SUPPORTING INFORMATION

Quantitative 3D Structure Linear Interaction Energy Model of 5'-*O*-[*N*-(Salicyl)sulfamoyl]adenosine and the Aryl Acid Adenylating Enzyme MbtA

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A. HPLC purity and conditions for compound S8.

Table S1. HPLC purity and methods

Compound	Conditions	Retention time (min)	Purity (%)
S8	Method 1	6.45	98.0
	Method 2	5.52	96.5

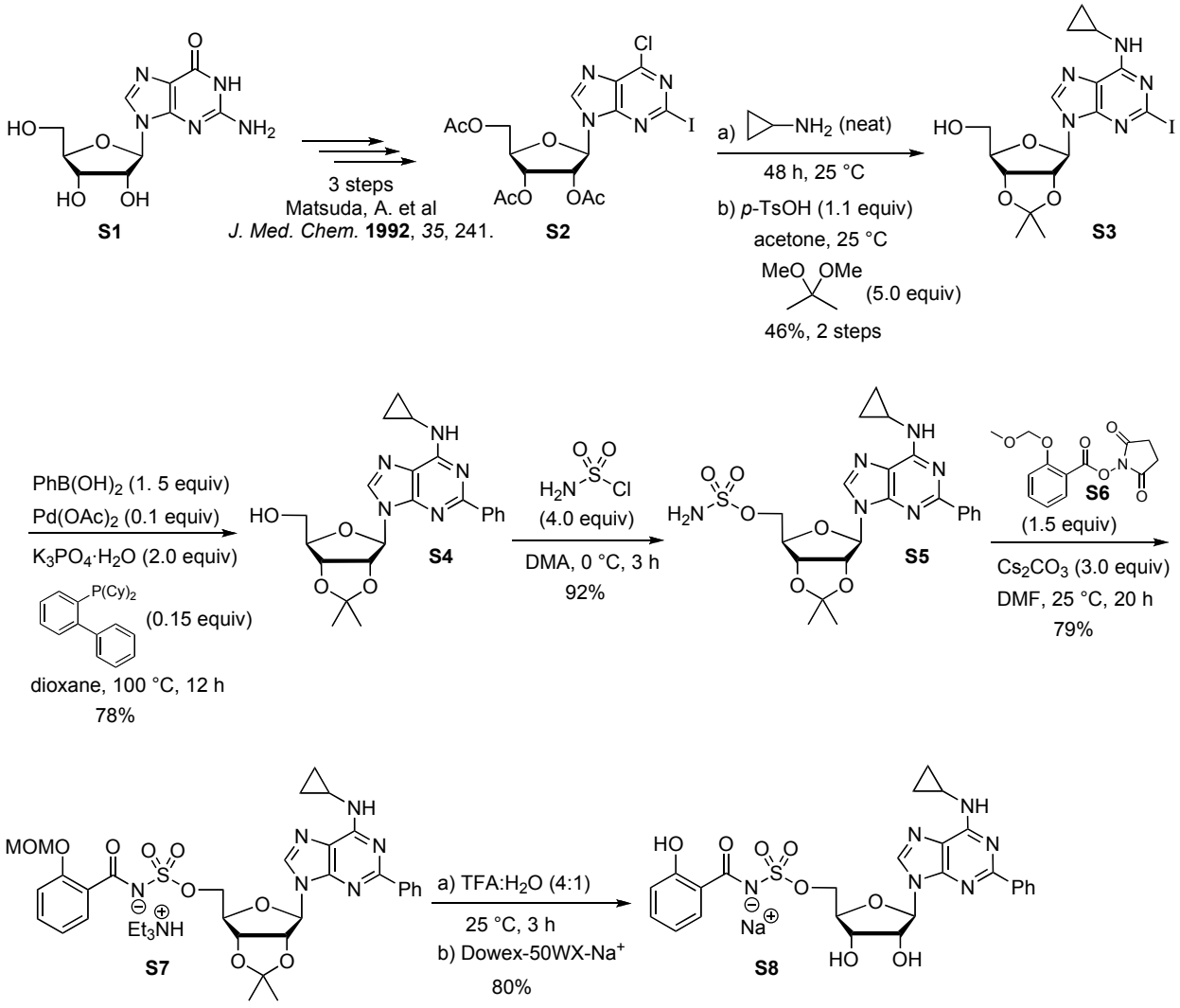
HPLC solvents:
A: 50 mM Triethylammonium bicarbonate
B: Methanol

HPLC column:
Analytical: Varian Microsorb MV100-5 C18, 250 × 4.6 mm, flowrate 0.5 mL/min

Methods:
Method 1: Isocratic elution with 30% B, 30 min, 220 nm detection
Method 2: Isocratic elution with 40% B, 30 min, 220 nm detection

B. Synthesis of N^6-Cyclopropyl-2-phenyl-Sal-AMS (S8).

Scheme S1.



Chemistry General Procedures. All commercial reagents (Sigma-Aldrich, Acros, Strem) were used as provided unless otherwise indicated. Sulfamoyl chloride was prepared by the method of Heacock except that this was used directly without recrystallization.[1] *N*-Hydroxysuccinimidyl 2-(methoxymethoxy)benzoate **S6** was prepared as described.[2] Anhydrous DMF, DMA, and 1,4-dioxane were purchased from Aldrich. Flash chromatography was performed with an ISCO Combiflash Companion® purification system with the prepacked silica gel cartridges with the indicated solvent system. All reactions were performed under an inert atmosphere

of dry Ar or N_2 in oven-dried (150 °C) glassware. ^{1}H and ^{13}C NMR spectra 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, integration. High resolution mass spectra were obtained on Agilent TOF/MS instrument equipped with either an ESI or APCI interface. Optical rotations were measured on Rudolph Autopol III polarimeter.

***N*^{6}-Cyclopropyl-2-iodo-2′,3′-*O*-isopropylideneadenosine (S3).** Compound **S2**[3] (657 mg, 1.22 mmol) was stirred in neat cyclopropylamine (2.0 mL) for 48 h at 25 °C. The reaction was concentrated in vacuo and the residue taken up in acetone (10 mL) and re-concentrated by rotary evaporation. The viscous orange oil was dried under hi-vacuum overnight.

To a solution of the crude product prepared above in acetone (5 mL) were added dimethoxypropane (749 μL, 6.10 mmol, 5.0 equiv) and *p*-TsOH·H_2O (255 mg, 1.34 mmol, 1.1 equiv). The reaction was stirred 24 h at 25 °C whereupon the product precipitated as the *p*-TsOH salt. Solid $NaHCO_3$ (537 mg, 6.1 mmol, 5.0 equiv) was added to the reaction and the heterogenous mixture stirred 20 min. The reaction was concentrated in vacuo and the residue partitioned between EtOAc (25 mL) and 1:1 H_2O/saturated aqueous $NaHCO_3$. The aqueous layer was further extracted with EtOAc (2 × 25 mL) and the combined organic extracts were washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4), and concentrated to a viscous orange oil. Flash chromatography (70:30 EtOAc/hexane) afforded the title compound (266 mg, 46%) as a colorless oil: R_f = 0.50 (80:20 EtOAc/hexane); ^{1}H NMR (600 MHz, CD_3OD) δ 8.10 (s, 1H), 6.03 (d, J = 3.0 Hz, 1H), 5.19 (dd, J = 6.0, 3.0 Hz, 1H), 4.96 (dd, J = 6.0, 2.4 Hz, 1H), 4.27–4.30 (m, 1H), 3.74 (dd, J = 12.0, 3.0 Hz, 1H), 3.68 (dd, J = 12.0, 4.8 Hz, 1H), 2.90–3.06 (br s, 1H), 1.55 (s, 3H), 1.33 (s, 3H), 0.80–0.83 (m, 2H), 0.57–0.60 (m, 2H); ^{13}C

NMR (150 MHz, CD_3OD) δ 156.5 (br), 149.7 (br), 139.8, 119.8, 114.1, 91.3, 87.2, 84.1, 81.8, 62.4, 26.5, 24.5, 24.7 (br), 7.3 (br), missing 1 aryl carbon (C-2); MS (ESI+) calcd for $C_{16}H_{21}IN_5O_4$ $[M + H]^+$ 474.1, found 474.1.

***N*^{6}-Cyclopropyl-2′,3′-*O*-isopropylidene-2-phenyladenosine (S4).** To a solution of compound **S3** (182 mg, 0.38 mmol, 1.0 equiv) in 1,4-dioxane (5 mL) were added phenylboronic acid (70 mg, 0.58 mmol, 1.5 equiv), $Pd(OAc)_2$ (8.5 mg, 0.038 mmol, 0.10 equiv), 2-(biphenyl)dicyclohexylphosphine (20.1 mg, 0.0576 mmol, 0.15 equiv), and K_3PO_4 (163 mg, 0.77 mmol, 2.0 equiv) and the reaction heated at 100 °C for 12 h. After cooling to rt, the mixture was diluted with EtOAc (50 mL), filtered thru a short pad of silica gel (1/3”), and concentrated onto Celite. Purification by flash chromatography (gradient from 60–100% EtOAc/hexane) afforded the title compound (128 mg, 78%) as a colorless oil: R_f = 0.42 (80:20 EtOAc/hexane); ^{1}H NMR (600 MHz, CD_3OD) δ 8.40 (d, *J* = 7.8 Hz, 2H), 8.16 (s, 1H), 7.39–7.43 (m, 3H), 6.21 (d, *J* = 3.0 Hz, 1H), 5.52 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.13 (dd, *J* = 6.0, 3.0 Hz, 1H), 4.31–4.33 (m, 1H), 3.74 (dd, *J* = 12.0, 4.2 Hz, 1H), 3.68 (dd, *J* = 12.0, 4.8 Hz, 1H), 3.10 (br s, 1H), 1.62 (s, 3H), 1.39 (s, 3H), 0.86–0.89 (m, 2H), 0.63–0.65 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 160.7, 156.8, 141.5, 139.9, 130.8, 129.3, 129.1, 119.7, 115.2, 92.1, 88.6, 85.3, 83.1, 63.4, 27.6, 25.6, 24.7 (br), 7.5 (br) missing 1 aryl carbon (C-2); MS (ESI+) calcd for $C_{22}H_{26}N_5O_4$ $[M + H]^+$ 424.2, found 474.2.

***N*^{6}-Cyclopropyl-2′,3′-*O*-isopropylidene-2-phenyl-5′-*O*-(sulfamoyl)adenosine (S5).** Sulfamoyl chloride (114 mg, 0.99 mmol, 4.0 equiv) was added to a solution of **S4** (105 mg, 0.25 mmol, 1.0 equiv) in dimethylacetamide (2.0 mL) at 0 °C. The solution was stirred 4 h at 0 °C then partitioned between EtOAc (25 mL) and H_2O (25 mL). The organic layer was separated, washed with H_2O (3 × 25 mL), saturated aqueous NaCl (25 mL), dried (Na_2SO_4) and concentrated to afford the title compound (114 mg, 92%) as a white foam: R_f = 0.20 (50:50 EtOAc/benzene); $[\alpha]_D^{23}$ = +19.4 (*c* 0.853, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 8.43 (d, *J* = 7.2 Hz, 2H), 8.15 (s, 1H), 7.41–7.45 (m, 3H), 6.30 (d, *J* = 1.8 Hz, 1H), 5.57 (dd, *J* = 6.0, 1.8 Hz, 1H), 5.23 (dd, *J* = 6.0, 3.0 Hz, 1H), 4.52–4.56 (m, 1H), 4.33 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.25 (dd, *J* = 10.8, 6.0 Hz, 1H), 3.14 (br s, 1H), 1.63 (s, 3H), 1.41 (s, 3H), 0.89–0.92 (m, 2H), 0.65–0.68 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 160.7,

156.9, 141.3, 139.8, 130.9. 129.3, 129.2, 119.6, 115.6, 91.8, 85.9, 85.5, 83.1, 81.8, 69.9, 27.5, 25.6, 24.7 (br), 7.5 (br) missing 1 aryl carbon (C-2); HRMS (APCI+) calcd for $C_{22}H_{27}N_6O_6S$ $[M + H]^+$ 503.1707, found 503.1769 (error 12.3 ppm).

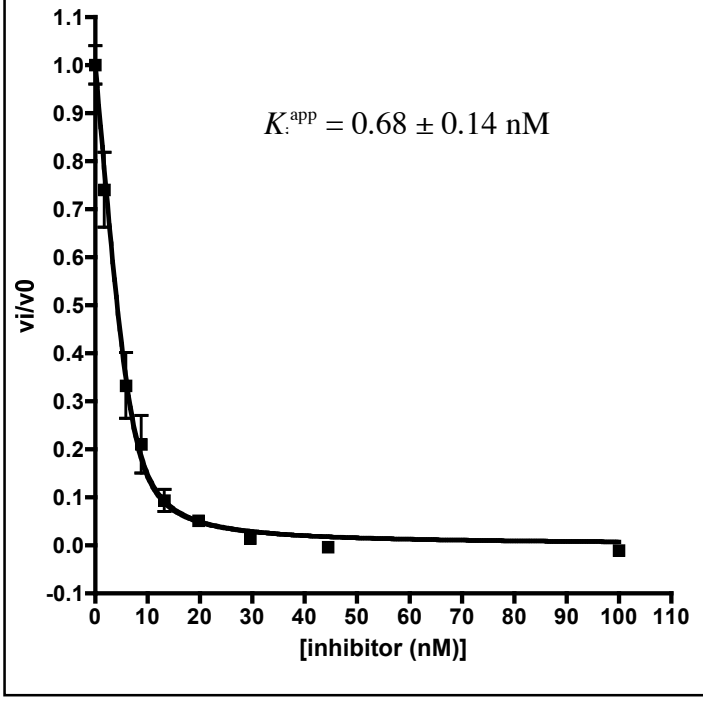
***N*^{6}-Cyclopropyl-2′,3′-*O*-isopropylidene-5′-*O*-{*N*-[2-(methoxymethoxy)benzoyl]sulfamoyl}-2-phenyladenosine Triethylammonium Salt (S7).** To a solution of **S5** (34 mg, 0.0677 mmol, 1.0 equiv) in DMF (3 mL) at 0 °C was added **S6** (28 mg, 0.10 mmol, 1.5 equiv) and Cs_2CO_3 (46 mg, 0.20 mmol, 3.0 equiv) and the reaction stirred 21 h at 25 °C. The reaction was filtered and the filtrate concentrated by rotary evaporation (P ~5 mbar, water bath ~35 °C). Purification by flash chromatography (EtOAc–10% MeOH/EtOAc + 1% Et_3N) afforded the title compound (40 mg, 79%) as a colorless oil: R_f = 0.6 (96:4 EtOAc/MeOH); $[\alpha]_D^{23}$ = –7.07 (*c* 1.98, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 8.47 (d, *J* = 7.2 Hz, 2H), 8.38 (s, 1H), 7.42–7.46 (m, 3H), 7.35 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.25 (td, *J* = 7.8, 1.8 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 6.93 (t, *J* = 7.2 Hz, 1H), 6.36 (d, *J* = 3.0 Hz, 1H), 5.52 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.29 (dd, *J* = 6.0, 1.8 Hz, 1H), 5.12 (s, 2H), 4.62–4.64 (m, 1H), 4.35–4.40 (m, 2H), 3.40 (s, 3H), 3.16 (br s, 1H), 3.10 (q, *J* = 7.2 Hz, 6H), 1.65 (s, 3H), 1.41 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 9H), 0.90–0.93 (m, 2H), 0.66–0.69 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.7, 160.7, 156.9 (br), 155.6, 151.3 (br), 141.2, 139.9, 132.4, 131.0, 130.9, 129.7, 129.3, 129.2, 122.5, 119.4, 116.9, 115.3, 96.3, 91.7, 85.8, 85.7, 83.4, 70.0, 56.5, 47.8, 27.6, 25.7, 24.7 (br), 9.2, 7.5 (br); HRMS (ESI–) calcd for $C_{31}H_{33}N_6O_9S$ $[M – H]^-$ 665.2035, found 665.2013 (error 3.3 ppm).

***N*^{6}-Cyclopropyl-5′-*O*-[*N*-(2-hydroxybenzoyl)sulfamoyl]-2-phenyladenosine Sodium Salt (S8).** To a solution of **S7** (40 mg, 0.052 mmol, 1.0 equiv) in H_2O (1 mL) was added trifluoroacetic acid (4 mL) and the reaction stirred 3 h at 25 °C. The reaction was concentrated in vacuo and placed under hi-vacuum for 16 h to remove traces of TFA. Purification by flash chromatography (EtOAc–20% MeOH/EtOAc + 1% Et_3N) provided the title compound as the triethylammonium salt. Ion-exchange (Dowex-50WX-100 resin in the sodium form, 10 × 100 mm, elution with 50% aqueous methanol) afforded the title compound (25 mg, 80%) as a white solid:

^{1}H NMR (600 MHz, CD_3OD) δ 8.46 (d, *J* = 6.6 Hz, 2H), 8.42 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.39–7.44 (m, 3H), 7.28 (t, *J* = 7.2 Hz, 1H), 6.78 (ovlp d, *J* = 8.4 Hz, 1H), 6.76 (ovlp t, *J* = 7.8 Hz, 1H), 6.23 (d, *J* = 6.0 Hz, 1H), 4.82 (t, *J* = 4.8 Hz, 1H), 4.49 (t, *J* = 4.2 Hz, 1H), 4.46 (dd, *J* = 10.8, 5.4 Hz, 1H), 4.38 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.33–4.36 (m, 1H), 3.15 (br s, 1H), 0.91–0.94 (m, 2H), 0.68 (s, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.3, 162.1, 160.7, 156.8, 140.9, 140.0, 134.4, 131.4, 130.8, 129.4, 129.3, 129.1, 120.5, 119.5, 119.3, 117.9, 89.5, 84.2, 75.8, 72.3, 69.8, 24.6 (br), 7.6 (br); HRMS (ESI+) calcd for $C_{26}H_{25}N_6O_8S$ $[M]^-$ 581.1460, found 581.1438 (error 3.8 ppm).

C. Enzyme Kinetic Studies. ATP–PPi Exchange Assay to Determine K_i^{app} for S8. The inhibition assays were performed as described in duplicate.[4] In brief the reaction was initiated by adding 10 μL [^{32}P]PP_i (0.5 μCi) to 7 nM MbtA in 90 μL reaction buffer (278 μM salicylic acid, 11.1 mM ATP, 1.11 mM PPi, 83.3 mM Tris-HCl, pH 7.5, 11.1 mM $MgCl_2$, 2.22 mM DTT) at 37 °C containing **S8** (1.73–100 nM). The reaction was terminated by the addition of 200 μL of quenching buffer (350 mM $HClO_4$, 100 mM PPi, 1.8 % *w*/*v* activated charcoal). The charcoal was pelleted by centrifugation and washed once with 500 μL H_2O and analyzed by liquid scintillation counting. The K_I^{app} value was calculated using the Morrison equation.[5]

Figure S1. Concentration-response plot of **S8**.



D. References

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