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Targeting *Mycobacterium tuberculosis* Biotin Protein Ligase (MtBPL) with Nucleoside-Based Bisubstrate Adenylation Inhibitors

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Abstract

Mycobacterium tuberculosis (*Mtb*) responsible for both latent and symptomatic tuberculosis (TB) remains the second leading cause of mortality among infectious diseases worldwide.

Mycobacterial biotin protein ligase (*MtBPL*) is an essential enzyme in *Mtb* and regulates lipid metabolism through the post-translational biotinylation of acyl coenzyme A carboxylases. We report the synthesis and evaluation of a systematic series of potent nucleoside-based inhibitors of *MtBPL* that contain modifications to the ribofuranosyl ring of the nucleoside. All compounds were characterized by isothermal titration calorimetry (ITC) and shown to bind potently with K_D 's below 2 nM. Additionally, we obtained high-resolution co-crystal structures for a majority of the compounds. Despite fairly uniform biochemical potency, the whole-cell *Mtb* activity varied greatly with minimum inhibitory concentrations (MIC) ranging from 0.78 to >100 μ M. Cellular accumulation studies showed a nearly 10-fold enhanced accumulation of a C-2'- α analog over the corresponding C-2'- β analog, consistent with their differential whole-cell activity.

Keywords

Biotin protein ligase; *Mycobacterium tuberculosis*; tuberculosis; bisubstrate adenylation inhibitors

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Supporting Information. Full ¹H and ¹³C NMR spectra of all described compounds, as well as direct and indirect raw ITC data for all final analogs included as Figures S1–14 and Table S1. A summary of observed diffraction and refinement statistics, along with figures depicting ligand omit difference density is included as Table S2.

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Ancillary Information: Atomic coordinates and diffraction data for *MtBPL* complexes with compounds **36**, **46**, **57**, **63**, **69**, **73**, **82**, **87** and **90** have been deposited with the Protein Data Bank with accession codes 4xtv, 4xtw, 4xtx, 4xty, 4xtz, 4xu0, 4xu1, 4xu2 and 4xu3, respectively.

Introduction

Tuberculosis (TB) is a leading cause of bacterial infectious disease mortality and morbidity. The bacillus *Mycobacterium tuberculosis* (*Mtb*) is responsible for both latent and symptomatic TB and belongs to the greater *Mycobacterium tuberculosis* complex, which includes *M. bovis*, *M. africanum*, *M. canettii*, and *M. microti*.¹ The current treatment for drug sensitive TB requires combination therapy with the first line drugs isoniazid, rifampin, pyrazinamide and ethambutol for 6–9 months.² Successful treatment of TB necessitates (1) stringent supervision by well-trained care givers, (2) an ample and consistent supply of high quality medicines and (3) drug intake fidelity by patients to minimize the risks of recrudescence and drug resistance. These are already tall obstacles to surmount in remote and under developed regions where TB is endemic. Consequently, the emergence of multidrug- and extensively drug-resistant TB (MDR-TB and XDR-TB) strains which are minimally resistant to the two most effective drugs, isoniazid and rifampin, has alarmed clinicians and threatens the positive gains made in combatting TB.³ Hence, it is imperative to develop new classes of anti-tubercular agents for this worldwide clinical need that are effective against drug-resistant TB and ideally possess a novel mode of action.

From the onset *Mtb* has both intrigued and frustrated researchers. Its cellular envelope contains a panoply of structurally diverse lipids that provide a protective barrier and modulate host-pathogen interactions.^{4,5} The fortified waxy cell envelope in turn makes TB a particularly challenging and intransigent infection to treat. The mycolic acids (**1**) are the most abundant lipids in *Mtb* comprising approximately 30% of its dry cellular weight and also the largest lipids known in nature containing up to 90 carbon atoms (Figure 1).^{6,7} These essential lipids are covalently attached to the arabinogalactan core of the cell wall and anchor the outer membrane. Other lipids and lipidated metabolites such as the phenolic glycolipids (PGLs, **2**), phthiocerol dimycocerosate A (PDIM-A, **3**), sulfolipids (SLs, **4**) and mycobactins (MBTs, **5**) are non-covalently associated with the cell envelope and critical for virulence.^{8–10}

All mycobacterial lipids are derived from simple malonyl coenzyme A (CoA) building blocks that are synthesized by acyl CoA carboxylases (ACCs). Distinct from most bacteria, *Mtb* possesses multiple ACCs for preparation of malonyl CoA, methylmalonyl CoA, and long-chained malonyl CoAs needed for synthesis of simple fatty acids, methyl branched lipids, and the mycolic acids, respectively.^{11–13} The multimeric ACCs require post-translational biotinylation in order to become functionally active, which is catalyzed by biotin protein ligase (BPL) encoded by *birA* (Rv3273c) in *Mtb*.¹⁴ The biotin cofactor mediates carboxy group transfer onto the acyl CoA substrates (Figure 2B). Consequently, *MtBPL* universally regulates lipid metabolism in *Mtb* and therefore represents an attractive biochemical target for therapeutic development.¹⁵

MtBPL catalyzes a two-step reaction initiated when the substrate biotin (**6**) and ATP bind to form an acyl-adenylate intermediate (**7**, Bio-AMP) and pyrophosphate (Figure 2A).¹⁶ In the succeeding step, *MtBPL* binds the ACC and catalyzes the transfer of biotin from **7** onto the terminal amine of a conserved lysine residue to afford an amide bound biotinylated-ACC

complex (**8**). We have reported the design of a bisubstrate inhibitor of *Mt*BPL termed Bio-AMS (**14**) as well as several related analogs including **15**, **16**, and **17**.^{15, 17} These analogs mimic **7** through substitution of the labile acyl-phosphate linkage in **7** with stable acyl-sulfamide moieties (Figure 3). Compound **14** is exceptionally potent, binding *Mt*BPL with a K_D of approximately 0.5 nM or more than 1,700 times more tightly than biotin, and derives its potency through interactions with the biotin and adenosine substrate binding pockets.¹⁷ The sulfamide is a near perfect phosphate isostere and is also negatively charged at pH 7 (the estimated pK_a of the acyl-sulfamide NH group that is *N*-acylated is ~3), which ensures preservation of the critical electrostatic interactions with R69, R72, and K138 of *Mt*BPL. Several other related bisubstrate biotin-adenosine inhibitors of BPLs from other organisms have also been described including: alkylphosphate **10**,¹⁸ β -ketophosphonate **11**,¹⁹ 1,2,3-triazole **12**,²⁰ and acylsulfamate **13**. More recently, Tieu, Abell and coworkers identified a novel series of *Staphylococcus aureus* BPL inhibitors containing a heterocyclic benzoxazoline moiety exemplified by **18** wherein the adenosine nucleobase has been replaced altogether.^{21,22} Furthermore, biotin analogs (**19** and **20**) with selective *S. aureus* BPL inhibitory activity have also been reported.²³

In addition to its potent biochemical activity, **14** was shown to possess promising antimycobacterial activity against ten MDR-TB and XDR-TB strains with minimum inhibitory concentrations (MICs) ranging from 0.16 to 0.625 μ M.¹⁵ Proteomic analysis demonstrated **14** inhibited biotinylation in whole-cell *Mtb* while overexpression of *Mt*BPL conferred resistance in *Mtb* and *M. smegmatis*. Collectively, these results provide strong evidence for the designed mechanism of action.¹⁵ The structure of **14** and **7** bound to *Mt*BPL reveals a large number of electrostatic interactions with the biotin, adenine, and acylsulfamate linkage as well as limited space in the active site to further extend the ligand at these positions.^{15,24} By contrast, the ribose moiety of the nucleoside has relatively few interactions with the protein and an adjacent accessible crevice to grow the ligand. As a result, we believe modification of the ribose offers the greatest opportunity to further increase potency and improve drug disposition properties. Based on these considerations, we describe herein the design, synthesis and evaluation of a systematic series of rationally designed bisubstrate inhibitors of *Mt*BPL that probe the consequences of modifications to the ribofuranosyl ring of **14**.

RESULTS AND DISCUSSION

Chemistry

The 4'-ribofuranosyl oxygen of the lead compound **14** does not appear to have any interactions with *Mt*BPL.¹⁵ To investigate the impact of deleting the 4'-ribofuranosyl oxygen we proposed the synthesis of a trio of analogs that include carbocyclic Bio-AMS **28**, carbocyclic 2',3'-dideoxy-Bio-AMS **36** and carbocyclic 2',3'-dideoxy-2',3'-dehydro-Bio-AMS **34**. Analogs **28**, **34** and **36** are all derived from the common intermediate **23**. Scheme 1 illustrates the synthesis of **28**. The aristeromycin derivative **24** was synthesized from Vince lactam (**21**),^{25,26} which was first elaborated to the cyclopentenylamine intermediate **22** in three steps following methodology developed by Jung and Rhee.^{27,28} The installation of the adenine moiety was accomplished via a palladium catalyzed allylic amination to

afford adenine intermediate **23**. The use of $[\text{PdCl}(\text{C}_3\text{H}_5)]_2/\text{triisopropylphosphite}$ as the catalyst and Cs_2CO_3 as the base was found to be superior to previous reported conditions for related compounds.²⁸ Pivalate hydrolysis of **23** followed by dihydroxylation and acetonide protection afforded a mixture of diastomeric carbocyclic nucleosides **24 α** and **24 β** in an approximately 1:1 ratio. The relative stereochemistry was confirmed by NOESY studies, comparison of ^1H NMR with literature reported values, and co-injection with an authentic standard of **24 α** obtained from the natural product aristeromycin.^{29–31} The aristeromycin derivative **24 α** was then converted into the bis-Boc-protected intermediate **25** in two steps, followed by desilylation to afford **26**. A Mitsunobu reaction with *tert*-butyl sulfamoylcarbamate was employed for direct installation of the sulfamide moiety at C-5'. It was discovered that excess triphenylphosphine and DIAD resulted in a significant generation of an iminophosphorane byproduct of **27** (not shown). This was avoided using three equivalents of *tert*-butyl sulfamoylcarbamate (3.0 equiv) and slow addition via syringe pump of a slight excess of DIAD (1.05 equiv). Biotinylation employing $\text{D-}(+)\text{-biotin } N\text{-hydroxysuccinimide ester}$ (Bio-NHS) mediated by Cs_2CO_3 followed by global deprotection with 50% aqueous TFA, reverse-phase HPLC purification and lyophilization afforded the carbocyclic Bio-AMS **28** in 56% over two steps as the triethylammonium salt.

The retention of the C2'-C3' olefinic bond in analog **34** and saturation of the olefin in cyclopentyl analog **36**, were designed to explore the impact of deletion of the corresponding hydroxyl groups and examine the subtle conformational implications on binding that result from saturation of the olefin. Continuing from intermediate **23**, hydrolysis of the C-5' pivaloyl ester proceeded with facility to afford **29** (Scheme 2). Reprotection of the C-5' alcohol as the silyl ether **30** followed by bis-Boc protection of the N^6 amine provided **31**. Desilylation of the primary alcohol yielded **32** that was utilized in the succeeding Mitsunobu reaction to give sulfamide intermediate **33**. Biotinylation provided the penultimate crude material, which was followed by global deprotection, reverse-phase HPLC purification, and lyophilization to achieve analog **34** in 52% over two steps. Analog **36** was synthesized over three synthetic steps from previously synthesized unsaturated intermediate **33**. Hydrogenation using 10 wt% Pd/C in EtOAc supplied the saturated carbocyclic Boc-sulfamide intermediate **35**. Biotinylation followed by direct global deprotection of the crude material with 80% aqueous TFA, reverse-phase HPLC purification and lyophilization furnished final analog **36** in 58% over two steps.

To directly target and manipulate the interactions that exist between the 2'-hydroxyl moiety of our lead compound **14** and an Asp167 residue in the binding pocket and provide a functional handle to grow ligands into the adjacent crevice, we envisioned the introduction of alternate functional groups at the C-2' carbon. Analogs **46**, **47**, **57**, **63** and **69** are representative archetypal 2'-deoxy-molecules that contain α -azido, α -amino, β -azido, α -fluoro and β -fluoro substituents, respectively. The synthesis of 2'- α -azido (**46**) and 2'- α -amino (**47**) analogs is shown in Scheme 3. Vidarabine (**37**) was protected simultaneously at the C-3' and C-5' alcohols using the Markiewicz protecting group yielding cyclic siloxane **38**.³² Conversion of the C-2' alcohol into the corresponding triflate **39** occurred smoothly. $\text{S}_{\text{N}}2$ displacement of the triflate by NaN_3 in DMF at 65 °C proceeded with inversion of configuration to provide **40**.³³ bis-Boc protection of the N^6 amine furnished **41**.³⁴ The

selective mono-deprotection of either the C-3' or C-5' alcohol nucleosides protected by the Markiewicz group is extensively described in the literature.³⁵ Utilizing a TFA, H₂O and THF (1:1:4) solution it is possible to exclusively deprotect the C-5' alcohol while leaving the C-3' alcohol protected.³⁶ We were successful in the aforementioned deprotection only to discover that any subsequent chemistry on the C-5' alcohol was hampered by the steric bulk of the Markiewicz group remnants still attached to C-3'. It was decided that the complete removal of the Markiewicz protecting group with TBAF and 1% AcOH followed by reprotection was the best avenue to pursue. Thus, removal of the Markiewicz group afforded **42** followed by TBS reprotection of the C-3' and the C-5' alcohols using standard protocols achieved **43**. Regioselective removal of the C-5' TBS group employing a 3:1:1 AcOH–THF–H₂O mixture provided the free C-5' alcohol **44**.³⁷ Despite the formation of inseparable side products, formation of sulfamide intermediate **45** via the Mitsunobu reaction proceeded smoothly. The standard biotinylation reaction described previously yielded the biotinylated product that was immediately followed by global deprotection in 80% aqueous TFA. The crude product was purified via reverse phase HPLC in a yield of 62% over two steps to furnish **46**. Further elaboration of the 2'- α -azido to the 2'- α -amine, an isostere of the C2'-hydroxyl on **14**, was affected by the Staudinger reduction to provide **47**.^{38,39}

The only difference between 2'- α -azido (**46**) described above and 2'- β -azido (**57**) is the β -facing appendage at the C-2' carbon. This minor alteration precluded us from utilizing the more sterically demanding Mitsunobu reaction to install the sulfamide arm. Therefore, we were forced to pursue sulfamate analogs for our β -C-2' analogs. The sulfamates were installed using the Okada protocol.⁴⁰ Sulfamoyl chloride is sterically smaller and vastly more reactive than *tert*-butyl sulfamoylcarbamate. Towards the synthesis of analog **57** shown in Scheme 3, adenosine (**48**) was analogously elaborated to **55**, which was treated with sulfamoyl chloride in dimethylacetamide (DMA) to afford sulfamate **56**. Biotinylation of the sulfamate intermediate was followed by acidic global deprotection, HPLC purification and lyophilization to yield **57** in 76% over two steps. Our previous experiences with a biotinylated acyl-sulfamate analog had been marred with rapid decomposition via an intramolecular displacement reaction to form the biologically inactive 3',5'-cyclo-5'-deoxyadenosine and *N*-(biotinyl)sulfamic acid.¹⁵ Remarkably in the cases of analogs **57** as well as **69** and **73** (*vide infra*), we were pleasantly surprised to discover that they resisted any cyclonucleoside formation. Their stability can be attributed to the β -facing C-2' moieties, which preclude the adenine base from adopting the *syn*-conformation around the glycosidic linkage from which the intramolecular displacement reaction occurs.

Introduction of a fluorine atom at the C-2' position of nucleosides is well known to increase anomeric stability, modulate ring pucker and binding affinity as well as enhance pharmacokinetic properties.^{41,42} Scheme 4 illustrates our synthesis of the 2'-deoxy-2'- α -fluoro analog **63** starting with commercially available 2'-deoxy-2'- α -fluoro-adenosine **58**. TBS protection of the C-3' and C-5' alcohols furnished **59**. Again, bis-Boc protection of the *N*⁶ amine cleanly afforded **60** and mono-desilylation, using our previously described conditions, achieved the desired product **61**. Formation of the sulfamide intermediate **62** using our optimized Mitsunobu procedure gave slightly impure product, which was used in the next reaction. Biotinylation, followed by global deprotection in 80% aqueous TFA,

furnished the final analog **63** in 56% over the final two steps. We also pursued the synthesis of the 2'-deoxy-2'- β -fluoro biotinylated analog **69** (Scheme 4). Compound **64** was analogously elaborated to **67**. Sulfamate formation (**68**) was followed by biotinylation and then global deprotection with 80% aqueous TFA. Reverse phase HPLC purification and lyophilization furnished the final analog **69** in a three-step yield of 51%.

Further modifications of the glycoside unit steered us towards the synthesis of analogs **73** in Scheme 5 and **82** in Scheme 6. Addition of the β -methyl to C-2' facilitated our investigation into whether the application of a small lipophilic arm would have any effect on binding. Analog **73** was synthesized over 9-steps from the tribenzoyl protected ribose **70**. Compound **71** was attained over six arduous chemical steps.⁴³ Sulfamate formation in DMA afforded **72** in good yield.^{28,30} Subsequent biotinylation was followed by global deprotection to afford analog **73** in 45% yield over three steps.

An even more extreme alteration can be seen in derivative **82** in which the ribosyl ring has been altogether removed in favor of a morpholine ring. Analog **82** was designed primarily as a platform for preparation of derivatives based upon modification of the morpholine nitrogen. The synthetic route to morpholine analog **82** (Scheme 9) began with commercially available *N*⁶-benzoyl adenosine (**74**). After selective TBDPS protection of the C-5' hydroxyl to afford **75**, reaction with sodium *meta*-periodate in MeOH resulted in oxidative cleavage of the C-2'–C-3' bond to reveal the nonisolable dihemiacetal intermediate **76**, which underwent *in situ* reaction with ammonium baborate to afford dihemiaminal **77**. Reduction with sodium cyanoborohydride in the presence of glacial acetic acid provided crude morpholine **78**.^{44,45} *N*-Tritylation furnished **79** in 43% yield over four synthetic steps.^{44,46} Desilylation followed by sulfamoylation of **80** in DME–MeCN afforded sulfamate **81** that was biotinylated using our standard protocol. The synthesis was completed by ammonolysis to remove the *N*⁶-benzoyl group and by subsequent treatment with 80% aqueous acetic acid to deprotect the *N*-trityl group to give **82** in 36% yield over three steps from **81**.

To examine the importance of the 2' and 3'-hydroxyls for biological activity, we attempted to prepare 2'-deoxy- and 3'-deoxy-Bio-AMS analogs. We initially synthesized 2'-deoxy-Bio-AMS (not shown), but discovered the molecule was innately unstable and rapidly decomposed at room temperature. We attribute this instability to a vastly less pronounced anomeric effect which is derived from poor orbital overlap between C-1' and C-2' resulting in a high propensity to depurinate.⁴⁷ However, 3'-deoxy-Bio-AMS **87** was successfully synthesized over six steps from commercially available 3'-deoxy-adenosine (**83**, Scheme 7). TBS protection of the C-2' and C-5' alcohols was followed by bis-Boc protection of *N*⁶ furnished **84** in 82% over two steps. Mono-desilylation yielded the desired product **85**. Formation of the sulfamide intermediate using our optimized Mitsunobu protocol afforded slightly impure product **86**, which was used directly in next reaction. Biotinylation was followed by global deprotection in 80% aqueous TFA to supply the final analog **87** in 62% over the last three steps.

To complete our narrative we examined the consequence of altogether eliminating the ribose moiety with acyclo-Bio-AMS analog **90** (Scheme 8). Acyclo-sulfamide analog **90** was prepared from the reported acycloadenosine (**88**),⁴⁸ that was converted to sulfamide

intermediate **89**, which was biotinylated and deprotected to provide **90** in 52% over three steps.

Ligand characterization by co-crystallization with *MtBPL* and isothermal titration calorimetry

The previously described co-crystal structures with **14**¹⁵ and **7**²⁴ revealed a binding site with two deep and largely enclosed subsites for the biotinyl and adenosyl groups, and the sidechain of the Trp74 stacked between them. The ribose lies across the open mouth of this pocket. The 2'-hydroxyl of **14** is engaged in a hydrogen bond with the carboxylate of Asp167, while the 3'-hydroxyl makes a water mediated contact with Gly73 (Figure 4A). In order to characterize possible structural consequences of glycosyl substitutions, co-crystal structures with compounds **36**, **46**, **57**, **63**, **69**, **73**, **82**, **87**, and **90** have been determined at high resolution (Diffraction data and summary refinement statistics are provided in Table S2). The various complexes with bisubstrate inhibitors are remarkably similar. The presence of an acylsulfamate with the 5' oxygen in **57**, **69**, **73**, and **82** instead of an acylsulfamide with the 5' nitrogen in **14**, **36**, **46**, **63**, **87**, and **90** seems structurally inconsequential; a hydrogen bond between the deprotonated acylsulfamate/acylsulfamide nitrogen and the amide NH of Arg69 is conserved in all structures.

A rotameric shift of Asp167 might have been anticipated upon loss of the 2'-hydroxyl, but no change is observed. A hydrogen bond from OD2⁶⁵ to the amide NH of Asp169 holds the carboxylate in position in all structures (Figure 4A). No shift occurs even in the case of analogs missing the 2'-hydroxyl (**36** or **90**). Altered stereochemistry at the 2'-position is also well tolerated. The 2'- α -fluoro and 2'- β -fluoro compounds (**63** and **69**, respectively) bind so similarly that all other atoms in the vicinity of the ligands almost perfectly align (Figure 4B, C). Both azido analogs **46** and **57** also compare similarly. In cases where a 2'-substituent is aliphatic, as in the 2'- β -methyl of **73** or the morpholine ring of **82**, the Asp167 side chain shifts just far enough (without changing rotamers) to extend the contact distance between OD1⁶⁵ and the carbon to the sum of their van der Waals radii (3.4 Å). The similarity of the structures is reflected in the flat SAR in binding affinity observed by ITC for these compounds.

Analog **82** and **90** are interesting in that they represent the most diverse departures from the ribofuranosyl ring, yet are well tolerated. Even the acyclic analog (**90**) follows a trajectory through C-1', O, and C-5' adopted by the ribose in Bio-AMS (**14**) (Figure 4D). The morpholine of **82** also superimposes well upon this triad. The conformation is likely necessary to allow the adenosine and biotin moieties to retain the same relative orientations upon binding.

Little density is observed for the azido functional group in complexes with **46** and **57**. Sufficient density confirms the stereochemistry of the 2' substitution, but the remainder of the azido group appears disordered in rotation about the C-2'-N bond. In each complex, this group has been modeled in the conformation that gives rise to at least (-) density in a $F_o - F_c$ difference maps, but any assignment of a preferred rotamer based on diffraction data is uncertain at best.

Because of the exceedingly tight-binding nature (K_D values ≈ 2 nM) of our family of analogs to *MtBPL* we were unable to determine their potency using a steady-state kinetic assay. Thus we enlisted a technique that has gained popularity to determine ligand protein binding affinities. Originally described in 1965 by Christensen and coworkers, isothermal titration calorimetry (ITC) was first used to concurrently determine the K_{eq} and H of various weak acid–base equilibria and metal ion complexation reactions.⁴⁹ Today, ITC has become a powerful and commonplace tool to directly characterize the thermodynamics of ligand-protein interactions. ITC enables determination of the dissociation constant (K_D), Gibbs free energy (G), enthalpy (H) and entropy (S) of ligand-protein interactions allowing researchers to tease out nuanced information of ligand binding.⁵⁰ Using **14** as our benchmark we evaluated a series of analogs for their activity against *MtBPL* using ITC (Table 1). Unfortunately, the remarkably tight-binding nature of our analogs exceeded the limit ($K_D = 10^{-8}$ M) of accurate determination for binding constants by ITC.⁵¹ Therefore, we employed a more reliable approach for determining the dissociation constants less than 10^{-8} M by using displacement ITC, in which our high affinity ligands are titrated into a solution of protein that is bound to a weaker ligand (in this case, biotin). Enthalpies (H) were determined by direct titration for all ligands while dissociation constants (K_D) and Gibbs free energies (G) were measured by displacement ITC experiments (see methods, Figures S1–S14, and Table S1).

All ligands bound tightly to *MtBPL* with K_D 's ranging from 27 pM to 2.14 nM. Binding was enthalpically driven and the H 's ranged from -18.8 to -29.3 kcal/mol (Table 1). Carbocycle **28** that varies by replacement of the ribofuranosyl ring oxygen with a CH_2 moiety was nearly equipotent to **14** with a K_D of 0.75 nM. **28** had a 1.2 kcal/mol decrease in H that was compensated by an equal increase in the entropic term, resulting in an identical G of -12.4 kcal/mol. Remarkably, cyclopentenyl **34**, cyclopentanyl **36** and acyclo **90** all are missing key hydrogen bond donors and acceptors (C-2' and C-3' hydroxyls), yet still bound tightly with K_D 's of 2.14, 0.54, and 0.315 nM, respectively. These analogs confirmed our notion that the ribofuranosyl oxygen alone played a limited role in the binding pocket. The results also suggest that deletion of the C-2' and C-3' hydroxyls in addition to the removal of the 4'-ribofuranosyl oxygen is also well tolerated inferring that the numerous other electrostatic hydrogen bonding interactions between the ligand and protein are sufficient to maintain affinity. Interestingly, compound **90** has improved enthalpic contributions, which could be attributed to improved interactions with *MtBPL* as a result of its increased flexibility. Cyclopentyl **34** is the least potent of all ligands evaluated. The presumed constrained C_s envelope conformation of the cyclopentene ring likely does not allow optimal interactions within the binding pocket. Compounds **46**, **47**, and **57** all replace the hydrogen bond donating 2'-hydroxyl with a 2'-amino or 2'-azido group. Both 2'- α substituted compounds **46** and **47** were nearly equipotent to **14**, with **47** being slightly better than **46**. Surprisingly, the 2'- β -azido analog (**57**) proved to be the tightest binder, with a K_D of 27 pM, which is 32-fold more potent than **14**. Additionally, **57** bound with the highest enthalpy of -29.3 kcal/mol, which cannot be easily reconciled. We speculate the azide may directly interact with *MtBPL*, however, it is not clear from the crystallographic data since there is little electron density for the azide. Both compounds **63** and **69** are epimeric with respect to their C-2'-fluoro substituents and bind with K_D 's of 0.614 nM and 0.153 nM. Although **63** and **69** are

similar in potency to **14**, they exhibit unique thermodynamic binding signatures driven by large enthalpic contributions of -24.3 kcal/mol and -28.4 kcal/mol. This could be attributed to the improved dipole-dipole interactions with *MtBPL* by the incorporation of the fluorine atom into the structure. Compounds **73** and **87** both had slight ring modifications, incorporating a 2'- β -methyl into the ring or removal of the 3'-hydroxyl, respectively. Both compounds exhibited similar binding profiles in terms of K_D with **87** displaying slightly tighter binding affinities. The improved enthalpic contribution of **73** can most likely be attributed to van der Waals interactions from the 2'- β -methyl group. Finally, the morpholine **82** analog with the most significant structural change to the ring structure was well tolerated and bound with a K_D of 0.324 nM as well as an identical thermodynamic binding signature compared to **14**. This result indicates *MtBPL* is quite tolerant to the perturbations of the glycosyl ring.

We also investigated how well our analogs stabilize *MtBPL* through a general binding experiment using differential scanning fluorimetry (DSF).⁵² They all had significant stabilization properties as evident by their change in melting temperature ranging from a change of 12.2 to 19.2 °C (Table 1). There seems to be limited correlation between the change in melting temperature and binding affinity, and these data could only be interpreted as a qualitative measure of ligand stabilization to *MtBPL*.

Anti-tubercular Activity and Cytotoxicity

We next determined the minimum inhibitory concentration that inhibited greater than 90% of cell growth (MIC) of our analogs against *Mtb* H37Rv. We previously published the MIC for **14** against H37Rv as 0.78 μ M.¹⁵ The MIC values of our Bio-AMS derivatives are as diverse as the molecules themselves (Table 1). The parent **14** still reigns supreme as the most potent inhibitor as determined by its whole-cell activity. The carbocyclic molecules **28**, **34** and **36** are all deprived of the 4'-ribofuranosyl oxygen. Analog **34** incorporates an olefin while **36** is a saturated cyclopentane. Interestingly, analog **28** does not show strong activity against *Mtb*, even at 100 μ M, but to our amazement both analogs **34** and **36** displayed MIC values that were comparable to **14**.

Analogs **46** and **57** along with compounds **63** and **69** are respective C-2' epimers, but differ in their substituents. Yet this minor modification in the stereochemistry had significant implications on the anti-tubercular activity of the molecules. The C-2'- α substituted compounds **46**, **47** and **63** are roughly as active as **14** against *Mtb* H37Rv, most likely due to the linearity and directionality of the new appendages which mimic that of the C-2' hydroxyl in Bio-AMS. This can be further attested by comparing the subtle changes in analogs **46** and **47**; 2'- α -amino analog **47** is slightly more potent than 2'- α -azido analog **46**, likely due to amino groups being significantly superior bioisosteres of hydroxyls than azides. Alternatively, compounds **57** with the 2'- β -azido and **69** with the 2'- β -fluoro handles are void of any biological activity. The poor anti-tubercular activity of compounds **57** and **69** is mirrored by analog **73** which also contains a β -facing attachment (CH_3) at the C-2' position. These results suggest a steric clash between the β -facing C-2' appendages and a putative nucleoside transporting protein responsible for uptake or association.

Of the current set of Bio-AMS analogs, the morpholine analog **82** and acyclo analog **90**, which exhibited the greatest structural changes, also exhibited potent antitubercular activity. The increased flexibility of the acyclo moiety in **90** and 6-membered ring in **82**,⁵³ as opposed to a 5-membered ribofuranosyl ring, could allow for perturbations without sacrificing whole-cell activity or binding affinity. We were also confident that deletion of the C-3' hydroxyl would result in a marginal change to the MIC when juxtaposed to Bio-AMS. As expected, compound **87** showed an equipotent MIC value of 0.78 μM to that of **14**. Moreover, the synthesis of **87** from 3'-deoxy-adenosine was facile in comparison by circumventing the obligatory C-3' and C-5' hydroxyl protection with the Markiewicz protecting group which rendered the syntheses of **46**, **47** and **57** cumbersome.

To assess the potential cytotoxicity of our compounds, Bio-AMS and a subset of the most active analogs (**14**, **36**, **63**, **82**, and **90**) were evaluated against HepG2 cells using an MTT assay. No inhibition of growth was observed ($\text{IC}_{50} > 100 \mu\text{M}$) for any of the tested compounds.

Cellular Uptake of C-2'-fluoro-Bio-AMS Analogs

The discordant whole-cell MIC data compared to binding data for C-2' epimers **63** and **69** prompted investigation of their cellular accumulation. Utilizing the uptake methodology developed by Rhee and coworkers,^{54,55} the amounts of compounds **63** and **69** associated with the cells were quantified by LC-MS/MS. The amount of cell-associated compound **63** was determined to be approximately 7-fold higher than compound **69** when *Mtb* H37Rv was incubated with 25 μM of each analog. This method, however, does not discern between the amounts of compound that is intracellular versus cell-associated. Nevertheless, there is still a substantial difference between the amounts of compound **63** and **69** that are accumulated giving credence to the hypothesis that the disparate MIC and binding data of the C-2' fluoro analogs can be potentially explained by differential cellular accumulation.

CONCLUSION

We have successfully designed and synthesized a focused series of *Mt*BPL bisubstrate inhibitors by strategically modifying the ribofuranosyl ring. All analogs bound tightly to *Mt*BPL, with K_D values ranging from 27 pM to 2.14 nM. Aside from the weakest binding **34** and strongest binding **57**, all compounds exhibited a narrow range of potency from 0.153 to 0.933 nM. ITC also illustrated the subtleties in the thermodynamic binding signatures of each analog that cannot be easily reconciled. These data, along with X-ray co-crystal structures, demonstrate modification or substitution of the ribofuranosyl ring does not substantially impact the binding of the analogs to *Mt*BPL. However, the relatively flat biochemical potencies sharply contrast with whole-cell antitubercular activity. Notably, all of the analogs containing a C-2'- β substituent including **57**, **69** and **73** had substantially reduced whole-cell activities with MIC ranging from 50 to $>100 \mu\text{M}$ despite potent binding affinities. This can only be attributed to differences in cellular accumulation through enhanced efflux or reduced uptake, and when examining the differences in cellular accumulation between **63** and **69**, there was substantial preference for cellular association of compound **63** over **69**. The other analogs in the series, except **28**, possessed whole-cell

activity commensurate with **14** with MIC values ranging from 0.78–3.12 μM . Both **47** and **82** represent promising ligands for further optimization since they can be readily functionalized through 'click chemistry' or substitution of the endocyclic nitrogen, respectively.

EXPERIMENTALS

General materials and methods

Chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Sigma-Aldrich, and TCI America and were used as received. The nucleosides 9- β -D-arabinofuranosyladenine **37** and 3-deoxyadenosine **83** were obtained from Berry & Associates (Dexter, MI) while 2'-deoxy-2'-fluoro-adenosine **58** and 9-(2'-deoxy-2'-fluoro- β -D-arabinofuranosyl)adenine **64** were obtained from Metkinen Chemistry (Kuopio, Finland), and N^6 -benzoyladenine **74** was obtained from Carbosynth (Berkshire, UK). *tert*-Butyl sulfamoylcarbamate,⁵⁶ sulfamoyl chloride,⁵⁷ D-(+)-biotin *N*-hydroxysuccinimide ester,⁵⁸ 5'-[*N*-(D-biotinoyl)sulfamoyl]amino-5'-deoxyadenosine triethylammonium salt (Bio-AMS) **14**,¹⁵ {(1*S*,4*R*)-4-[*N*-(phenylsulfonyl)pivalamido]cyclopent-2-enyl}methyl pivalate **22**,²⁸ 2',3'-*O*-isopropylidene- β -2'-methyladenosine **71**,⁵⁹ and 9-[(2-hydroxyethoxy)methyl]adenine **88**,⁴⁸ were prepared as described. 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 gas (Ar). Anhydrous grade MeOH, MeCN, pyridine, and DMA were purchased from Aldrich. EtOAc and hexanes were purchased from Fisher Scientific. All reactions were performed under an inert atmosphere of dry argon gas (Ar) in oven-dried (180 °C) glassware. TLC analyses were performed on TLC silica gel plates 60F254 from EMD Chemical Inc. and were visualized with UV light. Optical rotations values were obtained on a polarimeter using a 1 dm cell. Purification by flash chromatography was performed using a medium-pressure flash chromatography system equipped with flash column silica cartridges with the indicated solvent system. Preparative reversed-phase HPLC purification was performed on a Phenomenex Gemini 10 μm C18 250 \times 20 mm column operating at 30.0 mL/min with detection at 254 nm with the indicated solvent system. Analytical reversed-phase HPLC purification was performed on a Phenomenex Gemini 5 μm C18 250 \times 4.6 mm column operating at 1 mL/min with detection at 254 nm employing a linear gradient from 5% to 50% MeCN in 50 mM aqueous triethylammonium bicarbonate (TEAB) at pH 7.5 for 30 min (Method A). ^1H and ^{13}C spectra were recorded on 400, 600 or 900 MHz NMR spectrometers. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26), methanol (3.31), 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 (77.0), methanol (49.1), or dimethyl sulfoxide (39.5). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dt = doublet of triplets, t = triplet, q = quartet, pentet = pent, m = multiplet, ap = apparent, br = broad, ovlp = overlapping), coupling constant(s), integration. High-resolution mass spectra were obtained on an LTQ Orbitrap Velos (Thermo Scientific, Waltham, MA). All compounds were determined to be >95 % by analytical reverse-phase HPLC (purities for each final compound are given in the experimental section below).

General Procedure for Mitsunobu Reaction

To a solution of nucleoside (1.0 equiv), *tert*-butyl sulfamoylcarbamate (3.0 equiv) and PPh₃ (1.5 equiv) in THF (0.05 M for the limiting reagent) at 0 °C was added DIAD (1.05 equiv) dropwise over 15 min. The solution was stirred at 0 °C for 15 min, then gradually warmed to 23 °C over 3 h and stirred for 12 h at 23 °C. The reaction was concentrated *in vacuo* to afford the crude sulfamide, which was purified by flash chromatography using the indicated solvent system. The product was typically contaminated with triphenylphosphine oxide (TPPO). In this case the calculated yield was obtained by a simple linear equation relating the total isolated weight to the ratio of product:TPPO and formula weights for each compound.

General Procedure for Okada Sulfamoylation Protocol

To a solution of nucleoside (1.0 equiv) in DMA (0.4 M) was added freshly prepared sulfamoyl chloride (4.0 equiv) at 0 °C. The reaction was slowly warmed to 23 °C over 6 h and then stirred an additional 18 h at 23 °C. The reaction was diluted with EtOAc (200 mL/mmol) and washed successively with H₂O (5 × 100 mL/mmol), saturated aqueous NaCl (200 mL/mmol), then dried (Na₂SO₄) and concentrated under vacuum to afford the crude sulfamate that was typically greater than 90% pure contaminated only with residual DMA and used directly in the biotinylation step.

General Procedure for Biotinylation

To a solution of the intermediate nucleoside sulfamate/sulfamide (1.0 equiv) and Cs₂CO₃ (2.5 equiv) in DMF (0.5 M for the limiting reagent) at 0 °C was added *D*-(+)-biotin *N*-hydroxysuccinimide ester (1.5 equiv). The reaction mixture was stirred for 24 h at 23 °C during which time all starting material was consumed as monitored by electrospray mass spectrometry in the negative mode. DMF was removed by rotary evaporation under high vacuum (P = 0.01 torr) with mild heating (water bath 35 °C) to afford the crude biotinylated nucleoside. The crude product was used in the next global deprotection reaction directly without further purification.

General Procedure for TFA Global Deprotection

To a solution of the crude biotinylated nucleoside prepared above in H₂O (10 mL/mmol) at 0 °C was added TFA (40 mL/mmol) dropwise to achieve a final concentration of 0.02 M. The reaction mixture was stirred for 8 h at 23 °C during which time all starting material was consumed as monitored by electrospray mass spectrometry in the negative mode. The solvent was removed under vacuum. The crude material was re-dissolved in 1:1 MeCN–50 mM TEAB (10–20 mg/mL) and filtered to remove insoluble solids. The resulting solution was purified by preparative reverse phase HPLC with a Phenomenx Gemini C18 (250 × 20 mm) column at a flow rate of 30.0 mL/min employing a linear gradient of 5–15% acetonitrile (solvent B) in 50 mM aqueous triethylammonium bicarbonate (TEAB) at pH 7.5 (solvent A) for 30 min. The appropriate fractions were pooled and lyophilized to afford the final compound as the triethylammonium salt as a white foam.

General Procedure for TBS Protection

To a solution of nucleoside (1.0 equiv), imidazole (6.6 equiv), and DMAP (0.1 equiv) in DMF (0.2 M of limiting nucleoside substrate) at 0 °C was added TBSCl (3.3 equiv). The mixture was warmed to 23 °C over 3 h then stirred at 23 °C for an additional 13 h. The reaction was quenched with a saturated aqueous NH₄Cl (40 mL/mmol). The crude mixture was extracted with EtOAc (5 × 100 mL/mmol). The combined organic layers were washed with H₂O (100 mL/mmol), saturated aqueous NaCl (100 mL/mmol) and then concentrated *in vacuo*. Purification by flash chromatography (EtOAc–hexanes) afforded the title compound.

General Procedure for bis-Boc Protection

To a solution of nucleoside (1.0 equiv), Et₃N (3.0 equiv) and DMAP (0.5 equiv) in DMF (0.2 M in the limiting reagent) at 0 °C was added Boc₂O (3.0 equiv). The reaction was warmed to 23 °C over 3 h then stirred at 23 °C for an additional 10 h. The reaction was quenched with ice cold 1 N aqueous HCl (50 mL/mmol) and extracted with EtOAc (5 × 40 mL mL/mmol). The combined organic extracts were washed with saturated aqueous NaCl (60 mL mL/mmol), dried (MgSO₄), and concentrated *in vacuo*. Purification by flash chromatography (EtOAc–hexanes) afforded the title compound.

(1' R,4' S)-9-[(4'-Pivaloyloxy)methyl]cyclopent-2'-en-1'-yladenine (23)

To a round bottom flask charged with adenine (65 mg, 0.50 mmol, 1.2 equiv) and Cs₂CO₃ (156 mg, 0.50 mmol, 1.2 equiv) was added DMF (2.0 mL) and the mixture stirred at 22 °C for 30 min. To another flask charged with **22** (169 mg, 0.40 mmol, 1.0 equiv), [PdCl(C₃H₅)₂] (1.5 mg, 0.0040 mmol, 0.010 equiv) and P(O*i*Pr)₃ (8.3 mg, 10.0 μL, 0.040 mmol, 0.10 equiv) was added DMF (2.0 mL) and the mixture stirred at 23 °C for 30 min. The solution containing the substrate **22** was transferred via cannula over 5 min at 0 °C into the flask containing the cesium salt of adenine. The reaction mixture was stirred for another 5 h at 23 °C then quenched with saturated aqueous NH₄Cl (5 mL). The crude mixture was extracted with EtOAc (5 × 15 mL) and the combined organic layers were washed with saturated aqueous NaCl (20 mL) and then concentrated under vacuum. Purification by flash chromatography (10:1 CH₂Cl₂–MeOH) afforded the title compound (116 mg, 92%) as white foam: *R*_f = 0.45 (9:1 CH₂Cl₂–MeOH); [α]_D²³ +11.3 (*c* 0.50, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 1.19 (s, 9H), 1.64–1.68 (m, 1H), 2.87–2.92 (m, 1H), 3.17–3.19 (m, 1H), 4.09–4.15 (m, 2H), 5.73–5.76 (m, 1H), 5.92 (br s, 2H), 5.96–5.97 (m, 1H), 6.16–6.17 (m, 1H), 7.82 (s, 1H), 8.36 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 27.3, 35.9, 38.9, 44.7, 59.4, 66.5, 120.0, 130.4, 137.6, 138.3, 149.9, 153.0, 155.9, 178.4; HRMS (ESI+) calcd for C₁₆H₂₂N₅O₂ [M + H]⁺ 316.1768, found 316.1774 (error 1.9 ppm).

(1' R,2' S,3' S,5' S)-9-[(1',2'-Dihydroxy-1',2'-O-isopropylidene-5'-[(pivaloyloxy)methyl]cyclopent-3-yl)adenine (24a) or 2',3'-O-(Isopropylidene)aristeromycin (24a) and (1' S,2' R,3' S,5' S)-9-[(1,2-Dihydroxy-1',2'-O-isopropylidene-5'-[(pivaloyloxy)methyl]cyclopent-3-yl)adenine (24β)

To a solution of **23** (315 mg, 1.0 mmol, 1.0 equiv) in MeOH (5 mL) at 0 °C was added K₂CO₃ (414 mg, 3.0 mmol, 3.0 equiv) in one portion. The suspension was stirred at 0 °C for

30 min then heated at 60 °C for 4 h. The solution was concentrated *in vacuo* and re-suspended in 10:1 CH₂Cl₂–MeOH (20 mL). Filtration over Celite and concentration afforded the crude product. Purification by flash chromatography (CH₂Cl₂ to 10:90 MeOH–EtOAc, linear gradient) afforded (1'*R*,4'*S*)-9-[(4'-hydroxymethyl)cyclopent-2'-en-1'-yl]adenine (215 mg, 89%) as colorless oil: *R*_f = 0.10 (CH₂Cl₂–MeOH); ¹H NMR, ¹³C NMR, and HRMS data matched literature values.²

To a solution of (1'*R*,4'*S*)-9-[(4'-hydroxymethyl)cyclopent-2'-en-1'-yl]adenine prepared above (92.4 mg, 0.40 mmol, 1.0 equiv) in 1:1 THF–H₂O (8 mL) at 0 °C was added OsO₄ (250 μL of 4 wt% in *t*BuOH, 10 mol%) and NMO (93.7 mg, 0.80 mmol, 2.0 equiv). The dark brown mixture was stirred at 0 °C for 48 h then quenched with 10% aqueous Na₂S₂O₅ (5.0 mL). The resulting mixture was extracted with EtOAc (5 × 15 mL) and the combined organic extracts were concentrated *in vacuo* to afford the crude diol, which was used in next reaction directly.

To a solution of the crude diol prepared above in THF (4.0 mL) was added 2,2-dimethoxypropane (2.0 mL, 16.3 mmol, 41 equiv) and *p*-TsOH•H₂O (76.1 mg, 0.40 mmol, 1.0 equiv) at 0 °C. The mixture was allowed to gradually warm up to 23 °C and stirred for 15 h, then quenched with solid NaHCO₃ (3.4 g, 40 mmol, 100 equiv) and the suspension stirred for 30 min at 23 °C. The mixture was filtered and the solid washed with EtOAc (20 mL). The filtrate was concentrated *in vacuo* to afford the crude product. Purification by flash chromatography (CH₂Cl₂ to 10:90 MeOH–CH₂Cl₂, linear gradient) afforded the title compound **24a** and the undesired diastereomer **24β** as an inseparable 1:1 mixture (65 mg, 53% combined yield over 2 steps): *R*_f = 0.25 (1:9 MeOH–CH₂Cl₂). The diastereomers (**24a/β**) were separated by reverse-phase HPLC (22:77 CH₃CN–H₂O, isocratic condition, 1.5 mL/min, *t*_R(**24β**) = 18 min, *t*_R(**24a**) = 21 min) with a Phenomenx Gemini 5 μm C18 (250 × 20 mm) column. ¹H NMR, ¹³C NMR, HRMS data for **24a** are consistent with previously reported values.³ Data for **24β**: ¹H NMR (400 MHz, MeOH-*d*₄) δ 1.27 (s, 3H), 1.47 (s, 3H), 2.12–2.22 (m, 3H), 3.71 (dd, *J* = 10.8, 5.6 Hz, 1H), 3.88 (dd, *J* = 10.8, 5.6 Hz, 1H), 4.76–4.83 (m, 3H), 8.21 (br s, 2H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 24.0, 25.8, 30.4, 44.3, 57.0, 61.7, 79.5, 80.9, 111.9, 119.7, 142.2, 150.8, 153.6, 157.2. All other characterization data are identical to reported data.

***N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-(isopropylidene)aristeromycin (**25**)**

Compound **24a** (47.0 mg, 0.154 mmol) was converted to 5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-(isopropylidene)aristeromycin using the general procedure for TBS protection, which was used in the next step without further purification: *R*_f = 0.3 (1:9 MeOH–CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 0.06 (s, 6H), 0.88 (s, 9H), 1.28 (s, 3H), 1.56 (s, 3H), 2.35–2.51 (m, 3H), 3.77 (d, *J* = 4.4 Hz, 2H), 4.65 (dd, *J* = 7.2, 4.4 Hz, 1H), 4.76–4.80 (m, 1H), 5.01 (t, *J* = 6.0 Hz, 1H), 5.68 (br s, 2H), 7.85 (s, 1H), 8.33 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ –5.45, –5.43, 18.3, 25.1, 25.8, 29.7, 33.4, 45.7, 61.9, 63.0, 80.7, 83.8, 113.5, 121.9, 139.6, 150.2, 152.8, 155.; All other characterization data are identical to reported data.

Crude 5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-(isopropylidene)aristeromycin prepared above (60.5 mg, 0.14 mmol, 1.0 equiv) was converted to the title compound using the general procedure for bis-Boc protection. Purification by flash chromatography (3:1 to 1:1 hexane–EtOAc, linear gradient) afforded the title compound (82 mg, 92% over 2 steps) as a colorless oil: $R_f = 0.4$ (1:1 EtOAc–hexanes); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.92 (s, 9H), 1.32 (s, 3H), 1.47 (s, 18H), 1.57 (s, 3H), 2.39–2.42 (m, 2H), 2.55–2.60 (m, 1H), 3.76–3.79 (m, 2H), 4.68 (dd, $J = 6.8, 4.4$ Hz, 1H), 4.83–4.85 (m, 1H), 5.03 (t, $J = 6.4$ Hz, 1H), 8.11 (s, 1H), 8.83 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ –5.47, –5.45, 18.3, 25.1, 25.8, 27.6, 27.8, 33.1, 45.6, 62.3, 62.7, 80.6, 83.7, 83.8, 113.6, 129.5, 143.9, 150.4, 150.6, 151.8, 153.3; HRMS calcd for $\text{C}_{30}\text{H}_{50}\text{N}_5\text{O}_7\text{Si}$ $[\text{M} + \text{H}]^+$ 620.3474, found 620.3452 (error 3.5 ppm).

***N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-2',3'-*O*-(isopropylidene)aristeromycin (26)**

To a solution of **25** (124 mg, 0.20 mmol, 1.0 equiv) in THF (4 mL) at 0 °C was added a 1.0 M TBAF solution in THF (0.30 mL, 0.30 mmol, 1.5 equiv) dropwise. The solution was warmed to 23 °C and stirred for 1 h then quenched with saturated aqueous NaHCO_3 (20 mL). The aqueous layer was extracted with EtOAc (5×15 mL) and the combined organic extracts were washed with H_2O (30 mL), saturated aqueous NaCl (30 mL), dried (MgSO_4) and concentrated *in vacuo*. Purification by flash chromatography (2:1 to 1:1 hexane–EtOAc, linear gradient) afforded the title compound (94 mg, 93%) as a white foam: $R_f = 0.25$ (1:1 EtOAc–hexanes); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.33 (s, 3H), 1.46 (s, 18H), 1.51 (s, 3H), 2.44–2.49 (m, 3H), 3.82–3.86 (m, 2H), 4.74 (dd, $J = 6.8, 4.0$ Hz, 1H), 4.83–4.86 (m, 1H), 5.05 (t, $J = 6.4$ Hz, 1H), 8.13 (s, 1H), 8.84 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 25.1, 27.6, 27.8, 33.0, 45.5, 62.9, 63.8, 82.0, 83.8, 84.0, 113.7, 129.7, 144.3, 150.6 (2 C), 151.8, 153.1; HRMS calcd for $\text{C}_{24}\text{H}_{36}\text{N}_5\text{O}_7$ $[\text{M} + \text{H}]^+$ 506.2609, found 506.2588 (error 4.1 ppm).

***N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-5'-[*N*-(*tert*-butoxycarbonyl)-*N*-(sulfamoyl)amino]-5'-deoxy-2',3'-*O*-(isopropylidene)aristeromycin (27)**

Compound **26** (50.5 mg, 0.10 mmol, 1.0 equiv) was converted to the title compound using the general procedure for the Mitsunobu reaction. Purification by flash chromatography (3:1 to 1:1 hexane–EtOAc, linear gradient) afforded the title compound (62 mg, 83% based on $^1\text{H NMR}$ analysis) partially contaminated with triphenylphosphine oxide: $R_f = 0.28$ (1:1 EtOAc–hexanes); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 1.30 (s, 3H), 1.48 (s, 9H), 1.54 (s, 3H), 1.58 (s, 18H), 2.39–2.51 (m, 3H), 3.87 (dd, $J = 14.4, 4.4$ Hz, 1H), 4.00 (dd, $J = 14.4, 9.2$ Hz, 1H), 4.11 (q, $J = 7.2$ Hz, 2H), 4.83–4.87 (m, 1H), 4.92 (t, $J = 7.2$ Hz, 1H), 5.05–5.09 (m, 1H), 5.66 (br s, 2H), 8.07 (s, 1H), 8.84 (s, 1H).

5'-[*N*-(ϵ -Biotinoyl)sulfamoyl]amino-5'-deoxyaristeromycin triethylammonium salt (28)

Compound **27** (34 mg, 0.050 mmol, 1.0 equiv) was converted to the title compound using the general procedure for biotinylation and TFA global deprotection to afford (17 mg, 52% in 2 steps): HPLC purity: 95.0%, $t_R = 11.9$ min, $k' = 2.3$ (method A); $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 1.28 (t, $J = 6.8$ Hz, 9H), 1.34–1.48 (m, 3H), 1.54–1.75 (m, 5H), 1.95–2.01 (m, 1H), 2.25 (t, $J = 6.8$ Hz, 2H), 2.29–2.32 (m, 1H), 2.47–2.50 (m, 1H), 2.65–2.70 (m, 1H), 2.90 (dd, $J = 12.8, 4.8$ Hz, 1H), 3.14 (q, $J = 6.8$ Hz, 6H), 3.10–3.25 (m, 1H), 4.03 (t, $J = 5.2$

Hz, 1H), 4.29 (dd, $J = 12.4, 4.4$ Hz, 1H), 4.45 (dd, $J = 12.4, 4.4$ Hz, 1H), 4.57 (dd, $J = 8.4, 5.6$ Hz, 1H), 4.79–4.86 (m, 1H), 8.17 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (CD_3OD , 100 MHz) δ 9.5, 26.7, 29.6, 29.9, 31.2, 38.0, 41.2, 44.4, 47.70, 47.95, 57.2, 61.7, 62.4, 63.4, 74.9, 76.5, 120.8, 142.4, 151.0, 153.7, 157.4, 166.3, 178.2; HRMS (ESI $^-$) calcd for $\text{C}_{21}\text{H}_{30}\text{N}_9\text{O}_6\text{S}_2$ [$\text{M} - \text{H}$] $^-$ 568.1766, found 568.1784 (error 3.2 ppm).

(1'*R*,4'*S*)-9-[4'-(Hydroxymethyl)cyclopent-2'-en-1'-yl]adenine (29)

To a solution of **23** (1.43 g, 4.53 mmol, 1.0 equiv) in MeOH (10 mL) was added 1 M aqueous NaOH (10 mL) and the mixture stirred at 80 °C for 4 h. The reaction was concentrated *in vacuo* and the resulting crude solid residue was purified by flash chromatography (3:7 MeOH–EtOAc) to provide the title compound (0.953 mg, 95%) as a white solid: $R_f = 0.40$ (3:7 MeOH–EtOAc); ^1H NMR, ^{13}C NMR, and HRMS data matched literature values for the compound prepared by an alternate synthetic route.³⁰

(1'*R*,4'*S*)-9-[4'-(*tert*-Butyldimethylsilyloxymethyl)cyclopent-2'-en-1'-yl]adenine (30)

Compound **29** (0.953 g, 4.12 mmol) was converted to the title compound using the general procedure for TBS protection. Purification by flash chromatography (1:5 MeOH–EtOAc) afforded the title compound (1.19 g, 83%) as a white solid: $R_f = 0.35$ (1:5 MeOH–EtOAc); ^1H NMR (600 MHz, CDCl_3) δ 0.04 (s, 6H), 0.88 (s, 9H), 1.69 (dt, $J = 13.8, 6.0$, 1H), 2.80 (dt, $J = 13.8, 9.0$ Hz, 1H), 2.98–3.05 (m, 1H), 3.61 (dd, $J = 9.6, 6.0$ Hz, 1H), 3.71 (dd, $J = 9.6, 5.4$ Hz, 1H), 5.74 (td, $J = 5.4, 1.8$ Hz, 1H), 5.86–5.88 (m, 2H), 5.89 (br s, 2H), 6.16–6.17 (m, 1H), 7.89 (s, 1H), 8.36 (s, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ –5.25, –5.24, 18.5, 26.0, 34.9, 48.0, 59.4, 65.7, 119.8, 129.6, 138.9, 139.2, 149.8, 152.9, 155.9; HRMS (ESI $^+$) calcd for $\text{C}_{17}\text{H}_{28}\text{N}_5\text{OSi}$ [$\text{M} + \text{H}$] $^+$ 346.2058, found 346.2066 (error 2.3 ppm).

(1'*R*,4'*S*)-*N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-9-[4'-(*tert*-butyldimethylsilyloxymethyl)cyclopent-2'-en-1'-yl]adenine (31)

Compound **30** (1.25 g, 3.60 mmol) was converted to the title compound using the general procedure for bis-Boc protection. Purification by flash chromatography (2:8 EtOAc–hexanes) afforded the title compound (1.79 g, 91%) as a colorless oil: $R_f = 0.50$ (3:7 EtOAc–hexanes); ^1H NMR (600 MHz, CDCl_3) δ 0.03 (s, 6H), 0.86 (s, 9H), 1.43 (s, 18H), 1.71 (dt, $J = 13.8, 6.0$ Hz, 1H), 2.82 (dt, $J = 13.8, 8.4$ Hz, 1H), 3.00–3.05 (m, 1H), 3.62 (dd, $J = 9.6, 5.4$ Hz, 1H), 3.72 (dd, $J = 10.2, 4.8$ Hz, 1H), 5.82 (t, $J = 6.0$ Hz, 1H), 5.86–5.87 (m, 1H), 6.18–6.19 (m, 1H), 8.17 (s, 1H), 8.84 (s, 1H); ^{13}C NMR (150 MHz, CDCl_3) δ –5.21, –5.19, 18.6, 26.1, 27.9, 34.8, 48.0, 59.9, 65.7, 83.8, 129.2, 129.2, 139.8, 143.4, 150.3, 150.7, 152.0, 153.3; HRMS (ESI $^+$) calcd for $\text{C}_{27}\text{H}_{44}\text{N}_5\text{O}_5\text{Si}$ [$\text{M} + \text{H}$] $^+$ 546.3106, found 546.3130 (error 4.4 ppm).

(1'*R*,4'*S*)-*N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-9-[4'-(hydroxymethyl)cyclopent-2'-en-1'-yl]adenine (32)

To a solution of **31** (1.76 g, 3.22 mmol, 1.0 equiv) in THF (15 mL) at 0 °C was added a solution of TBAF (1.0 M in THF, 3.90 mL, 3.86 mmol, 1.2 equiv). The reaction mixture was stirred for 2 h at 0 °C, then quenched with ice cold aqueous 1 N HCl (10 mL). The crude mixture was extracted with EtOAc (5 \times 30 mL) and the combined organic extracts

were washed with saturated aqueous NaCl (30 mL) and concentrated under vacuum. Purification by flash chromatography (1:9 MeOH–EtOAc) afforded the title compound (1.14 g, 94%) as a colorless oil: $R_f = 0.40$ (1:9 MeOH–EtOAc); $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 1.41 (s, 18H), 1.88–1.93 (dt, $J = 13.8, 5.4$ Hz, 1H), 2.84 (dt, $J = 13.8, 9.6$ Hz, 1H), 3.08 (br s, 1H), 3.67 (dd, $J = 10.8, 4.2$ Hz, 1H), 3.81 (dd, $J = 10.8, 4.2$ Hz, 1H), 5.77–5.78 (m, 1H), 5.79 (br s, 1H), 5.83–5.84 (m, 1H), 6.17–6.18 (m, 1H), 8.30 (s, 1H), 8.80 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 27.9, 33.8, 47.7, 60.6, 64.6, 83.8, 129.3, 129.6, 139.3, 144.3, 150.2, 150.6, 151.7, 153.0; HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{30}\text{N}_5\text{O}_5$ $[\text{M} + \text{H}]^+$ 432.2241, found 432.2255 (error 3.2 ppm).

(1' R,4' S)-9[4-({[N-[(D-Biotinoyl)sulfamoyl]amino)methyl]cyclopent-2'-en-1'-yl]adenine triethylammonium salt (34)

Compound **32** (1.11 g, 2.58 mmol, 1.0 equiv), was converted to **33** using the general procedure for the Mitsunobu reaction. Purification by flash chromatography (1:1 to 7:3% EtOAc–hexane, linear gradient) afforded **33** (1.30 g, 70%, ~85% purity by weight, contaminated with PPh_3O) that was used in the next step without further purification. Compound **33** (0.378 g, 0.621 mmol) was converted to **34** using the general procedure for biotinylation and TFA deprotection to provide the title compound (0.246 g, 57% over 2 steps) as a white solid as the triethylammonium salt (1.9 equiv of Et_3N): HPLC purity:

96.9%, $t_R = 12.90$ min, $k' = 2.4$ (method A); $[\alpha]_D^{23} + 23.3$ (c 1.33, MeOH); $^1\text{H NMR}$ (400 MHz, MeOH- d_4) δ 1.19 (t, $J = 7.3$ Hz, 17H, excess Et_3N), 1.38–1.48 (m, 2H), 1.51–1.66 (m, 4H), 1.68–1.82 (m, 2H), 2.17 (t, $J = 7.8$ Hz, 2H), 2.67 (d, $J = 12.2$ Hz, 1H), 2.91 (q, $J = 7.1$ Hz, 13H, excess Et_3N), 2.98–3.15 (m, 4H), 3.16–3.22 (m, 1H), 4.30 (dd, $J = 8.0, 4.2$ Hz, 1H), 4.43–4.49 (m, 1H), 5.66–5.73 (m, 1H), 5.95 (d, $J = 5.5$ Hz, 1H), 6.21 (d, $J = 5.5$ Hz, 1H), 8.15 (s, 1H), 8.22 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 10.8, 25.0, 28.1, 28.2, 34.9, 36.2, 39.8, 44.4, 45.7, 47.2, 55.4, 59.1, 59.4, 61.1, 119.0, 130.2, 137.6, 138.9, 149.0, 152.3, 156.0, 162.8, 173.5; HRMS (ESI-) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_9\text{O}_4\text{S}_2$ $[\text{M} - \text{Et}_3\text{NH}]^-$ 534.1711, found 534.1711 (error 0 ppm).

(1' R,3' S)-9-[3'-({[N-[(D-Biotinoyl)sulfamoyl]amino)methyl]cyclopentan-1'-yl]adenine triethylammonium salt (36)

To a solution of **33** (0.800 g, 1.31 mmol, 1.0 equiv) in EtOAc (15 mL) was added 10% w/w Pd/C (0.23 g). The nitrogen atmosphere was exchanged for a hydrogen atmosphere (1 atm) and the reaction stirred for 16 h at 23 °C. The reaction was filtered through a pad of Celite washing with MeOH (30 mL). The filtrate was concentrated under reduced pressure.

Purification by flash chromatography (30% EtOAc–hexanes) afforded **35** that was used in the next coupling reaction directly without further purification. Compound **35** (0.650 g, 1.06 mmol, 1.0 equiv) was converted to **36** using the general procedure for biotinylation and TFA deprotection to afford the title compound (0.302 g, 58% over 3 steps) as the triethylammonium salt (2.4 equiv of Et_3N) as a white solid: HPLC purity: 92.0%, $t_R = 14.83$ min, $k' = 2.9$, (method A); $[\alpha]_D^{23} + 24.0$ (c 1.08, MeOH); $^1\text{H NMR}$ (400 MHz, MeOH- d_4) δ 1.17 (t, $J = 7.2$ Hz, 22H, excess Et_3N), 1.40–1.49 (m, 2H), 1.54–1.69 (m, 4H), 1.71–1.79 (m, 2H), 1.82–1.91 (m, 1H), 1.94–2.04 (m, 1H), 2.08–2.16 (m, 1H), 2.20 (t, $J = 7.4$ Hz, 2H), 2.26–2.37 (m, 2H), 2.42–2.51 (m, 1H), 2.67 (d, $J = 12.5$ Hz, 1H), 2.85 (q, $J = 7.0$ Hz, 15H,

excess Et₃N), 2.98–3.05 (m, 2H), 3.16–3.22 (m, 1H), 4.30 (dd, *J* = 7.8, 4.4 Hz, 1H), 4.43–4.50 (m, 1H) 4.90–4.96 (m, 1H), 8.20 (s, 1H), 8.25 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 11.2, 25.2, 27.5, 28.1, 28.2, 30.9, 36.6, 37.2, 39.9, 45.7, 48.0, 54.9, 55.5, 59.2, 61.1, 119.1, 139.3, 149.3, 152.1, 156.0, 162.8, 174.2 (missing 1 carbon); HRMS (ESI⁻) calcd for C₂₁H₃₀N₉O₄S₂ [M – Et₃NH]⁻ 536.1868, found 536.1880 (error 2.2 ppm).

9-[3',5'-O-(1,1,3,3-Tetraisopropylidisiloxy)]-β-D-arabinofuranosyl]adenine (38)

To a solution of **37** (0.200 g, 0.796 mmol, 1.0 equiv) in pyridine (10 mL) at 0 °C was added TIPDSCl₂ (0.395 g, 2.63 mmol, 3.3 equiv). The mixture was slowly warmed to 23 °C over 3 h and then stirred for 40 h at 23 °C. The reaction was concentrated *in vacuo* and the crude oil was taken up in EtOAc (100 mL) and washed successively with ice cold 1 N aqueous HCl (5 × 15 mL) and saturated aqueous NaCl (80 mL). The organic layer was dried (MgSO₄) and concentrated under vacuum. Purification by flash chromatography (4:1 EtOAc–hexane to 1:9 MeOH–EtOAc) afforded the title compound (0.35 g, 86%) as a white solid: *R*_f = 0.40 (10% MeOH–EtOAc); ¹H NMR (400 MHz, DMSO-d₆) δ 0.92–1.08 (m, 28H), 3.76–3.84 (m, 1H), 3.88–3.96 (m, 1H), 4.10 (dd, *J* = 12.3, 3.4 Hz, 1H), 4.46–4.61 (m, 2H), 5.77 (d, *J* = 5.8 Hz, 1H), 6.20 (d, *J* = 6.4 Hz, 1H), 7.27 (br s, 2H), 8.04 (s, 1H), 8.10 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 12.0, 12.21, 12.23, 12.4, 12.8, 16.80, 16.81, 16.85, 16.9, 17.16, 17.18, 17.3, 61.5, 74.9, 75.1, 79.6, 81.7, 118.5, 139.6, 149.5, 152.3, 156.0; HRMS (ESI⁺) calcd for C₂₂H₄₀N₅O₅Si₂ [M + H]⁺ 510.2562, found 510.2539 (error 4.5 ppm).

9-[3',5'-O-(1,1,3,3-Tetraisopropylidisiloxy)]-2'-O-trifluoromethylsulfonyl-β-D-arabinofuranosyl]adenine (39)

To a solution of **38** (0.327 g, 0.685 mmol, 1.0 equiv) and DMAP (42.0 mg, 0.343 mmol, 3.0 equiv) in CH₂Cl₂ (7 mL) at 0 °C was added Tf₂O (0.140 mL, 0.822 mmol, 1.2 equiv). The reaction was stirred for 1.5 h at 0 °C then concentrated *in vacuo* to provide an oil that was purified directly by flash chromatography (4:1 EtOAc–hexane to EtOAc, linear gradient) to afford the title compound (0.272 g, 62%) as a white foam: *R*_f = 0.55 (EtOAc); ¹H NMR (400 MHz, DMSO-d₆) δ 1.00–1.36 (m, 28H), 3.89–4.01 (m, 2H), 4.21 (dd, *J* = 12.0, 5.4 Hz, 1H), 5.60–5.71 (m, 1H) 6.05 (t, *J* = 7.6 Hz, 1H), 6.49 (d, *J* = 7.0 Hz, 1H), 7.39 (br s, 2H), 8.07 (s, 1H), 8.28 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 12.20, 12.22, 12.3, 12.4, 16.61, 16.64, 16.66, 17.06, 17.07, 17.08, 17.2, 62.0, 74.1, 78.4, 78.9, 89.2, 119.1, 119.2 (q, ¹*J*_{C-F} = 310 Hz), 140.4, 149.0, 152.4, 156.1; HRMS (ESI⁺) calcd for C₂₃H₃₉F₃N₅O₇SSi₂ [M + H]⁺ 642.2055, found 642.2025 (error 4.6 ppm).

2'-Azido-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxy)adenosine (40)

A solution of **39** (0.272 g, 0.424 mmol, 1.0 equiv) and sodium azide (83.0 mg, 1.27 mmol, 3.0 equiv) in DMF (10 mL) was stirred at 60 °C for 15 h. The reaction was concentrated *in vacuo* and the crude oil was purified by flash chromatography (3:2 to 4:1 EtOAc–hexane) to afford the title compound (0.199 g, 88%) as a white foam: *R*_f = 0.35 (3:2 EtOAc–hexanes); [*α*]_D²¹ – 33.8 (c 0.06, CHCl₃); ¹H NMR (400 MHz, DMSO-d₆) δ 0.96–1.29 (m, 28H), 3.88–4.08 (m, 3H), 5.01 (d, *J* = 6.2 Hz, 1H), 5.44 (t, *J* = 6.5 Hz, 1H) 5.83 (s, 1H), 7.35 (br s, 2H), 8.06 (s, 1H), 8.22 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 12.10, 12.16, 12.4, 12.7,

16.73, 16.78, 16.9, 17.03, 17.09, 17.10, 17.3, 60.8, 64.6, 71.9, 81.0, 86.1, 119.3, 140.0, 148.5, 152.4, 156.1; HRMS (ESI+) calcd for C₂₂H₃₉N₈O₄Si₂ [M + H]⁺ 535.2627, found 535.2611 (error 3.0 ppm).

2'-Azido-N⁶,N⁶-bis(*tert*-butoxycarbonyl)-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxy)adenosine (41)

Compound **40** (0.199 g, 0.373 mmol) was converted to **41** using the general procedure for bis-Boc protection. Purification by flash chromatography (1:4 EtOAc–hexane) afforded the title compound (0.205 g, 75%) as an off white foam: *R_f* = 0.65 (3:7 EtOAc–hexane); ¹H NMR (400 MHz, DMSO-*d*₆) δ 0.96–1.14 (m, 28H), 1.39 (s, 18H), 3.92–4.06 (m, 3H), 5.19 (d, *J* = 5.6 Hz, 1H), 5.36 (dd, *J* = 6.4, 4.8 Hz, 1H), 5.98 (s, 1H), 8.73 (s, 1H), 8.78 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 12.13, 12.18, 12.3, 12.7, 16.71, 16.76, 16.8, 17.03, 17.04, 17.1, 17.2, 27.2, 64.2, 65.2, 71.4, 81.0, 83.5, 86.5, 128.3, 145.8, 149.4, 149.9, 151.4, 151.9; HRMS (ESI+) calcd for C₃₂H₅₅N₈O₈Si₂ [M + H]⁺ 735.3676, found 735.3662 (error 1.9 ppm).

2'-Azido-N⁶,N⁶-bis(*tert*-butoxycarbonyl)-2'-deoxyadenosine (42)

To a solution of **41** (0.205 g, 0.280 mmol, 1.0 equiv) in THF (5 mL) at 0 °C was added glacial acetic acid (50 μL to provide a 1% v/v solution). The cooling bath was removed and the mixture was stirred at 23 °C for 1.5 h. The reaction was quenched with ice cold 1 N aqueous HCl (15 mL) then extracted with EtOAc (5 × 20 mL). The combined organic extracts were washed with saturated aqueous NaCl (30 mL), dried (MgSO₄), and concentrated. Purification by flash chromatography (EtOAc) afforded the title compound (0.099 g, 79%) as a colorless oil: *R_f* = 0.40 (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 1.44 (s, 18H), 3.56 (br s, 1H), 3.79 (d, *J* = 12.5 Hz, 1H), 3.98 (d, *J* = 12.5 Hz, 1H), 4.30 (s, 1H), 4.61–4.67 (m, 1H), 4.81–4.88 (m, 1H), 5.54 (br s, 1H), 6.01 (d, *J* = 7.5 Hz, 1H), 8.26 (s, 1H), 8.85 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 27.7, 62.7, 65.4, 72.6, 84.4, 87.9, 88.9, 130.3, 144.6, 150.2, 151.2, 151.7, 151.9; HRMS (ESI+) calcd for C₂₀H₂₉N₈O₇ [M + H]⁺ 493.2154, found 493.2141 (error 2.6 ppm).

2'-Azido-N⁶,N⁶-bis(*tert*-butoxycarbonyl)-3',5'-O-bis(*tert*-butyldimethylsilyl)-2'-deoxyadenosine (43)

Compound **42** (0.099 g, 0.202 mmol) was converted to **43** using the general procedure for TBS protection. Purification by flash chromatography (1:4 EtOAc–hexanes) afforded the title compound (0.105 g, 72%) as a yellow oil: *R_f* = 0.60 (3:7 EtOAc–hexanes); ¹H NMR (400 MHz, DMSO-*d*₆) δ -0.11 (s, 3H), -0.03 (s, 3H), 0.20 (s, 6H), 0.79 (s, 9H), 0.95 (s, 9H), 1.38 (s, 18H), 3.74 (dd, *J* = 11.7, 3.1 Hz, 1H), 3.90 (dd, *J* = 11.7, 3.6 Hz, 1H), 3.95–4.01 (m, 1H), 4.95–5.02 (m, 2H), 6.16 (d, *J* = 3.9 Hz, 1H), 8.81 (s, 1H), 8.86 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ -5.64, -5.20, -4.89, 12.8, 17.18, 17.21, 17.7, 17.9, 25.59, 25.62, 27.2, 61.1, 64.1, 71.2, 79.1, 83.4, 84.3, 85.8, 145.4, 149.4, 149.9, 151.7, 152.3; HRMS (ESI+) calcd for C₃₂H₅₇N₈O₇Si₂ [M + H]⁺ 721.3883, found 721.3858 (error 3.5 ppm).

2'-Azido-*N*⁶,*N*⁶-bis(*tert*-butoxycarbonyl)-3'-*O*-*tert*-butyldimethylsilyl-2'-deoxyadenosine (44)

To a solution of **43** (0.105 g, 0.145 mmol, 1.0 equiv) in 1:1 THF–H₂O (10 mL) at 0 °C was added glacial AcOH (15 mL) dropwise and the mixture stirred at 0 °C for 36 h. The reaction mixture was extracted with EtOAc (5 × 25 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL) and then concentrated. Purification by flash chromatography (3:2 EtOAc–hexane) afforded the title compound (0.056 g, 63%) as a white foam: *R*_f = 0.45 (1:1 EtOAc–hexane); ¹H NMR (400 MHz, MeOH-*d*₄) δ 0.21–0.24 (m, 6H), 1.00 (s, 9H), 1.40 (s, 18H), 3.75 (dd, *J* = 12.3, 3.1 Hz, 1H), 3.90 (dd, *J* = 12.3, 3.4 Hz, 1H), 4.17 (q, *J* = 3.2 Hz, 1H), 4.71 (t, *J* = 5.6 Hz, 1H), 4.79–4.82 (m, 1H), 6.32 (d, *J* = 6.0 Hz, 1H), 8.83 (s, 1H), 8.88 (s, 1H); ¹³C NMR (150 MHz, MeOH-*d*₄) δ –4.63, –4.45, 19.1, 26.4, 28.1, 62.1, 66.8, 74.3, 85.5, 88.2, 88.5, 130.8, 146.8, 151.4, 151.6, 153.3, 154.3; HRMS (ESI +) calcd for C₂₆H₄₃N₈O₇Si [M + H]⁺ 607.3018, found 607.2991 (error 4.4 ppm).

2'-Azido-5'-[*N*-(*p*-biotinoyl)sulfamoyl]amino-3',5'-dideoxyadenosine triethylammonium salt (46)

Compound **44** (0.282 g, 0.464 mmol, 1.0 equiv), was converted to **45** using the general procedure for the Mitsunobu reaction to afford (0.312 g, 85%, ~90% purity, contaminated with PPh₃O) that used in the next step without further purification. The intermediate nucleoside sulfamide **45** (0.312 g, 0.356 mmol) was converted to **46** using the general procedure for biotinylation and TFA deprotection to afford the title compound (0.230 g, 28% over 3 steps) as the triethylammonium salt (2.7 equiv of Et₃N) as a white solid: HPLC purity: 96.5%, *t*_R = 14.25 min, *k*' = 2.7 (method A); [α]_D²³ +20.2 (c 0.50, MeOH); ¹H NMR (400 MHz, MeOH-*d*₄) δ 1.15 (t, *J* = 7.3 Hz, 25H, excess Et₃N), 1.38–1.45 (m, 2H), 1.52–1.65 (m, 3H), 1.66–1.79 (m, 1H), 2.16 (t, *J* = 7.3 Hz, 2H), 2.66 (d, *J* = 12.3 Hz, 1H), 2.81 (q, *J* = 7.3 Hz, 17H, excess Et₃N), 3.12–3.28 (m, 4H), 4.18–4.22 (m, 1H), 4.25–4.33 (m, 1H), 4.40–4.45 (m, 1H), 4.68–4.73 (m, 1H), 4.82 (t, *J* = 5.9 Hz, 1H), 6.02 (d, *J* = 6.7 Hz, 1H), 8.31 (s, 1H), 8.32 (s, 1H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 10.6, 27.7, 29.7, 30.1, 40.0, 41.3, 46.5, 47.5, 57.2, 61.7, 63.5, 66.2, 74.0, 86.3, 88.7, 121.0, 142.0, 150.6, 154.5, 157.7, 166.4, 182.5; HRMS (ESI–) calcd for C₂₀H₂₇N₁₂O₆S₂ [M – Et₃NH][–] 595.1623, found 595.1609 (error 2.4 ppm).

2'-Amino-5'-[*N*-(*p*-biotinoyl)sulfamoyl]amino-3',5'-dideoxyadenosine (47)

To a solution of **46** (0.120 g, 0.133 mmol, 1.0 equiv) in 2:1 THF–H₂O (6 mL) at 0 °C was added PPh₃ (0.140 g, 0.534 mmol, 4 equiv). The ice bath was removed and the reaction was stirred for 6 h at 23 °C during which time all starting material was consumed as monitored by electrospray mass spectrometry in the negative mode. The reaction was concentrated *in vacuo*. The crude material was re-dissolved in 1:1 MeCN:50 mM TEAB (10–20 mg/mL) and filtered to remove insoluble solids. The resulting solution was purified by preparative reverse phase HPLC with a Phenomenx Gemini C18 (250 × 20 mm) column at a flow rate of 30.0 mL/min employing a linear gradient of 5–15% acetonitrile (solvent B) in 50 mM aqueous triethylammonium bicarbonate (TEAB) at pH 7.5 (solvent A) for 30 min. The appropriate fractions were pooled and lyophilized to afford the title compound (0.090 g, 75%) as the triethylammonium salt (3.0 equiv of Et₃N) as a white solid: HPLC purity:

97.5%, $t_R = 11.53$ min, $k' = 2.1$, (method A); $[\alpha]_D^{23} + 19.7$ (c 0.50, MeOH); $^1\text{H NMR}$ (400 MHz, MeOH- d_4) δ 1.15 (t, $J = 7.3$ Hz, 27H excess Et₃N), 1.38–1.47 (m, 2H), 1.52–1.65 (m, 3H), 1.66–1.74 (m, 1H), 2.19 (t, $J = 7.6$ Hz, 2H), 2.66 (d, $J = 13.2$ Hz, 1H), 2.83 (q, $J = 7.3$ Hz, 19H excess Et₃N), 3.12–3.18 (m, 1H), 3.19–3.27 (m, 3H), 4.20–4.29 (m, 4H), 4.41 (dd, $J = 7.7$, 5.0 Hz, 1H), 5.78 (d, $J = 8.3$ Hz, 1H), 8.26 (s, 1H), 8.33 (s, 1H); $^{13}\text{C NMR}$ (150 MHz, DMSO- d_6) δ 11.1, 25.0, 28.0, 28.1, 36.3, 39.8, 45.3, 45.7, 55.5, 56.4, 59.1, 61.0, 72.1, 84.6, 89.5, 119.5, 140.6, 149.9, 152.4, 156.2, 162.7, 173.9; HRMS (ESI⁻) calcd for C₂₀H₂₉N₁₀O₆S₂ [M – Et₃NH]⁻ 569.1718, found 569.1725 (error 1.1 ppm).

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxy)adenosine (49)

To a solution of adenosine **48** (0.200 g, 0.748 mmol, 1.0 equiv) in pyridine (5 mL) at 0 °C was added TIPDSCl₂ (0.248 g, 0.786 mmol, 1.05 equiv). The mixture slowly warmed to 23 °C over 3 h and was stirred at 23 °C for 45 h. The reaction was concentrated *in vacuo* and the crude oil was taken up in EtOAc (100 mL) and washed successively with ice cold 1 N aqueous HCl (5 × 25 mL) and saturated aqueous NaCl (100 mL). The organic layer was dried (MgSO₄) and concentrated under vacuum. Purification by flash chromatography (4:1 EtOAc–hexane to 1:9 MeOH–EtOAc) afforded the title compound (0.340 g, 89%) as a white solid: $R_f = 0.25$ (1:19 MeOH–EtOAc); $^1\text{H NMR}$ (400 MHz, CDCl₃) δ 0.99–1.07 (m, 28H), 3.86–3.94 (m, 1H), 3.98–4.10 (m, 2H) 4.52 (t, $J = 4.4$ Hz, 1H), 4.79 (dd, $J = 8.7$, 5.1 Hz, 1H), 5.61 (d, $J = 4.4$ Hz, 1H), 5.88 (s, 1H), 7.32 (br s, 2H), 8.08 (s, 1H), 8.21 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 12.0, 12.2, 12.4, 12.7, 16.7, 16.83, 16.86, 16.95, 17.12, 17.13, 17.15, 17.3, 60.8, 69.8, 73.6, 80.8, 89.3, 119.3, 139.2, 148.6, 152.5, 156.1; HRMS (ESI⁺) calcd for C₂₂H₄₀N₅O₅Si₂ [M + H]⁺ 510.2562, found 510.2543 (error 3.7 ppm).

3',5'-O-(1,1,3,3-Tetraisopropylidisiloxy)-2'-O-(trifluoromethylsulfonyl)adenosine (50)

To a solution of **49** (5.76 g, 11.3 mmol, 1.0 equiv) and DMAP (4.14 g, 33.9 mmol, 3.0 equiv) in CH₂Cl₂ (150 mL) at 0 °C was added Tf₂O (2.47 mL, 14.7 mmol, 1.3 equiv). The reaction was stirred for 1.5 h at 0 °C then concentrated *in vacuo* to provide an oil that was purified by flash chromatography (5:95 triethylamine–EtOAc) to afford the title compound (4.20 g, 58% yield) as a white foam: $R_f = 0.40$ (1:19 MeOH–CH₂Cl₂); $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 0.90–1.28 (m, 28H), 3.94–4.10 (m, 3H), 5.37 (dd, $J = 8.6$, 5.0 Hz, 1H), 6.07 (d, $J = 4.4$ Hz, 1H), 6.46 (s, 1H), 7.43 (br s, 2H), 8.04 (s, 1H), 8.27 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO- d_6) δ 12.1, 12.2, 12.3, 12.6, 16.53, 16.54, 16.6, 17.00, 17.02, 17.04, 17.2, 59.7, 68.3, 80.3, 85.4, 89.4, 119.2 (q, $^1J_{\text{C-F}} = 320$ Hz), 140.0, 148.5, 152.30, 152.33, 156.0; HRMS (ESI⁺) calcd for C₂₃H₃₉F₃N₅O₇SSi₂ [M + H]⁺ 642.2055, found 642.2021 (error 5.6 ppm).

9-[2'-Azido-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxy)-β-D-arabinofuranosyl]adenine (51)

A solution of **50** (0.272 g, 0.424 mmol, 1.0 equiv) and sodium azide (83.0 mg, 1.27 mmol, 3.0 equiv) in DMF (10 mL) was stirred at 60 °C for 15 h. The reaction was concentrated *in vacuo* and the crude oil was concentrated onto silica gel. Purification by flash chromatography (3:2 to 4:1 EtOAc–hexane, linear gradient) afforded the title compound (0.221 g, 98%) as a light yellow foam: $R_f = 0.40$ (3:2 EtOAc–hexane); $^1\text{H NMR}$ (400 MHz,

DMSO- d_6) δ 1.00–1.32 (m, 28H), 3.87–3.98 (m, 2H), 4.23 (dd, J = 13.7, 5.4 Hz, 1H), 4.84–4.95 (m, 2H), 6.36–6.43 (m, 1H), 7.36 (br s, 2H), 8.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 12.0, 12.1, 12.3, 12.8, 16.6, 16.7, 16.75, 16.77, 17.11, 17.13 (2C), 17.28, 61.4, 67.1, 73.9, 80.6, 81.1, 118.9, 138.9, 149.1, 152.4, 156.1; HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{39}\text{N}_8\text{O}_4\text{Si}_2$ $[\text{M} + \text{H}]^+$ 535.2627, found 535.2610 (error 5.0 ppm).

9-[2'-Azido-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropylidisiloxy)]- β -D-arabinofuranosyl]- N^6,N^6 -bis(*tert*-butoxycarbonyl)adenine (52)

Compound **51** (0.221 g, 0.413 mmol) was converted to **52** using the general procedure for bis-Boc protection. Purification by flash chromatography (2:8 EtOAc–hexane) afforded the title compound (0.230 g, 76%) as a cloudy oil: R_f = 0.55 (3:7 EtOAc–hexane); ^1H NMR (400 MHz, DMSO- d_6) δ 0.98–1.08 (m, 22H), 1.12 (t, J = 7.2 Hz, 6H), 1.34 (s, 18H), 3.92–4.04 (m, 2H), 4.21–4.31 (m, 1H), 4.75 (t, J = 8.8 Hz, 1H), 4.98 (t, J = 8.8 Hz, 1H), 6.58 (d, J = 7.1 Hz, 1H), 8.63 (s, 1H), 8.82 (s, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 12.0, 12.30, 12.35, 12.6, 16.4, 16.5, 16.66, 16.69, 17.03 (2C), 17.05, 17.2, 27.1, 27.4, 61.3, 67.0, 73.7, 80.1, 80.8, 81.7, 83.3, 128.2, 144.5, 149.5, 149.6, 151.6, 152.5; HRMS (ESI+) calcd for $\text{C}_{32}\text{H}_{55}\text{N}_8\text{O}_8\text{Si}_2$ $[\text{M} + \text{H}]^+$ 735.3676, found 735.3666 (error 1.4 ppm).

9-(2'-Azido-2'-deoxy- β -D-arabinofuranosyl)- N^6,N^6 -bis(*tert*-butoxycarbonyl)adenine (53)

To a solution of **52** (0.230 g, 0.313 mmol, 1.0 equiv) in THF (5 mL) was added glacial acetic acid (50 μL to provide a 1% v/v solution). The reaction was warmed to 23 $^\circ\text{C}$ and stirred for 6 h then quenched with cold 1 N aqueous HCl (15 mL). The crude mixture was extracted with EtOAc (5 \times 20 mL) and the combined organic layers were washed with saturated aqueous NaCl (30 mL), dried (MgSO_4) and concentrated under vacuum. Purification by flash chromatography (1:1 EtOAc–hexane) afforded the title compound (0.111 g, 72%) as a light yellow oil: R_f = 0.40 (1:1 EtOAc–hexane); ^1H NMR (400 MHz, DMSO- d_6) δ 1.36 (s, 18H), 3.66–3.91 (m, 3H), 4.40 (q, J = 8.0 Hz, 1H), 4.71, (t, J = 7.4 Hz, 1H), 5.22 (t, J = 5.1 Hz, 1H), 6.05 (d, J = 5.5 Hz, 1H), 6.59 (d, J = 7.1 Hz, 1H), 8.85 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 27.2, 59.5, 67.3, 71.3, 82.1, 83.3, 83.9, 127.8, 144.5, 149.9, 149.3, 149.8, 151.8, 152.7; HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{29}\text{N}_8\text{O}_7$ $[\text{M} + \text{H}]^+$ 493.2154, found 493.2149 (error 1.0 ppm).

9-[2'-Azido-3',5'-O-bis(*tert*-butyldimethylsilyl)-2'-deoxy- β -D-arabinofuranosyl]- N^6,N^6 -bis(*tert*-butoxycarbonyl)adenine (54)

Compound **53** (0.804 g, 1.63 mmol) was converted to **54** using the general procedure for TBS protection. Purification by flash chromatography (2:8 EtOAc–hexane) afforded the title compound (0.893 g, 76%) as a white solid: R_f = 0.55 (3:7 EtOAc–hexane); ^1H NMR (400 MHz, DMSO- d_6) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.12 (s, 6H), 0.90 (s, 18H), 1.35 (s, 18H), 3.78–3.89 (m, 1H), 3.90–4.02 (m, 2H), 4.47 (t, J = 8.0 Hz, 1H), 4.86 (t, J = 7.6 Hz, 1H), 6.64 (d, J = 6.8 Hz, 1H), 8.73 (s, 1H), 8.90 (s, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ –5.57, –5.53, –4.93, –4.92, 17.5, 18.1, 25.4, 25.8, 27.1, 61.1, 67.9, 72.1, 82.1, 82.5, 83.3, 127.6, 144.2, 149.4, 149.7, 151.9, 152.6; HRMS (ESI+) calcd for $\text{C}_{32}\text{H}_{57}\text{N}_8\text{O}_7\text{Si}_2$ $[\text{M} + \text{H}]^+$ 721.3883, found 721.3867 (error 2.2 ppm).

9-(2'-Azido-3'-O-tert-butylidimethylsilyl-2'-deoxy-β-D-arabinofuranosyl)-N⁶,N⁶-bis(tert-butoxycarbonyl)adenine (55)

To a solution of **54** (0.893 g, 1.24 mmol, 1.0 equiv) in 1:1 H₂O–THF (20 mL) at 0 °C was added glacial AcOH (30 mL) dropwise. The reaction was stirred at 0 °C for 46 h then extracted with EtOAc (5 × 60 mL). The combined organic layers were washed with saturated aqueous NaCl (100 mL), dried (MgSO₄), and concentrated under vacuum. Purification by flash chromatography (3:7 EtOAc–hexane) afforded the title compound (0.489 g, 65%) as a yellow foam: *R*_f = 0.25 (3:7 EtOAc–hexane); ¹H NMR (400 MHz, CDCl₃) δ 0.13 (s, 3H), 0.39 (s, 3H) 0.89 (s, 9H), 1.36 (s, 18H), 3.62–3.69 (m, 1H), 3.76–3.83 (m, 1H), 3.85–3.94 (m, 1H), 4.49 (t, *J* = 7.98 Hz, 1H), 4.82 (t, *J* = 7.28 Hz, 1H), 5.29–5.35 (m, 1H), 6.63 (d, *J* = 6.7 Hz, 1H) 8.86 (s, 1H), 8.89 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ –5.03, –4.82, 17.6, 25.5, 27.2, 59.1, 68.1, 72.4, 82.1, 82.5, 83.2, 127.7, 144.7, 149.3, 149.8, 151.8, 152.7; HRMS (ESI+) calcd for C₂₆H₄₃N₈O₇Si [M + H]⁺ 607.3018, found 607.3000 (error 3.0 ppm).

9-{2'-Azido-5'-O-[N-(D-biotinoyl)sulfamoyl]-2'-deoxy-β-D-arabinofuranosyl}adenine triethylammonium salt (57)

Compound **55** (0.267 g, 0.440 mmol) was converted to **56** using the general procedure for sulfamylation using the Okada protocol that was greater than 90% pure contaminated with residual DMA and used directly in the next step. The intermediate sulfamate **56** was converted to **57** using the general procedure for biotinylation and TFA deprotection to afford the title compound (0.193 g, 55% over 3 steps) as the triethylammonium salt (4.4 equiv of Et₃N) as a white solid: HPLC purity: 97.2%, *t*_R = 14.66 min, *k'* = 2.9 (method A);

[α]_D²³ +36.1 (*c* 1.08, MeOH); ¹H NMR (400 MHz, MeOH-*d*₄) δ 1.11 (t, *J* = 7.2 Hz, 40H, excess Et₃N), 1.39–1.47 (m, 2H), 1.53–1.67 (m, 3H), 1.68–1.77 (m, 1H), 2.22 (t, *J* = 7.2 Hz, 2H), 2.63–2.67 (m, 1H), 2.86 (dd, *J* = 12.8, 5.1 Hz, 1H), 2.71 (q, *J* = 7.3 Hz, 28H, excess Et₃N), 3.11–3.17 (m, 1H), 4.09–4.14 (m, 1H), 4.28 (dd, *J* = 7.8, 4.2 Hz, 1H), 4.36–4.40 (m, 2H), 4.41–4.46 (m, 1H), 4.47–4.52 (m, 1H), 4.54–4.59 (m, 1H), 6.47 (d, *J* = 6.0 Hz, 1H), 8.21 (s, 1H), 8.34 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 9.35, 25.5, 25.9, 28.1, 28.4, 39.9, 45.7, 55.5, 59.1, 61.1, 66.3, 66.9, 72.7, 80.7, 81.6, 118.5, 139.5, 149.1, 152.5, 155.9, 162.8, 177.9; HRMS (ESI–) calcd for C₂₀H₂₆N₁₁O₇S₂ [M – Et₃NH][–] 596.1464, found 596.1472 (error 1.3 ppm).

3',5'-O-bis(tert-Butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine (59)

Compound **58** (2.05 g, 7.62 mmol) was converted to the title compound using the general procedure for TBS protection. Purification by flash chromatography (1:19 MeOH–EtOAc) afforded the title compound (3.23 g, 85%) as a white solid: *R*_f = 0.45 (1:19 MeOH–EtOAc); ¹H NMR (600 MHz, CDCl₃) δ –0.11 (s, 3H), –0.02 (s, 3H), 0.14 (s, 3H), 0.15 (s, 3H), 0.77 (s, 9H), 0.91 (s, 9H), 3.71 (d, *J* = 11.2 Hz, 1H), 3.91 (d, *J* = 11.7 Hz, 1H), 3.94–3.98 (m, 1H), 4.86–4.94 (m, 1H), 5.63 (d, ²*J*_{C-F} = 53.5 Hz, 1H), 6.22 (d, ³*J*_{C-F} = 19.2 Hz, 1H), 7.33 (br s, 2H), 8.12 (s, 1H), 8.25 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ –5.71, –5.68, –5.23, –4.89, 17.7, 18.8, 25.54, 25.57, 60.8, 68.8 (d, ²*J*_{C-F} = 15.6 Hz), 82.5, 85.9 (²*J*_{C-F} = 33.6 Hz), 92.5 (¹*J*_{C-F} = 187 Hz), 119.0, 139.6, 148.7, 152.6, 156.1; HRMS (ESI+) calcd for C₂₂H₄₁FN₅O₃Si₂ [M + H]⁺ 498.2726, found 498.2709 (error 3.4 ppm).

***N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxy-2'-fluoroadenosine (60)**

Compound **59** (3.20 g, 6.43 mmol) was converted to the title compound using the general procedure for bis-Boc protection. Purification by flash chromatography (1:3 EtOAc–hexane) afforded the title compound (4.13 g, 92%) as a clear oil: R_f = 0.60 (3:7 EtOAc–hexane); ¹H NMR (600 MHz, CDCl₃) δ -0.15 (s, 3H), -0.03 (s, 3H), 0.15 (s, 6H), 0.77 (s, 9H), 0.92 (s, 9H), 1.38 (s, 18H), 3.75 (dd, J = 12.2, 3.1 Hz, 1H), 3.94 (dd, J = 12.0, 2.4 Hz, 1H), 3.98–4.03 (m, 1H), 4.94 (ddd, J = 22.0, 8.1, 4.4 Hz, 1H), 5.69 (dd, J = 52.8, 3.3 Hz, 1H), 6.39 (d, J = 19.2 Hz, 1H) 8.76 (s, 1H), 8.83 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ -5.73, -5.70, -5.25, -4.91, 17.7, 17.8, 25.51, 25.54, 27.2, 60.5, 68.6 (d, ² J_{C-F} = 15.3 Hz), 82.6, 83.4, 86.3 (d, ² J_{C-F} = 34.4 Hz), 92.5 (d, ¹ J_{C-F} = 187 Hz), 128.0, 145.3, 149.3, 149.9, 151.5, 152.0; HRMS (ESI+) calcd for C₃₂H₅₇FN₅O₇Si₂ [M + H]⁺ 698.3775, found 698.3760 (error 2.1 ppm).

***N*⁶,*N*⁶-bis(*tert*-Butoxycarbonyl)-3'-*O*-*tert*-butyldimethylsilyl-2'-deoxy-2'-fluoroadenosine (61)**

To a solution of **60** (3.90 g, 5.60 mmol, 1.0 equiv) in 1:1 H₂O–THF (20 mL) at 0 °C was added glacial AcOH (30 mL). The mixture was stirred at 0 °C for 13 h then quenched with ice cold 1 N aqueous HCl (50 mL). The mixture was extracted with EtOAc (5 × 50 mL) and the combined organic layers were washed with saturated aqueous NaCl (50 mL) and concentrated under vacuum. Purification by flash chromatography (1:1 EtOAc–hexane) afforded the title compound (2.09 g, 66% yield) as a white foam: R_f = 0.40 (1:1 EtOAc–hexane); ¹H NMR (600 MHz, CDCl₃) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.41 (s, 18H), 3.55–3.60 (m, 1H), 3.74–3.79 (m, 1H), 4.00–4.04 (m, 1H), 4.70–4.77 (m, 1H), 5.15–5.19 (m, 1H), 5.65 (d, J = 52.8 Hz, 1H), 6.40 (d, J = 16.6 Hz, 1H), 8.86 (s, 1H), 8.87 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ -5.16, -4.95, 17.8, 25.6, 27.3, 59.7, 69.6 (d, ² J_{C-F} = 15.4 Hz), 83.5, 84.5, 85.9 (d, ² J_{C-F} = 33.6 Hz), 92.3 (d, ¹ J_{C-F} = 189 Hz), 128.1, 145.1, 149.3, 150.0, 151.7, 152.3; HRMS (ESI+) calcd for C₂₆H₄₃FN₅O₇Si [M + H]⁺ 584.2910, found 584.2898 (error 2.1 ppm).

5'-[*N*-(*o*-Biotinoyl)sulfamoyl]amino-2',5'-dideoxy-2'-fluoroadenosine triethylammonium salt (63)

Compound **61** (2.13 g, 3.65 mmol) was converted to **62** using the general procedure for the Mitsunobu reaction. Purification by flash chromatography (30% EtOAc–hexanes) afforded **62** (>85% purity, contaminated with PPh₃O) that used in the next step without further purification. Compound **62** (2.78 g, 3.65 mmol) was converted to **63** using the general procedures for biotinylation and TFA deprotection to afford the title compound (0.861 g, 35% over 3 steps) as the triethylammonium salt (1.7 equiv of Et₃N) as a white solid: HPLC purity: 96.9%, t_R = 12.90 min, k' = 2.4 (method A); $[\alpha]_D^{23}$ +20.4 (*c* 0.49, MeOH); ¹H NMR (400 MHz, MeOH-*d*₄) δ 1.18 (t, J = 7.4 Hz, 16H, excess Et₃N), 1.37–1.45 (m, 2H), 1.52–1.60 (m, 3H), 1.66–1.75 (m, 1H), 2.16 (t, J = 7.7 Hz, 2H), 2.67 (d, J = 12.6 Hz, 1H), 2.89 (q, J = 7.7 Hz, 12H, excess Et₃N), 3.14–3.24 (m, 3H), 4.18–4.23 (m, 1H), 4.28 (dd, J = 8.5, 4.6 Hz, 1H), 4.42–4.46 (m, 1H), 4.71 (dt, J = 15.8, 5.8 Hz, 2H), 5.53 (dt, J = 53.1, 4.1 Hz, 1H), 6.23 (dd, J = 17.0, 3.4 Hz, 1H), 8.28 (s, 1H), 8.29 (s, 1H); ¹³C NMR (150 MHz, MeOH-*d*₄)

δ 9.5, 26.7, 29.6, 29.9, 38.0, 40.5, 41.2, 46.1, 48.0, 57.0, 61.7, 63.4, 71.7 (d, $^2J_{C-F}$ = 15.8 Hz), 84.2, 89.2 (d, $^2J_{C-F}$ = 33.3 Hz), 94.2 (d, $^1J_{C-F}$ = 190 Hz), 121.0, 142.0, 150.2, 154.5, 157.7, 166.3, 178.1; HRMS (ESI⁻) calcd for C₂₀H₂₆FN₈O₇S₂ [M - H]⁻ 573.1355, found 573.1524 (error 3.0 ppm).

9-[3',5'-O-bis(*tert*-Butyldimethylsilyl)-2'-deoxy-2'-fluoro- β -D-arabinofuranosyl]adenine (65)

Compound **64** (0.230 g, 0.853 mmol) was converted to the title compound using the general procedure for TBS protection. Purification by flash chromatography (1:19 MeOH–EtOAc) afforded the title compound (0.39 g, 92%) as a white foam: R_f = 0.45 (1:19 MeOH–EtOAc); 1H NMR (600 MHz, DMSO-*d*₆) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.13 (s, 6H), 0.89 (s, 18H), 3.79–3.91 (m, 3H), 4.67–4.71 (m, 1H), 5.27–5.40 (m, 1H), 6.44 (dd, J = 12.4, 4.7 Hz, 1H), 7.36 (br s, 2H), 8.15 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-*d*₆) δ -5.52 (2C), -5.26, -4.89, 17.6, 18.0, 25.5, 25.7, 61.4, 73.5 (d, $^2J_{C-F}$ = 23.3 Hz), 80.6 (d, $^2J_{C-F}$ = 17.2 Hz), 82.0 (d, $^2J_{C-F}$ = 7.0 Hz), 95.2 (d, $^1J_{C-F}$ = 193 Hz), 118.1, 138.90, 149.2, 152.9, 156.0; HRMS (ESI⁺) calcd for C₂₂H₄₁FN₅O₃Si₂ [M + H]⁺ 498.2726, found 498.2716 (error 2.0 ppm).

N⁶,N⁶-bis(*tert*-Butoxycarbonyl)-9-[3',5'-O-bis(*tert*-butyldimethylsilyl)-2'-deoxy-2'-fluoro- β -D-arabinofuranosyl]adenine (66)

Compound **65** (0.390 g, 0.784 mmol) was converted to the title compound using the general procedure for bis-Boc protection. Purification by flash chromatography (1:3 EtOAc–hexane) afforded the title compound (0.443 g, 81%) as a colorless oil: R_f = 0.55 (3:7 EtOAc–hexane); 1H NMR (600 MHz, DMSO-*d*₆) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.12 (s, 6H), 0.89 (s, 18H), 1.36 (s, 18H), 3.85 (dd, J = 12.0, 4.0 Hz, 1H), 3.89–3.97 (m, 2H), 4.72 (dt, J = 18.7, 5.5 Hz, 1H), 5.47 (dt, J = 59.2, 4.9 Hz, 1H), 6.62 (dd, J = 10.1, 5.4 Hz, 1H), 8.71 (s, 1H), 8.88 (s, 1H); ^{13}C NMR (150 MHz, DMSO-*d*₆) δ -5.59, -5.58, -5.23, -4.95, 17.6, 18.0, 25.4, 25.7, 27.1, 63.4, 73.0 (d, $^2J_{C-F}$ = 23.0 Hz), 81.1 (d, $^2J_{C-F}$ = 17.1 Hz), 82.1 (d, $^2J_{C-F}$ = 7.6 Hz), 83.3, 95.2 (d, $^1J_{C-F}$ = 194 Hz), 127.5, 144.60, 144.62, 149.4, 149.7, 151.9, 152.4; HRMS (ESI⁺) calcd for C₃₂H₅₇FN₅O₇Si₂ [M + H]⁺ 698.3775, found 698.3765 (error 1.4 ppm).

N⁶,N⁶-bis(*tert*-Butoxycarbonyl)-9-(3'-O-*tert*-butyldimethylsilyl-2'-deoxy-2'-fluoro- β -D-arabinofuranosyl)adenine (67)

To a solution of **66** (0.443 g, 0.635 mmol, 1.0 equiv) in H₂O–THF (10 mL) at 0 °C was added glacial AcOH (15 mL). The reaction was stirred at 0 °C for 23 h then extracted with EtOAc (5 × 50 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL) and concentrated under vacuum. Purification by flash chromatography (1:1 EtOAc–hexane) afforded the title compound (0.245 g, 66%) as a white foam: R_f = 0.25 (1:1 EtOAc–hexane); 1H NMR (600 MHz, DMSO-*d*₆) δ 0.06 (s, 6H), 0.88 (s, 9H), 1.36 (s, 18H), 3.86–3.96 (m, 3H), 4.51 (dt, J = 19.7, 4.5 Hz, 1H), 5.39 (dt, J = 59.2, 4.7 Hz, 1H), 6.08 (s, 1H), 6.61 (dd, J = 10.5, 5.0 Hz, 1H), 8.72 (s, 1H), 8.89 (s, 1H); ^{13}C NMR (226 MHz, MeOH-*d*₄) δ -4.71, -4.56, 19.0, 26.3, 28.1, 61.5, 75.6 (d, $^2J_{C-F}$ = 24.3 Hz), 84.4 (d, $^3J_{C-F}$ = 18.2 Hz), 85.5, 85.8 (d, $^2J_{C-F}$ = 5.3 Hz), 97.2 (d, $^1J_{C-F}$ = 194.0 Hz), 130.1, 147.0, 151.2,

151.5, 153.5, 154.5; HRMS (ESI+) calcd for C₂₆H₄₃FN₅O₇Si [M + H]⁺ 584.2910, found 584.2902 (error 1.4 ppm).

9-{5'-O-[N-(*o*-Biotinoyl)sulfamoyl]-2'-deoxy-2'-fluoro- β -D-arabinofuranosyl}adenine triethylammonium salt (69)

Compound **67** (0.245 g, 0.419 mmol) was converted to **68** using the general procedure for sulfamoylation employing the Okada protocol. Purification by flash chromatography (30% EtOAc–hexanes) afforded **68** that was greater than 90% pure contaminated with residual DMA and used directly in the next step. The intermediate sulfamate was converted to **69** using the general procedure for biotinylation and TFA deprotection to afford the title compound (0.182 g, 56% over 3 steps) as the triethylammonium salt (4.0 equiv of Et₃N) as a white solid: HPLC purity >99%, *t*_R = 14.23 min, *k*' = 2.8 (method A); [α]_D²³ +43.3 (*c* 1.43, MeOH); ¹H NMR (400 MHz, MeOH-d₄) δ 1.12 (t, *J* = 7.2 Hz, 36H, excess Et₃N), 1.40–1.48 (m, 2H), 1.53–1.67 (m, 3H), 1.68–1.77 (m, 1H), 2.21 (t, *J* = 7.3 Hz, 2H), 2.66 (d, *J* = 12.5 Hz, 1H), 2.74 (q, *J* = 7.3 Hz, 24H, excess Et₃N), 2.87 (dd, *J* = 12.8, 4.9 Hz, 1H), 3.13–3.19 (m, 1H), 4.18–4.23 (m, 1H), 4.28 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.30–4.34 (m, 2H), 4.41–4.46 (m, 1H), 4.61 (dt, *J* = 16.9, 3.2 Hz, 1H), 5.12 (dt, *J* = 52.3, 3.5 Hz, 1H), 6.51 (dd, *J* = 16.5, 3.8 Hz, 1H), 8.21 (s, 1H), 8.33 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 9.35, 25.2, 25.9, 28.1, 28.4, 39.8, 45.6, 55.5, 59.1, 61.0, 66.4, 73.3 (d, ²*J*_{C-F} = 23.5 Hz), 81.0, 81.6, 94.8 (d, ¹*J*_{C-F} = 191 Hz), 118.1, 139.5, 149.1, 152.8, 156.0, 162.8, 177.7; HRMS (ESI⁻) calcd for C₂₀H₂₆FN₈O₇S₂ [M – Et₃NH]⁻ 573.1355, found 573.1364 (error 1.5 ppm).

5'-O-[N-(*o*-Biotinoyl)sulfamoyl]- β -2'-methyladenosine triethylammonium salt (73)

Compound **71** (100 mg, 0.311 mmol) was converted to **72** using the general procedure for sulfamoylation employing the Okada protocol that was used directly without further purification. The intermediate sulfamate **72** was converted to **73** using the general procedures for biotinylation and TFA deprotection to afford the title compound (101 mg, 45% over 3 steps) as the triethylammonium salt (6.2 equiv of Et₃N) as a white solid: HPLC purity: 89.3%, *t*_R = 13.04 min, *k*' = 2.5 (method A); ¹H NMR (400 MHz, MeOH-d₄) δ 0.92 (s, 3H), 1.10 (t, *J* = 7.6 Hz, 56H, excess Et₃N), 1.38–1.46 (m, 2H), 1.59–1.66 (m, 3H), 1.69–1.76 (m, 1H), 2.22 (t, *J* = 7.0 Hz, 2H), 2.71 (q, *J* = 7.0 Hz, 40H, excess Et₃N), 2.83–2.89 (m, 2H), 3.11–3.16 (m, 1H), 4.19–4.29 (m, 3H), 4.41–4.51 (m, 3H), 6.10 (s, 1H), 8.19 (s, 1H), 8.44 (s, 1H); NOE ¹H NMR (600 MHz, DMSO-d₆) (Irr, H-1'), enhancement at 0.75 (CH₃) and 8.29 (H-2); ¹³C NMR (150 MHz, DMSO-d₆) δ 11.1 (Et₃NH), 20.03 (CH₃), 26.3 (C-9''), 28.4 (C-7''), 28.8 (C-8''), 39.1 (C-10''), 40.4 (Et₃NH), 46.4 (C-4''), 56.1 (C-6''), 59.2 (C-3'), 61.4 (C-4'), 66.6 (C-5'), 72.8 (C-6a), 80.4 (C-3a), 87.4 (C-2'), 91.3 (C-1'), 119.1 (C-5), 139.5 (C-2), 149.3 (C-8), 153.0 (C-4), 156.1 (C-6), 163.4 (C-2''), 179.1 (C-11''); HRMS (ESI⁻) calcd for C₂₁H₂₉N₈O₈S₂ [M – Et₃NH]⁻ 585.1555, found 585.1512 (error 7.3 ppm).

N⁶-Benzoyl-5'-O-(*tert*-butyldiphenylsilyl)adenosine (75)

To a suspension of N⁶-benzoyladenosine **74** (2.50 g, 6.73 mmol, 1.0 equiv) and DMAP (82 mg, 0.67 mmol, 0.1 equiv) in pyridine (50 mL) at 23 °C was added *tert*-butylchlorodiphenylsilane (1.93 mL, 7.41 mmol, 1.1 equiv) dropwise. The reaction was stirred for 19 h at 23 °C. Solvent was removed *in vacuo* and the residue was re-dissolved in

EtOH (10 mL). A white precipitate started to form, and further precipitation was induced by the addition of Et₂O (200 mL). The gummy solid was filtered, washed with H₂O (2 × 50 mL) and recrystallized from MeOH (50 mL) to afford the title compound (4.03 g, 98%) as a white solid: mp: 113–116 °C; *R*_f = 0.31 (1:19 MeOH–CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) 0.94 (s, 9H), 3.78–3.96 (m, 2H), 4.33–4.43 (m, 1H), 4.51–4.59 (m, 1H), 4.78 (t, *J* = 5.5 Hz, 1H), 6.06 (d, *J* = 5.9 Hz, 1H), 7.29–7.46 (m, 6H), 7.48–7.66 (m, 7H), 8.02 (d, *J* = 7.4 Hz, 2H), 8.26 (s, 1H), 8.71 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 19.1, 26.7, 63.9, 72.2, 75.9, 86.9, 90.3, 122.9, 127.81, 127.83, 127.9, 128.9 (2C), 129.9, 130.0, 132.5, 132.6, 133.5, 135.4, 135.5 (2C), 141.3, 149.5, 150.9, 152.2; HRMS (ESI+) calcd for C₃₃H₃₆N₅O₅Si [M + H]⁺ 610.2480, found 610.2473 (error 1.1 ppm).

(2′*R*,6′*S*)-*N*⁶-Benzoyl-9-{6′-[(*tert*-butyldiphenylsilyloxy)methyl]-*N*-tritylmorpholin-2′-yl}adenine (79)

To a suspension of **75** (1.47 g, 2.41 mmol, 1.0 equiv) in methanol (25 mL) at 23 °C was added sodium *meta*-periodate (570 mg, 2.66 mmol, 1.1 equiv) and ammonium baborate tetrahydrate (700 mg, 2.66 mmol, 1.1 equiv). The reaction mixture was stirred at 23 °C for 1.5 h during which time the starting material was consumed and a cluster of closely-spaced ninhydrin-active spots (TLC) was generated. The reaction mixture was filtered and to the filtrate was added successively activated powdered 4 Å molecular sieves (300 mg/mmol of **75**), sodium cyanoborohydride (330 mg, 5.3 mmol, 2.2 equiv) and glacial acetic acid (0.30 mL, 5.30 mmol, 2.2 equiv) dropwise. The reaction mixture was stirred for another 5 h at 23 °C. The mixture was then filtered through Celite and evaporated to dryness. The residue was dissolved in CHCl₃ (50 mL) and washed with H₂O (30 mL), saturated aqueous NaCl (30 mL), dried (Na₂SO₄) and concentrated *in vacuo* to yield crude **78** as a white solid, which was used in the next step directly without purification.

The crude **78** prepared above was co-evaporated with DMF (2 × 20 mL). Crude **78** was suspended in DMF (10 mL) and to the suspension was added triethylamine (0.50 mL, 3.62 mmol, 1.5 equiv), and trityl chloride (810 mg, 2.89 mmol, 1.2 equiv) portion-wise at 0 °C. After 2 h, the reaction mixture was quenched with MeOH (1 mL), and evaporated to dryness. The residue was then dissolved in EtOAc (50 mL) and washed with H₂O (30 mL), saturated aqueous NaCl (30 mL), dried (Na₂SO₄) and concentrated under vacuum. Purification by flash chromatography (0:1 to 1:9 MeOH–CH₂Cl₂, linear gradient) afforded the title compound (0.86 g, 43% over 4 steps) as a white foam: *R*_f = 0.49 (1:19 MeOH–CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) 0.95 (s, 9H), 1.62 (t, *J* = 11.0 Hz, 1H), 1.75 (t, *J* = 11.0 Hz, 1H), 3.36 (d, *J* = 11.7 Hz, 1H), 3.47 (d, *J* = 11.4 Hz, 1H), 3.61 (dd, *J* = 10.5, 5.9 Hz, 1H), 3.77 (dd, *J* = 10.6, 4.3 Hz, 1H), 4.29–4.41 (m, 1H), 6.38 (d, *J* = 8.6 Hz, 1H), 7.16–7.63 (m, 28H), 7.84 (s, 1H), 8.00 (d, *J* = 7.4 Hz, 2H), 8.81 (s, 1H), 8.96 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 19.2, 26.7, 49.9, 53.3, 64.5, 77.2, 80.2, 122.6, 126.5, 127.6, 127.8, 127.9, 128.8, 129.2, 129.68, 129.73, 132.7, 133.1, 133.2, 133.6, 135.5, 140.6, 149.3, 151.2, 152.8, 164.5; HRMS (ESI+) calcd for C₅₂H₅₁N₆O₃Si [M + H]⁺ 835.3786, found 835.3785 (error 0.1 ppm).

(2' R,6' S)-N⁶-Benzoyl-9-[6'-(hydroxymethyl)-N-tritylmorpholin-2'-yl]adenine (80)

To a solution of **79** (1.67 g, 2.00 mmol, 1.0 equiv) in THF (20 mL) at 23 °C was added a solution of TBAF (1.0 M in THF, 3.00 mL, 3.00 mmol, 1.5 equiv). The reaction mixture was stirred for 2 h at 23 °C then concentrated and diluted with EtOAc (30 mL), washed with H₂O (25 mL), saturated aqueous NaCl (25 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Purification by flash chromatography (0:1 to 1:19 MeOH–CH₂Cl₂, linear gradient) afforded the title compound (1.15 g, 96%) as a white solid: *R_f* = 0.29 (5% MeOH–CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 1.59 (t, *J* = 11.2 Hz, 1H), 1.79 (t, *J* = 10.8 Hz, 1H), 3.10 (d, *J* = 11.7 Hz, 1H), 3.50 (d, *J* = 11.7 Hz, 1H), 3.53–3.64 (m, 2H), 3.81 (br s, 1H), 4.29 (br s, 1H), 6.34 (d, *J* = 9.4 Hz, 1H), 7.01–7.23 (m, 3H), 7.29 (t, *J* = 7.4 Hz, 7H), 7.38–7.53 (m, 8H), 7.53–7.65 (m, 1H), 7.83 (s, 1H), 8.03 (d, *J* = 7.4 Hz, 1H), 8.77 (s, 1H), 9.60 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 45.8, 48.7, 53.0, 63.4, 76.9, 77.2, 77.6, 80.4, 122.1, 126.6, 127.9, 128.0, 128.7, 129.1, 132.6, 133.8, 140.1, 149.6, 150.8, 152.9, 164.8; HRMS (ESI+) calcd for C₃₆H₃₃N₆O₃ [M + H]⁺ 597.2609, found 597.2622 (error 2.2 ppm).

(2' R,6' S)-N⁶-Benzoyl-9-(6'-((sulfamoyl)oxy)methyl)-N-tritylmorpholin-2'-yl)adenine (81)

Compound **80** (200 mg, 0.335 mmol) was converted to the title compound using the Okada general procedure for sulfamoylation. Purification by flash chromatography (0:1 to 3:2 EtOAc–hexane, linear gradient) afforded the title compound (180 mg, 78%) as a white solid: *R_f* = 0.49 (5% MeOH–CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 1.60 (t, *J* = 11.4 Hz, 1H), 1.80 (t, *J* = 10.6 Hz, 1H), 3.20 (d, *J* = 11.7 Hz, 1H), 3.43 (d, *J* = 11.7 Hz, 1H), 4.08–4.23 (m, 2H), 4.55 (br s, 1H), 5.85 (br s, 2H), 6.28 (d, *J* = 8.2 Hz, 1H), 7.13–7.56 (m, 18H), 7.81 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 8.68 (s, 1H), 9.02 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃) 48.6, 52.7, 56.8, 70.0, 74.3, 80.1, 122.2, 126.8, 126.9, 127.8, 128.1, 128.7, 128.8, 129.1, 132.8, 133.3, 140.7, 148.9, 150.8, 152.7, 164.8; HRMS (ESI–) calcd for C₃₆H₃₂N₇O₅S [M – H][–] 674.2191, found 674.2192 (error 0.1 ppm).

(2' R,6' S)-9-[6'-((N-(*o*-Biotinoyl)sulfamoyl)oxy)methyl)morpholin-2'-yl]adenine triethylammonium salt (82)

Compound **81** (50 mg, 0.073 mmol) was biotinylated using the general procedure for biotinylation. To a solution of this crude biotinylated nucleoside in pyridine (1 mL) at 23 °C was added concentrated aqueous ammonia (1 mL). The reaction mixture was stirred for 25 h at 23 °C during which time all starting material was consumed as monitored by electrospray mass spectrometry in the negative mode. The solvent was removed under vacuum. The crude debenzoylated material was deprotected using the general procedure for TFA deprotection to afford the title compound (18 mg, 36% over 2 steps) as the triethylammonium salt (1.6 equiv of Et₃N) as a white solid: HPLC purity: 92.0%, *t_R* = 13.2 min (method A), *k'* = 1.4 (method A); ¹H NMR (600 MHz, MeOH-*d*₄) δ 1.14 (t, *J* = 7.2 Hz, 15H, excess Et₃N), 1.24 (t, *J* = 7.0 Hz, 2H), 1.32–1.46 (m, 2H), 1.46–1.75 (m, 5H), 2.15 (t, *J* = 7.2 Hz, 2H), 2.67 (d, *J* = 12.5 Hz, 1H), 2.79 (q, *J* = 7.0 Hz, 11H, excess Et₃N), 2.89 (dd, *J* = 12.9, 5.1 Hz, 2H), 3.02 (d, *J* = 12.5 Hz, 1H), 3.18 (d, *J* = 6.7 Hz, 2H), 4.04–4.16 (m, 2H), 4.28 (dd, *J* = 7.6, 4.5 Hz, 1H), 4.39–4.51 (m, 1H), 5.85 (t, *J* = 6.3 Hz, 1H), 7.46 (d, *J* = 7.4 Hz, 1H), 7.54 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 8.20 (s, 1H), 8.34 (s, 1H); ¹³C NMR (100 MHz, MeOH-*d*₄) 25.8, 28.1, 28.4, 38.4, 39.7, 45.2, 45.9, 46.8, 47.9, 48.1, 55.6, 60.2, 61.9,

68.3, 75.8, 80.1, 127.1, 128.1, 131.5, 139.2, 148.7, 152.5; HRMS (ESI⁻) calcd for C₂₀H₂₈N₉O₆S₂ [M – Et₃NH]⁻ 554.1609, found 554.1612 (error 0.5 ppm).

N⁶,N⁶-bis(*tert*-Butoxycarbonyl)-2',5'-*O*-bis(*tert*-butyldimethylsilyl)-3'-deoxyadenosine (84)

Compound **83** (0.200 g, 0.796 mmol) was converted to 2',5'-*O*-bis(*tert*-butyldimethylsilyl)-3'-deoxyadenosine using the general procedure for TBS protection. Purification by flash chromatography (5% MeOH–EtOAc) afforded 2',5'-*O*-bis(*tert*-butyldimethylsilyl)-3'-deoxyadenosine (0.327 g, 86%), which was used in the next step without further purification. 2',5'-*O*-bis(*tert*-Butyldimethylsilyl)-3'-deoxyadenosine (0.327 g, 0.685 mmol) was converted to the title compound using the general procedure for bis-Boc protection. Purification by flash chromatography (2:3 EtOAc–hexane) afforded the title compound (0.466 g, 82%, 2 steps) as a colorless oil: *R_f* = 0.50 (3:7 EtOAc–hexane);

[α]_D²³ +9.43 (*c* 1.0, MeOH); ¹H NMR (400 MHz, DMSO-*d*₆) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.04 (s, 6H), 0.83 (s, 9H), 0.88 (s, 9H), 1.36 (s, 18H), 1.95–2.03 (m, 1H), 2.27–2.37 (m, 1H), 3.79 (d, *J* = 9.5 Hz, 1H), 3.97 (d, *J* = 10.9 Hz, 1H), 4.41–4.48 (m, 1H), 4.80–4.85 (m, 1H), 6.04 (s, 1H), 8.81 (s, 1H), 8.86 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ –5.52, –5.49, –5.10, –5.08, 17.6, 18.0, 25.5, 25.8, 27.2, 33.9, 63.9, 76.3, 80.5, 83.3, 90.7, 128.1, 144.2, 149.2, 149.8, 151.7, 152.3; HRMS (ESI⁺) calcd for C₃₂H₅₈N₅O₇Si₂ [M + H]⁺ 680.3869, found 680.3852 (error 2.5 ppm).

N⁶,N⁶-bis(*tert*-Butoxycarbonyl)-2'-*O*-*tert*-butyldimethylsilyl-3'-deoxyadenosine (85)

To a solution of **84** (0.466 g, 0.685 mmol, 1.0 equiv) in 1:1 H₂O–THF (10 mL) at 0 °C was added glacial AcOH (15 mL). The mixture was stirred at 0 °C for 7 h then extracted with EtOAc (5 × 50 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL) and then concentrated under vacuum. Purification by flash chromatography (2:3 EtOAc–hexane) afforded the title compound (0.244 g, 63%) as a white foam: *R_f* = 0.50 (1:1 EtOAc–hexane); [α]_D²³ +8.44 (*c* 0.79, MeOH); ¹H NMR (400 MHz, DMSO-*d*₆) δ –0.02 (s, 3H), 0.01 (s, 3H), 0.82 (s, 9H), 1.38 (s, 18H), 1.90–2.01 (m, 1H), 2.24–2.36 (m, 1H), 3.51–3.63 (m, 1H), 3.69–3.79 (m, 1H), 4.35–4.46 (m, 1H), 4.82 (br s, 1H), 5.11 (t, *J* = 5.1 Hz, 1H), 6.03 (d, *J* = 1.7 Hz, 1H), 8.87 (s, 1H), 8.91 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ –5.10, –5.08, 17.6, 25.5, 27.2, 34.1, 62.1, 76.2, 80.5, 83.4, 90.7, 128.1, 144.6, 149.2, 149.9, 151.6, 152.4; HRMS (ESI⁺) calcd for C₂₆H₄₄N₅O₇Si₂ [M + H]⁺ 566.3005, found 566.2991 (error 2.5 ppm).

5'-[N-(*p*-Biotinoyl)sulfamoyl]amino-3',5'-dideoxyadenosine triethylammonium salt (87)

Compound **85** (0.238 g, 0.421 mmol, 1.0 equiv) was converted to **86** using the general procedure for the Mitsunobu reaction. Purification by flash chromatography (45% EtOAc–hexanes) afforded **86** (>85% purity, contaminated with PPh₃O) that used in the next step without further purification. The intermediate sulfamide **86** was converted to **87** using the general procedures for biotinylation and TFA deprotection to afford the title compound (171 mg, 62% over 3 steps) as the triethylammonium salt (3.3 equiv of Et₃N) as a white solid:

HPLC purity: 96.3%, *t_R* = 12.44 min, *k'* = 2.3 (method A); [α]_D²³ +10.6 (*c* 0.56, MeOH); ¹H NMR (400 MHz, MeOH-*d*₄) δ 1.13 (t, *J* = 7.3 Hz, 30H, excess Et₃N), 1.19–1.30 (m, 2H),

1.39–1.48 (m, 2H), 1.53–1.66 (m, 3H), 1.68–1.77 (m, 1H), 2.18 (t, $J = 7.1$ Hz, 2H), 2.33–2.42 (m, 1H), 2.66 (d, $J = 12.2$ Hz, 1H), 2.75 (q, $J = 7.1$ Hz, 20H, excess Et₃N), 2.87 (dd, $J = 13.0, 5.0$, 1H), 3.11–3.16 (m, 1H), 3.23–3.26 (m, 1H), 4.28 (dd, $J = 8.4, 4.9$ Hz, 1H), 4.41–4.46 (m, 1H), 4.54–4.61 (m, 1H), 4.74–4.75 (m, 1H), 5.91 (d, $J = 2.3$ Hz, 1H), 8.26 (s, 1H), 8.28 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 9.01, 21.9, 24.4, 27.98, 35.0, 35.7, 45.7, 46.7, 55.3, 59.1, 61.0, 67.8, 73.2, 77.3, 91.4, 119.4, 139.8, 148.5, 152.5, 156.2, 162.7, 171.7; HRMS (ESI⁻) calcd for C₂₀H₂₈N₉O₆S₂ [M – Et₃NH]⁻ 554.1609, found 554.1618 (error 1.7 ppm).

9-[(*N*-[D-Biotinoyl])ulfamoyl]amino}ethoxy)methyl]adenine triethylammonium salt (**90**)

Compound **88** (0.213 g, 1.02 mmol) was converted to **89** using the general procedure for the Mitsunobu reaction. Purification by flash chromatography (60% EtOAc–hexanes) afforded **89** (>85% purity, contaminated with PPh₃O) that used in the next step without further purification. The intermediate sulfamide **89** was converted to **90** using the general procedures for biotinylation and TFA deprotection to afford the title compound (438 mg, 52% over 3 steps) as the triethylammonium salt (3.5 equiv of Et₃N) as a white solid: HPLC purity: 97.2%, $t_R = 12.37$ min, $k' = 2.3$ (method A); [α]_D²³ +25.4 (c 1.06, MeOH); ¹H NMR (400 MHz, MeOH-d₄) δ 1.11 (t, $J = 7.3$ Hz, 46H, excess Et₃N), 1.39–1.47 (m, 2H), 1.53–1.66 (m, 3H), 1.68–1.78 (m, 1H), 2.14 (t, $J = 7.4$ Hz, 2H), 2.70 (q, $J = 7.3$ Hz, 32H, excess Et₃N), 2.89 (dd, $J = 12.9, 5.3$ Hz, 1H), 3.06 (t, $J = 5.6$ Hz, 2H), 3.16–3.22 (m, 1H), 3.66 (t, $J = 5.4$ Hz, 2H), 4.30 (dd, $J = 7.7, 4.4$ Hz, 1H), 4.47 (dd, $J = 7.7, 4.9$ Hz, 1H), 5.64 (s, 2H), 8.23 (s, 1H), 8.27 (s, 1H); ¹³C NMR (150 MHz, DMSO-d₆) δ 10.9, 25.1, 28.0, 28.2, 36.8, 39.8, 42.3, 45.7, 55.4, 59.2, 61.0, 67.6, 71.9, 118.4, 141.1, 149.7, 152.9, 156.0, 162.7, 175.1; HRMS (ESI⁻) calcd for C₁₈H₂₆N₉O₅S₂ [M – Et₃NH]⁻ 512.1504, found 512.1512 (error 1.6 ppm).

MtBPL cloning, expression and purification

MtBPL was cloned, expressed and purified as previously described.¹⁵

Isothermal titration calorimetry

All ITC experiments were conducted on an automated microcalorimeter (Malvern Instruments). The experiments were performed at 25 °C in ITC buffer (10 mM Tris pH 7.5, 200 mM KCl, 2.5 mM MgCl). *MtBPL* was exchanged (2 × 10 mL) into ITC buffer using an Amicon Ultra concentrator, and the final filtrate was used to prepare a solution of the analogs from a 10 mM stock in DMSO. Protein concentrations were 10 μM for *MtBPL* (determined by active site titration with **14**), 100 μM analog and 100 μM biotin (determined by weighing sample on an ultramicrobalance [Mettler Toledo] accurate to 0.001 mg). In the direct titration experiments, the analog was injected into a solution of the enzyme. In the competitive titration experiments, the analog was injected into a solution of the enzyme and biotin. Titrations were carried out with a stirring speed of 750 rpm and 200 s interval between 4 μL injections. The first injection for each sample was excluded from data fitting. Titrations were run past the point of enzyme saturation to correct for heats of dilution. The experimental data were fitted to a theoretical titration curve using the Origin software package (version 7.0) provided with the instrument to afford values of K_A^{app} (the apparent

binding constant of the ligands in the presence of biotin in M^{-1}), n (stoichiometry of binding) and H (the binding enthalpy change in kcal/mol). The K_A values for each ligand was obtained from the K_A^{app} value using equation 1:

$$K_A = K_A^{app} (1 + K_A^B [B]) \quad (1)$$

where $[B]$ is the concentration of biotin, and K_A^B is the association constant for biotin experimentally determined to be 7.29×10^5 . The thermodynamic parameters (G and S) were calculated from K determined from the displacement titration and H from the direct binding titration using equation 2:

$$\Delta G = -RT \ln(K) = \Delta H - T\Delta S \quad (2)$$

where G , H , and S are the changes in free energy, enthalpy, and entropy of binding, respectively; $R = 1.98 \text{ cal mol}^{-1} \text{ K}^{-1}$; and T is the absolute temperature (298 K). The affinity of the ligands for the protein is given as the dissociation constant ($K_D = 1/K_A$). ITC experiments were run in triplicate and analyzed independently, and the thermodynamic values were averaged.

Differential Scanning Fluorimetry

Differential scanning fluorimetry was performed using a Bio-Rad CFX96 according to established protocols.⁵² In brief, purified *MtBPL* was diluted to give 40 μL solution consisting of: 17.3 μM (0.5 mg/mL) *MtBPL*, 43 μM analog (diluted from a 2 mM stock in DMSO), 10 mM Tris pH 8.0, 200 mM KCl, 2.5 mM MgCl, 10% (v/v) glycerol, and 5 \times SYPRO Orange (Life Technologies). Each analog was run in triplicate. The fluorescence response (melting curve) was measured across a temperature range between 10 and 85 $^\circ\text{C}$ with 1 min incubation per 0.5 $^\circ\text{C}$ temperature increase. The melting temperature (T_m) was determined from the peak of the first derivatives of the melting curve; calculations were made using the Bio-Rad *CFX Manager* software and plots were produced using the program *Plot* (<http://plot.micw.eu/>).

M. tuberculosis MIC Assay

All compounds MICs were experimentally determined as previously described.¹⁵ Minimum inhibitory concentrations (MICs) were determined in quadruplicate in standard GAST medium according to the broth micro dilution method using compounds from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. Plates were incubated at 37 $^\circ\text{C}$ (100 μL /well) and growth was recorded by measurement of optical density at 580 nm. All measurements reported herein used an initial cell density of 10^5 cells/assay and growth monitored at 10–14 days, with the untreated and DMSO-treated control cultures reaching an $\text{OD}_{620} \sim 0.8$ –1.0.

Co-Crystallization of Ligands with *MtBPL*

MtBPL at 1.5 mg/ml (10 mM HEPES pH 7.5, 50 mM NaCl, 1 mM DTT) was combined with inhibitors (200 μM in DMSO) at 1:30 molar ratio, incubated at 37 $^\circ\text{C}$ for 30 min, and then concentrated using Amicon centrifugal filters (EMD Millipore) to 10 mg/ml. The

concentrated protein-compound solution was utilized to obtain crystals via vapor-diffusion using apo crystals as micro-seeds in hanging drops as previously described.¹⁵ Crystals grew within 1 week. Mother liquor included 17–24% PEG MME 2000, 100 mM Tris pH 8.5 and 100 mM trimethylamine *N*-oxide. Crystals were frozen in liquid nitrogen after a quick soak in cryo-protectant solution containing mother liquor supplemented with 20% PEG 400.

Crystallographic Data collection and structure solution

All diffraction data was collected at synchrotron X-ray sources under cryogenic conditions of 100 K (–173 °C). Data for the complex with compound **82** was collected at beamline 23-ID-B (GM-CA CAT) of the Advanced Photon Source (APS; Argonne, IL) equipped with a MAR 300 CCD detector. Data was integrated and scaled with HKL3000 (HKL Research, Inc). Data for the complex with compound **90** were collected at beamline 4.2.2 of the Advanced Light Source (ALS) in Berkeley, CA equipped with a NOIR-1 CCD detector. HKL-2000 was used to index, integrate and scale the data.⁶⁰ All other data were collected at APS beamline 17-ID (IMCA-CAT) equipped with a Dectris PILATUS 6M detector. The program autoPROC was utilized to index, integrate and scale the data.⁶¹ The complex with compound **36** was first to be phased by molecular replacement,⁶² using the *Mt*BPL complex with **14** (PDB-id 3RUX)¹⁵ stripped of inhibitor and solvent as a search model. The refined structure with **36** without inhibitor was subsequently used as the search model in molecular replacement model to phase complexes with the remaining compounds. All structures were subjected to subsequent rounds of model building using COOT⁶³ and refinement using phenix.refine⁶⁴ to acquire the final refined structures. Topology and parameter files for all ligands were generated using JLigand.⁶⁵

Cell Cytotoxicity-MTT Assay

Human liver cells (HepG2, ATCC) cells were plated in 96-well plates at $(2.5\text{--}5.0) \times 10^4$ cells per well (200 μL). HepG2 cells were maintained in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Compounds were prepared as 10 mM stock solutions in DMSO, and 1 μL of the compound stock solution was added to each well in 200 μL of EMEM, yielding a final compound concentration of 100 μM . Control wells contained either 1% DMSO (negative control) or 40 μM phenol (positive control), and all reactions were done in triplicate. The plate was incubated for 48 h at 37 °C in a 5% $\text{CO}_2/95\%$ air humidified atmosphere. Measurement of cell viability was carried out using a modified method of Mosmann based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).⁶⁶ The MTT solution was prepared fresh at 1 mg/mL in serum-free, phenol red free DMEM. MTT solution (200 μL) was added to each well, and the plate was incubated as described above for 3 h. The MTT solution was removed, and the formazan crystals were solubilized with 200 μL of 2 N KOH. The plate was read on a M5e spectrophotometer (Molecular Devices) at 570 nm for formazan and 650 nm for background subtraction. Cell viability was estimated as the percentage absorbance of sample relative to the DMSO control.

LC-MS/MS Quantitation of Compound Accumulation

Compounds **63** and **69** were evaluated for cellular accumulation using the method reported by Rhee and coworkers.^{54,55} Briefly, *M. tuberculosis* H37Rv cultures were grown in GAST medium containing tween 80 for 5 days, then transferred to 7H10 agar plates containing 0, 5, and 25 μM **63** and **69** for 24 hours. Samples were isolated using the method previously reported.⁵⁴ Samples were then analyzed by LC-MS/MS (Shimadzu UFLC XR-AB SCIEX QTRAP 5500) and quantified by reverse-phase LC on a Kinetix C18 column (50 mm \times 2.1 mm, 2.6 μm particle size; Phenomenex, Torrance, CA) at a flow rate of 0.5 mL/min. Initial conditions were 5% acetonitrile containing 0.1% formic acid (solvent B) and 95% water containing 0.1% formic acid (solvent A) from 0 to 0.5 min, after which solvent B was increased to 95% from 0.5 to 3 min. The column was washed in 95% solvent B for 2 min, returned to 5% solvent B over 0.2 min, and allowed to re-equilibrate for 2.8 min in 5% solvent B to provide a total run time of 8 min. The column oven was maintained at 40 $^{\circ}\text{C}$ and the injection volume was 10 μL . All analytes were analyzed by MS in positive ionization mode by Multiple Reaction Monitoring (MRM). To determine the optimum MRM settings, compounds **63** and **69** were infused at a concentration of 10 μM (in 1:1 water/acetonitrile containing 0.1% formic acid) onto the MS by a syringe pump at a flow of 10 $\mu\text{L}/\text{min}$. The MS parameters are listed in table S3.

Analyte and internal standard peak areas were calculated (MultiQuant, version 2.0.2, AB SCIEX). Analyte peak areas were normalized to internal standard peak areas and the analyte concentrations were determined with an appropriate standard curve (Figure S15). The protein concentration of the lysate in each of the samples were calculated by Bradford assay using Bovine Serum Albumin (BSA) as a standard. The average protein concentration in the lysate samples was determined to be 5.74 ± 1.35 $\mu\text{g}/\text{mL}$. The normalized concentration is given as a ratio of analyte concentration over protein concentration and were found to be 0.035 ± 0.014 μg analyte/ μg protein for **63** at 25 μM and 0.005 ± 0.003 μg analyte/ μg protein for **69** at 25 μM .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACCs acyl CoA carboxylases

| | |
|----------------|---|
| AMP | adenosine monophosphate |
| Bio-AMP | biotinyl-5'-adenosine monophosphate |
| Bio-AMS | biotinyl-5'-adenosine monosulfamide |
| Bio-NHS | D-(+)-biotin <i>N</i> -hydroxysuccinimide ester |
| Boc | <i>tert</i> -butyloxycarbonyl |
| BPL | biotin protein ligase |
| DIAD | diisopropyl azodicarboxylate |
| DMA | dimethylacetamide |
| DSF | differential scanning fluorimetry |
| HPLC | high performance liquid chromatography |
| ITC | isothermal titration calorimetry |
| MtBPL | Biotin protein ligase |
| MIC | minimum inhibitory concentration |
| Mtb | <i>Mycobacterium tuberculosis</i> |
| MtBPL | <i>Mycobacterium tuberculosis</i> biotin protein ligase |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide TB, tuberculosis |
| TBDPS | <i>tert</i> -butyldiphenylsilyl |
| TBS | <i>tert</i> -butyl dimethylsilyl |
| TFA | trifluoroacetic acid |
| MDR-TB | multi drug resistant tuberculosis |
| XDR-TB | extensively drug resistant tuberculosis |

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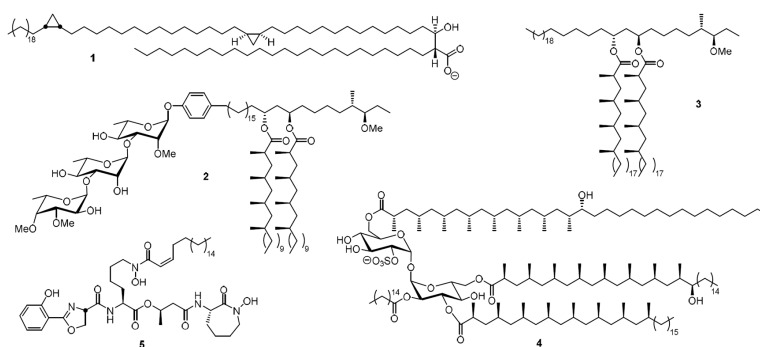


Figure 1. Unique lipids and lipidated metabolites found in cell envelope of *Mycobacterium tuberculosis*. All of the molecules shown exist as a suite of related isomers that vary in the lipid chain length. If reported, the major isomer is shown otherwise a representative molecule is depicted. The mycolic acids are represented by the most abundant a mycolic acid (α -MA, **1**), the phenolic glycolipids are represented by **2**, the phthiocerol dimycocerosates are exemplified by PDIM-A (**3**), the sulfolipids are represented by SL-1 (**4**) and the mycobactins by **5**.

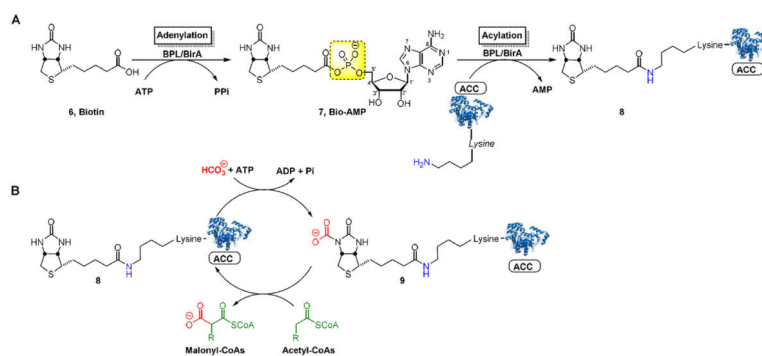
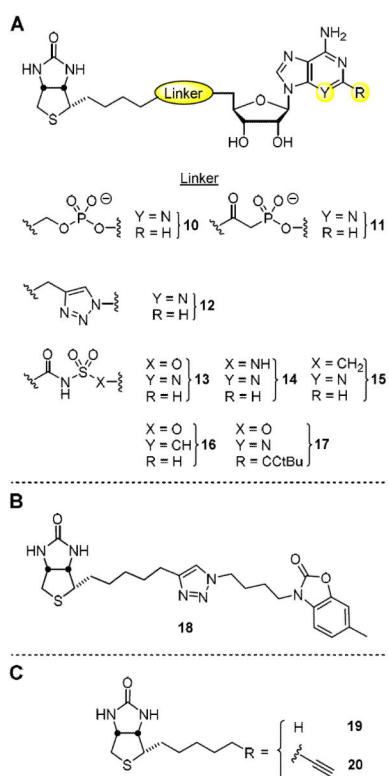


Figure 2.

(A) *Mt*BPL catalyzed biotinylation of ACC, a biotin dependent enzyme proceeds in two steps by sequential adenylation of biotin (6) to form Bio-AMP (7) followed by acylation of the biotin carboxylase carrier protein domain of ACCs to furnish holo-ACC (8). (B) Biotinylated ACC proteins mediate carboxyl group transfer onto acyl-CoAs forming malonyl-CoAs for lipid biosynthesis. The N-1 atom of biotin is carboxylated in an ATP-dependent manner by the biotin-carboxylase domain of ACCs to furnish 9. In the second half-reaction, ACCs transfer the carboxy group onto an acyl-CoA, mediated by the biotin carboxylase domain of ACCs.

**Figure 3.**

(A) Representative examples of biotin-AMP nucleoside-based bisubstrate inhibitors of BPL including: alkylphosphate **10**, β -ketophosphonate **11**, 1,2,3 triazole **12**, acylsulfamate **13**, acylsulfamide **14**, acylsulfonamide **15**, 3-deaza acylsulfamate **16**, and 2-*tert*-butylethynyl-acylsulfamate **17**. (B) Benzoxazoline moiety **18**, a selective inhibitor of *S. aureus* BPL. (C) Biotin based inhibitors **19** and **20** of *S. aureus* BPL.

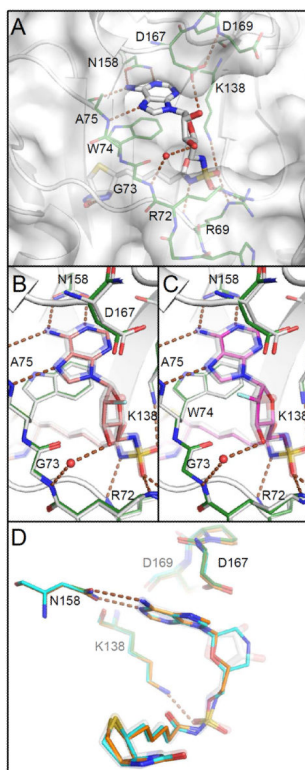
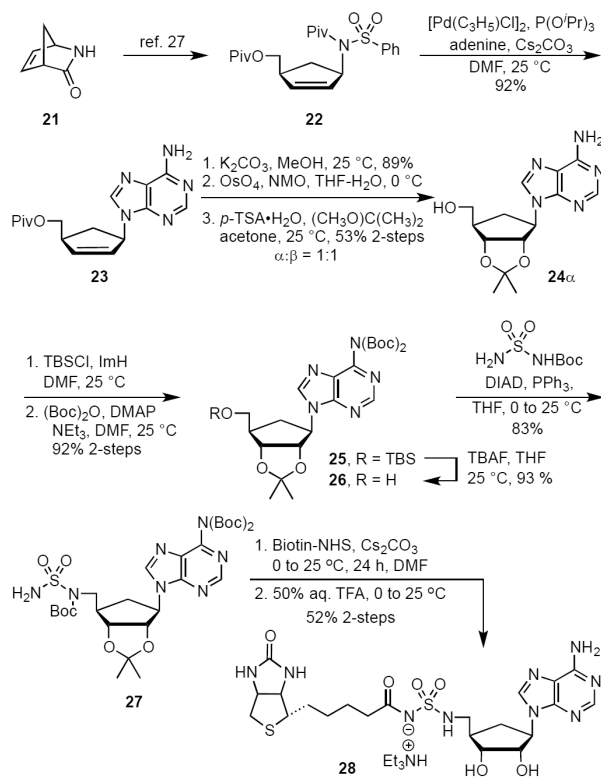
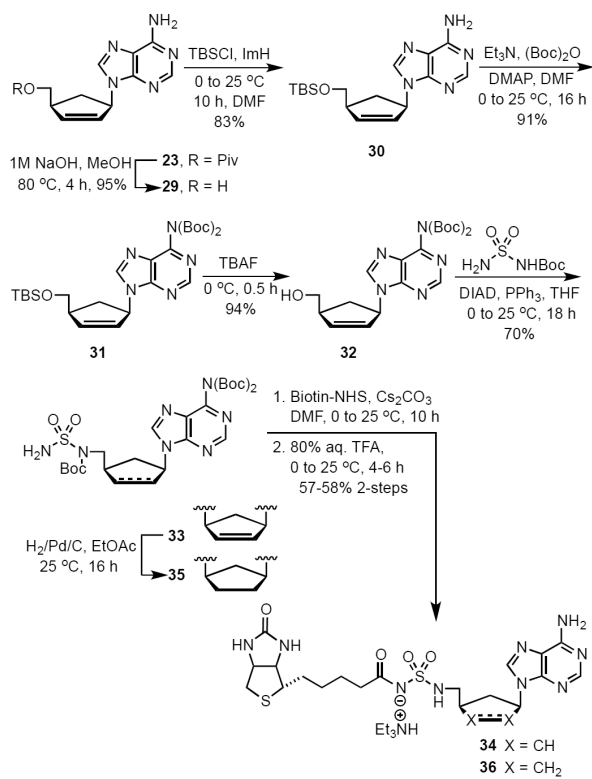


Figure 4.

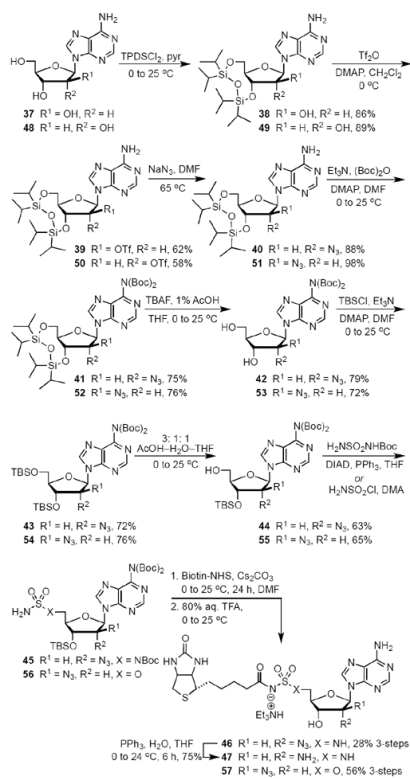
Comparison of co-crystal structures. A) Bio-AMS (**14**, white) binds with adenosyl and biotinyl groups in deep pockets with Trp74 stacked between them. Protein carbon atoms of this complex are green included in other panels for comparison. Hydrogen bonds are illustrated with brown dashed lines. The ribose is positioned across the open mouth of a larger unoccupied space. B and C) Binding site and ribose ring pucker are unchanged upon binding of the 2' fluoro analogs with standard (**63**, salmon) or reversed (**69**, magenta) stereochemistry, despite the loss of the hydrogen bond to Asp167. Azido analogs (**46**, **57**) bind similarly (not shown). D) Morpholino (**82**, cyan) and acyclo (**90**, orange) analogs adopt conformations that mimic the ribose.



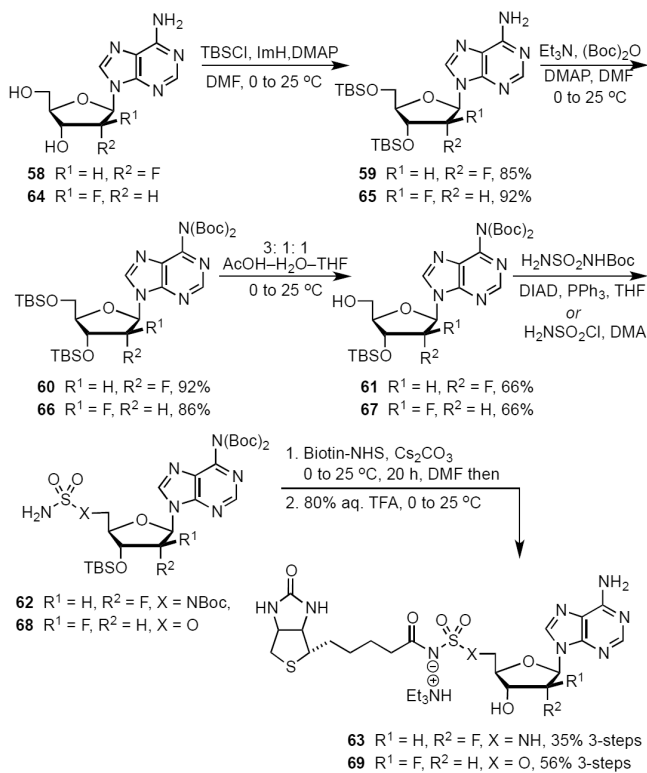
Scheme 1.
 Synthesis of Carbocyclic-Bio-AMS (**28**).



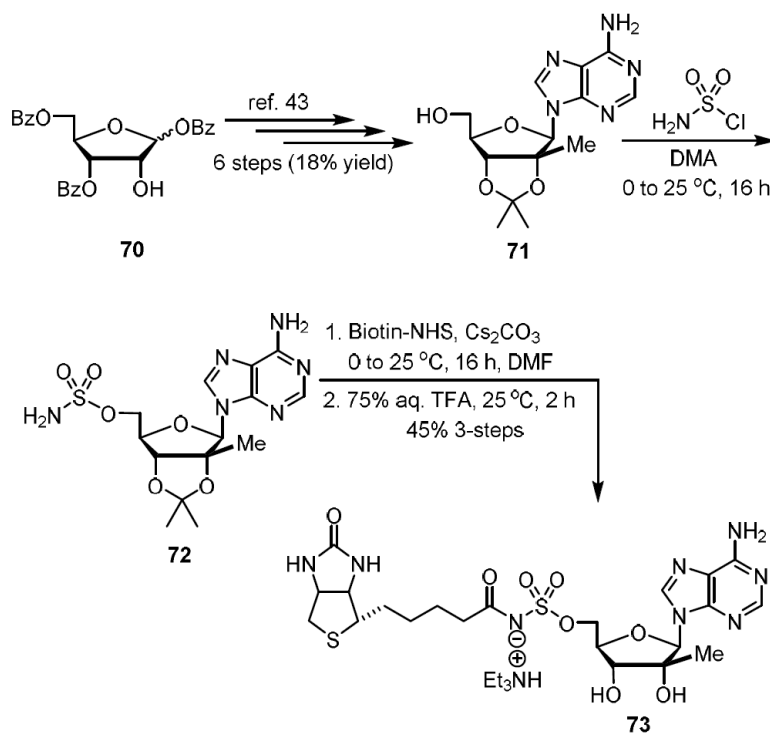
Scheme 2.
Synthesis of Cyclopentenyl Bio-AMS (**34**) and Cyclopentenyl Bio-AMS (**36**).

**Scheme 3.**

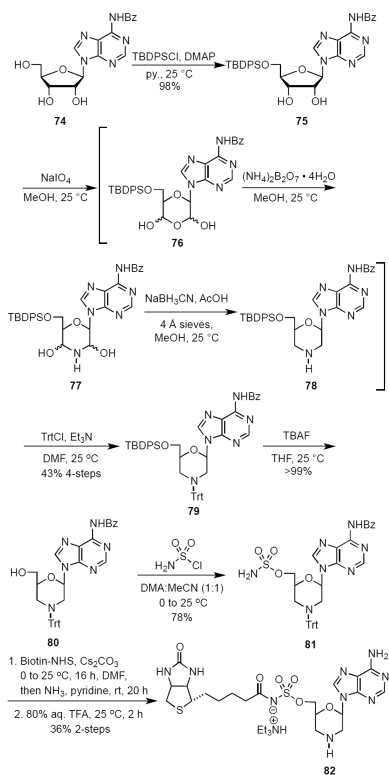
Synthesis of 2'- α -Azido-2'-deoxy-Bio-AMS (**46**), 2'- α -Amino-2'-deoxy-Bio-AMS (**47**) and 2'- β -azido-2'-deoxy-Bio-AMS (**57**).

**Scheme 4.**

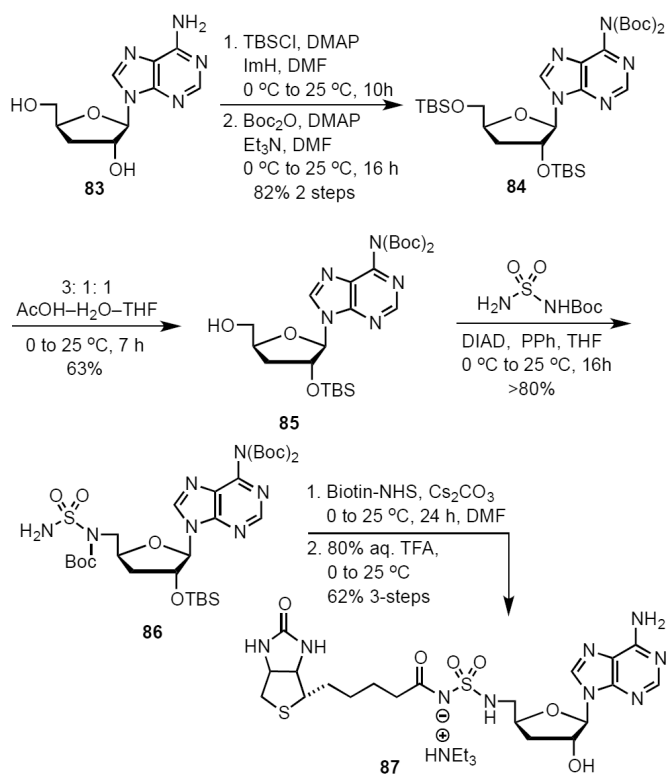
Synthesis of 2'-Deoxy-2'-α-fluoro-Bio-AMS (**63**) and C2'-deoxy-2'-β-fluoro-Bio-AMS (**69**).



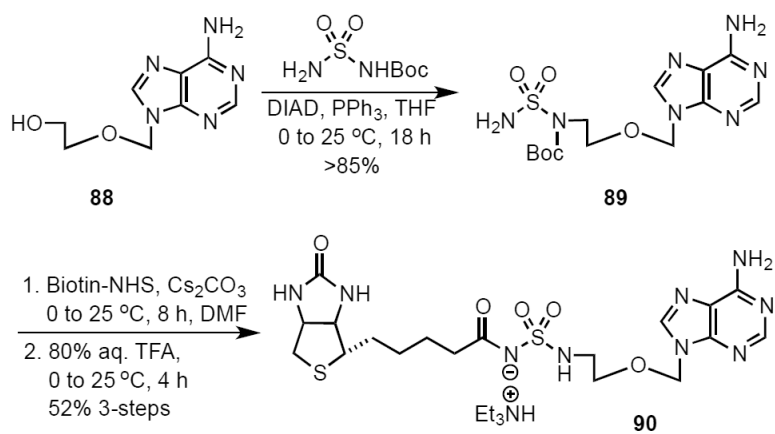
Scheme 5.
Synthesis of C-2'-β-methyl-Bio-AMS (**73**).



Scheme 6.
Synthesis of Morpholinyl-Bio-AMS (**82**).

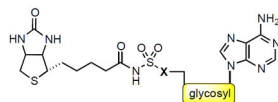


Scheme 7.
 Synthesis of 3'-Deoxy-Bio-AMS (**87**).

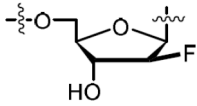
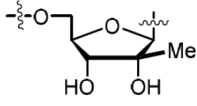
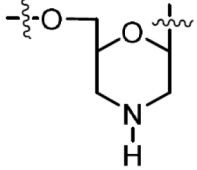
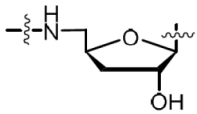
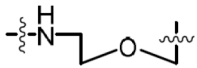


Scheme 8.
Synthesis of Acyclo-Bio-AMS (**90**).

Table 1

MIC₉₀ and thermodynamic data of biotinylated nucleosides.

| Compound | MIC ₉₀ , μM ^b | K _D , nM ^a | G, kcal/mol ^a | H, kcal/mol ^a | -T S, kcal/mol | T _m , °C | PDB |
|-----------------|-------------------------------------|----------------------------------|--------------------------|--------------------------|----------------|---------------------|-------------------|
| 14, Bio-AMS | 0.78 | 0.865 ± 0.040 | -12.4 ± 0.5 | -18.9 ± 0.1 | 6.6 ± 0.1 | 14.5 ± 1.0 | 3rux |
| 28 | >100 | 0.753 ± 0.002 | -12.4 ± 0.0 | -20.2 ± 0.2 | 7.8 ± 0.2 | 12.2 ± 1.0 | n.d. ^c |
| 34 | 3.12 | 2.14 ± 0.08 | -11.8 ± 0.2 | -22.1 ± 0.2 | 10.3 ± 0.2 | 12.5 ± 0.5 | n.d. ^c |
| 36 | 1.56 | 0.540 ± 0.007 | -12.6 ± 0.2 | -23.5 ± 0.4 | 10.7 ± 0.4 | 14.5 ± 1.0 | 4xtv |
| 46 | 3.12 | 0.933 ± 0.045 | -12.3 ± 0.6 | -23.2 ± 0.2 | 10.9 ± 0.3 | 13.0 ± 0.5 | 4xyw |
| 47 | 1.25 | 0.627 ± 0.016 | -12.6 ± 1.0 | -19.1 ± 0.1 | 6.5 ± 0.2 | 19.2 ± 0.6 | n.d. ^c |
| 57 | >100 | 0.027 ± 0.003 | -14.4 ± 0.2 | -29.3 ± 0.1 | 14.9 ± 0.1 | 17.0 ± 1.0 | 4xtx |
| 63 | 1.25 | 0.614 ± 0.018 | -12.6 ± 0.3 | -24.3 ± 0.5 | 11.8 ± 0.5 | 15.2 ± 0.8 | 4xty |

| Compound | MIC ₉₀ , μM ^b | K _D , nM ^a | G, kcal/mol ^a | H, kcal/mol ^a | -T S, kcal/mol | T _m , °C | PDB | |
|----------|---|----------------------------------|--------------------------|--------------------------|----------------|---------------------|------------|------|
| 69 |  | 50.0 | 0.153 ± 0.000 | -13.4 ± 0.0 | -28.4 ± 0.6 | 14.7 ± 0.5 | 17.7 ± 0.8 | 4xtz |
| 73 |  | 50.0 | 0.769 ± 0.006 | -12.4 ± 0.4 | -20.3 ± 0.4 | 8.1 ± 0.5 | 14.2 ± 0.3 | 4xu0 |
| 82 |  | 3.12 | 0.324 ± 0.023 | -12.9 ± 0.9 | -18.8 ± 0.0 | 5.9 ± 0.1 | 13.8 ± 0.8 | 4xu1 |
| 87 |  | 0.78 | 0.592 ± 0.009 | -12.6 ± 0.1 | -21.4 ± 0.5 | 8.8 ± 0.6 | 15.0 ± 1.0 | 4xu2 |
| 90 |  | 3.12 | 0.315 ± 0.003 | -13.0 ± 0.1 | -25.5 ± 0.7 | 12.6 ± 0.7 | 14.7 ± 0.3 | 4xu3 |

^a Competitive ITC experiments to determine K_D and G were performed at least in triplicate with 2 different enzyme batches while direct titration experiments were done in triplicate. All analogs showed n values of 1 ± 0.2.

^b Minimum inhibitory concentrations (MIC) that resulted in >90% growth inhibition of *M. tuberculosis* H37Rv were determined by a broth microdilution assay in GAS medium. Experiments were performed twice independently in triplicate. The MIC is defined as the lowest concentration of inhibitors that prevented growth, as determined by measuring the endpoint OD₆₀₀ values.

^c Not determined