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Aromatic Farnesyl Diphosphate Analogues: Vinyl Triflate-Mediated Synthesis and Preliminary Enzymatic Evaluation

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Abstract—A stereocontrolled vinyl triflate-based synthetic route has been used to prepare four analogues of farnesyl diphosphate (FPP) where the terminal isoprene units have been replaced with aromatic moieties. Two of these analogues exhibit no productive interaction with protein farnesyltransferase, but the 2-naphthyl derivative **2** is a modest inhibitor of the enzyme, and the *para*-biphenyl derivative **4** is a surprisingly effective alternative substrate.

Protein farnesylation is a common post-translational modification, which plays a key role in a variety of signal transduction pathways. It is carried out by an enzyme, protein-farnesyl transferase (FTase), which recognizes the CAAX box (where X = Ser, Met, Thr, or Gln) at the carboxyl terminus of a protein substrate and then attaches the farnesyl group from farnesyl diphosphate (FPP, Fig. 1) to the free sulfhydryl of the cysteine residue.[1] Initial studies on this process demonstrated that the key signal transduction protein and oncogene product Ras is farnesylated. The potential of farnesylation inhibitors as anticancer agents led to extensive and successful efforts to develop such compounds.[1] This intense effort has been rewarded recently with the introduction of FTase inhibitors into human clinical trials for the treatment of a variety of neoplasias.[1,2]

Efforts in the area of FTase inhibitors have concentrated on substrate-based and non-substrate-based compounds that bind to the CAAX peptide site on FTase. In contrast, previous work in this laboratory has been directed toward the synthesis and evaluation of FPP analogues modified in the α-isoprene unit (Fig. 1),[3] and isomeric FPP and GGPP analogues.[4] These studies have resulted in the discovery of potent, low nanomolar FTase inhibitors, and effective alternative substrates for this enzyme. Recent structural studies on mammalian FTase have suggested an alternative route to the development of FPP-based inhibitors. These structural studies, by the Duke group,[5] the Hoffman-LaRoche group,[6] and the Schering group,[7] have demonstrated that FPP binds to a deep hydrophobic pocket in FTase. This pocket is primarily lined with the electron-rich aromatic side chains of Tyr and Trp residues. This finding immediately suggested that aromatic analogues of FPP, particularly where the β- and ω-isoprene units were replaced with aromatic moieties, would be potent FTase inhibitors. Such compounds could take advantage of favorable aromatic-aromatic interactions that would promote tight binding to the FTase active site.[8]

Several other groups in the FTase arena have made the same observation, and have reported their initial results directed toward the development of aromatic-substituted FPP-based FTase inhibitors (vide infra).[9] We anticipated

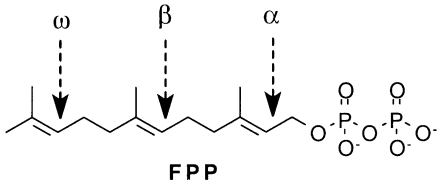

Figure 1. Structure of FPP, with the α, β, and ω isoprene units indicated.

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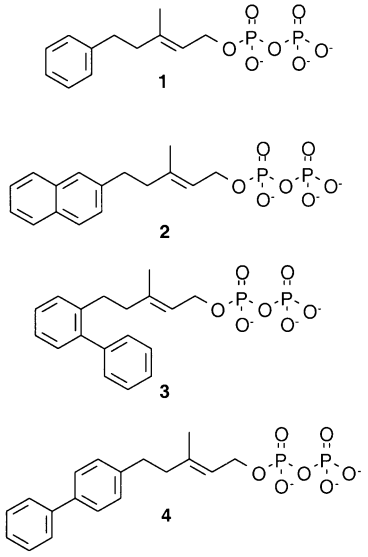

Figure 2. Structures of the four aromatic FPP analogues synthesized and evaluated versus FTase in this study.

that our stereoselective vinyl triflate-mediated route to isoprenoid analogues[1,3,4] could be readily adapted to the synthesis of FPP analogues where both the β- and ω-isoprene units were replaced with aromatic moieties. The four analogues shown in Figure 2 were chosen as the initial targets for synthesis and evaluation. These compounds were chosen in part due to the ready availability of the necessary benzylic starting materials. However, in addition to addressing the general question regarding the suitability of aromatic moieties as isoprenoid mimics, analogues **1**–**4** also provide insight into the hydrophobic bulk and overall geometry necessary for an FPP analogue to bind to the FTase active site.

The syntheses of **1**–**4** began with the commercially available benzyl bromides **5a**–**c** (X = Br) and the benzyl chloride **5d** (X = Cl) (Fig. 3). The selective mono-alkylation of the ethyl acetoacetate dianion **6** was attempted following the procedure previously developed by Weiler[10] and extensively utilized in our laboratory.[3,4] Surprisingly, very poor yields of the desired β-ketoesters **7a**–**d** were obtained. In order to obtain satisfactory yields of **7a**–**d**, it was necessary to conduct the alkylation of **6** at room temperature, rather than 0 °C.[11] The kinetic triflation of **7a**–**d** also proved to be more problematic than in the parent isoprenoid case, and it was necessary to conduct the enolate formation and triflation at room temperature to obtain sufficient amounts of **8a**–**d**. In contrast to the first two steps, we found that the Pd/CuI-catalyzed methylation of the triflates to afford **9a**–**d**, and their subsequent reduction to **10a**–**d**, proceeded smoothly in the same manner as observed in the parent system. The farnesol analogues **10a**–**d** were then converted to the desired aromatic FPP analogues **1**–**4** following the two-step phosphorylation procedure of Davisson et al.[12]

The four aromatic analogues of FPP, having been prepared, were then evaluated as potential substrates for or inhibitors of mammalian protein-farnesyl transferase

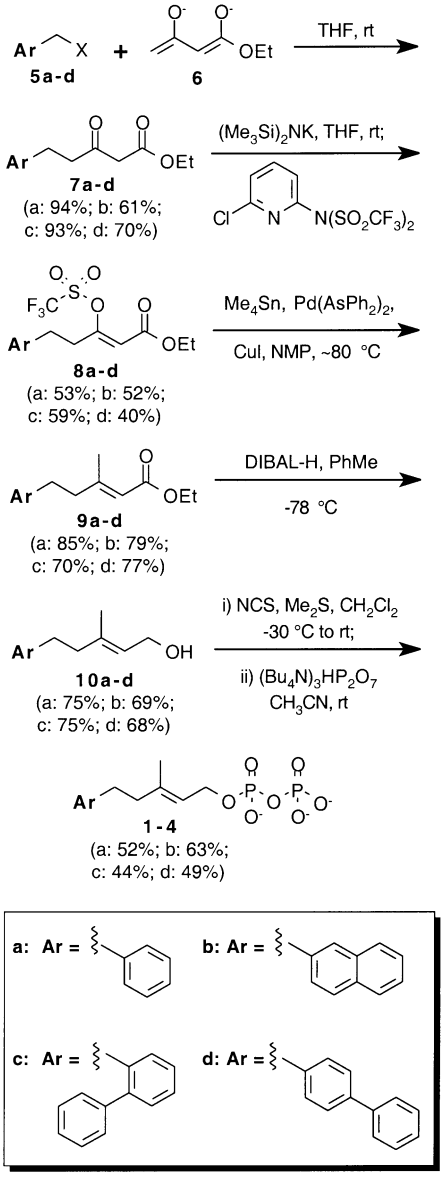

Figure 3. Syntheses of FPP analogues **1**–**4**.

(mFTase). A modified version of the continuous spectrofluorimetric assay for FTase was employed to test the ability of **1**, **2**, **3**, and **4** to act as mFTase substrates with the peptide cosubstrate dansyl-GlyCysValLeuSer-OH.[13,14] Initial studies indicated that **1**, **2**, and **3** were not substrates, while **4** was a substrate for mFTase. More detailed evaluation demonstrated that the *para*-biphenyl analogue **4** is a surprisingly effective mFTase substrate. Analogue **4** exhibited a submicromolar (800 ± 28 nM) K_m value for the enzyme, less than 3-fold higher than that observed with the natural substrate E,E-FPP under the same assay conditions (300 nM).[4] Moreover, the approximate V_{max} value observed with **4** was only ~three fold less than that seen with E,E-FPP. Note that the K_m value does not provide a true indication of affinity for the enzyme due to the complexity of the mFTase kinetic mechanism.[15] In contrast to the results obtained with **4**, analogues **1**–**3** were exceptionally poor mFTase substrates. Therefore, these compounds were

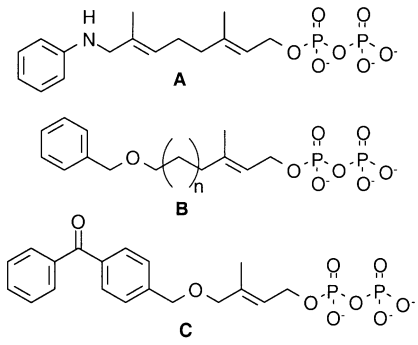

Figure 4. Structures of aromatic FPP analogues synthesized and evaluated versus FTase in other laboratories.

evaluated as inhibitors of mFTase. The phenyl analogue **1** and the *ortho*-biphenyl analogue **3** exhibited no inhibitory activity, and thus no apparent interaction with mFTase, ($IC_{50} \gg 10\,\mu M$; essentially no inhibition of mFTase was observed at 10 μM with either compound). However, the naphthyl analogue **2** proved to be a modest inhibitor of mFTase, with an IC_{50} value of 5.4 μM.

Several other groups in the FTase arena have reported their initial results directed toward the development of aromatic-substituted FPP-based FTase inhibitors (Fig. 4). In particular, the Spielmann laboratory has described several ω-substituted FPP analogues and their interaction with FTase. The aniline-substituted analogue **A** is not an FTase inhibitor, but instead is a surprisingly effective alternative substrate that is transferred to Ras with the same efficiency as the natural substrate FPP.[9] The set of analogues represented by structure **B** ($n = 1$–5) were found by Spielmann et al. to be modestly effective alternative substrates ($V_{rel} = 0.022$–0.22; $K_m = 56$–890 nM).[9] Distefano and co-workers have recently prepared the benzophenone-derived photoaffinity label **C**, and found that it is a poor substrate for mammalian and yeast FTase, but an effective inhibitor of these enzymes [IC_{50}(mFTase) = 2.3 μM].[9] They have also determined the structure of the mFTase–**C** complex, which confirms that the aromatic moiety in this analogue serves as a good mimic of the β- and ω-isoprene units of FPP. Note that a novel synthetic route to other aromatic substituted farnesoids has been developed by Wiemer and co-workers,[9] although no biological data on these analogues has yet been reported.

The results reported here on the biological activity of **1**–**4** are consistent with our structural knowledge of the mFTase FPP binding site, and are also consistent with results previously reported on other aromatic FPP analogues. The complete lack of binding of **1** to mFTase is consistent with the lack of hydrophobic bulk of this analogue, and with the fact that the smaller isoprenoid diphosphate GPP[9] and other FPP analogues of similar size[16] exhibit very poor binding to mFTase. An increase in the size of **1** leads to the naphthyl analogue **2**, which is a modest inhibitor of mFTase. Extensive structural studies on mFTase have demonstrated that FPP binds to the enzyme in an extended conformation. Thus, it is not surprising that **3**, which mimics a folded conformation of FPP, does not bind to mFTase. The active site of mFTase is quite large, with the peptide and FPP binding sites adjacent to each other,[7] and thus one might anticipate that **3** might utilize a portion of the peptide binding site and still bind to mFTase, but this was not the case. The most significant biological result presented here is the ability of **4** to act as an excellent substrate for mFTase. The recently reported work of Spielmann (**A** and **B**, Fig. 4) has established that an aromatic moiety can act as an effective mimic for the ω-isoprene unit, but it is striking that an appropriate aromatic moiety can effectively replace *both* the β- and ω-isoprene units of FPP. Prenylation of CAAX box proteins with **4** and possibly certain related analogues could lead to the modification of Ras and other key signal transduction proteins with a prenyl group distinct in structure from the naturally occuring farnesyl moiety. Such unnatural prenyl groups may be valuable tools to interrogate the biological functions of protein prenylation.[17]

Acknowledgements

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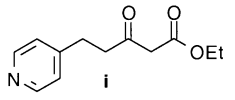
11. The β-ketoesters **i** and **ii** were prepared via the alkylation of the corresponding benzylic chlorides with the dianion **6**, in an attempt to synthesized the heteroaromatic versions of **1** and **2**. However, low yields of **i** and **ii** were obtained (<30%), even with a large excess of dianion **6** and extended reaction times at room temperature. Moreover, attempts to convert the small amounts of **i** and **ii** obtainted to the corresponding triflates were completely unsuccessful, and thus the attempted synthesis of the corresponding FPP analogues was abandoned.



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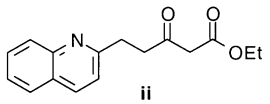

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14. Assay Procedure: Briefly, dansyl-GCVLS (4.7 μM) was employed as a peptide substrate. E,E-FPP, **1**, **2**, **3**, or **4** (~15 mM stock solution in 25 mM ammonium bicarbonate, pH 7.5; final concentration 0.10 to 5 μM) was added to the assay buffer solution [52 mM Tris–HCl, pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$ with added detergent solution (0.4% *n*-dodecyl-β-D-maltoside)]. The reaction was initiated with the addition of purified recombinant mFTase, and 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). The velocity was determined by converting the rate of increase in fluorescence intensity (cps/s) to μM/s. For the determination of the IC_{50} values, the E,E-FPP concentration was maintainted at a fixed level (1.0 μM) and the rate of farnesylation was determined with varying amounts of **3** added (0.3–30 μM).
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