Synthesis and Structure−Activity Relationships of Truncated Bisubstrate Inhibitors of Aminoglycoside 6′-*N*-Acetyltransferases

Feng Gao,[†] Xuxu Yan,[†] Tushar Shakya,[‡] Oliver M. Baettig,[§] Samia Ait-Mohand-Brunet,[†] Albert M. Berghuis,[§] Gerard D. Wright,[‡] and Karine Auclair*,[†]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Québec H3A 2K6, Canada, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada, and Department of Biochemistry, Room 5202, McGill University, 740 Dr. Penfield, Montréal, Québec H3A 2K6, Canada

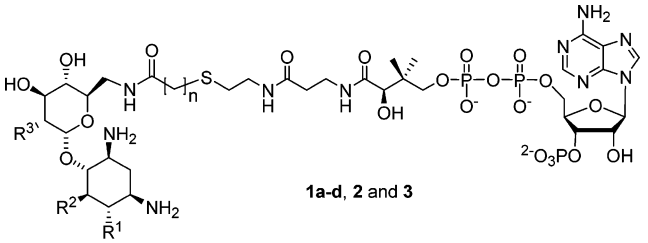
Received June 16, 2006

Truncated aminoglycoside−coenzyme A bisubstrate analogues were efficiently prepared using a convergent approach where the amine and the thiol are coupled in one pot with the addition of a linker, without the need for protecting groups. These derivatives were tested for their effect on the activity of the resistance-causing enzyme aminoglycoside 6′-*N*-acetyltransferase Ii, and key structure−activity relationships are reported. Moreover, one of the inhibitors is able to block aminoglycoside resistance in cells expressing this enzyme.

Introduction

Aminoglycosides are potent antibiotics particularly active against aerobic, Gram-negative bacteria.[1,2] Unfortunately, the emergence of aminoglycoside resistance is increasingly restricting their use as antibacterials.[3,4] The most common mechanism of resistance to aminoglycosides is enzymatic modification by acetyltransferases, phosphoryltransferases, or adenylyltransferases. Of these, the aminoglycoside 6′-*N*-acetyltransferase (AAC(6′)[a]) family, is one of the most widespread determinants of aminoglycoside resistance. *Enterococcus faecium* AAC(6′)-Ii was selected for this study based on its clinical importance as the reason for loss of *β*-lactam/aminoglycoside synergy in this important pathogen and because of the availability of detailed mechanistic and structural information for the enzyme.[5−12] Investigations by Wright and co-workers suggest that AAC-(6′)-Ii proceeds via an ordered bi bi mechanism where AcCoA binds first to the enzyme, followed by the aminoglycoside.[9] Crystallographic[7,8,11] and mutagenic[10] studies have failed to reveal specific amino acid side chains available to stabilize the proposed tetrahedral intermediate, suggesting that the role of this enzyme may be to bind and orient the two substrates in a geometry that favors the acyl transfer, without direct stabilization of an aminoglycoside intermediate.

In our efforts to study the catalytic mechanism of AAC(6′)s, we have developed a one-pot regio- and chemoselective method for the direct N-6′-derivatization of unprotected aminoglycosides.[12,13] This facilitated the preparation of the first generation of synthetic AAC(6′)-Ii inhibitors, the bisubstrate analogues **1a**−**c**, **2**, and **3** (Figure 1). These nanomolar inhibitors have allowed crystallization of AAC(6′)-Ii with aminoglycoside derivatives for the first time, although they lacked biological activity.[12] After the success of these molecules as structural and mechanistic probes of AAC(6′)s, we envisaged the preparation of a second generation of AAC(6′) inhibitors of smaller size to determine structure−activity relationships. Bisubstrate analogues **1a**, **2**, and **3** show similar K_i values against AAC(6′)-Ii, suggesting that aminoglycoside rings III and IV are not essential for inhibition. Bisubstrate **1a** was therefore used as the starting point in the design of our second generation of AAC(6′) inhibitors. Thus, the role of the different functionalities of **1a** for binding to AAC-(6′)-Ii was investigated with the preparation of shorter analogues.



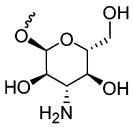
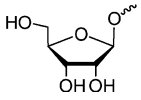
Inhibitor	n	R^1	R^2	R^3	K_i (nM)
1a	1	OH	OH	NH_2	76 ± 25
1b	2	OH	OH	NH_2	43 ± 23
1c	3	OH	OH	NH_2	161 ± 98
1d	4	OH	OH	NH_2	8000 ± 2700
2	1		OH	OH	110 ± 28
3	1	OH		NH_2	119 ± 14

Figure 1. First generation of bisubstrate inhibitors and K_i values against AAC(6′)-Ii.

Results

The synthesis of aminoglycoside−CoA bisubstrates is a challenging task, in part because of the complex protection/deprotection schemes often required for aminoglycoside de-

* To whom correspondence should be addressed. Phone: (+1) 514-398-2822. Fax: (+1) 514-398-3797. E-mail: karine.auclair@mcgill.ca.
† Department of Chemistry, McGill University.
‡ McMaster University.
§ Department of Biochemistry, McGill University.

[a] Abbreviations: AAC(6′), aminoglycoside 6′-*N*-acetyltransferase; AAC-(6′)-Ii, aminoglycoside 6′-*N*-acetyltransferase type Ii; AcCoA, acetyl coenzyme A.; CoA, coenzyme A.; 2-DOS, 2-deoxystreptamine; DTT, dithiothreitol; NBD bromoacetate, *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide bromoacetate.

10.1021/jm060732n CCC: $33.50
Published on Web 07/29/2006

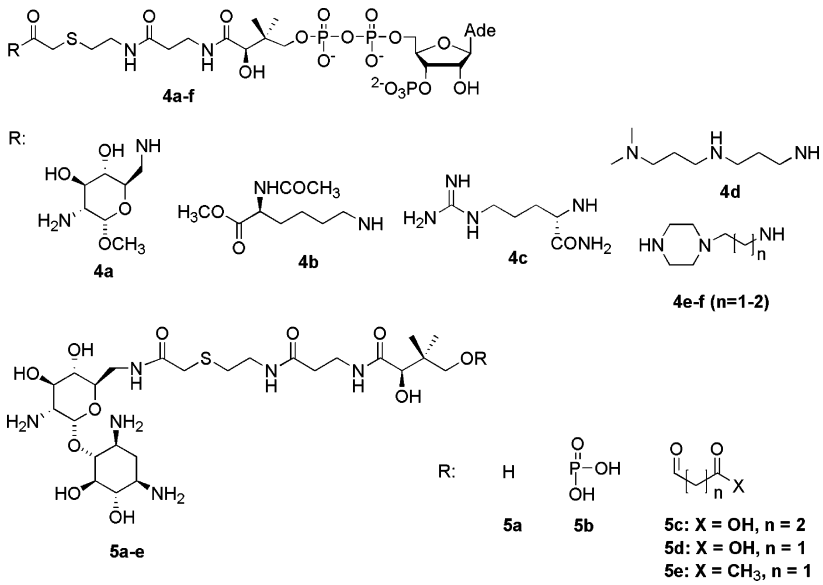
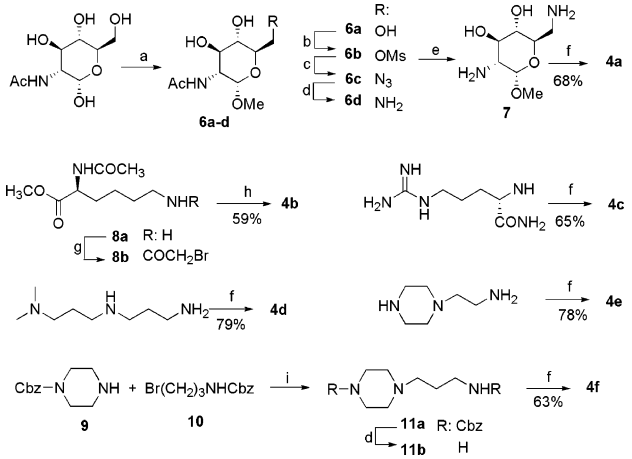


Figure 2. Second generation of AAC(6′) inhibitors.

Scheme 1. Synthetic Route to Inhibitors **4a**-**f**[a]



[a] Reagents and conditions: (a) Amberlite IR-120 (H), MeOH, reflux, 16 h (89%); (b) MsCl, py, 0 °C, 6 h (73%); (c) NaN_3, acetone/H_2O, reflux, 16 h (78%); (d) H_2/Pd(OH)$_2$, MeOH, room temp, 16 h (94%); (e) Ba(OH)$_2$, MeOH/H_2O, reflux, 16 h (81%); (f) 0.95 equiv of NBD bromoacetate, acetone/H_2O, room temp, 10 min, then add 0.9 equiv of CoA in TEA−H_2CO_3 buffer, pH 8.5, room temp, 1 h; (g) 1.05 equiv of bromoacetic acid, DCC, DMAP, DCM, room temp, 3 h (78%); (h) 0.95 equiv of CoA, TEA−H_2CO_3 buffer, pH 8.5, room temp, 1 h; (i) K_2CO_3, DMF, room temp, 16 h (97%).

rivatization and because of the water solubility of aminoglycosides and coenzyme A (CoA) derivatives. It was envisaged that our previously reported method to assemble aminoglycoside−CoA bisubstrates in one pot would be a great asset in the synthesis of truncated derivatives.[12] The syntheses described below all converge to the one-pot chemo- and regioselective coupling of an amine to a thiol with incorporation of an acetyl linker. From there, retrosynthetic analyses are reduced to the preparation of the corresponding amines and thiols, with no need for protecting groups. The first target, **4a** (Figure 2), is an analogue of **1a** lacking the 2-deoxystreptamine ring (2-DOS, ring II of the aminoglycoside). The amine moiety of target **4a** was prepared from *N*-acetylglucosamine (Scheme 1). Thus, after glycosylation to methyl 2-acetamido-α-D-glucopyranoside (**6a**),[14] the N-6′-amine was introduced by selective O-6′-mesylation and azidation, followed by reduction to methyl 2-acetamido-6-amino-α-D-glucopyranoside (**6d**). The *N*-2-acetyl group was removed using Ba(OH)$_2$ to yield **7**. This step could not be reported until later because of reagent incompatibility with the bromoacetyl group. As reported for aminoglycosides,[12] the use of *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide bromoacetate (NBD bromoacetate) followed by addition of CoA afforded **4a** in a one-pot reaction. Interestingly, the removal of ring II from the aminoglycoside moiety of **1a** led to a 50-fold decrease in affinity for AAC(6′)-Ii (Table 1). This result emphasizes the strength of the salt bridges and hydrogen bonds observed between the 2-DOS of **1a** and the enzyme in the crystal structure of the **1a**−AAC(6′)-Ii complex.[15]

Table 1. Patterns of Inhibition and Inhibition Constants for **4a**−**f** and **5a**−**e** Measured against AAC(6′)-Ii

inhibitor[a]	inhibition pattern[b]	K_i (μM)[c]
4a	competitive	3.4 ± 2.0
4b	competitive	∼130
4c	competitive	1.2 ± 0.2
4d	competitive	3.4 ± 1.7
4e	competitive	3.6 ± 0.4
4f	competitive	7.4 ± 2.9
5a	no inhibition	≥200
5b	competitive	12 ± 8
5c	no inhibition	≥1000
5d	competitive	2.2 ± 1.1
5e	competitive	11 ± 6

[a] All molecules were tested as the TFA salt. [b] Inhibition pattern versus AcCoA. [c] As a method validation, K_m and k_{cat} were determined for neamine, kanamycin A, and ribostamycin. They were comparable to those reported in the literature.[5]

AAC(6)′-Ii has been shown to acetylate polylysines and histones at the ε-NH_2.[5] To better understand the specificity of AAC(6′)-Ii for aminoglycosides, the neamine moiety of **1a** was replaced with lysine (target **4b**). Commercial N^{α}-acetyl-L-lysine methyl ester (**8a**) was selected to better mimic peptidic lysines. Compound **8a** was bromoacetylated to **8b** before reaction with CoA to yield **4b** (Scheme 1). To our surprise, **4b** showed 1700-fold reduced inhibition against AAC(6′)-Ii compared to **1a**. This result was attributed to the lack of positive charges available to interact with the negatively charged aminoglycoside binding site of this enzyme. To further confirm this assumption, we next prepared an arginine−CoA analogue. The use of NBD bromoacetate, here to selectively bromoacetylate arginine at the α-NH_2, followed by addition of CoA led to the formation of **4c** in one

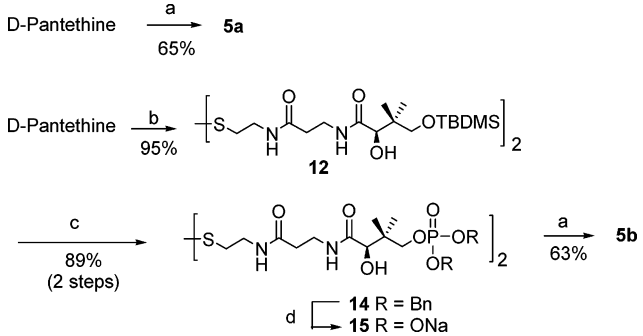
pot (Scheme 1). Remarkably, the arginine−CoA analogue **4c** was at least 2 orders of magnitude more potent than the lysine−CoA analogue **4b** and even slightly more potent than **4a** (Table 1). This result confirms the importance of positive charges for binding the aminoglycoside pocket of AAC(6′)-Ii, consistent with the electrostatic properties of the active site.[7]

Propylenediamine derivatives have been shown to imitate well the diamine portion of 2-DOS in the design of non-carbohydrate mimics of aminoglycosides.[16] This concept was used to conceive target **4d**, which includes one more positive charge compared to **4c**. Compound **4d** was assembled from commercial *N*,*N*-dimethyldipropylenetriamine without the need for protection, using NBD bromoacetate and CoA as described above (Scheme 1). Despite the extra positive charge, **4d** shows inhibition comparable to that observed for **4c** (Table 1). The larger entropic penalty needed for binding the polyamine may compensate for the higher enthalpy of binding expected with an additional electrostatic interaction.

To reduce the flexibility of the linear polyamine, the preparation of piperazine derivatives **4e** and **4f** was next envisaged. Indeed, piperazine is a common pharmacophore that may resemble 2-DOS.[17] Compound **4e** was synthesized directly from commercially available 1-(2-amino)piperazine (Scheme 1). Synthesis of **4f** began with a reaction between benzyl 1-piperazinecarboxylate (**9**) and benzyl 3-bromopropylcarbamate (**10**) in the presence of K_2CO_3 to form **11a** in quantitative yield (Scheme 1). Global Cbz deprotection of **11a** to **11b** was followed by our standard one-pot reaction with NBD bromoacetate and CoA to yield **4f**. Inhibition studies with **4e** and **4f** revealed similar potency compared to **4c** and **4d**. The longer chain of **4f** is somewhat detrimental. Overall these results not only confirm the importance of the positive charges for interaction with the aminoglycoside pocket of AAC(6′)-Ii but also confirm the necessity of proper orientation of the amino groups. Our results are also in agreement with a previous report suggesting that neamine is the minimal substrate of AAC(6′)-Ii.[5] Retaining neamine in the inhibitor is rather convenient because of the low cost of this aminoglycoside, the ease of derivatization using our one-pot methodology, and the known active transport of aminoglycosides across the cell membranes of most bacterial strains.[1] Neamine is also the smallest aminoglycoside able to cause mistranslation in bacterial protein synthesis.[18]

After modifications at the neamine moiety of **1a** (targets **4a**−**f**) we next envisaged truncation of the CoA end of **1a** (targets **5a**−**e**, Figure 2). The multiple negative charges of CoA are believed to prevent bisubstrates **1**−**4** from crossing bacterial cell membranes. Inhibitors that can penetrate cells are desirable not only for cell-based experiments but also for potential use as pharmaceuticals. Binding to the pantetheinyl portion of AcCoA to acetyltransferases is relatively conserved.[19−22] We hypothesized that with an intact pantetheinyl group, the molecules would more likely be general AAC(6′) inhibitors. Target **5a** was prepared in one-pot by regioselective N-6′-derivatization of neamine in the presence of NBD bromoacetate and D-pantetheine (Scheme 2). D-Pantetheine was produced in situ by reduction of commercial D-pantethine using dithiothreitol (DTT). Compound **5a** was next tested at concentrations up to 200 μM for its effect on the activity of AAC(6′)-Ii but did not show any significant inhibition. A considerable reduction in affinity was expected on the basis of the crystal structures of AAC(6′)-Ii,[7,8,11] which reveal that of the hydrogen bonds formed between the enzyme and AcCoA, five are between the phosphate groups and the protein. These hydrogen bonds likely serve as strong anchoring points for the cofactor. The absence of inhibition is likely also explained by the high flexibility of the pantetheinyl group.

Scheme 2. Synthetic Route to Inhibitors **5a**,**b**[a]

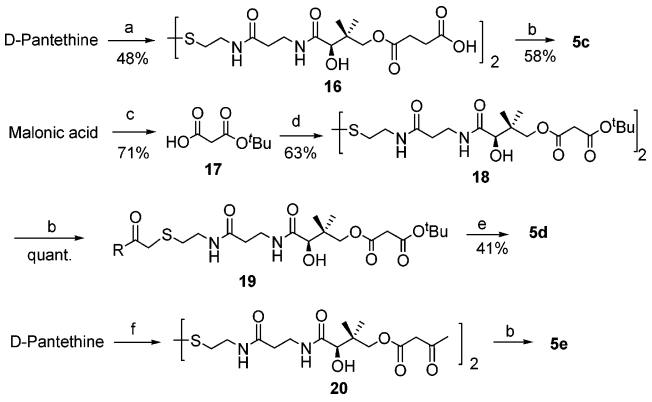


[a] Reagents and conditions: (a) *N*-6′-bromoacetylneamine, acetone/H_2O, DTT, 20 equiv of DIPEA, room temp, 1 h; (b) *tert*-butyldimethylsilyl chloride, imidazole, DMF, room temp, 16 h (99%); (c) dibenzylchlorophosphate (**13**), CsF (2.5 equiv), py, room temp, 3 h (97%); (d) Na, naphthalene, THF, −40 °C to room temp, 2 h (91%).

Target **5b** was next prepared to examine the importance of the pantetheinyl 4′-phosphate group. Synthesis of **5b** began with the preparation of known dibenzylphosphoryl chloride (**13**) using a modification of the reported procedure.[23] Direct phosphorylation of D-pantethine using **13** under conditions described previously for other alcohols[24] was not successful. This may be due to the low nucleophilicity of the 4′-hydroxyl group of D-pantethine, likely resulting from an intramolecular hydrogen bond with the nearby hydroxyl group. Thus, a silyl ether was formed to temporarily break this hydrogen bond. Cleavage of the silyl ether with CsF to generate the alcohol in situ facilitated the reaction with **13** to afford **14** (Scheme 2). Intermediate **14** was reduced using sodium naphthalenide[25] to remove the benzyl groups and to generate the free thiol. With the desired thiol in hand, our one-pot methodology was next applied using neamine and NBD bromoacetate to afford **5b** in 63% yield after HPLC purification. To our surprise, the simple addition of one phosphate group was sufficient to recover low micromolar inhibition.

The relatively good activity of **5b** encouraged us to add a second phosphate group; however, phosphates have negative charges that reduce their applicability. Two strategies may be envisaged to overcome this issue. One is to temporarily mask the phosphoric acids as esters or amides (e.g., as in some prodrugs).[26] A second approach is to use a mimic of the phosphate group.[27] The crystal structures reveal that the interactions between the CoA pyrophosphate group and the enzyme involve mainly hydrogen bonds as opposed to salt bridges.[7,8,11] This implies that it may be possible to mimic the pyrophosphate moiety with two carbonyl groups, as in targets **5c**−**e**. Synthesis of **5c** first involved the coupling of succinic anhydride to the 4′-hydroxyl group of D-pantethine to yield **16** (Scheme 3). Compound **16** was transformed into **5c** in one pot in the presence of DTT (in situ reduction of the disulfide), neamine, and NBD bromoacetate. The succinate moiety turned out to be a bad mimic of the pyrophosphate group, as suggested by the absence of AAC(6′)-Ii inhibition in the presence of **5c**. Synthesis of **5d** began with the coupling of *tert*-butyl malonate (**17**) and D-pantethine to yield **18** (Scheme 3). Removal of the *tert*-butyl protecting group at this point was not successful but proceeded more easily when carried after disulfide reduction and reaction with NBD bromoacetate and neamine. We were pleased to see that **5d** is a more potent inhibitor of AAC(6′)-Ii than **5b**, which

Scheme 3. Synthetic Route to Inhibitors **5c**−**e** (R = *N*-6′-Neamine)[a]



[a] Reagents and conditions: (a) succinic anhydride, py, room temp, 16 h; (b) *N*-6′-bromoacetylneamine, acetone,/H_2O, DTT, 20 equiv of DIPEA, room temp, 1 h; (c) MsCl, py, *tert*-butanol, THF, 0 °C, 2 h, room temp, 1 h; (d) D-pantethine, DCC, DMAP, THF, room temp, 16 h; (e) TFA/DCM/anisole, room temp, 16 h; (f) lithium acetoacetate, HATU, 2 equiv of DIPEA, DMF, room temp, 16 h.

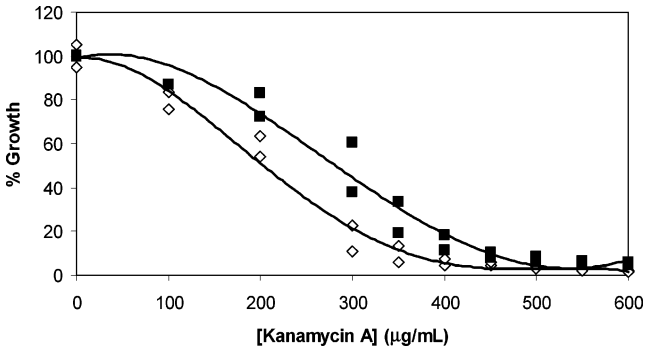


Figure 3. Potentiation of kanamycin A antibiotic activity by compound **5e**. Growth of *E. faecium* C238 in the absence (solid square) and presence of 512 μg/mL **5e**.

contained a phosphate group on 4′-OH. This result suggests that malonate is a good mimic of the pyrophosphate. The two carbonyl groups of succinate are likely too far apart to mimic the pyrophosphate group, as suggested by the absence of inhibition observed for **5c**. The malonate group of **5d**, on the other hand, seems to properly position the two carbonyls for interaction with the enzyme. It is interesting to note that truncation of ring II of neamine (see **4a**) has the same effect on the inhibition as the deletion of the entire ADP moiety of CoA (see **5b** and **5d**). This was unexpected based on the K_m values of neamine (6 μM) and CoA (9 μM) for this enzyme.[5] Next, **5e** was prepared and tested to verify the importance of the negative charge of the malonate group (the methyl malonate derivative was prepared but found to be unstable). Synthesis of **5e** involved the coupling of acetoacetate to pantethine, followed by disulfide reduction and reaction with *N*-6′-bromoacetylneamine as before (Scheme 3). We were pleased to find that replacement of a negatively charged oxygen with a methyl group did not cause a considerable reduction in the affinity for AAC(6′)-Ii. This result confirms that, for this enzyme, the pyrophosphate moiety of the CoA substrate can be conveniently mimicked with a β-dicarbonyl group.

Molecules **5d** and **5e** were tested in combination with the aminoglycoside kanamycin A (a substrate for AAC(6′)) against *E. faecium* C238 harboring the AAC(6′)-Ii encoding gene. Compound **5d** did not show any activity, whereas **5e** displayed a synergistic effect with kanamycin A and effectively decreased its MIC as predicted for an inhibitor of antibiotic resistance (Figure 3). This supports our hypothesis that the negative charge of the carboxylate group may prevent cell penetration.

Discussion

In conclusion, we have demonstrated that aminoglycoside−CoA bisubstrate analogues can be rapidly and efficiently assembled using NBD bromoacetate directly with unprotected amines and truncated CoA analogues. Our methodology is chemo- and regioselective, efficient and applicable to water-soluble molecules. By use of this method, 11 analogues of bisubstrate **1a** were rapidly synthesized. These molecules were tested for inhibition of the aminoglycoside resistance-causing enzyme AAC(6′)-Ii. This enzyme represents a good model not only for AAC(6′)s but also for the numerous other acetyltransferases that adopt the same fold and/or proceed via a similar catalytic mechanism (e.g., some histone acetyltransferases[28−30] involved in gene regulation and cancer, serotonine acetyltransferase[31] involved in circadian rhythm, and spermidine/spermine *N*-1-acetyltransferase[32] involved in cancer and HIV infection).

The bisubstrate analogues were tested in vitro for their effect on the activity of AAC(6′)-Ii. This led to the elucidation of several structure−activity relationships (Figure 4). Our results indicate that inhibition by aminoglycoside−CoA bisubstrates is more sensitive to truncation at the aminoglycoside than at the CoA end. The smaller neamine is, however, as efficient as larger aminoglycosides to maintain the affinity of the bisubstrate for AAC(6′)-Ii. This was expected on the basis of the reported K_m for various AAC(6′)-Ii substrates.[5] Our results with analogues **4a**−**e** have also emphasized the importance of properly oriented positively charged amino groups for interaction with the aminoglycoside binding pocket of AAC(6′)-Ii.

Truncation of the CoA moiety of bisubstrate **1a** revealed that the pantetheinyl group is not sufficient for recognition by the enzyme and at least one phosphate group is needed. More potent inhibitors are obtained when the pyrophosphate moiety of CoA

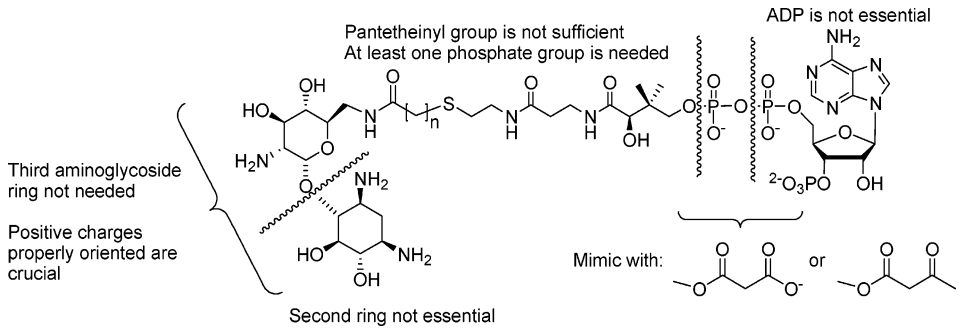


Figure 4. Summary of SARs.

is mimicked with β-dicarbonyl groups such as malonate or acetoacetate. To our knowledge, this is the first time that a pyrophosphate group is successfully mimicked with acetoacetate. Compound **5e** is also the first inhibitor of an aminoglycoside resistance-causing enzyme active in cells. This provides a proof of concept for the pharmaceutical potential of AAC(6′) inhibitors.

These structure−activity relationships, combined with our structural studies of bisubstrate-AAC(6′)-Ii complexes,[12] provide crucial information for the design of AAC(6′) inhibitors and novel antibacterials effective against resistant strains. Compound **5e** represents a new lead in the development of pharmaceuticals able to block aminoglycoside resistance caused by AAC(6′).

Experimental Section

General. All reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada) except for protected lysine **8a**, which was purchased from Chem-Impex International Inc. (Wood Dale, IL), and HATU, which was purchased from GL Biochem Ltd. (Shanghai, China). Coenzyme A was purchased as the trilithium salt (∼95%). Reagents and solvents were used without further purification unless otherwise stated. Flash chromatography and analytical TLC (F-254) were performed with 60 Å silica gel from Silicycle (Quebec, Canada). All the target molecules were purified by reversed-phase HPLC using an Agilent 1100 modular system equipped with an autosampler, a quaternary pump system, a photodiode array detector, a thermostated column compartment, and a ChemStation (for LC 3D A.09.03) data system. The column used was a semipreparative Zorbax SB-CN of 4.6 mm × 250 mm and 5 μm (Agilent, Palo Alto, CA). Samples were eluted at a flow rate of 3 mL/min, using a combination of mobile phase A (0.05% TFA in water at pH ∼3.5) and mobile phase B (acetonitrile containing 0.05% TFA). After 2 min at 1% mobile phase B, the eluent was brought to 40% mobile phase B in a linear gradient over 20 min. For molecules containing an adenine group, the detector was set to 260 nm; otherwise, 214 nm was used. The purity of the targets was evaluated by HPLC using the same equipment and column described above but using an isocratic elution with ratios of phase A/phase B of either 65/35 (method A) or 55/45 (method B). The purity of all target molecules used in the biological assays was >95%. HRMS data were obtained by direct infusion electrospray ionization from a solution of 50 mM formic acid: methanol, 90:10, at 2 μL/min in an IonSpec 7 T FTICR instrument. The resolving power was approximately 80 000. HRMS data of all intermediates were obtained on a Kratos MS 25RFA mass spectrometer at a source temperature of 200 °C and 70 eV. LRMS was performed using a Finnigan LCQDUO mass spectrometer with ESI without fragmentation. ^{1}H and ^{13}C NMR spectra were recorded using a Varian mercury 400 or 300 or a Unity 500 spectrometer. For all ^{1}H NMR spectra of target molecules, presaturation (presat) is used to suppress the water peak. The chemical shifts (δ) were reported in parts per million (ppm) and are referenced to either the internal standard TMS (when $CDCl_3$ is used) or the deuterated solvent used. The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; dt, doublet of triplet; ddd, doublet of doublet of doublet; td, triplet of doublet; m, multiplet; q, quartet; p, pentet; br s, broad singlet. The coupling constants J are reported in hertz (Hz). The NMR spectra were obtained from the TFA salts of **4a**−**f** and **5a**−**e** in D_2O (pD ∼4). Reactions with air- or moisture-sensitive reagents and solvents were carried out under an atmosphere of argon. All the reactions were carried out in round-bottom flasks capped with a septum unless otherwise stated.

Synthetic Procedures and Compound Characterization. *N*-6′-Bromoacetylneamine Solution. Neamine free base (32 mg, 0.1 mmol) was dissolved in water (2 mL) in a vial, and *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide bromoacetate[1] (18 mg, 0.06 mmol) was dissolved in acetone (2 mL) in another vial. The two solutions were mixed, sonicated for 1 min, and stirred for 15 min. This solution is referred to as the *N*-6′-bromoacetylneamine solution.

General Procedure for the Preparation of 4a,c−f. The general procedure for the preparation of **4a,c**−**f** is exemplified with the following procedure for **4a**. Compound **7** (20 mg, 0.1 mmol) was dissolved in water (2 mL) in a vial. *endo*-*N*-Hydroxy-5-norbornene-2,3-dicarboximide bromoacetate[1] (18 mg, 0.06 mmol) was dissolved in acetone (2 mL) in another vial. The two solutions were mixed, sonicated for 1 min, and stirred for an extra 15 min before addition of CoA−SH (15 mg, 0.02 mmol) in TEA H_2CO_3 buffer (4 mL, pH 8.5). The mixture was stirred for another hour and then evaporated to dryness under vacuum. The crude powder was dissolved in a 0.05% solution of TFA in water and purified by HPLC.

Compound 4a. Yield: 11 mg, 68%. ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.51 (s, 1H), 8.29 (s, 1H), 6.06 (d, J = 5.0, 1H), 4.83 (d, J = 3.5, 1H), 4.78−4.71 (m, 1H), 4.46 (br s, 1H), 4.14 (m, 2H), 3.88 (s, 3H), 3.73 (m, 1H), 3.66 (t, J = 10.0, 1H), 3.56 (m, 1H), 3.51−3.46 (m, 2H), 3.33 (m, 3H), 3.26 (s, 3H), 3.21 (t, J = 6.0, 2H), 3.18 (dd, J = 30, 9.0, 1H), 3.15 (s, 3H), 2.54 (t, J = 6.5, 2H), 2.33 (t, J = 6.5, 2H), 0.80 (s, 3H), 0.68 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.9, 174.1, 173.1, 150.0, 144.9, 142.6, 118.7, 117.6, 109.5, 103.5, 96.2, 87.7, 83.6, 74.4, 74.0, 72.2, 71.2, 70.3, 69.9, 65.3, 55.4, 54.1, 40.2, 38.4, 35.6, 35.5, 34.8, 31.4, 21.0, 18.5. HRMS for $C_{30}H_{52}N_9O_{21}P_3S$ $(M + 2H)^{2+}$ calcd 500.6178, found 500.6177. Purity, 98% (method A, t_R = 3.13 min; method B, t_R = 3.00 min).

Compound 4b. Compound **8b** (16 mg, 0.05 mmol) and CoA (25 mg, ∼0.03 mmol of the lithium salt) were dissolved in triethylammonium bicarbonate buffer (pH ∼8.5, 4 mL). The mixture was stirred overnight at room temperature and evaporated to dryness. The resulting white powder was dissolved in water containing 0.05% TFA and purified by HPLC to yield **4b** as a white powder (yield: 19.8 mg, 59%). ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.31 (s, 1H), 8.13 (s, 1H), 6.05 (d, J = 5.0, 1H), 4.46 (s, 1H), 4.20 (dd, J = 8.8, 3.6, 1H), 4.13 (s, 2H), 3.91 (s, 1H), 3.68 (d, J = 9.2, 1H), 3.64 (s, 3H), 3.44 (d, J = 9.2, 1H), 3.35 (t, J = 6.4, 2H), 3.25 (t, J = 6.4, 2H), 3.14 (s, 2H), 3.08 (t, J = 6.4, 2H), 2.56 (t, J = 6.4, 2H), 2.35 (t, J = 6.4, 2H), 1.93 (s, 3H), 1.81 (m, 1H), 1.61 (m, 1H), 1.56 (m, 2H), 1.40 (p, J = 7.2, 2H), 1.24 (m, 2H), 0.76 (s, 3H), 0.63 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.7, 174.4, 174.3, 174.2, 174.0, 154.1, 150.8, 149.3, 140.5, 119.2, 95.4, 86.8, 84.0, 74.2, 74.0, 73.8, 72.1, 71.9, 68.0, 67.6, 65.7, 53.5, 52.9, 39.7, 38.2, 35.8, 34.6, 31.4, 27.5, 21.3, 20.4, 18.2. HRMS for $C_{32}H_{54}N_9O_{20}P_3S$ (M + H) calcd 1010.2423, found 1010.2425. Purity, 98% (method A, t_R = 3.44 min; method B, t_R = 3.29 min).

Compound 4c. Yield: 10.2 mg, 65%. ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.49 (d, J = 1.6, 1H), 8.26 (s, 1H), 6.05 (d, J = 5.2, 1H), 4.74 (m, 1H), 4.43 (br s, 1H), 4.11 (m, 3H), 3.87 (s, 1H), 3.71 (m, 1H), 3.46 (br d, J = 9.6, 1H), 3.32 (t, J = 6.4, 2H), 3.20 (t, J = 6.4, 2H), 3.16 (s, 2H), 3.01 (t, J = 6.4, 3H), 2.54 (t, J = 6.4, 2H), 2.31 (t, J = 6.4, 2H), 1.75−1.65 (m, 1H), 1.65−1.55 (m, 1H), 1.55−1.40 (m, 2H), 0.79 (s, 3H), 0.68 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 175.8, 174.4, 174.2, 174.0, 158.2, 154.4, 151.2, 149.7, 141.0, 118.9, 86.8, 84.0, 74.2, 73.8, 72.1, 71.9, 67.6, 65.7, 53.5, 52.9, 39.5, 38.2, 35.8, 34.6, 31.4, 27.5, 21.3, 20.4, 18.0. HRMS for $C_{29}H_{51}N_{12}O_{18}P_3S$ (M + H) calcd 981.2451, found 981.2475. Purity, 98% (method A, t_R = 3.13 min; method B, t_R = 3.00 min).

Compound 4d. Yield: 13 mg, 78%. ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.52 (s, 1H), 8.33 (s, 1H), 6.09 (d, J = 5.0, 1H), 4.78 (m, 1H), 4.48 (br s, 1H), 4.16 (m, 2H), 3.89 (s, 1H), 3.74 (m, 1H), 3.65−3.50 (m, 12H), 3.41−3.32 (m, 4H), 3.26−3.19 (m, 4H), 2.57 (t, J = 6.4, 2H), 2.34 (t, J = 6.4, 2H), 0.81 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.6, 174.2, 173.9, 150.0, 149.3, 145.0, 143.1, 119.5, 88.6, 82.8, 74.0, 73.8, 72.0, 70.2, 67.9, 56.8, 49.3, 42.0, 41.4, 39.0, 36.1, 36.0, 35.2, 34.7, 32.1, 21.2, 18.8. HRMS for $C_{29}H_{51}N_{10}O_{17}P_3S$ (M + H) calcd 937.2367, found 937.2456. Purity, 96% (method A, t_R = 3.11 min; method B, t_R = 2.98 min).

Compound 4e. Yield: 9.7 mg, 63%. ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.55 (s, 1H), 8.35 (s, 1H), 6.11 (d, J = 5.0, 1H), 4.79 (m, 1H), 4.46 (br s, 1H), 4.19 (m, 2H), 3.92 (s, 1H), 3.79 (m, 3H), 3.70−3.50 (m, 6H), 3.50−3.38 (m, 4H), 3.28−3.15 (m, 8H), 2.61

(t, J = 6.4, 2H), 2.39 (t, J = 6.4, 2H), 1.94 (m, 2H) 0.81 (s, 3H), 0.71 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.5, 174.0, 173.6, 150.1, 149.4, 145.0, 143.2, 119.5, 88.0, 83.1, 74.2, 73.8, 72.2, 69.5, 64.8, 54.7, 46.6, 40.4, 39.8, 38.4, 36.4, 35.3, 35.1, 34.7, 31.2, 23.4, 20.9, 18.3. HRMS for $C_{30}H_{53}N_{10}O_{17}P_3S$ (M + H) calcd 951.2524, found 951.2528. Purity, 97% (method A, t_R = 3.27 min; method B, t_R = 3.08 min).

Compound 4f. Yield: 13 mg, 79%. ^{1}H NMR (D_2O, 400 MHz, presat): δ 8.47 (s, 1H), 8.25 (s, 1H), 6.04 (d, J = 5.0, 1H), 4.41 (br s, 1H), 4.16−4.03 (m, 2H), 3.83 (s, 1H), 3.67 (dd, J = 9.6, 4.4, 1H), 3.43 (dd, J = 9.6, 4.4, 1H), 3.30 (m, 2H), 3.19−3.09 (m, 5H), 3.09−3.02 (m, 3H), 3.02−2.98 (m, 2H), 2.98−2.88 (m, 4H), 2.72 (s, 6H), 2.50 (t, J = 6.8, 2H), 2.29 (t, J = 6.8, 2H), 2.03−1.95 (m, 2H), 1.80−1.71 (m, 2H), 0.76 (s, 3H), 0.65 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.6, 174.0, 173.0, 150.0, 148.5, 147.4, 144.8, 142.5, 118.7, 118.33, 87.1, 83.5, 74.5, 74.2, 72.2, 65.3, 54.5, 46.9, 44.5, 43.0, 38.6, 36.7, 35.6. 35.1, 31.5, 25.8, 23.4, 21.6, 21.2, 18.7. HRMS for $C_{31}H_{57}N_{10}O_{17}P_3S$ (M + H) calcd 967.2827, found 967.2917. Purity, 98% (method A, t_R = 3.32 min; method B, t_R = 3.11 min).

Compound 5a. In a vial, D-pantethine (15 mg, 0.03 mmol), DTT (5 mg, 0.03 mmol), and DIPEA (1 mL, 5 mmol) were mixed in acetone/H_2O (2 mL, 1/1 v/v) and sonicated for 2 min. This solution was transferred to the *N*-6′-bromoacetylneamine solution (0.06 mmol, 4 mL), and the resulting mixture was sonicated for another 2 min and then stirred for 1 h. After evaporation of the solvent under vacuum, the residue was dissolved in 0.1% aqueous TFA (10 mL) and washed with ethyl acetate (2 × 3 mL). The aqueous layer was evaporated to dryness under vacuum and redissolved in 0.05% TFA water (3 mL) before purification by HPLC to give **5a** (22 mg, 65% based on D-pantethine). In general the final workup process and purification for **5b**−**d** are the same as described here for **5a**. ^{1}H NMR (D_2O, 400 MHz, presat): δ 5.56 (d, J = 3.2, 1H), 3.82 (s, 1H), 3.74 (m, 3H), 3.52−3.30 (m, 10H), 3.30−3.12 (m, 6H), 2.58 (t, J = 6.4, 2H), 2.35 (m, 3H), 1.73 (q, J = 12.4, 1H), 0.76 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.4, 173.9, 173.7, 96.8, 79.0, 75.9, 75.0, 72.6, 71.5, 70.5, 68.8, 68.5, 53.8, 49.7, 48.5, 40.0, 39.5, 38.3, 35.4, 35.3, 34.7, 31.2, 28.3, 20.5, 19.2. HRMS for $C_{25}H_{48}N_6O_{11}S$ (M + H) calcd 641.3175, found 641.3167. Purity, 95% (method A, t_R = 3.25 min; method B, t_R = 3.06 min).

Compound 5b. 14 (25 mg, 0.05 mmol) and DIPEA (1 mL, 5 mmol) were mixed in acetone/H_2O (2 mL, 1/1 v/v) and sonicated for 2 min. This solution was transferred into the *N*-6′-bromoacetylneamine solution (0.06 mmol, 4 mL). The resulting mixture was sonicated for another 2 min and stirred for 1 h. Workup as described for **5a** was followed by HPLC to give **5b** (24 mg, 63% based on **14**). ^{1}H NMR (D_2O, 400 MHz, presat): δ 5.60 (br s, 1H), 3.90 (br s, 1H), 3.70−3.85 (m, 3H), 3.70−3.60 (m, 1H), 3.52−3.39 (m, 9H), 3.39−3.19 (m, 6H), 2.60 (t, J = 6.5, 2H), 2.38 (m, 3H), 1.78 (q, J = 13.2, 1H), 0.85 (s, 3H), 0.78 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 177.4, 175.0, 173.7, 96.1, 78.2, 77.1, 75.4, 72.7, 70.8, 70.2, 69.5, 68.4, 53.8, 49.9, 48.6, 41.5, 40.6, 39.7, 36.0, 33.1, 30.4, 29.1, 28.4, 21.0, 18.6. ^{31}P NMR (D_2O, 81 MHz): δ −0.05. HRMS for $C_{25}H_{49}N_6O_{14}PS$ (M + H) calcd 721.2838, found 721.2846. Purity, 97% (method A, t_R = 3.10 min; method B, t_R = 2.99 min).

Compound 5c. 15 (25 mg, 0.03 mmol), DTT (5 mg, 0.03 mmol), and DIPEA (1 mL, 5 mmol) were mixed in acetone/H_2O (3 mL, 1/1 v/v). The mixture was sonicated for 2 min and stirred overnight. This solution was transferred to the *N*-6′-bromoacetylneamine solution (4 mL). The resulting mixture was sonicated for 2 min and stirred for 1 h. Workup as described for **5a** was followed by HPLC to give **5d** (26 mg, 58%). ^{1}H NMR (D_2O, 400 MHz, presat): δ 5.57 (d, J = 3.5, 1H), 3.89 (d, J = 10.5, 1H), 3.83 (s, 1H), 3.79−3.70 (m, 4H), 3.51 (d, J = 9.0, 1H), 3.48 (t, J = 4.0, 1H), 3.45 (t, J = 4.0, 1H), 3.43−3.40 (m, 1H), 3.39 (m, 1H), 3.38−3.34 (m, 3H), 3.28−3.22 (m, 4H), 3.17 (m, 2H), 2.56 (m, 6H), 2.40−2.32 (m, 3H), 2.06 (s, 2H), 1.73 (q, J = 13.2, 1H), 0.81 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 174.4, 173.2, 172.2, 171.6, 170.6, 96.9, 79.3, 75.3, 75.2, 72.7, 71.8, 70.7, 70.6, 70.4, 68.9, 54.0, 48,8, 41.8, 39.8, 39.4, 38.5, 37.3, 35.0, 31.5, 30.5, 29.1, 28.5, 20.7, 19.6. HRMS for $C_{29}H_{52}N_6O_{14}S$ (M + H) calcd 741.3335, found 741.3333. Purity, 98% (method A, t_R = 3.55 min; method B, t_R = 3.34 min).

Compound 5d. Crude **18** (80 mg, 0.10 mmol) was dissolved in TFA/DCM/anisole (3 mL, 90/9/1 v/v) in a plastic bottle and stirred overnight. After workup as described for **5a** and evaporation of the TFA and DCM, the residue was purified by HPLC to give **5c** (18 mg, 41%). ^{1}H NMR (D_2O, 500 MHz, presat): δ 5.57 (d, J = 3.5, 1H), 3.94 (d, J = 10.5, 1H), 3.84 (s, 1H), 3.80 (d, J = 10.5, 1H), 3.79−3.70 (m, 3H), 3.54−3.30 (m, 9H), 3.30−3.22 (m, 4H, 3.21−3.13 (m, 3H), 2.56 (t, J = 6.4, 2H), 2.40−2.30 (m, 3H), 1.72 (q, J = 13.2, 1H), 0.82 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (D_2O, 125 MHz): δ 174.4, 173.2, 172.2, 171.6, 170.6, 97.2, 79.3, 75.5, 75.2, 72.6, 72.0, 71.2, 70.6, 69.0, 54.0, 50.0, 48.8, 40.1, 39.4, 38.5, 35.6, 35.5, 35.0, 34.8, 31.5, 28.5, 20.7, 20.0. HRMS for $C_{28}H_{50}N_6O_{14}S$ (M + H) calcd 727.3246, found 727.3237. Purity, 98% (method A, t_R = 3.47 min; method B, t_R = 3.25 min).

Compound 5e. D-Pantethine (0.27 g, 0.5 mmol) and lithium acetoacetate (0.18 g, 1.5 mmol) were dissolved in DMF (10 mL). The mixture was stirred for 10 min followed by the addition of HATU (0.38 g, 1.0 mmol) and DIPEA (0.4 mL, 2.0 mmol). The mixture was stirred for 16 h at room temperature before evaporation under vacuum. The residue was triturated with dichloromethane (2 × 10 mL) to yield a paste. This paste was dissolved in $CHCl_3$/MeOH (30 mL, 10/1, v/v) and washed with H_2O (2 × 10 mL). The organic layer was dried over sodium sulfate and evaporated to dryness to give **20** as a yellowish powder. Compound **20** (80 mg, ∼0.1 mmol), DTT (15 mg, 0.1 mmol), and DIPEA (1 mL, 5 mmol) were mixed in acetone/H_2O (5 mL, 1/1 v/v). The mixture was sonicated for 2 min and stirred overnight. This solution was transferred to the *N*-6′-bromoacetylneamine solution (4 mL). The resulting mixture was sonicated for 2 min and stirred for 1 h. Workup was carried out as described for **5a** and followed by HPLC purification to afford **5e** (4.6 mg, 10%). **1**H NMR (D_2O, 400 MHz, presat): δ 5.57 (s, 1H), 3.87 (s, 1H), 3.79 (t, J = 9.6, 2H), 3.69 (t, J = 9.6, 1H), 3.53−3.19 (m, 18 H), 2.62 (t, J = 6.0, 2 H), 2.41−2.34 (m, 3H), 2.16 (s, 3H), 1.71 (q, J = 13.2, 1H), 0.80 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (D_2O, 100 MHz): δ 215.2, 174.4, 174.0, 173.8, 173.5, 98.9, 88.5, 84.8, 76.2, 75.8, 73.2, 73.0, 72.0, 71.8, 69.3, 69.0, 55.8, 53.7, 51.2, 50.0, 48.8, 40.0, 39.6, 36.0, 35.8, 35.0, 32.0, 21.1, 19.3. HRMS for $C_{29}H_{53}N_6O_{13}S$ (M + H) calcd 741.3386, found 741.3384. Purity, 98% (method A, t_R = 3.35 min; method B, t_R = 3.21 min).

Compound 6a. *N*-Acetyl-D-glucosamine (3 g, 13.5 mmol) was dissolved in methanol (70 mL), and Amberlite IR 120 (H) resin (5 g) was added. The mixture was refluxed overnight, cooled to room temperature, and filtered to remove the resin. The filtrate was evaporated to dryness to give **6a** as a white powder (2.83 g, 89%, highly hygroscopic). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (d, J = 4.0, 1H), 4.6 (br s, 3H), 4.50 (d, J = 3.0, 1H), 3.62 (m, 2H), 3.38−3.46 (m, 2H), 3.28 (m, 1H), 3.22 (s, 3H), 3.10 (m, 1H), 1.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 98.6, 73.5, 71.6, 71.5, 61.3, 55.0, 54.5, 23.5. ESI-MS (M + H) for $C_9H_{17}NO_6$, calcd 236.1, found 236.3.

Compound 6b. 6a (1 g, 4.25 mmol) was dissolved in pyridine (15 mL), the mixture was cooled to −40 °C, and methanesulfonyl chloride (0.35 mL, 4.5 mmol) was added. The mixture was stirred at −40 °C for 2 h and at room temperature for 10 h. After evaporation of the solvent, the residue was purified by flash chromatography (R_f = 0.40, $CHCl_3$/MeOH 99/1) to give **6b** as a white powder (0.97 g, 73%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77 (d, J = 5.6, 1H), 5.36 (d, J = 5.6, 1H), 4.88 (d, J = 6.0, 1H), 4.54 (d, J = 3.6, 1H), 4.38 (d, J = 9.8, 1H), 4.27 (dd, J = 10.8, 5.6, 1H), 3.66 (td, J = 10.8, 3.6, 1H), 3.57 (dd, J = 10.8, 5.6, 1H), 3.34 (s, 3H), 3.43 (m, 1H), 3.23 (s, 3H), 3.15 (m, 1H), 1.81 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.0, 98.8, 79.9, 71.2, 71.0, 70.4, 55.4, 54.3, 37.5, 23.4. ESI-MS (M + H) for $C_{10}H_{19}NO_8S$ calcd 314.3, found 314.2.

Compound 6c. 6b (0.63 g, 2.11 mmol) and NaN_3 (0.68 g, 10.55 mmol) were mixed in acetone/H_2O (30 mL, 3/2 v/v). The mixture was refluxed for 16 h. After evaporation of the solvent, the residue

was purified by flash chromatography (R_f = 0.66, $CHCl_3$/MeOH 95/5) to give **6c** as a yellowish powder (0.43 g, 78%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.76 (d, J = 5.6, 1H), 5.26 (d, J = 5.6, 1H), 4.85 (d, J = 5.6, 1H), 4.54 (d, J = 3.6, 1H), 3.66 (td, J = 11.2, 5.4, 1H), 3.38−3.49 (m, 4H), 3.26 (s, 3H), 3.12 (m, 1H), 1.82 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.0, 98.8, 72.3, 71.9, 71.1, 55.3, 54.3, 52.0, 23.4. ESI-MS (M + H) for $C_9H_{16}N_4O_5$: 261.1, found 261.3.

Compound 6d. 6c (0.33 g, 1.27 mmol) and $Pd(OH)_2$/C (10%, 25 mg) were mixed in methanol (20 mL), and hydrogenolysis was performed under 1 atm of H_2 overnight. The mixture was run through a short pad of Celite and purified by flash chromatography (R_f = 0.28, $CHCl_3$/MeOH, 10/1 containing 2% TEA) to give **6d** as a yellowish paste (0.30 g, quant.). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.76 (d, J = 5.4, 1H), 5.38 (br s, 1H), 4.90 (br s, 1H), 4.52 (d, J = 3.6, 1H), 4.38 (d, J = 9.8, 1H), 4.27 (dd, J = 10.2, 5.6, 1H), 3.40−3.60 (m, 3H), 3.00−3.40 (m, 6H), 1.80 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 170.0, 99.0, 74.6, 71.2, 71.0, 55.4, 54.3, 38.1, 23.4. ESI-MS (M + Na) for $C_9H_{18}N_2O_5$: 257.1, found 257.2.

Compound 7. 6d (0.28 g, 1.20 mmol) was dissolved in MeOH/H_2O (20 mL, 1/1 v/v). $Ba(OH)_2$ (1.71 g, 10 mmol) was added, and the mixture was refluxed for 12 h before concentration to ∼10 mL. Following slow addition of 10% H_2SO_4 until pH ∼3, the solution was left in the refrigerator overnight. The next day, the solution was filtered to remove the precipitate ($BaSO_4$). The filtrate was evaporated to dryness to afford **7** as a white foamy powder (0.20 g, 81%). ^{1}H NMR (D_2O, 400 MHz): δ 4.62 (d, J = 3.4, 1H), 3.63 (m, 1H), 3.40 (t, J = 9.6, 1H), 3.23 (s, 3H), 3.17 (dd, J = 13.2, 3.6, 1H), 3.13 (t, J = 9.6, 1H), 2.92 (dd, J = 13.2, 8.8, 1H), 2.72 (dd, J = 9.6, 3.2, 1H). ^{13}C NMR (D_2O, 75 MHz): δ 99.3, 72.8, 71.7, 68.4, 55.7, 54.7, 40.8. HRMS (M + H) for $C_7H_{16}N_2O_4$, calcd 193.1110, found 193.1102.

Compound 8b. 8a (0.95 g, 4.72 mmol) and bromoacetic acid (0.69 g, 4.96 mmol) were dissolved in dry CH_2Cl_2 (40 mL). With stirring, DCC (1.02 g, 4.96 mmol) and DMAP (20 mg) were added, and the mixture was stirred overnight at room temperature. The mixture was filtered to remove DCU, and the filtrate was evaporated to dryness before purification by flash chromatography (R_f = 0.30, $CHCl_3$/MeOH, 95/5 v/v) to yield **8b** as a white and very fine powder (0.96 g, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.30 (br s, NH), 6.09 (br s, NH), 4.55 (m, 1H), 3.86 (s, 2H), 3.74 (s, 3H), 3.28 (m, 2H), 2.03 (s, 3H), 1.88 (m, 1H), 1.75 (m, 1H), 1.56 (m, 2H), 1.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.0, 170.3, 165.9, 52.9, 52.0, 39.9, 32.3, 29.6, 28.9, 23.6, 22.6. HRMS for $C_{11}H_{19}BrN_2O_4$ (M + H) calcd 322.0528 (100%) and 324.0508 (97%), found 322.0520 (100%) and 324.0502 (97%).

Compound 10. 3-Bromopropylamine hydrobromide (2.5 g, 11.4 mmol) was dissolved in 15% aqueous NaOH (40 mL). After the mixture was cooled to 0 °C, benzyl chloroformate (3.26 mL, 22.8 mmol) was added with vigorous stirring. The mixture was stirred overnight at room temperature. The precipitate was collected, washed with warm water (200 mL), and purified by flash chromatography (R_f = 0.35, ethyl acetate/hexane, $^1/_4$ v/v) to afford **10** as a colorless oil (3.4 g, 98%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31(m, 5H), 5.42 (br s, 1H), 5.06 (s, 2H), 3.38 (t, J = 6.5, 2H), 3.28 (m, 2H), 2.01 (m, 2H). ^{13}C NMR ($CDCl_3$,75 MHz): δ 156.8, 146.4, 128.7, 128.3, 127.6, 67.0, 39.8, 32.9, 31.2. ESI-MS (M + Na) for $C_{11}H_{14}BrNO_2$, calcd 294.0, found 294.2 (100%), 296.2 (97%).

Compound 11a. 9 (1.1 g, 5.0 mmol), **10** (1.36 g, 5.0 mmol), and K_2CO_3 (1.38 g, 10 mmol) were mixed in DMF (20 mL). The mixture was stirred at 90 °C for 5 h and then evaporated to dryness. The residue was dissolved in water (100 mL) and extracted with ethyl acetate (3 × 50 mL) to yield **11a** as a colorless oil (1.45 g, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34 (m, 10H), 5.62 (br s, 1H), 5.19 (s, 2H), 5.08 (s, 2H), 3.49 (t, J = 5.2, 4H), 3.26 (m, 2H), 2.39 (m, 6H), 1.68 (p, J = 5.2, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.6, 155.3, 136.9, 136.8, 128.7, 128.6, 128.2, 128.0, 127.6, 127.1, 67.4, 66.8, 57.0, 53.2, 44.1, 40.7, 26.6. ESI-MS (M + H) for $C_{23}H_{29}N_3O_4$, calcd 412.2, found 412.5.

Compound 11b. 11a (1.40 g, 3.4 mmol) was dissolved in methanol (10 mL). $Pd(OH)_2$/C (10% Pd, 0.15 g) was added, and hydrogenolysis was performed under 1 atm of hydrogen at room temperature for 12 h. The mixture was filtered and evaporated to give a yellowish oil (0.52 g), which was dissolved in water (10 mL). The aqueous solution was filtered through cotton and lyophilized to yield **11b** as a white powder (0.46 g, 94%). ^{1}H NMR (D_2O, 300 MHz): δ 2.56−2.64 (m, 6H), 2.20−2.30 (m, 6H), 1.54 (p, J = 5.4, 2H). ^{13}C NMR (D_2O, 75 MHz): δ 55.6, 52.6, 44.2, 38.8, 25.8. ESI-MS (M + H) for $C_7H_{17}N_3$, calcd 144.1, found 144.3.

Compound 12. D-Pantethine (0.22 g, 0.4 mmol) was dissolved in dry DMF (10 mL). Imidazole (75 mg, 1.1 mmol) and *tert*-butyldimethylchlorosilane (140 mg, 1.0 mmol) were added. The mixture was stirred overnight at room temperature. DMF was evaporated under vacuum. The resulting viscous liquid was extracted with $CHCl_3/H_2O$ to yield **13** as a transparent glasslike solid (0.30 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28 (t, J = 6.8, 1H), 6.94 (t, J = 6.8, 1H), 4.72 (br s, 1H), 3.99 (s, 1H), 3.53 (m, 6H), 2.80 (t, J = 6.8, 2H), 2.48 (t, J = 6.8, 2H), 0.95 (s, 3H), 0.94 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.9, 171.7, 79.1, 73.3, 40.0, 38.8, 38.7, 38.1, 36.9, 25.4, 22.1, 19.8, 18.5, −5.2. HRMS (M + H) for $C_{34}H_{70}N_4O_8S_2Si_2$ calcd 783.4174, found 783. 4166.

Compound 13. NCS (0.30 g, 2.2 mmol) was dissolved in dry toluene (10 mL). Benzyl phosphite (0.47 mL, 2.0 mmol) was added, and the mixture was stirred for 2 h at room temperature. After filtration to remove the precipitate, the filtrate was evaporated to yield **13** as a colorless oil (0.58 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36 (br s, 10H), 5.19 (d, J^3_{PH} = 10.2, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 141.1, 132.2, 130.8, 128.1, 70.1. ^{31}P NMR ($CDCl_3$, 81 MHz): δ 6.04. ESI-MS (M + H) for $C_{14}H_{15}ClO_3P$ calcd 297.0, found 297.2.

Compound 14. 13 (0.23 g, 0.29 mmol) was dissolved in dry THF (10 mL). CsF (0.22 g, 1.45 mmol) was added, and the mixture was refluxed for 1 h before cooling to room temperature. To this solution, freshly prepared dibenzylchlorophosphate (**13**, 0.19 g, 0.65 mmol) was added, and the mixture was stirred overnight at room temperature. This mixture was evaporated to dryness under high vacuum to afford a fine powder, which was triturated with $CHCl_3$/MeOH (5 × 20 mL, 10/1 v/v). The combined solutions were evaporated to dryness to yield **14** as a fine white powder (0.29 g, ∼97%). This crude product was used directly in the Birch reduction to prepare **16**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36 (br s, 10H), 7.28 (t, J = 6.8, 1H), 7.01 (t, J = 6.8, 1H), 5.12 (d, J^3_{PH} = 10.2, 4H), 4.75 (br s, 1H), 4.01 (s, 1H), 3.54 (m, 6H), 2.81 (t, J = 6.8, 2H), 2.50 (t, J = 6.8, 2H), 0.93 (s, 3H), 0.88 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.2, 171.9, 141.4, 132.5, 131.1, 128.4, 79.3, 73.5, 70.3, 39.8, 38.4, 38.0, 37.3, 35.6, 22.0, 19.2. ^{31}P NMR ($CDCl_3$, 81 MHz): δ 0.85. ESI-MS (M + H) for $C_{50}H_{68}N_4O_{14}P_2S_2$ calcd 1075.4, found 1075.3.

Compound 15. Sodium metal (0.12 g, 5 mmol, washed with hexane, THF, methanol, and THF) and naphthalene (0.67 g, 5.2 mmol) were dissolved in dry THF (20 mL) at −40 °C. The color of the mixture changed from clear to blue after stirring for 10 min and then to black. This mixture was stirred for a total of 1 h before slow transfer (by cannula) into a solution of **14** (0.25 g, 0.23 mmol) in THF (10 mL) until the solution turned pink (transfer was stopped after ∼15 mL). This mixture was stirred for another hour and then quenched with water (2 mL). After evaporation to dryness the residue was triturated with water (100 mL). The aqueous solution was washed with DCM (2 × 20 mL) and ether (1 × 20 mL) and lyophilized to yield crude product **15** (0.31 g, ∼91%). ^{31}P NMR (D_2O, 81 MHz): δ 5.27. ESI-MS (M + H) for $C_{11}H_{21}N_2O_7PSNa_2$ calcd 403.1, found 403.3.

Compound 16. D-Pantethine (0.275 g, 0.5 mmol), succinic anhydride (0.11 g, 1.1 mmol), and DMAP (25 mg) were dissolved in pyridine (10 mL). The solution was stirred at 60 °C overnight. After evaporation of the pyridine, the residue was purified by flash chromatography (R_f = 0.22, $CHCl_3$/MeOH, 10/1 v/v) to give **16** as a white powder (0.18 g, 48%). ^{1}H NMR (D_2O, 300 MHz): δ 3.86 (d, J = 11, 1H), 3.84 (s, 1H), 3.71 (d, J = 11, 1H), 3.33 (m, 4H),

2.66 (t, $J = 6.4$, 2H), 2.47 (t, $J = 6.4$, 2H), 2.33 (m, 4H), 0.81 (s, 3H), 0.76 (s, 3H). ^{13}C NMR (D_2O, 75 MHz): δ 175.9, 175.1, 174.8, 174.0, 74.8, 70.4, 38.2, 37.8, 36.7, 35.6, 35.5, 31.6, 30.6, 20.8, 19.5. HRMS (M + H) for $C_{30}H_{50}N_4O_{14}S_2$, calcd 755.2766, found 755.2759.

Compound 17. Malonic acid (11.88 g, 114 mmol) was dissolved in dry THF (70 mL). Pyridine (20 mL) and *tert*-butanol (20 mL, 209 mmol) were added with stirring. The mixture was cooled to 0 °C, and methanesulfonyl chloride (9 mL, 116 mmol) was added over 30 min. The mixture was stirred at room temperature for 3 h and then filtered to remove the pyridine hydrochloride salt. The filtrate was concentrated to ~20 mL and diluted in water (200 mL). This solution was brought to pH ~11 using 4 N NaOH and washed with DCM (2 × 25 mL). The aqueous layer was acidified to pH ~3 with concentrated HCl and extracted with DCM (4 × 50 mL) before evaporation to give **17** as a colorless oil **(**13.5 g, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.12 (br s, 1H), 3.34 (s, 2H), 1.47 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.2, 166.4, 82.8, 42.4, 28.3. ESI-MS (M + H) for $C_7H_{12}O_4$ calcd 161.1, found 161.3.

Compound 18. D-Pantethine (0.55 g, 1 mmol) and **17** (0.32 g, 2 mmol) were dissolved in THF/DCM (50 mL, 4/1 v/v). DCC (0.4 g, 2 mmol) and DMAP (25 mg) were added, and the mixture was stirred for 36 h at 60 °C. After filtration to remove DCU, the filtrate was evaporated to dryness and purified by flash chromatography ($R_f = 0.35$, $CHCl_3$/MeOH 10/1) to yield **18** as a white powder (0.53 g, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.48 (t, $J = 5.6$, 1H), 7.32 (t, $J = 5.6$, 1H), 4.58 (br s, 1H), 4.10 (d, $J = 9.2$, 1H), 3.88 (s, 2H), 3.56−3.41 (m, 4H), 3.30 (s, 2H), 2.76 (t, $J = 6.8$, 2H), 2.46 (t, $J = 6.8$, 2H), 1.44 (s, 9H), 1.00 (s, 3H), 0.93 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.1, 172.2, 167.2, 166.3, 82.8, 75.2, 71.6, 43.2, 38.9, 38.8, 38.0, 36.1, 35.6, 28.4, 21.6, 20.6. HRMS (M + H) for $C_{36}H_{62}N_4O_{14}S_2$ calcd 839.3722, found 839. 3712.

Compound 19. 18 (55 mg, 0.065 mmol), DTT (10 mg, 0.065 mmol), and DIPEA (1 mL, 5 mmol) were mixed in acetone (5 mL). The mixture was sonicated for 2 min and vigorously stirred overnight. ESI-MS revealed the disappearance of **18** and the formation of a product with peaks at 421.2 and 443.3 (*m*/*e*), corresponding to [M + H] and [M + Na] of reduced **18** (free thiol). This mixture was added to the *N*-6′-bromoacetylneamine solution (0.06 mmol, 4 mL). The resulting mixture was sonicated for 2 min, stirred for 1 h, and evaporated to dryness to give crude **19** as a white powder (106 mg, quant.).

AAC(6′)-Ii Inhibition Assays. AAC(6′)-Ii was expressed and purified as previously described elsewhere.[12] Enzyme activity was monitored using a procedure reported earlier.[5] Thus, the assays were performed in HEPES buffer (25 mM, pH 7.5) containing EDTA (1 mM), 4,4′-dithiodipyridine (DTDP, 400 μM), neamine (100 μM), and various concentrations of AcCoA. Reaction volumes were typically 400 μL. The assay mixtures were preincubated for 3 min at 37 °C, and the reaction was initiated by the addition of AAC(6′)-Ii (3.6 μM). The initial reaction velocities obtained at various concentrations of inhibitor were fit to eq 1 for competitive inhibition, eq 2 for noncompetitive inhibition, or eq 3 for uncompetitive inhibition, where [S] is the concentration of AcCoA, K_m is the Michaelis−Menten constant, V_{max} is the maximal velocity, [I] is the concentration of inhibitor, and K_i is the inhibition constant.

$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)} \quad (1)$$

$$v = \frac{V_{max}[S]}{([S] + K_m)\left(1 + \frac{[I]}{K_i}\right)} \quad (2)$$

$$v = \frac{V_{max}[S]}{[S]\left(1 + \frac{[I]}{K_i}\right) + K_m} \quad (3)$$

For all molecules tested eq 1 provided a much better fit than eq 2 or eq 3, which suggests competitive inhibition. However, the low K_i values obtained imply that these molecules are tight binding inhibitors (K_i of less than 1000 times the concentration of AAC(6′)-Ii).[33] Thus, eqs 4, 5, and 6[33] were applied for tight binding competitive inhibition, tight binding noncompetitive inhibition, and tight binding uncompetitive inhibition, respectively.[33]

$$K_i^{app} = K_i\left(1 + \frac{[S]}{K_m}\right) \quad (4)$$

$$K_i^{app} = K_i \quad (5)$$

$$K_i^{app} = K_i\left(1 + \frac{K_m}{[S]}\right) \quad (6)$$

The K_i^{app} was determined from Morrison eq 7,[33] where [E] is the AAC(6′)-Ii concentration, v_i is the initial velocity in the presence of inhibitor at concentration [I], and v_0 is the initial velocity in the absence of inhibitor, respectively.

$$\frac{v_i}{v_0} = 1 - \frac{([E] + [I] + K_i^{app}) - \sqrt{([E] + [I] + K_i^{app})^2 - 4[E][I]}}{2[E]} \quad (7)$$

The experimental data fit eq 4 much better than eq 5 or eq 6, confirming that the aminoglycoside derivatives are tight binding competitive inhibitors. Calculated kinetic constants are listed in Table 1.

Evaluation of Biological Activity of Compound 5e. A two-dimensional checkerboard MIC assay was conducted with **5e** and kanamycin A (Bioshop Canada) versus *Enterococcus faecium* C238 in brain heart infusion media (Difco) using NCCLS protocols in duplicate. Compound **5e** was diluted using a 2-fold dilution to create a gradient from 8 to 512 μg/mL, and the range of concentrations for kanamycin A was 100−600 μg/mL. The cultures were grown at 37 °C for 16 h in 96-well microtiter U-bottom MIC plates, and growth was monitored in a SPECTRAmax Plus plate reader at OD_{625}. The data from each set were averaged and normalized against the mean of the sterility controls and reported as a percentage of growth with respect to the mean of the positive growth controls.

Acknowledgment. This work was supported by the Natural Science and Engineering Research Council of Canada (NSERC grant to K.A.) and the Canadian Institutes of Health Research (CIHR grants to G.D.W. and A.M.B.). F.G., O.M.B., and S.A.-M.-B. were supported by scholarships or fellowships from the Chemical Biology Strategic Training Initiative of CIHR.

References

(1) Wright, G. D.; Berghuis, A. M.; Mobashery, S. Aminoglycoside Antibiotics: Structures, Functions, and Resistance. In *Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics*; Rosen, B. P., Mobashery, S., Eds; Plenum: New York, 1998; pp 27−69.

(2) Vakulenko, S. B.; Mobashery, S. Versatility of aminoglycosides and prospects for their future. *Clin. Microbiol. Rev.* **2003**, *16*, 430−450.

(3) Boehr, D. D.; Moore, I. F.; Wright, G. D. Aminoglycoside Resistance Mechanisms. In *Frontiers in Antimicrobial Resistance: A Tribute to Stuart B. Levy*; White, D. G., Alekshun, M. N., McDermott, P. F., Eds; ASM Press: Washington, DC, 2005; Chapter 7, pp 85−100.

(4) Magnet, S.; Blanchard, J. S. Molecular insights into aminoglycoside action and resistance. *Chem. Rev.* **2005**, *105*, 477−498.

(5) Wright, G. D.; Ladak, P. Overexpression and characterization of the chromosomal aminoglycoside 6′-*N*-acetyltransferase from *Enterococcus faecium*. *Antimicrob. Agents Chemother.* **1997**, *41*, 956−960.

(6) DiGimmarino, E. L.; Draker, K.-A.; Wright, G. D.; Serpersu, E. H. Solution studies of isepamicin and conformational comparisons between isepamicin and butirosin A when bound to an aminoglycoside 6′-*N*-acetyltransferase determined by NMR spectroscopy. *Biochemistry* **1998**, *37*, 3638−3644.

(7) Wybenga-Groot, L. E.; Draker, K.-A.; Wright, G. D.; Berghuis, A. M. Crystal structure of an aminoglycoside 6′-*N*-acetyltransferase: Defining the GCN5-related *N*-acetyltrasferase superfamily fold. *Structure* **1999**, *7*, 497−507.
(8) Burk, D. L.; Ghuman, N.; Wybenga-Groot, L. E.; Berghuis, A. M. X-ray structure of the AAC(6′)-Ii antibiotic resistance enzyme at 1.8 Å resolution: examination of oligomeric arrangements in GNAT superfamily members. *Protein Sci.* **2003**, *12*, 426−437.
(9) Draker, K.-A.; Northrop, D. B.; Wright, G. D. Kinetic mechanism of the GCN5-related chromosomal aminoglycoside acetyltransferase AAC(6′)-Ii from *Enterococcus faecium*: Evidence of dimer subunit cooperativity. *Biochemistry* **2003**, *42*, 6565−6574.
(10) Draker, K.-A.; Wright, G. D. Molecular mechanism of the enterococcal aminoglycoside 6′-*N*-acetyltransferase: Role of GNAT-conserved residues in the chemistry of antibiotic inactivation. *Biochemistry* **2004**, *43*, 446−454.
(11) Burk, D. L.; Xiong, B.; Breitbach, C.; Berghuis, A. M. Structures of aminoglycoside acetyltransferase AAC(6′)-Ii in a novel crystal form: structural and normal-mode analyses. *Acta Crystallogr.* **2003**, *D61*, 1273−1279.
(12) Gao, F.; Yan, X.; Baettig, O. M.; Berghuis, A. M.; Auclair, K. Regio- and chemo-selective 6′-*N*-derivatization of aminoglycosides: bisubstrate inhibitors as probes to study aminoglycoside 6′-*N*-acetyltransferase. *Angew. Chem., Int. Ed.* **2005**, *44*, 6859−6862.
(13) Auclair, K.; Gao, F.; Yan, X. U.S. Patent Application 11/359,274, filed February 21, 2006.
(14) Liang, H.; Grindley, T. B. An efficient synthesis of derivatives of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose. *J. Carbohydr. Chem.* **2004**, *23*, 71−82.
(15) Personal communication with Baettig and Berghuis.
(16) Welch, K. T.; Virga, K. G.; Whittemore, N. A.; Ozen, C.; Wright, E.; Brown, C. L.; Lee, R. E.; Serpersu, E. H. Discovery of non-carbohydrate inhibitors of aminoglycoside-modifying enzymes. *Bioorg. Med. Chem.* **2005**, *13*, 6252−6263.
(17) Olsen, C. A.; Witt, M.; Jaroszewski, J. W.; Franzyk, H. Solid-phase polyamine synthesis using piperazine and piperidine building blocks. *Org. Lett.* **2003**, *5*, 4183−4185.
(18) Konno, T.; Takahashi, T.; Kurita, D.; Muto, A.; Himeno, H. A minimum structure of aminoglycoside that causes an initiation shift of trans-translation. *Nucleic Acids Res.* **2004**, *32*, 4119−4126.
(19) Vetting, M. W.; De Carvalho, L. P. S.; Yu, M.; Hegde, S. S.; Magnet, S.; Roderick, S. L.; Blanchard, J. S. Structure and functions of the GNAT superfamily of acetyltransferases. *Arch. Biochem. Biophys.* **2005**, *433*, 212−226.
(20) Modis, Y.; Wierenga, R. Two crystal structures of *N*-acetyltransferases reveal a new fold for CoA-dependent enzymes. *Structure* **1998**, *6*, 1345−1350.
(21) Dyda, F.; Klein, D. C.; Hickman, A. B. GCN5-related *N*-acetyltransferases: a structural overview. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 81−103.
(22) Marmorstein, R. Structure of histone acetyltransferases. *J. Mol. Biol.* **2001**, *311*, 433−444.
(23) Kenner, G. W.; Todd, A. R.; Weymouth, F. J. Nucleotides. XVII. *N*-Chloroamides as reagents for the chlorination of diesters of phosphorous acid. New synthesis of uridine-5′-pyrophosphate. *J. Chem. Soc.* **1952**, 3675−3681.
(24) Michelson, A. M. Synthesis of coenzyme A. *Biochim. Biophys. Acta* **1964**, *93*, 71−77.
(25) Liu, H.; Yip, J.; Shia, K.-S. Reductive cleavage of benzyl ethers with lithium naphthalenide. A convenient method for debenzylation. *Tetrahedron Lett.* **1997**, *38*, 2253−2256.
(26) Liang, Y.; Narayanasamy, J.; Schinazi, R. F.; Chu, C. K. Phosphoramidate and phosphate prodrugs of (−)-β-D-(2*R*,4*R*)-dioxolanthymine: synthesis, anti-HIV activity and stability studies. *Bioorg. Med. Chem.* **2006**, *14*, 2178−2189.
(27) Rye, C. S.; Baell, J. B. Phosphate isosteres in medicinal chemistry. *Curr. Med. Chem.* **2005**, *12*, 3127−3141.
(28) Roth, S. Y.; Denu, J. M.; Allis, C. D. Histone acetyltransferases. *Annu. Rev. Biochem.* **2001**, *70*, 81−120.
(29) Langer, M. R.; Fry, C. J.; Peterson, C. L.; Denu, J. M. Modulating acetyl−CoA binding in the GCN5 family of histone acetyltransferases. *J. Biol. Chem.* **2002**, *277*, 27337−27344.
(30) Poux, A. N.; Cebrat, M.; Kim, C. M.; Cole, P. A.; Marmorstein, R. Structure of the GCN5 histone acetyltransferase bound to a bisubstrate inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14065−14070.
(31) Zheng, W.; Cole, P. A. Serotonin *N*-acetyltransferase: mechanism and inhibition. *Curr. Med. Chem.* **2002**, *9*, 1187−1199.
(32) Zhu, Y.-Q.; Zhu, D.-Y.; Yin, L.; Zhang, Y.; Vonrhein, C.; Wang, D.-C. Crystal structure of human spermidine/spermine N(1)-acetyltransferase (hSSAT): The first structure of a new sequence family of transferase homologous superfamily. *Proteins* **2006**, *63*, 1127−1131.
(33) Copeland, R. A. *Enzymes*: *A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 2000; pp 305−317.

JM060732N