Articles

Cuprate-Mediated Synthesis and Biological Evaluation of Cyclopropyl- and *tert*-Butylfarnesyl Diphosphate Analogs

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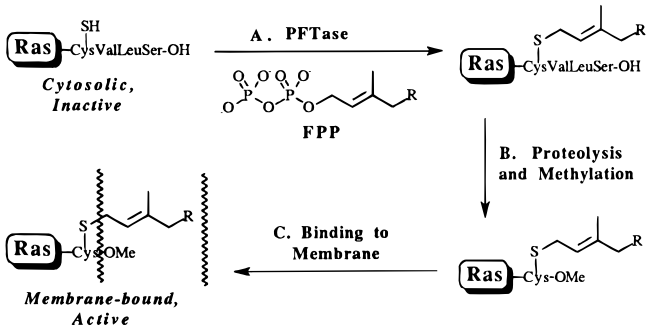
The novel farnesyl diphosphate (FPP) analog 3-cyclopropyl-3-desmethylfarnesyl diphosphate (3-cpFPP, **1**) was designed as a potential mechanism-based inhibitor of the FPP-utilizing enzyme protein−farnesyl transferase (PFTase). The key step in the synthesis of **1** involved the stereoselective coupling of vinyl triflate **8** with a lower order cyclopropyl cyanocuprate to afford the desired cyclopropyl ester **13**. The sterically encumbered analog 3-desmethyl-3-*tert*-butylfarnesyl diphosphate (3-tbFPP, **7**) was synthesized via a similar route. The use of the more reactive higher order *tert*-butyl cyanocuprate led to lower yields of ester **11**, the key intermediate in the synthesis of **7**. Biological evaluation of 3-cpFPP demonstrates that it is not a time-dependent inhibitor of recombinant yeast PFTase. Instead, 3-cpFPP is an alternative substrate for this enzyme that exhibits a K_m comparable to FPP and a k_{cat} only 5-fold lower than the natural substrate. In contrast, 3-tbFPP is an exceptionally poor substrate for yeast PFTase and acts as an inhibitor of this enzyme.

Introduction

Mutant Ras proteins, which are the products of *ras* oncogenes, are involved in ∼30% of all human cancers. It has been shown that, to be active, Ras proteins must be modified with a farnesyl moiety at a cysteine four residues from the carboxyl terminus (Scheme 1). This modification is carried out by an enzyme, protein−farnesyl transferase (PFTase), which uses farnesyl diphosphate (FPP) as the source of the farnesyl moiety.[1−4] PFTase has been the subject of intense research interest because inhibitors of this enzyme block the membrane localization and, thus, the action of mutant Ras proteins and possess significant potential as novel anticancer agents.[5−7] Notable progress has been made in the development of peptide-based PFTase inhibitors.[8−10] However, the design of more potent PFTase inhibitors would be facilitated by a greater understanding of the mechanism of this enzyme and more knowledge about the structure of its active site. We have therefore synthesized two new FPP analogs (**1** and **7**, Scheme 2) as probes of the FPP-binding site of PFTase. Such synthetic analogs are also of continuing interest for inhibitory and mechanistic studies of the sesquiterpene cyclases[11,12] and squalene synthase.[13]

Scheme 1



While the specificity of PFTase for its protein substrate has been extensively explored, there have only been limited reports on its specificity for FPP. Development of an FPP-based mechanism-based inhibitor of PFTase could provide information on the active site of the enzyme.[14] There is strong evidence that the first step in the PFTase-catalyzed transfer of the farnesyl group involves the ionization of FPP to give the stabilized allylic farnesyl carbocation.[15] If 3-cyclopropyl-3-desmethyl FPP (**1**, 3-cpFPP) reacts via this pathway to give the allylic carbocation **2**, then the cyclopropylcarbinyl resonance

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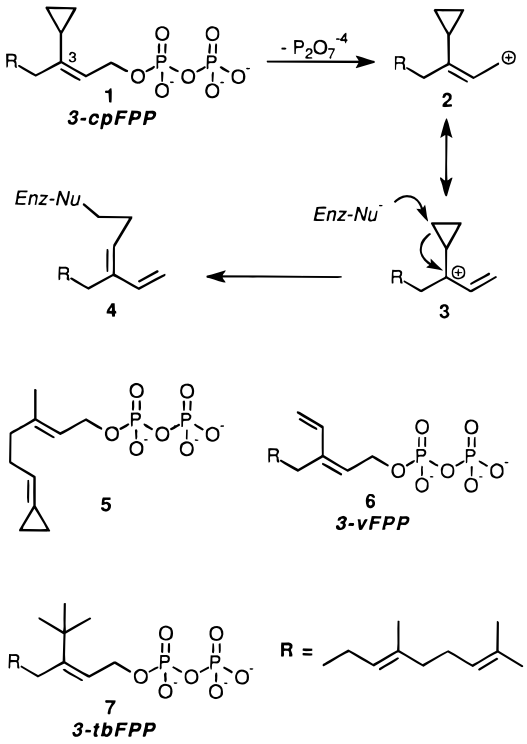
Scheme 2



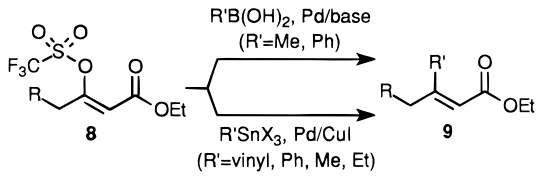
isomer **3** may react with a nucleophile in the enzyme active site to give adduct **4**, which would result in the irreversible inactivation of PFTase. It is well established that cyclopropylcarbinyl cations such as **3** can react with nucleophiles to give homoallylic products such as **4**.[16] Recently, Croteau and Cane reported that a cyclopropyl derivative of geranyl diphosphate (**5**) is a mechanism-based inhibitor of limonene synthase.[17] We have very recently demonstrated that 3-vFPP (**6**), also designed as a mechanism-based inhibitor of PFTase, is an alternative substrate and not an irreversible inhibitor.[18] However, the different shape of the cyclopropyl moiety of **1** and its potentially higher reactivity may lead to a greater chance of mechanism-based inhibition.[19]

Herein we report the synthesis of **1** and its biological evaluation with recombinant yeast PFTase. In the course of the synthetic studies the novel 3-desmethyl-3-*tert*-butyl FPP analog (**7**, 3-tbFPP) was prepared, and this compound was evaluated as a sterically demanding probe of the FPP binding site of PFTase.

Results and Discussion

Cuprate-Mediated Synthesis of 3-cpFPP and 3-tbFPP. We have recently described the use of the vinyl triflate **8** as a key intermediate for the synthesis of a variety of 3-substituted farnesyl and geranylgeranyl

Scheme 3



analogs (Scheme 3).[18,20,21] Triflate **8** readily couples with a variety of organotin nucleophiles through the Stille reaction[18,20] and with methyl and phenyl boronic acids via the Suzuki reaction.[21] Unfortunately, neither of these palladium-catalyzed coupling reactions is amenable to the introduction of a cyclopropyl or *tert*-butyl nucleophile. Therefore, the reaction of **8** with organocuprate nucleophiles was investigated. Weiler and co-workers have previously reported that the parent isoprenoid esters (**9**, where R′ = Me) can be prepared by coupling the vinyl phosphate analog of **8** with Me_2CuLi.[22] However, this vinyl phosphate does not undergo successful coupling with *t*-Bu_2CuLi[22] or a variety of vinyl cuprates.[20]

McMurry and Scott have found that vinyl triflates can be coupled with a variety of alkyl, vinyl, and aryl cuprates to give substituted olefins in a regio- and stereospecific manner.[23] With the less reactive aryl triflates, substitution required the use of higher order cyanocuprates ($R_2Cu(CN)Li_2$).[24] We therefore investigated the use of these cyanocuprates, developed by Lipshutz,[25] to introduce a *tert*-butyl group stereoselectively into the 3-position of the farnesyl structure. Treatment of vinyl triflate **8** with 1.5 equiv of *t*-$Bu_2Cu(CN)Li_2$ in THF at −78 °C led to a mixture of the desired ester **11** and reduced product **12** (Scheme 4). We hypothesized that **12** results from the protonation of the putative vinyl copper intermediate **10**, which may be slow to undergo conversion to **11**.[26] However, quenching the reaction at −78 °C with CD_3OD did not result in deuterium incorporation, suggesting that **12** may instead result from hydrogen transfer to a radical intermediate.[27,28] Stirring **8** and the cyanocuprate at −78 °C for 10 min afforded 19% of **11** and 57% of **12**. Extending the reaction time to 3 h produced a complex mixture. An improved yield of **11** (46%, along with 27% of **12**) was obtained by running the reaction for 1 h and using ether rather than THF as the solvent. Surprisingly, the best yield of **11** (68%, along with 14% of **12**) was obtained using the *lower order* cyanocuprate *t*-BuCu(CN)Li.[29,30] The reasons for the improved yield with *t*-BuCu(CN)Li are unclear; typically,

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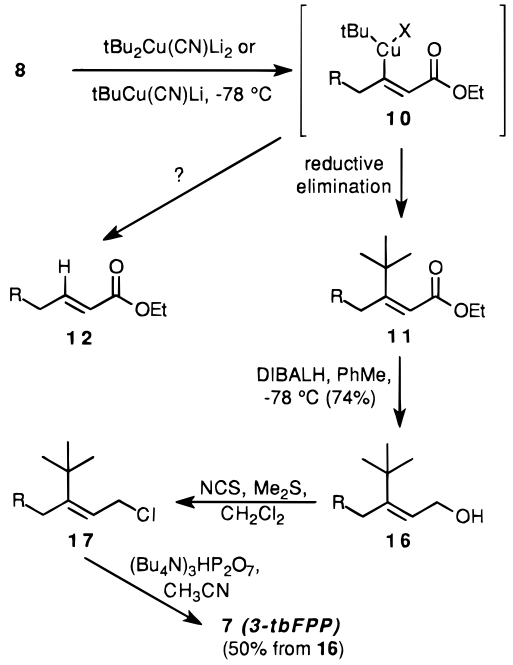
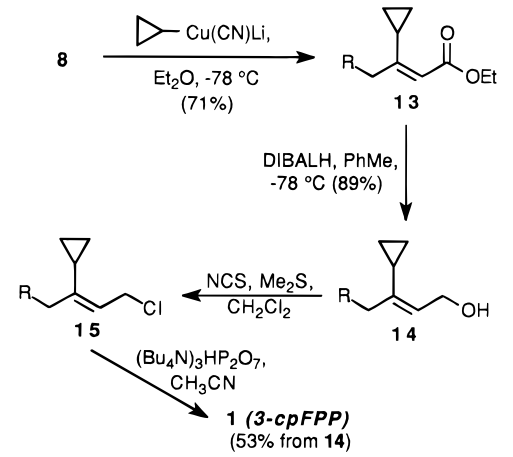
(30) Note that MeCu(CN)Li does not react with a vinyl phosphate,[29b] emphasizing the very reactive nature of vinyl triflate **8**.

Scheme 4



Scheme 5



higher order cyanocuprates are much more reactive than their lower order counterparts.[31] Apparently the more reactive reagent is incompatible with the sensitive vinyl triflate **8**. Note that mild and carefully controlled reaction conditions were required to effect the palladium-catalyzed coupling of **8** and boronic acids.[21]

With the reaction conditions for **11** optimized, we next turned to the synthesis of the cyclopropyl ester **13** (Scheme 5). The cyclopropyl cyanocuprate reagent was prepared *in situ* as described below. Treatment of cyclopropyl bromide with two equivalents of *tert*-butyllithium at −78 °C afforded 1 equiv of cyclopropyllithium,[32,33] which was then added to a slurry of CuCN in ether at −78 °C. Coupling of vinyl triflate **8** with the resulting lower order cyanocuprate at −78 °C for 1.5 h afforded **13** in a 71% yield. With the cyclopropyl cuprate, none of the reduction byproduct **12** was obtained. Due to the lower yield of **11** obtained with the higher order *tert*-butyl cyanocuprate, we did not investigate the reaction of the higher order cyclopropyl cyanocuprate reagent with **8**. Note that the double bond geometry was maintained during all the cuprate coupling reactions, and none of the more stable, undesired trans isomers of **11** and **13** were isolated.

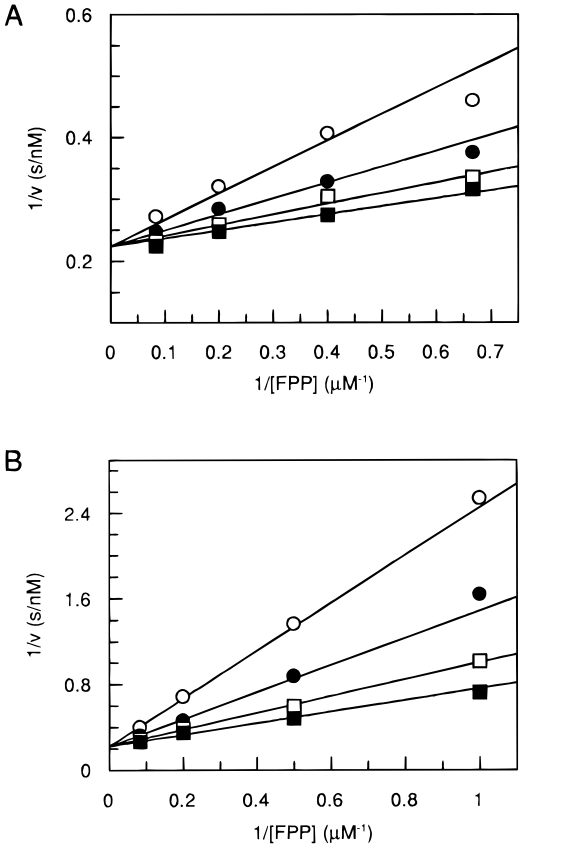


Figure 1. Inhibition of PFTase by 3-cpFPP and 3-tbFPP. Assays were conducted as described in the Experimental Section for reaction mixtures that contained 2.4 μM dansyl-GCVIA, 1−12 μM FPP, and 1−1.5 nM PFTase. A: 3-cpFPP ((■) 0.25, (□) 0.5, (●) 1.0, (○) 2.0 μM). B: 3-tbFPP ((■) 0.25, (□) 0.5, (●) 1.0, (○) 2.0 μM).

Reduction of esters **11** and **13** with diisobutylaluminum hydride (DIBALH) led to the efficient product of the desired alcohols **14** and **16**. We have previously found that DIBALH is a mild and very selective reducing reagent that led to the efficient production of other 3-substituted farnesols from the corresponding esters.[18,20,21] The diphosphorylation of alcohols **14** and **16** to 3-cpFPP and 3-tbFPP was accomplished using the two-step procedure developed by Poulter and co-workers.[34,35] The Corey−Kim procedure[36] was used to produce allylic chlorides **15** and **17**, which were not purified but instead taken directly on to the next step. Rapid handling was particularly important in the case of the unstable chloride **15**. Treatment of the chlorides with tris(tetrabutylammonium) hydrogen diphosphate then gave the desired diphosphates **1** and **7**. The recently developed straightforward reversed-phase HPLC procedure of Zhang and Poulter was used to purify the diphosphates.[37]

Biological Evaluation of 3-cpFPP and 3-tbFPP. The potential mechanism-based inhibitor 3-cpFPP (**1**) did

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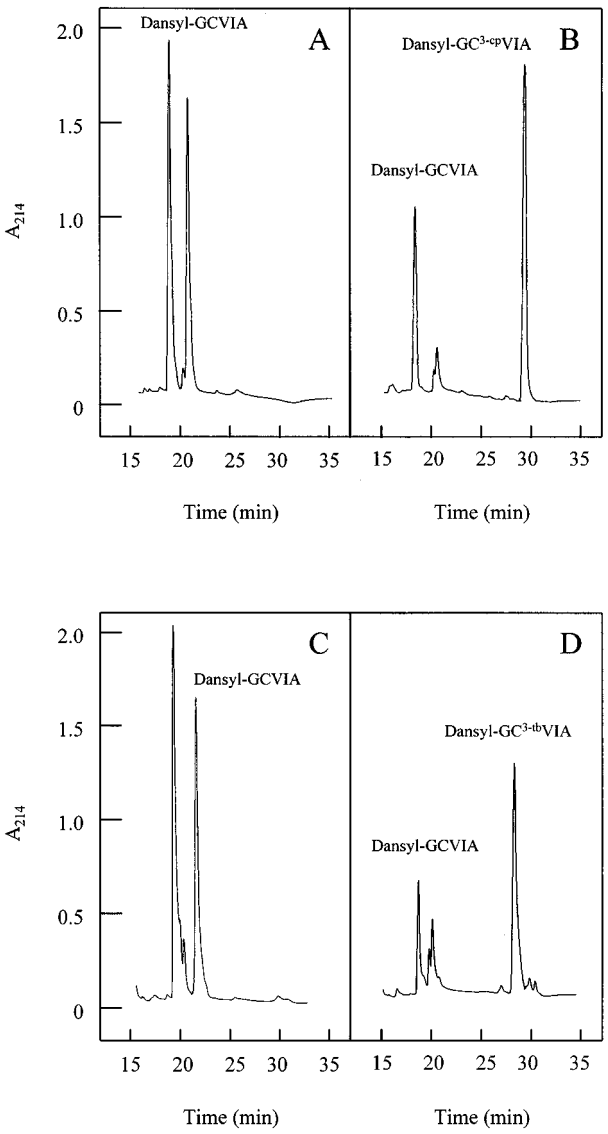

Figure 2. HPLC of the products from the PFTase-catalyzed condensation of dansyl-GCVIA with **1** and **7** on a Bondasil SP C18 reversed-phase column eluted with a 20 min (5−25 min) linear gradient from 0.025% TFA/5% CH_3CN/95% H_2O (v/v/v) to 0.025% TFA in CH_3CN (v/v) for **1** and 0.05% TFA/5% CH_3CN/95% H_2O (v/v/v) to 0.05% TFA in CH_3CN (v/v) for **7**. A: A control containing dansyl-GCVIA and 23 μg of PFTase. B: Products from incubation of dansyl-GCVIA with **1** and 23 μg of PFTase. C: A control containing dansyl-GCVIA and 15 μg of PFTase. D: Products from incubation of dansyl-GCVIA with **7** and 15 μg of PFTase.

not exhibit time-dependent inactivation of recombinant yeast PFTase. Instead, **1** was an alternative substrate for PFTase when incubated with dansyl-GCVIA as a cosubstrate. The K_m value measured for **1** (0.41 ± 0.02 μM) was comparable to that of the natural substrate, and the $k_{cat} = 0.83\ s^{-1}$ was only ca. five times less than that for FPP. Kinetic studies of 3-tbFPP (**7**) following standard assay procedures[38] (reaction initiated by PFTase) resulted in a substantial lag time before a linear rate was reached. In order to overcome this lag, **7** was preincubated with PFTase and the reaction was initiated by the addition of the peptide substrate. The sterically encumbered analog 3-tbFPP was also an alternative substrate of PFTase, but one with $k_{cat} = 0.0032\ s^{-1}$, 1500 times less than for FPP.

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The two FPP analogs, 3-cpFPP (**1**) and 3-tbFPP (**7**), were tested as inhibitors of PFTase. The cyclopropyl analog **1** behaves as a competitive inhibitor against FPP with a $K_I = 0.50 \pm 0.13\ \mu$M. Apparently, the competing conversion of 3-cpFPP to product is not sufficient to alter the Lineweaver−Burk profile, although the faster than "predicted" rates measured at the lowest concentration of FPP (1 μM) and two concentrations of 3-cpFPP (1.0 and 2.0 μM; see Figure 1A) may reflect the participation of the analog as an alternative substrate. The competitive profile in a double reciprocal plot (Figure 1A) indicates that the compound binds reversibly to the same site on PFTase as FPP. Since 3-tbFPP (**7**) is converted to product 1500 times more slowly than FPP, it can be treated as a dead-end inhibitor. Compound **7** was also a competitive inhibitor against FPP with $K_I = 0.31 \pm 0.04$ μM (Figure 1B).

In order to confirm that FPP analogs **1** and **7** were alternative substrates for yeast PFTase, the products from alkylation of dansyl-GCVIA upon incubation of the substrates with PFTase were isolated by reversed-phase HPLC. In a preparative scale reaction, 35 nmol of **1** and 25 nmol of dansyl-GCVIA were incubated with 23 μg of PFTase, and 24 nmol of **7** and 20 nmol of dansyl-GCVIA were incubated with 15 μg of PFTase. Due to substrate inhibition by dansyl-GCVIA,[38] it was necessary to add the peptide in several portions. The reaction mixtures were analyzed by HPLC, and the new peaks with retention times characteristic for alkylated pentapeptides were observed (Figure 2). The new peaks were isolated and gave negative ion FAB mass spectra with characteristic molecular ions at m/z 924 (M − 1) for dansyl-GC^{3-cp}VIA and m/z 940 (M − 1) for dansyl-GC^{3-tb}VIA.

The inability of 3-cpFPP to inhibit yeast PFTase irreversibly was presumably due to the lack of a suitable nucleophile in the active site to react with the putative cyclopropylcarbinyl moiety in 3-cpFPP. Not only must the nucleophile be positioned favorably, it must be powerful enough to compete with the thiolate moiety in the protein substrate. The failure to detect a time-dependent inactivation of PFTase by **1** suggests that such a nucleophile is not available. Instead, 3-cpFPP (**1**) was an alternative substrate of PFTase and competitive inhibitor against FPP. Preliminary results from product analysis studies involving 3-cpFPP determined that at TFA concentrations of 0.1% no product was detected by HPLC. This was presumably due to the degradation of the cyclopropyl product under these acidic conditions. The instability of dansyl-GC^{3-cp}VIA may be due to the rearrangement of the highly reactive vinyl-substituted cyclopropyl moiety following protonation of the product under the HPLC conditions used. The TFA concentration was lowered to 0.025% in subsequent studies, and a small product peak was detected by HPLC (Figure 2B). A sufficient amount of the material was collected and shown to be dansyl-GC^{3-cp}VIA by mass spectrometry.

Experimental Section[39]

Ethyl 3-*tert*-Butyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (11). In a flame-dried, argon-flushed flask were placed CuCN (0.47 mmol, 42 mg) and 1.0 mL of ether (distilled from Na/benzophenone). The resulting slurry was cooled to

(39) All synthetic reagents were from Aldrich Chemical Co. Solvents were from Aldrich or Fisher Scientific and were used as received unless otherwise indicated. HPLC work was carried out with HPLC grade solvents.

−78 °C, where *tert*-butyllithium (1.7 M in pentane, 0.47 mmol, 0.28 mL) was added dropwise. The mixture was allowed to warm to 0 °C and then recooled to −78 °C. A solution of the triflate **8** (0.32 mmol, 129 mg; prepared as previously described[18]) in 1.0 mL of ether was added dropwise, and the reaction was stirred for 1 h at −78 °C. The mixture was warmed to 0 °C and quenched with 2 mL of saturated NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography (20:1 hexanes/ethyl acetate) gave **11** as a colorless oil (67 mg, 68%) along with the more polar byproduct **12** (11 mg, 14%). **11:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 9H), 1.31 (t, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.01 (m, 4H), 2.14 (s, 4H), 4.15 (q, 2H), 5.11 (m, 2H), 5.59 (s, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.17, 16.08, 17.69, 25.69, 26.65, 27.88, 29.37, 35.42, 36.78, 39.66, 60.17, 115.80, 123.30, 124.24, 131.38, 135.82, 161.86, 168.74; HRMS calcd for $C_{20}H_{34}O_2$ 306.2559, found 306.2554. **12:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.03 (m, 4H), 2.20 (m, 4H), 4.18 (q, J = 6.9 Hz, 2H), 5.10 (m, 2H), 5.82 (d, J = 15.9 Hz, 1H), 6.97 (dt, J_1 = 15.6 Hz, J_2 = 6.3 Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 14.26, 16.04, 17.67, 25.67, 26.46, 26.63, 32.44, 39.62, 60.01, 121.36, 122.72, 124.17, 131.42, 136.33, 148.96, 166.74. Ester **12** has been previously prepared.[40]

Ethyl 3-Cyclopropyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (13). To a solution of cyclopropyl bromide (0.38 mmol, 31 μL) in 1.0 mL ether was added *tert*-butyllithium (1.7 M in pentane, 0.77 mmol, 0.45 mL) under argon at −78 °C. The resulting solution was stirred for 30 min, transferred to a slurry of CuCN (0.38 mmol, 34 mg) in 1.0 mL of ether at −78 °C, and then stirred for 15 min. Triflate **8** (0.26 mmol, 102 mg) in 1.0 mL of ether was added to the mixture at that temperature, and the reaction was stirred for 1.5 h. Workup and purification as described above for **11** afforded 53 mg (71%) of **13** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70 (m, 2H), 0.88 (m, 2H), 1.29 (t, 3H), 1.60 (s, 3H), 1.68 (s, 6H), 2.12 (m, 8H), 3.14 (m, 1H), 4.16 (q, 2H), 5.08 (t, 2H), 5.72 (s, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 6.75, 13.10, 14.37, 16.04, 17.67, 25.68, 26.62, 27.52, 30.98, 39.65, 59.42, 115.61, 122.85, 124.18, 129.96, 136.17, 164.02, 167.38; HRMS calcd for $C_{19}H_{30}O_2$ 290.2246, found 290.2246.

3-Cyclopropyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trien-1-ol (14). To a solution of **13** (0.22 mmol, 63 mg) in 1.0 mL of toluene (HPLC grade, stored over 4 Å sieves) was added DIBALH (1.0 M in toluene, 0.54 mmol, 0.54 mL) at −78 °C under argon. After being stirred for 1 h at −78 °C, the reaction was quenched by pouring it into saturated sodium potassium tartrate (20 mL). The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with water (2 × 15 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (4:1 hexanes/ethyl acetate), and 40 mg (74%) of **14** was obtained as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.50 (m, 2H), 0.68 (m, 2H), 1.60 (s, 6H), 1.68 (s, 3H), 1.78 (t, 1H), 2.10 (m, 8H), 4.33 (d, J = 6.9 Hz, 2H), 5.10 (m, 2H), 5.48 (t, J = 6.6 Hz, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 4.95, 11.58, 16.03, 18.68, 25.69, 26.69, 27.20, 32.94, 39.68, 59.23, 123.78, 124.28, 124.79, 131.35, 135.36, 143.16; HRMS calcd for $C_{17}H_{28}O$ 248.2140, found 248.2137.

3-*tert*-Butyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trien-1-ol (16). Ester **11** (0.14 mmol, 42 mg) was treated with DIBALH (1.0 M in toluene, 0.35 mmol, 0.35 mL) at −78 °C under argon for 1 h. Workup and purification as described above for **14** afforded 33 mg (89%) of **16** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 9H), 1.28 (t, 1H), 1.61 (s, 6H), 1.68 (s, 3H), 2.06 (m, 8H), 4.37 (t, 2H), 5.14 (m, 2H), 5.25 (t, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 16.08, 17.69, 25.70, 26.69, 29.01, 30.65, 35.78, 35.87, 39.69, 60.61, 124.08, 124.31, 125.21, 131.35, 135.14, 148.85; HRMS calcd for $C_{18}H_{32}O$ 264.2453, found 264.2417.

3-Cyclopropyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene 1-Chloride (15). A solution of *N*-chlorosuccinimide (0.089 mmol, 12 mg) in 1.0 mL of CH_2Cl_2 (distilled from CaH_2) was cooled to −30 °C in an acetonitrile/dry ice bath, and dimethyl sulfide (0.10 mmol, 8.0 μL) was added dropwise at this temperature. The milky white mixture was briefly warmed to 0 °C and recooled to −30 °C where the alcohol **14** (0.068 mmol, 17 mg, in 1.0 mL of CH_2Cl_2) was added dropwise. The reaction was stirred at 0 °C for 2 h and at room temperature for 15 min. The mixture was taken up with 20 mL of hexanes, washed with brine (2 × 15 mL), and dried over $MgSO_4$. After solvent removal, 14 mg (77%) of chloride **15** was obtained and used directly in the next step.

3-*tert*-Butyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene 1-Chloride (17). Alcohol **16** (0.11 mmol, 29 mg) was treated with *N*-chlorosuccinimide (0.22 mmol, 30 mg) and dimethyl sulfide (0.22 mmol, 16 μL) in 2.0 mL of CH_2Cl_2. Workup as above gave 29 mg (93%) of chloride **17**, which was used directly in the next step.

3-Cyclopropyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene Diphosphate (3-cpFPP, 1). A solution of chloride **15** (0.05 mmol, 14 mg) in 1.0 mL of acetonitrile (distilled from P_2O_5) was added to a suspension of tris(tetrabutylammonium) hydrogen pyrophosphate (0.10 mmol, 90 mg) in 0.5 mL of acetonitrile at 0 °C. The reaction was then warmed to room temperature, stirred for 2 h, and solvent was removed by a rotary evaporation at 30 °C. The residue was dissolved in 1 mL of ion-exchange buffer (1:49 v/v 2-propanol and 25 mM NH_4HCO_3), loaded onto a 1 × 5 cm ion-exchange column (Dowex AG50 × 8, NH_4^+ form), and eluted with 10 mL of ion-exchange buffer. The eluant was concentrated by lyophilization and then dissolved in 1 mL of 25 mM ammonium bicarbonate. This resulting mixture was purified by reversed-phase HPLC using a program of 5 min of 100% A followed by a linear gradient of 100% A to 100% B over 30 min (A: 25 mM aqueous NH_4HCO_3 (pH 8.0); B: CH_3CN; Vydac pH-stable C_8 4.6 mm × 250 mm columm; flow rate: 1.0 mL; UV monitoring at 214 and 230 nm). The retention time of the diphosphate **1** was 20 min. The fractions containing the product were pooled, the acetonitrile was removed by rotary evaporation, and the aqueous solution was then lyophilized to give 15 mg (65%) of 3-cpFPP **1** as a white, fluffy solid: ^{1}H NMR (300 MHz, D_2O) δ 0.51 (m, 2H), 0.72 (m, 2H), 1.60 (s, 6H), 1.67 (s, 3H), 2.11 (m, 8H), 4.63 (m, 2H), 5.20 (m, 2H), 5.54 (t, 1H); ^{31}P NMR (300 MHz, D_2O) −6.31 (J_{PP} = 21 Hz), −10.08 (J_{PP} = 21 Hz).

3-*tert*-Butyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene Diphosphate (3-tbFPP, 7). Chloride **17** (0.10 mmol, 29 mg) was treated with tris(tetrabutylammonium) hydrogen pyrophosphate (0.20 mmol, 185 mg) in 0.5 mL of acetonitrile for 2 h. The residue was converted to the ammonium form with 5 mL of resin and eluted with 10 mL of ion-exchange buffer. After purification by reversed-phase HPLC as described above for **1**, 27 mg (57%) of 3-tbFPP **7** (retention time: 20 min) was obtained as a white, fluffy solid. The sample contained a small residual amount of the tetrabutylammonium organic counterion, as evidenced by characteristic peaks at 0.89 and 3.09 ppm: ^{1}H NMR (300 MHz, D_2O) δ 1.08 (s, 9H), 1.55 (s, 6H), 1.60 (s, 3H), 2.04 (m, 8H), 4.63 (m, 2H), 5.11 (m, 1H), 5.19 (b, 1H), 5.27 (b, 1H); ^{31}P NMR (300 MHz, D_2O) −6.67 (J_{PP} = 21.05 Hz), −10.20 (J_{PP} = 21.05 Hz).

Prenyltransferase Assays. Recombinant yeast PFTase was produced in *E. coli* (JM101/pGP114) and was purified by ion-exchange and immunoaffinity chromatography as previously described.[41] Catalytic rate constants (k_{cat}) were measured using a fluorescence assay that continuously monitored farnesylation of the dansylated pentapeptide dansyl-GCVIA[38,42] using a Spex FluoroMax Model spectrofluorimeter with λ_{ex} = 340 (slit width = 5.1 nm) and λ_{em} = 486 (slit width = 5.1 nm) and 3 mm square cuvettes. Assays (250 μL total volume) were conducted at 30 °C in 50 mM Tris HCl, 10 mM $MgCl_2$, 10 μM

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$ZnCl_2$, 5 mM dithiothreitol, 0.04% (w/v) *n*-dodecyl β-D-maltoside, pH 7.0. Due to substrate inhibition,[38] Michaelis and inhibition constants were measured at 2.4 μM peptide concentrations, where inhibition was minimal. For determinations of k_{cat}, a large excess of the diphosphate substrate (100−200 μM) overcame substrate inhibition,[43] and a saturating concentration of the peptide substrate (5 μM) was used.

PFTase (1.0−4.0 nM) was used to initiate the 3-cpFPP reactions. This procedure gave a lag with 3-tbFPP. For this substrate 180 nM PFTase was preincubated with 3-tbFPP for 5 min, and the reaction was then initiated by the addition of dansyl-GCVIA. When 3-tbFPP was present as an inhibitor, the reaction was initiated by the addition of enzyme. Initial rates were measured from the linear region of each run, and all measurements were made in duplicate. Rates were measured in counts per second per second and converted to units of s^{-1} using a conversion factor (m) calculated from the slope of a line generated in a plot of concentration of synthetic dansyl-G(S-farnesyl)CVIA (dansyl-GC^{F}VIA) versus fluorescence intensity.[40]

Analysis of Enzymatic Reactions by HPLC and Isolation of the Prenylated Peptide. Reaction mixtures were chromatographed on a Bondasil SP C_{18} column eluted at 0.5 mL/min, and the products were detected by UV observance at 214 nm. Seven individual reactions containing FPP analog **1** (35 nmol), dansyl-GCVIA (25 nmol), and PFTase (23 μg) in 50 mM Tris HCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM dithiothreitol, 0.04% (w/v) *n*-dodecyl β-D-maltoside, pH 7.0, were incubated at 30 °C for 5 h. Due to substrate inhibition, the peptide was added in five separate 5 nmol portions. PFTase was added in five 2.9−5.7 μg portions. An HPLC gradient of 5% solvent B (CH_3CN/0.025% TFA) and 95% solvent A (H_2O/0.025% TFA) to 100% B over 20 min was used.

Five individual reactions containing FPP analog **7** (24 nmol), dansyl-GCVIA (20 nmol), and PFTase (15 μg) in 50 mM Tris HCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM dithiothreitol, 0.04% (w/v) *n*-dodecyl β-D-maltoside, pH 7.0, were incubated at 30 °C for 12 h. The peptide was added in five 4 nmol portions, and PFTase was added in five 3 μg portions. A similar HPLC gradient was used, but with solvents containing 0.05% TFA. The prenylated peptides eluted at 100% B. The peaks from the individual reactions were collected and pooled, and the solvent was removed under reduced pressure. The products were characterized by negative ion FAB mass spectrometry.

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Supporting Information Available: ^{1}H-NMR spectra of **1**, **7**, **11**−**14**, and **16** (7 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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