Synthesis of Allylic Isoprenoid Diphosphates by S_N2 Displacement of Diethyl Phosphate

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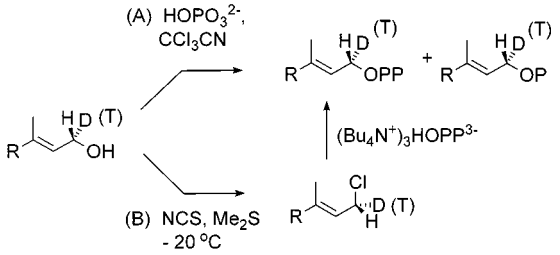
Allylic polyenyl diphosphates such as geranyl and (*E*,*E*,*E*)-geranylgeranyl diphosphates are ubiquitous substrates for monoterpene and diterpene synthases and transferases in isoprenoid biosynthesis. These enzyme substrates were prepared in asymmetrically labeled form by reduction of 1-deuterio aldehyde precursors with (*R*)- and (*S*)-Alpine boranes®, conversion into diethyl phosphates, and S_N2 displacements with tris(tetrabutylammonium) pyrophosphate which occurred slowly with essentially complete inversion of configuration over 2–5 days. The 8α- and 8β-hydroxy-17-nor analogs (**13** and **14**) of copalyl diphosphate as well as the (15*R*)-deuterium-labeled form of **13** were similarly prepared from nor-diols **11**, (15*S*)-[15-$^2H_1$]-**11**, and **12** by means of regioselective phosphorylation of the allylic hydroxy groups and displacements with pyrophosphate anion. The configurations and enantiopurities of the deuterium-labeled geraniols, before and after the S_N2 displacements, and the diastereopurity of the bicyclic keto alcohol intermediate (15*S*)-[15-$^2H_1$]-**15** were determined by conversion into (1*S*)-camphanate esters and ^{1}H-NMR analysis. Amino alcohol **18** was similarly converted into amino diphosphate **19**, 15-aza-14,15-dihydro GGPP, a potential aza analog inhibitor for diterpene synthases which generate stereoisomeric forms of copalyl diphosphate.

Introduction

Investigations on isoprenoid biosynthesis[1,2] as well as studies on chemical model reactions[3] and enzyme inhibitors[4] in this area depend critically on the synthesis of diphosphate derivatives of alcohol precursors. Although preparative-scale enzymatic syntheses have been sometimes employed to obtain normal biosynthesis intermediates,[5] chemical methods are more widely used, and they are usually the only choice for unnatural diphosphates.[6] The original Cramer–Böhm phosphorylation procedure involving condensation of the alcohol with inorganic phosphate and trichloroacetonitrile[3a,7] affords mixtures of monophosphate (major product), diphosphate (10–30%), triphosphate, and inorganic phosphates which are separated by various chromatographic methods[6] [Scheme 1 (A)]. Improved yields have been obtained by a modified procedure using tetrabutylammonium phosphate as phosphate source and other variations.[8] Primary allylic diphosphates may be more efficiently synthesized by S_N2 displacements of the corresponding allylic chloride or bromides with tris (tetrabutylammonium) pyrophosphate in anhydrous acetonitrile [Scheme 1 (B)].[6,9] Non-allylic diphosphates of homoallylic, β-aminoethyl, epoxy, and aziridino alcohols[4] have also been prepared by S_N2 displacements.

Elucidation of the stereochemistry of enzyme-catalyzed substitution and cyclization reactions that take place at the allylic C1 position in isoprenoid biosynthesis requires synthesis of the diphosphate substrates bearing deuterium or tritium label in one prochiral position.[1,2] These labeled diphosphates have been obtained from the corresponding labeled alcohols with retention of configuration by the Cramer–Böhm condensation[10] and by Poulter's S_N2 displacement method.[6,11] However, significant extents of racemization (6–31%) accompanied the two displacements involved in the latter case.[6,11]



Scheme 1. Synthesis of isoprenoid diphosphates by (A) Cramer–Böhm condensation and (B) by Poulter S_N2 displacement; PP = diphosphate, P = monophosphate

We now report that the isoprenoid alcohols, geraniol and (*E*,*E*,*E*)-geranylgeraniol, substituted with deuterium in the pro (*R*) or pro (*S*) position may be converted cleanly to the enantiomeric diphosphates by a single S_N2 displacement of the diethyl phosphate derivatives with inorganic pyrophosphate. The use of allylic monophosphate activation also enabled the formation of diphosphates of diol and amino alcohol precursors (**11**, (15*S*)-[15-$^2H_1$]-**11**, **12**, and **18**). Literature precedent for allylic substitutions of monophosphate esters exists in aryl oxide displacements of diphenyl phosphate[12] and in various organometallic couplings and substitutions.[13]

Results and Discussion

The suitability of phosphate as a leaving group for displacement with pyrophosphate anion, and the stereoselectivity of substitution, were evaluated with (*R*)- and (*S*)-[1-

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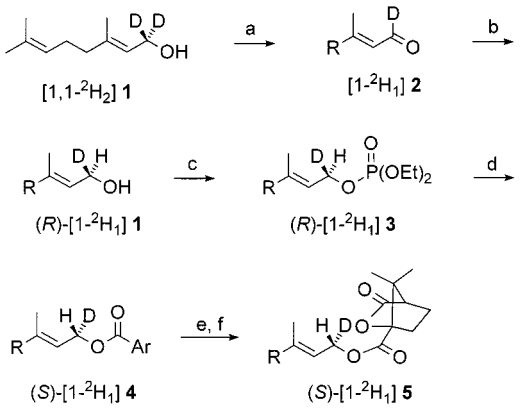
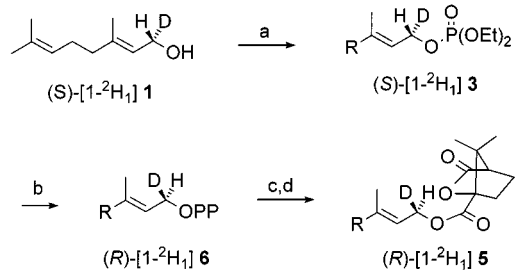
$^2H_1$]geranyl diethyl phosphates (Schemes 2 and 3). The required labeled forms of geraniol were synthesized in 3 steps by $LiAlD_4$ reduction of methyl geranoate, Swern[14] or MnO_2 oxidation to [1-$^2H_1$]geranial (**2**), and reductions with commercial (*S*)- and (*R*)-Alpine borane® (*B*-isopinocampheyl-9-borabicyclo[3.3.1]nonane)[15] in THF.[16] The labeled geraniol enantiomers (*R*)- and (*S*)-[1-$^2H_1$]-**1** were liberated from the borinate intermediate either by precipitation of the 9-BBN–ethanolamine complex,[15b,15c] or by H_2O_2 oxidation[17] (62% and 50%, respectively). The oxidative procedure was found to be experimentally more convenient for the 0.5–1.5-mmol scale reductions carried out in this work. Formation of the camphanate esters[18a] with (1*S*)-(–)-camphanoyl chloride[18b] and ^{1}H-NMR deconvolution analysis of the δ_H = 4.7 region for the allylic C*H*DOR proton of the geranyl moiety [(*R*): δ_H = 4.72; (*S*): δ_H = 4.73] showed the enantiomeric purities to be 97–99%.[19]



Scheme 2. a: $(COCl)_2$, DMSO, CH_2Cl_2; Et_3N; b: (*S*)-Alpine borane, THF; $NH_2CH_2CH_2OH$ (62% for 2 steps); c: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2 (96%); d: α-$C_{10}H_8CO_2 \cdot NBu_4$, MeCN, room temp., 5 d (44%); e: $LiAlH_4$, diethyl ether (45%); f: (1*S*)-camophanoyl chloride, pyr (83%); R = 4-methyl-3-pentenyl, Ar = α-naphthyl

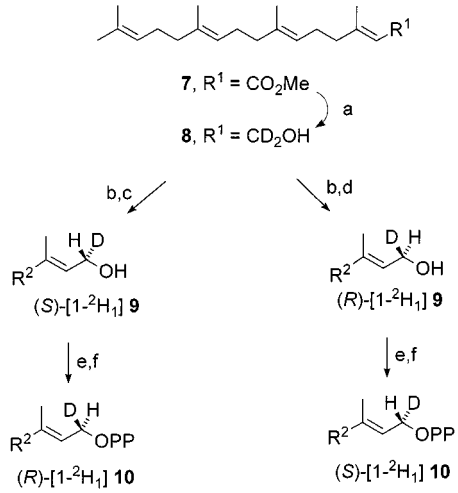


Scheme 3. a: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2 (84%); b: $(Bu_4N)_3 \cdot HOPP$, MeCN, room temp., 4 d; (HPLC, 78%; 28%); c: bacterial alkaline phosphatase, pH 8, 5 h (71%); d: (1*S*)-camophanoyl chloride, pyr (45%); R = 4-methyl-3-pentenyl

Conversion into (*R*)- and (*S*)-[1-$^2H_1$]geranyl diethyl phosphates (**3**) was accomplished in high yield by reactions with diethyl chlorophosphate in the presence of pyridine (CH_2Cl_2, 0 °C, 30 min, 84–96%).[13d] These allylic phosphates, known previously in unlabeled form,[13b,13d,13e] proved to be stable to purification by rapid chromatography on silica gel and storage at –20 °C. Displacement of phosphate from the (*R*) enantiomer with tetrabutylammonium α-naphthoate (2 equiv., 0.3 M) in acetonitrile proceeded slowly with a half-life estimated to be ca. 60 h. After 120 h the labeled geranyl α-naphthoate (**4**)[20] was isolated (44%) and cleaved to (*S*)-[1-$^2H_1$]geraniol which was converted into the camphanate, (*S*)-[1-$^2H_1$]-**5.**[18] The higher field position of the C1 proton (δ_H = 4.73) in the ^{1}H-NMR spectrum confirmed the (*S*) stereochemistry and deconvolution analysis[19] gave an enantiomeric purity of 99%.

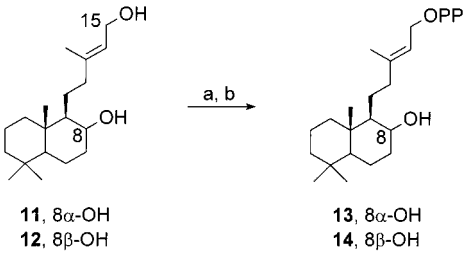
In similar fashion S_N2 displacement of the enantiomeric (*S*)-[1-$^2H_1$]geranyl diethyl phosphate with tris(tetrabutylammonium) pyrophosphate (1.6 equiv., 1 g/1 mL, 1.1 M)[6,9] in acetonitrile with monitoring by TLC for 4 d afforded (*R*)-[1-$^2H_1$]geranyl diphosphate (**6**). Ion exchange to the ammonium salts and purification to separate the diethyl phosphate anion and other by-products were accomplished either by selective precipitations to remove inorganic diphosphate,[6,9] fractionation on a Dowex 1-X8 ion exchange resin using an ammonium formate gradient elution, and hydrophobic adsorption on Amberlite XAD-2 resin[21] (28% yield) or more efficiently by ion exchange on Dowex 50 W-X8 resin with ammonium bicarbonate, cellulose chromatography to separate inorganic diphosphate,[6,9] and preparative reverse-phase HPLC[22] (78% yield). The (*R*) configuration and enantiomeric purity (98%) were established by hydrolysis of the diphosphate to (*R*)-[1-^{2}H]geraniol with bacterial alkaline phosphatase, derivatization with (*S*)-camphanoyl chloride[18] to (*R*)-[1-$^2H_1$]-**5**, and ^{1}H-NMR analysis.[19] Thus, both the α-naphthoate and pyrophosphate anion displacements of diethyl phosphate proceeded slowly with essentially complete inversion of configuration. The absence of significant racemization of (*S*)-[1-$^2H_1$]-**3** during the reaction indicates that the potentially competing displacement with the diethyl phosphate anion $(EtO)_2PO_2^-$ by-product is much slower than displacement with hydrogen pyrophosphate trianion $HP_2O_7^{3-}$. The greater nucleophilicity of $HP_2O_7^{3-}$ is readily understandable from the following pK_a values: $(MeO)_2PO_2H$, pK_a = 1.29; $H_2P_2O_7^{2-}$, pK_a = 6.60.[23]

For an investigation on the stereochemistry of the enzyme-catalyzed biosynthesis of taxadiene,[24] we required (*R*)- and (*S*)-[1-$^2H_1$]geranylgeranyl diphosphates (GGPP, **10**).[10c,10d,10e] These enantiomerically labeled substrates were prepared by the same methods (Scheme 4), i.e. asymmetric reductions of [1-$^2H_1$]geranylgeranial with (*S*)- and (*R*)-Alpine boranes,[15] conversion into the diethyl phosphates,[13d,13e] and displacement with tris(tetrabutylammonium) pyrophosphate (2.2 equiv., 0.5 M in MeCN)[6,9] for 90 h. Purifications by ion exchange, cellulose chromatography, preparative reverse-phase HPLC, and lyophilization afforded the (*R*) and (*S*) enantiomers **10** in 17% and 21% yields. The chemical purity of the ammonium diphosphate salts was verified by ^{1}H- and ^{31}P NMR spectra. The reason for the lower yields of the HPLC-purified C_{20} diphosphates **10** and the related amino diphosphate **19** described below is unclear.



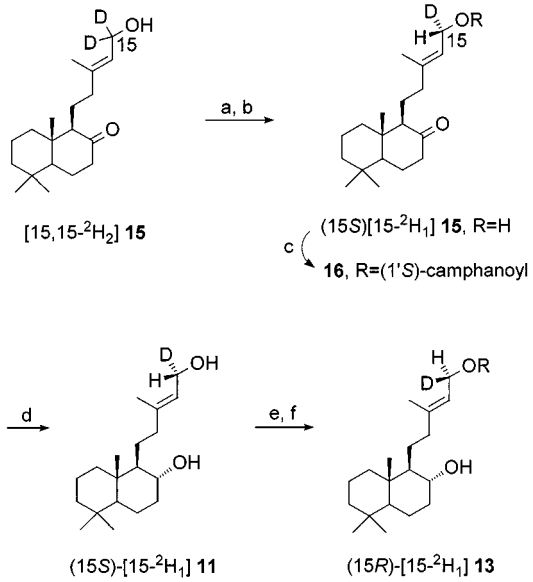
Scheme 4. a: $LiAlD_4$, diethyl ether (96%); b: $(COCl)_2$; DMSO, CH_2Cl_2; Et_3N; c: (*R*)-Alpine borane, THF; H_2O_2, OH^- (74% for 2 steps); d: (*S*)-Alpine borane, THF; H_2O_2, OH^- (75% for 2 steps); e: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2 (72% and 83%); f: $(Bu_4N)_3 \cdot HOPP$, MeCN, room temp., 3 d; HPLC (17% and 21%); R^2 = (*E*,*E*)-4,8,12-trimethyl-3,7,11-tetradecatrienyl

The phosphate displacement method was also found useful for formation of allylic diphosphates in the presence of other functionality. The 8α-hydroxy-17-nor analog **13** of the normal diterpene biosynthesis intermediate, copalyl diphosphate (CPP),[25] its labeled form bearing deuterium in the allylic 15-*pro*-(*S*) position, and the 8β-hydroxy-17-nor stereoisomer **14** were required for research in these laboratories on the stereochemistry and mechanism of abietadiene biosynthesis.[26,27] Reductions of the unlabeled keto alcohol **15**[28] with lithium in ammonia and $NaBH_4$ in methanol provided the crystalline diol isomers **11** (83%) and **12** (74%), respectively. However, attempts to convert the 8α,15-diol **11** selectively to the allylic chloride by Meyer's procedure (MsCl, LiCl, collidine, DMF)[29] afforded a mixture of products, presumably owing to facile S_N' cyclization. The primary allylic hydroxy group was selectively phosphorylated with diethyl chlorophosphate (46%) (Scheme 5).[13d,13e] Displacement with tris(tetrabutylammonium) pyrophosphate (4 equiv., 0.6 M, [$^2H_3$]MeCN)[6,9] with monitoring by ^{31}P-NMR spectroscopy for 4 days and purification by ion exchange, cellulose chromotography, and preparative HPLC afforded the desired 8α-hydroxy-17-nor CPP ammonium salt **13** in 65% yield. The 8β,15-diol epimer **12** was similarly converted into the mono(diethyl phosphate) (61%) and 8β-hydroxy-17-nor CPP ammonium salt **14** (67%).



Scheme 5. a: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2, 0 °C (46% and 61%); b: $(Bu_4N)_3 \cdot HOPP$, MeCN, 4 d; HPLC (65% and 67 %); PP = diphosphate

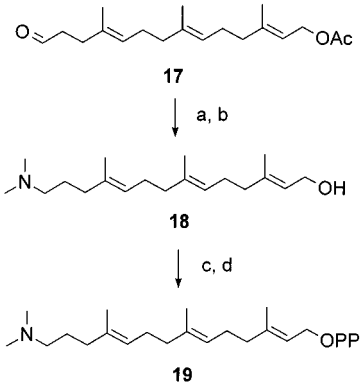
The stereoselectively deuterated form, (15*S*)-[15-$^2H_1$]-**11** of the 8α,15-diol was synthesized in three steps (Scheme 6) by oxidation of dideuterio keto alcohol [15,15-$^2H_2$]-**15** with MnO_2, stereo- and regioselective reduction of the keto aldehyde with (*R*)-Alpine borane (53% over 2 steps), and dissolving metal reduction[30] of the ketone carbonyl (Li, EtOH, NH_3, ether, 85%). The diastereomeric purity of the labeled keto alcohol (15*S*)-[15-$^2H_1$]-**15** was estimated at 99% by ^{1}H-NMR analysis[19] of the corresponding camphanate derivative (**16**)[17] (H15, δ_H = 4.62), and by comparisons with a (15*R*) + (15*S*) mixture of diastereomers (H15, δ_H = 4.60 and 4.62) obtained by stereorandom $NaBH_4$ reduction of the deuterio keto aldehyde and subsequent camphanate derivatization. Assignment of the (15*S*) configuration expected from the *re* face selective reduction by (*R*)-Alpine borane[15] is consistent with the greater ^{1}H-NMR chemical shift (δ_H = 4.62) observed for the stereoselectively labeled camphanate alone, or when added to the (15*R*) + (15*S*) diastereomer mixture. Conversion into the diethyl phosphate and displacement with tris(tetrabutylammonium) pyrophosphate as before afforded (15*R*)-[15-$^2H_1$]-**13** (43% overall).



Scheme 6. a: MnO_2, hexane; b: (*R*)-Alpine borane, THF; H_2O_2, OH^- (52% for 2 steps); c: (1*S*)-camophanoyl chloride, pyr (90%); d: Li, NH_3, EtOH, ether, –33 °C (85%); e: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2, 0 °C (71%); f: $(Bu_4N)_3 \cdot HOPP$, MeCN, room temp., 4 d; HPLC (60%)

Amino diphosphate **19,** a novel aza analog of the normal diterpene synthase substrate GGPP (**10**),[25] is expected to inhibit the enzymes[31] that catalyze the cyclization of GGPP to CPP (e.g. abietadiene synthase),[26] *syn*-CPP (9,10- and 8,10-*syn*-diterpene synthases),[32] and *ent*-CPP (e.g. kaurene synthase A).[33] The known acetoxy aldehyde **17**[34] (Scheme 7) was prepared by $HClO_4$-catalyzed hydrolysis of 14,15-epoxygeranylgeranyl acetate[35] to the 14,15-diol (85%) followed by periodate cleavage (78%). Reductive amination of **17** with Me_2NHHCl and $NaBH_3CN$ in MeOH (66%),[36] and subsequent methanolysis provided amino alcohol **18** (15-aza-14,15-dihydrogeranylgeraniol).

Attempts to convert **18** to the corresponding amino chloride by Meyer's procedure (MsCl, LiCl, collidine, DMF)[29] evidently afforded polymeric quaternary ammonium salts arising from intermolecular *N*-alkylations which were inert to inorganic pyrophosphate. However, reaction of **18** with diethyl chlorophosphate by the usual procedure furnished the corresponding allylic phosphate (85%) which was stable to chromatography on silica gel. Conversion into amino diphosphate **19** was accomplished by displacement with tris (tetrabutylammonium) pyrophosphate (3.5 equiv., 0.8 M in MeCN) over 5 days. 15-Aza-14,15-dihydroGGPP (**19**), a regioisomer of the known diterpene synthase inhibitor, 3-aza-2,3-dihydro GGPP,[4c] was purified by the same methods already mentioned (21%) and characterized by ^{1}H- and ^{31}P-NMR spectroscopy.



Scheme 7. a: $Me_2NH \cdot HCl$, $NaBH_3CN$, MeOH (66%); b: K_2CO_3, MeOH (60%); c: $ClP(O)(OEt)_2$, pyr, CH_2Cl_2, 0 °C (85%); d: $(Bu_4N)_3 \cdot HOPP$, MeCN, room temp., 5 d; HPLC (21%)

In summary, preparation of allylic isoprenoid diphosphates stereoselectively labeled with deuterium in the *pro*-(*R*) or *pro*-(*S*) position from the enantiomeric allylic alcohol precursors were carried out by conversion into the diethyl phosphates and single S_N2 displacements with pyrophosphate anion which proceeded slowly with complete inversion of configuration. This method complements the known procedures, i.e. Cramer–Böhm condensation and Poulter's allylic halide displacement, both of which afford allylic diphosphates of retained stereochemistry. Thus, enantiomeric diphosphates labeled with deuterium or tritium at Cl can be prepared from one labeled alcohol precursor. The likelihood of partial racemization in the formation, handling, and displacement of the labile allylic chlorides[6,11] is avoided by this phosphate displacement method. Selective conversions of the difunctional diols **11** and **12** as well as amino alcohol **18** to the diphosphates were also carried out by OH activation as the diethyl phosphates and S_N2 displacements with pyrophosphate anion.

Experimental Section

General: Melting points were determined in open capillaries and are uncorrected. NMR reference values: δ_H = 7.26 ($CDCl_3$), 7.15 (C_6D_6), or 4.67 (D_2O); δ_C = 77.27 ($CDCl_3$) or 128.0 (C_6D_6); δ_P = 0.0 (85% H_3PO_4) by external referencing. – IR: IBM FT IR/32. – Elemental analyses: UI Microanalytical Laboratory. – Mass spectra: UI Mass Spectroscopy Laboratory. – Column chromatography:[37] Woelm 32–64 mm grade silica gel. HPLC: Waters Model M-6000A and Schoeffel SF770 variable wavelength UV detector. TLC: Merck glass plates with indicator (0.25 mm 60 F-254 silica gel).

All reactions were carried out under nitrogen, unless otherwise noted. Solvents (distilled from; stored over): THF (Na/benzophenone), CH_2Cl_2 (CaH_2), DMF (P_2O_5; 4-Å sieves),[38a] DMSO (distilled; 4-Å sieves),[38a] acetonitrile (P_2O_5; 3-Å sieves),[38b] pyridine (CaH_2; KOH). All products except diphosphate salts were obtained as colourless oils unless specified otherwise.

Dowex AG 50 W-8X (100–200 mesh) cation exchange resin: (H^+ form: Bio-Rad Laboratories; NH_4^+ form, ref.[9b], Dowex 1 × 8 (200–400 mesh) anion exchange resin (Cl^- form): Bio-Rad Laboratories; formate form, refs.[9]). Whatman CF11fibrous cellulose powder Whatman Inc.; prepared according to ref.[9b]

(*E*)-[1,1-$^2H_2$]-3,7-Dimethyl-2,6-octadien-1-ol ([1,1-$^2H_2$]-1): A solution of methyl geranoate[39] (120 mg, 0.66 mmol) in Et_2O (2 mL) was stirred and cooled at 0 °C as $LiAlD_4$ (103 mg, 2.45 mmol) was added. The slurry was stirred at room temp. for 2 h and cooled to 0 °C. H_2O (0.10 mL), 15% NaOH (0.10 mL), and H_2O (0.30 mL) were added at 10-min intervals, and the white slurry was stirred at room temp. for 20 min. Filtration, drying (Na_2SO_4), and concentration gave a colourless oil (106 mg) which was purified by column chromatography with 4:1 hexane/EtOAc to give 89 mg (86%) of [1,1-$^2H_2$]geraniol. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) was identical to that of unlabelled geraniol except for the absence of the doublet at δ_H = 4.15 (C*H*$_2$OH).

(1*R*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadien-1-ol {(*R*)-[1-$^2H_1$]-1}: The oxidation and reduction were carried out according to Marshall's[14a] and Midland's procedures.[15b] A solution of oxalyl chloride (130 mg, 1.03 mmol) in CH_2Cl_2 (0.8 mL) was stirred and cooled at –78 °C as DMSO (158 mg, 2.02 mmol) in CH_2Cl_2 (0.7 mL) was added. After 15 min at –78 °C [1,1-$^2H_2$]-**1** (70 mg, 0. 45 mmol) in CH_2Cl_2 (0.7 mL) was added. After 30 min at –78 °C, Et_3N (0.35 mL, 2.51 mmol) was added, and the white suspension was allowed to warm to room temp. The suspension was filtered through $MgSO_4$ (10 g) and washed with 1:1 Et_2O/hexane (20 mL). The cloudy filtrate was filtered through Celite (10 g), dried (Na_2SO_4), and concentrated to give [1-$^2H_1$]-**2** (69 mg, quantitative) as a yellow oil. A solution of the aldehyde (69 mg, 0.45 mmol) in THF (2 mL) was stirred at room temp. as 0.5 M (*S*)-Alpine borane® in THF (1.8 mL, 0.90 mmol) was added. After 16 h, acetaldehyde (0.5 mL, 0.39 g, 8.9 mmol) was added. The solution was stirred for 10 min at room temp., the solvent was removed by N_2 stream, and the pinene was removed under high vacuum at 40 °C for 2 h. The residue was dissolved in Et_2O (10 mL) at 0 °C, and ethanolamine (1.0 mL, 1.01 g, 16.6 mmol) was added. The white precipitate was filtered, and the filtrate was diluted with Et_2O (40 mL). The ethereal solution was washed with H_2O (2 × 20 mL) and satd. NaCl (1 × 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash chromatography with 4:1 hexane/EtOAc gave 43 mg (62%) of (*R*)-[1-$^2H_1$]-**1**. The ^{1}H- and ^{13}C-NMR data spectra were identical to those of unlabeled geraniol except for the following: ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 4.12 (d, 1 H, *J* = 6.3 Hz; C*H*DOH), 5.40 (br dq, *J* = 7.1, 1.0 Hz, =C*H*CHD). – ^{13}C NMR (101 MHz, $CDCl_3$): δ_C = 58.9 (t, *J* = 21.7 Hz, CHD).

(1*R*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl (*S*)-Camphanate {(*R*)-[1-$^2H_1$]-5}: Esterification of alcohol (*R*)-[1-$^2H_1$]-**1** (6.0 mg, 0.04 mmol) with camphanoyl chloride (76 mg, 0.35 mmol) in pyridine (0.5 mL) for 16 h was carried out as described below for (*S*)-[1-

$^2H_1$]-**1**.[6,18] The yield of the camphanate was 16 mg as a yellow oil. ^{1}H-NMR analysis indicated 80% purity; deconvolution and integration[19] of the major doublet for the Cl proton (4.72) gave a diastereomeric ratio of 99:1 ± 1%: ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.95 (s, 3 H, CH_3), 1.05 (s, 3 H, CH_3), 1.11 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 1.67 (d, 3 H, J = 0.9 Hz, CH_3), 1.70 (m, 1 H, CH_2), 1.72 (d, 3 H, J = 0.7 Hz, CH_3), 1.91 (ddd, 1 H, J = 13.2, 10.8, 4.6 Hz,CH_2), 2.00–2.12 (m, 5 H, CH_2), 2.42 (ddd, 1 H, J = 13.5, 10.8, 4.2 Hz, CH_2), 4.72 (d, 1 H, J = 7.1 Hz, CHD), 5.07 (t of quint, 1 H, J = 7.0, 1.4 Hz, vinyl H), 5.37 (d, 1 H, J = 7.3 Hz).

(1*R*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl Diethyl Phosphate {(*R*)-[1-$^2H_1$]-3}: Murahashi's procedure for the unlabeled compound was followed.[13d] A solution of (*R*)-[1-$^2H_1$]-**1** (200 mg, 1.30 mmol) and pyridine (123 mg, 1.56 mmol) in CH_2Cl_2 (2 mL) was stirred and cooled at 0 °C as diethyl chlorophosphate (236 mg, 1.37 mmol) was added. After 30 min at 0 °C, Et_2O (20 mL) was added, and the solution was washed with 1 M HCl (3 × 10 mL), satd. $NaHCO_3$ (3 × 10 mL), and satd. NaCl (1 × 15 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a colourless oil (415 mg). Purification by rapid flash chromatography with 3:2 hexane/ethyl acetate gave (*R*)-[1-$^2H_1$]-**3** (363 mg, 96%). Slow decomposition of the phosphate on silica gel TLC plates was observed during fraction analysis. The ^{1}H-NMR spectrum was the same as that of the unlabelled compound except for peaks at δ_H = 4.53 and 5.38;[13d] ^{1}H NMR (400 MHz, $CDCl_3$):δ_H = 1.31 (td, 6 H, J = 7.1, 1.0 Hz, OCH_2CH_3), 1.58 (s, 3 H, CH_3), 1.66 (d, 3 H, J = 1.0 Hz, CH_3), 1.68 (d, 3 H, J = 1.0 Hz, CH_3), 2.00–2.12 (m, 4 H, CH_2), 4.09 (quint, 4 H, J = 7.3 Hz, OCH_2), 4.53 (t, 1 H, J = 7.6 Hz, CHD), 5.06 (t of quint, 1 H, J = 6.8, 1.5 Hz, vinyl H), 5.38 (d, 1 H, J = 7.1 Hz, vinyl H). – ^{13}C NMR (101 MHz, $CDCl_3$): δ_C = 16.0 (d, J = 6.9 Hz), 16.3, 17.5, 25.5, 26.0, 39.3, 63.4 (d, J = 6.1 Hz), 63.8 (td, J = 22.1, 5.3 Hz), 118.7 (d, J = 6.1 Hz), 123.5, 131.7, 142.5.

(1*S*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl 1-Naphthoate {(*S*)-[1-$^2H_1$]-4}: A suspension of powdered 3-Å molecular sieves (100 mg) in a solution of (*R*)-[1-$^2H_1$]-**3** (43 mg, 0.15 mmol) and tetrabutylammonium α-naphthoate (122 mg, 0.30 mmol) in MeCN (0.5 mL) was stirred at room temp. for 115 h. The reaction progress was followed by TLC (30% EtOAc in hexane), and the half life was estimated to be 60 h. The solvent was removed by a stream of N_2, and the oil was dissolved in Et_2O (30 mL). The molecular sieves were filtered, and the filtrate was washed with H_2O (3 × 10 mL) and satd. NaCl (1 × 10 mL). Drying (Na_2SO_4) and concentration gave a colourless oil which was purified by flash chromatography using 4:1 hexane/CH_2Cl_2 to give 20 mg (44%) of (*S*)-[1-$^2H_1$]-**4**, known previously in the unlabelled form:[20] ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.61 (s, 3 H, CH_3), 1.68 (d, 3 H, J = 1.0 Hz, CH_3), 1.81 (d, 3 H, J = 1.0 Hz, CH_3), 2.07–2.18 (m, 4 H, CH_2), 4.92 (d, 1 H,J = 7.1 Hz, CHD), 5.11 (t of quint, 1 H, J = 6.6, 1.3 Hz, vinyl H), 5.54 (d,1 H, J = 7.1 Hz, vinyl H), 7.47–7.55 (m, 2 H, aryl H), 7.61 (ddd, 1 H, J = 8.3, 6.8, 1.5 Hz, aryl H), 7.88 (d, 1 H, J = 8.1 Hz, aryl H), 8.01 (d, 1 H, J = 8.3 Hz, aryl H), 8.18 (dd, 1 H, J = 7.3, 1.2 Hz, aryl H), 8.91 (dd, 1 H, J = 8.8, 0.7 Hz, aryl H). – ^{13}C NMR (101 MHz, $CDCl_3$): δ_C = 16.5, 17.6,25.6, 26.2, 39.4, 61.5 (t, J = 22.5 Hz, CHD), 118.2, 123.6, 124.4, 126.0, 127.4, 127.5, 128.4, 130.0, 131.2, 131.7, 133.0, 133.7, 142.5, 167.5.

(1*S*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadien-1-ol {(*S*)-[1-$^2H_1$]-1}. – A. By Cleavage of (*S*)-[1-$^2H_1$]-4: $LiAlH_4$ (70 mg, 1.94 mmol) was added to a stirred solution of ester (*S*)-[1-$^2H_1$]-**4** (20 mg, 0.065 mmol) in Et_2O (1 mL). After 2 h at room temp., the solution was cooled to 0 °C, and H_2O (0.070 mL), 15% NaOH (0.070 mL), and H_2O (0.21 mL) were added at 15-min intervals. The resulting white suspension was stirred at 0 °C for 20 min and filtered. Drying (Na_2SO_4) and concentration gave a colourless oil (28 mg), which was purified by flash chromatography using 3:7 Et_2O/pentane to give 4.5 mg (45%) of the alcohol (*S*)-[1-$^2H_1$]-**1**. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) was identical to that of (*R*)-[1-$^2H_1$]-**1**.

B. By Asymmetric Reduction of [1-$^2H_1$]-2: A solution of alcohol [1,1-$^2H_2$]-**1** (802 mg, 5.17 mmol) in hexane (100 mL) was stirred at room temp. as activated MnO_2 (26.1 g, 30.0 mmol) was added. After 1 h, the suspension was filtered through Celite and washed with hexane (3 × 50 mL). The filtrate was concentrated under reduced pressure to give crude aldehyde [1-$^2H_1$]-**2**. The oil was stirred and cooled at 0 °C as 11.0 mL (5.5 mmol) of (*R*)-Alpine borane® (0.5 M in THF, Aldrich) was slowly added.[15b] After 14 h at room temp., acetaldehyde (1.0 mL) was added. After 1 h, the solution was diluted with 10% NaOH (10 mL), and stirred and cooled at 0 °C as 30% H_2O_2 (15 mL) was slowly added, maintaining the internal temperature below 25°C.[17] After 30 min, water (100 mL) was added, and the product was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a thick oil. Purification by column chromatography using 3:1 hexane/EtOAc gave (*S*)-[1-$^2H_1$]-**1** (401 mg, 50%) contaminated by a minor amount of pinol (ca. 10%). The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) and physical properties matched those reported above. The (*E*/*Z*) purity was estimated at ca 97% (*E*). (*S*)-[1-$^2H_1$]-**1** was previously prepared by reduction of [1-$^2H_1$]-**2** with horse liver alcohol dehydrogenase.[6]

(1*S*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl (1*S*)-Camphanate {(*S*)-[1-$^2H_1$]-5}: Poulter's procedure was followed with some modifications.[6] A solution of (*S*)-[1-$^2H_1$]-**1** (2.0 mg, 0.01 mmol) from Part A above and (*S*)-camphanoyl chloride (108 mg, 0.50 mmol)[18] in pyridine (0.5 mL) was stirred at room temp. for 18 h. The solution was cooled to 0 °C, and water (10 drops) was added. After 1 h at room temp., CH_2Cl_2 (5 mL) and Et_2O (25 mL) were added, and the solution was washed with cold 1 M HCl (5 × 10 mL), satd. $NaHCO_3$ (5 × 10 mL), and satd. NaCl (1 × 10 mL). Drying (Na_2SO_4) and concentration under reduced pressure gave a yellow oil (8 mg), which was purified by flash chromatography (7:3 hexane/EtOAc) to give the camphanate (*S*)-[1-$^2H_1$]-**5** (3.6 mg, 83%). The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) was identical to that of (*R*)-[1-$^2H_1$]-**5**, and the data corresponded well with the published values.[6]

Reaction of (*S*)-[1-$^2H_1$]-**1** (8.1 mg, 0.052 mmol) from Part B above with (*S*)-camphanoyl chloride (55 mg, 0.25 mmol) in pyridine (1.0 mL) for 1 h as described above gave crude camphanate. Purification by column chromatography using 4:1 hexane/EtOAc gave (*S*)-[1-$^2H_1$]-**5** (15 mg, 86%). The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) and physical properties matched those above. Deconvolution[19] of the major doublet at δ_H = 4.73 in the ^{1}H-NMR spectrum gave a diastereomeric ratio of 99 ± 0.5%.

(1*S*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl Diethyl Phosphate {(*S*)-[1-$^2H_1$]-3}: Reaction of (*S*)-[1-$^2H_1$]-**1** from Part B above (115 mg, 0.74 mmol) and pyridine (182 μL, 179 mg, 2.3 mmol) with diethyl chlorophosphate (216 μL, 259 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) at 0°C for 3 h followed by loading directly onto silica gel and elution with 2:1 hexane/EtOAc gave the phosphate (180 mg, 84%). Data and physical properties matched those reported above.

(1*R*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienyl Diphosphate, Ammonium Salt {(*R*)-[1-$^2H_1$]-6}: A solution of (*S*)-[1-$^2H_1$]-**3** (180 mg, 0.62 mmol) in dry MeCN (1 mL) was stirred as $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ [6][9b] (1.00 g, 1.05 mmol) was added as a solid. After dissolution, powdered, predried 3-Å molecular sieves

(ca. 150 mg) were added, and the flask was placed in a desiccated jar. After 4 d, the solution was concentrated under reduced pressure, the residue was suspended in water (6 mL), and the suspension was loaded onto an ion exchange column (Dowex 50 W-X8 NH_4^+ form, 1.4 × 30 cm). Elution with 0.1 M NH_4HCO_3/2% 2-propanol (silica plates, 6:3:1 2-propanol/NH_4OH/water, R_f = 0.2) followed by cellulose chromatography (2.4 × 15 cm, Whatman CF-11) using 2:1:1 2-propanol/acetonitrile/0.1 M NH_4HCO_3 provided a 1:1 mixture of diphosphate **6** and diethyl phosphate anions as NH_4^+ salts (220 mg). This mixture was purified by preparative HPLC[22] in three portions (0–100% MeCN in 25 mM NH_4HCO_3, 59 min linear ramp, 16 mL/min, 214 nm UV detection, Luna C8 (2) preparative column, 1-mL injections) to give diphosphate (*R*)-[1-$^2H_1$]-**6** (176 mg, 78%). The ^{1}H- and ^{31}P-NMR data agreed with the literature values.[6] – ^{1}H NMR (400 MHz, D_2O): δ_H = 4.28 (t, 1 H, *J* = 6.6 Hz, CHDOPP). – ^{31}P NMR (161 MHz, D_2O): δ = –5.79 (d, *J* = 23.2 Hz), –9.63 (d, *J* = 22.0 Hz).

In another run, (*R*)-[1-$^2H_1$]-**3** (200 mg, 0.69 mmol) was converted into (*S*)-[1-$^2H_1$]-**6**, Bu_4N^+ salt, as described above. After 4 d, the sieves were filtered and washed with MeOH (10 mL). The filtrate was concentrated under reduced pressure, dissolved in MeOH (10 mL), and divided into two centrifuge tubes. Inorganic pyrophosphate was precipitated with satd. NH_3/MeOH (15 mL) and centrifuged. The liquid was decanted and the solids were dissolved in MeOH (5 mL). After a second precipitation with satd. NH_3/MeOH (15 mL), the combined supernatants were concentrated and fractionated on a Dowex 1-X8 ion exchange column[21a] (1.4 × 24 cm, formate form, 50–100 mesh) using a linear gradient of ammonium formate (0.05 M to 0.5 M, 500 mL each) in methanol, to give the diphosphate. The ammonium formate was removed by two adsorptions onto Amberlite XAD-2 (2.2 × 30 cm, Aldrich) and elution with water (96 mL) followed by elution with MeOH (8-mL fractions).[21b] Ammonium formate was eluted in the initial fractions followed by diphosphate (*S*)-[1-$^2H_1$]-**6** (70 mg, 28%). The ^{1}H- and ^{31}P-NMR spectra matched those obtained previously.

(1*R*,2*E*)-[1-$^2H_1$]-3,7-Dimethyl-2,6-octadienol {(*R*)-[1-$^2H_1$]-1}: A solution of (*R*)-[1-$^2H_1$]-**6** (18 mg, 0.06 mmol) in buffer (0.1 M glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH = 8.0) was gently mixed with alkaline phosphatase (Sigma, Bacterial Type L-III, ca. 15 IU). After 5 h at 33°C, the cloudy solution was extracted with hexane (3 × 2 mL). The combined organic extracts were concentrated to give (*R*)-[1-$^2H_1$]-**1** (6 mg, 71%). Data and physical properties of the product matched those for (*R*)- and (*S*)-[1-$^2H_1$]-**1** obtained previously.

Reaction of (*R*)-[1-$^2H_1$]-**1** (6.0 mg, 0.04 mmol) with (*S*)-camphanoyl chloride (55 mg, 0.25 mmol) in pyridine (1.0 mL) for 0.5 h as described above followed by column chromatography gave (*R*)-[1-$^2H_1$]-**5** (6 mg, 45%). Data and physical properties matched those reported above. Deconvolution[19] of the major doublet at δ_H = 4.73 in the ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum gave a diastereomer ratio of 98:2 ± 0.5%.

(2*E*,6*E*,10*E*)-Methyl 3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenoate (7): (*E*,*E*,*E*)-Geranylgeraniol (1.50 g, 5.16 mmol) was converted into the methyl ester in 2 steps (MnO_2, hexane; MnO_2, NaCN, AcOH, MeOH) according to a literature procedure for oxidation of geraniol to methyl geranoate.[39] Purification by flash chromatography using 4:1 hexane/EtOAc as eluent afforded 1.16 g (71%) of methyl geranylgeranoate (**7**). – ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.56–1.59 (m, 9 H, 3 CH_3), 1.65 (d, 3 H, *J* = 1.0 Hz, CH_3), 1.92–2.00 (m, 6 H, CH_2), 2.00–2.09 (m, 6 H, CH_2), 2.15 (d, 3 H, *J* = 1.2 Hz, CH_3), 3.65 (s, 3 H, CH_3), 5.08 (m, 3 H, vinyl H), 5.65 (d, 1 H, *J* = 1.0 Hz, vinyl H). – ^{13}C NMR (101 MHz, $CDCl_3$): δ_C = 15.79, 15.84, 17.5, 18.6, 25.5, 25.8, 26.4, 26.6, 39.50, 39.54, 40.8, 50.5, 115.0, 122.7, 123.9, 124.2, 131.0, 134.8, 136.0, 159.9, 167.0. The ^{1}H-NMR data correspond with the literature values at lower field.[41]

(2*E*,6*E*,10*E*)-[1,1-$^2H_2$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-ol (8): Reduction of ester **7** (500 mg, 1.57 mmol) with $LiAlD_4$ (300 mg, 7.14 mmol) in Et_2O (8 mL) for 6 h as described above for methyl geranoate gave 439 mg (96%) of **8**. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) was identical to that of unlabelled geranylgeraniol except that the triplet at δ_H = 4.14 was absent, and the C2 vinyl proton signal was a singlet at δ_H = 5.41.

(1*S*,2*E*,6*E*,10*E*)-[1-$^2H_1$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-ol {(*S*)-[1-$^2H_1$]-9}: Dideuterio alcohol **8** (700 mg, 2.39 mmol) was oxidized to the aldehyde as described above for (*R*)-[1-$^2H_1$]-**1**. The crude product was dissolved in 0.5 M (*R*)-Alpine borane in THF (9.6 mL, 4.8 mmol), the solution was kept at room temp. for 16 h, and acetaldehyde (2 mL) was added. After 30 min, the THF and excess acetaldehyde were evaporated at reduced pressure, and α-pinene was removed by heating at 40 °C under high vacuum for 2 h. The brown oil was dissolved in THF (20 mL), and 15% NaOH (20 mL) and 30% H_2O_2 were added. The exothermic oxidation caused the solvent to boil spontaneously for ca. 20 min. After 30 min more at room temp., another aliquot of 30% H_2O_2 (20 mL) was added. After 18 h at room temp., hexane (20 mL) was added, and the aqueous layer was extracted with hexane (4 × 50 mL). The combined organic layers were washed with satd. NaCl (2 × 50 mL), dried (Na_2SO_4), and concentrated. Purification by flash column chromatography (4:1 hexane/EtOAc) afforded 518 mg (74%) of (*S*)-[1-$^2H_1$]-**9**. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) was identical to that of unlabelled geranylgeraniol except that the 2-H triplet at δ_H = 4.12 (*J* = 7.0 Hz) changed to 1-H doublet at δ_H = 4.13 (*J* = 7.1 Hz) and the triplet of sextuplets at δ_H = 5.39 (*J* = 7.0, 1.3 Hz) changed to a doublet at δ_H = 5.41 (*J* = 6.3 Hz).

(1*R*,2*E*,6*E*,10*E*)-[1-$^2H_1$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-ol {(*R*)-[1-$^2H_1$]-9}: Oxidation of **8** (684 mg, 2.34 mmol) to [1-$^2H_1$]geranylgeraniol followed by (*S*)-Alpine borane reduction as described above gave 509 mg (75%) of (*R*)-[1-$^2H_1$]-**9**. The ^{1}H-NMR spectrum was identical to that of the (*S*) enantiomer.

(1*S*,2*E*,6*E*,10*E*)-[1-$^2H_1$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl Diethyl Phosphate: Phosphorylation of (*S*)-[1-$^2H_1$]-**9** (100 mg, 0.34 mmol) as described above for (*R*)-[1-$^2H_1$]-**1** gave 106 mg (72%) of the phosphate. – ^{1}H NMR (400 MHz, [$^2H_6$]acetone): δ_H = 1.27 (td, 6 H, *J* = 7.1, 0.7 Hz, C*H*$_3$), 1.58 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 1.61 (s, 3 H, CH_3), 1.65 (s, 3 H, CH_3), 1.72 (s, 3 H, CH_3), 1.94–2.17 (m, 12 H, CH_2), 4.03 (quint, 4 H, *J* = 7.3 Hz, 2 OC*H*$_2$CH$_3$), 4.50 (t, 1 H, *J* = 7.8 Hz, C*H*D), 5.13 (m, 3 H, vinyl *H*), 5.40 (d, 1 H, *J* = 6.8 Hz, vinyl H). – ^{13}C NMR (101 MHz, [$^2H_6$]acetone): δ_C = 0 15.1, 15.4, 15.5, 16.7, 24.9, 25.9, 26.3, 26.4, 39.2, 39.40, 39.44, 62.9 (td, *J* = 22.1, 5.3 Hz CHD), 62.8 (d, *J* = 5.3 Hz), 119.3 (d, *J* = 6.1 Hz), 123.6, 124.0, 124.1, 130.6, 134.4, 134.9, 141.7.

(1*R*,2*E*,6*E*,10*E*)-[1-$^2H_1$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-yl Diphosphate, Ammonium Salt {(*R*)-[1-$^2H_1$]-10}: The displacement was carried out as described above for (*R*)-[1-$^2H_1$]-**6**. A suspension of powdered 3-Å molecular sieves (200 mg) in a solution of the preceding diethyl phosphate (*S*)-[1-$^2H_1$]-**9**-OP(O)(OEt)$_2$ (106 mg, 0.25 mmol) and $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ (483 mg, 0.506 mmol) in MeCN (1 mL) was stirred at room temp. for 90 h. The solids were filtered and washed with MeCN (30 mL).

The filtrate was washed with hexane (4 × 10 mL), and the MeCN layer was concentrated to give a yellow oil (772 mg). The oil was subjected to ion exchange chromatography (12 mL of resin, 24 mL of ion exchange buffer), and lyophilization of the eluent gave a white solid (96 mg). Purification by cellulose chromatography (30 mL cellulose) gave a 1:1 mixture (39 mg) of (*R*)-[1-$^2H_1$]-**10** and diethyl phosphate as NH_4^+ salts according to the ^{31}P-NMR spectrum. Purification by preparative reverse phase HPLC as described for (*R*)-[1-$^2H_1$]-**6** and lyophilization gave 21 mg (17%) of (*R*)-[1-$^2H_1$]-**10** that was pure according to ^{1}H- and ^{31}P-NMR spectra and TLC: R_f 0.40 (cellulose TLC, 2:1:1 2-propanol/acetonitrile/0.1 M NH_4HCO_3). The ^{1}H-NMR spectrum (400 MHz, D_2O) was the same as that of unlabelled **10** except for the following: δ_H = 4.27 (d, 1 H, *J* = 6.5 Hz, CHDO), 5.28 (d, 1 H, *J* = 7.3 Hz, =CH). – ^{31}P NMR (162 MHz, D_2O): δ_P = –7.19, –9.72 (2d, *J* = 19.5 Hz).

(1*S*,2*E*,6*E*,10*E*)-[1-$^2H_1$]-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-yl Diphosphate, Ammonium Salt {(*S*)-[1-$^2H_1$]-10}: Conversion of (*R*)-[1-$^2H_1$]-**9** (100 mg, 0.34 mmol) to the diethyl phosphate (121 mg, 83%) and displacement with $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ (640 mg, 0.67 mmol) in MeCN (1 mL) at room temp. for 90 h as described for the enantiomer gave 820 mg of a yellow oil. Purification of 200 mg by ion exchange, lyophilization, and preparative HPLC afforded 8.8 mg (21%) of (*S*)-[1-$^2H_1$]-**10**. The ^{1}H- and ^{31}P-NMR spectra were identical to those of the (*R*) enantiomer.

(5*S*,8*R*,9*R*,10*S*,13*E*)-17-Norlabda-13-ene-8,15-diol (11): A solution of unlabeled keto alcohol **15**[28] (290 mg, 0.99 mmol) in Et_2O (30 mL), EtOH (30 mL), and liquid NH_3 (ca 140 mL) was stirred at reflux (–33°C) as two small pieces of lithium (ca 55 mg, ca 7.92 mmol, EtOH washed) were added. After several seconds, a deep blue color formed which dispersed after ca. 1 min. The NH_3 was evaporated by a stream of nitrogen, water (250 mL) was added, and the product was extracted with Et_2O (4 × 100 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a thick oil. Purification by column chromatography using 1:1 hexane/EtOAc gave diol **11** as a solid (240 mg, 83%). Recrystallization from Et_2O/pentane (ca. 3–4 mL) gave the analytical sample as colourless needles; m.p. 85–86 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.76, 0.77, 0.84 (3 s, 9 H, 3 CH_3), 0.89 (dd, 1 H, *J* = 12.1, 2.4 Hz), 0.90 (td, 1 H, *J* = 13.0, 3.5 Hz), 1.11 (td, 1 H, *J* = 13.4, 3.7 Hz), 1.1–1.3 (m, 1 H), 1.25 (qd, 1 H, *J* = 11.5, 3.5 Hz), 1.30 (dd, 1 H, *J* = 12.4, 3.5 Hz), 1.3–1.5 (m, 3 H), 1.4–1.6 (m, 4 H), 1.6–1.7 (m, 1 H), 1.66 (s, 3 H, CH_3), 1.71 (dtd, 1 H, *J* = 12.8, 3.5, 1.7 Hz), 1.98 (ddd, 1 H, *J* = 14.3, 10.6, 5.3 Hz,), 2.03 (ddt, 1 H, *J* = 11.7, 4.9, 2.8 Hz), 2.17 (ddd, 1 H, *J* = 14.3, 10.8, 6.2 Hz), 3.41 (td, 1 H, *J* = 10.6, 4.9 Hz, C*H*OH), 4.10 and 4.14 (d ABq, 2 H, *J* = 7.0 Hz, J_{AB} = 12.3, 1 H, CH_2OH), 5.40 (t sext, *J* = 7.0, 1.3 Hz, 1 H, =CH). – ^{13}C NMR (126 MHz, $CDCl_3$): δ_C = 14.6 (CH_3), 16.7 (CH_3), 18.6 (CH_2), 21.1 (CH_2), 21.9 (CH_3), 26.2 (CH_2), 33.5 (C), 33.7 (CH_3), 37.3 (CH_2), 38.9 (CH_2), 39.1 (C), 42.26 (CH_2), 42.31 (CH_2), 54.9 (CH), 58.5 (CH), 59.6 (CH_2), 73.4 (CH), 123.3 (CH), 141.2 (C). – IR (CCl_4): $\tilde{\nu}$ = 3620, 3378 (OH) cm^{-1}. – $[\alpha]_D^{20}$ = +1.8 (*c* = 0.85, EtOH). – $C_{19}H_{34}O_2$ (294.48): calcd. C 77.50, H 11.64; found C 77.69, H 11.63.

(5*S*,8*R*,9*R*,10*S*,13*E*)-8-Hydroxy-17-norlabda-13-en-15-yl Diphosphate, Ammonium Salt (13): A solution of diol **11** (185 mg, 0.63 mmol) and pyridine (61 μL, 60 mg, 0.75 mmol) in CH_2Cl_2 (2 mL) was stirred and cooled at 0°C as diethyl chlorophosphate (95 μL, 114 mg, 0.66 mmol) was added neat by syringe. After 3 h at 0 °C, TLC indicated the reaction was nearly complete, and the CH_2Cl_2 solution was loaded directly onto a silica gel column. Elution with 4:1 EtOAc/hexane gave starting material (59 mg, 32%) and primary phosphate (125 mg, 46% or 68% based on unrecovered **11**). A solution of the phosphate (100 mg, 0.23 mmol) in dry [$^2H_3$]MeCN (1 mL) was stirred as $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ (885 mg, 0.93 mmol) was added as a solid. After dissolution, predried, powdered 3-Å molecular sieves (ca. 50 mg) were added, and the flask was placed in a desiccator. The reaction progress was monitored by comparison of the intensities of the inorganic diphosphate singlet (δ_P = –6.32) and the organic diphosphate doublet of doublets in the ^{31}P-NMR spectrum. The half-life was approximately 20 h, and the ratio remained constant after 88 h. The solution was concentrated under reduced pressure, the residue was suspended in water (5 mL), and the suspension was loaded onto an ion exchange column (NH_4^+ form, 1.4 × 30 cm). Ion exchange followed by cellulose chromatography and lyophilization as described above for (*R*)-[1-$^2H_1$]-**6** gave 114 mg of a 1:1:0.5 mixture of organic diphosphate **13**, diethyl phosphate, and an unknown impurity. A 30-mg portion was further purified by preparative HPLC to give diphosphate **13** (20 mg, 65% based on 76 mg from diethyl phosphate). – ^{1}H NMR (400 MHz, D_2O): δ_H = 0.64, 0.64, 0.70 (3 s, 9 H, 3CH_3), 0.9–1.6 (m, 13 H), 1.57 (s, 3 H, CH_3), 1.8–2.2 (m, 3 H), 3.35 (td, 1 H, *J* = 10.7, 4.6 Hz), 4.32 (t, 2 H, *J* = 6.6 Hz, CH_2OPP), 5.31 (t, 1 H, *J* = 6.8 Hz, =CH). – ^{31}P NMR (161 MHz, D_2O): δ_P = –7.02, –9.76 (2 d, *J* = 22.0 Hz).

(5*S*,8*S*,9*R*,10*S*,13*E*)-17-Norlabda-13-en-8,15-diol (12): A solution of unlabeled keto alcohol **15** (405 mg, 1.38 mmol) in MeOH (15 mL) was stirred as solid $NaBH_4$ (102 mg, 2.75 mmol) was added. After 3 h, the solution was diluted with water (100 mL) and extracted with diethyl ether (3 × 100 mL). The combined ethereal extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a thick oil. Purification by column chromatography using 1:1 hexane/EtOAc gave 301 mg (74%) of 8β,15-diol **12**. A minor amount of diol **11** identified by TLC comparisons was not isolated. Data for **12**: m.p. 96–98 °C. – ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 0.80–0.90 (m, 1 H), 0.81 (dd, 1 H, *J* = 12.8, 3.7 Hz), 0.83, 0.85, 0.97 (3 s, 9 H, 3 CH_3), 0.90–1.0 (m, 1 H), 1.12 (td, 1 H, *J* = 14.3, 4.0 Hz), 1.3–1.4 (m, 6 H), 1.4–1.6 (m, 4 H), 1.66 (s, 3 H, =CCH_3), 1.71 (dq, 1 H, *J* = 12.8, 2.0 Hz), 1.8–2.0 (m, 2 H), 2.12 (dt, 1 H, *J* = 13.5, 7.0 Hz), 3.95 (q, 1 H, *J* = 2.6 Hz, C*H*OH), 4.14 (d, 2 H,*J* = 7.0 Hz, CH_2OH), 5.40 (t of sext, 1 H, *J* = 7.0, 1.3 Hz, =C*H*). – ^{13}C NMR (126 MHz, $CDCl_3$): δ_C = 16.3 (CH_3), 16.5 (CH_3), 17.1 (CH_2), 18.5 (CH_2), 22.0 (CH_3), 22.5 (CH_2), 33.5 (C), 33.8 (CH_3), 35.6 (CH_2), 38.1 (C), 38.2 (CH_2), 39.4 (CH_2), 42.3 (CH_2), 53.8 (CH), 56.2 (CH), 59.6 (CH_2), 68.0 (CH), 123.7 (CH), 140.4 (C). – IR (CCl_4): $\tilde{\nu}$ = 3620, 3392 (OH): cm^{-1}. – $[\alpha]_D^{20}$ = +39.9 (*c* = 0.97, EtOH). – $C_{19}H_{34}O_2$ (294.48): calcd. C 77.50, H 11.64; found C 77.27, H 11.93.

(5*S*,8*S*,9*R*,10*S*,13*E*)-8-Hydroxy-17-nor-labda-13-en-15-yl Diphosphate, Ammonium Salt (14): Conversion of diol **12** (105 mg, 0.36 mmol) to the diethyl phosphate intermediate with pyridine (38 μL, 37 mg, 0.46 mmol) and diethyl chlorophosphate (57 μL, 68 mg, 0.39 mmol) in CH_2Cl_2 (1 mL) was carried out for 1 h at 0°C as described above for **13**. Purification by column chromatography using 4:1 EtOAc/hexane gave the phosphate (95 mg, 0.22 mmol, 61%). Displacement with $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ (650 mg, 0.68 mmol) and molecular sieves (ca 50 mg) in MeCN (1.5 mL) proceeded for 3.5 d. Purification by ion exchange chromatography, cellulose chromatography, and lyophylization as described above for (*R*)-[1-$^2H_1$]-**6** gave 100 mg (67% from the diethyl phosphate) as a 1:1 mixture of diphosphate **14** and diethyl phosphate as NH_4^+ salts. Data for **14** from the mixture: ^{1}H NMR (400 MHz, D_2O): δ_H = 0.74, 0.76, 0.84 (3 s, 9 H, 3 CH_3), 1.0–1.9 (m, 13 H), 1.65 (s, 3 H, CH_3), 1.8–2.2 (m, 3 H), 3.85 (s, 1 H, OCH), 4.3–4.4 (m, 2 H, CH_2OPP), 5.37 (t, 1 H, *J* = 6.8 Hz, =CH). – ^{31}P NMR (161 MHz, D_2O): δ_P = –5.67, –9.58 (2d, *J* = 22.0 Hz).

(5*S*,9*R*,10*S*,13*E*,15*S*)-[15-$^2H_1$]-17-Nor-8-oxolabda-13-en-15-ol-{(15*S*)-[15-$^2H_1$]-15}: The oxidation and asymmetric reduction were carried out as described above for (*S*)-[1-$^2H_1$]-**1**. Reaction of [15,15-$^2H_2$]-**15** (810 mg, 2.75 mmol) with activated MnO_2 (14 g, 161 mmol) in hexane (40 mL) at room temp. for 1 h gave crude deuterio aldehyde which was reduced with 10.0 mL (5.0 mmol) of (*R*)-Alpine borane (0.5 M in THF, Aldrich) at room temp. for 1.5 h followed by workup with THF (10 mL), 10% NaOH (15 mL), and 30% H_2O_2 (6 mL) in an ice bath to maintain the internal temperature below 25 °C. Extraction and purification by column chromatography using 2:1 hexane/EtOAc gave pinol (379 mg) and (15*S*)-[15-$^2H_1$]-**15** (429 mg, 53%) as a 13:1 mixture of (*E*) and (*Z*) isomers which were separated after reduction to the diol. The ^{1}H-NMR spectrum matched unlabeled **15** except for the following: ^{1}H NMR (500 Mz, $CDCl_3$): δ_H = 0 4.09 (d, 1 H, *J* = 6.8 Hz, C*H*DOH), 5.32 (d, 1 H, *J* = 6.8 Hz, =CH). – MS (FI); *m*/*z*: 293 (100) [M^+], 292 (1.0), 291 (1.5). Up to 99% [$^2H_1$], 1% [$^2H_0$].

The diastereopurity of (15*S*)-[1-$^2H_1$]-**15** was assessed by conversion into the (1′*S*)-camphanate derivative **16** (7 mg, 90%) and ^{1}H-NMR analysis as described above for (*S*)-[1-$^2H_1$]-**5**. The ^{1}H-NMR spectrum (500 MHz, C_6D_6) of a (15*R*) + (15*S*) mixture of [15-$^2H_1$]-**16** diastereomers prepared by $NaBH_4$ of the [15-$^2H_1$] keto aldehyde intermediate and conversion into the camphanate showed two overlapped doublets at δ_H = 4.60 (d, 1 H, *J* ≈ 6 Hz) and 4.62 (d, 1 H, *J* ≈ 7 Hz) for the CHDOR group. The amount of (15*R*) diastereomer in (15*S*)-[1-$^2H_1$]-**16** was estimated to be ca. 2%. – ^{1}H NMR (500 MHz, C_6D_6): δ_H = 0.59, 0.68, 0.73, 0.83, 0.85, 0.87, 1.60 (7 s, 21 H, 7CH_3), 0.80–0.90 (m, 2 H), 1.0–1.5 (m, 8 H), 1.48 (dq, 1 H, *J* = 13.2, 2.0 Hz), 1.6–1.8 (m, 3 H), 1.85 (dt, 1 H, *J* = 13.4, 7.6 Hz), 1.97 (td, 1 H, *J* = 13.2, 8.1 Hz), 2.01 (ddd, 1 H, *J* = 12.9, 9.8, 8.5 Hz), 2.12 (ddd, 2 H, *J* = 13.4, 10.0, 4.6 Hz), 2.35 (ddd, 1 H, *J* = 12.9, 4.7, 2.0 Hz,), 4.62 (d, 1 H, *J* = 6.8 Hz, CDHOR), 5.42 (dd, 1 H, *J* = 7.1, 1.0 Hz, =CH).

(5*S*,8*R*,9*R*,10*S*,13*E*,15*S*)-[15-$^2H_1$]-17-Norlabda-13-ene-8,15-diol {(15*S*)-[15-$^2H_1$]-11}: The Li/NH_3 reduction of (15*S*)-[15-$^2H_1$]-**15** (320 mg, 1.09 mmol) was carried out as described above for unlabeled **15**. Purification by column chromatography using 1:1 hexane/EtOAc gave 285 mg (85%) of (15*S*)-[15-$^2H_1$]-**11** as a solid. The m.p. and ^{1}H-NMR spectrum matched unlabeled diol **11** except for the following: ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 4.12 (d, 1 H, *J* = 6.9 Hz,C*H*DOH), 5.41 (d, *J* = 6.4, 1 H, =CH).

(5*S*,8*R*,9*R*,10*S*,13*E*,15*R*)-[15-$^2H_1$]-17-Nor-8-hydroxylabda-13-en-15-yl Diphosphate, Ammonium Salt {(15*R*)-[15-$^2H_1$]-13}: Conversion of (15*S*)-[15-$^2H_1$]-**11** (124 mg, 0.42 mmol) to the corresponding diethyl phosphate (128 mg, 71%) and displacement with $(Bu_4N)HP_2O_7 \cdot (H_2O)_3$ (1.11 g, 1.16 mol) in CH_3CN (2 mL) for 4 d were conducted as described above for unlabeled **11**. Purification by ion exchange chromatography followed by cellulose chromatography and lypohilization gave a 1:1 mixture of organic diphosphate and diethyl phosphate (197 mg). Further purification by preparative HPLC in two portions gave 90 mg (60% from the diethyl phosphate) of (15*R*)-[15-$^2H_1$]-**13**. The ^{1}H- and ^{31}P-NMR spectra matched those of unlabeled **13** except for the following: ^{1}H NMR (400 MHz, D_2O): δ_H = 4.26 (d, 1 H, *J* = 6.6 Hz, CHDOPP), 5.26 (d, 1 H, *J* = 6.8 Hz, =CH).

(6*E*,10*E*,14*E*)-16-Acetoxy-2,6,10,14-tetramethyl-6,10,14-hexadecatriene-2,3-diol: Hydrolysis conditions were based on those described by Grigor'eva, et al.[40] A solution of 14,15-oxidogeranylgeranyl acetate[35] (2.36 g crude, ca 6.77 mmol) in THF/H_2O (48 mL/30 mL) was stirred at room temp. as $HClO_4$ (70% in H_2O, ca. 0.5 mL, 20 drops) was added. After 1.2 h, the solution was diluted with satd $NaHCO_3$ (200 mL) and extracted with diethyl ether (4 × 75 mL). The combined ethereal extracts were washed with satd $NaHCO_3$ (40 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give a thick oil (2.71 g). Purification by column chromatography using 1:1 hexane/EtOAc gave 2.12 g (ca 85%) of the diol acetate. – ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 1.12, 1.16, 1.56, 1.58, 1.67 (5 s, 15 H, 5 CH_3), 1.37 (dddd, 1 H, *J* = 14.1, 10.6, 8.8, 5.7 Hz), 1.5–1.6 (m, 1 H), 2.02 (s, 3 H, O_2CCH_3), 1.9–2.3 (m, 12 H), 3.31 (dd, 1 H, *J* = 10.6, 1.8 Hz, C*H*OH), 4.55 (d, 2 H, *J* = 7.1 Hz, CH_2OH), 5.06 (t of sext, 1 H, *J* = 7.0, 1.3 Hz,=CH), 5.14 (t of sext, 1 H, *J* = 7.0, 1.3 Hz, =CH), 5.31 (t of sext, 1 H,*J* = 7.1, 1.3 Hz, H15 =CH). – ^{13}C NMR (126 MHz, $CDCl_3$): δ_C = 16.1 (CH_3), 16.2 (CH_3), 16.7 (CH_3), 21.3 (CH_3), 23.5 (CH_3), 26.3 (CH_2), 26.6 (CH_3), 26.7 (CH_2), 29.9 (CH_2), 37.0 (CH_2), 39.7 (CH_2), 39.8 (CH_2), 61.6 (CH_2), 73.2 (C), 78.5 (CH), 118.5 (CH), 124.0 (CH), 125.2 (CH), 135.1 (C), 135.5 (C), 142.5 (C), 171.5 (C). – IR (CCl_4): $\tilde{\nu}$ = 3451 (OH), 1740 (C=O): cm^{-1}. – $C_{22}H_{38}O_4$ (366.54): calcd. C 72.09, H 10.45; found C 71.93, H 10.49.

(*E*,*E*,*E*)-14-Acetoxy-4,8,12-trimethyl-4,8,12-tetradecatrienal (17): Conditions were based on those described by Grigor'eva, et al.[40] A solution of the preceding diol acetate (757 mg, 2.07 mmol) in THF (15 mL) was stirred at room temp. as $NaIO_4$ (1.10 g, 5.14 mmol) was added followed by sufficient water (ca. 10 mL) to dissolve the salt. After 2.5 h, the resulting suspension was diluted with water (100 mL) and extracted with diethyl ether (3 × 50 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a clear oil (510 mg). Purification by column chromatography using 5:1 hexane/EtOAc gave 496 mg (78%) of acetoxy aldehyde **17**. – ^{13}C NMR (126 MHz, $CDCl_3$): δ_C = 16.05 (CH_3), 16.16 (CH_3), 16.5 (CH_3), 21.1 (CH_3), 26.2 (CH_2), 26.6 (CH_2), 31.9 (CH_2), 39.55 (CH_2), 39.58 (CH_2), 42.2 (CH_2), 61.4 (CH_2), 118.5 (CH), 124.0 (CH), 125.4 (CH), 133.1 (C), 135.3 (C), 142.3 (C), 171.2 (C), 202.7 (CH). – IR (CCl_4): $\tilde{\nu}$ = 1740 (ester C=O), 1717 (aldehyde C=O) cm^{-1}. The ^{1}H-NMR data agreed with the literature values.[34]

(*E*,*E*,*E*)-14-(Dimethylamino)-3,7,11-trimethyl-2,6,10-hexadecatrienol (18): A solution of acetoxy aldehyde **17** (335 mg, 1.1 mmol) in anhydrous MeOH (8 mL) was stirred at room temp. as $Me_2NH{\cdot}HCl$ (168 mg, 2.1 mmol) and $NaBH_3CN$ (413 mg, 6.6 mmol) were added.[36] After 2 d, the solution was diluted with 1% NaOH (45 mL) and extracted with diethyl ether (4 × 50 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography using 10:1 CH_2Cl_2/MeOH gave hydroxy acetate (16 mg, 5%) and amino acetate (244 mg, 66%). A suspension of solid K_2CO_3 (500 mg) in a solution of the amino acetate (240 mg, 0.69 mmol) in MeOH (2 mL) was stirred at room temp. for 24 h. Water (25 mL) was added, and the product was extracted with CH_2Cl_2 (4 × 20 mL). The combined organic extracts were concentrated under reduced pressure to a cloudy oil (200 mg). Purification by column chromatography using 8:1 CH_2Cl_2/MeOH gave 121 mg (60%) of amino alcohol **18**. – ^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 1.52 (s, 6 H, 2 CH_3), 1.5–1.6 (m, 2 H), 1.59 (s, 3 H, CH_3),1.9–2.1 (m, 10 H), 2.18 [s, 6 H, $N(CH_3)_2$], 2.2–2.3 (m, 2 H), 3.84 (s, 1 H, OH), 4.05 (d, 2 H, *J* = 6.8 Hz, CH_2OH), 5.04 (t of sext, 2 H, *J* = 7.0, 1.3 Hz, = CH), 5.32 (t of sext, 1 H, *J* = 6.8, 1.3 Hz, C-2 =CH). – ^{13}C NMR (126 MHz, $CDCl_3$): δ_C = 16.05 (CH_3), 16.07 (CH_3), 16.4 (CH_3), 25.7 (CH_2), 26.3 (CH_2), 26.5 (CH_2), 37.4 (CH_2), 39.67 (CH_2), 39.72 (CH_2), 45.3 (2 CH_3), 59.1 (CH_2), 59.5 (CH_2), 124.2 (CH), 124.3 (CH), 124.6 (CH), 134.5 (C), 135.2 (C), 138.5 (C). – IR (CCl_4): $\tilde{\nu}$ = 3623, 3241 cm^{-1}. – $C_{19}H_{35}NO$ (293.50): calcd. C 77.76, H 12.02, N 4.77; found C 76.46, H 12.18, N 4.63.

(2*E*,6*E*,10*E*)-14-(Dimethylamino)-3,7,11-trimethyl-2,6,10-hexadecatrienyl Diphosphate, Ammonium Salt (19): Diphosphate **19** was prepared as described above for (*R*)-[1-$^2H_1$]-**6**. Reaction of amino alcohol **18** (116 mg, 0.40 mmol) with diethyl chlorophosphate (228 μL, 273 mg, 1.58 mmol) and pyridine (144 μL, 141 mg, 1.78 mmol) in CH_2Cl_2 (8 mL) at 0 °C for 2.5 h followed by extraction and purification by column chromatography using 6:1 CH_2Cl_2/MeOH gave the diethyl phosphate (144 mg, 0.34 mmol, 85%). Displacement with $(Bu_4N)_3 \cdot HP_2O_7 \cdot (H_2O)_3$ (1.01 g, 1.06 mmol) in MeCN (1.5 mL) containing powdered 3-Å molecular sieves (ca. 50 mg) for 5 d, followed by ion exchange, cellulose chromatography, and lyophilization gave 110 mg of a 6:1:2:1 mixture of diethyl phosphate **19** and two unknown impurities. Purification of this mixture by preparative HPLC in two portions gave 35 mg (21%) of amino diphosphate **19**. – ^{1}H NMR (400 MHz, D_2O): δ_H = 1.46 (s, 6 H, 2 CH_3), 1.56 (s, 3 H, CH_3), 1.6–1.7 (m, 2 H), 1.8–2.1 (m, 10 H), 2.69 [s, 6 H, $N(CH_3)_2$], 2.8–3.0 (m, 2 H, CH_2N), 4.23 (t, 2 H, J = 6.6 Hz, CH_2OPP), 5.04 (t, 1 H, J = 6.4 Hz, =CH), 5.05 (t, 1 H, J = 6.8 Hz, =CH), 5.29 (t, 1 H, J = 7.1 Hz, C-2, =CH). – ^{31}P NMR (162 MHz, D_2O): δ_P = –7.38, –9.87 (2 d, J = 22.0 Hz).

Acknowledgments

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