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Synthesis of Non-Natural, Frame-Shifted Isoprenoid Diphosphate Analogs

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Abstract

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

Graphical abstract

Isoprenoids are found in nearly all life forms and are the largest and most structurally diverse class of natural products.^{1,2} 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).³ 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.³ Isoprenoids can also serve as lipid anchors for proteins and carbohydrates.^{4,5} Perhaps the most interesting and complex

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Supporting Information

Spectral data (¹H NMR, ¹³C NMR, ³¹P NMR, LRMS, HRMS) for all newly synthesized compounds; detailed experimental procedures for the synthesis of **9**, **16**, **21**, **28**, **32**, **39a**, **39b**, and **39c**. ¹H NMR and ¹³C NMR spectra for all compounds excluding diphosphates; ¹H NMR and ³¹P NMR spectra for all diphosphates; detailed protocols for determination of analog reactivity parameters with FTase and/or GGTase-I. The Supporting Information is available free of charge on the ACS Publications website.

Author Contributions

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group of isoprenoid biosynthetic products is the vast set of cyclic terpene natural products such as monoterpenes, sesquiterpenes, and diterpenes.^{2,6} Due to 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.³ Not only are cyclic isoprenoids of importance to the pharmaceutical industry, but they are also of great interest in the materials, chemical, and fuel industries.⁷ Therefore, there is significant interest in generating novel isoprenoid diphosphate analogs 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 analogs as chemical tools to explore the enzyme specificity and requirements of FTase and GGTase-I. Recently, our laboratory explored the GGTase-I substrate requirements by evaluation of aryl and saturated GGPP analogs.^{8,9} Prior to these studies, our laboratory developed a method for the preparation of a small library of frame-shifted FPP analogs.¹⁰ The design of these frame-shifted FPP analogs increase and/or decrease 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 of the eight FPP frame-shifted analogs are substrates of FTase (2,2,1,1-OPP; 1,2,1-OPP; 1,3,1-OPP, 3,1,1-OPP) and one analog, homofarnesyl diphosphate (2,2,2-OPP), was an inhibitor of FTase with an IC₅₀ below 1 μ M. This inhibitory activity is believed to be attributed to the lower nucleophilicity at the non-allylic C₁ (the C attached to the -OPP). 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 analogs 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 analogs, these analogs 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 between the α - and β -isoprene units as well as install the α -isoprene unit using Negishi's zirconium-catalyzed asymmetric carbo-alumination (ZACA) reaction. To begin, 4-pentyn-1-ol (**1**) was transformed into the corresponding iodide which underwent Negishi's ZACA reaction and quenched with paraformaldehyde to afford iodo-alcohol **2**.¹¹ Similarly, 5-hexyn-1-ol (**4**) underwent a ZACA reaction and was quenched with iodide to yield the vinyl-iodide **5**. To install the last isoprene unit, **5** was subjected to Swern oxidation conditions to yield aldehyde **6** followed by a Wittig reaction to generate vinyl-iodide **7**. Previous efforts in our laboratory to generate frame-shifted FPP analogs 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.¹⁰ Thus, following THP-protection of alcohol **2**, alkyl iodide **3** could be converted into the

organoborane and then 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 was accomplished in a similar manner as described by Davisson *et al.* to produce pyrophosphate **9** (3,3,1-OPP).^{12,13}

Attempts to synthesize compounds **16** and **21** in a similar manner as compound **9** via aldehyde formation followed by a Wittig reaction proved unsuccessful. Decreasing the number of carbons between the isoprene unit and the aldehyde from three 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 bishomogeranyl 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.^{14,15} Briefly, LDA was added to an equimolar amount of ethyl acetate in the presence of Cu(I)I at $-110\text{ }^{\circ}\text{C}$. The solution was then allowed to slowly warm to $-30\text{ }^{\circ}\text{C}$ 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 utilized by Snyder and colleagues (Scheme 3).¹⁶ First, alcohol **17** was generated following published protocols from commercially available (2E, 6E)-farnesol.¹⁷ Next, diethyl chlorophosphate underwent a displacement reaction in the presence of alcohol **17** and DIEA to generate diethyl phosphate **18**. Previously, we found using the diethyl phosphate derivative rather than the 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 $\text{S}_{\text{N}}2$ and $\text{S}_{\text{N}}2'$ products that could not easily be separated via traditional chromatography methods.⁸ 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 (2E,6E)-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 borohydride reduction and iodination afforded intermediate **25** and subsequent deprotection with TBAF afforded iodo alcohol **26**. Substitution of alkyl iodide **26** was done in the presence of Cu(I)I and (2-methylprop-1-en-1-yl) magnesium bromide to produce 3,2,1-OH (**27**).^{10,18} Subsequent chlorination and pyrophosphorylation resulted in compound **28**.

One compound targeted for synthesis was a GGPP analog containing a nonallylic diphosphate. We hypothesized that homogeranylgeranyl pyrophosphate will behave in a

similar manner as the corresponding farnesyl derivative.¹⁰ When choosing the route to synthesize compound **32**, we wanted to explore the possibility of quenching Negishi's ZACA reaction with oxirane to generate homoallylic isoprenoid alcohols. To synthesize homogeranylgeranyl pyrophosphate (2,2,2,2-OPP, **32**), 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**).^{19,20} The alcohol was first converted into the mesylate and subsequently pyrophosphorylated to yield compound **32**.^{12,13}

With the above alcohols in hand, we turned to synthesizing the remaining three target analogs (Scheme 6). Conversion of commercially available bromo-ethylesters (**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 and colleagues and later used by Kocienski and colleague.^{10,21-23} In order to transform the carbon-oxygen bonds into carbon-carbon bonds, this method relies upon the nickel-catalyzed ring opening of dihydrofurans with Grignard reagents to produce stereo-defined trisubstituted alkenes. First, iodides **34a-c** underwent alkylation with 5-lithio-2,3-dihydrofuran using a modified procedure of Placzek and coworkers.¹⁰ The newly prepared alkylated dihydrofuran was immediately treated with MeMgBr in the presence of NiCl₂(PPh₃)₂ to produce alcohols **36a-c**. Conversion of the corresponding iodides (**37a-c**) to their organoborane derivatives followed by 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**.

Previously, Yu and coworkers synthesized the four olefin stereoisomers of farnesol and identified each isomer based on comparisons of ¹³C NMRs with literature values. Identification was then verified using HMBC experiments.³² This study, as well as known literature values, allow us to readily identify the olefin stereochemistry of our frame-shifted isoprenoid compounds based on ¹³C NMR spectra. Most telling are the C-4 and C-8 ¹³C NMR chemical shifts of farnesol (as well as the C-12 of geranylgeraniol). If the neighboring olefin has *cis* stereochemistry, the chemical shifts observed are between 31-33 ppm when taken in CDCl₃; however, *trans* stereochemistry affords a noticeable difference with chemical shifts between 38-40 ppm. Prior to the conversion to diphosphates, analysis of our frame-shifted isoprenoid alcohol precursors by ¹³C NMR confirmed all new compounds exhibited *E*-olefin stereochemistry.

A preliminary evaluation of these frame-shifted analogs utilized an *in vitro* continuous spectrofluorometric assay with either FTase or GGTase-I.^{24,25} With this data, it was evident that seven of the eight frame-shifted analogs were substrates of FTase and six were substrates of GGTase-I to varying degrees (Figures 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 analog

and the enzyme and/or peptide substrate. Alternatively, changes in flexibility of the frame-shifted analogs could potentially hinder product release from the enzyme, which is dependent on the movement of the isoprene chain into an exit groove.

The second observation is the length of the carbon chain appears to be more important than flexibility in regards 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 analogs 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 analog has two carbons between the α - and β -isoprene units, 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. Analog **39c** also reveals the γ -isoprene unit of GGPP is not necessary for enzyme recognition but it is desirable for higher enzyme turnover. Adding a methylene unit between the diphosphate moiety and the α -isoprene unit led to the homoallylic analog **32**. Preliminary data suggests analog **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 analogs. 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. Further analysis would reveal if these analogs could be selective substrates; meaning that although these analogs 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 analogs originally designed to probe the isoprenoid binding pockets of GGTase-I and FTase; however, these analogs 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.²⁶ Several non-natural isoprenoids have been shown to serve as substrates for terpene cyclases resulting in unique cyclic products.^{27–31} In a similar manner, several of the frame-shifted analogs shown in Figure 1 could potentially exhibit unusual interactions with terpene cyclases, perhaps allowing future researchers to develop unique cyclic terpenoid products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Evaluation of frame-shifted isoprenoid diphosphate analog substrate ability versus GGTase-I and FTase. GGTase-I assays utilized the co-substrate 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 co-substrate 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**).

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

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

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

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

Scheme 6.

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