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Synthesis of Non-natural, Frame-Shifted Isoprenoid Diphosphate Analogues

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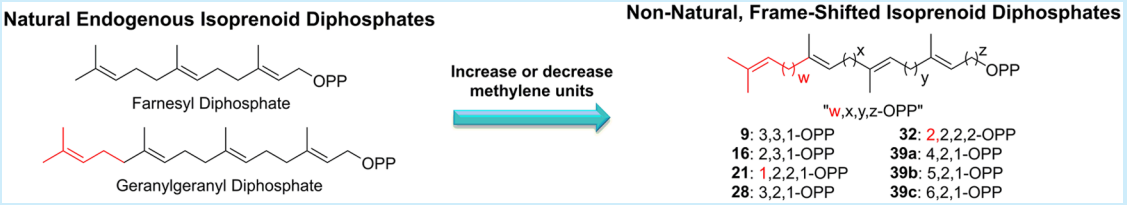
Supporting Information



ABSTRACT: A set of synthetic approaches was developed and applied to the synthesis of eight frame-shifted isoprenoid diphosphate analogues. These analogues were designed to increase or decrease the methylene units between the double bonds and/or the pyrophosphate moieties of the isoprenoid structure. Evaluation of mammalian GGTase-I and FTase revealed that small structural changes can result in substantial changes in substrate activity.

Isoprenoids are found in nearly all life forms and are the largest and most structurally diverse class of natural products.[1] As such, they are responsible for a multitude of biochemical functions, including their use as hormones (e.g., steroids, gibberellins, and abscisic acid) and roles in cell membrane structure (e.g., cholesterol), electron transfer (e.g., quinones), and photosynthesis (e.g., carotenoids).[2] As precursors to a myriad of lipid moieties, isoprenoids are important biosynthetic intermediates that lead to the production of sterols, triterpenes (e.g., squalene), carotenoids, and hopanoids.[2] Isoprenoids can also serve as lipid anchors for proteins and carbohydrates.[3] Perhaps the most interesting and complex group of isoprenoid biosynthetic products is the vast set of cyclic terpene natural products such as monoterpenes, sesquiterpenes, and diterpenes.[1b,4] Because of the extensive diversity of isoprenoid natural products, it is not surprising that many promising and effective pharmaceuticals such as Taxol (cancer), artemisinin (malaria), vinblastine (cancer), and prostratin (HIV) have been discovered.[2] Cyclic isoprenoids are not only of importance to the pharmaceutical industry but also of great interest in the materials, chemical, and fuel industries.[5] Therefore, there is significant interest in generating novel isoprenoid diphosphate analogues to use as chemical tools to further explore these multifarious processes.

Our laboratory has a long-standing interest in the design and synthesis of non-natural FPP and GGPP analogues as chemical tools to explore the enzyme specificity and requirements of FTase and, more recently, GGTase-I.[6] Prior to these studies, our laboratory developed a method for the preparation of a small library of frame-shifted FPP analogues.[7] The design of these FPP analogues increases and/or decreases the carbon spacers of the FPP backbone to examine the relevance of chain length and flexibility in relation to FTase activity. Preliminary evaluation revealed four analogues are substrates of FTase (2,2,1,1-OPP; 1,2,1-OPP; 1,3,1-OPP, 3,1,1-OPP) and one analogue, homofarnesyl diphosphate (2,2,2-OPP), is an inhibitor of FTase with an IC_{50} below 1 μM. The naming scheme refers to the number of carbon spacers between the double bonds and between the first isoprene double bond and the pyrophosphate group (Figure 1).

With the preliminary data in hand, our goal was to expand upon this theme and develop non-natural, frame-shifted isoprenoid diphosphate analogues in an effort to explore the enzyme specificity and requirements of GGTase-I versus FTase. The target compounds were 3,3,1-OPP, 2,3,1-OPP, 1,2,2,1-OPP, 2,2,2,2-OPP, 3,2,1-OPP, 4,2,1-OPP, 5,2,1-OPP, and 6,2,1-OPP. Unlike the previously synthesized frame-shifted analogues, these analogues are much more flexible and vary greatly in length between FPP and GGPP. Additionally, 6,2,1-OPP is essentially the same as GGPP with the exception that the third (γ) isoprene unit has been removed. We believe that increased flexibility may aid in binding ability.

The synthesis began with the preparation of 3,3,1-OPP (Scheme 1). By taking advantage of commercially available alkynyl alcohols represented by compound **1**, we could easily incorporate the desired carbon chain length as well as install the α-isoprene unit using Negishi's zirconium-catalyzed asymmetric carbo-alumination (ZACA) reaction. Quenching with paraformaldehyde afforded iodo alcohol **2**.[8] Similarly, 5-hexyn-1-ol (**4**) underwent a ZACA reaction and quenching with iodine yielded the vinyl iodide **5**. To install the last isoprene unit, **5** was subjected to a Swern oxidation to yield aldehyde **6** followed by a Wittig

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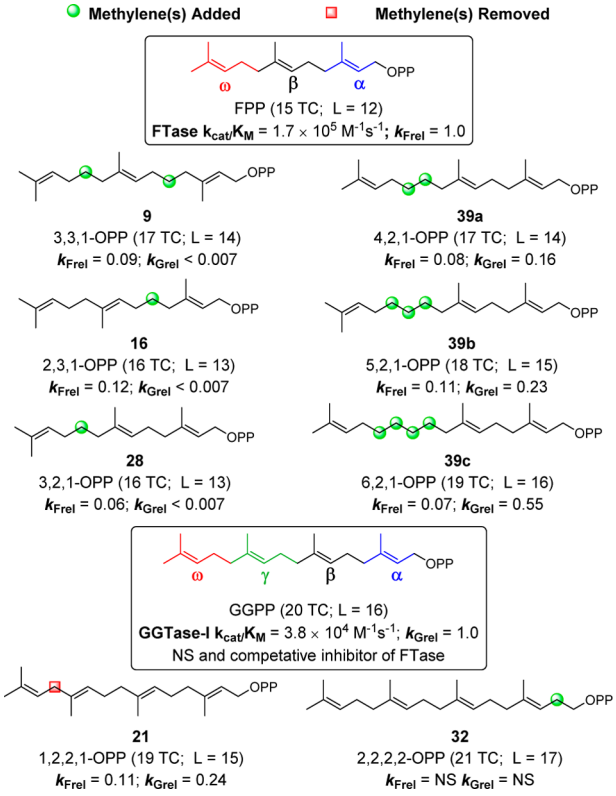
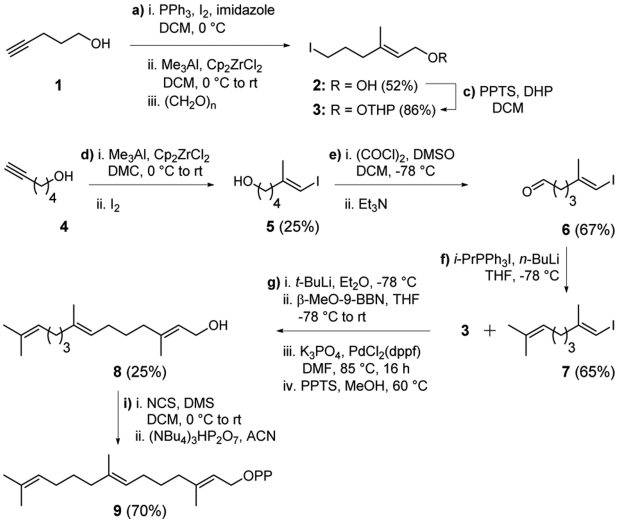


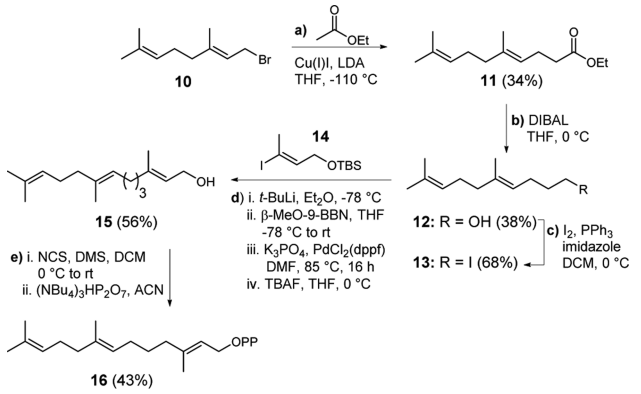
Figure 1. Evaluation of frame-shifted isoprenoid diphospate analogue substrate ability versus GGTase-I and FTase. GGTase-I assays utilized the cosubstrate dansyl-GCVLL peptide, and rates were compared to the native substrate, GGPP; k_{Grel} = relative rate derived from the preliminary GGTase-I screen by setting k_{cat}/K_M of GGPP = 1.0. FTase assays utilized the cosubstrate dansyl-GCVLS peptide, and rates were compared to the native substrate, FPP; k_{Frel} = relative rate derived from the preliminary FTase screen by setting k_{cat}/K_M of FPP = 1.0 (NS = nonsubstrate; nd = not determined; TC = total carbons; L = length of carbon chain).

Scheme 1. Synthesis of 3,3,1-OPP (9)



reaction to generate vinyl iodide **7**. Previous synthetic efforts in our laboratory relied on both Stille and Negishi couplings; however, we wished to eliminate the use of toxic tin-based reagents as well as employ more aqueous-friendly cross-coupling reactions.[7] Thus, following THP protection of alcohol **2**, alkyl iodide **3** could be converted into the organoborane and then

Scheme 2. Synthesis of 2,3,1-OPP (16)

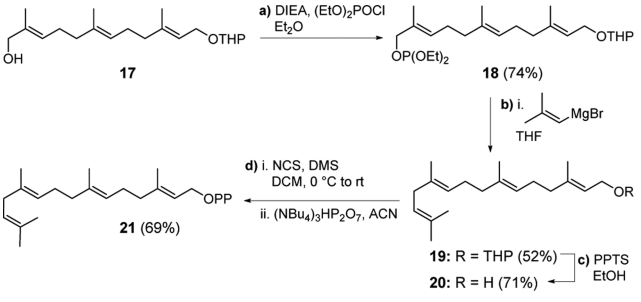


coupled with vinyl iodide **7** under Suzuki cross-coupling conditions. The THP ether was cleaved under mild acidic conditions to afford 3,3,1-OH (**8**). Chlorination and pyrophosphorylation were accomplished in a similar manner as described by Davisson et al. to produce pyrophosphate **9** (3,3,1-OPP).[9]

Attempts to synthesize compounds **16** and **21** in a similar manner as compound **9** proved unsuccessful. Shortening the carbon chain prior to aldehyde formation resulted in intermediates that quickly decomposed. Therefore, this route was abandoned in order to explore alternate methods that would allow the installation of shorter chain ω-isoprene units. To synthesize 2,3,1-OH (Scheme 2), we first synthesized alcohol, **12**, which was obtained in one step from commercially available (*E*)-geranyl bromide by employing a method first described by Kuwajima and Doi and later used in our laboratory with great success.[10] Briefly, LDA was added to an equimolar amount of ethyl acetate in the presence of Cu(I)I at −110 °C. The solution was then allowed to slowly warm to −30 °C, at which point geranyl bromide (**10**) was added to the reaction to give ester **11** in 34% yield. Subjecting the ester to DIBAL reduction followed by iodination afforded bishomogeranyl iodide **13**, which was converted into the corresponding organoborane and coupled to vinyl iodide **14** via Suzuki cross-coupling. Following deprotection of the TBS group with TBAF, alcohol **15** (2,3,1-OH) was produced in 56% yield. Subsequent chlorination and pyrophosphorylation resulted in compound **16** in moderate yield.

The strategy for the synthesis of 1,2,2,1-OH was based on the displacement of allylic diethyl phosphates with Grignard reagents as utilized by Snyder and colleagues (Scheme 3).[11] First, alcohol **17** was generated following published protocols from commercially available (2*E*,6*E*)-farnesol.[12] Next, diethyl chlorophosphate underwent a displacement reaction in the presence of alcohol **17** and DIEA to generate diethyl phosphate **18**. Previously, we found that using the diethyl phosphate derivative rather than the

Scheme 3. Synthesis of 1,2,2,1-OPP (21)



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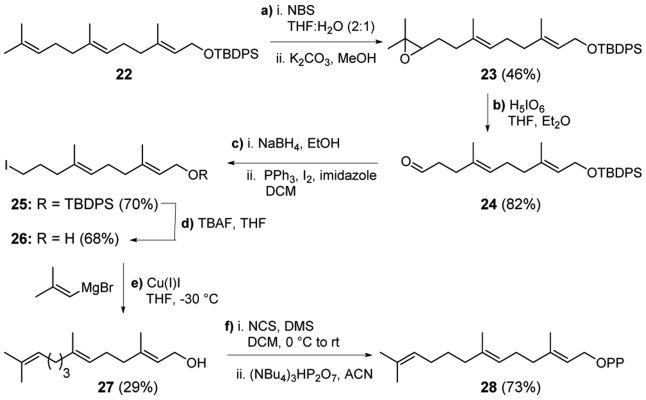
Scheme 4. Synthesis of 3,2,1-OPP (28)

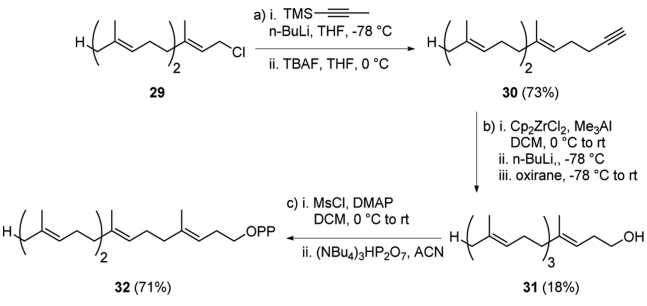


corresponding halide afforded several advantages. For example, the diethyl phosphate derivatives could be stored for longer periods. Additionally, the corresponding halides generally underwent Grignard displacement reactions to give mixtures of the S_N2 and S_N2' products that could not easily be separated via traditional chromatography methods.[6a] Displacement of the phosphate group with (2-methylprop-1-en-1-yl)magnesium bromide followed by THP deprotection produced allylic alcohol **20**. Subsequent chlorination and pyrophosphorylation resulted in compound **21**.

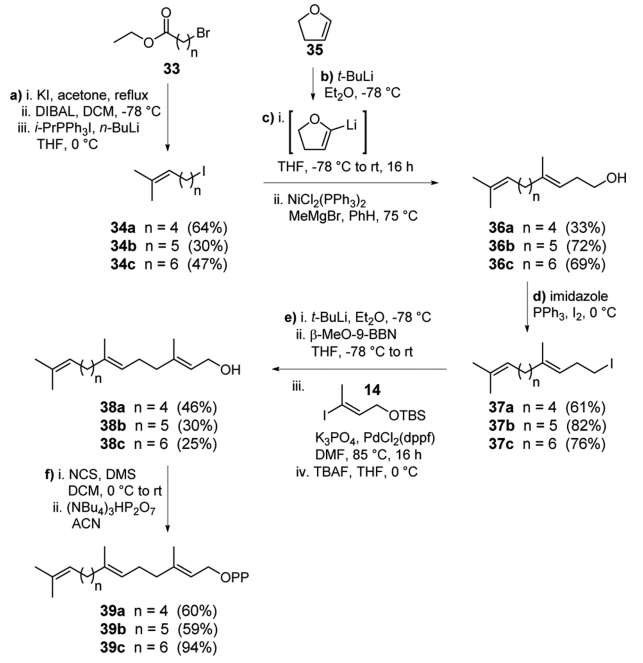
In the synthesis of 3,2,1-OH (Scheme 4), we decided to take advantage of the "2,1-OH" motif inherent to commercially available (2*E*,6*E*)-farnesol. Iodide **26** was obtained by first converting the TBDPS ether of farnesol (**22**) to epoxide **23** via a bromohydrin intermediate. Cleavage of the epoxide with periodic acid followed by sodium borhydride reduction and iodination afforded intermediate **25**, and subsequent deprotection with TBAF afforded iodo alcohol **26**. Substitution of alkyl iodide **26** was performed in the presence of Cu(I)I and (2-methylprop-1-en-1-yl) magnesium bromide to produce 3,2,1-OH (**27**).[7,13] Subsequent chlorination and pyrophosphorylation resulted in compound **28**.

We hypothesized that homogeranylgeranyl pyrophosphate would behave in a similar manner as the corresponding farnesyl derivative.[7] When choosing the route to synthesize compound **32** (Scheme 5), we wanted to explore the possibility of quenching Negishi's ZACA reaction with oxirane to generate homoallylic isoprenoid alcohols. To synthesize compound **32**, commercial (2*E*,6*E*)-farnesyl chloride (**29**) was treated with TMS-propynyl anion followed by TMS deprotection with TBAF to afford intermediate **30**. This alkyne underwent a ZACA reaction and quenching with oxirane afforded homogeranylgeraniol (**31**).[14]

Scheme 5. Synthesis of 2,2,2,2-OPP (32)



Scheme 6. Synthesis of 4,2,1-OPP, 5,2,1-OPP, and 6,2,1-OPP (39a–c)



The alcohol was first converted into the mesylate and subsequently pyrophosphorylated to yield compound **32**.[9]

Finally, we turned to synthesizing the remaining three target analogues (Scheme 6). Conversion of commercially available bromoethyl esters (**33**) into the corresponding iodoalkenes (**34a–c**) was accomplished utilizing a series of Finkelstein, DIBAL reduction, and Wittig reactions. In order to complete the unique transformation of **34** to **36**, our laboratory has successfully employed a strategy first developed by Wenkert et al. and later used by Kocienski et al.[7,15] This method relies upon the nickel-catalyzed ring opening of dihydrofurans with Grignard reagents to produce stereodefined trisubstituted alkenes. First, iodides **34a–c** underwent alkylation with 5-lithio-2,3-dihydrofuran using the modified procedure of Placzek et al.[7] Next, Suzuki cross-coupling with vinyl iodide **14** and then TBS deprotection with TBAF afforded alcohols **38a–c**. Subsequent chlorination and pyrophosphorylation produced compounds **39a–c**. Prior to the conversion to diphosphates, analysis of our frame-shifted isoprenoid alcohol precursors by ^{13}C NMR confirmed all new compounds exhibited (*E*)-olefin stereochemistry (see Supporting Information for brief discussion).

A preliminary evaluation of these frame-shifted analogues utilized an in vitro continuous spectrofluorometric assay with either FTase or GGTase-I.[16] With this data, it was evident that seven of the eight frame-shifted analogues were substrates of FTase and six were substrates of GGTase-I to varying degrees (Figure 1). The preliminary evaluation of these compounds revealed several interesting trends. The first observation was that increasing or decreasing the number of carbons by one methylene unit between the α- and β-isoprenes resulted in a significant decrease in both FTase and GGTase-I substrate activity (**16** vs **28**; **9** vs **39a**). One possible reason for this observation could be that extending the β-isoprene into the binding pocket of GGTase-I could result in unfavorable interactions between the analogue and the enzyme and/or peptide substrate. Alternatively, changes in flexibility of the frame-shifted analogues could potentially hinder

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product release from the enzyme, which is dependent on the movement of the isoprene chain into an exit groove.

The second observation is that the length of the carbon chain appears to be more important than flexibility in regard to GGTase-I substrate activity. Comparing compounds of 15 carbons in length, **39b** (k_{Grel} = 0.23; k_{Frel} = 0.11) lacks the γ-isoprene unit of **21** (k_{Grel} = 0.24; k_{Frel} = 0.11); however, both analogues have very similar reactivity. Thus, the lack of flexibility in the ω-isoprene of **21** seems not to be an important factor. The similarity between these two compounds also indicates the γ-isoprene is not required to produce substrate activity.

Moreover, when an analogue has two carbons between the α- and β-isoprene units (i.e. y = 2), increasing the carbon chain length from 13 to 15 carbons has a more pronounced effect on GGTase-I activity versus FTase (**28** vs **39b**). Increasing the length to 16 carbons (**39c**) has a ~3-fold increase in k_{cat}/K_M for GGTase-I substrate activity; however, this trend does not carry over to FTase. Analogue **39c** also reveals the γ-isoprene unit of GGPP is not necessary for enzyme recognition but is desirable for higher enzyme turnover. Adding a methylene unit between the diphosphate moiety and the α-isoprene unit led to the homoallylic analogue **32**. Preliminary data suggests analogue **32** is an inhibitor of GGTase-I.

This study addresses the effects of decreasing or increasing both flexibility and length of non-natural, frame-shifted isoprenoid diphosphate analoges. Our results indicated that a key factor in frame-shifted isoprenoid diphosphate substrate reactivity was the chain length and the position of the β-isoprene unit (y = 2 vs y = 3). Further analysis would reveal if these analogues could be selective substrates, meaning that although these analogues are poor co-substrates with dansyl-GCVLL in our preliminary GGTase-I assay they could potentially be great co-substrates with other CaaX sequences.

We successfully generated a small library of novel frame-shifted isoprenoid diphosphate analogues originally designed to probe the isoprenoid binding pockets of GGTase-I and FTase; however, these analogues and the synthetic routes described in this work also provide researchers with unique non-natural isoprenoids to further explore the limits of terpene cyclases. Terpene cyclases catalyze the key cyclization step in the biosynthesis of the largest class of natural products, terpenoids.[17] Several non-natural isoprenoids have been shown to serve as substrates for terpene cyclases resulting in unique cyclic products.[18] In a similar manner, several of the frame-shifted analogues shown in Figure 1 could potentially exhibit unusual interactions with terpene cyclases, perhaps allowing future researchers to develop unique cyclic terpenoid products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02977.

Spectral data (^{1}H NMR, ^{13}C NMR, ^{31}P NMR, LRMS, HRMS) for all newly synthesized compounds; detailed experimental procedures for the synthesis of **9**, **16**, **21**, **28**, **32**, **39a**−**39c**. Detailed protocols for determination of analog reactivity parameters with FTase and/or GGTase-I (PDF)

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Notes

The authors declare no competing financial interest.

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