Supplemental information

Pioneer synthesis of 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate and kinetic studies of *Mycobacterium tuberculosis* IspF, a potential drug target

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Experimental procedures

Starting materials were purchased from Aldrich chemicals. Flash chromatography was performed using EM Science silica gel 60(230-400 mesh). All glassware was oven dried, assembled hot and cooled under a stream of nitrogen before use. Reactions with air sensitive materials were carried out by standard syringe techniques. ^{1}H-NMR were recorded on a Varian Unity/Inova-400 NB (400 MHz). Chemical shifts are reported in parts per million (ppm) down field from TMS, using residual CDCl3 (7.27 ppm) as an internal standard. Data are reported as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of a doublet, m = multiplet, br = broad), coupling constants and integration. ^{13}C-NMR was recorded in a Varian Unity/Inova-400 NB (100 MHz). Using broadband proton decoupling. Chemical shifts are reported in parts per million (ppm) downfield from TMS, using the middle resonance of CDCl3

(77.0 ppm) as an internal standard. Mass Spectra (MS-ESI) were obtained from Mass Spectrometry Laboratory, Chemistry department, Colorado state university.

Data for **16**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.32 (m, 15H), 5.52 (s, 1H), 5.02 (m, 4H), 4.37 (d, 2H, *J* = 10.8 Hz), 4.08 (m, 6H), 3.98 (d, 1H, *J* = 11.2 Hz), 1.65 (s, 3H), 1.36 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 136.9, 129.2, 128.5, 128.3, 127.9, 126.2, 101.9, 81.2 (d, *J* = 4 Hz), 75.3, 69.3 (d, *J* = 6 Hz), 65.6 (d, *J* = 4 Hz), 63.9 (d, *J* = 6 Hz), 17.8, 16.1, 16.0.; IR (neat, cm^{-1}): 2965, 2360, 2332, 1651, 1619. MS (ESI) $C_{30}H_{39}O_{10}P_2$ (M+H): 621

Data for **17**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.14 (m, 9H), 1.62 (s, 3H), 1.36 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz): 83.7, 77.2 (d, *J* = 7 Hz), 76.5, 65.8 (d, *J* = 7 Hz), 65.4 (d, *J* = 6 Hz), 64.4 (d, *J* = 6 Hz), 18.4, 16.6, 16.5.; IR (neat, cm^{-1}): 3350, 2964, 2360, 2331, 1654. MS (ESI) $C_9H_{24}O_{10}P_2NNa$ ($M+NH_4+Na$): 391

Data for **18**: ^{1}H-NMR (D_2O, 400 MHz): δ 7.67 (d, 1H, *J* = 8.0 Hz), 5.91 (d, 1H, *J* = 8.0 Hz), 5.78 (d, 1H, *J* = 3.6 Hz), 3.99 (m, 14H), 1.55 (s, 3H), 1.17 (m, 6H).; ^{13}C-NMR (D_2O, 100 MHz): 164.1, 163.7, 161.5, 103.3, 97.5, 90.5, 84.4, 83.2 (2 C), 75.3 (d, *J* = 6 Hz), 70.8 (d, *J* = 6 Hz), 66.9 (d, *J* = 7 Hz), 65.9 (d, *J* = 4 Hz), 64.1 (2 C), 18.2, 16.4 (2 C); ^{31}P-NMR (D_2O, 121 MHz): 5.0, -9.6 (d, *J* = 10 Hz), -12.3 (d, *J* = 10 Hz); IR (neat, cm^{-1}): 3350, 2966, 2360, 2339, 1654, 1613. MS (ESI) $C_{18}H_{33}N_3O_{17}P_3$ (M+H): 656

Data for **6**: ^{1}H-NMR (D_2O, 400 MHz): δ 8.04 (d, 1H, *J* = 10.0 Hz), 5.98 (d, 1H, *J* = 10.0 Hz), 5.86 (d, 1H, *J* = 3.6 Hz), 4.04 (m, 10H), 1.75 (s, 3H); ^{31}P-NMR (D_2O, 121 MHz): -2.8, -8.0 (d, *J* = 11 Hz), -10.0 (d, *J* = 11 Hz); MS (ESI) $C_{14}H_{30}N_5O_{17}P_3$ ($M+2NH_4$): 633

PCR amplification and cloning of *Rv3581c* from *M. tuberculosis.* *Rv3581c* was amplified using the oligonucleotide primers designed from the sequences available on the TubercuList website (http://genolist.pasteur.fr/TubercuList/). The oligonucleotides used (Rv3581c-F, cat atg aat cag ctg ccc cgc gtt and Rv3581c-R, aag ctt cta ccg caa cga aac ca) contain *Nde*I and *Hin*dIII restriction enzyme sites (underlined). The PCR products were digested with *Nde*I and *Hin*dIII, and ligated into the pET28a(+) vector (EMD Biosciences, Inc.). Ligation mixtures were used to transform *E. coli* DH5α cells (Life Technologies) creating DH5α[pET28a(+)::*Rv3581c*], in which the target construct (pET28a(+)::*Rv3581c*) was propagated. The plasmid was isolated using Qiagen Plasmid Miniprep Kits and sequenced by Macromolecular Resources (Colorado State University).

Expression and purification of the recombinant Rv3581c. Recombinant Rv3581c was expressed and purified as previously described for other enzymes in the MEP pathway (Eoh et al., 2007; Mao et al., 2008). Briefly, transformation of BL21 (DE3) (Novagen) with pET28a(+)::*Rv3581c* afforded the recombinant strain BL21(DE3)[pET28a(+)::*Rv3581c*]. Protein expression was induced by adding 0.5 mM isopropyl–ß–D–thiogalactopyranoside (IPTG) and incubating at 20 ºC for 10 hr. Cells were harvested by centrifugation at 8000 x *g* for 15 min and stored at –80°C. The frozen cells were resuspended in a lysis buffer (2 ml/g) containing 50 mM 4-morpholine propane sulfonic acid (MOPS, pH 7.9), 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 10% glycerol, and 1 mM β-mercaptoethanol, broken by sonication on ice and then centrifuged for 40 min at 20,000 x *g*. A packed volume of 0.5 ml of Ni-NTA resin (Sigma-Aldrich) pre-equilibrated with

lysis buffer was added to the resulting solution and rocked at 4°C for 30 min. A small column was packed with the slurry and the recombinant Rv3581c carrying a His_6–tag was purified by immobilized metal affinity chromatography using a linear gradient of 50 to 200 mM imidazole in washing buffer [50 mM 4–morpholine propane sulfonic acid (MOPS, pH 7.9), 1 mM $MgCl_2$, 10 % glycerol and 1 mM β–mercaptoethanol]. Eluted fractions were analyzed by SDS–PAGE and Western blot visualized with Coomassie Brilliant Blue 250R and an anti–His antibody (Sigma), respectively. Fractions containing recombinant Rv3581c estimated to be at least 95% pure by Coomassie Brilliant Blue 250R staining of SDS–PAGE gels were pooled, desalted on a PD–10 column (Millipore), and stored at -70 ºC.

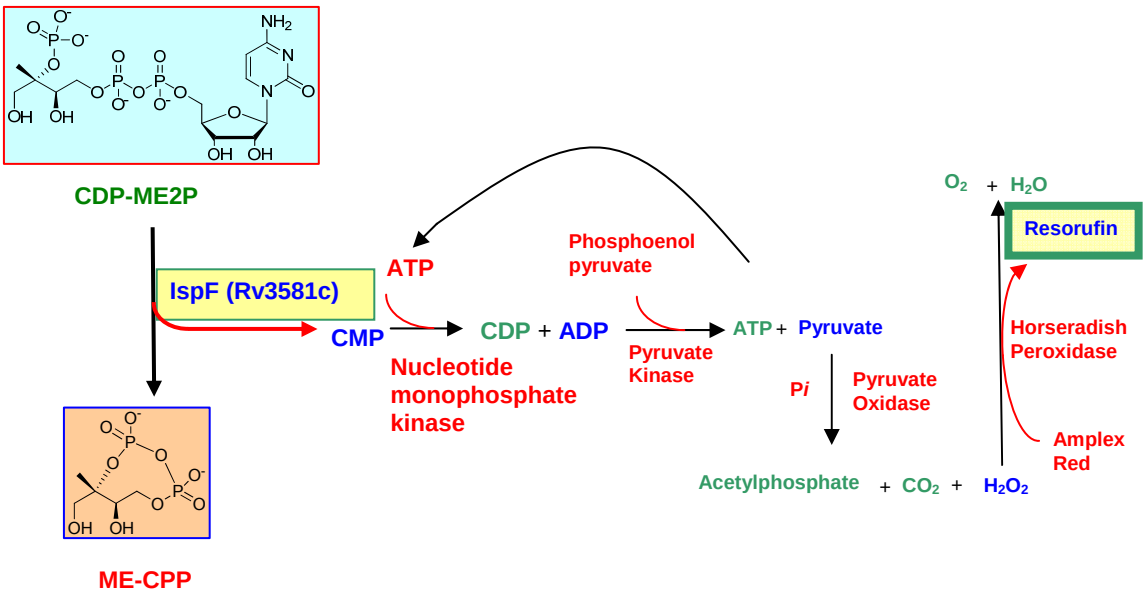

Figure S1, related to assay. Schematic showing of the reaction catalysed by IspF and coupling to generate fluorescence assay. NMK(nucleotide monophosphate kinase) is utilized to generate ADP in the presence of ATP and CMP. Pyruvate kinase, pyruvate oxidase, and horseradish peroxidase utilize ADP to produce resorufin, which is detected at an excitation wavelength 530 nm and emission wavelength 590 nm using a Synergy™ HT Multi-Detection spectrophotometer.