SYNTHESIS AND EVALUATION OF CONFORMATIONALLY CONSTRAINED ANALOGUES OF SAL-AMS, A POTENT BISUBSTRATE INHIBITOR OF THE MYCOBACTERIAL ARYL ACID ADENYLATING ENZYME MbtA INVOLVED IN MYCOBACTIN BIOSYNTHESIS

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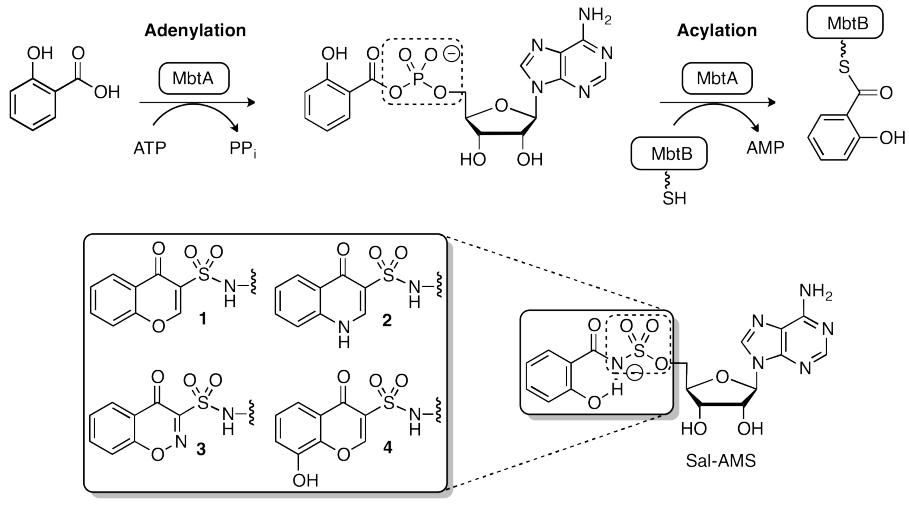
Abstract

Tuberculosis, caused primarily by the bacillus *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of bacterial infectious disease mortality. *Mtb* scavenges the essential micronutrient iron from its host via the synthesis, secretion, and reuptake of small-molecule iron chelators known as siderophores. Siderophores in *Mtb*, termed mycobactins, have been linked to virulence through targeted genetic disruption of the mycobactin biosynthetic pathway, validating inhibition of mycobactin biosynthesis for the development of novel antitubercular agents. Mycobactins are synthesized by mixed nonribosomal peptide synthetiase/polyketide synthase (NRPS/PKS) machinery in *Mtb*. The NRPS assembly line is primed by an aryl acid adenylating enzyme (AAAE) from *Mtb* known as MbtA. MbtA is an attractive therapeutic target due to lack of a human homologue, availability of structural information from homologous AAAEs, and extensive knowledge of the enzymatic mechanism of a functionally- and structurally-similar AAAE. The MbtA mechanism and a potent MbtA bisubstrate inhibitor, 5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine (Sal-AMS), are shown in the figure below. While demonstrating some adequate pharmacokinetic parameters, Sal-AMS is ultimately plagued by poor oral bioavailability. Previous studies in our lab indicate that an internal hydrogen bond is formed between the phenol and charged sulfamate nitrogen atom (estimated pK_a around 2) of Sal-AMS, enforcing a coplanar arrangement of the salicyl group when bound to the MbtA active site. We thus proposed the synthesis of conformationally constrained analogues **1**–**3** to mimic the bound conformation of Sal-AMS and potentially improve the oral bioavailability of the parent Sal-AMS compound by removing two rotatable bonds and the charged sulfamate moiety. Oral bioavailability studies are dependent on the proposed analogues' relative biochemical and antitubercular potencies versus those of Sal-AMS. Herein is reported the synthesis, biochemical and antitubercular evaluation of conformationally constrained analogues of Sal-AMS **1**–**3**, as well as its 2,3-dihydroxybenzoyl variant **4**.



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List of Abbreviations

AAAE Aryl Acid Adenylating Enzyme

AcOH Acetic Acid

AE Adenylating Enzyme

Anti-TB Antitubercular

APCI Atmospheric Pressure Chemical Ionization

Aq Aqueous

Ar Argon

ArCP Aryl Carrier Protein

ATP Adenosine Triphosphate

Benzoxazinone-AMS 5′-Amino-5′-*N*-[(4-oxo-4*H*-benzo[*e*][1,2]oxazin-3-yl)sulfonyl]-adenosine

Bicyclic-AMS Bicyclic Adenosine Monosulfonamide

Bn Benzyl

Boc *tert*-Butylcarbamate

***n*-BuLi** Butyllithium

***t*-Bu** *tert*-Butyl

***t*-BuOH** *tert*-Butyl Alcohol

Calcd Calculated

Cat. Catalytic

Chromone-AMS 5′-Amino-5′-*N*-[(chromon-3-yl)sulfonyl]adenosine

Cbz Benzyl Carbamate

Cs_2CO_3 Cesium Carbonate

d Day(s)

DCM Dichloromethane

DDQ 2,3-Dichloro-5,6-dicyano-1,2-benzoquinone

DHB 2,3-Dihydroxybenzoyl

DHBA 2,3-Dihydroxybenzoic Acid

DHB-AMP 1,2-Dihydroxybenzoyl Adenosine Monophosphate

DHB-AMS 1,2-Dihydroxybenzoyl Adenosine Monosulfamate

DIAD Diisopropyl Azodicarboxylate

DIPEA Diisopropylethylamine

DMAP 4-(Dimethylamino)pyridine

1,2-DME 1,2-Dimethoxyethane

DMF *N*,*N*-Dimethylformamide

DMF-DMA *N*,*N*-Dimethylformamide Dimethyl Acetal

Equiv Equivalent(s)

ESI Electrospray Ionization

Et_3N Triethylamine

EtOAc Ethyl Acetate

Fl-Sal-AMS 2′-*O*-{2-[2-(2-{[(Fluorescein-5-yl)carbonyl]amino}ethoxy)ethoxy]ethoxy}-Sal-AMS

FP Fluorescence Polarization

h Hour(s)

HCl Hydrogen Chloride/Hydrochloric Acid

HPLC High-Performance Liquid Chromatography

HRMS High Resolution Mass Spectrometry

K_D Equilibrium Dissociation Constant

K_i^{app} Apparent Equilibrium Dissociation Constant of Enzyme–Inhibitor Complex

LDA Lithium Diisopropylamide

LRMS Low-Resolution Mass Spectrometry

MDR-TB Multidrug Resistant Tuberculosis

MeCN Acetonitrile

MeOH Methanol

Me-Sal Methyl Salicylate

MIC Minimum Inhibitory Concentration

MIC_{99} Minimum Inhibitory Concentration Inhibiting >99% of Cell Growth

min Minute(s)

MOM Methoxymethyl

MS Mass Spectrometry

Mtb *Mycobacterium tuberculosis*

m/z Mass-to-Charge Ratio (in MS Analysis)

N_2 Nitrogen

NaH Sodium Hydride

$NaNO_2$ Sodium Nitrite

NH_4Cl Ammonium Chloride

NMR Nuclear Magnetic Resonance Spectroscopy

NRPS Nonribosomal Peptide Synthetase

PDB Protein Data Bank

Pd/C Palladium on Carbon

PK Pharmacokinetic

PKS Polyketide Synthase

PMB *para*-Methoxybenzyl

PPh_3 Triphenylphosphine

PP_i Pyrophosphate

ppm Parts Per Million (Unit of NMR Chemical Shift or Experimental Mass Error in MS)

$[^{32}P]PP_i$ ^{32}P-labeled Pyrophosphate

psi Pounds per Square Inch (Unit of Pressure)

Quant. Quantitative

Quinolone-AMS 5′-Amino-5′-*N*-[((quinol-4-on)-3-yl)sulfonyl]adenosine

RP-HPLC Reversed-Phase HPLC

SAL Salicylic Acid

Sal-AMS Salicyl Adenosine Monosulfamate (5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine)

SAR Structure–Activity Relationship

Satd Saturated

$SOBr_2$ Thionyl Bromide

$SOCl_2$ Thionyl Chloride

TB Tuberculosis

TBAF Tetrabutylammonium Fluoride

TBS *tert*-Butyldimethylsilyl

Temp. Temperature

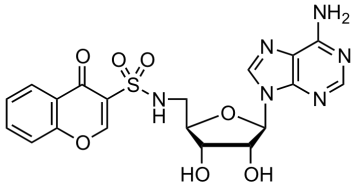
TFA Trifluoroacetic Acid

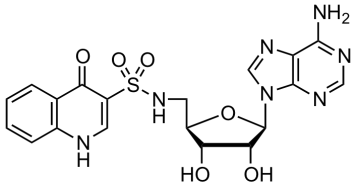
THF Tetrahydrofuran

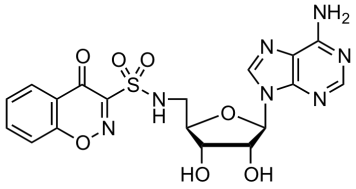
TLC Thin-layer Chromatography

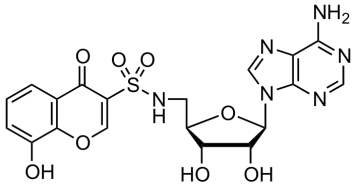
TsOH *para*-Toluenesulfonic Acid

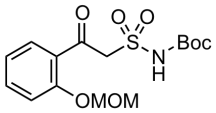
List of Numbered Compounds

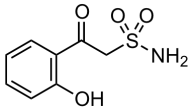
1 

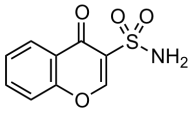
2 

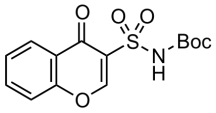
3 

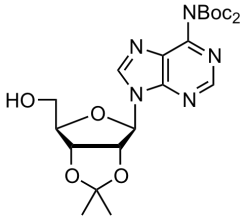
4 

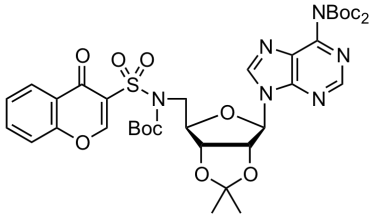
5 

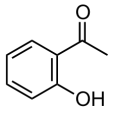
6 

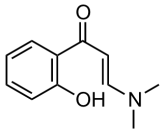
7 

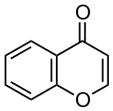
8 

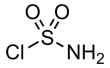
9 

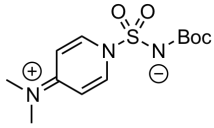
10 

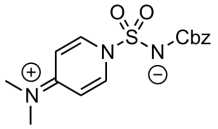
11 

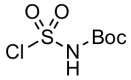
12 

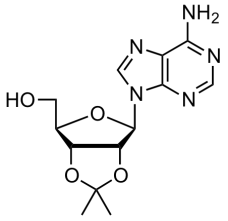
13 

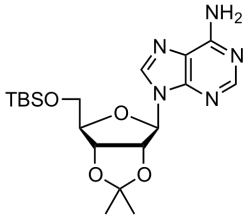
14 

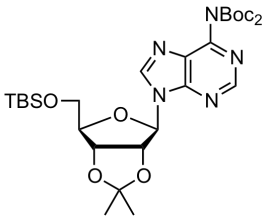
15 

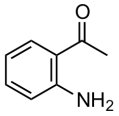
16 

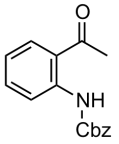
17 

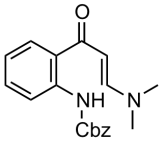
18 

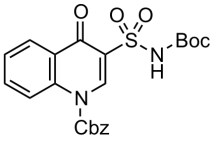
19 

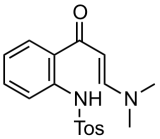
20 

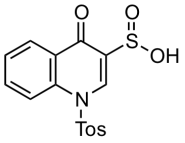
21 

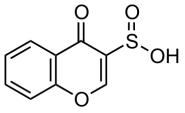
22 

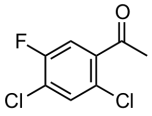
23 

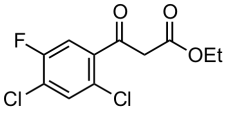
24 

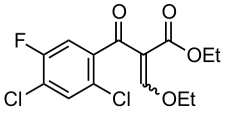
25 

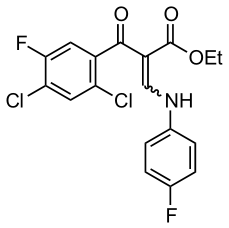
26 

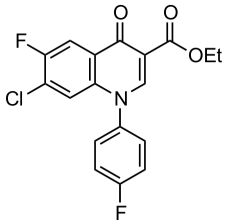
27 

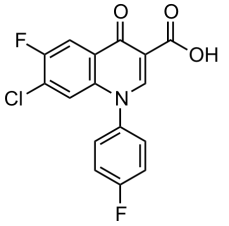
28 

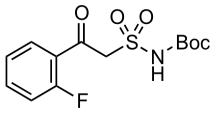
29 

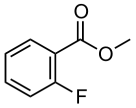
30 

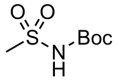
31 

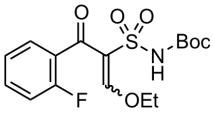
32 

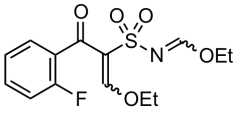
33 

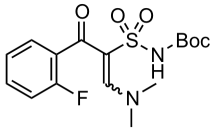
34 

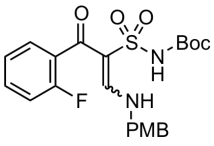
35 

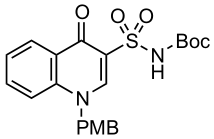
36 

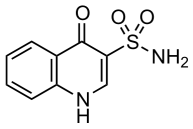
37 

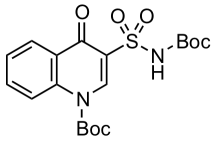
38 

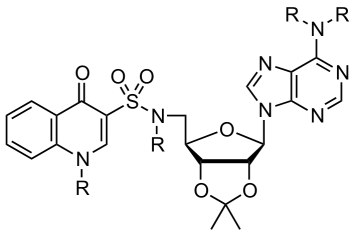
39 

40 

41 

42 

43 

44 

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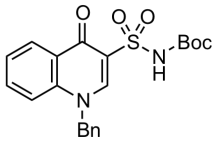
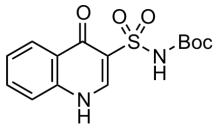
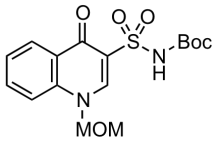
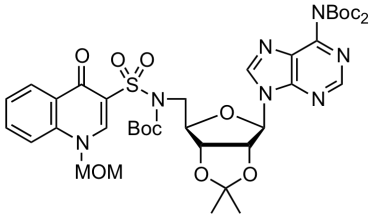
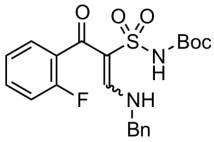
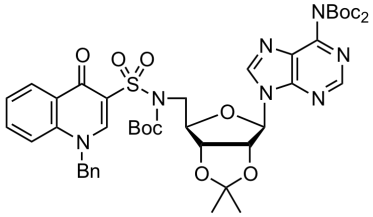
45 

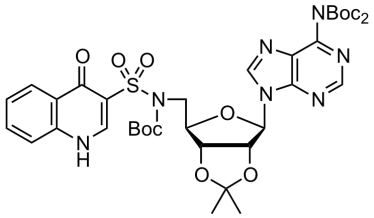
46 

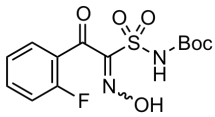
47 

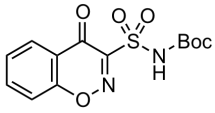
48 

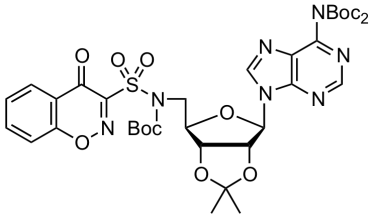
49 

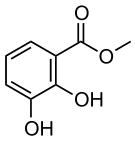
50 

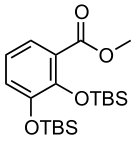
51 

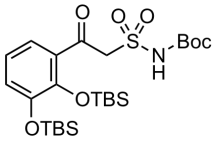
52 

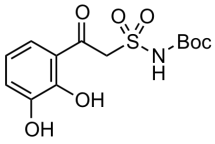
53 

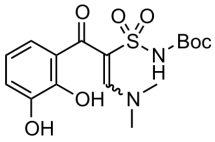
54 

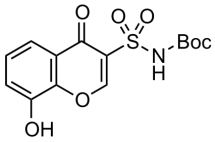
55 

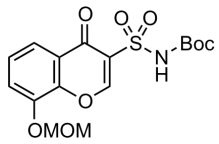
56 

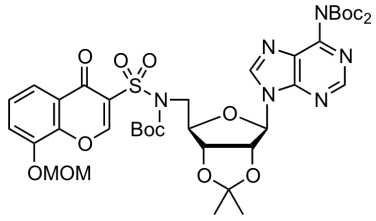
57 

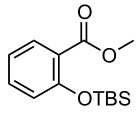
58 

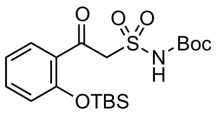
59 

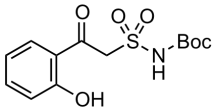
60 

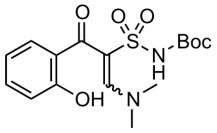
61 

62 

63 

64 

65 

66 

Introduction

Tuberculosis (TB) is caused primarily by the bacillus *Mycobacterium tuberculosis* (*Mtb*) and is the leading cause of bacterial infectious disease mortality, responsible for about 1.4 million worldwide deaths in 2011.[1,2] It is estimated that one-third of the world's population has latent TB and 8.7 million new infections occurred in 2011.[1] Current treatment of TB, known as Directly Observed Treatment Short-course, requires six to nine months of combination chemotherapy.[3] The emergence of multidrug resistant and extensively drug resistant TB strains and a lack of approval of any new antitubercular (anti-TB) agent in over four decades underscore the urgency of drug development for this worldwide clinical need.[3]

Iron is an essential micronutrient for almost all known organisms. However iron is extremely insoluble in biological fluids and the concentration of free iron is further repressed in a mammalian host by lactoferrin and transferrin to an astonishing 10^{-24} M, which is far too low to support bacterial colonization and growth.[4] Many pathogenic bacteria obtain iron via the synthesis, secretion, and reuptake of small-molecule iron chelators known as siderophores (Figure 1).[4] Siderophores in *Mtb*, termed mycobactins, have been linked to virulence through targeted genetic disruption of the mycobactin biosynthetic pathway, validating inhibition of mycobactin biosynthesis for the development of novel anti-TB agents.[5]

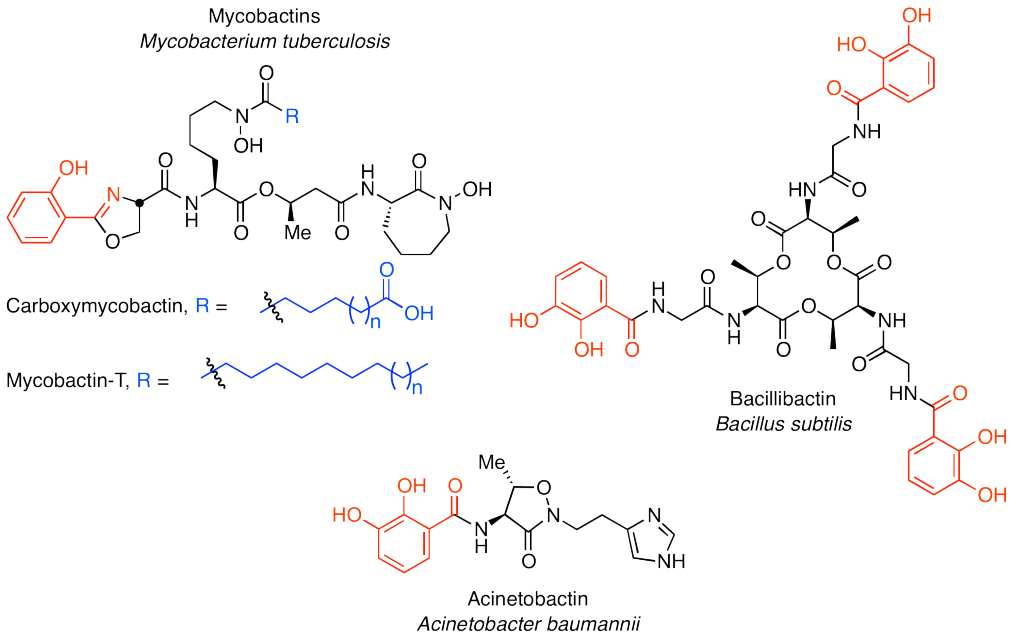


Figure 1. Representative aryl-capped siderophores, with iron-chelating regions highlighted in red.

Mycobactins are biosynthesized by a mixed nonribosomal peptide synthetase/polyketide synthase (NRPS/PKS) pathway in *Mtb* (Figure 2).[6] The NRPS assembly line is primed by an aryl acid adenylating enzyme (AAAE) from *Mtb* known as MbtA.[6] MbtA catalyzes a two-step adenylation/acylation reaction. In the adenylation half-reaction, salicylate and ATP are bound and condensed, releasing pyrophosphate but keeping the acyladenylate intermediate tightly bound. MbtA then binds the downstream aryl carrier protein (ArCP) domain of MbtB and transfers the aryl acid moiety onto the terminal thiol of the phosphopantetheinyl cofactor arm of the ArCP domain, thereby priming the NRPS assembly line for mycobactin biosynthesis. MbtA is an attractive therapeutic target[7] due to lack of a human homologue, availability of high-resolution co-crystal structures of related AAAEs with bound acyladenylate intermediate[8] and bisubstrate inhibitor,[9] and knowledge of the enzymatic mechanism of structurally- and functionally-similar EntE that has been extensively studied (EntE bears 40% amino acid

identity to MbtA and adenylates 2,3-dihydroxybenzoic acid [DHBA] versus salicylic acid [SAL]).[10] MbtA is also a member of the highly studied superfamily of adenylate-forming enzymes[11] for which inhibitors have already been developed and are in clinical use.[12] The MbtA mechanism and a prototypical AAAE bisubstrate inhibitor, 5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine (Sal-AMS), are shown in Figure 3 below.[13,14] Sal-AMS is a rationally designed nucleoside inhibitor of MbtA whereby the hydrolytically labile acylphosphate moiety of the tightly-bound intermediate is replaced by a stabile acylsulfamate linker, inspired by the natural product ascamycin, an alanyladenylate mimic isolated from an unknown *Streptomyces* species in Japan.[15]

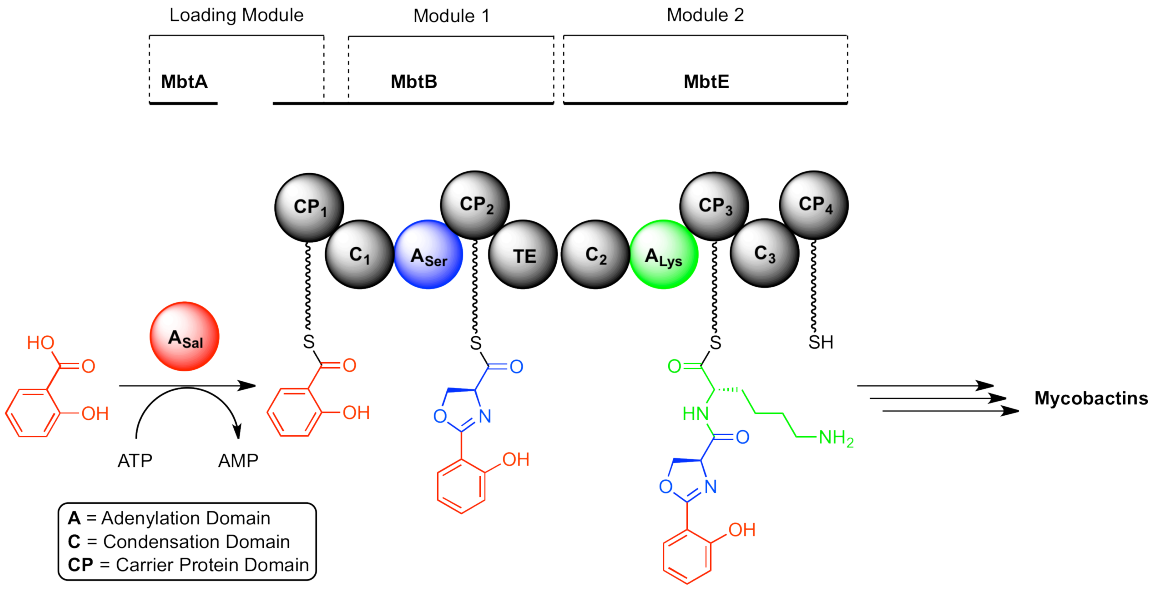


Figure 2. Mycobactin biosynthetic initiation and elongation.

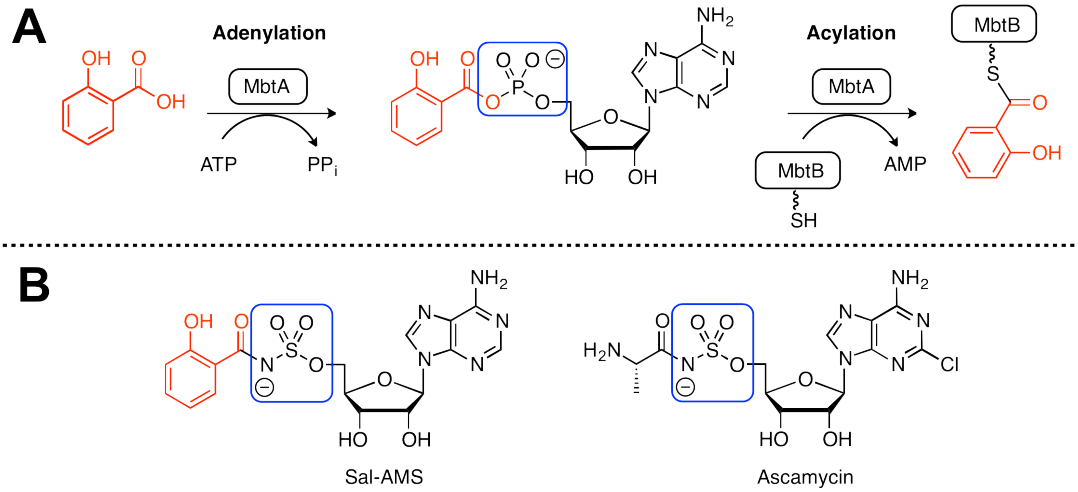


Figure 3. A. Enzymatic reactions catalyzed by MbtA, with hydrolytically labile acylphosphate outlined in blue. **B.** Sal-AMS and natural product ascamycin, from which Sal-AMS draws its inspiration for the hydrolytically stabile acylsulfamate bioisostere, outlined in blue.

Sal-AMS is a tight-binding inhibitor of MbtA with an equilibrium dissociation constant (K_D) value on the order of 1 pM and an apparent equilibrium dissociation constant of the enzyme–inhibitor complex (K_i^{app}) of 6.6 nM.[16] Furthermore, Sal-AMS displays potent whole cell activity against *Mtb* clinical isolate H37Rv under relevant iron-limiting conditions, with a minimum inhibitory concentration that inhibits >99% cell growth (MIC_{99}) of 0.39 μM, rivaling the first-line clinical agent isoniazid.[14,16] To date, our laboratory has conducted extensive structure–activity relationship (SAR) studies on Sal-AMS, systematically exploring its aryl,[16] linker,[14,17] glycosyl,[18] and nucleobase domains.[19,20] These SAR findings, in conjunction with a quantum mechanical study in which Sal-AMS was docked in the binding site of an MbtA homology model,[21] indicate that an internal hydrogen bond is formed between the phenol and sulfamate nitrogen atom (estimated pK_a around 2) of Sal-AMS. This enforces a coplanar arrangement of the salicyl group when bound to the MbtA active site. Further evidence in support of this

notion is observed in the co-crystal structures of related AAAEs from *Bacillus subtilis* (DhbE) and *Acinetobacter baumannii* (BasE) (Figure 4).

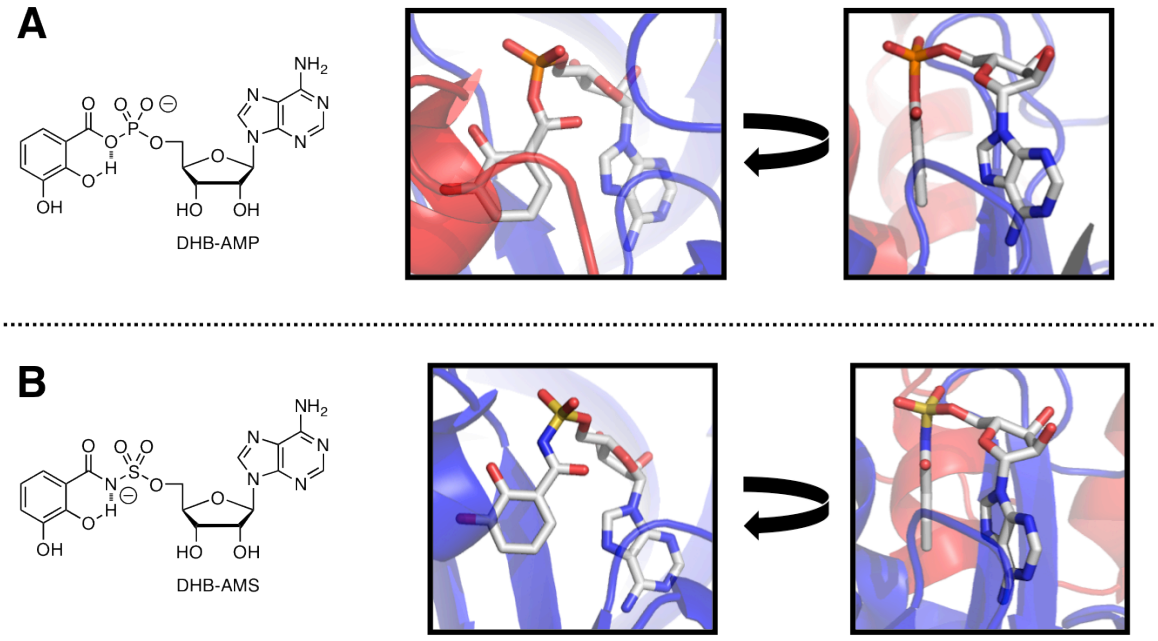


Figure 4. A (Left; center; right). Molecular structure of DHB-AMP; co-crystal structure of enzymatic intermediate DHB-AMP bound to AAAE DhbE from *B. subtilis* (PDB ID: 1MDB) with aryl ring in plane of paper; same co-crystal structure, rotated 90°, with aryl ring perpendicular to paper. **B (Left; center; right).** Molecular structure of DHB-AMS; co-crystal structure of bisubstrate mimic DHB-AMS bound to AAAE BasE from *A. baumannii*. (PDB ID: 3O82), with aryl ring in plane of paper; same co-crystal structure, rotated 90°, with aryl ring perpendicular to paper.

Preliminary pharmacokinetic (PK) parameters of Sal-AMS were obtained using deuterium-labeled Sal-AMS as internal standard in a tandem liquid chromatography–mass spectrometry method to measure plasma concentrations.[22] Initial studies with 300 mg/kg administration by oral gavage afforded C_{max}, t_{max}, $t_{1/2}$, and CL^{app} values of 28.2 ± 1.9 μM, 30 min, 1.97 ± 0.62 h, and 16.2 mL/min•kg respectively. The findings demonstrate that Sal-AMS possesses some adequate PK parameters (for example, the C_{max} is about 72-fold higher than the MIC_{99} value) but is ultimately plagued by poor oral bioavailability.[23] We have proposed a number of structural modifications to Sal-AMS to improve its oral bioavailability; one set of modifications is the subject of this master's

thesis. Based on the aforementioned SAR, quantum mechanical, and co-crystal studies, we proposed the synthesis of conformationally constrained analogues **1**–**3**, shown below in Figure 5. The proposed analogues mimic the hypothesized MbtA-bound conformation of Sal-AMS, removing two rotatable bonds and the charged sulfamate moiety. We expect these changes to improve the oral bioavailability of the parent Sal-AMS compound, studies depending on the proposed analogues' relative biochemical and antitubercular potencies versus those of Sal-AMS. Herein is reported the synthesis, biochemical and antitubercular evaluation of conformationally constrained analogues of Sal-AMS **1**–**3**, as well as its DHB variant **4**.

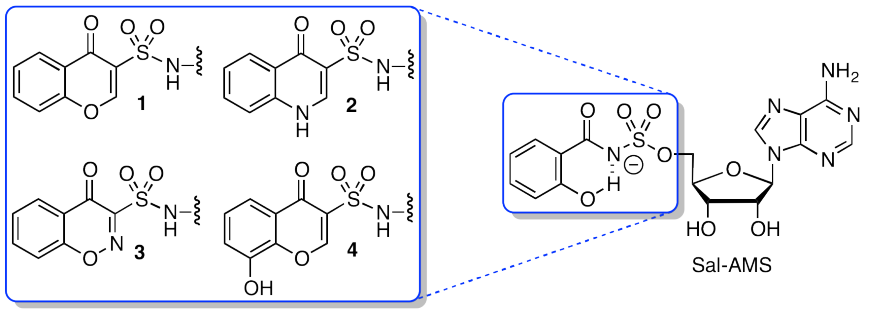


Figure 5. Conformationally constrained analogues of Sal-AMS **1**–**3**, and conformationally constrained analogue of DHB-AMS **4**.

Results and Discussion

Synthesis.

The most concise synthesis we envisioned toward the proposed bicyclic-AMS analogues involves disconnection of **1**–**4** by Mitsunobu reaction to bicyclic sulfonamides, inspired by our previous work on *β*-ketosulfonamide-based Sal-AMS analogues (Figure 6).[17] Further retrosynthesis leads to acetophenone or benzoic acid derivatives.

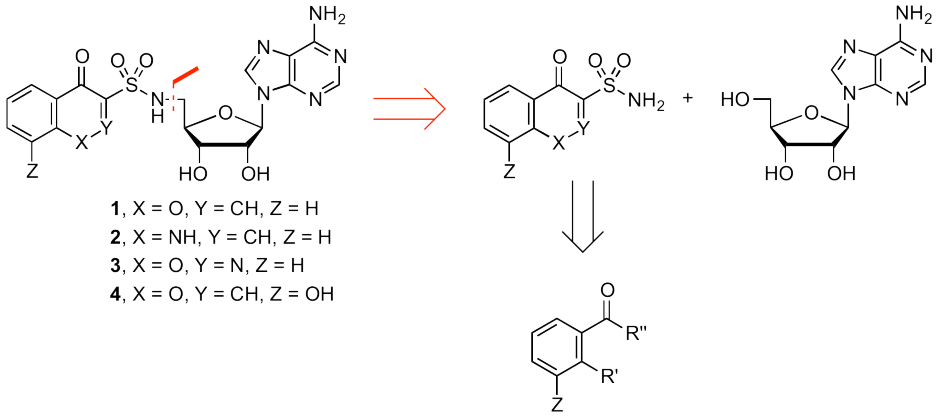


Figure 6. Retrosynthetic analysis.

Our original route to chromone-AMS **1** proposed five steps from *β*-ketosulfonamide **5** (Figure 7), prepared according to our published method.[17] Trifluoroacetic acid (TFA) deprotection followed by one-carbon homologation with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) and cyclization under acidic conditions (for example, *para*-toluenesulfonic acid [TsOH]) was anticipated to afford chromone-3-sulfonamide **7**.[24,25] Necessary[26,27] *tert*-butylcarbamate (Boc) protection of the sulfonamide[28] to give **8** followed by Mitsunobu coupling[17] with appropriately protected adenosine **9**[29] would afford penultimate protected chromone-AMS **10**. Global deprotection under 80% aqueous (aq) TFA conditions would provide chromone-AMS **1**.

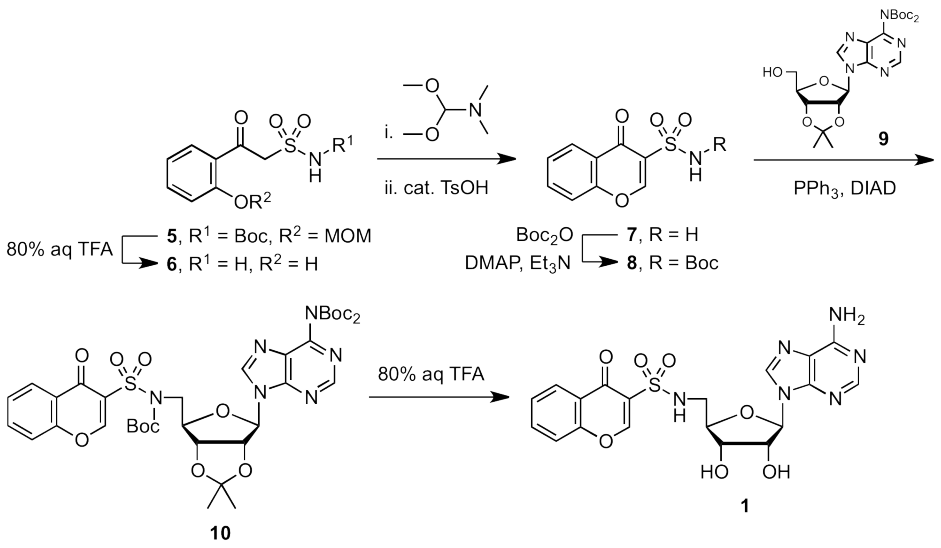


Figure 7. Initially proposed route to chromone-AMS **1**.

A review of the literature before initiating synthetic efforts suggested a potentially shorter route to **1**. It was found that isolable intermediate enaminone **12** from the 1971 synthesis of chromone **13** (Figure 8A)[24] was successfully used in a tandem sulfamoylation–cyclization with chlorosulfonylureas to produce chromone-3-sulfonylureas in 1996 (Figure 8B).[30] Based on our lab's experience with the sulfamoylating reagent sulfamoyl chloride **14**,[14,16,18,19,31] it seemed entirely feasible to arrive at intermediate chromone-3-sulfonamide **7** (Figure 8C) in short order from known enaminone **12**,[24] itself derived from commercial starting materials in one step (versus three steps to *β*-ketosulfonamide **6**).

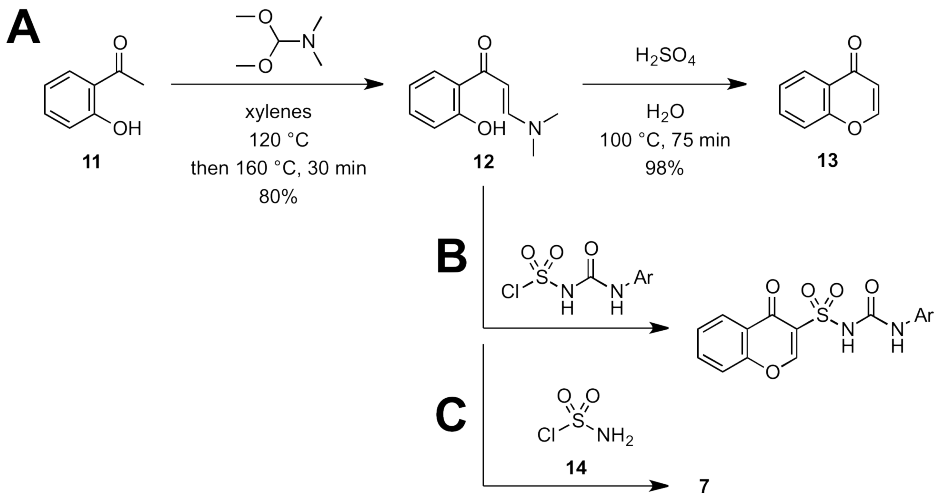


Figure 8. A. 1971 route to chromone **13**. **B.** 1996 route to chromone-3-sulfonylureas. **C.** Proposed shortened route to chromone-3-sulfonamide **7**.

Initial efforts to synthesize chromone-3-sulfonamide **7** through tandem sulfamoylation–cyclization with enaminone **12** and sulfamoyl chloride **14** proved nontrivial. After several small-scale attempts and purifying **14** through recrystallization,[32] the highest isolated yield of desired **7** was a mere 5%. Thin-layer chromatography (TLC) monitoring of the reaction displayed a high degree of streaking, even upon dilution of the reaction mixture before spotting. This was indicative of the formation of multiple undesired side products. The other isolable and characterizable reaction products were chromone **13** (32%) and starting enaminone **12** (15% recovered). A majority of the remaining mass was a highly insoluble material that could not be characterized. Its identity was hypothesized to be that of polymerized desired product: upon concentration of a sample of crystalline **7** prepared for proton nuclear magnetic resonance spectroscopy (NMR), an insoluble solid similar in appearance to that isolated from the reaction developed.

Attention was turned toward the reactivity and thus instability of reagent **14** itself by these preliminary results. Another literature search was conducted to find similar, but less reactive sulfamoylating reagents. Two were found, both 4-(dimethylamino)pyridine (DMAP) stabilized but each differentially protected on their sulfonamide moieties (Figure 9). Each could be synthesized in one pot like sulfamoyl chloride, but they offered a couple of advantages over the analogous use of that reagent: 1) the sulfonamide functionality was already protected, and 2) each was stable for prolonged periods at room temperature under normal atmosphere. In short order, sulfamoylating reagents **15**[33] and **16**[34] were prepared in ample amounts from their respective literature reports.

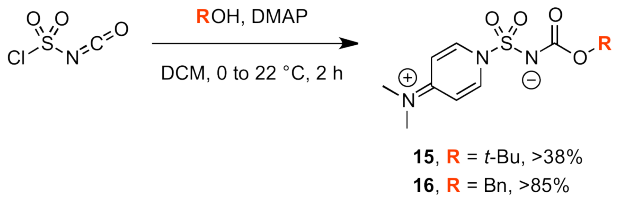
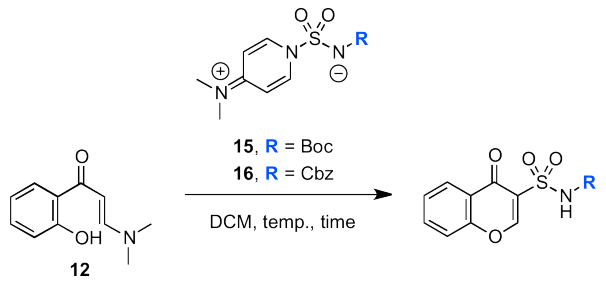


Figure 9. Synthesis of sulfamoylating reagents **15** and **16**.

With the new sulfamoylating reagents in hand, conditions were screened to see whether they could participate in the desired tandem sulfamoylation–cyclization reaction. Unfortunately, even with heating, the reagents proved unable to effect the transformation (Table 1). Instead, enaminone **12** cyclized to chromone **13** under acid-free conditions at high temperature as monitored by TLC and low-resolution mass spectrometry (LRMS) and compared to authentic commercially obtained **13**. The outcome suggested the reagents were far too bulky to give the desired outcome. Indeed, their described use is for the sulfamoylation of primary amines and hydroxyls.



Temp. (°C)	Time (h)	Reaction?
22	8	No
40	16	No
80[a]	24	Yes

[a]DCM exchanged for 1,4-dioxane

Table 1. Synthetic efforts to arrive at protected chromone-3-sulfonamides via sulfamoylating agents **15** and **16**.

A look back at the work of Löwe and Matzanke[30] suggested a hybrid approach of the first two failed routes to obtain the desired protected chromone-3-sulfonamide. They had utilized chlorosulfonylureas generated in situ for their tandem sulfamoylation–cyclization. The chlorosulfonylureas themselves were derived from reaction of an appropriate amine with chlorosulfonyl isocyanate, strikingly reminiscent of the route to agents **15** and **16**. Would it be possible to generate and use a carbamate-protected sulfamoyl chloride reagent in the same manner?

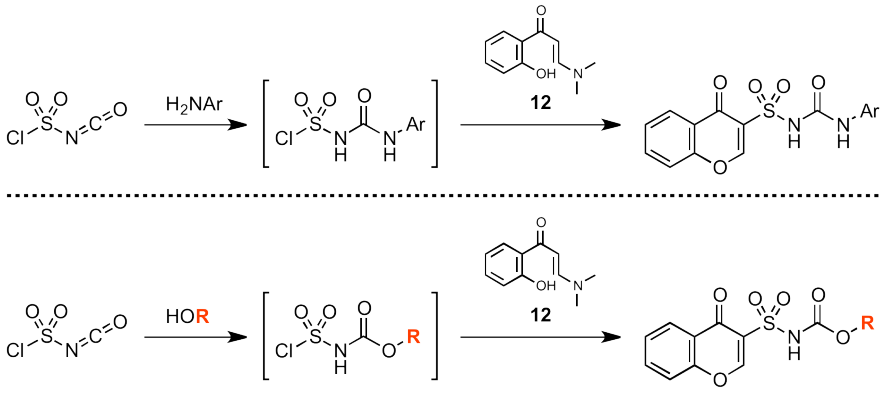


Figure 10. Löwe and Matzanke's tandem reaction (upper) and the potential route it inspired (lower).

tert-Butyl alcohol (*t*-BuOH) was chosen as it would yield a Boc-protected sulfonamide, presumably easily removed from the penultimate compound under the global deprotection conditions. Boc-protected sulfamoyl chloride **17** was generated in situ by addition of chlorosulfonyl isocyanate to a stirring solution of *t*-BuOH in dichloromethane (DCM). To this was added a stoichiometric equivalent of enaminone **12**. Initial attempts proved promising as the desired mass-to-charge ratio (m/z) was observed in LRMS analysis, although purification appeared daunting as the reaction was initially as messy as that with unprotected sulfamoyl chloride when monitored by TLC. An unusual solvent system of hexanes and DCM proved invaluable to purify and isolate the desired Boc-protected chromone-3-sulfonamide **8** in an astonishingly low yield of 3%. Having that 3% however was more than enough to identify and isolate product from improved reaction conditions.

As was the case with the use of sulfamoyl chloride, conditions improved with the use of recrystallized reagent. Although it added time to the synthetic scheme, and removed the in situ ease of reagent generation, recrystallizing **17** paid off with a seven-fold

increase in yield of **8**. An optimized but still quite modest 21% yield was primarily the result of competitive cyclization of the enaminone **12** to chromone **13** under the acidic conditions afforded by release of hydrogen chloride (HCl) during reaction. It was hypothesized that changing the order and time length of addition of reagents could improve this yield even further, however that approach was not pursued. The facile nature of Mitsunobu coupling between nucleophile **8** and appropriately protected adenosine **9** rendered the low yield of **8** virtually inconsequential. Resultant penultimate, fully protected **10** underwent global deprotection facilitated by aqueous TFA to yield chromone-AMS **1** in just three linear steps from known enaminone **12**.

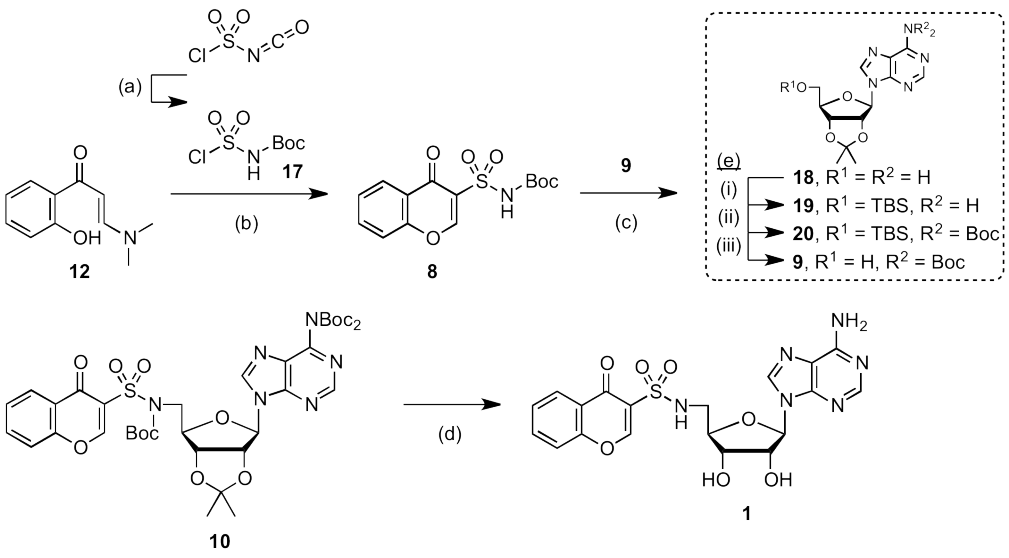


Figure 11. Final optimized scheme for the synthesis of **1** via key tandem sulfamoylation–cyclization.[a]
[a]Reaction Conditions: (a) *t*-BuOH, DCM, 0 to 22 °C, 83%; (b) DCM, 22 °C, 13 h, 21%; (c) PPh_3, DIAD, THF, 0 to 22 °C, 6 h, 80%; (d) 80% aq TFA, 0 to 22 °C, 4 h, 76%; (e) (i) TBSCl, imidazole, DMF, 0 °C, 2 h then 0 to 22 °C, 15 min, (ii) Boc_2O, DMAP, Et_3N, DMF, 0 to 22 °C, 87% over two steps, (iii) TBAF, THF, 22 °C, 1.5 h, 84%.

Attention was then focused on the synthesis of quinolone-AMS **2**. Initially, a route analogous to that of **1** was envisioned, whereby 2-aminoacetophenone **21** would be reacted with DMF-DMA to give the desired enaminone. However, a thorough inspection

of the structure as well as a literature search halted execution of those plans but did suggest a possible route.[35] A protecting group strategy could potentially be used to attenuate the nucleophilicity of the aniline nitrogen, thereby allowing the desired transformation to be affected. Knowing that refluxing conditions were likely necessary to provide the enaminone, heat-stabile Cbz was chosen. The aniline nitrogen was Cbz-protected under mildly basic conditions to afford carbamate **22** in quantitative yield (Figure 12A).[36] Reaction with DMF diethyl acetal under refluxing conditions provided enaminone **23** in 45% yield.[35] Attempts to affect tandem sulfamoylation–cyclization with Boc-sulfamoyl chloride **17** proved fruitless, unfortunately. Unlike with analogous hydroxy-enaminone **12**, no trace of desired product **24** was ever detected. The reaction would not proceed as monitored by TLC, likely due to reduced aniline nucleophilicity following Cbz protection. Nearly quantitative recovery of the starting enaminone **23** was the result of every attempt.

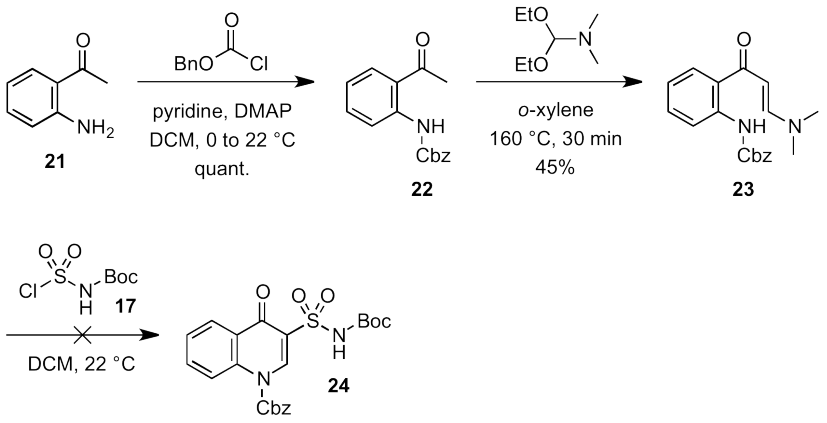


Figure 12. Attempted tandem sulfamoylation–cyclization via enaminone **23**.

Indeed, Löwe and Kietzmann were unable to affect reaction of tosylamino-enaminone **25** with thionyl bromide ($SOBr_2$) to sulfinic acid **26** (Figure 13A),[35] analogous to the

reported conversion of hydroxy-enaminone **12** to sulfinic acid **27** via thionyl chloride ($SOCl_2$) (Figure 13B).[37]

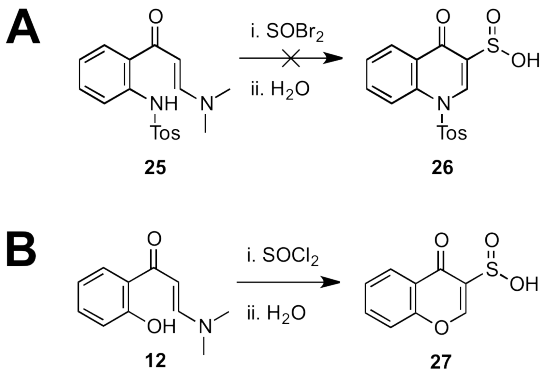


Figure 13. A. Reported unsuccessful sulfination of tosylamine-enaminone **25**.[35] **B.** Reported sulfination of hydroxy-enaminone **12**.[37]

Briefly, the route originally proposed to achieve chromone-3-sulfonamide **8** (Figure 7) was considered to afford an appropriately protected quinolone-3-sulfonamide (Figure 14). The largest problem with this idea was the arrival at the *β*-ketosulfonamide without an extensive and exhaustive protecting group strategy. It was thus necessary to consult the literature.

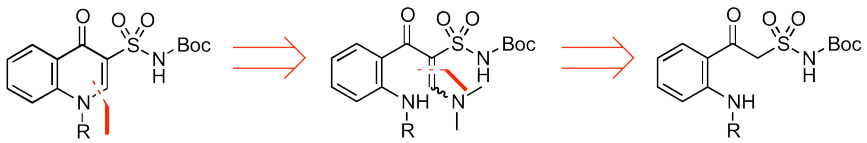


Figure 14. Retrosynthetic disconnections of quinolone-3-sulfonamide leading to a *β*-ketosulfonamide synthon.

Structural similarity searches pointed toward the quinolone antibacterial agents,[38] specifically toward quinolone-3-carboxylic acids synthesized from *β*-ketoesters.[39] A representative scheme is shown below in Figure 15. 2,4-Dichloro-5-fluoroacetophenone **28** was condensed with diethyl carbonate under basic conditions to afford *β*-ketoester **29**. Reaction with triethyl orthoformate in acetic anhydride afforded one-carbon

homologation product **30**. Evaporation to dryness and exposure to slight excess of 4-fluoroaniline resulted in addition–elimination product enaminone **31**. Cyclization to quinolone ester **32** was realized through nucleophilic aromatic substitution under basic conditions with heating in dimethoxyethane. Saponification of ester **32** followed by modifications to carboxylic acid **33** yielded a series of 7-substituted quinolones.

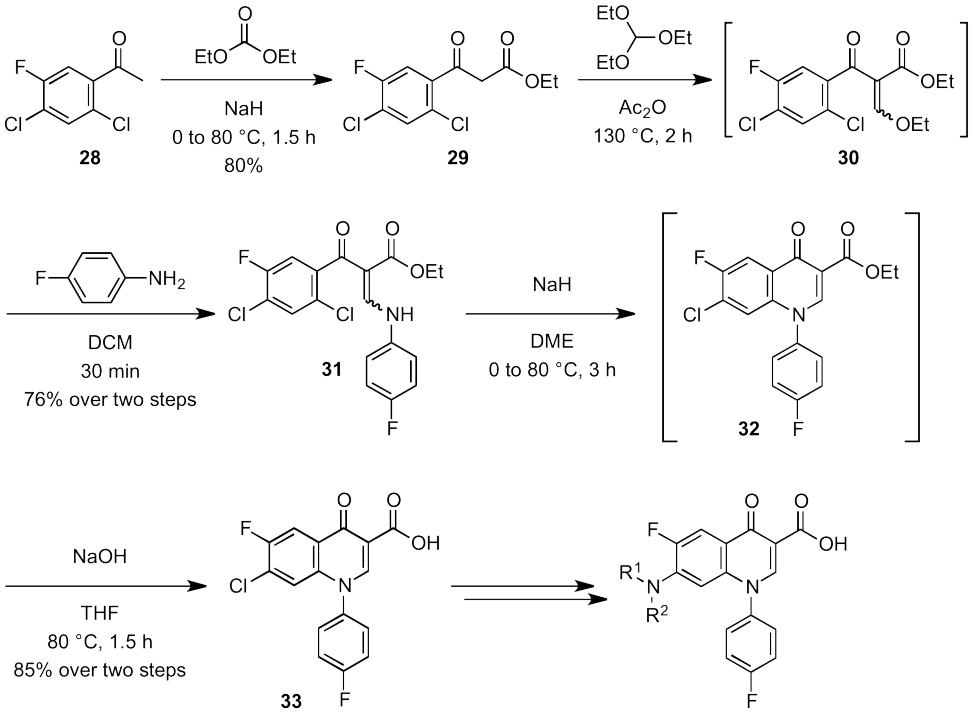


Figure 15. 1985 synthesis of arylfluoroquinolone-3-carboxylic acids from *β*-ketoesters.

Acid **33** bore a striking resemblance to the desired quinolone-3-sulfonamide. The necessary *β*-ketosulfonamide **34**, analogous to *β*-ketoester **29**, could be realized through a novel Claisen-like condensation developed by our lab.[17] A modified retrosynthetic scheme is shown below in Figure 16.

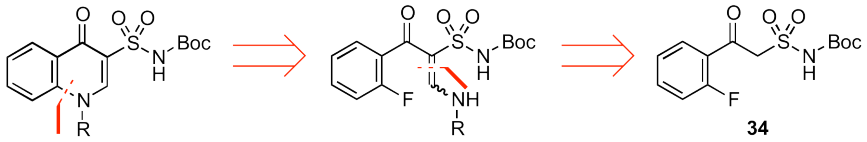


Figure 16. Revised retrosynthetic disconnections of quinolone-3-sulfonamide leading to an *ortho*-fluoro *β*-ketosulfonamide synthon.

Synthesis of the desired quinolone-3-sulfonamide commenced with esterification of 2-fluorobenzoic acid under classic Fischer conditions in methanol with catalytic sulfuric acid to afford methyl ester **35**. *β*-ketosulfonamide **34** was realized through the aforementioned Claisen-like condensation[17] of the lithium diisopropylamide (LDA)-generated dianion of *N*-Boc-methanesulfonamide **36**[28] with ester **35**.

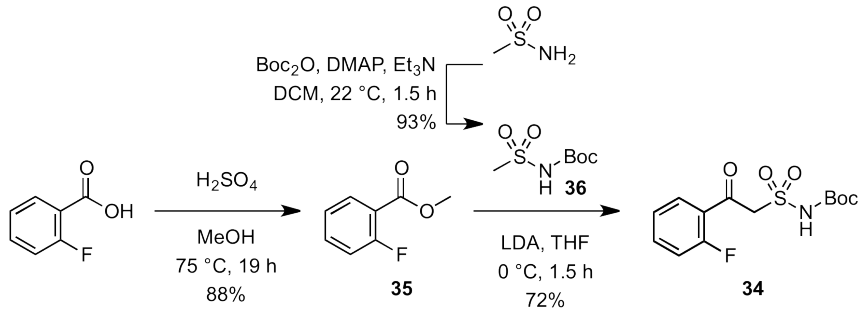
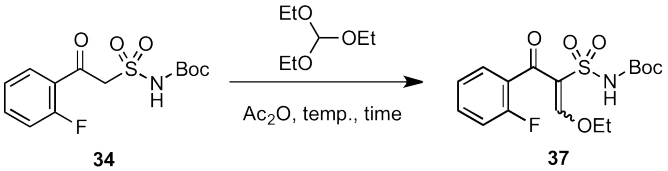


Figure 17. Synthesis of *β*-ketosulfonamide **34**.

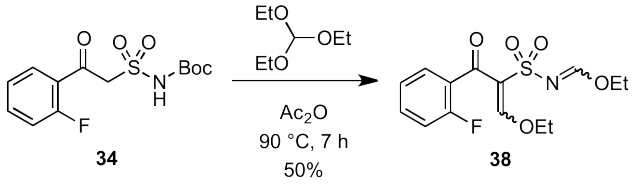
Reaction of *β*-ketosulfonamide **34** with triethyl orthoformate in acetic anhydride was attempted according to conditions outlined above,[39] albeit at lower temperatures given the thermal lability of Boc protecting groups. Steadily increased heating resulted in reaction progression as monitored by TLC (Table 2). However, purification and characterization by LRMS and ^{1}H NMR showed not only the desired homologation but also simultaneous Boc deprotection and Schiff base formation at the sulfonamide (Figure 18).



34 37

Temp. (°C)	Time (h)	Reaction?
22	0.5	No
77	1.5	No
90	7	Yes

Table 2. Synthetic efforts to arrive at enol ether **37** through formylation with triethyl orthoformate.



34 38

Figure 18. Outcome of reaction of *β*-ketosulfonamide **34** with triethyl orthoformate.

An alternate protecting group strategy was considered whereby thermally stabile Cbz could be used instead of Boc. However, a recollection of Reiter's mild conditions for homologation (introduced in Figure 7 above)[25] focused attention on that strategy. Reaction of *β*-ketosulfonamide **34** with DMF-DMA resulted in full conversion as monitored by TLC to enaminone **39**, as verified by LRMS and ^{1}H NMR of the crude material after evaporation to dryness (Figure 19).[25] Crude **39** was taken up in DCM and reacted with excess *para*-methoxybenzylamine ($PMBNH_2$) to give an inconsequential mixture of transamination product *E*- and *Z*-PMB-enaminone **40**,[39] an exciting find because it meant the desired protecting group strategy could be retained. Under basic conditions with sodium hydride (NaH), cyclization of **40** via nucleophilic aromatic

substitution provided PMB-quinolone sulfonamide **41**.[39] Unfortunately, deprotection of the PMB under standard oxidative cleavage conditions with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[40] could not be achieved. Harsh conditions in neat TFA[41] afforded quinolone sulfonamide **42**. Bis-Boc protection of **42**[42,43] followed by Mitsunobu coupling with adenosine **9** unexpectedly yielded a tris-Boc (rather than tetrakis-Boc) product **44**. This underwent global deprotection facilitated by aqueous TFA to arrive at quinolone-AMS **2**.

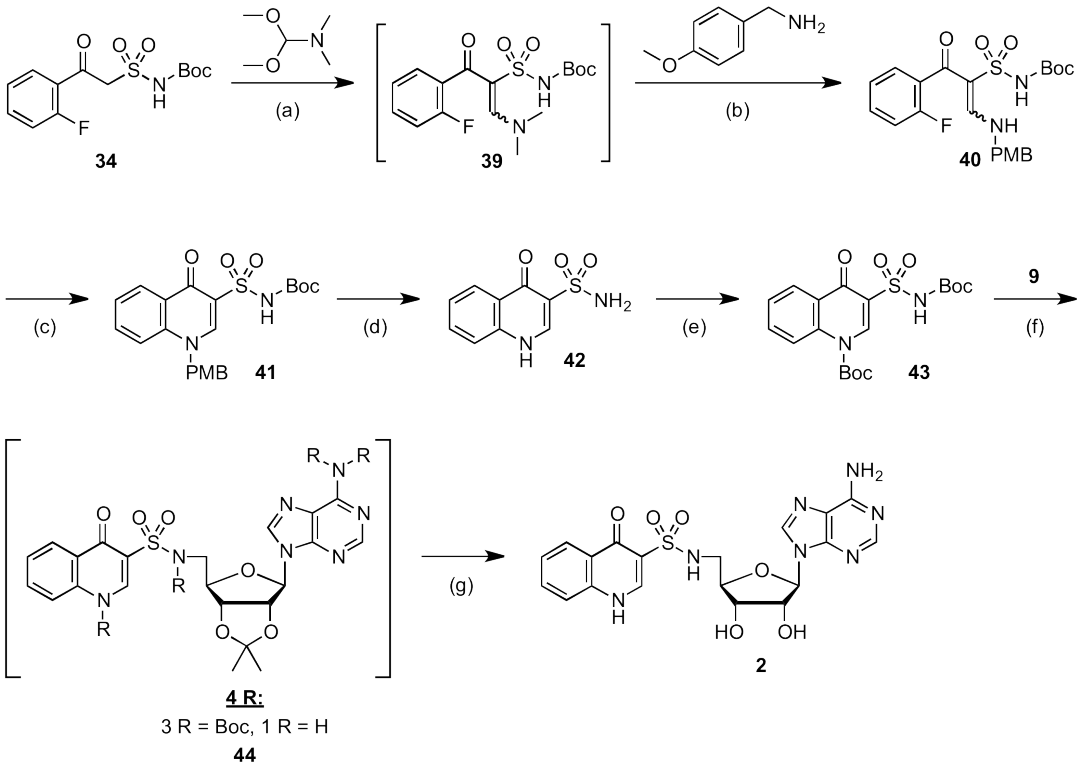


Figure 19. Scheme for the synthesis of **2** via key nucleophilic aromatic substitution.[a]
[a]Reaction Conditions: (a) THF, 22 °C, 17 h; (b) DCM, 22 °C, 5 min, 81% over two steps; (c) NaH, 1,2-DME, 0 to 22 °C, 19.5 h, 81%; (d) TFA, sealed tube, 72 °C, 17 h then 100 °C, 23 h, 58%; (e) Boc_2O, DMAP, Et_3N, DCM, 0 to 22 °C, 15 h, 18%; (f) PPh_3, DIAD, THF, 0 to 22 °C, 17.5 h; (g) 80% aq TFA, 0 °C, 24 h, 66% over two steps.

The bis-deprotection followed by bis-reprotection was less than desirable because it added steps to the sequence and inherently caused major hits to the overall yield of **2**. An

alternate protecting group strategy was investigated whereby the Boc-protected sulfonamide could be retained (Figure 20). Benzyl-quinolone sulfonamide **45** was realized in three steps from **34** without isolation of the intermediates. Reductive debenzylation with stoichiometric palladium on carbon[44,45] afforded mono-deprotected quinolone sulfonamide **46**. Efforts to protect quinolone **46** as its MOM amine[46] proved quite difficult; potential product **47** was not isolated and conclusively verified despite multiple synthetic attempts. The facile nature of quinolone debenzylation of **45** in conjunction with our lab's observed stability of adenosine derivatives to hydrogenation conditions inspired an alternate strategy. The benzyl protecting group of **45** was retained and successful Mitsunobu coupling with adenosine **9** afforded fully protected quinolone-AMS **50**. Debenzylation under standard conditions yielded protected **51**, itself deprotected under acidic conditions to afford quinolone-AMS **2**. This optimized route proved more efficient and higher yielding than the previous route outlined in Figure 19 as it removed intermediate deprotection–reprotection steps.

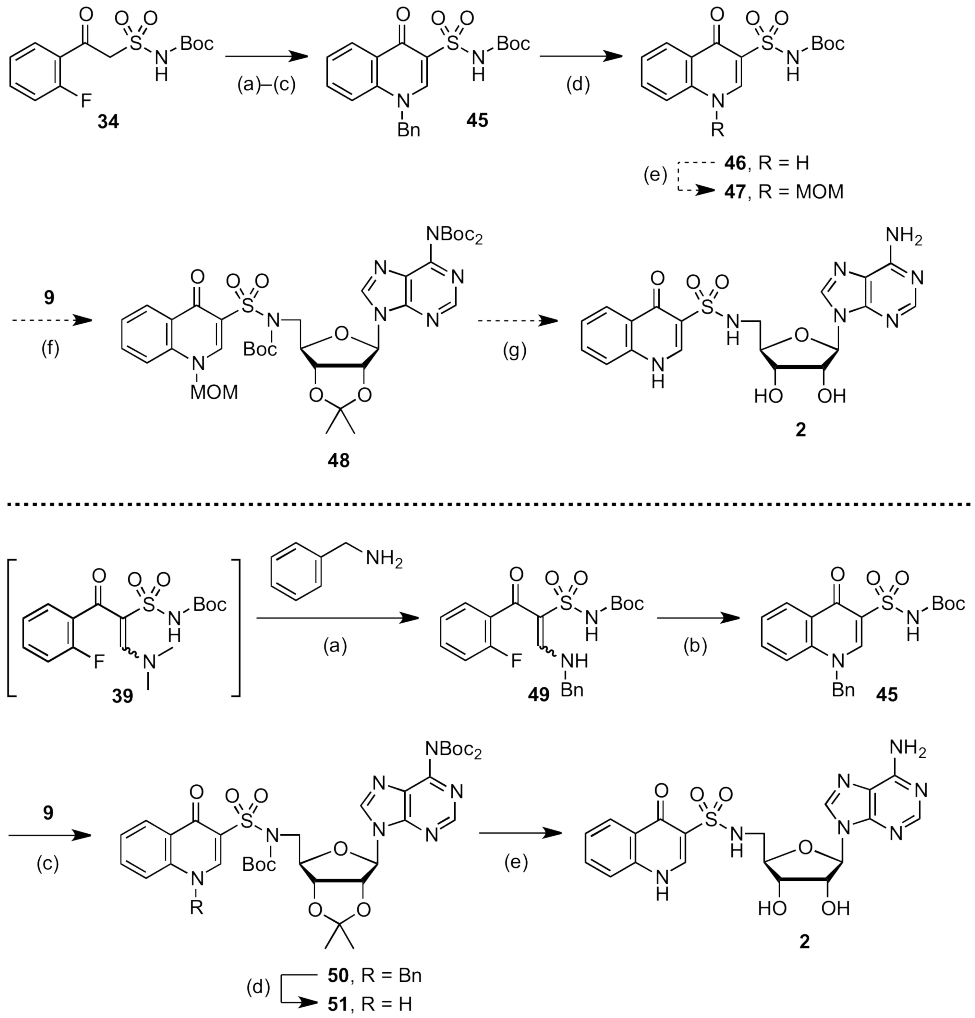


Figure 20. Upper. Modified scheme for the synthesis of **2** via key nucleophilic aromatic substitution.[a] **Lower.** Final optimized scheme for the synthesis of **2**.[b]
[a]Reaction Conditions: (a) THF, 22 °C, 2 h; (b) $BnNH_2$, THF, 22 °C, 10 min; (c) NaH, THF, 22 °C, 30 min, 38% over three steps; (d) H_2 (40 psi), Pd/C, AcOH, MeOH, 1 h, 32%; (e [hypothetical]) NaH, MOMCl, DMF, 0 to 22 °C; (f [hypothetical]) PPh_3, DIAD, THF, 22 °C; (g [hypothetical]) 80% aq TFA, 0 °C.
[b]Reaction Conditions: (a) THF, 22 °C, 10 min, 85% over two steps; (b) NaH, THF, 22 °C, 1 h, 51%; (c) PPh_3, DIAD, THF, 22 °C, 85%; (d) H_2 (40 psi), Pd/C, AcOH, MeOH, 22 °C, 4 h, 58%; (e) 80% aq TFA, 0 °C, 19 h, 70%.

Given the facile cyclization of enaminone **40** via nucleophilic aromatic substitution, it was envisioned that an oxime derivative of *β*-ketosulfonamide **34** could also easily cyclize to afford the Mitsunobu nucleophile for the synthesis of benzoxazinone-AMS **3** (Figure 21). Reaction of **34** with sodium nitrite under acidic conditions afforded oxime **52**,[47] which was cyclized under mildly basic conditions with cesium carbonate[48] to

provide benzoxazinone-sulfonamide **53**. Mitsunobu coupling with adenosine **9** provided penultimate benzoxazinone-AMS **54**, which was subsequently deprotected with aqueous TFA to afford **3**.

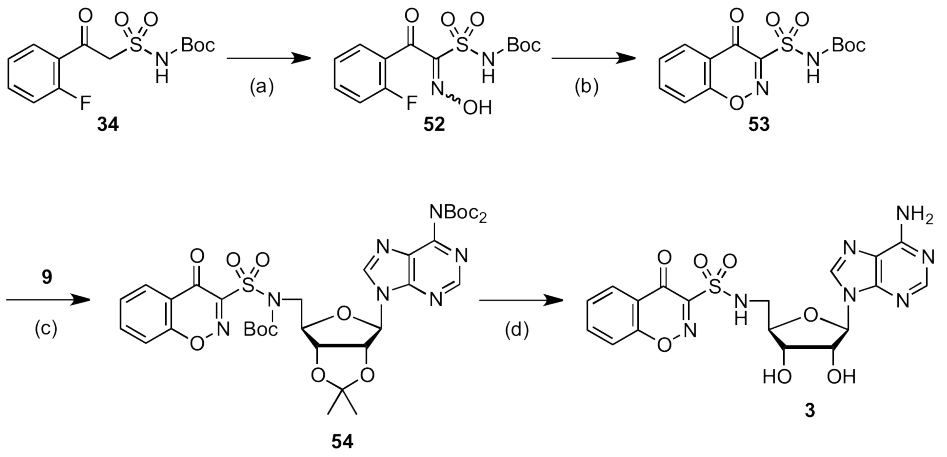


Figure 21. Synthesis of benzoxazinone-AMS **3**.[a]
[a]Reaction Conditions: (a) $NaNO_2$, AcOH/H_2O/THF (1:1:2), 0 °C, 19 h, 74%; (b) Cs_2CO_3, DMF, 22 °C, 4.5 h, 58%; (c) PPh_3, DIAD, THF, 22 °C, 28.5 h, 45%; (d) 80% aq TFA, 19 h, 81%.

Lastly, attention was turned toward the synthesis of conformationally constrained analogue of DHB-AMS, **4**. The ease with which *β*-ketosulfonamide **34** could undergo homologation with DMF-DMA and the competitive acid-catalyzed cyclization of **12** prompted a revisit to the initially proposed synthesis of **1** (Figure 7 above). This route, although more steps, could alternatively provide a higher yield of **1** as well as its 8″-hydroxy variant **4** (Figure 22). The necessary *β*-ketosulfonamide to test this hypothesis was synthesized from DHBA, which was esterified with $SOCl_2$ in refluxing methanol.[49] TBS was chosen as protecting group for the hydroxyl functionalities based on its ability to be removed under fairly mild conditions. Claisen-like condensation of the aforementioned *N*-Boc-methanesulfonamide **36** with ester **56**[17] resulted in the formation of two primary products: the bis-silyl ether shown and a mono-deprotected variant, both

isolable after workup and flash chromatography. To ease the purification process, and because the hydroxyl protection was no longer necessary, the crude material from reaction workup was directly subjected to TBAF deprotection, affording *β*-ketosulfonamide **58** in an acceptable 82% yield over the two discrete steps. Homologation with DMF-DMA to **59**[25] and cyclization via acidic wash of the reaction[24] provided 8-hydroxy chromone sulfonamide **60**. Mitsunobu coupling with the unprotected hydroxyl group proved nontrivial, so it was protected as its MOM ether **61**.[50] Reaction with adenosine **9** afforded fully protected **62**, which was deprotected under aqueous TFA conditions to yield the desired 8″-hydroxy chromone-AMS **4**.

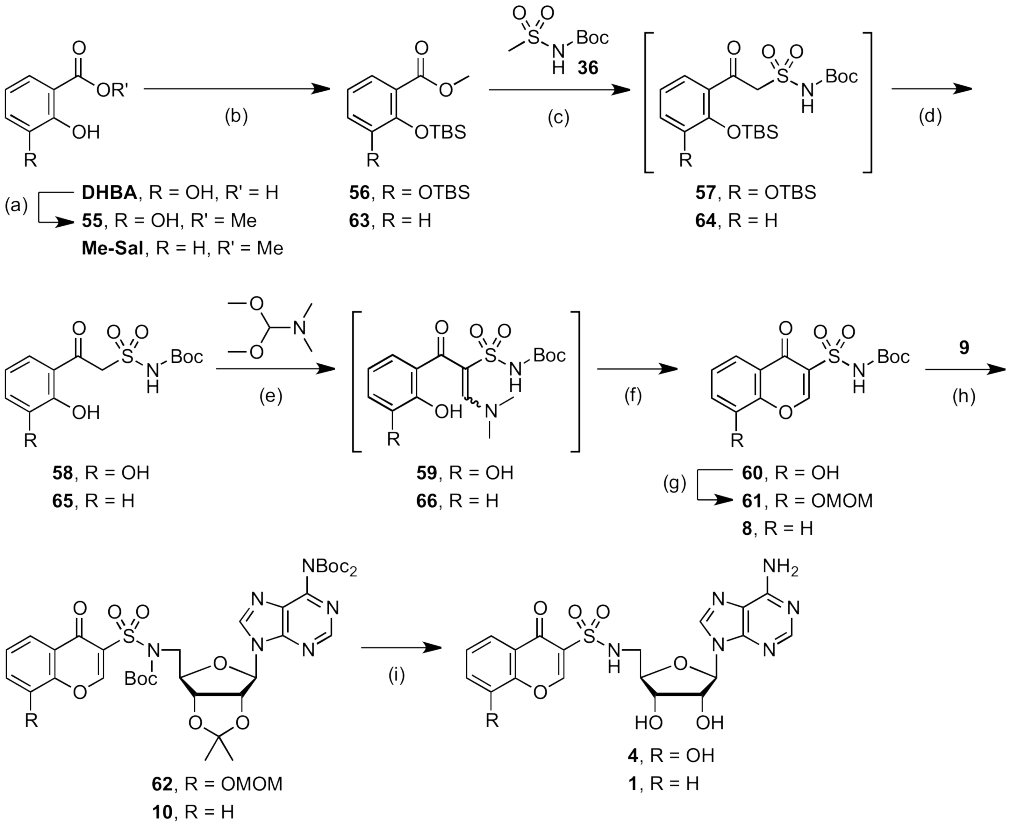


Figure 22. Synthesis of 8″-OH chromone-AMS **4**[a] and revised synthesis of chromone-AMS **1**.[b]
[a]Reaction Conditions for **4**: (a) $SOCl_2$, MeOH, 80 °C, 21 h, 90%; (b) TBSCl, imidazole, DMF, 60 °C, 99%; (c) LDA, THF, 0 °C, 2 h; (d) TBAF, THF, 0 °C, 1.5 h, 82% over two steps; (e) THF, 22 °C, 18 h; (f) Satd aq NH_4Cl, 79% over two steps; (g) MOMCl, DIPEA, DCM, 0 to 22 °C, 22 h, 58%; (h) PPh_3, DIAD, THF, 22 °C, 4.5 h, 28%; (i) 80% aq TFA, 0 °C, 24 h, 5%.
[b]Reaction Conditions for **1**: (a) Not applicable; (b) TBSCl, imidazole, DMAP, DMF, 0 to 22 °C, 21.5 h, 79%; (c) LDA, THF, 0 °C, 3 d; (d) TBAF, THF, 0 °C, 17 h, 86% over two steps; (e) THF, 22 °C, 18 h; (f) Satd aq NH_4Cl, 74% over two steps; (g) Not applicable; (h) As before (see Figure 11); (i) As before (see Figure 11).

Biochemical Evaluation.

The compounds were evaluated for their binding to MbtA as well as related AAAEs BasE, EntE, and VibE (from *A. baumannii*, *E. coli*, and *V. cholerae* respectively) through employment of a fluorescence polarization (FP) assay developed in our laboratory (Table 3).[51] This assay is a competitive binding assay in which our probe molecule Fl-Sal-AMS (courtesy João Neres, Figure 23) is displaced from its binding site with addition of inhibitory compounds. The experimental data affords the K_D for each compound.

	MbtA K_D (μM)	**BasE** K_D (μM)	**EntE** K_D (μM)	**VibE** K_D (μM)
Fl-Sal-AMS	0.0093[b]	0.093	0.23	0.13
1	3.6 ± 0.1	108 ± 8	280 ± 60	98 ± 7
2	0.0024 ± 0.0001	0.029 ± 0.002	0.32 ± 0.04	0.017 ± 0.002
3	0.37 ± 0.01	1.19 ± 0.07	12 ± 1	2.5 ± 0.7
4	290 ± 70	>290	>290	>290

Table 3. K_D values determined against MbtA and other AAAEs.[a]
[a]Assays performed by/with Daniel J. Wilson.
[b]Previously reported[51] with assay development.

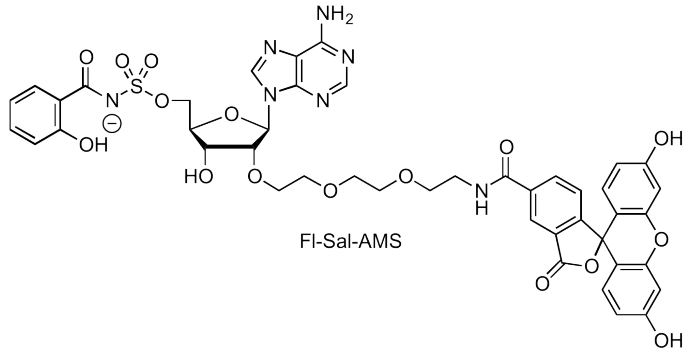


Figure 23. Structure of FP Probe Fl-Sal-AMS.

For those compounds yielding K_D values below that of the FP probe, only **2** in this case, our lab employs a [^{32}P]PP$_i$-ATP exchange assay that takes advantage of the equilibrium nature of MbtA's adenlyation half-reaction.[18] Here, compound **2** afforded a K_i^{app} of 120 ± 20 nM.

Although disheartening, the enzymatic data for **1**–**3** do align with previous SAR findings of Sal-AMS-based inhibitors. When the linker region of Sal-AMS was investigated, significant losses in potency were seen with complete removal of the parent sulfamate negative charge.[14,17] The total absence of negative character from both chromone **1** and benzoxazinone **3** is reflected in their approximately 400- and 40-fold losses in binding affinity versus that of the FP probe respectively. The higher affinity of benzoxazinone **3** versus **1** is most likely due to its more isosteric design when compared to the likely bound conformation of Sal-AMS. Quinolone **2** reintroduces the seemingly necessary partial negative charge through its acidic quinolone nitrogen, however possesses an 18-fold loss in $[^{32}P]PP_i$-ATP exchange assay activity versus that of Sal-AMS. These notions are further supported by the results from the other AAAEs tested because the trends align with MbtA. A surprising find of the FP assay was the complete lack of activity of compound **4**. Such an outcome was expected for MbtA as the substrate it accepts is SAL but rescued potency was expected for the remaining AAAEs because their accepted substrate is DHBA. A hypothesis explaining this disparity could be the compound's inability to achieve the predicted binding mode to thereby mimic the bound conformation as observed structurally (see Figure 4 above).

Antitubercular Evaluation.

The compounds were tested against *Mtb* under iron-deficient and iron-replete conditions as previously described (Table 4).[14]

	Iron-deficient (GAST)	Iron-replete (GAST/Fe)
INH[a]	0.11	0.11
Sal-AMS[b]	0.39	1.56
1	>50	>50
2	>50	>50
3	>50	>50
4	>50	>50

Table 4. MIC_{99} determined against *Mtb* clinical isolate H37Rv (μM).[c]
[a]INH is isoniazid positive control.
[b]Previously reported.[14]
[c]Assays performed by Helena I. Boshoff.

Unexpectedly, compounds **1**–**3** all showed total losses in appreciable activity. Although the partial negative charge afforded by quinolone **2** rescued its enzymatic potency versus **1** and **3**, it still lacked activity seen with Sal-AMS-based inhibitors containing charged linker moieties. As expected and demonstrated by loss of enzymatic potency in FP assay, compound **4** also showed almost no appreciable whole cell activity. It is hypothesized that unknown intracellular mechanisms and/or metabolism are responsible for the total loss of activity seen with **1**–**3**, thereby preventing the compounds from reaching their desired target. It is believed that cellular penetration issues are not responsible for the loss of activity; the increased lipophilicity of **1**–**3** should preclude that conclusion because charged parent Sal-AMS has substantial whole cell activity.

Conclusion and Potential Future Directions

Herein has been reported the synthesis, biochemical and antitubercular evaluation of conformationally constrained analogues of Sal-AMS **1**–**3**, as well as synthesis of DHB variant **4**. A concise synthesis to each has been developed whereby aryl *β*-ketosulfonamides are rapidly elaborated and coupled to appropriately protected adenosine via Mitsunobu reaction. Compounds **1**–**3** displayed low- to sub-micromolar binding to MbtA in our FP assay. Subsequent analysis of **2** showed 18-fold loss in potency versus that of Sal-AMS in our PP_i exchange assay. Unexpectedly, **1**–**3** displayed >130-fold loss in potency versus Sal-AMS in our whole cell assay under relevant iron-limiting conditions, even though the partial negative charge afforded by quinolone **2** somewhat rescued its enzymatic potency. All that considered, the syntheses of **2** and **3** provided a generalizable, higher-yielding route to **1** and sparked inspiration and interest in the synthesis of **4**. Based on our collaborators' previous crystallographic successes with BasE from *A. baumannii*, a co-crystal structure of **2** with BasE could provide necessary insight for the binding mode of these conformationally constrained analogues of Sal-AMS. Furthermore, the pK_a of quinolone **2** could be experimentally determined and subsequently modulated through appropriate installation of electron-withdrawing groups about the aryl ring of the compound. This would allow a detailed dissection of the role the acidic quinolone plays in binding affinity.

Experimental Details

Synthesis.

General Experimental Procedures. All commercial reagents 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 and CH_2Cl_2, while two packed columns of molecular sieves were used to dry DMF, and the solvents were dispensed under Ar. Anhydrous grade MeOH was purchased from Aldrich. Flash chromatography was performed using Combiflash Companion equipped with flash column silica cartridges with the indicated solvent system. Reversed-phase HPLC (RP-HPLC) purification was performed on a Phenomenex Gemini 10 micron C18 250 × 10.00 mm column operating at 5.0 mL/min with detection at 254 nm with the indicated solvent system. All reactions were performed under an inert atmosphere of dry Ar in oven-dried (150 °C) glassware. ^{1}H, ^{13}C, and ^{19}F 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), methanol (3.31), dichloromethane (5.32), dimethyl sulfoxide (2.50), or mono-deuterated water (HDO, 4.79); carbon chemical shifts are reported in ppm from an internal standard of residual chloroform (77.16), methanol (49.00), dichloromethane (54.00), or dimethyl sulfoxide (39.52); and fluorine chemical shifts are reported in ppm from an internal standard of 2-fluorobenzoic acid (-112.05).[52] Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ovlp = overlapping), coupling constant(s), integration. High-resolution mass spectra were obtained on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface.

Compounds from Figure 8

(*E*)-3-(Dimethylamino)-1-(2-hydroxyphenyl)prop-2-en-1-one (12).[24] A solution of *o*-hydroxyacetophenone (6.0 mL, 50 mmol, 1.0 equiv) and *N*,*N*-dimethylformamide dimethylacetal (8.0 mL, 60 mmol, 1.2 equiv) in *o*-xylene (50 mL) was refluxed at 160 °C for 5 h. The reaction was concentrated in vacuo. Crystallization of the resulting residue in toluene afforded the title compound (7.53 g, 79%) as a yellow crystalline solid: R_f 0.39 (1:1 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.93 (s, 3H), 3.15 (s, 3H), 5.75 (d, *J* = 12.0 Hz, 1H), 6.80 (t, *J* = 7.8 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.85 (d, *J* = 12.0 Hz, 1H), 13.97 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 37.5, 45.5, 90.1, 105.8, 118.1, 118.3, 120.4, 128.3, 134.0, 154.9, 163.0, 191.5; HRMS (APCI–) calcd for $C_{11}H_{12}NO_2^-$ $[M - H]^-$ 190.0874, found 190.0880 (error 3.2 ppm).

Chromone-3-sulfonamide (7). To a stirred solution of recrystallized sulfamoyl chloride **14**[32] (347 mg, 3.00 mmol, 1.00 equiv) in DCM (6 mL) at 0 °C was added enaminone **12** (574 mg, 3.00 mmol, 1.00 equiv) in one portion. After stirring 5 h, reaction was quenched with addition of satd aq $NaHCO_3$ (50 mL). The layers were separated and aqueous pH adjusted to neutral (~7 by pH paper). The aqueous was then extracted with EtOAc (3 × 75 mL). The combined organic layer was dried ($MgSO_4$), concentrated, and chromatographed (20:80 to 40:60 EtOAc/hexanes gradient) yielding cyclized chromone **13** (140 mg, 32%; characterization data matched that of authentic commercially obtained sample) and starting enaminone **12** (86 mg, 15%). LRMS showed the possibility of product remaining in the aqueous layer (saw principle peak of m/z = 224 in negative mode), so the aqueous was further extracted with *n*-BuOH (3 × 75 mL). The combined *n*-

BuOH layer was dried ($MgSO_4$) and concentrated. The resultant residue was taken up in MeOH/MeCN and insoluble solids filtered away. Upon sitting overnight, a precipitate had formed in the MeOH/MeCN filtrate. Isolation and drying of that precipitate afforded the title compound (34 mg, 5%) as off-white crystals: ^{1}H NMR (600 MHz, D_2O) δ 7.60 (t, J = 7.6 × 2 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.90 (ddd, J = 8.5, 7.6, 1.5 Hz, 1H), 8.21 (dd, J = 7.6, 1.5 Hz, 1H), 8.84 (s, 1H); LRMS (ESI–) calcd for $C_9H_6NO_4S^-$ $[M - H]^-$ 224, found 224.

Compounds from Figure 9

***N*-(*tert*-Butoxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide (15).**[33] To a stirred solution of *t*-BuOH (1.04 g, 14.0 mmol, 1.00 equiv) in DCM (10 mL) at 0 °C was added chlorosulfonyl isocyanate (1.2 mL, 14 mmol, 1.0 equiv) dropwise. The solution was stirred 5 min. DMAP (3.42 g, 28.0 mmol, 2.00 equiv) was added to the solution in one portion causing gas evolution and precipitate formation. The reaction mixture was maintained at 0 °C a further 10 min then was allowed to warm to 22 °C and stirred an addition 1.5 h. The mixture was diluted with DCM (25 mL) and was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated to an amorphous off-white solid. Recrystallization of the solid from MeCN afforded the title compound (1.63 g, 38%) as off-white crystals: Characterization data matched that as reported. The concentrated mother liquor could likely have been recrystallized, however this was not pursued because more than enough material was in hand for use.

***N*-(Benzyloxycarbonyl)-*N*-[4-(dimethylazaniumylidene)-1,4-dihydropyridin-1-ylsulfonyl]azanide (16).**[34] To a stirred solution of BnOH (1.45 mL, 14.0 mmol, 1.00 equiv) in DCM (10 mL) at 0 °C was added chlorosulfonyl isocyanate (1.2 mL, 14 mmol,

1.0 equiv) dropwise. The solution was stirred 5 min. DMAP (3.42 g, 28.0 mmol, 2.00 equiv) was added to the solution in one portion causing gas evolution and precipitate formation. The reaction mixture was maintained at 0 °C a further 10 min then was allowed to warm to 22 °C and stirred an addition 1.5 h. The mixture was diluted with DCM (25 mL) and was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated to an amorphous off-white solid. Recrystallization of the solid from MeCN afforded the title compound (4.02 g, 85%) as off-white crystals: Characterization data matched that as reported. The concentrated mother liquor could likely have been recrystallized, however this was not pursued because more than enough material was in hand for use.

Chromone (13) via attempted reaction of enaminone **12** with Boc-protected sulfamoylating reagent **15.** To a solution of enaminone **12** (19 mg, 0.10 mmol, 1.0 equiv) in DCM (1 mL) at 22 °C was added sulfamoylating reagent **15** (30 mg, 0.10 mmol, 1.0 equiv). After 8 h stirring at 22 °C, TLC monitoring showed no reaction progress, so reaction temperature increased to 40 °C. After 16 h stirring at 40 °C, TLC still showed no reaction progress. DCM was removed in vacuo and replaced with dioxane (1 mL). The solution was heated at 80 °C. After 24 h stirring, TLC showed total consumption of enaminone **12** and sole formation of chromone **13** as verified by LRMS ([ESI+] calcd for $C_9H_7O_2^+$ $[M + H]^+$ 147, found 147).

Chromone (13) via attempted reaction of enaminone **12** with Cbz-protected sulfamoylating reagent **16.** To a solution of enaminone **12** (19 mg, 0.10 mmol, 1.0 equiv) in DCM (1 mL) at 22 °C was added sulfamoylating reagent **16** (30 mg, 0.10 mmol, 1.0 equiv). After 8 h stirring at 22 °C, TLC monitoring showed no reaction progress, so reaction temperature increased to 40 °C. After 16 h stirring at 40 °C, TLC still showed no

reaction progress. DCM was removed in vacuo and replaced with dioxane (1 mL). The solution was heated at 80 °C. After 24 h stirring, TLC showed total consumption of enaminone **12** and sole formation of chromone **13** as verified by LRMS ([ESI+] calcd for $C_9H_7O_2^+$ $[M + H]^+$ 147, found 147).

Compounds from Figure 11

***tert*-Butyl chlorosulfonylcarbamate (17).**[33] To a stirred solution of *tert*-butanol (1.9 mL, 20 mmol, 1.3 equiv) in DCM (12 mL) at 0 °C was added chlorosulfonyl isocyanate (1.4 mL, 15 mmol, 1.0 equiv) dropwise over the course of 10 min. The reaction was removed from the 0 °C bath after 5 min of additional stirring. After warming to 22 °C, stirring was stopped and the reaction was concentrated in vacuo just until precipitate formation was observed. The flask was placed back into the 0 °C bath. After 50 min, the precipitate was filtered and washed with hexanes yielding the title compound (1.5 g, 46%) as a colorless solid. More product (1.2 g, 37%) was obtained by crystallizing the concentrated mother liquor in DCM at 0 °C. The precipitate was used directly without further purification: ^{1}H NMR (600 MHz, CD_2Cl_2) δ 1.56 (s, 9H), 8.50 (s, 1H).

***tert*-Butyl (chromon-3-yl)sulfonylcarbamate (8).**[30] To a stirred solution of Boc-protected sulfamoyl chloride **17** (1.5 g, 7.0 mmol, 1.0 equiv) in DCM (14 mL) at 22 °C was added enaminone **12** (1.3 g, 7.0 mmol, 1.0 equiv). The reaction was stirred 13 h then concentrated in vacuo. Purification by flash chromatography (30:70 to 100:0 DCM/hexanes gradient) afforded the title compound (377 mg, 21%) as a yellow amorphous solid: R_f 0.29 (1:5:95 Et_3N/MeOH/DCM); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.40 (s, 9H), 7.54 (t, J = 7.8 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.80 (t, J = 8.3 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 8.82 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.0, 84.4, 118.9, 123.6,

124.4, 126.4, 127.1, 135.4, 149.2, 156.3, 162.0, 171.8; HRMS (ESI–) calcd for $C_{14}H_{14}NO_6S^-$ $[M - H]^-$ 324.0547, found 324.0559 (error 3.7 ppm).

***N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine (9).**[29] To a stirred solution of 2′,3′-*O*-isopropylideneadenosine **18** (5.00 g, 16.3 mmol, 1.00 equiv) in DMF (50 mL) at 0 °C was added imidazole (2.83 g, 41.6 mmol, 2.55 equiv) followed by TBSCl (2.95 g, 19.6 mmol, 1.20 equiv). After 4 h the reaction was warmed to 22 °C and the mixture was concentrated. The resulting residue was partitioned between EtOAc (200 mL) and H_2O (100 mL). The organic layer was washed with H_2O (100 mL) and satd NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (0:100 to 100:0 EtOAc/hexanes gradient) afforded 5′-*O*-*tert*-butyldimethylsilyl-2′,3′-*O*-isopropylideneadenosine **19** in quantitative yield as an off-white solid: R_f 0.39 (4:1 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.015 (s, 3H), 0.022 (s, 3H), 0.85 (s, 9H), 1.41 (s, 3H), 1.64 (s, 3H), 3.77 (dd, *J* = 11.4, 4.2 Hz, 1H), 3.88 (dd, *J* = 11.4, 4.2 Hz, 1H), 4.42 (q, *J* = 4.2 Hz, 1H), 4.96 (dd, *J* = 6.6, 3.0 Hz, 1H), 5.29 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.71 (br s, 2H), 6.17 (d, *J* = 3.0 Hz, 1H), 8.05 (s, 1H), 8.38 (s, 1H).

To a stirred solution of adenosine derivative **19** (6.87 g, 16.3 mmol, 1.00 equiv) prepared above, DMAP (418 mg, 3.42 mmol, 0.210 equiv) and Et_3N (4.8 mL, 34 mmol, 2.1 equiv) in DMF (150 mL) at 0 °C was added Boc_2O (7.46 g, 34.2 mmol, 2.10 equiv) in one portion. After 1 h the reaction was warmed to 22 °C. Reaction monitoring by TLC showed no noticeable change over 18 h of stirring at 22 °C. More DMAP (219 mg, 1.79 mmol, 0.110 equiv) and Et_3N (2.5 mL, 18 mmol, 1.1 equiv) were added and the solution cooled to 0 °C. More Boc_2O (3.91 g, 17.9 mmol, 1.10 equiv) was added and the solution

was allowed to warm to 22 °C of its own accord while stirring for a further 26 h. The reaction was then concentrated yielding a thick brown oil (12.58 g). Purification by flash chromatography (20:80 EtOAc/hexanes) afforded N^6,N^6-bis(*tert*-butoxycarbonyl)-5′-*O*-*tert*-butyldimethylsilyl-2′,3′-*O*-isopropylideneadenosine **20** (8.81 g, 87%) as an off-white solid: R_f 0.73 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.85 (s, 9H), 1.41 (s, 3H), 1.44 (s, 18H), 1.65 (s, 3H), 3.78 (dd, J = 11.4, 4.1 Hz, 1H), 3.89 (dd, J = 11.4, 3.7 Hz, 1H), 4.45 (ap q, J = 3.7 Hz, 1H), 4.96 (dd, J = 6.3, 2.5 Hz, 1H), 5.22 (dd, J = 6.1, 2.5 Hz, 1H), 6.25 (d, J = 2.5 Hz, 1H), 8.34 (s, 1H), 8.88 (s, 1H).

To a stirred solution of silyl ether **20** (7.35 g, 11.8 mmol, 1.00 equiv) prepared above in THF (100 mL) was added TBAF (16 mmol, 1.4 equiv) in one portion. The solution was concentrated in vacuo after 1.5 h. Purification by flash chromatography (50:50 to 70:30 EtOAc/hexanes gradient) afforded the title compound (5.05 g, 84%) as an off-white solid: R_f 0.33 (1:2 acetone/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.34 (s, 3H), 1.39 (s, 18H), 1.56 (s, 3H), 3.53 (ap q, J = 8.8, 4.7 Hz, 2H), 4.29 (ap q, J = 6.5, 4.1 Hz, 1H), 5.00 (dd, J = 5.9, 2.4 Hz, 1H), 5.11 (t, J = 5.3 Hz, 1H), 5.45 (dd, J = 5.9, 2.4 Hz, 1H), 6.29 (d, J = 2.4 Hz, 1H), 8.82 (s, 1H), 8.88 (s, 1H).

N^6,N^6-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)-5′-*N*-(chromon-3-yl)sulfonyl-2′,3′-*O*-isopropylideneadenosine (10). To a stirred solution of Boc-protected chromone sulfonamide **8** (270 mg, 0.83 mmol, 1.1 equiv), bis-Boc adenosine **9** (381 mg, 0.75 mmol, 1.0 equiv) and PPh_3 (218 mg, 0.83 mmol, 1.1 equiv) in THF (20 mL) at 0 °C was added DIAD (0.16 mL, 0.83 mmol, 1.1 equiv) dropwise over the course of 10 min. The reaction was stirred 2.5 h at 0 °C and was then allowed to warm to 22 °C. The reaction was stirred another 1.5 h then was concentrated in vacuo.

Purification by flash chromatography (40:60 EtOAc/hexanes) afforded the title compound (491 mg, 80%) as a colorless oil: R_f 0.63 (3:1 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.34 (s, 9H), 1.41 (s, 18H), 1.65 (s, 3H), 4.25 (dd, *J* = 15.3, 6.5 Hz, 1H), 4.36 (dd, *J* = 15.3, 6.5 Hz, 1H), 4.67 (td, *J* = 6.5 × 2, 3.5 Hz, 1H), 5.21 (dd, *J* = 6.2, 3.5 Hz, 1H), 5.42 (dd, *J* = 6.2, 2.3 Hz, 1H), 6.23 (d, *J* = 2.3 Hz, 1H), 7.51 (t, *J* = 7.9 × 2 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.77 (ddd, *J* = 8.5, 7.9, 1.5 Hz, 1H), 8.19 (dd, *J* = 7.9, 1.5 Hz, 1H), 8.28 (s, 1H), 8.69 (s, 1H), 8.91 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.6, 27.4, 27.9, 28.0, 49.5, 82.5, 83.8, 84.6, 85.2, 85.5, 90.8, 114.9, 118.7, 124.4, 125.4, 126.3, 127.1, 129.1, 129.6, 135.2, 144.1, 150.4, 150.58, 150.61, 152.4, 152.7, 161.8, 171.3; HRMS (ESI+) calcd for $C_{37}H_{47}N_6O_{13}S^+$ $[M + H]^+$ 815.2916, found 815.2926 (error 1.2 ppm).

5′-Amino-5′-*N*-[(chromon-3-yl)sulfonyl]adenosine (1). To Mitsunobu product **10** at 0 °C was added ice-cold 80% aq TFA (5 mL). Reaction was allowed to warm to 22 °C after 1.5 h stirring and was stirred an additional 3 h. The reaction was concentrated in vacuo and subsequent purification by flash chromatography (10:90 MeOH/$CHCl_3$) afforded the title compound (210 mg, 76%) as a colorless amorphous solid: R_f 0.33 (1:9 MeOH/$CHCl_3$); ^{1}H NMR (600 MHz, 1:10 D_2O/DMSO-d_6) δ 3.20 (dd, *J* = 14.1, 3.5 Hz, 1H), 3.27 (dd, *J* = 14.1, 4.7 Hz, 1H), 4.05 (m, 1H), 4.07 (dd, *J* = 5.0, 1.6 Hz, 1H), 4.68 (td, *J* = 6.4 × 2, 1.6 Hz, 1H), 5.74 (d, *J* = 6.4 Hz, 1H), 7.53 (t, *J* = 7.6 × 2 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.84 (td, *J* = 7.6 × 2, 1.2 Hz, 1H), 8.05 (dd, *J* = 7.6, 1.2 Hz, 1H), 8.22 (s, 1H), 8.26 (s, 1H), 8.83 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 45.0, 71.3, 72.3, 84.0, 88.3, 118.7, 119.6, 123.7, 124.3, 125.2, 126.5, 135.1, 140.5, 148.6, 152.5, 155.6,

156.2, 160.3, 171.6; HRMS (ESI+) calcd for $C_{19}H_{19}N_6O_7S^+$ $[M + H]^+$ 475.1030, found 475.1025 (error 1.1 ppm).

Compounds from Figure 12

Benzyl (2-acetylphenyl)carbamate (22).[36] To a solution of *o*-aminoacetophenone **21** (1.2 mL, 10 mmol, 1.0 equiv), pyridine (3.2 mL, 40 mmol, 4.0 equiv), and DMAP (61 mg, 0.50 mmol, 0.050 equiv) in DCM (30 mL) at 0 °C was added benzyl chloroformate (2.9 mL, 20 mmol, 2.0 equiv) dropwise over 25 min. After 30 min stirring at 0 °C, H_2O (10 mL) was added to the mixture. The layers were separated and the aqueous extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with satd aq NaCl (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (50:50 to 100:0 EtOAc/hexanes gradient) afforded the title compound (2.69 g, quant.) as a pale yellow amorphous solid: Characterization data matched that as reported.[53]

(*E*)-Benzyl (2-(3-(dimethylamino)acryloyl)phenyl)carbamate (23). A solution of acetophenone **22** (1.89 g, 7.00 mmol, 1.00 equiv) and *N*,*N*-dimethylformamide diethylacetal (1.5 mL, 8.4 mmol, 1.2 equiv) in *o*-xylene (58 mL) in a flask topped with a short-path distillation apparatus was heated at 160 °C. After 15 min, the solution had gone from colorless to yellow and distillate (presumably EtOH side product) began collecting in the receiving flask. Heating was continued a further 15 min then was stopped. Precipitate formed upon sitting at 22 °C overnight. The precipitate was removed by filtration and the resultant solution concentrated. Recrystallization of the solid from toluene afforded the title compound (724 mg, 32%) as golden yellow crystals; the mother liquor was concentrated. Recrystallization of that solid from toluene afforded more of the title compound (299 mg, 13%) as golden yellow crystals: R_f 0.24 (1:99 MeOH/DCM); ^{1}H

NMR (600 MHz, $CDCl_3$) δ 2.92 (s, 3H), 3.16 (s, 3H), 5.19 (s, 2H), 5.66 (d, *J* = 12.0 Hz, 1H), 7.01 (t, *J* = 7.6 × 2 Hz, 1H), 7.30–7.41 (ovlp m, 5H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 12.0 Hz, 1H), 8.36 (d, *J* = 8.8 Hz, 1H), 11.36 (s, 1H); LRMS (APCI–) calcd for $C_{19}H_{19}N_2O_3^-$ $[M - H]^-$ 323, found 323.

(*E*)-Benzyl (2-(3-(dimethylamino)acryloyl)phenyl)carbamate (23) recovered from attempted synthesis of benzyl 3-(*N*-(*tert*-butoxycarbonyl)sulfamoyl)-4-oxoquinoline-1(4*H*)-carboxylate (**24**) via enaminone **23** and Boc-protected sulfamoyl chloride **17.** To a solution of Boc sulfamoyl chloride **17** (474 mg, 2.10 mmol, 1.50 equiv) in DCM (6 mL) at 0 °C was added enaminone **23** (454 mg, 1.40 mmol, 1.00 equiv) in one portion. After 30 min, TLC showed no reaction progress, so the solution was allowed to warm to 22 °C of its own accord overnight. After 16 h, the reaction was quenched with addition of satd aq $NaHCO_3$ (10 mL). The layers were separated and the aqueous extracted with EtOAc (3 × 25 mL). The combined organic layer was dried ($MgSO_4$) and concentrated affording starting enaminone **23** (428 mg, 94%): Characterization data matched that as given above.

Compounds from Figure 17

Methyl 2-fluorobenzoate (35). To 2-fluorobenzoic acid (1.4 g, 10 mmol, 1.0 equiv) in methanol (20 mL) was added a catalytic amount of concentrated H_2SO_4. The solution was heated at 75 °C. After 19 h, the solution was cooled to 0 °C and the acid quenched by addition of solid $NaHCO_3$ (1.5 g). The mixture was concentrated in vacuo, taken up in EtOAc (10 mL) and filtered away from the insoluble material. Concentration of the EtOAc yielded the title compound (1.36 g, 88%) as a colorless oil, used directly without further purification: R_f 0.64 (1:9 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.93 (s,

3H), 7.13 (m, 1H), 7.20 (ap t, J = 7.6 Hz, 1H), 7.52 (ap q, J = 7.6 Hz, 1H), 7.93 (ap t, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 52.4, 117.1 (d, $^2J_{C\text{-}F}$ = 23.1 Hz), 118.8 (d, $^2J_{C\text{-}F}$ = 10.4 Hz), 124.1 (d, $^3J_{C\text{-}F}$ = 4.6 Hz), 132.3, 134.6 (d, $^3J_{C\text{-}F}$ = 9.2 Hz), 162.0 (d, $^1J_{C\text{-}F}$ = 258.9 Hz), 165.0 (d, $^3J_{C\text{-}F}$ = 4.6 Hz); ^{19}F NMR (564 MHz, $CDCl_3$) δ -113.4; HRMS (APCI+) calcd for $C_8H_8FO_2^+$ $[M + H]^+$ 155.0503, found 155.0513 (error 6.4 ppm).

***tert*-Butyl methylsulfonylcarbamate (36).**[17,28] To a stirred suspension of methanesulfonamide (238 mg, 2.50 mmol, 1.00 equiv), Et_3N (0.38 mL, 2.8 mmol, 1.1 equiv), and DMAP (30 mg, 0.25 mmol, 0.10 equiv) in DCM (5 mL) at 22 °C was added a solution of Boc_2O (654 mg, 3.00 mmol, 1.20 equiv) in DCM (2 mL) dropwise over 10 min. After 2 h stirring, the reaction was concentrated to an oily residue. The residue was taken up in EtOAc (15 mL). The organic layer was washed with 1 N HCl (10 mL), H_2O (15 mL), and satd aq NaCl (15 mL), dried ($MgSO_4$) and concentrated affording the title compound (454 mg, 93%) as an off-white amorphous solid: Characterization data matched that as reported.

***tert*-Butyl (2-(2-fluorophenyl)-2-oxoethyl)sulfonylcarbamate (34).**[17] Freshly titrated *n*-BuLi (2.1 M in hexane, 12.4 mL, 26.1 mmol, 3.10 equiv) was added dropwise to freshly distilled (*i*-Pr)$_2$NH (3.9 mL, 27.8 mmol, 3.30 equiv) in THF (24 mL) at 0 °C. The mixture was stirred for 1 h, then sulfonamide **36** (1.65 g, 8.43 mmol, 1.00 equiv) in THF (24 mL) was added and the reaction stirred for a further 1 h at 0 °C. Next, methyl ester **35** (1.43 g, 9.28 mmol, 1.10 equiv) in THF (5 mL) was added and the reaction was stirred for 1.5 h at 0 °C. The reaction mixture was quenched with satd aq NaCl (25 mL) and 0.5 M aq NaH_2PO_4 (25 mL). The layers were separated and the aqueous layer acidified to pH ~5-6 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (3 × 75

mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/ hexanes) afforded the title compound (1.92 g, 72%) as a yellow solid: R_f 0.74 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.50 (s, 9H), 5.03 (s, 2H), 7.19 (m, 1H), 7.29 (ap t, J = 7.6 Hz, 1H), 7.62 (m, 1H), 7.91 (ap t, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.1, 61.6 (d, $^2J_{C\text{-}F}$ = 9.2 Hz), 85.0, 117.2 (d, $^2J_{C\text{-}F}$ = 23.1 Hz), 124.3, 125.1 (d, $^3J_{C\text{-}F}$ = 3.5 Hz), 131.2, 136.6 (d, $^3J_{C\text{-}F}$ = 9.2 Hz), 149.6, 162.2 (d, $^1J_{C\text{-}F}$ = 256.6 Hz), 186.2 (d, $^3J_{C\text{-}F}$ = 3.5 Hz); ^{19}F NMR (564 MHz, $CDCl_3$) δ -112.7; HRMS (ESI–) calcd for $C_{13}H_{15}FNO_5S^-$ $[M - H]^-$ 316.0660, found 316.0654 (error 1.9 ppm).

Compound from Figure 18

Ethyl *N*-(1-ethoxy-3-(2-fluorophenyl)-3-oxoprop-1-en-2-yl)sulfonylformimidate (38).[39] To a solution of *β*-ketosulfonamide **34** (64 mg, 0.20 mmol, 1.0 equiv) in Ac_2O (3.8 mL, 40 mmol, 200 equiv) at 22 °C was added triethyl orthoformate (0.33 mL, 2.0 mmol, 10 equiv). After 30 min, TLC monitoring showed no reaction progress. The reaction was then heated at 77 °C. TLC monitoring (at 15 min, 30 min, and 1.5 h) still showed no reaction progress. The reaction was then heated at 90 °C. TLC monitoring (at 30 min, 1 h, and 1.5 h) showed reaction progress. A further 6 h stirring at 90 °C showed total consumption of starting ketosulfonamide **34** by TLC. The reaction was concentrated yielding a brown oil (75 mg). Purification by flash chromatography (30:70 EtOAc/hexanes) afforded the title compound (33 mg, 50%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.16 (t, J = 7.0 Hz, 3H), 1.29 (t, J = 7.4 Hz, 3H), 4.17 (q, J = 7.0 Hz, 2H), 4.20 (q, J =

7.4 Hz, 2H), 7.07 (ap t, J = 9.4 × 2 Hz, 1H), 7.20 (t, J = 7.3 × 2 Hz, 1H), 7.49 (ap qd, J = 6.5 × 3, 1.2 Hz, 1H), 7.56 (t, J = 7.3 × 2 Hz, 1H), 7.67 (s, 1H), 8.10 (s, 1H).

Compounds from Figure 19

***tert*-Butyl (3-(2-fluorophenyl)-1-((4-methoxybenzyl)amino)-3-oxoprop-1-en-2-yl)sulfonylcarbamate (40).**[25,39] *N*,*N*-Dimethylformamide dimethylacetal (136 μL, 1.02 mmol, 1.20 equiv) was added to a solution of *β*-ketosulfonamide **34** (269 mg, 0.848 mmol, 1.00 equiv) in THF (5 mL) at 22 °C. The solution was stirred for 17 h, then was concentrated under reduced pressure yielding *tert*-butyl (1-(dimethylamino)-3-(2-fluorophenyl)-3-oxoprop-1-en-2-yl)sulfonylcarbamate **39** as a yellow oily residue used directly without further purification: R_f 0.25 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.80 (s, 3H), 3.32 (s, 3H), 7.07 (t, J = 8.8 × 2 Hz, 1H), 7.19 (t, J = 7.6 × 2 Hz, 1H), 7.44 (ap qd, J = 7.0 × 3, 1.8 Hz, 1H), 7.49 (ap td, J = 7.6 × 2, 1.8 Hz), 7.98 (s, 1H); LRMS (ESI–) calcd for $C_{16}H_{20}FN_2O_5S^-$ $[M - H]^-$ 371, found 371.

To a stirred solution of enaminone **39** prepared above in DCM (5 mL) at 22 °C was added $PMBNH_2$ (165 μL, 1.27 mmol, 1.50 equiv). After stirring 5 min the reaction was concentrated under reduced pressure yielding a golden foamy oil. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded a mixture of isomers (~2:1) of the title compound (319 mg, 81%) as an off-white amorphous solid: R_f 0.70 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 1.46 (s, 9H), 3.79 (*minor*, s, 1H), 3.80 (*major*, s, 2H), 4.39 (*minor*, d, J = 5.3 Hz, 0.67H), 4.57 (*major*, d, J = 5.9 Hz, 1.33H), 6.87 (*minor*, d, J = 8.2 Hz, 0.67H), 6.93 (*major*, d, J = 8.5 Hz, 1.33H), 7.069 (*major*, t, J = 8.8 Hz, 0.67H), 7.075 (*minor*, t, J = 8.5 Hz, 0.33H),

7.14–7.19 (ovrlp m, 2H), 7.24 (*major*, t, J = 7.0 Hz, 0.67H), 7.28 (*major*, d, J = 8.5 Hz, 1.33H), 7.36–7.40 (ovrlp m, 1H), 7.43 (*minor*, ap q, J = 7.0 Hz, 0.33H), 7.49 (br s, 1H), 8.27 (*major*, d, J = 14.1 Hz, 0.67H), 8.69 (*minor*, m, 0.33H), 10.97 (*major*, m, 0.67H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 14.5, 21.3, 28.2, 28.3, 53.6, 54.3, 55.8, 60.8, 83.5, 83.6, 107.7, 108.5, 114.8, 114.9, 115.8, 116.0, 116.4, 116.6, 124.09, 124.11, 124.99, 125.01, 128.59, 128.61, 129.3, 129.4, 129.77, 129.80, 132.39, 132.44, 150.2, 150.9, 157.9, 158.3, 158.4, 159.5, 159.9, 160.2, 160.3, 162.0, 185.4, 188.7; ^{19}F NMR (564 MHz, $CDCl_3$) δ -119.9 (*major*), -117.3; HRMS (ESI–) calcd for $C_{22}H_{24}FN_2O_6S^-$ $[M - H]^-$ 463.1345, found 463.1353 (error 1.7 ppm).

***tert*-Butyl (1-(4-methoxybenzyl)-quinol-4-on-3-yl)sulfonylcarbamate (41).**[39] NaH (60% dispersion in mineral oil, 48 mg, 1.2 mmol, 2.4 equiv) was added to enaminone **40** (232 mg, 0.50 mmol, 1.0 equiv) in 1,2-DME (4 mL) at 0 °C. The mixture was stirred 19.5 h and allowed to warm to 22 °C of its own accord. The reaction was diluted with EtOAc (20 mL) and quenched with satd aq NH_4Cl (10 mL). The layers were separated and the aqueous layer acidified to pH ~4-5 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (2 × 20 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding an off-white residue (249 mg). Purification by flash chromatography (0.4:0.4:39.2:60 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (181 mg, 81%) as an amorphous off-white solid: R_f 0.48 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, CD_3OD) δ 1.37 (s, 9H), 3.74 (s, 3H), 5.59 (s, 2H), 6.87 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.48 (t, J = 7.3 Hz, 1H), 7.70–7.75 (ovlp m, 2H), 8.35 (d, J = 8.2 Hz, 1H), 8.88 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 28.2, 55.7, 58.0, 83.7, 115.5, 118.4, 119.4, 127.0, 127.5, 128.0,

129.4, 134.7, 141.0, 151.2, 152.4, 161.3, 174.0, 174.2; HRMS (ESI–) calcd for $C_{22}H_{23}N_2O_6S^-$ $[M - H]^-$ 443.1282, found 443.1277 (error 1.1 ppm).

Quinol-4-on-3-sulfonamide (42).[41] Quinolone sulfonamide **41** (44 mg, 0.1 mmol, 1.0 equiv) in TFA (2 mL) was heated at 72 °C in a sealed tube for 17 h. The reaction was then heated at 100 °C for 23 h. The mixture was allowed to cool to 22 °C and was concentrated under reduced pressure yielding a light-brown solid (32 mg). Purification by flash chromatography (0:0:100 to 1:10:89 HCO_2H/MeOH/$CHCl_3$) afforded the title compound (13 mg, 58%) as a colorless residue: ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.77 (br s, 2H), 7.46 (dd, J = 8.2, 7.6 Hz, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.76 (dd, J = 8.2, 7.6 Hz, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.50 (s, 1H), 12.46 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 119.4, 122.1, 124.8, 125.0, 126.3, 132.8, 140.0, 140.4, 172.1; LRMS (ESI–) calcd for $C_9H_7N_2O_3S^-$ $[M - H]^-$ 223, found 223.

***tert*-Butyl (1-(*tert*-butoxycarbonyl)-quinol-4-on-3-yl)sulfonylcarbamate (43).**[42,43] To a suspension of quinolone sulfonamide **42** (47 mg, 0.21 mmol, 1.0 equiv) in DCM (2 mL) was added Et_3N (64 μL, 0.46 mmol, 2.2 equiv) and DMAP (5 mg, 0.04 mmol, 0.2 equiv). Boc_2O (100 mg, 0.46 mmol, 2.2 equiv) was added to the suspension at 0 °C. The mixture was stirred 15 h and allowed to warm to 22 °C of its own accord, after which time it was concentrated under reduced pressure yielding a yellow residue (132 mg). Purification by flash chromatography afforded recovered quinolone sulfonamide **42** (5.7 mg, 12%), mono-protected product **46** (39 mg, 57%, characterization data below), and the title compound (16 mg, 18%) as a colorless residue: ^{1}H NMR (600 MHz, CD_3OD) δ 1.37 (s, 9H), 1.74 (s, 9H), 7.58 (dd, J = 7.6 × 2 Hz, 1H), 7.83 (ddd, J = 8.8, 7.6, 1.8 Hz,

1H), 8.35 (dd, J = 7.6, 1.2 Hz, 1H), 8.58 (d, J = 8.8 Hz, 1H), 9.20 (s, 1H); LRMS (ESI–) calcd for $C_{19}H_{23}N_2O_7S^-$ $[M - H]^-$ 423, found 423.

5′-Amino-5′-*N*-[((quinol-4-on)-3-yl)sulfonyl]adenosine (2). To a solution of adenosine **9** (15 mg, 0.030 mmol, 1.0 equiv), sulfonamide **43** (14 mg, 0.033 mmol, 1.1 equiv), and PPh_3 (8.7 mg, 0.033 mmol, 1.1 equiv) in THF (2 mL) at 0 °C was added DIAD (6.5 μL, 0.033 mmol, 1.1 equiv). The reaction was stirred and allowed to warm to 22 °C of its own accord over 15 h. TLC monitoring of the reaction showed presence of remaining **9** and **43**. More PPh_3 (8.7 mg, 0.033 mmol, 1.1 equiv) followed by DIAD (6.5 μL, 0.033 mmol, 1.1 equiv) was added to the reaction. TLC monitoring of the reaction after 1.5 h additional stirring showed complete consumption of **9**. The reaction was concentrated to a yellow residue (105 mg). Purification by flash chromatography (0.6:0.6:58.8:40 HCO_2H/MeOH/EtOAc/hexanes) afforded tris-Boc-protected product **44** (19 mg, 78%) as a colorless residue: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.28 (s, 9H), 1.36 (s, 18H), 1.41 (s, 3H), 1.66 (s, 3H), 4.37 (m [2 ovlp m], 2H), 4.69 (m, 1H), 5.12 (m, 1H), 5.29 (m, 1H), 6.30 (d, J = 2.3 Hz, 1H), 7.41 (ap t, J = 7.9, 7.6 Hz, 1H) 7.66 (ap t, J = 8.2, 7.6 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 7.9 Hz, 1H), 8.60 (s [2 ovrl s], 2H), 8.92 (s, 1H); LRMS (ESI+) calcd for $C_{37}H_{48}N_7O_{12}S^+$ $[M + H]^+$ 814, found 814.

Tris-Boc-protected **44** (9.1 mg, 0.011 mmol, 1.0 equiv) prepared above was stirred in 80% aq TFA (5 mL) at 0 °C for 24 h. Concentration in vacuo yielded a colorless residue (7.7 mg). Purification by RP-HPLC (15:85 MeCN/H_2O) and lyophilization of appropriate fractions afforded the title compound (4.4 mg, 85%, 66% over two steps) as a fluffy colorless solid: R_f 0.07 (1:10:89 HCO_2H/MeOH/EtOAc); ^{1}H NMR (600 MHz, 1:10 D_2O/DMSO-d_6) δ 3.04 (dd, J = 13.8, 4.1 Hz, 1H), 3.10 (dd, J = 13.8, 4.1 Hz, 1H), 4.05

(ap br s [ddd], 1H), 4.11 (ap d [dd], J = 4.7 Hz, 1H), 4.72 (ap t, J = 6.4, 4.7 Hz, 1H), 5.28 (D_2O-exchangeable, br s, 1H), 5.44 (D_2O-exchangeable, br s, 1H), 5.79 (d, J = 6.4 Hz, 1H), 7.45 (ap t, J = 7.6, 7.4 Hz, 1H), 7.62 (D_2O-exchangeable, br s, 2H), 7.64 (d, J = 7.8 Hz, 1H), 7.76 (ap t, J = 7.8, 7.4 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 8.30 (ap d [2 ovlp s], 2H), 8.46 (s, 1H), 12.55 (D_2O-exchangeable, d, J = 6.5 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 44.9, 71.3, 72.2, 84.0, 88.4, 118.2, 119.1, 119.6, 124.9, 125.2, 126.3, 132.9, 139.6, 140.6, 142.4, 148.8, 152.6, 156.2, 171.9; HRMS (ESI+) calcd for $C_{19}H_{20}N_7O_6S^+$ $[M + H]^+$ 474.1190, found 474.1181 (error 1.9 ppm).

Compounds from Figure 20

***tert*-Butyl (1-benzyl-quinol-4-on-3-yl)sulfonylcarbamate (45).**[25,39] *N*,*N*-Dimethylformamide dimethylacetal (399 μL, 3.00 mmol, 1.50 equiv) was added to a solution of *β*-ketosulfonamide **34** (635 mg, 2.00 mmol, 1.00 equiv) in THF (10 mL) at 22 °C. The solution was stirred for 2 h, then was concentrated under reduced pressure yielding enaminone **39** as a yellow oily residue used directly without purification.

To a stirred solution of enaminone **39** prepared above in THF (6 mL) at 22 °C was added $BnNH_2$ (328 μL, 3.00 mmol, 1.50 equiv). After stirring 10 min the reaction was concentrated under reduced pressure yielding transamination product (Bn-enaminone variant of PMB-enaminone **40**) as a golden foamy oil used directly without purification.

To a stirred solution of Bn-enaminone prepared above in THF (8 mL) at 22 °C was added NaH (60% dispersion in mineral oil, 176 mg, 4.40 mmol, 2.20 equiv) portion-wise (~10 mg portions) such that noticeable gas evolution had ceased before next addition. After stirring 30 min, the reaction was quenched by addition of satd aq NH_4Cl (25 mL). The aqueous mixture was acidified to pH ~4 (pH paper) with aq 6 N HCl and was then

extracted with EtOAc (3 × 25 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding an off-white solid. Purification by flash chromatography (0.3:0.3:29.4:70 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (315 mg, 38% over three steps) as an amorphous off-white solid: R_f 0.44 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.29 (s, 9H), 5.80 (s, 2H), 7.25-7.35 (ovlp m, 5H), 7.49 (m, 1H), 7.74 (ap d [ovlp m], 2H), 8.26 (d, J = 7.6 Hz, 1H), 8.99 (s, 1H), 11.60 (D_2O-exchangeable, br s, 1H); ^{13}C NMR (150 MHz, 1:9 D_2O/DMSO-d_6) δ 27.9, 56.2, 82.2, 118.6, 126.2, 126.4, 126.8, 126.9, 128.0, 128.5, 129.2, 129.3, 133.9, 135.9, 139.5, 150.3, 171.3; HRMS (ESI–) calcd for $C_{21}H_{21}N_2O_5S^-$ $[M - H]^-$ 413.1177, found 413.1174 (error 0.7 ppm).

***tert*-Butyl (quinol-4-on-3-yl)sulfonylcarbamate (46).** To a Parr flask flushed with Ar was added Pd/C (10% by weight, 460 mg, 0.432 mmol, 1.00 equiv), *N*-benzyl quinolone **45** (179 mg, 0.432 mmol, 1.00 equiv), anhydrous MeOH (43 mL), and AcOH (27 μL, 0.48 mmol, 1.1 equiv). The reaction vessel was evacuated, then backfilled with H_2 to 40 psi, and the mixture was shaken at 22 °C for 1 h. The reaction mixture was filtered through celite and concentrated to an off-white amorphous solid. Purification by flash chromatography (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (46 mg, 32%) as an off-white solid: R_f 0.20 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.25 (s, 9H), 7.48 (dd, J = 8.2, 7.0 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 8.2, 7.0 Hz, 1H), 8.18 (d, J = 8.2 Hz, 1H), 8.57 (s, 1H), 11.44 (br s, 1H), 12.66 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 27.7, 81.3, 117.1, 119.4, 125.3, 125.4, 126.6, 133.3, 139.3, 144.8, 150.4,

171.3; HRMS (ESI–) calcd for $C_{14}H_{15}N_2O_5S^-$ $[M - H]^-$ 323.0707, found 323.0715 (error 2.5 ppm).

***tert*-Butyl (1-(methoxymethyl)-quinol-4-on-3-yl)sulfonylcarbamate (49).**[25,39] Dimethylformamide dimethylacetal (399 μL, 3.00 mmol, 1.50 equiv) was added to a solution of *β*-ketosulfonamide **34** (635 mg, 2.00 mmol, 1.00 equiv) in THF (10 mL) at 22 °C. The solution was stirred for 1.5 h, then was concentrated under reduced pressure yielding enaminone **39** as a yellow oily residue used directly without purification.

To a stirred solution of enaminone **39** prepared above in THF (6 mL) at 22 °C was added $BnNH_2$ (328 μL, 3.00 mmol, 1.50 equiv). After stirring 10 min the reaction was concentrated under reduced pressure yielding a golden foamy oil. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded a mixture of isomers (~2:1) of the title compound (739 mg, 85% over two steps) as a golden foamy oil: R_f 0.13 (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.46 (*minor*, s, 3H), 1.47 (*major*, s, 6H), 4.47 (*minor*, d, J = 5.3 Hz, 0.67H), 4.66 (*major*, d, J = 5.9 Hz, 1.33H), 6.65 (*major*, m, 0.67H), 7.10-7.25 (ovlp m, 1H), 7.14-7.19 (ovlp m, 1H), 7.24 (*major*, d, J = 7.0 Hz, 0.67H), 7.28 (*major*, t, J = 7.0 × 2 Hz, 0.67H), 7.31-7.43 (ovlp m, 6H), 7.63 (*minor*, m, 0.33H), 8.31 (*major*, d, J = 14.1 Hz, 0.67H); 8.70 (*minor*, br s, 0.33H), 11.03 (*major*, br s, 0.67H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.08, 28.14, 54.0, 54.7, 83.4, 83.6, 108.4, 115.5, 115.6, 116.1, 116.3, 123.77, 123.80, 124.68, 124.70, 127.7, 127.8, 128.51, 128.54, 128.64, 128.65, 128.77, 128.80, 129.2, 129.3, 129.85, 129.88, 131.2, 131.3, 132.15, 132.20, 135.0, 135.2, 149.5, 150.3, 157.5, 157.9, 158.25, 158.26, 159.2, 159.6, 161.9, 185.2, 188.8; ^{19}F NMR (564 MHz,

$CDCl_3$) δ -120.1 (*major*), -117.4; HRMS (APCI–) calcd for $C_{21}H_{22}FN_2O_5S^-$ $[M - H]^-$ 433.1239, found 433.1265 (error 6.0 ppm).

***tert*-Butyl (1-benzyl-quinol-4-on-3-yl)sulfonylcarbamate (45)** via *tert*-butyl (1-(methoxymethyl)-quinol-4-on-3-yl)sulfonylcarbamate (**49**)**.**[39] To a stirred solution of Bn-enaminone **49** (725 mg, 1.67 mmol, 1.00 equiv) in THF (7 mL) at 22 °C was added NaH (60% dispersion in mineral oil, 147 mg, 3.67 mmol, 2.20 equiv) portion-wise (~10 mg portions) such that noticeable gas evolution had ceased before next addition. After stirring 1 h, the reaction was quenched by addition of satd aq NH_4Cl (25 mL). The aqueous mixture was acidified to pH ~4 (pH paper) with aq 6 N HCl and was then extracted with EtOAc (4 × 25 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding an off-white solid (436 mg). Purification by flash chromatography (0.4:0.4:39.2:60 to 1:10:89:0 HCO_2H/MeOH/EtOAc/hexanes gradient) afforded the title compound (351 mg, 51%) as an amorphous off-white solid: Characterization data match that as given above.

***N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)- 5′-*N* -(1-benzyl-quinol-4-on-3-yl)sulfonyl- 2′,3′-*O*-isopropylideneadenosine (50).** To a stirred solution of quinolone **45** (228 mg, 0.550 mmol, 1.10 equiv), bis-Boc isopropylidene adenosine **9** (254 mg, 0.500 mmol, 1.00 equiv), and PPh_3 (144 mg, 0.550 mmol, 1.10 equiv) in THF (50 mL) at 22 °C was added DIAD (108 μL, 0.550 mmol, 1.10) dropwise over 5 min. After stirring 1 h, the reaction was concentrated in vacuo yielding an off-white foamy residue (816 mg). Purification by flash chromatography (40:60 EtOAc/hexanes) afforded the title compound (385 mg, 85%) as an off-white foamy residue: R_f 0.27 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz,

$CDCl_3$) δ 1.32 (s, 9H), 1.41 (s, 18H), 1.47 (s, 3H), 1.66 (s, 3H), 4.34 (dd, *J* = 15.0, 6.2 Hz, 1H), 4.46 (dd, *J* = 15.0, 6.2 Hz, 1H), 4.67 (td, *J* = 6.2 × 2, 3.5 Hz, 1H), 5.21 (dd, *J* = 6.5, 3.5 Hz, 1H), 5.36 (dd, *J* = 6.5, 2.6 Hz, 1H), 5.43 (s, 2H), 6.25 (d, *J* = 2.6 Hz, 1H), 7.19 (d, *J* = 7.0 Hz, 2H), 7.34 (ovlp m, 3H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.45 (t, *J* = 8.2 × 2 Hz, 1H), 7.61 (t, *J* = 7.0 × 2 Hz, 1H), 8.36 (s, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 8.61 (s, 1H), 8.92 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.7, 27.5, 27.9, 28.0, 49.5, 58.0, 82.4, 83.8, 84.3, 84.6, 85.2, 90.6, 115.0, 117.2, 118.6, 126.0, 126.3, 127.5, 128.5, 129.0, 129.5, 129.6, 133.6, 133.8, 139.4, 143.9, 149.4, 150.4, 150.6, 151.1, 152.4, 152.8, 172.2; HRMS (ESI+) calcd for $C_{44}H_{54}N_7O_{12}S^+$ $[M + H]^+$ 904.3546, found 904.3537 (error 1.0 ppm).

***N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)-5′-*N*-(quinol-4-on-3-yl)sulfonyl-2′,3′-*O*-isopropylideneadenosine (51).** To a Parr flask flushed with Ar was added Pd/C (10% by weight, 436 mg, 0.410 mmol, 1.00 equiv), a solution of *N*-benzyl quinolone-AMS **50** (371 mg, 0.410 mmol, 1.00 equiv) in anhydrous MeOH (10 mL), and AcOH (23 μL, 0.41 mmol, 1.0 equiv) respectively. The reaction vessel was evacuated, then backfilled with H_2 to 40 psi, and the mixture was shaken at 22 °C for 4 h. The reaction mixture was filtered through celite and concentrated to an off-white amorphous solid (454 mg). Purification by flash chromatography (65:35 EtOAc/hexanes) afforded the title compound (195 mg, 58%) as an off-white amorphous solid: R_f 0.54 (0.75:0.75:73.5:25 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.12 (s, 9H), 1.35 (s, 18H), 1.37 (s, 3H), 1.57 (s, 3H), 4.09 (dd, *J* = 15.0, 7.6 Hz, 1H), 4.14 (dd, *J* = 15.0, 6.8 Hz, 1H), 4.48 (ap td, *J* = 7.6, 6.8, 3.4 Hz, 1H), 5.24 (dd, *J* = 6.2, 3.4 Hz, 1H), 5.60 (dd, *J* = 6.2, 1.6 Hz, 1H), 6.40 (d, *J* = 1.6 Hz, 1H), 7.47 (t, *J* = 8.2 × 2 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.77 (t, *J* = 8.2 × 2 Hz, 1H), 8.12 (d, *J* = 8.2

Hz, 1H), 8.30 (s, 1H), 8.58 (D_2O-exchangeable [collapses to singlet], d, J = 6.2 Hz, 1H), 8.81 (s, 1H), 8.88 (s, 1H), 12.79 (D_2O-exchangeable, d, J = 6.2 Hz, 1H); ^{13}C NMR (150 MHz, 1:10 D_2O/DMSO-d_6) δ 25.5, 27.2, 27.6, 49.4, 79.3, 82.2, 83.8, 83.90, 83.93, 86.3, 89.5, 113.9, 117.8, 119.5, 125.5, 126.1, 126.5, 128.6, 133.9, 139.2, 144.8, 146.0, 149.6, 150.3, 150.8, 152.0, 152.8, 171.8; HRMS (ESI+) calcd for $C_{37}H_{48}N_7O_{12}S^+$ $[M + H]^+$ 814.3076, found 814.3071 (error 0.6 ppm).

5′-Amino-5′-*N*-[((quinol-4-on)-3-yl)sulfonyl]adenosine (2) via N^6,N^6-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)- 5′-*N* -(quinol-4-on-3-yl)sulfonyl-2′,3′-*O*-isopropylideneadenosine (**51**)**.** To debenzyl quinolone-AMS **51** (97 mg, 0.12 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aq TFA (2 mL). The reaction was stirred 19 h at 0 °C. The reaction was concentrated in vacuo and subsequent purification by flash chromatography (1:10:98 HCO_2H/MeOH/EtOAc) afforded the title compound (40 mg, 70%) as a colorless amorphous solid. Further purification of a portion (4.3 mg) by RP-HPLC (12.5:87.5 MeCN/H_2O) and lyophilization of appropriate fractions afforded the title compound (2 mg) as a fluffy colorless solid: Characterization data matched that as given above.

Compounds from Figure 21

***tert*-Butyl (2-(2-fluorophenyl)-1-(hydroxyimino)-2-oxoethyl)sulfonylcarbamate (52).**[47] To a solution of *β*-ketosulfonamide **34** (635 mg, 2.00 mmol, 1.00 equiv) in AcOH/H_2O/THF (2.5 mL, 2.5 mL, 5.0 mL respectively) at 0 °C was added $NaNO_2$ (276 mg, 4.00 mmol, 2.00 equiv). After stirring 19 h, the reaction mixture was diluted with H_2O (50 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layer was dried ($MgSO_4$) and concentrated, yielding a light yellow oil. Purification by flash

chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (513 mg, 74%) as an off-white foamy residue: R_f 0.40 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, CD_3OD) δ 1.48 (s, 9H), 7.25 (dd, J = 10.6, 8.8 Hz, 1H), 7.36 (ap t, J = 7.6 Hz × 2, 1H), 7.71 (dddd, J = 8.8, 7.0, 5.3, 1.8 Hz, 1H), 7.92 (td, J = 7.6 × 2, 1.8 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 28.2, 84.6, 118.0 (d, $^2J_{C\text{-}F}$ = 22.0 Hz), 124.7 (d, $^2J_{C\text{-}F}$ = 8.1 Hz), 125.9 (d, $^3J_{C\text{-}F}$ = 3.5 Hz), 132.7, 138.1 (d, $^3J_{C\text{-}F}$ = 9.2 Hz), 151.5, 155.3, 163.5 (d, $^1J_{C\text{-}F}$ = 261.3 Hz), 184.9; ^{19}F NMR (564 MHz, $CDCl_3$) δ -115.3; HRMS (ESI–) calcd for $C_{13}H_{14}FN_2O_6S^-$ $[M - H]^-$ 345.0562, found 345.0569 (error 2.0 ppm).

***tert*-Butyl (4-oxo-4*H*-benzo[*e*][1,2]oxazin-3-yl)sulfonylcarbamate (53).**[48] To a solution of oxime **52** (228 mg, 0.657 mmol, 1.00 equiv) in DMF (3.5 mL) at 22 °C was added Cs_2CO_3 (471 mg, 1.45 mmol, 2.20 equiv). After stirring 4.5 h the reaction mixture was quenched with satd aq NH_4Cl (20 mL). The aqueous solution was acidified to pH ~4-5 (pH paper) with aq 6 N HCl and was then extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a pale yellow residue. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (124 mg, 58%) as a pale yellow residue: R_f 0.57 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.42 (s, 9H), 7.54 (ap t, J = 7.9, 7.3 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.90 (ap t, J = 8.2, 7.3 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.58 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 27.8, 85.3, 116.9, 121.2, 125.4, 127.5, 137.3, 149.1, 156.2, 162.0, 163.6; HRMS (ESI–) calcd for $C_{13}H_{13}N_2O_6S^-$ $[M - H]^-$ 325.0500, found 325.0507 (error 2.2 ppm).

N^6,N^6-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)- 5′-*N* -(4-oxo-4*H*-benzo[*e*][1,2]oxazin-3-yl)sulfonyl- 2′,3′-*O*-isopropylideneadenosine (54). To a solution of benzoxazine sulfonamide **53** (45.4 mg, 0.139 mmol, 1.10 equiv), bis-Boc isopropylidene adenosine **9** (64 mg, 0.13 mmol, 1.0 equiv), and PPh_3 (36 mg, 0.14 mmol, 1.1 equiv) in THF (5 mL) was added DIAD (27 μL, 0.14 mmol, 1.1 equiv). After 4 h stirring, LRMS monitoring of the reaction suggested limiting reagent **9** remained; more PPh_3 (36 mg, 0.14 mmol, 1.1 equiv) and DIAD (27 μL, 0.14 mmol, 1.1 equiv) were added. After a further 19 h stirring, LRMS monitoring of the reaction suggested limiting reagent **9** still remained; more PPh_3 (36 mg, 0.14 mmol, 1.1 equiv) and DIAD (27 μL, 0.14 mmol, 1.1 equiv) were added. After a final 7.5 h stirring, LRMS monitoring suggested the total consumption of **9**. The reaction was concentrated to an off-white residue. Purification by flash chromatography (30:70 EtOAc/hexanes) afforded the title compound (46 mg, 45%) as an off-white oily residue: R_f 0.53 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.40 (s, 18H), 1.41 (s, 3H), 1.64 (s, 3H), 4.18 (dd, J = 14.7, 7.6 Hz, 1H), 4.25 (dd, J = 14.7, 5.3 Hz, 1H), 4.69 (m, 1H), 5.23 (dd, J = 6.2, 2.9 Hz, 1H), 5.49 (dd, J = 5.9, 1.8 Hz, 1H), 6.22 (d, J = 1.8 Hz, 1H), 7.53 (t, J = 7.9 × 2 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.88 (ap td, J = 8.5, 7.9, 1.2 Hz, 1H), 8.11 (dd, J = 7.9, 1.2 Hz, 1H), 8.22 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.6, 27.3, 27.89, 27.93, 49.5, 82.6, 83.8, 84.6, 85.8, 86.3, 91.0, 114.8, 116.9, 121.2, 125.4, 127.4, 128.4, 129.7, 137.1, 144.3, 150.4, 150.6, 152.2, 152.7, 157.1, 161.9, 163.3; HRMS (ESI+) calcd for $C_{36}H_{46}N_7O_{13}S^+$ $[M + H]^+$ 816.2869, found 816.2851 (error 2.2 ppm).

5′-Amino-5′-*N*-[(4-oxo-4*H*-benzo[*e*][1,2]oxazin-3-yl)sulfonyl]adenosine (3). To Mitsunobu product **54** (47 mg, 0.058 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aq TFA (2 mL). The reaction was stirred 19 h at 0 °C. The reaction was concentrated in vacuo and subsequent purification by flash chromatography (1:1:98 HCO_2H/MeOH/EtOAc) afforded the title compound (22 mg, 81%) as a colorless amorphous solid. Further purification by RP-HPLC (17.5:82.5 MeCN/H_2O) and lyophilization of appropriate fractions afforded the title compound (12 mg) as a fluffy colorless solid: R_f 0.32 (1:10:89 HCO_2H/MeOH/EtOAc); ^{1}H NMR (600 MHz, 1:10 D_2O/DMSO-d_6) δ 3.42-3.46 (ovlp m, 2H), 4.03 (m, 1H), 4.07 (dd, J = 5.6, 2.9 Hz, 1H), 4.58 (t, J = 6.2, 5.6 Hz, 1H), 5.67 (d, J = 6.2 Hz, 1H), 7.51 (dd, J = 8.2, 7.6 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.86 (dd, J = 8.2, 7.6 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 8.12 (s, 1H), 8.19 (s, 1H); ^{13}C NMR (150 MHz, 1:10 D_2O/DMSO-d_6) δ 46.0, 71.2, 73.0, 84.0, 88.2, 116.7, 119.5, 120.8, 124.8, 127.2, 137.2, 140.6, 148.9, 152.4, 156.0, 157.4, 161.4, 164.4; HRMS (ESI+) calcd for $C_{18}H_{18}N_7O_7S^+$ $[M + H]^+$ 476.0983, found 476.0996 (error 2.7 ppm).

Compounds from Figure 22

Methyl 2,3-dihydroxybenzoate (55).[49] To a solution of 2,3-dihydroxybenzoic acid (3.08 g, 20.0 mmol, 1.00 equiv) in methanol (20 mL) at 0 °C was added $SOCl_2$ (1.6 mL, 22 mmol, 1.1 equiv). The solution was heated at 80 °C. After 21 h, the solution was cooled to 22 °C and diluted with EtOAc (20 mL). The solution was then cooled to 0 °C and the acidity quenched by addition of satd aq $NaHCO_3$ (20 mL). The layers were separated and more satd aq $NaHCO_3$ added to the aqueous layer to increase its pH (~9-10). The aqueous layer was subsequently extracted with EtOAc (3 × 20 mL). The

combined organic layer was dried ($MgSO_4$) and concentrated, yielding a colorless amorphous solid. Purification by flash chromatography (0:100 to 1:99 MeOH/DCM gradient) afforded the title compound (3.02 g, 90%) as an off-white amorphous solid: R_f 0.69 (0.25:0.25:24.5:75 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.88 (s, 3H), 6.74 (t, J = 7.9 × 2 Hz, 1H), 7.03 (dd, J = 7.9, 1.5 Hz, 1H), 7.23 (dd, J = 7.9, 1.5 Hz, 1H), 9.40 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 52.4, 113.0, 118.9, 119.6, 120.7, 146.1, 149.5, 169.9; HRMS (ESI–) calcd for $C_8H_7O_4^-$ [M – H]$^-$ 167.0350, found 167.0342 (error 4.8 ppm).

Methyl 2,3-bis((*tert*-butyldimethylsilyl)oxy)benzoate (56).[54] To a solution of catechol **55** (2.95 g, 17.5 mmol, 1.00 equiv) in DMF (35 mL) was added imidazole (6.20 g, 91.1 mmol, 5.20 equiv) and TBSCl (7.92 g, 52.6 mmol, 3.00 equiv). The solution was heated at 65 °C. After 19 h, the solution was cooled to ambient temperature and diluted with 5% aq $NaHCO_3$ (100 mL), extracted with hexanes (3 × 100 mL), dried ($MgSO_4$) and concentrated yielding a colorless oil (8.77 g). Purification by flash chromatography (2:98 EtOAc/hexanes) afforded the title compound (6.93 g, 99%) as a colorless oil: R_f 0.37 (0.025:0.025:2.45:97.5 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.03 (s, 6H), 0.22 (s, 6H), 0.94 (s, 9H), 0.95 (s, 9H), 3.76 (s, 3H), 6.93 (ap t/dd, J = 7.6, 8.2 Hz, 1H), 7.07 (d, J = 8.2 Hz, 1H), 7.19 (d, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ -4.4, -3.9, 17.9, 18.3, 25.7, 25.9, 51.8, 121.4, 122.9, 123.9, 125.3, 145.1, 147.6, 166.7; HRMS (ESI+) calcd for $C_{20}H_{37}O_4Si_2^+$ [M + H]$^+$ 397. 2225, found 397.2226 (error 0.3 ppm).

***tert*-Butyl (2-(2,3-dihydroxyphenyl)-2-oxoethyl)sulfonylcarbamate (58).**[17] Freshly titrated *n*-BuLi (2.1 M in hexane, 11.0 mL, 23.2 mmol, 3.10 equiv) was added dropwise

to freshly distilled (i-Pr)$_2$NH (3.5 mL, 24.8 mmol, 3.30 equiv) in THF (24 mL) at 0 °C. The mixture was stirred for 1.5 h, then sulfonamide **36** (1.46 g, 7.50 mmol, 1.00 equiv) in THF (24 mL) was added and the reaction stirred for a further 1.5 h at 0 °C. Next, methyl ester **56** (3.25 g, 8.20 mmol, 1.10 equiv) was added and the reaction was stirred for 1.5 h at 0 °C. The reaction mixture was quenched with satd aq NaCl (25 mL) and 0.5 M aq NaH_2PO_4 (25 mL) and was diluted with EtOAc (25 mL). The layers were separated and the aqueous layer acidified to pH ~3-4 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a golden foamy residue (4.73 g). The residue was dissolved in THF (24 mL) and cooled to 0 °C. TBAF (1.0 M in THF, 18.0 mL, 18.0 mmol, 2.40 equiv) was added and the solution stirred at 0 °C for 1 h. The reaction mixture was quenched with satd aq NH_4Cl (25 mL). The layers were separated and the aqueous layer acidified to pH ~3-4 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (3 × 40 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a dark oily residue (7.25 g). Purification by flash chromatography (0.3:0.3:29.4:70 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (2.05 g, 82%) as a yellow solid: R_f 0.69 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.43 (s, 9H), 5.16 (s, 2H), 6.79 (ap t, J = 7.6, 8.2 Hz, 1H), 7.09 (d, J = 7.6 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 9.69 (br s, 1H), 10.70-11.90 (ovlp br s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 27.8, 59.7, 82.5, 119.1, 121.5, 121.6, 121.7, 146.3, 150.0, 150.7, 193.0; HRMS (ESI–) calcd for $C_{13}H_{16}NO_7S^-$ $[M - H]^-$ 330.0653, found 330.0662 (error 2.7 ppm).

***tert*-Butyl (8-hydroxychromon-3-yl)sulfonylcarbamate (60).**[24,25] *N*,*N*-Dimethylformamide dimethylacetal (638 μL, 4.80 mmol, 2.40 equiv) was added to a solution of *β*-ketosulfonamide **58** (663 mg, 2.00 mmol, 1.00 equiv) in THF (20 mL) at 22 °C. The solution was stirred for 18 h, then was acidified with satd aq NH_4Cl (25 mL) and diluted with EtOAc (25 mL). The layers were separated and the aqueous layer acidified to pH ~5-6 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (2 × 25 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a golden foamy residue. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (536 mg, 79%) as an off-white amorphous solid: R_f 0.48 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.29 (s, 9H), 7.35 (dd, J = 7.6, 1.2 Hz, 1H), 7.39 (t, J = 7.6 × 2 Hz, 1H), 7.52 (dd, J = 7.6, 1.2 Hz, 1H), 9.07 (s, 1H), 10.91 (s, 1H), 11.94 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 27.5, 82.2, 114.3, 120.6, 122.8, 125.0, 127.0, 145.1, 147.2, 149.7 162.6, 170.7; HRMS (ESI–) calcd for $C_{14}H_{14}NO_7S^-$ $[M - H]^-$ 340.0496, found 340.0529 (error 9.7 ppm).

***tert*-Butyl (8-(methoxymethoxy)chromon-3-yl)sulfonylcarbamate (61).**[50] To a solution of hydroxy chromone **60** (465 mg, 1.36 mmol, 1.00 equiv) and DIPEA (0.30 mL, 1.7 mmol, 1.3 equiv) in DMF (10 mL) at 0 °C was added MOMCl (124 μL, 1.63 mmol, 1.20 equiv). The reaction was stirred 25 h and was diluted with DCM (100 mL). The solution was washed with H_2O (2 × 100 mL) and saturated aq NaCl (100 mL). The organic layer was dried ($MgSO_4$) and concentrated yielding a yellow-orange solid (480 mg). Purification by flash chromatography (0.3:0.3:29.4:70 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (166 mg, 32%) as an off-

white amorphous solid: R_f 0.51 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.30 (s, 9H), 3.35 (s, 3H), 5.22 (s, 2H), 7.37 (dd, J = 7.6, 1.2 Hz, 1H), 7.40 (t, J = 7.6 × 2 Hz, 1H), 7.50 (dd, J = 7.6, 1.2 Hz, 1H), 9.21 (s, 1H), 10.99 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 27.3, 56.1, 78.8, 84.6, 114.2, 120.8, 123.2, 124.8, 127.2, 145.2, 147.3, 150.0, 162.8, 171.0; HRMS (ESI–) calcd for $C_{16}H_{18}NO_8S^-$ $[M - H]^-$ 384.0759, found 384.0790 (error 8.1 ppm).

***N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)- 5′-*N* -((8-(methoxymethoxy)chromon-3-yl)sulfonyl- 2′,3′-*O*-isopropylideneadenosine (62).** To a solution of chromone sulfonamide **61** (42 mg, 0.11 mmol, 1.1 equiv), adenosine **9** (51 mg, 0.10 mmol, 1.0 equiv), and PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) in THF (5 mL) at 22 °C was added DIAD (22 μL, 0.11 mmol, 1.1 equiv). After 2 h stirring, TLC monitoring of the reaction suggested limiting reagent **9** remained; more PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) and DIAD (22 μL, 0.11 mmol, 1.1 equiv) were added. After another 2.5 h stirring, the reaction appeared complete by TLC. The reaction mixture was concentrated to an off-white residue. Purification by flash chromatography (40:60 EtOAc/hexanes) afforded the title compound (24.4 mg, 28%) as an off-white amorphous solid: R_f 0.40 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.41 (s, 9H), 1.47 (s, 18H), 1.68 (s, 3H), 3.51 (s, 3H), 4.34 (br s, 1H), 4.46 (br s, 1H), 4.76 (br s, 1H), 5.27 (br s, 1H), 5.38 (s, 2H), 5.45 (br s, 1H), 6.31 (br s, 1H), 7.18 (br s, 1H), 7.37 (br s, 1H), 7.77 (br d, J = 6.5 Hz, 1H), 8.28 (br s, 1H), 8.79 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.6, 27.4, 28.0, 28.1, 49.5, 57.0, 77.5, 78.2, 79.9, 81.6, 84.2, 85.2, 91.0, 115.2, 117.4, 117.8, 124.8, 125.8, 126.9, 130.9, 134.9, 146.5, 147.8, 150.6, 150.7,

151.0, 152.4, 152.5, 161.6, 171.4; HRMS (ESI+) calcd for $C_{39}H_{51}N_6O_{15}S^+$ $[M + H]^+$ 875.3128, found 875.3136 (error 0.9 ppm).

5′-Amino-5′-*N*-[(8-hydroxychromon-3-yl)sulfonyl]adenosine (4). To Mitsunobu product **62** (24 mg, 0.027 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aq TFA (2.5 mL). The reaction was stirred 18 h at 0 °C. The reaction was concentrated in vacuo and subsequent purification by flash chromatography (1:1:98 to 1:4:95 HCO_2H/MeOH/EtOAc gradient) afforded the title compound (8.9 mg, 66%) as a colorless amorphous solid. Further purification by RP-HPLC (12.5:87.5 MeCN/H_2O) and lyophilization of appropriate fractions afforded the title compound (7.7 mg) as a fluffy colorless solid: R_f 0.22 (1:20:79 HCO_2H/MeOH/EtOAc); ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.33 (td, J = 5.3 × 2, 2.9 Hz, 1H), 4.41 (q, J = 5.3 × 3 Hz, 1H), 4.45 (dd, J = 11.2, 5.3 Hz, 1H), 4.49 (dd, J = 11.2, 2.9 Hz, 1H), 4.75 (q, J = 5.3 × 3 Hz, 1H), 5.44 (D_2O-exchangeable, d, J = 5.3 Hz, 1H), 5.63 (D_2O-exchangeable, d, J = 5.3 Hz, 1H), 6.01 (d, J = 5.3 Hz, 1H), 7.27 (D_2O-exchangeable, br s, 2H), 7.38 (D_2O-exchangeable, br s, 2H), 7.49 (t, J = 7.6 × 2 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 7.6 Hz, 1H), 8.15 (s, 1H), 8.38 (s, 1H), 8.97 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 69.2, 70.4, 73.3, 82.0, 87.5, 115.9, 117.4, 124.9, 126.5, 127.4, 132.7, 145.9, 147.7, 149.5, 152.7, 156.0, 158.0, 171.2, 171.6; HRMS (ESI+) calcd for $C_{19}H_{19}N_6O_8S^+$ $[M + H]^+$ 491.0980, found 491.0995 (error 3.1 ppm).

Methyl 2-((*tert*-butyldimethylsilyl)oxy)benzoate (63).[55] TBSCl (3.01 g, 20.0 mmol, 3.00 equiv) was added to a solution of methyl salicylate (862 μL, 6.65 mmol, 1.00 equiv), imidazole (1.81 g, 26.6 mmol, 4.00 equiv), and DMAP (8 mg, 0.07 mmol, 0.01 equiv) in DMF (10 mL) at 0 °C. After stirring 1 h, the homogeneous solution had become a thick

suspension. The ice bath was removed and the reaction stirred a further 21.5 h. 5% aq $NaHCO_3$ (100 mL) was added to the reaction and the resulting aqueous solution was extracted with Et_2O (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated yielding a colorless oil which was purified by flash chromatography (1:99 to 10:90 Et_2O/hexanes) yielding the title compound (1.41 g, 79%) as a colorless oil: R_f 0.19 (1:99 EtOAc/hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.21 (s, 6H), 1.01 (s, 9H), 3.86 (s, 3H), 6.87 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.98 (ddd, *J* = 7.6 × 2, 1.2 Hz, 1H), 7.35 (ddd, *J* = 8.2, 7.6, 1.8 Hz, 1H), 7.75 (dd, *J* = 7.6, 1.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ -4.2, 18.4, 25.8, 52.0, 121.0, 121.3, 123.0, 131.7, 133.1, 155.2, 167.5; HRMS (ESI+) calcd for $C_{14}H_{23}O_3Si^+$ $[M + H]^+$ 267.1411, found 267.1424 (error 4.9 ppm).

***tert*-Butyl (2-(2-hydroxyphenyl)-2-oxoethyl)sulfonylcarbamate (65).**[17] Freshly titrated *n*-BuLi (2.1 M in hexane, 5.0 mL, 11 mmol, 3.1 equiv) was added dropwise to freshly distilled (*i*-Pr)$_2$NH (1.6 mL, 11 mmol, 3.3 equiv) in THF (10 mL) at 0 °C. The mixture was stirred for 30 min, then sulfonamide **36** (666 mg, 3.41 mmol, 1.00 equiv) in THF (10 mL) was added and the reaction stirred for a further 1 h at 0 °C. Next, methyl ester **63** (1.00 g, 3.75 mmol, 1.10 equiv) in THF (2 mL) was added and the reaction was stirred for 3 d at 0 °C. The reaction mixture was quenched with satd aq NaCl (10 mL) and 0.5 M aq NaH_2PO_4 (10 mL) and was diluted with EtOAc (10 mL). The layers were separated and the aqueous layer acidified to pH ~5-6 (pH paper) with 6 N aq HCl. The aqueous was then extracted with EtOAc (2 × 100 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a golden oily residue (2.19 g). The residue was dissolved in THF (10 mL) and cooled to 0 °C. TBAF (1.0 M in THF, 8.2 mL, 8.2 mmol, 2.4 equiv) was added and the solution stirred at

0 °C for 17 h. The reaction mixture was quenched with satd aq NH_4Cl (25 mL) and was diluted with Et_2O (25 mL). The layers were separated and the aqueous layer acidified to pH ~5-6 (pH paper) with 6 N aq HCl. The aqueous was then extracted with Et_2O (25 mL) and EtOAc (50 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a golden oil (2.40 g). Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (930 mg, 86%) as an off-white solid: R_f 0.83 (0.5:0.5:49:50 HCO_2H/MeOH/EtOAc/hexanes); ^1H NMR (600 MHz, $CDCl_3$) δ 1.53 (s, 9H), 4.99 (s, 2H), 6.98 (dd, J = 8.2, 7.6 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.57 (dd, J = 8.2, 7.6 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 11.67 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.1, 57.9, 85.3, 119.0, 119.1, 119.9, 131.1, 138.4, 149.5, 163.5, 193.2; HRMS (APCI–) calcd for $C_{13}H_{16}NO_6S^-$ $[M - H]^-$ 314.0704, found 314.0732 (error 8.9 ppm).

***tert*-Butyl (chromon-3-yl)sulfonylcarbamate (8)** via *tert*-butyl (2-(2-hydroxyphenyl)-2-oxoethyl)sulfonylcarbamate (**65**).[24,25] *N*,*N*-Dimethylformamide dimethylacetal (638 μL, 4.80 mmol, 2.40 equiv) was added to a solution of *β*-ketosulfonamide **65** (631 mg, 2.00 mmol, 1.00 equiv) in THF (20 mL) at 22 °C. The solution was stirred for 18 h, then was acidified with satd aq NH_4Cl (25 mL) and diluted with EtOAc (25 mL). The layers were separated and the aqueous layer acidified to pH ~2-3 (pH paper) with aq 6 N HCl. The aqueous was then extracted with EtOAc (2 × 25 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated under reduced pressure yielding a golden foamy residue. Purification by flash chromatography (0.2:0.2:19.6:80 HCO_2H/MeOH/EtOAc/hexanes) afforded the title compound (484 mg,

74%) as an off-white amorphous solid: Characterization data matched that as given above.

***N*^{6},*N*^{6}-Bis(*tert*-butoxycarbonyl)-5′-amino-5′-*N*-(*tert*-butoxycarbonyl)- 5′-*N* -(chromon-3-yl)sulfonyl- 2′,3′-*O*-isopropylideneadenosine (10)** via *β*-ketosulfonamide **65.** To a stirred solution of Boc-protected chromone sulfonamide **8** (36 mg, 0.11 mmol, 1.1 equiv), bis-Boc adenosine **9** (51 mg, 0.10 mmol, 1.0 equiv) and PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) in THF (5 mL) at 0 °C was added DIAD (22 μL, 0.11 mmol, 1.1 equiv) in four portions over 5 min. The reaction was stirred 30 min at 0 °C and was then allowed to warm to 22 °C. TLC monitoring showed remaining adenosine after 4.5 h, so more PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) and DIAD (22 μL, 0.11 mmol, 1.1 equiv) were added. The reaction was stirred a further 4 h. TLC monitoring showed remaining adenosine, so more PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) and DIAD (22 μL, 0.11 mmol, 1.1 equiv) were added. The reaction was stirred 17.5 hours overnight. TLC monitoring showed remaining adenosine, so more PPh_3 (29 mg, 0.11 mmol, 1.1 equiv) and DIAD (22 μL, 0.11 mmol, 1.1 equiv) were added. The reaction was stirred another 4 h then was concentrated in vacuo. Purification by flash chromatography (40:60 EtOAc/hexanes) afforded the title compound (59 mg, 73%) as a colorless oil: Characterization data matched that as given above.

5′-Amino-5′-*N*-[(chromon-3-yl)sulfonyl]adenosine (1) via *β*-ketosulfonamide **65.** To Mitsunobu product **10** (54 mg, 0.066 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aq TFA (2.5 mL). The reaction was stirred 17 h at 0 °C. The reaction was concentrated in vacuo and subsequent purification by flash chromatography (1:1:98 to 1:3:96 HCO_2H/MeOH/EtOAc gradient) afforded the title compound (18 mg, 59%) as a colorless

amorphous solid. Further purification by RP-HPLC (15:85 MeCN/H_2O) and lyophilization of appropriate fractions afforded the title compound (12 mg) as a fluffy colorless solid: Characterization data matched that as given above.

Biochemical Evaluation.

Cloning, Overexpression, and Purification of MbtA. Cloning of the *mbtA* gene, creation of plasmid pCDD003, and electroporation of pCDD003 into *E. coli* BL21(DE3) containing the *groEL groES* chaperone plasmid pGRO7 (Takara) has been previously described.[18] Representative procedure for overexpression and purification, performed with the assistance of Daniel J. Wilson: Glycerol stocks of the aforementioned *E. coli* were plated on LB media supplemented with kanamycin (50 μg/mL) and chloramphenicol (25 μg/mL) and were incubated at 37 °C overnight. LB (2 × 5 mL) supplemented with kanamycin (50 μg/mL) and chloramphenicol (25 μg/mL) was inoculated with above colonies and was incubated with shaking at 37 °C overnight. LB (1 L) supplemented with kanamycin (50 μg/mL), chloramphenicol (25 μg/mL), $MgCl_2$ (10 mM), and arabinose (0.5 mg/mL) was inoculated with the 10 mL of overnight culture and was incubated with shaking at 37 °C to an OD_{600} of 0.67. The culture was induced with 0.4 mM IPTG and grown for an additional 3 h at 30 °C. The cultures were then centrifuged for 10 min at 5000 × g and 4 °C and resultant pellets frozen at -20 °C overnight. The pellets were resuspended in 22.5 mL GroEL stripping buffer A (100 mM Bicine, 170 mM NaCl, pH 7.6) and sonicated (Branson Sonifier 250, output 8, 30% for 4 × 2 min). The lysate was centrifuged for 10 min at 45000 × g and 4 °C. Subsequently, 2.5 mL GroEL stripping buffer B (100 mM Bicine, 200 mM $MgCl_2$, pH 7.6) and 138 mg ATP were added to the supernatant and the pH was adjusted from ~5.5 to 7.6 with 1 N

NaOH. The lysate was incubated for 30 min at 4 °C. To ensure removal of GroEL, 140 μL of denatured *E. coli* proteins prepared as previously described[56] was added and the lysate was allowed to incubate for another 1 h at 4 °C. 2 mL of 50% Ni–NTA (Qiagen) was added to the lysate and it was incubated on an end-over-end mixer for 1 h at 4 °C. The lysate/Ni–NTA mixture was poured into a column and the flow through collected. The column was washed with 14 mL wash buffer (50 mM HEPES, 300 mM NaCl, 20 mM imidazole, pH 8.0) and MbtA was then eluted with 3 mL of elution buffer (50 mM HEPES, 300 mM NaCl, 250 mM imidazole, pH 8.0) of which the first 0.5 mL (half of the dead volume) was discarded and the final 2.5 mL was collected. The MbtA solution was desalted on a PD-10 column (GE Healthcare) into MbtA storage buffer (10 mM Tris•HCl, 1 mM EDTA, 5% glycerol, pH 8.0) and stored overnight at -80 °C. To the 3.4 mL solution of MbtA-SUMO in storage buffer was added 21 mg Tris•HCl to increase its concentration from 10 to 50 mM, 30 mg NaCl resulting in a concentration of 150 mM, 1.5 mg $MgCl_2$ to suppress the effects of 1 mM EDTA, and 680 μL 1% Igepal CA-630 (Sigma) resulting in a final concentration of 0.2%. The solution's pH was adjusted to 8.0. SUMO protease[57] was added to make the solution 0.01 μM protease for each 1 μM MbtA-SUMO. The reaction was incubated for 15 h at 4 °C. After digestion, 0.5 mL of 50% Ni–NTA was added to the sample to remove the SUMO tag, SUMO protease, and other *E. coli* proteins carried through purification. The mixture was incubated for 1 h at 4 °C on an end-over-end mixer. The mixture was poured into a column and the flow through was collected. The resin was washed with SUMO buffer (50 mM Tris•HCl, 0.2% Igepal CA-630, 150 mM NaCl) and fractions containing MbtA were pooled. The protein was desalted on a PD-10 column into MbtA storage buffer. The protein concentration

was measured by the Bradford Assay (Bio-Rad) as 4.1 μM. The enzyme was stored at -80 °C.

Cloning, Overexpression, and Purification of BasE, EntE, and VibE. The cloning, overexpression, and purification of BasE,[51] EntE,[51,58] and VibE[51,59] was performed by Daniel J. Wilson and has been previously described.

Fluorescence Polarization Assays. Some of these assays were performed by Daniel J. Wilson using a modification of our previously described protocol.[51] Briefly, FP measurements were performed on a Molecular Devices SpectraMax M5e with excitation and emission wavelengths of 485 and 530 nm, respectively, using PMT sensitivity set to high and 100 readings per well. Assays were performed in triplicate in flat bottom, black polystyrene 384-well plates (3575 Corning Inc.) in a final volume of 50 μL. To determine the equilibrium dissociation constant K_{D1} of our fluorescent probe Fl-Sal-AMS, a direct binding experiment in which the probe was titrated with enzyme was performed. Specifically, a three-fold serial dilution of enzyme (10 μL, from ~0.1-1000 nM MbtA and EntE and ~2-2000 nM BasE and VibE final concentrations) was added to a 40 μL solution of Fl-Sal-AMS (20 nM final concentration), FP buffer (30 mM Tris•HCl [pH 7.5], 1 mM $MgCl_2$, 0.0025% Igepal CA-630, and 1 mM final concentrations), and water. The fluorescence anisotropy was measured after a 30 min incubation at 22 °C. Experimentally measured anisotropies A_{OBS} were fit to Equations 1 and 2 (below) using Mathmatica 8 (Wolfram Research Inc.) to give the K_{D1}. To determine the equilibrium dissociation constant K_{D2} of each compound, a competitive binding experiment in which each was titrated into Fl-Sal-AMS and enzyme was performed. Specifically, a three-fold serial dilution of each compound (0.5 μL, ~1-100000 nM final concentrations) was added

to a 49.5 μL solution of Fl-Sal-AMS (20 nM final concentration), enzyme (50 nM MbtA and 200 nM BasE, EntE, and VibE final concentrations), FP buffer, and water. The fluorescence anisotropy was measured after a 30 min incubation at 22 °C. Displacement curves of measured fluorescent anisotropies versus varied compound concentrations were fit to Equations 1 and 3 to give the K_{D2}.

$$(1)\ A_{OBS} = \frac{QF_{SB}A_B + (1-F_{SB})A_F}{1-(1-Q)F_{SB}}$$

$$(2)\ F_{SB} = \frac{K_{D1} + L_{ST} + R_T - \sqrt{(K_{D1} + L_{ST} + R_T)^2 - 4L_{ST}R_T}}{2L_{ST}}$$

$$(3)\ F_{SB} = \frac{2\sqrt{(a^2 - 3b)}\cos(\theta/3) - a}{3K_{D1} + 2\sqrt{(a^2 - 3b)}\cos(\theta/3) - a}$$

with

$$a = K_{D1} + K_{D2} + L_{ST} + L_T - R_T$$

$$b = (L_T - R_T)K_{D1} + (L_{ST} - R_T)K_{D2} + K_{D1}K_{D2}$$

$$c = K_{D1}K_{D2}R_T$$

$$\theta = \arccos\left[\frac{-2a^3 + 9ab - 27c}{2\sqrt{(a^2 - 3b)^3}}\right]$$

[^{32}P]PP$_i$-ATP Exchange Assay. This assay was performed by Daniel J. Wilson as previously described.[18] Briefly, reactions were performed under initial velocity conditions in a total volume of 101 μL. The reaction was set up in a volume of 90 μL and contained 250 μM salicylic acid (SAL), 10 mM ATP, 1 mM PP$_i$, and 7 nM MbtA in assay buffer (75 mM Tris•HCl [pH 7.5], 10 mM $MgCl_2$, 2 mM DTT). The inhibitors (1 μL) in DMSO or DMSO only as a control were added. The reaction components were allowed to equilibrate for 10 min at 22 °C. Reactions were initiated by the addition of 10 μL (0.5 μCi ^{32}PP$_i$, Perkin-Elmer 84.12Ci/mmol) in 50 mM sodium phosphate buffer (pH

7.8) and placed at 37 °C for 20 min. Reactions were quenched by the addition of 200 μL quenching buffer (350 mM $HClO_4$, 100 mM PP_i, 1.8% w/v activated charcoal). The charcoal was pelleted by centrifugation and washed once with 500 μL water. The washed pellet was resuspended in 200 μL water, transferred to a scintillation vial, mixed with 15 mL scintillation fluid (RPI), and counted on a Beckman LS6500. The counts from the bound γ-[^{32}P]-ATP were directly proportional to initial velocity of the reaction and the data were fit to Morrison's quadratic equation (Equation 4 below) for fitting concentration–response data for tight binding inhibitors as described by Copeland.[60]

$$(4)\ \frac{v_i}{v_0} = 1 - \frac{\left([E]_T + [I]_T + K_i^{app}\right) - \sqrt{([E]_T + [I]_T + K_i^{app})^2 - 4[E]_T[I]_T}}{2[E]_T}$$

Antitubercular Evaluation.

***M. tuberculosis* H37Rv MIC Assay.** This assay was performed by Helena I. Boshoff as previously described.[14] Briefly, MICs were determined in quadruplicate in iron-deficient GAST according to the broth microdilution method[5] using drugs from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. All measurements reported herein used an initial cell density of 10^4–10^5 cells/assay, and growth was monitored at 10 and at 14 days, with the untreated and DMSO-treated control cultures reaching an OD_{620} ~0.2–0.3. Plates were incubated at 37 °C (100 μL/well) and growth was recorded by measurement of optical density at 620 nm.

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