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Surprising Alteration of Antibacterial Activity of 5″-Modified Neomycin against Resistant Bacteria

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Abstract

A facile synthetic protocol for the production of neomycin B derivatives with various modifications at the 5″ position has been developed. Structural activity relationship (SAR) against aminoglycoside resistant bacteria equipped with various aminoglycoside-modifying enzymes (AME's) was investigated. Enzymatic and molecular modeling studies reveal that the superb substrate promiscuity of AME's allows the resistant bacteria to cope with diverse structural modifications despite the observation that several derivatives show enhanced antibacterial activity than the parent neomycin. Surprisingly, when testing synthetic neomycin derivatives against other human pathogens, two leads exhibit prominent activity against both Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) that are known to exert high level of resistance against clinically used aminoglycosides. These findings can be extremely useful in developing new aminoglycoside antibiotics against resistant bacteria. Our result also suggests that new biological and antimicrobial activities can be obtained by chemical modifications of old drugs.

Introduction

Chemical modification of aminoglycosides has been an effective way of reviving the antibacterial activity of aminoglycosides against resistant bacteria, especially against those that are equipped with aminoglycoside-modifying enzymes (AME's).[1-3] For example, attachment of (*S*)-4-amino-2-hydroxybutanoyl (AHB) and (*S*)-3-amino-2-hydroxypropanoyl groups at N-1 position has led to the production of clinically useful aminoglycosides, amikacin and isepamicin, respectively (Figure 1). In general, protection of amino groups as carbamates, or transformation of amino groups into azido groups on aminoglycosides are the most commonly employed strategies for introducing desired structural motifs regiospecifically.

Our group has been working on modifications of aminoglycoside antibiotics via the amine/azide transformation approach.[4,5] The polyazido intermediates employed in the synthesis can be conveniently manipulated in organic media using traditional synthetic methods, which is ideal for constructing a library of aminoglycosides for identifying leads. However, the safety

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Supporting Information Available: A table listing the appropriate analytical data including spectra of ^{1}H and ^{13}C NMR for the synthesized compounds, and ^{1}H, ^{13}C NMR, HPLC and HRMS of the assayed compounds. This material is available free of charge via the internet at http://pubs.acs.org.

concern of handling azido compounds, the higher cost involved in the synthesis of azidoaminoglycosides, and the reduction of azido groups impose challenges in scale-up production of the leads for subsequent animal studies and clinical trials. By comparison, direct modification of commercially available aminoglycosides using carbamate type of protecting groups, which can be carried out in large quantity, appear to be a more practical approach for generating clinically useful novel aminoglycosides. Thus, we began to ponder the possibility of developing diversity-oriented synthesis of aminoglycoside derivatives via the use of carbamate type of protecting groups.

Design and Synthesis of 5″-Modified Neomycin B

Recently, several examples of 5″-modified neomycin class of aminoglycosides have been reported including conformational constrained aminoglycosides with intramolecular linkage between N-2′ and C-5″[6,7] and between N-3 and N-6′,[8] dimer of neomycin with spacing linkage via C-5″[9], neomycin with 2,3-diaminopropanoyl group at 5″ position,[10] and orthogonal divergent synthesis of neomycin derivatives with modifications at various amine functionalities.[11] Although the constructed conformationally constrained aminoglycoside manifested much lower turn-over rate toward the AME of interest, the antibacterial activity also decreased significantly.[6] The design of orthogonal divergent synthesis appears to have the potential of being employed for diversity-oriented synthesis. Nevertheless, the reported synthesis did not include the deprotection of carbamate protecting groups and the production of neomycin derivatives that can be assayed for their antibacterial activity.

In addition, it has also been suggested that aminoglycosides bearing deoxygenation at 3′ position, such as in the case of tobramycin, display relatively higher cytotoxicity than those that still contain 3′-OH.[12] Neomycin B, which contains 3′-OH, exerts broad spectrum antibacterial activity and can be available in large quantity at relatively low cost. Thus, we reason that derivatizing neomycin with 5″-modifications could be valuable in investigating the structure-activity relationship (SAR) against resistant bacteria, the efficacy of avoiding enzyme-catalyzed modifications from aminoglycoside phosphotransferases (APH) that targets 3′-OH or, perhaps, aminoglycoside nucleotidyltransferases (ANT) that targets 4′-OH, and even the structure-cytotoxicity relationship. Neomycin derivatives bearing carbohydrate moieties at 5″-OH have also been shown to have good activity against a panel of bacteria.[13,14]

We prefer to employ the carbobenzyloxy (Cbz or Z) group as the protecting group of amines on neomycin since it can be tracelessly removed using hydrogenolysis. The synthetic strategy should be simple, which is suitable in producing a library of such derivatives for expedient screening and subsequent gram-scale synthesis of the leads. From our molecular modeling studies and SAR of others,[13,14] we have also noticed that diverse functional groups can be attached at C-5″ without significantly obstructing the antibacterial activity. Finally, 5″-OH is the only primary hydroxyl group on neomycin B which can be regioselectively converted to azido group and serve as the site of diversity-oriented modifications via “Click” chemistry or amide linkage. Our initial design was to randomly introduce structural moieties containing alkyl, aryl, cationic and anionic substitutents for probing the SAR. The diversity-oriented synthesis started from a known Cbz-protected neomycin B derivative, **1**[8] followed by regioselective substitution of 5″-OH with azide (Scheme 1). The 5″-azido group can undergo 1,3-dipolar cyclization with alkynes (class I design).[15] Alternatively, the 5″-azido group of **1** can be reduced to an amino group and coupled with various carboxylic acids or amino acids (class II design). Global deprotection of the Cbz groups of these derivatives from both routes provided 5″-modified neomycin derivatives ready for antibacterial assay. The yields and structural designs are summarized in Tables 1 and 2. With the goal to expand the structural diversity, a dimeric neomycin derivative, **33** can be prepared by reacting the 5″-NH_2 group with succinic anhydride providing **32**, and then coupled to another molecule of **3** (Scheme 2).

Antibacterial Activity of 5″-Modified Neomycin B

The synthesized aminoglycosides were assayed against susceptible and resistant bacterial strains using neomycin B as the control. Aminoglycoside susceptible *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used as standard reference strains. *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 phosphorylation at the 3′-OH of both neomycin and kanamycin classes of aminoglycosides. The second one is equipped with the pSF815 plasmid encoded for a bifunctional enzyme, AAC(6′)/APH(″), which catalyzes acetylation of the amino group at 6′-NH_2 and phosphorylation of the hydroxyl group at 2″-OH. These enzymes are among the most prevalent modes of resistance found in aminoglycoside resistant strains. The minimum inhibitory concentrations (MIC's) are summarized in Table 3.

From the MIC results, it appears that the 5″ position can tolerate the incorporation of diverse structural components, which is consistent with previous literature results.[13,14] Derivatives with 1,2,3-triazole linkage manifested modest antibacterial activities comparable to neomycin B with **4e** and **4g** as the most active derivatives. Most of the derivatives bearing amide-based linkages were less active than the parent neomycin B. Nevertheless, derivatives incorporated with long linear alkyl chains (**5b** and **5c**) showed better antibacterial activity than neomycin B, and the one with shorter linear alkyl chain (**5a**), even against resistant strains. Among the derivatives attached with amino acids, glycine (**5f**), alanine (**5g** and **5m**) and proline (**5h** and **5o**) manifested similar levels of antibacterial activity with no particular difference between D and L amino acids. On average, it appears that these 5″-modified neomycin derivatives are more active against G+ bacteria (*S. aureus*) than G− bacteria (*E. coli*). For example, there is only 4-fold MIC difference for neomycin B between G+ and G− bacteria while, in most of the synthetic derivatives, the differences are in the range of 4 to 16 fold.

As expected, compound **5l** incorporated with a negatively-charged carboxylate group showed much lower antibacterial activity as compared to other derivatives. With the exception of **5b** and **5c**, all the derivatives are inactive against resistant strain equipped with APH(3′)-I while retaining moderate activities against the susceptible control strain. This finding implies that APH(3′)-I has much broader substrate promiscuity than the RNA binding site for 5″-modified neomycin derivatives, which, again, exemplifies the challenges in developing aminoglycosides against drug-resistant bacteria. The activity of **5b** and **5c** against resistant bacteria is of particular interest, which prompts further enzymatic and molecular modeling investigations.

Enzymatic and Antibacterial Studies of 5″-Modified Neomycin B against APH (3′)-IIIa

Although no significant activity against bacteria equipped with APH(3′)-I was observed for most of the derivatives, several derivatives did appear to have improved or similar activity as compared to the parent neomycin B. Thus, effort was devoted to investigation of the relationship between enzyme kinetic studies and whole-cell based assay. Our initial strategy is to introduce structural variants at the 5″-position, which is close to the 3′-OH targeted by many APH(3′). There are as many as seven isoforms of APH(3′) (I-VII) which have been identified with various substrate specificity among gram-negative and gram-positive bacteria.[16-19] We selected APH(3′)-IIIa for enzymatic study due to consideration of its availability and clinical significance. For example, studies of the epidemiology of AME's have revealed the prevalence of the APH(3′)-IIIa in Methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis* and *Enterococcus faecium*.[20-23]

The enzyme APH(3′)-IIIa was expressed and purified according to the literature procedure.[24] Enzyme kinetic studies for 5″-modified neomycin derivatives, kanamycin A, amikacin and neomycin B were performed as reported.[25] The laboratory resistant strain was generated using *E. coli* (TG1) as the host harboring pET28a plasmid encoded for APH(3′)-IIIa. Neomycin B, kanamycin B (Kan B) and amikacin were used as controls in the antibacterial assay. The results from enzymatic studies and the MIC are summarized in Table 4.

By comparing the kinetic parameters from the commercially available aminoglycosides (kanamycin A, amikacin and neomycin B), the presence of AHB group increases K_M but lowers V_{max} and k_{cat} indicating that amikacin has lower binding affinity and turnover rate toward APH(3′)-IIIa. Interestingly, most of the 5″-modified neomycin derivatives manifest much higher K_M and lower V_{max} and k_{cat} implying that these derivatives can better evade the enzyme-catalyzed modification as well. The high K_M indicates that these 5″-modified neomycin derivatives are poor substrates for APH(3′)-IIIa. The low k_{cat} suggests that the added functionalities at 5″ position could disrupt the rate of phosphorylation at 3′-OH even in the case of **5f** where a relatively smaller glycine is incorporated. From the kinetic data, no obvious correlation between the k_{cat}/K_M and MIC can be deduced. For example, **4a** has a relatively low k_{cat}/K_M but still exerts no significant activity in the whole-cell based antibacterial assay against the resistant strain. Several possible reasons can account for these observations: 1) binding of the 5″-modified neomycin derivatives to RNA targeted site could have been disrupted by the structural modifications leading to lower antibacterial activity; 2) APH(3′)-IIIa can still inactivate the 5″-modified neomycin derivatives. Although inactivation occurs at a much slower rate, the higher concentration of APH(3′)-IIIa as compared to the targeted rRNA site renders the lower k_{cat}/K_M values insignificant for most of the neomycin derivatives in regaining their antibacterial activity; and 3) the uptake of the 5″-modified neomycin derivatives by bacteria could have also been hampered by the structural modifications.

To better compare the changes of antibacterial efficiency, we calculated the MIC ratio by dividing the MIC against susceptible strain with the MIC against resistant one (right column, Table 4). Amikacin, which has excellent activity against resistant strain has MIC ratio of 1, which means there is no loss of activity against the resistant strain. Several interesting findings were noted. While neomycin and most of the neomycin derivatives have MIC ratio range from 4 to 8, two compounds, **5b** and **5c** have ratios of 0.5 and 1, respectively. The kinetic studies also suggested that these derivatives should be “poor substrates” for the enzyme. Thus, these results point to an interesting question: why are these two compounds still active against resistant strains while other derivatives with similar or low k_{cat}/K_M values are inactive or less active?

Two possible reasons could account for the observed activity of **5b** and **5c** having similar antibacterial efficiency as compared to amikacin and superior efficiency as compared to neomycin. First: the structural modifications at N-5″ of **5b** and **5c** may have similar effects as the AHB group at N-1 of amikacin. From the X-ray crystallography study,[26] it has been demonstrated that AHB side chain can be accommodated by the binding site of rRNA while rendering amikacin a “poor substrate” for APH(3′)-IIIa. Second: these two derivatives may have *different modes of antibacterial action*. We favor the second postulate. Since the enzyme-catalyzed modifications occur within bacteria, neomycin derivatives with similar k_{cat}/K_M values should still undergo phosphorylation leading to the inactivation of these modified aminoglycosides. Therefore, **5b** and **5c** may exert their antibacterial activity not by binding to the rRNA which occurs within bacteria, but by a different mode of antibacterial action.

Compounds **5f** and **4d** exhibit better activity against resistant strains than does neomycin without suffering significant activity loss against susceptible strains. Since protected glycine can be readily prepared in large-scale and, in particular, **5f** has excellent activity against *S.*

aureus, we decided to treat compound **5f** as a lead for further chemical modifications to be discussed later. Finally, despite having very low k_{cat}/K_M value, the dimer, **33** displayed only modest activities against susceptible strains and low activity against resistant strains. Once again, this result implies the broader substrate promiscuity of APH(3′)-IIIa than the binding pocket of targeted rRNA site. Overall, our results from enzymatic studies suggest that a low k_{cat}/K_M value may not be applicable to predict the antibacterial activity of aminoglycoside constructs against resistant bacteria equipped with AME's.

Molecular Modeling of 5″-Modified Neomycin B

The bactericidal activity of neomycin and kanamycin arises from the binding of aminoglycosides to the A-site decoding region of 16S ribosomal RNA. To examine whether the targeted site of rRNA can accommodate the added functional groups at 5″ position, molecular modeling[27] was carried out using the reported X-ray structures of APH(3′)-IIIa and neomycin-bound rRNA as the templates.[28,29] Two particularly active compounds: **5f** and **4d**, representative of classes I and II, were selected for the molecular modeling analysis (Figure 2). While the part of the APH (3′)-IIIa enzyme responsible for the catalysis and the tight binding of the neamine portion of the molecule (rings I and II) is shaped in the form of a deep cleft, other fragments of the aminoglycoside molecule are bound to the rather sterically unhindered surface of the enzyme. This allows for the enzyme's unique substrate promiscuity, which is facilitated also by the substrate's conformational flexibility. Due to the steric consideration, the 5″-sidechain of **4d** is pointed away from the enzyme implying poor recognition and turnover from APH(3′)-IIIa, which is supported by the high K_M and low k_{cat}/K_M.

Both **5f** and **4d** can also bind to the targeted rRNA site although the 5″-sidechain appears to have significant flexibility by adopting different conformations in both cases. In contrast to the binding of neomycin derivatives to APH(3′)-IIIa, all four rings from neomycin scaffold interact with the RNA suggesting a lesser tolerance to structural modifications. It is expected that the larger 5″-sidechain of **4d** will lower the binding affinity thus accounting for the lower antibacterial activity. Nevertheless, as mentioned previously, the superior substrate promiscuity and higher concentration of APH(3′) will render most of the structural modifications at rings III and IV less effective. Unfortunately, the 5″-sidechain of **5b** and **5c** exhibit too much freedom to be properly predicted by molecular modeling.

Although scoring was attempted, the scores obtained do not correlate well with the experimental MICs. We have noticed earlier that theoretical scores are better correlated with experiment for less active compounds.[4] The correlation deteriorates for more active compounds. This suggests that the predictions are accurate when the ligand-macromolecule fit is the limiting factor. When it is not, other factors, such as solubility, transport or degradation of the aminoglycosides, cannot be neglected when constructing a quantitative, linear SAR.

Antibacterial Assay of Selected Derivatives against Other Pathogens

As mentioned previously, 5″-modified neomycin derivatives seem to be more active against G+ bacteria (*S. aureus*) than G− bacteria (*E. coli*), and **5f** appears to be one of the most prominent leads having similar MIC ratio as amikacin. We decided to further derivatize **5f** by preparing **5q** by extending one glycidal unit (Figure 3). In addition, we also intend to explore the spectrum of antibacterial activity of **5b** and **5c** against other clinically significant pathogens including *Klebsiella pneumoniae* (Gram negative), Methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* (Gram negative) and *Enterococcus faecalis* (Gram positive).

K. pneumoniae (ATCC 700603) is a clinical isolate that is resistant to ceftazidime, other β-lactams, and several aminoglycosides (ANT(2″).[30] *K. pneumoniae* (ATCC 13883) is resistant to ampicillin but susceptible to aminoglycosides. *P. aeruginosa* (ATCC 27853) that expresses

APH(3′)-IIb manifests modest resistance toward aminoglycosides.[31] Methicillin-resistant *S. aureus* (ATCC 33591) (MRSA) is the leading cause of bacterial infections and many MRSA strains harbor genes encoded for APH(3′), ANT(4′), and AAC(6′)/APH(2″), which render the bacteria resistant to various aminoglycosides.[20] It has been reported that microorganisms like enterococci and other anaerobes are intrinsically resistant to aminoglycosides due to the dull uptake of aminoglycosides as a result of the deficiency in their membrane-associated electron transport systems.[19] Like MRSA, vancomycin-resistant enterococci (VRE) that cause various types of nosocomial infections also represent a great threat to the public health.[32] *Enterococcus faecalis* (ATCC51299) which is one of the vancomycin-resistant enterococci (VRE), contains *vanB, ant(6)-I,* and *aac(6′)-aph(2″)* resistance genes and exerts high level of resistance to aminoglycosides and vancomycin.[33] *E. faecalis* (ATCC 29212), which is susceptible to vancomycin but manifests moderate resistance to aminoglycosides, is used as comparison.

The results from the antibacterial assay are summarized in Table 5. As expected, the derivatives with relatively smaller structural modifications (**5f** and **5q**) display similar antibacterial profile as neomycin suggesting unaltered modes of bactericidal and resistant mechanisms. **5f** and **5q** are more active against *P. aeruginosa* as compared to neomycin but are less active as compared to amikacin and gentamicin. Overall, **5f** is slightly more active than neomycin implies that the structural modification with glycidyl group is beneficial. Nonetheless, such a modification is insufficient in reviving the antibacterial activity against aminoglycoside resistant bacteria.

Intriguing results were obtained from the screening of **5b** and **5c** that have drastic difference in their antibacterial activity profile from other aminoglycosides. In general, **5b** and **5c** are also more active against G+ bacteria than G− bacteria as indicated in the MIC values. However, **5b** and **5c** exhibit prominent activity against *E. faecalis* (ATCC29212) while other aminoglycosides, including clinically used amikacin and gentamicin, are much less active. More impressively, **5b** and **5c** show excellent activity against VRE as compared to amikacin, gentamicin and even vancomycin. As mentioned previously, enterococci have intrinsic resistance against aminoglycosides. This result strongly supports our speculation that **5b** and **5c** have different modes of antibacterial action. We have also introduced similar structural modifications on kanamycin class aminoglycosides.[34] However, from the preliminary screening, such modifications are ineffective in reviving the activity of these kanamycin analogs against aminoglycoside resistant bacteria. Thus, to our knowledge, the modifications on **5b** and **5c** are one of the very few examples of aminoglycoside derivatives with clinically useful activity against MRSA, VRE and other resistant strains.

Discussion and Conclusion

We have developed a facile and cost-effective method for constructing neomycin derivatives. Although the original strategy was to avoid the inactivation from APH(3′), unexpected and useful outcomes were obtained. First of all, structural modifications, which can be accommodated by the binding site of rRNA, can be readily accommodated by AME's as well. Results from our enzymatic studies show that low k_{cat}/K_M values for the structurally modified aminoglycosides may not applicable to suggest that these aminoglycosides could avoid the enzyme-catalyzed inactivation and regain their antibacterial activity. Molecular modeling results indicate that AME's recognition on neamine ring is sufficient for enzymes to inactivate aminoglycoside derivatives bearing diverse modifications at rings III and IV of neomycin or ring III of kanamycin. Structural modifications targeting on neamine, such as the attachment of AHB group at N-1 and 3′,4′-dideoxygenation, remain to be the few effective approaches. Although several examples from our work show enhanced activity is possible, it could be challenging to reproduce the effectiveness of N-1 AHB group on kanamycin or ribostamycin. In summary, it may not be easy to develop structurally modified aminoglycosides active against

resistant bacteria while *maintaining the same mode of antibacterial action* of traditional aminoglycosides.

Nonetheless, our results also reveal another strategy to combat the problem of resistance. It is possible to revive the antibacterial activity of aminoglycoside using structural modifications that can alter the original mode of action. For example MRSA and VRE are known to exert high level resistance to aminoglycosides. The discovery of two leads active against both MRSA and VRE is particularly significant since the transfer of resistance from enterococci to opportunistic *S. aureus* has been recognized as one of the stringent threats to public health.[33] Ongoing efforts have been devoted into the investigation of possible antibacterial modes of action for **5b** and **5c**, and further structural optimization from these two leads.

Experimental Section

General Procedure for Coupling of Compound 5 with Carboxylic Acids

To a solution of compound **3** (0.20 g, 0.14 mmol) and carboxylic acids (0.28 mmol) in DMF (10 mL) and Et_3N (0.04 mL, 0.28 mmol), HOBt (0.030 g, 0.21 mmol) and EDC (0.040 g, 0.21 mmol) were added. The reaction was stirred at room temperature overnight. After completion of the reaction, the reaction was concentrated and diluted with EtOAc. The organic solution was washed with water, saturated $NaHCO_{3(aq)}$, brine and dried over anhydrous Na_2SO_4. After removal of the solvent followed by a fast gradient column chromatography (eluted from hexane/EtOAc = 1/1 to EtOAc/MeOH = 9:1), the product was usually obtained as a solid, which was characterized with ^1H and/or ^{13}C NMR and subjected to hydrogenation without further purification.

General Procedure for the 1,3-Dipolar Cycloaddition

A solution of compound **3** (0.028 mmol), alkyne (0.05 mmol), $Cu(OAc)_2$ (0.05 mmol) and sodium ascorbate (0.05 mmol) in a mixed solution of MeOH (1.7 mL), THF (0.46 mL) and water (0.3 mL) was sonicated at ambient temperature for 14 minutes (7 min.×2). After completion of the reaction, the reaction mixture was diluted with CH_2Cl_2 then filtered through Celite. After removal of the solvent followed by a fast gradient column chromatography purification (CH_2Cl_2:MeOH = 100:0 to 60:40), the product was typically obtained as a solid, which was characterized with ^1H and/or ^{13}C NMR and subjected to hydrogenation without further purification.

General Procedure for Hydrogenation and Purification

The solids from 1,3-dipolar cycloaddition or acid/amine coupling reaction (0.1 – 0.2 mmol) were dissolved in degassed MeOH (9 mL) followed by the addition of 1 mL HOAc : H_2O (1/4 ratio) solution. Catalytic amount of $Pd(OH)_2/C$ powder was added and the system was well sealed and further degassed. The system was stirred under atmospheric H_2 at room temperature for 10 hours. The reaction was then quenched by filtering through Celite and the residue washed with H_2O and the combined solutions were concentrated. The crude product was purified with Amberlite CG50 (NH_4^+) eluted with a gradient of NH_4OH solution (0% – 20%). After collection of the desired fractions and removal of solvent, the product was re-dissolved in water and loaded to an ion-exchange column packed with Dowex 1X8-200 (Cl^- form), and eluted with water. After removal of solvent, the product was obtained as white solid.

N-Propargyl-2-phenyl-4-quinolinecarboxamide (9)

To a solution of 2-phenyl-4-quinolinecarboxylic acid (2.09 g, 8.02 mmol) DMF (30 mL), Et_3N (2.3 mL), EDC (2.31 g, 12.0 mmol) and HOBt (1.63 g, 12.0 mmol) were added. The reaction was stirred at room temperature for 2 days. After completion of the reaction, reaction

was concentrated and then diluted with EtOAc. The organic solution was washed with 1N HCl, H_2O, $NaHCO_3$ and brine, and then dried over Na_2SO_4. After removal of solvent and recrystallization from ether, the desired product was obtained as a crystal (1.69 g, 5.90 mmol, 74%). ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.17 (d, *J* = 9.5 Hz, 1H), 8.1 (m, 2H), 7.81 (s, 1H), 7.72 (td, *J* = 9.2 Hz, *J* = 1.1 Hz, 1H), 7.4 – 7.5 (m, 5H), 6.54 (s, 1H), 4.36 (dd, *J* = 5.9 Hz, *J* = 2.9 Hz, 2H), 2.35 (t, *J* = 2.9Hz, 1H); ^{13}C ($CDCl_3$, 100MHz) δ166.6, 156.1, 148.0, 141.1, 138.1, 129.8, 129.5, 129.3, 128.4 (2 carbons), 127.0, 126.9(2 carbons), 124.3, 122.7, 116.0, 78.3, 72.0, 29.3; ESI/APCI Calcd for $C_{19}H_{15}N_2O$ ($[M+H]^+$ m/e 287.1179; found m/e 287.1176.

N-Propargylisonicotinamide (13)

The product was synthesized similarly as compound **9** except the purification was accomplished using gradient column chromatography (eluted from Hexane/EtOAc = 60/40 to Hexane/EtOAc = 0/100, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.71 (dd, *J* = 4.5 Hz, *J* = 1.7 Hz, 2H), 7.61 (dd, *J* = 4.5 Hz, *J* = 1.6 Hz, 2H), 6.81 (s, 1H), 4.23 (dd, *J* = 5.3 Hz, *J* = 2.6 Hz, 2H), 2.27 (dd, *J* = 2.4 Hz, *J* = 1.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.5, 150.8 (2 carbons), 136.0, 121.2 (2 carbons), 79.0, 72.5, 30.1; ESI/APCI Calcd for $C_9H_9N_2O$ ($[M+H]^+$) m/e 161.0709; found m/e 161.0713.

N-propargylcarbobenzyloxy-L-prolinamide (14)

The product was synthesized similarly as compound **9** except the purification was accomplished using gradient column chromatography (eluted from Hexane/EtOAc = 90/10 to Hexane/EtOAc = 30/70, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.3 (m,, 5H), 5.1 (m, 2H), 4.26 (s, 1H), 3.90 (d, *J* = 9.7 Hz, 2H), 3.4 (m, 2H), 1.8 – 2.2 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 156.1, 136.6, 128.7 (2 carbons), 128.2, 128.0 (2 carbons), 79.9, 71.6, 60.8, 47.7, 31.3, 29.2, 28.9, 24.7; ESI/APCI Calcd for $C_{16}H_{19}N_2O_3^+$ ($[M+H]^+$) m/e 287.1390; found m/e 287.1401.

Compound 2

A solution of compound **1** (1.50 g, 0.89 mmol) and NaN_3 (0.12 g, 1.78 mmol) in DMF was stirred at 80°C overnight. TLC showed the completion of the reaction (R_f = 0.50 eluted with EtOAc/MeOH = 97/3). The reaction was filtered through Celite and the residue was washed with EtOAc. After the removal of the solvent followed by purification with a gradient column chromatography (CH_2Cl_2:MeOH = 100:0 to 90:10), the product was obtained as a white solid (0.81 g, 63%). ^{1}H NMR (CD_3COCD_3, 400 MHz) δ 7.2 - 7.4 (m, 30H), 6.56 (m, 2H), 6.36 (d, *J* = 9.6 Hz, 1H), 4.8 – 5.3 (m, 18H), 4.5 (m, 4H), 3.8 – 4.1 (m, 6H), 3.3 – 3.9 (m, 20H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 157.6 (2 carbons), 157.4, 156.8, 156.5 (2 carbons), 137.4 – 137.7 (6 carbons), 127.7 – 128.7 (30 carbons), 110.0, 100.3, 99.1, 86.6, 80.5, 79.4, 74.8, 74.4, 73.1, 72.6, 72.3, 71.7, 70.3, 67.9, 66.0 – 67.9 (8 carbons), 56.6, 53.2, 51.9, 51.3, 42.6, 41.3, 34.8; ESI/APCI Calcd for $C_{71}H_{81}N_9O_{24}Na$ ($[M+Na]^+$) m/e 1466.5292; found m/e 1466.5263.

Compound 3

To a solution of compound **3** (1.98 g, 1.37 mmol) in THF (20 mL) and several drops of water, PMe_3 in THF (8.2 mL, 8.2 mmol) was added. The reaction was stirred at 50°C for an hour and TLC showed the completion of the reaction (R_f = 0.02 eluted with EtOAc/MeOH = 97/3). After the removal of the solvent followed by purification with a gradient column chromatography (CH_2Cl_2:MeOH = 100:0 to 70:30), the product was obtained as a white solid (1.80 g, 93%). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.3 – 7.4 (m, 30H), 5.1 (m, 10H), 3..3 – 3.9 (m, 37H); ^{13}C NMR (CD_3OD, 100 MHz) δ 157.6 – 158.0 (6 carbons), 136.9 – 137.1 (6 carbons), 127.7 – 128.4 (30 carbons), 110.9, 98.8, 98.1, 86.6, 79.4, 78.7, 74.8, 74.0, 73.3, 71.9, 71.8, 71.1, 70.3, 67.8, 56.2, 52.9, 51.7, 50.8, 43.4, 42.0, 41.1, 39.0, 33.6, 30.4, 29.6, 28.9, 24.4, 23.7, 22.8; ESI/APCI Calcd for $C_{71}H_{84}N_7O_{24}$ ($[M+H]^+$) m/e 1418.5568; found m/e 1418.5536.

Compound 4a

^{1}H NMR (D_2O, 400 MHz) δ 8.08 (s, 1H), 5.95 (d, *J* = 3.4 Hz, 1H), 5.34 (s, 1H), 4.3 (t, *J* = 5.0 Hz, 2H), 4.14 (s, 2H), 4.06 (t, *J* = 9.7 Hz, 1H), 3.8 – 4.0 (m, 6H), 3.8 (m, 2H), 3.6 – 3.8 (m, 3H), 3.4 – 3.5 (m, 4H), 3.2 – 3.4 (m, 11H), 2.3 (m, 1H), 2.0 (m, 1H), 1.82 (s, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 174.8, 159.4, 142.8, 125.5, 110.1, 95.5, 95.0, 84.9, 79.9, 76.8, 75.3, 73.5, 73.1, 72.4, 70.6, 70.4, 69.9, 68.2, 67.8, 67.5, 53.5, 52.2, 51.0, 49.9, 48.7, 47.0, 41.9, 40.7, 40.2, 38.6, 28.3, 22.0; ESI/APCI Calcd for $C_{32}H_{58}N_{11}O_{15}$ ($[M+H]^+$) m/e 836.4108; found m/e 836.4107.

Compound 4b

^{1}H NMR (D_2O, 400 MHz) δ 7.80 (s, 1H), 5.78 (d, *J* = 3.7 Hz, 1H), 5.30 (d, *J* = 3.8 Hz, 1H), 5.21 (m, 1H), 4.5 (m, 2H), 4.2 (m, 1H), 3.9 (m, 1H), 3.7 – 3.9 (m, 6H), 3.5 (m, 2H), 3.3 (m, 5H), 3.2 (m, 3H), 2.62 (t, *J* = 7.4 Hz, 2H), 2.22 (m, 1H), 1.97 (m, 1H), 1.6 (m, 2H), 1.1 – 1.2 (m, 8H), 0.75 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 149.4, 123.9, 109.9, 95.9, 95.4, 85.4, 79.9, 78.0, 77.2, 73.2, 73.0, 70.9, 69.3, 68.9, 67.8, 67.5, 53.8, 51.7, 51.0, 50.3, 48.8, 46.8, 40.7, 40.3, 30.8, 30.4, 28.8, 27.9, 24.5, 22.1,13.5; ESI/APCI Calcd for $C_{31}H_{60}N_9O_{12}$ ($[M+H]^+$) m/e 750.4356; found m/e 750.4333.

Compound 4c

^{1}H NMR (D_2O, 400 MHz) δ 8.39 (s, 1H), 5.97 (d, *J* = 3.6 Hz, 1H), 5.40 (d, *J* = 2.9 Hz, 1H), 5.33 (s, 1H), 4.9 (dd, *J* = 14 Hz, *J* = 1.0 Hz, 1H), 4.81 (dd, *J* = 15 Hz, *J* = 7.4 Hz, 1H), 4.61 (t, *J* = 5.7 Hz, 1H), 4.5 (m, 4H), 4.5 (s, 2H), 4.35 (t, *J* = 5.3 Hz, 1H), 4.22 (t, *J* = 3.0 Hz, 1H), 4.12 (dd, *J* = 7.6 Hz, *J* = 2.9 Hz, 1H), 4.0 (m, 1H), 3.98 (t, *J* = 9.2 Hz, 2H), 3.8 – 3.9 (m, 3H), 3.7 (m, 2H), 3.6 (m, 1H), 3.4 – 3.5 (m, 6H), 3.2 – 3.3 (m, 3H), 2.3 (m, 1H), 1.7 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 137.2, 128.4, 110.4, 95.7, 95.4, 85.3, 79.8, 77.0, 76.9, 73.1, 73.0, 71.0, 70.6, 69.5, 69.0, 67.9, 67.6, 53.9, 52.9, 51.3, 51.1, 50.4, 49.0, 42.5 (2 carbons), 40.8, 40.5, 30.1; ESI/APCI Calcd for $C_{28}H_{55}N_{10}O_{12}$ ($[M+H]^+$) m/e 723.3995; found m/e 723.4022.

Compound 4d

^{1}H NMR (D_2O, 400 MHz) δ 8.14 (s, 1H), 7.88 (t, *J* = 8.6 Hz, 2H), 7.73 (m, 3H), 7.68 (t, *J* = 8.2 Hz, 1H), 7.49 (t, *J* = 8.1 Hz, 1H), 7.4 (m, 3H), 5.94 (d, *J* = 3.9 Hz, 1H), 5.32 (d, *J* = 3.5 Hz, 1H), 5.21 (d, *J* = 1.5 Hz, 1H), 4.5 (m, 2H), 4.2 (m, 2H), 4.1 (m, 1H), 3.8 – 3.9 (m, 6H), 3.7 (m, 1H), 3.6 (m, 1H), 3.5 (m, 2H), 3.2 – 3.4 (m, 9H), 2.31 (dt, *J* = 8.4 Hz, *J* = 4.1 Hz, 1H), 1.73 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 169.7, 157.7, 147.5, 144.8, 142.2, 137.9, 131.3, 130.4, 129.2 (2 carbons), 128.3, 128.1, 127.9 (2 carbons), 125.0, 124.8, 123.0, 118.2, 110.0, 95.7, 95.2, 85.0, 79.9, 76.9, 76.2, 73.1, 72.6, 70.7, 70.3, 69.7, 68.4, 67.8, 67.5, 53.6, 52.2, 50.9, 50.0, 48.7, 40.7, 40.2, 35.1, 28.9; ESI/APCI Calcd for $C_{42}H_{59}N_{11}O_{13}$ ($[M+H]^+$) m/e 948.4186; found m/e 948.4208.

Compound 4e

^{1}H NMR (D_2O, 400 MHz) δ 8.22 (s, 1H), 5.85 (d, *J* = 3.5 Hz, 1H), 5.30 (d, *J* = 2.9 Hz, 1H), 5.24 (d, *J* = 1.5 Hz, 1H), 4.8 (dd, *J* = 15Hz, *J* = 2.8 Hz, 2H), 4.52 (t, *J* = 5.9 Hz, 1H), 4.5 (m, 1H), 4.32 (s, 2H), 4.27 (t, *J* = 5.1 Hz, 1H), 4.14 (t, *J* = 3.0 Hz, 1H), 4.05 (dd, *J* = 4.6 Hz, *J* = 2.9 Hz, 1H), 3.96 (m, 1H), 3.85 (t, *J* = 9.3 Hz, 2H), 3.8 (m, 1H), 3.6 (m, 1H), 3.51 (s, 1H), 3.3 -3.4 (m, 5H), 3.2 (m, 4H), 2.66 (m, 3H), 2.20 (m, 1H), 1.57 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 138.5, 127.1, 110.3, 95.6, 95.4, 85.4, 79.7, 78.0, 76.8, 73.0, 72.9, 71.0, 70.5, 69.3, 69.2, 67.9, 67.6, 53.9, 52.7, 51.0, 50.4, 48.9, 42.7, 40.7, 40.4, 32.3, 31.2; ESI/APCI Calcd for $C_{27}H_{52}N_{10}O_{12}Na$ ($[M+Na]^+$) m/e 731.3658; found m/e 731.3693.

Compound 4f

^{1}H NMR (D_2O, 400 MHz) δ 8.18 (s, 1H), 5.90 (d, *J* = 3.2 Hz, 1H), 5.31 (s, 1H), 5.21 (s, 1H), 4.53 (m, 1H), 4.46 (d, *J* = 8.9 Hz, 2H), 4.27 (m, 1H), 4.1 (m, 2H), 3.8 – 3.9 (m, 5H), 3.1 – 3.6 (m, 14H), 2.20 (m, 2H), 1.9 (m, 3H), 1.6 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 144.4, 124.9, 110.4, 95.6, 95.3, 85.4, 82.0, 79.7, 77.0, 76.9, 75.2, 72.9, 71.0, 70.5, 69.3, 69.0, 67.9, 67.6, 53.8, 52.0, 51.0, 50.4, 48.9, 45.1, 40.7, 40.4, 30.5, 28.3, 21.7, 21.6; ESI/APCI Calcd for $C_{30}H_{57}N_{10}O_{12}$ ($[M+H]^+$) m/e 749.4152; found m/e 749.4165.

Compound 4g

^{1}H NMR (D_2O, 400 MHz) δ 8.19 (s, 1H), 6.04 (d, *J* = 3.9 Hz, 1H), 5.36 (d, *J* = 3.1 Hz, 1H), 5.26 (d, *J* = 1.6 Hz, 1H), 4.53 (t, *J* = 5.2 Hz, 1H), 4.39 (m, 1H), 4.26 (m, 3H), 4.0 – 4.1 (m, 2H), 3.9 – 4.0 (m, 4H), 3.7 – 3.8 (m, 2H), 3.2 – 3.5 (m, 10H), 2.42 (m, 1H), 1.97 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 140.3, 126.1, 110.2, 95.5, 95.1, 85.0, 80.0, 76.8, 75.0, 73.0, 72.5, 70.6, 70.4, 70.0, 68.2, 67.8, 67.5, 53.4, 52.7, 51.0, 49.9, 48.6, 40.7, 40.3, 34.2, 28.1; ESI/APCI Calcd for $C_{26}H_{50}N_{10}O_{12}$ ($[M+Na]^+$) m/e 717.3502; found m/e 717.3525.

Compound 4h

^{1}H NMR (D_2O, 400 MHz) δ 7.96 (s, 1H), 5.90 (d, *J* = 3.8 Hz, 1H), 5.31 (d, *J* = 3.3 Hz, 1H), 5.24 (d, *J* = 1.6 Hz, 1H), 4.48 (m, 2H), 4.39 (s, 2H), 4.26 (t, *J* = 4.6 Hz, 1H), 4.14 (t, *J* = 3.1 Hz, 1H), 3.8 – 3.9 (m, 5H), 3.74 (m, 1H), 3.6 (dd, *J* = 9.0 Hz, *J* = 7.0 Hz, 1H), 3.51 (m, 1H), 3.2 – 3.4 (m, 12H), 2.96 (td, *J* = 12.9 Hz, *J* = 3.0 Hz, 2H), 2.6 (m, 1H), 23 (dt, *J* = 8.6 Hz, *J* = 4.2 Hz, 1H), 2.0 (m, 2H), 1.7 – 1.8 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 176.5, 145.2, 124.6, 110.2, 95.6, 95.2, 85.2, 79.9, 76.9, 76.8, 73.0, 72.8, 70.8, 70.4, 69.5, 68.6, 67.8, 67.5, 53.7, 52.3, 51.0, 50.1, 48.7, 43.3 (2 carbons), 40.7, 40.3, 39.8, 34.6, 29.0, 25.2 (2 carbons); ESI/APCI Calcd for $C_{32}H_{59}N_{11}O_{13}Na$ ($[M+Na]^+$) m/e 828.4186; found m/e 828.4170.

Compound 4i

^{1}H NMR (D_2O, 400 MHz) δ 8.01 (s, 1H), 5.98 (d, *J* = 4.0 Hz, 1H), 5.34(d, *J* = 3.5 Hz, 1H), 5.24 (s, 1H), 4.4 – 4.5 (m, 4H), 4.31 (t, *J* = 2.9 Hz, 1H), 4.26 (t, *J* = 5.4 Hz, 1H), 4.14 (t, *J* = 3.0 Hz, 1H), 3.97 (t, *J* = 9.0 Hz, 1H), 3.95 (dd, *J* = 10.5 Hz, *J* = 9.0 Hz, 1H), 3.9 (m, 3H), 3.8 (s, 1H), 3.64 (t, *J* = 10 Hz, 1H), 3.2 – 3.5 (m, 13H), 2.4 (m, 2H), 2.0 (m, 3H), 1.84 (dd, *J* = 14.0 Hz, *J* = 12.0 Hz. 1H); ^{13}C NMR (D_2O, 100 MHz) δ 169.8, 144.6, 124.7, 110.1, 95.7, 95.1, 84.9, 80.0, 77.0, 75.1, 73.1, 72.5, 70.6, 70.4, 69.8, 68.2, 67.8, 67.5, 59.9, 53.4, 52.3, 50.9, 49.8, 48.6, 46.7, 40.7, 40.2, 34.8, 29.8, 28.1, 24.0; ESI/APCI Calcd for $C_{31}H_{58}N_{11}O_{13}$ ($[M+H]^+$) m/e 792.4210; found m/e 792.4236.

Compound 5a

^{1}H NMR (D_2O, 400 MHz) δ5.80 (d, *J* = 3.8 Hz, 1H), 5.30 (d, *J* = 10.7 Hz, 1H), 5.19 (d, *J* = 1.3 Hz. 1H), 4.33 (t, *J* = 4.9 Hz, 1H), 4.22 (t, *J* = 5.0 Hz, 1H), 4.16 (t, *J* = 2.9 Hz, 1H), 3.8 – 3.9 (m, 4H), 3.7 (m, 1H), 3.65 (t, *J* = 9.6 Hz, 1H), 3.55 (dd, *J* = 12.8 Hz, *J* = 3.8 Hz, 1H), 3.5 (m, 1H), 3.2 – 3.4 (m, 10H), 2.61 (m, 1H), 2.17 (t, *J* = 7.2 Hz, 2H), 1.5 (m, 1H), 1.2 (m, 2H), 1.2 (m, 8 H), 0.74 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) 178.0, 109.3, 95.9, 95.2, 85.2, 80.8, 77.5, 76.5, 73.6, 72.7, 70.7, 70.3, 69.3, 68.5, 67.8, 67.5, 53.6, 51.0, 49.9, 48.8, 41.5, 40.7, 40.3, 36.1, 30.9, 27.8, 28.2, 25.6, 22.1, 13.5; ESI/APCI Calcd for $C_{30}H_{60}N_7O_{13}$ ($[M+H]^+$) m/e 726.4244; found m/e 726.4226.

Compound 5b

^{1}H NMR (D_2O, 400 MHz) δ 5.72 (d, *J* = 3.6 Hz, 1H), 5.28 (d, *J* = 4.1 Hz, 1H), 5.18 (d, *J* = 1.4 Hz, 1H), 4.31 (t, *J* = 3.6 Hz, 1H), 4.22 (t, *J* = 4.0 Hz, 1H), 4.17 (t, *J* = 4.9 Hz, 2H), 4.12 (t, *J* = 3.0 Hz, 1H), 3.95 (m, 1H), 3.7 – 3.8 (m, 3H), 3.4 – 3.6 (m, 3H), 3.3 – 3.4 (m, 6H), 3.2 (m,

3H), 2.2 (m, 3H), 1.6 (m, 1H), 1.48 (m, 2H), 1.2 (m, 26H), 0.74 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 178.1, 109.4, 96.0, 95.5, 85.4, 80.6, 77.5, 73.6, 73.0, 70.9, 70.3, 69.4, 69.1, 67.8, 67.6, 53.9, 51.0, 50.2, 49.0, 41.2, 40.7, 40.3, 36.1, 31.4, 28.5 – 29.0 (12 carbons), 25.6, 22.2, 13.6; ESI/APCI Calcd for $C_{39}H_{78}N_7O_{13}$ ($[M+H]^+$) m/e 852.5652; found m/e 852.5633.

Compound 5c

^{1}H NMR (D_2O, 400 MHz) δ 5.75 (d, *J* = 3.3 Hz, 1H), 5.31 (s, 1H), 5.17 (s, 1H), 4.35 (t, *J* = 4.6 Hz, 1H), 4.1 – 4.2 (m, 4H), 3.95 (m, 1H), 3.8 -3.9 (m, 3H), 3.62 (t, *J* = 8.8 Hz, 1H), 3.5 (m, 3H), 3.1 – 3.4 (m, 8H), 2.2 (m, 3H), 1.6 (m, 1H), 1.49 (m, 2H), 1.16 (m, 30H), 0.76 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 177.6, 109.4, 96.0, 95.6, 85.1, 80.3, 77.6, 73.4, 72.9, 71.1, 70.3, 69.4, 67.9, 67.7, 54.0, 51.1, 50.3, 49.0, 41.5, 40.7, 40.4, 39.0, 36.2, 31.7, 28.9 – 29.9 (14 carbons), 25.8, 22.5, 13.9; ESI/APCI Calcd for $C_{41}H_{82}N_7O_{13}$ ($[M+H]^+$) m/e 880.5965; found m/e 880.5959.

Compound 5d

^{1}H NMR (D_2O, 400 MHz) δ 8.13 (t, *J* = 8.8 Hz, 2H), 7.98 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), 7.95 (s, 1H), 7.87 (t, *J* = 8.4 Hz,1H), 7.70 (t, *J* = 7.2 Hz, 1H), 7.6 (m, 3H), 7.4 (m, 1H), 5.71 (m, 1H), 5.36 (d, *J* = 3.7 Hz, 1H), 5.2 (m, 1H), 4.55 (t, *J* = 5.3 Hz, 1H), 4.3 (m, 3H), 4.2 (m, 1H), 4.1 (m, 1H), 4.0 (m, 2H), 3.7 – 3.9 (m, 3H), 3.6 (m, 3H), 3.5 (m, 2H), 3.3 – 3.4 (m, 2H), 3.1 - 3.3 (m, 2H), 3.0 - 3.1 (m, 2H), 2.8 (m, 1H), 2.22 (m, 1H), 1.6 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 170.6, 158.1, 147.0, 142.8, 137.0, 131.6, 130.6, 129.5 (2 carbons), 128.6, 128.4, 128.0 (2 carbons), 124.9, 123.0, 118.7, 109.8, 95.8, 95.5 (2 carbons), 85.3, 80.3, 77.8, 73.6, 73.2, 70.8, 70.4, 69.2, 67.6, 67.2, 66.0, 53.9, 51.1, 50.3, 49.0, 41.5, 40.4, 40.2, 32.0; ESI/APCI Calcd for $C_{41}H_{82}N_7O_{13}$ ($[M+H]^+$) m/e 845.4040; found m/e 845.4021.

Compound 5e

^{1}H NMR (D_2O, 400 MHz) δ 5.80 (s, 1H), 5.31 (d, *J* = 3.3 Hz, 1H), 5.22 (s, 1H), 4.40 (t, *J* = 5.2 Hz, 1H), 4.2 – 4.3 (m, 6H), 4.13 (t, *J* = 3.1 Hz, 1H), 3.8 – 3.9 (m, 5H), 3.74 (s, 1H), 3.65 (t, *J* = 9.7 Hz, 1H), 3.5 (m, 4H), 3.2 – 3.4 (m, 4H), 3.07 (t, *J* = 7.2 Hz, 2H), 2.3 (m, 1H), 2.1 (m, 1H), 1.9 (m, 1H), 1.7 (m, 1H); ^{13}C (D_2O, 100 MHz) δ 176.1, 109.5, 95.8, 95.3, 85.0, 80.4, 77.4, 73.5, 72.7, 70.8, 70.3, 69.7, 69.5, 69.0, 67.9, 67.6, 53.8, 51.0, 50.2, 48.9, 41.3, 40.7, 40.3, 36.9, 31.2, 30.1, 30.0; ESI/APCI Calcd for $C_{27}H_{54}N_8O_{14}Na$ ($[M+Na]^+$) m/e 737.3652; found m/e 737.3682.

Compound 5f

^{1}H NMR (D_2O, 400 MHz) δ 5.88 (d, *J* = 3.9 Hz, 1H), 5.32 (d, *J* = 3.5 Hz, 1H), 5.21 (d, *J* = 1.5 Hz, 1H), 4.35 (t, *J* = 5.4 Hz, 1H), 4.26 (t, *J* = 5.3 Hz, 1H), 4.22 (d, *J* = 3.7 Hz, 1H), 4.1 (m, 2H), 4.03 (t, *J* = 9.0 Hz, 1H), 3.9 (m, 2H), 3.78 (s, 1H), 3.74 (m, 2H), 3.71 (t, *J* = 9.4 Hz, 1H), 3.6 (m, 1H), 3.2 – 3.5 (m, 11H), 2.35 (m, 1H), 1.80 (dd, *J* = 24.0 Hz, *J* = 14.6 Hz, 1H); ^{13}C (D_2O, 100 MHz) δ 170.0, 109.2, 95.9, 95.6, 84.8, 80.6, 78.3, 77.0, 73.3, 72.8, 70.8, ,70.3, 69.5, 69.1, 67.9, 67.6, 53.8, 51.0, 50.3, 49.0, 41.8, 40.7 (2 carbons), 40.3, 30.0; ESI/APCI Calcd for $C_{25}H_{51}N_8O_{13}$ ($[M+H]^+$) m/e 671.3570; found m/e 671.3591.

Compound 5g

^{1}H NMR (D_2O, 400 MHz) δ 5.88 (d, *J* = 3.9 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 1H), 5.22 (s, 1H), 4.31 (t, *J* = 5.3 Hz, 1H), 4.26 (t, *J* = 2.0 Hz, 1H), 4.18 (t, *J* = 4.9 Hz, 2H), 4.13 (t, *J* = 3.0 Hz, 1H), 4.0 – 4.1 (m, 2H), 3.9 – 4.0 (m, 3H), 3.74 (dd, *J* = 3.1 Hz, *J* = 1.5 Hz, 1H), 7.72 (d, *J* = 9.7 Hz, 1H), 3.2 – 3.6 (m, 11H), 2.36 (dt, *J* = 8.5 Hz, *J* = 4.2 Hz, 1H), 1.81 (m, 1H), 1.45 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 171.6, 108.8, 95.9, 95.2, 84.6, 80.9, 77.0, 73.4, 72.4, 70.6, 70.3, 69.8, 68.6, 67.8, 67.7, 53.5, 51.0, 50.0, 49.3, 48.8, 41.7, 40.8, 40.2, 27.0, 16.9; ESI/APCI Calcd for $C_{25}H_{53}N_8O_{13}$ ($[M+H]^+$) m/e 685.3727; found m/e 685.3735.

Compound 5h

^{1}H NMR (D_2O, 400 MHz) δ 5.91 (d, *J* = 4.0 Hz, 1H), 5.35 (d, *J* = 3.9 Hz, 1H), 5.21 (d, *J* = 1.7 Hz, 1H), 4.30 (t, *J* = 9.2 Hz, 1H), 4.31 (d, *J* = 10.8 Hz, 1H), 4.3 (m, 1H), 4.1 – 4.2 (m, 2H), 3.9 – 4.0 (m, 3H), 3.7 (m, 2H), 3.3 – 3.6 (m, 15H), 2.4 (m, 2H), 2.0 (m, 3H), 1.86 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 170.3, 108.8, 95.9, 95.2, 84.5, 81.0, 77.1, 75.2, 73.4, 72.3, 70.5, 70.2, 70.0, 68.4, 67.8, 67.7, 60.1, 53.3, 50.9, 49.8, 48.7, 46.6, 41.8, 40.7, 40.2, 29.9, 28.0, 24.1; ESI/APCI Calcd for $C_{28}H_{54}N_8O_{13}Na$ ([M+Na]$^+$) m/e 733.3703; found m/e 733.3725.

Compound 5i

^{1}H NMR (D_2O, 400 MHz) δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.26 (s, 1H), 7.17 (t, *J* = 7.1 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 5.66 (d, *J* = 3.7 Hz, 1H), 5.19 (d, *J* = 3.4 Hz, 1H), 4.94 (s, 1H), 4.26 (t, *J* = 6.9 Hz, 2H), 4.1 (m, 2H), 3.6 – 4.0 (m, 8H), 3.1 – 3.5 (m, 12H), 2.22 (dt, *J* = 8.8 Hz, *J* = 4.6 Hz, 1H), 1.6 (m, 1H), 1.2 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 170.7, 136.3, 126.8, 125.3, 122.5, 119.8, 118.4, 112.4, 108.5, 106.7, 96.0, 95.9, 84.7, 80.6, 78.0, 76.7, 70.9, 71.3, 70.0, 69.4, 69.3, 67.8, 67.5, 53.9, 53.8, 51.2, 50.8, 50.3, 49.1, 41.2, 40.7, 40.3, 30.2, 27.1; ESI/APCI Calcd for $C_{34}H_{58}N_9O_{13}$ ([M+H]$^+$) m/e 800.4149; found m/e 800.4139.

Compound 5j

^{1}H NMR (D_2O, 400 MHz) δ 5.95 (d, *J* = 3.9 Hz, 1H), 5.35 (d, *J* = 3.1 Hz, 1H), 4.38 (t, *J* = 5.4 Hz, 1H), 4.26 (t, *J* = 3.2 Hz, 2H), 4.1 – 4.2 (m, 4H), 3.9 – 4.0 (m, 5H), 3.6 – 3.8 (m, 3H), 3.2 – 3.5 (m, 11H), 2.42(dt, *J* = 8.5 Hz, *J* = 4.3 Hz, 1H), 1.86 (q, *J* = 12.6 Hz, 1.H); ^{13}C NMR (D_2O, 100 MHz) δ 168.8, 109.3, 95.6, 95.2, 84.6, 80.3, 76.7, 75.1, 73.3, 72.4, 70.6, 70.3, 69.9, 68.3, 67.8, 67.6, 60.5, 54.5, 53.3, 51.0, 49.9, 48.7, 41.8, 40.7, 40.2, 28.1; ESI/APCI Calcd for $C_{26}H_{53}N_8O_{14}$ ([M+H]$^+$) m/e 701.3676; found m/e 701.3695.

Compound 5k

^{1}H NMR (D_2O, 400 MHz) δ 5.75 (d, *J* = 3.5 Hz, 1H), 5.32 (d, *J* = 4.1 Hz, 1H), 5.21 (d, *J* = 1.5 Hz, 1H), 4.30 (t, *J* = 5.3 Hz, 1H), 4.26 (t, *J* = 3.7 Hz, 1H), 4.1 – 4.2 (m, 3H), 3.9 – 4.0 (m, 2H), 3.8 – 3.9 (m, 3H), 3.7 (m, 1H), 3.6 – 3.7 (m, 2H), 3.2 – 3.5 (m, 11H), 2.9 (t, *J* = 7.7 Hz, 1H), 2.2 (m, 1H), 1.8 (m, 2H), 1.6 (m, 3H), 1.38 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 170.9, 109.0, 96.0, 95.9, 84.8, 80.9, 77.1, 73.2, 72.8, 70.8, 70.2, 69.5, 69.1, 67.8, 67.7, 53.9, 53.3, 51.0, 50.2, 49.0, 42.0, 40.8, 40.3, 39.2 (2 carbons), 30.8, 30.0, 26.6, 21.7; MALDI Calcd for $C_{29}H_{59}N_9O_{13}Na$ ([M+Na]$^+$) m/e 764.4125; found m/e 764.4163.

Compound 5l

This compound was prepared by subjecting compound **32** to hydrogenation and sequential purification. ^{1}H NMR (D_2O, 300 MHz) δ 5.85 (d, *J* = 4.2 Hz, 1H), 5.31 (d, *J* = 4.1 Hz, 1H), 5.20 (d, *J* = 1.4 Hz, 1H), 4.35 (t, *J* = 4.8 Hz, 1H), 4.1 – 4.2 (m, 4H), 3.8 – 4.0 (m, 4H), 3.7 (m, 2H), 3.2 – 3.6 (m, 11H), 2.4 – 2.6 (m, 5H), 1.90 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 177.4, 175.6, 84.8, 80.8, 77.2, 75.3, 75.1, 73.5, 72.2 (2 carbons), 70.4, 70.2, 69.8 (2 carbons), 68.1, 67.7, 67.4, 53.3, 50.8, 49.7, 48.6, 41.0, 40.6, 40.1, 30.4, 29.5, 28.0; MALDI Calcd for $C_{21}H_{57}N_7O_{15}Na$ ([M+Na]$^+$) m/e 736.3335; found m/e 736.3331.

Compound 5m

^{1}H NMR (D_2O, 300 MHz) δ 5.87 (d, *J* = 7.9 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 1H), 5.20 (s, 1H), 4.40 (t, *J* = 5.5 Hz, 1H), 3.8 – 4.3 (m, 10H), 3.7 (m, 2H), 3.2 – 3.7 (m, 10H), 2.41 (dt, *J* = 8.6 Hz, *J* = 4.1 Hz, 1H), 1.90 (m, 1H), 1.46 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) δ 171.5, 108.8, 95.7, 95.2, 84.5, 80.9, 76.8, 75.2, 73.4, 72.2, 70.5, 70.2, 69.8, 68.2, 67.7, 67.5,

53.3, 50.9, 49.7, 49.2, 48.6, 40.6, 40.2, 28.0, 16.9; MALDI Calcd for $C_{26}H_{52}N_8O_{13}Na$ ([M +Na]$^+$) m/e 707.3546; found m/e 707.3518.

Compound 5n

^{1}H NMR (D_2O, 300 MHz) δ 5.69 (d, *J* = 3.8 Hz, 1H), 5.28 (d, *J* = 5.0 Hz, 1H), 5.20 (s, 1H), 4.33 (t, *J* = 5.1 Hz, 1H), 4.1 – 4.2 (m, 5H), 3.6- 3.8 (m, 9H), 3.1 – 3.5 (m, 10H), 2.19 (dt, *J* = 11.0 Hz, *J* = 4.5 Hz, 1H), 1.55 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 170.2, 109.0, 95.9, 84.8, 80.4, 79.1, 77.0, 73.2, 72.9, 70.9, 70.2, 69.4, 69.2, 67.8, 67.6, 61.1, 54.9, 53.9, 52.0, 50.9, 50.3, 49.0, 41.5, 40.6, 40.3, 30.7; MALDI Calcd for $C_{26}H_{52}N_8O_{14}Na$ ([M+Na]$^+$) m/e 723.3495; found m/e 723.3494.

Compound 5o

^{1}H NMR (D_2O, 400 MHz) δ 5.86 (d, *J* = 3.9 Hz, 1H), 5.33 (d, *J* = 3.6 Hz, 1H), 5.22 (s, 1H), 4.3 – 4.4 (m, 2H), 4.3 (m, 1H), 4.1 – 4.2 (m, 4H), 3.9 – 4.0 (m, 3H), 3.2 – 4.0 (m, 14H), 2.4 (M, 2H), 2.0 (m, 4H), 1.9 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 170.4, 109.1, 95.8, 95.3, 84.7, 80.9, 77.0, 75.3, 73.5, 72.4, 70.7, 70.3, 70.0, 68.3, 67.8, 67.6, 60.0, 53.4, 51.0, 49.8, 48.7, 46.8, 41.8, 40.7, 40.4, 30.2, 28.1, 24.1; ESI/APCI Calcd for $C_{28}H_{55}N_8O_{13}$ ([M+H]$^+$) m/e 711.3883; found m/e 711.3873.

Compound 5p

^{1}H NMR (D_2O, 400 MHz) δ 5.90 (d, *J* = 4.0 Hz, 1H), 5.34 (d, *J* = 3.4 Hz, 1H), 5.21 (d, *J* = 1.5 Hz, 1H), 4.39 (t, *J* = 6.5 Hz, 1H), 4.26 (m, 1H), 4.1 – 4.3 (m, 4H), 3.9 – 4.0 (m, 4H), 3.7 (m, 2H), 3.2 – 3.6 (m, 11H), 2.91 (t, *J* = 7.6 Hz, 2H), 2.41 (dt, *J* = 8.4 Hz, *J* = 4.0 Hz, 1H), 1.8 – 2.0 (m, 3H), 1.6 – 1.7 (m, 2H), 1.4 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 170.6, 109.1, 95.6, 95.3, 84.6, 80.8, 76.6, 75.2, 73.3, 72.4, 70.7, 70.4, 69.9, 68.4, 67.8, 67.6, 53.4, 53.3, 51.0, 49.9, 48.7, 41.7, 40.8, 40.3, 39.2, 30.7, 28.1, 26.6, 21.7; ESI/APCI Calcd for $C_{29}H_{59}N_9O_{13}Na$ ([M +Na]$^+$) m/e 764.4125; found m/e 764.4116.

Compound 33

To a solution of compound **3** (0.20 g, 0.14 mmol) in anhydrous DMF (5 mL), succinic anhydride (0.02 g, 0.20 mmol) was added. After being stirred overnight at room temperature overnight, the reaction mixture was concentrated. After removal of the solvent followed by a fast gradient column chromatography (eluted from CH_2Cl_2/MeOH = 100/0 to 80/20), the product, **32** was obtained as a solid and subjected to the next step without further purification. The acid crude product was re-dissolve in DMF (8 mL) and added with compound **3** (0.20 g, 0.14 mmol), Et_3N (0.04 mL, 0.28 mmol), HOBt (0.030 g, 0.21 mmol) and EDC (0.040 g, 0.21 mmol). After being stirred overnight at room temperature overnight, the reaction mixture was concentrated and diluted with EtOAc. The organic solution was washed with saturated $NaHCO_{3(aq)}$, water, brine and dried over anhydrous Na_2SO_4. After removal of the solvent followed by a fast gradient column chromatography (eluted from CH_2Cl_2/MeOH = 100/0 to 80/20), the product was obtained as a solid subjected to hydrogenation without further purification. ^{1}H NMR (D_2O, 400 MHz) δ 5.94 (d, *J* = 3.8 Hz, 2H), 5.37 (d, *J* = 3.5 Hz, 2H), 5.22 (s, 2H), 4.40 (t, *J* = 5.2 Hz, 2H), 4.2 – 4.3 (m, 8H), 3.9 (m, 6H), 3.8 (m, 6H), 3.2 – 3.6 (m, 22H), 2.5 (s, 4H), 2.43 (dt, *J* = 8.8 Hz, *J* = 4.5 Hz, 2H), 1.87 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 175.5 (2 carbons), 109.4 (2 carbons), 95.7 (2 carbons), 95.0 (2 carbons), 84.7 (2 carbons), 80.5 (2 carbons), 77.3 (2 carbons), 75.2 (2 carbons), 73.4 (2 carbons), 72.4 (2 carbons), 70.6 (2 carbons), 70.4 (2 carbons), 69.9 (2 carbons), 68.3 (2 carbons), 67.8 (2 carbons), 67.5 (2 carbons), 53.5 (2 carbons), 51.0 (2 carbons), 49.9 (2 carbons), 48.8 (2 carbons), 41.8 (2 carbons), 40.8 (2 carbons), 40.3 (2 carbons), 31.1 (2 carbons), 28.1 (2 carbons); ESI/APCI Calcd for $C_{50}H_{97}N_{14}O_{26}$ ([M+H]$^+$) m/e 1309.6693; found m/e 1309.6684.

Compound 5q

^{1}H NMR (D_2O, 400 MHz) δ 5.95 (d, *J* = 3.9 Hz, 1H), 5.33 (d, *J* = 3.2 Hz, 1H), 5.20 (s, 1H), 4.42 (t, *J* = 5.2 Hz, 1H), 4.28 (m, 2H), 4.14 (m, 3H), 3.8 – 4.0 (m, 7H), 3.7 (m, 2H), 3.3 – 3.5 (m, 11H), 2.42 (dt, *J* = 12.4 Hz, *J* = 4.0 Hz, 1H), 1.91 (dd, *J* = 12.2 Hz, *J* = 3.6 Hz, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 172.0, 168.2, 109.7, 95.6, 94.8, 84.8, 80.2, 77.5, 75.1, 73.4, 72.4, 70.6, 70.4, 69.8, 68.3, 67.8, 67.4, 53.5, 51.0, 49.9, 48.8, 42.9, 41.9, 40.8, 40.7, 40.2, 28.2; ESI/APCI Calcd for $C_{27}H_{54}N_9O_{14}$ ($[M+H]^+$) m/e 728.3785; found m/e 728.3780.

Procedure for MIC determination

A solution of selected bacteria was inoculated in the Trypticase Soy broth at 35°C for 1 - 2hrs. After which, the bacteria concentration was found, and diluted with broth, if necessary, to an absorption value of 0.08 to 0.1 at 625 nm. The adjusted inoculated medium (100 μL) was diluted with 10 mL broth, and then applied to a 96-well microtilter 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 hrs. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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a Abbreviations

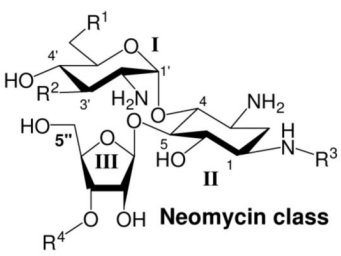
SAR, Structural activity relationship
AME, aminoglycoside-modifying enzyme
MRSA, Methicillin-resistant *Staphylococcus aureus*
VRE, vancomycin-resistant enterococci
AHB, (*S*)-4-amino-2-hydroxybutanoyl
APH, aminoglycoside phosphotransferases
ANT, aminoglycoside nucleotidyltransferases
Cbz or Z, carbobenzyloxy
AAC, aminoglycoside acetyltransferase
MIC, minimum inhibitory concentration
rRNA, ribosomal RNA
EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt, 1-Hydroxybenzotriazole

References

1. Umezawa, S.; Tsuchiya, T. Aminoglycoside Antibiotics. Umezawa, H.; Hooper, IR., editors. Springer-Verlag; New York: 1982. p. 37-110.
2. Haddad, J.; Kotra, LP.; Mobashery, S. Glycochemistry Principles, Synthesis, and Applications. Wang, PG.; Bertozzi, CR., editors. Marcel Dekker, Inc.; New York/Basel: 2001. p. 353-424.
3. Wang, J.; Chang, C-WT. Aminoglycoside Antibiotics. Arya, DP., editor. John Wiley & Sons, Inc.; 2007. p. 141-180.

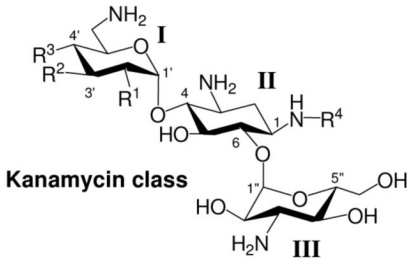
4. Wang J, Li J, Chen H-N, Chang H, Tanifum CT, Liu H-H, Czyryca PG, Chang C-WT. Glycodiversification for optimization of the kanamycin class aminoglycosides. J. Med. Chem 2005;48:6271–6285. [PubMed: 16190754]
5. Li J, Chiang F-I, Chen H-N, Chang C-WT. Investigation of the regioselectivity for Staudinger reaction and its application for the synthesis of aminoglycosides with N-1 modification. J. Org. Chem 2007;72:4055–4066. [PubMed: 17465564]
6. Asensio JL, Hidalgo A, Bastida A, Torrado M, Corzana F, Chiara JL, Garcia-Junceda E, Canada J, Jimenez-Barbero J. A simple structural-based approach to prevent aminoglycoside inactivation by bacterial defense proteins. conformational restriction provides effective protection against neomycin-B nucleotidylation by ANT4. J. Am. Chem. Soc 2005;127:8278–8279. [PubMed: 15941249]
7. Blount KF, Zhao F, Hermann T, Tor Y. Conformational constraint as a means for understanding RNA-aminoglycoside specificity. J. Am. Chem. Soc 2005;127:9818–9829. [PubMed: 15998086]
8. Kling D, Hesek D, Shi Q, Mobashery S. Design and synthesis of a structurally constrained aminoglycoside. J. Org. Chem 2007;72:5450–5453. [PubMed: 17579461]
9. Luedtke NW, Liu Q, Tor Y. RNA-ligand interactions: affinity and specificity of aminoglycoside dimers and acridine conjugates to the HIV-1 Rev response element. Biochemistry 2003;42:11391–11403. [PubMed: 14516190]
10. Boer J, Blount KF, Luedtke NW, Elson-Schwab L, Tor Y. RNA-Selective Modification by a Platinum (II) Complex Conjugated to Amino- and Guanidinoglycosides. Angew. Chem. Int. Ed 2005;44:927–932.
11. Quader S, Boyd SE, Jenkins ID, Houston TA. Multisite modification of neomycin B: combined Mitsunobu and click chemistry approach. J. Org. Chem 2007;72:1962–1979. [PubMed: 17298096]
12. Fujisawa K, Hashiya T, Kawaguchi H. Aminoglycoside antibiotics. VII. Acute toxicity. of aminoglycoside antibiotics. J. Antibiot 1974;27:677–681. [PubMed: 4436153]
13. Hainrichson M, Pokrovskaya V, Shallom-Shezifi D, Fridman M, Belakhov V, Shachar D, Yaronb S, Baasova T. Branched aminoglycosides: Biochemical studies and antibacterial activity of neomycin B derivatives. Bioorg. Med. Chem 2005;13:5797–5807. [PubMed: 15993084]
14. Fridman M, Belakhov V, Yaron S, Baasov T. A new class of branched aminoglycosides: pseudo-pentasaccharide derivatives of neomycin B. Org. Lett 2003;5:3575–3578. [PubMed: 14507176]
15. Huisgen, R. 1,3-Dipolar Cycloaddition Chemistry. Padwa, A., editor. Wiley; New York: 1984. p. 1-176.
16. Mingeot-Leclercq M-P, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. Antimicrob. Agents Chemother 1997;43:727–737. [PubMed: 10103173]
17. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspective on mechanisms of action and resistance and strategies to counter resistance. Antimicrob. Agents Chemother 2000;44:3249–3256. [PubMed: 11083623]
18. Wright GD. Aminoglycoside-modifying enzymes. Curr. Opin. Microbiol 1999;2:499–503. [PubMed: 10508725]
19. Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. Clin. Microbiol. Rev 2003;16:430–450. [PubMed: 12857776]
20. Ida T, Okamoto R, Shimauchi C, Okubo T, Kuga A, Inoue M. Identification of aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. J. Clin. Microbiol 2001;39:3115–3121. [PubMed: 11526138]
21. Schmitz FJ, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, Verhoef J, Heinz HP, Jones ME. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J. Antimicrob. Chemother 1999;43:253–259. [PubMed: 11252331]
22. Udo EE, Dashti AA. Detection of genes encoding aminoglycoside-modifying enzymes in staphylococci by polymerase chain reaction and dot blot hybridization. Int. J. Antimicrob. Agents 2000;13:273–279. [PubMed: 10755241]
23. Werner G, Hildebrandt B, Witte W. Aminoglycoside-streptothricin resistance gene cluster aadE-sat4-aphA-3 disseminated among multiresistant isolates of *Enterococcus faecium*. Antimicrob. Agents Chemother 2001;45:3267–3269. [PubMed: 11600397]

24. McKay GA, Thompson PR, Wright GD. Broad spectrum aminoglycoside phosphotransferase Type III from Enterococcus: overexpression, purification, and substrate specificity. Biochemistry 1994:6936–6944. [PubMed: 8204627]
25. McKay GA, Wright GD. Kinetic mechanism of aminoglycoside phosphotransferase type IIIa. J. Biol. Chem 1995:24686–24692. [PubMed: 7559583]
26. Russell RJM, Murray JB, Lentzen G, Haddad J, Mobashery S. The complex of a designer antibiotic with a model aminoacyl site of the 30S ribosomal subunit revealed by X-ray crystallography. J. Am. Chem. Soc 2003;125:3410–3411. [PubMed: 12643685]
27. Structures of 5″-substituted neomycins were docked to APH(3′)-IIIa and 16S ribosomal RNA. Models of the molecules of derivatized neomycins were prepared using HyperChem 7.5. Atomic charges were calculated using the AM1 semi-empirical method *in vacuo*. The conformations were based on the X-ray structures of complexes of neomycin with respective molecules. Orientations of the 5″ chains were adjusted manually and the structures were subject to geometry optimization (ligand molecules only). Then, the structures of the complexes were refined, by using AutoDock 4 as local minimum optimizer (Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. J. Comput. Chem 1998;19:1639–1662.). Visualizations were prepared using AutoDock Tools (Sanner MF. J. Mol. Graphics Mod 1999;17:57–61.).
28. Francois B, Russell RJ, Murray JB, Aboul-ela F, Masquida B, Vicens Q, Westhof E. Crystal structures of complexes between aminoglycosides and decoding A site oligonucleotides: role of the number of rings and positive charges in the specific binding leading to miscoding. Nucleic Acids Res 2005;33:5677–5690. [PubMed: 16214802]
29. Fong DH, Berghuis AM. Substrate promiscuity of an aminoglycoside antibiotic resistance enzyme via target mimicry. EMBO J 2002;21:2323–2331. [PubMed: 12006485]
30. Rasheed JK, Anderson GJ, Yigit H, Queenan AM, Domenech-Sanchez A, Swenson JM, Biddle JW, Ferraro MJ, Jacoby GA, Tenover FC. Characterization of the extended-spectrum β-lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC 700603), which produces the novel enzyme SHV-18. Antimicrob. Agents Chemother 2000;44:2382–2388. [PubMed: 10952583]
31. Hachiler H, Santanam P, Kayser FH. Sequence and characterization of a novel chromosomal aminoglycoside phosphotransferase gene, aph (3′)-IIb, in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother 1996;40:1254–1256. [PubMed: 8723476]
32. Fridkin SK, Lawton R, Edwards JR, Tenover FC, McGowan JE Jr. Gaynes RP. Monitoring antimicrobial use and resistance: comparison with a national benchmark on reducing vancomycin use and vancomycin-resistant enterococci. Emerg. Infect. Dis 2002;8:702–707. [PubMed: 12095438]
33. Swenson JM, Clark NC, Sahm DF, Ferraro MJ, Doern G, Hindler J, Jorgensen JH, Pfaller MA, Reller LB, Weinstein MP, Zabransky RJ, Tenover FC. Molecular characterization and multilaboratory evaluation of *Enterococcus faecalis ATCC 51299* for quality control of screening tests for vancomycin and high-level aminoglycoside resistance in Enterococci. J. Clin. Microbiol 1995;33:3019–3021. [PubMed: 8576364]
34. Fosso, M.; Zhang, J.; Wang, J.; Chang, C-WT. Unpublished results
35. Zhang J, Chen H-N, Chiang F-I, Takemoto JY, Bensaci M, Chang C-WT. Sonication-assisted library synthesis of oxazolidinone-carbohydrate conjugates. J. Comb. Chem 2007;9:17–19. [PubMed: 17206828]
36. Sajiki H, Hattori K, Hirota K. The formation of a novel Pd/C-ethylenediamine complex catalyst: chemoselective hydrogenation without deprotection of the *O*-benzyl and *N*-cbz groups. J. Org. Chem 1998;63:7990–7992.
37. Li J, Chen H-N, Chang H, Wang J, Chang C-WT. Tuning the regioselectivity of Staudinger reaction for the facile synthesis of kanamycin and neomycin class antibiotics with *N*-1 modification. Org. Lett 2005;7:3061–3064. [PubMed: 15987205]



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

DAG: 2,6-diamino-2,6-dideoxy–β-L-glucopyranosyl



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

AHB: (S)-4-amino-2-hydroxybutyryl

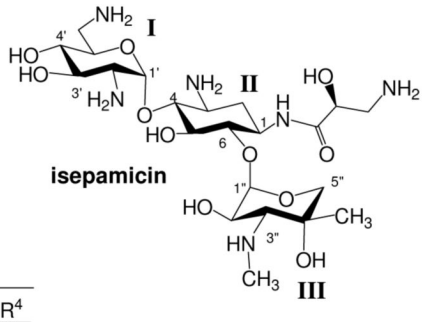
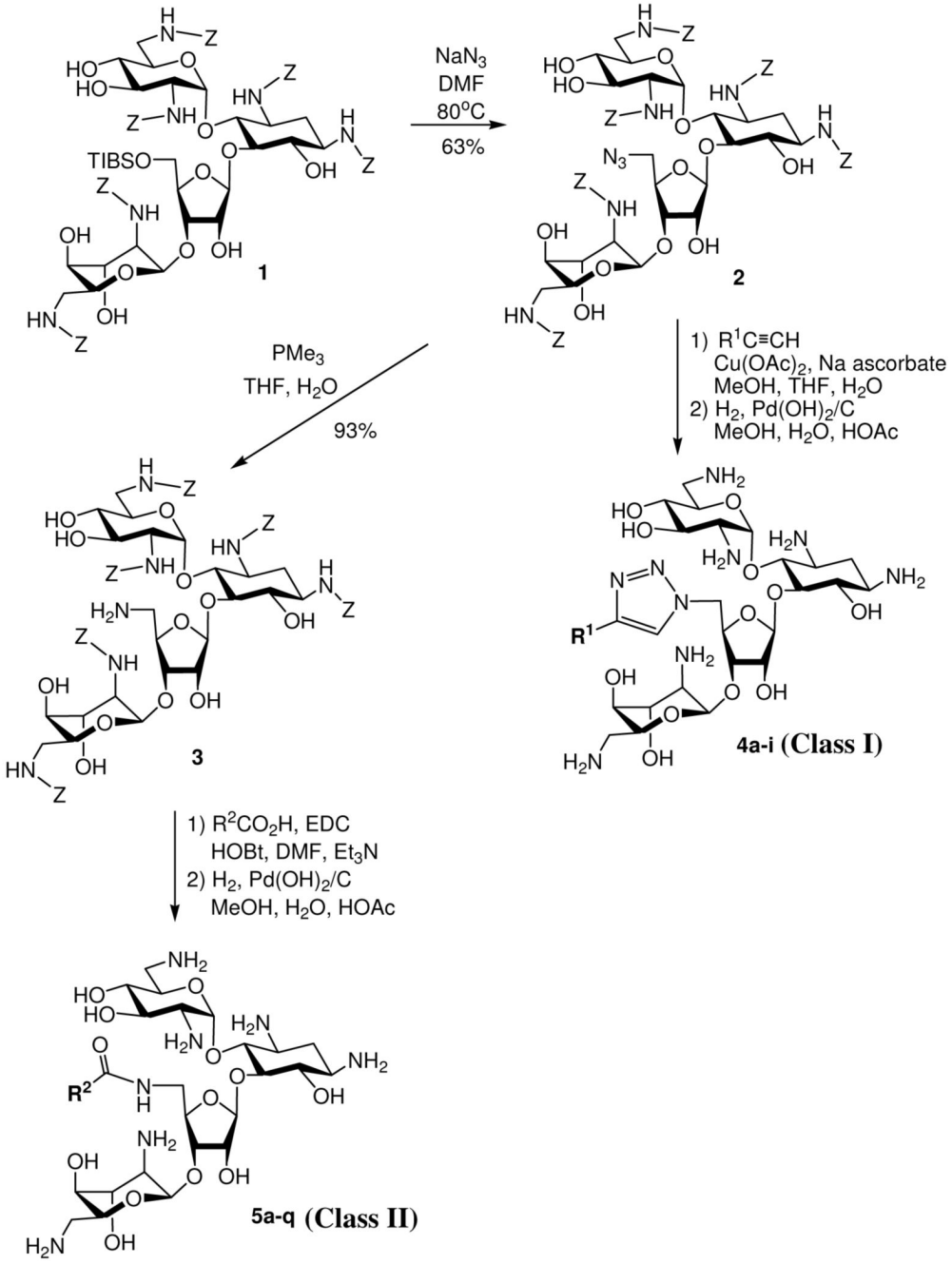
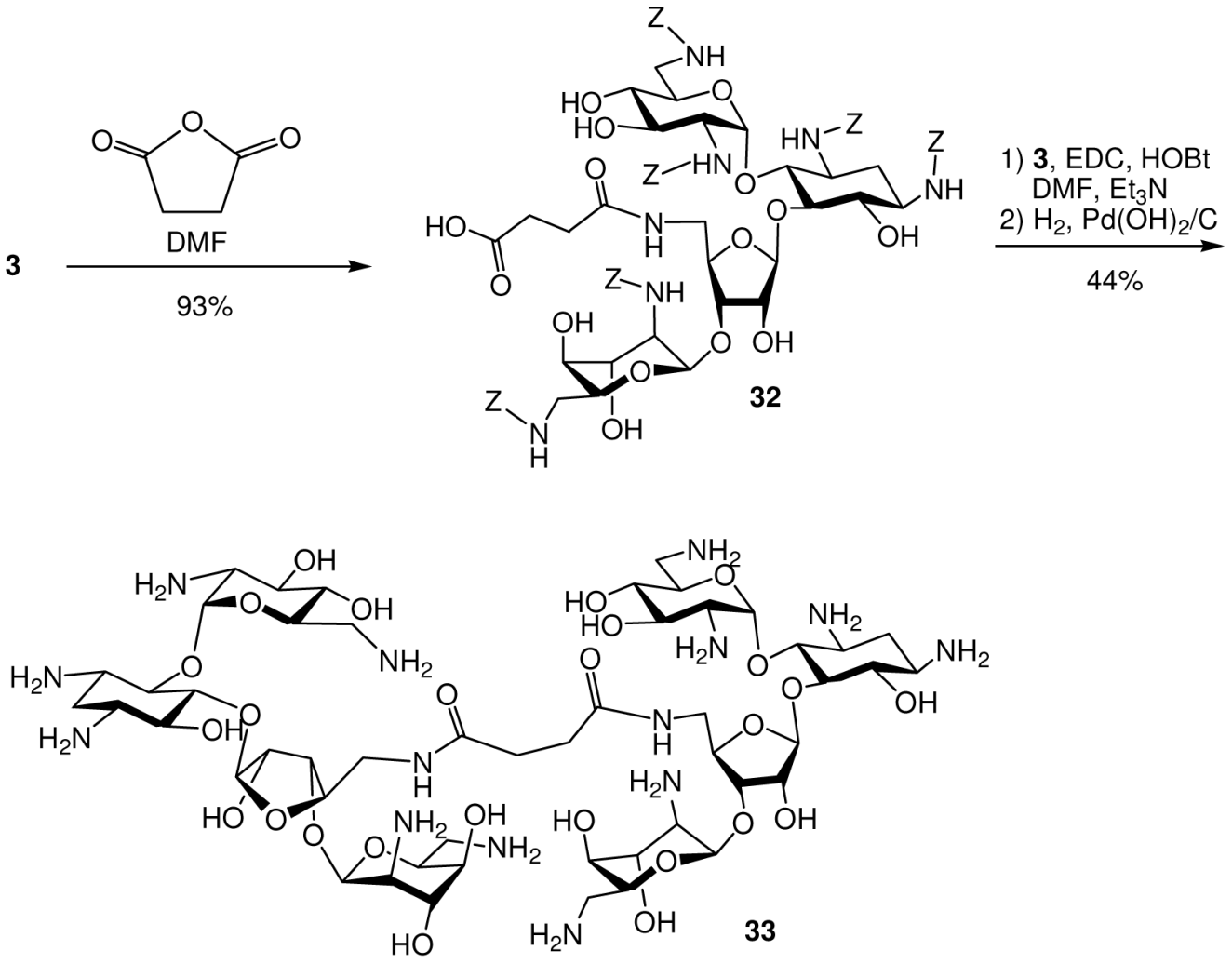


Figure 1.
Structures of Neomycin and Kanamycin Classes Aminoglycosides



Scheme 1.
Synthesis of Neomycin Derivatives



Scheme 2.
Synthesis of Neomycin Dimer

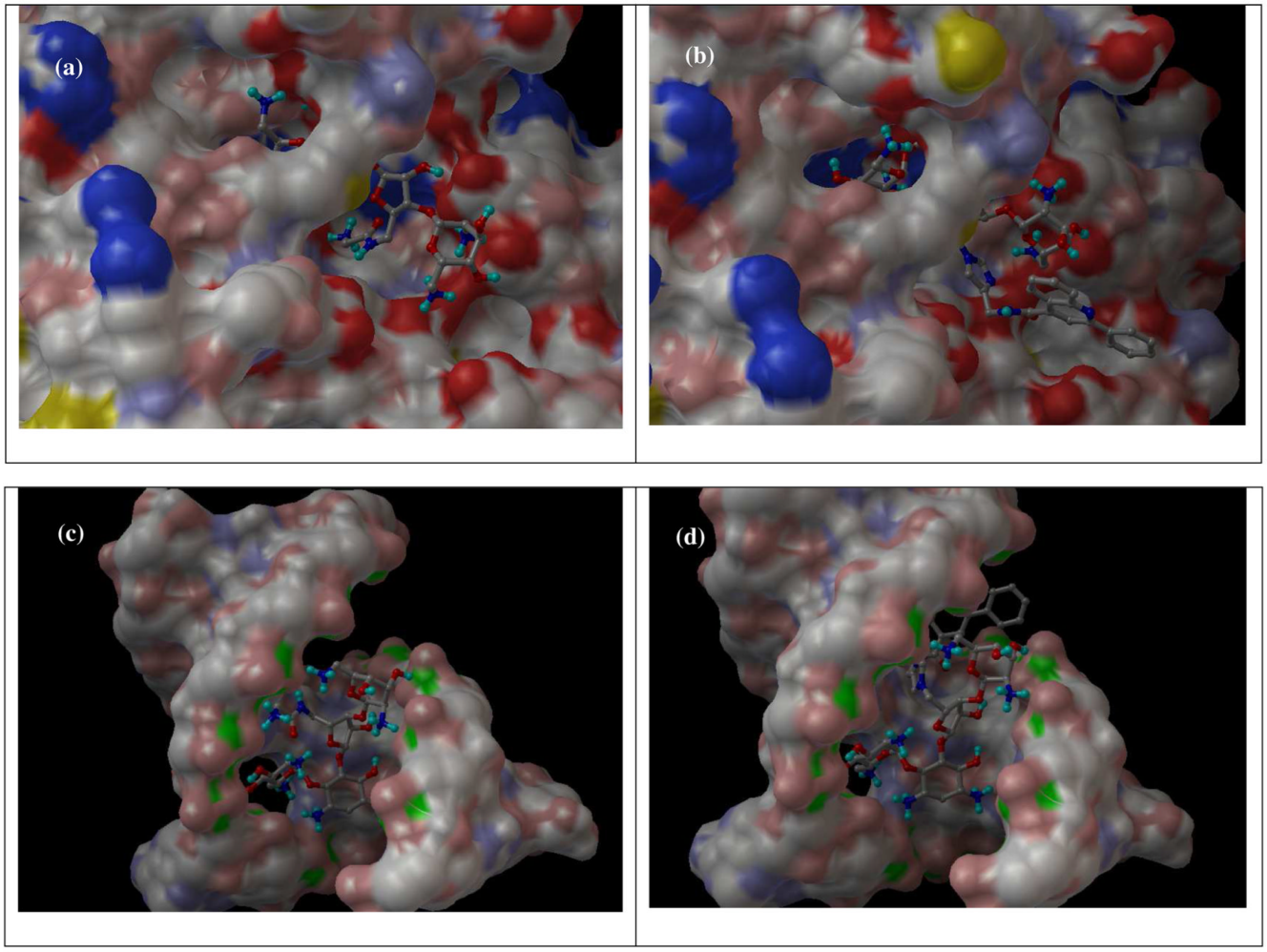
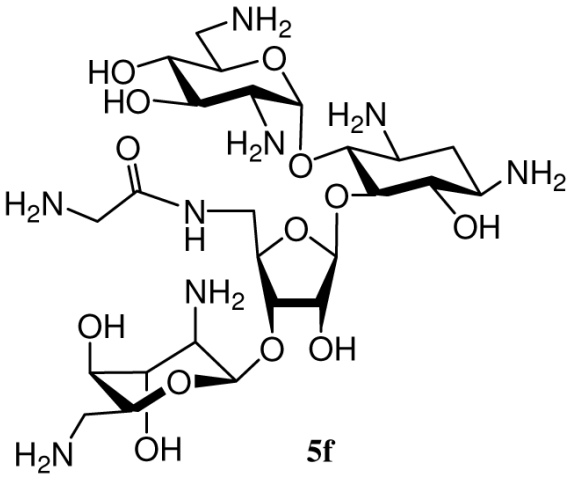


Figure 2.
(a) APH(3′)-IIIa with **5f**, (b) APH(3′)-IIIa with **4d**, (c) RNA with **5f**, (d) RNA with **4d**.



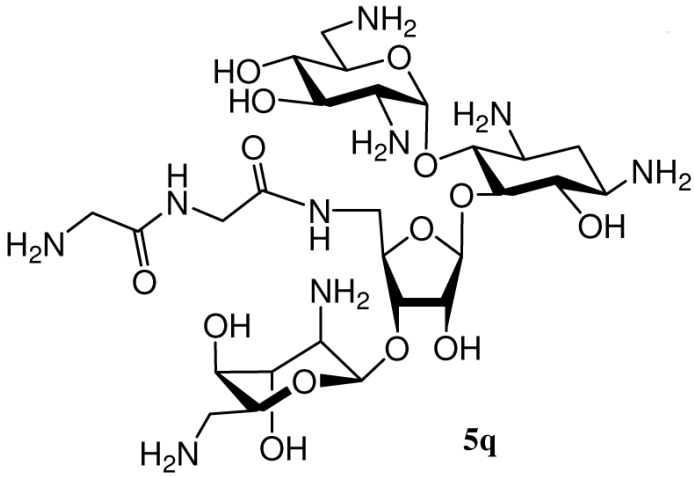


Figure 3.
Structures of **5f** and **5q**

Table 1

Yields and structural designs of class I 5″-modified neomycin derivatives

Compounds	Alkynes	Yield (%)	Source of alkynes
4a	*N*-propargyl-5-acetamidomethyl-2-oxazolidinone (**6**)	86%	Ref. 35
4b	1-octyne (**7**)	69%	Commercially available
4c	*N*,*N*-dimethyl propargylamine (**8**)	66%	Commercially available
4d	*N*-propargyl-2-phenyl-4-quinolinecarboxamide (**9**)	69%	Prepared in this work
4e	*N*-dimethyl propargylamine (**10**)	61%	Commercially available
4f	2-ethynylpyridine (**11**)	68%	Commercially available
4g	*N*-carbobenzyloxypropargylamine (**12**)	77%	Ref. 36
4h	*N*-propargylisonicotinamide (**13**)	40%	Prepared in this work
4i	*N*-carbobenzyloxy-L-proline *N*′-propargylamine (**14**)	49%	Prepared in this work

Table 2

Yields and structural designs of class II 5″-modified neomycin derivatives

Compounds	Carboxylic acids or amino acids	Yield (%)	Source of carboxylic acids or amino acids
5a	heptanoic acid (**15**)	88%	Commercially available
5b	palmitic acid (**16**)-	63%	Commercially available
5c	stearic acid (**17**)-	54%	Commercially available
5d	2-phenyl-4-quinolinecarboxylic acid (**18**)	30%	Commercially available
5e	(*S*)-Z-4-amino-2-benzyloxybutyric acid (**19**)	90%	Ref. 37
5f	Z-Gly (**20**)	27%	Commercially available
5g	Z-L-Ala (**21**)	36%	Commercially available
5h	Z-L-Pro (**22**)	50%	Commercially available
5i	Z-L-Trp (**23**)	67%	Commercially available
5j	Z-L-Ser (**24**)	52%	Commercially available
5k	Z-L-Lys (**25**)	61%	Commercially available
5l	succinic anhydride (**26**)	85%	Commercially available
5m	Z-D-Ala (**27**)	69%	Commercially available
5n	Z-D-Ser (**28**)	57%	Commercially available
5o	Z-D-Pro (**29**)	53%	Commercially available
5p	Z-D-Lys (**30**)	25%	Commercially available
5q	Z-Gly-Gly (**31**)	42%	Commercially available

Table 3

MIC of the 5″-Modified Neomycin Derivatives [f]

Compounds	*E. coli*[a]	*S. aureus*[b]	*E. coli* (TG1) [c]	*E. coli* (pSF815) [d]	*E. coli* (pTZ19U-3) [e]
Neomycin B	4	1	4-8	4-8	≥2000
4a	32	4-8	16	32	≥2000
4b	32	4	8-16	16	500
4c	8	2	8-16	16	≥2000
4d	32	8	8-16	32	500-1000
4e	8	1-2	16	16	≥2000
4f	8-16	2	16	16	≥2000
4g	8-16	1-2	16	16	1000
4h	16-32	4	16	32	≥2000
4i	16	2	16	16	≥2000
5a	16-32	4	8	16-32	≥2000
5b	**4**	**2**	**2-4**	**16**	**8**
5c	**4-8**	**4**	**2-4**	**32**	**8**
5d	8-16	4-8	8-16	16	≥2000
5e	8-16	1-2	16	16-32	≥2000
5f	16	1	8-16	8-16	500-1000
5g	8-16	0.5	8-16	16	≥2000
5h	16-32	0.5-1	16	16-32	≥2000
5i	16	2	8-16	16	≥2000
5j	8	1	8-16	16	≥2000
5k	16-32	2	16	32	125-250
5l	64	8	32	64	≥2000
5m	16-32	1	16	16-32	≥2000
5n	8-16	1	8-16	16	≥2000
5o	8-16	0.5-1	8	16	≥2000
5p	32	1-2	16	64	≥2000
5q	16-32	2-4	16-32	16	125-250
33	32	2-4	8-16	16-32	500

[a] *Escherichia coli* (ATCC 25922),

[b] *Staphylococcus aureus* (ATCC 25923),

[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] Unit: μg/mL.

Table 4

Kinetic Parameters and MIC of Aminoglycosides for APH(3′)-IIIa

Compound	K_M (μM)	V_{max} (μmol/mg/min)	k_{cat} (S^{-1})	$k_{cat}/K_M/10^4$ ($S^{-1}M^{-1}$)	MIC against bacterium with APH(3′)-IIIa [a]	MIC ratio [b]
Kanamycin A	8.2±0.7	6.96±0.12	3.48±0.06	42.4	125 (Kan B)	32 (Kan B)
Amikacin	57.4±5.7	2.75±0.13	1.38±0.07	24.0	1	**1**
Neomycin B	6.0±0.8	3.22±0.17	1.61±0.09	26.8	64	8
4a	160±15	0.41±0.03	0.21±0.02	0.13	125	8
4b	127±13	0.75±0.04	0.38±0.02	0.30	32-64	4
4c	120±14	0.65±0.05	0.33±0.03	0.28	64-125	8
4d	410±38	0.30±0.03	0.15±0.02	0.037	16-32	2
4e	100±9	0.72±0.07	0.36±0.04	0.36	125	8
4f	150±16	0.54±0.05	0.27±0.03	0.18	125	8
4g	111±12	0.68±0.04	0.34±0.02	0.3	64	4
4h	171±18	0.41±0.04	0.21±0.02	0.12	64-125	8
4i	175±18	0.40±0.04	0.20±0.02	0.14	64-125	8
5a	48±6	0.95±0.06	0.48±0.05	1.0	64	8
5b	58±4	0.85±0.08	0.42±0.05	0.72	**1-2**	**0.5**
5c	60±7	0.84±0.05	0.42±0.03	0.7	**2**	**1-2**
5d	400±35	0.38±0.04	0.19±0.02	0.048	32-64	4
5e	50±4	0.93±0.07	0.47±0.05	0.94	125	8
5f	75±8	0.88±0.06	0.44±0.03	0.6	32	2
5g	60±5	0.90±0.09	0.45±0.05	0.75	64	4
5h	80±9	0.63±0.07	0.32±0.03	0.4	64	4
5i	322±31	0.57±0.06	0.29±0.03	0.1	64	4
5j	42±4	1.01±0.09	0.51±0.05	1.2	32-64	4
5k	78±8	0.79±0.08	0.40±0.03	0.51	32-64	4
5l	53±6	0.86±0.09	0.43±0.04	0.81	250	8
5m	63±7	0.82±0.09	0.41±0.04	0.65	64-125	8
5n	57±6	0.93±0.10	0.47±0.05	0.82	64	4
5o	70±8	0.72±0.07	0.36±0.04	0.51	64	8
5p	75±8	0.70±0.07	0.35±0.04	0.47	64-125	8
33	>500	<0.1	<0.05	<0.01	64-125	8

Compound	K_M (μM)	V_{max} (μmol/mg/min)	k_{cat} (S^{-1})	$k_{cat}/K_M/10^4$ ($S^{-1}M^{-1}$)	MIC against bacterium with APH(3′)-IIIa [a]	MIC ratio [b]

[a] Unit: μg/mL,

[b] MIC ratio = (MIC against *E. coli* (TG1))/(MIC against *E. coli* (TG1) with APH(3′)-IIIa)

Table 5

MIC of the 5″-Modified Neomycin Derivatives against Other Strains of Bacteria [a]

entry	Compound	*K. pneumoniae* [b]	*K. pneumoniae* [c]	*S. aureus* [d]	*P. aeruginosa* [e]	*E. faecalis* [f]	*E. faecalis* [g]
1	**5b**	8-16	16-32	2-4	4	2-4	4-8
2	**5c**	8-16	32	4-8	8-16	4-8	8-16
3	**5f**	4	8	16-32	8-16	32-64	≥ 250
4	**5q**	4	8	32	16-32	64-125	≥ 250
5	Neomycin B	4	16-32	125	64	64-125	≥ 250
6	Amikacin	1	0.5	8-16	0.5-1	32-64	≥ 250
7	Gentamicin	1	8	2-4	0.5-1	8-16	≥ 250
8	Vancomycin	ND	ND	ND	ND	ND	125

[a] Unit: μg/mL, ND: Not Determined,

[b] *Klebsiella pneumoniae* (ATCC 13883),

[c] *K. pneumoniae* (ATCC 700603),

[d] *Staphylococcus aureus* (ATCC 33591) (MRSA),

[e] *Pseudomonas aeruginosa* (ATCC 27853),

[f] *Enterococcus faecalis* (ATCC 29212),

[g] *E. faecalis* (ATCC51299) (VRE).