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Synthesis of 7-Substituted Farnesyl Diphosphate Analogues

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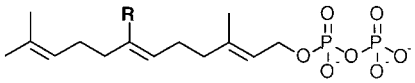
ABSTRACT



4 (R=ethyl); **5** (R=vinyl); **6** (R=allyl);
7 (R=isopropyl); **8** (R=isobutyl)

Six farnesyl diphosphate analogues modified in the central isoprene unit have been prepared via our stereoselective vinyl triflate-mediated route to isoprenoids. The 7-allyl compound 6 is a modest inhibitor of mammalian protein-farnesyl transferase, but surprisingly the other five analogues are effective alternative substrates for this enzyme.

Protein farnesylation, which is carried out by the enzyme protein-farnesyl transferase (FTase), is an essential post-translational modification of many proteins.[1] Initial studies on this process demonstrated that the key signal transduction protein and oncogene product Ras is farnesylated. The intense effort directed toward the development of FTase inhibitors[2,3] has been rewarded recently with the introduction of several such compounds into human clinical trials for the treatment of a variety of neoplasias.[4] Previous work in this laboratory has been directed toward the synthesis and evaluation of farnesyl diphosphate (FPP, Figure 1) analogues primarily modified in the α-isoprene unit.[5,6] These studies have resulted in the discovery of effective alternative substrates for this enzyme such as 3-vinylFPP (**2**) and potent, low-nanomolar FTase inhibitors such as 3-allylFPP (**3**).[5b] The fact that this subtle change in functionality leads to large differences in biological activity has provided an impetus for the synthesis

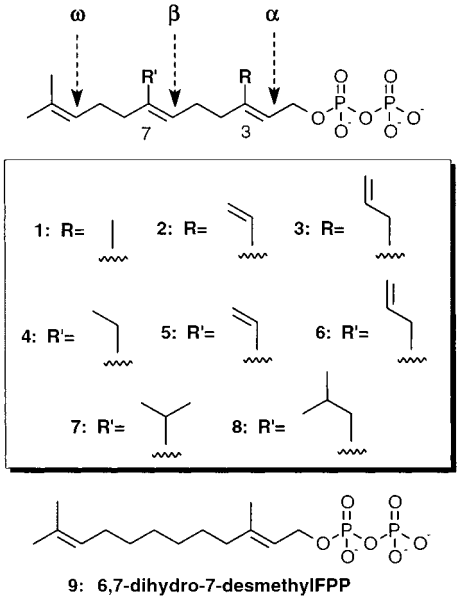


Figure 1. Structure of FPP, with the α-, *β*-, and *ω*-isoprene units indicated, and the previously prepared (**2** and **3**) and newly targeted (**4**−**9**) FPP analogues (R and R′ = Me unless indicated).

(1) Gibbs, R. A.; Zahn, T. J.; Sebolt-Leopold, J. S. *Curr. Med. Chem.* **2001**, *8*, 1437.
(2) Gibbs, J. B.; Oliff, A.; Kohl, N. E. *Cell* **1994**, *77*, 175.
(3) Leonard, D. M. *J. Med. Chem.* **1997**, *40*, 2971.
(4) (a) Rowinsky, E. K.; Windle, J. J.; VonHoff, D. D. *J. Clin. Oncol.* **1999**, *17*, 3631. (b) Karp, J. E.; Kaufmann, S. H.; Adjei, A. A.; Lancet, J. E.; Wright, J. J.; End, D. W. *Curr. Opin. Oncol.* **2001**, *13*, 470.
(5) (a) Gibbs, R. A.; Krishnan, U.; Dolence, J. M.; Poulter, C. D. *J. Org. Chem.* **1995**, *60*, 7821. (b) Gibbs, B. S.; Zahn, T. J.; Mu, Y. Q.; Sebolt-Leopold, J. S.; Gibbs, R. A. *J. Med. Chem.* **1999**, *42*, 3800. (c) Zahn, T. J.; Weinbaum, C.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1763. (d) Mu, Y.; Eubanks, L. M.; Poulter, C. D.; Gibbs, R. A. *Bioorg. Med. Chem.* **2002**, *10*, 1207.
(6) (a) Shao, Y.; Eummer, J. T.; Gibbs, R. A. *Org. Lett.* **1999**, *1*, 627. (b) Zahn, T. J.; Whitney, J.; Weinbaum, C.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1605.

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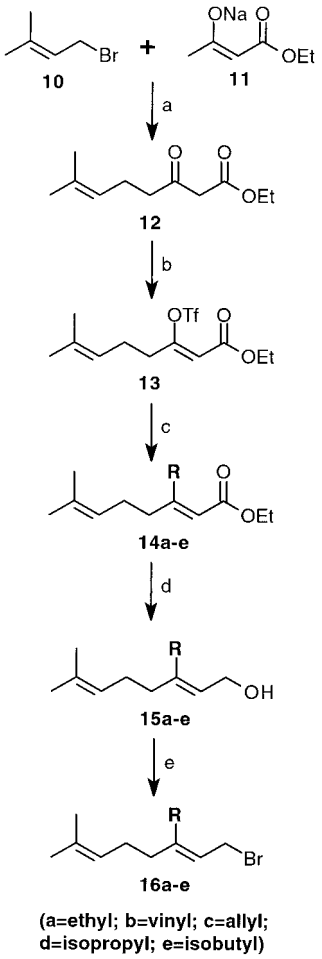
Scheme 1. Synthesis of 3-Substituted Geranyl Bromides[a]



[a] Reagents and yields: (a) **11**, ^{n}BuLi, THF, 0 °C; **10** (86%); (b) $(Me_3Si)_2NK$, THF, −78 °C; 2-(5-chloropyridyl)$N(SO_2CF_3)_2$, from −78 °C to rt (82%); (c) RMgBr, CuCN, Et_2O, −78 °C (R = Et, 75%; R = ^{i}Pr, 78%; R = ^{i}Bu, 76%) or $RSnBu_3$, $Pd(AsPh_3)_2$, CuI, *N*-methylpyrrolidinone, 70 °C or rt (R = vinyl, 82%; R = allyl, 86%); (d) DIBAL-H, toluene, −78 °C (78−88%); (e) CBr_4, Ph_3P, CH_2Cl_2, rt (85−94%).

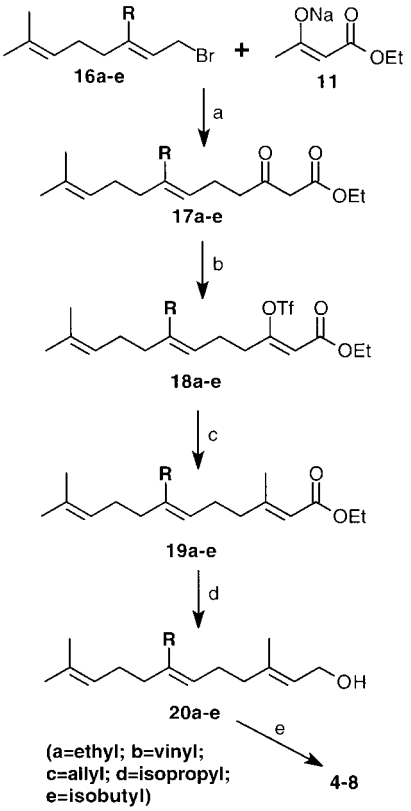
of FPP analogues modified in the β- and ω-isoprene units.[7] Thus, we have prepared a series of FPP analogues modified at the 7-position (**4**−**8**),[8] along with the saturated desmethyl analogue **9**. These compounds have also undergone preliminary evaluation as FTase inhibitors or alternative substrates.

We have developed improved synthetic methods for the preparation of isoprenoids during the past several years.[5,6] Our vinyl triflate-based route has proven to be suitable for the synthesis of a wide variety of FPP and GGPP analogues. We have now adapted this method to provide a linear but concise route for the synthesis of 7-substituted FPP analogues. The advantage of this route is that it allows for the ready preparation of analogues modified in both the α- and β-isoprene units. The strategy involves the initial synthesis of the appropriate 3-substituted geranyl bromide analogues, followed by their homologation to the 7-substituted farnesol analogues and subsequent conversion to the diphosphates.

The vinyl triflate-mediated synthesis of the 3-substituted geranyl bromide analogues is illustrated in Scheme 1. Prenyl bromide **10** and the dianion of ethyl acetoacetate were coupled to afford the β-ketoester **12**, which was readily transformed to the vinyl triflate **13**. The vinyl and allyl analogues **14b** and **14c**, respectively, were prepared via our original Stille coupling protocol.[5a] The ethyl, isopropyl, and isobutyl analogues were all prepared via our copper-mediated Grignard coupling procedure.[5c] All five of these esters were reduced to the corresponding alcohols and then transformed to the desired bromides **16a**−**e**.

The key, difficult step in the synthesis of 7-substituted FPP analogues (Scheme 2) is the coupling of the dianion of ethyl acetoacetate (derived from the sodium salt **11** and butyllithium) with the 3-substituted geranyl bromides (**16a**−**e**). Despite the remote nature of the R-groups in **16a**−**e**, these bromides are significantly less reactive than the parent

Scheme 2. Synthesis of 7-Substituted FPP Analogues[a]



[a] Reagents and yields: (a) **11**, ^{n}BuLi, THF, 0 °C; **16a**−**e** (52−61%). (b) $(Me_3Si)_2NK$, THF, −78 °C; 2-(5-chloropyridyl)-$N(SO_2CF_3)_2$, from −78 °C to rt (71−85%). (c) $SnMe_4$, $Pd(AsPh_3)_2$, CuI, *N*-methylpyrrolidinone, 70 °C (R = Et, 82%; R = allyl, 71%; R = ^{i}Pr, 81%; R = ^{i}Bu, 92%) or MeMgBr, CuCN, Et_2O, −78 °C (R = vinyl, 77%). (d) DIBAL-H, toluene, −78 °C (76−90%). (e) (i) NCS, Me_2S, CH_2Cl_2, from −30 °C to rt; (ii) $(Bu_4N)_3HP_2O_7$, MeCN, rt (62−70% for two steps).

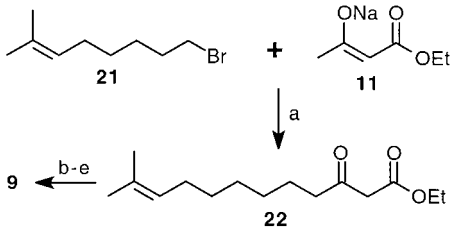
(7) For a recent study in this area, see: Zhou, C.; Shao, Y.; Gibbs, R. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1417.

(8) FPP analogue **4** has been prepared previously by another route: Sen, S. E.; Ewing, G. J. *J. Org. Chem.* **1997**, *62*, 3529.

geranyl bromide (where R = Me). Under the standard reaction conditions developed in our earlier studies, coupling of **11** with **16a** resulted in ~5% yield of the β-ketoester **17a**, and extended reaction periods led to isomerization and decomposition of the starting material **16a**.[9] In an attempt to optimize the yield of **17a**, it was found that the coupling of the dianion with **16** is very sensitive to the molar excess of **11** and butyllithium used in the reaction. An increase in the molar ratio of **11**/butyllithium to **16a** to 3.5:1.0 resulted in a 61% yield of β-ketoester **17a**. We have confirmed in the past that this type of alkylation reaction proceeds with retention of geometry at the allylic double bond,[6] and there was no indication of isomerization in this case as well. The other β-ketoesters (**17b**−**e**) were prepared in satisfactory yield by the same synthetic procedure developed for the preparation of **16a**. Triflation of the β-ketoesters produced the corresponding triflates **18a**−**e** in very good yield. Stille methylation of **18a** and **18c**−**e** proceeded smoothly, but surprisingly the reaction of 7-vinyl triflate **18b** with tetramethyltin under the same conditions led to the formation of a mixture of coupling products. Although we were not able to purify and fully characterize the byproducts, it was clear that the integrity of the diene moiety in the β-unit of the isoprene chain was lost. It is possible that the palladium catalyst in the Stille reaction complexed with the diene and mediated isomerization or migration of the diene. Thus, we turned to an alternative methylation procedure, utilizing a CuCN mediated reaction of methylmagnesium bromide with triflate **18b**. This afforded an excellent yield of the desired ester **19b**. Moreover, this reaction is very fast and proceeds to completion within 2 h, in comparison to Stille coupling, which generally requires 16−18 h for completion. DIBAL reduction of esters **19a**−**e** resulted in alcohols **20a**−**e** in nearly quantitative yields. Chlorination followed by diphosphorylation afforded the desired compounds **4**−**8**. Purification by ion exchange followed by cellulose column flash chromatography afforded the pure 7-substituted FPP analogues.

The dihydro FPP analogue **9** was synthesized using bromide **21** as a starting material (Scheme 3). Bromide **21** was derived from the corresponding alcohol, which was prepared by a modified version of the literature method.[10] Again coupling of **21** with the dianion of ethyl acetoacetate was problematic and did not work using the forcing reaction conditions that had been developed for the preparation of β-ketoesters **17a**−**e**. This is not surprising, given that the saturated bromide **21** should be less reactive than the corresponding allylic variants. However, carrying out the reaction at room temperature and increasing the molar ratio of **11** to **21** to 5.0:1.0 afforded β-ketoester **22** in 65% yield.

Scheme 3. Synthesis of 6,7-Dihydro-7-desmethyl FPP[a]



[a] Reagents and yields: (a) **11**, ^{n}BuLi, THF, 0 °C; **21**, from 0 °C to rt (65%). (b) $(Me_3Si)_2NK$, THF, −78 °C; 2-(5-chloropyridyl)-$N(SO_2CF_3)_2$, from −78 °C to rt (79%). (c) $SnMe_4$, $Pd(AsPh_3)_2$, CuI, *N*-methylpyrrolidinone, 70 °C (72%). (d) DIBAL-H, toluene, −78 °C (78%). (e) (i) NCS, Me_2S, CH_2Cl_2, from −30 °C to rt; (ii) $(Bu_4N)_3HP_2O_7$, MeCN, rt (79% for two steps).

Standard transformations of **22**, as illustrated in Scheme 3, afforded the desired FPP analogue **9** in five steps.

The six new analogues of FPP illustrated in Figure 1 were then evaluated as potential substrates for or inhibitors of mammalian protein-farnesyl transferase (mFTase). A continuous spectrofluorimetric assay for FTase was employed to test the ability of **4**−**9** to act as mFTase substrates with the peptide cosubstrate dansyl-GlyCysValLeuSer-OH.[5b,6a] Initial studies indicated that all of the analogues, with the exception of **6**, were substrates. In particular, the 7-ethyl, 7-vinyl, and 7-isopropyl analogues were nearly as effective as substrates as FPP itself under the same assay conditions. In contrast to the results obtained with the other analogues, 7-allylFPP (**6**) was an exceptionally poor mFTase substrate. Therefore, it was evaluated as an inhibitor of mFTase. It proved to be a modest inhibitor of mFTase, with an IC_{50} value of 1.6 μM. These preliminary biological results demonstrate the surprising substrate flexibility of FTase and its ability to accept a wide variety of hydrocarbon-substituted FPP analogues. The recently reported work of Spielmann and others has established that an aromatic moiety can act as an effective mimic for the ω-isoprene unit,[11] but it is striking that significant modifications can be made to the β-isoprene unit of FPP without altering its ability to act as a substrate. These results also contrast sharply with the results obtained when the corresponding substituents were placed at the 3-position; in that case, the ethyl, isopropyl, and isobutyl analogues were potent inhibitors, rather than alternative substrates.[5b,c] This implies that the 7-position is more amenable to substitution than the 3-position of the isoprenoid in the context of FTase alternative substrates.

Acknowledgment. We thank Drs. Zhengmao Guo and Zhiqian Wu for performing preliminary experiments in this area, Dr. Weidong Wu for assistance with the purification of the diphosphates, Sarah Reigard for assistance with the FTase assays, Jennifer S. Pickett, Katherine A. Hicks, and Prof. Carol A. Fierke (University of Michigan) for providing the FTase, and the NIH (R01 CA78819) for funding this project.

(9) (a) In our earlier studies,[5a] we utilized a 2-fold excess of the dianion derived from **11** and butyllithium for the alkylation of geranyl bromide. In the original Weiler procedure for the alkylation of acetoacetate dianions,[9b] only a slight excess of the dianion was necessary to alkylate simple primary halides. However, Weiler and Huckin generated the sodium salt of methyl (rather than ethyl) acetoacetate in situ using sodium hydride and used this to generate the dianion. In contrast, we employ the commercially available sodium salt of ethyl acetoacetate (**11**) in our coupling reactions. (b) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082.

(10) Soichi, S.; Maruoka, K.; Yamamoto, H. *Tetrahedron Lett.* **1986**, *42*, 2203.

(11) Micali, E.; Chehade, K. A. H.; Isaacs, R. J.; Andres, D. A.; Spielmann, H. P. *Biochemistry* **2001**, *40*, 12254. See also ref 7.

Supporting Information Available: Spectral data (^{1}H NMR, ^{13}C NMR, LRMS, and in some cases HRMS and GC-MS) for all new compounds; detailed experimental procedures for the synthesis of **12**, **13**, **14a**,**c**, **15c**, **16c**, **17c**, **19a**,**c**, **22**, and **6** (along with its chloride precursor); ^{1}H NMR spectra for all farnesol analogues (**20a**−**e** and **25**, the alcohol precursor of **9**), **14b**, **15b**, **16b**, **17b**, **18b**, **19b**, and **5**; elemental analyses for farnesol analogues **20a**−**e**; and a figure demonstrating the inhibition of mFTase by **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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