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Development of small-molecule inhibitors of fatty acyl-AMP and fatty acyl-CoA ligases in *Mycobacterium tuberculosis*

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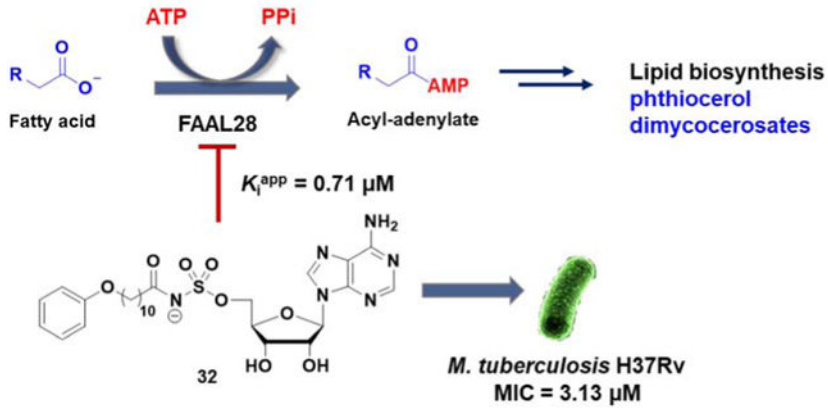
Abstract

Lipid metabolism in *Mycobacterium tuberculosis* (*Mtb*) relies on 34 fatty acid adenylating enzymes (FadDs) that can be grouped into two classes: fatty acyl-CoA ligases (FACLs) involved in lipid and cholesterol catabolism and long chain fatty acyl-AMP ligases (FAALs) involved in biosynthesis of the numerous essential and virulence-conferring lipids found in *Mtb*. The precise biochemical roles of many FACLs remain poorly characterized while the functionally nonredundant FAALs are much better understood. Here we describe the systematic investigation of 5′-*O*-[*N*-(alkanoyl)sulfamoyl]adenosine (alkanoyl adenosine monosulfamate, alkanoyl-AMS) analogs as potential multitarget FadD inhibitors for their antitubercular activity and biochemical selectivity towards representative FAAL and FACL enzymes. We identified several potent compounds including 12-azidododecanoyl-AMS **28**, 11-phenoxyundecanoyl-AMS **32**, and nonyloxyacetyl-AMS **36** with minimum inhibitory concentrations (MICs) against *M. tuberculosis* ranging from 0.098–3.13 μM. Compound **32** was notable for its impressive biochemical selectivity against FAAL28 (apparent K_i = 0.7 μM) versus FACL19 (K_i >100 μM), and uniform activity against a panel of multidrug and extensively drug-resistant TB strains with MICs ranging from 3.13–12.5 μM in minimal (GAST) and rich (7H9) media. The SAR analysis provided valuable insights for further optimization of **32** and also identified limitations to overcome.

GRAPHICAL ABSTRACT

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Keywords

Mycobacterium tuberculosis; fatty acyl-AMP ligases; FAAL28; fatty acyl-CoA ligases; FACL19; acyl-AMS analogs

1. Introduction

Tuberculosis (TB) has afflicted humans for thousands of years and is the leading cause of death from a single infectious agent.[1] One quarter of the world's population is infected asymptomatically with the pathogenic bacteria *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB.[1] According to a World Health Organization (WHO) estimate, approximately 10.4 million individuals fell ill with TB in 2018 resulting in 1.5 million deaths.[1] Chemotherapy for the simplest drug-susceptible TB requires a four-drug regimen comprised of isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months, followed by a continuation phase of isoniazid and rifampicin for another 4 months. Drug resistant TB (DR-TB) is considerably more challenging to treat and involves more complicated and longer drug regimens from 9–20 months with treatment success rates declining to 56% for multidrug resistant TB and 39% for extensively drug resistant TB.[2] To combat the growing crisis of DR-TB and achieve the WHO End TB Strategy, improved diagnostics, more effective vaccines and new drugs will be required to stop TB transmission.

Mycobacteria produce a tremendously diverse repertoire of lipophilic molecules. These molecules range from simple short chain fatty acids to the very complex mycolic acids.[3-5] Lipids in the cell envelope of *Mtb* include the essential mycolic acids as well as the virulence-conferring phthiocerol dimycocerosates (PDIMs), phenolic glycolipids (PGL), sulfolipids, and conditionally essential mycobactins (Fig. 1).[6-9] Lipid degradation is also critical and mycobacteria utilize host lipids (fatty acids and cholesterol) to fuel central metabolic pathways and as substrates for many of the aforementioned complex mycobacterial lipids. [10-13]

The *fadD* family of genes are involved in both lipid biosynthesis and catabolism. Highlighting the importance of lipid metabolism in mycobacteria the *Mtb* genome contains an astonishing 34 *fadD* genes, whereas *E. coli* encodes for a single *fadD* involved in fatty

acid degradation. Based on their function, the mycobacterial *fadD*'s are grouped into two subclasses: fatty acyl-CoA ligases (FACLs) involved in lipid degradation and fatty acyl-AMP ligases (FAALs) dedicated to lipid biosynthesis.[11-19] Gokhale has proposed to rename the FadDs by their functional classification, for example FadD19, a fatty acyl-CoA ligase, is renamed FACL19 and FadD28, a fatty acyl-AMP ligase, is renamed FAAL28.[17] Both FACLs and FAALs catalyze the ATP-dependent activation of fatty acid substrates to an intermediate acyl-adenylate, but transfer the acyl group onto different substrates: coenzyme A (CoA) for FACLs and polyketide synthases (PKS) for FAALs (Fig. 2).

The precise biochemical roles of the 20 FACLs are largely unknown. Four acyl-CoA synthetases FACL3, FACL17, FACL18 and FACL19 were shown to be up-regulated during growth of *Mtb* on cholesterol.[20, 21] Subsequently, FACL3, FACL17 and FACL19 have been biochemically validated as CoA ligases involved in cholesterol degradation. FACL3 is required in catabolism of the steroid rings C and D metabolism whereas FACL17 and FACL19 are involved in degradation of the C17 side chain of cholesterol.[20, 22-25] The sequence of FACL18 is nearly identical to FACL19 and Sampson has hypothesized FACL18 may have arisen from gene duplication.[23] FACL13 is part of the *mymA* operon in remodeling the cell envelope of intracellular *Mtb* under acidic conditions and exhibits a distinct preference for C24 and C26 fatty acids.[26] Transposon mutagenesis studies suggest most FACLs are not essential, which may be due to their functional redundancy.[27] Indeed, FACL6, FACL15, and FACL19 were shown to possess broad substrate specificity.[14]

By contrast, the FAAL class of FadDs appears to be functionally nonredundant and serve to link fatty acid and polyketide synthesis in mycobacteria.[13, 16] FadD10, involved in the synthesis of a virulence-related lipopeptide, was misannotated as a FACL; however, it is in fact a FAAL that transfers fatty acids to an acyl carrier protein (Rv0100).[28] FAAL21 is the fatty acyl AMP ligase that provides the activated fatty acyl starter unit to Pks3/4.[29] FAAL22 is essential for synthesis of the phenolic glycolipids and is responsible for the activation and transfer of 4-hydroxybenzoic acid onto PKS15/1.[30-32] FAAL23 was found to be involved in sulfolipid production.[33] There are three acyl-AMP ligases: FAAL26, FAAL28 and FAAL29 that are required for the biosynthesis of PDIM, a major virulence lipid in the cell wall.[34-38] FAAL26 initiates phthiocerol synthesis by loading the polyketide synthase PpsA with long-chained fatty acids while FAAL28 initiates mycocerosic acid synthesis by loading the PKS protein mycocerosic acid synthase.[7, 17, 31, 39-43] Finally, FAAL32 is required for activation of the long meromycolic chain and is essential for mycobacterial growth. [44-47]

The identification of specific inhibitors as tool compounds against each class of FadDs (FACL or FAAL) or selective inhibitors of an individual FadD enzyme can help to decipher the functional role of FadDs in lipid metabolism. Inhibitors that target crucial nodes or simultaneously disrupt the lipid metabolic network through multitarget inhibition could lead to the development of new class of antitubercular agents.[11, 12] Since FadDs are a newly discovered family of adenylate-forming enzymes in *Mtb* there are few reported inhibitors.[48] Among them, 5′-*O*-[*N*-(dodecanoyl)sulfamoyl]adenosine **9** (Fig. 3) was shown to inhibit FAAL28 and FACL19 with apparent K_i values of 1.5 μM and 4.9 μM respectively and possessed very modest antitubercular activity with a minimum inhibitory concentration

(MIC) of only 100 μM against *Mtb*.[17] The corresponding dodecylphosphate-AMP analogue **10** (Fig. 3) inhibited FAAL32 with an apparent K_i of 0.11 μM and was also a weak inhibitor of growth of *M. smegmatis* with an MIC of 20 μM.[45] Niu et al. hypothesized that non-hydrolyzable analogues of steroid metabolites (e.g., cholestenoic acyl-AMP or choloyl-AMP) could act as a class-specific inhibitor of the acyl-CoA synthetases responsible for steroid side chain degradation. [49] They designed 5′-*O*-[*N*-lithocholoyl)sulfamoyl]adenosine (LCA-AMS) **11** (Fig. 3) which exhibited highly selective inhibition toward mycobacteria. LCA-AMS **11** inhibited *M. smegmatis* FadD17 and FadD1 with apparent K_i values at 23 and 67 nM, respectively, but again displayed weak growth inhibition with an MIC of 50 μM against *Mtb.* The only non-substrate inhibitor of an FadD was described by Hung and co-workers, who discovered a coumarin analog as a FadD32 inhibitor from phenotypic high-throughput screening and identified the target through whole-genome sequencing.[50-52] The optimized inhibitor CCA34 **12** (Fig. 3) did not block the adenylation activity of FadD32, but rather lipid transfer onto Pks13 with an IC_{50} of ~5 μM.[52]

Herein we describe our efforts to prepare nucleoside inhibitors of FAAL enzymes using decanoyl-AMS **9** as a template. All compounds were initially evaluated against representative FACL and FAAL enzymes as well as for their whole-cell activity against *Mtb* H37Rv in minimal and rich media. We examined the importance of the acyl chain length, introduced conformational constraints into the acyl chain, and explored substituents in the vicinal and distal ends of the acyl side chain of **9**. These efforts led to the identification of 11-phenoxyundecanoyl-AMS **32** with significantly improved anti-mycobacterial activity and selectivity (Fig. 4). We then performed two independent SAR campaigns of **32** to investigate both the placement and flexibility of the terminal phenoxy group as well as the importance of acyl-sulfamate linker. Finally, the most promising analogs were evaluated against a panel of MDR-TB and XDR-TB strains.

2. Results and discussion

2.1. Chemistry

All inhibitors were synthesized following the general approach described in the Scheme 1. Fatty acids were converted to the corresponding *N*-hydroxysuccinimide ester (**9c**, **13c-56c**) and then coupled with 2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine **57** in the presence of cesium carbonate to afford protected acyl-sulfamoyladenosine intermediates **9d** and **13d–56d**. Deprotection of the isopropylidene group with aqueous TFA afforded the desired bisubstrate inhibitors **9d** and **13d–56d** in low to moderate yields in greater than 95% purities (Scheme 1).

Catalytic hydrogenation of the 12-azido analogue **28** afforded its 12-amino counterpart **29** in good yield while saponification of 12-methoxy carbonyl derivative **30** provided the corresponding acid **31**. Attempts to prepare the (*Z*)-2-dodecanoyl-AMS analogue **23** revealed that the α,β-unsaturated carbonyl moiety of **23d** readily isomerizes to the more stable *trans*-3-dodecanoyl-AMS intermediate providing **23d** as a 2:1 *E*:*Z* mixture. Moreover, attempts to synthesize pure (*E*)-2-dodecenoyl-**23d** from the corresponding *N*-

hydroxysuccinimide ester of *cis*-2-dodecenoic, provided the same 2:1 *E*:*Z* mixture of products (not shown), which was inseparable by flash chromatography conditions suggesting the 2:1 *E*:*Z* mixture represents the equilibrium ratio of products under the basic reaction conditions.

Fatty acids that were not available commercially were synthesized as described in Schemes 2-6. (*Z*)-2-Dodecanoic acid **23a** was prepared using the improved method of Rappe by bromination of 2-dodecanone **58** to yield 1,3-dibromoketone **59**, which was converted to **23a** in good yield by Favorskii rearrangement mediated by sodium hydroxide (Scheme 2A).[53] The constitutional isomer (*E*)-5-dodecanoic acid **24a** was synthesized from 1-octyne **60** by hydrozirconation with Schwarz's reagent and Negishi coupling of the corresponding vinylzirconium intermediate with ethyl 4-bromobutyrate employing $Pd_2(dba)_3$. Saponification of the ethyl ester **61** provided (*E*)-5-dodecanoic acid (Scheme 2B).[54] (*Z*)-5-Undecanoic acid **25a** was synthesized in one step by Wittig reaction between hexanal **62** and (4-carboxybutyl)triphenylphosphonium bromide **63** in the presence of sodium bis(trimethylsilyl)amide (Scheme 2C).[55] (1*S*,2*R*)-2-Nonyl-cyclopropanecarboxylic acid **26a** was synthesized from 2-dodecen-1-ol **64** using the method of Charette for the enantioselective cyclopropanation of allylic alcohols.[56] The resulting alcohol **65** was then oxidized by Jones reagent to the desired carboxylic acid **26a** (Scheme 2D). The (*Z*)-2-dodecenoic acid **23a** is not stable at room temperature and readily converts to the more stable *E* isomer.

12-Azidododecanoic acid **28a** was prepared from 12-bromododecanoic acid **66** by nucleophilic substitution with sodium azide (Scheme 3A).[57] 12-Methoxy-12-oxododecanoic acid **30a** was synthesized by Baeyer-Villiger oxidation of cyclododecanone **67** with potassium peroxydisulfate to afford 12-hydroxy methyl ester **68** followed by Jones oxidation (Scheme 3B).[58, 59]

The enantiopure 2-methyldodecanoic acids **33a** and **34a** (Scheme 4A-B) were prepared by Evan's asymmetric alkylation methodology.[60] The requisite *N*-acyloxazolidinones **69** and **71** were prepared by lithiation of (*S*) and (*R*)-4-benzyloxazolidin-2-one and subsequent reaction with the mixed pivalic dodecanoic anhydride. Methylation of the *Z*-enolates of **69** and **71** derived by deprotonation with sodium bis(trimethylsilyl)amide furnished **70** and **72**, respectively. The chiral auxiliary was removed with lithium hydroperoxide to afford the desired (*R*) and (*S*)-2-methyldodecanoic acids **33a** and **34a**.[61, 62] Two cycles of methylation of the lithiated dodecanoyl enolate of **9b** afforded the desired 2,2-dimethyl ester **73**, which was then saponified with lithium hydroxide to provide the desired 2,2-dimethyldodecanoic acid **35a** (Scheme 4C).[63]

2-(Nonyloxy)acetic acid **36a** and 2-[2-(2-butoxyethoxy)ethoxy]acetic acid **37a** were synthesized in moderate yields by the coupling of nonanol **75** and 2-(2-butoxyethoxy)ethanol **76**, respectively with chloroacetic acid **74** in the presence of NaH (Scheme 5A-B).[64]

Fatty acyl methyl esters **38b**, **39b**, and **42b–56b** were prepared from 10, 12, or 11-hydroxy-undecanoic acid methyl ester **77**, **68**, or **27b** respectively under Mitsunobu coupling

conditions with the appropriate phenol (Scheme 6A). Mesylation of 11-hydroxyundecanoic acid methyl ester **27b** followed by coupling with thiophenol in the presence of sodium hydride provided methyl 11-(phenylthio)undecanoate **40b** (Scheme 6B). 11-(Phenylamino)undecanoic acid **41a** was synthesized from 11-aminoundecanoic acid **79** via coupling with iodobenzene **80** in the presence of copper iodide and potassium phosphate to afford the desired acid in good yield (Scheme 6C).

Previous work in our group with other adenylating enzyme inhibitors showed that the acylsulfamide is also an excellent bioisostere of the acyl-adenylate and is more stable.[65] Consequently, we prepared acylsulfamide **83** by coupling 11-phenoxyundecanoate *N*-hydroxysuccinimide **32c** with 2′,3′-*O*-isopropylidene-5′-*N*-sulfamoylamideadenosine **81** in the presence of cesium carbonate followed by the removal of the isopropylidene group with aqueous TFA (Scheme 7).

Alkyl sulfamate **89** was created to explore the importance of the carbonyl group for activity. Reduction of 11-phenoxyundecanoic acid **32a** via $LiAlH_4$ provided the alcohol **84**, which was subsequently tosylated to supply ether **86**. Alkylation of N^6,N^6-bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine **87** with ether **86** employing Cs_2CO_3 gave sulfamate **88**, which was deprotected with aqueous TFA to yield the desired alkyl sulfamate **89** (Scheme 8).

We also prepared the reverse alkyl sulfamate by first tosylating N^6,N^6-bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine **90** to provide compound **91**. Alkylation of **91** with sulfamate **85** utilizing Cs_2CO_3 followed by deprotection with aqueous TFA afforded the desired compound **93** (Scheme 9).

2.2. Antimicrobial Activity

Acyl-AMS analogues **9**, **13–56**, **83**, **89** and **93** were evaluated for their whole-cell activity against *Mycobacterium tuberculosis* H37Rv in GAST and 7H9 media. GAST medium is a minimal medium where the sole carbon source is glycerol. We also evaluated compounds in 7H9 medium containing glycerol and glucose as the carbon sources supplemented with and without palmitate. The minimum inhibitory concentration (MIC) defined here is the concentration that results >99% inhibition of cell growth. The MICs for the acyl-AMS analogues exhibit a high media dependence with the best activity observed in GAST medium and poor activity in 7H9 medium either with and without palmitate (Table 1). Hence in the discussion of SAR trends below, the relative microbiological activity refers to the activity in GAST medium unless otherwise noted. The attenuated activity in 7H9 medium may be due to the high protein binding of these acidic lipophilic molecules since 7H9 contains bovine serum albumin. Alternatively, nutrient-rich media 7H9 contain lipids and other fatty acids that can potentially rescue activity of some FadD's.

Compounds **9** and **13–20** consisting of lipid chains ranging from C4 to C20 were evaluated initially to determine the optimum chain length. The activity followed a parabolic relationship with activity monotonically increasing with length of the lipid chain from C4 to C12, peaking at C12, then decreasing from C14 to C20. Among them, the three analogs bearing C_{10}-, C_{12}- and C_{14}-side alkyl chains, corresponded to the highest potency in the

series, having a MIC in the range 0.19–1.56 μM in GAST medium. Altering the lipid chain by shortening to C8 or lengthening to C16 displayed 64– and 16–fold lower activity respectively when compared to C12 compound. Analogs containing the shortest C4 chain (**13**) and the longest C20 lipid (**20**) were completely inactive.

Based on the impressive whole cell activity of dodecanoyl-AMS **9**, we conducted a systematic SAR analysis to further refine the acyl chain. Introduction of unsaturation with a terminal alkyne (**21**) or terminal alkene (**22**) was well tolerated resulting in a modest 2–4-fold loss in activity. However, incorporation of *trans*-unsaturation or *trans*-cyclopropanes within the chain in *trans*-Δ^2-**23**, *trans*-Δ^5-**24** and *trans*-2-cyclopropyl **26** almost completely abolished activity with MICs increasing to 50–100 μM. By contrast, *cis*-Δ^5-**25** retained considerable activity with an MIC of 6.25 μM. Introduction of alcohol, amino, methyl ester, and phenoxy functional groups on the terminus of the lipid chain (**27**, **29**, **30** and **32**) led to 8–16-fold reduction in potency relative to **9**, but these analogs still retained appreciable activity with MICs of 3.13–6.25 μM demonstrating some flexibility at this position. The lipophilic terminal azide **28** was equipotent while the polar carboxylic acid was 128-fold less active. Taken together, these results suggest only neutral or positively charged groups are permitted at the lipid terminus. Introduction of *R* or *S*-configured α-methyl substituents in **33** and **34** or an α-gem-dimethyl in **35** resulted in a greater than 64–fold loss in activity indicating steric bulk is poorly tolerated at this position. Lastly, introduction of oxygen atoms in the lipid chain was explored with mono-ether **36** and tri-ether **37**. Amazingly, mono-ether showed a 2–fold increase in activity (MIC = 0.098–0.19 μM), but this gain in activity was lost by incorporation of additional oxygen atoms in the lipid chain.

Although not the most potent in terms of MIC, we discovered that 11-phenoxyundecanoyl-AMS **32** exhibited biochemical selectivity for FAAL28 over FACL19 (vide infra) and thus elected to perform additional SAR of this new lead molecule with the goal to further improve antimicrobial activity while retaining selectivity for the acyl-AMP ligase FAAL28. We first evaluated homologs **38–39** one carbon shorter and longer, respectively (Table 2) and observed the parent compound **32** possessed optimal activity. Replacement of the ether oxygen atom for a sulfur atom obliterated activity. However, exchanging the oxygen for nitrogen led to a modest 4-fold loss in activity, a result consistent with 12-amino derivative **29** affirming the tolerance for a positively charged group at the lipid terminus. We next explored modification of the aryl ring with a range of electron donating and withdrawing substituents at the *ortho*-, *meta*-, and *para*-positions (**42–54**) as well as two ring-fused analogs (**55–56**). In general, the SAR was remarkably flat with MICs ranging from 12.5–50 μM for most active compounds, but some trends were observed. In general, *para*-substituted analogs were more potent (MIC = 6.25–50 μM), *meta*-substituted analogs were less active (MIC = 25–50 μM) and *ortho*-substituted analogs were inactive (MIC = > 50 μM). Among the *para*-substituted analogs potency was found to increase as the size of the atom/functional group decreased (MIC trend: F = Cl < CF_3 < Br = fused benzo = OMe).

Substitution of the sulfamate 5′-oxygen in **32** provided acyl sulfamide **83**, which has comparable antimicrobial activity with a MIC of 1.56–3.13 μM (Table 3). Removal of the carbonyl group to yield alkyl sulfamate **89** caused a greater than 16–fold loss in activity

revealing the importance of the carbonyl group. Finally, the reverse alkyl sulfamate **93** was also inactive displaying no inhibition of growth up to 50 μM.

Acyl-AMS derivatives displaying the most potent antimicrobial activity against *M. tuberculosis* H37Rv were tested against a panel of multidrug resistant (MDR) and extensively drug resistant (XDR) TB strains in GAST and 7H9 medium (Table 4).[66] These drug-resistant pathogens displayed less differential activity in media than the reference drug susceptible strain. As a result, most of the selected analogues were more active against the MDR-TB and XDR-TB strains in 7H9 medium compared to the reference H37Rv strain, but less active in GAST medium relative to the reference H37Rv strain. For example, compounds **9** and **17** containing C12 and C14 saturated fatty acyl chains displayed MICs in 7H9 medium ranging from 3.13–12.5 μM against this MDR and XDR *Mtb* panel, whereas their activity against *M. tuberculosis* H37Rv in 7H9 medium was only 50 μM. By contrast, the activity of **9** and **17** against the MDR/XDR strains in GAST medium was diminished (MICs 3.13–25 μM) compared to their activity against *M. tuberculosis* H37Rv (MIC: 0.19–0.78 μM). The lead 11-phenoxyundecanoyl-AMS **23** was notable for its fairly uniform activity against the MDR-TB and XDR-TB panel in both media with MICs of 3.13–12.5 μM for 8 of the 9 tested conditions.

2.2. Enzyme Inhibition

All compounds were tested for their inhibitory activity against two FadD enzymes: fatty acyl CoA ligase (FACL19) and fatty AMP ligase (FAAL28). These enzymes were selected as representative members of the FACL and FAAL class of enzymes since they have been structurally and biochemically well characterized and can be readily overexpressed and purified from *E. coli*.[17, 67] Shorter chain (C4 to C10) analogues **13–16** were essentially inactive against both enzymes. However, once the alkyl chain length reached C12 in dodecanoyl-AMS **9**, enzyme inhibition was observed with an apparent K_i of 67 μM for FACL19 and 6 μM for FAAL28 (Table 1). Potency monotonically increased as the alkyl chain increased reaching a K_i of 2.4 μM for FACL19 and 0.48 μM for FAAL28 with steroyl-AMS **19**. These results are consistent with previous observations demonstrating FACL19 and FAAL28 favor medium to long chain fatty acids.[17]

Introduction of unsaturation within the alkyl chain at the terminal, internal or vicinal position in analogs **21–25** ablated all biochemical inhibition of FACL19 and FAAL28. The *trans*-cyclopropane derivative **26** displayed weak inhibition of FAAL28 indicating some tolerance to conformational constraints within the alkyl chain of these acyl-AMS inhibitors. Substitution on the terminus of the lipid chain with hydroxy, amino, azido, ester and carboxylic acid functional groups in **27–31** was poorly tolerated. However, 11-phenoxyundecanoyl-AMS **32** displayed potent inhibition of FAAL28 with an apparent K_i of 0.7 μM, but was completely inactive against FACL19 demonstrating the highest level of biochemical selectivity observed for any compound evaluated. Introduction of methyl groups at the α-carbon in **33–35** and oxygen atoms within the alky chain in **36–37** obliterated activity.

Based on the promising biochemical selectivity and potent antitubercular activity of 11-phenoxyundecanoyl-AMS **32**, a series of related analogs was synthesized and evaluated. Decreasing the alkyl chain of **32** by one carbon in **38** led to a 10-fold decrease in potency for FAAL28 while increasing the chain length by one carbon resulted in a modest 4-fold decrease in potency for FAAL28. These biochemical results with FAAL28 were closely mirrored in the microbiological activity of **38** and **39**, which were 16-fold and 4-fold less active than **32** against *M. tuberculosis* H37Rv (Table 2). Both compounds maintained excellent biochemical selectivity with no inhibition of FACL19. Exchanging the terminal oxygen atom of **32** for a sulfur atom or nitrogen atom resulted in a 10–fold decrease in activity for FAAL28 and loss of biochemical selectivity as both analogs exhibited modest inhibition of FACL19. Among the phenyl substituted analogs **42–56**, *ortho*-substituted analogues tended to be more selective for FAAL28 in this series of compounds; however, biochemical selectivity for most compounds was severely degraded relative to **32** as the majority of analogs also showed good-to-moderate inhibition of FACL19 (Table 2).

The impact of the acyl sulfamate moiety of **32** on biochemical inhibition was examined with acyl sulfamide **83** and alkyl sulfamates **89** and **93** (Table 3). Acyl sulfamide **83** maintained biochemical selectivity with no inhibition of FACL19 and a modest 4-fold diminished inhibition of FAAL28 compared to **32**. Removal of the carbonyl in alkyl sulfamate **89** and reverse alkyl sulfamate **93** derivatives ablated biochemical inhibition of FAAL28.

2.3. Cytotoxicity

The cytotoxicity of compounds **9** and **13**–**37** was evaluated against Vero cells (ATCC) using the MTT assay with DMSO as a positive control (Table 1). Every compound except **31** containing a terminal carboxylic acid in the lipid tail was cytotoxic with IC_{50} values for inhibition of 50% cell viability between 0.07–81 μM. Unlike the antitubercular activity, which exhibited a parabolic relationship peaking at C12, the cytotoxicity monotonically increases with chain length from 81 μM with **13** containing a C4 lipid to 0.07 μM for **20** containing a C20 lipid. The SAR of the remaining analogs **21–26**, **28**, **30**, and **34–37** containing unsaturation, conformational constraints, α-methyl groups, and ether functional groups was relatively flat with IC_{50}'s between 5-17 μM. Compounds with polar and hydrogen-bond donating functional groups including alcohol **27** and amino **29** were slightly less cytotoxic with IC_{50} of 27 and 42 μM, respectively. Ether **36** exhibited moderate cytotoxicity (IC_{50} = 8.7 μM), but had the highest therapeutic index [MIC/IC_{50} = 46–92] of all analogues due to its exceptional potency (MIC = 0.098–0.19 μM). The lead compound 11-phenoxyundecanoyl-AMS **32** possesses notable cytotoxicity with an IC_{50} of 2 μM, a result consistent with the hydrophobic trend observed with the C4–C20 lipid analogues, resulting in a therapeutic index of less than one.

3. Conclusions

The goal of this study was to define the structure-activity relationships of 5′-*O*-[*N*-(alkanoyl)sulfamoyl]adenosine (alkanoyl adenosine monosulfamate, alkanoyl-AMS) inhibitors that govern antitubercular activity. A secondary goal was to identify a chemical probe selective for either FAAL or FACL enzymes using FAAL28 and FACL19 as proxies

for each class, recognizing that such an analysis is intrinsically limited since these two enzymes cannot capture the substrate specificities of all 12 FAALs and 24 FACLs.[16] This was accomplished through the synthesis of a systematic series of 48 compounds. We initially explored the impact of the acyl chain length activity with a series of even-chained analogs from C4 to C20 and observed a parabolic relationship between chain length and antitubercular activity where antitubercular activity was optimal at a chain length of C12-C14. Our second round of SAR focused on the tolerance of the lipid chain of dodecanoyl-AMS **9** to modification through introduction of alkene and cyclopropane conformation constraints at terminal and internal positions throughout the lipid chain. We also explored the impact of a range of polar and nonpolar functional groups at the lipid terminus, the effect of sterics at the α-carbon, and the ability to tolerate oxygen atoms in the lipid chain. These efforts led to the identification of several promising analogs including 12-azidodecanoyl-AMS **28**, 12-aminodecanoyl-AMS **29**, 11-phenoxyundecanoyl-AMS **32**, and nonyloxyacetyl-AMS **36** whose MICs in GAST medium are 0.19, 3.13, 3.13, and 0.19 μM, respectively. Concurrent biochemical evaluation against FACL19 and FAAL28 revealed 11-phenoxyundecanoyl-AMS **32** exhibited greater than 142-fold selectivity for FAAL28 over FACL19 providing the first compound with high biochemical selectivity. We had initially hypothesized that analogs selective for the FAAL class of enzymes would allow the separation of antitubercular activity from cytotoxicity since FAAL enzymes are functionally unique to mycobacteria whereas FACL enzymes are ubiquitous in mammals.[17] However, compound **32** retained appreciable cytotoxicity suggesting the alkanoyl-AMS scaffold is intrinsically cytotoxic. Indeed, all of the synthesized compounds **13–37** with the exception of the double ionized 11-carboxyundecanoyl-AMS **31** displayed some cytotoxicity. Our third series of compounds studied the SAR of the terminal phenoxy group of 11-phenoxyundecanoyl-AMS **32** on antitubercular activity and biochemical selectivity. Substitution on the terminal phenoxy group in all cases reduced antitubercular activity and lowered FAAL28 potency as well as decreased biochemical selectivity (K_i^{FAAL28}/K_i^{FAC19}). Finally, we investigated the role of the acylsulfamate linkage of **32** demonstrating the carbonyl moiety is absolutely critical for activity, but the 5′-oxygen atom can be replaced with a NH moiety, results consistent with previous studies of acyl-adenylate inhibitors.[68, 69]

4. Experimental section

4.1. Chemistry

All commercial reagents (Sigma-Aldrich, Acros, Fisher) were used as provided. Sulfamoyl chloride was prepared by the method of Heacock except that it was used directly without recrystallization.[70] 2′,3′-*O*-Isopropylidene-5′-*O*-sulfamoyladenosine **57** and **5’-deoxy-2’,3’-*O*-isopropylidene-5’-*N*-(sulfamoyl)aminoadenosine 81** were prepared as previously described.[69] N^6,N^6-Bis(*tert*-butoxycarbonyl)-5’-*O*-sulfamoyl-2’,3’-*O*-isopropylideneadenosine **87** and N^6,N^6-bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine **90** were prepared by the method of Ikeuchi et al.[68] An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying tetrahydrofuran (THF), CH_2Cl_2, and *N*,*N*-dimethylformamide (DMF), and the solvents were dispensed under Argon. Anhydrous grade 1,2-dimethoxyethane (DME),

methanol (MeOH), and acetonitrile (MeCN) were purchased from Sigma-Aldrich and used as provided. All reactions were performed under an inert atmosphere of dry Argon in oven-dried glassware (150 °C). Flash chromatography was performed with an ISCO Combiflash Companion® purification system with prepacked silica gel cartridges with the indicated solvent system. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual dimethyl sulfoxide (2.50 ppm), methanol (3.31 ppm) or chloroform (7.21 ppm) and carbon chemical shifts are reported using an internal standard of residual dimethyl sulfoxide (39.52 ppm) or methanol (49.19 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad), coupling constant, integration. Low and high resolution mass spectra were acquired on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. The purity of the final compounds was greater than 95%.

4.1.1. General Procedure for Synthesis of NHS Esters (Method A)—To a solution of the appropriate fatty acid **9b**, **13b–37b** (1.0 equiv) in CH_2Cl_2 (0.1 M) at 0°C was added *N*-hydroxysuccinimide (NHS, 1.0 equiv) and *N,N′*-dicyclohexylcarbodiimide (1.0 equiv). The reaction mixture was warmed to 23 °C and stirred 16 h. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure to provide NHS esters **9c**, **13c–37c** without further purification.

4.1.2. General Procedure for Synthesis of NHS Esters (Method B)—To the appropriate fatty acid methyl ester **38b–56b** (1.0 equiv) was added NaOH/MeOH (aqueous 1N NaOH, 1:1). The resulting solution was refluxed at 100 °C for 2 h. Next, the mixture was acidified with aqueous 1N HCl until pH < 2 and then extracted with EtOAc (5×10 mL). The combined extracts were washed with aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated. To the resulting crude mixture was added NHS (1.1 equiv), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1.1 equiv) and CH_2Cl_2 (8 mL). The reaction mixture was allowed to stir for 6 h and then quenched with aqueous 1N HCl (5 mL). The aqueous solution was extracted with CH_2Cl_2 (3×15 mL). The combined extracts were washed with aqueous NaCl (20 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (4:1 Hexanes/EtOAc) afforded **38c–56c** as white solids.

4.1.2.1. *N*-Hydroxysuccinimidyl 10-phenoxydecanoate (38c).: The title compound was prepared from **38b** using the general procedure for the synthesis of NHS esters, method B (90%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.37 (m, 6H), 1.40–1.46 (m, 4H), 1.72–1.79 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.83 (br s, 4H), 3.95 (t, *J* = 7.2 Hz, 2H), 6.89–6.90 (m, 2H), 6.91–6.94 (m, 1H), 7.26–7.28 (m, 2H); HRMS (ESI+) calcd for $C_{20}H_{28}NO_5$ $[M+H]^+$ 362.1962, found 362.1950 (error 3.3 ppm).

4.1.2.2. *N*-Hydroxysuccinimdyl 12-phenoxydodecanoate (39c).: The title compound was prepared from **39b** using the general procedure for the synthesis of NHS esters, method B (89%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30–1.36 (m, 10H), 1.39–1.46 (m, 4H), 1.73–1.79 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.81 (br s, 4H), 3.95 (t, *J* = 7.2 Hz, 2H), 6.89–6.93 (m,

3H), 7.26–7.28 (m, 2H); HRMS (ESI+) calcd for $C_{22}H_{32}NO_5$ $[M+H]^+$ 390.2275, found 390.2257 (error 4.6 ppm).

4.1.2.3. *N*-Hydroxysuccinimdyl 11-(phenylthio)undecanoate (40c).: The title compound was prepared from **40b** using the general procedure for the synthesis of NHS esters, method B (78%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.20–1.23 (m, 8H), 1.32–1.38 (m, 4H), 1.57–1.62 (m, 2H), 1.66–1.71 (m, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.76 (br s, 4H), 2.86 (t, *J* = 7.2 Hz, 2H), 7.09–7.12 (m, 1H), 7.21–7.23 (m, 2H), 7.26–7.27 (m, 2H); HRMS (ESI+) calcd for $C_{21}H_{30}NO_4S$ $[M+H]^+$ 392.1890, found 392.1893 (error 0.8 ppm).

4.1.2.4. *N*-Hydroxysuccinimdyl 11-(phenylamino)undecanoate (41c).: The title compound was prepared from **41a** using the general procedure for the synthesis of NHS esters, method B (74%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.26–1.31 (m, 8H), 1.37–1.42 (m, 4H), 1.59–1.64 (m, 2H), 1.72–1.77 (m, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.82 (br s, 4H), 3.10 (t, *J* = 7.2 Hz, 2H), 6.59–6.60 (m, 2H), 6.66–6.69 (m, 1H), 7.15–7.18 (m, 2H); HRMS (ESI+) calcd for $C_{21}H_{31}N_2O_4$ $[M+H]^+$ 375.2278, found 375.2267 (error 2.9 ppm).

4.1.2.5. *N*-Hydroxysuccinimdyl 11-(4-fluorophenoxy)undecanoate (42c).: The title compound was prepared from **42b** using the general procedure for the synthesis of NHS esters, method B (73%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30–1.36 (m, 8H), 1.37–1.46 (m, 4H), 1.71–1.77 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.82 (br s, 4H), 3.89 (t, *J* = 7.2 Hz, 2H), 6.80–6.82 (m, 2H), 6.93–6.96 (m, 2H); HRMS (ESI+) calcd for $C_{21}H_{29}FNO_5$ $[M+H]^+$ 394.2024, found 394.2009 (error 3.8 ppm).

4.1.2.6. *N*-Hydroxysuccinimdyl 11-(3-fluorophenoxy)undecanoate (43c).: The title compound was prepared from **43b** using the general procedure for the synthesis of NHS esters, method B (82%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.33 (m, 8H), 1.39–1.45 (m, 4H), 1.71–1.78 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.81–2.82 (br s, 4H), 3.92 (t, *J* = 7.2 Hz, 2H), 6.58–6.64 (m, 2H), 6.66–6.67 (m, 1H), 7.17–7.21 (m, 1H); MS HRMS (ESI+) calcd for $C_{21}H_{29}FNO_5$ $[M+H]^+$ 394.2024, found 394.2003 (error 5.3 ppm).

4.1.2.7. *N*-Hydroxysuccinimdyl 11-(2-fluorophenoxy)undecanoate (44c).: The title compound was prepared from **44b** using the general procedure for the synthesis of NHS esters, method B (76%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.35 (m, 8H), 1.37–1.41 (m, 2H), 1.43–1.48 (m, 2H), 1.71–1.76 (m, 2H), 1.78–1.83 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.81–2.82 (br s, 4H), 4.01 (t, *J* = 7.2 Hz, 2H), 6.85–6.88 (m, 1H), 6.94–6.97 (m, 1H), 7.02–7.26 (m, 2H); HRMS (ESI+) calcd for $C_{21}H_{29}FNO_5$ $[M+H]^+$ 394.2024, found 394.2010 (error 3.6 ppm).

4.1.2.8. *N*-Hydroxysuccinimdyl 11-(4-chlorophenoxy)undecanoate (45c).: The title compound was prepared from **45b** using the general procedure for the synthesis of NHS esters, method B (74%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30–1.33 (m, 8H), 1.38–1.44 (m, 4H), 1.71–1.78 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.82–2.83 (br s, 4H), 3.90 (t, *J* = 7.2 Hz, 2H), 6.80–6.82 (m, 2H), 7.20–7.22 (m, 2H); HRMS (ESI+) calcd for $C_{21}H_{29}C1NO_5$ $[M+H]^+$ 410.1729, found 410.1710 (error 4.6 ppm).

4.1.2.9. *N*-Hydroxysuccinimdyl 11-(3-chlorophenoxy)undecanoate (46c).: The title compound was prepared from **46b** using the general procedure for the synthesis of NHS esters, method B (70%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.39 (m, 8H), 1.39–1.45 (m, 4H), 1.72–1.78 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.82–2.83 (br s, 4H), 3.92 (t, *J* = 7.2 Hz, 2H), 6.76–6.78 (m, 1H), 6.88–6.91 (m, 2H), 7.16–7.19 (m, 1H); HRMS (ESI+) calcd for $C_{21}H_{29}C1NO_5$ $[M+H]^+$ 410.1729, found 410.1708 (error 5.1 ppm).

4.1.2.10. *N*-Hydroxysuccinimdyl 11-(2-chlorophenoxy)undecanoate (47c).: The title compound was prepared from **47b** using the general procedure for the synthesis of NHS esters, method B (72%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.41 (m, 10H), 1.46–1.51 (m, 2H), 1.71–1.76 (m, 2H), 1.80–1.85 (m, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.82–2.83 (br s, 4H), 4.02 (t, *J* = 7.2 Hz, 2H), 6.85–6.88 (m, 1H), 6.90–6.92 (m, 1H), 7.17–7.20 (m, 1H), 7.34–7.35 (m, 1H); HRMS (ESI+) calcd for $C_{21}H_{29}C1NO_5$ $[M+H]^+$ 410.1729, found 410.1717 (error 2.9 ppm).

4.1.2.11. *N*-Hydroxysuccinimdyl 11-(4-bromophenoxy)undecanoate (48c).: The title compound was prepared from **48b** using the general procedure for the synthesis of NHS esters, method B (77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30–1.32 (m, 8H), 1.37–1.44 (m, 4H), 1.71–1.78 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.81–2.83 (br s, 4H), 3.90 (t, *J* = 7.2 Hz, 2H), 6.75–6.78 (m, 2H), 7.33–7.36 (m, 2H) calcd for $C_{21}H_{29}BrNO_5^+$ [M+H] 454.1229, 456.1209, found 454.1208, 456.1188 (error 4.6 ppm).

4.1.2.12. *N*-Hydroxysuccinimdyl 11-[(4-trifluoromethyl)phenoxy] undecanoate (49c).: The title compound was prepared from **49b** using the general procedure for the synthesis of NHS esters, method B (83%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.34 (m, 8H), 1.38–1.47 (m, 4H), 1.71–1.81 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.82–2.83 (br s, 4H), 3.98 (t, *J* = 7.2 Hz, 2H), 6.93–6.94 (m, 2H), 7.51–7.53 (m, 2H).

4.1.2.13. *N*-Hydroxysuccinimdyl 11-[(3-trifluoromethyl)phenoxy] undecanoate (50c).: The title compound was prepared from **50b** using the general procedure for the synthesis of NHS esters, method B (77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.32–1.35 (m, 8H), 1.40–1.47 (m, 4H), 1.73–1.80 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.82–2.83 (br s, 4H), 3.98 (t, *J* = 7.2 Hz, 2H), 7.05–7.06 (m, 1H), 7.11 (s, 1H), 7.17–7.18 (m, 1H), 7.35–7.38 (m, 1H).

4.1.2.14. *N*-Hydroxysuccinimdyl 11-[(2-trifluoromethyl)phenoxy] undecanoate (51c).: The title compound was prepared from **51b** using the general procedure for the synthesis of NHS esters, method B (97%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.25–1.41 (10H, m), 1.44–1.49 (m, 2H), 1.71–1.76 (m, 2H), 1.78–1.82 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.81 (br s, 4H), 4.03 (t, *J* = 7.2 Hz, 2H), 6.95–6.98 (m, 2H), 7.44–7.46 (m, 1H), 7.54–7.55 (m, 1H).

4.1.2.15. *N*-Hydroxysuccinimdyl 11-(4-methoxyphenoxy)undecanoate (52c).: The title compound was prepared from **52b** using the general procedure for the synthesis of NHS esters, method B (75%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.30–1.33 (m, 8H), 1.37–1.45 (m, 4H), 1.71–1.76 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.81–2.83 (br s, 4H), 3.76 (s, 3H), 3.89 (t, *J*

= 7.2 Hz, 2H), 6.81–6.83 (s, 4H); HRMS (ESI+) calcd for $C_{22}H_{32}NO_6$ $[M+H]^+$ 406.2224, found 406.2207 (error 4.2 ppm).

4.1.2.16. *N*-Hydroxysuccinimdyl 11-(3-methoxyphenoxy)undecanoate (53c).: The title compound was prepared from **53b** using the general procedure for the synthesis of NHS esters, method B (77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.31–1.33 (m, 8H), 1.39–1.45 (m, 4H), 1.72–1.79 (m, 4H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.81–2.82 (br s, 4H), 3.78 (s, 3H), 3.93 (t, *J* = 7.2 Hz, 2H), 6.45–6.46 (m, 1H), 6.48–6.50 (m, 2H), 7.15–7.17 (m, 1H); HRMS (ESI+) calcd for $C_{22}H_{32}NO_6$ $[M+H]^+$ 406.2224, found 406.2241 (error 4.2 ppm).

4.1.2.17. *N*-Hydroxysuccinimdyl 11-(2-methoxyphenoxy)undecanoate (54c).: The title compound was prepared from **54b** using the general procedure for the synthesis of NHS esters, method B (74%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.26–1.34 (m, 8H), 1.37–1.47 (m, 4H), 1.71–1.76 (m, 2H), 1.81–1.86 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.82 (br s, 4H), 3.86 (s, 3H), 4.00 (t, *J* = 7.2 Hz, 2H), 6.89 (s, 4H); HRMS (ESI+) calcd for $C_{22}H_{32}NO_6$ $[M+H]^+$ 406.2224, found 406.2224 (error 0 ppm).

4.1.2.18. *N*-Hydroxysuccinimdyl 11-(1-naphthalenyloxy)undecanoate (55c).: The title compound was prepared from **55b** using the general procedure for the synthesis of NHS esters, method B (77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.33–1.41 (m, 10H), 1.54–1.59 (m, 2H), 1.72–1.77 (m, 2H), 1.90–1.95 (m, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.80–2.83 (br s, 4H), 4.13 (t, *J* = 7.2 Hz, 2H), 6.80–6.81 (m, 1H), 7.35–7.38 (m, 1H), 7.40–7.42 (m, 1H), 7.45–7.50 (m, 2H), 7.78–7.80 (m, 1H), 8.28–8.30 (m, 1H); HRMS (ESI+) calcd for $C_{25}H_{32}NO_5$ $[M+H]^+$ 426.2275, found 426.2251 (error 5.6 ppm).

4.1.2.19. *N*-Hydroxysuccinimdyl 11-(2-naphthalenyloxy)undecanoate (56c).: The title compound was prepared from **56b** using the general procedure for the synthesis of NHS esters, method B (75%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.33–1.41 (m, 10H), 1.49–1.52 (m, 2H), 1.74–1.76 (m, 2H), 1.84–1.86 (m, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.78 (br s, 4H), 4.07 (t, *J* = 7.2 Hz, 2H), 7.13–7.16 (m, 2H), 7.31–7.34 (m, 1H), 7.42–7.44 (m, 1H), 7.72–7.77 (m, 3H); HRMS (ESI+) calcd for $C_{25}H_{32}NO_5$ $[M+H]^+$ 426.2275, found 426.2265 (error 2.3 ppm).

4.1.3. General Procedure for Acylation—To a solution of 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine (1.0 equiv) in anhydrous DMF (0.1 M) at 0 °C were added cesium carbonate (3.0 equiv) and the appropriate *N*-hydroxysuccinimyl ester **9c**, **13c–56c** (1.5 equiv). The mixture was warmed to 23 °C and stirred for 16 h. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (0–20% EtOAc/MeOH + 0.5% Et_3N) afforded the title compounds **9d**, **13d–56d** as colorless oils.

4.1.3.1. 5′-*O*-[*N*-(Dodecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (9d).: NHS ester **9c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (69%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.21–1.30 (m, 25H), 1.37 (s, 3H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.16 (t,

J = 7.2 Hz, 2H), 3.15 (q, *J* = 7.2 Hz, 6H), 4.21–4.26 (m, 2H), 4.52–4.54 (m, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.20 (s, 1H), 8.45 (s, 1H); MS (ESI–) calcd for $C_{25}H_{39}N_6O_7S$ $[M - H]^-$ 567.3, found 567.3.

4.1.3.2. 5′-*O*-[*N*-(Butanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (13d).:

NHS ester **13c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (64%): R_f 0.4 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.92 (t, *J* = 7.2 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 9H), 1.39 (s, 3H), 1.57–1.61 (m, 2H), 1.61 (s, 3H), 2.14 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.21–4.24 (m, 2H), 4.53 (br s, 1H), 5.13 (d, *J* = 6.0 Hz, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 6.24 (br s, 1H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{17}H_{23}N_6O_7S$ $[M - H]^-$ 455.1354, found 455.1340 (error 3.1 ppm).

4.1.3.3. 5′-*O*-[*N*-(Hexanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (14d).:

NHS ester **14c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (68%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.27–1.30 (m, 13H), 1.38 (s, 3H), 1.58 (p, *J* = 7.2 Hz, 2H), 1.61 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.21–4.24 (m, 2H), 4.54 (br s, 1H), 5.12 (d, *J* = 6.0 Hz, 1H), 5.35 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{19}H_{27}N_6O_7S$ $[M - H]^-$ 483.1667, found 483.1666 (error 0.2 ppm).

4.1.3.4. 5′-*O*-[*N*-(Octanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (15d).:

NHS ester **15c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (74%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.85 (t, *J* = 7.2 Hz, 3H), 1.24–1.28 (m, 17H), 1.38 (s, 3H), 1.58 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.22–4.28 (m, 2H), 4.53 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.43 (s, 1H); HRMS (ESI−) calcd for $C_{21}H_{31}N_6O_7S$ $[M - H]^-$ 511.1980, found 511.1993 (error 2.5 ppm).

4.1.3.5. 5′-*O*-[*N*-(Decanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (16d).:

NHS ester **16c** was coupled with 5′-O-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (60%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.21–1.31 (m, 21H), 1.38 (s, 3H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.20–4.25 (m, 2H), 4.52 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0Hz, 1H), 5.35 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{23}H_{35}N_6O_7S$ $[M - H]^-$ 539.2, found 539.3.

4.1.3.6. 5′-*O*-[*N*-(Tetradecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (17d).:

NHS ester **17c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (68%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.24–1.29 (m, 29H), 1.37 (s, 3H), 1.58 (p, *J* = 7.2 Hz, 2H), 1.59 (s, 3H), 2.16 (t,

J = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.20–4.25 (m, 2H), 4.52 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.20 (s, 1H), 8.43 (s, 1H); MS (ESI−) calcd for $C_{27}H_{43}N_6O_7S$ [M − H]$^-$ 595.3, found 595.3.

4.1.3.7. 5′-*O*-[*N*-(Palmitoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (18d).:

NHS ester **18c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (65%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.24–1.32 (m, 33H), 1.38 (s, 3H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.21–4.26 (m, 2H), 4.52–4.54 (m, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.6, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{29}H_{47}N_6O_7S$ [M − H]$^-$ 623.3232, found 623.3204 (error 4.5 ppm).

4.1.3.8. 5′-*O*-[*N*-(Stearoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (19d).:

NHS ester **19c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (59%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.25–1.28 (m, 37H), 1.38 (s, 3H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.22–4.27 (m, 2H), 4.53 (br s, 1H), 5.12 (d, *J* = 6.0 Hz, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{31}H_{51}N_6O_7S$ [M − H]$^-$ 651.4, found 651.4.

4.1.3.9. 5′-*O*-[*N*-(Icosanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (20d).:

NHS ester **20c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (48%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.24–1.30 (m, 41H), 1.38 (s, 3H), 1.56 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.21–4.27 (m, 2H), 4.52 (br s, 1H), 5.11 (d, *J* = 6.0 Hz, 1H), 5.34 (dd, *J* = 6.0, 3.0 Hz, 1H), (d, *J* = 3.0 Hz, 1H), 8.20 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{33}H_{55}N_6O_7S$ [M − H]$^-$ 679.4, found 679.4.

4.1.3.10. 5′-*O*-[*N*-(Undec-10-ynoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (21d).:

NHS ester **21c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (70%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.30–1.36 (m, 8H), 1.38 (s, 3H), 1.43–1.50 (m, 2H), 1.56 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.11–2.17 (m, 5H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.20–4.26 (m, 2H), 4.52–4.54 (m, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.35 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{24}H_{33}N_6O_7S$ [M − H]$^-$ 549.2, found 549.2.

4.1.3.11. 5′-*O*-[*N*-(10-Undecenoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (22d).:

NHS ester **22c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the tile compound (58%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.30–1.36 (m, 19H), 1.39 (s, 3H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 1.99–2.02 (m, 2H), 2.20 (t, *J* =

7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.20–4.26 (m, 2H), 4.52–4.55 (m, 1H), 4.89 (d, *J* = 9.6 Hz, 1H), 4.96 (d, *J* = 17.4 Hz, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.35 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 5.74–5.82 (m, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 3H), 8.49 (s, 1H); MS (ESI−) calcd for $C_{24}H_{35}N_6O_7S$ [M − H]$^-$ 551.2, found 551.3.

4.1.3.12. 5′-*O*-{*N*-[(*E*)-Dodec-2-enoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (23d).: NHS ester **23c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (62%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.24–1.30 (m, 23H), 1.38 (s, 3H), 1.60 (s, 3H), 1.96 (q, *J* = 7.2 Hz, 2H), 2.87 (d, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.19–4.24 (m, 2H), 4.52–4.53 (m, 2H), 5.10 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.33 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.49 (dt, *J*=15.0, 7.2 Hz, 1H), 5.57 (dt, *J* = 15.0, 7.2 Hz, 1H) 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.45 (s, 1H); MS (ESI−) calcd for $C_{25}H_{37}N_6O_7S$ [M − H]$^-$ 565.3, found 565.3.

4.1.3.13. 5′-*O*-{*N*-[(*E*)-Dodec-5-enoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (24d).: NHS ester **24c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound: R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.26–1.31 (m, 17H), 1.38 (s, 3H), 1.60 (s, 3H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.94–1.99 (m, 4H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.22–4.24 (m, 2H), 4.53 (br s, 1H), 5.12 (d, *J* = 6.0 Hz, 1H), 5.35–5.37 (m, 3H), 6.24 (br s, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{25}H_{37}N_6O_7S$ [M − H]$^-$ 565.2, found 565.3.

4.1.3.14. 5′-*O*-{*N*-[(*Z*)-Undec-5-enoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (25d).: NHS ester **25c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (65%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.25–1.33 (m, 15H), 1.38 (s, 3H), 1.60 (s, 3H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.99–2.06 (m, 4H), 2.18 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2, 6H), 4.22–4.24 (m, 2H), 4.53 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.32–5.34 (m, 3H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.20 (s, 1H), 8.47 (s, 1H); MS (ESI−) calcd for $C_{24}H_{35}N_6O_7S$ [M − H]$^-$ 551.2, found 551.2.

4.1.3.15. 5′-*O*-{*N*-[(1*S*,2*R*)-2-Nonylcyclopropanecarbonyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (26d).: NHS ester **26c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (59%): R_f 0.7 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.50–0.54 (m, 1H), 0.87 (t, *J* = 7.2 Hz, 3H), 0.99–1.04 (m, 1H), 1.24–1.35 (m, 27H), 1.38 (s, 3H), 1.60 (s, 3H), 3.17 (q, *J* = 7.2 Hz, 6H), 4.20–4.26 (m, 2H), 4.52 (br s, 1H), 5.09 (d, *J* = 6.0 Hz, 1H), 5.33 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.43 (s, 1H); MS (ESI−) calcd for $C_{26}H_{39}N_6O_7S$ [M − H]$^-$ 579.3, found 579.3.

4.1.3.16. 5′-*O*-[*N*-(11-Hydroxyundecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (27d).: NHS ester **27c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (69%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz,

CD_3OD) δ 1.26–1.30 (m, 21H), 1.38 (s, 3H), 1.51 (p, *J* = 7.2 Hz, 2H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.61 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 3.53 (t, *J* = 7.2 Hz, 2H), 4.22–4.23 (m, 2H), 4.54 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.33 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.47 (s, 1H); MS (ESI−) calcd for $C_{24}H_{37}N_6O_8S$ $[M - H]^-$ 569.2, found 569.2.

4.1.3.17. 5′-*O*-[*N*-(12-Azidododecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (28d).: NHS ester **28c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (53%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26–1.31 (m, 23H), 1.38 (s, 3H), 1.53–1.59 (m, 4H), 1.60 (s, 3H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 3.26 (t, *J* = 7.2 Hz, 2H), 4.19–4.25 (m, 2H), 4.53 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.22 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{25}H_{38}N_9O_7S$ $[M - H]^-$ 608.3, found 608.2.

4.1.3.18. 5′-*O*-[*N*-(12-Methoxy-12-oxododecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (30d).: NHS ester **30c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (62%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.20–1.26 (m, 21H), 1.37 (s, 3H), 1.53–1.58 (m, 2H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.59 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 2.27 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.19–4.26 (m, 2H), 4.52 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{26}H_{39}N_6O_9S$ $[M - H]^-$ 611.3, found 611.2.

4.1.3.19. 5′-*O*-[*N*-(11-Phenoxyundecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (32d).: NHS ester **32c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (66%): R_f 0.5 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.24–1.31 (m, 21H), 1.38 (s, 3H), 1.43 (p, *J* = 7.2 Hz, 2H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.15 (q, *J* = 7.2 Hz, 6H), 4.19–4.25 (m, 2H), 4.52 (br s, 1H), 5.11 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.34 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.22 (d, *J* = 3.0 Hz, 1H), 6.86–6.88 (m, 3H), 7.22 (t, *J* = 7.2 Hz, 2H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{30}H_{41}N_6O_8S$ $[M - H]^-$ 645.3, found 645.3.

4.1.3.20. 5′-*O*-{*N*-[(*R*)-2-Methyldodecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (33d).: NHS ester **33c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine (0.52 mmol, 200 mg) using the general procedure for acylation to afford the title compound (57%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 7.2 Hz, 3H), 1.23–1.28 (m, 25H), 1.36 (s, 3H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.25–2.29 (m, 1H), 3.15 (q, *J* = 7.2 Hz, 6H), 4.19–4.27 (m, 2H), 4.51 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{26}H_{41}N_6O_7S$ $[M - H]^-$ 581.3, found 581.3.

4.1.3.21. 5′-*O*-{*N*-[(*S*)-(+)-2-Methyldodecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (34d).: NHS ester **34c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (62%): R_f 0.6 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.05 (d, *J* = 7.2 Hz, 3H), 1.21–1.27 (m, 25H), 1.36 (s, 3H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 2.24–2.29 (m, 1H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.20–4.26 (m, 2H), 4.52 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.34 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.45 (s, 1H); MS (ESI−) calcd for $C_{26}H_{41}N_6O_7S$ $[M - H]^-$ 581.3, found 581.3.

4.1.3.22. 5′-*O*-[*N*-(2,2-Dimethyldodecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (35d).: NHS ester **35c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (48%): R_f 0.7 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.08 (s, 6H), 1.21–1.29 (m, 25H), 1.37 (s, 3H), 1.47 (p, *J* = 7.2 Hz, 2H), 1.59 (s, 3H), 3.15 (q, *J* = 7.2 Hz, 6H), 4.18–4.24 (m, 2H), 4.51 (br s, 1H), 5.13 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.34 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.45 (s, 1H); MS (ESI−) calcd for $C_{27}H_{43}N_6O_7S$ $[M - H]^-$ 595.3, found 595.3.

4.1.3.23. 5′-*O*-[*N*-(Nonyloxy)acetylsulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (36d).: NHS ester **36c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (59%): R_f 0.4 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.20–1.32 (m, 21H), 1.36 (s, 3H), 1.55 (p, *J* = 7.2 Hz, 2H), 1.60 (s, 3H), 3.11 (q, *J* = 7.2 Hz, 6H), 3.45 (t, *J* = 7.2 Hz, 2H), 3.90 (s, 2H), 4.21–4.27 (m, 2H), 4.52 (br s, 1H), 5.12 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.37 (dd, *J* = 6.0, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{24}H_{37}N_6O_8S$ $[M - H]^-$ 569.2, found 569.3.

4.1.3.24. 5′-*O*-{*N*-2-[2-(2-Butoxyethoxy)ethoxy]acetylsulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium salt (37d).: NHS ester **37c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (56%): R_f 0.3 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.92 (t, *J* = 7.2 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 9H), 1.33–1.37 (m, 2H), 1.39 (s, 3H), 1.51–1.54 (m, 2H), 1.61 (s, 3H), 3.19 (q, *J* = 7.2 Hz, 6H), 3.46 (t, *J* = 7.2 Hz, 2H), 3.55–3.56 (m, 2H), 3.60–3.62 (m, 2H), 3.63–3.65 (m, 4H), 3.96 (s, 2H), 4.24–4.25 (m, 2H), 4.54–4.55 (m, 1H), 5.13 (dd, *J* = 6.0, 3.0 Hz, 1H), 5.36 (dd, *J* = 6.6, 3.0 Hz, 1H), 6.24 (d, *J* = 3.0 Hz, 1H), 8.21 (s, 1H), 8.46 (s, 1H); MS (ESI−) calcd for $C_{23}H_{35}N_6O_{10}S$ $[M - H]^-$ 587.2, found 587.3.

4.1.3.25. 5′-*O*-[*N*-(10-Phenoxydecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (38d).: NHS ester **38c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (85%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.26–1.32 (m, 8H), 1.38 (s, 3H), 1.40–1.44 (m, 2H), 1.60 (s, 3H), 1.56–1.61 (m, 2H), 1.70–1.75 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 3.92 (t, *J* = 7.2

Hz, 2H), 4.20-4.26 (m, 2H), 4.52–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.86–6.88 (m, 3H), 7.22–7.24 (m, 2H), 8.21 (s, 1H), 8.44 (s, 1H); MS (ESI−) calcd for $C_{29}H_{39}N_6O_8S$ [M − H]$^-$ 631.2556, found 631.2540 (error 2.5 ppm).

4.1.3.26. 5′-*O*-[*N*-(12-Phenoxydodecanoyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (39d).: NHS ester **39c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (88%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.26–1.33 (m, 12H), 1.38 (s, 3H), 1.42–1.46 (m, 2H), 1.55–1.59 (m, 2H), 1.60 (s, 3H), 1.71–1.76 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 3.94 (t, *J* = 7.2 Hz, 2H), 4.22–4.24 (m, 2H), 4.52–4.53 (m, 1H), 5.11–5.13 (m, 1H), 5.34–5.36 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.86–6.88 (m, 3H), 7.22–7.24 (m, 2H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{43}N_6O_8S$ [M − H]$^-$ 659.2869, found 659.2894 (error 3.7 ppm).

4.1.3.27. 5′-*O*-{*N*-[11-(Phenylthio)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (40d).: NHS ester **40c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (86%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, *J* = 7.2 Hz, 9H), 1.24–1.28 (m, 10H), 1.37–1.38 (m, 2H), 1.38 (s, 3H), 1.54–1.60 (m, 4H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 2.88–2.91 (m, 2H), 3.14–3.19 (m, 6H), 4.20–4.26 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.12 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 7.14–7.16 (m, 1H), 7.24–7.27 (m, 2H), 7.29–7.30 (m, 2H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{41}N_6O_7S_2^-$ [M − H]$^-$ 661.2484, found 661.2458 (error 3.9 ppm).

4.1.3.28. 5′-*O*-{*N*-[11-(Phenylamino)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (41d).: NHS ester **41c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (91%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26 (t, *J* = 7.2 Hz, 9H), 1.25–1.29 (m, 10H), 1.34–1.38 (m, 2H), 1.38 (s, 3H), 1.55–1.60 (m, 4H), 1.60 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.03 (t, *J* = 7.2 Hz, 2H), 3.14 (q, *J* = 7.2 Hz, 6H), 4.20–4.26 (m, 2H), 4.52–4.54 (m, 1H), 5.11–5.12 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.57–6.62 (m, 3H), 7.06–7.09 (m, 2H), 8.21 (s, 1H), 8.43 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{42}N_7O_7S$ [M − H]$^-$ 644.2872, found 644.2879 (error 1.1 ppm).

4.1.3.29. 5′-*O*-{*N*-[11-(4-Fluorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (42d).: NHS ester **42c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (90%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, *J* = 7.2 Hz, 9H), 1.28–1.32 (m, 10H), 1.38 (s, 3H), 1.40–1.45 (m, 2H), 1.55–1.60 (m, 2H), 1.60 (s, 3H), 1.70–1.75 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 3.91 (t, *J* = 7.2 Hz, 2H), 4.21–4.26 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.12 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.85–6.88 (m, 2H), 6.95–6.98 (m, 2H),

8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}FN_6O_8S$ [M − H]$^-$ 663.2618, found 663.2586 (error 4.8 ppm).

4.1.3.30. 5′-*O*-{*N*-[11-(3-Fluorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (43d).: NHS ester **43c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (95%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.38 (s, 3H), 1.41–1.45 (m, 2H), 1.55–1.59 (m, 2H), 1.60 (s, 3H), 1.71–1.76 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 3.94 (t, *J* = 7.2 Hz, 2H), 4.20–4.26 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.60–6.65 (m, 2H), 6.70–6.71 (m, 1H), 7.20–7.24 (m, 1H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}FN_6O_8S$ [M − H]$^-$ 663.2618, found 663.2641 (error 3.4 ppm).

4.1.3.31. 5′-*O*-{*N*-[11-(2-Fluorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (44d).: NHS ester **44c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (86%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.38 (s, 3H), 1.48–1.43 (m, 2H), 1.59–1.55 (m, 2H), 1.60 (s, 3H), 1.78–1.74 (m, 2H), 2.16 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.25–4.20 (m, 2H), 4.54–4.53 (m, 1H), 5.12–5.11 (m, 1H), 5.35–5.34 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.89–6.86 (m, 1H), 7.07–7.03 (m, 3H), 8.21 (s, 1H), 8.46 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}FN_6O_8S$ [M − H]$^-$ 663.2618, found 663.2632 (error 2.1 ppm).

4.1.3.32. 5′-*O*-{*N*-[11-(4-Chlorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (45d).: NHS ester **45c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (80%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.38 (s, 3H), 1.40–1.45 (m, 2H), 1.55–1.58 (m, 2H), 1.60 (s, 3H), 1.70–1.75 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 3.92 (t, *J* = 7.2 Hz, 2H), 4.21–4.26 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.86–6.87 (m, 2H), 7.20–7.22 (m, 2H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}ClN_6O_8S$ [M − H]$^-$ 679.2322, found 679.2337 (error 2.2 ppm).

4.1.3.33. 5′-*O*-{*N*-[11-(3-Chlorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (46d).: NHS ester **46c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (90%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26 (t, *J* = 7.2 Hz, 9H), 1.25–1.33 (m, 10H), 1.38 (s, 3H), 1.40–1.45 (m, 2H), 1.55–1.58 (m, 2H), 1.60 (s, 3H), 1.70–1.75 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.13 (q, *J* = 7.2 Hz, 6H), 3.93 (t, *J* = 7.2 Hz, 2H), 4.20–4.25 (m, 2H), 4.53–4.55 (m, 1H), 5.11–5.12 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.82–6.83 (m, 1H), 6.88–6.89 (m, 2H),

7.20–7.22 (m, 1H), 8.21 (s, 1H), 8.46 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}ClN_6O_8S$ [M − H]$^-$ 679.2322, found 679.2300 (error 3.2 ppm).

4.1.3.34. 5′-*O*-{*N*-[11-(2-Chlorophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (47d).: NHS ester **47c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (93%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, *J* = 7.2 Hz, 9H), 1.26–1.35 (m, 10H), 1.38 (s, 3H), 1.45–1.50 (m, 2H), 1.55–1.58 (m, 2H), 1.60 (s, 3H), 1.75–1.80 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.15 (q, *J* = 7.2 Hz, 6H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.20–4.25 (m, 2H), 4.52–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.86–6.88 (m, 1H), 7.00–7.02 (m, 1H), 7.20–7.23 (m, 1H), 7.31–7.32 (m, 1H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}ClN_6O_8S$ [M − H]$^-$ 679.2322, found 679.2340 (error 2.6 ppm).

4.1.3.35. 5′-*O*-{*N*-[11-(4-Bromophenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (48d).: NHS ester **48c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (97%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.38 (s, 3H), 1.40–1.44 (m, 2H), 1.56–1.59 (m, 2H), 1.60 (s, 3H), 1.70–1.75 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 3.92 (t, *J* = 7.2 Hz, 2H), 4.22–4.24 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.81–6.83 (m, 2H), 7.34–7.36 (m, 2H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{30}H_{40}BrN_6O_8S$ [M − H]$^-$ 723.1812, 725.1791, found 723.1813, 725.1797 (error 0.1 ppm).

4.1.3.36. 5′-*O*-{*N*-[11-(4-Trifluoromethylphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (49d).: NHS ester **49c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (94%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.33 (m, 10H), 1.38 (s, 3H), 1.42–1.48 (m, 2H), 1.54–1.58 (m, 2H), 1.60 (s, 3H), 1.74–1.78 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.23–4.28 (m, 2H), 4.52–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 7.02–7.03 (m, 2H), 7.54–7.55 (m, 2H), 8.21 (s, 1H), 8.43 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{40}F_3N_6O_8S$ [M − H]$^-$ 713.2586, found 713.2614 (error 3.9 ppm).

4.1.3.37. 5′-*O*-{*N*-[11-(3-Trifluoromethylphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (50d).: NHS ester **50c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (78%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.38 (s, 3H), 1.44–1.50 (m, 2H), 1.56–1.58 (m, 2H), 1.60 (s, 3H), 1.74–1.78 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 4.00 (t, *J* = 7.2 Hz, 2H), 4.21–4.26 (m, 2H), 4.53–4.54 (m, 1H), 5.11–5.13 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 7.13–7.19 (m, 3H), 7.42–7.45 (m, 1H),

8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{40}F_3N_6O_8S$ [M − H]$^-$ 713.2586, found 713.2608 (error 3.1 ppm).

4.1.3.38. 5′-*O*-{*N*-[11-(2-Trifluoromethylphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (51d).: NHS ester **51c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (89%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.25 (t, *J* = 7.2 Hz, 9H), 1.24–1.32 (m, 10H), 1.38 (s, 3H), 1.45–1.51 (m, 2H), 1.55–1.59 (m, 2H), 1.60 (s, 3H), 1.75–1.80 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.10 (q, *J* = 7.2 Hz, 6H), 4.05 (t, *J* = 7.2 Hz, 2H), 4.20–4.24 (m, 2H), 4.52–4.53 (m, 1H), 5.11–5.12 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.99–7.02 (m, 1H), 7.12–7.13 (m, 1H), 7.51–7.54 (m, 2H), 8.21 (s, 1H), 8.46 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{40}F_3N_6O_8S$ [M − H]$^-$ 713.2586, found 713.2587 (error 0.1 ppm).

4.1.3.39. 5′-*O*-{*N*-[11-(4-Methoxyphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (52d).: NHS ester **52c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (82%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.25 (t, *J* = 7.2 Hz, 9H), 1.24–1.36 (m, 10H), 1.38 (s, 3H), 1.40–1.44 (m, 2H), 1.55–1.59 (m, 2H), 1.60 (s, 3H), 1.68–1.73 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.10 (q, *J* = 7.2 Hz, 6H), 3.72 (s, 3H), 3.88 (t, *J* = 7.2 Hz, 2H), 4.19–4.25 (m, 2H), 4.53–4.55 (m, 1H), 5.11–5.12 (m, 1H), 5.33–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.81–6.82 (m, 4H), 8.21 (s, 1H), 8.46 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{43}N_6O_9S$ [M − H]$^-$ 675.2818, found 675.2789 (error 4.2 ppm).

4.1.3.40. 5′-*O*-{*N*-[11-(3-Methoxyphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (53d).: NHS ester **53c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (87%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, *J* = 7.2 Hz, 9H), 1.26–1.36 (m, 10H), 1.38 (s, 3H), 1.39–1.44 (m, 2H), 1.55–1.58 (m, 2H), 1.60 (s, 3H), 1.69–1.74 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 3.74 (s, 3H), 3.90 (t, *J* = 7.0 Hz, 2H), 4.20–4.27 (m, 2H), 4.51–4.53 (m, 1H), 5.11–5.12 (m, 1H), 5.35–5.36 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.42–6.43 (m, 1H), 6.45–6.47 (m, 2H), 7.10–7.13 (m, 1H), 8.22 (s, 1H), 8.42 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{43}N_6O_9S$ [M − H]$^-$ 675.2818, found 675.2812 (error 0.9 ppm).

4.1.3.41. 5′-*O*-{*N*-[11-(2-Methoxyphenoxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (54d).: NHS ester **54c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (83%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, *J* = 7.2 Hz, 9H), 1.25–1.34 (m, 10H), 1.38 (s, 3H), 1.41–1.45 (m, 2H), 1.56–1.58 (m, 2H), 1.60 (s, 3H), 1.73–1.76 (m, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 3.81 (s, 3H), 3.96 (t, *J* = 7.2 Hz, 2H), 4.20–4.24 (m, 2H), 4.52–4.53 (m, 1H), 5.11–5.12 (m, 1H), 5.34–5.35 (m, 1H), 6.23 (d, *J* = 3.0 Hz, 1H), 6.86–6.88 (m, 2H), 6.90–

6.93 (m, 2H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{31}H_{43}N_6O_9S$ $[M - H]^-$ 675.2818, found 675.2808 (error 1.5 ppm).

4.1.3.42. 5′-*O*-{*N*-[11-(Naphthalen-1-yloxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt Triethylammonium (55d).: NHS ester **55c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (81%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.22 (t, J = 7.2 Hz, 9H), 1.29–1.32 (m, 10H), 1.37 (s, 3H), 1.36–1.40 (m, 2H), 1.59 (s, 3H), 1.54–1.60 (m, 2H), 1.88–1.91 (m, 2H), 2.17 (t, J = 7.2 Hz, 2H), 3.04 (q, J = 7.2 Hz, 6H), 4.12 (t, J = 7.2 Hz, 2H), 4.21–4.23 (m, 2H), 4.52–4.54 (m, 1H), 5.10–5.12 (m, 1H), 5.32–5.34 (m, 1H), 6.22 (d, J = 3.0 Hz, 1H), 6.84–6.86 (m, 1H), 7.33–7.38 (m, 2H), 7.42–7.45 (m, 2H), 7.76–7.77 (m, 1H), 8.19–8.20 (m, 1H), 8.21 (s, 1H), 8.45 (s, 1H); HRMS (ESI−) calcd for $C_{34}H_{43}N_6O_8S$ $[M - H]^-$ 695.2869, found 695.2893 (error 3.4 ppm).

4.1.3.43. 5′-*O*-{*N*-[11-(Naphthalen-2-yloxy)undecanoyl]sulfamoyl}-2′,3′-*O*-isopropylideneadenosine Triethylammonium Salt (56d).: NHS ester **56c** was coupled with 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneadenosine using the general procedure for acylation to afford the title compound (93%): R_f 0.3 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26 (t, J = 7.2 Hz, 9H), 1.24–1.35 (m, 10H), 1.37 (s, 3H), 1.44–1.50 (m, 2H), 1.55–1.59 (m, 2H), 1.59 (s, 3H), 1.77–1.81 (m, 2H), 2.17 (t, J = 7.2 Hz, 2H), 3.14 (q, J = 7.2 Hz, 6H), 4.04 (t, J = 7.2 Hz, 2H), 4.20–4.25 (m, 2H), 4.52–4.53 (m, 1H), 5.10–5.12 (m, 1H), 5.33–5.34 (m, 1H), 6.22 (d, J = 3.0 Hz, 1H), 7.09–7.11 (m, 1H), 7.17–7.18 (m, 1H), 7.26–7.29 (m, 1H), 7.37–7.40 (m, 1H), 7.70–7.73 (m, 3H), 8.21 (s, 1H), 8.44 (s, 1H); HRMS (ESI−) calcd for $C_{34}H_{43}N_6O_8S$ $[M - H]^-$ 695.2869, found 695.2893 (error 3.4 ppm).

4.1.4. General Procedure for TFA Deprotection—To 5′-*O*-[*N*-(acyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine triethylammonium salt **9d**, **13d–56d** was added aqueous 80% TFA. The reaction was allowed to stir at 0 °C for 0.5 h, then warmed to 23 °C and stirred for an additional 1.5 h. The resulting mixture was concentrated under reduced pressure to remove all traces of TFA. Purification by flash chromatography (0–20% EtOAc/MeOH + 0.5% NEt_3) afforded the title compounds **9** and **13–56**.

4.1.4.1. 5′-*O*-[*N*-(Dodecanoyl)sulfamoyl]adenosine Triethylammonium Salt (9).: The title compound was prepared from **9d** using the general procedure for TFA deprotection (32%): R_f 0.28 (9:1 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, J = 7.2 Hz, 3H), 1.21–1.32 (m, 25H), 1.58–1.61 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.28–4.31 (m, 2H), 4.35–4.40 (m, 2H), 4.67 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.47 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 14.5, 23.7, 27.3, 30.45, 30.52, 30.6, 30.68, 30.74, 30.76, 33.1, 39.9, 47.9, 69.6, 72.3, 76.0, 84.4, 89.3, 120.2, 141.1, 150.9, 153.9, 157.3, 182.2; HRMS (ESI−) calcd for $C_{22}H_{35}N_6O_7S$ $[M - H]^-$ 527.2293, found 527.2316 (error 4.3 ppm).

4.1.4.2. 5′-*O*-[*N*-(Butanoyl)sulfamoyl]adenosine Triethylammonium Salt (13).: The title compound was prepared from **13d** using the general procedure for TFA deprotection

(20%): R_f 0.12 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.93 (t, J = 7.2 Hz, 3H), 1.29 (t, J = 7.2 Hz, 9H), 1.62 (p, J = 7.2 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.27–4.29 (m, 2H), 4.32–4.34 (m, 1H), 4.37–4.39 (m, 1H), 4.69 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 14.4, 20.8, 42.3, 48.0, 72.5, 76.2, 84.7, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 183.1; HRMS (ESI−) calcd for $C_{14}H_{19}N_6O_7S$ $[M - H]^-$ 415.1041, found 415.1049 (error 1.9 ppm).

4.1.4.3. 5′-*O*-[*N*-(Hexanoyl)sulfamoyl]adenosine Triethylammonium Salt (14).: The title compound was prepared from **14d** using the general procedure for TFA deprotection (34%): R_f 0.15 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, J = 7.2 Hz, 3H), 1.29–1.31 (m, 13H), 1.58–1.61 (m, 2H), 2.19 (t, J = 7.8 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.27–4.29 (m, 2H), 4.31–4.33 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.5, 14.4, 23.7, 27.3, 32.9, 40.4, 47.9, 69.3, 72.5, 76.2, 84.7, 89.2, 120.3, 141.3, 151.0, 154.0, 157.4, 183.4; HRMS (ESI−) calcd for $C_{16}H_{23}N_6O_7S$ $[M - H]^-$ 443.1354, found 443.1354 (error 0 ppm).

4.1.4.4. 5′-*O*-[*N*-(Octanoyl)sulfamoyl]adenosine Triethylammonium Salt (15).: The title compound was prepared from **15d** using the general procedure for TFA deprotection (61%): R_f 0.18 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.86 (t, J = 7.2 Hz, 3H), 1.23–1.31 (m, 17H), 1.59 (p, J = 7.2 Hz, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.28–4.30 (m, 2H), 4.33–4.35 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.5, 23.8, 27.5, 30.4, 30.6, 33.0, 40.2, 48.0, 69.5, 72.4, 76.2, 84.6, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 182.8; HRMS (ESI−) calcd for $C_{18}H_{27}N_6O_7S$ $[M - H]^-$ 471.1667, found 471.1659 (error 1.7 ppm).

4.1.4.5. 5′-*O*-[*N*-(Decanoyl)sulfamoyl]adenosine Triethylammonium Salt (16).: The title compound was prepared from **16d** using the general procedure for TFA deprotection (29%): R_f 0.25 (9:1 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, J = 7.2 Hz, 3H), 1.24–1.30 (m, 21H), 1.59 (p, J = 7.2 Hz, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.28–4.30 (m, 2H), 4.32–4.35 (m, 1H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.5, 23.8, 27.5, 30.5, 30.6, 30.7, 30.8, 33.1, 40.3, 48.0, 69.4, 72.4, 76.2, 84.6, 89.3, 120.3, 141.2, 151.0, 154.0, 157.4, 183.2; HRMS (ESI−) calcd for $C_{20}H_{31}N_6O_7S$ $[M - H]^-$ 499.1980, found 499.1971 (error 1.8 ppm).

4.1.4.6. 5′-*O*-[*N*-(Tetradecanoyl)sulfamoyl]adenosine Triethylammonium Salt (17).: The title compound was prepared from **17d** using the general procedure for TFA deprotection (30%): R_f 0.18 (8:2 EtOAc/MeOH): ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, J = 7.2 Hz, 3H), 1.24–1.31 (m, 29H), 1.59 (p, J = 7.2 Hz, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.28–4.30 (m, 2H), 4.32–4.35 (m, 1H), 4.37–4.39 (m, 1H), 4.67 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 14.5, 23.8, 27.5, 30.5, 30.6, 30.7, 30.8, 30.86, 30.87, 30.88. 30.89, 33.2,

40.3, 48.0, 69.4, 72.5, 76.2, 84.7, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 183.2; HRMS (ESI −) calcd for $C_{24}H_{39}N_6O_7S$ [M − H]$^-$ 555.2606, found 555.2587 (error 3.4 ppm).

4.1.4.7. 5′-*O*-[*N*-(Palmitoyl)sulfamoyl]adenosine Triethylammonium Salt (18).: The title compound was prepared from **18d** using the general procedure for TFA deprotection (50%); R_f 0.21 (8:2 EtOAc/MeOH): ^{1}H NMR (600 MHz, CD_3OD) δ 0.90 (t, J = 7.2 Hz, 3H), 1.24–1.32 (m, 33H), 1.57–1.59 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.21 (q, J = 7.2 Hz, 6H), 4.28–4.33 (m, 2H), 4.35–4.39 (m, 2H), 4.67 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 14.6, 23.9, 27.6, 30.4, 30.62, 30.67, 30.73, 30.77, 30.80, 30.85, 30.90, 30.92, 30.93, 33.2, 40.4, 48.0, 69.4, 72.5, 76.3, 84.7, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 183.3; HRMS (ESI−) calcd for $C_{24}H_{39}N_6O_7S$ [M − H]$^-$ 583.2919, found 583.2884 (error 6.0 ppm).

4.1.4.8. 5′-*O*-[*N*-(Stearoyl)sulfamoyl]adenosine Triethylammonium Salt (19).: The title compound was prepared from **19d** using the general procedure for TFA deprotection (24%): R_f 0.24 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, J = 7.2 Hz, 3H), 1.24–1.30 (m, 37H), 1.59 (p, J = 7.8 Hz, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.29–4.31 (m, 2H), 4.33–4.36 (m, 1H), 4.38–4.40 (m, 1H), 4.67 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.5, 23.8, 27.47, 27.49, 30.4, 30.56, 30.62, 30.66, 30.72, 30.75, 30.79, 30.85 (2C), 30.88 (2C), 33.2, 40.2, 48.0, 69.4, 72.4, 76.2, 84.6, 89.3, 120.3, 141.2, 151.0, 154.0, 157.4, 183.0; HRMS (ESI −) calcd for $C_{28}H_{47}N_6O_7S$ [M − H]$^-$ 611.3232, found 611.3202 (error 4.9 ppm).

4.1.4.9. 5′-*O*-[*N*-(Icosanoyl)sulfamoyl]adenosine Triethylammonium Salt (20).: The title compound was prepared from **20d** using the general procedure for TFA deprotection (18%): R_f 0.27 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.90 (t, J = 7.2 Hz, 3H), 1.24–1.32 (m, 41H), 1.55–1.60 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.21 (q, J = 7.2 Hz, 6H), 4.29–4.30 (m, 1H), 4.32–4.34 (m, 1H), 4.37–4.39 (m, 2H), 4.66 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.46 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.6, 14.6, 23.8, 23.9, 27.7, 30.6, 30.74, 30.78, 30.86, 30.89, 30.92 (3C), 30.93 (3C), 32.9, 33.2, 40.5, 48.0, 69.3, 72.5, 76.3, 84.8, 89.3, 120.2, 141.3, 151.1, 154.0, 157.4, 183.4 (Missing 1 C in the alkyl chain); HRMS (ESI−) calcd for $C_{30}H_{51}N_6O_7S$ [M − H]$^-$ 639.3545, found 639.3535 (error 1.6 ppm).

4.1.4.10. 5′-*O*-[*N*-(Undec-10-ynoyl)sulfamoyl]adenosine Triethylammonium Salt (21).: The title compound was prepared from **21d** using the general procedure for TFA deprotection (51%): R_f 0.55 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.22–1.32 (m, 17H), 1.42 (p, J = 7.2 Hz, 2H), 1.54 (p, J = 7.2 Hz, 2H), 2.08 (t, J = 7.2 Hz, 2H), 2.10–2.16 (m, 3H), 3.13 (q, J = 7.2 Hz, 6H), 4.23–4.30 (m, 3H), 4.35–4.37 (m, 1H), 4.66 (t, J = 6.0 Hz, 1H), 6.05 (d, J = 6.0 Hz, 1H), 8.16 (s, 1H), 8.45 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 19.0, 27.4, 29.6, 29.7, 30.0, 30.39, 30.43, 40.2, 47.7, 69.2, 69.4, 72.3, 76.0, 84.4, 85.1, 89.2, 120.1, 141.1, 150.8, 153.9, 157.2, 183.1; HRMS (ESI−) calcd for $C_{21}H_{29}N_6O_7S$ [M − H]$^-$ 509.1824, found 509.1830 (error 1.2 ppm).

4.1.4.11. 5′-*O*-[*N*-(Undec-10-enoyl)sulfamoyl]adenosine Triethylammonium Salt (22).: The title compound was prepared from **22d** using the general procedure for TFA

deprotection (39%): R_f 0.65 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26–1.30 (m, 19H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.99–2.02 (m, 2H), 2.20 (t, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.28–4.30 (m, 2H), 4.32–4.35 (m, 1H), 4.38–4.40 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 4.89 (d, *J* = 9.6 Hz, 1H), 4.96 (d, *J* = 17.4 Hz, 1H), 5.74–5.82 (m, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 3H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 27.5, 30.20, 30.27, 30.30, 30.60, 30.65, 35.0, 40.3, 48.0, 69.4, 72.4, 76.2, 84.7, 89.3, 114.7, 120.3, 140.3, 141.3, 151.0, 154.0, 157.4, 183.0; HRMS (ESI−) calcd for $C_{21}H_{31}N_6O_7S$ $[M - H]^-$ 511.1980, found 511.1973 (error 1.3 ppm).

4.1.4.12. 5′-*O*-{*N*-[(*E*)-Dodec-2-enoyl]sulfamoyl}adenosine Triethylammonium Salt (23).: The title compound was prepared from **23d** using the general procedure for TFA deprotection (25%); R_f 0.65 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, J = 7.2 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 9H), 1.23–1.30 (m, 14H), 1.98 (q, *J* = 7.2 Hz, 2H), 2.91 (d, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.25–4.30 (m, 2H), 4.37–4.39 (m, 2H), 4.68 (t, *J* = 6.0 Hz, 1H), 5.49 (dt, *J* = 15.0, 7.2 Hz, 1H), 5.57 (dt, *J* = 15.0, 7.2 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 14.4, 23.7, 30.2, 30.3, 30.4, 30.5, 30.6, 32.7, 33.1, 47.9, 69.4, 72.3, 76.1, 84.5, 89.2, 120.2, 125.5, 134.3, 141.1, 150.9, 153.9, 157.3, 182.0; HRMS (ESI−) calcd for $C_{22}H_{33}N_6O_7S$ $[M - H]^-$ 525.2137, found 525.2146 (error 1.7 ppm).

4.1.4.13. 5′-*O*-{*N*-[(*E*)-Dodec-5-enoyl]sulfamoyl}adenosine Triethylammonium Salt (24).: The title compound was prepared from **24d** using the general procedure for TFA deprotection (53%): R_f 0.25 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.23–1.32 (m, 17H), 1.63 (p, *J* = 7.2 Hz, 2H), 1.93–2.00 (m, 4H), 2.20 (t, *J* = 7.2 Hz, 2H), 3.20 (q, *J* = 7.2 Hz, 6H), 4.28–4.34 (m, 2H), 4.37–4.39 (m, 2H), 4.67 (t, *J* = 6.0 Hz, 1H), 5.33–5.41 (m, 2H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 1H), 8.45 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.6, 23.8, 25.1, 26.2, 26.4, 30.1, 30.8, 30.9, 33.2, 48.1, 69.9, 71.9, 75.7, 85.4, 89.9, 121.4, 130.6, 132.6, 140.7, 150.7, 153.9, 157.2, 182.3; HRMS (ESI−) calcd for $C_{22}H_{33}N_6O_7S$ $[M - H]^-$ 525.2137, found 525.2137 (error 0 ppm).

4.1.4.14. 5′-*O*-{*N*-[(Z)-Undec-5-enoyl]sulfamoyl}adenosine Triethylammonium Salt (25).: The title compound was prepared from **25d** using the general procedure for TFA deprotection (38%): R_f 0.26 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.23–1.32 (m, 15H), 1.63 (p, *J* = 7.8 Hz, 2H), 1.98–2.06 (m, 4H), 2.21 (t, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.28–4.30 (m, 2H), 4.32–4.35 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 5.32–5.34 (m, 2H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 14.6, 23.8, 27.6, 28.1, 28.3, 30.7, 32.8, 39.8, 48.1, 69.6, 72.5, 76.2, 84.7, 89.4, 120.4, 130.3, 131.6, 141.3, 151.0, 154.0, 157.4, 182.4; HRMS (ESI−) calcd for $C_{21}H_{31}N_6O_7S$ $[M - H]^-$ 511.1980, found 511.1958 (error 4.3 ppm).

4.1.4.15. 5′-*O*-{*N*-[(1*S*,2*R*)-2-Nonylcyclopropanecarbonyl]sulfamoyl} adenosine Triethylammonium Salt (26).: The title compound was prepared from **26d** using the general procedure for TFA deprotection (41%): R_f 0.38 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.51–0.54 (m, 1H), 0.89 (t, *J* = 7.2 Hz, 3H), 1.00–1.03 (m, 1H), 1.21–1.33 (m, 23H), 1.34–1.41 (m, 4H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.24–4.30 (m, 2H), 4.30–4.32 (m,

1H), 4.36–4.38 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.21 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.5, 15.7, 23.3, 23.7, 25.8, 30.39, 30.41, 30.5, 30.67, 30.72, 33.0, 34.4, 47.7, 69.2, 72.5, 76.0, 84.6, 89.1, 120.2, 141.1, 150.9, 153.9, 157.3, 182.7; HRMS (ESI−) calcd for $C_{23}H_{35}N_6O_7S$ $[M - H]^-$ 539.2293, found 539.2290 (error 0.6 ppm).

4.1.4.16. 5′-*O*-[*N*-(11-Hydroxyundecanoyl)sulfamoyl]adenosine Triethylammonium Salt (27).: The title compound was prepared from **27d** using the general procedure for TFA deprotection (56%): R_f 0.28 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.26–1.31 (m, 21H), 1.51 (p, J = 7.2 Hz, 2H), 1.57–1.61 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 3.52 (t, J = 7.2 Hz, 2H), 4.27–4.29 (m, 2H), 4.32–4.35 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 27.0, 27.5, 30.67, 30.70, 30.75, 30.80, 30.84, 33.8, 40.3, 48.0, 63.1, 69.4, 72.4, 76.1, 84.6, 89.3, 120.3, 141.2, 150.9, 154.0, 157.4, 183.2; HRMS (ESI−) calcd for $C_{21}H_{33}N_6O_8S$ $[M - H]^-$ 529.2086, found 529.2078 (error 1.5 ppm).

4.1.4.17. 5′-*O*-[*N*-(12-Azidododecanoyl)sulfamoyl]adenosine Triethylammonium Salt (28).: The title compound was prepared from **28d** using the general procedure for TFA deprotection (33%): R_f 0.18 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.25–1.35 (m, 23H), 1.54–1.61 (m, 4H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 3.26 (t, J = 7.2 Hz, 2H), 4.27–4.30 (m, 2H), 4.33–4.35 (m, 1H), 4.37–4.39 (m, 1H), 4.67 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.4, 27.8, 29.9, 30.2, 30.53, 30.57, 30.60, 30.62 (2C), 40.2, 47.9, 52.5, 69.2, 72.3, 76.1, 84.5, 89.2, 120.2, 141.2, 150.9, 153.9, 157.3, 183.2; HRMS (ESI−) calcd for $C_{22}H_{34}N_9O_7S$ $[M - H]^-$ 568.2307, found 568.2190 (error 20.6 ppm).

4.1.5. 5′-*O*-[*N*-(12-Aminododecanoyl)sulfamoyl]adenosine (29)—To compound **28** (0.13 mmol, 90 mg) in MeOH (20 mL) was added Pd/C (20 mg) at 23 °C. The mixture was shaken under H_2 (g) (40 psi) for 3 h. The crude mixture was filtered through a short pad of Celite. The filtrate was concentrated under reduced pressure, dissolved in MeOH, and concentrated onto Celite. Purification by flash chromatography (0–50% MeOH/EtOAc + 0.5% NEt_3) afforded the title compound (52 mg, 62%) as a colorless oil: R_f 0.11 (7:3 EtOAc/MeOH);^{1}H NMR (600 MHz, CD_3OD) δ 1.26–1.36 (m, 23H), 1.57–1.63 (m, 4H), 2.19 (t, J = 7.2 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H), 4.26–4.29 (m, 2H), 4.31–4.34 (m, 1H), 4.37–4.39 (m, 1H), 4.67 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 27.2, 27.4, 28.6, 29.85, 29.89, 30.10, 30.12, 40.3, 41.0, 60.3, 69.3, 72.5, 76.3, 84.7, 89.4, 120.4, 141.3, 151.0, 154.0, 157.5, 183.6 (missing 1C in alkyl chain); HRMS (ESI−) calcd for $C_{22}H_{36}N_7O_7S$ $[M - H]^-$ 542.2402, found 542.2367 (error 6.4 ppm).

4.1.4.18. 5′-*O*-[*N*-(12-Methoxy-12-oxododecanoyl)sulfamoyl]adenosine Triethylammonium Salt (30).: The title compound was prepared from **30d** using the general procedure for TFA deprotection (40%): R_f 0.37 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.24–1.31 (m, 21H), 1.54–1.60 (m, 4H), 2.18 (t, J = 7.2 Hz, 2H), 2.29 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 3.64 (s, 3H), 4.26–4.29 (m, 2H), 4.31–3.34 (m, 1H),

4.37–4.39 (m, 1H), 4.67 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.19 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 26.2, 27.5, 30.3, 30.5, 30.59, 30.67, 30.69, 30.7, 35.0, 40.3, 48.0, 52.1, 69.5, 72.4, 76.2, 84.6, 89.4, 120.3, 141.3, 150.9, 154.0, 157.4, 176.2, 183.1; HRMS (ESI−) calcd for $C_{23}H_{35}N_6O_9S$ $[M - H]^-$ 571.2192, found 571.2180 (error 2.1 ppm).

4.1.6. 5′-*O*-[*N*-(11-Carboxyundecanoyl)sulfamoyl]adenosine Triethylammonium Salt (31)—

To compound **30** (0.13 mmol, 80 mg) in MeOH/H_2O (1:1, 4 mL) was added LiOH (0.39 mmol, 17mg). The mixture was stirred at 23 °C for 16 h. The resulting mixture was concentrated under reduced pressure. The title compound was prepared from the crude mix using the general procedure for TFA deprotection (41%): R_f 0.38 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.25–1.28 (m, 21H), 1.56–1.59 (m, 4H), 2.15 (t, *J* = 7.2 Hz, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.14 (q, *J* = 7.2 Hz, 6H), 4.26–4.29 (m, 2H), 4.31–3.34 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 6.09 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 27.5, 27.6, 30.60, 30.64, 30.70, 30.73, 30.79, 30.82, 38.8, 40.4, 47.6, 69.1, 72.4, 76.1, 84.6, 89.2, 120.2, 141.2, 150.9, 153.9, 157.3, 182.5, 183.5; HRMS (ESI−) calcd for $C_{22}H_{32}N_6O_9S$ $[M - H]^-$ 557.2035, found 557.2064 (error 5.2 ppm).

4.1.1.19. 5′-*O*-[*N*-(11-Phenoxyundecanoyl)sulfamoyl]adenosine Triethylammonium Salt (32).: . The title compound was prepared from **32d** using the general procedure for TFA deprotection (62%): R_f 0.29 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.25–1.32 (m, 21H), 1.43 (p, *J* = 7.2 Hz, 2H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.73 (p, *J* = 7.2 Hz, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.17 (q, *J* = 7.2 Hz, 6H), 3.93 (t, *J* = 7.2 Hz, 2H), 4.26–4.29 (m, 2H), 4.31–4.34 (m, 1H), 4.38–4.40 (m, 1H), 4.69 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 3H), 7.23 (t, *J* = 7.2 Hz, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.5, 30.4, 30.47, 30.53, 30.55, 30.58, 30.64, 40.3, 47.8, 68.9, 69.2, 72.4, 76.1, 84.5, 89.2, 115.5 (2C), 120.2, 121.4, 130.4 (2C), 141.1, 150.9, 153.9, 157.3, 160.6, 183.2; HRMS (ESI−) calcd for $C_{27}H_{37}N_6O_8S$ $[M - H]^-$ 605.2399, found 605.2391 (error 1.3 ppm).

4.1.4.20. 5′-*O*-{*N*-[(*R*)-2-Methyldodecanoyl]sulfamoyl}adenosine Triethylammonium Salt (33).: The title compound was prepared from **33d** using the general procedure for TFA deprotection (51%): R_f 0.31 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.08 (d, *J* = 6.6 Hz, 3H), 1.23–1.30 (m, 25H), 1.59–1.65 (m, 2H), 2.27–2.31 (m, 1H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.26–4.28 (m, 2H), 4.31–4.34 (m, 1H), 4.38–4.40 (m, 1H), 4.69 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.4, 14.6, 18.8, 23.9, 28.9, 30.60, 30.61, 30.90, 30.94, 31.1, 33.2, 36.0, 44.7, 48.1, 69.5, 72.6, 76.2, 84.8, 89.3, 120.4, 141.3, 151.1, 154.0, 157.5, 183.0; HRMS (ESI−) calcd for $C_{23}H_{37}N_6O_7S$[M − H]$^-$ 541.2450, found 541.2459 (error 1.7 ppm).

4.1.4.21. 5′-*O*-{*N*-[(*S*)-2-Methylodecanoyl]sulfamoyl}adenosine Triethylammonium Salt (34).: The title compound was prepared from **34d** using the general procedure for TFA deprotection (57%): R_f 0.28 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 7.2 Hz, 3H), 1.23–1.28 (m, 25H), 1.59–1.63 (m, 4H), 2.27–2.31

(m, 1H), 3.16 (q, *J* = 7.2 Hz, 6H), 4.26–4.28 (m, 2H), 4.31–4.34 (m, 1H), 4.38–4.40 (m, 1H), 4.69 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.19 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.6, 18.8, 23.9, 28.6, 30.6, 30.62, 30.89, 30.93, 31.1, 33.2, 36.0, 44.7, 48.0, 69.4, 72.4, 76.2, 84.5, 89.3, 120.4, 141.3, 151.4, 154.0, 157.4, 183.2; HRMS (ESI−) calcd for $C_{23}H_{37}N_6O_7S$ $[M - H]^-$ 541.2450, found 541.2435 (error 2.8 ppm).

4.1.4.22. 5′-*O*-[*N*-(2,2-Dimethyldodecanoyl)sulfamoyl]adenosine Triethylammonium Salt (35).: The title compound was prepared from **35d** using the general procedure for TFA deprotection (40%): R_f 0.27 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.11 (s, 6H), 1.19–1.24 (m, 16H), 1.29 (t, *J* = 7.2 Hz, 9H), 1.47–1.51 (m, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.27–4.30 (m, 2H), 4.32–4.35 (m, 1H), 4.37–4.40 (m, 1H), 4.69 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 14.4, 23.7, 26.2, 26.5 (2C), 30.4, 30.7 (2C), 30.8, 31.6, 33.1, 42.7, 45.1, 47.9, 69.6, 72.4, 76.1, 84.5, 89.2, 120.2, 141.2, 150.9, 153.9, 157.3, 182.9; HRMS (ESI−) calcd for $C_{24}H_{39}N_6O_7S$ $[M - H]^-$ 555.2606, found 555.2579 (error 4.9 ppm).

4.1.4.23. 5′-*O*-[*N*-2-(Nonyloxy)acetylsulfamoyl]adenosine Triethylammonium Salt (36).: The title compound was prepared from **36d** using the general procedure for TFA deprotection (54%): R_f 0.3 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.24–1.32 (m, 21H), 1.56 (p, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 3.46 (t, *J* = 7.2 Hz, 2H), 3.93 (s, 2H), 4.27–4.29 (m, 2H), 4.31–4.34 (m, 1H), 4.37–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.20 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 14.4, 23.7, 27.1, 30.4, 30.58, 30.61, 30.7, 33.0, 47.9, 69.4, 72.3, 72.5, 72.6, 76.0, 84.4, 89.3, 120.2, 141.1, 150.9, 153.8, 157.2, 178.3; HRMS (ESI−) calcd for $C_{21}H_{33}N_6O_8S$ $[M - H]^-$ 529.2086, found 529.2083 (error 0.6 ppm).

4.1.4.24. 5′-*O*-{*N*-2-[2-(2-Butoxyethoxy)ethoxy]acetylsulfamoyl}adenosine Triethylammonium Salt (37).: The title compound was prepared from **37d** using the general procedure for TFA deprotection (46%): R_f 0.10 (8:2 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.90 (t, *J* = 7.2 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 9H), 1.33–1.37 (m, 2H), 1.51–1.54 (m, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 3.45 (t, *J* = 7.2 Hz, 2H), 3.54–3.64 (m, 8H), 3.98 (s, 2H), 4.27–4.30 (m, 2H), 4.32–4.34 (m, 1H, 4.37–4.40 (m, 1H), 4.67 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 8.19 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 14.3, 20.4, 32.9, 48.0, 69.3, 71.1, 71.4, 71.50, 71.55, 72.2, 72.4, 73.0, 76.2, 84.6, 89.3, 120.3, 141.3, 151.0, 154.0, 157.4, 178.6; HRMS (ESI−) calcd for $C_{20}H_{31}N_6O_{10}S$ $[M - H]^-$ 547.1828, found 547.1806 (error 4.0 ppm).

4.1.4.25. 5′-*O*-[*N*-(10-Phenoxydecanoyl)sulfamoyl]adenosine Triethylammonium Salt (38).: The title compound was prepared from **38d** using the general procedure for TFA deprotection (87%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, *J* = 7.2 Hz, 9H), 1.26–1.34 (m, 8H), 1.39–1.43 (m, 2H), 1.57–1.61 (m, 2H), 1.69–1.74 (m, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 6H), 3.92 (t, *J* = 7.2 Hz, 2H), 4.26–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 6.09 (d, *J* = 6.0 Hz, 1H), 6.86–6.89 (m, 3H), 7.22–7.24 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.1, 27.1, 27.4, 30.39, 30.46, 30.50, 30.58, 40.2, 47.7, 68.8, 69.2, 72.2, 76.0, 84.4, 89.2, 115.4,

120.1, 121.4, 130.3, 141.1, 150.7, 153.8, 157.2, 160.5, 183.2; HRMS (ESI−) calcd for $C_{26}H_{35}N_6O_8S$ [M − H]$^-$ 591.2243, found 591.2224 (error 3.2 ppm).

4.1.4.26. 5′-*O*-[*N*-(12-Phenoxydodecanoyl)sulfamoyl]adenosine Triethylammonium Salt (39).: The title compound was prepared from **39d** using the general procedure for TFA deprotection (52%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, J = 7.2 Hz, 9H), 1.24–1.34 (m, 12H), 1.42–1.46 (m, 2H), 1.58–1.60 (m, 2H), 1.71–1.76 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 3.93 (t, J = 7.2 Hz, 2H), 4.25–4.34 (m, 3H), 4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 6.87–6.88 (m, 3H), 7.22–7.24 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.0, 27.0, 27.3, 30.28, 30.36, 30.40, 30.45, 30.51, 30.53, 30.55, 40.1, 47.6, 68.87, 69.1, 72.1, 75.9, 84.3, 89.1, 115.3, 120.0, 121.3, 130.2, 141.0, 150.7, 153.7, 157.1, 160.4, 183.1; HRMS (ESI −) calcd for $C_{28}H_{39}N_6O_8S$ [M − H]$^-$ 619.2556, found 619.2565 (error 1.4 ppm).

4.1.4.27. 5′-*O*-{*N*-[11-(Phenylthio)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (40).: The title compound was prepared from **40d** using the general procedure for TFA deprotection (74%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, J = 7.2 Hz, 9H), 1.20–1.30 (m, 13H), 1.36–1.39 (m, 2H), 1.55–1.60 (m, 5H), 2.19 (t, J = 7.2 Hz, 2H), 2.89 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 4.27–4.35 (m, 3H), 4.38–4.40 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 7.13–7.15 (m, 1H), 7.25–7.30 (m, 2H), 7.30–7.31 (m, 2H), 8.19 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.2, 23.1, 27.4, 29.7, 30.21, 30.26, 30.52, 30.54, 34.29, 40.3, 47.8, 69.3, 72.3, 76.1, 84.5, 89.3, 119.5, 120.2, 126.7 (2C), 129.9, 130.1, 138.3, 141.2, 150.9, 153.9, 157.3, 183.2 (missing 1C in alkyl chain); HRMS (ESI−) calcd for $C_{27}H_{37}N_6O_7S_2$ [M − H]$^-$ 621.2171, found 621.2145 (error 4.2 ppm).

4.1.4.28. 5′-*O*-{*N*-[11-(Phenylamino)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (41).: The title compound was prepared from **41d** using the general procedure for TFA deprotection (89%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.27–1.31 (m, 12H), 1.35–1.38 (m, 2H), 1.57–1.60 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.04 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 6.59–6.63 (m, 3H), 7.07–7.10 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.2, 23.7, 27.4, 28.3, 30.36, 30.52, 30.57, 30.61, 30.68, 40.3, 45.1, 47.7, 69.2, 72.3, 76.1, 84.5, 89.3, 114.1 (2C), 117.9, 129.9 (2C), 141.15, 141.16, 150.4, 150.8, 153.9, 157.3, 183.5; HRMS (ESI−) calcd for $C_{27}H_{38}N_7O_7S$ [M − H]$^-$ 604.2559, found: 604.2551 (error 1.3 ppm).

4.1.4.29. 5′-*O*-{*N*-[11-(4-Fluorophenoxy)undecanoyl]sulfamoyladenosine Triethylammonium Salt (42).: The title compound was prepared from **42d** using the general procedure for TFA deprotection (77%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, J = 7.2 Hz, 9H), 1.26–1.32 (m, 10H), 1.40–1.45 (m, 2H), 1.57–1.61 (m, 2H), 1.70–1.74 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.12 (q, J = 7.2 Hz, 6H), 3.91 (t, J = 7.2 Hz, 2H), 4.26–4.33 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 6.86–6.88 (m, 2H), 6.95–6.98 (m, 2H), 8.19 (s, 1H), 8.50 (s, 1H); ^{13}C NMR

(150 MHz, CD_3OD) δ 9.2, 27.1, 27.4, 30.4, 30.47, 30.52, 30.56, 30.58, 30.7, 40.2, 47.9, 69.2, 69.6, 72.4, 76.1, 84.6, 89.2, 116.5, (d, *J* = 23.2 Hz, 2C), 116.6 (d, *J* = 8.0 Hz, 2C), 120.3, 141.2, 150.9, 153.9, 156.9, 157.3, 159.3 (d, *J* = 234.9 Hz), 183.0; HRMS (ESI−) calcd for $C_{27}H_{36}FN_6O_8S$ $[M - H]^-$ 623.2305, found 623.2278 (error 4.3 ppm).

4.1.4.30. 5′-*O*-{*N*-[11-(3-Fluorophenoxy)undecanoyl]sulfamoyladenosine Triethylammonium Salt (43).: The title compound was prepared from **43d** using the general procedure for TFA deprotection (81%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, *J* = 7.2 Hz, 9H), 1.26–1.30 (m, 10H), 1.39–1.44 (m, 2H), 1.56–1.60 (m, 2H), 1.70–1.74 (m, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.18 (q, *J* = 7.2 Hz, 6H), 3.93 (t, *J* = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.36–4.40 (m, 1H), 4.66 (t, *J* = 6.0 Hz, 1H), 6.09 (d, *J* = 6.0 Hz, 1H), 6.59–6.64 (m, 2H), 6.68–6.70 (m, 1H), 7.20–7.24 (m, 1H), 8.19 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.1, 26.9, 27.3, 30.1, 30.3, 30.37, 30.40, 30.43, 30.5, 40.1, 47.7, 69.1 (d, *J* = 11.8 Hz), 72.1, 75.9, 84.3, 89.1, 102.7 (d, *J* = 25.0 Hz), 107.8 (d, *J* = 21.5 Hz), 111.3 (d, *J* = 2.9 Hz), 120.0, 131.2 (d, *J* = 10.4 Hz), 141.0, 150.7, 153.7, 157.1, 162.0, 163.1 (d, *J* = 314.0 Hz), 165.7 183.1; HRMS (ESI−) calcd for $C_{27}H_{36}FN_6O_8S$ $[M - H]^-$ 623.2305, found 623.2312 (error 1.1 ppm).

4.1.4.31. 5′-*O*-{*N*-[11-(2-Fluorophenoxy)undecanoyl]sulfamoyladenosine Triethylammonium Salt (44).: The title compound was prepared from **44d** using the general procedure for TFA deprotection (83%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, *J* = 7.2 Hz, 9H), 1.28–1.32 (m, 10H), 1.42–1.46 (m, 2H), 1.58–1.60 (m, 2H), 1.73–1.78 (m, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 4.01 (t, *J* = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 6.86–6.89 (m, 1H), 7.03–7.07 (m, 3H), 8.20 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 27.1, 27.5, 30.46, 30.55, 30.65, 30.67, 30.69, 30.75, 40.4, 47.9, 69.4, 70.5, 72.4, 76.2, 84.6, 89.4, 116.3, 116.9 (d, *J* = 18.4 Hz), 120.3, 122.1 (d, *J* = 6.9 Hz), 125.6 (d, *J* = 3.9 Hz), 141.3, 148.6 (d, *J* = 10.4 Hz), 150.9, 154.0, 154.5 (d, *J* = 242.7 Hz), 157.4, 183.3; HRMS (ESI−) calcd for $C_{27}H_{36}FN_6O_8S$ $[M - H]^-$ 623.2305, found 623.2320 (error 2.4 ppm).

4.1.4.32. 5′-*O*-{*N*-[11-(4-Chlorophenoxy)undecanoyl]sulfamoyl} adenosine Triethylammonium Salt (45).: The title compound was prepared from **45d** using the general procedure for TFA deprotection (82%): R_f 0.1 (7:3 EtOAc/MeOH);^{1}H NMR (600 MHz, CD_3OD) δ 1.30 (t, *J* = 7.2 Hz, 9H), 1.27–1.33 (m, 10H), 1.40–1.45 (m, 2H), 1.58–1.60 (m, 2H), 1.70–1.75 (m, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 6H), 3.92 (t, *J* = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 6.86–6.88 (m, 2H), 7.21–7.23 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.4, 30.2, 30.3, 30.46, 30.51, 30.55, 30.57, 30.63, 40.2, 47.9, 69.28, 69.34, 72.3, 76.1, 84.5, 89.2, 117.0 (2C), 120.2, 130.2 (2C), 141.2, 150.9, 153.9, 157.3, 159.4, 183.1; HRMS (ESI−) calcd for $C_{27}H_{36}ClN_6O_8S$ $[M - H]^-$ 639.2009, found 639.2019 (error 1.6 ppm).

4.1.4.33. 5′-*O*-{*N*-[11-(3-Chlorophenoxy)undecanoyl]sulfamoyl} adenosine Triethylammonium Salt (46).: The title compound was prepared from **46d** using the

general procedure for TFA deprotection (88%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, J = 7.2 Hz, 9H), 1.27–1.31 (m, 10H), 1.40–1.45 (m, 2H), 1.57–1.62 (m, 2H), 1.71–1.75 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 3.93 (t, J = 7.2 Hz, 2H), 4.26–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 6.82–6.83 (m, 1H), 6.88–6.90 (m, 2H), 7.20–7.22 (m, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.3, 27.1, 27.4, 30.3, 30.46, 30.52, 30.56, 30.58, 30.64, 40.3, 47.9, 69.26, 69.33, 72.3, 76.1, 84.5, 89.3, 114.1, 115.8, 120.2, 121.5, 131.4, 135.8, 141.2, 150.9, 153.9, 157.3, 161.5, 183.3; HRMS (ESI−) calcd for $C_{27}H_{36}ClN_6O_8S$ $[M - H]^-$ 639.2009, found 639.1982 (error 4.2 ppm).

4.1.4.34. 5′-*O*-{*N*-[11-(2-Chlorophenoxy)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (47).: The title compound was prepared from **47d** using the general procedure for TFA deprotection (80%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.25–1.32 (m, 10H), 1.45–1.50 (m, 2H), 1.56–1.60 (m, 2H), 1.75–1.80 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 4.01 (t, J = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 6.86–6.89 (m, 1H), 7.01–7.02 (m, 1H), 7.21–7.23 (m, 1H), 7.31–7.33 (m, 1H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.2, 27.1, 27.4, 30.2, 30.38, 30.45, 30.56, 30.63, 40.3, 47.8, 69.2, 70.1, 72.3, 76.1, 84.5, 89.2, 114.7, 120.2, 122.2, 123.8, 128.9, 131.1, 141.2, 150.8, 153.9, 156.0, 157.3, 183.3 (missing 1C in alkyl chain); HRMS (ESI−) calcd for $C_{27}H_{36}ClN_6O_8S$ $[M - H]^-$ 639.2009, found 639.2004 (error 0.8 ppm).

4.1.4.35. 5′-*O*-{*N*-[11-(4-Bromophenoxy)undecanoyl]sulfamoyl} adenosine Triethylammonium Salt (48).: The title compound was prepared from **48d** using the general procedure for TFA deprotection (72%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.27–1.31 (m, 10H), 1.39–1.43 (m, 2H), 1.57–1.60 (m, 2H), 1.70–1.75 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 3.92 (t, J = 7.2 Hz, 2H), 4.28–4.36 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 6.81–6.84 (m, 2H), 7.34–7.37 (m, 2H), 8.20 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.1, 27.1, 27.3, 30.3, 30.42, 30.48, 30.52, 30.55, 30.61, 40.1, 47.7, 69.3, 69.4, 72.2, 76.0, 84.4, 89.3, 113.3 (2C), 117.5, 120.2, 133.2 (2C), 141.1, 150.8, 153.9, 157.3, 159.8, 182.9; HRMS (ESI−) calcd for $C_{27}H_{36}BrN_6O_8S$ $[M - H]^-$ 683.1499, 685.1478, found 683.1500, 685.1483 (error 0.1 ppm).

4.1.4.36. 5′-*O*-{*N*-[11-(4-Trifluoromethylphenoxy)undecanoyl] sulfamoyl}adenosine Triethylammonium Salt (49).: The title compound was prepared from **49d** using the general procedure for TFA deprotection (68%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.30 (t, J = 7.2 Hz, 9H), 1.27–1.33 (m, 10H), 1.42–1.48 (m, 2H), 1.57–1.62 (m, 2H), 1.74–1.79 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.20 (q, J = 7.2 Hz, 6H), 4.02 (t, J = 7.2 Hz, 2H), 4.26–4.36 (m, 3H), 4.38–4.40 (m, 1H), 4.67 (t, J = 5.4 Hz, 1H), 6.08 (d, J = 5.8 Hz, 1H), 7.03 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.3, 30.4, 30.50, 30.52, 30.54, 30.56, 30.62, 40.2, 47.9, 69.3, 69.4, 72.3, 76.1, 84.5, 89.2, 115.7, 120.2, 123.4 (q, J = 32.4 Hz), 126.1 (q, J = 269.4 Hz), 127.9 (q, J = 3.6 Hz), 140.6, 141.2, 150.3, 150.9, 153.9, 157.3, 163.3, 182.9;

HRMS (ESI−) calcd for $C_{28}H_{36}F_3N_6O_8S$ [M − H]$^-$ 673.2273, found 673.2260 (error 1.9 ppm).

4.1.4.37. 5′-*O*-{*N*-[11-(3-Trifluoromethylphenoxy)undecanoyl]sulfamoyladenosine Triethylammonium Salt (50).: The title compound was prepared from **50d** using the general procedure for TFA deprotection (70%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.28–1.33 (m, 10H), 1.42–1.47 (m, 2H), 1.58–1.60 (m, 2H), 1.74–1.79 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.19 (q, J = 7.2 Hz, 6H), 4.00 (t, J = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 7.13–7.19 (m, 3H), 7.43 (t, J = 7.9 Hz, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.5, 30.2, 30.46, 30.53, 30.56, 30.59, 30.64, 40.3, 47.8, 69.2, 69.4, 72.3, 76.1, 84.5, 89.2, 112.2 (q, J = 3.4 Hz), 117.9 (q, J = 3.7 Hz), 119.17, 120.2, 124.62 (q, J = 270.3 Hz), 131.4, 132.9 (q, J = 32.1 Hz), 141.1, 150.8, 153.9, 157.3, 160.9, 183.3; HRMS (ESI−) calcd for $C_{28}H_{36}F_3N_6O_8S$ [M − H]$^-$ 673.2273, found 673.2295 (error 3.3 ppm).

4.1.4.38. 5′-*O*-{*N*-[11-(2-Trifluoromethylphenoxy)undecanoyl]sulfamoyladenosine Triethylammonium Salt (51).: The title compound was prepared from **51d** using the general procedure for TFA deprotection (61%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.28–1.32 (m, 10H), 1.44–1.49 (m, 2H), 1.57–1.61 (m, 2H), 1.75–1.79 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 4.05 (t, J = 7.2 Hz, 2H), 4.27–4.35 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 7.00 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 8.3 Hz, 1H), 7.51–7.54 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 26.9, 27.4, 30.1, 30.2, 30.3, 30.48, 30.53, 30.6, 40.2, 47.8, 69.2, 69.6, 72.3, 76.1, 84.5, 89.2, 114.1, 119.6 (q, J = 29.8 Hz), 120.2, 120.8, 125.3 (q, J = 270.3 Hz), 127.7 (q, J = 5.7 Hz), 134.7, 141.1, 150.8, 153.9, 157.3, 158.5, 183.2; HRMS (ESI−) calcd for $C_{28}H_{36}F_3N_6O_8S$ [M − H]$^-$ 673.2273, found 673.2263 (error 1.5 ppm).

4.1.4.39. 5′-*O*-{*N*-[11-(4-Methoxyphenoxy)undecanoyl]sulfamoyl} adenosine Triethylammonium Salt (52).: The title compound was prepared from **52d** using the general procedure for TFA deprotection (86%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.29 (t, J = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.39–1.44 (m, 2H), 1.57–1.62 (m, 2H), 1.68–1.73 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2 Hz, 6H), 3.73 (s, 3H), 3.88 (t, J = 7.2 Hz, 2H), 4.29–4.36 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 6.81–6.82 (m, 4H), 8.20 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.4, 30.49, 30.51, 30.53, 30.56, 30.59, 30.7, 40.3, 47.8, 56.1, 69.2, 69.6, 72.3, 76.1, 84.5, 89.2, 115.6 (2C), 116.5 (2C), 120.2, 141.2, 150.9, 153.9, 154.7, 155.2, 157.3, 183.2; HRMS (ESI−) calcd for $C_{28}H_{39}N_6O_9S$ [M − H]$^-$ 635.2505, found 635.2476 (error 4.6 ppm).

4.1.4.40. 5′-*O*-{*N*-[11-(3-Methoxyphenoxy)undecanoyl]sulfamoyl} adenosine Triethylammonium Salt (53).: The title compound was prepared from **53d** using the general procedure for TFA deprotection (67%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, J = 7.2 Hz, 9H), 1.27–1.32 (m, 10H), 1.40–1.45 (m, 2H), 1.57–

1.61 (m, 2H), 1.70–1.74 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.17 (q, J = 7.2 Hz, 6H), 3.75 (s, 3H), 3.91 (t, J = 7.2 Hz, 2H), 4.26–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.09 (d, J = 6.0 Hz, 1H), 6.43–6.44 (m, 1H), 6.46–6.48 (m, 2H), 7.11–7.14 (m, 1H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.2, 27.1, 27.4, 30.4, 30.48, 30.52, 30.56, 30.58, 30.65, 40.2, 47.8, 55.6, 68.9, 69.3, 72.3, 76.1, 84.5, 89.2, 101.9, 107.1, 107.8, 120.2, 130.8, 141.1, 150.8, 153.9, 157.3, 161.8, 162.3, 183.1; HRMS (ESI−) calcd for $C_{28}H_{39}N_6O_9S$ [M − H]$^-$ 635.2505, found 635.2475 (error 4.7 ppm).

4.1.4.41. 5′-*O*-{*N*-[11-(2-Methoxyphenoxy)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (54).: The title compound was prepared from **54d** using the general procedure for TFA deprotection (74%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.30 (t, J = 7.2 Hz, 9H), 1.27–1.31 (m, 10H), 1.42–1.46 (m, 2H), 1.56–1.60 (m, 2H), 1.73–1.77 (m, 2H), 2.20 (t, J = 7.2 Hz, 2H), 3.20 (q, J = 7.2 Hz, 6H), 3.82 (s, 3H), 3.96 (t, J = 7.2 Hz, 2H), 4.29–4.36 (m, 3H), 4.37–4.38 (m, 1H), 4.67 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 6.87–6.88 (m, 2H), 6.91–6.94 (m, 2H), 8.20 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.2, 27.1, 27.4, 30.4, 30.48, 30.54, 30.57, 30.59, 30.7, 40.3, 47.8, 56.6, 69.2, 70.3, 72.3, 76.1, 84.5, 89.3, 113.6, 115.0, 120.2, 122.2, 122.3, 141.2, 150.1, 150.9, 151.0, 153.9, 157.3, 183.2; HRMS (ESI−) calcd for $C_{28}H_{39}N_6O_9S$ [M − H]$^-$ 635.2505, found 635.2477 (error 4.4 ppm).

4.1.4.42. 5′-*O*-{*N*-[11-(Naphthalen-1-yloxy)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (55).: The title compound was prepared from **55d** using the general procedure for TFA deprotection (66%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.27 (t, J = 7.2 Hz, 9H), 1.26–1.31 (m, 8H), 1.36–1.40 (m, 2H), 1.53–1.60 (m, 4H), 1.88–1.92 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.14 (q, J = 7.2 Hz, 6H), 4.13 (t, J = 7.2 Hz, 2H), 4.26–4.33 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 6.86–6.87 (m, 1H), 7.33–7.39 (m, 2H), 7.41–7.46 (m, 2H), 7.76–7.77 (m, 1H), 8.20 (s, 1H), 8.20–8.21 (m, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 9.1, 27.3, 27.4, 30.3, 30.46, 30.52, 30.54, 30.56, 30.63, 40.2, 47.8, 69.2, 69.2, 72.2, 76.0, 84.4, 89.3, 105.7, 120.2, 120.9, 122.9, 125.9, 127.0, 127.1, 127.2, 128.4, 135.9, 141.1, 150.8, 153.8, 156.0, 157.2, 183.2; HRMS (ESI−) calcd for $C_{31}H_{39}N_6O_8S$ [M − H]$^-$ 655.2556, found 655.2529 (error 4.1 ppm).

4.1.4.43. 5′-*O*-{*N*-[11-(Naphthalen-2-yloxy)undecanoyl]sulfamoyl}adenosine Triethylammonium Salt (56).: The title compound was prepared from **56d** using the general procedure for TFA deprotection (60%): R_f 0.1 (7:3 EtOAc/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28 (t, J = 7.2 Hz, 9H), 1.27–1.38 (m, 10H), 1.46–1.51 (m, 2H), 1.57–1.61 (m, 2H), 1.78–1.83 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H), 3.17 (q, J = 7.2 Hz, 6H), 4.06 (t, J = 7.2 Hz, 2H), 4.27–4.34 (m, 3H), 4.38–4.39 (m, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.08 (d, J = 6.0 Hz, 1H), 7.10–7.12 (m, 1H), 7.19–7.19 (m, 1H), 7.27–7.30 (m, 1H), 7.38–7.41 (m, 1H), 7.71–7.74 (m, 3H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 9.2, 27.2, 27. 5, 30.40, 30.52, 30.54, 30.57, 30.60, 30.64, 30.68, 40.3, 47.8, 69.0, 69.2, 72.4, 76.1, 84.5, 89.2, 107.6, 119.9, 124.4, 127.2, 127.7, 128.5, 130.2, 130.3, 136.2, 141.1, 150.8, 153.8, 157.2, 158.5, 183.2; HRMS (ESI−) calcd for $C_{31}H_{39}N_6O_8S$ [M − H]$^-$ 655.2556, found 655.2526 (error 4.6 ppm).

4.1.7. 5′-*N*-(*N*-(11-Phenoxyundecanoyl)sulfamoyl)-2′,3′-*O*-isopropylideneadenosine (82)—To a solution of 5′-deoxy-2′,3′-*O*-isopropylidene-5′-[(sulfamoyl)amino]adenosine **81** (0.42 mmol, 1.0 equiv, 160 mg) in DMF (4 mL) was added 11-phenoxyundecanoate *N*-hydroxysuccinimide **32c** (0.63 mmol, 1.5 equiv, 233 mg) and cesium carbonate (1.26 mmol, 3.0 equiv, 405 mg). The reaction mixture was stirred at 23 °C for 16 h. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was used in next step without further purification.

4.1.4.44. 5′-deoxy-5′-{*N*-[(11-Phenoxyundecanoyl)sulfamoyl] amino} adenosine Triethylammonium Salt (83).: The title compound was prepared from **82** using the general procedure for TFA deprotection (42% over 2 steps): R_f 0.3 (9:1 CH_2Cl_2/MeOH + 1% NEt_3); ^{1}H NMR (400 MHz, CD_3OD) 1.28 (t, J = 7.3 Hz, 9H), 1.24–1.36 (m, 10H), 1.44 (m, 2H), 1.60 (m, 2H), 1.70–1.77 (m, 2H), 2.20 (t, J = 7.4 Hz, 2H), 3.18 (q, J = 7.3 Hz, 6H), 3.93 (t, J = 6.4 Hz, 2H), 4.25–4.36 (m, 3H), 4.39 (m, 1H), 4.69 (t, J = 5.6 Hz, 1H), 6.09 (d, J = 5.8 Hz, 1H), 6.85–6.91 (m, 3H), 7.20–7.26 (m, 2H), 8.20 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (101 MHz, CD_3OD): δ 9.2, 27.1, 27.4, 30.42, 30.49, 30.52, 30.56, 30.59, 30.66, 40.2, 47.9, 68.9, 69.3, 72.4, 76.1, 84.6, 89.2, 115.5, 121.4, 121.3, 130.4, 141.1, 150.9, 153.9, 157.3, 160.6, 182.9; HRMS (ESI+) calcd for $C_{27}H_{39}N_7O_7S$ $[M + H]^+$ 606.2704, found 606.2709 (error 0.8 ppm).

4.1.8. 1-(11-Phenoxy)undecanol (84)—Lithium aluminum hydride (2.0 mmol, 2.0 equiv, 76 mg) was suspended in THF (8 mL) and cooled to 0°C. 11-Phenoxyundecanoic acid **32a** (1.0 mmol, 1.0 equiv, 278 mg) was added in THF (2 mL). The reaction mixture was allowed to warm to 23 °C and stirred for 16 h. The reaction mixture was cooled to 0 °C and water (3 mL) was added slowly. The resulting slurry was filtered through Celite, dried ($MgSO_4$) and concentrated under reduced pressure to yield the title compound (211 mg, 80%) as a white solid: R_f 0.25 (2:8 EtOAc/Hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.2–1.4 (m, 12H), 1.45 (p, J = 7.2 Hz, 2H), 1.56 (p, J = 7.2 Hz, 2H), 1.78 (p, J = 7.2 Hz, 2H), 3.63 (t, J = 7.2 Hz, 2H), 3.95 (t, J = 7.2 Hz, 2H), 6.89 (d, J = 7.2 Hz, 2H), 6.93 (t, J = 7.2 Hz, 1H), 7.27 (t, J = 7.2 Hz, 2H).

4.1.9. 11-Phenoxyundecyl sulfamate (85)—11-Phenoxyundecan-1-ol **84** (0.35 mmol, 1.0 equiv, 100 mg) was dissolved in THF (5 mL) and cooled to 0°C. Sodium hydride (60% in mineral oil, 0.36 mmol, 1.03 equiv, 14 mg) was added and the reaction was stirred for 10 minutes. Sulfamoyl chloride (0.36 mmol, 1.03 equiv, 41 mg) was added and the reaction was allowed to warm 23 °C and stirred for 16 h. Water (30 mL) was added to the reaction mixture and the mixture was extracted with EtOAc (3 × 20 ml). The combined organic extracts were washed with saturated aqueous NaCl (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield the title compound (96 mg, 80%) as a colorless oil: R_f; 0.18 (2:8 EtOAc/Hexanes); ^{1}H NMR (600 MHz, CD_3OD) δ 1.2–1.4 (m, 12H), 1.45 (p, J = 7.2 Hz, 2H), 1.51 (p, J = 7.2 Hz, 1H), 1.69 (p, J = 7.2 Hz, 1H), 1.74 (p, J = 7.2 Hz, 2H), 3.52 (t, J = 7.2 Hz, 1H), 3.92 (t, J = 7.2 Hz, 2H), 4.09 (t, J = 7.2 Hz, 1H), 6.8–6.9 (m, 3H), 7.22 (t, J = 7.2 Hz, 2H); MS (ESI +) cald for $C_{17}H_{29}NO_4S$ $[M + H]^+$ 344.2, found 344.2.

4.1.10. 11-Phenoxyundecyl 4-methylbenzenesulfonate (86)—

11-Phenoxyundecan-1-ol **84** (2.0 mmol, 1.0 equiv, 528 mg), 4-dimethylaminopyridine (6.0 mmol, 3.0 equiv, 733 mg), and 4-toluenesulfonyl chloride (2.4 mmol, 1.2 equiv, 0.458 g) were dissolved in CH_2Cl_2 (20 mL) and allowed to stir for 16 h at 23 °C. The reaction mixture was diluted with additional CH_2Cl_2 (20 mL) and washed with water (30 mL), aqueous 1M HCl (30 mL), and saturated aqueous $NaHCO_3$ (30 mL). The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure to yield the title compound: R_f: 0.45 (2:8 EtOAc/Hexanes); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.2–1.4 (m, 12H), 1.45 (p, J = 7.2 Hz, 2H), 1.63 (p, J = 7.2 Hz, 2H), 1.78 (p, J = 7.2 Hz, 2H), 2.45 (s, 3H), 3.53 (t, J = 6.8 Hz, 1H), 3.95 (t, J = 6.8 Hz, 2H), 4.02 (t, J = 6.8 Hz, 1H), 6.88–6.95 (m, 3H), 7.28 (t, J = 7.2 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.2 Hz, 2H).

4.1.11. *N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)-5′-*O*-[*N*-(11-phenoxyundecyl)sulfamoyl]-2′,3′-*O*-isopropylideneadenosine (88)—

To a solution of *N*^{6},*N*^{6}-bis(*tert*-butoxycarbonyl)-2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine **87** (1.0 mmol, 1.0 equiv, 386 mg) in DMF (25 mL) was added 11-phenoxyundecyl 4-methylbenzenesulfonate **86** (1.5 mmol, 1.5 equiv, 628 mg) and cesium carbonate (2.5 mmol, 2.5 equiv, 341 mg). The reaction mixture was stirred at 23°C for 16 h and then concentrated under reduced pressure. Purification by flash chromatography (0–10% CH_2Cl_2/MeOH) afforded the title compound (106 mg, 21%) as a white solid: R_f 0.5 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.24–1.35 (m, 14H), 1.38 (s, 3H), 1.45 (p, J = 7.2 Hz, 2H), 1.56–1.58 (m, 18H), 1.59 (s, 3H), 1.74 (p, J = 7.2 Hz, 2H), 2.79 (m, 2H), 3.93 (t, J = 7.2 Hz, 2H), 4.21 (m, 1H), 4.24 (m, 1H), 4.48 (q, J = 3.0 Hz, 1H), 5.13 (dd, J = 6.0, 3.0, 1H), 5.44 (dd, J = 6.0, 3.0 Hz, 1H), 6.24 (d, J = 3.0 Hz, 1H), 6.85–6.89 (m, 3H), 7.22 (t, J = 7.2 Hz, 2H), 8.21 (s, 1H), 8.25 (s, 1H); MS (ESI+) calcd for $C_{40}H_{60}N_6O_{11}S$ [M + H]$^+$ 833.4, found 833.4.

4.1.12. 5′-*O*-[*N*-(11-Phenoxyundecyl)sulfamoyl]adenosine (89)—

The title compound was prepared from **88** using the general procedure for TFA deprotection (97%): R_f 0.26 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.28–1.35 (m, 12H), 1.40–1.47 (m, 4H), 1.74 (p, J = 7.2 Hz, 2H), 2.92 (t, J = 7.2 Hz, 2H), 3.94 (t, J = 7.2 Hz, 2H), 4.28–4.32 (m, 2H), 4.34–4.36 (m, 1H), 4.43 (t, J = 6.0 Hz, 1H), 4.68 (t, J = 6.0 Hz, 1H), 6.07 (d, J = 6.0 Hz, 1H), 6.85–6.90 (m, 3H), 7.23 (t, J = 7.2 Hz, 2H), 8.21 (s, 1H), 8.28 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 27.2, 28.2, 30.2, 30.4, 30.51, 30.54, 30.67, 30.70, 30.8, 45.1, 59.7, 68.9, 71.7, 77.2, 85.2, 95.3, 115.5 (2C), 121.2, 121.5, 130.4 (2C), 140.6, 140.9, 150.3, 158.7, 160.6; HRMS (ESI+) calcd for $C_{27}H_{40}N_6O_7S$ [M + H]$^+$ 593.2757, found 593.2756 (error 0.2 ppm).

4.1.13. *N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)-5′-*O*-4-methylbenzenesulfonate-2′,3′-*O*-isopropylideneadenosine (91)—

N^6,*N*^{6}-bis(*tert*-Butoxycarbonyl)-2′,3′-*O*-isopropylideneadenosine **90** (0.25 mmol, 1.0 equiv, 129 mg), 4-toluenesulfonyl chloride (0.31 mmol, 1.2 equiv, 59 mg), and 4-dimethylaminopyridine (0.76 mmol, 3.0 equiv, 93 mg) were dissolved in CH_2Cl_2 (8 mL) and stirred at 23 °C for 16 h. The reaction mixture was diluted with additional CH_2Cl_2 (22 mL) and washed with water (30 mL), aqueous 1M HCl (30 mL), and saturated aqueous $NaHCO_3$ (30 mL). The organic extract was dried ($MgSO_4$)

and concentrated under reduced pressure to give the title compound (162 mg, >98%) as a white solid: R_f 0.44 (1:1 Hexanes/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.49 (s, 18H), 1.61 (s, 3H), 2.41 (s, 3H), 4.23 (qd, J = 10.8, 5.0 Hz, 2H), 4.46–4.69 (m, 1H), 5.04 (dd, J = 6.3, 3.1 Hz, 1H), 5.33 (dd, J = 6.3, 2.5 Hz, 1H), 6.14 (d, J = 3.0 Hz, 1H), 7.25 (d, J = 7.2 Hz, 2H), 7.65 (d, J = 7.2 Hz, 2H), 8.11 (s, 1H), 8.77 (s, 1H); MS (ESI+) cald for $C_{30}H_{39}N_5O_{10}S$ $[M + H]^+$ 662.2490, found 662.2496 (error 0.9 ppm).

4.1.14. *N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)-5′-deoxy-5′-[(11-phenoxyundecyloxy)sulfonyl]amino-2′,3′-*O*-isopropylideneadenosine (92)—

To a solution of *N*^{6},*N*^{6}-bis(*tert*-butoxycarbonyl)-5′-*O*-4-methylbenzenesulfonate-2′,3′-*O*-isopropylideneadenosine **91** (0.23 mmol, 1.0 equiv, 149 mg) in DMF (10 mL) was added 11-phenoxyundecyl sulfamate **85** (0.28 mmol, 1.2 equiv, 96 mg) and cesium carbonate (0.69 mmol, 3.0 equiv, 225 mg). The reaction was stirred at 23°C for 16 h. The resulting mixture was heated to 50 °C for an additional 24 h. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was used in next step without further purification.

4.1.15. 5′-Deoxy-5′-{[(11-phenoxyundecyloxy)sulfonyl]amino}adenosine (93)—

The title compound was prepared from **92** using the general procedure for TFA deprotection (11% over 2 steps): R_f 0.4 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.24–1.36 (m, 12H), 1.45 (p, J = 7.2 Hz, 2H), 1.68 (p, J = 7.2 Hz, 2H), 1.74 (p, J = 7.2 Hz, 2H), 3.42 (d, J = 3.3 Hz, 1H), 3.94 (t, J = 6.4 Hz, 2H), 4.04–4.11 (m, 2H), 4.28–4.30 (m, 1H), 4.32 (dd, J = 5.4, 2.6 Hz, 1H), 4.82–4.84 (m, 1H), 5.90 (d, J = 7.2 Hz, 1H), 6.86–6.90 (m, 3H), 7.23 (t, J = 7.2 Hz, 2H), 8.21 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 25.1, 25.7, 28.3, 28.6, 28.8, 28.9, 29.0, 29.0, 29.1, 45.2, 67.4, 69.7, 71.3, 72.6, 83.3, 88.4, 114.5 (2C), 119.7, 120.4, 129.6 (2C), 140.6, 148.9, 152.4, 156.4, 158.8; HRMS (ESI+) calcd for $C_{27}H_{39}N_6O_7S$ $[M + H]^+$ 593.2752, found 593.2759 (error 1.2 ppm).

4.2 Minimum Inhibitory Concentration Assay

Minimum inhibitory concentrations (MICs) were determined in quadruplicate in GAST[71] or 7H9 broth base supplemented with 5g/L BSA fraction V, 0.8g/L NaCl, 0.05% Tyloxapol and the appropriate carbon source according to the broth microdilution method[72] using compounds from DMSO stock solutions or with control wells treated with an equivalent amount of DMSO. The carbon sources were either 0.05 mM sodium palmitate or 20 mM glycerol/10 mM glucose. All MIC experiments were performed in triplicate and used an initial cell density of 10^4-10^5 cells/assay in a volume of 100 μL and growth was monitored at 7 and 14 days. The strains used were *Mtb* H37Rv ATCC 27294 or a subset of previously reported clinical strains.[73]

4.3. Enzyme Kinetic Assay

Apparent K_i values were determined using a coupled continuous assay employing FAAL28 or FACL19, pyrophosphatase, and nucleoside phosphorylase under initial velocity conditions as described.[74] Reactions contained 0.45 μM FAAL28 or 0.167 μM FACL19 in a buffer of 50 mM Trizma·HCl pH 8.0, 2.5 mM ATP, 5 mM $MgCl_2$, 0.5 mM DTT, 150 mM hydroxylamine pH 7, 0.1 U nucleoside phosphorylase, 0.04 U pyrophosphatase, 0.2 mM 7-

methylthioguanosine (MesG), and 33 μM tetradecanoic acid (K_M = 5.3 μM FAAL28) or 250 μM dodecanoic acid. Reactions were run in triplicate in 96-well half-area UV Star plates (Greiner) and the cleavage of MesG was monitored at A_{360} on a Molecular Devices Spectramax M5e plate reader. K_i^{app} values were determined by fitting the concentration-response plots to the Morrison equation since the inhibitors exhibited tight-binding behavior ($K_i^{app} \leq 100 \times [E]$).[75]

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

4.4. Cytotoxicity Assay

African green monkey *Cercopithecus aethiops* kidney cells (Vero, ATCC) cells were plated in 96-well plates at (2.5–5.0) × 10^4 cells per well (200 μL). Vero cells were maintained in minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Compounds were prepared as 20 mM stock solutions in DMSO, and 1 μL of the compound stock solution was added to each well in 200 μL of MEM, yielding a final compound concentration of 100 μM. Control wells contained either 50% DMSO (negative control) or 0.5% DMSO (positive control), and all reactions were done in triplicate. The plate was incubated for 72 h at 37 °C in a 5% 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).[76] MTT was prepared fresh at 1 mg/mL in serum-free, phenol red free RPMI 1640 media. 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 isopropanol. 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.

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Abbreviations

TB	tuberculosis
Mtb	Mycobacterium tuberculosis
FadD	fatty acid adenylating enzyme
FAAL	fatty acyl-AMP ligase
FACL	fatty acyl-CoA ligase
PKS	polyketide synthase

PDIM	phthiocerol dimycocerosate
FAS	fatty acid synthase
MDR	multi-drug resistant
XDR	extensively drug resistant
SAR	structure-activity relationship

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Highlights

- 5′-*O*-[*N*-(alkanoyl)sulfamoyl]adenosine (alkanoyl adenosine monosulfamate, alkanoyl-AMS) analogues were synthesized and evaluated for their antitubercular activity.
- 11-Phenoxyundecanoyl-AMS **32** inhibits Mycobacterium tuberculosis with a minimum inhibitory concentration of 3 μM.
- Compound **32** selectively inhibits the fatty acyl AMP ligase (FAAL28) over the fatty acyl CoA ligase (FACL19).

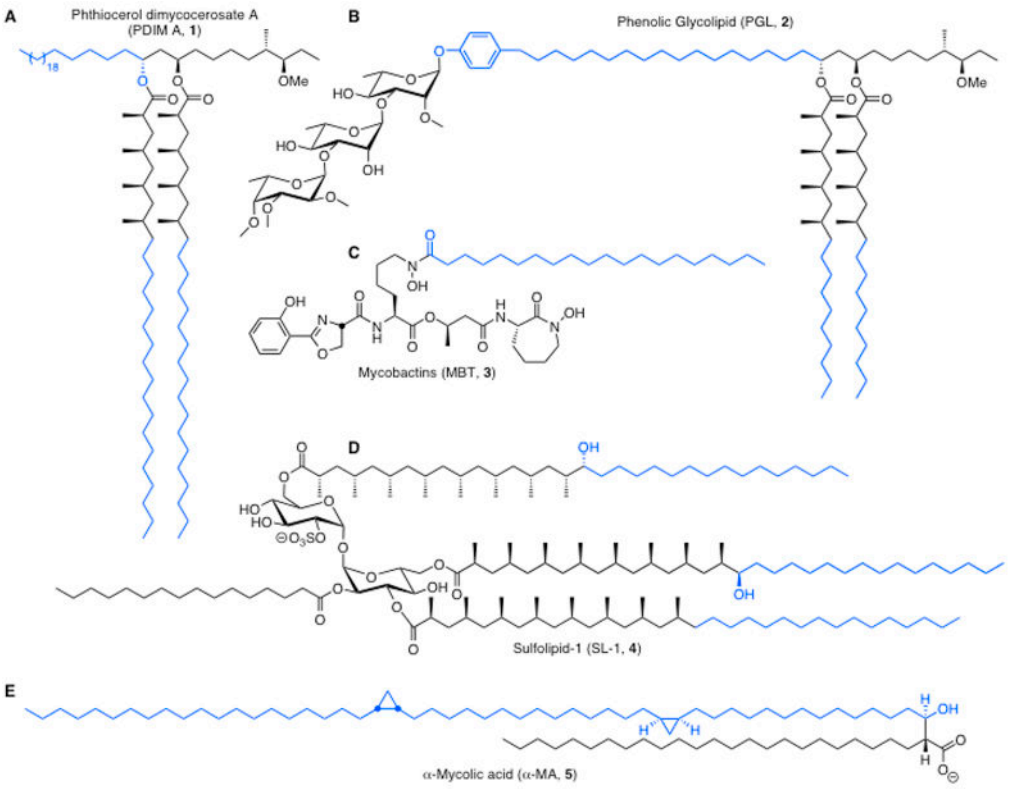


Fig. 1. Unique lipids found in cell envelope of *Mtb*.

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. Specific FadDs are responsible for installation of the lipid chains highlighted in blue. **A.** PDIM A (**1**) biosynthesis requires FAAL26 and FAAL28 for synthesis of phthiocerol and mycocerosic acid moieties, respectively. **B.** PGLs (**2**) require FAAL22 and FAAL29 for assembly of the phenolphthiocerol lipid as well as FAAL28 for the two mycocerosic acids. **C.** MBTs (**3**) employ FAAL33 for installation of the C-20 lipid residue on the central lysine moiety. **D.** Sulfolipids represented by SL-1 (**4**) require FAAL23 for biosynthesis of the phthioceranic acid and two hydroxyphthioceranic acid groups. **E.** The mycolic acids represented by the most abundant α-MA (**5**) employ FAAL32 for introduction of the meromycolic acid subunit.

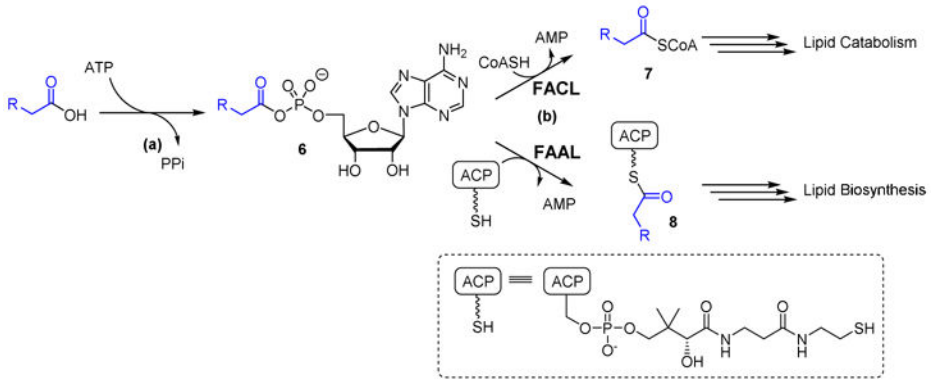


Fig. 2. FadD enzyme mechanism.

FadDs catalyze a two-step reaction. In the first step (a) FadDs catalyze the adenylation of a fatty acid to afford an intermediate acyladenylate **6**. In the second reaction (b) FadDs catalyze the acylation of an acceptor molecule resulting in thioester products **7** or **8**. FadDs that form CoA esters **7** are classified as fatty acyl CoA ligases (FACLs) whereas FadDs that load the ACP domain of polyketide synthase enzymes to provide **8** are known as fatty acyl AMP ligases (FAALs).

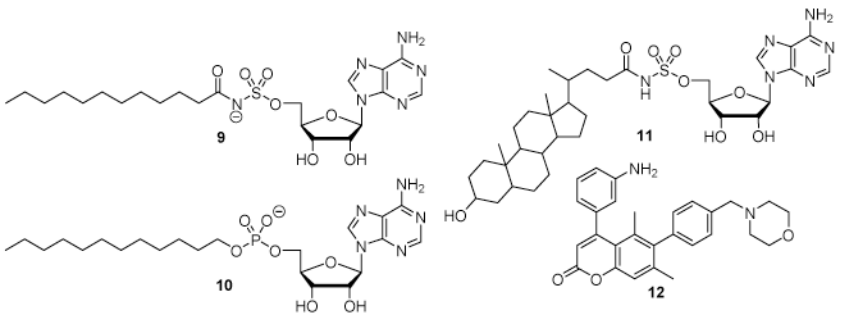


Fig. 3. Previously described nucleoside-based FadD inhibitors.

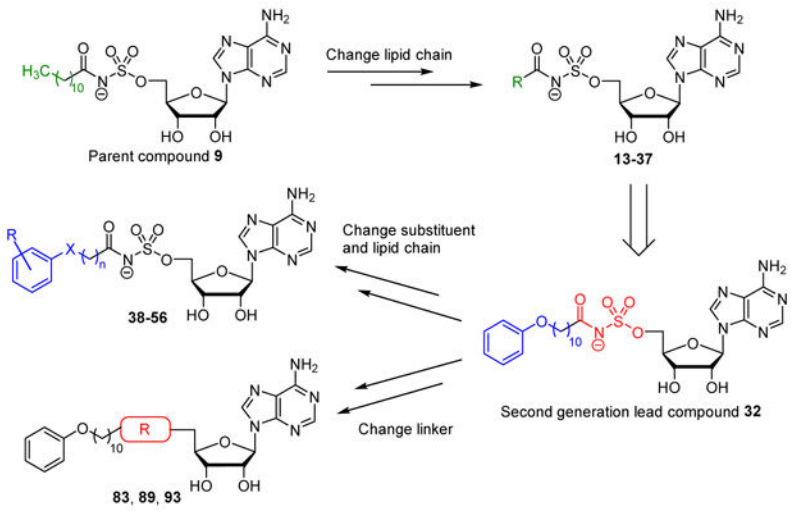
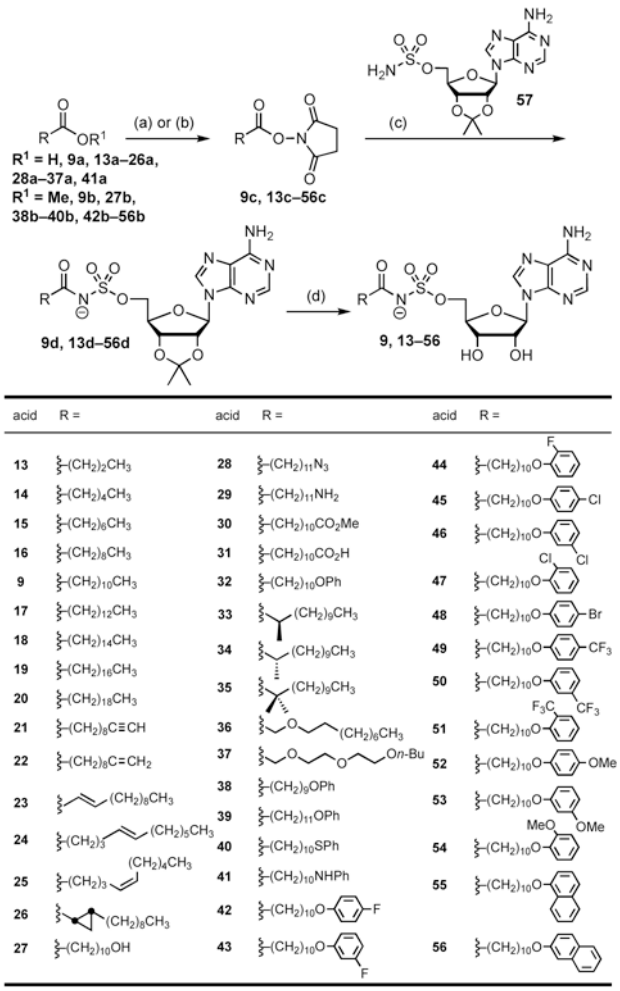


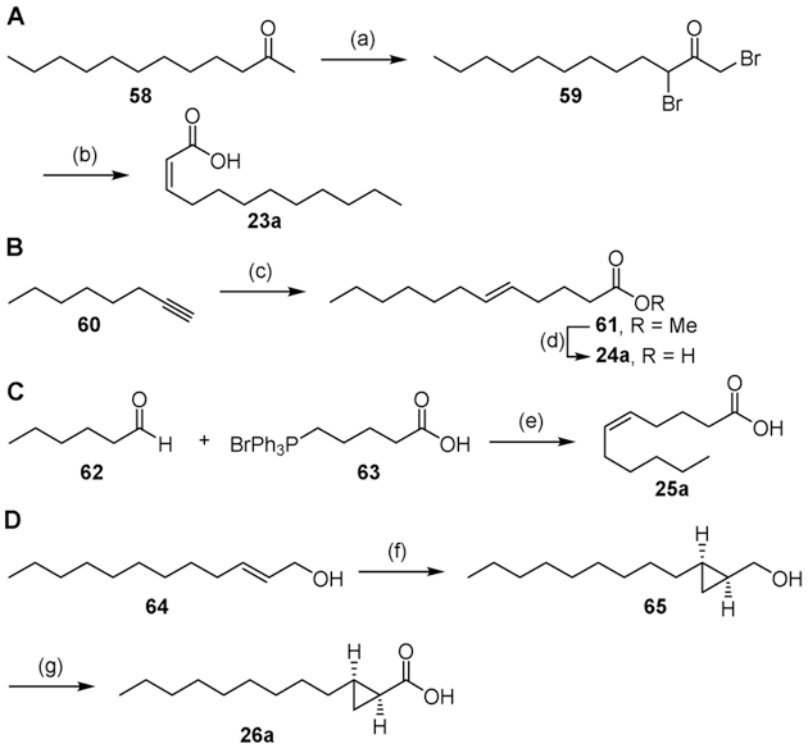
Fig. 4. Rational design of the new acyl-sulfamoyl adenosine-based inhibitors.



acid	R =	acid	R =	acid	R =
13	$-(CH_2)_2CH_3$	28	$-(CH_2)_{11}N_3$	44	$-(CH_2)_{10}O$-(2-F-Ph)
14	$-(CH_2)_4CH_3$	29	$-(CH_2)_{11}NH_2$	45	$-(CH_2)_{10}O$-(4-Cl-Ph)
15	$-(CH_2)_6CH_3$	30	$-(CH_2)_{10}CO_2Me$	46	$-(CH_2)_{10}O$-(2,4-Cl$_2$-Ph)
16	$-(CH_2)_8CH_3$	31	$-(CH_2)_{10}CO_2H$		
9	$-(CH_2)_{10}CH_3$	32	$-(CH_2)_{10}OPh$	47	$-(CH_2)_{10}O$-(3,4-Cl$_2$-Ph)
17	$-(CH_2)_{12}CH_3$	33	$-(CH_2)_9CH_3$ (branched)	48	$-(CH_2)_{10}O$-(4-Br-Ph)
18	$-(CH_2)_{14}CH_3$	34	$-(CH_2)_9CH_3$ (branched)	49	$-(CH_2)_{10}O$-(4-CF$_3$-Ph)
19	$-(CH_2)_{16}CH_3$	35	$-(CH_2)_9CH_3$ (branched)	50	$-(CH_2)_{10}O$-(3,5-(CF$_3$)$_2$-Ph)
20	$-(CH_2)_{18}CH_3$	36	$-O(CH_2)_6CH_3$	51	$-(CH_2)_{10}O$-(4-OMe-Ph)
21	$-(CH_2)_8C\equiv CH$	37	$-O(CH_2)_2O(n\text{-}Bu)$	52	$-(CH_2)_{10}O$-(3,4-(OMe)$_2$-Ph)
22	$-(CH_2)_8C=CH_2$	38	$-(CH_2)_9OPh$	53	$-(CH_2)_{10}O$-(1-naphthyl)
23	$-(CH_2)_8CH_3$ (cis)	39	$-(CH_2)_{11}OPh$	54	$-(CH_2)_{10}O$-(2-naphthyl)
24	$-(CH_2)_3$-(cis)-$(CH_2)_5CH_3$	40	$-(CH_2)_{10}SPh$	55	$-(CH_2)_{10}O$-(2-quinolyl)
25	$-(CH_2)_3$-(cis)-$(CH_2)_4CH_3$	41	$-(CH_2)_{10}NHPh$		
26	$-$(cyclopropyl)$-(CH_2)_8CH_3$	42	$-(CH_2)_{10}O$-(3-F-Ph)	56	$-(CH_2)_{10}O$-(1-naphthyl)
27	$-(CH_2)_{10}OH$	43	$-(CH_2)_{10}O$-(2-F-Ph)		

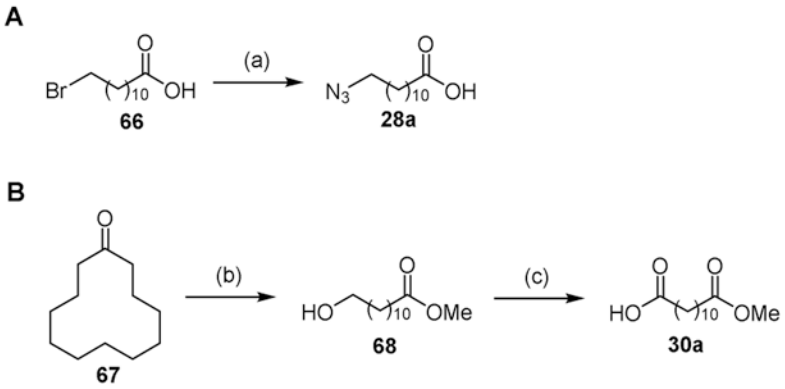
Scheme 1.

Synthesis of acyl sulfamate inhibitors **9** and **13–56**. Reaction conditions: (a) *N*-hydroxysuccinimide, DCC, CH_2Cl_2; (b) 1 N aqueous NaOH/MeOH, 100 °C, then *N*-hydroxysuccinimide, EDC, CH_2Cl_2; (c) Cs_2CO_3, DMF, 78–93%; (d) 80% aqueous TFA, 18–89%.



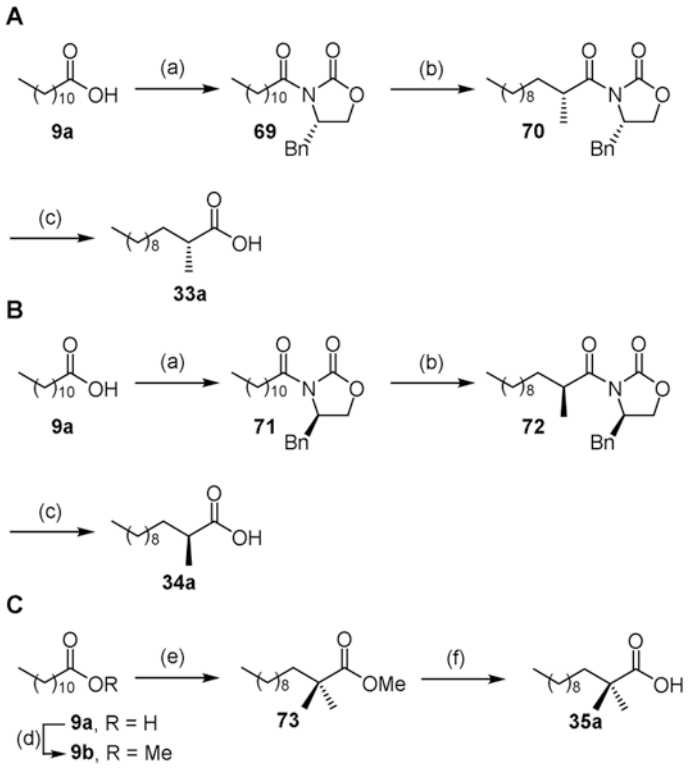
Scheme 2.

Synthesis of acids **23a–26a**. Reaction conditions: (a) HBr (aq), Br_2, 99%; (b) NaOH (aq), then HCl (aq), 87%; (c) Cp_2ZrHCl, $Pd_2(dba)_3$, LiBr, NMP/THF, then ethyl 4-bromobutyrate, 79%; (d) LiOH, MeOH/H_2O, 70%; (e) NaHMDS, THF, 40%; (f) (4*R*,5*R*)-2-butyl-*N*,*N*,*N*′*N*′-tetramethyl-1,3,2-dioxaborolane-4,5-dicarboxamide, $Zn(CH_2I)_2$, CH_2Cl_2, 66%; (g) CrO_3, H_2SO_4, H_2O/acetone, 75%.



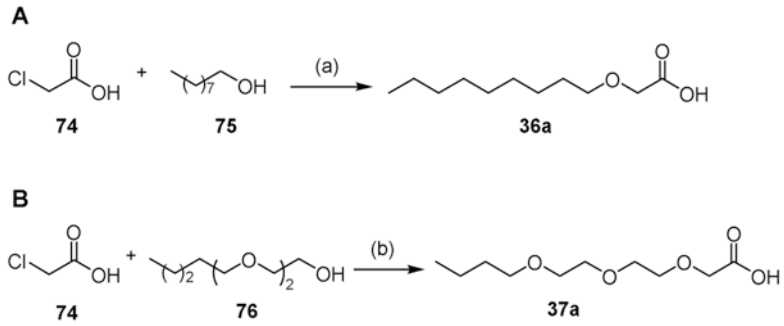
Scheme 3.

Synthesis of acids **28a** and **30a**. Reaction conditions: (a) NaN_3, DMSO, 97%; (b) H_2SO_4, $K_2S_2O_8$, MeOH, 83%; (c) CrO_3, H_2SO_4, H_2O/acetone, 57%.



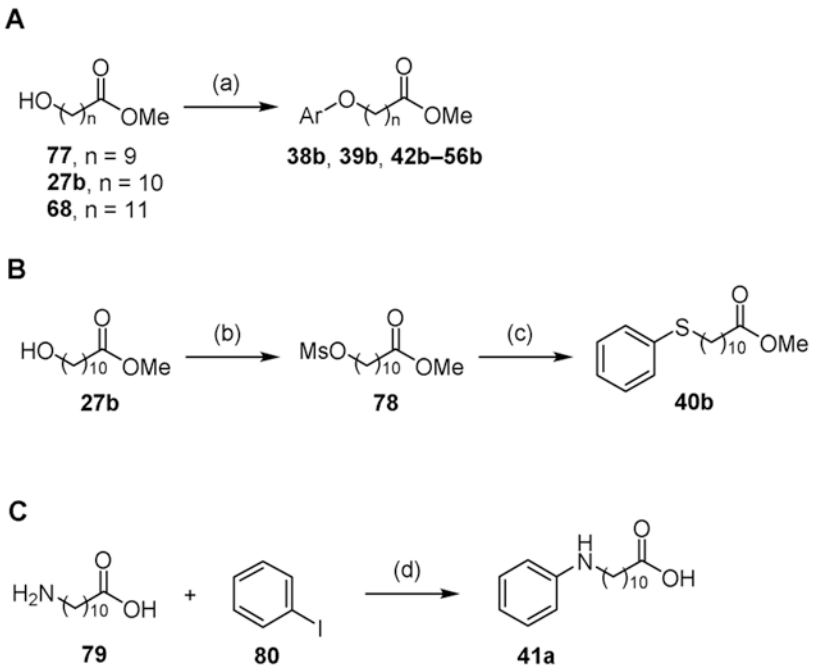
Scheme 4.

Synthesis of acids **33a–35a**. Reaction conditions: (a) PivCl, DIPEA, THF, then *n*-BuLi, (*S*) or (*R*)-4-benzyl-2-oxazolidinone, THF, 97%; (b) NaHMDS, MeI, THF, 78%; (c) 30% H_2O_2, LiOH, THF/H_2O, 99%; (d) H_2SO_4, MeOH, 99%; (e) LDA, THF, then MeI, repeated 2×, 53% (2 steps), (f) LiOH, THF/MeOH/H_2O, 88%.



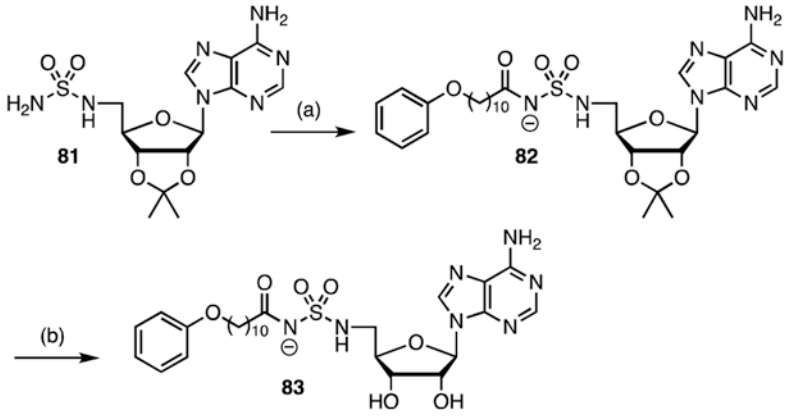
Scheme 5.

Synthesis of **36a** and **37a**. Reaction conditions: (a) NaH, THF: 30% (**36a**), 55% (**37a**).



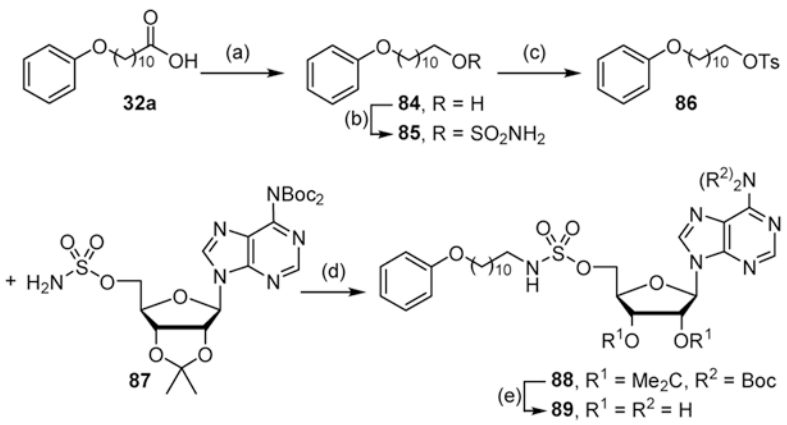
Scheme 6.

Synthesis of **41a**, **38b**–**56b**. Reaction conditions: (a) ArOH, DIAD, PPh_3, THF, 65–95%; (b) MsCl, Et_3N, THF, 97%; (c) NaH, THF, thiophenol, 98%; (d) 10 mol% CuI, $K_3PO_4 \cdot H_2O$, H_2O/decanol, 84%.



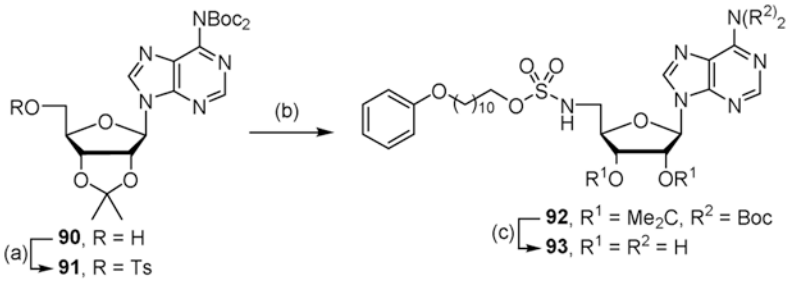
Scheme 7.

Synthesis of acylsulfamide **83**. Reaction conditions: (a) Cs_2CO_3, DMF, **32c**; (b) 80% aqueous TFA, 42% (2 steps).



Scheme 8.

Synthesis of sulfamate **89**. Reaction conditions: (a) $LiAlH_4$, THF, 80%; (b) $ClSO_2NH_2$, NaH, THF, 100%; (c) TsCl, DMAP, CH_2Cl_2, 100%; (d) Cs_2CO_3, DMF, 22%; (e) 80% aqueous TFA, 97%.

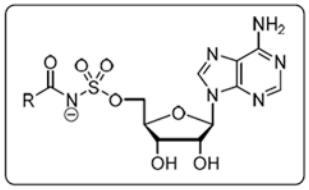


Scheme 9.

Synthesis of reverse sulfamate **93**. Reaction conditions: (a) TsCl, DMAP, CH_2Cl_2, 98%; (b) Cs_2CO_3, DMF, **85**; (c) 80% aqueous TFA, 11% (2 steps).

Table 1.

Biological activity and cytotoxicity of 5′-*O*-[*N*-(acyl)sulfamoyl]adenosine analogues 9, 13–37.



		MIC (μM)			K_i^{app} (μM)		Cytotox
Cmpd	**R=**	**GAST**[a]	**7H9**[b]	**7H9/palmitate**[c]	**FACL19**[d]	**FAAL28**[e]	**IC_{50} (μM)**[f]
13	$CH_3(CH_2)_2$-	>100	>100	>100	>100	>100	81.4
14	$CH_3(CH_2)_4$-	100	>100	>100	>100	>100	48.6
15	$CH_3(CH_2)_6$-	12.5–25	100	>100	>100	>100	6.0
16	$CH_3(CH_2)_8$-	1.56	100	>100	>100	88	10.1
9	$CH_3(CH_2)_{10}$-	0.19–0.39	50	50	67.4	6.4	28.7
17	$CH_3(CH_2)_{12}$-	0.39–0.78	50	100	10.0	1.4	0.9
18	$CH_3(CH_2)_{14}$-	6.25	100	>100	3.2	1.0	0.3
19	$CH_3(CH_2)_{16}$-	100	100	>100	2.4	0.5	0.1
20	$CH_3(CH_2)_{18}$-	>100	>100	>100	nd[g]	nd	0.1
21	$HCC(CH_2)_8$-	1.56	50	50	>100	>100	9.7
22	$H_2CHC(CH_2)_8$-	0.39–0.78	25	25	>100	>100	11.6
23	$CH_3(CH_2)_7CH_2CHCH$-*trans*-	100	>100	>100	>100	>100	10.4
24	$CH_3(CH_2)_5CHCH(CH_2)_3$-*trans*-	100	>100	>100	>100	>100	16.8
25	$CH_3(CH_2)_4CHCH(CH_2)_3$-*cis*-	6.25	100	100	>100	>100	14.3
26	$CH_3(CH_2)_8CH(CH_2)CH$-*S,S*-	50	>100	>100	>100	69.8	6.1
27	$HO(CH_2)_{10}$-	6.25	100	100	>100	92.7	42.7
28	$N_3(CH_2)_{11}$-	0.19	12.5–25	25	>100	28.5	5.6
29	$NH_2(CH_2)_{11}$-	3.13	50	12.5–25	93.3	>100	27.1
30	$MeOOC(CH_2)_{10}$-	3.13	100	>100	>100	>100	14.0
31	$HOOC(CH_2)_{10}$-	25–50	>100	>100	>100	>100	>100
32	$C_6H_5O(CH_2)_{10}$-	3.13	50	50	>100	0.71	2.0
33	$CH_3(CH_2)_9CH(CH_3)$-*R*-	12.5–25	>100	>100	>100	>100	39.4
34	$CH_3(CH_2)_9CH(CH_3)$-*S*-	50	>100	>100	>100	>100	14.5
35	$CH_3(CH_2)_9C(CH_3)_2$-	12.5–25	>100	>100	54.7	>100	6.7
36	$CH_3(CH_2)_8OCH_2$-	0.098–0.19	25	25–50	>100	>100	8.7
37	$CH_3(CH_2)_3O(CH_2)_2O(CH_2)_2OCH_2$-	6.25	>100	>100	>100	>100	16.0

Microbiological and biochemical data is from 3 replicates.

[a] Whole cell inhibitory activity against *Mtb* H37Rv in GAST medium.

[b] Whole cell inhibitory activity against *Mtb* H37Rv in 7H9 medium.

[c] Whole cell inhibitory activity against *Mtb* H37Rv in 7H9/palmitate medium.

[d] Inhibitory activity against FACL19. The standard deviation was ≤10% of the mean.

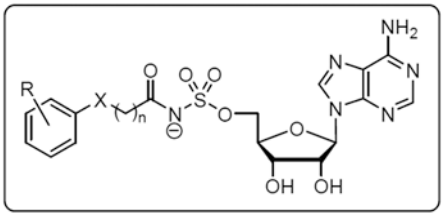
[e] Inhibitory activity against FAAL28. The standard deviation was ≤10% of the mean.

[f] Cytotoxicity against Vero monkey cells using the MTT assay.

[g] nd = no data.

Table 2.

The enzymatic and antimicrobial activity of 5′-*O*-[*N*-(phenoxyacyl)sulfamoyl]adenosine and related analogues 32, 38–56.



				MIC (μM)[a]	K_i^{app} (μM)[b]	K_i^{app} (μM)[c]
Cmpd	**R =**	**X**	**n =**	**GAST**	**FACL19**	**FAAL28**
32	H	O	10	3.13	>100	0.7
38	H	O	9	50	>100	6.7
39	H	O	11	12.5–25	>100	2.6
40	H	S	10	>50	41.6	6.3
41	H	NH	10	12.5	63.4	7.3
42	*p*-F	O	10	12.5–25	11.4	0.3
43	*m*-F	O	10	25–50	7.3	1.6
44	*o*-F	O	10	>50	>100	1.5
45	*p*-Cl	O	10	6.25–12.5	13.7	2.2
46	*m*-Cl	O	10	25–50	26.4	0.5
47	*o*-Cl	O	10	>50	>100	3.6
48	*p*-Br	O	10	>50	10.4	4.0
49	*p*-CF_3	O	10	25–50	34.4	15.6
50	*m*-CF_3	O	10	25–50	13.5	4.4
51	*o*-CF_3	O	10	>50	28.5	1.3
52	*p*-OMe	O	10	>50	>100	6.6
53	*m*-OMe	O	10	25–50	>100	1.7
54	*o*-OMe	O	10	>50	>100	>100
55	2,3-benzo	O	10	>50	9.64	25.24
56	3,4-benzo	O	10	>50	20.39	1.88

Microbiological data is from 3 replicates.

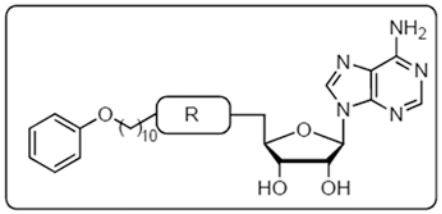
[a]Whole cell inhibitory activity against *Mtb* H37Rv in GAST medium.

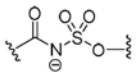
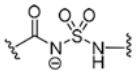
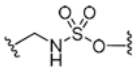
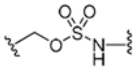
[b]Inhibitory activity against FACL19.

[c]Inhibitory activity against FAAL28.

Table 3.

The enzymatic and antimicrobial activity of linker analogues **83**, **89** and **93**.



Cmpd	R =	MIC (μM)[a] GAST	K_i^{app} (μM)[b] FACL19	K_i^{app} (μM)[c] FAAL28
32		3.13	>100	0.7
83		1.56–3.13	>100	3.1
89		>50	>100	>100
93		>50	>100	>100

Microbiological and biochemical data is from 3 replicates.

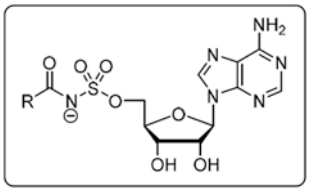
[a]Whole cell inhibitory activity against *Mtb* H37Rv in GAST medium.

[b]Inhibitory activity against FACL19.

[c]Inhibitory activity against FAAL28.

Table 4.

The activity against MDR-TB and XDR-TB strains of most potent compounds.



		MIC (μM)									
		HREZSKPTh[a,b]		HREZSKPTh[a,b]		HREKO[a,b]		HREP[a,c]		HRESPOCTh[a,c]	
Cmpd	**R=**	**7H9[d]**	**GAST[e]**	**7H9**	**GAST**	**7H9**	**GAST**	**7H9**	**GAST**	**7H9**	**GAST**
9	$CH_3(CH_2)_{10}$-	12.5	ng[f]	12.5	25	6.25	3.13	6.25	6.25	12.5	12.5
17	$CH_3(CH_2)_{12}$-	12.5	ng	25	12.5	3.13	6.25	6.25	6.25	12.5	12.5
22	$H_2CHC(CH_2)_8$-	6.25	ng	50	3.13	6.25	3.13	12.5	3.13	25	3.13
28	$N_3(CH_2)_{11}$-	6.25	ng	50	3.13	6.25	3.13	12.5	3.13	25	6.25
32	$C_6H_6O(CH_2)_{10}$-	12.5	ng	12.5	6.25	12.5	3.13	12.5	3.13	50	6.25
36	$CH_3(CH_2)_8OCH_2$-	12.5	ng	12.5	1.56	6.25	0.39	6.25	0.78	50	6.25
Isoniazid[g]		2	nt	2	>8	2	4	2	4	8	8

[a] Whole cell inhibitory activity against *Mtb* with resistance pattern listed as such: H, isoniazid; R, rifampicin; E, ethambutol; Z, pyrazinamide; S, streptomycin; K, kanamycin; P, para-aminosalicylic acid; Th, prothionamide; O, ofloxacin.

[b] MDR-TB strains.

[c] XDR-TB strains.

[d] Whole cell inhibitory activity against *Mtb* in 7H9 medium/tyloxapol.

[e] Whole cell inhibitory activity against *Mtb* in GAST medium.

[f] ng = no growth.

[g] The critical concentration for high-level resistance is 1 μM.