JOC Article

Application of the Synthetic Aminosugars for Glycodiversification: Synthesis and Antimicrobial Studies of Pyranmycin

Bryan Elchert,[†] Jie Li,[†] Jinhua Wang,[†] Yu Hui,[†] Ravi Rai,[†] Roger Ptak,[‡] Priscilla Ward,[‡] Jon Y. Takemoto,[§] Mekki Bensaci,[§] and Cheng-Wei Tom Chang*[,†]

Department of Chemistry and Biochemistry, and Department of Biology, Utah State University, 0300 Old Main Hill, Logan, Utah 84322-0300, and Infectious Disease Research Department, Southern Research Institute, 431 Aviation Way, Frederick, Maryland 21701

chang@cc.usu.edu

Received September 2, 2003

A divergent approach was employed for the synthesis of aminosugars, from which a novel library of aminoglycoside antibiotics (pyranmycins) was synthesized. Pyranmycins have comparable antibacterial activity as neomycin, a clinically used aminoglycoside antibiotic, against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*. In addition, pyranmycins, like streptomycin, are bacteriocidal while isoniazid (INH) is bacteriostatic. Therefore, pyranmycins may provide new therapeutic options in the treatment against tuberculosis. Several members of pyranmycins also manifest modest anti-Tat and anti-Rev activities, which may aid in the development of new anti-HIV agents. Although the antibacterial activity of pyranmycins against aminoglycoside resistant bacteria is less than expected, the synthetic methodologies of utilizing a library of aminosugars can be a model for future studies of glycodiversification or glycorandomization.

Introduction

Aminosugars have attracted a growing interest due to their broad spectrum of application in chemistry, biochemistry, medicine, and pharmaceutical fields.[1−6] Since aminosugars are often found in the naturally occurring antibiotics, one of the applications of aminosugars will be the library construction of naturally occurring antibiotic analogues with the original sugar replaced by a synthetic one leading to the concept of glycodiversification or glycorandomization. Extensive studies have been done toward the synthesis of various aminosugars.[7−11] However, there are two problems often associated with these results. The first one is the synthetic challenge involved in the preparation of a library of aminosugars. The second problem is the lack of methodologies for converting the synthetic aminosugars into glycosyl donors. Most of the glycosyl donors, such as glycosyl halides, glycosyl acetates, and glycosyl trichloroacetimidates, are not suitable for the procedures of aminosugar synthesis. Therefore, phenylthio or ethylthio groups, which are stable enough to endure the conditions for amino group incorporation and can be activated for glycosylation directly, are often used for the synthesis of aminosugars and corresponding derivatives.[12−17] Nevertheless, the starting materials, ethyl thioglucopyranoside or phenyl thioglucopyranoside, are inconvenient to prepare in large quantities for the synthesis of an aminosugar library. Commercially available thioglucopyranose is too expensive to be a practical option.

To provide solutions for these two problems, we designed a divergent approach for the expedient synthesis of an azidosugar (aminosugar) library with designed

* To whom correspondence should be addressed. Fax: 435-797-3390.
[†] Department of Chemistry and Biochemistry, Utah State University.
[‡] Southern Research Institute.
[§] Department of Biology, Utah State University.

(1) Kirschning, A.; Bechthold, A. F.-W.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 1−84.
(2) Johnson, D. A.; Liu, H.-w. *Curr. Opin. Chem. Biol.* **1998**, *2*, 642−649.
(3) Weymouth-Wilson, A. C. *Nat. Prod. Rep.* **1997**, 99−110.
(4) Hooper, I. R. *Aminoglycoside Antibiotics*; Springer-Verlag: New York, 1982.
(5) Priebe, W.; Van, N. T.; Burke, T. G.; Perez-Soler, R. *Anticancer Drugs* **1993**, *4*, 37−48.
(6) Lothstein, L.; Sweatman, T, W.; Priebe, W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1807−1812.
(7) For reviewing: (a) Brimacombe, J. S. *Angew. Chem., Int. Ed.* **1971**, *10*, 236−248. (b) Umezawa, S.; Tsuchiya, T. *Aminoglycoside Antibiotics*; Springer-Verlag: New York, 1982; pp 37−110.
(8) Zunszain, P. A.; Varela, O. *Tetrahedron: Asymmetry* **1998**, *9*, 1269−1276.
(9) Saotome, C.; Ono, M.; Akita, H. *Tetrahedron: Asymmetry* **2000**, *11*, 4137−4151.
(10) For the synthesis of 1,3-diaminopentose see: Yuasa, H.; Hashimoto, H. *J. Am. Chem. Soc.* **1999**, *121*, 5089−5090.
(11) For examples see: (a) Knapp, S.; Kukkola, P. J.; Sharma, S.; Dhar, T. G. M.; Naughton, A. B. J. *J. Org. Chem.* **1990**, *55*, 5700−5710. (b) Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2751−2755. (c) Dent, B. R.; Furneaux, R. H.; Gainsford, G. J.; Lynch, G. P. *Tetrahedron* **1999**, *55*, 6977−6996.
(12) Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965−1978.
(13) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435−436.
(14) Crich, D.; Sun, S. *J. Org. Chem.* **1997**, *62*, 1198−1199.
(15) Crich, D.; Sun, S. *J. Org. Chem.* **1997**, *62*, 4506−4507.
(16) Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926−4930.
(17) Garegg, P. J.; Olsson, L.; Oscarson, S. *J. Org. Chem.* **1995**, *60*, 2200−2204.

10.1021/jo035290r CCC: $27.50
Published on Web 01/28/2004

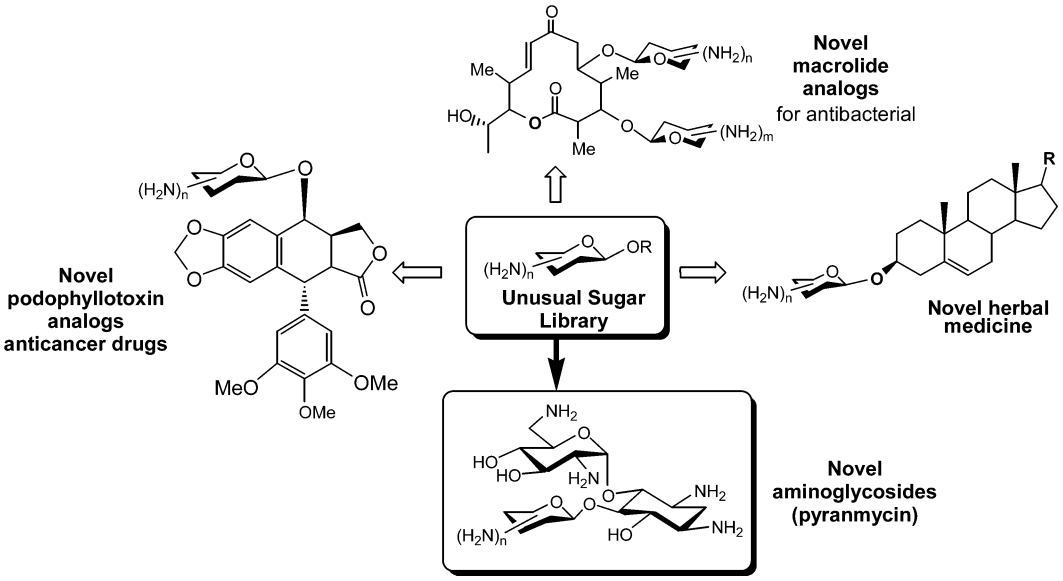


FIGURE 1. Prospective applications of glycodiversification.

binding motifs from commercially available and inexpensive methyl glucoside. The anomeric methoxyl group of methyl glucoside is stable enough in the conditions for amino-substitution or deoxygenation. However, it is too stable to be activated for glycosylation. Hence, we have also developed a general procedure for converting synthetic sugar derivatives into glycosyl donors ready for glycosylation. The amino groups are installed as azido groups. After completion of the aminosugar synthesis, we further utilize this library for the synthesis of a novel class of aminoglycoside antibiotics, which we have termed pyranmycins. Through our research, a model for the study of carbohydrate-containing compounds has been established. This includes the divergent synthesis of unusual sugars with designed binding motifs, the library construction of unusual sugar-containing compounds, and the glycodiversification approach for the structure−activity relationship studies of these compounds (Figure 1).

Given the complexity of these aminosugars and deoxysugars found in the naturally occurring aminoglycoside antibiotics, it is arduous to outline or predict the conclusions regarding the structure−activity relationship of unusual sugars among the vast numbers of unusual sugar-containing antibiotics. Therefore, Wong and coworkers have proposed several binding motifs based on the commonly observed structural features of unusual sugars on aminoglycosides (Figure 2).[18,19] These include *gluco*-/*galacto*-1,3-hydroxylamino and *cis*-/*trans*-1,2-hydroxylamino substructures. Following these binding motifs, we propose four new natural and nonnatural binding motifs, including *gluco*-/*galacto*-1,3-diamino and variants of *gluco*-/*galacto*-1,3-hydroxylamino substructures (Figure 2).[20] These scaffolds will be the targets for our synthesis of unusual sugars (Figure 1).

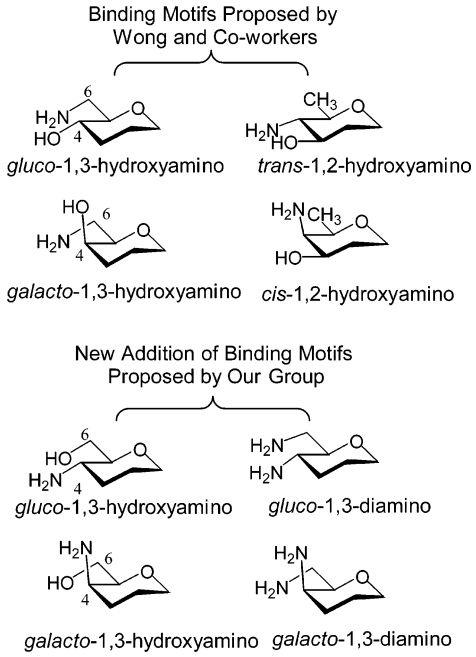


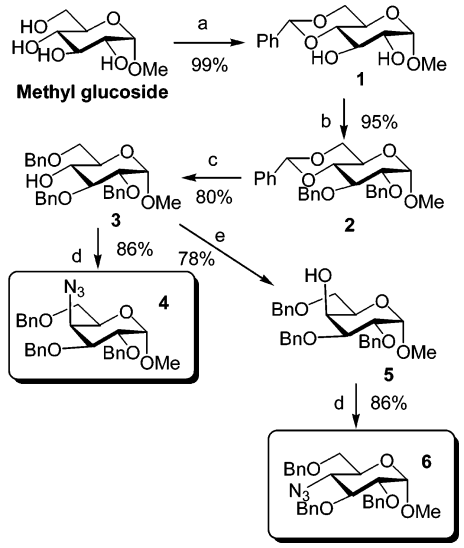
FIGURE 2. Proposed binding motifs of aminosugars.

Results and Discussion

Divergent Synthesis of Unusual Sugars. The synthesis of our aminosugar library began with the commercially available methyl glucopyranoside. Benzylation of compound **1**[21] yielded compound **2**[21] (Scheme 1). Compound **2** was treated with $NaBH_3CN$/HCl in THF to selectively deprotect the C-4 hydroxyl group providing compound **3**.[21] Triflation followed by azide substitution of compound **3** generated azidosugar **4** with intrinsic 1,3-hydroxyamine binding motif in the *galacto* configuration. Epimerization of the 4-OH of **3** was achieved with Swern oxidation and $NaBH_4$ reduction offering compound **5**, which allowed an S_N2 azide substitution that furnished

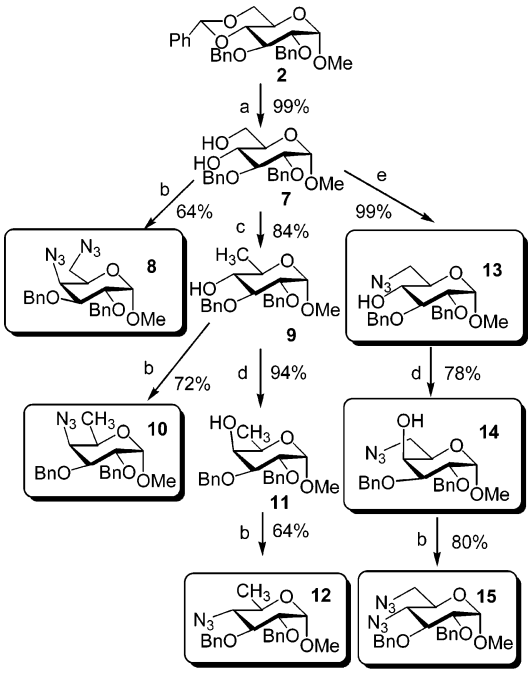
(18) Wong, C.-H.; Hendrix, M.; Manning, D. D.; Rosenbohm, C.; Greenberg, W. A. *J. Am. Chem. Soc.* **1998**, *120*, 8319−8327.
(19) Hendrix, M.; Alper, P. B.; Priestley, S.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 95−98.
(20) Hui, Y.; Chang, C.-W. T. *Org. Lett.* **2002**, *4*, 2245−2248.
(21) Hanessian, S. *Preparative Carbohydrate Chemistry*; Marcel Dekker: New York, 1996; pp 53−67.

SCHEME 1[a]



[a] Reagents: (a) $PhCH(OMe)_2$, PPTS, DMF; (b) BnBr, NaH, TBAI, THF; (c) $NaBH_3CN$, HCl, THF; (d) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF; (e) (1) $(COCl)_2$, DMSO, DIPEA, (2) $NaBH_4$, MeOH.

SCHEME 2[a]

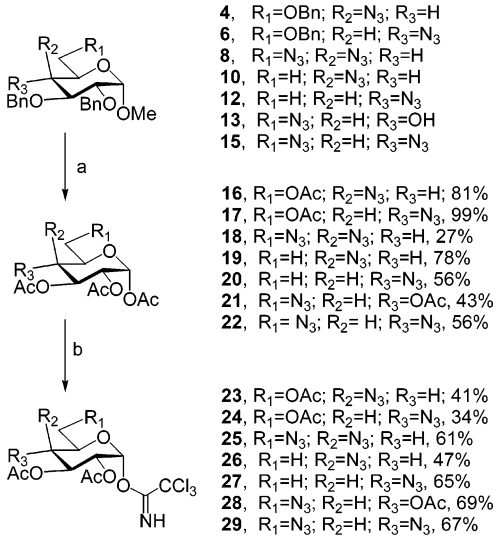


[a] Reagents: (a) TsOH−H_2O, MeOH; (b) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF; (c) (1) TsCl, py, (2) $LiAlH_4$, THF; (d) (1) $(COCl)_2$, DMSO, DIPEA, (2) $NaBH_4$, MeOH; (e) (1) TsCl, py, (2) NaN_3, DMF.

azidosugar **6** with intrinsic 1,3-hydroxylamino binding motif in the desired *gluco* configuration.

In another route (Scheme 2), compound **2** was treated with TsOH to give compound **7** with free hydroxyl groups at the C-4 and C-6 positions, which branched into three distinct routes. In the first route, triflation followed by azide substitution of compound **7** provided azidosugar **8** with a novel intrinsic *galacto*-1,3-diamino binding motif. In the second route, compound **7** was selectively deoxygenated at the C-6 position by sequential tosylation and $LiAlH_4$ reduction. Compound **9** was subjected to azide

SCHEME 3[a]



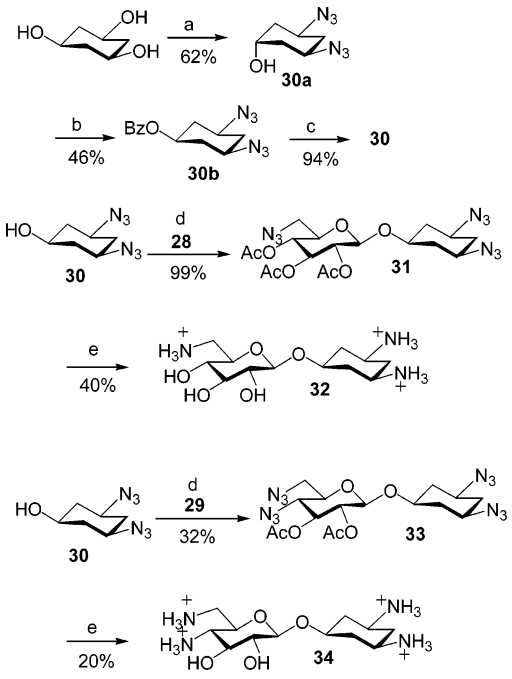
[a] Reagents: (a) Ac_2O, concentrated H_2SO_4; (b) (1) H_2NNH_2−HOAc, DMF, (2) CCl_3CN, DBU, CH_2Cl_2.

substitution generating azidosugar **10** with the intrinsic *cis*-1,2-hydroxylamino motif in the *galacto* configuration. Alternatively, compound **9** underwent the Swern oxidation/$NaBH_4$ reduction protocol to invert the C-4 hydroxyl group that allowed the synthesis of azidosugar **12** with the intrinsic *trans*-1,2-hydroxylamino motif in the *gluco* configuration. In the last path, compound **7** was treated with TsCl, followed by azide substitution to selectively place an azido group on the C-6 position. The free C-4 hydroxyl group in the equatorial position of compound **13** was converted to the axial position yielding compound **14**, which enabled the synthesis of azidosugar **15** with a novel intrinsic *gluco*-1,3-diamino binding motif.

We tried various methods[22−26] to selectively hydrolyze the anomeric methoxyl group into the hydroxyl group. In every attempt, low yield and complex mixtures were often encountered due to the concomitant deprotection of the benzyl groups. Therefore, we decided to convert the anomeric methoxyl group and all the benzyl groups into acetyl groups using Ac_2O with a catalytic amount of H_2SO_4 (Scheme 3). The resulting acetyl glycosides, however, failed to undergo direct glycosylation with various Lewis acids as catalysts or stoichiometric agents. As a result, we transformed the acetyl glucosides to the glycosyl trichloroacetimidate as the glycosyl donor. Treatment of the acetyl glucosides with hydrazine acetate resulted in the unprotected hydroxyl group at the anomeric position. Further treatment with trichloroacetonitrile and DBU as a base yielded the glycosyl trichloroacetimidate donor,[28] which could then be coupled to the acceptor of our choice. The constructed aminosugar

(22) Demuynck, M.; De Clercq, P.; Vandewalle, M. *J. Org. Chem*. **1979**, *44*, 4863.
(23) Hanessian, S.; Guindon, Y. *Tetrahedron Lett*. **1980**, *21*, 2305.
(24) Bhatt, M. V.; El-Morey, S. S. *Synthesis* **1982**, 1048.
(25) Ganem, B.; Small, V. R., Jr. *J. Org. Chem*. **1974**, *39*, 3728.
(26) Tsunoda, T.; Amaike, M.; Tambunan, U. S. F.; Fujise, T.; Ito, S.; Kodama, M. *Tetrahedron Lett*. **1987**, *28*, 2537.
(27) Wang, J.; Li, J.; Tuttle, D.; Takemoto, J.; Chang, C.-W. T. *Org. Lett*. **2002**, *4*, 3997−4000.
(28) Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett*. **2002**, *4*, 4603−4606.

SCHEME 4[a]



[a] Reagents: (a) (1) MsCl, pyr, (2) NaN_3, DMF; (b) BzOH, PPh_3, DEAD, THF; (c) NaOMe, MeOH; (d) TMSOTf, CH_2Cl_2, 4 Å MS; (e) (1) K_2CO_3, MeOH, (2) H_2, Pd/C.

library will undergo glycosylation in favor of the formation of the β-glycosidic bond due to the presence of an acetyl group at the O-2 position.

After completion of the synthesis of aminosugars with designed binding motifs, we carried out a model study of glycosylation (Scheme 4). One glycosyl acceptor, *cis*,*cis*-3,5-diazidocyclohexanol (**30**) with an intrinsic naturally occurring *cis*-1,3-diamino binding motif that can be found in the 2-deoxystreptamine of clinically used aminoglycoside antibiotics, such as neomycin, was selected for the model glycosylation and final syntheses. c*is*,*cis*-3,5-diazidocyclohexanol was synthesized from *cis*,*cis*-cyclohexanetriol via selective azido incorporation and Mitsunobu reaction. The glycosylations were carried out with the catalysis of TMSOTf or BF_3-OEt_2 (Scheme 4). The acetyl groups were hydrolyzed with K_2CO_3. Final hydrogenation with Pd/C and H_2 at atmospheric pressure reduced the azido groups into amino groups.

Library Construction of Pyranmycins. Previously, we have reported the synthesis of 26 pyranmycins (Scheme 5).[27−29] Pyranmycins contain a neamine core (rings I and II) and a variable ring III pyranose component attached at the O-5 of neamine via a β linkage. Herein, we wish to report the synthesis of five additional members (Figure 3) based on our structure−activity relationship (SAR) results, which reveals O-3″ could be the optimal position for more structural modifications. We wish to explore the effect of fluoro substitution at O-3″ and O-6″ positions on the activity of pyranmycins since fluorination is one of the common strategies of derivatizing aminoglycosides.[32]

The construction of the new pyranmycins begins with the synthesis of a common intermediate, compound **39**. Compound **39** was synthesized by the glycosylation of neamine derivative (Schemes 5 and 6) **35** and 3-*O*-allyl glycosyl donor **38**, which can be synthesized from **36** via a similar procedure as in our previous report.[29] The glycosylated precursor, **39**, was then directed into different synthetic routes yielding various final products (Scheme 7). Treatment of **39** with our final synthesis protocol provides **TC040**. Hydroboration of **39** gave **40**, which was converted to **TC041** with the final synthesis protocol. Alternatively, fluorination of the 3-hydroxylpropyl group with DAST offers **TC044** after completed deprotection. Glycosylation of **40** with the glycosyl donors **41**[29] generated **42**, which led to the synthesis of **TC045**.

The 6″-fluronated analogue was synthesized from methyl 6-fluoroglucopyranoside[30] with the same protocols (Scheme 8).

Antibacterial Activity of Pyranmycins. The previous synthesized and newly constructed pyranmycins were assayed against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus. subtilis*, and *Mycobacterium smegmatis* following the standard procedure.[31] The minimum inhibitory concentrations (MIC) were determined with use of neomycin, ribostamycin, neamine, or isoniazid (INH) as the controls. Previously, we have reported the SAR of pyranmycins against *E. coli*.[27−29] We observed a similar trend of activity among pyranmycins against *S. aureus*, *B. subtilis*, and *M. smegmatis*, which implies that the ribosomal target of pyranmycins is highly conserved and, likely, to be the 16S rRNA (Table 1 and Figure 4). For example, **TC005**, **TC006**, and **TC010** (entries 9, 10, and 14, respectively) are typically the most active members against all four strains of bacteria. Compounds from the model studies, **32** and **34**, are found to be inactive although they resemble the scaffold of rings II and III of pyranmycins. This result indicates the indispensable role of ring I in the neamine core-containing antibiotics. From our previous SAR studies, we learned that 6″-CH_3 group is crucial for the increased antibacterial activity. We speculated that the 6″-CH_3 group may contribute to an unusual hydrophobic interaction. Therefore, a 6″-fluornated analogue, **TC033**, was produced and evaluated with the expectation that fluoride may form better hydrophobic interaction than the hydroxyl group. However, we discovered that **TC033** (entry 31) is completely inactive against *E. coli*, and less active than **TC001**, the nonfluorinated counterpart, against *S. aureus*. We also noticed that, in the disk assay against *M. smegmatis*, pyranmycins, like streptomycin, are bacteriocidal in which the inhibition remained clear throughout the testing period while INH is bacteriostatic where significant bacterial grow back was observed after 2 days. Therefore, pyran-

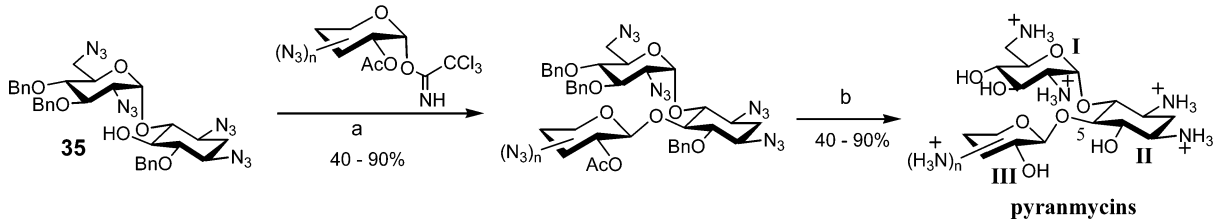
(29) Li, J.; Wang, J.; Hui, Y.; Chang, C.-W. T. *Org. Lett*. **2003**, *5*, 431−434.

(30) Card, P. J. *J. Org. Chem*. **1983**, *48*, 393−395.

(31) *Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria that Grow Aerobically*; Approved standard M7-A5, and Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved standard M2-A7, National Committee for Clinical Laboratory Standards: Wayne, PA.

(32) For reviewing: (a) Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother*. **1997**, *43*, 727−737. (b) Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother*. **2000**, *44*, 3249−3256. (c) Wright, G. D. *Curr. Opin. Microbiol*. **1999**, *2*, 499−503.

SCHEME 5[a]



[a] Reagents: (a) BF_3-OEt_2, CH_2Cl_2, 4 Å MS; (b) (1) K_2CO_3, MeOH, (2) PMe_3, THF, H_2O, (3) H_2, $Pd(OH)_2/C$.

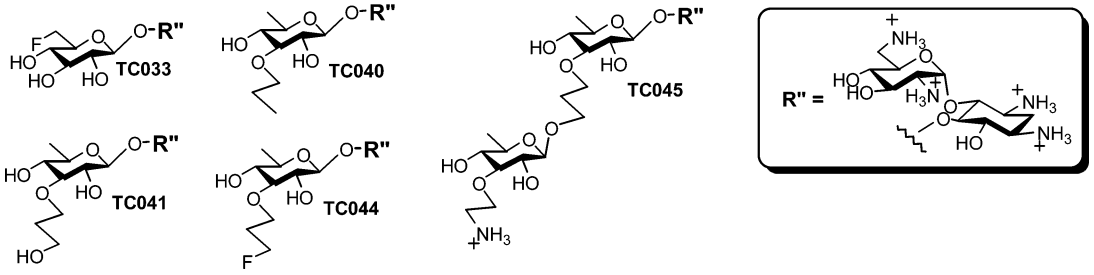
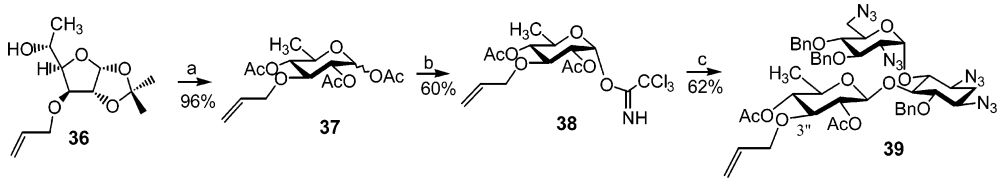


FIGURE 3. Newly synthesized pyranmycins.

SCHEME 6[a]



[a] Reagents: (a) (1) HOAc, TFA, H_2O; (2) Ac_2O, cat. H_2SO_4; (b) (1) H_2NNH_2-HOAc, (2) CCl_3CN, DBU, CH_2Cl_2; (c) neamine acceptor, BF_3-OEt_2, CH_2Cl_2, 4 Å MS.

mycins may provide new therapeutic options in the treatment against tuberculosis.

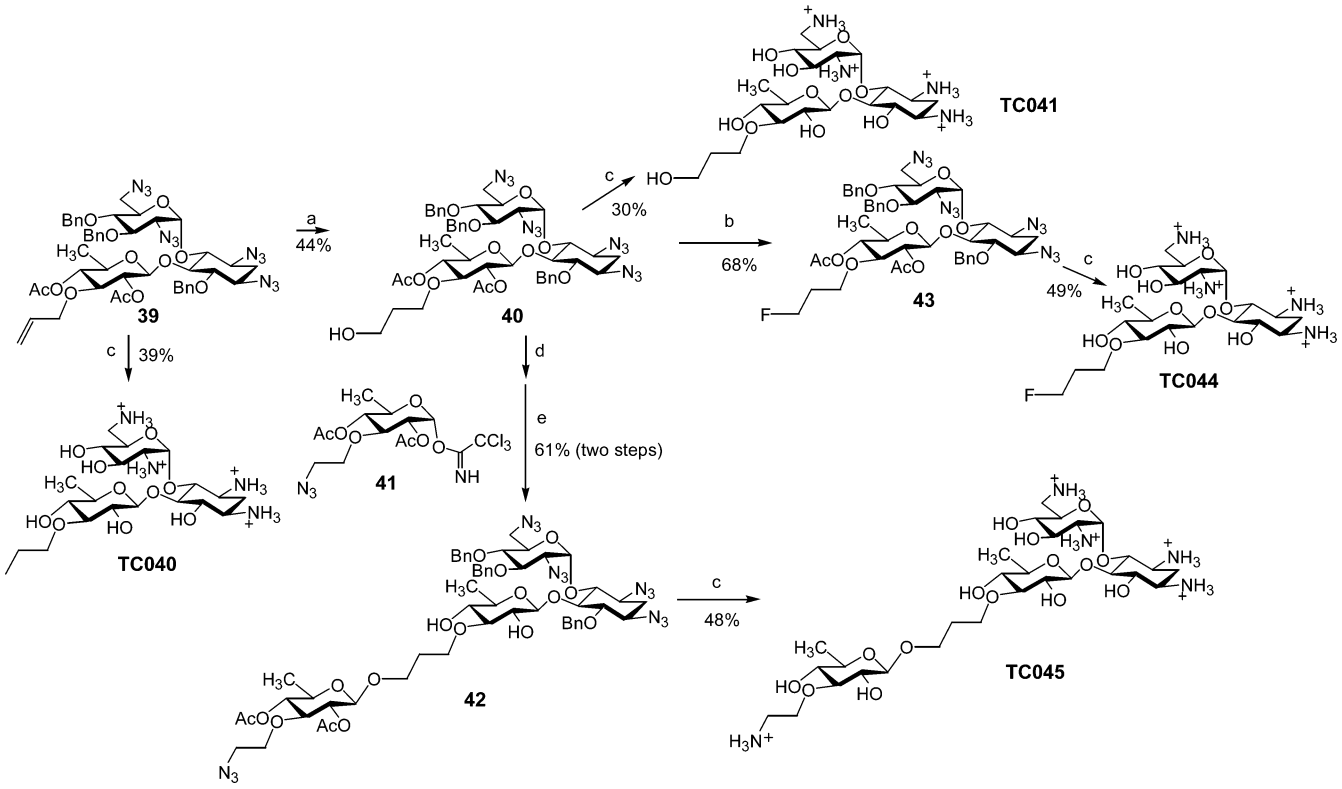
We have discovered that the O-3″ position is the only place that can tolerate chemical modification without losing significant antibacterial activity.[29] Therefore, we anticipated that the attachment of complex functionalities at the O-3″ position would result in new antibiotics that would be poor substrates for the aminoglycoside-modifying enzymes, and thereby avoid enzyme-catalyzed inactivation. However, the newly synthesized members with diverse modification at the O-3″ position are less active or inactive compared to the lead compound, **5**. It is clear to us that the O-3″ position can tolerate only small structural modifications. For example, even a small extension of a CH_2 unit (**TC026** vs **TC041**) results in the loss of activity.

The most prevalent mode of aminoglycoside resistance is enzyme-catalyzed modification. Over 50 different types of aminoglycoside-modifying enzymes, found in resistant bacteria, are known to be responsible for the inactivation of aminoglycosides (Figure 5).[32] These aminoglycoside-modifying enzymes, which are grouped into three classes including aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs), and aminoglycoside nucleotidyltransferases (ANTs), introduce chemical groups almost exclusively on the neamine core (rings I and II) of the aminoglycosides including neomycin and kanamycin.

We also examined the antibacterial activity of pyranmycins against resistant strains of bacteria, including *Escherichia coli* (TG1) containing pTZ19-3 plasmid coded with APH-3′ and *Escherichia coli* (TG1) containing pSF815 plasmid coded with AAC6′/APH2″. Pyranmycins showed no significant antibacterial activity as compared to amikacin and streptomycin. Therefore, only the diffusion assays were carried out (Table 2). However, pyranmycins (**TC005**, **TC006**, and **TC010**) manifest similar activity against *E. coli* (TG1) (AAC6′/APH2″) as neomycin (entries 5, 6, 7, and 8), and are also slightly more active than gentamicin and kanamycin (entries 2 and 3). The results suggest that the aminoglycoside with 4,5-disubstituted 2-deoxystreptamine (ring II) could be a better template for the modifications aiming to regain activity against resistant bacteria equipped with AAC6′ and APH2″ aminoglycoside-modifying enzymes than the aminoglycoside with 4,6-disubstituted 2-deoxystreptamine. In general, pyranmycins are prominent in serving as the lead structures for additional modification, which may pave the way to the development of a new library of antibiotics against drug-resistant bacteria.

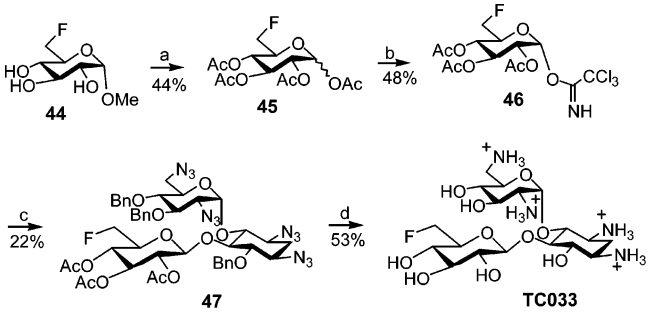
Anti-HIV-1 Tat and Rev Activity of Pyranmycins. Two HIV-1 regulatory proteins, Tat and Rev, mediate their functions in HIV-1 replication through binding to the viral TAR and RRE RNA stem−loop structures, respectively. Tat transactivates the 5′-long terminal repeat (LTR) promoter of the viral RNA genome, thereby increasing the level of viral mRNA transcription and

SCHEME 7[a]



[a] Reagents: (a) H_3B, THF then NaOH, H_2O_2; (b) DAST, CH_2Cl_2; (c) (1) K_2CO_3, MeOH, (2) PMe_3, THF, NaOH, (3) H_2, $Pd(OH)_2/C$; (d) K_2CO_3, MeOH; (e) BF_3-Et_2O, CH_2Cl_2.

SCHEME 8[a]



[a] Reagents: (a) Ac_2O, cat. H_2SO_4; (b) (1) H_2NNH_2-HOAc, (2) CCl_3CN, DBU, CH_2Cl_2, (c) **35**, BF_3-OEt_2, CH_2Cl_2, 4 Å MS; (d) (1) K_2CO_3, MeOH, (2) PMe_3, THF, NaOH, (3) H_2, $Pd(OH)_2/C$.

protein synthesis.[33] Rev protein prevents the splicing of viral mRNA and promotes the nuclear export of unspliced and incompletely spliced forms of the mRNA to the cytoplasm. Both proteins play essential roles in the proliferation of HIV-1, and the bindings of Tat and Rev proteins to viral RNA's are, therefore, targeted for the development of drugs against HIV-1. Several reports have explored or suggested the potential of using aminoglycosides as RNA binding surrogates for the inhibition of HIV-1 replication and potential therapeutic applications.[34,35] We therefore evaluated the library of pyranmycins as potential inhibitors of HIV-1 Tat and/or Rev function via cell-based assay. We observed several candidates that showed modest activity against HIV-1 Tat and/or Rev (Table 3). For example, **TC009** showed the highest activity against Rev among the pyranmycin members. Interestingly, **TC009** is almost inactive against both gram-positive and gram-negative bacteria. **TC016**, **TC018**, and **TC021** manifested the highest activities against Tat among the pyranmycin members. It should also be noted that with the exception of **TC045**, which appeared to be highly toxic at 100 μM in both assays, the pyranmycin members had minimal to no cytotoxicity in these tissue culture systems. Although we did not observe prominent results since the activities demonstrated required relatively high test concentrations of 50 to 100 μM (significant inhibitors would be expected to reduce the Tat or Rev function by >90% at concentrations of <1.0 μM), such a novel aminoglycoside library does present a unique model for screening of possible lead structures for future modifications.

Conclusion

We have prepared a library of novel aminosugars with natural and nonnatural binding motifs employing an efficient divergent synthetic strategy starting from a common starting material. We have demonstrated the feasibility of using such an aminosugar library for glycorandomization or glycodiversification research, leading to one of the prospective applications, the library

(33) For reviewing: Ptak, R. G. *Expert Opin. Invest. Drugs*, **2002**, *11*, 1099−1115.

(34) Baker, T. J.; Luedtke, N. W.; Tor, Y.; Goodman, M. *J. Org. Chem.* **2000**, *65*, 9054−9058.

(35) Luedtke, N. W.; Baker, T. J.; Goodman, M.; Tor, Y. *J. Am. Chem. Soc.* **2000**, *122*, 12035−12036.

TABLE 1. Minimum Inhibitory Concentration (μM) of Pyranmycins against Various Strains of Bacteria

entry	compd	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 25923)	*Bacillus subtilis*	*M. smegmatis*
1	Neomycin B	**2**	**0.3**		
2	Ribostamycin	**5**	**2**		
3	Neamine	36			
4	Isoniazid (INH)				**7**
5	**TC001**	42	12		51
6	**TC002**	16	4	3	**3**
7	**TC003**	19	8		**6**
8	**TC004**	25	>32		12
9	**TC005**	**9**	**3**	**2**	**6**
10	**TC006**	**9**	**3**		**2**
11	**TC007**	26	16	3	12
12	**TC008**	29	inactive		
13	**TC009**	inactive	>50	inactive	
14	**TC010**	**9**	**3**	**2**	**6**
15	**TC012**	20	8		24
16	**TC015**	inactive			51
17	**TC016**	28	11		**6**
18	**TC017**	45	18		
19	**TC018**	12	8		**3**
20	**TC019**	inactive			
21	**TC020**	19	13		52
22	**TC021**	inactive			
23	**TC022**	inactive	26		
24	**TC023**	38	inactive		
25	**TC024**	40	inactive		
26	**TC025**	inactive	inactive		
27	**TC026**	27	inactive		190
28	**TC028**	13	4		58
29	**TC031**	inactive	inactive		
30	**TC032**	39	8		
31	**TC033**	inactive	25		
32	**TC040**	>50			
33	**TC041**	inactive			
34	**TC044**	inactive			
35	**TC045**	inactive			
36	**32**	inactive	inactive		
37	**34**	inactive	inactive		

synthesis of pyranmycins. Pyranmycins have comparable antibacterial activity to neomycin. We believe this new addition of aminoglycoside can also lead to the development of antibiotics against drug-resistant bacteria. We are currently constructing another library of aminosugars that will favor the formation of the α-glycosidic bond. When combined with our current library of aminosugars, the newly constructed library will offer the opportunity for complete evaluation of the roles of carbohydrate components on molecules of biological importance.

Experimental Section

Methyl 4-Azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-galactopyranoside (4). To a solution of **3** (3.0 g, 6.4 mmol) and pyridine (0.83 mL, 10.3 mmol) in anhydrous CH_2Cl_2 at 0 °C was added Tf_2O (1.5 mL, 9.0 mmol) slowly. After being stirred for 30 min, the reaction mixture was diluted with CH_2Cl_2, washed with water, saturated $NaHCO_{3(aq)}$, and brine, and dried over $Na_2SO_{4(s)}$. The solution was filtered through Celite and transferred into a solution of excess NaN_3 and DMF. The reaction mixture was stirred overnight while the solvents were slowly evaporated with an aspirator. After completion of the reaction, the reaction mixture was filtered through Celite. The residue was washed with EtOAc. The combined solutions were concentrated and purified with gradient column chromatography (hexane:EtOAc = 90:10 to 40:60) providing the product as a light yellowish oil (2.70 g, 5.5 mmol, 86%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 4.82 (d, J = 11.9 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.72 (d, J = 11.5 Hz, 1H), 4.62 (d, J = 11.9 Hz, 1H), 4.564 (d, J = 3.6 Hz, 1H), 4.562 (d, J = 11.5 Hz, 1H), 4.49 (d, J = 11.9 Hz, 1H), 4.00 (dd, J = 9.0 Hz, J = 3.6 Hz, 1H), 3.99 (s, 1H), 3.92 (dd, J = 6.3 Hz, J = 6.6 Hz, 1H), 3.81 (dd, J = 9.0 Hz, J = 3.6 Hz, 1H), 3.57 (dd, J = 9.1 Hz, J = 6.6 Hz, 1H, H-6), 3.51 (dd, J = 9.1 Hz, J = 6.3 Hz, 1H), 3.33 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.3 (s), 138.2 (s), 137.8 (s), 128.54 (s), 128.48 (s), 128.2 (s), 127.96 (s), 127.89 (s), 127.8 (s), 98.8 (s), 77.8 (s), 76.2 (s), 73.9 (s), 73.7 (s), 73.3 (s), 69.0 (s), 67.1 (s), 61.5 (s), 55.5 (s); LRFAB *m/e* 507 $[M + NH_4]^+$; HRFAB calcd for $C_{28}H_{35}N_4O_5$ $[M + NH_4]^+$ *m/e* 507.2607, measured *m/e* 507.2611.

Methyl 2,3,6-Tri-*O*-benzyl-α-D-galactopyranoside (5). To a solution of $(COCl)_2$ (1.1 mL, 13.0 mmol) in 30 mL of anhydrous CH_2Cl_2 at −78 °C was added anhydrous DMSO (1.8 mL, 26.0 mmol) and the resulting solution was stirred for 15 min allowing the mixture to warm to −65 °C. To the reaction flask was added a solution of **3** (3.0 g, 6.5 mmol) in 20 mL of CH_2Cl_2. The reaction mixture was stirred for 0.5 h allowing the solution to warm to −45 °C. To this solution was added anhydrous DIPEA (9.1 mL, 52.0 mmol), and the reaction was allowed to warm to −10 to 0 °C in 0.5 h. After completion of the reaction, the reaction mixture was quenched with 1 N HCl and diluted with CH_2Cl_2. The organic layer was washed with pH 7 buffer (three times) and dried over Na_2SO_4. After removal of solvent, the crude product was obtained as a viscous oil. The crude product was dissolved in methanol (30 mL) and $NaBH_4$ (0.74 g, 19.5 mmol) was slowly added at 0 °C. The reaction was allowed to stir overnight. After the completion of the reaction, the reaction mixture was quenched with 1 N $HCl_{(aq)}$ then concentrated. The slurry crude product was dissolved in EtOAc and washed with water, saturated $NaHCO_{3(aq)}$, and brine, then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by gradient column chromatography (hexane:EtOAc = 90:10 to 40:60) afforded the product as a yellowish oil (2.4 g, 5.17 mmol, 80%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 4.81 (d, J = 11.9 Hz, 1H), 4.79 (d, J = 11.6 Hz, 1H), 4.69 (d, J = 11.6 Hz, 1H), 4.66 (d, J = 2.0 Hz, 1H), 4.64 (d, J = 11.9 Hz, 1H), 4.59 (d, J = 11.9 Hz, 1H), 4.52 (d, J = 11.9 Hz, 1H), 4.04 (s, 1H), 3.88 (dd, J = 5.3 Hz, J = 6.3 Hz, 1H), 3.85 (d, J = 2.0 Hz, 1H), 3.85 (s, 1H), 3.72 (dd, J = 9.9 Hz, J = 5.3 Hz, 1H), 3.65 (dd, J = 9.9 Hz, J = 6.3 Hz, 1H), 3.37 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.4 (s), 138.2 (s), 138.1 (s), 128.6 (s), 128.5 (s), 128.1 (s), 127.92 (s), 127.90 (s), 127.8 (s), 127.7 (s), 98.7 (s), 77.7 (s), 75.8 (s), 73.7 (s), 73.6 (s), 72.8 (s), 69.7 (s), 68.4 (s), 68.2 (s), 55.4 (s); LRFAB *m/e* 482 $[M + NH_4]^+$; HRFAB calcd for $C_{28}H_{36}N_1O_6$ $[M + NH_4]^+$ *m/e* 482.2543, measured *m/e* 482.2523.

Methyl 2,3-Di-*O*-benzyl-α-D-glucopyranoside (7). A solution of **2** (46 g, 0.10 mol) and TsOH-H_2O (ca. 2 g) in 500 mL of MeOH was stirred overnight. After completion of the reaction, the reaction was quenched by the addition of Et_3N (20 mL). The solvents were evaporated. The slurry crude product was dissolved in EtOAc and washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine, then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by gradient column chromatography (hexane:EtOAc = 65:35 to EtOAc:MeOH = 90:10) afforded the product as a yellowish oil, which solidify slowly at room temperature (37 g, 0.099 mol, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 10H), 5.04 (d, J = 11.7 Hz, 1H), 4.78 (d, J = 12.1 Hz, 1H), 4.70 (d, J = 12.1 Hz, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.60 (d, J = 3.7 Hz, 1H, 1-H), 3.79 (dd, J = 9.5 Hz, J = 8.8 Hz, 1H, 3-H), 3.7−3.8 (m, 2H), 3.62 (ddd, J = 4.0 Hz, J = 4.0 Hz, J = 9.9 Hz, 1H), 3.50 (dd, J = 9.5 Hz, J = 3.7 Hz, 1H, 2-H), 3.5−3.6 (m, 1H), 3.38 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.6 (s), 137.9 (s), 128.7 (s), 128.5 (s), 128.1 (s), 128.0 (s), 127.9 (s), 98.2 (s), 81.2 (s), 79.8 (s), 75.4 (s), 73.1 (s), 70.6 (s), 70.4 (s), 62.5 (s), 55.3 (s); LRCI *m/e* 392.4 $[M + NH_4]^+$; HRCI calcd for $C_{21}H_{30}NO_6$ $[M + NH_4]^+$ *m/e* 392.2073, measured *m/e* 392.2068.

Methyl 2,3-Di-*O*-benzyl-6-deoxy-α-D-glucopyranoside (9). To a solution of **7** (12.0 g, 32.0 mmol) in anhydrous

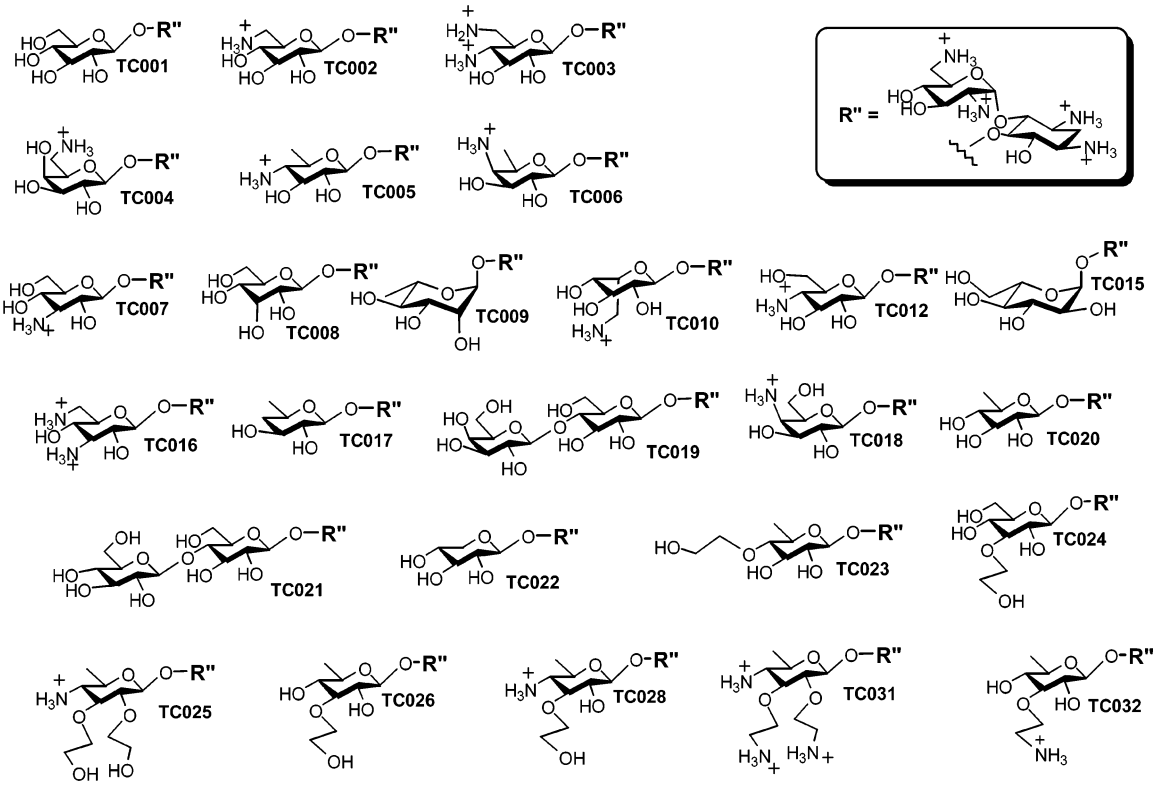


FIGURE 4. Structures of previously reported pyranmycins.

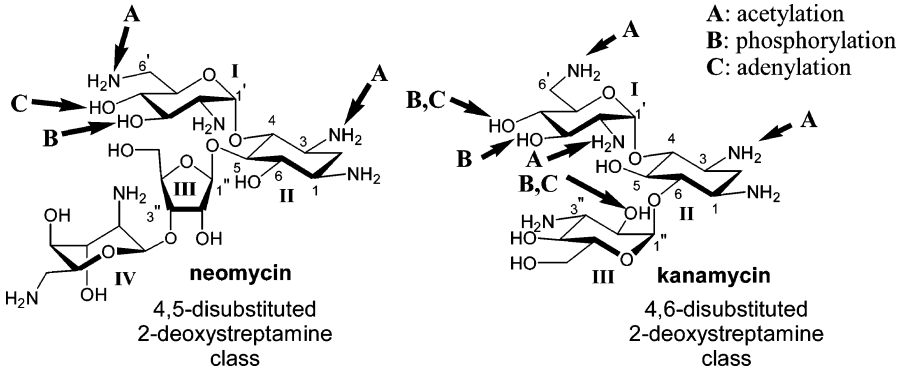


FIGURE 5. Modifications from resistance bacteria against aminoglycoside antibiotics.

TABLE 2. Zone of Inhibition (mm) against Drug-Resistant Strains

entry	compd	*E. coli* (TG1)	*E. coli* (TG1) (APH(3′)-I)	*E. coli* (TG1) (AAC6′/APH2″)
1	Amikacin	20	21	20
2	Gentamicin	20	18	12
3	Kanamycin	19	inactive	9
4	Streptomycin	22	18	24
5	Neomycin B	15	inactive	16
6	**TC005**	18	inactive	15
7	**TC006**	16	inactive	14
8	**TC010**	14	inactive	14
9	**TC028**	17	inactive	12

pyridine (30 mL) was added TsCl (7.34 g, 38.5 mmol) slowly at 0 °C. The reaction mixture was stirred overnight allowing the reaction to warm to room temperature. After the completion of the reaction, the reaction mixture was diluted with EtOAc. The combined organic layers were washed with 1 N $HCl_{(aq)}$ 3 times, saturated $NaHCO_{3(aq)}$, and brine, then dried over $Na_2SO_{4(s)}$. After removal of solvent, the tosylated crude product was dissolved in THF (30 mL) and $LiAlH_4$ (3.60 g, 96.0 mmol) was added. The reaction was stirred at room temperature overnight then refluxed for 2 h. After completion of the reaction, the reaction mixture was quenched by slow addition to ice then filtered through Celite. The filtered cake was washed with EtOAc, and the resulting cloudy yellowish solution was filtered through Celite. The combined organic solutions were washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine and dried over $Na_2SO_{4(s)}$. After removal of the solvent followed by purification with gradient column chromatography (hexane:EtOAc = 90:10 to 40:60), the product was obtained as a white powder (7.89 g, 22.0 mmol, 69%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 10H), 5.02 (d, J = 11.6 Hz, 1H), 4.75 (d, J = 11.9 Hz, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.65 (d, J = 11.9 Hz, 1H), 4.54 (d, J = 3.3 Hz, 1H), 3.71 (dd, J = 9.6 Hz, J = 9.0 Hz, 1H), 3.64 (dq, J = 9.2 Hz, J = 6.3 Hz, 1H), 3.50 (dd, J = 9.6 Hz, J = 3.3 Hz, 1H), 3.36 (s, 3H), 3.14 (dd, J = 9.2 Hz, J = 9.0 Hz, 1H), 2.12 (broad, 1H, 4-OH), 1.22 (d, J = 6.3 Hz, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.8 (s), 138.1 (s), 128.6 (s), 128.2 (s), 128.1 (s), 128.04 (s), 128.00 (s), 98.0 (s), 81.3 (s), 80.1 (s), 75.4 (s, 2 carbons), 73.1 (s), 66.9 (s),

TABLE 3. Anti-Rev and Anti-Tat Activities of Pyranmycins

			HIV-1 Rev assay		HIV-1 Tat assay	
entry	compd	test concn	% inhibition of Rev function[a]	% reduction in cell viability[b]	% inhibition of Tat function[a]	% reduction in cell viability[b]
1	**TC001**	50 μM	0.0	0.0	10.3	0.0
2	**TC002**	50 μM	0.0	0.0	36.6	0.0
3	**TC003**	50 μM	0.0	0.0	20.4	0.0
4	**TC004**	50 μM	0.0	0.0	15.2	0.0
5	**TC005**	50 μM	0.0	0.0	46.5	0.0
6	**TC006**	50 μM	0.0	0.0	17.1	0.0
7	**TC007**	50 μM	8.9	0.0	28.7	0.0
8	**TC009**	50 μM	49.6	12.3	14.1	0.0
9	**TC010**	50 μM	15.8	10.9	42.4	4.6
10	**TC012**	50 μM	21.9	16.1	4.9	11.6
11	**TC015**	50 μM	3.0	6.8	25.4	9.9
12	**TC016**	50 μM	16.6	13.2	71.1	13.2
13	**TC017**	50 μM	24.3	11.6	49.9	11.0
14	**TC018**	50 μM	38.4	18.4	70.4	13.6
15	**TC019**	50 μM	38.1	11.6	46.3	5.4
16	**TC021**	50 μM	14.7	0.0	65.2	0.0
17	**TC022**	50 μM	0.0	6.0	7.7	0.0
18	**TC038**	100 μM	29.4	22.5	20.8	21.0
19	**TC040**	100 μM	30.9	20.5	12.7	11.6
20	**TC041**	100 μM	21.9	14.4	20.4	21.4
21	**TC044**	100 μM	28.6	1.8	50.9	20.6
22	**TC045**	100 μM	100.0	99.9	100.0	100.0
23	**Leptomycin B**[c]	100 nM	100.0	60.6	98.3	73.8
24	**Ro24−7429**[d]	1 μM	53.4	35.5	100.0	42.8

[a] Renilla Luciferase analysis. [b] Firefly Luciferase analysis. [c] Reported inhibitor of the Rev function. [d] Reported inhibitor of the Tat function.

55.2 (s), 17.7 (s); LRFAB *m/e* 376 $[M + NH_4]^+$; HRFAB calcd for $C_{21}H_{30}N_1O_5$ $[M + NH_4]^+$ *m/e* 376.2124, measured *m/e* 376.2119.

Methyl 6-Azido-2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranoside (13). To a solution of **7** (6.01 g, 16.1 mmol) in anhydrous pyridine (30 mL) was added TsCl (4.06 g, 21.3 mmol) slowly at 0 °C. The reaction mixture was stirred overnight allowing the reaction to warm to room temperature. After the completion of the reaction, the reaction mixture was diluted with EtOAc. The combined organic layers were washed with 1 N $HCl_{(aq)}$ (3 times), water, saturated $NaHCO_{3(aq)}$, and brine, then dried over $Na_2SO_{4(s)}$. After removal of solvent, the tosylated crude product was dissolved in DMF (200 mL) and excess NaN_3 (3−5 equiv) was added. The reaction solution was stirred at 80 °C overnight. After removal of DMF, EtOAc was added, and the resulting cloudy yellowish solution was filtered through Celite. Removal of the solvent followed by gradient column chromatography (hexane:EtOAc = 90:10 to 50:50) afforded the product as a pale yellowish oil (6.41 g, 16.1 mmol, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 10H), 5.30 (d, J = 11.6 Hz, 1H), 4.76 (d, J = 11.9 Hz, 1H), 4.66 (d, J = 11.6 Hz, 1H), 4.65 (d, J = 11.9 Hz, 1H), 4.63 (d, J = 3.3 Hz, 1H), 3.74 (dd, J = 9.2 Hz, J = 9.0 Hz, 1H), 3.71 (m, 1H), 3.52 (dd, J = 9.6 Hz, J = 3.6 Hz, 1H), 3.4−3.5 (m, 2H), 3.40 (s, 3H), 3.3−3.4 (m, 1H), 2.18 (broad, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.6 (s), 137.9 (s), 128.8 (s), 128.6 (s), 128.2 (s), 128.1 (s), 98.1 (s), 81.1 (s), 79.8 (s), 75.5 (s), 73.2 (s), 70.7 (s), 70.3 (s), 55.5 (s), 51.6 (s); LRFAB *m/e* 417 $[M + NH_4]^+$; HRFAB calcd for $C_{21}H_{29}N_4O_5$ $[M + NH_4]^+$ *m/e* 417.2138, measured *m/e* 417.2140.

General Procedure for the Preparation of Acetyl Glycosides. To a solution of methyl glycoside in Ac_2O at 0 °C was added concentrated H_2SO_4 (catalytic amount) slowly. More concentrated H_2SO_4 can be added if the reaction does not go to completion in 5−6 h. After completion of the reaction, the reaction mixture was poured into a solution of saturated $NaHCO_{3(aq)}$ and EtOAc. After the mixture was stirred overnight, the organic layer was separated, washed with water, saturated $NaHCO_{3(aq)}$, and brine, and dried over $Na_2SO_{4(s)}$. After removal of the solvent followed by purification with gradient column chromatography (hexane:EtOAc = 90:10 to 40:60), the product was obtained as a yellowish oil containing α-anomer as the major product.

General Procedure for the Preparation of Glycosyl Trichloroacetimidate. To a solution of starting material in DMF was added hydrazine acetate (1.2 equiv). The solution was stirred at room temperature until the complete consumption of the starting material (4−5 h). The reaction mixture was filtered through a short column packed with a layer of silica gel on top of a layer of Celite. The column was eluted thoroughly with EtOAc. After removal of the solvents, the crude product can be used directly for the next step, or can be further purified with column chromatography providing a light yellowish oil. We do, however, recommend a purification of the crude product with flash column chromatography. To a solution of glycosyl hydroxide and trichloroacetonitrile (12 equiv) in anhydrous CH_2Cl_2 was added a catalytic amount of DBU dropwise. The solution was stirred at room temperature until the complete consumption of the starting material. The reaction mixture was added with charcoal and filtered through a short column packed with a layer of silica gel on top of a layer of Celite. The column was eluted thoroughly with ether. After removal of the solvents, the crude product was purified with column chromatography providing glycosyl trichloroacetimidate as a light yellowish oil. The glycosyl trichloroacetimidate may undergo slow decomposition at room temperature. Therefore, these compounds have been characterized only by ^{1}H and ^{13}C NMR.

***trans,trans*-3,5-Diazidocyclohexanol (30a).** To a solution of *cis,cis*-1,3,5-cyclohexanetriol (3.50 g, 0.0265 mol) in pyridine (15 mL) at 0 °C was added MsCl (6.38 g, 4.3 mL, 0.0557 mol). The reaction mixture was stirred overnight allowing the solution to warm to room temperature. After completion of the reaction, EtOAc was added, and the organic layer was washed with 1 N HCl, water, saturated Na_2CO_3, and brine then dried over Na_2SO_4. After removal of solvent, the crude mesylated compound was added with DMF (25 mL) and NaN_3 (7 g, 0.108 mol). The reaction mixture was stirred at 70 °C overnight. After removal of most of the DMF, the reaction mixture was diluted with EtOAc and filtered through Celite. After purification with gradient column chromatography (hexane:EtOAc = 90:10 to 40:60), the product was obtained as a volatile pale

yellowish oil (3 g, 0.0165 mol, 62%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.30 (t, J = 3.0 Hz, 1H), 3.74 (tt, J = 11.6 Hz, J = 3.9 Hz, 2H), 2.29 (dm, J = 14.2 Hz, $1H_{eq}$), 2.01 (dm, J = 14.2 Hz), 1.46 (td, J = 11.6 Hz, J = 3.0 Hz), 1.32 (q, J = 11.9 Hz); ^{13}C NMR (68 MHz, $CDCl_3$) δ 65.6 (s), 54.2 (s), 37.5 (s), 36.9 (s); LREI (compound degraded quickly in mass spec) *m/e* 155 ([MH $-$ N_2]$^+$, 10), 137 ([M $-$ OH $-$ N_2]$^+$, 10), 127 ([M $-$ H $-$ N_2 $-$ N_2]$^+$, 15), 112 ([M $-$ N_3 $-$ N_2]$^+$, 80).

***cis,cis*-3,5-Diazidocyclohexyl Benzoate (30b).** To a solution of **30a** (1 g, 0.0055 mol) in anhydrous THF (8 mL) at $-$50 °C were added $PhCO_2H$ (1.1 g 0.0088 mol), Ph_3P (2.30 g, 0.0088 mol), and DEAD (1.40 mL, 1.531 g, 0.0088 mol). The reaction mixture was allowed to warm to room temperature. After completion of the reaction, Et_2O was added, and the organic layer was washed with 1 N HCl, water, saturated Na_2CO_3, and brine then dried over Na_2SO_4. After purification with gradient column chromatography (hexane:EtOAc = 95:5 to 60:40), the product was obtained as a pale yellowish solid (0.72 g, 0.00252 mol, 46%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.4$-$8.1 (m, 5H), 5.01 (tt, J = 11.6 Hz, J = 4.4 Hz), 3.48 (tt, J = 12 Hz, J = 4.2 Hz), 2.49 (dm, J = 10.4 Hz), 2.37 (dm, J = 12 Hz), 1.56 (q, J = 12.4 Hz), 1.43 (q, J = 12.4 Hz); ^{13}C NMR (68 MHz, $CDCl_3$) δ 165.1 (s), 132.8 (s), 129.7 (s), 127.9 (s), 67.8 (s), 54.4 (s), 36.1 (s), 35.9 (s); LREI (compound degraded quickly in mass spec) *m/e* 137 ([M $-$ OBz $-$ N_2]$^+$, 5), 109 ([M $-$ OBz $-$ N_3 $-$ N_2]$^+$, 55).

***cis,cis*-3,5-Diazidocyclohexanol (30).** To a solution of **30b** (0.1 g, 0.35 mmol) in anhydrous methanol (2 mL) was added NaOMe/MeOH (0.1 mL, 0.5 M) slowly. After the solution was stirred for 4 h, Amberlite IR-120 (plus) was added, and the reaction mixture was filtered through glass wool. After removal of solvent and purification by gradient column chromatography (hexane:EtOAc = 90:10 to 40:60), the product was obtained as a volatile pale yellowish oil (0.06 g, 0.33 mmol, 94%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.72 (tt, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.35 (tt, J = 12.0 Hz, J = 4.0 Hz, 2H), 2.30 (m), 1.32 (m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 66.2 (s), 55.2 (s), 39.8 (s), 36.4 (s); LREI (compound degraded quickly in mass spec) *m/e* 155 ([MH $-$ N_2]$^+$, 10), 137 ([M $-$ OH $-$ N_2]$^+$, 10), 127 ([M $-$ H $-$ N_2 $-$ N_2]$^+$, 75), 112 ([M $-$ N_3 $-$ N_2]$^+$, 50).

***cis,cis*-3,5-Diaminocyclohexyl β-D-6-Amino-6-deoxyglucopyranoside (32).** A solution of **31** and K_2CO_3 (4$-$5 equiv) was stirred in MeOH (5 mL) at room temperature until the complete consumption of starting material (ca. 5 h). The solvent was removed, and the reaction mixture was diluted with EtOAc and filtered through a short column packed with TLC silica gel and Celite. After removal of solvents, the crude azido compound was added with a catalytic amount of Pd/C (10%) and 5 mL of degassed HOAc/H_2O (1/1). After being further degassed, the reaction mixture was stirred at room temperature under atmospheric H_2 pressure. After being stirred for 1 day, the reaction mixture was filtered through Celite. The residue was washed with water, and the combined solutions were concentrated affording product as an acetate salt. The final product with Cl^- salt can be prepared with an ion-exchange column packed with Dowex 1 $\times$ 8-200 (Cl^- form) and eluting with water. After the desired fractions were collected and the solven removed, the final products were subjected to bioassay directly. ^{1}H NMR (270 MHz, D_2O) (acetate salt) δ 4.63 (d, J = 8.0 Hz, 1H, H-1′), 4.00 (tt, J =12.0 Hz, J = 4.4 Hz, 1H), 3.68 (dd, J = 10.5 Hz, J = 3.2 Hz, 1H), 3.64 (dd, J = 10.5 Hz, J = 3.6 Hz, 1H), 3.50 (t, J = 9.5 Hz, 1H), 3.37 (t, J = 9.5 Hz, 1H), 3.25 (dd, J = 9.5 Hz, J = 8.0 Hz, 1H), 3.10$-$3.50 (m, 3H), 2.00$-$2.60 (m, 3H), 1.90 (s, 9H). 1.50 (q, J = 12.0 Hz, 2H), 1.40$-$1.60 (m, 1H); ^{13}C NMR (68 MHz, D_2O) δ 100.4 (s), 74.7 (s), 72.4 (s), 72.2 (s), 71.3 (s), 70.6 (s), 44.8 (s), 44.7 (s), 39.9 (s), 35.3 (s), 34.0 (s), 32.5 (s); LRFAB *m/e* 292 ([M^{3+} $-$ 2H$^+$]$^+$); HRFAB calcd for $C_{12}H_{26}N_3O_5$ ([M^{3+} $-$ 2H$^+$]$^+$) *m/e* 292.1872, measured *m/e* 292.1882.

Acetyl 2,4-Di-*O*-acetyl-3-*O*-allyl-6-deoxy-D-glucopyranoside (37). A solution of **36** (2.48 g, 10.2 mmol) in a 100 mL aqueous mixture of 1% TFA and 80% HOAc was stirred at 70 °C in a water bath for 1 h. The acids were removed by addition of 100 mL of H_2O, followed by coevaporation at 100 °C with a rotovap twice. After being further pump-dried under vacuum, the crude product was added to Ac_2O (40 mL) and 2 drops of concentrated H_2SO_4 at 0 °C. Upon completion of the reaction (monitored by TLC, hexane:EtOAc = 1:1), the reaction mixture was poured into a solution of saturated $NaHCO_{3(aq)}$ and EtOAc. After the solution was stirred for 1 D, the organic layer was washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine and dried over $Na_2SO_{4(s)}$. After removal of the solvent followed by purification with gradient column chromatography (hexane:EtOAc = 90:10 to 30:70), the product was obtained as a white powder (3.22 g, 9.75 mmol, 96%). ^{1}H NMR (270 MHz, $CDCl_3$) (mixture of α and β anomers) δ 6.22 (d, J = 4.0 Hz, 1H), 5.7$-$5.9 (m, 2H), 5.60 (d, J = 8.3 Hz, 1H), 5.1$-$5.2 (m, 4H), 5.07 (dd, J = 9.6 Hz, J = 8.3 Hz, 1H), 4.98 (dd, J = 9.9 Hz, J = 4.0 Hz, 1H), 4.83 (dd, J = 9.9 Hz, J = 9.6 Hz, 1H), 4.82 (dd, J = 9.9 Hz, J = 9.6 Hz, 1H), 4.0$-$4.1 (m, 3H), 3.89 (m, 1H), 3.77 (dd, J = 9.9 Hz, J = 9.6 Hz, 1H), 3.57 (dd, J = 9.6 Hz, J = 9.6 Hz, 1H), 3.5$-$3.6 (m, 2H), 2.13 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.1$-$1.3 (m, 6H); ^{13}C NMR (68 MHz, $CDCl_3$) (mixture of α and β anomers) δ 169.7 (s), 169.6 (s), 169.5 (s), 169.2 (s), 169.1 (s), 134.6 (s), 134.3 (s), 117.1 (s), 116.7 (s), 92.1 (s), 89.6 (s), 79.8 (s), 77.1 (s), 74.4 (s), 74.1 (s), 73.5 (s), 73.0 (s), 71.9 (s), 71.8 (s), 71.3 (s), 68.3 (s), 21.0 (s), 20.9 (s), 20.8 (s), 17.44 (s), 17.38 (s); LRFAB *m/e* 353 [M + Na]$^+$; HRFAB calcd for $C_{16}H_{26}O_8Na$ [M + Na]$^+$ *m/e* 353.1212, measured *m/e* 353.1198.

General Procedure for Glycosylation. A solution of glycosyl trichloroacetimidate, neamine acceptor, **35** (1.2 equiv) or *cis,cis*-3,5-diazidocyclohexanol (1.2 equiv), and activated powder 4Å molecular sieve was stirred in anhydrous CH_2Cl_2 (ca. 5 mL) at room temperature for 1 h then cooled to $-$50 °C. To this cloudy solution was added BF_3-OEt_2 or TMSOTf (0.3 equiv). The solution was stirred at low temperature until the complete consumption of the glycosyl trichloroacetimidate (ca. 40 min). The reaction mixture was quenched by the addition of $NaHCO_3$ powder. After being stirred for 15 min, the reaction mixture was filtered through Celite. The residue was washed thoroughly with CH_2Cl_2 and ethyl acetate. After removal of the solvents, the crude product was purified with column chromatography.

5-*O*-(2,4-Di-*O*-acetyl-3-*O*-(3-hydroxypropyl)-6-deoxy-β-D-glucopyranosyl)-6,3′4′-tri-*O*-benzyl-1,3,2′6′-tetraazidoneamine (40). To a solution of **39** (0.10 g, 0.10 mmol) in anhydrous THF (5 mL) was added borane/THF complex (1.0 M in THF; 0.10 mL, 0.10 mmol) at room temperature. The reaction bottle was stirred for 3 h and monitored with TLC (eluted with EtOAc:hexane = 50:50). After completion of the reaction, several drops of H_2O were added to destroy excessive borane followed by the addition of 3 N NaOH (5 mL) and H_2O_2 (30%) solution (5 mL). The solution was stirred for several minutes until no bubble was produced. The resulting solution was diluted with EtOAc and the organic layer was washed by brine solution and dried over Na_2SO_4. After removal of the solvents and purification by column chromatography, the product was afforded as a colorless oil (0.073 g, 0.071 mmol, 69%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2$-$7.4 (m, 15H), 5.69 (d, J = 3.6 Hz, 1H), 3.8$-$5.0 (m, 7H), 4.70 (d, J = 9.9 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 4.2$-$4.3 (m, 1H), 4.0$-$4.1 (m, 3H), 3.64 (dd, J = 5.6 Hz, J = 5.6 Hz, 4H), 3.2$-$3.6 (m, 9H), 2.28 (m, 1H), 2.15 (s, 3H), 2.08 (s, 3H), 1.4$-$1.7 (m, 3H), 1.20 (d, J = 6.3 Hz, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 169.9 (s), 169.2 (s), 137.9 (s, 2 carbons), 137.2 (s), 128.9 (s), 128.5 (s), 128.4 (s), 128.2 (s), 127.9 (s), 99.0 (s), 97.6 (s), 85.3 (s), 80.5 (s), 79.5 (s), 78.7 (s), 77.3 (s), 75.6 (s, 2C), 75.3 (s), 75.0 (s), 73.9 (s), 72.5 (s), 71.1 (s), 70.5 (s), 68.5 (s), 63.0 (s), 60.7(s), 60.4 (s), 59.5 (s), 51.2 (s), 32.6 (s), 29.8 (s), 21.2 (s), 21.0 (s), 16.9 (s); MALDI calcd for $C_{46}H_{56}N_{12}O_{13}K$ [M + K]$^+$ *m/e* 1023.3721, measured *m/e* 1023.3777.

5-*O*-(2,4-Di-*O*-acetyl-3-*O*-(3-fluoropropyl)-6-deoxy-β-D-glucopyranosyl)-6,3′4′-tri-*O*-benzyl-1,3,2′6′-tetraazido-

neamine (43). To a solution of **40** (0.34 g, 0.35 mmol) in anhydrous CH_2Cl_2 in a PE vial was added DAST (0.10 g, 0.62 mmol) at −30 °C. The reaction mixture was stirred for 3 h allowing the temperature to rise to −10 °C. After completion of the reaction (monitored by TLC, eluted with EtOAc:hexane = 50:50), the reaction was quenched by addition of 5 mL of MeOH. After removal of solvents and purification with column chromatography, the product was afforded as a light yellowish oil (0.23 g, 0.23 mmol, 68%). ^{1}H NMR (270 M Hz, $CDCl_3$) δ 7.2−7.6 (m, 15H), 5.70 (d, J = 4.0 Hz, 1H), 5.03 (d, J = 7.9 Hz, 1H), 4.97(d, J = 9.2 Hz, 1H), 4.8−4.9 (m, 5H), 4.70 (d, J = 9.9 Hz, 1H), 4.59 (d, J = 11.6 Hz, 1H), 4.53 (t, J = 5.6 Hz, 1H), 4.35 (t, J = 5.6 Hz, 1H), 4.1−4.2 (m, 1H), 4.07 (dd, J = 9.2 Hz, J = 8.9 Hz, 1H), 4.02 (dd, J = 10.2 Hz, J = 8.9 Hz, 1H), 3.1−3.6 (m, 12H), 2.30 (ddd, J = 13.2 Hz, J = 4.5 Hz, J = 4.5 Hz, 1H), 2.13 (s, 3H), 2.06 (s, 3H), 1.7−1.9 (m, 2H), 1.46 (ddd, J = 13.2 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H), 1.19 (d, J = 6.3 Hz, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 169.6 (s), 169.0 (s), 137.9 (s, 2C), 137.3 (s), 128.7 (s), 128.5 (s), 128.4 (s), 128.2 (s), 127.9 (s), 99.0 (s), 97.6 (s), 85.3 (s), 80.5 (d, J_{CF} = 163.5 Hz), 80.7 (s), 79.5 (s), 78.7 (s), 77.5 (s), 75.6 (s, 2 carbons), 75.3 (s), 75.0 (s), 74.6 (s), 73.1 (s), 71.1 (s), 70.6 (s), 67.4 (d, J_{CF} = 4.7 Hz), 63.0 (s), 60.7 (s), 59.5 (s), 51.2 (s), 32.6 (s), 31.1 (d, J_{CF} = 19.7 Hz), 21.1 (s, 2 carbons), 16.9 (s); MALDI calcd for $C_{46}H_{55}FN_{12}O_{12}$ $[M + Na]^+$ *m/e* 1009.3939,; measured *m/e* 1009.3882.

General Procedure for the Final Synthesis. A solution of starting material and K_2CO_3 (4−5 equiv) was stirred in MeOH (5 mL) at room temperature until the complete consumption of starting material (ca. 5 h). The solvent was removed, and the reaction mixture was diluted with EtOAc and filtered through a short column packed with a layer of silica gel on top of a layer of Celite. The column was eluted with EtOAc/MeOH (1/1 solution). Removal of solvents afforded crude product mixed with some K_2CO_3 solid. The crude product was added with THF (6 mL) and filtered through glasswool into the reaction flask for the next step. To the perbenzylated azidoaminoglycoside/THF solution in a reaction flask (or vial) equipped with a reflux condenser were added 0.1 M $NaOH_{(aq)}$ (0.5 mL) and PMe_3 (1 M in THF, 4−5 equiv). The reaction mixture was stirred at 50 °C for 2 h. The product has a R_f of 0 when eluted with EtOAc/MeOH (9/1) solution and a R_f of 0.9 when eluted with *i*PrOH/1 M NH_4OAc (2/1) solution. After completion of the reaction, the reaction mixture was cooled to room temperature and loaded to a short column (5 cm in height) packed with a layer of silica gel on top of a layer of Celite. The column was eluted with a series of solutions as follows: THF, THF/MeOH, MeOH, and MeOH/concentrated NH_4OH (from 0 to 28% of concentrated NH_4OH). The fractions containing the desired product were analyzed by TLC and collected. After removal of solvents, the crude perbenzylated aminoglycoside was added with a catalytic amount of $Pd(OH)_2/C$ (20% Degussa type) and 5 mL of degassed $HOAc/H_2O$ (1/1). After being further degassed, the reaction mixture was stirred at room temperature under atmospheric H_2 pressure. After being stirred for 1 day, the reaction mixture was filtered through Celite. The residue was washed with water, and the combined solutions were concentrated affording pure final product as an acetate salt. Most of the reported final products are characterized by ^{1}H and ^{13}C NMR at this stage. The product has a R_f of 0 when eluted with *i*PrOH/1 M NH_4OAc (2/1) solution and a R_f of 0.1−0.2 when eluted with concentrated NH_4OH/MeOH (2/5) solution. The final product with Cl^- salt can be prepared with an ion-exchange column packed with Dowex 1 × 8-200 (Cl^- form) and eluting with water. After the desired fractions were collected and the solvent removed, the final products were subjected to bioassay directly.

Procedure for Disk Assay of Antimicrobial Activity. *E. coli* ATCC 25922 was inoculated into Trypticase Soy Broth, and the resulting solution was incubated at 37 °C until it achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity of inoculated medium was adjusted to be the same as that of the 0.5 McFarland standard. This results in a suspension containing approximately 1×10^8 to 2×10^8 CFU/mL for *E coli* ATCC 25922, which was transferred into the Mueller−Hinton agar plate by swab. A disk containing 20 μL (1 mg/mL) of the pyranomycin solution was placed onto the plate. The plate was incubated at 37 °C for 16 h. The diameter of the zone of inhibition was measured, to the nearest whole millimeter.

Procedure for MIC Determination. *E. coli* ATCC 25922 was inoculated into Trypticase Soy Broth, and the resulting solution was incubated at 37 °C until it achieved or exceeded the turbidity of the 0.5 McFarland standard. The turbidity of inoculated medium was adjusted to an absorbance of 0.08 to 0.10 at 625 nm. The adjusted inoculated medium was diluted by 10-fold, then 5 μL of the inoculated medium was mixed with 45 μL of broth in the designated wells of a 96-well plate. A series of varying concentrations (2-fold dilution) of the tested compounds (50 μL) were added to the designated wells. The growth control was prepared by loading 50 μL of 0.9% brine onto the well inoculated with bacteria. The blank control was prepared by loading 0.1 mL of 0.9% brine. The plate was incubated at 37 °C for 16 h. The MIC is determined by the compound concentration that renders at least 90% inhibition of the growth of bacteria. The MIC results are repeated in duplicate.

Cell-Based Assays for HIV-1 Tat and Rev. Inhibition of the HIV-1 Rev function was determined by using the pDM128 Rev reporter plasmid previously described by Hope et al.[36] The pDM128 plasmid was modified by replacing the chloramphenicol acetyltransferase coding sequence with that of Renilla Luciferase. HeLa cells engineered to express HIV-1 Rev and Firefly Luciferase were subsequently transfected with the modified pDM128 plasmid to generate a cell line in which Renilla Luciferase expression is dependent upon Rev function. In contrast, Firefly Luciferase expression in this cell line is independent of Rev function and is used to assay for nonspecific or toxic compounds. Using this system, compounds that inhibit the Rev function are identified by their ability to reduce the expression of Renilla Luciferase with no effect on the expression of Firefly Luciferase. A similar cell line expressing HIV-1 Tat in place of Rev and that was transfected with a standard Tat/LTR-reporter gene system[37] (using Renilla Luciferase) instead of pDM128 was generated for testing inhibitors of HIV-1 Tat. Assays were performed in 96-well format by plating cells (2×10^4/well) in the presence of test compound (triplicate wells) and incubating for 24−48 h. Luciferase expression levels were subsequently determined with use of Dual-Luciferase assay reagents (Promega) following the manufacturer's instructions.

Acknowledgment. We acknowledge the Utah State University (CURI Grant), DARPA (DAAD 19-03-1-0050), and National Foundation for Infectious Diseases (New Investigator Matching Grant) for generous financial support. We also acknowledge the support from the Department of Chemistry and Biochemistry, Utah State University. We also thank Prof. Mobashery, Wayne State University, for the generous gift of the pTZ19-3 and pSF815 plasmids and Dr. Thomas J. Hope, University of Illinois at Chicago, for the generous gift of the pDM128 plasmid.

Supporting Information Available: ^{1}H and ^{13}C spectra of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035290R

(36) Hope, T. J.; Huang, X. J.; McDonald, D.; Parslow, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7787−7791.
(37) Jeeninga, R. E.; Hoogenkamp, M.; Armand-Ugon, M.; de Baar, M.; Verhoef, K.; Berkhout, B. *J. Virol.* **2000**, *74*, 3740−3751.