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# Exploration of GGTase-I substrate requirements. Part 1: Synthesis and biochemical evaluation of novel aryl-modified geranylgeranyl diphosphate analogs

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ABSTRACT

Protein geranylgeranylation is a type of post-translational modification that aids in the localization of proteins to the plasma member where they elicit cellular signals. To better understand the isoprenoid requirements of GGTase-I, a series of aryl-modified geranylgeranyl diphosphate analogs were synthesized and screened against mammalian GGTase-I. Of our seven-member library of compounds, six analogs proved to be substrates of GGTase-I, with **6d** having a $k_{rel}$ = 1.93 when compared to GGPP ($k_{rel}$ = 1.0).


Many proteins undergo prenylation, a type of posttranslational modification, which localizes the proteins to the plasma membrane.[1,2] Proteins such as small Ras and Rho GTPase superfamilies, nuclear lamins, and the kinesin motor proteins require prenylation in order to become biologically functional.[3]

Protein prenylation occurs on a cysteine four residues from the C-terminus. Prenylated proteins contain a C-terminal 'CaaX box' sequences, where 'C' denotes cysteine, 'a' is typically an aliphatic amino acid, and 'X' represents a small subset of amino acid residues.[4] This tetrapeptidic sequence allows recognition by prenyl transferase enzymes located in the cytosol and subsequent enzymatic catalysis to form a thioether bond between the Cys residue of the CaaX box and isoprenyl lipids.[5] There are two CaaX prenyltransferases in mammalian cells: (1) farnesyl transferase (FTase) catalyzes the covalent attachment of a 15-carbon farnesyl isoprenoid (farnesyl pyrophosphate, FPP) and (2) geranylgeranyl transferase-I (GGTase-I) catalyzes the attachment of a 20-carbon geranylgeranyl isoprenoid (geranylgeranyl pyrophosphate, GGPP) to cysteine (Fig. 1). After covalent attachment of the isoprenoid (s), the protein relocates to the endoplasmic reticulum where it undergoes proteolytic cleavage of the '-aaX' residues by the endoprotease Ras-converting enzyme-1 (Rce-1) followed by methyl-esterification by isoprenylcysteine carboxyl methyl transferase (Icmt). Upon completion of these modifications, the newly isoprenylated protein can be anchored in the plasma membrane and regulate various cellular functions.[6] (Fig. 1).

Estimations approximate that 0.5–2% of all mammalian proteins are prenylated; however, roughly only 60 proteins have been identified thus far.[7,8] Of the known prenylated proteins, many exhibit a plethora of cellular functions including cell signaling, cell mobility, cell division, organelle structure, and vascularization. Thus, targeting protein prenylation may prove to be a potential treatment not only for cancer but for a wide variety of other diseases as well.[9–15]

Many FTase studies have stemmed from structural investigations of FTase by the Beese group. Their work unveiled a hydrophobic binding pocket rich with aromatic amino acid residues such as Tyr, Trp, and Phe.[16–18] This sparked many researchers, our group included, to explore the possibility of pi–pi stacking interactions between these aromatic amino acids and FPP analogs containing aromatic motifs.[3,19–21] While these aryl-modifications have been greatly explored in relation to FTase, little has been done to investigate these modifications in relation to GGTase-I binding ability.

Previously, our laboratory has concentrated on generating GGPP analogs containing substitutions at the 3 and/or 7 positions of

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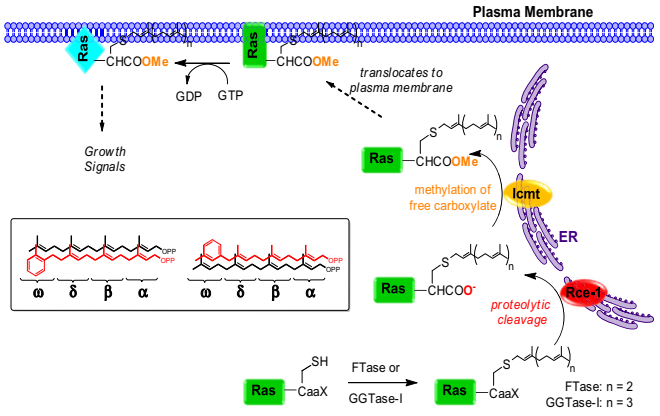


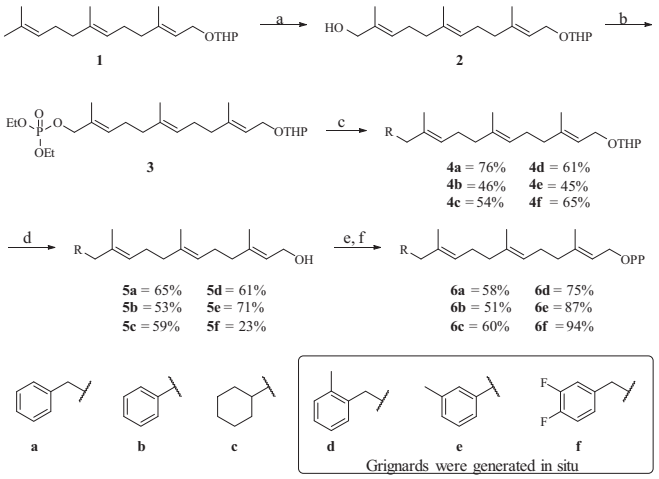

**Figure 1.** Ras protein prenylation pathways. Box: Overlay of GGPP (black) and aryl-modified analogs (red) is depicted.

GGPP.[22,23] Some of these analogs have been shown to act as efficient substrates of GGTase-I while others have high nanomolar $IC_{50}$ values. In order to investigate greater structural diversity in GGPP analogs, we synthesized a series of aryl ω-modified GGPP analogs. Some of the aromatic residues (W102, Y361) in FTase correspond to non-aromatic resides in GGTase-I (T49, Y361, respectively) in order to allow for a more spacious binding pocket to accommodate the longer isoprene chain of GGPP; however, structural studies have revealed that GGTase-I does have a hydrophobic binding pocket bountiful with aromatic residues.[5,24] The potential of aryl-containing GGPP analogs to participate in pi–pi stacking interactions with the aromatic amino acid residues of the GGTase-I binding pocket prompted us to synthesize and evaluate a small library of aryl-modified GGPP analogs.

When considering which analogs to evaluate, our goal was to select analogs that best mimicked the terminal isoprene unit. The two analogs that best simulate the isoprene unit both contain methyl-substituted benzene rings (Fig. 1). Analogs **6a** and **6b** also aligned well with GGPP, and though they lack the additional $CH_3$ of **6d**–**e**, both of these analogs provide the double bond of the terminal isoprene unit. Comparing the catalytic efficiency of analogs **6a**–**b** with **6d**–**e** may provide great insight into whether methyl substitution of the aromatic ring is beneficial. Analog **6c** was included to determine if hydrophobic bulk would be sufficient for GGTase-I catalysis or, as we hypothesize, if aromaticity would be more beneficial. Compound **6f** was chosen to evaluate the effects of electronics on the aromatic ring on catalytic efficiency.

Recently, our lab has synthesized a potent Icmt inhibitor designated 'TAB.'[25] This methyltransferase accepts both farnesylated and geranylgeranylated proteins that have been proteolyzed by Rce1 as substrates for methylation. Although a crystal structure has yet to be determined, it stands to reason that Icmt and the CaaX prenyltransferases have similar prenyl-binding pockets. Therefore, we wished to evaluate the corresponding diphosphate, **15**, as a potential inhibitor of GGTase-I to test this hypothesis and gain more insight into prenyl substrate binding requirements.

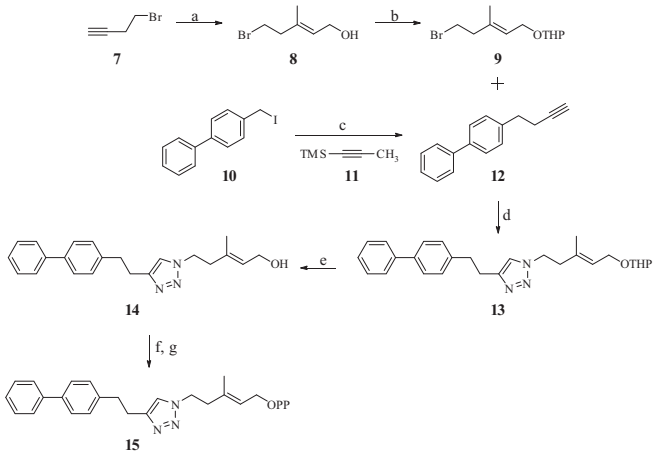
The synthesis of the aryl-modified GGPP analogs was designed in such a way that all compounds could be generated from a common intermediate, **3** (Scheme 1). Additionally, the availability of a wide variety of commercially available Grignard reagents and benzylic/phenylic halides in addition to the ease of introduction of the aryl-motifs motivated us to explore this synthetic route. To begin the synthesis, THP-protected farnesol (**1**) underwent oxidation in the presence of $SeO_2$ followed by a $NaBH_4$ reduction to generate alcohol **2**.[26–28] Next, diethyl chlorophosphate is subjected to a displacement reaction in the presence of **2** and DIEA to generate diethyl phosphate **3** in 74% yield. There were a few advantages of




**Scheme 1.** Synthesis of aryl-modified GGPP analogs. (a) i. DHP, PPTS, DCM; ii. $SeO_2$, t-BuOOH, salicylic acid, DCM; iii. $NaBH_4$, EtOH (37% – 3 steps); (b) DIEA, $(EtO)_2POCl$, $Et_2O$ (74%); (c) R-MgX, THF, 22 h; (d) PPTS, EtOH, 70 °C; (e) NCS, DMS, DCM, 2.5 h.; (f) $(NBu_4)_3HP_2O_7$, ACN, 3 h.

choosing this type of intermediate. One advantage to using diethyl phosphate **3** is that it can be stored for longer periods of time than the corresponding allylic halides which are unstable and easily degrade. More so, the corresponding allylic halides generally undergo Grignard displacement reactions to give a mixture of $S_N2$ and $S_N2'$ products usually in fairly equal quantities and isolations of one isomer are not facile.[29] Thus, with common intermediate **3** in hand, a similar method as Snyder and Treitler was employed and a variety of Grignard reagents could be utilized in an $S_N2$ displacement reaction to generate the aryl-modified analogs **4a**–**f**.[30] These analogs were first deprotected using PPTS in EtOH to generate alcohols **5a**–**f** and then converted into the corresponding pyrophosphates (**6a**–**f**) utilizing the method of Davisson et al.[31,32]

The synthesis of the 'TAB-pyrophosphate' **15** was accomplished according to the procedure of Bergman et al (Scheme 2).[25] It began with the conversion of 4-bromobut-1-yne (**7**) to alcohol **8** using Negishi's zirconium-catalyzed asymmetric carbo-alumination (ZACA) reaction.[33] Next, alcohol **8** was THP-protected using a standard procedure to generate compound **9**. The second half of the




**Scheme 2.** Synthesis of 'TAB' pyrophosphate. (a) $Me_3Al$, $Cp_2ZrCl_2$, DCM, 0 °C, 18 h then $(CH_2O)_n$, 3 h (83%); (b) PPTS, DHP, DCM (79%); (c) i. TMS-propyne, *n*-BuLi, THF, −78 °C; ii. $K_2CO_3$, MeOH, 12 h (36% – 2 Steps); (d) $NaN_3$, $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, DMF, 55 °C (20%); (e) PPTS, EtOH, 70 °C (85%); (f) MsCl, DMAP, DCM, 2.5 h; (g) $(NBu_4)_3HP_2O_7$, ACN, 3 h (89%).