Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Synthesis and evaluation of stable substrate analogs as potential modulators of cyclodiphosphate synthase IspF.

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SUPPORTING INFORMATION

Table of contents

General methods. All reagents and chemicals used were purchased from commercial sources and used without further purification. Dynamic Adsorbents 32 – 63 μm silica gel was used for flash column chromatography and 250 μm w/h F254 plates were used for thin layer chromatography (TLC). TLC plates were developed and visualized by staining with CAM (1% ceric ammonium nitrate and 2.5% ammonium molybdate in 10% sulfuric acid). ^{1}H and ^{31}P NMR spectra were recorded on Bruker 400 MHz, Varian 400 MHz or 500 MHz spectrometers. Chemical shifts (δ) are reported in parts per million. Beckman Coulter® System Gold HPLC equipped with low-retention PEEK tubing was used for HPLC analysis.

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Figure S1: **The methyl-D-erythritol 4-phosphate (MEP) pathway** for the biosynthesis of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) beginning from pyruvate and D-glyceraldehyde 3-phosphate (GAP). DXP is then converted to 2*C*-methyl-D-erythritol 4-phosphate (MEP) by the reductoisomerase IspC which represents the first committed step of the MEP pathway. MEP undergoes cytidylation by IspD and phosphorylation by IspE to form 4-diphosphocytidyl-2*C*-methyl- D-erythritol 2-phosphate (CDPME2P). IspF catalyzes the conversion of CDPME2P to the cyclic diphosphate 2*C*-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP) with concomitant release of CMP. MEcPP undergoes reductive ring opening catalyzed by IspG to form linear diphosphate (*E*)-4-hydroxy-3-methylbut-2-enyl pyrophosphate (HMBPP) which is finally converted into IPP and DMAPP by the reductase IspH.

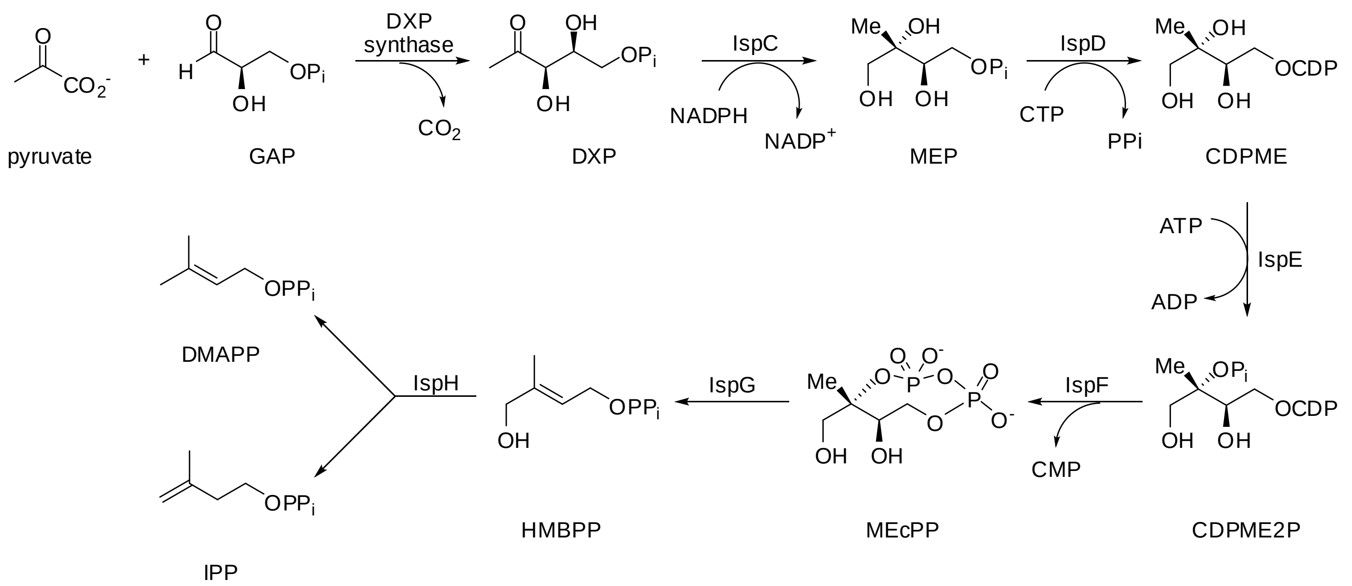
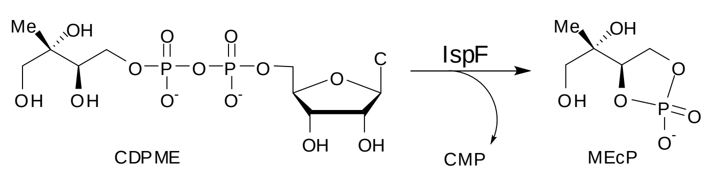
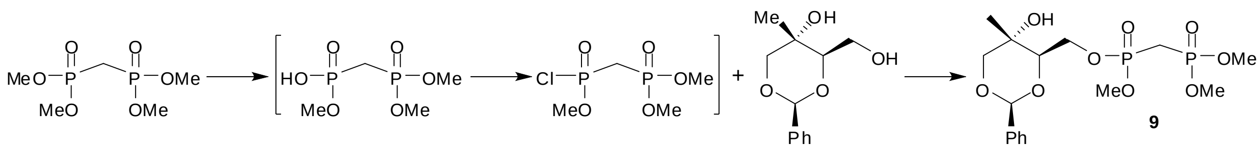


Figure S2: **IspF catalyzed formation of MEcP from CDPME.**[2]

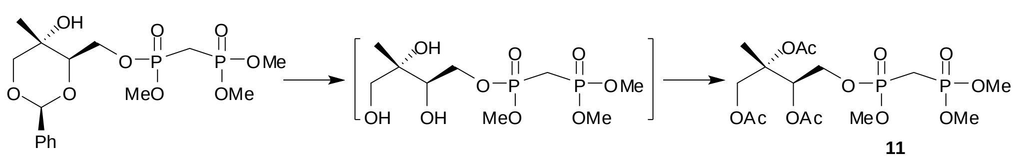


Measuring IspF-catalyzed CMP formation. *E. coli* IspF was purified and kinetically characterized as previously described.[1] For assays to measure the rate of CMP formation, IspF reactions contained 50 mM phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate (100 μM), 50 nM IspF and 50 μg/mL bovine serum albumin (BSA) in a total volume of 160 μL. *HPLC Sample preparation and analysis:* To terminate the IspF reaction, 40 μL of reaction mixture was added to 80 μL of cold 0.1% SDS at 2, 4 and 6 minutes. Quenched mixtures were briefly vortexed and incubated on ice for 15 minutes. To remove proteins prior to HPLC analysis, the quenched reaction mixture was passed through 3K MWCO (molecular weight cut off) Nanosep® centrifugal devices from Pall® Corporation. Samples (90 μL) were injected onto a Beckman HPLC equipped with low-retention PEEK tubing to reduce sample-to-metal interaction and analyzed by reversed-phase ion-pair HPLC using an Altima C18 3 μ, 53 × 7 mm Rocket® column. The column was developed with a linear gradient of 0 to 100% B at a flow rate of 3 mL/min (where A = 100 mM phosphate buffer, 5 mM tetrabutyl ammonium bisulfate, pH 6.0 and B = 100 mM phosphate buffer, 5 mM tetrabutyl ammonium bisulfate in 30% acetonitrile (Retention times: CMP = 1.10 minutes & CDPME2P = 3.53 minutes). The CMP and CDPME2P peak areas were measured, and the concentration of CMP was calculated as a fraction of the total peak area.

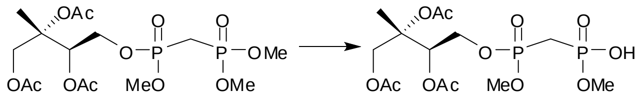
Synthesis of 4-bisphosphonocytidyl-2C-methyl-D-erythritol 2, CBPME and 4-bisphosphonocytidyl-2C-methyl-D-erythritol 2-phosphate, CBPME2P.



[(2S,4R,5S)-5-hydroxy-5-methyl-2-phenyl-1,3-dioxan-4-yl]methyl methyl[(dimethoxyphosphoryl)methyl]phosphonate (9). To tetramethyl bisphosphonate (1.00 g, 4.31 mmol) in 11 mL THF was added PhSH (522 mg, 484 μL, 4.74 mmol) and Et_3N (653 mg, 899 μL, 6.47 mmol), and the mixture was stirred at ambient temperature for 14 h. The volatiles were removed under reduced pressure, and the resultant ammonium salt was partitioned in 5 mL 5% HCl and 10 mL CH_2Cl_2. The layers were separated, and the water layer was washed 2 × 10 mL CH_2Cl_2 and passed through H^+-form DOWEX 50X8-200 resin. Solvents were removed *in vacuo* to give 845 mg of the crude trimethylbisphosphonic monoacid, which was used without further purification. Crude trimethylbisphosphonic monoacid (830 mg, 3.81 mmol) in 5 mL CH_2Cl_2 was added dropwise to a solution of Ghosez's reagent (557 mg, 551 μL, 4.15 mmol)[3,4] in 12 mL CH_2Cl_2 at 40°C, and the mixture was stirred for 5 minutes. The mixture was cooled to 0°C. To this mixture was added Hunig's base (1.34 g, 1.80 mL, 10.4 mmol) followed by 1,3-benzyledene-2*C*-methyl-D-erythritol[5] (775 mg, 3.46 mmol) and cat. DMAP in one portion. After 5 h, the mixture was quenched with 3 mL sat. NH_4Cl, diluted with 30 mL EtOAc, and the layers were separated. The water layer was extracted 4 × 25 mL EtOAc, and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash column chromatography using 5:95 MeOH:EtOAc to give 961 mg (65%) of the desired product as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$, 400MHz) for diastereomers δ 7.49 (m, 2H); 7.36 (m, 3H); 5.54 and 5.53 (s, 1H); 4.56 and 4.37 (m, 1H), 4.34 and 4.07 (m, 2H), 4.18 and 4.06 (m, 1H), 3.89 (m, 1H), 3.78 (m, 9H), 2.49 (m, 2H), 1.46 and 1.44 (s, 3H). **^{31}P NMR** (162MHz) for diastereomers δ –3.77 (d, *J* = 18.31 Hz and 7.32 Hz, 1P), –4.20 and –4.26 (d, *J* = 18.31 Hz and 7.32 Hz, 1P). **HRMS (ESI)**: Calcd. for $C_{16}H_{27}O_9P_2$: *m/z* 425.1125 $[M+H]^+$; Found 425.1130

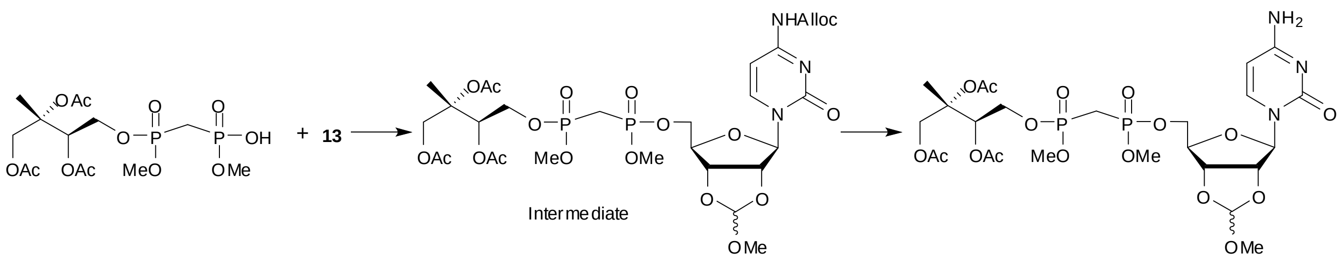


(2R,3S)-3,4-bis(acetyloxy)-1-({[(dimethoxyphosphoryl)methyl](methoxy)phosphoryl}oxy)-3-methylbutan-2-yl acetate (11). 1,3-benzylidene-2*C*-methyl-D-erythritol 4-trimethylbisphosphonate ester (240 mg, 0.57 mmol) in 5 mL MeOH was added to 10% Pd-C (72 mg) (pre-wet with 0.1 mL CH_2Cl_2) and hydrogenolyzed at 70-80 p.s.i. for 2hrs. The heterogeneous mixture was filtered, concentrated and re-dissolved in 2 mL CH_2Cl_2. To this mixture was added acetic anhydride (520 mg, 481 μL, 5.09 mmol) followed by Hunig's base (657 mg, 885 μL, 5.09 mmol) and cat. DMAP, and the mixture was stirred at 50°C for 3 h. The mixture was concentrated and purified by flash column chromatography using 10:90 MeOH:EtOAc to give 213 mg (82%) of a yellowish oil as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$, 400MHz) for diastereomers δ 5.50 (m, 1H), 5.53 (d, J = 12.47 Hz, 1H), 4.46 (m, 1H), 4.29 (dd, J = 12.46 and 2.2 Hz, 1H), 4.23 (m, 1H), 3.80 (m, 9H), 2.44 (m, 2H), 2.10 (s, 3H), 2.06 (s, 3H), 2.02 (m, 3H), 1.52 and 1.51 (s, 3H). **^{31}P NMR** (162MHz) δ –3.97 (m, 2P). **HRMS (ESI)**: Calcd. for $C_{15}H_{29}O_{12}P_2$: *m/z* 463.1129 $[M+H]^+$; Found 463.1134



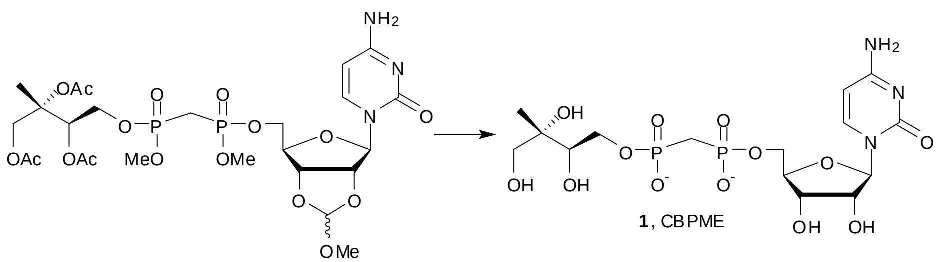
12

1,2,3-triacetoxy-2*C*-methyl-D-erythritol 4-*P*[1],*P*[2]-dimethyl-methylenebisphosphonic monoacid (**12**). To a mixture of triacetoxy methylerythrityl trimethylbisphosphonate ester (380 mg, 0.82 mmol) in 2 mL THF was added PhSH (136 mg, 126 μL, 1.23 mmol) followed by Et_3N (208 mg, 286 μL, 2.06 mmol), and the mixture was stirred at ambient temperature for 24 h. The solvents were removed under reduced pressure, and the crude material was purified by flash chromatography using 12:87:1 MeOH: CH_2Cl_2: Et_3N. The resultant ammonium salt was passed through 2 g of H^+-form DOWEX-50WX8-200, washed through with MEOH and concentrated to give (200 mg) 76% of the desired compound as a free acid. **^{1}H NMR** ($CDCl_3$, 400MHz) for diastereomers δ 7.49 (br s, O<u>H</u>, Exch. D_2O, 1H), 5.49 (m, 1H), 4.53 (d, *J* = 12.47, 1H), 4.42 (m, 1H), 4.30 (d, *J* = 11.73, 1H), 3.80 (m, 6H), 2.55 (m, 2H), 2.11 and 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.54 (s, 3H). **^{31}P NMR** (162MHz) δ –2.01 to –5.57 (m, 2P). **HRMS (ESI)**: Calcd. for $C_{14}H_{27}O_{12}P_2$: *m/z* 449.0972 $[M+H]^+$; Found 449.0968



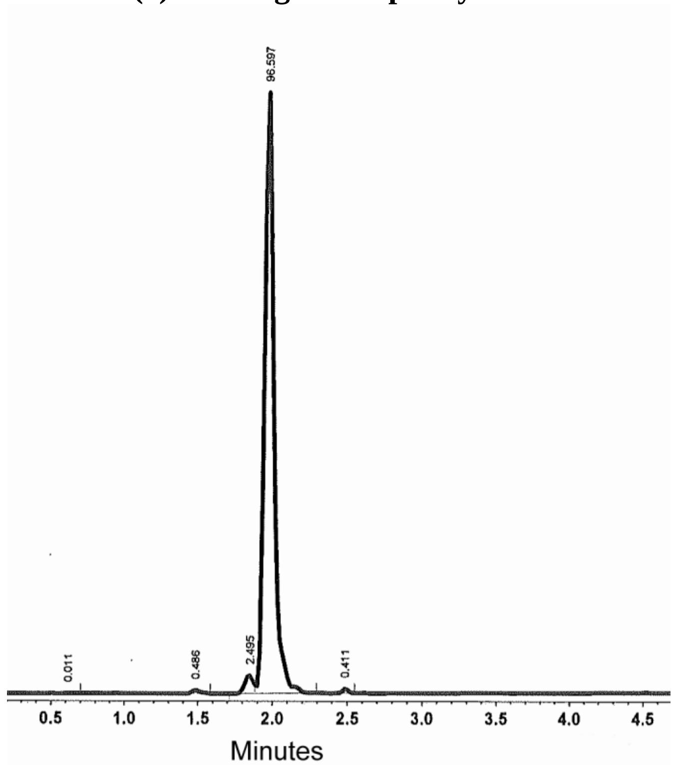
(2R,3S)-3,4-bis(acetyloxy)-1-{[methoxy({[methoxy({[(4R,6R)-2-methoxy-6-(2-oxo-4-{[(prop-2-en-1-yloxy)carbonyl]amino}-1,2-dihydropyrimidin-1-yl)-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy})phosphoryl]methyl})phosphoryl]oxy}-3-methylbutan-2-yl acetate (Intermediate). To triacetoxy methylerythrityl dimethylbisphosphonic monoacid (246 mg, 0.55 mmol), protected cytidine[6-9] (203 mg, 0.55 mmol) and PhP_3 (187 mg, 0.71 mmol) in 5.5 mL THF was added DIAD (144 mg, 140 μL, 0.71 mmol) dropwise, and the mixture was stirred for 5 h. The reaction mixture was concentrated and purified by flash chromatography using 7:93 MeOH:EtOAc to yield 343 mg (78%) of the desired intermediate compound as a complex mixture of diastereomers. **^{1}H NMR** ($CDCl_3$, 400MHz) δ 7.80 (m, 1H), 7.24 (m, 1H), 5.98 and 5.90 (s, C<u>H</u>OMe, 1H), 5.93 (m, 1H), 5.73 (m, 1H), 5.49, (m, 1H), 5.36 (d, *J* = 17.60, 1H), 5.29 (d, *J* = 10.27, 1H), 5.28 – 4.97 (m, 2H), 4.67 (d, *J* = 5.87, 1H), 4.61 – 4.06 (m, 8H), 3.79 (m, 6H), 3.40 and 3.30 (s, CHO<u>Me</u>, 3H), 2.47 (m, 2H), 2.10 (m, 3H), 2.06 (m, 3H), 2.02 (m, 3H), 1.51 (m, 3H). **^{31}P NMR** (162MHz) δ –3.91 to –4.90 (m, 2P). **HRMS (ESI)**: Calcd. for $C_{29}H_{44}N_3O_{19}P_2$: *m/z* 800.2039 $[M+H]^+$; Found 800.2054

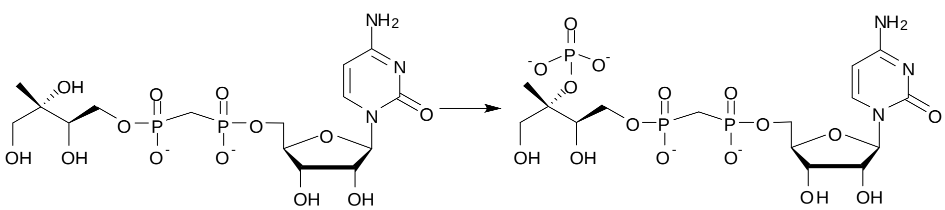
(2R,3S)-3,4-bis(acetyloxy)-1-({[({[(4R,6R)-6-(4-amino-2-oxo-1,2-dihydropyrimidin-1-yl)-2-methoxy-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy}(methoxy)phosphoryl)methyl](methoxy)phosphoryl}oxy)-3-methylbutan-2-yl acetate (14). To a mixture of the intermediate (186 mg, 0.23 mmol) and $Pd(Ph_3P)_4$ (11 mg, 0.009 mmol) in 1.6 mL THF was added pTS$O_2^-Na^+$ (46 mg, 0.26 mmol) followed by 0.6 mL H_2O. After 30 minutes, the solvents were removed *in vacuo*, and the resultant crude material was chromatographed using 7:92:1 MeOH:CH_2Cl_2:Et_3N to give 133 mg (80%) of the desired product as a complex mixture of diastereomers. **^{1}H NMR** ($CDCl_3$, 400MHz) δ 7.50 (m, 1H), 6.11 (m, 1H), 5.96 and 5.88 (s, C<u>H</u>OMe, 1H), 5.74 to 5.44 (m, 2H), 5.22 to 4.89 (m, 2H), 4.57 to 4.06 (m, 8H), 3.77 (m, 6H), 3.37 and 3.28 (s, CHO<u>Me</u>, 3H), 2.51 (m, 2H), 2.08 (m, 3H), 2.05 (m, 3H), 2.01 (m, 3H), 1.51 (m, 3H). **^{31}P NMR** (162MHz) δ –3.79 to –4.81 (m, 2P). **HRMS (ESI)**: Calcd. for $C_{25}H_{40}N_3O_{17}P_2$: *m/z* 716.1827 $[M+H]^+$; Found 716.1827



4-bisphosphonocytidyl-2*C*-methyl-D-erythritol, CBPME (1). Compound **14** (30 mg, 0.042 mmol), PhSH (46 mg, 0.42 mmol, 45 μL) and Et_3N (42 mg, 0.42 mmol, 58 μL) in 0.40 mL DMF was heated at 70°C for 9 days. Volatiles were removed under reduced pressure, and the resultant crude material was dissolved in 10% MeOH/H_2O (5 mL). The pH of the solution was adjusted to 2.0 using 1.0 M HCl, and the mixture was stirred for 24 h. The solvents were removed under reduced pressure, and the crude mixture was dissolved in 2 mL of a 2:1:0.5 mixture of MeOH:H_2O:Et_3N. After 18 hours, the solvents were removed by rotary evaporation, and the crude mixture was purified by reversed-phase ion-pair HPLC. *Reversed phase ion-pair HPLC chromatography*. This was accomplished using a Varian Dynamax C_{18} 250 × 21.4 mm prep column. The column was developed with a linear gradient of 0 to 30% B over 50 minutes at a flow rate of 10 mL/min (where A = 100 mM ammonium acetate buffer, 5 mM tetrabutyl ammonium bisulfate, pH 6.0, and B = acetonitrile containing 5 mM tetrabutyl ammonium bisulfate). Fractions containing the desired compound (λ_{max} = 272 nm, from 17.0 to 20.6 minutes) were combined and concentrated under reduced pressure to remove organic solvent, and the resultant solution was subjected to ion exchange chromatography to convert the product to the ammonium form, using 8 g of NH_4^+-form DOWEX WX8-200 resin. The resultant solution was lyophilized to give 9 mg (39%) of the desired compound as a white powder. **^{1}H NMR** ($CDCl_3$, 400MHz) δ 8.21 (d, J = 8.07 Hz, 1H), 6.24 (d, J = 8.07 Hz, 1H), 5.86 (d, J = 2.93 Hz, 1H), 4.30 (br, s, 1H), 4.23 (br, s, 1H), 4.19 (br, s, 2H), 4.10 (br, s, 2H), 3.87 (m, 1H), 3.75 (m, 1H), 3.53 (ν_A of ABq, d, J = 11.73 Hz, 1H), 3.39 (ν_B of ABq d, J = 11.73, 1H), 2.18 (t, 2H), 1.07 (s, 3H). **^{31}P NMR** (162MHz) δ –7.65 (br s, 2P). **HRMS (ESI)**: Calcd. for $C_{15}H_{28}N_3O_{13}P_2$: *m/z* 520.1092 $[M+H]^+$; Found 520.1097. **HPLC analysis**: An analytical sample of **1** was analyzed by reversed-phase ion-pair HPLC using an Altima C18 3 μ, 53 × 7 mm Rocket® column. The column was developed with a linear gradient of 0 to 100% B at a flow rate of 3 mL/min (where A = 100 mM phosphate buffer, 5 mM tetrabutyl ammonium bisulfate, pH 6.0 and B = 100 mM phosphate buffer, 5 mM tetrabutyl ammonium bisulfate in 30% acetonitrile). The desired compound was >95% pure as shown below.

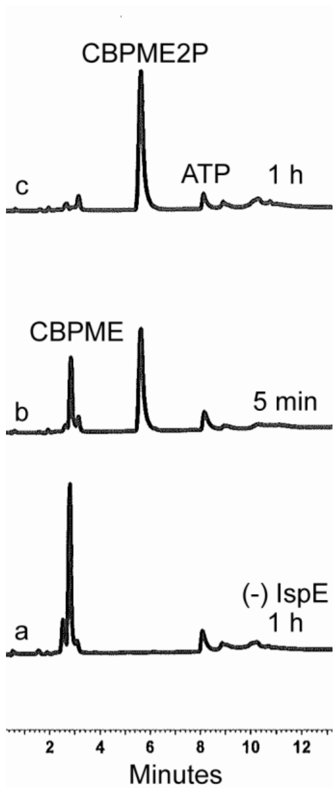
HPLC chromatogram of CBPME (1) showing >95% purity.



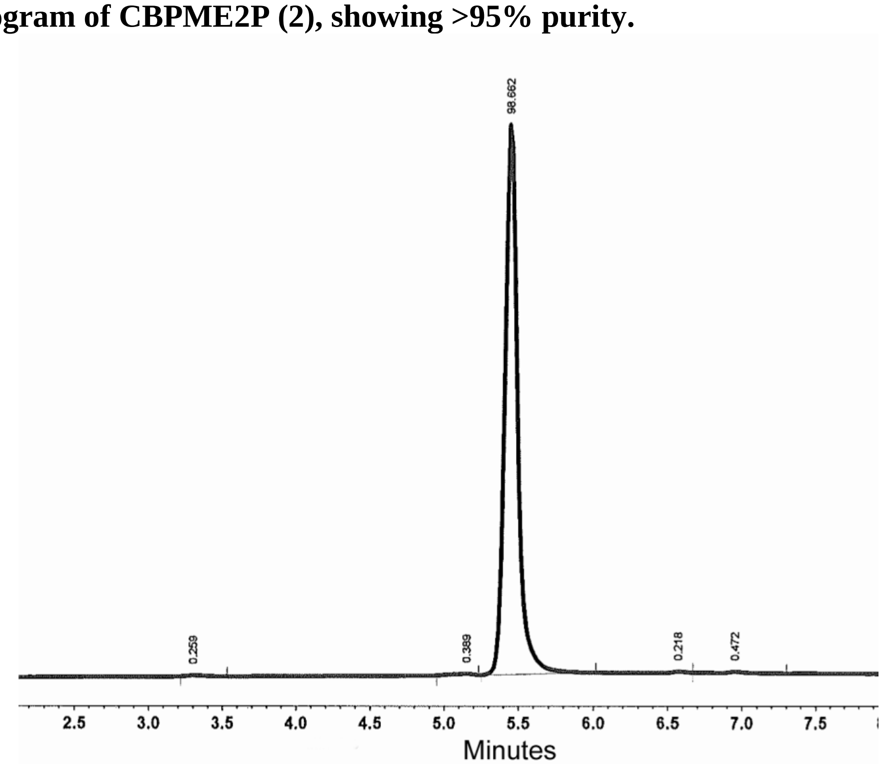


4-bisphosphonocytidyl-2*C*-methyl-D-erythritol 2-phosphate, CBPME2P (2). A mixture **1** (10 mM), ATP (1 mM), phosphoenol pyruvate (15 mM), $MgCl_2$ (2.5 mM), pyruvate kinase (3.5 units) and IspE (10 μM) in Tris pH 8.0 in a final volume of 744 μL was incubated at 37°C for 1 h, and the reaction progress was monitored by HPLC as shown below (Figure S4). After 1 h 10 min, the reaction was quenched with 700 μL MeOH, mixed by vortexing and incubated on ice for 10 min. The white precipitate was removed by centrifugation followed by removal of MeOH under reduced pressure. The resultant solution was purified by reversed phase ion-pair HPLC chromatography as described above. Fractions collected at λ_{max} = 272 nm, from 28.7 to 41.4 minutes. Yield: 2.1 mg (43%). **^{1}H NMR** ($CDCl_3$, 400MHz) δ 7.98 (d, *J* = 7.33 Hz, 1H), 6.10 (d, *J* = 7.33 Hz, 1H), 5.88 (d, *J* = 3.66 Hz, 1H), 4.27 (m, 2H), 4.16 (m, 2H), 4.07 (m, 1H), 3.86 (m, 1H), 3.69 (m, 1H), 3.66 (m, 1H), 3.57 (ν_A of ABq, d, *J* = 11.72 Hz, 1H), 3.47 (ν_B of ABq d, *J* = 11.72, 1H), 2.15 (t, 2H), 1.1.29 (s, 3H). **^{31}P NMR** (162MHz) δ –7.84 (br s, 2P), –28.31 (br s, 1P) **HRMS (ESI)**: Calcd. for $C_{15}H_{29}N_3O_{16}P_3$: 600.0761 *m/z* $[M+H]^+$; Found 600.0769. **HPLC analysis**: An analytical sample of **2** was analyzed as described for compound **1**. The desired compound was >95% pure as shown below. Compound **2** was also co-injected with the IspF substrate, CDPME2P, and exhibits similar chromatographic properties compared to CDPME2P as shown below.

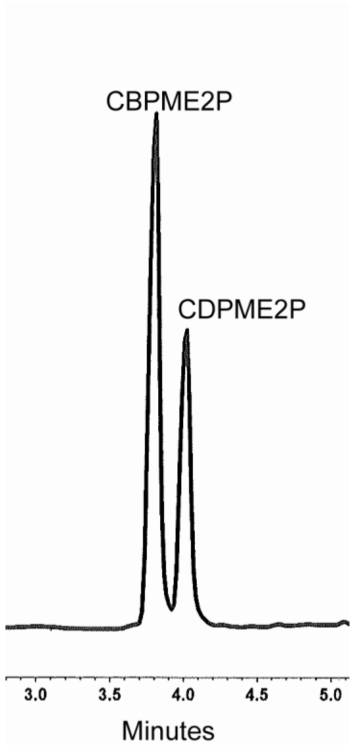
Figure S3. IspE-catalyzed phosphorylation of CBPME (1) to form CBPME2P (2).



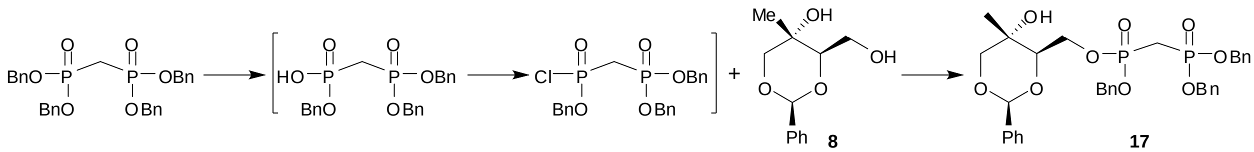
HPLC chromatogram of CBPME2P (2), showing >95% purity.



CDPME2P and CBPME2P (2), compared by HPLC co-injection, exhibit similar chromatographic properties.

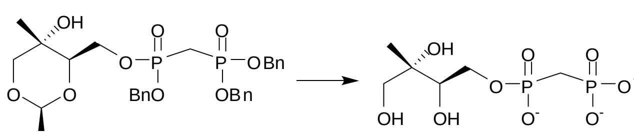


Synthesis of 2C-methyl-D-erythritol 4-bisphosphonic acid (MEBP)



8   17

Dibenzyl{[(benzyloxy)({[(2S,4R,5S)-5-hydroxy-5-methyl-2-phenyl-1,3-dioxan-4-yl]methoxy})phosphoryl]methyl}phosphonate (17). Methylene dibenzylbisphosphonate (2.40 g, 4.48 mmol)[10,11] and quinuclidine (497 mg, 4.48 mmol) in 15 mL toluene was refluxed for 2 hours. The reaction mixture was cooled to room temperature, diluted with 75 mL CH_2Cl_2 and washed 2 × 15 mL with 5% HCl. The organic layer was dried over Na_2SO_4, concentrated and dried and under high vac. The resultant oil (1.91 g) was used for the next step without further purification. A solution of tribenzylbisphosphonic mono acid (1.91 g, 4.28 mmol) in 4 mL CH_2Cl_2 was added dropwise over two minutes to a solution of Ghosez's reagent[3,4] (631 mg, 4.71 mmol) in 17 mL CH_2Cl_2 at 40˚C. The mixture was stirred at 40˚C for 10 minutes then cooled to room temperature, and *i*-Pr_2NEt (1.60 g, 12.84 mmol) was added in one portion followed by diol **8** (959 mg, 4.28 mmol) and cat. DMAP. After 2 hours, ^{31}P NMR showed consumption of the bisphosphonate. The reaction mixture was diluted with 10 mL CH_2Cl_2, washed 2 × 5 mL sat. NH_4Cl, dried over Na_2SO_4 and concentrated. The crude material was purified by flash chromatography using 70:30 EtOAc:Hexanes (elutes Ghosez's reagent by-product) then 3% MeOH in 1:1 EtOAc:CH_2Cl_2 to afford 1.47 g (53%) of the desired compound as a mixture of diastereomers. **^{1}H NMR** ($CDCl_3$, 400MHz) δ 7.45-7.23 (m, 20H), 5.46 and 5.47 (s, 1H), 5.20-4.88 (m, 6H, ArC<u>H$_2$</u>), 4.50 (m, 1H), 4.26 (m, 1H), 4.10 (m, 1H), 3.99 (m, 1H), 3.89 and 3.87 (d, *J* = 10.63 Hz, 1H), 3.68 and 3.66 (d, *J* = 11.00 Hz, 1H), 2.49 (mt, 2H), 1.43 and 1.41 (s, 3H). **^{31}P NMR** (162MHz) δ –4.96 to –5.44 (m, 2P). **HRMS (ESI)**: Calcd. for $C_{34}H_{39}O_9P_2$: *m/z* 653.2064 $[M+H]^+$; Found 653.2054



3, MECBP

2*C*-methyl-D-erythritol 4-bisphosphonic acid trisammonium salt, MEBP (3): To a solution of **17** (80 mg, 0.189 mmol) in 5 mL MeOH was added wet 10% Pd-C catalyst (24 mg), and the mixture was subjected to Parr shaker hydrogenolysis at 70-80 psi for 2.5 hrs. The mixture was filtered through filter paper and rinsed with MeOH. Solvents were removed *in vacuo* to yield 51 mg of crude colorless oil. The crude reaction mixture was purified by silica gel chromatography using 7:93 MeOH:CH_2Cl_2 to give 41 mg (66%) of bisphosphonate as a 1:1 mixture of diastereomers. **^{1}H NMR** (D_2O, 400MHz) (diastereomeric mix) δ 4.40 (m, 1H); 4.15 (m, 1H); 3.82 (m, 9H); 3.81 (t, 1H); 3.55 (ABq, 11.12 Hz, 1H); 3.42 (ABq, 11.12 Hz, 1H); 2.84 (m, 2H); 1.11 (s, 3H). **^{31}P NMR** (162MHz) (diastereomeric mix) δ –0.86 and –0.91 (d, *J* = 6.05 Hz and 5.69 Hz, 1P); –1.82 and –2.18 (d, *J* = 5.69 Hz, 1P). **HRMS (ESI)**: Calcd. for $C_9H_{22}O_9P_2Na$: *m/z* 359.0631 $[M+Na]^+$; Found 359.0637

Table S1: **Rates of CMP formation in the presence of CDPME, CBPME or CBPME2P**. Tabulated and normalized rates of CMP formation in the presence of 4-diphosphocytidyl-2*C*-methyl-D-erythritol 4-phosphate (CDPME, 500 μM), 4-bisphosphonocytidyl-2*C*-methyl-D-erythritol (CBPME, 500 μM) and 4-bisphosphonocytidyl-2*C*-methyl-D-erythritol 2-phosphate (CBPME2P, 500 μM). Enzyme reactions were initiated with IspF (0 min pre-incubation) or substrate (30 min pre-incubation), in the presence or absence of additives.

	[a]Time, (min)	Rate, μM • min^{-1}
Control	0	1.10 ± 0.15
	30	0.12 ± 0.04
[b]CDPME	0	1.24 ± 0.26
	30	0.19 ± 0.02
[b]CBPME	0	2.40 ± 0.12
	30	0.03 ± 0.03
[b]CBPME2P	0	1.52 ± 0.10
	30	0.91 ± 0.06
[b]MEP	0	2.25 ± 0.13
	30	1.99 ± 0.11
[b,c]CDPME	0	1.92 ± 0.10
	30	1.90 ± 0.03
[b,c]CBPME	0	3.01 ± 0.01
	30	2.49 ± 0.60
[b,c]CBPME2P	0	1.77 ± 0.28
	30	1.26 ± 0.12

(Brackets: Control 0 vs 30: ***; Control 30 / CBPME 0 vs CBPME2P 30: ****; MEP 0 vs 30: **; MEP 30 / CBPME 0 vs CBPME2P 30: **)

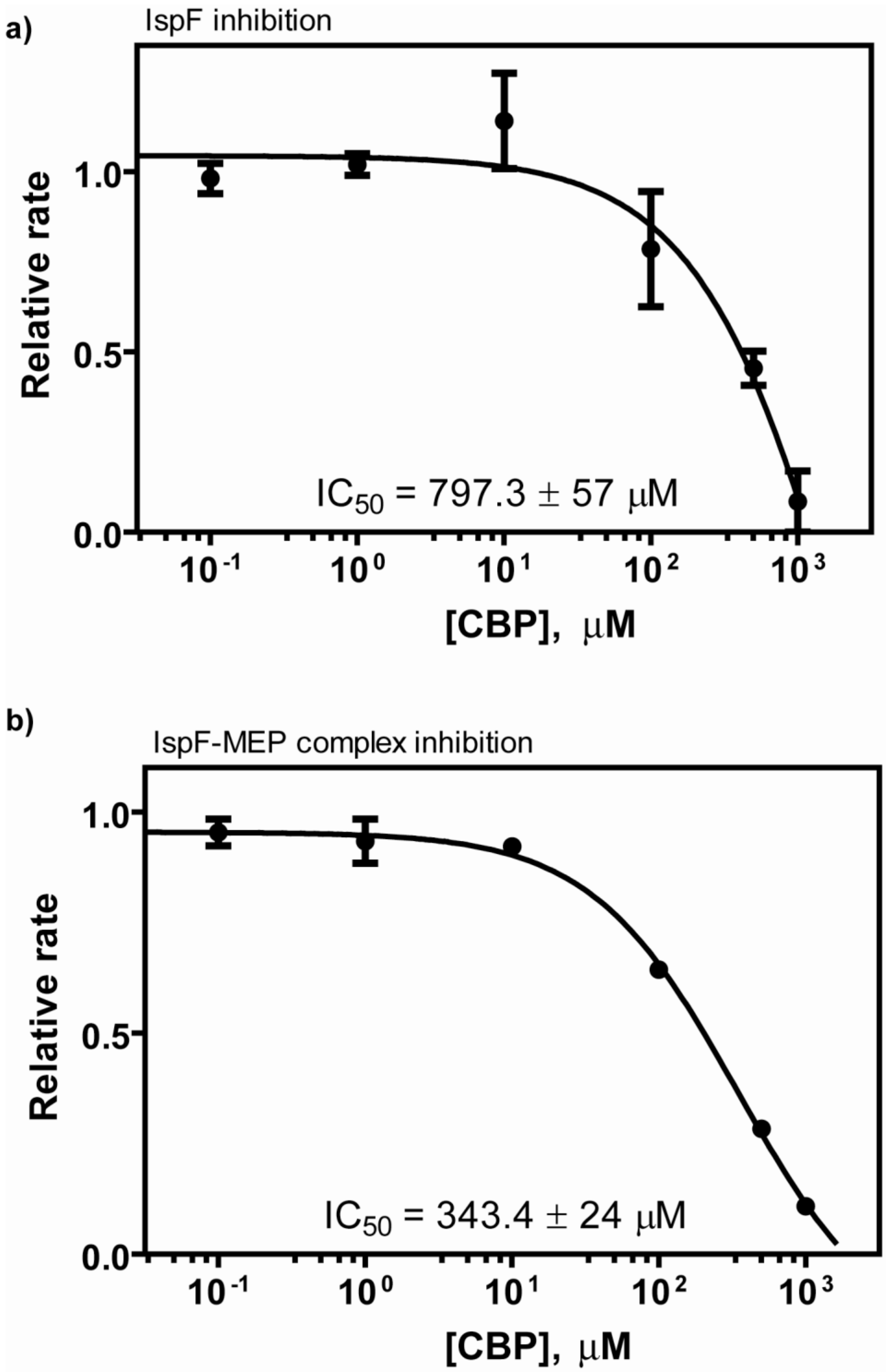
[a]Pre-incubation time, before the reaction was initiated with the substrate CDPME2P.[b]Used at a final concentration of 500 μM. [c]Reactions done in the presence of 500 μM MEP. **$p < 0.05$; ***$p < 0.01$; ****$p < 0.001$

Table S2: **Rates of CMP formation in the presence of MEBP, CDP, or CBP**. Tabulated and normalized rates of CMP formation by IspF or IspF-MEP complex in the presence of 2*C*-methyl-D-erythritol 4-bisphosphonate (MEBP, 500 and 1000 μM), cytidine 5`-diphosphate (CDP, 700 μM), or cytidine 5`-bisphosphonate (CBP, 700 μM). Enzyme reactions were initiated with IspF (0 min pre-incubation) or substrate (30 min pre-incubation), in the presence or absence of additives.

Control	0	1.10 ± 0.15	* (vs. Control 30); ** (vs. 500 μM MEBP 30)
	30	0.12 ± 0.04	* (vs. Control 0); *** (vs. 1000 μM MEBP 0)
[b]500 μM MEBP	0	1.71 ± 0.17	*
	30	0.29 ± 0.04	**
1000 μM MEBP	0	1.92 ± 0.20	***
	30	1.04 ± 0.20	***
700 μM CDP	0	0.45 ± 0.02	
	30	0	
[b]CBP	0	0.66 ± 0.08	
	30	0.21 ± 0.06	
[b]MEP	0	2.25 ± 0.13	
	30	1.99 ± 0.11	**
[b,c]500 μM MEBP	0	2.23 ± 0.21	**
	30	2.17 ± 0.20	**
[c]700 μM CDP	0	2.28 ± 0.21	**
	30	1.08 ± 0.18	**
[b,c]CBP	0	0.69 ± 0.31	
	30	0.51 ± 0.20	

[a]Pre-incubation time, before the reaction was initiated with the substrate CDPME2P. [b]Used at a final concentration of 500 μM. [c]Reactions done in the presence of 500 μM MEP. *$p = 0.05$; **$p < 0.05$; ***$p < 0.01$

Figure S4: Inhibition of IspF and the IspF-MEP complex by CBP. (a) IC_{50} curve showing CBP inhibition of IspF. (b) IC_{50} curve showing inhibition of the IspF-MEP complex by CBP. Reaction conditions for inhibition assays are identical to those previously described. [1]



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