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Synthesis of novel aminoglycosides via allylic azide rearrangement for investigating the significance of 2′-amino group

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ABSTRACT

Using allylic azide rearrangement, a convenient method has been developed for the synthesis of 2′,3′-dideoxyaminoglycosides that are, otherwise, difficult to be prepared. The antibacterial activity of these novel aminoglycosides also confirms the indispensable role of 2′-NH_2 group for both neomycin and kanamycin classes of aminoglycosides. A novel structural motif containing the hexylaminocarbonyl groups at *O*-5 and/or *O*-6 of 2′,3′-dideoxyneamine could lead to the production of new aminoglycosides against resistant bacteria.

1. Introduction

The neamine core contributes predominately to the antibacterial activity of neomycin and kanamycin classes of aminoglycosides (Fig. 1).[1] Neamine has four amino groups located at 1, 3, 2′, and 6′ positions. The presence of 1-NH_2 and 3-NH_2 are essential for the antibacterial activity of aminoglycosides that contain neamine. The 6′-NH_2 amino group can be replaced with OH without obstructing the activity as in the case of paromomycin.[1c] However, the roles of 2′-NH_2 amino group are multiplex and contradictory, especially, in association with the deoxygenation at 3′ position.

Modifications such as deoxygenation at 3′ and 4′ positions has been shown to be effective in reviving the activity of aminoglycoside constructs against aminoglycoside resistant bacteria that equipped with aminoglycoside modifying enzymes (AMEs) that introduce modifications at these two positions. For example, tobramycin that has deoxygenation at 3′ position is active against bacteria equipped with aminoglycoside phosphotransferases that catalyze phosphorylation at 3′-OH position (APH(3′)).[1a,b] On the other hand, aminoglycosides with deoxygenation at 3′ position also has been reported to exert higher cytotoxicity due to the increased basicity of 2′-NH_2.[2] It has been reported that an intramolecular hydrogen bond between 2′-NH_2 and 4″-oxygen atom on ring III of neomycin is crucial in orienting the conformation of neamine and, thus, optimizing the binding to the targeted rRNA site.[3] In addition, there is no significant difference in the antibacterial activity between kanamycin B and kanamycin A that have 2′-NH_2 and 2′-OH, respectively. Even the antibacterial results from the modified neamine derivatives were, in part, contradictory. For instance, neamine derivative, **1** with 2′-NH_2 replaced with 2′-OH shows much lower antibacterial activity than the parent neamine.[4] Interestingly, the neamine derivative, **2** with deamination at 2′ position manifests improved antibacterial activity.[5]

Judging from the results in the literature, it is unclear regarding the significance of 2′-NH_2 group. To this aim, we wish to explore the synthesis of aminoglycosides with deamination of the 2′-NH_2 group. Chemical deamination usually involves multiple steps and has not been noted to be accompanied with deoxygenation at 3′ position. Masking amino groups as azido groups (azido approach) is one of the commonly employed strategies for conducting structural modifications of aminoglycosides.[1c] In an effort to revive the activity of aminoglycosides, we have developed a mild method for introducing 3′,4′-dideoxygenation on aminoglycosides.[6] The process involves the formation of a double bond between 3′ and 4′ positions. With the presence of 2′-N_3 group, it is possible that an allylic azide rearrangement should offer a convenient access to deamination and deoxygenation at 2′ and 3′ positions, respectively. In addition, the 4′-OH will be replaced with 4′-NH_2, which should maintain the interactions involved in the parent neamine.[4] Adducts from allylic azide rearrangement could offer the information on the significance of having 2′-NH_2 group. Although allyl azide rearrangement has been reported,[7] such rearrangement has not been noted on azidoaminoglycosides.

2. Results and discussion

2.1. Initial discovery of the allylic azide rearrangement

The synthesis of azidoneamine core, **3** with a double bond between 3′ and 4′ positions was conducted according to our

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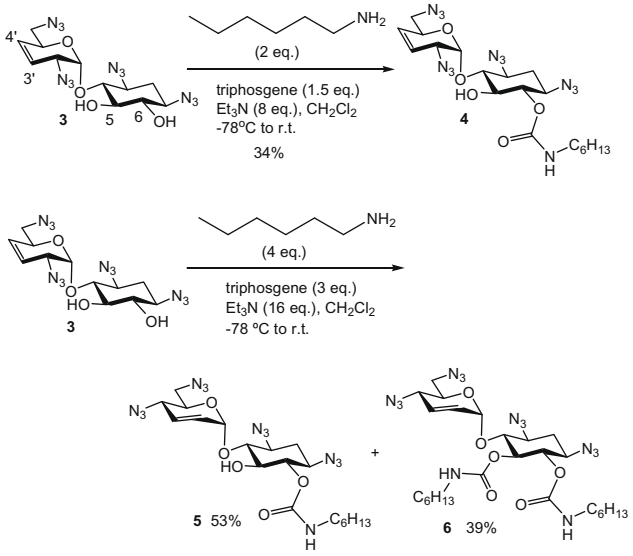
protocol.[6] The discovery of allylic azide rearrangement on **3** was actually an unexpected result (Scheme 1).[8] The initial attempts were designed to explore the antibacterial activity of non-carbohydrate moieties at *O*-6 and/or *O*-5 positions. The added functional groups were attached via carbamate linkage. When compound **3** reacted with *N*-hexylisocyanate generated in situ, the alkylcarbamate was incorporated at *O*-6 as expected. However, when excess *N*-hexylisocyanate was used, we isolated two products, **5** and **6** with mono- and di-alkylcarbamates attached, respectively. Nevertheless, compound **5** has different ^{1}H NMR from **4**. Upon further investigation, we confirmed that the double bond of both **5** and **6** were rearranged to 2′ and 3′ positions via an allylic azide rearrangement.

Although we did not know the details regarding why such a rearrangement occurred in these conditions, we envisioned that this allylic azide rearrangement can be triggered with no added reagents and conveniently lead to the production of neamine derivative with deamination and deoxygenation at 2′ and 3′ positions, respectively. More importantly, the aminoglycosides resulted from this allylic azide migration will provide direct evaluation on the significance of 2′-NH_2 group.



Scheme 1.

2.2. Allylic azide rearrangement of azidoneamine

The allylic azide rearrangement is expected to proceed under thermodynamic condition similarly to the [3,3]-sigmatropic rearrangement.[7] To our please, the allylic azide rearrangement using compound **3** can be carried out at 110 °C in toluene (Scheme 2). Since such rearrangement can be reversible, we obtained a mixture of **3** and **7**, which can be separated by column chromatography. Compound **7** was acetylated to yield **9** in order to make the proton signals discernable. The structure of the neamine derivative, **9** from allylic azide rearrangement was confirmed by 2D NMR (COSY and HSQC). Both **3** and **7** were subjected to Staudinger reduction and hydrogenation to yield the neamine derivatives, **10** and **11**, respectively. Direct glycosylation of **7** can provide the syntheses of 2′,3′-dideoxykanamycin classes of aminoglycosides as reported previously.[1c] Regioselective benzoylation of **7** offered compound **8**, which can be employed for the syntheses of 2′,3′-dideoxyneomycin classes of aminoglycosides also as reported previously.

2.3. Synthesis of novel aminoglycosides

Glycosylation of **7** using phenylthioglycoside, **12** offered the precursors of 2′,3′-dideoxykanamycin derivatives, **13** and **epi-13** as the minor product (Scheme 3). In order to compare the activity, the corresponding 3′,4′-dideoxykanamycin derivatives, **14** was also prepared. Based on our previous structure–activity relationship (SAR) studies of kanamycin B analogs,[9] compound **16** was synthesized. In our previous work, we have noticed that the presence of 6″-CH_3 is not essential for the antibacterial activity.[10] Therefore, due to the consideration of availability of glycosyl donor, compound **18** was synthesized for comparing the activity of aminoglycosides from **16**. The glycosyl donor, **17** can be prepared using similar procedures as reported (Scheme 4).[9]

Glycosylation of **8** using **22** yield the desired pyranmycin (a neomycin class aminoglycoside) derivative, **23** (Scheme 5). Based on our previous structure-activity relationship (SAR) studies of pyranmycin, compound **22** has the carbohydrate moiety that generates the most active aminoglycoside.[10] The corresponding 3′,4′-dideoxy pyranmycin derivatives has also been prepared previously. During the syntheses, we did notice that the rearrangement adducts with deamination at 2′ and double bond between 2′ and 3′ positions are very sensitive to acidic conditions. Therefore, all the processes were conducted with minimum exposure to acids or at lower temperature.

All the azidoaminoglycosides were subjected to similar global deprotection (Staudinger reduction followed by hydrogenation and ion-exchange column chromatography) to yield the designed aminoglycosides ready for assay (Scheme 6).

2.4. Antibacterial activity of aminoglycosides

The synthesized compounds were assayed against aminoglycoside susceptible bacteria *Escherichia coli* (ATCC 25922, G-) *Staphylococcus aureus* (ATCC 25923, G+) and using neamine, neomycin and

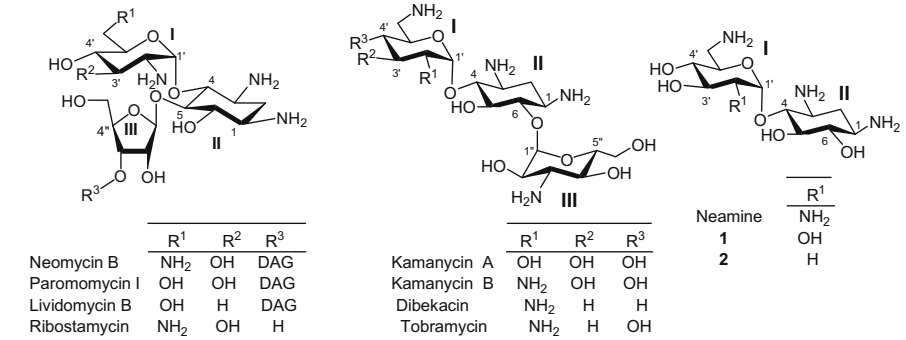
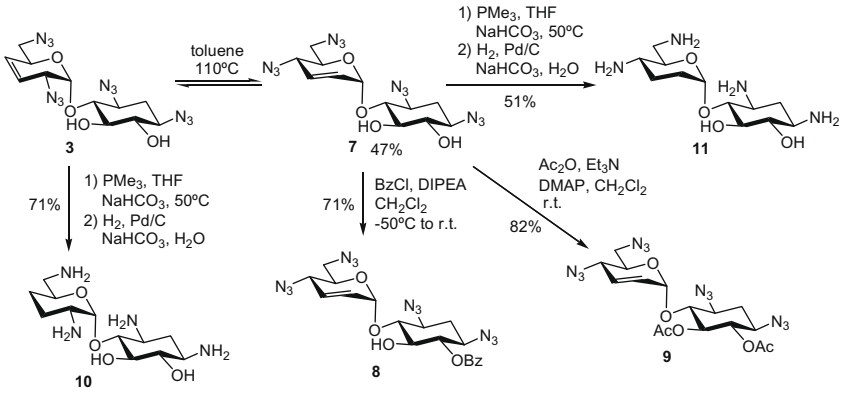
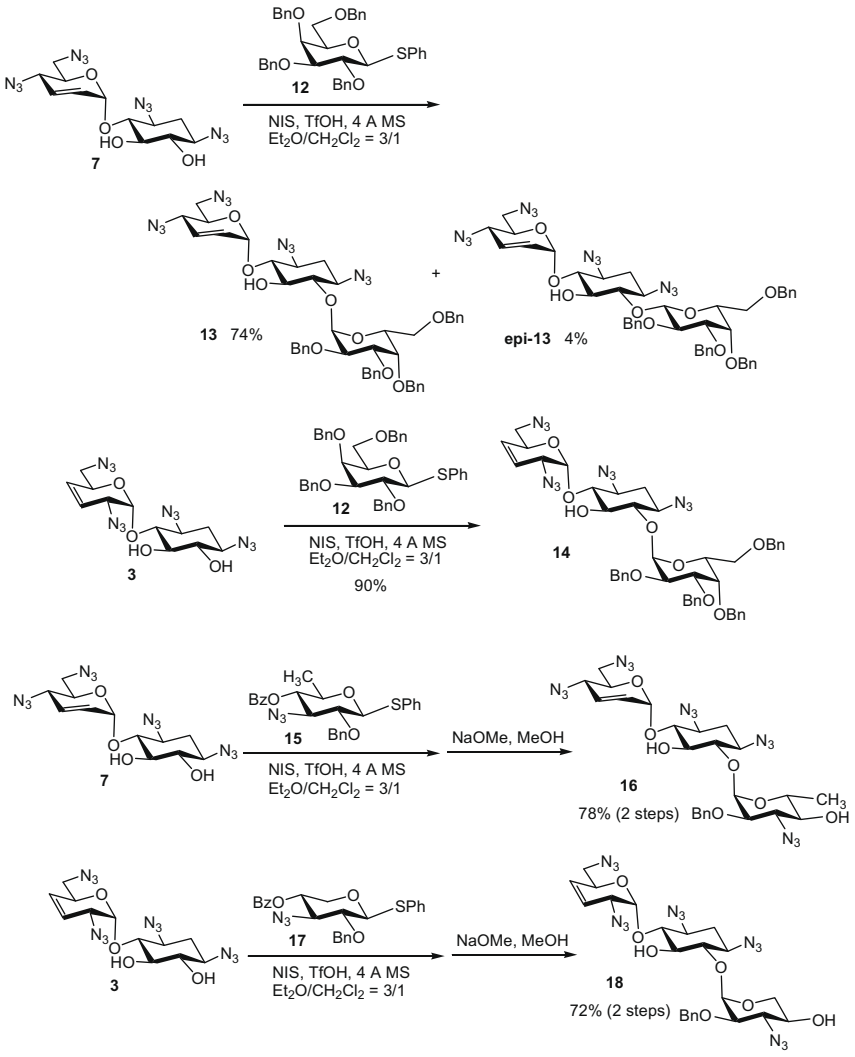


Figure 1. Structures of aminoglycosides.

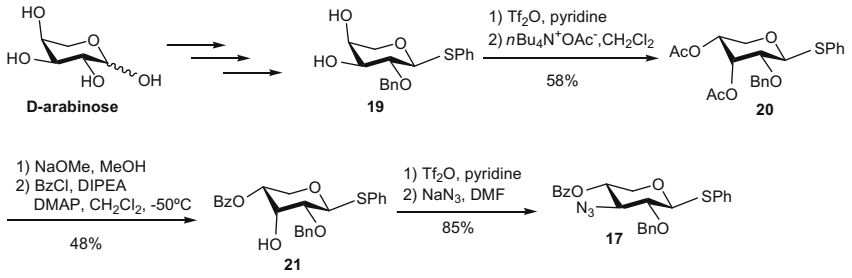


Scheme 2.

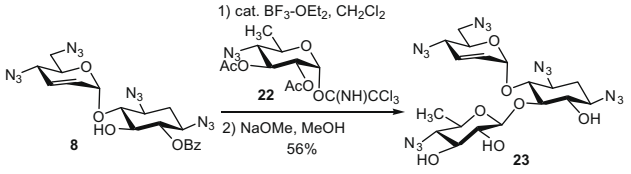


Scheme 3.

kanamycin as the controls (Table 1). From the minimum inhibitory concentration (MIC) results, the 2′-NH_2 group appears to have contradictory roles. The 2′-NH_2 group is important for the antibacterial activity of both neomycin and kanamycin classes of aminoglycosides since all the derivatives with 2′,3′-dideoxyaminoglycosides are less active than the corresponding 3′,4′-dideoxyaminoglycosides with the tendency of neamine > 3′,4′-dideoxyneamine > 2′,3′-dideoxyneamine. However, such tendency is less obvious or even reversed with the attachment of hexylaminocarbonyl group. For example, both **NRR101** and **NRR102** are slightly more active than **RR101** (entries 4–6). Further testing also reveals that **NRR102** manifests MIC's of 32–64 and 125 μg/mL against *E. coli* equipped with APH3′-I and AAC6′/APH2", respectively. In contrast, neamine, neomycin and kanamycin are inactive against these bacteria (MIC



Scheme 4.



Scheme 5.

>250 μg/mL). These results imply that 2′,3′-dideoxyneamine (no 2′-NH_2 group) combined with hexylaminocarbonyl group at *O*-5 or *O*-6 positions could be a novel motif for generating new aminoglycosides against resistant bacteria.

To explore the unexpected effect of hexylaminocarbonyl group, we decided to synthesize two addition neamine analogs, **RR103** and **RR104**, with mono- and di-hexylaminocarbonyl groups at *O*-6 and *O*-5/*O*-6 positions, respectively while keeping the scaffold of neamine (Scheme 7). The syntheses can be achieved via similar processes using compound **24**[10] as the starting material. A panel of aminoglycoside susceptible and resistant bacteria, including *Klebsiella pneumoniae* (ATCC 13883, susceptible, G-), *K. pneumoniae* (ATCC 700603, resistant, G-), *Pseudomonas aeruginosa* (ATCC 27853, resistant, G-), *S. aureus* (ATCC 33591, resistant, G+), were selected for evaluating the antibacterial activity.

From the result of antibacterial assay, it is obvious that **NRR102** is still the most active compound while other neamine derivatives with hexylaminocarbonyl group(s) are less active (Table 2). We have recently reported that incorporation of linear alkyl chains could alter the tradition antibacterial mode of aminoglycosides leading to the generation of novel antibiotics.[11] **RR103** and **RR104** are, however, much less active against *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 33591) that equipped with various APH(3′) (entries 5 and 6).[12,13] This result implies that these two analogs are still being inactivated by AME′s. Combining the antibacterial activity of these neamine analogs, we believe these analogs should exert traditional mode of antibacterial action. Therefore, the antibacterial activity of **NRR102** against bacteria equipped with AME′s should be attributed to the deoxygenation of 3′-OH. Nevertheless, in this new neamine motif, the presence of 2′-NH_2 group is no longer essential. Although the activity is less than what is needed to be clinically significant, it is possible that enhanced activity could be obtained by varying the length of the linear alkylamino chain.

3. Conclusion

We have developed a convenient method for synthesizing 2′,3′-dideoxyneomycin and 2′,3′-dideoxykanamycin derivatives. The reported allylic azide rearrangement can also be applicable of making aminosugar derivatives. For example, this rearrangement can serve as an alternative method to the reported Pd-catalyzed π-allyl method for the preparation of C-4 allylic azides that has been employed in the syntheses of several biologically interesting compounds.[14]

The antibacterial results confirm that 2′-NH_2 group is indispensable for the activity of neomycin and kanamycin classes of aminoglycosides, which also suggest that it may be difficult to compromise the contradictory roles of 2′-NH_2 group for these two classes of aminoglycosides. However, our results indicate that having hexylaminocarbonyl groups at *O*-5 and/or *O*-6 of neamine along with the 2′,3′-dideoxyneamine core may serve as an alternative structural motif for generating novel aminoglycosides against resistant bacteria. Further SAR investigation on similar functional groups is being carried out.

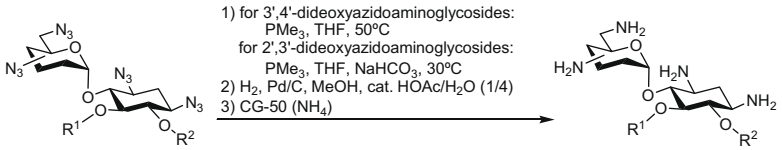
4. Experimental

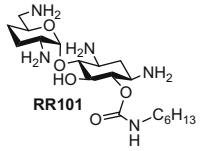
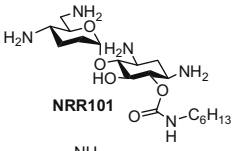
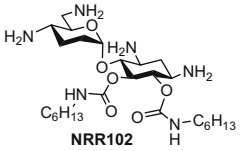
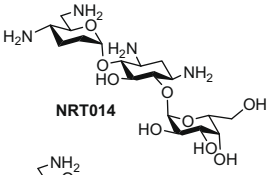
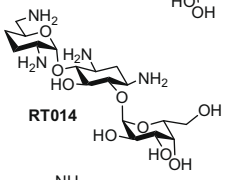
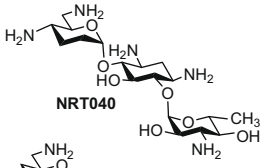
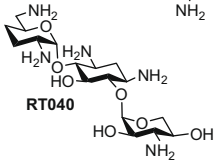
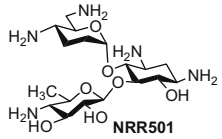
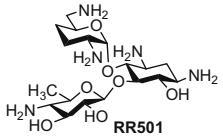
4.1. 6-*O*-Hexylaminocarbonyl-1,3,2′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (4)

To a solution of 1-heptamine (0.10 g, 1.0 mmol) and Et_3N (0.60 mL, 4.2 mmol) in anhydrous CH_2Cl_2 (5 mL) at −78 °C, triphosgene (0.22 g, 0.75 mmol) was added, and the reaction was stirred for several hours allowing the temperature to warm up to room temperature. The reaction mixture was cooled to 0 °C and added with compound **3** (0.20 g, 0.51 mmol). After being stirred overnight, the reaction was quenched with water and diluted with EtOAc. The combined organic solution was washed with 1 M HCl, water, saturated $NaHCO_3$, and brine, and then dried over anhydrous Na_2SO_4. After the removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 100/0 to 60/40), the product was obtained as an oil (0.08 g, 0.15 mmol, 34%) along with the recovered compound **3** (0.09 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.93 (d, J = 10.5 Hz, 1H), 5.89 (d, J = 10.4 Hz, 1H), 5.74 (d, J = 3.9 Hz, 1H), 4.85 (t, J = 6.2 Hz, 1H), 4.7 (m, 1H), 4.67 (t, J = 9.9 Hz, 1H), 3.9 (m, 1H), 3.8 (m, 1H), 3.68 (t, J = 9.1 Hz, 1H), 3.58 (t, J = 9.4 Hz, 1H), 3.4–3.5 (m, 2H), 3.3–3.4 (m, 2H), 3.2 (m, 2H), 2.27 (td, J = 13.3, 4.5 Hz, 1H), 1.6 (m, 1H), 1.5 (m, 2H), 1.2 (m, 6H), 0.85 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5, 129.6, 123.1, 97.4, 81.4, 75.9, 69.0, 59.2, 58.4, 55.4, 54.0, 41.7, 32.5, 32.0, 31.6, 29.9, 26.5, 22.7, 14.2; ESI/APCI Calcd for $C_{19}H_{30}N_{13}O_5^+$ ([M+H]$^+$) *m/e* 520.2487; measured *m/e* 520.2476.

4.2. 6-*O*-Hexylaminocarbonyl-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (5)

Compound **5** was prepared similarly to the synthesis of compound **4** except the following amount of reagents were used: 1-heptamine (0.21 g, 2.0 mmol), Et_3N (1.2 mL, 8.6 mmol) and triphosgene (0.45 g, 1.53 mmol) for reacting with compound **3** (0.20 g, 0.51 mmol). Two products were isolated after column chromatography (eluted with hexane/EtOAc from 100/0 to 60/40): compound **5** (0.14 g, 0.27 mmol, 53%) and compound **6**



Reactant	Product	Yield (%)
4	 **RR101**	50%
5	 **NRR101**	53%
6	 **NRR102**	89%
13	 **NRT014**	83%
14	 **RT014**	79%
16	 **NRT040**	72%
18	 **RT040**	59%
23	 **NRR501**	55%
-	 **RR501**	Ref. 6

Scheme 6.

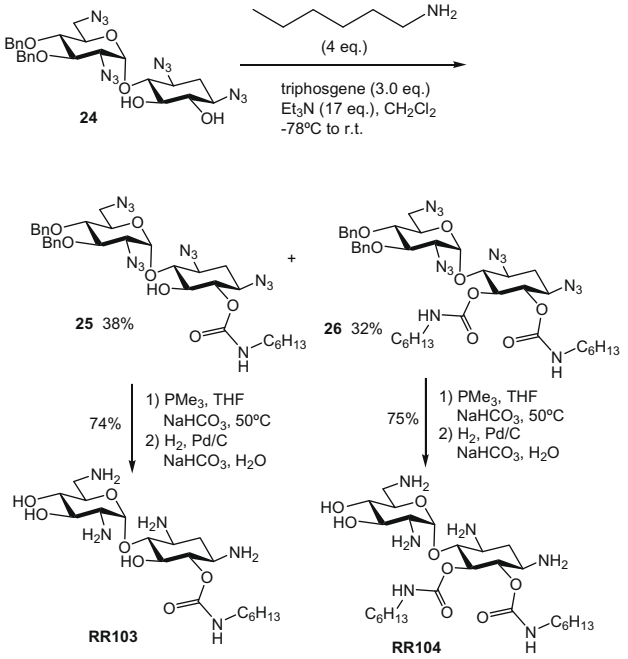
(0.13 g, 0.20 mmol, 39%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.9 (m, 2H), 5.38 (d, J = 4.0 Hz, 1H), 4.93 (t, J = 5.4 Hz, 1H), 4.8 (m, 2H), 3.8 (m, 1H), 3.6–3.7 (m, 2H), 3.4–3.5 (m, 3H), 3.2–3.3 (m, 3H), 2.25 (td, J = 8.1, 3.9 Hz, 1H), 1.4–1.6 (m, 4H), 1.2–1.3 (m, 6H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.1, 129.6, 123.3, 98.1, 78.7, 78.2, 76.5, 69.2, 60.8, 59.6, 54.9, 54.2, 41.8, 32.2, 32.0, 29.8, 26.6, 22.8, 14.2; ESI/APCI Calcd for $C_{19}H_{30}N_{13}O_5^+$ ([M+H]$^+$) m/e 520.2487; measured m/e 520.2481.

4.3. 5,6-Di-*O*-hexylaminocarbonyl-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (6)

Please refer to the procedure for the preparation of compound **5**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.9 (m, 2H), 5.35 (d, J = 4.5 Hz, 1H), 4.99 (t, J = 9.7 Hz, 1H), 4.8–4.9 (m, 3H), 3.70 (t, J = 9.6 Hz, 1H), 3.4–3.6 (m, 5H), 3.25 (dd, J = 13.0, 5.3 Hz, 1H), 3.1–3.2 (m, 4H), 2.3 (m, 1H), 1.6 (m, 5H), 1.2 (m, 12H), 0.85 (t, J = 4.3 Hz,

Table 1
MIC of aminoglycosides (μg/mL)

Entry	Compound	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 25923)
1	Neamine	32	4–8
2	**10**	32–64	16–32
3	**11**	⩾250	64–125
4	**RR101**	⩾250	125
5	**NRR101**	⩾250	32–64
6	**NRR102**	125	32
7	Neomycin	4	1
8	**RR501**	16	4–8
9	**NRR501**	125–250	125–250
10	Kanamycin	4	2
11	**RT014**	64–125	125–250
12	**NRT014**	⩾250	⩾250
13	**RT040**	4–8	2–4
14	**NRT040**	16–32	8–16



Scheme 7.

Table 2
MIC of aminoglycosides (μg/mL)

Entry	Compound	*K. pneumoniae* (ATCC 13883)	*K. pneumoniae* (ATCC 700603)	*P. aeruginosa* (ATCC 27853)	*S. aureus* (ATCC 33591)
1	Neamine	2–4	16–32	64–125	125–250
2	**RR101**	16–32	125	16–32	64–125
3	**NRR101**	16–32	⩾250	16–32	64–125
4	**NRR102**	4–8	⩾250	16–32	16–32
5	**RR103**	16–32	125–250	64–125	⩾250
6	**RR104**	32	125–250	125–250	125–250

6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 155.0, 129.5, 123.4, 97.6, 75.2, 74.9, 69.1 (2 carbons), 59.6, 58.2, 54.7, 54.1, 41.6, 41.5, 32.4, 31.7 (2 carbons), 29.99, 29.94, 26.6, 26.5, 22.8 (2 carbons), 14.2 (2 carbons); ESI/APCI Calcd for $C_{26}H_{43}N_{14}O_6^+$ ($[M+H]^+$) *m/e* 647.3485; measured *m/e* 647.3483.

4.4. 1,3,4′,6′-Tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (7)

A solution of compound **3** (0.30 g, 0.77 mmol) in toluene was refluxed overnight. After the removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 90/10 to 50/50), the product was obtained as an oil (0.14 g, 0.36 mmol, 47%) along with the recovered compound **3** (0.12 g, 0.31 mmol, 40%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (d, J = 10.2 Hz, 1H), 5.99 (dd, J = 12.0, 1.8 Hz, 1H), 5.57 (s, 1H), 4.0 (m, 1H), 3.91 (d, J = 9.8 Hz, 1H), 3.4–3.6 (m, 4H), 3.3–3.4 (m, 3H), 2.71 (s, 1H), 2.28 (td, J = 13.4, 4.3 Hz, 1H), 1.4–1.6 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 128.5, 128.2, 94.7, 79.8, 76.3, 76.2, 69.7, 60.5, 59.6, 54.8, 51.8, 32.4; ESI/APCI Calcd for $C_{12}H_{17}N_{12}O_4^+$ ($[M+H]^+$) *m/e* 393.1490; measured *m/e* 393.1502.

4.5. 6-*O*-Benzoyl-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (8)

To a solution of compound **7** (0.10 g, 0.26 mmol) and DIPEA (0.05 mL, 0.31 mmol) in anhydrous CH_2Cl_2 (10 mL) at −50 °C, BzCl (0.03 mL, 0.26 mmol) was added. After being stirred for 2 h, $NaHCO_{3(s)}$ was added and the solvent was removed. The residue was diluted with EtOAc and the organic solution was washed with saturated $NaHCO_{3(aq)}$, water and brine, and then dried over anhydrous Na_2SO_4. After the removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 100/0 to 70/30), the product was obtained as an oil (0.09 g, 0.18 mmol, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (dd, J = 8.0, 1.5 Hz, 2H), 7.61 (td, J = 7.2, 1.2 Hz, 1H), 7.48 (td, J = 7.7, 1.5 Hz, 2H), 6.0 (m, 2H), 5.64 (s, 1H), 5.07 (td, J = 9.6, 3.1 Hz, 1H), 4.0 (m, 1H), 3.9 (m, 1H), 3.5–3.7 (m, 7H), 2.39 (dt, J = 13.4, 4.5 Hz, 1H), 1.63 (dt, J = 12.6, 11.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 134.1, 130.2 (2 carbons), 128.9, 128.8 (2 carbons), 128.4, 128.2, 95.0, 80.5, 76.9, 75.9, 69.8, 59.4, 58.6, 54.8, 51.7, 32.7; ESI/APCI Calcd for $C_{19}H_{21}N_{12}O_5^+$ ($[M+H]^+$) *m/e* 497.1758 ; measured *m/e* 497.1575.

4.6. 5,6-Di-*O*-acetyl-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (9)

To a solution of compound **7** (0.02 g, 0.051 mmol), Et_3N (0.06 mL, 0.41 mmol) and DMAP (catalytic amount) in anhydrous CH_2Cl_2 (10 mL) at 0 °C, Ac_2O (0.02 mL, 0.26 mmol) was added. After being stirred for 2 h, the solvent was removed. After purification with column chromatography (eluted with hexane/EtOAc from 100/0 to 50/50), the product was obtained as an oil (0.02 g, 0.042 mmol, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.02 (d, J = 10.2 Hz, 1H), 5.72 (dt, J = 10.1, 2.1 Hz, 1H), 5.19 (s, 1H), 5.05 (t, J = 9.8 Hz, 1H), 4.92 (t, J = 10.0 Hz, 1H), 3.9 (m, 2H), 3.72 (t, J = 9.7 Hz, 1H), 3.4–3.6 (m, 4H), 2.35 (dt, J = 9.1, 4.6, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 1.5 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 169.7, 129.2, 127.4, 94.9, 78.4, 74.3, 74.0, 69.8, 59.5, 58.2, 54.5, 51.5, 32.4, 20.9, 20.8; ESI/APCI Calcd for $C_{16}H_{20}N_{12}O_6Na^+$ ($[M+Na]^+$) *m/e* 499.1521; measured *m/e* 499.1519.

4.7. 3′,4′-Dideoxyneamine (10)

This compound was prepared similarly to the synthesis of compound **11**. ^{1}H NMR (D_2O, 400 MHz) δ 5.66 (t, J = 3.4 Hz, 1H), 4.1 (m, 1H), 3.86 (t, J = 9.4 Hz, 1H), 3.56 (t, J = 9.2 Hz, 1H), 3.4–3.5 (m, 3H), 3.26 (dd, J = 12.5, 4.3 Hz, 1H), 3.22 (dd, J = 10.3, 4.2 Hz, 1H), 3.14 (dd, J = 13.5, 3.4 Hz, 1H), 2.98 (dd, J = 13.5, 7.2 Hz, 1H), 2.41 (td, J = 8.2, 4.1 Hz, 1H), 1.8–1.9 (m, 4H); ^{13}C NMR (D_2O, 75 MHz) δ 95.2, 77.2, 75.3, 72.6, 65.8, 49.8, 48.8, 48.7, 42.6, 28.3, 25.6, 20.5; ESI/APCI Calcd for $C_{12}H_{27}N_4O_4Na^+$ ($[M+Na]^+$) *m/e* 313.1846; measure *m/e* 313.1853.

4.8. 4′-Amino-2′,3′,4′-trideoxyneamine (11)

To a solution of compound **7** (0.21 g, 0.54 mmol), $NaHCO_{3(s)}$ (catalytic amount), several drops of water in THF (15 mL) at

50 °C, PMe_3 (2.0 mL, 1 M in THF) was added. After being stirred for 30 min., the solvent was removed. The residue was added with degassed water, Pd/C (catalytic amount) and $NaHCO_{3(s)}$ (catalytic amount), and then charged with atmospheric H_2. After being stirred for two days, the reaction was quenched by filtering through Celite and the residue was washed with H_2O and the combined solutions were concentrated. The crude product was purified with Amberlite CG50 (NH_4^+) eluted with a gradient of NH_4OH solution (0–20%). After collection of the desired fractions and removal of solvent, the product was re-dissolved in water and loaded to an ion-exchange column packed with Dowex 1X8-200 (Cl^- form), and eluted with water. After removal of solvent, the product was obtained as white solid (0.08 g, 0.27 mmol, 51%). ^1H NMR (D_2O, 400 MHz) δ 5.4 (m, 1H), 4.2 (m, 1H), 3.81 (t, J = 9.1 Hz, 1H), 3.2–3.5 (m, 7H), 2.38 (td, J = 8.8, 4.5 Hz, 1H), 1.8–2.9 (m, 5H); ^{13}C NMR (D_2O, 100 MHz) δ 96.4, 77.8, 74.9, 72.6, 69.6, 50.0, 48.9, 47.1, 39.4, 28.3, 26.1, 21.1; ESI/APCI Calcd for $C_{12}H_{27}N_4O_4Na^+$ ([M+Na]$^+$) *m/e* 313.1846; measured *m/e* 313.1853.

4.9. 6-*O*-(1,2,3,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (13)

A solution of compound **7** (0.20 g, 0.57 mmol), compound **12** (0.39 g, 0.61 mmol), (1.2 equiv), and activated powder 4 Å molecular sieve 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. The reaction mixture was cooled to −70 °C and *N*-iodosuccinimide (0.14 g, 0.61 mmol) was quickly added into the reaction mixture. After the temperature of the solution warmed up to −30 °C, trifluoromethanesulfonic acid (catalytic amount) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor (ca. 4 h, monitored by TLC, eluted with Hexane/EtOAc = 65/35). The reaction mixture was quenched by the addition of Et_3N (3 mL). After being stirred for 10 min, the reaction mixture was filtered through Celite and the residue was washed with EtOAc. The combined organic solution washed with 10% aqueous $Na_2S_2O_3$, saturated $NaHCO_{3(aq)}$ and brine, and dried over anhydrous Na_2SO_4. After removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 100/0 to 65/35), the product was obtained as an oil (0.34 g, 0.38 mmol, 74%). The other product, **epi-13** was obtained as a minor product (0.02 g, 0.022 mmol, 4%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.3 (m, 20H), 5.90 (d, J = 10.0 Hz, 1H), 5.70 (d, J = 12.4 Hz, 1H), 5.49 (s, 1H), 4.7–5.0 (m, 7H), 4.57 (d, J = 11.7 Hz, 1H), 4.41 (t, J = 11.3 Hz, 2H), 3.8–4.1 (m, 6H), 3.2–3.6 (m, 9H), 2.3 (td, J = 8.6, 3.8 Hz, 1H), 1.4 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.9, 138.5, 138.3, 137.7, 128.8 (2 carbons), 128.7 (2 carbons), 128.6 (2 carbons), 128.5 (4 carbons), 128.4 (2 carbons), 128.2 (2 carbons), 128.0 (2 carbons), 127.9, 127.8 (2 carbons), 127.7 (2 carbons), 127.4, 99.4, 95.0, 87.2, 80.0, 78.4, 77.6, 76.7, 76.0, 75.3, 74.7, 73.7 (2 carbons), 71.3, 70.0, 69.5, 59.8, 59.5, 54.7, 51.6, 32.8; ESI/APCI Calcd for $C_{46}H_{50}N_{12}O_9Na^+$ ([M+Na]$^+$) *m/e* 937.3721; measured *m/e* 937.3702.

4.10. 6-*O*-(1,2,3,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-1,3,4′,6′-tetraazido-2′,3′,4′-trideoxy-2′-enoneamine (epi-13)

Please refer to the procedure for the preparation of compound **13**. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.3 (m, 20H), 5.90 (d, J = 10.0 Hz, 1H), 5.74 (dt, J = 12.4, 2.1 Hz, 1H), 5.52 (s, 1H), 5.05 (d, J = 3.5 Hz, 1H), 4.94 (t, J = 11.7 Hz, 1H), 4.91 (d, J = 8.3 Hz, 1H), 4.7–4.8 (m, 3H), 4.58 (d, J = 11.3 Hz, 1H), 4.4 (m, 3H), 4.1 (m, 2H), 4.0 (m, 1H), 3.9 (m, 2H), 3.81 (d, J = 1.7 Hz, 1H), 3.2–3.6 (m, 9H), 2.24 (td, J = 13.4, 4.5 Hz, 1H), 1.4 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9, 138.5, 138.3, 137.7, 128.8 (2 carbons), 128.7, 128.6 (2 carbons), 128.5 (4 carbons), 128.4 (2 carbons), 128.3 (2 carbons), 128.0 (2 carbons), 127.9, 127.8 (2 carbons), 127.7 (2 carbons), 127.4 (2 carbons), 99.4, 95.0, 87.2, 80.1, 78.3, 76.7, 76.0, 75.3, 74.7, 73.7 (2 carbons), 71.3, 70.0, 69.5, 59.8, 59.6, 54.7, 51.7, 32.8, 14.3; ESI/APCI Calcd for $C_{46}H_{50}N_{12}O_9Na^+$ ([M+Na]$^+$) *m/e* 937.3721; measured *m/e* 937.3699.

4.11. 6-*O*-(1,2,3,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-1,3,4′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (14)

This compound was prepared similarly to the synthesis of compound **13**. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.2–7.4 (m, 20H), 5.9 (m, 2H), 5.08 (d, J = 3.4 Hz, 1H), 4.94 (t, J = 11.7 Hz, 1H), 4.7–4.9 (m, 5H), 4.4–4.6 (m, 4H), 4.1 (m, 1H), 4.10 (d, J = 3.8 Hz, 1H), 3.95 (dd, J = 8.3, 2.8 Hz, 1H), 3.8 (m, 1H), 3.7 (m, 2H), 3.2–3.5 (m, 8H), 2.27 (td, J = 13.4, 4.5 Hz, 1H), 1.6 (s, 1H), 1.46 (td, J = 12.9, 12.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0, 138.7, 138.5, 137.9, 129.5, 128.7 (2 carbons), 128.6 (2 carbons), 128.6 (4 carbons), 128.5 (2 carbons), 128.4 (2 carbons), 128.1 (2 carbons), 128.1, 128.0, 127.9, 127.8 (2 carbons), 127.7 ,123.7, 99.6, 96.9, 87.2, 79.1, 78.5, 76.7, 76.4, 75.3, 74.8, 73.8 (2 carbons), 73.7, 71.2, 69.4, 69.0, 59.9, 59.8, 54.9, 54.0, 32.9; ESI/APCI Calcd for $C_{46}H_{50}N_{12}O_{9+}Na^+$ ([M+Na]$^+$) *m/e* 937.3721; measured *m/e* 937.3704.

4.12. 6-*O*-(3-Azido-2-*O*-benzyl-3,6-dideoxy-α-D-glucopyranosyl)-1,3,4′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (16)

The first step for the preparation of this compound was similar to the synthesis of compound **13**. The product from the first step was dissolved in methanol (5 mL), and sodium methoxide (0.5 M in MeOH, 1 mL) was added. The reaction mixture was stirred at room temperature till the completion of the reaction (ca. 2 hours, monitored by TLC, eluted with hexane/EtOAc = 50/50). The reaction mixture was filtered through Celite and the residue was washed with MeOH. After the removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 90/10 to 35/65), the product was obtained as a solid (0.26 g, 0.40 mmol, 78%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.4 (m, 5H), 6.0 (m, 2H), 5.60 (s, 1H), 4.6–4.9 (m, 4H), 2.9–4.2 (m, 13H), 2.4 (m, 1H), 2.26 (td, J = 13.1, 4.5 Hz, 1H), 1.4 (m, 1H), 1.27 (d, J = 3.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.4, 128.8 (2 carbons), 128.7, 128.5, 128.4 (2 carbons), 128.0, 97.7, 95.0, 87.2, 79.8, 78.3, 76.0, 74.0, 73.4, 69.7, 69.0, 64.9, 59.5, 59.4, 54.8, 51.8, 32.9, 17.9; ESI/APCI Calcd for $C_{25}H_{35}N_{16}O_7^+$ ([M+NH$_4$]$^+$) *m/e* 671.2896; measured *m/e* 671.2852.

4.13. 6-*O*-(3-Azido-2-*O*-benzyl-3-deoxy-α-D-xylopyranosyl)-1,3,4′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (18)

This compound was prepared similarly to the synthesis of compound **16**. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.4–7.5 (m, 5H), 5.95 (d, J = 10.5 Hz, 1H), 5.890 (d, J = 10.8 Hz, 1H), 5.8 (d, 4.3 Hz, 1H), 4.9 (d, J = 3.0 Hz, 1H), 4.6–4.7 (m, 2H), 4.23 (d, J = 3.0 Hz, 1H), 4.09 (m, 1H), 3.6–3.9 (m, 6H), 3.2–3.5 (m, 8H), 2.32 (ddd, J = 13.2, 4.5, 4.5 Hz, 1H), 1.36 (ddd, J = 13.2, 12.5, 12.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.5, 128.6, 128.0, 127.7, 127.6, 122.7, 122.4, 97.1, 96.3, 85.0, 81.3, 79.0, 75.1, 73.0, 68.3, 67.5, 63.8, 62.7, 58.8, 58.7, 54.4, 53.3, 32.0, 29.1; MALDI Calcd for $C_{24}H_{29}N_{15}O_7Na$ ([M+Na]$^+$) *m/e* 662.2267; measured *m/e* 662.2232.

4.14. 3,4-Di-*O*-acetyl-2-*O*-benzyl-1-phenylthio-β-D-ribopyranoside (20)

To a solution of **19**[15] (1.1 g, 3.3 mmol) and pyridine (1.22 ml, 15 mmol) in anhydrous CH_2Cl_2 at 0 °C, Tf_2O (4.3 ml, 15 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. The solution was filtered through glass wool and transferred into a solution of tetrabutylammonium acetate (4.0 g, 13 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 (eluted with hexane/EtOAc from 100/0 to 50/50) afforded the desired product (0.80 g, 1.9 mmol, 58%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.4–7.5 (m, 2H), 7.2–7.3 (m, 8H), 5.69 (m, 1H), 5.06 (d, J = 8.2 Hz, 1H), 4.96 (ddd, J = 9.2, 4.9, 3.3 Hz, 1H), 4.60 (d, J = 11.2 Hz, 1H), 4.45 (d, J = 11.2 Hz, 1H), 3.99 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 11.2, 9.2 Hz, 1H), 3.49 (dd, J = 8.2, 3.0 Hz, 1H), 2.12 (s, 3H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 170.0, 137.3, 132.8, 132.7 (2 carbons), 129.1 (2 carbons), 128.6 (2 carbons), 128.4 (2 carbons), 128.2, 128.0, 84.6, 75.1, 72.3, 67.4, 67.1, 63.9, 21.0, 20.9; HRFAB Calcd for $C_{22}H_{24}O_6SNa^+$ ([M+Na]$^+$) m/e 439.1186; measure m/e 439.1191.

4.15. 4-*O*-Benzoyl-2-*O*-benzyl-1-phenylthio-β-D-ribopyranoside (21)

To a solution of **20** (0.79 g, 1.9 mmol) in MeOH (50 mL) and THF (10 mL), few drops of 1 M NaOMe was added. The reaction was stirred for 2 h. TLC indicated the completion of the reaction. The reaction was quenched by Amberlite IR-120 (H^+) and filtration. Removal of the solvent afforded the diol which was dissolved in anhydrous CH_2Cl_2 (30 mL) and then DMAP (catalytic amount), DIPEA (0.54 ml, 3.2 mmol), and BzCl (0.20 ml, 1,8 mmol) were added 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 layer was 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 (eluted with hexane/EtOAc from 100/0 to 60/40) afforded the desired product (0.40 g, 0.92 mmol, 48%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.1 (m, 2H), 7.59 (tt, J = 7.2, 1.4 Hz, 1H), 7.4–7.5 (m, 4H), 7.2–7.4 (m, 8H), 5.63 (d, J = 2.4 Hz, 1H), 5.32 (t, J = 3.1 Hz, 1H), 4.74 (d, J = 11.7 Hz, 1H), 4.66 (d, J = 11.7 Hz, 1H), 4.41 (dd, J = 12.4, 1.0 Hz, 1H), 4.15 (s, 1H), 4.08 (s, broad, 1H), 3.93 (dd, J = 12.4, 2.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0, 136.9, 133.9, 133.69 (2 carbons), 131.7 (2 carbons), 130.2, 129.9, 129.4 (2 carbons), 128.8 (2 carbons), 128.7 (2 carbons), 128.2 (2 carbons), 127.9 (2 carbons), 86.2, 78.0, 73.4, 69.4, 68.4, 66.2; ESI/APCI Calcd for $C_{25}H_{24}O_5SNa^+$ ([M+Na]$^+$) m/e 459.1237; measure m/e 459.1235.

4.16. 3-Azido-4-*O*-benzoyl-2-*O*-benzyl-3-deoxy-1-phenylthio-β-D-xylopyranoside (17)

To a solution of **21** (0.39 g, 0.9 mmol) and pyridine (0.18 mL, 39 mmol) in anhydrous CH_2Cl_2 at 0 °C, Tf_2O (0.3 mL, 2.3 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. After the removal of the solvent, the crude product was dissolved into DMF (20 mL) and to this NaN_3 (0.46 g, 7.1 mmol) was added. The reaction was stirred for 24 h at room temperature. Removal of the solvent followed by purification with gradient column chromatography (eluted with hexane/EtOAc from 100/0 to 50/50) afforded the desired product (0.35 g, 0.76 mmol, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.0–8.1 (m, 2H), 7.2–7.6 (m, 13H), 5.04 (ddd, J = 9.6, 9.5, 5.3 Hz, 1H), 4.95 (d, J = 10.2 Hz, 1H), 4.80 (d, J = 10.2 Hz, 1H), 4.75 (d, J = 9.1 Hz, 1H), 4.35 (dd, J = 11.5, 5.3 Hz, 1H), 3.85 (dd, J = 9.3, 9.6 Hz, 1H), 3.45 (dd, J = 9.1, 9.3 Hz, 1H), 3.40 (dd, J = 11.5, 9.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6, 137.5, 133.8, 133.2, 132.5, 130.0 (2 carbons), 129.3 (3 carbons), 128.74 (3 carbons), 128.70 (3 carbons), 128.4 (2 carbons), 128.2, 88.7, 79.1, 75.6, 70.3, 67.7, 66.9; EI Calcd for $C_{25}H_{27}N_4O_4S^+$ ([M+NH$_4$]$^+$) m/e 479.1748; measure m/e 479.1757.

4.17. 5-*O*-(4-Azido-4-deoxy-β-D-glucopyranosyl)-1,3,4′,6′-tetraazido-3′,4′-dideoxy-3′-enoneamine (23)

A solution of compound **22** (0.83 g, 2.0 mmol), compound **8** (0.83 g, 1.7 mmol), and activated powder 4 Å molecular sieve was stirred in anhydrous CH_2Cl_2 (8 mL) at 65 °C for 1 hr and then added with BF_3–OEt_2 (0.05 mL, 0.42 mmol). After the reaction was warmed to room temperature, the reaction was quenched by adding few drops of water. After being stirred for 20 min, $NaHCO_{3(s)}$ was added, and then the reaction mixture was filtered through a short column packed with layers of Celite and silica gel. The column was washed with EtOAc. After removal of the solvent and purification with column chromatography (eluted with hexanes/EtOAc from 100/0 to 60/40) afforded the intermediate product. The intermediate product was dissolved in methanol (5 mL) and few drops of water, and NaOMe (catalytic amount) was added. The reaction mixture was stirred at room temperature till the completion of the reaction (ca. 1 h, monitored by TLC, eluted with hexane/EtOAc = 50/50). The reaction mixture was filtered through Celite and the residue was washed with EtOAc. After the removal of the solvents and purification with column chromatography (eluted with hexane/EtOAc from 90/10 to 30/70), the product was obtained as a solid (0.52 g, 0.92 mmol, 56%). ^{1}H NMR (CD_3OD, 300 MHz) δ 6.10 (dt, J = 10.3, 2.4 Hz, 1H), 5.99 (d, J = 10.0 Hz, 1H), 5.63 (s, 1H), 4.60 (d, J = 8.2 Hz, 1H), 4.01 (dt , J = 10.0, 4.1 Hz, 1H), 3.87 (dd, J = 10.0, 1.7 Hz, 1H), 3.6 (m, 2H), 3.2–3.5 (m, 8H), 2.99 (t, J = 9.6 Hz, 1H), 2.16 (td, J = 13.4, 4.5 Hz, 1H), 1.33 (d, J = 6.2 Hz, 3H), 1.2 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 128.9, 127.2, 104.4, 94.3, 85.5, 78.1, 77.0, 75.9, 75.0, 70.9, 69.9, 68.3, 60.0, 59.9, 55.1, 51.6, 32.2, 17.7; ESI/APCI Calcd for $C_{18}H_{26}N_{15}O_7^+$ ([M+H]$^+$) m/e 564.2134; measure m/e 564.2125.

4.18. 6-*O*-Hexylaminocarbonyl-3′,4′-dideoxyneamine (RR101)

This compound was prepared similarly to the synthesis of compound **11** except no $NaHCO_{3(s)}$ was used in the first step, but MeOH and couple drops of HOAc/H_2O (1/4) was used as the solvent in the second step. ^{1}H NMR (D_2O, 300 MHz) δ 5.66 (d, J = 3.5 Hz, 1H), 2.9–4.1 (m, 13H), 2.44 (td, J = 12.4, 8.6 Hz, 1H), 1.9 (m, 1H), 1.7–1.8 (m, 2H), 1.3 (m, 2H), 1.2 (m, 6H), 0.73 (t, J = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 157.2, 95.2, 76.5, 74.1, 73.4, 66.0, 48.9, 48.6, 48.3, 42.8, 41.0, 30.9, 28.8, 28.4, 25.8, 25.7, 22.1, 20.5, 13.5; ESI/APCI Calcd for $C_{19}H_{42}N_5O_6^+$ ([M+H_2O]$^+$) m/e 436.3130; measured m/e 436.2763.

4.19. 4′-Amino-6-*O*-hexylaminocarbonyl-1,3,4′,6′-tetraamino-2′,3′,4′-trideoxyneamine (NRR101)

This compound was prepared similarly to the synthesis of compound **11** but using MeOH and couple drops of HOAc/H_2O (1/4) as the solvent. ^{1}H NMR (D_2O, 300 MHz) δ 5.66 (d, J = 3.5 Hz, 1H), 2.9–4.3 (m, 11H), 2.4 (m, 1H), 1.5–1.9 (m, 5H), 1.4 (m, 2H), 1.2 (m, 6H), 0.73 (t, J = 6.2 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 157.3, 95.2, 74.3, 73.8, 73.0, 66.0, 48.9, 48.6, 48.3, 42.8, 41.0, 40.8, 30.9, 28.8, 28.3, 25.8, 22.1, 20.6, 13.5; ESI/APCI Calcd for $C_{19}H_{41}N_5O_5^+$ ([M+H]$^+$) m/e 418.3024; measured m/e 418.3027.

4.20. 4′-Amino-5,6-di-*O*-hexylaminocarbonyl-1,3,4′,6′-tetraamino-2′,3′,4′-trideoxyneamine (NRR102)

This compound was prepared similarly to the synthesis of compound **11** but using MeOH and couple drops of HOAc/H_2O (1/4) as the solvent in the second step. ^{1}H NMR (D_2O, 300 MHz) δ 5.24 (d, J = 3.1 Hz, 1H), 4.9 (m, 2H), 4.33 (t, J = 10.0 Hz, 1H), 4.1 (m, 1H), 3.6 (m, 3H), 3.4 (m, 1H), 3.15 (d, J = 5.9 Hz, 2H), 2.9–3.0 (m, 3H), 2.48 (td, J = 12.7, 4.1 Hz, 1H), 2.0 (m, 4H), 1.8 (m, 1H), 1.3 (m, 4H), 1.2 (m, 12H), 0.75 (t, J = 4.5 Hz, 6H); ^{13}C NMR (D_2O, 100 MHz) δ 156.8, 156.5, 93.2, 75.2, 73.8, 72.6, 67.9, 48.7, 48.4, 48.1, 41.9, 41.2 (2 carbons), 31.2 (2 carbons), 29.1, 29.0, 28.0, 26.2 (2 carbons), 24.0, 22.3 (2 carbons), 20.7, 13.7 (2 carbons); ESI/APCI Calcd for $C_{26}H_{54}N_6O_6^+$ ([M+H]$^+$) *m/e* 545.4021; measured *m/e* 545.4021.

4.21. 6-*O*-(α-D-Galactopyranosyl)-2′,3′,4′-trideoxy-4′-aminoneamine (NRT014)

This compound was prepared similarly to the synthesis of compound **11** but using MeOH and couple drops of HOAc/H_2O (1/4) as the solvent in the second step. ^{1}H NMR (D_2O, 300 MHz) δ 5.32 (t, J = 4.8 Hz, 1H), 4.97 (d, J = 3.4 Hz, 1H), 4.2 (m, 1H), 4.01 (t, J = 5.9 Hz, 1H), 3.88 (d, J = 2.4 Hz, 1H), 3.2–3.8 (m, 11H), 2.7 (m, 1H), 2.37 (td, J = 12.4, 4.1 Hz, 1H), 1.7–2.0 (m, 5H); ^{13}C NMR (D_2O, 100 MHz) δ 101.8, 96.6, 83.6, 77.9, 73.9, 72.1, 69.5(2 carbons), 69.1, 68.9, 61.1, 50.0, 48.7, 47.1, 39.4, 27.8, 26.2, 21.1; ESI/APCI Calcd for $C_{18}H_{37}N_4O_9^+$ ([M+H]$^+$) *m/e* 453.2555; measured *m/e* 453.2549.

4.22. 6-*O*-(α-D-Galactopyranosyl)-3′,4′-dideoxyneamine (RT014)

This compound was prepared similarly to the synthesis of compound **11** except no $NaHCO_{3(s)}$ was used in the first step. ^{1}H NMR (D_2O, 300 MHz) δ 5.65 (d, J = 3.3 Hz, 1H), 5.01 (d, J = 3.6 Hz, 1H), 4.06 (t, J = 6.3 Hz, 2H), 3.6–3.9 (m, 9H), 3.4–3.5 (m, 2H), 3.13 (dd, J = 13.4, 3.1, 1H), 2.98 (dd, J = 13.4, 7.2 Hz, 1H), 2.38 (dt, J = 12.7, 4.1 Hz, 1H), 1.7–1.9 (m, 4H), 1.5 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 101.7, 95.7, 83.8, 77.4, 74.4, 72.6, 69.4, 69.1, 68.7, 66.0, 61.3, 49.7, 48.9, 48.6, 42.7, 28.0, 25.6, 20.6; ESI/APCI Calcd for $C_{18}H_{37}N_4O_9^+$ ([M+H]$^+$) *m/e* 453.2555; measured *m/e* 453.2565.

4.23. 6-*O*-(3-Amino-3,6-dideoxy-α-D-glucopyranosyl)-2′,3′,4′-trideoxy-4′-aminoneamine (NRT040)

This compound was prepared similarly to the synthesis of compound **11** but using MeOH and couple drops of HOAc/H_2O (1/4) as the solvent in the second step. ^{1}H NMR (D_2O, 400 MHz) δ 5.31 (s, 1H), 4.91 (s, 1H), 4.2 (m, 1H), 3.2–3.8 (m, 11H), 3.1 (m, 1H), 2.4 (m, 1H), 1.8–2.0 (m, 5H), 1.14 (d, J = 5.6 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 100.9, 96.8, 83.5, 78.2, 73.6, 71.0, 70.0, 68.9, 68.6, 55.0, 50.3, 48.8, 47.0, 39.3, 27.8, 25.8, 21.0, 16.5; ESI/APCI Calcd for $C_{18}H_{38}N_5O_7^+$ ([M+H]$^+$) *m/e* 436.2766; measured *m/e* 436.2771.

4.24. 6-*O*-(3-Amino-3-deoxy-α-D-xylopyranosyl)-3′,4′-dideoxyneamine (RT040)

This compound was prepared similarly to the synthesis of compound **11** except no $NaHCO_{3(s)}$ was used in the first step. ^{1}H NMR (D_2O, 400 MHz) δ 5.79 (d, J = 3.5 Hz, 1H), 5.04 (d, J = 3.6 Hz, 1H), 4.3 (m, 1H), 3.5–4.0 (m, 9H), 3.0–3.5 (m, 8H), 2.54 (ddd, J = 13.2, 4.5, 4.5 Hz, 1H), 1.70 (ddd, J = 13.2, 12.5, 12.5 Hz, 1H,); ^{13}C NMR (D_2O, 100 MHz) δ 100.8, 95.2, 84.0, 77.1, 74.5, 68.3, 66.2, 65.6, 57.1, 55.3, 49.9, 48.9, 48.7, 42.8, 27.1, 25.6, 20.7; HRFAB Calcd for $C_{17}H_{36}N_5O_7^+$ ([M+H]$^+$) *m/e* 422.2615; measured *m/e* 422.2603.

4.25. 6-*O*-(4-Amino-4,6-dideoxy-β-D-glucopyranosyl)-2′,3′,4′-trideoxy-4′-aminoneamine (NRR501)

This compound was prepared similarly to the synthesis of compound **11** but using MeOH and couple drops of HOAc/H_2O (1/4) as the solvent in the second step. ^{1}H NMR (D_2O, 300 MHz) δ 5.83 (t, J = 3.4 Hz, 1H), 4.6 (m, 1H), 3.89 (td, J = 8.6, 3.5 Hz, 1H), 2.8–3.6 (m, 11H), 2.61 (t, J = 10.0 Hz, 1H), 2.11 (td, J = 12.4, 3.8 Hz, 1H), 1.4–1.9 (m, 5H), 1.19 (d, J = 6.2 Hz, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 102.5, 96.8, 81.8, 78.3, 74.3, 73.8, 73.4, 70.4, 69.5, 57.4, 50.3, 49.0, 47.7, 40.4, 31.2, 27.0, 23.2, 17.2; ESI/APCI Calcd for $C_{18}H_{38}N_5O_7^+$ ([M+H]$^+$) *m/e* 436.2766; measured *m/e* 436.2762.

4.26. 3′,4′-Di-*O*-benzyl-6-*O*-hexylaminocarbonyl-1,3,4′,6′-tetraazidoneamine (25)

This compound was prepared similarly to the synthesis of compound **5**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2–7.4 (m, 10H), 5.33 (d, J = 3.8 Hz, 1H), 4.8–4.9 (m, 5H), 4.62 (d, J = 11.0 Hz, 1H), 4.1–4.2 (m, 2H), 3.98 (t, J = 9.6 Hz, 1H), 3.2–3.6 (m, 10H), 2.3 (m, 1H), 1.4–1.5 (m, 3H), 1.3 (m, 6H), 0.88 (t, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.1, 137.8, 137.7, 128.79 (2 carbons), 128.75 (2 carbons), 128.3 (2 carbons), 128.0 (2 carbons), 99.3, 83.2, 80.8, 78.8, 76.4, 75.8, 75.7, 75.5, 71.4, 64.2, 59.2, 58.5, 51.2, 41.7, 32.2, 31.8, 29.9, 29.8, 26.8, 26.6, 22.8, 14.2; ESI/APCI Calcd for $C_{33}H_{43}N_{13}O_7^+$ ([M+H]$^+$) *m/e* 734.3481; measure *m/e* 734.3485.

4.27. 3′,4′-Di-*O*-benzyl-5,6-Di-*O*-hexylaminocarbonyl-1,3,4′,6′-tetraazidoneamine (26)

This compound was prepared similarly to the synthesis of compound **5**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2–7.4 (m, 10H), 5.33 (d, J = 3.4 Hz, 1H), 4.89 (d, J = 12.7 Hz, 1H), 4.86 (d, J = 1.7 Hz, 2H), 4.60 (d, J = 11.0 Hz, 1H), 4.09 (t, J = 9.6 Hz, 1H), 3.8–4.0 (m, 8H), 3.2–3.7 (m, 8H), 2.49 (td, J = 8.9, 5.2 Hz, 1H), 1.5–1.6 (m, 5H), 1.3 (m, 12H), 0.88 (t, J = 4.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6, 152.7, 137.69, 137.66, 128.8 (3 carbons), 128.3 (3 carbons), 128.2 (2 carbons), 128.1 (2 carbons), 96.5, 81.0 (2 carbons), 79.9, 78.5, 75.8, 75.4, 75.2, 71.9, 63.1, 60.2, 56.3, 51.1, 43.5, 40.9, 33.9, 31.7, 29.8, 29.0, 26.9 (2 carbons), 26.8, 22.8 (2 carbons), 14.2 (2 carbons); ESI/APCI Calcd for $C_{40}H_{56}N_{14}O_8Na^+$ ([M+Na]$^+$) *m/e* 883.4289; measured *m/e* 883.4288.

4.28. 6-*O*-Hexylaminocarbonylneamine (RR103)

This compound was prepared similarly to the synthesis of compound **11**. ^{1}H NMR (D_2O, 300 MHz) δ 5.77 (d, J = 3.8 Hz, 1H), 3.7–3.9 (m, 4H), 2.9–3.5 (m, 9H), 2.40 (td, J = 8.6, 4.1 Hz, 1H), 1.84 (td, J = 12.5, 3.4 Hz, 1H), 1.35 (q, J = 6.5 Hz, 2H), 1.1 (m, 6H), 0.71 (t, J = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 157.3, 96.0, 77.7, 74.4, 73.7, 70.9, 69.3, 68.4, 53.7, 48.4, 48.3, 40.9, 40.3, 30.8, 28.9, 28.8, 25.8, 22.1, 13.5; ESI/APCI Calcd for $C_{19}H_{40}N_5O_7^+$ ([M+H]$^+$) *m/e* 450.2922; measured *m/e* 450.2928.

4.29. 5,6-Di-*O*-hexylaminocarbonylneamine (RR104)

This compound was prepared similarly to the synthesis of compound **11**. ^{1}H NMR (D_2O, 300 MHz) δ 5.20 (d, J = 3.8 Hz, 1H), 4.85 (t, J = 9.3 Hz, 1H), 4.74 (t, J = 10.0 Hz, 1H), 3.6–3.9 (m, 3H), 2.8–3.4 (m, 10H), 2.15 (td, J = 8.9, 4.1 Hz, 1H), 1.55 (td, J = 12.6, 12.5 Hz, 1H), 1.3 (m, 4H), 1.1 (m, 12H), 0.70 (t, J = 6.9 Hz, 6H); ^{13}C NMR (D_2O, 100 MHz) δ 160.0, 156.7, 94.1, 75.6, 75.2, 73.3, 70.7, 69.3, 68.5, 53.5, 48.6, 48.3, 41.2, 41.1, 40.2, 30.9 (2 carbons), 29.8, 28.8 (2 carbons), 25.9, 25.8, 22.1 (2 carbons), 13.5 (2 carbons); ESI/APCI Calcd for $C_{26}H_{53}N_6O_8^+$ ([M+H]$^+$) *m/e* 577.3919, measured *m/e* 577.3929.

4.30. Procedure for MIC determination

A solution of selected bacteria was inoculated in the Trypticase Soy broth at 35 °C for 1–2 h. After which, the bacteria concentration was found, and diluted with broth, if necessary, to an absorption value of 0.08 to 0.1 at 625 nm. The adjusted inoculated medium (100 μL) was diluted with 10 mL broth, and then applied to a 96-well microtiter plate (50 μL). A series of solutions (50 μL each in 2-fold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35 °C for 12–18 h. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.027.

References and notes

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8. We have conducted serial reactions for the purpose of revealing the factors that cause the allylic azide rearrangement. However, no allylic azide rearrangement was observed except in the presence of triethyl ammonium salt, which triggered very minimum rearrangement. Please refer Supplementary data for details. Our conclusion is that thermodynamic condition is the most reliable method to conduct allylic azide rearrangement in our system.
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15. Compound **19** was prepared according to the same procedure as the preparation of 2-*O*-benzyl-1-phenylthio-β-D-fucopyranoside described in Ref. 9.