Research Article

Synthesis of high specific activity ^{35}S-labelled *N*-methanesulfonyl farnesylcysteine and a photoactive analog

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Summary

Prenylated cysteine analogs, which mimic the prenylated cysteine residue of prenylated GTP-binding proteins (G-proteins), have been used in a variety of contexts for the study of prenylated G-protein behavior. In earlier work in this area, we prepared the photoactive analog [^{35}S]**4** and showed that it labelled RhoGDI upon photolysis; those results were consistent with the idea that GDI contains an isoprenoid binding site. Here, we describe the preparation of [^{35}S]*N*-methanesulfonyl labelled analogs (**1a** and **2a**) of *N*-acetyl farnesylcysteine and its methyl ester together with an improved synthetic procedure for photoactive analogs **3** and **4**; specific activities of ~ 1100 Ci/mmol were achieved. Compounds **1a** and **2a** in unlabelled form were used as competitors in photolysis reactions to show that the methanesulfonamido group is a reasonable acetamide substitution. Additional experiments show that the photoactive ester [^{35}S]**3** can cross-link GDI in both purified form and crude bacterial extract. However, the extent of cross-linking obtained with the ester ([^{35}S]**3**) is significantly less than that observed with the free acid ([^{35}S]**4**) despite the fact that the esterified form probably more closely reflects the structure of

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the C-terminus of a prenylated protein; using the GDI·Cdc42 co-crystal structure, the structural basis for these results is discussed. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: ^{35}S; prenylcysteine; photoaffinity labelling; isoprenoid binding site; RhoGDI

Introduction

The process of protein prenylation, whereby a C_{15} or C_{20} isoprenoid is covalently attached to a specific C-terminal cysteine residue of certain GTP-binding proteins (G-proteins) via a thioether linkage, has been widely studied since the discovery that certain prenylated proteins play a key role in the development of a range of cancers.[1–4] Mutations in Ras proteins, which undergo farnesyl (C_{15}) addition, have been found in roughly 30% of human tumors[3] and recently, members of the Rho protein family, the majority of which undergo geranylgeranyl (C_{20}) addition, have been implicated in the progression of cancer.[5–7] Prenylation is required for both normal and oncogenic biological activity of these proteins as prenylation enhances their hydrophobicity, allowing for translocation from the cytosol to membranes where further signaling events transpire.[8]

Prenyltransferases catalyze the addition of an isoprenoid moiety from a prenyl diphosphate to the G-protein substrate. These enzymes have been extensively examined for their therapeutic potential and numerous inhibitors have been generated towards this end.[9, 10] Following the initial prenyl attachment, additional processing occurs: an endoprotease removes those residues distal to the modified cysteine, then methylation of the free carboxylic terminus follows. These latter processing steps are gaining more attention[11–13] since an alternative means of stopping cancerous signal transduction may lie in the disruption of prenylated protein–protein interactions. The contacts between particular prenylated proteins and their cognate target protein may be more specific than the broad specificity shown by the prenyltransferases for G-proteins. Several studies have suggested or confirmed the existence of isoprenoid binding sites within proteins which interact with prenylated proteins, implying a purpose for the prenyl group beyond the simple facilitation of hydrophobic interactions with

membranes.[1,8,14–21] The design of compounds which interfere with key interactions between a prenylated protein and a cognate receptor protein offers a promising, and potentially more specific, alternative to the current approaches that target inhibition of the prenylation step.

With the goal of understanding how prenylated proteins interact with other proteins, we and others have designed compounds which mimic the prenylated cysteine residue of G-proteins. Prenylated cysteine analogs such as farnesylated cysteine, *N*-acetyl farnesylcysteine and its methyl ester (FC, AFC (**2b**) and AFCE (**1b**), respectively, shown below), and the geranylgeranylated counterparts (GGC, AGGC, AGGCE) have been used in a variety of experiments for the study of prenylated protein properties and behavior.[19–41] Noteworthy in the context of studying specific prenylated protein–protein interactions are their use in examining the endoprotease and methyltransferase enzymes, both of which operate only on prenylated substrates.[22,24,30,34,36,38,40]
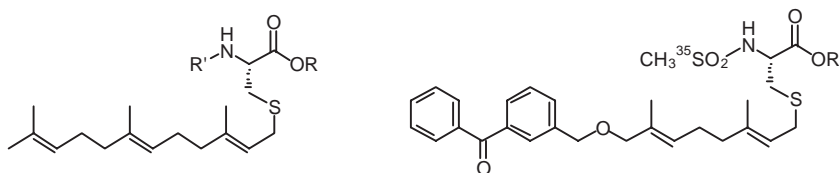
1a R = CH$_3$, R' = ^{35}SO$_2$CH$_3$
1b R = CH$_3$, R' = CH$_3$CO
2a R = H, R' = ^{35}SO$_2$CH$_3$
2b R = H, R' = CH$_3$CO

3 R = CH$_3$
4 R = H

In the studies mentioned above, the cysteine amine was typically acetylated. However, in our work, we were interested in exploring the use of analogs that incorporate a methanesulfonyl moiety in lieu of the more typical acetamide. The rationale for this modification was based on work reported by Dean *et al.*[42] who showed that methanesulfonyl chloride incorporating an ^{35}S radiolabel could be used to generate high specific activity (1100 Ci/mmol) compounds from amine precursors. In our initial work in this area,[41] we prepared a photoactive analog, [^{35}S]**4**, of *N*-acetyl farnesylcysteine, **2b**, that contained a benzophenone photophore and an ^{35}S radiolabel. Compound [^{35}S]**4** was used in cross-linking experiments and was shown to label the protein RhoGDI (Rho GDP-dissociation inhibitor) at physiological meaningful concentrations (<1 μM). That biochemical data was consistent with recent structural information which suggested that GDI contains an isoprenoid binding site and that the site is occupied by the prenylated C-terminus of Cdc42 in the GDI·Cdc42 binary complex.[19–21]

The above cross-linking results suggested that analog [^{35}S]**4** and related compounds should be useful tools for probing for isoprenoid bindings sites in GDI homologs and other proteins that are known to interact with prenylated proteins. However, those studies were incomplete in the sense that they did not allow us to compare the relative binding efficiencies of the sulfonamido- and acetamido-containing analogs to assess whether the sulfonamide was a reasonable substitution. More importantly, compound [^{35}S]**4** is a cysteine analog containing a free carboxylate whereas most prenylated proteins exist as C-terminal methyl esters.[4,11,13] Thus, we felt compelled to examine the ester analog in experiments similar to those performed previously with [^{35}S]**4**.

In this paper, we first describe the preparation of [^{35}S]**1a** and [^{35}S]**2a** which are sulfonylated analogs of *N*-acetyl farnesylcysteine methyl ester **1b** and the corresponding free acid **2b**. Those compounds, in unlabelled form, were then used as competitors in photolysis reactions containing the previously described acid-containing analog, [^{35}S]**4**, to assess the relative affinities of the sulfonamides versus the acetamides. Those experiments demonstrate that the sulfonamido- and acetamido-containing analogs reduce the extent of photocross-linking of GDI by [^{35}S]**4** by comparable amounts, suggesting that the methanesulfonamido group is a reasonable acetamide substitution. A revised synthetic procedure for ester [^{35}S]**3** and acid [^{35}S]**4** is also presented here. Significant improvements in the radiochemical purity of [^{35}S]**3** (an increase of 37 to 95%) and radiochemical yield for [^{35}S]**4** (an increase from 5 to 26%) have been obtained using the modified synthesis and purification conditions. Finally, we demonstrate that the photoactive ester [^{35}S]**3** can be used to cross-link GDI in both purified form and crude bacterial extract. However, the extent of cross-linking obtained with the ester ([^{35}S]**3**) is significantly less than that observed with the free acid ([^{35}S]**4**) despite the fact that the esterified form probably more closely reflects the structure of the C-terminus of a prenylated protein; using the GDI·Cdc42 co-crystal structure, the structural basis for these results is discussed.

Results and discussion

Synthesis of [^{35}S]N-Methanesulfonyl Farnesylcysteine Analogs ([^{35}S]1a, [^{35}S]2a)

The alkylation of cysteine, within a peptide or alone, by a halogenated allylic isoprenoid has been performed in a variety of ways[43–47] but we
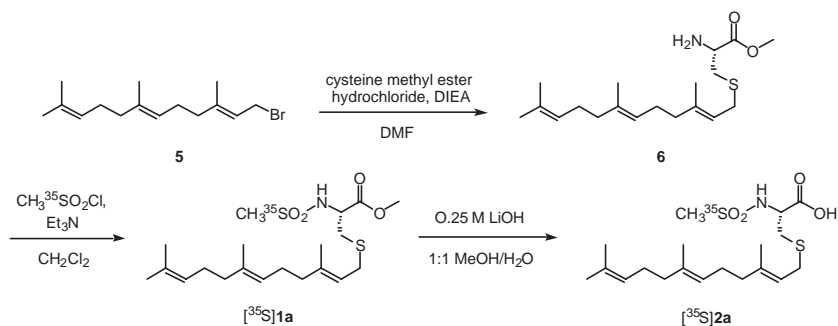
Scheme 1. Synthesis of ^{35}S-labelled farnesylated cysteine analogs

have found the method by Kania and co-workers to be the most efficient for the purpose of prenylating a single cysteine methyl ester in its free amine form.[43] Thus, farnesyl bromide was used under basic conditions with cysteine methyl ester hydrochloride to generate farnesylated cysteine methyl ester, **6** (FCE, Scheme 1).

To introduce the [^{35}S]sulfonamide linkage, the method of Dean *et al.* was followed.[42] A dilute solution of [^{35}S]methanesulfonyl chloride (10 mCi, 1198 Ci/mmol) in anhydrous CH_2Cl_2 was concentrated and added to a solution of FCE and Et_3N in anhydrous CH_2Cl_2. Following quenching of the reaction with MeOH, part of the crude reaction mixture was used directly in the hydrolysis reaction while the remainder was purified by reversed-phase HPLC to afford compound [^{35}S]**1a** in 20% radiochemical yield (92.1% radiochemical purity). Generation of the free acid was carried out using LiOH, and the product, [^{35}S]**2a**, was purified (85% radiochemical yield from crude [^{35}S]**1a**) and analyzed by reversed-phase HPLC (93.0% radiochemical purity). The reversed-phase HPLC chromatograms of the radiolabelled products, the retention times of which matched those of unlabelled **1a** and **2a**, are shown in Figure 1.

Synthesis of [^{35}S]N-Methanesulfonyl Cysteine (S-(E,E)-8-O-(3-Benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) Analogs ([^{35}S]3, [^{35}S]4)

Photoaffinity labelling can provide structural information for proteins and is an especially useful technique when NMR and/or X-ray

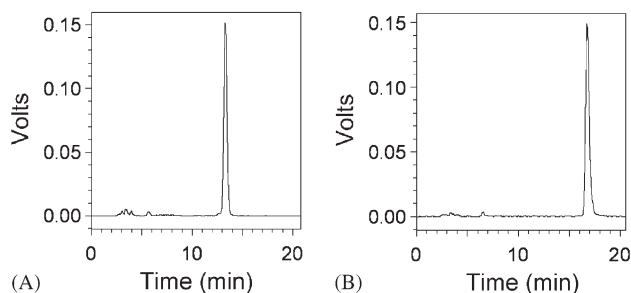**Figure 1. Reversed-phase HPLC chromatograms showing the radioactivity associated with the purified products [^{35}S]1a (panel A) and [^{35}S]2a (panel B). Radioactivity is reported in units of volts. HPLC elution conditions for[^{35}S]1a: 28% 0.1% aq. HClO$_4$/72% CH$_3$CN, isocratic. HPLC elution conditions for[^{35}S]2a: 40% 0.1% aq. HClO$_4$/60% CH$_3$CN, isocratic**

crystallographic data are difficult to obtain.[48–50] The high specific activity that can be obtained using the [^{35}S]sulfonamide group is particularly attractive for photoaffinity labelling experiments. Given this useful feature, we elected to prepare compounds [^{35}S]3 and [^{35}S]4 in which the prenyl group has been replaced with a photoactive isoprenoid analog incorporating a benzophenone photophore. Compounds [^{35}S]3 and [^{35}S]4 can be photolyzed with putative prenyl binding site-containing proteins for the purpose of exploring the makeup of the binding site.[51] Identification and characterization of cross-linked residues can be greatly facilitated by the presence of the sensitive ^{35}S radiolabel. The isoprenoid-linked benzophenone unit, while somewhat bulkier than either the farnesyl or geranylgeranyl isoprenoids, is still an effective mimic of the natural substrates.[52–56] As previously mentioned, a crystal structure of a geranylgeranylated Rho protein, Cdc42, in complex with GDI, was recently obtained (see Photoaffinity Labelling discussion below).[21] Examination of Figure 2, showing the super-positioning of [^{35}S]4 with the crystal structure of only the geranylger-anylated cysteine residue of Cdc42, reveals good complementarity between the structure of **4** and the conformation of the bound geranylgeranyl group.

The preparation of bromide **8** (Scheme 2) was reported in earlier work,[41,57] and the alkylation of cysteine methyl ester under basic conditions to yield **9** was accomplished as described for the farnesylated analogs above.[41] The following reactions, using first [^{35}S]methanesulfo-nyl chloride to generate the radiolabelled sulfonamide linkage ([^{35}S]3),
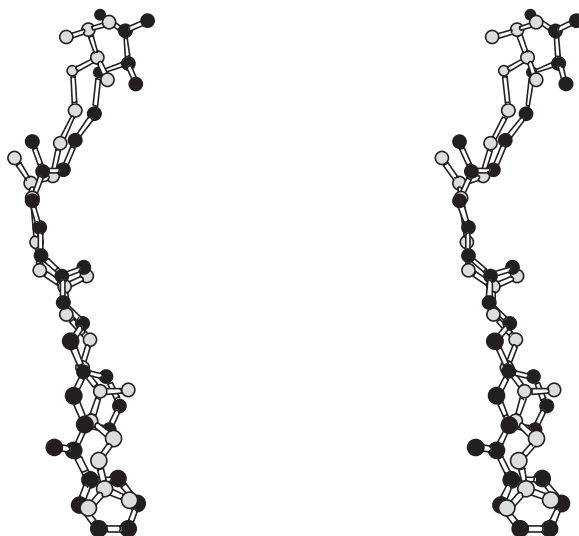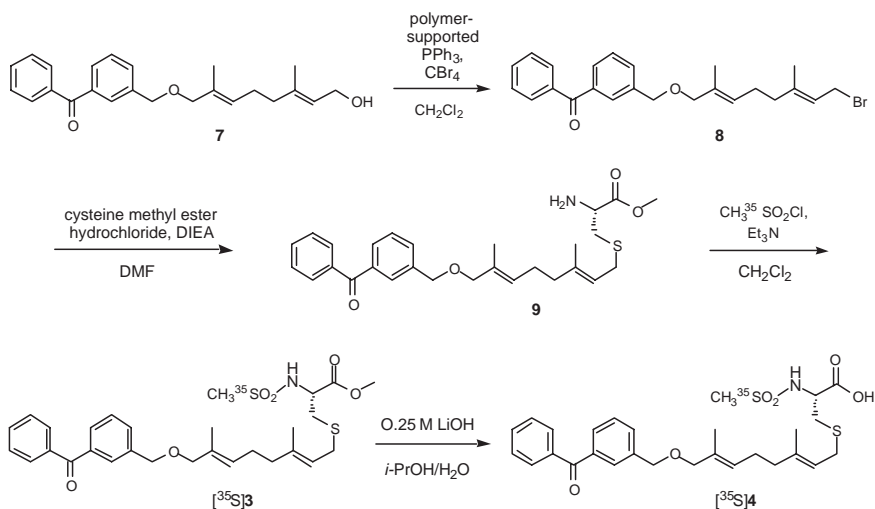
Figure 2. Stereo representation of the superposition of [^{35}S]4 (black atoms) and a geranylgeranylated cysteine residue from Cdc42 bound to RhoGDI[21] (gray atoms)

Scheme 2. Synthesis of photoaffinity labelled ^{35}S-labelled prenylcysteine analogs

and the subsequent ester hydrolysis to produce [^{35}S]4, proceeded in the same fashion as for the farnesylated compounds [^{35}S]1a and [^{35}S]2a. Although products [^{35}S]3 and [^{35}S]4 were obtained in lower radio-chemical yields, their radiochemical purities were improved (Table 1).

Furthermore, in comparison with a previously described preparation of these materials,[41] an improved purity of [35S]**3** was obtained and the radiochemical yield of [35S]**4** was greatly increased while a high degree of radiochemical purity was preserved.

Figure 3 shows the HPLC chromatograms of the radiolabelled benzophenone-containing prenylcysteines, the retention times of which matched those of the unlabelled counterparts. Radiochemical yields and purities for all radioactive products ([35S]**1**-[35S]**4**) are presented in Table 1.

[35S]**1a** and [35S]**2a** as Mimics of AFCE (**1b**) and AFC (**2b**)

As mentioned above, farnesylcysteines **1b** and **2b** have been used extensively to study prenylated protein behavior. The radiolabelled *N*-methanesulfonamide compounds [35S]**1a** and [35S]**2a** were prepared as a means to access highly radioactive analogs of these commonly used

Table 1. Radiochemical yields and purities of synthetic prenylcysteine analogs [35S]1–[35S]4.

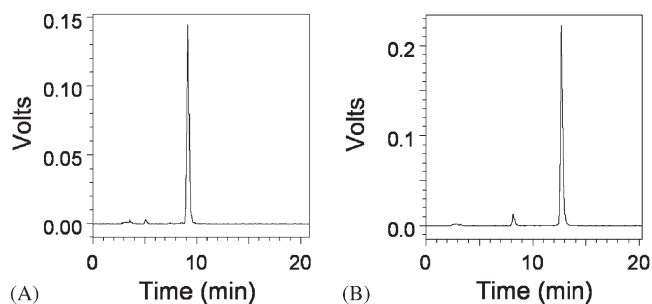| Product	Radiochemical Yield (%)	Radiochemical Purity (%)
[35S]**1a**	20	92.1
[35S]**2a**	85	93.0
[35S]**3**	5	95.3
[35S]**4**	26	93.4

Figure 3. Reversed-phase HPLC chromatograms showing the radioactivity associated with the purified products [35S]3 (panel A) and [35S]4 (panel B). Radioactivity is reported in units of volts. HPLC elution conditions for [35S]3; 30% 0.1% aq. HClO₄/70% CH₃CN, isocratic. HPLC elution conditions for [35S]4: 50–100% CH₃CN in 0.1% aq. HClO₄ in 20 min

protein prenylcysteine mimics. To test their effectiveness and to examine the influence — if any — of the methanesulfonyl substitution, competition experiments were performed by separately irradiating unlabelled compounds **1a**, **2a**, **1b**, and **2b** in the presence of [^{35}S]**4** and GDI. In earlier work, Rando and co-workers developed a fluorescence assay to study GDI in which the dissociation constants of a number of alkylated cysteines were determined.[39] GDI is a multifunctional cytosolic protein which operates only on Rho proteins in their prenylated form.[58] Rho proteins themselves have been shown to be necessary for oncogenic Ras transformation.[5–7,59,60] A distinctive feature of GDI is its ability to extract membrane-bound prenylated Rho proteins from membranes; therefore, GDI likely plays a key role in regulating the signaling activities of these G-proteins.[61] NMR studies suggest not only the presence of an isoprenoid binding site within GDI, but that the prenylated cysteine analog AFC binds in the pocket.[19,20] The X-ray crystal structure of GDI complexed with a geranylgeranylated Rho protein (Cdc42) provided additional confirmation: the Rho protein prenyl moiety was found to be localized to a hydrophobic pocket of GDI.[21]

Figure 4(a) shows the results of the competition experiments. Because [^{35}S]**4** was found to be a more effective cross-linking reagent than its ester counterpart, [^{35}S]**3** (Figure 4(b); see also the Photoaffinity Labelling discussion below), [^{35}S]**4** was used to test these compounds. At competitor concentrations of 200 μM, one can see that the *N*-methanesulfonyl farnesylcysteines compare favorably with the *N*-acetylated analogs. Cross-linking by [^{35}S]**4** is similarly reduced in the presence of each of the four compounds (lanes 2-5), and is significantly reduced by increasing the concentration of **1a** (lane 6). These results suggest that compounds **1a** and **2a** are effective agents for investigating activity associated with prenylated protein behavior, and imply that the use of the highly radioactive forms of these compounds will be beneficial in a variety of future applications.

Photoaffinity Labelling of RhoGDI with [^{35}S]3 and [^{35}S] 4

Previous photolysis work with [^{35}S]**4** and GDI implied that this photoaffinity labelled compound was an effective probe for studying isoprenoid binding sites.[41] Because of the preponderance of C-terminal cysteine carboxymethylated prenylated proteins,[4,11,13] the ester precursor to this prenylcysteine free acid, [^{35}S]**3**, was examined for its
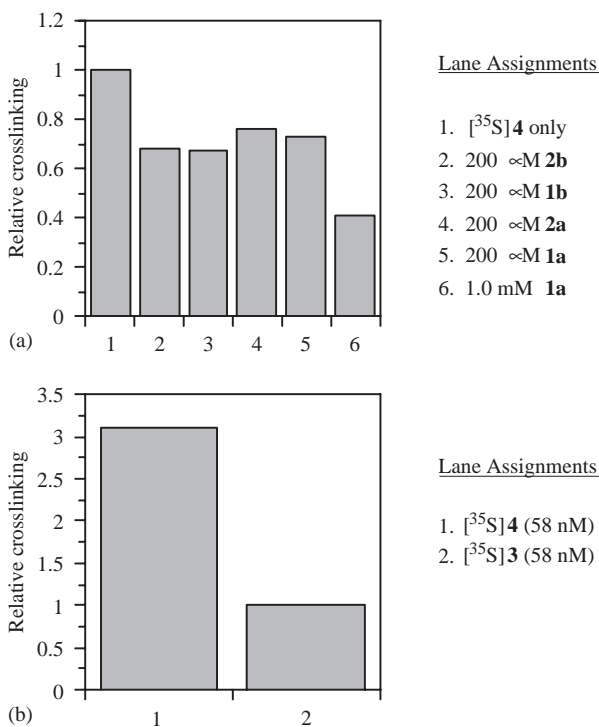
Figure 4. Phosphorimaging analyses following photolabelling of GDI by either [^{35}S]3 or [^{35}S]4 in the presence and absence of various competitors. Reactions, irradiated for 0.5 h, were performed using GDI (4.0 μM) and 58 nM of either [^{35}S]3 (Figure 4(b)) or [^{35}S]4 (Figures 4(a) and 4(b)). Lane assignments for 4(a): (1) [^{35}S]4 (2) [^{35}S]4, 200 μM 2b (3) [^{35}S]4, 200 μM 1b, (4) [^{35}S]4, 200 μM 2a (5) [^{35}S]4, 200 μM 1a (6) [^{35}S]4, 1.0 mM 1a. Lane assignments for 4(b): (1) [^{35}S]4 (2) [^{35}S]3

cross-linking abilities to GDI in several contexts. Purified GDI[62,63] was first irradiated in the presence of the esterified probe [^{35}S]3, followed by competition experiments with AGGCE and AGGC. Figure 5 shows the results of photolyzing 24 nM probe with 4.0 μM protein compared with the cross-linking seen when competitor (100 μM) was added.

Almost all competition studies shown in this present study use cysteine analogs in concentrations of 200 μM or less: this is important when one considers the possible detergent-like effects these compounds could display.[25] In another study showing the usefulness of prenylated cysteine analogs, Béliveau and co-workers have estimated the critical micellular concentration of AGGC to be 340 μM, substantially above the amounts used in Figure 5.[31] Interestingly, AGGCE was a
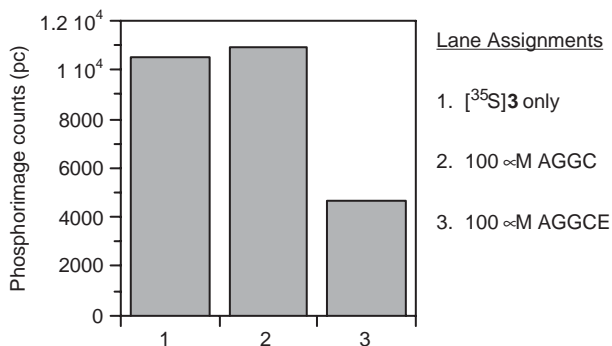
Figure 5. **Phosphorimaging analyses following photolabelling of GDI by [^{35}S]3 in the presence of AGGC and AGGCE. Reactions, irradiated for 0.5 h, were performed using GDI (4.0 μM) and [^{35}S]3 (24 nM). Lane assignments are as follows: (1) no competitor (2) 100 μM AGGC (3) 100 μM AGGCE**

significantly better competitor than AGGC: the latter offered no protection while AGGCE reduced GDI cross-linking to 44% of that of GDI irradiated with [^{35}S]3 alone. Competition experiments previously performed using GDI and [^{35}S]4 with these geranylgeranylcysteines also indicated a similar pattern of cross-linking reduction, with AGGCE being the more effective competitor.[41] Furthermore, in their study of the affinity of various prenylated cysteines for GDI, Rando and co-workers found that esters bound more strongly than the acid counterparts.[39] Recalling that methylation of the prenylated cysteine of a G-protein ends the threefold prenylation process,[4,11,13] these results could prove useful for the design of prenylated protein−protein disruption agents.

Next, competition experiments were performed to see if all cross-linking could be eliminated upon the addition of increasing amounts of appropriate unlabelled probe (Figure 6). Significantly less cross-linking of GDI by [^{35}S]3 (24 nM) is seen with the addition of 5 μM unlabelled ester (lane 4′), and signal is nearly eliminated upon the inclusion of 100 μM unlabelled probe (lane 2′).

With successful cross-linking of purified GDI by the photoaffinity labelled cysteine analogs [^{35}S]3 and [^{35}S]4[41] achieved, we then sought to preferentially label GDI within crude *E. coli* extract overexpressing GDI as a glutathione-*S*-transferase (GST)−GDI fusion protein.[62] Figure 7 shows the sodium dodecylsulfate polyacrylamide electrophoretic (SDS−PAGE) treatment of the extract (35 μg) following photolysis with [^{35}S]3 (58 nM). The protein stain shows numerous proteins at a range of molecular masses, with the predominant band corresponding
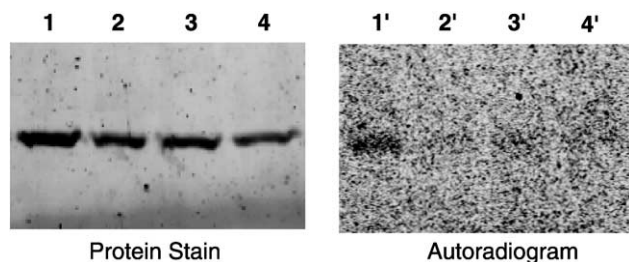
Figure 6. Analysis of photolabelling of GDI by [^{35}S]3 using SDS-polyacrylamide gel electrophoresis (PAGE). Lanes 1−4 show protein staining while lanes 1′−4′ show the radiolabelled proteins. All lanes contain 4.0 μM GDI and 24 nM [^{35}S]3. Lanes 1 and 1′ contain a sample of GDI irradiated in the presence of [^{35}S]3 only; lanes 2 and 2′ contain a sample of GDI irradiated in the presence of [^{35}S]3 and 3 (100 μM); lanes 3 and 3′ contain a sample of GDI irradiated in the presence of [^{35}S]3 and 3 (50 μM); lanes 4 and 4′ contain a sample of GDI irradiated in the presence of [^{35}S]3 and 3 (5 μM). Irradiation time was 0.5 h

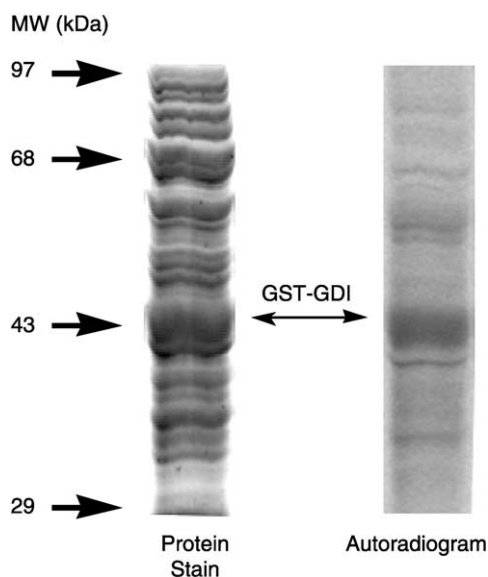**Figure 7. Analysis of photolabelling by [^{35}S]3 of a crude *E. coli*/pGEX-KG cell extract of GST−GDI after induction with IPTG by SDS−PAGE. Crude extract (35 μg) was irradiated in the presence of [^{35}S]3 (58 nM) for 0.5 h**

to the desired fusion protein near the 43 kDa protein standard.[64] The corresponding autoradiogram indicates the fusion protein is, indeed, preferentially cross-linked.

The graphs in Figure 8 are densitometric scans of the gel data similar to that presented in Figure 7 along with similar data from the crude extract labelling by [^{35}S]**4** (each probe present at a concentration of 58 nM), distinctly showing the strong radiolabelling by both probes of GST−GDI while showing significantly less labelling of other proteins. While [^{35}S]**3** is not as an efficient cross-linker as [^{35}S]**4** (Figure 4(b)), both probes appear to selectively cross-link to the GDI fusion protein: several proteins present in fairly high concentration are not targeted by
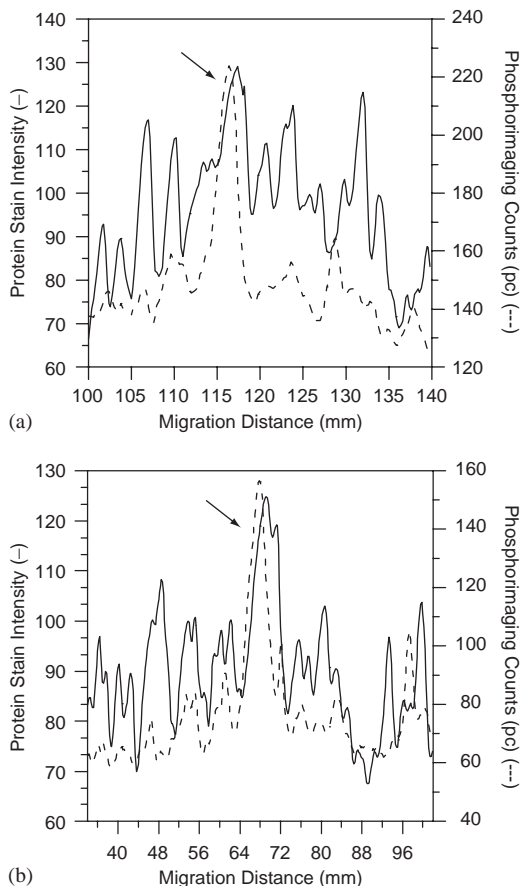
Figure 8. Densitometric representations of protein staining and radiolabelling by [^{35}S]3 8(a) and [^{35}S]4 8(b) of *E coli*. crude cell extracts after fractionation by SDS−PAGE. [^{35}S]3 (58 nM); [^{35}S]4 (58 nM); irradiation time: 0.5 h. The extent of cross-linking is expressed as the amount of radioactivity determined by phosphorimaging analysis (phosphorimage counts, pc). Protein stain intensity (solid line); phosphorimaging counts (dashed line). The arrow indicates the band corresponding to GST−GDI

the benzophenone-containing cysteines. Regarding the cross-linking efficiencies of the two probes, it is important to keep in mind that their binding affinities need not correlate with cross-linking effectiveness since there may be subtle conformational differences in each of the probe−protein complexes.

It is unlikely that presence of the methyl ester moiety directly alters the photochemical properties of the benzophenone unit since these functional groups are located relatively far from each other in **3**. Instead, the reduced cross-linking efficiency obtained with **3** relative to **4** is likely due to small differences in the way these probes bind to GDI. In this regard, it is interesting to note that the isoprenoid binding site of GDI, shown in Figure 9[21], contains a lysine residue (K167) that may interact with the carboxylate-containing probe, **4**, but not the ester form, **3**. Such an interaction would alter the positioning of the C-terminal cysteine which would in turn reorient the isoprenoid group within the binding site. Given that the lipid binding pocket is composed of residues with widely varying photochemical reactivity, even small structural differences could greatly influence the observed cross-linking efficiency.
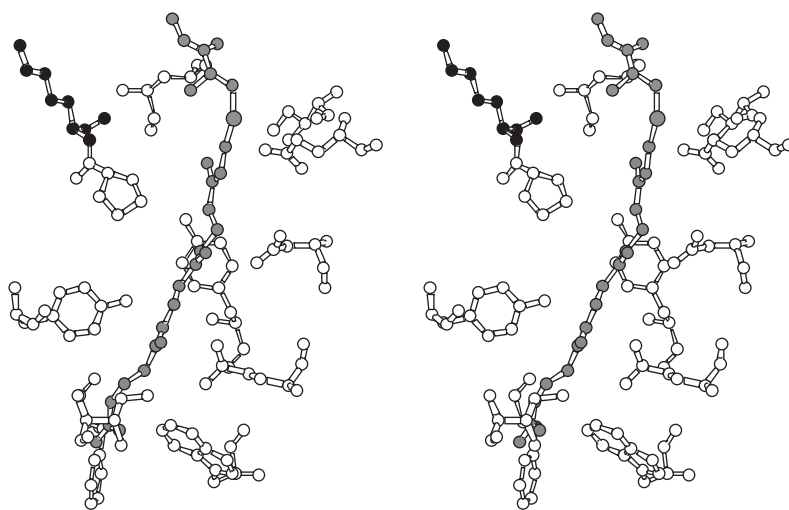
Figure 9. Stereo representation showing the C-terminal geranylgeranylcysteine methyl ester residue (grey) of Cdc42 bound in the isoprenoid binding pocket of GDI[21]. Lysine 167 is shown in black. Residues in close proximity to the prenylated cysteine are shown in white. Clockwise from the upper right: I14, E17, D140, Y175, Q130, W194, F102, I112, Y110, P166, K167, L170. The cysteine methyl ester methyl group is at the top center of each image

Experimental

Materials and Methods

All synthetic reactions were conducted under a nitrogen atmosphere, stirred magnetically, and carried out at room temperature unless otherwise noted. Analytical TLC was performed on precoated (250 μm) silica gel 60 F-254 plates from E. Merck. Visualization of plates was done under UV irradiation or by staining with an ethanolic phosphomolybdic acid solution followed by heating. Flash chromatography silica gel (60−120 mesh) was obtained from E. M. Science or Scientific Adsorbents, Inc. CH_2Cl_2 was distilled from CaH_2. Deuterated NMR solvents were used as obtained from Cambridge Isotope Laboratories, Inc. NMR spectra were obtained at 300 MHz (^{1}H) or 75 MHz (^{13}C) on Varian instruments. Chemical shifts are reported in ppm and J values are given in Hertz. Liquid scintillation fluid used was Cytoscint obtained from ICN. Protein concentrations were determined by the Biorad method, which employs the Bradford procedure,[65] using BSA as a standard. Molecular masses of GDI and GST−GDI were determined by comparison with high molecular weight standards (GIBCO BRL Life Technologies: myosin (200 kDa), phosphorylase b (97.4 kDa), BSA (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), β-lactoglobulin (18.4 kDa), lysozyme (14.3 kDa)) using SDS−PAGE. Unless otherwise noted, HPLC analyses were carried out using a Beckman model 127/166 instrument equipped with a diode array UV detector and a Varian Dynamax C_{18} column (8.0 μm, 4.6×250 mm) equipped with a 5 cm guard column (flow rate: 1.0 ml/min, 500 μl injection loop, 5 to 100% B in 40 min. Solvent A: 95% H_2O, 5% CH_3CN, 0.2% TFA; solvent B: 100% CH_3CN, 0.2% TFA) with monitoring at 220 and 260 nm. Phosphorimaging analysis was performed with a Bio-Rad Molecular Imager FX instrument using Quantity One version 4.1.0 software.

Computer Modeling

The stereopicture in Figure 2 was constructed using the coordinates of the geranylgeranylcysteine residue from Cdc42 present in the complex with RhoGDI.[21] The cysteine and first two isoprenoid units from compound 4 were initially superimposed on the geranylgeranylcysteine residue using the 'superimpose' feature in the program CS Chem3D Pro

(CambridgeSoft Corp., V4.0). The conformation of the benzophenone moiety was then manually positioned to maximize overlap with the space occupied by the last two isoprenoid units from the geranylgeranyl group.

Nonradioactive Syntheses

*Cysteine (S-Farnesyl) Methyl Ester (**6**).* To a solution of cysteine methyl ester hydrochloride (172 mg, 1.0 mmol, 1.5 eq) dissolved in DMF was added 1 eq of farnesyl bromide (190 mg, 0.67 mmol).[43] DIEA was then added (0.19 ml, 1.1 mmol, 1.6 eq) and the mixture was allowed to stir overnight. After diluting the solution with water, it was extracted three times with EtOAc and the combined organic layers were washed once with saturated NaCl and then dried over anhydrous MgSO$_4$. After rotary evaporation, the crude product was purified via flash chromatography (1:1 toluene/EtOAc, v/v; $R_f = 0.28$) to afford the desired product in 43% yield. ^{1}H NMR (CDCl$_3$) δ 1.59 (s, 3 H), 1.67 (s, 6 H), 1.78 (s, 3 H), 1.94–2.11 (m, 8 H), 2.64 (dd, $J = 13.5$, 1.5, 1 H), 2.87 (dd, $J = 13.5$, 4.5, 1 H), 3.17 (dd, $J = 13.6$, 4.5, 2 H), 3.62 (dd, $J = 6.0$, 1.5, 1 H), 3.74 (s, 3 H), 5.08 (m, 2 H), 5.23 (t, $J = 7.6$, 1 H). ^{13}C-DEPT NMR (CDCl$_3$) δ 16.0, 16.1, 17.7, 25.7, 52.3 (primary), 26.4, 26.7, 29.8, 36.4, 39.6, 39.7 (secondary), 54.1, 120.0, 123.8, 124.3 (tertiary), 131.3, 135.3, 139.6, 174.6 (quaternary). HR-CI-MS calculated for C$_{19}$H$_{34}$NO$_2$S [M + H]$^+$ 340.2310, was found 340.2288.

*N-Methanesulfonyl (S-Farnesyl) Cysteine Methyl Ester (unlabelled **1a**).* Cysteine methyl ester (*S*-farnesyl) **6** (85.6 mg, 0.25 mmol, 1 eq) was dissolved in 175 μl dry CH$_2$Cl$_2$. Et$_3$N, dried over molecular sieves for 48 h (69.5 μl, 0.5 mmol, 2 eq), was then added and the yellow mixture was stirred under N$_2$. CH$_3$SO$_2$Cl (10.9 mg, 7.4 μl, 95.1 μmol, 1.5 eq) was added slowly by syringe to the amine solution. Dry CH$_2$Cl$_2$ was added as needed throughout the reaction. The reaction was stirred for 1 h at which time it was diluted with CH$_2$Cl$_2$. This organic layer was washed twice with saturated NaHCO$_3$ and the organic layers were combined and dried over anhydrous Na$_2$SO$_4$. After rotary evaporation, the crude product was purified via flash chromatography (5:2 toluene/EtOAc, v/v; $R_f = 0.48$) to afford unlabelled **1a** in 58% yield. Purity was determined by reversed-phase HPLC: $t_R = 54.4$ min, 92% pure. ^{1}H NMR (CDCl$_3$) δ 1.57 (s, 6 H), 1.66 (s, 6 H), 1.95–2.09 (m, 8 H), 2.86 (dd, $J = 13.9$, 6.3, 1 H), 2.91 (dd, $J = 13.9$, 5.1, 1 H), 3.03 (s, 3 H), 3.19 (m, 2 H), 4.32 (ddd,

$J = 8.4$, 6.0, 5.1, 1 H), 5.06 (m, 2 H), 5.19 (t, $J = 7.8$, 1 H), 5.31 (d, $J = 8.4$, 1 H). ^{13}C-DEPT NMR (CDCl$_3$) δ 16.1, 16.2, 17.7, 25.7, 42.0, 53.0 (primary), 26.4, 26.7, 30.1, 34.4, 39.6, 39.7 (secondary), 55.8, 119.4, 123.7, 124.3 (tertiary), 131.4, 135.4, 140.4, 171.3 (quaternary). HR-CI-MS calculated for $C_{20}H_{36}O_4NS_2$ [M + H]$^+$ 418.2086, was found 418.2098.

*N-Methanesulfonyl (S-Farnesyl) Cysteine (unlabelled **2a**)*. *N*-Methanesulfonyl (*S*-farnesyl) cysteine methyl ester (42.2 mg, 0.10 mmol, 1 eq) was dissolved in 4 ml of a 0.25 M LiOH solution in 1:1 MeOH/H$_2$O (1.0 mmol, 10 eq). Addition of *i*-PrOH was useful if solubility problems were encountered. After stirring overnight, the reaction was judged to be complete by TLC. The mixture was adjusted to pH 3 with 10% HCl and then extracted with EtOAc and saturated NaCl. The organic layers were combined and dried over anhydrous Na$_2$SO$_4$. Following rotary evaporation, 38.4 mg of product were recovered, a 95% yield (NMR analysis indicated the reaction had gone to roughly 98% completion). Chemical purity, as analyzed by reversed-phase HPLC, was 90% ($t_R = 50.4$ min). ^{1}H NMR (CD$_3$OD) δ 1.59 (s, 6 H), 1.65 (s, 3 H), 1.68 (s, 3 H), 1.93–2.11 (m, 8 H), 2.74 (dd, $J = 13.8$, 7.2, 1 H), 2.86 (dd, $J = 13.8$, 4.8, 1 H), 2.96 (s, 3 H), 3.24 (d, $J = 7.8$, 2 H), 3.96 (dd, $J = 6.9$, 4.8, 1 H), 5.08 (m, 2 H), 5.23 (t, $J = 7.8$, 1 H). ^{13}C-DEPT NMR (CD$_3$OD) δ 14.8, 15.0, 16.4, 24.7, 39.6 (primary), 26.1, 26.4, 29.4, 35.1, 39.5, 39.6 (secondary), 50.3, 120.4, 123.8, 124.1 (tertiary), 130.7, 134.8, 138.8 (quarternary, one unobserved). HR-CI-MS calculated for $C_{19}H_{34}O_4NS_2$ [M + Na]$^+$ 426.1740, was found 426.1744.

*N-Methanesulfonyl Cysteine (S-(E,E)-8-O-(3-Benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) Methyl Ester (unlabelled **3**)*. The following procedure is a modification of a published protocol.[42] Cysteine (*S*-(*E*,*E*)-8-*O*-(3-benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) methyl ester **9** (190 mg, 0.39 mmol, 1 eq) was dissolved in 200 µl of freshly distilled CH$_2$Cl$_2$ followed by addition of Et$_3$N (109 µl, 0.78 mmol, 2 eq). CH$_3$SO$_2$Cl was then added slowly (51.3 µl, 0.66 mmol, 1.7 eq) over a period of 1 min and the reaction quickly became more yellow. Additional CH$_2$Cl$_2$ (0.5 ml) was added and TLC analysis showed that the reaction was essentially complete in 10 min. After 1 h, the reaction was diluted with CH$_2$Cl$_2$ and washed two times with saturated NaHCO$_3$. The aqueous layers were combined and washed one time with CH$_2$Cl$_2$. All organic layers were then combined, dried over

anhydrous Na_2SO_4, and evaporated. Flash chromatography (5:2 toluene/EtOAc, v/v; $R_f = 0.42$) was performed and 155 mg of product were recovered, a 71% yield. ^{1}H NMR ($CDCl_3$) δ 1.67 (s, 6 H), 2.03–2.21 (m, 4 H), 2.83 (dd, $J = 13.8, 6.3, 1$ H), 2.90 (dd, $J = 13.9, 5.2, 1$ H), 3.00 (s, 3 H), 3.15 (dd, $J = 13.1, 7.6, 1$ H), 3.22 (dd, $J = 13.2, 7.8, 1$ H), 3.76 (s, 3 H), 3.91 (s, 2 H), 4.32 (dt, $J = 8.6, 5.8, 1$ H), 4.51 (s, 2 H), 5.21 (t, $J = 7.7, 1$ H), 5.39 (t, $J = 6.3, 1$ H), 5.48 (d, $J = 8.4, 1$ H), 7.42–7.49 (m, 3 H), 7.55-7.60 (m, 2 H), 7.68–7.70 (m, 1 H), 7.78–7.80 (m, 3 H). ^{13}C-DEPT NMR ($CDCl_3$) δ 14.0, 16.2, 41.9, 53.0 (primary), 26.0, 30.0, 34.4, 39.2, 71.0, 76.5 (secondary), 55.8, 119.8, 127.9, 128.3 (overlap of two signals), 129.2, 129.4, 130.1 (overlap of two signals), 131.8, 132.5 (tertiary), 132.3, 137.6, 137.7, 139.1, 139.9, 171.2, 196.8 (quaternary). HR-FAB-MS calculated for $C_{29}H_{38}NO_6S_2$ $[M + H]^+$ 560.2131, was found 560.2106. Purity analysis by reversed-phase HPLC indicated >85% pure material when the reaction was performed at this scale ($t_R = 40.5$ min). An extinction coefficient (254 nm) value of 14 800 M^{-1} cm^{-1} was determined in CH_3CN.

N-Methanesulfonyl Cysteine (S-(E,E)-8-O-(3-Benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) (unlabelled **4***)*. *N*-Methanesulfonyl cysteine (*S*-(*E,E*)-8-*O*-(3-benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) methyl ester **3** (18.7 mg, 44.8 μmol, 1 eq) was dissolved in a 0.25 M LiOH solution consisting of 1:1 *i*-PrOH/H_2O (1.8 ml, 0.45 mmol LiOH, 10 eq). After stirring for 24 h, the reaction was acidified to pH 3 with 10% HCl and then dissolved in EtOAc, followed by three washings with water. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated, leaving the product as 17.7 mg of a thick, dark yellow-colored oil (98% yield). ^{1}H NMR (CD_3OD) δ 1.64 (s, 3 H), 1.67 (s, 3 H), 2.13 (m, 4 H), 2.73 (dd, $J = 13.8, 7.2, 1$ H), 2.88 (dd, $J = 13.8, 5.1, 1$ H), 2.96 (s, 3 H), 3.18 (dd, $J = 12.9, 7.8, 1$ H), 3.24 (dd, $J = 12.9, 8.0, 1$ H), 3.91 (s, 2 H), 4.05 (m, 1 H), 4.47 (s, 2 H), 5.22 (t, $J = 7.8, 1$ H), 5.39 (t, $J = 7.0, 1$ H), 7.46–7.70 (m, 9 H). ^{13}C-DEPT NMR (CD_3OD) δ 12.8, 14.9, 39.9 (primary), 25.6, 29.3, 34.4, 38.9, 70.2, 76.1 (secondary), 57.4, 120.7, 128.1, 128.2 (overlap of two signals), 128.8, 128.9, 129.7 (overlap of two signals), 131.7, 132.5 (tertiary), 131.9, 137.5 (overlap of two signals), 138.6, 139.2, 174.1, 197.1 (quaternary). HR-FAB-MS calculated for $C_{28}H_{36}NO_6S_2$ $[M + H]^+$ 546.1975, was found 546.1975; for $[M + Na]^+$: calculated 568.1795, was found 568.1781. Purity analysis by reversed-phase HPLC indicated a purity of >90% when the reaction was

performed at this scale ($t_R = 37.0$ min). An extinction coefficient (256 nm) value of 17 700 M^{-1} cm^{-1} was determined in MeOH.

N-Acetyl Cysteine (S-Geranylgeranyl) Methyl Ester (AGGCE). ACE (*N*-acetyl cysteine methyl ester, 31.7 mg, 178 μmol, 1.5 eq) and Zn(OAc)$_2$ (77.9 mg, 356 μmol, 3 eq) were dissolved 200 μl of 2:1:1 DMF/CH$_3$CN/ 0.025% TFA with stirring.[44] Geranylgeranyl bromide (42.1 mg, 119 μmol, 1 eq) was then added but formed an oil layer at the top of the 2:1:1 solution. Additional solvent was added (DMF and 0.025% TFA separately) until all components were dissolved. The reaction was then allowed to stir overnight at which time the mixture was diluted with EtOAc. This organic layer was washed once with water and following separation of the layers, the water layer was washed once with EtOAc. The organic layers were combined, dried over anhydrous Na$_2$SO$_4$, and solvent was removed *in vacuo*. The oily residue was applied to a 5:2 toluene/EtOAc (v/v) silica gel flash column ($R_f = 0.50$) and 29.9 mg of product were recovered, a 56% yield. ^{1}H NMR (CDCl$_3$) δ 1.61 (s, 6 H), 1.67 (s, 3 H), 1.69 (s, 6 H), 1.99–2.11 (m, 12 H), 2.06 (s, 3 H), 2.88 (dd, $J = 13.8$, 5.7, 1 H), 2.97 (dd, $J = 13.9$, 4.6, 1 H), 3.13 (dd, $J = 13.6$, 7.8, 1 H) 3.21 (dd, $J = 13.4$, 8.1, 1 H), 3.78 (s, 3 H), 4.82 (dt, $J = 7.8$, 5.4, 1 H), 5.10 (m, 3 H), 5.21 (t, $J = 7.8$, 1 H), 6.27 (d, $J = 7.5$, 1 H). HR-FAB-MS calculated for C$_{26}$H$_{43}$NO$_3$S [M + H]$^+$ 450.3031, was found 450.3017.

N-Acetyl Cysteine (S-Geranylgeranyl) (AGGC). AGGCE (15.8 mg, 35.2 μmol, 1 eq) was dissolved in 0.25 M LiOH in 1:1 MeOH/H$_2$O (1.41 ml, 352 μmol, 10 eq) and the mixture was allowed to react for 24 h before 1 N HCl was added. Upon reaching pH 3, EtOAc was added and the material was transferred to a separatory funnel. Saturated NaCl was used to twice wash the organic layer and the combined aqueous layers were washed once with EtOAc. The organic layers were combined, dried over anhydrous Na$_2$SO$_4$, and evaporated *in vacuo*. No chromatography was performed and the material (13.7 mg; 89% yield) appeared as one baseline spot via TLC (5:2 toluene/EtOAc, v/v). ^{1}H NMR (CD$_3$OD) δ 1.57 (s, 6 H), 1.64 (s, 3 H), 1.66 (s, 6 H), 1.96–2.11 (m, 12 H), 1.97 (s, 3 H), 2.64 (dd, $J = 8.7$, 2.4, 1 H), 2.69 (dd, $J = 8.7$, 2.4, 1 H), 3.10 (dd, $J = 13.2$, 7.3, 1 H), 3.24 (m, 1 H), 4.54 (m, 1 H), 5.07 (m, 3 H), 5.18 (t, $J = 7.2$, 1 H). HR-FAB-MS calculated for C$_{25}$H$_{41}$NO$_3$S [M + Na]$^+$ 458.2695, found 458.2679.

Radioactive Syntheses

*[^{35}S]N-Methanesulfonyl Cysteine (S-Farnesyl) Methyl Ester [^{35}S]***1a**.
The title compound was prepared using the procedure of Dean *et al.*[42] A
solution of [^{35}S]methanesulfonyl chloride (10 mCi, 1198 Ci/mmol) in
anhydrous CH_2Cl_2 (2 ml) was dried over Na_2SO_4 for 1 h, filtered, then
concentrated by atmospheric distillation to 120 µl. This solution was
added to a solution of cysteine methyl ester (*S*-farnesyl) (**6**, 4.7 mg,
13.8 µmol) and Et_3N (5 ml) in anhydrous CH_2Cl_2 (20 µl). After stirring
for 1 h at RT, the reaction was quenched by the addition of MeOH
(2 ml). Half of the solution (1 ml) was used directly in the next reaction.
The remaining solution (1 ml) was concentrated to 500 µl and purified
by reversed-phase HPLC (Zorbax RX-C$_{18}$, 9.4 × 250 mm, 5 mm, 5 ml/
min, 20°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq.
$HClO_4$, solvent B = CH_3CN, 32% A/68% B isocratic, elution t_R
[^{35}S]**1a** = 20 min). The purified [^{35}S]**1a** was isolated by solid phase
extraction, concentrated, dissolved in MeOH (1.5 ml), counted (990 µCi,
20% radiochemical yield) and analyzed by HPLC (Zorbax RX-C$_{18}$,
4.6 × 250 mm, 5 mm, 1 ml/min, 30°C, 215/254 nm, Packard flow
monitor; solvent A = 0.1% aq. $HClO_4$, solvent B = CH_3CN, 28%
A/72% B isocratic, elution t_R [^{35}S]**1a** = 13.3 min, 92.1% radiochemical
purity). The identity of the [^{35}S]**1a** was confirmed by co-elution with an
authentic sample of unlabelled **1a**.

[^{35}S]N-Methanesulfonyl Cysteine (S-Farnesyl) [^{35}S] **2a**. To the
methanolic solution of crude [^{35}S]**1a** described above (1.0 ml) was
added THF (1.0 ml), followed by a solution of $LiOH \cdot H_2O$ (10 mg) in
H_2O (0.5 ml). The reaction mixture was stirred at rt for 5 h, at which
stage HPLC indicated that reaction was complete. The reaction mixture
was concentrated to 500 µl, acidified with $HClO_4$ (20 µl), and purified by
reversed-phase HPLC (Zorbax RX-C$_{18}$, 9.4 × 250 mm, 5 mm, 5 ml/min,
20°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq. $HClO_4$,
solvent B = CH_3CN, 45% A/55% B isocratic, elution t_R [^{35}S]**2a** =
20 min). The purified [^{35}S]**2a** was isolated by solid phase extraction,
concentrated, dissolved in CH_3CN (1.5 ml), counted (1.55 mCi, 85%
radiochemical yield from crude [^{35}S]**1a**) and analyzed by HPLC (Zorbax
RX-C$_{18}$, 4.6 × 250 mm, 5 mm, 1 ml/min, 30°C, 215/254 nm, Packard
flow monitor; solvent A = 0.1% aq. $HClO_4$, solvent B = CH_3CN, 40%
A/60% B isocratic, elution t_R [^{35}S]**2a** = 16.7 min, 93.0% radiochemical

purity). The identity of the $[^{35}S]$**2a** was confirmed by co-elution with an authentic sample of unlabelled **2a**.

$[^{35}S]$N-Methanesulfonyl Cysteine (S-(E,E)-8-O-(3-Benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) Methyl Ester $[^{35}S]$ **3**. The title compound was prepared using the procedure of Dean *et al.*[42] A solution of $[^{35}S]$methanesulfonyl chloride (15 mCi, 1080 Ci/mmol) in anhydrous CH_2Cl_2 (12 ml) was dried over Na_2SO_4 for 1 h, filtered, then concentrated by atmospheric distillation to 120 μl. This solution was added to a solution of cysteine methyl ester (S-(E,E)-8-O-(3-benzoyl-benzyl)-3,7-dimethyl-2,6-octadiene) (**9**, 5.0 mg, 10.4 μmol) and Et_3N (5 ml) in anhydrous CH_2Cl_2 (20 ml). After stirring 1 h at RT, the reaction was quenched by the addition of MeOH (2 ml). Half of the solution (1 ml) was used directly in the next reaction. The remaining solution (1 ml) was concentrated to 500 μl and purified by reversed-phase HPLC (Zorbax RX-C_8, 9.4 × 250 mm, 5 mm, 5 ml/min, 20°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq. TFA, solvent B = CH_3CN, 40% A/60% B isocratic, elution t_R $[^{35}S]$**3** = 20 min). The purified $[^{35}S]$**3** was isolated by solid phase extraction, concentrated, dissolved in MeOH (1.5 ml), counted (380 μCi, 5% radiochemical yield) and analyzed by HPLC (Zorbax RX-C_8, 4.6 × 250 mm, 5 mm, 1 ml/min, 30°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq. TFA, solvent B = CH_3CN, 30% A/70% B isocratic, elution t_R $[^{35}S]$**3** = 9.2 min, 95.3% radiochemical purity). The identity of the $[^{35}S]$**3** was confirmed by co-elution with an authentic sample of unlabelled **3**.

$[^{35}S]$N-Methanesulfonyl Cysteine (S-(E,E)-8-O-(3-Benzoylbenzyl)-3,7-dimethyl-2,6-octadiene) $[^{35}S]$ **4**. To the methanolic solution of crude $[^{35}S]$**3** described above (1.0 ml) was added THF (1.0 ml), followed by a solution of LiOH·H_2O (10 mg) in H_2O (0.5 ml). The reaction mixture was stirred for 5 h, at which stage HPLC indicated that the reaction was complete. The reaction mixture was concentrated to 500 μl, acidified with TFA (20 μl), and purified by reversed phase-HPLC (Zorbax SB-Phenyl, 9.4 × 250 mm, 5 mm, 5 ml/min, 20°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq. TFA, solvent B = CH_3CN, 52% A/48% B isocratic, elution t_R $[^{35}S]$**2** = 25 min). The purified $[^{35}S]$**4** was isolated by solid phase extraction, concentrated, dissolved in CH_3CN (1.5 ml), counted (130 μCi, 26% radiochemical yield from crude **3**) and analyzed by HPLC (Zorbax SB-Phenyl, 4.6 × 250 mm, 5 mm, 1 ml/min, 30°C, 215/254 nm, Packard flow monitor; solvent A = 0.1% aq. TFA, solvent

B = CH$_3$CN, 50% A/50% B to 0% A/100% B over a 20 min linear gradient, elution t_R [^{35}S]**4** = 12.7 min, 93.4% radiochemical purity). The identity of the [^{35}S]**4** was confirmed by co-elution with an authentic sample of unlabelled **4**.

Preparation of GST−GDI (E. coli.)/pGEX-KG Crude Cell Lysate; Purification of GDI. A truncated GDI whose first 25 amino-terminal residues had been removed was used in these experiments. This modification increases the stability of the protein (G. R. Hoffman, personal communication) and behaves similarly to the wild-type protein.[63] Both the expression of GST−GDI in *E. coli* and the purification of GDI alone were performed using a published protocol[41] based on the work of Cerione and co-workers.[62, 63]

General Photolysis Procedure. All reactions (typically 50 μl final volume) were performed in silinized quartz test tubes covered with Parafilm at 4°C in a UV Rayonet minireactor equipped with 8 RPR-3500° lamps and a circulating platform that allowed for irradiation of up to eight samples simultaneously. The amount of [^{35}S]**3** or [^{35}S]**4** added to photolysis mixtures came from stock solutions in DMSO. Competitor stock solutions were also prepared as DMSO solutions and the percent DMSO in all photolysis reactions remained under 10%. GDI buffer (20 mM HEPES, pH 8.0, 5 mM MgCl$_2$, 1 mM NaN$_3$, 100 mM NaCl) was used for all reactions. All reagents (buffer, protein, [^{35}S]**3** or [^{35}S]**4**, competitor as applicable) were combined, mixed gently, and immediately photolyzed. In experiments where results of photolyses with [^{35}S]**3** and [^{35}S]**4** are in direct comparison, their concentrations were the same and their specific activities were either identical or, if different, the data was adjusted to allow for a direct comparison. Irradiation times up to 10.7 h resulted in no decomposition of GDI as detected by SDS−PAGE and cross-linking only occurred when the mixtures were irradiated. Following photolysis, unless otherwise indicated, loading buffer (50 mM *Tris*-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol) was added to each sample and samples were heated for 2 min at 100°C followed by analysis by SDS−PAGE using *Tris*-glycine gels (12 or 15% acrylamide). Unincorporated [^{35}S]**3** or [^{35}S]**4** passed through the gel and did not interfere with analysis of most cross-linked protein products, but upper and lower portions of each gel were

removed before autoradiography in order to facilitate analysis. Gels were then stained with Coomassie brilliant blue or SYPRO stain (Bio-Rad), dried, and subjected to autoradiography. Intensities of radiolabelled products were determined by phosphorimaging analysis of the dried gels.

Conclusion

Alkylated single cysteine analogs have proved to be useful tools in the G-protein arena due in part to their effectiveness in serving as mimics of the C-termini of prenylated proteins. This has allowed investigation of both the multistep prenylation pathway and of subsequent interactions between fully processed G-proteins and other proteins such as RhoGDI.[19–41] In experiments involving the isoprenoid binding site-containing protein GDI,[21] the farnesylcysteine analogs presented here (**1a** and **2a**) displayed behavior similar to that of AFCE and AFC, compounds frequently used to assess prenylated protein behavior. The introduction of the [^{35}S]N-methanesulfonyl moiety of high specific activity ($\sim$1100 Ci/mmol) late in the synthesis of these farnesylated analogs ([^{35}S]**1a** and [^{35}S]**2a**) is a significant benefit over the traditional use of ^{3}H for such compounds.[22,26,36,38] For some applications, the [^{35}S]sulfonamide moiety may prove more practical and useful than [^{35}S]cysteine[66] and its high specific activity eclipses typical values found with both ^{3}H and ^{14}C (0.1–150 and 1–100 mCi/mmol, respectively).

   We took advantage of these benefits by incorporating the [^{35}S]sulfonamide into photoaffinity labelled cysteines [^{35}S]**3** and [^{35}S]**4**, analogs which closely mimic the prenylated C-terminal cysteine of G-proteins (see Figure 2). Photolysis experiments using these compounds with GDI demonstrate not only the utility of the radiolabel, but also the potential use of these probes for studying binding sites within other proteins. Both [^{35}S]**3** and [^{35}S]**4** showed comparable cross-linking profiles in competition experiments using AGGCE and AGGC with purified GDI, and each preferentially cross-linked to the GST–GDI fusion protein in crude bacterial extract.[41] However, the acid photoprobe, [^{35}S]**4**, more efficiently cross-linked to purified GDI than its ester counterpart, [^{35}S]**3**. This phenomenon may not hold true for other prenylated protein–protein systems, as some prenylated proteins are methylesterified while some retain their prenylcysteine carboxylate C-terminus. Furthermore, the physiologically relevant state of a prenylated protein in complex

with a cognate receptor may not reflect the final, methylesterified form of certain proteins. Thus, the two photoprobes described here provide complementary tools to explore the significance of prenylcysteine carboxymethylation in other prenylated protein−protein interactions.

Finally, from a global perspective, prenylated G-proteins need to be membrane-bound to exhibit their biological activity,[4] and therefore it is likely that numerous membranous receptor proteins exist which contain binding sites for the isoprenoid moiety. Photoaffinity labels such as those described here may be particularly useful for the study of these receptors, since structural analysis via NMR and X-ray crystallographic methods is quite difficult to obtain in membrane associated systems. In short, the [^{35}S]sulfonamide feature with its high specific activity, stability, and the advantageous ease of synthetic introduction into desired prenylated cysteines will provide researchers in the prenylation field, as well as others, with a strategically placed and potent radiolabel for a variety of applications.

Acknowledgements

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