Fe is externally bound or it readily passes into and out of the C_{60}. This latter possibility is unlikely, given the radius of Fe^+ of $\simeq$0.96 Å and that of Fe of $\simeq$1.24 Å.[14] Furthermore, these results also provide strong support for internally bound "eggshell" MC_{60}^+ (M = La, K, Cs) species reported earlier, "grown" in a supersonic expansion following laser desorption from a metal-impregnated graphite target, since these latter species are highly stable and lose C_2 molecules when sufficiently activated.[3,4] In summary, if Fe is indeed bound externally, generation of MC_{60}^+ in this way with other metal ions such as La^+ should result in externally bound isomeric complexes.[7] We are currently attempting to generate this new family of ions.

The ligand displacement reactions imply $D°(Fe^+-C_nH_{2n}) \simeq$ 40 kcal/mol[15] < $D°(Fe^+-C_{60})$. Similar displacement reactions should provide a bracket for $D°(Fe^+-C_{60})$ and, likewise, for other metal cations. Finally, reactions other than displacement may occur with these externally bound species and are also being investigated in our laboratory.

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(14) Taken from the following: *Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1972. The value for Fe^+ is an estimate based on the values for Fe, Fe^{2+}, and Fe^{3+}.

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Heteroatom Requirements for Substrate Recognition by GTP-Binding Protein Methyltransferase

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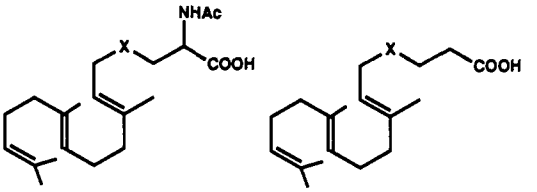
Signal transducing G proteins are posttranslationally modified by isoprenylation.[1-7] This process occurs at protein carboxyl termini ending in a CAAX motif (C = cysteine, A = aliphatic amino acid, and X = any amino acid).[1-3,8] The cysteine residue is enzymatically farnesylated or geranylgeranylated at sulfur in an isoprenyltransferase-mediated process using an isoprenyl pyrophosphate as substrate.[9-11] This reaction is followed by a protease-mediated cleavage that occurs after the modified cysteine, and finally by a *S*-adenosylmethionine- (SAM-) linked methylation at the newly generated cysteine carboxyl terminus.[12-18]

In studies on retinal rod outer segment GTP-binding protein methyltransferase, it was found that the enzyme can process relatively simple substrate molecules. For example, it can methylate *N*-acetyl-*S*-farnesyl-L-cysteine[14] (AFC, **1**) and *S*-farnesylthiopropionic acid (FTP, **2**), with K_M values of 23 and 14 μM, respectively.[19] In both cases all remnants of the protein backbone are gone, suggesting that the enzyme recognizes only the 3-farnesylthiopropionic acid moiety, and not the carrier protein. In this communication we further investigate the nature of the specificity requirements for the enzyme and show the importance of the unmodified sulfur atom in defining whether a molecule is a substrate or inhibitor of the G protein methyltransferase.



1: X=S **2**: X=S
3: X=S=O **5**: X=Se
4: X=Se **7**: X=O
6: X=O **8**: X=NH

To probe the role of the sulfur in **1**, the sulfoxides **3** (1:1 mixture of diastereomers) were studied. These sulfoxide analogues were previously shown to be completely inactive as substrates for the enzyme[19] and are shown here to be potent competitive inhibitors of it (Figure 1), with a K_i of 13.2 μM for the mixture. Moreover, no stereoselection at sulfur was observed. The diastereomeric sulfoxides were separated by HPLC on silica.[20] The less polar diastereomer was equipotent with the diastereomeric mixture as an inhibitor. These observations show that the nucleophilic sulfur atom is crucial for substrate activity, but not for binding to the enzyme. The nature of the sulfur requirement was further explored by studying the racemic selenocysteine analogue **4**. This molecule proved to be a substrate for the enzyme (Table I). The K_M was close to that of **1**, assuming stereospecificity, but the V_{max} was substantially reduced. In addition **5**, the selenopropionate analogue of **2**, was also a substrate for the enzyme, but had a larger K_M and a smaller V_{max} than found with **2** (Table I).

Several attempts were made to synthesize *N*-acetyl-*O*-farnesyl-L-serine (**6**) with little success due to the great lability of this molecule. However, **7**, the oxo analogue of **2**, proved to be an exceedingly weak substrate for the methyltransferase, but bound to the enzyme approximately as well as the other propionate analogues (Table I). *Thus, the major differences between the S, S=O, Se, and O substrates appear primarily in the V_{max} term, rather than in the K_M term.*

One possible mechanism to account for the observations described above is one in which initial full or partial transmethylation occurs between SAM and the heteroatom of the methyltransferase

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(20) The retention times were 7.8 and 8.3 min eluting at 2 mL/min with 1:3 2-propanol/hexane containing 0.1% 1,1,1-trifluoroacetic acid.

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Table I. Substrate Activities of Analogues[23a]

substrate analogue	K_M, μM	V_{max}, pmol min^{-1} mg^{-1}
1	23.1 ± 1.4	14.0 ± 0.3
2	14.0 ± 1.1	14.0 ± 0.3
4	50.6 ± 5.4	3.4 ± 0.1
5	29.0 ± 4.7	7.4 ± 0.4
7	25.8 ± 3.6	0.6 ± 0.02

[a] Methyltransferase activity was determined as described in the legend of Figure 1. Kinetic constants for **1** and **2**[19] are given here for comparison.

substrate. Intramolecular transfer of the methyl group to the free carboxyl moiety produces the neutral ester. A mechanism of this type would develop positive charge on the sulfur atom, suggesting that β-alanine derivatives like **8** be studied as possible substrates or transition-state inhibitors of the enzyme. However, **8** proved to be neither a substrate nor an inhibitor of the methyltransferase. Therefore, there is currently no evidence for a mechanism involving initial transfer of the methyl group to the sulfur atom of the substrate. Mechanisms of this type also seem unlikely since model studies suggest that an "in-line" displacement mechanism is required in methyl group transfers from sulfur.[21,22]

The studies described here demonstrate a great deal of specificity of the methyltransferase for the farnesylthiopropionic acid moiety. Here we show a clear preference for the presence of a nucleophilic sulfur atom in an active substrate. Alterations in this heteroatom progressively lead from substrate molecules to competitive inhibitors to an inert molecule in the case of **8**. Although the methyltransferase's function is to modify a large protein, in seeking the protein the enzyme looks not for the protein itself but for a small prosthetic unit and, indeed, not for the entire prosthetic unit but for a specific moiety within it.

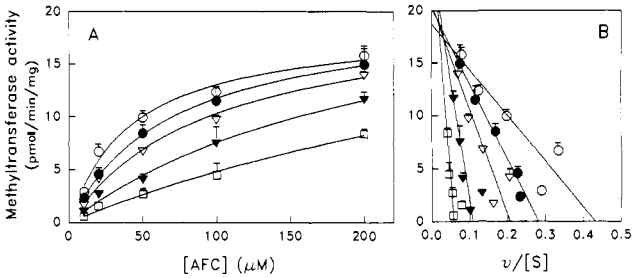

Figure 1. Inhibition of **1** methylation by **3**. Michaelis-Menten (A) and Eadie–Hofstee (B) plots of the formation of **1** [^{3}H]methyl ester as a function of **1** concentration in the presence of increasing concentrations of **3**: (○) no **3** and (●) 10, (△) 20, (▼) 50, and (□) 100 μM **3**. Assays were performed as described in ref 14. Briefly: **1** and **3**, were dissolved in Me_2SO and incubated with washed rod outer segment membranes (0.5 mg of protein/mL) and *S*-adenosyl[*methyl*-^{3}H]methionine (10 μM, 15 Ci/mmol) in 100 mM Hepes (pH 7.4), 100 mM NaCl, 5 mM $MgCl_2$ for 30 min at 37 °C. Final Me_2SO concentration in the assay was 4% (v/v). The amount of the corresponding *methyl*-^{3}H esters was determined by HPLC analysis of the chloroform extracts obtained from the incubation mixtures. Samples were injected in 15% 2-propanol/hexane on a normal-phase HPLC column (Dynamax 60) connected to an on-line Berthold radioactivity monitor and eluted with the same solvent at 1.5 mL/min. Symbols represent average values of four determinations and error bars indicate the standard deviation of mean.

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(23) **4** was prepared from DL-selenocystine by esterification followed by N-acetylation, reduction with zinc, S-alkylation with *trans,trans*-farnesyl bromide, and saponification. Chromatography of the ester on a flash silica column, eluting with hexane/ethyl acetate (4:1–1:2), gave the methyl ester of **4**, as a light yellow oil. [NMR (300 MHz, $CDCl_3$) δ 1.57 (6 H, s), 1.63 (3 H, s), 1.65 (3 H, s), 1.8–2.1 (8 H, m), 2.01 (3 H, s), 2.79 (1 H, dd, J = 5.6, 12.9 Hz), 2.89 (1 H, dd, J = 5.6, 12.9 Hz), 3.20 (2 H, m), 3.73 (3 H, s), 4.85 (1 H, dt, J_d = 7.5 Hz, J_t = 5.1 Hz), 5.05 (2 H, br t, J = 6.5 Hz), 5.26 (1 H, t, J = 8.1 Hz), 6.28 (1 H, br d, J = 7.5 Hz)]. The methyl ester of **4** (0.10 g, 0.2 mmol) was treated with 5% potassium hydroxide/methanol (10 mL) for 30 min, and the mixture was acidified to approximately pH 5 with 2 M HCl, diluted with 30 mL of water, and extracted with chloroform. The organic extract was dried, concentrated to a small volume, and chromatographed on a silica preparative layer, eluting with ethyl acetate/methanol (4:1) to give **4** as a waxy solid. The NMR spectrum of **4** was essentially identical with that of the methyl ester except that the singlet resonance at δ 3.73 ppm was absent. **5** was prepared by treating *trans,trans*-farnesyl bromide with selenourea, hydrolysis of the selenoronium salt, and conjugate addition of the selenol to methyl acrylate. Chromatography of the ester on silica eluting with ethyl acetate/hexane (1:5) gave the methyl ester of **5** as a colorless oil [NMR (300 MHz, $CDCl_3$) δ 1.58 (6 H, s), 1.65 (3 H, s), 1.66 (3 H, s), 1.9–2.2 (8 H, m), 2.6–2.8 (4 H, m), 3.24 (2 H, d, J = 8.2 Hz), 3.67 (3 H, s), 5.07 (2 H, br t, J = 6.2 Hz), 5.31 (1 H, t, J = 8.2 Hz)]. Saponification of the methyl ester as described above and chromatography on silica, eluting with ethyl acetate/hexane (1:4–1:1), gave the product as a thick colorless oil. The NMR spectrum of **5**, was identical with that of the methyl ester except that the singlet resonance at δ 3.67 ppm was absent. **6** was prepared by a conjugate addition of *trans,trans*-farnesol to methyl acrylate followed by saponification. This ester was chromatographed on flash silica eluting with ethyl acetate/hexane (1:9–1:3). The methyl ester was obtained as a colorless oil [NMR (300 MHz, $CDCl_3$) δ 1.58 (6 H, s), 1.65 (3 H, s), 1.66 (3 H, s), 1.9–2.2 (8 H, m), 2.57 (2 H, t, J = 6.3 Hz), 3.68 (3 H, s), 3.68 (2 H, t, J = 6.3 Hz), 3.98 (2 H, d, J = 6.6 Hz), 5.07 (2 H, m), 5.31 (1 H, t, J = 6.6 Hz)]. Saponification of the methyl ester (50 mg) was carried out as described above. Chromatography on silica eluting with ethyl acetate/hexane (1:4–1:1) gave the product as a thick colorless oil. The NMR spectrum of **6** was identical with that of the methyl ester except that the singlet resonance at δ 3.68 ppm was absent. **7** was prepared by formation of the Schiff base between β-alanine methyl ester and *trans,trans*-farnesal followed by reduction with sodium borohydride and saponification. This ester was chromatographed on a preparative silica layer, eluting with methanol/ethyl acetate (1:2). The methyl ester of **7** had an R_f of 0.5 and was isolated as a colorless oil in 19% yield [NMR (300 MHz, $CDCl_3$) δ 1.58 (6 H, s), 1.75 (6 H, s), 1.9–2.1 (8 H, m), 2.47 (2 H, t, J = 7.5 Hz), 2.83 (2 H, br t, J = 6.5 Hz), 3.18 (2 H, br d, J = 6.0 Hz), 3.64 (3 H, s), 5.05 (2 H, br m), 5.22 (1 H, br t, J = 6.0 Hz)]. Saponification of the methyl ester using the procedure described above and chromatography on a preparative silica layer, eluting with methanol/ethyl acetate (1:1), gave the product (R_f = 0.3) as a waxy solid [NMR (300 MHz, $CDCl_3$) δ 1.58 (6 H, s), 1.75 (6 H, s), 1.9–2.1 (8 H, m), 2.88 (2 H, br t, J = 7.5 Hz), 3.18 (2 H, br m), 3.65 (2 H, br d, J = 5.9 Hz), 5.05 (2 H, br m), 5.39 (1 H, br t, J = 5.9 Hz)].

A Highly Efficient Multienzyme System for the One-Step Synthesis of a Sialyl Trisaccharide: In Situ Generation of Sialic Acid and *N*-Acetyllactosamine Coupled with Regeneration of UDP-Glucose, UDP-Galactose, and CMP-Sialic Acid[1]

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Increasing interests in sialyl oligosaccharides[2] necessitate the development of practical methodology for the synthesis of this class of molecules. Although chemical methods for the synthesis of sialosides have been highly developed,[3] the necessary multistep protection/deprotection procedure makes large-scale process

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