# Computational and Conformational Evaluation of FTase Alternative Substrates: Insight into a Novel Enzyme Binding Pocket

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Protein farnesyltransferase (FTase) is an important anticancer drug target. In an effort to develop isoprenoid diphosphate-based FTase inhibitors, striking variations have been observed in the ability of conservatively modified analogues to bind to the enzyme. For example, 2*Z*-GGPP is an alternative substrate with high binding affinity, while GGPP is not an alternative substrate. Using the availability of high-resolution FTase crystal structures, we have used pharmacophore and docking studies to elucidate a new binding pocket for isoprenoid analogues. The unique conformations between the first two isoprene units of 2*Z*-GGPP, but not GGPP, allows 2*Z*-GGPP to exploit this new binding pocket. The discovered conformation allows the molecule to adopt a reactive conformation while placing hydrophobic groups within the predominately hydrophobic binding pocket. This computational finding is supported by NMR studies on $^{13}$C-labeled 2*Z*-farnesol, which confirm that the computationally predicted conformation is also favored in solution. These discoveries suggest that ligand conformational flexibility may be an important design consideration for the development of both inhibitors and alternative substrates of FTase.

## INTRODUCTION

Protein farnesyltransferase (FTase) is a crucial post-translational modification enzyme that catalyzes the transfer of a farnesyl group from farnesyl diphosphate (FPP) to the cysteine in a carboxyl-terminal −Cys-aaX box (where a is any amino acid and X is typically Ser or Met) of many important signal transduction proteins (Figure 1).[1,2] In the case of the important cancer target Ras, the farnesyl moiety provides a hydrophobic anchor that is required in the proper localization of Ras to the plasma membrane and its participation in the MAP kinase cascade. Mutations in Ras account for approximately 30% of all cancers, and this makes FTase an attractive target for chemotherapeutic intervention.[2,3] Therefore, FTase inhibitors (FTIs) have been developed and are under active investigation as anticancer agents.[2−4] While these agents have exhibited some promising results in the clinical arena, they do not act as "anti-Ras" agents as expected.[3,4] It is clear that there is much to learn about the biological consequences of protein prenylation and the potential clinical uses of FTIs.

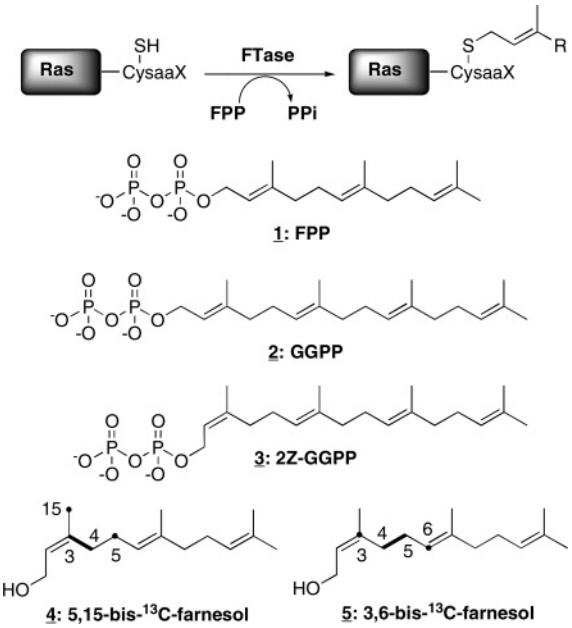


**Figure 1.** The reaction catalyzed by FTase and the structures of FTase ligands and spectroscopic probes investigated in this work.

Our laboratory has a long-standing interest in the development of FPP analogues as potential FTIs.[2] We have found that subtle modifications in the structure of FPP can lead to alternative substrates, potent inhibitors, or compounds that bind poorly to FTase. Recently, our laboratory has explored a variety of geometric isomers of FPP and geranylgeranyl diphosphate (GGPP) to determine their ability to act as alternative substrates and/or inhibitors of FTase. Isomers of

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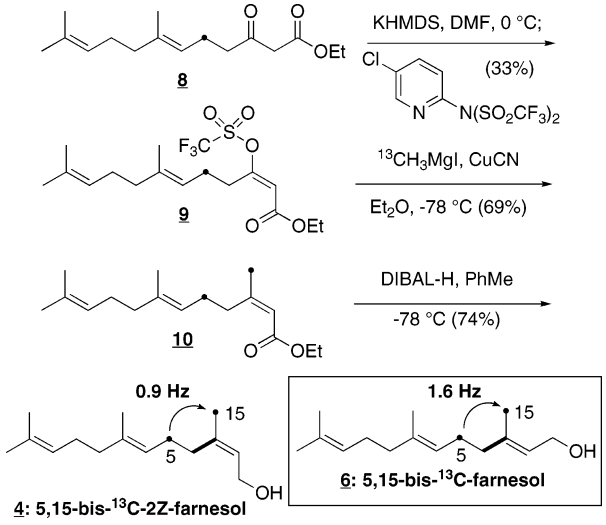
FPP were determined to be excellent alternative substrates for FTase.[5] The naturally occurring homologue GGPP is a potent inhibitor of FTase but is not an effective substrate for the enzyme.[6] Surprisingly, the 2Z-isomer of geranylgeranyl diphosphate (2*Z*-GGPP) bound well to FTase and was also capable of acting as an alternative substrate for this enzyme.[7] In contrast, it was an inhibitor of geranylgeranyl transferase I (GGTase I).[7] With the availability of high-resolution crystal structures for mammalian FTase,[8−10] and binding data on a small library of FPP analogues,[2] we utilized docking simulations and pharmacophore computations to explore the binding of 2*Z*-GGPP to FTase. In tandem with this work, we have also synthesized two bis-$^{13}$C-labeled variants of 2*Z*-farnesol and used them as spectroscopic probes to evaluate the conformational consequences of the 2*Z*-geometry. The corresponding labeled variants of 2*E*-farnesol proved to be valuable conformational probes in this regard.[11,12] The model created in this paper provides an explanation for the inhibitory activity of FPP analogues previously published and specifically, a rationale for the surprising ability of 2*Z*-GGPP to both bind tightly to the FTase active site and to act as an effective substrate for the enzyme.[7]

## METHODS

**Pharmacophore Screening of 2*Z*-GGPP Conformational Libraries.** The Molecular Operating Environment 2004 (MOE) software package (Chemical Computing Group, Inc., Montreal, Canada) was utilized in the creation of conformational libraries of 2*Z*-GGPP and in pharmacophore searching. A library of 2*Z*-GGPP conformations was created using the stochastic conformational search feature as executed by MOE. This method is similar to the RIPS method but differs in that the energy is minimized in dihedral angle space followed by Cartesian perturbation and minimization.[13] Energy minimization was limited to 200 steps for each conformation. The stochastic conformations were calculated with chiral inversion turned off but bond rotation of 30° was allowed. Individual conformations that displayed a heavy-atom RMSD of less than or equal to 0.1 Å from any other conformation were removed as duplicates and conformations that had an energy value 7 kcal/mol greater than the minimum were discarded. The unique conformations were collated into a library database, which was subsequently screened against the pharmacophore (see Results) using the pharmacophore searching feature as implemented by MOE. Briefly, the pharmacophore was created using polarity-charge-hydrophobicity (PCH) features in which an essential acceptor feature was placed on the oxygen adjacent to $C_1$ of FPP and an essential hydrophobic feature was placed at $C_{11}$. All other features were not required for a match to the pharmacophore. The conformational library was screened against the pharmacophore, and conformations that placed appropriate features into the pharmacophore model within 1.7 Å or less were determined to be plausible binding conformations.

**Docking of FPP to FTase.** Docking studies were performed using the ternary FTase complex (1D8D).[10] To determine the optimal force field and partial charges for docking, FPP was removed from the active site and placed in the extended conformation. Four different force fields

**Scheme 1.** Synthesis and Conformational Analysis of 5,15-Bis-$^{13}$C-2*Z*-farnesol (**4**)




(CHARMM, MMFF94s, MMFF94x, and OPLS-aa) were applied using partial charges (PEOE),[14−26] to the docking of FPP to the FTase prenyl binding site. A 69 × 47 × 47 Å docking box was created centered on the prenyl binding site and a Tabu search docking method of 50 runs at 1000 steps per run and 10 attempts per step was performed.[27] The initial runs were performed using the default settings in MOE, but the number of docking runs was later expanded from 25 to 50 to ensure better coverage. The results were examined by calculating the heavy-atom RMSD between the docked structure and the experimentally determined FPP-FTase (1FT2) complex.[9] Of the force fields and partial charges tested, the CHARMM force field and PEOE partial charges yielded the best RMSD difference between the docking results and the crystal structure of the FPP-FTase complex (1.7 Å). Thus, these conditions were utilized in subsequent docking studies.

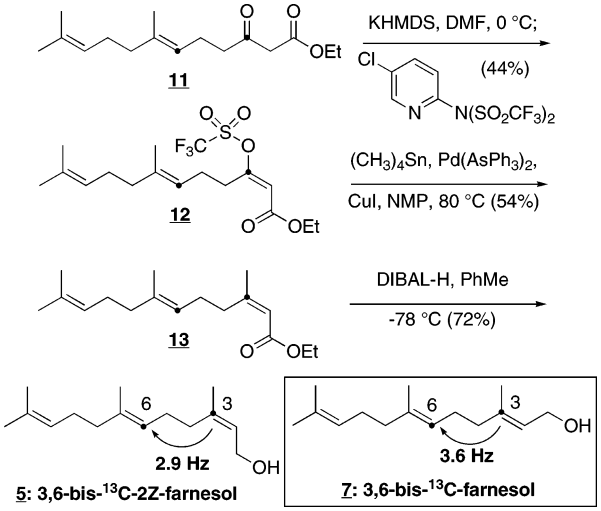
**Docking of 2*Z*-GGPP to FTase.** Docking studies were performed using the ternary FTase complex (1D8D).[10] The compound 2*Z*-GGPP was constructed in MOE using the builder program contained within MOE, in both an extended conformation and the skewed conformation indicated by the pharmacophore search. 2*Z*-GGPP was energy minimized using the CHARMM force field with PEOE partial charges, and the resulting minimized structures were placed into a docking box (69 × 47 × 47 Å) centered on the prenyl binding site of FTase. The Tabu Search docking method was run as described previously. The resulting structures displaying the lowest total and lowest van der Waals energy were then analyzed to determine the structural features of binding.

**Synthesis of $^{13}$C-Labeled 2*Z*-Farnesol Derivatives.** The 2*Z*-farnesol derivatives **4** and **5** were synthesized from the previously prepared beta-ketoesters **8** and **11**,[12] as illustrated in Schemes 1 and 2. Compounds **4** and **5** were prepared in the same manner as the 2*E*-farnesol derivatives **6** and **7**,[12] with the exception that the stereochemistry of the intermediate triflates was installed using our stereoselective method to prepare 2*Z*-isoprenoids.[5]

## RESULTS

**Pharmacophore Modeling of 2*Z*-GGPP**. Recently, we developed a pharmacophore model for FTase (unpublished

**Scheme 2.** Synthesis and Conformational Analysis of 3,6-Bis-$^{13}$C-2*Z*-farnesol (**5**)




results) using MOE and a library of 34 biologically active FPP analogues. The pharmacophore has six features, five of which were required for a molecule to be considered as a potential ligand (Figure 2). The pharmacophore model was built to exploit six features. First, a hydrogen bond acceptor was centered on the oxygen adjacent to $C_1$ to account for the requirement for an ionic group to take the place of the diphosphate group. This feature was deemed essential since it forced ligands to be in close proximity to the catalytic zinc. Hydrophobic elements were placed at the $C_3$- and $C_{11}$-methyl groups to account for critical interactions observed in numerous compounds. The $C_{11}$-hydrophobic element was also essential in the screen. We chose this in order to identify only molecules that existed in a more extended conformation which have been experimentally observed. Additional hydrophobic features were placed along with the prenyl binding region in recognition of the common hydrophobic core of most substrates and inhibitors. Nine void volumes, determined from a contour plot of the binding site and thus key active site residues, were placed around the lipid binding pocket to represent molecular surfaces and to prevent "binding" of conformations of 2*Z*-GGPP that would place substantial portions of the molecule into space occupied by the protein.

Using this model we used the pharmacophore searching feature of MOE to place the extended conformation of 2*Z*-GGPP within the pharmacophore model; however, the extended conformation of 2*Z*-GGPP failed to fit within the defined model. We next created a stochastic conformational library of 2*Z*-GGPP that contained the most plausible and energetically favorable conformations of 2*Z*-GGPP. The library contained 707 different conformations of 2*Z*-GGPP, many of which were expected to be incompatible with binding to FTase. To determine which conformations were capable of binding to FTase, we screened the library against the pharmacophore model. Of the 707 members of the library, 5 were capable of fitting the pharmacophore model within an RMSD of 1.2 Å. All five conformations displayed an extended conformation for the last two isoprene units mimicking the conformation seen in the crystal structure of FPP analogues bound to FTase. However, the relative orientations between the first two isoprene units were different from FPP indicating the importance of alternative

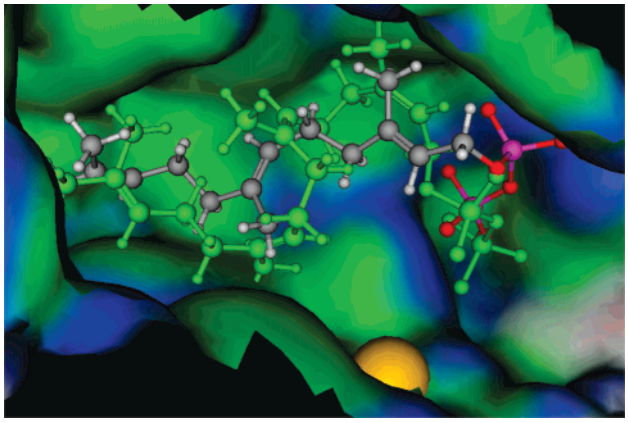


**Figure 2.** The orientation of the best RMSD conformation of 2*Z*-GGPP in the FTase active site determined by fitting the stochastic conformation library of 2*Z*-GGPP to the pharmacophore model. The conformation is shown in green and along with the FPP. The molecular surface of the active site of FTase is shown to delineate the binding pocket. The active site zinc is shown as an orange sphere. The nine void volumes that were used to construct the surface of the active site were determined from the van der Waals radii of the side chains of the following residues that line the surface of the active site. The residues that correspond to each void volume are as follows: V1 − His 201α; V2 − Tyr 205*β*; V3 − Trp 102*β*; V4 − Tyr154*β*; V6 − Tyr 361*β*; V7 − Trp 303*β*; V8 − Gly 250*β*; V9 − Tyr 200α.

conformations in this region of the molecule (Figure 2). Only conformations in which the $C_3-C_4$ and $C_4-C_5$ dihedral angles were not 180° were observed. This contrasts the corresponding bond angles in FPP and GGPP, which exist in primarily trans configurations. This conformation helps to place $C_1$ into a reaction location within the active site and also served to place hydrophobic portions of 2*Z*-GGPP into a hydrophobic region of the enzyme.

**Docking of 2*Z*-GGPP.** The pharmacophore model analysis of 2*Z*-GGPP indicated that only conformations of 2*Z*-GGPP that contained a skewed conformation between the first and second isoprene units were capable of binding to FTase. While instructive, this analysis utilized a stochastic conformational library that does not contain all possible low-energy conformers. Since the pharmacophore model treats conformations as being rigid, it is possible, though improbable, that the conformers selected would not necessary exist when bound to the enzyme. To address this issue, we turned to docking studies of the 2*Z*-GGPP since this method only generates potential ligand−protein complexes ranked relative to the energy of the complex. To initiate the docking study, we first examined force fields and partial charges to determine which combination would provide the best docking results and thus first explored docking of FPP into the active site of FTase. We chose this ligand because the availability of several high-resolution crystal structures of the binary complex of FPP and FTase would allow us to compare our docking results with experiment. Using a Tabu search method, we found that CHARMM with the PEOE partial charges provided docking results in which the docked FPP had an all heavy-atom RMSD of less than 2 Å from the crystal structure.

Using these parameters, we docked 2*Z*-GGPP into the active site of FTase. The docking results generated 50 possible 2*Z*-GGPP−FTase complexes; however, only 15 out of the 50 structures had an RMSD of less than 2 Å compared to the crystal structure of FPP. Although FPP and 2*Z*-GGPP

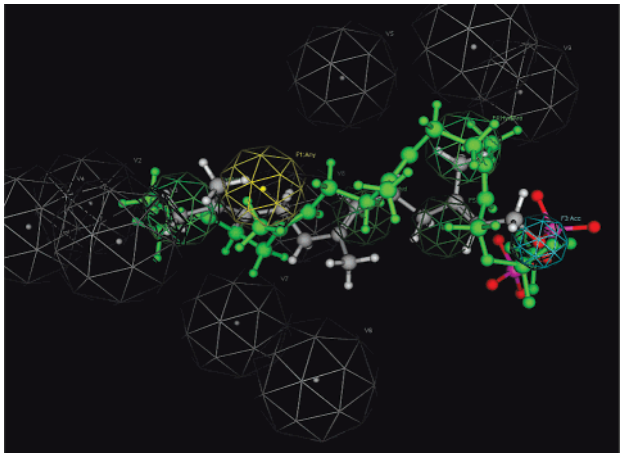


**Figure 3.** Overlay of the 2*Z*-GGPP conformation (shown in green), as determined by docking, with FPP in the FTase active site. The presence of the "kink" in the first isoprene unit of 2*Z*-GGPP can be clearly seen. The required hydrophobic pharmacophore features, centered around $C_3$ and $C_{11}$ of FPP, and the acceptor feature at $C_1$ are also shown.

have different chain lengths and structures, we chose to utilize this comparison, where all atoms in 2*Z*-GGPP were compared to all atoms in FPP, because of the experimental result that 2*Z*-GGPP was an alternative substrate and it thus must have common structural features with the naturally occurring substrate FPP.

Examination of the best-fitting docked structures revealed that conformation about the $C_3-C_4$ and $C_4-C_5$ bonds were similar to conformations determined using the pharmacophore model (Figure 3). This altered conformation served to place the pyrophosphate moiety of 2*Z*-GGPP into an orientation similar to that of FPP where the $C_1$ atom of both compounds could react with the nucleophilic sulfur of cysteine. This altered conformation of 2*Z*-GGPP cannot be adopted by the *all-trans*-GGPP. In addition, the conformation identified by docking places hydrophobic first isoprene unit of 2*Z*-GGPP into a hydrophobic pocket in the enzyme (see Discussion), thus enhancing the binding affinity to FTase. This result highlights the importance of altered conformational constraints within the first isoprene unit as a valuable tool in the design of new inhibitors and substrates for FTase.

**Solution Phase, Experimental Conformational Evaluation of 2*Z*-Farnesol**. We have previously shown that NMR analysis of bis-$^{13}$C-labeled isoprenoids can provide insight into the conformational preferences of isoprenoids and thus confirm and extend computational and structural studies on these molecules.[11,12] In view of the intriguing biological activity of 2*Z*-GGPP, we synthesized the labeled molecules **4** and **5** (Figure 1). The synthetic routes to **4** and **5** are outlined in Schemes 1 and 2. The Karplus coupling constant relationship observed between the two $^{13}$C-labeled carbon atoms in **4** and **5** ($^3J_{CC}$) provides information about the conformational preferences of the $C_3-C_4$ and $C_4-C_5$ bonds, respectively. The $^3J_{CC}$ coupling between labeled carbons is at a maximum (~4−5 Hz) in the trans (180°) or eclipsed (0°) conformers and is at a minimum (~0−2 Hz) in the gauche (60°) or skew (90°) conformers.[28,29] As indicated in Schemes 1 and 2 (see also Table 1), the coupling constant observed in **4** is 0.9 Hz, while the coupling constant observed in **5** is 2.9 Hz. These numbers are particularly instructive in comparison to those previously reported for the corresponding labeled 2*E*-farnesol isomers **6** (1.6 Hz; Scheme 1) and **7** (3.6 Hz; Scheme 2).[11,12] This comparison, summarized in Table 1, indicates that the 2*Z*-geometry leads to a greater preference for a skew (90°) geometry about the $C_3-C_4$ bond in **4** than that observed in **6** (the skew conformer leads to the smallest $^3J_{CC}$).[28] The smaller coupling constant observed in **5** is consistent with either a lower preference for the extended conformation about the $C_4-C_5$ bond than that seen with **7** or a mixture of eclipsed and gauche conformers in **5**. The latter explanation is consistent with the 43° angle observed about this bond in the docked structure of 2*Z*-GGPP (Figure 3 and Table 1).

**Table 1.** Computational and Experimental Torsional Angle Preferences of Isoprenoids

| torsional angle | 2*Z*-GGPP (docked structure)[a] | 2*Z*-farnesol $^3J_{CC}$ values | 2*E*-farnesol $^3J_{CC}$ values | FPP (crystal structure)[f] |
|---|---|---|---|---|
| $C_5-C_4-C_3-C_{15}$ | 107° | 0.9 ± 0.2 Hz[b] | 1.6 ± 0.2 Hz[d] | 69° |
| $C_6-C_5-C_4-C_3$ | 43° | 2.9 ± 0.2 Hz[c] | 3.6 ± 0.2 Hz[e] | 171° |

[a] This study; values from the docked 2*Z*-GGPP conformation shown in Figure 3. [b] This study; carbon-13 NMR coupling constant determined from 5,15-bis-$^{13}$C-2*Z*-farnesol. Consistent with a primarily skew (90°) conformation (**4**). The error in the $^3J_{C-C}$ coupling constant measurements is estimated on the basis of the digital resolution of the NMR spectra.[12] [c] This study; carbon-13 NMR coupling constant determined from 3,6-bis-$^{13}$C-2*Z*-farnesol (**5**). Consistent with a mixture of eclipsed (0°) and gauche (60°) [or trans (180°) and gauche] conformers. [d] Carbon-13 NMR coupling constant previously determined from 5,15-bis-$^{13}$C-2*Z*-farnesol (**6**).[12] Consistent with a gauche conformational preference. [e] Carbon-13 NMR coupling constant previously determined from 5,15-bis-$^{13}$C-2*E*-farnesol (**7**).[12] Consistent with a trans conformational preference. [f] Values from the indicated bonds in the FPP in the experimentally determined FPP-FTase (1FT2) complex.[9]

## DISCUSSION

The conformational preferences of isoprenes play an important role in the chemistry and biology of these agents. Previous work from our laboratory using $^{13}$C labeled FPP and GGPP have shown that these agents exist in an extended conformation in water and when bound to the enzyme FTase.[12] However, a study of the reaction pathway of FTase has shown that FPP must adopt a different conformation around the first two isoprene units in order for the $C_1$ group to be placed near the reactive cysteine residue.[8] Thus, while structural studies have shown an extended conformation for FPP and GGPP, it is clear that conformational flexibility is required for catalysis.

There has been considerable speculation on how relatively minor modifications to the structure of FPP can lead to either inhibitory analogues with $IC_{50}$ values in the nanomolar range or compounds that bind very poorly to FTase. For example, 3-vinyl FPP binds tightly to the FTase active site and is an effective alternative substrate ($K_m$ = 173 nM), [30] while 3-isobutenyl FPP and 3-*tert*-butylFPP are potent, low nanomolar ($IC_{50}$ = 15 and 31 nM, respectively) FTIs,[30] but 3-neopentylFPP is an exceptionally poor FTI ($IC_{50}$ = 6200 nM).[31] We were puzzled to find that the compound 2*Z*-GGPP is a tight binding and effective alternative substrate for FTase.[7] In contrast, the natural all-trans configuration of GGPP is a poor alternative FTase substrate, although it binds quite tightly to the enzyme.[6] Thus, it would appear that 2*Z*-GGPP is able to adopt alternative conformations that allow

the $C_1$ carbon to be within striking distance of the sulfhydryl group of the cysteine. Given this contention, we explored the conformational flexibility of 2*Z*-GGPP, using both computational and spectroscopic methods. We have shown that 2*Z*-GGPP can adopt an alternative conformation that enables its $C_1$ carbon to be within striking distance of the sulfhydryl group of cysteine. While the altered conformation of 2*Z*-GGPP explains the capability of this molecule to be an alternative substrate, it does not explain the apparent tight binding of this compound ($K_m = 21$ nM) to the enzyme when compared to either *all-trans*-GGPP ($K_m = 40$ nM) or FPP ($K_m = 46$ nM).[32] It is important to note here that FTase is a kinetically complex enzyme,[33] and substrate $K_m$ values may bear little relationship to $K_D$ values.

One intriguing possibility is that many analogues utilize a potential cleft (formed by the side chains of Tyr200alpha, His201alpha, His248beta, and Tyr251beta) visible in crystal structures that exists in proximity to the 3-position of FPP. Side chains that project into this cleft could pick up additional van der Waals interactions and as a result lead to tighter binding by the ligand. The pharmacophore model generated using the existing FPP analogue binding data demonstrated that the putative 3-substituent cleft is an important hydrophobic binding site, as illustrated in Figure 2. Surprisingly, this cleft, which accommodates 3-substituents, may also provide an explanation for the ability of 2*Z*-GGPP to bind to the FTase active site as the conformation identified by both docking and pharmacophore modeling contains a kink in the region of the second isoprene unit. This kink places significant additional hydrophobic residues from 2*Z*-GGPP into the putative cleft and thus may account for its efficacy as a substrate ($K_m = 21$ nM; $k_{rel} = 0.67$).[7] A comparison of the binding modes of 2*Z*-GGPP and *all-trans*-GGPP led to the conclusion that the *all-trans*-GGPP was unable to adopt an altered conformation that would allow it to act as an alternative substrate. We propose that this, rather than the "molecular ruler" hypothesis proposed by Long, Beese, and Casey,[9] may explain the inability of *all-trans*-GGPP to act as a substrate and suggest that the "molecular ruler" hypothesis may not fully explain the molecular discrimination of isoprenoids by FTase. In agreement with this, a recently determined crystal structure of the FTase−GGPP binary complex led Distefano and co-workers to propose that a "second site exclusion model", in which FTase binds larger isoprenoids in a fashion that prevents the subsequent productive binding of peptide substrate, explains the lack of substrate ability of GGPP.[34] Our findings here suggest that the greater conformational flexibility and consequent higher preference of 2*Z*-GGPP to exist in a folded conformation allow it to avoid binding in a manner that excludes peptide from the second site. Note that the dihedral angles for the docked 2*Z*-GGPP structure depicted in Figure 3 ($C_3-C_4$: 107°; $C_4-C_5$: 43°) are consistent with an experimental $C_3-C_4$ preference for skew conformers and a $C_4-C_5$ preference for a mixture of eclipsed and gauche conformers, as determined by the $^{13}C$ NMR analysis of **4** and **5** (Table 1).

This study has highlighted two considerations for the continued design of FTIs and FTase alternative substrates. First, a consideration of the conformational flexibility, especially around $C_1$, is necessary for a determination of the ability of agents to act as alternative substrates. In addition, conformations that differ from extended conformations may be able to take advantage of additional contacts with the enzyme. Second, the presence of a hydrophobic cleft adjacent to the $C_3$ substituents can provide additional binding energy by forming hydrophobic and steric contacts with the ligand. Further computational and experimental analysis will be required to validate the hypotheses described in this report, and efforts along these lines are currently underway.

## ACKNOWLEDGMENT

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