Supporting Information

Bacillus anthracis o-succinylbenzoyl-CoA synthetase: reaction kinetics and a novel inhibitor mimicking its reaction intermediate

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MATERIALS AND METHODS

Cloning, overexpression and purification of (his)$_6$-tagged E. coli 1,4-dihydroxy-2-naphthoate-CoA (DHNA-CoA) synthetase. The *E. coli* DHNA-CoA synthetase gene (*menB*) sequence was obtained from Genbank by accession number NC_002695. The *menB* gene was obtained by PCR amplification from *E. coli* strain DH5α genomic DNA using *Pfx* DNA polymerase. Two primers (5'-GCGGCGCCATATGATTTATCCTGATGAAGCA-3'; 5'-CGGGATCCTTACGGATTCCGTTTGAATTTGCT-3') containing *Nde*I and *Bam*HI sites (underscored in the primer sequence) were used for the PCR reactions. The PCR products were cloned into a modified pET15b plasmid (containing a TEV protease cleavage site instead of the original thrombin cleavage site) using the above two restriction sites, which placed *menB* gene in frame with an N-terminal (his)$_6$-tag sequence. The plasmid was sequenced at the DNA sequencing facility at Research Resource Center at University of Illinois at Chicago. The mutation-free construct was transformed into *E. coli* strain BL21 (DE3). The cells were grown at 37 ^{o}C in 4 L LB medium until the OD_{600} reached 0.6. IPTG was added to the cell culture at a final concentration of 0.5 mM for induction of protein expression. After four hours of induction, cells were collected by centrifugation at 3,300*g* for 10 minutes at 4 ^{o}C. For purification, 12 grams of cell pellet were resuspended in 30 mL of Buffer E (50 mM Tris-HCl, 10 mM imidazole, pH 7.5) and lysed by sonication using a GEX-600 sonics ultrasonic processor (Sonics and Materials, Inc., Newtown, CT) with a 0.5'' probe. The sonication lasted for 6 minutes with a repeating pulse of 6.6 seconds on and 9.9 seconds off at 65% amplitude. The cell lysate was centrifuged at 39,000*g* for 40 minutes at 4 ^{o}C, after which the supernatant was collected and filtered through a 0.22 μm filter (Millipore, Carrigtwohill, Co.Cork, Ireland). The clear filtrates (~30 mL) were loaded onto a 5 mL HiTrap affinity column (1.6 × 2.5 cm) (Amersham Biosciences, Piscataway, NJ) charged with cobalt and equilibrated with Buffer E. The column was washed with 100 mL Buffer E to remove the weakly bound proteins. *E. coli* DHNA-CoA synthetase was eluted by running a linear gradient of 0-100% Buffer F (50 mM Tris-HCl, 1 M imidazole, pH 7.5) in 120 mL. Fractions were analyzed by SDS-PAGE. The fractions containing pure *E. coli* DHNA-CoA synthetase were pooled. Protein concentrations were determined using a BIO-RAD protein assay kit (BIO-RAD Laboratories Inc., Hercules, CA) with BSA as a standard.

Determination of pH optimum. The pH optimum of *B. anthracis* OSB-CoA synthetase activity was studied in a pH range of 6.0 to 8.5. Bis-Tris-HCl (50 mM) buffer was used for the pH range of 6.0 to 7.25 and Tris-HCl (50 mM) buffer was used for the pH range of 7.25 to 8.5. The phosphate detection assay was used in this study. We first tested the enzyme stability in buffers of pH 6.0 to 8.5. OSB-CoA synthetase (100 μM) was pre-incubated in the buffers of different pH for 30 minutes. The enzyme was then diluted into 50 mM Tris-HCl buffer (pH 7.5) to measure the residual activity in the standard assay mixture containing 50 mM Tris-HCl, 20 mM NaCl, 2 mM $MgCl_2$, 50 nM OSB-CoA synthetase, 0.5 U PNP, 0.075 U IPP, 300 μM MESG, pH 7.5, 256 μM OSB, 512 μM ATP, and 1024 μM CoA. As shown in Figure S2, the enzyme showed similar activities after incubating in buffers of pH 6.0 to 8.5, suggesting that the enzyme was stable in the pH range.

The specific activity ($V_{maxapp.}$) of the enzyme was then measured directly in the buffers of different pH using a modified assay condition, which containing 50 mM buffer at indicated pH, 20 mM NaCl, 2 mM $MgCl_2$, 50 nM OSB-CoA synthetase, 0.225 U IPP, 1.5 U PNP, 300 μM MESG, 256 μM OSB, 512 μM ATP, and 1024 μM CoA. Excess amounts of coupling enzymes IPP and PNP were included in this modified assay to minimize the effect

of pH on them. Little buffer effect was observed on enzyme activity at pH 7.25. The *Vmaxapp.* Vs pH data showed a best fit a bell shaped pH profile equation

$$V_{maxapp.} = V_{max} / (1+ 10^{(pKa-pH)}+10^{(pH-pKb)})$$

where Vmaxapp. is the specific activity of the enzyme at different pH, Vmax is the maximum reaction rate, pKa is the negative logarithm (base 10) of the acid dissociation constant of the first ionization, pKb is the negative logarithm (base 10) of the acid dissociation constant of the second ionization, pH is negative logarithm (base 10) of the proton concentration in the assay. As shown in Figure S2, the enzyme exhibited a bell shaped pH profile with optimum activity between pH 7.25 to 7.5 (pKa=6.6 ± 0.07, pKb=8.0 ± 0.07).

Effect of Mg^{2+} on B. anthracis OSB-CoA synthetase activity. The OSB-CoA synthetase activity was measured at different concentrations of $MgCl_2$ (0-4 mM) in the phosphate detection assay containing 50 mM Tris-HCl, pH 7.5, 20 mM NaCl, 50 nM OSB-CoA synthetase, 0.5 U PNP, 0.075 U IPP, 300 μM MESG, 256 μM OSB, 512 μM ATP, and 1024 μM CoA. The enzyme showed the highest activity at 1-2 mM Mg^{2+} (Table S1).

Calculation of the inherent lag phase (τ) of the coupled assay in the pre-steady-state kinetic studies of E+ATP+OSB+CoA. There is a theoretical, inherent lag phase for all coupled enzyme assays. A common practice to shorten this lag phase and minimize its effect on the coupled assay is to add an excess amount of coupled enzymes (Rudolph et al., 1979, page 30, Methods Enzymology, Volume 63, part A). In Figure 7, Panel A, we added 1 U of IPP and 2.5 U of PNP in the 100 uL assay, which means the final concentration is 10 U / mL for IPP and 25 U / mL for PNP. The concentration of OSB-CoA synthetase is 1 μM in the assay, which is 0.15 U / mL. The predicted inherent lag phase (τ) can be calculated for the coupled assay based on Cleland (*1*):

$$\tau = K_{mIPP}/V_{maxIPP} + K_{mPNP}/V_{maxPNP} \qquad (S1)$$

where τ is the calculated lag phase (minute), K_{mIPP} is the apparent Michaelis constant of IPP on substrate PPi (mM), V_{maxIPP} is the maximum velocity of IPP on substrate PPi (units / mL), K_{mPNP} is the apparent Michaelis constant of PNP on substrate Pi (mM), V_{maxPNP} is the maximum velocity of PNP on substrate Pi (units / mL).

Based on equation S1 and the kinetic constants in Table S4, the lag phase (τ) is calculated as:

τ = (0.01 / 10) + (0.026 / 25) = 0.001 + 0.00104 = 0.002 minute = 0.12 seconds

Therefore, the lag phase is about 0.12 second, or only 120 milliseconds, for this coupled assay, which is too short to have any significant and observable effects on the time course of the reaction presented in Figure 7.

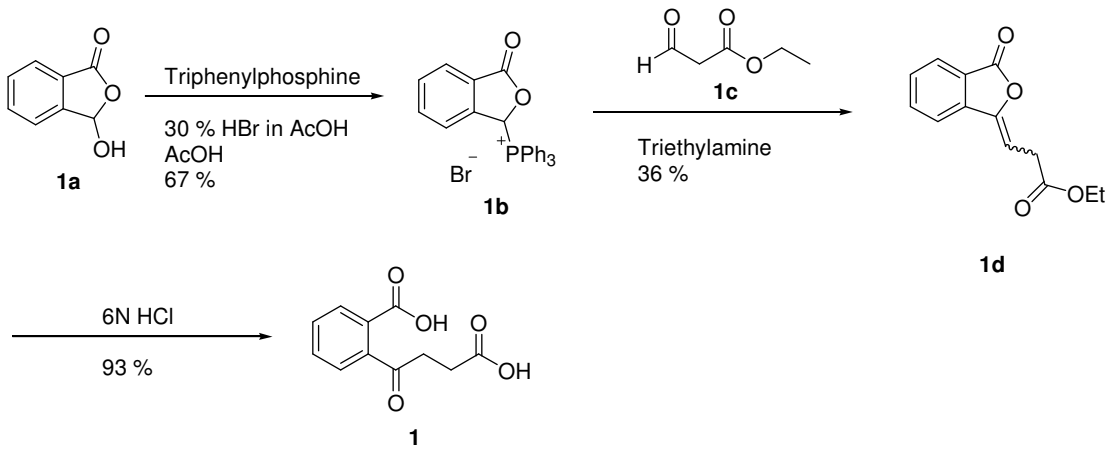
Syntheses of Compounds 1 - 6

General procedures

^{1}H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively, with a Bruker Advance DRX-500 spectrometer in either $CDCl_3$, D_2O, CD_3OD or acetone-d_6. 2D COSY and NOESY studies were performed with a Bruker Advance DRX-500 500 MHz spectrometer. Infrared spectra were obtained with a Genesis II FTIR^{TM} spectrophotometer. High resolution mass spectroscopy was obtained by the Research Resources Center of the University of Illinois at Chicago. All reactions were performed under a nitrogen atmosphere. Melting points were measured with a Thomas hotstage microscope instrument.

Improved synthesis of compound 1 (*o*-succinylbenzoate, OSB)

The synthesis procedure is summarized in Scheme S1



Synthesis of compound 1b (*2*)**.** A solution of 2-carboxybenzaldehyde **1a** (35.7 g, 0.24 mol), triphenylphosphine (62.4 g, 0.24 mol), 30 % hydrobromic acid in acetic acid (47.6 mL, 0.24 mol) and acetic acid (47.6 mL) was stirred at 90 ^oC under nitrogen for three days. The reaction mixture was evaporated and dried under vacuum and acetonitrile (260 mL) was added to the residue, which was then heated to reflux for 1 h. After cooling in an ice-bath, the reaction mixture was filtered and washed with acetonitrile. After drying overnight under vacuum, the title compound **1b** was obtained as a white solid (56.6 g). Additional product (19.5 g) was obtained from the filtrate (67 % total yield). ^{1}H NMR ($CDCl_3$) δ: 7.00-7.01 (m, 1H), 7.50-7.62 (m, 9H), 7.73-7.77 (m, 9H), 9.76 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 74.6, 75.0, 114.2, 114.9, 124.3, 125.8, 126.4, 130.5, 130.6, 131.2, 134.6, 134.7, 135.3, 136.0, 141.1, 167.4.

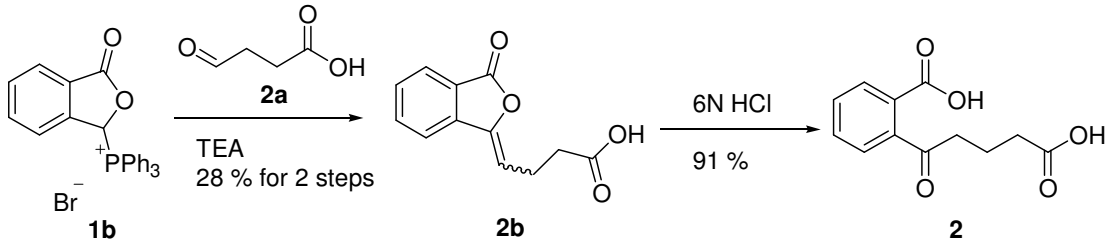
Synthesis of compound 1c (*3*)**.** Trifluoroacetic acid (2 mL) and water (2 mL) was added to a solution of ethyl 3,3-diethoxypropionate (1.3 g, 7 mmol) in dichloromethane (8 mL). The solution was stirred for 2.5 h at room temperature and then separated into two layers. The aqueous layer was extracted with dichloromethane (10 mL × 3). The combined dichloromethane layers were dried over Na_2SO_4, filtered and used for the next step without further purification. ^{1}H NMR ($CDCl_3$) (*4*) δ: 1.30 (t, *J* = 7.2 Hz, 3H), 3.39 (d, *J* = 2.4 Hz, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 9.82 (t, *J* = 2.4 Hz, 1H).

Synthesis of compound 1d (*2*)**.** Triethylamine (0.97 mL, 7 mmol) was added dropwise to a solution of triphenyl(3-phthalidyl)phosphonium bromide **1b** (2.9 g, 7 mmol) and ethyl 3-oxopropanoate **1c** in dichloromethane (40 mL). The reaction mixture was stirred for 4 h, washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Concentration under vacuum, followed by purification by column chromatography over silica gel (hexanes:ethyl acetate, 20:1 to 5:1) gave a 1:6 mixture **1d** of *E* and *Z* isomers (0.51 g, 36% for 2 steps) as a yellow oil. ***Z*-1d**: ^{1}H NMR ($CDCl_3$) (*4*) δ: 1.28 (t, *J* = 7.0 Hz, 3H), 3.52 (d, *J* = 7.5 Hz, 2H), 4.18 (q, *J* = 7.0 Hz, 2H), 5.81 (t, *J* = 7.5 Hz, 1H), 7.51-7.88 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 14.2, 31.3, 61.2, 100.3, 120.2, 124.6, 125.4, 130.1, 134.5, 139.0, 147.0, 166.6, 170.7. ***E*-1d**: ^{1}H NMR ($CDCl_3$) δ: 1.09 (t, *J* = 7.2 Hz, 3H), 3.49 (d, *J* = 8.1 Hz, 2H), 4.01 (q, *J* = 7.2 Hz, 2H), 5.94 (t, *J* = 8.1 Hz, 1H), 7.51-7.88 (m, 4H).

Synthesis of compound 1 (OSB). HCl (6N, 10 mL) was added to the phthalide **1d** (245 mg) and the mixture was heated to reflux for 2 h, then concentrated under vacuum and purified by column chromatography over silica gel (ethyl acetate to ethyl acetate:methanol, 1:1) to give **1** (218 mg, 93 %) as a white solid. Mp = 136 °C (ref. (*5*), (*6*) mp=137 °C). ^{1}H NMR (D_2O) δ: 2.27 (t, *J* = 7.2 Hz, 2H), 2.52, (t, *J* = 7.2 Hz, 2H), 7.49-7.71 (m, 4H); ^{13}C NMR (acetone-d_6) (*5*) δ: 28.0, 34.8, 123.5, 125.9, 127.3, 130.3, 134.1, 148.1, 167.4, 173.1, 205.7.

Compound 2 (5-(2-carboxyphenyl)-5-oxopentanoic acid).

The synthesis procedure is summarized in Scheme S2

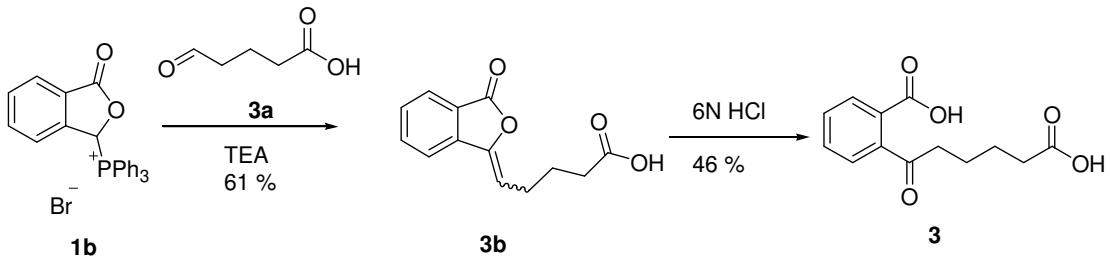


Synthesis of compound 2b. A solution of 4-pentenoic acid (0.49 g, 4.9 mmol) in dichloromethane (30 mL) was treated with a stream of ozone at -78 °C for 20 min until a persistent blue color was observed, and then with a stream of nitrogen until the blue color was discharged. Methyl sulfide (1 mL, 9.8 mmol) was then added and the reaction mixture was warmed to room temperature and stirred for an additional 6 h. A solution of triphenyl(3-phthalidyl)phosphonium bromide **1b** (4.7 g, 9.80 mmol) and triethylamine (2.3 mL, 16.2 mmol) in dichloromethane (30 mL) was added dropwise to the so-obtained solution of 4-oxobutyric acid **2a** (*2*). After stirring overnight, the reaction mixture was acidified by 3N HCl to pH 1. Water (60 mL) was added, and the mixture was extracted with dichloromethane (30 mL × 3), washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Purification by column chromatography over silica gel (hexanes:ethyl acetate, 5:1 to ethyl acetate) gave an inseparable 1:1 mixture **2b** of *E* and *Z* isomers (0.3 g, 28% for two steps) as a yellow solid, which was used directly in the next step. ***Z*-2b**: ^{1}H NMR ($CDCl_3$) δ: 2.62 (t, *J* = 7.5 Hz, 2H), 2.78 (q, *J* = 7.5 Hz, 2H), 5.68 (t, *J* = 7.5 Hz, 1H), 7.51-7.94 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 20.9, 33.3, 106.4, 119.9, 124.5, 125.3, 129.8, 134.4, 139.2, 146.5, 166.9, 178.7. ***E*-2b**: ^{1}H NMR ($CDCl_3$) δ: 2.66 (t, *J* = 7.5 Hz, 2H), 2.90 (q, *J* = 7.5 Hz, 2H), 5.82 (t, *J* = 8.0 Hz, 1H), 7.55-7.94 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 21.2, 33.6, 111.0, 123.2, 125.6, 126.2, 130.0, 134.5, 137.8, 146.6, 166.8, 178.3.

Synthesis of compound 2. A mixture of carboxylic acid **2b** (265 mg) and 6N HCl (12 mL) was heated to reflux for 2 h and then at 80 °C overnight, before it was concentrated under vacuum, and the residue purified by column chromatography over silica gel (hexanes:ethyl acetate, 1:1 to ethyl acetate:methanol, 3:1) to give the title product **2** (260 mg, 91 %) as a white solid. Mp = 123-126 °C. ^{1}H NMR (CD_3OD) δ: 1.68 (quintet, *J* = 7.5 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 4H), 7.56-7.86 (m, 4H); ^{13}C NMR (CD_3OD) δ: 23.0, 37.0, 42.7, 127.3, 130.0, 131.3, 134.2, 138.2, 152.1, 173.3, 180.0. ESI HRMS Calculated for $C_{12}H_{11}O_5$ (*7*): 235.0612. Found: 235.0611.

Compound 3 (6-(2-carboxyphenyl)-6-oxohexanoic acid).

The synthesis procedure is summarized in Scheme S3

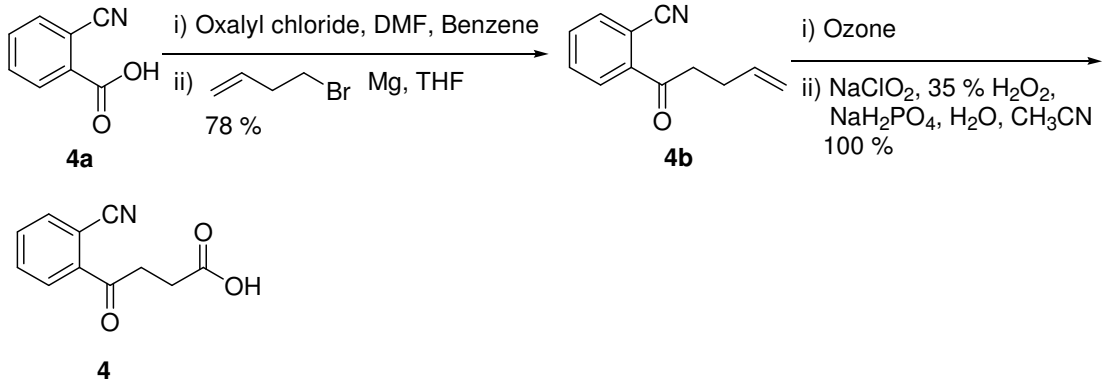


Synthesis of compound 3b. A solution of 5-hexenoic acid (0.6 g, 5.26 mmol) in dichloromethane (36 mL) was treated with a stream of ozone at -78 °C for 20 min until a persistent blue color was observed and then with a stream of nitrogen until the blue color was discharged. Methyl sulfide (0.77 mL, 10.5 mmol) was added and the reaction mixture was warmed to room temperature and stirred overnight. To the so-obtained solution of 5-oxopentanoic acid **3a** was added a solution of triphenyl(3-phthalidyl)phosphonium bromide **1b** (5.0 g, 10.5 mmol) and triethylamine (2.4 mL, 17.4 mmol) in dichloromethane (24 mL) dropwise. After stirring for 3.5 h, the reaction mixture was acidified with 3N HCl to pH 1, water (60 mL) was added, and the mixture was extracted with dichloromethane (30 mL × 3), washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Purification by column chromatography over silica gel (hexanes:ethyl acetate, 5:1 to ethyl acetate) gave **3b** as a 3: 2 mixture of *E* and *Z* isomers (0.74 g, 61 % for two steps) as a yellow solid, which was used in the next step directly. Mp = 73-75 °C. ^{1}H NMR ($CDCl_3$) δ: 1.90 (quintet, *J* = 7.5 Hz, 2H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.55 (q, *J* = 7.5 Hz, 2H), 5.62 (t, *J* = 7.5 Hz, 1H), 7.52-7.93 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 24.2, 25.1, 33.2, 107.8, 119.8, 124.5, 125.4, 129.7, 134.4, 139.3, 146.4, 167.0, 178.2.

Synthesis of compound 3. A mixture of **3b** (0.74 g) and 6N HCl (20 mL) was heated to reflux for 6 h, then concentrated under vacuum, and the residue purified by column chromatography on silica gel (ethyl acetate to ethyl acetate:methanol, 1:1) to give the title compound **3** (0.37 g, 46 %) as a white solid. Mp = 127-128 °C. ^{1}H NMR (CD_3OD) δ: 1.47 (bs, 2H), 1.61 (t, *J* = 7.3 Hz, 2H), 2.24 (bs, 2H), 2.27 (t, *J* = 7.3 Hz, 2H), 7.59-7.85 (m, 4H); ^{13}C NMR (CD_3OD) δ: 22.9, 24.3, 33.3, 38.2, 108.4, 122.6, 124.6, 126.9, 130.1, 134.3, 149.5, 169.2, 176.0. ESI HRMS calculated for $C_{13}H_{13}O_5$: 249.07685. Found: 249.07678.

Compound 4 (4-(2-cyanophenyl)-4-oxobutyric acid)

The synthesis procedure is summarized in Scheme S4

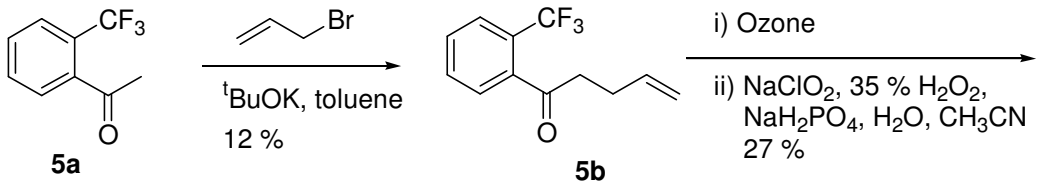


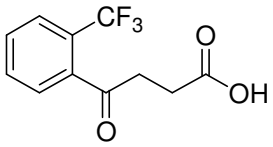
Synthesis of compound 4b. A solution of 2-cyanobenzoic acid **4a** (0.29 g, 1.97 mmol) and DMF (4 drops) in benzene (6 mL) at 0 ^{o}C was treated with oxalyl chloride (1.5 mL), then was warmed up to room temperature slowly. After stirring overnight, the reaction mixture was concentrated under vacuum, benzene was added to the residue, and removed under vacuum. The residue was taken up in THF (5 mL) and treated at –78 ^{o}C with 3-butenylmagnesium bromide in THF (3.4 mL), prepared by refluxing 4-bromo-1-butene (0.2 mL, 1.97 mmol) and Mg (0.6 g) in THF (3.4 mL) for 25 min, drop wise. After slowly warming up to room temperature overnight with stirring, the reaction mixture was quenched with 1N HCl and extracted with ethyl acetate. The organic layer was washed by brine and dried over $MgSO_4$. The title ketone **4b** (0.28 g, 78 %) was obtained as a yellow oil after purification by column chromatography over silica gel (hexanes:ethyl acetate, 5:1) and was used directly in the next step. ^{1}H NMR ($CDCl_3$) δ: 2.53 (q, *J* = 7.5 Hz, 2H), 3.12 (t, *J* = 7.5 Hz, 2H), 5.01-5.12 (m, 2H), 5.85-5.93 (m, 1H), 7.63-7.81 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 27.9, 39.1, 111.0, 115.9, 118.1, 129.3, 132.3, 132.6, 135.3, 136.6, 140.0, 197.9.

Synthesis of compound 4. A stream of ozone was passed into a solution of ketone **4b** (0.18 g, 0.97 mmol) in dichloromethane (20 mL) at -78 ^{o}C for 15 min until the mixture turned blue. Dry nitrogen was passed for 5 min until the blue color had dissipated. The reaction mixture was concentrated, taken up in acetontirile (10 mL), and treated with a solution of NaH_2PO_4 (800 mg) in water (10 mL), with 35 % H_2O_2 (10 mL), and drop wise with a solution of sodium chlorite (590 mg) in water (10 mL). The reaction mixture was stirred overnight before Na_2SO_3 (10 g) was added. The reaction mixture was acidified to pH 1 by adding 1N HCl and extracted with ethyl acetate (25 mL × 3). The combined organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvents, the white crystalline **4** was obtained in quantitative yield (0.2g). Mp = 131-134 ^{o}C. ^{1}H NMR (CD_3OD) δ: 2.75 (t, *J* = 6.5 Hz, 2H), 3.33 (t, *J* = 6.5 Hz, 2H), 7.73- 8.20 (m, 4H); ^{13}C NMR (CD_3OD) δ: 27.5, 34.0, 110.2, 117.7, 129.7, 132.5, 132.8, 135.1, 139.3, 174.9, 197.6. ESI HRMS calculated for $C_{11}H_8NO_3$: 202.05097. Found: 202.05100.

Compound 5 (4-(2-trifluoromethylphenyl)-4-oxobutyric acid)

The synthesis procedure is summarized in Scheme S5





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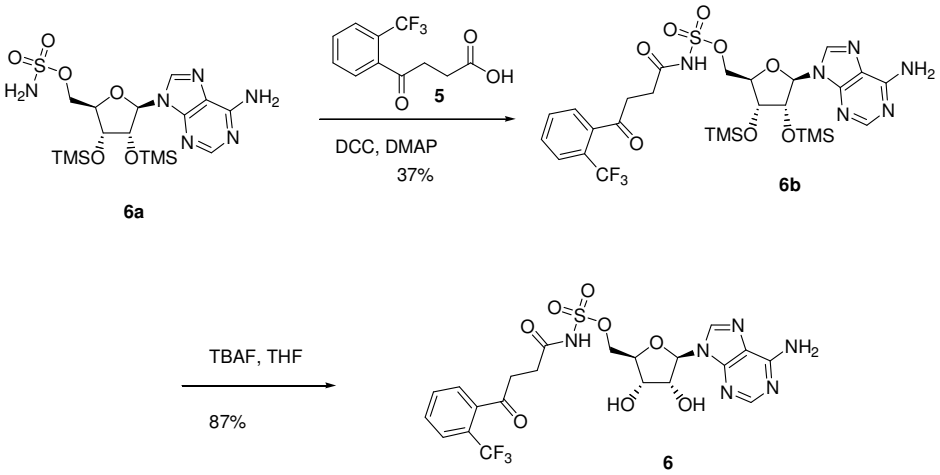
Synthesis of compound 5b. A solution of trifluoromethylacetophenone **5a** (1.25 g, 6.6 mmol) and allyl bromide (0.62 mL, 7.3 mmol) in toluene (20 mL) was treated with ^{t}BuOK (0.89 g, 7.3 mmol) and stirred at room temperature for 19 h, then heated to reflux for 1.5 h. Water was added to the cooled reaction mixture, which was then was extracted with ethyl acetate. The combined organic layer was washed by saturated ammonium chloride solution and brine, and dried over $MgSO_4$ After concentration, purification by column chromatography over silica gel (hexanes to hexanes:ethyl acetate, 20:1) gave **5b** (0.18 g, 12 %) as a colorless oil, which was used directly in the next step. ^{1}H NMR ($CDCl_3$) δ: 2.47 (q, *J* = 6.9 Hz, 2H), 2.94 (t, *J* = 7.2 Hz, 2H), 5.00-5.11 (m, 2H), 5.86 (ddt, *J* = 16.8, 10.5, 6.6 Hz, 1H), 7.41 (d, *J* = 6.9 Hz, 1H), 7.51-7.63 (m, 2H), 7.70 (d, *J* = 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 27.9, 42.5, 107.0, 115.8, 126.8, 126.9, 127.0, 130.1, 132.0, 136.8, 140.6, 203.7; ^{19}F NMR ($CDCl_3$) δ: 14.37.

Synthesis of compound 5. Alkene **5b** (0.22 g, 0.96 mmol) was converted to the product **5** (64 mg, 27 %) by ozonolysis and chlorate oxidation as described for **4**. After purification by column chromatography over silica gel (hexanes:ethyl acetate, 1:4 to ethyl acetate) it was obtained as a white solid Mp = 65-69 ^{o}C. ^{1}H NMR ($CDCl_3$) δ: 2.83, (t, *J* = 6.5 Hz, 2H), 3.16 (t, *J* = 6.5 Hz, 2H), 7.53-7.72 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 27.9, 37.5, 122.5, 124.6, 126.7, 127.3, 130.3, 132.0, 139.7, 178.6, 202.1; ^{19}F NMR ($CDCl_3$) δ: 14.25. ESI HRMS calculated for $C_{11}H_8F_3O_3$: 245.0431. Found: 249.0431.

Compound 6 (acyl sulfamoyl adenosine, Acyl-AMS)

The synthesis procedure is summarized in Scheme S6



Synthesis of compound 6b. Sulfonamide adenosine **6a** (*8*) (115 mg, 0.20 mmol) was added to a stirred solution of carboxylic acid **5** (55 mg, 0.22 mmol), DCC (54 mg, 0.26 mmol), and DMAP (2 mg, 0.02 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 ^oC under argon. After stirring overnight the reaction mixture was filtered and the filter cake was washed by CH_2Cl_2. The combined filtrates and washings were concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (dichloromethane: methanol, 20:1) to give **6b** (59 mg, 37%), which was used directly in the next step. ^1H NMR (500 MHz, CH_3OD) δ: -0.37 (s, 3H), -0.07 (s, 3H), 0.11 (s, 3H), 0.12 (s, 3H), 0.71 (s, 9H), 0.93 (s, 9H), 2.67 (t, *J* = 6.5 Hz, 2H), 3.17 (t, *J* = 6.5 Hz, 2H), 3.30 (m, 2H), 4.27 (m, 1H), 4.32 (dd, *J* = 4.0, 11.0 Hz, 1H), 4.39 (m, 2H), 4.83 (dd, *J* = 4.5, 7.5 Hz, 1H), 6.08 (d, *J* = 7.0 Hz, 1H), 7.59 (t, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.71-7.77 (m, 2H), 8.18 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (125 MHz, CH_3OD) δ: -5.8, -5.7, 17.3, 17.5, 24.8 (3C), 25.0 (3C), 32.4, 38.6, 68.2, 73.1, 75.8, 84.3, 87.2, 118.8, 126.2, 127.6, 128.2 (q, *J* = 195 Hz, 1C), 129.9, 130.3, 132.0, 132.1, 140.1 (q, *J* = 70 Hz, 1C), 149.6, 152.6, 155.9, 180.0, 203.9.

Synthesis of compound 6. A 1.0 M TBAF solution in THF (0.10 mL, 0.10 mmol) was added to a stirred solution of **6b** (15 mg, 0.02 mmol) in THF (2 mL) at room temperature. After stirring for 4 h the solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (dichloromethane: methanol, 10:1) then RP-HPLC (Acetonitrile: water, 3:97 to 50:50) to give the nucleoside analog **6** (10 mg, 87%). ^1H NMR (500 MHz, CH_3OD) δ: 2.68 (t, *J* = 6.6 Hz, 2H), 3.15 (t, *J* = 6.7 Hz, 2H), 4.29 (m, 1H), 4.35-4.38 (m, 3H), 4.65 (t, *J* = 6.0Hz, 1H), 6.07 (d, *J* = 6.0Hz, 1H), 7.64-7.78 (m, 4H), 8.19 (s, 1H), 8.47 (s, 1H); ^{13}C NMR (125 MHz, CH_3OD) δ: 32.4, 42.1, 67.7, 71.0, 74.7, 83.2, 87.7, 126.0, 127.6, 129.8, 131.9, 134.1, 139.7, 143.2, 152.4, 179.8, 203.7; ESI HRMS calculated for $C_{21}H_{20}F_3N_6O_8S$ (*7*): 573.10154. Found 573.1028.

TABLES

Table S1. Effect of Mg^{2+} on *B. anthracis* OSB-CoA synthetase activity[a]

Metal ion	Specific activity (μmol/min/mg) at different concentration of Mg^{2+}				
	0 mM	0.5 mM	1 mM	2 mM	4 mM
Mg^{2+}	0.02 ± 0.006	2.25 ± 0.07	2.39 ± 0.06	2.39 ± 0.05	1.82 ± 0.15

[a] Data presented as the mean ± standard deviation of a triplicate measurement.

Table S2. Goodness-of-fit of the experimental data to various equations in the bisubstrates initial velocity studies[a]

Kinetic mechanisms	AIC_C	Mean ± standard error of K_m (μM)			Best fit
		OSB	ATP	CoA	
OSB = 8 μM, CoA vs ATP (Figure S3A in the report)					
Ping-pong (equation 2)	-295.9	-	17.8 ± 1.3	229 ± 19.9	Yes
Rapid equilibrium ordered (equation 3)	-193.1	-	0.3 ± 2.3	20916 ± 159600	No
Steady-state ordered (equation 4)	-293.9	-	1.2 ± 1.7	15.9 ± 22.7	No
Rapid equilibrium random (equation 5)	-293.9	-	16.6 ± 23.5	219 ± 313	No
ATP = 8 μM, CoA vs OSB (Figure S3B in the report)					
Ping-pong (equation 2)	-382.9	10.4 ± 0.8	-	164 ± 15.8	Yes
Rapid equilibrium ordered (equation 3)	-250.0	2.9 ± 0.9	-	605 ± 265	No
Steady-state ordered (equation 4)	-326.3	0.4 ± 1.0	-	6.3 ± 17.1	No
Rapid equilibrium random (equation 5)	-326.3	4.1 ± 25.4	-	160 ± 434	No
CoA = 256 μM, ATP vs OSB (Figure S3C in the report)					
Ping-pong (equation 2)	-731.6	2.6 ± 0.2	8.2 ± 0.7	-	No
Rapid equilibrium ordered (equation 3)	-713.8	48.5 ± 10.4	3.2 ± 0.5	-	No
Steady-state ordered (equation 4)	-780.4	1.5 ± 0.3	5.2 ± 1.3	-	Yes
Rapid equilibrium random (equation 5)	-780.4	14.7 ± 3.2	51.1 ± 12.6	-	Yes
OSB = 64 μM, CoA vs ATP (Figure 2A in the report)					
Ping-pong (equation 2)	-326.2	-	10.6 ± 0.7	279 ± 19.3	Yes
Rapid equilibrium ordered (equation 3)	-171.9	-	0.3 ± 2.3	141400±9754000	No
Steady-state ordered (equation 4)	-323.5	-	8.8 ± 29.0	272 ± 776	No
Rapid equilibrium random (equation 5)	-323.5	-	0.4 ± 0.9	9.3 ± 26.5	No
ATP = 128 μM, CoA vs OSB (Figure 2B in the report)					
Ping-pong (equation 2)	-264.5	5.8 ± 0.5	-	269 ± 24.9	Yes
Rapid equilibrium ordered (equation 3)	-168.5	0.005 ± 0.7	-	35656 ± 481300	No
Steady-state ordered (equation 4)	-262.2	5.2 ± 5.2	-	244 ± 248	No
Rapid equilibrium random (equation 5)	-262.2	33.9 ± 34.6	-	0.7 ± 0.7	No
CoA = 1024 μM, ATP vs OSB (Figure 2C in the report)					
Ping-pong (equation 2)	-275.0	10.6 ± 1.2	13.5 ± 1.3	-	No
Rapid equilibrium ordered (equation 3)	-221.2	0.029 ± 1.4	18853 ± 944400	-	No
Steady-state ordered (equation 4)	-313.6	1.4 ± 0.3	5.1 ± 1.2	-	Yes
Rapid equilibrium random (equation 5)	-313.6	23.6 ± 5.4	41.5 ± 13.0	-	Yes

[a]AIC_C refers to Akaike Information Criterion corrected for small sample size. If the absolute difference between the lowest AIC_C values and the second lowest AIC_C value is more than 7, the equation with the lowest AIC_C values is the best fit. If the absolute difference is smaller than 7, the equation with the lowest AIC_C vaules that gives the best estimate of the kinetic parameters is the best fit.

Table S3. Goodness of fit of the experimental data to various equations in the product inhibition studies[a].

OSB = 8 μM, CoA = 128 μM, ATP vs AMP (Figure 4A in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (mM)	αK_i (mM)	
Competitive (equation 8)	-476.6	13.1 ± 0.9	3.6 ± 0.4	-	Yes
Uncompetitive (equation 9)	-400.0	25.8 ± 2.3	-	22.1 ± 3.2	No
Mixed-type (equation 10)	-465.9	13.9 ± 1.1	4.1 ± 0.6	204 ± 29.9	No
Non-competitive (equation 11)	-425.4	22.9 ± 1.6	-	25.7 ± 2.8	No
OSB = 8 μM, ATP = 16 μM, CoA vs AMP (Figure 4B in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (mM)	αK_i (mM)	
Competitive (equation 8)	-351.8	9.3 ± 8.7	0.4 ± 0.4	-	No
Uncompetitive (equation 9)	-488.7	49.9 ± 4.9	-	10.3 ± 0.6	Yes
Mixed-type (equation 10)	-475.3	49.4 ± 5.8	292 ± 1955	14576 ± 97378	No
Non-competitive (equation 11)	-463.1	38.9 ± 4.0	-	12.2 ± 0.7	No
OSB = 8 μM, ATP = 16 μM, CoA vs benzoyl-CoA (Figure 4C in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (mM)	αK_i (mM)	
Competitive (equation 8)	-494.2	54.5 ± 5.4	1.9 ± 0.2	-	No
Uncompetitive (equation 9)	-431.8	127 ± 12.4	-	13.8 ± 1.5	No
Mixed-type (equation 10)	-504.6	66.2 ± 6.3	3.0 ± 0.5	15.3 ± 2.6	Yes
Non-competitive (equation 11)	-460.8	110 ± 8.4	-	16.7 ± 1.3	No
ATP = 16 μM, CoA = 128 μM, OSB vs AMP (Figure 4D in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (mM)	αK_i (mM)	
Competitive (equation 8)	-504.9	1.9 ± 0.2	1.9 ± 0.2	-	No
Uncompetitive (equation 9)	-475.6	4.8 ± 0.4	-	14.7 ± 1.3	No
Mixed-type (equation 10)	-576.4	2.8 ± 0.2	4.6 ± 0.6	34.5 ± 4.5	Yes
Non-competitive (equation 11)	-524.7	4.1 ± 0.2	-	18.2 ± 1.1	No
OSB = 8 μM, ATP = 16 μM, CoA vs TFMP-butyl-AMS (Figure 6A in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (μM)	αK_i (μM)	
Competitive (equation 8)	-437.5	8.8 ± 4.8	0.7 ± 0.4	-	No
Uncompetitive (equation 9)	-716.3	39.3 ± 1.8	-	8.9 ± 0.5	Yes
Mixed-type (equation 10)	-719.0	37.3 ± 2.0	54.1 ± 27.2	8.6 ± 4.4	No
Non-competitive (equation 11)	-636.4	29.1 ± 2.0	-	16.4 ± 0.8	No
OSB = 8 μM, CoA = 128 μM, ATP vs TFMP-butyl-AMS (Figure 6B in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (μM)	αK_i (μM)	
Competitive (equation 8)	-682.9	5.1 ± 0.4	5.6 ± 0.6	-	No
Uncompetitive (equation 9)	-535.0	12.6 ± 1.2	-	69.4 ± 8.5	No
Mixed-type (equation 10)	-690.6	5.4 ± 0.5	5.2 ± 0.8	108 ± 16.7	Yes
Non-competitive (equation 11)	-571.5	11.4 ± 0.9	-	78.1 ± 7.6	No
ATP = 16 μM, CoA = 128 μM, OSB vs TFMP-butyl-AMS (Figure 6C in the report)					
Kinetic mechanisms	AIC_C	Mean ± standard error of			Best fit
		K_m (μM)	K_i (μM)	αK_i (μM)	
Competitive (equation 8)	-649.0	2.8 ± 0.2	4.5 ± 0.5	-	No
Uncompetitive (equation 9)	-604.8	5.8 ± 0.4	-	38.7 ± 3.4	No

Mixed-type (equation 10)	-730.7	3.6 ± 0.2	5.6 ± 0.8	24.6 ± 3.5	Yes
Non-competitive (equation 11)	-649.9	5.2 ± 0.3	-	44.7 ± 3.1	No

[a]AIC_C refers to Akaike Information Criterion corrected for small sample size. If the absolute difference between the lowest AIC_C values and the second lowest AIC_C value is more than 7, the equation with the lowest AIC_C values is the best fit. If the absolute difference is smaller than 7, the equation with the lowest AIC_C vaules that gives the best estimate of the kinetic parameters is the best fit.

Table S4. The kinetic constants of the coupled enzymes

	K_m (mM)	V_{max} (units / mL)	references[a]
IPP	~ 0.01	10	(*9*)
PNP	~ 0.026	25	(*10*)

[a] Only the K_m values were obtained from the references.

FIGURES

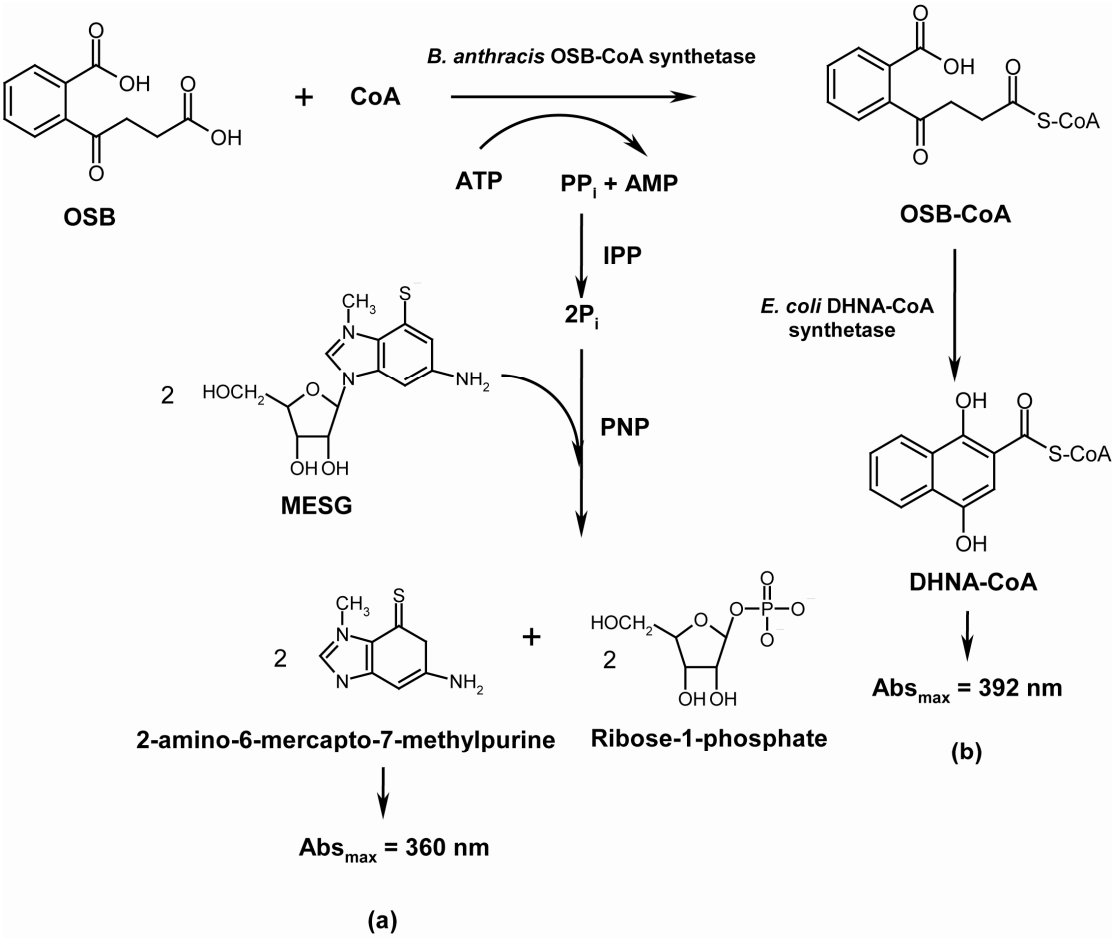


Figure S1. Coupled enzyme assays utilized for the detection of *B. anthracis* OSB-CoA synthetase activity. (a) The product of the first half-reaction, PP_i, is coupled to the reaction of inorganic pyrophosphatase (IPP) which cleaves PP_i into two molecules of inorganic phosphate (P_i). The production of P_i is coupled to the reaction of purine nucleoside phosphorylase (PNP) which converts 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG) to ribose-1-phosphate and 2-amino-6-mercapto-7-methylpurine which absorbes strongly at 360 nm ($\varepsilon = 11{,}000\ M^{-1}cm^{-1}$). (b) The product of the overall reaction of OSB-CoA synthetase, OSB-CoA, is coupled to the reaction catalyzed by DHNA-CoA which is the next reaction in the menaquinone biosynthetic pathway. The DHNA-CoA synthetase reaction produces DHNA-CoA which absorbs strongly at 392 nm ($\varepsilon = 4{,}000\ M^{-1}cm^{-1}$).

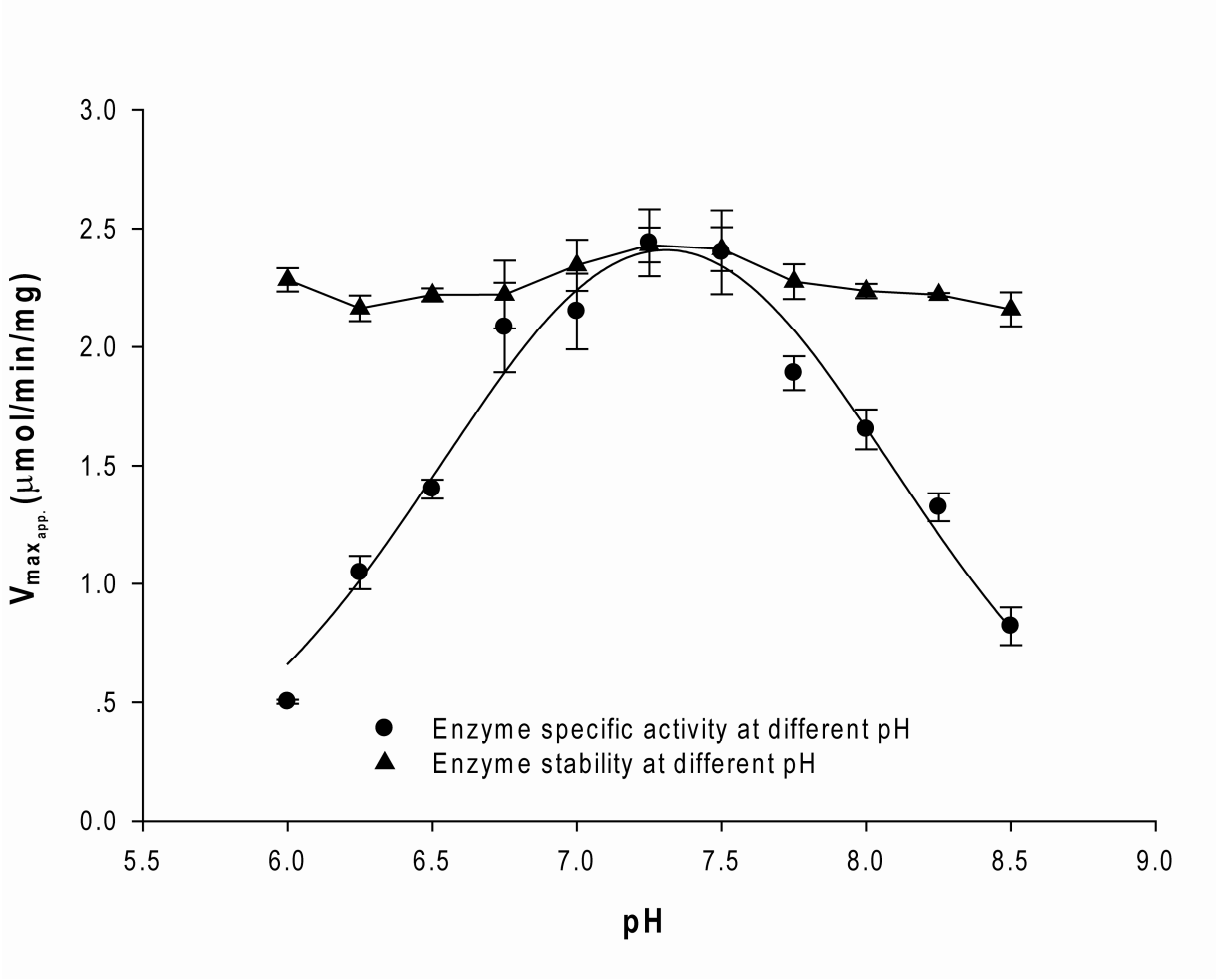


Figure S2. ***B. anthracis*** **OSB-CoA synthetase stability and activity vs pH**. Each data point in the figures represents the average of a triplicate test with standard deviation as the error bar. The enzyme is stable in the pH range tested and the enzyme shows the highest activity at pH 7.25-7.5.

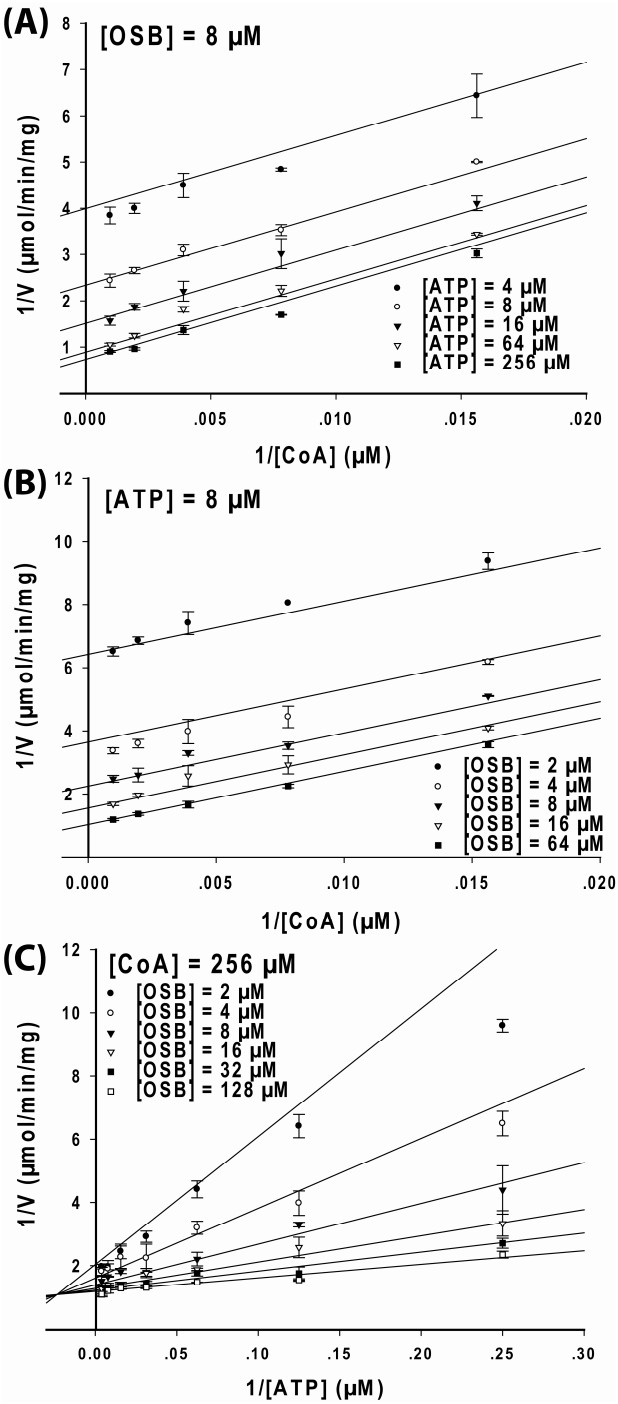


Figure S3. Initial velocity study of *B. anthracis* OSB-CoA synthetase catalyzed reaction. The kinetic data were displayed as Lineweaver-Burk plots of the reaction rate vs substrate concentrations. For each plot, one substrate was kept at constant level while varying the other two substrate concentrations. (A) OSB was kept constant at 8 µM while varying CoA and ATP concentrations (●=4, ○=8, ▼=16, □=64, ■=256 µM). (B) ATP was kept constant at 8 µM while varying CoA and OSB concentrations (●=2, ○=4, ▼=8, □=16, ■=64 µM). (C) CoA was kept constant at 256 µM while varying ATP and OSB concentrations (●=2, ○=4, ▼=8, □=16, ■=32, □=128 µM). Each data point in the figures represents the mean of a duplicate test. The upper and lower bars represent the duplicate measurements. The kinetic parameters and patterns are summarized in Table 1.

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