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Synthesis of 4-Diphosphocytidyl-2-*C*-methyl-D-erythritol and 2-*C*-Methyl-D-erythritol-4-phosphate

Andrew T. Koppisch and C. Dale Poulter*

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

poulter@chem.utah.edu

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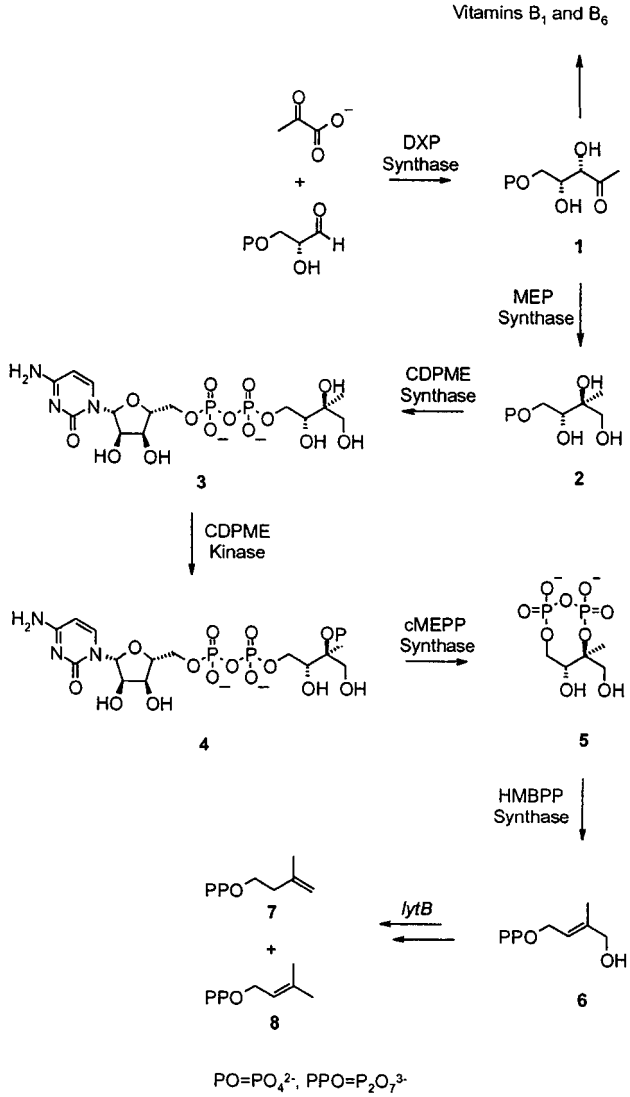
Abstract: 2-*C*-Methyl-D-erythritol 4-phosphate (MEP, **2**) and 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDPME, **3**) are metabolites in the MEP pathway for biosynthesis of isoprenoid compounds in bacteria, plant chloroplasts, and algae. The free phosphoacid of **2** was prepared from benzyloxyacetone in five steps with an overall yield of 27% and an enantiomeric ratio (er) of 75:25. Following titration to the corresponding tributylammonium salt, **2** was coupled to cytidine 5′-monophosphate using a protocol originally developed for synthesis of base-sensitive nucleoside diphosphate sugars to give **3** in 40% yield, following purification by size exclusion chromatography.

After the discovery that isoprenoids were synthesized from mevalonate in fungi and mammals in the late 1950s, it was assumed this was the major route to the fundamental isoprenoid building blocks isopentenyl diphosphate (IPP[1], **7**) and dimethylallyl diphosphate (DMAPP, **8**) in all organisms.[2] More recently, labeling studies conducted independently in the laboratories of Rohmer[3] and Arigoni[4] uncovered a novel pathway for the synthesis of IPP and DMAPP in bacteria, plant chloroplasts, and algae. Since then most of the transformations in the "mevalonate-independent" or methylerythritol phosphate (MEP) pathway have been established (Scheme 1). [5]

The first committed step in the MEP pathway is the conversion of 1-D-deoxyxylulose-5-phosphate (DXP, **1**) to 2-*C*-methyl-D-erythritol-4-phosphate (MEP, **2**).[6] MEP is then coupled with cytidine triphosphate (CTP) to produce 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDPME, **3**),[7,8] which is subsequently phosphorylated and cyclized to give 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (cMEPP, **5**).[9,10] The cyclic diphosphate is converted to 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate (HMBPP, **6**) by the enzyme encoded by *gcpE*,[11,12] and recent evidence indicates the *lytB* gene product is involved in the conversion of **6** into both IPP and DMAPP.[13]

Because enzymes in the MEP pathway are not found in animals,[14] they are attractive targets for the develop-

SCHEME 1. Methylerythritol Phosphate Pathway to IPP and DMAPP



* To whom correspondence should be addressed. Phone: (801) 581-6685. Fax: (801) 581-4391.

(1) Abbreviations used: CDPME, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol; CDPMEP, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol-2-phosphate; cMEPP, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate; CMP, cytidine monophosphate; CTP, cytidine triphosphate; DMA, dimethylaniline; DMAPP, dimethylallyl diphosphate; DXP, 1-D-deoxyxylulose-5-phosphate; equiv, equivalents; er, enantiomeric ratio; FPLC, fast protein liquid chromatography; HMBPP, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate; MeIm, methylimidazole; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; MSA, methane sulfonamide; MS(EI), electron impact mass spectrometry; TBA, tributylamine; TEA, triethylamine; TFAA, trifluoroacetic anhydride.

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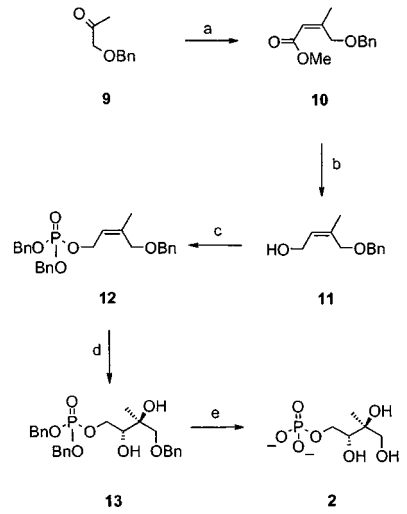
ment of herbicides and antimicrobial agents. The MEP synthase inhibitor fosmidomycin is effective as a treatment for malaria in an animal model.[15] At this time only a few of the enzymes in the MEP pathway have been studied in detail,[5] and a major limitation to this research has been the unavailability of substrates. Although large-scale enzymatic preparations of several MEP pathway intermediates have been reported,[16−19] there are no efficient organic syntheses available for many of the advanced intermediates. Reliable syntheses of DXP[20] and MEP[21] were important in our studies of DXP synthase[22] and MEP synthase.[23] We now report a chemical synthesis CDPME (**3**), the next intermediate in the MEP pathway, and an improved procedure for the synthesis of MEP (**2**).

Synthesis of 2-*C*-Methyl-D-erythritol-4-phosphate (2). We recently reported the synthesis of the monosodium salt of **2** in seven steps from 1,2-propane diol.[21] Although the synthesis provided ample quantities of **2** for our enzymatic studies, the limited solubility of the sodium salt in organic solvents compromised its use as a precursor for the synthesis of **3** by coupling with CMP. The nucleophilicity of unprotected phosphates is extremely dependent on the solvent used in coupling reactions[24] and the nature of the counterion. Alkylammonium salts of the phosphoacids are often employed to increase their solubility in organic solvents.[25] Since replacement of alkali metal cations with alkylammonium ions is an unfavorable process for most commercial ion-exchange resins, we chose to modify our synthesis of **2** to obtain the free acid as shown in Scheme 2.

The Still modification of the Horner−Emmons reaction[26] was used to convert commercially available benzyloxyacetone **9** to the protected olefin **10**, with increased selectivity for the Z-isomer. The isomers were separated by flash column chromatography, and the Z-ester was reduced with $LiAlH_4$ to give allylic alcohol **11**. The ^1H and ^{13}C NMR spectra of **11** were identical to those reported by Sato and co-workers.[27] Phosphorylation of **11** with iodine and tribenzyl phosphite[28] provided the fully benzylated olefin phosphate **12** in good yield.

SCHEME 2. Synthesis of Free Acid of MEP (2)[a]



[a] (a) KH, (2,2,2-trifluoroethyl) methoxycarbonylmethyl-phosphonate, THF, −20 °C (67%, 7:3 *c*:*t*); (b) $LiAlH_4$, CH_2Cl_2, −40 °C (85%); (c) tribenzyl phosphite/iodine, CH_2Cl_2 0 °C (88%); (d) AD-mix *β*, *t*-BuOH/H_2O, 0 °C; (55%); (e) Pd/C, MeOH/H_2O (95%).

The asymmetric dihydroxylation of **12** to form **13** is an adaptation of a similar procedure first reported for the synthesis of methylerythritol.[29] We had previously observed that standard AD-mix conditions for dihydroxylation of allylic phosphates frequently gave low yields, accompanied by substantial degradation. For example, in the previously reported synthesis of **2**, dihydroxylation of an analogue of **12**, where the primary alcohol was protected as a TBS ether and the phosphate was a dimethyl ester,[21] required a substantial increase in the concentrations of $(DHQD)_2PHAL$ and OsO_4 and addition of $NaHCO_3$ to buffer the reaction.[30] In addition, the usual rate enhancement of dihydroxylation associated with using (methanesulfonamide, NMO) as a co-oxidant was minimal for the TBS containing allylic phosphate. Compound **12** was more robust to the standard AD-mix conditions than the TBS derivative. Both modified conditions and buffering had little effect on yield of the dihydroxylation. By using standard conditions, **12** was converted to **13** in 55% yield. The er of the dihydroxylation was estimated to be 75:25 by integration of the diastereomeric ^{19}F resonances in the Mosher's ester of **13**.[31] The benzyl groups in **13** were removed by hydrogenation over Pd/C in water/methanol to give **2** as the free acid in five steps and an overall yield of 27%. Fontana[32] recently described a synthesis of MEP from dimethyl fumarate where dihydroxylation was accomplished by asymmetric epoxidation of the trans-isomer of allylic alcohol **10**, followed by opening of the epoxide with perchloric acid. The E-isomer from our olefination step could be converted to **2** using this strategy.

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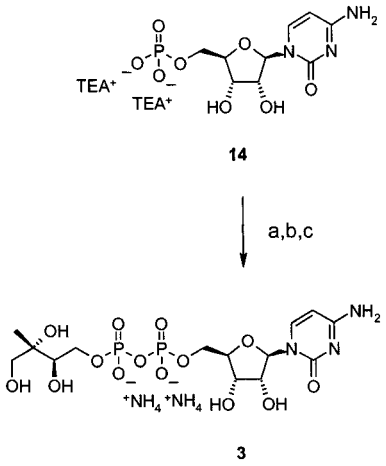
SCHEME 3. Coupling of CMP (14) and MEP (2) To Form CDPME (3)[a]



[a] (a) TFAA, TEA, CH_3CN; (b) MeIm, DMA, TEA, CH_3CN; (c) tributylammonium-2, TEA, CH_3CN, 2 Å mol sieves (40%).

Often preparations of **2** contained small quantities of paramagnetic contaminants from the debenzylation step, as indicated by broadening of the ^1H NMR resonances. Contaminated samples gave poorer yields of the corresponding alkylammonium salt, and attempts to purify the debenzylated product over cellulose[33] did not remove the contaminants. The metal contaminants were removed by ion exchange over CHELEX. Typically the resin was converted to the proton form by repeatedly washing with 2 M HCl, followed by exhaustive washing with deionized water. However, the material obtained by titration of **2** following treatment with CHELEX typically contaminated by 0.8−1.2 equiv of an extraneous tri-*n*-butylammonium species. We had previously observed degradation of **2** and migration of the phosphate moiety when the protecting groups were removed with acid and the resulting solution was neutralized with NH_4HCO_3.[21] In contrast, titration of an aqueous solution of the free acid of **2** to pH 7 with tri-*n*-butylamine did not promote intramolecular phosphate migration, and the tributylammonium salt was obtained upon lyophilization.

Synthesis of 4-Diphosphocytidyl-2-*C*-methyl-D-erythritol (3). Our synthesis of **3** (Scheme 3) follows a recently reported procedure that produces the nucleoside/carbohydrate diphosphate rapidly, with a minimum of undesired side products.[34]

The overall procedure involves transient activation of the phosphoester moiety as a trifluoroacetyl anhydride, followed by its conversion to the corresponding phosphoramide by treatment with methylimidazole. This procedure was first developed for the synthesis of nucleoside triphosphates[35] and was subsequently adapted for the synthesis of sugar/nucleoside diphosphates by Marlow and Kiessling.[34] The TFAA/methylimidazole protocol provided a higher yield of coupled nucleotide in a much shorter time than a previously reported synthesis based on morpholidate/tetrazole activation, where significant degradation can occur in base-sensitive carbohydrate moieties during the time required for the reaction.[36] We anticipated similar problems for the conversion of **2** to **3**, which were confirmed in small-scale reactions.

Triethylammonium cytidine 5′-monophosphate (**14**) was prepared by titration of the corresponding phosphoacid with triethylamine. The nucleoside phosphate was activated by treatment, in succession, with trifluoroacetic anhydride and methylimidazole[34,35] and then coupled with the tributylammonium salt of **2**. The reaction was complete after 2 h as judged by ^{31}P NMR and was then quenched to minimize formation of side products. The crude mixture was chromatographed over Biogel P2-fine[35] with elution by 100 mM $(NH_4)_2HCO_3$, pH 7.8. Fractions were monitored both by UV and ^{31}P NMR spectroscopy. Those containing product were combined and lyophilized to give a 40% yield of **3** free of buffer salts. Our yield is comparable to that reported for UDP-Gal*f*.[33] ^1H, ^{13}C, and ^{31}P NMR spectra for **3** agreed with those reported for a sample prepared enzymatically.[6,7] Overall, **3** was obtained in 11% yield in six steps from benzyloxyacetone.

Experimental Section

4-Diphosphocytidyl-2-*C*-methyl-D-erythritol (3).[6,7] To a solution of triethylammonium cytidine monophosphate **14** (103 mg, 0.23 mmol) in CH_3CN was added dimethylaniline (117 μL, 0.93 mmol) and triethylamine (32 μL, 0.23 mmol). The solution was cooled to 0 °C before trifluoroacetic anhydride (164 μL, 1.16 mmol) in CH_3CN was added over 30 s. The reaction was stirred for 15 min before excess anhydride and trifluoroacetic acid were removed at reduced pressure. A solution of methylimidazole (55 μL, 0.69 mmol) and triethylamine (164 μL, 1.16 mmol) in CH_3CN was added over 3 min, and stirring was continued for 30 min before the mixture was transferred to a solution of tributylammonium **2** (125 mg, 0.20 mmol) and 50 mg of finely crushed and activated 4 Å molecular sieves in CH_3CN. The reaction was stirred at 0 °C for 4 h before cold 1 M NH_4HCO_3, pH 8, was added. The mixture was extracted with $CHCl_3$, and residual alkylammonium salts were exchanged with NH_4^+ by passing the aqueous layer over a column of DOWEX 50WX8-200 resin (NH_4^+ form), and eluting with 100 mM NH_4HCO_3, pH 7.8. After lyophilization, the crude material was suspended in a minimum volume of elution buffer (100 mM NH_4HCO_3, pH 7.8) and applied to a FPLC column (1.5 cm × 100 cm, Bio-gel P2 fine) that had been preequilibrated in elution buffer. The column was eluted at a flow rate of 0.14 mL/min, and fractions were monitored by UV spectroscopy and ^{31}P NMR. Fractions containing product were lyophilized to give 45 mg (40%) of a white solid. ^1H NMR (D_2O): δ 1.10 (s, 3H), 3.46 (d, J = 12.2 Hz, 1H), 3.55 (d, J = 12.2 Hz, 1H), 3.81 (d, J = 6.8 Hz, 1H), 3.94−3.96 (m, 1H), 4.16−4.33 (m, 6H), 5.94 (d, J = 4 Hz, 1H), 6.14 (d, J = 7.3 Hz, 1H), 8.02 (d, J = 7.3 Hz, 1H). ^{13}C NMR (D_2O): δ 18.5, 64.9 (d, J_{CP} = 6.0 Hz), 66.4, 66.5, 67.3 (d, J_{CP} = 6.0 Hz), 69.5, 73.7 (d, J_{CP} = 7.5 Hz), 74.1, 74.5, 78.0, 83.0 (d, J_{CP} = 9 Hz), 89.5, 96.4, 141.7, 158.0, 166.4. ^{31}P NMR (D_2O): δ −10.59 (d, J = 20.6 Hz), −11.28 (d, J = 21.1 Hz). IR (KBr pellet): 3515 (br), 3159 (br), 1642, 1619, 1580, 1401, 1160 cm^{-1}. MS (EI, 70 eV): m/z 551 (M + NH_4, 1), 275 (M − MEP + PO_4, 10), 184 (MEP − 2OH, 5). $[\alpha]^{20}_D$ (c 0.015) +10.2 °.

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Supporting Information Available: General methods and experimental procedures for the synthesis of **2** and **10**−**13**, as well as ^1H, ^{13}C, and ^{31}P NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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