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THE DESIGN AND SYNTHESIS OF FARNESYL "TAIL" ANALOGUES INCORPORATING AROMATIC RINGS: A COMPARISON OF WITTIG AND GRIGNARD REACTION SEQUENCES

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Abstract

Mutant RAS proteins have been linked to over 30% of all human cancers. It has been shown that mutant RAS proteins that cannot be farnesylated do not induce malignant transformation. Therefore, farnesyl protein transferase (FPTase) inhibitors have become attractive targets as potential chemotherapeutic agents. Two farnesyl "tail" analogues have been prepared that incorporate aromatic rings. One of the compounds, *trans*-9-phenyl-8-nonen-1-ol, could only be prepared pure using a Grignard reaction sequence. This sequence is compared to the initially attempted Wittig reaction sequence that results in an inseparable mixture of *cis*/*trans* isomers. It is anticipated that when coupled with polar diphosphate "head" mimetics, the "tails" prepared in this paper will help illuminate the importance of nonbonding interactions in the binding of farnesyl pyrophosphate analogues to the FPTase enzyme active site.

Keywords: RAS proteins, Farnesyl Protein Transferase Inhibitors, Farnesyl Pyrophosphate Mimetics, Wittig Reaction, Grignard Reaction

Introduction

RAS proteins play an essential role in the signal transduction pathways that regulate cell proliferation (1). In order for RAS proteins to demonstrate signaling activity, they must first become membrane bound. This occurs through a series of post-translational modifications, the key step being the addition of a fifteen carbon, farnesyl chain to a cysteine amino acid residue located near the RAS carboxy-terminus (2). Farnesyl protein transferase (FPTase) is the enzyme that catalyzes this transfer of a farnesyl group from farnesyl pyrophosphate to the cysteine residue (3) (Figure 1). Modified in this manner, RAS proteins are sufficiently lipophilic to associate with the inner surface of the plasma membrane. Once associated with the membrane, RAS proteins act as a molecular switch for cell growth.

The association between RAS and cancer arises from the fact that over 30% of all human malignancies contain mutant RAS proteins (4). These mutant proteins show excessive "on" signaling activity and thus promote tumor formation. The RAS farnesylation process has become an attractive target to prevent this unregulated cell proliferation. Mutant RAS proteins

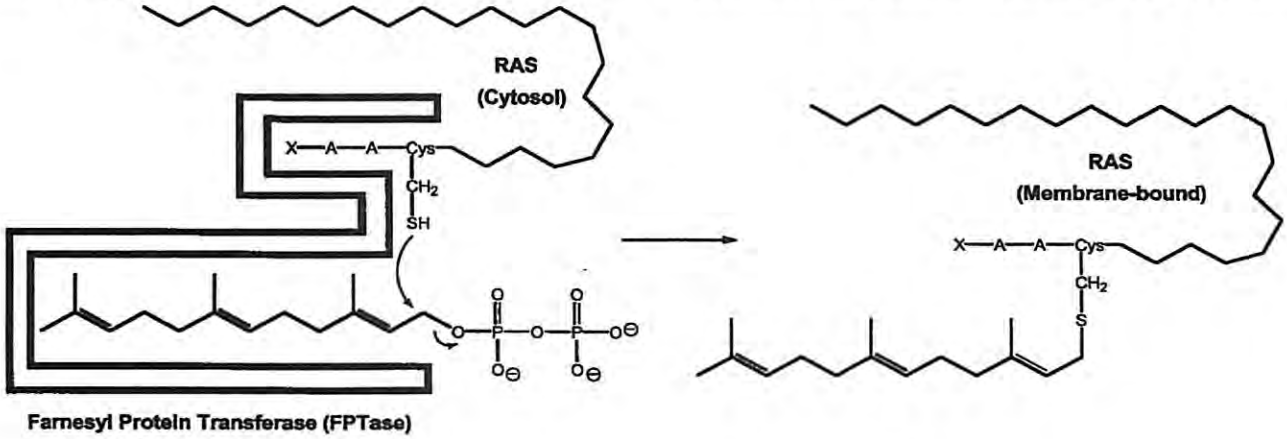


Figure 1. The RAS farnesylation process catalyzed by FPTase.

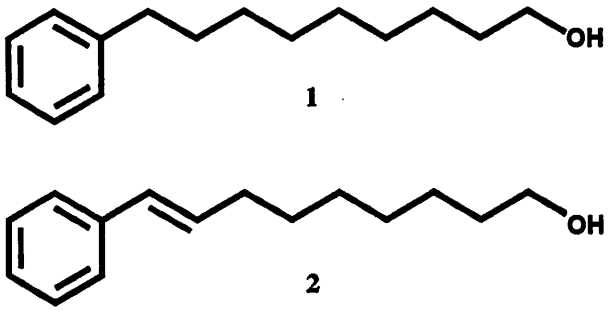


Figure 2. Two targeted farnesyl "tail" mimetics.

that cannot be farnesylated do not induce malignant transformations because they can never become membrane bound (5).

One method to inhibit RAS farnesylation is the design of farnesyl pyrophosphate mimetics. While very little work has been performed on modifications of the farnesyl skeleton (the "tail"), numerous compounds have been prepared by replacing the biologically labile diphosphate group with a variety of phophonic and/or carboxylic acid "heads" (6-10). Many of these compounds have demonstrated nanomolar potency against FPTase.

This paper describes the synthesis of two farnesyl "tail" mimetics incorporating aromatic rings (Figure 2). It is anticipated that when linked with previously reported "heads", these "tails" will increase the binding affinity of the farnesyl pyrophosphate analogues to the enzyme active site. The crystal structure of FPTase supports this theory. The crystal structure reveals a hydrophobic pocket lined with ten aromatic amino acid residues that presumably accepts the farnesyl diphosphate terpenoid chain (11). The discovery of this cleft lends credibility to the design of modified farnesyl "tails" incorporating aromatic rings. It is predicted that nonbonding interactions between the aromatic groups in the "tail" and the amino acids in the cleft (i.e. pi stacking interactions) will lead to enhanced binding to the enzyme pocket. Some evidence of aromatic rings causing increased binding has been observed in the literature (12,13).

The two targeted "tails", 9-phenyl-1-nonanol [**1**] and *trans*-9-phenyl-8-nonen-1-ol [**2**], were expected to be prepared via a five step, linear sequence. The key step in this sequence was envisioned to be a Wittig reaction (Scheme 1, Wittig Route). This paper outlines the problems encountered while performing the Wittig route. It also illustrates how these problems were solved using a Grignard reaction (Scheme 1, Grignard Route).

Experimental

Column chromatography was performed on 230-400 mesh silica gel. NMR spectra (^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz) were recorded on a Jeol 300 MHz NMR spectrometer with $CDCl_3$ solvent. All starting chemicals were purchased from Aldrich and used as received.

Step 1 (14)

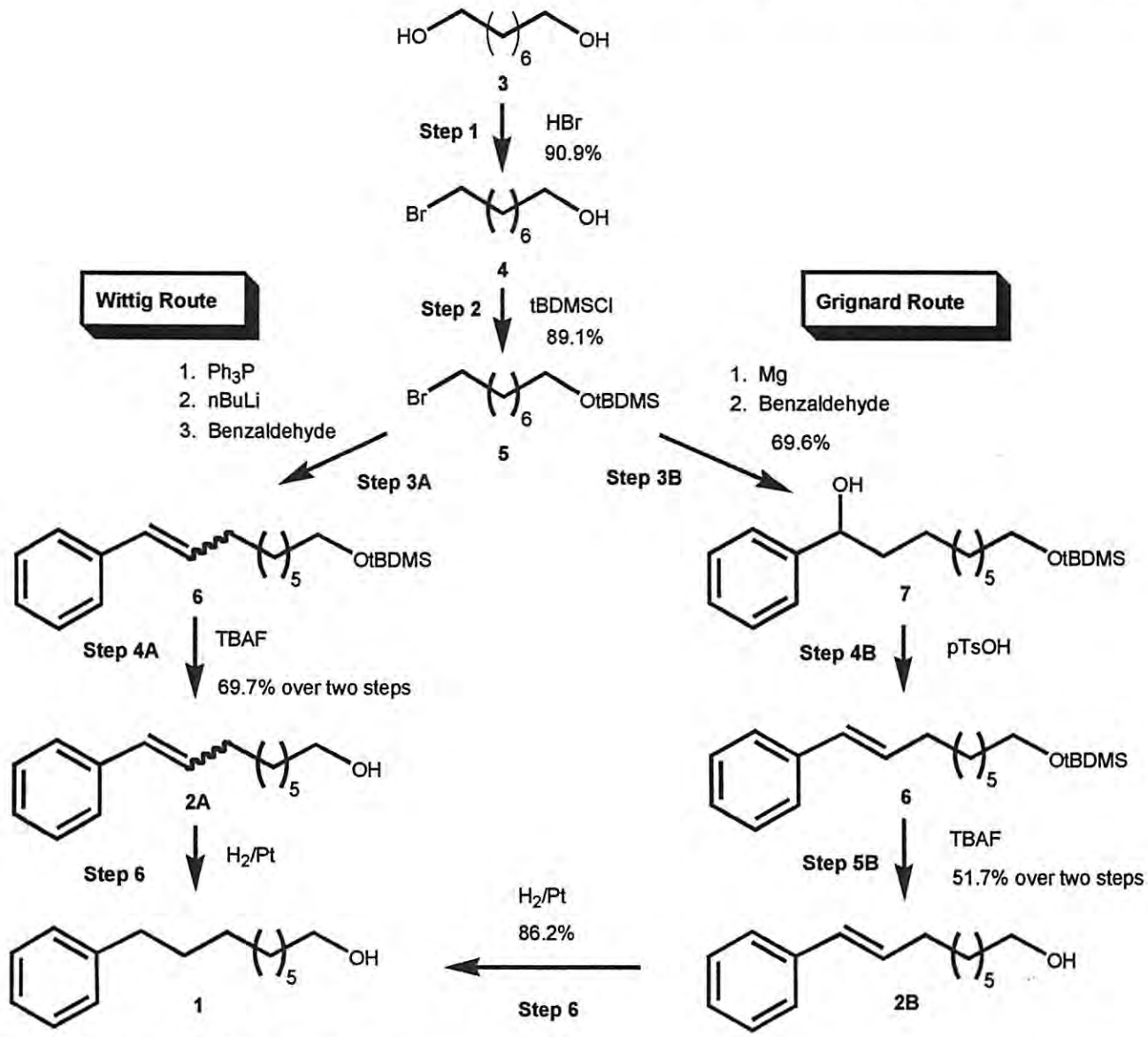
1,8-octanediol [**3**] (1.46 g, 10.0 mmol) was dissolved in toluene (25 mL). HBr (4.35 mL of 48%, 24.9 mmol) was then added and the solution was refluxed for 30 min at 120°C. The solution was subsequently cooled to room temperature and brine (25 mL) was added. The product was extracted with hexanes and dried over sodium sulfate to afford the monobrominated product [**4**] (1.90 g, 90.9% yield). No further purification was necessary. ^{1}H NMR (300 MHz) δ 3.58 (t, *J*=6.6 Hz, 2H), 3.37 (t, *J*= 6.6 Hz, 2H), 2.15 (br s, 1H), 1.87-1.76 (m, 2H), 1.59-1.42 (m, 2H), 1.42-1.21 (m, 8H); ^{13}C NMR δ 62.9, 34.1, 32.9, 32.7, 29.3, 28.8, 28.2, 25.7.

Step 2

8-bromo-1-octanol [**4**] (1.59 g, 7.61 mmol) was placed in CH_2Cl_2 (21 mL). Imidazole (0.621 g, 9.13 mmol) and *tert*-butyldimethylsilylchloride (*t*BDMSCl) (1.26 g, 8.37 mmol) were added and the reaction was stirred for two hours. The resulting mixture was diluted with water and a diethyl ether extraction was performed. The organic extract was dried over sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography (95:5 hexanes:ethyl acetate) to afford silyl ether [**5**] (2.19 g, 89.1%). ^{1}H NMR (300 MHz) δ 3.59 (t, *J*=6.6 Hz, 2H), 3.40 (t, *J*=6.8 Hz, 2H), 1.90-1.79 (m, 2H), 1.56-1.36 (m, 4H), 1.35-1.26 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR δ 63.3, 34.0, 32.9(2C), 29.3, 28.8, 28.2, 26.1(3C), 25.8, 18.5, -5.2(2C).

Steps 3A and 4A

Silyl ether [**5**] (2.19g, 6.78 mmol) and triphenylphosphine (2.13 g, 8.13 mmol) were mixed at 120°C for one hour. The reaction mixture was then cooled to approximately 70°C and 10 mL tetrahydrofuran (THF) was added. Butyllithium (2.5M in hexanes, 3.25 mL, 8.13 mmol) was added slowly to the solution. The



Scheme 1. Wittig and Grignard reaction sequences.

resulting mixture was stirred for 10 minutes; a red color was observed indicating ylide generation. After 10 minutes, benzaldehyde (0.82 mL, 8.10 mmol) was added dropwise. The reaction was allowed to cool to room temperature and stirred for approximately one hour. The resulting reaction mixture was quenched with 1M HCl (20 mL) and extracted with diethyl ether. The organic layer was dried over sodium sulfate and concentrated to afford the Wittig reaction product **[6]** which was carried directly into the next step.

Crude silyl ether **[6]** was dissolved in THF (10 mL) and treated with tetrabutylammonium fluoride (TBAF) (1M in THF, 8.13 mL, 8.13 mmol). The resulting solution was mixed at room temperature for 45 minutes. The reaction was then quenched with saturated NH_4Cl(aq) and extracted with diethyl ether. The organic layer was dried over sodium sulfate, filtered, and concentrated. Purification by column chromatography (50:50 hexanes:ethyl acetate) provided the desired deprotected product **[2A]** (1.03 g, 69.7% over the two steps). The product was a heavy mixture of *cis*/*trans* isomers (1.3 *trans*: 1.0 *cis*) and the isomers could not be separated by column chromatography. ^{1}H NMR (300 MHz) δ 7.40-7.15 (m, 5H), 6.44 (*cis*, d, J = 11.6 Hz, 1H), 6.40 (*trans*, d, J = 15.7 Hz, 1H), 6.24 (*trans*, dt, J = 15.7, 6.6 Hz, 1H), 5.68 (*cis*, dt, J = 11.6, 7.1 Hz, 1H), 3.64 (*trans*, t, J = 6.6 Hz, 2H), 3.53 (*cis*, t, J = 6.6 Hz, 2H), 2.40-2.30 (*cis*, m, 2H), 2.27-2.18 (*trans*, m, 2H), 1.65-1.27 (m, 10H); ^{13}C NMR was messy due to the *cis*/*trans* mixture.

Step 3B

Crushed magnesium turnings (0.41 g, 16.7 mmol) were placed in a round bottom flask and flame-dried. Dry ether (8.4 mL) was added to the magnesium followed by a crystal of iodine. Bromide **[5]** (2.69 g, 8.40 mmol) was then dissolved in ether (5.0 mL) and added dropwise via cannula to the magnesium. Once Grignard reagent formation was observed, benzaldehyde (0.85 mL, 8.4 mmol) was added dropwise to the reaction mixture. After 30 min, the solution was quenched with NH_4Cl(aq). The product was extracted with ether, dried over sodium sulfate, filtered, and concentrated. Purification by column chromatography (70:30 hexanes:ethyl acetate) provided pure alcohol **7** (2.05 g, 69.6% yield). ^{1}H NMR (300 MHz) δ 7.36-7.24 (m, 5H), 4.66 (t, J= 6.7 Hz, 1H), 3.58 (t, J= 6.6 Hz, 2H), 1.85-1.66 (m, 2H), 1.53-1.40 (m, 2H), 1.35-1.20 (m, 10H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR δ 145.0, 128.5(2C), 127.6, 126.0(2C), 74.8, 63.4, 39.2, 32.9, 29.6, 29.5, 29.4, 26.1(3C), 25.9, 25.8, 18.5, -5.2(2C).

Steps 4B and 5B

In a round bottom flask fitted with a Dean-Stark trap, a solution of alcohol **[7]** (0.305 g, 0.870 mmol), *para*-toluenesulfonic acid (pTsOH) (0.066 g, 0.347 mmol), and toluene (10 mL) was heated to reflux for five hours. The reaction mixture was subsequently diluted with water (10 mL) and an ether extraction was performed. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide crude alkene **[6]** which was carried on into the next step without further purification.

Crude alkene **[6]** was dissolved in THF (5 mL) and TBAF (1M in THF, 2.61 mL, 2.61 mmol) was added. The reaction mixture was allowed to stir overnight (16 hours). The reaction was quenched with saturated NH_4Cl(aq), extracted with diethyl ether, and concentrated. Purification by column chromatography (50:50 hexanes:ethyl acetate) afforded alcohol **[2B]** (0.098 g, 51.7% over the two steps). Unlike the Wittig reaction product, alcohol **[2B]** was prepared as the pure *trans* isomer. ^{1}H NMR (300 MHz) δ 7.36-7.14 (m, 5H), 6.37 (d, J= 15.7 Hz, 1H), 6.21 (dt, J= 15.7, 6.6Hz, 1H), 3.64 (t, J = 6.6 Hz, 2H), 2.25-2.15 (m, 2H), 1.62-1.18 (m, 10H); ^{13}C NMR δ 138.0, 131.2, 129.9, 128.6(2C), 126.9, 126.0(2C), 63.1, 33.1, 32.9, 29.4(2C), 29.3, 25.8.

Step 6

Alkene **[2A]** or **[2B]** (0.046 g, 0.211 mmol) was dissolved in methanol (1.0 mL) and treated with palladium (5 wt. % on carbon, 45 mg, 0.021 mmol). A hydrogen balloon was inserted into the flask and the reaction was allowed to stir for 20 hours. The resulting reaction mixture was filtered through a frit funnel containing celite and silica gel. Concentration of the filtrate provided alcohol **[1]** (0.040 g, 86.2%). No further purification was necessary. ^{1}H NMR (300 MHz) δ 7.31-7.24 (m, 2H), 7.21-7.14 (m, 3H), 3.64 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.69-1.50 (m, 4H), 1.41-1.23 (m, 10H); ^{13}C NMR δ 143.0, 128.5(2C), 128.3(2C), 125.6, 63.2, 36.1, 32.9, 31.6, 29.6, 29.5, 29.5, 29.4, 25.8.

Results and Discussion

The synthesis of both "tail" analogues, **[1]** and **[2]**, began with the monohydrobromination of 1,8-octanediol **[3]**. The major problem with this reaction was the formation of dibrominated product. The reaction was monitored by gas chromatography and it was determined that a reaction time of thirty minutes provided the most favorable ratio of monobromide to dibromide product (16:1). The product of this reaction was pure enough to be taken on crude.

The second step of the sequence was a standard silyl ether protection of an alcohol using *tert*-butyldimethylsilylchloride and imidazole. The resulting bromide **[5]** was purified and taken into the Wittig reaction step. Addition of bromide **[5]** to triphenylphosphine provided the phosphonium salt, which was immediately treated with *n*-butyllithium to provide the phosphorus ylide. Nucleophilic addition of the ylide to benzaldehyde afforded the expected product, alkene **[6]**. Separation of alkene **[6]** from reaction side-products (i.e. triphenylphosphine oxide) was difficult due to both compounds being very nonpolar. Deprotection of the silyl ether with tetrabutylammonium fluoride, however, provided a much more polar compound, alcohol **[2A]**, which could easily be separated from all reaction by-products. Alcohol **[2A]** was found to be a heavy mixture of *trans*/*cis* isomers. The two isomers were identified by their vinyl hydrogen splitting patterns. The *trans* isomer was observed to have a coupling constant of 15.7 Hz; the *cis* isomer was 11.6 Hz (Figure 3). By integrating the area under the peaks, the *trans*:*cis* ratio was calculated to be 1.3:1.0. Unfortunately, the two stereoisomers

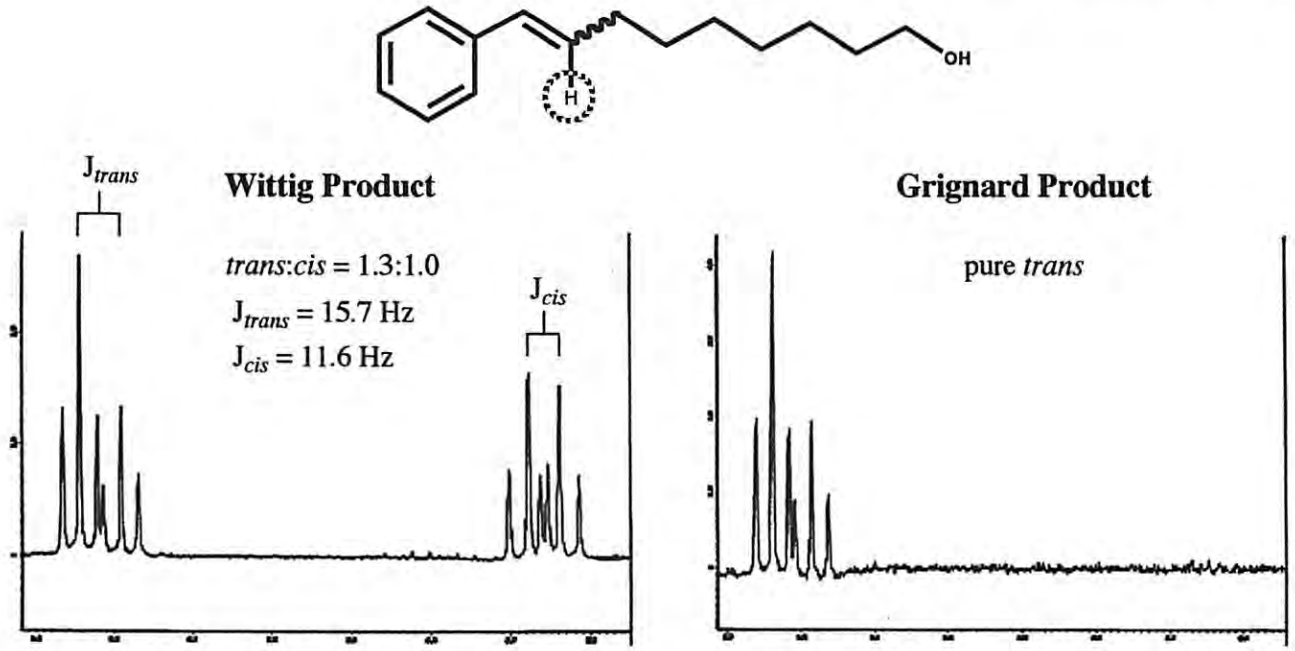


Figure 3. Doublet of triplets representing the circled vinyl hydrogen.*
*Coupling constants shown represent the doublet splitting pattern in the doublet of triplets.

could not be separated by column chromatography. This was a major problem because literature precedence clearly illustrates that farnesyl pyrophosphate mimetics incorporating *Z* double bonds are much poorer FPTase inhibitors than the corresponding *E* isomers (15). A new strategy was required to circumvent this problem.

The new strategy involved making bromide **[5]** into a Grignard reagent instead of an ylide. When treated with benzaldehyde, the Grignard reaction provided alcohol **[7]**. This alcohol was then subjected to acid-catalyzed dehydration conditions to provide the desired alkene **[6]**. The dehydration conditions also caused partial deprotection of the silyl group, so the crude product was carried directly into the TBAF deprotection step to optimize the yield. ^{1}H NMR analysis of the product clearly illustrated that pure *trans*-9-phenyl-8-nonen-1-ol [**2B**] had been prepared (Figure 3). Alcohol **[2B]** was then hydrogenated under standard conditions to provide the second farnesyl "tail" analogue **[1]**.

Conclusion

In summary, the pure *trans* farnesyl "tail" analogue [**2B**] could only be prepared via the Grignard reaction sequence. It was synthesized in five linear steps with an overall yield of 29.1%. Polar "heads" can now be added to the "tails" prepared in this paper and the FPTase activity of these novel compounds can be measured. It is anticipated that these analogues will help to illuminate the importance of nonbonding interactions in the binding of farnesyl pyrophosphate mimetics to the enzyme active site.

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