Farnesyl-Diphosphate Synthase. Catalysis of an Intramolecular Prenyl Transfer with Bisubstrate Analogs

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Abstract: Bisubstrate analogs for isopentenyl diphosphate and dimethylallyl diphosphate were examined as substrates for farnesyl-diphosphate synthase. The hydrocarbon moieties of the normal substrates were joined by a one-carbon bridge that permits reaction between the centers normally involved in the 1'-4 condensation. An essential feature of the syntheses of **1-OPP** and **2-OPP** was the regioselective activation of the allylic and homoallylic centers in the analogs for simultaneous conversion to diphosphate esters by displacement with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate. The enzyme catalyzes stereoselective cyclizations of both analogs to give products **8-OPP**, **9-OPP**, and **10-OPP** with skeletons like those in the *p*-menthane isoprenoids. Each analog produced a unique distribution of products. Steady-state kinetic analyses of the enzymatic cyclization indicated that the catalytic efficiencies (k_{cat}/K_m) for these reactions are similar to those of the normal substrates. A 1,2-hydrogen migration during enzyme catalyzed cyclization was established by a deuterium labeling study of the bisubstrate analogs and provides the first evidence for sequential condensation and elimination steps in the enzyme-catalyzed prenyl chain elongation process.

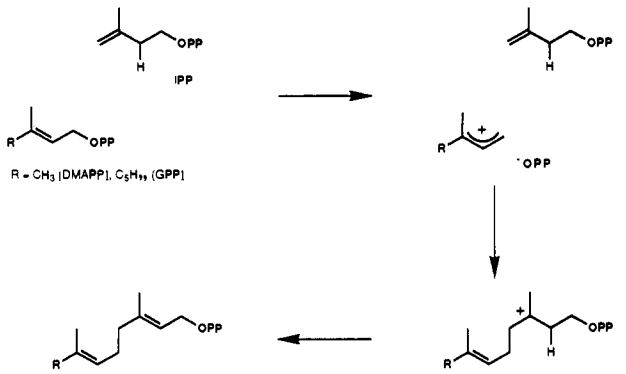
Introduction

The isoprene biosynthetic pathway is unrivaled in metabolism for the diversity of the structures and its products. The main carbon chain elongation reactions in the pathway are sequential 1'-4 condensations between isopentenyl diphosphate (IPP) and allylic diphosphates. Biosynthesis of linear isoprenoids is ubiquitous, and every organism contains a family of enzymes which differ in their specificities for double-bond stereochemistry and chain length.[1] These compounds, in turn, serve as substrates for prenyltransferases that catalyze reactions at branch points in the pathway. In addition to the case of the prenyltransferases, structural diversity in isoprene metabolism is introduced by the cyclase class of enzymes, which transform linear substrates into the numerous cyclic mono-, sesqui-, and diterpenes found in nature.[2] Although the products are different, the general mechanistic features of prenyl transfer and cyclization are similar.[1,3] Both involve attack by an electrophilic allylic moiety on an electron-rich center. For 1'-4 condensation, electrophilic condensation of the allylic unit with IPP is followed by elimination of a proton.[1] For cyclization, condensation may be followed by elimination, but a variety of other reactions are seen, including the addition of nucleophiles and hydrogen, methyl, or skeletal rearrangements.[2,3]

Farnesyl-diphosphate synthase from yeast and higher vertebrates has served as a prototypical enzyme for biochemical studies of the prenyl-transfer reaction. This particular prenyltransferase catalyzes the irreversible, sequential condensation of IPP with dimethylallyl diphosphate (DMAPP) and with geranyl diphosphate (GPP) to yield farnesyl diphosphate (FPP), a pivotal C_{15} metabolite in the pathway.[1] Mechanistic studies with this enzyme indicate that the first step in prenyl transfer is rupture of the carbon-oxygen bond in the allylic substrate,[4-6] as shown in Scheme I; however, the timing of subsequent condensation and elimination steps has not been unambiguously established.

Our interest in the relationship between substrate conformation and mechanism in prenyltransferases has led to the design of a new class of molecules that serve as bisubstrate analogs for avian liver FPP synthase. Both reactive moieties for the 1'-4 condensation are contained within a single carbon chain by placing a methylene bridge between the *Z* methyl group at C(3') of DMAPP and the C(4) position of IPP, as illustrated in Figure 1. This positions the nucleophilic acceptor for prenyl transfer six carbons away from (C1') of the allylic moiety. Thus, the normal chain

Scheme I. An Electrophilic Mechanism for 1'-4 Condensation



elongation activity of FPP synthase is transformed by "substrate mutagenesis" into a catalytic activity similar to that of limonene cyclase.[7] There are relatively few conformations for the bisubstrate analogs **1-OPP** and **2-OPP** that juxtapose the reactive

1-OPP 2-OPP

centers in a manner suitable for the intramolecular carbon-carbon bond forming step. Although the hydrocarbon chains of **1-OPP** and **2-OPP** fold into compact shapes for these productive conformations, significant differences exist between the atomic positioning within the carbon chain which may have important influences upon the binding and subsequent catalytic events of the enzyme-mediated process. To test this postulate, we synthesized **1-OPP** and **2-OPP** and investigated their reactions in the presence of avian liver FPP synthase. We now describe kinetic and product studies for **1-OPP** and **2-OPP** which provide new insight concerning the mechanism of prenyl transfer.[8] In the following paper, we address the issue of substrate topology and

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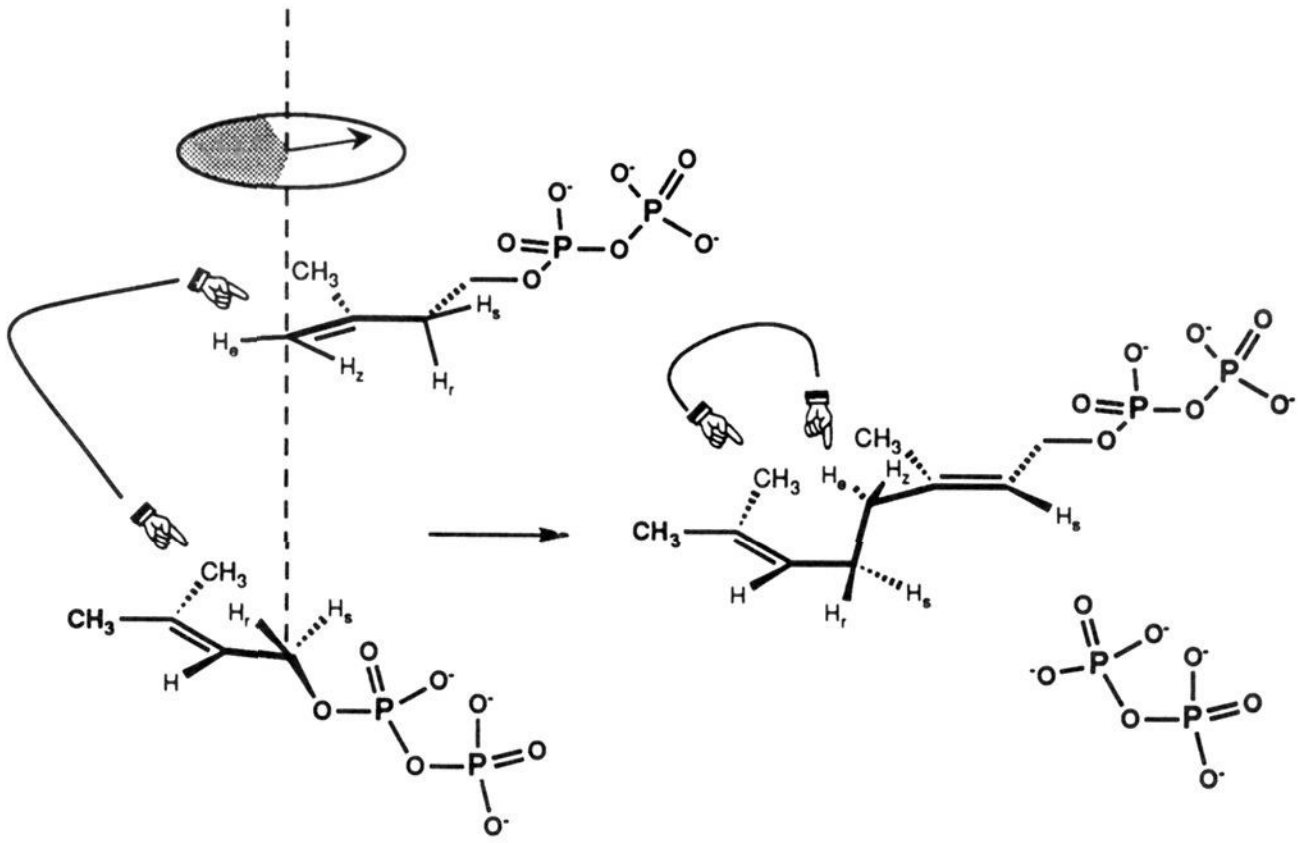


Figure 1. Overview of the major substrate topological relationships in the FPP synthase catalyzed reaction.

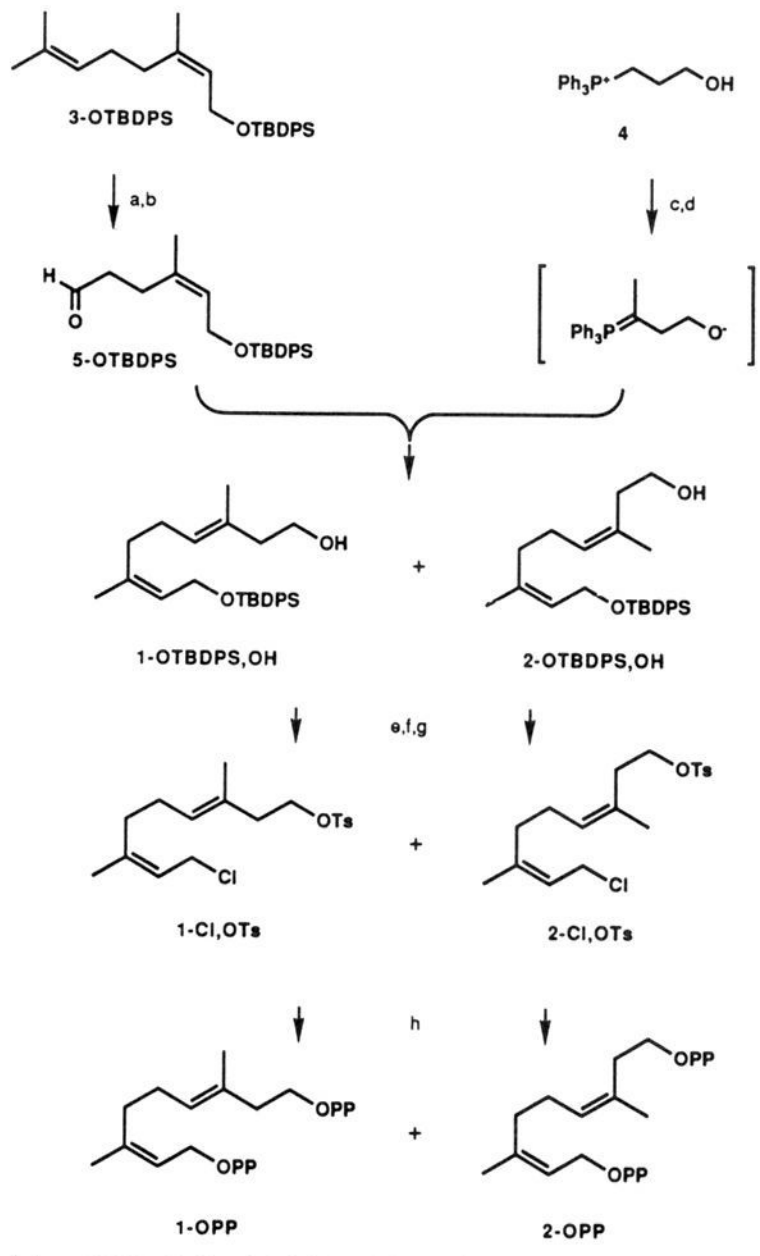
additional aspects of mechanism for the cyclization reactions.

Results

Syntheses of Bisubstrate Analogs. The overall plan for the synthesis of **1**-OPP and **2**-OPP relied upon the ability to introduce both diphosphate esters simultaneously in a final step by nucleophilic displacement at the appropriate allylic and homoallylic centers with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate.[9] As outlined in Scheme II, we used aldehyde **5**-CTBDPS as a common precursor to assemble the carbon skeletons for the analogs with differentially activated allylic and homoallylic carbons. The (*tert*-butyldiphenylsilyl)oxy group was stable to treatment with periodate and gave a 70% overall yield of pure aldehyde on an 8–10-g scale.[10,11] The remainder of the carbon backbone was assembled in a single step using the Wittig reagent derived from (3-hydroxypropyl)triphenylphosphonium iodide (**4**).[12] Alternative stereoselective methods were considered;[13] however, the increased number of steps and lower ultimate yields were not competitive with the nonselective route. Efforts made to optimize the yields of the Wittig reactions by changing time and temperature, and by adding cosolvents, did not give significant improvements. Formation of the ylide and subsequent methylation were easily monitored by TLC and usually required overnight to reach completion. Addition of *n*-butyl lithium solutions made at −70 °C instead of 0 °C gave less decomposition of the phosphonium salt. Addition of up to 4 molar equiv of HMPA[14] resulted in homogeneous solutions of ylide but gave lower mass recoveries. When the corresponding phosphine oxide ylide[15] was used, the overall yield for the two-step process was only 35%. Protection of the hydroxyl group with 2-methoxypropene[16] also offered no advantage.

The stereochemistry of the C(6)–C(7) double bonds in **1**-OTBDPS,OH and **2**-OTBDPS,OH was assigned from ^{1}H NMR NOE difference spectra for the two isomers.[17] A positive NOE

Scheme II. Synthesis of Bisubstrate Analogs **1**-OPP and **2**-OPP



(a) *m*-CPBA/ether/−20 °C; (b) H_5IO_6; (c) *n*BuLi/THF/CH_3I/−70 °C; (d) *n*BuLi/−70 °C; (e) TsCl/DMAP/0 °C; (f) $nBu_4NF\cdot 3H_2O$/0 °C; (g) NCS/DMS/−30 °C; (h) $(nBu_4N)_3HP_2O_7\cdot 3H_2O$/$CH_3CN$.

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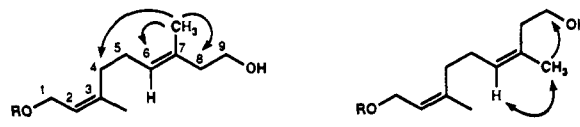
was observed for **2**-OTBDPS,OH at 5.18 ppm (H(6)) upon



1-OTBDPS,OH **2**-OTBDPS,OH

preirradiation of the resonance for the C(7) methyl group at 1.64 ppm. Preirradiation of the C(7) methyl group in **1**-OTBDPS,OH resulted in positive NOEs at H(8) (3.60 ppm) and H(4)/H(6) (2.17, 2.01 ppm). These assignments are consistent with the trends of ^1H and ^{13}C chemical shifts from a series of trisubstituted olefins. In the ^1H NMR spectra, upfield shifts were observed for the *Z* olefinic methyl relative to its *E* methyl counterpart,[18] while ^{13}C resonances for substituents attached to the sp^2 carbon centers were shielded when cis to an alkyl chain.[19-21] The methyl group at C(7) has a ^1H chemical shift of 1.55 ppm for **1**-OTBDPS,OH and 1.64 ppm for **2**-OTBDPS,OH. ^{13}C NMR resonances for C(8) and the methyl group at C(7) appear at 42.4 and 15.5 ppm, respectively. Similar resonances in **1**-OTBDPS,OH show a −7.6 ppm shift for the C(8) methylene and a +7.7 ppm shift for the C(7) methyl group.

During the course of a related investigation,[22] a mild and efficient method for synthesis of sulfonate esters was developed using an equal molar mixture of DMAP/TsCl in dichloromethane and was utilized to prepare the tosylates of **1**-OTBDPS,OH and **2**-OTBDPS,OH. The *tert*-butyldiphenylsilyl protecting group was removed in the presence of the tosylate moiety by addition of tetra-*n*-butylammonium fluoride to a solution of **1**-OTBDPS,OTs or **2**-OTBDPS,OTs at 0 °C for 2 h. The allylic C(1) carbons in the resulting hydroxy tosylate were then activated by chlorination with dimethyl sulfide and *N*-chlorosuccinimide at −30 °C[23] to afford **1**-Cl,OTs and **2**-Cl,OTs. These compounds were >95% pure, as estimated by ^1H and ^{13}C NMR, and were used directly in the next step.

The diphosphate moieties were introduced by treating **1**-Cl,OTs or **2**-Cl,OTs with the recrystallized tris(tetra-*n*-butylammonium) hydrogen pyrophosphate in acetonitrile at 0 °C followed by warming to room temperature. Complete displacement occurred at both carbons within 3–4 h, as indicated by ^1H NMR. Use of the standard cation-exchange protocol to exchange tetrabutylammonium for ammonium counterions gave quantitative recoveries of phosphorylated materials. Medium-pressure chromatography of the crude mixtures on cellulose provided the diphosphate esters, free of excess inorganic pyrophosphate, in overall yields of 65% and 55% from compounds **1**-OH,OTs and **2**-OH,OTs, respectively. For **2**-OPP, the ^1H NMR resonances for the C(1) and C(9) methylenes at 4.42 and 3.97 ppm are displayed in Figure 2a. The ^{31}P NMR spectrum for **2**-OPP, presented in Figure 2b, shows two pairs of doublets at chemical shifts similar to those for IPP and DMAPP. The absolute chemical shifts of the ^{31}P resonances were highly sample dependent. The ^{13}C NMR spectra had doublets due to three- and four-bond phosphorus couplings for **1**-OPP at 123.5, 67.3, 65.0, and 42.7 ppm and for **2**-OPP at 123.5, 66.9, 64.9, and 35.2 ppm.

Mass analysis of the isoprenoid diphosphates was possible by negative-ion fast atom bombardment (FAB) mass spectrometry using the hexaammonium salts of **1**-OPP and **2**-OPP. Molecular ions corresponding to the acid form of the bisdiphosphates ($C_{11}H_{23}P_4O_{14}$) were observed at *m/z* 503 $[M - H]^-$, and major

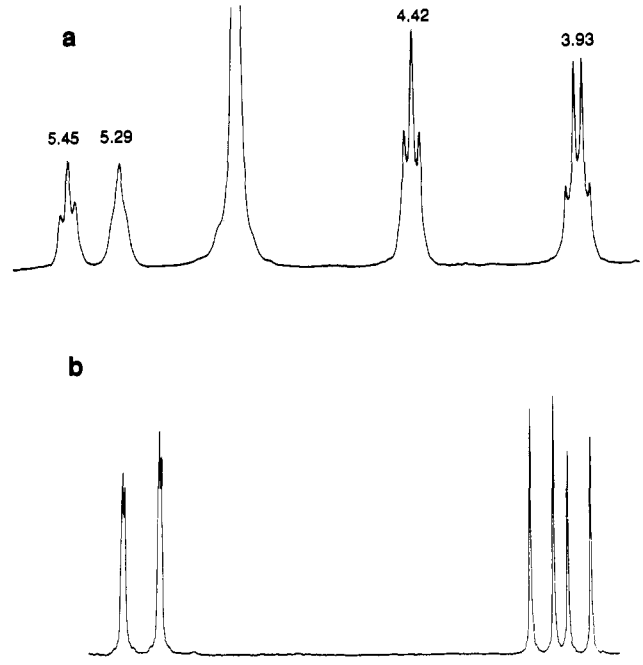


Figure 2. NMR spectra of **2**-OPP. (a) A region of the ^1H NMR spectrum in D_2O that shows the phosphorus-coupled C(1) (4.42 ppm) and C(9) (3.93 ppm) methylene resonances. (b) A ^{31}P NMR spectrum from −10 to −14 ppm.

fragment ions were seen at *m/z* 423 $[M - H - HPO_3]^-$, 325 $[M - H - H_4P_2O_7]^-$, 177 $[H_3P_2O_7]^-$, and 159 $[HP_2O_6]^-$.[24] This pattern is consistent with the observed fragmentation of other isoprenoid diphosphates, except that the peak at *m/z* 423 was more intense, suggesting more phosphorus–oxygen-bond cleavage. Loss of the diphosphate moiety (*m/z* 325) is characteristic of the reactivity of the 3,3-dialkyl allylic system.[25] A glycerol adduct observed at *m/z* 417 $[M - H - H_4P_2O_7 + \text{glycerol}]^-$ may be the result of allylic diphosphate ester solvolysis and substitution by a nucleophilic glycerol in the matrix.

Tritium-labeled derivatives of bisdiphosphates **1**-OPP and **2**-OPP were synthesized for use in enzyme assays. The syntheses were first developed for [1-^2H]**1**-OPP and [1-^2H]**2**-OPP and then extended to the radiolabeled derivatives. Tosyl alcohols **1**-OH,OTs and **2**-OH,OTs were oxidized with manganese oxide to afford the corresponding aldehydes, which were immediately reduced with sodium borodeuteride. The levels of isotope incorporation were determined to be at least 98% by ^1H NMR. The procedure was duplicated with sodium borotriteride, and radiochemical specific activity was confirmed by converting small quantities of [1-^3H]**1**-OH,OTs and [1-^3H]**2**-OH,OTs to the corresponding naphthoate esters. The high UV molar extinction coefficients of purified samples were used to determine the concentration of solutions containing microgram quantities of the tritium-containing material.[26] Specific activities were then calculated from radioisotopic measurements made by liquid scintillation spectrometry. This convenient procedure has proven useful for similar measurements in other alcohols.

Bisubstrate analogs **1**-OPP and **2**-OPP were labeled with deuterium at C(6) by using deuterated aldehyde [6-^2H]**5**-OTBDPS in the Wittig step shown in Scheme II. Deuterium was introduced by first oxidizing C(6) of **5**-OTBDPS and reducing the corresponding methyl ester with lithium aluminum deuteride, followed by oxidation to [6-^2H]**5**-OTBDPS.[27] The [6-^2H]bisdiphosphates were then prepared as described for the unlabeled

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Table I. Steady-State Kinetic Constants for FPP Synthase with Normal Substrates GPP/IPP and Bisubstrate Analogs 1 and 2

substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
IPP/DMAPP	0.13/0.50	1.60	$1.2 \times 10^7/3.2 \times 10^6$
1-OPP	0.082	0.160	2.0×10^6
2-OPP	0.046	0.145	3.2×10^6

materials. Incorporation of deuterium at C(6) in 1-OPP and 2-OPP was greater than 97%, as judged from ^1H, ^2H, and ^{13}C NMR spectra and negative-ion FABMS (m/z 504).

Enzymatic Studies. A. Steady-State Kinetic Analysis. The assay for conversion of 1-OPP or 2-OPP during incubation with FPP synthase is based on the acid-lability procedure used for the normal substrates.[28] This procedure utilizes radiolabeled IPP and relies on selective decomposition of the allylic product when the incubation mixture is acidified with methanolic HCl. Since the anticipated products of intramolecular prenyl transfer have acid-labile allylic diphosphates and 1-OPP and 2-OPP each contain the acid-stable homoallylic diphosphate moiety, treatment of the enzymatic reaction mixture with HCl should yield materials derived from radioactive enzymatic products that are extractable with ligroine. However, the starting linear diphosphate esters remain in the aqueous layer, and quantitation of the radioactivity in the extracts provides a measure of the extent of each reaction. The subsequent discovery that non-allylic products are also formed (see below) required us to correct the measured extractable radioactivity for the proportion of non-allylic product generated from each bisubstrate analog.

Table I summarizes the kinetic constants determined from Lineweaver–Burk analysis of initial velocities measured by the acid-lability assay and corrected for formation of non-allylic products. Although k_{cat} values for both analogs were 10-fold lower than for the condensation of IPP and DMAPP, K_m values for 1-OPP and 2-OPP were substantially lower than for either IPP or DMAPP. Thus, the catalytic efficiencies for FPP synthase, as determined by k_{cat}/K_m, were similar for 1-OPP, 2-OPP, and the normal substrates.

B. Product Studies. The diphosphate esters were hydrolyzed to the corresponding alcohols with alkaline phosphatase to facilitate identification of the water-soluble products from incubation of 1-OPP and 2-OPP with FPP synthase. However, attempts to determine structures at this stage were hampered by loss of materials due to their volatility and difficulties in separating the isomeric materials. These problems were circumvented by converting the mixtures of isomeric alcohols to the corresponding naphthoate esters.

In a typical experiment, 0.15 mmol of 1-OPP or 2-OPP was incubated with FPP synthase in pH 7.0 buffer containing Mg^{2+} for 9 h. The reactions were terminated by adjusting the pH to 10.5 with lysine, and alkaline phosphatase was added to hydrolyze the diphosphate moieties in the products and unconsumed analogs. The mixtures were extracted with CH_2Cl_2, and the products were analyzed by comparison with authentic samples on GLPC. Typically, small quantities of 1-OH or 2-OH were seen, and these materials were removed from cyclic products by rapid chromatography on silica gel. After esterification with 2-naphthoic acid, the isomeric esters were separated by HPLC, and their structures (see Scheme III) were deduced from ^1H and ^{13}C NMR and UV spectra. The percentages of 8-OPP, 9-OPP, and 10-OPP were calculated from the relative ratios of the alcohols determined by GLPC analysis using samples where care had been taken to prevent losses due to evaporation. The overall yields of the naphthoate esters from 1-OPP and 2-OPP ranged from 60–70% using this isolation procedure. ORD spectra were recorded for naphthoate esters for solutions where concentrations were calculated from microanalytical weights and UV absorbance. Ultimately, the structures were verified by synthesis of authentic samples, as outlined in the following paper.

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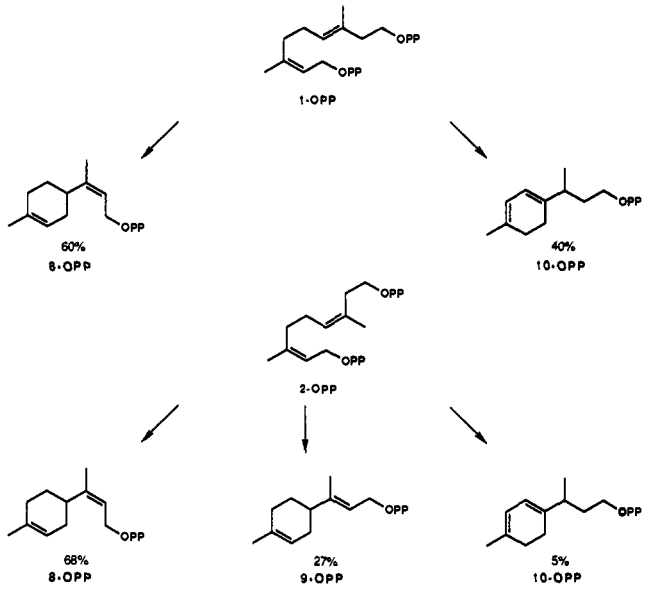
Scheme III. Structures of Cyclized Products from Reactions of 1-OPP and 2-OPP Catalyzed by Avian FPP Synthase



Bisubstrate analog 1-OPP gave two products. Cyclohexenyl diphosphate 8-OPP has the carbon skeleton expected from an intramolecular 1′–4 condensation; however, the stereochemistry of the new C(2)–C(3) double bond is *Z*, rather than *E* as seen for the normal condensation of IPP with DMAPP. The other product, cyclohexadiene 10-OPP, results from a hydrogen rearrangement. Analog 2-OPP also gave 8-OPP and 10-OPP, along with 9-OPP, the anticipated *E* isomer for a normal prenyl transfer catalyzed by FPP synthase. In preliminary experiments, we thought that a small amount (<5%) of 9-OPP was also being produced from 1-OPP.[8] However, closer examination revealed that the peak originally assigned to 9-OH in GLPC traces contained two components, and the NMR spectra of the naphthoate derivatives did not correspond to that for 9-ONp. These materials were also observed in blank reaction mixtures when 1-OPP was incubated in buffer without enzyme. Later preparations of 1-OPP did not give rise to these unknown alcohols.

The rearrangement required to form diene 10-OPP from 1-OPP is most consistent with a 1,2-migration of hydrogen, from the cyclohexenyl ring into the side chain. Enzyme-catalyzed hydrogen migrations are observed for monoterpene cyclases,[29,30] but this is the first example encountered with a prenyltransferase. To verify that the rearrangement is a 1,2-migration, [6-^2H]1-OPP and [6-^2H]2-OPP were prepared from [6-^2H]5-OTBDPS, as described for 1-OPP. The products from incubation of [6-^2H]1-OPP with FPP synthase were converted to naphthoate esters, and GC/MS analysis of the alcohols obtained after treatment with phosphatase indicated that all of the cyclic products contained a single deuterium. Further characterization of naphthoates 8-ONp, 9-ONp, and 10-ONp by ^1H NMR spectroscopy established the location of the deuterium atom in each product. For 8-ONp, the signal at 2.81 ppm for the tertiary proton at C(1′) in the cyclohexenyl ring of the unlabeled material was absent in the spectra of the materials from cyclization of [6-^2H]1-OPP and [6-^2H]2-OPP. Similar results were obtained for 9-ONp derived from 2-OPP, where the proton signal at 2.01 ppm for the tertiary proton at C(1′) was absent. Parts a and b of Figure 3 show the ^1H NMR spectra between 1.7 and 2.5 ppm for 10-ONp from cyclization of 1-OPP and [6-^2H]1-OPP. The signal at 2.42 ppm for the tertiary proton at C(3) is absent in labeled 10-ONp, and the pattern centered at 1.9 ppm for the C(2) methylene hydrogens is simplified. In addition, the signal for the C(4) methyl group at 1.13 ppm in

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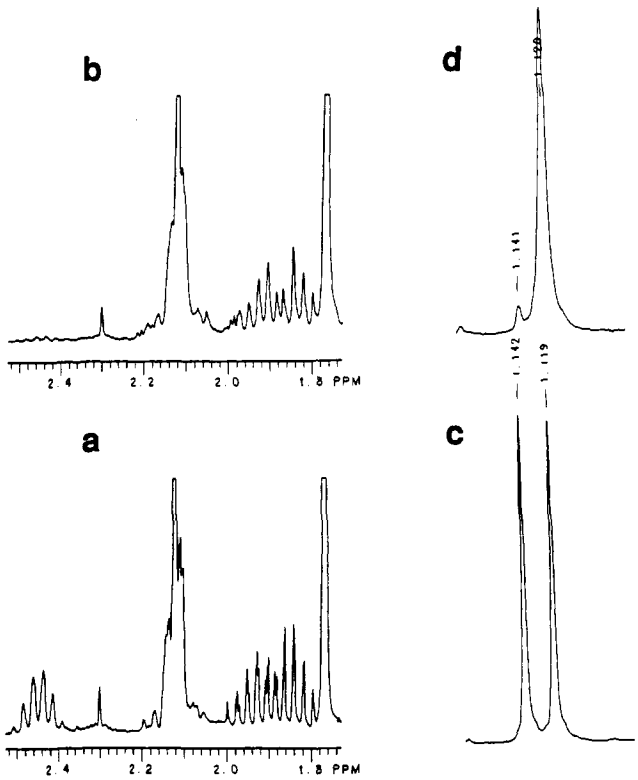


Figure 3. ^{1}H NMR spectra expanded from 1.7 to 2.5 ppm for (a) **10**-ONp and (b) [3-^{2}H]**10**-ONp from cyclization of [6-^{2}H]**1**-OPP. C(4) methyl resonance for (c) **10**-ONp and (d) [3-^{2}H]**10**-ONp from cyclization of [6-^{2}H]**1**-OPP.

10-ONp has collapsed to a broad singlet in the product from cyclization of [6-^{2}H]**1**-OPP, as shown in part d of Figure 3. Thus, the deuterium resides in the cyclohexane ring of **8**-ONp and **9**-ONp and in the side chain of **10**-ONp, thereby establishing the 1,2-hydride migration.

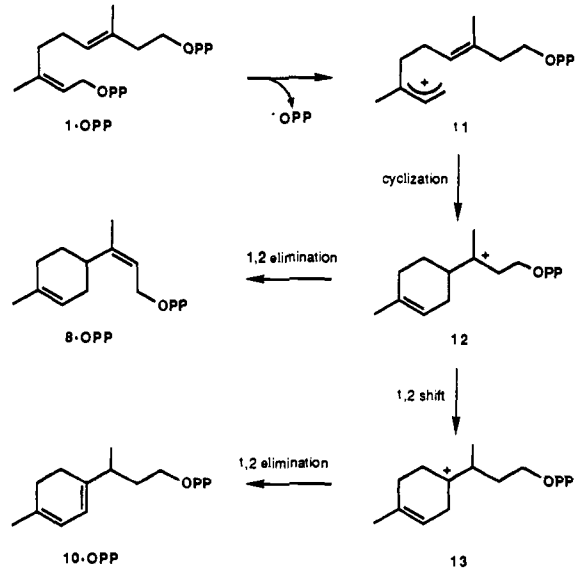
Discussion

Many prenyltransferases and isoprenoid cyclases catalyze their respective intermolecular and intramolecular carbon–carbon bond forming reactions via condensation of an allylic diphosphate with a remote double bond. Mechanistic studies, including linear free energy correlations[4-6] and inhibition experiments with analogs of reactive intermediates,[31] indicate that the reactions are electrophilic alkylations. The products of 1′–4 condensations catalyzed by prenyltransferases are all formed by elimination of a proton in the final step.[1] However, the mechanistically related cyclizations generate a rich variety of structures from further cyclizations and rearrangements[2,3,29,30] similar to those observed for carbocationic intermediates in non-enzymatic reactions.

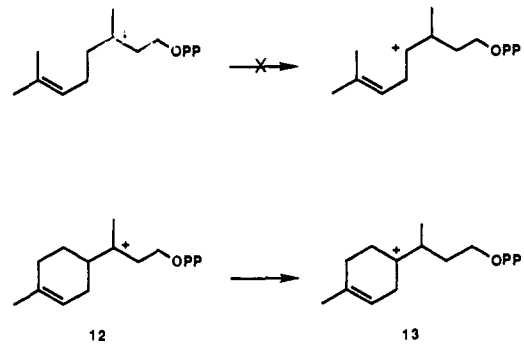
One of the fundamental unresolved mechanistic questions for the 1′–4 condensation is the number of chemical steps in the enzyme-catalyzed transformation. A completely stepwise mechanism is shown in Scheme I. However, the entire reaction or any two consecutive steps could, in theory, be concerted. Linear free energy comparisons of enzymatic and non-enzymatic activities for allylic analogs are consistent with the formation of an allylic cation as a discrete species prior to the condensation step.[4-6] This proposal is also consistent with the recent observation that the lifetime of the dimethylallyl cation in water is 3×10^{-9} s.[32] If one assumes that the double bond in IPP is less nucleophilic than water, this value represents a lower limit for the lifetime of the allylic cation during the enzyme-catalyzed alkylation.

The products from **1**-OPP and **2**-OPP provide new insight about the remaining transformations in Scheme I. In addition to the

Scheme IV. A Mechanism for Formation of **8**-OPP and **10**-OPP



normal elimination products, both analogs give cyclohexadiene **10**-OPP. As outlined in Scheme IV, **10**-OPP is produced by the 1,2-rearrangement **12** → **13**, followed by elimination. Since **12**



partitions between elimination and rearrangement, the cation must be an intermediate along the reaction path, and alkylation of the homoallylic double bond in **11**, thus, represents a discrete step. Alkylation of the homoallylic double bond in IPP generates a tertiary cation with a directly attached methylene carbon. The α-methine moiety in **12** should stabilize the tertiary cation by only 2–3 kcal/mol[33,34] relative to the case of the α-methylene in the carbocation produced by alkylation of IPP. However, the substituent exerts a powerful stabilizing effect on rearranged cation **13** relative to the putative secondary carbocation produced by a related 1,2-rearrangement during the normal prenyl-transfer reaction with IPP. A related stabilization of the transition state should facilitate the process of **12** → **13**. Although we were not able to detect rearrangement during the normal reaction and thereby obtain direct evidence that alkylation of IPP gives an intermediate, our results with **1**-OPP and **2**-OPP show that formation of such a species within the catalytic site of FPP synthase is energetically feasible. A minimal mechanism for 1′–4 condensation requires that FPP synthase bind IPP and DMAPP in the proper orientation, catalyze heterolytic rupture of the carbon–oxygen bond in the allylic substrate, shield the carbocationic intermediates from adventitious nucleophiles during condensation, and deprotonate the penultimate species. Concerted variants are unsupported embellishments.

The intramolecular 1′–4 condensations of **1**-OPP and **2**-OPP are analogous to the cyclizations of GPP (**14**) to *p*-menthane and

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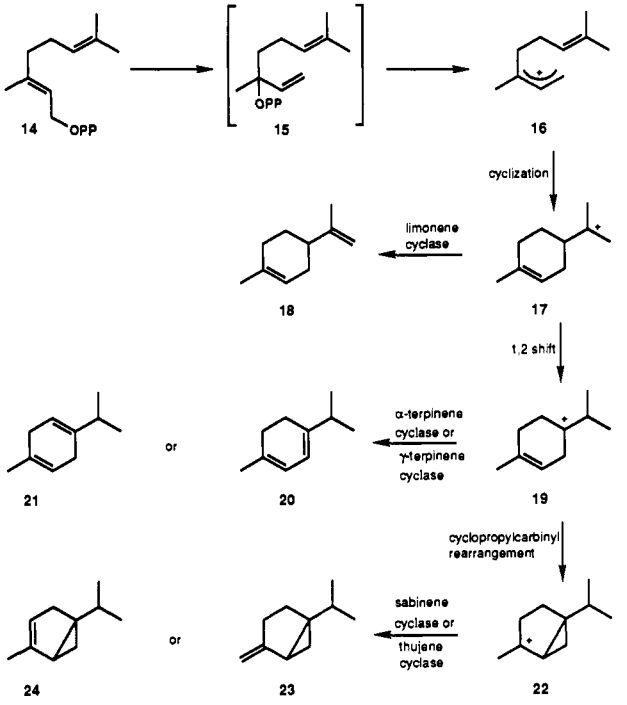
Scheme V. Mechanisms for Formation of *p*-Menthane and Sabinane Monoterpenes



sabinane monoterpenes.[7,29,30,35] Scheme V outlines a set of reactions for the monoterpenes that are remarkably similar to those shown for the bisubstrate analogs in Scheme IV. The cyclizations **11** → **12** and **16** → **17** followed by eliminations **12** → **8**-OPP and **17** → limonene (**18**), respectively, are mechanistically identical, as are 1,2-hydrogen rearrangements **12** → **13** and **17** → **19**, followed by eliminations **13** → **10**-OPP and **19** → γ-terpinene (**20**). In addition to catalizing cyclizations, monoterpene cyclases possess the ability to catalyze isomerization of **14** to linalyl diphosphate (**15**) without release of the allylic isomer from the active site. Monoterpene cyclases are also known which catalyze **19** → α-terpinene (**21**) and the homoallylic to cyclopropylcarbinyl rearrangement **19** → **22**, with subsequent eliminations to thujene (**23**) and sabinene (**24**). Although monoterpene cyclases produce a family of metabolites from GPP, it is clear that the reactions catalyzed by individual enzymes are normally regioselective.[29,30] This selectivity could readily be achieved by the appropriate placement of basic moieties to catalyze elimination, by electrostatic negative fields to encourage rearrangement, or by the conformations of the bound substrates.[3] However, like FPP synthase, regiochemical constraints are reduced when cyclases are provided with abnormal substrates.[36]

The homoallylic diphosphate moieties in **1**-OPP and **2**-OPP are probably not essential for production of cyclic compounds. Several years ago Saito and Rilling[37,38] discovered that cyclic hydrocarbons were formed along with farnesol and nerol during the enzyme-catalyzed solvolysis of FPP. However, FPP is not a very efficient substrate for these reactions. The Michaelis constant for solvolysis of FPP is slightly higher than the values for solvolysis of DMAPP and GPP, which in turn are 30–60 times larger than K_m for condensation of the two substrates with IPP. In addition, V_{max} for cyclization of FPP is $^1/_{20\,000}$th of the normal rate for condensation between IPP and DMAPP or GPP, giving an overall catalytic efficiency (V/K) for solvolysis that is $<10^{-6}$ of the value for the normal prenyl-transfer reaction. In comparison, **1**-OPP and **2**-OPP are excellent alternate substrates with catalytic efficiencies approaching those for 1′–4 condensation. On the basis of these comparisons, we conclude that the diphosphate moieties in the bisubstrate analogs interact strongly with the homoallylic and allylic regions of the catalytic site.

Examination of models suggests that the methylene tether which joins IPP and DMAPP in the bisubstrate analogs is not innocuous. There is no single optimal conformation for forming a planar allylic cation *and* for a least-motion cyclization. In addition, ring strain develops in the cyclohexenyl moiety during cyclization, and the shapes of the cyclic products are different from that of GPP. These factors undoubtedly influence E·S and E·P binding and may well change the rate-limiting step (k_{cat}) from release of product[39] to formation of the allylic carbocation. A dissection of the effects of these perturbations in terms of the kinetic constants shown in Table I is beyond the scope of this study. Suffice it to say that the catalytic efficiencies (k_{cat}/K_m) for FPP synthase with both bisubstrate analogs are similar to those evaluated for either IPP or DMAPP when the concentration of the other substrate is saturating. Thus, **1**-OPP and **2**-OPP are bound by FPP synthase in productive conformations that allow each of the steps shown in Scheme IV to proceed at efficiencies approaching those of the normal substrates. ORD measurements revealed that all of the products are optically active and that optical antipodes of **8**-OPP and **10**-OPP are obtained from **1**-OPP and **2**-OPP. Topological considerations, which provide important insights into the pivotal role of binding upon stereochemistry and regiochemistry in these reactions, are explored in more detail in the following paper.

Experimental Section

Materials. Dihydrogen disodium pyrophosphate was purchased from Sigma Chemical Co. Dowex AG 50W-X8 cation-exchange resin (100–200 mesh) was purchased from Bio Rad. tris(Tetra-*n*-butylammonium) hydrogen pyrophosphate trihydrate was prepared as described[9] using tetra-*n*-butylammonium hydroxide solution (40%) from Fluka Chemical Co. (3-Hydroxypropyl)triphenylphosphonium iodide was prepared as described[12] and used within one month. Dimethyl sulfide, toluene, and triethylamine were distilled under nitrogen from CaH before use. Dimethyl sulfoxide and *N*,*N*-dimethylformamide were distilled from CaH at reduced pressure and stored over 4-Å molecular sieves. Methanol was first distilled from Mg turnings and then stored over 3-Å molecular sieves. Diethyl ether and THF were freshly distilled from sodium benzophenone under a nitrogen atmosphere. Acetonitrile and CH_2Cl_2 were freshly distilled from P_2O_5 under a nitrogen atmosphere. NaB^2H_4, $LiAl^2H_4$, and alkyllithium reagents were purchased from Aldrich Chemical Co. Bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid (BHDA) was prepared from the corresponding anhydride which was recrystallized from hexanes and CH_2Cl_2.

General Methods. Analytical TLC on silica gel was performed on EM Reagents silica gel 60 F-254, 0.25-mm layer, 2.5 × 10 cm glass plates which were visualized by either UV light, 4% phosphomolybdic acid in ethanol, or 2% vanillal/sulfuric acid in ethanol. Flash chromatography on 235–400-mesh silica gel 60 (J.T. Baker) was performed as described by Still.[40] Analytical TLC on cellulose was performed on EM Reagents, 0.1-mm layer, 2.5 × 10 cm glass plates and were visualized by sulfosalicylic acid/$FeCl_3$ stain. Medium-pressure cellulose chromatography was performed in glass columns slurry packed at 4 mL min^{-1} with Whatman CC-31 microgranular cellulose. Reactions that required a dry argon or nitrogen atmosphere were performed with oven-dried (140 °C) glassware unless otherwise stated. Analytical HPLC separations were performed with a 0.78 × 30 cm column packed with 5 μm Hypersil silica gel. Gas chromatography was performed with a 30 M SE-54 or DB-5 glass capillary column with helium carrier gas.

^1H, ^{13}C, ^{31}P, and ^{19}F NMR spectra were recorded at 22 °C. ^1H and ^{13}C spectra are referenced in parts per million (ppm) from a solvent lock signal or 3-(trimethylsilyl)-1-propanesulfonic acid (TPS). ^{31}P and ^{19}F spectra are referenced with external phosphoric acid or trifluoroacetic acid, respectively. Samples for ^{31}P spectra were usually 1 mM in Na_2-EDTA. All ^{13}C and ^{31}P NMR data are reported for broad-band decoupled spectra. ^{13}C multiplicities were derived from attached proton experiments (APT).[41] Routine ^{13}C, ^{31}P, and ^{19}F experiments were acquired with a sufficient number of data points to achieve 0.4, 0.24, and 0.2 Hz digital resolution, respectively.

Optical rotary dispersion data were recorded in a 10-cm light path, 1-mL, jacketed, microcell thermostated at the desired temperature with

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a circulating water bath. Negative-ion fast atom bombardment mass spectra (FABMS) were obtained at −5.0 kV and a resolving power of 2000 using argon bombardment of the sample in a glycerol matrix. A direct inlet with a glass capillary column was used for GC/MS runs. Elemental analysis was performed by Desert Analysis of Tucson, Arizona. Melting points are uncorrected.

Farnesyl-diphosphate synthase was purified from frozen avian liver (Pel Freeze) by the procedure of Rilling.[42] Pure enzyme was stored at protein concentrations of >6 mg mL^{-1} in a buffer containing 100 mM potassium phosphate (pH 7.0), 1 mM EDTA, 2 mM dithiothreitol, and 35% glycerol at −20 °C. These preparations were used directly for incubation experiments. *Escherichia coli* Type III alkaline phosphatase was purchased from Sigma Chemical Co. Enzyme assays were conducted as previously described.[28]

Synthesis. (*Z*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-octadiene (3-OTBDMS). To a solution of 6.66 g (40 mmol) of nerol and 6.26 g (92.0 mmol) of imidazole in CH_2Cl_2 was added over 1 h 12.88 g (46.0 mmol) of *tert*-butyldiphenylsilyl chloride in 1:1 (v/v) DMF/CH_2Cl_2. The mixture was stirred at room temperature for 3 h, diluted with diethyl ether, and washed with 0.1 N HCl, saturated $NaHCO_3$, and saturated NaCl. The mixture was dried over $MgSO_4$, and solvent was removed by rotary evaporation. The residue was purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 14.9 g (95%) of a colorless oil: TLC (R_f 0.45; 9:1 hexanes/CH_2Cl_2); IR (CCl_4) 3070, 2930, 1475, 1430, 1375, 1110, 1060, 705; ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (4, m, Ph H), 7.47 (6, m, Ph H), 5.49 (1, t, J = 6.35 Hz, H at C(2)), 5.07 (1, t, J = 5.85 Hz, H at C(6)), 4.28 (2, d, J = 6.35 Hz, H at C(1)), 1.99 (4, m), 1.79 (3, s, methyl at C(3)), 1.69 (3, s, methyl), 1.59 (3, s, methyl), 1.13 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.4, 135.4, 134.0, 129.5, 127.7, 127.6, 124.9, 123.9, 60.8, 32.2, 26.8, 26.6, 25.6, 23.4, 19.2, 17.6 ppm; MS (EI, 70 eV) m/z 392 (2.1) $[M]^+$, 335 (13.3) $[M - C_4H_9]^+$, 239 (16.4), 199 (100), 183 (58.2), 105 (11). Anal. Calcd for $C_{26}H_{36}OSi$: C, 79.53; H, 9.24. Found: C, 79.53; H, 9.35.

(*Z*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3-methyl-2-hexen-6-al (5-OTBDPS). A suspension of 4 g (10.0 mmol) of 3-OTBDPS and 2.12 g (20.0 mmol) of Na_2CO_3 in 45 mL of a 2:1 (v/v) mixture of $CHCl_3$ and diethyl ether was cooled to −78 °C before the addition of 2.18 g (16.0 mmol) of *m*-chloroperbenzoic acid (95%) in 40 mL of $CHCl_3$ over a 2 h period. The mixture was kept at −78 °C for 6 h, warmed to −10 °C for 1 h, and then quenched with 60 mL of 10% $NaHSO_3$ solution. The resulting mixture was diluted with 100 mL of CH_2Cl_2, washed with 50% saturated $NaHCO_3$, and dried over Na_2SO_4. Solvent was removed by rotary evaporation to give 4.5 g (98%) of a pale-yellow oil which was routinely used without further purification. A pure sample of a colorless oil was obtained by flash chromatography (8:2 hexanes/diethyl ether): TLC (R_f 0.34; 8:2 hexanes/diethyl ether); IR (CCl_4) 3070, 2960, 2860, 1475, 1430, 1375, 1110, 1060, 825, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (4, m, Ph H), 7.43 (6, m, Ph H), 5.48 (1, t, J = 6.50 Hz, H at C(2)) 4.24 (2, d, J = 6.50 Hz, H at C(1)) 2.60 (1, t, J = 6.35 Hz), 2.03 (2, m), 1.61 (1, m), 1.75 (3, s, methyl at C(3)), 1.43 (1, m), 1.25 (3, s, CH_3), 1.19 (3, s, CH_3), 1.08 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.5, 135.6, 133.9, 129.5, 127.6, 125.4, 63.8, 60.6, 58.2, 28.7, 27.4, 26.8, 24.7, 23.3, 19.1, 18.6 ppm. Anal. Calcd for $C_{26}H_{36}O_2Si$: C, 76.42; H, 8.88. Found: C, 76.72; H, 8.96.

A solution of the epoxide (4.05 g, 9.9 mmol) from above in 100 mL of diethyl ether was cooled to −10 °C before addition of 2.51 g (11.0 mmol) of periodic acid. When the starting material was consumed (4–5 h at −10 °C), the mixture was quenched by the addition of 2 g of $NaHCO_3$ and filtered through Celite. The filtrate was dried over $MgSO_4$, and solvent was removed by rotary evaporation. The residue was purified by flash chromatography (90:5:5 hexanes/diethyl ether/ethyl acetate) to yield 2.28 g (63%) of a colorless oil: TLC (R_f 0.28; 8:2 hexanes/diethyl ether); IR (CCl_4) 3070, 2935, 2860, 1730, 1470, 1430, 1265, 1110, 1070, 825, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.68 (1, s, H at C(6)), 7.72 (4, m, Ph H), 7.44 (6, m, Ph H), 5.49 (1, b t, J = 6.35 Hz, H at C(2)), 4.24 (2, d, J = 6.35 Hz, H at C(1)), 2.4 (2, b t, J = 7.74 Hz, H at C(5)), 2.21 (2, t, J = 7.74 Hz, H at C(4)), 1.72 (3, s, CH_3 at C(3)), 1.09 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.8, 135.6, 135.5, 133.7, 129.6, 127.6, 126.0, 60.4, 42.1, 26.8, 24.4, 23.0, 19.1 ppm; MS (EI, 70 eV) m/z 309 (5.8) $[M - C_4H_9]^+$, 241 (6.2), 231 (14.2), 199 (100), 183 (6.1), 163 (11.5), 139 (9.4). Anal. Calcd for $C_{23}H_{30}O_2Si$: C, 75.36; H, 8.25. Found: C, 74.20; H, 8.30.

(2*Z*,6*E*)- and (*Z*,*Z*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-nonadienyl-9-ol (1-OTBDPS,OH) and (2-OTBDPS,OH). A suspension of 6.72 g (15.0 mmol) of (3-hydroxypropyl)triphenylphosphonium iodide (**4**) in 110 mL of THF was cooled to −70 °C before the dropwise addition of 11.1 mL (30.0 mmol) of nBuLi (2.7 M in hexanes) over a 45-min period. This suspension was allowed to warm to 0 °C over 4 h and maintained at that temperature for 1.5 h. The resulting orange/red solution was recooled to −78 °C, and 1.23 mL (2.80 g, 19.7 mmol) of CH_3I in 8.0 mL of THF was added over 1 h followed by warming to 0 °C over 6 h. A creamy white suspension formed after an additional 4 h at room temperature.

The suspension was cooled to −70 °C before addition of 5.7 mL (15.4 mmol) of nBuLi (2.7 M in hexanes) followed by warming to 0 °C for 2 h and to room temperature for 15 min with sonication. During this period, the solution turned a deep red. The solution was cooled to −78 °C, and a solution of aldehyde **5**-OTBDPS (4.40 g, 12.0 mmol) in 50 mL of THF was added over 1 h. The red mixture was warmed to −40 °C for 8 h, and then the reaction was quenched with 20 mL of saturated NH_4Cl. The mixture was extracted with 1:1 (v/v) hexanes/diethyl ether and washed with saturated NH_4Cl. The combined aqueous phases were extracted again with diethyl ether, and the combined organic layers were washed in succession with saturated $NaHCO_3$ and saturated NaCl before drying over $MgSO_4$. Solvent was removed at reduced pressure to afford a semisolid that was diluted with 100 mL of 2:1 hexanes/diethyl ether and allowed to stand overnight. After filtration through Celite and removal of solvent, the residue was purified by chromatography (6:4 hexanes/diethyl ether) to yield 3.5 g (69%) of a pale-yellow oil. Analysis by normal-phase HPLC (65:35 hexane/*tert*-butyl methyl ether) showed two components in a ratio of 55:45, and the compounds were separated on a Waters preparative silica gel cartridge (85:15 hexanes/diethyl ether) to yield colorless oils.

1-OTBDPS,OH (1.41 g, 28%): TLC (R_f 0.22; 1:1 hexanes/diethyl ether); IR (neat) 3360, 3070, 2930, 2860, 1470, 1430, 1115, 1060, 825, 740, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (4, m, Ph H), 7.41 (6, m, Ph H), 5.43 (1, b t, J = 6.40 Hz, H at C(2)), 5.11 (1, b t, J = 6.90 Hz, H at C(6)), 4.21 (2, t, J = 6.40 Hz, H at C(1)), 3.60 (2, t, J = 6.23 Hz, H at C(9)), 2.17 (2, t, J = 6.23 Hz, H at C(8)), 2.01 (2, m), 1.92 (2, m), 1.72 (3, b s, methyl at C(3)), 1.55 (3, s, CH_3 at C(7)), 1.07 ppm (9, s, tBu); NOE difference, irradiating at δ 1.55 alters resonances at 3.60, 2.17, 2.01, and 1.92 ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3 (s), 135.7 (d), 134.0 (s), 131.8 (s), 129.6 (d), 127.7 (d), 127.1 (d), 125.2 (d), 60.6 (t), 59.7 (t), 42.4 (t), 31.8 (t), 26.6 (q), 26.2 (t), 23.1 (q), 18.9 (s), 15.5 ppm (q); MS (EI, 70 eV) m/z 422 (1.0) $[M]^+$, 365 (2.0) $[M - C_4H_9]^+$, 267 (3.1), 229 (6.0), 199 (100), 181 (6.1), 149 (24.0), 93 (19), 81 (15). Anal. Calcd for $C_{27}H_{38}O_2Si$: C, 76.75; H, 9.06. Found: C, 76.65; H, 9.16.

2-OTBDPS,OH (1.73 g, 34%): TLC (R_f 0.27; 1:1 hexanes/diethyl ether); IR (neat) 3360, 3070, 2935, 2860, 1470, 1430, 1115, 1060, 825, 740, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (4, m, Ph H), 7.41 (6, m, Ph H), 5.43 (1, tq, J = 6.41, 1.10 Hz, H at C(2)), 5.18 (1, m, H at C(6)), 4.20 (2, d, J = 6.41 Hz, H at C(1)), 3.58 (2, t, J = 6.61 Hz, H at C(9)), 2.22 (2, t, J = 6.61 Hz, H at C(8)), 2.02 (2, m), 1.91 (2, m), 1.71 (3, b d, J = 1.10 Hz, CH_3 at C(3)), 1.64 (3, b d, J = 1.24 Hz, CH_3 at C(7)), 1.06 ppm (9, s, tBu); NOE difference, irradiating at δ 1.64 alters resonances at 5.18, and 3.58 ppm, irradiating at δ 5.18 alters resonances at 2.02, 1.91, and 1.64 ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3 (s), 135.7 (d), 134.1 (s), 131.6 (s), 129.6 (d), 127.8 (d), 127.7 (d), 125.2 (d), 60.7 (t), 60.4 (t), 34.8 (t), 32.1 (t), 26.6 (q), 26.2 (t), 23.2 (q), 23.1 (q), 18.9 ppm (s); MS (EI, 17 eV) m/z 422 (0.8) $[M]^+$, 365 (1.6) $[M - C_4H_9]^+$, 267 (3.1), 229 (6.0), 199 (100), 181 (6.1), 149 (24), 135 (5.9), 121 (5.9), 93 (18.9), 81 (15.0). Anal. Calcd for $C_{27}H_{38}O_2Si$: C, 76.75; H, 9.06. Found: C, 76.76; H, 9.24.

(2*Z*,6*E*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-nonadien-9-yl 4-Methylbenzenesulfonate (1-OTBDPS,OTs). To a solution of 227 mg (1.19 mmol) of *p*-toluenesulfonyl chloride and 161 mg (1.32 mmol) of 4-(*N*,*N*-dimethylamino)pyridine in 5 mL of CH_2Cl_2 at 0 °C was added 420 mg, 0.99 mmol of **1**-OTBDPS,OH in 1.5 mL of CH_2Cl_2. After 0.5 h at 0 °C, the reaction mixture was allowed to warm to room temperature for 2.5 h before it was diluted with pentane. The resulting suspension was stirred for 15 min; the precipitate was removed by gravity filtration and rinsed with 1:1 (v/v) hexanes/diethyl ether. Solvent was removed by rotary evaporation, and the resulting oil was dissolved in hexanes and filtered through Celite. Solvent was removed again, and the residue was purified by flash chromatography (7:3 hexanes/diethyl ether) to yield 536 mg (94%) of a colorless oil: TLC (R_f 0.37; 7:3 hexanes/diethyl ether); IR (neat) 3070, 2930, 2865, 1600, 1470, 1430, 1365, 1190, 1180, 1115, 1060, 965, 910, 825, 740, 705, 665, 610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (2, d, J = 8.30 Hz, sulfonate Ph H), 7.68 (4, m, Ph H), 7.48 (6, m, Ph H), 7.31 (2, d, J = 8.30 Hz, sulfonate Ph H), 5.38 (1, tq, J = 6.47, 1.31 Hz, H at C(2)), 5.00 (1, m, H at C(6)), 4.18 (2, d, J = 6.47 Hz, H at C(1)), 4.01 (2, t, J = 6.99 Hz, H at C(9)), 2.43 (3, s, CH_3 at Ph), 2.20 (2, t, J = 6.99 Hz, H at C(8)), 1.90 (2, m), 1.84 (2, m), 1.68 (3, d, J = 1.31 Hz, CH_3 at C(3)), 1.42 (3, s, CH_3 at C(7)), 1.04 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$), δ. 144.6 (s), 137.1 (s), 135.6 (d), 133.9 (s), 133.3 (s), 129.8 (s), 129.7 (d), 129.5 (d), 127.8 (d), 127.6

(42) Reed, B. C.; Rilling, H. C. *Biochemistry* **1975**, *14*, 50–54.

(d), 127.5 (d), 125.0 (d), 68.9 (t), 60.7 (t), 38.6 (t), 31.8 (t), 26.8 (q), 26.5 (t), 23.4 (q), 21.6 (q), 19.1 (s), 15.8 ppm (q).

(*Z*,*Z*)-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-nonadien-9-yl 4-Methylbenzenesulfonate (2-OTBDPS,OTs). Using a procedure similar to that described for 1-OTBDPS,OTs, 343 mg (1.8 mmol) of *p*-toluenesulfonyl chloride and 244 mg (2.0 mmol) of 4-(*N*,*N*-dimethylamino)pyridine in 6 mL of CH_2Cl_2 were allowed to react with (633 mg, 1.5 mmol) of 2-OTBDPS,OH. The product was purified by flash chromatography (8:2 hexanes/diethyl ether) to yield 829 mg (97%) of a colorless oil: TLC (R_f 0.37; 7:3 hexanes/diethyl ether); IR (neat) 3070, 2930, 2860, 1600, 1470, 1430, 1365, 1190, 1180, 1110, 1060, 965, 825, 745, 710, 665, 610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (2, d, J = 8.58 Hz, sulfonate Ph H), 7.68 (4, m, Ph H), 7.39 (6, m, Ph H), 7.30 (2, d, J = 8.58 Hz, sulfonate Ph H), 5.37 (1, t, J = 6.47 Hz, H at C(2)), 5.07 (1, m, H at C(6)), 4.14 (2, d, J = 6.47 Hz, H at C(1)), 3.96 (2, t, J = 7.20 Hz, H at C(9)), 2.43 (3, s, CH_3 at Ph), 2.26 (2, t, J = 7.20 Hz, H at C(8)), 1.84 (4, m), 1.65 (3, b s, CH_3 at C(3)), 1.52 (3, b s, CH_3 at C(7)), 1.04 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6 (s), 136.9 (s), 135.6 (d), 133.9 (s), 133.2 (s), 129.8 (d), 129.5 (d), 129.4 (s), 128.3 (d), 127.9 (d), 127.6 (d), 125.2 (d), 68.4 (t), 60.7 (t), 31.9 (t), 31.3 (t), 26.8 (q), 26.2 (t), 23.3 (q), 23.2 (q), 21.6 (q), 19.2 ppm (s).

(2*Z*,6*E*)-3,7-Dimethyl-9-(((4-methylphenyl)sulfonyl)oxy)-2,6-nonadien-1-ol (1-OH,OTs). To a solution of 458 mg (0.853 mmol) of 1-OTBDPS,OTs in 9 mL of THF at 0 °C was added 296 mg (0.938 mmol) of solid tetrabutylammonium fluoride trihydrate. After 3 h, the mixture was diluted with 35 mL of hexanes/diethyl ether, and the organic layer was washed in succession with 0.05 N HCl and saturated NaCl solution. The solution was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The residue was purified by flash chromatography (7:3 hexanes/diethyl ether) to yield 240 mg (84%) of a colorless liquid: TLC (R_f 0.24; 3:7 diethyl ether/hexanes); IR (neat) 3400, 2970, 2860, 1600, 1450, 1360, 1190, 1180, 1100, 965, 910, 820, 775, 665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (2, d, J = 8.51 Hz, Ph H), 7.34 (2, d, J = 8.51 Hz, Ph H), 5.42 (1, t, J = 7.09 Hz, H at C(2)), 5.13 (1, m, H at C(6)), 4.09 (2, d, J = 7.09 Hz, H at C(1)), 4.06 (2, t, J = 6.85 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.29 (2, t, J = 6.85 Hz, H at C(8)), 2.06 (4, m), 1.72 (3, s, CH_3 at C(3)), 1.52 ppm (3, s, methyl at C(7)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6 (s), 138.5 (s), 132.7 (s), 129.9 (s), 129.6 (s), 127.5 (d), 127.1 (d), 124.6 (d), 68.6 (t), 58.6 (t), 38.3 (t), 31.3 (t), 26.2 (t), 23.1 (q), 21.4 (q), 15.6 ppm (q); MS (CI, CH_4) m/z 339 (3.7) $[M + 1]^+$, 320 (34), 210 (15.6), 187 (18.8), 177 (25), 173 (100), 155 (90), 148 (92), 121 (89), 107 (98).

(*Z*,*Z*)-3,7-Dimethyl-9-(((4-methylphenyl)sulfonyl)oxy)-2,6-nonadien-1-ol (2-OH,OTs). Following the procedure described above, 395 mg, 0.69 mmol of 2-OTBDPS,OTs was treated with 240 mg (0.76 mmol) of solid tetrabutylammonium fluoride trihydrate to yield 186 mg (80%) of a colorless liquid: TLC (R_f 0.24; 3:7 hexanes/diethyl ether); IR (neat) 3400, 2960, 2860, 1600, 1450, 1360, 1190, 1180, 1100, 965, 910, 820, 775, 675 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (2, d, J = 8.50 Hz, Ph H), 7.32 (2, d, J = 8.50 Hz, Ph H), 5.42 (1, t, J = 6.90 Hz, H at C(2)), 5.18 (1, m, H at C(6)), 4.05 (2, d, J = 6.90 Hz, H at C(1)), 4.00 (2, t, J = 6.90 Hz, H at C(9)), 2.42 (3, s, CH_3 at Ph), 2.36 (2, t, J = 6.90 Hz, H at C(8)), 2.03 (4, m), 1.68 (3, s, CH_3 at C(3)), 1.56 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6 (s), 138.5 (s), 132.7 (s), 129.6 (d), 129.5 (s), 128.0 (s), 127.5 (d), 124.6 (d), 68.2 (t), 58.5 (t), 31.6 (t), 31.1 (t), 26.2 (t), 23.1 (q), 22.9 (q), 21.3 ppm (s); MS (CI, CH_4) m/z 339 (3.6) $[M + 1]^+$, 321 (23), 201 (8.9), 179 (6.9), 173 (100), 167 (12.1), 157 (15.0), 155 (24.0), 150 (92), 148 (98), 121 (89), 107 (95).

(2*Z*,6*E*)-1-Chloro-3,7-dimethyl-2,6-nonadien-9-yl 4-Methylbenzenesulfonate (1-Cl,OTs). A solution of 99 mg (0.74 mmol) of *N*-chlorosuccinimide in 3.5 mL of CH_2Cl_2 was cooled to 0 °C before addition of 73 μL (62 mg, 1.0 mmol) of dimethyl sulfide. The resulting white suspension was cooled to −30 °C before 227 mg (0.67 mmol) of 1-OH,OTs in 2.5 mL of CH_2Cl_2 was added over a 5-min period. After being stirred at −20 °C for 1.5 h, the reaction mixture was allowed to warm to −5 °C and was quenched with saturated NaCl solution. The mixture was diluted with 1:1 (v/v) hexanes/diethyl ether. The organic layer was washed with saturated NaCl solution and dried over $MgSO_4$. Solvent was removed to give 215 mg (90%) of a colorless liquid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (2, d, J = 8.13 Hz, Ph H), 7.35 (2, d, J = 8.13 Hz, Ph H), 5.44 (1, t, J = 7.54 Hz, H at C(2)), 5.13 (1, m, H at C(6)), 4.08 (2, d, J = 7.00 Hz, H at C(1)), 4.05 (2, t, J = 6.85 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.31 (2, t, J = 6.85 Hz, H at C(8)), 2.1 (4, m), 1.75 (3, s, CH_3 at C(3)), 1.54 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6, 142.2, 133.1, 130.4, 129.7, 127.8, 126.9, 121.3, 68.8, 40.8, 38.5, 31.4, 26.2, 23.3, 21.6, 15.9 ppm.

(*Z*,*Z*)-1-Chloro-3,7-dimethyl-2,6-nonadien-9-yl 4-Methylbenzenesulfonate (2-Cl,OTs). Using a procedure similar to that described above, 81 mg (0.605 mmol) of *N*-chlorosucciniimide, 60 μL (51 mg, 0.82 mmol) of dimethyl sulfide, and 186 mg (0.55 mmol) of 2-OH,OTs were combined to yield 185 mg (94%) of a pale-yellow liquid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (2, d, J = 8.23 Hz, Ph H), 7.29 (2, d, J = 8.23 Hz, Ph H), 5.37 (1, t, J = 8.05 Hz, H at C(2)), 5.20 (1, m, H at C(6)), 3.98 (2, d, J = 8.70 Hz, H at C(1)), 3.96 (2, t, J = 7.20 Hz, H at C(9)), 2.39 (3, s, CH_3 at Ph), 2.31 (2, t, J = 7.20 Hz, H at C(8)), 2.02 (4, m), 1.67 (3, s, CH_3 at C(3)), 1.55 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6, 142.1, 133.1, 130.0, 129.7, 127.9, 127.8, 121.4, 68.3, 40.8, 31.7, 31.3, 26.2, 23.4, 23.2, 21.6 ppm.

(2*Z*,6*E*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate (1-OPP). A solution of 172 mg (0.48 mmol) of 1-Cl,OTs in 2.0 mL of acetonitrile was cooled in an ice bath before the addition of 1.38 g (1.45 mmol) of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate trihydrate in three equal portions over a 10-min period. After 30 min, the ice bath was removed, and the reaction was continued for an additional 4 h before solvent was removed by rotary evaporation. The residue was dissolved in 3 mL of 25 mM NH_4HCO_3 and applied to a 2 × 14 cm column of DOWEX AG 50W-X8 cation ion-exchange resin (NH_4^+ form). The product was eluted with 85 mL of water and dried by lyophilization to yield a white crusty solid. The product was purified by medium-pressure chromatography on cellulose (1.5 × 22 cm; 3:3:4 1-propanol/THF/0.1 M NH_4HCO_3) at a flow rate of 2.0 mL min^{-1}. Fractions containing the desired material were pooled and dried by lyophilization. Final traces of carbonate anion were removed by dissolving the recovered material in 100 mM NH_4HCO_3, pH 7.0, and drying by lyophilization. A total of 195 mg (65%) of a hygroscopic white powder was obtained: TLC (cellulose, R_f 0.4; 2.5:2.5:5 1-propanol/THF/0.1 M NH_4HCO_3); ^1H NMR (300 MHz, D_2O) δ 5.41 (1, m, H at C(2)), 5.26 (1, m, H at C(6)), 4.40 (2, dd, $J_{H,H}$ = 6.72 Hz, $J_{H,P}$ = 6.72 Hz, H at C(1)), 3.92 (2, dt, $J_{H,H}$ = 6.66 Hz, $J_{H,P}$ = 6.66 Hz, H at C(9)), 2.30 (2, b t, J = 6.66 Hz, H at C(8)), 2.11 (4, m), 1.71 (3, s, CH_3), 1.61 ppm (3, s, CH_3); ^{13}C NMR (300 MHz, D_2O) δ 145.6, 136.0, 129.1, 123.5 (d, $J_{C,P}$ = 7.8 Hz), 67.3 (d, $J_{C,P}$ = 5.5 Hz), 65.0 (d, $J_{C,P}$ = 5.1 Hz), 42.7 (d, $J_{C,P}$ = 7.4 Hz), 34.0, 29.0, 25.5, 18.1 ppm; ^{31}P NMR (121 MHz, D_2O) δ −9.76 (1, d, $J_{P,P}$ = 21.39 Hz), −9.79 (1, d, $J_{P,P}$ = 21.40 Hz), −13.00 (1, d, $J_{P,P}$ = 21.39 Hz), −13.10 ppm (1, d, $J_{P,P}$ = 21.40 Hz); FABMS (−Ve, glycerol) m/z 503 (44.9) $[M - 1]^-$, 423 (9.3), 417 (4.8), 325 (6.0), 258 (5.7), 190 (9.8), 177 (75.3), 165 (6.6), 159 (55.9), 151 (13), 97 (5.6).

(*Z*,*Z*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate (2-OPP). As described above, 146 mg (0.41 mmol) of 2-Cl,OTs was treated with 1.37 g (1.44 mmol) of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate trihydrate to yield 135 mg (55%) of a hygroscopic white solid: TLC (cellulose, R_f 0.42; 2.5:2.5:5 1-propanol/THF/0.1 M NH_4HCO_3); ^1H NMR (300 MHz, D_2O) δ 5.45 (1, t, J = 6.90 Hz, H at C(2)), 5.29 (1, m, H at C(6)), 4.42 (2, dd, $J_{H,H}$ = 6.90 Hz, $J_{H,P}$ = 6.90 Hz, H at C(1)), 3.93 (2 dt, $J_{H,H}$ = 6.89 Hz, $J_{H,P}$ = 6.89 Hz, H at C(9)), 2.39 (2, b t, J = 6.89 Hz, H at C(8)), 2.16 (4, m), 1.74 (3, s, CH_3), 1.71 ppm (3, s, CH_3); ^{13}C NMR (75 MHz, D_2O), δ 145.3, 135.9, 129.6, 123.5 (d, $J_{C,P}$ = 8.10 Hz), 66.9 (d, $J_{C,P}$ = 6.00 Hz), 64.9 (d, $J_{C,P}$ = 5.30 Hz), 35.2 (d, $J_{C,P}$ = 7.20 Hz), 33.9, 28.5, 25.5, 25.3 ppm; ^{31}P NMR (121 MHz, D_2O) δ −9.77 (1, d, $J_{P,P}$ = 21.29 Hz), −9.80 (1, d, $J_{P,P}$ = 21.43 Hz), −13.02 (1, d, $J_{P,P}$ = 21.29 Hz), −13.14 ppm (1, d, $J_{P,P}$ = 21.43 Hz); FABMS (−Ve, glycerol) m/z 503 (44.9) $[M - 1]^-$, 423 (16.1), 417 (6.6), 387 (5.1), 327 (8.7), 325 (11.0), 258 (7.9), 190 (10.9), 177 (46.8), 159 (39.5), 151 (5.6), 97 (22.7).

(2*Z*,6*E*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl 9-(4-Methylbenzenesulfonate) 1-(2-Naphthoate) (1-ONp,OTs). To a solution of 17 mg (0.1 mmol) of 2-naphthoic acid, 12 mg (0.1 mmol) of 4-(*N*,*N*-dimethylamino)pyridine, and 16 mg (0.1 mmol) of 4-(*N*,*N*-dimethylamino)-pyridine hydrochloride in 0.5 mL of CH_2Cl_2 was added 133 μL (20.6 mg, 0.1 mmol) of 1,3-dicyclohexylcarbodiimide (0.74 M in CH_2Cl_2). The mixture was stirred at room temperature for 1.5 h before addition of 17 mg (0.05 mmol) of 1-OH,OTs in 1 mL of CH_2Cl_2. The reaction was allowed to continue overnight before solvent was removed under a nitrogen stream. The residue was extracted with hexanes and purified by preparative TLC (R_f 0.27; 7:3 hexanes/diethyl ether) to yield 22 mg (88%) of a colorless oil: UV (CH_3CN) λ_{max} 232 (ϵ 68 410), 273 (5262), 281 nm (6000); ^1H NMR (300 MHz, $CDCl_3$) δ 8.60 (1, b s, naphthyl H), 8.04 (1, b s, naphthyl H), 7.95 (1, m, naphthyl H), 7.89 (2, m, naphthyl H), 7.76 (2, d, J = 8.25 Hz, sulfonate Ph H), 7.55 (2, m, naphthyl H), 7.32 (2, d, J = 8.25 Hz, sulfonate Ph H), 5.54 (1, t, J = 7.24 Hz, H at C(2)), 5.17 (1, m, H at C(6)), 4.84 (2, d, J = 7.24 Hz, H at C(1)), 4.06 (2, t, J = 6.90 Hz, H at C(9)), 2.43 (3, s, CH_3 at Ph), 2.30 (2, t, J = 6.90 Hz, H at C(8)), 2.15 (4, m), 1.80 (3, s, CH_3 at C(3)), 1.54 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (300 MHz, $CDCl_3$) δ 166.6, 144.5, 142.3, 135.4, 133.1, 132.3, 130.9, 130.2, 129.7, 129.2, 128.1, 128.0, 127.7, 127.6, 127.1, 126.5, 125.2, 119.5, 68.9, 61.6, 38.7, 32.0, 26.8, 23.6, 21.7, 16.0 ppm.

(*Z*,*Z*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl 9-(4-Methylbenzenesulfonate) 1-(2-Naphthoate) (2-ONp,OTs). Using the protocol from

above, **2**-OH,OTs was converted to its naphthoate ester in 76% yield: TLC (R_f 0.27; 7:3 hexanes/diethyl ether); UV (CH_3CN) λ_{max} 234 (ϵ 74000), 274 (6850), 279 nm (7872); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (1, b s, naphthyl H), 8.06 (1, m, naphthyl H), 7.95 (1, m, naphthyl H), 7.87 (2, m, naphthyl H), 7.77 (2, d, J = 8.25 Hz, sulfonate Ph H), 7.58 (2, m, naphthyl H), 7.32 (2, d, J = 8.25 Hz, sulfonate Ph H), 5.53 (1, t, J = 7.24 Hz, H at C(2)), 5.26 (1, m, H at C(6)), 4.84 (2, d, J = 7.24 Hz, H at C(1)), 4.03 (2, t, J = 7.14 Hz, H at C(9)), 2.42 (3, s, CH_3 at Ph), 2.38 (2, t, J = 7.14 Hz, H at C(8)), 2.14 (4, m), 1.78 (3, s, CH_3 at C(3)), 1.59 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 144.5, 142.1, 135.4, 133.1, 132.4, 130.9, 129.9, 129.7, 129.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 126.5, 125.2, 119.6, 68.4, 61.6, 32.2, 31.5, 26.5, 23.6, 23.4, 21.7 ppm.

(2*Z*,6*E*)-[1-^{2}H]-3,7-Dimethyl-9-(((4-methylphenyl)sulfonyl)oxy)-2,6-nonadien-1-ol ([1-^{2}H]1-OH,OTs). A suspension of 313 mg (3.6 mmol) of activated MnO_2 in 1.5 mL of CH_2Cl_2 was treated with 30 mg (0.09 mmol) of **1**-OH,OTs in 0.3 mL of CH_2Cl_2. After 0.5 h, the suspension was filtered through Celite, and the Celite was rinsed with CH_2Cl_2. Solvent was removed from the filtrate, and the residue was redissolved in THF. The flask was purged with nitrogen, and the solution was cooled to 0 °C before 3.8 mg (0.09 mmol) of sodium borodeuteride in a total of 0.2 mL of methanol was added. After 30 min, the reaction was quenched with 50% saturated NH_4Cl solution, and the ice bath was removed. Water was added and the mixture was extracted with diethyl ether. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation to yield 24 mg (77%) of a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (2, d, J = 8.27 Hz, Ph H), 7.34 (2, d, J = 8.27 Hz, Ph H), 5.43 (1, d, J = 6.49 Hz, H at C(2)), 5.13 (1, b m, H at C(6)), 4.08 (1, b d, J = 6.49 Hz, H at C(1)), 4.06 (2, t, J = 6.82 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.30 (2, t, J = 6.82 Hz, H at C(7)), 2.07 (4, m), 1.73 (3, s, CH_3 at C(3)), 1.53 ppm (3, s, CH_3 at C(7)).

(*Z*,*Z*)-[1-^{2}H]-3,7-Dimethyl-9-(((4-methylphenyl)sulfonyl)oxy)-2,6-nonadien-1-ol ([1-^{2}H]2-OH,OTs). Using the protocol described above, **2**-OH,OTs gave 22 mg (71%) of a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (2, d, J = 8.27 Hz, Ph H), 7.34 (2, d, J = 8.27 Hz, Ph H), 5.42 (1, d, J = 6.73 Hz, H at C(2)), 5.22 (1, m, H at C(6)), 4.05 (1, b d, J = 6.73 Hz, H at C(1)), 4.02 (2, t, J = 7.07 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.37 (2, t, J = 7.07 Hz, H at C(7)), 2.06 (4, m), 1.71 (3, s, CH_3 at C(3)), 1.61 ppm (3, s, CH_3 at C(7)).

(2*Z*,6*E*)-[1-^{3}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([1-^{3}H]1-OPP). The oxidation as described above and subsequent reduction were performed for **1**-OH,OTs by substituting sodium borotrideride (25 mCi, 500 mCi mmol^{-1}) for sodium borodeuteride. After the material was dried under vacuum (0.5 mmHg) for 2–3 h, the residue was dissolved in 0.4 mL of CH_2Cl_2.

The specific activity of the sample obtained from the oxidation–reduction sequence was determined by the procedure of Davisson et al.[26] A 20-μL portion of the CH_2Cl_2 solution containing the radioactive alcohol was added to 3.5 mg (0.02 mmol) of 2-naphthoic acid, 2.5 mg (0.02 mmol) of 4-(*N*,*N*-dimethylamino)pyridine, 3.0 mg (0.02 mmol) of 4-(*N*,*N*-dimethylamino)pyridine hydrochloride, and 4 mg (0.02 mmol) of 1,3-dicyclohexylcarbodiimide in 230 μL of CH_2Cl_2. The mixture was worked up and purified as described for **1**-ONp,OTs. The product was dried in a 2-mL volumetric flask and redissolved in acetonitrile. Appropriate dilutions of this solution were made to quantify radioactivity by liquid scintillation spectrometry (Optifluor) and concentration by UV spectroscopy. The specific activity of the product was 55.8 μCi μmol^{-1}. The labeled alcohol was processed to the bisdiphosphate in an overall chemical yield of 28%.

(*Z*,*Z*)-[1-^{3}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([1-^{3}H]2-OPP). [1-^{3}H]**2**-OPP was prepared from **2**-OH,OTs as described above with a specific activity of 34.6 μCi μmol^{-1} in 19% overall chemical yield.

(*Z*)-6-((*tert*-Butyldiphenylsilyl)oxy)-4-methyl-4-hexenoic Acid (26-OTBDPS). To a solution of 1.6 g (4.37 mmol) of **5**-OTBDPS in 20 mL of *N*,*N*-dimethylformamide was added at 0 °C 3.3 g (8.74 mmol) of freshly prepared pyridinium dichromate, and the solution was stirred at 4 °C overnight. The solution was poured into 500 mL of diethyl ether and washed in succession with 0.1 N HCl, water, and saturated NaCl. The material was dried over $MgSO_4$, and solvent was removed by rotary evaporation to afford 1.66 g (99%) of a pale-yellow liquid: TLC (R_f 0.31; 96:4 CH_2Cl_2/isopropanol); IR (CCl_4) 3075, 2930, 2856, 1708, 1465, 1425, 1105, 1055, 820, 675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (4, m, Ph H), 7.39 (6, m, Ph H), 5.44 (1, t, J = 6.48 Hz, H at C(5)), 4.21 (2, d, J = 6.48 Hz, H at C(6)), 2.30 (2, m), 2.18 (2, m), 1.70 (3, s, CH_3 at C4), 1.04 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 135.6, 135.2, 133.8, 129.6, 127.6, 126.3, 60.5, 32.4, 26.9, 26.6, 22.9, 19.1 ppm.

(*Z*)-Methyl-6-((*tert*-butyldiphenylsilyl)oxy)-4-methyl-4-hexenoate (27-OTBDPS). A solution of 1.63 g (4.26 mmol) of **26**-OTBDPS in 50 mL of diethyl ether was treated with 250 mL of a 0.16 M solution of CH_2N_2 in diethyl ether. After standing overnight, the solution was concentrated to half volume under a stream of nitrogen, diluted with diethyl ether, washed with water, and dried over $MgSO_4$. Solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 1.18 g (70% from **5**-OTBDPS) of a colorless liquid: TLC (R_f 0.32; 9:1 hexanes/diethyl ether); IR (CCl_4) 3075, 2930, 2850, 1735, 1469, 1426, 1105, 1055, 820, 695 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (4, m, Ph H), 7.41 (6, m, Ph H), 5.43 (1, t, J = 6.47 Hz, H at C(5)), 4.20 (2, t, J = 6.47 Hz, H at C(6)), 3.60 (3, s, *O*-methyl), 2.24 (4, m), 1.69 (3, s, CH_3 at C(4)), 1.05 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 135.5, 135.4, 133.7, 129.4, 127.5, 126.1, 60.5, 51.1, 32.6, 27.4, 26.9, 22.9, 19.2 ppm; MS (EI, 70 eV) m/z 339 (100) [M – C_4H_9]$^+$, 307 (5.6), 271 (20.6), 261 (13.1), 231 (10.0), 100 (199), 109 (5.0), 81 (10.5). Anal. Calcd for $C_{24}H_{32}O_3Si$: C, 72.68; H, 8.13. Found: C, 73.05; H, 8.27.

(*Z*)-[6,6-$^2H_2$]-1-((*tert*-Butyldiphenylsilyl)oxy)-6-hydroxy-3-methyl-2-hexenol ([6,6-$^2H_2$]7-OTBDPS). A solution of 1.2 g (2.57 mmol) of **6**-OTBDPS in 15 mL of diethyl ether was cooled to −10 °C, and 86 mg (2.06 mmol) of lithium aluminum deuteride was added. After 3 h, the reaction mixture was warmed to room temperature and filtered through Celite. The filtrate was dried over Na_2SO_4, and solvent was removed by rotary evaporation to yield 900 mg (94%) of a colorless oil. An analytical sample was purified by flash chromatography (1:1 hexanes/diethyl ether): TLC (R_f 0.34; 1:1 hexanes/diethyl ether); IR (CCl_4) 3470, 3070, 2920, 2850, 1470, 1425, 1108, 1055, 820, 650 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (4, m, Ph H), 7.41 (6, m, Ph H), 5.41 (1, t, J = 6.96 Hz, H at C(2)), 4.18 (2, d, J = 6.96 Hz, H at C(1)), 2.04 (2, t, J = 7.25 Hz, H at C(5)), 1.70 (3, s, CH_3 at C(3)), 1.58 (2, t, J = 7.25 Hz), 1.04 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.2, 135.5, 133.7, 129.5, 127.5, 124.7, 60.9 (quintet, $J_{C,D}$ = 21.6 Hz), 60.4, 30.1, 27.8, 26.9, 23.2, 19.2 ppm; ^{2}H NMR (46 MHz, CH_2Cl_2) δ 3.42 ppm (b s); MS (EI, 17 eV) m/z 313 (61.4) [M – C_4H_9]$^+$, 283 (9.2), 234 (4.4), 205 (16), 199 (95), 184 (100), 139 (4.8), 119 (44.7), 97 (16). Anal. Calcd for $C_{23}H_{30}{}^2H_2O_2Si$: C, 74.54; H, 8.70. Found: C, 74.54; H, 8.97.

(*Z*)-[6-^{2}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3-methyl-2-hexen-6-al ([6-$^2H_2$]5-OTBDPS). To a solution of 7 mL of CH_2Cl_2 and 400 mg (0.275 mL, 3.15 mmol) of oxalyl chloride at −60 °C was added 394 mg (0.36 mL, 5.04 mmol) of dimethyl sulfoxide. After cooling to −78 °C, a solution of 934 mg (2.52 mmol) of [6,6-$^2H_2$]7-OTBDPS in 3.0 mL of CH_2Cl_2 was added over 10 min followed by 1.14 g (1.57 mL, 11.25 mmol) of triethylamine. The resulting suspension was stirred for 2 h and allowed to warm to −60 °C. Water (5 mL) was added, and the mixture was extracted with diethyl ether. The organic layer was washed in succession with water, saturated NH_4Cl, and saturated $NaHCO_3$. The solution was dried over $MgSO_4$, and the solvent was removed at reduced pressure. The residue was purified by flash chromatography (8:2 hexanes/diethyl ether) to yield 710 mg (77% from ester) of a colorless oil: TLC (R_f 0.28; 8:2 hexanes/diethyl ether); IR (CCl_4) 3070, 2960, 2930, 2855, 1715, 1470, 1428, 1105, 1055, 820, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (4, m, Ph H), 7.43 (6, m, Ph H), 5.48 (1, t, J = 6.50 Hz, H at C(2)), 4.23 (2, t, J = 6.50 Hz, H at C(1)), 2.40 (2, t, J = 7.74 Hz, H at C(4)), 2.21 (2, t, J = 7.74 Hz, H at C(5)), 1.72 (3, s, CH_3 at C(3)), 1.06 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.3 (t, $J_{C,D}$ = 26.2 Hz), 135.5, 135.4, 133.7, 129.5, 127.5, 126.0, 60.4, 42.0, (t, $J_{C,D}$ = 7.4 Hz), 26.9, 24.4, 23.1, 19.2 ppm; ^{2}H NMR (46 MHz, CH_2Cl_2) δ 9.7 ppm (s); MS (EI, 17 eV) m/z 310 (25.6) [M – C_4H_9]$^+$. 232 (62.5), 199 (100), 139 (30.0), 94 (95). Anal. Calcd for $C_{23}H_{29}{}^2HO_2Si$: C, 75.15; H, 8.23. Found: C, 75.10; H, 8.23.

(2*Z*,6*E*)-[6-^{2}H]- and (*Z*,*Z*)-[6-^{2}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-nonadien-9-ol ([6-^{2}H]1-OTBDPS,OH and [6-^{2}H]2-OTBDPS,OH). Following the procedure for unlabeled materials, 552 mg (1.5 mmol) of [6-^{2}H]**5**-OTBDPS was converted to 169 mg of [6-^{2}H]**1**-OTBDPS,OH and 103 mg of [6-^{2}H]**2**-OTBDPS,OH (total yield of 42%).

[6-^{2}H]**1**-OTBDPS,OH: TLC (R_f 0.22; 1:1 hexanes/diethyl ether); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.71 (4, m, Ph H), 7.43 (6, m, Ph H), 5.43 (1, t, J = 6.49 Hz, H at C(2)), 4.19 (2, d, J = 6.49 Hz, H at C(1)), 3.61 (2, t, J = 6.22 Hz, H at C(9)), 2.18 (2, t, J = 6.22 Hz, H at C(8)), 2.01 (2, m), 1.92 (2, m), 1.72 (3, s, CH_3 at C(3)), 1.56 (3, s, CH_3 at C(7)), 1.07 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.1, 135.5, 133.9, 131.4, 129.4, 127.5, 126.7 (t, $J_{C,D}$ = 22.8 Hz), 125.0, 60.7, 60.0, 42.5, 32.0, 26.9, 26.4, 24.0, 19.2, 15.7 ppm; ^{2}H NMR (46 MHz, CH_2Cl_2) δ 5.01 ppm (b s); MS (EI, 70 eV) m/z 423 (8.2) [M]$^+$, 366 (4.7) [M – H_2O]$^+$, 348 (4.5), 336 (3.6), 256 (6.6), 229 (11.7), 199 (100), 167 (121), 150 (95.3), 149 (46.9), 136 (14), 122 (16.4), 108 (14.4), 94 (27.3), 93 (43.4). Anal. Calcd for $C_{27}H_{37}{}^2HO_2Si$: C, 76.54; H, 9.28. Found: C, 76.42; H, 9.31.

[6-^{2}H]2-OTBDPS,OH: TLC (R_f 0.27; 1:1 hexanes/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (4, m, Ph H), 7.40 (6, m, Ph H), 5.41 (1, t, J = 6.36 Hz, H at C(2)), 4.18 (2, d, J = 6.36 Hz, H at C(1)), 3.59 (2, t, J = 6.67 Hz, H at C(9)), 2.21 (2, t, J = 6.67 Hz, H at C(8)), 2.01 (2, m), 1.92 (2, m), 1.71 (3, s, CH_3 at C(3)), 1.64 (3, s, CH_3 at C(7)), 1.06 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.1, 135.6, 134.0, 131.3, 129.45, 129.4 (m), 127.5, 125.1, 60.9, 60.6, 35.1, 32.4, 26.9, 26.4, 23.6, 23.4, 19.3 ppm, appropriate signal to noise was not achieved for estimation of $J_{C,D}$; ^{2}H NMR (46 MHz, CH_2Cl_2) δ 5.15 ppm (b s); MS (EI, 70 eV) m/z 423 (7.8) $[M]^+$, 366 (6.0) $[M - C_4H_9]^+$, 336 (5.7), 256 (4.6), 229 (12.5), 199 (100), 167 (8.0), 150 (87.5), 149 (35.1), 122 (12.6), 107 (11.7), 94 (20.3), 93 (38.3). Anal. Calcd for $C_{27}H_{37}{}^2HO_2Si$: C, 76.54; H, 9.28. Found: C, 76.57; H, 9.40.

(2*Z*,6*E*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadienyl-9-(((4-methylphenyl)-sulfonyl)oxy)-1-ol ([6-^{2}H]1-OH,OTs). As described for unlabeled material, 84 mg (0.44 mmol) of *p*-toluenesulfonyl chloride and 58 mg (0.476 mmol) of 4-(*N*,*N*-dimethylamino)pyridine in 3.0 mL of CH_2Cl_2 were treated with 152 mg (0.359 mmol) of [6-^{2}H]1-OTBDPS,OH to yield 207 mg (100%) of a colorless oil. This material was immediately treated with 130 mg (0.413 mmol) of tetra-*n*-butylammonium fluoride trihydrate to yield 110 mg (90%) of a colorless oil: TLC (R_f 0.24; 3:7 hexanes/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (2, d, J = 8.30 Hz, Ph H), 7.34 (2, d, J = 8.30 Hz, Ph H), 5.43 (1, t, J = 7.20 Hz, H at C(2)), 4.07 (2, d, J = 7.20 Hz, H at C(1)), 4.04 (2, t, J = 6.79 Hz, H at C(8)), 2.45 (3, s, CH_3 at Ph), 2.30 (2, t, J = 6.79 Hz, H at C(8)), 2.06 (4, m), 1.73 (3, s, CH_3 at C(3)), 1.52 ppm (3, s, CH_3 at C(7)).

(*Z*,*Z*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadienyl-9-(((4-methylphenyl)-sulfonyl)oxy)-1-ol ([6-^{2}H]2-OH,OTs). As described for [6-^{2}H]1-OH,-OTs, 92 mg (0.48 mmol) of *p*-toluenesulfonyl chloride and 66 mg (0.54 mmol) of 4-(*N*,*N*-dimethylamino)pyridine were treated with 170 mg (0.40 mmol) of [6-^{2}H]2-OTBDPS,OH to give 230 mg (100%) of a colorless oil which was further treated with 130 mg (0.413 mmol) of tetra-*n*-butylammonium fluoride trihydrate to yield 100 mg (75%) of a colorless oil: TLC (R_f 0.24; 3:7 hexanes/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2, d, J = 8.51 Hz, Ph H), 7.30 (2, d, J = 8.51 Hz, Ph H), 5.41 (1, t, J = 7.10 Hz, H at C(2)), 4.06 (2, d, J = 7.10 Hz, H at C(1)), 4.00 (2, t, J = 7.13 Hz, H at C(8)), 2.40 (3, s, CH_3 at Ph), 2.33 (2, t, J = 7.13 Hz, H at C(8)), 2.02 (4, m), 1.68 (3, s, CH_3 at C(3)), 1.56 ppm (3, s, CH_3 at C(7)).

(2*Z*,6*E*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([6-^{2}H]1-OPP). As described for unlabeled material, 110 mg (0.32 mmol) of [6-^{2}H]1-OH,OTs was treated with 49 mg (0.386 mmol) of *N*-chlorosuccinimide and 35 μL (30 mg, 0.28 mmol) of dimethyl sulfide to afford 96 mg (84%) of a pale-yellow liquid. This material was mixed with 898 mg (0.94 mmol) of tris(tetra-*n*-butylammonium) hydrogen pyrophosphate to yield 79 mg (48%) of a white solid: TLC (R_f 0.4; 2.5:2.5:5 1-propanol/THF/0.1 M NH_4HCO_3); ^{1}H NMR (300 MHz, D_2O) δ 5.45 (1, t, J = 6.69 Hz, H at C(2)), 4.45 (2, dd, $J_{H,H}$ = 6.69 Hz, $J_{H,P}$ = 6.69 Hz, H at C(1)), 3.97 (2, dt, $J_{H,H}$ = 6.89 Hz, $J_{H,P}$ = 6.89 Hz, H at C(9)), 2.34 (2, t, J = 6.89 Hz, H at C(8)), 2.15 (4, m), 1.76 (3, s, CH_3 at C(3)), 1.65 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, D_2O) δ 145.7, 135.8, 128.8 (t, $J_{C,D}$ = 20.7 Hz), 123.4 (d, $J_{C,P}$ = 7.4 Hz), 67.3 (d, $J_{C,P}$ = 5.1 Hz), 65.1 (d, $J_{C,P}$ = 5.0 Hz), 42.7 (d, $J_{C,P}$ = 7.2 Hz), 34.1, 29.1, 25.7, 18.2 ppm; ^{2}H NMR (46 MHz, D_2O) δ 5.3 ppm (b s); ^{31}P NMR (121 MHz, D_2O) δ -11.37 (1, d, $J_{P,P}$ = 20.9 Hz), -11.39 (1, d, $J_{P,P}$ = 20.8 Hz), -13.27 (1, d, $J_{P,P}$ = 20.9 Hz), -13.38 ppm (1, d, $J_{P,P}$ = 20.8 Hz); FABMS (-Ve, glycerol) m/z 504 (100) $[M - 1]^-$, 406 (16.6) $[M - H_3PO_4]^-$, 388 (3.1), 177 (59.6).

(*Z*,*Z*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([6-^{2}H]2-OPP). Following the procedure described for [6-^{2}H]1-OPP, 100 mg (0.295 mmol) of [6-^{2}H]2-OH,OTs was converted to 61 mg (34%) of a fine white powder: TLC (R_f 0.4; 2.5:2.5:5 1-propanol/THF/0.1 M NH_4HCO_3); ^{1}H NMR (300 MHz, D_2O) δ 5.45 (1, d, J = 6.70 Hz, H at C(2)), 4.43 (2, dd, $J_{H,H}$ = 6.70 Hz, $J_{H,P}$ = 6.70 Hz, H at C(1)), 3.94 (2, dt, $J_{H,H}$ = 6.99 Hz, $J_{H,P}$ = 7.20 Hz, H at C(9)), 2.40 (2, t, J = 6.99 Hz, H at C(8)), 2.16 (4, m), 1.75 (3, s, CH_3 at C(3)), 1.72 ppm (3, s, CH_3 at C(7)); ^{13}C NMR (75 MHz, D_2O) δ 145.3, 135.9, 123.7 (d, $J_{C,P}$ = 8.04 Hz), 67.2 (d, $J_{C,P}$ = 5.69 Hz), 65.2 (d, $J_{C,P}$ = 4.99), 35.4 (d, $J_{C,P}$ = 6.71 Hz), 34.1, 28.6, 25.7, 25.5 ppm; an appropriate level of signal to noise was not achieved to assign the multiplet for the carbon bearing deuterium; ^{2}H NMR (46 MHz, D_2O) δ 5.30 ppm (b s); ^{31}P NMR (121 MHz, D_2O) δ -10.60 (1, d, $J_{P,P}$ = 21.33 Hz), -10.69 (1, d, $J_{P,P}$ = 21.18 Hz), -13.25 (1, d, $J_{P,P}$ = 21.33 Hz), -13.42 ppm (1, d, $J_{P,P}$ = 21.18 Hz); FABMS (-Ve, glycerol) m/z 504 (100) $[M - 1]^-$, 406 (2.0) $[M - H_3PO_4]^-$, 344 (3.3), 295 (4.1), 177 (12.2).

Product Studies. (2*Z*,6*E*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate (1-OPP). A solution of 0.8 mL of BHDA buffer (0.1 M, pH 7.0), 0.2 mL of $MgCl_2$ (0.1 M), 1.6 mL of deionized water, and 1.0 mL (94 mg, 0.155 mmol) of **1**-OPP was kept on ice until addition of 0.41 mL (0.74 mg, 2.6 units) of FPP synthase. The mixture was incubated at 37 °C for 9 h before addition of lysine hydrochloride buffer (1 mL, 0.2 M, pH 10.5) and 0.4 mL (0.92 mg, 55.2 units) of *E. coli* type III alkaline phosphatase. After an additional 13 h at 37 °C the mixture was diluted with 10 mL of water and extracted with two 30-mL portions of CH_2Cl_2. The organic extracts were dried over Na_2SO_4, and the solvent was carefully removed by rotary evaporation. The mixture was analyzed by GC on a 30 M DB-5 column (operating at 120 °C isothermally for 12 min followed by a 10 °C min^{-1} ramp to 200 °C) to estimate the product ratio and amount of residual uncyclized material. Under these conditions, 3% of the uncyclized diol was recovered. Cyclized products were further analyzed by GCMS, and retention times of the products were established by conjection with authentic materials.

Residual diol **1**-OH was removed by chromatography on a silica gel column (1.5 × 7 cm; 6:4 hexanes/ethyl ether). To 1 mL of CH_2Cl_2 were added 39 mg (0.229 mmol) of 2-naphthoic acid, 28 mg (0.229 mmol) of 4-(*N*,*N*-dimethylamino)pyridine, and 36 mg (0.229 mmol) of 4-(*N*,*N*-dimethylamino)pyridine hydrochloride. To this solution was added 47 mg (0.229 mmol) of 1,3-dicyclohexylcarbodiimide, and the heterogeneous mixture was stirred for 1.5 h before the mixture of alcohols (from above) dissolved in 3 mL of CH_2Cl_2 was added. After 15 h, solvent was removed by a stream of nitrogen, and the residue was extracted with hexanes and filtered. The products were purified by flash chromatography (95:5 hexanes/diethyl ether) to yield 33 mg (67% from the bisdiphosphate) of a colorless oil. Each component was separated by preparative HPLC on a Rainin silica gel column (98.5:1.5 hexane/*tert*-butyl methyl ether). The esters were dried for 48 h (0.01 mmHg) in 5-mL volumetric flasks. NMR spectra were recorded in $CDCl_3$. The samples were passed through silica gel, dried under vacuum again, and redissolved in acetonitrile for ORD and UV measurements.

(+)-**8**-ONp: $[\alpha]^{22}_D$ +7.46°, $[\alpha]^{22}_{546}$ +9.7°, $[\alpha]^{22}_{436}$ +29.1°, $[\alpha]^{22}_{365}$ +75° (*c* 0.268, CH_3CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (1, b s, naphthyl H), 8.09 (1, m, naphthyl H), 7.95 (1, m, naphthyl H), 7.88 (2, m, naphthyl H), 7.57 (2, m, naphthyl H), 5.53 (1, b t, J = 6.83 Hz, H at C(2)), 5.42 (1, m, H at C(3′)), 4.95 (1, dd, J = 6.83 Hz, J = 12.58 Hz, H_a at C(1)), 4.89 (1, dd, J = 6.83 Hz, J = 12.58 Hz, H_b at C(1)), 2.80 (1, m, H at C(10′)), 2.18–1.82 (4, m), 1.75 (3, s), 1.67 (3, s), 1.66–1.59 ppm (2, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 146.8, 135.3, 133.8, 132.4, 130.9, 129.2, 128.0, 127.97, 127.7, 127.6, 126.5, 125.2, 120.4, 118.8, 61.2, 35.9, 30.5, 29.9, 27.5, 23.6, 19.5 ppm; MS (EI, 70 eV) m/z 172 (27.8) [McLafferty rearrangement], 155 (96.4), 148 (100), 133 (46.5), 127 (42.6), 119 (12.3), 106 (34.2), 93 (42.1), 81 (32.2).

Careful inspection of the minor product (7% total), initially thought to be **9**-ONp, indicated that it was a mixture. The ^{1}H NMR spectra (400 MHz, $CDCl_3$) of both components were different from that of **9**-ONp. Control experiments from incubations which contained no enzyme indicated that alcohol components with the same retention times (GC) were formed.

(+)-**10**-ONp: $[\alpha]^{22}_D$ +111.8°, $[\alpha]^{22}_{546}$ +120.6°, $[\alpha]^{22}_{436}$ +279.6°, $[\alpha]^{22}_{365}$ +564.7° (*c* 0.0912, CH_3CN); UV (CH_3CN) λ_{max} 235 (ϵ 62300), 268 (18790), 278 (18000), difference of *Z* isomer λ_{max} 266 nm (ϵ 5500); IR (film) 2958, 2925, 1717, 1286, 1228, 1196, 1130, 1095, 1014, 762 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (1, b s, naphthyl H), 8.06 (1, m, naphthyl H), 7.96 (1, m, naphthyl H), 7.88 (2, m, naphthyl H), 7.56 (2, m, naphthyl H), 5.67 (1, d, J = 5.42 Hz, H at C(2′)), 5.62 (1, m, H at C(3′)), 4.39 (1, dt, J = 7.02 Hz, J = 11.03 Hz, H_a at C(1)), 4.31 (1, dt, J = 7.02 Hz, J = 11.03 Hz, H_b at C(1)), 2.45 (1, m, H at C(3)), 2.12 (4, m), 1.93 (1, m), 1.85 (1, m), 1.78 (3, s, CH_3 at C(4′)), 1.13 ppm (3, d, J = 6.83 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 139.1, 135.4, 133.8, 132.4, 130.9, 129.3, 128.1, 128.0, 127.7, 127.6, 126.5, 125.2, 119.3, 119.0, 63.9, 37.6, 33.7, 28.9, 24.0, 23.0, 19.7 ppm; MS (EI, 70 eV) m/z 320 (19.3) $[M]^+$, 172 (9.9) [McLafferty rearrangement], 155 (32.7), 148 (45.8), 133 (100), 127 (40.0), 119 (28.5), 105 (32.7), 93 (12.3), 91 (21.6), 77 (11.5). HRMS. Calcd for $C_{22}H_{24}O_2$: 320.1770. Found: 320.1778.

(*Z*,*Z*)-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate (2-OPP). Using the protocol described for **1**-OPP, 0.5 mL (0.062 mmol) of **1**-OPP was treated with 0.04 mL (0.4 mg, 1.2 units) of FPP synthase. After analysis by GCMS, the recovered alcohol products were converted to the corresponding naphthoate esters and separated by HPLC.

(−)-**8**-ONp: $[\alpha]^{22}_{436}$ -27.4°, $[\alpha]^{22}_{365}$ -75.3° (*c* 0.073, CH_3CN); ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectra were identical to those for the (+)-**8**-ONp isolated from the **1**-OPP reaction.

(+)-**9**-ONp: $[\alpha]^{22}_D$ +50.9°, $[\alpha]^{22}_{546}$ +63.7°, $[\alpha]^{22}_{436}$ +108.3°, $[\alpha]^{22}_{365}$ +159° (*c* 0.031, CH_3CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (1, b s, naphthyl H), 8.09 (1, m, naphthyl H), 7.93 (1, m, naphthyl H), 7.88 (2, m, naphthyl H), 7.59 (2, m, naphthyl H), 5.56 (1, b t, J = 6.98 Hz, H at C(2)), 5.42 (1, m, H at C(3′)), 4.94 (2, d, J = 6.98 Hz, H at C(1)), 2.23–1.91 (5, m), 1.82 (1, m), 1.80 (3, s), 1.66 (3, s), 1.55 ppm (1, m);

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 146.2, 135.4, 133.7, 132.4, 130.9, 129.2, 128.1, 128.0, 127.9, 127.7, 126.5, 125.2, 120.4, 117.1, 62.2, 42.8, 30.6, 27.7, 23.6, 14.9 ppm.

(-)-**10**-ONp: $[\alpha]^{22}_{436}$ -240.4°, $[\alpha]^{22}_{365}$ -519.2° (*c* 0.014, CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) spectra were identical to those stated for the (+)-**10**-ONp isolated from the **1**-OPP reaction.

(2*Z*,6*E*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([6-^{2}H]1-OPP). In a 5-mL reaction vial were combined 0.6 mL of BHDA buffer (0.1 M, pH 7.0), 0.15 mL of 0.1 M $MgCl_2$, 1.4 mL of water, and 0.77 mL (0.04 mmol) of [6-^{2}H]**1**-OPP. The mixture was kept on ice until the addition of 0.095 mL (0.828 mg, 2.4 units) of FPP synthase. The mixture was incubated at 37 °C for 9.5 h. Lysine hydrochloride buffer (1.0 mL, 0.2 M, pH 10.5) was added, followed by 0.4 mL (0.92 mg, 55 units) of *E. coli* Type III alkaline phosphatase. After an additional 13 h at 37 °C, the organic soluble material was extracted with two 30-mL portions of CH_2Cl_2 and dried over Na_2SO_4. Rotary evaporation was used to concentrate the sample to 3 mL, and the composition of the mixture was analyzed by GC (30 M DB-5, 120 °C for 12 min followed by a 10 °C min^{-1} ramp to 200 °C). Uncyclized material was estimated to be 3% of the mixture and was removed by passage through silica gel with elution by CH_2Cl_2. The deuterium content of each component was analyzed by GCMS.

[^{2}H]**8**-OH: MS (EI, 70 eV) *m/z* 167 (3.1) $[M]^+$, 149 (75.5) $[M - H_2O]^+$, 148 (24.6), 134 (75.5), 121 (9.9), 120 (17.0), 107 (25.5), 106 (72.6), 95 (11.3), 94 (77.4), 93 (84.8), 92 (71.7), 91 (23.1), 84 (46.8), 81 (100).

[^{2}H]**10**-OH: MS (EI, 70 eV) *m/z* 167 (47.7) $[M]^+$, 166 (6.7), 134 (5.6), 122 (100), 121 (47.3), 120 (50.9), 106 (52.7), 94 (35.0), 93 (80.0), 92 (50.9), 91 (42.3), 84 (14.1).

The alcohols were esterified as described for the unlabeled materials, and the mixture of naphthoates was purified by flash chromatography to yield 6.8 mg (53% from bisdiphosphate) of a colorless oil. Products were separated by HPLC as described above.

[1′-^{2}H]**8**-ONp: ^1H NMR (300 MHz, $CDCl_3$) δ 8.64 (1, b s, naphthyl H), 8.09 (1, m, naphthyl H), 7.95 (1, m, naphthyl H), 7.88 (2, m, naphthyl H), 7.57 (2, m, naphthyl H), 5.53 (1, b t, *J* = 7.30 Hz, H at C(2)), 5.42 (1, b s, H at G(3′)), 4.95 (1, dd, *J* = 7.30 Hz, *J* = 12.60 Hz, H_a at C(1)), 4.89 (1, dd, *J* = 7.30 Hz, *J* = 12.60 Hz, H_b at C(1)), 2.20-1.80 (4, m), 1.75 (3, s), 1.66 (3, s), 1.66-1.48 ppm (2, m).

[3-^{2}H]**10**-ONp: ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (1, b s, naphthyl H), 8.06 (1, m, naphthyl H), 7.95 (1, m, naphthyl H), 7.88 (2, m, naphthyl H), 7.56 (2, m, naphthyl H), 5.67 (1, d, *J* = 5.42 Hz, H at C(2)), 5.61 (1, m, H at C(3′)), 4.39 (1, dt, *J* = 11.03 Hz, *J* = 7.07 Hz, H_a at C(1)), 4.32 (1, dt, *J* = 11.03 Hz, *J* = 7.07 Hz, H_b at C(1)), 2.12 (4, m), 1.93 (1, dt, *J* = 14.10 Hz, *J* = 7.07 Hz, H_a at C(2)), 1.82 (1, dt, *J* = 14.10 Hz, *J* = 7.07 Hz, H_b at C(2)), 1.77 (3, s), 1.12 ppm (3, s).

(*Z*,*Z*)-[6-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ([6-^{2}H]2-OPP). As described for [6-^{2}H]**1**-OPP, a mixture of 0.6 mL of BHDA buffer (0.1 M, pH 7.0), 0.15 mL of 0.1 M $MgCl_2$, 1.37 mL of water, and 0.8 mL (0.04 mmol) of [6-^{2}H]**2**-OPP was treated with FPP synthase. After alkaline phosphatase hydrolysis, the alcohols were analyzed by GCMS. Following the procedure described above, the alcohols were converted to the naphthoate esters and separated by HPLC.

[1′-^{2}H]**8**-OH: (EI, 70 eV) *m/z* 167 (2.1) $[M]^+$, 149 (6.5) $[M - H_2O]^+$, 134 (46.0), 121 (7.4), 120 (11.0), 119 (5.2), 108 (12.5), 107 (32.0), 106 (26.5), 105 (11.5), 98 (14.7), 94 (40.0), 93 (41.0), 92 (32.0), 91 (18.0), 84 (50.0).

[1′-^{2}H]**9**-OH: (EI, 70 eV) *m/z* 167 (10.3) $[M]^+$, 149 (28.9) $[M - H_2O]^+$, 136 (12.0), 134 (14.9), 125 (4.8), 122 (6.2), 120 (8.0), 108 (16.5), 107 (16.1), 94 (36.6), 93 (41.3), 92 (28.7), 91 (12.0), 79 (30.0), 69 (100).

[3-^{2}H]**10**-OH: (EI, 70 eV) *m/z* 167 (43.9) $[M]^+$, 166 (2.7), 134 (11.9), 133 (11.1), 122 (100), 106 (43.4), 94 (40.8), 93 (65.2), 92 (69.3), 91 (34.7), 84 (51.0).

[1′-^{2}H]**8**-ONp: ^1H NMR (300 MHz, $CDCl_3$) all resonances were identical to those found for the material isolated from the [6-^{2}H]**1**-OPP reaction.

[1′-^{2}H]**9**-ONp: ^1H NMR (300 MHz, $CDCl_3$) δ 8.62 (1, b s, naphthyl H), 8.08 (1, m, naphthyl H), 7.96 (1, m, naphthyl H), 7.87 (2, m, naphthyl H), 7.56 (2, m, naphthyl H), 5.55 (1, b t, *J* = 6.93 Hz, H at C(2)), 5.30 (1, m, H at C(3′)), 4.93 (2, d, *J* = 6.93 Hz, H at C(1)), 2.12-1.90 (4, m), 1.81 (1, m), 1.78 (3, s), 1.66 (3, s), 1.53 ppm (1, m).

[3-^{2}H]**10**-ONp: An insufficient amount of material was obtained for NMR analysis at 300 MHz.

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Farnesyl-Diphosphate Synthase. Interplay between Substrate Topology, Stereochemistry, and Regiochemistry in Electrophilic Alkylations

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Abstract: The absolute stereochemistries of **8**-OPP, **9**-OPP, and **10**-OPP obtained from incubation of bisubstrate analogs **1**-OPP and **2**-OPP with farnesyl-diphosphate synthase were determined by correlation of ORD spectra with those of synthetic materials. Only one enantiomer was found for each of the enzymatic products. The products from **1**-OPP were (*S*)-**8**-OPP and (*S*)-**10**-OPP, while those from **2**-OPP were (*R*)-**8**-OPP, (*R*)-**9**-OPP, and (*R*)-**10**-OPP. Conformational analysis of **1**-OPP and **2**-OPP, which considers topological limitations imposed by FPP synthase, indicates that the products from the enzymatic reactions are formed from discrete E·S complexes formed from different conformers of the substrates. Overlays of the conformations that give the observed products are consistent with a model where the hydrocarbon moieties occupy a central volume flanked on top and bottom by their diphosphate residues.

Introduction

The fine-tuning of enzymes under the pressure of natural selection has resulted in efficient catalysts that impart high regioselectivity and stereoselectivity to the reactions they promote. This phenomenon is amply illustrated by the isoprenoid biosynthetic pathway with its vast array of over 20 000 metabolites. Much of the structural diversity in isoprene metabolism lies in the ability of a family of enzymes, each acting at the same branch point, to convert a common simple achiral substrate into a unique product. For example, there are at least six prenyltransferases in man that catalyze condensations with farnesyl diphosphate (FPP) to produce precursors for the biosynthesis of sterols,[1]

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