^{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

V. Jo Davisson† and C. Dale Poulter*

Contribution from the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112.
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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 20000 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]

* Address correspondence to this author.
† Present address: Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Science, Purdue University, West Lafayette, IN 47907.

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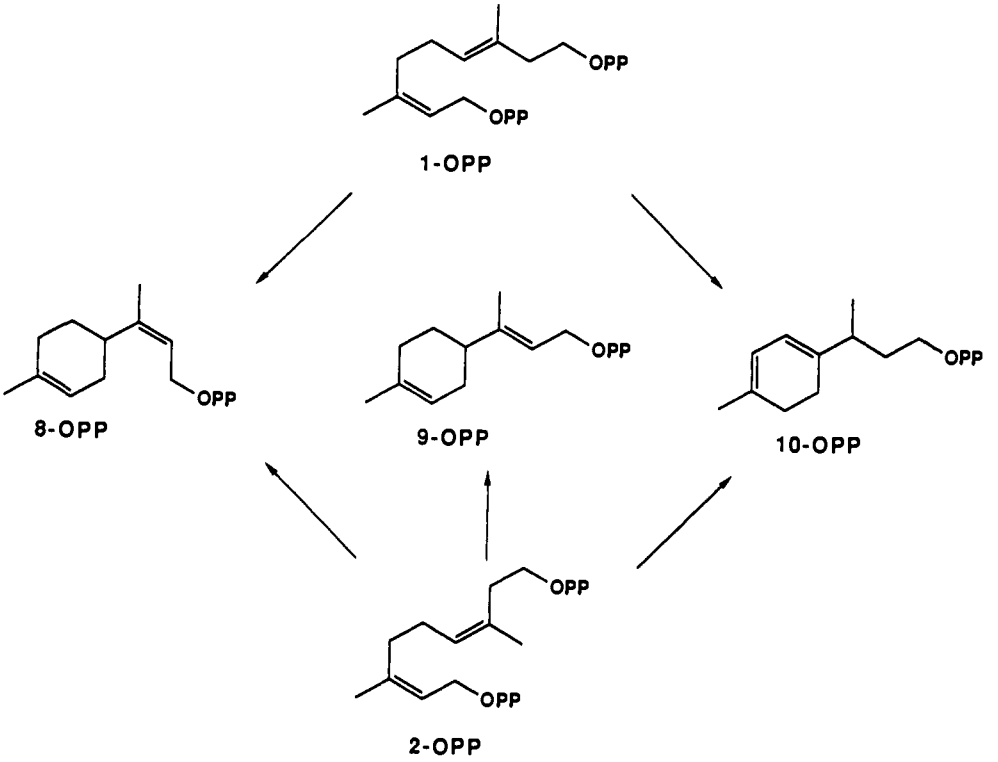
Scheme I. Products from Incubation of Bisubstrate Analogs **1**-OPP and **2**-OPP with Avian Farnesyl-Diphosphate Synthase



dolichols,[2] ubiquinone,[3] heme a,[4] and prenylated proteins.[5] Even more dramatic illustrations of product diversity from single precursors are found in the mono- and sesquiterpene pathways in higher plants, where the essential oils of some species contain an incredible array of isoprenoids derived from either geranyl diphosphate (GPP) or FPP.[6,7] The carbon skeleta of these compounds are produced by cyclases that catalyze branch point reactions, and it is common for an essential oil to contain several different structural classes of terpenes, including both optical antipodes.

Carbocations typical of those generated by enzymes in isoprenoid metabolism are notoriously unselective in their reactions.[1,8] In theory, an enzyme could capitalize on this low selectivity to generate a variety of structures from a single enzyme–intermediate (E·I) complex. However, as more mono- and sesquiterpene cyclases are purified and characterized, it has become evident that the individual enzymes are selective, both regio- and stereochemically.[7,9,10] While some minor components of essential oils appear to be "by-products" of reactions that produce major metabolites, most components in the complex mixtures appear to arise selectively from individual enzyme-catalyzed transformations. Thus, a family of branch point enzymes has evolved, each of which is responsible for producing a specific metabolite.

During work with bisubstrate analogs for isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), we discovered that FPP synthase catalyzed the cyclizations of **1**-OPP and **2**-OPP shown in Scheme I.[11] Diphosphates **8**-OPP and **9**-OPP[12] were formed in transformations analogous to those for the normal substrate, but **10**-OPP was the product of a novel 1,2-rearrangement of a hydrogen atom. Although similar 1,2-rearrangements are common in mono- and sesquiterpene biosynthesis, they had not been previously detected in reactions catalyzed by prenyltransferases. The mixture of products from **1**-OPP and **2**-OPP could result from partitioning of a common cationic intermediate between rearranged and unrearranged products, as is typically assumed for related non-enzymatic reactions, or each could be formed selectively via individual reaction paths for specific E·S complexes of FPP synthase and different conformers of **1**-OPP and **2**-OPP. We now describe stereochemical studies which show that the products from bisubstrate analogs **1**-OPP and **2**-OPP are formed from unique enzyme-bound conformations.

Results

Absolute Configurations of 8-OPP, 9-OPP, and 10-OPP. In the preceding paper, we reported that the cyclic diphosphates produced from **1**-OPP and **2**-OPP by FPP synthase were optically active.[11] Absolute configurations and optical purities of the compounds are now established by comparisons of ORD spectra for naphthoates **8**-ONp, **9**-ONp, and **10**-ONp derived from the enzymatic products with those of authentic samples.

Naphthoates **8**-ONp and **9**-ONp of known absolute stereochemistry were synthesized from cyclohexenyl esters **28** and **29**, which can be prepared from limonene (**18**), as reported by Delay and Ohloff[13] (see Scheme II). Reduction of **28** and **29** with DIBAL followed by conversion of the resulting alcohols to **8**-ONp and **9**-ONp gave optically active materials for comparison with the enzymatic products. Upper limits for the optical purities of the synthetic naphthoates were established by conversion of (*R*)- and (*S*)-limonene to α-terpineol (**30**-OH) by oxymercuration[14] and analysis of the corresponding (methoxytrifluorophenyl)acetyl (MTPA) esters by ^{19}F NMR spectroscopy. The ^{19}F spectrum of

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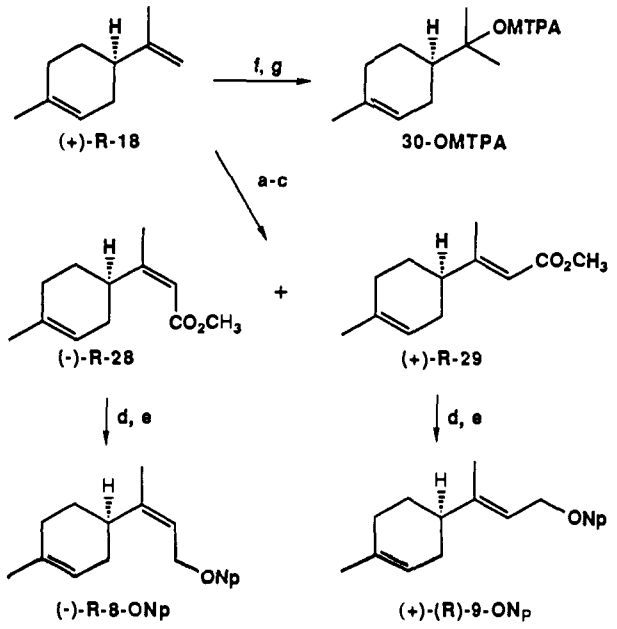
Scheme II. Synthesis of (*R*)-**8**-ONp and (*R*)-**9**-ONp from (*R*)-Limonene



(a) *m*-CPBA/O_3; (b) Zn/KI/AcOH; (c) $(CH_3O)_2POCH_2CO_2CH_3$/NaH; (d) DIBAL/0 °C; (e) DCC/DMAP/2-naphthoic acid; (f) $Hg(OAc)_2/NaBH_4$; (g) (*S*)-MTPA chloride/DMAP/1,2-dichloroethane/70 °C.

(*S*,*S*)-**30**-OMTPA had peaks at 3.73 and 3.68 ppm in a relative ratio of 98:2. We assign these resonances to the *S*,*S* and *R*,*S* diastereomers, respectively, and their intensities reflect the optical purity of the sample of (*S*)-limonene used in the synthesis of (*R*)-**8**-ONp. A ^{19}F spectrum of (*R*,*S*)-**30**-OMTPA also had resonances at 3.73 and 3.68 ppm; however, the ratio of intensities was 7:93, respectively.

Our synthesis of (*R*)-**10**-ONp is outlined in Scheme III. Lithium ammonia reduction of *p*-tolylbutanoic acid **31** gave 1,4-cyclohexadiene **32**. The 1,4-diene was isomerized to **33** by treatment with potassium *tert*-butoxide in DMSO/toluene according to the procedure of Taveres and Katten[15] to give a 7:3 mixture of the 1,3 and 1,4 isomers. The isomerization was run under argon with degassed solutions to prevent **33** from oxidizing back to **31**. The 1,3 and 1,4 isomers were separated as methyl esters on silver nitrate impregnated silica gel plates. 1,3-Dienyl ester **33** was hydrolyzed to the corresponding acid and then treated with ((*R*)-naphthylethyl)amine and DCC to give a mixture of (*R*,*R*)-**34** and (*S*,*R*)-**34**. The diastereomeric amides were separated by HPLC before methylation of (*R*,*R*)-**34**, followed by reduction with the butyllithium "ate" complex of diisobutylaluminum hydride to yield enantiomerically pure (*R*)-**10**-OH.[16] Esterification of the alcohol with 2-naphthoic acid gave (*R*)-**10**-ONp for comparison with samples from the enzyme-catalyzed reactions.

The absolute stereochemistry of (−)-(*R*)-**10**-ONp was established by optical correlation with aromatic ester (−)-(*R*)-**36**-ONp of known absolute configuration and optical purity. Amides (*R*,*R*)- and (*S*,*R*)-**35** are known compounds, and their absolute stereochemistries have been correlated with that of (S)-(+)-β-hydroxybutyric acid derived from yeast reduction of ethyl acetoacetate.[17] (−)-(*R*)-**36**-OH was obtained from (*R*,*R*)-**35**, as described for (*R*)-**10**-OH, and its rotation was correlated with the (+)-(*S*)-enantiomer derived from degradation of naturally occurring α-turmerone.[18,19] Both correlations indicate that (−)-**10**-OH is the (*R*)-antipode. The absolute configurations of 1,3-cyclohexadienyl alcohol (−)-(*R*)-**10**-OH and the corresponding ester (−)-(*R*)-**10**-ONp were assigned by oxidation of (−)-(*R*)-**10**-ONp to (−)-(*R*)-**36**-ONp.

The absolute configurations and enantiomeric excesses for the products of enzyme-catalyzed cyclizations of **1**-OPP and **2**-OPP are summarized in Scheme IV. The cyclic diphosphates from incubation of the analogs with FPP synthase were hydrolyzed with alkaline phosphatase, esterified, and purified as naphthoate esters.[11] Samples for optical rotations were weighed on a microbalance, and the concentrations of the solutions were calculated in the usual manner. The concentrations determined from the weighed samples agreed with those calculated from absorbance measurements based upon extinction coefficients for the synthetic materials. Within the accuracy of our determinations, diphosphates **8**-OPP, **9**-OPP, and **10**-OPP from enzyme-catalyzed cyclization were single enantiomers.

Stereochemistry at C(1) during Cyclization of 1-OPP and 2-OPP. Synthesis of (*S*)-[1-^{2}H]1-OPP and (*S*)-[1-^{2}H]2-OPP. Syntheses for (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP are similar to those described for unlabeled materials and rely on a Wittig condensation with aldehyde **5**-OTBDPS to construct the carbon chain. C(1) in (*S*)-[1-^{2}H]**5**-OTBDPS was labeled with deuterium as illustrated in Scheme V. Acetylenic ester **38**-OEE was obtained from **37**-OH in a straightforward manner and treated with lithium dimethyl cuprate to give the α,β-unsaturated ester **39**-OEE.

Deuterium was introduced at C(1) by reduction with $LiAl^2H_4$. The ensuing oxidation with MnO_2[20] was sluggish because of a substantial primary kinetic isotope effect and required overnight to proceed to completion. In comparison, oxidation of unlabeled **40**-OH,OEE took approximately 30 min. The slow oxidation of the deuterated alcohol was accompanied by some isomerization of the C(2)–C(3) double bond. The mixture was treated with (*R*)-1-β-isopinocampheyl-9-borabicyclo[3.3.1]nonane to give (*S*)-[1-^{2}H]**40**-OH,OEE. The stereochemistry for this reduction was established by Midland and Parry for related compounds.[21-23] The alcohol was purified by chromatography to remove unwanted *E* isomer introduced during the oxidation–reduction sequence.

The optical purity of (*S*)-[1-^{2}H]**40**-OH,OEE was determined by conversion to the corresponding (*S*)-O-acetyl mandelate (AMA) ester and removal of the ethoxyethyl moiety. In an unlabeled sample, the *pro*-*R* and *pro*-*S* protons at C(1) resonated at 4.44 and 4.67 ppm, respectively. Integration of the corresponding peaks in the ^1H NMR spectrum of (*S*)-[1-^{2}H]**40**-OAMA,OH indicated the material was a 91:9 mixture of (*S*,*S*)- and (*R*,*S*)-diastereomers. (*S*)-[1-^{2}H]**40**-OH,OEE was protected with *tert*-butyldiphenylsilyl chloride, the ethoxyethyl moiety was removed with dilute hydrochloric acid, and the resulting alcohol was oxidized to give (*S*)-[1-^{2}H]**5**-OTBDPS.

The final steps in the conversion of (*S*)-[1-^{2}H]**5**-OTBDPS to (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP proceeded as previously described for the unlabeled derivatives.[11] The downfield region of the ^1H NMR spectrum for (*S*)-[1-^{2}H]**2**-OPP had a one-proton, three-line pattern at 4.41 ppm for the hydrogen at C(1) and a doublet at 5.44 ppm for the hydrogen at C(2). Similar features were seen in the NMR spectrum of (*S*)-[1-^{2}H]**1**-OPP. ^{13}C NMR spectra of the labeled diphosphates had multiplets centered at 64.6 and 64.5 ppm, respectively, for the C(1) methylenes. These resonances appeared as six-line patterns, consistent with couplings to ^{31}P and ^2H. The negative-ion FAB mass spectra of the analogs had the molecular ions expected at m/z 504.

Cyclization and ^1H NMR Analysis of Products. Labeled bis-diphosphates (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP were incubated with avian liver FPP synthase, as described in the paper

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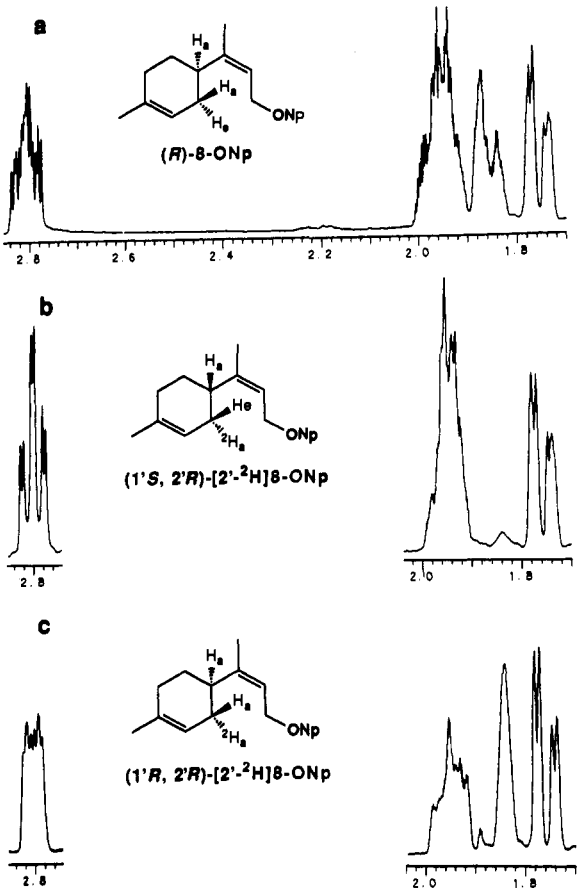


Figure 1. ^{1}H NMR (500-MHz) spectra in C_6D_6 of the region from 1.39 to 2.83 ppm for (a) (*R*)-**8**-ONp; (b) (1′*S*,2′*R*)-[2′-^{2}H]**8**-ONp from cyclization of (*S*)-[1-^{2}H]**1**-OPP; and (c) (1′*R*,2′*R*)-[2′-^{2}H]**8**-ONp from cyclization of (*S*)-[1-^{2}H]**2**-OPP.

preceding this one. The products were hydrolyzed with alkaline phosphatase, and the resulting alcohols were esterified with 2-naphthoic acid. GCMS spectra of the cyclic alcohols had molecular ions at m/z 167, demonstrating that deuterium was retained in these products.

The location of the labels in **8**-OH and **9**-OH was deduced from ^{1}H NMR spectra of the corresponding naphthoates. Figure 1 shows the region between 1.4 and 3.0 ppm for unlabeled **8**-ONp (part a) and the analogous deuterium-labeled naphthoates from (*S*)-[1-^{2}H]**1**-OPP (part b) and (*S*)-[1-^{2}H]**2**-OPP (part c), respectively. The four allylic hydrogens in the cyclohexane ring at C(2′) and C(6′) in unlabeled **8**-ONp appeared as a complex set of peaks between 1.7 and 2.0 ppm, while the allylic hydrogen at C(1′) gave a multiplet at 2.8 ppm. In the spectrum shown in part b, the asymmetric doublet at approximately 1.84 ppm was missing, and the multiplet at 2.8 ppm was a well-defined triplet of doublets. Similarly, in part c, the complex signal centered at 1.95 ppm had lost considerable intensity, the asymmetrical doublet at 1.84 ppm became a broad singlet, and the multiplet at 2.88 ppm had simplified to a closely spaced doublet of triplets. These changes demonstrate that the deuterium resides at C(2′). Similar data are shown in Figure 2 for unlabeled **9**-ONp and labeled **9**-ONp from (*S*)-[1-^{2}H]**2**-OPP. The major differences between spectra for labeled and unlabeled materials are the absence of a broad doublet centered at about 1.97 ppm and simplification of multiplets at 1.88 and 2.02 ppm upon deuteration. The pattern at 2.02 ppm from the hydrogen at C(1′) became a triplet of doublets. These comparisons clearly show that the deuterium in **9**-ONp is also at C(2′).

The location of deuterium in **10**-ONp was deduced from the ^{1}H NMR spectra shown in Figure 3. Part a gives the olefinic region of unlabeled **10**-ONp. The hydrogens at C(2′) and C(3′) appeared as a closely spaced AB system. The doublet at 5.67 ppm was broadened by long-range coupling to allylic hydrogens at

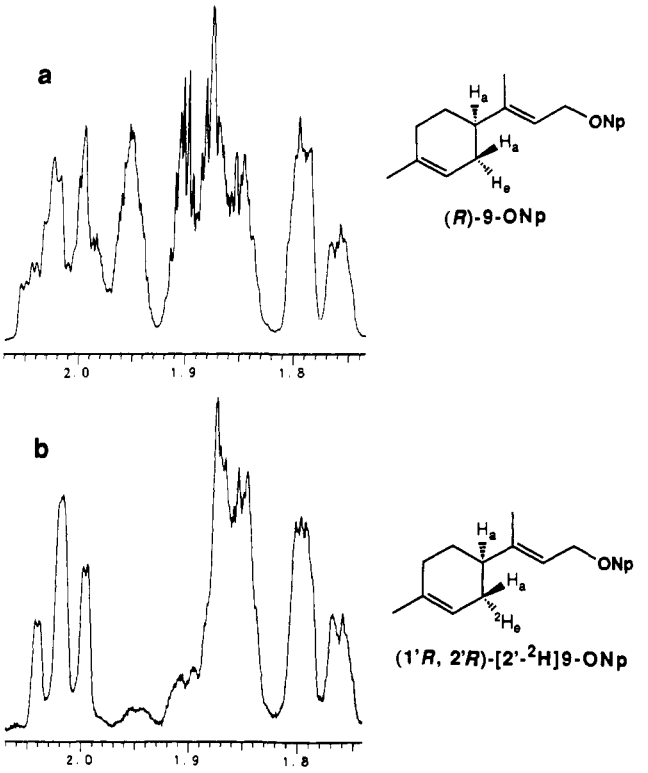


Figure 2. ^{1}H NMR (500-MHz) spectra in C_6D_6 of the region from 2.07 to 1.38 ppm for (a) (*R*)-**9**-ONp and (b) (1′*R*,2′*R*)-[2′-^{2}H]**9**-ONp from cyclization of (*S*)-[1-^{2}H]**2**-OPP.

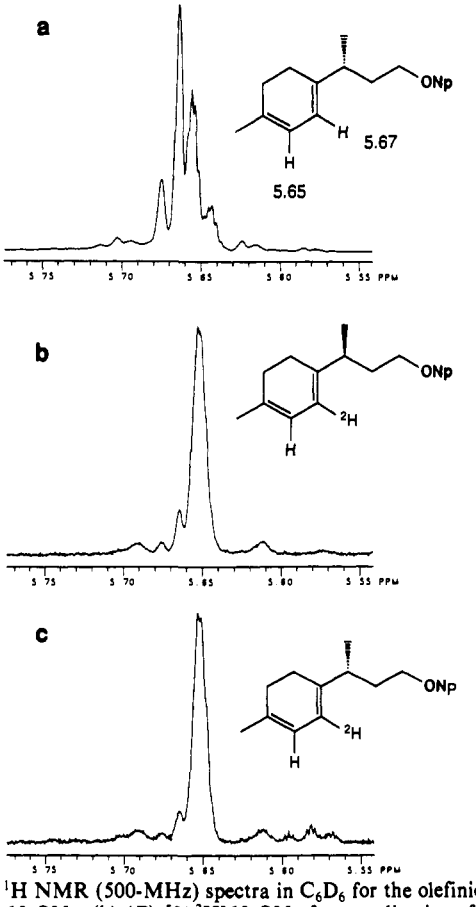
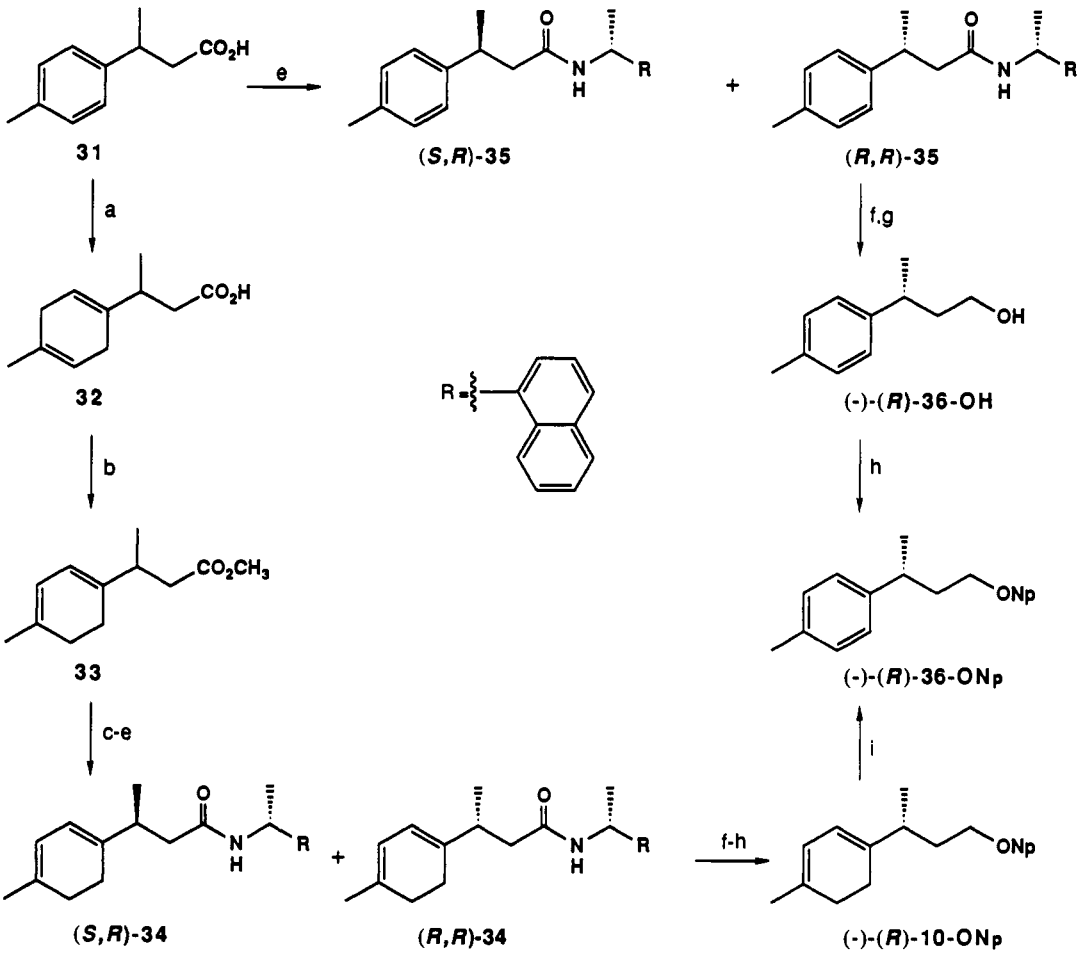


Figure 3. ^{1}H NMR (500-MHz) spectra in C_6D_6 for the olefinic region of (a) (*R*)-**10**-ONp; (b) (*R*)-[2′-^{2}H]**10**-ONp from cyclization of (*S*)-[1-^{2}H]**2**-OPP; and (c) (*S*)-[2′-^{2}H]**10**-ONp from cyclization of (*S*)-[1-^{2}H]-**1**-OPP.

C(6′), while the doublet at 5.65 ppm was further broadened into an unresolved multiplet due to long-range coupling to the methyl

Scheme III. Synthesis of (*R*)-**10**-ONp



(a) $Li/NH_3/THF$; (b) tBuOK/DMSO; (c) CH_2N_2; (d) NaOH/THF; (e) DCC/DMAP-(*R*)-1-(1-naphthylethyl)amine; (f) [i] $KH/CH_3I/THF$; (g) $nBuLi/DIBAL/NaBH_4$; (h) DCC/DMAP/2-naphthoic acid; (i) *m*-CPBA/Na_2CO_3/−30 °C.

at C(4′). The olefinic regions of **10**-ONp from both bisubstrate analogs (parts b and c) are identical. The broad doublet centered at 5.67 ppm was absent in the deuterated sample, and the broad doublet at 5.65 ppm became a broad singlet. Analysis of NMR and mass spectra indicated that >98% of the deuterium at C(2′) was retained upon elimination, to give the 1,3-diene.

The preferred conformations of dienes **8**-ONp and **9**-ONp, including absolute stereochemistry, and chemical shift assignments for all of the hydrogens on the cyclohexane rings are shown in Figure 4. The absolute configuration at C(2′) in deuterium-labeled **8**-ONp and **9**-ONp was determined by relating the stereochemistry of that center to the stereochemistry at C(1′). The absolute configuration of the latter was established by synthesis as described above. To determine the relative configuration of the chiral centers at C(1′) and C(2′), we assigned chemical shifts for the hydrogens in the cyclohexene ring and determined the conformation of the ring. In general, measurements of chemical shifts and coupling constants in cyclohexene rings are difficult because of second-order effects in the ^1H NMR spectra.[24] For our compounds, the chemical shifts of the allylic hydrogens at C(2′) are similar to those of the C(1′) methine and C(5′) methylene protons. An additional level of complexity is introduced by allylic and homoallylic couplings between protons at C(1′) and C(2′) and those of the methyl group. We were, however, able to make assignments and determine coupling constants for the ring protons in **8**-ONp and **9**-ONp with a combination of $^1H/^{13}C$ heteronuclear correlation (HETCOR), double quantum filtered correlation (DQCOSY), and homocorrelation 2D-J (HOM2DJ) experiments. The data are summarized in Table I.

A $^1H/^{13}C$ HETCOR spectrum[25] of **8**-ONp taken in C_6D_6 indicated that the allylic methylene carbons at 30.2 and 30.6 ppm bore hydrogens that resonate between 1.7–2.0 ppm. The nonallylic carbon at 28.0 ppm C(6′) correlated with the ^1H spin system for two protons centered at 1.45 ppm. The downfield signal at 2.81 ppm correlated with the ^{13}C resonances at 36.2 ppm for the tertiary carbon at C(1′).

A DQCOSY spectrum[26,27] of **8**-ONp at 500 MHz reveals several important features. An expansion of the upfield region showed that the ^1H resonances at 1.85 and 1.75 ppm were not strongly spin coupled and, thus, resided on separate allylic carbons. Both resonances gave crosspeaks to the two-proton multiplet centered at 1.94 ppm. The resonance at 1.85 ppm was assigned to C(2′) by its coupling to the C(1′) proton at 2.81 ppm, while the resonance at 1.75 ppm was assigned to the hydrogen at C(5′) by correlation to the multiplet at 1.44 ppm for the C(6′) hydrogens. A crucial observation was the strong crosspeak between the C(3′) vinyl proton at 5.36 ppm and the C(2′) hydrogen at 1.85 ppm and a weak crosspeak to the resonance at 1.95 ppm. Single-frequency ^1H decoupling experiments confirmed the assignments discussed above.

Signals for individual protons in the cyclohexene ring of **8**-ONp were assigned from HOMO2DJ spectra with and without single-frequency decoupling.[28,29] The resonances for the hydrogens

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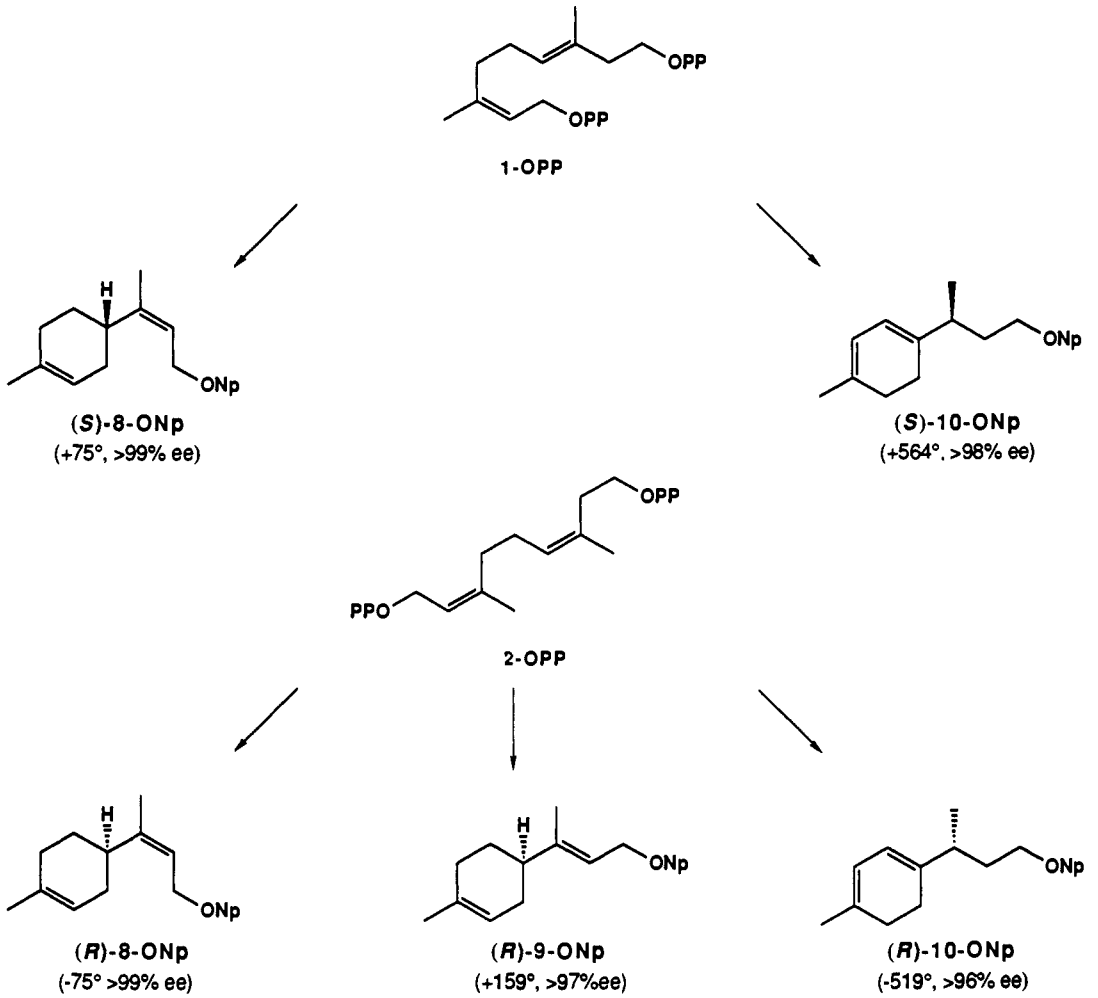
Scheme IV. Absolute Stereochemistries and Enantiomeric Excess for Cyclization of **1**-OPP and **2**-OPP by Avian FPP Synthase[a]



[a] Following incubation of each bisubstrate analog with the enzyme, the phosphate esters were hydrolyzed with alkaline phosphatase, and the products were isolated as the corresponding naphthoate esters.

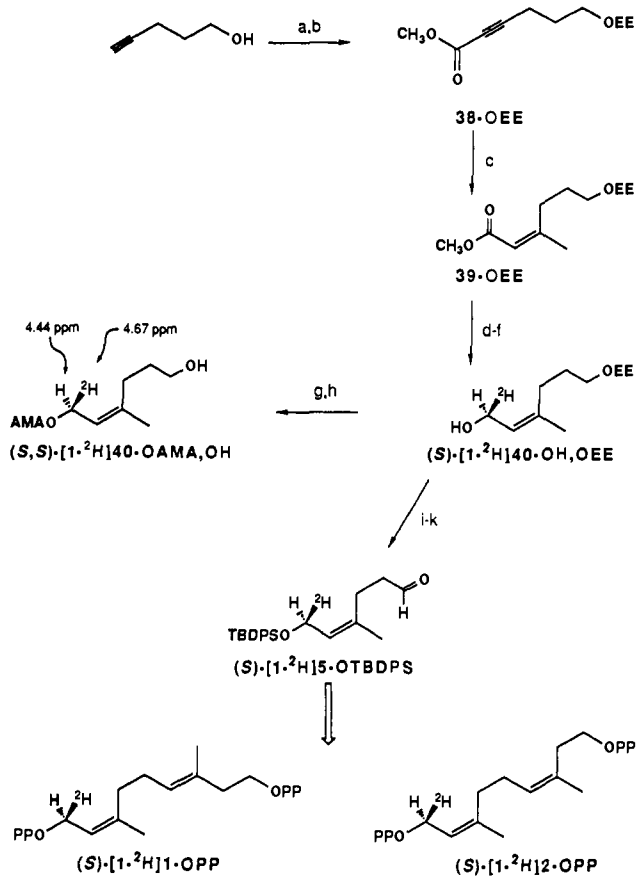
at C(2′) were assigned from vicinal couplings to the resonances at 5.37 and 2.81 ppm. Analysis of the spin system at 1.44 ppm indicated three large coupling constants (>11 Hz), consistent with one geminal and two vicinal diaxial relationships, indicating it is the resonance for the axial proton at C(6′). Two diaxial, two equatorial, and a small allylic coupling were found for the methine proton at C(1′), indicating it is in a pseudo-axial position and the cyclohexane ring is in a pseudochair conformation. Irradiation at 2.81 ppm collapsed the signal at 1.97 ppm for the pseudo-axial hydrogen at C(2′) to a broad doublet. Irradiation of the signal at 5.37 ppm caused the resonance at 1.85 ppm to collapse and confirmed its assignment as the pseudo-equatorial proton at C(2′). Only a minor change in the pattern at 1.97 ppm was observed in this experiment, consistent with a very small coupling between the olefinic proton and the axial proton at C(2′) due to a vicinal dihedral angle near 90°. This observation agrees with the DQCOSY spectrum, which has a crosspeak between the peaks at 1.85 and 5.37 ppm but not between the olefinic signal and the multiplet at 1.97 ppm. Estimates of the major coupling constants in the ring system are summarized in Figure 4.

A similar series of experiments was conducted for **9**-ONp. ^{13}C resonances for the two allylic cyclohexenyl carbons at 30.9 and 30.8 ppm were resolved in C_6D_6. HETCOR data were used to assign the C(6′) resonances at 1.61 and 1.37 ppm and the C(1′) tertiary resonance at 2.02 ppm. The four other allylic hydrogens were clustered between 1.7–2.0 ppm. A 500-MHz DQCOSY spectrum showed crosspeaks from the tertiary proton at 2.02 ppm to the two resonances centered at 1.87 ppm. The resonance at 1.77 ppm gave crosspeaks to the C(6′) proton resonances at 1.61 and 1.37 ppm. The C(3′) olefinic proton gave a strong crosspeak to the signal at 1.96 ppm and a weak interaction with the resonances at 1.87 ppm. Based on these data, the peak at 1.77 ppm was assigned to a proton at C(5′), and the signal at 1.96 ppm to a proton at C(2′). HOMO2DJ experiments at 500 MHz, with and without single-frequency decoupling, were used to determine the multiplicity of the pattern at 1.37 ppm and permitted us to assign this signal to the pseudo-axial proton at C(6′). Unraveling the multiplet structure at 2.02 ppm by decoupling at 1.37 and 1.61 ppm indicated that the C(1′) methine hydrogen was axial and confirmed the pseudochair conformation of the cyclohexenyl ring. Unfortunately, the two ^1H resonances at 1.87 ppm were not resolved, and decoupling experiments at 2.02 ppm were not informative due to the proximity of the resonances at 1.96 ppm for the other proton at C(2′). However, irradiation of the olefinic proton at 5.35 ppm provided an estimate of its coupling constant to the proton at 1.96 ppm. This suggests that the C(2′) proton at 1.96 ppm is pseudo-equatorial, and because there is no coupling to the allylic resonance at 1.87 ppm, this hydrogen must reside in a pseudo-axial position.

The conformations for (1′*R*,2′*R*)-[2′-^2H]**8**-ONp and (1′*R*,2′*R*)-[2′-^2H]**9**-ONp from (*S*)-[1-^2H]**1**-OPP and (*S*)-[1-^2H]**2**-OPP shown in Figure 4 include the absolute configurations for C(1′) established by total synthesis and those for C(2′) deduced from ^1H–^1H coupling constants between hydrogens at C(1′) and C(2′). The hydrogens at C(1′) in both naphthoates have large diaxial couplings to protons at C(2′) and C(6′). Thus, the side chains at C(1′) are equatorial, as expected. The axial and equatorial hydrogens at C(2′) are readily assigned from their

(29) Nagayama, K. *J. Chem. Phys.* **1979**, *71*, 4404–4415.

Scheme V. Synthesis of (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP

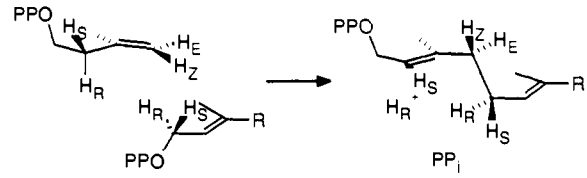


(a) Ethyl vinyl ether/PPTS; (b) nBuLi/CH_3O_2CCl; (c) $(CH_3)_2Cu$-Li; (d) $LiAl^2H_4$; (e) MnO_2; (f) (*R*)-Pinanyl-9BBN; (g) DCC/(*S*)-(*O*-acetyl)mandelic acid; (h) 0.1 N HCl; (i) *tert*-butyldiphenylsilyl chloride/imidazole; (j) HCl/THF; (k) DMSO/$COCl_2$/Et_3N.

Table I. Chemical Shifts and Coupling Constants for the Cyclohexenyl Carbons and Hydrogens in **8**-ONp and **9**-ONp in C_6D_6 at 25 °C

position	^{13}C (ppm)	^{1}H (ppm)	$J_{1H,1H}$ (Hz)
		8-ONp	
1′	36.2	2.81	11.98 (2′ ax)
			5.23 (2′ eq)
			11.10 (6′ ax)
			3.24 (6′ eq)
2′	30.2	1.85 (eq)	14.40 (2′ ax)
			6.40 (3′)
		1.97 (ax)	
3′	120.5	5.37	
5′	30.6	1.75 (eq)	14.07 (5′ ax)
			5.29 (6′ ax)
			5.15 (6′ eq)
		1.94 (ax)	11.30 (6′ ax)
			5.58 (6′ eq)
6′	27.9	1.44 (ax)	12.78 (6′ eq)
		1.47 (eq)	
		9-ONp	
1′	43.1	2.02	11.64 (2′ ax)
			5.11 (2′ eq)
			11.72 (6′ ax)
			3.77 (6′ eq)
2′		1.87 (ax)	
		1.96 (eq)	5.64 (3′)
3′	120.4	5.35	
5′	30.8	1.77 (eq)	5.56 (6′ ax)
			3.94 (6′ eq)
		1.87 (ax)	11.15 (6′ ax)
			4.22 (6′ eq)
6′	28.0	1.37 (ax)	12.71 (6′ eq)
		1.61 (eq)	

Scheme VI. Stereochemistry of the Prenyl-transfer reaction catalyzed by FPP synthase



diaxial (11.6–12.0 Hz) and axial–equatorial (5.1–5.2 Hz) couplings. For **8**-ONp from (*S*)-[1-^{2}H]**1**-OPP, the axial resonance at 1.97 ppm is absent. Since C(1′) has the (*S*)-configuration, **8**-ONp is the (1′*S*,2′*R*)-diastereomer. Using a similar rationale, the absence of a signal at 1.85 ppm for the equatorial C(2′) hydrogen in **8**-ONp shows that the naphthoate from (*S*)-[1-^{2}H]**2**-OPP is the (1′*R*,2′*R*)-diastereomer. Finally, the C(2′) equatorial resonance at 1.96 ppm is absent in the spectrum of **9**-ONp, again demonstrating that the (1′*R*,2′*R*)-diastereomer is formed from labeled **2**-OPP. It is important to note that the relative values of the chemical shifts of the axial and equatorial hydrogens at C(2′) in **8**-ONp and **9**-ONp reverse when the stereochemistry of the double bond in the side chain changes from *Z* to *E*. Thus, evaluation of the individual coupling constants between the hydrogens at C(1′) and C(2′) was essential for assigning the relative stereochemistry at those centers. *The combination of our results shows that C(1) of* ***1*****-OPP and** ***2*****-OPP underwent inversion upon enzyme-mediated cyclization.*

Discussion

Eukaryotic farnesyl-diphosphate synthase is a homodimer.[30-33] The subunits possess a single active site which catalyzes both steps in the chain elongation of DMAPP to FPP.[34] These reactions are highly stereoselective. As illustrated in Scheme VI, C(1) of the allylic substrate (DMAPP or GPP) adds to the *re*-face of C(4) of IPP with inversion of configuration, and the new *E* double bond in the product is generated by loss of the *pro-R* hydrogen from C(2) of IPP. These observations are sufficient to fix the location of C(1) in the allylic substrates relative to C(4) in IPP in the E·S complex and to determine that the dihedral angle between the C(1)–C(2) and C(3)–C(4) bonds in IPP is greater than 90°.[35,36]

The 1′–4 condensations of a substantial number of derivatives of IPP, DMAPP, and GPP have also been reported. These include IPP analogs with substitutions at C(2), C(3), or C(4) and cyclic derivatives.[37-39] Over 30 allylic alternate substrates are known with substituents attached to C(4) or the methyl at C(3).[40-44] Typically, these compounds give only one product, whose stereochemistry is consistent with that observed for the normal substrates. One notable exception is 4-methyl-4-pentenyl diphosphate,

(30) Eberhardt, N. L.; Rilling, H. C. *J. Biol. Chem.* **1975**, *250*, 863–866.
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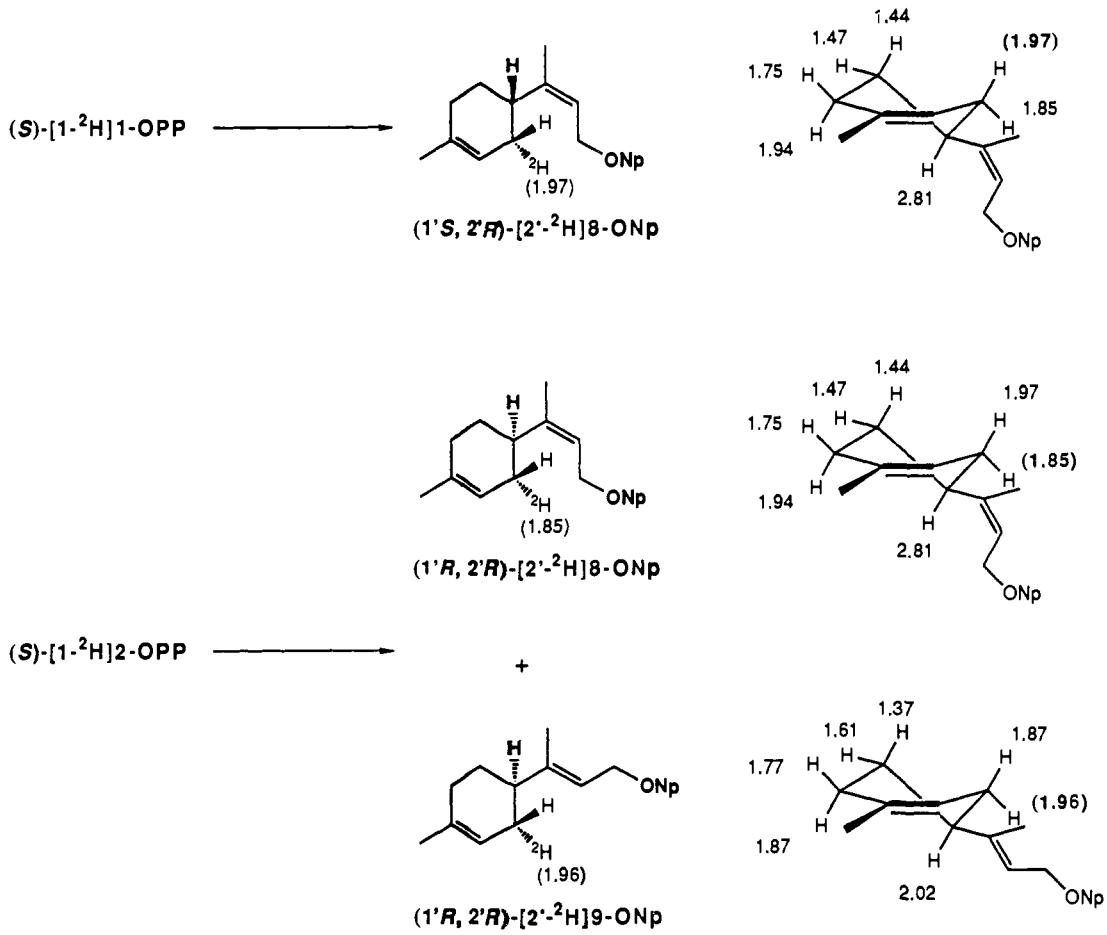
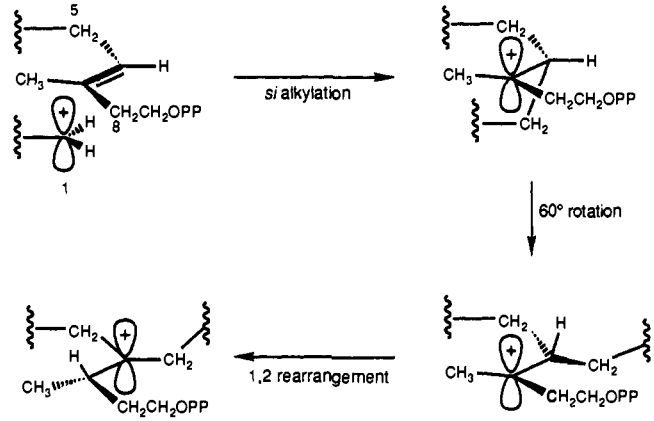


Figure 4. Summary of the ^1H chemical shifts and stereochemical assignments for 8-ONp and 9-ONp from (*S*)-[1-^{2}H]1-OPP and (*S*)-[1-^{2}H]2-OPP. Chemical shifts of the missing resonances are given in parenthesis.

an elongated analog of IPP. The compound was a prenyl acceptor for DMAPP and GPP, but the stereochemistry of the new double bond was *Z* rather than *E*.[39] Thus, FPP synthase is rather tolerant toward substitutions in the hydrocarbon moieties of its substrates. In contrast, the diphosphate moieties are important for tight binding and essential for catalysis.[45,46]

The behavior of our bisubstrate analogs is more complex than was observed for other alternate substrates. Both **1**-OPP and **2**-OPP gave mixtures of products. In addition, **9**-OPP, the compound most closely related to the normal intermolecular condensation products, was not formed from **1**-OPP and was a minor component in the mixture of products from **2**-OPP. Since prenyltransferases typically catalyze condensations with high regio- and stereocontrol, the observation that the bisubstrate analogs gave mixtures raises questions about how the individual products are formed. A cursory examination of their structures indicates that FPP synthase binds at least two different conformers of **2**-OPP in order to accommodate formation of the isomeric double bonds in **8**-OPP and **9**-OPP. However, our discovery that the cyclic products were, within experimental error, optically pure suggests a more complex mode of binding.

(*S*)-**8**-OPP and (*S*)-**10**-OPP are the sole products from **1**-OPP. In order to generate the *S* stereocenter in **8**-OPP, cyclization must occur at the *re*-face of the remote C(6)–C(7) double bond. This is the same facial selectivity seen for alkylation of the double bond in IPP during the normal prenyl-transfer reaction. However, there must be some distortion of the isopentenyl region in **1**-OPP, since **8**-OPP has a *Z* double bond. Cyclohexadiene **10**-OPP also has an *S* stereocenter. However, in this case we suggest that the (*S*)-configuration is the result of a *si*-facial alkylation to generate (*R*)-**12**, followed by a suprafacial 1,2-hydrogen migration. As illustrated below, the hydrogen and methylene attached to C(6)



move from the original plane of the double bond as C(6) becomes tetrahedral. A rotation is required to bring the C(6) hydrogen into optimal alignment for the ensuing 1,2-rearrangement. A least-motion rotation of 60° positions the hydrogen for a suprafacial migration to the *re*-face of the adjacent trigonal cationic center to generate the *S* stereocenter in **10**-OPP. The alternative process, cyclization at the *re*-face of the C(6)–C(7) double bond in **1**-OPP, requires a 120° rotation to produce an *S* stereocenter. The 120° process results in a substantially larger change in topology with a larger required volume in the active site than the 60° rotation. We believe the more intrusive rotation is less probable within the confines of the active site of FPP synthase.

In order for **1**-OPP to cyclize to **8**-OPP and **10**-OPP, carbons 1–6 must adopt a helical conformation with the remote C(6)–C(7) double bond either exo or endo to the helix. There are four

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(46) Rilling, H. C. *Pure Appl. Chem.* **1979**, *51*, 597–608.

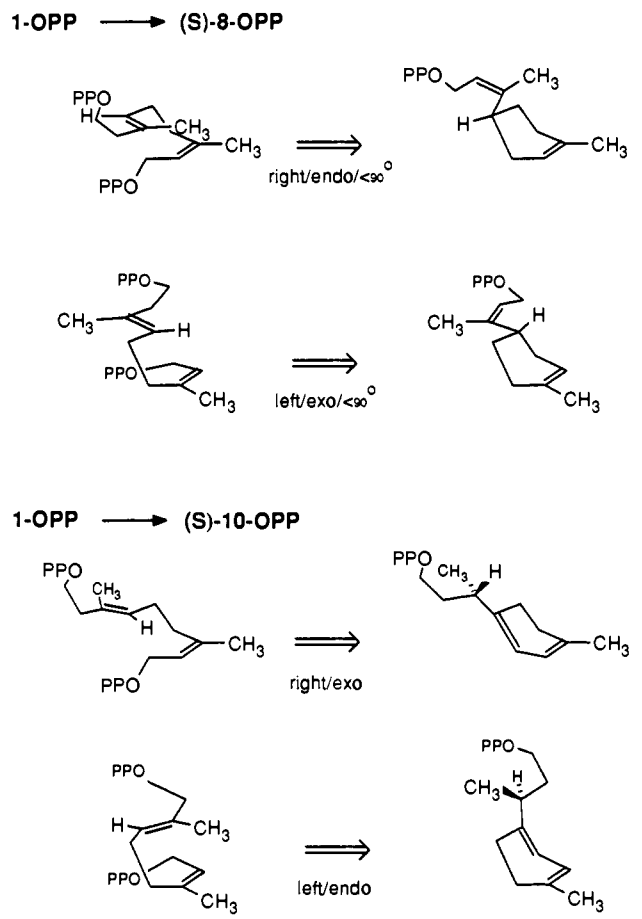


Figure 5. Conformational isomers of **1**-OPP that can cyclize to (*S*)-**8**-OPP and (*S*)-**10**-OPP by a *si*-facial alkylation.

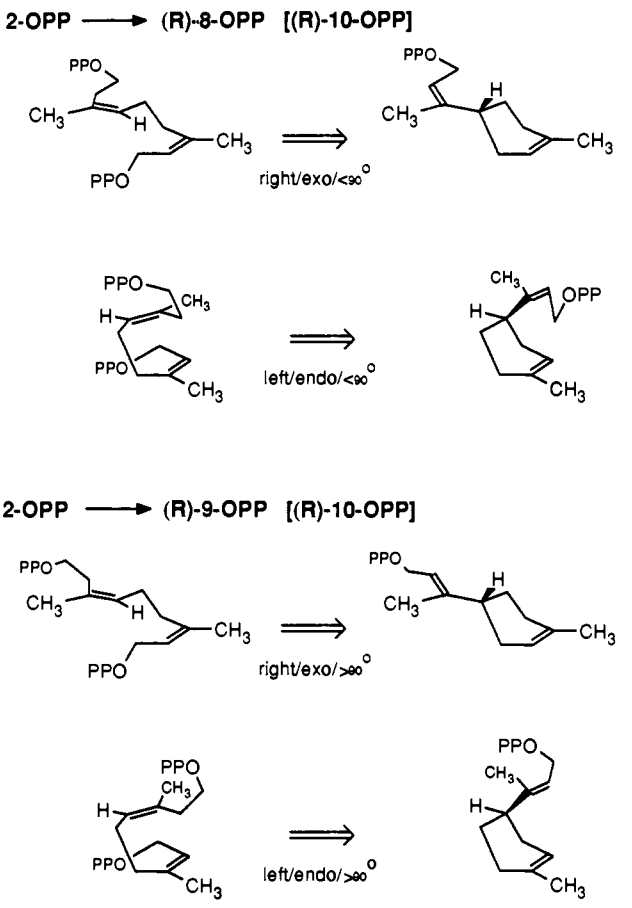
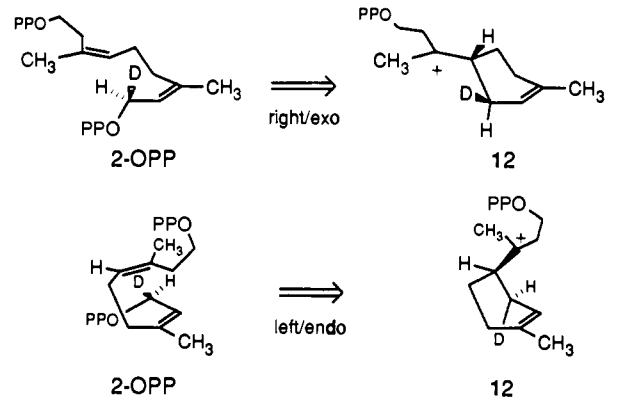


Figure 6. Conformational isomers of **2**-OPP that can cyclize to (*R*)-**8**-OPP, (*R*)-**9**-OPP, and (*R*)-**10**-OPP by a *re*-facial alkylation.

conformational diastereomers consisting of combinations of right- or left-handed helices and endo or exo double bonds. Of these only two, right/endo and left/exo, fold into a "boat-like" orientation for a *re*-facial alkylation needed to generate the *S* stereocenter in **8**-OPP (see Figure 5). Likewise, the enantiomeric right/exo and left/endo conformers can undergo a *si*-facial least-motion cyclization–rearrangement to give (*S*)-**10**-OPP. In addition, the C(6)–C(7)/C(8)–C(9) dihedral angle in **1**-OPP must be less than 90° in order to form the *Z* double bond in (*S*)-**8**-OPP. Two orientations would satisfy this criterion. We chose to position C(9) on the "top" face of the structure so the C(8) hydrogen can be removed from the "bottom" face, in accord with the normal stereochemistry for FPP synthase. Unfortunately, the absence of a double bond in the side chain of (*S*)-**10**-OPP makes it impossible to define the C(7)–C(8) stereochemistry in the conformer that yields the cyclohexadiene.

The *R* stereocenters in **8**-OPP and **9**-OPP obtained from the FPP synthase-catalyzed cyclization of **2**-OPP result from a *si*-facial cyclization of the right/exo or left/endo diastereomers shown in Figure 6. For (*R*)-**8**-OPP the C(6)–C(7)/C(8)–C(9) dihedral angle must be greater than 90°, while, for (*R*)-**9**-OPP, the dihedral angle must be less than 90°. We suggest that diene (*R*)-**10**-OPP is the product of a *si*-facial alkylation least-motion rearrangement analogous to that discussed for (*S*)-**10**-OPP.

The labeling studies with (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP provide essential information about the conformations that give rise to the diene products. For both (*S*)-**10**-OPP and (*R*)-**10**-OPP, >98% of the deuterium was retained in the cyclohexadiene ring. If we assume that discrimination between protium and deuterium is the result of stereoelectronic factors rather than a primary kinetic isotope effect, the "axial-like" hydrogen on the bottom face of the ring in carbocation **13** should be removed preferentially. This interpretation is also stereochemically compatible with using the base that deprotonates C(2) of IPP in the normal prenyl-transfer reaction to assist with the elimination in **13**. It is the right-handed conformers of (*S*)-[1-^{2}H]**1**-OPP and (*S*)-[1-^{2}H]**2**-OPP that give a "boat-like" cyclohexenyl intermediate **12** with "axial" protium and "equatorial" deuterium, which then rearranges to **13** before the elimination steps. Thus, we propose that (*S*)-**10**-OPP and (*R*)-**10**-OPP come from the right/exo conformers of **1**-OPP and **2**-OPP, respectively, as illustrated below for **2**-OPP.



Taken as a group, the conformational isomers shown in Figures 5 and 6 have substantially different topologies. In order to further define the shape of the active site of FPP synthase, we sought to identify a subset whose composite shape was consistent with the known constraints for substrate binding by the enzyme. These include a conserved location for the diphosphate moieties, an overall topology consistent with the Popjak–Cornforth stereochemistry, and a net volume for the hydrocarbon chains that falls within the space defined by the known alternate substrates for IPP and DMAPP. In addition, the structures must be compatible with the regiochemical and stereochemical properties of our bisubstrate analogs.

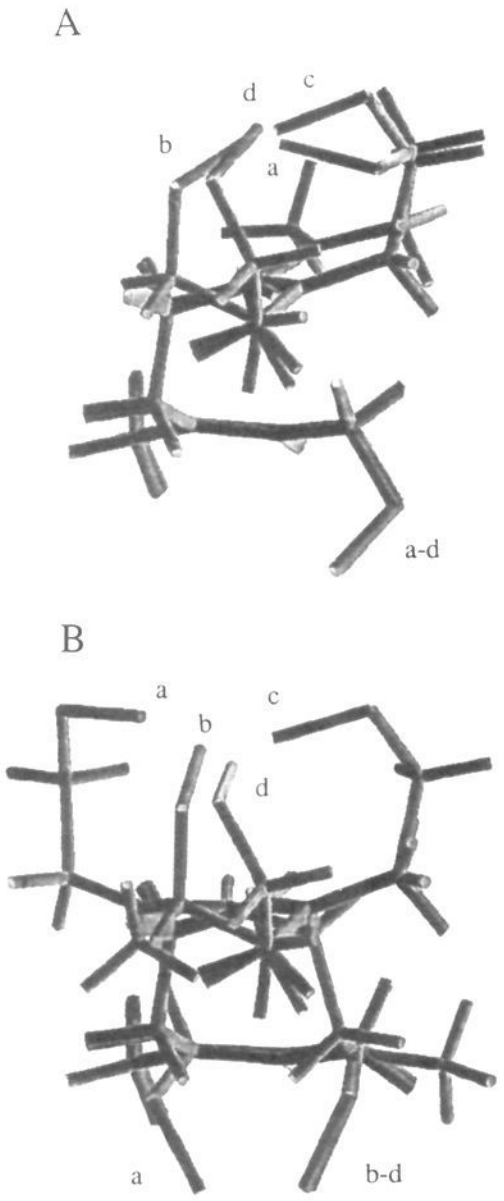


Figure 7. Superimposed conformations of **1**-OPP and **2**-OPP that could cyclize to **8**-OPP, **9**-OPP, and **10**-OPP. The structures shown encompass the P(1) phosphorus atoms of the diphosphate moieties and the intervening hydrocarbon chains. The remainder of the diphosphate units are not shown. Part A: (a) right/endo/<90° and (b) right/exo/<90° conformers of **1**-OPP; (c) right/exo/<90°; and (d) right/exo/>90° conformers of **2**-OPP. Part B: (a) left/exo/<90° and (b) right/exo/<90° conformers of **1**-OPP; (c) right/exo/<90° and (d) right/exo/>90° conformers of **2**-OPP.

Two collections of conformations that satisfy these criteria are shown in Figure 7. The first set shown in part A consists of the right/endo/<90° ((*S*)-**8**-OPP) and right/exo/<90° ((*S*)-**10**-OPP) conformers of **1**-OPP and the right/exo/<90° ((*R*)-**8**-OPP) and right/exo/>90° ((*R*)-**9**-OPP) for **2**-OPP. Although (*S*)-**10**-OPP could arise from either <90° or >90° right/exo conformer of **1**-OPP, the <90° one superimposes more closely on the other structures in the set. (*R*)-**10**-OPP could come from any of the right/exo conformers of **2**-OPP. For these conformers, the lower parts of **1**-OPP and **2**-OPP superimpose identically. The structures diverge from C(6) through the end of the hydrocarbon chain and reconverge at P(1) of the homoallylic diphosphate residue. While the overall superimposition is excellent, the endo orientation for **1**-OPP may present a problem for the elimination of a proton from C(8) after cyclization. For the normal substrates, the hydrogen on the bottom face of C(2) in IPP is removed following condensation with the allylic moiety of DMAPP and, presumably, so is the catalytic site base that assists the removal. Cyclization of **1**-OPP from an endo conformer moves C(8), which is equivalent to C(2) in IPP, upward from the allylic region where this base should be located.

An alternative set of conformers, consisting of left/exo/<90° ((*S*)-**8**-OPP) and right/exo/<90° ((*S*)-**10**-OPP) orientations of **1**-OPP and right/exo/<90° ((*R*)-**8**-OPP) and right/exo/>90° ((*R*)-**9**-OPP) orientations of **2**-OPP is shown in part B. As before, (*R*)-**10**-OPP could come from any right/exo conformer of **2**-OPP. For this set, the right- and left-handed conformations can be superimposed to form a "box-like" structure for carbons 1–6 which converges on top at C(6) and has opposing C(1)s on the bottom. The structures diverge in the isopentenyl region to reconverge at P(1) of the homoallylic diphosphate.

In order for the allylic diphosphate moieties to overlap, the C(1)–O bonds must rotate inward. Although this orientation directs the backside of the C(1)–O bonds away from the C(6)–C(7) double bonds in **1**-OPP and **2**-OPP, the prenyl-transfer reaction does not appear to be concerted,[11] and a precise alinement of C(1) with respect to the remote double bond is not essential. The degree of overlap between the π-bond and the developing p-orbital at C(1) during rupture of the carbon–oxygen bond follows a cosine dependence, and the reactivity of an allylic moiety doesn't decrease dramatically until the C(1)–oxygen bond nears the plane of the adjacent double bond. Similar arguments apply at C(8) during the elimination step.

The structures in part B are less compact than those shown in part A and require a more "tolerant" active site. A substantial expansion of the volume of the homoallylic region is necessary to handle C(7) to the P(1) homoallylic phosphorus atom for the left/exo/<90° conformer of **1**-OPP. Additional volume is also needed in the allylic region for the P(1) phosphorus and the methyl group at C(3). However, the conformations for the lower half of the right- and left-handed helices are similar to those proposed by Cane and Croteau for monoterpene cyclase catalyzed cyclization of enantiomeric forms of linalyl diphosphate (LPP).[9,10,47-51] These enzymes possess two activities: an isomerase activity, which catalyzes the 1,3-allylic rearrangement of GPP to LPP, followed by rotation about the C(2)–C(3) bond in LPP, and a cyclase activity, which closes the ring. The conversion of GPP to LPP is highly stereoselective. However, the other enantiomer of LPP is an alternate substrate, which the cyclases process to give the optical antipode of their normal product. They propose that cyclization of enantiomers of LPP proceeds through antipodal "boat-like" α-terpinyl intermediates. As a consequence, the absolute configuration of *p*-menthane monoterpenes is governed by the stereoselectivity of isomerization, not cyclization. In view of the observations of Cane and Croteau and the ability of FPP synthase to process a variety of allylic diphosphates, the right- and left-handed conformers of **1**-OPP and **2**-OPP might be accommodated by the allylic region of the catalytic site.

In addition to the orientation of the hydrocarbon chains in **1**-OPP and **2**-OPP, we feel our experiments with **1**-OPP and **2**-OPP provide information about the relative location of the diphosphate moieties in the E·S complex. On the basis of binding studies, Reed and Rilling suggested that the diphosphate units of the substrates were stacked in a manner so they shared the two magnesium ions bound in the catalytically competent E·S complex.[34,46] However, attempts by us to find a collection of conformers that satisfied the conformational constraints placed on the hydrocarbon chains in **1**-OPP and **2**-OPP and permitted both diphosphate moieties to interact with the same magnesium ion were unsuccessful. We find it more likely that the diphosphate residues in IPP and DMAPP lie above and below the volume of space occupied by the hydrocarbon units. In this orientation, charge–charge repulsions between the negative diphosphates are minimized when the substrates are sequestered within their individual binding domains.

Prenyltransferases and cyclases catalyze electrophilic condensations on remote double bonds through allylic carbocationic intermediates. The mechanistic similarity between the two classes of enzymes presents a strong circumstantial case for divergent evolution. Our results with FPP synthase reveal that the reactions of the carbocationic intermediates are very sensitive to the conformation of the substrate in the E·S complex. It is easy to envision

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how mutations in a prenyltransferase could produce conformational changes in its allylic substrate that transform the enzyme into a cyclase. One can also rationalize the evolution of a variety of cyclase and multicyclase activities, including synthesis of both optical antipodes, from an ancestral prenyltransferase or cyclase by mutations that alter substrate conformation in the E·S complex.

During the past few years, genes for farnesyl-diphosphate synthase, geranylgeranyl diphosphate synthase, and hexaprenyl diphosphate synthase have been cloned.[52-55] The amino acid sequences of the encoded proteins show two conserved asparate-rich domains which Ashby et al. proposed as the binding loci for IPP and DMAPP.[56,57] Other prenyltransferases that attach allylic moieties to non-isoprenoid acceptors contain a single conserved aspartate-rich domain.[57] One would anticipate that isoprenoid cyclases might have similar aspartate-rich domains if they evolved from prenyltransferases. Unfortunately, there are no primary structures for any monoterpene cyclases available for comparison. However, genes for two sesquiterpene cyclases, trichodiene synthase[58,59] and aristolochene synthase,[60] were recently sequenced and found to encode aspartate-rich regions similar to those in prenyltransferases. Although these results are tantalizing, Cane[61] recently pointed out the consensus sequence (I,L,V,H) X(D,-N)DXX(D,E) proposed for the allylic binding domain of prenyltransferases would be statistically expected in 1 of 20 proteins. Although the asparate-rich sequences appear in prenyltransferases at a much higher than expected frequency, their functional significance has not been established, and as of now there are too few cyclase sequences known to establish a link between prenyltransferases and cyclases at the genetic level.

Experimental Section

Materials. Materials not unique to this paper were described previously.[11] Pyridinium dichromate was prepared from potassium dichromate[62] and used fresh after a second recrystallization from acetone. Solutions of CH_2N_2 in diethyl ether were used immediately and were not distilled prior to use. CH_3I was freshly distilled before use. Freshly precipitated CuI was washed with tetrahydrofuran and diethyl ether and dried over P_2O_5. (*S*)-O-Acetyl mandelic acid[63] was dried over P_2O_5 in a vacuum desiccator.

General Methods. Chromatographies were described previously.[11] $AgNO_3$-impregnated silica gel plates were prepared by dipping in a 2% solution in acetonitrile and drying for 3–4 h. The plates were used immediately. ^1H and ^{13}C spectra are referenced in parts per million (δ, ppm) from a solvent lock signal or 3-(trimethylsilyl)-1-propanesulfonic acid (TPS). ^{31}P and ^{19}F spectra are referenced to external phosphoric acid or trifluoroacetic acid, respectively. Double quantum filtered correlation spectra[27] were acquired using a 3955-Hz sweep width, a Fourier number of 2048, and 512 increments. A 1.5-s pulse delay was used, and data were processed with 0.1-Hz line broadening. Homocorrelation 2D-J spectroscopy[28] was performed with a 4543-Hz sweep in the F2 dimension, 60 Hz in the F1 dimension, 384 increments, and a 2-s pulse delay. The fully decoupled spectra were acquired as described by Nagayama.[29] ^{13}C–^1H heterocorrelation spectra[25] were acquired with a sweep of 12135 Hz and Fourier number of 8192 in the F2 dimension; a 2365-Hz sweep and Fourier number of 1024 was used in the F1 dimension with 256 increments. Data were processed with line broadening in F2 and F1 dimensions of 1 and 0.1 Hz, respectively.

(*R*,*Z*)- and (*R*,*E*)-Methyl 3-(4-Methyl-3-cyclohexenyl)-2-butenoate ((*R*)-28 and (*R*)-29). (*S*,*Z*)- and (*S*,*E*)-Methyl 3-(4-Methyl-3-cyclohexenyl)-2-butenoate ((*S*)-28 and (*S*)-29). (*R*)- and (*S*)-1-methyl-4-acetylcyclohex-1-ene were prepared from 34 g (0.25 mol) of freshly distilled (+)-(*R*)-limonene ((+)-(*R*)-**18**) [Aldrich Chemical Co., $[\alpha]^{24}_D$ +106° (*c* 1.0, methanol), lit[13] $[\alpha]^{24}_D$ +118° (*c* 1.1, $CHCl_3$)] or (−)-(*S*)-limonene ((−)-(*S*)-**18**) [$[\alpha]^{19}_D$ −100° (*c* 10, ethanol), lit[13] $[\alpha]^{24}_D$ −106° (*c* 1.0, $CHCl_3$)] and purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 10.5 g (38%) of a colorless liquid: (*R*)-enantiomer $[\alpha]^{22}_D$ +120° (*c* 1.34, $CHCl_3$), lit[13] $[\alpha]^{24}_D$ +122.3°; (*S*) enantiomer $[\alpha]^{22}_D$ −92° (*c* 6.33, $CHCl_3$), lit[13] $[\alpha]^{24}_D$ −122.7°; IR (CCl_4) 2910, 2830, 1708, 1425, 1345, 1160, 945, 905 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.38 (1, b s, H at C(3′)), 2.51 (1, m, H at C(1′)), 2.16 (4, m), 1.98 (3, m), 1.64 (3, s, CH_3 at C(4)), 1.58 ppm (2, m).

Following the procedure of Delay,[13] (*R*)-1-methyl-4-acetylcyclohex-1-ene (6.6 g, 48.0 mmol) was treated with 10.9 g (60.0 mmol) of sodium dimethyl methyl phosphonoacetate and the products were purified by preparative HPLC on silica gel (96:4 hexanes/diethyl ether). A total of 3.65 g of (*R*)-**29** and 0.913 g of (*R*)-**28** were recovered as colorless liquids (61% total based on recovered ketone). A similar procedure was used to isolate the (*S*)-enantiomers.

(*R*)-**28** $[\alpha]^{22}_D$ −19.1° (*c* 0.23, ethanol), lit[13] $[\alpha]^{20}_D$ −25.2°; (*S*)-**28** $[\alpha]^{22}_D$ +13.5° (*c* 0.2, ethanol), lit[13] $[\alpha]^{20}_D$ +14.8°; IR (CCl_4) 2910, 2830, 1715, 1630, 1430, 1372, 1225, 1195, 1155, 910, 865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.65 (1, m, H at C(2)), 5.44 (1, m, H at C(4′)), 3.79 (1, m, H at C(1′)), 3.67 (3, s, OCH_3), 2.10 (1, m), 2.0–1.85 (3, m), 1.82 (3, s, CH_3 at C(3)), 1.65 (3, b s, CH_3 at C(4′)), 1.64–1.5 ppm (2, m); MS (EI, 70 eV) *m*/*z* 194 (77.6) $[M]^+$, 179 (7.9) $[M - CH_3]^+$, 163 (26.5), 162 (55.1), 147 (70.3), 139 (19.5), 135 (26.7), 125 (40.4), 119 (29.9), 111 (28.3), 107 (18.3), 105 (29.2), 94 (100), 81 (39.9).

(*R*)-**29** $[\alpha]^{22}_D$ +88° (*c* 0.23, ethanol), lit[13] $[\alpha]^{20}_D$ +84.8°; (*S*)-**29** $[\alpha]^{22}_D$ −85° (*c* 0.275, ethanol), lit[13] $[\alpha]^{20}_D$ −65.7°; IR (CCl_4) 2915, 2830, 1715, 1640, 1430, 1220, 1198, 1155, 1140, 865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.69 (1, dq, *J* = 1.0 Hz, *J* = 1.2 Hz, H at C(2)), 5.40 (1, m, H at C(3′)), 3.69 (3, s, OCH_3), 2.23 (1, m), 2.16 (3, d, *J* = 1.2 Hz, CH_3 at C(3)), 2.15–1.90 (4, m), 1.85–1.75 (1, m), 1.66 (3, b s, CH_3 at C(4′)), 1.62–1.48 ppm (1, m); MS (EI, 70 eV) *m*/*z* 194 (32.7) $[M]^+$, 163 (13.6), 162 (15.0), 147 (9.5), 135 (26.0), 134 (13.6), 125 (11.1), 120 (13.8), 101 (81.1), 94 (100).

(*R*,*Z*)- and (*S*,*Z*)-3-(4-Methyl-3-cyclohexenyl)-2-buten-1-ol ((*R*) and (*S*)-8-OH). A solution of 388 mg (2.0 mmol) of (*R*)-**28** in 5 mL of CH_2Cl_2 at 0 °C was treated with 5 mL (711 mg, 5.0 mmol) of DIBAL (1.0 M in hexanes). After 40 min at 0 °C, the mixture was poured into methanol, and the resulting gel was stirred with 25 mL of saturated sodium potassium tartrate solution for 1 h. The organic material was extracted with CH_2Cl_2 and dried over Na_2SO_4. Solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (7:3 hexanes/diethyl ether) to yield 285 mg (85%) of a colorless liquid. A similar procedure was used to prepare (*S*)-**8**-OH from (*S*)-**28**. (*R*)-**8**-OH $[\alpha]^{22}_D$ +27° (*c* 0.18, ethanol), lit[13] $[\alpha]^{20}_D$ +26.5° (*c* 0.1, ethanol); (*S*)-**8**-OH $[\alpha]^{22}_D$ −23° (*c* 0.21, ethanol), lit[13] $[\alpha]^{20}_D$ −21.5° (*c* 0.1, ethanol); IR (CCl_4) 3320, 2920, 1655, 1435, 1370, 1005, 910, 797, 780 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.40 (2, m), 4.16 (2, m, H at C(1)), 2.65–2.60 (1, m, H at C(1′)), 2.17–1.77 (4, m), 1.68 (3, s), 1.65 (3, s), 1.62–1.57 ppm (2, m); MS (EI, 70 eV) *m*/*z* 148 (61.1) $[M - H_2O]^+$, 133 (62.4), 119 (19.6), 106 (37.6), 97 (13.1), 93 (58.2), 91 (50.1), 83 (63.7), 79 (88.6), 69 (100).

(*R*,*E*)- and (*S*,*E*)-3-(4-Methyl-3-cyclohexenyl)-2-buten-1-ol ((*R*)- and (*S*)-9-OH). The protocol described for (*R*)- and (*S*)-**8**-OH was used to prepare 314 mg of (*R*)- or (*S*)-**9**-OH in 94% yield from (*R*)- or (*S*)-**29**. (*R*)-**9**-OH $[\alpha]^{22}_D$ +89.5° (*c* 0.22, ethanol), lit[13] $[\alpha]^{20}_D$ +84.8° (*c* 0.1, ethanol); (*S*)-**9**-OH $[\alpha]^{22}_D$ −82° (*c* 0.23, ethanol), lit[13] $[\alpha]^{20}_D$ −65.7° (*c* 0.1, ethanol); IR (CCl_4) 3380, 2910, 1655, 1435, 1370, 1140, 985, 910 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.45 (1, b d, *J* = 6.76 Hz, H at C(2)), 5.41 (1, m, H at C(4′)), 4.17 (2, d, *J* = 6.8 Hz, H at C(1)), 2.16–1.85 (5, m), 1.80–1.70 (1, m), 1.67 (3, s), 1.65 ppm (3, s); MS (EI, 70 eV) *m*/*z* 166 (5.9) $[M]^+$, 148 (20.8) $[M - H_2O]^+$, 135 (11.7), 121 (8.3), 119 (9.9), 107 (16.7), 105 (18.7), 97 (10.5), 93 (49.9), 83 (54.0), 79 (41.6), 69 (100).

(*R*,*Z*)-3-(4-Methyl-3-cyclohexenyl)-2-buten-1-yl 2-Naphthoate ((*R*)-8-ONp). To a solution of 52 mg (0.3 mmol) of 2-naphthoic acid, 37 mg (0.3 mmol) of 4-(*N*,*N*-dimethylamino)pyridine, and 48 mg (0.3 mmol) of 4-(*N*,*N*-dimethylamino)pyridine hydrochloride in 1 mL of CH_2Cl_2 was added 62 mg (0.3 mmol) of DCC followed by (*R*)-**8**-OH (33 mg, 0.2 mmol) in 3 mL of CH_2Cl_2. After 16 h at room temperature, solvent was removed and the resultant solid was extracted with hexanes.

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The material recovered from the filtrate was purified by flash chromatography (6:4 hexanes/CH_2Cl_2) to yield 62 mg (97%) of a colorless oil. A sample was transferred to a volumetric flask, dried at 0.02 mmHg for 48 h, and diluted with acetonitrile. This standard solution was used to measure the UV extinction coefficient and optical rotation: $[\alpha]^{22}_D$ −3.33°, $[\alpha]^{22}_{546}$ −7.33°, $[\alpha]^{22}_{436}$ −26.0°, $[\alpha]^{22}_{365}$ −74.0° (*c* 0.15, acetonitrile); UV (acetonitrile) λ_{max} 235 (ε 61 260), 271 (11 200), 280 (11 800); IR (CCl_4) 2920, 2830, 1715, 1435, 1275, 1220, 1190, 1125, 1075, 955, 860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.63 (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), 5.54 (1, b t, *J* = 7.04 Hz, H at C(2)), 5.43 (1, m, H at C(3′)), 4.96 (1, dd, *J* = 7.04, 12.60 Hz, H_a at C(1)), 4.90 (1, dd, *J* = 7.04, 12.60 Hz, H_a at C(1)), 2.81 (1, m, H at C(1′)), 2.16–1.84 (4, m), 1.76 (3, s), 1.69 (3, b s), 1.68–1.60 ppm (2, m); ^{13}C NMR (75 MHz, $CDCl_3$) 166.7, 146.9, 135.4, 133.9, 132.5, 131.0, 129.3, 128.15, 128.1, 127.74, 127.69, 126.6, 125.3, 120.5, 118.8, 61.3, 36.0, 30.6, 29.9, 27.6, 23.8, 19.6 ppm; MS (EI, 70 eV) *m/z* 172 (26.8) [McLafferty rearrangement], 155 (99.7), 148 (100), 133 (47.2), 127 (46.5), 119 (11.4), 106 (34.4), 93 (41.2), 81 (25.9). Anal. Calcd for $C_{22}H_{24}O_2$: C, 82.46; H, 7.55. Found: C, 82.62; H, 7.56.

(*R*,*E*)-3-(4-Methyl-3-cyclohexenyl)-2-buten-1-yl 2-Naphthoate ((*R*)-9-ONp). Using the same protocol described for (*R*)-8-ONp, the (*R*)-9-OH was converted to its naphthoate ester (57 mg, 89%): $[\alpha]^{22}_D$ +51.1°, $[\alpha]^{22}_{546}$ +58.3°, $[\alpha]^{22}_{436}$ +978°, $[\alpha]^{22}_{365}$ +153° (*c* 0.223, acetonitrile); UV (acetonitrile) λ_{max} 235 (ε 64 182), 282 (6500), 292 nm (4800); IR (CCl_4) 3070, 2920, 1715, 1625, 1435, 1275, 1220, 1190, 1125, 1080, 955, 910, 865, 820 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 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* = 6.92 Hz, H at C(2)), 5.41 (1, m, H at C(4′)), 4.93 (2, d, *J* = 6.92 Hz, H at C(1)), 2.24–1.89 (5, m), 1.82 (1, m), 1.79 (3, s), 1.66 (3, s), 1.54 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; MS (EI, 70 eV) *m/z* 172 (35.5) [McLafferty], 155 (100), 148 (59.2), 133 (35.5), 127 (53.9), 106 (24.7), 93 (38.8). Anal. Calcd for $C_{22}H_{24}O_2$: C, 82.46; H, 7.55. Found: C, 82.75; H, 7.59.

(*S*)-α-Methoxy-α-(((trifluoromethyl)phenyl)acetyl) Esters of (*R*)- and (*S*)-Terpineol ((*R*,*S*)- and (*S*,*S*)-30-OMTPA). (*R*)-30-OH was prepared[14] from (+)-(*R*)-18 (409 mg, 3.0 mmol, Aldrich Chemical, $[\alpha]^{24}_D$ +106° (*c* 1.0, methanol), lit[13] $[\alpha]^{24}_D$ +118° (*c* 1.1, $CHCl_3$)) and isolated by flash chromatography (65:35 hexanes/diethyl ether) to yield 220 mg (48%) of a colorless liquid. This material comigrated on TLC with authentic terpineol. In a similar fashion, (*S*)-30-OH was prepared from (*S*)-(−)-18. (*R*)-30-OH $[\alpha]^{21}_D$ +86.8° (*c* 0.65, methanol), lit[64] $[\alpha]_D$ +98.4 (ethanol); (*S*)-30-OH $[\alpha]^{21}_D$ −85.8° (*c* 1.43, methanol); IR (CCl_4) 3470, 2960, 2920, 1435, 1375, 1360, 1280, 910, 830 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.37 (1, b s, H at C(3′)), 2.01 (3, m), 1.72 (2, m), 1.63 (3, b s, CH_3 at C(4′)), 1.48 (1, m), 1.25 (1, m), 1.17 (3, s, CH_3 at C(1)), 1.15 ppm (3, s, CH_3 at C(1)).

To a solution of 293 mg (1.25 mmol) of (*S*)-MPTA in 2 mL of CH_2Cl_2 at 0 °C was added 0.13 mL (190 mg, 1.5 mmol) of oxalyl chloride and 3 μL of DMF. After 1.5 h, the mixture was warmed to room temperature, and solvent was removed by rotary evaporation to yield a yellow oil. The oil was redissolved in 2 mL of 1,2-dichloroethane and used in the esterification step.

The acid chloride was added to 1.8 mL of 1,2-dichloroethane followed by 160 mg (1.31 mmol) of DMAP and 158 mg (0.625 mmol) of (*R*)-30-OH in 1.5 mL of 1,2-dichloroethane. The solution was warmed to 70 °C for 18 h, diluted with CH_2Cl_2, washed with 1 N HCl, and dried over Na_2SO_4. Solvent was removed by rotary evaporation, and the product was purified by flash chromatography (97:3 hexanes/diethyl ether) to yield 92 mg (50%) of a colorless oil: TLC (R_f 0.33; 97:3 hexanes/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$) 7.55 (2, m, Ph H), 7.38 (3, m, Ph H), 5.32 (1, m, H at C(3′)), 3.52 (3, q, $J_{H,F}$ = 1.2 Hz, OCH_3), 2.02–1.87 (4, m), 1.82–1.73 (1, m), 1.72–1.69 (2, m), 1.61 (3, s, CH_3 at C(4′)), 1.54 (3, s, CH_3 at C(1)), 1.52 ppm (3, s, CH_3 at C(1)); ^{19}F NMR (282 MHz, C_6D_6) δ 3.73, (*R*)/(*S*) ratio = 98:2.

Following the procedure described above, 62 mg (0.4 mmol) of (*S*)-30-OH was esterified in the presence of 316 mg (1.25 mmol) of (*S*)-MPTA and 244 mg (2.0 mmol) of DMAP in 3.5 mL of 1,2-dichloroethane to yield 113 mg (76%) of a colorless oil: TLC (R_f 0.33; 97:3 hexanes/diethyl ether); IR (CCl_4) 2920, 2840, 1735, 1445, 1380, 1365, 1270, 1180, 1165, 1115, 1020, 710, 695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.55 (2, m, Ph H), 7.38 (3, m, Ph H), 5.32 (1, m, H at C(3′)), 3.52 (3, q, $J_{H,F}$ = 1.2 Hz, OCH_3), 2.0–1.83 (4, m), 1.82–1.69 (2, m), 1.60 (3, b s, CH_3 at C(4′)), 1.55 (3, s, CH_3 at C(1)), 1.53 ppm (3, s, CH_3 at C(1)); ^{19}F NMR (282 MHz, C_6D_6) δ 3.68 (*S*)/(*R*) ratio = 93:7.

3-(4-Methylphenyl)butanoic Acid (31). To 1.82 g (0.075 mol) of Mg turnings and 10 mL of THF was added 8.55 g (0.05 mol) of 4-methylphenyl bromide in 30 mL of THF over a 1.5–2.0-h period at room temperature. The resultant greenish suspension was stirred for 1 h before use. To a suspension of 4.76 g (0.025 mol) of copper(I) iodide in 30 mL of THF was added 8.35 mL (7.07 g, 0.114 mol) of dimethyl sulfide. After the reaction mixture was cooled to −35 °C, the Grignard solution was introduced via cannula over 1.5 h. After addition of 1.63 mL (1.72 g, 0.02 mol) of β-propiolactone over 5 min, the mixture was allowed to warm to 0 °C over the next 3 h. The reaction was quenched with saturated NH_4Cl (made basic with NH_4OH), and the resulting suspension was extracted with hexanes. The aqueous layer was cooled in an ice bath and acidified with concentrated HCl. Organic material was extracted with diethyl ether, washed with saturated NH_4Cl, and dried over $MgSO_4$. Solvent was removed by rotary evaporation, and the residue was purified by Kugelrohr distillation (60–90 °C/0.3 mmHg) to yield 1.67 g (47%) of a white solid: TLC (R_f 0.44; 6:4 CH_2Cl_2/2-propanol); IR (CCl_4) 3200, 2970, 2920, 1705, 1510, 1405, 1310, 1295, 1200, 1010, 930 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 11.07 (1, b s, OH), 7.06 (4, m, Ph H), 3.20 (1, tq, *J* = 5.80 Hz, *J* = 7.00 Hz, H at C(3)), 2.50 (2, m, H at C(2)), 2.35 (3, s, CH_3 at Ph), 1.27 ppm (3, d, *J* = 7.00 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 178.4, 142.2, 136.0, 129.2, 126.5, 42.6, 35.7, 21.9, 21.0 ppm; MS (EI, 70 eV) *m/z* 178 (18.8) $[M]^+$, 132 (7.9), 119 (100), 91 (18.4). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.30; H, 7.99.

3-(4-Methyl-1,4-cyclohexadienyl)butanoic Acid (32). To 90 mL of NH_3 at −65 °C under argon was added **31** (1.5 g, 8.4 mmol) in 30 mL of THF followed by 2.33 g (33.6 mmol) of Li wire over 1 h. The reaction mixture was warmed to −35 °C, and 5 mL of ethanol was added over 1 h. Excess Li was decomposed by further addition of ethanol, and NH_3 was allowed to evaporate under a stream of nitrogen. The resulting suspension was poured into water and extracted with hexanes. The aqueous layer was cooled with ice and acidified with concentrated HCl. The organic material was extracted with diethyl ether and dried over $MgSO_4$. Solvent was removed by rotary evaporation to give 1.38 g (91%) of a white solid: TLC (R_f 0.46; 96:4 CH_2Cl_2/2-propanol); IR (CCl_4) 3100, 3010, 2960, 2870, 2810, 1705, 1445, 1425, 1405, 1300, 1290, 1200, 945 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.50 (1, b s), 5.42 (1, m), 2.60 (5, m), 2.50 (1, dd, *J* = 6.61 Hz, *J* = 14.90 Hz, H_a at C(2)), 2.30 ppm (1, dd, *J* = 8.13 Hz, *J* = 14.90 Hz, H_b at C(2)); ^{13}C NMR (75 MHz, $CDCl_3$) 178.8, 137.5, 131.2, 118.4, 118.3, 40.0, 36.8, 31.5, 27.3, 22.9, 19.2 ppm; MS (EI, 70 eV) *m/z* 180 (53.4) $[M]^+$, 121 (81.5), 119 (35.8), 105 (61.6), 93 (100), 91 (40), 77 (34.4). HRMS. Calcd for $C_{11}H_{16}O_2$: 180.1151. Found: 180.1112.

Methyl 3-(4-Methyl-1,3-cyclohexadienyl)butanoate (33). To a solution of 3.49 g (31.1 mmol) of potassium *tert*-butoxide in 35 mL of DMSO (degassed, kept under argon for 25 min, and cooled to 0 °C) was added 1.4 g (7.78 mmol) of **32** in 25 mL of toluene over 10 min. The mixture was allowed to warm to room temperature for 2.5 h and poured into 3 mL of concentrated HCl in 80 mL of ice water. The mixture was extracted with diethyl ether, and the organic layer was dried over $MgSO_4$. Solvent was removed by rotary evaporation, to afford 1.4 g of a yellow solid: TLC (R_f 0.37; 96:4 CH_2Cl_2/2-propanol); ^1H NMR (90 MHz) indicated a 7:3 ratio of the 1,3- to 1,4-cyclohexadienyl isomers.

The residue was dissolved in diethyl ether and treated with an ethereal solution of CH_2N_2 prepared from 8.01 g (77.8 mmol) of *N*-nitroso-*N*-methylurea and 156 mL of 50% (w/v) KOH solution. The reaction mixture was allowed to stand overnight before it was washed with 50% saturated $NaHCO_3$ and saturated NaCl. The material 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 1.25 g (83%) of a colorless liquid. Separation of the isomers was achieved by preparative TLC on 2% $AgNO_3$-impregnated silica gel plates with three developments (3:7:0.4 hexanes/CH_2Cl_2/diethyl ether). A total of 695 mg (46%) of a colorless liquid was recovered: TLC (R_f 0.36; 9:1 hexanes/diethyl ether); UV (ethanol) λ_{max} 265 nm (ε 11 000); IR (CCl_4) 2960, 2920, 2870, 1735, 1650, 1432, 1345, 1265, 1190, 1160, 1070, 1010, 826 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.60 (2, b s, H at C(2′) and C(3′)), 3.65 (3, s, OCH_3), 2.68 (1, ddq, *J* = 6.78, 6.86, 8.10 Hz, H at C(3)), 2.46 (1, dd, *J* = 6.78, 14.63 Hz, H_a at C(2)), 2.27 (1, dd, *J* = 8.10, 14.63 Hz, H_b at C(2)), 1.76 (3, s, CH_3 at C(4′)), 1.06 ppm (3, d, *J* = 6.86 Hz, CH_3 at C(3)); ^{13}C NMR (75 MHz, $CDCl_3$) 173.3, 138.9, 134.1, 119.3, 118.4, 51.5, 40.2, 37.2, 28.9, 24.8, 23.0, 19.1 ppm; MS (EI, 70 eV) *m/z* 194 (49.6) $[M]^+$, 162 (3.9), 147 (6.9), 121 (100), 105 (82.3), 93 (68.7), 77 (12.0). HRMS. Calcd for $C_{12}H_{18}O_2$: 194.1307. Found: 194.1294.

(3*R*,1′*R*)- and (3*S*,1′*R*)-*N*-(1-Naphthylethyl)-3-(4-methyl-1,3-cyclohexadienyl)butanamide ((*R*,*R*)- and (*S*,*R*)-34). To a solution of 412 mg (10.3 mmol) of NaOH in 30 mL of water was added 500 mg (2.58 mmol) of **33** in 8 mL of THF. After 4.5 h at room temperature, the heterogeneous solution was cooled in an ice bath and acidified with concentrated HCl. The suspension was extracted with diethyl ether and

dried over $MgSO_4$. After removal of solvent by rotary evaporation, 452 mg (97%) of a white solid was obtained: IR (CCl_4) 3250, 2960, 2915, 2860, 2820, 1705, 1650, 1430, 1405, 1275, 1260, 1200, 920, 825 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.64 (1, b d, J = 6.20 Hz, H at C(2')), 5.61 (1, m, H at C(3')), 2.69 (1, ddq, J = 6.70, 8.11, 6.85 Hz, H at C(3)), 2.51 (1, dd, J = 6.70, 14.86 Hz, H_a at C(2)), 2.31 (1, dd, J = 8.11, 14.86 Hz, H_b at C(2)), 2.10 (4, m), 1.77 (3, s, CH_3 at Ph), 1.11 ppm (3, d, J = 6.85 Hz, CH_3 at C(3)); ^{13}C NMR (300 MHz, $CDCl_3$) 178.6, 138.4, 134.1, 119.1, 118.1, 40.1, 36.9, 28.9, 24.8, 23.0, 19.2 ppm; MS (EI, 70 eV) m/z 180 (24.0) $[M]^+$, 121 (98.9), 119 (57.5), 105 (62.2), 93 (100), 79 (10.3), 77 (15.2).

A 418-mg (2.32 mmol) portion of the acid from above in 12 mL of CH_2Cl_2 at room temperature was treated with 0.45 mL (477 mg, 2.78 mmol) of (*R*)-(1-naphthylethyl)amine followed by 28 mg (0.22 mmol) of solid DMAP. DCC (573 mg, 2.78 mmol) was added, and a white precipitate formed during the next 2 h. The suspension was concentrated by rotary evaporation, and the residue was extracted with diethyl ether. After filtration through Celite, solvent was removed by rotary evaporation. The residue was purified by flash chromatography (7:3:1 hexanes/diethyl ether/ethyl acetate) to yield 545 mg (71%) of a white foam. Diastereomers (*R,R*)- and (*S,R*)-**34** were separated by preparative HPLC on a Rainin preparative silica column (6:2:2 hexane/*tert*-butyl methyl ether/ethyl acetate).

(*R,R*)-**34**: mp 136–138 °C; TLC (R_f 0.34; 7:3 hexanes/ethyl acetate); UV (ethanol) λ_{max} 224 (ϵ 57 000), 270 nm (11 500); IR (KBr) 3280, 2960, 2910, 2865, 1635, 1546, 1525, 1445, 1360, 1200, 1170, 820, 795, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.07 (1, m, naphthyl H), 7.85 (1, m, naphthyl H), 7.80 (1, m, naphthyl H), 7.54 (4, m, naphthyl H), 5.92 (1, dq, J = 8.19, 6.68 Hz, H at C(1) of Et), 5.78 (1, b d, J = 8.19 Hz, NH), 5.54 (1, b d, J = 5.65 Hz, H at C(2')), 5.49 (1, dq, J = 5.65, 1.43 Hz, H at C(3')), 2.67 (1, ddq, J = 7.20, 7.45, 6.84 Hz, H at C(3)), 2.32 (1, dd, J = 7.45, 14.26 Hz, H_a at C(2)), 2.11 (1, dd, J = 7.20, 14.26 Hz, H_b at C(2)), 1.99 (4, m), 1.72 (3, d, J = 1.43 Hz, CH_3 at C(4')), 1.64 (3, d, J = 6.68 Hz, H at C(2) of Et), 1.06 ppm (3, d, J = 6.84 Hz, H at C(4)); ^{13}C NMR (300 MHz, $CDCl_3$) 170.8, 138.8, 138.2, 134.0, 133.9, 131.1, 129.2, 128.6, 128.3, 126.5, 125.8, 125.1, 123.5, 122.5, 119.1, 44.4, 42.4, 37.5, 28.7, 24.5, 22.9, 20.6, 19.2 ppm; MS (EI, 70 eV) m/z 333 (5.8) $[M]^+$, 241 (8.1), 178 (4.6), 155 (100), 119 (69.1), 91 (4.1).

(*S,R*)-**34**: mp 130–131 °C; TLC (R_f 0.4; 7:3 hexanes/ethyl acetate); IR (KBr) 3280, 3060, 2960, 2915, 2860, 1635, 1550, 1440, 1175, 1085, 995, 960, 905, 860, 815, 795, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.09 (1, m, naphthyl H), 7.85 (1, m, naphthyl H), 7.80 (1, m, naphthyl H), 7.50 (4, m, naphthyl H), 5.92 (1, dq, J = 8.09, 6.68 Hz, H at C(1) of Et), 5.77 (1, b d, J = 8.09 Hz, NH), 5.55 (1, d, J = 5.53 Hz, H at C(2')), 5.49 (1, dq, J = 5.53, 1.43 Hz, H at C(3')), 2.67 (1, ddq, J = 7.22, 7.46, 6.85 Hz, H at C(3)), 2.33 (1, dd, J = 7.46, 14.26 Hz, H_a at C(2)), 2.11 (1, dd, J = 7.22, 14.26 Hz, H_b at C(2)), 1.99 (4, m), 1.72 (3, d, J = 1.43 Hz, CH_3 at C(4')), 1.64 (3, d, J = 6.68 Hz, H at C(2) of Et), 1.06 ppm (3, d, J = 6.85 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 170.1, 138.8, 138.2, 133.9, 131.0, 129.1, 128.6, 128.2, 126.6, 126.4, 125.8, 125.0, 123.5, 122.5, 119.0, 44.3, 42.3, 37.5, 28.7, 24.4, 22.8, 20.5, 19.2 ppm; MS (EI, 70 eV) m/z 333 (5.6) $[M]^+$, 241 (8.0), 178 (4.5), 155 (100), 119 (68.6). Anal. Calcd for $C_{23}H_{27}ON$: C, 82.84; H, 8.16; N, 4.20. Found: C, 83.02; H, 8.17; N, 4.11.

(*R*)-3-(4-Methyl-1,3-cyclohexadienyl)butan-1-ol ((*R*)-10-OH). A suspension of 18 mg (0.45 mmol) of KH in 1.9 mL of THF and 100 mg (0.3 mmol) of (*R,R*)-**34** was stirred for 1.5 h at room temperature before addition of 37 μL (85 mg, 0.6 mmol) of CH_3I. After 20 min, 1 mL of saturated NH_4Cl was added, and the mixture was extracted with diethyl ether. The organic layer was washed with water and saturated NaCl and dried over $MgSO_4$. Solvent was removed by rotary evaporation to give 95 mg (92%) of an oil: TLC (R_f 0.41; 4:6 hexanes/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$) 8.09 (1, m), 7.85 (1, m), 7.80 (1, m), 7.49 (4, m), 6.63 (1, q, J = 6.88 Hz, H at C(1) of Et), 5.60 (2, b s), 2.80 (1, m), 2.49 (3, s, NCH_3), 2.46 (1, m), 2.27 (1, dd, J = 8.00 Hz, J = 14.73 Hz, H_a at C(2)), 2.09 (4, m), 1.77 (3, b s), 1.59 (3, d, J = 6.88 Hz, H at C(2) of Et), 1.14 ppm (3, d, J = 6.83 Hz, H at C(4)); MS (EI, 17 eV) m/z 347 (21.8) $[M]^+$, 346 (8.1), 191 (2.4), 170 (7.1), 156 (14.8), 154 (5.3), 119 (3.6), 86 (55.1), 84 (100).

A solution of 10 mL (10 mmol) of DIBAL (1 M in hexanes) and 5 mL of THF was cooled to 0 °C before addition of 4.6 mL (10.0 mmol) of *n*-butyllithium (2.18 M in hexanes).

N-Methyl amide from above was treated with 3.0 mL (1.53 mmol) of the hydride reagent. After 2 h at 0 °C, 11 mg (0.3 mmol) of $NaBH_4$ in 2 mL of methanol was added, and after 10 min, the mixture was poured into 40 mL of CH_2Cl_2. A clear biphasic mixture was obtained after 2 h of vigorous mixing with 40 mL of saturated sodium potassium tartrate solution. The organic layer was dried over Na_2SO_4 before the solvent was removed at reduced pressure. The residue was purified by flash chromatography (4:6 hexanes/diethyl ether) to yield 27 mg (60%) of a colorless liquid: TLC (R_f, 0.27; 1:1 hexanes/diethyl ether); UV (ethanol) λ_{max} 265 nm (ϵ 4800); $[\alpha]^{22}_D$ −30° (*c* 0.2, ethanol); IR (neat) 3370, 2958, 2925, 2870, 2820, 1450, 1050, 824 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.64 (1, d, J = 5.62 Hz, H at C(2')), 5.61 (1, m, H at C(3')), 3.63 (2, t, J = 6.58 Hz, H at C(1)), 2.34 (1, m), 2.09 (4, m), 1.77 (3, b s, CH_3 at C(4')), 1.62 (2, m), 1.04 ppm (3, d, J = 6.68 Hz, CH_3 at C(3)); ^{13}C NMR (75 MHz, $CDCl_3$) 140.2, 133.8, 129.1, 126.7, 61.7, 37.7, 37.6, 28.9, 23.9, 23.0, 19.7 ppm; MS (EI, 70 eV) m/z 166 (44.2) $[M]^+$, 151 (2.1), 135 (2.8), 133 (8.3), 121 (100), 105 (38.2), 93 (80). HRMS. Calcd for $C_{11}H_{18}O$: 166.1358. Found: 166.1345.

(*R*)-3-(4-Methyl-1,3-cyclohexadienyl)butan-1-yl 2-Naphthoate ((−)-(*R*)-10-ONp). To a solution of 17 mg (0.1 mmol) of 2-naphthoic acid, 12 mg (0.1 mmol) of DMAP, and 16 mg (0.1 mmol) of DMAP-HCl in 0.5 mL of CH_2Cl_2 was added 21 mg (0.1 mmol) of DCC. The mixture was stirred for 1.5 h before a solution of 10 mg (0.06 mmol) of (*R*)-**10**-OH was added in 0.50 mL of CH_2Cl_2. After 4 h, the mixture was concentrated by a stream of nitrogen, and the residue was extracted with hexanes and filtered. Solvent was removed at reduced pressure, and the residue was purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 17 mg (89%) of a colorless film: TLC (R_f 0.28; 6:4 hexanes/diethyl ether); $[\alpha]^{22}_D$ −101.2°, $[\alpha]^{22}_{546}$ −124.°, $[\alpha]^{22}_{436}$ −259.8°, $[\alpha]^{22}_{365}$ −546° (*c* 0.082, acetonitrile); UV (acetonitrile) λ_{max} 236 (ϵ 62 000), 269 (14 300), 278 (14 000); IR (neat) 2957, 2924, 1716, 1284, 1277, 1195, 1154, 1014, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.59 (1, b s), 8.06 (1, dd, J = 1.70, 8.71 Hz), 7.96 (1, b dd, J = 1.61, 8.71 Hz), 7.88 (2, b d, J = 8.52 Hz), 7.56 (2, m), 5.64 (1, b d, J = 5.15 Hz, H at C(3')), 5.61 (1, m, H at C(2')), 4.39 (1, dt, J = 10.99, 7.01 Hz, H_a at C(1)), 4.33 (1, dt, J = 10.99 Hz, J = 7.04 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), 1.77 (3, s, CH_3 at C(4')), 1.13 ppm (3, d, J = 6.95 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 166.6, 139.0, 135.3, 133.8, 132.3, 130.8, 129.2, 128.1, 127.9, 127.6, 127.5, 126.5, 125.1, 119.2, 119.0, 63.9, 37.6, 33.7, 28.9, 23.9, 23.0, 19.7 ppm. HRMS. Calcd for $C_{22}H_{24}O_2$: 320.1770. Found: 320.1774.

(*R*)-3-(4-Methylphenyl)butan-1-yl 2-Naphthoate ((−)-(*R*)-36-ONp). A solution of 6 mg (0.0188 mmol) of (−)-(*R*)-**10**-ONp in 0.35 mL of CH_2Cl_2 was cooled to −30 °C before addition of 3.8 mg (0.022 mmol) of Na_2CO_3 followed by 2 mg (0.020 mmol) of *m*CPBA (95%). After 1.5 h at −30 °C, the mixture was diluted with water and pipetted into CH_2Cl_2. The organic layer was dried over Na_2SO_4, and solvent was removed by rotary evaporation to yield 5.5 mg of an oil. The material was purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 1 mg (17%) of a colorless oil: TLC (R_f 0.33; 9:1 hexanes/diethyl ether), (R_f 0.55; 75:25 CH_2Cl_2/hexanes, 2% $AgNO_3$ TLC plate); $[\alpha]^{22}_{365}$ −240° (*c* 0.01, acetonitrile); ^1H NMR (300 MHz, $CDCl_3$) 8.53 (1, b s), 8.01 (1, m), 7.95 (1, m), 7.88 (2, m), 7.57 (2, m), 7.13 (4, m, Ph H), 4.33 (1, dt, J = 11.01 Hz, J = 6.90 Hz, H_a at C(1)), 4.26 (1, dt, J = 11.01 Hz, J = 6.90 Hz, H_b at C(1)), 2.95 (1, tq, J = 7.01 Hz, J = 7.02 Hz, H at C(3)), 2.30 (3, s, CH_3 at Ph), 2.11 (2, m), 1.34 ppm (3, d, J = 7.02 Hz, H at C(4)); MS (EI, 70 eV) m/z 318 (0.5) $[M]^+$, 172 (4.5), 155 (11.5), 146 (77.1), 131 (100), 127 (39.9), 119 (32.0), 91 (7.2).

(3*R*,1'*R*)- and (3*S*,1'*R*)-*N*-(1-Naphthylethyl)-3-(4-methylphenyl)-butanamide ((*R,R*)- and (*S,R*)-35). Using a protocol similar to that described for amides (*R,R*)- and (*S,R*)-**34**, 356 mg (2.0 mmol) of **31** was dissolved in 10 mL of CH_2Cl_2, and the solution was treated in sequence with 0.39 mL (411 mg, 2.4 mmol) of (*R*)-(1-naphthylethyl)amine, 24 mg (0.196 mmol) of DMAP, and 494 mg (2.4 mmol) of DCC. Diastereomers were isolated by flash chromatography (4:6 hexanes/diethyl ether) to yield a total of 563 mg (85%) of white solids.

(*R,R*)-**35**: TLC (R_f 0.33; 1:1 hexanes/diethyl ether); UV (ethanol) λ_{max} 224 (ϵ 73 000), 282 (9530), 291 nm (7600); IR (KBr) 3280, 3015, 1625, 1525, 1445, 1365, 1205, 1165, 1095, 915, 810, 795, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.16 (1, m), 8.02 (1, m), 7.94 (1, m), 7.66 (2, m), 7.54 (1, m), 7.39 (1, m), 7.16 (4, m, Ph H), 6.05 (1, dq, J = 7.81, 6.79 Hz, H at C(1) of Et), 5.77 (1, d, J = 7.81 Hz, NH), 3.42 (1, tq, J = 7.02, 6.77 Hz, H at C(3)), 2.58 (2, m), 2.45 (3, s, CH_3 at Ph), 1.75 (3, d, J = 6.79 Hz, H at C(2) of Et), 1.46 ppm (3, d, J = 7.02 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 170.6, 142.6, 138.2, 135.8, 133.8, 131.0, 129.2, 128.7, 128.2, 126.7, 126.6, 125.8, 125.1, 123.5, 122.4, 45.8, 44.5, 36.6, 22.0, 21.8, 20.8 ppm; MS (EI, 70 eV) m/z 331 (70.4) $[M]^+$, 212 (27.2), 170 (11.5), 155 (100), 134 (36.4), 133 (38.8), 119 (44.9). HRMS. Calcd for $C_{23}H_{25}ON$: 331.1938. Found: 331.1956.

(*S,R*)-**35**: TLC (R_f 0.41; 1:1 hexanes/diethyl ether); IR (KBr) 3240, 3040, 2955, 2915, 1625, 1542, 1505, 1445, 1365, 815, 798, 775, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.03 (1, m), 7.85 (1, m), 7.78 (1, m), 7.51 (2, m), 7.41 (1, m), 7.36 (1, m), 7.08 (4, s, Ph H), 5.83 (1, dq, J = 7.04 Hz, J = 6.74 Hz, H at C(1) of Et), 5.40 (1, b d, J = 7.04 Hz, NH), 3.27 (1, tq, J = 6.98, 7.54 Hz, H at C(3)), 2.37 (2, d, J = 7.54 Hz, H at C(2)), 2.31 (3, s, CH_3 at Ph), 1.44 (3, d, J = 6.74 Hz, H at C(2) of Et), 1.25 ppm (3, d, J = 6.98 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 170.5, 142.6, 138.1, 135.9, 133.8, 131.0, 129.2, 128.7, 128.3, 126.7, 126.5,

125.8, 125.1, 123.5, 122.5, 46.0, 44.4, 36.8, 21.8, 21.0, 20.3 ppm; MS (EI, 70 eV) m/z 331 (73.4) $[M]^+$, 212 (32.7), 176 (4.6), 170 (9.4), 155 (100), 134 (38.5), 133 (40.9), 119 (36.2).

(*R*)-3-(4-Methylphenyl)butan-1-ol ((−)-(*R*)-36-OH). As described for (*R*)-**10**-OH, 166 mg (0.5 mmol) of (*R*,*R*)-**35** was added to a suspension of 30 mg (0.75 mmol) of KH (free from oil) in 2 mL of THF. After 1.5 h, the yellow solution was treated with 62 μL (142 mg, 1.0 mmol) of CH_3I. The product was purified by flash chromatography (6:4 hexanes/diethyl ether) to yield 160 mg (92%) of a white powder: TLC (R_f 0.34; 4:6 hexanes/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$) 7.85 (2, m), 7.48 (3, m), 7.35 (1, m), 7.12 (5, m), 6.61 (1, q, J = 6.89 Hz, H at C(2) of Et), 3.40 (1, m, H at C(3)), 2.63 (1, dd, J = 6.48, 15.10 Hz), 2.54 (1, dd, J = 7.92, 15.10 Hz), 2.45 (3, s, NCH_3), 2.32 (3, s, CH_3 at Ph), 1.60 (3, d, J = 6.89 Hz, H at C(2) of Et), 1.37 ppm (3, d, J = 6.92 Hz, H at C(4)); MS (EI, 70 eV) m/z 345 (77.1) $[M]^+$, 190 (8.6), 170 (10.4), 155 (69.8), 133 (100), 119 (54.9).

The material (160 mg, 0.46 mmol) from above was treated with 4.8 mL (1.95 mmol) of DIBAL/*n*-butyl lithium solution (0.4 M in THF) at 0 °C. After 1.2 h, 3 mL of methanol was added followed by 19 mg (0.50 mmol) of $NaBH_4$. The mixture was kept at 0 °C for 1 h before workup with 10 mL of saturated sodium potassium tartrate solution for 4 h. The material was purified by flash chromatography (1:1 hexanes/diethyl ether) to yield 51 mg (67%) of a colorless liquid: TLC (R_f 0.30; 1:1 hexanes/diethyl ether); $[\alpha]^{22}_D$ −32.2° (*c* 0.50, $CHCl_3$), lit[19] $[\alpha]^{22}_D$ +33°; ^1H NMR (300 MHz, $CDCl_3$) 7.11 (4, b s, Ph H), 3.55 (2, m, H at C(1)), 2.85 (1, tq, J = 7.86, 6.99 Hz, H at C(3)), 2.32 (3, s, CH_3 at Ph), 1.84 (2, m, H at C(2)), 1.25 ppm (3, d, J = 6.99 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 143.8, 135.6, 129.2, 126.9, 61.4, 41.1, 36.1, 22.6, 21.0 ppm; MS (EI, 70 eV) m/z 164 (10.0) $[M]^+$, 131 (8.8), 119 (100), 105 (6.8), 91 (7.0). HRMS. Calcd for $C_{11}H_{16}O$: 164.1202. Found: 164.1197.

(*R*)-3-(4-Methylphenyl)butan-1-yl 2-Naphthoate ((−)-(*R*)-36-ONp). Following the esterification of (*R*)-(−)-**10**-OH, 10 mg (0.061 mmol) of the alcohol (−)-(*R*)-**36**-OH was added to a reaction flask which contained 12 mg (0.068 mmol) of 2-naphthoic acid, 9 mg (0.068 mmol) of DMAP, 11 mg (0.068 mmol) of DMAP·HCl, and 15 mg (0.068 mmol) of DCC in 0.7 mL of CH_2Cl_2. After workup, the residue was purified by flash chromatography (9:1 hexanes/diethyl ether) to yield 16 mg (84%) of a colorless oil: TLC (R_f 0.25; 6:4 hexanes/CH_2Cl_2); $[\alpha]^{22}_{365}$ −229° (*c* 0.048, acetonitrile); ^1H NMR (300 MHz, $CDCl_3$) 8.53 (1, b s), 8.00 (1, m), 7.95 (1, m), 7.89 (2, m), 7.56 (2 m), 7.13 (4, m, Ph H), 4.33 (1, dt, J = 11.00, 6.83 Hz, H_a at C(1)), 4.25 (1, dt, J = 11.00, 6.83 Hz, H_b at C(1)), 2.95 (1, tq, J = 6.83, 6.98 Hz, H at C(3)), 2.30 (3, s, CH_3 at Ph), 2.13 (2, m), 1.34 (ppm (3, d, J = 6.98 Hz, H at C(4)); ^{13}C NMR (75 MHz, $CDCl_3$) 166.7, 143.2, 135.7, 135.5, 132.5, 130.9, 129.3, 129.2, 128.1, 128.0, 127.7, 127.6, 126.8, 126.6, 125.2, 63.7, 37.0, 36.6, 22.6, 20.9 ppm; MS (EI, 70 eV) m/z 318 (0.4) $[M]^+$, 199 (6.8), 155 (11.2), 146 (92.2), 131 (100), 127 (38.2), 119 (32.9). Anal. Calcd for $C_{22}H_{22}O_2$: C, 82.99; H, 6.96. Found: C, 82.83; H, 6.00.

Methyl 6-(1-Ethoxyethyl)-2-hexynoate (38-OEE). To a solution of 6.1 g (72.6 mmol) of 4-pentyn-1-ol (**37**, OH) and 50 mL (0.524 mmol) of freshly distilled ethyl vinyl ether in 75 mL of CH_2Cl_2 was added 0.25 g (1.0 mmol) of pyridinium *p*-toluenesulfonate at 0 °C, and the reaction mixture was warmed to room temperature after 0.5 h. After 2 h, the volatile materials were removed by rotary evaporation. The residue was treated with 50 mL of hexanes and filtered through Celite. Solvent was removed under vacuum to give 11.2 g (98%) of a colorless liquid judged to be 98% a single component by GC; ^1H NMR (300 MHz, $CDCl_3$) 4.65 (1, q, J = 5.35 Hz, H at C(7)), 3.63 (2, m), 3.45 (2, m), 2.27 (2, dt, J = 2.67, 6.96 Hz), 1.91 (1, t, J = 2.67 Hz, H at C(1)), 1.75 (2, tt, J = 6.28, 6.96 Hz, H at C(3)), 1.27 (3, d, J = 5.35 Hz, CH_3 at C(7)), 1.17 ppm (3, t, J = 7.06 Hz, H at C(10)); ^{13}C NMR (75 MHz, $CDCl_3$) 99.5, 83.8, 68.4, 63.2, 60.7, 28.7, 19.8, 15.32, 15.29 ppm.

The material from above (11.2 g, 71.8 mmol) in 120 mL of THF was cooled to −70 °C before addition of 33 mL (83.5 mmol) of *n*-butyl lithium (2.5 M in hexanes) over 15 min. After 3 h, 7.0 mL (8.5 g, 90.0 mmol) of methyl chloroformate was added, and the solution was allowed to warm to 0 °C over a 10-h period before quenching with saturated NH_4Cl solution. Diethyl ether was added, and the suspension was washed in succession with water and saturated $NaHCO_3$. The organic layer was dried over a 1:1 (w/w) mixture of $MgSO_4/Na_2CO_3$, and solvent was removed by rotary evaporation. The residue was purified by flash chromatography (7:3 hexanes/diethyl ether) to yield 13.08 g (84%) of a colorless liquid. An analytical sample was purified by Kugelrohr distillation: TLC (R_f 0.42; 7:3 hexanes/diethyl ether); IR (neat) 2970, 2910, 2230, 1708, 1430, 1370, 1250, 1125, 1065, 955, 860, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 4.65 (1, q, J = 5.35 Hz, H at C(8)), 3.73 (3, s, OCH_3), 3.62 (2, m), 3.47 (2, m), 2.44 (2, t, J = 7.09 Hz, H at C(4)), 1.81 (2, tt, J = 7.09, 6.07 Hz, H at C(5)), 1.27 (3, d, J = 5.35 Hz, H at C(8)), 1.17 ppm (3, t, J = 7.06 Hz, CH_3 at C(11)); ^{13}C NMR (75 MHz, $CDCl_3$) 154.1, 99.6, 89.0, 73.0, 62.9, 60.8, 52.5, 27.8, 19.7, 15.5, 15.2 ppm; MS (EI, 17 eV) m/z 199 (9.3) $[M - CH_3]^+$, 169 (11.1) $[M - C_2H_5O]^+$, 137 (10.3), 125 (28.8), 111 (8.3), 73 (100). Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.79; H, 8.65.

(*Z*)-Methyl-6-(1-Ethoxyethyl)-3-methyl-2-hexenoate (39-OEE). A suspension of 10.9 g (57.4 mmol) of CuI in 250 mL of THF was cooled to −30 °C before the addition of 80 mL (12.0 mmol) of CH_3Li (1.5 M in diethyl ether) over 25 min. The clear, colorless solution was then cooled to −78 °C, and 10.68 g (49.9 mmol) of **38**-OEE in 30 mL of THF was added over 40 min, while the temperature was maintained at less than −75 °C. The mixture was stirred at −78 °C for an additional 1 h before the reaction was quenched with absolute ethanol. The contents of the flask were poured into saturated NH_4Cl (made basic with NH_4OH), and the mixture was stirred vigorously for 2.5 h. Organic material was extracted with diethyl ether and washed with 50% saturated NH_4Cl. The organic layer was dried over a 1:1 (w/w) mixture of $MgSO_4/K_2CO_3$, solvent was removed by rotary evaporation, and the residue was diluted with hexanes before filtration through Celite. Rotary evaporation of solvent, followed by flash chromatography (8:2 hexanes/diethyl ether), gave 9.82 g (86%) of a colorless liquid. Analysis by GC indicated that the material consisted of a 96:4 isomeric ratio of products: TLC (R_f 0.4; 7:3 hexanes/diethyl ether); IR (neat) 2985, 2965, 1716, 1640, 1435, 1375, 1230, 1185, 1140, 1020, 950, 915, 855 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.66 (1, b s, H at C(2)), 4.66 (1, q, J = 5.31 Hz, H at C(8)), 3.65 (3, s, OCH_3), 3.63 (2, m), 3.45 (2, m), 2.67 (2, b t, J = 7.99 Hz, H at C(4)), 1.88 (3, s, CH_3 at C(3)), 1.73 (2, m), 1.28 (3, d, J = 5.31 Hz, CH_3 at C(8)), 1.18 ppm (3, t, J = 7.07 Hz, H at C(11)); ^{13}C NMR (75 MHz, $CDCl_3$) 166.4, 160.4, 115.9, 99.6, 65.1, 60.8, 50.7, 30.1, 28.4, 25.1, 19.9, 15.3 ppm; MS (EI, 17 eV) m/z 215 (1.2) $[M - CH_3]^+$, 199 (4.2), 185 (9.2) $[M - C_2H_5O]^+$, 169 (3.9), 158 (62.9), 157 (65.1), 141 (90) $[M - C_4H_9O_2]^+$, 127 (78.9), 125 (68), 112 (48.6), 109 (87.5), 81 (58.6), 73 (100). Anal. Calcd for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.69; H, 9.76.

(*Z*)-[1-$^2H_2$]-6-(1-Ethoxyethyl)-3-methyl-2-hexen-1-ol ([1-$^2H_2$]40-OH,OEE). A solution of 3.22 g (14.0 mmol) of **39**-OEE in 70 mL of diethyl ether was cooled to −10 °C. Solid $LiAl^2H_4$ (0.59 g, 14.0 mmol) was added, and the contents of the flask were maintained at −10 °C for 3–4 h. The reaction was quenched with a 2:1 (w/w) mixture of $Na_2SO_4{\cdot}10H_2O$/Celite, allowed to warm to room temperature, and filtered. Solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (3:7 hexanes/diethyl ether) to give 2.54 g (89%) of a colorless liquid: TLC (R_f 0.3; 3:7 hexanes/diethyl ether); IR (neat) 3400, 2970, 2930, 2870, 1655, 1440, 1375, 1335, 1125, 1080, 1050, 950 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.49 (1, b s, H at C(2)), 4.64 (1, q, J = 5.34 Hz, H at C(8)), 3.59 (2, m), 3.43 (2, m), 2.27 (1, dt, J = 7.57, 5.59 Hz, H_a at C(4)), 2.10 (1, dt, J = 7.57, 6.73 Hz, H_b at C(4)), 2.05 (1, b s, OH), 1.72 (3, d, J = 1.46 Hz, CH_3 at C(3)), 1.69 (2, m), 1.30 (3, d, J = 5.34 Hz, CH_3 at C(8)), 1.19 ppm (3, t, J = 7.06 Hz, H at C(11)); ^{13}C NMR (75 MHz, $CDCl_3$) 138.9, 125.4, 99.8, 64.1, 61.3, 58.3, 27.9, 27.5, 23.0, 20.0, 15.3 ppm. An adequate level of signal to noise was not achieved to measure $J_{C,D}$. ^2H NMR (46 MHz, CH_2Cl_2) 3.94 (d, J = 4.29 Hz) ppm. Anal. Calcd for $C_{11}H_{20}O_3$: C, 64.67; H, 10.85. Found: C, 64.53; H, 11.03.

(*S*,*Z*)-[1-^2H]-6-(1-Ethoxyethyl)-3-methyl-2-hexen-1-ol ((*S*)-[1-^2H]-40-OH,OEE). To a solution of 2.5 g (12.25 mmol) of [1-$^2H_2$]**40**-OH,-OEE in 80 mL of CH_2Cl_2 was added 43.5 g (0.5 mmol) of activated MnO_2 in three equal portions over 3 h. The black suspension was stirred for 21 h at room temperature before it was filtered through Celite. The filtrate was dried over Na_2SO_4, and the solvent was removed by rotary evaporation to yield 1.75 g (71%) of a pale-yellow liquid. A sample was retained for spectral analysis, and the bulk of the material was used immediately in the following reduction: TLC (R_f 0.47; 4:6 hexanes/diethyl ether); IR (neat) 2970, 2930, 2870, 1655, 1625, 1435, 1370, 1325, 1120, 1050, 985, 950, 890 cm^{-1}; ^1H NMR 5.79 (1, b s, H at C(2)), 4.48 (1, q, J = 5.35 Hz, H at C(8)), 3.34 (3, m), 3.12 (1, m), 2.24 (2, dt, J = 2.48, 7.44 Hz, H at C(4)), 1.40 (2, m), 1.73 (3, d, J = 1.34 Hz, CH_3 at C(3)), 1.21 (3, d, J = 5.35 Hz, CH_3 at C(8)), 1.10 ppm (3, t, J = 7.04 Hz, CH_3 at C(11)); ^2H NMR (46 MHz, CH_2Cl_2) 9.74 ppm.

To a solution of 2.99 g (12.25 mmol) of 9-borabicyclo[3.3.1]nonane in 30 mL of THF was added 4.28 mL (3.67 g, 26.95 mmol) of 1-(*R*)-α-pinene (98% ee, Aldrich Chemical Co.). The solution was heated at gentle reflux for 3 h and cooled to room temperature. Solvent was removed by a positive argon stream, followed by reduced pressure (25 mmHg). The colorless solution was cooled to 0 °C, and 1.75 g (8.7 mmol) of the aldehyde from above in 13 mL of THF was added over 7 min. During the next 9 h, the solution was allowed to warm to room temperature and was then cooled to 0 °C. Acetaldehyde (7 mL) was added and the solution warmed to room temperature. Solvent was removed by rotary evaporation, and pinene was partially removed at reduced pressure (0.1 mmHg for 1.5 h). The residue was dissolved in 30

mL of diethyl ether, cooled to 0 °C, and treated with 1.6 g (1.6 mL, 26 mmol) of ethanolamine. A heavy white precipitate was removed by filtration through Celite. The filtrate was washed with saturated $NaHCO_3$, dried over 2:1 (w/w) $MgSO_4/Na_2CO_3$, and concentrated by rotary evaporation. The product was purified by flash chromatography (3:7 hexanes/diethyl ether) to yield 1.54 g (87%) of a colorless liquid: TLC (R_f 0.30; 3:7 hexanes/diethyl ether); IR (neat) 3450, 2970, 2920, 1660, 1440, 1375, 1335, 1290, 1125, 1050, 945, 865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 5.49 (1, d, J = 7.17 Hz, H at C(2)), 4.64 (1, q, J = 5.35 Hz, H at C(8)), 4.12 and 4.07 (1, d, J = 7.17 Hz, H at C(1)) ratio of 94:6, 2.28 (1, dt, J = 6.89, 6.73 Hz, H_a at C(4)), 2.10 (1, dt, J = 6.89, 6.89 Hz, H_b at C(4)), 2.06 (1, b s, OH), 1.72 (3, s, CH_3 at C(3)), 1.29 (3, d, J = 5.35 Hz, CH_3 at C(8)), 1.19 (3, t, J = 7.05 Hz, CH_3 at C(11)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 138.8, 125.3, 64.1, 61.3, 58.3 (t, $J_{C,D}$ = 21.5 Hz), 53.4, 27.8, 27.5, 23.1, 20.0, 15.3 ppm; ^2H NMR (46 MHz, CH_2Cl_2) 4.05 ppm.

(*Z*)-1-[(*S*)-*O*-Acetylmandelyl]-3-methyl-2-hexen-6-ol (40-OAMA,-OH). A 1.5-mL solution of 69 mg (0.3 mmol) of **39**-OEE in diethyl ether was cooled to −10° and treated with 17 mg (0.45 mmol) of $LiAlH_4$. After 2 h at 0°, the mixture was quenched with $Na_2SO_4 \cdot 10H_2O$/Celite and dried over $MgSO_4/K_2CO_3$. Solvent was removed by rotary evaporation to yield 53 mg (90%) of a clear colorless liquid.

A solution of 20 mg (0.099 mmol) of the above alcohol, 23 mg (0.114 mmol) of (*S*)-*O*-acetylmandelic acid, 2 mg (0.016 mmol) of DMAP, and 100 μL (23.7 mg, 0.115 mmol) of DCC (1.15 M in CH_2Cl_2) in 1 mL of CH_2Cl_2 was stirred at room temperature for 3 h. The mixture was diluted with hexanes and filtered through glass wool. Solvent was removed by rotary evaporation, and the residue was purified by preparative TLC (7:3 hexanes/diethyl ether) with three developments to yield 24 mg (63%) of a colorless oil: TLC (R_f 0.31; 6:4 hexanes/diethyl ether).

The material was diluted in 1.2 mL of THF, 0.4 mL of 0.1 N HCl was added, and the mixture was stirred for 9 h. Diethyl ether was added, and the organic layer was washed with water and saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, solvent was removed by rotary evaporation, and the residue was purified by preparative TLC (1:1 hexanes/ethyl acetate). The sample was carefully dried at 0.02 mmHg for 24 h before analysis; 18 mg (87%): TLC (R_f 0.28; 6:4 ethyl acetate/hexanes); IR (neat) 3420, 2915, 2870, 1735, 1490, 1440, 1365, 1225, 1200, 1170, 1045 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) 7.46 (2, m, Ph H), 7.05 (3, m, Ph H), 6.04 (1, s, H at C(2')), 5.21 (1, t, J = 6.13 Hz, H at C(2)), 4.67 (1, dd, J = 7.49, 12.22 Hz, H_a at C(1)), 4.44 (1, dd, J = 7.31, 12.22 Hz, H_b at C(2)), 3.28 (2, t, J = 6.13 Hz, H at C(7)), 1.90 (2, m), 1.71 (3, s, CH_3 at C(3)), 1.40 (3, s, acetyl), 1.33 ppm (2, m); ^{13}C NMR (75 MHz, $CDCl_3$) 170.3, 168.7, 143.3, 133.6, 129.1, 128.6, 127.6, 118.6, 74.6, 62.2, 61.8, 30.5, 28.0, 23.3, 20.8 ppm.

(*S*,*Z*)-[1-^{2}H]-1-[(*S*)-*O*-Acetylmandelyl]-3-methyl-2-hexen-6-ol ((*S*,-*S*)-[1-^{2}H]40-OAMA,OH). Following the procedure for **40**-OAMA,OH, (*S*)-[1-^{2}H]**40**-OH,OEE was esterified with (*S*)-*O*-acetylmandelic acid and the ethoxyethyl group was removed with 0.1 N HCl to yield 17 mg (55%) of a colorless oil: ^1H NMR (300 MHz, C_6D_6) 7.46 (2, m, Ph H), 7.06 (3, m, Ph H), 6.04 (1, s, H at C(2')), 5.20 (1, d, J = 7.12 Hz, H at C(2)), 4.63 (0.09, b d, J = 6.78 Hz, H_a at C(1)), 4.41 (0.91, b d, J = 6.78 Hz, H_b at C(1)), 3.28 (2, t, J = 6.13 Hz, H at C(7)), 1.90 (2, m), 1.71 (3, s, CH_3 at C(3)), 1.40 (3, s, acetyl), 1.33 ppm (2, m); ^{13}C NMR (75 MHz, $CDCl_3$) 170.3, 168.7, 143.4, 133.6, 129.1, 128.6, 127.6, 118.6, 74.6, 61.9 (t, $J_{C,D}$ = 22.7 Hz), 61.7, 30.5, 28.0, 23.4, 20.8 ppm.

(*S*,*Z*)-[1-^{2}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3-methyl-2-hexen-6-ol ((*S*)-[1-^{2}H]40-OTBDPS,OH). A solution of 2.23 g (8.13 mmol) of *tert*-butyldiphenylsilyl chloride in 5 mL of CH_2Cl_2 was added to 1.5 g (7.39 mmol) of (*S*)-[1-^{2}H]**40**-OH,OEE, 1.11 g (16.26 mmol) of imidazole, and 1.5 mL of *N*,*N*-dimethylformamide in 20 mL of CH_2Cl_2 over a 25-min period. After 3 h at room temperature, the mixture was diluted with diethyl ether and washed in succession with water and saturated $NaHCO_3$. The organic layer was dried over a 2:1 (w/w) mixture of $MgSO_4/Na_2CO_3$. Solvent was removed by rotary evaporation to yield a colorless oil: TLC (R_f 0.53; 6:4 hexanes/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$) 7.69 (4, m, Ph H), 7.40 (6, m, Ph H), 5.40 (1, b d, J = 6.26 Hz, H at C(2)), 4.59 (1, q, J = 5.30 Hz, H at C(8)), 4.18 (1, b d, J = 6.26 Hz, H at C(7)), 3.58 (1, m), 3.42 (2, m), 3.27 (1, m), 1.94 (2, m), 1.71 (3, b s, CH_3 at C(3)), 1.57 (2, m), 1.22 (3, d, J = 5.30 Hz, CH_3 at C(8)), 1.16 (3, t, J = 7.07 Hz, CH_3 at C(11)), 1.05 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) 137.0, 135.5, 133.9, 129.4, 127.5, 125.0, 99.5, 64.8, 60.6, 60.4 (t, $J_{C,D}$ = 22.1 Hz), 28.7, 28.2, 26.9, 23.3, 19.9, 19.2, 15.4 ppm.

The material from above was dissolved in 123 mL of THF and treated with 0.1 N HCl at room temperature for 9 h. The mixture was diluted with hexanes, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. Solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (1:1 hexanes/diethyl ether) to afford 1.88 g (69%) of a colorless liquid: TLC (R_f 0.29; 6:4 hexanes/diethyl ether); IR (neat) 3360, 1658, 1585, 1465, 1421, 1100, 1050, 820, 735, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.70 (4, m, Ph H), 7.41 (6, m, Ph H), 5.43 (1, b d, J = 6.71 Hz, H at C(2)), 4.16 (1, b d, J = 6.71 Hz, H at C(1)), 3.54 (2, t, J = 6.21 Hz, H at C(6)), 2.06 (2, t, J = 7.30 Hz, H at C(4)), 2.01 (1, b s, OH), 1.71 (3, s, CH_3 at C(3)), 1.59 (2, tt, J = 6.21, 7.30 Hz, H at C(5)), 1.05 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) 138.3, 135.5, 133.6, 129.5, 127.5, 124.6, 61.6, 60.1 (t, $J_{C,D}$ = 21.4 Hz), 30.3, 27.8, 26.9, 23.2, 19.2 ppm; MS (EI, 17 eV) m/z 312 (6.8) [M − C_4H_9]$^+$, 281 (2.1), 234 (2.1), 199 (100), 135 (2.0), 119 (4.0), 98 (17.2). Anal. Calcd for $C_{23}H_{31}{}^2HO_2Si$: C, 74.74; H, 8.73. Found: C, 74.73; H, 8.88.

(*S*,*Z*)-[1-^{2}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3-methyl-2-hexen-1-al ((*S*)-[1-^{2}H]5-OTBDPS). To a solution of 0.35 mL (0.508 g, 4.0 mmol) of oxalyl chloride in 14 mL of CH_2Cl_2 at −30 °C was added 0.45 mL (0.5 g, 6.4 mmol) of DMSO. The flask was cooled to −75 °C, and 1.18 g (3.2 mmol) of (*S*)-[1-^{2}H]**40**-OTBDPS,OH in 6 mL of CH_2Cl_2 was added over 10 min. After 20 min, 2.23 mL (1.62 g, 16.0 mmol) of triethylamine was added, and the mixture was stirred for 1 h, warmed to 0 °C, and quenched with 5 mL of 50% saturated NH_4Cl. The mixture was diluted with diethyl ether and washed in succession with 50% saturated NH_4Cl and $NaHCO_3$. The organic layer was dried over $MgSO_4$, solvent was removed by rotary evaporation, and the residue was purified by flash chromatography (8:2 hexanes/diethyl ether) to yield 910 mg (78%) of a colorless oil: TLC (R_f 0.28; 8:2 hexanes/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$) 9.65 (1, b s, H at C(6)), 7.69 (4, m, Ph H), 7.40 (6, m, Ph H), 5.44 (1, b d, J = 6.46 Hz, H at C(2)), 4.18 (1, b d, J = 6.46 Hz, H at C(1)), 2.38 (2, tq, J = 7.59 Hz, J = 1.57 Hz, H at C(4)), 2.19 (2, t, J = 7.59 Hz, H at C(5)), 1.71 (3, s, CH_3 at C(3)), 1.06 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) 201.6, 135.5, 135.4, 133.7, 129.5, 127.5, 125.9, 60.1 (t, $J_{C,D}$ = 21.5 Hz), 42.2, 26.9, 24.5, 23.1, 19.2 ppm; MS (EI, 17 eV) m/z 310 (5.8) [M − C_4H_9]$^+$, 232 (11.2), 199 (100), 139 (4.9), 94 (3.5).

(*S*,*Z*,*E*)- and (*S*,*Z*,*Z*)-[1-^{2}H]-1-((*tert*-Butyldiphenylsilyl)oxy)-3,7-dimethyl-2,6-nonadienyl-9-ol ((*S*)-[1-^{2}H]1-OTBDPS,OH and (*S*)-[1-^{2}H]2-OTBDPS,OH). Using the protocol described for the unlabeled **1**-OTBDPS,OH and **2**-OTBDPS,OH,[11] 602 mg (1.5 mmol) of (3-hydroxypropyl)triphenylphosphonium bromide was treated with 1.24 mL (3.15 mmol) of nBuLi (2.55 M in hexanes). The solution was treated with 0.11 mL (256 mg, 1.8 mmol) of CH_3I followed by 0.65 mL (1.65 mmol) of nBuLi (2.55 M in hexanes). The ylide solution was cooled to −75 °C before addition of (*S*)-[1-^{2}H]**5**-OTBDPS. Following workup, the material was purified by flash chromatography (6:4 hexanes/diethyl ether) to yield 227 mg (46% total) of a mixture of isomers. The isomers were separated by preparative HPLC on silica (7:3 hexane/*tert*-butyl methyl ether).

(*S*)-[1-^{2}H]**1**-OTBDPS,OH: 97 mg; ^1H NMR (300 MHz, $CDCl_3$) 7.68 (4, m, Ph H), 7.40 (6, m, Ph H), 5.40 (1, b d, J = 6.34 Hz, H at C(2)), 5.10 (1, qt, J = 1.25, 6.86 Hz, H at C(6)), 4.16 (1, b d, J = 6.34 Hz, H at C(1)), 3.59 (2, t, J = 6.26 Hz, H at C(9)), 2.16 (2, t, J = 6.26 Hz, H at C(8)), 2.01 (2, m), 1.90 (2, m), 1.71 (3, s, CH_3 at C(3)), 1.54 (3, d, J = 1.25 Hz, CH_3 at C(7)), 1.04 ppm (9, s, tBu); ^{13}C NMR (75 MHz, $CDCl_3$) 137.2, 135.5, 133.9, 131.5, 129.5, 129.4, 127.5, 127.1, 125.0, 60.4 (t, $J_{C,D}$ = 21.6 Hz), 60.1, 42.6, 32.1, 26.9, 26.6, 23.4, 19.2, 15.7 ppm; ^2H NMR (46 MHz, CH_2Cl_2) 4.15 ppm (b s); MS (EI, 17 eV) m/z 423 (0.9) [M]$^+$, 366 (0.7) [M − C_4H_9]$^+$, 229 (3.0), 199 (100), 150 (37.5), 94 (48.4).

(*S*)-[1-^{2}H]**2**-OTBDPS,OH: 137 mg; ^1H NMR (300 MHz, $CDCl_3$) 7.70 (4, m, Ph H), 7.41 (6, m, Ph H), 5.41 (1, b d, J = 5.98 Hz, H at C(2)), 5.19 (1, m, H at C(6)), 4.17 (1, b d, J = 5.98 Hz, H at C(1)), 3.60 (2, b t, J = 6.21 Hz, H at C(9)), 2.23 (2, t, J = 6.21 Hz, H at C(8)), 2.01 (2, m), 1.90 (2, m), 1.72 (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, $CDCl_3$) 137.2, 135.5, 134.0, 131.4, 129.4, 127.7, 127.5, 125.0, 60.55, 60.50 (t, $J_{C,D}$ = 21.0 Hz), 35.0, 32.4, 26.9, 26.5, 23.5, 23.4, 19.3 ppm; ^2H NMR (46 MHz, CH_2Cl_2) 4.15 ppm (b s); MS (EI, 17 eV) m/z 423 (1.8) [M]$^+$, 366 (3.3) [M − C_4H_9]$^+$, 310 (12.1), 277 (5.1) 255 (6.2), 242 (6.4), 232 (51.5), 218 (10.3), 199 (100), 167 (10.7), 150 (90.7).

(*S*,*Z*,*E*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadienyl-9-[((4-methylphenyl)-sulfonyl)oxy]-1-ol ((*S*)-[1-^{2}H]1-OH,OTs). As described for **1**-OH,OTs,[11] 361 mg (0.85 mmol) of (*S*)-[1-^{2}H]**1**-OTBDPS,OH was treated with 245 mg (1.25 mmol) of *p*-toluenesulfonyl chloride and 172 mg (1.41 mmol) of DMAP in 5.5 mL of CH_2Cl_2. After dilution with hexanes, filtration, and removal of solvent, the sample was treated with 379 mg (1.2 mmol) of tetra-*n*-butylammonium fluoride trihydrate in 5.5 mL of THF at 0 °C. Following purification of the product by flash chromatography (3:7 hexanes/diethyl ether), 188 mg (65%) of a colorless oil was obtained: ^1H NMR (300 MHz, $CDCl_3$) 7.78 (2, d, J = 8.55 Hz, Ph H), 7.34 (2, d, J = 8.55 Hz, Ph H), 5.43 (1, b d, J = 7.25 Hz, H at C(2)), 5.14 (1, m, H at C(6)), 4.08 (1, b d, J = 7.25 Hz, H at C(1)), 4.07 (2, t, J = 6.79 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.30 (2, t, J = 6.79 Hz, H at

C(8)), 2.07 (4, m), 1.73 (3, s, CH_3 at C(3)), 1.53 ppm (3, s, CH_3 at C(7)).

(*S*,*Z*,*Z*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadienyl-9-[((4-methylphenyl)-sulfonyl)oxy]-1-ol ((*S*)-[1-^{2}H]2-OH,OTs). As described for **1**-OH,OTs,[11] 130 mg (0.307 mmol) of [1-^{2}H]**2**-OTBDPS,OH was treated with 69 mg (0.353 mmol) of *p*-toluenesulfonyl chloride and 52 mg (0.423 mmol) of DMAP in 1.7 mL of CH_2Cl_2. After dilution with hexanes, filtration, and removal of solvent, the sample was treated with 107 mg (0.338 mmol) of tetra-*n*-butylammonium fluoride trihydrate in 2.0 mL of THF at 0 °C. Following purification of the product by flash chromatography (3:7 hexanes/diethyl ether), 85 mg (82%) of a colorless oil was obtained: ^{1}H NMR (300 MHz, $CDCl_3$) 7.79 (2, d, J = 8.45 Hz, Ph H), 7.34 (2, d, J = 8.45 Hz, Ph H), 5.43 (1, b d, J = 7.08 Hz, H at C(2)), 5.23 (1, m, H at C(6)), 4.06 (1, b d, J = 7.08 Hz, H at C(1)), 4.03 (2, t, J = 7.07 Hz, H at C(9)), 2.45 (3, s, CH_3 at Ph), 2.38 (2, t, J = 7.07 Hz, H at C(8)), 2.06 (4, m), 1.71 (3, s, CH_3 at C(3)), 1.61 ppm (3, s, CH_3 at C(7)).

(*S*,*Z*,*E*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ((*S*)-[1-^{2}H]1-OPP). As described for unlabeled **1**-OPP,[11] 188 mg (0.555 mmol) of (*S*)-[1-^{2}H]**1**-OH,OTs was treated with a mixture of 85 mg (0.638 mmol) of *N*-chlorosuccinimide and 52 mg (61 μL, 0.833 mmol of dimethyl sulfide in 5.5 mL of CH_2Cl_2 to give 194 mg (97%) of a colorless liquid. A solution of the chloro tosylate in 2.25 mL of acetonitrile was treated with 1.8 g (1.90 mmol) of tris(tetra-*n*-butylammonium hydrogen diphosphate. Purification of the NH_4^+ salt by medium-pressure chromatography on cellulose (3:3:4 2-propanol/THF/0.1 M NH_4HCO_3) yielded 220 mg (67%) of a white powder: ^{1}H NMR (300 MHz, D_2O) 5.45 (1, b d, J = 6.82 Hz, H at C(2)), 5.30 (1, m, H at C(6)), 4.42 (1, dd, $J_{H,H}$ = 6.82 Hz, $J_{H,P}$ = 7.09 Hz, H at C(1)), 3.97 (2, dt, $J_{H,H}$ = 6.86 Hz, $J_{H,P}$ = 7.00 Hz, H at C(9)), 2.34 (2, t, J = 6.86 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(6)); ^{13}C (75 MHz, D_2O) 145.4, 135.8, 129.0, 123.3 (d, $J_{C,P}$ = 7.9 Hz), 67.2 (d, $J_{C,P}$ = 5.7 Hz), 64.6 (dt, $J_{C,P}$ = 5.3 Hz, $J_{C,D}$ = 20.5 Hz), 42.6 (d, $J_{C,P}$ = 7.4 Hz), 34.0, 29.1, 25.5, 18.1 ppm; ^{2}H NMR (46 MHz, D_2O) 4.38 ppm (b s); ^{31}P NMR (121 MHz, D_2O) −9.40 (d, $J_{P,P}$ = 21.85 Hz), −9.43 (d, $J_{P,P}$ = 21.85 Hz), −13.10 (d, $J_{P,P}$ = 21.85 Hz), −13.26 ppm (d, $J_{P,P}$ = 21.85 Hz); FAB MS (−Ve, glycerol) *m*/*z* 504 (80.0) [M − 1]$^-$, 424 (3.5), 418 (3.0), 326 (8.0), 258 (7.5), 190 (13.5), 177 (96.0), 159 (100).

(*S*,*Z*,*Z*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ((*S*)-[1-^{2}H]2-OPP). As described for the unlabeled **2**-OPP,[11] 85 mg (0.251 mmol) of (*S*)-[1-^{2}H]**2**-OH,OTs was treated with a mixture of 38.5 mg (0.289 mmol) of *N*-chlorosuccinimide and 28 μL (23 mg, 0.377 mmol) of dimethyl sulfide in 3.0 mL of CH_2Cl_2. The chlorotosylate (87 mg, 96%) was dissolved in 1.0 mL of acetonitrile and treated with tris-(tetra-n-butylammonium) hydrogen diphosphate to yield 72 mg (49%) of a white powder: ^{1}H NMR (300 MHz, D_2O) 5.44 (1, b d, J = 6.67 Hz, H at C(2)), 5.29 (1, m, H at C(6)), 4.41 (1, dd, $J_{H,H}$ = 6.67 Hz, $J_{H,P}$ = 6.82 Hz, H at C(1)), 3.94 (2, dt, $J_{H,H}$ = 7.02 Hz, $J_{H,P}$ = 7.12 Hz, H at C(9)), 2.40 (2, t, J = 7.02 Hz, H at C(8)), 2.17 (4, m), 1.75 (3, s, CH_3 at C(6)), 1.71 ppm (3, s, CH_3 at C(3)); ^{13}C NMR (75 MHz, D_2O) 145.1, 135.8, 129.5, 123.5 (d, $J_{C,P}$ = 7.9 Hz), 66.9 (d, $J_{C,P}$ = 5.4 Hz), 64.6 (dt, $J_{C,P}$ = 5.5 Hz, $J_{C,D}$ = 21.4 Hz), 35.3 (d, $J_{C,P}$ = 7.3 Hz), 34.0, 28.4, 25.6, 25.3 ppm; ^{2}H NMR (46 MHz, H_2O) 4.36 ppm (b s); ^{31}P NMR (121 MHz, D_2O) −12.12 (d, $J_{P,P}$ = 20.64 Hz), −12.20 (d, $J_{P,P}$ = 20.52 Hz), −13.53 (d, $J_{P,P}$ = 20.52 Hz), −13.54 ppm (d, $J_{P,P}$ = 20.64 Hz); FAB MS (−Ve, glycerol) *m*/*z* 504 (51.4) [M − 1]$^-$, 418 (12.0), 328 (9.5), 326 (7.8), 292 (44), 183 (100) [glycerol], 177 (65), 159 (59.4).

Enzyme-Catalyzed Cyclizations. (*S*,*Z*,*E*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ((*S*)-[1-^{2}H]1-OPP). Following the procedure described previously for unlabeled **1**-OPP,[11] 1.2 mL (0.06 mmol) of (*S*)-[1-^{2}H]**1**-OPP (50 mM in 25 mM NH_4HCO_3) was incubated with 0.15 mL (0.9 mg, 2.0 units) of farnesyl-diphosphate synthase at 37 °C for 14 h. The resulting cloudy suspension was treated with 1 mL of lysine buffer (0.2 M, pH 10.4) followed by 0.15 mL (0.83 mg, 47 units) of *Escherichia coli* Type III alkaline phosphatase. After 6 h at 37 °C, the white suspension was diluted with water and extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4. Solvent was removed by rotary evaporation and the residue dissolved in 2 mL of CH_2Cl_2. A sample from the solution was analyzed by GC (30 M DB-5, 115 °C isothermal for 12 min followed by a 10 °C min^{-1} ramp to 200 °C). No uncyclized diol was detected. The deuterium content of the products was estimated by GCMS.

[2′-^{2}H]**8**-OH: MS (EI, 70 eV) *m*/*z* 167 (1.2) [M]$^+$, 149 (59.5) [M − H_2O]$^+$, 148 (12.3), 134 (34.4), 133 (12.7), 121 (4.1), 119 (24.9), 107 (48.3), 106 (92.5), 105 (36.2), 97 (10.5), 96 (31.6), 95 (13.0), 94 (57.6), 93 (40.4), 92 (32.6), 80 (73.9), 79 (100).

[2′-^{2}H]**10**-OH: MS (EI, 70 eV) *m*/*z* 167 (55.5) [M]$^+$, 166 (2.0), 152 (5.4), 134 (6.0), 122 (73.8), 120 (9.5), 107 (5.7), 106 (36.7), 105 (10.3), 94 (100), 93 (84.1), 92 (57.8).

As described previously,[11] the mixture of alcohols was converted to naphthoate esters, which were purified by preparative TLC (95:5 hexanes/diethyl ether) to yield 12 mg (63% from bisdiphosphate), and each isomer was separated by HPLC.

[2′-^{2}H]**8**-ONp: ^{1}H NMR (500 MHz, C_6D_6) 8.75 (1, b s, naphthyl H), 8.27 (1, m, naphthyl H), 7.52 (3, m, naphthyl H), 7.20 (2, m, naphthyl H), 5.52 (1, ddq, J = 7.02, 7.32, 0.57, 1.48 Hz, H at C(2)), 5.37 (1, m, H at C(3′)), 4.97 (1, ddq, J = 7.32, 12.45, 1.03 Hz, H_a at C(1)), 4.01 (1, ddq, J = 7.02, 12.45, 1.03 Hz, H_b at C(2)), 2.81 (1, m, H at C(1′)), 1.95 (1, m, H_{ax} at C(5′)), 1.84 (1, b s, H_{eq} at C(2′)), 1.76 (q, b dd, H_{eq} at C(5′)), 1.61 (3, b s, CH_3 at C(4′)), 1.55 (3, b s, CH_3 at C(3)), 1.48 (1, m, H_{eq} at C(6′)), 1.44 ppm (1, m, H_{ax} at C(6′)).

[2′-^{2}H]**10**-ONp: ^{1}H NMR (500 MHz, C_6D_6) 8.78 (1, b s, naphthyl H), 8.26 (1, m, naphthyl H), 7.56 (3, m, naphthyl H), 7.19 (2, m, naphthyl H), 5.65 (1, b s, H at C(3′)), 4.35 (1, dt, J = 11.03, 7.33 Hz, H_a at C(1)), 4.31 (1, dt, J = 11.03, 7.33 Hz, H_b at C(1)), 2.24 (1, m, H at C(3)), 1.95 (4, m), 1.75 (1, m, H_a at C(2)), 1.66 (3, b s, CH_3 at C(4′)), 1.63 (1, m, H_b at C(2)), 0.96 ppm (3, d, J = 6.95 Hz, H at C(4)).

(*S*,*Z*,*Z*)-[1-^{2}H]-3,7-Dimethyl-2,6-nonadien-1,9-diyl Bisdiphosphate ((*S*)-[1-^{2}H]2-OPP). The procedures for incubation and isolation were identical to those described for the reaction of (*S*)-[1-^{2}H]**1**-OPP. The deuterium content of each isomer was determined by GCMS.

[2′-^{2}H]**8**-OH: MS (EI, 70 eV) *m*/*z* 167 (1.7) [M]$^+$, 149 (99.4) [M − H_2O]$^+$, 128 (5.4), 134 (48.0), 120 (10.3), 107 (39.4), 106 (51.0), 105 (12.8), 95 (11.0), 94 (66.4), 93 (52.9), 92 (56.0), 91 (13.7), 80 (51.3), 79 (55.4), 69 (100).

[2′-^{2}H]**9**-OH: MS (EI, 70 eV) *m*/*z* 167 (4.2) [M]$^+$, 149 (95.7) [M − H_2O]$^+$, 134 (22.5), 120 (15.3), 108 (10.3), 107 (20.9), 106 (30.5), 105 (15.5), 96 (13.2), 95 (18.4), 94 (81.6), 93 (28.0), 92 (26.8), 80 (31.5), 79 (38.9), 69 (100).

[2′-^{2}H]**10**-OH: MS (EI, 70 eV) *m*/*z* 167 (57.3) [M]$^+$, 166 (4.9), 152 (12.4), 134 (13.1), 122 (100), 121 (25.9), 119 (63.3), 108 (16.5), 106 (51.0), 105 (13.3), 95 (21.9), 94 (62.9), 93 (49.2), 92 (40.2).

The mixture of alcohols was converted to naphthoate esters as described above to give 11 mg (57% from bisdiphosphate) of a colorless oil. Each isomer was separated by HPLC.

[2′-^{2}H]**8**-ONp: ^{1}H NMR (500 MHz, C_6D_6) 8.76 (1, b s, naphthyl H), 8.27 (1, m, naphthyl H), 7.52 (3, m, naphthyl H), 7.20 (2, m, naphthyl H), 5.52 (1, dddd, J = 7.02, 7.32, 0.57, 1.41 Hz, H at C(2)), 5.37 (1, b s, H at C(3′)), 4.97 (1, ddq, J = 7.32, 12.45, 1.03 Hz, H_a at C(1)), 4.91 (1, ddq, J = 7.02 Hz, J = 12.45 Hz, J = 1.03 Hz, H_b at C(1)), 2.81 (1, m, H at C(1′)), 2.0–1.9 (2, m, H_{ax} at C(2′), H_{ax} at C(5′)), 1.76 (1, m, H_{eq} at C(5′)), 1.61 (3, s), 1.55 (3, s, CH_3 at C(3)), 1.48 (1, m, H_{eq} at C(6′)), 1.44 (1, m, H_{ax} at C(6′)).

[2′-^{2}H]**9**-ONp: ^{1}H NMR (500 MHz, C_6D_6) 8.78 (1, m, naphthyl H), 8.29 (1, m, naphthyl H), 7.54 (2, m, naphthyl H), 7.48 (1, m, naphthyl H), 7.19 (1, m, naphthyl H), 7.14 (1, m, naphthyl H), 5.58 (1, dtq, J = 1.25, 6.98, 1.10 Hz, H at C(2′)), 5.36 (1, b s, H at C(3′)), 4.93 (2, d, J = 6.96 Hz, H at C(1)), 2.02 (1, m, H at C(1′)), 1.92–1.83 (2, m, H_{ax} at C(2′), H_{ax} at C(5′)), 1.78 (1, m, H_{eq} at C(5′)), 1.61 (1, m, H_{eq} at C(6′)), 1.59 (3, b s, CH_3 at C(4′)), 1.55 (3, b s, CH_3 at C(3)), 1.38 (1, m, H_{ax} at C(6′)).

[2′-^{2}H]**10**-ONp: ^{1}H NMR (500 MHz, $CDCl_3$) 8.79 (1, b s, naphthyl H), 8.27 (1, m, naphthyl H), 7.56 (3, m, naphthyl H), 7.20 (2, m, naphthyl H), 5.65 (1, b s, H at C(3′)), 4.35 (1, dt, J = 10.95, 7.35 Hz, H_a at C(1)), 4.31 (1, dt, J = 10.95, 7.35 Hz, H_b at C(1)), 2.24 (1, m, H at C(3)), 1.95 (4, m), 1.76 (1, m, H_a at C(2)), 1.66 (3, b s, CH_3 at C(4′)), 1.62 (1, m, H at C(2)), 0.96 (3, d, J = 6.90 Hz, H at C(4)).

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