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Grignard-Mediated Synthesis and Preliminary Biological Evaluation of Novel 3-Substituted Farnesyl Diphosphate Analogues

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Abstract—A series of substituents was installed at the 3 position of farnesyl diphosphate through a copper-cyanide mediated coupling of a vinyl triflate with various Grignard reagents. These novel FPP mimetics were then evaluated as inhibitors of or substrates for mammalian protein farnesyl transferase. The IC_{50} values for these compounds range from 18 to 10,100 nm, with the 3-isopropenyl analogue being one of the most potent FPP-mimetic mFTase inhibitors yet synthesized. ©2000 Elsevier Science Ltd. All rights reserved. © 2000 Elsevier Science Ltd. All rights reserved.

Protein farnesyl transferase (FTase) is an enzyme that carries out the isoprenoid modification of proteins containing a CAAX box motif. In particular, this ca. 90 kDa heterodimeric enzyme catalyzes the addition of a 15-carbon farnesyl moiety to the terminal cysteine sulfhydryl of several key signal transduction proteins, including Ras proteins. This farnesylation of Ras is the first and obligate step for its participation in a signal transduction cascade. Mutated Ras proteins have been implicated in ca. 30% of all human cancers, with the highest incidence found in pancreatic tumors (ca. 90%).[1] Therefore, inhibition of FTase has become the object of intense research efforts.[1] Significant progress has been made in the development of peptidomimetic and non-substrate based FTase inhibitors, and in vivo evaluation of some of these compounds has indicated promising potential for their development into anticancer agents.[2] However, less work has been initiated on farnesyl diphosphate based inhibitors of FTase (FPP; **1a**, Figure 1). Consequently, less is known regarding the specificity of FTase for its isoprenoid substrate. Recently, we have found that 3-vFPP (**1b**) is an alternative substrate for mammalian FTase, while 3-alFPP (**1c**) is a potent and selective inhibitor of this enzyme.[3] The introduction of a *tert*-butyl group at the 3-position leads to one of the most potent FPP-based mFTase inhibitors, 3-tbFPP (**1d**). Moreover, the alcohol precursors of **1b** and **1c** inhibit the growth of ras transformed cells, apparently via two different mechanisms.[3] The fact that this subtle change in functionality leads to large differences in activity has provided the impetus for the preparation and testing of a wide variety of 3-substituted FPP analogues. Herein, we report the Grignard-mediated synthesis and preliminary biological evaluation of several novel 3-substituted FPP analogues as potential mFTase inhibitors.

The natural biosynthetic route to isoprenoids is well-characterized, but is of little use for the synthesis of analogues. Therefore, synthetic chemical routes must be used instead. The procedures used for the synthesis of isoprenoids via various synthetic methodologies have been continually improved and updated. Methods that have been employed for chain elongation of isoprenyl derivatives include Wittig condensations,[4] Biellman couplings,[5] and palladium-catalyzed cross-coupling reactions.[6] We have developed a novel and efficient synthetic route leading to a wide variety of isoprenoids, in particular 3-substituted farnesyl and geranylgeranyl analogues.[7a,b] This method is based on a vinyl phosphate route used to synthesize isoprenoids developed by Sum and Weiler.[8]

Previously we have reported the coupling of **3** with a wide variety of organotin,[7] organoboron,[9] and organocopper[10] reagents. We have now enhanced our arsenal of synthetic methodologies with a copper-cyanide mediated coupling of **3** with Grignard reagents. This new method has been used to introduce various substituents

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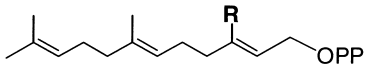
at the 3 position of FPP (Scheme 1). The synthetic route begins, as described previously, with the conversion of β-ketoester **2** to the vinyl triflate **3** in a highly stereoselective fashion. We have recently established that the cuprate reagent derived from [^{13}C]-methylmagnesium iodide and copper cyanide couples in an effective fashion with triflate **3**, and this was employed as a key step in the preparation of [5,15-bis-^{13}C]-FPP.[11a] Since a wide variety of alkyl, alkenyl, and aryl Grignard reagents are commercially available, this appeared to be an attractive procedure for the synthesis of a wide variety of 3-substituted FPP analogues.

Preliminary studies indicated that the previously prepared *tert*-butyl ester **4d** could be synthesized in excellent yield from the organocopper reagent prepared from *tert*-butylmagnesium chloride and a slight excess of copper cyanide. Therefore we have used the same protocol for the synthesis of the eight 3-substituted esters **4e–l** (Scheme 1).[11b] The yields of the esters are modest in many cases; however, note that these coupling reactions (and the subsequent steps) are unoptimized and in most cases the reported yield is from a single reaction attempt. The majority of the Grignard reagents tried produced the desired ester. However, the attempted coupling of the cuprate reagent derived from 2-methyl-(1-propenyl)magnesium bromide and triflate **3** afforded only starting material. Moreover, several attempts with allylmagnesium chloride yielded at best only a few percent of the desired ester. Note also that the reaction appears to proceed much better in pure ether than in ether/THF solvent mixtures.[10b] Chloride, bromide and iodide Grignard reagents all can be used effectively in the coupling reaction. Reduction of the 3-substituted esters, followed by the two-step phosphorylation procedure of Poulter and co-workers,[12] afforded the desired 3-substituted FPP analogues **1d–l**.



1a (FPP; R= CH_3) (K_m = 107nM)[a,b]

1b (3-vFPP; R= vinyl) (K_m = 156nM)[a]

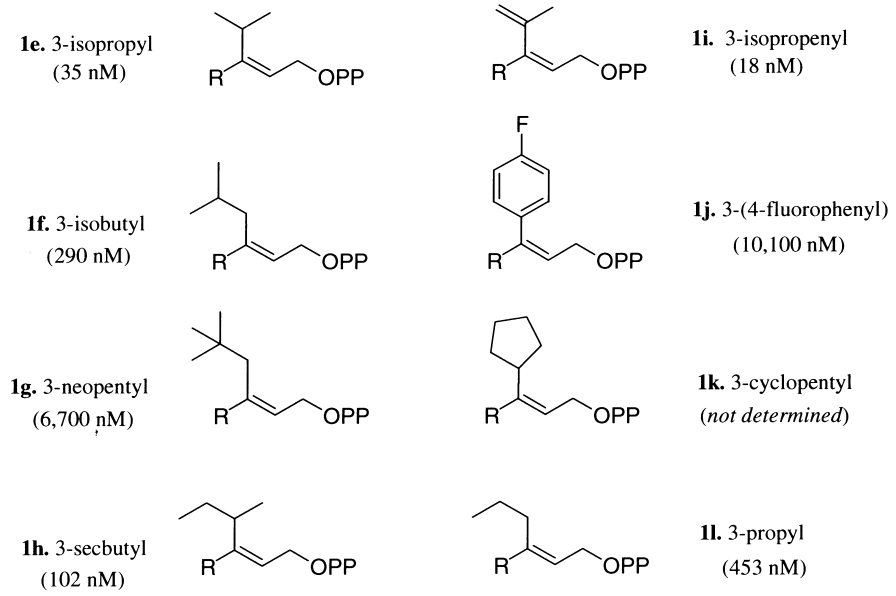
1c (3-aIFPP; R= allyl) (IC_{50} =189nM)[a]

1d (3-tbFPP; R= *tert*-butyl) (IC_{50} = 31nM)[a]

Figure 1. Previously prepared 3-substituted FPP analogues. (a) Values from ref 3. (b) Under the assay conditions used in this paper.

The newly synthesized FPP analogues were evaluated as potential substrates or for inhibitors of mammalian protein–farnesyl transferase. Kinetic values were determined using a modified version of a continuous spectrofluorometric mFTase assay with the peptide cosubstrate dansyl-GCVLS-OH.[13,14] This assay was employed because it can be readily used to determine if new FPP analogues are substrates, or inhibitors. The assay was used first to determine an IC_{50} value for the previously characterized analogue **1c**, and this was in reasonably good agreement with the previously determined value.

The results observed with analogues **1e–1l** confirm that subtle changes in functionality at the 3 position can lead to dramatic changes in activity (Table 1 and Figure 2). Removal of one methyl group from the potent *tert*-butyl compound **1d** leads to the equally potent isopropyl analogue **1e**. Interestingly, the similar isobutyl compound **1f** is ca. eight times less potent than **1e**. Perhaps the extra carbon in the isobutyl group of **1f** interferes with its tight binding to the active site of mFTase. To expand on this hypothesis, we synthesized the neopentyl analogue **1g**. Consistent with this trend, **1g** is a strikingly poor inhibitor of mFTase. Note in particular the several 100-fold difference in binding affinity seen between the *tert*-butyl analogue **1d** and the neopentyl analogue **1g**. The *sec*-butyl analogue **1h** was prepared to generate an analogue intermediate in size between **1e** and **1f**. It is evident that **1h** retains high affinity for the



Scheme 1. Grignard-mediated synthesis of new FPP analogues.

Table 1. Relative abilities of compounds **1b**–**1l** to act as inhibitors of or substrates for mammalian protein–farnesyl transferase[a]

Analogue (3-substituent)	IC_{50} (nM)	k_{rel}[b]	K_m (nM)
1b (vinyl)	173[c]	0.521[c]	156[c]
1c (allyl)	119 (189)[c]	0.053[c]	nd
1d (*tert*-butyl)	31[c]	nd	nd
1e (isopropyl)	35	0.013	nd
1f (isobutyl)	290	0.000	nd
1g (neopentyl)	6600	0.042	nd
1h (*sec*-butyl)	102	0.000	nd
1i (isopropenyl)	18	0.000	nd
1j (4-fluorophenyl)	10,100	0.002	nd
1k (cyclopentyl)	nd	0.072	102
1l (propyl)	453	0.078	nd

[a]Values were determined using a fluorescence assay as previously described in ref 3 (nd = not determined). Note that the concentrations of all analogues were verified by phosphate analysis.[14] Recombinant mFTase was expressed in baculovirus and purified as previously described.[18]
[b]Relative V_{max} determined for each analogue at 10 μM, compared to the V_{max} determined for FPP (10 μM) under the same conditions.
[c]Values previously reported in ref 3.

active site of mFTase, with an IC_{50} value between those of **1e** and **1f**. Note that all four of these analogues (**1e**-**1h**) are very poor substrates for the enzyme.

Previous work in our laboratory has demonstrated that the 3-vinyl FPP analogue **1b** is an alternative substrate for mFTase.[3] It is possible that the additonal conjugated double bond in **1b** makes it more reactive and thus a better prenyl donor than the other FPP analogues we have prepared. In view of this proposal, we designed the isopropenyl analogue **1i**, which has a combination of the functionalities of both the potent isopropyl substituted inhibitor **1e** and the alternative substrate **1b**. Apparently the combination of the pi bond of a vinyl group and the tight fitting isopropyl group leads to a synergistic enhancement of binding to the active site of mFTase. In fact, **1i** is one of the most potent FPP based inhibitors of mFTase developed.[15] It is quite surprising, however, that the isopropenyl analogue **1i** is not an alternative substrate for the enzyme like the vinyl analogue **1b**. The *n*-propyl FPP analogue **1l** was synthesized to evaluate further the effect of unsaturation at the 3-position, in comparison to the allyl derivative **1c**. In agreement with the increased potency of **1i** in comparison to **1e**, the allyl derivative **1c** is significantly more potent than **11**. Note also the beneficial effects of branching on the 3-alkyl chain; compare the *n*-propyl analogue **1l** with the more potent **1e** and **1h**.

To further probe the steric constraints of the active site of FTase, additional bulky functional groups were added to the 3-position of FPP. The 3-(4-fluorophenyl) analogue **1j** was chosen for its hydrophobic phenyl group, and the additional electron withdrawing characteristics of the fluorine atom.[16] Surprisingly, this compound was the least potent FPP analogue examined thus far, and in particular it should be noted that it binds more than an order of magnitude more weakly to mFTase than the corresponding unsubstituted 3-phenyl FPP analogue (IC_{50} = 299 nM).[3] To generate an analogue intermediate in bulk between the potent isopropyl analogue **1e** and the ineffective 3-(4-fluorophenyl) analogue **1j**, we synthesized the cyclopentyl compound **1k**. One might assume, on the basis of steric considerations, that **1k** would exhibit a potency somewhere between **1e** and **1j**. However, **1k** was not an inhibitor at all, but was instead a substrate. The K_m for **1k** is 102 nM, suggesting that it binds tightly to mFTase, but its V_{max} appears to be relatively modest. Surprisingly, an IC_{50} value for this

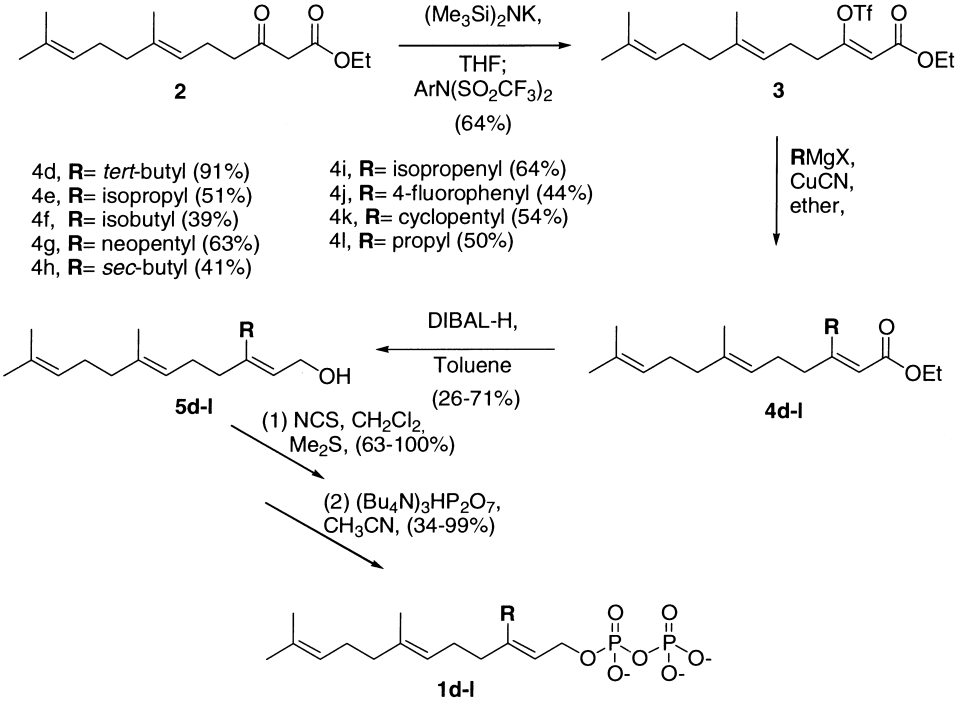


Figure 2. The 3-substituted FPP analogues synthesized in this study, with the IC_{50} values determined for them in parentheses.

analogue could not be determined, as no concentration dependent decrease in mFTase activity was observed with increasing concentrations of **1k**. This finding is a sobering reminder of the complicated kinetic mechanism of mFTase,[17] and its potentially complex mode of interaction with a substrate analogue like 3-cyclopentyl FPP in the presence of FPP and a peptide substrate.

In conclusion, we have extended the utility and applicability of the vinyl triflate route for the synthesis of 3-substituted FPP analogues. In particular, we have expanded our synthetic capability through the use of a copper-cyanide mediated coupling of a vinyl triflate with alkyl, alkenyl and aryl magnesium halides. Furthermore, the preliminary biological assessment of these FPP mimetics has demonstrated that subtle changes in functionality at the three position can lead to large and surprising differences in activity. In addition, we report one of the most potent FPP based inhibitors designed thus far, the isopropenyl derivative **1i**. It is evident that mimics of the natural isoprenoid substrate of mFTase present unique and useful targets for the design of potent inhibitors of the enzyme.

Acknowledgements

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