Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7711–7719
Bioorganic & Medicinal Chemistry

Synthesis and antibacterial activity of pyranmycin derivatives with *N*-1 and *O*-6 modifications

Jie Li, Fang-I Chiang, Hsiao-Nung Chen and Cheng-Wei Tom Chang*

Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300, USA

Received 2 July 2007; revised 24 August 2007; accepted 28 August 2007
Available online 1 September 2007

Abstract—Continuing from our ongoing effort in modifying aminoglycoside antibiotics with the goal of counteracting drug resistant bacteria, we have further derivatized pyranmycin, a neomycin class aminoglycoside antibiotic, with modifications at *O*-6 and *N*-1 positions. The revealed SAR results demonstrated that the antibacterial activity of pyranmycin can be modulated by different acylic substituents at *O*-6. Among these results, the 6-*O*-aminoethyl derivative, JT050, showed effective activity against resistant strain *Escherichia coli* (pTZ19U-3) and *E. coli* (pSF815), which provides insight into further structural modifications.

1. Introduction

Aminoglycoside antibiotics have been a valuable resource against infectious diseases due to their broad spectrum activity against both Gram positive and negative bacteria.[1–3] Nevertheless, the prevalence of aminoglycoside resistant bacteria has significantly reduced their effectiveness.[4–6] Advancing discoveries from the studies of resistant mechanism and the structural information from the binding of aminoglycoside toward the target, the A-site decoding region of 16S rRNA,[7–9] have inspired new strategies for structural modifications of aminoglycosides aiming to revive the antibacterial activity against aminoglycoside resistant bacteria.[10] For example, attaching functionalities at the *N*-1 position of the 2-deoxystreptamine of kanamycin A has led to the development of semi-synthetic amikacin that has an (*S*)-4-amino-2-hydroxybutanoyl (AHB) group at *N*-1 position (Fig. 1). The added AHB group has been suggested to cause steric interactions toward the aminoglycoside-modifying enzymes leading to the regain of antibacterial activity of aminoglycosides.[13,19]

Our laboratory has been working on the structural optimization of a novel class of aminoglycoside antibiotic, pyranmycin (Fig. 2).[11,12] From the studies of structure–activity relationship, we have noted that the attachment of AHB group at *N*-1 can also revive the antibacterial activity of pyranmycin against resistant bacteria. Therefore, we began to explore other possible site(s) for introducing further structural modifications with the aim to enhance the activity.

Recently, there are two reports focusing on the structural modifications at *O*-5 and/or *O*-6 of 1-*N*-(*S*)-4-amino-2-hydroxybutyryl neamine derivatives.[13,14] The SAR studies showed that both *O*-5 and *O*-6 positions can be the effective sites for structural modifications, if appropriate functional groups were incorporated. Inspired by the results, we began to investigate whether the addition of functional groups at *O*-6 can also be beneficial for the antibacterial activity of pyranmycin containing AHB group at *N*-1. Since trisaccharide-based aminoglycoside, such as ribostamycin, is much more active than disaccharide-based aminoglycoside like neamine, it is expected that pyranmycin bearing both *N*-1 AHB group and *O*-6 functionalities may yield improved antibacterial activity as shown in previous reports. In this paper, we describe the synthesis of the pyranmycin derivatives with both *O*-6 and *N*-1 modifications and the antibacterial activity study of these derivatives (Fig. 3).

2. Results

We have recently developed a protocol for regioselective reduction of the *N*-1 azido group from polyazidoaminoglycoside derivatives.[15] The synthesis of pyranmycin

Keywords: Aminoglycoside; Neomycin; Pyranmycin; Aminoglycoside resistant bacteria.
* Corresponding author. Tel.: +1 435 797 3545; fax: +1 435 797 3390; e-mail: chang@cc.usu.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.08.059

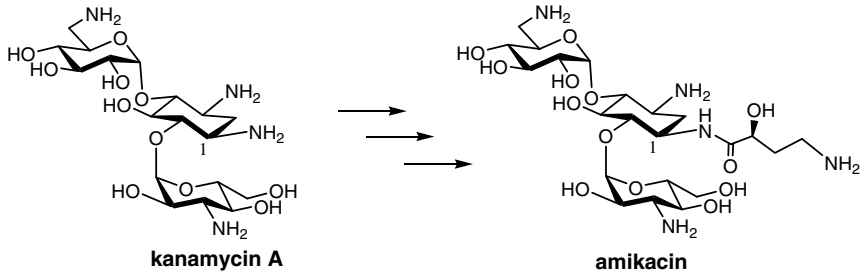


Figure 1. Structures of amikacin and kanamycin A.

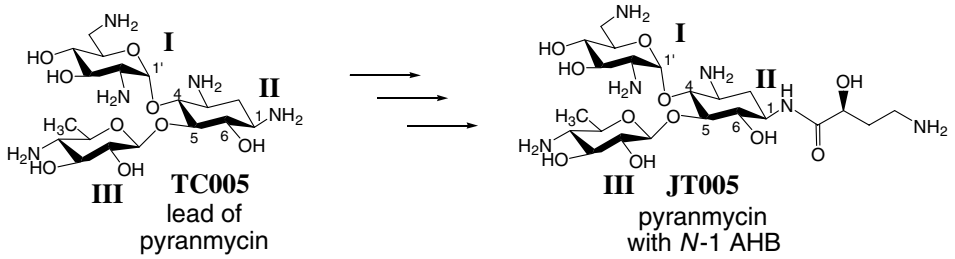


Figure 2. Design of pyranmycin with *N*-1 AHB.

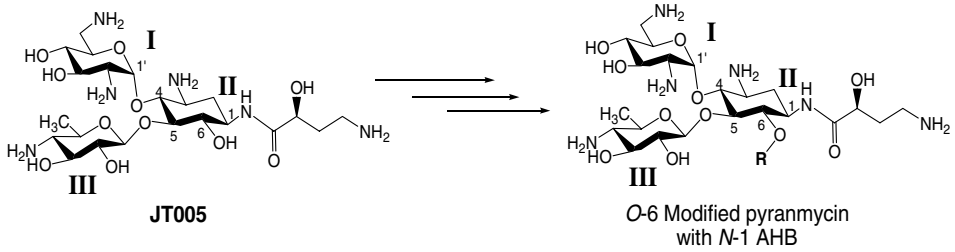


Figure 3. Design of pyranmycin with *O*-6 modification and *N*-1 AHB.

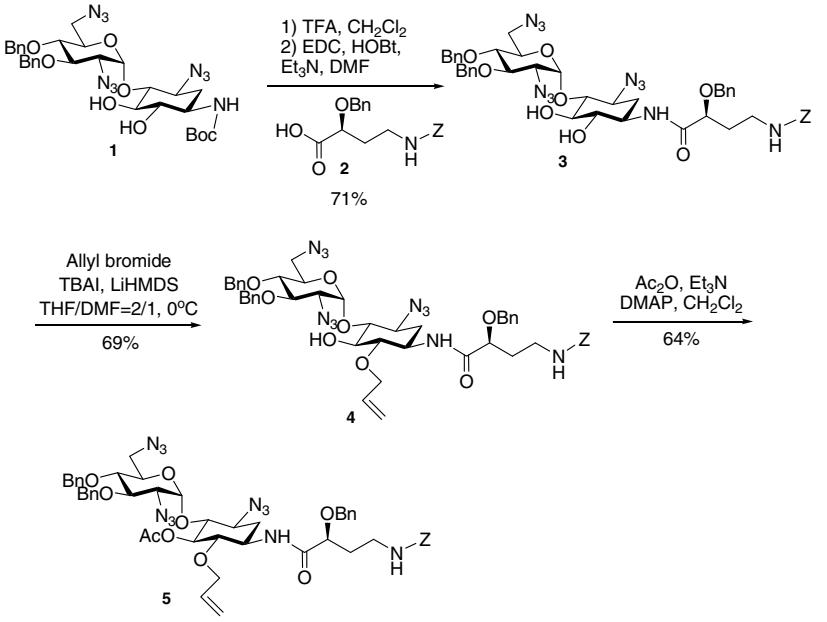
with both *O*-6 and *N*-1 AHB began with the synthesis of neamine derivative bearing *O*-6 allyl group and *N*-1 AHB (Scheme 1). The *N*-1 AHB group can be installed from deprotection of the Boc group of **1**,[15] followed by coupling with the protected AHB derivative, **2**, which can be prepared using the reported procedure.[16] Interestingly, using the bulky base under the specific ratio of mixture solvents for the allylation of **3**, the *O*-6 mono-allylated adduct, **4**, was obtained as the major product.[17] The regioselectivity was confirmed by the acetylation of *O*-5 hydroxyl group of **4**. The characteristic down-field shift of *H*-5 of **5** can be discerned by ^{1}H–^{1}H COSY.

After verifying the regioselectivity of allylation, a glycosylation of **4** with **6**[12] followed by hydrolysis of acetyl groups furnished **7**, which led to the synthesis of **JT052** after the global deprotection. In an alternative route, compound **7** was subjected to ozonolysis and $NaBH_4$ reduction (Scheme 2). The resulting compound **8** was converted to **9** via substitution of hydroxyl group with azido group. Both **8** and **9** were transformed to the corresponding **JT051** and **JT050**, respectively, in similar fashion.

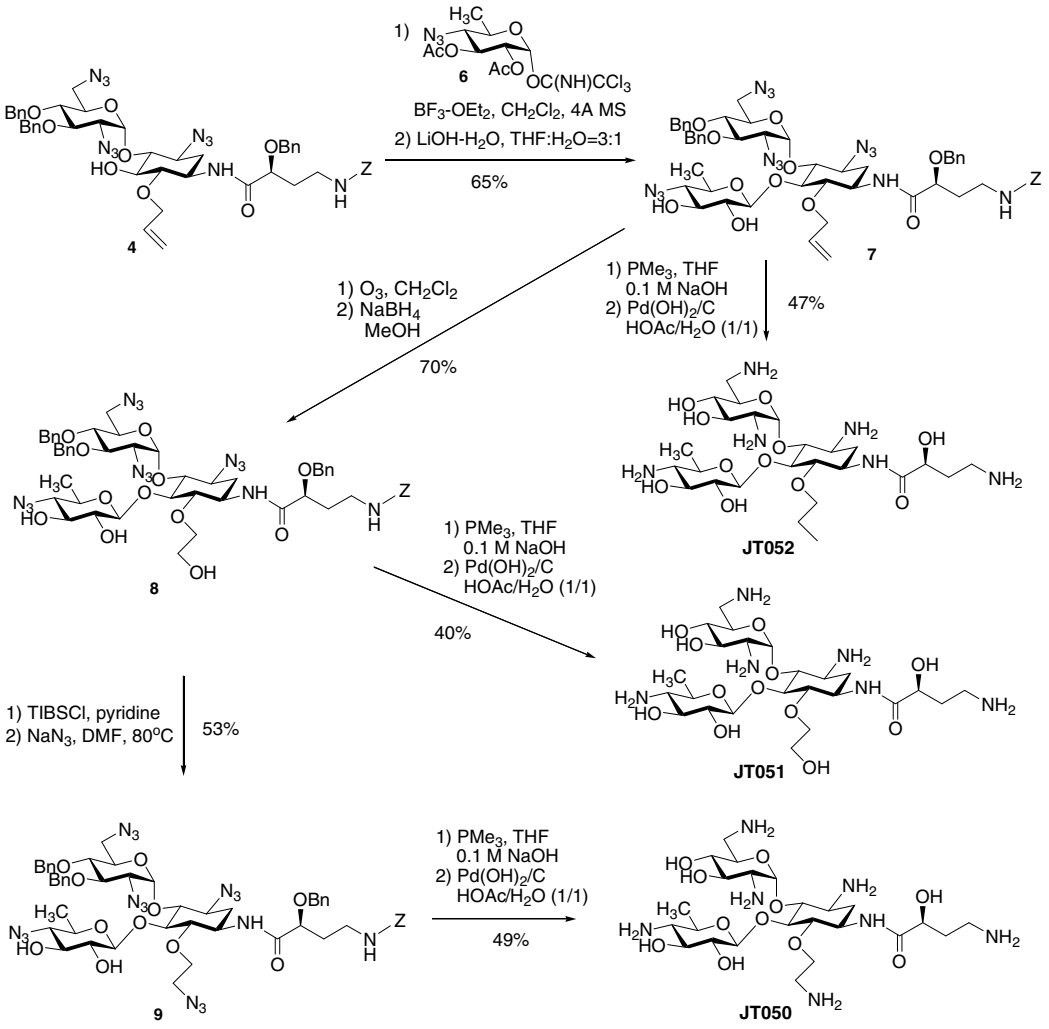
The above synthesized aminoglycosides were assayed against susceptible and resistant strains using neomycin B, ribostamycin, and butirosin A as the controls (Fig. 4).[18] Butirosin A is the only control bearing *N*-1 AHB group. Aminoglycoside susceptible *Escherichia coli* (ATCC 25922) was used as a standard reference strain. *E. coli* (pTZ19U-3) and *E. coli* (pSF815) are laboratory resistant strains using *E. coli* (TG1) as the host. The first one is equipped with the pTZ19U-3 plasmid encoded for APH(3′)-I, which catalyzes a phosphorylation at the 3′-OH of both neomycin and kanamycin classes of aminoglycosides. The second one is equipped with the pSF815 plasmid encoded for AAC(6′) and APH(2″), which produces a bifunctional enzyme that catalyzes acetylation of amino group at *C*-6′ and phosphorylation of hydroxyl group at *C*-2″ position. These enzymes are among the most prevalent modes of resistance found in aminoglycoside resistant strains.

3. Discussion

From the minimum inhibitory concentrations (MIC), the synthesized aminoglycosides with the attachment of AHB group at *N*-1 regain the activity against both resistant strains of bacteria with the exception of **JT052**, which has an *n*-propyl group attached at *O*-6 of 2-deoxystreptamine ring (Table 1). By comparing



Scheme 1.



Scheme 2.

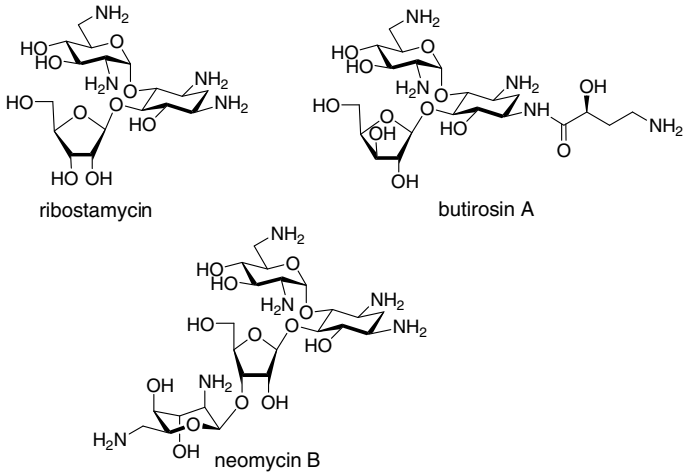


Figure 4. Structures of ribostamycin, butirosin A, and neomycin B.

Table 1. Minimum inhibitory concentrations (MIC)[a]

Compound	Strains of bacteria			
	E. coli[b]	*E. coli* (TG1)[c]	*E. coli* (pSF815)[d]	*E. coli* (pTZ19U-3)[e]
Neomycin B	4–8	4–8	4	Inactive[f]
Ribostamycin	8	4	4–8	Inactive
Butirosin A	4–8	1–2	0.5–1	0.5–1
TC005	8–16	8	8–16	Inactive
JT005	8	4	2–4	4
JT050	8	8	2	1
JT051	16	2	8	16
JT052	Inactive	8	Inactive	Inactive

N.D., not determined.
[a] Unit: μg/mL.
[b] *Escherichia coli* (ATCC 25922).
[c] *E. coli* (TG1) (aminoglycoside susceptible strain).
[d] *E. coli* (TG1) (pSF815 plasmid encoded for (AAC(6′)/APH(2″))).
[e] *E. coli* (TG1) (pTZ19U-3 plasmid encoded for APH(3′)-I).
[f] Inactive is defined as MIC $\geqslant$ 32 μg/mL.

the functionalities at *O*-6, 2-aminoethyl group as in the case of **JT050** appears to be the optimal structural scaffold. In contrast, 2-hydroxyethyl and *n*-propyl groups as in the case of **JT051** and **JT052**, respectively, appear to have negative effect leading to lower antibacterial activity. However, from the results of **JT005** and **JT050**, it is clear that *N*-1 AHB and *O*-6 modifications can function cooperatively to achieve better antibacterial activity. **JT050** is slightly more active than **JT005** suggesting a possible beneficial role of 2-aminoethyl group at *O*-6, which prompts the further molecular modeling investigation that will be discussed later.

Finally, even without the attachment of AHB group at *N*-1, the neomycin class antibiotic, ribostamycin, seems to be modestly active against the *E. coli* (pSF815). The results imply that neomycin class antibiotics with the AHB group at *N*-1 position represent a better template for further modification. All the synthetic aminoglycosides are less active than butirosin A, which may suggest a furanose for ring III is superior to a pyranose. Nonetheless, a pyranose scaffold as in pyranmycin is more stable in acidic condition which could be advantageous in generating orally active antibiotics.[11]

JT050 and related pyranmycin adducts (**TC005** and **JT005**) were further assayed against other clinically significant strains of bacteria (Table 2). None of the aminoglycosides including butirosin A and clinically used neomycin was active against methicillin-resistant *Staphylococcus aureus* (ATCC 33591) (MRSA) although we did notice that **JT050** showed the lowest MIC of 32 μg/mL. Interestingly, the added *O*-6 functional group

Table 2. MIC of **JT050** against other strains of bacteria[a]

Compound	Strains of bacteria			
	S. aureus[b]	*K. pneumoniae*[c]	*K. pneumoniae*[d]	*P. aeruginosa*[e]
Neomycin B	Inactive[f]	16–32	8	0.5
Butirosin A	Inactive	1–2	1–2	1
TC005	Inactive	16–32	4	4
JT005	Inactive	1	1–2	2
JT050	Inactive	2	4	0.5–1

[a] Unit: μg/mL.
[b] *Staphylococcus aureus* (ATCC 33591) (MRSA).
[c] *Klebsiella pneumoniae* (ATCC 700603).
[d] *K. pneumoniae* (ATCC 13883).
[e] *Pseudomonas aeruginosa* (ATCC 27853).
[f] Inactive is defined as MIC $\geqslant$ 32 μg/mL.

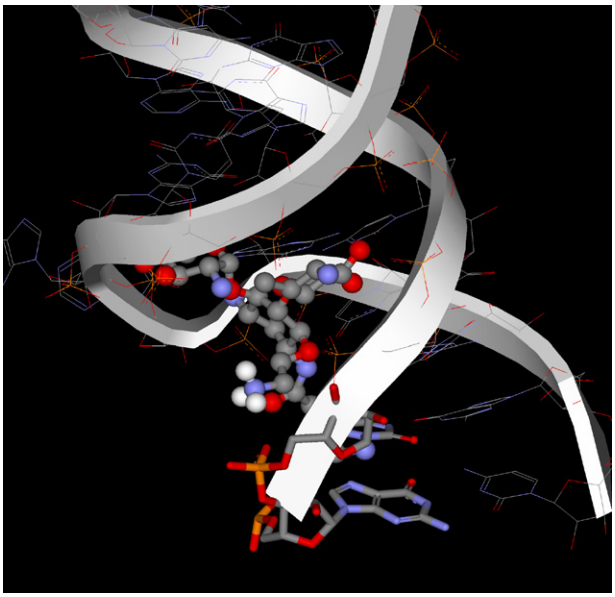


Figure 5. Molecular modeling for the binding of **JT050** toward model rRNA. The hydrogen atoms on **JT050** except for the ammonium group at the *O*-6 aminoethyl group are omitted for clarity. The phosphodiester backbone, C1404 and G1403 on the RNA backbones are highlighted in wide stick.

seems to slightly reduce the activity of **JT050** against *Klebsiella pneumoniae* albeit the difference was within the margin of error. The addition of AHB is clearly essential for reviving the activity against resistant *K. pneumoniae* (ATCC 700603). Finally, **JT050** manifested the best activity against *Pseudomonas aeruginosa* which suggest a collaboration effect of having *N*-1 AHB and *O*-6 2-aminoethyl group.

Examination of the binding of **JT050** toward rRNA using molecular modeling based on the published X-ray structural study reveals an interesting finding (Fig. 5).[19] The trisaccharide skeleton adopts similar conformation as what we have found previously. The *N*-1 AHB resides at the same location as reported by Mobashery and co-workers.[20] The terminal ammonium group of *O*-6 aminoethyl group appears to interact closely with the phosphodiester backbone between C1404 and G1403 forming a hydrogen-bonding network which may explain the improved activity of **JT050** as compared to its parent analog, **JT005**. In addition, the hydroxyethyl group at *O*-6 of **JT051** is expected to provide weaker interaction with the same phosphodiester backbone leading to slightly lower antibacterial activity. Finally, it is conceivable that a hydrophobic *n*-propyl group at *O*-6 of **JT052** will yield disfavored interaction causing drastic decrease in activity (Fig. 6).

4. Conclusion

In conclusion, we have completed the synthesis of several pyranmycin adducts with *N*-1 AHB and various *O*-6 functional groups. The antibacterial assay reveals that the antibacterial activity of 1-*N*-(*S*)-4-amino-2-hydroxybutyryl pyranmycin can be modulated and improved by

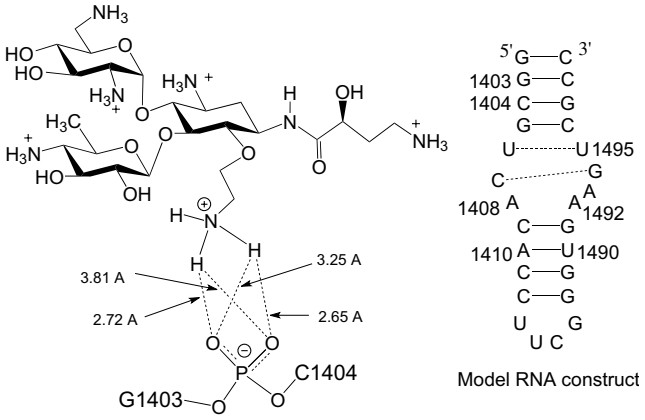


Figure 6. Interactions of the *O*-6 aminoethyl group of **JT050** with rRNA.

different acylic substituents at *O*-6 position. Based on the revealed SAR and molecular modeling results, 2-aminoethyl group at *O*-6 is the optimal functionality. Further modifications based on the lead, 2-aminoethyl group at *O*-6, are currently being carried out.

5. Experimental

Proton nuclear magnetic resonance spectra were recorded using a Bruker ARX 400 spectrometer. Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane in δ unit, and coupling constants were given in cycles per second (Hz). Splitting patterns were designed as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C spectra were obtained using a Bruker ARX 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. High-resolution electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) was 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 × 450 mesh, Sorbent Tech.) unless otherwise noted.

5.1. 3′,4′-Di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4-(benzyloxycarbonylamino)butanoyl]-3,2′,6′-triazidoneamine (3)

Compound **1** (670 mg, 0.98 mmol) was treated with a mixture solution of 99% trifluoroacetic acid (5 mL) and dichloromethane (5 mL) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with triethylamine and concentrated to dryness as crude product which was directly used for peptide coupling. A mixture of (*S*)-2-benzyloxy-4-*N*-

(benzyloxycarbonylamino)butyric acid (370 mg, 1.08 mmol) and EDC (280 mg, 1.48 mmol), HOBt (200 mg, 1.48 mmol), Et_3N (1 mL), and the above crude product in anhydrous DMF (5 mL) was stirred under nitrogen at room temperature for overnight. After completion of the reaction mixture (R_f = 0.42, monitored by TLC, hexane/ethyl acetate = 35:65), the reaction mixture was concentrated and extracted with EtOAc. The organic layer was washed by H_2O, brine, and then dried with $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexane/ethyl acetate = 60:40 to 0:100) afforded the product as white crystals after vacuum pump (640 mg, 0.71 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3–7.4 (m, 20H), 6.74 (d, J = 7.4 Hz, 1H, NH), 5.34 (m, 1H, H-1′), 5.2 (m, 1H), 5.05 (s, 2H, $PhCH_2O$), 4.91 (s, 2H, $PhCH_2O$), 4.90 (d, J = 10.1 Hz, 1H, $PhCH_2O$), 4.65 (d, J = 11.1 Hz, 1H, $PhCH_2O$), 4.59 (d, J = 11.6 Hz, 1H, $PhCH_2O$), 4.49 (d, J = 11.6 Hz, 1H, $PhCH_2O$), 4.19 (m, 1H, H-5′), 3.9–4.0 (m, 2H), 3.8–3.9 (m, 2H), 3.6 (m, 1H), 3.60 (dd, J = 9.4, 9.4 Hz, 1H), 3.4–3.6 (m, 3H), 3.42 (dd, J = 13.3, 4.3 Hz, 1H), 3.2–3.3 (m, 3H), 2.2 (m, 1H, H-2_{eq}), 2.0–2.1 (m, 2H), 1.3 (m, 1H, H-2_{ax}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 156.9, 137.9, 137.8, 137.1, 136.9, 129.0, 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 98.9, 82.1, 80.4, 78.9, 78.7, 77.0, 75.7, 75.3, 74.8, 73.2, 71.3, 66.8, 64.0, 59.4, 51.2, 48.8, 37.2, 32.9, 32.4; ESI/APCI Calcd for $C_{45}H_{52}N_{11}O_{10}$ $[M+H]^+$ m/z 906.3898; measure m/z 906.3914.

5.2. 6-*O*-Allyl-3′,4′-di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4-benzyloxycarbonylamino-butanoyl]-3,2′,6′-triazidoneamine (4)

To a solution of **3** (670 mg, 0.73 mmol) and tetrabutylammonium iodide (590 mg, 1.60 mmol) in a mixed anhydrous solution of THF/DMF (2/1) was added allylbromide (0.15 mL, 1.74 mmol). The reaction mixture was stirred at room temperature for 5 min; while the reaction mixture was vigorously stirred, lithium bis(trimethyl silyl) amide (1.9 mL, 1.89 mmol) was added at once under ice-water bath. The reaction was monitored by TLC until the complete consumption of the starting material (ca. overnight, R_f = 0.20, EtOAc/hexane = 35:65). The reaction was quenched with 1 N AcOH and concentrated by compressed air. The crude product was extracted with EtOAc, washed with H_2O, brine and dried over anhydrous Na_2SO_4. Removal of the solvent followed by purification with gradient column chromatography (hexane/ethyl acetate = 85:15 to 45:55) afforded the product (400 mg, 0.42 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–7.3 (m, 20H), 6.67 (d, J = 7.3 Hz, 1H, N*H*), 5.8 (m, 1H), 5.20 (d, J = 3.6 Hz, 1H, H-1′), 5.0–5.2 (m, 5H), 4.8–4.9 (m, 2H), 4.6 (m, 2H), 4.49 (d, J = 11.3 Hz, 1H, $PhCH_2O$), 4.35 (dd, J = 10.5, 5.1 Hz, 1H), 4.15 (ddd, J = 9.8, 3.6, 2.7 Hz, 1H, H-5′), 4.08 (dd, J = 12.6, 6.6 Hz, 1H), 4.00 (dd, J = 9.9, 9.1 Hz, 1H), 3.96 (dd, J = 5.5, 5.5 Hz, 1H), 3.7 (m, 1H), 3.5–3.6 (m, 4H), 3.4 (m, 2H), 3.2–3.3 (m, 3H), 3.15 (dd, J = 4.9, 4.7 Hz, 1H), 2.37 (ddd, J = 13.2, 4.0, 4.0 Hz, 1H, H-2_{eq}), 2.0 (m, 2H), 1.3 (m, 1H, H-2_{ax}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 156.6, 137.7, 137.6, 137.0, 136.8, 134.5, 129.0, 128.8, 128.7, 128.4, 128.2, 127.9, 118.3, 99.6, 84.6, 80.9, 80.2, 78.9, 78.5, 77.1, 75.9, 75.4, 73.4, 73.3, 71.4, 66.8, 64.5, 59.0, 51.1, 48.3, 37.4, 32.9, 32.6. ESI/APCI Calcd for $C_{48}H_{56}N_{11}O_{10}$ $[M+H]^+$ m/z 946.4211; measure m/z 946.4219.

5.3. 5-*O*-Acetyl-6-*O*-allyl-3′,4′-di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4-(benzyloxy-carbonylamino)butanoyl]-3,2′,6′-triazidoneamine (5)

To the solution of **4** (30 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5 mL), Et_3N (0.01 mL, 0.1 mmol) and DMAP (50 mg, catalyst) were added slowly, followed by Ac_2O (6 μL, 0.06 mmol) at room temperature. After completion of the reaction (R_f = 0.2, monitored by TLC, EtOAc/hexane = 35:65), the reaction mixture was quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic solution was washed with water, brine and dried over $Na_2SO_{4(s)}$. After removal of the solvent, the crude product was subjected to a gradient column chromatography (hexane/EtOAc = 100:0 to 50:50), afforded product (20 mg, 0.02 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–7.3 (m, 20H), 6.74 (d, J = 7.4 Hz, 1H, N*H*), 5.8 (m,1H), 5.0–5.2 (m, 6H), 4.89 (d, J = 10.5 Hz, 1H, PhCH_2O), 4.88 (s, 2H, PhCH_2O), 4.63 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.62 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.48 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.26 (ddd, J = 10.0, 3.4, 2.6 Hz, 1H, H-5′), 3.9–4.0 (m, 4H), 3.8 (m, 1H, H-1), 3.5–3.6 (m, 4H), 3.3–3.4 (m, 4H), 3.2 (m, 1H), 2.39 (ddd, J = 12.7, 4.3, 3.9 Hz, 1H, H-2_{eq}), 2.14 (s, 3H, CH_3), 2.0 (m, 2H), 1.45 (ddd, J = 12.6, 12.4, 12.2 Hz, 1H, H-2_{ax}). ^{13}C NMR (100 MHz, $CDCl_3$) δ172.4, 169.4, 156.5, 137.8 (s, 2C), 136.9, 136.8,133.9, 129.0, 128.7 (s, 2C), 128.5, 128.3, 128.2, 128.1, 128.0, 118.4, 99.1, 79.8, 79.2, 79.1, 78.9, 78.4, 77.4, 75.7, 75.4, 74.6, 73.4, 72.1, 71.5, 66.8, 63.5, 59.3, 51.1, 47.9, 37.5, 32.5, 30.0, 21.2. ESI/APCI Calcd for $C_{50}H_{58}N_{11}O_{11}$ $[M+H]^+$ m/z 988.4317; measure m/z 988.4333.

5.4. 5-*O*-(4-Azido-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-allyl-3′,4′-di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4-(benzyl-oxycarbonylamino)butanoyl]-3, 2′, 6′-triazidoneamine (7)

A solution of glycosyl trichloroacetimidate, **6** (250 mg, 0.58 mmol), neamine acceptor, **4** (460 mg, 0.48 mmol), and activated powder 4 Å molecular sieve was stirred in anhydrous CH_2Cl_2 (ca. 12 mL) at room temperature for 1 h then cooled to −50 °C. To this cloudy solution was added BF_3–OEt_2 (0.07 mL, 0.58 mmol). The solution was stirred at low temperature until the complete consumption of the glycosyl trichloroacetimidate (ca. 5 h). The reaction mixture was quenched by the addition of $NaHCO_3$ powder. After being stirred for 15 min, the reaction mixture was filtered through Celite. The residue was washed thoroughly with CH_2Cl_2 and ethyl acetate. After removal of the solvents, the crude product was directly used for the hydrolysis reaction.

A solution of above crude product and LiOH monohydrate (120 mg, 2.9 mmol) in THF/H_2O (12 mL/4 mL) was stirred at room temperature until the complete con-

sumption of starting material after overnight. (R_f = 0.15, monitored by TLC, EtOAc/hexane = 35:65). The reaction mixture was filtered through Celite and was concentrated. After extracted with EtOAc, the organic layer was washed by H_2O, brine, and then dried with $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexane/ethyl acetate = 80:20 to 20:80) afforded the product (380 mg, 0.03 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–7.4 (m, 20H), 6.91 (d, J = 8.8 Hz, 1H, N*H*), 5.8 (m, 1H), 5.67 (d, J = 3.4 Hz, 1H, H-1′), 5.3 (m, 1H), 5.2 (m, 1H), 5.17 (s, 1H), 5.12 (d, J = 11.6 Hz, 1H, PhCH_2O), 5.1 (s, 1H, H-1″), 5.0 (m, 1H), 4.91 (d, J = 11.3 Hz, 1H, PhCH_2O), 4.80 (m, 3H, PhCH_2O), 4.5–4.6 (m, 3H), 4.49 (d, J = 11.2 Hz, 1H, PhCH_2O), 4.2 (m, 2H), 3.9–4.0 (m, 5H), 3.8 (s, 1H), 3.70 (dd, J = 8.9, 7.9 Hz, 1H), 3.4–3.6 (m, 5H), 3.2–3.3 (m, 6H), 3.11 (dd, J = 9.2, 7.9 Hz, 1H), 3.05 (dd, J = 9.6, 9.6 Hz, 1H), 2.24 (ddd, J = 13.2, 4.9, 4.4 Hz, 1H, H-2$_{eq}$), 1.9–2.0 (m, 2H), 1.4 (m, 1H, H-2$_{ax}$), 1.39 (d, J = 6.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 156.8, 137.9 (s, 2C), 136.8 (s, 2C), 134.0, 129.1, 128.8, 128.7 (s, 2C), 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 127.2, 118.5, 102.1, 96.9, 81.2, 79.7, 79.6, 78.9, 78.3, 76.6, 75.6, 75.2 (s, 2C), 73.5, 73.4, 71.4, 71.2, 69.9, 67.5, 66.9, 63.3, 59.6, 55.1, 51.4, 47.6, 37.6, 32.7, 32.4, 18.2. ESI/APCI Calcd for $C_{54}H_{65}N_{14}O_{13}$ $[M+H]^+$ m/z 1117.4855; measure m/z 1117.4879.

5.5. 5-*O*-(4-Azido-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-(2- hydroxyethyl)-3′,4′-di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4- (benzyloxycarbonylamino)butanoyl]-3,2′,6′-triazidoneamine (8)

To a solution of **7** (210 mg, 0.19 mmol) in CH_2Cl_2 (6 mL), under −35 °C, O_3 was bubbling through till the complete consumption of starting material (1 min). After removal of excess O_3 by bubbling of N_2, the reaction mixture was cooled to 0 °C, diluted with MeOH, and slowly added with $NaBH_4$ (excess). After being stirred for 3 h, TLC of the reaction showed the complete consumption of the starting material. (R_f = 0.2, monitored by TLC, EtOAc/hexane = 65:35). The reaction mixture was concentrated, diluted with EtOAc and was washed with 1 N $HCl_{(aq)}$, H_2O, saturated $NaHCO_{3(aq)}$ Brine, then dried with $Na_2SO_{4(s)}$. Removal of the solvent followed by purification with gradient column chromatography (hexane/ethyl acetate = 65:35 to 0:100) afforded the product (150 mg, 0.13 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–7.4 (m, 20H), 7.15 (d, J = 8.5 Hz, 1H, N*H*), 5.81 (d, J = 3.6 Hz, 1H, H-1′), 5.0–5.1 (m, 3H), 4.90 (d, J = 11.9 Hz, 1H, PhCH_2O), 4.89 (s, 2H, PhCH_2O), 4.86 (d, J = 10.7 Hz, 1H, PhCH_2O), 4.78 (d, J = 7.7 Hz, 1H, H-1″), 4.62 (d, J = 11.4 Hz, 1H, PhCH_2O), 4.58 (d, J = 15.0 Hz, 1H, PhCH_2O), 4.47 (d, J = 11.1 Hz, 1H, PhCH_2O), 4.2 (m, 1H), 4.08 (dd, J = 10.1, 8.9 Hz, 1H), 4.00 (dd, J = 10.1, 8.2 Hz, 1H), 3.96 (dd, J = 6.5, 5.1 Hz, 1H), 3.7 (m, 1H), 3.6–3.7 (m, 3H), 3.4–3.5 (m, 4H), 3.2–3.3 (m, 8H), 3.09 (dd, J = 9.7, 9.6 Hz, 1H), 2.14 (ddd, J = 13.2, 4.8, 4.5 Hz, 1H, H-2$_{eq}$), 1.9–2.0 (m, 2H), 1.39 (d, J = 6.1 Hz, 3H, CH_3), 1.3 (m, 1H, H-2$_{ax}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 156.8, 137.9, 137.8, 136.7, 136.6, 129.1, 128.9, 128.7 (s, 2C), 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 102.3, 96.6, 83.1, 79.9, 79.7, 78.8, 78.2, 75.8, 75.6 (s, 2C), 75.3, 75.1, 73.9, 73.5, 71.3, 71.0, 67.6, 66.9, 63.3, 62.2, 59.9, 51.4, 48.1, 37.6, 32.8, 32.6, 18.1. ESI/APCI Calcd for $C_{53}H_{65}N_{14}O_{14}$ $[M+H]^+$ m/z 1121.4804; measure m/z 1121.4816.

5.6. 5-*O*-(4-Azido-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-(2-azidoethyl)-3′,4′-di-*O*-benzyl-1-*N*-[(*S*)-2-benzyloxy-4-(benzyloxycarbonylamino)butanoyl]-3,2′,6′-triazidoneamine (9)

To a solution of **8** (110 mg, 0.09 mmol) in anhydrous pyridine (5 mL) was added into triisopropylbenzenesulfonyl chloride (TIBSCl) (900 mg, 3.14 mmol) at room temperature. After the completion of the reaction (ca. 3 days, R_f = 0.5, EtOAc/hexanes = 50:50), the reaction mixture was extracted with EtOAc and was washed by 1 N HCl (three times), water and brine, then dried over $Na_2SO_{4(s)}$. After removal of solvent, the crude product was dissolved in DMF (5 mL), and added with NaN_3 (60 mg, 0.98 mmol). The reaction was refluxed under 80 °C oil bath for overnight and TLC of the reaction showed the complete consumption of the starting material (R_f = 0.3, EtOAc/hexanes = 50:50). After removal of DMF, the crude product was extracted with EtOAc and washed with water, brine, then dried over anhydrous $Na_2SO_{4(s)}$. Removal of solvent followed by purification with gradient column chromatography (hexane/ethyl acetate = 60:40 to 0:100) afforded the product (60 mg, 0.05 mmol, 53.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3–7.4 (m, 20H), 7.14 (d, J = 8.8 Hz, 1H, N*H*), 5.68 (d, J = 3.1 Hz, 1H, H-1′), 5.22 (dd, J = 5.5, 5.2 Hz, 1H), 5.1 (s, 2H), 4.90 (d, J = 13.1 Hz, 1H, PhCH_2O), 4.8–4.9 (m, 3H), 4.5–4.7 (m, 2H), 4.51 (d, J = 11.0 Hz, 1H, PhCH_2O), 4.2 (m, 1H), 3.9–4.1 (m, 5H), 3.8 (m, 1H), 3.74 (dd, J = 8.5, 7.4 Hz, 1H), 3.6 (m, 2H), 3.4–3.5 (m, 3H), 3.43 (dd, J = 7.6, 7.4 Hz, 1H), 3.2–3.4 (m, 6H), 3.15 (dd, J = 8.6, 8.1 Hz, 1H), 3.06 (dd, J = 9.7, 9.6 Hz, 1H), 2.21 (ddd, J = 13.4, 4.9, 4.7 Hz, 1H, H-2$_{eq}$), 1.9–2.1 (m, 2H), 1.47 (ddd, J = 12.9, 10.9, 10.8 Hz, 1H, H-2$_{ax}$),1.39 (d, J = 6.0 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 156.8, 137.9 (2C), 136.7 (2C), 129.2, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 101.9, 96.8, 81.9, 79.7, 78.9, 78.8, 78.4, 77.5, 76.3, 75.8, 75.6, 75.2, 74.8, 73.7 (2C), 67.5, 67.0, 63.3, 59.3, 51.4 (3C), 47.6, 37.6, 32.9, 29.9, 18.2. ESI/APCI Calcd for $C_{53}H_{64}N_{17}O_{13}$ $[M+H]^+$ m/z 1146.4869; measure m/z 1146.4895.

5.7. General procedure for the synthesis of pyranmycin derivatives

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 a R_f of 0 when eluted with EtOAc/MeOH (9/1) solution and a 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 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 reac-

tion mixture was stirred at room temperature under atmospheric H_2pressure. 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^+ eluted with a gradient of NH_4OH 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 bioassay directly. The reported final products are characterized by ^1H and ^{13}C NMR at this stage.

5.8. 5-*O*-(4-Amino-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-(2-aminoethyl)-1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-neamine (JT050)

Please refer to the general procedure for the preparation of pyranmycin derivatives. ^1H NMR (400 MHz, D_2O) (chloride salt) δ 5.82 (d, J = 4.0 Hz, 1H, H-1′), 5.00 (d, J = 8.2 Hz,, 1H, H-1″), 4.28 (dd, J = 9.7, 3.5 Hz, 1H), 4.17 (dd, J = 9.2, 9.2 Hz, 1H), 3.8–4.1 (m, 7H), 3.74 (dd, J = 13.7, 5.8 Hz, 1H), 3.67 (dd, J = 10.1, 9.3 Hz, 1H), 3.5–3.6 (m, 4H), 3.39 (dd, J = 9.2, 9.2 Hz, 1H), 3.35 (dd, J = 9.2, 9.2 Hz, 1H), 3.0–3.2 (m, 5H), 2.1–2.3 (m, 2H), 1.8 1.9 (m, 2H), 1.42 (d, J = 6.3 Hz, 3H, H-6″). ^{13}C NMR (100 MHz, D_2O) (chloride salt) δ 175.7, 103.2, 96.4, 82.8, 80.1, 76.5, 73.5, 72.0, 70.4, 70.0, 69.80, 69.75, 68.9, 68.4, 57.0, 53.6, 49.4, 48.9, 40.0, 39.95, 37.2, 31.2, 30.3, 16.9. ESI/APCI Calcd for $C_{24}H_{50}N_7O_{11}$ $[M+H]^+$ m/z 612.3568; measure m/z 612.3551.

5.9. 5-*O*-(4-Amino-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-(2-hydroxyethyl)-1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-neamine (JT051)

Please refer to the general procedure for the preparation of pyranmycin derivatives. ^1H NMR (400 MHz, D_2O) δ 5.87 (d, J = 4.1 Hz, 1H), 5.17 (d, J = 8.2 Hz, 1H), 4.27 (dd, J = 9.4, 3.6 Hz, 1H), 4.17 (dd, J = 9.1, 9.0 Hz, 1H), 4.08 (dd, J = 9.8, 9.3 Hz, 1H), 4.0 (m, 1H), 4.01 (dd, J = 10.7, 9.0 Hz, 1H), 3.8–3.9 (m, 3H), 3.4–3.7 (m, 10H), 3.33 (dd, J = 13.8, 5.9 Hz, 1H), 3.1–3.2 (m, 3H), 2.20 (ddd, J = 12.8, 4.6, 4.3 Hz, 1H, H-2_{eq}), 2.1 (m, 1H), 1.8–1.9 (m, 2H), 1.42 (d, J = 6.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, D_2O) δ 175.7, 102.3, 96.1, 82.6, 79.5, 76.1, 73.9, 73.6, 72.0, 70.6, 69.9, 69.8 (2C), 68.3, 61.2, 57.1, 53.6, 49.3, 48.6, 40.1, 37.1, 31.1, 30.2, 16.9. ESI/APCI Calcd for $C_{24}H_{49}N_6O_{12}$ $[M+H]^+$ m/z 613.3408; measure m/z 613.3401.

5.10. 5-*O*-(4-Amino-4,6-dideoxy-β-D-glucopyranosyl)-6-*O*-*n*-propyl-1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-neamine (JT052)

Please refer to the general procedure for the preparation of pyranmycin derivatives. ^1H NMR (400 MHz, D_2O) δ 5.72 (d, J = 3.9 Hz, 1H, H-1′), 4.86 (d, J = 8.2 Hz, 1H, H-1″), 4.17 (dd, J = 9.6, 3.3 Hz, 1H), 4.01 (dd, J = 9.2, 9.1 Hz, 1H), 3.8–3.9 (m, 4H), 3.73 (dd, J = 6.9, 6.9 Hz, 1H), 3.70 (dd, J = 9.8, 3.2 Hz, 1H), 3.57 (dd, J = 9.9, 9.6 Hz, 1H), 3.3–3.5 (m, 6H), 3.2 (m, 1H), 3.19 (dd, J = 13.7, 6.4 Hz, 1H), 3.06 (t, J = 7.3 Hz, 2H), 3.00 (dd, J = 10.0, 10.0 Hz, 1H), 2.0–2.1 (m, 1H), 2.01 (ddd, J = 12.9, 4.4, 3.8 Hz, 1H, H-2_{eq}), 1.7–1.8 (m, 1H), 1.67 (ddd, J = 12.8, 12.7, 12.7 Hz, 1H, H-2_{ax}), 1.3–1.5 (m, 2H), 1.31 (d, J = 6.2 Hz, 3H, CH_3), 0.75 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 175.4, 102.3, 96.3, 82.8, 79.4, 77.4, 75.1, 73.4, 72.3, 70.8, 70.2, 69.7, 69.5, 68.7, 57.2, 53.7, 49.3, 48.6, 40.2, 37.2, 31.2 (2C), 23.1, 16.9, 10.3. ESI/APCI Calcd for $C_{25}H_{51}N_6O_{11}$ $[M+H]^+$ m/z 611.3615; measure m/z 611.3596.

5.11. 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 measured, and diluted with broth, if necessary, to an absorption value of 0.08–0.1 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 twice.

Acknowledgments

We acknowledge National Institutes of Health (AI053138) for financial support. We thank Prof. Mobashery of Notre Dame University for providing the pTZ19U-3 and pSF815 plasmids.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.059.

References and notes

1. Haddad, J.; Kotra, L. P.; Mobashery, S. In *Glycochemistry Principles, Synthesis, and Applications*; Wang, P. G., Bertozzi, C. R., Eds.; Marcel Dekker: New York, 2001; pp 307–424.
2. Vakulenko, S. B.; Mobashery, S. *Clin. Microbiol. Rev.* **2003**, *16*, 430–450.
3. Hooper, I. R. *Aminoglycoside Antibiotics*; Springer-Verlag: New York, 1982.
4. Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 727–737.
5. Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249–3256.
6. Wright, G. D. *Curr. Opin. Microbiol.* **1999**, *2*, 499–503.
7. Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367–1371.
8. Fourmy, D.; Recht, M. I.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347–362.
9. Fong, D. H.; Berghuis, A. M. *EMBO J.* **2002**, *21*, 2323–2331.

10. For reviewing Li, J.; Chang, C.-W. T. *Anti-Infect. Agents Med. Chem.* **2006**, *5*, 255–271.
11. Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603–4606.
12. Elchert, B.; Li, J.; Wang, J.; Hui, Y.; Rai, R.; Ptak, R.; Ward, P.; Takemoto, J. Y.; Bensaci, M.; Chang, C.-W. T. *J. Org. Chem.* **2004**, *69*, 1513–1523.
13. Haddad, J.; Kotra, L. P.; Llano-Sotelo, B.; Kim, C.; Azucena, E. F., Jr.; Liu, M.; Vakulenko, S. B.; Chow, C. S.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 3229–3237.
14. Minowa, N.; Akiyama, Y.; Hiraiwa, Y.; Maebashi, K.; Usui, T.; Ikeda, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6351–6354.
15. Li, J.; Chen, H.-N.; Chang, H.; Wang, J.; Chang, C.-W. T. *Org. Lett.* **2005**, *7*, 3061–3064.
16. Moon, M. S.; Jun, S. J.; Lee, S. H.; Cheong, C. S.; Kim, K. S.; Lee, B. S. *Bull. Korean Chem. Soc.* **2003**, *24*, 163–164.
17. Various conditions were attempted. However, the poor regioselectivity and yield were achieved: (1) Using LiHMDS as the base and THF as the solvent, no reaction occurred; (2) Using LiHMDS as the base and DMF as the solvent, no mono-*O*-6 allylated product was obtained; (3) Using NaH as the base, no mono-*O*-6 allylated product was obtained.
18. The procedure was modified from *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.
19. Molecular modeling was conducted using HyperChem 7.0 based on the X-ray structure reported by Mobashery and co-workers (Ref. 20).
20. Russell, R. J. M.; Murray, J. B.; Lentzen, G.; Haddad, J.; Mobashery, S. *J. Am. Chem. Soc.* **2003**, *125*, 3410–3411.