

Prenyl sulfates as alkylating reagents for mercapto amino acids

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A new methodology for prenylation of thiol compounds has been developed. The approach is based on the use of prenyl sulfates as new reagents for S-prenylation of benzenethiol and cysteamine in aqueous systems. The C₁₀-prenols geraniol and nerol that differ in the configuration (E or Z, correspondingly) of the α -isoprene unit were efficiently O-sulfated in the presence of a pyridine-SO₃ complex. The obtained geranyl and neryl sulfates were tested as alkylating agents. These compounds were chosen to reveal the influence of the α -isoprene unit configuration on their alkylation (prenylation) ability. S-Geranyl cysteine was prepared to demonstrate the applicability of this method for prenylation of peptides containing mercapto amino acids.

Keywords: S-prenylation, lipidated amino acids, geraniol, nerol, cysteamine, cysteine

INTRODUCTION

Prenylated proteins play important roles in eukaryotic cells (Omer & Gibbs, 1994; Takai *et al.*, 2001). The first prenylated polypeptide, the α -mat-ting factor of yeast, was discovered 25 years ago (Kamiya *et al.*, 1978). Studies during the last decades have shown that the proper functioning of many proteins requires a series of post-translational lipid modifications (Volkert *et al.*, 2001; Pechlivanis & Kuhlmann, 2006). The interest in protein lipidation increased rapidly after recognition that Ras and Rab proteins are subject to post-translational lipidation (Hancock *et al.*, 1989; Farnsworth *et al.*, 1991). Both proteins belong to the Ras superfamily and are key regulators of cell growth in all eukaryotic cells. Gene expression, apoptosis and remodelling of the cytoskeleton are all controlled by Ras proteins. Ras proteins were originally identified as the products of oncogenes capable of inducing cell transformation. Over the last twenty-five years they have been studied in great detail because mutant Ras proteins are associated with many types of human cancer (Bu-

day & Downward, 2008). It has been shown that the attached lipid groups function not only as anchors linking the modified protein to the cytoplasmic or vesicular membranes, but could also be involved in signal transduction by facilitating protein–protein and protein–lipid interactions (Wittinghofer & Waldmann, 2000; Kuhn *et al.*, 2001). The post-translational modification of proteins with lipids is a key mechanism in the regulation of protein localization and function. Prenylation is irreversible but it is frequently associated with reversible post-translational modifications, such as palmitoylation and phosphorylation. Both of them act like switches that modulate the dynamics of protein–membrane or protein–protein interactions and target the modified proteins to specific membrane compartments (Perez-Sala, 2007). Recent work (Abankwa *et al.*, 2007) has shown that Ras proteins are laterally segregated into non-overlapping, dynamic domains of the plasma membrane called nanoclusters. This lateral segregation is important in determining Ras interactions with membrane-associated proteins, effectors and scaffolding proteins, and is critical for Ras signal transduction

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Abbreviations: DMF, N,N-dimethylformamide; FCC, flash column chromatography; TLC, thin-layer chromatography.

(Eisenberg & Henis, 2008; Vögler *et al.*, 2008). Prenylated proteins of which geranylgeranylated ones represent 80% (Rilling *et al.*, 1990), can comprise up to 2% of total cellular protein (Epstein *et al.*, 1991).

In vivo S-prenylation of proteins is achieved by enzymatic formation of S-geranylgeranyl or S-farnesyl cysteine sulfides, the cysteine residues being located at (or close to) the C-terminus. This involves either one or two geranylgeranyl transferases or a farnesyl transferase (Zhang & Casey, 1996; Lane & Beese, 2006). Prenyltransferases attach the isoprenoid lipids to the C-terminus of several guanosine triphosphate-binding proteins. The prenyl groups are essential for the biological activity of these proteins. The prenyltransferases and other components of the mevalonate pathway are either present or potential drug targets for cancer, osteoporosis, restenosis and high serum cholesterol-level-associated diseases (Simonen *et al.*, 2008; Baron & Seabra, 2008). Control of Ras GTPase signalling might reduce the aberrant DNA methylation and, accordingly, may reduce the risk of cancer development (Patra, 2008).

A regulatory role of prenylation of plant proteins in developmental processes and hormone signal transduction has been suggested (Rodriguez-Concepcion *et al.*, 1999; Galichet & Gruissem, 2006). In addition to farnesyl and geranylgeranyl derivatives, various polyprenyl and dolichyl as well as phytyl residues were also discovered to covalently modify plant proteins (Swiezewska *et al.*, 1993; Ship-ton *et al.*, 1995; Gutkowska *et al.*, 2004).

Synthetic strategies that give access to lipidated peptides have been developed in the last ten years both for solution (Silvius & Lheureux, 1994; Shahinian & Silvius, 1995; Kadereit *et al.*, 2001) and solid-support approaches (Ludolph & Waldmann, 2003; Kragol *et al.*, 2004; Lumbierres *et al.*, 2005). For both methods (Brunsveld *et al.*, 2006), two basic techniques are used for introduction of the lipid functionalities. In the first technique, the lipid groups are introduced through coupling of prelipidated amino acids, whereas in the second one, they are introduced through lipidation of a selectively deprotected peptide (i.e., a peptide with a free thiol group). Quite frequently, a combined solution/solid-phase approach is used (Xue *et al.*, 1992; Naider & Becker, 1997; Reents *et al.*, 2005, Brunsveld *et al.*, 2005). In this approach, the majority of the peptide sequence is, first, rapidly synthesized on a solid support, then cleaved off and coupled with lipidated amino acids in solution or lipidated by alkylation of free thiol side chains of cysteines. In addition, the use of prelipidated building blocks has been suggested for the synthesis of lipidated peptides on a solid support (Kragol *et al.*, 2004; Lumbierres *et al.*, 2005).

In thioalkylation reactions, cysteine or cysteine residues of peptides are reacted with pre-

nyl bromide or prenyl chloride in organic or mixed organic/aqueous media. On-resin prenylation of the cysteines requires a large excess of prenyl halide in order to achieve complete conversion. Depending on the solubility of the cysteine-containing peptide, prenylation is accomplished either in alkaline, neutral or acidic conditions. Synthesis under alkaline conditions is usually performed in *N,N*-dimethylformamide (DMF) with aqueous KHCO_3 (Naider & Becker, 1997) or in tetrahydrofuran/liquid ammonia mixtures (Schroeder *et al.*, 1997, Kuhn *et al.*, 2001, Ludolph *et al.*, 2002). Reaction under neutral conditions proceeds in DMF and $\text{KF}\cdot 2\text{H}_2\text{O}$ in the presence of an equimolar amount of *N,N*-diisopropylethylamine (Silvius & Lheureux, 1994; Shahinian & Silvius, 1995). S-Prenylation procedure under acidic conditions is carried out in a DMF/aqueous trifluoroacetic acid/*n*-butanol (or acetonitrile) mixture in the presence of $\text{Zn}(\text{OAc})_2$ as a catalyst (Xue *et al.*, 1992). All these procedures provide the required compounds in good yields for short peptides. However, the use of alkyl halides for thioalkylation of natural long-chain peptides and proteins requires a mixed organic/aqueous medium, which is problematic for solubility reasons. The bivalent character of the lipidated peptides, featuring both the highly hydrophobic lipid groups and polar amino acids, makes them difficult to handle. The intrinsic instability of prenyl moieties in acidic conditions limits the number of applicable methods for S-prenylation of cysteine residues.

The successful development of solid-phase synthesis of lipidated Ras and Rab proteins demands new techniques for the incorporation of the required lipid groups. In this paper we demonstrate a new method for the chemical prenylation of mercapto amino acids using prenyl sulfates as alkylating reagents. This approach avoids the use of organic solvents and allows the S-prenylation reaction to be performed in aqueous systems.

MATERIALS AND METHODS

Geraniol and nerol were purchased from Lancaster (UK); cysteamine and L-cysteine from Aldrich (USA); pyridine- SO_3 complex, triethylamine and benzenethiol from Fluka (Germany). The equilibrium mixture of *n*-butanol and water was prepared by shaking equal volumes of both components. The upper layer of the equilibrium mixture contained *n*-butanol and water in a ratio of 86:14 (v/v) and the bottom layer of the mixture contained *n*-butanol and water in a ratio of 14:86 (v/v). Flash column chromatography (FCC) was performed on a column (1.4×18 cm) with Kieselgel 60 (0.040–0.063 mm) (Merck). TLC was performed on Kieselgel 60 F_{254} (Merck) developed with chloroform/methanol/

water (60:25:4, by vol., solvent A) and light petroleum (40–60°C)/ethyl acetate (95:5, v/v, solvent B). Unsaturated compounds were detected on TLC-plates with iodine vapor with subsequent charring by spraying with 4% sulfuric acid in methanol and heating. Compounds with free amino group were detected by spraying with 1% ninhydrin in ethanol and heating to 100°C. UV spectra were measured on a Specord UV-VIS. ¹H and ¹³C NMR spectra (¹H at 500 MHz, ¹³C at 125 MHz) were recorded on a Bruker AMX-500 spectrometer for solutions in CDCl₃, unless otherwise indicated. Chemical shifts (δ in ppm) are given relative to those for tetramethylsilane, *J* values are given in Hz. ES-MS spectra were obtained with a Finnigan LCQ spectrometer, EI-MS spectra were measured with a Jeol DX-300 apparatus.

Geranyl sulfate, triethylammonium salt [3]. Triethylamine (3.5 ml) was added to a mixture of geraniol (311 mg, 2.02 mmol) and pyridine-SO₃ complex (650 mg, 4.05 mmol) and the solution was stirred at room temp. for 1.5 h. Water (0.12 ml) was added, stirring was continued for 1 h and, after phase separation, the upper layer was discarded. The residue was concentrated, dissolved in the upper layer of the equilibrium mixture n-butanol/water (6.5 ml) and the solution was washed with the lower phase of the same equilibrium mixture (6×1.5 ml). The upper layer solution was concentrated. n-Heptane (4 ml) was added to the residue and the mixture was kept at 0°C for 2 h; the upper layer was removed and the bottom layer was concentrated to give compound **3** as an oil. Yield 509 mg, 75%. *R*_f 0.58 (solvent A); δ_{H} 9.47 (s, 1 H, SO₃H), 5.34 (dt, 1 H, *J*_{2,1} 7.0, *J*_{2,Me-3} 1.5, 2-H), 5.01 (dt, 1 H, *J*_{6,5} 7.0, *J*_{6,8} 1.3, 6-H), 4.53 (d, 2 H, *J*_{1,2} 7.1, 1-H₂), 3.12 (q, 6 H, *J* 7.3, CH₂N), 2.02 (m, 2 H, 4-H₂), 1.96 (m, 2 H, 5-H₂), 1.63 (s, 3 H, 3-CH₃), 1.61 (s, 3 H, 8-H₃), 1.54 (s, 3 H, 9-H₃), 1.30 (t, 9 H, *J* 7.4, CH₃CH₂N); δ_{C} 141.2 (C-3), 131.6 (C-7), 123.8 (C-6), 119.0 (C-2), 64.7 (C-1), 46.5 (CH₂N), 39.5 (C-4), 26.3 (C-5), 25.6 (C-9), 17.6 (C-8), 16.4 (CH₃-3), 8.7 (CH₃CH₂N).

Neryl sulfate, triethylammonium salt [4]. Neryl sulfate **4** was obtained from nerol (264 mg, 1.71 mmol) and pyridine-SO₃ complex (616 mg, 3.87 mmol) following the above procedure and isolated as an oil. Yield 413 mg, 72%. *R*_f 0.65 (solvent A); δ_{H} 9.53 (s, 1 H, SO₃H), 5.40 (dt, 1 H, *J*_{2,1} 7.5, *J*_{2,Me-3} 1.5, 2-H), 5.08 (dt, 1 H, *J*_{6,5} 7.0, *J*_{6,8} 1.2, 6-H), 4.57 (d, 2 H, *J*_{1,2} 7.5, 1-H₂), 3.18 (q, 6 H, *J* 7.5, CH₂N), 2.06 (m, 2 H, 4-H₂), 2.01 (m, 2 H, 5-H₂), 1.70 (s, 3 H, 3-CH₃), 1.68 (s, 3 H, 8-H₃), 1.60 (s, 3 H, 9-H₃), 1.37 (t, 9 H, *J* 7.5, CH₃CH₂N); δ_{C} 141.6 (C-3), 131.9 (C-7), 123.8 (C-6), 119.6 (C-2), 65.0 (C-1), 46.5 (CH₂N), 39.0 (C-4), 26.4 (C-5), 25.6 (C-9), 23.5 (CH₃-3), 17.6 (C-8), 8.7 (CH₃CH₂N).

Geranyl benzenethiol [6]. Benzenethiol **5** (1.35 ml, 13.1 mmol) and 5 M aq. KOH (3.28 ml, 16.4 mmol) were added to a stirred solution of the sulfate **3** (550 mg, 1.64 mmol) in the lower phase of the equilibrium mixture n-butanol/water (2.0 ml), the mixture was stirred at room temp. for 2 h. Light petroleum (15 ml) was added and, after phase separation, the upper phase was stored and the lower phase was extracted with light petroleum (10 ml). The combined organic layers were filtered through a Celite pad, and the solution was concentrated. FCC (light petroleum) of the residue gave the target compound **6** as an oil. Yield 193 mg, 48%. *R*_f 0.72 (solvent B); UV λ_{max} (n-hexane)/nm 234 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 4460), 269 (315); δ_{H} 7.26 (m, 2 H, 3'- and 5'-H), 7.19 (m, 2 H, 2'- and 6'-H), 7.09 (m, 1 H, 4'-H), 5.24 (dt, 1 H, *J*_{2,1} 6.5, *J*_{2,Me-3} 1.1, 2-H), 4.98 (m, 1 H, 6-H), 3.48 (d, 2 H, *J*_{1,2} 6.5, 1-H₂), 1.95 (m, 4 H, 4-H₂ and 5-H₂), 1.60 (d, 3 H, 3-CH₃), 1.52 (s, 3 H, 8-H₃), 1.50 (s, 3 H, 9-H₃); δ_{C} 139.9 (C-3), 136.8 (C-1'), 131.6 (C-7), 129.9 (C-3' and -5'), 128.7 (C-2' and -6'), 126.0 (C-4'), 123.9 (C-6), 119.2 (C-2), 39.6 (C-1), 32.2 (C-4), 26.4 (C-5), 25.7 (C-9), 17.7 (C-8), 16.0 (CH₃-3). EI-MS (+): *m/z* 246.42 (M⁺); calc. for C₁₆H₂₂S, *m* = 246.41.

Neryl benzenethiol [7]. Neryl benzenethiol **7** was obtained from neryl sulfate **4** (420 mg, 1.25 mmol), benzenethiol **5** (1.03 ml, 10.0 mmol) and 5 M aq. KOH (2.5 ml, 12.5 mmol) as described above for the preparation of **6**. The solution was stirred at room temp. for 75 min. The procedure of extraction and isolation was the same as for compound **6**. FCC (n-hexane) gave compound **7** as an oil. Yield 166 mg, 54%. *R*_f 0.82 (solvent B); UV λ_{max} (n-hexane)/nm 234 ($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ 4470), 269 (316); δ_{H} 7.31 (dd, 2 H, *J* 7.1, *J* 0.9, 3'- and 5'-H), 7.24 (m, 2 H, *J* 7.6, 2'- and 6'-H), 7.14 (dd, 1 H, *J* 7.3, *J* 0.6, 4'-H), 5.30 (dt, 1 H, *J*_{2,1} 7.6, *J*_{2,Me-3} 1.5, 2-H), 5.08 (m, 1 H, 6-H), 3.53 (d, 2 H, *J*_{1,2} 7.7, 1-H₂), 2.02 (m, 4 H, 4-H₂ and 5-H₂), 1.70 (d, 3 H, *J* 1.0, 3-CH₃), 1.66 (s, 3 H, 8-H₃), 1.58 (s, 3 H, 9-H₃); δ_{C} 140.0 (C-3), 137.0 (C-1'), 132.0 (C-7), 129.4 (C-3' and -5'), 128.6 (C-2' and -6'), 125.9 (C-4'), 123.9 (C-6), 119.8 (C-2), 39.5 (C-1), 31.9 (C-4), 26.5 (C-5), 25.7 (C-9), 23.3 (CH₃-3), 17.7 (C-8). EI-MS (+): *m/z* 246.15 (M⁺); calc for C₁₆H₂₂S, *m* = 246.41.

Geranyl cysteamine [9]. solution of cysteamine (527 mg, 6.83 mmol) in 2.5 M aq. KOH (3.98 ml, 9.87 mmol) was stirred for 2 min. Then, geranyl sulfate **3** (507 mg, 1.52 mmol) in the lower phase of the equilibrium mixture n-butanol/water (3.5 ml) was added. The solution was stirred at room temp. for 1.5 h and light petroleum (6 ml) was added to the mixture. After phase separation, the upper phase was stored and the lower phase was extracted with light petroleum (2×6 ml). The combined organic layers were concentrated. FCC (chloroform (40 ml), then chloroform-methanol, 9:1 (250 ml)) provided fractions with the desired product, which were com-

bined and concentrated. The residue was dissolved in light petroleum (5 ml) and the solution was filtered through a PTFE filter (0.22 μm , Acrodisc) and concentrated to give compound **9** as an oil. Yield 174 mg, 54%. R_f 0.53 (solvent A); δ_H 5.17 (dt, 1 H, $J_{2,1}$ 7.7, $J_{2,\text{Me-3}}$ 1.0, 2-H), 5.00 (m, 1 H, 6-H), 3.08 (d, 2 H, $J_{1,2}$ 7.7, 1-H₂), 2.72 (m, 2 H, CH₂N), 2.50 (t, 2 H, J 6.4, SCH₂CH₂N), 2.01 (t, 2 H, J 7.0, 4-H₂), 1.98 (m, 2 H, 5-H₂), 1.61 (s, 3 H, 3-CH₃), 1.58 (s, 3 H, 8-H₃), 1.52 (s, 3 H, 9-H₃); δ_C 139.2 (C-3), 132.0 (C-7), 124.3 (C-6), 120.8 (C-2), 41.5 (C-1), 35.3 (SCH₂CH₂N), 31.9 (C-4), 29.3 (CH₂N), 26.8 (C-5), 26.0 (C-9), 18.0 (C-8), 16.4 (CH₃-3). ES-MS (+): m/z 214.16 [M+H]⁺; calc. for C₁₂H₂₃NS, $m = 213.38$.

Neryl cysteamine [10]. A solution of cysteamine (359 mg, 4.65 μmol) in 2.5 M aq. KOH (2.4 ml, 5.88 mmol) was stirred for 2 min. The sulfate **4** (413 mg, 1.23 mmol) was dissolved in the lower phase of the equilibrium mixture n-butanol/water (3 ml) and then added to the solution. The mixture was stirred at room temp. for 45 min and light petroleum (20 ml) was added. After phase separation, the upper layer was stored and the lower phase was extracted with diethyl ether (2 \times 10 ml). The combined organic layers were concentrated. FCC (chloroform (40 ml), then chloroform/methanol, 9:1 (250 ml)) of the residue gave **10** as an oil. Yield 200 mg, 76%. R_f 0.55 (solvent A); δ_H 5.21 (dt, 1 H, $J_{2,1}$ 7.5, $J_{2,\text{Me-3}}$ 1.5, 2-H), 5.09 (m, 1 H, 6-H), 3.14 (d, 2 H, $J_{1,2}$ 7.5, 1-H₂), 2.81 (m, 2 H, CH₂N), 2.52 (t, 2 H, J 6.2, SCH₂CH₂N), 2.01 (t, 2 H, J 7.0, 4-H₂), 2.00 (m, 1 H, 5-H₂), 1.65 (s, 3 H, 3-CH₃), 1.62 (s, 3 H, 8-H₃), 1.56 (s, 3 H, 9-H₃); δ_C 139.3 (C-3), 132.0 (C-7), 124.1 (C-6), 121.0 (C-2), 41.5 (C-1), 39.9 (C-4), 35.5 (SCH₂CH₂N), 29.34 (CH₂N), 26.8 (C-5), 26.1 (C-9), 24.0 (CH₃-3), 18.1 (C-8). ES-MS (+): m/z 214.23 [M+H]⁺; calc. for C₁₂H₂₃NS, $m = 213.38$.

Geranyl cysteine (potassium salt) [12]. A solution of geranyl sulfate **3** (320 mg, 0.953 mmol) in the lower phase of the equilibrium mixture n-butanol/water (2 ml) was added to a stirred solution of L-cysteine **11** (462 mg, 3.81 mmol) in 2 M aq. KOH (4.3 ml, 8.58 mmol). The solution was stirred at room temp. for 2.5 h and diethyl ether (7 ml) was added to the mixture. After phase separation (30 min), the ethereal layer was stored and the lower layer was extracted with the upper phase of the equilibrium mixture n-butanol/water (3 \times 4 ml). The combined or-

ganic layers were concentrated and the residue was dissolved in methanol (2 ml). The solution was kept at 4°C for 16 h, then filtered through a PTFE filter (0.22 μm , Acrodisc) and concentrated to give **12** as white crystals. Yield 120.6 mg, 43%. R_f 0.60 (solvent A); δ_H (CD₃OD) 5.20 (t, 1 H, $J_{2,1}$ 7.5, 2-H), 5.04 (t, 1 H, $J_{6,5}$ 6.5, 6-H), 3.30 (m, 2 H, 1-H₂), 3.14 (dd, 1 H, J 4.0, J 7.6, CHNH₂), 2.90 (dd, 1 H, 2J 13.4, 3J 4.1, SCH₂-CHNH₂), 2.59 (dd, 1 H, 2J 13.4, 3J 7.6, SCH₂CHNH₂), 2.04 (m, 2 H, 5-H₂), 1.96 (m, 2 H, 4-H₂), 1.62 (s, 3 H, 8-H₃), 1.61 (s, 3 H, 3-CH₃), 1.54 (s, 3H, 9-H₃); δ_C (CD₃OD) 180.0 (C=O), 140.0 (C-3), 132.4 (C-7), 125.1 (C-6), 121.8 (C-2), 56.5 (CHNH₂), 40.7 (C-1), 38.7 (SCH₂CHNH₂), 30.4 (C-4), 27.6 (C-5), 25.9 (C-9), 17.8 (C-8), 16.2 (CH₃-3). ES-MS (-): m/z 255.30 [M-H]⁻; calc for C₁₃H₂₂O₂NS, $m = 256.38$.

RESULTS AND DISCUSSION

In prenylated peptides (Omer & Gibbs, 1994), geranylgeraniol (*E,E,E* - C₂₀ isoprenoid alcohol) and farnesol (*E,E* - C₁₅ isoprenoid alcohol) are the major prenols that are covalently attached to the thiol groups of cysteine residues at (or near) the C-terminus (see Fig. 1). Some proteins from the Ras superfamily are post-translationally converted into C-terminal methyl esters.

In this work, we used short C₁₀-prenols geraniol **1** and nerol **2** (Scheme 1) that differ in the configuration (*E* or *Z*, correspondingly) of the α -isoprene unit. These compounds were chosen to reveal the influence of the α -isoprene unit configuration on the alkylation (prenylation) reaction. Geranyl sulfate **3** and neryl sulfate **4** were tested as alkylating agents. They were synthesized by the method that we had developed previously for long-chain polyprenyl sulfates (Maltsev *et al.*, 2001). The method was modified for the synthesis of short-chain prenyl sulfates **3** and **4** because of the different solubility of long and short prenols and their sulfates in organic solvents. Prenols **1** and **2** were transformed into their sulfates **3** and **4** using triethylamine (instead of DMF, as in Maltsev *et al.*, 2001) as a solvent (see Scheme 1).

We discovered that prenols **1** and **2** could be efficiently O-sulfated in the presence of a two-fold molar excess of pyridine-SO₃ complex. After treatment of the reaction mixtures with water, the de-

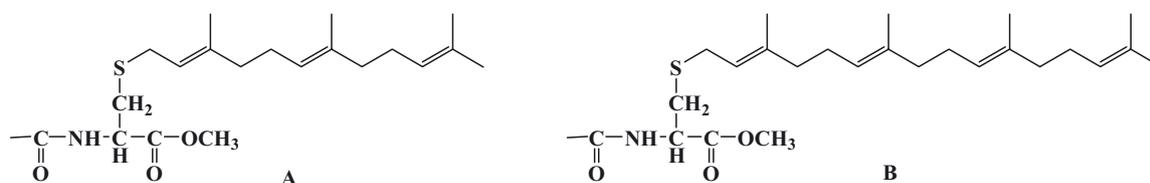
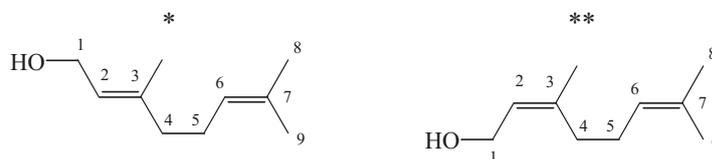


Figure 1. Farnesyl (A) and geranylgeranyl (B) sulfide linkages found in peptides and proteins. Note that double bonds are all in *trans* (*E*) configuration.

Table 1. Selected ^1H NMR and ^{13}C NMR data for compounds 1–4, 6, 7, 9, 10 and 12

Compound	δ_{H} (ppm)		δ_{C} (ppm)	
	H-1	S-OH	C-1	C=O
Ger-SO ₄ H-Et ₃ N (3)	4.53	9.47	64.7	
Ner-SO ₄ H-Et ₃ N (4)	4.57	9.53	65.0	
Ger-S-Ph (6)	3.48		39.6	
Ner-S-Ph (7)	3.53		39.5	
Ger-S-C ₂ H ₄ -NH ₂ (9)	3.08		41.5	
Ner-S-C ₂ H ₄ -NH ₂ (10)	3.14		41.5	
Ger-S-CH ₂ CH(NH ₂)COOK (12)	3.30		40.7	180.0
Ger-OH* (1)	4.05		58.6	
Ner-OH** (2)	4.05		58.7	



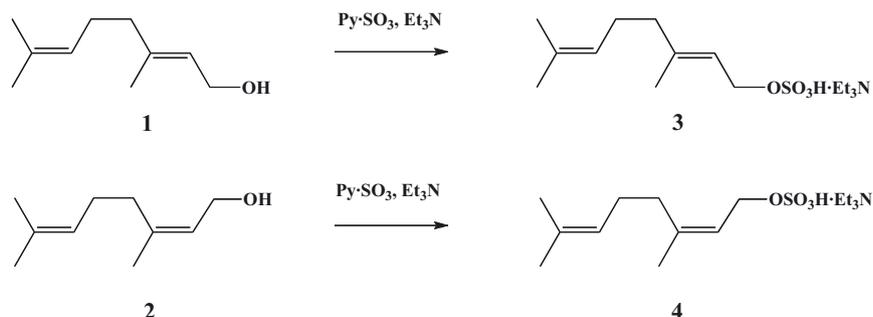
sired prenyl sulfates precipitated together with the non-consumed prenols. The latter were then extracted from the residue with cold n-heptane to provide pure geranyl sulfate 3 (75%) and neryl sulfates 4 (72%) as triethylammonium salts. The products were characterized by NMR. ^1H -NMR spectra of 3 and 4 virtually coincided with those of prenols 1 and 2 (Crombie *et al.*, 1975), apart from a characteristic downfield shift of the CH₂O group signal (δ 4.53 for 3 and 4.57 for 4) and the presence of diagnostic signals at δ 9.47 (3) and 9.53 (4) of the hydrogen sulfate groups (see Table 1). ^{13}C -NMR spectra of 3 and 4 completely coincided with those of the starting alcohols except for the signals of the α -terminal units. Introduction of the sulfate group into prenyl derivatives resulted in a large downfield shift of C1 signals (see Table 1).

Triethylammonium salts of prenyl sulfates 3 and 4 were used for S-prenylation of benzenethiol, cysteamine and L-cysteine in aqueous solutions in the presence of KOH. For the preparation of S-prenyl benzenethiols 6 and 7, a solution of geranyl sulfate 3 (or neryl sulfate 4) in the lower layer of the equilibrium mixture n-butanol/water was treated

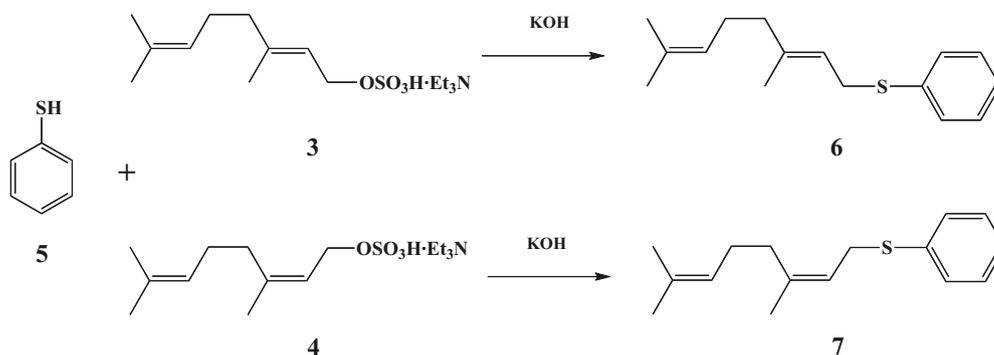
with benzenethiol 5 and aq. KOH (Scheme 2). The prenylation products 6 and 7 were extracted with light petroleum and isolated in 48 and 54% yields, respectively. The products were characterized by NMR, UV and EI-MS. We observed a large characteristic upfield shift of the signals for H-1 (δ 3.48 for 6 and 3.53 for 7) as compared with the signals for the sulfates 3 and 4 (δ 4.53 and 4.57, respectively) in ^1H -NMR spectra (see Table 1). ^{13}C -NMR spectra showed a large upfield shift of the C1 signal. Signals of pseudo-molecular ions (m/z 246.42 for 6 [M⁺] and 246.15 for 7 [M⁺]) for both thioethers in mass spectra were close to the calculated value of m , 246.41.

Cysteamine 8 was S-prenylated under similar conditions (Scheme 3), thus providing S-geranyl 9 (54%) and S-neryl 10 (76%) derivatives of cysteamine. Their structures were confirmed by the characteristic chemical shifts for H-1 signal in the ^1H -NMR spectra (see Table 1) and by MS data. The main signals in the mass spectra corresponded to the pseudo-molecular ions for compounds 9 (m/z 214.16 [M+H]⁺) and 10 (m/z 214.23 [M+H]⁺).

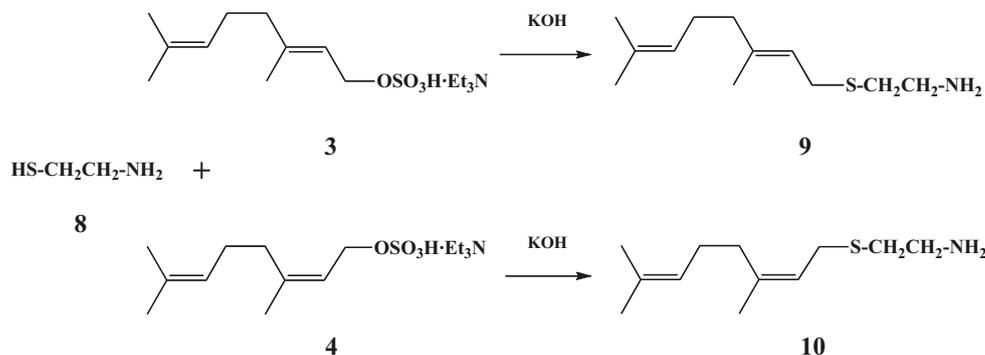
Worth noting is the observation that the yields of S-neryl derivatives of benzenethiol (7) and



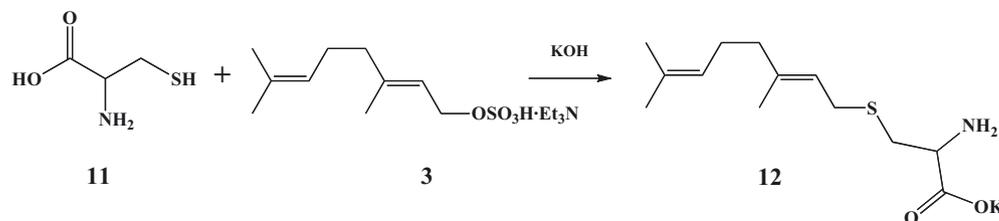
Scheme 1. Synthesis of prenyl sulfates 3 and 4.



Scheme 2. Synthesis of benzenethiol derivatives 6 and 7.



Scheme 3. Synthesis of cysteamine derivatives 9 and 10.

Scheme 4. Synthesis of *S*-geranyl cysteine 12.

cysteamine (10) were higher than those for the corresponding *S*-geranyl derivatives (6 and 9), thus indicating that prenyl sulfate 4 containing (*Z*)- α -isoprene unit seems to be a more efficient prenylating reagent than the (*E*)-isomer 3. However, since prenyl residues in natural prenylated proteins contain (*E*)-isoprene units, only geranyl sulfate 3 was used for *S*-alkylation of L-cysteine 11 (Scheme 4).

After the standard *S*-prenylation procedure, the reaction mixture was extracted with diethyl ether to remove triethylamine and geraniol (which was formed, probably, due to partial hydrolysis of 3). The desired geranyl cysteine 12 was easily purified and isolated as potassium salt in 43% yield. Structure of the product was confirmed by NMR (see Table 1) and MS. The high-field position (compared to 3) of the H-1 signal in the $^1\text{H-NMR}$ spectrum and the presence of the signal of the C=O group at δ_{C} 180.0 in the $^{13}\text{C-NMR}$ spectrum are characteristic of *S*-geranyl cysteine 12. The main signal in the mass spectrum corresponded to the pseudo-molecular ion of 12 (m/z 255.30 [M-H] $^-$).

In conclusion, a rather simple and convenient method has been developed for *S*-prenylation of thiols. Prenyl sulfates were used as alkylating reagents, which allowed the *S*-prenylation reactions to be carried out in aqueous solutions at room temperature. This provides a new chemical tool for introduction of lipid functionalities into cysteine-containing peptides.

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