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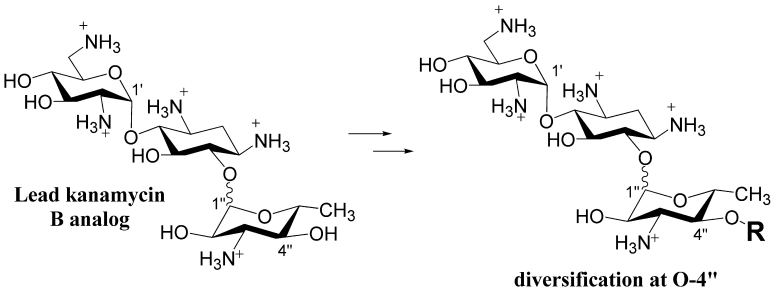

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Glycodiversification for the Optimization of the Kanamycin Class Aminoglycosides

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Received April 19, 2005

In an effort to optimize the antibacterial activity of kanamycin class aminoglycoside antibiotics, we have accomplished the synthesis and antibacterial assay of new kanamycin B analogues. A rationale-based glycodiversification strategy was employed. The activity of the lead is comparable to that of commercially available kanamycin. These new members, however, were found to be inactive against aminoglycoside resistant bacteria. Molecular modeling was used to provide the explanation. Thus, a new strategy for structural modifications of kanamycin class aminoglycosides is suggested.

Introduction

Aminoglycoside antibiotics have been used as a treatment against infectious diseases for over 60 years,[1] although the prevalence of aminoglycoside resistant bacteria has significantly reduced their effectiveness.[2] Nevertheless, aminoglycoside antibiotics are still a valuable resource against serious infections. With the unraveled structural information involving the aminoglycoside-bound rRNA molecules[3] and the details of the resistance mechanisms, especially the information obtained from the X-ray structural studies of aminoglycoside-modifying enzymes,[4] a growing interest has resurfaced into the development of new aminoglycoside antibiotics to counteract the problem caused by aminoglycoside resistant bacteria.[5]

Design and Synthesis of New Kanamycin B Analogues

Our group has prepared libraries of aminosugars (azidosugars), which enable a modular approach for the construction of libraries of novel aminosugar-containing glycoconjugates with the original carbohydrate component replaced by a synthetic one. This strategy is termed glycodiversification.[6] Following this concept, our group has synthesized a library of kanamycin B analogues with structural variation at ring III (Figure 1).[7] We have established the preliminary structure activity relationship (SAR) (Figure 2): (i) An equatorial amino group is preferred over an equatorial hydroxyl group at C-3″. (ii) At the C-4″ position, the presence of an axial NH_2 decreases the activity. (iii) Deoxygenation at C-6″ (6″-CH_3) provides better activity than CH_2NH_2 and CH_2-OH groups. However, there are some structural features whose effectiveness is still required to be established, for example, the effect of having an equatorial OH versus an axial one at the C-4″ position, the importance of having 6″-CH_3 group, and the effect of 4″-deoxygenation. To address these questions and to confirm the observed activity, we decided to synthesize more kanamycin B analogues by glycosylation of the O-6 OH of neamine (rings I and II) (Figure 3).

The design of **17** is to examine the importance of 4″-OH group. The designs of **18** and **19** are to confirm the advantage of 6″-CH_3. The designs of **16** and **20** can be used to establish the effectiveness of an axial 4″-OH. Incorporation of D-fucose as in the design of **21** can be used to further demonstrate the effect of axial 4″-OH group, while **23** containing L-fucose can be used as a comparison. If the importance of 6″-CH_3 is established, **22** should be the most active compound compared to kanamycin.

The syntheses of the corresponding glycosyl donors for the preparation of **16**−**19**, **21**, and **23** are analogous to the reported procedures.[7] The synthesis of the glycosyl donor for the preparation of **20** began from **24** (Scheme 1).[6,8] Hydrolysis of the acetyl groups, followed by protection of 4,6-diol with benzylidene, afforded **25**. After benzyl protection of 2-OH, compound **26** was treated with $Me_3N{-}BH_3$ in THF generating **27**.[9] A two-step epimerization of 4-OH using Tf_2O and then *t*-Bu_4-NOAc yielded the designed glycosyl donor **28**.

The synthesis of glycosyl donor **35** started from phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-D-fucopyranose, **29** (Scheme 2).[8] Hydrolysis of the acetyl groups, followed by the isopropylidene protection of the 3,4-diol, gave **30**. Protection of 2-OH followed by acid-mediated deprotection generated **31**, which was subjected to a two-step epimerization to produce **32**. Hydrolysis of the acetyl groups of **32**, followed by selective benzoylation of the 4-OH, generated **34**. Incorporation of azido group at the C-3 position completed the synthesis of designed glycosyl donor.

The kanamycin B analogues from the corresponding glycosyl donor were prepared according to the reported procedure (Schemes 3 and 4).[7] The designed kanamycin B analogues were tested against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) using kanamycin B as the control (Table 1).[10]

The SAR of the kanamycin B analogues in Table 1 demonstrates that an axial OH group is superior to an equatorial OH at the C-4″ position (entries 7 and 4, **21**

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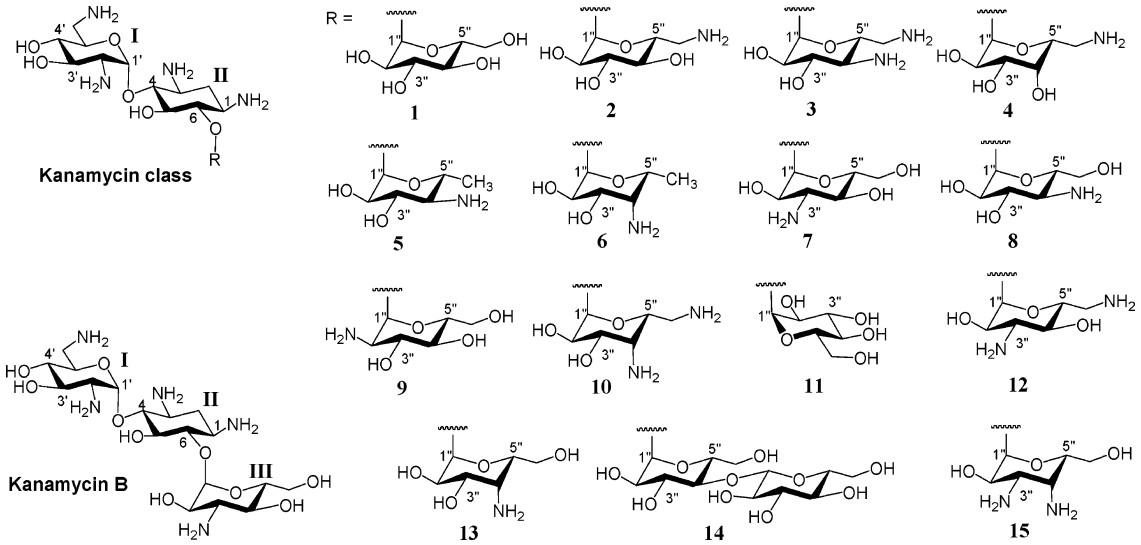


Figure 1. Structures of Kanamycin class aminoglycosides.

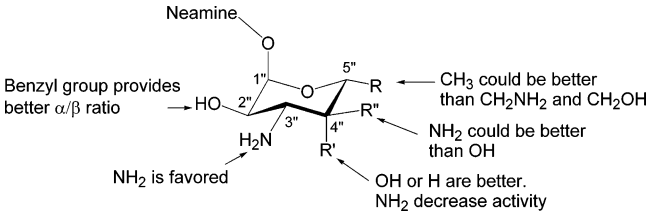


Figure 2. Summary of the SAR of ring iii of kanamycin B analogues.

vs **18**). A hydroxyl group (or amino group) at the C-4″ position is essential for activity (entries 3 and 10, **17** vs **5**). The presence of the 6″-CH_3 group appears to be important. However, replacing the 6″-CH_3 group with H does not abolish the antibacterial activity. Rather, such modification seems to enhance the activity (entries 4 and 5, **18** vs **19**). The observation, however, requires further investigation. Finally, as predicted, **22** is the most active compound, which could be employed as the lead for further modification.

Combined SAR information allows us to identify 4″-OH as the optimal site for further modification on ring III. At the beginning, four designs including **45**–**48**, where the R group represents the point of diversifica-

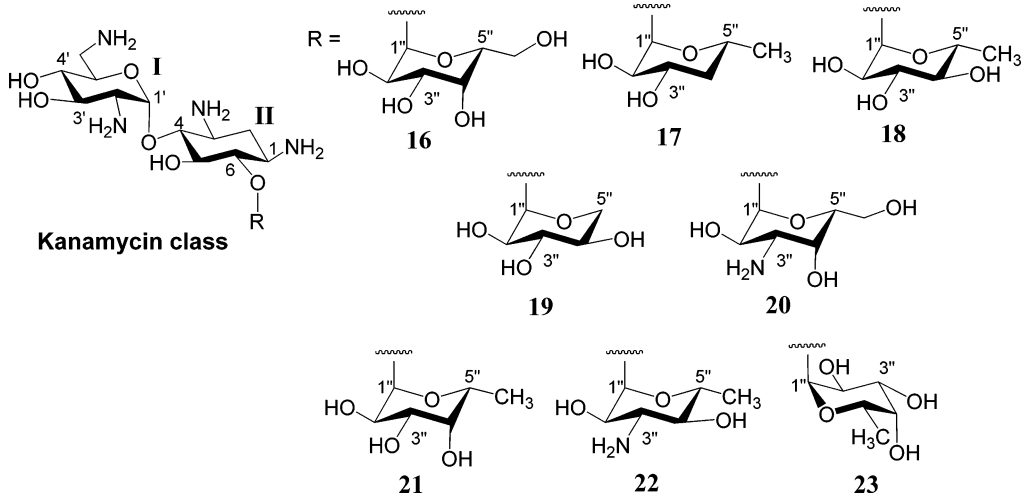
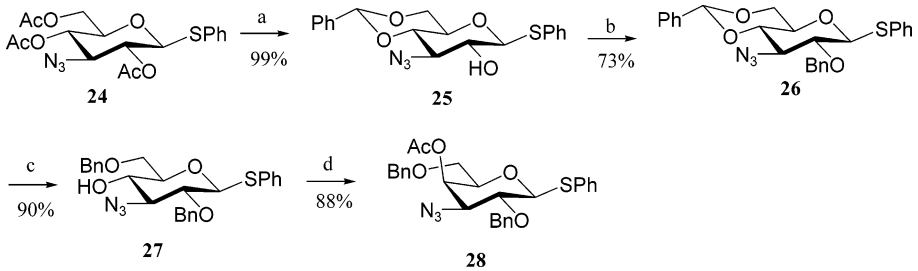


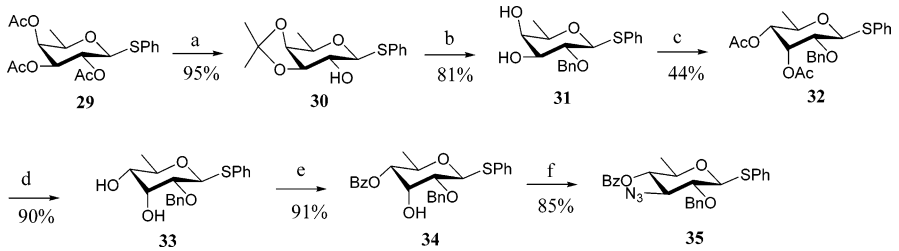
Figure 3. Structures of additional kanamycin class aminoglycosides.

Scheme 1. Synthesis of Glycosyl Donor[a]



[a] (a) (1) NaOMe, MeOH, (2) $PhCH(OMe)_2$, TsOH, DMF; (b) BnBr, NaH, TBAI, THF; (c) BH_3–Me_3N, $AlCl_3$, 4 Å molecular sieves, THF; (d) (1) Tf_2O, CH_2Cl_2, pyridine, (2) Bu_4NOAc, CH_2Cl_2.

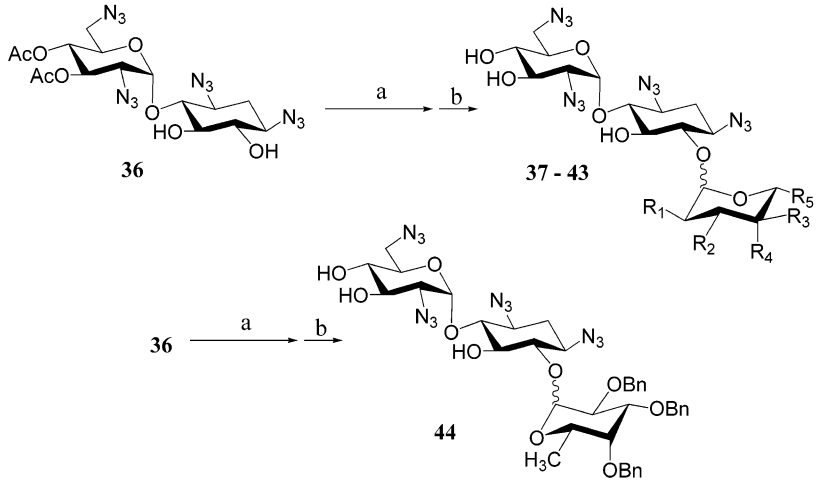
Scheme 2. Synthesis of Glycosyl Donor[a]



29 30 31 32 33 34 35

[a] (a) (1) NaOMe, MeOH, (2) $Me_2C(OMe)_2$, TsOH·H_2O, acetone; (b) (1) BnBr, NaH, TBAI, THF, (2) HOAc, TFA, H_2O; (c) (1) Tf_2O, py, $CHCl_2$, (2) *n*-$Bu_4N^+-AcO^-$; (d) NaOMe, MeOH; (e) BzCl, DIPEA, DMAP, CH_2Cl_2; (f) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF.

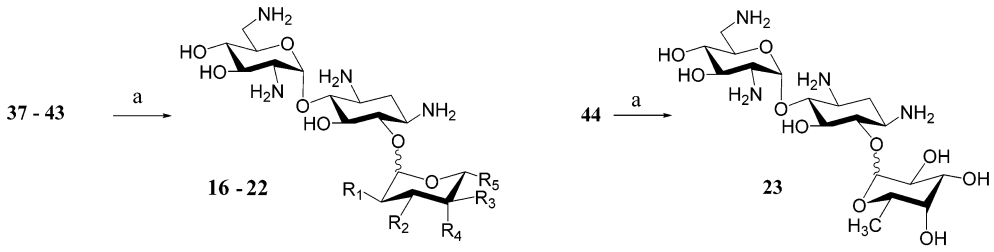
Scheme 3[a]



Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	α : β[a]
37	OBn	OBn	H	OBn	CH_2OBn	52	only α
38	OBn	OBn	H	H	CH_3	29	10:1
39	OBn	OBn	OBn	H	CH_3	46	4.5:1
40	OBn	OBn	OBn	H	H	40	5:1
41	OBn	N_3	H	OH	CH_2OBn	53	10:1
42	OBn	OBn	H	OBn	CH_3	42	only α
43	OBn	N_3	OH	H	CH_3	46	only α
44	-	-	-	-	-	71	5:1

[a] The ratios, including those in the following tables, are measured on the basis of the integral ratio of the ring I anomeric proton (H-1′). (a) Glycosyl donor, NIS, TfOH, Et_2O:CH_2Cl_2 (3:1); (b) NaOMe, MeOH:THF (5:1).

Scheme 4[a]



Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	α :β
16	OH	OH	H	OH	CH_2OH	99	only α
17	OH	OH	H	H	CH_3	89	10:1
18	OH	OH	OH	H	CH_3	61	4.5:1
19	OH	OH	OH	H	H	87	10:1
20	OH	NH_2	H	OH	CH_2OH	67	only α
21	OH	OH	H	OH	CH_3	66	only α
22	OH	NH_2	OH	H	CH_3	59	only α
23	-	-	-	-	-	71	5:1

[a] (a) (1) PMe_3, NaOH, THF, (2) H_2, $Pd(OH)_2$/C, HOAc, H_2O (3) Dowex 1X8-200 (Cl^- form).

tion, were envisioned (Figure 4). Nevertheless, there are several problems associated with the first three designs. For example, it is very difficult to introduce an equatorial 4″-NH_2 group. Attempts to use reductive amination and nucleophilic substitution for the synthesis of **45** (R = $(CH_2)_4N_3$) were unsuccessful. The presence of an acid-

Table 1. Minimum Inhibitory Concentration (MIC)

		MIC (μg/mL)	
entry	compd	*E. coli*	*S. aureus*
1	kanamycin B	2	2
2	**16**	32	32
3	**17**	inactive	inactive
4	**18**	inactive	inactive
5	**19**	32	64
6	**20**	4	1
7	**21**	32	16
8	**22**	2	2
9	**23**	inactive	inactive
10	**5** (ref 7)	12	2

labile tertiary 4″-OH in the design of **46** may hinder its synthesis. On the basis of our and others' [5e] experience, kanamycin analogues with a *galacto*-configuration, as in the design of **47**, will degrade under acidic conditions more easily than those with the *gluco*-configuration. Therefore, we decided to employ **48** as the template for introducing modifications at O-4″.

Kanamycin exerts its antibacterial activity by binding to rRNA and is a highly negatively charged molecule due to its phosphodiester backbone. It is our expectation that by introducing a more positively charged side chain at the O-4″ position, an increase in the antibacterial activity can be obtained. Therefore, we propose the synthesis of several new kanamycin B analogues with modification at O-4″ position following the design of **48** (Figure 5). Since 6-deoxy-3-aminoglucopyranose is harder to prepare, we used 6-deoxyglucopyranose for the model studies (**49**−**53**). After the identification of the optimal structural component at O-4″, the desired 6-deoxy-3-aminoglucopyranose was prepared (**55**). The design of **54** contains an enlarged side chain that hopefully may render it a poor substrate for the aminoglycoside-modifying enzymes. Therefore, if the high potency of such an analogue can be maintained, this new aminoglycoside antibiotic may generate activity against resistant strains of bacteria (Figure 6).

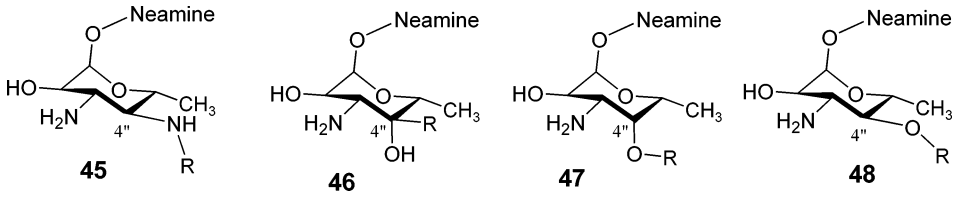


Figure 4. Possible designs of kanamycin B analogues with O-4″ modifications.

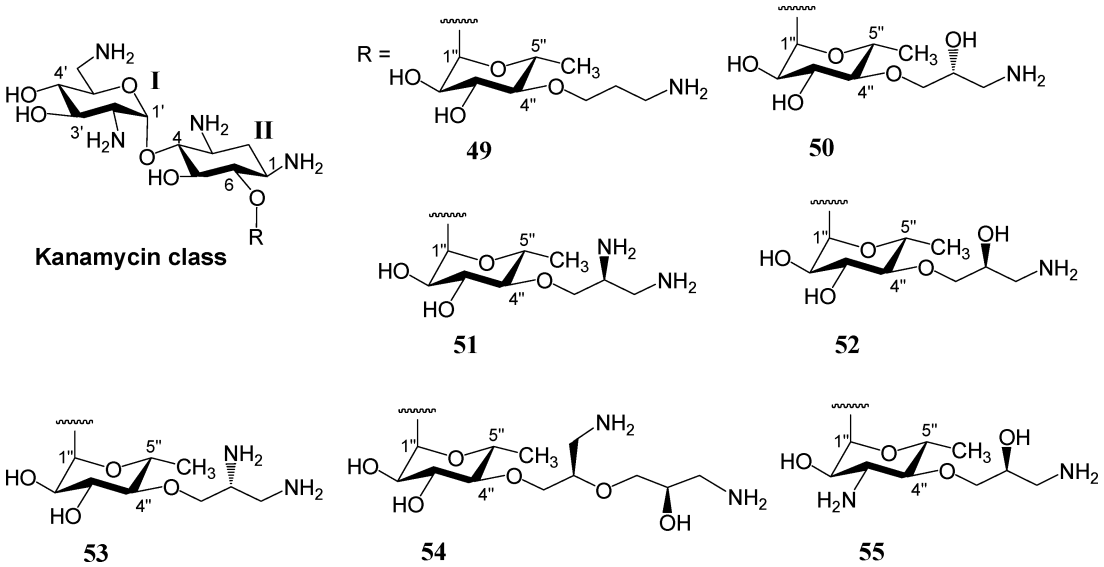


Figure 5. Structures of kanamycin class aminoglycosides bearing O-4″ modification.

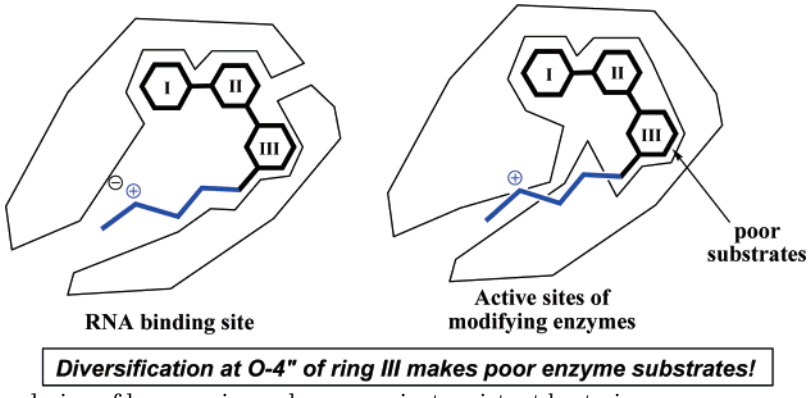
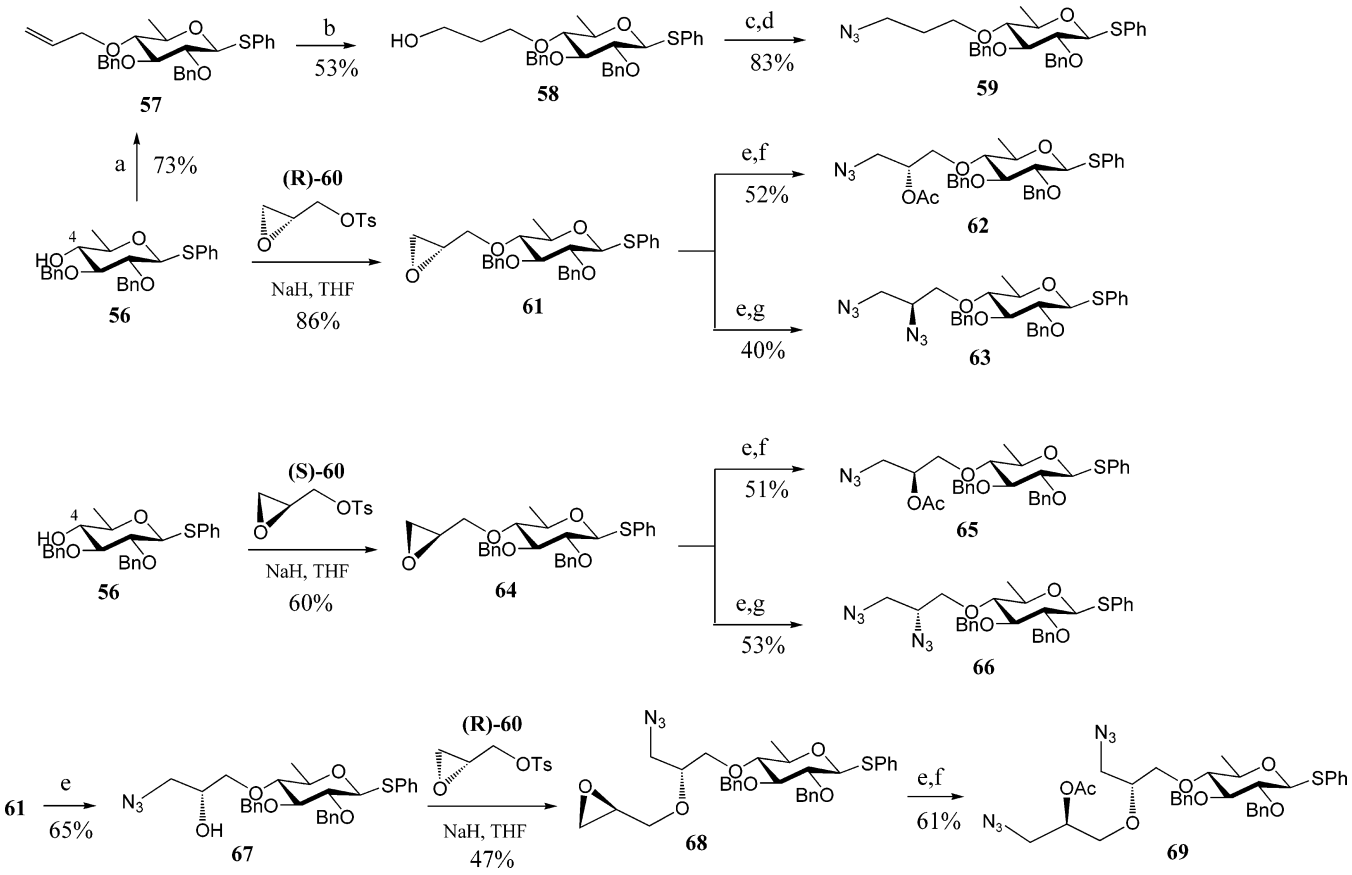


Figure 6. Concept for the design of kanamycin analogues against resistant bacteria.

Scheme 5. Synthesis of the Donors for the Preparation of Kanamycin B with O-4″ Modifications[a]



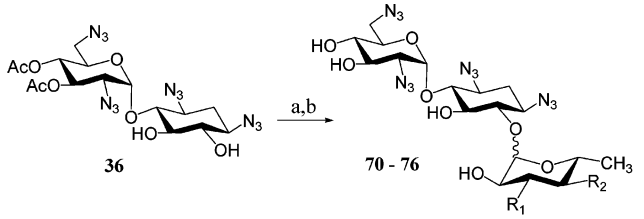
(a) AllBr, NaH, THF, TBAI; (b) (1) BH_3, THF, (2) NaOH, H_2O_2; (c) TSCl, CH_2Cl_2, Et_3N, DMAP; (d) NaN_3, DMF; (e) NaN_3, $CeCl_3 \cdot 7H_2O$, CH_3CN/H_2O (9:1); (f) Ac_2O, CH_2Cl_2, Et_3N, DMAP; (g) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF.

The syntheses of glycosyl donors for the model studies of the designed kanamycin analogues started from **56** (Scheme 5).[6] The 4-OH can be alkylated with allyl, (*R*)-glycidyl, and (*S*)-glycidyl groups, yielding **57**, **61**, and **64**, respectively. The designed glycosyl donor, **59**, can be synthesized via hydroboration followed by azido substitution from **57**, while the glycosyl donors **62**, **63**, **65**, and **66** can be prepared from **61** and **64** via azide-induced ring opening and acetylation or azido substitution. The designed donor **69** can be synthesized by repeating the glycidylation and the azide-induced ring-opening processes. The kanamycin B analogues from the corresponding glycosyl donors were prepared as before (Schemes 6 and 7).

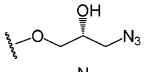
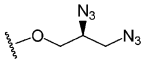
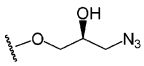
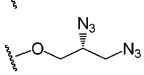
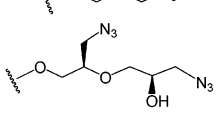
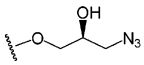
Results and Conclusion

The additional kanamycin B analogues were assayed as described previously (Scheme 7).[10] From the results of antibacterial assay, we noticed that there is no significant difference in the activity of analogues with various side chains at O-4″, although such side chains can revive the activity compared to the corresponding inactive parent compound **18**. However, to our surprise, when one of the side chains was attached to the lead, no increase in antibacterial activity (entry 8, **55** vs **22**) was observed. No activity was obtained when these analogues were tested against aminoglycoside resistant bacteria.[11] The results were disappointing; thus, we used molecular modeling studies of the kanamycin analogues for providing a rationale. The kanamycin analogues were docked to the RNA binding site, and

Scheme 6

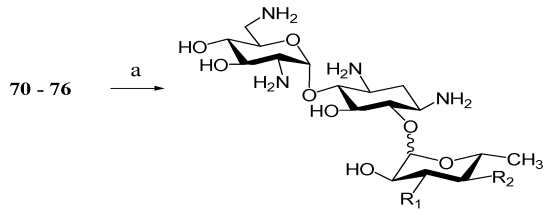


(a) glycosyl donor, NIS, TfOH, Et_2O:CH_2Cl_2 (3:1); (b) NaOMe, MeOH:THF (5:1).

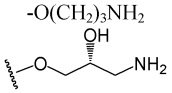
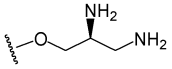
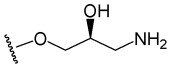
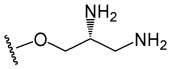
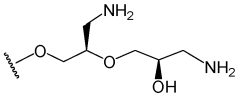
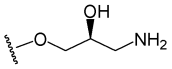
Compound	R_1	R_2	Yield (%)	α : β
70	OBn	$O(CH_2)_3N_3$	46	5:1
71	OBn		53	Only α
72	OBn		56	20:1
73	OBn		42	Only α
74	OBn		42	12:1
75	OBn		59	35:1
76	N_3		32	25:1

selected structures were docked to the kanamycin kinase type III (APH(3′)-IIIa) as well.[12] The scoring function was based on the electrostatic interactions, the molecular mechanics using the Amber 96 force field as

Scheme 7[a]



(a) (1) PMe_3, NaOH, THF, (2) $Pd(OH)_2/C$, HOAc, H_2O, (3) Dowex 1X8-200 (Cl^- form).

Entry	Compound	R_1	R_2	Yield (%)	α : β	Binding score (No. of NH_2)[a]	MIC (μg/mL) *E. coli*	MIC (μg/mL) *S. aureus*
1	Kanamycin B	-	-	-	-	-429.78 (5)	2	2
2	**49**	OH	$-O(CH_2)_3NH_2$	99	30:1	-426.18 (5)	4	4
3	**50**	OH		99	Only α	-426.02 (5)	4	4
4	**51**	OH		66	20:1	-527.00 (6)	8	8
5	**52**	OH		51	Only α	-421.12 (5)	4	4
6	**53**	OH		97	Only α	-515.71 (6)	8	8
7	**54**	OH		56	35:1	-519.34 (6)	8	4
8	**55**	NH_2		52	25:1	-529.63 (6)	4	2

[a] The lower are the values, the better is the binding affinity to rRNA.

implemented in HyperChem 7.0, and the solvent-accessible surface methodology to account for the hydration effects. The function was developed as a part of the de novo drug design package and not specifically for the calculation of absolute binding affinity. Therefore, it evaluates the relative binding affinities rather than the absolute binding scores.

From the binding scores, we noticed that there is no significant difference among compounds with the same total number of amino groups although the extra amino group on the side chain seems to lower the activity, while the binding score suggests otherwise. When fitting **54** into the binding site of the kinase, we found that the compound can still bind to the active site, following a conformational change in the side chain attached at O-4″. The conformations of rings I and II remain largely unchanged. The result suggests that the sites of modifications, primarily on rings I and II, are still susceptible to enzyme-catalyzed reactions such as acetylation, phosphorylation, and adenylation. This can explain the ineffectiveness of **54** that is equipped with an enlarged but flexible side chain (Figure 7).

In conclusion, we have synthesized complex analogues of kanamycin B. Through a chemical glycodiversification strategy, a library of structurally diverse glycosyl donors can be readily incorporated onto a given aglycon (neamine derivative) whereas an enzymatic method of synthesis may not be viable because of the constrained substrate acceptance by glycosyltransferase. Although the expected activity against aminoglycoside resistant bacteria was not observed, we have outlined a rationale-based model for the development of novel kanamycin class antibiotics. Results from molecular modeling and antibacterial assay both suggest that there is no significant difference in the antibacterial activity due to the variation of functional groups (OH or NH_2) and stereocenter. However, we did notice a discrepancy: an extra amino group on the side chain seems to lower the activity while increasing the binding score. The importance of employing real molecules in a whole cell based assay has also been highlighted because binding affinity studies using aminoglycoside and fragment of RNA molecules will likely generate results as predicted by molecular modeling and, thus, overemphasize the importance of amino group(s). Finally, the lack of activity of **54** against aminoglycoside resistant bacteria suggests that the attachment of a large but flexible side chain at the O-4″ position is an ineffective design. Perhaps a rigid functionality where we are currently devoting our effort will be a better design.

Experimental Section

Proton magnetic resonance spectra were recorded using a JEOL 270 or Bruker 400 spectrometer. Chemical shifts (δ) were reported as parts per million (ppm) downfield from tetramethylsilane, and coupling constants were given in cycles per second (Hz). Splitting patterns were designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C spectra were obtained using the JEOL 270 spectrometer at 68 MHz or Bruker 400 spectrometer at 100 MHz. Routine ^{13}C NMR spectra were fully decoupled by broad-band waltz decoupling. All NMR spectra were recorded at ambient temperature unless otherwise noted. Results from low-resolution fast atom bombardment (LRFAB) and high-resolution fast atom bombardment (HRFAB) or high-resolution matrix-as-

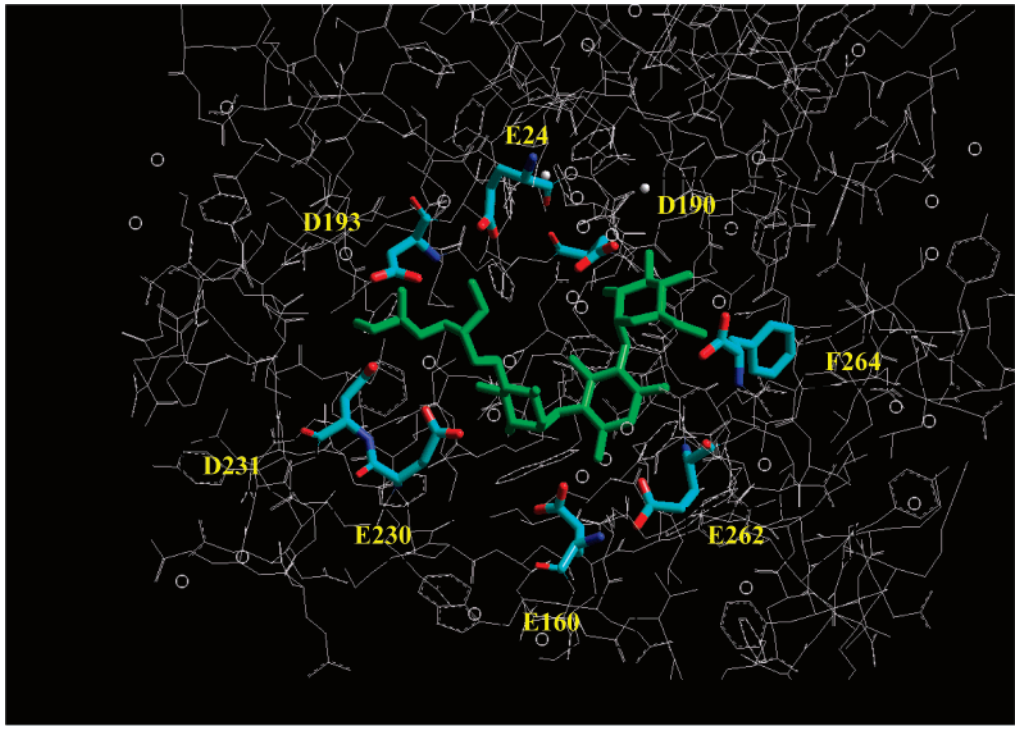


Figure 7. Binding of **54** in APH(3′)-IIIa.

sisted laser desorption ionization (MALDI) were provided by the Mass Spectrometry Facilities, University of California, Riverside.

Chemical reagents and starting materials were purchased from Aldrich Chemical Co. or Acros Chemical Co. and were used without purification unless otherwise noted. Dichloromethane was distilled over CaH_2. Other solvents were used without purification. Column chromatography was carried out by using silica gel (60 Å, 230 mm × 450 mm mesh, Sorbent Tech.) unless otherwise noted.

Phenyl 3-Azido-4,6-*O*-benzylidene-3-deoxy-1-thio-*β*-D-glucopyranoside (25). To a solution of **24** (1.2 g, 2.86 mmol) in anhydrous MeOH (10 mL), 1 mL of NaOMe (2 M in MeOH) was added. After completion of the reaction (1 h), the reaction was quenched by addition of Amberlite IR-120 (H^+) and filtered. After removal of solvent, the crude triol was dissolved in DMF. To the mixture were then added $PhCH(OM_2)_2$ (2.0 mL, 13.3 mmol) and a catalytic amount of $TsOH{\cdot}H_2O$. The reaction mixture was stirred at 60 °C for 0.5 h, and then the solvent was removed with a rotovap. The product was precipitated by addition of saturated $NaHCO_{3(aq)}$ and collected with a Hirsch funnel as a light-yellowish solid (1.1 g, 2.86 mmol, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3−7.5 (m, 10H), 5.55 (s, 1H), 4.62 (d, J = 9.6 Hz, 1H, H-1), 4.39 (dd, J = 10.2 Hz, J = 4.3 Hz, 1H), 3.76 (dd, J = 10.2 Hz, J = 9.9 Hz, 1H), 3.71 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 3.5−3.6 (m, 2H), 3.38 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 136.7 (s), 133.4 (s), 130.8 (s), 129.3 (s), 128.8 (s), 128.4 (s), 126.1 (s), 101.6 (s), 89.2 (s), 79.2 (s), 71.7 (s), 71.5 (s), 68.6 (s), 65.9 (s); LRFAB *m/e* 386 ([M + Na]$^+$); HRFAB calcd for $C_{19}H_{20}N_3O_4S$ ([M + H]$^+$) *m/e* 386.1175, measured *m/e* 386.1184.

Phenyl 3-Azido-2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-1-thio-*β*-D-glucopyranoside (26). To a solution of compound **25** (1.03 g, 2.67 mmol) in anhydrous THF (10 mL), BnBr (0.64 mL, 5.35 mmol), NaH (0.53 g, 13.4 mmol), and a catalytic amount of TBAI were added. The reaction mixture was stirred overnight. The excess BnBr was quenched by addition of MeOH (0.5 mL). Then the reaction mixture was poured into a solution of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine and then dried over $Na_2SO_{4(s)}$. After removal of solvents, the product was crystallized and collected with a Hirsch funnel. The crystal was washed with a solution of hexanes/ether (95/5) and collected as a light-yellowish solid (0.92 g, 1.94 mmol, 73%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 5.57 (s, 1H), 4.93 (d, J = 10.0 Hz, 1H, $PhCH_2O$), 4.81 (d, J = 10.0 Hz, 1H, $PhCH_2O$), 4.75 (d, J = 9.6 Hz, 1H, H-1), 4.38 (dd, J = 10.9 Hz, J = 4.3 Hz, 1H), 3.7−3.8 (m, 2H), 3.4−3.5 (m, 2H), 3.36 (dd, J = 8.9 Hz, J = 9.6 Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 137.3 (s), 136.8 (s), 132.9 (s), 132.4 (s), 129.2 (s), 128.66 (s), 128.58 (s), 128.4 (s), 128.28 (s), 128.18 (s), 126.1 (s), 101.5 (s), 88.7 (s), 79.7 (s), 79.1 (s), 75.8 (s), 71.1 (s), 68.7 (s), 67.0 (s); LRFAB *m/e* 476 ([M + H]$^+$); HRFAB calcd for $C_{26}H_{26}N_3O_4S$ ([M + H]$^+$) *m/e* 476.1644, measured *m/e* 476.1665.

Phenyl 3-Azido-2,6-di-*O*-benzyl-3-deoxy-1-thio-*β*-D-glucopyranoside (27). A solution of **26** (0.2 g, 0.42 mmol) in THF (15 mL) was stirred for 10 min over 4 Å molecular sieves. Borane−trimethylamine complex (0.18 g, 2.52 mmol) was added in one portion, followed by aluminum chloride (0.34 g, 2.52 mmol). After 4.5 h, additional borane−trimethylamine complex (0.12 g, 1.68 mmol) and aluminum chloride (0.17 g, 1.26 mmol) were added, and the mixture was stirred overnight at ambient temperature. The reaction mixture was filtered through Celite, neutralized with 1 M H_2SO_4, diluted with EtOAc, and washed with water, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by gradient column chromatography (hexanes/EtOAc = 100:0 to 55:45) afforded the product (0.18 g, 0.38 mmol, 90%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.5 (m, 15H), 4.91 (d, J = 10.2 Hz, 1H, $PhCH_2O$), 4.74 (d, J = 10.2 Hz, 1H, $PhCH_2O$), 4.66 (d, J = 9.6 Hz, 1H, H-1), 4.59 (d, J = 11.9 Hz, 1H, $PhCH_2O$), 4.53 (d, J = 11.9 Hz, 1H, $PhCH_2O$), 3.7−3.8 (m, 2H), 3.4−3.6 (m, 3H), 3.32 (dd, J = 9.2 Hz, J = 8.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7 (s), 137.5 (s), 133.6 (s), 132.3 (s), 129.2 (s), 128.8 (s), 128.74 (s), 128.67 (s), 128.3 (s), 128.2 (s), 128.0 (s), 88.2 (s), 79.3 (s), 78.1 (s), 75.5 (s), 74.0 (s), 71.2 (s), 70.7 (s), 70.6 (s); LRFAB *m/e* 500 ([M + Na]$^+$); HRFAB calcd for $C_{26}H_{27}N_3O_4SNa$ ([M + Na]$^+$) *m/e* 500.1620, measured *m/e* 500.1640.

Phenyl 4-*O*-Acetyl-3-azido-2,6-di-*O*-benzyl-3-deoxy-1-thio-*β*-D-galactopyranoside (28). To a solution of **27** (0.81 g, 1.69 mmol) and pyridine (0.41 mL, 5.08 mmol) in anhydrous CH_2Cl_2 at 0 °C, Tf_2O (0.57 mL, 3.38 mmol) was added 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 then dried over $Na_2SO_{4(s)}$. The solution was filtered through glass wool and transferred into a solution of tetrabutylammonium acetate (1.02 g, 3.38 mmol) in CH_2Cl_2. The reaction mixture was stirred overnight while the solvent was slowly evaporated with an aspirator. After completion of the reaction, the reaction mixture was diluted with EtOAc, washed with 1 N HCl, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 50:50) afforded the product (0.77 g, 1.48 mmol, 88%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 5.48 (dd, J = 2.6 Hz, J = 0.7 Hz, 1H, H-4), 4.97 (d, J = 9.9 Hz, 1H, PhCH_2O), 4.74 (d, J = 9.9 Hz, 1H, PhCH_2O), 4.73 (d, J = 9.0 Hz, 1H, H-1), 4.54 (d, J = 11.6 Hz, 1H, PhCH_2O), 4.47 (d, J = 11.6 Hz, 1H, PhCH_2O), 3.79 (ddd, J = 6.2 Hz, J = 6.2 Hz, J = 0.7 Hz, 1H, H-5), 3.6−3.7 (m, 2H), 3.60 (dd, J = 9.8 Hz, J = 6.2 Hz, 1H, H-6), 3.52 (dd, J = 9.8 Hz, J = 6.2 Hz, 1H, H-6), 2.12 (s, 3H, CH_3CO$_2$); ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.1 (s, CH_3CO_2), 137.8 (s), 137.8 (s), 133.8 (s), 132.1 (s), 129.2 (s), 128.8 (s), 128.68 (s), 128.64 (s), 128.4 (s), 128.2 (s), 128.1 (s), 127.9 (s), 88.6 (s), 76.9 (s), 75.7 (s), 73.9 (s), 68.7 (s), 68.5 (s), 65.6 (s), 20.9 (s, CH_3CO_2); LRFAB *m/e* 542 ([M + Na]$^+$); HRFAB calcd for $C_{28}H_{29}N_3O_5SNa$ ([M + Na]$^+$) *m/e* 542.1726, measured *m/e* 542.1732.

Phenyl 3,4-*O*-Isopropylidene-1-thio-β-D-fucopyranoside (30). To a solution of compound **29**[8] (7.04 g, 18.4 mmol) in anhydrous MeOH (30 mL), 2 mL of NaOMe (2 M in MeOH) was added. After completion of the reaction (1 h), the reaction was quenched by addition of Amberlite IR-120 (H^+) and the mixture was filtered. After removal of solvent, the crude triol was dissolved in a solution of acetone (20 mL) and $Me_2C(OMe)_2$ (20 mL) containing a catalytic amount of TsOH·H_2O. The reaction mixture was stirred overnight, and the reaction was quenched by addition of Et_3N (5 mL). After removal of solvents, the oily crude produced was redissolved in EtOAc. The organic solution was washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 50:50) afforded the product as a clear oil (5.19 g, 17.5 mmol, 95%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.4−7.5 (m, 2H), 7.2−7.3 (m, 3H), 4.40 (d, J = 10.2 Hz, 1H, H-1), 4.01 (m, 2H, H-3, H-4), 3.85 (qd, J = 6.6 Hz, J = 1.3 Hz, 1H, H-5), 3.52 (dd, J = 10.2 Hz, J = 6.3 Hz, 1H, H-2), 1.41 (d, J = 6.6 Hz, 3H, H-6), 1.41 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 132.7 (s), 132.2 (s), 129.0 (s), 128.1 (s), 109.9 (s), 87.9 (s), 79.1 (s), 76.4 (s), 72.9 (s), 71.4 (s), 28.2 (s), 26.4 (s), 17.0 (s); LRFAB *m/e* 296 (M^+); HRFAB calcd for $C_{15}H_{20}O_4S$ (M^+) *m/e* 296.1082, measured *m/e* 296.1078.

Phenyl 2-*O*-Benzyl-1-thio-β-D-fucopyranoside (31). To a solution of compound **30** (5.19 g, 17.5 mmol) in anhydrous THF (30 mL), BnBr (3.1 mL, 26.3 mmol), NaH (2.1 g, 87.5 mmol), and catalytic amount of TBAI were added. The reaction mixture was stirred overnight. The excess BnBr was quenched by addition of MeOH (2 mL). Then the reaction mixture was slowly poured into a solution of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. After removal of solvents, the crude product was redissolved in an aqueous solution (30 mL) of HOAc/TFA/H_2O (80/1/20). The reaction mixture was stirred at 60 °C for 1 h, and then the solvent was removed with a rotovap. Water was added to the oily crude product and removed again. Purification of the crude product with gradient column chromatography (hexanes/EtOAc = 100:0 to 50:50) afforded the product as a clear oil (4.89 g, 14.1 mmol, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.6 (m, 10H), 4.94 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.68 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.59 (d, J = 9.6 Hz, 1H, H-1), 3.6−3.7 (m, 3H), 3.53 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 1.33 (d, J = 6.6 Hz, 3H, H-6); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.3 (s), 134.2 (s), 132.0 (s), 129.1 (s), 128.8 (s), 128.5 (s), 128.3 (s), 127.7 (s), 87.6 (s), 78.3 (s), 75.5 (s, 2 carbons), 74.7 (s), 71.9 (s), 16.8 (s); LRFAB *m/e* 345 ([M − H]$^+$); HRFAB calcd for $C_{19}H_{21}O_4S$ ([M − H]$^+$) *m/e* 345.1160, measured *m/e* 345.1145.

Phenyl 3,4-Di-*O*-acetyl-2-*O*-benzyl-6-deoxy-1-thio-β-D-allopyranoside (32). Refer to the procedure for the preparation of **28**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 10H), 5.79 (dd, J = 3.0 Hz, J = 2.7 Hz, 1H, H-3), 5.00 (d, J = 9.7 Hz, 1H, H-1), 4.62 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.56 (dd, J = 10.2 Hz, J = 2.7 Hz, 1H, H-4), 4.41 (d, J = 11.1 Hz, 1H, PhCH_2O), 3.98 (dq, J = 10.2 Hz, J = 6.2 Hz, 1H, H-5), 3.44 (dd, J = 9.7 Hz, J = 3.0 Hz, 1H, H-2), 2.12 (s, 3H, CH_3CO$_2$), 2.03 (s, 3H, CH_3CO$_2$), 1.24 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (s, CH_3CO_2), 169.9 (s, CH_3CO_2), 137.2 (s), 133.0 (s), 132.8 (s), 129.0 (s), 128.6 (s), 128.4 (s), 128.2 (s), 128.0 (s), 83.8 (s), 75.0 (s), 72.15 (s), 72.06 (s), 70.9 (s), 67.4 (s), 21.0 (s, CH_3CO_2), 20.9 (s, CH_3CO_2), 17.8 (s); LRFAB *m/e* 453 ([M + Na]$^+$); HRFAB calcd for $C_{23}H_{26}O_6SNa$ ([M + Na]$^+$) *m/e* 453.1348, measured *m/e* 453.1358.

Phenyl 2-*O*-Benzyl-6-deoxy-1-thio-β-D-allopyranoside (33). A solution of **32** (0.76 g, 1.77 mmol) and NaOMe (1 M, 1.0 mmol) in MeOH (5 mL) was stirred at room temperature till the complete consumption of starting material (~2 h). Then Amberlite 120H$^+$ was added to quench the reaction. The reaction mixture was filtered through Celite and washed with EtOAc and MeOH. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 90:10 to 40:60) afforded the product (0.55 g, 1.59 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 10H), 4.95 (d, J = 9.8 Hz, 1H, H-1), 4.78 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.62 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.18 (dd, J = 3.0 Hz, J = 3.0 Hz, 1H, H-3), 3.70 (dq, J = 9.4 Hz, J = 6.2 Hz, 1H, H-5), 3.40 (dd, J = 9.8 Hz, J = 3.0 Hz, 1H, H-2), 3.21 (dd, J = 9.4 Hz, J = 3.0 Hz, 1H, H-4), 1.33 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5 (s), 132.0 (s), 129.0 (s), 128.8 (s), 128.5 (s), 128.4 (s), 127.6 (s), 83.6 (s), 73.1 (s), 72.91 (s), 72.89 (s), 69.3 (s), 18.1 (s); LRFAB *m/e* 369 ([M + Na]$^+$); HRFAB calcd for $C_{19}H_{22}O_4SNa$ ([M + Na]$^+$) *m/e* 369.1137, measured *m/e* 369.1155.

Phenyl 4-*O*-Benzoyl-2-*O*-benzyl-6-deoxy-1-thio-β-D-allopyranoside (34). To a solution of **33** (0.55 g, 1.59 mmol) in anhydrous CH_2Cl_2 (30 mL) was added DMAP (catalytic amount), DIPEA (0.53 mL, 3.18 mmol), and BzCl (0.20 mL, 1.75 mmol) at −50°C. The reaction mixture was stirred and allowed to warm to −10°C. Water was added to quench the reaction. After removal of the solvent, the reaction mixture was diluted with EtOAc. The organic layers were washed with 1 N HCl, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 60:40) afforded the product (0.65 g, 1.44 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 10H), 4.95 (d, J = 9.8 Hz, 1H, H-1), 4.78 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.62 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.18 (dd, J = 3.0 Hz, J = 3.0 Hz, 1H, H-3), 3.70 (dq, J = 9.4 Hz, J = 6.2 Hz, 1H, H-5), 3.40 (dd, J = 9.8 Hz, J = 3.0 Hz, 1H, H-2), 3.21 (dd, J = 9.4 Hz, J = 3.0 Hz, 1H, H-4), 1.33 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2 (s), 137.3 (s), 133.56 (s), 133.49 (s), 132.5 (s), 130.0 (s), 129.9 (s), 129.1 (s), 128.79 (s), 128.68 (s), 128.44 (s), 128.34 (s), 127.81 (s), 83.5 (s), 76.9 (s), 74.5 (s), 72.8 (s), 70.1 (s), 67.2 (s), 18.0 (s); LRFAB *m/e* 473 ([M + Na]$^+$); HRFAB calcd for $C_{26}H_{26}O_5SNa$ ([M + Na]$^+$) *m/e* 473.1400, measured *m/e* 473.1380.

Phenyl 3-Azido-4-*O*-benzoyl-2-*O*-benzyl-3,6-dideoxy-1-thio-β-D-glucopyranoside (35). To a solution of **34** (0.79 g, 1.76 mmol) and pyridine (0.31 mL, 3.86 mmol) in anhydrous CH_2Cl_2 at 0 °C, Tf_2O (0.53 mL, 3.17 mmol) was added slowly. After the mixture was stirred for 1 h, TLC was performed. If the reaction did not go to completion, more pyridine (0.15 mL, 1.90 mmol) and Tf_2O (0.26 mL, 1.55 mmol) were added. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2, washed with water, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. The solution was filtered through glass wool and transferred into a solution of NaN_3 (1.14 g, 17.6 mmol) in 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 diluted with EtOAc and filtered through Celite.

Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 65:35) afforded the product (0.71 g, 1.49 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.0−8.1 (m, 2H), 7.3−7.6 (m, 13H), 4.97 (d, J = 10.1 Hz, 1H, PhCH_2O), 4.93 (dd, J = 9.5 Hz, J = 9.6 Hz, 1H, H-4), 4.79 (d, J = 10.1 Hz, 1H, PhCH_2O), 4.72 (d, J = 9.7 Hz, 1H, H-1), 3.76 (dd, J = 9.7 Hz, J = 9.4 Hz, 1H, H-2), 3.65 (dq, J = 9.6 Hz, J = 6.2 Hz, 1H, H-5), 3.48 (dd, J = 9.4 Hz, J = 9.5 Hz, 1H, H-3), 1.30 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (s), 137.5 (s), 133.7 (s), 132.5 (s), 130.0 (s), 129.44 (s), 129.26 (s), 128.80 (s), 128.75 (s), 128.68 (s), 128.4 (s), 128.1 (s), 88.0 (s), 79.7 (s), 75.6 (s), 75.2 (s), 73.7 (s), 68.6 (s), 18.0 (s); LRFAB *m/e* 498 ([M + Na]$^+$); HRFAB calcd for $C_{26}H_{25}N_3O_4SNa$ ([M + Na]$^+$) *m/e* 498.1463, measured *m/e* 498.1453.

General Procedure for Glycosylation and Hydrolysis. A solution of glycosyl donor, neamine derivative (1.2 equiv), and activated powder 4 Å molecular sieves was stirred in anhydrous Et_2O and CH_2Cl_2 (Et_2O, 4.5 mL; CH_2Cl_2, 1.5 mL) at room temperature overnight. *N*-Iodosuccinimide (1.2 equiv) was quickly added into the above solution, and the reaction mixture was cooled to −70 °C. After the solution was warmed to −40 °C, trifluoromethanesulfonic acid (0.15 equiv) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor (∼4 h, monitored by TLC, hexane/EtOAc = 65: 35). The reaction was quenched by the addition of triethylamine (3 mL). After being stirred for 10 min, the reaction mixture was filtered through Celite and the solvent was removed. The crude product was extracted with EtOAc, washed with 10% aqueous $Na_2S_2O_3$, saturated $NaHCO_{3(aq)}$, and brine, and dried over $Na_2SO_{4(s)}$. After removal of the solvents, the crude product was purified by column chromatography. The glycosylated compounds were often mixed with inseparable impurities and were fully characterized after hydrolysis. The glycosylated product was dissolved in tetrahydrofuran (1 mL) and methanol (5 mL), and sodium methoxide (0.5 M in methanol, 1 mL) was added. The reaction mixture was stirred at room temperature till the completion of the reaction (∼2 h, monitored by TLC, EtOAc/hexane = 50: 50). The reaction mixture was neutralized with Amberlite IR-120 (H^+) and filtered through Celite, and the solvent was removed. The residue was purified via column chromatography to provide the product as a colorless oil.

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,2′,6′-tetraazidoneamine (37). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 20H), 5.56 (d, J = 3.3 Hz, 1H, H-1′), 5.11 (d, J = 3.3 Hz, 1H, H-1″), 4.91 (d, J = 11.6 Hz, 1H, PhCH_2O), 4.84 (d, J = 12.5 Hz, 1H, PhCH_2O), 4.80 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.72 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.71 (d, J = 11.5 Hz, 1H, PhCH_2O), 4.53 (d, J = 12.5 Hz, 1H, PhCH_2O), 4.51 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.39 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.1−4.2 (m, 3H), 3.8−3.9 (m, 3H), 3.2−3.7 (m, 10H), 3.09 (dd, J = 10.6 Hz, J = 3.6 Hz, 1H), 2.28 (ddd, J = 13.0 Hz, J = 4.3 Hz, J = 4.3 Hz, 1H, H-2$_{eq}$) 1.47 (ddd, J = 13.0 Hz, J = 12.2 Hz, J = 12.2 Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.0 (s), 138.56 (s), 138.54 (s), 137.9 (s), 128.60 (s), 128.56 (s), 128.48 (s), 128.2 (s), 128.0 (s), 127.9 (s), 127.8 (s), 99.3 (s), 98.3 (s), 85.8 (s), 79.9 (s), 78.6 (s), 76.6 (s), 76.0 (s), 75.3 (s), 74.8 (s), 73.8 (s), 73.7 (s), 73.6 (s), 71.8 (s), 71.7 (s), 71.2 (s), 71.1 (s), 69.3 (s), 63.1 (s), 59.8 (s), 59.4 (s), 51.4 (s), 32.5 (s); MALDI calcd for $C_{46}H_{52}N_{12}O_{11}Na$ ([M + Na]$^+$) *m/e* 971.3771, measured *m/e* 971.3808.

6-*O*-(2,3-Di-*O*-benzyl-4,6-dideoxy-α-D-*xylo*-hexopyranosyl)-1,3,2′,6′-tetraazidoneamine (38). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3−7.4 (m, 10H), 5.66 (d, J = 3.9 Hz, 1H, H-1′), 5.00 (d, J = 3.6 Hz, 1H, H-1″), 4.81 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.75 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.72 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.66 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.1−4.2 (m, 2H), 3.8−3.9 (m, 2H), 3.2−3.7 (m, 10H), 2.29 (ddd, J = 13.5 Hz, J = 3.9 Hz, J = 3.9 Hz, 1H, H-2$_{eq}$), 2.07 (m, 1H, H-4″$_{eq}$), 1.49 (ddd, J = 13.5 Hz, J = 12.9 Hz, J = 12.9 Hz, 1H, H-2$_{ax}$), 1.39 (ddd, J = 13.2 Hz, J = 10.9 Hz, J = 10.9 Hz, 1H, H-4″$_{ax}$), 1.17 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.1 (s), 138.5 (s), 128.6 (s), 128.56 (s), 128.1 (s), 128.0 (s), 127.8 (s), 127.7 (s), 99.3 (s), 98.2 (s), 86.0 (s), 80.6 (s), 79.9 (s), 75.8 (s), 74.6 (s), 73.6 (s), 72.7 (s), 71.8 (s), 71.5 (s), 71.3 (s), 66.0 (s), 63.3 (s), 59.5 (s), 59.3 (s), 51.5 (s), 39.1 (s), 32.6 (s), 21.0 (s); MALDI calcd for $C_{32}H_{40}N_{12}O_9Na$ ([M + Na]$^+$) *m/e* 759.2933, measured *m/e* 759.2977.

6-*O*-(2,3,4-Tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (39). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 5.65 (d, J = 3.6 Hz, 1H, H-1′), 4.96 (d, J = 10.9 Hz, 1H, PhCH_2O), 4.94 (d, J = 4.3 Hz, 1H, H-1″), 4.89 (d, J = 10.9 Hz, 1H, PhCH_2O), 4.78 (d, J = 10.9 Hz, 1H, PhCH_2O), 4.76 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.70 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.61 (d, J = 10.9 Hz, 1H, PhCH_2O), 3.9−4.1 (m, 3H), 3.1−3.7 (m, 12H), 2.30 (ddd, J = 12.9 Hz, J = 4.0 Hz, J = 4.0 Hz, 1H, H-2$_{eq}$), 1.48 (ddd, J = 12.9 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$), 1.25 (d, J = 5.9 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (s), 138.2 (s, two carbons), 128.7 (s), 128.6 (s), 128.24 (s), 128.23 (s), 128.19 (s), 128.14 (s), 98.6 (s), 98.3 (s), 86.3 (s), 83.4 (s), 81.2 (s), 80.1 (s), 79.9 (s), 76.1 (s), 75.9 (s), 75.7 (s), 73.6 (s), 71.8 (s), 71.5 (s), 71.3 (s), 68.7 (s), 63.3 (s), 59.6 (s), 59.2 (s), 51.5 (s), 32.5 (s), 18.0 (s); MALDI calcd for $C_{39}H_{46}N_{12}O_{10}Na$ ([M + Na]$^+$) *m/e* 865.3352, measured *m/e* 865.3340.

6-*O*-(2,3,4-Tri-*O*-benzyl-α-D-xylopyranosyl)-1,3,2′,6′-tetraazidoneamine (40). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 5.60 (d, J = 3.6 Hz, 1H, H-1′), 5.01 (d, J = 3.3 Hz, 1H, H-1″), 4.86 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.80 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.7−4.8 (m, 3H, PhCH_2O), 4.61 (d, J = 11.5 Hz, 1H, PhCH_2O), 3.8−4.2 (m, 4H), 3.2−3.7 (m, 12H), 2.31 (ddd, J = 13.0 Hz, J = 4.3 Hz, J = 4.3 Hz, 1H, H-2$_{eq}$), 1.49 (ddd, J = 13.0 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (s), 138.18 (s), 138.15 (s), 128.72 (s), 128.69 (s), 128.63 (s), 128.59 (s), 128.26 (s), 128.24 (s), 128.17 (s), 128.13 (s), 128.0 (s), 98.50 (s), 98.43 (s), 84.8 (s), 80.4 (s), 80.2 (s), 79.1 (s), 77.4 (s), 75.6 (s, two carbons), 73.9 (s), 73.8 (s), 72.0 (s), 71.6 (s), 71.3 (s), 63.3 (s), 61.7 (s), 59.7 (s), 59.2 (s), 51.5 (s), 32.6 (s); MALDI calcd for $C_{38}H_{44}N_{12}O_{10}Na$ ([M + Na]$^+$) *m/e* 851.3196, measured *m/e* 851.3158.

6-*O*-(3-Azido-2,6-di-*O*-benzyl-3-deoxy-α-D-galactopyranosyl)-1,3,2′,6′-tetraazidoneamine (41). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.4 (m, 10H), 5.47 (d, J = 3.7 Hz, 1H, H-1′), 5.25 (d, J = 2.9 Hz, 1H, H-1″), 4.74 (s, 2H, PhCH_2O), 4.61 (d, J = 12.1 Hz, 1H, PhCH_2O), 4.57 (d, J = 12.1 Hz, 1H, PhCH_2O), 4.27 (dd, J = 5.0 Hz, J = 5.0 Hz, 1H), 3.9−4.1 (m, 4H), 3.3−3.5 (m, 11H), 3.23 (dd, J = 10.3 Hz, J = 3.7 Hz, 1H), 2.33 (ddd, J = 12.9 Hz, J = 4.2 Hz, J = 4.2 Hz, 1H, H-2$_{eq}$), 1.54 (ddd, J = 12.9 Hz, J = 12.8 Hz, J = 12.8 Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6 (s), 137.5 (s), 128.7 (s), 128.4 (s), 128.2 (s), 128.1 (s), 98.7 (s), 97.7 (s), 83.9 (s), 81.0 (s), 75.7 (s), 75.2 (s), 74.0 (s), 73.3 (s), 72.3 (s), 71.6 (s), 71.4 (s), 69.9 (s), 69.6 (s, two carbons), 63.4 (s), 61.5 (s), 59.8 (s), 59.3 (s), 51.4 (s), 32.3 (s); MALDI calcd for $C_{32}H_{39}N_{15}O_{10}Na$ ([M + Na]$^+$) *m/e* 816.2897, measured *m/e* 816.2889.

6-*O*-(2,3,4-Tri-*O*-benzyl-α-D-fucopyranosyl)-1,3,2′,6′-tetraazidoneamine (42). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 5.67 (d, J = 3.9 Hz, 1H, H-1′), 5.01 (d, J = 4.6 Hz, 1H, H-1″), 4.98 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.86 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.80 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.72 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.71 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.63 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.1−4.2 (m, 3H), 3.9−4.0 (m, 2H), 3.3−3.6 (m, 8H), 3.2−3.3 (m, 2H), 2.29 (ddd, J = 12.9 Hz, J = 4.6 Hz, J = 4.6 Hz, 1H, H-2$_{eq}$), 1.48 (ddd, J = 12.9 Hz, J = 12.9 Hz, J = 12.9 Hz, 1H, H-2$_{ax}$), 1.11 (d, J = 6.6 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.2 (s), 138.57 (s), 138.55 (s), 128.6 (s), 128.5 (s), 128.2 (s), 127.9 (s), 127.7 (s), 99.1 (s), 98.1 (s), 86.1 (s), 79.8 (s), 78.7 (s), 77.9 (s), 76.1 (s), 75.9 (s), 75.1 (s), 73.8 (s), 73.6 (s), 71.8 (s), 71.6 (s), 71.2 (s), 68.6 (s), 63.4 (s), 59.5 (s), 59.3 (s), 51.5 (s), 32.6 (s),

16.7 (s); MALDI calcd for $C_{39}H_{46}N_{12}O_{10}Na$ ([M + Na]$^+$) *m/e* 865.3352, measured *m/e* 865.3317.

6-*O*-(3-Azido-2-*O*-benzyl-3,6-dideoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (43). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3−7.4 (m, 5H), 5.57 (d, J = 3.6 Hz, 1H, H-1′), 5.01 (d, J = 3.6 Hz, 1H, H-1″), 4.72 (s, 2H, PhCH_2O), 4.12 (m, 1H), 3.9−4.0 (m, 2H), 3.79 (dd, J = 9.9 Hz, J = 9.9 Hz, 1H), 3.2−3.7 (m, 10H), 3.02 (dd, J = 9.6 Hz, J = 9.6 Hz, 1H), 2.33 (ddd, J = 13.0 Hz, J = 4.3 Hz, J = 4.3 Hz, 1H, H-2$_{eq}$), 1.51 (ddd, J = 13.0 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$), 1.25 (d, J = 5.6 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.4 (s), 128.8 (s), 128.4 (s), 128.3 (s), 98.5 (s), 97.5 (s), 85.3 (s), 80.6 (s), 78.3 (s), 75.9 (s), 74.1 (s), 73.3 (s), 72.1 (s), 71.6 (s), 71.3 (s), 68.8 (s), 65.0 (s), 63.5 (s), 59.6 (s), 59.2 (s), 51.5 (s), 32.4 (s), 17.7 (s); MALDI calcd for $C_{25}H_{33}N_{15}O_9Na$ ([M + Na]$^+$) *m/e* 710.2478, measured *m/e* 710.2485.

6-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-1,3,2′,6′-tetraazidoneamine (44). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.4 (m, 15H), 5.67 (d, J = 4.0 Hz, 1H, H-1′), 5.01 (d, J = 3.6 Hz, 1H, H-1″), 4.94 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.92 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.76 (s, 2H, PhCH_2O), 4.74 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.64 (d, J = 11.2 Hz, 1H, PhCH_2O), 3.9−4.2 (m, 4H), 3.5−3.8 (m, 7H), 3.92−3.4 (m, 4H), 2.32 (ddd, J = 13.2 Hz, J = 4.0 Hz, J = 4.0 Hz, 1H, H-2eq), 1.51 (ddd, J = 13.2 Hz, J = 11.9 Hz, J = 11.9 Hz, 1H, H-2ax), 1.16 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.66 (s), 138.55 (s), 137.4 (s), 128.9 (s), 128.7 (s), 128.44 (s), 128.38 (s), 127.90 (s), 127.87 (s), 127.6 (s), 102.0 (s), 97.7 (s), 85.0 (s), 80.2 (s), 78.6 (s), 77.5 (s), 76.9 (s), 76.0 (s), 75.1 (s), 75.0 (s), 72.7 (s), 71.7 (s), 71.6 (s), 71.2 (s), 67.9 (s), 63.2 (s), 59.5 (s, two carbons), 51.5 (s), 32.7 (s), 16.8 (s); MALDI calcd for $C_{39}H_{46}N_{12}O_{10}Na$ ([M + Na]$^+$) *m/e* 865.3352, measured *m/e* 865.3322.

General Procedure for the Synthesis of Kanamycin B Analogues. To a starting material/THF solution in a reaction vial equipped with a reflux condenser, 0.1 M $NaOH_{(aq)}$ (0.5 mL) and PMe_3 (1 M in THF, 5−7 equiv) were added. The reaction mixture was stirred at 50 °C for 2 h. The product has an R_f of 0 when eluted with an EtOAc/MeOH (9/1) solution and has an R_f of 0.6 when eluted with *i*-PrOH/1 M NH_4OAc (2/1) solution. After completion of the reaction, the solvents were removed, and the crude benzylated aminoglycoside was added with a catalytic amount of $Pd(OH)_2$/C (20% Degussa type) and 5 mL of degassed HOAc/H_2O (1/3). 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. The crude product was purified with Amberlite CG50(NH_4^+) and was eluted with a gradient of NH_4-OH solution (0−20%). The final product with Cl^- salt can be prepared with an ion-exchange column packed with Dowex 1X8-200 (Cl^- form) and eluting with water. After collection of the desired fractions and removal of solvent, the final products are subjected to a bioassay directly. The reported final products are characterized by ^{1}H and ^{13}C NMR at this stage.

6-*O*-(α-D-Galactopyranosyl)neamine (16). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 5.91 (d, J = 3.3 Hz, 1H, H-1′), 5.10 (d, J = 3.3 Hz, 1H, H-1″), 4.15 (J = 6.3 Hz, J = 5.9 Hz, 1H), 3.9−4.1 (m, 7H), 3.7−3.8 (m, 3H), 3.4−3.6 (m, 5H), 3.26 (dd, J = 13.5 Hz, J = 7.3 Hz, 1H), 2.52 (d, J = 11.9 Hz, 1H, H-2$_{eq}$), 1.96 (ddd, J = 11.9 Hz, J = 12.9 Hz, J = 12.9 Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.7 (s), 96.3 (s), 83.9 (s), 78.0 (s), 74.4 (s), 72.7 (s), 71.0 (s), 69.44 (s), 69.40 (s), 69.28 (s), 68.8 (s), 68.5 (s), 61.4 (s), 53.8 (s), 49.8-(s), 48.5 (s), 40.5 (s), 28.3 (s); LRFAB *m/e* 485 ([M + H]$^+$); HRFAB calcd for $C_{18}H_{37}N_4O_{11}$ ([M + H]$^+$) *m/e* 485.2459, measured *m/e* 485.2467.

6-*O*-(4,6-Dideoxy-α-D-*xylo*-hexopyranosyl)neamine (17). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 5.95 (d, J = 3.6 Hz, 1H, H-1′), 5.01 (d, J = 3.3 Hz, 1H, H-1″), 4.23 (m, 1H), 4.0−4.1 (m, 4H), 3.85 (dd, J = 9.2 Hz, J = 8.9 Hz, 1H), 3.71 (dd, J = 10.2 Hz, J = 8.6 Hz, 1H), 3.4−3.6 (m, 6H), 3.29 (dd, J = 13.5 Hz, J = 6.9 Hz, 1H), 2.53 (d, J = 12.5 Hz, 1H, H-2$_{eq}$), 2.04 (m, 1H, H-4″$_{eq}$), 1.96 (ddd, J = 12.5 Hz, J = 12.2 Hz, J = 12.2 Hz, 1H, H-2$_{ax}$), 1.37 (ddd, J = 12.5 Hz, J = 11.9 Hz, J = 11.9 Hz, 1H, H-4″$_{ax}$), 1.18 (d, J = 6.4 Hz, 1H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 102.8 (s), 96.1 (s), 83.7 (s), 77.7 (s), 74.3 (s), 73.7 (s), 70.9 (s), 69.5 (s), 68.4 (s), 67.2 (s), 66.7 (s), 53.7 (s), 50.1 (s), 48.6 (s), 40.4 (s), 39.7 (s), 28.2 (s), 20.1 (s); LRFAB *m/e* 453 ([M + H]$^+$); HRFAB calcd for $C_{18}H_{37}N_4O_9$ ([M + H]$^+$) *m/e* 453.2561, measured *m/e* 453.2580.

6-*O*-(6-Deoxy-α-D-glucopyranosyl)neamine (18). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.95 (d, J = 3.6 Hz, 1H, H-1′), 4.98 (s, 1H, H-1″), 3.9−4.0 (m, 4H), 3.84 (dd, J = 9.2 Hz, J = 9.0 Hz, 1H), 3.71 (dd, J = 9.8 Hz, J = 9.2 Hz, 1H), 3.65 (m, 1H), 3.4−3.5 (m, 6H), 3.27 (dd, J = 13.4 Hz, J = 7.1 Hz, 1H), 3.15 (dd, J = 8.6 Hz, J = 8.2 Hz, 1H), 2.49 (m, 1H, H-2$_{eq}$), 1.91 (ddd, J = 12.8 Hz, J = 12.3 Hz, J = 12.3 Hz, 1H, H-2$_{ax}$), 1.22 (d, J = 6.1 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 102.0 (s), 96.0 (s), 83.9 (s), 78.0 (s), 74.9 (s), 74.4 (s), 72.8 (s), 72.2 (s), 71.0 (s), 69.4 (s), 69.2 (s), 68.6 (s), 53.8 (s), 50.2 (s), 48.6 (s), 40.4 (s), 28.5 (s), 17.0 (s); LRFAB *m/e* 469 ([M + H]$^+$); HRFAB calcd for $C_{18}H_{37}N_4O_{10}$ ([M + H]$^+$) *m/e* 469.2510, measured *m/e* 469.2512.

6-*O*-(α-D-Xylopyranosyl)neamine (19). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 5.95 (s, 1H, H-1′), 5.01 (s, 1H, H-1″), 3.9−4.0 (m, 3H), 3.86 (dd, J = 8.6 Hz, J = 8.6 Hz, 1H), 3.4−3.8 (m, 11H), 3.28 (dd, J = 13.2 Hz, J = 6.9 Hz, 1H), 2.53 (d, J = 12.2 Hz, 1H, H-2$_{eq}$), 1.91 (m,1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 102.1 (s), 96.2 (s), 83.9 (s), 77.8 (s), 74.4 (s), 73.1 (s), 71.9 (s), 70.9 (s), 69.4 (s), 69.1 (s), 68.5 (s), 62.7 (s), 53.7 (s), 50.0 (s), 48.5 (s), 40.4 (s), 28.2 (s); LRFAB *m/e* 455 ([M + H]$^+$); HRFAB calcd for $C_{17}H_{35}N_4O_{10}$ ([M + H]$^+$) *m/e* 455.2353, measured *m/e* 455.2337.

6-*O*-(3-Amino-3-deoxy-α-D-galactopyranosyl)neamine (20). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.90 (d, J = 3.6 Hz, 1H, H-1′), 5.14 (d, J = 3.6 Hz, 1H, H-1″), 4.0−4.2 (m, 7H), 3.92 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.82 (dd, J = 9.9 Hz, J = 8.6 Hz, 1H), 3.4−3.7 (m, 7H), 3.26 (dd, J = 13.9 Hz, J = 6.9 Hz, 1H), 2.53 (ddd, J = 12.5 Hz, J = 4.3 Hz, J = 4.3 Hz, 1H, H-2$_{eq}$), 1.96 (ddd, J = 12.5 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 100.8 (s), 96.4 (s), 83.9 (s), 77.9 (s), 74.5 (s), 72.0 (s), 70.9 (s), 69.5 (s), 68.4 (s), 65.6 (s), 65.5 (s), 60.9 (s), 53.8 (s), 52.1 (s), 49.7 (s), 48.5 (s), 40.4 (s), 28.0 (s); LRFAB *m/e* 484 ([M + H]$^+$); HRFAB calcd for $C_{18}H_{38}N_5O_{10}$ ([M + H]$^+$) *m/e* 484.2619, measured *m/e* 484.2596.

6-*O*-(α-D-Fucopyranosyl)neamine (21). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 5.96 (d, J = 3.3 Hz, 1H, H-1′), 5.00 (s, 1H, H-1″), 4.26 (q, J = 6.6 Hz, 1H, H-5″), 3.8−4.1 (m, 7H), 3.71 (dd, J = 9.9 Hz, J = 8.9 Hz, 1H), 3.4−3.6 (m, 5H), 3.28 (dd, J = 13.5 Hz, J = 6.9 Hz, 1H), 2.52 (m, 1H, H-2$_{eq}$), 1.94 (ddd, J = 12.5 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$), 1.18 (d, J = 6.6 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 102.3 (s), 96.1 (s), 83.6 (s), 77.7 (s), 74.3 (s), 71.9 (s), 70.9 (s), 69.59 (s), 69.47 (s), 68.66 (s), 68.45 (s), 68.3 (s), 53.7 (s), 50.1 (s), 48.6 (s), 40.4 (s), 28.2 (s), 15.7 (s); LRFAB *m/e* 469 ([M + H]$^+$); HRFAB calcd for $C_{18}H_{37}N_4O_{10}$ ([M + H]$^+$) *m/e* 469.2510, measured *m/e* 469.2533.

6-*O*-(3-Amino-3,6-dideoxy-α-D-glucopyranosyl)neamine (22). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 6.01 (d, J = 3.6 Hz, 1H, H-1′), 5.05 (d, J = 3.3 Hz, 1H, H-1″), 3.9−4.1 (m, 6H), 3.81 (dd, J = 9.6 Hz, J = 8.9 Hz, 1H), 3.4−3.7 (m, 7H), 3.30 (dd, J = 13.9 Hz, J = 6.6 Hz, 1H), 2.55 (ddd, J = 12.6 Hz, J = 4.0 Hz, J = 4.0 Hz, 1H, H-2$_{eq}$), 1.98 (ddd, J = 12.6 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$), 1.28 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride

salt) δ 101.0 (s), 96.0 (s), 83.9 (s), 77.5 (s), 74.5 (s), 71.0 (s), 70.9 (s), 69.5 (s), 69.4 (s), 68.6 (s), 68.5 (s), 55.0 (s), 53.7(s), 50.0 (s), 48.6 (s), 40.4 (s), 28.1 (s), 16.7 (s); LRFAB *m/e* 468 ($[M + H]^+$); HRFAB calcd for $C_{18}H_{38}N_5O_9$ ($[M + H]^+$) *m/e* 468.2670, measured *m/e* 468.2677.

6-*O*-(α-L-Fucopyranosyl)neamine (23). Refer to the general procedure for the synthesis of kanamycin B analogues. ^{1}H NMR (270 MHz, D_2O) (chloride salt) δ 5.92 (d, J = 3.6 Hz, 1H, H-1′), 5.27 (s, 1H, H-1″), 4.23 (m, 1H), 3.8−4.1 (m, 8H), 3.4−3.6 (m, 5H), 3.28 (dd, J = 13.5 Hz, J = 6.9 Hz, 1H), 2.58 (ddd, J = 12.9 Hz, J = 4.6 Hz, J = 4.6 Hz, 1H, H-2_{eq}), 1.97 (ddd, J = 12.9 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2_{ax}), 1.21 (d, J = 6.6 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 100.4 (s), 96.3 (s), 81.8 (s), 77.8 (s), 74.8 (s), 71.8 (s), 71.0 (s), 69.43 (s), 69.36 (s), 68.48 (s), 68.32 (s), 68.18 (s), 53.8 (s), 48.63 (s), 48.46 (s), 40.4 (s), 28.4 (s), 15.8 (s); LRFAB *m/e* 469 ($[M + H]^+$); HRFAB calcd for $C_{18}H_{37}N_4O_{10}$ ($[M + H]^+$) *m/e* 469.2510, measured *m/e* 469.2485.

Phenyl 4-*O*-Allyl-2,3-di-*O*-benzyl-6-deoxy-1-thio-β-D-glucopyranoside (57). To a solution of **56**[6] (0.4 g, 0.92 mmol), NaH (0.07 g, 1.83 mmol, 60% dispersion in mineral oil), and a catalytic amount of TBAI in anhydrous THF, allyl bromide (0.44 mL, 5.09 mmol) was added slowly at 0 °C. After the mixture was stirred for 24 h, the excess allyl bromide was quenched by addition of MeOH (1.0 mL). After removal of solvent, the reaction mixture was diluted with EtOAc, washed with 1 N HCl, 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 (hexanes/EtOAc =100:0 to 65:35), the product was obtained as a light-yellowish solid (0.32 g, 0.67 mmol, 73%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.6 (m, 15H), 5.90 (ddd, J = 17.5 Hz, J = 10.2 Hz, J = 5.9 Hz, 1H), 5.25 (d, J = 17.5 Hz, 1H), 5.16 (d, J = 10.2 Hz, 1H), 4.88 (d, J = 10.6 Hz, 1H, PhCH_2O), 4.86 (d, J = 10.6 Hz, 1H, PhCH_2O), 4.81 (d, J = 10.6 Hz, 1H, PhCH_2O), 4.73 (d, J = 10.6 Hz, 1H, PhCH_2O), 4.64 (d, J = 9.6 Hz, 1H, H-1), 4.32 (dd, J = 12.2 Hz, J = 5.9 Hz, 1H), 4.15 (dd, J = 12.2 Hz, J = 5.9 Hz, 1H), 3.60 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.44 (dd, J = 8.9 Hz, J = 9.6 Hz, 1H), 3.36 (m, 1H, H-5), 3.08 (dd, J = 8.9 Hz, J = 9.2 Hz, 1H), 1.35 (d, J = 5.9 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7 (s), 138.3 (s), 134.9 (s), 134.2 (s), 132.1 (s), 129.1 (s), 128.62 (s), 128.5 (s), 128.4 (s), 128.1 (s), 128.0 (s), 127.9 (s), 127.6 (s), 117.4 (s), 87.7 (s), 86.7 (s), 83.4 (s), 81.4 (s), 76.0 (s), 75.9 (s), 75.6 (s), 74.3 (s), 18.4 (s); MALDI calcd for $C_{29}H_{32}O_4SNa$ ($[M + Na]^+$) *m/e* 499.1914, measured *m/e* 499.1937.

Phenyl 2,3-Di-*O*-Benzyl-6-deoxy-4-*O*-(3-hydroxypropyl)-1-thio-β-D-glucopyranoside (58). To a solution of **57** (0.31 g, 0.65 mmol) in anhydrous THF, borane/THF (0.98 mL, 1 M solution) was added. The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction, H_2O_2 (0.30 mL, 30%) and a couple of drops of NaOH solution (3 M) were added at 0 °C. After being stirred for 10 min, the reaction mixture was diluted with EtOAc. The combined organic layers were washed with brine and dried over $Na_2SO_{4(s)}$. After removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 90:10 to 50:50), the product was obtained (0.17 g, 0.34 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 4.90 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.90 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.81 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.73 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.64 (d, J = 9.7 Hz, 1H, H-1), 3.99 (dt, J = 9.0 Hz, J = 5.6 Hz, 1H), 3.77 (dt, J = 9.0 Hz, J = 5.6 Hz, 1H), 3.73 (t, J = 5.9 Hz, 2H), 3.58 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.46 (dd, J = 9.7 Hz, J = 9.0 Hz, 1H), 3.36 (m, 1H, H-5), 3.03 (dd, J = 9.2 Hz, J = 9.3 Hz, 1H), 1.81 (m, 2H), 1.38 (d, J = 6.1 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7 (s), 138.3 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.67 (s), 128.61 (s), 128.4 (s), 128.0 (s), 127.9 (s), 127.7 (s), 87.7 (s), 86.5 (s), 84.2 (s), 81.5 (s), 75.9 (s), 75.8 (s), 75.6 (s), 72.2 (s), 61.5 (s), 33.1 (s), 18.4 (s); MALDI calcd for $C_{29}H_{34}O_5SNa$ ($[M + Na]^+$) *m/e* 517.2019, measured *m/e* 517.2030.

Phenyl 4-*O*-(3-Azidopropyl)-2,3-di-*O*-benzyl-6-deoxy-1-thio-β-D-glucopyranoside (59). To a solution of **58** (0.15 g, 0.30 mmol), Et_3N (0.10 mL, 0.75 mmol), and DMAP (catalytic amount) in anhydrous CH_2Cl_2 (5 mL), TsCl (0.12 g, 0.61 mmol) was added slowly at 0 °C. The reaction mixture was stirred overnight and allowed 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, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. After removal of solvent, the tosylated crude product was dissolved in anhydrous DMF (5 mL), and NaN_3 (0.20 g, 3.0 mmol) was added. The reaction mixture was stirred at 0 °C overnight. After removal of the solvent, the residue was diluted with EtOAc and filtered through Celite. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 65:35) afforded the product (0.13 g, 0.25 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.5−7.6 (m, 2H), 7.3−7.4 (m, 13H), 4.91 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.90 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.79 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.73 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.67 (d, J = 9.6 Hz, 1H, H-1), 3.87 (dt, J = 9.3 Hz, J = 5.8 Hz, 1H), 3.67 (m, 1H), 3.58 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.46 (dd, J = 9.6 Hz, J = 9.0 Hz, 1H, H-2), 3.37 (m, 1H), 3.33 (t, J = 6.4 Hz, 2H), 3.02 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 1.81 (m, 2H), 1.36 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6 (s), 138.3 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.0 (s), 127.9 (s), 127.7 (s), 87.7 (s), 86.6 (s), 84.0(s), 81.5 (s), 75.88 (s), 75.76 (s), 75.62 (s), 70.1 (s), 48.6 (s), 29.9 (s), 18.4 (s); MALDI calcd for $C_{29}H_{33}N_3O_4SNa$ ($[M + Na]^+$) *m/e* 542.2084, measured *m/e* 542.2066.

Phenyl 2,3-Di-*O*-benzyl-6-deoxy-4-*O*-((*R*)-glycidyl)-1-thio-β-D-glucopyranoside (61). To a solution of **56**[6] (0.85 g, 1.95 mmol), NaH (0.31 g, 7.80 mmol, 60% dispersion in mineral oil), and a couple of drops of DMF in anhydrous THF, 2*R*-(−)-glycidyl tosylate (1.11 g, 4.87 mmol) was added. After the mixture was stirred for 24 h, the reaction was quenched by addition of NH_4Cl (saturated) and the reaction mixture was diluted with EtOAc. The combined organic layers were washed with brine and dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 70:30) afforded the product (0.83 g, 1.68 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 4.92 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.91 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.84 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.74 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.66 (d, J = 9.8 Hz, 1H, H-1), 4.05 (dd, J = 11.3 Hz, J = 3.0 Hz, 1H), 3.62 (dd, J = 8.9 Hz, J = 9.0 Hz, 1H), 3.54 (dd, J = 11.3 Hz, J = 6.6 Hz, 1H), 3.47 (dd, J = 9.8 Hz, J = 9.0 Hz, 1H, H-2), 3.40 (dq, J = 9.4 Hz, J = 6.2 Hz, 1H), 3.11 (dd, J = 9.2 Hz, J = 9.4 Hz, 1H), 3.09 (m, 1H), 3.76 (dd, J = 5.1 Hz, J = 4.4 Hz, 1H), 2.53 (dd, J = 5.1 Hz, J = 2.7 Hz, 1H), 1.40 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (s), 138.7 (s), 134.1 (s), 132.1 (s), 131.9 (s), 129.1 (s), 128.64 (s), 128.61 (s), 128.4 (s), 128.3 (s), 128.11 (s), 128.0 (s), 127.9 (s), 127.7 (s), 87.7 (s), 86.6 (s), 84.3 (s), 81.5 (s), 75.9 (s), 75.7 (s), 75.6 (s), 74.6 (s), 51.1 (s), 44.5 (s), 18.3 (s); MALDI calcd for $C_{29}H_{32}O_5SNa$ ($[M + Na]^+$) *m/e* 515.1863, measured *m/e* 515.1854.

Phenyl 4-*O*-((R)-2-Acetoxyl-3-azidopropyl)-2,3-di-*O*-benzyl-6-deoxy-1-thio-β-D-glucopyranoside (62). For the first step of the procedure, refer to the synthesis of **67**. To a solution of **67** (0.15 g, 0.28 mmol) in anhydrous CH_2Cl_2 (5 mL) were added DMAP (catalytic amount), Et_3N (0.12 mL, 0.84 mmol), and Ac_2O (0.053 mL, 0.56 mmol) at room temperature. After the reaction was completed (∼4 h), the reaction was quenched by addition of $NaHCO_3$ (saturated). After removal of solvent, the reaction mixture was diluted with EtOAc. The organic layer was washed with brine and dried over $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 65:35) afforded the product (0.13 g, 0.23 mmol, 80%, two-step yield: 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 5.01 (m, 1H), 4.92 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.90 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.79 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.71 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.64 (d, J = 9.7 Hz, 1H, H-1), 3.93 (dd, J = 10.2 Hz, J = 4.8 Hz, 1H), 3.74 (dd, J = 10.2 Hz,

J = 5.6 Hz, 1H), 3.58 (dd, J = 8.1 Hz, J = 8.1 Hz, 1H), 3.3−3.5 (m, 4H), 3.04 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 2.01 (s, 3H, CH_3CO$_2$), 1.36 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (s, CH_3CO_2), 138.6 (s), 138.2 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.1 (s), 128.0 (s), 127.8 (s), 127.7 (s), 87.7 (s), 86.5 (s), 84.3 (s), 81.5 (s), 75.8 (s), 75.6 (s), 75.5 (s), 71.8 (s), 71.7 (s), 50.9 (s), 21.0 (s, CH_3CO_2), 18.3 (s); MALDI calcd for $C_{31}H_{35}N_3O_6SNa$ ([M + Na]$^+$) *m/e* 600.2139, measured *m/e* 600.2144.

Phenyl 2,3-di-*O*-Benzyl-6-deoxy-4-*O*-((*S*)-2,3-diazidopropyl)-1-thio-*β*-D-glucopyranoside (63). For the first step of the procedure, refer to the synthesis of **67**. To a solution of **67** (0.20 g, 0.37 mmol) and pyridine (0.048 mL, 0.60 mmol) in anhydrous CH_2Cl_2 at 0 °C, Tf_2O (0.088 mL, 0.52 mmol) was added slowly. After the mixture was stirred for a half hour, TLC was performed. After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2, washed with water, saturated $NaHCO_{3(aq)}$, and brine, and then dried over $Na_2SO_{4(s)}$. The solution was filtered through glass wool and transferred into a solution of NaN_3 (0.20 g, 3.0 mmol) in DMF. The reaction mixture was stirred overnight while the solvents were slowly evaporated with aspirator. After most of the solvent was removed, the reaction mixture was diluted with EtOAc and filtered through Celite. Removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 65:35) afforded the product (0.13 g, 0.23 mmol, 62%, two-step yield: 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 4.96 (d, J = 10.6 Hz, 2H, PhCH_2O), 4.77 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.74 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.66 (d, J = 9.8 Hz, 1H, H-1), 3.94 (dd, J = 9.7 Hz, J = 6.0 Hz, 1H), 3.69 (dd, J = 9.7 Hz, J = 5.2 Hz, 1H), 3.62 (dd, J = 8.9 Hz, J = 8.8 Hz, 1H), 3.54 (m, 1H), 3.49 (dd, J = 9.6 Hz, J = 8.9 Hz, 1H), 3.39 (dq, J = 9.4 Hz, J = 6.2 Hz, 1H), 3.3−3.4 (m, 2H), 3.04 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 1.38 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5 (s), 138.1 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.8 (s), 128.6 (s), 128.4 (s), 128.2 (s), 128.1 (s), 128.0 (s), 127.7 (s), 87.7 (s), 86.4 (s), 84.1 (s), 81.7 (s), 75.8 (s), 75.6 (s), 75.4 (s), 72.6 (s), 61.0 (s), 51.7 (s), 18.4 (s); MALDI calcd for $C_{29}H_{32}N_6O_4SNa$ ([M + Na]$^+$) *m/e* 583.2098, measured *m/e* 583.2084.

Phenyl 2,3-Di-*O*-benzyl-6-deoxy-4-*O*-((*S*)-glycidyl)-1-thio-*β*-D-glucopyranoside (64). Refer to the synthesis of **61**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 4.93 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.90 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.86 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.76 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.67 (d, J = 9.8 Hz, 1H, H-1), 3.88 (dd, J = 11.1 Hz, J = 3.3 Hz, 1H), 3.77 (dd, J = 11.1 Hz, J = 6.2 Hz, 1H), 3.63 (dd, J = 8.9 Hz, J = 9.0 Hz, 1H), 3.48 (dd, J = 8.9 Hz, J = 9.7 Hz, 1H), 3.40 (m, 1H), 3.1−3.2 (m, 2H), 2.79 (dd, J = 4.6 Hz, J = 4.9 Hz, 1H), 2.57 (dd, J = 4.9 Hz, J = 2.6 Hz, 1H), 1.40 (d, J = 6.1 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6 (s), 138.3 (s), 134.1 (s), 132.1 (s), 131.9 (s), 129.1 (s), 128.6 (s), 128.4 (s), 128.2 (s), 127.9 (s), 127.7 (s), 87.7 (s), 86.5 (s), 84.3 (s), 81.4 (s), 76.0 (s), 75.7 (s, two carbons), 74.2 (s), 50.8 (s), 44.7 (s), 18.3 (s); LRFAB *m/e* 515 ([M + Na]$^+$); HRFAB calcd for $C_{29}H_{32}O_5S$ Na ([M + Na]$^+$) *m/e* 515.1868, measured *m/e* 515.1871.

Phenyl 4-*O*-((*S*)-2-Acetoxyl-3-azidopropyl)-2,3-di-*O*-benzyl-6-deoxy-1-thio-*β*-D-glucopyranoside (65). Refer to the synthesis of **62**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 5.05 (m, 1H), 4.93 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.91 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.77 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.72 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.65 (d, J = 9.7 Hz, 1H, H-1), 3.99 (dd, J = 10.1 Hz, J = 5.1 Hz, 1H), 3.73 (dd, J = 10.1 Hz, J = 5.3 Hz, 1H), 3.58 (dd, J = 9.9 Hz, J = 8.9 Hz, 1H), 3.3−3.5 (m, 4H), 3.03 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 2.08 (s, 3H, CH_3CO$_2$), 1.36 (d, J = 6.1 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (s, CH_3CO_2), 138.4 (s), 138.2 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.1 (s), 127.7 (s), 87.7 (s), 86.4 (s), 84.2 (s), 81.5 (s), 75.8 (s), 75.6 (s), 75.5 (s), 71.6 (s), 71.4 (s), 50.9 (s), 21.1 (s, CH_3CO_2), 18.3 (s); LRFAB *m/e* 600 ([M + Na]$^+$); HRFAB calcd for $C_{31}H_{35}N_3O_6SNa$ ([M + Na]$^+$) *m/e* 600.2144, measured *m/e* 600.2168.

Phenyl 2,3-di-*O*-Benzyl-6-deoxy-4-*O*-((*R*)-2,3-diazidopropyl)-1-thio-*β*-D-glucopyranoside (66). Refer to the synthesis of **63**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 4.95 (d, J = 10.8 Hz, 2H, PhCH_2O), 4.75 (d, J = 10.8 Hz, 1H, PhCH_2O), 4.73 (d, J = 10.8 Hz, 1H, PhCH_2O), 4.66 (d, J = 9.8 Hz, 1H, H-1), 3.91 (dd, J = 9.6 Hz, J = 4.1 Hz, 1H), 3.6−3.7 (m, 2H), 3.5−3.6 (m, 2H), 3.40 (m, 1H), 3.29 (dd, J = 12.7 Hz, J = 4.9 Hz, 1H), 3.22 (dd, J = 12.7 Hz, J = 6.9 Hz, 1H), 3.03 (dd, J = 9.2 Hz, J = 9.1 Hz, 1H), 1.38 (d, J = 6.1 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6 (s), 138.1 (s), 134.1 (s), 132.0 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.1 (s), 128.0 (s), 127.96 (s), 127.7 (s), 87.7 (s), 86.6 (s), 84.2 (s), 81.7 (s), 75.9 (s), 75.6 (s), 75.4 (s), 73.3 (s), 61.3 (s), 51.7 (s), 18.4 (s); LRFAB *m/e* 583 ([M + Na]$^+$); HRFAB calcd for $C_{29}H_{32}N_6O_4SNa$ ([M + Na]$^+$) *m/e* 583.2103, measured *m/e* 583.2085.

Phenyl 4-*O*-((*R*)-3-Azido-2-hydroxypropyl)-2,3-Di-*O*-benzyl-6-deoxy-1-thio-*β*-D-glucopyranoside (67). To a solution of **64** (0.1 g, 0.20 mmol), $CeCl_3 \cdot 7H_2O$ (0.04 g, 0.10 mmol) in CH_3CN/H_2O (4.5 mL/0.5 mL), NaN_3 (0.02 g, 0.22 mmol) was added. The reaction mixture was stirred for 24 h under reflux till the completion of the reaction. After removal of solvent, the residue was diluted with EtOAc. The organic layer was washed with 1 N HCl, water, saturated $NaHCO_{3(aq)}$, and brine and then dried over $Na_2SO_{4(s)}$. After removal of the solvent followed by purification with gradient column chromatography (hexanes/EtOAc = 100:0 to 60:40), the product was obtained (0.07 g, 0.13 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.5 (m, 15H), 4.97 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.96 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.76 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.72 (d, J = 10.3 Hz, 1H, PhCH_2O), 4.65 (d, J = 9.7 Hz, 1H, H-1), 3.7−3.8 (m, 2H), 3.67 (m, 1H), 3.61 (dd, J = 8.9 Hz, J = 9.0 Hz, 1H), 3.50 (dd, J = 8.9 Hz, J = 9.6 Hz, 1H), 3.37 (dq, J = 9.3 Hz, J = 6.2 Hz, 1H), 3.22 (m, 2H), 3.09 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 1.37 (d, J = 6.2 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1 (s), 138.0 (s), 134.0 (s), 132.1 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.1 (s), 128.0 (s), 127.8 (s), 87.8 (s), 86.0 (s), 84.3 (s), 81.7 (s), 75.9 (s), 75.8 (s), 75.5 (s), 74.5 (s), 70.2 (s), 53.3 (s), 18.4 (s); MALDI calcd for $C_{29}H_{32}N_3O_5$-SNa ([M + Na]$^+$) *m/e* 558.2033, measured *m/e* 558.2024.

Phenyl 4-*O*-((*R*)-3-Azido-2-((*R*)-glycidyl)propyl)-2,3-di-*O*-benzyl-6-deoxy-1-thio-*β*-D-glucopyranoside (68). Refer to the synthesis of **61**. ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2−7.5 (m, 15H), 4.90 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.88 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.76 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.69 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.62 (d, J = 9.6 Hz, 1H, H-1), 3.8−3.9 (m, 2H), 3.3−3.7 (m, 8H), 3.09 (m, 1H), 3.00 (dd, J = 9.2 Hz, J = 9.2 Hz, 1H), 2.73 (dd, J = 4.9 Hz, J = 4.0 Hz, 1H), 2.51 (dd, J = 4.9 Hz, J = 3.0 Hz, 1H), 1.35 (d, J = 6.3 Hz, 3H, H-6); ^{13}C NMR (68 MHz, $CDCl_3$) δ 138.5 (s), 138.0 (s), 133.9 (s), 131.9 (s), 129.0 (s), 128.54 (s), 128.50 (s), 128.3 (s), 127.95 (s), 127.86 (s), 127.6 (s), 87.5 (s), 86.3 (s), 84.1 (s), 81.3 (s), 78.7 (s), 75.6 (s), 75.5 (s, two carbons), 72.9 (s), 71.5 (s), 51.9 (s), 50.9 (s), 44.2 (s), 18.3 (s); LRFAB *m/e* 614 ([M + Na]$^+$); HRFAB calcd for $C_{32}H_{37}N_3O_6SNa$ ([M + Na]$^+$) *m/e* 614.2300, measured *m/e* 614.2293.

Phenyl 4-*O*-((*R*)-2-((*R*)-2-Acetoxyl-3-azidopropyl)-3-azidopropyl)-2,3-di-*O*-benzyl-6-deoxy-1-thio-*β*-D-glucopyranoside (69). Refer to the synthesis of **62**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 15H), 5.02 (m, 1H), 4.93 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.91 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.76 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.71 (d, J = 10.2 Hz, 1H, PhCH_2O), 4.65 (d, J = 9.7 Hz, 1H, H-1), 3.82 (dd, J = 9.9 Hz, J = 4.8 Hz, 1H), 3.6−3.7 (m, 3H), 3.58 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.4−3.5 (m, 4H), 3.37 (m, 1H), 3.2−3.3 (m, 2H), 2.98 (dd, J = 9.1 Hz, J = 9.1 Hz, 1H), 2.08 (s, 3H, CH_3CO$_2$), 1.36 (d, J = 6.1 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (s, CH_3CO_2), 138.7 (s), 138.2 (s), 134.1 (s), 132.1 (s), 129.1 (s), 128.7 (s), 128.6 (s), 128.4 (s), 128.1 (s), 127.9 (s), 127.7 (s), 87.7 (s), 86.5 (s), 84.3 (s), 81.6 (s), 79.4 (s), 75.7 (s), 75.5 (s), 73.2 (s), 71.4 (s), 69.0 (s), 52.0 (s), 50.9 (s), 21.1 (s, CH_3CO_2), 18.4 (s); MALDI calcd for $C_{34}H_{40}N_6O_7SNa$ ([M + Na]$^+$) *m/e* 699.2571, measured *m/e* 699.2541.

6-*O*-(4-*O*-(3-Azidopropyl)-2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (70). Refer to

the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.4 (m, 10H), 5.70 (d, J = 3.7 Hz, 1H, H-1′), 4.95 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.94 (d, J = 3.6 Hz, 1H, H-1″), 4.76 (d, J = 11.8 Hz, 1H, PhCH_2O), 4.74 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.70 (d, J = 11.8 Hz, 1H, PhCH_2O), 4.17 (m, 1H), 3.9−4.0 (m, 3H), 3.3−3.7 (m, 14H), 2.96 (dd, J = 9.3 Hz, J = 9.3 Hz, 1H), 2.34 (ddd, J = 13.0 Hz, J = 4.4 Hz, J = 4.4 Hz, 1H, H-2$_{eq}$), 1.7−1.8(m, 2H), 1.53 (ddd, J = 13.0 Hz, J = 12.8 Hz, J = 12.8 Hz, 1H, H-2$_{ax}$), 1.27 (d, J = 6.1 Hz, 3H,H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (s), 138.2 (s), 128.7 (s), 128.6 (s), 128.1 (s), 127.9 (s), 98.6 (s), 98.3 (s), 86.5 (s), 85.1 (s), 80.9 (s), 80.1 (s), 79.8 (s), 76.1 (s), 75.7 (s), 73.6 (s), 71.8 (s), 71.6 (s), 71.3 (s), 70.2 (s), 68.7 (s), 63.3 (s), 59.5 (s), 59.3 (s), 51.5 (s), 48.5 (s), 32.5 (s), 29.9 (s), 18.0 (s); MALDI calcd for $C_{35}H_{45}N_{15}O_{10}Na$ ([M + Na]$^+$) *m/e* 858.3366, measured *m/e* 858.3392.

6-*O*-(4-*O*-((*R*)-3-Azido-2-hydroxypropyl)-2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (71). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.4 (m, 10H), 5.69 (d, J = 3.7 Hz, 1H, H-1′), 5.01 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.95 (d, J = 3.5 Hz, 1H, H-1″), 4.74 (d, J = 11.7 Hz, 1H, PhCH_2O), 4.72 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.70 (d, J = 11.7 Hz, 1H, PhCH_2O), 4.19 (m, 1H), 3.9−4.0 (m, 3H), 3.2−3.8 (m, 15H), 3.03 (dd, J = 9.3 Hz, J = 9.4 Hz, 1H), 2.35 (ddd, J = 13.0 Hz, J = 4.5 Hz, J = 4.5 Hz, 1H, H-2$_{eq}$), 1.52 (ddd, J = 13.0 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2$_{ax}$), 1.29 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1 (s), 137.7 (s), 128.54 (s), 128.49 (s), 128.2 (s), 128.1 (s), 128.0 (s), 127.9 (s), 98.2 (s), 98.1 (s), 86.3 (s), 84.2 (s), 80.2 (s, two carbons), 79.7 (s), 75.9 (s), 75.6 (s), 74.4 (s), 73.4 (s), 71.6 (s), 71.4 (s), 71.1 (s), 70.0 (s), 68.6 (s), 63.2 (s), 59.4 (s), 59.0 (s), 53.0 (s), 51.3 (s), 32.3 (s), 17.8 (s); MALDI calcd for $C_{35}H_{45}N_{15}O_{11}$-Na ([M + Na]$^+$) *m/e* 874.3315, measured *m/e* 874.3298.

6-*O*-(2,3-Di-*O*-benzyl-6-deoxy-4-*O*-((*S*)-2,3-diazidopropyl)-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (72). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.6 (m, 10H), 5.69 (d, J = 3.7 Hz, 1H, H-1′), 4.99 (d, J = 11.3 Hz, 1H, PhCH_2O), 4.95 (d, J = 3.5 Hz, 1H, H-1″), 4.75 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.72 (d, J = 12.2 Hz, 1H, PhCH_2O), 4.71 (d, J = 11.3 Hz, 1H, PhCH_2O), 4.18 (m, 1H), 3.9−4.0 (m, 3H), 3.3−3.7 (m, 15H), 2.97 (dd, J = 9.4 Hz, J = 9.1 Hz, 1H), 2.35 (ddd, J = 13.0 Hz, J = 4.3 Hz, J = 4.3 Hz, 1H, H-2$_{eq}$), 1.52 (ddd, J = 13.0 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2$_{ax}$), 1.28(d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7 (s), 138.0 (s), 128.72 (s), 128.69 (s), 128.26 (s), 128.19 (s), 128.0 (s), 98.4 (s), 98.3 (s), 86.5 (s), 84.0 (s), 80.7 (s), 80.2 (s), 79.9 (s), 76.1 (s), 75.7 (s), 73.6 (s), 72.6 (s), 71.8 (s), 71.6 (s), 71.3 (s), 68.4 (s), 63.4 (s), 60.9 (s), 59.5 (s), 59.3 (s), 51.8 (s), 51.5 (s), 32.5 (s), 18.0 (s); MALDI calcd for $C_{35}H_{44}N_{18}O_{10}Na$ ([M + Na]$^+$) *m/e* 899.3380, measured *m/e* 899.3353.

6-*O*-(4-*O*-((*S*)-3-Azido-2-hydroxypropyl)-2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (73). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.5 (m, 10H), 5.70 (d, J = 3.8 Hz, 1H, H-1′), 5.00 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.93 (d, J = 3.6 Hz, 1H, H-1″), 4.75 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.74 (d, J = 11.6 Hz, 1H, PhCH_2O), 4.70 (d, J = 11.6 Hz, 1H, PhCH_2O), 4.19 (m, 1H), 3.9−4.0 (m, 3H), 3.80 (m, 1H), 3.5−3.7 (m, 10H), 3.39 (m, 1H), 3.2−3.3 (m, 3H), 3.11 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H), 2.35 (ddd, J = 13.3 Hz, J = 4.5 Hz, J = 4.5 Hz, 1H, H-2$_{eq}$), 1.52 (ddd, J = 13.3 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2$_{ax}$), 1.28 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1 (s), 137.9 (s), 128.74 (s), 128.67 (s), 128.54 (s), 128.28 (s), 128.22 (s), 128.17 (s), 98.5 (s), 98.3 (s), 86.7 (s), 84.5 (s), 80.3 (s, two carbons), 79.9 (s), 76.1 (s), 76.0 (s), 75.5 (s), 73.6 (s), 71.8 (s), 71.6 (s), 71.2 (s), 70.8 (s), 68.9 (s), 63.4 (s), 59.6 (s), 59.2 (s), 53.1 (s), 51.5 (s), 32.5 (s), 17.9 (s); MALDI calcd for $C_{35}H_{45}N_{15}O_{11}Na$ ([M + Na]$^+$) *m/e* 874.3315, measured *m/e* 874.3293.

6-*O*-(2,3-Di-*O*-benzyl-6-deoxy-4-*O*-((*R*)-2,3-diazidopropyl)-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (74). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.5 (m, 10H), 5.70 (d, J = 3.8 Hz, 1H, H-1′), 4.99 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.94 (d, J = 3.6 Hz, 1H, H-1″), 4.76 (d, J = 11.8 Hz, 1H, PhCH_2O), 4.72 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.71 (d, J = 11.8 Hz, 1H, PhCH_2O), 4.18 (m, 1H), 3.9−4.0 (m, 3H), 3.68 (dd, J = 8.9 Hz, J = 8.9 Hz, 1H), 3.4−3.6 (m, 10H), 3.2−3.3 (m, 4H), 2.96 (dd, J = 9.2 Hz, J = 9.4 Hz, 1H), 2.55 (ddd, J = 13.3 Hz, J = 4.0 Hz, J = 4.0 Hz, 1H, H-2$_{eq}$), 1.49 (ddd, J = 13.3 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2$_{ax}$), 1.29 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8 (s), 138.0 (s), 128.72 (s), 128.66 (s), 128.51 (s), 128.24 (s), 128.17 (s), 127.98 (s), 98.5 (s), 98.3 (s), 86.6 (s), 84.0 (s), 80.9 (s), 80.2 (s), 79.9 (s), 76.1 (s), 75.7 (s), 73.6 (s), 73.3 (s), 71.8 (s), 71.6 (s), 71.2 (s), 68.5 (s), 63.4 (s), 61.3 (s), 59.5 (s), 59.3 (s), 51.7 (s), 51.5 (s), 32.5 (s), 18.0 (s); MALDI calcd for $C_{35}H_{44}N_{18}O_{10}Na$ ([M + Na]$^+$) *m/e* 899.3380, measured *m/e* 899.3387.

6-*O*-(4-*O*-((*R*)-3-Azido-2-((*R*)-3-azido-2-hydroxypropyl)-propyl)-2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (75). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.4 (m, 10H), 5.69 (d, J = 3.7 Hz, 1H, H-1′), 5.00 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.97 (d, J = 3.6 Hz, 1H, H-1″), 4.74 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.71 (d, J = 11.4 Hz, 2H, PhCH_2O), 4.18 (m, 1H), 3.8−4.0 (m, 6H), 3.69 (dd, J = 9.0 Hz, J = 8.8 Hz, 1H), 3.4−3.6 (m, 10H), 3.2−3.4 (m, 6H), 2.97 (dd, J = 9.3 Hz, J = 9.3 Hz, 1H), 2.34 (ddd, J = 12.7 Hz, J = 4.5 Hz, J = 4.5 Hz, 1H, H-2$_{eq}$), 1.51 (ddd, J = 12.7 Hz, J = 12.7 Hz, J = 12.7 Hz, 1H, H-2$_{ax}$), 1.28 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (s), 138.0 (s), 128.7 (s), 128.6 (s), 128.20 (s), 128.16 (s), 128.0 (s), 127.9 (s), 127.6 (s), 98.3 (s, two carbons), 86.2 (s), 84.1 (s), 80.8 (s), 80.1 (s), 80.0 (s), 79.7 (s), 76.0 (s), 75.6 (s), 73.5 (s), 73.4 (s), 72.3 (s), 71.8 (s), 71.6 (s), 71.3 (s), 70.1 (s), 68.3 (s), 63.4 (s), 59.5 (s), 59.2 (s), 53.4 (s), 52.2 (s), 51.5 (s), 32.5 (s), 18.1 (s); MALDI calcd for $C_{38}H_{50}N_{18}O_{12}Na$ ([M + Na]$^+$) *m/e* 973.3748, measured *m/e* 973.3740.

6-*O*-(3-Azido-4-*O*-((*S*)-3-azido-2-hydroxypropyl)-2-*O*-benzyl-3,6-dideoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (76). Refer to the general procedure for glycosylation and hydrolysis. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4−7.5 (m, 5H), 5.60 (d, J = 3.6 Hz, 1H, H-1′), 4.98 (d, J = 3.5 Hz, 1H, H-1″), 4.76 (d, J = 11.7 Hz, 1H, PhCH_2O), 4.73 (d, J = 11.7 Hz, 1H, PhCH_2O), 4.36 (d, J = 2.0 Hz, 1H), 4.17 (m, 1H), 3.9−4.0 (m, 3H), 3.87 (dd, J = 10.0 Hz, J = 10.0 Hz, 1H), 3.7−3.8 (m, 2H), 3.65 (m, 1H), 3.3−3.6 (m, 9H), 3.27 (dd, J = 9.4 Hz, J = 9.5 Hz, 1H), 2.83 (dd, J = 9.7 Hz, J = 9.7 Hz, 1H), 2.36 (ddd, J = 13.5 Hz, J = 4.2 Hz, J = 4.2 Hz, 1H, H-2$_{eq}$), 1.53 (ddd, J = 13.5 Hz, J = 12.9 Hz, J = 12.9 Hz, 1H, H-2$_{ax}$), 1.27 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.3 (s), 128.8 (s), 128.5 (s), 128.4 (s), 98.5 (s), 97.2 (s), 85.2 (s), 83.2 (s), 80.7 (s), 78.3 (s), 75.8 (s), 75.0 (s), 73.4 (s), 72.1 (s), 71.6 (s), 71.3 (s), 70.4 (s), 68.3 (s), 64.2 (s), 63.5 (s), 59.6 (s), 59.1 (s), 53.4 (s), 51.5 (s), 32.4 (s), 18.0 (s); MALDI calcd for $C_{28}H_{38}N_{18}O_{10}Na$ ([M + Na]$^+$) *m/e* 809.2911, measured *m/e* 809.2929.

6-*O*-(4-*O*-(3-Aminopropyl)-6-deoxy-α-D-glucopyranosyl)-neamine (49). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.96 (d, J = 3.1 Hz, 1H, H-1′), 4.96 (d, J = 2.8 Hz, 1H, H-1″), 3.8−4.0 (m,8H), 3.4−3.7 (m, 9H), 3.26 (dd, J = 13.6 Hz, J = 6.9 Hz, 1H), 3.10 (dd, J = 7.3 Hz,, J = 7.0 Hz, 2H), 3.04 (dd, J = 9.6 Hz, J = 9.4 Hz, 1H), 2.50 (ddd, J = 12.3 Hz, J = 4.2 Hz, J = 4.2 Hz, 1H, H-2$_{eq}$), 1.92 (m, 1H, H-2$_{ax}$), 1.25 (d, J = 5.9 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.8 (s), 95.9 (s), 83.8 (s), 83.7 (s), 77.6 (s), 74.3 (s), 72.5 (s), 72.2 (s), 70.9 (s), 70.6 (s), 69.5 (s), 68.5 (s), 68.3 (s), 53.7 (s), 50.2 (s), 48.6 (s), 40.4 (s), 37.9 (s), 28.3 (s), 27.4 (s), 17.3 (s); LRFAB *m/e* 526 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{44}N_5O_{10}$ ([M + H]$^+$) *m/e* 526.3088, measured *m/e* 526.3065.

6-*O*-(4-*O*-((*R*)-3-Amino-2-hydroxylpropyl)-6-deoxy-α-D-glucopyranosyl)neamine (50). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.99 (d, J = 3.9 Hz, 1H, H-1′), 4.98 (d, J = 3.8 Hz, 1H, H-1″), 4.0−4.1 (m, 5H), 3.7−3.9 (m, 6H),

3.5−3.6 (m, 5H), 3.28 (dd, J = 13.6 Hz, J = 6.9 Hz, 1H), 3.19 (dd, J = 13.2 Hz, J = 3.3 Hz, 1H), 3.09 (dd, J = 9.5 Hz, J = 9.4 Hz, 1H), 3.03 (dd, J = 13.2 Hz, J = 9.2 Hz, 1H), 2.53 (ddd, J = 12.6 Hz, J = 4.0 Hz, J = 4.0 Hz, 1H, H-2_{eq}), 1.97 (ddd, J = 12.6 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2_{ax}), 1.27 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.8 (s), 95.9 (s), 84.1 (s), 83.8 (s), 77.4 (s), 74.3 (s, two carbons), 72.6 (s), 72.2 (s), 70.9 (s), 69.5 (s), 68.5 (s), 68.3 (s), 67.1 (s), 53.7 (s), 50.2 (s), 48.7 (s), 42.2 (s), 40.5 (s), 28.1 (s), 17.3 (s); LRFAB *m/e* 542 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{44}N_5O_{11}$ ([M + H]$^+$) *m/e* 542.3037, measured *m/e* 542.3055.

6-*O*-(6-Deoxy-4-*O*-((*S*)-2,3-diaminopropyl)-α-D-glucopyranosyl)neamine (51). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.98 (d, J = 3.7 Hz, 1H, H-1′), 4.98 (d, J = 3.6 Hz, 1H, H-1″), 4.0−4.1 (m, 7H), 3.7−3.9 (m, 4H), 3.3−3.6 (m, 7H), 3.29 (dd, J = 13.5 Hz, J = 7.0 Hz, 1H), 3.14 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H), 2.53 (ddd, J = 12.7 Hz, J = 3.9 Hz, J = 3.9 Hz, 1H, H-2_{eq}), 1.97 (ddd, J = 12.7 Hz, J = 12.5 Hz, J = 12.5 Hz, 1H, H-2_{ax}), 1.27 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.9 (s), 95.9 (s), 83.81 (s), 83.78 (s), 77.4 (s), 74.3 (s), 72.7 (s), 72.2 (s), 70.9 (s), 69.5 (s, two carbons), 68.4 (s), 68.2 (s), 53.7 (s), 50.2 (s), 49.2 (s), 48.7 (s), 40.4 (s), 38.8 (s), 28.1 (s), 17.4 (s); LRFAB *m/e* 541 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{45}N_6O_{10}$ ([M + H]$^+$) *m/e* 541.3197, measured *m/e* 541.3192.

6-*O*-(4-*O*-((*S*)-3-Amino-2-hydroxylpropyl)-6-deoxy-α-D-glucopyranosyl)neamine (52). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.93 (d, J = 3.9 Hz, 1H, H-1′), 4.96 (d, J = 3.7 Hz, 1H, H-1″), 3.9−4.1 (m, 5H), 3.7−3.8 (m, 6H), 3.4−3.5 (m, 5H), 3.26 (dd, J = 13.6 Hz, J = 6.9 Hz, 1H), 3.18 (dd, J = 13.2 Hz, J = 3.3 Hz, 1H), 3.06 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H), 3.02 (dd, J = 13.2 Hz, J = 8.8 Hz, 1H), 2.47 (ddd, J = 12.6 Hz, J = 3.9 Hz, J = 3.9 Hz, 1H, H-2_{eq}), 1.88 (ddd, J = 12.6 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2_{ax}), 1.26 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.8 (s), 96.0 (s), 84.0 (s), 83.9 (s), 78.1 (s), 74.4 (s), 74.3 (s), 72.6 (s), 72.1 (s), 70.9 (s), 69.4 (s), 68.6 (s), 68.3 (s), 66.9 (s), 53.8 (s), 50.2 (s), 48.6 (s), 42.2 (s), 40.4 (s), 28.6 (s), 17.3 (s); LRFAB *m/e* 542 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{44}N_5O_{11}$ ([M + H]$^+$) *m/e* 542.3037, measured *m/e* 542.3018.

6-*O*-(6-Deoxy-4-*O*-((*R*)-2,3-diaminopropyl)-α-D-glucopyranosyl)neamine (53). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.97 (d, J = 3.9 Hz, 1H, H-1′), 4.96 (d, J = 3.7 Hz, 1H, H-1″), 4.0−4.1 (m, 6H), 3.8−3.9 (m, 3H), 3.7−3.8 (m, 2H), 3.5−3.6 (m, 2H), 3.4−3.5 (m, 5H), 3.26 (dd, J = 13.6 Hz, J = 7.0 Hz, 1H), 3.13 (dd, J = 9.4 Hz, J = 9.4 Hz, 1H), 2.50 (ddd, J = 12.6 Hz, J = 3.9 Hz, J = 3.9 Hz, 1H, H-2_{eq}), 1.96 (ddd, J = 12.6 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2_{ax}), 1.25 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 101.8 (s), 95.8 (s), 83.8 (s, two carbons), 77.4 (s), 74.3 (s), 72.4 (s), 72.2 (s), 70.9 (s), 69.6 (s), 69.5 (s), 68.5 (s), 68.1 (s), 53.8 (s), 50.2 (s), 49.2 (s), 48.7 (s), 40.5 (s), 39.1 (s), 28.2 (s), 17.4 (s); LRFAB *m/e* 541 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{45}N_6O_{10}$ ([M + H]$^+$) *m/e* 541.3197, measured *m/e* 541.3190.

6-*O*-(4-*O*-((*R*)-3-Amino-2-((*R*)-3-amino-2-hydroxylpropyl)-propyl)-6-deoxy-α-D-glucopyranosyl)neamine (54). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.95 (d, J = 3.9 Hz, 1H, H-1′), 4.94 (d, J = 3.8 Hz, 1H, H-1″), 3.9−4.0 (m, 6H), 3.7−3.9 (m, 8H), 3.5−3.6 (m, 2H), 3.2−3.3 (m, 4H), 3.0−3.1 (m, 2H), 2.49 (ddd, J = 12.6 Hz, J = 4.1 Hz, J = 4.1 Hz, 1H, H-2_{eq}), 1.92 (ddd, J = 12.6 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2_{ax}), 1.22 (d, J = 6.2 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 102.8 (s), 95.9 (s), 83.8 (s), 77.4 (s), 75.4 (s), 74.3 (s), 72.5 (s), 72.3 (s), 71.1 (s), 70.9 (s), 70.8 (s), 69.5 (s), 68.4 (s), 68.1 (s), 66.9 (s), 53.7 (s), 50.1 (s), 48.6 (s), 42.0 (s, two carbons), 40.9 (s), 40.5 (s), 28.1 (s), 17.4 (s); LRFAB *m/e* 615 ([M + H]$^+$); HRFAB calcd for $C_{24}H_{51}N_6O_{12}$ ([M + H]$^+$) *m/e* 615.3565, measured *m/e* 615.3589.

6-*O*-(3-Amino-4-*O*-((*S*)-3-amino-2-hydroxylpropyl)-3,6-dideoxy-α-D-glucopyranosyl)neamine (55). Refer to the general procedure for the synthesis kanamycin B analogues. ^{1}H NMR (400 MHz, D_2O) (chloride salt) δ 5.98 (d, J = 3.9 Hz, 1H, H-1′), 5.03 (d, J = 3.7 Hz, 1H, H-1″), 3.9−4.2 (m, 6H), 3.8−3.9 (m, 2H), 3.79 (dd, J = 10.1 Hz, J = 9.1 Hz, 1H), 3.70 (dd, J = 10.5 Hz, J = 6.5 Hz, 1H), 3.4−3.6 (m, 6H), 3.37 (dd, J = 9.9 Hz, J = 10.0 Hz, 1H), 3.30 (dd, J = 13.6 Hz, J = 6.6 Hz, 1H), 3.17 (dd, J = 13.3 Hz, J = 9.9 Hz, 1H), 3.01 (dd, J = 13.3 Hz, J = 9.1 Hz, 1H), 2.53 (ddd, J = 12.6 Hz, J = 4.2 Hz, J = 4.2 Hz, 1H, H-2_{eq}), 1.96 (ddd, J = 12.6 Hz, J = 12.6 Hz, J = 12.6 Hz, 1H, H-2_{ax}), 1.32 (d, J = 6.3 Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 100.8 (s), 96.0 (s), 83.9 (s), 80.0 (s), 77.4 (s), 74.5 (s), 73.6 (s), 70.8 (s), 69.5 (s), 68.52 (s), 68.44 (s, 2 carbons), 67.1 (s), 53.9 (s), 53.7 (s), 50.0 (s), 48.6 (s), 41.8 (s), 40.3 (s), 28.1 (s), 17.3 (s); LRFAB *m/e* 541 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{45}N_6O_{10}$ ([M + H]$^+$) *m/e* 541.3197, measured *m/e* 541.3191; LRFAB *m/e* 541 ([M + H]$^+$); HRFAB calcd for $C_{21}H_{45}N_6O_{10}$ ([M + H]$^+$) *m/e* 541.3197, measured *m/e* 541.3191.

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Supporting Information Available: HPLC and mass spectrometric results for kanamycin B analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reviews: (a) Haddad, J.; Kotra, L. P.; Mobashery, S. In *Glycochemistry: Principles, Synthesis, and Applications*; Wang, P. G., Bertozzi, C. R., Ed.; Marcel Dekker: New York, 2001; p307−424. (b) Kondo, S. Development of arbekacin and synthesis of new derivatives stable to enzymatic modifications by methicillin-resistant *Staphyloccus aureus*. *Jpn. J. Antibiot.* **1994**, *47*, 561−574. (c) Vakulenko, S. B.; Mobashery, S. Versatility of aminoglycosides and prospects for their future. *Clin. Microbiol. Rev*. **2003**, *16*, 430−450. (d) Hooper, I. R. *Aminoglycoside Antibiotics*; Springer-Verlag: New York, 1982.

(2) (a) Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M. Aminoglycosides: activity and resistance. *Antimicrob. Agents Chemother*. **1999**, *43*, 727−737. (b) Kotra, L. P.; Haddad, J.; Mobashery, S. Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob. Agents Chemother*. **2000**, *44*, 3249−3256. (c) Wright, G. D. Aminoglycoside-modifying enzymes. *Curr. Opin. Microbiol*. **1999**, *2*, 499−503.

(3) (a) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. Structure of the A Site of *Escherichia coli* 16*S* ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* **1996**, *274*, 1367−1371. (b) Ma, C.; Baker, N. A.; Joseph, S.; McCammon, J. A. Binding of aminoglycoside antibiotics to the small ribosomal subunit: A continuum electrostatics investigation. *J. Am. Chem. Soc.* **2002**, *124*, 1438−1442. (c) Fourmy, D.; Recht, M. I.; Puglisi, J. D. Binding of neomycin-class aminoglycoside antibiotics to the A-site of 16S rRNA. *J. Mol. Biol*. **1998**, *277*, 347−362.

(4) (a) Fong, D. H.; Berghuis, A. M. Substrate promiscuity of an aminoglycoside antibiotic resistance enzyme via target mimicry. *EMBO J.* **2002**, *21*, 2323−2331. (b) Owston, M. A.; Serpersu, E. H. Cloning overexpression, and purification of aminoglycoside antibiotic 3-acetyltransferase-IIIb: conformational studies with bound substrates. *Biochemistry* **2002**, *41*, 10764−10770. (c) Pedersen, L. C.; Benning, M. M.; Holden, H. M. Structural investigation of the antibiotic and ATP-binding sites in kanamycin nucleotidyltransferase. *Biochemistry* **1995**, *34*, 13305−13311. (d) Cox, J. R.; McKay, G. A.; Wright, G. D.; Serpersu, E. H. Arrangement of substrates at the active site of an aminoglycoside antibiotic 3′-pohosphotransferase as determined by NMR. *J. Am. Chem. Soc*. **1996**, *118*, 1295−1301. (e) Sakon, J.; Liao, H. H.; Kanikula, A. M.; Benning, M. M.; Rayment, I.; Holden, H. M. Molecular structure of kanamycin nucleotidyltransferase determined to 3.0 Å resolution. *Biochemistry* **1993**, *32*, 11977−11984. (f) DiGiammarino, E. L.; Draker, K.-a.; Wright, G. D.; Serpersu, E. H. Solution studies of isepamicin and conformational comparisons between isepamicin and buritiroson A when

bound to an aminoglycoside 6′-*N*-acetyltransferase determined by NMR spectroscopy. *Biochemistry* **1998**, *37*, 3638−3644. (g) Burk, D. L.; Hon, W. C.; Leung, A. K.-W.; Berghuis, A. M. Structural analyses of nucleotide binding to an aminoglycoside phosphotransferase. *Biochemistry* **2001**, *40*, 8756−8764.

(5) For examples, see the following. (a) Tanaka, H.; Nishida, Y.; Furuta, Y.; Kobayashi, K. A convenient synthesis pathway for multivalent assembly of aminoglycoside antibiotics starting from amikacin. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1723−1726. (b) Hanessian, S.; Tremblay, M.; Swayze, E. E. Tobramycin analogs with C-5 aminoalkyl ether chains intended to mimic rings III and IV of paromomycin. *Tetrahedron* **2003**, *59*, 983−993. (c) Hanessian, S.; Kornienko, A.; Swayze, E. E. Probing the functional requirements of the L-haba side-chain of amikacin-synthesis, 16S A-site rRNA binding, and antibacterial activity. *Tetrahedron* **2003**, *59*, 995−1007. (d) Seeberger, P. H.; Baumann, M.; Zhang, G.; Kanemitsu, T.; Swayze, E. E.; Hofstadler, S. A.; Griffey, R. H. Synthesis of neomycin analogs to investigate aminoglycoside−RNA interactions. *Synlett* **2003**, 1323−1326. (e) Chou, C.-H.; Wu, C.-S.; Chen, C.-H.; Lu, L.-D.; Kulkarni, S. S.; Wong, C.-H.; Hung, S.-C. Regioselective glycosylation of neamine core: a facile entry to kanamycin B related analogues. *Org. Lett*. **2004**, *6*, 585−588. (f) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Risen, L.; Griffey, R. H. Design and Synthesis of paromomycin-related heterocycle-substituted aminoglycoside mimetics based on a mass spectrometry RNA-binding assay. *Angew. Chem., Int. Ed*. **2003**, *42*, 3409−3412. (g) Francois, B.; Szychowski, J.; Adhikari, S. S.; Pachamuthu, K.; Swayze, E. E.; Griffey, R. H.; Migawa, M. T.; Westhof, E.; Hanessian, S. Antibacterial aminoglycosides with a modified mode of binding to the ribosomal-RNA decoding site. *Angew. Chem., Int. Ed*. **2004**, *43*, 6735−6738. (h) Fridman, M.; Belakhov, V.; Lee, L. V.; Liang, F.-S.; Wong, C.-H.; Baasov, T. Dual effect of synthetic aminoglycosides: antibacterial activity against *Bacillus anthracis* and inhibition of anthrax lethal factor. *Angew. Chem., Int. Ed*. **2005**, *44*, 447−452. (i) Fridman, M.; Belakhov, V.; Yaron, S.; Baasov, T. A new class of branched aminoglycosides: Pseudo-pentasaccharide derivatives of neomycin B. *Org, Lett*. **2003**, *5*, 3575−3578. (j) Liang, F.-S.; Wang, S.-K.; Nakatani, T.; Wong, C.-H. Targeting RNAs with tobramycin analogues. *Angew. Chem., Int. Ed*. **2004**, *43*, 6496−6500. (k) Agnelli, F.; Sucheck, S. J.; Marby, K. A.; Rabuka, D.; Yao, S.-L.; Sears, P. S.; Liang, F.-S.; Wong, C.-H. Dimeric aminoglycosides as antibiotics. *Angew. Chem., Int. Ed*. **2004**, *43*, 1562−1566. (l) Bryan, M. C.; Wong, C.-H. Aminoglycoside array for the high-throughput analysis of small molecule−RNA interactions. *Tetrahedron Lett.* **2004**, *45*, 3639−3642.

(6) Elchert, B.; Li, J.; Wang, J.; Hui, Y.; Rai, R.; Ptak, R.; Ward, P.; Takemoto, J. Y.; Bensaci, M.; Chang, C.-W. T. Application of the synthetic aminosugars for glycodiversification: Synthesis and antimicrobial studies of pyranmycin. *J. Org. Chem.* **2004**, *69*, 1513−1523.

(7) Li, J.; Wang, J.; Czyryca, P. G.; Chang, H.; Orsak, T. W.; Evanson, R.; Chang, C.-W. T. Application of glycodiversification: Expedient synthesis and antibacterial evaluation of a library of kanamycin B analogs. *Org. Lett.* **2004**, *6*, 1381−1384.

(8) Phenylthioglycopyranosides can be prepared from treating the corresponding acetyl glycopyranosides with HSPh and BF_3−OEt_3. For reference, see the following. Tai, C.-A.; Kulkarni, S. S.; Hung, S.-C. Facile $Cu(OTf)_2$-catalyzed preparation of per-*O*-acetylated hexopyranoses with stoichiometric acetic anhydride and sequential one-pot anomeric substitution to thioglycosides under solvent-free conditions. *J. Org. Chem.* **2003**, *68*, 8719−8722.

(9) Garegg, P. J. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 53−67.

(10) *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.

(11) One resistant strain is *E. coli* (TG1) equipped with the pTZ19U-3 plasmid encoded for APH(3′)-I. The other resistant strain is *E. coli* (TG1) equipped with the pSF815 plasmid encoded for AAC-(6′) and APH(2″).

(12) The structure is based on PDB 1L8U with bound neomycin B and ADP: Fong, D. H.; Berghuis, A. M. Substrate promiscuity of an aminoglycoside antibiotic resistance enzyme via target mimicry. *EMBO J*. **2002**, *21*, 2323−2331.

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