Concise Synthesis of (+)-2-*C*-Methyl-D-erythritol-4-phosphate

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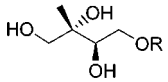
2-*C*-Methyl-D-erythritol-4-phosphate **1** (MEP) is believed to be one of the key intermediates in the biosynthesis of terpenoids by the mevalonate independent pathway (MIP) in bacteria, green algae, and plant chloroplasts.[1,2] Although the final steps of the MIP are



1 R = -PO_3H_2
2 R = -H

still unclear, it has been recently proposed that MEP (**1**) may be converted into isopentenyl diphosphate (IPP) via some phosphorylated derivatives including 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME), 2-phospho-4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME2P), and 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MECDP) (Scheme 1).[3−5] However, although much evidence has been published to support the biosynthesis of terpenes via the MIP,[1,2] very few direct observations on the role of MEP (**1**) have been presented.[6] The main obstacle to such studies is obtaining labeled compounds for feeding studies. In the last few months three efficient syntheses of MEP have been independently reported,[7−9] and more recently, Rohmer and co-workers have described the preparation and incorporation of deuterium labeled 2-*C*-methylerythritol in *E. coli*.[10,11]

Following the approach recently developed in our laboratory for the synthesis of 2-methyl-erythritol (**2**),[12] this paper describes the preparation of MEP (**1**) by a convenient method that is suitable for incorporation of carbon and hydrogen isotopes from commercially available precursors. The general strategy relies on the functionalization of a five-carbon skeleton (compound **6**), which has been prepared starting from dimethyl fumarate (**3**) and 2-(triphenylphosphoranylidene)propionaldehyde (**4**) (Scheme 2).[12] In fact, under the conditions described by Hon and Lu,[13] ozonolysis of **3** followed by Wittig reaction with **4** gave an intermediate aldehyde from which the alcohol **5** was obtained by reduction with $NaBH_4$ in a one-pot reaction.[12] Benzylation with benzyl-2,2,2-trichloroacetamidate in acidic medium[14] converted the product in the key intermediate **6**, which contains the desired isoprenoid skeleton. Reaction of **6** with an excess of DIBAL at −78 °C resulted in a nearly quantitative reduction of the ester yielding the *E* alkene **7** together with a small amount of the saturated alcohol derived by conjugated addition of hydride. Column chromatography on silica resolved the reaction mixture to give the pure allylic alcohol **7** in 92% yield. The *E* stereochemistry of the double bond was confirmed by NOE experiments that showed a clear enhancement of the methylene group at C-4 (δ 4.10) when the vinyl methyl group at δ 1.62 was irradiated. Sharpless epoxidation of **7** with titanium isopropoxide and (−)-diethyl tartrate in CH_2Cl_2 at −23 °C afforded the *threo* epoxide **8**, which was easily phosphorylated by using dibenzyl phosphoroiodidate (DBPI) generated in situ from freshly prepared tribenzyl phosphite and iodine.[15] The reaction proceeded smoothly at room temperature, and the epoxy alcohol **8** was almost quantitatively transformed into the dibenzyl phosphate **9** when an excess of DBPI was used. Ring opening of the protected epoxide was achieved under acidic hydrolysis by catalytic $HClO_4$ in 60% DMSO−water. The reaction is very sensitive to solvent, and lower yields were observed when DMSO was replaced with THF or dioxane. The reaction proceeded by enantioselective inversion of the configuration at the quaternary carbon[16] to give the desired *erythro* derivative **10** {$[\alpha]_D$ +2.8° (*c* 2.7, $CHCl_3$)} in 64% yield. Reaction of the diol **10** with (*S*)-MTPA−Cl in CH_2Cl_2 yielded the diastereomeric mixture of mono-MTPA derivatives, the analysis of which supported a 72% ee for the entire synthesis percentage (measured on the methyl group of the (*R*)-MTPA derivatives of **10**; δ 0.99 for the minor diastereomer and 1.09 for the major diastereomer). Finally, removal of the benzyl groups with 10% Pd/C in MeOH/H_2O 2:1 gave free 2-*C*-methyl-D-erythritol 4-phosphate (**1**) {$[\alpha]^{20°}_D$ +13.4° (*c* 0.8, H_2O)}[17] whose NMR spectra were very similar to those reported in the literature.[7] Methylerythritol 4-phosphate (**1**) was prepared in five steps from the readily available ester **6** with an overall yield of 51%.

In conclusion, the paper describes the preparation of **1** by the general approach recently applied to the synthesis of 2-*C*-methylerythritol (**2**).[12] Starting from fumarate and commercially available (2-triphenylphosphoranylidene) propionaldehyde,[12] the method affords an

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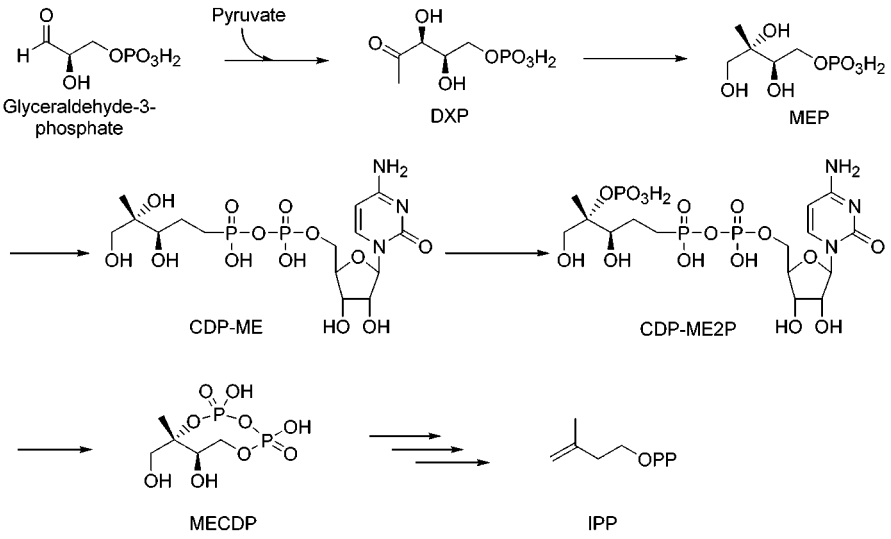
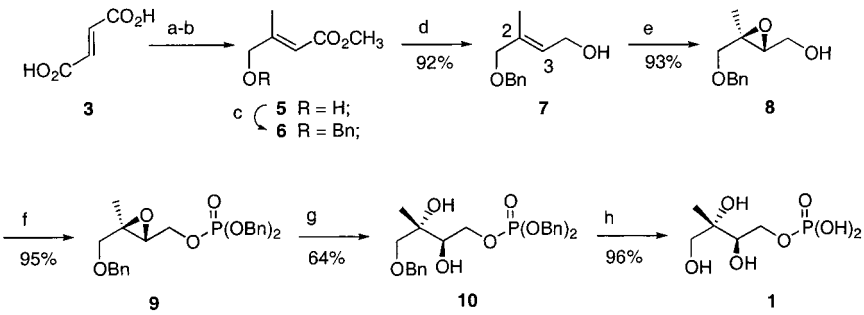
Scheme 1



Scheme 2[a]



[a] (a) HCl−MeOH at reflux; (b) O_3, CH_2Cl_2, −78 °C; 2-(triphenylphosphoranyliden) propionaldehyde (**4**), −78 °C; $NaBH_4$, MeOH (79% from **4**); (c) benzyl trichloroacetamidate, triflic acid, rt (55%); (d) DIBAL, THF, −78 °C; (e) (−)-DET, $Ti(OPr^i)_4$, *t*-BuOOH, −23 °C; (f) I_2−$P(OBn)_3$, Pyridine, CCL_4; (g) cat. $HClO_4$, 60% DMSO/H_2O; (g) H_2, 10% Pd/C.

easy way of preparing isotopically labeled compounds. The methodology compliments other synthetic procedures[7−9] and, although it gives lower overall enantioselectivity, offers the possibility of introducing labeling isotopes in different positions of the 2-methylerythritol.[18] This is certainly necessary for future biosynthetic studies, as well as to search new inhibitors for antibacterial application.

Experimental Section

Materials. Chemicals were obtained from Aldrich Chemicals and were used without further purification. All the organic solvents were distilled prior their use. NMR spectra were recorded at room temperature on Bruker 400 and 300 MHz spectrometers. ^1H and ^{13}C chemical shifts are reported in ppm from $CHCl_3$ (δ 7.26 and 77.0) or DMSO (δ 49.5). ^{31}P chemical shifts are referred to H_3PO_4 (δ 0.0) used as internal standard. Optical rotations were measured on a JASCO DIP370 polarimeter. Column chromatography was performed on Merck kieselgel 60.

One-Pot Procedure for the Synthesis of 5 from Fumarate. Dimethyl fumarate (**3**, 5 g, 34.7 mmol) in dry CH_2Cl_2 (200 mL) was reacted with ozone at −78 °C. When the starting material had totally disappeared by TLC (approximately 3 h), 10 g of 2-(triphenylphosphoranylidene) propionaldehyde (**4**, 31.1 mmol) and 4.8 mL of Et_3N (34.5 mmol) were added slowly, and stirring was continued for 30 min. The reaction mixture was left to warm to room temperature and stirred for another 4 h. The clear mixture was concentrated at reduced pressure. The slurry residue was dissolved in MeOH (250 mL) and an excess (140 mmol, 5.3 g) of $NaBH_4$ was added over a period of 30 min. The reaction was stirred at room temperature overnight. After evaporation of the solvent and addition of 1N HCl (65 mL), the mixture was extracted with Et_2O three times. The organic layers were dried over Na_2SO_4, filtered, and evaporated. The resulting colorless oil was fractionated on a SiO_2 column (petroleum ether/Et_2O 80:20) to give 3.18 g of **5** (pale yellow oil, 24.5 mmol, 79%). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.79 (s, 3H), 3.42 (s, 3H), 3.84 (s, 2H), 5.23 (s, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 15.5 (C-5), 50.9 (OCH_3), 66.2 (C-4), 111.3 (C-2), 157.8 (C-3), 167.3 (C-1); IR (film) 3444, 1707, 1660 cm^{-1}; CIMS (NH_3) 131 (90, M + H^+), 112 (100), 99 (95).

4-*O*-Benzyl-3-methyl-but-2-enoic Acid (6). To a solution of **5** (1.4 g, 10.8 mmol) in 2 mL of dry CH_2Cl_2 and 2.4 mL of cyclohexane were added 5.5 g benzyl 2,2,2-trichloroacetamidate (21.6 mmol) and a catalytic amount of triflurometh anesulfonic acid (25 μL, 42.5 mg) at room temperature. The reaction mixture was stirred under argon for approximately 3 h and then filtered. The filter cake was washed with CH_2Cl_2/hexane (50:50 v/v). The filtrates were combined and extracted with sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The oily residue was purified on a silica column (*n*-hexane/EtOAc 95:5) to give 1.3 g (5.91 mmol, 54.7%) of **6** (pale yellow oil). ^1H NMR ($CDCl_3$, 300 MHz) δ 2.11 (s, 3H), 3.69 (s, 3H), 3.97 (s, 2H), 4.51 (s, 2H), 6.04 (bs, 1H), 7.24−7.35 (bs, 5H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 15.7 (C-5), 50.7 (OCH_3), 72.3 (C-4), 73.8 (Bn), 114.6 (C-2), 127.4 (Bn), 127.6 (Bn), 128.3 (Bn), 137.7 (Bn), 154.8 (C-3), 166.8 (C-1); IR (film) 1722, 1660 cm^{-1}; CIMS (NH_3) 238 (40, M + NH_4^+), 221 (30, M + H^+), 91 (100).

(18) The enantio-enrichment of **1** may be addressed by partial resolution of intermediates **9** and **10** on chiral HPLC (Phenomenex columns: Chirex S-VAL & R-NEA cat. 3014 or 3020; *n*-hexane/1,2-dichloroethane/EtOH 50: 15:1).

4-Benzyloxy-3-methyl but-2-en-1-ol (7). Under argon, 11.6 mL (11.6 mmol) of 1 M DIBAL in *n*-hexane was added to a solution of **6** (320 mg, 1.45 mmol) in THF (10 mL) at −78 °C. The reaction was stirred for 5 h at the same temperature, and then the mixture was warmed to 0 °C over 1 h. After addition of MeOH (5 mL) and H_2O (5 mL), the white suspension was vigorously stirred for 10 min at room temperature. Then, Et_2O (15 mL) was added to the slurry solution and the resulting mixture was filtered through a thin layer of silica. The clear filtrate was evaporated to dryness at reduced pressure, and the residue was purified by a SiO_2 column (10 g, *n*-hexane/ethyl acetate 80:20) to give **7** (oil, 257 mg, 92%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.62 (s, 3H), 3.83 (s, 2H), 4.10 (d, J = 6.8 Hz, 2H), 4.39 (s, 2H), 5.59 (t, J = 6.8 Hz, 1H), 7.21−7.35 (bs, 5H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.1 (C-5), 58.9 (C-4), 71.9 (C-1), 75.3 (Bn), 126.2 (C-3), 127.5 (Bn), 127.6 (Bn), 128.3 (Bn), 135.4 (C-2), 138.2 (Bn); IR (film) 3455; CIMS (NH_3) 210 (40, M+NH_4^+), 192 (15, M^+), 91 (100).

(2*R*,3*R*)-4-Benzyloxy-2,3-epoxy-3-methyl butan-1-ol (8). Under argon atmosphere, 497 mg (2.41 mmol) of (−)-diethyl tatrate (DET) was dissolved in 4 mL of dry CH_2Cl_2 in a flask dried in oven. Through a septum, neat Ti(IV) isopropoxide (533 mg, 1.88 mmol) was added to the solution at −23 °C. After the resulting mixture was stirred for 10 min at the same temperature, **7** (239 mg, 1.24 mmol) in dry CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred for another 10 min at −23 °C prior the addition of 0.83 M *t*-BuOOH in nonane (763 μL, 4.02 mmol). The resulting mixture was stirred at the same temperature for 1h and then kept in the freezer (−20 °C) for 14 h without stirring. The reaction was quenched with 3 mL of MeOH. After stirring at −23 °C for 45 min, the mixture was treated with 15% NaOH (4 mL) and stirred at room temperature for another 45 min. The resulting suspension was partitioned between CH_2CL_{12} and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, hexane/EtOAc 80:20) to give **8** (240 mg, 93%) as a colorless oil, $[\alpha]^{20}{}_D$ +0.21° (*c* 10, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 3H), 3.13 (dd, J = 6.5 and 4.3 Hz, 1H), 3.47 (d, J = 10.9 Hz, 1H), 3.52 (d, J = 10.9 Hz, 1H), 3.70 (dd, J = 12.2 and 6.8 Hz, 1H), 3.82 (dd, J = 12.2 and 4.4 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.59 (d, J = 12.0 Hz, 1H), 7.30−7.35 (bs, 5H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.5 (C-5), 60.0 (C-2), 60.3 (C-3), 60.9 (C-4), 73.2 (C-1), 74.1 (Bn), 127.7 (Bn), 127.8 (Bn), 128.4 (Bn), 137.8 (Bn); IR (film) 3455; CIMS (NH_3) 226 (30, M+NH_4^+), 210 (10), 91 (100).

Dibenzyl (2*R*,3*R*)-4-Benzyloxy-2,3-epoxy-3-methyl-but-1-yl phosphate (9). To a solution of tribenzyl phosphite (715 mg, 2.03 mmol) in dry CH_2Cl_2 (18 mL) was added iodine (469 mg, 1.84 mmol) at 0 °C in an argon atmosphere. The resulting mixture was cooled to −78 °C, and treated with **8** (192 mg, 0.92 mmol) and dry pyridine (588 μL). The reaction mixture was stirred at the same temperature for 20 min and then warmed to room temperature over 2 h. When the starting material was not detectable any longer by TLC, the mixture was washed subsequently by 0.6 M potassium hydrogen sulfate, sat. $NaHCO_3$, and brine. The organic layers were dried (Na_2SO_4) and evaporated. The resulting oil was purified by column chromatography (SiO_2, CH_2Cl_2/acetone 97.5:2.5) to give **9** (410 mg, 95%) as a colorless oil, $[\alpha]^{20}{}_D$ +0.19° (*c* 3, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 3H), 3.14 (dd, J = 6.1 and 5.0 Hz, 1H), 3.40 (d, J = 11.1 Hz, 1H), 3.49 (d, J = 11.1 Hz, 1H), 4.11 (m, 2H), 4.51 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 5.06 (d, J = 12.2 Hz, 6H), 7.31−7.33 (m, 15H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.3 (C-5), 57.5 (d, J_{CP} = 8.6 Hz, C-3), 59.7 (C-2), 65.9 (d, J_{CP} = 5.2 Hz, C-4), 69.4 (m, J_{CP} = 2.8 Hz), 73.1, 73.4 (C-1), 127.6−128.5, 135.6 (d, J_{CP} = 5.2 Hz), 137.7; ^{31}P NMR ($CDCl_3$, 300 MHz) δ −0.39 (s); CIMS (NH_3) *m*/*z* 469 (38, M + H^+), 108; HRCIMS *m*/*z* 469.1782 ($C_{26}H_{30}O_6P$, calcd 469.1780).

Dibenzyl (2*S*,3*R*)-1-benzyloxy-2-*C*-methylerythritol-4-phosphate (10). Perchloric acid (20 μL) in 800 μL of distilled water was added to a solution of **9** (95 mg, 0.195 mmol) in 1.2 mL DMSO at 0 °C over 5 min. The reaction mixture was stirred at room temperature overnight, and then neutralized with a saturated solution (2.6 mL) of $NaHCO_3$. The aqueous solution was frozen and freeze-dried. The resulting residue was fractionated by column chromatography (SiO_2, $CHCl_3$/acetone 95:5) to give **10** (57 mg, 64%) as a colorless oil, $[\alpha]^{20}{}_D$ +2.8° (*c* 2.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.09 (s, 3H), 3.31 (d, J = 9.1 Hz, 1H), 3.56 (d, J = 9.1 Hz, 1H), 3.80 (bm, 1H), 4.03 (m, 1H), 4.25 (ddd, J = 10.7, 10.7 and 2.2 Hz, 1H), 4.52 (s, 2H), 5.05 (d, J = 8.5 Hz, 4H), 7.30−7.35 (m, 15H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 19.6 (C-5), 69.5 (bs), 72.6 (C-2), 73.5 (C-1), 74.3 (d, J_{CP} = 4.9 Hz, C-4), 74.7 (C-3), 121.7, 127.9, 128.0, 128.2, 128.5, 128.6, 135.6 (d, J_{CP} = 6.3 Hz), 137.6; ^{31}P NMR ($CDCl_3$, 300 MHz) δ 1.00 (s); FAB+ MS (glycerol) *m*/*z* 509 (28, M+Na^+), 487 (38, M + H^+); FAB + HRMS *m*/*z* 487.1881 ($C_{26}H_{32}O_7P$, calcd 487.1885).

2-*C*-Methylerythritol 4-phosphate (1). A solution of **10** (28 mg, 0.058 mol) in 1.5 mL of 50% MeOH in water was hydrogenated by H_2 and catalytic 10% Pd/C at room temperature overnight. The catalyst was filtered off, and the filtrate was evaporated to dryness to give 12 mg (0.056 mmol, 96%) of **1** as an amorphous solid, $[\alpha]^{20}{}_D$ +13.4° (*c* 0.8, H_2O) [lit.[17] $[\alpha]^{20}{}_D$ +6.4° (*c* 0.1, H_2O)]. ^{1}H NMR (D_2O, 300 MHz) δ 1.13 (s, 3H), 3.47 (d, J = 11.8 Hz, 1H), 3.59 (d, J = 11.8 Hz, 1H), 3.80 (dd, J = 8.2 and 2.4 Hz, 1H), 3.90 (m, 1H), 4.13 (m, 1H); ^{13}C NMR (D_2O + 1 drop DMSO-d_6, 300 MHz) δ 19.4 (C-5), 67.5 (C-4 and C-1), 74.6 (d, J_{CP} = 8.9 Hz, C-3), 75.1 (C-2); ^{1}H NMR (D_2O + 1 drop DCl, 300 MHz) δ 1.06 (s, 3H), 3.40 (d, J = 11.8 Hz, 1H), 3.53 (d, J = 11.8 Hz, 1H), 3.76 (dd, J = 8.2 and 2.4 Hz, 1H), 3.91 (m, 1H), 4.16 (m, 1H); ^{31}P NMR (D_2O + 1 drop DCl, 300 MHz) δ 1.11 (s) (phosphoric acid as internal standard, δ = 0.00).

Preparation of the Mono-(*R*)-MTPA Derivative of 10. Under dry conditions, (*S*)-MTPA Cl (Chiraselect, Fluka) (10.8 mg, 8 μL) was added to a solution of **10** (3.5 mg) and a catalytic amount DMAP in 400 μL of CH_2Cl_2. The reaction mixture was stirred at room temperature overnight and then evaporated to dryness. The oil residue was dissolved in $CHCl_3$ and purified by column chromatography (SiO_2, $CHCl_3$/acetone 95:5) to give quantitatively the mono MTPA ester of **10**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (s, 3H, minor isomer), 1.09 (s, 3H, major isomer), 3.07 (d, J = 9.4 Hz, 1H, minor isomer), 3.15 (d, J = 9.4 Hz, 1H, minor isomer), 3.19 (d, J = 9.4 Hz, 1H, major isomer), 3.30 (d, J = 9.4 Hz, 1H, major isomer), 3.47 (bs, 3H, MTPA, minor compound), 3.51 (bs, 3H, MTPA, major compound), 4.07 (m), 4.56 (m), 4.98 (m), 5.49 (dd, J = 8.3 and 2.5 Hz, 1H, major isomer), 5.52 (dd, J = 8.3 and 2.5 Hz, 1H, minor isomer), 7.15−7.65 (m).

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Supporting Information Available: Data about identification (^{1}H and ^{13}C NMR) for newly synthesized compounds (**1**, **5**−**10**) are available free of charge via the Internet at http://pubs.acs.org.

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