A Stereoselective Palladium/Copper-Catalyzed Route to Isoprenoids: Synthesis and Biological Evaluation of 13-Methylidenefarnesyl Diphosphate

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The novel farnesyl diphosphate (FPP) analog 13-methylidenefarnesyl diphosphate (3-VFPP, **4**) was designed as a potential mechanism-based inhibitor of the FPP-utilizing enzyme protein-farnesyl transferase (PFTase). A six-step stereoselective route to 3-VFPP is described. The key step in the synthetic sequence involved the stereoselective coupling of vinyl triflate **16** with vinyltributyltin using $Pd(AsPh_3)_2$ and CuI as catalysts to afford primarily the desired (*Z*)-divinyl ester **15**. It was also demonstrated that other 3-substituted farnesyl analogs can be prepared in a highly stereoselective manner by this Pd(0)/CuI-catalyzed route. The presence of CuI significantly increases the stereoselectivity of the coupling reaction, and a possible mechanistic rationale for this observation is presented. Biological evaluation of 3-VFPP demonstrates that it is not a time-dependent inhibitor of recombinant yeast PFTase. Instead, 3-VFPP is an alternative substrate for this enzyme that exhibits a K_m comparable to FPP but a k_{cat} significantly lower than the natural substrate.

Introduction

The mevalonate pathway has attracted intense biological and medicinal interest over the past decade.[1] The medical significance of this pathway is exemplified by the clinical utility of HMG-CoA reductase inhibitors as cholesterol-lowering agents. Farnesyl diphosphate (FPP, **1**, Figure 1) is a biosynthetic intermediate which occupies a key branch point in the mevalonate pathway. The primary route for FPP metabolism is its conversion into squalene by the enzyme squalene synthase (**2**).[2] Squalene is then transformed by a series of enzymatic steps to cholesterol. Therefore, squalene synthase has attracted significant interest as a potential additional target for cholesterol-lowering agents.[3] FPP is also converted in the cell to other important isoprenoids, such as dolichol and ubiquinone, which are utilized in protein glycosylation and electron transport, respectively.

More recently it has been recognized that FPP plays an additional crucial role in the cell. It is utilized by the enzyme protein-farnesyl transferase (PFTase) as the source of a farnesyl moiety that is attached to the cysteine sulfhydryl on the ras G proteins and certain other proteins which bear a carboxyl-terminal Cys-AAX-OH sequence (**3**).[4-6] This farnesylation event and the subsequent proteolysis and carboxymethylation modifications serve to increase the hydrophobicity of these proteins and thus convert them to peripheral membrane proteins.[7] Some very recent and intriguing reports have indicated that the farnesyl moiety plays a more active role in the interaction of G proteins with their intracellular activators and effectors.[7,8]

Ras proteins must be farnesylated, and thus directed to the cell membrane, to exert their biological action.[9] Since mutant forms of ras proteins are involved in 30% of human carcinomas, the development of PFTase inhibitors as anticancer agents has been an area of intense pharmaceutical interest.[10,11] Significant progress has been made in the development of peptide-based PFTase inhibitors,[12,13] and the Merck group has recently reported a compound that is effective *in vivo* in a mouse pancreatic carcinoma model system.[14] While the specificity of PFTase for its protein substrate has been extensively explored,[15,16] there have only been limited reports on its specificity for FPP.[17-19]

Mechanism-based inhibition is a well-established paradigm for the selective, irreversible inactivation of en-

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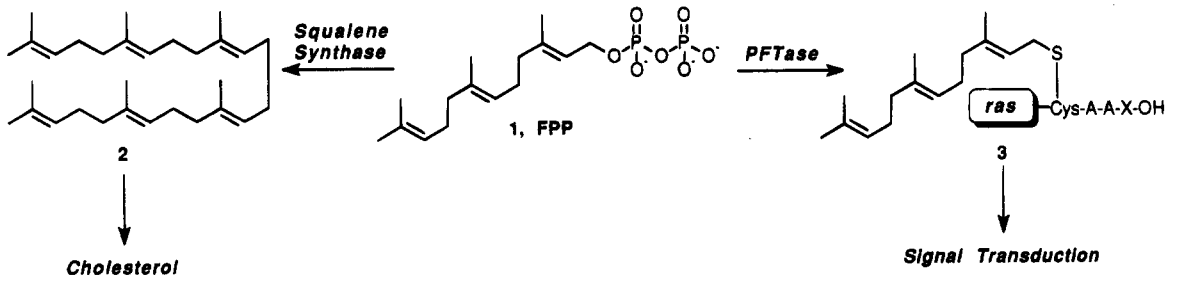

Figure 1.

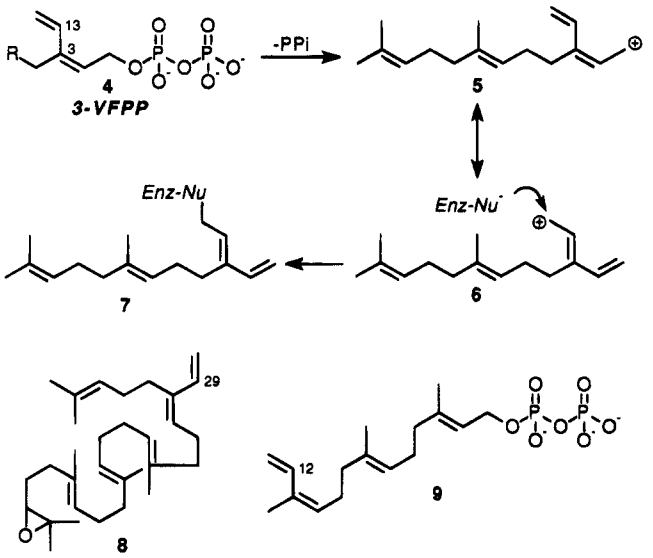

Figure 2.

zymes,[20] but there have been few reports of mechanism-based inhibitors of isoprenoid-utilizing enzymes.[21-24] We designed the vinyl FPP analog **4** (3-VFPP; Figure 2) as a potential mechanism-based inhibitor of PFTase. There is strong evidence that the first step in the PFTase-catalyzed transfer of the farnesyl group, as with many prenyltransferase enzymes, involves the ionization of FPP to give the stabilized allylic farnesyl carbocation.[25] If **4** reacts via this pathway to give the allylic carbocation **5**, then the resonance isomer **6** may react with a nucleophile in the enzyme active site to give adduct **7**, which would result in the irreversible inactivation of PFTase. There is some evidence that such a nucleophile is present in the active site of other prenyltransferases.[26,27] Furthermore, Prestwich and his co-workers have demonstrated that 29-methylideneoxidosqualene (**8**) is a mechanism-based inhibitor of oxidosqualene cyclase,[22,28] and very recently Cane and his co-workers have shown that 12-methylidene-FPP (**9**) is an irreversible inhibitor of aristolochene synthase.[24] Both of these compounds are proposed to react with their target enzymes in a similar manner to that shown for 3-VFPP in Figure 2.

Herein we report in detail the synthesis of **4**[29] and its biological evaluation with recombinant yeast PFTase.[30] In the course of these studies a new method for the synthesis of farnesyl analogs was developed,[31] and these results are presented first.

Results and Discussion

Pd(0)/CuI-Catalyzed Coupling of Organostannanes with Isoprenoid Triflates. The development of stereoselective routes for the synthesis of isoprenoids and their analogs is a subject of continuing interest,[32] as evidenced by four recent reports of improved methods for the synthesis of *all-trans*-geranylgeraniol.[33-35] In particular, there has been significant activity recently directed toward the preparation of farnesyl analogs as potential squalene synthase inhibitors,[3,26,36-38] and farnesyl analogs continue to be prepared as mechanistic probes for other enzymes.[24,25,39,40] For our purposes we required a method that would allow for the ready, stereoselective introduction of a vinyl group into the 3-position of the isoprenoid skeleton. The synthetic strategy thus chosen was the homologation sequence developed by Weiler and co-workers.[41] As shown in Figure 3, coupling of the dianion **10** (readily prepared by treatment of commercially available sodium acetoacetate with butyllithium) with geranyl bromide leads to the selectively alkylated product **12** (R = geranyl). This

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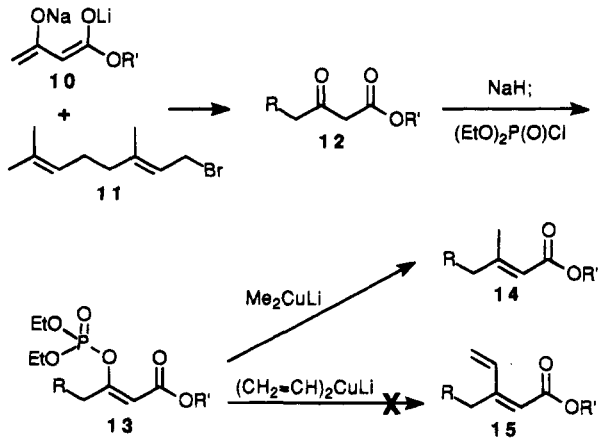

Figure 3.

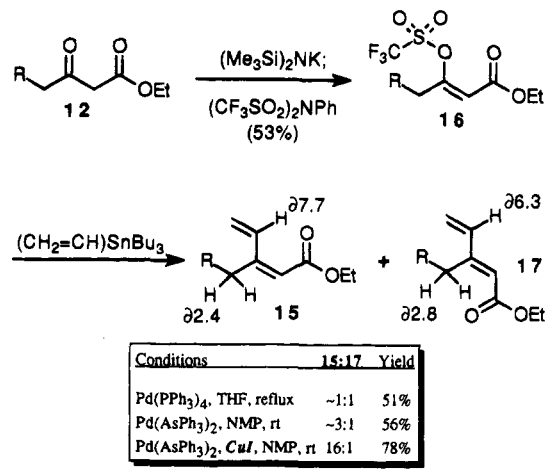

Figure 4.

β-keto ester was then transformed into the enol phosphate **13**. Previously, Weiler had reported that **13** could be readily and stereoselectively coupled with dimethylcuprate to afford methyl farnesoate (**14**; R′ = Me).[41] We anticipated that **13** could be coupled in the same manner with a divinylcuprate to afford ethyl 13-methylidenefarnesoate (**15**; R′ = Et), which would then be reduced and pyrophosphorylated to give the desired potential inhibitor **4**. Unfortunately, all attempts to couple **13** with either a lower order cuprate ($(CH_2{=}CH)_2CuLi$) or a more reactive higher order cyanocuprate ($(CH_2{=}CH)_2Cu(CN)Li_2$)[42] were unsuccessful, leading to recovery of the starting enol phosphate. It has been shown in other systems that vinyl cuprates are less reactive than the corresponding alkyl cuprates.[42] Similarly, **13** was also recovered unreacted from treatment with vinylmagnesium chloride in the presence of a nickel catalyst.[43] It was apparent that a more reactive leaving group was necessary to introduce a vinyl group into the 3 position of the farnesyl structure.

Numerous studies have established vinyl triflates as superior intermediates for the stereoselective synthesis of substituted vinyl compounds.[44] It has been demonstrated that they can undergo palladium-catalyzed cross-coupling reactions with a variety of nucleophiles, including organostannane,[45] organoalane,[46] and organoboron compounds.[47] Particularly encouraging for our purpose were recent reports that vinyl triflates derived from β-keto esters couple with vinylstannanes to give dienyl esters.[48-50] We therefore attempted to prepare vinyl triflate **16** from **12**, using a variety of different conditions (Figure 4). Houpis had reported that methyl acetoacetate could be transformed into the corresponding vinyl triflate by deprotonation with NaH followed by treatment with *N*,*N*-bis(trifluoromethanesulfonyl)-*N*-phenylamine (Tf_2NPh).[49] However, in our case, treatment of **12** with NaH followed by Tf_2NPh led to recovery of the starting material, even after extended reaction at room temperature. The same result was obtained when **12** was treated with triflic anhydride and 2,6-di-*tert*-butylpyridine in dichloromethane.[51] An attempt to produce **16** by treatment of **12** with triflic anhydride in the presence of triethylamine led to the production of a mixture of uncharacterized products. This method was not pursued further because it leads to the production of mixture of (*E*)- and (*Z*)-vinyl triflate isomers from ethyl acetoacetate.[46,52] Success was finally achieved using potassium bis(trimethylsilyl)amide as the base to generate the potassium enolate, followed by quenching of the reaction with Tf_2NPh, as described by Crisp and Meyer.[52] The yield of the desired triflate **16** is modest; however, the reaction is stereoselective and primarily the desired *Z* isomer of the vinyl triflate is obtained. [53]

The initial palladium-catalyzed coupling of triflate **16** with vinyltributyltin was performed using the procedure of Scott and Stille.[45] The desired coupling product **15** was obtained in a ~1:1 mixture with the undesired *E* isomer **17**. This appears surprising, in view of the well-established stereoselectivity of the Stille coupling reaction. However, Houpis has previously reported the loss of stereochemical integrity of a double bond in the Pd(II)-catalyzed coupling of a vinyl triflate derived from benzyl acetoacetate and vinyltributyltin.[49] Esters **15** and **17** were not readily separable by flash chromatography, but they were separated by normal phase HPLC and then characterized. The assignment of stereochemistry was based on the chemical shifts observed for the vinyl protons and C4 protons indicated in Figure 4. The ester carbonyl has an anisotropic deshielding effect that results in a downfield shift of protons adjacent to it.[54] A similar, but less pronounced deshielding effect has been observed in other farnesyl ester analogs.[41,55]

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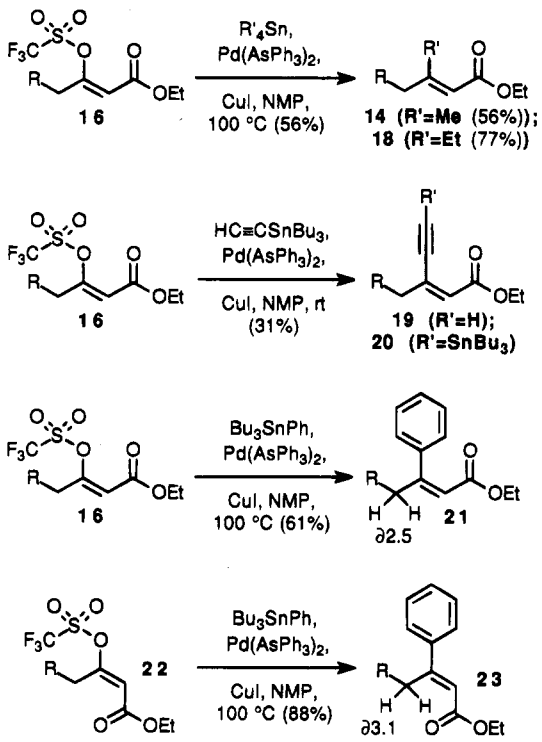

Figure 5.

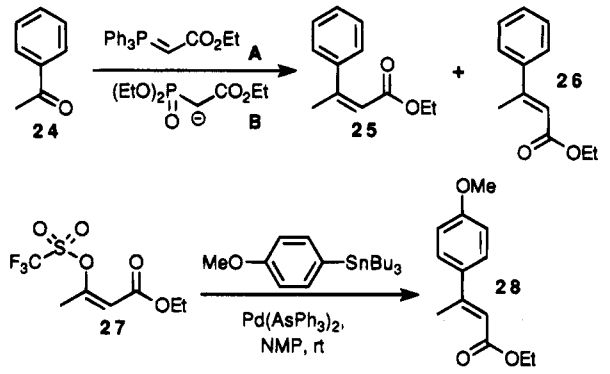

Figure 6.

Farina and his co-workers have recently developed a protocol, which involves the use of weaker palladium ligands such as triphenylarsine and more polar solvents such as NMP, that provides superior results for a variety of Pd(0)-catalyzed coupling reactions.[56,57] In our case, the use of "$Pd(AsPh_3)_2$" (prepared in situ from $Pd(PhCN)_2Cl_2$ and $AsPh_3$) in *N*-methylpyrrolidone (NMP) afforded a higher ratio of the desired *Z* isomer **15**. However, the addition of CuI as a cocatalyst *significantly* increased the stereoselectivity of the reaction and increased the yield as well. Liebeskind and his co-workers were the first to document the beneficial effect of CuI on the Stille reaction.[58] More recently, Johnson and his co-workers[59] have described the combined use of CuI and $Pd(AsPh_3)_2$ to effect some difficult Stille reactions. However, this is the first report that it can increase stereoselectivity of this process.[60]

The Pd(0)/CuI-catalyzed coupling of organostannane reagents with vinyl triflate **16** has also been used to synthesize other farnesyl ester derivatives (Figure 5). In all cases, the reaction was highly stereoselective, affording exclusively the desired isomer. Tetramethyltin did not couple with **16** at room temperature, but at elevated temperatures the parent compound ethyl farnesoate (**14**) was produced in an entirely stereoselective fashion. Ethyl farnesoate has been previously converted to FPP; thus this provides a highly stereoselective route to **1**. This is a significant observation in view of the recent interest in synthesis of the farnesyl homolog *all-trans*-geranylgeraniol.[33-35] Tetraethyltin was also coupled with **16** under the same conditions to give the previously reported 13-methyl derivative of ethyl farnesoate (**18**).[55] The ethynyl derivative **19** was also prepared from **16**, although in this case the coupling occurred at room temperature. Surprisingly, the tributyltin compound **20** was also obtained, possibly through *in situ* generation of $Bu_3SnC{\equiv}CCu$ from ethynyltributyltin followed by its Pd(0)-catalyzed coupling with **16**.[61] Fluoride-induced destannylation of **20** produced additional **19**,[62] thus confirming the structural assignment of **20**.[63a] The 3-phenyl-3-desmethyl derivative **21** was readily synthesized from **16**,[63b] and the stereospecificity of the Pd/CuI protocol was demonstrated by converting the 2(*E*)-triflate isomer **22**[53a] to the 2(*E*)-phenyl isomer **23**. We also attempted to prepare the 3-desmethyl analog of ethyl farnesoate by coupling **16** with tributyltin hydride under the same Pd/CuI catalyst conditions. However, none of the desired product was produced; instead, the β-keto ester **12** was obtained along with other unidentified products, and this matter was not pursued further.

Particularly noteworthy is the synthesis of the 3-phenyl derivative **21** in a completely stereoselective manner. Figure 6 depicts a prototypical condensation of a hindered ketone with a stabilized phosphorous reagent. Other workers have reported that reaction of acetophenone **24** with Wittig reagent **A** or Horner–Emmons reagent **B** either leads to a ~1:1 ratio of **25** and **26** or primarily the *E* isomer **26**.[64-66] Alternative methods for the synthesis

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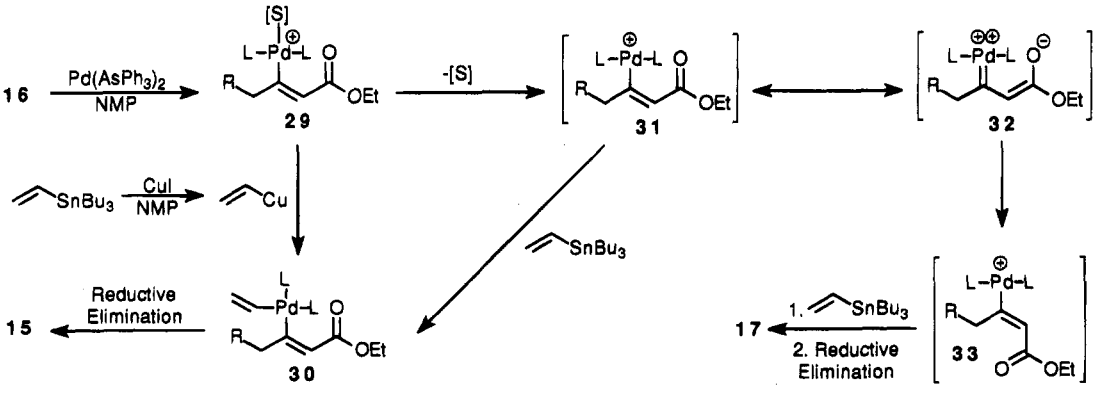

Figure 7.

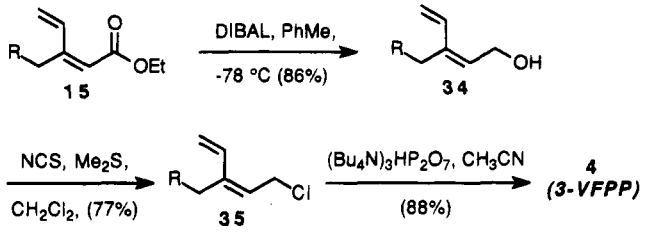

Figure 8.

of **25/26** also lead to mixtures of *E* and *Z* products.[67-69] The coupling method described herein may be uniquely useful for the stereoselective preparation of hindered *Z*-β,β-disubstituted-α,β-unsaturated esters such as **21** or **25**. Recently Farina and co-workers have reported the very similar coupling of (*p*-methoxyphenyl)tributyltin and the (*Z*)-vinyl triflate **27**.[57] Note that, under very similar conditions to those described in this paper except *in the absence of copper iodide*, only the isomerized (*E*)-3-aryl derivative **28** was obtained. This complete reversal of stereochemical outcome underlines the significant role that copper iodide plays in the coupling reactions of organostannanes and triflate **16**.

Recently Farina and Liebeskind have published a paper describing a series of mechanistic experiments exploring the "copper effect" on the Stille cross-coupling reaction.[70] These experiments provide evidence that, in polar solvents such as NMP, CuI reacts with organostannanes to afford an iodostannane and an organocopper derivative.[58] This organocopper derivative then presumably reacts with the organopalladium derivative of the vinyl triflate. On the basis of this report, we have proposed the mechanistic scheme shown in Figure 7 which could account for the observed stereochemical results in the coupling of vinyl triflate **16** and vinyltributyltin. Oxidative addition of palladium to vinyl triflate **16** leads to the vinylpalladium intermediate **29** (where L = $AsPh_3$ and [S] = solvent). Farina and Liebeskind propose that an organocopper species could directly attack **29** (via a pentacoordinate transition state) to give **30**. Since transmetalation is the slow step in palladium-catalyzed cross-couplings,[56] **30** should then rapidly undergo reductive elimination to give the desired *Z* olefin **15**. However, it has been proposed that organostannanes may not undergo direct coupling with species such as **29**.[56] Instead, **29** may first dissociate to give the highly reactive intermediate **31**, which then couples with vinyltributyltin to give the vinylpalladium intermediate **30**. We propose that the presence of the intermediate **31** leads to isomerization, via the enolate/Pd–carbene resonance isomer **32**, to the (*E*)-vinylpalladium compound **33**. A similar Pd–carbene species has very recently been invoked to explain a double-bond isomerization seen in a different palladium-induced process.[71a] Intermediate **33** would then couple with vinyltributyltin to give **17**. Evidence for this mechanism is provided by the fact that coupling of **31** with a vinylstannane (in the absence of CuI) leads to a mixture of *E* and *Z* olefins (Figure 4), whereas coupling of an arylstannane with an analogous vinyl triflate leads exclusively to the more stable, isomerized *E* olefin **28** (Figure 6). This would be explained by the fact that transmetalations from vinylstannanes are much faster than transmetalation from arylstannanes. Thus if the reaction of **31** with arylstannanes is slower, it would have a greater opportunity to isomerize via **32**, which would lead to the production of the more stable *E* olefin isomer.[71b]

Synthesis of 3-VFPP. The development of a highly selective route to the key 3-vinyl intermediate **15** allowed us to return to the original goal of the project, the synthesis of 3-VFPP. The first step in the preparation of **4** involved the reduction of **15** to the corresponding alcohol **34**. Previously, with other farnesyl analogs, this reduction step was accomplished with $LiAlH_4$[55] or $LiAl(OR)H_3$.[40] However, with the more sensitive dienyl ester **15** treatment with $LiAl(OEt)H_3$ led to none of the desired alcohol. Instead, a mixture was obtained which appeared to contain primarily products that resulted from the reduction of the conjugated double bonds as well as the ester functionality. Red-Al is a milder reducing agent than $LiAlH_4$ that can selectively reduce certain α,β-unsaturated esters to the corresponding allylic alcohols,[72] but treatment of **15** with Red-Al led to a mixture similar

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to that obtained with $LiAl(OEt)H_3$. Diisobutylaluminum hydride (DIBAL-H) is a mild and very selective reducing reagent that is also known to selectively reduce α,β-unsaturated esters to the corresponding allylic alcohols.[73] We were particularly attracted by its use in the selective reduction of a dienyl ester to a dienyl allylic alcohol in the course of the synthesis of a vitamin D A-ring synthon.[74] The conditions described by the Hoffman-LaRoche group[74] were used to selectively reduce **15** to **34**.[75] Attempts to increase the yield by using longer reduction times or additional equivalents of DIBAL-H led to a mixture of uncharacterized over-reduction products.

The diphosphorylation of alcohol **34** to 3-VFPP was accomplished using the two-step procedure developed by Poulter and co-workers.[76,77] Compound **34** was converted via the Corey–Kim procedure[78] into allylic chloride **35**, which was not purified but instead taken directly on to the next step. Chloride **35** was then treated with tris(tetrabutylammonium) hydrogen pyrophosphate to give the desired diphosphate **4**. However, the yield for this last step was quite low (ca. 20%). The reason for this low yield is unclear, but losses of material appeared to occur during the purification of the diphosphate by cellulose chromatography. We therefore utilized the recently developed, straightforward reversed-phase HPLC purification procedure of Zhang and Poulter.[79] This allowed us to isolate 3-VFPP in very good yield and in sufficient quantities for biological evaluation.

Biological Evaluation of 3-VFPP. Incubation of 3-VFPP (**4**) with yeast PFTase by itself or in the presence of the peptidomimetic inhibitor Cys-AMBA-Met[80] did not show time-dependent inactivation of the enzyme. Instead, **4** was an alternative substrate for PFTase when incubated with dansyl-GCVIA as a cosubstrate. The K_m value measured for **4** (0.46 ± 0.02 μM) was similar to that for FPP (1.0 μM); however, k_{cat} (0.085 s^{-1}) was ~60-fold less than for FPP ($k_{cat} = 5.2$ s^{-1}).[81] 3-VFPP (**4**) was then tested as an inhibitor of PFTase. Since **4** is converted to product much more slowly than FPP, it can be treated as a dead end inhibitor. Analysis of the data via a Lineweaver–Burke plot (Figure 9) indicated that 3-VFPP is a competitive inhibitor against FPP with a $K_i = 2.7 \pm 0.5$ μM. This type of inhibition is consistent with **4** binding to the same site on the enzyme as FPP.

To confirm that FPP analog **4** was an alternative substrate for yeast PFTase, the product from alkylation of dansyl-GCVIA upon incubation of 3-VFPP with PFTase was isolated by reversed-phase HPLC. In a preparative scale reaction, 25 nmol of **4** and 10 nmol of dansyl-GCVIA were incubated with 4.5 μg of PFTase. Due to substrate inhibition by dansyl-GCVIA, it was necessary to add the peptide in several portions. The reaction mixture was analyzed by HPLC, and a new peak with a retention time characteristic for alkylated pentapeptides was observed. In a control where **4** was not present, this peak was not seen (Figure 10). The new peak was isolated and gave a negative FAB mass spectrum with a characteristic molecular ion at m/z 909 (M − 1).

The ability of 3-VFPP to inhibit PFTase irreversibly depends on having a suitably positioned nucleophile in the active site to intercept the putative dienyl cation (**6**, Figure 2) generated during catalysis. The failure to detect a time-dependent inactivation of the enzyme during turnover suggests that such a nucleophile is not present in yeast PFTase. Alternatively, since the sulf-

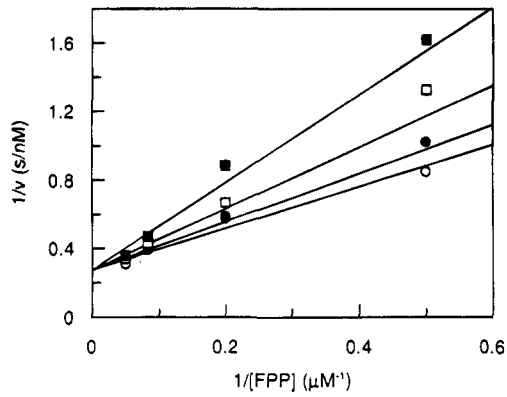

Figure 9. Inhibition of PFTase by 3-VFPP. Assays were conducted as described in the Experimental Section for reaction mixtures that contained 2.4 μM dansyl-GCVIA, 1–20 μM FPP, 3-VFPP ((○) 0.5, (●) 1.0, (□) 2.0, (■) 4.0 μM), and PFTase (1.0–2.0 nM).

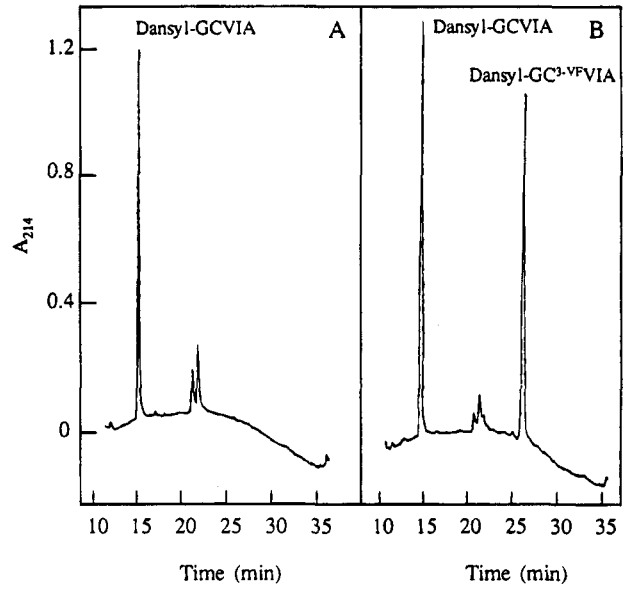

Figure 10. HPLC of the products from the PFTase-catalyzed condensation of dansyl-GCVIA with **4** on a Bondasil SP C18 reversed-phase column eluted with a linear gradient from 5% solvent B to 100% B over 35 min was used (solvent A, 0.1% TFA/H_2O; solvent B, 0.1% TFA/CH_3CN; UV detection at 214 nm). Incubations were performed as described in the Experimental Section. A: A control containing dansyl-GCVIA and 4.5 μg of PFTase. B: Products from incubation of dansyl-GCVIA with **4** and 4.5 μg of PFTase. The peaks eluting between 21 and 23 min were not seen in a sample containing all ingredients except PFTase and are attributed to the enzyme preparation.

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hydryl (or perhaps thiolate) moiety in the protein substrate is such a powerful nucleophile, it is possible that less nucleophilic groups in the catalytic site cannot effectively compete for the electrophilic dienyl substrate. However, the enzyme was stable when incubated with only 3-VFPP or with 3-VFPP and a nonreactive peptide analog that is a competitive inhibitor for the normal protein substrate. While it is not certain which explanation is correct, clearly yeast PFTase is capable of using 3-VFPP as an alternative substrate for prenyl transfer without suffering covalent modification.

Experimental Section[82]

Ethyl 7,11-Dimethyl-3-oxododeca-6,10-dienoate (12). The sodium salt of ethyl acetoacetate (20.0 mmol, 3.04 g) was dissolved in 40 mL of THF (distilled from sodium/benzophenone ketyl), cooled to 0 °C, and *n*-butyllithium (2.0 M in cyclohexane, 21.0 mmol, 10.6 mL) was added dropwise. After 20 min geranyl bromide **11** (10.0 mmol, 1.98 mL, 2.16 g) was added to the solution of dianion **10** and stirring was continued for 30 min at 0 °C. The mixture was then poured into a cold saturated solution of potassium hydrogen phosphate and extracted with ether (3 × 25 mL). The combined organic layers were washed with water (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (9:1 hexane/ethyl acetate) afforded 2.05 g (77%) of **12** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.30 (t, 3H), 1.62 (two s, 6H), 1.70 (s, 3H), 2.01 (m, 2H), 2.11 (m, 2H), 2.20 (q, 2H), 2.37 (t, 2H), 3.54 (s, 2H), 4.21 (q, 2H), 5.10 (m, 2H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.07, 15.95, 17.62, 22.12, 25.62, 26.55, 39.59, 42.99, 49.33, 61.26, 122.07, 124.11, 131.35, 136.66, 167.15, 202.51. MS-EI: *m/e* 266 (M^+).

Ethyl 3-(((Trifluoromethyl)sulfonyl)oxy)-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (16). A solution of the β-keto ester **12** (4.0 mmol, 1.064 g) in THF (10 mL; distilled from sodium/benzophenone ketyl) was added to potassium bis(trimethylsilyl)amide (0.5 M in toluene, 4.8 mmol, 9.6 mL) at −78 °C. While at −78 °C, *N*,*N*-bis(trifluoromethanesulfonyl)-*N*-phenylamine (4.8 mmol, 1.72 g) was added and the mixture was allowed to warm to room temperature overnight. The mixture was taken up in 30 mL of ether and washed with a 10% citric acid solution (2 × 20 mL) and water (1 × 20 mL). The ether layer was dried over $MgSO_4$ and the solvent removed in vacuo. Purification by flash chromatography (95:5 hexane/ethyl acetate) gave 0.846 g (53%) of compound **16** as an oil.[53] ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.30 (t, 3H), 1.59 (two s, 6H), 1.67 (s, 3H), 2.02 (m, 4H), 2.29 (q, 2H), 2.41 (t, 2H) 4.24 (q, 2H), 5.06 (m, 2H), 5.74 (s, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.00, 16.01, 17.66, 24.39, 25.65, 26.49, 34.58, 39.58, 61.23, 112.01, 120.58, 123.89, 131.64, 138.15, 158.42, 162.43. MS-CI: *m/e* 399 (M + 1).

Ethyl 3-Vinyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (15) and Ethyl 3-Vinyl-7,11-dimethyldodeca-2(*E*),6(*E*),10-trienoate (17). Triflate **16** (1.09 mmol, 434 mg), Ph_3As (0.11 mmol, 34 mg), bis(benzonitrile)palladium(II) chloride (0.054 mmol, 21 mg), and CuI (0.11 mmol, 21 mg) were placed in an argon-flushed flask and dissolved in *N*-methylpyrrolidone (NMP, 1.1 mL). Vinyltributyltin (1.3 mmol, 412 mg, 0.38 mL) was then added, and the reaction was stirred for ~15 h at rt. The mixture was then dissolved in 1:1 EtOAc/hexanes (100 mL), washed with aqueous KF (2 × 30 mL) and H_2O (20 mL), dried over $MgSO_4$, and then filtered and concentrated. Purification by flash chromatography (98:2 hexane/EtOAc) afforded the desired vinyl ester (234 mg; 78%). The ratio of *E* (**15**) and *Z* (**17**) isomers was determined to be 94:6 by integration of the NMR peaks at ∂ 7.74 and 6.32. HPLC purification (solvent 98:2 hexanes/MeOtBu; column Waters NovaPak silica gel 25 mm × 100 mm Radial-Pak cartridge; flow rate 8 mL/min; UV monitoring at 230 nm) of a sample produced via the original Stille procedure (where **15**: **17** = ~1:1) afforded analytical samples of each isomer. **15.** ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.28 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.6 (two s, 6H, two vinylic CH_3), 1.69 (s, 3H, vinylic CH_3), 2.1−1.9 (m, 4H, C_8 and C_9 CH_2), 2.20 (q, *J* = 7 Hz, 2H, C_5 CH_2), 2.37 (t, *J* = 7 Hz, 2H, C_4 CH_2), 4.21 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 5.12 (m, 2H, H_6 and H_{10}), 5.48 (d, *J* = 11.0 Hz, 1H, CH_2=CH−(cis H)), 5.61 (d, *J* = 17.9 Hz, 1H, CH_2=CH−(trans H)), 5.68 (s, 1H, H_2), 7.74 (dd, *J* = 17.9, 11.0 Hz, 1H, CH_2=CH−). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.29, 16.04, 17.66, 25.64, 26.70, 27.45, 33.60, 39.64, 59.80, 117.47, 119.76, 123.07, 124.26, 131.38, 133.19, 136.26, 154.31, 166.3. UV: (hexanes) λ_{max} 252 nm (ε 13 400). MS-EI: *m/e* 276 (M^+), 233, 161, 133, 109, 93, 81, 69, 55. HRMS: calcd for $C_{18}H_{28}O_2$ 276.2089, found 276.2093. **17.** ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.28 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.60 (s, 6H, two vinylic CH_3), 1.68 (s, 3H, vinylic CH_3), 2.1−1.9 (m, 4H, C_8 and C_9 CH_2), 2.18 (q, *J* = 7.8 Hz, 2H, C_5 CH_2), 2.80 (t, *J* = 7.9 Hz, 2H, C_4 CH_2), 4.18 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 5.10 (m, 1H, H_{10}), 5.22 (t, *J* = 7.3 Hz, 1H, H_6), 5.39 (d, *J* = 10.8 Hz, 1H, CH_2=CH−(cis H)), 5.64 (d, *J* = 17.4 Hz, 1H, CH_2=CH−(trans H)), 5.76 (s, 1H, H_2), 6.32 (dd, *J* = 17.4, 10.8 Hz, 1H, CH_2=CH−). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.30, 15.98, 17.65, 25.65, 26.75, 27.23, 28.01, 39.71, 59.74, 118.96, 119.60, 123.59, 124.40, 131.28, 135.72, 139.09, 156.19, 166.51. UV: (hexanes) λ_{max} 255 nm (ε 31 000).

Ethyl 3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-trienoate (14). Triflate **16** (0.33 mmol, 133 mg), CuI (0.033 mmol, 6.3 mg), Ph_3As (0.033 mmol, 10.2 mg), and bis(benzonitrile)-palladium(II) chloride (0.0165 mmol, 6.3 mg) were placed in an argon-flushed flask and dissolved in NMP (0.40 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tetramethyltin (0.66 mmol, 0.09 mL, 157 mg) was added, and the reaction mixture was stirred for 18 h. It was then cooled, taken up in EtOAc (25 mL), and washed with aqueous KF (2 × 20 mL) and H_2O (2 × 20 mL). The aqueous layers were back extracted with EtOAc (30 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (hexanes/ethyl acetate 95:5) gave 49 mg (56%) of **14**. ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.29 (t, 3H), 1.62 (s, 6H), 1.70 (s, 3H), 2.07 (m, 4H), 2.19 (s, 7H), 4.18 (q, 2H), 5.11 (m, 2H), 5.69 (s, 1H).[41] ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.33, 16.00, 17.66, 18.79, 25.65, 25.94, 26.65, 39.65, 40.95, 59.42, 115.63, 122.88, 124.20, 131.38, 136.11, 159.76, 166.87.

Ethyl 3-Ethyl-7,11-dimethyldodeca-2(*E*),6(*E*),10-trienoate (18). Triflate **16** (0.33 mmol, 133 mg), CuI (0.033 mmol, 6.3 mg), Ph_3As (0.033 mmol, 10.2 mg) and bis(benzonitrile)palladium(II) chloride (0.0165 mmol, 6.3 mg) were placed in an argon-flushed flask and dissolved in NMP (0.40 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tetraethyltin (0.66 mmol, 0.13 mL, 155 mg) was added, and the reaction mixture was stirred for 18 h. It was then cooled and worked up as described for **14**. Purification by flash chromatography (95:5 hexanes/ethyl acetate) gave 70 mg (77%) of **18**. ^{1}H NMR (300 MHz, $CDCl_3$): ∂ 1.08 (t, 3H), 1.28 (t, 3H), 1.60 (two s, 6H), 1.68 (3H, s), 1.95−2.15 (m, 4H), 2.17 (narrow m, 4H), 2.63 (q, 2H), 4.13 (q, 2H), 5.09 (m, 2H), 5.62 (s, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 13.00, 14.29, 16.04, 17.69, 25.33, 25.69, 26.13, 26.65, 37.94, 39.66, 59.45, 114.82, 123.01, 124.20, 128.44, 128.63, 133.71, 166.51. MS-CI: *m/e* 279 (M + 1). The ^{1}H NMR data obtained agree with those reported previously for this compound by Ortiz de Montellano and co-workers.[55]

Ethyl 3-Ethynyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (19). Triflate **16** (0.33 mmol, 133 mg), CuI (0.033 mmol, 6.3 mg), Ph_3As (0.033 mmol, 10.2 mg), and bis(benzonitrile)palladium(II) chloride (0.0165 mmol, 6.3 mg) were placed in an argon-flushed flask and dissolved in NMP (0.40 mL). The mixture was stirred for 10 min, and ethynyltributyltin (0.66 mmol, 0.19 mL, 210 mg) was added. The reaction mixture was stirred for 18 h at room temperature and worked up as described for **14**. Flash chromatography (95:5 hexane/ethyl acetate) afforded 15 mg of **19** and 60 mg of a less polar fraction identified as **20**. This less polar fraction was

(82) 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.

treated with nBu_4NF (1 mL, 1.0 M in THF) for 1 h at room temperature. The reaction mixture was diluted with ether, washed with 5% aqueous HCl and H_2O, dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (91:9 hexane/ethyl acetate) afforded an additional 13 mg of **19** (combined yield 31%). ^1H NMR (300 MHz, $CDCl_3$): ∂ 1.29 (t, 3H) 1.60 (two s, 6H), 1.68 (s, 3H), 2.00 (m, 2H), 2.05 (m, 2H), 2.31 (app s, 4H), 3.62 (s, 1H), 4.19 (q, 2H), 5.09 (m, 2H), 6.05 (s, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.17, 16.04, 17.66, 25.65, 26.33, 26.64, 38.95, 39.65, 60.26, 81.25, 88.46, 122.17, 124.17, 125.88, 131.42, 136.79, 138.05, 164.8. MS-CI 275 (M + 1).

Ethyl 3-Phenyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trienoate (21). Triflate **16** (0.33 mmol, 133 mg), CuI (0.033 mmol, 6.3 mg), Ph_3As (0.033 mmol, 10.2 mg), and bis(benzonitrile)palladium(II) chloride (0.0165 mmol, 6.3 mg) were placed in an argon-flushed flask and dissolved in NMP (0.40 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tributylphenyltin (0.66 mmol, 0.20 mL, 240 mg) was added, and the reaction mixture was stirred for 18 h. It was then cooled and worked up as described for **14**. Flash chromatography (95:5 hexanes/ethyl acetate) afforded 66 mg (61%) of **21** as an oil. ^1H NMR (300 MHz, $CDCl_3$): ∂ 1.09 (t, 3H), 1.55 (s, 3H), 1.63 (s, 3H), 1.71 (s, 6H), 2.02 (m, 2H), 2.10 (m, 4H), 2.50 (m, 2H), 4.00 (q, 2H), 5.11 (m, 2H), 5.91 (s, 1H), 7.19 (m, 2H), 7.35 (m, 3H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 13.94, 16.04, 17.68, 25.37, 25.68, 26.62, 39.63, 40.43, 59.74, 117.38, 122.69, 124.20, 127.11, 127.64, 127.78, 131.54, 136.24, 140.12, 159.11, 166.06. MS-CI: 327 (M^+ + 1).

Ethyl 3-Phenyl-7,11-dimethyldodeca-2(*E*),6(*E*),10-trienoate (23). The isomeric 2(*E*)-triflate **22** (0.17 mmol, 68 mg), CuI (0.017 mmol, 3.2 mg), Ph_3As (0.017 mmol, 5.2 mg), and bis(benzonitrile)palladium(II) chloride (0.0085 mmol, 3.3 mg) were placed in an argon-flushed flask and dissolved in NMP (0.3 mL). The mixture was immersed in an oil bath maintained at a temperature of 100 °C, tributylphenyltin (0.26 mmol, 0.084 mL, 96 mg) was added, and the reaction mixture was stirred for 18 h. It was then cooled and worked up as described for vinyl ester **15**. Flash chromatography (98:2 hexanes/ethyl acetate) afforded 49 mg (88%) of **23** as an oil. ^1H NMR (300 MHz, $CDCl_3$): ∂ 1.03 (t, 3H), 1.49 (s, 3H), 1.60 (s, 3H), 1.68 (s, 6H), 1.90–2.05 (m, 4H), 2.12 (app q, 2H), 3.14 (app t, 2H), 4.21 (q, 2H), 5.08 (narrow m, 1H), 5.15 (narrow m, 1H), 6.03 (s, 1H), 7.3–7.45 (m, 5H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 14.3, 16.0, 17.7, 25.7, 26.6, 27.4, 31.0, 39.6, 59.8, 117.6, 123.3, 124.3, 126.7, 128.4, 128.8, 131.2, 135.8, 141.4, 160.2, 166.4.

3-Vinyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-trien-1-ol (34). A solution of the vinyl ester **15** (0.85 mmol, 234 mg) in toluene (4.2 mL; HPLC grade dried over 4 Å sieves) was treated at −78 °C under argon with diisobutylaluminum hydride (1.0 M in toluene; 2.38 mmol, 2.38 mL). After the addition the mixture was stirred for 1 h at −78 °C. The reaction was quenched by adding the solution to saturated aqueous potassium sodium tartrate (40 mL), the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL) and dried ($MgSO_4$). Filtration and concentration followed by flash chromatography (hexane/ethyl acetate 9:1) gave 173 mg (86%) of vinyl alcohol **34**. ^1H NMR (300 MHz, $CDCl_3$): ∂ 1.59 (two s, 6H), 1.69 (s, 3H), 2.06 (m, 4H), 2.22 (m, 4H), 4.31 (d, 2H), 5.17 (m, 2H), 5.30 (d, 1H), 5.36 (d, 1H), 5.59 (m, 1H), 6.58 (dd, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): ∂ 16.07, 17.69, 25.71, 26.78, 27.17, 33.35, 39.72, 58.57, 118.51, 123.85, 124.36, 128.02, 131.35, 132.29, 135.55, 139.40.

1-Chloro-3-vinyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene (35). NCS (*N*-chlorosuccinimide; 0.42 mmol, 60 mg) was dissolved in 1.75 mL of CH_2Cl_2 (distilled from CaH_2), and the resulting solution was cooled to −30 °C with a dry ice/acetonitrile bath. Dimethyl sulfide (0.45 mmol, 0.03 mL, 27 mg) was added dropwise by a syringe, and the mixture was warmed to 0 °C, maintained at that temperature for 5 min, and cooled to −40 °C. To the resulting milky white suspension was added dropwise the vinyl alcohol **34** (0.38 mmol, 90 mg) dissolved in 5 mL of distilled CH_2Cl_2. The suspension was warmed to 0 °C and stirred for 2 h. The ice bath was removed, and the reaction mixture was allowed to warm to room temperature and stirred for an additional 15 min. The resulting solution was washed with hexane (2 × 20 mL). The hexane layers were then washed with brine (2 × 10 mL) and dried over $MgSO_4$. Concentration afforded 74 mg (77%) of vinyl chloride **35** as an oily liquid which was used directly in the next step without purification. ^1H NMR (300 MHz, $CDCl_3$): ∂ 1.61 (two s, 6H), 1.71 (s, 3H), 2.09 (m, 2H), 2.11 (m, 2H), 2.19 (m, 2H), 2.26 (m, 2H), 4.26 (d, 2H), 5.15 (m, 2H), 5.32 (d, 1H), 5.61 (m, 1H), 6.66 (dd, 1H).

3-Vinyl-7,11-dimethyldodeca-2(*Z*),6(*E*),10-triene 1-Diphosphate (13-Methylidenefarnesyl Diphosphate, 3-VFPP) (4). Tris(tetrabutylammonium) hydrogen pyrophosphate (0.40 mmol, 365 mg) was dissolved in acetonitrile (3 mL; freshly distilled from P_2O_5) under an argon atmosphere. Vinyl chloride **35** (0.10 mmol, 25 mg) was added to the resulting milky white suspension. The mixture was stirred at room temperature for 2.5 h, and the solvent was removed in a rotary evaporator at room temperature. The residue was dissolved in deionized water, and the resulting solution was passed through a 2 × 8 cm Dowex AG50 × 8 ion exchange column (NH_4^+ form). The eluant was then concentrated in vacuo (Speedvac) to yield a pale yellow solid which was then dissolved in 2 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; column, Waters μBondapak C_{18} 25 mm × 100 mm Radial-Pak cartridge; flow rate, 5 mL; UV monitoring at 214 and 230 nm). The retention time of the diphosphate **4** was 25 min. The fractions containing the product were pooled, the acetonitrile was removed by rotary evaporation, and the aqueous solution was then lyophilized to afford 39 mg (88%) of pure **4** as a white, fluffy solid. ^1H NMR (300 MHz, D_2O/NH_4OH): ∂ 1.6 (two s, 6H, two vinylic CH_3), 1.69 (s, 3H, vinylic CH_3), 2.0–2.35 (m, 8H, C_4, C_5, C_8 and C_9, four CH_2), 4.65 (d, 2H, C_1 CH_2 (partially overlaps with HDO peak)), 5.12 and 5.25 (two m, 2H, H_6 and H_{10}), 5.27 (d, J = 11.7 Hz, 1H, CH_2=CH−(cis H)), 5.43 (d, J = 17.4 Hz, 1H, CH_2=CH−(trans H)), 5.65 (t, J = 6.8 Hz, H_2), 6.75 (dd, J = 17.4 Hz, 11.7 Hz, 1H, CH_2=C*H*−). ^{31}P NMR (100 MHz, D_2O/NH_4OH): ∂ −6.0 (d, J = 18.7 Hz), −10.1 (d, J = 18.7 Hz).

Prenyltransferase Assays. Recombinant yeast PFTase was expressed and purified by immunoaffinity chromatography as previously described.[30] Catalytic rate constants (k_{cat}) were measured using a fluorescence assay that continuously monitored farnesylation of dansylated pentapeptide dansyl-GCVIA[83,84] using a Spex FluoroMax model spectrofluorimeter with λ_{ex} = 340 (slit width = 5.1 nm) and λ_{em} = 486 nm (slit width = 5.1 nm) and 3 mm square cuvettes. Assays (250 μL) were conducted at 30 °C in 50 mM Tris·HCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0. Due to substrate inhibition, a saturating concentration of the peptide substrate was not used and the concentration that gave a maximal rate (2.4 μM) was chosen instead.

PFTase was used to initiate the reactions. When **4** was used as the substrate, 15 nM of PFTase was used, and when **4** was present as an inhibitor, 1.0–2.0 nM of PFTase was present. Initial rates were measured from the linear region of each run, and all measurements were made in duplicate. Rates were measured in counts/second per second and converted to units of s^{-1} using a conversion factor calculated from the slope of a line generated in a plot of concentration of synthetic dansyl-G(S-farnesyl)CVIA versus fluorescence intensity.

Analysis of Enzymatic Reactions by HPLC and Isolation of the Prenylated Peptide. Ten individual reactions containing FPP analog **4** (25 nmol), dansyl-GCVIA (10 nmol), and PFTase (4.5 μg) in 50 mM Tris·HCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0, were incubated at 30 °C for 10 h. Due to substrate inhibition, the peptide was added in five portions so that a

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concentration of 3 μM was maintained in the reactions. PFTase was added in three 1.5 μg portions. Reaction mixtures were chromatographed on a Bondasil SP C18 column eluted at 1.0mL/min, and the products were detected by UV at 214 nm. A gradient of 5% solvent B to 100% B over 35 min was used (solvent A, 0.1% TFA/H_2O; solvent B, 0.1% TFA/CH_3CN). The farnesylated product eluted between 90–94% B. The peaks from the individual reactions were collected, pooled, frozen, lyophilized, and then analyzed by negative ion MS.

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Supporting Information Available: ^1H NMR spectra of **4**, **15–17**, **21–23**, and **34** (9 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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