

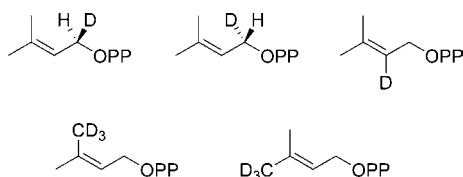
Synthesis of Deuterium-Labeled Derivatives of Dimethylallyl Diphosphate

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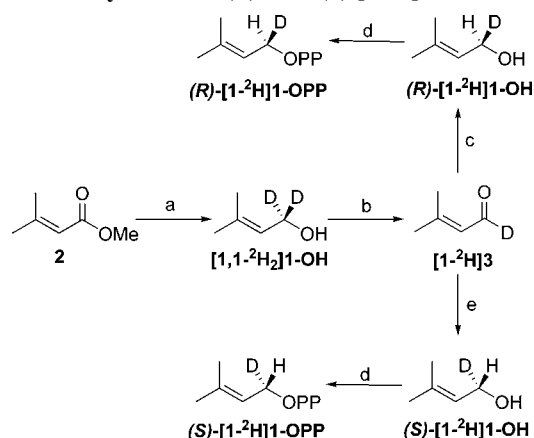


Short practical syntheses for five deuterium-labeled derivatives of dimethylallyl diphosphate (DMAPP) useful for enzymological studies are reported. These include the preparation of the C1-labeled derivatives (*R*)-[1-²H]3-methylbut-2-enyl diphosphate ((*R*)-[1-²H]1-OPP) and (*S*)-[1-²H]3-methylbut-2-enyl diphosphate ((*S*)-[1-²H]1-OPP), the C2-labeled derivative [2-²H]3-methylbut-2-enyl diphosphate ([2-²H]1-OPP), and the methyl-labeled derivatives (*E*)-[4,4,4-²H₃]3-methylbut-2-enyl diphosphate ((*E*)-[4,4,4-²H₃]1-OPP) and (*Z*)-[4,4,4-²H₃]3-methylbut-2-enyl diphosphate ((*Z*)-[4,4,4-²H₃]1-OPP).

Isoprenoid compounds constitute a large diverse class of “small molecule” natural products with over 35 000 individual known metabolites. The vast majority of these compounds are ultimately derived from two fundamental isoprenoid building blocks, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP, **1-OPP**). DMAPP is the electrophilic primer required to initiate the 1'–4 (head-to-tail) chain elongation reactions for the biosynthesis of polyisoprenoid compounds,¹ which are generated by successive alkylations of IPP by a growing allylic diphosphate chain.^{2,3} DMAPP is also the substrate for biosynthesis of monoterpenes with non-head-to-tail skeletons⁴ and a variety of metabolites where the dimethylallyl moiety is attached to nonisoprenoid fragments.¹

The prenyl-transfer enzymes that catalyze electrophilic alkylation reactions with DMAPP are typically highly stereoselective. Because many enzymes do not tolerate substitutions that substantially increase the steric bulk of the substrates, the

SCHEME 1. Synthesis of (*R*)- and (*S*)-[1-²H]1-OPP^a



^a Key: (a) LiAlD₄, rt, 2 h, 86%; (b) PCC, CH₂Cl₂, rt, 3 h, 78%; (c) *S*-BITIP, Bu₃SnH, Et₂O, –20 °C, 24 h, 70%; (d) bis-triethylammonium phosphate (TEAP), CCl₃CN, CH₃CN, rt, 15 min, 30%; (e) *R*-BITIP, Bu₃SnH, Et₂O, –20 °C, 24 h, 69%.

stereochemistries of these reactions are best studied with isotopically labeled derivatives of the normal substrates. We now report short practical routes for the stereoselective synthesis of deuterated derivatives of DMAPP, which collectively place labels at each of the carbons bearing hydrogen atoms.

The syntheses of (*R*)-[1-²H]3-methyl-2-butenyl diphosphate ((*R*)-[1-²H]1-OPP) and (*S*)-[1-²H]3-methyl-2-butenyl diphosphate ((*S*)-[1-²H]1-OPP) are outlined in Scheme 1. [1-²H]3-Methyl-2-butenal ([1-²H]3)⁵ was reduced with BITIP catalysts derived from (*S*)- and (*R*)-BINOL and Bu₃SnH⁶ to give (*R*)-[1-²H]1-OH and (*S*)-[1-²H]1-OPP, respectively. The ¹H NMR spectra of (*R*)- and (*S*)-[1-²H]1-OH were similar to that reported for the *S* enantiomer obtained by a fermenting yeast reduction of [1-²H]3.⁷ A portion of each alcohol was converted to the corresponding Mosher ester.^{8,9} The C1 protons in the ¹H NMR spectra of the diastereomeric esters from (*R*)- and (*S*)-[1-²H]1-OH and (*R*)-(-)-Mosher's chloride were cleanly resolved, giving peaks at 4.77 and 4.82 ppm, respectively. A comparison of peak intensities indicated that the enantiomeric ratios for (*R*)- and (*S*)-[1-²H]1-OH were 96/4 and 94.5/5.5, respectively. Diphosphates (*R*)- and (*S*)-[1-²H]1-OPP were synthesized from corresponding chiral alcohols by treatment with trichloroacetonitrile and inorganic phosphate,¹⁰ a procedure that does not alter the absolute stereochemistry of C1.

[2-²H]3-methyl-2-butenyl diphosphate ([2-²H]1-OPP) was prepared from ethyl acetoacetate (**4**) as shown in Scheme 2. Deuterium was introduced at the C2 position of the β-ketoester by two exchanges with D₂O to give ~96% exchange of the two methylene hydrogens. The labeled keto ester was then

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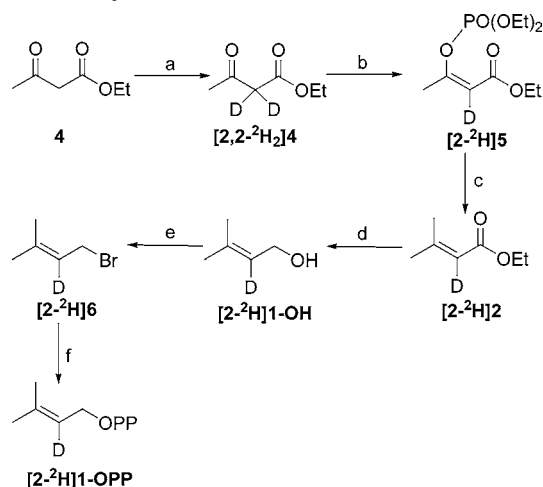
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SCHEME 2. Synthesis of [2-²H]1-OH^a

^a Key: (a) D₂O, rt, 24 h, two times, 92%; (b) NaH, CIPO(OEt)₂, Et₂O, 0 °C, 2 h, 89%; (c) Me₂CuLi, Et₂O, -47 °C, 2 h, 93%; (d) DIBAL, Et₂O, -70 °C, 3 h, 84%; (e) PBr₃, Et₂O, 2 h, 81%; (f) tris(tetra-*n*-butylammonium) hydrogen diphosphate, CH₃CN, 34%.

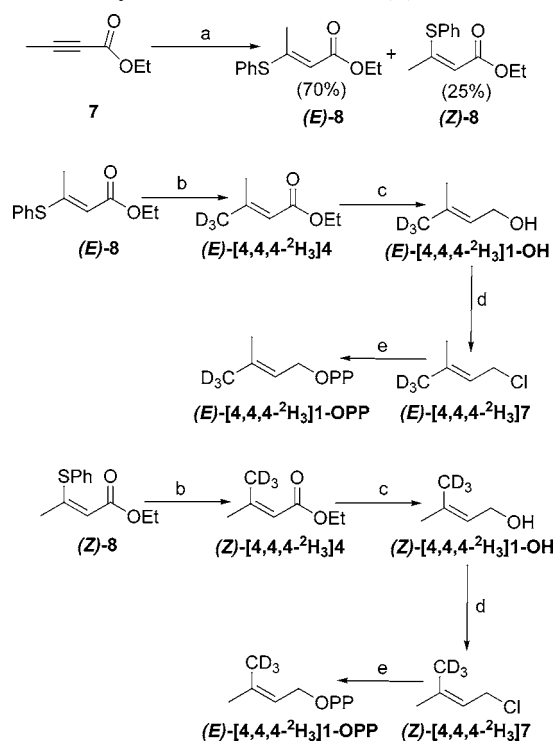
converted to enol phosphate [2-²H]5 followed by treatment with lithium dimethyl cuprate to give [2-²H]2¹¹ with a ²H content of 93%. The allylic ester was treated with DIBAL to give [2-²H]1-OH. The allylic alcohol was converted into the corresponding diphosphate by the well-established two-step halogenation/phosphorylation procedure for synthesis of allylic diphosphates.¹²

Syntheses of (*E*)-[4-²H₃]3-methyl-2-butenyl diphosphate ((*E*)-[4,4,4-²H₃]1-OPP) and (*Z*)-[4-²H₃]3-methyl-2-butenyl diphosphate ((*Z*)-[4,4,4-²H₃]1-OPP) are shown in Scheme 3. Although both compounds have been prepared previously,^{13,14} the route we report is an efficient divergent synthesis that yields both stereoisomers. Benzenethiol was added to acetylenic ester **7** to give a 95% yield of a ~3:1 mixture of (*E*)- and (*Z*)-**8**, which were readily separated by column chromatography. The geometries of (*E*)- and (*Z*)-**8** were assigned from the chemical shift of the methyl¹⁵ and olefinic protons. In the ¹H NMR spectrum of the major isomer (*E*)-**8**, the olefinic proton and methyl protons gave peaks at 5.26 and 2.43 ppm, respectively. For (*Z*)-**8**, the corresponding resonances were observed at 5.85 and 1.81 ppm, respectively. Treatment of the isomeric thioenol ethers with CD₃-MgBr in the presence of CuI gave the corresponding α,β-unsaturated esters. Treatment of (*E*)- and (*Z*)-[4,4,4-²H₃]4 with LAH gave allylic alcohols (*E*)- and (*Z*)-[4,4,4-²H₃]1-OH, respectively. The corresponding diphosphates were prepared by halogenation/phosphorylation.¹²

In summary, we report short practical syntheses for five labeled derivatives of DMAPP where deuterium is stereospecifically incorporated at specific protonated carbons in the molecule.

Experimental Section

(*R*)- and (*S*)-[1-²H]3-Methyl-2-buten-1-ol ((*R*)- and (*S*)-[1-²H]1-OH). A mixture of (*S*)-BINOL (1.14 g, 4.0 mmol), Ti(O-*i*Pr)₄ (1.2 mL, 1.2 mmol), CF₃COOH (1.2 mL of 0.5 M in CH₂Cl₂),

SCHEME 3. Synthesis of (*E*)- and (*Z*)-[4,4,4-²H₃]1-OPP^a

^a Key: (a) PhSH, NaOH, EtOH, rt, 4 h; (b) CD₃MgBr, CuI, THF, -65 °C, 3 h, 81%/87%; (c) LiAlH₄, Et₂O, 0 °C, 2.5 h, 82%/75%; (d) NCS, CH₂Cl₂, DMS, 78%/82%; (e) Tris(tetra-*n*-butylammonium) hydrogen diphosphate, CH₃CN, 60%/63%.

and oven-dried 4 Å molecular sieves (8 g) in ether was heated at reflux for 1 h. The solution was cooled to room temperature, and [1-²H]3 (1.02 g, 12 mmol) was added. The mixture was stirred for 5 min and cooled to -78 °C before Bu₃SnH (4.19 g, 14.4 mmol) was added. The mixture was stirred for 10 min and then placed in a -20 °C freezer for 24 h. The usual workup⁶ and flash chromatography on silica gel with 3% ether in pentane gave (*R*)-[1-²H]1-OH as a colorless oil: 0.73 g (70%; >99% ²H); ¹H NMR (CDCl₃) δ 1.67 (s, 3H) 1.74 (s, 3H), 4.10 (d, *J* = 6.9 Hz, 1H), 5.40 (d, *J* = 7.2 Hz, 1H); ¹³C NMR (CDCl₃) δ 18.0, 25.9, 59.2, 123.7, 136.7; mass spectrum *m/z* (rel intensity) 87 (28), 72 (100), 54 (20), 49 (25), 42 (23); HRMS (EI) calcd for C₅H₉²HO (M⁺) 87.0793, found 87.0796.

Following the same procedure with (*R*)-BINOL gave (*S*)-[1-²H]1-OH⁷ as a colorless oil: 0.72 g (69% yield; >99% ²H); ¹H NMR (CDCl₃) δ 1.67 (s, 3H) 1.73 (s, 3H), 4.10 (d, *J* = 6.9 Hz, 1H), 5.39 (d, *J* = 7.2 Hz, 1H); ¹³C NMR (CDCl₃) δ 18.0, 25.9, 59.1, 123.7, 136.6; mass spectrum *m/z* (rel intensity) 87 (29), 72 (100), 54 (18), 42 (23); HRMS (EI) calcd for C₅H₉²HO (M⁺) 87.0793, found 87.0799.

Ethyl [2-²H]3-Methyl-2-butenate ([2-²H]2). To a solution of lithium dimethylcuprate (40 mmol, 2 equiv) in ether, cooled to -47 °C, was added enol phosphate [2-²H]5 (5.3 g, 20.0 mmol), and the mixture was stirred at -47 °C. After 2 h, the mixture was poured into an ice-cold mixture of 50% aqueous NH₄Cl and concd NH₄-OH (5:1) and the aqueous phase was extracted with ethyl ether. The combined ether extracts were washed with brine, dried over MgSO₄, and concentrated. Flash chromatography on silica gel (CH₂-

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Cl₂) gave 2.13 g (93% yield, 93% ²H) of a colorless oil: ¹H NMR (CDCl₃) δ 1.27 (t, *J* = 7.2 Hz, 3H), 1.88 (s, 3H), 2.16 (s, 3H), 4.13 (2H, q, *J* = 7.2 Hz); ¹³C NMR (CDCl₃) δ 14.5, 20.3, 27.5, 59.6, 116.3, 156.5, 166.9; mass spectrum *m/z* (rel intensity) 129 (4), 72 (18), 57 (16), 43 (100); HRMS (EI) calcd for C₇H₁₁²HO₂ (*M*⁺) 129.0899, found 129.0920.

Ethyl (*E*)- and (*Z*)-[4-²H₃]3-Methyl-2-butenolate ((*E*)- and (*Z*)-[4,4,4-²H₃]4). To a stirred suspension of CuI (5.1 g, 0.03 mol) in dry THF at -63 °C was added CD₃MgI (67 mL of 1 M, 0.07 mol) in ether. After the mixture was stirred for 15 min at -63 °C, a solution of *E*-**8** (3.00 g, 0.014 mol) in dry THF was added, and stirring was continued for 3 h. The mixture was then poured into satd NH₄Cl and stirred for 15 min at rt. The aqueous layer was extracted with ether. The combined organic layers were washed with 5% NaOH, water, and brine, dried over MgSO₄, filtered, and concentrated in vacuo. The residue was chromatographed over silica (9:1 hexane/ether) to give 1.43 g (81% yield, >99% ²H₃) of a colorless oil: ¹H NMR (CDCl₃) δ 1.27 (t, *J* = 7.2 Hz, 3H), 2.17 (d, *J* = 1.2 Hz, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 5.67 (d, *J* = 1.2 Hz, 1H); ¹³C NMR (CDCl₃) δ 14.5, 20.3, 59.6, 116.3, 156.6, 166.9; HRMS (CI) [*M* + 1]⁺ calcd for C₇H₉²H₃O₂ 132.1100, found 132.1108.

Following the same procedure, 3.00 g (0.014 mol) of (*Z*)-**8** gave (*Z*)-[4,4,4-²H₃]4 as a colorless oil: 1.54 g (87% yield, >99% ²H₃); ¹H NMR^{14,15} (CDCl₃) δ 1.28 (t, *J* = 7.2 Hz, 3H), 1.89 (d, *J* = 1.0 Hz, 3H), 4.15 (t, *J* = 7.2 Hz, 2H), 5.68 (d, *J* = 1 Hz, 1H); ¹³C NMR (CDCl₃) δ 14.5, 27.5, 59.6, 116.4, 156.7, 166.9; HRMS (CI) [*M* + 1]⁺ calcd for C₇H₉²H₃O₂ 132.1100, found 132.1107.

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Supporting Information Available: General experimental details, characterization data for all compounds (except (*R*)- and (*S*)-[1-²H]1-OH, [2-²H]2, and (*E*)- and (*Z*)-[4,4,4-²H₃]4) and ¹H and ¹³C or ³¹P spectra for (*R*)- and (*S*)-[1-²H]1-OH, (*R*)- and (*S*)-[1-²H]1-OPP, [2-²H]5, [2-²H]2, [2-²H]1-OH, [2-²H]1-OPP, (*E*)-**8**, (*Z*)-**8**, and (*E*)-[4,4,4-²H₃]4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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