Published in final edited form as:
J Org Chem. 2006 February 17; 71(4): 1739–1741.

Synthesis of Deuterium Labeled Derivatives of Dimethylallyl Diphosphate

Hirekodathakallu V. Thulasiram, **Richard M. Phan**, **Susan B. Rivera**, and **C. Dale Poulter***
315 South 1400 East RM 2020; Department of Chemistry; University of Utah, Salt Lake City, UT, USA 84112

Abstract

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-^{2}H]3-methylbut-2-enyl diphosphate (**(*R*)-[1-^{2}H]1-OPP**) and (*S*)-[1-^{2}H]3-methylbut-2-enyl diphosphate (**(*S*)-[1-^{2}H]1-OPP**), the C2-labeled derivative [2-^{2}H]3-methylbut-2-enyl diphosphate (**[2-^{2}H]1-OPP**), and the methyl-labeled derivatives (*E*)-[4,4,4-$^2H_3$]3-methylbut-2-enyl diphosphate (**(*E*)-[4,4,4-$^2H_3$]1-OPP**) and (*Z*)-[4,4,4-$^2H_3$]3-methyl-but-2-enyl diphosphate (**(*Z*)-[4,4,4-$^2H_3$]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 skeletons[4] and a variety of metabolites where the dimethylallyl moiety is attached to non-isoprenoid fragments.[1]

The prenyltransfer enzymes that catalyze electrophilic alkylation reactions with DMAPP are typically highly stereoselective. Because many enzymes do not tolerate substitutions that substantially increase their steric bulk of the substrates, the 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 label at each of the carbons bearing hydrogen atoms.

The syntheses of (*R*)-[1-^{2}H]3-methyl-2-butenyl diphosphate (**(*R*)-[1-^{2}H]1-OPP**) and (*S*)-[1-^{2}H]3-methyl-2-butenyl diphosphate (**(*S*)-[1-^{2}H]1-OPP**) are outlined in Scheme 1. [1-^{2}H]-3-Methyl-2-butenal (**[1-^{2}H]3**)[5] was reduced with BITIP catalysts derived from (*S*)- and (*R*)-BINOL and Bu_3SnH[6] to give **(*R*)-[1-^{2}H]1-OH** and **(*S*)-[1-^{2}H]1-OPP**, respectively. The ^{1}H NMR spectra of **(*R*)-** and **(*S*)-[1-^{2}H]1-OH** were similar to that reported for the ***S*** enantiomer obtained by a fermenting yeast reduction of **[1-^{2}H]3**.[7] A portion of each alcohol was converted to the corresponding Mosher ester.[8,9] The C1 protons in the ^{1}H NMR spectra of the diastereomeric esters from **(*R*)-** and **(*S*)-[1-^{2}H]1-OH** and (*R*)-(-)-Mosher’s chloride were

*Corresponding author: Phone: 801-581-6685; Fax: 801-581-4391; e-mail: poulter@chemistry.utah.edu.

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-^{2}H]1-OH** were 96/4 and 94.5/5.5, respectively. Diphosphates **(*R*)- and (*S*)-[1-^{2}H]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-^{2}H]3-methyl-2-butenyl diphosphate (**[2-^{2}H]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 D_2O, to give ~96% exchange of the two methylene hydrogens. The labeled keto ester was then converted to enol phosphate **[2-^{2}H]5** followed by treatment with lithium dimethyl cuprate to give **[2-^{2}H]2** [11] with a ^{2}H content of 93%. The allylic ester was treated with DIBAL to give **[2-^{2}H]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-$^2H_3$]3-methyl-2-butenyl diphosphate (**(*E*)-[4,4,4-$^2\mathbf{H_3}$]1-OPP**) and (*Z*)-[4-$^2H_3$]3-methyl-2-butenyl diphosphate **((*Z*)-[4,4,4-$^2\mathbf{H_3}$]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. Benzene thiol 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 methyl [15] and olefinic protons.[16] In the ^{1}H NMR spectrum of the major isomer **(*E*)-8**, the olefinic proton and methyl protons gave peaks at 5.26 ppm and 2.43 ppm, respectively. For **(*Z*)-8**, the corresponding resonances were observed at 5.85 ppm and 1.81 ppm, respectively. Treatment of the isomeric thioenol ethers with CD_3MgBr in presence of CuI gave corresponding α,β-unsaturated esters. Treatment of ***(E)*-** and ***(Z)*-[4,4,4-$^2\mathbf{H_3}$]4** with LAH gave allylic alcohols ***(E)*-** and ***(Z)*-[4,4,4-$^2\mathbf{H_3}$]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-^{2}H]3-Methyl-2-buten-1-ol ((*R*)- and (*S*)-[1-^{2}H]1-OH)

A mixture of (*S*)-BINOL (1.14 g, 4.0 mmol), $Ti(O\text{-}iPr)_4$ (1.2 mL, 1.2 mmol), CF_3COOH (1.2 mL of 0.5 M in CH_2Cl_2) 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-^{2}H]3** (1.02 g, 12 mmol) was added. The mixture was stirred for 5 min and cooled to −78 °C before Bu_3SnH (4.19g, 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 workup [6] and flash chromatography on silica gel with 3% ether in pentane gave **(*R*)-[1-^{2}H]1-OH** as a colorless oil; 0.73 g (70%; >99 % ^{2}H); ^{1}H NMR ($CDCl_3$), 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); ^{13}C NMR ($CDCl_3$) 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 $C_5H_9\,^2HO$ (M^+) 87.0793, found 87.0796.

Following the same procedure with (R)-BINOL gave **(*S*)-[1-^{2}H]1-OH** [7] as a colorless oil; 0.72 g (69 % yield; >99 % ^{2}H); ^{1}H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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 $C_5H_9\,^2HO$ (M^+) 87.0793, found 87.0799.

Ethyl [2-^{2}H]3-methyl-2-butenoate ([2-^{2}H]2)

To a solution of lithium dimethylcuprate (40 mmol, 2 eq) in ether, cooled to −47 °C, was added enol phosphate **[2-^{2}H]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 NH_4Cl and concd NH_4OH (5:1) and the aqueous phase was extracted with ethyl ether. The combined ether extracts were washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography on silica gel (CH_2Cl_2) gave 2.13 g (93% yield, 93% ^{2}H) of a colorless oil; ^{1}H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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 $C_7H_{11}\ ^2HO_2$ (M^+) 129.0899, found 129.0920.

Ethyl (*E*)- and (*Z*)-[4-$^2H_3$]3-methyl-2-butenoate ((*E*)- and (*Z*)-[4,4,4-$^2H_3$]4)

To a stirred suspension of CuI (5.1 g, 0.03 mol) in dry THF at −63 °C was added CD_3MgI (67 mL of 1 M, 0.07 mol) in ether. After stirring 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 sat NH_4Cl and stirred for 15 min at room temp. The aqueous layer was extracted with ether. The combined organic layers were washed with 5% NaOH, water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed over silica (9:1 hexane/ether) to give 1.43 g (81 % yield, >99 % $^2H_3$) of a colorless oil; ^{1}H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) δ 14.5, 20.3, 59.6, 116.3, 156.6, 166.9; HRMS (CI) [M +1]$^+$ calculated for $C_7H_9\ ^2H_3O_2$ 132.1100, found 132.1108.

Following the same procedure, 3.00 g (0.014 mol) of ***(Z)*-8** gave **(*Z*)-[4,4,4-$^2H_3$]4** as a colorless oil; 1.54 g (87% yield, >99 % $^2H_3$); ^{1}H NMR [14,15] ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) δ 14.5, 27.5, 59.6, 116.4, 156.7, 166.9; HRMS (CI) [M+1]$^+$ calculated for $C_7H_9\ ^2H_3O_2$ 132.1100, found 132.1107.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

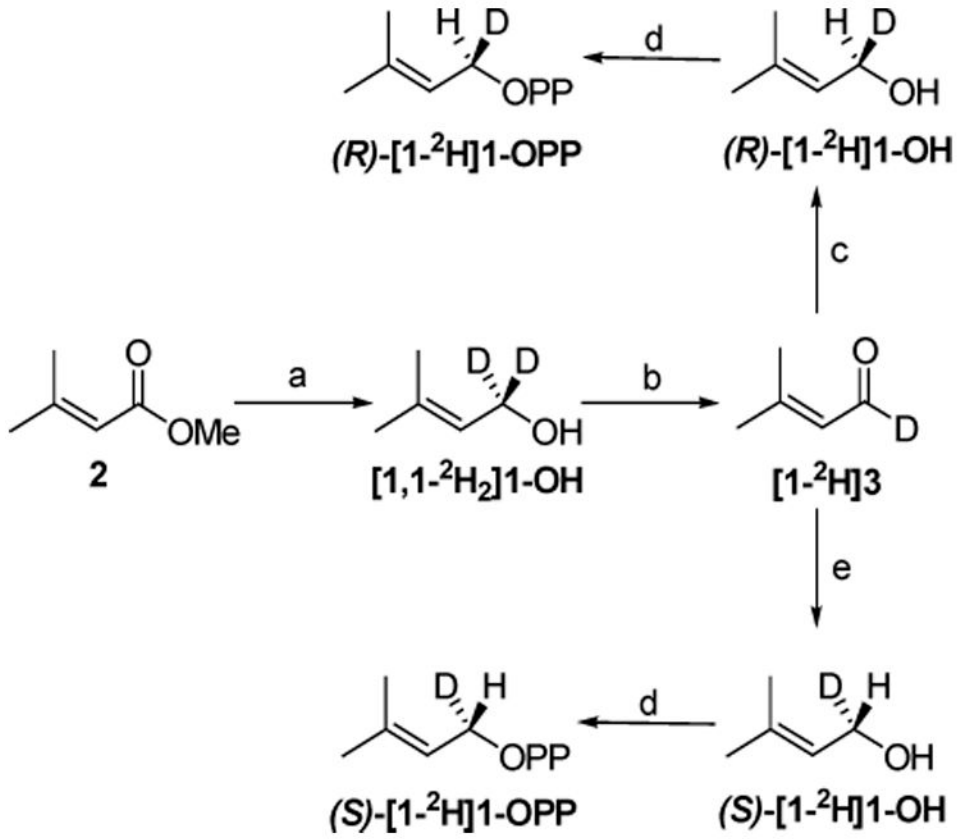
Acknowledgment

This study was supported by NIH Grant GM 21328.

References

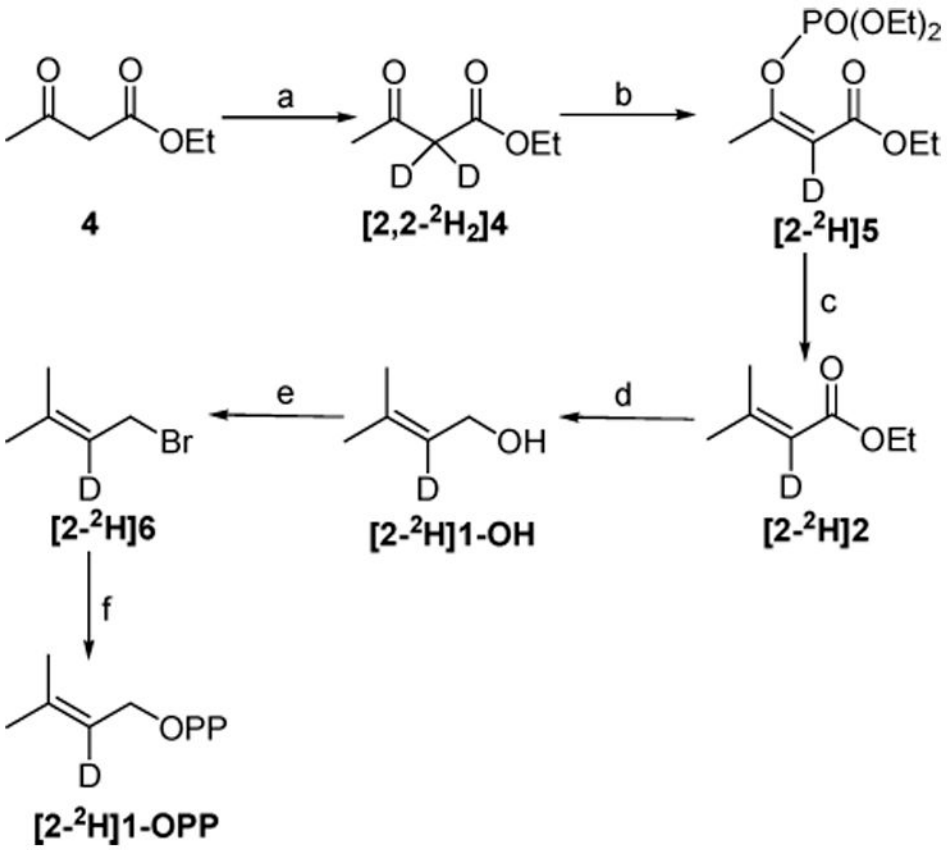
1. Poulter CD. Acc. Chem. Res 1990;23:70.
2. Kellogg BA, Poulter CD. Curr. Opin. Chem. Biol 1997;4:570–578. [PubMed: 9667899]
3. Koyama, T.; Ogura, K. Comprehensive Natural Products Chemistry: Isoprenoids. Cane, DE., editor. Vol. 2. Oxford: Elsevier Science; 1998. p. 73
4. Rivera SB, Swedlund BD, King GJ, Bell RN, Hussey CE, Shattuck-Eidens DM, Wrobel WM, Peiser GD, Poulter CD. Proc. Natl. Acad. Sci. U.S.A 2001;98:4373. [PubMed: 11287653]
5. Vassilikogiannakis G, Chronakis N, Orfanopoulos M. J. Am. Chem. Soc 1998;120:9911.
6. Keck GE, Krishnamurthy D. J. Org. Chem 1996;61:7638. [PubMed: 11667705]
7. King CHR, Poulter CD. J. Am. Chem. SOC 1982;104:1413.
8. Dale JA, Dull DL, Mosher HS. J. Org. Chem 1969;34:2543.
9. Gao Y, Hanson RM, Klunder JM, KO SY, Masamune H, Sharpless KB. J. Am. Chem. Soc 1987;109:5765.
10. Keller RK, Thompson R. J. Chromatogr 1993;645:161.
11. Sum FM, Weiler L. Tetrahedron 1981;37:303.

12. Davisson VJ, Woodside AB, Neal TR, Stremler KE, Muehlbacher M, Poulter CD. J. Org. Chem 1986;51:4768.
13. Mohanty SS, Uebelhart P, Eugster CH. Helv. Chim. Acta 2000;83:2036.
14. Shibuya M, Chou HM, Fountoulakis M, Hassam S, Kim SU, Kobayashi K, Otsuka H, Rogalska E, Cassady JM, Floss HG. J. Am. Chem. Soc 1990;112:297.
15. Hill RK, Yan S, Arfin SM. J. Am. Chem. Soc 1973;95:7857. [PubMed: 4586190]
16. Mori K, Mori H. Tetrahedron 1987;43:4097.



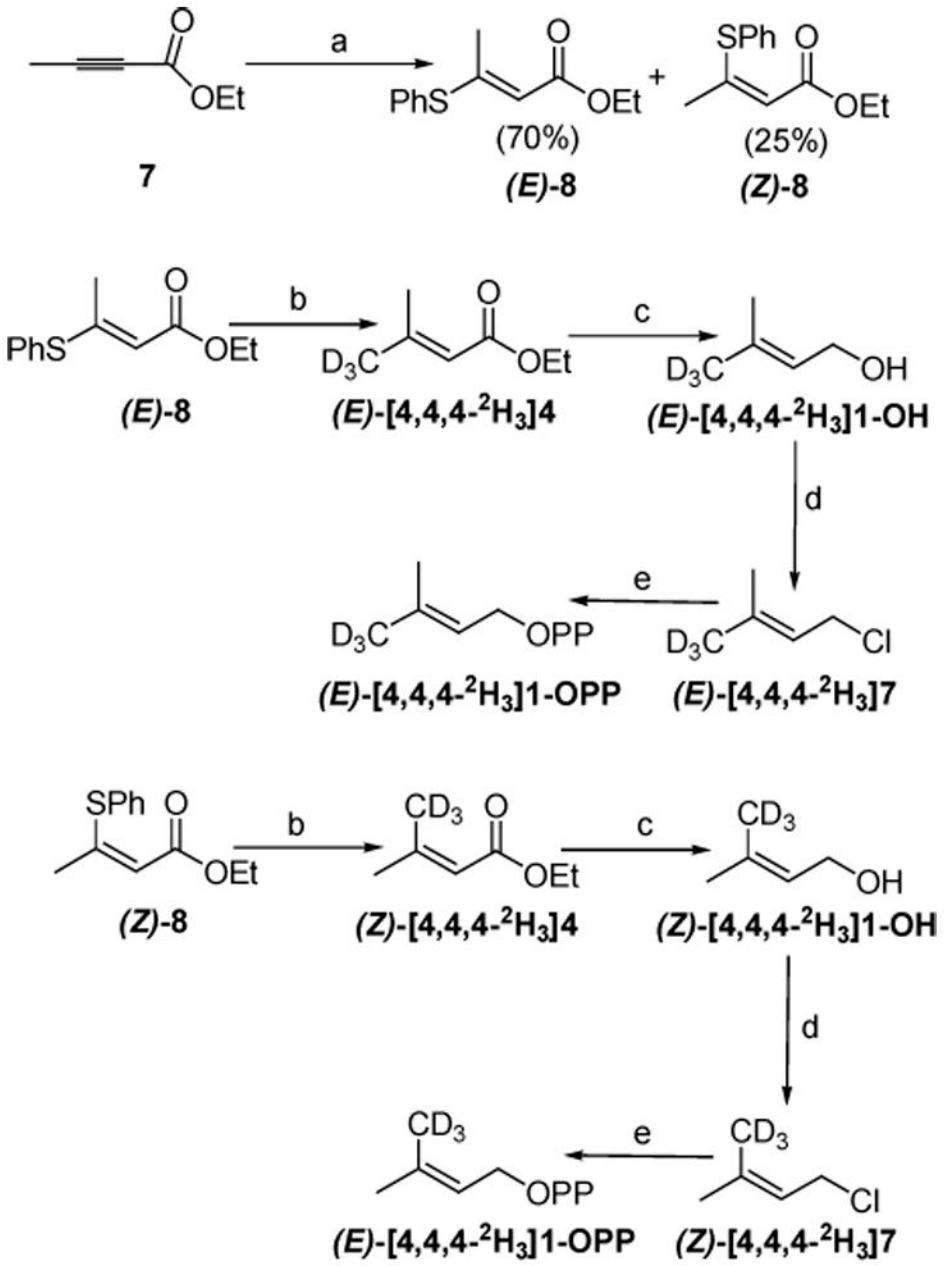
Scheme 1. Synthesis of *(R)*- and *(S)*-[1-^{2}H]1-OPP

(a) $LiAlD_4$, rt, 2 h, 86%; (b) PCC, CH_2Cl_2, rt, 3 h, 78%; (c) *S*-BITIP, Bu_3SnH, Et_2O, −20 °C, 24 h, 70%; (d) bis-triethylammonium phosphate (TEAP), CCl_3CN, CH_3CN, rt, 15 min, 30%; (e) *R*-BITIP, Bu_3SnH, Et_2O, −20 °C, 24 h, 69%.



Scheme 2. Synthesis of [2-^{2}H]1-OH

(a) D^2O, rt, 24 h, 2 times, 92%; (b) NaH, $ClPO(OEt)_2$, Et_2O, 0 °C, 2 h, 89 %; (c) Me_2CuLi, Et_2O, −47 °C, 2 h, 93%; (d) DIBAL, Et_2O, −70 0 °C, 3 h, 84%; (e) PBr_3, Et_2O, 2 h, 81%; (f) Tris(tetra-n-butylammonium) hydrogen diphosphate, CH_3CN, 34%.



Scheme 3. Synthesis of *(E)*- and *(Z)*-[4,4,4-$^2H_3$]1-OPP

(a) PhSH, NaOH, EtOH, rt, 4 h; (b) CD_3MgBr, CuI, THF, −65 °C, 3 h, 81%/87%; (c) $LiAlH_4$, Et_2O, 0 °C, 2.5 h, 82%/75%; (d) NCS, CH_2Cl_2, DMS, 78%/82%; (e) Tris(tetra-n-butylammonium) hydrogen diphosphate, CH_3CN, 60%/63%.