Synthesis of characteristic lipopeptides of lipid modified proteins employing the allyl ester as protecting group

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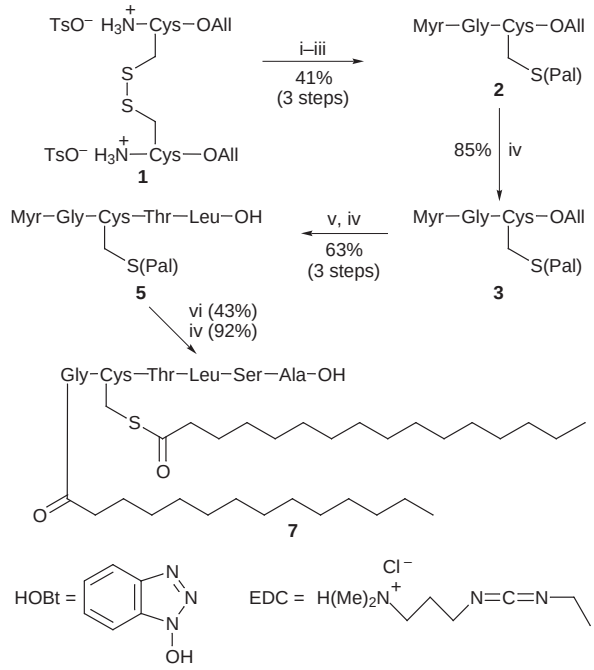
Lipidated peptides which represent characteristic lipid modified substructures of lipidated human $G_{\alpha O}$ protein and the human N- and R-*Ras* proteins were built up efficiently by employing the selective Pd^0-mediated removal of allyl esters under very mild conditions as the key step.

Lipid modified proteins are critically involved in biological signal transduction.[1] Thus, the transmembrane G protein coupled receptors are *S*-palmitoylated, their downstream effectors, the heterotrimeric G proteins are *N*-myristoylated, *S*-palmitoylated and *S*-farnesylated, the H- and the N-*Ras* proteins carry palmitoyl and farnesyl groups and the R-*Ras* protein is palmitoylated and geranylgeranylated. For the proper execution of their biological functions the correct lipidation of these classes of proteins is paramount.[2–4]

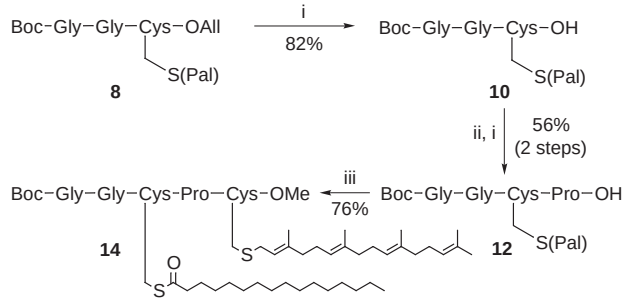
For the study of the biological significance of lipidated proteins, in particular their possible roles in signal transduction processes, lipidated peptides that contain the characteristic structural elements of their parent proteins are very useful reagents.[5,6] Therefore, the development of efficient methods for the construction of lipidated peptides is of particular interest. However, the thioesters present in these peptide conjugates are readily hydrolyzed at pH > 7 and may be lost *via* base-mediated β-elimination; also the double bonds present in farnesyl and geranylgeranyl groups are attacked under acidic conditions.[7,8] Consequently, protecting groups have to be employed that can be removed under neutral conditions.[5,8] We now report that characteristic lipidated peptides which represent lipid modified substructures of lipidated proteins can efficiently be built up by employing the allyl ester[9] as a protecting group.

In order to construct the *N*-myristoylated and *S*-palmitoylated *N*-terminus **7** of a human $G_{\alpha O}$ protein which embodies the characteristic Myr-Gly-Cys(Pal)-AA-AA-Ser/Thr-AA motif found in many G proteins,[10] cystine bisallyl ester **1** was coupled with *N*-myristoylglycine and after reductive cleavage of the disulfide bond the liberated mercapto groups were *S*-palmitoylated to give the protected thioester **2** (Scheme 1). From **2** the allyl ester was removed with complete selectivity in high yield by Pd^0-mediated transfer of the allyl group to morpholine as the accepting nucleophile. Subsequent elongation of the peptide chain by the dipeptide **4** yielded a lipidated tetrapeptide allyl ester from which the allyl group was also cleaved to give the selectively unmasked lipotetrapeptide **5** in high yield.‡ Further extension of the peptide chain by the dipeptide allyl ester **6** and final Pd^0-mediated removal of the allyl group delivered the desired myristoylated and palmitoylated *N*-terminal G protein fragment **7**. The three allyl ester cleavages performed in this sequence proceeded with complete selectivity and without any undesired side reactions. The conditions of the noble-metal complex mediated allyl transfer are so mild that neither an attack on the base-sensitive thioester nor a base-induced β-elimination of the palmitoyl group occurred.

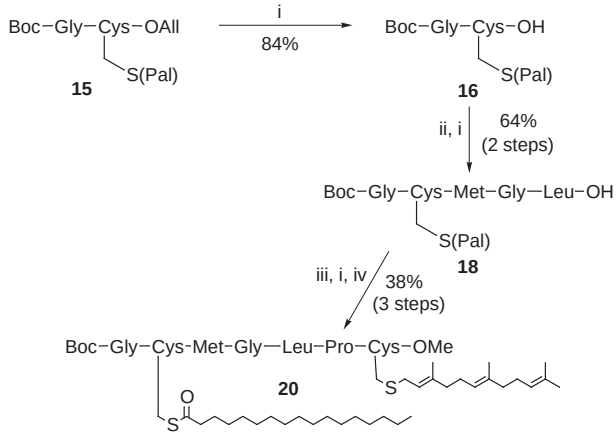
By means of this advantageous technique also the *S*-palmitoylated and *S*-geranylgeranylated *C*-terminus **14** of the human R-*Ras* protein[11] was efficiently built up (Scheme 2). To this end, the *S*-palmitoylated cysteinyl tripeptide allyl ester **8** was synthesized from $(BocGlyGlyCysOAll\text{-}S)_2$ by reductive cleavage of the disulfide and *S*-acylation, as described for **2** in Scheme 1 [the cystinyl precursor was built up from BocGly-GlyOH and cystine bis(allyl ester) by peptide coupling]. In this case 1,3-dimethylpyrimidine-2,4,6-trione (*N*,*N*′-dimethylbarbituric acid) **9**[12] was employed as accepting C-nucleophile in the Pd^0-mediated reaction. The C-terminal deprotection proceeded smoothly to give the desired carboxylic acid **10** in high yield. Elongation of the peptide chain by proline allyl ester **11** and a further Pd^0-mediated allyl ester cleavage in the presence of **9** delivered the *S*-palmitoylated tetrapeptide carboxylic acid **12** in high yield.‡ Finally, the synthesis was completed by coupling of **12** with *S*-geranylgeranylated cysteine methyl ester **13** to give the desired R-*Ras* peptide **14**.



Scheme 1 *Reagents and conditions*: i, Myr-Gly-OH, EEDQ, NEt_3, CH_2Cl_2, Pr^iOH; ii, DTT, NEt_3, CH_2Cl_2; iii, palmitoyl chloride, DMAP, NEt_3, CH_2Cl_2; iv, $Pd(PPh_3)_4$, morpholine, THF; v, H-Thr-Leu-OAll **4**, EDC, HOBt, CH_2Cl_2; vi, H-Ser-Ala-OAll **6**, EDC, HOBt, CH_2Cl_2



Scheme 2 *Reagents and conditions*: i, $Pd(PPh_3)_4$, **9**, THF; ii, ProOAll **11**, EDC, HOBt, CH_2Cl_2; iii, Cys(GerGer)OMe **13**, EDC, HOBt, CH_2Cl_2



Scheme 3 *Reagents and conditions*: i, $[Pd(PPh_3)_4]$, **9**, THF; ii, MetGly-LeuOAll **17**, EDC, HOBt, CH_2Cl_2; iii, **11**, EDC, HOBt, CH_2Cl_2; iv, Cys(Far)OMe **19**, EDC, HOBt, CH_2Cl_2

In a third application the *S*-palmitoylated and *S*-farnesylated C-terminus **20** of the human N-*Ras* protein[11] was built up by employing the Pd^0 mediated cleavage of allyl esters as the key step (Scheme 3). In order to construct this molecule, the *S*-palmitoylated allyl ester **15** was deprotected in high yield to give the selectively unmasked *S*-palmitoylated dipeptide **16**. Coupling with the tripeptide **17** and subsequent allyl transfer to **9** yielded the pentapeptide carboxylic acid **18** in an efficient manner.‡ A chain elongation with proline allyl ester **11**, a further allyl removal and subsequent coupling with *S*-farnesylated cysteine methyl ester **19** delivered the N-*Ras* peptide **20**.

All Pd^0-catalyzed allyl ester cleavage reactions proceeded without any undesired side reaction. In no case could a β-elimination or a nucleophilic attack on the activated thioesters be observed.

The use of the allyl ester blocking function thus makes sensitive lipidated peptides available in an efficient manner which up to now has only been matched by enzymatic protecting techniques proceeding under extremely mild conditions.[8] This useful strategy readily gives access to further functionalized lipidated peptides.

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Notes and References

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‡ *General procedure*. To a solution of the peptide allyl ester (1 mmol) in THF or CH_2Cl_2 (30 ml) under Ar was added $Pd(PPh_3)_4$ (0.01 equiv.) and morpholine or **9** (1.2 equiv.). The solution was stirred at room temperature for 30–60 min. If morpholine was used as the nucleophile the solution was extracted with 10 ml of 1 M HCl and 10 ml of brine. The organic layer was dried with $MgSO_4$, the solvent was evaporated *in vacuo* and the product was isolated from the residue by flash chromatography on silica gel.

According to this procedure **5**, **12** and **18** were obtained.

Selected data for **5**: 84%; mp 127–131 °C; R_f 0.56 [CH_2Cl_2–MeOH–AcOH 90 : 10 : 1 (v/v/v)]; $[\alpha]_D^{25}$ −6 (*c* 0.5, DMF); δ_H(400 MHz, CD_3OD) 0.86 (t, *J* 7, 6 H, Me Pal, Me Myr), 0.90 (d, *J* 5.5, 3 H, Me Leu), 0.94 (d, *J* 5.5, 3 H, Me Leu), 1.17 (d, *J* 6.3, 3 H, Me Thr), 1.23 (s, 44 H, 12 CH_2 Pal, 10 CH_2 Myr), 1.40–1.51 (m, 7 H, β-CH_2 Pal, β-CH_2 Myr, β-CH_2 Leu, γ-CH Leu), 2.27 (t, *J* 8, 2 H, α-CH_2 Myr), 2.55 (t, *J* 8, 2 H, α-CH_2 Pal), 3.13 (dd, *J* 14, *J* 8, 1 H, α-CH Cys), 3.23 (dd, *J* 14, 4, 1 H, β-CH Cys), 3.75 (d, *J* 17, 1 H, α-CH Gly), 3.95 (d, *J* 17, 1 H, β-CH Gly), 4.21 (t, *J* 5.8, 1 H, α-CH Leu), 4.33 (d, *J* 3.5, α-CH Thr), 4.40 (dd, *J* 8, 4, 1 H, α-CH Cys), 4.40–4.47 (m, 1 H, β-CH Thr); δ_C(100.6 MHz, CD_3OD) 14.15 (Me Pal, Me Myr), 18.79 (Me Thr), 21.65 (Me Leu), 22.79 (CH_2 Pal, CH_2 Myr), 23.00 (Me Leu), 24.97 (γ-CH Leu), 25.61 (CH_2 Cys), 29.00–30.00 (12 CH_2 Pal, 10 CH_2 Myr), 32.04 (α-CH_2 Pal), 36.22 (α-CH_2 Myr), 40.71 (CH_2 Leu), 44.10 (CH_2 Cly), 51.67 (α-CH Leu), 54.45 (α-CH Cys), 58.53 (α-CH Thr), 67.25 (β-CH Thr), 170.53 (C=O), 170.79 (C=O), 175.41 (3 C=O), 201.22 (C=O, thioester); *m/z* [FABMS (glycerol)] 842.1 $[M + H]^+$. For **12**: 70%; R_f 0.20 [EtOAc–MeOH: 7 : 3 (v/v)]; $[\alpha]_D^{25}$ −29 (*c* 1.65, MeOH), mp 110–113 °C; δ_H(400 MHz, $CDCl_3$) 0.84 (t, *J* 6.7, 3 H, Me Pal), 1.19 (s, 24 H, 12 CH_2 Pal), 1.41 (s, 9 H, 3 CH_3 Boc), 1.52–1.64 (m, 3 H, β-CH_2 Pal), 1.89–2.23 (m, 2 H, 2 CH_2 Pro, CH_2Me Pal), 2.51 (t, *J* 7.1, 2 H, α-CH_2 Pal), 2.71–2.84 (m, 1 H, β-CH_2 Cys), 3.32–3.48 (m, 1 H, β-CH_2 Cys), 3.57–3.72 (m, 2 H, CH_2N Pro), 3.78–4.04 (m, 4 H, 2 CH_2 Gly), 4.30–4.40 (m, 1 H, α-CH Cys), 5.05–5.12 (m, 2 H, NH urethane, α-CH Pro), 8.35–8.46 (m, 2 H, 2 NH). For **18**: 79%; R_f 0.18 [EtOAc–MeOH 7 : 3 (v/v)]; $[\alpha]_D^{25}$ −33 (*c* 1.25, CH_3OH); δ_H(400 MHz, CD_3OD) 0.86 (t, *J* 6.6, 3 H, Me Pal), 0.94 (d, *J* 4.9, 6 H, Me Leu), 1.23 (s, 24 H, 12 CH_2 Pal), 1.44 (s, 9 H, 3 Me Boc), 1.51–1.76 (m, 3 H, β-CH_2 Pal, γ-CH Leu), 2.00–2.12 (m, 2 H, CH_2Me Pal), 2.08 (s, 3 H, Me Met), 2.17–2.25 (m, 2 H, γ-CH_2 Met), 2.50–2.57 (m, 4 H, α-CH_2 Pal, β-CH_2 Met), 3.25–3.34 (m, 2 H, β-CH_2 Cys), 3.85–3.98 (m, 2 H, CH_2 Gly), 4.05–4.22 (m, 2 H, CH_2 Gly), 4.62–4.74 (m, 3 H, α-CH Cys, α-CH Leu, α-CH Met); δ_C(100.6 MHz, CD_3OD) 14.13 (Me Pal), 15.24 (Me Met), 21.89 (Me Leu), 22.69 (CH_2 Pal), 22.90 (Me Leu), 24.80 (γ-CH Leu), 25.60 (CH_2 Pal), 28.42 (Me Boc), 29.29–31.40 (12 CH_2 Pal), 31.91 (β-CH_2 Cys), 41.21 (β-CH_2 Leu), 43.15 (β-CH_2 Cys), 44.04 (CH_2 Gly), 44.32 (CH_2 Gly), 79.99 (Cq Boc), 156.45 (C=O, urethane), 168.80 (C=O), 169.77 (C=O), 170.41 (C=O), 171.26 (C=O), 172.71 (C=O), 199.57 (C=O, thioester) (Calc. for $C_{39}H_{71}N_5O_9S_2$: C, 57.25; H, 8.75; N, 8.56. Found: C, 57.28; H, 8.97; N, 8.79%.)

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