Pergamon

Tetrahedron Letters, Vol. 37, No. 28, pp. 4845-4848, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)00972-0

Analogs of Farnesyl Pyrophosphate Incorporating Internal Benzoylbenzoate Esters: Synthesis, Inhibition Kinetics and Photoinactivation of Yeast Protein Farnesyltransferase

Tammy C. Turek, Igor Gaon and Mark D. Distefano*

Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

Abstract: The syntheses of two analogs (**1a** and **1b**) of farnesyl pyrophosphate incorporating photoactive benzoylbenzoate esters are described. Both **1a** and **1b** are competitive inhibitors of yeast protein farnesyltransferase with respect to farnesyl pyrophosphate and have Ki values of 3300 nM and 880 nM, respectively. Upon photolysis for two hours, **1a** and **1b** inactivate the enzyme by 46% and 11%, respectively. These compounds should be useful for a variety of studies of protein prenyltransferases.

To aid in the design of possible anticancer agents that inhibit the farnesylation of Ras,[1] it is important to establish the identity of amino acids that constitute the active sites of protein prenyltransferases; photoaffinity labeling experiments can be useful for this purpose.[2] In 1988, Baba and Allen introduced a substrate analog that incorporated a photoactive diazo-ester moiety into a derivative of geraniol;[3] this is currently the only photoactive farnesyl pyrophosphate (FPP) analog described in the literature and it has been used extensively.[4] While the compound is nearly superimposible with FPP, the pendant diazotrifluoropropionate crosslinking group possesses a number of less desirable features including low crosslinking efficiency and the requirement for short wavelength UV irradiation for photoactivation. Molecules that incorporate benzophenone moieties are attractive alternatives to diazo- and azide-containing compounds for photoaffinity labeling experiments.[5] Benzophenone-based crosslinking agents function via radical intermediates that are not quenched by solvent molecules and are hence highly efficient. In comparison to simple azides and diazo compounds, they are activated by irradiation at longer wavelengths where protein damage is less likely, they are chemically more stable, and can they be manipulated in ambient light. In this paper, the syntheses of two photoactive benzophenone-based FPP analogs, **1a** and **1b** (Fig. 1) are presented together with inhibition kinetics and photoinactivation experiments with yeast farnesyltransferase (PFTase). The design of **1a** and **1b** originated with our observation of significant overlap in a comparison of the structures of FPP and benzophenone; FPP is superimposed with **1a** and **1b** in Fig. 2.

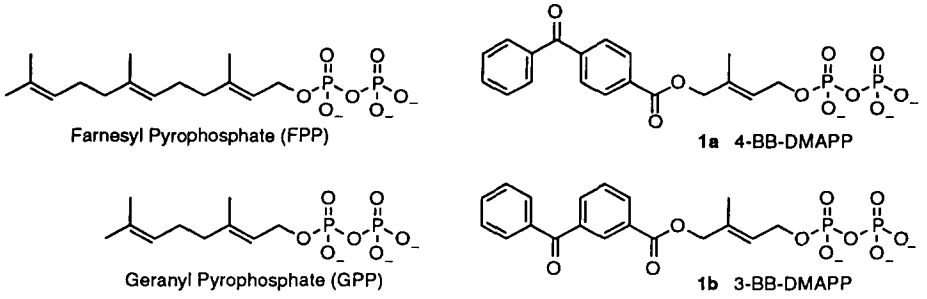


Figure 1

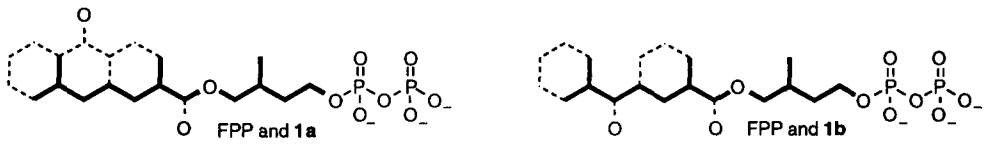
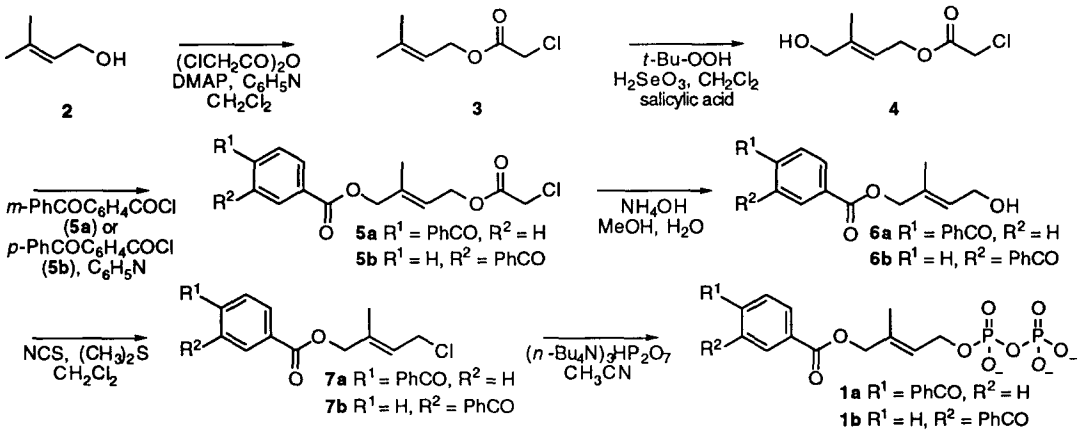


Figure 2. Superposition of FPP (bold) and **1a** and **1b** (dashed lines). Double bonds are not shown for clarity.

Synthesis. The syntheses of compounds **1a** and **1b** were each accomplished in six steps as illustrated in Scheme 1. Dimethylallyl alcohol (**2**) was first protected by esterification with chloroacetic anhydride. The resulting chloroacetate (**3**) was then oxidized with *t*-butyl hydroperoxide and catalytic H_2SeO_3 to yield **4**. The *E*-stereoselectivity for the hydroxylation reaction was confirmed by the disappearance of the C-4 methyl group in the ^{13}C NMR spectrum of **4**.[6] Photoactive benzoylbenzoates were then coupled to **4** by acylation with 4-benzoylbenzoyl chloride and 3-benzoylbenzoyl chloride to yield **5a** and **5b**, respectively.[7] The chloroacetate protecting groups of **5a** and **5b** were selectively hydrolyzed in the presence of the benzoylbenzoates with a $NH_3/MeOH/H_2O$ mixture to reveal the free alcohols, **6a** and **6b**.[8] Finally, **6a** and **6b** 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 coworkers.[9] Diphosphates **1a** and **1b** were purified by reversed-phase chromatography and characterized by ^1H NMR , ^{31}P NMR, and FAB mass spectrometry.[10]



Scheme 1

Enzyme Kinetics. To evaluate their potential as enzyme inhibitors, the rate of PFTase[11] catalyzed farnesylation of a peptide substrate[12] was measured in the presence of fixed concentrations of **1a** and **1b** at various concentrations of FPP. Double reciprocal plots of these data for **1a** (Fig. 3A) and **1b** (Fig. 3B) both give patterns of lines that intersect on the 1/v axis, consistent 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 **1a** yields a value of 3300 nM, while **1b** gives a value of 880 nM. Comparison of these data with the K_D value of 75 nM obtained for FPP by Dolence *et al.*[13] indicates that **1a** and **1b** bind effectively to PFTase; the presence of the benzoylbenzoates in **1a** and **1b** results in a 43 and 12 fold respective decrease in binding affinity for PFTase when compared to FPP; the 3-benzoylbenzoate in **1b** appears to be a good mimic for the second and third isoprene units of FPP (see Fig. 2). It is also interesting to compare the K_i

values for **1a** and **1b** with a similar pair of compounds based on geraniol instead of dimethyl allyl alcohol previously described.[14] Diphosphates **1a** and **1b** bind only 3.6 and 2.3 fold less tightly than their respective C_{10} homologs.

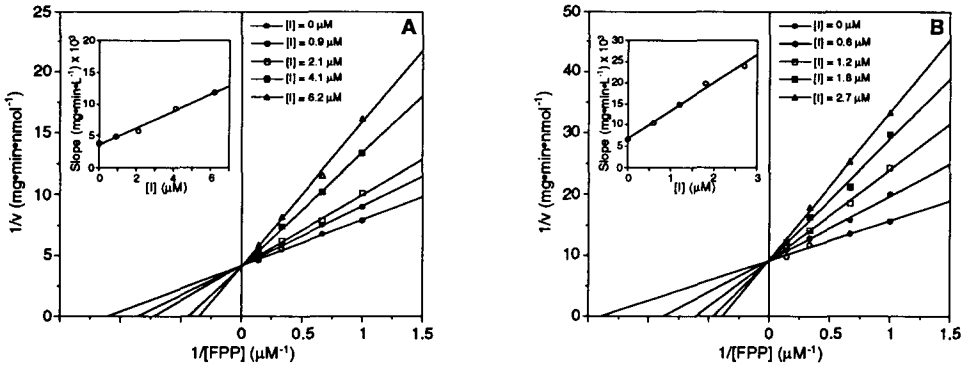


Figure 3

Photochemistry. Compounds **1a** and **1b** 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.[15] The results of these experiments are summarized in Fig. 4A (**1a**) and 4B (**1b**). Irradiation of PFTase alone for up to two hours resulted in no decrease in enzyme activity. In contrast, irradiation for two hours in the presence of the inhibitors at saturating concentrations led to a 46% decrease in enzyme activity with **1a** and a 11% decrease in activity for **1b**. This inactivation could be partially inhibited by the addition of high concentrations of the substrate, FPP. In the case of reactions with **1a** and FPP only 20% inactivation was observed after two hours, whereas similar reactions containing **1b** and FPP yielded 7% inactivation. It should be noted that more extensive inactivation could be obtained upon prolonged irradiation (74% for **1a** and 63% for **1b** after 12 hours) although some (9%) inactivation of the enzyme was observed under these conditions. It is interesting that **1a** is a more efficient photoinactivation agent than **1b** despite its lower affinity for PFTase. Given these excellent photochemical properties, **1a** and **1b** should be useful reagents for studies of protein prenyltransferases.

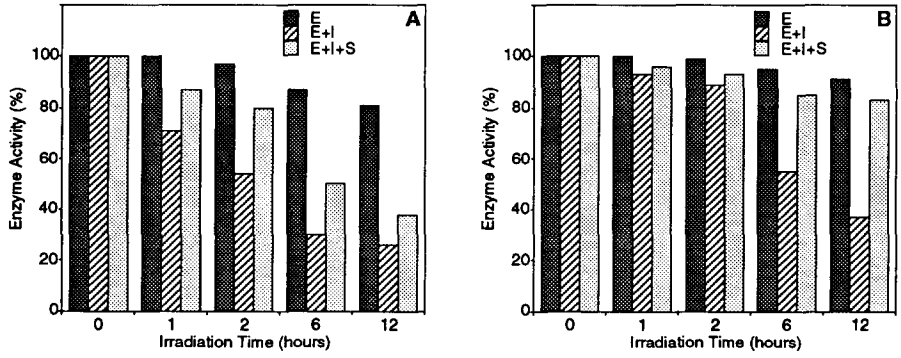


Figure 4

Acknowledgments: This research was supported by funds from the American Cancer Society (BE-222, IN-13-33-47, and JFRA-584). We thank C. D. Poulter for providing *E. coli* DH5α/pGP114.

References and Notes

1. (a) Buss, J. E.; Marsters, J. C. *Chemistry and Biology* **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.
2. Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479-12520.
3. (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.
4. (a) Das, N. P.; Allen, C. M. *Biochem. Biophys. Res. Comm.* **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.
5. Dorman, G.; Prestwich, G. D. *Biochemistry* **1994**, *33*, 5661-5673.
6. Compound **4**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.73 (s, 3H); 4.09 (s, 4H); 4.73 - 4.77 (d, 2H, *J* = 8.0); 5.58 - 5.67 (m, 1H). ^{13}C NMR (52.3 MHz, $CDCl_3$): δ = 13.9 (primary C); 41.0, 62.6, 67.3 (secondary C); 117.3 (tertiary C); 142.2, 167.5 (quarternary C). HRFAB-MS: $[M+H]^+$, calcd. 179.0471, found 179.0475, $[M+NH_4]^+$, calcd. 196.0735, found 196.0740. Yield (from **2**): 25%.
7. Compound **5a**: ^{1}HNMR (200 MHz, $CDCl_3$): δ = 1.81 (s, 3H); 4.04 (s, 2H); 4.73 (s, 2H); 4.75 (s, 2H); 5.66 - 5.73 (m, 1H); 7.41 - 7.58 (m, 3H); 7.74 - 7.78 (d, 2H, *J* = 8.0); 7.78 - 7.82 (d, 2H, *J* = 8.0); 8.11 - 8.15 (d, 2H, *J* = 8.0). ^{13}C NMR (52.3 MHz,$CDCl_3$): δ = 14.4 (primary C); 40.9, 62.2, 69.2 (secondary C); 121.0, 128.6, 129.6, 129.9, 130.2 (tertiary C); 133.1, 137.0, 141.7, 165.4, 167.3, 196.0 (quarternary C). HRCI-MS: $[M+H]^+$, calcd. 387.0993, found 387.0999; $[M+NH_4]^+$, calcd. 404.1257, found 404.1265. Yield: 61%.
   Compound **5b**: ^{1}H NMR (200MHz, $CDCl_3$): δ = 1.81 (s, 3H); 4.06 (s, 2H); 4.74 (s, 2H); 4.76 (s, 2H); 5.66 - 5.73 (m, 1H); 7.45 - 7.62 (m,4H); 7.77 - 7.81 (d, 2H, *J* = 8.0); 7.98 - 8.02 (d, 1H, *J* = 8.0); 8.24 - 8.28 (d, 1H, *J* = 8.0); 8.45 (s, 1H). ^{13}C NMR (52.3 MHz): δ = 14.2 (primary C); 40.7, 62.0, 68.9 (secondary C); 120.8, 128.4, 128.5, 129.9, 130.2, 130.9, 132.7, 133.1 (tertiary C); 134.1, 136.8, 136.9, 138.0, 165.2, 167.0, 195.5 (quarternary C). HRCI-MS: $[M]^+$: calcd. 386.0915, found 386.0916. Yield: 57%.
8. Compound **6a**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.78 (s, 3H); 4.23 - 4.27 (d, 2H, *J* = 8.0); 4.76 (s, 2H); 5.74 - 5.81 (m, 1H); 7.44 - 7.64 (m, 3H); 7.76 - 7.80 (d, 2H, *J* = 8.0); 7.80 - 7.84 (d, 2H, *J* = 8.0); 8.13 - 8.17 (d, 2H, *J* = 8.0). ^{13}C NMR (52.3 MHz, $CDCl_3$): δ = 14.0 (primary C); 58.8, 69.7 (secondary C); 127.4, 128.4, 129.4, 129.7, 130.0 (tertiary C); 132.9, 133.1, 136.8, 141.4, 165.4, 195.9 (quarternary C). HRCI-MS: $[M+H]^+$, calcd. 311.1283, found 311.1283; $[M+NH_4]^+$, calcd 328.1547, found 328.1549. mp: 44 - 46°C. Yield: 73%.
   Compound **6b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.76 (s, 3H), 4.21 - 4.24 (d, 2H, *J* = 6.0), 4.74 (s, 2H); 5.72 - 5.78 (m, 1H); 7.48 - 7.61 (m, 4H); 7.77 - 7.81 (d, 2H, *J* = 8.0); 7.97 - 8.01 (d, 1H, *J* = 8.0); 8.24 - 8.28 (d, 1H, *J* = 8.0); 8.45 (s, 1H). ^{13}C NMR (52.3 MHz, $CDCl_3$): δ = 14.0 (primary); 58.9, 69.6 (secondary); 127.3, 128.4, 128.5, 130.0, 130.4, 130.9, 132.8, 133.0 (tertiary); 133.1, 134.1, 136.9, 137.9, 165.4, 195.5 (quarternary). HRCI-MS: $[M]^+$, calcd. 310.1205, found 310.1218. mp: 63-65°C. Yield: 69%.
9. Davisson, V. J.; Woodside, A. B. Poulter, C. D. *Methods Enzymol.* **1985**, *110*, 130-144.
10. Compound **1a**: ^{1}H NMR (300 MHz, D_2O, pH 8/ND_4OD): δ = 1.64 (s, 3H); 4.38 (s, 2H); 4.55 (s, 2H); 5.65 (s, 1H); 7.37 - 7.40 (m, 2H); 7.53 - 7.56 (m, 1H); 7.61 - 7.62 (d, 2H, *J* = 3.0); 7.66 - 7.67 (d, 2H, *J* = 3.0); 7.97 - 7.99 (d, 2H, *J* = 6.0) ^{31}P NMR (121.4 MHz, D_2O, pH 8/ND_4OD) δ = -7.69 (d, 1P, *J* = 22), -10.89 (d, 1P, *J* = 22); FAB-MS: $[M+H]^+$, calcd. 471.1, found 471.1; $[M+Na]^+$, calcd. 493.0, found 493.0. UV (H_2O), λ_{max} = 262 nm, ε = 17,200 $M^{-1}cm^{-1}$. Yield (from **6a**): 51%.
    Compound **1b**: ^{1}H NMR (300 MHz, D_2O, pH 8/ND_4OD): δ = 1.63 (s, 3H); 4.40 (s, 2H); 4.62 (s, 2H); 5.65 (s, 1H); 7.39 - 7.60 (m, 4H); 7.63 - 7.65 (d, 2H, *J* = 6.0); 7.86 - 7.89 (d, 1H, *J* = 9.0); 8.15 - 8.18 (d, 1H, *J* = 9.0); 8.20 (s, 1H). ^{31}P NMR (121.4 MHz, D_2O, pH 8/ND_4OD) δ -7.84 (d, 1P, *J* = 22), -10.96 (d, 1P, *J* = 22). UV (H_2O), λ_{max} = 224 nm, ε = 27,900 $M^{-1}\cdot cm^{-1}$, λ_{max} = 258 nm, ε = 17,500 $M^{-1}\cdot cm^{-1}$. FAB-MS: $[M+H]^+$, calcd. 471.1, found 471.0; $[M+Na]^+$, calcd. 493.0, found 492.9. Yield (from **6b**): 48%.
11. 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.
12. 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.
13. Dolence, J. M.; Cassidy, P. B.; Mathis, J. R.; Poulter, C. D. *Biochemistry* **1995**, *34*, 16687-16694.
14. Distefano, M. D. (Unpublished results).
15. Photolysis reactions were conducted at 4 °C in a UV Rayonet Mini-Reactor equiped with 8 RPR-3500° lamps. All reactions (1 mL) were performed in silinized quartz test tubes (10 x 45 mm) with PFTase (38 nM). Where appropriate, reactions contained inhibitor (**1a**, 33 μM; **1b**, 8.8 μM) and FPP (100 μM).

(*Received in USA* 12 *April* 1996; *revised* 8 *May* 1996; *accepted* 10 *May* 1996)