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Synthesis of 2-*C*-Methyl-D-erythritol 4-Phosphate: The First Pathway-Specific Intermediate in the Methylerythritol Phosphate Route to Isoprenoids

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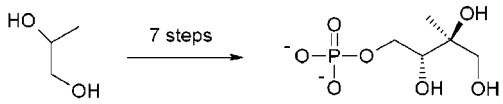
ABSTRACT



2-*C*-Methyl-D-erythritol 4-phosphate (4), formed from 1-deoxy-D-xylulose 5-phosphate (3), is the first pathway-specific intermediate in the methylerythritol phosphate route for the biosynthesis of isoprenoid compounds in bacteria, algae, and plant chloroplasts. In this report, 4 was synthesized from 1,2-propanediol (7) in seven steps with an overall yield of 32% and in an enantiomeric excess of 78%.

Isoprenoid compounds constitute one of the most chemically diverse families in Nature,[1] with over 30 000 identified representatives to date. Isopentenyl diphosphate (IPP, **6**) serves as the universal precursor to the members of this family, whose carbon skeletons are typically synthesized by condensing IPP with allylic diphosphates. Recent isotopic labeling studies have established that bacteria,[2] plant chloroplasts,[3] and green algae[4] synthesize **6** from 1-deoxy-D-xylulose 5-phosphate (**3**). In contrast to the well-established mevalonate (MVA) pathway operating in most eukaryotes and archaebacteria,[5] this "mevalonate-independent" or methylerythritol phosphate (MEP) pathway uses glyceraldehyde 3-phosphate (**1**) and pyruvate (**2**) as the primary substrates for the biogenesis of **6** (Scheme 1). Although the exact sequence of transformations in the MEP route to IPP has yet to be established, the first pathway-specific step is an enzyme-catalyzed rearrangement/reduction of **3** to 2-*C*-methyl-D-erythritol 4-phosphate (MEP, **4**).[6] Additional labeling studies have established the direct incorporation of methylerythritol into isoprenoid compounds,[7] and MEP has been identified as a critical metabolite in the development of the parasite responsible for malaria, *Plasmodium falciparum*.[8]

Recently, Zenk and co-workers reported the isolation of a recombinant *Escherichia coli* enzyme that catalyzes the conversion of MEP to a 5′-cytidine diphosphate derivative (**5**), as well as the incorporation of **5** into the carotenoids of *Capsicum annuum*.[9] Evidence exists which indicates that the gene responsible for this transformation (*ygbP*) is conserved

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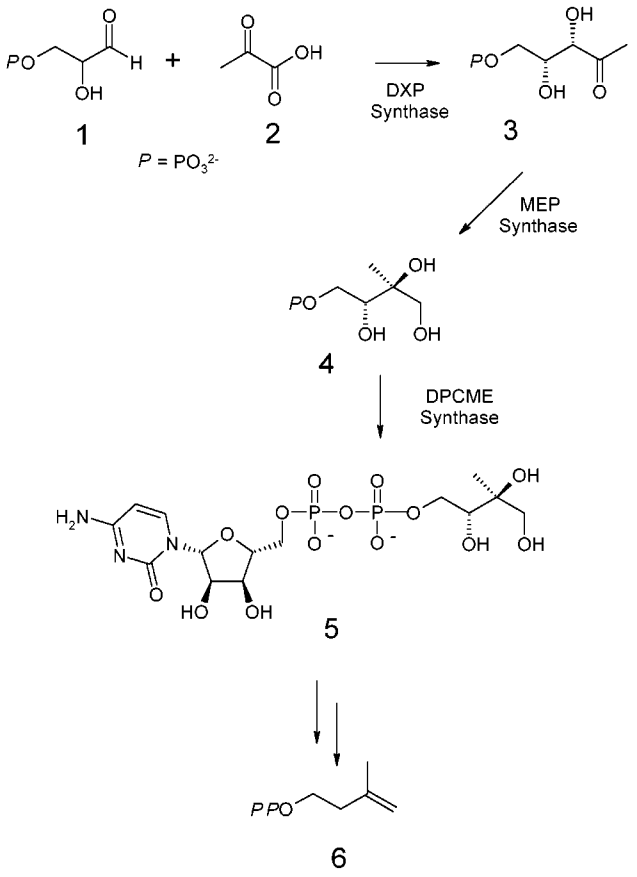
Scheme 1

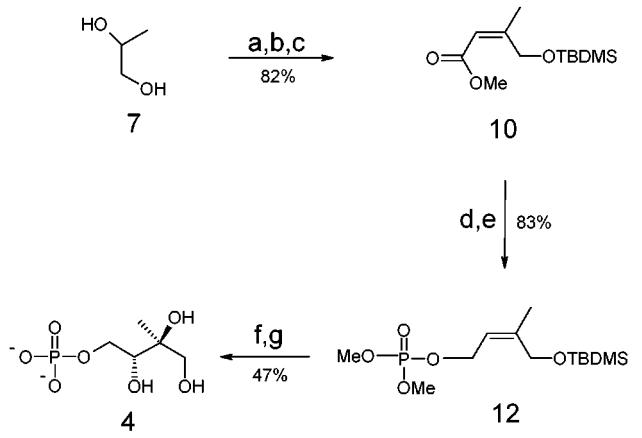


within numerous organisms known to contain the MEP pathway, and the formation of **5** may represent the next biosynthetic step toward IPP. Additionally, Lange and Croteau[10] have reported the isolation of a kinase responsible for catalyzing the conversion of isopentenyl monophosphate (IP) to IPP. Incorporation of IP into isoprenoids of peppermint suggests that it is a viable intermediate in the MEP route, and the phosphorylation of IP may represent the final step in the biosynthesis of IPP from MEP. The remaining transformations in the MEP pathway have yet to be identified, although both the MVA and MEP pathways converge at IPP.[11]

Although Rohmer and co-workers have reported the synthesis of 2-*C*-methyl-D-erythritol,[7] an efficient route to the 4-phosphate has not been described. We now describe a synthesis of optically active 2-*C*-methyl-D-erythritol 4-phosphate from 1,2-propanediol in 78% enantiomeric excess and an overall yield of 32%.

The route to 2-*C*-methyl-D-erythritol 4-phosphate is outlined in Scheme 2. Treatment of 1,2 propanediol (**7**) with 1 equiv of *tert*-butyldimethylsilyl chloride afforded the mono-

Scheme 2[a]



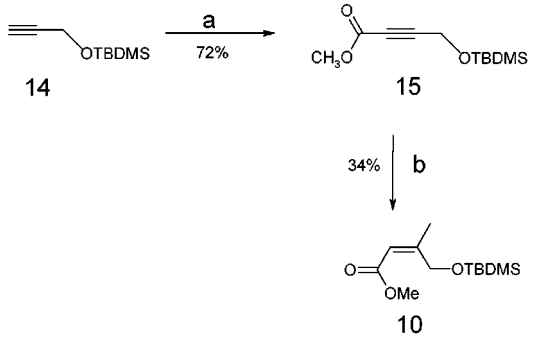
[a] Key: (a) TBDMSCl, *N*-ethyldiisopropylamine, CH_2Cl_2; (b) TPAP, NMO, CH_2Cl_2; (c) KH, (2,2,2-trifluoroethyl)methoxycarbonylmethyl phosphonate, THF, −20 °C; (d) DIBALH, CH_2Cl_2, −40 °C; (e) dimethyl chlorophosphate, DMAP, CH_2Cl_2; (f) modified AD-mix *β*, *t*-BuOH/H_2O, $NaHCO_3$, 0 °C; (g) TMSBr/H_2O; HCl.

silylated alcohol **8**[12] in high yield. Subsequent oxidation of **8** with TPAP/NMO[13] gave the protected ketone **9**.[14]

Still modifications[15] of the Horner−Emmons reaction were used to convert **9** to the protected olefin **10** with increased selectivity for the *Z* isomer. Warming to −40 °C accelerated the reaction without affecting the yield, but *E*/*Z* selectivity was reduced. *Z*/*E* isomers were separated by flash column chromatography at this stage or after installation of the phosphate moiety (**12**). The stereochemistry of the double bond was established by a NOESY experiment, where the vinylic proton of the *Z* isomer had a strong cross-peak with the protons on the methyl group.

Synthesis of **10** from **15** (Scheme 3) using the method of Corey and Katzenellenbogen[16] gave the *Z* isomer exclusively. Propargyl ester **15**[17] was obtained from methyl chloroformate and the TBDMS-protected propargyl alcohol **14**. Although this approach gives an overall lower yield of **10**, it does

Scheme 3[a]



[a] Key: (a) *n*-BuLi, methyl chloroformate, THF, −78 °C; (b) $CuBr_2$−DMS, MeLi, THF, −78 °C.

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provide a convenient method for specific incorporation of carbon or hydrogen isotopes into **4** from commercially available precursors.

Olefin **10** was reduced with 2 equiv of DIBALH followed by reaction with dimethyl chlorophosphate to give **12**. The phosphotriester was then dihydroxylated via the method of Sharpless to give **13**.[18] Preliminary experiments indicated that the phosphotriester moiety imposed several limitations on use of the standard AD-mix conditions. We found the basic nature of the commercial AD-mix reagents promoted the intramolecular migration of the phosphotriester to the secondary and tertiary hydroxyl groups in **13** and promoted hydrolysis to form the corresponding triol. In addition, **12** reacted sluggishly. These problems were circumvented by the use of "modified" AD-mix conditions with buffering by $NaHCO_3$.[19] We observed that the usual rate enhancement of the dihydroxylation upon the addition of a co-oxidant (methanesulfonamide, NMO) to the mixture was minimal and also led to substantial degradation. For this reason the asymmetric dihydroxylations were conducted in the absence of co-oxidant. Our modifications gave a 60% yield of **13** from **12** with a 78% enantiomeric excess.[20] The phosphotriester methyl groups in **13** were removed by treatment with trimethylsilyl bromide followed by hydrolysis.[21] The *tert*-butyldimethylsilyl moiety was removed in the same pot by increasing the acidity of the aqueous solution. The mixture was neutralized with $NaHCO_3$ and lyophilized, and the solid residue was chromatographed on cellulose to give MEP.[22] The NMR spectra as well as the optical rotation of the synthetic **4** are consistent with those reported for an enzymatically prepared sample.[6b] In summary, the synthetic routes to MEP, which compliment our previous synthesis of 1-deoxy-D-xylulose phosphate,[23] offer a straightforward approach for synthesizing isotopically labeled materials for use in biosynthetic experiments.

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Supporting Information Available: Complete experimental procedures for the synthesis of compounds **4**, **8**−**13**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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