Enantioselective Synthesis of (+)-Presqualene Diphosphate

Daniel H. Rogers, Eugene C. Yi, and C. Dale Poulter*

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

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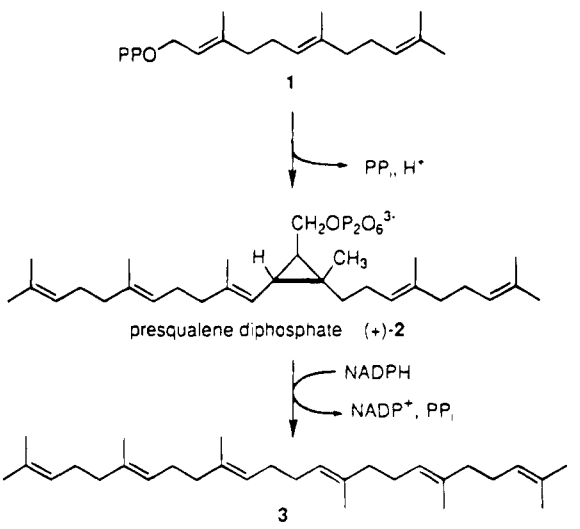
(+)-(1*R*,2*R*,3*R*)-Presqualene diphosphate (PSPP) is an intermediate in the biosynthesis of squalene from farnesyl diphosphate (FPP). The natural 1*R*,2*R*,3*R* enantiomer was synthesized from farnesol in nine steps with ≥98% ee. The key step in the synthesis was the stereoselective intramolecular cyclopropanation of farnesyl diazoacetate (**5**) upon treatment with a chiral rhodium catalyst to give (−)-lactone **6**.

Squalene synthase (SQSase, farnesyl diphosphate: farnesyl diphosphate farnesyl transferase, EC 2.5.1.2.1) catalyzes the first pathway specific step of cholesterol biosynthesis.[1] Because of its location at a branch point, the enzyme is considered to be an attractive target for development of drugs to control hypercholesterolemia.[2-4] Squalene is synthesized from FPP in two distinct steps (see Scheme 1).[1] In the first, two molecules of FPP are coupled to form a stable cyclopropylcarbinyl triterpene, (+)-(1*R*,2*R*,3*R*) presqualene diphosphate (PSPP, **2**). Under normal conditions, only trace amounts of PSPP are detected when FPP is converted to squalene.[5] Although PSPP accumulates in the absence of NADPH, it is difficult to obtain sufficient PSPP for mechanistic studies.

In connection with steady state and pre-steady state kinetic experiments with SQSase, we required sources of both cold and radiolabeled (+)-(1*R*,2*R*,3*R*)-PSPP. Although several syntheses of racemic presqualene alcohol (PSOH) have been reported,[6-9] including one case where the alcohol was resolved,[9] the yields of racemic PSOH were typically less than 5%. For enzymatic experiments, the alcohol must then be resolved and phosphorylated. Of the various possibilities for developing a useful synthesis of (+)-(1*R*,2*R*,3*R*)-PSPP, we were drawn to the approach reported by Coates *et al.*[6] based on an intramolecular cyclopropanation of farnesyl diazoacetate to construct the cyclopropane ring in PSPP. By using a chiral catalyst for the cyclopropanation, as recently described by Doyle and co-workers,[10-13] we thought it might be possible to fix the three stereocenters in the cyclopropane ring during the cyclization. We now describe the use of chiral Rh_2[5(*R*)-MEPY]$_4$ (**4**) to prepare optically enriched PSOH and its subsequent conversion to enantiomerically pure PSPP.

Scheme 1



Results and Discussion

Dirhodium tetrakis[methyl 2-pyrrolidone-5(*R*)-carboxylate] (Rh_2[5(*R*)-MEPY]$_4$, **4**) was selected as a catalyst based on a previous report of the stereoselective intramolecular cyclopropanation of 3-methyl-2-buten-1-yl diazoacetate, an excellent model of the Coates lactone.[13] The catalyst was synthesized by ligand exchange of rhodium(II) acetate ($Rh_2(OAc)_4$) with methyl 2-pyrrolidone-5(*R*)-carboxylate using a continuous extraction and purified on cyanide-capped silica gel.[13] Farnesyl diazoacetate (**5**) was prepared in 94% yield from commercially available *trans,trans*-farnesol and glyoxylic acid chloride *p*-toluenesulfonate by standard methods.[14]

Slow addition of diazoester **5** to a solution of **4** (0.01 equiv) in dichloromethane at reflux, followed by filtration

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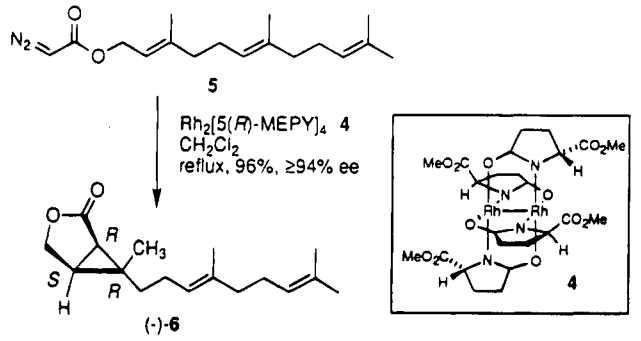
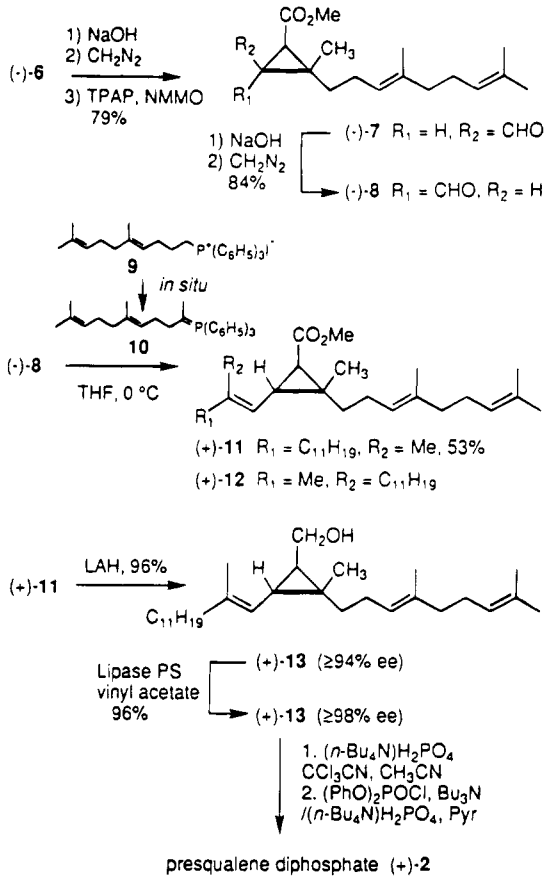
(1) Poulter, C. D. *Acc. Chem. Res.* **1990**, *23*, 70–77.
(2) Ciosek, C. P., Magnin, D. R.; Harrity, T. W.; Logan, J. V. H.; Dickson, J. K., Jr.; Gordon, E. M.; Hamilton, K. A.; Jolibois, K. G.; Kunselman, L. K.; Lawrence, R. M.; Mookhtiar, K. A.; Rich, L. C.; Slusarchyk, D. A.; Sulsky, R. B.; Biller, S. A. *J. Biol. Chem.* **1993**, *268*, 24832–24837.
(3) Bergstrom, J. D.; Kurtz, M. M.; Rew, D. J.; Amend, A. M.; Karkas, J. D.; Bostedor, R. G.; Bansal, V. S.; Dufresne, C.; VanMiddlesworth, F. L.; Hensens, O. D.; Liesch, J. M.; Zink, D. L.; Wilson, K. E.; Onishi, J.; Milligan, J. A.; Bills, G.; Kaplan, L.; Omstead, M. N.; Jenkins, R. G.; Huang, L.; Meinz, M. S.; Quinn, L.; Burg, R. W.; Kong, Y. L.; Mochales, S.; Mojena, M.; Martin, I.; Pelaez, F.; Diez, M. T.; Alberts, A. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 80–84.
(4) Baxter, A.; Fitzgerald, B. J.; Hutson, J. L.; McCarthy, A. D.; Motteram, J. M.; Ross, B. C.: Sapra, M.; Snowden, M. A.; Watson, N. S.; Williams, R. J.; Wright, C. *J. Biol. Chem.* **1992**, *267*, 11705–11708.
(5) Muscio, F.; Carlson, J. P.; Kuehl, L.; Rilling, H. C. *J. Biol. Chem.* **1974**, *249*, 3746–3749.
(6) (a) Coates, R. M.; Robinson, W. H. *J. Am. Chem. Soc.* **1971**, *93*, 1785–1786. (b) Robinson, W. H. Ph.D. Thesis, University of Illinois, Diss. Abstr.
(7) Altman, L. R.; Kowerski, R. C.; Rilling, H. C. *J. Am. Chem. Soc.* **1971**, *93*, 1782–1783.
(8) Campbell, R. V. M.; Crombie, L.; Pattenden, G. *Chem. Commun.* **1971**, 218–219.
(9) Altman, L. J.; Kowerski, R. C.; Laungani, D. R. *J. Am. Chem. Soc.* **1978**, *100*, 6174–6182.
(10) Doyle, M. P.; Dorow, R. L.; Terpstra, J. W.; Rodenhouse, R. A. *J. Org. Chem.* **1985**, *50*, 1663–1666.
(11) Doyle, M. P.; Brandes, B. D.; Kazala, A. P.; Pieters, R. J.; Jarstfer, M. B.; Watkins, L. M.; Eagle, C. T. *Tetrahedron Lett.* **1990**, *31*, 6613- 6616.
(12) Doyle, M. P.; Pieters, R. J.; Martin, S. F.; Austin, R. E.; Oalman, C. J.; Müller, P. *J. Am. Chem. Soc.* **1991**, *113*, 1423- 1424.
(13) Doyle, M. P.; Winchester, W. R.; Hoorn, J. A. A.; Lynch, V.; Simonsen, S. H.; Ghosh, R. *J. Am. Chem. Soc.* **1993**, *115*, 9968–9978.
(14) Corey, E. J.; Myers, A. G. *Tetrahedron Lett.* **1984**, *25*, 3559–3562.

0022-3263/95/1960-0941$09.00/0

Scheme 2



Scheme 3



and flash chromatography, gave a 96% isolated yield of (−)-lactone **6** (Scheme 2).[15] The enantioselectivity of the cyclopropanation was ≥94% ee, as determined by analysis of ^{1}H NMR spectra of samples containing increasing amounts of the chiral shift reagent tris[3-((heptafluoropropyl)hydroxymethylene)-(−)-camphorato]europium (Eu(hfc)$_3$). Addition of 0.3−0.5 equiv of the shift reagent was sufficient to isolate and to resolve the cyclopropyl methyls (shift from 0.87 ppm to *ca.* 2.8 ppm) of both enantiomers. The relative intensity of the peaks was determined from an expanded spectrum by cutting and weighing. The ratio of (−)-(4*S*,5*R*,6*R*)-**6** to the minor enantiomer was *ca.* 50:1. The presence of (+)-(4*R*,5*S*,6*S*)-**6** was confirmed by its synthesis using Rh$_2$[5(*S*)-MEPY]$_4$.

Completion of the Carbon Skeleton. The cyclopropanation established three stereogenic centers, two of which were incorporated unchanged into (+)-(1*R*,2*R*,3*R*)-PSPP. We used the oxidation/epimerization strategy (Scheme 3) reported by Coates[6] to invert the third center. Alkaline hydrolysis of (−)-lactone **6**, followed by treatment of the acidified product with diazomethane, gave an intermediate hydroxy ester, which was immediately treated with tetra-*n*-propylammonium perruthenate (TPAP)[16,17] in the presence of *N*-methylmorpholine *N*-oxide (NMO) to provide (−)-aldehyde **7** in good yield (79%). The aldehyde was epimerized under basic conditions and re-esterified with diazomethane to give (−)-*epi*-aldehyde[18] **8** in 85% overall yield.

Phosphonium salt **9** (Scheme 3) was synthesized from geranyl bromide in five steps and 80% overall yield. Ylide **10** was generated *in situ* by treatment of phosphonium salt **9** with *n*-BuLi, alkylation of the ylide with MeI, and treatment of the resulting salt with *n*-BuLi.[19] Addition of 0.5 equiv of aldehyde **8** at 0 °C to the ylide gave a *ca.* 2:1 mixture of (*E*)-(+)-**11** and its (*Z*)-isomer **12**. Isomer (+)-**11** was obtained in 53% yield after purification by flash chromatography. Attempts to improve the *E*/*Z* ratio by changing the solvent, temperature, or base were unsuccessful.

(+)-(1*R*,2*R*,3*R*)-Presqualene alcohol (**13**) was obtained by reduction of (+)-ester **11** with LiAlH$_4$. The optical purity was ≥94% ee on the basis of ^{1}H NMR experiments using chiral shift reagents, as well as optical rotation.[20] Upon addition of increasing amounts of Eu(hfc)$_3$ to a sample of racemic presqualene alcohol in C$_6$D$_6$, the peaks for two olefinic methyls around 1.83 ppm moved downfield to *ca.* 3.38 and 2.51 ppm, respectively, and resolved into pairs separated by 10−21 Hz. The upfield signals in each pair were assigned to the (1*R*,2*R*,3*R*)-enantiomer from comparisons of these spectra with those for enantiomerically enriched samples of PSOH. The ratio of enantiomers was *ca.* 50:1. (+)-**PSOH** was further enriched by an enantioselective enzyme-catalyzed acetylation of the unnatural (1*S*,2*S*,3*S*)-enantiomer. In preliminary studies with racemic presqualene alcohol, a higher enantioselectivity was obtained with lipase from *Pseudomonas sp.* (PSL)[21] than the more commonly used lipases from porcine pancreas[22] or *Candida cyclindracea*.[23] Alcohol (−)-(1*S*,2*S*,3*S*)-**13** was preferrentially acylated by PSL in dry vinyl acetate, and the ee of recovered (+)-PSOH was ≥70% at 50% conversion. Resubjecting the partially resolved alcohol to the same conditions gave completely resolved (+)-PSOH in 29% overall yield. In a similar manner, treatment of the synthetic (1*R*,2*R*,3*R*)-PSOH (~94% ee) with PSL gave (+)-PSOH (≥98% ee), as determined by ^{1}H NMR using Eu(hfc)$_3$ and by optical rotation, in 96% yield.

Phosphorylation of Presqualene Alcohol. An efficient method for the conversion of primary isoprenoid

(15) This material contained a minor impurity (*ca.* 5%) believed to come from *trans,cis*-farnesol present in the starting material. The impurity was not observed in subsequent intermediates. Faster addition of **7** resulted in a lower yield of **6** and increased formation of unidentified byproducts.

(16) Griffith, W. P.; Ley, S. V. *Aldrichim. Acta* **1990**, *23*, 13−20.

(17) Griffith, W. P.; Whitecombe, G. P.; White, A. D.; Ley, S. V. *J. Chem. Soc., Chem. Commun.* **1987**, 1625−1627.

(18) Aldehyde **10**, as the methyl ester, tended to epimerize to a *ca.* 1:1 mixture under mildly acidic conditions, e.g., prolonged exposure to traces of silica gel, even at −20 °C.

(19) Coates, R. M.; Ley, D. A.; Cavender, P. L. *J. Org. Chem.* **1978**, *43*, 4915−4922.

(20) Other spectral data were in agreement with those previously reported.

(21) Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 3127−3129.

(22) Laroyre, J.; Verrier, J.; Baratti, J. *Biotech. Bioeng.* **1982**, *24*, 2175−2177.

(23) Abramowics, D. A.; Keese, C. R. *Biotech. Bioeng.* **1989**, *33*, 149−156.

alcohols to their diphosphates by nucleophilic substitution with tris(tetrabutylammonium) hydrogen diphosphate was reported by Davisson *et al.*[24,25] However, this procedure failed for several different derivatives of PSOH, presumably because of steric hindrance created by the highly substituted cyclopropane ring, as well as the tendency of the cyclopropylcarbinyl moiety to rearrange. As a consequence, the diphosphate moiety was introduced by a Cramer phosphorylation.[26] This reaction typically gives poor yields of isoprenoid diphosphates; however, Danilov *et al.*[27] recently reported a substantial improvement using tetra-*n*-butylammonium phosphate as the source of phosphate for the condensation. In our hands, the modified version of the Cramer phosphorylation consistently gave acceptable (22%) isolated yields of PSPP. (1*R*,2*R*,3*R*)-PSOH was phosphorylated by the slow addition of 6 equiv of tetra-*n*-butylammonium dihydrogen phosphate (TBADP) in acetonitrile to a mixture of the alcohol dissolved in excess trichloroacetonitrile. The reaction gave both di- and triphosphates esters, and we had difficulty in separating the compounds. Pure PSPP was more reliably prepared by a two-step process. The alcohol was first treated with 1.2 equiv of TBADP in the presence of excess trichloroacetonitrile to give a mixture of monophosphorylated (52%) and diphosphorylated (10%) products, along with recovered starting material (35%), after purification on a hydrophobic LH-20 sephadex column. The monophosphate was condensed with diphenylchlorophosphate, treated with TBADP, and purified by chromatography on a LH-20 sephadex column to afford PSPP (68%). For enzymatic studies the tetrabutylammonium cation was replaced with NH_4^+ by cationic exchange chromatography, and (+)-(1*R*,2*R*,3*R*)-PSPP was obtained in 29% overall yield from PSOH after preparative-scale reversed-phase HPLC.[28]

Conclusions. Following a strategy originally reported by Coates *et al.* for racemic material,[6] enantiomerically pure (+)-(1*R*,2*R*,3*R*)-presqualene alcohol was synthesized from farnesol in seven steps in an overall yield of 29%. The key step in our synthesis of the alcohol was an enantioselective rhodium-catalyzed intramolecular cyclopropanation of farnesyl diazoacetate. Our approach represents a significant improvement over previous reports for synthesis of racemic alcohol. Presqualene alcohol was converted to naturally occurring (+)-(1*R*,2*R*,3*R*) presqualene diphosphate in 68% yield. The overall yield for conversion of PSOH to ammonium PSPP was 29% (based on recovered starting material). These procedures give reasonable quantities of natural material for enzymatic studies.

Experimental Section[29]

General Methods. All nonaqueous reactions were performed under a dry Ar atmosphere, with dry solvents and reaction vessels. "Dried and concentrated" refers to removal of residual quantities of water with anhydrous Na_2SO_4 followed by evaporation of solvent on a rotary evaporator. Flash chromatography[30] was conducted on silica gel grade 60, 230–400 mesh with the solvent system indicated. Thin layer chromatography was performed on silica gel 60 F254. The plates were visualized by UV light and developed with phosphomolybdic acid. ^1H and ^{13}C NMR spectra were recorded in C_6D_6 at 300 MHz and 75 MHz, respectively, unless otherwise stated, using the residual C_6D_6 (^1H NMR, 7.20 ppm; ^{13}C NMR, 128.0 ppm, center line) signal as an internal standard. ^{31}P NMR spectra were measured using phosphoric acid in D_2O (0 ppm) as an external standard. Optical rotations were determined at 20 °C (*c* in g per 100 mL solvent). Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded using electron impact (EI) at 70 eV unless otherwise stated.

Materials. All solvents and volatile reagents were distilled prior to use. Diethyl ether (ether) and THF were distilled from sodium or potassium/benzophenone; benzene, CH_2Cl_2, CH_3CN, *N*,*N*-dimethylaniline, and triethylamine were distilled from CaH_2; vinyl acetate was distilled from anhydrous $CaCl_2$. Lipases (Sigma) were stored at −20 °C. Other reagents were purchased from Aldrich.

***(E,E)*-Farnesyl Diazoacetate (5).** To a solution of 1.70 g (6.51 mmol, 1.9 equiv) of glyoxylic acid chloride (*p*-toluenesulfonyl)hydrazone[31] and 0.81 g (3.48 mmol) of *trans*,*trans*-farnesol in 17 mL dry CH_2Cl_2 at 0 °C was added 0.77 g (6.39 mmol, 1.8 equiv) of *N*,*N*-dimethylaniline dropwise. The mixture was stirred for 15 min, 1.79 g (17.7 mmol, 5.1 equiv) of triethylamine was added to the green solution, and the resulting orange brown mixture was stirred at 0 °C for 10 min and at rt for 15 min, before 10 mL of water was added. The volatile components were removed *in vacuo*, and 1:9 (v/v) ethyl acetate in hexanes was added. The organic phase was washed with water, saturated citric acid solution, water, and saturated NaCl solution, dried, and concentrated. Purification by flash chromatography (1:19 (v/v) ethyl acetate in hexane) provided 950 mg (94%) of a bright yellow oil: R_f 0.38 (1:9 (v/v) ethyl acetate in hexane); ^1H NMR δ 5.43, 5.26, 5.20 (3 × m, 3 × 1H), 4.69 (m, 2H), 4.07 (s(br), 1H), 2.25–1.93 (m, 8H), 1.72, 1.61, 1.58, 1.53 (4 × s(br), 4 × 3H); ^{13}C NMR δ 142.1, 135.4, 131.2, 124.8, 124.2, 119.2, 61.6, 45.7, 40.2, 39.8, 27.2, 26.5, 25.9, 17.8, 16.4, 16.1.

(−)-(4*R*,5*R*,6*S*)-Lactone 6. To a mixture of 100 mg (0.11 mmol, 0.01 equiv) of dirhodium(II) tetrakis(methyl 2-pyrrolidone-5(*R*)-carboxylate) (Rh_2[5(*R*)-MEPY)$_4$, **4**)[13] in 18 mL of dry CH_2Cl_2 at reflux was added 3.15 g (10.9 mmol) of diazoester **5** in 53 mL of CH_2Cl_2 dropwise over 11 h by syringe pump. The reaction mixture was concentrated and purified by flash chromatography (step gradient from 1:13 to 1:5.6 (v/v) ethyl acetate in hexane, 300 mL silica gel) to give 2.73 g (96%; contains *ca.* 5% impurity, believed to have arisen from *trans*,*cis*-farnesol present in the starting material) of a colorless oil: ^1H NMR using Eu(hfc)$_3$ indicated ≥94% ee; $[\alpha]^{20}_D$ −48.7° (*c* 20.6, $CHCl_3$); R_f 0.38 (1:4 (v/v) ethyl acetate in hexane); ^1H NMR δ 5.27, 5.07 (2 × m, 2 × 1H), 3.60 (dd, 1H, J = 9.8, 5.6 Hz), 3.48 (d(br), 1H, J = 9.8 Hz), 2.27–2.06 (m, 4H), 1.89 (m, 2H), 1.73, 1.62 (2 × s(br), 2 × 3H), 1.61 (d, 1H, J = 5.6 Hz), 1.56 (s(br), 3H), 1.10 (t(br), 1H, J = 5.6 Hz), 0.93 (m, 2H), 0.87 (s, 3H); ^{13}C NMR δ 173.5, 135.6, 131.4, 124.7, 123.8, 65.4, 40.1, 39.1, 29.7, 29.0, 27.1, 25.8, 25.0, 17.7, 16.0, 11.5. The (+)-lactone, $[\alpha]^{20}_D$ +48.2° (*c* 0.45, $CHCl_3$), synthesized by treating **5** with Rh_2[5(*S*)-MEPY]$_4$, was used to identify the minor enantiomer.

(−)-(1*R*,2*R*,3*S*)-Aldehyde 7. A solution of 2.00 g (7.63 mmol) of lactone **6** and 610 mg (15.3 mmol) of NaOH in 20 mL of methanol was stirred at rt for 21 h and then concentrated *in vacuo*. The mixture was carefully acidified with saturated aqueous citric acid solution and extracted with ether. The organic phase was washed with water and saturated NaCl solution and then dried. Excess freshly prepared diazomethane in ether[32] was added to the acid in 100 mL of ether.

(24) Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D *J. Org. Chem.* **1986**, *51*, 4768–4779.
(25) Davisson, V. J., Neal, T. R., and Poulter, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 1235–1245.
(26) Cramer, F.; Rittersdorf, W. *Tetrahedron* **1967**, *23*, 3015–3022.
(27) Danilov, L. L.; Druzghinina, T. N.; Kalinchuk, N. A.; Maltsev, S. D.; Shibaev, V. N. *Chem. Phys. Lipids* **1989**, *51*, 191–203.
(28) Zhang, D.; Poulter, C. D. *Anal. Biochem.* **1993**, *213*, 356–361.
(29) All compounds, except **9**, have been synthesized previously as racemates. Previously unreported data for these compounds are now provided.
(30) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(31) Blankley, C. J.; Sauter, F. J.; House, H. O. *Organic Syntheses;* John Wiley: New York, 1973; Collect Vol. V, p 258.
(32) Vogel, A. I. *A Textbook of Practical Organic Chemistry*, 4th ed.; Longman: Birmingham, AL, 1978; p 291.

After 5 min, unreacted diazomethane was removed under a stream of argon, and the ether was removed *in vacuo*.

A mixture of the crude ester, 1.25 g (10.7 mmol, 1.5 equiv) of NMO, and 1 g of freshly activated lightly crushed 4 Å molecular sieves in 15 mL of dry CH_2Cl_2 was stirred at 23 °C for 10 min. TPAP (125 mg, 0.36 mmol, 0.05 equiv) was added carefully (CAUTION! *Exothermic reaction can cause the solvent to boil*), and the resulting black mixture was stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2, filtered through a plug of silica gel, concentrated, and purified by flash chromatography (1:19 (v/v) ethyl acetate in hexane, 150 mL silica gel) to afford 1.75 g (79% overall yield) of a colorless oil: $[\alpha]^{20}_D$ −75.0° (*c* 4.7, $CHCl_3$); R_f 0.38 (1:5.6 (v/v) ethyl acetate in hexane); ^{1}H NMR δ 10.02 (d, 1H, *J* = 6.1 Hz), 5.26, 5.04 (2 × m, 2 × 1H), 3.32 (s, 1H), 2.25−2.04 (m, 4H), 1.93 (m, 2H), 1.79 (d, 1H, *J* = 8.7 Hz), 1.72 (s(br), 3H), 1.69 (dd, 1H, *J* = 8.7, 6.1 Hz), 1.65, 1.63 (2 × s(br), 2 × 3H), 1.33 (s, 3H), 1.05 (m, 2H); ^{13}C NMR δ 198.4, 170.2, 135.8, 131.3, 124.8, 123.4, 51.6, 41.7, 40.4, 40.1, 35.2, 32.8, 27.1, 25.9, 24.8, 17.8, 16.1, 12.1.

(−)-(1*R*,2*R*,3*R*)-Aldehyde 8. A solution of 1.65 g (5.65 mmol) of aldehyde **7** and 65 mL of a 10% aqueous NaOH solution in 80 mL of methanol was stirred at rt for 24 h. Methanol was removed *in vacuo*. The aqueous residue was diluted with ether and carefully acidified with saturated aqueous citric acid solution. The organic phase was washed with saturated NaCl solution and dried. Freshly prepared diazomethane in ether was added. After 5 min, unreacted diazomethane was removed under a stream of argon and the solution concentrated under vacuum. Chromatography of the residue (1:19 (v/v) ethyl acetate in hexane, 200 mL silica gel) gave 1.41 g (85%) of a colorless oil: $[\alpha]^{20}_D$ −12.7° (*c* 13.8, $CHCl_3$); R_f 0.38 (1:5.6 (v/v) ethyl acetate in hexane); ^{1}H NMR δ 9.26 (d, 1H, *J* = 2.0 Hz), 5.19, 5.07 (2 × m, 2 × 1H,), 3.39 (s, 3H), 2.41 (d, 1H, *J* = 5.6 Hz), 2.32 (dd, 1H, *J* = 5.6, 2.0 Hz), 2.18−1.97 (m, 4H), 1.87 (m, 2H), 1.69, 1.58, 1.53 (3 × s(br), 3 × 3H), 1.52 (m, 1H), 1.36 (m, 1H), 1.20 (s, 3H); ^{13}C NMR δ 196.7, 169.9, 135.9, 131.2, 124.8, 123.4, 51.5, 42.1, 40.1, 36.9, 34.0, 33.7, 27.0, 25.9, 25.8, 17.8, 17.5, 16.0.

[(*E*)-5,9-Dimethyl-4,8-decadienyl]triphenylphosphonium Iodide (9). A solution of 4.76 g (16.3 mmol) of 5,9-dimethyl-4,8-(*E*)-decadienyl iodide[19] and 5.60 g (21.2 mmol, 1.3 equiv) of triphenylphosphine in 3 mL of dry benzene was shielded from light and stirred at rt for 4 d. The cloudy suspension was added to 150 mL of ether and the white solid removed by filtration. The solid was washed with ether and dried *in vacuo* to give 3.2 g of a white powder. The filtrate was concentrated and repeatedly resubjected to the same reaction conditions to ultimately provide 7.98 g (88% total yield) of the solid: mp 96−97 °C (lit.[19] mp 97−98 °C); ^{1}H NMR (75% C_6D_6, 25% $CDCl_3$): δ 7.59 (m, 2H), 7.34 (m, 3H), 5.11, 5.05 (2 × m, 2 × 1H), 3.74 (m, 2H), 2.42 (m, 2H), 2.02−1.96 (m, 4H), 1.64, 1.60, 1.54 (3 × m, 3 × 3H), 1.53 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 137.9, 135.0 (d, *J* = 2.8 Hz), 133.1 (d, *J* = 10.0 Hz), 131.2, 130.3 (d, *J* = 12.5 Hz), 123.7 (d, *J* = 2.0 Hz), 121.5, 118.3, 117.2, 39.4, 28.1 (d, *J* = 16.4 Hz), 26.3, 25.4, 22.4 (d, *J* = 4.4 Hz), 21.8, 17.5, 16.2; ^{31}P NMR ($CDCl_3$) δ 22.3 (s(br), 1P).

(+)-(1*R*,2*R*,3*R*)-Methyl Ester 11 and (+)-Methyl Ester 12. Phosphonium salt **9** (1.03 g, 1.85 mmol, 2.0 equiv) was dried by repeated evaporation from dry benzene (2 × 5 mL) and dissolved in 20 mL of dry THF. The colorless solution was cooled to 0 °C, and 1.15 mL (1.85 mmol, 2.0 equiv, 1.6 M in hexanes) of *n*-butyllithium was added *via* syringe. After 15 min, 115 mL (1.85 mmol, 2.0 equiv) of methyl iodide was added to the orange solution, and stirring was continued for 15 min before a second addition of 1.15 mL (1.85 mmol, 2.0 equiv) of *n*-butyllithium. After 15 min, 266 mg (0.911 mmol) of aldehyde **8** in 5 mL of THF was added dropwise to the blood red mixture. Stirring was continued at 0 °C for 2 h and at rt for 12 h. The mixture was quenched with solid NH_4Cl, diluted with 150 mL of ether, filtered through a plug of silica gel, and concentrated. Purification by repeated flash chromatography (step gradient from 1:2.3 to 1:1 (v/v) toluene in hexane, 300 mL silica gel) gave 218 mg (53%) of (*E*)-olefin **11** as a colorless oil: $[\alpha]^{20}_D$ +20.8° (*c* 2.9, $CHCl_3$); R_f 0.40 (1:1 (v/v) toluene in hexane); ^{1}H NMR δ 5.28 (t(br), 4H), 5.05 (d(br), 1H, *J* = 7.8 Hz), 3.46 (s, 3H), 2.41 (dd, 1H, *J* = 7.8, 5.4 Hz), 2.28−2.04 (m, 14H), 1.72 (s(br), 2 × 3H), 1.69 (s(br), 3H), 1.66 (d, 1H, *J* = 5.4 Hz), 1.63 (s(br), 6H), 1.62 (s(br), 6H), 1.46 (s, 3H), 1.44 (m, 2H); ^{13}C NMR δ 172.3, 139.0, 135.1, 131.1, 131.1, 124.9, 124.9, 124.6, 124.5, 121.7, 51.1, 40.2, 39.9, 36.6, 34.9, 33.1, 32.4, 30.2, 27.2, 27.1, 27.0, 25.8, 25.5, 17.7, 17.6, 16.8, 16.1, 16.0.

***(Z)*-Olefin 12** (166 mg) co-eluted with an uncharacterized byproduct (~15%): $[\alpha]^{20}_D$ +20.5° (*c* 1.1, $CHCl_3$); R_f 0.40 (1:1 (v/v) toluene in hexane); FTIR (film) cm^{-1} 2926 (s), 2857 (m), 1730 (s), 1439 (m), 1377 (w), 1196 (w), 1163 (m), 860 (w); ^{1}H NMR δ 5.28 (m, 4H), 5.01 (d(br), 1H, *J* = 7.3 Hz), 3.46 (s, 3H), 2.46 (dd, 1H, *J* = 7.3, 5.4 Hz), 2.28−2.08 (m, 14H), 1.73 (s(br), 6H), 1.66 (d, 1H, *J* = 5.4 Hz), 1.71 (s(br), 3H), 1.68 (s(br), 3H), 1.61 (m, 9H), 1.46 (s, 3H), 1.44 (m, 2H); ^{13}C NMR δ 172.2, 138.9, 135.4, 135.2, 131.2, 131.1, 124.9, 124.9, 124.5, 124.4, 122.5, 51.1, 40.2, 40.2, 36.6, 35.0, 33.0, 32.8, 32.4, 27.2, 27.1, 26.8, 25.8, 25.5, 23.5, 17.7, 17.6, 16.1, 16.0; MS 455 (M^+ + 1, 7), 454 (M^+, 20), 385 (7), 317 (6), 285 (4), 257 (11), 247 (7), 137 (13); HRMS calcd for $C_{31}H_{50}O_2$ 454.3813, found 454.3782.

(+)-(1*R*,2*R*,3*R*)-Presqualene Alcohol (13). To a suspension of 50 mg (1.3 mmol, 3.5 equiv) of $LiAlH_4$ in 5 mL dry ether was added 168 mg (0.370 mmol) of ester **12** in 2 mL of ether, dropwise. The mixture was stirred at rt for 3 h before slow addition of saturated NaCl solution to destroy excess $LiAlH_4$, followed by addition of 5 mL of saturated aqueous citric acid solution. The gray suspension was extracted with ether. The combined organic phases were dried, concentrated, and purified by flash chromatography (1:9 (v/v) ethyl acetate in hexane, 70 mL silica gel) to give 152 mg (96%) of a colorless oil: $[\alpha]^{20}_D$ +49.2° (*c* 4.8, $CHCl_3$); R_f 0.44 (1:5.6 (v/v) ethyl acetate in hexane); ^{13}C NMR δ 137.0, 135.3, 135.1, 131.4, 131.4, 125.6, 125.2, 125.1, 124.6, 63.5, 40.6, 40.5, 37.6, 36.0, 29.6, 27.6, 27.6, 27.5, 26.9, 26.3, 26.1, 19.0, 18.2, 17.2, 16.5, 16.5.

Resolution of Racemic Presqualene Alcohol (13). To a solution of 180 mg (0.433 mmol) of racemic **13** in 4.0 mL of freshly distilled vinyl acetate (*it is important to avoid distilling over any residual traces of acetic acid to prevent acid-catalyzed decomposition of the alcohol*) was added 22 mg (462 units, EC 3.1.1.3) of *Pseudomonas sp.* lipase (PSL). The mixture was stirred vigorously at rt for 4 h, filtered through a plug of Celite, concentrated, and purified by chromatography (1:9 (v/v) ethyl acetate in hexane, 75 mL of silica gel) to give 85 mg (47%) of partially resolved (+)-PSOH. The ee of (+)-alcohol was ≥70% as determined by ^{1}H NMR using Eu(hfc)$_3$.

(−)-Acetate **13**-OAc was collected in a separate fraction: R_f 0.40 (1:1 toluene in hexane); FTIR (film) cm^{-1} 2924 (s), 2855 (m), 1743 (s), 1448 (m), 1376 (m), 1239 (s), 1027 (m); ^{1}H NMR δ 5.31 (m, 4H), 5.06 (d(br), 1H, *J* = 7.6 Hz), 3.46 (s, 3H), 4.38 (dd, 1H, *J* = 11.8, 6.7 Hz), 4.00 (dd, 1H, *J* = 11.8, 8.7 Hz), 2.28−2.08 (m, 14H), 1.78 (s, 3H), 1.72 (m, 12H), 1.67, 1.64, 1.62 (3 × s(br), 9H), 1.43 (m, 2H), 1.23 (dd, 1H, *J* = 7.6, 5.4 Hz), 1.10 (s, 3H), 1.04 (ddd, 1H, *J* = 8.7, 6.7, 5.4 Hz); MS (M^+ + 1, 21), 262 (M^+, 58), 409 (56), 339 (32), 271 (57), 203 (93), 69 (100); HRMS calcd for $C_{32}H_{52}O_2$ 468.3970, found 468.3951.

The partially resolved (+)-alcohol (81 mg, 0.19 mmol) was resubjected to the above reaction conditions [PSL (10 mg, 210 units), vinyl acetate (1 mL)] for 3 h. Similar workup and chromatography (1:9 (v/v) ethyl acetate in hexane, 30 mL silica gel) provided 50 mg (29% overall yield) of (+)-alcohol; ≥98% ee as determined by ^{1}H NMR with the shift reagent, and by optical rotation; $[\alpha]^{20}_D$ +49.8° (*c* 2.3, $CHCl_3$), [lit.[6] $[\alpha]^{20}_D$ +47° (*c* 1.0, $CHCl_3$)]. PSOAc was recovered in a separate fraction.

Resolution of Enriched (+)-Presqualene Alcohol (13). Alcohol **13** (140 mg, 0.329 mmol, ≥94% ee) was treated with lipase as described for the resolution of (±)-**13** [PSL (19 mg, 400 units), vinyl acetate (2 mL), 3 h]. Workup and chromatography (1:9 (v/v) ethyl acetate in hexane, 100 mL silica gel) gave 134 mg (96%) of (+)-**13** ≥98% ee as determined by ^{1}H NMR and optical rotation: $[\alpha]^{20}_D$ +49.8° (*c* 6.2, $CHCl_3$).

(1*R*,2*R*,3*R*)-Presqualene Diphosphate (2). Method One. To a stirred solution of 55 mg (0.129 mmol) of (+)-13 and 0.155 mL (1.55 mmol, 12 equiv) of CCl_3CN in 2 mL of dry CH_3CN at rt was added 53 mg (0.155 mmol, 1.2 equiv) of dry tetra-*n*-butylammonium dihydrogen phosphate in one portion. After 1 h the volatile components were removed *in vacuo* and then

concentrated further from 1 mL of 10% water in acetonitrile. The residue was dissolved in 2 mL of methanol and loaded onto the top of a 1.5 cm × 30 cm column containing LH-20 sephadex beads pre-equilibrated with methanol. Elution of the column with methanol and collecting 3.5 mL fractions using an automated fraction collector provided 50 mg of monophosphate (52%), 14 mg of diphosphate (10%), and 19 mg of recovered starting alcohol (35%).

To a solution of 50 mg of monophosphate (0.067 mmol) and 0.032 mL of dry tributylamine (0.13 mmol, 2 equiv) in 2 mL of dry CH_2Cl_2 at rt was added 0.014 mL of diphenyl chloro phosphate (0.068 mmol, 1.0 equiv). After 4.5 h, the mixture was concentrated *in vacuo* over 30 min. Pyridine (1 mL) was added, followed by 75 mg of tetrabutylammonium dihydrogen phosphate (0.26 mmol, 3.4 equiv), and stirring was continued for 14 h. The mixture was concentrated over 10 h. The residue was dissolved in 2 mL of methanol and was eluted from the LH-20 sephadex column, collecting 3.5 mL fractions, to provide (when combined with material from the previous step) 62 mg of diphosphate **2** (68% based on recovered starting material; ca. 90% pure). A solution of the diphosphate in 2 mL of *1*-propanol/1 M NH_4HCO_3 (1:3.3 (v/v), buffer A) was applied to a 2-cm × 10-cm Dowex ion exchange column (NH_4^+ form) pre-equilibrated with buffer A. Presqualene diphosphate eluted with one column volume of buffer A. ^1H NMR analysis of the concentrated residue indicated that no tetra-*n*-butylammonium counterion remained in the sample. The cloudy mixture was frozen with liquid nitrogen and lyophilized (10 h) to give a white, fluffy solid. A 25 mM solution of NH_4HCO_3 (8 mL) was added, followed by sufficient *1*-propanol to clarify the mixture (ca. 2 mL). HPLC purification utilizing a 2.1-cm × 30-cm preparative C18 reversed phase column followed by lyophilization provided 15 mg of pure **2** (19%, or 29% based on recovered starting material): $[\alpha]^{20}_D$ +32.2° (*c* 1.4, $CHCl_3$); FTIR cm^{-1} 3190 (s, br), 2926 (s), 1448 (s), 1383 (w), 1207 (s), 1101 (s), 926 (m), 849 (w); ^1H NMR (CD_3OD/CD_2Cl_2 (2:1 v/v)) δ 5.18 (m, 4H), 4.90 (d(br), 1H), 4.70 (m, ~9H), 4.08 (m, 1H), 3.83 (m, 1H), 1.59–2.1 (m, 14 H), 1.67 (s, 3H), 1.64 (s, 6H), 1.57 (s, 15H), 1.40 (m, 1H), 0.93 (m, 1H); ^{13}C NMR (CD_3OD/CD_2Cl_2 (2:1 v/v)) δ 137.4, 135.6, 135.3, 131.7, 125.4, 125.0, 124.9, 124.2, 67.3 (m), 40.4, 37.8, 33.4 (m), 29.6, 27.4, 27.4, 25.9, 25.6, 18.7, 17.8, 16.9, 16.2, 16.1; ^{31}P NMR (CD_3OD/CD_2Cl_2 (2:1 v/v)) δ −10.1 (s, 1P), −19.3 (s, 1P); MS ((−)-FAB): 585 ($M^+ - (3 \times NH_4^+) + H^+$, 100), 346 (22), 329 (100), 164 (42), 163 (34); HRMS calcd for $C_{30}H_{51}P_2O_7$ 585.3110, found 585.3091.

Racemic Presqualene Diphosphate (2): Method Two. To a solution of 21.5 mg of racemic alcohol **13** (0.05 mmol) in 290 mg of CCl_3CN (2.02 mmol, 40 equiv) was added dropwise to a solution of 68 mg of tetra-*n*-butylammonium dihydrogen phosphate (0.2 mmol, 4 equiv) in 2 mL of CH_3CN over 30 min, via syringe pump, at rt. The pale yellow mixture was stirred for a further 2 h and then concentrated *in vacuo*. The residue was dissolved in a minimal amount of 1-propanol in 25 mM NH_4HCO_3 solution (3:7 (v/v), buffer A) and applied to a 2-cm × 22-cm Dowex ion exchange column (NH_4^+ form) pre-equilibrated with buffer A. Presqualene diphosphate eluted with 1.5 column volumes of buffer A. ^1H NMR analysis of the concentrated residue indicated that no tetra-*n*-butylammonium counterion remained in the sample. The cloudy mixture was frozen with liquid nitrogen and lyophillized to give a yellow solid. A minimal volume of 25 mM NH_4HCO_3 was added, followed by sufficient 1-propanol to clarify the mixture. HPLC purification utilizing a 1-cm × 25-cm preparative C18 reversed phase column followed by lyophillization provided 5.2 mg of racemic **2** (22%).

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Supplementary Material Available: ^1H NMR spectra of **2**, **5**–**9**, **11**–**13**, and **13**-OAc; ^{13}C NMR spectra of **2**, **5**–**9**, and **11**–**13**; ^{31}P NMR spectra of **2** and **9**; IR spectra of **2**, **12**, and **13**-OAc; MS and HRMS of **2**, **12**, and **13**-OAc (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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