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Rational design and synthesis of potent aminoglycoside antibiotics against resistant bacterial strains

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ABSTRACT

Based on the structural information of biomacromolecule–aminoglycoside complexes, a series of kanamycin B analogues were rationally designed and synthesized. A convenient approach to the construction of kanamycin derivatives, in which the C4′-position on ring I of neamine moiety was modified, was developed. Most synthetic analogues exhibited good to excellent antibiotic activity against some typical drug-resistant bacteria. The disclosed results suggested that the C4′-position of aminoglycosides such as kanamycin may be an ideal site for modification to gain new modifying enzyme-resistant aminoglycoside antibiotics.

1. Introduction

Antibiotic resistance has become a severe problem in clinic throughout the world nowadays. Since the first antituberculosis agent, streptomycin, was discovered in 1940s, aminoglycoside antibiotics have been used in therapy over sixty years. Like other classes of antibiotics, however, aminoglycosides also face the challenges of resistance because of extensive use. Among resistance mechanisms, the most common mode is overexpression of aminoglycoside-modifying enzymes.[1] These enzymes include three groups: aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs), and aminoglycoside nucleotidyltransferases (ANTs). In order to surmount resistance from enzymatic modification, researchers have developed two strategies so far. One way is to develop enzyme inhibitors,[2] and the other is to develop enzyme-resistant analogues of natural aminoglycosides.[3] Over the past decade many structural analogues of natural aminoglycosides were synthesized, and some of the designed structures showed considerable antibacterial activities.[4] But in some cases, the modified drugs lost their activity.[5] One possible reason resulting in antibacterial activity failure may be that aminoglycosides bind to drug target (16S rRNA A-site) in a highly sequence-selective and specified style, and inappropriate modification would bring adverse influence on the natural binding mode, thus leading to the decrease or even complete loss of activity. In recent years, since some 3D structures of drug target or drug-modifying enzymes in complex with aminoglycosides have been disclosed, the knowledge about the interaction details between aminoglycosides and biomacromolecules affords medicinal chemists brand-new insights for rational design of new aminoglycoside analogues, and some advances have been achieved.[4c,6] Herein, we report a new approach to aminoglycoside modifications based on the structural information.

2. Results and discussion

2.1. Design

Neamine (**1**) is a conserved pseudodisaccharide which constitutes the core structure of many aminoglycosides such as paromomycin (**2**), neomycin B (**3**), tobramycin, and kanamycin A (**4**) and B (**5**) (Fig. 1). Previous investigations proved that it is the optimal pharmacophore to manifest the antibiotic activity.[7] The studies on 3D structures of RNA–aminoglycoside complexes revealed that these aminoglycosides bind to RNA in a similar way: the sugar rings have equivalent orientations and the relatively same binding site and most of the conserved contacts involve the neamine moiety, even in the case of apramycin (**6**), a larruping aminoglycoside.[8] In contrast to the neamine unit, the binding of the other segments of aminoglycosides looks disorderly (Fig. 2). All these results suggest that neamine moiety might be an ideal target for modifications to gain new antibacterial characteristics. Among the natural aminoglycosides containing neamine core, the common substituent fashions are 4,5- and 4,6-disubstitution on 2-deoxystreptamine (2-DOS, ring II),

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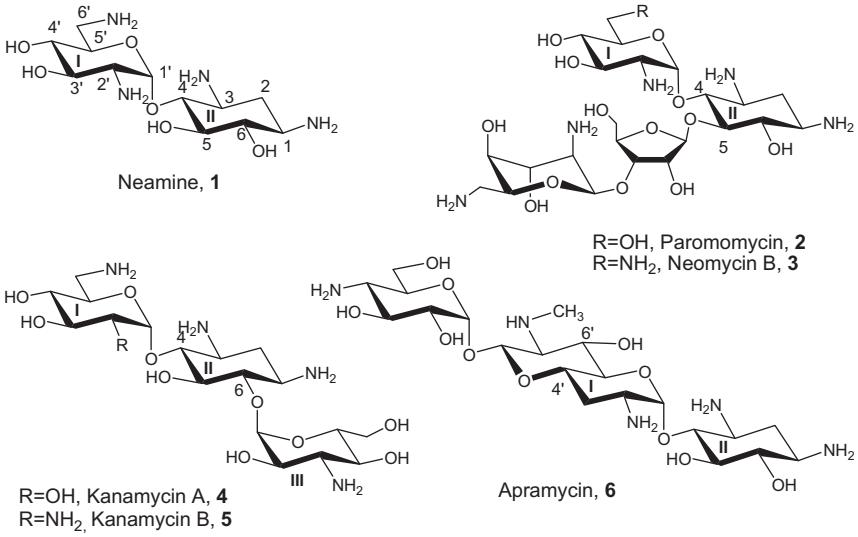


Figure 1. Structures of several representative aminoglycosides. The neamine core is composed of ring I and ring II.

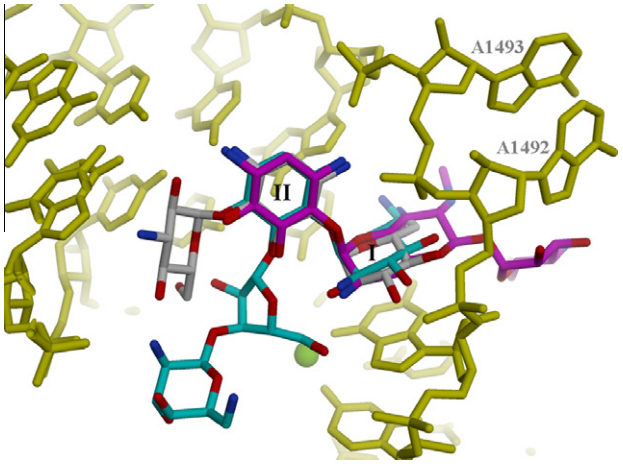


Figure 2. Crystal structures of three neamine-containing aminoglycosides bound to A site. The complexes of apramycin (magenta, 1YRJ), kanamycin A (gray, 2ESI) and neomycin B (cyanic, 2ET4) with the decoding site are superimposed (based on nucleic acid chains). Only the RNA (yellow) of the kanamycin A complex is shown. The neamine moiety is marked with Roman numerals I and II. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).[9]

one component of neamine. An exceptional case is apramycin, in which the substituents are attached to the C4′- and C6′-positions of ring I, another component of neamine. Though the chemical structure of apramycin is so dissimilar to that of the other aminoglycoside members, the 3D structure shows that the neamine moiety still binds to A site with a very similar mode, whether the binding position or the binding conformation. That is to say, substituents attached to C4′- or C6′-position of ring I in neamine segment have little effect on the binding between drugs (aminoglycosides) and the drug target (RNA); so it is likely that modification at the corresponding positions in other aminoglycosides would not affect the original binding modes, thus the resulting analogues would keep antibacterial activity. On the other hand, for those structures of inhomogeneous resistant enzymes in complex with similar aminoglycosides, the binding modes are different. An obvious fact is that the neamine moiety, in binding sites, binding groups, binding force, binding conformations, etc., makes different contacts with these enzymes.[8a]

Despite the lack of resemblance among various enzymes, the binding specificity for neamine unit still could be observed, for instance in the cases of AAC(2′)-Ic or APH(3′)-IIIa.[10] Because of the strict specificity existing in the recognition between substrates and enzymes, if some modifications are made on the neamine segment, the introduced functional groups would probably hinder the recognition between the resulting analogues and some resistant enzymes, thus they could resist the attack from these enzymes and keep antibiotic activity against those bacteria expressing aminoglycoside-modifying enzymes. To verify these hypotheses, after considering the synthetic accessibility, we designed and synthesized a series of kanamycin B derivatives, in which the original 4′-hydroxyl group on neamine segment was replaced by some other functional groups. In the crystal structure of kanamycin A–RNA complex, a H-bond can be observed between the 4′-hydroxyl group of kanamycin A and the phosphate oxygen atom of A1493 (Fig. 3). In addition, the cavity formed by A site can accommodate a bulky substituent on neamine ring I of apramycin. In order to retain these specialties, a series of amide functionalities including small and bulky groups were served as substituents which were arbitrarily designated as acetamido, 4-amino-butanoylamino, cyclohexylcarbonylamino, 2-(2-methyl-benzamido)-acetamido, and piperidinyl-4-carbonylamino groups.

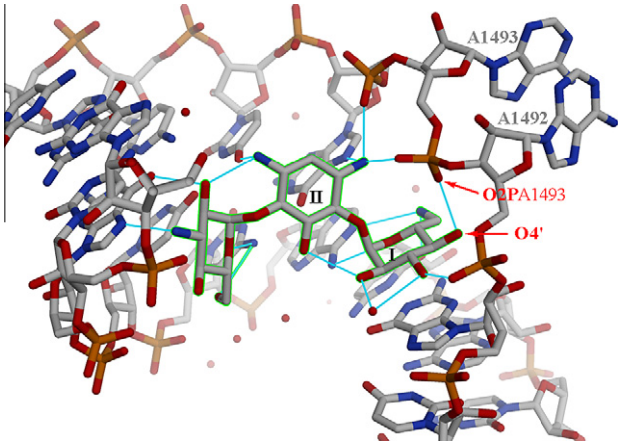


Figure 3. The crystal structure of kanamycin A–A site complex. Hydrogen bonds between aminoglycoside and RNA are shown with cyanic lines. Water molecules are indicated as red balls.

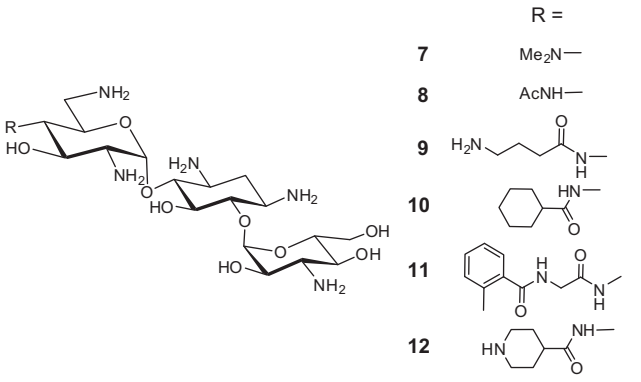


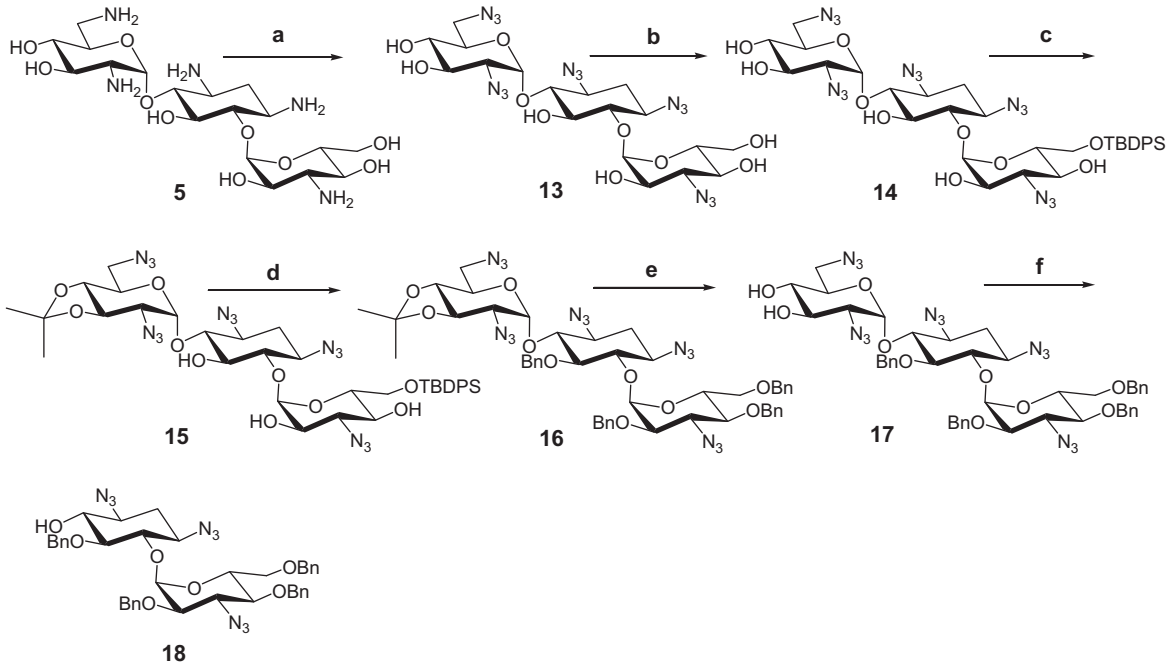
Figure 4. Designed kanamycin B analogues.

Besides, dimethylamino group was also used as a substituent to investigate the influence of the positively charged replacement (Fig. 4).

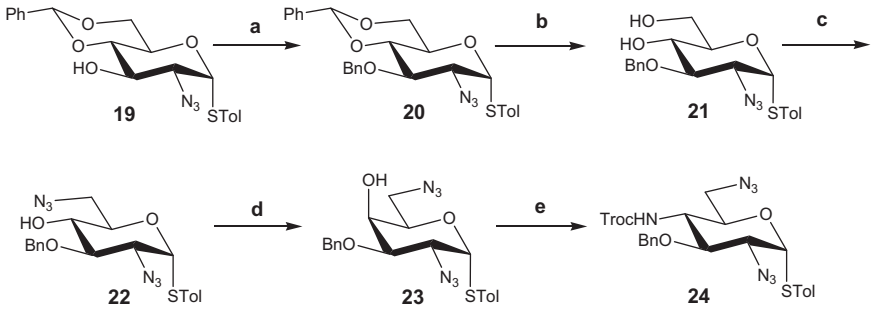
2.2. Chemistry

After undergoing many frustrations in direct modifications on kanamycin B, an alternative approach to modify the ring I of kanamycin was developed. Our modification strategy included the following steps: (1) disconnecting the ring I from kanamycin B to achieve a glycosyl acceptor containing ring II and ring III, (2) preparing 4-amino-protected thioglycoside donor as the building block of ring I, (3) reconstructing kanamycin B skeleton by the coupling of glycosyl donor and acceptor, and (4) further modifications to yield the target compounds.

For this purpose, as shown in Scheme 1, the commercially available kanamycin B (**5**) was firstly converted into perazido species **13** by the modified diazotransfer procedure.[11] The primary hydroxyl group at the C6″-position of **13** was selectively protected by treatment with *tert*-butyldiphenylchlorosilane in pyridine to afford compound **14**. The protection of C6″-OH was proved to be necessary for decreasing byproducts and convenient purification in the next step. The selective protection of 3′,4′-diol in **14** with isopropylidene provided compound **15**. The removal of silyl protection at C6″-OH of



Scheme 1. Synthesis of glycosyl acceptor **18**. Reagents and conditions: (a) (i) TfN_3, NEt_3, Cu^{2+}, CH_3CN, H_2O; (ii) acetic anhydride, pyridine; (iii) NaOMe, MeOH; (b) TBDPSCl, pyridine, DMAP, 59% over four steps; (c) 2,2-dimethoxypropane, CH_2Cl_2, CSA, 52%; (d) (i) TBAF, THF; (ii) NaH, BnBr, DMF, 82% over two steps; (e) AcOH, MeOH, 100%; (f) (i) $NaIO_4$, MeOH; (ii) *n*-$BuNH_2$, MeOH, 88% over two steps.



Scheme 2. Synthesis of glycosyl donor **24**. Reagents and conditions: (a) NaH, BnBr, DMF, 92%; (b) CSA, MeOH; (c) (i) TsCl, pyridine; (ii) NaN_3, DMF, 81% over three steps; (d) (i) Dess–Martin's periodinane, CH_2Cl_2; (ii) $NaBH_4$, MeOH, 95% based on the recovery of starting material; (e) (i) Tf_2O, pyridine, CH_2Cl_2; (ii) NH_3, MeOH; (iii) TrocCl, $NaHCO_3$, THF, 55% over three steps.

15 and the subsequent benzylation of the free hydroxyl groups afforded compound **16**, which was treated with acetic acid to give the diol **17**. Finally, the desired glycosyl acceptor **18** was prepared in 88% isolated yield from **17** by two steps according to the reported procedure.[12]

The synthesis of glycosyl donor was started from thioglycoside **19**.[13] As displayed in Scheme 2, benzylation of **19** gave compound **20** smoothly. Removal of the benzylidene group from **20** led to diol **21**. Tosylation of the C6-hydroxyl group of **21** and the subsequent nucleophilic displacement with sodium azide produced compound **22**. Oxidation of **22** with Dess–Martin's periodinane and subsequent reduction of the resulting 4-ketone with sodium borohydride gave the C4-configuration-inversed compound **23**. Compound **23** reacted with triflyl anhydride to afford a triflate intermediate, which was successively treated with ammonia and 2,2,2-trichloroethoxycarbonyl chloride (TrocCl), providing the desired thioglycoside donor **24**. This glycosyl donor possesses an azido group at the C-2 position, which would facilitate forming α-glycosidic bond in the following glycosylation under the influence of anomeric effect.

With the glycosyl donor and acceptor in hand, coupling was conducted by *N*-iodosuccinimide (NIS)/TfOH-promoted glycosylation (Scheme 3). As expected, the α-anomeric product **25** was the major product. Treatment of **25** with zinc powder in the presence of acetic acid to remove Troc group unfortunately resulted in the formation of a mess of complicated products. Therefore, compound **25** was subjected to hydrolysis under basic conditions to furnish the key pseudotrisaccharide **26**.

Compound **26** was treated with iodomethane in the presence of sodium hydride to obtain the dimethylated product **27**, while acetylation of **26** with acetic anhydride gave compound **28**. Reaction of **26** with 4-chlorobutanoyl chloride using sodium bicarbonate as acid scavenger followed by treatment with sodium azide provided **29** in 80% isolated yield. On the other hand, compound **26** was coupled with different carboxylic acids using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as the condensation reagent in the presence of Hünig's base (*N*,*N*-diisopropylethylamine, DIPEA), affording compounds **30**, **31**, and **32**, respectively (Scheme 3). Thus, all precursors for final deprotection were achieved. After reduction of azido groups with H_2S followed by debenzylation via catalytic hydrogenolysis, all these precursors were smoothly transformed into final products **7–12**.

2.3. Biological assay

All compounds were tested against American Type Culture Collection (ATCC) reference strains as well as clinical isolates of both Gram-positive and Gram-negative bacteria, including pathogenic and resistant strains, and the minimum inhibitory concentrations (MIC) in μg/mL were determined by the microdilution assay. Kanamycin A and B served as controls (Table 1). Resistant strains covered low to high level resistant microbe, including *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), methicillin-resistant *Staphylococcus aureus* (ATCC 33591), *Enterococcus faecalis* (ATCC 29212), *Mortierella alpine* (ATCC32221), methicillin-resistant *Staphylococcus epidermidis* 08–18, *Staphylococcus epidermidis* 07–9, and *Escherichia coli* BL21 (pET29a). *Staphylococcus epidermidis* 08–18 and *Staphylococcus epidermidis* 07–9 are clinical isolated drug-resistant strains. *E. coli* BL21 (pET29a) is a recombinant strain harboring pET29a expressing vector which contains the gene encoding APH(3′)-Ia as a marker.

As seen in Table 1, with the exception of **7**, all new compounds exhibited good to excellent antibacterial activity against nonresistant bacteria. A remarkable 2- to 16-folds enhancement against the selected four reference strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228) was observed for compound **9** when compared with the controls, whereas compounds **8**, **10**, **11**, **12** had MIC values close to that of controls. The results showed that the antibacterial activities of these compounds were retained or even enhanced in some cases. The most significant fact was that these compounds can inhibit the growth of drug-resistant bacteria, just as expected.

Klebsiella pneumoniae is a common nosocomial pathogen causing severe morbidity and mortality in paediatric patients. *K. pneumoniae* K6 (ATCC 700603) is a clinical isolate that is resistant to some aminoglycosides (caused by ANT(2″)).[14] Our antimicrobial susceptibility test showed that it was also resistant to kanamycin A (MIC = 64 μg/mL) and kanamycin B (MIC = 16 μg/mL). In our research, compounds **8** and **9** showed fairly good execution, with the MIC values of 2 and 1 μg/mL, respectively; while compound **12** had somewhat better activity than kanamycin.

P. aeruginosa is an opportunistic human pathogen and the leading cause of nosocomial infection, which is frequently isolated from patients with complicated urinary tract infections. The ubiquity of genes encoding aminoglycoside-modifying enzymes including *aph(3′)-IIb* in *P. aeruginosa* leads to aminoglycoside resistance.[15] Using *P. aeruginosa* ATCC 27853 as evaluation object, all newly synthesized compounds except **7** displayed better bactericidal effect than controls. Again, compound **9** showed preeminent antibiotic efficacy (MIC = 0.5 μg/mL).

Methicillin-resistant *S. aureus* (MRSA) is the leading cause of bacterial infections. Many MRSA strains contain genes encoded for APH(3′), ANT(4′), and AAC(6′)/APH(2″), which render the bacteria resistant to many aminoglycosides.[16] Once again, the antimicrobial susceptibility test gave satisfactory results. With the exception of **7**, all new derivatives displayed better bactericidal effect against ATCC 33591 than controls. Compounds **9**, **10**, **12** were able to inhibit the growth of the tested strains in low concentrations (2, 4 and 2 μg/mL, respectively).

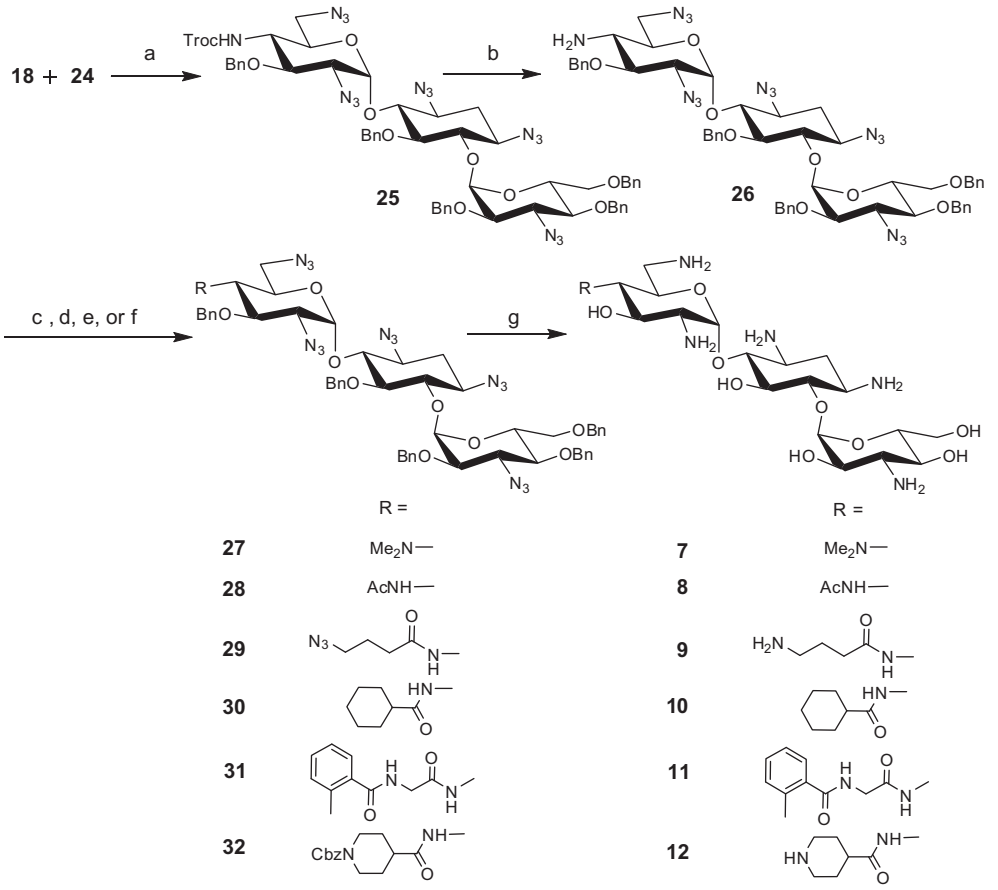
Staphylococcus epidermidis 08–18 is a methicillin-resistant clinical isolate obtained from a patient at the Beijing Friendship Hospital in 2008. The susceptibility test results showed that it was also resistant to kanamycin B. Except **7**, all newly synthesized derivatives of kanamycin B showed good antibiotic effect, especially, compounds **9** and **12** inhibited the growth of this strain at a very low concentration (MIC = 0.25 μg/mL in both cases).

Staphylococcus epidermidis 07–9 is also a clinical isolate that was obtained from a patient at the Beijing Friendship Hospital in 2007. PCR assay indicated it contains *aph(3′)-IIIa* gene. The susceptibility test results showed that it was resistant to both new compounds and controls. However, compared with controls, compound **9** still showed moderate antibiotic activity.

In the case of *E. coli* BL21 (pET29a), a laboratory strain expressing APH(3′)-Ia, the susceptibility test results clearly indicated that two derivatives of kanamycin B, compounds **9** and **12**, can tolerate the attack from APH(3′)-Ia.

Enterococcus faecalis ATCC 29212 is a microbe expressing efflux pump responsible for resistance to many structurally unrelated antimicrobial agents.[17] Unfortunately, except **9**, which showed somewhat better activity, all new compounds showed similar or higher MIC values when compared with kanamycin. It seemed that the efflux pump could extrude all these compounds. The antifungal activity against *Mortierella alpine* with ATCC 32221 as the reference strain was also checked, however, none of the compounds showed obvious inhibitory efficacy at the concentration of 128 μg/mL.

Generally speaking, all 4′-amide derivatives displayed considerable antibiotic activity. It indicated that the proper modification at C4′-position of neamine segment would not impart negative effect on the bioactivity. Among these analogues, compounds **9** and **12** displayed the most potent activity. Interestingly, in spite of the difference in structures of 4′-substituents, these two compounds have some commonness. That is to say, there is a nitrogen atom at the end of substituent, and three carbon–carbon bonds



Scheme 3. Reconstruction of kanamycin B skeleton and further modifications. Reagents and conditions: (a) NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, −70 °C to rt, 57%, α/β = 1.74/1; (b) NaOH, H_2O, 1,4-dioxane, reflux, 64%; (c) NaH, MeI, DMF, 50% for **27**; (d) Ac_2O, pyridine, 80% for **28**; (e) (i) 4-chloro-butanoyl chloride, $NaHCO_3$, THF; (ii) NaN_3, DMF, 80% for **29**; (f) TBTU, carboxylic acids, DIPEA, DMF, 78% for **30**, 75% for **31**, 81% for **32**; (g) (i) H_2S, pyridine, triethylamine, H_2O; (ii) H_2, Pd/C, MeOH, H_2O, HCl, 95% for **7**, 87% for **8**, 66% for **9**, 61% for **10**, 64% for **11**, 60% for **12**.

Table 1
The results of antibacterial activities of target compounds[a]

Compound	MIC (μg/mL)											
	E. coli (G−)		*S. aureus* (G+)		*S. epidermidis* (G+)			*K. pneumoniae* (G−)	*P. aeruginosa* (G−)	*E. faecalis* (G+)	Fungus	*E. coli* BL21
	a	b	c	d	e	f	g	h	i	j	k	l
Kan A	2	4	2	128	1	8	>128	64	>128	64	>128	>128
Kan B	4	4	1	>128	0.25	32	>128	16	64	64	>128	64
7	128	64	32	>128	16	32	>128	64	>128	>128	>128	>128
8	2	8	2	16	0.5	2	>128	2	4	>128	>128	128
9	1	1	0.25	2	<0.06	0.25	32	1	0.5	32	>128	8
10	2	2	1	4	0.25	1	>128	32	8	64	>128	64
11	4	4	2	64	0.5	8	>128	64	32	64	>128	>128
12	2	2	1	2	0.25	0.25	128	8	2	128	>128	4

[a] a: ATCC 25922; b: ATCC 35218; c: ATCC 29213; d: ATCC 33591; e: ATCC 12228; f: 08–18, methicillin-resistant clinical isolate; g: 07–9, clinical isolate expressing APH(3′)-IIIa; h: ATCC 700603; i: ATCC 27853; j: ATCC 29212; k: *Mortierella alpine*, ATCC 32221; l: pET29a, engineering strain expressing APH(3′)-Ia. G+: Gram-positive; G−: Gram-negative.

lie between the end nitrogen atom and carbonyl group in each case. In contrast, compound **7**, the sole 4′-amine derivative of kanamycin B, only showed poor activity occasionally. The possible reason for this abnormality might be that the additional dimethylamino group can be protonated under test conditions, which would result in a different binding mode to drug target (A site). In the cases of 4′-amide derivatives, however, the ability of 4′-N–H group forming hydrogen bond is still retained; as a result these compounds would be able to bind to the A site in the similar way to that of kanamycin, thus the antibiotic activity remained. More importantly, several compounds exhibited remarkable activity against some typical drug-resistant bacteria that can express aminoglycoside-modifying enzymes. Obviously, the introduced functional groups at C4′ exerted some impact on the interactions between these analogues and some resistant enzymes, probably by interference with the course of recognition. Therefore, it would make the analogues no longer appropriate substrates for the enzymes; as a result they could resist the attack from these enzymes and keep antibiotic activity. The structural diversification of 4′-amide manifested excellent latitude for modification at this position, holding the potential to discover more analogues with better activity.

3. Conclusion

In conclusion, we have developed an accessible approach for the construction of kanamycin derivatives based on the modifications of ring I in neamine moiety. By using this protocol, a series of kanamycin analogues were rationally designed and synthesized. Among these synthetic analogues, some compounds such as **9** and **12** exhibited remarkable antibiotic activity against drug-resistant bacteria. These results also designated that the C4′-position on neamine is a noticeable site for modifications to gain new antibacterial agents with resistance to aminoglycoside-modifying enzymes.

4. Experimental

4.1. Chemistry

General. Non-aqueous reactions were performed under nitrogen atmosphere at room temperature, unless otherwise noted. All commercial reagents were used without further purification unless otherwise stated. Anhydrous pyridine and dichloromethane were obtained by distilling commercial ones over CaH_2 and anhydrous DMF was obtained by distilling over P_2O_5 under reduced pressure. Specific rotations were determined on RUDOLPH RESEARCH ANALYTICAL AUTOPOL III automatic polarimeter at 20 °C. Routine ^1H and ^{13}C nuclear magnetic resonance spectra were recorded on the Varian INOVA-500. Samples were dissolved in deuterated chloroform ($CDCl_3$) or deuterium oxide (D_2O) and tetramethylsilane (TMS) was used as reference. Elemental analyses were performed in PE-2400C analyzer. Mass spectra were recorded on PE SCIEX QSTAR mass spectrometer (for low resolution ESI-MS) or Bruker APEX IV mass spectrometer (for high resolution ESI-MS). Analytical thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F_{254}. Compounds were visualized by UV light (254 nm) and/or by staining with a yellow solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) or ninhydrin solution in ethyl acetate (5%) followed by heating.

4.1.1. 6″-*O*-*tert*-Butyldiphenylsilyl-pentaazidokanamycin B (14)

To a solution of kanamycin B (2.00 g, 4.14 mmol) in water (6 mL) was added $CuSO_4$ (33 mg, 0.21 mmol) and the resulting mixture was stirred for 15 min, then triethylamine (4.18 g, 41.4 mmol) was added in one portion. After the mixture was stirred for 20 min at ice-bath temperature, triflyl azide solution in acetonitrile (21 mL, 1.5 N, 31.5 mmol) was added dropwise in a period of 15 min. After 2 h, the ice-bath was removed and the mixture was stirred for another 10 h at room temperature. Then the solvent was removed and the crude product was treated with the mixture of acetic anhydride (6 mL) and pyridine (9 mL). After stirring overnight, the reaction mixture was poured into 150 mL of brine, and the resulting precipitates were collected by filtration. The crude product was purified by column chromatography on silica gel, using petroleum ether/ethyl acetate (6:1 to 3:1) as eluent. The resulting product was dissolved in 60 mL of methanol, and catalytic amount of sodium methoxide was added. When TLC assay showed that the reaction was completed, the reaction mixture was neutralized with cation-exchange resin and the solvent was removed. The yielded product (1.66 g, 2.71 mmol) was dissolved in 30 mL of pyridine, *tert*-butyldiphenylchlorosilane (1.50 g, 5.5 mmol) and catalytic amount of DMAP were added to the solution. After stirring for 48 h at room temperature, the solvent was removed and the crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (3:1) as eluent. Finally, 2.08 g (2.44 mmol, 59% overall yield) of title product was obtained as a colorless semisolid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.70–7.65 (m, 4H), 7.47–7.39 (m, 6H), 5.54 (d, J = 3.5 Hz, 1H), 5.09 (d, J = 3.5 Hz, 1H), 4.13–4.09 (m, 1H), 4.01–3.90 (m, 4H), 3.84 (dd, J_1 = 4.0 Hz, J_2 = 11.0 Hz, 1H), 3.68–3.45 (m, 9H), 3.42–3.27 (m, 4H), 2.94 (br s, 3H), 2.42 (ddd, $J_1 = J_2$ = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.77 (br s, 2H), 1.62 (ddd, $J_1 = J_2 = J_3$ = 12.5 Hz, 1H), 1.08 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.60, 135.55, 132.74, 132.64, 130.00, 129.95, 127.88, 127.83, 99.40, 98.02, 84.06, 80.12, 75.29, 72.10, 72.01, 71.37, 71.22, 71.18, 70.03, 66.30, 63.80, 63.12, 58.90, 58.78, 51.17, 31.54, 26.82, 19.21. HRMS (ESI) calcd for $C_{34}H_{49}N_{16}O_{10}Si$ ($M+NH_4^+$): 869.3581; found: 869.3584.

4.1.2. 3′,4′-*O*-Isopropylidene-6″-*O*-*tert*-butyldiphenylsilyl-pentaazidokanamycin B (15)

To a solution of **14** (0.71 g, 0.83 mmol) in dichloromethane (20 mL) and 2,2-dimethoxypropane (20 mL) was added camphorsulfonic acid (10 mg, 0.04 mmol) and the mixture was stirred for 24 h at room temperature, then it was quenched with triethylamine (0.5 mL). After removal of the solvent, the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 8:1 to 3:1) to afford three fractions: 0.24 g of **15**, the byproduct with a higher R_f value than **15** (which should be the over-protected product based on the ^1H NMR spectrum analysis) and byproduct with a lower R_f value than **15**. The byproducts with higher R_f value was dissolved in the mixed solvent of dichloromethane/methanol (15 mL/5 mL) and 20 mg of cation-exchange resin was added into the mixture. The reaction mixture was stirred at room temperature. After 3 h, TLC showed the reaction was completed. The mixture was filtered, and the filtrate was added triethylamine (0.5 mL). After removal of solvent, the crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (6:1 to 3:1) as eluent to give 0.14 g of **15**. Thus, 0.38 g (0.43 mmol, 52% yield) of desired product was obtained in all. ^1H NMR (500 MHz, $CDCl_3$) δ 7.68–7.63 (m, 4H), 7.47–7.38 (m, 6H), 5.71 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 3.0 Hz, 1H), 4.31–4.28 (m, 1H), 3.96–3.81 (m, 5H), 3.65–3.52 (m, 7H), 3.47–3.40 (m, 3H), 3.31–3.27 (m, 2H), 2.83 (d, J = 9.5 Hz, 1H), 2.60 (d, J = 3.0 Hz, 1H), 2.43 (ddd, $J_1 = J_2$ = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.62 (ddd, $J_1 = J_2 = J_3$ = 13.0 Hz, 1H), 1.47 (s, 3H), 1.46 (s, 3H), 1.06 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.60, 135.55, 132.67, 132.60, 130.00, 129.94, 127.88, 127.83, 111.76, 100.06, 98.06, 85.37, 79.15, 75.68, 75.20, 74.98, 72.37, 72.07, 71.43, 69.92, 66.34, 63.81, 61.56, 59.05, 58.60, 51.68, 31.48, 26.81, 26.74, 26.43, 19.19. ESI-MS: 909 $M+NH_4^+$. Anal. Calcd for $C_{37}H_{49}N_{15}O_{10}Si$: C, 49.82; H, 5.54; N, 23.55. Found: C, 50.11; H, 5.46; N, 23.81.

4.1.3. 3′,4′-*O*-Isopropylidene-5,2″,4″,6″-tetra-*O*-benzyl-pentaazidokanamycin B (16)

To a solution of **15** (0.63 g, 0.71 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 0.85 mL, 0.85 mmol). The resulting yellow solution was stirred for 30 min and then the solvent was removed. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (3:1) as eluent to afford a colorless semisolid, which was treated with sodium hydride (0.16 g, 4.2 mmol, 60% dispersed in mineral oil) and benzyl bromide (0.70 g, 4.1 mmol) in DMF (10 mL) for 4 h. The reaction mixture was poured into 80 mL of water, and the aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine, dried over $NaSO_4$ and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give compound **16** (0.59 g, 0.58 mmol, 82%) as a yellow semisolid. $[\alpha]_D$ +133.3 (c 0.45, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.43–7.41 (m, 2H), 7.39–7.36 (m, 2H), 7.33–7.20 (m, 11H), 7.14–7.06 (m, 3H), 6.98–6.96 (m, 2H), 5.63 (d, J = 4.0 Hz,

1H), 5.61 (d, J = 4.0 Hz, 1H), 4.95 (s, 2H), 4.81 (d, J = 12.0 Hz, 1H), 4.74 (d, J = 12.5 Hz, 1H), 4.62 (d, J = 11.0 Hz, 1H), 4.47 (d, J = 12.5 Hz, 1H), 4.41–4.38 (m, 1H), 4.27–4.23 (m, 2H), 3.98 (dd, J_1 = 9.0 Hz, J_2 = 11.0 Hz, 1H), 3.82–3.78 (m, 2H), 3.72 (t, J = 9.5 Hz, 1H), 3.65 (t, J = 9.5 Hz, 1H), 3.58–3.52 (m, 3H), 3.47–3.23 (m, 7H), 3.16 (dd, J_1 = 3.0 Hz, J_2 = 11.0 Hz, 1H), 2.40 (ddd, $J_1 = J_2$ = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.62 (ddd, $J_1 = J_2 = J_3$ = 12.5 Hz, 1H), 1.45 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.85, 137.59, 137.33, 137.20, 128.49, 128.30, 128.23, 128.15, 128.10, 128.06, 127.75, 127.72, 127.47, 127.21, 126.16, 111.82, 97.74, 95.83, 83.23, 77.81, 77.48, 77.19, 75.89, 75.06, 74.99, 74.77, 74.46, 73.48, 73.08, 72.21, 70.10, 67.74, 65.31, 61.22, 60.18, 59.37, 51.70, 31.97, 26.70, 26.38. ESI-MS: 1052 M+K$^+$. Anal. Calcd for $C_{49}H_{55}N_{15}O_{10}$: C, 58.04; H, 5.47; N, 20.72. Found: C, 57.83; H, 5.55; N, 20.46.

4.1.4. 5,2″,4″,6″-Tetra-*O*-benzyl-pentaazidokanamycin B (17)

A mixture of **16** (2.92 g, 2.88 mmol), acetic acid (1.5 mL) and methanol (60 mL) was heated under reflux for 4 h. The solvent was removed and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 6:1 to 3:1). Finally, 2.80 g (2.87 mmol, 100%) of title compound was obtained as a yellow semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.36 (m, 4H), 7.34–7.18 (m, 11H), 7.12–7.07 (m, 3H), 6.96–6.94 (m, 2H), 5.59 (d, J = 4.0 Hz, 1H), 5.47 (d, J = 4.0 Hz, 1H), 4.98 (d, J = 12.0 Hz, 1H), 4.92 (d, J = 11.5 Hz, 1H), 4.80 (d, J = 11.5 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.26 (d, J = 12.0 Hz, 1H), 4.22 (d, J = 11.0 Hz, 1H), 4.19–4.15 (m, 1H), 3.91 (dd, J_1 = 9.0 Hz, J_2 = 10.0 Hz, 1H), 3.81–3.77 (m. 2H), 3.67–3.63 (m, 2H), 3.58–3.33 (m, 8H), 3.29 (dd, J_1 = 1.5 Hz, J_2 = 11.0 Hz, 1H), 3.13 (dd, J_1 = 2.5 Hz, J_2 = 11.0 Hz, 1H), 3.07 (dd, J_1 = 4.0 Hz, J_2 = 10.5 Hz, 1H), 2.82 (br s, 1H), 2.66 (br s, 1H), 2.37 (ddd, $J_1 = J_2$ = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.63 (ddd, $J_1 = J_2 = J_3$ = 12.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.79, 137.56, 137.30, 128.53, 128.33, 128.26, 128.20, 128.13, 128.10, 127.77, 127.52, 127.17, 125.92, 97.48, 95.90, 82.81, 77.78, 77.49, 75.92, 74.86, 74.47, 73.51, 73.18, 71.97, 71.20, 71.07, 70.09, 67.74, 65.28, 62.86, 60.07, 59.18, 51.21, 31.75. HRMS (ESI) calcd for $C_{46}H_{52}N_{15}O_{10}$ (M+H$^+$): 974.4016; found: 974.3997.

4.1.5. 1,3-Diazido-5-*O*-benzyl-6-*O*-(3-deoxy-3-azido-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-2-deoxystreptamine (18)

To a solution of **17** (4.60 g, 4.72 mmol) in methanol (80 mL) was added $NaIO_4$ (2.02 g, 9.44 mmol) in one portion at room temperature. After stirring for 8 h, TLC showed that the starting material was consumed. Then the reaction mixture was poured into water (200 mL) and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined organic phase was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was dissolved in methanol (200 mL), and *n*-butylamine (300 mg) was added. The reaction was stirred overnight, and the solvent was removed. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (6:1) as eluent to give the desired product (3.18 g, 4.17 mmol, 88%) as a colorless semisolid. $[\alpha]_D$ +56.8 (c 0.95, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.09 (m, 20H), 5.62 (d, J = 3.5 Hz, 1H), 4.84–4.71 (m, 5H), 4.51 (d, J = 12.0 Hz, 1H), 4.35 (d, J = 11.0 Hz, 1H), 4.28 (d, J = 12.5 Hz, 1H), 4.07–4.05 (m, 1H), 3.90 (t, J = 10.0 Hz, 1H), 3.60–3.53 (m, 2H), 3.47–3.30 (m, 7H), 2.48 (br s, 1H), 2.30–2.25 (m, 1H), 1.53–1.45 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.87, 137.66, 137.39, 137.28, 128.49, 128.32, 128.24, 128.18, 128.09, 127.97, 127.75, 127.70, 95.61, 82.45, 77.34, 76.91, 76.86, 76.32, 76.14, 74.80, 73.41, 72.93, 69.88, 67.74, 65.48, 60.72, 59.84, 31.76. ESI-MS: 784 M+Na$^+$. Anal. Calcd for $C_{40}H_{43}N_9O_7$: C, 63.06; H, 5.69; N, 16.55. Found: C, 62.86; H, 5.69; N, 16.22.

4.1.6. *p*-Tolyl 2-deoxy-2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside (20)

To a stirred solution of **19** (1.98 g, 4.95 mmol) in DMF (25 mL) was added sodium hydride (0.40 g, 60% in mineral oil, 9.9 mmol) and the resulting mixture was stirred for 20 min at room temperature, then benzyl bromide (1.69 g, 10 mmol) was added dropwise. After stirring for 3 h, the reaction mixture was poured into water (150 mL) and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel using the mixed solvent (petroleum ether/ethyl acetate/dichloromethane 8:1:1 to 5:1:1) as eluent to give product **20** (2.23 g, 4.55 mmol, 92%) as a colorless oil. $[\alpha]_D$ +130.6 (c 0.75, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52–7.49 (m, 2H), 7.42–7.28 (m, 10H), 7.13 (d, J = 8.5 Hz, 2H), 5.59 (s, 1H), 5.49 (d, J = 5.0 Hz, 1H), 4.97 (d, J = 10.5 Hz, 1H), 4.83 (d, J = 11.0 Hz, 1H), 4.44 (dt, $J_1 = J_2$ = 5.0 Hz, J_3 = 10.0 Hz, 1H), 4.23 (dd, J_1 = 5.0 Hz, J_2 = 10.5 Hz, 1H), 3.99–3.93 (m, 2H), 3.78–3.73 (m, 2H), 2.34 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.34, 137.65, 137.11, 133.10, 129.97, 129.08, 128.42, 128.30, 128.23, 127.94, 125.99, 101.47, 88.14, 82.74, 77.81, 75.17, 68.59, 63.69, 63.57, 21.14. ESI-MS: 528 M+K$^+$. Anal. Calcd for $C_{27}H_{27}N_3O_4S$: C, 66.24; H, 5.56; N, 8.58. Found: C, 66.08; H, 5.55; N, 8.50.

4.1.7. *p*-Tolyl 2,6-dideoxy-2,6-diazido-3-*O*-benzyl-1-thio-α-D-glucopyranoside (22)

A mixture of **20** (4.25 g, 8.68 mmol) and camphorsulfonic acid (100 mg) in dichloromethane (20 mL) and methanol (80 mL) was heated under reflux for 6 h. The solvent was removed, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (4:1 to 2:1) as eluent to give the corresponding diol intermediate, which was subsequently treated with *p*-toluene sulfonyl chloride (2.37 g, 12.4 mmol) in the presence of pyridine (30 mL) overnight. Then the solvent was removed, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (6:1 to 4:1) as eluent to afford the tosylate intermediate, which was mixed with sodium azide (2.37 g, 12.4 mmol) and DMF (40 mL). After stirring at 60 °C for 8 h, the reaction mixture was poured into 150 mL of water and the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8:1 to 4:1) as eluent to afford **22** (3.00 g, 7.03 mmol, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.38 (m, 6H), 7.37–7.33 (m, 1H), 7.14 (d, J = 8.0 Hz, 2H), 5.52 (d, J = 5.5 Hz, 1H), 5.01 (d, J = 11.0 Hz, 1H), 4.71 (d, J = 11.5 Hz, 1H), 4.33–4.29 (m, 1H), 3.89 (dd, J_1 = 5.5 Hz, J_2 = 10.5 Hz, 1H), 3.60 (t, J = 8.5 Hz, 1H), 3.54 (dt, $J_1 = J_2$ = 3.5 Hz, J_3 = 9.5 Hz, 1H), 3.49 (dd, J_1 = 3.0 Hz, J_2 = 13.0 Hz, 1H), 3.49 (dd, J_1 = 5.5 Hz, J_2 = 13.5 Hz, 1H), 2.34 (s, 3H), 2.15 (d, J = 3.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.26, 137.69, 132.73, 129.98, 129.05, 128.85, 128.38, 128.15, 87.52, 81.33, 75.42, 71.55, 71.17, 63.78, 51.30, 21.11. ESI-MS: 444 M+NH_4^+. Anal. Calcd for $C_{20}H_{22}N_6O_3S$: C, 56.32; H, 5.20; N, 19.70. Found: C, 56.30; H, 4.98; N, 19.90.

4.1.8. *p*-Tolyl 2,6-dideoxy-2,6-diazido-3-*O*-benzyl-1-thio-α-D-galactopyranoside (23)

To a stirred solution of **22** (1.20 g, 2.81 mmol) in anhydrous dichloromethane (30 mL) was added Dess–Martin's periodinane (1.44 g, 3.39 mmol) and the resulting mixture was stirred for 3 h at room temperature. Subsequently, a solution of sodium dithionite (1.50 g) and sodium bicarbonate (3.0 g) in water (30 mL) was added. After stirring for 30 min, the organic layer was separated out and the aqueous layer was extracted with dichlorometh-

ane (2 × 10 mL).The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was suspended in methanol (20 mL) and the resulting mixture was cooled by ice-bath, then sodium borohydride (0.12 g, 3.16 mmol) was added in several portions. With the addition of sodium borohydride, the reaction mixture turned clear soon. When TLC showed the reaction was completed, the solvent was removed, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8:1 to 4:1) as eluent to give product **23** (0.99 g, 2.32 mmol, 95% based on the recovery of the starting material) as a colorless oil, and 0.16 g of **22** was also recovered. $[\alpha]_D$ +87.6 (*c* 1.05, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.38 (m, 6H), 7.37–7.35 (m, 1H), 7.14 (d, *J* = 8.0 Hz, 2H), 5.54 (d, *J* = 5.5 Hz, 1H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.69 (d, *J* = 11.5 Hz, 1H), 4.44–4.42 (m, 1H), 4.22 (dd, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 1H), 3.99 (t, *J* = 1.5 Hz, 1H), 3.76 (dd, J_1 = 3.5 Hz, J_2 = 10.5 Hz, 1H), 3.59 (dd, J_1 = 7.5 Hz, J_2 = 12.5 Hz, 1H), 3.36 (dd, J_1 = 5.0 Hz, J_2 = 13.0 Hz, 1H), 2.41 (t, *J* = 1.5 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.20, 136.75, 132.77, 129.97, 128.94, 128.78, 128.49, 128.10, 87.65, 77.54, 72.48, 69.95, 66.57, 59.52, 51.06, 21.12. ESI-MS: 444 M+NH_4^+. Anal. Calcd for $C_{20}H_{22}N_6O_3S$: C, 56.32; H, 5.20; N, 19.70. Found: C, 56.39; H, 5.18; N, 19.85.

4.1.9. *p*-Tolyl 2,6-dideoxy-2,6-diazido-3-*O*-benzyl-4-deoxy-4-(2,2,2-trichloroethoxycarbonylamino)-1-thio-α-D-glucopyranoside (24)

To a solution of **23** (2.96 g, 6.94 mmol) in anhydrous dichloromethane (60 mL) was added pyridine (2.4 mL), then the solution was cooled in a ice-bath while stirring. After 20 min, triflyl anhydride (3.92 g, 2.4 mL, 13.9 mmol) was added to the mixture dropwise over 2 h by a syringe. After completion of addition, the mixture was stirred for another 2 h. The reaction mixture was poured into the ice–water mixture (200 mL), the pH was adjusted to ~3 by the addition of solid $KHSO_4$. The organic layer was separated out and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was dissolved in THF (30 mL), then the resulting solution was transferred into a sealed tube, and to the tube was added ammonia solution in methanol (7 N, 10 mL). The sealed tube was kept at 50 °C for 48 h. The solvent was removed, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (6:1 to 3:1) as eluent. The resulting product (1.55 g, 3.64 mmol) was mixed with THF (30 mL), sodium bicarbonate (1.22 g, 14.52 mmol), and 2,2,2-trichloroethoxycarbonyl chloride (1.54 g, 7.28 mmol). The mixture was stirred for 4 h. After removal of the solvent, the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (10:1 to 8:1) as eluent to afford the title compound **24** (2.07 g, 3.44 mmol, 55% based on the recovery of the starting material) as a colorless syrup, and the starting alcohol **23** (0.28 g) was also recovered. For compound **24**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.31 (m, 7H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.55 (d, *J* = 5.0 Hz, 1H), 4.90 (d, *J* = 11.0 Hz, 2H), 4.77–4.66 (m, 3H), 4.49–4.46 (m, 1H), 3.96 (dd, J_1 = 5.5 Hz, J_2 = 10.0 Hz, 1H), 3.76 (t, *J* = 10.0 Hz, 1H), 3.65 (t, *J* = 10.0 Hz, 1H), 3.44–3.36 (m, 2H), 2.34 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.10, 138.39, 137.01, 132.66, 130.04, 128.76, 128.65, 128.40, 128.29, 95.19, 87.30, 77.13, 74.96, 74.62, 71.13, 64.47, 54.55, 51.56, 21.12. Anal. Calcd for $C_{23}H_{24}Cl_3N_7O_4S$: C, 45.97; H, 4.03; N, 16.32. ESI-MS: 622 M+Na^+. Found: C, 46.12; H, 4.16; N, 16.04.

4.1.10. 4′-Deoxy-4′-(2,2,2-trichloroethoxycarbonylamino)-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (25)

To a cold (−70 °C) mixture of glycosyl donor **24** (0.18 g, 0.30 mmol), acceptor **18** (0.30 g, 0.39 mmol), NIS (88 mg, 0.39 mmol), and 4Å molecular sieves (0.40 g) was added anhydrous CH_2Cl_2 (5 mL) by a syringe. After the mixture was stirred for 20 min, TfOH (10 μL) was added. The mixture was stirred at −70 °C for 10 min and then gradually raised to room temperature over a period of 1.5 h. Then triethylamine (0.2 mL) was added into the reaction mixture, and the mixture was diluted with CH_2Cl_2 (10 mL), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1 to 5:1) to give α-anomeric product (134 mg, 0.11 mmol, 36%) as well as β-anomeric product (77 mg, 0.06 mmol, 21%) as white semisolids. For α-anomer **25**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.19 (m, 20H), 7.13–7.09 (m, 3H), 6.95–6.93 (m, 2H), 5.60 (d, *J* = 3.5 Hz, 1H), 5.56 (d, *J* = 3.5 Hz, 1H), 5.00 (d, *J* = 11.5 Hz, 1H), 4.94 (d, *J* = 12.0 Hz, 1H), 4.83–4.71 (m, 5H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.61 (d, *J* = 11.0 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.35–4.32 (m, 1H), 4.26 (d, *J* = 12.0 Hz, 1H), 4.23 (d, *J* = 11.0 Hz, 1H), 3.89 (t, *J* = 10.0 Hz, 1H), 3.81–3.77 (m, 2H), 3.70–3.61 (m, 3H), 3.58–3.27 (m, 10H), 3.14 (dd, J_1 = 3.0 Hz, J_2 = 11.0 Hz, 1H), 2.38 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.63 (ddd, J_1 = J_2 = J_3 = 12.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.02, 137.85, 137.59, 137.34, 137.26, 137.02, 128.55, 128.52, 128.32, 128.18, 128.15, 128.11, 127.72, 127.48, 127.21, 125.81, 97.14, 95.93, 82.93, 77.83, 77.77, 77.48, 76.15, 75.88, 75.04, 74.62, 74.51, 74.45, 73.51, 73.13, 70.79, 70.12, 67.75, 65.32, 63.42, 60.18, 59.31, 54.09, 51.58, 31.92. ESI-MS: 1259 M+Na^+. Anal. Calcd for $C_{56}H_{59}Cl_3N_{16}O_{11}$: C, 54.31; H, 4.80; N, 18.09. Found: C, 54.59; H, 4.91; N, 17.88. For β-anomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.06 (m, 20H), 5.53 (d, *J* = 4.0 Hz, 1H), 4.90–4.85 (m, 3H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.72–4.62 (m, 7H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.27 (d, *J* = 11.0 Hz, 1H), 4.20 (d, *J* = 12.0 Hz, 1H), 3.93 (d, *J* = 10.0 Hz, 1H), 3.83 (t, *J* = 10.0 Hz, 1H), 3.73 (t, *J* = 9.5 Hz, 1H), 3.61–3.51 (m, 4H), 3.44–3.33 (m, 5H), 3.29–3.23 (m, 1H), 3.19–3.12 (m, 2H), 3.06–3.03 (m, 1H), 2.37 (ddd, J_1 = J_2 = 4.0 Hz, J_3 = 13.5 Hz, 1H), 1.61 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.06, 138.01, 137.88, 137.71, 137.42, 137.14, 128.68, 128.46, 128.26, 128.19, 128.10, 128.00, 127.89, 127.68, 127.59, 127.43, 101.03, 96.01, 80.47, 80.01, 77.71, 77.39, 76.04, 75.70, 74.69, 74.60, 74.13, 73.44, 72.86, 69.94, 67.48, 66.86, 65.47, 60.91, 60.44, 54.55, 51.22, 32.00. HRMS (ESI) calcd for $C_{56}H_{59}Cl_3N_{16}O_{11}Na$ (M+Na^+): 1259.3507, found: 1259.3517.

4.1.11. 4′-Deoxy-4′-amino-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (26)

To a solution of **25** (0.48 g, 0.39 mmol) in 1,4-dioxane (10 mL) were added water (1.5 mL) and sodium hydroxide (0.10 g, 2.5 mmol), then the reaction mixture was heated under reflux for 3 h. After cooling to room temperature, the reaction mixture was poured into brine (150 mL), and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent (5:1 to 1:1) to afford the title compound (0.26 g, 0.24 mmol, 64%) as a colorless semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.23 (m, 16H), 7.22–7.19 (m, 4H), 7.14–7.08 (m, 3H), 6.96–6.93 (m, 2H), 5.61 (d, *J* = 3.5 Hz, 1H), 5.56 (d, *J* = 4.0 Hz, 1H), 5.03 (d, *J* = 12.0 Hz, 1H), 4.94 (d, *J* = 12.0 Hz, 1H), 4.91 (d, *J* = 11.0 Hz, 1H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.74 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 11.0 Hz, 1H), 4.60 (d, *J* = 10.5 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.25 (d, *J* = 12.5 Hz, 1H), 4.22 (d, *J* = 11.5 Hz, 1H), 3.99–3.96 (m, 1H), 3.81–3.77 (m, 2H), 3.71–3.53 (m, 6H), 3.49–3.44 (m, 2H), 3.39 (dd, J_1 = 3.5 Hz, J_2 = 10.5 Hz, 1H), 3.35 (t, *J* = 10.0 Hz, 1H), 3.29 (dd, J_1 = 2.0 Hz, J_2 = 11.0 Hz, 1H), 3.21 (dd, J_1 = 4.0 Hz, J_2 = 10.0 Hz, 1H), 3.13 (dd, J_1 = 2.5 Hz, J_2 = 11.0 Hz, 1H), 2.70 (t, *J* = 10.0 Hz, 1H), 2.37 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.5 Hz, 1H), 1.61 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.87, 137.58, 137.35, 137.32, 128.64, 128.52, 128.31, 128.28, 128.18, 128.10, 127.74, 127.46,

127.15, 125.84, 97.54, 95.95, 83.04, 80.92, 77.87, 77.48, 75.89, 75.20, 75.00, 74.44, 73.51, 73.11, 73.03, 70.09, 67.74, 65.32, 63.69, 60.24, 59.49, 54.11, 51.83, 32.05. ESI-MS: 1063 M+H$^+$. Anal. Calcd for $C_{53}H_{58}N_{16}O_9$: C, 59.88; H, 5.50; N, 21.08. Found: C, 59.71; H, 5.36; N, 20.99.

4.1.12. 4′-Deoxy-4′-dimethylamino-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (27)

To a solution of **26** (64 mg, 0.06 mmol) in DMF (3 mL) was added sodium hydride (60% in mineral oil, 20 mg, 0.5 mmol), followed by the addition of iodomethane (60 mg, 0.42 mmol). After stirring for 4 h, more sodium hydride (60% in mineral oil, 20 mg, 0.5 mmol) and iodomethane (60 mg, 0.42 mmol)) were added and the mixture was stirred overnight. The reaction mixture was poured into brine (50 mL), extracted with ethyl acetate (3 × 15 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8:1 to 5:1) as eluent to give the title compound (33 mg, 0.03 mmol, 50%) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.18 (m, 15H), 7.11–7.07 (m, 3H), 6.95–6.93 (m, 2H), 5.60 (d, J = 3.5 Hz, 1H), 5.50 (d, J = 4.0 Hz, 1H), 5.02 (d, J = 11.5 Hz, 1H), 4.93 (d, J = 11.5 Hz, 1H), 4.83–4.72 (m, 4H), 4.59 (d, J = 11.0 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.37–4.33 (m, 1H), 4.24 (d, J = 12.0 Hz, 1H), 4.21 (d, J = 11.0 Hz, 1H), 4.03 (t, J = 10.0 Hz, 1H), 3.80–3.76 (m, 2H), 3.68–3.63 (m, 2H), 3.59–3.45 (m, 5H), 3.39 (dd, J_1 = 3.5 Hz, J_2 = 10.5 Hz, 1H), 3.35 (t, J = 10.0 Hz, 1H), 3.28 (dd, J_1 = 2.0 Hz, J_2 = 11.0 Hz, 1H), 3.24 (dd, J_1 = 4.0 Hz, J_2 = 10.0 Hz, 1H), 3.10 (dd, J_1 = 11.0 Hz, J_2 = 2.5 Hz, 1H), 2.64 (t, J = 10.0 Hz, 1H), 2.47 (s, 6H), 2.40 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.5 Hz, 1H), 1.66 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.88, 137.71, 137.59, 137.34, 137.30, 128.52, 128.37, 128.31, 128.26, 128.19, 128.10, 127.97, 127.73, 127.45, 127.11, 125.78, 97.35, 95.97, 83.00, 77.85, 77.47, 75.88, 75.49, 75.02, 74.43, 73.51, 73.10, 70.11, 70.07, 67.72, 65.34, 65.13, 65.04, 60.24, 59.44, 52.67, 41.95, 32.10, 29.68. HRMS (ESI) calcd for $C_{55}H_{63}N_{16}O_9$ (M+H$^+$): 1091.4958; found: 1091.4960.

4.1.13. 4′-Deoxy-4′-acetamido-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (28)

Compound **26** (54 mg, 0.05 mmol) was dissolved in pyridine (1.5 mL) and acetic anhydride (1 mL), and the mixture was stirred for 6 h. The reaction mixture was poured into brine (30 mL) while stirring, and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent (4:1 to 1:1) to afford the title compound (45 mg, 0.04 mmol, 80%) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.19 (m, 15H), 7.15–7.09 (m, 3H), 6.96–6.94 (m, 2H), 5.60 (d, J = 3.5 Hz, 1H), 5.58 (d, J = 3.5 Hz, 1H), 5.06 (d, J = 8.5 Hz, 1H), 4.99 (d, J = 11.5 Hz, 1H), 4.94 (d, J = 11.5 Hz, 1H), 4.81 (d, J = 11.5 Hz, 1H), 4.77 (d, J = 11.5 Hz, 1H), 4.74 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 11.0 Hz, 1H), 4.51 (d, J = 11.5 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.25 (d, J = 12.5 Hz, 1H), 4.23 (d, J = 11.0 Hz, 1H), 4.17–4.13 (m, 1H), 3.89–3.77 (m, 4H), 3.71 (t, J = 9.5 Hz, 1H), 3.65 (t, J = 9.5 Hz, 1H), 3.58–3.47 (m, 3H), 3.40–3.34 (m, 3H), 3.31–3.27 (m, 3H), 3.14 (dd, J_1 = 2.5 Hz, J_2 = 11.0 Hz, 1H), 2.36 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.76 (s, 3H), 1.61 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.16, 137.83, 137.57, 137.38, 137.32, 137.19, 128.66, 128.50, 128.31, 128.17, 128.09, 127.72, 127.46, 127.22, 125.85, 96.93, 95.92, 82.99, 77.76, 77.65, 77.47, 76.22, 75.86, 75.02, 74.44, 73.75, 73.50, 73.10, 71.66, 70.10, 67.71, 65.30, 63.21, 60.23, 59.51, 51.94, 51.65, 31.99, 23.29. HRMS (ESI) calcd for $C_{55}H_{61}N_{16}O_{10}$ (M+H$^+$): 1105.4751, found: 1105.4746.

4.1.14. 4′-Deoxy-4′-(4-azidobutoylamino)-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (29)

To a stirred solution of **26** (85 mg, 0.08 mmol) was added sodium bicarbonate (27 mg, 0.32 mmol), followed by the addition of 4-chlorobutanoyl chloride (45 mg, 0.32 mmol). After stirring for 4 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and stirred for 1 h. The aqueous layer was extracted with ethyl acetate (3 × 15 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo to afford crude product, which was mixed with DMF (4 mL) and sodium azide (20 mg, 0.31 mmol). After stirring at 80 °C overnight, the reaction mixture was poured into brine (50 mL) while stirring and the aqueous layer was extracted with ethyl acetate (3 × 15 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (5:1 to 2:1) to give **29** (75 mg, 0.06 mmol, 80%) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.11 (m, 23H), 7.12–7.00 (m, 4H), 6.95–6.94 (m, 2H), 5.60 (d, J = 3.5 Hz, 1H), 5.58 (d, J = 3.5 Hz, 1H), 5.13 (d, J = 8.5 Hz, 1H), 5.00–4.93 (m, 2H), 4.82–4.69 (m, 3H), 4.61 (d, J = 11.0 Hz, 1H), 4.51 (d, J = 11.5 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.25 (d, J = 12.0 Hz, 1H), 4.23 (d, J = 11.0 Hz, 1H), 4.18–4.14 (m, 1H), 3.93–3.77 (m, 4H), 3.71 (t, J = 9.5 Hz, 1H), 3.65 (t, J = 9.5 Hz, 1H), 3.59–3.48 (m, 3H), 3.42–3.34 (m, 3H), 3.32–3.25 (m, 4H), 3.16–3.13 (m, 2H), 2.37 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.0 Hz, 1H), 2.06–1.96 (m, 2H), 1.82–1.76 (m, 2H), 1.61 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.87, 137.83, 137.57, 137.37, 137.32, 137.19, 128.64, 128.51, 128.44, 128.31, 128.17, 128.14, 128.09, 127.90, 127.73, 127.46, 127.23, 125.84, 96.96, 95.91, 82.97, 77.73, 77.47, 76.25, 75.86, 75.01, 74.44, 73.64, 73.50, 73.11, 71.60, 70.10, 67.72, 65.31, 63.12, 60.21, 59.49, 51.95, 51.52, 50.66, 32.91, 31.98, 24.33, 13.80. HRMS (ESI) calcd for $C_{57}H_{63}N_{19}O_{10}Na$ (M+Na$^+$): 1196.4898; found: 1196.4894.

4.1.15. 4′-Deoxy-4′-cyclohexylcarbonylamino-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (30)

Compound **26** (65 mg, 0.06 mmol), cyclohexylcarboxylic acid (16 mg, 0.12 mmol), DMF (4 mL), DIPEA (22 mg, 0.17 mmol), and TBTU (47 mg, 0.17 mmol) were added to a flask in sequence, and the mixture was stirred for 12 h. Then the reaction mixture was poured into brine (30 mL) while stirring, and the aqueous layer was extracted with ethyl acetate (3 × 6 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent (4:1 to 1:1) to afford the title compound (56 mg, 78%) as a white semisolid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.19 (m, 20H), 7.14–7.08 (m, 3H), 6.95–6.94 (m, 2H), 5.60 (d, J = 3.5 Hz, 1H), 5.59 (d, J = 4.0 Hz, 1H), 5.20 (d, J = 8.0 Hz, 1H), 4.99 (d, J = 12.0 Hz, 1H), 4.94 (d, J = 12.0 Hz, 1H), 4.81 (d, J = 12.0 Hz, 1H), 4.74 (d, J = 11.5 Hz, 1H), 4.71 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 11.0 Hz, 1H), 4.53 (d, J = 11.5 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.25 (d, J = 12.0 Hz, 1H), 4.22 (d, J = 11.0 Hz, 1H), 4.18–4.15 (m, 1H), 3.95–3.86 (m, 2H), 3.81–3.77 (m, 2H), 3.72 (t, J = 9.5 Hz, 1H), 3.65 (d, J = 9.5 Hz, 1H), 3.58–3.48 (m, 3H), 3.40–3.34 (m, 3H), 3.31–3.24 (m, 3H), 3.14 (dd, J_1 = 2.5 Hz, J_2 = 11.0 Hz, 1H), 2.36 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.86–1.80 (m, 1H), 1.74–1.57 (m, 6H), 1.37–1.14 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.14, 137.84, 137.57, 137.33, 137.28, 137.20, 128.51, 128.52, 128.31, 128.17, 128.14, 128.10, 127.73, 127.46, 127.22, 125.86, 97.01, 95.91, 83.00, 77.76, 77.47, 76.25, 75.86, 75.01, 74.44, 73.58, 73.50, 73.10, 71.76, 70.09, 67.71, 65.31, 62.97, 60.23, 59.53, 51.98, 51.25, 45.31, 32.01, 29.68, 29.18, 25.58, 25.53, 25.48. HRMS (ESI) calcd for $C_{60}H_{68}N_{16}O_{10}Na$ (M+Na$^+$): 1195.5197; found: 1195.5177.

4.1.16. 4′-Deoxy-4′-(2-(2-methylbenzamido)-acetamido)-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (31)

This compound was synthesized by the same procedure as described in the preparation of **30**, providing **31** as a crystalline powder in 75% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.18 (m, 24H), 7.13–7.08 (m, 3H), 6.95–6.93 (m, 2H), 6.60 (d, J = 8.5 Hz, 1H), 6.48 (t, J = 4.5 Hz, 1H), 5.61 (d, J = 3.5 Hz, 1H), 5.51 (d, J = 3.5 Hz, 1H), 5.02 (d, J = 12.0 Hz, 1H), 4.92 (d, J = 12.0 Hz, 1H), 4.82–4.73 (m, 3H), 4.60 (d, J = 11.0 Hz, 1H), 4.53 (d, J = 11.5 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.30–4.21 (m, 3H), 4.00–3.85 (m, 3H), 3.81–3.73 (m, 3H), 3.65–3.61 (m, 2H), 3.52–3.46 (m, 2H), 3.40–3.22 (m, 7H), 3.12 (dd, J_1 = 3.0 Hz, J_2 = 11.0 Hz, 1H), 2.45 (s, 3H), 2.27 (ddd, J_1 = J_2 = 4.5 Hz, J_3 = 13.0 Hz, 1H), 1.58 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.36, 168.80, 137.84, 137.71, 137.56, 137.32, 136.47, 134.75, 131.23, 130.52, 128.51, 128.46, 128.30, 128.26, 128.17, 128.09, 127.91, 127.73, 127.70, 127.44, 127.20, 127.13, 125.88, 125.72, 97.28, 95.90, 82.87, 78.06, 77.71, 77.44, 77.09, 75.86, 75.02, 74.42, 74.24, 73.49, 73.10, 70.99, 70.06, 67.71, 65.32, 63.35, 60.17, 59.04, 51.88, 51.65, 43.84, 31.79, 20.05. HRMS (ESI) calcd for $C_{63}H_{67}N_{17}O_{11}Na$ (M+Na$^+$): 1260.5098; found: 1260.5088.

4.1.17. 4′-Deoxy-4′-(*N*-benzoxycarbonyl-piperidinyl)-4-carbonylamino-5,3′,2″,4″,6″-penta-*O*-benzyl-pentaazido-kanamycin B (32)

This compound was synthesized by the same procedure as described in the preparation of **30**, providing **32** as a white semisolid in 81% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.09 (m, 28H), 6.96–6.93 (m, 2H), 5.60 (d, J = 3.5 Hz, 1H), 5.59 (d, J = 3.5 Hz, 1H), 5.27 (d, J = 8.0 Hz, 1H), 5.11 (s, 2H), 4.98 (d, J = 12.0 Hz, 1H), 4.94 (d, J = 12.0 Hz, 1H), 4.81 (d, J = 12.0 Hz, 1H), 4.74 (d, J = 12.0 Hz, 1H), 4.73 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 11.0 Hz, 1H), 4.49 (d, J = 12.0 Hz, 1H), 4.47 (d, J = 12.0 Hz, 1H), 4.25 (d, J = 12.0 Hz, 1H), 4.23 (d, J = 11.0 Hz, 1H), 4.17–4.14 (m, 3H), 3.94–3.84 (m, 2H), 3.81–3.77 (m, 2H), 3.72 (t, J = 9.5 Hz, 1H), 3.65 (t, J = 9.5 Hz, 1H), 3.58–3.48 (m, 3H), 3.40–3.28 (m, 5H), 3.24 (dd, J_1 = 2.5 Hz, J_2 = 13.0 Hz, 1H), 3.14 (dd, J_1 = 3.0 Hz, J_2 = 11.0 Hz, 1H), 2.73 (br, 2H), 2.38–2.32 (m, 1H), 1.96–1.90 (m, 1H), 1.70–1.57 (m, 3H), 1.56–1.46 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.28, 155.04, 137.82, 137.55, 137.30, 137.16, 136.62, 128.58, 128.49, 128.30, 128.15, 128.07, 127.86, 127.70, 127.45, 127.22, 125.83, 96.95, 95.88, 82.95, 77.76, 77.70, 77.46, 76.30, 75.84, 74.99, 74.42, 73.48, 73.09, 71.65, 70.09, 67.71, 67.14, 65.29, 62.95, 60.19, 59.50, 51.93, 51.25, 43.21, 43.16, 42.85, 31.97, 28.38, 27.99. HRMS (ESI) calcd for $C_{67}H_{73}N_{17}O_{12}Na$ (M+Na$^+$): 1330.5517; found: 1330.5516.

General procedure for deprotection of compounds **27–32**. The starting compound (0.03–0.08 mmol) was dissolved in the mixture of pyridine (3 mL), triethylamine (2 mL), and water (0.5 mL). Then, H_2S gas was introduced into the mixture until it turned deep-green. The mixture was stirred for 8–12 h at room temperature. After TLC showed the reaction was completed, the mixture was concentrated and the residue was passed through a short column (silica gel) with eluents as the following: petroleum ether/ethyl acetate (25 mL/25 mL), ethyl acetate (50 mL), ethyl acetate/methanol (25 mL/25 mL), methanol (50 mL), and methanol/concentrated aqueous ammonia (100 mL/10 mL). The crude product was then dissolved in 5 mL of methanol, and pH value of the resulting solution was adjusted to 3–4 with hydrochloric acid (1 M). Then Pd/C (10%, 100 mg) was added. The mixture was subjected to hydrogenolysis for 3–7 days. After TLC showed that only one spot appeared, the mixture was filtered through a pad of Celite. The filtrate was evaporated, and the crude product was dissolved in deionized water (3 mL) and the resulting solution was passed through a short column (packed with C18 silica gel), the fraction was collected to yield the product after lyophilization.

4.1.18. 4′-Deoxy-4′-dimethylamino-kanamycin B (7)

Yield 95%, white amorphous powder. $[\alpha]_D$ +71.3 (c 0.75, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 6.09 (d, J = 4.0 Hz, 1H), 5.15 (d, J = 4.0 Hz, 1H), 4.66 (dt, J_1 = 3.0 Hz, J_2 = J_3 = 10.0 Hz, 1H), 4.58 (t, J = 10.5 Hz, 1H), 4.16 (t, J = 10.0 Hz, 1H), 3.98–3.92 (m, 3H), 3.90–3.86 (m, 2H), 3.82–3.61 (m, 7H), 3.54–3.46 (m, 2H), 3.12 (s, 6H), 2.56 (ddd, J_1 = 4.0 Hz, J_2 = J_3 = 12.5 Hz, 1H), 2.02 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 101.37, 96.34, 84.37, 79.03, 74.83, 73.74, 68.74, 67.13, 66.14, 65.48, 63.22, 60.66, 55.56, 55.02, 50.25, 48.68, 43.14, 41.06, 28.56. HRMS (ESI) calcd for $C_{20}H_{43}N_6O_9$ (M+H$^+$): 511.3086; found: 511.3067.

4.1.19. 4′-Deoxy-4′-acetamido-kanamycin B (8)

Yield 87%, white amorphous powder. $[\alpha]_D$ +80.6 (c 0.80, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 6.06 (d, J = 3.5 Hz, 1H), 5.14 (d, 3.5 Hz, 1H), 4.25 (t, J = 10.0 Hz, 1H), 4.20–4.16 (m, 1H), 4.09 (t, J = 10.0 Hz, 1H), 3.98–3.93 (m, 3H), 3.89–3.77 (m, 4H), 3.72 (t, J = 10.0 Hz, 1H), 3.68–3.59 (m, 2H), 3.55 (dd, J_1 = 4.0 Hz, J_2 = 10.5 Hz, 1H), 3.52 (t, J = 10.5 Hz, 1H), 3.36 (dd, J_1 = 2.5 Hz, J_2 = 14.0 Hz, 1H), 3.16 (dd, J_1 = 6.5 Hz, J_2 = 14.0 Hz, 1H), 2.58–2.55 (m, 1H), 2.08 (s, 3H), 2.02 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 176.11, 101.39, 96.48, 84.44, 77.72, 75.04, 73.66, 68.87, 68.80, 66.13, 60.63, 55.60, 54.84, 53.27, 50.33, 49.09, 40.96, 28.53, 22.78. HRMS (ESI) calcd for $C_{20}H_{41}N_6O_{10}$ (M+H$^+$): 525.2879; found: 525.2865.

4.1.20. 4′-Deoxy-4′-(4-amino-butanoylamino)-kanamycin B (9)

Yield 66%, white amorphous powder. $[\alpha]_D$ +73.4 (c 0.35, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 6.04 (d, J = 3.5 Hz, 1H), 5.14 (d, 3.5 Hz, 1H), 4.23 (t, J = 10.5 Hz, 1H), 4.21–4.18 (m, 1H), 4.08 (t, J = 9.0 Hz, 1H), 3.98–3.50 (m, 12H), 3.36 (dd, J_1 = 3.0 Hz, J_2 = 14.0 Hz, 1H), 3.16 (dd, J_1 = 5.5 Hz, J_2 = 13.5 Hz, 1H), 3.04 (t, J = 7.5 Hz, 2H), 2.58–2.55 (m, 1H), 2.49 (t, J = 7.5 Hz, 2H), 2.03–1.94 (m, 3H); ^{13}C NMR (125 MHz, D_2O) δ 176.80, 101.42, 96.75, 84.46, 78.08, 74.98, 73.70, 68.92, 68.76, 66.13, 60.61, 55.58, 54.91, 53.06, 50.28, 49.05, 40.89, 39.59, 33.10, 28.54, 23.40. HRMS (ESI) calcd for $C_{22}H_{46}N_7O_{10}$ (M+H$^+$): 568.3301; found: 568.3300.

4.1.21. 4′-Deoxy-4′-cyclohexylcarbonylamino-kanamycin B (10)

Yield 61%, white amorphous powder. $[\alpha]_D$ +67.2 (c 0.95, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 6.04 (d, J = 4.0 Hz, 1H), 5.14 (d, J = 3.5 Hz, 1H), 4.22 (t, J = 10.0 Hz, 1H), 4.20–4.17 (m, 1H), 4.07 (t, J = 10.0 Hz, 1H), 3.99–3.78 (m, 7H), 3.73 (t, J = 10.0 Hz, 1H), 3.69–3.60 (m, 3H), 3.57–3.50 (m, 2H), 3.32 (dd, J_1 = 2.5 Hz, J_2 = 13.5 Hz, 1H), 3.11 (dd, J_1 = 5.5 Hz, J_2 = 14.0 Hz, 1H), 2.59–2.55 (m, 1H), 2.37–2.31 (m, 1H), 2.02–1.95 (m, 1H), 1.84–1.75 (m, 4H), 1.67 (d, J = 12.0 Hz, 1H), 1.43–1.14 (m, 5H); ^{13}C NMR (125 MHz, D_2O) δ 182.22, 101.43, 96.85, 84.48, 78.12, 74.99, 73.72, 68.99, 68.75, 66.08, 63.27, 60.60, 55.59, 54.98, 52.69, 50.27, 49.05, 45.68, 40.79, 29.91, 28.56, 25.93, 25.80. HRMS (ESI) calcd for $C_{25}H_{49}N_6O_{10}$ (M+H$^+$): 593.3505; found: 593.3504.

4.1.22. 4′-Deoxy-4′-(2-(2-methylbenzamido)-acetamido)-kanamycin B (11)

Yield 64%, yellow amorphous powder. $[\alpha]_D$ +64.9 (c 1.30, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.47–7.42 (m, 2H), 7.35–7.29 (m, 2H), 6.07 (d, J = 3.5 Hz, 1H), 5.14 (d, J = 3.0 Hz, 1H), 4.31(t, J = 10.0 Hz, 1H), 4.29–4.25 (m, 1H), 4.18–4.08 (m, 3H), 3.97–3.83 (m, 6H), 3.79 (dd, J_1 = 5.0 Hz, J_2 = 12.5 Hz, 1H), 3.72 (t, J = 10.0 Hz, 1H), 3.70–3.61 (m, 2H), 3.59–3.50 (m, 2H), 3.40 (d, J = 12.0 Hz, 1H), 3.20 (dd, J_1 = 6.0 Hz, J_2 = 13.5 Hz, 1H), 2.58–2.56 (m, 1H), 2.37 (s, 3H), 2.01 (ddd, J_1 = J_2 = J_3 = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 174.76, 173.29, 136.59, 135.15, 131.66, 131.49, 127.83, 126.65, 101.42, 96.65, 84.47, 77.91, 75.03, 73.71, 68.79, 66.12, 65.98, 60.63, 55.60, 54.88, 53.46, 50.31, 49.09, 43.77, 40.91, 28.55, 19.44. HRMS (ESI) calcd for $C_{28}H_{48}N_7O_{11}$ (M+H$^+$): 658.3406; found: 658.3413.

4.1.23. 4′-Deoxy-4′-(piperidinyl-4-carbonyl)amino-kanamycin B (12)

Yield 60%, white amorphous powder. $[\alpha]_D$ +65.6 (*c* 0.85, H_2O). ^1H NMR (500 MHz, D_2O) δ 6.08 (d, *J* = 4.0 Hz, 1H), 5.14 (d, *J* = 3.5 Hz, 1H), 4.28 (t, *J* = 10.5 Hz, 1H), 4.24–4.17 (m, 1H), 4.09 (t, *J* = 10.0 Hz, 1H), 3.97–3.82 (m, 5H), 3.78 (dd, J_1 = 5.0 Hz, J_2 = 12.5 Hz, 1H), 3.71 (t, *J* = 10.0 Hz, 1H), 3.67–3.59 (m, 3H), 3.55–3.44 (m, 4H), 3.33–3.26 (m, 1H), 3.18 (dd, J_1 = 7.0 Hz, J_2 = 14.5 Hz, 1H), 3.13–3.05 (m, 1H), 2.77–2.72 (m, 1H), 2.56–2.54 (m, 1 H), 2.10–1.98 (m, 2H), 1.91–1.83 (m, 2H), 1.58–1.56 (m, 1H); ^{13}C NMR (125 MHz, D_2O) δ 178.18, 101.42, 96.61, 84.47, 77.84, 75.03, 73.70, 68.89, 68.79, 66.12, 66.08, 60.63, 55.61, 54.92, 53.02, 50.31, 49.10, 43.81, 43.76, 40.97, 40.67, 28.55, 25.91, 25.60. HRMS (ESI) calcd for $C_{24}H_{48}N_7O_{10}$ ($M+H^+$): 594.3457; found: 594.3453.

4.2. Antibacterial assay

All newly synthesized compounds were tested in the form of hydrochloride. The purity of all tested compounds was over 98% according to the HPLC analysis results. Minimum inhibitory concentrations (MIC) in μg/mL were determined by the microdilution broth method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Inoculum was prepared by diluting actively growing broth cultures to a McFarland value of 0.5 (1–108 cfu/mL). Antibiotic dilutions were made by dispensing 0.1 mL into each of the 96 wells of a standard microtitre tray in 2-fold dilutions. The inoculum was added to give a final concentration of 5–105 cfu/mL. The trays were covered and incubated at 37 °C for 16–20 h. The MIC was defined as the lowest concentration of drug inhibiting visible growth. The microdilution broth susceptibility testing was performed in triplicate.

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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.2010.11.065.

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