

5'-O-[(N-Acyl)sulfamoyl]adenosines as Antitubercular Agents that Inhibit MbtA: An Adenylation Enzyme Required for Siderophore Biosynthesis of the Mycobactins

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A study of the structure–activity relationships of 5'-O-[(N-(salicyl)sulfamoyl]adenosine (**6**), a potent inhibitor of the bifunctional enzyme salicyl-AMP ligase (MbtA, encoded by the gene *Rv2384*) in *Mycobacterium tuberculosis*, is described, targeting the salicyl moiety. A systematic series of analogues was prepared exploring the importance of substitution at the C-2 position revealing that a hydroxy group is required for optimal activity. Examination of a series of substituted salicyl derivatives indicated that substitution at C-4 was tolerated. Consequently, a series of analogues at this position provided 4-fluoro derivative, which displayed an impressive MIC₉₉ of 0.098 μ M against whole-cell *M. tuberculosis* under iron-limiting conditions. Examination of other heterocyclic, cycloalkyl, alkyl, and aminoacyl replacements of the salicyl moiety demonstrated that these nonconservative modifications were poorly tolerated, a result consistent with the fairly strict substrate specificities of related non-ribosomal peptide synthetase adenylation enzymes.

Introduction

Tuberculosis (TB^a) is the leading cause of infectious disease mortality by a bacterial pathogen.¹ TB is extremely difficult to treat since the etiological agent, *Mycobacterium tuberculosis*, is slow growing, is capable of switching its metabolism to a latent or nonreplicating state, and possesses a nearly impenetrable cell wall, which provides a permeability barrier that limits the uptake of many antibiotics. As a result of these factors, effective therapy requires prolonged treatment with 3–4 antibiotics. A combination of poor patient compliance and the inevitable evolution of drug resistance has resulted in the development of multidrug resistant tuberculosis (MDR-TB) defined as resistance to both the first-line agents isoniazid and rifampin. Further, co-infection with HIV/AIDS is a deadly combination, and over one-third of HIV-related deaths are due to TB or related mycobacterial infections. Despite the tremendous advances in TB chemotherapy made in the 20th century, TB has now reemerged as one of the most significant threats to global public health; thus there is a great demand for new drugs to combat TB. The Global Alliance for TB Drug Development (<http://www.tballiance.org>) recommends that an ideal new TB drug should shorten the duration of effective treatment, improve the treatment of MDR-TB, and provide more effective treatment of latent TB infection. Additionally, drugs that are specific antitubercular agents as opposed to broad-spectrum antibiotics may be advantageous to prevent disturbance to beneficial commensal bacteria as well as minimize the potential development of cross-resistance.

Iron is an essential micronutrient for almost all known organisms including *M. tuberculosis* where it serves as an obligate cofactor for numerous metalloproteins.² A particular exception is *Borrelia burgdorferi*, the causative agent of Lyme disease, which apparently has evolved to utilize manganese instead of iron.³ Notwithstanding this anomaly, iron acquisition is critical for bacterial pathogenesis, and bacteria have evolved a variety of mechanisms to obtain this vital nutrient. The most common mechanism involves the synthesis, secretion, and reuptake of small molecule iron-chelators termed siderophores.² In a mammalian host, siderophore production is crucial since the concentration of free iron is approximately 10⁻²⁴ M, which is far too low to support bacterial colonization and growth.⁴ Thus, inhibition of siderophore biosynthesis represents a logical strategy for the development of a new class of antibiotics.⁵ An alternate strategy pioneered by Miller and co-workers involves antagonism of siderophore function using synthetic siderophore analogues that likely cannot be taken up by the bacterial siderophore transport system.^{3,2}

M. tuberculosis produces two series of structurally related siderophores, collectively known as the mycobactins, that are critical for virulence and growth (Figure 1).^{6,7} Mycobactin biosynthesis is initiated by MbtA, an adenylate-forming enzyme that catalyzes a two-step reaction and is responsible for incorporating salicylic acid into the mycobactins (Figure 1).⁸ MbtA first binds its substrates salicylic acid and ATP then catalyzes their condensation to afford acyladenylate **2** and pyrophosphate. The acyladenylate remains tightly bound whereas pyrophosphate dissociates. Next, MbtA binds the N-terminal aryl carrier domain of MbtB and catalyzes the transfer of the salicyl moiety onto the nucleophilic sulfur atom of the phosphopantetheinyl cofactor of MbtB to afford thioester tethered-MbtB **3** that is elaborated in the mycobactins by a mixed nonribosomal peptide synthetase polyketide synthase (NRPS-PKS) assembly line.⁸

Acyladenylates have been shown to bind several orders of magnitude more tightly than the substrate acids since they simultaneously occupy both substrate binding pockets.^{9,10} Thus

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^a Abbreviations: CDI, carbonyl diimidazole; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; MDR-TB, multidrug resistant tuberculosis; MIC, minimum inhibitory concentration; NHS, *N*-hydroxysuccinimide; NRPS, nonribosomal peptide synthetase; PAS, *para*-aminosalicylic acid; PDB, protein data bank; PKS, polyketide synthase; SAR, structure–activity relationships; TB, tuberculosis; TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid.

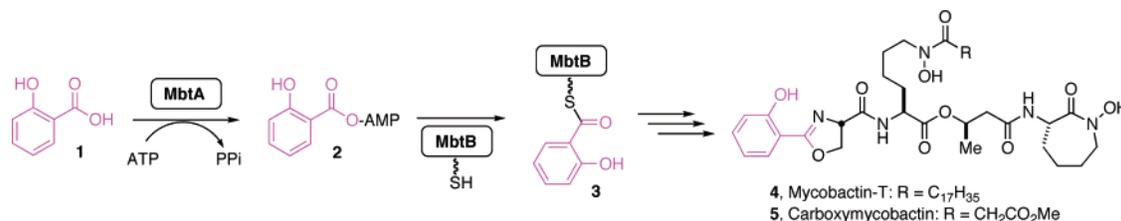


Figure 1. Biosynthesis of the mycobactins and carboxymycobactins.⁶ The depicted lipid side chain is a representative as both **4** and **5** are isolated as a suite of compounds with various length lipid residues.

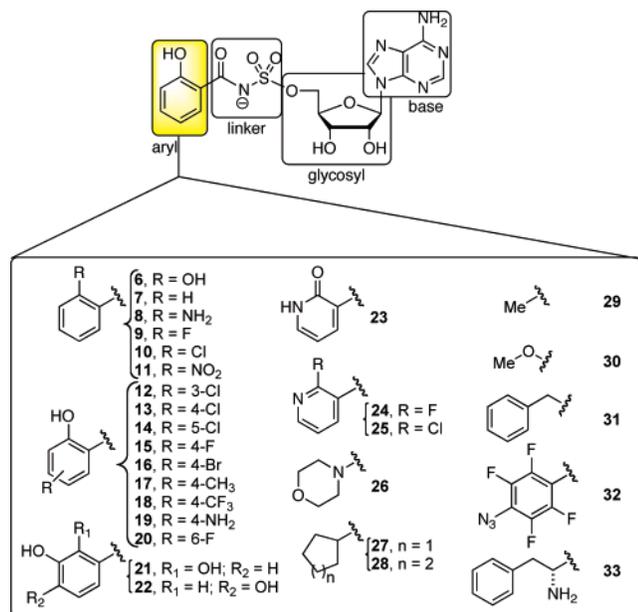


Figure 2. Bisubstrate inhibitors of MbtA. The expanded portion of the figure shows the Ar modifications described herein.

acyladenylate analogues that incorporate a stable linker as a bioisostere of the labile acylphosphate function provide potent adenylation enzyme inhibitors. The general inhibitor scaffold is comprised of four domains (aryl, linker, glycosyl, and base) as depicted in Figure 2. The most crucial portion of the inhibitor scaffold is the linker domain since this must be metabolically stable and appropriately position both the aryl and nucleoside moieties in their respective binding pockets. We have previously explored both the molecular geometry and polarity of the linker pharmacophore with the preparation of β -ketophosphonate, acylsulfamate, acylsulfamide, sulfamate, β -ketosulfonamide, α,α -difluoro- β -ketosulfonamide, acyltriazole, and vinylsulfonamide linkages as surrogates for the labile acylphosphate linkage.^{11–13} Inhibitors incorporating the acylsulfamate and acylsulfamide linkages were found to be the most potent with low nanomolar apparent inhibition constants and possessed sub-micromolar antitubercular activity against whole-cell *M. tuberculosis* rivaling the first-line agent isoniazid.^{11,14} Next, we systematically examined the glycosyl domain and found that both the 3'-hydroxy and 4'-ribofuranose ring oxygen were dispensable for bioactivity while modifications making the sugar either more or less flexible were detrimental.¹⁵ In this article, we explore the importance of the aryl ring of the bisubstrate inhibitor scaffold.

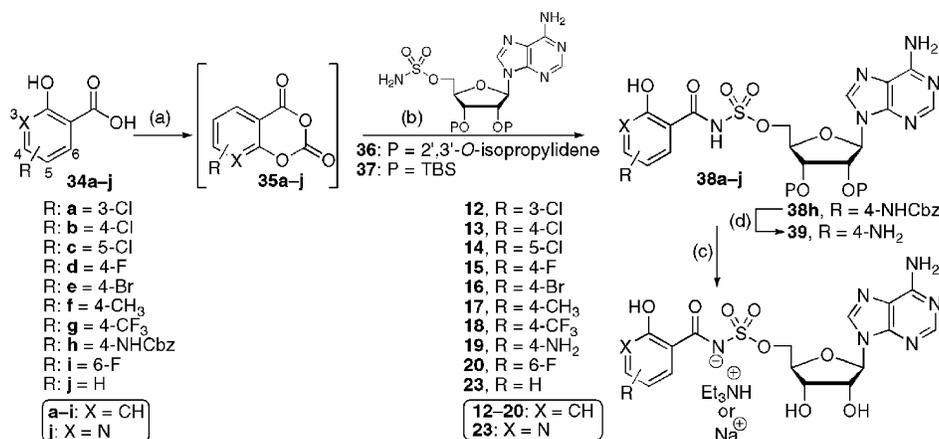
Results and Discussion

Chemistry. Since NRPS adenylation domains exhibit a fairly strict substrate specificity, bisubstrate inhibitors containing a number of conservative aryl modifications were prepared to

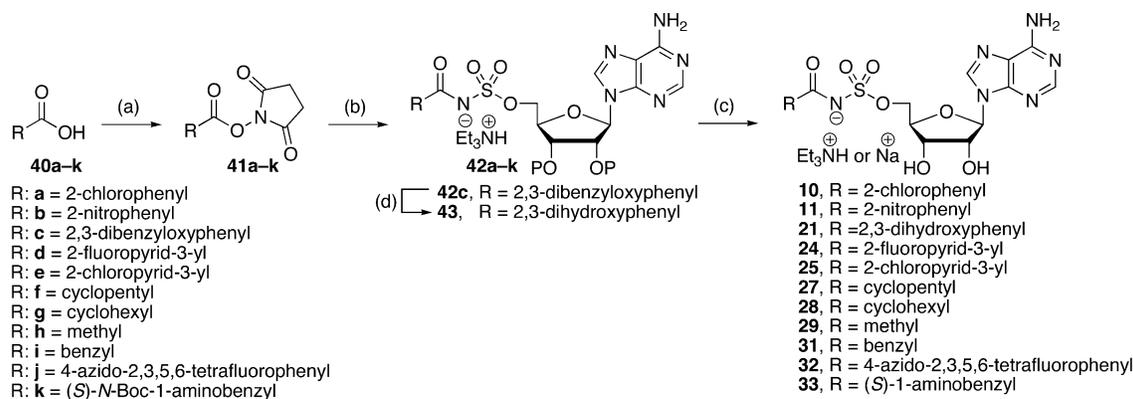
explore the importance of the *ortho*-hydroxy group, to define the steric requirements of the shallow aryl acid binding pocket of MbtA, and to assess the potential for interacting with specific residues within the active site such as C240. Additionally, a number of heteroaryl, cycloalkyl, alkyl, and aminoalkyl modifications were targeted. The syntheses of **6**,^{11,14,16} **7**,^{11,16} **8**,¹¹ **9**,¹⁶ **21**,¹⁶ and **22**¹⁷ were disclosed previously, but **8** and **21** were synthesized by alternate routes in improved overall yields. The other 22 inhibitors were synthesized through three related synthetic routes as described below.

Salicylic acid derivatives **12–20** and **23** were prepared according to the method described by Tan, Quadri, and co-workers from the corresponding acids **34a–j** by direct activation with CDI to afford intermediate cyclic anhydrides **35a–j**, which were not isolated but subsequently reacted with either protected 5'-*O*-(sulfamoyl)adenosine derivatives **36** or **37** employing either DBU or Cs₂CO₃ as base to afford **38a–j** (Scheme 1).¹⁴ Catalytic hydrogenation of **38h** provided *p*-aminosalicylic derivative **39**. Final deprotection of the isopropylidene acetal (80% aq TFA) or TBS ethers (TBAF) furnished bisubstrate inhibitors **12–20** and **23**. Anthranoyl analogue **8** was prepared analogously from isatoic anhydride and **36** (not shown, see Experimental Section). The *tert*-butyldimethylsilyl (TBS) protected nucleosides were preferred for compounds, such as nicotinylic derivative **23**, that decomposed under acidic conditions. Compounds **15–18** and **23**, which were initially isolated as the tetrabutylammonium salts, were converted to the corresponding sodium salts by ion-exchange with a strong cation exchange resin in the sodium form.

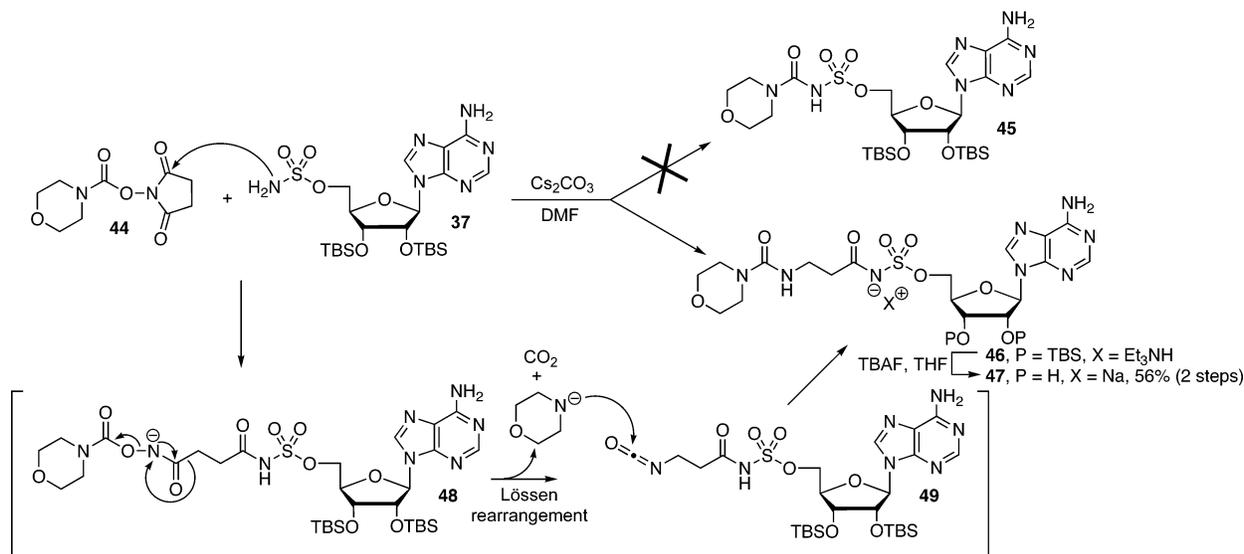
Direct CDI activation of aryl acids lacking a 2-hydroxy group was much less efficient. In these cases the acyl imidazolide that forms in situ is substantially less reactive than the cyclic anhydride species (see compound **35**, Scheme 1) formed with salicylic acid derivatives. For most other aryl-, heteroaryl-, and alkyl carboxylic acids (**40a–k**), the corresponding preformed *N*-hydroxysuccinimide (NHS) esters provided superior coupling yields. Thus, coupling of NHS esters **41a–k** to **36** or **37** mediated by either DBU or Cs₂CO₃ afforded acylsulfamate derivatives **42a–k** (Scheme 2).¹⁸ Catalytic hydrogenation of **42c** provided 2,3-dihydroxybenzoyl derivative **43**. In general Cs₂CO₃ as base is favored since in a few instances, such as **42a**, DBU salts of the coupled products were obtained—the DBU could not be removed by an acid wash, ion-exchange, or by chromatography (coeluting with 1% Et₃N). Deprotection of the isopropylidene acetal (80% aq TFA) or the TBS ethers (TBAF) of **42a–b**, **42d–k**, and **43** yielded the final bisubstrate inhibitors **10**, **11**, **21**, **24**, **25**, **27–29**, and **31–33**. Compounds **27–29** and **31**, which were initially isolated as tetrabutylammonium salts, were converted to the corresponding sodium salts by ion-exchange chromatography. The acetonide protected nucleosides were preferred for compounds, such as 4-azido-2,3,5,6-tetrafluorophenyl derivative **32**, that decomposed under the fluoride mediated conditions (TBAF or HF·pyr) required for TBS deprotection.

Scheme 1^a

^a Reaction conditions: (a) CDI, MeCN, 60 °C; (b) **36** or **37**, DBU or Cs₂CO₃, DMF; (c) 80% aq TFA, 0 °C; or TBAF, THF; (d) H₂, Pd/C.

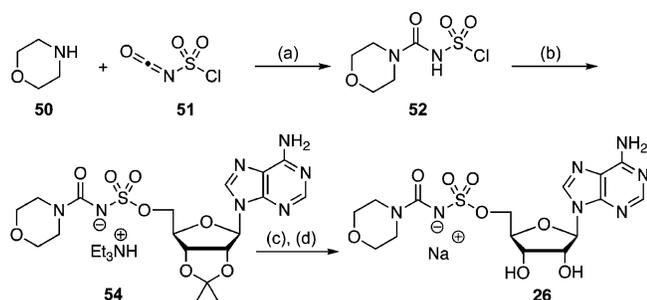
Scheme 2^a

^a Reaction conditions: (a) DCC, NHS, THF; (a) **36** or **37**, DBU or Cs₂CO₃, DMF; (c) 80% aq TFA or TBAF, THF; (d) H₂, Pd/C.

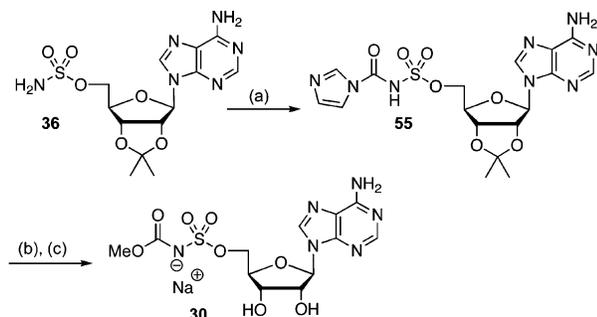
Scheme 3^a

Attempts to prepare a morpholine derivative from the corresponding NHS ester **44** and 5'-O-sulfamoyladenine derivative **37** did not afford the anticipated product **45** but rather **46** which was deprotected with TBAF to provide **47** in 56% overall yield from **44** (Scheme 3). The β -alanine fragment in **47** was confirmed by HRMS and NMR.¹⁹ The β -alanine moiety of compound **46** is hypothesized to arise from a sequential series of reactions as depicted in Scheme 3 above. Initial nucleophilic

attack of sulfamate **37** onto the succinimidyl carbonyl of **44** followed by opening of the imide affords acylhydroxamate intermediate **48**, which in turn undergoes Lössen rearrangement to provide isocyanate **49** and expulsion of the carbamic acid of morpholine. Decarboxylation of the carbamic acid affords morpholine that condenses with isocyanate **49** to provide the observed β -alanine urea derivative **46**. Precedent exists for opening of the succinimide by a nucleophile.²⁰ Failure to obtain

Scheme 4^a

^a Reaction conditions: (a) CH₂Cl₂, pPyr; (b) 53, MeCN; (c) 80% aq TFA; (d) ion-exchange, 4% overall.

Scheme 5^a

^a Reaction conditions: (a) MeCN, CDI; (b) MeOH, Et₃N; (c) 80% aq TFA; (d) ion-exchange, 64% overall.

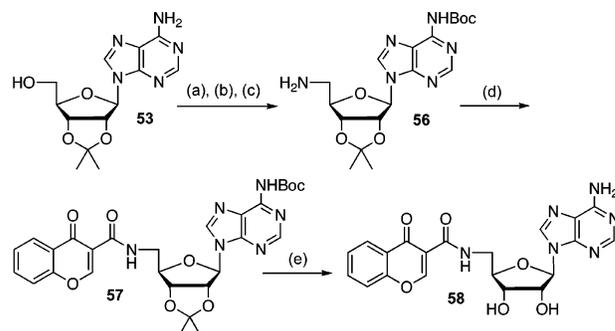
the desired product arises from the attenuated electrophilicity of the *N*-(morpholinyl)carbonyl group, which favors addition at the more reactive imide carbonyls.

On the basis of the above synthetic considerations, morpholine analogue 26 was successfully prepared by simply interchanging the electrophile. Thus, treatment of chlorosulfonylisocyanate 51 with morpholine 50 provided chlorosulfonylurea 52 (Scheme 4). This intermediate was not isolated but condensed with 2',3'-*O*-isopropylideneadenosine 53 to afford 54; subsequent acetonide deprotection (80% aq TFA) and ion-exchange to the sodium salt afforded 26.

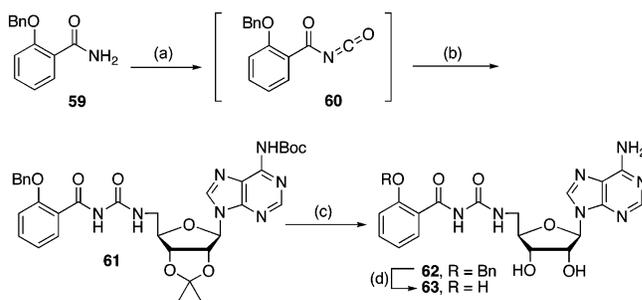
Treatment of 5'-*O*-(sulfamoyl)adenosine derivative 36 with carbonyl diimidazole afforded [(imidazolyl)carbonyl]sulfamate 55 (Scheme 5). This intermediate was not isolated but treated with methanol and triethylamine, and subsequent acetonide deprotection (80% aq TFA) and ion-exchange to the sodium salt afforded 30.

Bicyclic chromone 58 was prepared as both structural and modeling studies suggested that the *ortho*-hydroxy group of 6 internally hydrogen bonds with the sulfamate nitrogen.^{12,21} This analogue simultaneously explores modifications to both the aryl and linker domains of the inhibitor scaffold and was synthesized from 2',3'-*O*-isopropylideneadenosine 53 by interconversion of the 5'-alcohol to an azide, Boc protection of the *N*⁶-amine of adenine, and hydrogenation to afford 56 (Scheme 6).²² CDI mediated coupling of chromone-3-carboxylic acid provided 57 that was deprotected with aqueous TFA to furnish 58.

Finally, acylurea analogue 63 was prepared in order to examine the importance of the sulfonyl group of the linker domain of 6 (Figure 2). Acylurea 63 was prepared from 2-benzyloxybenzamide 59 by treatment with oxalyl chloride to provide intermediate acylisocyanate 60 (Scheme 7). After complete removal of the solvent and excess oxalyl chloride, a solution of 5-aminoadenosine derivative 56 in acetonitrile was added to provide 61, which was sequentially deprotected with aqueous TFA to 62 and hydrogenated to afford acylurea 63.

Scheme 6^a

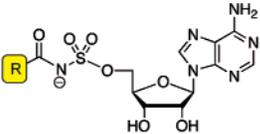
^a Reaction conditions: (a) ref 22, 92%; (b) Boc₂O, NaH, DMF/THF (4:1), 16 h, 76%; (c) H₂ (1 atm), Pd/C, MeOH, 95%; (d) (i) CDI, DBU, DMF; (ii) chromone-3-carboxylic acid; (e) 80% aq TFA, 20% (2 steps).

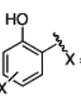
Scheme 7^a

^a Reaction conditions: (a) (COCl)₂, CH₂Cl₂, 50 °C, 3 h; (b) 56, MeCN, 16 h, 88%; (c) H₂, Pd/C, 51%; (d) 80% aq TFA, 70%.

Enzyme Assay. Enzyme assays were performed at 37 °C with recombinant MbtA expressed in *E. coli* in a buffer of 75 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 2 mM DTT, 250 μM salicylic acid, 10 mM ATP, and 1 mM PPI.¹⁵ The initial rates of pyrophosphate exchange (≤10% reaction) were monitored using an enzyme concentration (typically 5–10 nM) by measuring the amount of [³²P]ATP formed after addition of [³²P]PPI. The enzyme concentration was determined by active-site titration with inhibitor 6. The apparent inhibition constants (*K*₁^{app}) were determined by fitting the concentration–response plots either to the Hill equation (eq 1, see Experimental Section) or to the Morrison equation (eq 2, see Experimental Section). The Morrison equation was employed in cases where the inhibitors exhibited tight-binding behavior (*K*₁^{app} ≤ 100[E]). This class of inhibitors has been shown to exhibit reversible and competitive inhibition toward MbtA with respect to both salicylic acid and ATP.¹⁵ Attempts to determine the true inhibition constants (*K*₁) have been hampered by the tight-binding nature of the inhibitors, which has precluded assessment of the *K*₁ by traditional steady-state kinetic methods.²³ The *K*₁^{app} of the parent inhibitor 6 is 6.6 nM (measured at 250 μM salicylic acid, 10 mM ATP). If one accounts for the competitive substrate salicylic acid, which is held at 250 μM or 120*K*_M, then the *K*₁ is 55 pM. However, this analysis still neglects the effect of the nonvaried substrate ATP, which is held at 10 mM, or 55*K*_M, thus the true *K*₁ for 6 is estimated at approximately 1 pM, a value that has been independently confirmed by isothermal calorimetry (Daniel Wilson, Thomas Anderson, unpublished results). All of the *K*₁^{app} values reported are uncorrected for substrate concentrations and represent an upper limit of the true dissociation constant. Although the *K*₁^{app} reported herein are not a measure of the true inhibitor potency, the differences are reflective of free energy differences associated with inhibitor binding to MbtA, presuming equivalent modalities of inhibition.

Table 1



Compound, R =	K_i^{app} (μM) ^a	MIC ₉₉ (μM) ^b (iron-deficient)	MIC ₉₉ (μM) ^c (iron-rich)	Compound, R =	K_i^{app} (μM) ^a	MIC ₉₉ (μM) ^b (iron-deficient)	MIC ₉₉ (μM) ^c (iron-rich)	
 X = <ul style="list-style-type: none"> 6 OH 7 H 8 NH₂ 9 F 10 Cl 11 NO₂ 	0.0066 ± 0.00015 ^d	0.39	1.56	 X = <ul style="list-style-type: none"> 24 F 25 Cl 	2.9 ± 0.4	> 200	> 200	
	0.092 ± 0.007	12.5	50		0.175 ± 0.019	> 200	12.5	50
	0.77 ± 0.12	> 200	> 200	 26	> 100	> 200	> 200	
	0.038 ± 0.008	12.5	50		 27 n = 1 28 n = 2	49.8 ± 2.0	> 200	> 200
	18.1 ± 1.7	> 200	> 200			17.3 ± 2.8	> 200	> 200
	7.32 ± 0.9	> 200	> 200		 29	> 100	> 200	> 200
 X = <ul style="list-style-type: none"> 12 3-Cl 13 4-Cl 14 5-Cl 15 4-F 16 4-Br 17 4-CH₃ 18 4-CF₃ 19 4-NH₂ 20 6-F 	0.061 ± 0.003	50	> 200			 30	2.5 ± 0.4	> 200
	0.012 ± 0.0006	12.5	50		 31		> 100	> 200
	0.020 ± 0.0014	12.5	50	 32			> 100	> 200
	0.012 ± 0.0016	0.098	0.39		 33		36.5 ± 2.2	> 200
	0.021 ± 0.001	6.25–12.5	50	3.7 ± 0.5			> 200	> 200
	0.014 ± 0.0007	1.56	25					

^a Value ± std error. ^b Grown in GAST media without added Fe³⁺. ^c Grown in GAST media supplemented with Fe³⁺. ^d See ref 15.

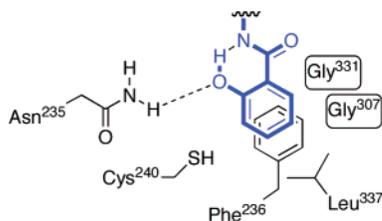


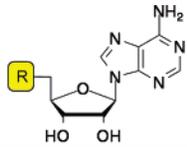
Figure 3. Schematic diagram of key nucleoside/protein interactions for analogue **6** based on docking.

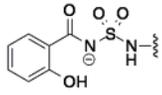
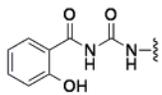
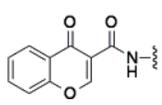
Aryl Ring SAR Study. To explore the significance of the *ortho*-hydroxy group, a systematic series of analogues was prepared bearing substitution at the 2-position. Several important trends emerged from this series. Deletion of the hydroxy afforded benzoyl analogue **7** and resulted in a concomitant 14-fold loss of binding affinity (Table 1). Substitution of the *ortho*-hydroxy group reduced potency in all cases but by widely varying amounts: by 6-fold for 2-fluoro **9**, 117-fold for 2-amino **8**, 1100 fold for 2-nitro **11**, and 2700-fold for 2-chloro **10**. Docking studies of the parent ligand **6** into a homology model of MbtA as well as quantum mechanics calculations free from the constraints of the active site show that **6** adopts a coplanar conformation that is stabilized by an internal hydrogen bond where the phenol acts as a hydrogen bond donor to the sulfamate nitrogen ($pK_a \sim 1$), which is deprotonated (Figure 3).¹¹ The X-ray cocrystal structure of DhbE with adenylated 2,3-dihydroxybenzoic acid also shows a similar coplanar arrangement.²¹ Molecular mechanics simulations of **10** and **11** show that the bulky nitro- and chloro- substituents disfavor the required coplanar conformation and additionally are unable to form an internal hydrogen bond. The 117-fold loss of activity when the aryl hydroxy is replaced by an amino group suggests that the carboxamide side chain of N235 in MbtA must present the amino group to the inhibitor (for ease of interpretation using

the DhbE X-ray structure, residues are numbered as in DhbE). The 2-fluoro analogue, which notably lacks the metabolically labile phenol function, displayed the smallest destabilization of binding, consistent with its ability to adopt a coplanar arrangement in quantum calculations. Unfortunately, the bicyclic chromone analogue **58** that mimics the postulated planar conformation of **6** was inactive. The related acyclic acylurea derivative **63** exhibited a profound 329-fold loss in potency relative to acylsulfamide **64** (Table 2). Modeling suggests that twisting of the urea functionality of **63** is required for binding, partially explaining its decreased activity relative to **6**. Cyclization as in **58** imposes even greater energy penalties for this twisting, which likely explains the further loss of potency of this compound.

Analogues **12–14** were prepared to define the steric requirements of the shallow binding pocket and to identify potential sites for further modification. A homology model of MbtA reveals a shallow hydrophobic binding pocket for salicylic acid consisting of F236, C240, G307, V329, G331, and L337. The chlorine atom is isosteric with a methyl group and importantly can be modified by standard palladium mediated coupling reactions. The SAR from this chloro-scan showed that substitution at the 4-position of the aryl ring was most tolerated, resulting in a modest 2-fold decrease in potency relative to **6** whereas substitution at the 3- and 5-positions reduced inhibitor potency 3- and 9-fold, respectively (Table 1). Since the optimal position was found to be the 4-position, several additional substituents (F, Br, CH₃, CF₃, and NH₂) were examined at this position. None of the analogues in this series (**15–19**) exceeded the potency of 4-Cl **13**, and strongly electron-withdrawing substituents were strongly disfavored. Thus, 4-CF₃ **18** exhibited a remarkable 313-fold loss of potency relative to the 4-CH₃ **17**. Additionally, modeling suggested that a 6-substituent might be able to accept a poor-geometry hydrogen bond from the

Table 2



Compound, R =	K_1^{app} (μM) ^a	MIC ₉₉ (μM) ^b (iron-deficient)	MIC ₉₉ (μM) ^c (iron-rich)
	0.0038 ± 0.0006 ^d	0.19 ^e	0.39
	1.25 ± 0.05	200	> 200
	> 100	12.5–25	50

^a Value ± std error. ^b Grown in GAST media without added Fe³⁺. ^c Grown in GAST media supplemented with Fe³⁺. ^d See ref 15. ^e See ref 11.

backbone amide of G307. The only 6-substituted compound in the series, 6-fluorosalicil **20**, showed activity nearly identical to the parent compound.

Since many siderophores contain an aryl-capped residue derived from 2,3-dihydroxybenzoic acid, inhibitor **21** was evaluated against MbtA and found to possess a 20-fold loss in potency relative to the parent compound **6**. *Bacillus subtilis* uses the adenylating enzyme DhbE in the synthesis of the 2,3-dihydroxybenzoic acid-capped siderophore bacillibactin, and compounds **6** and **21** were shown to possess K_1^{app} values toward DhbE of 106 and 85 nM respectively; however, this analysis neglected the tight-binding nature of the inhibitors and thus underestimated both the inhibitor potency as well as selectivity.^{16,24} MbtA shares 42% overall sequence identity to DhbE but significantly higher homology in the ligand binding site. Of the 21 residues contacting the adenylate ligand in the X-ray cocrystal structure of DhbE (within 4 Å), 16 are identical in MbtA and the remaining 5 residues represent conservative changes (Y236F, S240C, A308S, V337L, T411S).²¹ In DhbE, the 3-hydroxy hydrogen bonds to S240, which is a cysteine in MbtA and therefore a weaker hydrogen-bonding partner. In addition, the nearby V337L substitution reduces the space available for the *meta*-hydroxyl. Therefore residues S240 and V337 probably contribute to the specificity differences between DhbE and MbtA. *Bacillus anthracis* incorporates native substrate 3,4-dihydroxybenzoic acid to make the siderophore petrobactin.²⁵ Accordingly, compound **22** was prepared to probe the MbtA active site compatibility for 3,4-dihydroxybenzoic acid. 3,4-Dihydroxybenzoyl analogue **22** was a modest nanomolar inhibitor of AsbC (IC₅₀ = 250 nM¹⁷), but displayed no activity toward MbtA, indicating an important active site difference between the two adenylation enzymes.

Incorporation of a nitrogen at the 3-position in pyridyl analogue **23–25** was explored because of the S240C and V337L substitutions. A nitrogen internal to the aryl ring would avoid any steric issues present with a 3-hydroxyl group, and molecular modeling of **24** and **25** showed that C240 can potentially donate a hydrogen bond to the 3-nitrogen. The carbon to nitrogen substitutions in 2-Cl pyridyl **25** provided an 103-fold increase in potency relative to 2-Cl phenyl **10**, while 2-F pyridyl **24** decreased activity 76-fold relative to 2-F phenyl **9**. Additionally,

the 2-OH pyridyl **23** suffered a 560-fold loss in potency relative to **6**, a result that can be partially reconciled since **23** exists exclusively as the keto tautomer. The lack of any apparent trend with **24** and **25** suggests that multiple effects are responsible for the observed activity.

In order to explore the tolerance for nonaromatic groups, a small set of alkyl derivatives **27–29** were prepared. The trend from this small series shows that larger alkyl substituents led to an increase activity. This series was not further explored systematically since the best compound **28** exhibited a 2600-fold loss in potency relative to **6**. In docking studies, all low-energy conformations of **28** showed unfavorable contacts between the cyclohexyl hydrogens and residues Phe236 and Gly331, which are on opposite faces of the aryl binding pocket, suggesting the pocket is not tall enough to accommodate saturated ring systems. However, methoxy analogue **30** was found to exhibit a greater than 40-fold increase in activity relative to methyl analogue **29**, and this unexpected increase in activity warrants further investigation.

Incorporation of a methylene spacer between the linkage carbonyl and the phenyl group in benzyl analogue **31** led to complete loss of activity (>1000-fold decrease in potency relative to **7**). 4-Azido-2,3,5,6-tetrafluorophenyl derivative **32** was prepared as a potential photoaffinity probe, but it was found to be completely inactive.²⁶ Finally, the phenylalanine derived inhibitor **33** was evaluated to probe the selectivity of MbtA toward aminoacyl adenylate inhibitors. Surprisingly, compound **33** exhibited moderate micromolar activity suggesting that the amino group of **33** is able to favorably interact with MbtA.

Inhibition of *M. tuberculosis* H37Rv. Compounds **6–33**, **58**, and **63–64** were evaluated for whole-cell activity against *M. tuberculosis* H37Rv under iron-limiting and iron-rich conditions. The minimum inhibitory concentrations (MIC₉₉) that inhibited >99% of cell growth are shown in Tables 1 and 2. The MIC₉₉ for **6–8** and **64** have been previously reported under iron-deficient conditions, but the MIC₉₉ under iron-rich conditions have not yet been disclosed.¹¹ In general, the MIC₉₉ values were approximately 100-fold greater than the corresponding K_1^{app} values. The strong correlation between in vitro enzyme inhibition and whole-cell biological activity provides support for the designed mechanism of action. 4-Fluorosalicil derivative **15** displayed the most potent activity with an impressive MIC₉₉ of 0.098 μM under iron-deficient conditions, which is 4-fold more potent than the parent compound **6**. Although the 2-hydroxy group is required for optimal activity, the finding that this can be replaced with a fluoro in analogue **9** and still maintain respectable biological activity may be useful to increase the metabolic stability due to potential glucuronidation of the phenol. Interestingly, chromone derivative **58** displayed modest activity with a MIC₉₉ of 12.5–25 μM under iron-deficient conditions despite no apparent enzyme inhibition. However, it should be recognized that the inhibition studies were conducted under supersaturating concentrations of substrates, which underestimate compound potency by more than 1000-fold.

Another consideration is inhibitor selectivity since siderophore production is not essential under iron-rich conditions, suggesting that activity under iron-rich conditions is due to off-target binding and subsequent inhibition of alternate biochemical pathways.¹⁴ The parent inhibitor **6** has an MIC₉₉ of 0.39 μM under iron-deficient conditions and 1.56 μM under iron-rich conditions. The ratio of MIC₉₉s of iron-rich/iron-deficient (MIC₉₉^{Fe+}/MIC₉₉^{Fe-}) is a measure of the inhibitor selectivity, and the selectivity ratio for **6** is only 4. By contrast, the first-line antitubercular agent isoniazid, which disrupts cell-wall

biosynthesis, was found to be equipotent under iron-rich and iron-deficient conditions ($MIC_{99}^{Fe^+} = MIC_{99}^{Fe^-} = 0.19 \mu M$). *p*-Aminosalicyl derivative **19** displayed improved selectivity showing a 16-fold increase in potency under iron-deficient conditions, suggesting that this modification reduced off-target binding. Compound **6** was previously shown to exhibit moderate activity against *Yerinia pseudotuberculosis* with an MIC_{99} of 20 μM and >400 μM under iron-deficient and iron-rich conditions respectively.¹⁵ Bioinformatic analysis of *M. tuberculosis* and *Y. pseudotuberculosis* suggests that the observed phenotypic difference between these organisms elicited by **6** may be due to the presence of over 30 functionally related fatty acid adenylating (FadD) enzymes found in *M. tuberculosis*.²⁷ Many of these FadD enzymes catalyze essential reactions in the biosynthesis *M. tuberculosis* lipids and may represent potential off-target proteins inhibited by this class of adenylation inhibitors.²⁸

Conclusion

A series of 5'-*O*-[(*N*-acyl)sulfamoyl]adenosines was prepared and evaluated for inhibition of MbtA and activity against whole-cell *M. tuberculosis* under iron-deficient and iron-rich conditions. A comprehensive and systematic evaluation of the acyl group revealed that a benzoyl substituent is required for antitubercular activity and potent enzyme inhibition. Modifications of the benzoyl group indicated that a hydroxy or fluoro group at C-2 is necessary and that small substituents at C-4 are tolerated. 4-Fluorosallyl **15** was found to exhibit the most antitubercular activity, while *p*-aminosalicyl **19** was the first analogue that exhibited improved selectivity against *M. tuberculosis* under iron-rich conditions. Overall, the collective SAR demonstrated that the aryl domain is poorly tolerant to modification in accord with the fairly strict specificity of NRPS adenylation enzymes; however, minor modifications were found to provide incremental enhancement in both potency and selectivity of this new class of adenylation inhibitors.

Experimental Section

General Procedures. All commercial reagents (Sigma-Aldrich, Acros), 4-bromosalicylic acid (ABCR), and 4-trifluoromethylsalicylic acid (TCI America) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF, Et₂O, and CH₂Cl₂ while two 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; Et₃N was distilled from CaH₂. 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 N₂ in oven-dried (150 °C) glassware. ¹H and ¹³C 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 (77.3 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), coupling constant, integration. High-resolution mass spectra were obtained on an 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. Optical rotations were measured on Rudolph Autopol III polarimeter. Melting points were measured on electrothermal Mel-Temp manual melting point apparatus and are uncorrected.

General Procedure for CDI Mediated Acylation.^{14,18} A solution of **34a–j** (3.0 mmol, 3.0 equiv) and CDI (3.6 mmol, 3.6 equiv) in DMF (10 mL) was stirred at 60 °C for 2 h. The solution was cooled to rt, a mixture of either **36**¹¹ or **37**¹⁴ (1.0 mmol, 1.0 equiv) and either DBU or Cs₂CO₃ (1.5 mmol, 1.5 equiv) was then added, and the reaction mixture was stirred a further 3 h at rt. The reaction mixture was concentrated in vacuo and purified by flash chromatography to afford the title compound.

General Procedure for Synthesis of NHS Esters. Method A: To a solution of carboxylic acid **40a–e** and **40j–k**, (1.0 mmol, 1.0 equiv) in THF (10 mL) at 0 °C were added *N*-hydroxysuccinimide (1.0 mmol, 1.0 equiv) and DCC (1.0 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 afforded the desired *N*-hydroxysuccinimidyl aryl ester.

Method B: To a solution of acid chloride/anhydride **40f–i** (1.0 mmol, 1.0 equiv) in THF (10 mL) at 0 °C were added *N*-hydroxysuccinimide (1.0 mmol, 1.0 equiv) and pyridine (1.0 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, and the filtrate was concentrated under reduced pressure. The product was used without further purification.

General Procedure for NHS Ester Mediated Acylation.^{11,18} To a solution of *N*-hydroxysuccinimidyl ester **41a–k** (1.0 mmol) in DMF (10 mL) at 0 °C were added **36** or **37** (1.5 mmol, 1.5 equiv) and DBU or Cs₂CO₃ (2.0 mmol, 2.0 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 and filtered. The solids were washed with additional EtOAc (100 mL), and the combined filtrate was concentrated. Purification by flash chromatography (EtOAc/MeOH/Et₃N) afforded the title compound.

General Procedure for TFA Deprotection. To a solution of 5'-*O*-[*N*-acyl(sulfamoyl)]-2',3'-*O*-isopropylideneadenosine triethylammonium salt (0.2 mmol) was added 80% aq TFA (2.5 mL). The resulting solution was stirred for 30 min at 0 °C then concentrated under reduced pressure. Purification by flash chromatography (EtOAc/MeOH/Et₃N) afforded the title compound.

General Procedure for TBS Deprotection. To a solution of 5'-*O*-[*N*-acyl(sulfamoyl)]-2',3'-*O*-di-(*tert*-butyldimethylsilyl)adenosine triethylammonium salt (0.1 mmol, 1 equiv) in THF (5 mL) at rt was added TBAF (1.0 M in THF, 0.25 mmol, 2.5 equiv). The reaction mixture was stirred 30 min then concentrated in vacuo. Purification by flash chromatography (EtOAc/MeOH/Et₃N) afforded the title compound.

General Procedure for Ion-Exchange. A solution of a nucleoside tetrabutylammonium or triethylammonium salt (0.25 mmol, 1 equiv) in H₂O (0.5 mL) was added to a short column (10 × 50 mm, Dowex 50WX8-100-Na+) and incubated for 10 min before eluting with H₂O (20 mL). The fractions containing the product were lyophilized to afford the sodium salt as a flocculant white solid. The Dowex cation exchange resin was converted to the sodium form by sequentially washing the column with MeOH (50 mL), H₂O (50 mL), 1 N aq NaOH (25 mL), and H₂O (100 mL).

5'-*O*-[*N*-(2-Aminobenzoyl)sulfamoyl]-2',3'-*O*-isopropylideneadenosine Triethylammonium Salt. To a solution of isatoic anhydride (47 mg, 0.284 mmol, 1.1 equiv) in DMF (1.0 mL) at rt was added **36** (100 mg, 0.26 mmol, 1.0 equiv) and Cs₂CO₃ (169 mg, 0.52 mmol, 2.0 equiv). The reaction was stirred 12 h at rt then concentrated in vacuo. Purification by flash chromatography (100:25:1 EtOAc/MeOH/TEA) afforded the title compound (135 mg, 86%). ¹H NMR, ¹³C NMR, and HRMS agreed with literature values.¹¹ This compound was deprotected as described.¹¹

***N*-Hydroxysuccinimidyl 2-chlorobenzoate (41a).** This was prepared from 2-chlorobenzoic acid (780 mg, 5.0 mmol) using the general procedure for NHS ester synthesis. Purification by flash chromatography (1:1 hexanes/EtOAc) afforded the title compound (880 mg, 70%): *R*_f 0.5 (1:1 hexanes/EtOAc); ¹H NMR (600 MHz, CDCl₃) δ 2.91 (s, 4H), 7.39 (ddd, *J* = 7.8, 6.0, 1.8 Hz, 1H), 7.53–

7.57 (m, 2H), 8.10 (dd, $J = 7.8, 1.8$ Hz, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 25.9, 124.8, 127.1, 131.8, 132.6, 134.8, 135.8, 160.4, 169.2; MS (ESI+) calcd for $\text{C}_{11}\text{H}_9\text{ClNO}_4$ [$\text{M} + \text{H}$] $^+$ 254.0, found 254.0.

5'-O-[N-(2-Chlorobenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine (42a). This was prepared from **41a** (126 mg, 0.50 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (100:10:1 EtOAc/MeOH/TEA) afforded the title compound (58 mg, 22%): R_f 0.2 (100:10:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -102$ (c 2.79, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.36 (s, 3H), 1.59 (s, 3H), 4.35 (d, $J = 1.8$ Hz, 2H), 4.58 (d, $J = 1.8$ Hz, 1H), 5.20 (d, $J = 6.0$ Hz, 1H), 5.37 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.24 (d, $J = 3.0$ Hz, 1H), 7.24 (t, $J = 7.2$ Hz, 1H), 7.28 (t, $J = 7.2$ Hz, 1H), 7.33 (d, $J = 7.8$ Hz, 1H), 7.46 (d, $J = 7.8$ Hz, 1H), 8.18 (s, 1H), 8.46 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.4, 26.3, 68.9, 82.1, 84.5, 84.6, 90.7, 114.1, 118.9, 126.4, 128.9, 129.6, 129.8, 130.7, 139.5, 140.3, 149.3, 152.8, 156.1, 174.7; HRMS (APCI+) calcd for $\text{C}_{20}\text{H}_{22}\text{ClN}_6\text{O}_7\text{S}$ [$\text{M} + \text{H}$] $^+$ 525.0959, found 525.0953 (error 1.1 ppm).

5'-O-[N-(2-Chlorobenzoyl)sulfamoyl]adenosine (10). This was prepared from **42a** (29 mg, 0.050 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography afforded the title compound (22 mg, 93%): R_f 0.18 (100:15:2 MeOH/EtOAc/TEA); $[\alpha]_D^{21} -18.7$ (c 0.83, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 4.37 (br s, 1H), 4.49 (t, $J = 4.8$ Hz, 1H), 4.62–4.70 (m, 3H), 6.11 (d, $J = 4.8$ Hz, 1H), 7.34–7.37 (m, 1H), 7.44–7.47 (m, 3H), 8.36 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 70.3, 71.5, 74.5, 82.4, 89.3, 119.3, 127.1, 128.9, 130.2, 130.9, 132.4, 133.5, 142.7, 144.3, 148.9, 150.8, 165.7; HRMS (ESI+) calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_6\text{O}_7\text{S}$ [$\text{M} + \text{H}$] $^+$ 485.0641, found 485.0649 (error 0.2 ppm).

N-Hydroxysuccinimidyl 2-Nitrobenzoate (41b). This was prepared from 2-nitrobenzoic acid (835 mg, 5.0 mmol) using the general procedure for NHS ester synthesis. Purification by flash chromatography (6:4 hexanes/EtOAc) afforded the title compound (1.10 g, 85%): R_f 0.2 (6:4 hexanes/EtOAc); ^1H NMR (600 MHz, CDCl_3) δ 2.89 (s, 4H), 7.76–7.79 (m, 2H), 7.92–7.95 (m, 1H), 8.06–8.08 (m, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 25.9, 123.2, 124.8, 130.9, 133.6, 133.7, 147.9, 161.2, 168.7; MS (ESI+) calcd for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 265.0, found 264.9.

2',3'-O-Isopropylidene-5'-O-[N-(2-nitrobenzoyl)sulfamoyl]adenosine Triethylammonium Salt (42b). This was prepared from **41b** (133 mg, 0.50 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (100:10:1 EtOAc/MeOH/TEA) afforded the title compound (75 mg, 28%): R_f 0.17 (100:10:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -142$ (c 2.38, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.23 (t, $J = 7.2$ Hz, 9H), 1.36 (s, 3H), 1.59 (s, 3H), 3.13 (q, $J = 7.2$ Hz, 6H), 4.32–4.35 (m, 2H), 4.58 (br s, 1H), 5.18 (d, $J = 6.0$, 1H), 5.37–5.39 (m, 1H), 6.23 (d, $J = 2.4$, 1H), 7.52 (t, $J = 7.2$ Hz, 1H), 7.62 (t, $J = 8.4$ Hz, 1H), 7.66 (d, $J = 7.2$ Hz, 1H), 7.82 (d, $J = 8.4$ Hz, 1H), 8.18 (s, 1H), 8.45 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.0, 24.4, 26.3, 46.7, 68.8, 82.2, 84.5, 84.6, 90.7, 114.1, 118.9, 123.4, 129.3, 129.7, 132.6, 135.5, 140.2, 148.1, 149.3, 152.8, 156.1, 172.6; HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{22}\text{N}_7\text{O}_9\text{S}$ [$\text{M} + \text{H}$] $^+$ 536.1199, found 536.1201 (error 0.4 ppm).

5'-O-[N-(2-Nitrobenzoyl)sulfamoyl]adenosine Triethylammonium Salt (11). This was prepared from **42b** (55 mg, 1.0 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:25:2.5 EtOAc/MeOH/TEA) afforded the title compound (11 mg, 22%): R_f 0.15 (100:25:2.5 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -99.7$ (c 0.290, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.24 (t, $J = 7.8$ Hz, 9H), 3.14 (q, $J = 7.8$ Hz, 6H), 4.31–4.34 (m, 1H), 4.40 (t, $J = 3.6$ Hz, 2H), 4.45 (dd, $J = 3.6$ Hz, 1H), 4.72 (t, $J = 6.0$ Hz, 1H), 6.09 (d, $J = 6.0$ Hz, 1H), 7.52 (t, $J = 8.4$, 1H), 7.61 (t, $J = 7.2$ Hz, 1H), 7.68 (d, $J = 7.2$ Hz, 1H), 7.83 (d, $J = 8.4$ Hz, 1H), 8.18 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.0, 46.7, 68.5, 71.3, 74.9, 83.5, 87.9, 118.9, 123.4, 129.3, 129.5, 132.7, 135.7, 139.8, 147.9, 149.7, 152.7, 156.1, 172.7; HRMS (APCI+) calcd for $\text{C}_{17}\text{H}_{18}\text{N}_7\text{O}_9\text{S}$ [$\text{M} + \text{H}$] $^+$ 496.0876, found 496.0904 (error 5.6 ppm).

5'-O-[N-(3-Chloro-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine (38a). This was prepared from 3-chlorosalicylic acid (170 mg, 1.0 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:5:1 EtOAc/MeOH/TEA) afforded the title compound (83 mg, 15%): R_f 0.15 (100:5:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -32.3$ (c 2.00, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.31 (s, 3H), 1.55 (s, 3H), 4.32–4.34 (m, 2H), 4.54 (br s, 1H), 5.09–5.11 (m, 1H), 5.34 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.20 (d, $J = 3.0$ Hz, 1H), 6.71 (t, $J = 7.8$ Hz, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.83 (d, $J = 7.8$ Hz, 1H), 8.14 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.3, 26.2, 68.9, 82.1, 84.5, 84.6, 90.8, 114.1, 118.0, 119.0, 120.8, 121.3, 128.7, 133.3, 140.2, 149.2, 152.7, 156.1, 156.8, 172.9; HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{22}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 541.0903, found 541.0903 (error 0 ppm).

5'-O-[N-(3-Chloro-2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (12). This was prepared from **38a** (30 mg, 0.060 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:15:1 EtOAc/MeOH/TEA) afforded the title compound (20 mg, 65%): R_f 0.17 (100:15:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -32.8$ (c 0.570, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.28 (t, $J = 7.2$ Hz, 9H), 3.18 (q, $J = 7.2$ Hz, 6H), 4.34–4.35 (m, 1H), 4.39–4.46 (m, 3H), 4.74 (t, $J = 5.4$ Hz, 1H), 6.11 (d, $J = 6.0$ Hz, 1H), 6.76 (t, $J = 7.8$ Hz, 1H), 7.40 (dd, $J = 7.8, 1.2$ Hz, 1H), 7.91 (dd, $J = 7.8, 1.2$ Hz, 1H), 8.20 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.1, 46.6, 68.5, 71.1, 74.8, 83.3, 87.9, 117.9, 118.9, 120.9, 121.3, 128.7, 133.1, 139.8, 149.6, 152.6, 156.0, 156.7, 172.6; HRMS (ESI+) calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 501.0595, found 501.0585 (error 2.0 ppm).

5'-O-[N-(4-Chloro-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine Triethylammonium Salt (38b). This was prepared from 4-chlorosalicylic acid (43 mg, 0.25 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:10:1 EtOAc/MeOH/TEA) afforded the title compound (94 mg, 70%): R_f 0.15 (100:10:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -88.4$ (c 1.86, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.24 (t, $J = 7.8$ Hz, 9H), 1.34 (s, 3H), 1.58 (s, 3H), 3.09 (q, $J = 7.8$ Hz, 6H), 4.29 (dd, $J = 10.8, 4.2$ Hz, 1H), 4.32 (dd, $J = 10.8, 3.6$ Hz, 1H), 4.53–4.54 (m, 1H), 5.11 (dd, $J = 6.0, 1.8$ Hz, 1H), 5.37 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.21 (d, $J = 3.0$ Hz, 1H), 6.72–6.80 (m, 2H), 7.83 (d, $J = 9.0$ Hz, 1H), 8.14 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.3, 24.2, 26.2, 46.6, 68.8, 82.2, 84.51, 84.53, 90.8, 114.1, 115.9, 116.6, 118.3, 118.4, 131.6, 138.3, 140.2, 149.2, 152.8, 156.1, 161.7, 172.6; MS (ESI+) calcd for $\text{C}_{20}\text{H}_{22}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 541.09, found 541.08.

5'-O-[N-(4-Chloro-2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (13). This was prepared from **38b** (35 mg, 0.060 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:15:1 EtOAc/MeOH/TEA) afforded the title compound (19 mg, 60%): R_f 0.14 (100:15:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -23.3$ (c 1.81, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.24 (t, $J = 7.8$ Hz, 9H), 3.13 (q, $J = 7.8$ Hz, 6H), 4.30 (dd, $J = 6.6, 3.0$ Hz, 1H), 4.34–4.42 (m, 3H), 4.70 (t, $J = 5.4$ Hz, 1H), 6.07 (d, $J = 5.4$ Hz, 1H), 6.74 (dd, $J = 8.4, 1.2$ Hz, 1H), 6.79 (d, $J = 1.2$ Hz, 1H), 7.88 (d, $J = 8.4$ Hz, 1H), 8.16 (s, 1H), 8.47 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.1, 46.7, 60.3, 68.5, 71.2, 74.8, 83.3, 88.1, 116.6, 118.3, 119.0, 131.5, 138.3, 139.9, 149.7, 152.7, 156.1, 161.7, 172.6; HRMS (ESI+) calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 501.0595, found 501.0592 (error 0.6 ppm).

5'-O-[N-(5-Chloro-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine (38c). This was prepared from 5-chlorosalicylic acid (86 mg, 0.50 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:5:1.5 EtOAc/MeOH/TEA) afforded the title compound (33 mg, 12%): R_f 0.18 (100:5:1.5 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -96.0$ (c 1.26, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.33 (s, 3H), 1.56 (s, 3H), 4.30–4.33 (m, 2H), 4.53 (br s, 1H), 5.10 (d, $J = 6.0$ Hz, 1H), 5.35 (dd, $J = 6.0, 2.4$ Hz, 1H), 6.19 (d, $J = 2.4$ Hz, 1H), 6.75 (d, $J = 8.4$ Hz, 1H), 7.22 (dd, $J = 8.4, 2.4$ Hz, 1H), 7.84 (d, $J =$

2.4 Hz, 1H), 8.13 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.2, 26.2, 60.3, 68.9, 82.1, 84.5, 84.6, 90.8, 104.7, 114.1, 118.5, 120.5, 122.7, 129.3, 132.8, 140.2, 152.7, 156.0, 159.5, 172.4; HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{22}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 541.0903, found 541.0914 (error 2.0 ppm).

5'-O-[N-(5-Chloro-2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (14). This was prepared from **38c** (28 mg, 0.050 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:20:1.5 EtOAc/MeOH/TEA) afforded the title compound (15 mg, 59%); R_f 0.15 (100:20:1.5 EtOAc/MeOH/TEA); $[\alpha]_{\text{D}}^{21}$ -23.3 (c 1.72, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.17 (t, $J = 7.2$ Hz, 9H), 2.90 (q, $J = 7.2$ Hz, 6H), 4.31–4.42 (m, 4H), 4.68 (t, $J = 5.4$ Hz, 1H), 6.06 (d, $J = 5.4$ Hz, 1H), 6.76 (d, $J = 8.4$ Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 1H), 7.87 (s, 1H), 8.15 (s, 1H), 8.44 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.8, 46.3, 60.4, 68.5, 71.1, 74.8, 83.2, 88.3, 116.8, 118.0, 118.4, 120.6, 129.4, 132.8, 139.9, 152.7, 156.1, 159.5, 172.4; HRMS (ESI+) calcd for $\text{C}_{17}\text{H}_{18}\text{ClN}_6\text{O}_8\text{S}$ [$\text{M} + \text{H}$] $^+$ 501.0595, found 501.0594 (error 0.2 ppm).

5'-O-[N-(4-Fluoro-2-hydroxybenzoyl)sulfamoyl]adenosine Sodium Salt (15). This was prepared from 4-fluorosalicic acid (16 mg, 0.10 mmol) using the general procedure for CDI mediated acylation to afford **38d**. The crude product was used directly for the next step.

Crude **38d** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (25 mg, 48% overall yield): R_f 0.21 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21}$ -0.05 (c 0.33, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.29–4.31 (m, 1H), 4.35 (dd, $J = 10.8, 3.0$ Hz, 1H), 4.38–4.40 (m, 2H), 4.69 (t, $J = 5.4$ Hz, 1H), 6.07 (d, $J = 6.0$ Hz, 1H), 6.47–6.50 (m, 2H), 7.94 (t, $J = 7.8$ Hz, 1H), 8.15 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 69.5, 72.4, 76.0, 84.6, 89.2, 104.1 (d, $^2J_{\text{C-F}} = 23.4$), 106.5 (d, $^2J_{\text{C-F}} = 21.8$), 120.1, 133.44, 133.51, 141.1, 150.9, 153.8, 157.2, 164.1, 167.2 (d, $J_{\text{C-F}} = 247.8$), 174.0; HRMS (ESI $-$) calcd for $\text{C}_{17}\text{H}_{16}\text{FN}_6\text{O}_8\text{S}$ [$\text{M} - \text{H}$] $^-$ 483.0739, found 483.0758 (error 3.9 ppm).

5'-O-[N-(4-Bromo-2-hydroxybenzoyl)sulfamoyl]adenosine Sodium Salt (16). This was prepared from 4-bromosalicylic acid (60 mg, 0.28 mmol) using the general procedure for CDI mediated acylation to afford **38e**. The crude product was used directly for the next step.

Crude **38e** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (116 mg, 74% overall yield): R_f 0.23 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21}$ -0.13 (c 0.53, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.31–4.32 (m, 1H), 4.35–4.37 (m, 1H), 4.40–4.42 (m, 2H), 4.70 (t, $J = 5.4$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 6.91 (dd, $J = 7.8$ Hz, 1.8 Hz, 1H), 6.97 (d, $J = 1.8$, 1H), 7.81 (d, $J = 7.8$ Hz, 1H), 8.17 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 69.7, 72.4, 76.1, 84.6, 89.3, 119.9, 120.2, 120.9, 122.5, 127.8, 132.8, 141.1, 150.9, 153.9, 157.3, 162.9, 174.1; HRMS (ESI $-$) calcd for $\text{C}_{17}\text{H}_{16}\text{BrN}_6\text{O}_8\text{S}$ [$\text{M} - \text{H}$] $^-$ 542.9939, found 542.9945 (error 1.1 ppm).

5'-O-[N-(2-Hydroxy-4-methylbenzoyl)sulfamoyl]adenosine Sodium Salt (17). This was prepared from 4-methylsalicylic acid (76 mg, 0.50 mmol) using the general procedure for CDI mediated acylation to afford **38f**. The crude product was used directly for the next step.

Crude **38f** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (113 mg, 45% overall yield): R_f 0.20 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21}$ -0.15 (c 0.56, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 2.27 (s, 3H), 4.31–4.32 (m, 1H), 4.34–4.37 (m, 1H), 4.39–4.42 (m, 2H), 4.71 (t, $J = 5.4$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 6.59–6.61 (m, 2H), 7.81 (d, $J = 7.8$ Hz, 1H), 8.17 (s,

1H), 8.51 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 20.7, 68.6, 71.5, 75.2, 83.7, 88.3, 117.1, 119.3, 119.5, 130.4, 140.2, 144.4, 150.0, 152.9, 156.4, 161.1, 174.4 (missing 1 C); HRMS (ESI $-$) calcd for $\text{C}_{18}\text{H}_{19}\text{N}_6\text{O}_8\text{S}$ [$\text{M} - \text{H}$] $^-$ 479.0990, found 479.1009 (error 4.0 ppm).

5'-O-[N-(4-Trifluoromethyl-2-hydroxybenzoyl)sulfamoyl]adenosine Sodium Salt (18). This was prepared from 4-trifluoromethylsalicylic acid (20.6 mg, 0.10 mmol) using the general procedure for CDI mediated acylation to afford **38g**. The crude product was used directly for the next step.

Crude **38g** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (27 mg, 48% overall yield): R_f 0.25 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21}$ -0.04 (c 0.25, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 4.30–4.33 (m, 1H), 4.37–4.45 (m, 3H), 4.70 (t, $J = 5.4$ Hz, 1H), 6.09 (d, $J = 6.0$ Hz, 1H), 7.02 (d, $J = 7.8$ Hz, 1H), 7.04 (s, 1H), 8.08 (d, $J = 7.8$ Hz, 1H), 8.17 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 68.8, 71.5, 75.2, 83.7, 88.3, 114.0, 114.3, 119.3, 123.0, 131.4, 136.3, 140.2, 150.0, 153.0, 156.4, 161.3, 172.5 (missing 1 aryl C and CF_3); HRMS (ESI $-$) calcd for $\text{C}_{18}\text{H}_{16}\text{F}_3\text{N}_6\text{O}_8\text{S}$ [$\text{M} - \text{H}$] $^-$ 533.0707, found 533.0714 (error 1.1 ppm).

4-Benzyloxycarbonylamino-2-hydroxybenzoic Acid (34h). To a solution of 4-aminosalicylic acid (2.0 g, 13.1 mmol) in THF (40 mL) was added saturated aq NaHCO_3 (40 mL) followed by benzyl chloroformate (2.04 mL, 14.4 mmol, 1.1 equiv). The reaction was stirred for 16 h at rt then concentrated to remove THF. The remaining aqueous layer was acidified with 6 N HCl to pH 3–4 then extracted with EtOAc (3 \times 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford the title compound (2.30 g, 61%) as a light brown solid: ^1H NMR (600 MHz, CD_3OD) δ 5.17 (s, 2H), 6.91 (dd, $J = 9.0, 1.8$ Hz, 1H), 7.14 (d, $J = 1.8$ Hz, 1H), 7.29–7.39 (m, 5H), 7.72 (d, $J = 9.0$ Hz, 1H), 9.57 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 66.6, 105.2, 107.2, 109.3, 127.9, 128.0, 128.3, 131.1, 136.6, 145.9, 154.0, 163.1, 172.0; HRMS (ESI $-$) calcd for $\text{C}_{15}\text{H}_{12}\text{NO}_5$ [$\text{M} - \text{H}$] $^-$ 286.0721, found 286.0727 (error 2.1 ppm).

5'-O-[N-(4-Benzyloxycarbonylamino-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine (38h). This was prepared from **34h** (71 mg, 0.25 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:7 MeOH/EtOAc) afforded the title compound (75 mg, 38%) as a white solid: R_f 0.20 (100:7 MeOH/EtOAc); $[\alpha]_{\text{D}}^{21}$ 86.1 (c 1.29, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 1.32 (s, 3H), 1.56 (s, 3H), 4.30 (t, $J = 4.2$ Hz, 2H), 4.54 (br s, 1H), 5.10 (d, $J = 5.4$ Hz, 1H), 5.15 (s, 2H), 5.35 (br s, 1H), 6.20 (d, $J = 3.0$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 1H), 7.28 (t, $J = 7.2$ Hz, 1H), 7.34 (t, $J = 7.2$ Hz, 2H), 7.38 (d, $J = 7.2$ Hz, 2H), 7.66 (s, 1H), 7.80 (d, $J = 8.4$ Hz, 1H), 8.13 (s, 1H), 8.40 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 24.2, 26.2, 66.4, 68.7, 82.2, 84.5, 90.8, 105.6, 108.8, 114.1, 119.0, 121.4, 127.8, 127.9, 128.3, 130.9, 135.1, 136.8, 140.2, 143.9, 149.2, 152.7, 154.2, 156.1, 161.8, 173.9; HRMS (ESI+) calcd for $\text{C}_{28}\text{H}_{30}\text{N}_7\text{O}_{10}\text{S}$ [$\text{M} + \text{H}$] $^+$ 656.1769, found 656.1707 (error 9.4 ppm).

5'-O-[N-(4-Amino-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine Triethylammonium Salt (39). To a solution of compound **38h** (120 mg, 0.18 mmol) in MeOH (5 mL) was added 10% Pd/C (12 mg, 10% by wt), and the reaction mixture was stirred under H_2 (1 atm) for 12 h. The reaction mixture was then filtered through a plug of Celite. Purification by flash chromatography (100:10:1 MeOH/EtOAc/TEA) afforded the title compound (84 mg, 89%) as a white solid: R_f 0.10 (100:10:1 MeOH/EtOAc/TEA); $[\alpha]_{\text{D}}^{21}$ -153 (c 1.88, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.18 (t, $J = 7.2$ Hz, 9H), 1.33 (s, 3H), 1.58 (s, 3H), 2.97 (q, $J = 7.2$ Hz, 6H), 4.27 (d, $J = 3.6$ Hz, 2H), 4.55 (br s, 1H), 5.11 (d, $J = 6.0$ Hz, 1H), 5.37 (dd, $J = 6.0, 2.4$ Hz, 1H), 6.02 (d, $J = 2.4$ Hz, 1H), 6.11 (dd, $J = 8.4, 1.8$ Hz, 1H), 6.20 (d, $J = 1.8$ Hz, 1H), 7.64 (d, $J = 8.4$ Hz, 1H), 8.15 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 8.6, 24.2, 26.2, 46.4, 68.6, 82.3, 84.4, 84.5, 90.8, 100.3, 106.2, 108.9, 114.1, 118.9, 131.5, 131.7, 140.2, 149.3, 152.8, 153.8,

156.1, 162.7; HRMS (ESI+) calcd for $C_{20}H_{24}N_7O_8S$ [M + H]⁺ 522.1402, found 522.1385 (error 3.3 ppm).

5'-O-[N-(4-Amino-2-hydroxybenzoyl)sulfamoyl]adenosine (19). This was prepared from **39** (50 mg, 0.090 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:30:3 MeOH/EtOAc/TEA) afforded the title compound (15 mg, 35%) as a white solid: R_f 0.10 (100:30:3 MeOH/EtOAc/TEA); $[\alpha]_D^{21} -55.1$ (c 3.75, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 1.18 (t, $J = 7.2$ Hz, 9H), 3.17 (q, $J = 7.2$ Hz, 6H), 4.29–4.40 (m, 4H), 4.72 (t, $J = 6.0$ Hz, 1H), 6.02 (br s, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 6.10 (d, $J = 9.0$ Hz, 1H), 7.66 (d, $J = 9.0$ Hz, 1H), 8.16 (s, 1H), 8.50 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ 8.0, 46.4, 68.2, 71.3, 74.9, 83.5, 88.0, 100.3, 106.2, 109.0, 118.9, 131.7, 139.9, 149.7, 152.7, 153.8, 156.1, 162.7, 177.8; HRMS (ESI-) calcd for $C_{17}H_{18}N_7O_8S$ [M - H]⁻ 480.0943, found 480.0941 (error 0.4 ppm).

5'-O-[N-(6-Fluoro-2-hydroxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine Triethylammonium Salt (38i). This was prepared from 6-fluorosalicic acid (70 mg, 0.50 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:7.5:1 EtOAc/MeOH/TEA) afforded the title compound (157 mg, 60%): R_f 0.15 (100:7.5:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -52.8$ (c 0.420, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 1.23 (t, $J = 7.2$ Hz, 9H), 1.35 (s, 3H), 1.58 (s, 3H), 3.08 (q, $J = 7.2$ Hz, 6H), 4.31 (dd, $J = 10.8, 4.2$ Hz, 1H), 4.35 (dd, $J = 10.8, 4.2$ Hz, 1H), 4.54 (dd, $J = 4.2, 1.8$ Hz, 1H), 5.14 (dd, $J = 3.0, 1.8$ Hz, 1H), 5.37 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.22 (d, $J = 3.0$ Hz, 1H), 6.44–6.50 (m, 1H), 6.58–6.62 (m, 1H), 7.17–7.22 (m, 1H), 8.15 (s, 1H), 8.42 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ 8.2, 24.3, 26.3, 46.6, 68.9, 82.2, 84.5, 90.8, 106.1 (d, $J_{C-F} = 24$ Hz), 111.9, 112.6, 114.1, 119.0, 131.8 (d, $J_{C-F} = 11.7$ Hz), 132.3 (d, $J_{C-F} = 12.2$ Hz), 140.2, 149.3, 152.8, 156.1, 162.0 (d, $J = 131$ Hz), 164.2, 170.7 (d, $J_{C-F} = 2.7$ Hz); HRMS (ESI+) calcd for $C_{20}H_{22}FN_6O_8S$ [M + H]⁺ 525.1198, found 525.1215 (error 3.2 ppm).

5'-O-[N-(6-Fluoro-2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (20). This was prepared from **38i** (50 mg, 0.10 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:16:3 EtOAc/MeOH/TEA) afforded the title compound (36 mg, 76%): R_f 0.09 (100:16:3 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -47.0$ (c 0.340, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 1.14 (t, $J = 7.2$ Hz, 9H), 2.85 (q, $J = 7.2$ Hz, 6H), 4.33 (dd, $J = 6.6, 3.0$ Hz, 1H), 4.38–4.45 (m, 3H), 4.69 (t, $J = 5.4$ Hz, 1H), 6.08 (d, $J = 5.4$ Hz, 1H), 6.47 (dd, $J = 11.4, 8.4$ Hz, 1H), 6.60 (d, $J = 8.4$ Hz, 1H), 7.16–7.21 (m, 1H), 8.15 (s, 1H), 8.41 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ 8.9, 46.2, 68.6, 71.1, 74.8, 83.2, 88.3, 106.1 (d, $J_{C-F} = 24$ Hz), 109.8 (d, $J_{C-F} = 9.0$ Hz), 112.7 (d, $J_{C-F} = 3.3$ Hz), 119.1, 132.4 (d, $J_{C-F} = 12.3$ Hz), 139.9, 149.5, 152.7, 156.1, 162.0 (d, $J_{C-F} = 125$ Hz), 164.1, 171.1 (d, $J = 3.3$ Hz); HRMS (ESI+) calcd for $C_{17}H_{18}FN_6O_8S$ [M + H]⁺ 485.0891, found 485.0906 (error 3.1 ppm).

N-Hydroxysuccinimidy-2,3-dibenzyloxybenzoate (41c). This was prepared from 2,3-dibenzyloxybenzoic acid²⁹ (1.00 g, 2.99 mmol, 1.0 equiv) using the general procedure for NHS ester synthesis. Purification by flash chromatography (4:1 EtOAc/hexanes) afforded the title compound (0.80 g, 62%): R_f 0.85 (EtOAc); ¹H NMR (600 MHz, CDCl₃) δ 4.49 (m, 4H), 5.13 (s, 4H), 7.12 (t, $J = 8.4$ Hz, 1H), 7.20–7.30 (m, 4H), 7.32–7.39 (m, 4H), 7.39–7.44 (m, 3H), 7.85 (dd, $J = 8.4, 1.2$ Hz, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 25.9, 71.6, 76.1, 120.4, 121.3, 123.9, 124.4, 127.8, 128.2, 128.4, 128.5, 128.8, 128.9, 136.5, 137.2, 150.1, 153.2, 160.9, 169.4.

5'-O-[N-(2,3-Dibenzyloxybenzoyl)sulfamoyl]-2',3'-O-isopropylideneadenosine Triethylammonium Salt (42c). This was prepared from **41c** (837 mg, 1.88 mmol, 2.50 equiv) using the general procedure for NHS mediated acylation. Purification by flash chromatography (95:5:1 MeOH/EtOAc/Et₃N) afforded the title compound (460 mg, 77%): R_f 0.30 (1:5 MeOH/EtOAc); $[\alpha]_D^{20} -43.5$ (c 0.540, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 1.08 (t, $J = 7.2$ Hz, 9H), 1.32 (s, 3H), 1.57 (s, 3H), 2.77 (q, $J = 7.2$ Hz, 6H), 4.24 (d, $J = 3.6$ Hz, 2H), 4.40–4.48 (m, 1H), 5.04–5.12 (m, 5H), 5.29 (dd, $J = 5.4, 3.0$ Hz, 1H), 6.19 (d, $J = 3.0$ Hz, 1H),

7.00–7.06 (m, 2H), 7.06–7.10 (m, 1H), 7.14–7.22 (m, 3H), 7.26–7.36 (m, 3H), 7.38–7.46 (m, 4H), 8.17 (s, 1H), 8.44 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ 10.2, 25.6, 27.5, 47.3, 69.8, 72.0, 76.6, 83.2, 85.6, 85.7, 91.8, 115.2, 116.1, 120.2, 121.8, 125.1, 128.6, 128.9, 129.0, 129.1, 129.5, 129.6, 137.7, 138.5, 139.4, 141.4, 146.8, 150.5, 153.5, 153.9, 157.3, 176.4; HRMS (ESI-) calcd for $C_{34}H_{33}N_6O_9S$ [M - H]⁻ 701.2035, found 701.2082 (error 6.7 ppm).

5'-O-[N-(2,3-Dihydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt (21). Compound **42c** (0.45 g, 0.56 mmol) was treated with 10% Pd/C (90 mg, 20% by wt) at rt under H₂ (1 atm) in MeOH (20 mL) for 12 h. The reaction mixture was filtered through Celite and concentrated to afford **43**. The crude product was used directly for the next step.

Compound **43** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (25:75:1 MeOH/EtOAc/Et₃N) afforded the title compound (287 mg, 88%) as a white solid: mp 138–140 °C; R_f 0.2 (1:1 MeOH/EtOAc); $[\alpha]_D^{20} -23.5$ (c 0.480, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 1.24 (t, $J = 7.8$ Hz, 9H), 3.12 (q, $J = 7.8$ Hz, 6H), 4.26–4.50 (m, 4H), 4.71 (t, $J = 4.8$ Hz, 1H), 6.08 (d, $J = 5.4$ Hz, 1H), 6.61 (t, $J = 7.8$ Hz, 1H), 6.84 (d, $J = 7.8$ Hz, 1H), 7.43 (d, $J = 7.8$ Hz, 1H), 8.16 (s, 1H), 8.50 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ 9.3, 47.8, 69.6, 72.4, 76.0, 84.6, 89.2, 118.6, 119.3, 120.2, 120.9, 121.9, 141.1, 146.1, 146.8, 150.9, 153.9, 157.3, 175.0; HRMS (ESI+) calcd for $C_{17}H_{19}N_6O_9S$ [M + H]⁺ 483.0929, found 483.0917 (error 2.5 ppm).

2',3'-O-Bis(*t*-butyldimethylsilyl)-5'-O-[N-(2-pyridon-1-yl)sulfamoyl]adenosine Triethylammonium Salt (38j). This was prepared from 2-hydroxynicotinic acid (83 mg, 0.60 mmol) using the general procedure for CDI mediated acylation. Purification by flash chromatography (100:10:2 MeOH/EtOAc/TEA) afforded the title compound (62 mg, 15%): R_f 0.27 (100:10:2 MeOH/EtOAc/TEA); $[\alpha]_D^{21} -148$ (c 4.20, MeOH); ¹H NMR (600 MHz, CD₃OD) δ -0.39 (s, 3H), -0.07 (s, 3H), 0.13 (s, 3H), 0.15 (s, 3H), 0.69 (s, 9H), 0.95 (s, 9H), 1.27 (t, $J = 7.2$ Hz, 9H), 3.18 (q, $J = 7.2$ Hz, 6H), 4.31 (br s, 1H), 4.44–4.47 (m, 3H), 4.87 (dd, $J = 7.2, 4.8$ Hz, 2H), 6.10 (d, $J = 7.2$ Hz, 1H), 6.76 (br s, 1H), 7.98 (br s, 1H), 8.18 (s, 1H), 8.34 (d, $J = 6.0$ Hz, 1H), 8.57 (s, 1H); ¹³C NMR (150 MHz, CD₃OD) δ -5.1, -4.2, -4.17, -4.11, 9.4, 18.7, 19.0, 26.3, 26.5, 47.8, 69.7, 74.8, 77.5, 86.1, 88.6, 105.9, 114.1 (br), 120.3, 141.6 (br), 142.9, 148.0 (br), 151.1, 154.0, 157.5, 166.0 (br), 172.6; HRMS (ESI-) calcd for $C_{28}H_{44}N_7O_8SSi_2$ [M - H]⁻ 694.2516, found 694.2548 (error 4.6 ppm).

5'-O-[N-(2-Pyridon-1-yl)sulfamoyl]adenosine Triethylammonium Salt (23). This was prepared from **38j** (35 mg, 0.050 mmol) using the general procedure for TBAF deprotection. Purification by flash chromatography (90:5:7 MeOH/EtOAc/TEA) afforded the title compound (6.1 mg, 87%): R_f 0.03 (90:5:7 MeOH/EtOAc/TEA); $[\alpha]_D^{21} -105$ (c 0.760, MeOH); ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.15 (t, $J = 6.6$ Hz, 9H), 2.95 (q, $J = 6.6$ Hz, 6H), 4.11–4.17 (m, 2H), 4.19 (dd, $J = 7.8, 4.2$ Hz, 1H), 4.24 (dd, $J = 10.8, 4.2$ Hz, 1H), 4.60 (q, $J = 6.0$ Hz, 1H), 5.38 (d, $J = 4.2$ Hz, 1H), 5.53 (d, $J = 6.0$ Hz, 1H), 5.92 (d, $J = 6.0$ Hz, 1H), 6.82 (br s, 1H), 7.31 (br s, 2H), 8.11 (s, 1H), 8.12–8.18 (br m, 1H), 8.39 (s, 1H); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 9.3, 45.7, 68.3, 70.7, 73.5, 82.4, 86.9, 115.3, 118.9, 139.1 (br), 139.3, 149.6, 151.4 (br), 152.7, 156.1, 165.4, 169.6 (missing one aryl carbon); HRMS (ESI-) calcd for $C_{16}H_{16}N_7O_8S$ [M - H]⁻ 466.0787, found 466.0767 (error 4.3 ppm).

N-Hydroxysuccinimidy-2-Fluoropyridine-3-carboxylate (41d). This was prepared from 2-fluoronicotinic acid (300 mg, 2.1 mmol) using the general procedure for NHS ester synthesis. Purification by flash chromatography (1:1 EtOAc/hexane) afforded the title compound (250 mg, 50%): R_f 0.18 (1:1 EtOAc/hexane); ¹H NMR (600 MHz, CDCl₃) δ 2.91 (s, 4H), 7.38 (t, $J = 6.0$ Hz, 1H), 8.49–8.52 (m, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 23.3, 107.0 (d, $^2J_{C-F} = 25.1$ Hz), 119.4 (d, $^4J_{C-F} = 5.0$ Hz), 141.4, 151.4 (d, $^3J_{C-F} = 15.6$ Hz), 155.9 (d, $^3J_{C-F} = 15.6$ Hz), 159.0 (d, $^1J_{C-F} = 251$ Hz), 166.4; HRMS (ESI+) calcd for $C_{10}H_8FN_2O_4$ [M + H]⁺ 239.0463, found 239.0469 (error 2.5 ppm).

5'-O-[N-[(2-Fluoropyridin-3-yl)carbonyl]sulfamoyl]-2',3'-O-isopropylideneadenosine (42d). This was prepared from **41d** (100

mg, 0.40 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (1:7 MeOH/EtOAc) afforded the title compound (58 mg, 27%): R_f 0.17 (1:7 MeOH/EtOAc); $[\alpha]_D^{21} -63.0$ (c 2.50, MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 1.36 (s, 3H), 1.59 (s, 3H), 4.32 (d, $J = 3.6$ Hz, 2H), 4.56–4.58 (m, 1H), 5.15 (dd, $J = 6.0, 2.4$ Hz, 1H), 5.37 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.22 (d, $J = 3.0$ Hz, 1H), 7.26–7.30 (m, 1H), 8.16 (s, 1H), 8.17–8.23 (m, 2H), 8.45 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 24.3, 26.3, 68.7, 82.1, 84.5, 84.6, 90.7, 114.1, 118.9, 121.4, 140.2, 142.2, 148.2, 148.3, 149.3, 152.7, 156.1, 170.1 (d, $^1J_{\text{C-F}} = 252$ Hz), 173.7; HRMS (ESI $^-$) calcd for $\text{C}_{19}\text{H}_{19}\text{FN}_7\text{O}_7\text{S}$ [$\text{M} - \text{H}$] $^-$ 508.1056, found 508.1059 (error 0.6 ppm).

5'-O- $\{N-[(2\text{-Fluoropyridin-3-yl})\text{carbonyl}]\text{sulfamoyl}\}$ -adenosine Triethylammonium Salt (24). This was prepared from **42d** (30 mg, 0.060 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:20:1.5 MeOH/EtOAc/TEA) afforded the title compound (14 mg, 50%): R_f 0.17 (100:20:1.5 MeOH/EtOAc/TEA); $[\alpha]_D^{21} -69.6$ (c 2.55, MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 1.24 (t, $J = 7.2$ Hz, 9H), 3.13 (q, $J = 7.2$ Hz, 6H), 4.31–4.32 (m, 1H), 4.32–4.43 (m, 3H), 4.70 (t, $J = 4.8$ Hz, 1H), 6.08 (d, $J = 5.4$ Hz, 1H), 7.27–7.29 (m, 1H), 8.16 (s, 1H), 8.18 (d, $J = 4.2$ Hz, 1H), 8.24–8.27 (m, 1H), 8.50 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 8.1, 46.5, 68.4, 71.2, 74.8, 83.4, 87.9, 118.9, 121.5 (d, $J_{\text{C-F}} = 4.5$ Hz), 122.1 (d, $J_{\text{C-F}} = 25.7$ Hz), 139.9, 142.2 (d, $J_{\text{C-F}} = 2.7$ Hz), 148.3 (d, $J_{\text{C-F}} = 14.0$ Hz), 149.6, 152.7, 156.1, 160.6 (d, $J_{\text{C-F}} = 243$ Hz), 170.1 (d, $J_{\text{C-F}} = 6.2$ Hz); HRMS (ESI $^-$) calcd for $\text{C}_{16}\text{H}_{15}\text{FN}_7\text{O}_7\text{S}$ [$\text{M} - \text{H}$] $^-$ 468.0743, found 468.0740 (error 0.6 ppm).

N-Hydroxysuccinimide 2-Chloropyridine-3-carboxylate (41e). This was prepared from 2-chloronicotinic acid (780 mg, 5.00 mmol) using the general procedure for NHS ester synthesis. Purification by flash chromatography (1:1 EtOAc/hexane) afforded the title compound (660 mg, 52%): R_f 0.21 (1:1 EtOAc/hexane); $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 2.92 (s, 4H), 7.41 (t, $J = 7.8$ Hz, 1H), 8.42 (dd, $J = 7.8, 1.8$ Hz, 1H), 8.63 (dd, $J = 7.8, 1.8$ Hz, 1H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 25.9, 122.1, 122.4, 141.4, 151.7, 153.9, 159.6, 168.9; HRMS (ESI $^+$) calcd for $\text{C}_{10}\text{H}_8\text{ClN}_2\text{O}_4$ [$\text{M} + \text{H}$] $^+$ 255.0167, found 255.0163 (error 1.6 ppm).

5'-O- $\{N-[(2\text{-Chloropyridin-3-yl})\text{carbonyl}]\text{sulfamoyl}\}$ -2',3'-O-isopropylideneadenosine Triethylammonium Salt (42e). This was prepared from **41e** (50 mg, 0.20 mmol) using the general procedure for NHS mediated acylation. Purification by flash chromatography (100:10:1 EtOAc/MeOH/TEA) afforded the title compound (86 mg, 82%): R_f 0.15 (100:10:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -94.3$ (c 0.340, MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 1.27 (t, $J = 7.8$ Hz, 9H), 1.36 (s, 3H), 1.59 (s, 3H), 3.16 (q, $J = 7.8$ Hz, 6H), 4.33–4.35 (m, 2H), 4.57 (dd, $J = 5.4, 2.4$ Hz, 1H), 5.20 (dd, $J = 6.0, 2.4$ Hz, 1H), 5.39 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.24 (d, $J = 3.0$ Hz, 1H), 7.35 (dd, $J = 7.8, 4.8$ Hz, 1H), 7.90 (dd, $J = 7.8, 1.8$ Hz, 1H), 8.19 (s, 1H), 8.32 (dd, $J = 4.8, 1.8$ Hz, 1H), 8.45 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 8.0, 24.4, 26.3, 46.7, 68.9, 82.1, 84.5, 84.6, 90.7, 114.2, 119.0, 122.7, 136.1, 138.3, 140.2, 147.3, 149.0, 149.3, 152.8, 156.1, 172.3; HRMS (ESI $^+$) calcd for $\text{C}_{19}\text{H}_{21}\text{ClN}_7\text{O}_7\text{S}$ [$\text{M} + \text{H}$] $^+$ 526.0906, found 526.0909 (error 0.6 ppm).

5'-O- $\{N-[(2\text{-Chloropyridin-3-yl})\text{carbonyl}]\text{sulfamoyl}\}$ -adenosine Triethylammonium Salt (25). This was prepared from **42e** (40 mg, 0.080 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (100:20:1 EtOAc/MeOH/TEA) afforded the title compound (21 mg, 53%): R_f 0.10 (100:20:1 EtOAc/MeOH/TEA); $[\alpha]_D^{21} -64.7$ (c 0.340, MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 1.26 (t, $J = 7.8$ Hz, 9H), 3.15 (q, $J = 7.8$ Hz, 6H), 4.33 (dd, $J = 6.6, 3.0$ Hz, 1H), 4.39–4.46 (m, 3H), 4.73 (t, $J = 6.0$ Hz, 1H), 6.09 (d, $J = 4.8$ Hz, 1H), 7.35 (dd, $J = 7.2, 4.2$ Hz, 1H), 7.94 (dd, $J = 7.2, 1.8$ Hz, 1H), 8.19 (s, 1H), 8.31 (dd, $J = 4.2, 1.8$ Hz, 1H), 8.49 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 8.1, 46.7, 68.5, 71.2, 74.8, 83.4, 88.1, 119.0, 122.6, 136.2, 138.3, 139.9, 147.3, 148.9, 149.7, 152.7, 156.1, 172.4; HRMS (ESI $^+$) calcd for $\text{C}_{16}\text{H}_{17}\text{ClN}_7\text{O}_7\text{S}$ [$\text{M} + \text{H}$] $^+$ 486.0593, found 486.0598 (error 1.0 ppm).

5'-O- $\{N-[(\text{Morpholino})\text{carbonyl}]\text{sulfamoyl}\}$ adenosine Sodium Salt (26). Morpholine (54 μL , 3.0 mmol, 1.0 equiv) was added

dropwise to a solution of chlorosulfonyl isocyanate (54 μL , 3.0 mmol, 1.0 equiv) in CH_2Cl_2 (15 mL) at -20 °C. The reaction mixture was stirred at -15 °C to -20 °C for 4 h, then the solvent was evaporated in vacuo, and the crude product **52** was used for the subsequent step without any further workup.

To the above intermediate **52** was added a solution of 2',3'-O-isopropylideneadenosine (155 mg, 2 mmol, 0.66 equiv) and pyridine (48 μL , 3 mmol, 1 equiv) in MeCN (38 mL). The resulting solution was stirred at rt for 20 h. The solvent was evaporated in vacuo, and purification by flash column chromatography (70:30:1 EtOAc/MeOH/TEA) afforded 2',3'-O-isopropylidene-5'-O- $\{N-[(\text{morpholino})\text{carbonyl}]\text{sulfamoyl}\}$ adenosine triethylammonium salt **54** that was approximately 50–75% pure based on $^1\text{H NMR}$.

Crude **54** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash column chromatography (70:30:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (12 mg, 4% overall yield): R_f 0.29 (6:4 EtOAc/MeOH); $[\alpha]_D^{21} -0.06$ (c 0.22, CH_3OH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 3.40–3.60 (m, 4H), 3.58–3.60 (m, 4H), 4.25–4.30 (m, 3H), 4.41–4.43 (m, 1H), 4.70 (t, $J = 5.4$ Hz, 1H), 6.09 (d, $J = 6.0$ Hz, 1H), 8.19 (s, 1H), 8.54 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 49.7, 68.1, 68.8, 72.5, 76.3, 85.0, 89.1, 120.2, 141.4, 151.0, 154.0, 157.4, 162.4; HRMS (ESI $^-$) calcd for $\text{C}_{15}\text{H}_{20}\text{N}_7\text{O}_8\text{S}$ [$\text{M} - \text{H}$] $^-$ 458.1099, found 458.1106 (error 1.4 ppm).

2',3'-O-O-Bis(*t*-butyldimethylsilyl)-5'-O- $\{N-[(\text{cyclopentyl})\text{carbonyl}]\text{sulfamoyl}\}$ adenosine Triethylammonium Salt (42f). Cyclopentanecarbonyl chloride (920 μL , 7.54 mmol) was converted to the NHS ester **41f** using the general procedure B for NHS ester synthesis. The crude NHS ester was used directly for the next step.

The title compound was prepared from **41f** (126 mg, 0.60 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) afforded the title compound (270 mg, 67% overall yield): R_f 0.23 (6:4 EtOAc/MeOH); $[\alpha]_D^{21} -64.7$ (c 0.34, MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ -0.37 (s, 3H), -0.01 (s, 3H), 0.16 (s, 3H); 0.17 (s, 3H), 0.71 (s, 9H), 0.98 (s, 9H), 1.23 (t, $J = 7.2$ Hz, 9H), 1.54–1.57 (m, 2H), 1.70–1.72 (m, 2H), 1.80–1.84 (m, 4H), 2.65 (p, $J = 8.4$ Hz, 1H), 3.03 (q, $J = 7.2$ Hz, 6H), 4.29–4.34 (m, 3H), 4.45 (d, $J = 4.2$ Hz, 1H), 4.84–4.86 (m, 1H, obscured by CD_3OD : assigned by gCOSY as H-2'), 6.12 (d, $J = 7.2$ Hz, 1H), 8.18 (s, 1H), 8.57 (s, 1H); $^{13}\text{C NMR}$ (600 MHz, CD_3OD) δ -5.2, -4.2, -4.14 (2C), 9.8, 18.8, 19.0, 26.3, 26.5, 27.1, 31.9, 32.0, 47.8, 69.1, 75.4, 77.7, 86.6, 88.3, 120.2, 141.7, 151.2, 154.0, 157.5, 186.9; MS (ESI $^-$) calcd for $\text{C}_{28}\text{H}_{49}\text{N}_6\text{O}_7\text{SSi}_2$ [$\text{M} - \text{H}$] $^-$ 669.3, found 669.3.

5'-O- $\{N-[(\text{Cyclopentyl})\text{carbonyl}]\text{sulfamoyl}\}$ adenosine Sodium Salt (27). This was prepared from **42f** (270 mg, 0.40 mmol) using the general procedure for TBS protection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (96 mg, 55%): R_f 0.25 (8:2 EtOAc/MeOH); $[\alpha]_D^{21} -0.23$ (c 0.49, CH_3OH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ 1.52–1.54 (m, 2H), 1.66–1.68 (m, 2H), 1.72–1.77 (m, 2H), 1.80–1.83 (m, 2H), 2.63 (p, $J = 8.4$ Hz, 1H), 4.24–4.31 (m, 3H), 4.38–4.39 (m, 1H), 4.69 (t, $J = 6.0$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CD_3OD) δ 27.1, 27.1, 31.3, 31.9, 69.2, 72.5, 76.2, 84.8, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 186.3; HRMS (ESI $^-$) calcd for $\text{C}_{16}\text{H}_{21}\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$] $^-$ 441.1197, found 441.1183 (error 3.2 ppm).

2',3'-O-O-Bis(*t*-butyldimethylsilyl)-5'-O- $\{N-[(\text{cyclohexyl})\text{carbonyl}]\text{sulfamoyl}\}$ adenosine Triethylammonium Salt (42g). Cyclohexanecarbonyl chloride (930 μL , 6.82 mmol) was converted to the NHS ester **41g** using the general procedure B for NHS ester synthesis. The crude NHS ester was used directly for the next step.

The title compound was prepared from **41g** (88 mg, 0.39 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) afforded the title compound (230 mg, 87%): R_f 0.22 (6:4 EtOAc/MeOH); $^1\text{H NMR}$ (600 MHz, CD_3OD) δ -0.37 (s, 3H), -0.01 (s, 3H), 0.16 (s, 3H), 0.17 (s, 3H), 0.71 (s, 9H), 0.98 (s, 9H), 1.23 (t,

$J = 7.2$ Hz, 9H), 1.28–1.33 (m, 3H), 1.43–1.46 (m, 2H), 1.65–1.66 (m, 1H), 1.75–1.76 (m, 2H), 1.86–1.88 (m, 2H), 2.16 (tt, $J = 11.4$, 3.0 Hz, 1H), 3.06 (q, $J = 7.2$ Hz, 6H), 4.30–4.33 (m, 3H), 4.45 (d, $J = 4.2$ Hz, 1H), 4.84–4.86 (m, 1H, obscured by CD_3OD : assigned by gCOSY as H-2'), 6.11 (d, $J = 7.8$ Hz, 1H), 8.18 (s, 1H), 8.56 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ -5.3, -4.33, -4.28 (2C), 9.7, 18.6, 18.9, 26.2, 26.4, 27.0, 27.4, 31.25, 31.29, 47.6, 69.0, 74.9, 77.5, 86.4, 88.2, 120.1, 141.5, 151.1, 153.9, 157.3, 185.8; MS (ESI⁻) calcd for $\text{C}_{29}\text{H}_{51}\text{N}_6\text{O}_7\text{SSi}_2$ [$\text{M} - \text{H}$]⁻ 683.3, found 683.3.

5'-O-*N*-[(Cyclohexyl)carbonyl]sulfamoyl adenosine Sodium Salt (28). This was prepared from **42g** (230 mg, 0.33 mmol) using the general procedure for TBS protection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (82 mg, 51%): R_f 0.22 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.26$ (c 0.50, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 1.17–1.30 (m, 3H), 1.37–1.42 (m, 2H), 1.63–1.65 (m, 1H), 1.73–1.74 (m, 2H), 1.82–1.84 (m, 2H), 2.14 (tt, $J = 11.4$, 3.0 Hz, 1H), 4.23–4.30 (m, 3H), 4.37–4.39 (m, 1H), 4.69 (t, $J = 6.0$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 27.2, 27.3, 31.27, 31.28, 69.2, 72.6, 76.2, 84.8, 89.3, 120.3, 141.3, 151.0, 153.9, 157.4, 186.4; HRMS (ESI⁻) calcd for $\text{C}_{17}\text{H}_{23}\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$]⁻ 455.1354, found 455.1348 (error 1.3 ppm).

2',3'-*O*-Bis(*t*-butyldimethylsilyl)-5'-*O*-[(*N*-acetyl)sulfamoyl]adenosine Triethylammonium Salt (42h). Acetic anhydride (920 μL , 9.8 mmol) was converted to the NHS ester **41h** using the general procedure B for NHS ester synthesis. The crude NHS ester was used directly for the next step.

The title compound was prepared from **41h** (96 mg, 0.61 mmol) using the general procedure for NHS ester mediated acylation. Purification by flash chromatography (70:30:1 EtOAc/MeOH/TEA) afforded the title compound (350 mg, 93%): R_f 0.20 (85:15 EtOAc/MeOH); ^1H NMR (600 MHz, CD_3OD) δ -0.35 (s, 3H), -0.01 (s, 3H), 0.16 (s, 3H), 0.17 (s, 3H), 0.71 (s, 9H), 0.98 (s, 9H), 1.29 (t, $J = 7.2$ Hz, 9H), 1.96 (s, 3H), 3.17 (q, $J = 7.2$ Hz, 6H), 4.46 (d, $J = 4.2$ Hz, 1H), 4.30–4.35 (m, 3H), 4.82–4.84 (1H, m, obscured by CD_3OD : assigned by gCOSY as H-2'), 6.12 (d, $J = 7.2$ Hz, 1H), 8.19 (s, 1H), 8.56 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ -5.2, -4.24, -4.20, -4.17, 9.5, 18.8, 19.0, 26.2, 26.3, 26.5, 47.9, 69.2, 74.9, 77.7, 86.4, 88.3, 120.2, 141.6, 151.2, 154.0, 157.5, 180.4; MS (ESI⁻) calcd for $\text{C}_{24}\text{H}_{43}\text{N}_6\text{O}_7\text{SSi}_2$ [$\text{M} - \text{H}$]⁻ 615.2, found 615.2.

5'-O-*N*-[Acetyl]sulfamoyl adenosine Sodium Salt (29). This was prepared from **42h** (350 mg, 0.57 mmol) using the general procedure for TBS protection. Purification by flash chromatography (80:20:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (148 mg, 74%): R_f 0.26 (7:3 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.25$ (c 0.56, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 1.95 (s, 3H), 4.26–4.33 (m, 3H), 4.38–4.39 (m, 1H), 4.66 (t, $J = 6.0$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 8.19 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 26.2, 69.2, 72.4, 76.2, 84.7, 89.3, 120.3, 141.2, 151.0, 154.0, 157.4, 180.8, HRMS (ESI⁻) calcd for $\text{C}_{16}\text{H}_{21}\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$]⁻ 387.0728, found 387.0722 (error 1.5 ppm).

5'-O-*N*-[(Methoxy)carbonyl]sulfamoyl adenosine Sodium Salt (30). A solution of **41h** (80 mg, 0.2 mmol, 1.0 equiv) and CDI (65 mg, 0.4 mmol, 2 equiv) in MeCN (10 mL) was stirred at rt for 2 h. Next, MeOH (3 mL) and Et_3N (55 μL , 0.4 mmol, 2.0 equiv) were added, and the reaction mixture was stirred for an additional 2 h and then concentrated in vacuo. Purification by flash column chromatography (80:20:1 EtOAc/MeOH/ Et_3N) afforded 2',3'-*O*-isopropylidene-5'-*O*-*N*-[(methoxy)carbonyl]sulfamoyl adenosine triethylammonium salt.

2',3'-*O*-Isopropylidene-5'-*O*-*N*-[(methoxy)carbonyl]sulfamoyl adenosine triethylammonium salt prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (70:30:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (84 mg, 64% overall yield):

R_f 0.20 (7:3 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.16$ (c 0.58, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 3.56 (s, 3H), 4.23–4.31 (m, 3H), 4.36–4.38 (m, 1H), 4.65 (t, $J = 5.4$ Hz, 1H), 6.08 (d, $J = 6.0$ Hz, 1H), 8.18 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 51.8, 68.2, 71.4, 75.3, 83.8, 88.3, 119.3, 140.2, 145.0, 153.0, 156.4, 161.2; HRMS (ESI⁻) calcd for $\text{C}_{17}\text{H}_{23}\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$]⁻ 403.0677, found 403.0690 (error 3.1 ppm).

5'-O-*N*-[(Benzyl)carbonyl]sulfamoyl adenosine Sodium Salt (31). Phenylacetic acid (1.00 g, 7.3 mmol) was converted to the NHS ester **41i** using the general procedure A for NHS ester synthesis. The crude NHS ester was used directly for the next step.

Compound **41i** (85 mg, 0.37 mmol) prepared above was treated with **36** using the general procedure for NHS ester mediated acylation to afford crude 2',3'-*O*-*O*-bis(*t*-butyldimethylsilyl)-5'-*O*-*N*-[(benzyl)carbonyl]sulfamoyl triethylammonium salt that was used directly for the next step.

2',3'-*O*-Bis(*t*-butyldimethylsilyl)-5'-*O*-*N*-[(benzyl)carbonyl]sulfamoyl triethylammonium salt prepared above was deprotected using the general procedure for TBS deprotection. Purification by flash chromatography (75:25:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (110 mg, 65% overall yield): R_f 0.29 (7:3 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.14$ (c 0.43, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 3.48 (s, 2H), 4.18–4.20 (m, 1H), 4.22–4.26 (m, 2H), 4.29–4.30 (m, 1H), 4.62 (t, $J = 5.4$ Hz, 1H), 6.06 (d, $J = 6.0$ Hz, 1H), 7.13 (t, $J = 7.2$ Hz, 1H), 7.21 (t, $J = 7.8$ Hz, 2H), 7.30 (d, $J = 7.8$ Hz, 2H), 8.19 (s, 1H), 8.45 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 47.2, 69.3, 72.4, 76.2, 84.6, 89.3, 120.3, 127.3, 129.3, 130.4, 138.5, 141.2, 150.9, 154.0, 157.4, 181.1; HRMS (ESI⁻) calcd for $\text{C}_{18}\text{H}_{19}\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$]⁻ 463.1041, found 463.1046 (error 1.1 ppm).

5'-O-*N*-[4-Azido-2,3,5,6-tetrafluorobenzoyl]sulfamoyl adenosine Triethylammonium Salt (32). *N*-Hydroxysuccinimidyl-4-azidotetrafluorobenzoate²⁶ (235 mg, 1.0 mmol, 1.0 equiv) was treated with **36** using the general procedure for NHS ester mediated acylation. Purification by flash column chromatography (80:20:1 EtOAc/MeOH/ Et_3N) afforded 5'-*O*-*N*-[4-azido-2,3,5,6-tetrafluorobenzoyl]sulfamoyl-2',3'-*O*-isopropylideneadenosine triethylammonium salt (141 mg, 39%).

5'-*O*-*N*-[4-Azido-2,3,5,6-tetrafluorobenzoyl]sulfamoyl-2',3'-*O*-isopropylideneadenosine triethylammonium salt (105 mg, 0.2 mmol) prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash column chromatography (80:20:1 EtOAc/MeOH/ Et_3N) afforded the title compound (62 mg, 47%): R_f 0.29 (8:2 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.11$ (c 0.37, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 1.29 (t, $J = 7.8$ Hz, 9H), 3.21 (q, $J = 7.8$ Hz, 6H), 4.32–4.42 (m, 4H), 4.70 (t, $J = 5.4$ Hz, 1H), 6.09 (d, $J = 5.4$ Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 48.1, 69.9, 72.5, 76.1, 84.7, 89.3, 120.3, 141.2, 151.0, 154.0, 157.4, 166.1 (missing three aryl carbons and C=O); HRMS (ESI⁻) calcd for $\text{C}_{17}\text{H}_{12}\text{F}_4\text{N}_6\text{O}_7\text{S}$ [$\text{M} - \text{H}$]⁻ 562.0522, found 562.0548 (error 4.6 ppm).

***N*-Hydroxysuccinimidyl *N*-(*t*-Butyloxycarbonyl)-*L*-phenylalanine (41k).** This was prepared from *N*-(*t*-butyloxycarbonyl)-*L*-phenylalanine (795 mg, 3.00 mmol) using the general procedure for NHS ester synthesis. Purification by flash chromatography (2:5 EtOAc/hexane) afforded the title compound (800 mg, 74%): R_f 0.33 (3:1 hexane/EtOAc); $[\alpha]_{\text{D}}^{21} -37.1$ (c 0.730, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 1.39 (s, 9H), 2.84 (s, 4H), 3.18 (dd, $J = 13.2$, 5.4 Hz, 1H), 3.30 (dd, $J = 13.2$, 5.4 Hz, 1H), 4.90 (t, $J = 5.4$ Hz, 1H), 7.25–7.33 (m, 5H); ^{13}C NMR (150 MHz, CDCl_3) δ 25.8, 28.2, 38.4, 52.8, 80.8, 127.6, 128.9, 129.9, 134.9, 154.8, 167.9, 168.7; MS (ESI⁺) $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_4$ [$\text{M} + \text{H}$] calcd 263.1, found 263.1.

5'-O-*N*-[*N*-(*t*-Butyloxycarbonyl)-*L*-phenylalanyl]sulfamoyl-2',3'-*O*-isopropylideneadenosine (42k). This was prepared from **41k** (363 mg, 1.00 mmol) using the general procedure for NHS mediated acylation. Purification by flash chromatography (100:10:1 EtOAc/MeOH/TEA) afforded the title compound (600 mg, 95%): R_f 0.16 (10:1 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -19.1$ (c 1.00, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 1.33 (s, 9H), 1.36 (s, 3H), 1.58 (s, 3H), 2.84 (dd, $J = 13.2$, 9.0 Hz, 1H), 3.10 (dd, $J = 13.2$, 4.8 Hz,

1H), 4.16–4.23 (m, 3H), 4.47–4.51 (m, 1H), 5.06 (d, $J = 6.0$ Hz, 1H), 5.32 (br s, 1H), 6.21 (br s, 1H), 7.11–7.22 (m, 5H), 8.19 (s, 1H), 8.41 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 25.1, 26.3, 27.5, 57.9, 80.5, 82.0, 84.4, 84.6, 85.3, 85.9, 90.7, 92.3, 114.1, 118.9, 126.2, 128.1, 129.4, 139.4, 140.3, 149.1, 149.3, 152.7, 156.0; HRMS (ESI+) calcd for $\text{C}_{27}\text{H}_{36}\text{N}_7\text{O}_9\text{S}$ $[\text{M}]^+$ 634.2295, found 634.2309 (error 3.0 ppm).

5'-O-[N-(L-Phenylalanyl)sulfamoyl]adenosine Sodium Salt (33). This was prepared from **42k** (200 mg, 0.32 mmol) using the general procedure for TFA deprotection. Purification by HPLC (Microsorb C18 Dynamax preparative HPLC column, 41.4×250 mm, pore size 100 Å, 20–80% A in 45 min, $A = 40$ mM TEAB buffer, $B = 70\%$ CH_3CN in H_2O , flow rate = 40 mL/min, monitored at 254 nm) and lyophilization of the pooled fractions followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (90 mg, 58%); $t_{\text{R}} = 12.7$ min; $[\alpha]_{\text{D}}^{21} -7.5$ (c 0.74, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 2.87 (dd, $J = 13.2, 5.4$ Hz, 1H), 3.17 (dd, $J = 13.2, 5.4$ Hz, 1H), 3.64 (t, $J = 6.6$ Hz, 1H), 4.22–4.29 (m, 3H), 4.36 (t, $J = 4.2$ Hz, 1H), 4.62 (t, $J = 4.8$ Hz, 1H), 6.07 (d, $J = 5.4$ Hz, 1H), 7.13–7.15 (m, 1H), 7.22–7.25 (m, 4H), 8.18 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 41.6, 58.5, 67.9, 70.9, 75.1, 83.2, 88.3, 119.0, 126.2, 128.2, 129.3, 138.4, 140.1, 149.6, 152.7, 156.1, 181.6 (missing 2C's); HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{24}\text{N}_7\text{O}_7\text{S}$ $[\text{M} + \text{H}]^+$ 494.1458 found 494.1489 (error 0.6 ppm).

5'-O-[N-(3-[(Morpholino)carbonyl]amino)propanoyl]-sulfamoyl]adenosine Sodium Salt (47). Morpholinocarbonyl chloride (0.77 mg, 6.7 mmol) was converted to the NHS ester **44** using the general procedure B for NHS ester synthesis. The crude NHS ester was used directly for the next step.

Compound **44** (82 mg, 0.36 mmol) prepared above was treated with **37** using the general procedure for NHS mediated acylation. The crude product **46** was used directly for the next step.

Compound **46** prepared above was deprotected using the general procedure for TBS deprotection. Purification by flash chromatography (70:30:1 EtOAc/MeOH/TEA) followed by conversion to the sodium salt using the general procedure for ion-exchange afforded the title compound (107 mg, 56% overall yield): R_f 0.21 (6:4 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -0.08$ (c 0.42, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ 2.40 (t, $J = 6.6$ Hz, 2H), 3.29 (t, $J = 4.8$ Hz, 4H), 3.42 (t, $J = 6.6$ Hz, 2H), 3.58 (t, $J = 4.8$ Hz, 4H), 4.27–4.33 (m, 3H), 4.39 (t, $J = 4.2$ Hz, 1H), 4.66 (t, $J = 5.4$ Hz, 1H), 6.08 (d, $J = 5.4$ Hz, 1H), 8.20 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 38.8, 40.3, 45.2, 67.7, 69.2, 72.4, 76.3, 84.6, 89.4, 120.3, 141.3, 150.9, 154.0, 157.4, 160.2, 181.4; HRMS (ESI-) calcd for $\text{C}_{18}\text{H}_{25}\text{N}_8\text{O}_9\text{S}$ $[\text{M} - \text{H}]^-$ 529.1470, found 529.1489 (error 3.5 ppm).

5'-Azido-*N*⁶-butyloxycarbonyl-5'-deoxy-2',3'-O-isopropylideneadenosine. To a solution of 5'-azido-5'-deoxy-2',3'-O-isopropylideneadenosine²² (146 mg, 0.44 mmol, 1.0 equiv) in THF/DMF (4:1, 10 mL) was added sodium hydride (60% dispersion in mineral oil, 20 mg, 0.5 mmol, 1.15 equiv) at rt. The resulting solution was stirred for 30 min, and then solid di-*tert*-butyl dicarbonate (106 mg, 0.48 mmol, 1.1 equiv) was added in one portion. The reaction was monitored by TLC until complete consumption of the amine was observed (3 h). The solution was diluted with CH_2Cl_2 (20 mL) and washed with saturated aq NaHCO_3 (20 mL). The aqueous phase was back-extracted with CH_2Cl_2 (2×10 mL), and the combined organic phase was washed with H_2O (20 mL) and saturated aq NaCl (30 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (4:6 hexane/EtOAc) afforded the title compound (144 mg, 60%); R_f 0.55 (1:2 hexane/EtOAc); $[\alpha]_{\text{D}}^{21} -33.1$ (c 0.972, CH_3OH); ^1H NMR (600 MHz, CDCl_3) δ 1.35 (s, 3H), 1.53 (s, 9H), 1.58 (s, 3H), 3.54 (d, $J = 5.4$ Hz, 2H), 4.36 (dd, $J = 3.6, 5.4$ Hz, 1H), 5.01 (dd, $J = 6.6, 3.6$ Hz, 1H), 5.42 (dd, $J = 6.6, 2.4$ Hz, 1H), 6.13 (d, $J = 2.4$ Hz, 1H), 8.09 (s, 1H), 8.74 (s, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ 25.5, 27.3, 28.3, 52.4, 82.0, 82.6, 84.2, 85.8, 90.9, 115.1, 122.3, 141.8, 149.8, 150.3, 150.4, 153.2; MS (APCI+) calcd for $\text{C}_{18}\text{H}_{25}\text{O}_5\text{N}_8$ $[\text{M} + \text{H}]^+$ 433.2, found 433.2.

5'-Amino-*N*⁶-*t*-butoxycarbonyl-5'-deoxy-2',3'-O-isopropylideneadenosine (56). To a solution of 5'-azido-*N*⁶-*tert*-butoxycarbonyl-5'-deoxy-2',3'-O-isopropylideneadenosine (150 mg, 0.35 mmol, 1.0 equiv) in MeOH (5.0 mL) was added 10% Pd/C (10 mg, 7% by wt) in MeOH (0.5 mL), and the reaction mixture was stirred under H_2 (2.5 atm) for 12 h. The reaction mixture was then filtered through a plug of Celite. Purification by flash chromatography (100:17:1 EtOAc/MeOH/TEA) afforded the title compound 132 mg, 94%). ^1H NMR, ^{13}C NMR, and HRMS agreed with literature values.¹¹

5'-Amino-5'-N-[(chromon-3-yl)carbonyl]adenosine (58). A solution of chromone 3-carboxylic acid (74 mg, 0.39 mmol) and CDI (63.3 mg, 0.39 mmol, 1.0 equiv) in DMF (6 mL) was stirred at 60 °C for 2 h. The solution was cooled to rt, a mixture of **56** (100 mg, 0.32 mmol, 0.8 equiv) and DBU (92 μL , 0.62 mmol, 1.5 equiv) were then added, and the reaction mixture was stirred overnight at rt. The reaction mixture was then concentrated in vacuo and purified by flash chromatography to afford **57**. The crude product was used directly for the next step.

Crude **57** prepared above was deprotected using the general procedure for TFA deprotection. Purification by flash chromatography (60:40 EtOAc/MeOH) afforded the title compound (29 mg, 20% overall yield): R_f 0.22 (7:3 EtOAc/MeOH); $[\alpha]_{\text{D}}^{21} -31.7$ (c 0.11, MeOH); ^1H NMR (600 MHz, DMSO) δ 3.97 (br s, 1H), 4.08 (dd, $J = 5.4, 4.8$ Hz, 1H), 4.16 (dd, $J = 6.6, 3.6$ Hz, 1H), 4.22 (br s, 1H), 4.67–4.71 (m, 1H), 5.43 (br s, 1H), 5.57 (br s, 1H), 5.92 (d, $J = 6.0$ Hz, 1H), 7.26–7.30 (m, 4H), 7.63 (t, $J = 7.8$ Hz, 1H), 7.88 (t, $J = 7.8$ Hz, 1H), 8.10 (br s, 1H), 8.33 (s, 1H), 8.43–8.45 (m, 1H), 10.39 (br s, 1H); ^{13}C NMR (150 MHz, DMSO) δ 51.7, 70.8, 72.7, 82.1, 87.7, 96.0, 116.9, 119.3, 120.2, 123.9, 125.3, 134.3, 139.9, 149.3, 152.6, 154.2, 156.1, 162.6, 163.2, 179.4; MS (APCI-) calcd for $\text{C}_{20}\text{H}_{18}\text{N}_6\text{O}_6$ $[\text{M} - \text{H}]^-$ 437.1215, found 437.1378.

2-Benzyloxybenzamide (59). To a solution of 2-hydroxybenzamide (2.74 g, 20 mmol, 1.0 equiv) and K_2CO_3 (3.30 g, 24 mmol, 1.2 equiv) in acetone (100 mL) was added benzyl bromide (2.80 mL, 24 mmol, 1.2 equiv), and the reaction mixture was heated at reflux for 16 h. After cooling to rt, the solution was filtered and the filtrate was concentrated under reduced pressure. The crude solid was recrystallized from acetone to afford the title compound (2.89 g, 62%) as a white solid: ^1H NMR (CDCl_3 , 600 MHz) δ 5.19 (s, 2H), 5.92 (br s, 1H), 7.05 (d, $J = 8.4$ Hz, 1H), 7.09 (t, $J = 8.4$ Hz, 1H), 7.36–7.48 (m, 6H), 7.73 (br s, 1H), 8.23 (d, $J = 7.8$ Hz, 1H); ^{13}C NMR (CDCl_3 , 150 MHz) δ 71.5, 112.9, 121.3, 121.8, 128.1, 129.0, 129.2, 132.9, 133.6, 135.8, 157.4, 167.2; HRMS (APCI+) calcd for $\text{C}_{14}\text{H}_{14}\text{NO}_2$ $[\text{M} + \text{H}]^+$ 228.1019, found 228.1042 (error 10.0 ppm).

5'-Amino-5'-N-[(2-benzyloxybenzoyl)amino]carbonyl]-*N*⁶-*t*-butoxycarbonyl-5'-deoxy-2',3'-O-isopropylideneadenosine (61). To a stirred solution of **59** (114 mg, 0.50 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) was added oxalyl chloride (129 μL , 1.5 mmol, 3 equiv) dropwise at rt. The solution was then heated at 50 °C for 3 h. After cooling to rt, the reaction mixture was concentrated in vacuo to afford a light yellow solid. Next, a solution of **56** (244 mg, 0.6 mmol, 1.2 equiv) in CH_3CN (10 mL) was added, and the resulting mixture was stirred for 16 h at rt. The crude reaction was concentrated in vacuo, and the residue was partitioned between CH_2Cl_2 (25 mL) and H_2O (25 mL). The aqueous layer was extracted with CH_2Cl_2 (2×25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash chromatography (7:3 EtOAc/hexane) afforded the title compound (290 mg, 88%) as a white solid: R_f 0.10 (6:4 EtOAc/hexane); ^1H NMR (CDCl_3 , 600 MHz) δ 1.35 (s, 3H), 1.54 (s, 9H), 1.59 (s, 3H), 3.64–3.71 (m, 2H), 4.41–4.44 (m, 1H), 4.99 (dd, $J = 6.0, 3.6$ Hz, 1H), 5.28 (s, 2H), 5.37 (dd, $J = 6.0, 3.0$ Hz, 1H), 6.12 (d, $J = 3.0$ Hz, 1H), 7.05 (d, $J = 8.4$ Hz, 1H), 7.10 (t, $J = 7.8$ Hz, 1H), 7.35–7.37 (m, 1H), 7.40–7.43 (m, 4H), 7.48 (dt, $J = 8.4, 1.2$ Hz, 1H), 8.05 (br s, 1H, NH), 8.12 (dd, $J = 7.8, 1.2$ Hz, 1H), 8.18 (s, 1H), 8.74 (s, 1H), 8.85 (t, $J = 6.0$ Hz, 1H, NH), 9.97 (s, 1H, NH); ^{13}C NMR (CDCl_3 , 150 MHz) δ 25.7, 27.5, 28.3, 41.5, 71.8, 81.9, 82.5, 84.2, 85.3, 90.4, 113.5, 115.2, 119.9, 122.1, 122.3, 127.7, 129.1, 129.2, 132.9, 134.9, 135.0, 141.7, 149.7, 150.2, 150.7,

153.4, 153.9, 157.2, 165.9; HRMS (ESI+) calcd for C₃₃H₃₈N₇O₈ [M + H]⁺ 660.2776, found 660.2667 (error 16.5 ppm).

5'-Amino-5'-N-[(2-benzyloxybenzoyl)amino]carbonyl]-5'-deoxyadenosine (62). This was prepared from **61** (100 mg, 0.15 mmol) using the general procedure for TFA deprotection. Purification by flash chromatography (500:3 EtOAc/MeOH) afforded the title compound (55 mg, 70%): *R*_f 0.3 (500:3 EtOAc/MeOH); ¹H NMR (CD₃OD, 600 MHz) δ 3.64 (dt, *J* = 14.4, 4.8 Hz, 1H), 3.76 (dt, *J* = 14.4, 6.0 Hz, 1H), 4.18 (q, *J* = 4.8 Hz, 1H), 4.40 (t, *J* = 5.4 Hz, 1H), 4.72 (t, *J* = 5.4 Hz, 1H), 5.31 (s, 2H), 5.99 (d, *J* = 5.4 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 2H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 8.15 (s, 1H), 8.29 (s, 1H), 8.96 (br t, *J* = 6.0 Hz, 1H, 5'-NH); ¹³C NMR (CD₃OD, 150 MHz) δ 40.7, 71.2, 71.4, 74.0, 83.2, 89.2, 113.8, 119.4, 120.5, 121.5, 127.6, 128.3, 128.7, 131.6, 134.6, 135.8, 140.7, 149.4, 151.8, 154.7, 155.5, 157.2, 166.6; HRMS (ESI-) calcd for C₂₅H₂₄N₇O₆ [M - H]⁻ 518.1794, found 518.1795 (error 0.1 ppm).

5'-Amino-5'-deoxy-5'-N-[(2-hydroxybenzoyl)amino]carbonyl]-adenosine (63). To a solution of **62** (50 mg, 0.096 mmol) in MeOH (10 mL) was added 10% Pd/C (10 mg, 20% by wt), and the reaction mixture was stirred at rt under H₂ (1 atm). After 2 h, the reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (10:2 EtOAc/MeOH) afforded the title compound (21 mg, 51%): *R*_f 0.12 (100:20 EtOAc/MeOH); ¹H NMR (CD₃OD, 600 MHz) δ 3.66 (dd, *J* = 14.4, 4.2 Hz, 1H), 3.80 (dd, *J* = 14.4 Hz, 4.8 Hz, 1H), 4.20 (q, *J* = 4.8 Hz, 1H), 4.42 (t, *J* = 4.8 Hz, 1H), 4.74 (t, *J* = 4.8 Hz, 1H), 6.00 (d, *J* = 4.8 Hz, 1H), 6.90 (ovlp t, *J* = 7.8 Hz, 1H), 6.91 (ovlp d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 8.16 (s, 1H), 8.32 (s, 1H); ¹³C NMR (CD₃OD, 150 MHz) δ 40.7, 71.2, 73.9, 83.3, 89.0, 116.7, 117.4, 119.3, 119.4, 130.9, 134.8, 140.4, 149.5, 152.7, 155.1, 156.1, 158.9, 167.5; HRMS (ESI-) calcd for C₁₈H₁₈N₇O₆ [M - H]⁻ 428.1324, found 428.1327 (error 0.7 ppm).

Enzyme Kinetic Studies. ATP/PPi Exchange Assay.³⁰ MbtA was expressed in *E. coli* and purified as described.¹⁵ The inhibition assays were performed as described in duplicate.¹⁵ In brief the reaction was initiated by adding 10 μL of [³²P]PPi with 7 nM MbtA in 90 μL of reaction buffer (250 μM salicylic acid, 10 mM ATP, 1 mM PPi, 75 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 2 mM DTT) at 37 °C in the presence of five different concentrations of the inhibitor. The reaction was terminated by the addition of 200 μL of quenching buffer (350 mM HClO₄, 100 mM PPi, 1.8% *w/v* activated charcoal). The charcoal was pelleted by centrifugation, washed once with 500 μL of H₂O, and analyzed by liquid scintillation counting as described.³⁰ The *K*₁^{app} values were calculated using either the Hill Morrison (eq 1) or Morrison (eq 2) equations as described.^{15,31}

$$v_i/v_0 = \frac{1}{1 + ([I]/K_1^{app})^h} \quad (1)$$

$$v_i/v_0 = \frac{([E] - [I] - K_1^{app}) + \sqrt{([E] - [I] - K_1^{app})^2 + 4[E][K_1^{app}]}}{2[E]} \quad (2)$$

M. tuberculosis H37Rv MIC Assay. All compounds MICs were experimentally determined as previously described.¹¹ Minimum inhibitory concentrations (MICs) were determined in quadruplicate in iron-deficient GAST and GAST supplemented with 200 μM FeCl₃ according to the broth microdilution method using compounds from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. Isoniazid was used as the positive control while DMSO was employed as a negative control. All measurements reported herein used an initial cell density of 10⁴–10⁵ cells/assay, and growth was monitored at 10–14 days, with the untreated and DMSO-treated control cultures reaching an OD₆₂₀ of 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.

Docking Studies. The homology model and docking runs were configured as described,¹² except that the side chains of residues 235, 240, 329, and 337 were allowed to move along with the ligand.

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Supporting Information Available: Table of HPLC purities of the inhibitors disclosed herein. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (t, $J = 6.6$ Hz, 2H, H-2) and 3.42 (t, $J = 6.6$ Hz, 2H, H-3). ^{13}C NMR: 181.4 (C = O, C-1), 40.3 (C-2), 38.8 (C-3). HMBC H-2 (coupled to C-1, C-3), H-3 (coupled to C-1, H-2, and C=O at 160.2 from (morpholino)carbonyl fragment).
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