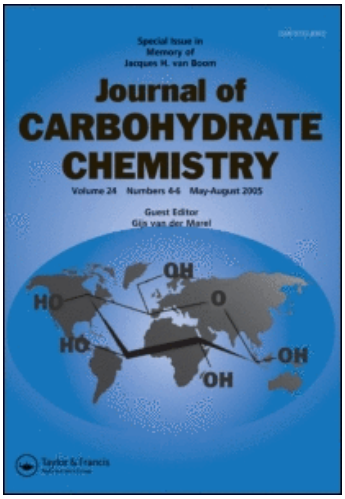

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Novel Method for the Synthesis of 3′,4′-Dideoxygenated Pyranmycin and Kanamycin Compounds, and Studies of Their Antibacterial Activity Against Aminoglycoside-Resistant Bacteria

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A novel protocol for converting a *trans*-diol to an alkene under mild conditions was developed. This method led to the synthesis of a 3′,4′-dideoxykanamycin (dibekacin) analog and a 3′,4′-dideoxypyranmycin that were found to be active against aminoglycoside-resistant bacteria.

Keywords Carbohydrate, Aminoglycoside antibiotics, Antibiotic, Drug resistance, Drug design, Pyranmycin, Kanamycin, Dideoxygenation

Aminoglycoside antibiotics have long been used as bactericidal drugs.[1] Unlike many antibiotics that are active only against gram-positive bacteria, aminoglycosides have broad-spectrum activity against both gram-positive and -negative bacteria. However, their clinical usage has often been limited only to serious infections due to the prevalence of aminoglycoside-resistant bacteria[1c,2] and

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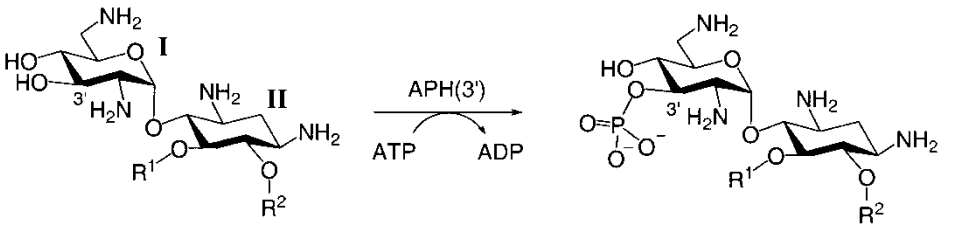
the high cytotoxicity of aminoglycosides.[3] In an effort to revive the effectiveness of aminoglycoside antibiotics against resistant bacteria, we have been working on modification and synthesis of neomycin[4] and kanamycin[5] classes of aminoglycosides. We wish to report two novel pyranmycin and kanamycin compounds with activity against resistant strains equipped with aminoglycoside-modifying enzymes.

Overexpression of aminoglycoside-modifying enzymes from resistant bacteria is the most commonly encountered mode of resistance.[2] Various aminoglycoside-modifying enzymes have been identified that catalyze a wide range of modifications including acetylation, phosphorylation, and adenylation. For example, one of the most prevalent modifying enzymes is APH(3′), which catalyzes phosphorylation at the 3′-OH of both neomycin and kanamycin classes of aminoglycosides, rendering the phosphorylated adduct incapable of binding toward the ribosomal target (Fig. 1).

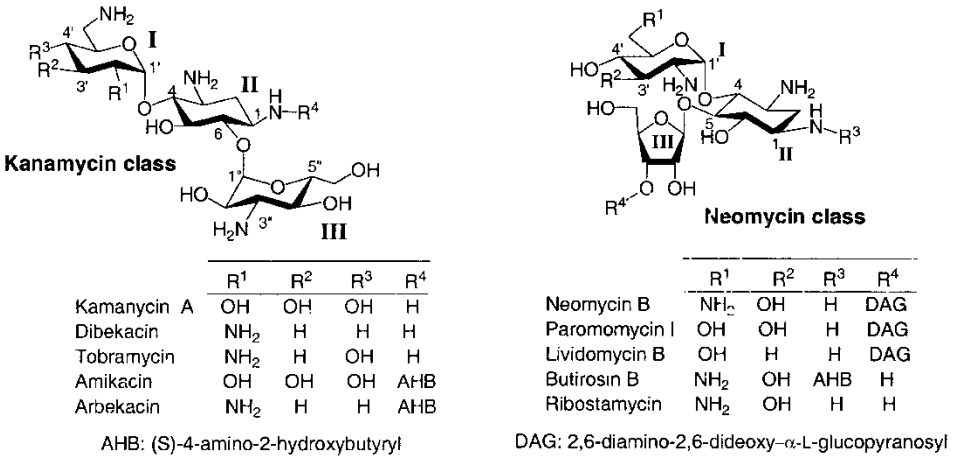
Aminoglycosides with deoxygenation at 3′-OH have been demonstrated to be effective against APH(3′) as reported by Umezawa[1b,6] and others.[7] The concept has led to the syntheses and discovery of tobramycin,[8] arbekacin,[9] and other similar aminoglycosides (Fig. 2).[1b] Despite the fruitful results from these studies, there are several shortcomings. First, most of the research uses carbamate-type protecting groups for the protection of amino groups on the aminoglycoside, resulting in the formation of polycarbamate compounds with low solubility in organic media. The poor solubility of these compounds poses difficulties in their purification and characterization. Second, most of the syntheses begin with the kanamycin scaffold. There are very few examples of deoxygenation on neomycin class antibiotics.[7d] Third, the reported syntheses of both classes of antibiotics usually derive from kanamycin or neomycin, which limits the options for introducing novel structural motifs at other desirable places of aminoglycosides.

In light of the existing deficiencies in the development of aminoglycosides with 3′-deoxygenation, we wish to report a novel method for convenient synthesis of both kanamycin and neomycin classes of aminoglycosides with



Kanamycin class (R^1 = H, R^2 = carbohydrate)
Neomycin class (R^1 = carbohydrate, R^2 = H)

Figure 1: Phosphorylation of kanamycin and neomycin classes aminoglycosides by APH(3′).



	R^1	R^2	R^3	R^4
Kamanycin A	OH	OH	OH	H
Dibekacin	NH_2	H	H	H
Tobramycin	NH_2	H	OH	H
Amikacin	OH	OH	OH	AHB
Arbekacin	NH_2	H	H	AHB

	R^1	R^2	R^3	R^4
Neomycin B	NH_2	OH	H	DAG
Paromomycin I	OH	OH	H	DAG
Lividomycin B	OH	H	H	DAG
Butirosin B	NH_2	OH	AHB	H
Ribostamycin	NH_2	OH	H	H

Figure 2: Structure of kanamycin and neomycin classes aminoglycosides bearing 3′ and/or 4′ deoxygenation.

3′,4′-dideoxygenation. Our group has developed a new family of neomycin class of aminoglycosides, pyranmycins, which bear a pyranose in the place of a furanose at the ring III position.[4] A lead structure has been identified. We have also developed an expedient method for preparation of kanamycin B analogs.[5] Combining our results from these two families of aminoglycosides, we intend to prepare the new aminoglycosides as indicated in Figure 3. Our goal is to utilize neamine as the common core for the synthesis of 3′,4′-dideoxygenation products of both classes of aminoglycosides.

To avoid the solubility problem, we used azido groups as the surrogate for amino groups. The synthesis of a key intermediate, 3′,4′-dideoxyneamine, began from neamine. Neamine was obtained from acid-hydrolysis of neomycin, then converted to tetraazidoneamine, **2**, using TfN_3 and $CuSO_4$ (Sch. 1).[5] The

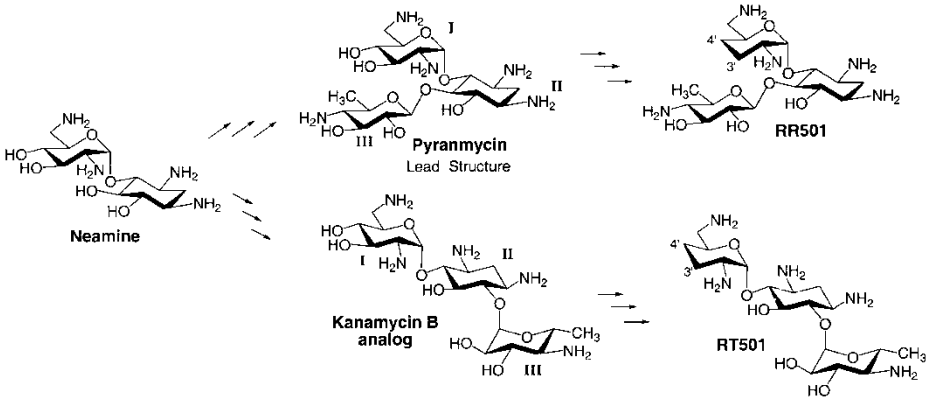
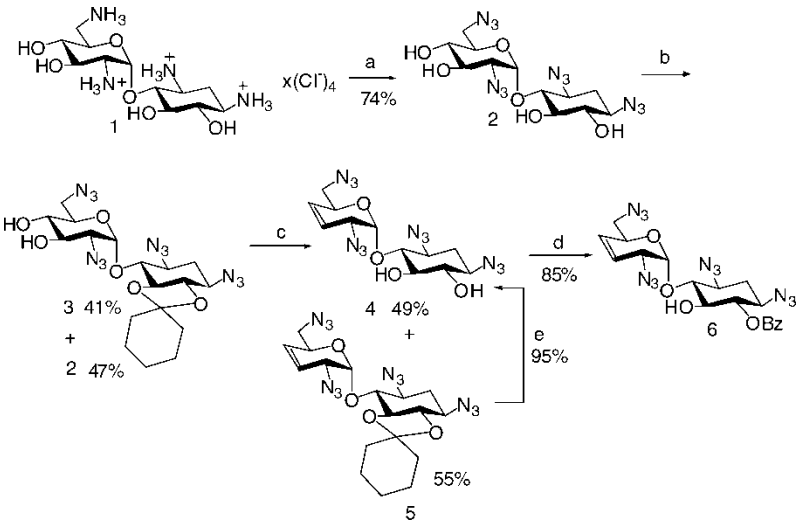


Figure 3: Proposed strategy for the synthesis of aminoglycoside with 3′,4′-dideoxygenation.



Scheme 1: Synthesis of neamine acceptor. Conditions: (a) TfN_3, $CuSO_4$, H_2O, CH_2Cl_2, (b) Cyclohexone dimethyl ketal, TsOH-H_2O, CH_3CN, (c) (1) Tf_2O, pyr., CH_2Cl_2; (2) $Na_2S_2O_3$, NaI, acetone, (d) BzCl, CH_2Cl_2, $-50^{\circ}C$, (e) H_2O, HOAc, dioxane, $65^{\circ}C$.

selective protection of the diol was achieved using cyclohexanone dimethylketal. The key transformation is the elimination of diol to alkene. To our surprise, we were unable to locate didexoygenation methods that are compatible with the presence of the azido group and the acid-labile glycoside bond despite numerous documentations. In general, the reported methods for dideoxygenation often require reductive or harsh conditions, for example, the presence of Zn, NaI, and heating from dimesylated compound (Tipson-Cohen method),[10] acid-catalyzed elimination from a diol using ethyl orthoformate (Crank-Eastwood method),[11] $LiAlH_4/TiCl_4$ (McMurry-Fleming method),[12] diphosphorous tetraiodide from diol (Kuhn-Winterstein reaction),[13] $SnCl_2/HCl$,[14] and PPh_3 and I_2.[15]

Among these reported methods, the method involving mesylated compound and Zn-mediated elimination appeared to be the most suitable one of being modified to meet our needs. Since a triflated hydroxyl group is more reactive than a mesylated hydroxyl group, we expected that the ditriflate could be replaced with a *trans* diiodide, in which the two iodides are in an antiparallel configuration (Fig. 4). Such a configuration can induce a facile elimination under the catalysis of I^-, producing the desired alkene and I_2. To avoid complication from the possible addition reaction between the alkene and I_2, $Na_2S_2O_3$ was added to reduce I_2 into I^-, allowing I_2/I^- to function as a catalyst. We were pleased to discover that the elimination occurred smoothly as expected, providing compounds **4** and **5**. Compound **5** was converted to **4**, giving an overall yield of $\sim$80%.

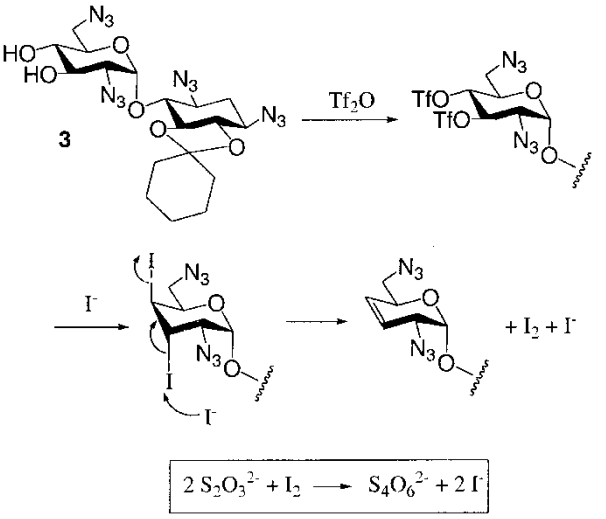
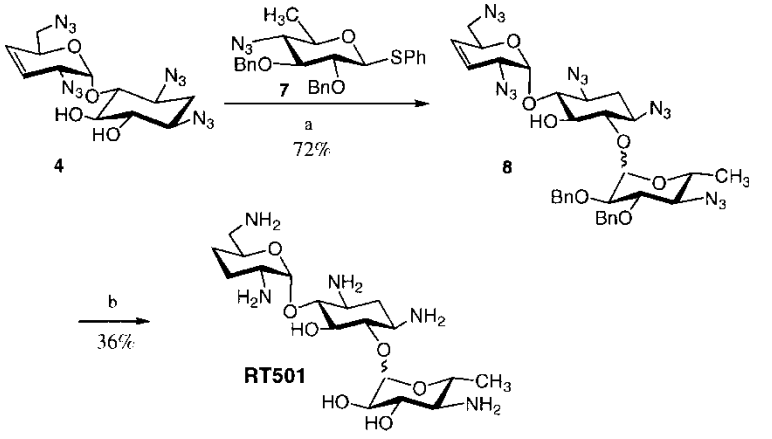


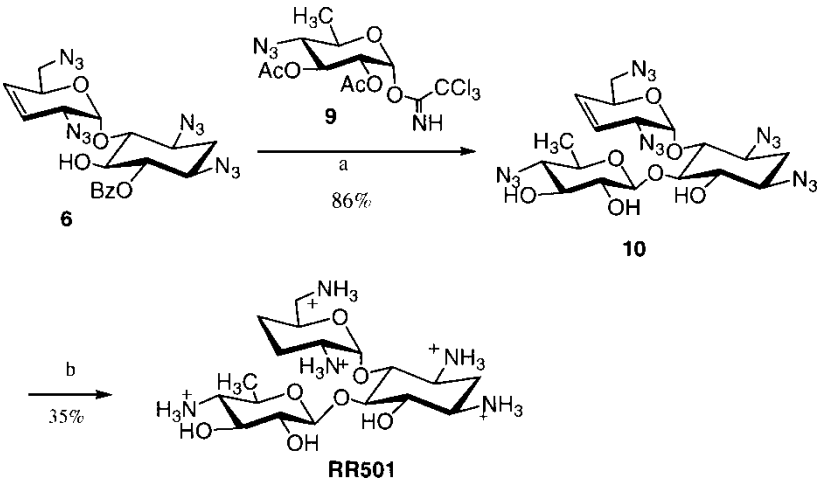
Figure 4: Proposed mechanism for elimination.

Regioselective glycosylation of **4** was obtained in a similar fashion as reported in our previous work generating compound **8** (Sch. 2). The glycosyl donor, **7**, was selected based on the ease of synthesis and the higher antibacterial activity from the same donor in our work on kanamycin B analogs. Compound **8** was subjected to Staudinger reaction, hydrogenation, and ion exchange, and the final product, **RT501** ($\alpha/\beta = 7/1$), was obtained as a chloride salt.

Regioselective protection of the C-6 hydoxyl group using benzoyl chloride furnished compound **6** in excellent yield (Sch. 3). Compound **6** was glycosylated



Scheme 2: Synthesis of 3′,4′-dideoxy kanamycin B analog. Conditions: (a) NIS, TfOH, Et_2O:CH_2Cl_2 (3:1), (b) (i) PMe_3, NaOH, THF, (ii) $Pd(OH)_2/C$, HOAc, H_2O, (iii) Dowex 1X8-200 (Cl^- form).



Scheme 3: Synthesis of 3′,4′-dideoxy pyranmycin. Conditions: (a) (i) BF_3-OEt_2, CH_2Cl_2, (ii) NaOMe, MeOH, (b) (i) PMe_3, THF, (ii) $Pd(OH)_2/C$, HOAc, H_2O, (iii) Dowex 1X8-200 (Cl^- form).

with the corresponding trichloroacetimidate donor, **9**, from the lead structure of our previous work, generating the 3′,4′-dideoxy pyranmycin adduct **RR501**.

After the synthesis was completed, both representative aminoglycosides were assayed against aminoglycoside-susceptible and -resistant strains of *Escherichia coli* using amikacin, kanamycin, ribostamycin, and butirosin as the controls. One resistant strain was equipped with the pTZ19U-3 plasmid encoded for APH(3′)-I, which rendered resistance to kanamycin, neomycin, lividomycin, paromomycin, and ribostamycin. The other resistant strain was equipped with the pSF815 plasmid encoded for AAC(6′) and APH(2″), which produced a bifunctional enzyme that catalyzed acetylation of amino group at C-6′ and phosphorylation of hydroxyl group at C-2″ position. This bifunctional enzyme enables bacteria to acquire resistance against gentamycin, tobramycin, netilmicin, and kanamycin.

The minimum inhibitory concentration (MIC) results are summarized in Table 1. As expected, both kanamycin and ribostamycin are either inactive or much less active against aminoglycoside-resistant bacteria. Kanamycin-class antibiotics are, however, less effective than neomycin-class antibiotics against bacteria equipped with AAC6′/APH2″. Incorporation of an (S)-4-amino-2-hydroxybutyryl (AHB) group at the *N*-1 position appears to be the superior design against both strains of resistant bacteria. Although both **RR501** and **RT501** contain no AHB group at *N*-1, they are both active against resistant bacteria equipped with APH(3′). They are, however, less active against bacteria equipped with AAC6′/APH2". **RR501** is slightly more active than **RT501** against both resistant strains, which is consistent with the results obtained from commercially available aminoglycosides. By

Table 1: Minimum inhibitory concentration of synthesized aminoglycosides[a]

Compounds	Strains		
	***E. coli* (TG1)**	***E. coli* (TG1) (pSF815)[b]**	***E. coli* (TG1) (pTZ19U-3)[c]**
Amikacin	1	1	0.5
Kanamycin B	4	Inactive	32
Ribostamycin	2	16	Inactive
Butirosin	0.5	0.5	0.5
RR501	8	4	4
RT501	8	Inactive	4

[a]Unit: μg/mL.
[b]Plasmid encoded for AAC6′/APH2″.
[c]Plasmid encoded for APH(3′)-I.

summarizing the information from MIC values, we believe a better design should be a neomycin class with the AHB group at *N*-1 and 3′,4′-dideoxygenation (or 3′-deoxygenation). Nevertheless, the problem of the acid-labile glycosidic bond between rings II and III is an obstacle that remains to be overcome. Therefore, our design of **RR501** that has better stability in acidic media could be valuable for designing new aminoglycosides against a broad spectrum of aminoglycoside-resistant bacteria.

In conclusion, we have developed a novel protocol for transforming a *trans*-diol to an alkene under mild conditions with high efficiency. Our method can be very useful for chemical modification of compounds with functional groups sensitive to reduction, acid, or heating. We have further demonstrated the efficacy of our method by synthesizing 3′,4′-dideoxykanamycin (dibekacin) analog and 3′,4′-dideoxypyranmycin. Antibacterial activity has been shown. Further work of synthesizing other potent analogs of these compounds is being carried out.

EXPERIMENTAL SECTION

5,6-Cyclohexylidene-1,3,2′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (5). To a solution of starting material (0.85 g, 1.67 mmol) in anhydrous CH_2Cl_2 (10 mL) and pyridine (0.76 mL, 9.3 mmol) at 0°C, triflic anhydride (1.36 mL, 8.0 mmol) was added slowly. The reaction mixture was stirred at 0°C for 45 min monitored by TLC (Hexane:EtOAc = 3:1). After completion of the reaction, the reaction is quenched by adding a few drops of water and diluted with CH_2Cl_2. The organic layer was washed with water, $NaHCO_3$, and brine, then dried over Na_2SO_4. After concentration, the crude product (1.06 g, 1.51 mmol) was dissolved in acetone, and then a catalytic amount of NaI and $Na_2S_2O_3$ (0.9 g, 5.95 mmol) was added. After being stirred overnight at rt, the reaction mixture was diluted with EtOAc. The organic layer was washed with

1 N $Na_2S_2O_3$, water, and brine, then dried over Na_2SO_4. Removal of solvents and purification with column chromatography afforded the desired product (0.39 g, 0.76 mmol, 49%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.91 (d, $J = 10.5$ Hz, 1H), 5.87 (d, $J = 10.8$ Hz, 1H), 5.69 (d, $J = 3.9$ Hz, 1H, H-1′), 4.59 (m, 1H, H-5′), 4.1 (m, 1H), 3.86 (m, 1H, H-4), 3.80 (m, 1H, H-1), 3.2–3.7 (m, 5H), 2.3 (m, 1H, H-2_{eq}), 1.2–1.6 (m, 11H).

1,3,2′,6′-Tetraazido-3′,4′-dideoxy-3′-enoneamine (4). A solution of starting material (2.58 g, 5.4 mmol) in a mixture of water (25 mL), 1,4 dioxane (25 mL), and acetic acid (36 mL) was stirred at 65°C for 24 hr. After completion of the reaction (monitored with TLC [Hexane:EtOAc = 65:35]), the reaction mixture was diluted with EtOAc and saturated $NaHCO_{3(aq)}$. After being stirred overnight, the organic layer was washed with $NaHCO_3$ and brine, then dried over Na_2SO_4. Removal of solvents and purification with column chromatography afforded the desired product (2.02 g, 5.2 mmol, 95%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.95 (d, $J = 10.5$ Hz, 1H), 5.90 (d, $J = 10.8$ Hz, 1H), 5.54 (d, $J = 3.9$ Hz, 1H, H-1′), 4.72 (m, 1H, H-5′), 4.09 (m, 1H), 3.2–3.6 (m, 7H), 2.3 (m, 1H, H-2_{eq}), 1.57 (d, $J = 3.2$ Hz 1H, H-2_{ax}); ^{13}C NMR (68 MHz, $CDCl_3$) δ 129.5 (s), 122.6 (s), 97.5 (s), 81.6 (s), 77.5 (s), 77.1 (s), 76.6 (s), 76.4 (s), 75.7 (s), 68.8 (s), 59.9 (s), 59.3 (s), 55.5 (s), 53.8 (s), 32.2 (s), 29.7 (s); HRESI Calcd for $C_{12}H_{16}N_{12}O_4Na$ $[M + Na]^+$ m/e 415.1315; measure m/e 415.1314.

5-*O*-(4″-Azido-4″,6″-dideoxy-β-D-glucopyranosyl)-1,3,2′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (10). A solution of glycosyl donor (0.17 g, 0.39 mmol) and the acceptor (0.17 g, 0.33 mmol) and activated powder 4 Å molecular sieve was stirred in anhydrous CH_2Cl_2 (8 mL) at rt for 1 hr and then cooled to −50°C. To this solution, $BF_3.OEt_2$ (0.05 mL) was added. After being stirred for 45 min, the reaction mixture was quenched by the addition of powder $NaHCO_3$. After being stirred for 15 min, the reaction mixture was filtered through Celite. The residue was washed thoroughly with EtOAc. Removal of solvent and purification with column chromatography provided desired product mixed with inseparable impurities. The crude product was dissolved in anhydrous methanol, and then a catalytic amount of NaOMe (2 M in MeOH) was added. After being stirred overnight at rt, the reaction mixture was added with Amberlite IR-120 (H^+) then filtered through Celite. The residue was washed thoroughly with MeOH. The combined solution was concentrated, then redissolved in EtOAc, and then washed with water and brine and dried over Na_2SO_4. Removal of solvents and purification with column chromatography afforded the desired product (0.16 g, 0.28 mmol, 86%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.96 (d, $J = 10.5$ Hz, 1H), 5.91 (d, $J = 10.8$ Hz, 1H), 5.70 (d, $J = 3.9$ Hz, 1H, H-1′), 5.6 (d, $J = 3.9$ Hz, 1H, H-1′), 4.26 (ddd, $J = 9.9$ Hz, $J = 4.8$ Hz, $J = 2.2$ Hz, 1H), 4.0–4.1 (dd, $J = 7.1$ Hz, $J = 6.9$ Hz, 1H), 3.2–3.7

(m, 11H), 3.09 (dq, $J = 9.9$ Hz, $J = 6.3$ Hz, 1H, H-5″), 2.32 (ddd, $J = 13.2$ Hz, $J = 4.5$ Hz, $J = 4.5$ Hz, 1H, H-2_{eq}), 1.36 (ddd, $J = 13.2$ Hz, $J = 12.5$ Hz, $J = 12.5$ Hz, 1H, H-2_{ax}), 1.21 (d, $J = 6.3$ Hz, H-6″); ^{13}C NMR (68 MHz, $CDCl_3$) δ 129.0 (s), 127.9 (s), 127.2 (s), 122.9 (s), 122.0 (s), 94.9 (s), 94.0 (s), 86.1 (s), 85.7 (s), 80.7(s), 79.1 (s), 76.8 (s), 76.5 (s), 76.1 (s), 75.9 (s), 75.8 (s), 75.1 (s), 74.4 (s), 74.3 (s), 70.8 (s), 69.2 (s), 68.9 (s), 66.9 (s), 59.7 (s), 59.1 (s), 58.8 (s), 54.0 (s), 53.5 (s), 53.2 (s), 51.0 (s), 45.8 (s), 31.7 (s), 29.1 (s), 17.9 (s); HRESI Calcd for $C_{18}H_{25}N_{15}O_7Na$ $[M + Na]^+$m/e 586.1959; measure m/e 586.1979.

6-*O*-Benzyol-1,3,2′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (6). To a solution of starting material (1.13 g, 2.88 mmol) and DIPEA (0.60 mL, 3.45 mmol) in anhydrous CH_2Cl_2, at -50°C, BzCl (0.33 mL, 2.87 mmol) was slowly added. After being stirred overnight, the reaction was quenched by adding a few drops of water and then diluted with EtOAc. The reaction mixture was washed with $NaHCO_3$ and brine, then dried over Na_2SO_4. Removal of solvents and purification with column chromatography afforded the desired product (1.21 g, 2.44 mmol, 85%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.3–8.0 (m, 5H) 5.95 (d, $J = 10.5$ Hz, 1H), 5.90 (d, $J = 10.8$ Hz, 1H), 5.64 (d, $J = 3.9$ Hz, 1H, H-1′), 5.17 (t, $J = 9.8$ Hz, 1H, H-6), 4.70 (m, 1H, H-5′), 4.0–4.1 (dd, $J = 7.1$ Hz, $J = 6.9$ Hz, 1H), 3.89 (m, 1H, H-2′), 3.80 (m, 1H, H-5), 3.69 (m, 1H, H-4), 3.58 (m, 1H, H-1), 3.38 (m, 1H, H-3), 3.2 (m, 1H, H-6′), 2.34 (m, 1H, H-2_{eq}), 1.69 (d, $J = 3.2$ Hz 1H, H-2_{ax}). ^{13}C NMR (68 MHz, $CDCl_3$) δ 171.3 (s), 166.2 (s), 133.6 (s), 130.0 (s), 129.8 (s), 129.5 (s), 129.7 (s), 129.5 (s), 129.4 (s), 129.2 (s), 128.5 (s), 122.9 (s), 97.8 (s), 81.2 (s), 77.8 (s), 77.6 (s), 77.2 (s), 76.7 (s), 75.6 (s), 68.8 (s), 60.4 (s), 59.2 (s), 58.3 (s), 55.3 (s), 53.8 (s), 32.2 (s), 21.0 (s), 14.2 (s).

6-*O*-(4″-Azido-4″,6″-dideoxy-α-D-glucopyranosyl)-1,3,2′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (8). A solution of glycosyl donor (0.6 g, 1.29 mmol), acceptor (0.56 g, 1.42 mmol), and activated 4 Å molecular sieves in anhydrous Et_2O (12 mL) and CH_2Cl_2 (4 mL) was stirred overnight. The reaction mixture was cooled to −78°C, and NIS (0.17 g, 0.75 mmol) was added. The reaction mixture was allowed to warm up to −45°C, and then TfOH (28.3 μL, 0.31 mmol) was added. After completion of the reaction (monitored by TLC [hexane/EtOAc = 3/1]), the reaction was quenched by adding a few drops of triethyl amine. After being stirred for several minutes, the reaction mixture was concentrated, diluted with EtOAc, and then filtered through Celite. The residue was washed with EtOAc. The combined organic solution was washed with 1 N $Na_2S_2O_3$, water, and brine, then dried over Na_2SO_4. Removal of solvents and purification with column chromatography afforded the desired product (0.69 g, 0.93 mmol, 72%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.2–7.4 (m, 10H), 6.2 (d, $J = 3.2$ Hz, 1H, H-1′), 5.9 (d, $J = 10.5$ Hz, 1H), 5.86 (d, $J = 1.3$ Hz, 1H), 5.83 (d, $J = 3.6$ Hz, 1H, H-1″), 4.94 (d, $J = 10.9$ Hz, 1H,

PhCH_2O), 4.80 (d, $J = 10.9$ Hz, 1H, PhCH_2O), 4.77 (d, $J = 11.9$ Hz, 1H, PhCH_2O), 4.70 (d, $J = 11.9$ Hz, 1H, PhCH_2O), 4.38 (d, $J = 1.6$ Hz, 1H), 4.13 (m, 1H), 3.8–4.0 (m, 2H), 3.3–3.7 (m, 10H), 3.11 (dd, $J = 9.9$ Hz, $J = 9.9$ Hz, 1H), 2.33 (ddd, $J = 13.0$ Hz, $J = 4.3$ Hz, $J = 4.3$ Hz, 1H, H-2_{eq}), 1.50 (ddd, $J = 13.0$ Hz, $J = 12.2$ Hz, $J = 12.2$ Hz, 1H, H-2_{ax}), 1.27 (d, $J = 5.9$ Hz, 3H, H-6″); ^{13}C NMR (68 MHz, $CDCl_3$) δ 171.3 (s), 166.2 (s), 137.9 (s), 137.7 (s), 129.1 (s), 128.6 (s), 128.4 (s), 128.1 (s), 128.0 (s), 127.9 (s), 123.4 (s), 98.23 (s), 96.9 (s), 86.2 (s), 79.7 (s), 77.5 (s), 77.1 (s), 76.6 (s), 75.9 (s), 60.4 (s), 59.3 (s), 50.0 (s), 53.9 (s), 32.6 (s), 29.7 (s), 18.4 (s), 14.2 (s).

General procedure for Staudinger reaction and hydrogenation. To the trisaccharides (**8** or **10**)/THF solution in a reaction flask (or vial) equipped with a reflux condenser, 0.1 M $NaOH_{(aq)}$ (0.5 mL) and PMe_3 (1M in THF, 6 equivalents) were added. The reaction mixture was stirred at 50°C for 2 hr. The product has an R_f of 0 when eluted with EtOAc/MeOH (9/1) solution and an R_f of 0.9 when eluted with *i*PrOH/1 M NH_4OAc (2/1) solution. After completion of the reaction, the reaction mixture was cooled to rt and loaded to a short column (5 cm in height) packed with a layer of silica gel on top of a layer of Celite. The column was eluted with a series of solutions as follows: THF, THF/MeOH, MeOH, and MeOH/conc. NH_4OH (from 0% to 28% of conc. NH_4OH). The fractions containing the desired product were analyzed by TLC and collected. After removal of solvents, the crude perbenzylated aminoglycoside was added with a catalytic amount of $Pd(OH)_2/C$ (20% Degussa type) and 5 mL of degassed HOAc/H_2O (1/1). After being further degassed, the reaction mixture was stirred at rt under atmospheric H_2 pressure. After being stirred for 1 d, the reaction mixture was filtered through Celite. The residue was washed with water, and the combined solutions were concentrated, affording pure final product as an acetate salt. Most of the reported final products are characterized by ^{1}H and ^{13}C NMR at this stage. The product has an R_f of 0 when eluted with *i*PrOH/1 M NH_4OAc (2/1) solution and an R_f of 0.1–0.2 when eluted with conc. NH_4OH/MeOH (2/5) solution. 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 desired fractions and removal of solvent, the final products are subjected to bioassay directly.

5-*O*-(4″-Amino-4″,6″-dideoxy-β-D-glucopyranosyl)-3′,4′-dideoxyneamine (RR501) contains about 10% inseparable byproduct. ^{1}H NMR (400 MHz, D_2O) (chloride salt), δ 5.73 (d, $J = 3.4$ Hz, 1H, H-1′), 5.2 (d, $J = 8.0$ Hz, 1H, H-1″), 4.0–4.1 (m, 1H), 3.8–4.0 (m, 4H), 3.4–3.8 (m, 4H), 3.36 (dd, $J = 13.5$ Hz, $J = 3.5$ Hz, 1H), 3.1–3.2 (m, 2H), 3.0 (dd, $J = 10.2$ Hz, $J = 9.9$ Hz, 1H), 2.2 (m, 1H, H-2_{eq}), 2.0–2.2 (m, 4H), 1.96 (dd, $J = 12.6$ Hz, $J = 12.6$ Hz, 1H, H-2_{ax}), 1.48 (dd, $J = 6.9$ Hz, $J = 6.6$ Hz, 3H); ^{13}C NMR

(68 MHz, D2O) (chloride salt) δ 102.6 (s, C-1″), 95.4 (s, C-1′), 81.0 (s), 76.3 (s), 73.5 (s), 73.0 (s), 71.7 (s), 69.6 (s), 66.2 (s), 56.5 (s), 49.7 (s), 48.9 (s), 48.7 (s), 42.4 (s), 28.3 (s), 25.2 (s), 20.4 (s), 16.7 (s). HRESI Calcd for $C_{18}H_{42}N_5O_7Na$ $[M + Na]^+$ m/e 458.2593; measure m/e 458.2590.

6-*O*-(4″-Amino-4″,6″-dideoxy-α-D-glucopyranosyl)-3′,4′-dideoxyneamine (RT501) ($\alpha/\beta = 7/1$, only the α epimer reported) ^{1}H NMR (400 MHz, D_2O) (Chloride salt) 5.90 (d, $J = 2.7$ Hz, 1H, H-1′), 5.10 (d, $J = 2.7$ Hz, 1H, H-1″), 4.4 (m, 1H), 3.8–4.1 (m, 7H), 3.5–3.7 (m, 5H), 3.36 (d, $J = 5.2$ Hz, 1H), 3.35 (d, $J = 5.2$ Hz, 1H), 3.2 (dd, $J = 7.8$ Hz, 1H), 3.1 (t, $J = 9.1$ Hz, 1H), 2.6 (m, 1H, H-2_{eq}), 2.0 (ddd, $J = 9.1$ Hz, $J = 9.1$ Hz, $J = 9.1$ Hz, 1H, H-2_{ax}), 1.42 (d, $J = 6.3$ Hz, 3H, H-6″); ^{13}C NMR (100 MHz, D_2O) (chloride salt) 104.6 (s), 97.7 (s), 86.6 (s), 79.5 (s), 76.9 (s), 74.5 (s), 71.6 (s), 68.8 (s), 68.6 (s), 59.5 (s), 52.9 (s), 51.5 (s), 51.4(s), 45.3 (s), 30.8 (s), 28.2 (s), 23.3 (s), 19.6 (s); HRESI Calcd for $C_{18}H_{38}N_5O_7$ $[MH]^+$m/e 436.2771; measure m/e 436.2751.

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