Experimental

General Aspects:

Melting points were determined in open capillary tubes using a Thomas Hoover oil melting point apparatus and are not corrected. Boiling points are uncorrected at the pressure noted as measured by a McLoed gauge.

NMR spectra were acquired at the VOICE NMR Laboratory at the University of Illinois on the Unity 400 or 500 spectrometers. ^{1}H NMR spectra were referenced to 7.24($CDCl_3$), 7.15(C_6D_6), or 4.67(D_2O) ppm. ^{13}C NMR spectra were referenced to the middle line of the 1:1:1 triplets from the solvents at 77.27($CDCl_3$), or 128.0(C_6D_6) ppm. ^{31}P NMR spectra were referenced to 0 ppm (85% H_3PO_4) by external referencing. Samples for NOE analysis were prepared by the freeze-thaw method using at least three cycles. A cycle consisted of sealing the NMR tube with a 5 mm septum and parafilm, freezing the sample with liquid nitrogen and, using a 20 gauge needle inserted through the septum, evacuating and filling the tube with nitrogen. IR spectra were taken as neat liquids or CCl_4 solutions on an IBM FTIR/32 spectrophotometer and absorption positions are presented as wave numbers (cm^{-1}). Optical rotations were taken in the solvent noted on a JASCO Model DIP-360 Digital Polarimeter using the sodium D line (589 nm).

Elemental analyses were performed by the Microanalytical Laboratory at the University of Illinois. Mass spectra were recorded on CH5, 731, or 70-VSE instruments, and isotope ratios were calculated by the Matrix program at the Mass Spectroscopy Laboratory at the University of Illinois. GC-MS were obtained on a 311A system.

GC analyses were carried out on a Shimadzu Model 14A-GC on either Rt_X-5 or Rt_X-200 30 m fused silica capillary columns with a split ratio of 100:1. Temperature program rates for lower ($<C_{11}$) and higher ($>C_{11}$) molecular weight compounds were as follows: 75°C start, hold 1 min, ramp 15°C/min to 275 and 150°C start, hold 1 min, ramp 15°C/min to 275. Injector temperatures

were set at 300°C and detector temperatures were 310°C. Analysis of chromatograms were carried out on a HP-3395 integrator.

Samples for quantitative liquid scintillation counting (LSC) analysis were prepared using Pipetteman repeating samplers set to an appropriate volume in either 7- or 18-mL scintillation vials. Scintrix scintillation fluid (toluene-PPO-POPOP) from J.T. Baker Chemical Co. was used for organic soluble compounds and Bray's scintillation fluid (dioxane based) from J.T. Baker Chemical Co. was used for water soluble compounds. Repeated trials showed the accuracy to be ±3%. Samples for qualitative LSC analysis were prepared by using Drummond "Microcaps" disposable micro-pipettes with vigorous mixing after addition of the scintillation fluid. Radio TLC analyses were conducted on a TM Analytic 6895 Betatrac or by division of the plate into zones and scraping of the plate into scintillation vials followed by mixing with the appropriate scintillation fluid.

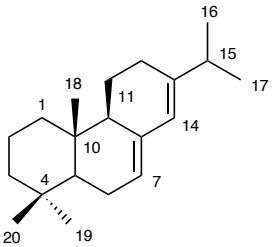
Column chromatography was performed as described by Still et al.[1] using a 50-100 times weight excess of Merck 60 Å, grade 9385, 230-400 mesh silica gel at a pressure of 4-8 psi. Experimental conditions are specified in the following format (grams of silica gel, diameter of column, fraction volume, void volume). HPLC was performed with a Waters Model M-6000A delivery system using a Schoeffel SF770 variable wavelength UV detector at 214 or 254 nm. Preparative scale HPLC was carried out on a Dynamax-60A 2.4x25cm silica column at a flow rate of 18mL/min. Reverse phase HPLC was carried out on a Phenomenex LUNA C8(2) analytical (250x4.6 mm) or preparative (250x21.2 mm) columns at a flow rate of 1 mL/min and 18 mL/min, respectively. Analytical TLC was conducted on Merck glass TLC plates (0.25mm 60 F-254 silica gel) with visualization by UV, I_2, phosphomolybdic acid (PMA) (0.2 M with 2.5% conc. H_2SO_4 (v/v) in EtOH), or anisaldehyde (2.5% (v/v) with 1% HOAc (v/v), 3.4% conc. H_2SO_4 (v/v) in EtOH).

All reactions were carried out under nitrogen unless otherwise noted. Glassware used in moisture sensitive reactions was dried at 120°C for > 24 h and cooled under a stream of nitrogen just prior to use. Technical grade pentane, hexane, and EtOAc were distilled for chromatographic

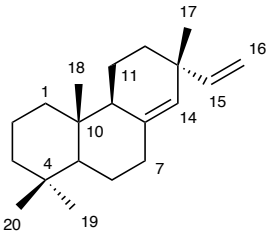
uses. Benzene, ether, THF, and toluene were distilled from sodium/benzophenone just prior to use. Methylene chloride was distilled from CaH_2 just prior to use. Methanesulfonyl chloride and DMF were distilled from P_2O_5 under reduced pressure and stored over 4Å sieves.[2] Triethylamine and s-collidine were distilled from CaH_2 and stored over 4Å sieves.[3] DMSO was distilled at atmospheric presssure through a 10-cm vigreaux column. The first 20% of the distillate was discarded, and the remaining distillate was collected and stored over 4Å sieves.[2] Acetonitrile was distilled from B_2O_5 and stored over 3Å sieves.[4] Molecular sieves were stored at 120°C for at least one week prior to use. *n*-Butyl- and *sec*-butyllithium were purchased from Strem and titrated using diphenylacetic acid prior to use.[5] All other reagents and solvents used were reagent grade unless otherwise noted.

Dowex AG 50W-8X (100-200mesh) cation exchange resin (H^+ form) and Dowex 1X-8 (200-400 mesh) anion exchange resin (Cl^- form) were purchased from Bio-Rad Laboratories. The ammonium form of the cation exchange resin was prepared by washing the resin with three portions of NH_4OH (5-6 times amount of resin (w/w)) and rinsing with deionized water until the eluent was neutral. The formate form of the anion exchange resin was prepared by washing the resin with three portions of 1 M NaOH (5-6 times amount of resin (w/w)), and three portions of 1 M formic acid and then rinsing with deionized water until the eluent was neutral. Whatman CF11 fibrous cellulose powder was purchased from Whatman Inc., and was prepared as noted in the literature.[6]

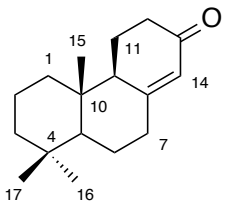
Compounds are named in accordance with IUPAC nomenclature. Number systems for the various ring systems are shown below (Figure **1**). Standard JOC abreviations are used unless otherwise given.[7]



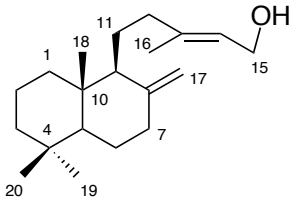
(5*S*,9*S*,10*R*)-Abieta-7,13-diene (**2**)



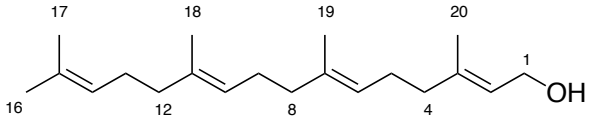
(5*S*,9*S*,10*R*,13*R*)-Sandracopimaradiene (**3b**)



(5*S*,9*R*,10*S*)-Podocarp-8(14)-en-13-one



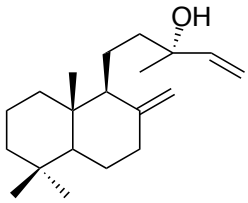
(5*S*,9*R*,10*S*,13*E*)-Labda-8(17),13-dien-15-ol (**12**) or (*E*)-copalol (**12**)



(*E*,*E*,*E*)-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenol or (*E*,*E*,*E*)-geranylgeraniol

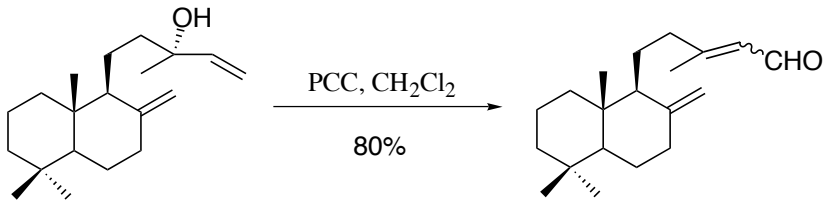
Figure 1. Numbering systems utilized in text.

The purity of all purified products was estimated to be ≥95% by GC analysis and/or examination of the ^{1}H and ^{13}C NMR spectra, unless explicitly stated otherwise.



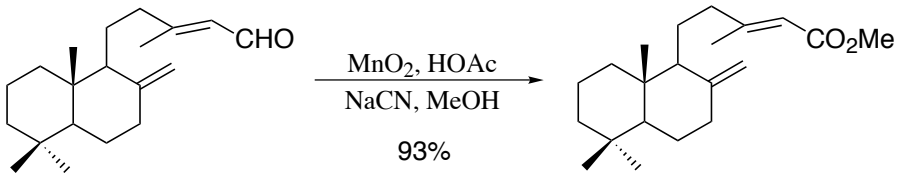
(5*S*,9*R*,10*S*,13*R*)-Labda-8(17),14-dien-13-ol. Manool was isolated from heartwood extract of *Halocarpus biforme* obtained from Westchem Industries Ltd, PO Box 708, Dunedin, NZ with assistance from Prof. Brian Robinson, Chemistry Department, University of Otago. In a

typical isolation, 13 g of resin in 6:1 hexane-EtOAc (50 mL) was loaded onto a silica gel column (500 g, 77 mm diameter) and eluted with 6:1 hexane-EtOAc. After a void fraction (500 mL), fractions (50 mL) were collected and analyzed by TLC (2:1 hexane-EtOAc, R_f = 0.65). Pure fractions (18-33) were combined and concentrated under reduced pressure to give a yellow oil which solidified on standing at -20°C (9g, 70%): mp 49-50°C [lit.[8] mp 53°C]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.64, 0.77, 0.84 (3s, 9H, $3CH_3$), 1.00 (td, J= 13.0, 3.8 Hz, 1H), 1.05 (dd, J=12.6, 2.8 Hz, 1H), 1.14 (td, J=13.4, 4.0 Hz, 1H), 1.67 (dd, J= 5.9, 3.3 Hz, 1H), 1.69 (ddd, J= 12.8, 5.3, 2.6 Hz, 1H), 1.74 (dtd, J= 12.6, 2.4, 1.1 Hz, 1H), 1.93 (td, J= 13.0, 5.1 Hz, 1H), 2.35 (ddd, J= 12.6, 4.2, 2.3 Hz, 1H), 4.45 (d, J=1.2 Hz, 1H, $=CH_2$), 4.77 (d, J=1.2 Hz, 1H, $=CH_2$), 5.03 (dd, J= 10.8, 1.3Hz, 1H, cis CH=CH_2), 5.18 (dd, J= 17.4, 1.3 Hz, 1H, trans CH=CH_2), 5.88 (dd, J= 17.4, 10.8 Hz, 1H, CH=CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.76 (CH_3), 17.87 (CH_2), 19.60 (CH_2), 21.94 (CH_3), 24.67 (CH_2), 28.27 (CH_3), 33.80 (C), 33.86 (CH_3), 38.59 (CH_2), 39.30 (CH_2), 40.08 (C), 41.58 (CH_2), 42.41 (CH_2), 55.79 (CH), 57.44 (CH), 73.91 (C), 106.58 (CH_2), 111.86 (CH_2), 145.39 (CH), 149.01 (C); $[\alpha]_D^{20}$ +31.4° (c 1.72, EtOH) [lit.[8] $[\alpha]_D^{20}$ +30.4°, EtOH]; IR (CCl_4) 3610 (OH). ^{1}H NMR and optical rotation data agreed with literature values.[8]



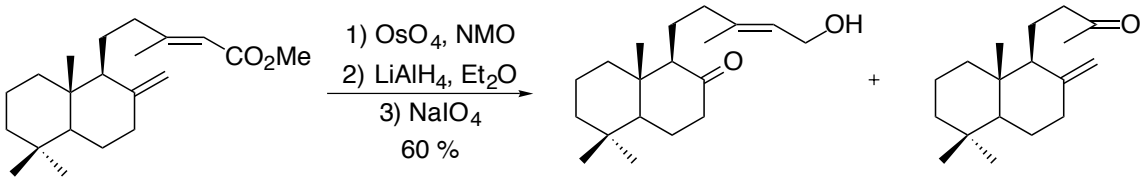
(*E*)- and (*Z*)-(5*S*,9*R*,10*S*)-Labda-8(17),13-dien-15-al . Conditions for the following reaction were based on those described by Herz.[8] A suspension of pyridinium chlorochromate[9] (28.6 g, 132 mmol) in CH_2Cl_2 (475 mL) was stirred as a solution of manool (11.5 g, 40 mmol) in CH_2Cl_2 (25 mL) was added. After 18 h, the brown solution with a layer of black sludge was

diluted with ether (500 mL) giving an orange ppt. The salts were filtered over Celite and washed with ether (500 mL). The filtrate was concentrated under reduced pressure to give an oil containing some solids. ^{1}H NMR analysis indicated a 1:2 ratio of (*Z*)- and (*E*)-copalal. Purification by column chromatography (450, 77, 50, 1000) using 10:1 hexane-ether gave mixed fractions 15-28 (4.69 g) and fractions 29-58 enriched in the E isomer (4.47 g, Z/E 1:10). The combined yield was 9.16 g (80%). Purification of the E rich fractions by another column chromatography (400, 77, 50, 1300) using 10:1 hexane-ether gave mixed fractions and pure (*E*)-copalal in fractions 19-28: yield, 2.22 g, (Z/E 4:96); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65, 0.76, 0.84 (3s, 9H, $3CH_3$), 0.97 (td, J= 12.9, 3.9 Hz, 1H), 1.05 (dd, J=12.6, 2.7 Hz, 1H), 1.14 (td, J=13.4, 4.1 Hz, 1H), 1.28 (qd, J= 12.9, 4.2 Hz, 1H), 1.36 (d sextet, J= 13.2, 1.4 Hz, 1H), 1.92 (td, J= 13.0, 5.1 Hz, 1H), 1.99 (ddd, J= 15.6, 9.1, 7.1 Hz, 1H), 2.12 (d, J=1.1 Hz, 3H, CH_3C=), 2.33 (ddd, J=14.3, 9.9, 3.8 Hz, 1H), 2.35 (ddd, J=12.9, 4.2, 2.5 Hz, 1H), 4.44 (d, J=1.0 Hz, 1H, $=CH_2$), 4.81 (d, J=1.0 Hz, 1H, $=CH_2$), 5.83 (d sextet, J= 8.1, 1.3 Hz, 1H, C=CH), 9.96 (d, J= 8.1 Hz, 1H, CHO); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.76 (CH_3), 17.94 (CH_3), 19.54 (CH_2), 21.87 (CH_2), 22.47 (CH_3), 24.62 (CH_2), 25.05 (C), 31.33 (C), 33.67 (CH_3), 38.35 (CH_2), 39.24 (CH_2), 39.82 (CH_2), 42.24 (CH_2), 55.69 (CH), 55.99 (CH), 106.78 (CH_2), 129.34 (CH), 148.39 (C), 165.04 (C), 191.29 (C); IR (CCl_4) 1680 (C=O). ^{1}H and ^{13}C NMR data agreed with literature values.[10]



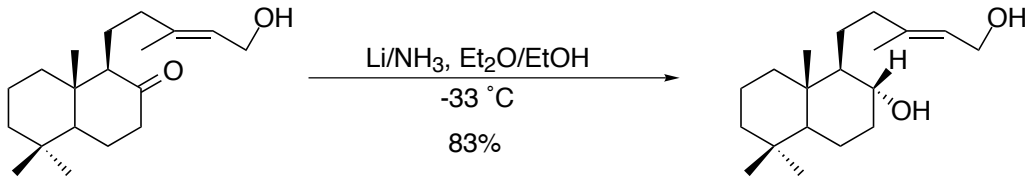
(5*S*,9*R*,10*S*,13*E*)-Methyl Labda-8(17),13-dien-15-oate (10). Conditions for the following reaction were based on those described by Corey.[11] A solution of (*E*)-copalal (2.10 g, 7.28 mmol) in MeOH (50 mL) was stirred as a solution of NaCN (1.79 g, 37 mmol) and glacial acetic acid (657 μL, 657 mg, 10.9 mmol) in MeOH (60 mL) was added followed by activated

MnO_2 (12.5 g, 145 mmol) after 10 min. After 19 h, the solids were filtered over Celite and washed with ether (200 mL). The filtrate was concentrated to dryness under reduced pressure, dissolved in 5% $NaHCO_3$ (150 mL), and extracted with hexane (3x100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography using 13:1 hexane-ether gave ester **10** as an oil: yield, 2.15 g, (93%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65, 0.77, 0.84 (3s, 9H, $3CH_3$), 0.98 (td, J= 12.5, 3.9 Hz, 1H), 1.06 (dd, J=12.5, 2.7 Hz, 1H), 1.15 (td, J=13.4, 4.1 Hz, 1H), 1.29 (qd, J= 13.0, 4.3 Hz, 1H), 1.36 (d sextet, J= 13.1, 1.3 Hz, 1H), 2.13 (d, J=1.1 Hz, 3H, CH_3C=), 2.26 (ddd, J=14.0, 9.9, 4.4 Hz, 1H), 2.36 (ddd, J=12.7, 4.2, 2.5 Hz, 1H), 3.66 (s, 3H, OCH_3), 4.46 (d, J=1.0 Hz, 1H, =CH_2), 4.82 (d, J=1.2 Hz, 1H, =CH_2), 5.63 (sextet, J= 1.1 Hz, 1H, C=CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.69 (CH_3), 19.13 (CH_3), 19.59 (CH_2), 21.73 (CH_2), 21.94 (CH_3), 24.66 (CH_2), 33.82 (C), 33.83 (CH_3), 38.52 (CH_2), 39.27 (CH_2), 39.92 (C), 40.04 (CH_2), 42.34 (CH_2), 51.02 (CH_3), 55.72 (CH), 56.36 (CH), 106.59 (CH_2), 115.11 (CH), 148.59 (C), 161.51 (C), 167.61 (C); IR (CCl_4) 1722 (C=O). ^{1}H and ^{13}C NMR data agreed with literature values.[10]



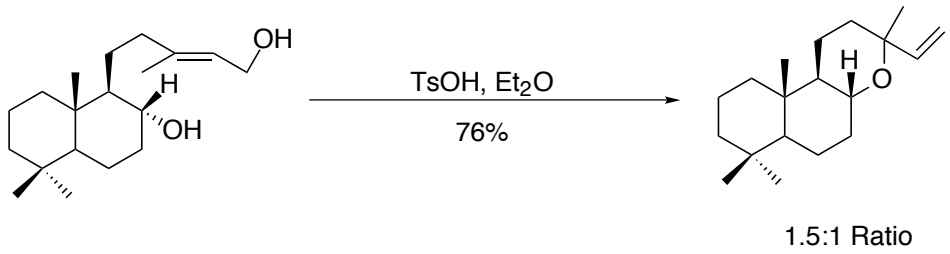
(5*S*,9*R*,10*S*,13*E*)-17-Nor-8-oxo-labda-13-en-15-ol (6a). Conditions for the catalytic osymylation and periodate cleavage were based on those described by Hartley[12] and Semenovskii,[13] respectively. A solution of methyl copalate (**10**) (3.60 g, 11.3 mmol), NMO (3.44 g, 29.4 mmol), and two crystals of OsO_4 (ca 10 mg, ca 0.039 mmol) in acetone (60 mL) was stirred for 3 h at rt. The solvent was removed under reduced pressure and the residue was purified by column chromatography (150,44,25,0) using 1:1 hexane-EtOAc. The yield of diols as a ca 3:1 (based on earlier runs) mixture of regioisomers was 3.10 g (78%). A suspension of $LiAlH_4$ (600 mg, 15.8 mmol) in ether (50 mL) was stirred and cooled at 0°C as a solution of diols

(2.29 g, 6.50 mmol) in ether (50 mL) was slowly added. The solution was allowed to warm to rt and after 10 h, excess reagent was hydrolyzed by addition of water (600 μL), 15% NaOH (600 μL) and water (1.8 mL). The solids were filtered through Celite and washed with ether (5x100 mL). The combined filtrates were concentrated under reduced pressure to give a mixture of triols which was dissolved in acetone (20 mL). The solution was stirred as $NaIO_4$ (4.15 g, 19.4 mmol) and enough water for complete dissolution were added. After 24 h (not followed), the resulting suspension was diluted with water (150 mL) and extracted with ether (3x100 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a thin oil. Purification by column chromatography (80, 34, 25, 0) using 4:1 hexane-EtOAc gave keto alcohol **6a** (1.14 g, 60%). The methyl ketone was identified by TLC comparison with previously prepared sample, but it was not isolated. Data for **6a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.69, 0.82, 0.94 (3s, 9H, $3CH_3$), 1.12 (td, J= 12.6, 4.6 Hz, 1H), 1.21 (qd, J= 13.2, 4.6 Hz, 1H), 1.2-1.4 (m, 2H), 1.42 (dtd, J= 13.0, 3.1, 1.5 Hz, 1H), 1.46 (dd, J= 13.0, 2.6 Hz, 1H), 1.4-1.6 (m, 2H), 1.62 (qd, J= 13.2, 4.9 Hz, 1H), 1.64 (s, 3H, CH_3), 1.7-1.9 (m, 3H), 1.9-2.1 (m, 3H), 2.27 (td, J= 13.2, 7.0 Hz, 1H), 2.38 (ddd, J= 13.2, 4.7, 2.0 Hz, 1H), 4.11 (d, J= 7.0 Hz, 2H, C*H*$_2$OH), 5.34 (t sextet, J= 7.0, 1.3 Hz, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.97 (CH_3), 16.41 (CH_3), 19.26 (CH_2), 19.84 (CH_2), 21.91 (CH_3), 24.35 (CH_2), 33.74 (CH_3), 33.94 (C), 39.13 (CH_2), 39.49 (CH_2), 42.18 (CH_2), 42.91 (CH_2), 42.94 (C), 54.54 (CH), 59.65 (CH_2), 63.64 (CH), 123.78 (CH), 140.32 (C), 212.45 (C); IR (CCl_4) 3622, 3483 (OH), 1710 (C=O) cm^{-1}; $[\alpha]_D^{20}$ -41.1° (c 1.27, EtOH); MS (FI) *m/z* 294 (M^++2, 7.4), 293 (M^++1, 24.0), 292 (M^+, 100), 291 (4.8), 290 (2.6); Anal. Calcd for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.65; H, 10.81.



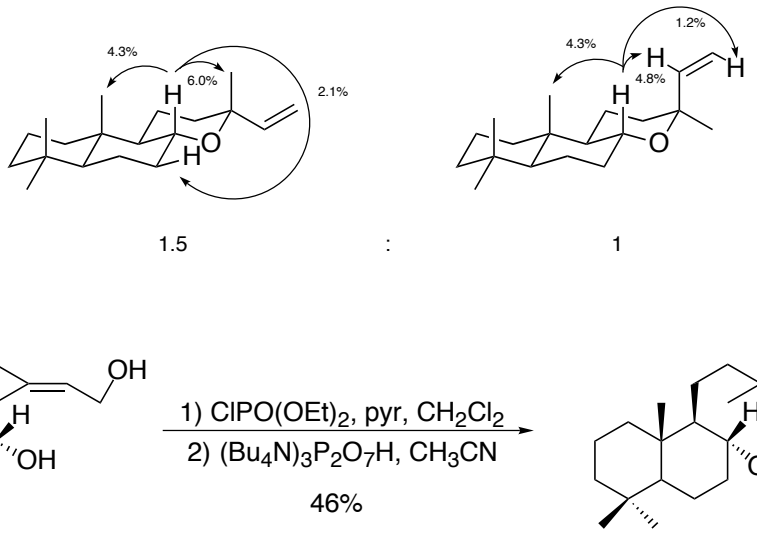
(5*S*,8*R*,9*R*,10*S*,13*E*)-17-Norlabda-13-en-8,15-diol (7a) - Conditions for the following reduction were based on those described by LaPrade.[14] A solution of keto alcohol **6a** (290 mg, 0.99 mmol) in Et_2O (30 mL), EtOH (30 mL), and ammonia (ca 140 mL) was prepared by condensation of ammonia at -78°C followed by warming to reflux (-33°C). This solution 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 50 sec. The ammonia was evaporated by a stream of nitrogen, and the solution was diluted with water (250 mL) and extracted with Et_2O (4x100 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a thick oil. Purification by column chromatography (20, 24, 8, 0) using 1:1 hexane-EtOAc gave diol **7a** as a solid (240 mg, 83%). Recrystallization of the analytical sample from Et_2O/pentane (ca 3-4 mL) gave white needles: mp 85-86°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.76, 0.77, 0.84 (3s, 9H, $3CH_3$), 0.89 (dd, J= 12.1, 2.4 Hz, 1H), 0.90 (td, J= 13.0, 3.5 Hz, 1H), 1.11 (td, J= 13.4, 3.7 Hz, 1H), 1.1-1.3 (m, 1H), 1.25 (qd, J= 11.5, 3.5 Hz, 1H), 1.30 (dd, J= 12.4, 3.5 Hz, 1H), 1.3-1.5 (m, 3H), 1.4-1.6 (m, 4H), 1.6-1.7 (m, 1H), 1.66 (s, 3H, CH_3), 1.71 (dtd, J= 12.8, 3.5, 1.7 Hz, 1H), 1.98 (ddd, J= 14.3, 10.6, 5.3 Hz, 1H), 2.03 (ddt, J= 11.7, 4.9, 2.8 Hz, 1H), 2.17 (ddd, J= 14.3, 10.8, 6.2 Hz, 1H), 3.41 (td, J= 10.6, 4.9 Hz, 1H, C*H*OH), 4.10 and 4.14 (d ABq, J= 7.0 Hz, J_{AB}=12.3, 2H, CH_2OH), 5.40 (t sextet, J= 7.0, 1.3 Hz, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.63 (CH_3), 16.68 (CH_3), 18.65 (CH_2), 21.12 (CH_2), 21.92 (CH_3), 26.20 (CH_2), 33.47 (C), 33.69 (CH_3), 37.33 (CH_2), 38.92 (CH_2), 39.06 (C), 42.26 (CH_2), 42.31 (CH_2), 54.90 (CH), 58.49 (CH), 59.59 (CH_2), 73.43 (CH), 123.30 (CH), 141.17 (C); IR (CCl_4) 3620, 3378 (OH) cm^{-1};

$[\alpha]_D^{20}$ +1.8° (c 0.85, EtOH); Anal. Calcd for $C_{19}H_{34}O_2$: C, 77.50; H, 11.64. Found: C, 77.69; H, 11.63



1.5:1 Ratio

(5*S*,8*R*,9*R*,10*R*,13*R*)- and (5*S*,8*R*,9*R*,10*R*,13*S*)-14-Oxapimara-15-enes. A solution of diol **7a** (16 mg, 0.054 mmol) and p-TsOH·H_2O (200 mg, 1.05 mmol) in ether (4 mL) was stirred at rt for 1.5 h, diluted with 5% $NaHCO_3$ (20 mL), and extracted with hexane (3x5 mL). The combined organic extracts were concentrated under reduced pressure. Purification by column chromatography (8,14,5,0) using 15:1 hexane-ethyl acetate gave a 1.5:1 mixture of 13*R* and 13*S* ethers (12 mg, 76%). The 13R (13β CH_3) and 13S (13α CH_3) stereochemistry was assigned by the results of NOE experiments illistrated below. Data for 13*R* (major isomer): ^{1}H NMR (500 MHz, C_6D_6) δ 0.74 (s, 3H, C20 CH_3), 0.78 and 0.81 (2s, 6H, C18 and C19 CH_3s or reverse), 1.21 (s, 3H, C17 CH_3), 3.52 (td, J= 10.8, 4.9 Hz, 1H, OCH), 5.01 (dd, J= 10.7, 1.7 Hz, 1H, cis CH=C*H*$_2$), 5.37 (dd, J= 17.6, 1.9 Hz, 1H, trans CH=C*H*$_2$), 6.04 (dd, 17.3, 11.0 Hz, 1H, C*H*=CH_2); Data for 13*S* (minor isomer): ^{1}H NMR (500 MHz, C_6D_6) δ 0.74 (s, 3H, C20 CH_3), 0.76 and 0.81 (2s, 6H, C18 and C19 CH_3s or reverse), 1.31 (s, 3H, C17 CH_3), 3.54 (td, J= 11.0, 5.1 Hz, 1H, OCH), 5.11 (dd, 16.8, 1.5 Hz, 1H, trans CH=C*H*$_2$), 5.11 (dd, J= 12.2, 1.5 Hz, 1H, cis CH=C*H*$_2$), 5.79 (dd, J= 18.1, 10.5 Hz, 1H, C*H*=CH_2); HRMS for 1.5:1 mixture $C_{19}H_{32}O$, 276.234316; found, 276.244786.



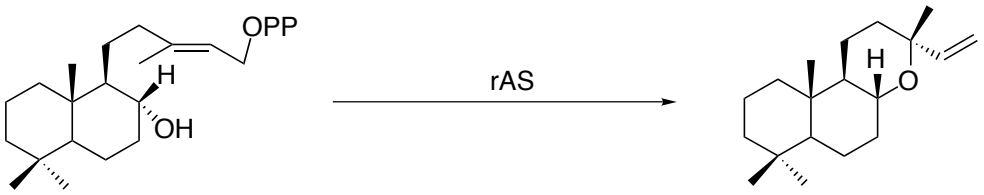
(5*S*,8*R*,9*R*,10*S*,13*E*)-8-Hydroxy-17-norlabda-13-en-15-yl Diphosphate, Ammonium Salt (8a). Conditions for preparation of the diethylphosphate and diphosphate esters were based on those described by Yamamoto[15] and Poulter,[16] respectively. A solution of diol **7a** (185 mg, 0.63 mmol) and pyridine (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. The CH_2Cl_2 solution was loaded directly on a silica gel column (30, 24, 20, 0). Elution with 4:1 EtOAc-hexane gave starting material in fractions 7-8 (59 mg, 32%) and primary phosphate in fractions 10-16 (125 mg, 46% or 68% based on recovered **7a**). A solution of the phosphate (100 mg, 0.23 mmol) in dry CD_3CN (1 mL) was stirred as tris(tetrabutylammonium) diphosphate (885 mg, 0.93 mmol) was added as a solid. After dissolution, predried 3Å powered molecular sieves (ca. 50 mg) were added and the flask was placed in a dissector to prevent absorption of moisture. The reaction progress was monitored by comparison of the intensities of the inorganic diphosphate singlet (δ_P -6.32 ppm) 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, 1.4x30 cm). Elution with 0.1M NH_4HCO_3/2%

2-propanol (silica plates, 6:3:1 2-propanol:NH_4OH:water, R_f = 0.2, 10-mL fractions) followed by cellulose chromatography (2.4x25 cm, 8-mL fractions, 1mL/min) and lyophilization gave a 1:1:0.5 mixture of organic diphosphate **8a**, diethyl phosphate and an unknown impurity (^{31}P NMR δ -9.72 (s): yield, 114 mg. A 30-mg portion was further purified by preparative HPLC[17] (0-100% CH_3CN in 25 mM NH_4HCO_3, 45 min linear ramp, 16 mL/min, 214 nm detection, Luna C8(2) preparative column, 1 mL injection) to gave diphosphate **8a** (20 mg, 68% based on 76 mg): ^{1}H NMR (400 MHz, D_2O) δ 0.64, 0.64, 0.70 (3s, 9H, $3CH_3$), 0.9-1.6 (m, 13H), 1.57 (s, 3H, CH_3), 1.8-2.2 (m, 3H), 3.35 (td, J= 10.7, 4.6 Hz, 1H), 4.32 (t, J= 6.6 Hz, 2H, CH_2OPP), 5.31 (t, J= 6.8 Hz, 1H, =CH); ^{31}P NMR (161 MHz, D_2O) δ -7.02, -9.76 (2d, J= 22.0 Hz).

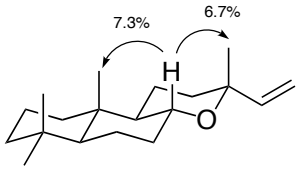
General Aspects for Incubations with Recombinant Abietadiene Synthase:

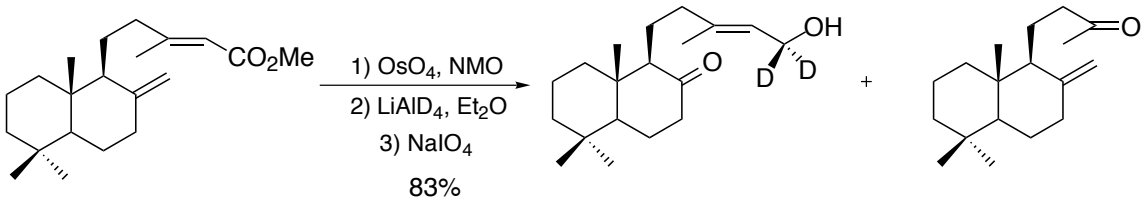
Conditions for the following enzymatic reactions were based on those described by Croteau.[18] All enzymatic reactions were run in teflon-capped glass tubes. Incubation buffer consisted of 30 mM HEPES (pH 7.2), 5.0 mM dithiothreitol, 7.5 mM $MgCl_2$, 20 μM $MnCl_2$, and 5% (v/v) glycerol. Conversions generally ranged from 2-5%, but conversions of 10-20% were occasionally seen. Parallel blank runs lacking enzyme gave negligible conversion (≤ 0.02%) in the extracts.

Enzymatic products were prepared for NMR analysis by dissolving in C_6D_6 (0.5 mL), concentrating to dryness, and dissolving in C_6D_6 (0.2-0.3 mL). Solvent susceptibility plugs (5 mm GFP #99928, obtained from Doty Scientific Inc.) were used to minimize solvent volumes. Acquisition times ranged from 1-5 h depending on concentration.

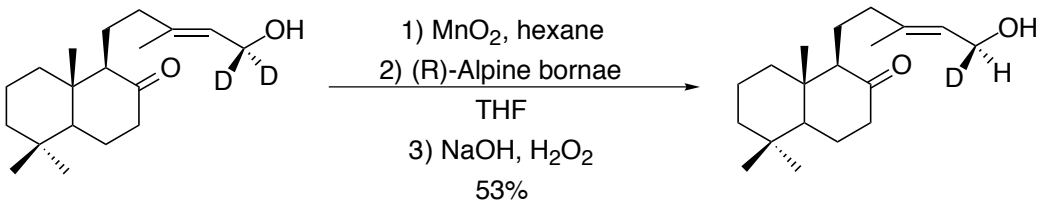


(5*S*,8*R*,9*R*,10*R*,13*S*)-14-Oxa-pimara-15-ene (9a). Three separate tubes containing enzyme (cell homogenate supernatant, 1.0 mL each) in buffer (4.0 mL) were gently mixed as 100 μL of 17-nor-8α-hydroxy-CPP (**8a**) (ca 4 mM in 0.1 M NH_4HCO_3, 0.400 μmol, 80 μM final concentration) was added followed by a hexane overlay (2 mL). The nine tubes were incubated at 31°C for 16 h, vigorously mixed on a vortex genie, and centrifuged to separate the emulsions. For each tube, the hexane layer was removed, and two extractions with hexane (2x1.5 mL) were performed. The hexane solutions were passed through pipettes containing dry silica gel (ca 4 cm) topped with anhydrous $MgSO_4$ (ca 0.5 cm) followed by a hexane wash (2 mL) and an ether wash (6 mL). After concentration under a stream of nitrogen, GC analysis of the hexane eluate showed only background peaks while analysis of the ether fraction showed a single major peak (8.3 min, purity by GC 76%): yield, ca 150 μg (46%) and ≥85% purity by ^{1}H NMR estimates; ^{1}H NMR (500 MHz, C_6D_6) δ 0.74, 0.78, 0.81, 1.21 (4s, 12H, 4xCH_3, C20, C18 or C19, C18 or C19, C17), 3.52 (td, J= 10.8, 4.9 Hz, 1H, OCH), 5.01 (dd, J= 10.7, 1.7 Hz, 1H, cis CH=CH_2), 5.37 (dd, J= 17.6, 1.9 Hz, 1H, trans CH=CH_2), 6.04 (dd, 17.3, 11.0 Hz, 1H, CH=CH_2). The ^{1}H NMR spectrum and data matched those for the major product resulting from acid-catalyzed cyclization of diol **7a**. The stereochemistry was established by NOE data both on the enzymatic product and on the mixture generated from the acid-catalyzed mixture mentioned above.



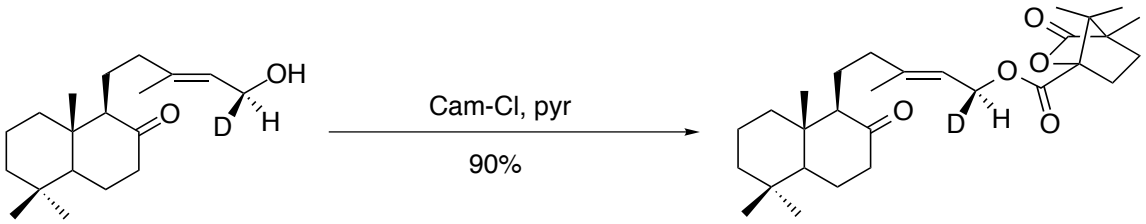


[15,15-$^2H_2$] (5*S*,9*R*,10*S*,13*E*)-17-Nor-8-oxolabda-13-en-15-ol (6b). [15,15-$^2H_2$] **6b** was prepared as described above (**6a**). A solution of methyl copalate (**10**) (3.71 g, 11.6 mmol) and NMO (3.42 g, 29.1 mmol) in acetone (60 mL) with two crystals of OsO_4 (ca 10 mg, ca 0.039) was stirred at rt for 21 h. The reaction still appeared incomplete by TLC. The acetone was removed under reduced pressure, and the residue was purified by column chromatography (140, 44, 25, 50) using 3:2 hexane-EtOAc to give crude starting material (3.15 g) and a 3:1 mixture of 8,17 and 13,14 diols (1.7 g) based on ^1H NMR analysis. The recovered starting material was dissolved in acetone (60 mL), NMO (3.41 g, 29.1 mmol) and OsO_4 (ca 10 mg) were added and the solution was stirred for 18 h. Purification by column chromatography as described above gave combined product (3.76 g). Reduction of the diol ester (3.76 g, 10.6 mmol) with $LiAlD_4$ (1.0 g, 24 mmol) in ether (270 mL) at rt for 1 h followed by workup and filtration gave crude dideutero triol. Oxidative cleavage of triol with $NaIO_4$ (4.22 g, 19 mmol) in acetone (150 mL) and water (ca. 60 mL) for 2.5 h, and purification by column chromatography (160, 44, 20, 100) using 2:1 hexane-EtOAc gave methyl ketone (531 mg, 76%) and [15,15-$^2H_2$] **6b** (1.93 g, 83%). The ^1H NMR spectrum matched unlabeled keto alcohol except for the following: ^1H NMR (500 MHz, $CDCl_3$) δ 4.11 absent (CD_2OH), 5.33 (s, 1H, =CH); MS(FI) MS (FI) *m*/*z* 294 (M^+, 100), 293 (1.0), 292 (0.56), 291 (0.41); 98% d_2, 2% d_1.



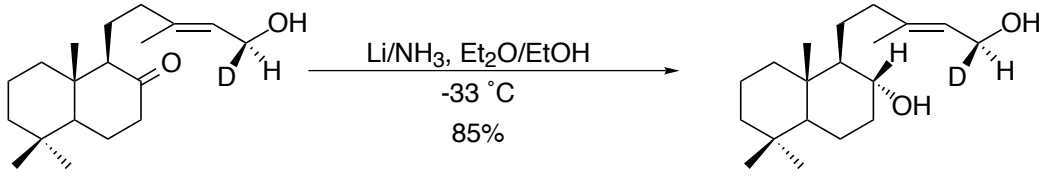
[15-$^2H_1$] (5*S*,9*R*,10*S*,13*E*,15*S*)-17-Nor-8-oxolabda-13-en-15-ol (6c). Con-ditions for the following oxidation and Alpine borane reduction were based on those described by Corey[11] and Midland,[19,20] respectively. A solution of [15,15-$^2H_2$] **6b** (810 mg, 2.75 mmol) in hexane (40 mL) was stirred at rt as activated MnO_2 (14 g, 161 mmol) was added. After 1 h (followed by TLC), the suspension was filtered through Celite and washed with hexane (3x50 mL). The filtrate

was concentrated under reduced pressure to give a thin oil. The oil was stirred and cooled at 0°C as 10.0 mL (5.0 mmol) of (*R*)-Alpine borane (0.5 M in THF, Aldrich) was slowly added, and the solution was allowed to warm to rt. After 1.5 h, THF (10 mL) and 10% NaOH (15 mL) were added and the resulting solution was stirred and cooled at 0°C as 30% H_2O_2 (6 mL) was slowly added maintaining the internal temperature below 25°C. After 30 min, water (100 mL) was added, and the product was extracted with EtOAc (3x100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a thick oil. Purification by column chromatography (80, 44, 25, 0) using 2:1 hexane-EtOAc gave pinol (379 mg) from oxidation of excess reagent and (15*S*)-[15-$^2H_1$] **6c** (429 mg, 53%) as a 13:1 ratio of E:Z isomers which were separated later after reduction to the diol. The ^1H NMR spectrum matched unlabeled keto alcohol except for the following: ^1H NMR (500 MHz, $CDCl_3$) δ 4.09 (d, J= 6.8 Hz, CHDOH), 5.32 (d, J= 6.8 Hz, 1H, =CH); MS (FI) *m/z* 293 (M^+, 100), 292 (1.0), 291 (1.5); 99% d_1, 1% d_0.

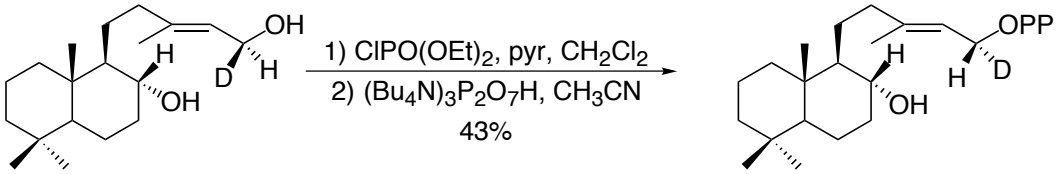


[15-$^2H_1$] (*E*)-(5*S*,9*R*,10*S*,15*S*)-17-Nor-8-oxolabda-13-en-15-yl (*S*)-Camphanate. Conditions for the following reaction were based of those described by Poulter.[12] A solution of (15*S*)-[15-$^2H_1$] **6c** (5 mg, 0.017 mmol) in pyridine (1 mL) was stirred as (*S*)-camphanoyl chloride (55 mg, 0.25 mmol) was added as a solid. After 1 h, the solution was diluted with satd $NaHCO_3$ (20 mL) and extracted with ether (3x10 mL). The combined ethereal extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give a thin oil. Purification by column chromatography (8, 14, 5, 0) using 4:1 hexane-EtOAc gave (15*S*)-[15-$^2H_1$] camphanate (7 mg, 90%). A small amount (ca 1 mg) of a diastereomeric mixture of monodeutero keto camphanates was prepared by stereorandom reduction of the [15-$^2H_1$] aldehyde with $NaBH_4$ and derivatization

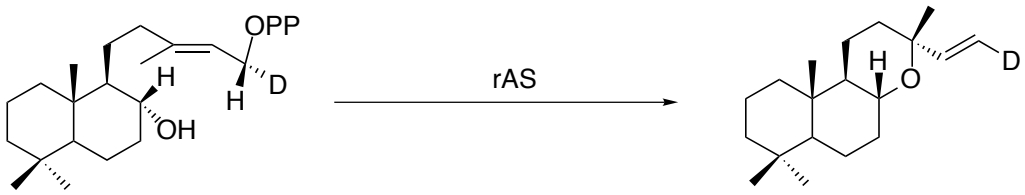
as above. The ^{1}H NMR spectrum (500 MHz, C_6D_6) of the 15R+15S camphanates shows the CHDOR as two overlapping doublets [^{1}H NMR (500 MHz, C_6D_6) δ 4.60 (d, J= 5.4 Hz), 4.62 (d, J= 7.1 Hz)]. Spiking experiments of the 15R/15S mixture and product showed an increase in the upfield doublet. In this way, the diasteromeric purity was estimated to be at least 98% de. Data for (15*S*)-[15-^{2}H$_1$] camphanate: ^{1}H NMR (500 MHz, C_6D_6) δ 0.59, 0.68, 0.73, 0.83, 0.85, 0.87, 1.60 (7s, 21H, 7CH_3), 0.80-0.90 (m, 2H), 1.0-1.5 (m, 8H), 1.48 (dq, J= 13.2, 2.0 Hz, 1H), 1.6-1.8 (m, 3H), 1.85 (dt, J= 13.4, 7.6 Hz, 1H), 1.97 (td, J= 13.2, 8.1 Hz, 1H), 2.01 (ddd, J= 12.9, 9.8, 8.5 Hz, 1H), 2.12 (ddd, J= 13.4, 10.0, 4.6 Hz, 2H), 2.35 (ddd, J= 12.9, 4.7, 2.0 Hz, 1H), 4.62 (d, J= 6.8 Hz, 1H, CDHOR), 5.42 (dd, J= 7.1, 1.0 Hz, 1H, =CH).



[15-^{2}H$_1$] (5*S*,9*R*,10*S*,13*E*,15*S*)-17-Nor-labda-13-en-8,15-diol (7b). (15*S*)-[15-^{2}H$_1$] **7b** was prepared as described above (**7a**). Reduction of (15*S*)-[15-^{2}H$_1$] **6c** (320 mg, 1.09 mmol) with lithium (13 mg, 1.9 mmol) in Et_2O (30 mL), EtOH (30 mL), and NH_3 (180 mL) for 30 min at -33°C and purification by column chromatography (20, 24, 8, 0) gave (15*S*)-[15-^{2}H$_1$] **7b** (285 mg, 85%) as a white solid. Proton NMR and mp matched unlabeled diol except for the following: mp 83-84°C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.12 (d, J= 6.9 Hz, 1H, C*H*DOH), 5.41 (d, J= 6.4, 1H, =CH).

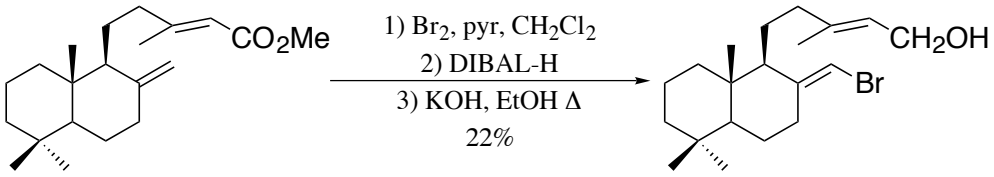


[15-$^2H_1$] (5*S*,9*R*,8*R*,10*S*,13*E*,15*S*)-17-Nor-8-hydroxy-labda-13-en-15-yl Diphosphate, Ammonium Salt (8b). (15*R*)-[15-$^2H_1$] **8b** was prepared as described above (**8a**). Reaction of (15*S*)-[15-$^2H_1$] **7b** (124 mg, 0.42 mmol) with diethyl chlorophosphate (214 μL, 255 mg, 1.48 mmol) and pyridine (137 μL, 134 mg, 1.69 mmol) in CH_2Cl_2 (8 mL) at 0ºC for 2 h was complete according to TLC. The solution was diluted with water (60 mL) and extracted with ether (4x20 mL). The combined ethereal extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography (28, 24, 8, 0) using 4:1 EtOAc-hexane gave diethyl phosphate (128 mg, 0.30 mmol, 71%). Displacement with tris(tetrabutylammonium) diphosphate (1.11 g, 1.16 mmol) in CH_3CN (2 mL) with predried 3Å powered molecular sieves (ca. 150 mg) for 4 d and purification by ion exchange chromatography (NH_4^+ form, 1.8x30 cm, 8-mL fractions) using 0.1M NH_4HCO_3/2% 2-propanol (silica plates, 6:3:1 2-propanol:NH_4OH:water, R_f = 0.2) followed by cellulose chromatography (2.4x20 cm, 8-mL fractions) and lypohilization gave a 1:1 mixture of organic diphosphate and diethyl phosphate (197 mg) as the ammonium salts. Further purification by preparative HPLC[17] in two portions (0-100% CH_3CN in 25 mM NH_4HCO_3, 45 min linear ramp, 16 mL/min, 214 nm detection, Luna C8(2) preparative column, 1 mL injection) gave (15*R*)-[15-$^2H_1$] **8b** (90 mg, 62%): ^1H NMR (400 MHz, D_2O) δ 0.62, 0.63, 0.70 (3s, 9H, $3CH_3$), 0.6-1.6 (m, 13H), 1.55 (s, 3H, CH_3), 1.7-1.9 (m, 2H), 2.0-2.1 (m, 1H), 3.3-3.4 (m, 1H, CHOH), 4.26 (t, J= 6.6 Hz, 2H, CH_2OPP), 5.26 (d, J= 6.8 Hz, 1H, =CH); ^{31}P NMR (161 MHz, D_2O) δ -5.63, -9.40 (2d, J= 22.0 Hz).



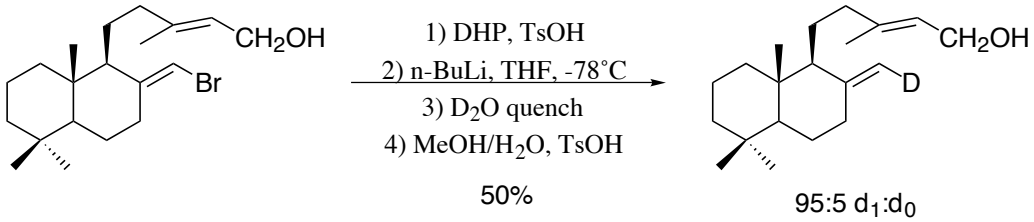
[16-^2H] (5*S*,8*R*,9*R*,10*R*,13*S*,15*E*)-14-Oxapimara-15-ene (9b). Enzyme incubations were run as described above using four tubes containing enzyme (cell homogenate supernatant, 1

mL each) in buffer (4 mL) with 75 μL of [15-$^2H_1$] (15*R*)-17-nor-8α-hydroxy CPP (**8b**) (5 mM, 0.375 μmol, 75 μM final concentration) at 33°C for 18 h. The organic extractable product was isolated and purified as outlined above. Concentration of the ether eluant gave (15*E*)-[16-$^2H_1$] **9b** (ca 70 μg (17%), ≥90% purity by ^{1}H NMR estimates). The ^{1}H NMR spectrum and data matched those of unlabelled **9a** except for the following: ^{1}H NMR (500 MHz, C_6D_6) δ 5.01 absent, 5.37 (d, J= 17.6 Hz, 1H, trans CH=C*H*D), 6.04 (d, 17.3 Hz, 1H, C*H*=CHD).



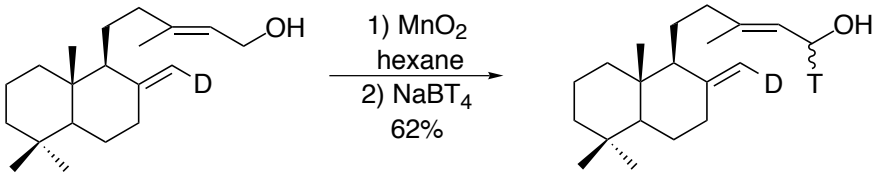
(5*S*,8(17)*E*,9R,10*S*,13*E*)-17-Bromolabda-8(17),13-dien-15-ol (11). Conditions for the following reaction were based on those described by Coates and Cavender for preparation of the enantiomer.[21] A solution of ester **10** (1.06 g, 3.32 mmol) and pyridine (200 μL, 197 mg, 2.49 mmol) in CH_2Cl_2 (5 mL) was stirred and cooled to 0°C as a precooled solution of bromine (188 μL, 582 mg, 3.64 mmol) and pyridine (200 μL, 197 mg, 2.49 mmol) in CH_2Cl_2 (15 mL) at 0°C was added by cannula. After 45 min, the solution was diluted with 5% $NaHCO_3$/5% $Na_2S_2O_3$ (100 mL) and CH_2Cl_2 (40 mL) and extracted with CH_2Cl_2 (2x30 mL). The combined organic extracts were washed with water (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give crude dibromide as a yellow oil. ^{1}H NMR analysis indicated no starting material remained. The solution of the crude dibromo ester in CH_2Cl_2 (30 mL) was stirred and cooled at 0°C as 10 mL (10 mmol) of 1.0 M iBu_2AlH in hexane (Aldrich) was slowly added. After 30 min, excess hydride was hydrolyzed by addition of MeOH (4 mL) and water (20 mL) to give a slurry of aluminum salts. The salts were suspended in CH_2Cl_2 (200 mL) and filtered through Celite. The filtrate was combined with water (30 mL) and extracted with CH_2Cl_2 (1x50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give crude dibromo alcohol as a yellow oil. A solution of crude dibromo alcohol and KOH (1.8 g) in water

(3.5 mL) and EtOH (30 mL) was heated at reflux for 4.5 h, diluted with satd NaCl (100 mL), and extracted with ether (3x75 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give an orange oil. Purification by column chromatography using 4:1 hexane-EtOAc gave bromocopalol **11** as a clear oil (270 mg, 22%). Other minor byroducts were not identified. Data for **11**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.64, 0.77, 0.85 (3s, 9H, $3CH_3$), 0.97 (td, J= 13.0, 4.1 Hz, 1H), 1.08 (dd, J=12.6, 2.8 Hz, 1H), 1.14 (td, J=14.0, 4.1 Hz, 1H), 1.28 (qd, J= 12.9, 4.2 Hz, 1H), 1.47 (tt, J= 13.9, 5.3 Hz, 1H), 1.54 (dt, J= 13.6, 3.4 Hz, 1H), 1.63 (s, 3H, CH_3C=), 2.09 (ddd, J=14.3, 10.3, 4.2 Hz, 1H), 3.05 (ddd, J=12.9, 3.9, 2.4 Hz, 1H), 4.12 (d, J=6.3 Hz, 2H, CH_2OH), 5.34 (t sextet, J= 6.3, 1.2 Hz, 1H, C=CH), 5.68 (s, 1H, =CHBr); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.78 (CH_3), 16.61 (CH_3), 19.53 (CH_2), 21.37 (CH_2), 21.86 (CH_3), 23.37 (CH_2), 33.78 (CH_3), 33.80 (C), 33.97 (CH_2), 38.13 (CH_2), 38.95 (CH_2), 40.57 (C), 42.24 (CH_2), 55.79 (C), 57.97 (CH), 59.56 (CH_2), 99.18 (CH), 123.63 (CH), 140.03 (C), 144.02 (C); IR (neat) 3316 (OH). ^{1}H and ^{13}C NMR data agreed with literature values for *ent*-**11**.[21,22]



[17-^{2}H$_1$] (5*S*,8(17)*E*,9*R*,10*S*,13*E*)-Labda-8(17),13-dien-15-ol (12). Conditions for the following reaction were based on those described by Coates and Cavender for the enantiomer.[21] A solution of bromo copalol **11** (400 mg, 1.1 mmol), dihydropyran (273 mg, 3.3 mmol), and a catalytic amount (ca 10 mg) of p-TsOH·H_2O in ether (4 mL) was stirred for 3 h after which the reaction was judged complete by TLC. Dilution with hexane (4 mL) and purification by column chromatography (50, 24, 8, 0) using 10:1 hexane-EtOAc provided the THP ether as a clear oil (372 mg, 76%). A solution of the ether (320 mg, 0.71 mmol) in THF (22 mL) was stirred and

cooled at -78°C as *sec*-BuLi in pentane (8.1 mL, 1.3M) was added slowly over 10 min. After 45 min at -78°C, over which time a dark orange color developed, 4 mL of 1:1 (v/v) D_2O (98% *d*) and THF was added in small portions. The suspension was allowed to warm slowly to rt, diluted with 5% $NaHCO_3$ (150 mL), and extracted with hexane (3x75 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography (20, 24, 8, 0) using 10:1 hexane-EtOAc as eluent provided the deuterated THP ether (202 mg, 76%) as a light yellow oil. A solution of the THP ether (200 mg, 0.53 mmol) and a catalytic amount (ca 20 mg) of p-TsOH·H_2O in MeOH (20 mL) was stirred for 1 h at rt, diluted with 5% $NaHCO_3$ (100 mL), and extracted with ether (3x80 mL). The combined ethereal extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography (20, 24, 8, 0) using 5:1 hexane-EtOAc provided [17-$^2H_1$] **12** (134 mg, 86%). The deuterium content was estimated to be 95% d_1 and 5% d_0 from the ^{1}H NMR spectrum. The physical and spectroscopic data matched unlabeled **12** except for the following: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.46 (s, 1H, =CHD), 4.80 (s, 0.05H, =CHD); ^{13}C NMR (126 MHz, $CDCl_3$) δ 106.21 (1:1:1 t, J=23.02 Hz, =CHD); IR (CCl_4) 3620 (OH), d_1:d_0 (Based on ^{1}H NMR analysis): 95:5. Data for unlabelled **12**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.65, 0.78, 0.84 (3s, 9H, $3CH_3$), 0.98 (td, J= 13.4, 3.8 Hz, 1H), 1.06 (dd, J=12.7, 2.8 Hz, 1H), 1.15 (td, J=13.4, 4.1 Hz, 1H), 1.29 (qd, J= 12.9, 4.2 Hz, 1H), 1.36 (d sextet, J= 13.3, 1.3 Hz, 1H), 1.65 (s, 3H, CH_3C=), 1.94 (td, J= 12.9, 4.9 Hz, 1H), 2.13 (ddd, J=14.2, 10.5, 4.4 Hz, 1H), 2.36 (ddd, J=12.7, 4.2, 2.4 Hz, 1H), 4.13 (d, J=6.8 Hz, 2H, CH_2OH), 4.49 (d, J= 1.1 Hz, 1H, =CH_2), 4.80 (d, J= 1.1 Hz, 1H, =CH_2), 5.36 (t sextet, J= 6.8, 1.2 Hz, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.75 (CH_3), 16.61 (CH_3), 19.64 (CH_2), 21.98 (CH_3), 22.02 (CH_2), 24.69 (CH_2), 33.83 (C), 33.87 (CH_3), 38.60 (C), 38.67 (CH_2), 39.33 (CH_2), 39.90 (C), 42.41 (CH_2), 55.76 (CH), 56.55 (CH), 59.69 (CH_2), 106.50 (CH_2), 123.20 (CH), 140.92 (C), 148.88 (C); IR (neat) 3329 (OH).



[15-^{3}H$_1$,17-^{2}H$_1$] (5*S*,9*R*,10*S*,13*E*,(8)17*E*)-Labda-8(17),13-dien-15-ol (12). Oxidation of [17-^{2}H$_1$] copalol (**12**) (16 mg, 0.055 mmol) with activated MnO_2 (250 mg, 2.88 mmol) in hexane (6 mL) for 45 min followed by reduction with $NaBT_4$ (ca 2 mg, 0.05 mmol, 5 mCi, 100 mCi/mmol, American Radiolabeled Chemicals) in EtOH (4 mL) for 1.5 h and purification by column chromatography (8, 14, 5, 0) using 5:1 hexane-EtOAc gave [15-^{3}H$_1$,17-^{2}H$_1$] copalol (**12**) in fractions 7-10 (10 mg, 62%, 0.75 mCi, 15 % radiochemical yield, 21.8 mCi/mmol).

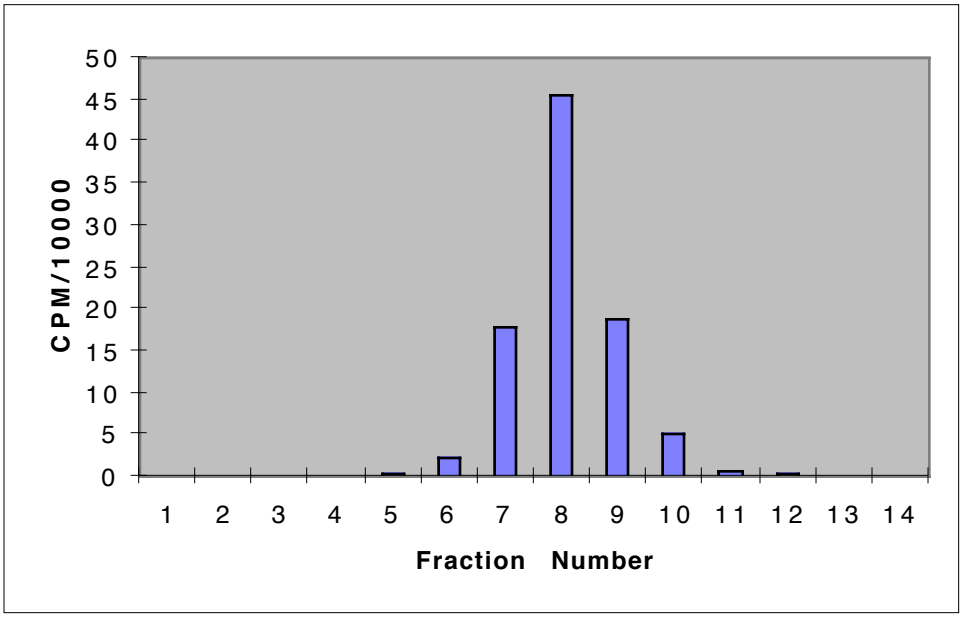
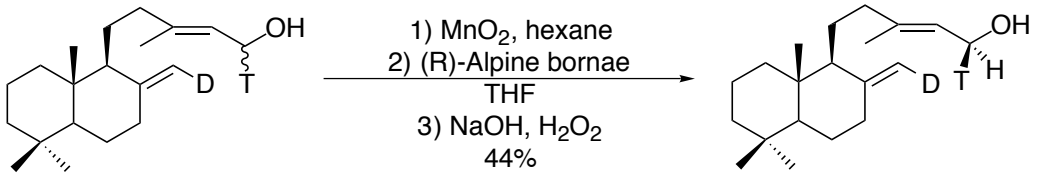


Figure 2. Radioactivity profile from chromatographic purification of [15-^{3}H$_1$,17-^{2}H$_1$]-((8)17*E*)-copalol (**12**) on silica gel.



[15-$^3H_1$,17-$^2H_1$] (5*S*,9*R*,10*S*,13*E*,15*S*,(8)17*E*)-Labda-8(17),13-dien-15-ol (12). (15*S*)-[15-$^3H_1$,17-$^2H_1$] **12** was prepared as described above for **6c**. A solution of alcohol (10 mg, 0.034 mmol, 0.75 mCi, 21.8 mCi/mmol) in hexane (6 mL) was stirred as activated MnO_2 (300 mg, 3.45 mmol) was added. After 1.5 h (followed by TLC), the suspension was filtered through Celite, the solids were washed with hexane (5 mL), and the filtrate was concentrated under a stream of N_2. A solution of the crude copalal in THF (2 mL) was stirred as 1.00 mL (0.50 mmol) of (*R*)-Alpine borane (0.5 M in THF, Aldrich) was slowly added. After 1.7 h, excess Alpine borane was consumed by the addition of acetaldehyde (1.58 g, 2 mL, 35.8 mmol). After 2.5 h, the reaction mixture was concentrated under a stream of N_2. A solution of the oil in THF (8 mL) and 10% NaOH (2 mL) was stirred and cooled at 0°C as 30% H_2O_2 (3 mL) was slowly added. After 1.5 h, water (20 mL) was added, and the product was extracted with ether (3x10 mL). The combined ethereal extracts were dried ($MgSO_4$) and concentrated under a stream of N_2 to give a yellow oil. Purification by column chromatography (8, 14, 5, 0) using 5:1 hexane-EtOAc gave (15*S*)-[15-$^3H_1$,17-$^2H_1$] **12** in fractions 6-10 (5 mg, 50%, 0.33 mCi, 44% radiochemical yield, 21.8 mCi/mmol). RadioTLC indicated a single peak (≥ 90% radiochemical purity, R_f = 0.6, 2:1 hexane-EtOAc).

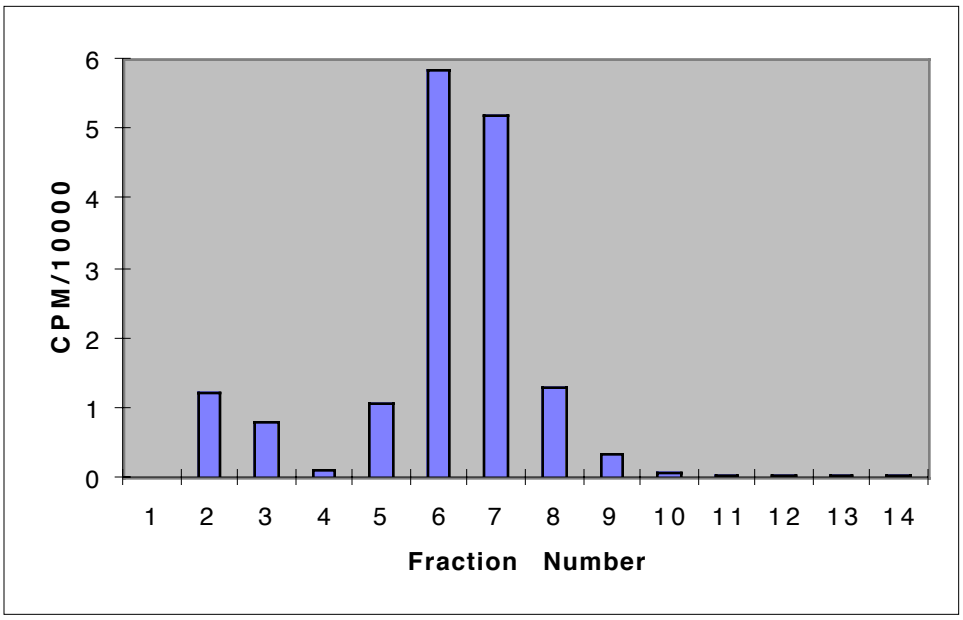
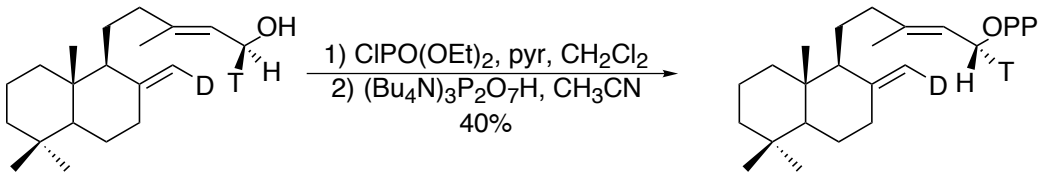


Figure 3. Radioactivity profile from chromatographic purification of (15*S*,(8)17*E*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] (15*S*,(8)17*E*) copalol (**12**) on silica gel.



[15-$^{3}H_{1}$,17-$^{2}H_{1}$] (5*S*,9*R*,10*S*,13*E*,15*R*,8(17)*E*)-Labda-8(17),13-dien-15-yl Diphosphate, Ammonium Salt (2). (15*R*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] **2** was prepared as described above for **8a**. Reaction of (15*S*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] copalol (**12**) (5 mg, 0.017 mmol, 0.33 mCi, 21.8 mCi/mmol) with diethyl chlorophosphate (103 μL, 123 mg, 0.72 mmol) in pyridine (87 μL, 85 mg, 1.07 mmol) and CH_2Cl_2 (5 mL) at 0°C for 1.5 h followed by column chromatography (6, 14, 5, 0) using 2:1 hexane-EtOAc gave diethyl phosphate in fractions 6-9.

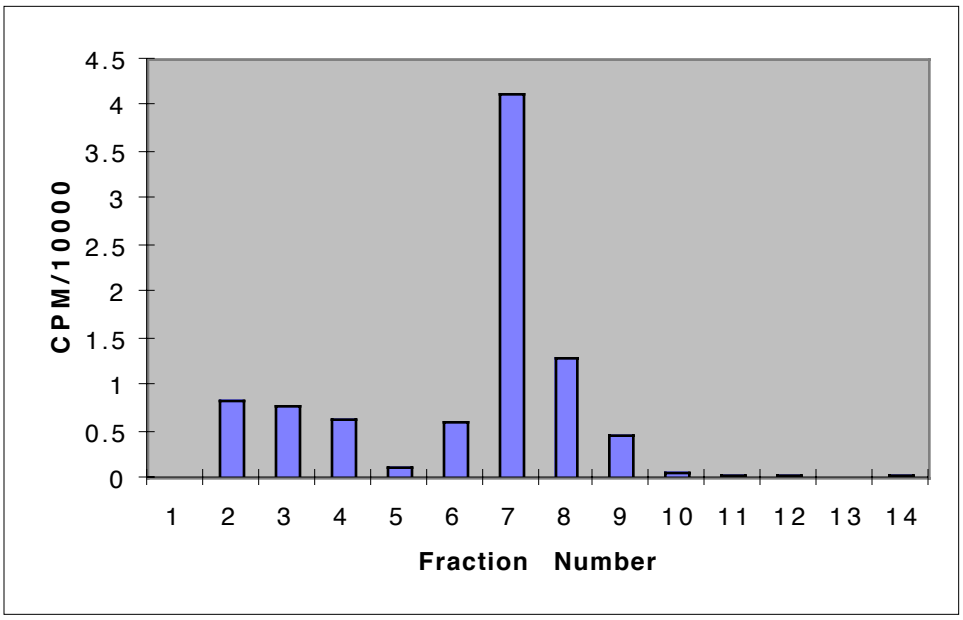


Figure 4. Radioactivity profile from chromatographic purification of (15*R*,(8)17*E*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] (15*R*,(8)17*E*) copalyl diethylphosphate (**2**) on silica gel.

Displacement of diethyl phosphate with tris(tetrabutylammonium) diphosphate (1.10 g, 1.15 mmol) in CH_3CN (1 mL) with predried 3Å powered molecular sieves (ca. 50 mg) for 4 d at rt gave crude diphosphate. The suspension was concentrated under a stream of N_2 and resuspended in water (6 mL), and the aqueous mixture was extracted with hexane (4 mL) to remove remaining diethyl phosphate. Purification of the diphosphate by ion exchange chromatography (NH_4^+ form, 2.4x20 cm, 5-mL fractions) using 0.1M NH_4HCO_3/2% 2-propanol followed by cellulose chromatography (2.4x15 cm, 8-mL fractions) and lyophilization gave (15*R*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] **2** in fractions 4-6 (4 mg, 47%, 0.13 mCi, 40% RCY). Radiochemical purity was determined by loading unlabelled CPP (**2**) followed by labelled CPP (**2**) (760000 dpm) onto a silica plate (2x5.5 cm) 1 cm from the base and develoment with 6:3:1 2-propanol-concd NH_4OH-water. After development, the plate was divided into three zones (0-2 cm, 2-3 cm, and 3-5.5 cm), scraped and analyzed by LSC (Bray's solution). The three zones gave 6100, 420091 and 62517 dpm, respectively (radiochemical purity 84%).

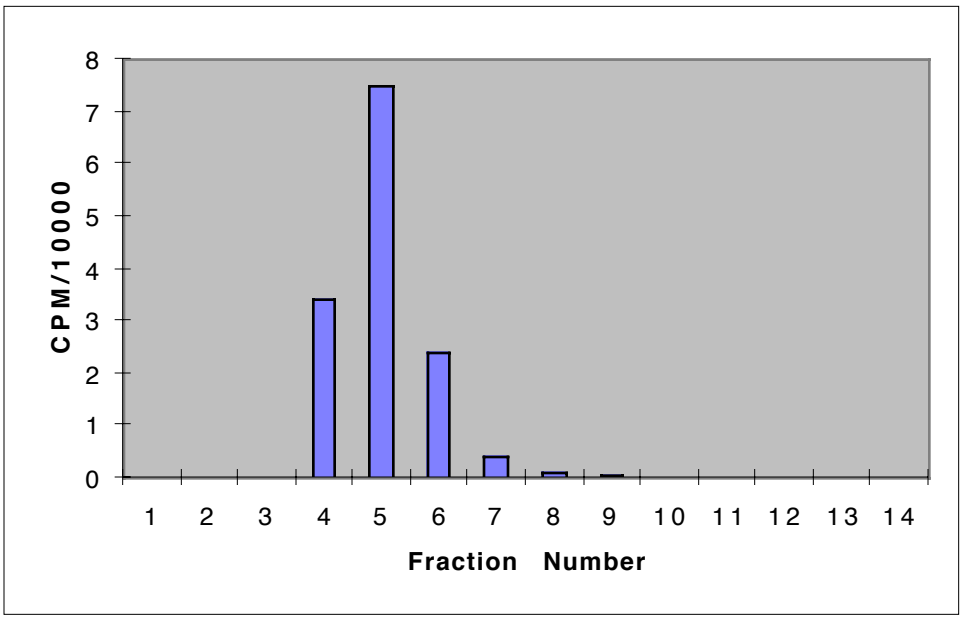
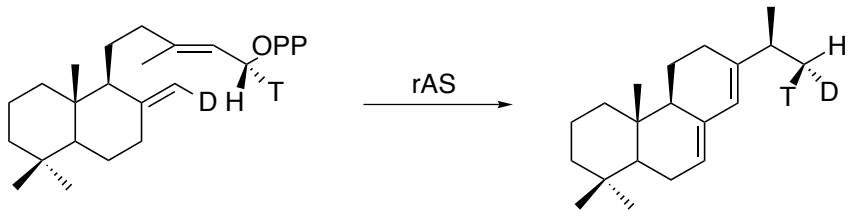
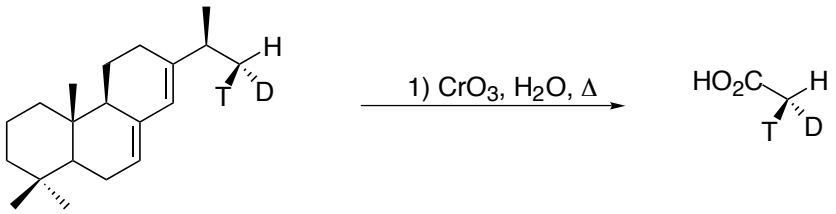


Figure 5. Radioactivity profile from chromatographic purification of (15*R*,(8)17*E*)-[15-$^{3}H_{1}$,17-$^{2}H_{1}$] copalyl diphosphate (**2**) on cellulose.



[16-^{2}H,16-^{3}H] (5*S*,9*R*,10*S*,15*R*,16*R*)-Abieta-7,13-diene (4). Six separate tubes containing enzyme (cell homogenate supernatant, 0.6 mL each) in buffer (2.4 mL) were gently mixed as 60 μL of [15-$^{3}H_{1}$,17-$^{2}H_{1}$] (15*R*,17*E*) CPP (**2**) (ca 2 mM in 0.1 M NH_4HCO_3, 0.120 μmol, 40 μM final concentration) was added followed by a hexane overlay (2 mL). The tubes were incubated at 31°C for 1.5 h, vigorously mixed on a vortex genie, and centrifuged to separate the emulsions. For each tube, the hexane layer was removed, and one extraction with hexane (2 mL) containing carrier abietadiene (**4**, 19 μg/mL) was performed. The hexane extracts were passed through pipettes containing dry silica gel (ca 4 cm) topped with anhydrous $MgSO_4$ (ca 0.5 cm) and rinsed with hexane (2 mL). LSC analysis of an aliquot from the combined eluents (39 mL) indicated 7.13 x 10^6 dpm (3.36 μCi, 21.4% radiochemical yield).



Kuhn-Roth Degradation of [16-^{2}H,16-^{3}H] (5*S*,9*R*,10*S*,15*R*,16*R*)-Abieta-7,13-diene (2). Conditions for the following degradation were based on those described by Clifford for the Kuhn-Roth oxidation of estrone.[23] A solution of [16-^{2}H,16-^{3}H] abietadiene **2** (1.8 mg, 0.0066 mmol, 1.12 mCi/mmol, 1.57x10^8 dpm) and carrier abietadiene **2** (4 mg, 0.015 mmol) in benzene (ca 20 mL) was concentrated under a stream of N_2 onto the bottom half of a 100-mL, round-bottomed flask to produce a thin layer. A solution of CrO_3 (5.5 g, 55 mmol) in water (13 mL) was added, and the mixture was heated to reflux. After 16 h, 85% H_3PO_4 (2 mL) and water (10 mL) were added, and volatile degradation products were isolated by steam distillation (100 mL total collected, water added to pot in 25-mL portions). The distillate was treated with indicator (phenol red, 1mg/mL, 2 drops) and titrated with 0.0509 M NaOH (1.25 mL, 0.0636 mmol). An additional aliquot of base (2 mL) was added and the water was removed under reduced pressure. The residue was dissolved in water (10 mL) and kept at -20°C until needed (3.1x10^7 dpm, 20% radiochemical yield). GC analysis of an aliquot was accomplished by concentration of the basic sample (0.2 mL) under a stream of N_2 followed by addition of DMSO (50 μL) and concd HCl (1-2 μL). Isothermal (35°C) splitless GC analysis of an aliquot (5 μL) showed several peaks (2.73 min, 29.6%; 3.14 min, 58.4%, acetic acid; 3.80 min, 1.8 %; 6.82 min, 7.2%, isobutyric acid; 7.92 min, 2.7%; 8 min, DMSO). The impure chiral methyl acetic acid was used directly for analysis.

Radiochemical purity of the acetate was determined by derivatization as the *p*-phenyl-phenacyl ester.[24] A solution of cold sodium acetate (272 mg, 3.31 mmol), labelled sodium acetate (150000 dpm) and p-phenyl-phenacyl bromide (Aldrich, 902 mg, 3.65 mmol) in dry DMF (6 mL) was stirred at rt for 14 h. The reaction mixture was diluted with water (100 mL), and the product was extracted with CH_2Cl_2 (3x50 mL). The combined organic extracts were dried ($MgSO_4$) and

concentrated under reduced pressure to give a white solid. Purification by column chromatography (38, 24, 8, 0) using 3:1 hexane-EtOAc followed by recrystallization from EtOH (15 mL) gave *p*-phenyl-phenacyl acetate (326 mg, 38.9%). The entire sample was analyzed by LSC (toluene/PPO/POPOP, 52,800 dpm, 90% radiochemical purity).

Enzymatic Chirality Analysis. The following procedures were modeled after published procedures and unpublished protocols developed by Floss and coworkers.[25] Malate synthase, acetate kinase, phosphotransacetylase, sodium glyoxylate, coenzyme A, EDTA, and fumarase were all purchased from Sigma and used within six months. Enzymes purchased as lyophilized powders (acetate kinase, phosphotransacetylase) were dissolved in water, and the solutions were stored at 4°C. Enzymes purchased as suspensions (malate synthase and fumarase) were stored as indicated on the vial. Standard samples of (*R*)- and (*S*)-chiral methyl acetic acid with respective F-values of 76 and 23 were provided to us by Dr. Heinz Floss at the University of Washington. [1-^{14}C]-Acetic acid was purchased from American Radiolabeled Chemicals. Verbal and procedural advice was obtained from Dr. Sungook Lee in Dr. Floss' labs. All solutions were measured using Pipetteman repeating samplers.

A sample of chiral acetate (3 mL, ca 1.0×10^7 dpm, ca 6-8 μmol) was mixed with ATP disodium salt (50 mg, 83 μmol), 0.2 M Na_2CO_3 buffer (pH 9.5, 5 mL), 0.2 M sodium glyoxylate (0.1 mL, 20 μmol), 0.2 M EDTA (pH 7.0, 0.05 mL, 10 μmol), 0.1 M $MgCl_2$ (0.5 mL, 50 μmol), 1 M sodium acetate carrier (2 μL, 2 μmol), [1-^{14}C] sodium acetate (54.3 mCi/mmol, 1.24×10^5 dpm, 0.001 μmol, ^{3}H/^{14}C ratio 8:1), phosphotransacetylase (100 IU), acetate kinase (25 IU), malate synthase (25 IU) and coenzyme A (8 mg, 10 μmol) and diluted with water (1 mL) to a total volume of 9 mL. The solution was incubated at 32°C for 24 h, acidified with 10% HCl (2 mL), mixed gently with a pipette, and loaded onto an ion exchange column (Dowex 50X-8, H^+ form, 2.4x25 cm, 100-200 mesh). The column was eluted with water (175 mL), 10 mM 2,4-dinitrophenylhydrazine in 10% HCl (2 mL, 20 μmol) was added to the eluent and after 30 min at rt the entire solution was loaded onto an anion exchange chromatography column (Dowex 1X-8,

formate form, 1.4x20 cm). The column was washed with water (175 mL) and the products were eluted overnight with a linear gradient of 1 M to 3 M formic acid (300 mL each, gradient mixing chambers, 4-5 min/fraction) while 33 8-mL fractions were collected. Every third fraction was analyzed by LSC of 0.5 mL aliquots and fractions 8-15 were pooled (radiochemical yields of ^{3}H and ^{14}C calculated below were 65% and 87%, respectively).

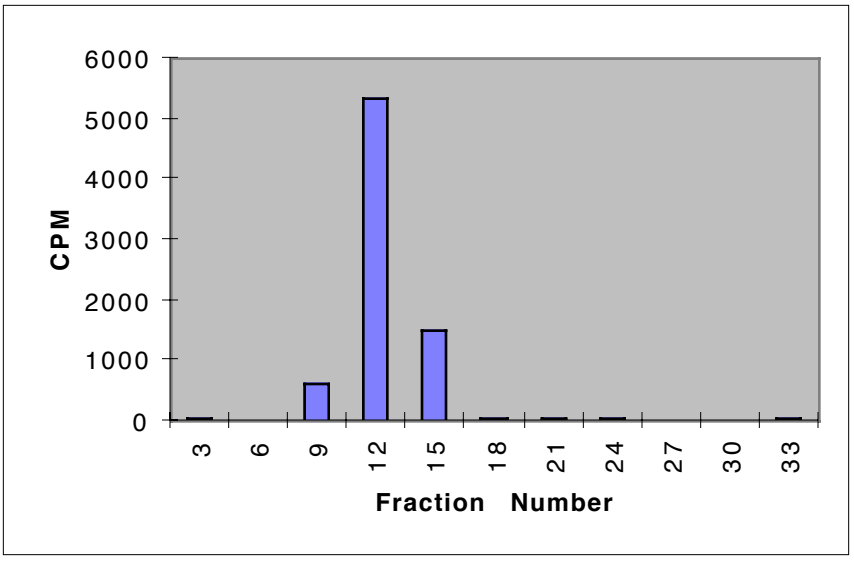


Figure 6. Chromatographic profile of anion exchange chromatography.

The combined fractions were concentrated under reduced pressure and left under reduced pressure for 5-6 h to remove traces of formic acid. The residue was dissolved in 0.2 M K_2HPO_4 buffer (4 mL, pH 7.6) and since the pH of the buffer was often affected by remaining traces of formic acid, the pH was readjusted to pH 7.6 by addition of 1 M KOH (ca 5-6 μL).

An aliquot of this solution (1.5 mL) was placed in a scintillation vial for later analysis (malate vial). A second aliquot of this solution (1.5 mL) was treated with fumarase (25 IU) for 3 h at rt, frozen onto the walls of a 200-mL, round-bottomed flask and lyophilized overnight. The residue was dissolved in 0.2 M K_2PO_4 buffer (8 mL, pH 7.6) and split into two scintillation vials for analysis.

Analysis of the samples was carried out using an open channel setting with the channel A counting window set to 800 to 1000 to act as a ^{14}C only channel and the channel B counting

window set to 0 to 700 to act as a mixed channel. Counting windows were determined empirically by using pure samples of labeled sodium acetate. Samples were repeat counted for 20 min (3-6 repeats) to establish error limits. Samples were then spiked with [1-^{14}C] sodium acetate (62010 ± 102 dpm) and recounted as above followed by [1-^{3}H] sodium acetate (51683 ± 151 dpm) and recounting as above. Spiking values were used to establish relative efficiencies of the channels.

Efficiencies for both channels were calculated by subtraction of the spiked cpm value from the previous value and dividing by the dpm value for the spike. The total ^{14}C dpm was calculated by dividing the initial channel A cpm (^{14}C only channel) by the ^{14}C efficiency for that channel. The total ^{3}H dpm was calculated by subtraction of the contribution of ^{14}C to channel B (total ^{14}C dpm x efficiency ^{14}C for channel B) followed by dividing with the ^{3}H for that channel. The percent ^{3}H lost was calculated by normalizing the ^{14}C values and calculation of the percent ^{3}H lost. From the calculated values, radiochemical yields of ^{3}H and ^{14}C were 65% and 87%, respectively.

Table 1. Liquid scintillation analysis of ^{14}C and ^{3}H content of [$^{3}H_1$,$^{14}C_1$] malate from unknown [2-$^{3}H_1$] acetate before and after fumarase exchange.

	Total cpm		Counting Efficiencies	
	Channel A (cpm)	Channel B (cpm)	Efficiency Channel A	Efficiency Channel B
Malate	10991 ± 42.3	68369 ± 72.6		
Fumarase	4261 ± 54.6	25178 ± 73.4		
Fumarase	4113 ± 34.7	27826 ± 25.7		
^{14}C Spike				
Malate	27710 ± 48.2	141042 ± 132	0.2696 ± 0.0007	1.1720 ± 0.0022
Fumarase	20312 ± 25.4	98712 ± 333	0.2588 ± 0.0006	1.1858 ± 0.0033
Fumarase	17062 ± 7.78	98535 ± 119	0.2088 ± 0.0004	1.1403 ± 0.0022
^{3}H Spike				
Malate	28266 ± 103	145383 ± 61.5	0.0108 ± 0.0011	0.0840 ± 0.0014
Fumarase	20281 ± 42.4	102696 ± 58.4	(0.0006) ± 0.0005	0.0771 ± 0.0033
Fumarase	17088 ± 80.7	101669 ± 87.7	0.0009 ± 0.0014	0.0606 ± 0.0014

Table 2. Calculated total radioactivity and F values for unknown [2-$^{3}H_1$] acetate.

	^{3}H (dpm)	^{14}C (dpm)		
Malate	245,189 ± 4437	40765 ± 188		
Fumarase	73,389 ± 3581	16462 ± 216		
Fumarase	88,502 ± 2682	19696 ± 171		
% ^{3}H Lost	25.9% ± 2.2		F Values	74.1 ± 2.1
	25.3% ± 1.7			74.7 ± 1.6

Analysis of the (*R*) [$^{2}H_{1}$,$^{3}H_{1}$] acetate standard was carried out as described above using (*R*) acetate ($3.18x10^{5}$ dpm, ca 2-3 μmol), 1 M sodium acetate carrier (5 μL, 5 μmol), [1-^{14}C] sodium acetate (54.3 mCi/mmol, $1.06x10^{5}$ dpm, 0.001 μmol, $^{3}H/^{14}C$ ratio 3:1) and malate synthase (8.3 IU) diluted with water (2 mL) to a total volume of 10 mL. The malate synthase incubation was conducted for 48 h. The ion exchange chromatography and fumarase exchange (10 IU) were carried out as described above. One half of the sample was used for the fumarase exchange and the other half was used for the malate LSC analysis.

Table 3. Liquid scintillation analysis of ^{14}C and ^{3}H content of [$^{3}H_{1}$,$^{14}C_{1}$] malate from (*R*) [2-$^{3}H_{1}$] acetate before and after fumarase exchange.

	Total cpm		Counting Efficiencies	
	Channel A (cpm)	Channel B (cpm)	Efficiency Channel A	Efficiency Channel B
Malate	7287 ± 80	36743 ± 80		
Fumarase	4953 ± 80	38740 ± 80		
^{3}H Spike				
Malate	6796 ± 80	44977 ± 80	0.0075 ± 0.0008	0.1263 ± 0.0009
Fumarase	4377 ± 80	43934 ± 80	0.0088 ± 0.0009	0.0797 ± 0.0009
^{14}C Spike				
Malate	28567 ± 60	135073 ± 60	0.3511 ± 0.0014	1.4529 ± 0.0048
Fumarase	16690 ± 60	130558 ± 60	0.1986 ± 0.0010	1.3969 ± 0.0046

Table 4. Calculated total radioactivity and F values for (*R*) [2-$^{3}H_{1}$] acetate.

	^{3}H (dpm)	^{14}C (dpm)		
Malate	52165 ± 1534	20755 ± 243		
Fumarase	48899 ± 3853	24944 ± 424		
% ^{3}H Lost	22.0% ± 3.6		F Value	78.0 ± 3.6

Analysis of the (*S*) [$^{2}H_{1}$, $^{3}H_{1}$] acetate standard was carried out as described above using (*S*) ($2.97x10^{5}$ dpm, ca 3-4 μmol), 1 M sodium acetate carrier (5 μL, 5 μmol), [1-^{14}C] sodium acetate (54.3 mCi/mmol, $1.21x10^{5}$ dpm, 0.001 μmol, $^{3}H/^{14}C$ ratio 2.45) and malate syntase (11 IU) diluted with water (2 mL) to a total volume of 10 mL. The malate synthase incubation was conducted for 48 h. The ion exchange chromatography and fumarase exchange (10 IU) were

carried out as described above. One quarter of the sample was used for the fumarase exchange and another quarter was used for the malate LSC analysis.

Table 5. Liquid scintillation analysis of ^{14}C and ^{3}H content of [$^{3}H_1$,$^{14}C_1$] malate from (*S*) [2-$^{3}H_1$] acetate before and after fumarase exchange.

	Total cpm		Counting Efficiencies	
	Channel A (cpm)	Channel B (cpm)	Efficiency Channel A	Efficiency Channel B
Malate	7250 ± 80	47620 ± 80		
Fumarase	6718 ± 80	42422 ± 80		
^{14}C Spike				
Malate	22970 ± 80	135393 ± 80	0.2535 ± 0.0012	1.4155 ± 0.0048
Fumarase	21669 ± 80	130641 ± 80	0.2411 ± 0.0012	1.4227 ± 0.0047
^{3}H Spike				
Malate	21895 ± 80	140202 ± 80	0.0165 ± 0.0009	0.0737 ± 0.0009
Fumarase	21810 ± 80	137492 ± 80	0.0022 ± 0.0009	0.1051 ± 0.0009

Table 6. Calculated total radioactivity and F values for (*S*) [2-$^{3}H_1$] acetate.

	^{3}H (dpm)	^{14}C (dpm)		
Malate	96811 ± 3659	28599 ± 343		
Fumarase	26481 ± 2552	27863 ± 359		
% ^{3}H Lost	71.9% ± 3.6		F Value	28.1 ± 3.6

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