JOC Article

Synthesis and Activity of Fluorescent Isoprenoid Pyrophosphate Analogues

MeeKyoung Kim,[†] Troy S. Kleckley,[†] Andrew J. Wiemer,[‡] Sarah A. Holstein,[‡] Raymond J. Hohl,[‡] and David F. Wiemer*,[†]

Departments of Chemistry, Pharmacology, and Internal Medicine, University of Iowa, Iowa City, Iowa 52242-1294

david-wiemer@uiowa.edu

Received May 28, 2004

New fluorescent analogues of farnesol and geranylgeraniol have been prepared and then converted to the corresponding pyrophosphates. These analogues incorporate anthranylate or dansyl-like groups anchored to the terpenoid skeleton through amine bonds that would be expected to be relatively stable to metabolism. After addition of the alcohols or the pyrophosphates to the culture medium, their fluorescence is readily observed inside a human-derived leukemia cell line. Enzyme assays have revealed that the farnesyl pyrophosphate analogue is an inhibitor of FTase, while the corresponding alcohol is not. These results, together with Western blot analyses of cell lysates, indicate that the farnesyl pyrophosphate analogue penetrates the cells as an intact pyrophosphate and that it does so at a biologically relevant concentration.

Introduction

Phosphate and pyrophosphate esters are ubiquitous intermediates in metabolism, and numerous efforts have been directed at preparation of analogues that may display useful biological activity.[1] In many cases, phosphonic acid analogues of the phosphate monoester have been prepared to provide increased metabolic stability[2] because phosphonates would not be expected to undergo hydrolysis catalyzed by common phosphatases. However, phosphonic acids, phosphate esters, and pyrophosphate esters all bear substantial negative charge at physiological pH, and it has long been recognized that this may limit the compounds' ability to penetrate the cell membrane.[2−4] In an effort to circumvent this perceived limitation, various programs have developed biodegradable protecting groups that will mask a negative charge until after penetration of the cell membrane.[5,6] For example, the pivaloyloxymethyl (POM) group has been used to generate neutral derivatives of acyclic nucleoside phosphonates that have anti-viral activity. The resulting derivatives have an improved profile of biological activity at least in part because of an improved ability for these nucleotide analogues to enter the body through the intestinal mucosa.[7] However, it would be easy to conclude that the majority of studies on masking phosphate and phosphonate anions have focused on nucleoside analogues and derivatives, and the extent to which these studies apply to more lipophilic organophosphorus compounds is not completely clear.

For some time, we have been interested in isoprenoid metabolism, including both synthesis of modified farnesols[8] and preparation of phosphonic acid analogues of farnesyl pyrophosphate (FPP, **1**) (Figure 1).[9−13] Several other research groups have reported preparation of pyrophosphate derivatives of farnesol analogues, some of which serve as serve either as alternate substrates or as inhibitors for the enzyme farnesyl:protein transferase (FTase, EC 2.5.1.58).[14−25] One of the simplest FTase inhibitors, α-hydroxy farnesylphosphonate (**2**), has been

* Corresponding author. Tel: 319-335-1365. Fax: 319-335-1270.
[†] Department of Chemistry.
[‡] Departments of Internal Medicine and Pharmacology.

(1) Engel, R. *Chem. Rev.* **1977**, *77*, 349−367.
(2) Leibman, K. C.; Heidelberger, C. *J. Biol. Chem.* **1955**, *216*, 823−830.
(3) Roll, P. M.; Weinfeld, H.; Carroll, E. *J. Biol. Chem.* **1956**, *220*, 455−465.
(4) Roll, P. M. *J. Biol. Chem.* **1958**, *231*, 183−186.
(5) Schultz, C.; Vajanaphanich, M.; Harootunian, A. T.; Sammak, P. J.; Barrett, K. E.; Tsien, R. Y. *J. Biol. Chem.* **1993**, *268*, 6316−6322.
(6) Jiang, T.; Sweeney, G.; Rudolf, M. T.; Klip, A.; Traynor-Kaplan, A.; Tsien, R. Y. *J. Biol. Chem.* **1998**, *273*, 11017−11024.
(7) Farquhar, D.; Chen, R.; Khan, S. *J. Med. Chem.* **1995**, *38*, 488−495.
(8) Mechelke, M. F.; Wiemer, D. F. *J. Org. Chem.* **1999**, *64*, 4821−4829.
(9) Hohl, R. J.; Lewis, K. A.; Cermak, D. M.; Wiemer, D. F. *Lipids* **1998**, *33*, 39−46.
(10) Holstein, S. A.; Cermak, D. M.; Wiemer, D. F.; Lewis, K.; Hohl, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 687−694.
(11) Cermak, D. M.; Du, Y.; Wiemer, D. F. *J. Org. Chem.* **1999**, *64*, 388−393.
(12) Du, Y.; Wiemer, D. F. *J. Org. Chem.* **2002**, *67*, 5701−5708.
(13) Du, Y.; Wiemer, D. F. *J. Org. Chem.* **2002**, *67*, 5709−5717.
(14) Gibbs, J. B.; Oliff, A. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 143−166.
(15) Sebti, S. M.; Hamilton, A. D. *Pharmacol. Ther.* **1997**, *74*, 103−114.
(16) Rowinsky, E. K.; Windle, J. J.; Von Hoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631−3652.
(17) Sebti, S. M.; Hamilton, A. D. *Expert Opin. Investig. Drugs* **2000**, *9*, 2767−2782.
(18) Gibbs, R. A.; Krishnan, U.; Dolence, J. M.; Poulter, C. D. *J. Org. Chem.* **1995**, *60*, 7821−7829.
(19) Mu, Y. Q.; Gibbs, R. A.; Eubanks, L. M.; Poulter, C. D. *J. Org. Chem.* **1996**, *61*, 8010−8015.
(20) Dudler, T.; Gelb, M. H. *Biochemistry* **1997**, *36*, 12434−12441.
(21) Shao, Y.; Eummer, J. T.; Gibbs, R. A. *Org. Lett.* **1999**, *1*, 627−630.

10.1021/jo049101w CCC: $27.50
Published on Web 10/05/2004

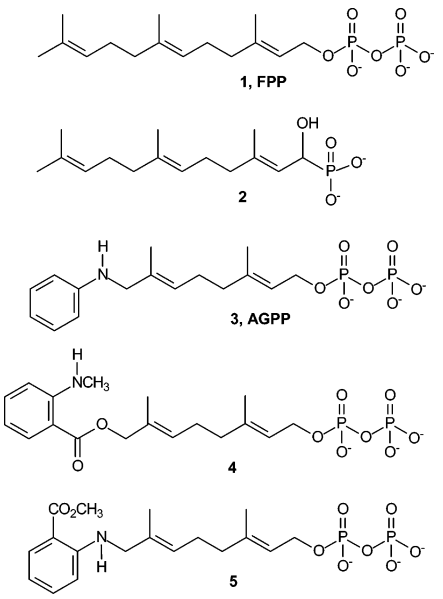


FIGURE 1. Structures of FPP and analogues.

shown to be very effective in enzyme assays,[26] but our studies have suggested that it is less effective in cell-based assays.[9] Whether this apparent decrease in activity is due to a limited ability of the phosphonic acid to penetrate the cell membrane, a heightened instability in the culture medium, or some other reason is not clear. However, it is known that under conditions of mevalonate depletion induced by an HMG-CoA reductase inhibitor, addition of FPP or GGPP can restore protein farnesylation or geranylgeranylation, respectively.[27] In addition, radiolabeled FPP or GGPP can become incorporated into isoprenylated proteins.[28] Such experiments can only be successful if the isoprenoid pyrophosphates penetrate the cell membrane at a level sufficient to restore a significant internal concentration. To provide more information on the ability of charged isoprenoids to enter cells at biologically relevant concentrations, we have prepared fluorescent analogues of FPP and GGPP and have studied their impact on isoprenoid metabolism in a human-derived leukemia cell line (RPMI-8402 cells). Both the synthesis of these compounds and select studies on their activity in cells are presented here.[29]

Results and Discussion

Design of the initial target compound was guided by recent studies of Spielmann[30,31] and Waldmann.[32,33] The Spielmann group has shown that an aniline ring can serve as a surrogate for a prenyl unit through preparation of compound **3**, while the Waldmann group has reported preparation of a fluorescent anthranilate ester of geranyl pyrophosphate (i.e., compound **4**) that can be viewed as an FPP analogue. Out of concern that the ester linkage of compound **4** might be cleaved by nonspecific esterases, which would release the fluorescent label from the isoprenoid chain, the presumably more stable amine **5** became our initial target.

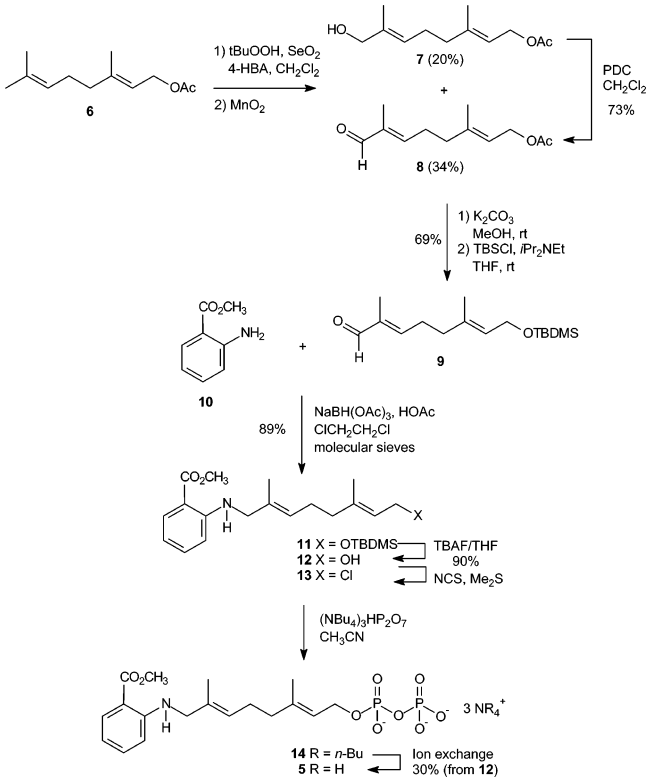
To begin the synthesis of the anthranylate derivative **5**, geranyl acetate (**6**) was oxidized with selenium dioxide and *tert*-butyl hydroperoxide as described by Sharpless.[31,34] Both the alcohol **7** and the aldehyde **8** were isolated after column chromatography, and the alcohol **7** was oxidized with PDC to afford additional aldehyde. Compound **8** could be used in a reductive amination with methyl anthranylate (**10**), but the yield was moderate and the resulting product proved difficult to isolate. After removal of the acetate group and treatment of the resulting alcohol with TBDMSCl gave the TBDMS-protected aldehyde **9**, reductive amination proceeded smoothly to afford the new amine **11**. This product was much less polar than the starting material **10**, so it was more readily purified by column chromatography and the yield was improved. Final reaction with TBAF gave the farnesol analogue **12** in 90% yield.[35]

The farnesol analogue **12** was converted to its corresponding chloride **13** through reaction with *N*-chlorosuccinimide and dimethyl sulfide in CH_2Cl_2.[36,37] The resulting allylic chloride (**13**) was not isolated because it may be unstable, so it was used directly for the next step. Reaction with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate[38,39] produced compound **14**. Purification by sequential ion exchange chromatography and C18 reversed-phase column chromatography afforded the farnesyl pyrophosphate analogue **5** in 30% yield from alcohol **12** (Scheme 1). Because compound **5** decomposes upon prolonged storage at rt, as monitored by ^{31}P NMR spectroscopy, it has been stored at 0 °C in a buffer solution (MeOH/10mM aq. NH_4OH, 7:3). As expected, compound **5** is highly fluorescent with an emission maximum of 422 nm.

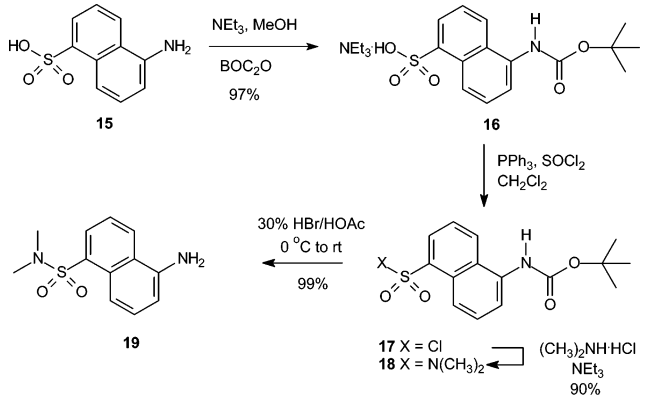
A geranylgeranyl pyrophosphate analogue **27** related to the dansyl group also has been prepared as a prototype for a second family of fluorescent isoprenoids.[40,41] For this synthesis, commercially available 5-aminonaphthalene

(22) Gibbs, B. S.; Zahn, T. J.; Mu, Y.; Sebolt-Leopold, J. S.; Gibbs, R. A. *J. Med. Chem.* **1999**, *42*, 3800−3808.
(23) Liu, X. H.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2137−2140.
(24) Kale, T. A.; Hsieh, S. J.; Rose, M. W.; Distefano, M. D. *Curr. Top. Med. Chem.* **2003**, *3*, 1043−1074.
(25) Turek-Etienne, T. C.; Strickland, C. L.; Distefano, M. D. *Biochemistry* **2003**, *42*, 3716−3724.
(26) Pompliano, D. L.; Rands, E.; Schaber, M. D.; Mosser, S. D.; Anthony, N. J.; Gibbs, J. B. *Biochemistry* **1992**, *31*, 3800−3807.
(27) Holstein, S. A.; Wohlford-Lenane, C. L.; Hohl, R. J. *Biochemistry* **2002**, *41*, 13698−13704.
(28) Van Dessel, G.; De Busser, H.; Lagrou, A. *Biochem. Biophys. Res. Commun.* **1997**, *238*, 7−11.
(29) Presented in part at the 221st National Meeting of the American Chemical Society, April 4, 2001, San Diego (302-MEDI). See also: US Patent No. 6,727,234 B2.
(30) Micali, E.; Chehade, K. A.; Isaacs, R. J.; Andres, D. A.; Spielmann, H. P. *Biochemistry* **2001**, *40*, 12254−12265.
(31) Chehade, K. A.; Andres, D. A.; Morimoto, H.; Spielmann, H. P. *J. Org. Chem.* **2000**, *65*, 3027−3033.
(32) Owen, D. J.; Alexandrov, K.; Rostkova, E.; Scheidig, A. J.; Goody, R. S.; Waldmann, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 509−512.
(33) Kuhn, K.; Owen, D. J.; Bader, B.; Wittinghofer, A.; Kuhlmann, J.; Waldmann, H. *J. Am. Chem. Soc.* **2001**, *123*, 1023−1035.
(34) Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526−5528.
(35) Barrett, A. G. M.; Hamprecht, D.; James, R. A.; Ohkubo, M.; Procopiou, P. A.; Toledo, M. A.; White, A. J. P.; Williams, D. J. *J. Org. Chem.* **2001**, *66*, 2187−2196.
(36) Corey, E. J.; Kim, C. U.; Takeda, M. *Tetrahedron Lett.* **1972**, *13*, 4339−4342.
(37) Hayashi, Y.; Mukaiyama, T. *Chem. Lett.* **1987**, *9*, 1811−1814.
(38) Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768−4779.
(39) Woodside, A. B.; Zheng, H.; Poulter, C. D. *Org. Synth.* **1988**, *66*, 211−219.

SCHEME 1. Synthesis of FPP Analogue 5



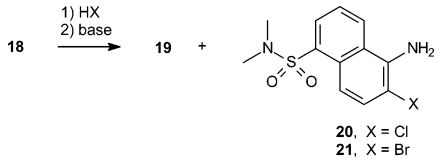
SCHEME 2. Synthesis of *N*,*N*-Dimethyl-5-aminonaphthalenesulfonamide (19)



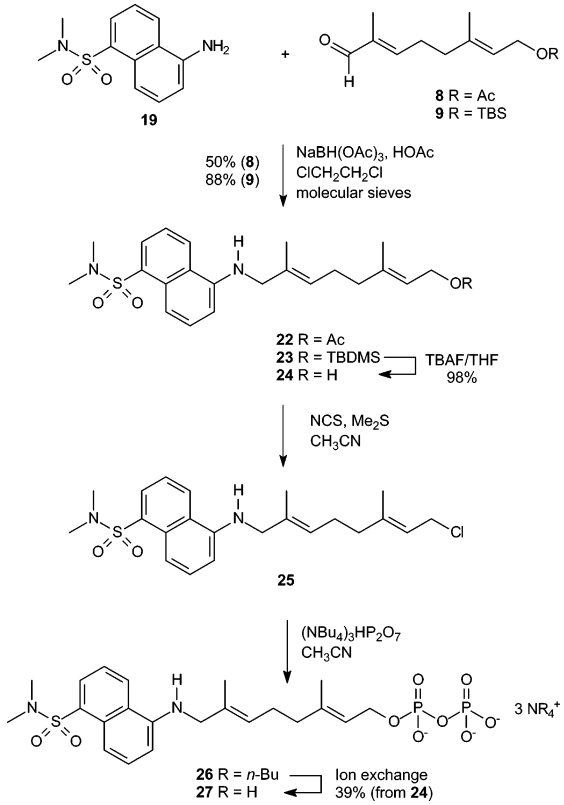
sulfonic acid (**15**) was first protected as its BOC derivative **16** through reaction with di-*tert*-butyl carbonate (Scheme 2). The sulfonic acid **16** then was converted to the sulfonyl chloride **17** by treatment with triphenylphosphine and thionyl chloride, and the acid chloride **17** was treated with dimethylamine to generate compound **18**.[42]

Cleavage of the BOC protecting group of compound **18** initially was problematic. Treatment with HCl/dioxane generated the desired compound as well as the unexpected byproduct **20** (Scheme 3). A similar byproduct (**21**) also was obtained by treatment of compound **18** with HBr/HOAc at room temperature and may result from

SCHEME 3. Byproduct Formation upon BOC Hydrolysis of Compound 18



SCHEME 4. Synthesis of Geranylgeranyl Pyrophosphate Analogue 27



BOC cleavage and subsequent reaction of the amine **19** with trace amounts of Br_2.[43] When compound **18** was treated with HBr at 0 °C, the deprotection was greatly improved and compound **19** was obtained as a single product (Scheme 4).

Reductive amination of compound **19** with aldehyde **8** gave the desired product **22**, but the parallel reaction of compound **19** with the TBDMS-protected aldehyde **9** gave a better yield and allowed more facile separation of the product **23** (Scheme 4)**.** Hydrolysis of the TBDMS group by treatment of compound **23** with TBAF in THF gave the geranylgeraniol analogue **24** in 98% yield. Alcohol **24** was a highly fluorescent green-yellow oil with an excitation maximum at 394 nm and an emission at 537 nm in MeOH.

Conversion of the alcohol **24** to the corresponding allylic chloride **25** was achieved upon treatment with *N*-chlorosuccinimide and DMS in CH_2Cl_2,[36,37] and the pyrophosphate **26** was obtained upon subsequent reaction with $(NBu_4)_3HP_2O_7$ in CH_3CN. The product pyrophosphate **26** was converted to the NH_4^+ salt by loading on a cation-exchange resin (NH_4^+ form) and eluting with a

(40) Hartwig, J. F.; Pil, P. M.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 8292−8293.
(41) Yamamoto, Y.; Katow, H.; Sofuku, S. *Chem. Lett.* **1994**, *8*, 1379−1382.
(42) Huang, J.; Widlanski, T. S. *Tetrahedron Lett.***1992**, *33*, 2657−2660.
(43) Kinsey, B. M.; Kassis, A. I. *Nucl. Med. Biol.* **1993**, *20*, 13−22.

buffer solution (*i*PrOH:25 mM aq NH_4HCO_3, 1:49) to yield the desired geranylgeranyl pyrophosphate analogue **27** in 39% overall yield from the allylic alcohol **24** (Scheme 4).

The terminal methyl groups of FPP fit the FTase pocket near the bottom of the active site.[44−46] If the larger steric demand of a terminal anthranylate or dansyl-like group limits the ability of the pyrophosphate analogues to fit into the prenyl transferases, either fluorescent group could be moved in to the middle of the prenyl chains where it might be better accommodated. This strategy is supported by a very recent study that contrasts the active site of GGTase with the FTase active site.[47]

Preparation of a GGPP analogue with a fluorescent reporter group in the interior of the chain began with conversion of compound **16** to the sulfonyl chloride, followed by condensation with methylamine to afford the sulfonamide **28** (Scheme 5). The sulfonamide **28** then was treated with NaH in DMF followed by reaction with 5-bromo-2-methyl-3-pentene to yield the dialkyl sulfonamide **29** in 55% yield. The BOC group was cleaved by treatment with 30% HBr/HOAc to afford the amine **30** in quantitative yield.

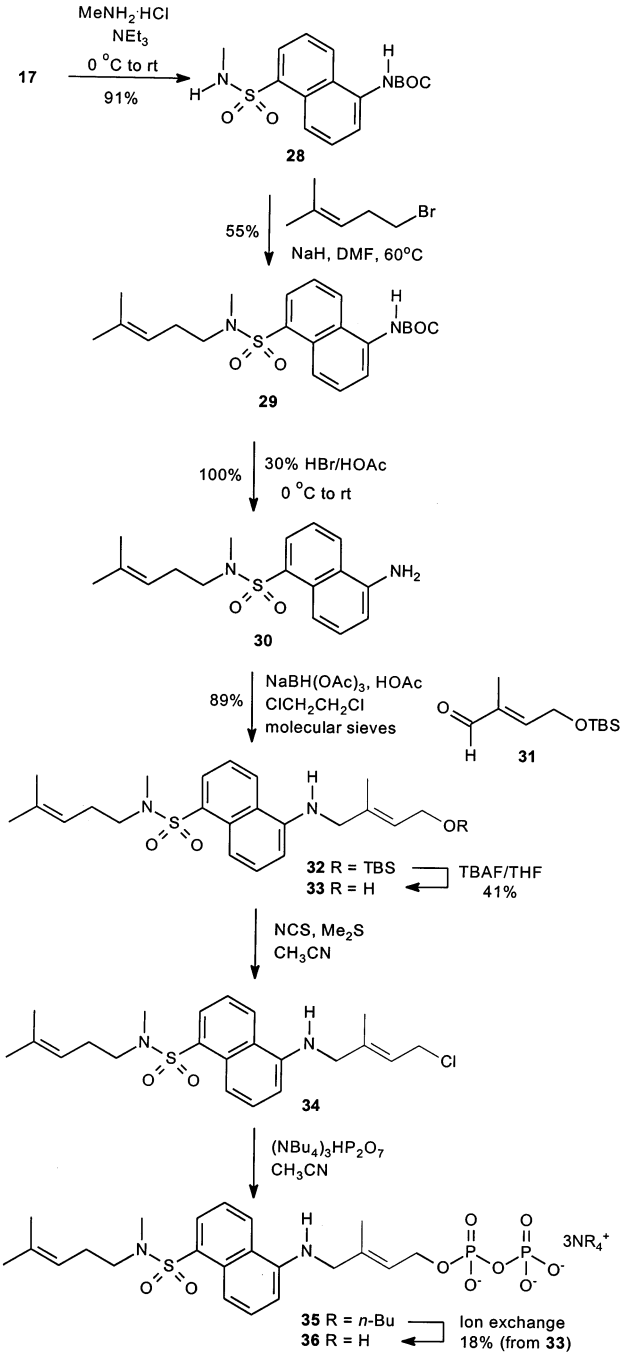
After removal of the BOC protecting group, reductive amination of compound **30** with the TBDMS-protected aldehyde **31**[48] generated compound **32** in good yield. Removal of the TBDMS group through reaction with TBAF afforded the geranylgeraniol analogue **33** as a green-yellow fluorescent oil in 41% yield after final purification by column chromatography. The geranylgeranyl pyrophosphate analogue **36** was prepared from alcohol **33** via allylic chloride **34** through standard reactions as described for compound **27**.[36,37]

The fluorescent farnesol/farnesyl pyrophosphate analogues and geranylgeraniol/geranylgeranyl pyrophosphate analogues have been the subject of a variety of bioassays. Using an in vitro enzyme assay, the ammonium salt of compound **5** was shown to inhibit FTase with an IC_{50} value of ~50 nM. This is comparable to the effects of compound **2** (IC_{50} = 39 nM).[26] As a control, anthranilic acid alone was shown not to have an inhibitory effect on FTase (data not shown) and the farnesol analogue **12** also did not inhibit FTase (IC_{50} >50 μM). Parallel studies assessing the ability of the geranylgeranyl analogues **24**, **27**, **33**, and **36** to inhibit GGTase are planned.

Consistent with the known cytotoxicity of farnesol,[49] the farnesol analogue **12** proved to be more toxic to cells than did the pyrophosphate **5**. DNA synthesis assays demonstrated that while the pyrophosphate **5** did not reach an IC_{50} at 100 μM, compound **12** inhibited [^{3}H]-thymidine incorporation with an IC_{50} of ~10 μM in RPMI-8402 human leukemia cells.

Fluorescent microscopy was performed to assess the cellular uptake of the fluorescent compounds. As shown in Figure 2, intracellular accumulation of compounds **5** and **12** occurred in a time-dependent manner, with greater uptake of the alcohol **12**. Similarly, the fluorescent geranylgeranyl analogues (compounds **24** and **27**) also could be visualized within cells (Figure 3). Uptake of the GGOH analogue **24** is more facile, presumably because it is neutral. However, microscopy clearly shows that the GGPP analogue **27** also penetrates cells. The mechanism by which the charged pyrophosphates enter the cell is not well-understood. Previous studies have shown that the uptake of radiolabeled FPP or GGPP is not significantly affected by the temperature of the incubation medium or by metabolic poisons,[28] suggesting that the process is energy-independent. It should be noted

SCHEME 5. Synthesis of Geranylgeranyl Pyrophosphate Analogue 36



(44) Park, H. W.; Boduluri, S. R.; Moomaw, J. F.; Casey, P. J.; Beese, L. S. *Science* **1997**, *275*, 1800−1805.
(45) Dunten, P.; Kammlott, U.; Crowther, R.; Weber, D.; Palermo, R.; Birktoft, J. *Biochemistry* **1998**, *37*, 7907−7912.
(46) Long, S. B.; Casey, P. J.; Beese, L. S. *Biochemistry* **1998**, *37*, 9612−9618.
(47) Taylor, J. S.; Reid, T. S.; Terry, K. L.; Casey, P. J.; Beese, L. S. *Embo. J.* **2003**, *22*, 5963−5974.
(48) de Lera, Angel R.; Torrado, A.; Iglesias, B.; Lopez, S. *Tetrahedron Lett.* **1992**, *33*, 6205−6208.

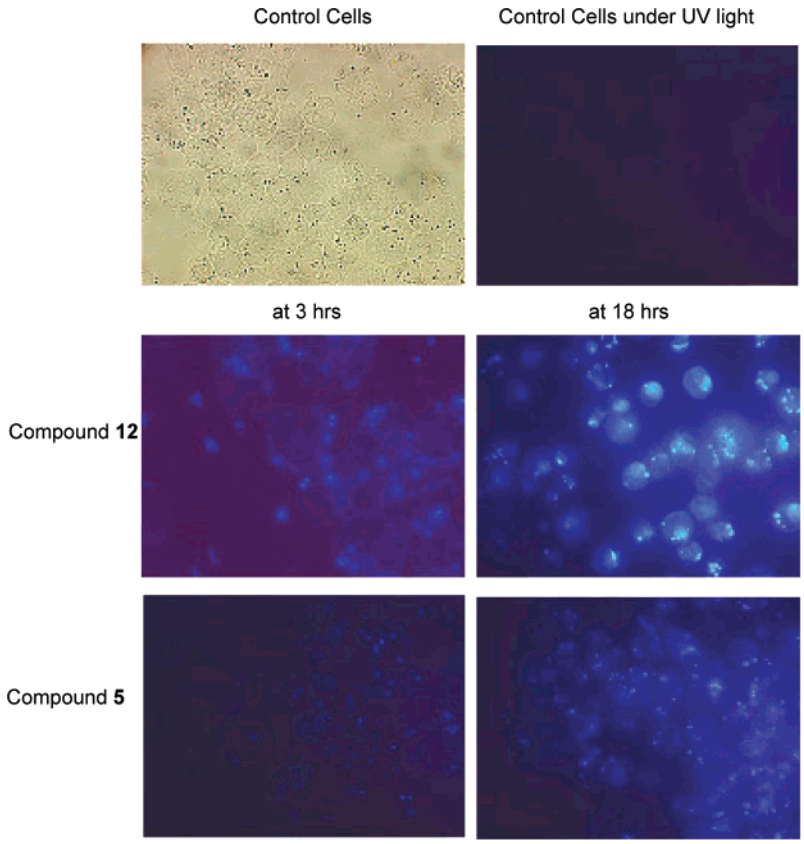


FIGURE 2. Uptake of farnesyl analogues **5** and **12** by RPMI-8402 cells. Cells were incubated with compound **12** (10 μM) or **5** (100 μM) for 3 or 18 h and were subsequently examined via fluorescent microscopy. Control cells under phase-contrast microscopy and under fluorescent microscopy are also shown.

that in plants, there appears to be a unidirectional transport system in the chloroplast envelope membrane which mediates the export of isoprenoid pyrophosphates (IPP, GPP > FPP, DMAPP) from the chloroplast to the cytosol.[50]

Further evidence of cellular uptake and biological activity of compound **5** was obtained through examination of Ras protein processing via western blot analysis (Figure 4). As a control, cells were treated with lovastatin, which resulted in the accumulation of unmodified Ras (the more slowly migrating band). Incubation of cells with the pyrophosphate **5** led to the appearance of unmodified Ras, consistent with the enzyme studies which demonstrated that compound **5** is an FTase inhibitor. In contrast, treatment of cells with the alcohol **12** alone did not increase unmodified Ras. The increase in unmodified Ras induced by compound **5** could be prevented by coincubation with FPP (**1**), indicating that compound **5** is likely a competitive inhibitor of FTase with respect to FPP (data not shown).

In summary, we have prepared fluorescent analogues of both FPP and GGPP. The FPP analogue **5** is a potent inhibitor of FTase in both enzymatic assays and cultured cells. These experiments provide additional evidence that isoprenoid pyrophosphates can cross cell membranes intact. It has been shown that cells can phosphorylate farnesol and geranylgeraniol to yield FPP and GGPP.[51−53] However, there are several findings in our studies which support the hypothesis that the activity of the pyrophosphate **5** is not a consequence of hydrolysis followed by phosphorylation once inside the cell. First, compounds **5** and **12** displayed differential toxicities in the DNA synthesis assays; the alcohol **12** is greater than 10 times more cytotoxic than the pyrophosphate **5**. Second, treatment of intact cells with the pyrophosphate **5** results in inhibition of Ras farnesylation but treatment with the alcohol **12** does not. It is interesting to note that while levels of FPP in human plasma have been estimated to be in the range of 5−7 ng/mL (~15 nM), levels of farnesol were below the detection limit of 0.5 ng/mL (~2 nM)[54] indicating stability of FPP under physiologic conditions. While these results do not bear on the potential clinical utility of isoprenoid pyrophosphate analogues, they do suggest a valuable role for such compounds as metabolic probes in cell cultures and encourage further efforts to design pyrophosphate-based inhibitors and alternate substrates of the prenyltransferase enzymes.

(49) Rioja, A.; Pizzey, A. R.; Marson, C. M.; Thomas, N. S. *FEBS Lett.* **2000**, *467*, 291−295.
(50) Bick, J. A.; Lange, B. M. *Arch. Biochem. Biophys.* **2003**, *415*, 146−154.
(51) Westfall, D.; Aboushadi, N.; Shackelford, J. E.; Krisans, S. K. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 562−568.
(52) Bentinger, M.; Grunler, J.; Peterson, E.; Swiezewska, E.; Dallner, G. *Arch. Biochem. Biophys.* **1998**, *353*, 191−198.
(53) Thai, L.; Rush, J. S.; Maul, J. E.; Devarenne, T.; Rodgers, D. L.; Chappell, J.; Waechter, C. J. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 13080−13085.
(54) Saisho, Y.; Morimoto, A.; Umeda, T. *Anal. Biochem.* **1997**, *252*, 89−95.

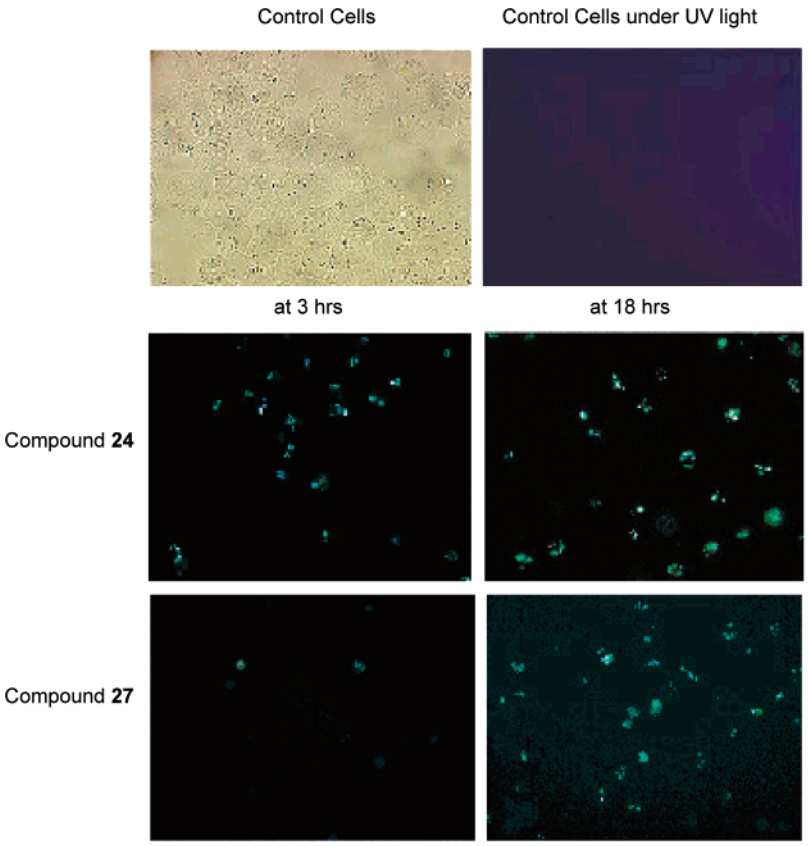


FIGURE 3. Uptake of geranylgeranyl analogues **24** and **27** by RPMI-8402 cells. Cells were incubated with compound **24** (10 μM) or **27** (100 μM) for 3 or 18 h and were subsequently examined via fluorescent microscopy. Control cells under phase-contrast microscopy and under fluorescent microscopy are also shown.

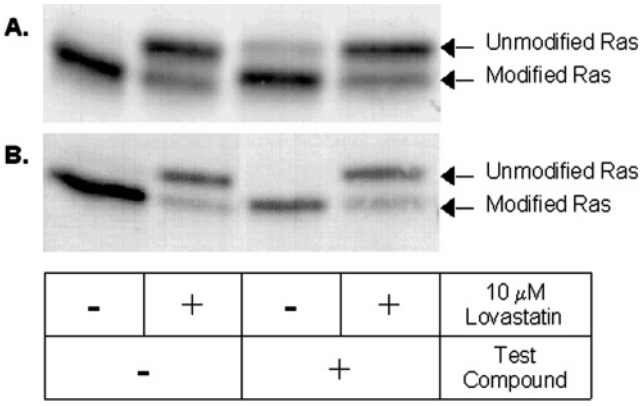


FIGURE 4. Effect of farnesyl analogues on Ras processing. Cells were treated for 24 h with (A) compound **5** (100 μM) or (B) compound **12** (10 μM) in the presence or absence of 10 μM lovastatin.

Experimental Section

8-(*tert*-Butyldimethylsilyloxy)-2,6-dimethylocta-2,6-dienal (9). Solid K_2CO_3 (7.13 g, 51.6 mmol) was added to a solution of compound **8**[34,55] (4.20 g, 20.0 mmol) in MeOH (40 mL) at rt, and the reaction mixture was stirred for 2.5 h and then was diluted with EtOAc. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$). After concentration in vacuo, the residue was dissolved in THF (10 mL), the solution was cooled to 0 °C, *i*-Pr_2NEt (13.9 mL, 79.9 mmol) was added, and the resulting mixture was stirred for 10 min. TBDMSCl (8.37 g, 0.12 mol) was added, and the reaction mixture was stirred for an additional 2 h. The mixture then was diluted with ether, and a white precipitant was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was washed with water and brine and dried ($MgSO_4$). Final purification by column chromatography (hexane/EtOAc, 95:5) gave compound **9** (3.89 g, 69%) as a colorless oil.[56]

Amine 11. Methyl anthranilate hydrochloride (1.00 g, 5.33 mmol) and aldehyde **9** (4.39 g, 15.5 mmol) were dissolved in $ClCH_2CH_2Cl$ (21 mL). Acetic acid (2.5 mL, 43.2 mmol) and 4 Å molecular sieves were added, and the reaction solution was stirred for 5 min at rt. After $NaBH(OAc)_3$ (6.89 g, 32.5 mmol) was added, the solution was stirred for 2.7 h at rt and then quenched by addition of 5% aq $NaHCO_3$ dropwise at 0 °C. The product was extracted with ether, and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to afford a colorless oil. Final purification by flash column chromatography (hexane/EtOAc, 95:5) gave compound **11** (2.51 g, 89%) as a colorless oil: ^{1}H NMR δ 7.89 (dd, J = 7.5, 1.6 Hz, 1H), 7.85 (br s, 1H), 7.31 (dq, J = 7.7, 1.4 Hz, 1H), 6.63 (d, J = 7.7 Hz, 1H), 6.58 (dq, J = 7.5, 1.4 Hz, 1H), 5.40 (dt, J = 6.8, 1.3 Hz, 1H), 5.29 (dt, J = 6.4, 1.4 Hz, 1H), 4.18 (d, J = 6.4 Hz, 2H), 3.85 (s, 3H), 3.73 (d, J = 5.3 Hz, 2H), 2.20−2.12 (m, 2H), 2.06−2.00 (m, 2H), 1.67 (s, 3H), 1.61 (s, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz) δ 169.1, 151.4, 136.6, 134.4, 131.5, 131.5, 125.8, 124.6, 114.4, 111.7, 109.8, 60.3, 51.4, 50.6, 39.2, 26.1, 26.0 (3C), 18.4, 16.3, 14.5, −5.0 (2C); HRMS (FAB) calcd for $C_{24}H_{40}NO_3Si$ $(M + H)^+$ 418.2777, found 418.2780.

Farnesol Analogue 12. Compound **11** (1.55 g, 3.70 mmol) was treated with TBAF (26.6 mL, 26.7 mmol) in THF (31 mL), and the reaction solution was stirred for 2 h at 0 °C. The

(55) Kim, M. K. Ph.D. Thesis, University of Iowa, 2002.
(56) Aldrich, J. R.; Oliver, J. E.; Waite, G. K.; Moore, C.; Waters, R. M. *J. Chem. Ecol.* **1996**, *22*, 729−738.

reaction solution was quenched by addition of saturated NH_4Cl (aq) and extracted with EtOAc. The combined organic layers were washed with brine and dried ($MgSO_4$). Final purification by column chromatography afforded the farnesol analogue **12** (1.01 g, 90%) as a colorless oil: ^{1}H NMR δ 7.89 (d, J = 8.0 Hz, 1H), 7.82 (br s, 1H), 7.31 (dd, J = 8.6, 7.1 Hz, 1H), 6.63 (d, J = 8.6 Hz, 1H), 6.58 (dd, J = 8.0, 7.1 Hz, 1H), 5.42−5.36 (m, 2H), 4.12 (d, J = 6.8 Hz, 2H), 3.85 (s, 3H), 3.72 (d, J = 5.1 Hz, 2H), 2.22−2.15 (m, 2H), 2.08−2.03 (m, 2H), 1.68 (s, 3H), 1.66 (s, 3H); ^{13}C NMR δ 169.2, 151.3, 138.7, 134.5, 131.6, 131.5, 125.3, 124.0, 114.4, 111.6, 109.5, 59.3, 51.5, 50.3, 39.0, 25.7, 16.1, 14.7; HRMS (FAB) calcd for $C_{18}H_{26}NO_3$ $(M + H)^+$ 304.1913, found 304.1907.

Chloride 13. *N*-Chlorosuccinimide (399 mg, 2.99 mmol) was dissolved in CH_2Cl_2 (11 mL), the solution was allowed to cool to −30 °C in an acetonitrile/dry ice bath and dimethyl sulfide (0.24 mL, 3.26 mmol) was added dropwise by syringe. The reaction mixture was allowed to warm to 0 °C in an ice bath and then stirred for 5 min. After the reaction mixture was cooled to −40 °C, a solution of the farnesol analogue **12** (824 mg, 2.72 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise by syringe at −40 °C. The suspension was allowed to warm to 0 °C in an ice bath and then stirred for 3 h. The reaction mixture was allowed to warm to rt, stirred an additional 15 min, then poured into a separatory funnel containing cold brine and extracted with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and filtered. After concentration on a rotary evaporator, the residue was dried in vacuo for 1 h to afford compound **13** as a colorless oil. This material was carried to the next step without further purification: ^{1}H NMR δ 7.9 (dd, J = 8.0, 1.6 Hz, 1H), 7.32 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 6.63 (dd, J = 8.6, 0.8 Hz, 1H), 6.59 (ddd, J = 8.0, 7.1, 0.8 Hz, 1H), 5.40 (m, 2H), 4.07 (d, J = 7.8 Hz, 2H), 3.83 (s, 3H), 3.74 (d, J = 4.4 Hz, 2H), 2.17 (m, 2H), 2.08 (m, 2H), 1.72 (s, 3H), 1.68 (s, 3H); ^{13}C NMR δ 169.0, 151.3, 142.2, 134.3, 131.9, 131.4, 125.0, 120.6, 114.4, 111.6, 109.7, 51.4, 50.3, 41.0, 39.0, 25.8, 16.0, 14.6; HRMS (FAB) calcd for $C_{18}H_{25}NO_2Cl$ $(M + H)^+$ 322.1574, found 322.1558.

Pyrophosphate 5. Tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (3.32 g, 3.68 mmol) was dissolved in CH_3CN (11 mL), compound **13** in CH_3CN (2 mL) was added, and the resulting mixture was stirred for 3 h at rt. After concentration in vacuo, the resulting residue was dissolved in ion exchange buffer (1/49 v/v, isopropyl alcohol and 25 mM NH_4HCO_3) and loaded onto a column (2.5 × 8.6 cm) containing 140 mequiv of cation-exchange resin (100−200 mesh 1.7 mequiv/mL). The eluant was collected, frozen, and lyophilized to give compound **5** as a white solid. The initial product was purified by C18 reverse phase column chromatography (25 mM NH_4HCO_3/CH_3CN, 10:0 to 4:6) to afford pyrophosphate **5** (422 mg, 30% from compound **12**) as a white solid: ^{1}H NMR (400 MHz, D_2O) δ 7.81 (d, J = 7.6 Hz, 1H), 7.29 (dd, J = 8.3, 7.4 Hz, 1H), 6.63 (d, J = 8.3 Hz, 1H), 6.58 (dd, J = 7.6, 7.4 Hz, 1H), 5.40 (br s, 1H), 5.29 (br s, 1H), 4.44 (br s, 2H), 3.80 (s, 3H), 3.63 (s, 2H), 2.14−2.10 (m, 2H), 2.01−1.99 (m, 2H), 1.66 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 170.3, 151.1, 142.7, 135.2, 132.3, 131.9, 125.8, 120.2 (d, J_{CP} = 8.7 Hz), 115.7, 113, 110.4, 62.8 (d, J_{CP} = 3.9 Hz), 52.2, 50.0, 39.0, 25.9, 16.0, 14.0; ^{31}P NMR (120 MHz, D_2O) δ −8.6 (d, J_{PP} = 20.0 Hz, 1P), −10.8 (d, J_{PP} = 20.0 Hz, 1P); HRMS (FAB) calcd for $C_{18}H_{28}NO_9P_2$ $(M - 3NH_4^+ + H)^{2-}$ 464.1239, found 464.1247.

5-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-naphthalenesulfonic Acid, Triethylamine Salt (16). To a solution of 5-aminonaphthalenesulfonic acid (**15,** 2.43 g, 10.9 mmol) and triethylamine (1.74 mL, 12.5 mmol) in anhydrous methanol (21 mL) was added di-*tert*-butyl dicarbonate (5.51 mL, 24.0 mmol). The reaction mixture was stirred for 36 h at rt and then concentrated by rotary evaporation. The residue was dissolved in water (100 mL) and washed with EtOAc. After the remaining organic solvent was removed in vacuo, the aqueous solution was frozen in a dry ice/acetone bath and lyophilized to afford compound **16** (4.48 g, 97%) as a light pink solid: ^{1}H NMR (400 MHz, D_2O) δ 8.56 (d, J = 8.4 Hz, 1H), 8.13 (dd, J = 7.2, 1.0 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H), 7.65 (dd, J = 8.4, 8.1 Hz, 1H), 7.54 (dd, J = 8.6, 7.2 Hz, 1H), 7.51 (dd, J = 8.1, 1.1 Hz, 1H), 3.04 (q, J = 7.3 Hz, 6H), 1.50 (s, 9H), 1.16 (t, J = 7.4 Hz, 9H); ^{13}C NMR (100 MHz, D_2O) δ 159.4, 141.2, 135.8, 132.3, 131.5, 129.9, 129.1, 129.0, 127.6, 126.4, 126.3, 84.7, 49.3 (3C), 30.3 (3C), 10.9 (3C). Anal. Calcd for $C_{21}H_{32}N_2O_5S$: C, 59.41; H 7.60; N, 6.60. Found: C, 59.31; H 7.51; N, 6.60.

Sulfonamide 18. Triphenylphosphine (2.60 g, 9.92 mmol) in CH_2Cl_2 (104 mL) was cooled in an ice bath to 0 °C, thionyl chloride (0.79 mL, 10.9 mmol) was added, and the reaction mixture was stirred for 20 min. The ice bath was removed, and compound **16** (2.03 g, 4.77 mmol) was added. The reaction mixture was stirred for 1 h at rt and cooled to 0 °C, and then dimethylamine hydrochloride (1.17 g, 14.30 mmol) and triethylamine (4.2 mL, 30.5 mmol) were added dropwise over 5 min. After the solution was stirred for 2 h at rt, it was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N HCl and brine and dried ($MgSO_4$). After concentration in vacuo, final purification by column chromatography (hexane/EtOAc, 7:3) gave compound **18** (1.50 g, 4.29 mmol, 90%) as a white solid: ^{1}H NMR δ 8.52 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 9.2 Hz, 1H), 8.13 (dd, J = 7.5, 1.1 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.52 (dd, J = 9.2, 7.5 Hz, 1H), 7.49 (dd, J = 8.6, 7.5 Hz, 1H), 6.94 (br s, 1H), 2.76 (s, 6H), 1.51 (s, 9H); ^{13}C NMR δ 153.6, 133.6, 133.2, 130.3, 129.6, 128.6, 127.7, 127.3, 124.0, 122.2, 121.3, 80.9, 37.3 (2C), 28.3 (3C). Anal. Calcd for $C_{17}H_{22}N_2O_4S$: C, 58.27; H 6.33; N, 7.99. Found: C, 57.93; H 6.24; N, 7.88.

5-Amino-*N,N*-dimethyl-1-naphthalenesulfonamide (19). To a solution of compound **18** (2.87 g, 8.91 mmol) in HOAc (11 mL) was added 30% HBr/HOAc (22 mL) at 0 °C. The reaction mixture was stirred for 20 min and then allowed to warm to rt and stand for 40 min. The reaction mixture was diluted with ether to obtain a white precipitate, which was collected by filtration and crystallized from ethanol/ether to generate compound **19** (2.92 g, 99%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.34 (d, J = 8.5 Hz, 1H), 8.12 (dd, J = 7.5, 1.0 Hz, 1H), 8.05 (d, J = 8.7 Hz, 1H), 7.50 (dd, J = 8.5, 7.5 Hz, 1H), 7.39 (dd, J = 8.7, 7.5 Hz, 1H), 6.91 (dd, J = 7.5, 1.0 Hz, 1H), 2.78 (s, 6H); ^{13}C NMR (CD_3OD) δ 145.3, 132.9, 130.5, 130.4, 128.9, 128.5, 125.1, 122.4, 114.7, 110.4, 36.9 (2C). Anal. Calcd for $C_{12}H_{14}N_2O_2S$: C, 57.58; H 5.64; N, 11.19. Found: C, 57.58; H 5.64; N, 11.03.

5-Amino-4-chloro-*N,N*-dimethyl-1-naphthalenesulfonamide (20). Compound **18** (153 mg, 0.44 mmol) was treated with 6 N HCl in dioxane (1.3 mL) for 1 h at rt. The reaction was quenched by addition of 1 N NaOH and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine and dried ($MgSO_4$). After removal of solvent under reduced pressure, the product was purified by column chromatography ($CHCl_3$/EtOAC, 9:1) to afford compound **19** (74 mg, 68%) and the byproduct **20** (6 mg, 5%) as yellow oils. For compound **20**: ^{1}H NMR δ 8.16 (d, J = 7.3 Hz, 1H), 8.13 (d, J = 9.3 Hz, 1H), 8.05 (d, J = 8.6 Hz, 1H), 7.51 (dd, J = 8.6, 7.3 Hz, 1H), 7.49 (d, J = 9.3 Hz, 1H), 4.69 (br s, 2H, exchanges with D_2O), 2.80 (s, 6H); ^{13}C NMR δ 138.9, 133.2, 130.2, 129.0, 128.5, 126.8, 124.8, 123.7, 116.0, 115.2, 37.4 (2C). Anal. Calcd for $C_{12}H_{13}N_2O_2SCl$: C, 50.62; H 4.60; N, 9.84; Cl, 12.45. Found: C, 50.56; H 4.55; N, 9.76; Cl, 12.69.

Pyrophosphate 27. To a solution of $(NBu_4)_3HP_2O_7$ (6.98 g, 7.74 mmol) in CH_3CN (16 mL) was added by syringe a solution of allylic chloride **25** in CH_3CN (3.4 mL), and the reaction mixture was stirred at rt for 3 h. The solvent was removed in vacuo, and the resulting residue was dissolved in an ion- exchange buffer (1/49 v/v, isopropyl alcohol and 25 mM NH_4HCO_3) and then loaded onto a column (2.5 × 16 cm) containing 262 mequiv of a cation-exchange resin (100−200 mesh). The eluant was frozen and lyophilized to give compound **27** as a yellow solid. Final purification by column chromatog-

raphy (cellulose powder) and C18 reversed-phase column chromatography (25 mM NH_4HCO_3/CH_3CN, 6:4) gave the pyrophosphate **27** (926 mg, 39% from compound **24**) as a yellow solid: ^{1}H NMR (ND_4OD/D_2O) δ 8.56 (d, J = 8.1 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.70 (dd, J = 8.1, 7.8 Hz, 1H), 7.62 (dd, J = 8.1, 7.6 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 5.72 (t, J = 6.6 Hz, 1H), 5.34 (br s, 1H), 4.83 (m, 2H), 3.92 (s, 2H), 2.93 (s, 6H), 2.31−2.25 (m, 2H), 2.19−2.16 (m, 2H), 1.94 (s, 3H), 1.83 (s, 3H); ^{13}C NMR (ND_4OD/D_2O) δ 147.8, 144.5, 135.0, 134.7, 133.1, 132.8, 132.3, 130.5, 128.4, 127.2, 125.6, 123.5 (d, J_{CP} = 9.7 Hz), 115.6, 108.7, 65.3 (d, J_{CP} = 4.5 Hz), 53.8, 41.9, 40.0 (2C), 29.0, 18.9, 17.0; ^{31}P NMR (D_2O) δ −6.0 (1P, d, J_{PP}= 22.0 Hz), −9.8 (1P, d, J_{PP} = 22.0 Hz); HRMS (FAB) calcd for $C_{22}H_{31}N_2O_9P_2S$ $(M - 3NH_4^+ + 2H)^-$ 561.1226, found 561.1206.

Sulfonamide 28. Dichlorotriphenylphosphorane (3.10 g, 9.30 mmol) and compound **17** (2.01 g, 4.73) were dissolved in CH_2Cl_2 (100 mL), and the solution was stirred for 1.5 h at r. The solution then was cooled to 0 °C in an ice bath, and NEt_3 was added dropwise. After the reaction mixture was stirred for 10 min, it was allowed to warm to rt and then allowed to stir for 2 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N HCl and brine and diluted with hexane to precipitate triphenylphosphine oxide as a white solid. The precipitate was removed by filtration, and the filtrate was dried ($MgSO_4$). After concentration in vacuo, final purification by column chromatography (hexane/EtOAc, 8:2) gave the desired product **28** (1.45 g, 91%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.56 (dt, J = 8.3, 1.0 Hz, 1H), 8.35 (dt, J = 8.6, 1.0 Hz, 1H), 8.24 (dd, J = 7.5, 1.1 Hz, 1H), 7.74−7.62 (m, 3H), 2.49 (s, 3H), 1.57 (s, 9H); ^{13}C NMR (CD_3OD) δ 156.8, 136.2, 136.1, 131.2, 130.9, 130.5, 129.7, 128.9, 125.3, 124.1, 123.4, 81.6, 29.1, 28.8 (3C); HRMS (FAB) calcd for $C_{16}H_{20}N_2O_4NaS$ $(M + Na)^+$ 359.1041, found 359.1055.

Sulfonamide 29. A mixture of compound **28** (405 mg, 1.20 mmol) and NaH (60% dispersion in mineral oil; 86.4 mg, 2.16 mmol) was suspended in DMF (3.3 mL) and stirred for 40 min at 60 °C. 5-Bromo-2-methyl-2-pentene (0.4 mL, 2.99 mmol) was added slowly, and the reaction mixture was stirred for 40 min at 60 °C. After the reaction mixture was allowed to cool to rt, it was diluted with water and extracted with chloroform, and the combined organic layers were dried ($MgSO_4$). After concentration in vacuo, purification of the residue by column chromatography (hexane/EtOAc, 8:2) afforded compound **29** as a colorless oil (274 mg, 55%): ^{1}H NMR δ 8.51 (d, J = 8.7 Hz, 1H), 8.21−8.16 (m, 2H), 7.86 (d, J = 7.4 Hz, 1H), 7.63−7.52 (m, 2H), 6.85 (s, 1H, exchanges with D_2O), 4.98−4.93 (m, 1H), 3.17 (t, J = 7.4 Hz, 2H), 2.84 (s, 3H), 2.24−2.17 (m, 2H), 1.61 (s, 3H), 1.55 (s, 12H); ^{13}C NMR δ 153.8, 135.1, 134.6, 133.8, 130.1, 129.7, 128.7, 128.0, 127.2, 124.3, 122.4, 121.3, 120.0, 81.2, 49.6, 34.3, 28.5 (3C), 26.9, 25.8, 17.9; HRMS (FAB) calcd for $C_{22}H_{31}N_2O_4S$ $(M + H)^+$ 419.2005, found 419.1986.

Sulfonamide 30. A solution of compound **29** in HOAc (0.8 mL) was treated with 30% HBr/HOAc (1.6 mL) at 0 °C. The reaction mixture was stirred for 5 min at 0 °C and then allowed to warm to rt and stand for 20 min. The reaction mixture was diluted with ether to precipitate the product. The white precipitate was collected by filtration, and the crude compound was crystallized from ethanol/ether to afford compound **30** (251 mg, 100%) as a white solid: ^{1}H NMR δ 8.20 (m, 3H), 7.53 (m, 2H), 7.03 (d, J = 6.9 Hz, 1H), 4.96 (t, J = 6.3 Hz, 1H), 3.22−3.17 (m, 2H), 2.83 (s, 3H), 2.24−2.19 (m, 2H), 1.65 (s, 3H), 1.60 (s, 3H); ^{13}C NMR δ 134.3, 134.7, 134.5, 130.2, 130.1, 129.1, 128.6, 127.1, 125.2, 124.1, 123.4, 120.1, 50.2, 36.6, 26.9, 25.8, 17.9; HRMS (FAB) calcd for $C_{17}H_{23}N_2O_2S$ $(M + H)^+$ 319.1480, found 319.1484.

Pyrophosphate 36. $(NBu_4)_3HPO_7$ (264 mg, 0.29 mmol) was dissolved in CH_3CN (0.5 mL) at rt. Compound **34** in CH_3CN (0.5 mL) was added, and the resulting mixture was stirred for 3 h at rt, when the milky suspension became clear. After the solvent was removed under reduced pressure, the resulting residue was dissolved in an ion-exchange buffer (1/49 v/v, isopropyl alcohol and 25 mM NH_4HCO_3) and loaded onto a column (2 × 5 cm) containing 26 mequiv of a cation-exchange resin (100−200 mesh 1.7 mequiv/mL). The eluant was collected, frozen, and lyophilized to give compound **36** as a yellow solid. The initial product was purified by C18 reversed-phase column chromatography (25 mM NH_4HCO_3/CH_3CN, 7:3) to afford the pyrophosphate **36** (20 mg, 18% from compound **33**) as a yellow solid: ^{1}H NMR (ND_4OD/D_2O) δ 8.89 (d, J = 8.7 Hz, 1H), 8.58 (d, J = 7.3 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 8.07−7.96 (m, 2H), 7.25 (d, J = 7.9 Hz, 1H), 6.17 (t, J = 6.6 Hz, 1H), 5.25 (t, J = 6.5 Hz, 2H), 4.97 (t, J = 6.5 Hz, 1H), 4.37 (s, 2H), 3.65−3.58 (m, 2H), 3.32 (s, 3H), 2.60−2.2.53 (m, 2H), 2.23 (s, 3H), 1.88 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (ND_4OD/D_2O) δ 147.9, 140.7, 138.5, 135.7, 133.2, 132.7, 132.4, 131.0, 127.6, 126.1, 124.8 (d, J_{CP} = 9.2 Hz), 123.2, 115.9, 109.6, 65.2 (d, J_{CP} = 5.0 Hz), 53.5, 52.4, 36.5, 28.8, 28.0, 20.0, 17.3; ^{31}P NMR (D_2O) δ −5.3 (1P, d, J_{PP}= 22.5 Hz), −9.4 (1P, d, J_{PP} = 22.5 Hz); HRMS (FAB) calcd for $C_{22}H_{31}N_2O_9P_2S$ $(M - 3NH_4^+ + 2H)^-$ 561.1226, found 561.1211.

Cell Culture. RPMI-8402 cells[57] were cultured at 37 °C in the presence of 5% CO_2 in RPMI-1640 media containing 10% heat-inactivated fetal bovine serum.

Enzyme Assays. FTase assays were performed using a method modified from Harwood.[58] The reaction mixture contained Tris (50 mM, pH7.5), DTT (5 mM), $ZnCl_2$ (20 μM), $MgCl_2$ (5 mM), H-Ras (4 μM), FPP (500 nM), and bovine brain homogenate (30 μg) containing partially purified FTase.[59,60]

[^{3}H]-Thymidine Assay. RPMI cells (2 × 10^5 cells/200 μL) were incubated in a 96-well plate with varying concentrations of test compounds for a total of 24 h. During the last four hrs, cells were labeled with [^{3}H]-thymidine (0.04 μCi/well). Cells were harvested, and the amount of [^{3}H]-thymidine incorporated into cellular DNA was determined by scintillation spectrophotometry as previously described.[61]

Fluorescence Microscopy. Following incubation of cells with the test compounds for 3 or 18 h, cells were examined and photographed using a fluorescence microscope with a CCD camera.

Western Blotting. Cells (5 × 10^6 cells/5 mL) were incubated for 24 h in the presence or absence of test compounds and lovastatin. Western blotting was performed as previously described.[57]

Acknowledgment. We thank Prof. Lei Geng and Gufang Wang for their assistance in obtaining the fluorescence spectra, Chris Wohlford-Lenane and the University of Iowa Central Microscopy Research Facility for their assistance with the fluorescence microscopy, and the University of Iowa Mass Spectrometry Facility. This project was supported by the Roy J. Carver Charitable Trust as a Research Program of Excellence and the Roland W. Holden Family Program for Experimental Cancer Therapeutics.

Supporting Information Available: General experimental protocols; the ^{1}H, ^{13}C, and (where relevant) ^{31}P NMR spectra for compounds **5**, **11, 12**, **19**, **23**, **24**, **27**, **29**, **33**, and **36**; and the experimental procedures for preparation of compounds **22**−**25** and **32**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO049101W

(57) Hohl, R. J.; Lewis, K. *J. Biol. Chem.* **1995**, *270*, 17508−17512.
(58) Harwood, H. J., Jr. *Anal. Biochem.* **1995**, *226*, 268−278.
(59) Moores, S. L.; Schaber, M. D.; Mosser, S. D.; Rands, E.; O'Hara, M. B.; Garsky, V. M.; Marshall, M. S.; Pompliano, D. L.; Gibbs, J. B. *J. Biol. Chem.* **1991**, *266*, 14603−14610.
(60) Khan, S. G.; Mukhtar, H.; Agarwal, R. *J. Biochem. Biophys. Methods* **1995**, *30*, 133−144.
(61) Larson, R. A.; Yachnin, S. *Blood* **1984**, *64*, 257−262.