SUPPORTING INFORMATION

Rationally Designed Nucleoside Antibiotics that Inhibit Siderophore Biosynthesis of *Mycobacterium tuberculosis*

Ravindranadh Somu[a]; Helena Boshoff[b]; Chunhua Qiao[a]; Eric Bennett[a]; Clifton E. Barry III[b]; Courtney C. Aldrich[a]*

[a]*Center for Drug Design, University of Minnesota, Minneapolis, Minnesota 55455,* [b]*Tuberculosis Research Section, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852-1742.*

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***M. tuberculosis* H37Rv MIC Assay.** Minimum inhibitory concentrations (MICs) were determined in quadruplicate in iron-deficient GAST according to the broth microdilution method[3] using drugs from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. All measurements reported herein used an initial cell density of 10^4-10^5 cells/assay and growth monitored at 10 and at 14 days, with the untreated and DMSO-treated control cultures reaching an OD_{620} ~ 0.2-0.3. Plates were incubated at 37 °C (100 μl/well) and growth was recorded by measurement of optical density at 620 nm. The corresponding dose-response curves generated from these data are shown in Figure S1 below. Inhibitors **11**-**13** are not shown in Figure S1 since these did not display any inhibition up to 100 μM, the maximum concentration evaluated. MIC_{50} values were determined by fitting the dose-response curves by nonlinear regression analysis to the four parameter sigmodial dose-response curve using Graphpad Prism version 4.0.

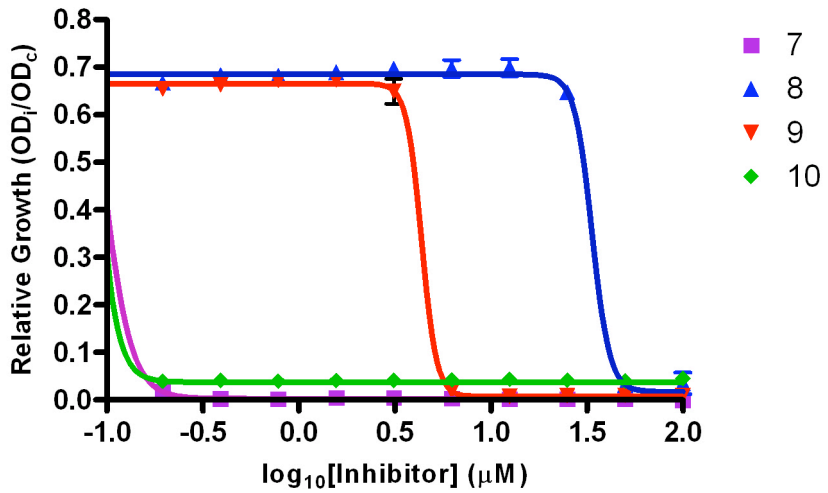


Figure S1. Dose Response Curves of Inhibitors **7**-**10**.

Siderophore Production Assay.[4,5] This assay uses a low iron GAST (glycerol-alanine-salts) medium except ferric ammonium citrate is omitted and Tween 80 is added to 0.05%. Low-iron GAST (50 mL) was inoculated with MTb H37Rv to an OD_{620} of 0.05 and incubated at 37 °C. Cultures were grown in this medium to an OD_{620} of 0.20 after which cells were harvested and resuspended in an equivalent volume of Chelex-deferrated GAST medium. Cultures (1 mL) were treated with either **7** or **10** from drug stocks in DMSO whereas a control culture was treated with an equivalent amount of DMSO (0.5%). To monitor mycobactin and carboxymycobactin biosynthesis, [7-^{14}C]salicylic acid was added to the cultures to a specific activity of 1.25 mCi/mL. After 3 d of incubation, the cultures were centrifuged. The siderophores in the supernatant were recovered as the iron complex by addition of $FeCl_3$ (0.555 mM final concentration) after acidifcation to pH 3.5 with HCl and extracted with $CHCl_3$ (3 x 0.75mL). The cell-associated siderophores were isolated from the cell pellet, which was suspended in EtOH (0.3 mL) and agitated 16 h. The supernatant was treated with $FeCl_3$ (2.2 mM final concentration), incubated for 1 h and partitioned between $CHCl_3$ (0.3 mL) and H_2O (0.3 mL). The organic layer was evaporated. The crude mycobactin- and carboxymycobactin-iron complexes were taken up in a minimum of chloroform

and subjected to TLC analysis developing with 2:3:3 petroleum ether:*n*-butanol:ethyl acetate. The plate was dried and analyzed using a Storm 860 (Molecular Dynamics) phosporimager (see Figure S2). The observed R_f of mycobactins and of carboxymycobactins agreed with those reported.[6]

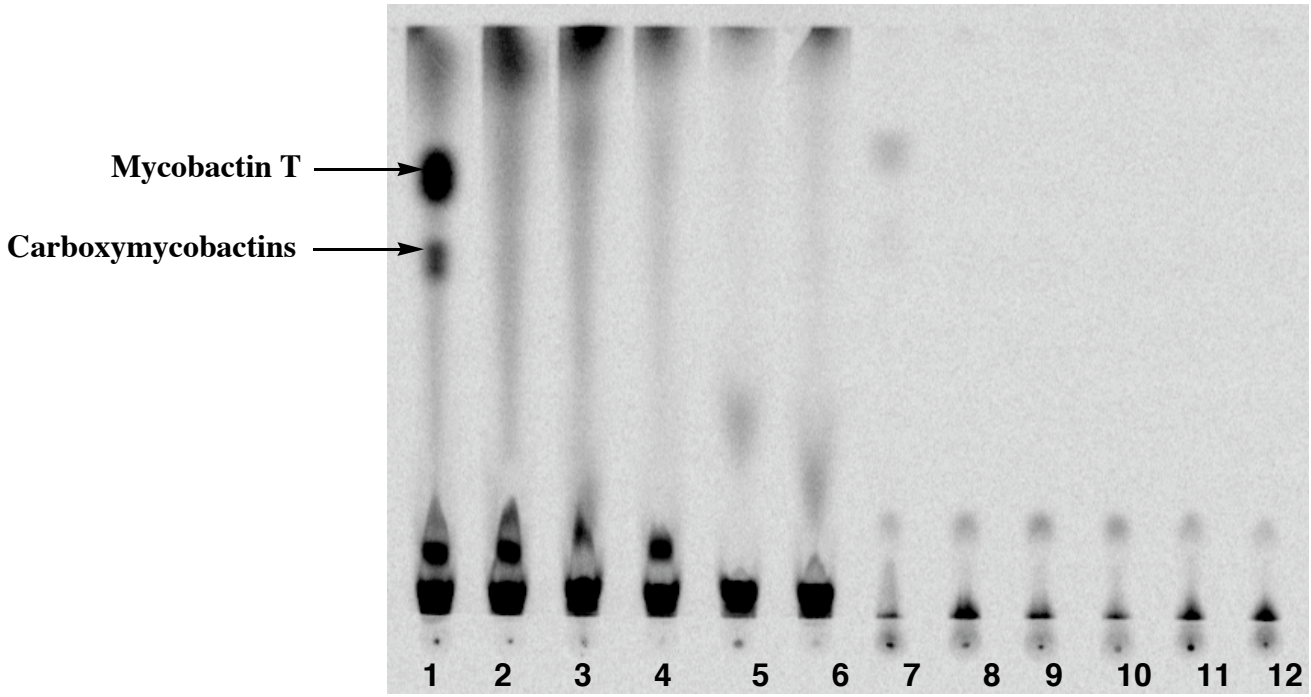


Figure S2. TLC Autoradiograph of [^{14}C]-mycobactin T and [^{14}C]-carboxymycobactins. *M. tuberculosis* H37Rv was treated with inhibitor **7** or **10** and [7-^{14}C]-salicylic acid. Developed with 2:3:3 petroleum ether:n-butanol:ethyl acetate. Lane 1–6 secreted mycobactins and carboxymycobatins. Lanes 7–12 cell associated mycobactins and carboxymycobactions Lane 1: DMSO treated control, Lane 2: 20 μM **7**, Lane 3: 200 μM **7**, Lane 4: 10 μM **10**, Lane 5: 20 μM **10**, Lane 6: 200 μM **10**, Lane 7: DMSO control, Lane 8: 20 μM **7**, Lane 9: 200 μM **7**, Lane 10: 10 μM **10**, Lane 11: 20 μM **10**, Lane 12: 200 μM **10**.

Cytotoxicity Assay.[7] P388 cells were plated in 12-well plates at 1.5 x 10^5 cells per well (2 mL). A two-fold serial dilution of inhibitors **7**–**13** was prepared to provide a final concentration from 100 μg/mL down to 1.56 μg/mL and a final concentration of 1% DMSO. The cells were incubated at 37 °C, 5% CO_2 for 3 d and the cells were counted using a hemocytometer and the data analyzed using Graphpad Prism Version 4.0. Puromycin was used as a positive control while DMSO was employed as the negative control.

pK_a Determination.[8] The acylsulfamate moiety has been reported as a neutral isostere of the acylphosphate function; however, it was expected that this function is considerably more acidic than suggested.[5] Among the inhibitors **7**–**9** incorporating the acylsulfamate linkage, only **9** was successfully isolated in the acid form as a pure compound. Although the free acid slowly decomposed at 25 °C over several days, it was sufficiently stable for pK_a measurements. Due to limited solubility of the conjugate base of **9**, it was necessary to employ 5% MeOH as a cosolvent. A Fisher Acumet AB15 pH meter equipped with a micro pH electrode (127 x 3 mm) and calomel reference was used for all titration experiments and was calibrated directly before use. All aqueous solutions were degassed for 30 min by

sparging with nitrogen. A 25 mM solution of **9** (500 μL) in H_2O:MeOH (95:5 v/v) was titrated with 0.100 M aq NaOH. The equivalence point was determined by analysis of the first-derivative plot of the titration curve.

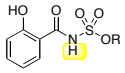
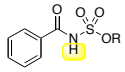
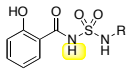
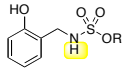
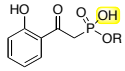
Molecular Modeling.

Docking. The active site of DhbE (PDB code 1MDB)[9] was used for modeling. Hydrogens were added and minimized, then the entire protein was relaxed with a heavy atom restraint of 1 kcal/mol-Å. A shell of methyl-capped complete residues within 14 Å of the ligand was retained for further modeling. Of 21 residues contacting the DHB-adenylate ligand (within 4 Å) five are not conserved in MbtA. The five residues were changed (Y236F, S240C, A308S, V337L, T411S), the dihydroxybenzyl group was converted to a salicyl group, and water 688 was deleted to make room for the V337L substitution. Substituted residues were optimized by a 20,000 step GB/SA solvated MMFFs conformational search in MacroModel, allowing only the changed residues and nearby waters to move. The resulting structure was used to dock **7**-**13**: 10,000 search steps were performed with only the ligand and nearby waters allowed to move. A 5 kcal/mol-Å restraint with a 2 Å half-width flat bottom was applied to the nearby waters for all simulations.

pKa calculations. Acidity constants were calculated on truncated model compounds using the pKa module[10] of Jaguar.[11] Model compounds were generated by cutting the bond between ribose atoms C4 and C5 and removing the ribose and adenine rings, then subjected to a GB/SA solvated MMFFs conformational search in MacroModel 9.[12] The lowest energy conformation was used to calculate the pKa. Different protonated and deprotonated conformations were used for the model compounds of **7**, **9**, and **10**.

Quantum geometry optimizations. Geometries of model compounds (see *pKa calculation*) were optimized at the CC-PVTZ(-F)/B3LYP level using Jaguar.

Table S1. pK_a Values

Inhibitor Scaffold	Linker[a]	pKa.
7		0.6[a]
9		3.4[a] (2.8)[b]
10		2.7[a]
11		7.5[a] (8.5)[c]
12		(2.0)[d]

Key: [a]Acidity constants were calculated on truncated model compounds using the pKa module (Klicic, 2002) of Jaguar; [b]Experimentally deterimined by titration of a 25 mM aqueous solution of **9** (R = 5'-adenosyl) at 23 °C containing 5% MeOH; [c]Experimentally determined for a molecule containing a sulfamate function[1]; [d]pKa of phosphonic acid monoester[2].

Chemistry General Procedures. All commercial reagents (Sigma-Aldrich, Acros) were used as provided unless otherwise indicated. 2-Benzyloxybenzoic acid was purchased from Alfa Aesar. Sulfamoyl chloride was prepared by the method of Heacock except that this was used directly without recrystallization.[13] An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2 while 2 packed columns of molecular sieves were used to dry DMF and the solvents were dispensed under argon. Anhydrous grade DME, MeOH, and MeCN were purchased from Aldrich. Pyridine was freshly distilled from KOH, Et_3N was distilled from CaH_2. Flash chromatography was performed with Silia P grade

silica gel 60 (Silicycle) 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 300 or 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 (77.3 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), integration, coupling constant. Overlapping carbon signals were assigned by HMQC or HMBC analysis. High resolution mass spectra were obtained on Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. Analytical HPLC were obtained on a Agilent 1100 Series HPLC system with a PDA detector.

Synthetic Procedures for the Preparation of Inhibitor 7

***N*-Hydroxysuccinimdyl 2-benzyloxybenzoate (22).**[14] To a solution of 2-benzyloxybenzoic acid (5.0 g, 21.9 mmol, 1.0 equiv) in THF (120 mL) at 0 °C was added *N*-hydroxysuccinimide (2.54 g, 21.9 mmol, 1.0 equiv) and DCC (4.53 g, 21.9 mmol, 1.0 equiv). The resulting mixture was stirred for 30 min at 0 °C then 2 h at rt. The reaction mixture was filtered to remove the DCU precipitate and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (4:1 EtOAc/hexane) afforded the title compound (5.91 g, 82%) as a white solid. R_f = 0.85 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (s, 4H), 5.22 (s, 2H), 6.98–7.12 (m, 2H), 7.22–7.42 (m, 3H), 7.44–7.60 (m, 3H), 8.20 (dd, 1H, J = 7.8, 2.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.1, 70.8, 114.0, 114.8, 120.8, 127.0, 128.0, 128.8, 133.0, 136.1, 136.3, 159.7, 164.9, 169.9.

***N*^{6}-*tert*-Butoxycarbonyl-2',3'-*O*-isopropylideneadenosine (14)**. To a solution of 2',3'-*O*-isopropylideneadenosine (10.0 g, 35.7 mmol, 1.0 equiv) in CH_2Cl_2 (250 mL) at 0 °C was added imidazole (3.64 g, 53.5 mmol, 1.5 equiv) and *tert*-butyldimethylsilyl chloride (16.3 g, 105 mmol, 3.5 equiv). After 16 h, the reaction mixture was filtered and the residue washed with acetone (100 mL). The combined filtrates were concentrated to afford 5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine as a white solid that was directly carried onto the next step. An analytically pure sample was obtained by flash chromatography (3:1 hexane/EtOAc). R_f = 0.85 (1:6 MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.02 (s, 3H), -0.01 (s, 3H), 0.8 (s, 9H), 1.37 (s, 3H), 1.60 (s, 3H), 3.73 (dd, 1H, J = 11.4, 4.2 Hz), 3.85 (dd, 1H, J = 11.4, 4.2 Hz), 4.36–4.40 (m, 1H), 4.92 (dd, 1H, J = 6.0, 2.1 Hz), 5.24 (dd, 1H, J = 6.0, 2.1 Hz), 6.14 (d, 1H, J = 2.4 Hz), 7.15–7.19 (m, 2H), 8.03 (s, 1H), 8.33 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ -5.4 (2C), 19.2, 25.5, 26.3, 27.4, 64.8, 83.0, 85.9, 89.1, 92.4, 115.1, 120.5, 136.3, 141.4, 150.3, 153.9, 157.4; HRMS (ESI+) calcd. for $C_{19}H_{32}N_5O_4Si$ $[M+H]^+$ 422.2220, found 422.2224 (error 0.9 ppm).

The crude product from above was suspended in a mixture of THF (600 mL) and DMF (100 mL), then NaH (1.77 g, 44.3 mmol, 1.1 equiv, 60% dispersion in oil) was added portionwise with vigorous stirring at 0 °C. After stirring the viscous mass for 15–20 min $(Boc)_2O$ (8.8 g, 40.3 mmol, 1.0 equiv) was added. Another portion of $(Boc)_2O$ (4.4 g. 20.2 mmol, 0.5 equiv) was added after 30 min. After 16 h, the reaction mixture was quenched with ice and concentrated under reduced pressure. The residue

was partitioned between H_2O (100 mL) and EtOAc (100 mL) and the organic layer was dried (Na_2SO_4) and concentrated. Purification by flash chromatography (EtOAc/hexanes) afforded the desired N^6-Boc product (5.5 g, 30%) along with the N^6-bis-Boc side-product (1.90 g, 8%) and recovered starting material (8.0 g, 53%). Characterization data for N^6,N^6-bis(*tert*-butoxycarbonyl)-5'-*O*-*tert*-Butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine: R_f = 0.85 (3:1 hexanes/EtOAc); Characterization data for N^6-*tert*-butoxycarbonyl-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylideneadenosine: R_f = 0.65 (3:1 hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.03 (s, 3H), -0.02 (s, 3H), 0.8 (s, 9H), 1.38 (s, 3H), 1.53 (s, 9H), 1.61 (s, 3H), 3.74 (dd, 1H, *J* = 11.2, 3.6 Hz), 3.87 (dd, 1H, *J* = 11.2, 3.6 Hz), 4.40–4.50 (m, 1H), 4.92 (dd, 1H, *J* = 6.0, 2.1 Hz), 5.24 (dd, 1H, *J* = 5.7, 2.1 Hz), 6.19 (d, 1H, *J* = 2.7 Hz), 8.18 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ -5.2, -5.1, 18.7, 25.7, 26.2, 27.5, 28.5, 63.9, 81.8, 82.5, 85.4, 87.8, 92.2, 114.3, 122.2, 141.2, 149.8, 150.0, 150.4, 153.3; HRMS (ESI+) calcd. for $C_{24}H_{40}N_5O_6Si$ $[M+H]^+$ 522.2742, found 522.2751 (error 1.7 ppm).

To a solution of the mono-N^6-Boc compound prepared above (1.40 g, 2.68 mmol, 1.0 equiv) in THF (100 mL) at rt was added TBAF (1.0 M solution in THF, 4.0 mL, 4.0 mmol, 1.5 equiv). After 4 h, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography (1–3% MeOH/EtOAc) afforded the title compound **14** (1.1 g, 100%). R_f = 0.30 (1:9 MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.52 (s, 9H), 1.61 (s, 3H), 3.76 (br d, 1H, *J* = 12.9 Hz), 3.94 (dd, 1H, *J* = 12.9, 1.8 Hz), 4.46–4.56 (m, 1H), 5.07 (dd, 1H, *J* = 6.0, 1.2 Hz), 5.17 (t, 1H, *J* = 5.1 Hz), 5.88 (d, 1H, *J* = 5.1 Hz), 8.0 (s, 1H), 8.47 (br s, 1H), 8.68 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 27.9, 28.5, 63.5, 81.9, 82.8, 83.3, 86.4, 94.4, 114.3, 123.2, 142.2 (2C), 145.7, 150.8, 152.7; HRMS (APCI-) calcd. for $C_{18}H_{24}N_5O_6$ $[M-H]^-$ 406.1732, found 406.1764 (error 7.8 ppm).

***N*^{6}-*tert*-Butoxycarbonyl-2',3'-*O*-isopropylidene-5'-*O*-(sulfamoyl)adenosine (15).**[13] To a solution of **14** (0.5 g, 1.23 mmol, 1.0 equiv) in DME (50 mL) at 0 °C was added NaH (74 mg, 1.84 mmol, 1.5 equiv, 60% suspension in mineral oil) and the solution stirred 30 min at 0 °C. Next, a solution of sulfamoyl chloride (213 mg, 1.84 mmol, 1.5 equiv) in DME (15 mL) was added dropwise over 5 min and the reaction stirred 16 h at rt. The reaction mixture was quenched at 0 °C with MeOH (30 mL) and concentrated under reduced pressure. Purification by flash chromatography (19:1 EtOAc/MeOH) afforded the title compound (0.49 g, 82%). R_f = 0.9 (19:1 EtOAc/MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.50 (s, 9H), 1.58 (s, 3H), 4.30 (dd, 1H, *J* = 10.8, 5.4 Hz), 4.37 (dd, 1H, *J* = 10.8, 3.6 Hz), 4.52–4.56 (m, 1H), 5.05 (dd, 1H, *J* = 6.0, 2.7 Hz), 5.36 (dd, 1H, *J* = 6.3, 2.4 Hz), 6.03 (br s, 2H), 6.18 (d, 1H, *J* = 2.4 Hz), 8.15 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 27.4, 28.5, 69.6, 81.4, 82.7, 84.4, 84.8, 91.4, 115.0, 122.1, 142.0, 150.0, 150.2, 150.6, 153.0; HRMS (ESI-) calcd. for $C_{18}H_{25}N_6O_8S$ $[M-H]^-$ 485.1460, found 485.1497 (error 7.6 ppm).

***N*^{6}-*tert*-Butoxycarbonyl-5'-*O*-(*N*-(2-benzyloxybenzoyl)sulfamoyl)-2',3'-isopropylideneadenosine triethylammonium salt (16).** To a solution of **15** (0.37 g, 0.76 mmol, 1.0 equiv) in DMF (8.0 mL) at -20 °C was added **22** (0.37 g, 1.14 mmol, 1.5 equiv) and Cs_2CO_3 (371 mg, 1.14 mmol, 1.5 equiv). The reaction mixture was warmed to rt and stirred 16 h. The reaction was concentrated under reduced pressure and the residue taken up in EtOAc (50 mL) and filtered. The solids

were washed with additional EtOAc (100 mL) and the combined filtrate was concentrated. Purification by flash chromatography (96:4:1 EtOAc/MeOH/Et_3N) afforded the title compound (0.48 g, 80%). R_f = 0.55 (EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.22 (t, 9H, *J* = 7.2 Hz), 1.34 (s, 3H), 1.58 (s, 9H), 1.59 (s, 3H), 3.11 (q, 6H, *J* = 7.2 Hz), 4.10–4.20 (m, 2H), 4.32–4.40 (m, 1H), 5.02–5.10 (m, 3H), 5.32 (dd, 1H, *J* = 6.3, 3.3 Hz), 6.25 (d, 1H, *J* = 3.3 Hz), 6.92 (td, 1H, *J* = 7.5, 0.9 Hz), 7.03 (d, 1H, *J* = 8.4 Hz), 7.14-7.32 (m, 4H), 7.36–7.50 (m, 3H), 8.54 (s, 1H), 8.64 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.2, 24.5, 26.5, 27.4, 46.7, 60.4, 68.7, 70.4, 81.6, 82.2, 84.7, 91.0, 113.0, 113.9, 120.3, 121.9, 127.4, 127.6, 128.2, 128.6, 130.1, 130.3, 137.4, 142.5, 149.9, 151.0, 151.2, 152.0, 155.9, 175.5; HRMS calcd. for $C_{32}H_{37}N_6O_{10}S$ $[M+H]^+$ 697.2292, found 697.2295 (error 0.4 ppm).

***N*^{6}-tert-Butoxycarbonyl-5'-*O*-(*N*-(2-hydroxybenzoyl)sulfamoyl)-2',3'-isopropylideneadenosine triethylammonium salt (17).** To **16** (106 mg, 0.13 mmol) in abs. EtOH (20 mL) was added 10% Pd/C (40 mg) and the reaction placed under a H_2 atmosphere. After 3 h, the reaction mixture was filtered through a plug of Celite, which was further washed with MeOH (60 mL) and the combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (100:10:1 EtOAc/MeOH/Et_3N) afforded the title compound (90 mg, 95%). R_f = 0.25 (EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.24 (t, 9H, *J* = 7.2 Hz), 1.34 (s, 3H), 1.56 (s, 3H), 1.57 (s, 9H), 3.14 (q, 6H, *J* = 7.2 Hz), 4.32 (d, 2H, *J* = 3.6 Hz), 4.56–4.62 (m, 1H), 5.12 (dd, 1H, *J* = 5.7, 1.8 Hz), 5.41 (dd, 1H, *J* = 6.0, 2.7 Hz), 6.27 (d, 1H, *J* = 2.4 Hz), 6.70–6.80 (m, 2H), 7.26 (td, 1H, *J* = 7.5, 0.6 Hz), 7.84 (dd, 1H, *J* = 7.5, 0.6 Hz), 8.52 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 8.2, 24.4, 26.4, 27.5, 46.6, 68.8, 81.6, 82.2, 84.7, 85.0, 91.2, 114.0, 116.7, 118.1, 119.2, 121.8, 130.0, 133,2, 142.6, 150.0, 150.8, 151.1, 152.0, 160.7, 173.6; HRMS (ESI+) calcd. for $C_{25}H_{31}N_6O_{10}S$ $[M+H]^+$ 607.1816, found 607.1819 (error 0.5 ppm).

5'-*O*-(*N*-(2-Hydroxybenzoyl)sulfamoyl)adenosine triethylammonium salt (7). To **17** (43 mg, 0.070 mmol, 1.0 equiv) was added 80% aq TFA (2 mL). After 2 h, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography (75:25:1 EtOAc/MeOH/Et_3N) afforded the title compound (30 mg, 74%). R_f = 0.15 (2:3 MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.24 (t, 9H, *J* = 7.2 Hz), 3.10 (q, 6H, *J* = 7.2 Hz), 4.28–4.38 (m, 1H), 4.38–4.48 (m, 3H), 4.72 (t, 1H, *J* = 5.1 Hz), 6.08 (d, 1H, *J* = 6.0 Hz), 6.72–6.84 (m, 2H), 7.28 (ddd, 1H, *J* = 8.1, 7.2, 1.8 Hz), 7.92 (dd, 1H, *J* = 7.8, 1.5 Hz), 8.16 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (CD_3OD, 75 MHz) δ 8.3, 46.7, 68.5, 71.3, 74.9, 83.4, 88.1, 116.7, 118.1, 118.9, 119.4, 130.1, 133.2, 139.9, 149.6, 150.9, 152.6, 160.8, 173.7; HRMS (ESI+) calcd. for $C_{17}H_{19}N_6O_8S$ $[M+H]^+$ 467.0979, found 467.0986 (error 1.4 ppm).

Synthetic Procedures for the Preparation of Inhibitor 8

***N*-Hydroxysuccinimdyl 2-carbobenzyloxyamino benzoate (23).** This compound was prepared from 2-(2-benzyloxycarbonyl)amino benzoic acid[15] (4.0 g, 14.8 mmol, 1.0 equiv) using the procedure described for the synthesis of **22** to afford the title compound (4.6 g, 84%) as a white solid. R_f = 0.85 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.88 (s, 4H), 5.19 (s, 2H), 7.09 (t, 1H, *J* = 7.8 Hz), 7.28–7.42 (m, 6H), 7.65 (t, 1H, *J* = 7.2 Hz), 8.17 (d, 1H, *J* = 7.8 Hz), 9.73 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ

25.9, 67.5, 109.9, 119.5, 122.3, 128.5, 128.6, 128.8, 131.4, 136.0, 137.1, 143.0, 153.2, 163.3, 169.3; MS (ESI+) calcd. for $C_{19}H_{16}N_2NaO_6$ $[M+Na]^+$ 391.1, found 391.2.

5'-*O*-(*N*-(2-Carbobenzyloxyaminobenzoyl)sulfamoyl)-2',3'-*O*-isopropylideneadenosine (19). To a solution of **18**[13] (0.25 g, 0.649 mmol, 1.0 equiv) in DMF (7.5 ml) were added **23** (0.21 g, 0.78 mmol, 1.20 equiv) and DBU (233 μl, 1.55 mmol, 2.4 equiv). After 12 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (1:10 MeOH/$CHCl_3$). Further purification by flash chromatography (1:9 MeOH/EtOAc) afforded the title compound (0.153 g, 36%) as an oil. R_f = 0.35 (1:9 MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (s, 3H), 1.55 (s, 3H), 4.22–4.40 (m, 2H), 4.45–4.55 (m, 1H), 5.00–5.20 (m, 3H), 5.32 (dd, 1H, *J* = 6.0, 3.0 Hz), 6.20 (d, 1H, *J* = 3.0 Hz), 6.92 (t, 1H, *J* = 7.8 Hz), 7.20–7.42 (m, 6H), 8.05 (dd, 1H, *J* = 7.8, 1.2 Hz), 8.10 (s, 1H), 8.22 (d, 1H, *J* = 7.8), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.3, 26.2, 66.3, 68.6, 82.1, 84.5, 84.6, 90.8, 114.1, 118.3, 119.0, 121.3, 122.9, 127.7, 127.8, 128.3, 128.4, 131.2, 136.8, 140.1, 140.2, 149.2, 152.7, 154.0, 156.0, 174.4; HRMS (ESI-) calcd. for $C_{28}H_{28}N_7O_9S$ $[M-H]^-$ 638.1674, found 638.1626 (error 7.5 ppm).

5'-*O*-(*N*-(2-Aminobenzoyl)sulfamoyl)-2',3'-isopropylideneadenosine triethylammonium salt (20). This was prepared from **19** (0.35 g, 0.55 mmol) using the procedure described for the synthesis of **17**. Purification by flash chromatography (84:14:1 EtOAc/MeOH/Et_3N) afforded the title compound (135 mg, 41%). R_f = 0.2 (1:6 MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.25 (t, 9H, *J* = 7.2 Hz), 1.35 (s, 3H), 1.59 (s, 3H), 3.12 (q, 6H, *J* = 7.2 Hz), 4.28 (d, 2H, *J* = 3.9 Hz), 4.50–4.60 (m, 1H), 5.14 (dd, 1H, *J* = 6.3, 2.1 Hz), 5.38 (dd, 1H, *J* = 6.3, 3.3 Hz), 6.23 (d, 1H, *J* = 3.3 Hz), 6.53 (ddd, 1H, *J* = 8.4, 7.2, 0.9 Hz), 6.67 (dd, 1H, *J* = 8.1, 0.9 Hz), 7.10 (ddd, 1 H, *J* = 9.0, 7.5, 1.8 Hz), 7.89 (dd, 1H, *J* = 8.1, 1.5 Hz), 8.15 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.1, 25.5, 27.5, 47.7, 69.6, 83.2, 85.6, 85.7, 91.8, 115.1, 116.7, 117.8, 120.0, 120.1, 132.4, 133.0, 141.3, 150.2, 151.1, 153.7, 157.0, 176.9; HRMS (APCI-) calcd. for $C_{20}H_{22}N_7O_7S$ $[M-H]^-$ 504.1306, found 504.1306 (error 0 ppm).

5'-*O*-(*N*-(2-Aminobenzoyl)sulfamoyl)adenosine triethylammonium salt (8). This was prepared from **20** (39 mg, 0.065 mmol) using the procedure described for the synthesis of **7**. Purification by flash chromatography (75:25:1 EtOAc/MeOH/Et_3N) afforded the title compound (23 mg, 64%). R_f = 0.4 (1:1 MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.55 (t, 9H, *J* = 7.2 Hz), 2.88 (q, 6H, *J* = 7.2 Hz), 4.26–4.33 (m, 1H), 4.34–4.39 (m, 2H), 4.39–4.46 (m, 1H) 4.73 (t, 1H, *J* = 5.7 Hz), 6.09 (d, 1H, *J* = 6 Hz), 6.54 (ddd, 1H, *J* = 8.1, 7.2, 1.2 Hz), 6.66 (dd, 1H, *J* = 7.8, 0.9 Hz) 7.1 (ddd, 1H, *J* = 8.4, 7.2, 1.5 Hz), 7.9 (dd, 1H, *J* = 8.1, 1.8 Hz), 8.16 (s, 1H), 8.55 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 47.9, 69.1, 72.6, 76.2, 84.9, 89.0, 116.9, 118.0, 118.3, 120.6, 132.6, 132.9, 142.2, 151.0, 151.1, 153.9, 157.3; 175.9. HRMS (ESI+) calcd. for $C_{17}H_{20}N_7O_7S$ $[M+H]^+$ 466.1139, found 466.1177 (error 8.1 ppm).

Synthetic Procedures for the Preparation of Inhibitor 9

***N*-Hydroxysuccinimdyl benzoate (24).** This was prepared starting from benzoic acid (3.66 g, 30 mmol) using the procedure described for the synthesis of **22**. Purification by flash chromatography (3:1 hexane/EtOAc) afforded the title compound (4.47 g, 68%) as a white solid. R_f = 0.25 (3:1 hexane/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91 (s, 4H), 7.48–7.54 (m, 2H), 7.65–7.75 (m, 1H),

8.12–8.15 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 125.3, 129.1, 130.8, 135.2, 162.1, 169.5; HRMS (APCI-) calcd. for $C_{11}H_8NO_4$ $[M-H]^-$ 218.0453, found 218.0457 (error 1.8 ppm).

5'-*O*-(*N*-(Benzoyl)sulfamoyl)-2',3'-isopropylideneadenosine (21). This was prepared from **24** (263 mg, 1.2 mmol) using the procedure desribed for the synthesis of **19**. Purification by flash chromatography (90:10:1 $CHCl_3$/MeOH/Et_3N) afforded the title compound (22 mg, 44%) as a white solid. R_f = 0.25 (10:1 $CHCl_3$/MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.34 (s, 3H), 1.58 (s, 3H), 4.30–4.33 (m, 2H), 4.52–4.56 (m, 1H), 5.15 (dd, 1H, *J* = 6.0, 2.4 Hz), 5.35 (dd, 1H, *J* = 6.0, 3.0 Hz), 6.21 (d, 1H, *J* = 3.0 Hz), 7.30–7.40 (m, 3H), 7.90–8.10 (m, 2H), 8.14 (s, 1H), 8.44 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.3, 26.3, 68.6, 82.1, 84.5, 84.6, 90.7, 114.1, 119.0, 127.6, 128.8, 131.1, 137.4, 140.3, 149.2, 152.8, 156.1, 174.2; MS (ESI+) calcd. for $C_{20}H_{23}N_6O_7S$ $[M+H]^+$ 491.13, found: 491.11.

5'-*O*-(*N*-(Benzoyl)sulfamoyl)adenosine (9). This was prepared from **21** (80 mg, 0.16 mmol) using the procedure described for the synthesis of **7** to afford the title compound (73 mg, 100%) as a white solid. R_f = 0.18 (6:1 EtOAc/MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 4.29–4.33 (m, 1H), 4.42 (t, 1H, *J* = 5.1 Hz), 4.56 (t, 2H, *J* = 3.6 Hz), 4.67 (t, 1H *J* = 5.1 Hz), 6.03 (d, 1H, *J* = 5.1 Hz), 6.80–7.43 (m, 3H), 7.84-7.88 (m, 2H), 8.18 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 68.1, 71.2, 74.9, 83.5, 88.1, 119.0, 127.6, 128.7, 130.9, 137.7, 140.0, 149.7, 152.7, 156.1, 174.2; HRMS (ESI+) calcd. for $C_{17}H_{19}N_6O_7S$ $[M+H]^+$ 451.1036, found 451.1051 (error 3.3 ppm).

Synthetic Procedures for the Preparation of Inhibitor 10

***N*^{6}-*tert*-Butoxycarbonyl-5'-azido-5'-deoxy-2',3'-*O*-isopropylideneadenosine (25).**[16] To a solution of alcohol **14** (2.15 g, 5.3 mmol, 1.0 equiv) in DMF (40 mL) at 0 °C was added PPh_3 (2.77 g, 10.6 mmol, 2.0 equiv), CBr_4 (3.5 g, 10.6 mmol, 2.0 equiv) and NaN_3 (6.8 g, 105.6 mmol, 20.0 equiv). The reaction was warmed to rt and stirred 16 h then concentrated *in-vacuo*. The residue was taken up in EtOAc (50 mL) and filtered. The filtrate was concentrated and purified by flash chromatography (EtOAc/hexane) to afford the title compound (1.1 g, 48%). R_f = 0.65 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.52 (s, 9H), 1.59 (s, 3H), 3.53 (d, 2H, *J* = 5.4 Hz), 4.36 (ddd, 1H, *J* = 9.0, 5.4, 3.6 Hz), 5.02 (dd, 1H, *J* = 6.6, 3.6 Hz), 5.43 (dd, 1H, *J* = 6.6, 2.4 Hz) 6.11 (d, 1H, *J* = 2.4 Hz), 8.05 (br s, 1H), 8.22 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.5, 27.3, 28.3, 52.2, 82.1, 82.6, 84.2, 85.8, 90.9, 115.1, 122.5, 141.9, 149.8, 150.4, 150.5, 153.3; HRMS (ESI+) calcd. for $C_{18}H_{25}N_8O_5$ $[M+H]^+$ 433.1942, found 433.1939 (error 0.6 ppm).

***N*^{6}-*tert*-Butoxycarbonyl-5'-amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine (26).** To a solution of azide **25** (0.64g, 1.48 mmol, 1.0 equiv) in MeOH (50 mL) was added 10% Pd/C (0.12 g) then the reaction was placed under a H_2 atm. After 16 h, the reaction mixture was filtered through a plug of Celite, washing with MeOH, and the combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (4:1 EtOAc/MeOH) afforded the title compound (0.42 g, 70%). R_f = 0.2 (4:1 EtOAc/MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.37 (s, 3H), 1.57 (s, 9H), 1.59 (s, 3H), 2.84–2.92 (m, 2H), 4.20–4.26 (m, 1H), 5.02 (dd, 1H, *J* = 5.8, 3.0 Hz), 5.49 (dd, 1H, *J* = 5.8, 1.8 Hz), 6.20 (d, 1H, *J* = 1.8 Hz), 8.44 (s, 1H), 8.57 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 25.6, 27.5, 28.5, 44.6, 82.8,

83.3, 85.0, 88.6, 91.8, 115.7, 123.7, 144.4, 151.6, 152.2, 152.4, 153.3; HRMS (APCI-) calcd. for $C_{18}H_{27}N_6O_5$ [M-H]$^-$ 405.1876, found 405.1891 (3.7 ppm).

***N*^{6}-*tert*-Butoxycarbonyl-5'-deoxy-2',3'-*O*-isopropylidene-5'-*N*-(sulfamoyl)aminoadenosine (27).** To a solution of amine **26** (0.2 g, 0.49 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) at 0 °C was added Et_3N (102 μL, 0.74 mmol, 1.5 equiv) followed by freshly prepared sulfamoyl chloride (85.2 mg, 0.74 mmol, 1.5 equiv). The reaction mixture was stirred for 24 h then concentrated *in-vacuo* under reduced pressure. Purification by flash chromatography (1–2% MeOH/EtOAc) afforded the title compound (80 mg, 34%) and further elution of the column (10% MeOH/EtOAc) led to recovery of unreacted **26** (70 mg, 35%). R_f = 0.9 (EtOAc); ^{1}H NMR (600 MHz, CD_3OD) δ 1.35 (s, 3H), 1.56 (s, 9H), 1.58 (s, 3H), 3.32 (dd, 1H, *J* = 13.2, 4.2 Hz); 3.36 (dd, 1H, *J* = 13.2, 4.2 Hz), 4.44 (dd, 1H, *J* = 6.6, 4.2 Hz), 5.10 (dd, 1H, *J* = 6.6, 3.0 Hz), 5.36 (dd, 1H, *J* = 6.6, 3.6 Hz), 6.11 (d, 1H, *J* = 3.6 Hz), 8.39 (s, 1H), 8.61 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.4, 26.4, 27.3, 44.8, 81.7, 82.1, 83.3, 84.6, 91.8, 114.5, 122.7, 143.3, 150.5, 151.1, 152.2 (2C); HRMS (ESI+) calcd. for $C_{18}H_{28}N_7O_7S$ [M+H]$^+$ 486.1771, found 486.1755 (error 3.2 ppm).

***N*^{6}-*tert*-Butoxycarbonyl-5'-deoxy-5'-*N*-(*N*-(2-hydroxybenzoyl)sulfamoyl)amino-2',3'-*O*-isopropylideneadenosine triethylammonium salt (28).**[17] Salicylic acid (43 mg, 0.31 mmol, 3.0 equiv) and CDI (61 mg, 0.37 mmol, 3.6 equiv) were suspended in CH_3CN (3.0 mL) and heated at 60 °C for 1 h to afford a clear homogenous solution. The reaction mixture was cooled to rt then a solution **27** (50 mg, 0.1 mmol, 1.0 equiv) and DBU (23 μL, 0.15 mol, 1.5 equiv) in CH_3CN (3.0 mL) were added to provide a yellow solution. The reaction mixture was heated at 60 °C for 1 h during which time the yellow color faded. The reaction was quenched with MeOH and concentrated *in-vacuo* under reduced pressure. Purification by flash chromatography (1–2% MeOH/EtOAc) led to recovery of unreacted starting **27** (16 mg, 33%). Further elution of the column (5–10% MeOH/EtOAc) afforded the title compound (25 mg, 40%), R_f = 0.5 (1:9 MeOH/EtOAc); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26 (t, 9H, *J* = 7.2 Hz), 1.32 (s, 3H), 1.55 (s, 3H), 1.57 (s, 9H), 3.15 (q, 6H, *J* = 7.2 Hz), 3.27 (d, 2H, *J* = 1.2 Hz), 4.38–4.44 (m, 1H), 5.10 (dd, 1H, *J* = 6.6, 2.4 Hz), 5.38 (dd, 1H, *J* = 6.0, 3.6 Hz), 6.14 (d, 1H, *J* = 3.0 Hz), 6.70–6.80 (m, 2H), 7.23 (t, 1H, *J* = 7.2 Hz), 7.77 (d, 1H, *J* = 7.2 Hz), 8.42 (s, 1H), 8.57 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.1, 24.3, 26.3, 27.3, 45.5, 46.7, 81.6, 82.4, 83.8, 85.3, 91.1, 114.4, 116.6, 118.0, 119.5, 122.3, 129.7, 132.8, 143.0, 150.2, 150.7, 151.1, 152.3, 160.6, 173.1; HRMS (ESI+) calcd. for $C_{25}H_{32}N_7O_9S$ [M+H]$^+$ 606.1976, found 606.1951 (error 4.1 ppm).

5'-Deoxy-5'-*N*-(*N*-(2-hydroxybenzoyl)sulfamoyl)aminoadenosine triethylammonium salt (10). This was prepared from **28** (25 mg, 0.035 mmol) using the procedure described for the synthesis of **7**. Purification by flash chromatography (60:40:1 EtOAc/MeOH/Et_3N) afforded the title compound (16.2 mg, 81%). R_f = 0.15 (3:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.23 (t, 9H, *J* = 7.2 Hz), 3.05 (q, 6H, *J* = 7.2 Hz), 3.30–3.34 (m, 2H), 4.20–4.25 (m, 1H), 4.34 (dd, 1H, *J* = 4.8, 2.4 Hz), 4.86 (t, 1H, *J* = 6.6 Hz), 5.90 (d, 1H, *J* = 6.6 Hz), 6.72–6.80 (m, 2H), 7.24 (t, 1H, *J* = 7.2 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 8.26 (s, 1H), 8.30 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.4, 45.3, 46.5, 71.9, 73.5, 84.8, 89.5, 116.6, 117.9, 119.7, 119.8, 129.7, 132.7, 140.8, 152.9, 156.1, 160.7, 171.9, 173.1; HRMS (ESI+) calcd. for $C_{17}H_{20}N_7O_7S$ [M+H]$^+$ 466.1139, found 466.1139 (error 0 ppm).

Synthetic Procedures for the Preparation of Inhibitor 11

2-Benzyloxybenzaldehyde (29).[18] To a solution of salicylaldehyde (5.0 g, 41.0 mmol, 1.0 equiv) in acetone (125 mL) was added K_2CO_3 (6.23 g, 45.1 mmol, 1.1 equiv) and benzyl bromide (4.87 mL, 41.0 mmol, 1.0 equiv). The reaction was heated at reflux for 5 h then cooled to rt, filtered, and the filtrate was concentrated under reduced pressure. Purification by distillation (P = 0.34 Torr) afforded the title compound (7.1 g, 95%) as a colorless oil. bp 140-145 °C, 0.34 Torr; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.18 (s, 2H), 7.0–7.07 (m, 2H), 7.32–7.46 (m, 5H), 7.52 (ddd, 1H, *J* = 8.4, 7.2, 1.8 Hz), 7.86 (dd, 1H, *J* = 7.8, 1.8 Hz), 10.57 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 70.7, 113.3, 121.3, 125.4, 127.5, 128.5, 128.7, 129.0, 136.2, 136.3, 161.3, 189.9.

2-Benzyloxybenzyl alcohol (30). To a solution of aldehyde **29** (1.00 g, 4.71 mmol, 1.0 equiv) in MeOH (25 mL) at 0 °C was added $NaBH_4$ (0.40 g, 9.43 mmol, 2.0 equiv) portionwise over 10 min. After gas evolution ceased, the reaction mixture was warmed to rt and stirred for 30 min. The reaction was concentrated and the residue partitioned between H_2O (50 mL) and EtOAc (50 mL). The organic phase was separated and the aqueous phase extracted with EtOAc (2×75 mL). The combined organic extracts were dried (Na_2SO_4) then concentrated to afford the title compound (0.98 g, 98%), which required no further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.22 (br s, 1H), 4.73 (s, 2H), 5.12 (s, 2H), 6.92–7.00 (m, 2H), 7.24–7.29 (m, 1H), 7.29–7.37 (m, 2H), 7.37–7.44 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 62.4, 70.3, 111.9, 121.3, 127.6, 128.4, 129.0, 129.1, 129.2, 129.8, 137.0, 156.9.

2-Benzyloxybenzyl bromide (31). To a solution of **30** (2.0 g, 9.3 mmol, 1.0 equiv) in CH_2Cl_2 (60 mL) at 0 °C was added PPh_3 (3.67 g, 14.0 mmol, 1.5 equiv) and CBr_4 (4.64 g, 14.0 mmol, 1.5 equiv). After 30 min the reaction was concentrated *in-vacuo*, then the residue was taken up in Et_2O (50 mL) and placed at -20 °C for 30 min. The solution was filtered to remove solid Ph_3PO and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc) afforded the title compound (2.28 g, 88%). R_f = 0.75 (9:1 Hexane/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.62 (s, 2H), 5.16 (s, 2H), 6.80–7.10 (m, 2H), 7.20–7.55 (m, 7H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 29.4, 70.3, 112.5, 121.2, 126.8, 127.4, 128.1, 128.8, 130.4, 131.2, 137.1, 156.8.

5'-*O*-(*N*-(2-Benzyloxybenzyl)sulfamoyl)-2',3'-*O*-isopropylideneadenosine (32).[19] To a solution of **18**[13] (400 mg, 1.04 mmol, 1.0 equiv) in a mixture of CH_2Cl_2 (25 mL) and DMF (3 mL) was added Cs_2CO_3 (675 mg, 2.1 mmol, 2.0 equiv) followed by a solution of **31** (574 mg, 2.1 mmol, 2.0 equiv) in CH_2Cl_2 (5 mL) and the reaction stirred 16 h at rt. The reaction was diluted with H_2O (5 mL) and quenched by the addition of solid $KHSO_4$ with vigorous stirring until the pH of the aqueous layer was acidic (pH ~ 3). The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (2×30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash chromatography (10:1 EtOAc/hexanes) afforded the title compound (289 mg, 48%) along with (150 mg, 20%) of the bis benzylated side-product. Characterization data for bis benzylated side-product: R_f = 0.5 (EtOAc). Characterization data for **32**: R_f = 0.41 (EtOAc); ^{1}H NMR: (600 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.58 (s, 3H), 3.96–4.08 (m, 2H), 4.22 (d, 2H, *J* = 5.4 Hz), 4.29 (dd, 1H, *J* = 7.8, 4.8 Hz), 4.86 (dd, 1H, *J* = 6.0, 3.0 Hz), 5.05 (s, 2H), 5.22 (dd, 1H, *J* = 6.0, 2.4 Hz), 5.64 (t, 1H, *J* = 6.0

Hz), 5.80 (br s, 2H), 6.02 (d, 1H, *J* = 2.4 Hz), 6.84–6.92 (m, 2H), 7.18 (d, 1H, *J* = 7.2 Hz), 7.22 (t, 1H, *J* = 7.2 Hz), 7.26–7.32 (m, 1H), 7.32–7.38 (m, 4H), 7.77 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 25.0, 26.5, 44.4, 59.0, 71.0, 81.7, 86.5, 87.1, 93.5, 112.9, 115.0, 121.4, 121.8, 128.3, 128.7, 128.8, 129.2, 129.6, 130.4, 138.8, 140.5, 141.4, 150.2, 157.8, 158.8; HRMS (APCI-) calcd. for $C_{27}H_{29}N_6O_7S$ $[M-H]^-$ 581.1823, found 581.1822 (error 0.1 ppm).

5'-*O*-(*N*-(2-Hydroxybenzyl)sulfamoyl)-2',3'-*O*-isopropylideneadenosine (33). This was prepared starting from **32** (50 mg, 0.090 mmol) using the procedure described for the synthesis of **17**. Purification by flash chromatography (MeOH/EtOAc) afforded the title compound (35 mg, 83%). R_f = 0.35 (EtOAc); ^{1}H NMR (600 MHz, CD_3OD) δ 1.35 (s, 3H), 1.37 (s, 3H), 4.00–4.20 (m, 4H), 4.30–4.40 (m, 1H), 4.89 (dd, 1H, *J* = 6.3, 2.4 Hz), 5.30 (dd, 1H, *J* = 6.3, 1.5 Hz), 6.17 (d, 1H, *J* = 1.5 Hz), 6.71 (t, 1H, *J* = 7.2 Hz), 6.74 (d, 1H, *J* = 8.4 Hz), 7.05 (t, 1H, *J* = 7.8 Hz), 7.11 (d, 1H, *J* = 7.2 Hz), 8.18 (s, 1H), 8.20 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.4, 26.2, 42.2, 68.7, 81.7, 84.3, 84.4, 90.7, 114.3, 114.8, 119.21, 119.23, 123.4, 129.0, 129.8, 140.2, 149.1, 152.9, 155.4, 156.2; HRMS (APCI-) calcd. for $C_{20}H_{23}N_6O_7S$ $[M-H]^-$ 491.1354, found 491.1370 (error 3.2 ppm).

5'-*O*-(*N*-(2-Hydroxybenzyl)sulfamoyl)adenosine (11). This was prepared starting from **33** (30 mg, 0.060 mmol) using the procedure described for the synthesis of **7**. Purification by flash chromatography (60:40:1 EtOAcMeOH:Et_3N) afforded the title compound (16.2 mg, 81%). R_f = 0.23 (9:1 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 4.15–4.21 (m, 4H), 4.21–4.30 (m, 2H), 4.59 (t, 1H, *J* = 4.8 Hz), 6.01 (d, 1H, *J* = 4.8 Hz), 6.71 (t, 1H, *J* = 7.2 Hz), 6.75 (d, 1H, *J* = 8.4 Hz), 7.06 (t, 1H, *J* = 7.8 Hz), 7.16 (d, 1H, *J* = 7.8 Hz), 8.18 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 42.2, 68.6, 70.7, 74.3, 82.5, 88.6, 114.8, 119.3, 123.3, 128.9, 129.7, 139.7 (2C), 149.5, 152.8, 155.4, 156.1; HRMS (APCI+) calcd. for $C_{17}H_{21}N_6O_7S$ $[M+H]^+$ 453.1197, found 453.1184 (error 2.8 ppm).

Synthetic Procedures for the Preparation of Inhibitor 12

Methyl 2-(benzyloxy)benzoate (35). To a solution of *O*-benzylsalycilic acid (5.0 g, 21.9 mmol, 1.0 equiv) in acetone (150 mL) was added solid K_2CO_3 (7.57 g, 54.8 mmol, 2.5 equiv) and MeI (2.05 mL, 32.9 mmol, 1.5 equiv) and the reaction heated at reflux for 4 h. The reaction mixture cooled to rt, filtered, and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (hexane/EtOAc) afforded the title compound (5.3 g, 100%) that was greater than 98% pure as judged by ^{1}H NMR and was used without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.90 (s, 3H), 5.18 (s, 2H), 6.80–7.04 (m, 2H), 7.35–7.48 (m, 4H), 7.50 (d, 2H, *J* = 7.8 Hz), 7.83 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 52.2, 70.8, 114.1, 120.8, 121.1, 127.1, 128.0, 128.8, 132.0, 133.6, 137.0, 158.4, 167.0.

Dimethyl 2-(2-(benzyloxy)phenyl)-2-oxoethylphosphonate (36).[20] To a solution of dimethyl methylphosphonate (0.537g, 4.33 mmol, 2.1 equiv) in THF (6 mL) at -78 °C was added a solution of *n*-BuLi (2.5 M in hexanes, 1.8 mL, 4.33 mmol, 2.1 equiv) to afford a white heterogeneous mixture, which was stirred 15 min. Next, a solution of **35** (500 mg, 2.06 mmol, 1.0 equiv) in THF (6 mL) was added slowly down the side of the flask to the lithium phosphonate solution at -78 °C to afford a clear solution. After stirring for 1 h, the cooling bath was removed and the solution was allowed to warm to 0 °C. The

reaction was partitioned between EtOAc (25 mL) and 1 M aq. HCl (10 mL) and sat'd aq. NaCl (10 mL). The organic layer was separated and the aq. layer was extracted with EtOAc (2×25 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to a colorless oil. Purification by flash chromatography (4:1 EtOAc/hexane) afforded the title compound (610 mg, 88%) as a colorless oil. R_f = 0.43 (EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (d, 6H, $^3J_{H,P}$ = 11.4 Hz), 3.77 (d, 2H, $^2J_{H,P}$ = 21.6 Hz), 5.16 (s, 2H), 6.96–7.04 (m, 2H), 7.30–7.50 (m, 6H), 7.71 (dd, 1H, *J* = 7.8, 1.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 41.9 (d, $^1J_{C,P}$ = 131 Hz), 53.6 (d, $^2J_{C,P}$ = 6.0 Hz), 71.2, 113.2, 121.4, 127.9, 128.1, 128.7, 129.1, 131.3, 134.6, 136.1, 158.1, 193.5 (d, $^2J_{C,P}$ = 7.3 Hz); ^{31}P NMR (195 MHz, $CDCl_3$) δ 25.1; HRMS (APCI-) calcd. for $C_{17}H_{18}O_5P$ $[M-H]^-$ 333.0897, found 333.0928 (error 9.3 ppm).

5'-*O*-[(Hydroxy)[(2-oxo-2(2-benzyloxyphenyl))ethyl)phosphinyl]-2',3'-*O*-isopropylideneadenosine triethylammonium salt (38). To a solution of **36** (2.0 g, 6.0 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL) was added TMSBr (3.89 mL, 29.9 mmol, 5.0 equiv).[21] The reaction was monitored by ^{31}P NMR and found to be complete at 7 h. The reaction was concentrated *in-vacuo* then treated with 5% aq pyridine. The reaction was concentrated under reduced pressure and the residue was azeotropically dried with anhydrous pyridine (3×30 mL) to provide crude **37**. To a solution of **37** in anhydrous pyridine (30 mL) was added trisyl chloride (1.69 g, 7.8 mmol, 1.5 equiv).[22] The reaction was slightly exothermic and stirred 15 min then 2',3'-*O*-isopropylideneadenosine (1.59 g, 5.7 mmol, 1.1 equiv) was added and the reaction was stirred 16 h. The reaction was concentrated *in-vacuo*. Purification by flash chromatography (MeOH/EtOAc + 1% Et_3N) afforded the title compound (184 mg, 6%). R_f = 0.75 (1:2 MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 1.22 (t, 9H, *J* = 7.2 Hz), 1.35 (s, 3H), 1.58 (s, 3H), 3.08 (q, 6H, *J* = 7.2 Hz), 3.50–3.70 (m, 2H), 3.81–3.92 (m, 2H), 4.28 (m, 1H), 4.95 (dd, 1H, *J* = 6.0, 2.4 Hz), 5.15 (s, 2H), 5.24 (dd, 1H, *J* = 6.0, 3.6 Hz), 6.14 (d, 1H, *J* = 3.0 Hz), 6.92 (t, 1H, *J* = 7.8 Hz), 7.06 (d, 1H, *J* = 8.4 Hz), 7.22–7.28 (m, 1H), 7.32 (t, 2H, *J* = 7.2 Hz), 7.35–7.41 (m, 1H), 7.41 (d, 2H, *J* = 7.8 Hz), 7.56 (dd, 1H, *J* = 7.8, 1.8 Hz), 8.12 (s, 1H), 8.40 (s, 1H); ^{13}C NMR (150 MHz, CD_3CN) δ 8.0, 24.7, 26.6, 45.4 (d, $^1J_{C,P}$ = 130 Hz), 45.5, 64.6, 70.5, 81.9, 85.0, 86.0 (d, $^2J_{C,P}$ = 7.2 Hz), 90.4, 113.3, 113.5, 119.2, 120.7, 127.8, 128.1, 128.6, 130.3, 130.4, 132.8, 136.9, 140.0, 149.6, 152.9, 156.0, 157.0, 198.3; ^{31}P NMR (195 MHz, CD_3CN) δ 14.9; HRMS (ESI+) calcd. for $C_{28}H_{31}N_5O_8P$ $[M+H]^+$ 596.1904, found 596.1914 (error 1.6 ppm).

5'-*O*-[(Hydroxy)(2-oxo-2-(2-hydroxyphenyl)ethyl)phosphinyl]-2',3'-*O*-isopropylideneadenosine triethylammonium salt (39). This was prepared starting from **38** (80 mg, 0.13 mmol) using the procedure described for the synthesis of **17**. Purification by flash chromatography (60:40:1 EtOAc/MeOH/Et_3N) afforded the title compound (25 mg, 40%). R_f = 0.16 (1:2 MeOH/EtOAc); ^{1}H NMR (600 MHz, CD_3OD) δ 1.21 (t, 9H, *J* = 7.2 Hz), 1.36 (s, 3H), 1.58 (s, 3H), 3.03 (q, 6H, *J* = 7.2 Hz), 3.42–3.62 (m, 2H), 4.00–4.10 (m, 2H), 4.40–4.46 (m, 1H), 5.06 (dd, 1H, *J* = 6.0, 1.8 Hz), 5.30 (dd, 1H, *J* = 6.0, 3.0 Hz), 6.17 (d, 1H, *J* = 3.0 Hz), 6.82–6.88 (m, 2H), 7.40–7.45 (m, 1H), 7.96 (dd, 1H, *J* = 7.8 Hz), 8.20 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.4, 24.5, 26.5, 46.4, 64.8 (d, $^2J_{C,P}$ = 5.7 Hz), 82.1, 85.5 (d, $^2J_{C,P}$ = 12 Hz), 85.6, 90.6, 114.0, 117.5, 118.8, 119.0, 120.1, 132.5, 136.2, 140.1,

149.2, 152.7, 156.1, 162.4, 202.1, (-CO<u>C</u>H_2PO_3- the underlined carbon [expected ~45 ppm] was not observed due to deuterium exchange, the resulting doublet of pentets due to coupling with deuterium and phosphorous nucleii precluded observation of this carbon signal. Acquisition of ^{13}C spectra in aprotic solvents was hindered by limited substrate solubility); ^{31}P NMR (195 MHz, CD_3OD) δ 13.8; HRMS (ESI+) calcd. for $C_{21}H_{25}N_5O_8P$ $[M+H]^+$ 506.1435, found 506.1446 (error 2.1 ppm).

5'-*O*-[(Hydroxy)(2-oxo-2-(2-hydroxyphenyl)ethyl)phosphinyl]adenosine triethylammonium salt (12). This was prepared starting from **39** (25 mg, 0.040 mmol) using the procedure described for the synthesis of **7**. Purification by flash chromatography (66:33:1 EtOAc/MeOH/Et_3N) afforded the title compound (21 mg, 90%). R_f = 0.15 (1:1 MeOH/EtOAc); ^1H NMR (600 MHz, CD_3OD) δ 1.25 (t, 9H, J = 7.2 Hz), 3.15 (q, 6H, J = 7.2 Hz), 3.42–3.62 (m, 2H), 4.10–4.16 (m, 2H), 4.16–4.22 (m, 1H), 4.35 (dd, 1H, J = 4.8, 3.6 Hz), 4.67 (t, 1H, J = 5.4 Hz), 6.05 (d, 1H, J = 6.0 Hz), 6.84–6.90 (m, 2H), 7.43 (t, 1H, J = 9.0 Hz), 8.01 (d, 1H, J = 7.8 Hz), 8.18 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.2, 46.5, 58.9, 64.5 (d, $^2J_{C,P}$ = 7.8 Hz), 71.1, 74.8, 84.6 (d, $^2J_{C,P}$ = 7.9 Hz), 87.8, 117.5, 118.8, 119.0, 120.1, 132.5, 136.2, 139.9, 149.7, 152.6, 156.0, 162.4, 201.9 (d, $^2J_{C,P}$ = 6.3 Hz), (-CO<u>C</u>H_2PO_3- the underlined carbon [expected ~45 ppm] was not observed due to deuterium exchange, the resulting doublet of pentets due to coupling with deuterium and phosphorous nucleii precluded observation of this carbon signal. Acquisition of ^{13}C spectra in aprotic solvents was hindered by limited substrate solubility); ^{31}P NMR (195 MHz, CD_3OD) δ 13.6; HRMS (ESI+) calcd. for $C_{18}H_{21}N_5O_8P$ $[M+H]^+$ 466.1122, found 466.1162 (error 8.5 ppm).

Synthetic Procedures for the Preparation of Inhibitor 13

2-(Benzyloxy)-*N*-methoxy-*N*-methylbenzamide (40).[23] To a solution of 2-benzyloxybenzoic acid (1.14 g, 5.0 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) and a catalytic amount of DMF (2 drops) freshly distilled oxalyl chloride (472 μL, 6.0 mmol, 1.2 equiv) was slowly added via syringe at 0 °C. The reaction was stirred for 2 h then concentrated *in-vacuo* under reduced pressure. The crude acid chloride obtained was dissolved in ethanol-free anhydrous chloroform[24] (50 mL) and *N, O*-dimethylhydroxylamine·HCl (0.5 g, 6 mmol, 1.2 equiv) was added. The reaction mixture was cooled to 0 °C and Et_3N (20 mL) was added dropwise. The mixture was stirred at rt for 2.5 h then concentrated *in vacuo*. The residue was partitioned between sat'd aq NaCl and 1:1 CH_2Cl_2/Et_2O. The organic layer was dried (Na_2SO_4) and concentrated to afford the title compound (1.19 g, 80%) as an oil that was greater than 98% pure based on ^1H NMR. R_f = 0.3 (3:1 hexanes/EtOAc); ^1H NMR (600 MHz, $CDCl_3$) δ 3.23 (br s, 3H), 3.40 (br s, 3H), 5.09 (s, 2H), 6.55–6.95 (m, 2H), 7.29–7.40 (m, 7H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 33.3, 61.4, 70.6, 112.9, 121.1, 127.3, 128.1, 128.6, 128.7, 129.2, 130.8, 137.0, 155.1, 169.5; MS (ESI+) calcd. for $C_{16}H_{18}NO_3$ $[M+H]^+$ 272.12, found 272.13.

1-(2-Benzyloxy)phenyl-3-(trimethylsilyl)prop-2-yn-1-one (41).[25] To a solution of ethynyltrimethylsilane (424 μL, 3 mmol, 1.0 equiv) in THF (30 mL) was added *n*-BuLi (2.5 M in hexane, 1.2 mL, 3.4 mmol, 1.13 equiv) at 0 °C. After stirring for 30 min, a solution of **40** (814 mg, 3.0 mmol, 1.0 equiv) in THF (3 mL) was added dropwise via syringe. The reaction was allowed to slowly warm to 25 °C over 2 h then diluted with Et_2O (30 mL) and poured onto sat'd aq NH_4Cl (30 mL). The

organic phase was separated and the aqueous phase extracted with Et_2O (2x30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash chromatography (15:1 hexanes/EtOAc) afforded the title compound (0.71 g, 76%) as a light yellow oil. R_f = 0.27 (5:1 hexane/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.00 (s, 9H), 5.01 (s, 2H), 6.78 (d, 1H, *J* = 8.4 Hz), 6.80–6.82 (m, 1H), 7.08–7.10 (m, 1H), 7.15–7.18 (m, 2H), 7.24 (dd, 1H, *J* = 7.8, 1.8 Hz), 7.29–7.32 (m, 2H), 7.76 (dd, 1H, *J* = 7.8, 1.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 0.00, 68.9, 71.1, 114.4, 121.3, 127.6, 128.5, 129.3, 129.5, 131.6, 133.4, 135.5, 137.2, 159.4, 177.4; HRMS (ESI+) calcd. for $C_{19}H_{21}O_2Si$ $[M+H]^+$ 309.1311, found 309.1316 (error 1.6 ppm).

1-(2-(Benzyloxy)phenyl)prop-2-yn-1-one (42).[26] To a solution of **41** (450 mg, 1.46 mmol, 1.0 equiv) in THF (20 mL) at 0 °C was added TBAF (1.0 M solution in THF, 1.46 mL, 1.46 mmol, 1.0 equiv.). After 20 min, the reaction was diluted with Et_2O (40 mL) and was washed with sat'd aq NaCl (2×40 mL). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography (20:1 hexane/EtOAc) afforded the title compound (272 mg, 79%) as colorless oil. R_f = 0.24 (20:1 hexanes/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.25 (s, 1H), 5.21 (s, 2H), 7.02 (d, 1H, *J* = 8.4 Hz), 7.03–7.04 (m, 1H), 7.30–7.32 (m, 1H), 7.36–7.38 (m, 2H), 7.40–7.50 (m, 3H), 8.02 (dd, 1H, *J* = 8.4, 1.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 70.8, 79.8, 82.7, 113.9, 120.9, 126.6, 127.4, 128.2, 128.8, 133.0, 135.5, 136.4, 159.2, 176.2; MS (ESI+) calcd. for $C_{16}H_{13}O_2$ $[M+H]^+$ 237.09, found 237.00.

***N*^{6}-*tert*-Butoxycarbonyl-5'-*N*-(4-(2-benzyloxybenzoyl)-1*H*-1,2,3-triazol-1-yl)-5'-deoxy-2',3'-*O*-isopropylideneadenosine (43).**[27] To a suspension of **42** (13 mg, 0.056 mmol, 1.0 equiv) and azide **25** (24 mg, 0.056 mmol, 1.0 equiv) in H_2O (600 μL) and *tert*-butyl alcohol (300 μL) was added sodium ascorbate (1.4 mg, 0.017 mmol, 0.30 equiv) followed by $CuSO_4 \cdot (H_2O)_5$ (1.4 mg, 0.0056 mmol, 0.1 equiv). The resulting heterogeneous mixture was stirred vigorously for 16 h. The reaction mixture was concentrated *in vacuo* and the residue was subjected to flash chromatography (3:1 EtOAc/hexane) to afford the title compound (33.0 mg, 89%) as a white crystalline solid. R_f = 0.26 (3:1 EtOAc/hexane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.37 (s, 3H), 1.56 (s, 9H), 1.59 (s, 3H), 4.57 (ddd, 1H, *J* = 8.4, 4.2, 3.6 Hz), 4.70 (dd, 1H, *J* = 14.4, 8.4 Hz), 4.82 (dd, 1H, *J* = 14.4, 3.6 Hz), 5.03 (s, 2H), 5.19 (dd, 1H, *J* = 6.0, 4.2 Hz), 5.38 (dd, 1H, *J* = 6.0, 1.8 Hz), 6.06 (d, 1H, *J* = 1.8 Hz), 7.01 (d, 1H, *J* = 7.8), 7.03 (t, 1H, *J* = 7.2 Hz), 7.16–7.25 (m, 5H), 7.43 (dt, 1H, *J* = 7.8, 1.8 Hz), 7.54 (dd, 1H, *J* = 7.2, 1.8 Hz), 7.86 (s, 1H), 7.99 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.5, 27.3, 28.3, 52.1, 70.7, 82.0, 83.0, 84.3, 85.6, 90.9, 113.5, 115.6, 121.1, 122.0, 127.2, 127.9, 128.1, 128.5, 128.8, 130.5, 133.1, 136.8, 142.2, 148.6, 149.8, 150.1, 150.4, 153.3, 157.4, 188.1; MS (ESI+) calcd. for $C_{34}H_{37}N_8O_7$ $[M+H]^+$ 669.28, found 669.28.

5'-Deoxy-5'-*N*-((4-(2-hydroxybenzoyl)-1*H*-1,2,3-triazol-1-yl)adenosine (**13**). To a solution of **40** (33 mg, 0.049 mmol) in MeOH (5 mL) was added 10% Pd/C (3.3 mg) and the reaction placed under an H_2 atm. After 16 h, the reaction was filtered through Celite and the filtrate concentrated under reduced pressure. The residue was treated with 80% aq TFA (2 mL) and stirred 30 min, then concentrated *in-vacuo* to afford the title compound (21.6 mg, 100%) as a white crystalline solid, which required no

further purification. ^{1}H NMR (600 MHz, CD_3OD) δ 4.40–4.44 (m, 1H), 4.50 (dd, 1H, *J* = 7.2, 6.0 Hz), 4.65 (dd, 1H, *J* = 4.8, 3.6 Hz), 4.88–4.95 (m, 2H), 4.95 (dd, 1H, *J* = 4.8, 3.0 Hz), 6.02 (d, 1H, *J* = 3.0 Hz), 6.93–6.98 (m, 1H), 7.54 (dd, 1H, *J* = 7.8, 7.2 Hz), 8.31 (s, 1H), 8.20 (s, 1H), 8.38 (s, 1H), 8.65–8.68 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 50.6, 70.5, 73.4, 81.9, 90.3, 117.8, 119.1, 119.3, 119.7, 131.1, 133.0, 136.6, 142.7, 146.6, 146.8, 148.5, 152.4, 163.2, 189.1; HRMS (ESI+) calcd. for $C_{19}H_{19}N_8O_5$ $[M+H]^+$ 439.1478, found 439.1449 (error 6.6 ppm).

HPLC Purity of Inhibitors. HPLC purity was determined for all final target inhibitors **7**–**13**. For inhibitors **7**–**10** that showed activity, two separate reverse-phase HPLC conditions were performed. An Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm particle size) with detection at 190–400 nm (The integrated absorbance signal from 190–400 nm was used to evaluate purity) and the indicated HPLC conditions (Methods A–C, Solvent A = H_2O, Solvent B = MeCN, flowrate = 0.8 mL/min) as described below was employed to determine the purity of inhibitors.

Method A: 0–20 min: gradient 10%–95% B; isocratic 20–27 min: 95% B
Method B: 0–20 min: isocratic 15% B; gradient 20–21 min: 15–95% B; isocratic 21–27 min: 95% B.
Method C: 0–20 min: isocatic 20% B; gradient 20–21 min: 15–95% B; isocratic 21–27 min: 95% B.
Method D: 0-3 min: isocratic 5% B; 3–30 min: gradient 5-70% B; 30–40 min: gradient 70–100% B.

Table S2. HPLC Purity of Inhibitors.

Inhibitor	Method	t_{ret} (min)	Purity (%)
7	A	4.48	94
	B	3.95	95
8	A	6.25	99
	B	3.26	95
9	A	5.67	96
	B	9.73	97
10	A	5.40	96
	C	3.83	97
11	A	7.94	93
12	A	4.38	97
13	D	19.2	96

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