synthesis and molecular biology, we have recently developed a general biological readout system that allows to determine and quantify the ability of a given lipidated peptide moiety to direct a given protein to the plasma membrane.[6] The key step of this method consists of the coupling of a mutant Ras protein to different selectively functionalized lipidated peptides. The scope of this system critically depends on the availability of efficient methods for the synthesis of multifunctional and sensitive farnesylated and palmitoylated peptides, even in selectively deprotected form, if so required. Farnesylated and palmitoylated peptides are acid- and base-sensitive[7] and often contain additional reactive amino acid side chain functions. Thus, their synthesis calls for the development of sophisticated protecting-group strategies employing blocking functions for carboxy, amino, thiol, and hydroxy groups, which are orthogonally stable to each other, yet can be removed selectively under very mild conditions.[8] Here we describe a protecting-group strategy for the synthesis of the characteristic C-terminal octapeptide of H-Ras in correctly modified and selectively unmasked form. The key feature of our strategy is the orchestration of the acid-labile *tert*-butyl ester function as carboxy protecting group, the Pd^0-sensitive allyloxycarbonyl (Aloc) urethane function as amino-blocking group, and the reduction-labile *tert*-butyl disulfide function for masking of thiol groups.

The C-terminal octapeptide **1a** of H-Ras contains one acid-sensitive farnesyl thioether and two base-labile thioester moieties. In addition, a serine OH group and, in particular, a lysine ε-amino group are located in the immediate vicinity of the thioester groups, increasing the danger of $S \rightarrow O$ and $S \rightarrow N$ acyl migrations in the course of the synthesis. In a retrosynthetic sense, triply lipidated peptide **1a** was divided into two selectively unmasked palmitoylated tripeptide building blocks **2a** and **3a**, N-terminally unmasked lysine derivative **4**, and *S*-farnesylated cysteine methyl ester **5** (Scheme 1). Similarly, it was planned to build up the farnesylated but nonpalmitoylated analogue **1b** by means of *S*-protected building blocks **2b** and **3b**. Octapeptide **1b** or its *S*-protected analogue might be used advantageously for the investigation of in vitro and in vivo palmitoylation[6] and the role of *S*-farnesylation.[9]

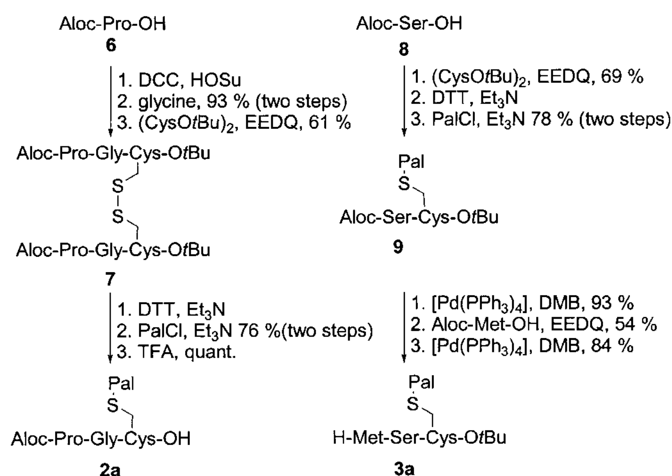


Scheme 1. Retrosynthetic strategy for the synthesis of C-terminal H-Ras octapeptides.

The synthesis of the *S*-acylated tripeptide **2a** (Scheme 2) started with an *N*,*N*-dicyclohexylcarbodiimide(DCC)- and *N*-hydroxysuccinimide(HOSu)-mediated coupling of Aloc-protected proline **6** with glycine and condensation of the resulting dipeptide with cystine bis(*tert*-butyl ester) to give disulfide **7**. Subsequent reduction of the disulfide bridge by treatment with dithiothreitol (DTT), *S*-acylation with palmitoyl chloride, and selective acid-mediated removal of the *tert*-butyl ester group

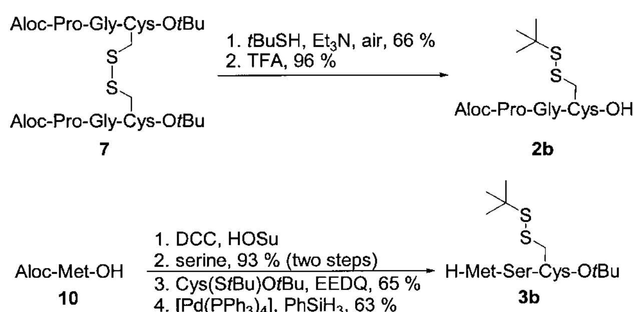
[a] Prof. Dr. H. Waldmann
Department of Chemical Biology
Max-Planck-Institut für molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2499
E-mail: herbert.waldmann@mpi-dortmund.mpg.de
[b] Dr. D. Kadereit
Universität Karlsruhe
Richard-Willstätter-Allee 2, 76128 Karlsruhe (Germany)

yielded palmitoylated tripeptide **2a**. For the synthesis of tripeptide **3a** (Scheme 2), Aloc-protected serine **8** was condensed with cystine bis(*tert*-butyl ester). Subsequent reduction of the disulfide bridge gave the free thiol, which had to be acylated at lower temperature with careful addition of palmitoyl chloride to suppress the competing *O*-palmitoylation at serine. Subsequent removal of the Aloc group proceeded smoothly through palladium(0)-catalyzed allyl transfer to *N,N'*-dimethylbarbituric acid (DMB)[8, 10] as allyl acceptor. Finally, condensation with Aloc-protected methionine followed by another treatment with [Pd(PPh$_3$)$_4$]/DMB yielded selectively unmasked tripeptide **3a**.



*Scheme 2. Synthesis of the palmitoylated tripeptides **2a** and **3a**. EEDQ = 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; TFA = trifluoroacetic acid.*

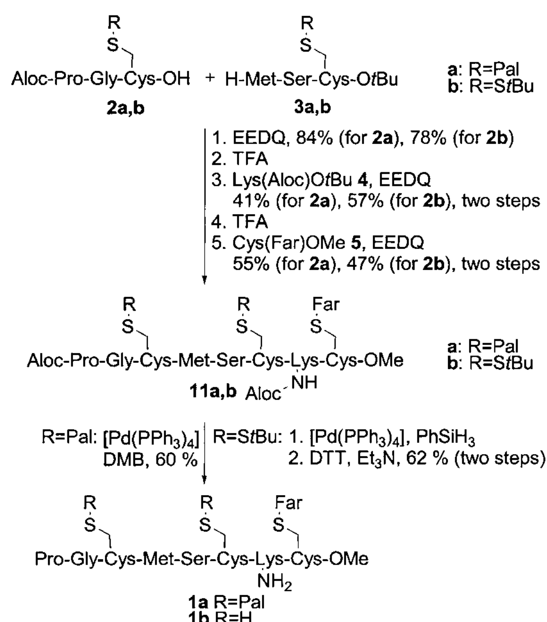
Similarly, *S*-protected dipeptides **2b** and **3b** were synthesized (Scheme 3). For the protection of the cysteine thiol group, the *S*-*tert*-butyl disulfide was employed which can readily be removed by reductive cleavage. Accordingly, fully masked cystine derivative **7** was treated with *tert*-butanethiol in the presence of air, followed by cleavage of the *tert*-butyl ester to give selectively deprotected dipeptide **2b**. For the synthesis of N-terminally unmasked tripeptide **3b**, Aloc-protected methionine was condensed with serine followed by coupling of the resulting dipeptide with Cys(S*t*Bu)O*t*Bu. The resulting protected tripeptide



*Scheme 3. Synthesis of the nonpalmitoylated tripeptide building blocks **2b** and **3b**.*

was converted to **3b** by cleaving the Aloc group with PhSiH$_3$[11] and catalytic amounts of Pd^0.

With the required selectively deprotected tripeptide building blocks in hand, the synthesis of the target H-Ras lipooctapeptides was approached. Thus, *S*-palmitoylated tripeptide **2a** bearing a free carboxylic acid group was condensed with N-terminally unmasked tripeptide ester **3a** (Scheme 4). The C-terminal *tert*-butyl ester moiety was selectively removed from the resulting hexapeptide by treatment with TFA. After chain elongation with lysine building block **4** and repeated *tert*-butyl ester cleavage with TFA, coupling of the doubly palmitoylated heptapeptide acid with *S*-farnesylated cysteine methyl ester **5** yielded fully protected lipooctapeptide **11a** in preparatively useful yield. Both Aloc groups were removed from this compound with complete selectivity by Pd^0-mediated allyl transfer to DMB to give the desired H-Ras peptide **1a**. By the same sequence of coupling and protection steps, bis-disulfide-masked and farnesylated octapeptide **11b** was synthesized (Scheme 4). Pd^0-catalyzed cleavage of the Aloc urethane moiety unmasked both the N-terminal and the lysine side chain amino groups. Finally, the two disulfide bridges shielding the cysteine SH groups were cleaved reductively by treatment with DTT to deliver farnesylated H-Ras octapeptide **1b** in appreciable yield.



Scheme 4. Block condensation and completion of the synthesis.

It should be noted that in all protecting-group manipulations the blocking functions were completely orthogonal to each other (i.e., they could be removed selectively without any harm to the other blocking functions present). In particular, both the Aloc urethane and the *tert*-butyl ester functions could be cleaved without any attack on the *tert*-butyl disulfide. Furthermore, the base-labile palmitic acid thioester and the acid-labile farnesyl thioether linkages remained completely intact during all transformations, and an *S*→*O* or *S*→*N* acyl shift could not be observed at all.

In conclusion, we have developed an efficient protecting-group strategy that allows for the selective synthesis of polyfunctional and sensitive palmitoylated and farnesylated peptides. It should open up new opportunities for bioorganic studies on the role of protein lipidation in various intracellular events.

Experimental Section

General: THF was distilled over potassium prior to use. Dichloromethane used for coupling reactions was distilled over CaH_2. The identity of the products was verified by either electron ionization mass spectrometry (EI-MS; including high-resolution data), fast atom bombardment mass spectrometry (FAB-MS; 3-nitrobenzyl alcohol (3-NBA) as matrix), or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; 2,5-dihydroxybenzoic acid (DHB)/TFA as matrix) and ^{1}H NMR spectroscopy (500 MHz).

Representative experimental procedures

Coupling reactions: A solution of C-terminally deprotected peptide (0.60 mmol), N-terminally deprotected peptide (0.60 mmol), and EEDQ (0.78 mmol) in dry dichloromethane (10 mL) was stirred for 15 h at ambient temperature. Then dichloromethane (90 mL) was added and the solution was extracted twice with 0.5 N hydrochloric acid (50 mL). The solution was dried over $MgSO_4$ and filtered, the solvent was evaporated under reduced pressure, and the product was purified by flash chromatography.

***tert*-Butyl ester cleavage:** A solution of peptide *tert*-butyl ester (1.0 mmol) in dichloromethane (2 mL) and TFA (2 mL) was stirred for 1 h at ambient temperature. The solvents were evaporated in vacuo, and the product was isolated by flash chromatography.

Aloc group removal: Phenylsilane (0.02 mmol) or DMB (0.02 mmol), respectively, and $[Pd(PPh_3)_4]$ (10 mol%) were added to a solution of the Aloc-protected peptide (0.01 mmol) in THF (0.5 mL) under an argon atmosphere. After stirring for 1 h at ambient temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (25 mL) and extracted twice with aqueous phosphate buffer (pH 6.5). The solution was dried over $MgSO_4$ and filtered, the solvent was evaporated under reduced pressure, and the product was purified by flash chromatography or, in two cases, by size exclusion chromatography (peptides **1 a** and **3 a**, Sephadex LH-20). If phenylsilane was used as allyl acceptor, the product was isolated by flash chromatography without aqueous workup.

Disulfide reduction: A solution of the disulfide (0.01 mmol), triethylamine (2 equiv), and DTT (4 equiv) in dichloromethane (2 mL) was stirred for 1 h at ambient temperature under an argon atmosphere. The solvent was evaporated under reduced pressure, and the product was isolated by flash chromatography or size exclusion chromatography (peptide **1 b**, Sephadex LH-20).

Characteristic spectroscopic data of selected peptides

Aloc-Pro-Gly-Cys(Pal)-OH (2 a): $[\alpha]_D^{20} = -13.8$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD, TMS): $\delta = 5.85-6.00$ (m, 1 H, $CH_2{=}CH$), 5.31 (d, 1 H, $J = 17.3$ Hz, $CH_{2a}{=}CH$), 5.19 (d, 1 H, $J = 10.6$ Hz, $CH_{2b}{=}CH$), 4.55 – 4.66 (m, 3 H, 2 $CH_2{=}CH\text{-}CH_2$, α-CH Cys), 4.22 – 4.26 (m, 1 H, α-CH Pro), 3.99 (d, 1 H, $J = 16.9$ Hz, α-CH_{2a} Gly), 3.77 (d, 1 H, $J = 17.0$ Hz, α-CH_{2b} Gly), 3.45 – 3.61 (m, 3 H, δ-CH_2 Pro, β-CH_{2a} Cys), 3.12 – 3.23 (m, 1 H, β-CH_{2b} Cys), 2.55 (t, 2 H, $J = 7.3$ Hz, 2 α-CH_2 Pal), 2.18 – 2.30 (m, 1 H, Pro), 1.98 – 2.13 (m, 2 H, Pro), 1.88 – 1.95 (m, 1 H, Pro), 1.59 – 1.68 (m, 2 H, 2 β-CH_2 Pal), 1.28 (s, 24 H, $CH_3(CH_2)_{12}(CH_2)_2COS$), 0.90 (t, 3 H, $J = 6.8$ Hz, ω-CH_3 Pal); ^{13}C NMR (125 MHz, CD_3OD, TMS): $\delta = 200.2$, 175.5, 173.2, 171.6, 156.9 (5 C=O), 134.2 ($H_2C{=}CH$), 117.8 ($H_2C{=}CH$), 67.4 ($CH_2{=}CH\text{-}CH_2O$), 62.2 (α-CH Pro), 53.5 (α-CH Cys), 48.1, 44.8, 43.5, 33.1, 32.5, 31.3, 31.1, 30.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.0, 26.7, 25.6, 24.6, 23.8 (18 CH_2), 14.1 (CH_3 Pal); MS (FAB, 3-NBA): *m/z*: 620.4 $[M+Na]^+$, 597.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{30}H_{51}N_3O_7S$: C 62.45, H 9.09, N 6.43; found: C 62.18, H 9.20, N 6.69.

Aloc-Pro-Gly-Cys(S*t*Bu)-OH (2 b): M.p. 61 °C; $[\alpha]_D^{20} = -65.2$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, TMS): $\delta = 8.1-8.5$ (br., 1 H, CO_2H), 7.42 – 7.69 (m, 2 H, NH), 5.88 – 5.97 (m, 1 H, $CH_2{=}CH$), 5.32 (d, 1 H, $J = 17.3$ Hz, $CH_{2a}{=}CH$), 5.21 (d, 1 H, $J = 10.3$ Hz, $CH_{2b}{=}CH$), 4.71 – 4.79 (m, 1 H, α-CH Cys), 4.52 – 4.65 (m, 2 H, 2 $CH_2{=}CH\text{-}CH_2$), 4.27 – 4.39 (m, 1 H, α-CH Pro), 4.20 (d, 1 H, $J = 16.7$ Hz, α-CH_{2a} Gly), 3.84 (d, 1 H, $J = 16.7$ Hz, α-CH_{2b} Gly), 3.45 – 3.62 (m, 2 H, δ-CH_2 Pro), 3.16 – 3.25 (m, 1 H, β-CH_{2a} Cys), 3.02 – 3.14 (m, 1 H, β-CH_{2b} Cys), 2.02 – 2.22 (m, 3 H, Pro), 1.83 – 1.96 (m, 1 H, Pro), 1.31 (s, 9 H, $SC(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): $\delta = 173.2$, 173.1, 170.4, 155.8 (4 C=O), 132.5 ($H_2C{=}CH$), 117.8 ($H_2C{=}CH$), 66.5 ($CH_2{=}CH\text{-}CH_2O$), 60.8 (α-CH), 60.4 (α-CH_2 Gly), 52.4 (α-CH), 48.1 ($SC(CH_3)_3$), 47.1, 42.9, 42.8 (3 CH_2), 29.9 ($SC(CH_3)_3$), 24.7 (CH_2); MS (FAB, 3-NBA): *m/z*: 470.1 $[M+Na]^+$, 448.1 $[M+H]^+$; HR-MS (FAB, 3-NBA): *m/z*: calcd for $C_{18}H_{30}N_3O_6S_2$ 448.1576, found 448.1604.

H-Met-Ser-Cys(Pal)-O*t*Bu (3 a): $[\alpha]_D^{20} = -20.9$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, TMS): $\delta = 8.06$ (d, 1 H, $J = 7.7$ Hz, NH), 7.27 (d, 1 H, $J = 7.3$ Hz, NH), 4.66 – 4.70 (m, 1 H, α-CH), 4.47 – 4.50 (m, 1 H, α-CH), 4.07 (dd, 1 H, $J = 11.5$, 5.3 Hz, β-CH_{2a} Ser), 3.60 (dd, 1 H, $J = 11.5$, 4.7 Hz, β-CH_{2b} Ser), 3.59 (dd, 1 H, $J = 8.1$, 4.7 Hz, α-CH Met), 3.47 (dd, 1 H, $J = 14.1$, 4.1 Hz, β-CH_{2a} Cys), 3.27 (dd, 1 H, $J = 14.1$, 6.1 Hz, β-CH_{2b} Cys), 2.60 – 2.65 (m, 2 H, γ-CH_2 Met), 2.56 (t, 2 H, $J = 7.6$ Hz, α-CH_2 Pal), 2.43 (br., 2 H, NH_2), 2.11 – 2.19 (m, 1 H, β-CH_{2a} Met), 2.11 (s, 3 H, SCH_3 Met), 1.77 – 1.84 (m, 1 H, β-CH_{2b} Met), 1.58 – 1.65 (m, 2 H, β-CH_2 Pal), 1.46 (s, 9 H, $CO_2C(CH_3)_3$), 1.25 (s, 24 H, $CH_3(CH_2)_{12}(CH_2)_2COS$), 0.87 (t, 3 H, $J = 6.9$ Hz, ω-CH_3 Pal); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): $\delta = 199.4$, 175.3, 170.7, 168.8 (4 C=O), 83.3 $[CO_2C(CH_3)_3]$, 62.8 (β-CH_2 Ser), 54.2, 54.0, 53.0 (3 α-CH), 44.1 (β-CH_2 Cys), 33.9, 31.9, 30.6, 30.2, 29.7, 29.7, 29.6, 29.4, 29.4, 29.2, 29.0 (11 CH_2), 27.9 ($CO_2C(CH_3)_3$), 25.6, 22.7 (2 CH_2), 15.3 (SCH_3 Met), 14.1 (CH_3 Pal); MS (FAB, 3-NBA): *m/z*: 634.4 $[M+H]^+$; elemental analysis calcd (%) for $C_{31}H_{59}N_3O_6S_2$: C 58.55, H 8.84, N 5.85; found:C 58.49, H 8.61, N 5.54.

H-Met-Ser-Cys(S*t*Bu)-O*t*Bu (3 b): $[\alpha]_D^{20} = -73.8$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, TMS): $\delta = 8.10$ (d, 1 H, $J = 7.6$ Hz, NH), 7.45 (d, 1 H, $J = 7.6$ Hz, NH), 4.70 – 4.76 (m, 1 H, α-CH Cys), 4.54 – 4.59 (m, 1 H, α-CH Ser), 4.06 (dd, 1 H, $J = 11.4$, 3.9 Hz, β-CH_{2a} Ser), 3.71 (dd, 1 H, $J = 11.4$, 5.6 Hz, β-CH_{2b} Ser), 3.60 (dd, 1 H, $J = 8.1$, 4.7 Hz, α-CH Met), 3.24 (dd, 1 H, $J = 13.6$, 4.4 Hz, β-CH_{2a} Cys), 3.11 (dd, 1 H, $J = 13.6$, 6.3 Hz, β-CH_{2b} Cys), 2.59 – 2.65 (m, 2 H, γ-CH_2 Met), 2.45 – 2.60 (br., 3 H, OH, NH_2), 2.12 – 2.22 (m, 1 H, β-CH_{2a} Met), 2.11 (s, 3 H, CH_3 Met), 1.77 – 1.86 (m, 1 H, β-CH_{2b} Met), 1.48 (s, 9 H, $CO_2C(CH_3)_3$), 1.32 (s, 9 H, $SC(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): $\delta = 175.5$ (C=O), 170.7 (C=O), 169.2 (C=O), 83.2 ($CO_2C(CH_3)_3$), 62.8 (β-CH_2 Ser), 54.2, 53.9, 53.0 (3 α-CH), 48.3 ($SC(CH_3)_3$), 42.3 (β-CH_2 Cys), 33.9 (γ-CH_2 Met), 30.6 (β-CH_2 Met), 29.8 ($SC(CH_3)_3$), 28.0 ($CO_2C(CH_3)_3$), 15.3 (SCH_3); MS (70 eV, EI): *m/z* (%): 483.2 (18) $[M]^+$, 427.2 (40) $[M-t\text{Bu}]^+$, 371.1 (71) $[M-2\,t\text{Bu}]^+$; elemental analysis calcd (%) for $C_{19}H_{37}N_3O_5S_3$: C 47.18, H 7.71, N 8.69; found: C 47.37, H 7.59, N 8.47.

Aloc-Pro-Gly-Cys(Pal)-Met-Ser-Cys(Pal)-Lys(Aloc)-Cys(Far)-OMe (11 a): M.p. 215 °C; $[\alpha]_D^{20} = -18.3$ ($c = 0.2$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ (10:1), TMS): $\delta = 5.79-5.89$ (m, 2 H, $H_2C{=}CH\text{-}CH_2O$), 5.09 – 5.28 (m, 5 H, $H_2C{=}CH\text{-}CH_2O$, CH=C Far), 4.99 – 5.06 (m, 2 H, CH=C Far), 4.18 – 4.9 (m, 11 H, α-CH, $H_2C{=}CH\text{-}CH_2O$), 3.81 – 3.90 (m, 2 H, β-CH_{2a} Ser, α-CH_{2a} Gly), 3.65 – 3.78 (m, 2 H, β-CH_{2b} Ser, α-CH_{2b} Gly), 3.74 (s, 3 H, $COOCH_3$), 3.45 – 3.55 (m, 2 H, 2 δ-CH_2 Pro), 3.33 – 3.39 (m,

2 H, ε-CH_2 Lys), 3.12 – 3.24 (m, 3 H, α-CH_{2a} Far, 2β-CH_{2a} Cys_{Pal}), 3.02 – 3.10 (m, 3 H, α-CH_{2b} Far, 2β-CH_{2b} Cys_{Pal}), 2.82 (dd, 1 H, $J = 13.9$, 5.2 Hz, β-CH_{2a} Cys_{Far}), 2.75 (dd, 1 H, $J = 13.9$, 7.9 Hz, β-CH_{2b} Cys_{Far}), 2.47 – 2.59 (m, 6 H, α-CH_2 Pal, γ-CH_2 Met), 2.11 – 2.18 (m, 2 H), 1.95 – 2.05 (m, 8 H), 2.05 (s, 3 H, SCH_3 Met), 1.78 – 1.92 (m, 4 H), 1.52 – 1.69 (m, 4 H, β-CH_2 Pal), 1.61 (s, 6 H, CH_3 Far), 1.54 (s, 6 H, CH_3 Far), 1.28 – 1.49 (m, 4 H, CH_2 Lys), 1.21 (s, 48 H, $H_3C(CH_2)_{12}(CH_2)_2COS$ Pal), 0.88 (t, 6 H, $J = 6.9$ Hz, ω-CH_3 Pal); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$ (10:1), TMS): $\delta = 200.4$, 174.5, 172.7, 171.3, 156.9, 156.0 (6 C=O), 140.1, 135.4 (quart, 2 Far-C), 133.1, 132.4 (2 $H_2C{=}CH$-CH_2O), 131.4 (quart, Far-C), 124.4, 123.9, 119.7 (3 Far-CH), 117.9, 117.5 (2 $H_2C{=}CH$-CH_2O), 66.7, 65.5 (2 $CH_2{=}CH$-CH_2O), 61.8 (β-CH_2 Ser), 61.2, 55.6, 54.4, 54.1 (4 α-CH), 53.6 ($COOCH_3$), 53.5, 52.6, 52.1 (3 α-CH), 47.2, 44.1, 40.6, 39.8, 32.6, 32.0, 31.1, 30.4, 30.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 29.1, 29.1, 26.8, 26.6 (20 CH_2), 25.7 (Far-CH_3), 25.6, 24.8, 22.7 (3 CH_2), 17.7, 16.1, 16.0 (3 Far-CH_3), 15.2 (SCH_3 Met), 14.1 (CH_3 Pal); MS (FAB, 3-NBA): m/z: 1713.4 $[M+Na]^+$, 1691.2 $[M+H]^+$.

Aloc-Pro-Gly-Cys(S*t*Bu)-Met-Ser-Cys(S*t*Bu)-Lys(Aloc)-Cys(Far)-OMe (11 b): M.p. 236 °C; $[\alpha]_D^{20} = -8.8$ ($c = 1.0$, $CHCl_3/CH_3OH$ (2:1)); ^1H NMR (500 MHz, $CDCl_3/CD_3OD$ (6:1), TMS): $\delta = 5.76 – 5.99$ (m, 2 H, $CH_2{=}CH$), 5.07 – 5.39 (m, 7 H, $CH_2{=}CH$ Aloc, 3 $CR_2{=}CH$ Far), 4.30 – 4.71 (m, 10 H), 4.17 – 4.21 (m, 1 H, α-CH Pro), 3.71 – 4.01 (m, 7 H, α-CH_2 Gly, β-CH_2 Ser, CO_2CH_3), 3.55 – 3.61 (m, 2 H, δ-CH_2 Pro), 2.90 – 3.32 (m, 9 H, 5 β-CH_2 Cys, α-CH_2 Far, ε-CH_2 Lys), 2.78 – 2.86 (m, 1 H, β-CH_{2b} Cys_{Far}), 2.52 – 2.66 (m, 2 H, γ-CH_2 Met), 1.85 – 2.29 (m, 16 H), 1.22 – 1.79 (m, 5 H), 1.68 (s, 6 H, Far-CH_3), 1.60 (s, 6 H, Far-CH_3), 1.34 (s, 9 H, $SC(CH_3)_3$), 1.33 (s, 9 H, $SC(CH_3)_3$); ^{13}C NMR (500 MHz, $CDCl_3/CD_3OD$ (6:1), TMS): $\delta = 171.3$, 170.9, 156.8, 156.0 (4 C=O), 139.9, 135.4 (quart, 2 Far-C), 133.2, 132.3 (2 $H_2C{=}CH$), 131.4 (quart, Far-C), 124.3, 123.9, 119.7 (3 Far-CH), 118.2, 117.5 (2 $H_2C{=}CH$), 66.9, 65.4 (2 $CH_2{=}CH$-CH_2O), 62.2 (β-CH_2 Ser), 61.7, 57.4, 54.4, 53.7 (4 α-CH), 53.5 ($COOCH_3$), 52.5, 52.3 (2 α-CH), 48.8, 48.2 (2 $SC(CH_3)_3$), 47.2, 43.9, 40.6, 39.8, 32.6, 30.9, 30.5 (7 CH_2), 30.0 ($SC(CH_3)_3$), 29.5, 29.1, 26.7, 26.6, 26.5 (5 CH_2), 25.7 (Far-CH_3), 24.8, 22.9 (2 CH_2), 17.7, 16.2, 16.0 (3 Far-CH_3), 15.1 (SCH_3 Met); MS (FAB, 3-NBA): m/z: 1412.4 $[M+Na]^+$, 1390.5 $[M+H]^+$.

H-Pro-Gly-Cys(Pal)-Met-Ser-Cys(Pal)-Lys-Cys(Far)-OMe (1 a): ^1H NMR (500 MHz, $CDCl_3/CD_3OD$ (6:1), TMS): Poor resolution, characteristic peaks: $\delta = 5.17 – 5.25$ (m, $CH{=}C$ Far), 5.02 – 5.11 (m, $CH{=}C$ Far), 3.76 (s, $COOCH_3$), 1.68 (s, CH_3 Far), 1.60 (s, CH_3 Far), 1.26 (br., $CH_3(CH_2)_{12}(CH_2)_2COS$ Pal), 0.88 (t, $J = 6.6$ Hz, ω-CH_3 Pal).

H-Pro-Gly-Cys-Met-Ser-Cys-Lys-Cys(Far)-OMe (1 b): ^1H NMR (500 MHz, $CDCl_3$, TMS): Poor resolution, characteristic peaks: $\delta = 5.05 – 5.25$ (m, 2 H, 3 $CR_2{=}CH$ Far), 3.78 (s, 3 H, CO_2Me), 1.68 (s, 6 H, Far-CH_3), 1.60 (s, 6 H, Far-CH_3); MS (MALDI-TOF, DHB/TFA matrix): 1046 $[M+H]^+$.

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

[1] L. Zhang, W. R. Tschantz, P. J. Casey, *J. Biol. Chem.* **1997**, *272*, 23 354 – 23 359.

[2] a) F. L. Zhang, P. J. Casey, *Annu. Rev. Biochem.* **1996**, *65*, 241 – 269; b) J. T. Dunphy, M. E. Linder, *Biochim. Biophys. Acta* **1998**, *1436*, 245 – 261, c) M. D. Resh, *Biochim. Biophys. Acta* **1999**, *1451*, 1 – 16.

[3] a) A. A. Siddiqui, J. R. Garland, M. B. Dalton, M. Simensky, *J. Biol. Chem.* **1998**, *273*, 3712 – 3717; b) R. Haklai, M. Gana-Weisz, G. Elad, A. Paz, D. Marciano, Y. Egozi, G. Ben-Baruch, Y. Kloog, *Biochemistry* **1998**, *37*, 1306 – 1314.

[4] a) C.-D. Hu, K.-I. Kariya, M. Tamaoka, K. Akasaka, M. Shirouzu, S. Yokoyama, T. Kataoka, *J. Biol. Chem.* **1995**, *270*, 30 274 – 30 277; b) J. D. Drugan, R. Khosravi-Far, M. A. White, C. J. Der, Y.-J. Sung, Y.-W. Hwang, S. L. Campbell, *J. Biol. Chem.* **1996**, *271*, 233 – 237; c) T. Okada, T. Masuda, M. Shinkai, K.-I. Kariya, T. Kataoka, *J. Biol. Chem.* **1996**, *271*, 4671 – 4678; d) Z. Luo, B. Diaz, M. S. Marshall, J. Avruch, *Mol. Cell. Biol.* **1997**, *17*, 46 – 53; e) D. Stokoe, F. McCormick, *EMBO J.* **1997**, *16*, 2384 – 2396; f) P. McGeady, S. Kuroda, K. Shimizu, Y. Takai, M. H. Gelb, *J. Biol. Chem.* **1995**, *270*, 26 347 – 26 351.

[5] I. Rubio, P. Rodriguez-Viciana, J. Downward, R. Wetzker, *Biochem. J.* **1997**, *326*, 891 – 895.

[6] B. Bader, K. Kuhn, D. J. Owen, H. Waldmann, A. Wittinghofer, J. Kuhlmann, *Nature* **2000**, *403*, 223 – 226.

[7] a) H. Waldmann, E. Nägele, *Angew. Chem.* **1995**, *107*, 2425 – 2428; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2259 – 2262; b) M. Koppitz, T. Spellig, R. Kahmann, H. Kessler, *Int. J. Pept. Protein Res.* **1996**, *48*, 377 – 390.

[8] D. Kadereit, J. Kuhlmann, H. Waldmann, *ChemBioChem* **2000**, *1*, 144 – 169.

[9] I. Rubio, U. Wittig, C. Meyer, R. Heinze, D. Kadereit, H. Waldmann, J. Downward, R. Wetzker, *Eur. J. Biochem.* **1999**, *266*, 70 – 82.

[10] H. Kunz, J. März, *Angew. Chem.* **1988**, *100*, 1424 – 1425; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1375 – 1377.

[11] M. Dessolin, M.-G. Guillerez, N. Thieriet, F. Guibé, A. Loffet, *Tetrahedron Lett.* **1995**, *36*, 5741 – 5744.

Received: April 19, 2000
Revised version: June 21, 2000 [Z 45]