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Synthesis of Chirally Pure 1-Deoxy-D-Xylulose-5-Phosphate: A Substrate for Ispc Assay to Determine MTP inhibitor

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Abstract

1-Deoxy-D-xylulsose-5-phosphate (DXP) is a key intermediate in the non-mevalonateor methyl erythritol phosphate (MEP) pathway for the biosynthesis of isoprenoid, which are essential building blocks involved in the construction of pathogens growth. Since the homologous enzymes of this pathway are not present in vertebrates, including humans, the MEP pathway presents a viable source for antimicrobial drug targets. However, an insight into the features of the enzymes involved in this pathway has been plagued by lack of chirally pure substrates. Here in, we report an efficient synthesis of enantiomerically pure 1-deoxy-D-xylulose-5-phosphatefrom commercially available 1, 2-*O*-isopropylidene-α-D-xylofuranose through Weinreb amide formation in shorter route.

Introduction

Isoprenoids are important building blocks in the formation of cell wall, electron transfer process intermediates and many other necessary metabolites for the survival of bacteria [1]. Isoprenoids are also one of the most diverse classes of natural products with over 30,000 different compounds ranging from essential primary metabolites, secondary metabolites and intermediates [2]. Although, the diversity of structure and function, all isoprenoids are made from the five carbon precursors, isopentenyldiphosphate (IPP) and its isomer, dimethylallyldiphosphate (DMAPP), through polymerization or repetitive condensation. To date, two distinct pathways have been identified for the biosynthesis of IPP and DMAPP: the mevalonate pathway found in animals and the non-mevalonate or MEP pathway found in many bacteria, protozoa and plants [3]. Generally, in the MEP pathway, DXP is produced through DXS catalyzed condensation of pyruvate, 1 and glyceraldehyde-3-phosphate, 2. The DXP, 3 is then converted to 2-C-methyl-D-erythritol-4-phosphate, 4 (MEP), where IspC catalyzed reducto-isomerization takes place. Subsequently MEP is coupled with cytidine triphosphate (CTP) using IspD to produce 4-diphosphocytidyl-2-C-methyl-D-erythritol, 5 (CDPME), which is concurrently phosphorylated at tertiary hydroxyl group by IspE to synthesize cytidine diphosphate methyl erythritol-2- phosphate, 6 (CDPME2P). In order CDPME2P undergoes cyclization using IspF to give 2-C-methyl-D-erythritol 2,4-cyclodiphosphate, 7 (ME-CPP). The cyclic diphosphate is converted to 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate, 8 (HMBPP) by the enzyme IspG. IspH then catalyzes the conversion of HMBPP into IPP, 9 and DMAPP, 10 through elimination reaction (Figure 1) [4].

Recently, many research groups explore the MEP pathway as a promising drug target for developing new antimicrobials, antimalarials and herbicidal agents [5,6]. For instance, fosmidomycinis a phosphonate drug that targets the enzyme in the MEP pathway [7]. Although biosynthetic preparations of several MEP pathway intermediates have been reported [3], there are only few efficient organic synthetic methods available for those intermediates and rare chirally pure intermediates. A few years ago we developed a reliable route to MEP, 4 from 1,2-O-isopropylidene-α-D-xylofuranose [8]. Earlier our group has also reported the synthesis of chirally pure CDPME, 5, (4) CDPME2P, 6, (9) ME-CPP, 7 [9,10] in good yield. In that regard and in keeping with our objective to uncover the kinetics of the MEP enzymes, we sought to prepare chirally pure DXP, 3, the first intermediate in the MEP pathway through short route and enantiomerically pure form.

Materials and Methods

General methods

All reactions were performed under a dry argon atmosphere unless otherwise noted. Reagents were obtained from commercial sources and used directly. Flash chromatography was performed using flash silica gel (32-63 μ) from Dynamic Adsorbents Inc. Reactions were followed by TLC on precoated silica plates (250 μm, F-254 from SiliCycle Inc.). The compounds were visualized by UV fluorescence or by staining with anisaldehyde or $KMnO_4$ stains. NMR spectra were recorded on a Varian INOVA 500 spectrometer. Proton NMR data is reported in ppm downfield from TMS as an internal standard. Mass spectra were recorded using ESI.

Amide, 16

To a solution of the aldehyde 15 (283 mg, 0.602 mmol) in ^{t}BuOH (4 mL) at 0°C, were added sequentially 2, 3-dimethyl-2-butene (392 μL), $NaClO_2$ (86 mg) and $NaH_2PO_4.H_2O$ (86 mg dissolved in 700 μL of H_2O). The mixture was stirred and gradually warmed to rt over 3 h, then partitioned between ethyl acetate (30 mL) and 1 M HCl (20 mL). The aqueous layer was extracted further with ethyl acetate (4×30 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to give the carboxylic acid that was used in the next step without further purification. N,O dimethylhydroxylamine hydrochloride (116 mg, 1.196 mmol, 2.0 equiv.), Et_3N (250 μL, 182 mg, 1.794 mmol, 3.0 equiv.), and HATU (454 mg, 1.196 mmol, 2.0 equiv.) were added to a solution of the carboxylic acid residue (292 mg, 0.598 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) at 0°C under argon. The mixture was stirred and warmed to rt over 6 h, concentrated, and the product

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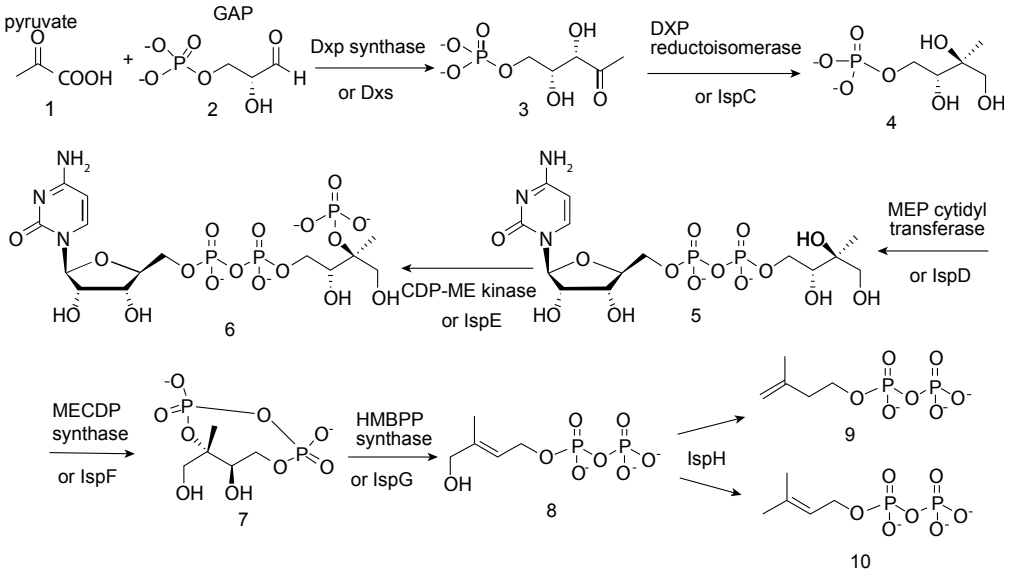

Figure 1: MEP pathway for synthesis of 9 and 10.

isolated by flash chromatography eluting with 70% EtOAc/Hex to give the Weinreb amide 16 as an oil (228 mg, 72%). Rf 0.38 (70% EtOAc/Hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.14 (s, 3H), 3.47 (s, 3H), 4.20-4.28 (m, 3H), 4.39 (d, *J*=11.5 Hz, 5H), 4.48 (br, 1H), 4.74 (d, *J*=11.5 Hz, 1H), 4.92-5.06 (m, 4H), 7.24-7.40 (m, 15 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 38.1, 61.3, 67.7, 69.4, 70.5, 72.0, 79.0, 127.6, 127.9, 128.2, 128.3, 128.4, 128.6, 135.9, 136.9, 169.3. MS (m/e) calcd for $C27H_{32}NO_8P$ (M + H)$^+$: 530.5020; obsd: 530.5016.

Ketone, 17

To a stirred solution of the amide 16 (80 mg, 0.151 mmol, 1.0 equiv.) in anhydrous THF (3 mL) at -78°C under argon was added MeLi (188 µL, 1.6 M in Et2O, 0.302 mmol, 2.0 equiv.). The mixture was stirred at this temperature for 1 h, then quenched with 0.5 M HCl (10 mL). The two layers were separated and the aqueous layer was extracted further with ethyl acetate (3×15 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography, eluting with 70% EtOAc/Hex to give 17 as colorless oil (50 mg, 68%). R_f0.44 (70% EtOAc/Hex). 1H NMR (500 MHz, $CDCl_3$) δ 2.19 (s, 3H), 3.90-4.20 (m, 3H), 4.42 (app. d, *J*=11.5 Hz, 1H), 4.66 (app. d, *J*=11.5 Hz, 1H), 4.94-5.12 (m, 6H), 7.20-7.60 (m, 15 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.5, 67.6, 69.6, 71.0, 73.5, 83.0, 127.9, 128.0, 128.3, 128.5, 128.6, 128.7, 135.9, 136.6, 209.4. MS (m/e) calcd for $C_{26}H_{29}O_7P$ (M + H) +: 485.1715; obsd: 485.1710.

DXP, 3

Pd/C (10% Pd, 12 mg) was added to a solution of 17(60 mg, 0.123 mmol) in MeOH/H_2O (1:1, 2 mL). The mixture was hydrogenated for 14 h, filtered through a pad of Celite and lyophilized with added $NaHCO_3$ (20 mg, 2 equiv.) to give the sodium salt of 3 as a colorless powder. MS (m/e) calcd for $C_5H_9O_7P$ (M + H)$^+$: 213.0164; obsd: 213.0168, $[\alpha]_D$=42.9 (c 0.5, H_2O).

Results and Discussion

Synthesis of chirally pure DXP, 3 was initiated from commercially available 1, 2-*O*-isopropylidene-α-D-xylofuranose, 11. However to phosphorylate the 11 we synthesized the phosphorylating reagent, dibenzylphosphochloridate, following a modified procedure of chlorinating dibenzylphosphite using N-chlorosuccinimide in toluene and benzene [8,10]. The primary hydroxyl group of 1,2-O-isopropylidene-α-D-xylofuranosewas selectively phosphorylated with the newly synthesized dibenzylphosphorochloridate in pyridine to give 5-dibenzylphosphate-1,2-O-isopropylidene-α-D-xylofuranose 12 in good yield (Figure 2). Secondary hydroxyl group in compound 12 was protected by activating the hydroxyl group by sodium hydride and benzylating using benzyl bromide in a pre-cooled mixture in acetonitrile in high yield. The protected xylofuranose, 13 also increased the solubility of the substrate in organic solvents used in the subsequent steps. Trifluoroacetic acid mediated acetonidede protection of 13 gave the corresponding two anomers of the vicinal diol14 that were routinely used in the next step without further purification. The vicinal diol in 14 was then introduced to undergo oxidative cleavage by sodium metaperiodate to give aldehyde 15 in high yield, which was immediately subjected to further oxidation using a mixture of sodium chlorite, 2,3-dimethyl-2-butene (scavenger) and buffer (sodium dihydrogen phosphate). Indeed, the above-mentioned conditions generated cleaner reactions compared to when the experiment was conducted in the absence of the scavenger and the buffer. We also explored oxidizing the aldehyde using bromine and buffered with sodium bicarbonate, nevertheless, partial oxidation of the alcohol in 15 reduced the chemical yield of the desired acid product.

Having synthesized the carboxylic acid in good yield, we sought to investigate the methods for ketone formation from the available carboxylic acid group. Weinreb amides have been extensively used for the preparation of ketones and their application in synthesis has been widely demonstrated [11]. We explored this strategy, to minimize the number of steps and the protecting group manipulations associated with the free hydroxyl group in 15. We also reasoned that nucleophilic addition of either organolithium or Grignard reagents on the amide would afford the corresponding ketone product. We therefore, prepared the Weinreb amide by coupling the free carboxylic acid with N-O-dimethylhydroxylamine hydrochloride using HATU in high yield. Subsequently, treatment of amide 16 with methyllithium

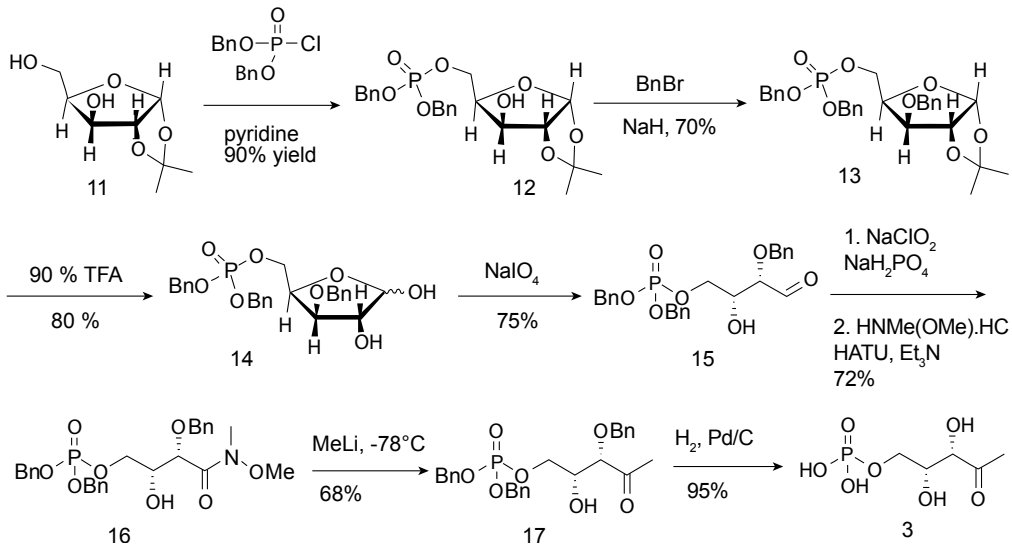

Figure 2: Synthesis of 3from 11using Weinreb amide.

in dry THF furnished compound 17 in excellent yield. Finally, DXP was synthesized by performing a global deprotection through hydrogenolysis with Pd/C and H2 affording the desired product in good yield and enantiomerically pure form that was stored as a sodium salt. Chirally pure compound 3, was analysed, characterized and confirmed by mass spectra and NMR. We also prepared some of the analogs of the DXP from a similar synthetic strategy and characterization and kinetic study of those compounds are in progress.

Conclusion

In summary, in continuation with the synthesis of chirally pure MEP pathway intermediates, we have demonstrated an effective method for the preparation of enantiomerically pure DXP, 3 from commercially available 1, 2-O-isopropylidene-α-D-xylofuranose, 11. The terminal carboxylic acid group was converted into methyl ketone on the final step via the Weinreb amide methodology in good yield, even without protection of the free hydroxyl group. Radiolabelled compounds can be also prepared on reduction step. However, studies on the kinetic properties of *Mycobacterium tuberculosis* DXS enzyme using compound 3and determination of IspC inhibitors against *Mycobacterium tuberculosis* will be reported in due course.

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