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Synthesis of Enantiopure 2-*C*-Methyl-D-erythritol 4-Phosphate and 2,4-Cyclodiphosphate from D-Arabitol

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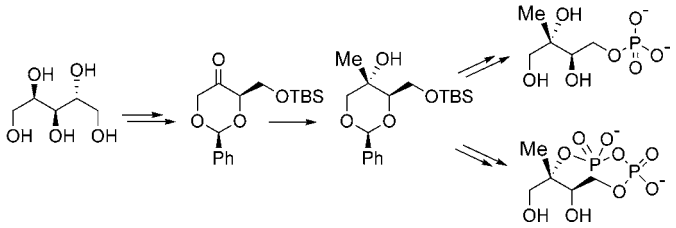
ABSTRACT



Two key intermediates of the newly discovered mevalonate-independent pathway for isoprenoid biosynthesis were prepared. Optically pure 2-*C*-methyl-D-erythritol 4-phosphate and 2,4-cyclodiphosphate were chemically synthesized from D-arabitol using a convenient benzylidene and *tert*-butyldimethylsilyl protection of polyhydroxylated intermediates. The new scheme offers a straightforward route to analogues and labeled forms.

Terpenes, one of the largest groups of natural products, are assembled from two precursors, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) by electrophilic elongations, cyclizations, and rearrangements. Since the first identification of the acetate-replacement factor in 1956, the mevalonate pathway was considered to be the universal source of these biosynthetic isoprene units.[1] However, the recent discovery of the mevalonate-independent (methylerythritol phosphate, MEP) pathway for isoprenoid biosynthesis in bacteria, plant chloroplasts, and algae has triggered an intense burst of research activity.[2] Since the MEP pathway is absent in animal cells, new opportunities have become available to develop herbicides and drugs against pathogenic bacteria and the malaria parasite.[3] Although many details of this novel route are unknown, some intermediates, mechanisms, enzymes, and genes have been identified (Scheme 1, Cy = cytidyl).[2,4]

The initial transketolase-like condensation between D-glyceraldehyde 3-phosphate (**1**) and pyruvate (**2**) forming 1-deoxy-D-xylulose 5-phosphate (**3**) is followed by semibenzilic rearrangement and reduction to 2-*C*-methyl-D-erythritol 4-phosphate (MEP, **4**), the first pathway-specific intermediate. MEP is then coupled with cytidine triphosphate (CTP) to produce 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME, **5**), which is subsequently phosphorylated to give 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate (**6**). Cyclization of **6** leads to 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MECDP, **7**), previously identified as an oxidative stress metabolite of gram-negative bacterial para-

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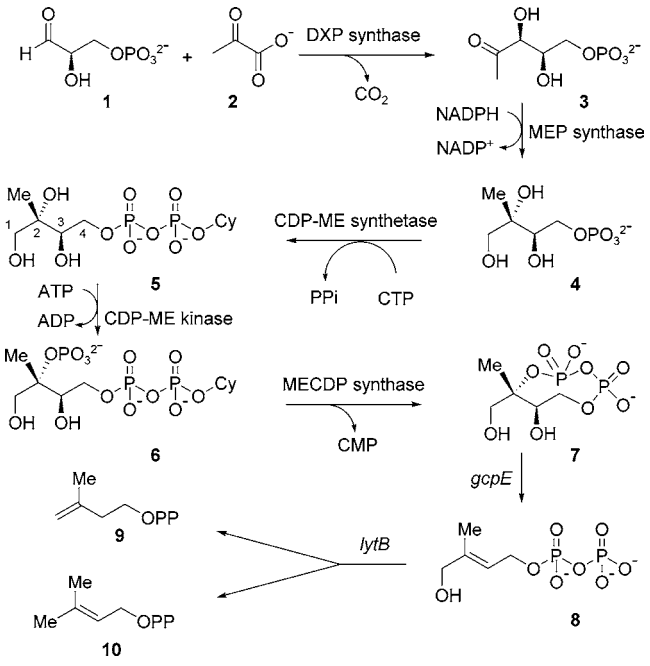
Scheme 1

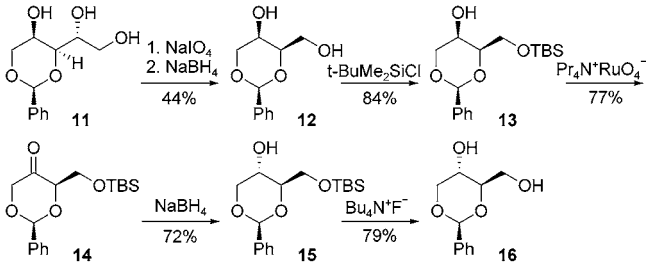


sites.[5] The last reductive steps leading to IPP (**9**) and DMAPP (**10**) via (*E*)-4-hydroxy-DMAPP (**8**) involving the *gcpE* and *lytB* genes are still a matter of investigation.

Efficient synthesis of MEP pathway intermediates, their labeled forms, and their derivatives is important in the mechanistic study of these unusual enzymes and for discovery of specific inhibitors and therapeutic agents. Enzymatic preparations of phosphate **4**[6,7] and cyclodiphosphate **7**[8−10] have been reported. Known chemical syntheses are either based on asymmetric oxidation methods of suitably functionalized 3-methylbut-2-enyl-1,4-diols[11−14] or utilize carbohydrate precursors.[15,16] We report a new, versatile approach to enantiomerically pure MEP and MECDP, which appears to be readily adaptable to access the cytidine intermediates, to preparation of various analogues, and to stereoselective labeling.[17]

Literature procedures[18,19] were used to prepare 1,3-benzylidene-D-threitol (**12**) from commercially available D-arabitol (Scheme 2). The primary hydroxyl group of the diol

Scheme 2



was protected with a *tert*-butyldimethylsilyl group (TBSCl, DMAP, Et_3N, DMF) to give monoether **13**. TPAP/NMO oxidation[20] afforded the key intermediate **14**. Alternatively, phase-transfer-promoted RuO_4 oxidation (RuO_2/$NaIO_4$, CCl_4, H_2O, $BnEt_3NCl$)[21] proved to be of similar efficiency with the advantage of lower cost for the oxidant. The required 2*S* configuration for 2-*C*-methyl-D-erythritol was expected to be obtained by nucleophilic additions to the protected D-erythrulose **14** since highly selective axial attack in the reactions of complex metal hydrides[22] and various

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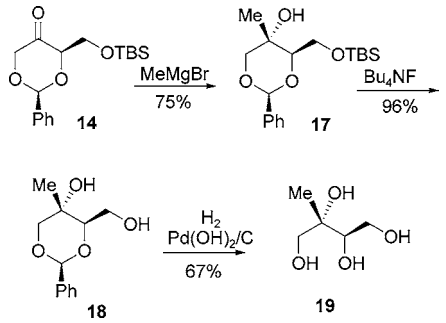
organometallic reagents with 1,3-dioxan-5-ones is well established.[23−25] In agreement with this precedent, reduction of **14** with $NaBH_4$/MeOH yielded exclusively equatorial alcohol **15**. Cleavage of the primary silyl ether gave 1,3-benzylidene-D-erythritol (**16**), the physical data of which are similar to those reported for the known L enantiomer.[26] In like manner, Grignard addition (MeMgBr, Et_2O, −78 °C) was accomplished in ≥20:1 selectivity, and silica gel chromatography afforded pure tertiary alcohol **17** (Scheme 3).

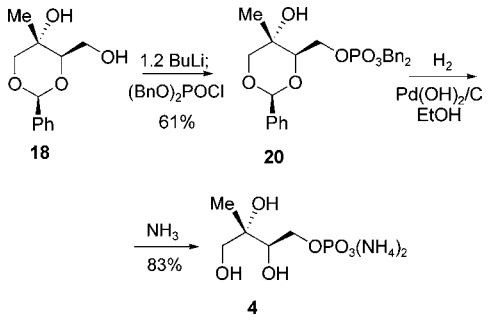
Scheme 3



Cleavage of the TBS protecting group (Bu_4NF, THF) afforded 1,3-benzylidene derivative **18**, mp 99−100 °C, and subsequent hydrogenolysis (H_2, 200 psi, $Pd(OH)_2$/C, EtOH, HCOOH) gave the parent C_5 tetrol, 2-*C*-methyl-D-erythritol (**19**), that had spectroscopic properties in agreement with those reported by Kis et al.[15]

Selective phosphorylation of the primary hydroxyl group of benzylidene diol **18** was effected by lithiation with BuLi (1.2 equiv, THF, −78 °C) and reaction with dibenzyl chlorophosphoridate (Scheme 4). The resulting monophos-

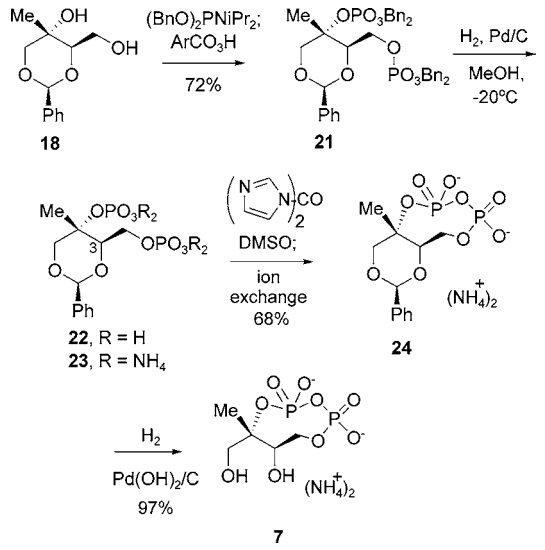
Scheme 4



phate **20**, mp 86−87 °C, underwent simultaneous hydrogenolysis of the benzyl and benzylidene protecting groups to form the phosphomonoester that was converted to the diammonium salt of MEP (**4**), $[\alpha]_D$ +11.3° (*c* 1.6, H_2O), with dry gaseous ammonia.

Double phosphorylation[27] of **18** by the phosphoramidite method led to the benzyl-protected diphosphate **21** (Scheme 5). Selective debenzylation (H_2, Pd/C, MeOH, −10 °C) gave

Scheme 5



bis-phosphomonoester **22**, which was characterized as its tetraammonium salt **23**. ^{13}C NMR spectra (126 MHz, D_2O) of **23** show three-bond $^{13}C-^{31}P$ coupling at the C-3 methine (85.6 ppm, dd, $^3J_{PC}$ = 10.5, $^3J_{P'C}$ = 8.1 Hz). Additional signals indicate the presence of one methyl (C-2′, 19.82 ppm), five benzylidene (104.68, 129.18, 131.47, 132.68, 139.04 ppm), and one quatermary and two methylene (65.60, 73.15, 78.35 ppm) carbons. The 2-*C*-methylerythritol part of the molecule is represented in the ^1H NMR spectrum (500 MHz, D_2O) by a singlet (2′-CH_3, 1.45 ppm), an AB pattern for the endocyclic methylene (3.92 and 4.13 ppm, J_{AB} = 10.8 Hz), and three clearly resolved signals (3.67, 4.00, 4.07 ppm) indicating the $POCH_2CH$ fragment.

We found that bisphosphate **22** is smoothly converted to cyclic diphosphate **24** under the influence of 1,1′-carbonyldiimidazole (anhyd DMSO, room temperature, 4 h).[28] The crude imidazolium salt was exchanged to the ammonium form (Dowex 50WX8-200) and purified by silica gel chromatography. The ^{31}P NMR spectrum of the product displays signals at −15.80 and −11.09 ppm as doublets with $^{31}P-^{31}P$ coupling (J = 25 Hz), confirming the presence of the phosphoryl anhydride linkage. The ^{13}C NMR spectrum reflects changes of the P−O−C−C dihedral angles by splitting of the C-2′ methyl (19.30 ppm, d, $^3J_{PC}$ = 6.4 Hz) and by disappearance of the strong $^{13}C-^{31}P$ coupling at C-3 (80.50 ppm). Final hydrogenolytic removal of the ben-

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zylidene group provided 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (**7**) as the diammonium salt, mp 220−222 °C (dec), $[\alpha]_D^{25}$ + 1.8° (*c* 1.00).

Benzylidene acetals **23** and **24** were also evaluated under conditions of acid-catalyzed hydrolysis. ^{1}H NMR kinetic experiments in HCOOH/D_2O demonstrated that cleavage of the benzylidene moiety of **23** is approximately two times faster than that of **24**. The diphosphate anhydride of **24** proved to be hydrolytically stable, and besides formation of **7**, no other products were observed in ^{1}H and ^{31}P NMR spectra. Thus, the hydrolytic lability of the benzylidene acetals can be utilized as an alternative method of deprotection.

In conclusion, this straightforward synthesis affords optically pure phosphates **4** and **7** in substantial amounts and satisfactory yields. The benzylidene protecting group proved to be stable to nucleophilic and basic conditions; its rigid structure favors cyclic diphosphate formation, and deprotections under neutral or weakly acidic conditions avoid facile cyclic phosphate rearrangements.[14] Schemes to access structural analogues and labeled forms[17] of **4**, **7**, and **19** can be readily devised. These compounds and labeled variants, like their mevalonate counterparts,[29] are certain to find useful applications in research on MEP pathway enzymes and terpene biosynthesis.

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Supporting Information Available: Details of experimental procedures, characterization data, and reproductions of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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