Photoactive Analogs of Farnesyl Pyrophosphate Containing Benzoylbenzoate Esters: Synthesis and Application to Photoaffinity Labeling of Yeast Protein Farnesyltransferase

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Farnesyl pyrophosphate (FPP) is involved in a large number of cellular processes including the prenylation of transforming mutants of Ras proteins implicated in cancer. Photoactive analogs could provide useful information about enzyme active sites that bind farnesyl pyrophosphate; however, the availability of such compounds is extremely limited. Molecules that incorporate benzophenone moieties are attractive photoaffinity labeling reagents because of their useful photochemical properties. Here, the syntheses of two compounds, **3a** and **3b**, containing *para*- and *meta*-substituted benzoylbenzoates are described. Compounds **3a** and **3b** are competitive inhibitors (with respect to FPP) of yeast protein farnesyltransferase (PFTase) with K_i values of 910 and 380 nM, respectively. Both compounds inactivate PFTase upon photolysis, resulting in as much as 44% inactivation of enzyme activity. Photolysis of PFTase in the presence of [^{32}P]**3a** or of [^{32}P]**3b** results in preferential labeling of the β subunit, suggesting that this subunit is involved in prenyl group recognition. These compounds should be valuable tools for studying enzymes that utilize FPP as a substrate.

Introduction

Chemical modification techniques are useful in studying the interactions between small molecules and proteins because they allow critical residues involved in ligand binding and catalysis to be identified. Photoaffinity labeling experiments utilize functional groups incorporated into ligand or substrate structures that can be activated by light; upon irradiation, they form reactive intermediates that undergo bond insertion reactions with a macromolecule.[1] Since the intermediates produced by photolysis are highly reactive, these species typically react with residues that are in close proximity. This lack of chemical specificity is useful because many different amino acids can be labeled, and hence identified, using these reagents.[2]

Farnesyl pyrophosphate (FPP, **1**) and its C_{20} homologue, geranylgeranyl pyrophosphate (**2**), shown in Figure 1, are isoprenoids that are involved in a number of cellular processes including cholesterol biosynthesis, glycoprotein synthesis, vitamin and cofactor synthesis, and protein prenylation.[3] Recently, the enzyme protein farnesyltransferase (PFTase), which utilizes FPP as a substrate, has attracted considerable attention as a possible target for the design of chemotherapeutic agents.[4] Photoaffinity labeling reagents could provide important information concerning the active site of this enzyme; however, the availability of such compounds is extremely limited; very few photoactive FPP analogs have been prepared.[5] In 1988, Baba and Allen introduced a substrate analog that incorporated a photoactive diazoester moiety into a derivative of geraniol.[6] While the resulting molecule was nearly superimposible with FPP, the pendant diazotrifluoropropionate cross-linking group possessed a number of less desirable features including low cross-linking efficiency and the requirement for prolonged short wavelength UV irradiation for photoactivation.[2,7] Molecules that incorporate benzophenone moieties are attractive alternatives to diazo- and azide-containing compounds for photoaffinity labeling experiments.[8] Benzophenone-based cross-linking agents function via diradical intermediates that are not quenched by solvent

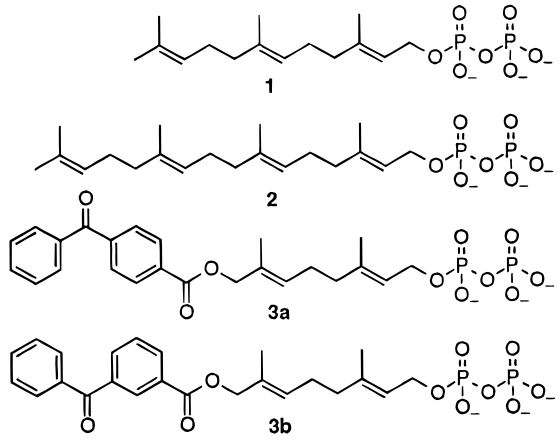

Figure 1. Structures of FPP, GGPP, and photoaffinity labeling analogs.

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(1) Chowdhry, V.; Westheimer, F. H. *Ann. Rev. Biochem.* **1979**, *48*, 293−325.

(2) Burnner, J. *Ann. Rev. Biochem.* **1993**, *62*, 483−514. (b) Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479−12520.

(3) (a) Goldstein, J.; Brown, M. S. *Nature* **1990**, *343*, 425−430. (b) Sinensky, M.; Lutz, R. J. *BioEssays* **1992**, *14*, 25−31.

(4) (a) Buss, J. E.; Marsters, J. C. *Chem. Biol.* **1995**, *2*, 787−791. (b) Gibbs, J. B.; Oliff, A.; Kohl, N. E. *Cell* **1994**, *77*, 175−178. (c) Tamanoi, F. *TIBS* **1993**, *18*, 349−353.

(5) (a) Das, N. P.; Allen, C. M. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 729−735. (b) Omer, C. A.; Kral, A. M.; Diehl, R. E.; Prendergast, G. C.; Powers, S.; Allen, C. M.; Gibbs, J. B.; Kohl, N. E. *Biochemistry* **1993**, *32*, 5167−5176. (c) Yokoyama, K.; McGeady, P.; Gelb, M. H. *Biochemistry* **1995**, *34*, 1344−1354. (d) Bukhtiyarov, Y. E.; Omer, C. A.; Allen, C. M. *J. Biol. Chem.* **1995**, *270*, 19035−19040.

(6) (a) Baba, T.; Allen, C. M. *Biochemistry* **1984**, *23*, 1312−1322. (b) Baba, T.; Muth, J.; Allen, C. M. *J. Biol. Chem.* **1985**, *260*, 10467−10473.

(7) Chowdhry, V.; Vaughan, R.; Westheimer, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1406−1408.

(8) Dorman, G.; Prestwich, G. D. *Biochemistry* **1994**, *33*, 5661−5673.

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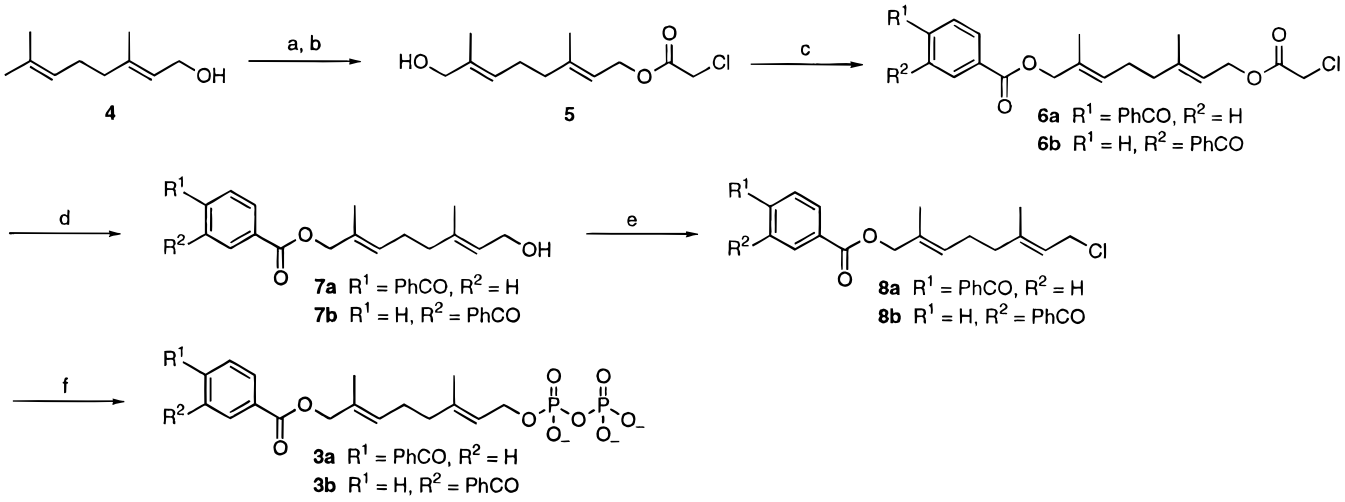
Scheme 1[a]



[a] Reaction conditions: (a) $(ClCH_2CO)_2O$, pyridine, DMAP, DMF; (b) *tert*-butyl hydroperoxide, H_2SeO_3, salicylic acid, CH_2Cl_2; (c) *p*-benzoylbenzoyl chloride (**6a**) or *m*-benzoylbenzoyl chloride (**6b**), pyridine; (d) NH_3, MeOH, H_2O; (e) *N*-chlorosuccinamide, Me_2S, CH_2Cl_2; (f) $[(n\text{-}Bu)_4N]_3HP_2O_7$, CH_3CN.

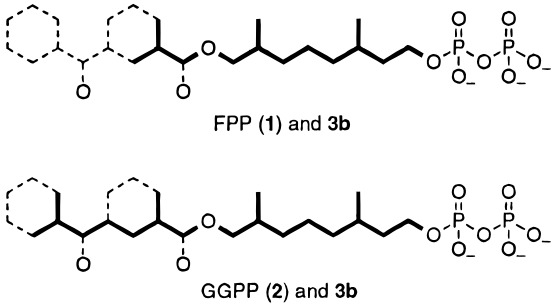


Figure 2. Superposition of FPP and GPP (bold) on **3b** (dashed lines). Double bonds are not shown for clarity.

molecules and are hence highly efficient. Furthermore, they are activated by irradiation at longer wavelengths where protein damage is less likely. Taking advantage of these features, Coleman and co-workers recently used peptides incorporating benzophenones to study the prenyl group acceptor binding site in PFTase.[9] In this paper, the syntheses of two FPP analogs, **3a** and **3b**, are presented together with inhibition kinetics and photoinactivation experiments with PFTase. The results clearly indicate that these compounds are useful photoaffinity labeling analogs for studying PFTase and other enzymes that employ FPP as a substrate.

Results and Discussion

Design and Synthesis. The design of **3a** and **3b** as analogs for FPP originated from our observation of significant overlap in a comparison of the structures of FPP and benzophenone. This complementarity is illustrated in Figure 2 where FPP is superimposed on **3b**. Also shown is a superposition of GGPP and **3b**; compounds **3a** and **3b** may be useful analogs of this longer isoprenoid. The syntheses of compounds **3a** and **3b** were each accomplished in six steps starting from commercially available geraniol as illustrated in Scheme 1. The hydroxyl group of geraniol was first protected by esterification with chloroacetic anhydride; other protecting groups including *tert*-butyldimethylsilyl and *tert*-butyldiphenylsilyl were also employed, but proved to be less stable later in the syntheses. The resulting chloroacetate was then oxidized with *tert*-butyl hydroperoxide and catalytic H_2SeO_3 to yield **5**.[6] The *E*-stereoselectivity for the hydroxylation reaction was confirmed by the disappearance of the C-8 methyl group in the ^{13}C NMR spectrum of **5**.[10] To attach photoactive benzophenone moieties to the C_{10} isoprenoid unit, **5** was acylated with 4-benzoylbenzoyl chloride and 3-benzoylbenzoyl chloride to yield **6a** and **6b**, respectively. The chloroacetate protecting groups of **6a** and **6b** were then selectively hydrolyzed in the presence of the benzoylbenzoates with a $NH_3/MeOH/H_2O$ mixture producing the free alcohols **7a** and **7b,** which are stable compounds that can be stored at −20 °C indefinitely. Finally, **7a** and **7b** were converted to their corresponding pyrophosphates by chlorination with *N*-chlorosuccinamide and dimethyl sulfide followed by displacement of the allylic chlorides with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate as described by Poulter and co-workers.[11] Diphosphates **3a** and **3b** were purified by reversed-phase chromatography and characterized by ^1H NMR, ^{31}P NMR, and FAB mass spectrometry.

Product Studies. To determine whether compounds **3a** and **3b** were substrates or inhibitors for PFTase, a continuous fluorescence assay using the peptide *N*-dansyl-GCVIA (**9**) was first employed. This assay, developed by Pompliano *et al.* for the human PFTase and adapted by Poulter and co-workers for the yeast PFTase, monitors the time-dependent increase in the fluorescence of the dansyl group due to the local increase in hydrophobicity as the adjacent cysteine residue is isoprenylated;[12] under the assay conditions an approximately 10-fold increase in the dansyl group fluorescence occurs upon farnesylation of **9** to **10**. In contrast, incubation of **3a** or **3b** with PFTase and **9** resulted in no discernible change in fluorescence suggesting that these compounds are not

(9) Ying, W.; Sepp-Lorenzino, L.; Cai, K.; Aloise, P.; Coleman, P. S. *J. Biol. Chem.* **1994**, *269*, 470−477.

(10) Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526−5527.

(11) Davisson, V. J.; Woodside, A. B.; Poulter, C. D. *Methods Enzymol.* **1985**, *110*, 130−144.

(12) (a) Bond, P. D.; Dolence, J. M.; Poulter, C. D. *Methods Enzymol.* **1995**, *250*, 30−43. (b) Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945−7946.

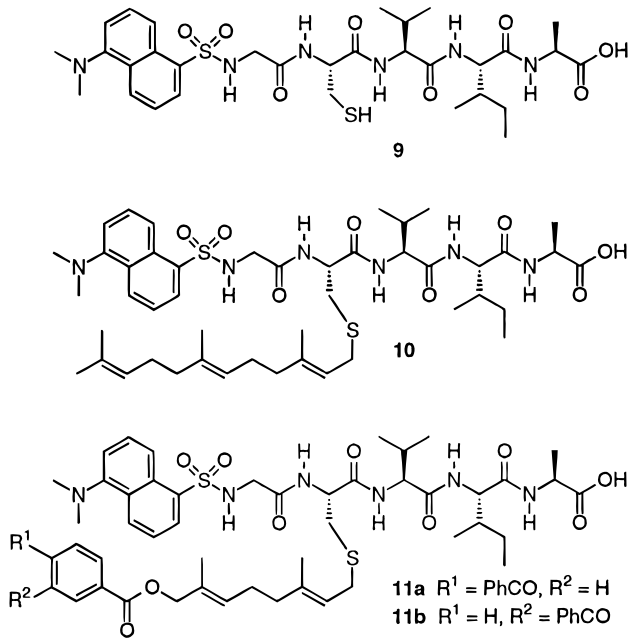


Figure 3. Structures of expected products of enzymatic prenylation of **9** by **3a** and **3b**.

enzyme substrates. To study this question in greater detail, the expected products of enzymatic prenylation of **9** with **3a** and **3b** were prepared by chemical synthesis using the method of Brown *et al.*[13] and purified by reversed-phase HPLC to yield **11a** and **11b** shown in Figure 3. Interestingly, the fluorescence intensities for **11a** and **11b** are 4−6-fold less than those for **9**, which suggests that the benzoylbenzoates present in **11a** and **11b** quench the dansyl group fluorescence and that a time dependent decrease in the fluorescence should occur if **11a** and **11b** are PFTase substrates; as noted above, no such effect was observed. The possibility that **3a** and **3b** were substrates was also investigated by HPLC analysis using authentic **10**, **11a**, and **11b** as standards. A large-scale enzyme reaction using **1** and **9** as substrates resulted in the conversion of **9** to **10** in quantitative yield (data not shown). In contrast, HPLC analysis of a reaction mixture containing **9** and **3a** did not result in the formation of significant amounts of **11a** (Figure 4A). A similar result was obtained with **3b** (Figure 4B). HPLC analysis with fluorescence detection of the same reaction mixtures did allow the detection of small amounts of products in both reactions (Figure 5A,B). In both cases, the yields of products formed were less than 1%. Since these large-scale reactions were performed with 18-fold greater amounts of enzyme and were allowed to proceed for 6-fold longer times than would be necessary to quantitatively convert a reaction containing **1** and **9** to **10**, it can be estimated that **3a** and **3b** react 10 000-fold slower than the natural substrate, **1**, and are hence not efficient substrates for PFTase.

Inhibition Kinetics. To evaluate their potential as enzyme inhibitors, the rate of PFTase-catalyzed farnesylation of *N*-dansyl-GCVIA was measured in the presence of fixed concentrations of **3a** and **3b** at various concentrations of FPP. Double reciprocal plots of these data for **3a** (Figure 6A) and **3b** (Figure 6B) both give patterns of lines that intersect on the 1/*v* axis, consistent

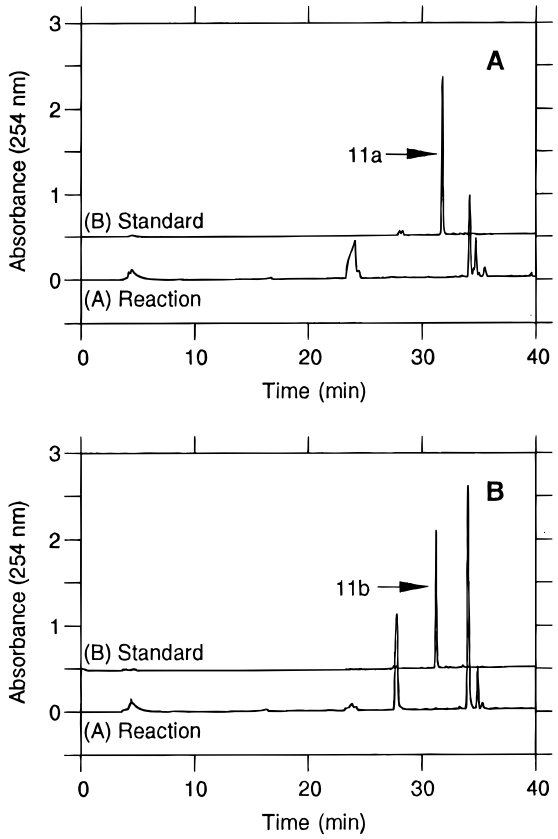


Figure 4. HPLC analysis of reactions containing PFTase, **9**, and **3a** or **3b**. Panel A: chromatogram A is from a 10 mL reaction mixture containing **9** and **3a**. Chromatogram B is from a sample of chemically synthesized **11a**. The amount of **11a** injected is equal to the amount of product that would be formed in the enzymatic reaction assuming 100% conversion. Panel B: chromatogram A is from a 10 mL reaction mixture containing **9** and **3b**. Chromatogram B is from a sample of chemically synthesized **11b**. The amount of **11b** injected is equal to the amount of product that would be formed in the enzymatic reaction assuming 100% conversion.

with competitive inhibition with respect to the substrate, FPP. The rate data were further analyzed by the method of Eadie-Hoftsee to determine K_i values for each inhibitor. Compound **3a** yields a value of 910 nM, while **3b** gives a value of 380 nM. Comparison of these data with the K_D value of 75 nM obtained for FPP by Dolence *et al.* indicates that **3a** and **3b** bind effectively to PFTase; the presence of the benzophenone moieties in **3a** and **3b** results in a 5−12-fold decrease in binding affinity for PFTase when compared to the natural ligand, **1**.[14]

Photolysis Kinetics. Compounds **3a** and **3b** were tested for their ability to inactivate PFTase upon UV irradiation. These experiments were performed by irradiating mixtures of the inhibitors and the enzyme, withdrawing aliquots at regular intervals, and assaying the resulting samples for residual activity. The results of these experiments are summarized in Figure 7A (**3a**) and B (**3b**). Irradiation of PFTase alone for up to 12 h resulted in no decrease in enzyme activity. In contrast, irradiation for 2 h in the presence of **3a** or **3b** at saturating concentrations led to a 12% decrease in enzyme activity with **3a** and a 9% decrease with **3b**. This inactivation could be partially reversed by the addition

(13) Brown, M. J.; Milano, P. D.; Lever, D. C.; Epstein, W. W.; Poulter, C. D. *J. Am. Chem. Soc.* **1991**, *113*, 3176−3177.

(14) Dolence, J. M.; Cassidy, P. B.; Mathis, J. R.; Poulter, C. D. *Biochemistry* **1995**, *34*, 16687−16694.

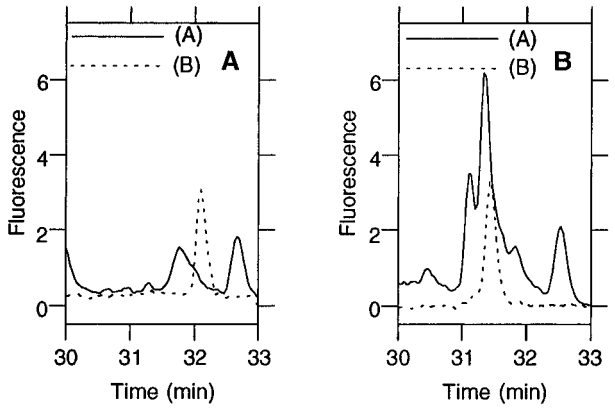


Figure 5. HPLC analysis employing fluorescence detection of reactions containing PFTase, **9**, and **3a** or **3b**. Panel A: chromatogram A is from a 10 mL reaction mixture containing **9** and **3a**. Chromatogram B is from a sample of chemically synthesized **11a**. The amount of **11a** injected is equal to the amount of product that would be formed in the enzymatic reaction assuming 0.5% conversion. Panel B: chromatogram A is from a 10 mL reaction mixture containing **9** and **3b**. Chromatogram B is from a sample of chemically synthesized **11b**. The amount of **11b** injected is equal to the amount of product that would be formed in the enzymatic reaction assuming 0.5% conversion.

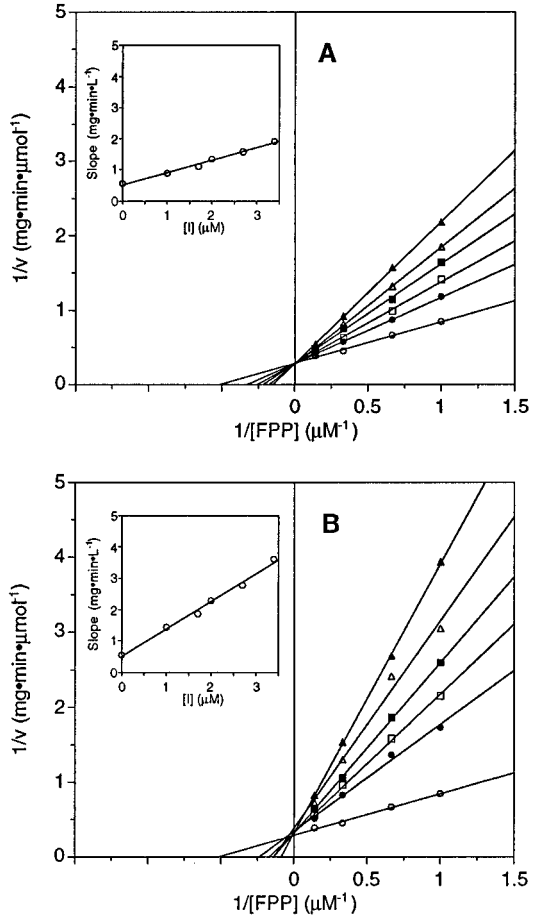


Figure 6. Double reciprocal plots showing the competitive inhibition of PFTase by **3a** and **3b**. The concentrations of **3a** (or **3b**) are as follows: (○) 0 μM, (●) 1.0 μM, (□) 1.7 μM, (■) 2.0 μM, (△) 2.7 μM, (▲) 3.4 μM. Inset: replot of slopes from double reciprocal plot versus [**3a**] (or [**3b**]). Panel A: inhibition by **3a**. Panel B: inhibition by **3b**.

of substrate, FPP; in the case of reactions containing **3a** or **3b** and FPP (100 μM), only 3% inactivation was observed with **3a** and 4% inactivation with **3b**. Greater levels of inactivation were achieved with longer incubation times. Photolysis of PFTase in the presence of **3a** resulted in 21% inactivation after 6 h and 44% inactivation after 12 h. Similar results were obtained with **3b**. It should be noted that no inactivation occurred when PFTase was incubated with **3a** or **3b** in the dark and that inactivation ceased when irradiation of reaction mixtures was stopped. These experiments indicate that **3a** and **3b** are true photoaffinity labeling reagents and that no long-lived reactive intermediates are involved in the inactivation process.

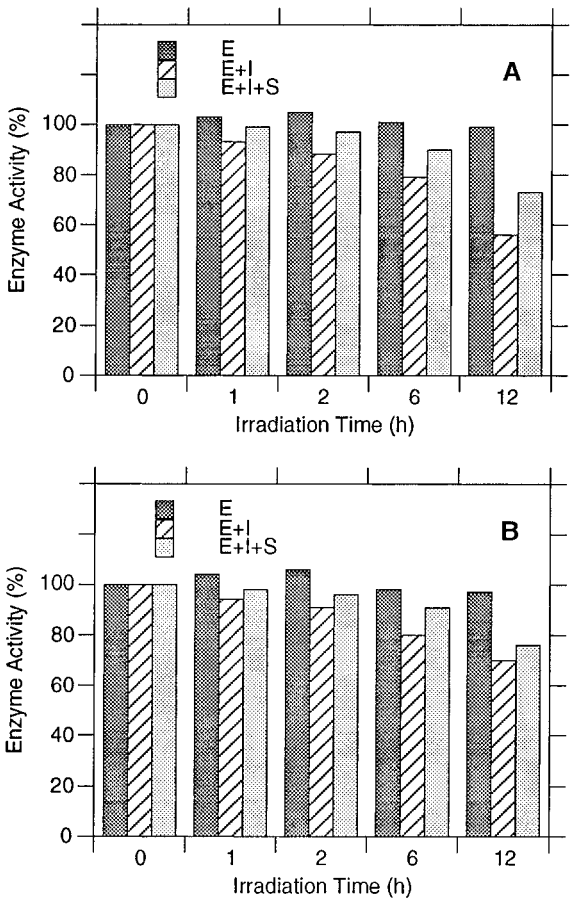


Figure 7. Time course for the photoinactivation of PFTase by **3a** and **3b**. Irradiation of PFTase alone (E), in the presence of **3a** (E + I), or in the presence of **3a** and FPP (E + I + S). Panel A: inhibition by **3a**. Panel B: inhibition by **3b**.

Photolysis Reactions with [^{32}P]3a and [^{32}P]3b. To determine the sites of cross-linking of PFTase with **3a** and **3b**, the radiolabeled analogs [^{32}P]**3a** and [^{32}P]**3b** were prepared and purified using a modification of the procedure developed by Bukhtiyarov.[5d] Photolysis of PFTase in the presence of [^{32}P]**3a** resulted in preferential labeling of the β subunit (Figure 8A, lane 3). Similar results were obtained with [^{32}P]**3b** (Figure 8B, lane 3). Addition of substrate, **1**, to photolysis reactions containing[^{32}P]**3a** or [^{32}P]**3b** resulted in substantial protection from labeling (Figure 8A, lane 4, and Figure 8B, lane 4). To quantitate the relative labeling efficiencies for each reaction, phosphorimaging analysis was employed (Figure 9). Using [^{32}P]**3a**, the β subunit was labeled 3.7-fold more than the α subunit. With [^{32}P]**3b**, the β subunit was labeled 4.2-fold more than the α subunit. Inclusion of substrate **1** in photolysis reactions containing PFTase and [^{32}P]**3a** resulted in a 4.5-fold decrease in β subunit labeling and a 3.1-fold decrease in α subunit labeling. Similar experi-

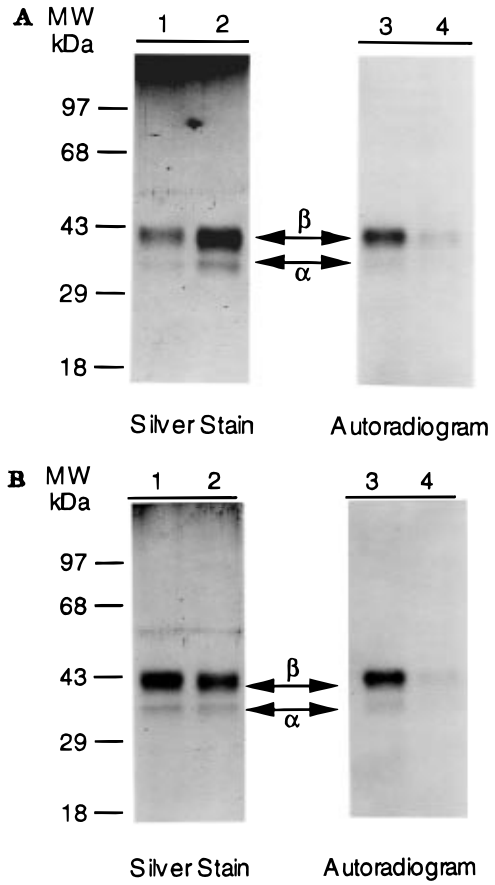


Figure 8. Analysis of photolabeling of PFTase by [^{32}P]**3a** and [^{32}P]**3b** by SDS−polyacrylamide gel electrophoresis. Lanes 1 and 3 contain samples of PFTase irradiated in the presence of [^{32}P]**3a** (or [^{32}P]**3b**), Lanes 2 and 4 contain samples of PFTase irradiated in the presence of [^{32}P]**3a** (or [^{32}P]**3b**) and substrate **1**. Lanes 1 and 2 show the silver-stained proteins and lanes 3 and 4 show the radiolabeled proteins. Panel A: labeling with [^{32}P]**3a**. Panel B: labeling with [^{32}P]**3b**. The lighter intensity of the α subunit compared to the β subunit is an artifact of silver staining. In gels stained with coomassie blue, both bands appear with equal intensities.

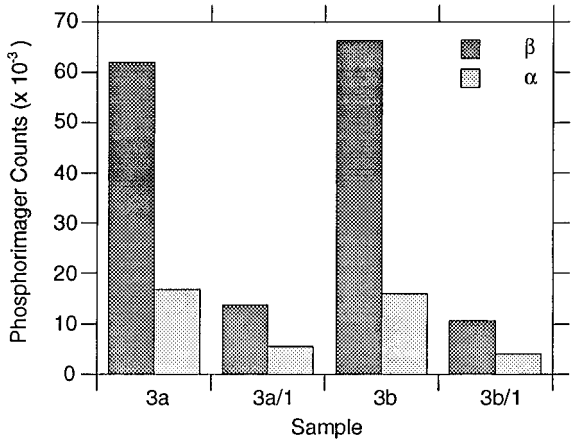


Figure 9. Phosphorimaging analysis of photolabeling of α and β subunits of PFTase by [^{32}P]**3a** and [^{32}P]**3b**. Column 3a: PFTase irradiated in the presence of [^{32}P]**3a**. Column 3a/1: PFTase irradiated in the presence of [^{32}P]**3a** and **1**. Column 3b: PFTase irradiated in the presence of [^{32}P]**3b**. Column 3b/1: PFTase irradiated in the presence of [^{32}P]**3b** and **1**.

ments with [^{32}P]**3b** yielded a 6.3-fold decrease in β subunit labeling and a 4.0-fold decrease in α subunit labeling. The preferential labeling of the β subunit of yeast PFTase is consistant with results obtained with human PFTase using other photoprobes.[5] Collectively, these results implicate the β subunit in prenyl group recognition and suggest that benzophenone-based photoprobes may allow specific amino acid residues in the prenyl group binding site to be identified. These experiments are currently in progress.

Stability Studies. Although the ^{32}P-labeled compounds employed here did allow the prenyl group binding subunit to be identified, these allylic pyrophosphates and their cross-linked products are not stable to the conditions normally used to separate cross-linked peptides. For peptide mapping, the radiolabel must be incorporated at a position that is stable to the purification conditions; consequently, the stability of the ester linkage in **7b** was evaluated. Incubation of **7b** in CD_3CN/D_2O (9:1) containing 0.2% CF_3CO_2D for 2 weeks at 37 °C resulted in no significant cleavage of the benzoylbenzoate ester linkage as determined by ^{1}H NMR. Since these conditions are more severe than those typically used in the HPLC analysis of tryptic digests of proteins that have been subjected to photocross-linking, these results suggest that radioactive labels can be incorporated into either the benzophenone or the isoprenoid portions of **3a** and **3b** without risking the loss of label during peptide analysis.

Conclusions

Compounds **3a** and **3b** are analogs of FPP that are competitive inhibitors of PFTase with K_i values in the submicromolar range. Upon photolysis, they inactivate PFTase in a time-dependent manner, resulting in up to 44% inactivation of enzyme activity. Photolysis of PFTase in the presence of [^{32}P]**3a** or of [^{32}P]**3b** resulted in preferential labeling of the β subunit, suggesting that this subunit is involved in prenyl group recognition. These compounds should be valuable tools for studying enzymes that utilize FPP as a substrate.

Experimental Section

General Procedures. All reactions were conducted under dry nitrogen and stirred magnetically. Reaction temperatures refer to external bath temperatures. Analytical TLC was performed on precoated (0.25 mm) silica gel 60F-254 plates purchased from E. Merck. Visualization was done under UV irradiation or by subjecting the plates to ethanolic phosphomolybdic acid solutions followed by heating. Flash chromatography silica gel (60−120 mesh) was obtained from E. M. Science. CH_2Cl_2, CH_3CN, and pyridine were distilled from CaH_2, and Et_2O was distilled from sodium/benzophenone ketyl. DMF was dried with 3 Å molecular sieves. Melting points were recorded in open capillaries and are uncorrected. NMR *J* values are given in Hz. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. UV spectra were obtained using a Hewlett-Packard 8452A spectrophotometer, and fluorescence measurements were performed with a Perkin-Elmer LS 50B luminescence spectrometer. HPLC analysis was carried out using a Beckman Model 127/166 instrument equiped with a diode array UV detector and a Beckman 6300A fluorescence detector (305−395 nm excitation filter and 480−520 nm emission filter). Preparative HPLC separations were performed with a Rainin Dynamax Microsorb C_{18} column (2.14 × 25 cm with a 5 cm guard column), while analytical separations employed a Phenomenex Luna C_{18} column (5 μm, 4.6 × 250 mm). Phosphorimaging analysis was performed with a Moleular Dynamics 445 SI Phosphorimager. Cell line YL1/2 (ECACC # 92092402) was obtained from the European Collection of Animal Cell Cultures. Fetal bovine serum was obtained from Hyclone, and DMEM/F-12 media and Protein-Free Hybridoma Media were obtained from Gibco/BRL. Gam-

maBind Plus Sepharose resin, DE-52 resin, and Dowex 50W-X8 resin were obtained from Pharmacia, Whatman, and BioRad, respectively. Sep-Pak columns were obtained from Waters. Asp-Phe and *N*-dansylglycine were purchased from Sigma. [^{32}P]H_3PO_4 (specific activity 8500−9120 Ci/mmol) was obtained from DuPont NEN. *E. coli* DH5α/pGP114 was a generous gift from Dr. C. D. Poulter, Department of Chemistry, University of Utah.

3,7-Dimethyl-1-(chloroacetoxy)-2,6-octandien-8-ol (5). To a solution of geraniol, **4** (1.54 g, 10 mmol), in DMF (10 mL) was added chloroacetic anhydride (2.57 g, 15 mmol), pyridine (2 mL), and DMAP (0.122 g, 1 mmol), and the resulting solution was stirred at rt for 30 min. The solvents were removed *in vacuo*, and the resulting oily residue was dissolved in Et_2O, washed with 1 M $NaHCO_3$, dried with Na_2SO_4, and filtered. The organic layer was then concentrated and purified by flash chromatography on silica gel (hexanes/toluene, 2:1, v/v), which afforded geranyl chloroacetate (2.26 g, 98%) as a colorless oil: R_f 0.1 (silica gel, hexanes/toluene (2:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.32 (1H, t), 5.04 (1H, t), 4.67 (2H, d, J = 7.2), 4.02 (2H, s), 2.13−1.97 (4H, m), 1.69 (3H, s), 1.65 (3H, s), l.57 (3H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 167.5, 143.8, 132.1, 123.9, 117.7, 63.2, 41.2, 39.8, 26.5, 25.9, 17.9, 16.7; HR-CI MS calcd for $C_{12}H_{23}ClNO_2$ $[M + NH_4]^+$ 248.1417, found 248.1411.

Geranyl chloracetate (2.30 g, 10 mmol) and *tert*-butyl hydroperoxide (4.0 mL, 36 mmol) were stirred in the presence of H_2SeO_3 (26 mg, 0.2 mmol) and salicylic acid (140 mg, 1 mmol) in CH_2Cl_2 (10 mL) for 20 h at rt. The CH_2Cl_2 was removed under reduced pressure, and the *tert*-butyl-hydroperoxide was removed by repeated (3×) addition of toluene and evaporation. The residue was dissolved in Et_2O, washed with 1 M $NaHCO_3$ to remove H_2SeO_3, dried with Na_2SO_4, and filtered. The organic layer was concentrated and the crude product purified by flash chromatography (toluene/EtOAc, 10:1, v/v) to yield **5** as a colorless oil (1.11 g, 48%): R_f 0.26 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.32 (2H, t, J = 6.9), 4,67 (2H, d, J = 7.2), 4.03 (2H, s), 3.95 (2H, s), 2.18-2.01 (4H, m), 1.69 (3H, s), l.63 (3H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 167.6, 143.3, 135.6, 125.1, 117.9, 68.8, 63.2, 41.1, 39.2, 25.8, 16.6, 13.8; HR-CI MS calcd for $C_{12}H_{23}ClNO_3$ $[M + NH_4]^+$ 264.1366, found 264.1363. Anal. Calcd for $C_{12}H_{19}O_3Cl$: C, 58.45; H, 7.70. Found: C, 58.26, H, 7.59.

(*E,E*)-8-*O*-(4-Benzoylbenzoyl)-1-(chloroacetoxy)-3,7-dimethyl-2,6-octadiene (6a). Compound **5** (492 mg, 2.0 mmol) was acylated with 4-benzoylbenzoyl chloride (700 mg, 2.8 mmol) in pyridine (2.5 mL) at rt for 6 h. The reaction mixture was then filtered, and the pyridine was removed under reduced pressure. Toluene was added to the resulting residue, and the mixture was filtered to remove excess 4-benzoylbenzoyl chloride. The solution was concentrated, dissolved in toluene/EtOAc (10:1, v/v), and purified by flash chromatography using the same solvent. Evaporation of the solvent gave **6a** as a light yellow oil (349 mg, 38%): R_f 0.58 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.11 (2H, d, J = 8.2), 7.78 (2H, d, J = 8.2), 7.74 (2H, d, J = 6.9), 7.56 - 7.39 (3H, m), 5.49 (1H, t, J = 6.00), 5.32 (1H, t, J = 7.2), 4.67 (2H, d, J = 7.2), 4.63 (2H, s), 4.00 (2H, s), 2.19−2.07 (4H, m), 1.70 (3H, s), 1.68 (3H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 196.0, 167.4, 165.8, 143.0, 141.5, 137.2, 133.7, 133.1, 130.7, 130.3, 130.0, 129.7, 129.2, 128.7, 118.2, 71.1, 63.1, 41.2, 39.0, 26.1, 16.7, 14.3; HR-EI MS calcd for $C_{26}H_{27}ClO_5$ $[M]^+$ 454.1547, found 454.1561. Anal. Calcd for $C_{26}H_{27}ClO_5$: C, 68.67; H, 5.94. Found: C, 68.78; H, 5.79.

(*E,E*)-8-*O*-(4-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadien-1-ol (7a). Compound **6a** (0.454 g, 1.0 mmol) was hydrolyzed with 4.5 mL of 0.1 M NH_4OH in aqueous methanol (90% MeOH, v/v) at rt for 1 h. The methanol was removed under reduced pressure, dissolved in Et_2O, and dried over Na_2SO_4. The crude product was then concentrated *in vacuo*, dissolved in toluene, and purified by flash chromatography by elution with toluene/EtOAc (10:1, v/v) to yield **7a** as a pale yellow oil (158 mg, 42%): R_f 0.16 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (2H, d, J = 8.20), 7.82 (2H, d, J = 8.28), 7.79 (2H, d, J = 8.2), 7.76−7.25 (3H, m) 5.53 (1H, t, J = 6.8), 5.40 (1H, t, J = 6.9), 4.72 (2H, s), 4.13 (2H, d, J = 6.8), 2.25−2.08 (4H, m), 1.73 (3H, s), 1.66 (3H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 196.3, 166.0, 141.6, 138.9, 137.2, 133.8, 133.2, 130.4, 130.0, 129.8, 129.7, 128.7, 124.3, 71.3, 59.6, 39.1, 26.2, 16.5, 14.3; HR-EI MS calcd for $C_{24}H_{26}O_4$ $[M]^+$ 378.1824, found 378.1816. Anal. Calcd for $C_{24}H_{26}O_4$: C, 76.21; H, 6.87. Found: C, 76.13; H, 7.05.

(*E,E*)-8-*O*-(4-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene 1-Chloride (8a). *N*-Chlorosuccinamide (0.147 g 1.1 mmol) was dissolved in CH_2Cl_2 (4.5 mL) and cooled to −30 °C. Dimethyl sulfide (74 mg, 1.2 mmol) was added dropwise, and the solution was then cooled to −40 °C. Compound **7a** (0.378 g, 1.0 mmol) dissolved in 1 mL of CH_2Cl_2 was slowly added, and the reaction mixture was allowed to warm to 0 °C over 1 h. After being stirred at 0 °C for an additional 15 min, the mixture was poured into a separatory funnel containing cold saturated NaCl solution (2.5 mL) and pentane (2.0 mL). After extraction, the pentane layer was removed and retained, and the aqueous layer was extracted two additional times with pentane (2 mL). The organic fractions were then combined, washed twice with cold saturated aqueous NaCl (10 mL each time), dried over Na_2SO_4, filtered, and evaporated to yield the allylic chloride as a pale yellow oil (190 mg, 48%): R_f 0.71, (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.15 (2H, d, J = 8.0), 7.83 (2H, d, J = 8.0), 7.79 (2H, d, J = 8.2), 7.64−7.45 (3H, m), 5.56−5.41 (2H, m), 4.86 (1H, s), 4.07 (2H, d, J = 7.8), 2.17−2.15 (4H, m), 1.7 (6H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 196.3, 165.8, 142.1, 141.5, 137.0, 133.6, 133.0, 130.6, 130.2, 129.9, 129.6, 129.1, 128.6, 121.0, 71.0, 41.0, 38.9, 26.0, 16.2, 14.2; HR-EI MS calcd for $C_{24}H_{25}ClO_3$ $[M]^+$ 396.1493, found 396.1489.

(*E,E*)-8-*O*-(4-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene1-Diphosphate (3a). The allylic chloride **8a** (41 mg, 0.103 mmol) was pyrophosphorylated with $[(n\text{-}Bu)_4N]_3HP_2O_7$ (196 mg, 0.218 mmol) in anhydrous CH_3CN (0.492 mL) for 3 h at rt. The product was converted to the NH_4^+ salt using an ion-exchange column (Dowex 50W-X8, NH_4^+ form) in 25 mM NH_4HCO_3/2-propanol (49:1, v/v), and the salt was obtained by lyophilization. The final product was purified employing a C_{18} reversed-phase column (Sep-pak cartridge) in a 25 mM NH_4HCO_3/CH_3CN solvent system. After lyophilization, **3a** (30 mg, 50% yield) was obtained as a white powder: ^{1}H NMR (300 MHz, D_2O, adjusted to pH 8 with ND_4OD) δ 8.03 (2H, d, J = 8.4), 7.73 (2H, d, J = 8.4), 7.68 (1H, d, J = 8.7), 7.58 (2H, d, J = 7.2), 7.43 (2H, t, J = 7.5), 5.50 (1H, t, J = 5.7), 5.33 (1H, t, J = 7.5), 4.73 (2H, s), 4.31 (2H t, , J = 7.5), 2.10 (2H, m), 1.99 (2H, m), 1.61 (3H, s), 1.57 (3H, s); ^{31}P NMR (121.4 MHz, D_2O, adjusted to pH 8 with ND_4OD) δ −6.84 (1P, d, J = 23), −10.87 (1P, d, J = 23); UV (H_2O), λ_{max} = 262 nm, ϵ = 18 600 $M^{-1}cm^{-1}$; FAB MS calcd for $C_{24}H_{27}O_{10}P_2$ $[M - H]^-$ 537.0, found 537.1, calcd for $C_{24}H_{26}O_{10}P_2Na$ $[M - 2H + Na]^-$ 559.0, found 559.0; HR-FAB MS calcd for $C_{24}H_{28}O_{10}P_2Na$ $[M + Na]^+$ 561.1048, found 561.1088.

(*E,E*)-8-*O*-(3-Benzoylbenzoyl)-1-(chloroacetoxy)-3,7-dimethyl-2,6-octadiene (6b). Compound **6b** was prepared and purified using the procedures described for the synthesis of **6a**. After chromatography, **6b** was obtained as a clear oil (38%): R_f 0.50 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.43 (1H, s), 8.25 (1H, d, J = 8.0), 7.97 (1H, d, J = 8.0), 7.79 (1H, s), 7.75 (1H, s), 7.60−7.25 (4H, m), 5.49 (1H, t), 5.34 (1H, t), 4.69 (2H, d, J = 6.0), 4.65 (2H, s), 4.03 (2H, s), 2.18−2.07 (4H, m), 1.78 (6H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 195.8, 167.4, 165.7, 143.0, 138.1, 134.1, 133.3, 132.9, 131.1, 130.2, 129.1, 128.7, 128.6, 128.4, 128.2, 127.4, 118.1, 70.9, 63.0, 41.1, 39.0, 26.0, 16.7, 14.2; HR-EI MS calcd for $C_{26}H_{27}ClO_5$ $[M]^+$ 454.1547, found 454.1558. Anal. Calcd for $C_{26}H_{27}ClO_5$: C, 68.67; H, 5.94. Found: C, 68.49; H, 5.77.

(*E,E*)-8-*O*-(3-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadien-1-ol (7b). The procedures described for the preparation of **7a** were used for the synthesis and purification of **7b**. After purification by flash chromatography, **7b** was isolated as a white crystalline solid (43%): mp 37−38 °C; R_f 0.13 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.44 (1H, s), 8.25 (1H, d, J = 8.0), 7.97 (1H, d, J = 8.0), 7.81 (1H, s), 7.77 (1H, s), 7.64−7.44 (4H, m), 5.48 (1H, t), 5.40 (1H, t), 4.71 (2H, s), 4.13 (2H, d, J = 6.8), 2.21−2.07 (4H, m), 1.70 (3H, s), 1.66 (3H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 198.8,

165.7, 138.9, 138.1, 137.0, 134.2, 133.3, 132.9, 131.1, 131.0, 130.3, 130.2, 129.4, 128.7, 128.6, 124.2, 71.0, 59.4, 38.9, 26.0, 16.3, 14.2; HR-EI MS calcd for $C_{24}H_{26}O_4$ $[M]^+$ 378.1824, found 378.1831. Anal. Calcd for $C_{24}H_{26}O_4$: C, 76.21; H, 6.87. Found: C, 76.10; H, 6.98.

(*E,E*)-8-*O*-(3-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene 1-Chloride (8b). Compound **8b** was prepared from **7b** using the procedure outlined for the synthesis of **8a**. The desired chloride (**8b**) was obtained as a pale yellow oil (52%): R_f 0.66 (silica gel, toluene/EtOAc, 10:1, v/v); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.44 (1H, s), 8.26 (1H, d, J = 8.0), 7.98 (1H, d, J = 7.8), 7.81 (1H, s), 7.78 (1H, s), 7.77−7.38 (4H, m), 5.53−5.40 (2H, m), 4.71 (2H, s), 4.07 (2H, d, J = 8.0), 2.21−2.10 (4H, m), 1.72 (6H, s); ^{13}C NMR (52.3 MHz, $CDCl_3$) δ 196.5, 165.7, 142.1, 138.1, 137.2, 134.0, 133.3, 132.9, 131.1, 130.6, 130.3, 130.2, 129.0, 128.7, 128.6, 120.9, 71.0, 41.1, 38.9, 26.0, 16.2, 14.2; HR-EI MS calcd for $C_{24}H_{25}ClO_3$ $[M]^+$ 396.1493, found 396.1493.

(*E,E*)-8-*O*-(3-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene 1-Diphosphate (3b). The allylic chloride **8b** was pyrophosphorylated as described for the preparation of **3a** to give **3b** as a white powder (13 mg, 33%): ^{1}H NMR (300 MHz, D_2O, adjusted to pH 8 with ND_4OD) δ 8.18 (1H, s), 8.14 (1H, d, J = 7.8), 7.88 (1H, d, J = 7.8), 7.66 (1H, m), 7.64 (1H, m), 7.55 (2H, m), 7.42 (2H, m), 5.42 (1H, m), 5.28 (1H, m), 4.78 (2H, s), 4.29 (2H, m), 2.04 (2H, m), 1.93 (2H, m), 1.54 (6H, s); ^{31}P NMR (121.4 MHz, D_2O, adjusted to pH 8 with ND_4OD) δ −6.92 (1P, d, J = 22), −10.84 (1P, d, J = 23); UV (H_2O), λ_{max} = 222 nm, ϵ = 39,100 $M^{-1}\cdot cm^{-1}$, λ_{max} = 258 nm, ϵ = 24 100 $M^{-1}\cdot cm^{-1}$; FAB MS calcd for $C_{24}H_{29}O_{10}P_2$ $[M + H]^+$ 539.0, found 539.2, calcd for $C_{24}H_{28}O_{10}P_2Na$ $[M + Na]^+$ 561.0, found 561.1, calcd for $C_{24}H_{27}O_{10}P_2$ $[M - H]^-$ 537.0, found 537.1.

(*E,E*)-[α,β(*n*)^{32}P]-8-*O*-(4-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene 1-Diphosphate ([^{32}P]3a). Alcohol **7a** (2.4 mg, 6.3 μmol) was reacted with anhydrous $[^{32}P]H_3PO_4$ (1.2 mg, 13 μmol) in CH_3CN (200 μL) containing 20% (v/v) CCl_3CN and triethylamine (2.5 mg, 26 μmol) for 2 h. The volatile components from the reaction were then evaporated, and the resulting residue was purified using a reversed-phase Sep-pak C_{18} cartridge and a NH_4HCO_3/CH_3CN step gradient to yield [^{32}P]**3a** in 3.3% yield (specific activity 480 Ci/mol); the concentration of solutions containing [^{32}P]**3a** were determined by UV using the wavelengths and extinction coefficients reported for **3a**. The radiochemical purity of [^{32}P]**3a** was assessed by thin layer chromatography in 2-propanol/NH_4OH/H_2O (6:3:1, v/v/v) followed by phosphorimaging analysis and was found to be 50%. [^{32}P]**3a** was further purified by preparative thin layer chromatography using the above solvent system to yield material whose radiochemical purity was greater than 90%. Non-radioactive **3a** prepared using this procedure and analyzed by HPLC coeluted with **3a** prepared via the chloride.

(*E,E*)-[α,β(*n*)^{32}P]-8-*O*-(3-Benzoylbenzoyl)-3,7-dimethyl-2,6-octadiene 1-Diphosphate ([^{32}P]3b). Compound [^{32}P]**3b** was prepared as described for [^{32}P]**3a**. After reversed-phase chromatography, [^{32}P]**3b** was obtained in 1.0% yield (specific activity 700 Ci/mol) and 56% radiochemical purity. Further purification by preparative thin layer chromatography yielded [^{32}P]**3b**, whose radiochemical purity was greater than 90%.

***N*-Dansyl-GCVIA (9).** *N*-Dansyl-GCVIA was synthesized by solid-phase methods using standard protocols for α-*N*-Boc-protected amino acids, beginning with *N*-Boc-Ala Merrifield resin and employing a *p*-methoxybenzyl protecting group for cysteine.[15] Dansylation was accomplished by treating the solid-phase resin with a solution of dansyl chloride in CH_2Cl_2 in the presence of TEA. The completed peptide was deprotected and cleaved from the resin with HF under standard conditions and purified by reversed-phase HPLC employing a H_2O/CH_3CN (containing 0.2% TFA) gradient: FAB MS calcd for $C_{31}H_{45}N_6O_8S_2$ $[M - H]^-$ 693.3, found 693.4, calcd for $C_{31}H_{47}N_6O_8S_2$ $[M + H]^+$ 695.3, found 695.4; fluorescence (H_2O/CH_3CN/TFA, 50/50/0.2) λ_{ex} = 322 nm, λ_{em} = 530 nm, intensity = 1.3 (relative to *N*-dansylglycine, 1.0).

***N*-Dansyl-GC(4-BBG)VIA (11a)**. Compound **11a** was prepared by reacting **8a** with **9** in liquid ammonia as described by Brown *et al.*[13] The crude product was purified by reversed-phase HPLC employing a H_2O/CH_3CN (containing 0.2% TFA) gradient: FAB MS calcd for $C_{55}H_{70}N_6NaO_{11}S_2$ $[M + Na]^+$ 1077.4, found 1077.5, calcd $C_{55}H_{69}N_6Na_2O_{11}S_2$ $[M - H + 2Na]^+$ 1099.4, found 1099.4; UV (H_2O/CH_3CN/TFA, 50/50/0.2) λ_{max} = 258 nm, ϵ = 21 600 $M^{-1}\cdot cm^{-1}$, λ_{max} = 320 nm, ϵ = 1780 $M^{-1}\cdot cm^{-1}$; fluorescence (H_2O/CH_3CN/TFA, 50/50/0.2) λ_{ex} = 322 nm, λ_{em} = 530 nm, intensity = 0.21 (relative to *N*-dansylglycine, 1.0).

***N*-Dansyl-GC(3-BBG)VIA (11b)**. Compound **11b** was prepared from **8b** and **9** as described for **11a**: FAB MS calcd for $C_{55}H_{70}N_6NaO_{11}S_2$ $[M + Na]^+$ 1077.4, found 1077.6, calcd $C_{55}H_{69}N_6Na_2O_{11}S_2$ $[M - H + 2Na]^+$ 1099.4, found 1099.6, calcd $C_{55}H_{69}N_6O_{11}S_2$ $[M - H]^-$ 1053.4, found 1053.6; UV (H_20/CH_3-CN/TFA, 50/50/0.2) λ_{max} = 254 nm, ϵ = 18 100 $M^{-1}\cdot cm^{-1}$, λ_{max} = 320 nm, ϵ = 1780 $M^{-1}\cdot cm^{-1}$; fluorescence (H_2O/CH_3CN/TFA, 50/50/0.2) λ_{ex} = 322 nm, λ_{em} = 530 nm, intensity = 0.29 (relative to *N*-dansylglycine, 1.0).

Preparation of *anti*-α-Tubulin Immunoaffinity Column. Cell line YL1/2 producing *anti*-α-tubulin was obtained as a frozen sample, cultured in DMEM/F-12 medium containing 10% fetal bovine serum to allow the cells to recover from cryopreservation. The production of *anti*-α-tubulin by the cells was measured by ELISA using a horseradish peroxidase conjugated rabbit anti-rat IgG secondary antibody and purified α-tubulin as a standard (purified α-tubulin was obtained by homogenization of a fresh bovine brain, fractionation by ammonium sulfate precipitation, and chromatography using DEAE Sephadex). The resulting cells, producing *anti*-α-tubulin at approximately 4 μg/mL of media, were weaned from fetal bovine serum-supplemented medium into protein-free medium and cultured in large roller bottles. *anti*-α-Tubulin was purified by ammonium sulfate fractionation of the culture medium and dialysis, which yielded approximately 40 mg of antibody per 400 mL of culture medium. For immunoaffinity column preparation, purified *anti*-α-tubulin was dissolved in binding buffer (10 mM sodium phosphate pH 7.0, 150 mM NaCl, 10 mM EDTA) and applied to a 1.5 × 10 cm column containing 5 mL of GammaBind Plus Sepharose resin. The column was washed with binding buffer, and the amount of bound antibody was quantitated by comparing the concentration of the applied solution with the concentration of antibody in the wash fractions; approximately 24 mg of antibody remained bound.

Purification of Yeast Protein Farnesyltransferase. PFTase was purified by a modification of the procedure described by Mayer *et al.*[16] A 50 mL overnight culture of *E. coli* DH5α/pGP114 cells grown in LB media was used to innoculate 4 L of SB media (32 g of Bactotryptone, 20 g of yeast extract, 5 g of NaCl, 5 mL of 1 M NaOH) containing ampicillin (100 μg/mL). The large culture was grown to an OD_{600} of 0.48, induced with IPTG (0.2 mM), grown for an additional 12 h, harvested by centrifugation, flash frozen in N_2 (l), and stored at −80 °C. Approximately 65 g of wet cells were obtained. For purification, 3.0 g of cells were thawed, suspended in 80 mL of disruption buffer (50 mM Tris·HCl pH 7.0, 5 mM 2-mercaptoethanol, 1 mM PMSF), and pulse-sonnicated for 2 min. The cell lysate was clarified by centrifugation (23 000*g* for 15 min) and dialyzed against disruption buffer (2 × 2 L) without PMSF and the resulting solution applied to a DE 52 anion exchange column (3 × 30 cm) equilibrated with buffer A (50 mM Tris·HCl, pH 7.0, 5 mM $MgCl_2$, 50 μM $ZnCl_2$, 10 mM 2-mercaptoethanol). The column was eluted with a stepwise gradient consisting of 100 mL aliquots of buffer A supplemented with 0 mM, 100 mM, 200 mM, and 1 M NaCl. PFTase eluted from the column in the 200 mM NaCl wash as determined by the kinetic assay described below. Active fractions were pooled (25 mL total) and applied to an *anti*-α-tubulin immunoaffinity column (described above) that was equilibrated with buffer B (10 mM sodium phosphate pH 7.0, 150 mM NaCl, 10 mM EDTA, 10 mM 2-mercaptoethanol). The

(15) Barany, G.; Merrifield, R. B. In *The Peptides*; Academic Press: New York, 1979; Vol. 2; pp 1−284.

(16) Mayer, M. P.; Prestwich, G. D.; Dolence, J. M.; Bond, P. D.; Wu, H.-y.; Poulter, C. D. *Gene* **1993**, *132*, 41−47.

column was washed with additional buffer B to remove contaminating proteins until the A_{280} returned to base line followed by elution with buffer B supplemented with 5.0 mM Asp-Phe to afford purified PFTase (yield: 1.6 mg, specific activity: 0.86 μmol·min^{-1}·mg^{-1}).

Enzyme Assays. Solutions containing *N*-dansyl-GCVIA were prepared by dissolving the solid peptide in buffer (20 mM Tris·HCl, pH 7.0 and 10 mM EDTA), and their concentrations determined by UV absorbance at 340 nm using *N*-dansylglycine as a standard. Solutions of FPP were prepared by dissolving the solid in 25 mM NH_4HCO_3 and their concentrations determined by phosphate analysis as described by Reed and Rilling employing KH_2PO_4 as a standard;[17] solutions containing **3a** and **3b** were prepared and standardized in the same manner. PFTase was prepared for use by diluting purified enzyme with buffer (52 mM Tris·HCl, pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$) containing 1 mg/mL bovine serum albumin. Enzyme assays contained 50 mM Tris·HCl, pH 7.0, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM DTT, 0.040% (w/v) *n*-dodecyl-β-D-maltoside, 2.4 μM *N*-dansyl-GCVIA, PFTase, FPP, and **3a** or **3b**, where appropriate, in a final reaction volume of 500 μL. The assay mixtures were equilibrated to 30 °C, initiated by the addition of PFTase, and monitored spectrofluorometrically (340 nm excitation and 505 nm emission) for 300 s. Initial velocities were obtained from linear regression analysis of the time-dependent fluorescence emission data using the fluorimeter software.

Product Studies. Large-scale reactions contained 50 mM Tris·HCl, pH 7.0, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM DTT, 2.4 μM *N*-dansyl-GCVIA, 45 nM PFTase, and FPP (10 μM), **3a** (10 μM), or **3b** (10 μM), where appropriate, in a final reaction volume of 10 mL. The reactions were equilibrated to 30 °C, initiated by the addition of PFTase, and allowed to react for 1 h. To desalt the samples, each reaction mixture was applied to a Sep-Pak C_{18} cartridge, washed with H_2O/CH_3CN/TFA (95:5:0.1, v/v/v), eluted with CH_3CN/TFA (100:0.1, v/v), concentrated *in vacuo*, dissolved in H_2O/CH_3CN/TFA (50:50:0.2, v/v/v), and analyzed by HPLC using a C_{18} reversed-phase column and a H_2O/CH_3CN/TFA gradient.

Enzyme Inhibition Experiments. To determine if **3a** and **3b** were competitive inhibitors of PFTase, a 4 × 6 grid of duplicate assays were run in which the substrate, FPP, was maintained at a set of fixed concentrations and the inhibitor (**3a** or **3b**) concentrations were varied at each FPP concentration. Concentrations of 1.0, 1.5, 3.0, and 7 μM for FPP and 0, 1.0, 1.7, 2.0, 2.7, and 3.4 μM for **3a** and **3b** were chosen (based on IC_{50} experiments performed first, data not shown); the PFTase concentration in these experiments was 2.5 nM. The rates were determined from initial velocity measurements performed as described above. K_i values were calculated from Eadie-Hofstee plots obtained from a Macintosh PowerMac 7100 computer running KaleidaGraph (v. 3.0.1) software.

(17) Reed, B. C.; Rilling, H. C. *Biochemistry* **1976**, *15*, 3739−3745.

Photolysis Kinetics. Photolysis reactions were conducted at 4 °C in a UV Rayonet mini-reactor equiped with 8 RPR-3500° lamps and a circulating platform that allows up to eight samples to be irradiated simultaneously. All reactions (0.5−1 mL) were performed in silinized quartz test tubes (10 × 45 mm) and contained 52 mM Tris·HCl, pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 25 mM NH_4HCO_3, and 3.8 nM PFTase. Where appropriate, reactions contained **3a** (9.1 μM) or **3b** (3.8 μM); these concentrations were chosen to be 10 times above their calculated K_i values. For substrate protection experiments, FPP was added to a final concentration of 100 μM. Reactions were photolyzed for up to 12 h, during which time duplicate samples (50 μL) were removed at various intervals, placed on ice, and assayed for activity.

Photolysis Reactions with [^{32}P]3a and [^{32}P]3b. All reactions (100 μL) were performed in silinized quartz test tubes (10 × 45 mm) and contained 52 mM Tris·HCl, pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 25 mM NH_4HCO_3, and 380 nM PFTase. Each reaction contained [^{32}P]**3a** (9.1 μM) or [^{32}P]-**3b** (3.8 μM); for substrate protection experiments, FPP was added to a final concentration of 100 μM. Reactions were photolyzed for 2 h using the apparatus described above. Loading buffer was then added to each sample, and the samples were heated to 70 °C for 5−10 min followed by analysis by SDS-polyacrylamide gel electrophoresis with a 10% Tris−tricine gel. Gels were silver stained, dried, and subjected to autoradiography at −80 °C with an intensifying screen for 6−24 h. The relative intensities of photolabeled products were determined by phosphorimaging analysis of the dried gels.

Stability Studies. Compound **7b** was dissolved in 0.5 mL of CD_3CN/D_2O (9:1, v/v) containing 0.2% CD_3CO_2D. These conditions were chosen to resemble the acidic conditions used in the HPLC purification of peptide fragments that would typically be used to identify photocross-linking sites. The above solution was incubated at 4 °C for 8 days, rt for 20 days, and finally 37 °C for 14 days. During this time the ratio of the integrated areas for the C1 and C8 methylene protons was monitored by ^{1}H NMR as an indicator of the integrity of the benzoylbenzoate ester. In the course of this experiment, no significant cleavage was observed.

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Supporting Information Available: Copies of ^{1}H- and ^{31}P-NMR spectra of **3a** and **3b** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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