

SUPPORTING INFORMATION

Aryl Acid Adenylating Enzymes Involved in Siderophore Biosynthesis: Fluorescence Polarization Assay, Ligand Specificity, and Discovery of Non-nucleoside Inhibitors via High-Throughput Screening

João Neres,¹ Daniel J. Wilson,¹ Laura Celia,² Brian J. Beck,² Courtney C. Aldrich^{1}*

1. Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, Minnesota 55455, USA.

2. Bacteriology Program, American Type Culture Collection, Manassas, Virginia 20110, USA.

Chemistry. All commercial reagents (Sigma-Aldrich, Acros) were used as provided unless otherwise indicated. Sulfamoyl chloride was prepared by the method of Heacock except that this was used directly without recrystallization (40). An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, DMF and CH₂Cl₂ and the solvents were dispensed under argon. Anhydrous DME was purchased from Aldrich and used as provided. Flash chromatography was performed on an ISCO Combiflash Companion® purification system with prepacked silica gel cartridges and the indicated solvent system. All reactions were performed under an inert atmosphere of dry Ar or N₂ in oven-dried (150 °C) glassware. ¹H, ¹³C, [¹H, ¹H]-gCOSY, [¹H, ¹³C]-gHMQC, and [¹H, ¹³C]-gHMBC experiments were recorded on a Varian 600 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm) or methanol (3.31 ppm), and carbon chemical shifts are reported using an internal standard of residual chloroform (77.0 ppm) or methanol (49.1 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s =

singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad), coupling constant, and integration. High-resolution mass spectra were obtained on Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. Preparative HPLC was performed on a Varian Microsorb MV 100-8 C18 column (41.4 × 250 mm, 8 μm particle size) operating at 40 mL/min with detection at 254 nm.

2'-O-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}adenosine (9). Adenosine **7** (300 mg, 1.12 mmol, 1.0 equiv.) was suspended in dry DMF (5 mL) at 0 °C and sodium hydride (63 mg 60% oil suspension, 1.57 mmol, 1.4 equiv.) was added. The mixture was stirred for 30 min at 0 °C and a solution of 2-[2-(2-azidoethoxy)ethoxy]-1-bromoethane **8** (41, 42) (400 mg, 1.68 mmol, 1.5 equiv.) in DMF (10 mL) was added. The mixture was stirred at 23 °C for 20 h, then MeOH (5 mL) was added and stirring continued for 30 min. The solvent was removed under reduced pressure. Purification by flash chromatography (0–10% MeOH/CH₂Cl₂) afforded the title compound as a thick oil (140 mg, 29%): *R_f* 0.41 (80:20 EtOAc/MeOH); ¹H NMR (600 MHz, CD₃OD) δ 3.34 (t, *J* = 4.8 Hz, 2H, H-6''), 3.49–3.60 (m, 8H, 2 × H-2'', 2 × H-3'', 2 × H-4'', 2 × H-5''), 3.66–3.69 (m, 1H, H-1''), 3.73–3.77 (m, 2H, H-1'', H-5'), 3.88 (dd, *J* = 12.6, 2.4 Hz, 1H, H-5'), 4.18–4.19 (m, 1H, H-4'), 4.47–4.49 (m, 1H, H-3'), 4.67 (t, *J* = 5.4 Hz, 1H, H-2'), 6.07 (d, *J* = 6.6 Hz, 1H, H-1'), 8.19 (s, 1H, H-8), 8.32 (s, 1H, H-2); gHMBC (600 MHz, CD₃OD) correlation from C-2' at 83.6 ppm to H-1'' at 3.66–3.69 ppm; ¹³C NMR (150 MHz, CD₃OD) δ 51.8 (C-6''), 63.5 (C-5'), 71.16, 71.24, 71.38, 71.42, 71.6, 71.7 (C-3', C-1'', C-2'', C-3'', C-4'', C-5''), 83.6 (C-2'), 88.5 (C-4'), 89.6 (C-1'), 121.1 (C-5), 142.2 (C-8), 150.1 (C-4), 153.7 (C-2), 157.7 (C-6); HRMS (ESI+) calcd for C₁₆H₂₅N₈O₆ [M + H]⁺ 425.1892, found 425.1901 (error 2.1 ppm).

2'-O-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}-3',5'-O-di(tert-butyldimethylsilyl)adenosine (10). To a solution of compound **9** (138 mg, 0.33 mmol, 1.0 equiv.), imidazole (135 mg, 1.98 mmol, 6.0 equiv.) and DMAP (6.1 mg, 0.05 mmol, 0.15 equiv.) in DMF (5 mL) at 0 °C was added a solution of TBDMSCl

(125 mg, 0.83 mmol, 2.5 equiv.) in DMF (5 mL) and the reaction stirred for 26 h at 23 °C. The reaction mixture was diluted with EtOAc (50 mL) and washed successively with H₂O (2 × 50 mL) and saturated aqueous NaCl (50 mL), then the organic layer was dried (MgSO₄) and concentrated under reduced pressure. Purification by flash chromatography (EtOAc) afforded the title compound as a thick oil (149 mg, 70%): *R_f* 0.23 (EtOAc); ¹H NMR (600 MHz, CDCl₃) δ 0.075 (s, 3H), 0.079 (s, 3H), 0.096 (s, 3H), 0.103 (s, 3H), 0.904 (s, 9H), 0.906 (s, 9H), 3.33 (t, *J* = 5.4 Hz, 2H), 3.53–3.63 (m, 8H), 3.73–3.77 (m, 3H), 3.96 (dd, *J* = 11.4, 3.6 Hz, 1H), 4.10 (dd, *J* = 7.8, 3.6 Hz, 1H), 4.39 (t, *J* = 4.2 Hz, 1H), 4.52 (t, *J* = 4.8 Hz, 1H), 6.06 (br s, 2H, NH₂), 6.14 (d, *J* = 3.6 Hz, 1H), 8.16 (s, 1H), 8.32 (s, 1H); ¹³C NMR (150 MHz, CDCl₃) δ -5.2, -5.1, -4.6, -4.4, 18.4, 18.7, 26.0, 26.2, 50.9, 62.2, 70.2, 70.3, 70.5, 70.76, 70.84, 70.9, 82.6, 85.2, 87.1, 120.3, 139.7, 149.9, 153.2, 155.8; HRMS (ESI+) calcd for C₂₈H₅₃N₈O₆Si₂ [M + H]⁺ 653.3621, found 653.3631 (error 1.5 ppm).

2'-O-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}-3'-O-(tert-butyldimethylsilyl)adenosine (11). To a solution of **10** (149 mg, 0.23 mmol) in THF (5 mL) at 0 °C was added a 50% aqueous solution of TFA (5 mL). The solution was stirred at 0 °C for 1.5 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (0–10% MeOH/EtOAc) affording the title compound as a thick oil (93 mg, 75%): *R_f* 0.49 (90:10 EtOAc/MeOH); ¹H NMR (600 MHz, CD₃OD) δ 0.158 (s, 3H), 0.162 (s, 3H), 0.95 (s, 9H), 3.29–3.30 (m, 2H), 3.38–3.46 (m, 4H), 3.48 (t, *J* = 4.8 Hz, 2H), 3.51–3.56 (m, 3H), 3.67 (dt, *J* = 10.8, 4.2 Hz, 1H), 3.71 (dd, *J* = 12.6, 2.4 Hz, 1H), 3.86 (dd, *J* = 12.6, 2.4 Hz, 1H), 4.13–4.14 (m, 1H), 4.58 (dd, *J* = 6.0, 1.8 Hz, 1H), 4.68 (dd, *J* = 6.0, 4.8 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.18 (s, 1H), 8.34 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ -4.7, -4.5, 19.1, 26.3, 51.7, 63.1, 71.1, 71.2, 71.4, 71.6, 71.7, 72.8, 83.1, 89.2, 89.4, 121.0, 142.3, 150.1, 153.5, 157.5; HRMS (ESI+) calcd for C₂₂H₃₉N₈O₆Si [M + H]⁺ 539.2756, found 539.2774 (error 3.3 ppm).

2'-O-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}-3'-O-(tert-butyldimethylsilyl)-5'-O-

(sulfamoyl)adenosine (12). To a solution of **11** (93 mg, 0.17 mmol) in dimethylacetamide (5 mL), at 0 °C, was added sulfamoyl chloride (30 mg, 0.26 mmol) and the reaction stirred for 30 min at 0 °C, then at 23 °C for 16 h. The reaction was quenched with MeOH (10 mL) then concentrated under reduced pressure. Purification by flash chromatography (0–10% MeOH/EtOAc) afforded the title compound as a thick oil (96 mg, 92%): R_f 0.56 (90:10 EtOAc/MeOH); ^1H NMR (600 MHz, CD_3OD) δ 0.179 (s, 3H), 0.183 (s, 3H), 0.96 (s, 9H), 3.32 (t, $J = 5.4$ Hz, 2H), 3.50–3.54 (m, 4H), 3.57–3.60 (m, 4H), 3.68–3.72 (m, 1H), 3.74–3.77 (m, 1H), 4.30–4.32 (m, 2H), 4.41 (dd, $J = 4.8, 6.0$ Hz, 1H), 4.63–4.67 (m, 2H), 6.20 (d, $J = 4.8$ Hz, 1H), 8.31 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ -4.7, -4.5, 18.9, 26.2, 51.7, 68.9, 71.1, 71.4 (2 C), 71.6, 71.8, 72.0, 83.2, 84.6, 88.8, 120.6, 143.7, 146.4, 150.0, 152.4; HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{40}\text{N}_9\text{O}_8\text{SSi}$ $[\text{M} + \text{H}]^+$ 618.2484, found 618.2461 (error 3.7 ppm).

2'-O-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}-3'-O-(tert-butyldimethylsilyl)-5'-O-{N-[2-

(methoxymethoxy)benzoyl]sulfamoyl}adenosine (14). To a solution of **12** (61 mg, 0.10 mmol) in DMF (10 mL) at 23 °C was added *N*-hydroxysuccinimidyl 2-methoxymethoxybenzoate **13** (**22**) (42 mg, 0.15 mmol) and Cs_2CO_3 (98 mg, 0.30 mmol) and the reaction stirred 16 h. The reaction mixture was filtered and the solids washed with additional DMF (5 mL). The filtrate was concentrated under reduced pressure ($P = 10$ mbar, $T = 35$ °C). Purification of the residue by flash chromatography (89.5:10:0.5 EtOAc/MeOH/ Et_3N) afforded the title compound (60 mg, 69%): R_f 0.64 (80:20 EtOAc/MeOH); ^1H NMR (600 MHz, CD_3OD) δ 0.16 (s, 3H), 0.17 (s, 3H), 0.96 (s, 9H), 3.29 (t, $J = 4.8$ Hz, 2H), 3.42–3.46 (m, 7H), 3.49–3.52 (m, 2H), 3.55 (t, $J = 4.8$ Hz, 2H), 3.59–3.63 (m, 1H), 3.69–3.73 (m, 1H), 4.31–4.33 (m, 1H), 4.37 (dd, $J = 11.4, 3.0$ Hz, 1H), 4.42 (dd, $J = 11.4, 4.2$ Hz, 1H), 4.70 (dd, $J = 4.8, 1.8$ Hz, 1H), 4.73 (dd, $J = 6.6, 4.8$ Hz, 1H), 5.17 (s, 2H), 6.20 (d, $J = 6.6$ Hz, 1H), 6.96 (t, $J = 7.2$ Hz, 1H), 7.11 (d, $J = 8.4$ Hz, 1H), 7.26 (dt, $J = 8.4, 1.8$ Hz, 1H), 7.49 (dd, $J = 7.2, 1.8$ Hz, 1H), 8.20 (s, 1H), 8.59 (s, 1H);

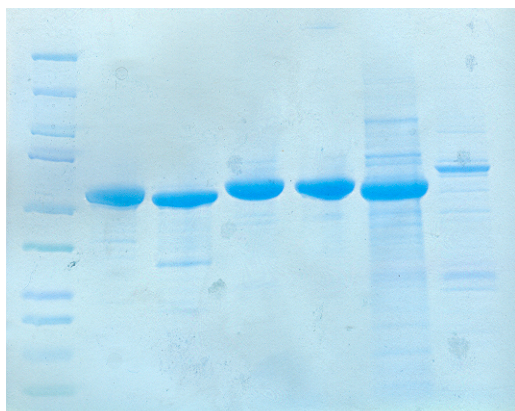
^{13}C NMR (150 MHz, CD_3OD) δ -4.6, -4.4, 19.1, 26.4, 51.7, 56.6, 69.2, 71.1, 71.3, 71.4, 71.5, 71.6, 73.0, 83.4, 86.1, 87.2, 96.6, 117.4, 120.2, 122.6, 130.1, 131.1, 132.5, 141.5, 151.0, 153.9, 156.0, 157.3, 176.6; HRMS (ESI $^-$) calcd for $\text{C}_{31}\text{H}_{46}\text{N}_9\text{O}_{11}\text{SSi}$ $[\text{M} - \text{H}]^-$ 780.2812, found 780.2814 (error 0.3 ppm).

2'-O-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}-3'-O-(tert-butyldimethylsilyl)-5'-O-[N-[2-(methoxymethoxy)benzoyl]sulfamoyl]adenosine (15). To a solution of **14** (40 mg, 0.051 mmol) in EtOAc (5 mL) was added 10% Pd/C (15 mg). The resulting suspension was shaken in a Parr hydrogenation apparatus under H_2 (40 psi) for 5 h. The reaction mixture was filtered through a bed of Celite, which was further washed with EtOAc (10 mL). The combined filtrate was concentrated under reduced pressure. Purification by flash chromatography afforded the title compound (20 mg, 66%) as a white solid: R_f 0.15 (50:50 EtOAc/MeOH); ^1H NMR (600 MHz, CD_3OD) δ 0.17 (s, 3H), 0.18 (s, 3H), 0.97 (s, 9H), 3.09–3.11 (m, 2H), 3.37–3.57 (m, 9H), 3.63–3.69 (m, 4H), 4.34–4.36 (m, 1H), 4.37–4.39 (m, 2H), 4.69 (dd, $J = 4.8, 1.8$ Hz, 1H), 4.77 (dd, $J = 7.2, 4.8$ Hz, 1H), 5.16 (s, 2H), 6.22 (d, $J = 7.2$ Hz, 1H), 6.97 (t, $J = 7.8$ Hz, 1H), 7.12 (d, $J = 7.8$ Hz, 1H), 7.28 (td, $J = 7.8, 1.2$ Hz, 1H), 7.47 (dd, $J = 7.8, 1.2$ Hz, 1H), 8.20 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ -4.5, -4.4, 19.1, 26.3, 40.8, 56.6, 68.1, 69.8, 70.7, 71.3, 71.7, 72.1, 73.2, 83.8, 86.2, 87.0, 96.7, 117.5, 120.1, 122.7, 130.1, 131.3, 132.4, 141.5, 151.0, 154.0, 155.8, 157.4, 176.7; HRMS (ESI $^-$) calcd for $\text{C}_{31}\text{H}_{48}\text{N}_7\text{O}_{11}\text{SSi}$ $[\text{M} - \text{H}]^-$ 754.2907, found 754.2913 (error 0.8 ppm).

2'-O-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}-5'-O-[N-(2-hydroxybenzoyl)sulfamoyl]adenosine (16). To compound **15** (20 mg, 0.026 mmol) was added 80% aqueous TFA (2.5 mL). The resulting solution was stirred for 4 h at 23 °C, then concentrated under reduced pressure to afford the title compound (15 mg, 97%) as a white solid: ^1H NMR (600 MHz, CD_3OD) δ 3.16 (t, $J = 4.8$ Hz, 2H), 3.60–3.66 (m, 6H), 3.70–3.73 (m, 2H), 3.79–3.85 (m, 2H), 4.37 (d, $J = 3.6$ Hz, 1H), 4.55–4.57 (m, 3H), 4.65 (t, $J = 4.8$ Hz, 1H), 6.20 (d, $J = 4.8$ Hz, 1H), 6.85–6.89 (m, 2H), 7.39 (t, $J = 7.8$ Hz, 1H), 7.81 (d, J

= 7.8 Hz, 1H), 8.33 (s, 1H), 8.57 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 40.7, 67.9, 71.1, 71.14, 71.15, 71.40, 71.44, 71.6, 83.6, 84.3, 88.5, 118.15, 118.24, 120.43, 120.47, 131.3, 135.9, 143.7, 146.1, 150.0, 152.4, 160.5, 170.1; HRMS (ESI $^-$) calcd for $\text{C}_{23}\text{H}_{30}\text{N}_7\text{O}_{10}\text{S}$ [$\text{M} - \text{H}$] $^-$ 596.1780, found 596.1778 (error 0.3 ppm).

2'-O-{2-[2-(2-[(Fluorescein-5-yl)carbonyl]amino)ethoxy]ethoxy}ethoxy}-5'-O-[N-(2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (6). To a solution of **16** (12 mg, 0.02 mmol) in DMF (4 mL) were added triethylamine (12 μL , 0.09 mmol, 4.5 equiv.) and 5-carboxyfluorescein *N*-hydroxysuccinimide ester **17** (12 mg, 0.025 mmol, 1.25 equiv.) and the reaction stirred 4 h at 23 $^\circ\text{C}$. The solvent was removed under reduced pressure and the crude residue purified by HPLC using a Varian Dynamax Microsorb 100-8 C18 column (250 \times 41.4 mm) and a gradient of 20–80% MeOH/50 mM triethylammonium bicarbonate over 25 min. The retention time of the product was 17.7 min and the respective fractions were pooled and lyophilized to afford the title compound (9.5 mg, 45%) as an orange solid: ^1H NMR (600 MHz, CD_3OD) δ 1.21 (t, $J = 7.2$ Hz, 9H), 3.08 (q, $J = 7.2$ Hz, 6H), 3.53–3.66 (m, 10H), 3.75 (t, $J = 4.2$ Hz, 2H), 4.23–4.25 (m, 1H), 4.32–4.37 (m, 2H), 4.52 (dd, $J = 4.8, 1.8$ Hz, 1H), 4.73 (dd, $J = 7.2, 4.8$ Hz, 1H), 6.14 (d, $J = 7.2$ Hz, 1H), 6.52 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.58 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.60 (d, $J = 2.4$ Hz, 1H), 6.62 (d, $J = 2.4$ Hz, 1H), 6.74–6.79 (m, 2H), 6.95 (d, $J = 9.0$ Hz, 1H), 6.98 (d, $J = 9.0$ Hz, 1H), 7.27 (dt, $J = 7.8, 1.8$ Hz, 1H), 7.41 (d, $J = 7.8$ Hz, 1H), 7.94 (dd, $J = 7.8, 1.8$ Hz, 1H), 8.05–8.07 (m, 2H), 8.48 (d, $J = 1.8$ Hz, 1H), 8.54 (s, 1H); HRMS (ESI $^-$) calcd for $\text{C}_{44}\text{H}_{40}\text{N}_7\text{O}_{16}\text{S}$ [$\text{M} - \text{H}$] $^-$ 954.2258, found 954.2263 (error 0.5 ppm).



1 2 3 4 5 6 7

Figure S1. SDS-PAGE Gel of AAAEs used in this study. A 4–20% polyacrylamide gel followed by staining with Coomassie Brilliant Blue R-250 in 45% methanol, 45% water, and 10% glacial acetic acid solution and destain. Lane 1: Precision Plus Protein Standards (Bio-Rad) 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa. Lane 2: YbtE. Lane 3: EntE. Lane 4: VibE. Lane 5: BasE. Lane 6: MbtA. Lane 7: DhbE.

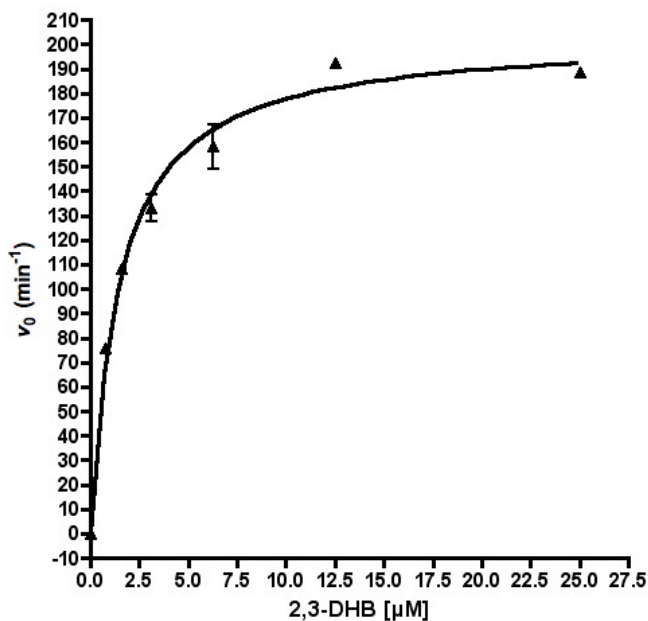


Figure S2. Normalized plot used to determine apparent steady state kinetic parameters for BasE with 2,3-dihydroxybenzoic acid (2,3-DHB).

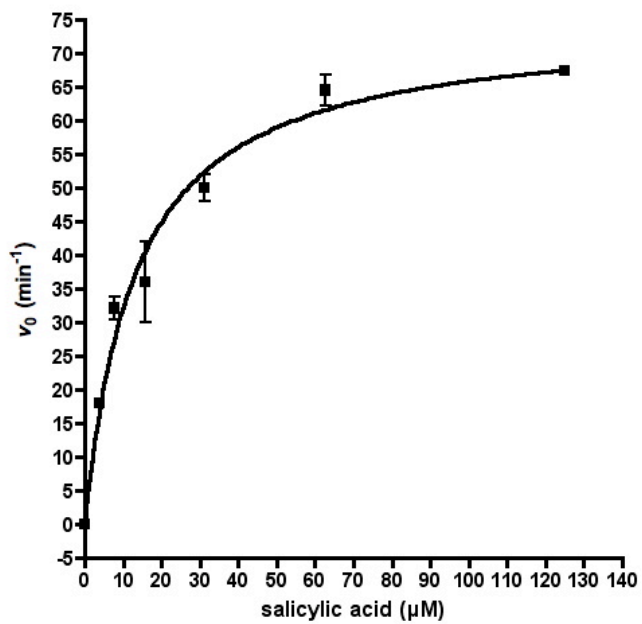


Figure S3. Normalized plot used to determine apparent steady state kinetic parameters for BasE with salicylic acid.