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Farnesyl and Geranylgeranyl Pyrophosphate Analogs Incorporating Benzoylbenzyl Ethers: Synthesis and Inhibition of Yeast Protein Farnesyltransferase

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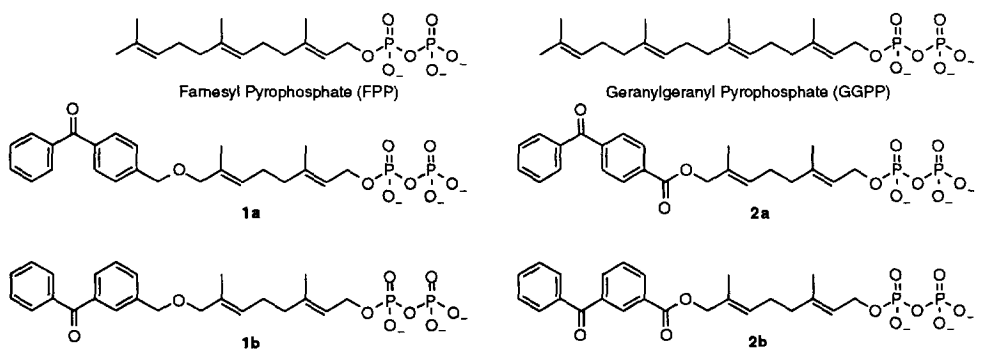
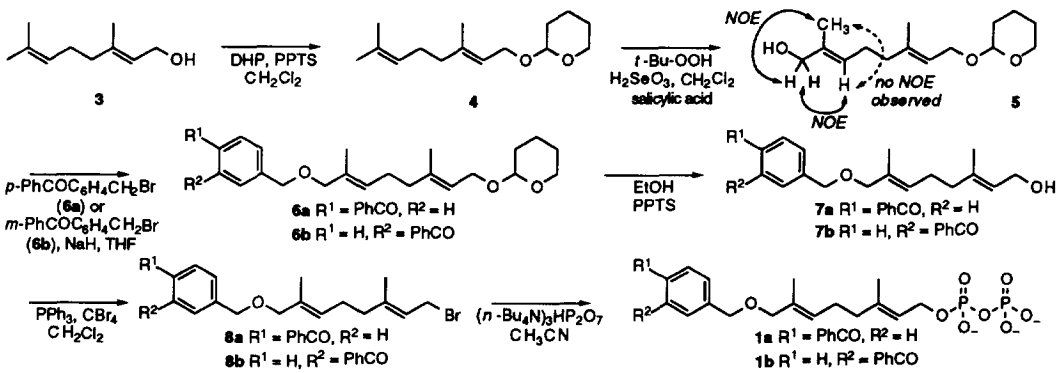
Abstract: The syntheses of two photoactive analogs (**1a** and **1b**) of farnesyl pyrophosphate that incorporate stable ether-linked benzophenones are described. Compounds **1a** and **1b** were prepared from geraniol in 17% overall yield. Both **1a** and **1b** are competitive inhibitors of yeast protein farnesyltransferase with respect to farnesyl pyrophosphate and have K_I values of 45 nM and 49 nM. Upon photolysis for twelve hours, **1a** and **1b** both inactivate the enzyme by 40%.

Protein prenylation, discovered in 1976 by Kamiya, is an important post-translational protein modification.[1] A large number of prenylated proteins including Ras have been identified which has stimulated considerable interest in this area and a number of groups are designing inhibitors of protein farnesyltransferase (PFTase) as potential anticancer agents.[2] Photoaffinity labeling is a useful technique for studying the interactions between small molecules and proteins.[3] To gain information about the interactions between proteins and prenyl pyrophosphates, we have synthesized a number of analogs of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) that incorporate benzophenone crosslinking groups.[4] Benzophenone-containing compounds have synthetic and photochemical properties[5] that complement existing diazoester-containing reagents.[6] Earlier work demonstrated the utility of benzophenone-containg analogs **2a** and **2b** by crosslinking these compounds to the β-subunit of yeast PFTase.[4b] This paper describes the preparation and use of new analogs, **1a** and **1b**, that possess ether-linked benzophenones in lieu of the ester-linkages present in compounds **2a** and **2b**. Compounds **1a** and **1b** can be prepared by a more efficient synthesis, bind to PFTase with higher affinity, and are more stable to a variety of conditions when compared with **2a** and **2b**.



Synthesis. The syntheses of compounds **1a** and **1b** were each accomplished in six steps as illustrated in Scheme 1. Geraniol (**3**) was first protected by reaction with 3,4-dihydropyran and pyridinium *p*-toluenesulfonate (PPTS);[7] other protecting groups including chloroacetoxy and TBDPS proved to be problematic in later steps of the synthesis. The resulting ether, **4**, was then oxidized with *t*-butyl hydroperoxide and catalytic H_2SeO_3 to yield **5** in 56% yield after purification by flash chromatography.[8] The *E*-stereoselectivity for the hydroxylation reaction was confirmed by pulsed field gradient NOE spectroscopy as indicated in Scheme 1.[9] Compound **5** was then treated with NaH and 4-(bromomethyl) benzophenone or 3-(bromomethyl) benzophenone to give **6a** and **6b** in 78% and 80% yields, respectively.[10] An alternative procedure based on Mitsunobu coupling[11] of **5** with 4-(hydroxymethyl) benzophenone gave **6a** in 47% yield and was consequently not pursued further. Deprotection of **6a** and **6b** was accomplished with PPTS in ethanol to yield **7a** and **7b** in quantitative yield.[12] In early experiments, alcohol **7a** was converted to the corresponding chloride by treatment with *N*-chlorosuccinamide and dimethyl sulfide followed by pyrophosphorylation. However, subsequent efforts using the method described by Davisson *et al.* to generate the more reactive allylic bromides produced the final pyrophosphates in higher yields.[13] Thus, **7a** and **7b** were treated with PPh_3 and CBr_4 to give **8a** and **8b**[14] which were then reacted, without purification, with tris (tetra-*n*-butylammonium) hydrogen pyrophosphate to give **1a** and **1b** in 41% and 38% yields. Diphosphates **1a** and **1b** were purified by reversed-phase chromatography and characterized by ^1H NMR, ^{31}P NMR, UV, and HR-FAB mass spectrometry.[15] The route described here produces **1a** and **1b** in 17% overall yield and thus provides an efficient means of preparing photoactive analogs of FPP and GGPP.



Scheme 1

Enzyme Inhibition and Photolysis. To evaluate their potential as enzyme inhibitors, the rate of PFTase[16] catalyzed prenylation of a peptide substrate (*N*-Dansyl-GCVIA) was measured in the presence of **1a** and **1b**. This was accomplished using an assay that measures the time dependent increase in the fluorescence of the dansyl group as the neighboring cysteine is farnesylated.[17] No change in the fluorescence of the peptide was observed in reactions containing **1a** or **1b** without FPP which suggests that these compounds are not substrates for PFTase. In the presence of FPP, **1a** and **1b** attenuate the rate of peptide farnesylation indicating that these compounds are enzyme inhibitors. Plots of 1/v versus [I], shown in Fig. 1, gave IC_{50} values of 1600 nM for **1a** and 850 nM for **1b**. Comparison of these values with data obtained in previous work with esters **2a** and **2b** suggests that the ether analogs are more potent inhibitors (Table 1). Further kinetic experiments and analysis by the method of Eadie-Hoftsee gave K_I values of 45 nM (**1a**) and 49 nM (**1b**) and indicated that these inhibitors are competitive with respect to FPP. These values are 7 to 20 fold less than those for the corresponding esters highlighting the deleterious effect on binding of the carbonyl groups present in **2a** and **2b** (Table 1). Comparison of these K_I values with the K_D of 75 nM obtained for FPP by Dolence *et al.*[18] indicates that **1a** and **1b** bind effectively to PFTase; the presence of the benzoylbenzyl groups in **1a** and **1b** result in a

ca. 1.5 fold increase in binding affinity for PFTase when compared to FPP. Finally, photolysis of PFTase in the presence of **1a** or **1b** resulted in 40% inactivation over 12 hours[19] comparable to results observed with **2a** and **2b**.[4b]

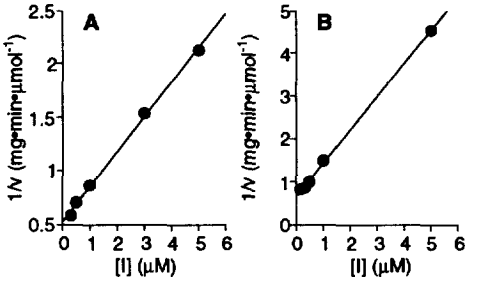


Figure 1

Compound	IC_{50} (nM)	K_I (nM)
1a	1600	45
1b	850	49
2a	3000[a]	910[a]
2b	2300[a]	380[a]
FPP	-	75 (K_D)[b]

[a]From reference 4b. [b]From reference 18.

Table 1

Implications for Studies of Protein Prenylation. This paper describes efficient syntheses of analogs of FPP that contain stable ether linkages between the photoactive benzophenone and isoprenoid moieties. In addition to photoaffinity labeling experiments with purified PFTase and PGGTase (that are now in progress), compounds **1a** and **1b** may be useful in experiments with crude cellular extracts. Radiolabeling of **1a** and **1b** with tritium should be straightforward since we have already determined that **7b** can be oxidized with MnO_2 and reduced back to **7b** with $NaBH_4$; this is in contrast to the ester-linked analogs which undergo degradation (probably via transesterification with solvent) in the presence of $NaBH_4$. The stable ether linkage present in intermediates **8a** and **8b** should also facilitate the preparation of photoactive prenylated peptides by solid phase synthesis or post-synthetic peptide modification. Finally, the ability of **1a** and **1b** to bind to PFTase, as well or better than FPP, suggests that elaboration of the third isoprene unit in FPP may be fruitful for the design of new inhibitors of PFTase.

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8. Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ =1.50 - 1.73 (m, 6H), 1.64 (s, 3H), 1.66 (s, 3H), 2.03 - 2.19 (m, 4H), 3.46 - 3.53 (m, 1H), 3.83 -3.90 (m, 1H), 3.95 (s, 2H), 4.02 (dd, 1H, *J* = 6.0, 6.0), 4.22 (dd, 1H, *J* = 6.0, 6.0), 4.61 (t, 1H, *J* = 6.0), 5.33 (t, 1H, *J* = 6.0), 5.35 (t, 1H, *J* = 6.0). ^{13}C NMR (75.5 MHz, DEPT, $CDCl_3$): δ = 13.6, 16.3 (primary), 19.5, 25.4, 25.7, 30.6, 39.1, 62.2, 63.6, 68.5 (secondary), 97.7, 120.8, 125.2 (tertiary), 135.1, 139.8 (quarternary). HRFAB-MS: $[M - H]^+$, calcd. 253.1797, found 253.1794.

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10. Compound **6a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.52 - 1.71 (m, 6H), 1.67 (s, 6H), 2.04 - 2.20 (m, 4H), 3.42 - 3.58 (m, 1H), 3.83 - 3.88 (m, 1H), 3.98 (s, 2H), 4.02 - 4.06 (m, 1H), 4.16 - 4.23 (m, 1H), 4.52 (s, 2H) 4.62 (t, 1H, *J* = 6.0), 5.33 - 5.40 (m, 2H), 7.42 - 7.81 (m, 9H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 13.8, 16.2 (primary), 19.5, 25.3, 25.8, 30.6, 39.0, 62.0, 63.5, 70.7, 76.3 (secondary), 97.7, 120.9, 127.1, 128.3, 128.9, 129.8, 131.4, 132.2 (tertiary), 132.2, 137.5, 137.9, 138.9, 139.4, 196.3 (quarternary). HRFAB-MS:$[M + H]^+$, calcd. 449.2682, found 449.2677.
Compound **6b**: ^{1}H NMR (200 MHz,$CDCl_3$): δ = 1.51 - 1.75 (m, 6H), 1.68 (s, 6H), 2.08 - 2.20 (m, 4H), 3.46 - 3.51 (m, 1H), 3.82 - 3.87 (m, 1H), 3.91 (s, 2H), 3.99 (dd, 1H, *J* = 12.0, 12.0), 4.24 (dd, 1H, *J* = 12.0, 12.0), 4.48 (s, 2H) 4.60 (t, 1H, *J* = 6.0), 5.38 (t, 1H, *J* = 12.0), 5.41 (t, 1H, *J* = 12.0), 7.36 - 7.80 (m, 9H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 13.7, 16.1 (primary), 19.4, 25.2, 25.7, 30.5, 38.9, 62.0, 63.4, 70.6, 76.2 (secondary), 97.6, 120.8, 127.1, 128.2, 128.6, 128.9, 129.0, 129.8, 131.4, 132.1 (tertiary), 132.0, 137.5, 137.9, 138.8, 139.4, 196.3 (quarternary). HRFAB-MS: $[M + H]^+$, calcd. 449.2682, found 449.2664.
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12. Compound **7a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.68 (s, 3H), 1.69 (s, 3H), 2.04 - 2.24 (m, 4H), 3.93 (s, 2H), 4.15 (d, 2H, *J* = 9.0), 4.52 (s, 2H), 5.42 (t, 2H, *J* = 9.0), 7.42 -7.49 (m, 4H), 7.56 - 7.61 (m, 1H), 7.72 - 7.81 (m, 4H). ^{13}C NMR (75.4 MHz, $CDCl_3$): 14.0, 16.3 (primary), 25.9, 39.1, 59.4, 70.8, 76.6 (secondary), 123.7, 127.2, 128.2, 128.3, 130.0, 130.3, 132.4 (tertiary), 132.2, 136.7, 137.7, 139.1, 143.5, 196.6 (quarternary). HRFAB-MS: $[M]^+$, calcd. 364.2031, found 364.2038, $[M + H]^+$, calcd. 365.2109, found 365.2116, $[M + NH_4]^+$, calcd. 382.2373, found 382.2382.
Compound **7b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.68 (s, 3H), 1.69 (s, 3H), 2.08 - 2.21 (m, 4H), 3.93 (s, 2H), 4.14 (d, 2H, *J* = 12.0), 4.56 (s, 2H), 5.42 (t, 2H, *J* = 12.0), 7.43 - 7.50 (m, 4H), 7.54 - 7.58 (m, 1H), 7.76 - 7.80 (m, 4H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 13.8, 16.0 (primary), 25.8, 38.9, 59.1, 70.7, 76.2 (secondary), 123.7, 127.9, 128.1, 128.6, 128.9, 129.1, 129.9, 131.5, 132.3 (tertiary), 132.0, 137.4, 137.5, 138.7, 138.9, 196.5 (quaternary). HRFAB-MS:$[M - H]^+$, calcd. 363.1953, found 363.1974, $[M - OH]^+$, calcd. 347.2004, found 347.2011.
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14. Compound **8a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.68 (s, 3H), 1.72 (s, 3H), 2.05 - 2.19 (m, 4H), 3.93 (s, 2H), 3.99 (d, 2H, *J* = 9.0), 4.51 (s, 2H), 5.37 - 5.44 (m, 1H), 5.53 (t, 1H, *J* = 9.0), 7.42 - 7.78 (m, 9H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.0, 16.0 (primary), 25.8, 29.6, 39.1, 70.8, 76.5 (secondary), 120.8, 127.2, 128.3, 128.9, 130.0, 130.3, 132.5 (tertiary), 132.4, 136.6, 137.6, 143.1, 1435, 196.5 (quaternary). HRFAB-MS: $[M + H]^+$ calcd. 427.1265, found 427.1273.
Compound **8b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.65 (s, 3H), 1.70 (s, 3H), 2.04 - 2.18 (m, 4H), 3.90 (s, 2H), 3.97 (d, 2H, *J* = 12.0), 4.47 (s, 2H), 5.37 (m, 1H), 5.50 (t, 1H, *J* = 12.0), 7.41 - 7.76 (m, 9H). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 13.8, 15.7 (primary), 25.6, 29.2, 38.8, 70.7, 76.2 (secondary), 120.7, 127.3, 128.2, 128.4, 128.9, 129.0, 129.8, 131.8, 132.2 (tertiary), 131.0, 133.1, 137.5, 138.8, 142.8, 196.4 (quaternary). HRFAB-MS: $[M + H]^+$, calcd. 427.1265, found 427.1251.
15. Compound **1a**: ^{1}H NMR (300 MHz, D_2O): δ = 1.47 (s, 3H), 1.54 (s, 3H), 1.88 - 2.03 (m, 4H), 3.79 (s, 2H), 4.25 (t, 2H, *J* = 9.0), 4.37 (s, 2H), 5.30 (t, 2H, *J* = 9.0), 7.31 - 7.42 (m, 5H), 7.50 - 7.62 (m, 4H). ^{31}P NMR (121.4 MHz, D_2O, pH = 8 with ND_4OD): δ = -6.86 (1P, d, *J* = 22), -10.76 (1P, d, *J* = 22). UV (H_2O), λ_{max} = 262 nm, ε = 23,300 $M^{-1}\cdot cm^{-1}$. HRFAB-MS: $[M + H]^+$, calcd. 525.1435, found 525.1449, $[M + Na]^+$, calcd. 547.1254, found 547.1285.
Compound **1b**: ^{1}H NMR (300 MHz, D_2O): δ = 1.42 (s, 3H), 1.48 (s, 3H), 1.86 - 2.01 (m, 4H), 3.76 (s, 2H), 4.24 (m, 2H), 4.34 (s, 2H), 5.20 - 5.28 (m, 2H), 7.35 - 7.63 (m, 9H). ^{31}P NMR (121.4 MHz, D_2O, pH = 8 with ND_4OD): δ = -6.92 (1P, d, *J* = 23), -10.91 (1P, d, *J* = 23). UV (H_2O), λ_{max} = 260 nm, ε = 25,400 $M^{-1}\cdot cm^{-1}$. HRFAB-MS: $[M + H]^+$, calcd. 525.1435, found 525.1445, $[M + Na]^+$, calcd. 547.1254, found 547.1312.
16. Yeast protein farnesyltransferase was purified from *E. coli* DH5α/pGP114 as described by Mayer, M. P.; Prestwich, G. D.; Dolence, J. M.; Bond, P. D.; Wu, H.-y.; Poulter, C. D. *Gene* **1993**, *132*, 41-47. Enzyme assays were performed spectrofluorometrically as described by Bond, P. D.; Dolence, J. M.; Poulter, C. D. *Methods Enzymol.* **1995**, *250*, 30-43. Solutions of **1a** and **1b** were prepared by dissolving the solid in 25 mM NH_4HCO_3 and their concentrations determined by phosphate analysis employing KH_2PO_4 as a standard as described by Reed, B. C.; Rilling, H. C. *Biochemistry* **1976**, *15*, 3739-3745.
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19. Photolysis reactions were conducted at 4 °C in a UV Rayonet Mini-Reactor equiped with 8 RPR-3500° lamps. All reactions (750 μL) were performed in silinized quartz test tubes (10 x 45 mm) with PFTase (38 nM). Where appropriate, reactions contained inhibitor (**1a**, 450 nM; **1b**, 490 nM) and FPP (100 μM).

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