JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Synthesis of Chromone, Quinolone, and Benzoxazinone Sulfonamide Nucleosides as Conformationally Constrained Inhibitors of Adenylating Enzymes Required for Siderophore Biosynthesis

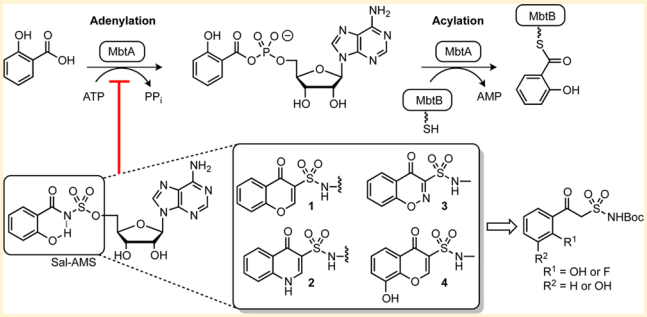
Curtis A. Engelhart and Courtney C. Aldrich*

Center for Drug Design, University of Minnesota, Minneapolis, Minnesota 55455, United States

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, United States

Supporting Information

ABSTRACT: MbtA catalyzes the first committed step of mycobactin biosynthesis in *Mycobacterium tuberculosis* (*Mtb*) and is responsible for the incorporation of salicylic acid into the mycobactin siderophores. 5′-*O*-[*N*-(Salicyl)sulfamoyl]-adenosine (Sal-AMS) is an extremely potent nucleoside inhibitor of MbtA that possesses excellent activity against whole-cell *Mtb* but suffers from poor bioavailability. In an effort to improve the bioavailability, we have designed four conformationally constrained analogues of Sal-AMS that remove two rotatable bonds and the ionized sulfamate group on the basis of computational and structural studies. Herein we describe the synthesis, biochemical, and microbiological evaluation of chromone-, quinolone-, and benzoxazinone-3-sulfonamide derivatives of Sal-AMS. We developed new chemistry to assemble these three heterocycles from common β-ketosulfonamide intermediates. The synthesis of the chromone- and quinolone-3-sulfonamide intermediates features formylation of a β-ketosulfonamide employing dimethylformamide dimethyl acetal to afford an enaminone that can react intramolecularly with a phenol or intermolecularly with a primary amine via addition−elimination reaction(s). The benzoxazinone-3-sulfonamide was prepared by nitrosation of a β-ketosulfonamide followed by intramolecular nucleophilic aromatic substitution. Mitsunobu coupling of these bicyclic sulfonamides with a protected adenosine derivative followed by global deprotection provides a concise synthesis of the respective inhibitors.



INTRODUCTION

Tuberculosis (TB) is caused primarily by the acid-fast bacillus *Mycobacterium tuberculosis* (*Mtb*) and is the leading cause of bacterial infectious disease mortality, responsible for 1.4 million deaths and 8.7 million new infections in 2011.[1] It is estimated that one-third of the world's population is infected with latent TB.[1] The current standard of treatment for drug-susceptible TB, known as directly observed treatment short-course, requires six to nine months of combination chemotherapy of the four frontline TB agents: isoniazid, rifampin, pyrazinamide, and ethambutol. The emergence of multidrug resistant and extensively drug resistant TB strains demands the development of new drugs ideally with novel mechanisms of action.[2]

Iron is an essential micronutrient for almost all known organisms; its redox tuneability makes it an indispensable cofactor for life processes and biochemistry.[3] However, the extreme insolubility of ferric hydroxide at physiological pH and the further sequestration of ferric iron in a mammalian host by iron transport proteins like lactoferrin and transferrin suppress its free concentration to an astonishing 10^{-24} M, which is far too low to support bacterial colonization and growth.[3] To overcome this lack of readily available iron, pathogenic bacteria have evolved the ability to obtain iron from the serum and/or tissues of their host via the synthesis, secretion, and reuptake of small-molecule iron chelators known as siderophores.[4] *Mtb* produces a suite of structurally related siderophores that are essential for iron acquisition.[5] Disruption of genes involved in mycobactin biosynthesis results in *Mtb* strains unable to replicate in vitro unless chemically complemented with exogenous mycobactin, which in turn suggests that inhibition of mycobactin biosynthesis may represent a novel strategy for the development of new antitubercular agents.[6]

Mycobactins (Figure 1A) are biosynthesized by a mixed nonribosomal peptide synthetatse−polyketide synthase (NRPS−PKS) pathway in *Mtb*.[7] MbtA, an aryl acid adenylating enzyme (AAAE), is responsible for initiating mycobactin biosynthesis by priming the NRPS−PKS assembly line.[7a] MbtA does this by catalyzing a two-step adenylation−acylation reaction (Figure 1B). In the adenylation half-reaction, salicylate and ATP are condensed to form an acyl-adenylate intermediate that remains tightly bound to prevent adventitious hydrolysis of this labile mixed anhydride. MbtA then catalyzes the transfer of the acyl moiety onto MbtB, the second protein in the

Received: May 4, 2013
Published: June 27, 2013

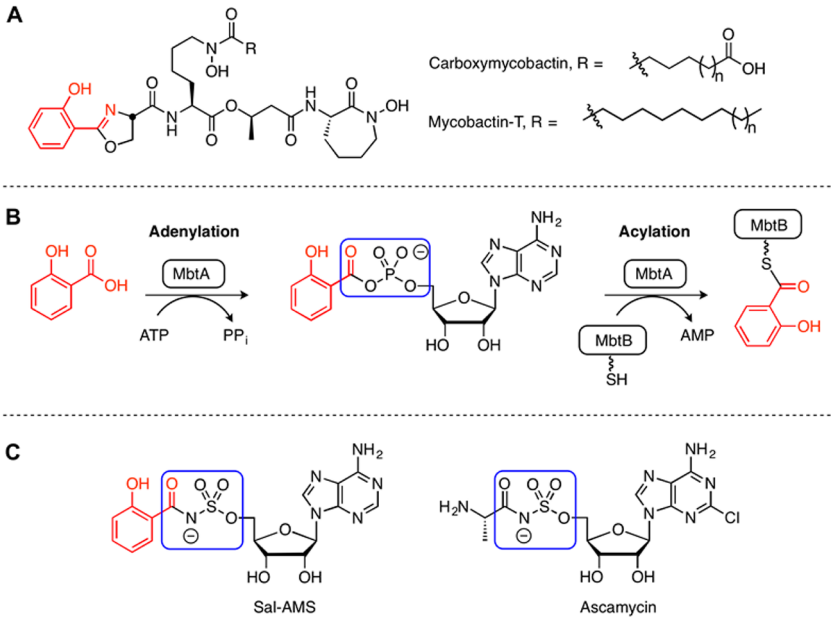


Figure 1. (A) Aryl-capped siderophores from *Mtb*, the mycobactins. (B) Enzymatic reactions catalyzed by MbtA, with hydrolytically labile acylphosphate outlined in blue. (C) Sal-AMS and natural product ascamycin. The hydrolytically stabile acylsulfamate bioisostere is outlined in blue.

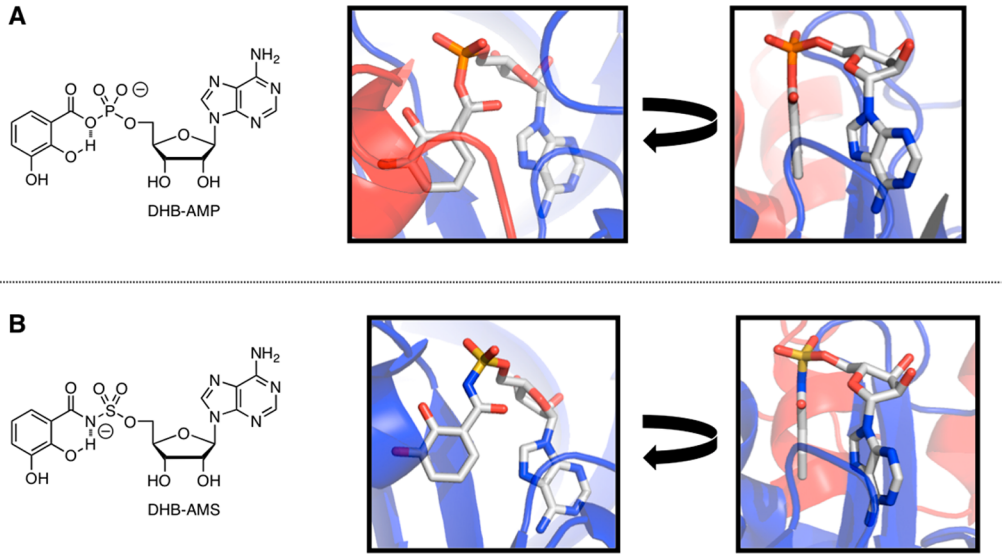


Figure 2. (A) (left; center; right) Molecular structure of DHB-AMP; cocrystal structure of enzymatic intermediate DHB-AMP bound to AAAE DhbE from *B. subtilis* (PDB ID: 1MDB) with aryl ring in plane of paper; same cocrystal structure, rotated 90°, with aryl ring perpendicular to paper. (B) (left; center; right) Molecular structure of DHB-AMS; cocrystal structure of bisubstrate mimic DHB-AMS bound to AAAE BasE from *A. baumannii* (PDB ID: 3O82) with aryl ring in plane of paper; same cocrystal structure, rotated 90°, with aryl ring perpendicular to paper.

mycobactin pathway. 5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine (Sal-AMS) is a rationally designed inhibitor of MbtA wherein the hydrolytically labile acyl-phosphate moiety of the acyl-adenylate intermediate is replaced by a stabile acyl-sulfamate linker (Figure 1C).[8] The sulfamate linker is inspired by the natural product ascamycin isolated from an unknown *Streptomyces* species in Japan.[9]

Sal-AMS is a potent inhibitor of MbtA with an apparent K_i in a functional kinetic assay of 7 nM.[10] Furthermore, Sal-AMS displays potent whole cell activity against *Mtb* H37Rv under iron-limiting conditions with a minimum inhibitory concentration (MIC) of 0.39 μM, rivaling the first-line clinical agent isoniazid.[8c] To date, our laboratory has conducted extensive structure−activity relationship (SAR) studies on the Sal-AMS scaffold, systematically exploring its aryl,[11] linker,[8c,12] glycosyl,[10] and nucleobase domains.[13] 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, indicate that an internal hydrogen bond is formed between the phenol and sulfamate nitrogen atom (estimated pK_a around 2) of Sal-AMS.[14] This enforces a coplanar arrangement of the salicyl group when bound in the MbtA active site. Further evidence in support of this binding mode is observed in the cocrystal structures of homologous AAAEs from *Bacillus subtilis* (DhbE)[15] and *Acinetobacter baumannii* (BasE)[16] with the acyl-adenylate ligands 5′-*O*-[*N*-(2,3-dihydroxybenzoyl)sulfamoyl]-adenosine (DHB-AMS) and 2,3-dihydroxybenzoyl adenosine monophosphate (DHB-AMP) (Figure 2).

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

Preliminary pharmacokinetic (PK) studies demonstrated that Sal-AMS has poor oral bioavailability.[17] Veber and co-workers have shown that oral bioavailibility inversely correlates with two criteria: the number of rotatable bonds and polar surface area.[18] In an attempt to improve the oral bioavailability of Sal-AMS, we designed conformationally constrained analogues **1**−**3**, shown in Figure 3. These analogues mimic the hypothesized MbtA-

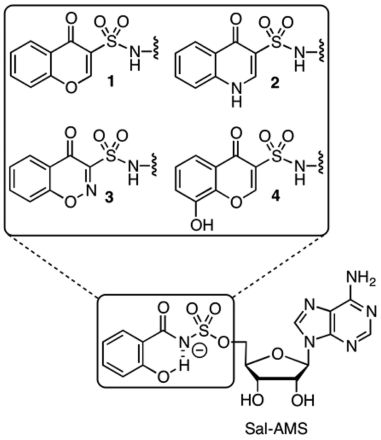


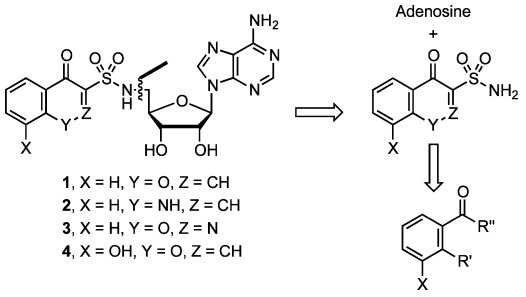
Figure 3. Conformationally constrained analogues of Sal-AMS (**1**−**3**) and DHB-AMS (**4**).

bound conformation of Sal-AMS and could potentially improve its oral bioavailability through removal of two rotatable bonds and the charged sulfamate moiety, which reduces the total polar surface area (tPSA). The calculated tPSA and octanol−water coefficient (cLogP) for all compounds are shown in Table 2. Herein we report the synthesis, biochemical and microbiological evaluation of conformationally constrained analogues **1**−**4** of Sal-AMS and DHB-AMS.

RESULTS AND DISCUSSION

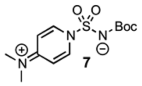
Synthesis. The most concise synthesis we envisioned to the proposed bicyclic sulfonamide adenosine analogues involves disconnection of **1**−**4** by Mitsunobu reaction to bicyclic sulfonamides and an appropriately protected adenosine derivative (Scheme 1). Further retrosynthetic disconnection leads to acetophenone or benzoic acid derivatives.

Scheme 1. Retrosynthetic Analysis



A review of the reported methods for synthesizing chromone and other derivatives caused our attention to focus on a potentially short route to chromone-3-sulfonamides. Enaminone **5** from Föhlisch's chromone synthesis[19] was successfully used by Löwe and Matzanke in a tandem sulfamoylation−cyclization with chlorosulfonylureas to produce chromone-3-sulfonylureas in modest yields.[20] We pursued this route by screening a few sulfamoylating reagents to effect the desired transformation (Table 1). After extensive experimentation with

Table 1. Optimization of Tandem Sulfamoylation−Cyclization of Enaminone 5

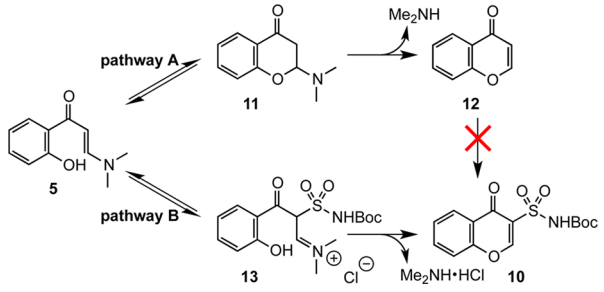
Entry	Reagent	Conditions	Results
1	 **6**	DCM, 0 °C	5% of **9** 32% of chromone 15% of **5** recovered
2	 **7**	1,4-dioxane, 80 °C	Total conversion to chromone
3	 **8**	DCM, 22 °C	21% of **10**

sulfamoyl chloride **6**, we successfully isolated the desired chromone-3-sulfonamide **9** in 5% yield (Table 1, entry 1). The use of recrystallized sulfamoyl chloride[21] was essential to obtain this meager yield. The remainder of material was cyclized, nonsulfamoylated chromone (32%), recovered enaminone **5** (15%), and a highly insoluble material that could not be characterized, potentially polymerized product and/or multi-sulfamoylated material. The less reactive DMAP-stabilized and Boc-protected sulfamoylating reagent **7**[22] was then tried. We considered it an attractive reagent because the sulfonamide functionality is already protected and unlike sulfamoyl chloride, it is stable for prolonged periods at room temperature and is not air-senstive. To our dismay, **7** was unable to effect the desired transformation even with heating, which resulted in cyclization to nonsulfamoylated chromone (Table 1, entry 2). On the basis of the presumed over-reactivity of **6** and the nonreactivity of **7**, we investigated *tert*-butyl chlorosulfonylcarbamate **8** that we hypothesized would possess intermediate reactivity. This was prepared by addition of chlorosulfonyl isocyanate to a stirring solution of *t*-BuOH in dichloromethane and successfully purified by recrystallization. Tandem sulfamoylation−cyclization of enaminone **5** with **8** afforded **10** in a final optimized yield of 21% (Table 1, entry 3), which represents a 4-fold improvement over sulfamoyl chloride **6**. This low yield was primarily the result of competitive cyclization of the enaminone to chromone but was commensurate with the yields achieved by Löwe and Matzanke.[20]

The modest yield of chromone-3-sulfonamide **10** is due to competitive direct cyclization of enaminone **5** to chromone **12** (Scheme 2, pathway A). Since we were unable to convert chromone **12** to chromone-3-sulfonamide **10** under any reaction conditions, we propose that sulfamoylation of enaminone **5** must occur first to afford an *α*-formyl-*β*-ketosulfonamide intermediate **13** followed by rapid cyclization to furnish **10** and $Me_2NH{\cdot}HCl$ (Scheme 2, pathway B), the latter of which could serve to catalyze either pathway. Addition of triethylamine or other bases suppresses both pathways. HCl can also be formed through decomposition of the sulfamoylating reagents **6** and **8**.

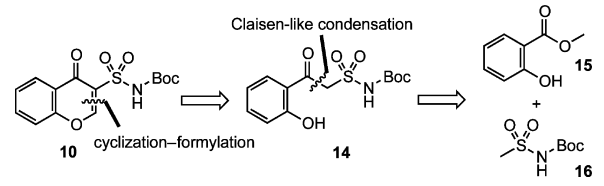
dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

Scheme 2. Proposed Mechanism for Tandem Sulfamoylation−Cyclization of Enaminone 5



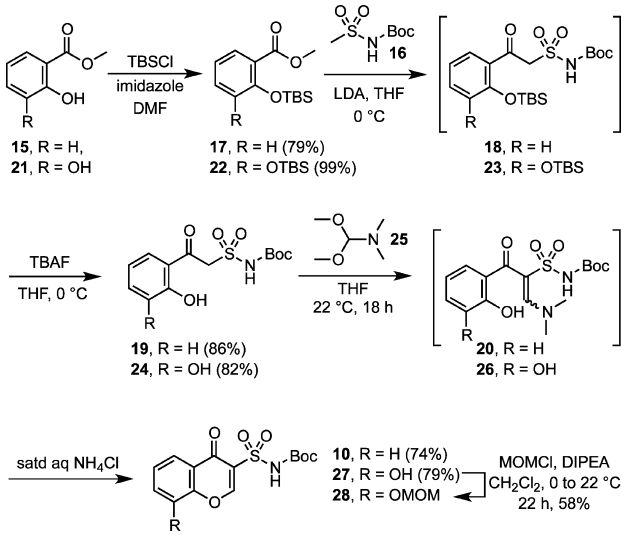
On the basis of the proposed mechanism shown in Scheme 2, we hypothesized that **10** could be obtained through α-formylation of a β-ketosulfonamide followed by cyclization (Scheme 3). The necessary β-ketosulfonamide was synthesized

Scheme 3. Revised Retrosynthetic Analysis of Chromone-3-sulfonamide



by TBS protection of methyl salicylate **15** followed by Claisen-like condensation[12] with the LDA-generated dianion of *N*-Boc-methanesulfonamide **16** (Scheme 4).[23] The intermediate **18**

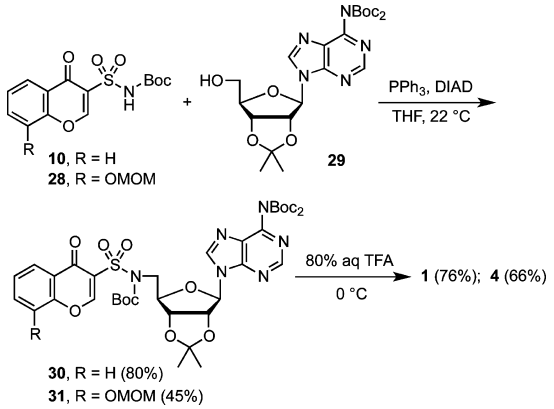
Scheme 4. Second Generation Chromone Synthesis



was not isolated, but directly treated with TBAF to furnish β-ketosulfonamide **19** in 86% overall yield. Formylation was initially attempted with triethyl orthoformate in acetic anhydride as described by Chu and co-workers[24] in their synthesis of quinolone-3-carboxylic acids; however, its sluggish reactivity required elevated temperatures (~100 °C) that led to Boc-deprotection and imidate formation at the sulfonamide in conjunction with the desired α-formylation. We next investigated the use of dimethylformamide dimethyl acetal (DMF−DMA, **25**) since this has been shown to formylate active methylenes under mild conditions.[25] Treatment of β-ketosulfonamide **19** with DMF-DMA at ambient temperature resulted in full conversion to enaminone **20** as monitored by TLC and indicated by ^{1}H NMR and MS of the crude material. Addition of saturated aqueous NH_4Cl to crude **20** in THF induced rapid cyclization (~5 min) to chromone **10**. This second generation synthesis of chromone-3-sulfonamide **10** requires 3 steps from methyl salicylate **15** and proceeds in 50% overall yield. The corresponding 8-hydroxychromone-3-sulfonamide **27** was prepared analogously from methyl 2,3-dihydroxybenzoate **21** in 3 steps in 64% overall yield. The free phenol in **27** was converted to MOM ether **28** in order to avoid complication with the subsequent Mitsunobu reaction.

Mitsunobu coupling of chromone **10** with bis-Boc-adenosine **29**[26] afforded chromone nucleoside **30** in 80% yield (Scheme 5).[12] Mitsunobu couplings of purine nucleosides at the 5′

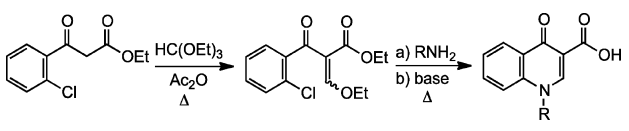
Scheme 5. Mitsunobu Coupling to Chromone 1



position are notoriously problematic as noted in Mitsunobu's seminal review[27] due to cyclonucleoside formation between purine N-3 and ribose C-5′. We avoided this competitive reaction through bis-Boc protection of the exocyclic N-6 amino group of adenosine, which served to attenuate the nucleophilicity at N-3. Global deprotection of the Boc and acetonide groups in **30** with 80% aqueous TFA provided the desired chromone analogue **1**. The 8-hydroxychromone analogue **4** was prepared analogously from **28**.

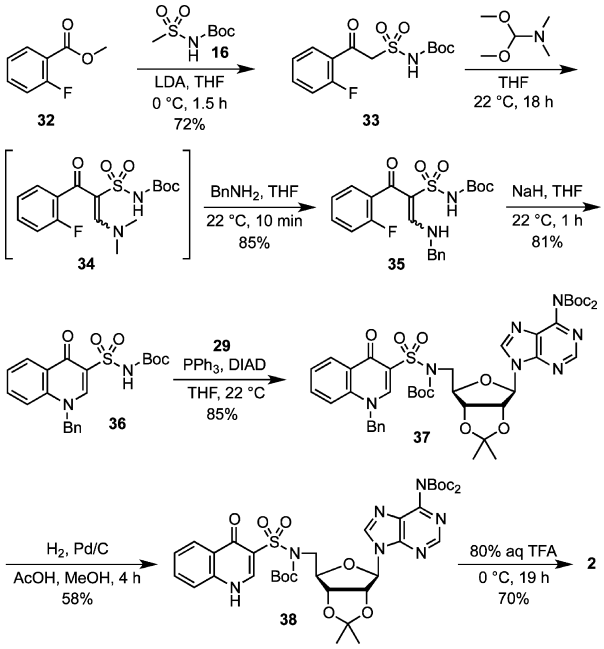
We developed a new synthesis of quinolone-3-sulfonamides from β-ketosulfonamides by adapting the classic Grohe−Heitzer route used for the preparation of the related quinolone-3-carboxylic acids, which involves formylation of a β-ketoester followed by introduction of the quinolone nitrogen through a tandem addition−elimination reaction of a primary amine (Scheme 6).[28] The requisite β-ketosulfonamide **33** was synthesized from methyl 2-fluorobenzoate **32** through Claisen-like condensation with the dianion of *N*-Boc-methanesulfonamide **16** (Scheme 7).[12] Reaction of β-ketosulfonamide **33** with DMF−DMA at room temperature for 18 h resulted in total conversion to enaminone **34**, which was not isolated but directly treated with excess benzylamine. Transamination of **34**

Scheme 6. Grohe−Heitzer Quinolone Synthesis



dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

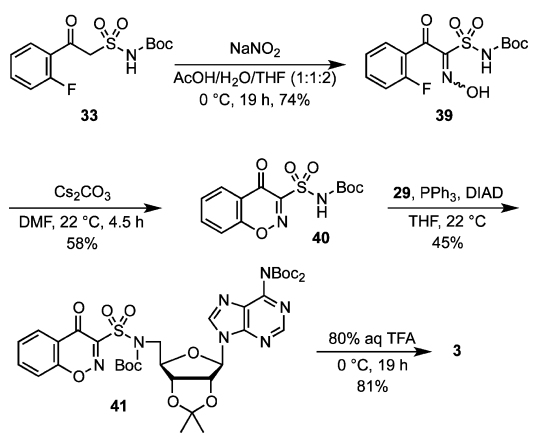
Scheme 7. Synthesis of Quinolone 2



via an addition−elimination pathway was complete in 10 min and furnished *N*-benzyl enaminone **35** as an inseperable 2:1 mixture of geometric isomers in 85% overall yield. Cyclization of enaminone **35** to *N*-benzyl quinolone **36** was achieved by treatment with sodium hydride at ambient temperature in 1 h. Mitsunobu coupling of **36** with adenosine derivative **29** afforded quinolone nucleoside **37**. Debenzylation under standard hydrogenolysis conditions yielded **38** and subsequent deprotection of the acetonide and Boc groups with aqueous TFA provided the desired quinolone **2**. Overall, this new route to quinolone-3-sulfonamides is notable for the facile installation of the nitrogen at N-1, which occures at 0 °C to ambient temperature as well as the use of DMF-DMA for formylation of the β-ketosulfonamide.

Given the facile cyclization of enaminone **35**, it was hypothesized that an oxime derivative (i.e., α-hydroxyimino-β-ketosulfonamide) could also easily cyclize to afford the 1,2-benzoxazin-4-one heterocycle of **3**. The key oxime precursor was prepared using the classic Meyer nitrosation reaction (Scheme 8).[29] Thus, treatment of β-ketosulfonamide **33** with

Scheme 8. Synthesis of Benzoxazinone 3



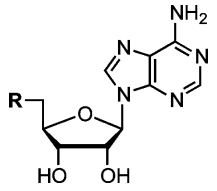
sodium nitrite in a mixed AcOH−H_2O−THF solvent system[30] yielded a nitroso intermediate that tautomerized to oxime **39** as a single undefined geometric isomer that was moderately stabile and was directly utilized in the next step following purification. We explored several methods to induce cyclization of **39** to the desired 1,2-benzoxazin-4-one **40** and ultimately identified cesium carbonate in DMF as the optimal conditions, which provided **40** in 58% yield at room temperature in 1 h. Under these conditions total consumption of the substrate was observed. We speculate that the modest yield is due to decomposition of the labile oxime under the basic reaction conditions. The only other precedence for this transformation (i.e., **39** → **40**) is found in a patent from Pharmacia & Upjohn that discloses a single example of an analogous α-hydroxyimino-β-ketoester.[31] However, the reported cyclization in this patent did not employ a base and required reflux in toluene for 48 h. The synthesis of target molecule **3** was completed by Mitsunobu coupling of **40** with adenosine derivative **29** to provide **41** followed by deprotection with aqueous TFA. Overall, this concise route provides 1,2-benzoxazin-4-one **3** in only 4 steps from β-ketosulfonamide intermediate **33** and employs extremely mild reaction conditions to assemble this rare heterocycle.[32]

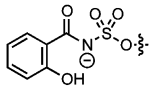
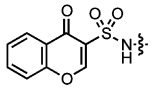
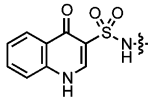
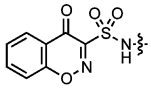
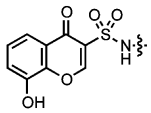
Biochemical Evaluation. The compounds were evaluated for their binding affinity to MbtA using a previously described fluorescence polarization (FP) assay.[33] FP assays are competitive binding assays in which a fluorescently labeled probe molecule is displaced from its receptor (i.e., MbtA) by a competitive ligand (i.e., **1**−**4**), and they allow direct determination of ligand dissociation constants. The binding affinity of chromone **1** toward MbtA using the FP assay is 3.6 μM. Replacement of the "CH" at C-2 in chromone **1** with a "N" atom in benzoxazinone **3** results in a nearly 10-fold increase in potency to 0.37 μM. The higher binding affinity of **3** versus **1** is most likely due to the more isosteric design of **3** when compared to the proposed bound conformation of Sal-AMS in the MbtA active site. The 8-hydroxychromone **4** shows very low affinity to MbtA with a K_D of 290 μM, which represents an 80-fold loss of potency relative to chromone **1**. This result is readily reconciled since MbtA binds salicylic acid preferably over 2,3-dihydroxybenzoic acid.[7a] The K_D of quinolone **2** is below the detection limit of the assay and is <0.01 μM. The acidity of the quinolone nitrogen (estimated pK_a ~ 7.3)[34] is low enough that it may be ionized when bound to MbtA, which might more closely mimic the electrostatics of the native acyl-adenylate and Sal-AMS, which are both negatively charged. Bicyclic sulfonamides **1**−**4** were also evaluated against other AAAE homologues including BasE, EntE, and VibE from *Acinetobacter baumannii*, *Escherichia coli*, and *Vibrio cholerae*, respectively. The native substrate of these AAAE's is 2,3-dihydroxybenzoic acid; hence, we expected 8-hydroxychromone **4** to have enhanced potency toward these enzymes. Surprisingly, **4** was inactive against all three AAAE's (K_D > 300 μM). The relative potency trends of **1**−**3** toward these other AAAE's are the same as observed with MbtA. Thus, quinolone **2** displays the highest potency with K_D's generally below the lower limit of the assay (<0.1 to 0.32 μM). Benzoxazinone **3** is the next most potent with K_D ranging from 1−12 μM, while chromone **1** binds the weakest with K_D's ranging from 98−280 μM, which represents more than a 1000-fold loss of potency relative to quinolone **2**.

FP assays are limited in the accuracy of binding constants they provide when an inhibitor's affinity is greater than that of

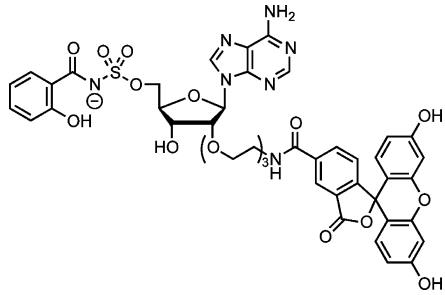
dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

Table 2. Combined Biochemical and Antitubercular Evaluation of 1−4



Compound	R	ClogP[b]	tPSA[b]	K_D (μM)				K_i^{app} (μM)	MIC_{50} (μM)	
				MbtA	BasE	EntE	VibE	MbtA	−Fe	+Fe
Fl-Sal-AMS[a]	n.a.	n.a.	n.a.	0.0093	0.093	0.23	0.13	—	—	—
Sal-AMS		-1.79	210.8	<0.01	<0.1	<0.2	<0.1	0.0066	0.39[c]	1.56[c]
1		-0.65	188.5	3.6 ± 0.1	108 ± 8	280 ± 60	98 ± 7	—	>50	>50
2		-0.57	193.4	<0.01	<0.1	0.32 ± 0.04	<0.1	0.12 ± 0.02	>50	>50
3		-1.26	200.9	0.37 ± 0.01	1.19 ± 0.07	12 ± 1	2.5 ± 0.7	—	>50	>50
4		-1.10	208.7	290 ± 70	>300	>300	>300	—	>50	>50

[a]Structure of Fl-Sal-AMS:



[b]Calculated using ChemBioDraw Ultra Version 13.0.0.3015. [c]Previously reported.

the probe.[35] We were unable to discriminate the potency of quinolone **2** versus Sal-AMS using our FP assay since both compounds bound more tightly than the fluorescent probe Fl-Sal-AMS. In order to address this shortcoming, we used a functional [^{32}P]PP_i-ATP steady-state kinetic exchange assay[10] employing supersaturating concentrations of substrates to determine apparent K_i values for Sal-AMS and quinolone **2** with respect to MbtA under identical assay conditions. This analysis afforded a K_i^{app} of 120 nM for **2** against MbtA, which is approximately 18-fold less active than Sal-AMS (K_i^{app} of 6.6 nM). The compromise in activity of **2** is offset by the substantially improved physicochemical properties of quinolone **2**.

Antitubercular Evaluation. All compounds were tested against *Mtb* under iron-deficient and iron-replete conditions as previously described (Table 2).[8c] Unexpectedly, compounds **1**−**4** are inactive with minimum inhibitory concentrations greater than 50 μM, the highest concentration evaluated. The lack of activity of quinolone **2** is disappointing and suggests it may not accumulate at sufficient concentrations intracellularly, despite improved ClogP and tPSA values relative to SalAMS, due to poor penetration or active efflux. Cellular accumulation studies will be required to address this possibility.

CONCLUSION

We have designed a divergent strategy for the synthesis of chromone-, quinolone-, and benzoxazinone-3-sulfonamides from common β-ketosulfonamide intermediates, which were assembled via a Claisen-like condensation between an appropriate benzoate ester derivative and the dianion of *N*-Boc-methanesulfonamide. Formylation of the active methylene of the β-ketosulfonamide was found to optimally proceed with

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

dimethylformamide dimethyl acetal at room temperature to afford an enaminone intermediate, which underwent intramolecular cyclization with a phenol to provide the chromone-3-sulfonamides. The quinolone-3-sulfonamide was accessed from a similar enaminone intermediate by transamination with benzylamine followed by intramolecular cyclization via nucleophilic aromatic substitution onto an *ortho*-fluorophenyl moiety. The versatility of the β-ketosulfonamide intermediate was further demonstrated by the electrophilic nitrosation of the active methylene to yield an intermediate oxime. Subsequent intramolecular cyclization onto an *ortho*-fluorophenyl group via nucleophilic aromatic substitution provided the benzoxazinone-3-sulfonamide. Each of the bicyclic-3-sulfonamides was efficiently coupled to a protected adenosine via a Mitsunobu reaction to furnish the desired inhibitors **1**–**4** following global deprotection. These compounds were designed as conformationally restricted analogues of 5′-*O*-[*N*-(salicyl)sulfamoyl]-adenosine (Sal-AMS) to improve oral bioavailability by removal of two rotatable bonds and the charged sulfamate moiety. Biochemical studies with MbtA showed that the negative charge of Sal-AMS appears critical to maintain potent activity as chromone **1** and benzoxazine **3** analogues that lack an ionizable function in the heterocycle display substantially reduced potency, while quinolone **2**, which contains an ionizable NH moiety at N-1, is only 18-fold less active than Sal-AMS toward MbtA as measured in a functional steady-state kinetic assay. This is likely a general phenomenon of adenylating enzymes and has been noted previously.[12,26] We hypothesized that the compromise in activity of **2** would be offset by the substantially improved physicochemical properties of quinolone **2**. Unfortunately, quinolone **2** is inactive against *Mtb* in a whole-cell assay with a minimum inhibitor concentration of greater than 50 μM, which represents more than a 128-fold loss of activity relative to Sal-AMS. Quinolone **2** shows significant biochemical potency; thus, the inactivity against *Mtb* may be due to reduced cellular accumulation. While our primary goal was to remove the ionizable and negatively charged sulfamate group, it may be necessary to lower the pK_a of quinolone **2** to ensure this is fully ionized. Additional studies will be required to understand the loss of biological activity, which is critical to further optimize this scaffold.

EXPERIMENTAL SECTION

General Synthetic Methods. All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system 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 Argon. Anhydrous grade MeOH was purchased from Aldrich. All reactions were performed under an inert atmosphere of dry Ar in oven-dried (150 °C) glassware. TLC analyses were performed on TLC silica gel plates and were visualized with UV light. Purification by flash chromatography was performed using a medium-pressure flash chromatograpy system equipped with flash column silica cartridges with the indicated solvent system. Reversed-phase HPLC (RP-HPLC) purification was performed on a Phenomenex Gemini 10 μm C18 250 × 10.00 mm column operating at 5.0 mL/min with detection at 254 nm with the indicated solvent system. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a 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 monodeuterated 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).[36] Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, ap = apparent, br = broad, ovlp = overlapping), coupling constant(s), integration. High-resolution mass spectra were obtained on a TOF/MS instrument equipped with either an ESI or APCI interface. Compounds **5**,[19] **6**,[21] **7**,[22] **15**, **16**,[23] **21**,[40] **29**,[26] and **32** were prepared as described.

Experimental for Compounds from Table 1. *tert-Butyl chlorosulfonylcarbamate (**8**).* To a stirred solution of *tert*-butanol (1.9 mL, 20 mmol, 1.3 equiv) in CH_2Cl_2 (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 mixture was removed from the 0 °C bath after 5 min of additional stirring. After warming to 22 °C, stirring was stopped, and the reaction mixture was concentrated in vacuo to one-third volume. The flask was placed back into the 0 °C bath, and the product crystallized out of solution. After 50 min, the product was filtered and washed with hexanes yielding the title compound (1.5 g, 46%) as a colorless solid. Additional product (1.2 g, 37%) was obtained by crystallizing the concentrated mother liquor in CH_2Cl_2 at 0 °C: mp 60–68 °C; ^{1}H NMR (600 MHz, CD_2Cl_2) δ 1.56 (s, 9H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 28.1, 87.4, 147.8; HRMS (ESI−) calcd for $C_5H_{10}NO_5S^-$ (hydrolysis product) [M − H]$^-$ 196.0285, found 196.0274 (error 5.6 ppm).

*Chromone-3-sulfonamide (**9**).* To a stirred solution of sulfamoyl chloride **6** (347 mg, 3.00 mmol, 1.00 equiv) in CH_2Cl_2 (6 mL) at 0 °C was added enaminone **5** (574 mg, 3.00 mmol, 1.00 equiv) in one portion. After stirring 5 h, the reaction was quenched with addition of saturated aqueous $NaHCO_3$ (50 mL). The layers were separated, and the pH of the aqueous layer was adjusted to neutral (∼7 by pH paper). The aqueous layer 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 chromone **12** (140 mg, 32%; characterization data matched that of authentic commercially obtained sample) and recovered enaminone **5** (86 mg, 15%). MS showed the possibility of product remaining in the aqueous layer (major peak of m/z = 224 in negative mode), so the aqueous layer was further extracted with *n*-BuOH (3 × 75 mL). The combined *n*-BuOH layers were dried ($MgSO_4$) and concentrated. The resultant residue was taken up in MeOH–MeCN (20 mL, 1:1), and insoluble solids filtered away. Upon sitting overnight, the product had crystallized to afford the title compound (34 mg, 5%) as off-white crystals: ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.34 (s, 2H), 7.60 (t, J = 7.6 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.90 (td, J = 7.6, 1.5 Hz, 1H), 8.14 (dd, J = 7.6, 1.5 Hz, 1H), 8.97 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 118.9, 123.8, 125.2, 126.7, 127.3, 135.3, 155.7, 158.6, 171.7; HRMS (ESI−) calcd for $C_9H_6NO_4S^-$ [M − H]$^-$ 224.0016, found 224.0023 (error 3.1 ppm).

*tert-Butyl (chromon-3-yl)sulfonylcarbamate (**10**). Method A: Sulfamoylation of **5**.* To a solution of **8** (1.5 g, 7.0 mmol, 1.0 equiv) in CH_2Cl_2 (14 mL) at 22 °C was added enaminone **5** (1.3 g, 7.0 mmol, 1.0 equiv). The reaction was stirred 13 h and then concentrated in vacuo. Purification by flash chromatography (30:70 to 100:0 CH_2Cl_2–hexanes, linear gradient) afforded the title compound (377 mg, 21%) as a yellow amorphous solid: R_f 0.29 (1:5:95 Et_3N–MeOH–CH_2Cl_2); ^{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).

Experimental for Compounds from Scheme 4. *Methyl 2-(tert-butyldimethylsilyloxy)benzoate (**17**).* TBSCl (3.01 g, 20.0 mmol, 3.00 equiv) was added to a solution of **15** (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 was stirred a further 24 h at room temperature. A 5% aqueous $NaHCO_3$ (100 mL) was added to the reaction mixture, and the resulting aqueous solution was extracted with

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470–7481

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) to afford 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 (td, J = 7.6, 1.2 Hz, 1H), 7.35 (td, J = 8.2, 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 (**19**).* Freshly titrated *n*-BuLi (2.1 M in hexane, 5.0 mL, 11 mmol, 3.1 equiv) was added dropwise to freshly distilled $(i\text{-Pr})_2NH$ (1.6 mL, 11 mmol, 3.3 equiv) in THF (10 mL) at 0 °C. The mixture was stirred for 30 min, and then sulfonamide **16** (666 mg, 3.41 mmol, 1.00 equiv) in THF (10 mL) was added, and the reaction was stirred for a further 1 h at 0 °C. Next, methyl ester **17** (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 saturated aqueous NaCl (10 mL) and 0.5 M aqueous NaH_2PO_4 (10 mL) and then diluted with EtOAc (10 mL). The layers were separated, and the aqueous layer was acidified to pH ∼5–6 (pH paper) with 6 N aqueous HCl. The aqueous layer was extracted with EtOAc (2 × 100 mL). The organic extracts 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 saturated aqueous NH_4Cl (25 mL) and was diluted with Et_2O (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼5–6 (pH paper) with 6 N aqueous HCl. The aqueous layer 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); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.53 (s, 9H), 4.99 (s, 2H), 6.98 (t, J = 8.2 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.57 (t, J = 8.2 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 (**10**). Method B: Formylation–Cyclization of **19**.* *N*,*N*-Dimethylformamide dimethylacetal (638 μL, 4.80 mmol, 2.40 equiv) was added to a solution of β-ketosulfonamide **19** (631 mg, 2.00 mmol, 1.00 equiv) in THF (20 mL) at 22 °C. The solution was stirred for 18 h and then was acidified with satdurated aqueous NH_4Cl (25 mL) and diluted with EtOAc (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼2–3 (pH paper) with aqueous 6 N HCl. The aqueous layer 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 for Method A.

*Methyl 2,3-bis(tert-butyldimethylsilyloxy)benzoate (**22**).* To a solution of methyl 2,3-dihydroxybenzoate **21** (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 room temperature and diluted with 5% aqueous $NaHCO_3$ (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 (t, J = 7.9 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 (**24**).* Freshly titrated *n*-BuLi (2.1 M in hexane, 11.0 mL, 23.2 mmol, 3.10 equiv) was added dropwise to freshly distilled $(i\text{-Pr})_2NH$ (3.5 mL, 24.8 mmol, 3.30 equiv) in THF (24 mL) at 0 °C. The mixture was stirred for 1.5 h, and then **16** (1.46 g, 7.50 mmol, 1.00 equiv) in THF (24 mL) was added, and the reaction was stirred for a further 1.5 h at 0 °C. Next, methyl ester **22** (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 saturated aqueous NaCl (25 mL) and 0.5 M aqueous NaH_2PO_4 (25 mL) and was diluted with EtOAc (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼3–4 (pH paper) with aqueous 6 N HCl. The aqueous layer 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 saturated aqueous NH_4Cl (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼3–4 (pH paper) with aqueous 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 (t, J = 7.9 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 (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 (**27**).* *N*,*N*-Dimethylformamide dimethyl acetal (638 μL, 4.80 mmol, 2.40 equiv) was added to a solution of β-ketosulfonamide **24** (663 mg, 2.00 mmol, 1.00 equiv) in THF (20 mL) at 22 °C. The solution was stirred for 18 h and then was acidified with saturated aqueous NH_4Cl (25 mL) and diluted with EtOAc (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼5–6 (pH paper) with aqueous 6 N HCl. The aqueous layer 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 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 (**28**).* To a solution of **27** (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 then diluted with CH_2Cl_2 (100 mL). The solution was washed with H_2O (2 × 100 mL) and saturated aqueous 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 (310 mg, 58%) 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 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,

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470–7481

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).

Experimental for Compounds from Scheme 5. *N^6,N^6-bis(tert-Butoxycarbonyl)-5′-amino-5′-N-(tert-butoxycarbonyl)-5′-N-[(chromon-3-yl)sulfonyl]-5′-deoxy-2′,3′-O-isopropylideneadenosine (**30**).* To a stirred solution of **10** (270 mg, 0.83 mmol, 1.1 equiv), **29** (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 and then 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, 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 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.77 (td, J = 8.5, 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, 124.8, 125.4, 126.3, 127.1, 129.1, 135.2, 144.1, 150.4, 150.6, 152.4, 152.7, 156.0, 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]-5′-deoxy-adenosine (**1**).* To solid **30** (474 mg, 0.582 mmol) at 0 °C was added ice-cold 80% aqueous TFA (5 mL). The reaction was stirred 1.5 h at 0 °C and then warmed to 22 °C and 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), 4.04−4.06 (m, 1H), 4.07 (dd, J = 5.0, 1.6 Hz, 1H), 4.68 (td, J = 6.4, 1.6 Hz, 1H), 5.74 (d, J = 6.4 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.84 (td, J = 7.6, 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).

*N^6,N^6-bis(tert-Butoxycarbonyl)-5′-amino-5′-N-(tert-butoxycarbonyl)-5′-deoxy-5′-N-{[(8-(methoxymethoxy)chromon-3-yl]-sulfonyl}-2′,3′-O-isopropylideneadenosine (**31**).* To a solution of **28** (17 mg, 0.044 mmol, 1.1 equiv), **29** (20 mg, 0.040 mmol, 1.0 equiv), and PPh_3 (12 mg, 0.044 mmol, 1.1 equiv) in THF (2 mL) at 22 °C was added DIAD (0.44 mL [100 mM in THF], 0.044 mmol, 1.1 equiv) dropwise. After 2 h stirring, TLC monitoring of the reaction suggested the limiting reagent **29** remained; therefore, additional PPh_3 (12 mg, 0.044 mmol, 1.1 equiv) and DIAD (0.44 mL [100 mM in THF], 0.044 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 (36 mg, 45%) 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 1.26 (s, 3H), 1.41 (s, 9H), 1.48 (s, 18H), 1.68 (s, 3H), 3.51 (s, 3H), 4.33 (dd, J = 9.0, 5.2 Hz, 1H), 4.47 (dd, J = 9.0, 5.2 Hz, 1H), 4.77 (dd, J = 9.0, 3.6 Hz, 1H), 5.26 (dd, J = 5.8, 3.6 Hz, 1H), 5.38 (s, 2H), 5.45 (dd, J = 5.8, 2.0 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.37 (t, J = 8.2 Hz, 1H), 7.77 (d, J = 8.2 Hz, 1H), 8.37 (br s, 1H), 8.77 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.6, 27.4, 28.0, 28.1, 53.2, 57.0, 77.5, 78.2, 79.9, 81.7, 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′-deoxy-5′-N-[(8-hydroxychromon-3-yl)sulfonyl]-adenosine (**4**).* To solid **31** (24 mg, 0.027 mmol) at 0 °C was added ice-cold 80% aqueous 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, linear 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 the 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, 2.9 Hz, 1H), 4.41 (q, J = 5.3 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 Hz, 1H), 5.44 (d, J = 5.3 Hz, 1H, D_2O-exchangeable), 5.63 (d, J = 5.3 Hz, 1H, D_2O-exchangeable), 6.01 (d, J = 5.3 Hz, 1H), 7.27 (br s, 2H, D_2O-exchangeable), 7.38 (br s, 2H, D_2O-exchangeable), 7.49 (t, J = 7.6 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).

Experimental for Compounds from Scheme 7. *tert-Butyl [2-(2-fluorophenyl)-2-oxoethyl]sulfonylcarbamate (**33**).* 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, and then **16** (1.65 g, 8.43 mmol, 1.00 equiv) in THF (24 mL) was added, and the reaction was stirred for a further 1 h at 0 °C. Next, methyl ester **32** (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 saturated aqueous NaCl (25 mL) and 0.5 M aqueous NaH_2PO_4 (25 mL). The layers were separated, and the aqueous layer was acidified to pH ∼5−6 (pH paper) with aqueous 6 N HCl. The aqueous layer 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), 7.18−7.21 (m, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.62−7.64 (m, 1H), 7.91 (t, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.1, 61.6 (d, $^2J_{C-F}$ = 9.2 Hz), 85.0, 117.2 (d, $^2J_{C-F}$ = 23.1 Hz), 124.3, 125.1 (d, $^3J_{C-F}$ = 3.5 Hz), 131.2, 136.6 (d, $^3J_{C-F}$ = 9.2 Hz), 149.6, 162.2 (d, $^1J_{C-F}$ = 256.6 Hz), 186.2 (d, $^3J_{C-F}$ = 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).

*tert-Butyl [3-(2-fluorophenyl)-1-((benzylamino)-3-oxoprop-1-en-2-yl]sulfonylcarbamate (**35**).* Dimethylformamide dimethyl acetal (399 μL, 3.00 mmol, 1.50 equiv) was added to a solution of β-ketosulfonamide **33** (635 mg, 2.00 mmol, 1.00 equiv) in THF (10 mL) at 22 °C. The solution was stirred for 1.5 h and then was concentrated under reduced pressure yielding enaminone **34** as a yellow oily residue used directly without purification: R_f 0.25 (0.5:0.5:49:50 HCO_2H−MeOH−EtOAc−hexanes); LRMS (ESI−) calcd for $C_{16}H_{20}FN_2O_5S^-$ [M − H]$^-$ 371, found 371.

To a stirred solution of enaminone **34** 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 (s, 3H, *minor*), 1.47 (s, 6H, *major*), 4.47 (d, J = 5.3 Hz, 0.67H, *minor*), 4.66 (d, J = 5.9 Hz, 1.33H, *major*), 6.64−6.67 (m, 0.67H, *major*), 7.10−7.25 (ovlp m, 1H), 7.14−7.19 (ovlp m, 1H), 7.24 (d, J = 7.0 Hz, 0.67H, *major*), 7.28 (t, J = 7.0 Hz, 0.67H, *major*), 7.31−7.43 (ovlp m, 5H), 7.62−7.64 (m, 0.33H, *minor*), 8.31 (d, J = 14.1 Hz, 0.67H, *major*); 8.70 (br s, 0.33H, *minor*), 11.03 (br s, 0.67H, *major*); ^{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,

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

188.8; ^{19}F NMR (564 MHz, $CDCl_3$) δ −120.1 (*major*), −117.4 (*minor*); 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-benzylquinol-4-on-3-yl)sulfonylcarbamate (**36**).* To a stirred solution of enaminone **35** (434 mg, 1.00 mmol, 1.00 equiv) in THF (4 mL) at 22 °C was added NaH (60% dispersion in mineral oil, 88 mg, 2.2 mmol, 2.2 equiv) portionwise (∼10 mg portions) such that noticeable gas evolution had ceased before next addition. After stirring 1 h, the reaction was quenched by addition of saturated aqueous NH_4Cl (25 mL). The aqueous mixture was acidified to pH ∼4 (pH paper) with 6 N aqueous HCl and 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 (0.4:0.4:39.2:60 to 1:10:89:0 HCO_2H−MeOH−EtOAc−hexanes, linear gradient) afforded the title compound (336 mg, 81%) 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), 7.48−7.50 (m, 1H), 7.74−7.76 (m, 2H), 8.26 (d, J = 7.6 Hz, 1H), 8.99 (s, 1H), 11.60 (br s, 1H, D_2O-exchangeable); ^{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).

*N^6,N^6-bis(tert-Butoxycarbonyl)-5′-amino-5′-N-(tert-butoxycarbonyl)-5′-N-[(1-benzylquinol-4-on-3-yl)sulfonyl]-5′-deoxy-2′,3′-O-isopropylideneadenosine (**37**).* To a stirred solution of quinolone **36** (228 mg, 0.550 mmol, 1.10 equiv), **29** (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, 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), 7.32−7.35 (ovlp m, 3H), 7.40 (d, J = 8.2 Hz, 1H), 7.45 (t, J = 8.2 Hz, 1H), 7.61 (t, J = 7.0 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′-deoxy-5′-N-[(quinol-4-on-3-yl)sulfonyl]-2′,3′-O-isopropylideneadenosine (**38**).* 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 **37** (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 and 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 (td, J = 7.2, 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 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.77 (t, J = 8.2 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 8.30 (s, 1H), 8.58 (d, J = 6.2 Hz, 1H, D_2O-exchangeable [collapses to singlet]), 8.81 (s, 1H), 8.88 (s, 1H), 12.79 (d, J = 6.2 Hz, 1H, D_2O-exchangeable); ^{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′-deoxy-5′-N-[(quinol-4-on-3-yl)sulfonyl]adenosine (**2**).* To solid **38** (97 mg, 0.12 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aqueous 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 the appropriate fractions afforded the title compound (2 mg) 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), 4.04−4.05 (m, 1H), 4.10−4.11 (m, 1H), 4.72 (t, J = 5.5 Hz, 1H), 5.28 (br s, 1H, D_2O-exchangeable), 5.44 (br s, 1H, D_2O-exchangeable), 5.79 (d, J = 6.4 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.62 (br s, 2H, D_2O-exchangeable), 7.64 (d, J = 7.8 Hz, 1H), 7.76 (t, J = 7.6 1H), 8.13 (d, J = 7.6 Hz, 1H), 8.30 (s, 1H), 8.46 (s, 1H), 12.55 (d, J = 6.5 Hz, 1H, D_2O-exchangeable); ^{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).

Experimental for Compounds from Scheme 8. *tert-Butyl [2-(2-fluorophenyl)-1-(hydroxyimino)-2-oxoethyl]sulfonylcarbamate (**39**).* To a solution of β-ketosulfonamide **33** (635 mg, 2.00 mmol, 1.00 equiv) in AcOH−H_2O−THF (1:1:2, 10 mL) 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 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 (t, J = 7.6 Hz, 1H), 7.71 (dddd, J = 8.8, 7.0, 5.3, 1.8 Hz, 1H), 7.92 (td, J = 7.6, 1.8 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 28.2, 84.6, 118.0 (d, $^2J_{C-F}$ = 22.0 Hz), 124.7 (d, $^2J_{C-F}$ = 8.1 Hz), 125.9 (d, $^3J_{C-F}$ = 3.5 Hz), 132.7, 138.1 (d, $^3J_{C-F}$ = 9.2 Hz), 151.5, 155.3, 163.5 (d, $^1J_{C-F}$ = 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-4H-benzo[e][1,2]oxazin-3-yl)sulfonylcarbamate (**40**).* To a solution of oxime **39** (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 saturated aqueous NH_4Cl (20 mL). The aqueous solution was acidified to pH ∼4−5 (pH paper) with 6 N aqueous HCl and 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 (t, J = 7.6 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.90 (t, J = 7.7 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′-deoxy-5′-N-[(4-oxo-4H-benzo[e][1,2]oxazin-3-yl)sulfonyl]-2′,3′-O-isopropylideneadenosine (**41**).* To a solution of **40** (45.4 mg, 0.139 mmol, 1.10 equiv), **29** (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, MS monitoring of the reaction suggested limiting reagent **29** remained; therefore, additional 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, MS monitoring of the reaction suggested limiting reagent **29** still remained; thus, 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, MS monitoring

dx.doi.org/10.1021/jo400976f | J. Org. Chem. 2013, 78, 7470−7481

suggested the total consumption of **29**. 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), 4.69–4.70 (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 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.88 (td, J = 8.2, 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′-deoxy-5′-N-[(4-oxo-4H-benzo[e][1,2]oxazin-3-yl)-sulfonyl]adenosine (**3**).* To solid **41** (47 mg, 0.058 mmol, 1.0 equiv) at 0 °C was added ice-cold 80% aqueous 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 the 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), 4.03–4.04 (m, 1H), 4.07 (dd, J = 5.6, 2.9 Hz, 1H), 4.58 (t, J = 5.9 Hz, 1H), 5.67 (d, J = 6.2 Hz, 1H), 7.51 (t, J = 7.9 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.86 (t, J = 7.9 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).

Fluorescence Polarization Assays. The overexpression and purification of MbtA,[10] BasE,[33,16] EntE,[33,37] and VibE[33,38] were performed as previously described. The FP assays were performed using a modification of our previously described protocol.[33] Briefly, FP measurements were performed on a microplate reader 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 3-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) in FP buffer (30 mM Tris·HCl [pH 7.5], 1 mM $MgCl_2$, 0.0025% Igepal CA-630, and 1 mM final concentrations). The fluorescence anisotropy was measured after a 30 min incubation at 22 °C. Experimentally measured anisotropies A_{OBS} were fit to eqs 1 and 2 using Mathematica 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 3-fold serial dilution of each compound (0.5 μL, ∼1–100 000 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 eqs 2 and 3 to give the K_{D2}.

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

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

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

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 as previously described.[10] 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 84.12 Ci/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 of 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 of water. The washed pellet was resuspended in 200 μL of water, transferred to a scintillation vial, mixed with 15 mL of scintillation fluid (RPI), and counted on a scintillation counter. 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 for fitting concentration–response data for tight binding inhibitors as described by Copeland.[39]

***M. tuberculosis* H37Rv MIC Assay.** This assay was performed as previously described.[8c] Briefly, MICs were determined in quadruplicate in iron-deficient GAST according to the broth microdilution method[6c] 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.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, and ^{19}F NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: aldri015@umn.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by a grant from the National Institutes of Health (AI070219). We thank João Neres for providing FP probe, Fl-Sal-AMS. We thank Daniel Wilson for performing the [^{32}P]PP$_i$-ATP exchange assay and Dr. Helena Boshoff for whole-cell screening against *M. tuberculosis* H37Rv.

REFERENCES

(1) World Health Organization, Tuberculosis Fact Sheet No. 104, October 2012. http://www.who.int/mediacentre/factsheets/fs104/en/index.html.

(2) Koul, A.; Arnoult, E.; Lounis, N.; Guillemont, J.; Andries, K. *Nature* **2011**, *469*, 483.

(3) Ratledge, C.; Dover, L. G. *Annu. Rev. Microbiol.* **2000**, *54*, 881.

(4) (a) Miethke, M.; Marahiel, M. A. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 413. (b) Crosa, J. H.; Walsh, C. T. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 223. (c) Sandy, M.; Butler, A. *Chem. Rev.* **2009**, *109*, 4580. (d) Hider, R. C.; Kong, X. *Nat. Prod. Rep.* **2010**, *27*, 637.

(5) Snow, G. A. *Bacteriol. Rev.* **1970**, *34*, 99. (b) Vergne, A. F.; Walz, A. J.; Miller, M. J. *Nat. Prod. Rep.* **2000**, *17*, 99. (c) De Voss, J. J.; Rutter, K.; Schroeder, B. G.; Barry, C. E., 3rd *J. Bacteriol.* **1999**, *181*, 4443.

(6) (a) Tullius, M. V.; Harmston, C. A.; Owens, C. P.; Chim, N.; Morse, R. P.; McMath, L. M.; Iniguez, A.; Kimmey, J. M.; Sawaya, M. R.; Whitelegge, J. P.; Horwitz, M. A.; Goulding, C. W. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 5051. (b) Jones, C. M.; Niederweis, M. *J. Bacteriol.* **2011**, *193*, 1767. (c) De Voss, J. J.; Rutter, K.; Schroeder, B. G.; Su, H.; Zhu, Y.; Barry, C. E., 3rd *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 1252.

(7) (a) Quadri, L. E.; Sello, J.; Keating, T. A.; Weinreb, P. H.; Walsh, C. T. *Chem. Biol.* **1998**, *5*, 631. (b) Chavadi, S. S.; Stirrett, K. L.; Edupuganti, U. R.; Vergnolle, O.; Sadhanandan, G.; Marchiano, E.; Martin, C.; Qiu, W. G.; Soll, C. E.; Quadri, L. E. *J. Bacteriol.* **2011**, *193*, 5905. (c) McMahon, M. D.; Rush, J. S.; Thomas, M. G. *J. Bacteriol.* **2012**, *194*, 2809.

(8) (a) Ferreras, J. A.; Ryu, J. S.; Di Lello, F.; Tan, D. S.; Quadri, L. E. *Nat. Chem. Biol.* **2005**, *1*, 29. (b) Miethke, M.; Bisseret, P.; Beckering, C. L.; Vignard, D.; Eustache, J.; Marahiel, M. A. *FEBS J.* **2006**, *273*, 409. (c) Somu, R. V.; Boshoff, H.; Qiao, C.; Bennett, E. M.; Barry, C. E., 3rd; Aldrich, C. C. *J. Med. Chem.* **2006**, *49*, 31.

(9) Isono, K.; Uramoto, M.; Kusakabe, H.; Miyata, N.; Koyama, T.; Ubukata, M.; Sethi, S. K.; McCloskey, J. A. *J. Antibiot.* **1984**, *37*, 670.

(10) Somu, R. V.; Wilson, D. J.; Bennett, E. M.; Boshoff, H. I.; Celia, L.; Beck, B. J.; Barry, C. E., 3rd; Aldrich, C. C. *J. Med. Chem.* **2006**, *49*, 7623.

(11) Qiao, C.; Gupte, A.; Boshoff, H. I.; Wilson, D. J.; Bennett, E. M.; Somu, R. V.; Barry, C. E., 3rd; Aldrich, C. C. *J. Med. Chem.* **2007**, *50*, 6080.

(12) Vannada, J.; Bennett, E. M.; Wilson, D. J.; Boshoff, H. I.; Barry, C. E., 3rd; Aldrich, C. C. *Org. Lett.* **2006**, *8*, 4707.

(13) (a) Neres, J.; Labello, N. P.; Somu, R. V.; Boshoff, H. I.; Wilson, D. J.; Vannada, J.; Chen, L.; Barry, C. E., 3rd; Bennett, E. M.; Aldrich, C. C. *J. Med. Chem.* **2008**, *51*, 5349. (b) Gupte, A.; Boshoff, H. I.; Wilson, D. J.; Neres, J.; Labello, N. P.; Somu, R. V.; Xing, C.; Barry, C. E., 3rd; Aldrich, C. C. *J. Med. Chem.* **2008**, *51*, 7495.

(14) Labello, N. P.; Bennett, E. M.; Ferguson, D. M.; Aldrich, C. C. *J. Med. Chem.* **2008**, *51*, 7154.

(15) May, J. J.; Kessler, N.; Marahiel, M. A.; Stubbs, M. T. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 12120.

(16) Drake, E. J.; Duckworth, B. P.; Neres, J.; Aldrich, C. C.; Gulick, A. M. *Biochemistry* **2010**, *49*, 9292.

(17) Nelson, K.; Aldrich, C. unpublished data.

(18) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.

(19) Föhlisch, B. *Chem. Ber.* **1971**, *104*, 348.

(20) Löwe, W.; Matzanke, N. *J. Heterocycl. Chem.* **1996**, *33*, 763.

(21) Heacock, D.; Forsyth, C. J.; Shiba, K.; Musier-Forsyth, K. *Bioorg. Chem.* **1996**, *24*, 273.

(22) Winum, J.-Y.; Toupet, L.; Barragan, V.; Dewynter, G.; Montero, J.-L. *Org. Lett.* **2001**, *3*, 2241.

(23) Neustadt, B. R. *Tetrahedron Lett.* **1994**, *35*, 379.

(24) Chu, D. T.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.

(25) (a) Abdulla, R. F.; Brinkmeyer, R. S. *Tetrahedron* **1979**, *35*, 1675. (b) Abu-Shanab, F. A.; Sherif, S. M.; Mousa, S. A. S. *J. Heterocycl. Chem.* **2009**, *46*, 801. (c) Reiter, L. A. *J. Org. Chem.* **1984**, *49*, 3494.

(26) Ikeuchi, M.; Meyer, M. E.; Ding, Y.; Hiratake, J.; Richards, N. G. *Bioorg. Med. Chem.* **2009**, *17*, 6641.

(27) Mitsunobu, O. *Synthesis* **1981**, 1.

(28) Mitscher, L. A. *Chem. Rev.* **2005**, *105*, 559.

(29) Touster, O. *Org. React.* **1953**, *7*, 327.

(30) Ferris, J. P.; Sanchez, R. A.; Mancuso, R. W. *Org. Synth.* **1968**, *48*, 1.

(31) Pharmacia & Upjohn Co. WO200200444, 2002.

(32) 1,2-Benzoxazin-4-ones are rare heterocycles with a mere 20 examples found in SciFinder, searched April 8, 2013.

(33) Neres, J.; Wilson, D. J.; Celia, L.; Beck, B. J.; Aldrich, C. C. *Biochemistry* **2008**, *47*, 11735.

(34) pK_a estimated using Marvin 5.12.3, 2013, ChemAxon (http://www.chemaxon.com).

(35) Roehrl, M. H. A.; Wang, J. Y.; Wagner, G. *Biochemistry* **2004**, *43*, 16056.

(36) Döbele, M.; Vanderheiden, S.; Jung, N.; Bräse, S. *Angew. Chem., Int. Ed.* **2010**, *49*, 5986.

(37) Drake, E. J.; Nicolai, D. A.; Gulick, A. M. *Chem. Biol.* **2006**, *13*, 409.

(38) Keating, T. A.; Marshall, C. G.; Walsh, C. T. *Biochemistry* **2000**, *39*, 15522.

(39) Copeland, R. A. *Evaluation of Enzyme Inhibitors in Drug Discovery*; Wiley: Hoboken, NJ, 2005.

(40) Sharma, S. K.; Miller, M. J.; Payne, S. M. *J. Med. Chem.* **1989**, *32*, 357.