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Synthesis and Evaluation of GGPP Geometric Isomers: Divergent Substrate Specificities of FTase and GGTase I

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Abstract—A stereocontrolled synthetic route has been used to prepare two of the geometric isomers of all-*trans*-GGPP. Neither of these isomers is effective substrates for mammalian GGTase I, but **3** is a potent inhibitor of this enzyme ($IC_{50} = 100$ nM). Surprisingly, both compounds are effective substrates for mammalian FTase.

Protein prenylation is a common post-translational modification, which plays a key role in a variety of signal transduction pathways. There are three different protein prenylation motifs—farnesylation, geranylgeranylation, and bis-geranylgeranylation. The first modification is carried out by an enzyme, protein-farnesyl transferase (FTase), which recognizes the CAAX box (where X = Ser or Met) at the carboxyl terminus of a protein substrate and then attaches the farnesyl group from farnesyl diphosphate (FPP) to the free sulfhydryl of the cysteine residue.[1–3] The second, closely related enzyme protein-geranylgeranyl transferase I (GGTase I) attaches a geranylgeranyl moiety from geranylgeranyl diphosphate (GGPP) to a cysteine in a similar CAAX box, where leucine is the carboxyl terminal residue. Initial studies demonstrated that the key signal transduction protein and oncogene product Ras is farnesylated. The intense biological interest in protein farnesylation has culminated recently in the introduction of FTase inhibitors into human clinical trials as anticancer agents.[3]

The mammalian form of GGTase I has been cloned and overexpressed,[4] and subjected to detailed kinetic and biochemical characterization. It is a heterodimer that is highly homologous to FTase, and in fact shares the same α subunit with FTase. GGTase I has received less attention than FTase, due to the demonstrated anticancer potential of FTase inhibitors. This is in spite of the fact that geranylgeranylation of proteins is more common than farnesylation.[1,2] However, recent biological studies on protein geranylgeranylation have resulted in increased interest in this enzyme. In fact, GGTase I inhibitors have recently exhibited potent anticancer activity in model systems.[5] Despite this, relatively few studies have been published on GGTase I inhibitors.[6] The comparative study of the prenyl diphosphate specificities of FTase and GGTase I is also of intrinsic interest due to the fact that GGTase I exhibits a much higher specificity for GGPP than FTase does for FPP.[7] This indicates that it may have a more restrictive prenyl diphosphate binding site.

Recently, we have synthesized the three geometric isomers of *E,E*-FPP via a novel stereocontrolled synthetic route.[8] Preliminary evaluation of these isomers versus mammalian FTase demonstrated that isomers **1** and **2** (Fig. 1) were excellent alternative substrates for FTase.[8a] In view of the increasing interest in GGTase I and its inhibitors, we have now synthesized the corresponding GGPP isomers **3** and **4**, and evaluated them versus both GGTase I and FTase.

Over the past several years, we have developed a novel and efficient vinyl triflate-based synthetic route leading to a wide variety of isoprenoids, in particular 3-substituted farnesyl and geranylgeranyl analogues.[9] This route was adapted for the synthesis of the unnatural *Z*-isomers of farnesol,[8] and has now been utilized for the synthesis of two *Z*-isomers of GGPP. The *Z,E,E*-isomer of GGPP was prepared starting from the previously described geranylgeranyl β-ketoester **5**, which we have

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used to prepare other GGPP analogues (Scheme 1).[9b,d,f] The key step in the synthesis is the completely stereoselective conversion of the β-ketoester **5** into the *E*-triflate **6**. This stereochemical outcome is readily achieved simply by using DMF as the solvent in place of the standard solvent THF, as previously described for the synthesis of *Z,E*-farnesol.[8] Methylation of the triflate via a Stille reaction afforded the ester **7**, which was then reduced to *Z,E,E*-geranylgeraniol **8**. Alcohol **8** was diphosphorylated in two steps, following the protocol of Poulter and co-workers,[10] to give the desired GGPP analogue **3**. The yields of the synthetic steps are modest in many cases; however, note that they are unoptimized and in most cases the reported yield is from a single reaction attempt.

The *E,Z,E*-GGPP isomer **4** was synthesized in six steps, starting from *Z,E*-farnesyl bromide **9**.[8] Again, the same synthetic procedures used for the preparation of the corresponding FPP isomer **2** were used in the synthesis of **4**. Coupling of **9** with the dianion of ethyl acetoacetate afforded the β-ketoester **10**. Previously, we have demonstrated that this type of alkylation reaction proceeds with retention of geometry at the allylic double bond,[8b] and there was no indication of isomerization in this case. Triflation of **10** using THF as the reaction solvent afforded the *Z*-triflate **11**. Stille methylation of **11**, followed by reduction of the resulting ester **12**, gave the desired *E,Z,E*-geranylgeraniol isomer **13**. Diphosphorylation of **13** gave the desired GGPP isomer **4**. Note that the stereochemistry of the intermediates in Schemes 1 and 2 were confirmed based on comparisons of their NMR spectra to the corresponding farnesyl derivatives.[8]

Isomers **3** and **4** were first evaluated as potential substrates for recombinant mammalian GGTase I, employing the peptide cosubstrate dansyl-Gly-Cys-Val-Leu-Leu in a spectrofluorometric assay as previously described.[11] Neither compound was a substrate for GGTase I under these conditions. Instead, these compounds are potent inhibitors of GGTase I. Indeed, **3** is the most potent

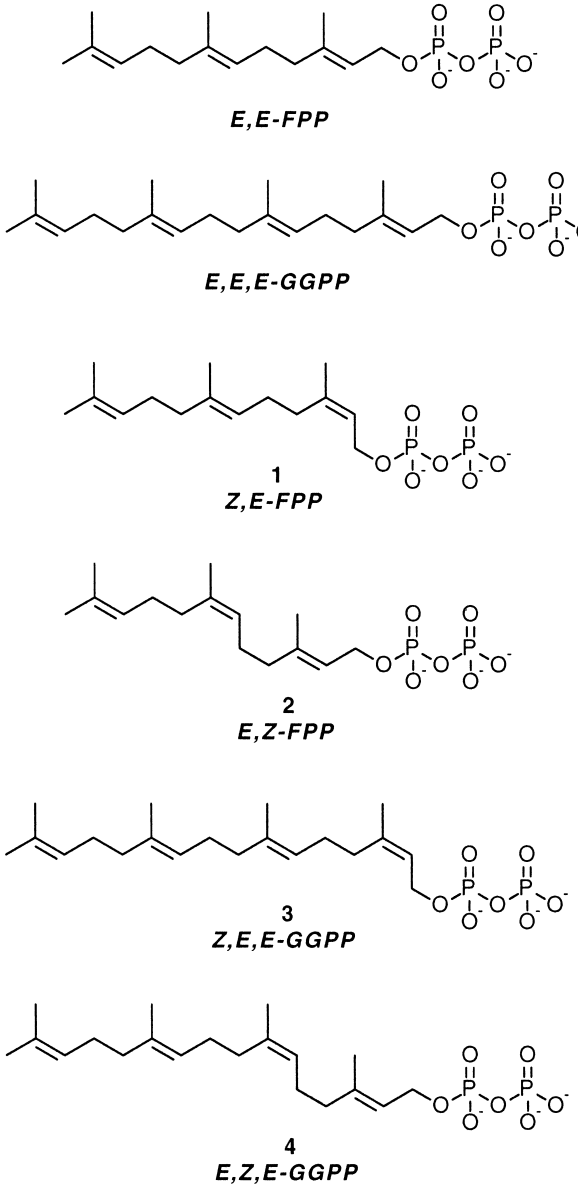
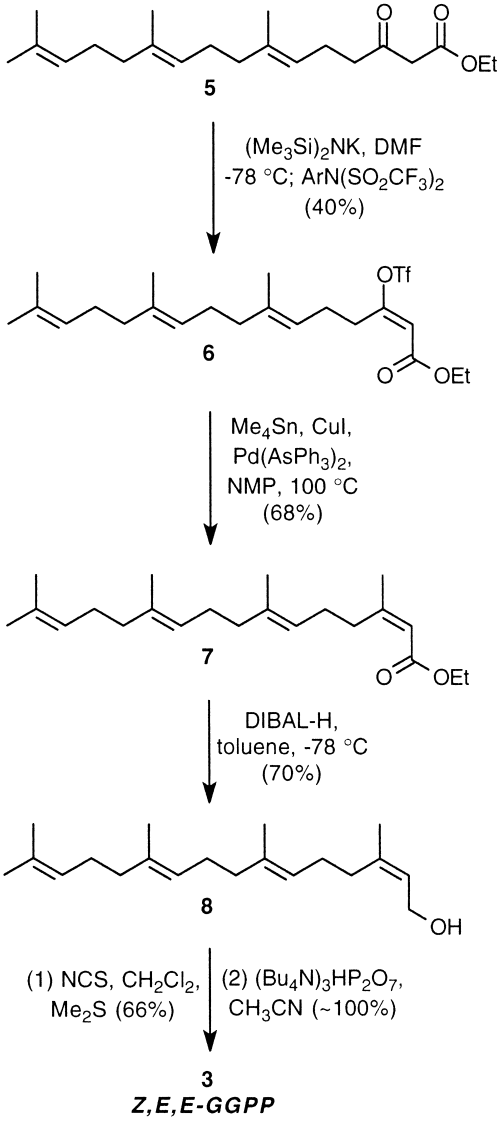


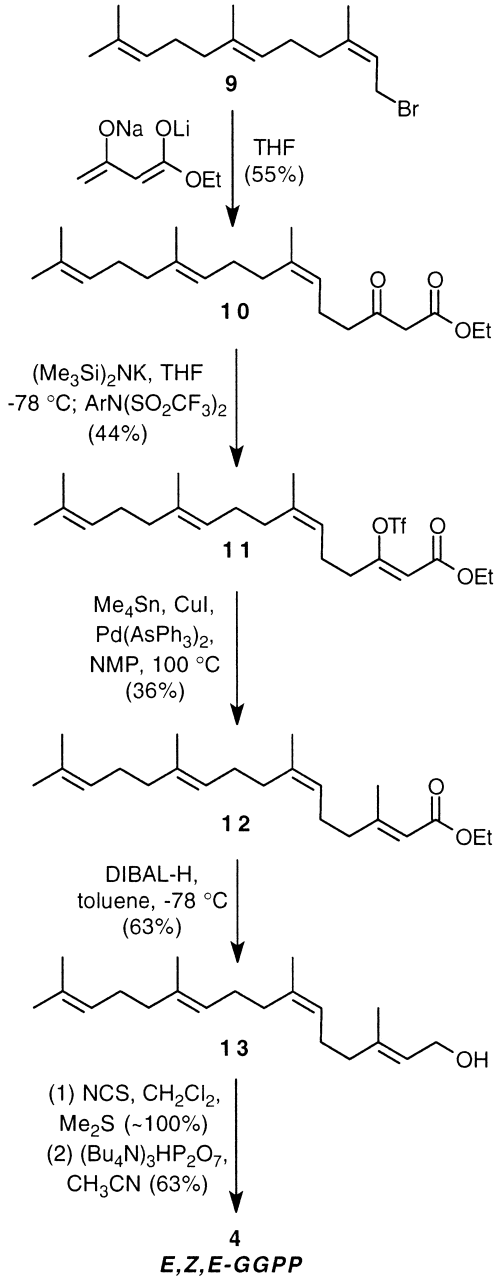
Figure 1. Natural substrates for FTase and GGTase I, and the isomeric derivatives evaluated in this study.



Scheme 1. Synthesis of 2*Z*,6*E*,10*E*-GGPP **3**.

isoprenoid-based inhibitor of GGTase I yet described, with an IC_{50} of 100 nM.

The relaxed substrate specificity of mammalian FTase, illustrated by its ability to effectively utilize **1** and **2**, suggested that **3** and **4** might bind to FTase. Thus, **3** and **4** were evaluated as potential substrates for FTase, using a continuous spectrofluorometric assay.[12] Surprisingly, both of these GGPP isomers are very effective substrates for FTase, despite the fact that the natural *E,E,E*-GGPP isomer is a very poor substrate for FTase. The *Z,E,E*-isomer **3** is a particularly good substrate, with K_m and V_{max} comparable to the natural substrate (Fig. 2). The ability of **3** to serve as a substrate for mFTase was confirmed by HPLC isolation of the prenylated peptide product (Table 1).[13]



Scheme 2. Synthesis of 2*E*,6*Z*,10*E*-GGPP **4**.

Mammalian (and yeast) FTase and GGTase I possess a high degree of sequence similarity, and share an identical α subunit. However, GGTase I possesses a significantly higher specificity for its isoprenoid substrate than does FTase. Thus, there appear to be significant differences between the isoprenoid binding sites of these two enzymes. This is consistent with the fact that mammalian[9d] and yeast[9f] GGTase I bind more weakly to 3-substituted GGPP analogues than would be expected based on the interaction of the corresponding FPP analogues with mammalian and yeast FTase.[9a,c,d]

Previous studies have shown that GGPP binds very tightly to FTase, but is a very poor substrate for this enzyme. Based on the structure of the mFTase–FPP complex, Long et al. have proposed that FPP and GGPP bind to the isoprenoid site of FTase in the same extended conformation.[15] If this is the case, then the C_1

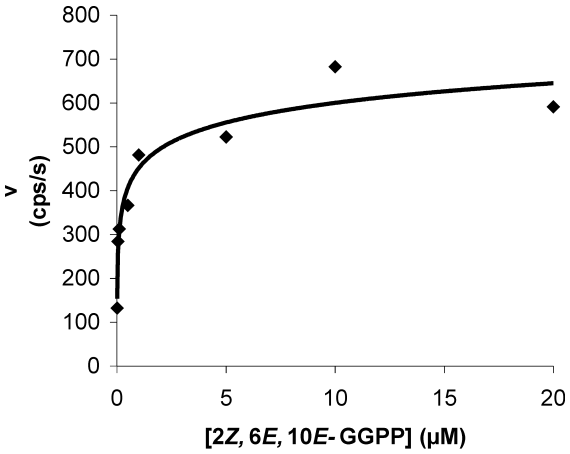


Figure 2. v/S Plot for the FTase-catalyzed coupling of **3** and dansyl-Gly-Cys-Val-Leu-Ser.

Table 1. Enzymatic evaluation of FPP and GGPP isomers as substrates or inhibitors of FTase and GGTase I

Compounds	FTase[a] K_m (nM)	FTase k_{rel}[b]	GGTase I IC_{50} (nM)[c]
1	225	0.47	nd
2	136	0.41	nd
3	21	0.67	100
4	481	0.40	340

[a]Values were determined using a fluorescence assay as previously described in refs 8a and 9e (nd = not determined). The values shown for **1** and **2** are those previously reported.[8a] Note that the concentrations of all diphosphates were verified by phosphate analysis.[12b] Recombinant mFTase and mGGTase I were expressed in baculovirus and purified as previously described.[14]

[b]Relative V_{max} determined for each analogue, compared to the V_{max} determined for *E,E*-FPP under the same assay conditions.

[c]A modified version of the continuous spectrofluorometric assay was utilized.[11] Briefly, dansyl-GCVLL (0.8 μM) was employed as a peptide substrate, 2*E*,6*E*,10*E*-GGPP (~15 mM stock solution in 25 mM ammonium bicarbonate, pH 7.5; final concentration 10 μM)[d] was added to the assay buffer solution [50 mM Tris, pH 7.5, 1 mM DTT, 5 mM $MgCl_2$, 50 μM $ZnCl_2$ with added detergent solution (0.2% octyl-β-D-glucoside)]. The reaction was initiated with the addition of mGGTase I. The change in fluorescence was detected using a time-based scan at 30 °C for a period of 300 s (excitation wavelength = 350 nm; emission wavelength = 486 nm), and the reaction velocity was obtained as cps/s.

carbon of GGPP is not in a position to react with the sulfhydryl of the bound peptide cosubstrate. Thus, **3** and **4** presumably are able to adopt more folded conformations in the FTase active site such that they can act as substrates. Our previous work with certain farnesol and geranylgeraniol analogues implies that **8** could be converted to **3** in cells.[9d] Therefore, it is possible that this compound could *simultaneously* block the prenylation of normally geranylgeranylated proteins, and lead to the abnormal geranylgeranylation of normally farnesylated proteins. Thus, alcohol **8** may be a unique tool to investigate the biological effects of these two related post-translational modifications.

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References and Notes

1. (a) Fu, H. W.; Casey, P. J. *Rec. Prog. Horm. Res.* **1999**, *54*, 315. (b) Leonard, D. M. *J. Med. Chem.* **1997**, *40*, 2971.
2. Gibbs, R. A.; Zahn, T. J.; Sebolt-Leopold, J. S. *Curr. Med. Chem.* **2001**, *8*, in press.
3. Rowinsky, E. K.; Windle, J. J.; VonHoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631.
4. Zhang, F. L.; Diehl, R. E.; Kohl, N. E.; Gibbs, J. B.; Giros, B.; Casey, P. J.; Omer, C. A. *J. Biol. Chem.* **1994**, *269*, 3175.
5. (a) Sun, J.; Blaskovich, M. A.; Knowles, D.; Qian, Y.; Ohkanada, J.; Bailey, R. D.; Hamilton, A. D.; Sebti, S. M. *Cancer Res.* **1999**, *59*, 4919. (b) GGTase I inhibitors are also of potential benefit in other clinical situations (e.g., cardiovascular disease, osteoporosis); for a review, see: Gibbs, R. A. *Curr. Opin. Drug Discov. Dev.* **2000**, *3*, 585.
6. (a) Macchia, M.; Jannitti, N.; Gervasi, G.; Danesi, R. *J. Med. Chem.* **1996**, *39*, 1352. (b) Vasudevan, A.; Qian, Y.; Vogt, A.; Blaskovich, M. A.; Ohkanada, J.; Sebti, S. M.; Hamilton, A. D. *J. Med. Chem.* **1999**, *42*, 1333. (c) Smalera, I.; Williamson, J. M.; Baginsky, W.; Leting, B.; Mazur, P. *Biochim. Biophys. Acta* **2000**, *1480*, 132.
7. Yokoyama, K.; Zimmerman, K.; Scholten, J.; Gelb, M. H. *J. Biol. Chem.* **1997**, *272*, 3944.
8. (a) Shao, Y.; Eummer, J. T.; Gibbs, R. A. *Org. Lett.* **1999**, *1*, 627. (b) Xie, H.; Shao, Y.; Becker, J. M.; Naider, F.; Gibbs, R. A. *J. Org. Chem.* **2000**, *65*, 8552.
9. (a) Gibbs, R. A.; Krishnan, U.; Dolence, J. M.; Poulter, C. D. *J. Org. Chem.* **1995**, *60*, 7821. (b) Mu, Y. Q.; Gibbs, R. A. *Tetrahedron Lett.* **1995**, *36*, 5669. (c) Mu, Y. Q.; Gibbs, R. A.; Eubanks, L. M.; Poulter, C. D. *J. Org. Chem.* **1996**, *61*, 8010. (d) Gibbs, B. S.; Zahn, T. J.; Mu, Y. Q.; Sebolt-Leopold, J.; Gibbs, R. A. *J. Med. Chem.* **1999**, *42*, 3800. (e) Zahn, T. J.; Weinbaum, C.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1763.
10. Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768.
11. Pickett, W. C.; Zhang, F. L.; Silverstrim, C.; Schow, S. R.; Wick, M. M.; Kerwar, S. S. *Anal. Biochem.* **1995**, *225*, 60.
12. (a) Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945. (b) Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. *Meth. Enzymol.* **1995**, *250*, 30.
13. Briefly, the peptide substrate dansyl-GCVLS (100 μM) and FPP or 2*Z*,6*E*,10*E*-GGPP (150 μM) were added to 1.0 mL of the assay buffer solution [50 mM Tris, pH 7.5, 1 mM DTT, 5 mM $MgCl_2$, 50 μM $ZnCl_2$ with added detergent solution (0.2% octyl-β-D-glucoside)]. The reaction was initiated with 15 μg of mFTase. After a 1 h incubation at 35 °C, the reaction was quenched by addition of an equal volume of CH_3CN, and then analyzed for product by reversed-phase HPLC. Chromatography was performed on a Vydac 4.6×250 mm C_8 column eluted at 1.0 mL/min, and the products were detected by UV at 330 nm. An isocratic elution of 75% A/25% B over 5 min, followed by a gradient of 25% solvent B to 100% B over 40 min was used (A: 0.025% TFA/H_2O; B: 0.020% TFA/CH_3CN). The non-prenylated peptide eluted at 17 min and the prenylated products eluted at 27 min.
14. Moomaw, J. F.; Zhang, F. L.; Casey, P. J. *Meth. Enzymol.* **1995**, *250*, 12.
15. Long, S. B.; Casey, P. J.; Beese, L. S. *Biochemistry* **1998**, *37*, 9612.