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New Synthetic Methodology for the Construction of 7-Substituted Farnesyl Diphosphate Analogs

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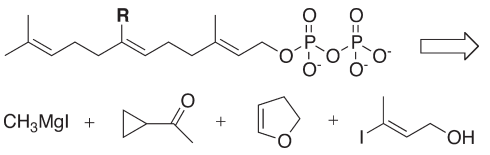
ABSTRACT



Through the use of a 1,2-metalate rearrangement, six 7-substituted farnesol analogs were generated in a concise manner. This new synthetic route allowed us to quickly prepare several diverse farnesyl diphosphate analogs with interesting biological activities against mammalian protein-farnesyl transferase.

Isoprenoid diphosphates play vital roles in many cellular processes, such as cholesterol biosynthesis, protein prenylation, and as substrates for sesquiterpene cyclases in plants. The post-translational prenylation of proteins is a required event in the targeting and function of many biologically important proteins. Farnesyl protein transferase (FTase) catalyzes the attachment of a 15 carbon isoprenoid moiety from (farnesyl diphosphate) onto over 60 targets bearing a carboxyl-terminal Ca_1a_2X sequence, including many signaling proteins that play a key role in cancer.[1] With the high prevalence of farnesylated proteins found in cancer, there is a need to characterize the biological activities of farnesylated proteins. The overall goal of this project is to devise new chemical tools that will enable a method to distinguish between the biological activities of different farnesylated proteins. The ability to differentiate the biological impact of one farnesylated protein over another may lead to the development and design of more powerful and robust anticancer therapeutics.

The crystal structure of FTase reveals that FPP adopts a conformation in the enzyme that leads to an interaction between the 7 position of the isoprenoid and the a_2 residue of the incoming peptide substrate.[2] Based on the crystal structure and the known conformational changes in the FTase active site, we evaluated several 7-substituted FPP compounds against a library of CaaX-containing peptides.[3,4] The biochemical screening revealed several 7-substituted FPP analogs that selectively farnesylated certain CaaX-box-containing peptides but not others.[4] Based on these previous results, there is a need for a larger and more diverse library of 7-substituted FPP compounds.

Previously we used the synthetic methodology for the synthesis of 7-substituted FPP analogs developed by Rawat and Gibbs.[3] This synthesis was highlighted by two consecutive rounds of vinyl triflate-mediated chain-elongation sequences to successfully complete 7-substituted FPP compounds in 10 linear steps. While successful, this route has several drawbacks. It is linear, with the diversity element (the 7-substituent) installed early in the route. Second, the triflimide reagent needed for each isoprenoid homologation step is expensive and utilized in excess. To facilitate an increase in the size and diversity of the 7-substituted FPP library we developed a new approach that would reduce the number of linear steps. This methodology

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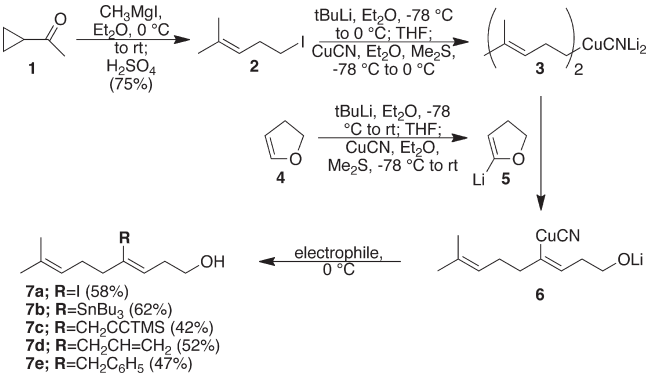
would also allow us to access other centrally modified farnesyl diphosphate analogs.

After an extensive investigation of various synthetic approaches that could lead to the synthesis of 7-substituted FPP compounds, we focused on a route that utilizes substituted dihydrofuran molecules for installing the 7-substituents into the farnesyl structure. The synthesis of trisubstituted olefins from a Ni-(0)-catalyzed coupling of 2,3-dihydrofurans with Grignard reagents was first reported by Wenkert and colleagues[5] and more extensively studied by Kocienski.[6] Kocienski and colleagues reported a copper(I)-catalyzed coupling of Grignard reagents and organolithiums with 5-lithio-2,3-dihydrofuran results in trisubstituted olefins[7,8] in a straightforward and stereoselective manner. Therefore, we applied the synthesis of 4-homogeraniol derivatives to the generation of substituted farnesyl analogs.

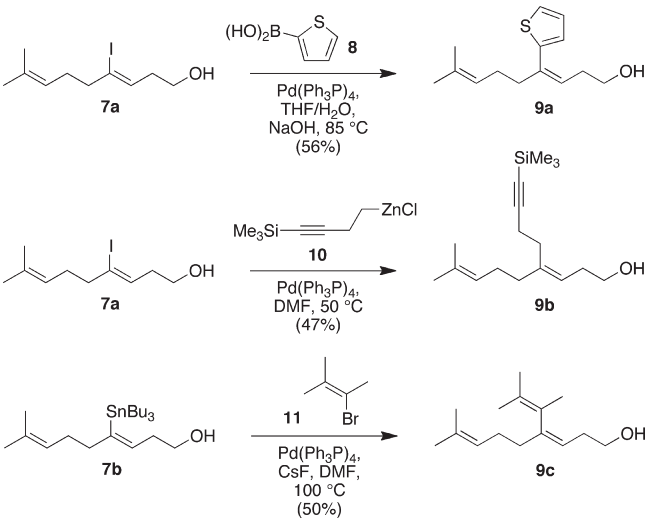
Scheme 1. Synthesis of 4-Homogeraniol Derivatives



To begin the synthesis of 4-homogeraniol derivatives (Scheme 1), we first prepared homoprenyl iodide (**2**) from cyclopropyl methyl ketone in a 75% yield.[9] Subsequent lithium-halogen exchange, followed by the addition of CuCN, resulted in **3**. With **3** in hand, we generated 5-lithio-2,3-dihydrofuran (**5**) from the action of *t*-BuLi on 2,3-dihydrofuran (**4**). Attempts to replicate the 1, 2-metalate rearrangement resulting from the addition of **3** to **5**, as reported by Kocienski and colleagues[8] for the synthesis of 4-homogeraniol derivatives, were largely unsuccessful because of the decomposition of organocuprate **3**. The problem was resolved when dimethylsulfide was added as a cosolvent, which presumably stabilizes the organocuprate, and as a result the expected 1,2-metalate rearrangement took place.[10] The 1,2-metalate rearrangement led to the production of the higher order alkenylcuprate **6**, a versatile intermediate for the synthesis of 7-substituted FPP analogs. The coupling of **6** with a variety of electrophiles ($SnBu_3Cl$, I_2, TMS-propargyl bromide, and allyl bromide) was achieved by recooling the solution of alkenylcuprate (**6**) to 0 °C and adding in the appropriate electrophiles. This led to the formation of 4-substituted homogeraniol derivatives (**7a**–**e**) in moderate yields (42–62%). Despite the modest yields, utilization of this synthetic transformation is beneficial in the synthesis of farnesol derivatives because it allows for the transformation of readily available starting materials into advanced synthetic intermediates in one step. We next focused on introducing various substituents into iodide **7a** and stannane **7b** that would eventually become the 7-substituent on the corresponding farnesyl diphosphate analog.

The facile nature of the Suzuki–Miyaura reaction and the commercial availability of a large library of organoboranes prompted us to examine their cross coupling of organoboranes with **7a**. We used the standard Suzuki–Miyaura coupling conditions[11] in which 2-thienyllboronic acid (**8**) was successfully coupled to vinyl iodide **7a** to result in **9a** in a 56% yield (Scheme 2). Similar Suzuki–Miyaura couplings should allow for the future installment of diverse aromatic and vinyl substituents at the 7-position of FPP. Once the methodology was developed to enable the synthesis of 7-substituted FPP compounds with aryl and vinyl moieties at the 7-position, we next examined methodology that would enable us to install alkyl moieties at the 7-position. A Negishi coupling was envisioned to replace the iodide of **7a** with a variety of commercially available or readily prepared alkyl zinc reagents.[12] The organozinc reagent **10** was prepared through a lithium-halogen exchange of

Scheme 2. Pd-Catalyzed Coupling Reactions with 4-Substituted Homogeraniol Derivatives



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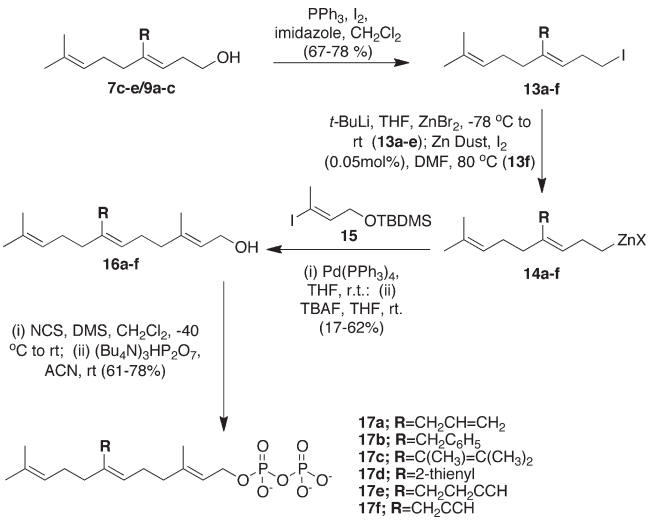
1-iodo-4-trimethylsily-1-butyne with *t*-BuLi, followed by the addition of a zinc chloride solution in THF. With **13** in hand, we employed a Negishi cross coupling procedure,[12] to join **7a** and the organozinc **10** resulting in the synthesis of the alkyl substituted 4-homogeraniol derivative **9b**. The vinyl stannane **7b** would serve to provide 4-substituted homogeraniol derivatives via the Stille reaction with commercially available vinyl and aryl halides. One substituent we wished to install at

Scheme 3. Conversion of 4-Homogeraniol Derivatives into Their Corresponding 7-Substituted FPP Analogs



the 7-position of FPP was the 1,3-dimethylpropenyl moiety. Using CsF as an additive, we successfully coupled **7b** to **11** using $Pd(PPh_3)_4$, in DMF to produce **9a** in a 50% yield (Scheme 2).

Once the synthesis of the 4-substituted homogeraniol derivatives (**7c**−**e** and **9a**−**c** (Schemes 1 and 2)) was complete, we performed the final steps necessary to generate 7-substituted FPP analogs (Scheme 3). Transformation of the homogeraniol derivatives **10c**−**e** and **11a**−**c** into the resulting iodides **13a**−**f** was accomplished in good yields.[13] With the alkyl iodides **13a**−**f** in hand, we next focused our attention on converting the alkyl iodides into their corresponding organozinc reagents. To achieve this, we employed two different procedures depending on the substituent at the four position of our homogeraniol derivatives. We successfully employed the same strategy to prepare the organozinc derivatives **14a**−**c, e**−**f** as were generated as previously described for the construction of **10** (Scheme 2). Preparation of organozinc derivative **14d**

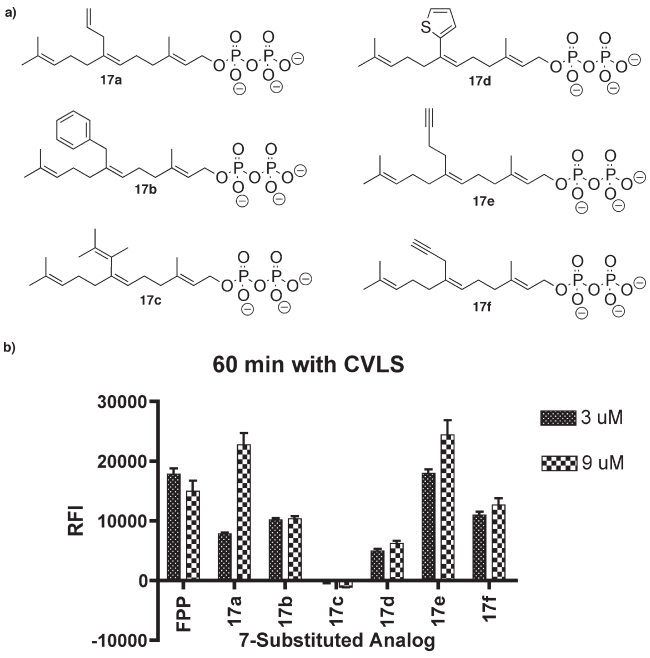


Figure 1. Structures and substrate activity of 7-substituted FPP analogs. (a) Structures of synthesized 7-substituted diphosphates. (b) Substrate activity of 7-substituted FPP analogs with dansyl-GCVLS.

was unsuccessful with the harsh conditions employed for constructing **10**. The slightly acidic proton or the conjugation of the thiophene to the double bond of the homogeraniol backbone may have caused unwanted side reactions with *t*-BuLi, thus preventing the preparation of **14d**. In order to circumvent this problem, we followed a procedure developed by Huo to convert unactivated halides into the corresponding organozincs without the use of strong bases,[14] which resulted in the preparation of the organozinc intermediate **14d**. The rest of the organozinc derivatives (**14a**−**c** and **14e**−**f**) were generated as previously described for the construction of **10** (Scheme 2). Vinyl iodide **15**[15,16] was then added to the organozinc solution of **14a**−**f**, followed by the addition of $Pd(PPh_3)_4$ at room temperature resulting in the synthesis of 7-substituted-farnesol derivatives (**16a**−**f**). With the 7-substituted farnesol derivatives synthesized we next converted them into their corresponding diphosphates (**17a**−**f**) by employing a Corey−Kim chlorination[17] of the allylic alcohol, followed by displacement of the resulting chloride with tetrabutylammonium diphosphate (Scheme 3).[18] Previously, our laboratory has shown that 7-substituted FPP analogs can display diverse biochemical activity with FTase and a

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variety of CaaX-peptides.[4] The 7-substituted FPP compounds synthesized in this study (**17a**−**f**, Figure 1a) were initially evaluated for their biological activity in an *in vitro* fluorescence-based assay[4] with FTase and the CaaX-peptide, dansyl-GCVLS (H-Ras). Among the 7-substituted compounds assayed for their *in vitro* biological activity, compounds **17 a,b,e,f** were found to behave as efficient substrates (Figure 1b). In addition to the substrate activity of the newly synthesized 7-substituted compounds, one compound (**17c**) was found to act as a weak inhibitor with an IC_{50} of 25 μM against FTase. Of particular importance are the substrate activities of the two alkynyl FPP compounds **17e** and **17f** with dansyl-GCVLS. Both compounds are efficient substrates as demonstrated in the fluorescence assay and in an HPLC confirmation assay (data not shown). These compounds were designed with the goal to be used as chemical tools for the tagging of prenylated proteins via "click chemistry".[19,20] This strategy has proved valuable for the dissection of the cellular biology of various ligands with protein targets,[21−24] including FTase.[25,26]

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The synthetic approaches developed in this study for the synthesis of 7-substituted FPP analogs have been used to develop several diverse FPP analogs with biological activities. The original synthetic plan[3] for these compounds required eleven steps for the completion of 7-allylfarnesyl diphosphate (**17a**; overall yield = 7%). The newly developed strategy allows for the rapid and efficient synthesis of 7-substituted FPP analogs; specifically, 7-allyl analog **17a** was prepared in six steps with a 12% overall yield. In addition to significantly shortening the route, we have also provided methodology that allows for the construction of FPP analogs unachievable by previous methods.

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Supporting Information Available. Spectral data (^{1}H NMR, ^{13}C NMR, LRMS, HRMS) for all newly synthesized compounds; detailed experimental procdedures for the synthesis of **2**, **7a**, **9a**−**c**, **13a**, **15**, **16a**, **16d**, and **17a**. ^{1}H and ^{13}C NMR spectra for all compounds excluding diphosphates (**17a**−**f**). This material is available free of charge via the Internet at http://pubs.acs.org.