Synthesis of prenylated cysteines from serine derivatives

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Prenylated cysteines are prepared by the reaction of serine β-lactone with prenyl thiolate.

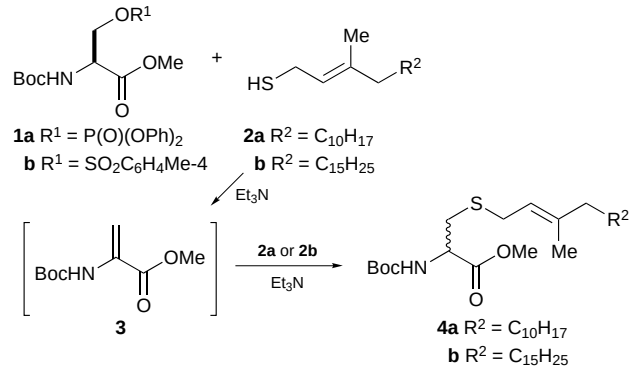
Prenylation, a recently discovered post-translational modification of proteins, has become a topic of substantial interest.[1–5] Prenylation involves covalent attachment of either farnesyl (C_{15}) or geranylgeranyl (C_{20}) isoprenoids to conserved carboxy-terminal cysteine residues by a family of prenyltransferase enzymes. Three types of carboxy-terminal cysteine sequences are substrates for this modification. The major class of these proteins has a CAAX motif (where C is a cysteine, A is usually an aliphatic amino acid and X is an amino acid that determines whether a protein is farnesylated or geranylgeranylated). The second and third types of proteins have CXC or CC motifs, respectively, in which both cysteines are geranylgeranylated. Subsequent to prenylation, proteins containing the CAAX motif are further processed by proteolytic removal of the three terminal amino acids (AAX) followed by methylation of the prenylcysteine carboxy group. Prenylation is common for many physiologically important proteins. Since oncogenic forms of Ras proteins require farnesylation for their ability to transform cells, inhibition of farnesylation has become a very attractive target for anti-cancer drug design.

Since Kamiya *et al.*[6] first synthesized farnesylcysteine, several methods for the preparation of prenylated cysteines and peptides have been reported.[7–9] These methods are based on alkylation of the sulfhydryl group of cysteine with prenyl halides. Despite the success achieved, there are two potential problems for prenylation of cysteine by this alkylation approach. Since basic conditions are generally required, free amino, hydroxy and carboxylate moieties, and especially other sulfide anions, are potential competing nucleophiles which can lead to major side reactions. The second and less important problem is that the required allylic prenyl halides are hard to purify. These potential difficulties led us to explore a new route to prenylated cysteines. Here we report a method for the synthesis of prenylated cysteines from a suitable serine precursor.

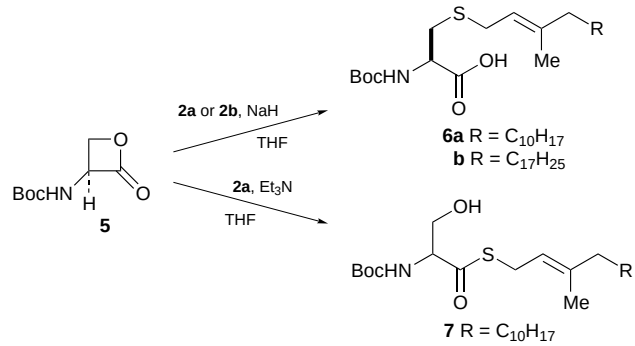
Our initial synthetic plan was based on activation of the serine hydroxy group and subsequent displacement with a prenyl thiol. *N*-Boc-*O*-diphenylphosphoryl-l-serine methyl ester **1a** and *N*- Boc-*O*-tosyl-l-serine methyl ester **1b** were prepared from Boc-l-serine methyl ester. The required prenyl thiols **2a** and **2b** were prepared from the C_{15} and C_{20} prenyl chlorides.[10] The low yields and lack of regiospecificity in the preparation of C_{15} and C_{20} prenyl halides were circumvented by using the method of Collington and Meyers.[11] Treatment of serine derivatives **1a** and **1b** with prenyl thiol **2a‡ or** 2b in the presence of triethylamine yielded racemic prenylated cysteine **4§** (Scheme 1). The isolation of **3** suggested to us that β-elimination may be the initial step of the reaction followed by Michael addition of the anion of **2a** or **2b** to the α,β-unsaturated substrate **3** to give the observed racemic mixture.

In order to avoid the above stereochemical problems, we turned to an alternative sequence. The synthetic methodology developed by Arnold *et al.*[12] for the preparation of β-substituted alanines and their derivatives seemed applicable to our problem. Thus we undertook the synthesis of prenylated cysteines by the stereospecific opening of serine β-lactone with prenyl thiols. The serine β-lactone **5** was prepared by reaction of Boc-serine with triphenylphosphine and diethyl azodicarboxylate (DEAD).[12]

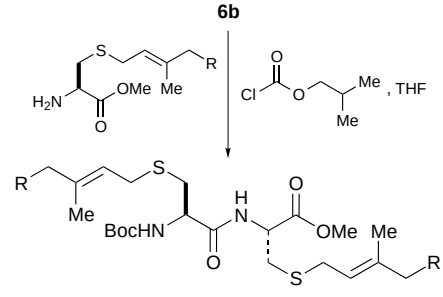
Although there are two possible ways of opening the β-lactone as shown in Scheme 2,[13] alkyl–oxygen cleavage occurred when farnesyl thiolate, prepared by the reaction of farnesyl thiol with sodium hydride, was used to give the desired stereochemically pure prenylated cysteines **6a¶** and **6b** in 52–60% yield. It should be noted that acyl–oxygen fission occurred when triethylamine was used as the base to give thioester **7** as the major product (61%). With **6b** in hand, the dipeptide **9‖** with two geranylgeranylated cysteines was pre-



Scheme 1



Scheme 2



Scheme 3

pared by coupling of **5b** with geranylgeranylated cysteine methyl ester **8** *via* the mixed anhydrides method.[14] Dipeptide **9** may serve as an important model compound to study geranylgeranylated Rab proteins.[2]

In summary we have described a practical and novel route to stereospecifically synthesize prenylated cysteines from serine β-lactone. The optical purity and simplicity of this approach to prenylated cysteines are attractive features compared with previous methods.[8]

Footnotes

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‡ All new compounds described herein have been fully characterized by ^{1}H and ^{13}C NMR spectral, HRMS and elemental analyses. *Selected data* for **2a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35 (m, 1 H), 5.10 (m, 2 H), 3.16 (dd, *J* 7.5, 0.6 Hz, 2 H), 2.11–1.97 (m, 8 H), 1.68 (d, *J* 0.9 Hz, 3 H), 1.66 (d, *J* 0.9 Hz, 3 H), 1.60 (s, 6 H), 1.40 (t, *J* 7.2 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.50, 135.30, 131.29, 124.31, 123.72, 123.28, 39.68, 39.38, 26.70, 26.27, 25.69, 22.11, 17.68, 16.02, 15.77; ν_{max}(neat)/cm^{-1} 2966.7, 2926.2, 1446.7, 1381.1 (Calc. for $C_{15}H_{26}S$: C, 75.56; H, 10.99. Found: C, 75.43; H, 11.03%).

§ *Selected data* for **4a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.31 (d, *J* 6.6 Hz, 1 H), 5.21 (t, *J* 7.8 Hz, 1 H), 5.11–5.06 (m, 2 H), 3.76 (s, 3 H), 3.18 (t, *J* 8.1 Hz, 2 H), 2.95–2.81 (m, 2 H), 2.07–2.00 (m, 8 H), 1.68 (d, *J* 1.2 Hz, 3 H), 1.67 (d, *J* 1.2 Hz, 3 H), 1.60 (s, 6 H), 1.45 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.94, 155.39, 140.16, 135.57, 131.51, 124.52, 123.93, 119.87, 80.27, 53.45, 52.67, 39.90, 39.83, 33.86, 30.23, 28.51, 26.92, 26.62, 25.91, 17.89, 16.34, 16.22. (Calc. for $C_{24}H_{41}NO_4S$: C, 65.57; H, 9.40; N, 3.19. Found: C, 65.63; H, 9.47; N, 3.15%).

¶ *Selected data* for **6a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35 (m, 1 H), 5.23 (t, *J* 7.8 Hz, 1 H), 5.09 (t, *J* = 6.9 Hz, 2 H), 4.48 (brs, 1 H), 3.21 (m, 2 H), 2.93 (m, 1 H), 2.09–2.00 (m, 8 H), 1.68 (d, *J* 0.9 Hz, 3 H), 1.67 (d, *J* 1.2 Hz, 3 H), 1.60 (s, 6 H),1.46 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.00, 155.74, 140.11, 135.44, 131.46, 124.52, 124.01, 119.73, 80.03, 54.17, 39.90, 39.83, 29.90, 28.62, 28.54, 26.92, 26.70, 25.91, 17.89, 16.37, 16.21 (HRMS FAB): calc. for $C_{23}H_{39}NO_4S$, 424.2522. Found, 424.2503).

‖ *Selected data* for **9**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (d, *J* 6.9 Hz, 1 H), 5.36 (brs, 1 H), 5.28–5.17 (m, 2 H), 5.11–5.07 (m, 6 H), 4.80–4.67 (m, 1 H), 4.30 (brs, 1 H), 3.23–3.07 (m, 4 H), 3.00–2.80 (m, 2 H), 2.10–1.98 (m, 24 H), 1.68 (s, 6 H), 1.60 (s, 18 H), 1.47 (s, 9 H); (HRMS CI): calc. for $C_{52}H_{86}N_2O_5S_2$ 882.5978. Found 882.5811).

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