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Stereospecific Synthesis and Biological Evaluation of Farnesyl Diphosphate Isomers

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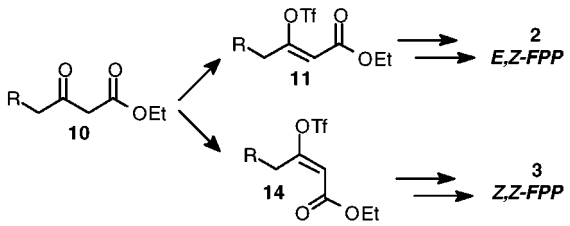
ABSTRACT



A unified, stereospecific synthetic route to the three geometric isomers of (*E*,*E*)-farnesyl diphosphate (*E*,*E*-FPP) (1, 2, and 3) has been developed. The key feature of this synthesis is the ability to control the stereochemistry of triflation of the *β*-ketoester 10 to give either 11 or 14. Preliminary evaluation of these compounds with protein-farnesyl transferase indicates that 1 and 2 are surprisingly effective substrates; however, *Z*,*Z*-FPP (3) is a poor substrate and a sub-micromolar inhibitor.

The development of stereoselective methods for the synthesis of isoprenoids is a subject of continuing interest. Isoprenoid phosphates act as intermediates in cholesterol biosynthesis. In addition, specialized isoprenoids such as dolichol and ubiquinone play key roles in cellular metabolism.[1] Particular recent interest has been attached to the development of inhibitors of protein farnesylation as potential anticancer agents.[2] We have synthesized several 3-substituted farnesyl diphosphate (FPP) analogues that are potent protein-farnesyl transferase (FTase) inhibitors.[3] These analogues were also used as probes for the function of the farnesyl group in farnesylated peptides, and this study demonstrated that an unnatural *cis*-farnesyl isomer differed significantly in its biological activity from the *all-trans* isomer.[4] Thus it is of interest to explore further the effects of double bond isomerization on the biological activity of farnesylated compounds. Numerous methods have been previously developed for the stereoselective synthesis of *trans*-isoprenoids,[5] and some methods have also been developed for the synthesis of *cis*-isoprenoids.[6] However, it would be desirable to develop a general, flexible stereospecific method that would allow for the preparation of analogues of both geometric farnesyl isomers from a common starting material. This paper reports the adaptation of our previously developed vinyl triflate method[3] to the preparation of *cis*-isoprenoids. As a demonstration of the utility of this method, the three geometric isomers of FPP have been synthesized (Figure 1),[7] which has then allowed for their characterization as potential

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(1) Gibbs, R. A. In *Comprehensive Biological Catalysis*; Sinnott, M. L., Ed.; Academic Press: London, 1998; Vol. 1, pp 31−118.

(2) Leonard, D. M. *J. Med. Chem.* **1997**, *40*, 2971−2990.

(3) (a) Gibbs, R. A.; Krishnan, U.; Dolence, J. M.; Poulter, C. D. *J. Org. Chem.* **1995**, *60*, 7821−7829. (b) Mu, Y. Q.; Gibbs, R. A.; Eubanks, L. M.; Poulter, C. D. *J. Org. Chem.* **1996**, *61*, 8010−8015.

(4) Dawe, A. L.; Becker, J. M.; Jiang, Y.; Naider, F.; Eummer, J. T.; Mu, Y. Q.; Gibbs, R. A. *Biochemistry* **1997**, *36*, 12036−12044.

(5) For some recent examples, see: (a) Bouzbouz, S.; Kirschleger, B. *Synlett* **1994**, 763−764. (b) Corey, E. J.; Shieh, W.-C. *Tetrahedron Lett.* **1992**, *33*, 6435−6438. (c) Klinge, S.; Demuth, M. *Synlett* **1993**, 783−784. (d) Mu, Y. Q.; Gibbs, R. A. *Tetrahedron Lett.* **1995**, *36*, 5669−5672. (e) For a leading reference to earlier work, see: Cainelli, G.; Cardillo, G. *Acc. Chem. Res.* **1981**, *14*, 89−94.

(6) Sato, K.; Miyamoto, O.; Inoue, S.; Furusawa, F.; Matsuhashi, Y. *Chem. Lett.* **1983**, 725−728.

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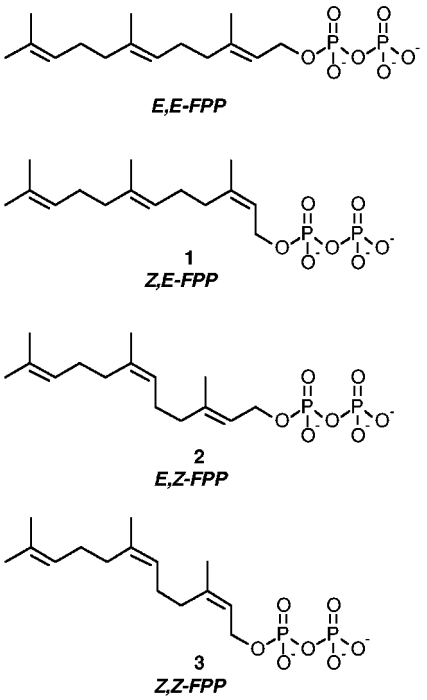


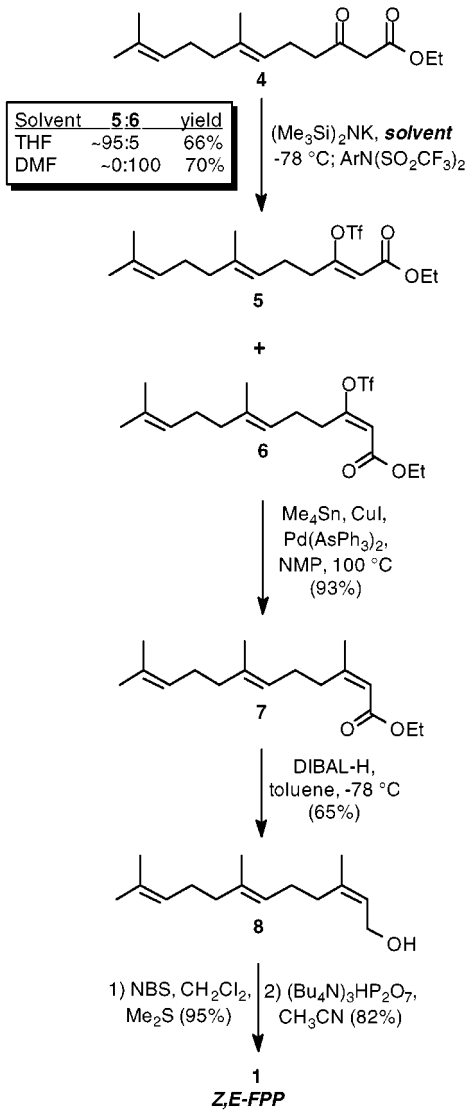
Figure 1. Structures of FPP geometric stereoisomers.

FTase substrates or inhibitors. This route may be of more general interest, in that it is potentially applicable to the stereoselective synthesis of a broad range of substituted allylic alcohols.

A key step in the triflate-mediated synthesis of isoprenoid analogues is the stereoselective synthesis of triflate **5** from the potassium enolate of β-ketoester **4** (Scheme 1). In an attempt to optimize the yields of **5** obtained in this process, DME was used in place of THF. However, this increase in solvent polarity resulted in a loss of stereocontrol leading to a mixture of stereoisomers. This may be due to the more polar solvent disrupting an intramolecular chelation of the potassium enolate by the ester carbonyl, allowing for enolate double bond isomerization. When the polar aprotic solvent DMF was used in the triflation reaction, only the stereoisomer **6** was obtained (note that **6** has been previously characterized).[3a] Triflation of **4** in DMF thus could be used as the key step in the preparation of *Z*,*E*-FPP in a stereocontrolled fashion. We have previously demonstrated that the Pd/CuI-catalyzed coupling of isoprenoid triflates **5** and **6** with organotin reagents proceeds in a stereospecific manner.[3a] Coupling of **6** with tetramethyltin afforded the *Z*,*E*-ester **7**, which was then reduced with DIBAL to the alcohol **8**. The proton NMR spectrum of **7** was consistent with that previously observed for *Z*,*E*-methyl farnesoate.[7b] Bromination followed by pyrophosphorylation[8] afforded the desired compound **1**.

(7) (a) The geometric isomers of farnesol have been previously prepared by other methods (for an earlier example, see: Pitzele, B. S.; Baran, J. S.; Steinman, D. H. *Tetrahedron* **1976**, *32*, 1347−1351). (b) For the first definitive report on the stereochemical assignment of the farnesol isomers, see: Burrell, J. W. K.; Garwood, R. F.; Jackman, L. M.; Oskay, E.; Weedon, B. C. L. *J. Chem. Soc. C* **1966**, 2144−2154.

Scheme 1. Synthesis of *Z*,*E*-FPP

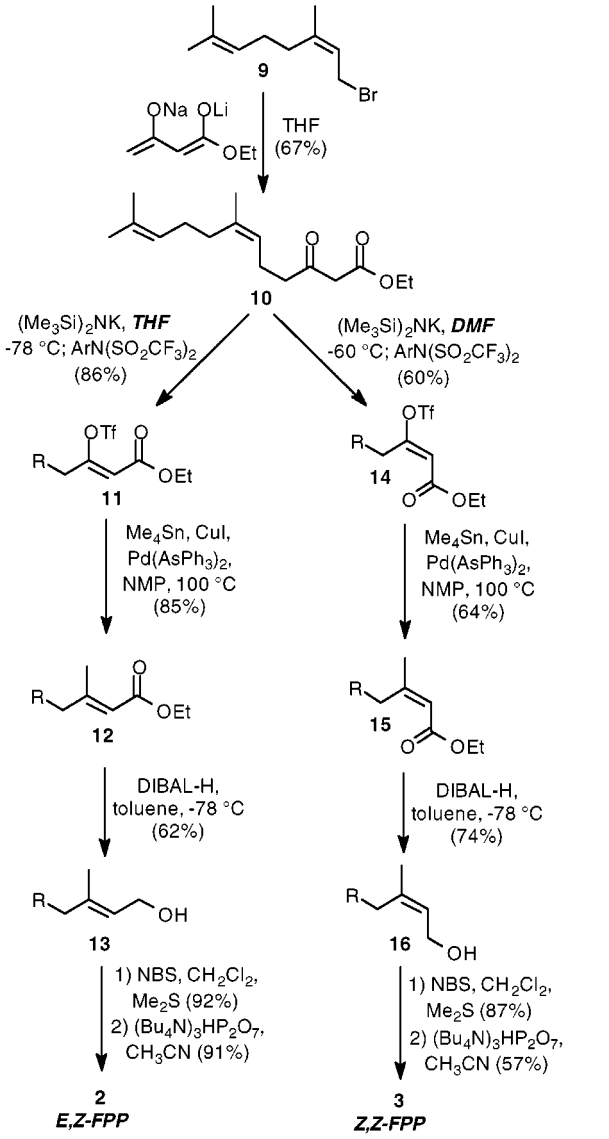


The syntheses of the *E*,*Z*- and *Z*,*Z*-FPP isomers required the preparation of the *Z*-β-ketoester **10** (Scheme 2). This compound was synthesized in a straightforward manner via the coupling of neryl bromide (prepared from commercially available nerol) with the dianion of ethyl acetoacetate. The geometry of the central double bond in **10** was confirmed by NMR comparison to **4**.[9] Triflation of **10** in THF afforded **11** in excellent yield and stereoisomeric purity after flash chromatography. Coupling with tetramethyltin produced ester **12**, again apparently in stereochemically pure form. Reduction of **12** to alcohol **13** followed by pyrophosphoylation

(8) Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768−4779.

(9) The ^{13}C NMR signals were assigned for both **4** and **10** using a 2D $^{1}H-^{13}C$ correlation in combination with a DEPT experiment. A comparison of the C_7-Me signals for **4** and **10** indicated that the chemical shift for the former (16.7 ppm) was significantly upfield from that of the latter (24 ppm), consistent with the shielding expected for a methyl group cis to a methylene group versus that cis to a proton. A similar but less pronounced shift was observed in the proton NMR signals for the C_7-Me groups (**4**, 1.62 ppm; **10**, 1.69 ppm).[7]

Scheme 2. Synthesis of *E,Z*- and *Z,Z*-FPP



afforded the desired compound *E,Z*-FPP. Note that the proton NMR spectrum of **12** was consistent with that reported for *E,Z*-methyl farnesoate.[7b] Triflation of **10** in DMF afforded the isomeric compound **14** as the sole product. Methylation of **14** gave the ester **15**, which was then reduced to alcohol **16**. Bromination followed by pyrophosphorylation afforded the desired diphosphate **3**.

The three unnatural stereoisomers of FPP, having been prepared, were then evaluated as potential substrates for or inhibitors of mammalian protein-farnesyl transferase (mFTase).[10] A previous report determined that *Z,E*-FPP inhibits protein farnesylation by crude mFTase.[11] However, its ability to act as a substrate was not investigated. A modified version of the continuous spectrofluorimetric assay[12] for FTase was employed to test the ability of **1**, **2**, and **3** to act as mFTase substrates with the peptide cosubstrate dansyl-GCVLS-OH. Preliminary studies indicated that **1** and **2** were substrates, while **3** was not. More detailed evaluation demonstrated that, surprisingly, both *Z,E*-FPP and *E,Z*-FPP were very effective mFTase substrates (Table 1). In fact,

Table 1. Evaluation of FPP Isomers versus mFTase[a]

compd	K_m (nM)	k_{rel}[b]
E,E-FPP	300[c]	1.0
Z,E-FPP	225	0.47
E,Z-FPP	136	0.41
Z,Z-FPP	778[d]	*e*

[a] A modified version of the continuous spectrofluorimetric assay was utilized.[12] Briefly, dansyl-GCVLS (4.7 μM) was employed as a peptide substrate. *E,E*-FPP, **1**, **2**, or **3** (~15 mM stock solution in 25 mM ammonium bicarbonate, pH 7.5; final concentration 0.10 to 5 μM)[f] was added to the assay buffer solution [52 mM Tris-HCl, pH 7.0, 5.8 mM DTT, 12 mM $MgCl_2$, 12 μM $ZnCl_2$ with added detergent solution (0.4% *n*-dodecyl-β-D-maltoside)]. The reaction was initiated with the addition of purified recombinant mFTase, and fluorescence was detected using a time-based scan at 30 °C for a period of 300 s (excitation wavelength = 350 nm; emission wavelength = 486 nm). The velocity was determined by converting the rate of increase in fluorescence intensity (cps/s) to μM/s. [b] Relative V_{max} determined for each analogue, compared to the V_{max} determined for *E,E*-FPP under the same assay conditions. [c] The K_m value reported here for *E,E*-FPP is significantly higher than that reported previously for this enzyme (e.g., 40 nM),[20] but this may be due to the higher concentration of peptide substrate used in the fluorescence assay. [d] IC_{50} value rather than K_m value. The IC_{50} value was determined from initial rates of assays with a fixed *E,E*-FPP concentration (1.0 μM) and varying *Z,Z*-FPP concentrations in each reaction. [e] A V_{max} value was not determined for this isomer.[13] [f] The concentration of the stock solution of each FPP isomer was determined by phosphate analysis as previously described.[12b]

under the assay conditions, both isomers exhibited *lower* K_m values for the enzyme than the natural substrate *E,E*-FPP. Moreover, their V_{max} values were only ~2-fold less than the V_{max} value determined with *E,E*-FPP. The K_m value does not provide a true indication of affinity for the enzyme due to the complexity of the mFTase kinetic mechanism.[10] Further experiments will be required to investigate the apparent modest, yet still surprising increases in affinity resulting from double bond isomerization. In contrast to the results obtained with *Z,E*-FPP and *E,Z*-FPP, the doubly isomerized analogue *Z,Z*-FPP is an exceptionally poor mFTase substrate.[13] Therefore, this compound was evaluated as an inhibitor of mFTase. The IC_{50} value determined indicates that *Z,Z*-FPP retains significant, submicromolar affinity for mFTase, despite its inability to act as an effective substrate.

The chemical results described here provide a general method for the synthesis of isoprenoid analogues with either double bond geometry. It has been previously demonstrated that a wide variety of β-ketoesters can be prepared via alkylation of acetoacetate dianions,[14] and isoprenoid triflates

(10) (a) Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241−269. (b) Casey, P. J.; Seabra, M. C. *J. Biol. Chem.* **1996**, *271*, 5289−5292.
(11) Das, N. P.; Allen, C. M. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 729−735.
(12) (a) Pompliano, D. L.; Gomez, R. P.; Anthony, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 7945−7946. (b) Cassidy, P. B.; Dolence, J. M.; Poulter, C. D. *Methods Enzymol.* **1995**, *250*, 30−43.
(13) Its mode of interaction with mFTase may be complex. At relatively high *Z,Z*-FPP concentrations, a small initial fluorescence increase was occasionally observed. However, no further turnover was observed, and thus an accurate rate for farnesylation with Z,Z-FPP could not be determined.
(14) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082−1087.

can be coupled with a wide variety of organotin,[3a] organoboron,[5d] and organocopper reagents.[15] Thus this route may also be of more general interest, in that it should be applicable to the stereoselective synthesis of a broad range of trisubstituted allylic alcohols.

The biological results, in particular the ability of *Z*,*E*-FPP and *E*,*Z*-FPP to act as effective mFTase substrates, are quite surprising. This is particularly true because the *E*,*E*-isomer of α-hydroxyfarnesyl phosphonate is a very potent mFTase inhibitor, while the *Z*,*E*-isomer is a very poor inhibitor.[16] Perhaps the hydroxyphosphonates bind to mFTase in a different manner than the FPP analogues. The results seen with the three FPP isomers also appear surprising in view of the exceptionally low K_d for FPP binding to mFTase (∼2 nM)[10b] along with the restrictive farnesyl binding site evident from recent crystallographic structures.[17] However, previous studies have indicated that several other structurally diverse FPP analogues can bind tightly to FTase, and a subset of these can act as alternative substrates.[18] Whatever the reason for the observed relaxed substrate specificity of mFTase, it is clear that this enzyme can be utilized to attach the unnatural *Z*,*E*- and *E*,*Z*-farnesyl moieties to cysteine residues in peptides and proteins that are normally modified with the natural *E*,*E*-farnesyl chain.[19]

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(15) (a) Reference 3b. (b) Zahn, T. J.; Ksebati, M. B.; Gibbs, R. A. *Tetrahedron Lett.* **1998**, *39*, 3991−3994. Zahn, T. J.; Gibbs, R. A., unpublished results. (c) For the copper-catalyzed coupling of organozinc reagents with **5**, see: Lipshutz B. H., Vivian R. W. *Tetrahedron Lett.* **1999**, *40*, 2871−2874.

(16) (a) Hohl, R. J.; Lewis, K.; Cermak, D. M.; Wiemer, D. F. *Lipids* **1998**, *33*, 39−46. (b) Note also that with the prenyltransferase FPP synthase, neryl diphosphate, the 2-*Z*-isomer of the natural substrate geranyl diphosphate is not a substrate: Popjak, G.; Holloway, P. W.; Bond, R. P. M.; Roberts, M. *Biochem. J.* **1969**, *111*, 333−343.

(17) (a) Park, H.; Boduluri, S.; Moomaw, J.; Casey, P.; Beese, L. *Science* **1997**, *275*, 1800−1804. (b) Strickland, C. L.; Windsor, W. T.; Syto, R.; Wang, L.; Bond, R.; Wu, Z.; Schwartz, J.; Le, H. V.; Beese, L. S.; Weber, P. C. *Biochemistry* **1998**, *37*, 16601−16611 and references therein.

(18) See, for example: (a) McGeady, P.; Kuroda, S.; Shimizu, K.; Takai, Y.; Gelb, M. H. *J. Biol. Chem.* **1995**, *270*, 26347−26351. (b) Edelstein, R. L.; Distefano, M. D. *Biochem. Biophys. Res. Commun.* **1997**, *235*, 377−382. (c) References 3a and 3b.

(19) All reported compounds were chromatographically homogeneous and exhibited appropriate NMR spectra. Representative procedures for the syntheses of **10**, **14**, and **15** are given below. **Ethyl 7,11-Dimethyl-3-oxododeca-6(*Z*),10-dienoate (10).** Monosodium ethyl acetoacetate (4.26 g, 28.0 mmol) in 56.0 mL of THF (distilled from Na/benzophenone) was cooled to 0 °C and treated with butyllithium (2.0 M in hexane, 14.7 mL, 29.4 mmol). After 20 min, neryl bromide (3.03 g, 14.0 mmol) was added to the resulting dianion. After 30 min at 0 °C, the reaction was quenched (∼10 mL of 10% aqueous citric acid) and extracted with ether (3 × 50 mL). The organic layers were combined, washed with saturated NaCl (2 × 30 mL), and dried ($MgSO_4$). Flash chromatography (hexane/EtOAc 9:1) afforded 2.50 g (67%) of **10**. ^{1}H NMR: δ 1.28 (t, 3H), 1.61 (s, 3H, CH_3 at C_{11}), 1.69 (app s, 6H, CH_3 at C_7 and C_{12}-CH_3), 2.04 (narrow m, 4H), 2.30 (m, 2H), 2.56 (app t, 2H), 3.43 (s, 2H), 4.19 (q, 2H), 5.08 (m, 2H). ^{13}C NMR: δ 14.8 (O−CH_2CH_3), 18.4 (CH_3 at C_{11}), 22.6 (C_5), 24.0 (CH_3 at C_7), 26.4 (C_{12}), 27.3 (C_{10}), 32.6 (C_9), 44.0 (C_4), 50.1 (C_2), 62.1 (O-CH_2-CH_3), 123.6 (C_6), 124.8 (C_{10}), 132.4, 137.5, 167.9, 203.3.[9] **Ethyl 3-(Trifluoromethylsulfonyloxy)-7,11-dimethyldodeca-2(*E*),6(*Z*),10-trienoate (14).** To a solution of **10** (867 mg, 3.26 mmol) in DMF (8.0 mL, HPLC grade, used as obtained) at −60 °C was added potassium bis(trimethylsilyl)amide (0.5 M in toluene; 7.7 mL, 3.85 mmol). After 2 h, 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]-5-chloropyridine (1.39 g, 3.54 mmol) in ∼5 mL of DMF was added to the resulting enolate solution, and stirring was continued at ca. −60 °C for 3.5 h. The reaction was then taken up in 30 mL of ether, washed with 10% aqueous citric acid (2 × 20 mL), and water (20 mL). The organic layer was dried over $MgSO_4$ and concentrated. Flash chromatography (20:1 hexane/EtOAc) afforded 797 mg (60%) of triflate **14**. None of the isomeric triflate **11** was observed by proton NMR. ^{1}H NMR: δ 1.28 (t, 3H), 1.60 (s, 3H), 1.69 (app s, 6H), 2.04 (app s, 4H), 2.30 (m, 2H), 2.91 (t, 2H), 4.20 (q, 2H), 5.11 (m, 3H), 5.93 (s, 1H). Note the characteristic strong deshielding of the C_4-CH_2 signal (δ 2.91) by the ester carbonyl (C_4-CH_2 signal for triflate **11**: δ 2.39). **Ethyl 3,7,11-Trimethyldodeca-2(Z),6(Z),10-trienoate (15).** Triflate **14** (580 mg, 1.46 mmol), $Pd(PhCN)_2Cl_2$ (28 mg, 0.073 mmol), $AsPh_3$ (45 mg, 0.146 mmol), and CuI (28 mg, 0.146 mmol) were dissolved in 1.8 mL of *N*-methylpyrrolidone (NMP; 99.5%, anhydrous). This solution was heated to ∼100 °C, and tetramethyltin (0.40 mL, 1.91 mmol. **CAUTION:** poisonous and volatile) was added. After 15 h, the reaction was cooled, taken up in 100 mL of EtOAc, and washed with aqueous KF (3 × 30 mL). The aqueous layer was back-extracted with EtOAc (2 × 15 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. Flash chromatography (hexane/EtOAc 20:1) afforded **15** (248 mg, 64%). ^{1}H NMR: δ 1.28 (t, 3H), 1.61 (s, 3H), 1.68 (s, 6H), 1.88 (s, 3H), 2.04 (m, 4H), 2.15 (m, 2H), 2.63 (t, 2H, C_4-CH_2), 4.12 (q, 2H), 5.16 (m, 2H), 5.65 (s, 1H). The identity, and in particular the stereochemistry, of this ester was confirmed by the similarity of the methyl peaks in its ^{1}H NMR spectrum to that of (*Z*,*Z*)-methyl farnesoate.[7b] Moreover, reduction of **15** afforded (*Z*,*Z*)-farnesol **16** with an ^{1}H NMR spectrum identical to that previously reported.[7a] GC-MS analysis of a sample of **16** indicated that it contained 98% (*Z*,*Z*)-farnesol, 2% (*E*,*Z*)-farnesol, and none of the other two geometric isomers.

(20) Pompliano, D. L.; Rands, E.; Schaber, M. D.; Mosser, S. D.; Anthony, N. J.; Gibbs, J. B. *Biochemistry* **1992**, *31*, 3800−3807.