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Effects of 5-*O*-Ribosylation of Aminoglycosides on Antimicrobial Activity and Selective Perturbation of Bacterial Translation

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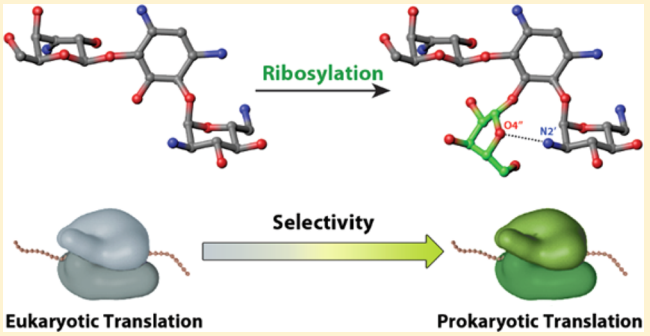
Supporting Information

ABSTRACT: We studied six pairs of aminoglycosides and their corresponding ribosylated derivatives synthesized by attaching a β-*O*-linked ribofuranose to the 5-OH of the deoxystreptamine ring of the parent pseudo-oligosaccharide antibiotic. Ribosylation of the 4,6-disubstituted 2-deoxystreptamine aminoglycoside kanamycin B led to improved selectivity for inhibition of prokaryotic relative to cytosolic eukaryotic in vitro translation. For the pseudodisaccharide aminoglycoside scaffolds neamine and nebramine, ribosylated derivatives were both more potent antimicrobials and more selective to inhibition of prokaryotic translation. On the basis of the results of this study, we suggest that modification of the 5-OH position of the streptamine ring of other natural or semisynthetic pseudodisaccharide aminoglycoside scaffolds containing an equatorial amine at the 2′ sugar position with a β-*O*-linked ribofuranose is a promising avenue for the development of novel aminoglycoside antibiotics with improved efficacy and reduced toxicity.



INTRODUCTION

For over eight decades, aminoglycosides (AGs) have been an important family of clinically used antibiotics. AGs are composed of pseudoamino-oligosaccharides and are commonly used to treat topical and, in severe cases, systemic bacterial infections.[1,2] AGs perturb the fidelity of bacterial protein synthesis by binding to the 16S ribosomal A-site decoding rRNA region of the prokaryotic ribosome.[3−6] Crystal structures of different AGs in complex with either the small subunit of the prokaryotic ribosome or an rRNA oligonucleotide model representing the AG binding domain in the prokaryotic A-site have revealed their mechanism of action in detail.[7,8] During the translation process, the A-site nucleotides assume two different conformations: the "off" state, in which two adenine residues, A1492 and A1493, fold into the A-site rRNA minor groove, and the "on" state, in which the two adenines are bulged out from the A-site (Figure 1).[9]

In the "on" state, the bulged adenines participate in the stabilization of the Watson−Crick base pairs between the codon of the mRNA and the anticodon of the tRNA. Binding interactions between the AG and the A-site nucleotides stabilize the "on" state and induce the incorporation of incorrect amino acids into the growing protein chain, thereby causing multiple errors in the translation process.[4]

The use of AGs to treat systemic infections is limited due to their toxicity. All AGs are both nephrotoxic and ototoxic, with the latter being the major drawback to their clinical use.[10−12] AG ototoxicity causes irreversible damage to the cochlear sensory hair cells of the inner ear, and ∼20% of patients experience irreversible hearing damage.[13] On the basis of in vitro translation experiments using bacterial hybrid ribosomes carrying mitochondrial ribosome decoding site nucleotides and on mitochondrial in organello translation experiments, it was previously demonstrated that AGs perturb mitochondrial translation.[14,15] The significant contribution of perturbation of mitochondrial translation to the ototoxicity of AGs is supported by the results of in vitro inhibition of mitochondrial translation experiments, in which inhibition by various AGs correlated with their relative ototoxicity.[14] In addition, it was previously shown that several AGs, including gentamicins, tobramycin (TOB), and kanamycin A (KAN-A), exhibited similar abilities to inhibit both in vitro mitochondrial translation and in vitro cytosolic eukaryotic translation.[16,17] These data suggest that depending on cell permeability levels and on the specific AG, toxicity may be attributed to perturbation of the fidelity of both mitochondrial and cytosolic eukaryotic translation. A possible explanation for the pronounced nephrotoxicity and ototoxicity of AGs is their increased uptake into epithelial cells by megalin, a multiligand endocytic receptor that facilitates increased cellular uptake of these antibiotics; megalin is expressed in most epithelial cells, including kidney and cochlea cells.[18]

An additional obstacle that prevents broader clinical use of AGs is the continuing increase in infections caused by AG-resistant bacteria.[19] Bacterial resistance to AGs can be divided into three major mechanisms: (1) decreased intracellular concentration of AGs due to bacterial membrane alterations or active efflux, (2) alteration of the target A-site rRNA through

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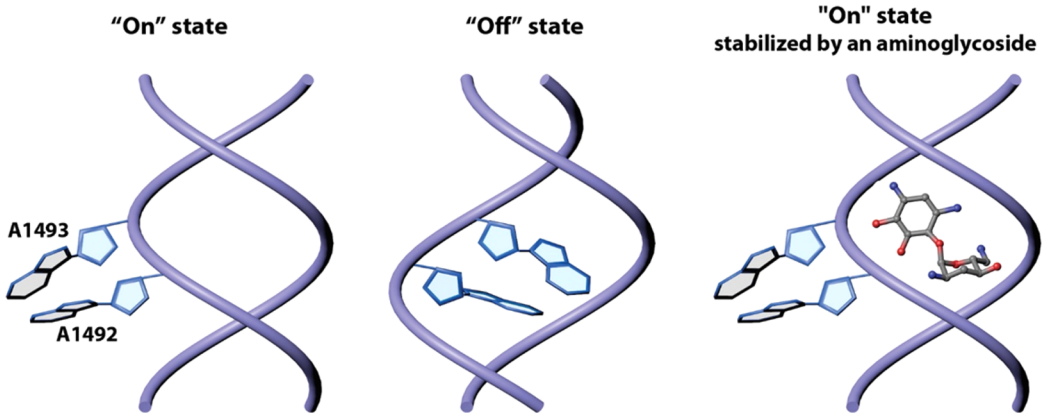

Figure 1. Illustration of the decoding A-site in "on" and "off" states and stabilization of the "on" state by an aminoglycoside.

mutations or base modifications such as methylation, and (3) inactivation of AGs through *N*-acetylation, *O*-nucleotidylation, or *O*-phosphorylation.[20,21]

Since the isolation of the first AG, streptomycin, by Chatz and Waksman in 1943, multiple AGs have been isolated and thousands of AG derivatives have been synthesized in an attempt to improve their pharmacological properties.[22,23] Only a handful of semisynthetic analogues have become clinically useful. The most well-known clinically used semisynthetic AG is amikacin (AMK), which was developed by Fugisawa and co-workers from the natural AG KAN-A and was first reported in 1972.[24] AMK is particularly effective against Gram-negative bacilli that are resistant to other AGs.[25] This important AG antibiotic is on the World Health Organization's list of essential medicines, but like all other AGs in clinical use it has dose-limiting toxicities, and resistance against this AG derivative is on the rise.[26−28]

To date, development of improved AGs has been focused mainly on devising strategies to overcome the various resistance mechanisms that evolved in bacteria against these antibiotics. Few studies have focused on developing strategies to reduce the toxicity of this important class of antibiotics. In searching for a novel direction for the design of less toxic AGs, we reasoned that increasing the number of interactions between the AG and the target prokaryotic decoding A-site would improve target specificity and reduce undesired perturbation of the fidelity of the cytosolic eukaryotic translation process.

Most natural and clinically used AG antibiotics vary significantly both in size and in structure, but they can be divided into two major subfamilies: 4,6-disubstituted 2-deoxystreptamines and 4,5-disubstituted 2-deoxystreptamines (Figure 2).

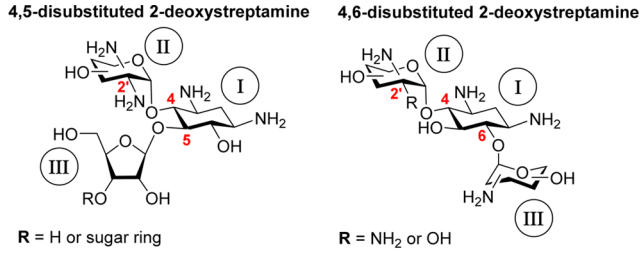

Figure 2. General structures of 4,5- and 4,6-disubstituted 2-deoxystreptamine aminoglycosides.

Careful structural analysis of all known natural 4,5-disubstituted 2-deoxystreptamine antibiotics revealed that these AGs contain a D-ribofuranose ring attached to the 5-OH of the 2-deoxystreptamine ring through a β-*O*-glycosidic bond and that the 2′-position on ring II in these AGs is substituted by an equatorial amine (Figure 2). In AGs belonging to the 4,6-disubstituted 2-deoxystreptamines, the 2′-position can be substituted with either an amine or an alcohol.

Westhof and co-workers reported crystal structures of complexes between an oligo-ribonucleotide representing the prokaryotic decoding A-site and the 4,5-disubstituted 2-deoxystreptamines ribostamycin (RIB), neomycin B, and paromomycin (Figure 3).[7,8] In all of these structures, the ribofuranose sugar ring interacts with the target A-site nucleotides through a set of hydrogen bonds. Notably, the 2′-amine in these AG structures is within range to form a hydrogen bond with the ribofuranose sugar ring oxygen, which may assist in orienting the positioning of the ribofuranose ring in the A-site.

Superimposed structures of 4,6-disubstituted and 4,5-disubstituted 2-deoxystreptamine AGs in complex with A-site rRNA nucleotides indicated that rings I and II of these antibiotics (Figure 2) occupy an almost identical space in the binding site. These superimposed AG structures also indicate that the ribofuranose ring III of the 4,5-disubstituted 2-deoxystreptamine subfamily and ring III of the 4,6-disubstituted 2-deoxystreptamine subfamily occupy different spaces.[29−31]

On the basis of the crystallographic data and the superimposed AG structures, we rationalized that attachment of a β-linked ribofuranose ring to the 5-OH of 4,6-disubstituted 2-deoxystreptamines with a 2′-equatorial amine would result in 4,5,6-trisubstituted 2-deoxystreptamine AGs with enhanced occupancy of the prokaryotic A-site rRNA binding domain. This should improve the specificity of the resultant AG derivatives to the perturbation of prokaryotic relative to cytosolic eukaryotic translation and thereby reduce the toxicity of these antibiotics. We expected that the same principle could be implemented to generate novel 4,5-disubstituted 2-deoxystreptamines via attachment of a β-linked ribofuranose ring to the 5-OH of various 4-monosubstituted deoxystreptamine pseudodisaccharide AGs with a 2′-equatorial amine. In this study, we evaluated the effects of 5-OH ribosylation of four 4,6-disubstituted 2-deoxystreptamine and two 4-monosubstituted deoxystreptamine AGs on antimicrobial activity and

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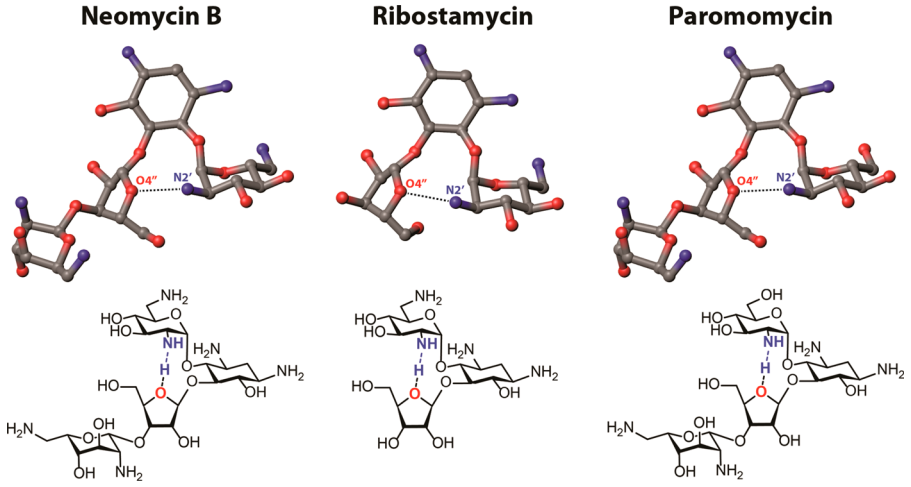

Figure 3. Structures of 4,5-disubstituted 2-deoxystreptamine AGs from crystal structures of complexes with a prokaryotic A-site oligonucleotide.[7,8]

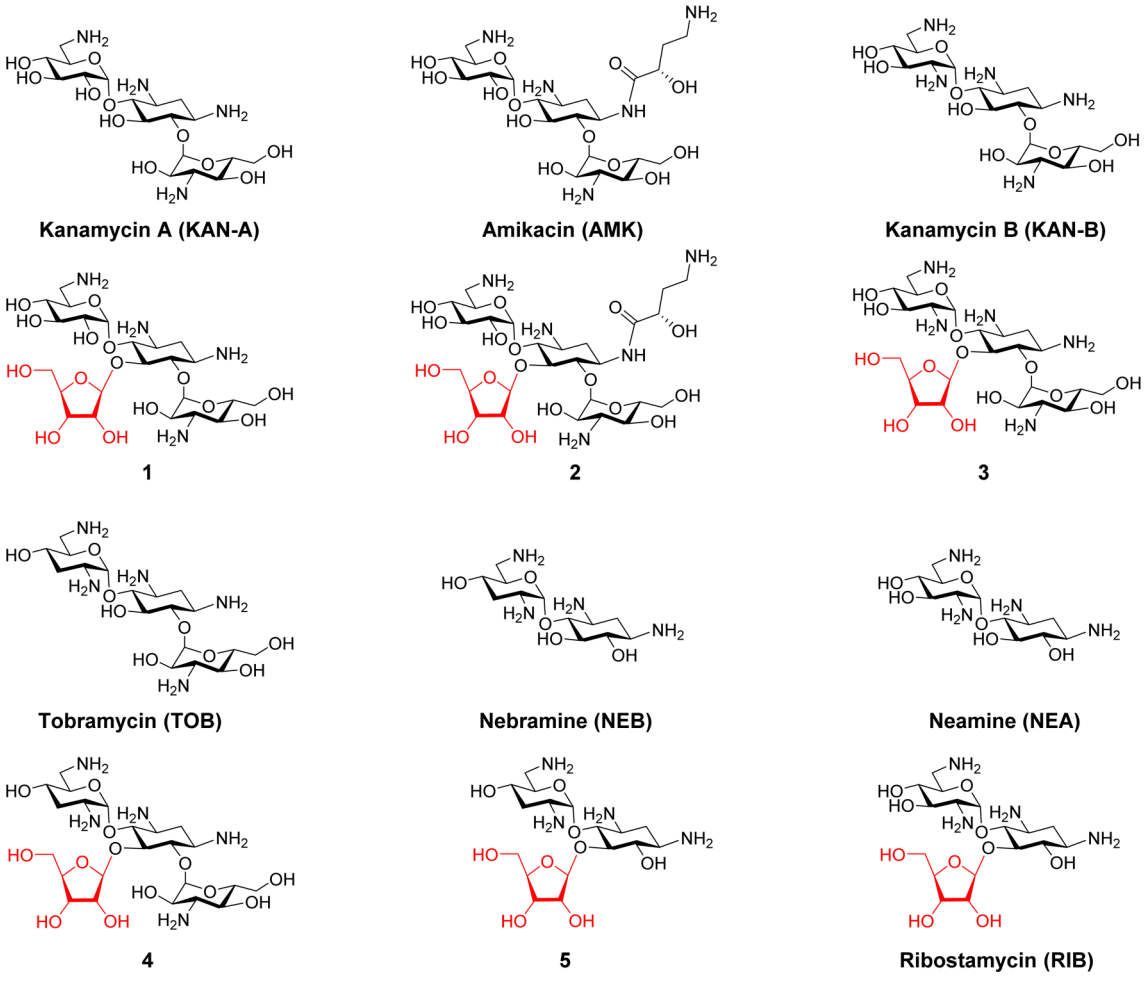

Figure 4. Structures of the parent AGs and of the corresponding ribosylated derivatives.

selective perturbation of prokaryotic relative to cytosolic eukaryotic translation.

RESULTS AND DISCUSSION

Synthesis. To test our hypothesis, we chose six AGs and their corresponding 5-*O*-ribosylated derivatives as shown in Figure 4.

Four AGs belonging to the 4,6-disubstituted 2-deoxystreptamine class were chosen: KAN-A and AMK, both with a 2′-equatorial alcohol, and kanamycin B (KAN-B) and TOB, which both possess a 2′-equatorial amine. In addition, we investigated the effect of ribosylation on two pseudodisaccharide-based AGs, neamine (NEA) and nebramine (NEB), both of which contain a 2′-equatorial amine. The natural AG RIB is the ribosylated

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Scheme 1. (A) Synthesis of Ribosylated Pseudo-Trisaccharide AGs; (B) Synthesis of Ribosylated NEB

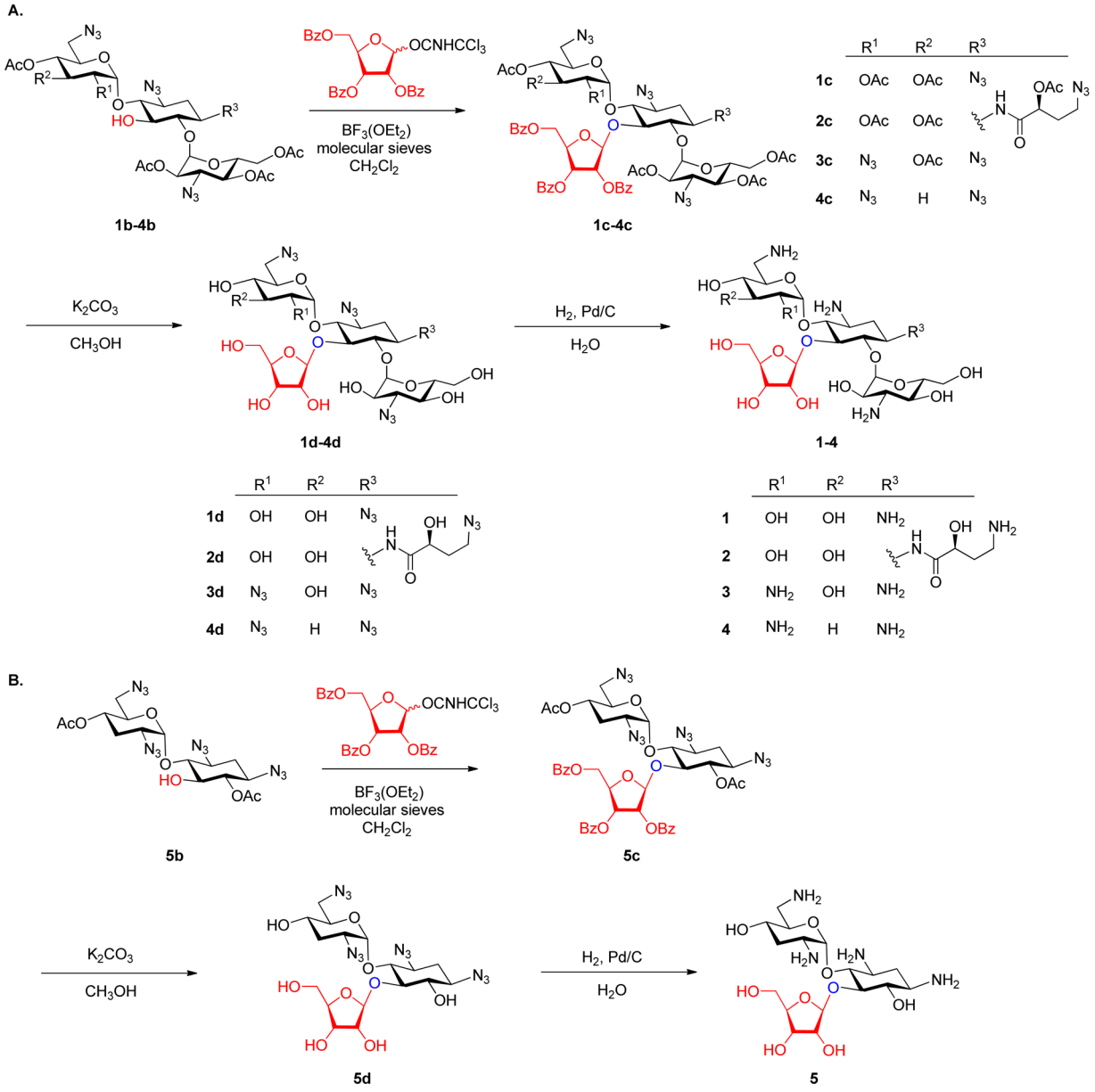

Table 1. Minimal Inhibitory Concentration (MIC) Values [μg/mL][a]

	KAN-A	1	AMK	2	KAN-B	3	TOB	4	NEA	RIB	NEB	5
(A) *P. aeruginosa* ATCC 47085	>32	>32	4	>32	>32	>32	1	2	>32	>32	>32	4
(B) *P. aeruginosa* ATCC 27853	>32	>32	8	>32	>32	>32	1	4	>32	>32	>32	4
(C) *H. influenzae* ATCC 49247	8	>32	32	>32	4	8	4	8	32	8	>32	4
(D) *H. influenzae* ATCC 10211	8	>32	32	>32	2	8	4	8	32	8	>32	4
(E) *K. pneumoniae* ATCC 13883	4	>32	4	>32	1	4	1	2	16	8	>32	8
(F) *K. pneumoniae* ATCC 10031	4	>32	4	>32	1	4	1	2	16	8	>32	4
(G) *E. coli* ATCC 25922	4	32	2	>32	4	4	2	4	16	4	16	2
(H) *A. baumannii* ATCC 19606	8	>32	4	>32	4	>32	2	32	>32	16	>32	32
(I) *B. cepacia* ATCC 25416	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
(J) *E. coli* BAA-2452	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
(K) *K. pneumonia* BAA-2470	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32

[a]MICs were determined using the double-dilution method. Each concentration of each AG was analyzed in triplicate in two independent sets of experiments.

derivative of NEA and was included in the collection of AGs in this study for comparison to the pseudodisaccharide NEA.[32]

Ribosylation of the chosen AGs was accomplished in five synthetic steps from the parent AGs. Briefly, the commercially

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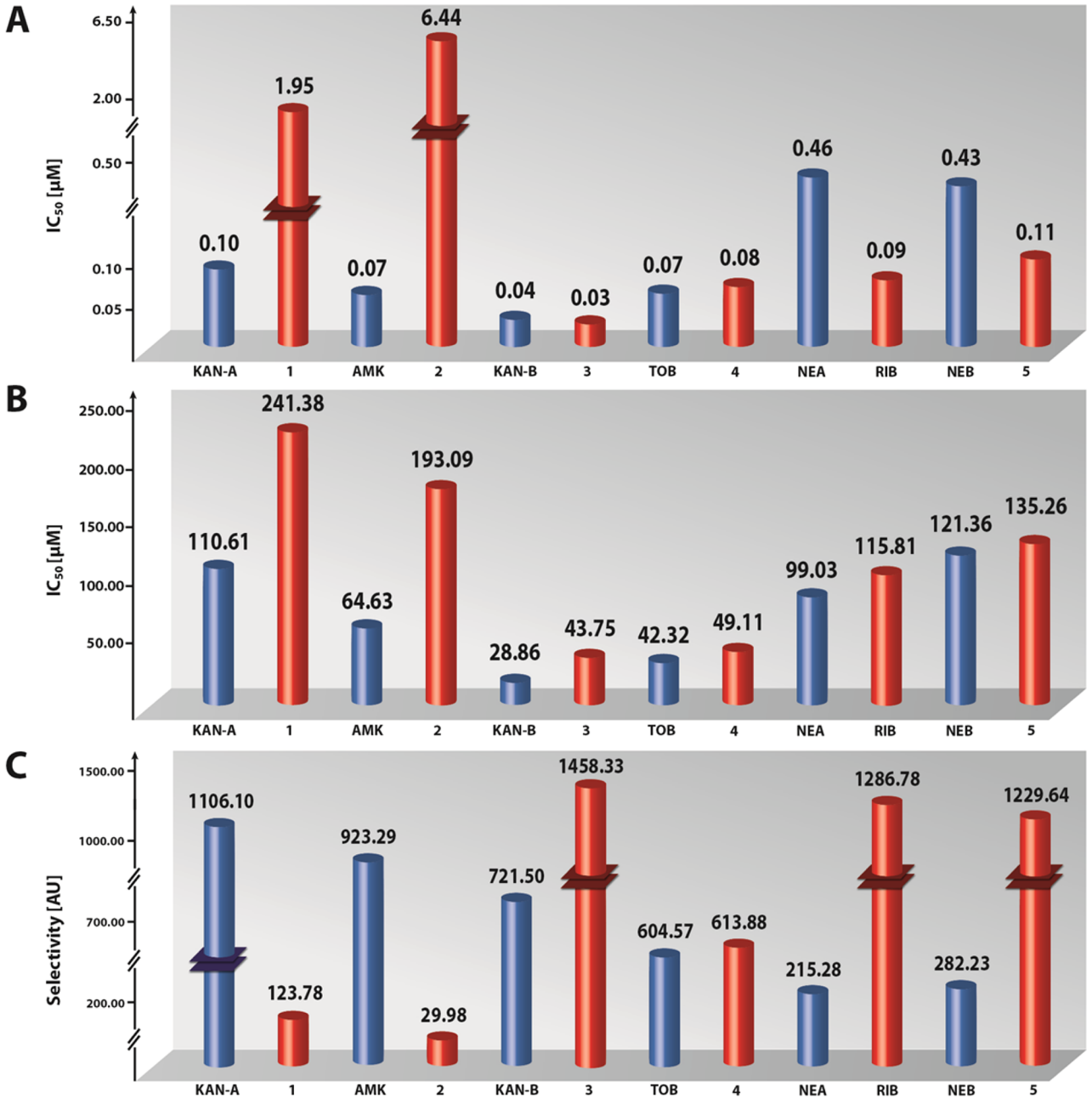

Figure 5. (A) IC_{50} values of inhibition of prokaryotic in vitro translation. (B) IC_{50} values of inhibition of cytosolic eukaryotic in vitro translation. (C) Ratios of the IC_{50} values of inhibition of cytosolic eukaryotic and prokaryotic translation: IC_{50} eukaryote/IC_{50} prokaryote. Experiments were performed in duplicate, and the results are the averages of two independent experiments. For exact values and standard deviation, see Supporting Information, Table S1.

available parent AGs were converted into pseudo-oligosaccharide 5-OH acceptors by converting primary amines to azides.[33] This was followed by acetylation of all alcohols of the resultant azido-protected AGs with the exception of the desired 5-OH by taking advantage of its lower reactivity toward anhydride-mediated esterification. The AG-derived glycosyl acceptors were glycosylated with the 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl-trichloroacetimidate glycosyl donor to afford the protected ribosylated derivative (Scheme 1).[34] Deprotection was accomplished through saponification under mild alkaline conditions followed by reduction of the azide groups by catalytic hydrogenation to afford the 5-*O*-ribosylated AGs **1**−**5**. Alternative synthetic routes for the preparation of compounds **1** and **5** and their evaluation as antimicrobial agents against several bacterial strains were previously reported.[35,36]

Antimicrobial Activity. We compared the antimicrobial activity of ribosylated AGs to that of the parent antibiotics by determining minimum inhibitory concentrations (MICs) against bacterial pathogens that are treated with AGs in severe cases of infection. AGs are used systemically to treat severe pulmonary infections that are especially frequent in cystic fibrosis patients.[37] A large percentage of these pulmonary infections involve Gram-negative pathogens such as *Pseudomonas aeruginosa* and *Haemophilus influenzae*.[38−40] We tested antimicrobial activity against two representative strains of each of these bacteria (strains A−D, Table 1). AGs are also used

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systemically to treat blood and pulmonary infections caused by *Klebsiella pneumoniae* (strains E and F); in many cases, this Gram-negative bacterium is inherently resistant to a number of antibiotics.[41,42] The Gram-negative bacterium *Escherichia coli* is part of the natural flora of the lower intestine; however, some serotypes can cause severe infections that are treated with AGs such as AMK.[43,44] We therefore tested antimicrobial activity of the collection of AGs against *E. coli* ATCC 29522, which is commonly used for antimicrobial susceptibility testing (strain G). *Acinetobacter baumannii* (strain H) is a Gram-negative pathogen of clinical importance that causes bacteremia, pneumoniae, and urinary tract and wound infections. *A. baumannii* isolates are usually highly antibiotic resistant, and, in severe cases, infections caused by this pathogen are treated by AGs such as TOB and AMK.[45−47] Finally, to determine if ribosylation affects AG resistance, the commercial and semisynthetic AGs in this study were evaluated for antimicrobial activity against three highly AG-resistant Gram-negative pathogens: *Burkholderia cepacia* ATCC 25416, *E. coli* ATCC BAA-2452, and *K. pneumoniae* ATCC BAA-2470 (strains I, J, and K, respectively, Table 1). Antimicrobial activity was determined using the double-dilution method, and the results are summarized in Table 1.

None of the commercial or semisynthetic ribosylated aminoglycosides in this study, including the clinically used AMK and TOB, were effective against the three AG-resistant Gram-negative pathogens with MICs ≥32 μg/mL, which suggests that this modification had no impact on the ability to overcome drug resistance. Unlike the parent AGs KAN-A and AMK, the corresponding ribosylated derivatives **1** and **2** were inactive against the tested strains (MIC values ≥32 μg/mL, Table 1). In contrast, the ribosylated derivatives of KAN-B and TOB, compounds **3** and **4**, maintained good antimicrobial activity with MIC values that were one to two double dilutions higher than those of the parent AGs. The exception was activity against the tested *A. baumannii* strain; unlike the parent AGs, the ribosylated derivatives were inactive, possibly due to AG-modifying enzymes that may target the ribofuranose ring of the ribosylated derivative.

A significant improvement in antimicrobial activity was observed for the ribosylated derivatives of the pseudodisaccharide AG scaffolds NEA and NEB. The naturally ribosylated NEA, RIB, and the ribosylated derivative of NEB, compound **5**, had significantly lower MIC values against the tested strains than did NEA and NEB (Table 1). The most significant improvement in antimicrobial activity in this study was observed for the ribosylated NEB derivative **5**. This AG derivative was at least two to four double dilutions more potent than the parent pseudodisaccharide NEB against all tested strains.

Selective Inhibition of in Vitro Translation. To study the direct effect of ribosylation of AGs on the impact of these antibiotics on the fidelity of the translation process, we evaluated the effects of the parent and ribosylated AGs on translation in commercially available cell-free extracts from *E. coli*, which represent prokaryotic ribosomes. Because access to extracts containing mitochondrial ribosomes or bacterial hybrid ribosomes carrying mitochondrial ribosome decoding site nucleotides is limited, in this study we used commercially available extracts from rabbit reticulocytes to represent eukaryotic cytosolic ribosomes. The concentrations at which the tested compounds inhibited 50% of functional luciferase translation (IC_{50}) were determined, and the results are summarized in Figure 5.

Compared to KAN-A and AMK, the corresponding ribosylated derivatives **1** and **2** exhibited a significant reduction in the inhibition of in vitro prokaryotic translation of luciferase (∼20-fold and ∼92-fold, respectively, Figure 5A). In contrast, the IC_{50} values of the ribosylated derivatives of KAN-B and TOB (compounds **3** and **4**) did not significantly differ from those of the parent AGs. RIB and compound **5**, the ribosylated derivatives of the pseudodisaccharides NEA and NEB, were significantly better inhibitors of prokaryotic translation than were the parent AGs. The IC_{50} value of **5** was ∼4-fold lower than that of the parent NEB and that of RIB was ∼5-fold lower than that of the parent NEA. These results support the hypothesis that the 2′-equatorial amine plays an important role in facilitating the binding of 4,5-disubstituted 2-deoxystreptamines to the prokaryotic A-site.

Unlike the observed effect on prokaryotic in vitro translation, in most cases the ribosylated derivatives inhibited cytosolic eukaryotic in vitro translation to similar extents as the parent AGs (Figure 5B). The exceptions were KAN-A and AMK. Compared to the parent KAN-A and AMK, the ribosylated derivatives **1** and **2** had ∼2-fold and ∼3-fold higher IC_{50} values, respectively. These two ribosylated derivatives also exhibited low affinity for both the prokaryotic and cytosolic eukaryotic ribosomes A-sites, as was also evident from their poor antimicrobial activity compared to that of the parent antibiotics KAN-A and AMK.

Finally, calculation of the ratio between the IC_{50} values in cytosolic eukaryotic vs prokaryotic translation assays revealed an interesting structure−activity relationship (Figure 5C).[17,48,49] The most significant improvement in selectivity for inhibition of prokaryotic translation was observed when NEA and NEB and their ribosylated derivatives were compared. NEA had a ∼6-fold lower ratio of eukaryotic to prokaryotic IC_{50} than was observed for the corresponding ribosylated AG RIB (Figure 5C). Compared to the ratio of the parent NEB, an increase of ∼4-fold was observed for compound **5**. It was previously shown that when measuring acute intravenous toxicity in rats, NEA had an LD_{50} range of 121−129 mg/kg, whereas that of RIB was significantly higher (in the range of 250−270 mg/kg).[50] This result suggests that there may be a connection between selectivity and acute toxicity and that the ∼6-fold improvement in selectivity of RIB relative to NEA may be associated with a reduction in acute intravenous toxicity. The improved selectivity ratio resulted from the significant improvement in the inhibition of in vitro prokaryotic translation on one hand and a modest reduction in the inhibition of in vitro cytosolic eukaryotic translation on the other hand when the ribosylated derivatives were compared to the parent pseudodisaccharide AGs. Taken together with the results of the antimicrobial activity tests, the results of the in vitro translation experiments suggest that ribosylation of other pseudodisaccharide-based AG scaffolds that contain a 2′-equatorial amine may result in novel AGs with improved antimicrobial activity and target selectivity.

CONCLUSION

In conclusion, on the basis of crystallographic structural information, we reasoned that attachment of a β-*O*-linked ribofuranose sugar unit to the 5-OH of the 2-deoxystreptamine ring of AG antibiotics can lead to improved target selectivity of the resultant AG derivative. Herein we showed that in several

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cases ribosylation enhanced inhibition of prokaryotic translation, increased selectivity for the prokaryotic relative to the cytosolic eukaryotic translation machinery, and enhanced antimicrobial activity. Like the parent AGs in this study, the ribosylated derivatives were ineffective against highly AG-resistant bacterial strains. Beneficial effects were observed only when the 2′-position of the parent AG contained an equatorial amine. Ribosylation of the 4,6-disubstituted 2-deoxystreptamine AGs KAN-A and AMK, which are substituted by a 2′-equatorial alcohol, abrogated inhibition of prokaryotic translation and antimicrobial activity. Ribosylation of the 4,6-disubstituted 2-deoxystreptamine AG scaffolds KAN-B and TOB, which contain 2′-amines, enhanced specificity of the resultant ribosylated AG derivatives for the prokaryotic translation machinery due to reduced inhibition of cytosolic eukaryotic translation compared to that of the parent AGs. The MIC values of these ribosylated AG derivatives were a modest one to two double dilutions less active against the tested strains than were the parent AGs. The most impressive improvement in all three of the tested biological activity aspects was achieved through ribosylation of pseudodisaccharide AG scaffolds, as was demonstrated by comparison of the biological activities of NEA and NEB with the corresponding ribosylated RIB and compound **5**. The effects of ribosylation of the 5-OH of AGs described here suggest that similar derivatives of other natural or semisynthetic pseudodisaccharide AG scaffolds that contain a 2′-equatorial amine group would have potential as AG antibiotics with improved clinical properties.

EXPERIMENTAL SECTION

General Chemistry Methods. ^{1}H NMR spectra (including 1D TOCSY) were recorded on Bruker Avance 400 or 500 spectrometers, and chemical shifts (reported in ppm) were calibrated to CD_3OD, D_2O, or $CDCl_3$ (d = 3.31, 4.79, and 7.26 ppm, respectively). ^{13}C NMR spectra were recorded on Bruker Avance 400 or 500 spectrometers at 100 or 125 MHz, respectively. For NMR samples, the free base form of compounds **1**−**5** was treated with 95% TFA and freeze-dried to afford the corresponding TFA salts. Low-resolution electron spray ionization mass spectra were measured on a Waters 3100 mass detector. High-resolution electron spray ionization mass spectra were measured on a Waters Synapt instrument. Chemical reactions were monitored by TLC analysis (Merck, silica gel60, F_{254}). TLC visualization was achieved using a cerium molybdate stain [$(NH_4)_2Ce(NO_3)_6$ (5g), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (120 g), H_2SO_4 (80 mL), and H_2O (720 mL)]. All reactions were carried out in an argon atmosphere with anhydrous solvents unless otherwise noted. All chemicals, unless otherwise stated, were obtained from commercial sources. Compounds were purified by means of flash chromatography (Merck, silica gel 60). The purity of compounds **1**−**5** was ≥95% as determined by ULC-MS (see Supporting Information).

Compound ***1c.*** To powdered, flame-dried 4-Å molecular sieves (1.4 g), anhydrous dichloromethane (10 mL) was added followed by the addition of acceptor **1b** (350 mg, 0.41 mmol) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl-trichloroacetimidate glycosyl donor (960 mg, 1.59 mmol). After stirring for 10 min at room temperature, the mixture was cooled to −50 °C and $BF_3\cdot OEt_2$ (40 μL) was added. The reaction was allowed to warm to room temperature and was analyzed by TLC (ethyl acetate/petroleum ether, 4:6). Upon completion, the reaction was diluted with ethyl acetate and filtered through Celite. After thorough washing of the Celite with ethyl acetate, the washes were combined, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography (SiO_2, ethyl acetate/dichloromethane) to yield **4c** (515 mg, 96%) as a white solid. LRMS (ESI): *m*/*z* calcd for $C_{56}H_{61}N_{12}O_{24}$, 1285.39 $[M + H]^+$; found, 1285.77. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 8.1, 1.0 Hz, 2H, Bz), 7.97 (dd, *J* = 8.1, 0.9 Hz, 2H, Bz), 7.92 (dd, *J* = 7.9, 0.8 Hz, 2H, Bz), 7.59−7.51 (m, 3H, Bz), 7.45−7.38 (m, 4H, Bz), 7.34 (t, *J* = 7.8 Hz, 2H, Bz), 5.82 (d, *J* = 5.2 Hz, 1H, H-1‴), 5.68 (dd, *J* = 7.4, 5.1 Hz, 1H, H-2‴), 5.63 (d, *J* = 3.7 Hz, 1H, H-1″), 5.61 (d, *J* = 4.1 Hz, 1H, H-1′), 5.60 (m, 1H, H-3‴), 5.35 (dd, *J* = 9.7 Hz, 1H, H-3′), 4.99 (dd, *J* = 10.3, 4.0 Hz, 1H, H-2′), 4.92 (dd, *J* = 9.6 Hz, 1H, H-4′), 4.89 (dd, *J* = 9.9 Hz, 1H, H-4″), 4.82 (dd, *J* = 10.7, 3.7 Hz, 1H, H-2″), 4.74−4.68 (m, 2H, H-4‴, H-5‴), 4.54 (m, 1H, H-5‴), 4.31 (dd, *J* = 10.4 Hz, 1H, H-3″), 4.24−4.15 (m, 3H, H-5′, H-5″, H-6″), 4.12 (dd, *J* = 12.3, 2.2 Hz, 1H, H-6″), 3.92−3.86 (m, 2H, H-4, H-5), 3.76−7.70 (m, 2H, H-3, H-6), 3.64 (m, 1H, H-1), 3.34−3.25 (m, 2H, H-6′, H-6′), 2.46 (ddd, *J* = 13.9, 5.7 Hz, 1H, H-2eq), 2.19 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.95 (m, 6H, 2x$COCH_3$), 1.92 (s, 3H, $COCH_3$), 1.62 (m, 1H, H-2ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.2, 169.9, 169.7, 169.5, 169.3, 166.2, 165.4, 165.2, 133.9, 133.8, 133.5, 129.9, 129.87, 129.84, 129.7, 128.9, 128.8, 128.7, 128.6, 108.4 (anomeric), 95.7 (anomeric), 95.6 (anomeric), 81.2, 79.8, 79.0, 78.0, 75.3, 72.2, 71.6, 69.9, 69.7, 69.6, 69.5, 68.8, 68.3, 65.1, 62.1, 60.4, 59.6, 58.3, 51.1, 31.7, 29.8, 20.8, 20.7, 20.6, 20.5.

Compound ***2c.*** Compound **2c** was prepared as described for compound **1c** using acceptor **2b** (360 mg, 0.37 mmol), donor (685 mg, 1.13 mmol), flame-dried 4-Å molecular sieves (1.5 g), anhydrous dichloromethane (10 mL), and $BF_3\cdot OEt_2$ (40 μL). Propagation of the reaction was monitored by TLC analysis (ethyl acetate/petroleum ether, 4:6). The crude was purified by flash chromatography (SiO_2, ethyl acetate/dichloromethane) to yield **2c** (440 mg, 84%) as a white solid. LRMS (ESI): *m*/*z* calcd for $C_{62}H_{70}N_{13}O_{27}$, 1428.45 $[M + H]^+$; found, 1428.80. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.4, 1.3 Hz, 2H, Bz), 7.93 (dd, *J* = 8.4, 1.3, 2H, Bz), 7.88 (dd, *J* = 8.4, 1.3 Hz, 2H, Bz), 7.58 (td, *J* = 7.5, 1.1 Hz, 2H, Bz), 7.53 (tt, *J* = 7.4, 1.2 Hz, 1H, Bz), 7.44 (t, *J* = 7.8 Hz, 2H, Bz), 7.40 (t, *J* = 7.8 Hz, 2H, Bz), 7.34 (t, *J* = 7.8 Hz, 2H, Bz), 6.99 (d, *J* = 9.0 Hz, 1H, N*H*), 5.79 (dd, *J* = 7.1, 5.1 Hz, 1H, H-3‴), 5.76 (d, *J* = 5.1 Hz, 1H, H-2‴), 5.45 (bs,1H, H-1‴), 5.38 (d, *J* = 3.7 Hz, 1H, H-1″), 5.34 (dd, *J* = 9.7 Hz, 1H, H-3′), 5.27 (d, *J* = 3.9 Hz, 1H, H-1′), 5.13 (dd, *J* = 6.2 Hz, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*α*)), 4.97 (dd, *J* = 10.1 Hz, 1H, H-4″), 4.95−4.90 (m, 2H, H-2′, H-4′), 4.86 (dd, *J* = 10.6, 3.7 Hz, 1H, H-2″), 4.76 (td, *J* = 6.3, 3.9 Hz, 1H, H-4‴), 4.71 (dd, *J* = 12.0, 3.8 Hz, 1H, H-5‴), 4.61 (dd, *J* = 12.0, 5.9 Hz, 1H, H-5‴), 4.26−4.20 (m, 2H, H-1, H-6″), 4.12 (dd, *J* = 17.2, 6.7 Hz, 1H, H-3″), 4.11−4.06 (m, 3H, H-5′, H-5″, H-6″), 4.03−4.00 (m, 2H, H-3, H-5), 3.92 (dd, *J* = 5.2, 3.5 Hz, 1H, H-4), 3.89 (dd, *J* = 5.4, 3.3 Hz, 1H, H-6), 3.37−3.32 (m, 3H, H-6′, H-6′, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*γ*)), 3.28 (dd, *J* = 13.2, 2.5 Hz, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*γ*)), 2.33 (ddd, *J* = 14.1, 6.0, 3.7 Hz, 1H, H-2eq), 2.17 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.08 (m, 2H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*β*)), 2.01 (m, 9H, 3x$COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.65 (ddd, *J* = 14.1, 6.7 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 169.9, 169.8, 169.7, 169.6, 169.5, 169.3, 168.8, 166.2, 165.4, 165.3, 134.0, 133.8, 133.6, 129.9, 129.8, 129.6, 128.8, 128.7, 128.6, 106.0 (anomeric), 97.6 (anomeric), 94.1 (anomeric), 80.6, 79.7, 78.5, 75.5, 73.2, 71.8, 71.3, 70.9, 70.1, 69.8, 69.7, 69.6, 68.6, 68.5, 64.6, 62.0, 60.7, 57.9, 51.4, 47.2, 45.7, 30.9, 27.8, 20.7, 20.6.

Compound ***3c.*** Compound **3c** was prepared as described for compound **1c** using acceptor **3b** (315 mg, 0.38 mmol), donor (580 mg, 0.96 mmol), flame-dried 4-Å molecular sieves (700 mg), anhydrous dichloromethane (6 mL), and $BF_3\cdot OEt_2$ (30 μL). Propagation of the reaction was monitored by TLC analysis (ethyl acetate/petroleum ether, 4:6). The crude was purified by flash chromatography (SiO_2, ethyl acetate/petroleum ether) to yield **3c** (460 mg, 95%) as a white solid. LRMS (ESI): *m*/*z* calcd for $C_{54}H_{57}N_{15}O_{22}Na$, 1290.37 $[M + Na]^+$; found, 1290.06. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 7.1, 1.3 Hz, 2H, Bz), 7.99 (dd, *J* = 7.1, 1.3 Hz, 2H, Bz), 7.89 (dd, *J* = 7.2, 1.3 Hz, 2H, Bz), 7.58 (m, 2H, Bz), 7.53 (m, 1H, Bz), 7.45 (dd, *J* = 7.9 Hz, 2H, Bz), 7.42 (dd, *J* = 7.7 Hz, 2H, Bz), 7.33 (dd, *J* = 7.8 Hz, 2H, Bz), 5.78 (dd, *J* = 5.0, 0.8 Hz, 1H, H-2‴), 5.75 (dd, *J* = 5.1 Hz, 1H, H-3‴), 5.70 (bs, 1H, H-1‴), 5.53 (d, *J* = 3.8 Hz, 1H, H-1′), 5.46 (d, *J* = 3.7 Hz, 1H, H-1″), 5.35 (dd, *J* = 10.5, 9.4 Hz, 1H, H-3′), 4.92 (dd, *J* = 10.1 Hz, 1H, H-4′), 4.89 (dd, *J* = 10.3 Hz, 1H, H-4″), 4.79−4.72 (m, 3H, H-2″, H-5‴, H-4‴), 4.61 (dd,

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J. Med. Chem. XXXX, XXX, XXX−XXX

J = 12.8, 7.2 Hz, 1H, H-5‴), 4.22−4.14 (m, 3H, H-5′, H-3″, H-5″), 4.14−4.11 (m, 2H, H-6″, H-6″), 4.01 (dd, J = 5.8, 4.7 Hz, 1H, H-5), 3.94 (dd, J = 7.1, 4.6 Hz, 1H, H-4), 3.77 (dd, J = 7.4 Hz, 1H, H-6), 3.68 (m, 1H, H-3), 3.59 (m, 1H, H-1), 3.45 (dd, J = 10.6, 3.8 Hz, 1H, H-2′), 3.36 (dd, J = 13.4, 2.6 Hz, 1H, H-6′), 3.29 (dd, J = 13.4, 5.7 Hz, 1H, H-6′), 2.36 (ddd, J = 13.6, 5.7 Hz, 1H, H-2eq), 2.18 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.63 (ddd, J = 13.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.0, 169.8, 169.5, 166.2, 165.3, 133.9, 133.8, 133.5, 129.9, 129.8, 129.0, 128.7, 128.6, 107.0 (anomeric), 96.3 (anomeric), 95.6 (anomeric), 80.4, 79.9, 79.0, 78.6, 75.4, 72.0, 71.5, 70.7, 69.8, 69.5, 68.8, 68.4, 65.0, 62.1, 61.7, 60.5, 58.6, 58.2, 50.9, 30.2, 20.8, 20.7.

Compound ***4c***. Compound **4c** was prepared as described for compound **1a** using acceptor **4b** (310 mg, 0.41 mmol), donor (550 mg, 0.91 mmol), flame-dried 4-Å molecular sieves (900 mg), anhydrous dichloromethane (6 mL), and $BF_3{\cdot}OEt_2$ (30 μL). Propagation of the reaction was monitored by TLC analysis (ethyl acetate/petroleum ether, 3:7). The crude was purified by flash chromatography (SiO_2, ethyl acetate:dichloromethane) to yield **4c** (480 mg, 98%) as a white solid. LRMS (ESI): m/z calcd for $C_{52}H_{55}N_{15}O_{20}Na$, 1232.36 $[M + Na]^+$; found, 1231.99. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07−8.03 (m, 2H, Bz), 8.00−7.95 (m, 2H, Bz), 7.89−7.85 (m, 2H, Bz), 7.59−7.47 (m, 3H, Bz), 7.40 (m, 4H, Bz), 7.31 (t, J = 7.8 Hz, 2H, Bz), 5.78 (m, 1H, H-2‴), 5.73 (m, 1H, H-3‴), 5.71 (bs, 1H, H-1‴), 5.48 (d, J = 3.7 Hz, 1H, H-1″), 5.38 (d, J = 3.4 Hz, 1H, H-1′), 4.86 (t, J = 9.9 Hz, 1H, H-4″), 4.79−4.73 (m, 2H, H-2″, H-4‴), 4.70 (dd, J = 11.7, 4.0 Hz, 1H, H-5‴), 4.67−4.59 (m, 2H, H-4′, H-5‴), 4.20−4.10 (m, 4H, H-3″, H-5″, H-6″, H-6″), 4.09−4.04 (m, 2H, H-4, H-5), 4.00 (ddd, J = 6.7, 6.2, 2.4 Hz, 1H, H-5′), 3.82 (dd, J = 8.2, 6.1 Hz, 1H, H-6), 3.74 (m, 1H, H-3), 3.67 (ddd, J = 10.4, 8.3, 6.6 Hz, 1H, H-1), 3.35 (dd, J = 13.2, 2.3 Hz, 1H, H-6′), 3.31−3.23 (m, 2H, H-2′, H-6′), 2.38 (ddd, J = 13.8, 5.9 Hz, 1H, H-2eq), 2.32 (ddd, J = 11.7, 4.3 Hz, 1H, H-3′eq), 2.15 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.93 (ddd, J = 11.6 Hz, H-3′ax), 1.64 (ddd, J = 13.8, 10.3 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.8, 169.6, 169.3, 166.0, 165.1, 133.7, 133.6, 133.3, 129.7, 129.6, 128.9, 128.7, 128.6, 128.5, 128.4, 106.7 (anomeric), 95.8 (anomeric), 95.4 (anomeric), 80.5, 79.5, 79.0, 77.9, 75.3, 72.0, 71.4, 70.2, 68.8, 68.2, 66.9, 65.1, 62.0, 60.4, 58.6, 58.4, 56.3, 50.9, 30.4, 28.2, 20.9, 20.6, 20.52, 20.49.

Compound ***5c***. Compound **5c** was prepared as described for compound **1c** using acceptor **5b** (285 mg, 0.58 mmol), donor (520 mg, 0.86 mmol), flame-dried 4-Å molecular sieves (1.5 g), anhydrous dichloromethane (10 mL), and $BF_3{\cdot}OEt_2$ (40 μL). Propagation of the reaction was monitored by TLC analysis (ethyl acetate/petroleum ether, 3:7). The crude was purified by flash chromatography (SiO_2, ethyl acetate/dichloromethane) to yield **5c** (465 mg, 86%) as a white solid. LRMS (ESI): m/z calcd for $C_{42}H_{42}N_{12}O_{14}Na$, 961.28 $[M + Na]^+$; found, 961.48. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, J = 7.1, 1.4 Hz, 2H, Bz), 7.95 (dd, J = 7.2, 1.2 Hz, 2H, Bz), 7.89 (dd, J = 7.2, 1.2 Hz, 2H, Bz), 7.61−7.54 (m, 2H, Bz), 7.53−7.45 (m, 3H, Bz), 7.40 (t, J = 7.8 Hz, 2H, Bz), 7.34 (t, J = 7.8 Hz, 2H, Bz), 5.71 (dd, J = 6.7, 4.9 Hz, 1H, H-3″), 5.68 (d, J = 3.6 Hz, 1H, H-1′), 5.63 (dd, J = 4.8, 1.3 Hz, 1H, H-2″), 5.56 (d, J = 1.1 Hz, 1H, H-1″), 4.91 (dd, J = 12.2, 3.4 Hz, 1H, H-5″), 4.85 (t, J = 9.9 Hz, 1H, H-6), 4.69 (dt, J = 6.7, 3.8 Hz, 1H, H-4″), 4.63 (td, J = 10.6, 4.7 Hz, 1H, H-4′), 4.42 (dd, J = 12.1, 4.0 Hz, 1H, H-5″), 4.23 (ddd, J = 9.9, 5.8, 2.5 Hz, 1H, H-5′), 3.84 (dd, J = 9.1 Hz, 1H, H-5), 3.47 (dd, J = 9.9 Hz, 1H, H-4), 3.45−3.36 (m, 2H, H-1, H-3), 3.32 (dd, J = 13.3, 2.4 Hz, 1H, H-6′), 3.27 (m, 1H, H-2′), 3.21 (dd, J = 13.2, 5.8 Hz, 1H, H-6′), 2.34−2.29 (m, 2H, H-2eq, H-3′eq), 2.27 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 1.95 (ddd, J = 11.6 Hz, 1H, H-3′ax), 1.46 (ddd, J = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.9, 166.3, 165.6, 165.3, 133.8, 133.6, 133.4, 130.2, 129.9, 129.8, 129.0, 128.9, 128.7, 128.6, 128.5, 107.4 (anomeric), 95.5 (anomeric), 80.7, 79.6, 77.1, 75.0, 74.3, 71.6, 69.6, 67.3, 63.5, 59.5, 58.5, 56.4, 51.4, 31.8, 28.5, 21.1, 21.0.

Compound ***1d***. The fully protected ribosylated compound **1c** (320 mg, 0.25 mmol) was dissolved in methanol/dichloromethane (9:1, 10 mL), and K_2CO_3 (62 mg, 0.45 mmol) was added. The mixture was stirred overnight at ambient temperature. Upon completion (as shown by TLC analysis, methanol/dichloromethane, 2:8), the solvent was removed by evaporation and the crude product was purified by flash column chromatography (SiO_2, methanol/dichloromethane) to yield **1d** (144 mg, 80%) as a white solid. LRMS (ESI): m/z calcd for $C_{23}H_{35}N_{12}O_{15}$, 719.24 $[M - H]^-$; found, 719.54. ^{1}H NMR (500 MHz, CD_3OD) δ 5.52 (d, J = 3.7 Hz, 1H, H-1″), 5.41 (d, J = 3.9 Hz, 1H, H-1′), 5.36 (d, J = 3.2 Hz, 1H, H-1‴), 4.03 (ddd, J = 9.9, 5.2, 2.3 Hz, 1H, H-5′), 3.99 (dd, J = 5.9 Hz, 1H, H-3‴), 3.95 (dd, J = 7.7 Hz, 1H, H-5), 3.94−3.90 (m, 2H, H-5″, H-2‴), 3.87 (td, J = 5.9, 2.9 Hz, 1H, H-4‴), 3.83−3.63 (m, 10H, H-1, H-3, H-4, H-6, H-3′, H-3″, H-6″,H-6″, H-5‴, H-5‴), 3.57 (dd, J = 13.2, 2.3 Hz, 1H, H-6′), 3.45−3.40 (m, 3H, H-2′, H-6′,H-2″), 3.37−3.29 (m, 2H, H-4′, H-4″), 2.40 (ddd, J = 12.7, 4.7 Hz, 1H, H-2eq), 1.62 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, CD_3OD) δ 109.4 (anomeric), 99.9 (anomeric), 98.5 (anomeric), 84.5, 81.4, 79.7, 78.3, 75.9, 74.5, 73.7, 73.6, 73.3, 72.3, 72.0, 70.9, 70.1, 67.9, 63.4, 62.1, 61.1, 60.9, 52.6, 32.5.

Compound ***2d***. Compound **2d** was prepared as described for compound **1d** using **2c** (200 mg, 0.14 mmol), methanol/dichloromethane (9:1, 10 mL), and K_2CO_3 (39 mg, 0.28 mmol). The reaction mixture was stirred at ambient temperature overnight. Upon completion (TLC analysis, methanol/dichloromethane, 2:8), solvent was evaporated and the crude was purified by flash column chromatography (SiO_2, methanol/dichloromethane) to yield **2d** (94 mg, 81%) as a white solid. LRMS (ESI): m/z calcd for $C_{27}H_{42}N_{13}O_{17}$, 820.28 $[M - H]^-$; found, 820.59. ^{1}H NMR (500 MHz, CD_3OD) δ 5.39 (d, J = 3.8 Hz, 1H, H-1′), 5.35 (d, J = 2.4 Hz, 1H, H-1‴), 5.22 (d, J = 3.4 Hz, 1H, H-1″), 4.14 (dd, J = 9.0, 3.5 Hz, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (Hα)), 4.06−4.00 (m, 3H, H-5′, H-2‴, H-3‴), 3.96−3.90 (m, 3H, H-1, H-5, H-4‴), 3.87−3.79 (m, 3H, H-5″, H-6″, H-5‴), 3.75−3.57 (m, 8H, H-3, H-4, H-6, H-3′, H-6′, H-3″, H-6″, H-5‴), 3.50−3.42 (m, 5H, H-2′, H-6′, H-2″, (*S*)-(−)-4-amino-2-hydroxybutyryl (2Hγ)), 3.34−3.27 (m, 2H, H-4′, H-4″), 2.47 (ddd, J = 12.9, 3.7 Hz, 1H, H-2eq), 2.09−2.02 (m, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (Hβ)), 1.84 (m, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (Hβ)), 1.45 (ddd, J = 12.0 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, CD_3OD) δ 176.6, 109.0 (anomeric), 100.0 (anomeric), 99.4 (anomeric), 84.7, 82.4, 79.9, 78.6, 76.1, 74.9, 74.5, 73.7, 73.3, 72.7, 72.1, 71.4, 70.7, 69.9, 67.6, 63.8, 62.5, 60.8, 52.6, 34.6, 31.9.

Compound ***3d***. Compound **3d** was prepared as described for compound **1d** using **3c** (80.5 mg, 0.06 mmol), methanol/dichloromethane (9:1, 10 mL), and K_2CO_3 (14 mg, 0.10 mmol). The reaction mixture was stirred at ambient temperature overnight. Upon completion (TLC analysis, methanol/dichloromethane, 15:85), solvent was evaporated, and the crude was purified by flash column chromatography (SiO_2, methanol/dichloromethane) to yield **3d** (44.7 mg, 94%) as a white solid. LRMS (ESI): m/z calcd for $C_{23}H_{35}N_{15}O_{14}Na$, 768.24 $[M + Na]^+$; found, 768.10. ^{1}H NMR (400 MHz, CD_3OD) δ 5.67 (d, J = 3.8 Hz, 1H, H-1′), 5.39−5.38 (m, 2H, H-1″, H-1‴), 4.08−3.69 (m, 16H, H-1, H-3, H-4, H-5, H-6, H-3′, H-5′, H-2″, H-5″, H-6″, H-6″, H-2‴, H-3‴, H-4‴, H-5‴, H-5‴), 3.57 (dd, J = 13.2, 2.1 Hz, 1H, H-6′), 3.49−3.34 (m, 4H, H-4′, H-6′, H-3″, H-4″), 3.24 (dd, J = 10.4, 3.8 Hz, 1H, H-2′), 2.40 (ddd, J = 13.0, 5.3 Hz, 1H, H-2eq), 1.59 (ddd, J = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD_3OD) δ 108.9 (anomeric), 98.9 (anomeric), 97.5 (anomeric), 84.6, 80.3, 78.4, 77.9, 76.1, 73.8, 73.3, 72.5, 72.2, 71.6, 69.9, 67.8, 64.5, 64.0, 62.0, 60.4, 60.2, 52.6, 31.8.

Compound ***4d***. Compound **4d** was prepared as described for compound **1d** using **4c** (118 mg, 0.09 mmol), methanol/dichloromethane (4:1, 5 mL), and K_2CO_3 (24 mg, 0.17 mmol). The reaction mixture was stirred at ambient temperature overnight. Upon completion (TLC analysis, methanol/dichloromethane, 2:8), solvent was evaporated and the crude was purified by flash column chromatography (SiO_2, methanol/dichloromethane) to yield **4d** (55 mg, 77%) as a white solid. LRMS (ESI): m/z calcd for $C_{23}H_{34}N_{15}O_{13}$, 728.25 $[M - H]^-$; found, 728.78. ^{1}H NMR (500 MHz, CD_3OD) δ 5.63 (d, J = 3.0 Hz, 1H, H-1′), 5.44 (d, J = 3.3 Hz, 1H, H-1″), 5.40 (s, 1H, H-1‴), 4.04−4.00 (m, 2H, H-5, H-3‴), 3.98−3.85 (m, 6H, H-5′, H-5″, H-4, H-6, H-2‴, H-4‴), 3.84−3.69 (m, 7H, H-3″, H-6″, H-6″, H-1, H-3, H-5‴, H-5‴), 3.61−3.53 (m, 2H, H-4′, H-6′), 3.46−3.34

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J. Med. Chem. XXXX, XXX, XXX−XXX

(m, 3H, H-6′, H-2″, H-4″), 3.30 (m, 1H, H-2′), 2.42 (ddd,*J* = 13.2, 5.0 Hz, 1H, H-2eq), 2.19 (ddd, *J* = 9.2, 3.3 Hz, 1H, H-3′eq), 2.05 (ddd, *J* = 9.4 Hz, 1H, H-3′ax), 1.59 (ddd, *J* = 12.5 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, CD_3OD) δ 108.9 (anomeric), 98.8 (anomeric), 96.3 (anomeric), 84.5, 80.7, 78.4, 77.4, 76.2, 74.2, 73.8, 72.2, 71.6, 69.9, 67.9, 66.5, 64.1, 61.9, 60.6, 57.7, 52.6, 32.3, 32.2.

Compound **5d**. Compound **5d** was prepared as described for compound **1d** using **5c** (409 mg, 0.44 mmol), methanol/dichloromethane (9:1, 15 mL), and K_2CO_3 (60 mg, 0.43 mmol). The reaction mixture was stirred at ambient temperature overnight. Upon completion (TLC analysis, methanol/dichloromethane, 15:85), solvent was evaporated and the crude was purified by flash column chromatography (SiO_2, methanol/dichloromethane) to yield **5d** (223 mg, 94%) as a white solid. LRMS (ESI): *m*/*z* calcd for $C_{17}H_{25}N_{12}O_9$, 541.19 $[M - H]^-$; found, 541.30. ^{1}H NMR (400 MHz, CD_3OD) δ 5.67 (d, *J* = 3.4 Hz, 1H, H-1′), 5.36 (s, 1H, H-1″), 4.15−4.11 (m, 2H, H-2″, H-3″), 4.07 (ddd, *J* = 9.4, 5.9, 2.0 Hz, 1H, H-5′), 3.94 (m, 1H, H-4″), 3.78 (dd, *J* = 11.9, 3.3 Hz, 1H, H-5″), 3.74−3.67 (m, 2H, H-4, H-5), 3.64 (dd, *J* = 11.9, 5.5 Hz, 1H, H-5″), 3.61−3.48 (m, 3H, H-4′, H-6′, H-3), 3.47−3.38 (m, 3H, H-6′, H-1, H-6), 3.18 (dt, *J* = 12.8, 4.1 Hz, 1H, H-2′), 2.23 (ddd, *J* = 12.8, 4.2 Hz, 1H, H-2eq), 2.16 (dt, *J* = 11.1, 4.5 Hz, 1H, H-3′eq), 2.04 (ddd, *J* = 11.4 Hz, 1H, H-3′ax), 1.40 (ddd, *J* = 12.4 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, CD_3OD) δ 109.0 (anomeric), 97.2 (anomeric), 84.8, 84.4, 77.3, 77.2, 76.9, 74.0, 71.6, 66.5, 63.6, 61.8, 61.3, 57.6, 52.5, 32.9, 32.2.

Compound **1**. The azide-protected ribosylated compound **1d** (47 mg, 0.06 mmol) was dissolved in water (6 mL), and palladium on charcoal (10% Pd, cat.) was added. Hydrogen was bubbled through the suspension for 10 min, and reaction mixture was stirred under hydrogen atmosphere at ambient temperature for 3 h. Progress of the reaction was monitored by low-resolution ESI-MS. Upon completion, the mixture was filtered through a syringe filter (diam 25 mm; pore size 0.2 μm; PTEF membrane) and freeze-dried to yield **1** (38 mg, 94%) as a white solid. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{43}N_4O_{15}$, 615.2725 $[M - H]^-$; found, 615.2718. ^{1}H NMR (400 MHz, D_2O) δ 5.44 (d, *J* = 4.2 Hz, 1H, H-1‴), 5.38 (d, *J* = 3.7 Hz, 1H, H-1″), 5.23 (d, *J* = 3.6 Hz, 1H, H-1′), 4.36 (dd, *J* = 8.5 Hz, 1H, H-6), 4.31 (dd, *J* = 8.6 Hz, 1H, H-5), 4.24 (dd, *J* = 9.8, 6.9 Hz, 1H, H-4), 4.19 (dd, *J* = 4.5 Hz, 1H, H-2‴), 4.14 (dd, *J* = 4.4 Hz, 1H, H-3‴), 4.07 (m, 1H, H-4‴), 4.02 (m, 1H, H-5″), 3.93−3.87 (m, 2H, H-2′, H-6′), 3.84−3.73 (m, 5H, H-1, H-5′, H-6′, H-3″, H-5‴), 3.73−3.65 (m, 3H, H-4′, H-2″, H-5‴), 3.60 (m, 1H, H-3), 3.48 (dd, *J* = 10.6 Hz, 1H, H-3′), 3.42 (dd, *J* = 13.4, 3.3 Hz, 1H, H-6″), 3.38 (dd, *J* = 9.9, 9.2 Hz, 1H, H-4″), 3.19 (dd, *J* = 13.4, 7.8 Hz, 1H, H-6″), 2.54 (ddd, *J* = 12.6, 4.6 Hz, 1H, H-2eq), 1.89 (ddd, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, D_2O) δ 163.3 (q, *J* = 35 Hz, CF_3COOH), 116.6 (q, *J* = 292 Hz, CF_3COOH), 104.7 (anomeric), 100.6 (anomeric), 95.0 (anomeric), 84.7, 82.9, 77.9, 75.3, 75.3, 73.2, 72.5, 71.1, 70.9, 69.9, 69.4, 68.7, 65.7, 62.2, 60.2, 55.2, 49.7, 47.6, 40.7, 27.3.

Compound **2**. Compound **2** was prepared as described for compound **1** using **2d** (52.0 mg, 0.06 mmol), water (5 mL), and 10% Pd/C (cat.). The reaction mixture was filtered and freeze-dried to yield **2** (42.9 mg, quantitative yield) as a white solid. HRMS (ESI): *m*/*z* calcd for $C_{27}H_{50}N_5O_{17}$, 716.3202 $[M - H]^-$; found, 716.3204. ^{1}H NMR (500 MHz, D_2O) δ 5.35 (d, *J* = 3.7 Hz, 1H, H-1′), 5.30 (d, *J* = 2.4 Hz, 1H, H-1‴), 5.25 (d, *J* = 3.7 Hz, 1H, H-1″), 4.33 (dd, *J* = 5.8, 3.0 Hz, 1H, H-5), 4.29 (dd, *J* = 9.2, 3.8 Hz, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*α*)), 4.26−4.15 (m, 5H, H-1, H-4, H-6, H-2‴, H-3‴), 4.06−4.02 (m, 1H, H-4‴), 3.95 (ddd, *J* = 10.1, 6.8, 3.5 Hz, 1H, H-5′), 3.89−3.76 (m, 7H, H-3, H-3′, H-6′, H-2″, H-5″, H-6″, H-5‴), 3.72−3.62 (m, 3H, H-2′, H-4″, H-5‴), 3.43−3.39 (m, 3H, H-4′, H-6′, H-3″), 3.27 (dd, *J* = 13.4, 6.7 Hz, 1H, H-6′), 3.16 (t, *J* = 7.3 Hz, 2H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*γ*)), 2.37−2.30 (m, 1H, H-2eq), 2.21−2.13 (m, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*β*)), 1.98−1.89 (m, 1H, (*S*)-(−)-4-amino-2-hydroxybutyryl (H*β*)), 1.85−1.75 (m, 1H, H-2ax). ^{13}C NMR (125 MHz, D_2O) δ 175.8, 163.0 (q, *J* = 35 Hz, CF_3COOH), 116.1 (q, *J* = 292 Hz, CF_3COOH), 107.0 (anomeric), 98.5 (anomeric), 97.5 (anomeric), 83.8, 78.5, 78.2, 76.3, 75.2, 73.0, 72.4, 71.1, 71.1, 70.2, 69.8, 69.3, 68.4, 66.0, 61.9, 60.2, 55.4, 49.1, 47.6, 40.5, 37.2, 31.3, 28.7.

Compound **3**. Compound **3** was prepared as described for compound **1** using **3d** (30.7 mg, 0.041 mmol), water (5 mL), and 10% Pd/C (cat.). The reaction mixture was filtered and freeze-dried to yield **3** (25.1 mg, quantitative yield) as a white solid. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{44}N_5O_{14}$, 614.2885 $[M - H]^-$; found, 614.2890. ^{1}H NMR (500 MHz, D_2O) δ 6.01 (d, *J* = 3.8 Hz, 1H, H-1′), 5.51 (d, *J* = 2.6 Hz, 1H, H-1‴), 5.31 (d, *J* = 3.4 Hz, 1H, H-1″), 4.24−4.18 (m, 2H, H-4, H-5), 4.17 (dd, *J* = 4.9, 2.6 Hz, 1H, H-2‴), 4.13−4.09 (m, 2H, H-6, H-3‴), 4.07−4.01 (m, 2H, H-3′, H-4‴), 4.01−3.95 (m, 2H, H-5′, H-2″), 3.94−3.87 (m, 3H, H-5″, H-6″, H-5‴), 3.79 (dd, *J* = 9.2, 5.0 Hz, 1H, H-6″), 3.71−3.65 (m, 3H, H-1, H-4″, H-5‴), 3.62−3.53 (m, 4H, H-3, H-2′, H-4′, H-3″), 3.45 (dd, *J* = 13.8, 3.7 Hz, 1H, H-6′), 3.38 (dd, *J* = 13.9, 6.3 Hz, 1H, H-6′), 2.54 (ddd, *J* = 12.7, 4.5 Hz, 1H, H-2eq), 2.04−1.94 (m, 1H, H-2ax). ^{13}C NMR (125 MHz, D_2O) δ 162.9 (q, *J* = 35 Hz, CF_3COOH), 116.3 (q, *J* = 292 Hz, CF_3COOH), 108.1 (anomeric), 99.2 (anomeric), 94.5 (anomeric), 83.8, 82.9, 81.2, 76.2, 74.8, 73.6, 70.4, 69.8, 69.1, 68.4, 68.1, 65.3, 61.5, 60.0, 54.6, 52.9, 48.8, 48.4, 39.7, 27.7.

Compound **4**. Compound **4** was prepared as described for compound **1** using **4d** (55 mg, 0.07 mmol), water (5 mL), and 10% Pd/C (cat.). The reaction mixture was filtered and freeze-dried to yield **4** (44 mg, 97%) as a white solid. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{44}N_{15}O_{13}$, 598.2936 $[M - H]^-$; found, 598.2930. ^{1}H NMR (500 MHz, D_2O) δ 5.60 (d, *J* = 2.6 Hz, 1H, H-1′), 5.26 (d, *J* = 1.3 Hz, 1H, H-1‴), 5.21 (d, *J* = 3.4 Hz, 1H, H-1″), 4.27 (dd, *J* = 4.6, 1.4 Hz, 1H, H-2‴), 4.20−4.13 (m, 2H, H-5′, H-4), 4.08 (dd, *J* = 7.3, 4.7 Hz, 1H, H-3‴), 4.02−3.94 (m, 3H, H-5, H-6, H-4‴), 3.91 (dd, *J* = 10.7, 3.4 Hz, 1H, H-2″), 3.89−3.82 (m, 5H, H-2′, H-4′, H-5″, H-6″, H-5‴), 3.77 (dd, *J* = 12.4, 4.8 Hz, 1H, H-6″), 3.67 (dd, *J* = 10.1 Hz, 1H, H-4″), 3.63 (dd, *J* = 12.6, 6.5 Hz, 1H, H-5‴), 3.58 (m, 1H, H-3), 3.53 (t, *J* = 10.7 Hz, 1H, H-3″), 3.48 (m, 1H, H-1), 3.37 (dd, *J* = 13.9, 8.6 Hz, 1H, H-6′), 3.25 (dd, *J* = 13.9, 3.8 Hz, 1H, H-6′), 2.49 (dt, *J* = 12.6, 4.3 Hz, 1H, H-2eq), 2.22 (dt, *J* = 14.4, 3.8 Hz, 1H, H-3′eq), 2.13 (ddd, *J* = 14.4, 6.3 Hz, 1H, H-3′ax), 1.95 (ddd, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, D_2O) δ 164.17 (q, *J* = 35 Hz, CF_3COOH), 117.61 (q, *J* = 291 Hz, CF_3COOH), 110.8 (anomeric), 100.9 (anomeric), 94.6 (anomeric), 85.6, 83.7, 82.5, 79.3, 76.4, 76.1, 74.9, 70.7, 69.9, 66.5, 64.6, 63.2, 61.2, 56.0, 50.4, 49.7, 48.1, 39.9, 29.3, 28.9.

Compound **5**. Compound **5** was prepared as described for compound **1** using **5d** (82 mg, 0.15 mmol), water (6 mL), and 10% Pd/C (cat.). The reaction mixture was filtered and freeze-dried to yield **5** (62 mg, 93%) as a white solid. HRMS (ESI): *m*/*z* calcd for $C_{17}H_{33}N_4O_9$, 437.2248 $[M - H]^-$; found, 437.2253. ^{1}H NMR (400 MHz, D_2O) δ 5.86 (d, *J* = 3.6 Hz, 1H, H-1′), 5.33 (d, *J* = 1.5 Hz, 1H, H-1″), 4.20 (dd, *J* = 4.7, 1.6 Hz, 1H, H-2″), 4.15 (dd, *J* = 6.9, 4.8 Hz, 1H, H-3″), 4.07 (dd, *J* = 9.6 Hz, 1H, H-4), 4.03 (td, *J* = 6.6, 2.7 Hz, 1H, H-4″), 3.89 (m, 1H, H-5′), 3.88−3.83 (m, 2H, H-5, H-5″), 3.72 (m, 1H, H-4′), 3.70−3.62 (m, 3H, H-2′, H-6, H-5″), 3.54 (ddd, *J* = 12.6, 10.7, 4.0 Hz, 1H, H-3), 3.40 (dd, *J* = 13.6, 3.6 Hz, 1H, H-6′), 3.34 (m, 1H, H-1), 3.25 (dd, *J* = 13.6, 6.8 Hz, 1H, H-6′), 2.49 (ddd, *J* = 12.5, 4.1 Hz, 1H, H-2eq), 2.26 (dt, *J* = 12.0, 4.2 Hz, 1H, H-3′eq), 2.03 (ddd, *J* = 12.0 Hz, 1H, H-3′ax), 1.89 (ddd, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (100 MHz, D_2O) δ 163.2 (d, *J* = 35 Hz, CF_3COOH), 116.6 (q, *J* = 291, CF_3COOH), 110.7 (anomeric), 93.9 (anomeric), 85.2, 82.9, 75.6, 72.9, 70.7, 69.4, 64.7, 61.5, 50.1, 48.8, 48.0, 40.1, 29.5, 28.3.

General Biology Methods. All bacterial strains utilized in this study were purchased from the American Type Culture Collection (ATCC). Bacteria were grown in cation-adjusted Mueller−Hinton broth (Sigma), overnight, at 37 °C in 5% CO_2 under aerobic conditions with the exceptions of *H. influenzae* and *E. coli*, which were grown in 814 GC broth medium (ATCC medium) and LB (Lennox), respectively. *B. cepacia* was grown in cation-adjusted Mueller−Hinton broth (Sigma), for 24 h at 30 °C.

Cell-Free Prokaryotic in Vitro Translation Inhibition Assay. Protein translation inhibition was quantified in a coupled transcription/translation assay using *E. coli* S30 extracts for circular DNA with the pBEST*luc* plasmid (Promega) according to the manufacturer's protocol. Briefly, reactions were carried out in a total volume of 10 μL. Each reaction contained 3 μL of S30 Extract Circular, 1 μL of

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J. Med. Chem. XXXX, XXX, XXX−XXX

pBEST*luc* plasmid (1 μg/μL), 4 μL of S30 premix, 1 μL of amino acid mixture, and 1 μL of the tested compound in various concentrations. All translation mixtures were incubated at 37 °C for 90 min, cooled on ice for 5 min, and diluted with 45 μL of a dilution reagent (25 mM Tris-phosphate buffer, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexanetetraacetate, 10% glycerol, 1% Triton X-100, and 1 mg/mL BSA) into white polystyrene 96-well flat-bottom plates (Corning). The luminescence was measured immediately after the addition of the luciferase assay reagent (25 μL, Promega) using a TECAN microplate reader (Infinite F200 Pro). The concentrations of half-maximal inhibition (IC_{50}) were obtained from concentration–response curves fitted to the data of at least two independent experiments using Grafit 5 software.

Cell-Free Cytosolic Eukaryotic in Vitro Translation Inhibition Assay. Protein translation inhibition was quantified in a coupled transcription/translation assay using S30 cell extract derived from rabbit reticulocytes supplemented with TNT coupled reticulocyte lysate systems (Promega) according to the manufacturer's protocol. Briefly, reactions were carried out in a total volume of 10 μL. Each reaction contained 8 μL of TnT Quick Master Mix, 0.8 μL of Luciferase T7 Control DNA plasmid (1 μg/μL), 0.2 μL of methionine, and 1 μL of the tested compound in various concentrations. All translation mixtures were incubated at 30 °C for 60 min, cooled on ice for 5 min and diluted with 45 μL of a dilution reagent (25 mM Tris-phosphate buffer, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexanetetraacetate, 10% glycerol, 1% Triton X-100, and 1 mg/mL BSA) into white polystyrene 96-well flat-bottom plates (Corning). The luminescence was measured immediately after the addition of the luciferase assay reagent (25 μL, Promega) using a TECAN microplate reader (Infinite F200 Pro). The IC_{50} values were obtained from concentration–response curves fitted to the data of at least two independent experiments using Grafit 5 software.

Minimal Inhibitory Concentration Experiments. Starter cultures were incubated for 24 h (37 °C, 5% CO_2, aerobic conditions) and then diluted in fresh medium to obtain an optical density of 0.004 (OD_{600}, Evolution 60, Thermo Scientific). All strains were tested using the double-dilution method starting at 32 μg/mL in 96-well flat-bottom plates (Corning). After 24 h of incubation, MTT (25 μL of a 1 mg/mL solution in H_2O) was added to each well followed by additional incubation at 37 °C for 1 h. MIC values were determined as the lowest concentration at which no bacterial growth was observed. Results were obtained from two independent experiments, and each experiment was performed in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00793.

Synthetic procedure for the preparation of AG-based 5-OH glycosyl acceptors and of the ribofuranose glycosyl donor, detailed results of in vitro translation experiments, ^1H and ^{13}C NMR spectra of compounds **1**–**5**, ULC-MS traces of compounds **1**–**5**, and compound purity information (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AG, aminoglycoside; AMK, amikacin; KAN-A, kanamycin A; KAN-B, kanamycin B; NEA, neamine; NEB, nebramine; RIB, ribostamycin; TOB, tobramycin

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J. Med. Chem. XXXX, XXX, XXX−XXX