VOLUME 122, NUMBER 30
AUGUST 2, 2000

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Evaluation of Isoprenoid Conformation in Solution and in the Active Site of Protein-Farnesyl Transferase Using Carbon-13 Labeling in Conjunction with Solution- and Solid-State NMR

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Received March 9, 2000

Abstract: The enzyme protein-farnesyl transferase (FTase) catalyzes the farnesylation of the Ras protein and other key signal transduction proteins, using farnesyl diphosphate (FPP) as the prenyl source. Inhibitors of FTase are thus of great interest as potential novel anticancer agents. The design of such agents would be informed by a detailed knowledge of the solution conformation of FPP, as well as its conformation in the active site of FTase. Four bis-^{13}C-labeled derivatives of farnesol and geranylgeraniol have been synthesized and used to prepare the corresponding FPP and GGPP derivatives. The labeled farnesyl and geranylgeranyl derivatives **2**−**7** were utilized in conjunction with solution ^{13}C NMR to probe the conformation of the prenyl chain in a variety of different solvents. These studies, along with molecular dynamics simulations, demonstrate that the prenyl chain exists primarily in an extended conformation. Surprisingly, this preference for the extended conformation is solvent-insensitive; no significant change in conformation is seen with all six solvents investigated, *including water*. The [6,15-bis ^{13}C]FPP analogue **8** was complexed with mammalian FTase, and this complex was utilized in conjunction with rotational resonance MAS NMR to investigate the prenyl chain conformation when bound in the active site of this enzyme. The conformation determined from these experiments is in good agreement with the structure determined from crystallographic studies on the FPP−FTase complex. Thus, the isoprenyl chain of FPP exhibits a strong preference for an extended conformation, both in a variety of solvents of different polarities and in the active site of mammalian FTase.

The mevalonate pathway, both the main trunk and its various branch points, has been the subject of intense pharmaceutical, biochemical, and chemical research activity.[1] The chemical mechanisms of steroid and terpene cationic cyclization reactions have been the subjects of long-standing and intensive research interest from bioorganic and biological chemists.[2−5] The recent

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10.1021/ja000860f CCC: $19.00
Published on Web 07/08/2000

discovery of protein farnesylation as a necessary step in the activation of mutant Ras proteins and certain other key signal transduction proteins has spurred intense interest in the development and evaluation of protein farnesyltransferase inhibitors as potential novel anticancer agents.[6−9] The farnesylation reaction is carried out by an enzyme, protein-farnesyl transferase (FTase), that uses farnesyl diphosphate (FPP) as the source of the isoprenoid moiety.[10,11] We have recently synthesized several FPP analogues that are effective FTase inhibitors.[12−14] The design of more potent inhibitors[15,16] would be facilitated by a greater understanding of the conformational preferences of FPP, both in solution and in the active site of FTase.[17]

Surprisingly, little is currently known about the solution structure of isoprenoid diphosphates, despite their central role in key biochemical pathways. Previous studies have used molecular mechanics, ab initio techniques, and solid-state structural studies to examine the conformation of prenyl derivatives.[18−20] There has been only one previous report on the solution-phase conformation of an isoprenoid.[21] A key conformational question concerning isoprenoids is the extent of chain folding in solution. As shown in Figure 1, the C_4-C_5 bond in farnesol (**1**) could assume a trans (structure **A**) or gauche conformation (**B**). The trans disposition about the C_4-C_5 bond leads to an overall extended conformation in **A**, while the gauche arrangement leads to a folded isoprenoid conformation in **B**. Carbon-13 NMR studies of a C_3-C_6 bis-^{13}C-labeled isoprenoid should be able to distinguish between conformation **A** and conformation **B**. A Karplus relationship is observed in the $^3J_{CC}$ coupling constants, and the coupling between the labeled carbons in **A** is significantly larger (∼4−5 Hz) than the coupling in the gauche conformer **B** (∼0−2 Hz).[22,23] Such a combination of specific ^{13}C labeling and ^{13}C NMR spectroscopy has been previously utilized[24,25] to evaluate the conformational preferences of flexible molecules.

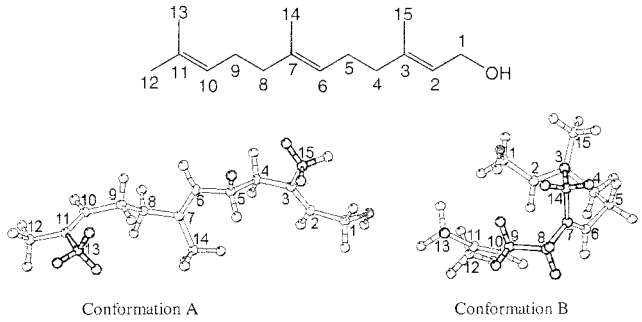

Figure 1. Extended (**A**) and folded (**B**) conformations of farnesol (**1**).

Isoprenoid folding in various environments is central to a number of issues concerning their chemistry and biological activity. Chemical reactivity studies by van Tamelen and Breslow with farnesol and geranylgeraniol derivatives (and other isoprenoids) have indicated that they frequently undergo preferential reaction at the terminal olefin moiety.[2,26] They have argued that these results indicate that isoprenoids prefer a folded or self-coiled conformation (such as **B** in Figure 1), particularly in hydrophilic solvents. Structural studies on dolichol and ubiquinone have implicated folded conformations for these isoprenoids,[20,27] and these folded conformations may have important implications for their biological activities. Isoprenoid folding is also key for the biogenesis of cyclic terpenes and steroids from acyclic isoprenoid precursors.[1,3,4] Recent studies have indicated that the membrane[28,29] or protein-bound[30−32] conformations of the prenyl group may be very important in influencing the biological activity of prenylated proteins. More generally, conformational studies on FPP may provide insight into the influence of hydrophobic effects on the conformation of hydrophobic molecules in water, a problem of significant general interest.[33]

Unfortunately, the approach described above cannot be used to study prenyl groups that are bound to membranes, membrane proteins, or large proteins such as FTase. However, one technique particularly useful for obtaining structural information in these environments is rotational resonance magic angle spinning (MAS) NMR.[34,35] This technique can provide accurate measurements of distance in the range of 3.0−6.8 Å with a

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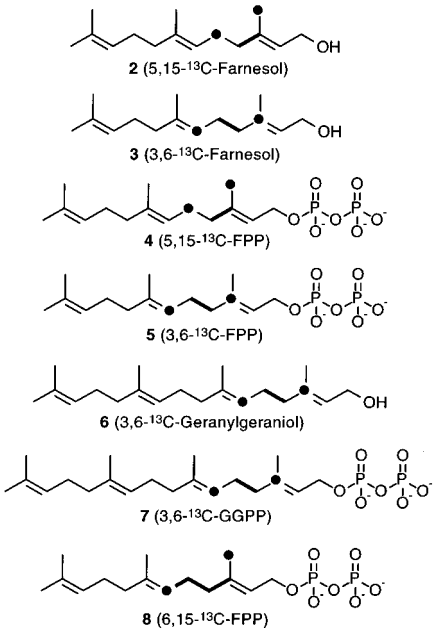

Figure 2. Bis-^{13}C-labeled farnesyl and geranylgeranyl analogues.

resolution of 0.1−0.2 Å,[36] and it has provided key information about the conformation of bis-^{13}C-labeled variants of the isoprenoid retinal in rhodopsin, bacteriorhodopsin, and other environments.[34,37,38] Thus, it could be used to provide accurate conformational information on the farnesyl chain in the active site of FTase, in other proteins, and in a variety of membrane environments.[39] Note that the synthetic objective (the selective introduction of two ^{13}C labels) is the same for the development of spectroscopic probes for both types of NMR studies, although the exact targets differ. Thus, the synthesis of the labeled isoprenoids described below (Figure 2) and their evaluation using solution and solid-state NMR has allowed for the determination of isoprenoid conformation in a wide variety of environments.

Results

Synthesis of Carbon-13-Labeled Farnesol and Geranylgeraniol Analogues. The various bis-carbon-13-labeled prenyl analogues targeted for synthetic design are shown in Figure 2. In each of the structures, the bold dots represent the incorporated ^{13}C labels, and the bold-type bond indicates the bond being probed for its conformation. The preparation of each of these compounds utilizes the vinyl triflate route to isoprenoids that has been developed in this laboratory.[12−14,40−42] The [5,15-bis-^{13}C]farnesol analogue **2** was designed to evaluate the solution-phase conformation of farnesol about the C_3-C_4 bond. Molecular mechanics and ab initio calculations on model systems concerning this bond angle are inconclusive, indicating that both skew and eclipsed conformations about this bond are accessible.[18,19,21,45] However, these two possibilities should be readily distinguished using **2**, as the eclipsed conformer would exhibit a ∼4−5-Hz coupling constant. The [3,6-bis-^{13}C]farnesol **3** provides useful insight into the conformation about the C_4-C_5 bond. The [5,15-bis-^{13}C]FPP analogue **4** and the [3,6-bis-^{13}C]-FPP analogue **5** were designed to provide evidence about the extent of isoprenoid chain folding in aqueous environments. The [3,6-bis-^{13}C]geranylgeranyl derivatives **6** and **7** allow for the evaluation of the effect of chain length on isoprenoid folding. The [6,15-bis-^{13}C]FPP **8** was designed and synthesized for use in the rotational resonance experiments. This compound allows for accurate distance determinations between the labeled methyl group (C_{15}) and the double-bond carbon (C_6) in the rotational resonance MAS technique, due to the large differences in chemical shift (>100 ppm) and the optimal distance between the two labels. Note that this compound also allows for the determination of the degree of folding between the C_2-C_3 and C_6-C_7 double bonds.

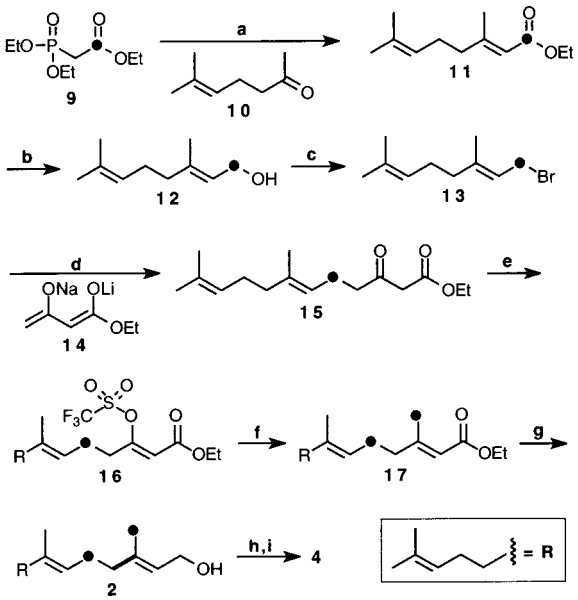

Figure 3. Synthesis of **2** and **4**. Reagents and conditions: (a) **9**, NaH, DME ($CH_3OCH_2CH_2OCH_3$), 0 °C; **10**, 0 °C to room temperature (57%); (b) DIBAL-H, toluene, −78 °C (71%); (c) CBr_4, PPh_3, CH_2Cl_2, (77% (crude yield)); (d) sodium ethyl acetoacetate, ^{n}BuLi, THF, 0 °C; **13**, 0 °C (51%); e) $(Me_3Si)_3NK$, toluene/THF, −78 °C; 2-[$(CF_3SO_2)_2$N]-5-chloropyridine, −78 °C to room temperature (34%); (f) $^{13}CH_3MgI$, CuCN, Et_2O; **16**, -78 °C to room temperature (46%); (g) DIBAL-H, toluene, −78 °C (96%); (h) *N*-chlorosuccinimide, Me_2S, CH_2Cl_2, −30 to 0 °C; **2**, −30 °C to room temperature; (i) (n-$Bu_4N)_3HP_2O_7$, CH_3CN, room temperature (27% from **2**).

The first compounds synthesized were the [5,15-bis-^{13}C]-farnesol and FPP analogues **2** and **4** (Figure 3). The required labeled intermediate geranyl bromide **13** was prepared from commercially available **9** essentially as previously described by

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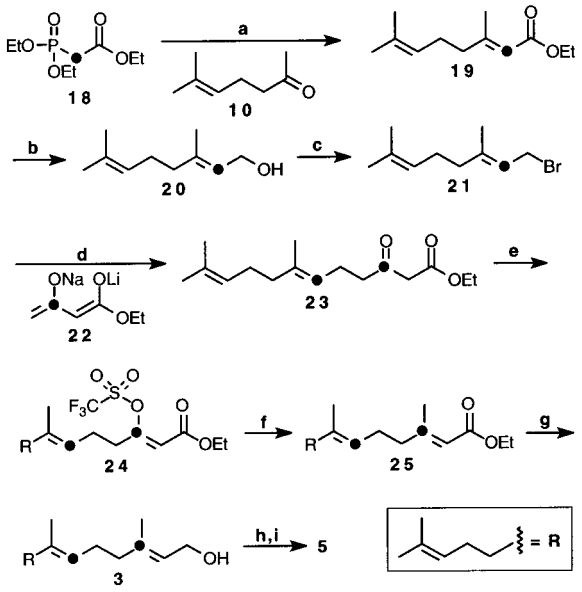

Figure 4. Synthesis of **3** and **5**. Reagents and conditions: (a) **18**, NaH, DME ($CH_3OCH_2CH_2OCH_3$), 0 °C; **10**, 0 °C to room temperature (35%); (b) DIBAL-H, toluene, −78 °C (91%); (c) CBr_4, PPh_3, CH_2Cl_2, (88% (crude yield)); (d) [3-^{13}C]ethyl acetoacetate, NaH, THF; ^{n}BuLi, 0 °C; **21**, 0 °C (26%); (e) $(Me_3Si)_3NK$, toluene/THF, −78 °C; 2-[$(CF_3SO_2)_2$N]-5-chloropyridine, −78 °C to room temperature (38%); (f) $(CH_3)_4Sn$, CuI, $(Ph_3As)_2Pd$, *N*-methylpyrrolidone, 100 °C (82%); (g) DIBAL-H, toluene, −78 °C (94%); (h) *N*-chlorosuccinimide, Me_2S, CH_2Cl_2, −30 to 0 °C; **3**, −30 °C to room temperature; (i) $(n\text{-}Bu_4N)_3HP_2O_7$, CH_3CN, room temperature (33% from **3**).

Urano and colleagues.[46] Coupling of **13** with the dianion of ethyl acetoacetate (**14**)[47] afforded the [5-^{13}C]-β-ketoester **15**. Compound **15** was transformed in a highly stereoselective fashion to the vinyl triflate **16**.[12,48] The key new chemical step in this synthesis involves the attachment of a carbon-13-labeled methyl group to the C_3 position. This was accomplished through a copper cyanide-mediated coupling of [^{13}C]MeMgI with the carbon-13-labeled triflate **16** to give ester **17**. The key advantage of this procedure for this route is the ready availability of the [^{13}C]methyl group from relatively inexpensive [^{13}C]methyl iodide.[49] Reduction of **17** using DIBAL-H gave the first targeted compound, the [5,15-bis-^{13}C]farnesol **2**. This compound was then converted into the final [5,15-bis-^{13}C]-**4** using the two-step chlorination/diphosphorylation procedure developed by Poulter and colleagues.[50]

The synthesis of the [3,6-bis-^{13}C]FPP **5** follows essentially the same synthetic route as for diphosphate **4**, with two distinctions (Figure 4). The geranyl bromide **21** with a ^{13}C label in the 2-position was used as the starting material, rather than **13**. Bromide **21** was then coupled with **22**, the dianion of [3-^{13}C]-ethyl acetoacetate, affording the bis-^{13}C-labeled-β-ketoester **23**. The stereoselective conversion of **23** to the vinyl triflate **24** utilized the same chemistry as shown above. The next step, a palladium-catalyzed, copper iodide-mediated coupling of tetramethyltin with **24** to obtain ester **25**, employed chemistry previously developed in this laboratory.[12,14] Reduction of **25** with DIBAL-H afforded the desired [3,6-bis-^{13}C]farnesol **3**, which was converted to **5** using the diphosphorylation procedure described above.

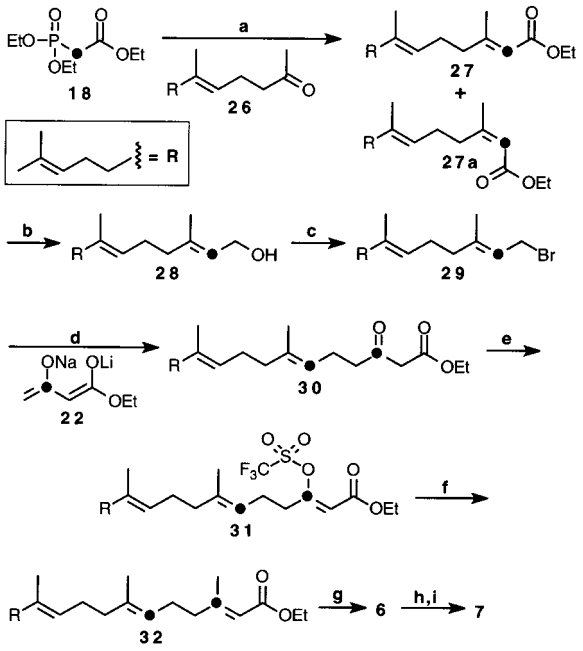

Figure 5. Synthesis of **6** and **7**. Reagents and conditions: (a) **18**, NaH, DME ($CH_3OCH_2CH_2OCH_3$), 0 °C; **26**, 0 °C to room temperature (26%); (b) DIBAL-H, toluene, −78 °C (68%); (c) CBr_4, PPh_3, CH_2Cl_2, (64% (crude yield)); (d) [3-^{13}C]ethyl acetoacetate, NaH, THF; ^{n}BuLi, 0 °C; **29**, 0 °C (43%); (e) $(Me_3Si)_3NK$, toluene/THF, −78 °C; 2-[$(CF_3SO_2)_2$N]-5-chloropyridine, −78 °C to room temperature (65%); (f) $(CH_3)_4Sn$, CuI, $(Ph_3As)_2Pd$, *N*-methylpyrrolidone, 100 °C (60%); (g) DIBAL-H, toluene, −78 °C (62%); (h) *N*-chlorosuccinimide, Me_2S, CH_2Cl_2, −30 to 0 °C; **6**, −30 °C to room temperature; (i) $(n\text{-}Bu_4N)_3HP_2O_7$, CH_3CN, room temperature (43% from **6**).

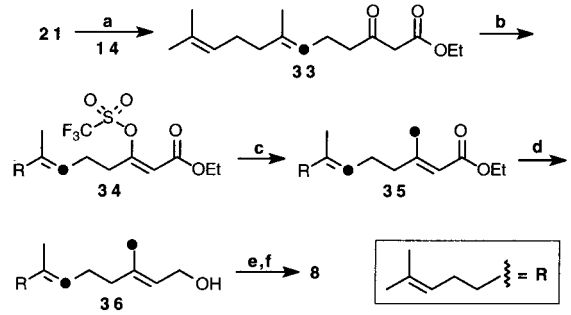

Figure 6. Synthesis of **8**. Reagents and conditions: (a) sodium ethyl acetoacetate, ^{n}BuLi, THF, 0 °C; **21**, 0 °C (33%); (b) $(Me_3Si)_3NK$, toluene/THF, −78 °C; 2-[$(CF_3SO_2)_2$N]-5-chloropyridine, −78 °C to room temperature (39%); (c) $^{13}CH_3MgI$, CuCN, Et_2O; **34**, −78 °C to room temperature (80%); (g) DIBAL-H, toluene, −78 °C (62%); (h) *N*-chlorosuccinimide, Me_2S, CH_2Cl_2, −30 to 0 °C; **36**, −30 °C to room temperature; (i) $(n\text{-}Bu_4N)_3HP_2O_7$, CH_3CN, room temperature (69% from **36**).

The [3,6-bis-^{13}C]geranylgeranyl diphosphate **7** was obtained via the same route used for the synthesis of diphosphate **5**. However, the [2-^{13}C]farnesyl bromide **29** was utilized in place of the geranyl bromide **21** (Figure 5). The preparation of the [6,15-bis-^{13}C]FPP **8** employed a combination of the procedures described for the syntheses of **4** and **5** (Figure 6). Geranyl bromide **21** was coupled with the dianion of unlabeled ethyl acetoacetate (**14**) to give the β-ketoester **33**. Triflation of **33** gave vinyl triflate **34**, which was then coupled with [^{13}C]MeMgI to give the bis-^{13}C-labeled ester **35**. Reduction of ester **35**,

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followed by diphosphorylation leads to diphosphate **8**, with the desired labels in the C_6 and C_{15} positions.

Solution NMR Studies. The incorporation of the appropriate labels in each of the bis-^{13}C-labeled compounds was verified by the intense ^{13}C NMR signals observed in the NMR spectra (see Experimental Section). In addition, in comparison to the known unlabeled farnesyl and geranylgeranyl derivatives, the large $^1J_{C-H}$ couplings observed in the proton spectra were particularly striking and in many cases provided further evidence of the correct incorporation of the desired ^{13}C labels. The labeled farnesol and geranylgeraniol derivatives were initially dissolved in chloroform ($CDCl_3$) and their ^{1}H and ^{13}C NMR spectra were obtained. The incorporation of the appropriate ^{13}C labels in [5,15-bis-^{13}C]farnesol **2** was confirmed by the intense, coupled ^{13}C NMR signals at 16.3 and 26.3 ppm, along with the observation of the large $^1J_{C-H}$ couplings with the $C_{15}-CH_3$ and C_5-CH_2 signals in the proton NMR spectrum. The incorporation of the appropriate ^{13}C labels in the [3,6-bis-^{13}C]farnesol **3** was also confirmed by the intense, coupled ^{13}C NMR signals at 123.8 and 139.8 ppm, along with the observation of ^{13}C coupling with the C_6 vinylic signal in the proton NMR spectrum. The same characteristic ^{13}C and ^{1}H NMR patterns were seen with the 3,6-labeled geranylgeraniol **6**. Following HPLC purification, the FPP and GGPP derivatives were dissolved in D_2O, and their solution ^{1}H and ^{13}C NMR spectra were taken at 500 and 125 MHz.[51a] The signals of the ^{13}C labels were observed to be at 16.3 and 26.3 ppm for the [5,15-bis-^{13}C]FPP **4**. The incorporation of the appropriate ^{13}C labels in **5** was confirmed by the intense, coupled (vide infra) ^{13}C NMR signals at 123.8 and 139.8 ppm, along with the observation of ^{13}C coupling with the C_6 vinylic signal in the proton NMR spectrum. The presence of the labels at the C_6 and C_{15} positions of the diphosphate **8** was confirmed by the intense ^{13}C NMR peaks observed at 123 and 26 ppm. Its proton spectra also demonstrated coupling of the C_6 label with the vinylic proton, like that seen in the ^{1}H NMR spectrum of the [3,6-bis-^{13}C]FPP **5**.

With the target compounds synthesized, the coupling constants of interest for the alcohol and diphosphate derivatives were determined in a straightforward fashion using standard solution ^{13}C NMR in various solvents, and these values are reported in Table 1. The 1.6-Hz coupling constant for the [5,15-bis-^{13}C]farnesol **2** is consistent with a gauche (~60°) or skew (~90°) conformation about the C_3-C_4 bond of farnesol. In contrast, the 3.6-Hz coupling observed for the [3,6-bis-^{13}C]-farnesol **3** indicates that the C_4-C_5 bond exists primarily in a trans (~180°) conformation. These values are identical to those determined by Facke and Berger for unlabeled farnesol using a pulse-transfer sequence for the determination of $^3J_{C-C}$ values,[21] and this confirms the validity of their method. Compound **6**, the [3,6-bis-^{13}C] version of the longer C_{20} isoprenoid geranylgeraniol, was also synthesized as a part of these studies; it was prepared to investigate the effect of chain length on the conformation and extent of folding of isoprenoid chains. Interestingly, the coupling constant for the [3,6-bis ^{13}C]-geranylgeraniol **6** was almost exactly the same (3.7 Hz) as that of the [3,6-bis ^{13}C]farnesol **3**. This indicates that the same preference for a trans conformation exists about the C_4-C_5 bond with longer and presumably more hydrophobic geranylgeranyl chain as well as with the farnesyl chain.

(51) (a) The solution ^{1}H and ^{13}C NMR spectra were taken on the ammonium salt forms of the diphosphates in unbuffered deuterium oxide. Preliminary attempts to obtain the ^{13}C NMR spectrum of **5** in mFTase buffer or as its sodium salt were unsucessful. (b) It has recently been reported that the cmc for FPP is 12 mM, and the cmc for GGPP is 0.45 mM: Bragg, B., Ph.D. Dissertation, University of Utah, 1999.

Table 1. $^3J_{C-C}$ Coupling Constants of Carbon-13-Labeled Isoprenoids

analogue	solvent	coupling const,[a] Hz
[5,15-bis-^{13}C]-farnesol (**2**)	$CDCl_3$	1.6 ± 0.2
[5,15-bis-^{13}C]-FPP (**4**)	D_2O	1.6 ± 0.2
[3,6-bis-^{13}C]-farnesol (**3**)	$CDCl_3$	3.6 ± 0.2
[3,6-bis-^{13}C]farnesol (**3**)	CD_3OD	3.3 ± 0.2
[3,6-bis-^{13}C]FPP (**5**)	D_2O	3.5 ± 0.2[b]
[3,6-bis-^{13}C]FPP (**5**)	D_2O/CD_3OD	3.5 ± 0.2
[3,6-bis-^{13}C]geranylgeraniol (**6**)	C_6D_{12}	3.4 ± 0.2
[3,6-bis-^{13}C]geranylgeraniol (**6**)	C_6D_6	3.5 ± 0.2
[3,6-bis-^{13}C]geranylgeraniol (**6**)	$CDCl_3$	3.7 ± 0.2
[3,6-bis-^{13}C]geranylgeraniol (**6**)	CD_3OD	3.7 ± 0.2
[3,6-bis-^{13}C]geranylgeraniol (**6**)	$CD_3S(O)CD_3$	3.4 ± 0.2
[3,6-bis-^{13}C]GGPP (**7**)	D_2O	3.7 ± 0.2[c]

[a] The error in the $^3J_{C-C}$ coupling constant measurements is estimated on the basis of the digital resolution of the NMR spectra (see Experimental Section). [b] The same $^3J_{C-C}$ coupling constant was obtained for both a ~5 mM solution and a ~10 mM solution of **5**.[51b] [c] The $^3J_{C-C}$ coupling constant was obtained from a ~10 mM solution of **7**.[51b]

All of the coupling constants discussed above were determined in deuteriochloroform, a relatively hydrophobic solvent. It might be expected that the isoprenoid chain would assume a more folded conformation in a more polar solvent. The ^{13}C NMR spectrum of farnesol **3** was thus also obtained in the significantly more polar solvent methanol-d_4, which indicated a slightly lower preference for a trans arrangement about the C_4-C_5 bond, although the magnitude of the change (0.3 Hz) was small. Thus, the C_4-C_5 bond in [3,6-bis-^{13}C]geranylgeraniol **6** was probed in several different solvent environments to determine in a more careful manner how solvent polarity influences the conformation of the prenyl chain. The results indicate that there are no significant differences in conformation about the C_4-C_5 bond between solvents with different polarity. In fact, the spectrum in the most polar solvent used, DMSO-d_6, gave an observed coupling constant exactly the same as that in the least polar solvent used, cyclohexane-d_{12} (Table 1).

The ^{13}C NMR spectra of diphosphates **4**, **5**, and **7** were determined in D_2O, as FPP and GGPP are water-soluble, and water is the biologically relevant solvent. It might be supposed that the hydrophobic farnesyl moiety of FPP would exist in a more folded, globular form in water and that the gauche conformation in the hydrocarbon chain would be observed. Such folded conformations correspond to those required for the cyclization of FPP to various sesquiterpene structures. However, the coupling constants observed for **4** (1.6 Hz) and **5** (3.5 Hz) in D_2O are *virtually identical* to those observed for **2** and **3** (respectively) in $CDCl_3$. The coupling constants for the [3,6-bis-^{13}C]FPP and GGPP analogues **5** and **7** were observed to be 3.5 and 3.7 Hz, respectively (Table 1). This confirms that the conformation of the C_4-C_5 bond in solution is primarily trans and that chain length does not influence the conformational preferences of the prenyl moiety. Again, there was no significant difference between the conformation of geranylgeraniol **6** and the diphosphate **7** in these experiments. The proton and carbon-13 NMR spectra of these two compounds provided no evidence of aggregation at the concentrations investigated in these experiments. Furthermore, essentially no change in the coupling constant of the [3,6-bis-^{13}C]FPP analogue **5** was seen when the concentration was varied from ~10 to ~5 mM,[51b] or with ~50:50 D_2O/CD_3OD as a solvent. Presumably, the more hydrophobic solvent methanol would have disrupted any aggregation by the hydrophobic farnesyl chains.

Molecular Dynamics Simulations of Farnesol Conformation. The conformational preferences of the isoprenoid chain

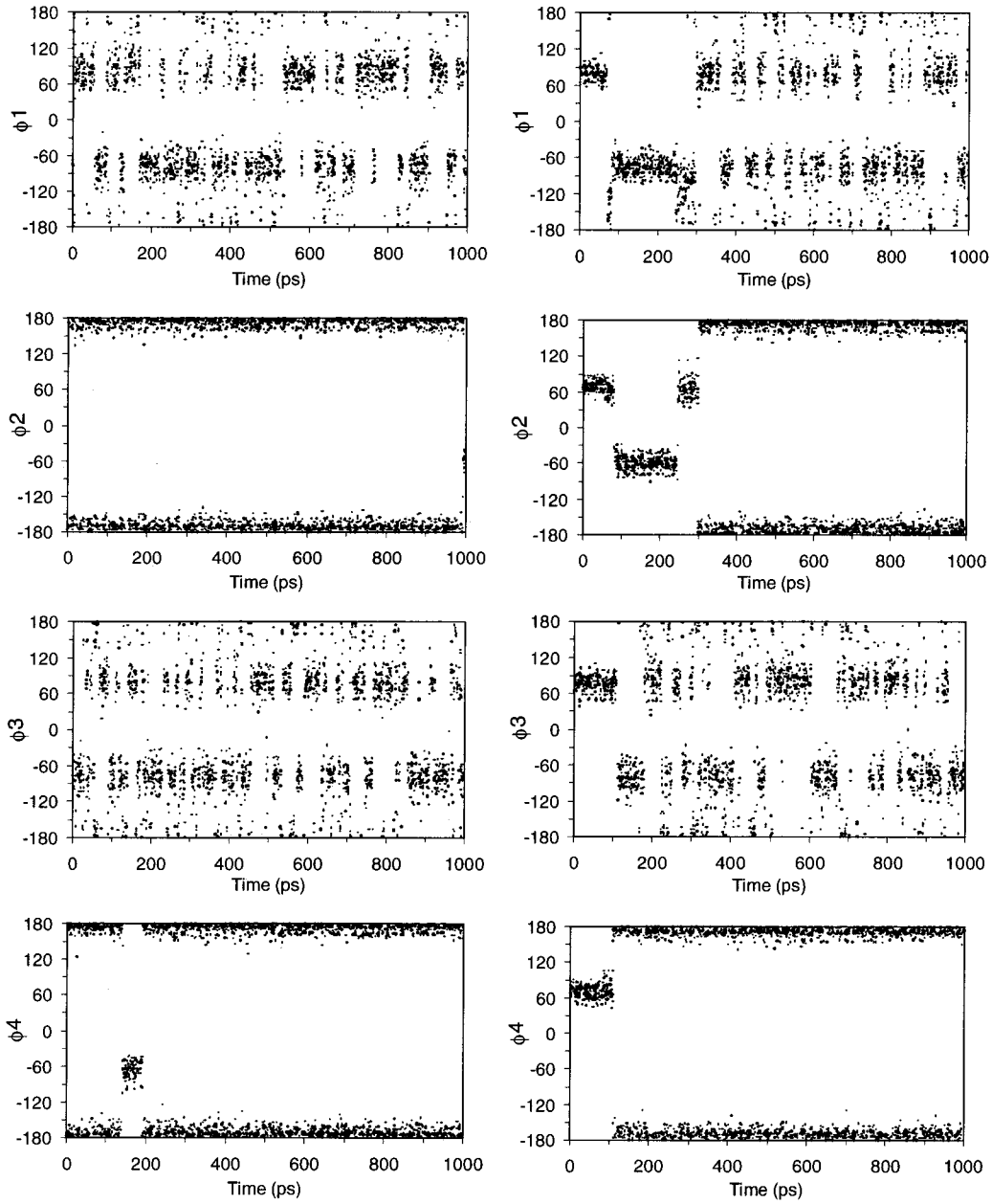

Figure 7. Profiles of the torsional angles $\phi1-\phi4$ of farnesol as a function of MD simulation time (left column, conformation **A** as a starting structure; right column, conformation **B** as a starting structure). The dihedral angles of farnesol are defined as following: $\phi1$ ($C_5-C_4-C_3-C_{15}$); $\phi2$ ($C_6-C_5-C_4-C_3$); $\phi3$ ($C_9-C_8-C_7-C_{14}$); $\phi4$ ($C_{10}-C_9-C_8-C_7$).

have been examined by several workers previously, with the most recent MM3 studies performed by Facke and Berger.[21] The calculated conformations determined by these workers were consistent with their (and thus our) NMR results. However, we wished to examine in more detail the conformational preferences of the isoprenoid chain by computational methods. Such studies were particularly important in view of the surprisingly solvent-insensitive nature of the NMR results described above. Note that traditional energy minimization alone (such as MM3 and related procedures) limits the structure to the local minimum in the region of conformation space afforded by the starting structure. The molecular dynamics (MD) method shows its advantage by allowing the system to surmount energy barriers that are of order of k_BT per degree of freedom. They thus extensively sample the conformational space and obtain structural details complementary to those elucidated from experiments.

Two starting conformations of farnesol, one (**A**; Figure 1) resembling the crystal structure of FPP bound with farnesyltransferase (PDB: 1ft1)[52] and the other (**B**) adopting a coiled conformation ($\phi2$ and $\phi4$: gauche) were used in this MD simulation study (Figure 7 and Table 2). Note that the dihedral angles of farnesol are defined as follows: $\phi1$ ($C_5-C_4-C_3-C_{15}$); $\phi2$ ($C_6-C_5-C_4-C_3$); $\phi3$ ($C_9-C_8-C_7-C_{14}$); $\phi4$ ($C_{10}-C_9-C_8-C_7$). The coiled conformation **B** was employed to determine the effect of the starting structure on the MD trajectory. Figure 8 shows 1-ns torsional trajectories of the farnesol molecule for both conformations **A** and **B**. In general, the extended form of farnesol is dynamically favored in both MD runs. In both cases, torsional angles $\phi2$ and $\phi4$ were distinctively locked into a trans conformation, which is consistent with the solution NMR conformational studies described above. It is also quite similar to the conformations observed by crystallography[52] and solid-state NMR (vide infra) in the active

(52) Long, S. B.; Casey, P. J.; Beese, L. S. *Biochemistry* **1998**, *37*, 9612−9618.

Table 2. Torsional Angles of $\phi1-\phi4$ in the FPP Crystal Structure, Conformations **A** and **B**[a]

	cryst struct	**A**	**B**
$\phi1$	−69	−68	86
$\phi2$	−171	−178	69
$\phi3$	112	−76	79
$\phi4$	−159	−178	65

[a] The dihedral angles of farnesol are defined as following: $\phi1$ ($C_5-C_4-C_3-C_{15}$); $\phi2$ ($C_6-C_5-C_4-C_3$); $\phi3$ ($C_9-C_8-C_7-C_{14}$); $\phi4$ ($C_{10}-C_9-C_8-C_7$).

site of FTase. For conformation **B**, the torsion of $\phi2$ shifted from gauche to trans at ~300 ps. Similarly, it was observed that the torsion of $\phi4$ experienced a significant migration from gauche to trans in the early stage of the simulation. This indicates that the conformational space of farnesol was extensively explored and sampled, and varying the starting conformation of farnesol does not affect its resulting dynamic profile.

The conformation between the second and third double bonds (i.e., $\phi4$) was not investigated experimentally through synthesis and NMR analysis of a specifically labeled farnesol analogue. However, the similarity of the MD simulations for $\phi2$ and $\phi4$ indicates that the conformation about $\phi4$ is similar to the conformation of $\phi2$ that was determined through analysis of the NMR spectra of **3**. The torsions of $\phi1$ and $\phi3$ were not as strictly confined within a narrow angle range as were torsions of $\phi2$ and $\phi4$. For both starting conformations, it is evident that the majority of the populations of $\phi1$ and $\phi3$ are near 90°, although they can easily swing between −60° and +60°. Thus, the MD studies appear to confirm the solution NMR results that indicate a preference of the $\phi1$ bond for a skew arrangement, but indicate that it still retains significant conformational flexibility about this average position.

Solid-State NMR Studies. The [6,15-bis-^{13}C]FPP **8** was complexed with recombinant mammalian protein-farnesyltransferase (mFTase),[53] and the resulting complex was lyophilized to give a powder. Control experiments demonstrated that lyophilized mFTase retains its activity when reconstituted, and thus presumably its active site retains its three-dimensional shape in the solid state. Note also that the affinity of mFTase for FPP is quite high ($K_D \sim 2$ nM),[53a] and thus (a) it is likely that the labeled FPP will remain bound to the active site of mFTase and (b) solid-state NMR techniques are clearly appropriate for studies on the structure of this tightly bound ligand. Rotational resonance MAS NMR was used to determine the conformation of FPP in the active site of the enzyme. Figure 9 presents the MAS NMR spectrum of mFTase with bound [6,15-bis-^{13}C]FPP **8**. The labeled ^{13}C resonances are observed as sharp lines at 124.1 and 16.6 ppm. The line width of 110 Hz is consistent with the prenyl chain of FPP having a single conformation. No dipolar splittings were observed in the rotationally coupled resonances, indicating that the labeled sites were separated by >3.8 Å.[38] To drive the rotational resonance magnetization exchange, the olefinic ^{13}C resonance at 124.1 ppm was inverted and the spinning speed was adjusted to 9716 Hz. This corresponds to the $n = 1$ rotational resonance condition. Magnetization exchange at the $n=1$ condition is largely insensitive to the chemical shift and dipolar tensor orientations.

Figure 10 presents the observed rotational resonance exchange curves for the FPP model compound **8** (filled circles) and for FPP **8** bound to mFTase (filled squares). The experimental data are plotted along with a set of simulated rotational resonance exchange curves corresponding to distances between 4.1 and 4.7 Å. The rotational resonance exchange curve for the FPP model compound (i.e., **8** in the solid form not bound to mFTase) corresponds to a distance of 4.5 ± 0.2 Å. The 6,15-bis-^{13}C labels are 4.5 Å apart when the farnesyl moiety of the FPP molecule is in a planar trans conformation. The exchange curve for FPP bound in the active site of mFTase corresponds to a distance of 4.4 ± 0.2 Å. This distance correlates very well with the 4.44-Å distance in the crystal structure of mFTase containing bound FPP reported by Long et al.[52] The RR-derived distance rules out FPP conformations with large deviations in torsions about the C_3-C_4 and C_4-C_5 bonds. Depending on the magnitude of these two torsion angles, the distance between the labeled ^{13}C sites can vary between ~2.6 and 4.5 Å.

Discussion

With the target compounds synthesized and the coupling constants determined for the alcohols and diphosphates, we could determine important information concerning the solution-phase conformation of the isoprenoid derivatives. The 1.6-Hz coupling constant observed with [5,15-bis-^{13}C]farnesol **2** and FPP (**4**) is consistent with a skew (90°) conformation about the C_3-C_4 bond, while the coupling constants of the [3,6-bis-^{13}C]-farnesyl and geranylgeranyl analogues (**3**, **5**, **6**, and **7**) indicate that C_4-C_5 bond exists primarily in the trans (~180°) conformation. These values are essentially identical to those determined by Facke and Berger for unlabeled farnesol using a pulse-transfer sequence for the determination of $^3J_{C-C}$ values[21] and thus provide further confirmation of the validity of their method. These values are also consistent with the molecular dynamics studies described above. Note in particular that the skew configuration about the C_3-C_4 bond and the trans configuration about the C_4-C_5 bond are reproduced in the MD simulations, irrespective of whether an extended or folded conformation of farnesol is used as a starting point (Figures 7 and 8).

Chemical reactivity studies by van Tamelen and Breslow with farnesol and geranylgeraniol derivatives (and other isoprenoids) have indicated that they frequently undergo preferential reaction at the terminal olefin moiety.[2,26] They have argued that these results indicate that isoprenoids prefer a folded or self-coiled conformation, particularly in hydrophilic solvents. However, the observed coupling constant for [3,6-bis-^{13}C]geranylgeraniol **6** is *essentially the same in five solvents of widely different polarities* (cyclohexane, benzene, chloroform, methanol, and DMSO: $^3J_{C-C} = 3.4-3.7$ Hz). The large $^3J_{C-C}$ values observed in all solvents are consistent with either a primarily trans or a primarily eclipsed conformation and inconsistent with a gauche or skew conformation.[22,23] Eclipsed conformations can be eliminated by the energetic barrier to such hindered conformations, as confirmed by molecular mechanics calculations, and are particularly unlikely in hydrophobic solvents such as cyclohexane. This indicates the conformational stability of the trans arrangement about the C_4-C_5 bond in the isoprenoid chain must outweigh any unfavorable interactions incurred by the hydrophobic group in D_2O. This apparently surprising result is consistent with Menger's findings that $^3J_{C-C}$ measurements for the C_3-C_4 bond in *n*-undecane do not vary as the solvent environment is changed from chloroform to water/ethanol mixtures.[54,55]

The studies on other solvents are interesting and instructive, particularly from a theoretical point of view, but water is a

(53) (a) Yokoyama, K.; Zimmerman, K.; Scholten, J.; Gelb, M. H. *J. Biol. Chem.* **1997**, *272*, 3944−3952. (b) Scholten, J. D.; Zimmerman, K. K.; Oxender, M. G.; Leonard, D. M.; Sebolt-Leopold, J.; Gowan, R.; Hupe, D. J.; *J. Biol. Chem.* **1997**, *272*, 18077−18081.

(54) (a) Menger, F. M.; Dulany, M. A.; Carnahan, D. W.; Lee, L. H. *J. Am. Chem. Soc.* **1987**, *109*, 6899−6900. (b) Menger, F. M.; D′Angelo, L. L. *J. Am. Chem. Soc.* **1988**, *110*, 8241−8242.

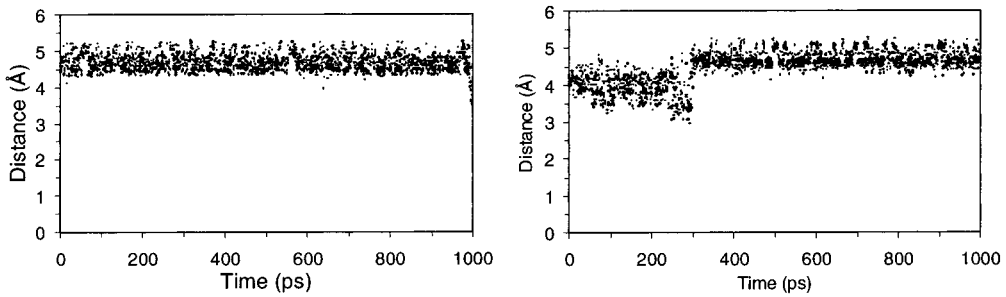

Figure 8. Profile of C_6-C_{15} distances in farnesol as a function of MD simulation time. The left figure represents the trajectory starting from an extended conformation (**A**, Figure 1), and the right figure represents the trajectory starting from a coiled conformation (**B**, Figure 1).

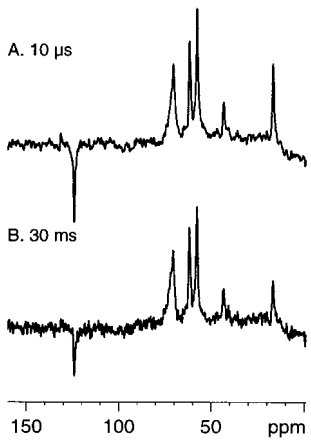

Figure 9. Representative rotational resonance MAS NMR spectra of the **8**−mFTase complex. The sharp, intense peaks at 124.1 and 16.6 ppm are due to the labeled atoms in [6,15-bis-^{13}C]FPP **8**. In the experiment, the peak at 124.1 ppm was inverted, and the spectra in panels A and B indicate the magnetization exchange between the labeled peaks at 10 μs and 30 ms, respectively.

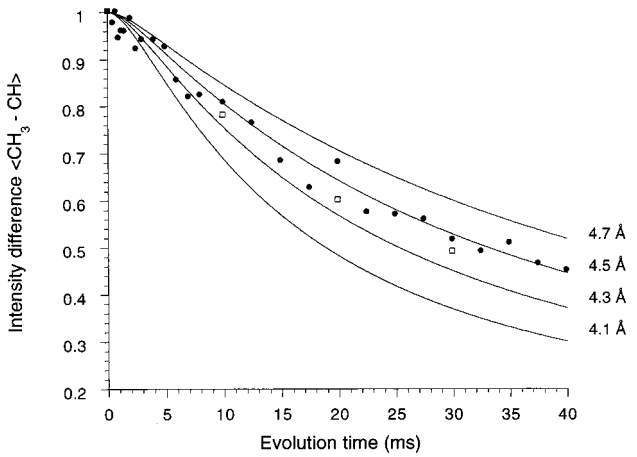

Figure 10. Rotational resonance magnetization exchange curves for [6,15-bis-^{13}C]FPP **8** (filled circles) and the[6,15-bis-^{13}C]FPP **8**−mFTase complex (open squares). Simulated curves are shown for different internuclear distances between the 6-^{13}C and 15-^{13}C labels. The curves were simulated using a T2ZQ of 1.5 ms derived from the best to the FPP model compound and an internuclear distance of 4.5 Å.

fundamentally different solvent.[33] Moreover, FPP and GGPP are water-soluble, they are the key biological compounds of interest, and water is the biologically relevant solvent for these molecules. Thus, conformational studies on labeled FPP and GGPP analogues in D_2O are central to our study. A comparison of the coupling constants for the labeled farnesol and geranylgeraniol analogues in various nonaqueous solvents, and the coupling constants for the related diphosphates in D_2O, indicates that there is *essentially no difference in conformation in water* (Table 1). This is particularly surprising for **5** and **7** in view of the expected preference of the prenyl chain for folded conformations in water. Micelle formation by FPP and GGPP in water could account for the lack of gauche coupling constants observed with the 3,6 bis-^{13}C-labeled FPP and GGPP analogues **5** and **7**. However, Menger found that the gauche conformational preference of hydrocarbon chains is increased, rather than decreased, in micelles.[54] Thus, it is unlikely that the observed coupling constants are due to micelle or aggregate formation. Moreover, the proton and carbon-13 NMR spectra of the diphosphates provide no evidence of aggregation in D_2O, and essentially no change in coupling constant of **5** was seen when methanol was used as a cosolvent. Furthermore, the lack of $^3J_{C-C}$ variation with solvent also argues strongly against extensive prenyl chain folding in polar solvents such as methanol or water.

These findings with the diphosphates are quite surprising because the hydrophobic farnesyl and geranylgeranyl moiety of FPP and GGPP would be expected to exist in a more folded, globular form in water, and gauche conformations in the hydrocarbon chains would be expected to predominate.[2,26] The determination of the preferred solution conformation of FPP has important additional implications, beyond the design of FPP-based FTase inhibitors. Structural studies on dolichol and ubiquinone have indicated the presence of folded conformations for these isoprenoids,[20,27] and these folded conformations may have important implications for their biological activities. It should also be noted that isoprenoid folding is key for the biogenesis of cyclic terpenes and steroids from acyclic isoprenoid precursors.[1,3,4] Thus, the issue of the extent of isoprenoid folding in solution is central to a number of issues concerning their chemistry and biological activity. It is important to note that NMR studies such as those described in this paper only provide a time-averaged view of the conformation of flexible molecules such as these isoprenoids.[61b] Thus, our results

(55) Note that the overall trans (extended) conformational preference seen in this study stands in sharp contrast to the significant proportion of gauche conformers calculated based on early structural studies on amorphous *trans*-1,4-polyisoprene: Mark, J. E. *J. Am. Chem. Soc.* **1966**, *88*, 4354−4359. Mark, J. E. *J. Am. Chem. Soc.* **1967**, *89*, 6829−6835. Flory, P. J. *Statistical Mechanics of Chain Molecules*; John Wiley & Sons: New York, 1969; p 192−198. However, the structures of crystalline *trans*-1,4-polyisoprene (Takahashi, Y.; Sato, T.; Tadokoro, H.; Tanaka, Y. *J. Polym. Sci.* **1973**, *11*, 233−248) and its inclusion complex with perhydrotriphenylene (Tonelli, A. E. *Macromolecules* **1990**, *23*, 3134−3137) indicate that in these environments this polymer does adopt an entirely trans, extended conformation. The relationship of these polymer studies to our solution studies is unclear, but we thank a reviewer for bringing them to our attention.

(56) Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 159−164.
(57) Engberts, J. B. F. N. *Pure Appl. Chem.* **1995**, *67*, 823−828.
(58) Gajewski, J. J. *Acc. Chem. Res.* **1997**, *30*, 219−225.
(59) Jiang, X.-K. *Acc. Chem. Res.* **1988**, *21*, 362−367.

certainly do not exclude the presence of conformers of FPP or GGPP that have a gauche configuration about the C_4-C_5 bond and thus are folded in solution. However, our results do demonstrate that (a) such conformers are a small minority of the total molecular ensemble and (b) the proportion of folded conformations does not increase as solvent polarity increases.

These studies on FPP and GGPP may also provide insight into the general problem of the influence of hydrophobic effects[33] on the conformation of small molecules in water. This is a subject of significant current interest, due to the role of hydrophobic effects in many biological processes such as protein folding. The acceleration of certain organic reactions in water has provided a key area for the study of hydrophobic effects on small molecules,[56] although the interpretation of these effects is not straightforward, and factors other than hydrophobic induced association may in part lead to the observed rate accelerations.[57,58] Several lines of evidence indicate that small flexible hydrophobic molecules can undergo self-coiling,[59,60] such as that proposed by van Tamelen and Breslow for farnesyl and geranylgeranyl derivatives. For example, NMR studies have indicated that the complex isoprenoid-derived anticancer drugs Taxol (paclitaxel) and Taxotere undergo a prominent conformational shift to a folded or "collapsed" form in polar/aqueous solvents,[61a,b] and this form may be of importance for its binding to tubulin.[39] Moreover, Wiley and Rich have extensively characterized the "hydrophobic collapse" exhibited by certain peptidic enzyme inhibitors and the important pharmacological consequences of this conformation shift.[62] However, in carefully designed model systems, detailed NMR studies by Gellman and co-workers have produced conflicting results concerning the ability of small molecules to undergo water-induced folding or self-coiling due to the operation of the hydrophobic effect.[63,64] In order for folding or self-coiling of a small flexible molecule to be preferred in solution, the unfavorable interactions of the hydrophobic chain with the hydrophilic solvent must outweigh the unfavorable conformational effects induced by folding of the isoprenoid chain, in particular, the introduction of gauche or eclipsing interactions into the chain. It does not appear from our studies that the hydrophobic effect is sufficient to overcome the basic conformational preferences of the farnesyl and geranylgeranyl moiety; however, note the caveat concerning time-averaged nature of NMR studies (vide supra).[61b]

Mammalian FTase is a key drug target, and it thus has been the subject of several solution NMR[65,66] and crystallographic structural studies,[52,67−70] but no previous MAS NMR studies. We have used rotation resonance MAS NMR in conjunction with the labeled FPP analogue **8** to determine the conformation of the prenyl chain of FPP in the active site of mFTase. These results demonstrated that FPP is in an extended trans conformation in the active site of the enzyme. The exchange curve corresponds to a 4.4-Å distance that is in excellent agreement with the 4.44-Å distance reported in the crystal structure of the FTase bound to FPP.[52] The rotational resonance-derived distance rules out FPP conformations with large deviations in torsions about the C_3-C_4 and C_4-C_5 bonds and provides further evidence that verifies the preferred extended conformation of the isoprenoid chains. The solution-phase structure of FPP and GGPP, as determined from our NMR studies, does not resemble the folded conformations that have been found to exist in the active site of various sesquiterpene cyclases.[71,72] Instead, the solution structure of FPP and GGPP seems to resemble the extended conformation found in the FPP binding site in mammalian FTase.[52,67] It is always difficult and risky to make arguments concerning the protein-bound conformations of molecules based on their preferred conformations in solution or *in silico*. However, because of the high affinity of FTase for FPP ($K_D \sim 2$ nM),[53a] it is perhaps not surprising that FPP apparently binds to this enzyme in its low-energy conformation.[62] The solid-state results presented here serve primarily to confirm the previous crystallographic studies on the FPP−mFTase complex. It is anticipated, however, that various prenylated peptides and proteins derived from **8** and related labeled isoprenoids will prove to be valuable tools to investigate, in conjunction with solid-state NMR techniques, the conformation of the farnesyl group in a variety of other environments not amenable to crystallographic studies.

Experimental Procedures

General Synthetic Procedures. Reactions that were sensitive to air and/or water were conducted in flame-dried glassware under an argon atmosphere. Moisture-sensitive reagents were transferred by syringe and placed in the reaction flask through rubber septum caps. All reactions were stirred with a magnetic bar. Solvents and organic and inorganic reagents were obtained from Aldrich Chemical Co., Fisher Scientific, and VWR Scientific unless otherwise noted. All reagents and solvents were used without further purification unless otherwise noted. To prepare anhydrous solvents used in reaction mixtures, the following procedures were used: THF and diethyl ether were distilled from Na/benzophenone; dichloromethane was distilled from CaH_2; toluene was dried and stored over activated 3-Å molecular sieves. Flash column chromatography was performed with silica gel 60 (230−440 mesh). Thin-layer chromatography was conducted on precoated alumina plates or glass plates, which were visualized with UV and/or a 10% phosphomolybdic acid/ethanol solution. HPLC was performed on a Waters system with a U6K injector, two 501 pumps, and a 490E multiwavelength UV detector.

[5-^{13}C]Ethyl 7,11-Dimethyl-3-oxododeca-6(*E*),10-dienoate (15). The sodium salt of ethyl acetoacetate (1.99 mmol, 303 mg) was dissolved in tetrahydrofuran (THF, 2.8 mL) and cooled to 0 °C. *n*-Butyllithium (2.0 M in cyclohexane, 2.08 mmol, 1.04 mL) was added dropwise and the reaction stirred at 0 °C for 25 min. The 1-^{13}C-labeled geranyl bromide **13** (0.99 mmol, 217 mg; prepared via the method of Urano and colleagues[46] as described in the Supporting Information) was added directly by syringe and the reaction stirred for 1.25 h at 0 °C. The mixture was then poured into a cold saturated solution of potassium hydrogen phosphate (20 mL) and was extracted with ether (3 × 20 mL). The combined organic layers were washed with water (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography using a 9:1 hexane/EtOAc solvent system afforded 136 mg

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(51%) of desired product **15** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.61 (s, 6H), 1.68 (s, 3H), 1.95−2.05 (m, 4H), 2.28 (app d of quartets; $^1J_{C-H} = \sim 160$ Hz; overlaps with peaks at ∼2.0 and 2.55 ppm), 2.55 (m, 2H), 3.43 (s, 2H), 4.19 (q, 2H), 5.05 (br, 2H).

[5-^{13}C]Ethyl 3-(Trifluoromethylsulfonyloxy)dodeca-7,11-dimethyl-2(*E*),6(*E*),10-trienoate. (16). The labeled β-ketoester **15** (0.511 mmol, 136 mg) was dissolved in THF (1.3 mL) and added to a solution of potassium bis(trimethylsilyl)amide (KHMDS; 0.5 M in toluene, 0.61 mmol, 1.23 mL) at −78 °C. After 1.5 h at −78 °C, a solution of 2-[*N*,*N*-bis(trifluoromethanesulfonyl)]amino-5-chloropyridine (0.613 mmol, 241 mg) in THF (0.32 mL) was added dropwise. The reaction mixture was stirred at room temperature overnight and then taken up in ∼20 mL of ether. The reaction mixture was then washed with a 10% citric acid solution (2 × 20 mL) and water (1 × 10 mL). The ether layer was then dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (95:5 hexane/ethyl acetate) afforded 69 mg (34%) of the desired product **16** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.0−2.1 (m, 4H), 2.29 (app d of quartets, $^1J_{C-H} \sim 126$ Hz; partially overlaps with peak at ∼2.05 ppm), 2.42 (m, 2H), 4.21 (q, 2H), 5.07 (br, 2H), 5.74 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ intense peak at 24.14 due to ^{13}C label.

[5,15-Bis-^{13}C]ethyl 3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-trienoate (17). In a flame-dried flask equipped with a condenser, magnesium turnings (170 mg) were placed in ether (4.0 mL, distilled from Na/benzophenone). [^{13}C]Methyl iodide (7.0 mmol, 1.0 g) was added to this solution and stirring was continued for 45 min at room temperature. In a separate flame-dried flask, CuCN (0.932 mmol, 83.5 mg) was dissolved in ether (1.0 mL) and cooled to −78 °C. A portion of the [^{13}C]methylmagnesium iodide (∼1.7 M, ∼1.7 mmol, 1.0 mL) prepared above was added dropwise, and the mixture was warmed to 0 °C for 5 min and then recooled to −78 °C. A solution of the [5-^{13}C]farnesyl triflate **16** (0.34 mmol, 137 mg) in 1.0 mL of ether was added dropwise and the reaction stirred for 2.5 h at −78 °C. The reaction mixture was warmed to 0 °C and quenched with NH_4Cl (2 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (20:1 hexane/ethyl acetate) afforded 39 mg (46%) of the desired product as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.60 (s, 6H), ∼2.1 (app d of multiplets, 2H, $^1J_{C-H} \sim 140$ Hz), 2.01 (m, 6H), 2.16 (d, 3H, $^1J_{C-H} \sim 126$ Hz), 4.10 (q, 2H), 5.08 (br, 2H), 5.65 (d, 1H).

[5,15-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-trien-1-ol ([5,15-Bis-^{13}C]farnesol, 2). A solution of **17** (0.21 mmol, 52 mg) in toluene (0.5 mL) was cooled to −78 °C and treated with DIBAL-H (1.0 M solution in toluene, 1.0 mmol, 1.0 mL). After 1 h at −78 °C, the reaction was quenched by addition to saturated aqueous potassium sodium tartrate (∼30 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (1 × 20 mL) and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (9:1 hexane/ethyl acetate) afforded 45 mg (96%) of the desired product as an oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (s, 3H), 1.65 (d, 3H, $^1J_{C-H} = 108$ Hz), 1.68 (s, 6H), 1.98−2.05 (m, 6H), 2.14 (C_4−CH_2, dq, 2H, $^1J_{C-H} = 105$ Hz), 4.14 (d, 2H), 5.08 (br, 2H), 5.41 (t, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 16.27 (large peak, $J = 1.6$ Hz), 17.81, 23.75, 26.12, 26.29 (large peak, $J = 1.6$ Hz), 26.50, 27.21, 28.21, 59.02, 122.85, 123.41, 123.69, 124.18, 131.51, 135.87; HRMS calculated for $^{13}C_2C_{13}H_{26}O$: 224.2051; found 224.2052.

[5,15-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-triene Diphosphate ([5,15-Bis-^{13}C]FPP, 4). In a flame-dried flask, *N*-chlorosuccinimide (0.134 mmol, 18 mg) was dissolved in 0.6 mL of dichloromethane and the resultant mixture cooled to −30 °C. This solution was treated with dimethyl sulfide (0.14 mmol, 11 μL) and warmed to 0 °C for 5 min. The mixture was recooled to −30 °C, a solution of [5,15-bis-^{13}C]farnesol **2** (0.121 mmol, 27 mg) in 1.0 mL of CH_2Cl_2 was added dropwise, and the reaction was then warmed back to 0 °C and stirred at this temperature for 2 h. The ice bath was removed, and the reaction was allowed to warm to room temperature and stirred for an additional 15 min. The mixture was added to hexane (20 mL) and washed with brine (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated to yield 9 mg (31%) of crude farnesyl chloride, which was used directly in the next step without purification. Tris(tetrabutylammonium) hydrogen pyrophosphate (0.16 mmol, 146 mg) and 5,15-bis-carbon-13 farnesyl chloride were dissolved in acetonitrile (1.2 mL) and the resultant mixture stirred for 2.5 h at room temperature. After removal of the acetonitrile solvent, the residue was dissolved in 1−2 mL of ion-exchange buffer (1:49 v/v 2-propanol and 25 mM NH_4HCO_3) and was passed through a column containing 3−10 mL of cation-exchange resin (Dowex AG 50W-X8, 100−200 mesh (Bio-Rad Laboratories, Richmond, CA), NH_4^+ form). The column was eluted with two column volumes of ion-exchange buffer at a flow rate of ∼1 mL/min. The eluent was dried by lyophilization, and a pale yellow solid was obtained. The crude product was dissolved in 1−3 mL of 25 mM NH_4HCO_3 and purified by reversed-phase HPLC using a program of 5 min of 100% A followed by a linear gradient of 100% A to 100% B over 30 min (A, 25 mM aqueous NH_4HCO_3, pH 8.0; B, CH_3CN; Vydac pH-stable C_8 10 × 250 mm column; flow rate, 3.0 mL; UV monitoring at 214 and 254 nm). The fractions containing the diphosphate (retention time of 17 min) were collected, pooled, and dried by lyophilization, to afford 13 mg (86%) of pure [5,15-bis-^{13}C]FPP **4** as a fluffy solid: ^{1}H NMR (300 MHz, D_2O) δ 1.60 (s, 6H), 1.68 (s, 6H), 1.27−2.1 (m, 8H), 4.55 (t, 2H), 5.15 (br, 2H), 5.53 (m, 1H); ^{31}P NMR (122 MHz, D_2O) δ −6.83, −10.81; ^{13}C NMR (125.6 MHz, D_2O) δ two intense peaks from ^{13}C-labeled carbons: 25.558 and 15.580 ppm ($^3J_{C-C} = 1.6$ Hz). Due to the hygroscopic and amorphous nature of the diphosphates, and the limited amounts available, these compounds were not characterized by elemental analysis. However, these compounds were always purified by reversed-phase HPLC, and their purity and identity was confirmed by analytical reversed-phase HPLC, ^{1}H NMR, ^{31}P NMR, and in some cases quantitative phosphate analysis.[73]

[3,6-Bis-^{13}C]ethyl 7,11-Dimethyl-3-oxododeca-6(*E*),10-dienoate (23). A suspension of sodium hydride (2.3 mmol, 92 mg, 60% in mineral oil) in THF (3.4 mL) was cooled to 0 °C. [3-^{13}C]Ethyl acetoacetate (Cambridge Isotope Laboratories; 2.0 mmol, 260 mg, 0.26 mL) was added; after 10 min *n*-butyllithium (2.0 M in cyclohexane, 1.6 mmol, 0.80 mL) was added dropwise and stirring was continued for an additional 10 min at 0 °C. To the resulting dianion of ethyl acetoacetate (**22**), a solution of the previously prepared geranyl bromide **21** (0.92 mmol, 200 mg; prepared via the method of Urano and colleagues[46] as described in the Supporting Information) in THF (0.54 mL) was added. The reaction was stirred for 2 h at 0 °C and was quenched with 2 N HCl (15 mL). The organic layer was separated, and the aqueous layer was extracted with *tert*-butyl methyl ether (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (9:1 hexane/ethyl acetate) gave 63 mg (26%) of the pure product **23**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.61 (s, 6H), 1.68 (s, 3H), 2.0−2.1 (m, 4H), 2.29 (m, 2H), 2.56 (m, 2H), 3.42 (d, 2H, $^2J_{C-H} \sim 5$ Hz), 4.18 (q, 2H), 5.06 (dt, 1H, $^1J_{C-H} = 153$ Hz), 5.06 (br t, 1H).

[3,6-Bis-^{13}C]ethyl 3-(Trifluoromethylsulfonyloxy)dodeca-7,11-dimethyl-2(*E*),6(*E*),10-trienoate (24). The 3,6-bis-^{13}C-labeled β-keto ester **23** (0.589 mmol, 157 mg) was triflated as described above for **15**, using KHMDS (0.5 M in toluene, 0.71 mmol, 1.42 mL), 2-[*N*,*N*-bis(trifluoromethanesulfonyl)amino]-5-chloropyridine (0.71 mmol, 280 mg), and THF (1.73 mL), followed by workup as described previously. Flash chromatography (95:5 hexane/ethyl acetate) afforded 90 mg (38%) of the desired product **24** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.03 (m, 4H), 2.20 (m, 2H), 2.42 (m, 2H), 4.29 (q, 2H), 5.06 (dt, 1H, $^1J_{C-H} = 153$ Hz), 5.06 (br, 1H), 5.73 (d, 1H, $^2J_{C-H} = 8.1$ Hz).

[3,6-Bis-^{13}C]ethyl 3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-trienoate (25). Triflate **24** (0.23 mmol, 90 mg), copper iodide (0.023 mmol, 4.4 mg), triphenylarsine (0.023 mmol, 7.0 mg), and bis(benzonitrile)-palladium(II) chloride (0.011 mmol, 4.4 mg) were placed in an argon-flushed flask and dissolved in *N*-methylpyrrolidone (anhydrous, 99.5%, 0.3 mL). The mixture was heated to 100 °C and stirred for 30 min. Tetramethyltin (0.46 mmol, 81.4 mg, 0.06 mL) was added to the solution and stirring continued for ∼18 h at 100 °C. The reaction mixture was cooled to room temperature and taken up in ethyl acetate

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(30 mL). The organic solution was washed with 10% potassium fluoride solution (2 × 20 mL) and water (2 × 20 mL). The aqueous layers were back extracted with ethyl acetate (25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (95:5 hexane/ethyl acetate) afforded 50 mg (82%) of the desired product **25** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.00 (m, 4H), 2.15 (m, 7 H), 4.23 (q, 2H), 5.09 (dt, 1H, $^1J_{C-H}$ = 151 Hz), 5.08 (t, 1H), 5.67 (s, 1H).

[3,6-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-trien-1-ol ([3,6-Bis-^{13}C]farnesol, 3). A solution of **25** (0.19 mmol, 50 mg) in toluene (0.9 mL) was cooled to −78 °C and treated with DIBAL-H (1.0 M solution in toluene, 0.52 mmol, 0.52 mL). The reaction was stirred for 1 h at −78 °C and was quenched by adding to saturated aqueous potassium sodium tartrate (40 mL). Workup and flash chromatography as described above for **2** afforded 39 mg (94%) of desired product as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (s, 6H), 1.68 (s, 6H), 1.96−2.11 (m, 8H), 4.14 (app t, 2H), 5.10 (t, 1H), 5.18 (dt, 1H, $^1J_{C-H}$ = 115 Hz), 5.40 (dt, 1H, $^2J_{C-H}$ = 6.8 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 16.24, 16.72, 17.93, 25.94, 26.92, 26.95, 39.55, 39.93, 59.65, 121.52, 123.13, 124.00 (d, $^3J_{C-C}$ = 3.60 Hz), 131.60, 135.22, 140.12 (d, $^3J_{C-C}$ = 3.60 Hz); HRMS calculated for $^{13}C_2C_{13}H_{26}O$: 224.2051; found 224.2050. Examination of the proton and carbon-13 NMR spectra indicated that a minor amount of the (2*Z*,6*E*)-farnesol isomer was present in this sample along with the desired (2*E*,6*E*)-isomer. This mixture was readily separated by isocratic normal-phase HPLC (solvent system, 9:1 hexane/methyl *tert*-butyl ether; Waters Nova-Pak HR Silica 7.8 × 300 mm columm; flow rate, 3.0 mL; UV monitoring at 214 and 230 nm) to give the pure cis and pure trans isomers.

[3,6-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-triene Diphosphate ([3,6 Bis-^{13}C]FPP, 5). The [3,6-bis-^{13}C]-farnesol **3** (0.07 mmol, 16 mg) was converted to the corresponding chloride using *N*-chlorosuccinimide (0.08 mmol, 11 mg), dimethyl sulfide (0.032 mmol, 2.0 μL), and 1.3 mL of dichloromethane as described above for **4**. This chloride was then treated with tris(tetrabutylammonium) hydrogen pyrophosphate (0.13 mmol, 126 mg) in acetonitrile (1.0 mL) for 2.5 h at room temperature. After solvent removal, the residue was subject to ion-exchange chromatography and then purified by reversed-phase HPLC as described above for **4**, to give 9 mg (72%) of pure [3,6-bis-^{13}C]FPP **5** as a fluffy solid: ^{1}H NMR (300 MHz, D_2O) δ 1.60 (s, 6H), 1.68 (s, 6H), 1.27−2.1 (m, 8H), 4.55 (t, 2H), 5.15 (br, 2H), 5.53 (m, 1H); ^{31}P NMR (122 MHz, D_2O) δ −11.36 (d), −12.76 (d); ^{13}C NMR (125.6 MHz, D_2O) δ 15.88, 16.08, 16.42, 17.60, 25.49, 26.05, 26.40, 39.40, 63.38, 124.68, 124.95 (d, $^3J_{C-C}$ = 3.30 Hz), 125.69, 134.17, 142.93, 143.45 (d, $^3J_{C-C}$ = 3.30 Hz).

[2-^{13}C]Ethyl 3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-trienoate (27). A suspension of NaH (60% dispersion in mineral oil; 8.99 mmol, 345 mg) in 14.9 mL of 1,2-dimethoxyethane (DME) was cooled to 0 °C. The commercially available 2-^{13}C-labeled triethyl phosphonoacetate **18** (8.88 mmol, 2.0 g) was added dropwise to the cold solution and stirred for 1 h at 0 °C. A solution of geranyl acetone **26** (9.99 mmol, 1.94 g, 2.22 mL) in 10.0 mL of DME was added dropwise to the reaction mixture, and stirring was continued for ∼23 h at room temperature. The reaction was cooled to 0 °C and quenched with water (24 mL). The aqueous layer was extracted with ether (3 × 40 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography using a 94:1 hexane/*tert*-butyl methyl ether solvent system afforded 605 mg (26%) of the pure desired 2(*Z*)-ester **27**. The isomer **27a** was present in the mixture as ∼20% of the crude: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 2.00−2.05 (m, 4H), 2.16 (narrow m, 7H), 4.16 (q, 2H), 5.09 (br, 2H), 5.67 (d, 1H; $^1J_{C-H}$ = 159.0 Hz).

[2-^{13}C]-3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-triene-1-ol ([2-^{13}C]-Farnesol, 28). A solution of **27** (1.76 mmoL, 466.0 mg) in toluene (6.3 mL) was cooled to −78 °C and treated with DIBAL-H (1.0 M solution in toluene, 3.57 mmol, 3.57 mL). The reaction was stirred for 1 h at −78 °C and was quenched by addition to saturated aqueous potassium sodium tartrate (40 mL). Workup and flash chromatography as described above for **2** afforded 266 mg (68%) of the desired product **28** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (s, 6H), 1.68 (s, 6H), 1.98−2.07 (m, 8H), 4.16 (narrow m, 2H), 5.09 (narrow m, 2H), 5.43 (dt, 1H, $^1J_{C-H}$ = 149 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) incorporation of the label was confirmed by the intense peak at 122.23 ppm.

[2-^{13}C]-3,7,11-Trimethyldodeca-2(*E*),6(*E*),10-triene 1-Bromide ([2-^{13}C]Farnesyl Bromide, 29). [2-^{13}C]Farnesol **28** (1.30 mmol, 290 mg) was dissolved in methylene chloride (12 mL), and to this solution, triphenylphosphine (1.82 mmol, 477 mg), and carbon tetrabromide (2.27 mmol, 753 mg) were added. The reaction was stirred for 2.5 h at room temperature, and the solvent was then removed. Hexane (1.5 mL) was added to the residue, and a white precipitate formed. The suspension was centrifuged, and the clear supernatant was concentrated. The resulting residue was taken up with hexane (∼1 mL), and the process was repeated. After three additional precipitation/centrifugation cycles, the desired carbon-13-labeled farnesyl bromide **29** was obtained (240 mg, 64%) as a clear liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (s, 6H), 1.68 (s, 3H), 1.73 (d, 3H, $^2J_{C-H}$ ∼ 5 Hz), 2.08 (m, 8H), 4.01 (dd, 2H, $^2J_{C-H}$ = 4 Hz), 5.08 (br, 2H), 5.53 (dt, 1H, $^1J_{C-H}$ = 159 Hz). The crude bromide was used directly in the next step.

[3,6-Bis-^{13}C]ethyl 7,11,15-Trimethyl-3-oxohexadeca-6(*E*),10(*E*),14-trienoate (30). A suspension of sodium hydride (4.0 mmol, 160 mg, 60% dispersion in mineral oil) in THF (6.5 mL) was cooled to 0 °C. [3-^{13}C]Ethyl acetoacetate (3.84 mmol, 50.3 mg, 0.49 mL) was added to solution, and after 15 min, *n*-butyllithium (4.0 mmol, 2.0 M in cyclohexane, 2.0 mL) was added dropwise and stirring was continued for an additional 15−20 min at 0 °C. To the resulting labeled dianion of ethyl acetoacetate (**22**), the previously prepared 2-^{13}C-labeled farnesyl bromide **29** (1.77 mmol, 508 mg) in THF (1.0 mL) was added directly. The reaction was stirred for 2 h at 0 °C and then quenched with 2 N HCl (15 mL). The organic layer was separated, and the aqueous layer was extracted with *tert*-butyl methyl ether (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (9:1 hexane/ethyl acetate) afforded 257 mg (43%) of the desired product **30**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H), 1.61 (s, 6H), 1.68 (s, 6H), 1.99 (m, 8H), 2.28 (m, 2H), 2.56 (m, 2H), 3.42 (d, $^2J_{C-H}$ ∼ 5 Hz), 4.18 (q, 2H), 5.06 (dt, 1H, $^1J_{C-H}$ = 153 Hz), 5.09 (t, 2H).

[3,6-Bis-^{13}C]ethyl 3-(Trifluoromethylsulfonyloxy)hexadeca-7,11,15-trimethyl-2(*E*),6(*E*),10(*E*),14-tetranoate (31). The 3,6 bis-^{13}C-labeled β-ketoester **30** (0.768 mmol, 257 mg) was triflated as described above for **15**, using KHMDS (0.5 M in toluene, 0.99 mmol, 2.0 mL), 2-[*N*,*N*-bis(trifluoromethanesulfonyl)amino]-5-chloropyridine (0.92 mmol, 362 mg), and THF (2.4 mL), followed by workup as described previously. Flash chromatography (95:5 hexane/ethyl acetate) afforded 235 mg (65%) of the desired product **31** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, 3H), 1.60 (s, 9H), 1.68 (s, 3H), 1.95−2.15 (m, 8H), 2.28 (m, 2H), 2.40 (m, 2H), 4.26 (q, 2H), 5.05 (dt, 1H, $^1J_{C-H}$ = 146 Hz), 5.09 (t, 2H), 5.73 (d, 1H, $^2J_{C-H}$ = 8.1 Hz).

[3,6-Bis-^{13}C]ethyl3,7,11,15-Tetramethylhexadeca-2(*E*),6(*E*),10(*E*),14-tetraenoate (32). Triflate **31** (0.5 mmol, 235 mg), copper iodide (0.05 mmol, 9.7 mg), triphenylarsine (0.05 mmol, 15.6 mg), and bis-(benzonitrile)palladium(II) chloride (0.025 mmol, 9.5 mg) were placed in an argon-flushed flask and dissolved in *N*-methylpyrrolidone (0.64 mL). The mixture was heated to 100 °C and stirred for 30 min. Tetramethyltin (0.99 mmol, 177 mg, 0.14 mL) was added to the solution, and stirring continued for ∼18 h at 100 °C. Workup and purification as described above for **25** afforded 100 mg (60%) of the desired product **32** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.60 (s, 9H), 1.68 (s, 3H), 2.00 (m, 8H), 2.15 (m, 2H), 2.16 (d, 3H, $^2J_{C-H}$ ∼ 6 Hz), 4.15 (q, 2H), 5.09 (dt, 1H, $^1J_{C-H}$ = 150 Hz), 5.10 (t, 2H), 5.66 (s, 1H).

[3,6-Bis-^{13}C]-3,7,11,15-tetramethylhexadeca-2(*E*),6(*E*),10(*E*),14-tetraen-1-ol ([3,6-bis-^{13}C]geranylgeraniol, 6). A solution of **32** (0.29 mmol, 100 mg) in toluene (1.5 mL) was cooled to −78 °C and treated with DIBAL-H (1.0 M solution in toluene, 0.84 mmol, 0.84 mL). The reaction was stirred for 1 h at −78 °C and was quenched by addition to saturated aqueous potassium sodium tartrate (30 mL). Workup and flash chromatography as described above for **2** afforded 54 mg (62%) of the desired product **6** as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (s, 9H), 1.68 (s, 6H), 1.95−2.15 (m, 12H), 4.15 (app t, 2H), 5.11 (dt, 1H, $^1J_{C-H}$ = 150 Hz), 5.10 (narrow m, 2H), 5.41 (t, 1H, J = 6.8 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 16.24, 16.72, 17.92, 25.94, 26.32, 26.76, 26.84, 26.86, 26.98, 39.57, 39.95, 59.67, 123.12, 123.81, 124.00

(d, $^3J_{C-C}$ = 3.70 Hz), 125.98, 131.53, 133.34, 135.21, 140.32 (d, $^3J_{C-C}$ = 3.70 Hz); HRMS calculated for $^{13}C_2C_{18}H_{34}O$: 292.2677; found 292.2679. Inspection of the flash chromatography-purified product indicated the presence of a minor amount of the (2*Z*,6*E*,10*E*)-isomer of the desired alcohol **6**. This mixture was readily separated by isocratic normal-phase HPLC (solvent system, 9:1 hexane/methyl *tert*-butyl ether; Waters Nova-Pak HR Silica 7.8 × 300 mm columm; flow rate, 3.0 mL; UV monitoring at 214 and 230 nm) to give the pure cis and pure trans isomers.

[3,6-Bis-^{13}C]ethyl3,7,11,15-Tetramethylhexadeca-2(*E*),6(*E*),10(*E*),14-tetraene Diphosphate ([3,6-Bis-^{13}C]GGPP, 7). The [3,6-bis-^{13}C]-geranylgeraniol **6** (0.07 mmol, 21 mg) was converted to the corresponding chloride using *N*-chlorosuccinimide (0.08 mmol, 11.0 mg), dimethyl sulfide (0.09 mmol, 6.0 μL), and 1.3 mL of dichloromethane as described above for **4**. This chloride was then treated with tris(tetrabutylammonium) hydrogen pyrophosphate (0.31 mmol, 281 mg) in acetonitrile (2.31 mL) at 2.5 h at room temperature. After solvent removal, the residue was subject to ion-exchange chromatography and then purified by reversed-phase HPLC as described above for **4**. The retention time of the more hydrophobic GGPP analogue was 19 min, and concentration of these fractions gave 19 mg (43%) of the desired [3,6-bis-^{13}C]GGPP **7** as a fluffy solid: ^{1}H NMR (500 MHz, D_2O) δ 1.60 (s, 9H), 1.68 (s, 6H), 1.8−2.0 (m, 12H), 4.31 (br, 2H), 5.00 (t, 1H, J = 150 Hz), 4.97 (m, 2H), 5.30 (t, 1H); ^{31}P NMR (122 MHz, D_2O) δ −6.82 (d), −3.24 (d); ^{13}C NMR (125.7 MHz, D_2O) δ 16.24, 16.72, 17.92, 25.94, 26.32, 26.76, 26.84, 26.86, 26.98, 39.57, 39.84, 62.55, 123.12, 123.81, 124.19 (d, $^3J_{C-C}$ = 3.70 Hz), 125.98, 131.53, 133.34, 135.21, 142.32 (d, $^3J_{C-C}$ = 3.70 Hz).

[6,15-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-trien-1-ol ([6,15-Bis-^{13}C]farnesol, 36). A solution of **35** (0.158 mmol, 42.0 mg; prepared using our standard protocols as fully described in the Supporting Information) in toluene (3.7 mL) was cooled to −78 °C and treated with DIBAL-H (1.0 M solution in toluene, 2.11 mmol, 2.11 mL). The reaction was allowed to stir for 1 h at −78 °C and was quenched by adding to saturated aqueous potassium sodium tartrate (30 mL). Workup and flash chromatography as described above for **2** afforded 22 mg (62%) of the desired alcohol **36** as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (s, 3H), 1.65 (d, 3H, $^1J_{C-H}$ = 108 Hz), 1.68 (s, 6H), 1.98−2.1 (m, 8H), 4.15 (d, 2H), 5.10 (dt, 1H, $^1J_{C-H}$ = 150 Hz), 5.10 (t, 1H), 5.42 (t, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) two intense peaks representing the carbon-13 labels were observed at 16.18 and 123.76 ppm; HRMS calculated for $^{13}C_2C_{13}H_{26}O$: 224.2051; found 224.2053.

[6,15-Bis-^{13}C]-3,7,11-trimethyldodeca-2(*E*),6(*E*),10-triene Diphosphate ([6,15-Bis-^{13}C]FPP, 8). The [6,15-bis-^{13}C]farnesol **36** (0.05 mmol, 12 mg) was converted to the corresponding chloride using *N*-chlorosuccinimide (0.06 mmol, 8.0 mg), dimethyl sulfide (0.02 mmol, 1.8 μL), and 0.96 mL of dichloromethane as described above for **4**. This chloride was then treated with tris(tetrabutylammonium) hydrogen pyrophosphate (0.14 mmol, 126 mg) in acetonitrile (1.0 mL) at 2.5 h at room temperature. After solvent removal, the residue was subject to ion-exchange chromatography and then purified by reversed-phase HPLC as described above for **4**, to give 15 mg (>100% due to bicarbonate salt impurities) of [6,15-bis-^{13}C]FPP **8** as a fluffy solid: ^{1}H NMR (300 MHz, D_2O) δ 1.60 (s, 6H), 1.69 (s, 6H), 1.8−2.0 (m, 8H), 4.38 (br, 2H), 5.07 (b, 2H), 5.36 (m, 1H); ^{31}P NMR (122 MHz, D_2O) δ −6.18, −9.89; ^{13}C NMR (75 MHz, D_2O) two large peaks identified the proper labels at 15.82 and 123.4 ppm. Due to the presence of the ammonium bicarbonate impurities, the concentration of the sample of **8** complexed with mFTase for the rotational resonance MAS NMR studies was confirmed by phosphate analysis.[73]

Solution-State NMR Methods. Proton and carbon-13 NMR data were obtained on General Electric QE-300, Varian Unity-400, and Varian Unity-500 spectrometers. Carbon−carbon ($^3J_{C-C}$) coupling constants were determined from standard carbon-13 spectra acquired on the Varian Unity-500 spectrometer at 125.4 MHz. The solution ^{1}H and ^{13}C NMR spectra were taken on the ammonium salt forms of the diphosphates in unbuffered deuterium oxide. Typically, spectra were acquired with 60° pulse tip angle, 5.1 s repetition rate, 144 scans, 30 418 Hz sweep window, 2.1 s acquisition time, and 256K processing with no exponential multiplication used before FT deconvolution. The final digital resolution was 0.23 Hz.

Computational Methods. Molecular mechanics calculations were carried out using the commercial program SYBYL (version 6.4, Tripos, Inc., St. Louis, MO (http://www.tripos.com)). All computational jobs were carried out on an SGI Octane workstation equipped with dual R12000 CPU processors, 512 MB memory, and 13 GB storage space (Silicon Graphics Inc., Mountain View, CA). The MM2 force field was used for 1-ns molecular dynamics simulations,[74] and the Gasteiger-Huckel charge method was applied to the molecule. The farnesol molecule **1** was sketched and energy-minimized using 3000 steps of Powell to RMSD of 0.01 kcal/Å. A 1-fs time step was used, and the nonbonded interactions were truncated at 8 Å to reduce the amount of nonbonded atom pairs and updated every 25 fs. A dielectric constant of 80 was set to screen the electrostatic interactions. The temperature of the system was maintained at 300 K using the Berendsen algorithm with 0.5-ps coupling constant. The snapshot of MD trajectory was recorded at a 500-fs interval.

Preparation of the [6,15 Bis-^{13}C]FPP−mFTase Complex. Rat FTase was overexpressed in Sf9 cells and purified to greater than 95% purity by affinity chromatography essentially as described[53b] except that the protein was concentrated and washed with the following buffer: 20 mM Tris-HCl (pH 7.4), 10 μM $ZnCl_2$, 10 mM DTT, and 20 mM KCl. PEG 8000 was omitted from the wash buffer. This protein was lyophilized on a FTS Stoppering lyophilizer. The enzyme concentration was determined by gel versus a BSA standard to be ~3 mg/mL. Thus, 3 mg of lyophilized FTase was brought up in water to make a 30 μM solution along with the addition of a 1:1.1 molar ratio of enzyme to [6,15-bis-^{13}C]FPP **8** (33 μM). This sample was then relyophilized and used for solid-state NMR analysis.

Solid-State NMR Methods. Rotational resonance NMR measurements were carried out on a Bruker Avance 360-MHz spectrometer using double-resonance probes (5-mm rotor diameters) from Doty Scientific (Columbia, SC). The pulse sequence used ramped amplitude cross polarization[75] and TPPM decoupling.[76] The decoupling field strength was 62 kHz. For the FPP model compound, 32 different rotational resonance exchange times were used, and 1024 scans were averaged for each exchange time point. For the FPP−FTase complex, five different rotational resonance exchange times were used and 65 536 scans were averaged for each exchange time point. Simulations were carried out using the program CC2Z (Malcolm Levitt, University of Stockholm). The chemical shift tensor values for the C=C and CH_3 groups were based on the values for the corresponding atom types in the isoprenoid retinal.[38] The zero quantum T2 relaxation time of 1.5 ms used in the simulations was derived from the best fit to experimental data from the FPP model compound and an internuclear distance of 4.5 Å.

Acknowledgment. This work was supported by the NIH (Grants CA 67292 and CA 78819 to R.A.G. and Grant GM 41412 to S.O.S.). This paper was taken in part from the dissertation submitted by T.J.Z. to WSU in partial fulfillment of the requirements for the Ph.D. degree. R.A.G. was supported in part by an American Cancer Society Junior Faculty Research Award (JFRA-609), and T.J.Z. was supported in part by a WSU GRA award.

Supporting Information Available: Experimental procedures for the synthesis of **11**−**13**, **19**−**21**, and **33**−**35**; and 300-, 400-, or 500-MHz ^{1}H NMR spectra of **2**−**8** and **36** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA000860F

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