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Coupling of Isoprenoid Triflates with Organoboron Nucleophiles: Synthesis and Biological Evaluation of Geranylgeranyl Diphosphate Analogues

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Abstract—The Suzuki coupling reaction has been used to introduce a methyl group derived from commercially available methylboronic acid into a vinyl triflate. This has led to a concise synthesis of all-*trans*-geranylgeraniol (**10**), with the key step being the palladium-catalyzed, silver-mediated methylation of triflate **8** to give ethyl geranylgeranoate **9**. This coupling protocol has also been used to produce the novel geranylgeranyl diphosphate (GGPP) analogue 3-phenyl-3-desmethylgeranylgeranyl diphosphate (3-PhGGPP, **2d**). Our previously developed organocuprate coupling protocol has been used to introduce the cyclopropyl and *tert*-butyl moieties into the 3-position of vinyl triflate **8**. The four GGPP analogues 3-vinyl-3-desmethylgeranylgeranyl diphosphate (3-vGGPP, **2a**), 3-cyclopropyl-3-desmethylgeranylgeranyl diphosphate (3-cpGGPP, **2b**), 3-*tert*-butyl-3-desmethyl-geranylgeranyl diphosphate (3-tbGGPP, **2c**), and **2d** were then evaluated as potential inhibitors of recombinant yeast protein-geranylgeranyl transferase I (PGGTase I). The potential mechanism-based inhibitors 3-vGGPP and 3-cpGGPP did not exhibit time-dependent inactivation of PGGTase I. Instead, both analogues were alternative substrates, in accord with the interaction of the corresponding farnesyl analogues 3-vFPP and 3-cpFPP with PFTase. The *tert*-butyl and phenyl analogues were not substrates, but were instead competitive inhibitors of PGGTase I. Note that all four of the GGPP analogues were bound less tightly by the enzyme than the natural substrate, in contrast to the behavior of the 3-substituted FPP analogues.

The initial evidence that proteins are modified with a mevalonate pathway intermediate was presented just over a decade ago. Preliminary biochemical studies quickly demonstrated that there are three different protein prenylation motifs—farnesylation, geranylgeranylation, and bis-geranylgeranylation. The first modification is carried out by an enzyme, protein-farnesyl transferase (PFTase), which recognizes the CAAX box (where X = Ser or Met) at the carboxyl terminus of the protein substrate and then attaches the farnesyl group from farnesyl diphosphate (FPP) to the free sulfhydryl of the cysteine residue (Scheme 1).[1–5] The second, closely related enzyme protein-geranylgeranyl transferase I (PGGTase I), attaches a geranylgeranyl moiety from geranylgeranyl diphosphate (GGPP) to a cysteine in a similar CAAX box, where leucine is the carboxyl terminal residue. The third enzyme, PGGTase II, attaches two geranylgeranyl residues to two cysteine residues at the carboxyl terminus of rab proteins.[6] Initial studies demonstrated that the key signal transduction protein and oncogene product Ras is farnesylated.[7] Thus, PFTase has been the subject of intense research interest. PFTase inhibitors block the action of mutant Ras proteins and halt the growth of many types of human cancer cells in vitro and in vivo, and therefore have great potential as novel anti-cancer agents.[8–11] The intense biological interest in protein farnesylation has culminated recently in the introduction of PFTase inhibitors into human clinical trials.[12]

The bulk of the progress in the area of PFTase inhibitors has been made in the development of peptide-based

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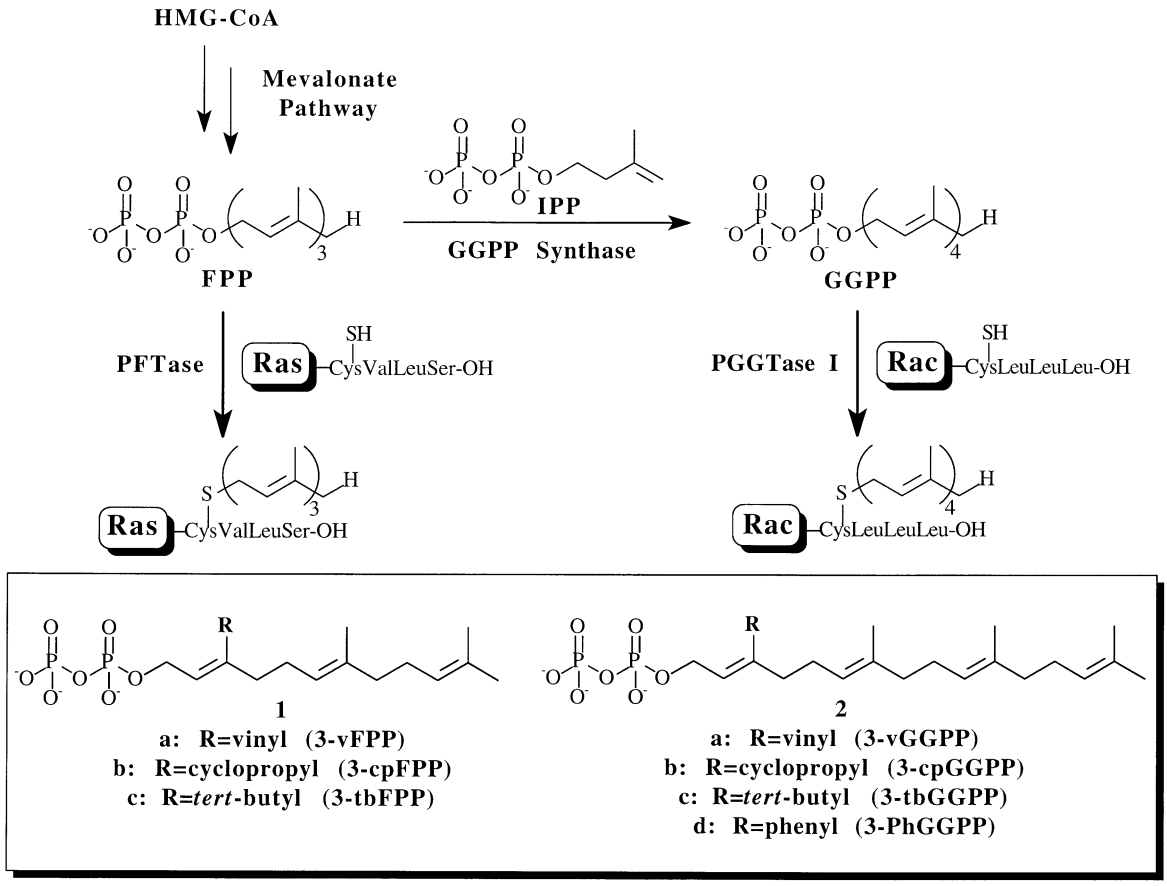
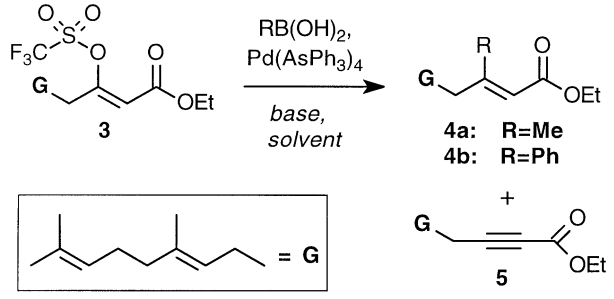


Scheme 1.



Scheme 2.

and other peptide competitive compounds.[13,14] However, less work has been done on FPP-based PFTase inhibitors,[15–22] and thus less is known about the specificity of PFTase for its isoprenoid substrate. We therefore synthesized three novel FPP analogues as probes of the FPP-binding site of PFTase, and characterized their interaction with recombinant yeast PFTase.[23,24] The vinyl and cyclopropyl analogues 3-vFPP (**1a**) and 3-cpFPP (**1b**) were designed as potential mechanism-based inhibitors, but were instead competent alternative substrates for yeast PFTase. In contrast, the sterically encumbered analogue 3-tbFPP (**1c**) is an exceptionally poor substrate and a potent competitive inhibitor of this enzyme. Herein, we report the evaluation of the three homologous 3-substituted GGPP analogues (**2a**, **2b**, and **2c**) with recombinant yeast PGGTase I. We have also developed a new route to isoprenoids based on a Suzuki coupling reaction of isoprenoid triflates,[25,26] and this route has been used to prepare all-*trans*-geranylgeraniol and the novel GGPP analogue **2d**, which has also been evaluated versus PGGTase I.

The yeast and mammalian forms of PGGTase I have been cloned and overproduced,[27] and they have also been subjected to detailed kinetic and biochemical characterization.[28–31] These enzymes are heterodimers that are highly homologous to PFTase, and in fact they share the same α subunit with PFTase. The geranylgeranyl transferases have received less attention than PFTase, due to the demonstrated anticancer potential of PFTase inhibitors, despite the fact that geranylgeranylation of proteins is more common than farnesylation.[1–3] However, recent biological studies on protein geranylgeranylation have resulted in increased interest in this enzyme. Earlier studies in yeast had demonstrated that the same peptide or protein substrate could be prenylated by either PFTase or PGGTase I.[32] More recently, two studies have demonstrated that the K-Ras oncogene product can be geranylgeranylated in cells by PGGTase I.[33] These studies suggest that PGGTase I also may be an important pharmacological target, since K-Ras is the most commonly found mutant Ras protein in human carcinomas. In fact, PGGTase I inhibitors have recently exhibited potent anticancer activity in model systems.[34] Moreover, other biological studies imply that inhibition of protein geranylgeranylation may be of benefit in the treatment of cardiovascular diseases[35] and osteoporosis.[36] Despite this, few

studies have been previously published on PGGTase I inhibitors.[37–39] The comparative study of the prenyl diphosphate specificities of PFTase and PGGTase I is also of intrinsic interest due to the fact that PGGTase I exhibits a much higher specificity for GGPP than PFTase does for FPP.[31] This indicates that it may have a more restrictive prenyl diphosphate binding site.

Results and Discussion

Coupling of isoprenoid triflates with boronic acids

Previously, we prepared 3-vFPP (**1a**; Scheme 1) for investigation as a possible mechanism-based inhibitor of PFTase. The key step in the synthetic sequence involved the stereoselective coupling of vinyl triflate **3**[40] with vinyltributyltin using $Pd(AsPh_3)_2$ and CuI as co-catalysts to afford the desired 3-vinyl ester.[41] It was also demonstrated that this route is applicable to the stereoselective synthesis of other farnesyl analogues.[41,42] However, the desirability of this method is tempered by (a) the restricted availability of the organotin coupling partners, (b) the inability to transfer most alkyl groups by this route, and (c) the toxic nature of the organotin compounds (including the byproducts, which are difficult to remove on a large scale). This last drawback is particularly significant in the case of the parent system, where synthesis of ethyl farnesoate from **3** requires the use of toxic and volatile tetramethyltin. The Suzuki reaction has been applied to the coupling of aryl boronic acids and alkyl boranes with vinyl triflates,[43] including activated vinyl triflates such as **3**.[44] We therefore decided to investigate the use of the Suzuki reaction for the prepartion of ethyl farnesoate (**4a**; Scheme 2). However, the only previous report of methylation via the Suzuki reaction involved first the preparation of the appropriate methylating reagent via a two-step procedure.[45] We have found that, under the correct conditions, commerically available methylboronic acid can be employed for the efficient Pd(0)-catalyzed methylation of **3** to give ethyl farnesoate. This procedure has also been used in an efficient, stereospecific synthesis of all-*trans*-geranylgeraniol.

The conditions investigated for the Suzuki coupling reaction of vinyl triflate **3** with phenylboronic acid and methylboronic acid are shown in Table 1. The primary variable which influences the success of the Suzuki reaction is the base used to convert the organoboron compound into the more reactive 'ate' complex. For this reason, the palladium catalyst selected, the triphenylarsine system introduced by Farina[46] and used successfully by numerous investigators,[41,42,47,48] was maintained in all of the reactions shown. The initial attempt to couple **3** and methylboronic acid was made with cesium carbonate in DMF/THF as described by Johnson and Braun in their PGE1 synthesis.[48] The starting material was completely consumed; however, instead of the desired product, the ynoate **5** was obtained, presumably via an elimination reaction (entry 1). The undesired product **5** was also obtained when the coupling of **3** and phenylboronic acid was attempted under the same conditions (entry 2), even though phenylboronic acid is known to participate in the Suzuki reaction.[49,50]

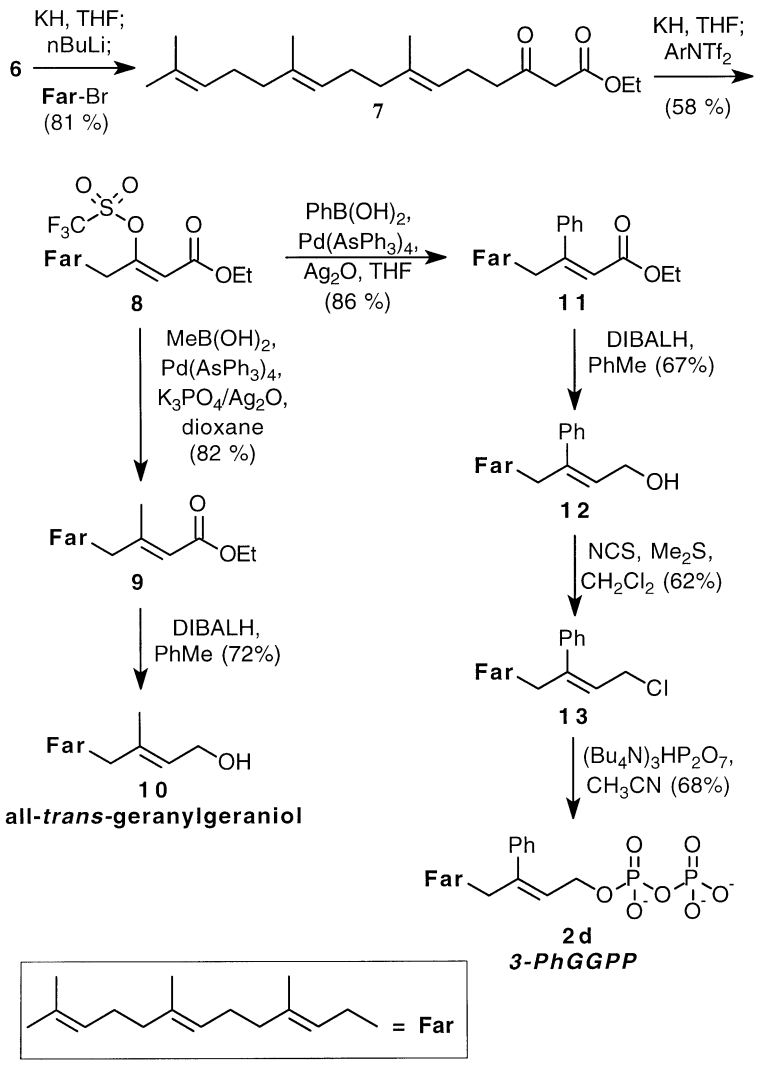
Table 1. Cross-coupling of triflate **3** with boronic acids[a]

Entry	R	Base	Solvent	Time (h)	Product	Yield (%)
1	Me	Cs_2CO_3	DMF/THF	3	**5**	47
2	Ph	Cs_2CO_3	DMF/THF	3	**5**	60
3	Ph	Ag_2O	THF	3	**4b**	72
4	Me	Ag_2O	THF	5	**3**	—
5	Me	K_3PO_4	THF	21	**4a** + **5**	—
6	Me	K_3PO_4, CuI	THF	71	**5**	62
7	Me	K_3PO_4, Ag_2O	Dioxane	19	**4a**	79

[a]Conditions: 1.5 equiv $RB(OH)_2$, 0.10 equiv $Pd(PhCN)_2Cl_2$, 0.40 equiv $AsPh_3$. All reactions were performed at room temperature.

There have been several reports, starting with Kishi's original observation,[51a] that the very mild base silver oxide can greatly accelerate the coupling of boronic acids with aryl or vinyl halides.[49,52,53] We were gratified to find that silver oxide in THF *at room temperature* smoothly promoted the coupling of phenylboronic acid with **3** to give ethyl 3-phenyl-3-desmethylfarnesoate (**4b**, entry 3). However, methylboronic acid did not couple with vinyl triflate **3** under these conditions after 5 h at room temperature (entry 4). Addition of potassium phosphate to this reaction mixture led to the sluggish conversion of **3** to the desired methylated ester (42% yield of **4a** after 71 h). Use of K_3PO_4 alone led to relatively rapid consumption of triflate **3**; unfortunately, a mixture of the desired ester **4a** and the elimination product **5** was isolated (entry 5). There have been several reports that copper iodide has a beneficial effect on the Stille coupling reaction of triflates with organotin compounds.[54] However, in this case copper has a deleterious effect on the coupling reaction; after 3 days at room temperature, the only product obtained was **5** (entry 6). The successful production of **4a** was finally achieved by changing the solvent to the more polar ether dioxane, and employing *both* Ag_2O *and* K_3PO_4 (entry 7). This requirement for both bases implies that silver oxide performs an additional function in this Suzuki coupling aside from the formation of the 'ate' complex from the boronic acid. It has been proposed that transmetallation of the R group from boron to silver may be responsible for this effect.[51b]

With the appropriate methylation conditions determined, we turned to their use in the synthesis of all-*trans*-geranylgeraniol, a subject of current interest[55–57] due in part to the recently recognized biological importance of geranylgeranylated proteins (vide supra).[58] The dianion derived from ethyl acetoacetate (**6;** Scheme 3) was coupled with farnesyl bromide to provide the β-ketoester **7** in very good yield.[41] The potassium enolate of **7** was then transformed into the required vinyl triflate **8**.[40] Coupling of methylboronic acid with **8** using the conditions described above led to the production of the desired ethyl geranylgeranoate **9** in 82% yield in only 5 h (the reaction was monitored by TLC). Reduction of **9** with DIBALH led to all-*trans*-geranylgeraniol **10** in four steps and 28% overall yield. Note that the key



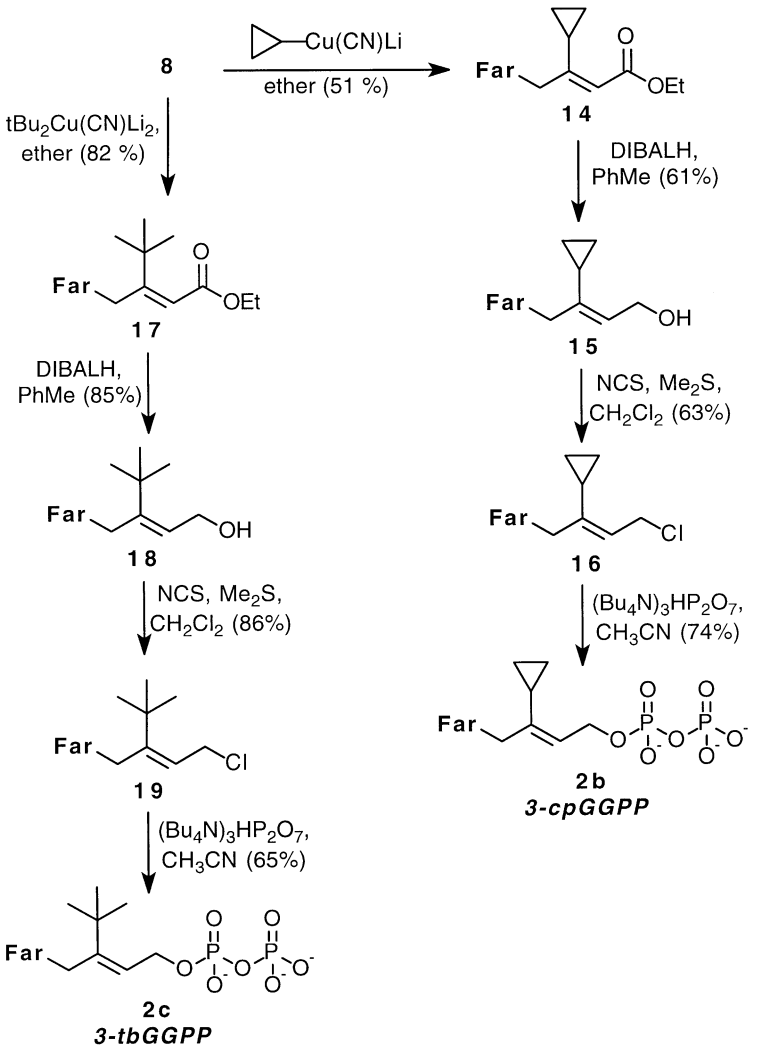
Scheme 3.

Suzuki coupling reaction was completely stereoselective; only the desired all-*trans* isomer of **10** was obtained. This route has been utilized very recently by Distefano and coworkers for the preparation of deuterium-labeled GGPP derivatives.[59]

Synthesis of 3-substituted GGPP analogues

The novel phenyl-substituted GGPP analogue **2d** was prepared using the modified Suzuki route as described above (Scheme 3). Coupling of triflate **8** with phenylboronic acid afforded the desired 3-phenyl ester **11** in excellent yield. Note that in this case silver oxide was the only base required to effect coupling.[25] Reduction of ester **11** with diisobutylaluminum hydride (DIBALH) led to the efficient production of the desired alcohol **12**. 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.[23,24] The diphosphorylation of alcohol **12** to 3-PhGGPP was accomplished using the two-step procedure developed by Poulter and co-workers.[60,61] The Corey–Kim procedure[62] was used to produce allylic chloride **13**, which was not purified but instead taken directly on to the next step. Treatment of **13** with tris(tetrabutylammonium)hydrogen pyrophosphate then gave the desired diphosphate **2d**. The straightforward reversed-phase HPLC purification procedure of Zhang and Poulter was used to purify 3-PhGGPP to homogeneity.[63]

The cyclopropyl and *tert*-butyl analogues **2b** and **2c** were prepared via our previously developed cuprate coupling protocol,[24] again starting from triflate **8** (Scheme 4). The lower order cyclopropyl cuprate reagent was prepared by lithiation of cyclopropyl bromide with *tert*-butyllithium, followed by addition of one equivalent of copper(I) cyanide. Coupling of this reagent with triflate **8** led to the desired ester **14**. Compound **14** was then reduced to alcohol **15**, converted to chloride **16**, and diphosphorylated to 3-cpGGPP **2b** in the same manner as described above for **2d**. Treatment of triflate **8** with the higher order *tert*-butyl cyanocuprate reagent led to the desired *tert*-butyl ester **17** in very good yield. Subsequent to this study, during the synthesis of 3-tbFPP,[24] we found that the corresponding lower order cyanocuprate (*t*BuCuCNLi) was more effective in coupling reactions with vinyl triflate **3** than



Scheme 4.

the higher order cyanocuprate. However, due to the very good yield of **17** obtained with the higher order cuprate, the coupling of *t*BuCuCNLi with triflate **8** was not examined. Note that the reaction yields given here are not optimized, and are the result of one to two attempts in each case; furthermore, the low yield in the cyclopropyl case may be due to the necessity of preparing the cyclopropyllithium reagent in situ. Ester **17** was then reduced to alcohol **18**, converted to chloride **19**, and diphosphorylated to give 3-tbGGPP **2c** in an efficient and straightforward manner. The vinyl analogue **2a** was prepared from triflate **8** via our Stille coupling protocol as previously described.[26a]

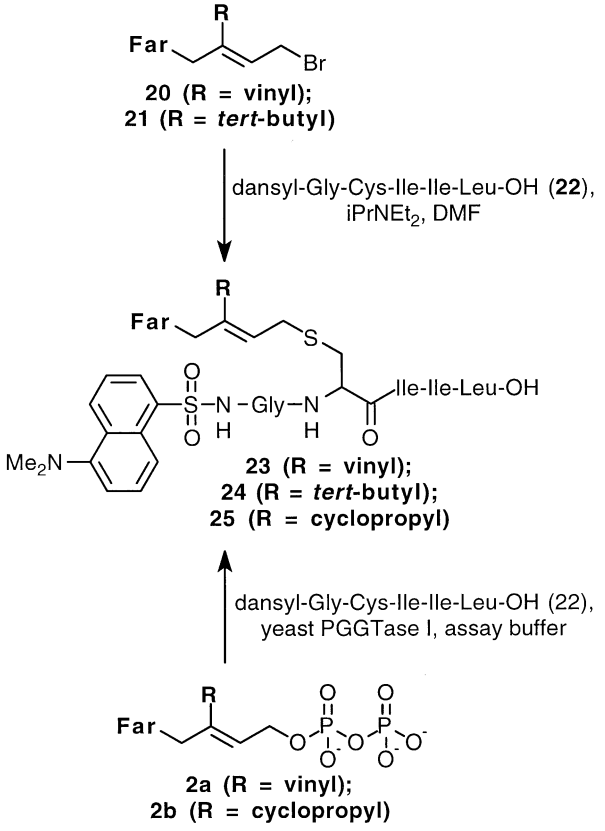
Synthesis of geranylgeranylated pentapeptides

The synthesis of dansyl-Gly-Cys^{3-vGG}-Ile-Ile-Leu (**23**) and dansyl-Gly-Cys^{3-tbGG}-Ile-Ile-Leu (**24**) was accomplished in a two-step procedure starting with the appropriate 3-substituted geranylgeraniol analogue (Scheme 5). The alcohols were converted to the corresponding bromides **20** and **21** using carbon tetrabromide and triphenylphosphine following the procedure developed by Dolence and Poulter.[64] The bromides were taken directly on to the next step without further purification. The sulfhydryl group of dansyl-Gly-Cys-Ile-Ile-Leu (**22**) was then alkylated with the bromide according to the procedure developed for farnesylation of dansylated pentapeptides.[65] Purification of the geranylgeranylated peptides was achieved by silica TLC as described in the experimental section. The overall yield of the two-step synthesis and purification (45–51%) was much higher than previously reported for the synthesis of dansyl-Gly-Cys^{GG}-Ile-Ile-Leu (10%), which was purified by C_{18} reversed-phase chromatography.[27a] Several attempts at the synthesis of dansyl-Gly-Cys^{3-cpGG}-Ile-Ile-Leu both through the bromide and chloride intermediate resulted in none of the desired product. This was presumably due to the loss of the cyclopropyl moiety during the attempted coupling reaction, although the byproducts were not isolated and characterized.

Biological evaluation of the GGPP analogues

The potential mechanism-based inhibitors 3-cpGGPP (**2b**) and 3-vGGPP (**2a**) did not exhibit time-dependent inactivation of recombinant yeast PGGTase I. Instead, **2b** and **2a** were alternative substrates for GGPP when dansyl-GCIIL was the cosubstrate (Table 2). The K_m

for **2b** (11.8±1.2 μM) was circa 12 times higher than that for the natural substrate, and the k_{cat} (0.032 s^{-1}) was 10 times lower. In comparison, K_m for **2a** (51.9±14.6 μM) was circa 52 times higher, and k_{cat} (0.052 s^{-1}) was only 6 times lower. The sterically encumbered analogue 3-tbGGPP (**2c**) was investigated as an alternative substrate using fluorescence and HPLC to monitor the reaction. Small changes in fluorescence were observed at very high concentrations of **2c** (0.5–1.2 mM) and PGGTase I (112–448 nM), although no evidence for formation of a prenylated peptide was observed by HPLC (Fig. 1). The other sterically encumbered analogue, 3-PhGGPP (**2d**), was also not a substrate for the reaction with dansyl-GCIIL.



Scheme 5.

Table 2. Kinetic constants for FPP and GGPP analogues[a]

Analogue	k_{cat} PFT	k_{cat} PGGT	K_m PFT	K_m PGGT	K_i PFT	K_i PGGT
3-vFPP	0.085[b]	—	0.46[b]	—	2.7[b]	—
3-cpFPP	0.83[c]	—	0.41[c]	—	0.50[c]	—
3-tbFPP	0.0032[c]	—	nd[c]	—	0.31[c]	—
3-vGGPP	—	0.052	—	51.9	—	4.7
3-cpGGPP	—	0.032	—	11.8	—	3.0
3-tbGGPP	—	nd[d]	—	nd[d]	—	3.7
3-phGGPP	—	nd[d]	—	nd[d]	—	6.1

[a]Conditions: All K_m and K_i values are in micromolar amounts, and k_{cat} values are in s^{-1}. K_m, k_{cat} and K_i values were determined using recombinant yeast PFTase ('PFT') or recombinant yeast PGGTase I ('PGGT') in a continuous spectrofluorimetric assay (see experimental procedures). Under the conditions of the fluorimetric PFTase assay, the K_m determined for FPP itself was 1.0 μM, and the k_{cat} value was 5.2 s^{-1}. Under the conditions of the fluorimetric PGGTase I assay, the K_m determined for GGPP itself was 1.0 μM, and the k_{cat} value was 0.3 s^{-1}.
[b]Values previously determined for 3-vFPP.[23]
[c]Values previously determined for 3-cpFPP and 3-tbFPP with yeast PFTase.[24] The limited turnover observed with 3-tbFPP precluded the determination of a K_m value for this analogue.
[d]These analogues were not substrates for yeast PGGTase I, and thus these kinetic values could not be determined.

In order to confirm that GGPP analogue **2b** was an alternative substrate for yeast PGGTase I, the product from the enzyme catalyzed alkylation of dansyl-GCIIL was isolated by reversed-phase HPLC and analyzed by mass spectrometry. In preparative scale reactions, 44–66 nmol of **2b** and 8–24 nmol of dansyl-GCIIL (**22**) were incubated with 10–35 μg of PGGTase I. The reaction mixtures were analyzed by HPLC, and a new peak with a retention time characteristic for alkylated pentapeptides was observed. This material was isolated and gave a negative ion FAB mass spectrum with a characteristic molecular ion m/z 1047 (M−1) for dansyl-GC^{3-cpGG}IIL (**25**).

Analogue **2a** was also an alternative substrate. The reaction mixture from incubation of **2a** and dansyl-GCIIL with PGGTase I was analyzed by HPLC, and a new alkylated pentapeptide peak at 32 min was observed (Fig. 1E). A sample of synthetic dansyl-GC^{3-vGG}IIL (3 nmol) was added to a second enzymatic reaction, and a more intense peak was observed (Fig. 2F), confirming the enzymatic product as dansyl-GC^{3-vGG}IIL (**23**).

All four GGPP analogues, 3-cpGGPP (**2b**), 3-vGGPP (**2a**), 3-tbGGPP (**2c**), and 3-PhGGPP (**2d**) were tested as inhibitors of yeast PGGTase. The cyclopropyl and vinyl analogues, **2b** and **2a**, respectively, were competitive inhibitors against GGPP, $K_I^{2b}=3.0\pm0.4$ μM and $K_I^{2a}=4.7\pm0.7$ μM, suggesting that both analogues bind reversibly to the same site on PGGTase I as GGPP. Analogues **2c** and **2d** were also competitive inhibitors against GGPP with $K_I^{2c}=3.7\pm0.5$ μM and $K_I^{2d}=6.1\pm0.8$ μM (Fig. 2). These results were compared with those seen for 3-cpFPP, 3-vFPP, and 3-tbFPP with yeast PFTase (Table 2).[23,24] While the overall pattern is similar—the vinyl and cyclopropyl analogues are both substrates, and the *tert*-butyl analogues are both very poor or non-substrates—there are significant differences in the abilities of the analogues to bind to the two different enzymes.

Conclusion

PGGTase I is similar to PFTase in both steady-state kinetic behavior and the requirement of Zn^{2+} for catalytic activity.[28–31] More detailed investigations of PFTase and its chemical mechanism provide evidence for a late associative transition state with substantial development of a positive charge in the farnesyl moiety.[4,66] Based on these studies and the similarities between PFTase and PGGTase I, it can be proposed that the enzymatic reaction of PGGTase proceeds through a related associative transition state involving a substrate-derived nucleophile. Therefore, the inability of 3-cpGGPP and 3-vGGPP to inhibit yeast PGGTase I

irreversibly is presumably due to the lack of a good nucleophile in the active site that would compete with this type of mechanism. This is comparable to the studies with yeast PFTase in which the analogous vinyl and cyclopropyl FPP analogues (**1a** and **1b**; Scheme 1) did not cause time-dependent inactivation.[23,24] In the same manner, **2b** and **2a** were alternative substrates of PGGTase I and competitive inhibitors against GGPP.

In contrast to the shared resistance of PFTase and PGGTase I to irreversible inhibition by the vinyl and cyclopropyl analogues, there are significant differences

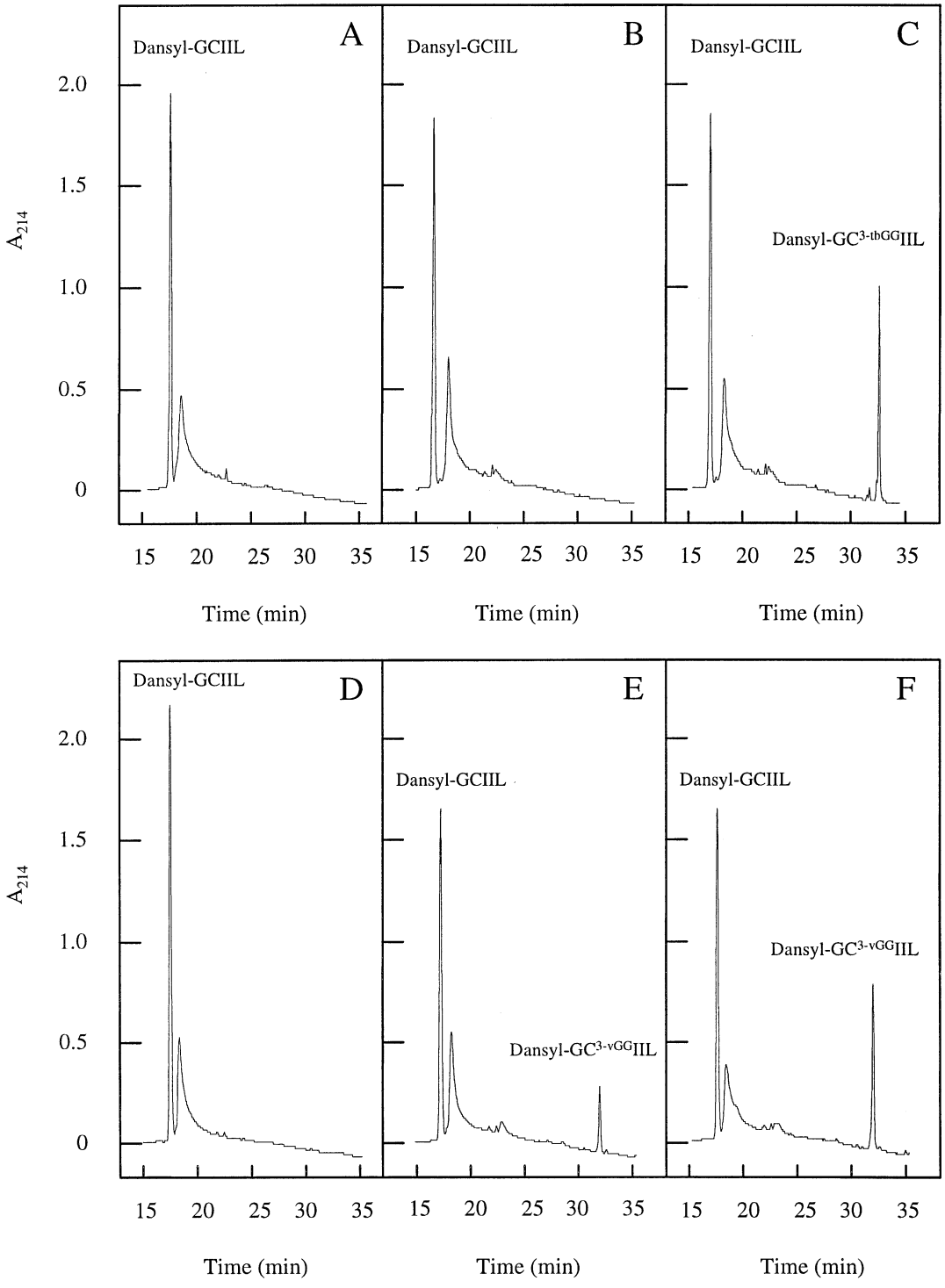


Figure 1. An HPLC trace of the products from the PGGTase I-catalyzed condensation of dansyl-GCIIL with **2c** and **2a** on a C_{18} reverse-phase column eluted with a 20 min (10–30 min) linear gradient from 0.10% TFA/30% CH_3CN/70% H_2O (v/v/v) to 0.10% TFA in CH_3CN (v/v): (A) a control sample containing dansyl-GCIIL and 3-tbGGPP (**2c**); (B) products from incubation of dansyl-GCIIL with **2c** and 10 μg of PGGTase I. (C) co-injection of products from reaction B and 6 nmol of chemically synthesized dansyl-$GC^{3\text{-tbGG}}$-IIL; (D) a control sample containing dansyl-GCIIL and 3-vGGPP (**2c**); (E) products from incubation of dansyl-GCIIL with **2a** and 10 μg of PGGTase I; (F) co-injection of products from reaction E and 3 nmol of chemically synthesized dansyl-$GC^{3\text{-vGG}}$-IIL.

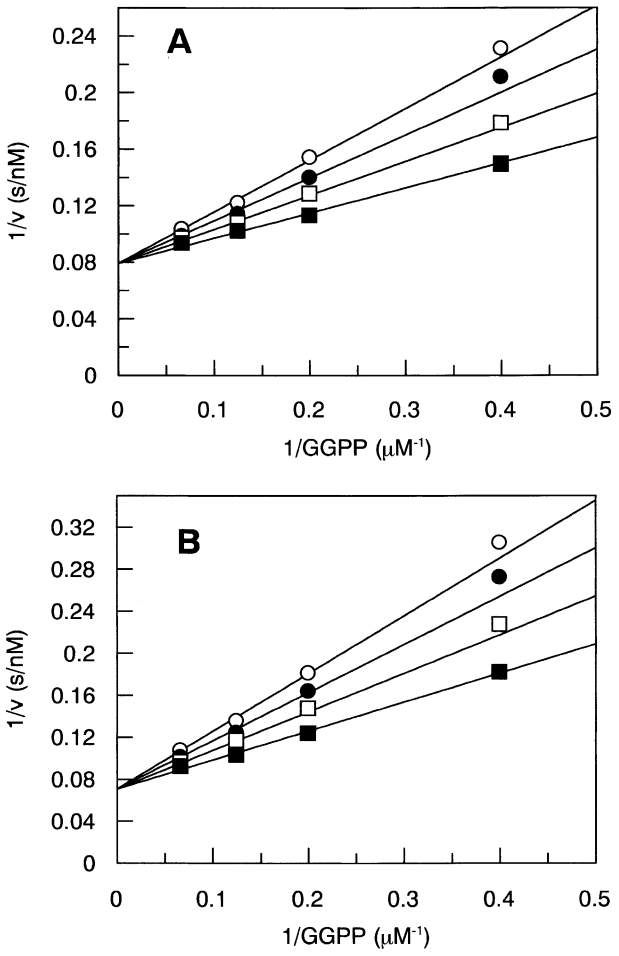


Figure 2. Double reciprocal plots for inhibition of PGGTase I by 3-tbGGPP and 3-PhGGPP. Assays were conducted as described in the Experimental for reactions mixtures that contained 4 μM dansyl-GCIIL, 2.5–15 μM GGPP and 30 nM PGGTase I; (A) 3-tbGGPP [(■) 2.0, (□) 4.0, (●) 6.0, (○) 8.0 μM]; (B) 3-PhGGPP [(■) 3.0, (□) 6.0, (●) 9.0, (○) 12.0 μM].

in their relative binding affinities for the FPP and GGPP analogues. The K_m values for GGPP analogues **2a** and **2b** with PGGTase I are 12–52-fold higher than that for the natural substrate (GGPP) while the K_m values for the same FPP analogues (3-cpFPP and 3-vFPP) with PFTase are similar to the natural substrate (FPP).[23,24] The k_{cat}'s determined for both the GGPP and FPP cyclopropyl analogue with PGGTase I and PFTase, respectively, are circa 5 times lower than those with GGPP and FPP. Interestingly, the k_{cat} for 3-vGGPP is only 10 times lower, while the k_{cat} for 3-vFPP is 60 times lower.[23,24] In the case of the *tert*-butyl analogues, 3-tbFPP binds to PFTase ~3-fold more tightly than the natural substrate FPP (as measured by a rough K_m vs K_i comparison), while 3-tbGGPP binds to PGGTase I roughly 4-fold less tightly than the natural substrate GGPP. In conclusion, both sets of analogues bind tightly to their respective enzyme active sites but the effect of the substituent at the C_3 position on enzyme catalysis is unique for each analogue and enzyme. However, these results tentatively indicate that PGGTase I has a more restricted isoprenoid binding site than PFTase. PGGTase and PFTase have identical α subunits but distinct β subunits.[67] The β subunits comprise the catalytic cores of these enzymes and are presumably responsible for the substrate selectivity of the enzymes and the influence of the C_3 substituent on catalysis and binding.

Experimental[68]

Ethyl 3,7,11,15-tetramethylhexadeca-2*E*,6*E*,10*E*,14-tetraenoate 9. Vinyl triflate **8** (0.24 mmol, 110 mg), $MeB(OH)_2$ (0.36 mmol, 22 mg), bis(benzonitrile)palladium (II) chloride (0.024 mmol, 9.2 mg), $AsPh_3$ (0.096 mmol, 29 mg), and Ag_2O (0.48 mmol, 111 mg) were placed in 2.0 mL of dioxane under argon. Potassium phosphate ($K_3PO_4 \cdot nH_2O$, 1:1.2 w/w, 144 mg) was then added, and the resulting mixture was stirred for 5 h at room temperature. The reaction was diluted with ether (20 mL), washed with water (15 mL), dried over $MgSO_4$, and concentrated. Purification by flash chromatography (hexanes/ethyl acetate, 20:1) afforded ethyl all-*trans*-geranylgeranoate **9** as a colorless oil (65 mg, 82%). ^{1}H NMR: δ 1.28 (t, $J \sim 7.2$ Hz, 3H), 1.60 (s, 9H), 1.63 (s, 3H), 1.99–2.16 (m, 12H), 2.16 (s, 3H), 4.14 (q, $J \sim 7.2$ Hz, 2H), 5.10 (m, 3H), 5.66 (s, 1H); ^{13}C NMR: δ 14.3, 16.0, 17.6, 18.2, 25.7, 26.0, 26.5, 26.8, 39.7, 41.0, 59.5, 115.6, 122.9, 124.1, 124.3, 128.5, 128.6, 135.0, 136.1, 159.8.

3,7,11,15-Tetramethylhexadeca-2*E*,6*E*,10*E*,14-tetraen-1-ol (all-*trans*-geranylgeraniol) 10. To a solution of ester **9** (73 mg, 0.22 mmol) in 1.0 mL of toluene was added DIBAL-H (1.0 M solution in toluene, 0.55 mL, 0.55 mmol) under argon at −78 °C. The reaction was stirred at −78 °C for 1 h, warmed to room temperature, and then quenched with 20 mL of Rochelle salt solution (saturated aqueous sodium potassium tartrate). The aqueous solution was extracted with ethyl acetate (2 × 20 mL), and the combined organic layers were washed with saturated NaCl (2 × 20 mL), and then dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by flash chromatography (hexanes/ethyl acetate, 4:1) to give alcohol **10** (46 mg, 72%) as a colorless oil. ^{1}H NMR: δ 1.60 (s, 9H), 1.69 (s, 6H), 1.95–2.15 (m, 12H), 4.16 (narrow m, 2H), 5.10 (narrow m, 3H), 5.42 (t, 1H); ^{13}C NMR: δ 16.01, 16.27, 17.66, 25.72, 26.29, 26.62, 26.75, 39.56, 39.72, 59.42, 123.27, 123.75, 124.15, 124.36, 131.29, 134.98, 135.40, 139.85. MS-EI: 290 (M^+). The ^{1}H and ^{13}C NMR data are in good accord with those previously reported in the literature.[69]

Ethyl 3-phenyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraenoate 11. A mixture of triflate **8** (103 mg, 0.22 mmol), phenylboronic acid (42 mg, 0.32 mmol), $Pd(PhCN)_2Cl_2$ (8.4 mg, 0.22 mmol), $AsPh_3$ (27 mg, 0.088 mmol) and Ag_2O (102 mg, 0.44 mmol) was placed in a flask that was then flushed with argon. This mixture was then dissolved in THF (2.0 mL, containing two drops of water). The reaction was stirred at room temperature for 2.5 h, 20 mL of ether was then added, and the solid material was removed by filtration. The ether

solution was washed with 15 mL of water and dried over $MgSO_4$. Following concentration the residue was purified by flash chromatography (hexanes/ethyl acetate 20:1, R_f 0.28), and ester **11** was obtained as a pale yellow oil (76 mg, 86%). The identity, and in particular the stereochemistry, of this ester was confirmed by the similarity of its ^{1}H NMR spectrum to the previously prepared ester **4b**.[23] ^{1}H NMR: δ 1.07 (t, J = 3.9 Hz, 3H), 1.53 (s, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.00–2.07 (m, 10H), 2.47 (t, 2H), 4.00 (q, J = 3.9 Hz, 2H), 5.09 (b, 3H), 5.88 (s, 1H), 7.15 (d, J = 7.2 Hz, 2H), 7.34 (m, 3H); ^{13}C NMR δ 13.94, 16.01, 16.07, 17.70, 25.72, 25.88, 26.53, 26.75, 39.66, 39.72, 40.46, 59.74, 117.31, 122.68, 124.08, 124.33, 127.11, 127.54, 127.79, 128.44, 128.64, 128.73, 131.29, 133.71, 135.04, 159.36, 166.05. MS-EI: 394 (M^+).

3-Phenyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraen-1-ol 12. A solution of ester **11** (70 mg, 0.18 mmol) in 1 mL of toluene was treated with DIBAL-H (1.0 M solution in toluene, 0.44 mL, 0.44 mmol) under argon at −78 °C for 1 h. The reaction was warmed to room temperature and quenched with 20 mL of Rochelle salt solution. The aqueous solution was extracted with ethyl acetate (2 × 20 mL), and the combined organic layers were washed with saturated NaCl (2 × 20 mL), and then dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by flash chromatography (hexanes/ethyl acetate, 4:1) to give 44 mg (67%) of alcohol **12**. ^{1}H NMR: δ 1.51 (s, 3H), 1.59 (s, 6H), 1.68 (s, 3H), 2.04 (m, 10H), 2.41 (t, 2H), 4.05 (t, 2H), 5.01 (m, 3H), 5.69 (t, 1H), 7.13 (d, J = 6.9 Hz, 2H), 7.31 (m, 3H); ^{13}C NMR: δ 16.02, 17.69, 25.70, 26.49, 26.61, 26.75, 39.00, 39.66, 39.71, 60.29, 123.50, 124.17, 124.37, 125..59, 127.05, 128.06, 128.18, 131.29, 135.56, 139.92, 144.58. MS-EI: 352 (M^+).

Ethyl 3-cyclopropyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraenoate 14. To a solution of cyclopropyl bromide (0.36 mmol, 0.03 mL) in 1.0 mL ether was added *tert*-butyllithium (1.7 M in pentane, 0.72 mmol, 0.42 mL) under argon at −78 °C. The resulting solution was stirred for 30 min, transferred to a slurry of CuCN (0.36 mmol, 32 mg) in 1.0 mL of ether at −78 °C and then stirred for 15 min. Triflate **8** (0.24 mmol, 114 mg) in 1.0 mL of ether was added to the mixture and the reaction was stirred for 1.5 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, the aqueous layer was extracted with ether (3 × 15 mL), and the combined organic layers were dried over $MgSO_4$. Concentration followed by flash chromatography (hexanes/ethyl acetate, 20:1) gave 43 mg (51%) of ester **14** as a colorless oil and 15 mg of triflate **16**. The identity, and in particular the stereochemistry, of **14** was confirmed by the similarity of its ^{1}H NMR spectrum to the previously prepared 3-cyclopropyl-3-desmethylfarnesyl ester.[24] ^{1}H NMR: δ 0.70 (m, 2H), 0.88 (m, 2H), 1.29 (t, 3H), 1.60 (s, 6H), 1.68 (s, 6H), 1.9–2.2 (m, 12H), 3.13 (m, 1H), 4.16 (q, 2H), 5.09 (m, 3H), 5.72 (s, 1H); ^{13}C NMR: δ 6.75, 13.09, 14.37, 16.06, 17.67, 19.21, 31.02, 39.68, 59.42, 115.62, 121.58, 122.85, 124.04, 124.33, 131.29, 136.19, 164.02, 167.35.

3-Cyclopropyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraen-1-ol 15. To a solution of **14** (0.12 mmol, 43 mg) in 1.0 mL of toluene (HPLC grade, dried over 4 Å sieves), at −78 °C under argon, was added DIBALH (1.0 M in toluene, 0.3 mmol, 0.3 mL). After 1 h at −78 °C, the reaction was quenched with Rochelle salt solution (10 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 (hexanes/ethyl acetate, 4:1) and 23 mg (61%) of **15** was obtained as a colorless oil. ^{1}H NMR: δ 0.50 (m, 2H), 0.67 (m, 2H), 1.60 (s, 9H), 1.68 (s, 3H), 1.76 (m, 1H), 1.9–2.1 (m, 12H), 4.33 (d, 2H), 5.12 (m, 3H), 5.48 (t, 1H); ^{13}C NMR: δ 4.95, 11.58, 16.03, 17.69, 25.71, 26.60, 27.24, 27.76, 32.97, 39.71, 59.23, 123.32, 124.14, 124.36, 124.29, 131.29, 134.98, 135.40, 143.62. EI-MS: 316 (M^+).

Ethyl 3-*tert*-butyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraenoate 17. In a flame dried, argon flushed flask were placed CuCN (0.40 mmol, 36 mg) and 1.0 mL of ether (distilled from Na/benzophenone). The resulting slurry was cooled to −78 °C where *tert*-butyllithium (1.7 M in pentane, 0.80 mmol, 0.47 mL) was added dropwise. The mixture was warmed to 0 °C and then recooled to −78 °C. A solution of triflate **8** (0.27 mmol, 124 mg) 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, the aqueous layer was extracted with ether (3 × 15 mL), and the combined organic layers were dried over $MgSO_4$. Concentration followed by flash chromatography (hexanes/ethyl acetate, 20:1) gave **17** as a colorless oil (81 mg, 82%). The identity, and in particular the stereochemistry, of this ester was confirmed by the similarity of its ^{1}H NMR spectrum to the previously prepared 3-*tert*-butyl-3-desmethylfarnesyl ester.[24] ^{1}H NMR: δ 1.22 (s, 9H), 1.32 (t, 3H), 1.60 (s, 9H), 1.68 (s, 3H), 1.9–2.3 (m, 12H), 4.16 (q, 2H), 5.11 (m, 3H), 5.59 (s, 1H); ^{13}C NMR: δ 14.14, 16.00, 17.69, 25.68, 26.58, 26.75, 27.94, 28.76, 29.37, 35.45, 39.24, 39.68, 60.16, 115.80, 123.31, 124.11, 124.25, 124.33, 135.00, 135.85, 161.41. MS-EI: 374 (M^+).

3-*tert*-Butyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraene-1-ol 18. To a solution of **17** (0.21 mmol, 79 mg) in 1.0 mL of toluene (HPLC grade, dried over 4 Å sieves) at −78 °C under argon, was added DIBALH (1.0 M in toluene, 0.53 mmol, 0.53 mL). After 1 h at-78 °C, the reaction was quenched with Rochelle salt solution (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 (hexanes/ethyl acetate, 4:1) and 59 mg (85%) of alcohol **18** was obtained as a colorless oil. ^{1}H NMR: δ 1.14 (s, 9H), 1.60 (s, 9H), 1.68 (s, 3H), 2.0–2.2 (m, 12H), 4.37 (d, 2H), 5.12 (m, 3H), 5.25 (t, 1H). ^{13}C NMR: δ 16.00, 16.08, 17.68, 25.69, 26.59,

26.75, 29.04, 29.22, 30.66, 35.81, 35.87, 39.69, 60.58, 124.08, 124.17, 124.36, 125.21, 131.26, 134.98, 135.16, 148.88. EI–MS: 332 (M+).

General procedure for preparation of chlorides

In a flame-dried, round-bottomed flask were placed with *N*-chlorosuccinimide (1.2 equiv) and dichloromethane (distilled from CaH_2). The solution was cooled to −30 °C in an acetonitrile/dry ice bath. Dimethyl sulfide (1.5 equiv) was added dropwise to the cold solution, and the resulting milky white mixture was warmed to 0 °C for 5 min and recooled to −30 °C. A solution of 1 equiv of the alcohol in 1 mL of dichloromethane was added dropwise to the mixture at −30 °C. The reaction was slowly warmed up to 0 °C and stirred for an additional hour at that temperature. The resulting clear, colorless solution was stirred at room temperature for 20 min and poured into 10 mL of cold brine solution. The aqueous layer was extracted with 2 × 15 mL hexanes, and the combined organic layers were washed with 10 mL of cold brine solution and dried over $MgSO_4$. Concentration (rotary evaporation followed by high vacuum at room temperature) afforded the chlorides as colorless or pale yellow oils which were used directly for the next reaction.

General procedure for preparation of diphosphates

In a flame-dried, round-bottom flask were placed two equivalents of tris(tetra-*n*-butylammonium)hydrogen pyrophosphate[60,61] and 1.0 mL of acetonitrile (distilled from P_2O_5). The mixture was cooled to 0 °C and 1 equiv of chloride in 0.5 mL of acetonitrile was added dropwise. The reaction was allowed to stir at room temperature for 2 h, and the solvent was removed by rotary evaporation at room temperature. The residue was dissolved in 1–2 mL of ion exchange buffer (1:49 v/v isopropyl alcohol and 25 mM NH_4HCO_3) and was passed through a column containing 3–10 mL cation-exchange resin (DOWEX AG 50W-X8, NH_4^+ form). The column was eluted with two column volumes of ion exchange buffer at a flow rate of ∼1 mL/min. The eluent was dried by lyophilization and a pale-yellow solid was obtained. The crude product was dissolved in 1–3 mL of 25 mM NH_4HCO_3 and purified by reverse-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 aq NH_4HCO_3, pH 8.0; B: CH_3CN; Vydac pH-stable C8 4.6 × 250 mm columm; flow rate: 1.0 mL; UV monitoring at 214 and 254 nm). The fractions were collected, pooled and dried by lyophilization, and the diphosphates were obtained as white fluffy solids. Occasionally, the diphosphates were obtained as mixed ammonium/tetrabutylammonium salts; the presence of the tetrabutylammonium cation was indicated by characteristic peaks at 0.93, 1.34, and 3.17 ppm in the proton NMR spectrum. However, the diphosphates were always purified by reversed phase HPLC, their identity was confirmed by analytical reverse-phase HPLC, ^{1}H NMR, ^{31}P NMR, and in certain cases the purity was confirmed quantitative phosphate analysis.[27a]

3-Phenyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraene diphosphate 2d. Alcohol **12** (40 mg, 0.11 mmol) was treated with *N*-chlorosuccinimide (20 mg, 0.15 mmol) and dimethyl sulfide (0.013 mL, 0.17 mmol) in 2.0 mL of methylene chloride. Following the general procedure, 26 mg (62%) of allylic chloride **13** was obtained as a colorless oil. ^{1}H NMR: 1.51 (s, 3H), 1.59 (s, 6H), 1.68 (s, 3H), 1.9–2.1 (m, 10H), 2.42 (t, 2H), 3.97 (d, 2H), 5.09 (m, 3H), 5.69 (t, 1H), 7.20 (d, 2H), 7.36 (m, 3H); ^{13}C NMR: 16.05, 17.68, 25.70, 26.20, 26.59, 26.75, 27.97, 38.97, 39.67, 39.71, 42.38, 122.39, 123.17, 124.16, 124.33, 124.95, 126.59, 127.37, 128.02, 128.29, 131.29, 134.98, 135.78, 139.12, 146.64.

Chloride **13** (47 mg, 0.084 mmol) was treated with tris (tetra-*n*-butylammonium) hydrogen pyrophosphate (151 mg, 0.17 mmol) in 1.0 mL of CH_3CN for 2 h at room temperature. The resulting material was converted to ammonium form by ion exchange with 3 mL of resin and 8 mL of ion exchange buffer. After lyophilization, the residue was purified by reverse-phase HPLC as described in the general procedure. 3-Phenyl GGPP **2d** (32 mg, 68%) was obtained as a white fluffy solid. ^{1}H NMR: 1.33 (br, 9H), 1.42 (br, 3H), 1.7–2.1 (br, 10H), 2.39 (br, 2H), 4.43 (br, 2H), ∼5.0 (m, 3H, overlaps with HDO peak), 5.83 (br t, 1H), 7.22 (br d, 2H), 7.38 (m, 3H); ^{31}P NMR: −5.8 (d, J = 18 Hz), −9.8 (d, J = 18 Hz).

3-Cyclopropyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraene diphosphate 2b. Cyclopropyl alcohol **15** (18 mg, 0.06 mmol) was treated with *N*-chlorosuccinimide (16 mg, 0.11 mmol) and dimethyl sulfide (0.009 mL, 0.12 mmol) in 1 mL of methylene chloride. Following workup as described in the general procedure, 12 mg (63%) of allylic chloride **16** was obtained as a colorless oil.

The chloride **16** (12 mg, 0.04 mmol) was treated with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (65 mg, 0.07 mmol) in 0.5 mL of CH_3CN for 2 h at room temperature. The resulting material was converted to ammonium form by ion exchange with 3 mL of resin and 8 mL of ion exchange buffer. After lyophilization, the residue was purified by reverse-phase HPLC as described in the general procedure, and 3-cyclopropyl GGPP **2b** (14 mg, 74%) was obtained as a white fluffy solid. ^{1}H NMR: 0.49 (m, 2H), 0.72 (m, 2H), 1.60 (s, 9H), 1.67 (s, 3H), 1.86 (m, 1H), 2.0–2.2 (m, 12H), 4.66 (br, 2H), 5.17 (m, 3H), 5.53 (m, 1H); ^{31}P NMR −6.25 (d, J = 16 Hz), −9.92 (d, J = 16 Hz).

3-*tert*-Butyl-7,11,15-trimethylhexadeca-2*Z*,6*E*,10*E*,14-tetraene diphosphate 2c. Alcohol **18** (22 mg, 0.07 mmol) was treated with *N*-chlorosuccinimide (18 mg, 0.13 mmol) and dimethyl sulfide (0.010 mL, 0.13 mmol) in 1.0 mL of methylene chloride. Following workup as described in the general procedure, 20 mg (86%) of chloride **19** was obtained as a colorless oil.

Chloride **19** (20 mg, 0.06 mmol) was treated with tris (tetra-*n*-butylammonium) hydrogen pyrophosphate salt (103 mg, 0.11 mmol) in 0.5 mL of CH_3CN for 2 h at room temperature. The resulting material was converted

to ammonium form by ion exchange with 3 mL of resin and 8 mL of ion exchange buffer. After lyophilization, the residue was purified by reverse-phase HPLC as described in the general procedure. 3-*tert*-Butyl GGPP **2c** (20 mg, 65%) was obtained as a white fluffy solid. ^{1}H NMR: 1.15 (s, 9H), 1.63 (m, 12H), 2.07 (m, 12H), 4.70 (partially buried under HDO peak), 5.15 (b, 1H), 5.33 (m, 3H); ^{31}P NMR −7.63 (d, $J=20$ Hz), −10.27 (d, $J=20$ Hz).

General procedure for preparation of bromides

In a flame-dried, argon flushed round-bottom flask was first placed one equivalent of the alcohol and then 0.50 mL of dichloromethane (distilled from CaH_2).[64] Carbon tetrabromide (1.2 equiv) was then added first followed by triphenylphosphine (1.1 equiv) while stirring. The reaction was allowed to stir at room temperature for 2 h under argon and quenched by the addition of 25 mL of pentane. The milky white solution was filtered through a plug of Celite and the excess pentane was removed with a gentle stream of nitrogen. Three additional 25 mL portions of pentane were added followed by filtration and removal of excess solvent. Concentration (rotary evaporation) afforded the bromides as pale-yellow oils that were used directly for the next reaction.

General procedure for preparation of geranylgeranylated pentapeptides

In a flame-dried, argon flushed round-bottom flask were placed 1 equiv of dansyl-GCIIL (**22**, 5.8–6.3 mg) and 0.35 mL of anhydrous dimethylformamide.[65] The bromide (0.82 equiv) followed by diisopropylethylamine (3.0 equiv) were added and the reaction mixture was stirred at room temperature for 2 h under nitrogen. Water (5.0 mL) was added, and the solution was frozen and lyophilized. The crude product was dissolved in methanol/chloroform 1:5 and purified by preparative silica TLC (methanol/chloroform, 1:5; R_f 0.57). The solvent was removed by rotary evaporation to afford the geranylgeranylated pentapeptides as a pale-yellow powder.

Dansyl-Gly-Cys$^{3\text{-vGG}}$-Ile-Ile-Leu (23). 3-Vinyl-3-desmethylgeranylgeraniol (4.9 mg, 0.016 mmol) was treated with carbon tetrabromide (6.5 mg, 0.020 mmol) and triphenylphosphine (4.7 mg, 0.018 mmol) in 0.50 mL of dichloromethane. Following the general procedure, 6.0 mg (65% by ^{1}H NMR) of the allylic bromide **20** was obtained as a pale-yellow oil that was used directly in the next step.

Bromide **20** (2.5 mg, 0.007 mmol) was then treated with dansyl-GCIIL (**22**; 6.3 mg, 0.008 mmol) and diisopropylethylamine (0.005 mL, 0.255 mmol) in 0.35 mL dimethylformamide. Following the general procedure and TLC purification described above, 7 mg (78%) of dansyl-Gly-Cys$^{3\text{-vGG}}$-Ile-Ile-Leu **23** was obtained as a pale-yellow solid. ^{1}H NMR: (500 MHz, DMSO) δ 0.75–0.88 (m, 18H), 0.98–1.73 (m, 9H), 1.53 (s, 3H), 1.54 (s, 6H), 1.62 (s, 3H), 1.89–2.18 (m, 12H), 2.42 (dd, $J=7.8$ 7.8 Hz, 1H), 2.64 (dd, $J=5.9$, 5.4 Hz, 1H), 2.82 (s, 6H), 3.25 (d, $J=7.8$ Hz, 2H), 3.51 (s, 2H), 4.20 (m, 3H), 4.47 (q, $J=7.8$ Hz, 1H), 5.08 (m, 4H), 5.26 (d, $J=17.1$ Hz, 1H), 5.38 (t, $J=8.2$ Hz, 1H), 6.62 (dd, $J=11.2$, 11.2 Hz, 1H), 7.25 (d, $J=7.3$, 1H), 7.57 (dd, $J=5.3$, 5.3 Hz, 1H), 7.59 (dd, $J=4.8$, 4.8 Hz, 1H), 7.79 (d, $J=9.3$ Hz, 1H), 7.98 (d, $J=8.8$ Hz, 1H), 8.03 (d, $J=8.3$ Hz, 1H), 8.06 (bd, $J=7.3$ Hz, 1H), 8.12 (d, $J=7.3$ Hz, 1H), 8.29 (d, $J=8.8$ Hz, 2H), 8.45 (d, $J=8.8$ Hz, 1H). MS (FAB): 1033 (M^-).

Dansyl-Gly-Cys$^{3\text{-tbGG}}$-Ile-Ile-Leu (24). Alcohol **18** (5.3 mg, 0.016 mmol) was treated with carbon tetrabromide (6.4 mg, 0.020 mmol) and triphenylphosphine (4.6 mg, 0.018 mmol) in 0.50 mL of dichloromethane. Following the general procedure, 6.3 mg (62% by ^{1}H NMR) of the allylic bromide **21** was obtained as a pale-yellow oil that was used directly in the next step.

Bromide **21** (2.5 mg, 0.006 mmol) was then treated with dansyl-GCIIL (**22**; 5.8 mg, 0.008 mmol) and diisopropylethylamine (0.005 mL, 0.232 mmol) in 0.35 mL dimethylformamide. Following the general procedure and TLC purification described above, 5 mg (75%) of dansyl-Gly-Cys$^{3\text{-tbGG}}$-Ile-Ile-Leu **24** was obtained as a pale-yellow solid. ^{1}H NMR: (500 MHz, DMSO) δ 0.75–0.88 (m, 18H), 1.09 (s, 9H), 0.98–1.73 (m, 9H), 1.54 (s, 6H) 1.55 (s, 3H), 1.62 (s, 3H), 1.87–2.06 (m, 12H), 2.45 (dd, $J=7.8$, 7.8 Hz, 1H), 2.72 (dd, $J=5.9$, 5.4 Hz, 1H), 2.82 (s, 6H), 3.51 (s, 2H), 4.20 (m, 3H), 4.46 (q, $J=8.3$ Hz, 1H), 5.07 (m, 4H), 7.25 (d, $J=6.8$ Hz, 1H), 7.57 (dd, $J=6.4$, 6.4 Hz, 1H), 7.59 (dd, $J=5.9$, 5.9 Hz, 1H), 7.78 (d, $J=8.8$ Hz, 1H), 7.98 (d, $J=8.8$ Hz, 1H), 8.05 (d, $J=8.3$ Hz, 2H), 8.12 (d, $J=7.3$ Hz, 1H), 8.27 (bs, 1H), 8.30 (d, $J=8.8$ Hz, 1H), 8.45 (d, $J=8.3$, 1H). MS (FAB): 1063 (M^-).

Prenyltransferase assays

Recombinant yeast PGGTase I was produced in *Escherichia coli* (JM101/pWGS-1-273B) and was purified by ion-exchange and immunoaffinity chromatography as previously described.[27a] Catalytic rate constants (k_{cat}) were measured using a fluorescence assay that continuously monitored geranylgeranylation of the dansylated pentapeptide dansyl-GCIIL[27a] using a Spex FluoroMax spectrofluorimeter. Excitation and emission wavelengths used were 340 and 486 nm, respectively, with a bandpass of 5.1 nm for both excitation and emission monochromators. Assays were carried out at 30 °C in prewarmed cuvettes (3 mm^2) using a thermostated cuvette holder. Assays (220 μL) were conducted in a final buffer concentration of 50 mM Tris–HCl, 1.0 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM dithiothreitol, 0.020% *n*-dodecyl β-D-maltoside, pH 7.5. Reaction mixture were preincubated at 30 °C for 5 min before the reaction was initiated with PGGTase I previously diluted with assay buffer to the appropriate concentration. Initial rates were measured from the linear region of each run, and all measurements were made in duplicates or triplicates. The rate of change in fluorescence intensity (cps s^{-1}) was converted to units of velocity (μM s^{-1}) with the previously determined conversion factor ($m=3.6\times10^6$ cps μM^{-1}) and fluorescence enhancement factor ($E=10$).[27a]

Inhibition studies were conducted at varying concentrations of GGPP (2.5–15 μM), fixed concentrations of dansyl-GCIIL (4 μM, approximately K_m), and varying concentrations of the GGPP analogue (3-cpGGPP (2.0–8.0 μM), 3-vGGPP (3.0–12.0 μM), 3-tbGGPP (2.0–8.0 μM), and 3-PhGGPP (3.0–12.0 μM). The reactions were initiated with 30 nM enzyme. Data were analyzed as double-reciprocal plots. K_i values were determined with the Michaelis–Menten equation including an inhibition term in the denominator for competitive inhibition.

Michaelis constants for 3-cpGGPP and 3-vGGPP were determined at a fixed concentration of the second substrate (dansyl-GCIIL). Reactions were initiated with 70–190 nM enzyme. K_m values were determined by fitting hyperbolic plots of initial velocities versus substrate concentration. The k_{cat} values were determined at saturating concentrations of the substrate 3-cpGGPP (75–80 μM) and 3-vGGPP (50–80 μM).

Analysis of prenylated peptides from enzymatic reactions by HPLC

Reaction mixtures were chromatographed on a 250 × 4.6 mm C_{18} column (Vydac™ or Phenomenex©) at a flow rate of 1.0 mL/min with detection by UV absorbance at 214 nm. Four individual reactions containing GGPP analogue **2b** (44–66 nmol), dansyl-GCIIL (8–24 nmol) and PGGTase I (10–35 μg) in assay buffer (50 mM Tris–HCl, 1.0 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM dithiothreitol, 0.020% *n*-dodecyl β-D-maltoside, pH 7.5), were incubated at 30 °C for 5 h. PGGTase I was added in 10 μg batches over the 5 h period. Compounds were eluted with a gradient of 30% solvent B (CH_3CN/0.025% TFA) and 70% solvent A (H_2O/0.025% TFA) to 100% B over 20 min. The prenylated peptide eluted at 100% B. The peaks from the individual reactions were collected and pooled, and the solvent was removed under reduced pressure. The residue was analyzed by negative ion FABMS.

In a second set of reactions, GGPP analogue **2a** (18 nmol) or **2c** (49–148 nmol), dansyl-GCIIL (11-33 nmol) and PGGTase I (10 μg) in assay buffer, were incubated at 30 °C for 12 h. These samples were coinjected with dansyl-Gly-Cys$^{3\text{-vGG}}$-Ile-Ile-Leu (3 nmol) and dansyl-Gly-Cys$^{3\text{-tbGG}}$-Ile-Ile-Leu (6 nmol) which were chemically synthesized and characterized as previously described. A similar HPLC gradient was used, except the solvents contained 0.10% TFA. The prenylated peptides eluted at 100% B.

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