Electronic Supplementary Information

Selective inhibition of *E. coli* 1-deoxy-D-xylulose-5-phosphate synthase by acetylphosphonates

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Materials and General Methods. Unless otherwise noted, all reagents were obtained from commercial sources. Spectrophotometric analyses were performed on a Beckman DU 800 UV/Vis spectrophotometer. Pyruvate dehydrogeanse from porcine heart and transketolase from baker's yeast (*S. cerevisiae*) were obtained from Sigma Aldrich. *E. coli* DXP synthase and DXP reductoisomerase were purified as previously described [1][2].

Kinetic Analyses. DXP synthase activity was measured spectrophotometrically using IspC as a coupling enzyme as previously reported [2][3] The concentration of D-GAP in stock solutions was determined as previously reported[1]. All experiments were performed in duplicate. Pyruvate dehydrogeanse activity was measured spectrophotometrically as previously reported[4] by measuring changes in optical density at 340 nm. The basic assay medium (30°C), contained 100 mM HEPES (pH = 8.0), 1 mg/mL BSA, 0.2 mM ThDP, 0.1 mM coenzyme A, 1 mM $MgCl_2$, 2 mM cysteine, 0.3 mM TCEP. The reaction was initiated with enzyme. Transketolase activity was measured as previously reported by measuring changes in optical density at 340 nm[5]. The basic assay medium (37°) contained 50 mM glycylglycine (pH = 7.7), 10 mM sodium arsenate, GADPH, 3.2 TCEP, 2.5 $CaCl_2$, 80 µM ThDP, 70 µM xylulose-5-phosphate, 0.5 mM ribose-5-phosphate, 0.4 mM β-nicotinamide adenine dinucleotide phosphate sodium salt hydrate (NAD+). The reaction was initiated with enzyme.

Inhibition Studies. Suppression of DXP synthase activity was measured spectrophotometrically using IspC as a coupling enzyme and monitoring NADPH consumption by IspC[3][2]. DXP synthase reaction mixtures containing 100 mM HEPES buffer, pH 8.0 (described above) were pre-incubated at 37°C for 5 minutes with compound **1** (2.5, 5, and 10 µM), compound **2** (10, 25, and 50 µM), compound **3** (10, 25, and 50 µM), and compound **4** (250, 500, and 750 µM). The reaction was initiated with enzyme. Inhibition of DXP synthase by compounds **5-6** was not observed. Suppression of pyruvate dehydrogenase activity was measured spectrophotometrically by measuring changes in optical density at 340 nm. Pyruvate dehydrogenase reaction mixtures containing 100 mM HEPES buffer, pH 8.0 (described above) were pre-incubated at 30°C for 5 minutes with compound **1** (50, 100, 250 µM), compound **2** (50, 100, 250 µM), compound **3** (200, 500, 1000 µM). Inhibition of porcine pyruvate dehydrogenase by compounds **4-6** was not observed up to 1 mM. Inhibition of the coupling enzyme (IspC) and transketolase from baker's yeast by compounds **1-6** was not observed up to 1 mM. All experiments were performed in duplicate. Double reciprocal analysis of data was carried out using GraFit version 7 from Erithacus Software.

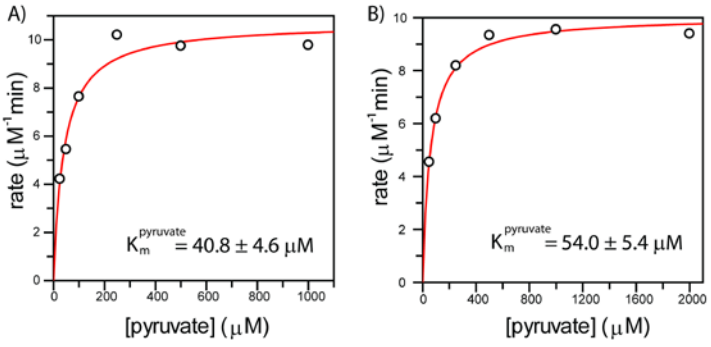


Figure S1. Determination of $K_m^{pyruvate}$ for A) DXP synthase and B) the E1 subunit of pyruvate dehydrogenase.

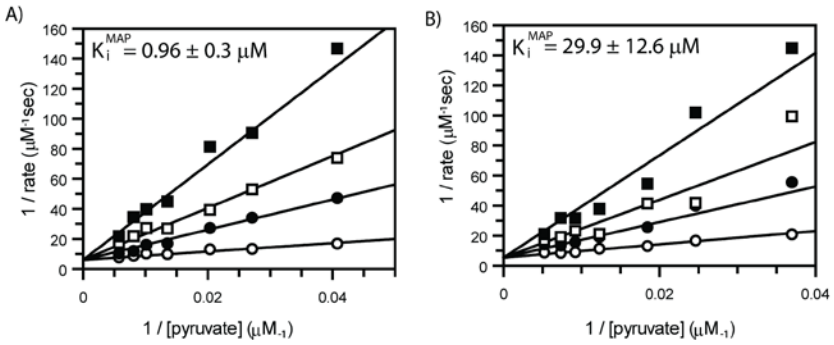


Figure S2. Competitive inhibition by methyl acetylphosphonate (MAP): **A) Inhibition of DXP synthase by MAP**. The concentration of pyruvate was varied with increasing concentrations of MAP: 0 (○), 2.5 (●), 5 (□), and 10 (■) µM MAP. **B) Inhibition of the E1 subunit of pyruvate dehydrogenase by MAP**. The concentration of pyruvate was varied at with increasing concentrations of MAP: 0 (○), 50 (●), 100 (□), and 200 (■) µM MAP.

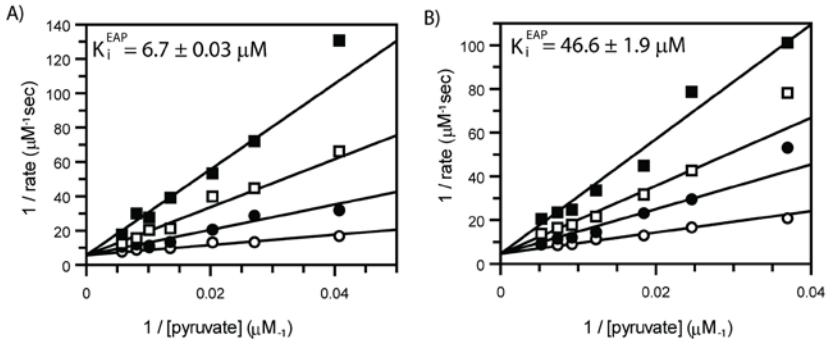
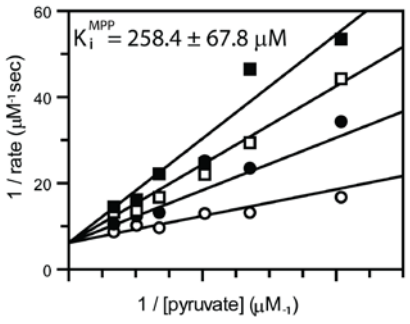


Figure S3. Competitive inhibition by ethyl acetylphosphonate (EAP): **A) EAP inhibition of DXP synthase**. The concentration of pyruvate was varied with increasing concentrations of EAP: 0 (○), 10 (●), 25 (□), and 50 (■) µM EAP. **B) EAP inhibition of E1 subunit of pyruvate dehydrogenase**. The concentration of pyruvate was varied at with increasing concentrations of EAP: 0 (○), 50 (●), 100 (□), and 200 (■) µM EAP.



Supplemental Figure S4. Competitive inhibition of DXP synthase by compound 4 (MPP). The concentration of pyruvate was varied with increasing concentrations of 4: 0 (○), 250 (●), 500 (□), and 750 (■) µM.

Synthesis

General Experimental. Trialkyl phosphites, acyl chlorides and lithium bromide were obtained from commercial sources and used without further purification. Anhydrous acetonitrile was purchased in Sure-Seal bottles. All reactions were carried out in oven-dried glassware under an inert argon atmosphere. NMR spectra were recorded on a Bruker 400 MHz spectrometer for dialkyl acyl phosphonate intermediates, or a Varian 500 MHz spectrometer for final compounds. Chemical shifts are reported in units of parts per million (ppm), relative to a standard reference point. ^{1}H NMR chemical shifts are reported relative to tetramethylsilane (TMS, δ = 0 ppm) as internal

reference. ^{31}P chemical shifts are reported relative to triphenylphosphine oxide (TPPO, δ = 0 ppm) as an external standard. Mass spectrometry analysis was carried out at University of Illinois at Urbana-Champagne, School of Chemical Sciences, Mass Spectrometry Laboratory. Chemical synthesis and characterization of compound (**1**) (MAP) was reported previously[2]. Compound (**5**) (MBP) was prepared according to published protocol[6].

Synthesis of ethyl acetyl phosphonate (2). An oven-dried flask was equipped with a stir-bar and charged with 0.21 mL of acetyl chloride (0.24g, 3.0 mmol). Triethyl phosphite (0.52 mL, 3.0 mmol) was added drop-wise at room temperature. The solution was stirred at room temperature for about 1 hour, until triethyl phosphite had disappeared (as indicated by ^{31}P NMR analysis). Diethyl acylphosphonate (**7**) was isolated as a pale yellow oil following vacuum distillation (241.6mg, 97% pure as determined by ^{31}P NMR, 45% yield) and was carried on without further purification. ^{31}P-NMR ($CDCl_3$): δ -27.84 (s) ^{1}H-NMR ($CDCl_3$): δ 1.08 (t, 6H), 2.182 (d, 3H), 4.02 (m, 4H).

Diethyl acylphosphonate (**7**) was dissolved in 1.7 mL of anhydrous acetonitrile. Lithium bromide (106.8 mg, 1.2 mmol) was added in one portion, and the reaction mixture was heated to 70 ^{o}C and stirred overnight. The resulting white solid was filtered and washed with two 10 mL portions of anhydrous acetonitrile followed by two 10 mL portions of diethyl ether. Ethyl acetylphosphonate (**2**) was isolated as a white solid (132.5 mg, 69% yield). ^{31}P-NMR (D_2O): δ -27.08 (s); ^{1}H-NMR (D_2O): 1.17 (t, 3H), 2.34 (d, 3H), 3.84 (m, 2H). HRMS (ESI), calculated m/z for $C_4H_9LiO_4P$ (free acid form), $[M+H]^+$ = 159.0399; observed: 159.0398.

Synthesis of butyl acetyl phosphonate (3). An oven-dried flask was equipped with a stir-bar and charged with 0.40 mL of acetyl chloride (0.44g, 5.6 mmol). Tributyl phosphite (1.5 mL, 6.0 mmol) was added drop-wise at room temperature. The solution was stirred at room temperature for about 1 hour, until tributyl phosphite had disappeared (as indicated by ^{31}P NMR analysis). Dibutyl acylphosphonate (**8**) was isolated as a pale yellow oil following vacuum distillation (754mg, 90% pure as determined by ^{31}P NMR, 64% yield) and was carried on without further purification. ^{31}P-NMR ($CDCl_3$): δ -28.75 (s) ^{1}H-NMR ($CDCl_3$): δ 0.94 (t, 6H), 1.42 (m, 4H), 1.71 (m, 4H), 2.49 (d, 3H), 4.16 (m, 4H).

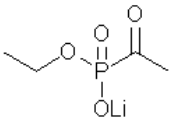
Dibutyl acylphosphonate (**8**) was dissolved in 4 mL of anhydrous acetonitrile. Lithium bromide (269.7 mg, 3.1 mmol) was added in one portion, and the reaction mixture was heated to 70 ^{o}C and stirred overnight. The resulting white solid was filtered and washed with two 10 mL portions of anhydrous acetonitrile followed by two 10 mL portions of diethyl ether. Butyl acetylphosphonate (**3**) was isolated as a white solid (261.1 mg, 44%). ^{31}P-NMR (D_2O): δ -27.01(s); ^{1}H-NMR (D_2O): 0.76 (t, 3H), 1.22 (m, 2H), 1.47 (m, 2H), 2.29 (d, 3H), 3.76 (m, 2H). HRMS (ESI), calculated m/z for $C_6H_{13}LiO_4P$ (free acid form), $[M+H]^+$ = 187.0712; observed: 187.0709.

Synthesis of methylpropionylphosphonate (4). An oven-dried flask was equipped with a stir-bar and charged with 0.50 mL of propionyl chloride (0.53g, 5.5 mmol). Trimethyl phosphite (0.60 mL, 5.1 mmol) was added drop-wise at room temperature. The solution was stirred at room temperature for about 1 hour, until trimethyl phosphite had disappeared (as indicated by ^{31}P NMR analysis). Dimethyl propionylphosphonate was isolated as a pale yellow oil following vacuum distillation (542.3 mg, 98% pure as determined by ^{31}P NMR, 64% yield) and was carried on without further purification. ^{31}P-NMR ($CDCl_3$): δ -26.77 (s)

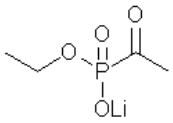
Dimethyl propionylphosphonate was dissolved in 4 mL of anhydrous acetonitrile. Lithium bromide (269.7 mg, 3.1 mmol) was added in one portion, and the reaction mixture was heated to 70 ^{o}C and stirred overnight. The resulting white solid was filtered and washed with two 10 mL portions of anhydrous acetonitrile followed by two 10 mL portions of diethyl ether. Methyl propionylphosphonate (**3**) was isolated as a white solid (241.0 mg, 47%). ^{31}P-NMR (D_2O): δ -27.01; ^{1}H-NMR (D_2O): 0.76 (t, 3H), 1.22 (m, 2H), 1.47 (m, 2H), 2.29 (d, 3H), 3.76 (m, 2H). ESI (M/S) calculated m/z for $C_4H_9LiO_4P$ (free acid form), $[M+H]^+$ = 159.0403; observed: 159.0399.

Synthesis of methylvaleroylphosphonate (6). An oven-dried flask was equipped with a stir-bar and charged with 0.3 mL of valeroyl chloride (0.3g, 2 mmol), to which 0.3mL of trimethyl phosphite (0.3g, 2 mmol) was added. After one hour, reaction mixture was diluted with 3.1 mL of anhydrous acetonitrile. To this solution, 220 mg of LiBr (2.53 mmol) was added in one portion. The reaction mixture was warmed to 70^{o}C overnight. The solid white precipitate was filtered with two 10 mL portions of anhydrous acetonitrile, followed by two 10 mL portions of diethyl ether. Methyl valeroylphosphonate was isolated as a white solid (110.3mg, 23% overall yield). ^{31}P-NMR (D_2O): δ -25.89 (s); ^{1}H-NMR (D_2O): 0.79 (t, 3H), 1.18 (m, 2H), 1.46 (m, 2H), 2.73 (t, 2H), 3.52 (d, 3H). HRMS (ESI), calculated m/z for $C_6H_{13}LiO_4P$ (free acid form), $[M+H]^+$ = 187.0712; observed: 187.0708.

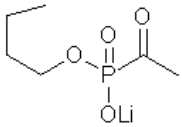
^{31}P NMR of **2**



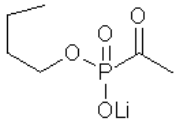
^{1}H NMR of **2**



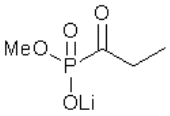
^{31}P NMR of **3**



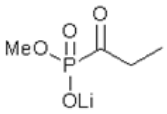
^{1}H NMR of **3**



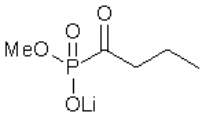
^{31}P NMR of **4**



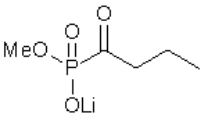
^{1}H NMR of **4**



^{31}P NMR of **5**



^{1}H NMR of **5**



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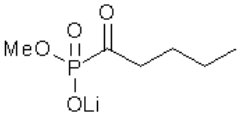
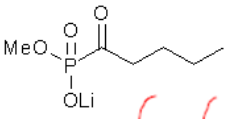
^{31}P NMR of **6**



^{1}H NMR of **6**



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