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ENANTIOMERIC SYNTHESIS OF 2-*C* -METHYL-D-ERYTHRITOL 2, 4-CYCLODIPHOSPHATE

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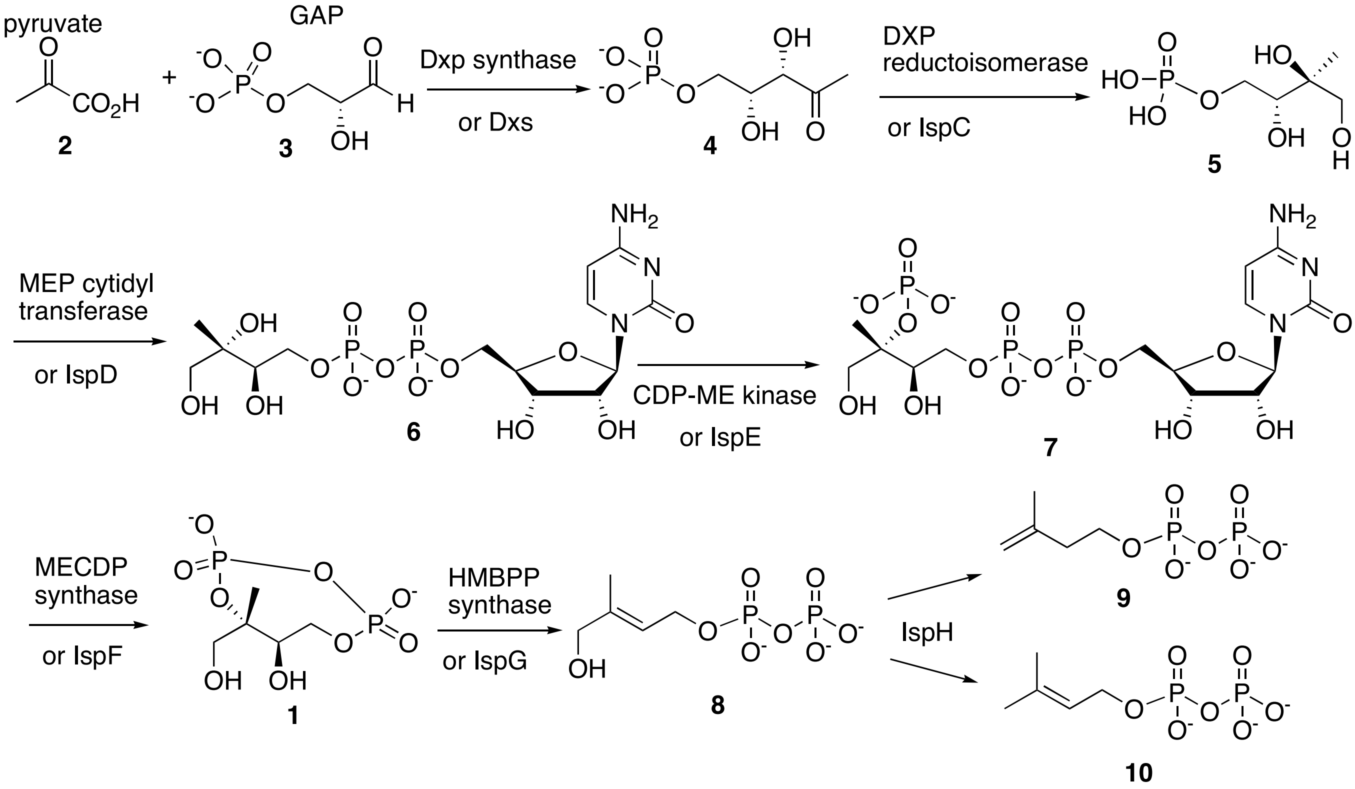
This paper is dedicated to Professor Ryoji Noyori, an inspirational scientist, on the occasion of his 70th birthday.

Abstract – Enantiomerically pure 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate **1** (ME-CPP) is synthesized from 1,2-*O*-isopropylidene-α-D-xylofuranose with facile phosphorylation in good yield. Subsequently, the synthesized enantiomerically pure **1** can be used as a substrate in IspG assays to identify inhibitors that may be developed into antibacterial drug leads.

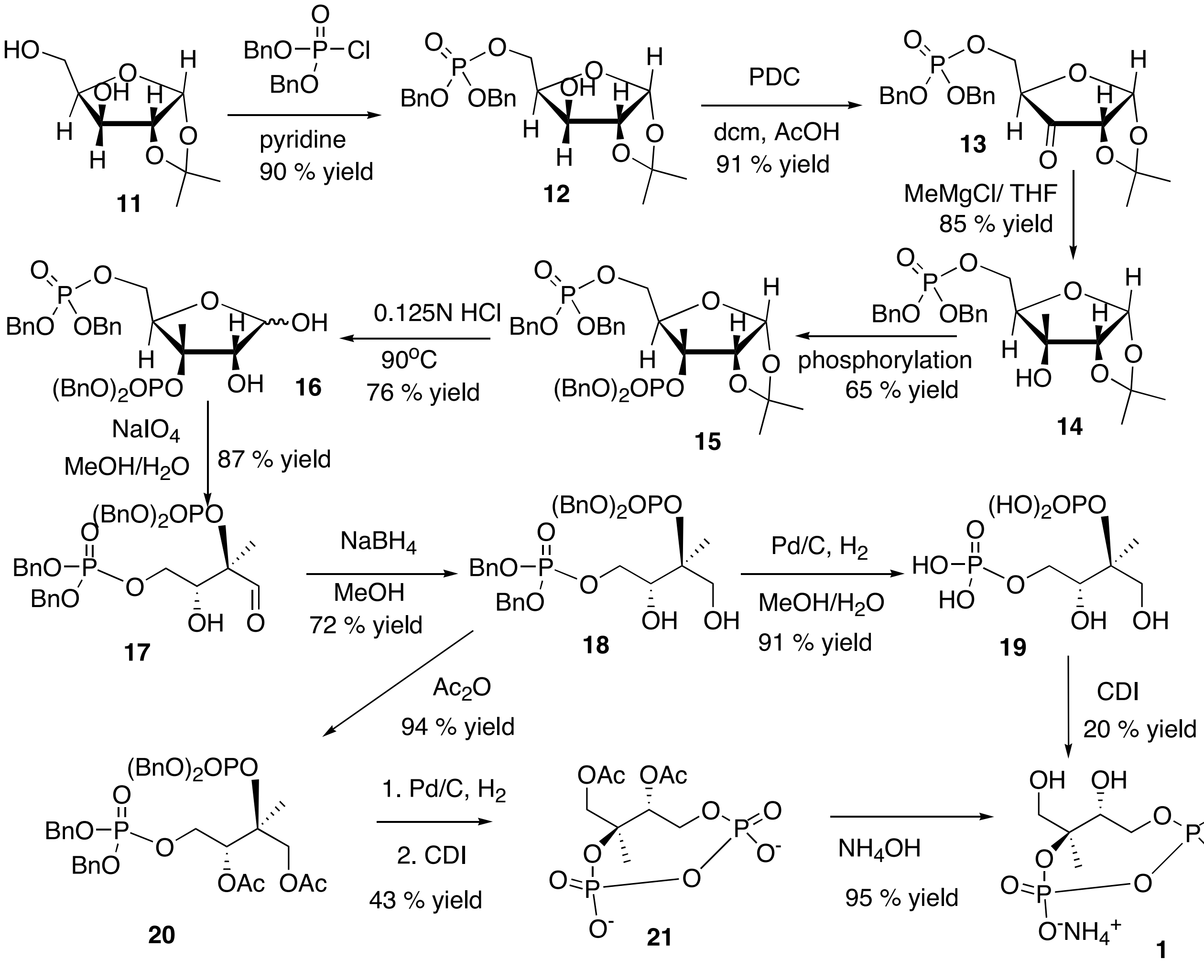
The World Health Organization estimates that 1 to 2 billion people are currently infected with tuberculosis (TB). Each year, 8 million people develop active disease and of these approximately 20% of the cases will be multiple drug resistant and 2% will be extensively drug resistant.[1a] In all, the annual death toll from TB is 2-3 million people worldwide.[1b] However, in spite of recent intensive research no new classes of anti-TB drugs have been developed for clinical use since the 1960's.[1] In this context, designing and developing a new drug is very important. To date, two different biosynthetic pathways leading to isopentenyl diphosphate and dimethylallyl diphosphate, the universal precursor of isoprenoids, have been reported. One is the mevalonate pathway,[2] first identified in mammals, and the other is the non-mevalonate or methyl erythritol phosphate (MEP) pathway (scheme 1) found in many bacteria, some protozoa and plants.[3] In the MEP pathway, 1-deoxy-D-xylulose 5-phosphate **4** (DXP) is made by condensing pyruvate **2** and glyceraldehyde 3-phosphate **3** catalysed by DXP synthase (DXS). Subsequently **5** is synthesized by intramolecular rearrangement and reduction of **4** with DXP reductoisomerase (IspC) enzyme. **5** is coupled with cytidine triphosphate (CTP) using MEP cytidyl transferase (IspD) to produce **6** as major product. **6** is subsequently phosphorylated by CDP-ME kinase (IspE) to form 4-diphosphocytidyl-2-*C*-methyl-D-erythritol- 2-phosphate **7** (CDP-ME2P) and cyclized by MECDP synthase (IspF) to form 2-*C*-methyl-D-erythritol 2, 4-cyclodiphosphate **1** (ME-CPP). The cyclic diphosphate is transformed into 1-hydroxy-2-methyl-2-*E*-butenyl 4-diphosphate **8** (HMBPP) by IspG and IspH (LytB) is utilized subsequently to generate isopentenyl diphosphate **9** (IPP) and dimethylallyl diphosphate **10** (DMAPP).



Scheme 1. MEP pathway of **9** and **10** biosynthesis.

Since the MEP pathway is not found in human cells it is considered as a good target for the development of antimicrobials, antimalarial and herbicidal agents, a hypothesis being explored by many researchers. However, a major impediment in this area is the lack of availability of pure substrates. Access to MEP pathway intermediates and their analogues are essential to ongoing biochemical investigations and development of new antibiotics targeting the respective enzymes. Recently we reported kinetic studies of mycobacterial DXS, IspC, IspD and IspE,[4] and the enantiomeric synthesis of **6**.[5] In order to extend our research to include IspG, we were in need of compound **1**. The reported chemical synthesis of **1** has numerous steps starting from commercially available substrate,[6] and the reported enzymatic synthesis of **1** is tedious, expensive and leads to very low yield.[7] Here in, we report the synthesis of enantiomerically pure **1**.

Interestingly, although reported for the synthesis of **5,** there is no procedure for synthesis of enantiomerically pure **1** from commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose **11**. To initiate the reactions leading to **1**, we extended the studies on the synthesis of **14** described in our last report.[4d,5] Dibenzyl phosphochloridate in pyridine was used to selectively protect the primary alcohol of commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose **11** to yield 5-dibenzylphosphate-1,2-*O*-isopropylidene-α-D-xylofuranose **12**. Then the free secondary alcohol **12** was oxidized to ketone **13** quantitatively with pyridinium dichromate and catalytic acetic acid. Dry toluene was added to **13** and evaporated three times prior to subsequent use (Scheme 2).[8]



Scheme 2. Enantiomeric synthesis of **1**.

Previously 3-*C*-alkyl ribofuranoses were obtained by a stereoselective addition reaction with an alkyl group on the β-face of the carbohydrate ring.[9] Accordingly, in the ketone **13**, addition of the methyl group occurs from the less hindered β-face, leading to the tertiary alcohol **14** with the desired stereochemistry. The tertiary alcohol **14** was phosphorylated using dibenzyl diisopropyl phosphoramidite and then oxidized to diphosphate **15**.[10]

Acetonide deprotection was carried out in 0.125 N hydrochloric acid at 90 °C giving rise to a 76 % yield of a mixture of two anomers **16** which underwent a sodium metaperiodate mediated glycol oxidative cleavage to the aldehyde **17** without further purification. The aldehyde was reduced using sodium borohydride to form alcohol **18,**[11] which was followed by hydrogenolysis in water/methanol medium without acid workup leading to **19** (Scheme 2). Further, **19** was stabilized by titrating to form the ammonium salt. Subsequently **19** was cyclized using *N*, *N'*-carbonyldiimidazole as reported in literature.[6] However we observed only 20 % yield due to the presence of the free hydroxyl groups in **19**. So, we utilized a different route, protecting the free hydroxyl groups of **18** with acetic anhydride leading to **20** which further underwent hydrogenolysis and cyclization to give protected ME-CPP **21**. Deprotection of **21** was carried out using ammonium hydroxide[6b] giving a 41 % yield for the cyclization and deprotection steps. **1** was characterized by HRMS, ^{1}H- NMR and found match with reported data and data generated with authentic **1**.[6]

In conclusion, we successfully synthesized enantiomerically pure **1**. Radiolabeling can be introduced during the methylation or reduction steps if required. Pure **1** can be used as a substrate for studying bacterial IspG activities and importantly for high throughput screening to identify IspG inhibitors.

ACKNOWLEDGEMENTS

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10. To 627 mg (1.35mmol) of alcohol **14** in 5 mL of dcm, 4 mmol of 0.45 M 1*H*-tetrazole solution was added at room temperature, followed by addition of 932 mg (2.7 mmol) of dibenzyl diisopropyl phosphoramidite. After 3 h of stirring, completion of reaction was checked by TLC and then cooled to 0°C. Oxidation was done by adding 280 mg (1.62 mmol) of *m*-chloroperbenzoic acid at 0°C. Reaction was stirred at room temperature for 5 h and then diluted with ether. Subsequently the organic phase was washed with 10 % sodium metabisulfite solution, saturated aq. sodium bicarbonate and brine solution. Organic mixture was dried with $MgSO_4$, solvents were evaporated at low pressure and the crude mixture was purified by flash column chromatography in silica gel using 80:20 (EtOAc : Hexane) eluent and gave 65 % yield of **15**. Data for **15**: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.32 (m, 20H), 5.72 (d, 1H, J = 3.3 Hz), 5.13 (d, 2H, J = 7.2 Hz), 5.04 (m, 6H), 4.58 (d, 1H, J = 3.6 Hz), 4.28 (dd, 1H, J = 3.9 & 7.2 Hz), 3.76 (m, 2H), 1.51 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H).; ^{13}C-NMR ($CDCl_3$, 75 MHz): 136.0, 135.9, 135.6, 135.5, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 113.0, 103.6, 83.7, 83.6, 83.1, 78.6, 78.5, 78.4, 78.3, 69.4, 69.3, 69.2, 69.1, 67.8, 65.1, 65.0, 60.3, 26.5, 20.9, 14.1.; IR (neat, cm^{-1}): 2965, 2360, 2332, 1651, 1619. HRMS (ESI) $C_{37}H_{42}O_{11}P_2Na$ (M+Na$^+$): calculated 747.2095 and found 747.2095; $[\alpha]_D$ 13.6 (c 0.25, $CHCl_3$).
11. Data for **18**: ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.36 (m, 20H), 5.05 (m, 8H), 4.06 (m, 2H), 3.75 (m, 1H), 3.59 (m, 1H), 3.52 (m, 1H), 1.19 (s, 3H).; ^{13}C-NMR ($CDCl_3$, 75 MHz):139.3, 139.2, 135.5, 128.7, 128.6, 128.0, 74.0, 73.9, 69.9, 69.7, 69.1, 68.8, 67.6, 66.8, 19.1; IR (neat, cm^{-1}): 3350, 2966, 2360, 2339, 1654, 1613. HRMS (ESI) $C_{33}H_{37}O_9P_2$ (M-OH): calculated 639.1907 and found 639.1946 ; $[\alpha]_D$ 3.0 (c 0.4, $CHCl_3$).