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Synthesis of Frame-Shifted Farnesyl Diphosphate Analogs

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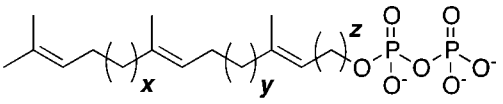
ABSTRACT



x = 0,1,2; ***y*** = 0,1,2; ***z*** = 1,2

A set of synthetic approaches were developed and applied to the synthesis of eight frame-shifted farnesyl diphosphate (FPP) analogs. These analogs bear increased or decreased methylene units between the double bonds and/or diphosphate moieties of the isoprenoid structure. Evaluation versus mammalian FTase revealed that small structural changes can lead to dramatic changes in substrate ability.

Isoprenoid diphosphates play major roles as biosynthetic intermediates in both prokaryotes and eukaryotes. They serve as precursors for a diverse set of lipid moieties, including sterols,[1] lipid anchors for carbohydrates,[2] and lipid anchors for proteins.[3] Perhaps the most interesting set of isoprenoid products are the cyclic sesquiterpenes generated from FPP (farnesyl diphosphate or pyrophosphate), due to their diversity,[4] their unique and promising pharmacological properties,[5] and the intriguing mechanisms of the sesquiterpene cyclases.[6] Thus, there is significant continuing interest in the preparation of FPP analogs as probes of these diverse and important processes, and the development of new synthetic routes to these analogs.

Our laboratory has a long-standing interest in the development of new methods for the preparation of FPP analogs and the use of these analogs as probes for the prenylation of proteins by protein-farnesyltransferase (FTase).[3] We have developed methods for the preparation of FPP analogs substituted at the 3 and 7 positions,[7,8] leading to both potent inhibitors of FTase[9] and novel chemical probes.[10] Our goal in this study was to alter the structure of FPP in a different manner, by increasing and/or decreasing the carbon spacers in its backbone. These analogs will probe the importance of interactions between the central olefin of FPP and FTase and the significance of the length and flexibility of the isoprenoid chain. The targeted compounds were 1,3,1-OPP, 1,2,1-OPP, 2,1,1-OPP, 1,1,1-OPP, 3,1,1-OPP, 2,2,1,1-OPP, where the numbering scheme refers to the methylene units between the double bonds or between the first double bond and diphosphate. By increasing or decreasing the number of methylene units, we generated both more flexible and more strained versions of FPP (2,2,1-OPP), which may provide significant information on the degree of flexibility needed for binding to and turnover by FTase. We also wanted to establish the inhibitory potency of compounds displaying a nonallylic diphosphate (2,2,2-OPP and 2,1,2-OPP). These

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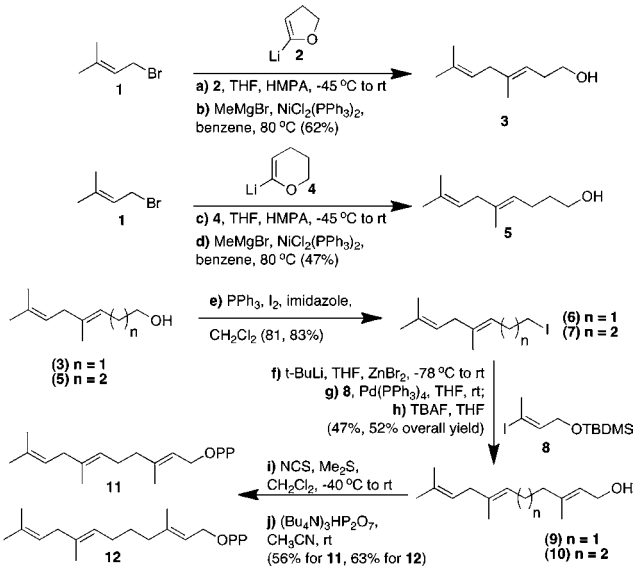
compounds share many common characteristics with FPP, but lack the allylic diphosphate. We do not expect 2,2,2-OPP and 2,1,2-OPP to behave as FTase substrates due to the lower nucleophilicity at C_1; however, we hypothesized that they will act as inhibitors.

Scheme 1. Synthesis of 1,2,1-OPP (**11**) and 1,3,1-OPP (**12**)



The strategy for the synthesis of these compounds was based on the transformation of carbon−oxygen bonds into carbon−carbon bonds developed by Wenkert and colleagues[11] and later used by Kocienski and colleagues.[12] This unique transformation is based on the nickel-catalyzed ring opening of both dihydrofurans and dihydropyrans with Grignard reagents to yield stereodefined trisubstituted alkenes. To utilize this powerful procedure, we first alkylated prenyl bromide **1** with either 5-lithio-2,3-dihydrofuran **2** or 6-lithio-2,3-dihydro-2*H*-pyran (**4**) according to procedures developed by Boeckman and colleagues[13] (Scheme 1). The alkylated dihydrofuran or dihydropyran was then immediately reacted with MeMgBr and $NiCl_2(PPh_3)_2$ to produce the homoallylic alcohols **3** or **5** in a 62% and 47% yield respectively.[12] The use of an additional 2 equiv of MeMgBr and a longer reaction time were necessary for the construction of **5**.

The iodides corresponding to **3** and **5** (**6** and **7**) were then converted into their organozinc derivatives. Negishi cross-coupling[14] of the organozincs with vinyl iodide **8**[8] followed by deprotection with TBAF afforded **9** and **10** in 47% and 52% overall yield respectively. This sequence of steps allowed us to quickly prepare two frame-shifted alcohols

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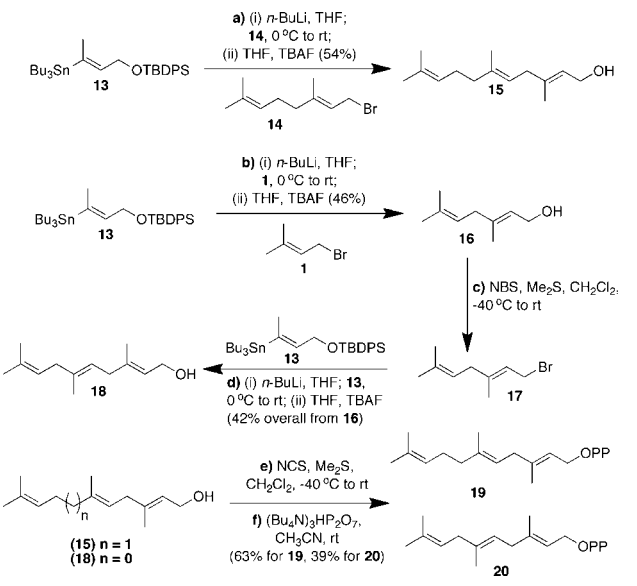
Scheme 2. Synthesis of 2,1,1-OPP (**19**) and 1,1,1-OPP (**20**)

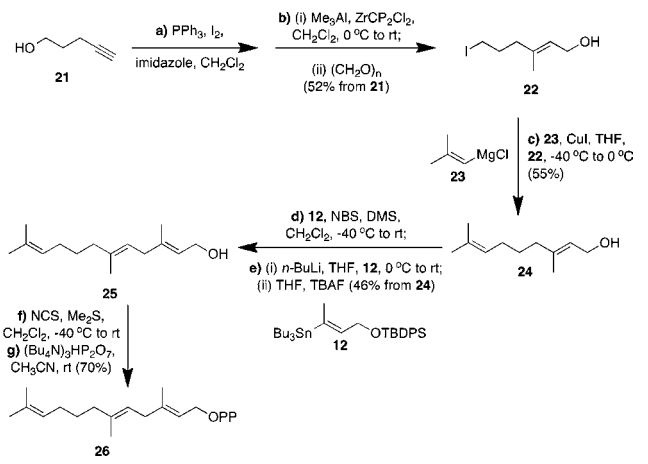


in good yields and with excellent stereoselectivity. We were not able to detect any of the corresponding Z isomer in either case. Diphosphorylation (Scheme 1) generated the two frame-shifted FPP analogs **11** and **12** in 56% and 63% overall yield.

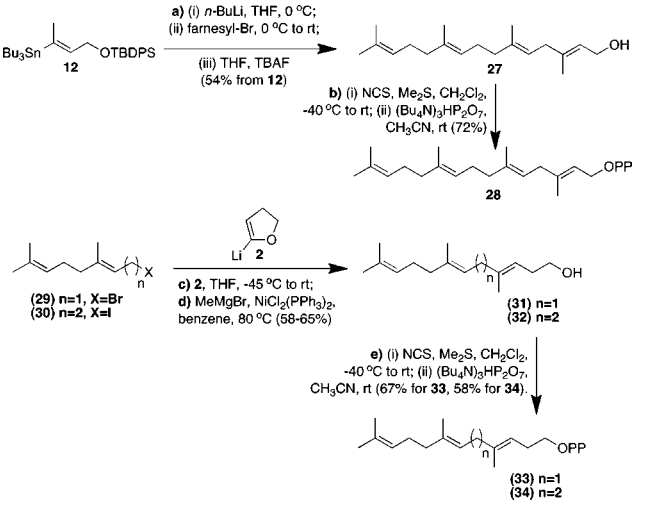
With the completion of 1,2,1-OPP and 1,3,1-OPP, we then targeted the synthesis of two FPP analogs with a shortened central isoprene unit (2,1,1-OPP and 1,1,1-OPP). Both FPP analogs contain a common 1*E*,4*E*-pentadiene structural motif. Initially, we planned on coupling the required allyl bromide with the vinyl lithium generated from **8** (Scheme 1). Unfortunately, following lithium−halogen exchange with either *t*-BuLi or *n*-BuLi, we were only able to generate the desired 1*E*,4*E*-pentadiene in very poor yield (< 10%). Several additives were tried, including copper salts, cosolvents, and Li chelators, all of which did not lead to any improvement in yields. The poor yields were later attributed to either the possibility of a lithium−silicon exchange or decomposition of the organolithium during preparation and/or an attempted coupling reaction.

These unsuccessful attempts prompted us to consider a Stille coupling between stannane **13** and geranyl bromide (**14**) (Scheme 2). Several different variations of the Stille coupling were tried; however, all reactions were found to be contaminated with the same S_N2' product which could not be separated from the desired product. In a third approach, we generated the originally desired vinyllithium via the actions of *n*-BuLi on vinyl-stannane **13** and performed the corresponding coupling with geranyl bromide (**14**) (Scheme 2). Fortunately, this third route was was found to be the superior method for generating the 1*E*,4*E*-pentadiene motif. We obtained the desired product **15** in a 54% yield. We then applied the same methodology used to generate **15** for the construction of **18** (Scheme 2). With the two alcohols **15** and **18** in hand,

Scheme 3. Synthesis of 3,1,1-OPP (**26**)



Scheme 4. Synthesis of 2,2,1,1-OPP (**28**), 2,1,2-OPP (**33**), and 2,2,2-OPP (**34**)



the standard chlorination/pyrophosphorylation procedure produced the corresponding diphosphates **19** and **20** in good yields.

We had targeted three frame-shifted FPP compounds that have the same overall length as FPP: 1,3,1-OPP, 3,1,1-OPP, and 2,1,2-OPP. With 1,3,1-OPP (**12**) prepared, we next turned our focus to the synthesis of 3,1,1-OPP (**26**), which would require an alternative approach (Scheme 3). This synthesis began with propargyl alcohol **21**, the precursor to the central isoprene unit of **26**. Following the iodination of **21**, we performed Negishi's ZACA reaction[15] to produce the iodo-alcohol **22** in a 52% yield. We then planned to install the terminal isoprene using the vinyl Grignard reagent **23**.[16] Coupling of the alkyl iodide of **22** with the vinyl Grignard reagent **23** in the presence of CuI produced **24** in a 55% yield. The geranyl analog **24** was then subjected to the same synthetic methodology developed for the synthesis of **19** and **20** (Scheme 2) for the production of 3,1,1-OPP (**26**). Corey–Kim bromination[17] of **24**, displacement of the resulting allylic bromide with the vinyl lithium derivative of **12**, and deprotection afforded alcohol **25** in a 46% yield. Conversion to the diphosphate was achieved as previously described, resulting in the synthesis of 3,1,1-OPP (**26**).

In addition to preparing molecules of similar length to FPP, we also targeted a molecule more similar to geranylgeranyl diphosphate (GGPP) in length. This molecule (2,2,1,1-OPP (**28**), Scheme 4) is only one CH_2 unit shorter than GGPP. To synthesize 2,2,1,1-OPP we started with farnesyl bromide, then performed our two-step vinyl lithium coupling/deprotection sequence (as in Scheme 2). This generated the precursor alcohol (**27**) of 2,2,1,1-OPP in a 54% yield (Scheme 4). The standard Corey–Kim chlorination/Poulter diphosphorylation procedure[18] was then applied to **27** to yield 2,2,1,1-OPP (**28**) in a 72% yield (Scheme 4).

The last two compounds targeted for synthesis were two FPP analogs containing nonallylic diphosphates (2,1,2-OPP (**33**) and 2,2,2-OPP (**34**), Scheme 4). These molecules were hypothesized to behave as nonsubstrates due to the significantly decreased leaving group ability of homoallylic diphosphate, which should not allow the prenylation of cysteine found on the CaaX sequence of FTase substrates. Homofarnesol (**32**) was previously synthesized,[12] but not converted into the corresponding diphosphate. Following Kocienski's procedure,[12] we synthesized **32** in good yield from iodide **30**. The diphosphorylation procedure developed by Davisson et al.[18] was used to successfully synthesize homofarnesyl diphosphate (**34**, Scheme 4) in excellent yield. The Wenkert–Kocienski homologation protocol allowed for the elongation of geranyl bromide **29** to alcohol **31**, the precursor to 2,1,2-OPP (**33**).

Preliminary evaluation of the eight frame-shifted FPP analogs versus mammalian FTase[7,10] revealed that four of the analogues are substrates (**11**, **12**, **26**, **28**) and four of the analogues are not accepted as substrates (**19**, **20**, **33**, **35**) (Figure 1). A preliminary inhibitory potency assay with the four nonsubstrates revealed that homofarnesyl diphosphate (**34**) was the only analog with an apparent IC_{50} below 1 μM, although an inseparable ammonium *p*-toluenesulfonate contaminant prevented the determination of an IC_{50} value. Analogs **19**, **20**, and **33** all exhibit very poor binding to FTase, demonstrating that modest changes to the FPP isoprenoid motif can lead to significant changes in binding. A more detailed evaluation of the four substrates provided further surprising effects of subtle structural changes in the isoprenoid moiety. For example, the conformationally restricted 1*E*,4*E*-pentadiene structural motif found in the "tail" of **11**, **12**, and **20** appears to translate

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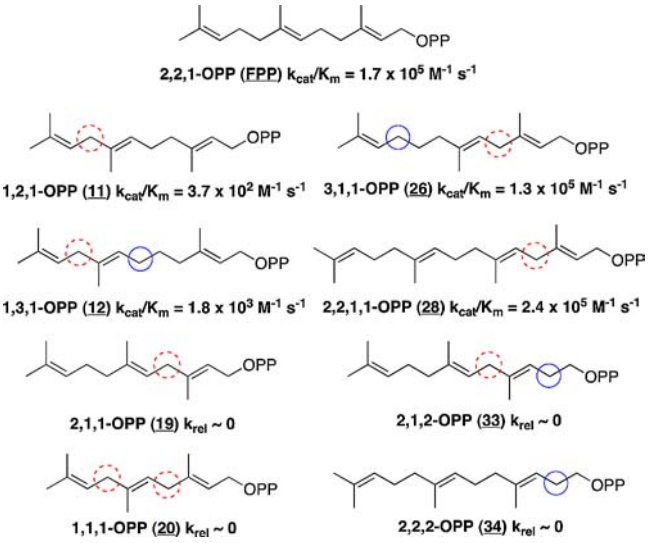

Figure 1. Evaluation of FPP analog substrate ability versus FTase (k_{rel} = relative rate in preliminary screen, with FPP = 1.0).

into either weak substrates (**11**, **12**) with k_{cat}/K_M values orders of magnitude lower than FPP or a nonsubstrate (**20**). This motif appears to destabilize analog binding to FTase, as the increased K_M values for analogs **11** and **12** are consistent with the poor binding exhibited by nonsubstrate **20**. Conversely, compounds **26** and **28**, with a methylene group removed between the first and second isoprene units, are very good substrates exhibiting k_{cat}/K_M values comparable to that of FPP with FTase. It should be noted that these comparable k_{cat}/K_M values for **26** and **28** result from ~10-fold decreases in both k_{cat} and K_M as compared to FPP. This indicates that removal of the methylene group impacts both the binding and reactivity of analogs **26** and **28**. The case of **28**, with a nor-GGPP structural motif, is particularly surprising. Our results are also in surprising contrast to the observation of substrate plasticity seen with aromatic isoprenoid FTase substrates by the Spielmann group.[19]

This study addressed the effects of increasing or decreasing both the length and flexibility of the frame-shifted FPP analogs with respect to FPP. Our results indicate that a key factor in frame-shifted FPP substrate ability was the flexibility of the isoprenoid, and in particular a lack of flexibility between the terminal double bonds in the molecule. The "nor-geranylgeranyl" analog **28** is a strikingly effective substrate, in view of the poor substrate ability of GGPP with FTase.[20] A rationale for its superior substrate ability to FPP is not clear. However, this analog may be a useful biological tool, as it could enable the prenylation of normally farnesylated proteins with a very close mimic of the geranylgeranyl group, allowing for an exploration of the biological impact of these two closely related modifications.[21] It has recently been demonstrated that tobacco 5-epi-aristolochene synthase can accept the 2*Z*,6*E*-FPP isomer as a substrate, leading to an entirely different set of cyclic sequiterpene products than the natural 2*E*,6*E*-FPP isomer.[22] In a similar manner, several of the frame-shifted analogs shown in Figure 1, and in particular diphosphates **12** and **26**, have the potential to exhibit unusual interactions with sequiterpene cyclases, perhaps leading to unique sequiterpene products.

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Supporting Information Available. Spectral data (^{1}H NMR, ^{13}C NMR, LRMS, HRMS) for all newly synthesized compounds; detailed experimental procedures for the synthesis of **3**, **5**, **8**–**11**, **13**, **15**, **24**, and **34**. ^{1}HNMR, ^{13}C NMR spectra for all compounds excluding diphosphates (**11**, **12**, **19**, **20**, **26**, **28**, **33**, and **34**); detailed protocols and V/E versus [analog] plots for determination of analog reactivity parameters with FTase. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.