A General Platform for Systematic Quantitative Evaluation of Small-Molecule Permeability in Bacteria

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A. SUPPORTING INFORMATION FIGURES S1–S14

Figure S1. Principal component analysis (PCA) indicates that many antibacterial drugs have distinct structural and physicochemical properties compared to other drug classes. PCA plots of 91 antibacterial drugs and 50 top-selling, brand-name non-antiinfective drugs (Drugs): (a) PC1 vs. PC2, (b) PC1 vs. PC3, (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; *Mtb* = *Mycobacterium tuberculosis*; AVG = hypothetical average for a given dataset.
Figure S2. Loading plots and component loading values indicate the influence of each structural and physicochemical parameter on the positioning of compounds in the PCA plot. Corresponding loading plots for PCA of 91 antibacterial drugs and 50 top-selling, brand-name non-antiinfective drugs in Figure 1a and Figure S1: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2, (d) Component loadings of 20 structural and physicochemical parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S3. Standard calibration curves of salicyl-AMS indicate that media and internal standard influence limits of detection. Calibration curves in: (a) LB media, L-alanyl-AMS internal standard; quantifiable from 0.025–10 μM (2.6 logs), (b) LB media, benzoyl-AMS internal standard; quantifiable from 0.050–500 μM (4.0 logs), and (c) PBS, benzoyl-AMS internal standard; quantifiable from 0.0025–100 μM (4.6 logs).
Figure S4. PCA indicates that sulfonyladenosines have structural and physicochemical properties similar to those of antibacterial drugs and distinct from non-antiinfective drugs. PCA plots of 25 sulfonyladenosines (AMS), 91 antibacterial drugs, and 50 top-selling, brand-name non-antiinfective drugs (Drugs): (a) PC1 vs. PC2, (b) PC1 vs. PC3, (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; Mtb = Mycobacterium tuberculosis; AVG = hypothetical average for a given dataset. Although the PC2 and PC3 axes were inverted compared to Figure S1, the signs of all PC axes are arbitrary. (d) Expanded view of panel (a) to visual positions of sulfonyladenosines.
Figure S5. Loading plots and component loading values indicate a similar influence of structural and physicochemical parameters on the positioning of compounds in the PCA plots with sulfoniyadenosines. Corresponding loading plots for PCA of 25 sulfoniyadenosines, 91 antibacterial drugs, and 50 top-selling, brand-name non-antiinfective drugs in Figure S4: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2, (d) Component loadings of 20 structural and physicochemical parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S6. Accumulation of sulfonyladenosine compounds across bacteria with different cellular envelopes. Alternative view of Figure 4 from the manuscript. Data are reported as mean ± SD for 4 experiments. Statistical significance was assessed using two-way ANOVA and Tukey’s multiple comparison test and 95% confidence intervals. *p < 0.05, **p < 0.01, ***p < 0.001.
Figure S7. PCA biplots indicate relationships between the 10 sulfonyladenosines and structural and physicochemical parameters. Combined PCA and loading plots: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyladenosine compounds; ● = physicochemical parameters. (d) Component loadings of 20 structural and physicochemical parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S8. PCA biplots of sulfonyl adenosines indicate structural and physicochemical parameters that correlate with accumulation in *E. coli*. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; mean sulfonyl adenosine intracellular concentrations (µM) are noted in parentheses; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyl adenosine compounds; ● = physicochemical parameters; ■ = accumulation parameters. (d) Component loadings of 21 structural, physicochemical, and accumulation parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S9. PCA biplots of sulfonyladenosine congeners indicate structural and physicochemical parameters that correlate with accumulation in B. subtilis. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; mean sulfonyladenosine intracellular concentrations (µM) are noted in parentheses; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyladenosine compounds; ● = physicochemical parameters; ■ = accumulation parameters. (d) Component loadings of 21 structural, physicochemical, and accumulation parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S10. PCA biplots of sulfonyladenosine congeners indicate structural and physicochemical parameters that correlate with accumulation in *M. smegmatis*. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; mean sulfonyladenosine intracellular concentrations (\(\mu\)M) are noted in parentheses; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyladenosine compounds; ● = physicochemical parameters; ■ = accumulation parameters. (d) Component loadings of 21 structural, physicochemical, and accumulation parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S11. Heatmap of Pearson pairwise correlation coefficients of 20 physicochemical properties from 10 sulfonlaldenosine compounds used in bacterial compound accumulation assays reveal correlations between the physicochemical parameters. Positive correlations in red; negative correlations in blue; correlations in bold are statistically significant as assessed using two-tailed unpaired t-test and 95% confidence intervals (p < 0.05).
Figure S12. Accumulation of C2-substituted salicyl-AMS analogues in *E. coli* is consistent with correlations identified using sulfonyladenosine variants in the acyl region. (a) Structures and physicochemical properties of C2-substituted sulfonyladenosines, synthesized as previously described.3d (b) Accumulation of C2-substituted sulfonyladenosines in *E. coli* (100 µM extracellular, 30 min, LB media). Data are reported as mean ± SD for 4 experiments. Statistical significance was assessed relative to cells treated with salicyl-AMS using one-way ANOVA and Tukey’s multiple comparison test and 95% confidence intervals: *p < 0.05, **p < 0.01.
Figure S13. PCA biplots of sulfonyladaenosine congeners indicate structural and physicochemical parameters that correlate with efflux sensitivity in *E. coli*. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyladaenosine compounds; ● = physicochemical parameters; ■ = efflux parameters (calculated for each compound as relative accumulation level compared to concentration in the absence of the efflux pump inhibitor). (d) Component loadings of 22 structural, physicochemical, and efflux parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S14. PCA biplots of sulfonyladenosine congeners indicate structural and physicochemical parameters that correlate with efflux sensitivity in *B. subtilis*. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyladenosine compounds; ● = physicochemical parameters; ▼ = efflux parameters (calculated for each compound as relative accumulation level compared to concentration in the absence of the efflux pump inhibitor). (d) Component loadings of 22 structural, physicochemical, and efflux parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
Figure S15. PCA biplots of sulfonyl-adenosine congeners indicate structural and physicochemical parameters that correlate with efflux sensitivity in *M. smegmatis*. Combined PCA and loading plots of: (a) PC1 vs. PC2, (b) PC1 vs. PC3, and (c) PC3 vs. PC2; percent contribution for each principal component is indicated on the axes; ▲ = sulfonyl-adenosine compounds; ● = physicochemical parameters; ■ = efflux parameters (calculated for each compound as relative accumulation level compared to concentration in the absence of the efflux pump inhibitor). (d) Component loadings of 22 structural, physicochemical, and efflux parameters on the first three principal components; the five most influential parameters for each principal component are highlighted (yellow).
### B. SUPPORTING INFORMATION TABLES S1–S4

**Table S1. Non-antiinfective drugs and antibacterials used in PCA**

| Series                  | Compounds                          |
|-------------------------|------------------------------------|
| **Top-selling Drugs**   |                                    |
| **Non-antiinfective**   |                                    |
| (50 entries)            |                                    |
| Nexium                  | Diovan                             |
| Abilify                 | Lyrica                             |
| Crestor                 | Lipitor                            |
| Advair                  | Celebrex                           |
| Cymbalta                | Gleevec                            |
| Singular                | Namenda                            |
| Plavix                  | Actos                              |
| Spiriva                 | Vyvanse                            |
| Oxycontin               | Seroquel                           |
| Januvia                 | Zetia                              |
| **β-Lactams**           |                                    |
| (38 entries)            |                                    |
| Amoxicillin             | Cefitoren pivoxil                   |
| Ampicillin              | Cefepime                           |
| Azlocillin             | Cefixime                           |
| Carbenicillin           | Cefmenoxime                         |
| Cefaclor                | Cefmetazole                         |
| Cefadroxil              | Ceforanide                         |
| Cefalotin               | Cefotaxime                          |
| Cefdinir                | Cefotiam                            |
| **Peptides**            |                                    |
| (4 entries)             |                                    |
| Colistin                | Daptomycin                          |
| **Aminoglycosides**     |                                    |
| (7 entries)             |                                    |
| Gentamicin              | Kanamycin                           |
| Amikacin                | Neomycin                            |
| **Quinolones**          |                                    |
| (15 entries)            |                                    |
| Besifloxacin            | Gatifloxacin                         |
| Ciprofloxacin           | Gemifloxacin                         |
| Enoxacin                | Grepafloxacin                       |
| **Macrolides**          |                                    |
| (7 entries)             |                                    |
| Azithromycin            | Dirithromycin                        |
| Clarithromycin          | Erythromycin                         |
| **Tetracyclines**       |                                    |
| (7 entries)             |                                    |
| Oxytetracycline         | Demeclocycline                       |
| Clomocycline            | Doxycycline                          |
| **Oxazolidinones**      |                                    |
| (2 entries)             |                                    |
| Linezolid               | Ranezolid                            |
| **Tuberculosis Drugs**  |                                    |
| (5 entries)             |                                    |
| Bedaquiline             | Ethambutol                           |
| Sulfa Drugs             |                                    |
| (6 entries)             |                                    |
| Sulfadiazine            | Sulfamethoxazole                     |
| Sulfamethizole          | Sulfanilamide                        |
### Table S2. Structural and physicochemical properties used in PCA

| Parameter  | Description                                                                 | Method of Determination |
|------------|------------------------------------------------------------------------------|-------------------------|
| MW         | molecular weight                                                             | Instant JChem           |
| SA         | surface area                                                                 | Instant JChem           |
| N          | number of nitrogens                                                          | Instant JChem           |
| O          | number of oxygens                                                            | Instant JChem           |
| HBD        | number of hydrogen bond donors                                               | Instant JChem           |
| HBA        | number of hydrogen bond acceptors                                            | Instant JChem           |
| LogD       | calc n-octanol/water partition coefficient (pH 7.4)                          | Instant JChem           |
| LogP       | calc n-octanol/water partition coefficient                                   | Instant JChem           |
| ALOGPs     | calc n-octanol/water partition coefficient (Tetko)                           | http://www.vcclab.org   |
| ALOGpS     | calc aqueous solubility (Tetko)                                              | http://www.vcclab.org   |
| tPSA       | topological polar surface area                                               | Instant JChem           |
| relPSA     | tPSA + SA                                                                    | Instant JChem           |
| nStereo    | number of chiral atoms                                                       | Instant JChem           |
| nStMW      | nStereo + MW (stereochemical density)                                        | Instant JChem           |
| Fsp3       | number of sp3 carbons + number of carbons                                    | Instant JChem           |
| RotB       | # rotatable bonds                                                            | Instant JChem           |
| Rings      | number of rings                                                              | Instant JChem           |
| RngAr      | number of aromatic rings                                                     | Instant JChem           |
| RngLg      | number of atoms in largest ring                                              | Instant JChem           |
| RngSys     | number of ring systems                                                       | Instant JChem           |
| RRRSys     | Rings + RngSys (ring complexity)                                             | Instant JChem           |

### Table S3. Average structural and physicochemical properties by compound class.

|                  | Non-antifungal Drugs | Sulfonamides | All Antibiotics | β-Lactams | Peptides | Aminoglycoside | Quinolones | Macrolides | Tetacyclines | Oxazolidinones | Tuberculosis Drugs | Sulfon Drugs |
|------------------|----------------------|--------------|-----------------|-----------|----------|----------------|------------|------------|---------------|-----------------|---------------------|--------------|
| MW               | 417.21               | 477.33       | 493.34          | 440.57    | 1495.26  | 526.72         | 355.92     | 813.66     | 480.88        | 399.39          | 368.60               | 240.44       |
| SA               | 593.38               | 597.80       | 664.30          | 538.55    | 2063.58  | 743.00         | 473.87     | 1327.71    | 613.71        | 562.00          | 539.60               | 321.83       |
| N                | 2.48                 | 6.32         | 4.07            | 2.99      | 13.25    | 5.29           | 3.27       | 1.29       | 2.57          | 4.00            | 2.80                | 3.17         |
| O                | 3.94                 | 7.88         | 6.86            | 6.53      | 22.50    | 10.29          | 3.33       | 14.00      | 8.14          | 5.00            | 3.60                | 2.50         |
| HBD              | 2.04                 | 4.45         | 4.45            | 3.03      | 20.50    | 10.71          | 1.80       | 5.14       | 6.29          | 1.00            | 2.80                | 1.67         |
| HBA              | 4.96                 | 11.84        | 8.88            | 7.18      | 24.50    | 15.43          | 6.60       | 14.00      | 9.57          | 6.00            | 5.60                | 4.33         |
| LogD             | 1.35                 | -2.44        | -3.23           | -3.51     | -12.06   | -13.14         | -0.33      | 1.88       | -4.86         | 1.06            | 0.84                | -0.17        |
| LogP             | 2.62                 | -2.62        | -1.12           | -1.04     | -7.01    | -6.32          | -0.08      | 3.63       | -3.68         | 1.08            | 1.59                | 0.26         |
| ALOGP            | 2.72                 | -0.53        | 0.44            | 0.63      | 0.42     | -2.51          | -0.04      | 3.19       | -0.31         | 1.06            | 1.74                | 0.31         |
| ALOGS            | -4.00                | -2.41        | -2.99           | -3.32     | -4.39    | -1.14          | -2.87      | -3.61      | -2.71         | -2.89           | -2.58               | -2.36        |
| tPSA             | 90.96                | 213.24       | 163.99          | 143.13    | 580.32   | 281.67         | 81.97      | 204.38     | 186.42        | 97.60           | 93.43               | 93.87        |
| relPSA           | 14.98                | 36.08        | 25.34           | 26.72     | 27.90    | 37.85          | 17.40      | 15.36      | 30.78         | 17.02           | 23.76               | 29.57        |
| nStereo          | 2.50                 | 4.16         | 5.13            | 3.00      | 15.50    | 14.71          | 0.93       | 17.71      | 5.00          | 1.00            | 2.60                | 0.00         |
| nStMW +         | 5.54                 | 8.68         | 8.68            | 7.20      | 10.49    | 27.90          | 2.41       | 22.26      | 10.56         | 2.57            | 4.87                | 0.00         |
| Fsp3             | 0.44                 | 0.43         | 0.47            | 0.40      | 0.57     | 0.96           | 0.42       | 0.91       | 0.43          | 0.46            | 0.35                | 0.14         |
| RotB             | 6.62                 | 5.80         | 6.31            | 6.00      | 26.50    | 7.86           | 3.07       | 9.86       | 3.00          | 5.50            | 4.80                | 2.33         |
| Rings            | 3.16                 | 3.80         | 3.47            | 3.50      | 6.25     | 3.14           | 3.73       | 3.29       | 4.00          | 3.50            | 2.40                | 1.67         |
| RngAr            | 1.98                 | 2.60         | 1.36            | 1.32      | 3.25     | 0.00           | 2.07       | 0.14       | 1.00          | 1.50            | 1.80                | 1.67         |
| RngLg            | 6.44                 | 7.04         | 7.45            | 5.92      | 21.50    | 6.00           | 6.07       | 14.71      | 6.00          | 6.00            | 8.40                | 8.83         |
| RngSys           | 2.22                 | 2.66         | 2.42            | 2.50      | 3.28     | 3.14           | 2.47       | 3.14       | 1.00          | 3.50            | 1.40                | 1.67         |
| RRRSys           | 1.60                 | 1.69         | 1.55            | 1.43      | 1.83     | 1.00           | 1.56       | 1.05       | 4.00          | 1.23            | 1.00                | 1.00         |

‡ For clarity, values shown are [nStMW x 1000]
Table S4. Structural and physicochemical properties of sulfonyladenosines evaluated in bacterial compound accumulation assays

|        | H-AMS (2) | L-alp-AMS (3) | L-lac-AMS (4) | Me-suc-AMS (5) | 4-antra-AMS (6) | OSB-AMS (7) | Me-AMS (1) | Bz-AMS (8) | 4-PHBz-AMS (9) | doc-AMS (10) |
|--------|-----------|---------------|---------------|---------------|-----------------|------------|-----------|-----------|----------------|-------------|
| MW     | 346       | 417           | 418           | 460           | 465             | 550        | 466       | 450       | 526            | 500         |
| SA     | 450       | 523           | 519           | 583           | 567             | 679        | 563       | 554       | 661            | 725         |
| N      | 6         | 7             | 6             | 6             | 7               | 6          | 6         | 6         | 6              | 6           |
| O      | 6         | 7             | 8             | 9             | 7               | 10         | 8         | 7         | 7              | 7           |
| HBD    | 4         | 4             | 4             | 4             | 5               | 5          | 5         | 4         | 4              | 4           |
| HBA    | 10        | 12            | 12            | 12            | 12              | 12         | 12        | 11        | 11             | 11          |
| LogD   | -2.81     | -3.91         | -3.85         | -3.60         | -1.89           | -5.41      | -1.43     | -1.71     | -0.07          | 0.25        |
| LogP   | -2.81     | -3.90         | -4.66         | -4.33         | -2.58           | -3.30      | -2.11     | -2.41     | -0.76          | -0.49       |
| ALogPs | -1.26     | -1.36         | -1.23         | -0.97         | -0.70           | -0.49      | -0.44     | -0.35     | 1.25           | 1.37        |
| ALogpS | -1.77     | -1.96         | -1.87         | -2.05         | -2.40           | -2.61      | -2.21     | -2.47     | -3.54          | -3.26       |
| tPSA   | 189       | 218           | 212           | 218           | 218             | 246        | 212       | 195       | 192            | 192         |
| relPSA | 45        | 42            | 41            | 37            | 38              | 36         | 38        | 35        | 29             | 26          |
| nStereo| 4         | 5             | 5             | 4             | 4               | 4          | 4         | 4         | 4              | 4           |
| nStMW ‡| 11.6      | 12            | 12            | 8.7           | 8.6             | 7.3        | 8.6       | 8.9       | 7.6            | 8           |
| Fsp3† | 0.50      | 0.54          | 0.54          | 0.53          | 0.29            | 0.33       | 0.29      | 0.29      | 0.22           | 0.70        |
| RotB   | 4         | 5             | 5             | 8             | 5               | 9          | 5         | 6         | 6              | 12          |
| Rings  | 3         | 3             | 3             | 3             | 4               | 4          | 4         | 4         | 5              | 3           |
| RngAr  | 2         | 2             | 2             | 2             | 3               | 3          | 3         | 3         | 4              | 2           |
| RngLg  | 6         | 6             | 6             | 6             | 6               | 6          | 6         | 6         | 6              | 6           |
| RngSys | 2         | 2             | 2             | 2             | 3               | 3          | 3         | 3         | 3              | 4           |
| RRSys  | 1.50      | 1.50          | 1.50          | 1.50          | 1.33            | 1.33       | 1.33      | 1.33      | 1.25           | 1.50        |

‡ For clarity, values shown are [nStMW x 1000]

Physicochemical properties shown are for the non-ionized forms of sulfonyladenosines (as shown in Figure 3 of the manuscript), because the algorithm used to compute ALogPs and ALogpS (vcclab.org) of the ionized compounds as they would exist at pH 7.4 neutralizes the negatively charged sulfamate nitrogen by addition of an ammonium counterion: for OSB-AMS, the algorithm adds two ammonium counterions to neutralize both the sulfamate and the carboxylate; for L-alanyl-AMS, the algorithm adds ammonium and chloride to neutralize the sulfamate and positively charge amine, respectively. Computation of the physicochemical properties of compounds in their ionized state at pH 7.4 did not substantially change the values. The largest observed differences between the non-ionized and pH 7.4 ionized compounds were the ALogPs values for L-alanyl-AMS (ALogPs –1.03 for ionized form vs. –1.36 for non-ionized form) and OSB-AMS (ALogPs 0.19 for ionized form vs. –0.49 for non-ionized form).
C. MATERIALS AND METHODS

Reagents

Reagents were obtained from Aldrich Chemical (www.sigma-aldrich.com) or Acros Organics (www.fishersci.com) and used without further purification. Phenylalanine-arginine-beta-naphthylamide (PAβN) was purchased from MP Biomedicals. Optima or HPLC grade solvents were obtained from Fisher Scientific (www.fishersci.com), degassed with Ar, and purified on a solvent drying system as described unless otherwise indicated.

Reactions

All reactions were performed in flame-dried glassware under positive Ar pressure with magnetic stirring unless otherwise noted. Liquid reagents and solutions were transferred through rubber septa via syringes flushed with Ar prior to use. Cold baths were generated as follows: 0 °C, wet ice/water; –10 °C, wet ice/brine; –20 °C, dry ice/isopropanol monitored with a thermometer; –44 °C, dry ice/CH$_3$CN; –63 °C, dry ice/chloroform; –78 °C, dry ice/acetone; –100 °C, dry ice/Et$_2$O.

Chromatography

TLC was performed on 0.25 mm E. Merck silica gel 60 F254 plates and visualized under UV light (254 nm) or by staining with potassium permanganate (KMnO$_4$), cerium ammonium molybdenate (CAM), phosphomolybdic acid (PMA), iodine (I$_2$), or p-anisaldehyde. Silica flash chromatography was performed on E. Merck 230–400 mesh silica gel 60. Lyophilization of small aqueous samples was performed using a GeneVac HT-4X centrifugal evaporator. Lyophilization of larger aqueous samples was performed using a Labconco Freezone 2.5 instrument.

Analytical Instrumentation

IR spectra were recorded on a Bruker Optics Tensor 27 FTIR spectrometer with peaks reported in cm$^{-1}$. NMR spectra were recorded on a Bruker UltraShield Plus 500 MHz Avance III NMR or UltraShield Plus 600 MHz Avance III NMR with DCH CryoProbe at 24 °C in CDCl$_3$ unless otherwise indicated. Chemical shifts are expressed in ppm relative to TMS ($^1$H, 0 ppm) or solvent signals: CDCl$_3$ ($^{13}$C, 77.0 ppm), C$_6$D$_6$ ($^1$H, 7.16 ppm; $^{13}$C, 128.0 ppm) or acetone-$d_6$ ($^{13}$C, 206.2 ppm); coupling constants are expressed in Hz. NMR spectra were processed using Bruker TopSpin or nucleomatica iNMR (www.inmr.net) software. Mass spectra were obtained at the MSKCC Analytical Core Facility on a Waters Acuity SQD LC-MS or PE SCIEX API 100 by electrospray (ESI) ionization or atmospheric pressure chemical ionization (AP-Cl). High resolution mass spectra were obtained on a Waters Acuity Premiere XE TOF LC-MS by electrospray ionization.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was carried out on an Agilent Technologies 6410 triple quad LC-MS/MS system with autosampler in electrospray ionization (ESI) mode, with an Agilent Zorbax Eclipse XDB-C18 reverse phase column (50 × 4.6 mm, 5 μm) using a flow rate of 0.4 mL/min and an isocratic mobile phase of CH$_3$CN in 0.1% aq formic acid (mixture optimized for each analyte, see Table S5) over 5 min.

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1 Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. Organometallics 1996, 15, 1518–1520.
**Nomenclature**

Atom numbers shown in chemical structures herein correspond to the standard nucleoside numbering system used in the text of the article and Supporting Information and not to IUPAC nomenclature, which was used solely to name each compound. Compounds not cited in the paper are numbered herein from S1.

**Microbiology**

*Bacillus subtilis subtilis* (ATCC 6051) was cultured at 30 °C in Lennox LB broth (Fisher). *Escherichia coli* (ATCC 25922) was cultured at 37 °C in tryptic soy broth (BD Biosciences). *Mycobacterium smegmatis* (ATCC 700084) was cultured at 37 °C in Middlebrook 7H9 (BD Biosciences) supplemented with 0.1% Tween and 10% ADN (5% BSA, 2% dextrose, 0.85% NaCl).²

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² Tatham, E.; Sundaram Chavadi, S.; Mohandas, P.; Edupuganti, U. R.; Angala, S. K.; Chatterjee, D.; Quadri, L. E. *BMC Microbiol* 2012, 12, 118.
D. SYNTHESIS OF ACYL-AMS ANALOGUES (2-10)

Figure S16. General synthetic approach to access acyl-AMS derivatives not previously reported in the literature. (TBS = t-butyldimethylsilyl; DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene; TFA = 2,2,2-trifluoroacetic acid)

Salicyl-AMS (1), sulfamoyladenosine (2), L-alanyl-AMS (3), anthranilyl-AMS (6), OSB-AMS (7), benzoyl-AMS (8), glycyl-AMS (Figure S17), 2-phenyl-salicyl-AMS, and 2-phenylamino-salicyl-AMS were prepared as previously described.3

General procedure for preparation of N-hydroxysuccinamide esters S1a–f

N-hydroxysuccinamide esters S1a–f were prepared using the procedures described by Anderson, et al.4

2,5-Dioxopyrrolidin-1-yl(S)-2-((tert-butyldimethylsilyl)oxy)propanoate (S1a). Synthesized from (S)-2-((tert-butyldimethylsilyl)oxy)propanoic acid,5 white solid (421 mg, 57%). TLC: Rf 0.56 (1:1 hexanes/EtOAc). IR (ZnSe, film): 2954, 1819, 1788, 1743, 1364, 1257, 1207, 1109, 1085, 1067, 968, 913, 836, 815, 783, 735. 1H-NMR (500 MHz): δ 4.65 (q, J = 6.8 Hz, 1H), 2.83 (s, 4H), 1.58 (d, J = 6.8 Hz, 3H), 0.92 (s, 9H), 0.13 (d, J = 6.5 Hz, 6H). 13C-NMR (125 MHz): δ 169.70, 169.00, 67.05, 25.84, 25.72, 21.80, 18.42, -5.20. ESI-MS m/z (rel int):

3 (a) Ferreras, J. A.; Ryu, J. S.; Di Lello, F.; Tan, D. S.; Quadri, L. E. Nat. Chem. Biol. 2005, 1, 29. (b) Cisar, J. S.; Ferreras, J. A.; Soni, R. K.; Quadri, L. E.; Tan, D. S. J. Am. Chem. Soc. 2007, 129, 7752. (c) Qiao, C.; Gupte, A.; Boshoff, H. I.; Wilson, D. J.; Bennett, E. M.; Somu, R. V.; Barry, C. E.; Aldrich, C. C. J. Med. Chem. 2007, 50, 6080. (d) Lu, X.; Zhou, R.; Sharma, I.; Li, X.; Kumar, G.; Swaminathan, S.; Tonge, P. J.; Tan, D. S. ChemBiochem 2012, 13, 129. (d) Neres, J., Labello, N. P., Somu, R. V., Boshoff, H. I., Wilson, D. J., Vannada, J., Chen, L., Barry, C. E., Bennett, E. M., and Aldrich, C. C. J. Med. Chem. 2008, 51, 5349.
4 Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. J. Am. Chem. Soc. 1964, 86, 1839.
5 Blonski, C.; Gefflaut, T.; Perie, J. Bioorg Med Chem 1995, 3, 1247.
(pos) 324.3 ([M+Na]^+, 100); (neg) 300.3 ([M–H]–, 100). \textbf{HRMS} \textit{m/z} \textit{calcd} for C\textsubscript{13}H\textsubscript{23}NO\textsubscript{5}NaSi ([M+H]^+) 324.1251; found 324.1243.

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\textbf{2,5-Dioxopyrrolidin-1-yl methyl succinate (S1b).} Synthesized from methyl succinate, white solid (795.0 mg, 92\%). \textbf{TLC:} \textit{R} \textsubscript{f} 0.24 (1:1 hexanes/EtOAc). \textbf{IR} (ZnSe, film): 2954, 2933, 2857, 1815, 1784, 1738, 1438, 1365, 1206, 1093, 1071, 996, 843, 747, 702. \textbf{\textit{^1}H-NMR} (500 MHz): $\delta$ 3.72 (d, \textit{J} = 2.5 Hz, 3H), 2.95 (td, \textit{J} = 7.0, 2.3 Hz, 2H), 2.83 (s, 4H), 2.75 (td, \textit{J} = 7.0, 2.4 Hz, 2H). \textbf{\textit{^{13}C-NMR} (125 MHz):} $\delta$ 171.6, 169.1, 167.9, 52.4, 28.8, 26.6, 25.8. \textbf{ESI-MS} \textit{m/z} (rel int): (pos) 252.0 ([M+Na]^+, 100), 267.9 ([M+K]^+, 20), 481.1 ([2M+Na]^+, 5). \textbf{HRMS} \textit{m/z} \textit{calcd} for C\textsubscript{9}H\textsubscript{11}NO\textsubscript{6}Na ([M+Na]^+) 252.0484; found 252.0485.

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\textbf{2,5-Dioxopyrrolidin-1-yl[1,1'-biphenyl]-4-carboxylate (S1c).} Synthesized from 4-phenylbenzoic acid, white solid (414.9 mg, 56\%). \textbf{TLC:} \textit{R} \textsubscript{f} 0.13 (3:1 hexanes/EtOAc). \textbf{IR} (ZnSe, film): 2952, 2930, 2856, 1770, 1740, 1606, 1367, 1258, 1248, 1211, 1707, 999, 848, 838, 778, 741. \textbf{\textit{^1}H-NMR} (500 MHz): $\delta$ 8.20 (dd, \textit{J} = 8.3, 3.4 Hz, 2H), 7.73 (dd, \textit{J} = 8.1, 3.3 Hz, 2H), 7.65-7.63 (m, 2H), 7.51-7.47 (m, 2H), 7.44-7.42 (m, 1H), 2.92 (s, 4H). \textbf{\textit{^{13}C-NMR} (125 MHz):} $\delta$ 169.5, 162.0, 147.9, 139.7, 131.4, 129.3, 128.9, 128.7, 127.7, 127.6, 124.0, 26.0.

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\textbf{2,5-Dioxopyrrolidin-1-yl decanoate (S1d).} Synthesized from decanoic acid, white solid (864.0 mg, 84\%). \textbf{TLC:} \textit{R} \textsubscript{f} 0.26 (3:1 hexanes/EtOAc). \textbf{IR} (ZnSe, film): 2955, 2923, 1853, 1787, 1727, 1378, 1212, 1073, 997, 912, 869, 815, 736. \textbf{\textit{^1}H-NMR} (500 MHz): $\delta$ 2.83 (d, \textit{J} = 0.6 Hz, 4H), 2.60 (t, \textit{J} = 7.5 Hz, 2H), 1.74 (t, \textit{J} = 7.6 Hz, 2H), 1.40 (t, \textit{J} = 7.6 Hz, 2H), 1.33-1.27 (m, 10H), 0.88 (t, \textit{J} = 6.9 Hz, 3H). \textbf{\textit{^{13}C-NMR} (125 MHz):} $\delta$ 169.4, 168.9, 32.1, 31.2, 29.56, 29.46, 29.33, 29.0, 25.8, 24.8, 22.9, 14.4. \textbf{ESI-MS} \textit{m/z} (rel int): (pos) 292.1 ([M+Na]^+, 20), 561.3 ([2M+Na]^+, 5); (neg) 268.1 ([M–H]–, 25), 537.3 ([2M–H]–, 25). \textbf{HRMS} \textit{m/z} \textit{calcd} for C\textsubscript{14}H\textsubscript{23}NO\textsubscript{5}Na ([M+Na]^+) 292.1525; found 292.1537.
2,5-Dioxopyrrolidin-1-yl 2-((tert-butyldimethylsilyl)oxy)acetate (S1e). Synthesized from 2-((tert-butyldimethylsilyl)oxy)acetic acid, white solid (894 mg, 54%). TLC: Rf 0.56 (3:1 hexanes/EtOAc). IR (ZnSe, film): 2956, 2932, 2859, 1841, 1741, 1469, 1430, 1362, 1255, 1205, 1101, 1070, 874, 838, 785, 702. \(^1\)H-NMR (500 MHz): \(\delta\) 4.58 (s, 2H), 2.85 (s, 4H), 0.92 (s, \(J = 5.9\) Hz, 9H), 0.13 (d, \(J = 6.2\) Hz, 6H). \(^13\)C-NMR (125 MHz): \(\delta\) 169.0, 167.5, 59.9, 25.8, 18.5. ESI-MS m/z (rel int): (pos) 310.3 ([M+Na]\(^+\), 100); (neg) 286.2 ([M–H]–, 100). HRMS m/z calcd for C\(_{12}\)H\(_{21}\)NO\(_5\)NaSi ([M+H]\(^+\)) 310.1088; found 310.1087.

2,5-Dioxopyrrolidin-1-yl hexanoate (S1f). Synthesized from hexanoic acid, white solid (1.4548 g, 85%). TLC: Rf 0.51 (3:1 hexanes/EtOAc). IR (ZnSe, film): 2960, 2874, 1815, 1785, 1741, 1730, 1209, 1069, 915, 866, 815, 734. \(^1\)H-NMR (500 MHz): \(\delta\) 2.83 (s, 4H), 2.60 (t, \(J = 7.5\) Hz, 2H), 1.74 (q, \(J = 7.5\) Hz, 2H), 1.41-1.34 (m, 4H), 0.91 (t, \(J = 7.1\) Hz, 3H). \(^13\)C-NMR (125 MHz): \(\delta\) 169.4, 168.9, 77.5, 31.2, 25.8, 24.5, 22.4, 14.1. ESI-MS m/z (rel int): (pos) 252.0 ([M+K]\(^+\), 50), 449.4 ([2M+Na]\(^+\), 10); (neg) 211.9 ([M–H]–, 75). HRMS m/z calcd for C\(_{10}\)H\(_{14}\)NO\(_4\) ([M+H]\(^+\)) 212.0923; found 212.0914.

General procedure for coupling N-hydroxysuccinamide esters S1a–f with 5’-O-sulfamoyl-adenosine S2a or S2b

In a 25 mL conical flask, protected sulfamoyl adenosine\(^3a\) S2a or S2b (1.3 mmol, 1.0 equiv) and the N-hydroxysuccinimidyl ester S1a–f (2.0 mmol, 1.5 equiv) were dissolved in 13 mL CH\(_3\)CN. DBU (0.28 mL, 2.0 mmol, 1.5 equiv) was added and the mixture was stirred at rt for 2 h. The reaction mixture was poured into 150 mL EtOAc, washed with satd aq NH\(_4\)Cl (1 x 15 mL), satd aq NaHCO\(_3\) (1 x 15 mL), and brine (1 x 15 mL), dried (MgSO\(_4\)), filtered, and concentrated by rotary evaporation. Purification by silica flash chromatography (95:5 → 4:1 CH\(_2\)Cl\(_2\)/MeOH) yielded the protected acyl sulfamoyl adenosines S3.

\(^6\) Vo, C. V.; Mitchell, T. A.; Bode, J. W. J Am Chem Soc 2011, 133, 14082.
\(^7\) Castro-Pichel, J.; Garcia-Lopez, M. T.; De las Heras, F. G. Tetrahedron 1987, 43, 383.
2',3'-O-Isopropylidene-5'-O-[N-(O-t-butyldimethylsilyl-L-lactyl)sulfamoyl]adenosine (S3a). Synthesized from S1a and S2a, white solid (549.2 mg, 76%). TLC: Rf 0.33 (4:1 CH₂Cl₂/MeOH). IR (ZnSe, film): 2954, 2932, 2858, 1654, 1471, 1254, 1213, 1130, 1103, 1058, 1045, 973, 832, 800, 782. ¹H-NMR (500 MHz, CD₃OD): δ 8.42 (s, 1H), 8.21 (s, 1H), 6.24 (d, J = 3.0 Hz, 1H), 5.38 (dd, J = 6.1, 3.0 Hz, 1H), 5.15 (dd, J = 6.1, 2.4 Hz, 1H), 4.54-4.52 (m, 1H), 4.27 (dd, J = 10.8, 4.2 Hz, 2H), 4.20 (q, J = 9.4, 4.8 Hz, 1H), 1.61 (s, 3H), 1.38 (s, 2H), 1.31 (d, J = 6.7 Hz, 3H), 0.89 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H). ¹³C-NMR (125 MHz): δ 183.5, 154.2, 150.4, 141.6, 140.6, 93.7, 92.0, 87.3, 86.7, 85.9, 83.5, 73.1, 69.9, 59.2, 27.7, 26.7, 26.4, 25.8, 22.8, 22.0, 19.5, 19.1, -4.3, -4.5, -4.79, -4.97. ESI-MS m/z (rel int): (pos) 611.1 ([M+K]+, 60), 595.1 ([M+Na]+, 20), 573.2 ([M+H]+, 10); (neg) 571.2 ([M–H]–, 100). HRMS m/z calcd for C₂₂H₃₇N₆O₈Si ([M+H]+) 573.2162; found 573.2163.

2',3'-O,O-Bis(t-butyldimethylsilyl)-5'-O-[N-(methyl-succinyl)sulfamoyl]adenosine (S3b). Synthesized from S1b and S2b, white solid (83.2 mg, 70%). TLC: Rf 0.55 (4:1 CH₂Cl₂/MeOH). IR (ZnSe, film): 2953, 2932, 2858, 1733, 1618, 1575, 1472, 1363, 1295, 1253, 1144, 1094, 989, 956, 834, 778, 713. ¹H-NMR (500 MHz, CD₃OD): δ 8.50 (s, 1H), 8.20 (s, 1H), 6.10 (d, J = 7.0 Hz, 1H), 4.78 (m, 1H), 4.44 (d, J = 4.4 Hz, 1H), 4.38 (dd, J = 11.1, 4.0 Hz, 1H), 4.32-4.27 (m, 2H), 3.63 (s, 3H), 2.62-2.59 (m, 2H), 2.56-2.54 (m, 2H), 0.97 (s, 9H), 0.73 (s, 9H), 0.17 (d, J = 9.0 Hz, 6H), -0.33 (s, 3H), -0.02 (s, 3H). ¹³C-NMR (125 MHz): δ 181.7, 175.7, 157.5, 154.1, 151.2, 141.7, 120.4, 88.8, 85.9, 77.4, 74.7, 69.5, 52.3, 50.0, 35.0, 31.0, 26.6, 26.4, 19.1, 18.9, -4.06, -4.13, -5.0. HRMS m/z calcd for C₂₇H₄₉N₆O₉Si₂S ([M+H]+) 689.2820; found 689.2800.
2',3'-O-[N-(4-phenylbenzoyl)sulfamoyl]adenosine (S3c). Synthesized from S1c and S2b, white solid (108 mg, 82%). TLC: Rf 0.61 (4:1 CH₂Cl₂/MeOH). IR (ZnSe, film): 2952, 2931, 2857, 1618, 1541, 1472, 1350, 1254, 1162, 1125, 990, 915, 843, 779, 750. \( ^1H\)-NMR (500 MHz, CD₃OD): \( \delta \) 8.52 (s, 1H), 8.18 (d, \( J = 8.5 \) Hz, 2H), 8.14 (s, 1H), 7.60-7.56 (m, 4H), 7.43-7.39 (m, 2H), 7.35-7.32 (m, 1H), 6.09 (d, \( J = 6.9 \) Hz, 1H), 4.89 (dd, \( J = 6.9, 4.4 \) Hz, 1H), 4.53 (dd, \( J = 11.3, 4.0 \) Hz, 1H), 4.46 (dd, \( J = 4.4, 1.6 \) Hz, 1H), 4.43 (dd, \( J = 11.3, 3.5 \) Hz, 1H), 4.30 (td, \( J = 3.6, 1.7 \) Hz, 1H), 0.93 (s, 9H), 0.70 (s, 9H), 0.13 (s, 9H), 0.37 (s, 3H), -0.06 (s, 3H). \( ^13C\)-NMR (125 MHz): \( \delta \) 175.3, 157.5, 154.1, 151.1, 145.7, 141.8, 141.6, 137.2, 131.1, 130.1, 128.2, 127.6, 120.4, 89.0, 86.0, 77.3, 74.7, 69.5, 26.5, 26.3, 19.1, 18.8, -4.0, -4.1, -4.2, -5.0. HRMS \( m/z \) calcd for C₃₅H₅₁N₆O₇Si₂S ([M+H]⁺) 755.3079; found 755.3041.

2',3'-O-[N-(decanoyl)sulfamoyl]adenosine (S3d). Synthesized from S1d and S2a, white solid (55.1 mg, 65%). TLC: Rf 0.24 (9:1 CH₂Cl₂/MeOH). IR (ZnSe, film): 2954, 2928, 2857, 1653, 1605, 1468, 1428, 1379, 1216, 1146, 1108, 1047, 999, 829, 782, 739, 704. \( ^1H\)-NMR (500 MHz, CD₃OD): \( \delta \) 8.40 (s, 1H), 8.21 (s, 1H), 6.23 (d, \( J = 2.9 \) Hz, 1H), 5.36 (dd, \( J = 6.1, 3.0 \) Hz, 1H), 5.12 (dd, \( J = 6.1, 2.4 \) Hz, 1H), 4.54-4.52 (m, 1H), 4.26 (qd, \( J = 12.9, 4.3 \) Hz, 2H), 2.16 (t, \( J = 7.6 \) Hz, 2H), 1.64-1.60 (m, 3H), 1.60 (s, 3H), 1.38 (s, 3H), 1.28 (s, 9H), 1.24 (s, 8H), 0.88 (q, \( J = 7.4 \) Hz, 5H). \( ^13C\)-NMR (125 MHz): \( \delta \) 184.0, 157.5, 154.2, 150.6, 141.7, 120.4, 115.6, 91.9, 85.9, 83.3, 69.9, 40.5, 33.2, 30.8, 30.7, 30.62, 30.59, 30.4, 27.5, 26.5, 25.8, 23.9, 14.6. ESI-MS \( m/z \) (rel int): (neg) 539.2 ([M–H]⁻, 100), 1079.3 ([2M–H]⁻, 5). HRMS \( m/z \) calcd for C₂₃H₃₇N₆O₇S ([M+H]⁺) 541.2431; found 541.2444.
2′,3′-O-Isopropylidene-5′-O-[(N-(O-t-butyldimethylsilyl-glycolyl)sulfamoyl)adenosine (S3e). Synthesized from S1e and S2a, white solid (519.6 mg, 75%). **TLC:** \(R_f\) 0.22 (4:1 CH₂Cl₂/MeOH). **IR** (ZnSe, film): 2954, 2938, 2858, 1642, 1473, 1428, 1385, 1296, 1256, 1213, 1149, 1112, 1060, 836, 785, 703. **\(^1H-NMR** (500 MHz, CD₃OD): \(\delta\) 8.44 (s, 1H), 8.21 (s, 1H), 6.24 (d, \(J = 3.1\) Hz, 1H), 5.37 (dd, \(J = 6.1, 3.1\) Hz, 1H), 5.13 (dd, \(J = 6.1, 2.2\) Hz, 1H), 4.55-4.52 (m, 1H), 4.24 (qd, \(J = 9.6, 4.0\) Hz, 2H), 1.61 (s, 3H), 1.39 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H). **\(^{13}C-NMR** (125 MHz): \(\delta\) 180.2, 154.2, 141.6, 115.5, 92.0, 85.93, 85.89, 83.5, 69.8, 66.4, 27.7, 26.6, 26.45, 25.8, 19.6, -4.9. **ESI-MS** \(m/z\) (rel int): (pos) 581.1 ([M+Na]+, 30), 559.1 ([M+H]+, 10); (neg) 557.1 ([M–H]–, 100). **HRMS** \(m/z\) calcd for C₂₁H₃₅N₆O₈S ([M+H]+) 559.2012; found 559.2006.

General procedure for deprotection of S3a–f

In a 20 mL vial, \(N\)-acyl sulfamoyl adenosine S3a–f (70 µmol), 344 µL H₂O was added and cooled to 0 °C. TFA (1.7 mL) was added and the reaction was warmed to rt and stirred for 2–24 h. The solution was cooled to 0 °C, then MeOH was added (5 mL) and the mixture was concentrated by rotary evaporation. The mixture was azeotroped from MeOH (2 x 5 mL) and cyclohexane (3 x 5 mL). Purification by silica flash chromatography (5:1 → 3:1 EtOAc/MeOH) yielded the acyl sulfamoyl adenosines.
5´-O-[N-(1-Lactyl)sulfamoyl]adenosine (4, L-lactyl-AMS). Synthesized from S3a, white solid (33.7 mg, 79%). TLC: Rf, 0.10 (3:1 EtOAc/MeOH). IR (ZnSe, film): 2473, 1622, 1121, 1093, 973, 902, 825, 763. 1H-NMR (500 MHz, CD3OD): δ 8.49 (s, 1H), 8.19 (s, 1H), 6.09 (d, J = 5.5 Hz, 1H), 4.66 (t, J = 5.2 Hz, 1H), 4.39 (t, J = 4.2 Hz, 1H), 4.35 (dd, J = 11.7, 3.8 Hz, 1H), 4.30 (dt, J = 7.8, 3.6 Hz, 2H), 4.12-4.08 (m, 1H), 1.35 (d, J = 6.7 Hz, 3H). 13C-NMR (125 MHz): δ 183.5, 157.4, 154.0, 151.0, 141.4, 94.1, 89.5, 84.6, 76.3, 72.3, 70.9, 69.4, 22.1. ESI-MS m/z (rel int): (pos) 419.0 ([M+H]+, 100), 441.2 ([M+Na]+, 100); (neg) 417.7 ([M– H]–, 100). HRMS m/z calcd for C13H19N6O8S ([M+H]+) 419.0982; found 419.0985.

5´-O-[N-(Methyl-succinyl)sulfamoyl]adenosine (5, methyl-succinyl-AMS). Synthesized from S3b, white solid (11.0 mg, 42%). TLC: Rf, 0.05 (4:1 CH2Cl2/MeOH). IR (ZnSe, film): 1725, 1681, 1647, 1603, 1581, 1298, 1208, 1147, 1002, 840, 800, 723. 1H-NMR (500 MHz, CD3OD): δ 8.48 (s, 1H), 8.20 (s, 1H), 6.09 (d, J = 5.7 Hz, 1H), 4.67 (t, J = 5.4 Hz, 1H), 4.38 (dd, J = 5.0, 3.1 Hz, 1H), 4.30 (dt, J = 13.0, 8.0 Hz, 3H), 3.62 (s, 3H), 2.59-2.56 (m, 2H), 2.53-2.51 (m, 2H). 13C-NMR (125 MHz): δ 175.7, 157.5, 154.0, 151.0, 141.3, 89.4, 84.7, 76.2, 72.5, 69.4, 52.3, 34.7, 30.9. HRMS m/z calcd for C15H21N6O9S ([M+H]+) 461.1091; found 461.1079.

5´-O-[N-(4-Phenylbenzoyl)sulfamoyl]adenosine (9, 4-phenylbenzoyl-AMS). Synthesized from S3c, white solid (30.7 mg, 100%). TLC: Rf, 0.10 (4:1 CH2Cl2/MeOH). IR (ZnSe, film): 1675, 1336, 1302, 1203, 1134, 978, 914, 845, 801, 751, 715, 725. 1H-NMR (500 MHz, CD3OD): δ 8.44 (s, 1H), 8.15-8.11 (m, 3H), 7.60 (dd, J = 16.1, 7.8 Hz, 4H), 7.44-7.41 (m, 2H), 7.35 (d, J = 7.3 Hz, 1H), 6.08 (d, J = 5.2 Hz, 1H), 4.73-4.71 (m, 1H), 4.63-4.60 (m, 2H), 4.50-4.41 (m, 3H), 4.36-4.35 (m, 1H). 13C-NMR (125 MHz): δ 175.5, 163.5, 163.3, 157.4, 154.0, 150.8, 145.6, 141.8, 141.2, 137.2, 130.9, 130.1, 129.0, 128.2, 127.6, 120.4, 119.5, 117.1, 89.8, 84.5, 76.0, 72.4,
ESI-MS m/z (rel int): (pos) 565.2 ([M+K]+, 100), 549.2 ([M+Na]+, 40), 527.2 ([M+H]+, 10).

HRMS m/z calcd for C23H23N6O7S ([M+H]+) 527.1349; found 527.1357.

5´-O-[(N-(Decanoyl)sulfamoyl)adenosine (10, decanoyl-AMS). Synthesized from S3d, white solid (17.1 mg, 76%). TLC: Rf 0.18 (4:1 CH2Cl2/MeOH). IR (ZnSe, film): 2925, 2853, 1684, 1630, 1355, 1301, 1208, 1143, 834, 802. ¹H-NMR (500 MHz, CD3OD): δ 8.45 (s, 1H), 8.20 (s, 1H), 6.08 (d, J = 5.5 Hz, 1H), 4.67 (t, J = 5.3 Hz, 1H), 4.40-4.34 (m, 2H), 4.29 (dt, J = 7.9, 3.8 Hz, 2H), 2.19 (t, J = 7.6 Hz, 2H), 1.60-1.57 (m, 2H), 1.29-1.25 (m, 6H), 1.23 (s, 7H), 0.87 (t, J = 7.0 Hz, 3H). ¹³C-NMR (125 MHz): δ 184.1, 154.1, 150.9, 141.3, 89.6, 84.5, 76.1, 72.4, 69.6, 40.5, 33.2, 30.83, 30.79, 30.74, 30.62, 27.6, 23.9, 14.6. ESI-MS m/z (rel int): (pos) 523.2 ([M+Na]+, 100), 501.2 ([M+H]+, 102), 251.6 ([2M+Na]+, 5). (neg) 499.1 ([M– H]–, 100), 999.4 ([2M–H]–, 20). HRMS m/z calcd for C20H33N6O7S ([M+H]+) 501.2131; found 501.2120.

5´-O-[N-(Glycolyl)sulfamoyl]adenosine (S4, glycoyl-AMS). Synthesized from S3e, white solid (30.0 mg, 78%). TLC: Rf 0.38 (3:1 EtOAc/MeOH). IR (ZnSe, film): 2925, 2853, 1619, 1122, 1092, 973, 901, 823. ¹H-NMR (500 MHz, CD3OD): δ 8.51 (s, 1H), 8.20 (s, 1H), 6.09 (d, J = 5.6 Hz, 1H), 4.66 (t, J = 5.3 Hz, 1H), 4.39 (dd, J = 4.9, 3.5 Hz, 1H), 4.31 (ddd, J = 13.2, 9.1, 3.7 Hz, 3H), 3.97 (s, 2H). ¹³C-NMR (125 MHz): δ 180.5, 157.5, 154.0, 151.0, 141.3, 120.5, 89.4, 84.6, 76.3, 72.3, 69.2, 64.5. ESI-MS m/z (rel int): (neg) 403.1 ([M– H]–, 100). HRMS m/z calcd for C12H15N6O8S ([M+H]+) 403.0674; found 403.0672.

5´-O-[N-(Hexanoyl)sulfamoyl]adenosine (S5, hexanoyl-AMS). Synthesized from S3f, white solid (50.0 mg, 100%). TLC: Rf 0.20 (3:1 EtOAc/MeOH). IR (ZnSe, film): 2960, 2934, 1678, 1453, 1379, 1333, 1300, 1207, 1143, 982, 843, 801, 725. ¹H-NMR (500 MHz, CD3OD): δ 8.39 (s, 1H), 8.20 (s, 1H), 6.08 (d, J = 5.3 Hz, 1H), 4.68 (t, J = 5.2 Hz, 1H), 4.42-4.40 (m, 2H), 4.36-4.30 (m, 2H), 2.22-2.19 (m, 2H), 1.59-1.54 (m, 2H), 1.27 (dt, J = 7.6, 3.9 Hz, 4H), 0.86 (t, J =
7.0 Hz, 3H). $^1$H-NMR (125 MHz): $\delta$ 157.4, 154.1, 150.9, 141.2, 120.5, 89.9, 84.2, 75.9, 72.3, 70.2, 40.0, 32.8, 26.9, 23.6, 14.4. HRMS $m/z$ calcd for C$_{16}$H$_{25}$N$_6$O$_7$S ([M+H]$^+$) 445.1505; found 445.1518.
E. LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

To quantify the amount of the sulfonyladenosine of interest in each sample, a standard curve (0.0025–100 µM) was constructed by mixing 200 µL analyte (in PBS) with 200 µL internal standard (2 µM, acetonitrile). Approximately 4 µL of sample were injected into the LC-MS/MS. For the purposes of platform methodology validation herein, we selected close structural analogues of each analyte for the internal standards to maximize sensitivity and linear range of detection. For example, we obtained a wider linear range of detection for salicyl-AMS when we used benzoyl-AMS, a close structural analogue as an internal standard, compared to when we used alanyl-AMS, a more structurally distinct compound (Figure S3). Full details regarding internal standards for each analyte and the multiple reaction monitoring (MRM) transitions are provided below in Table S5.

Table S5. Analytes, internal standards and MRM transitions used for LC-MS/MS

| Analyte   | Analyte MRM | Analyte ret. time (min) | Internal Standard | IS MRM transition | IS ret. time (min) | Eluent (% B) |
|-----------|-------------|--------------------------|-------------------|-------------------|--------------------|--------------|
| H-AMS (2) | 347/136     | 1.7                      | guanosine         | 284/152           | 1.6                | 10           |
| L-Ala-AMS (3) | 418/136 | 1.5                      | glycoly-AMS       | 404/136           | 1.4                | 10           |
| L-Lac-AMS (4) | 419/136 | 1.8                      | glycoly-AMS       | 405/136           | 1.7                | 10           |
| Me-suc-AMS (5) | 461/136 | 1.4                      | hex-AMS           | 445/136           | 2.0                | 35           |
| antha-AMS (6) | 466/136 | 1.4                      | Bz-AMS            | 451/136           | 1.5                | 30           |
| OSB-AMS (7) | 551/136 | 1.6                      | sal-AMS           | 467/136           | 1.6                | 30           |
| sal-AMS (1) | 467/136 | 1.5                      | Bz-AMS            | 451/136           | 1.4                | 35           |
| Bz-AMS (8) | 451/136 | 1.4                      | sal-AMS           | 467/136           | 1.5                | 35           |
| 4-PhBz-AMS (9) | 527/136 | 3.8                      | sal-AMS           | 467/136           | 1.6                | 35           |
| dec-AMS (10) | 501/136 | 1.8                      | hex-AMS           | 445/136           | 1.4                | 60           |

Multiple reaction monitoring (MRM) transitions were monitored in the positive ionization mode. Mobile phase: A=0.1% formic acid in water, B=acetonitrile.

Figure S17. Structures of internal standards used for LC-MS/MS. (Bz = benzoyl; sal = salicyl; hex = hexanoyl).
F. COMPOUND ACCUMULATION STUDIES

Salicyl-AMS influx experiments

E. coli (OD$_{600} = 0.5$) was treated with salicyl-AMS (0.01–1000 µM, 37 °C, 0–60 min) in PBS, centrifuged (15,000 rpm, 4 °C, 5 min), resuspended, washed with cold PBS (4 x 200 µL), and lysed by sonication. The lysate and all wash supernatants were analyzed separately by LC-MS/MS with benzoyl-AMS (1 µM) as an internal standard. The intracellular concentration of salicyl-AMS was calculated based on CFU determination of the culture prior to centrifugation.

Salicyl-AMS efflux experiments

E. coli (OD$_{600} = 0.5$) was treated with CCCP (100 µM, 37 °C, 10 min) in PBS and then with salicyl-AMS (100 µM, 37 °C, 15 min). Cells were washed and resuspended in cold PBS containing either glucose (0.2%) or CCCP (100 µM). The preloaded cells were incubated at 37 °C and aliquots were removed at various times (0–60 min) and processed as above.

Compound accumulation experiments

E. coli (OD$_{600} = 0.5$, tryptic soy broth, 37 °C), B. subtilis (OD$_{600} = 0.5$, LB media, 30 °C), or M. smegmatis (OD$_{620} = 0.5$, Middlebrook 7H9 + 10% ADN (5% BSA, 2% dextrose, 0.85% NaCl), 37 °C) was equilibrated (10 min) without or with indicated efflux pump inhibitors: CCCP (100 µM), reserpine (33 µM; 20 µg/mL), or PAβN (38 µM; 20 µg/mL). Then, bacteria were incubated with the appropriate sulfonyladenosine (100 µM, 30 min) and processed as above for salicyl-AMS influx experiments.

Calculation of cellular concentration

The total number of cells was determined via viable cell counts and plating of colony forming units (CFUs). After incubation with analyte, serial dilutions in fresh media were plated on agar. Colonies were grown for 16–24 h and plates containing 25–250 colonies were used to calculate the total number of cells. Individual cell volumes of B. subtilis,$^8$ E. coli,$^9$ and M. smegmatis$^{10}$ were taken from the literature and total cell volume and cellular analyte concentration was calculated using this information.

Optimization of Wash Protocol

To determine whether four washes were adequate to remove extracellular salicyl-AMS in the compound accumulation studies, we incubated B. subtilis with 1 mM salicyl-AMS for 1 h and varied the number of wash steps. We reasoned that cell-associated salicyl-AMS would reach an asymptote as the number of wash steps was increased. Indeed, the amount of cell associated salicyl-AMS decreased between no washes and three washes and reached an asymptote after three washes.

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$^8$ Sharpe, M. E.; Hauser, P. M.; Sharpe, R. G.; Errington, J. J Bacteriol 1998, 180, 547.
$^9$ Kubitschek, H. E.; Friske, J. A. J Bacteriol 1986, 168, 1466.
$^{10}$ Nguyen, L.; Scherr, N.; Gatfield, J.; Walburger, A.; Pieters, J.; Thompson, C. J J Bacteriol 2007, 189, 7896.
**Figure S18. Optimization of wash steps in B. subtilis.** Four washes are adequate to remove cell-associated salicyl-AMS. *B. subtilis* (OD$_{600}$ = 0.5) was treated with salicyl-AMS (1.0 mM, 30 °C, 1 h, LB media) and the number of wash steps was varied. Cell-associated salicyl-AMS was quantified by LC-MS/MS. The concentration of cell-associated salicyl-AMS decreased between no washes and three washes, and reached an asymptote thereafter.

**Assessment of day-to-day variation in accumulation experiments**

To assess the interday variation in accumulation of salicyl-AMS, we conducted accumulation experiments under the following conditions: (1) 100 μM salicyl-AMS, 37 °C; (2) 100 μM salicyl-AMS, 100 μM CCCP, 37 °C; and (3) 100 μM salicyl-AMS, 20 μg/mL PAβN, 37 °C. Pretreatment with CCCP significantly increased the accumulation of salicyl-AMS in *E. coli*, whereas treatment with a competitive inhibitor of AcrAB-TolC, phenylalanine-arginine-β-naphthylamide (PAβN) did not substantially increase cellular salicyl-AMS. These trends were consistent for experiments conducted on three separate days.

**Figure S19. Interday variation in salicyl-AMS accumulation in E. coli.** Trends in accumulation of salicyl-AMS are consistent across experiments on three separate days. (100 μM extracellular, 30 min, tryptic soy broth). Statistical significance compared to salicyl-AMS alone assessed using one-way ANOVA and Tukey’s multiple comparison test and 95% confidence intervals: ***p < 0.001. CCCP = carbonyl cyanide m-chlorophenylhydrazone; PAβN = phenylalanine arginine-β-naphthylamide. All data are reported as mean ± SD for four replicates.
G. **Principal Component Analysis**

To generate the plots shown in **Figure 1** of the manuscript, a total of 141 compounds (**Table S1**) were compared by principal component analysis (PCA).\(^{11}\)

The compounds analyzed by PCA included the following:

- 50 top selling brand-name, non-antiinfective small molecule drugs by revenue in 2012 (**Figure S20**),
- 91 antibacterials (**Figure S21–S29**), including:
  - 38 β-lactams (**Figure S21**)
  - 4 peptides (**Figure S22**)
  - 7 aminoglycosides (**Figure S23**)
  - 15 quinolones (**Figure S24**)
  - 7 macrolides (**Figure S25**)
  - 7 tetracyclines (**Figure S26**)
  - 2 oxazolidinones (**Figure S27**)
  - 5 *M. tuberculosis* targeting drugs (**Figure S28**)
  - 6 sulfa drugs (**Figure S29**)

The drug reference set consists of drugs with non-antiinfective indications and the different classes of antibacterials were chosen to demonstrate that the structural and physicochemical properties of most antibacterials are distinct from drugs targeting other therapeutic indications.

A set of 20 physicochemical descriptors (**Table S2**) were calculated using cheminformatics tools (Instant JChem, VCCLab\(^{14}\)). The descriptors were selected based on several criteria. First, Lipinski’s parameters\(^{15}\) (MW ≤ 500, HBD ≤ 10, HBA ≤ 5, LogP ≤ 5) and Veber’s parameters\(^{12}\) (RotB ≤ 10, tPSA ≤ 140 Å\(^2\)) have been correlated with oral bioavailability.\(^{16}\) While oral bioavailability is not an immediate goal of most academic screening campaigns, some attention to these parameters is useful to the extent that they partially correlate to cell permeability.\(^{6}\) Second, Tetko’s calculated logS aqueous solubility (ALogpS) and log P hydrophobicity (ALogPs) were included since a balance between compound solubility and hydrophobicity are critical for oral bioavailability.\(^{6}\) The distribution coefficient (logD\(_{7.4}\)) was included to approximate aqueous solubility of ionizable molecules at pH = 7.4. Third, several stereochemical parameters (nStereo, nStMW, Fsp\(^3\)) were included to approximate three-
dimensional complexity. Fourth, most antibacterials are natural products or derived from natural products, and additional parameters previously found to differentiate synthetic drugs and natural products were included. Synthetic drugs tend to have higher nitrogen content, while natural products tend to have higher oxygen content (N, O). Natural products tend to have fewer aromatic rings and more complex, fused ring systems (Rings, RngAr, RngSys, RngLg, RRSys). Lastly, relative polar surface area (relPSA) was included because it has been shown to be a distinguishing factor between Gram-positive and Gram-negative antibacterials.

These data were assembled in a Microsoft Excel spreadsheet and average values for each parameter were calculated within each compound series. The hypothetical average values for each antibacterial class, non-antiinfectives, and the sulfonyladenosine datasets were included in the PCA analysis (Table S3).

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17 Lovering, F.; Bikker, J.; Humblet, C. *J Med Chem* **2009**, *52*, 6752.
18 Feher, M.; Schmidt, J. M. *J Chem Inf Comput Sci* **2003**, *43*, 218.
19 O'Shea, R.; Moser, H. E. *J Med Chem* **2008**, *51*, 2871.
Figure S20. 2012 brand-name drug reference set for PCA (50 structures).
Figure S21. β-Lactam reference set for PCA (38 structures).
Figure S22. Peptide reference set for PCA (4 structures).

Figure S23. Aminoglycoside reference set for PCA (7 structures).
Figure S24. Quinolone reference set for PCA (15 structures).

Figure S25. Macrolide reference set for PCA (7 structures).

Figure S26. Tetracycline reference set for PCA (7 structures).
Detailed PCA Protocol (Windows)
To provide a visual representation of the position of each component in chemical space, we conducted PCA with the “R” open source statistical computing package\(^20\) to rotate the 20-dimensional vector corresponding to each compound to a 2-dimensional vector, with minimal loss of information. The detailed protocol is as follows:

1. In MS Excel, a “Raw” worksheet was created with compounds in rows and physicochemical descriptors in columns.

2. Mean and standard deviation values were calculated for each column.

3. A “Norm” worksheet was created and mean-centered, standardized values were generated for each column using the equation:

\[
\text{normval} = \frac{\text{val} - \text{column mean}}{\text{column standard deviation}}
\]

4. With the upper cell left blank (R requires this to recognize a header row), the Number format was designated for all data columns to 4 decimal places.

5. Excel workbook was saved.

\(^{20}\) The R Project for Statistical Computing; [http://www.r-project.org/](http://www.r-project.org/)
6. The “Norm” worksheet was saved as “AntibacterialsNorm.txt” (Text-Tab Delimited) on the Desktop (Windows).

7. Close Excel workbook and discard changes.

8. The “R” open source computing package was opened and the following commands were entered:

9. R>a<-read.table("C:\Users\Tony\Desktop\AntibacterialsNorm.txt", header=T, sep="t", row.names=1) 
   # read data into dataframe a

10. R>prcomp(a)->b 
    # PCA of dataframe b, results to c

11. R>summary(b) 
    # prints summary of % PC contributions

12. R>b 
    # prints the rotation (loading) matrix

13. R>b$x 
    # prints the rotated data (scores)

14. The command in step 13 prints the rotated data (scores). Select and copy the first section of this data (PC1–PC10, without top headers).

15. Paste results into a MS Word text file and change font to Courier New 5 pt.

16. Save MS Word file as: “PC Scores.txt” (Select “Windows (Default)” for text encoding, end lines with “CR/LF” from dropdown menu. Uncheck the boxes marked “insert line breaks” and “allow character substitution”).

17. Open MS Excel workbook from earlier, create a new worksheet “Scores” and import the .txt file by selecting “Get External Data” in the Data menu, then select “From Text.”

18. Check the delimited button, click next, and select the “space” delimiter. Check the box marked “treat consecutive delimiters as one.” Click Finish.

19. The first three columns (compound numbers, PC1, PC2, and PC3) were copied into a new worksheet “PCA”, and the Number format was designated to 4 decimal places.

20. Each group of compounds was sorted in order of ascending PC1 to facilitate location on the PCA plot.

21. PCA plots of PC1 vs. PC2, PC1 vs. PC3, and PC3 vs. PC2 were generated by importing the scores in GraphPad Prism.

22. To obtain loading plots, repeat steps 14–21 for the component loadings in step 12.
23. To generate biplots, overlay the PCA plots with loading plots using the Layout feature in GraphPad Prism.

Following PCA, all 141 compounds were plotted on newly generated, unitless, orthogonal axes (principal components) that are based on linear combinations of the original 20 parameters (Figure 1a, Figures S1). Summary information from R is shown below in Table S6.

Table S6. Standard deviation and percent contribution for each principal component in PCA plot of antibacterials (R summary).

|                  | PC1   | PC2   | PC3   | PC4   | PC5   | PC6   | PC7   | PC8   | PC9   | PC10  |
|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Standard deviation| 3.046 | 2.119 | 1.570 | 1.212 | 1.092 | 0.722 | 0.682 | 0.550 | 0.456 | 0.433 |
| Proportion of Variance | 0.442 | 0.214 | 0.117 | 0.070 | 0.057 | 0.025 | 0.022 | 0.014 | 0.010 | 0.009 |
| Cumulative Proportion | 0.442 | 0.656 | 0.773 | 0.843 | 0.900 | 0.924 | 0.947 | 0.961 | 0.971 | 0.980 |

These data indicate that 90% of the variation in the complete 20-dimensional dataset is accounted for by the five six principal components (PC1–PC5), due to correlations between some of the original 20 parameters. For visualization purposes, the first two principal components (PC1, PC2) were used to generate the plots shown in Figure 1a of the manuscript. Together, these principal components account for 66% of the variation in the complete dataset, with individual contributions of 44% and 21%, respectively (Table S6).

The component loadings generated from the PCA were used to construct loading plots using GraphPad Prism (Figure S2). These data indicate that MW, O, HBD, HBA, and tPSA have the largest loadings on PC1 and shift molecules to the right along PC1 in the PC1 vs. PC2 (Figures S2a, d) and PC1 vs. PC3 (Figure S2b, d) plots. The descriptors with the largest loadings on PC2 are ALogPs and RngAr, which shifts molecules to the top of the PC1 vs. PC2 (Figure S2a, d) and PC3 vs. PC2 (Figures S2c, d) plots, and ALogpS and relPSA, which shifts molecules to the bottom of these plots. The descriptors with the largest loadings on PC3 are nStMW and Fsp³, which shifts molecules to the left along PC3, and N and RngAr, which shifts molecules to the right along PC3.

To compare the physicochemical properties of the 10 sulfonyladenosines evaluated in bacterial compound accumulation assays (Figure 3), we conducted an additional PCA incorporating these sulfonyladenosines, 50 top selling, brand-name, non-antiinfectives, and 91 antibacterials (Figures S4–S5).

To evaluate the robustness of PCA on a smaller dataset, we conducted separate PCAs on the 10 sulfonyladenosines used in the compound accumulation assays (Figure S7) and the 9 sulfonyladenosines that accumulated to detectable levels in the compound accumulation assays (data not shown). The relative positions of each compound were not substantially affected when each plot was compared to the other and the PCA with non-antiinfectives and antibacterials.

For PCA analyses that included sulfonyladenosines, we used the physicochemical properties of sulfonyladenosines in their non-ionized forms. Using the physicochemical properties of the pH 7.4 ionized forms did not substantially alter the PCA results for correlations between properties and E. coli accumulation shown in Figure 5a and Figure S8.
H. \(^1\text{H}-\text{NMR AND }^{13}\text{C}-\text{NMR SPECTRA}\)

1. Preparation of \(N\)-hydroxysuccinamide esters (S1a–f) \(\text{S44}\)
2. Coupling reactions between \(N\)-hydroxysuccinamide esters (S1a–f) and sulfamoyl adenosines (S2a or S2b) \(\text{S50}\)
3. Deprotection to acyl-AMS compounds \(\text{S56}\)
   a. \(\text{L-Lactyl-AMS (4)}\) \(\text{S56}\)
   b. \(\text{Methyl-succinyl-AMS (5)}\) \(\text{S57}\)
   c. \(\text{4-Phenylbenzoyl-AMS (9)}\) \(\text{S58}\)
   d. \(\text{Decanoyl-AMS (10)}\) \(\text{S59}\)
   e. \(\text{Glycolyl-AMS (S4)}\) \(\text{S60}\)
   f. \(\text{Hexanoyl-AMS (S5)}\) \(\text{S61}\)
Me

S1d
