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Singly Modified Amikacin and Tobramycin Derivatives Show Increased rRNA A-site Binding and Higher Potency against Resistant Bacteria

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Abstract

Semi-synthetic derivatives of clinically useful aminoglycosides, tobramycin and amikacin, were prepared by selectively modifying their 6″ position with a variety of hydrogen bond donors and acceptors. Their binding to the rRNA A-site was probed using an in vitro FRET-based assay and their antibacterial activity against several resistant strains (e.g., P. aeruginosa, K. pneumonia, MRSA) was quantified by determining minimum inhibitory concentrations (MICs). The most potent derivatives were evaluated for their eukaryotic cytotoxicity. Most analogs displayed greater affinity for the bacterial A-site compared to the parent compounds. Although most tobramycin analogs exhibited no improvement antibacterial activity, several amikacin analogs showed potent and broad-spectrum antibacterial activity against resistant bacteria. Derivatives tested for eukaryotic cytotoxicity exhibited minimal toxicity, similar to the parent compounds.

Keywords

antibiotics; aminoglycosides; drug resistant bacteria; A-site rRNA

Introduction

The discovery of penicillin (a β-lactam) and streptomycin (an aminoglycoside) in the 1940s launched the golden age of antibiotics. Many of the antibiotics discovered in the ensuing decades are still used in the clinic today.[1] However, the extensive and frequently unnecessary use of antibiotics has contributed to the increase in resistant pathogens. Horizontal gene transfer between bacteria has played a significant role in conferring resistance.[2] Drug-resistant bacteria, especially the ESKAPE pathogens (*Enterococcus*

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faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter s*pecies), *Clostridium difficile*, and *Escherichia coli*, now infect not only immunocompromised hospital patients, but otherwise healthy individuals as well.[3] This trend has led to rising healthcare costs, often due to extended stay in hospitals, and increased mortality.[4] Problematically, the number of new antibiotics approved by the US Food and Drug Administration has been steadily decreasing, a reflection that many pharmaceutical companies have been abandoning or downsizing their antibacterial research and development.[5]

On a positive note, there have been a few new classes of antibiotics in recent years, all of which target Gram-positive bacteria.[6] Nevertheless, the emergence of multi-drug resistant bacteria, especially Gram-negative bacilli with no new treatment options, has led to reexamination of drugs from the early years of antibiotic discovery.[7] Aminoglycosides are effective against a broad range of bacteria, although the advent of safer, less toxic antibiotics resulted in their declined use. However, with the increase in resistant pathogens, especially severe Gram-negative infections, aminoglycosides remain clinically useful for certain infections.[8] Tobramycin (**1a**) is often used for *P. aeruginosa* infection in cystic fibrosis patients, amikacin (**2a**) prescribed for highly resistant Gram-negative infections, and gentamicin is used for preventative measures, as well as for sepsis (Figure 1).[8a]

Most aminoglycosides bind to the ribosomal RNA (rRNA) A-site, the site of mRNA decoding, and cause translation infidelity.[9] The mode of action and resistance mechanisms have been well studied and the aminoglycoside scaffold has been established to bind RNA.[10] With this as a starting point, derivatization could lead to compounds that bind the A-site more avidly and show activity against otherwise drug resistant bacteria.[11] Additionally, structural modifications could possibly diminish adverse side effects on host cells or physiology. With this in mind, we have pursued the preparation and evaluation of minimally modified aminoglycosides in order to test their A-site affinity and, importantly, evaluate their effectiveness as potential antibiotics against important contemporary multidrug resistant bacterial strains.

Here we selectively modify two of the most common clinically used aminoglycoside antibiotics, amikacin and tobramycin. The primary alcohol in the 6″ position on these molecules is accessible to modification and is substituted for a variety of hydrogen bond donors and acceptors of different sizes (Figure 1). Most of the compounds show increased in vitro affinity to the A-site as determined by a Förster resonance energy transfer (FRET) based binding assay. Additionally, some of the derivatives show equal to or better potency against certain resistant bacterial strains, while their eukaryotic cytotoxicity remains identical to that of the parent antibiotic.

Results

Design Strategy

The 6″ hydroxyl group is one of the few functional groups that appears to form no hydrogen bonds to the A-site RNA, neither direct or water-mediated, in the crystal structures of tobramycin (**1a**) and amikacin (**2a**), though both are in close proximity to U1406 and C1407

(Figure 2).[12] Analogs with guanidinium groups replacing the 6″ hydroxyl have been shown to display increased A-site affinity and in some cases superior antibacterial activity.[13] This suggests that certain modifications to the 6″ position may indeed increase the affinity for the A-site and confer desirable antibacterial efficacy. We set out to test this hypothesis by making derivatives of both **1a** and **2a** with a variety of substituents differing in size, basicity, and in number of hydrogen bond donors and acceptors. More basic functional groups could increase the overall positive charge of the analogs, creating favorable electrostatic interactions with the polyanionic A-site rRNA. Hydrogen bond donors and acceptors could create new contacts to the A-site not observed in the parent compounds. Beyond imparting greater affinity for the A-site, some modifications could potentially disrupt recognition by aminoglycoside modifying enzymes, the most common mechanism of aminoglycoside deactivation. Such derivatives may exhibit greater antibacterial potency against resistant bacteria.

Synthesis

The parent aminoglycosides were converted into three key intermediates using known procedures.[13,14] The synthetic approach for the conversion of the parent aminoglycosides into these intermediates is illustrated with tobramycin (**1a**) in Scheme 1). First, all amines were globally *tert*-butyloxycarbonyl (Boc)-protected using di-*tert*-butyl dicarbonate. The single primary alcohol of (Boc)$_5$tobramycin (**3**) was then selectively converted to a sterically demanding sulfonate by treatment with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in pyridine. Reflux in methanolic ammonia afforded 6″-deoxy-6″-amino(Boc)$_5$tobramycin (**5**). Alternatively, the TPS derivative could be converted to 6″-deoxy-6″-azido(Boc)$_5$tobramycin (**6**) by treating it with sodium azide.

6″-Deoxy-6″-triisopropylbenzylsulfonyl(Boc)$_5$tobramycin (**4**) can also undergo substitution reactions with a variety of other nucleophiles (Scheme 2). Reflux in ethanolic methylamine yielded 6″-deoxy-6″-methylamino(Boc)$_5$tobramycin (**7**). Reflux with dimethylamine in tetrahydrofuran (THF) and dimethylformamide (DMF) mixture gave 6″-deoxy-6″-dimethylamino(Boc)$_5$tobramycin (**8**). 6″-Deoxy-6″-(2-(aminoethyl)amino)-(Boc)$_7$tobramycin (**9**) was obtained by heating with ethylene diamine in methanol, followed by Boc protection using di-*tert*-butyl dicarbonate to facilitate purification of this intermediate.

The free amine of 6″-deoxy-6″-amino(Boc)$_5$tobramycin (**5**) was used nucleophilically to react with 2,4-dimethoxybenzyl isocyanate in the presence of pyridine to give a 2,4-dimethoxybenzyl (DMB) protected urea. The DMB and Boc protecting groups were concurrently removed using a one to one mixture of trifluoroacetic acid (TFA) and dichloromethane with a tri-*iso*-propyl silane (TIPS) cation scavenger. HPLC purification afforded the analytically pure 6″-deoxy-6″-ureidotobramycin (**1f**) (Scheme 3).

6″-Deoxy-6″-azido(Boc)$_5$tobramycin (**6**) was used in a cycloaddition reaction with propargyl (Boc)amine in the presence of copper sulfate and sodium ascorbate to give 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)-(Boc)$_6$tobramycin (**10**) (Scheme 4). The intermediates **5**, **7**, **8**, **9**, and **10** were all be deprotected using the aforementioned acidic

conditions and HPLC purified to yield the tobramycin analogs **1b**, **1c**, **1d**, **1e**, and **1g**. All the amikacin derivatives were made using the same reagents as the tobramycin analogs.

Affinity for the bacterial 16S A-site RNA construct

To determine the affinity of all derivatives to the bacterial 16S A-site, we used a modified version of a FRET-based assay that was previously developed in our lab (Figure 3).[15] This modified version has been previously used to measure A-site affinities of modified aminoglycosides.[13] It consists of an aminoglycoside-coumarin conjugate (FRET donor), which binds to a Dy547 labeled 16S A-site RNA hairpin construct (FRET acceptor) (Figure 3). The relative affinity of unlabeled ligands for the A-site can be measured in a competition experiment, where the compound of interest is titrated in and displaces the coumarin–aminoglycoside conjugate, resulting in a decreased emission of the FRET acceptor, Dy547. Different coumarin–aminoglycoside conjugates can be used to cover distinct affinity ranges of A-site ligands. Plotting the fractional fluorescent saturation versus compound concentration generates titration curves.

Amikacin has a much lower affinity to the A-site as compared to tobramycin, so initial titrations of amikacin analogs were performed with a coumarin–kanamycin derivative, the lowest affinity aminoglycoside conjugate (Table 1). Tobramycin derivatives and higher affinity amikacin analogs were titrated against a coumarin–neomycin derivative (Table 2). In all cases, binding curves were generated by plotting the fractional fluorescence saturation of the FRET acceptor against the concentration of the molecule of interest. Representative curves of kanamycin-coumarin and neomycin-coumarin are shown in Figure 3.

All amikacin derivatives showed improved A-site binding with the exception of 6″-deoxy-6″-ureidoamikacin (**2f**), which had a much lower affinity than any other aminoglycoside tested. All amikacin analogs with modifications containing a single amine moiety: **2b–e** and **2g** showed similar binding to each other and were also comparable to tobramycin (**1a**). 6″-Deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**) showed binding superior to any of the other amikacin derivatives.

All tobramycin analogs showed improved binding over tobramycin (**1a**). Like the amikacin derivatives, the urea modification resulted in the weakest binder. This urea tobramycin analog (**1f**) was the only one that was not superior to all of the amikacin derivatives. 6″-Deoxy-6″-aminotobramycin (**1b**) and 6″-deoxy-6″-(2-(aminoethyl)amino)tobramycin (**1e**) showed the highest affinities of all derivatives tested. The methylamino (**1c**) and dimethylamino (**1d**) modified derivatives were the next best binders. 6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)tobramycin (**1g**) was worse than these, but still significantly better than the urea modified analog (**1f**).

Antibacterial activities

To assess the relative antibacterial activities of the synthetic derivatives, minimum inhibitory concentration (MIC) values of both the modified and parent antibiotics were determined against an array of bacterial strains (Tables 3 and 4). Multiple Gram-positive and Gram-negative strains were chosen to establish a broad-spectrum representation of

antibacterial activity. The compounds were first tested against the antibacterial susceptible control *E. coli* strain ATCC25922. No derivatives showed improvement against this strain and only one compound, 6″-Deoxy-6″-aminoamikacin (**2b**), even showed equal activity to its parent aminoglycoside with an MIC value of 6.25 – 12.5 μg/mL.

The aminoglycosides were tested against three *P. aeruginosa* strains, P4, PA01, and ATCC27853. Tobramycin (**1a**) shows much better activity than amikacin (**2a**) against these *P. aeruginosa* strains. Unfortunately, only one tobramycin derivative, 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)tobramycin (**1g**), showed even equal activity to tobramycin (**1a**) against any of these strains. Both had MIC values of 0.39 μg/mL against PA01. However, the amikacin derivatives, 6″-deoxy-6″-aminoamikacin (**2b**), 6″-deoxy-6″-methylaminoamikacin (**2c**), and 6″-deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**) showed improved activity. 6″-Deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**) showed superior activity against all three strains including a four-fold improvement to 6.25 μg/mL against P4. 6″-Deoxy-6″-aminoamikacin (**2b**) was equal to amikacin (**2a**) with MIC values of 1.56 – 3.13 μg/mL against PA01, but was slightly improved against ATCC27853 with an MIC value of 1.56 – 3.13 μg/mL compared to a parent MIC value of 3.13 μg/mL. It also showed a four-fold improvement against P4.

The aminoglycosides were also tested against two *K. pneumoniae* strains, ATCC700603 and the highly drug resistant, *K. pneumoniae* carbapenemase producer GNR1100. Amikacin (**2a**) shows better activity than tobramycin (**1a)** against these strains. Again, the tobramycin derivatives were disappointing with only 6″-deoxy-6″-(2-(aminoethyl)amino)tobramycin (**1e**) showing even equal activity to the parent antibiotic. Both had MIC values of 6.25 μg/mL against ATCC700603. 6″-Deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**) and 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**) showed equal activity to amikacin (**2a**) against ATCC700603 with MIC values of 0.78 μg/mL. Interestingly, they both also improved from 50 μg/mL to 12.5 – 25 μg/mL against GNR1100.

To test efficacy against Gram-positive bacteria, the aminoglycosides were tested against MRSA strains TCH1516 and ATCC33591. No amikacin or tobramycin derivatives showed any improvements or even equal activity to their parents against TCH1516. There were, however, several compounds that showed improved activity against ATCC33591. 6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)tobramycin (**1g**) improved to an MIC value of 0.78 – 1.56 μg/mL from a parent value of 3.13 μg/mL. Several amikacin derivatives showed increased potency compared to the parent MIC value of 25 μg/mL for amikacin (**2a**). These included 6″-deoxy-6″-aminoamikacin (**2b**), with a slight improvement to 12.5 – 25 μg/mL and 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**) and 6″-deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**), which both had more significant improvements to 12.5 μg/mL.

Cytotoxicity

The amikacin and tobramycin derivatives with the most potent, broad-spectrum antibacterial activities (**1g**, **2b**, **2e**, **2g**) were tested for eukaryotic cytotoxicity against HeLa cells compared to the parent compounds (**1a**, **2a**). The derivatives showed minimal toxicity,

similar the parent compounds, with little detrimental effects to up to 100 μM (~55 – 66 μg/mL) (Table S1).

Discussion

Tobramycin and amikacin analogs, modified at the 6″ position, were synthesized and evaluated for their A-site affinities and antibacterial activity. All tobramycin analogs showed superior affinity for the A-site as compared to tobramycin (**1a**), the parent antibiotics. There were significant variations in A-site affinity among the tobramycin analogs. The tightest binders were 6″-deoxy-6″-aminotobramycin (**1b**) and 6″-deoxy-6″-(2-(aminoethyl)amino)tobramycin (**1e**) and the worst tobramycin analog was 6″-deoxy-6″-ureidotobramycin (**1f**). The general trend among the tobramycin analogs suggests that binders with smaller steric bulk or with greater overall potential charge show higher affinity.

All amikacin analogs showed improved A-site binding with the exception of 6″-deoxy-6″-ureidoamikacin (**2f**), which had by far the lowest A-site affinity of any compound tested. It is the only modification made without a basic functionality, which likely contributed to its lower RNA affinity. The amikacin analogs with one additional basic functional group showed similar IC_{50} values including the bulky 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**). 6″-Deoxy-6″-(2-(aminoethyl)amino)-amikacin (**2e**) has two additional basic amines as compared to amikacin (**2a**) and, indeed, it displayed the highest A-site affinity among the amikacin analogs. In contrast to the tobramycin analogs, the RNA affinity of the amikacin analogs appears to be mostly sensitive to electrostatic effects with no apparent steric preference among the analogs tested.

The tobramycin analogs generally showed disappointing antibacterial activity. The most successful analog was 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)tobramycin (**1g**), which showed no eukaryotic toxicity up to 100 μM, similar to the parent antibiotic (**1a**). It showed better activity than tobramycin (**1a**) against a MRSA strain and equal activity against one *P. aeruginosa* strain. In most other cases its MIC values were two fold worse. This particular modification was also one of the more successful ones among the amikacin analogs. 6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**) showed equal or better activity than amikacin (**2a**) against five out of nine strains tested against and in all other cases its MIC was within one serial dilution. It is intriguing that this modification was so efficacious, particularly since it was the most structurally significant alteration made.

6″-Deoxy-6″-aminoamikacin (**2b**) was also promising with equal or improved activity against six out of nine strains, including all three *P. aeruginosa* strains. It is interesting to note that the antibacterial activity was reduced across the entire panel for 6″-deoxy-6″-methylaminoamikacin (**2c**) and even more so for 6″-deoxy-6″-dimethylaminoamikacin (**2d**). This trend was also present in the tobramycin derivatives. This suggests hydrogen bonding may be playing a role in the increased activity of 6″-deoxy-6″-aminoamikacin (**2b**).

The most successful derivative made, however, was 6″-deoxy-6″-(2-(aminoethyl)amino)-amikacin (**2e**). This compound showed increased activity against five strains and equal

activity against one. It was universally better against the *P. aeruginosa* strains and it showed equal or better activity against both *K. pneumoniae* strains including an improvement against GNR1100. This makes the broad spectrum improvement of some of the amikacin derivatives particularly fascinating given that amikacin itself is a semi-synthetic aminoglycoside with an amino 2-hydroxybutyryl (AHB) side chain, which lowers its susceptibility to aminoglycoside-modifying enzymes.[16] It is possible that the AHB and 6″ modifications operate synergistically to further decrease its affinity for these enzymes. This is a hypothesis that we have previously posited when we observed increased antibacterial activity in an analog with a guanidinium group in this position.[13]

When analyzing MIC values, it is important to appreciate that the affinity of an antibiotic to the A-site does not necessarily correlate with ribosome susceptibility as determined by in vitro translation assays or with antibacterial potency.[17] Interestingly, all but one derivative, 6″-deoxy-6″-aminoamikacin (**2b**), showed inferior antibacterial activity against the control *E. coli* strain ATCC25922. This suggests that improvement seen in activity against resistant strains is at least partially due to overcoming bacterial resistance mechanisms.

Conclusion

A series of 6″ modified tobramycin and amikacin analogs were synthesized. In all cases the derivatives showed improved A-site affinity compared with their parent antibiotics when tested in an in vitro FRET-based assay with the exception of 6″-deoxy-6″-ureidoamikacin (**2f**), which showed greatly decreased binding affinity. The tobramycin analogs generally showed disappointing antibacterial activity. In contrast, several amikacin analogs exhibited promising antibacterial potency against resistant strains. The most potent antibacterial derivatives tested did not show toxicity toward eukaryotic cells. Most notably, 6″-deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**) showed greater potency than amikacin (**2a**) against the majority of strains that were tested in MIC assays. Our results illustrate the potential utility of modifying the native antibiotics, as well as their established semi-synthetic analogs, as a pathway to new agents of altered, yet effective therapeutic spectrum.

Experimental Section

Materials

Unless otherwise specified, materials purchased from commercial suppliers were used without further purification. Tobramycin (**1a**) and amikacin (**2a**) were obtained from Sigma–Aldrich as their free bases. Propargyl (Boc)amine was synthesized according to an established procedure.[18] Anhydrous NH_3 was purchased from Airgas. All other anhydrous solvents and reagents, and ion exchange resins were purchased from Sigma–Aldrich. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

The Dy-547-labeled A-site construct was purchased from Thermo Scientific and purified by gel electrophoresis[7d, 13, 15b]. Kanamycin–coumarin and neomycin–courmarin conjugates were synthesized and purified according to established procedures.[15] Chemicals for preparing buffer solutions (enzyme grade) were purchased from Fisher Biotech. Autoclaved water was used in all fluorescence titrations.

Mueller–Hinton broth used for sensitivity testing was obtained from Hardy Diagnostics (Santa Maria, CA, USA). Polystyrene 96-well microplates for MIC testing were purchased from Corning Inc. (Corning, NY, USA). Bacterial strains for sensitivity testing included five strains from the American Type Culture Collection (Manassas, VA, USA): hospital-associated MRSA strain 33591 rendered resistant to rifampicin by serial passage, USA300 MRSA strain TCH1516 (BAA-1717), *K. pneumoniae* strain 700603, *P. aeruginosa* strain 27853, and *E. coli* strain 25922. *P. aeruginosa* strain PA01 was used as a general antibiotic-sensitive *P. aeruginosa* strain.[19] Other Gram-negative strains used were clinical isolates obtained from a tertiary academic hospital in the New York metropolitan area; these were: *K. pneumoniae* strain GNR1100 (respiratory isolate) and *P. aeruginosa* strain P4 (sputum isolate).

Instrumentation

NMR spectra were recorded on Varian Mercury 300 and 400 MHz, Varian VX 500 MHz, and Jeol ECA 500 MHz spectrometers. Mass spectra (MS) were recorded at the University of California, San Diego, Chemistry and Biochemistry Mass Spectrometry Facility, utilizing an Agilent 6230 HR-ESI-TOF mass spectrometer. Reverse-phase HPLC (Vydac C18 column) purification and analysis were carried out using an Agilent 1200 series instrument. Products were lyophilized utilizing a Labconco FreeZone 2.5 freeze drier. Steady-state fluorescence experiments were carried out in a microfluorescence cell with a path length of 1.0 cm (Hellma GmH & Co KG, Mullenheim, Germany) on a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer. A background spectrum (buffer) was subtracted from each sample. A VersaMax plate reader (Molecular Devices, Mountain View, CA, USA) set at 600 nm wavelength was used for MIC assays.

Aminoglycoside Desalting

Aminoglycoside·TFA salts obtained upon global deprotection reactions (up to 40 mg) were dissolved in autoclaved H_2O (0.6 mL) in a sterile eppendorf tube. Dowex Monosphere 550 A (100 mg) was added, and the suspension was shaken lightly overnight. The resin was removed by centrifugal filtration and washed twice with autoclaved H_2O. The desalted solutions were lyophilized, and the complete removal of TFA counterions was confirmed by ^{13}C NMR spectroscopy.

A-site binding assay

Aminoglycoside titration procedures, binding curves, and the curve fitting equation can be found in the Supporting Information.

Minimum inhibitory concentration (MIC) determinations

MIC values for aminoglycosides were determined using broth microdilution in accordance with Clinical Laboratory Standards Institute guidelines.[20]

Cytotoxicity

The aminoglycosides were tested for mammalian cell cytotoxicity by measuring lactate dehydrogenase (LDH) release into the media. Briefly, HeLa cells (American Type Culture

Collection, Manassas, VA) were seeded at 2 × 104 per well in sterile tissue culture-treated microtiter plates (Sigma-Aldrich, St. Louis, MO). The cells were allowed to attach for 24 hours and were then incubated with the aminoglycosides in fresh media. LDH was assayed in the supernatant at 72 hours using the CytoTox 96 Non-Radioactive Cytotoxicity Assay according to manufacturer's instructions (Promega Corporation, Madison, WI, USA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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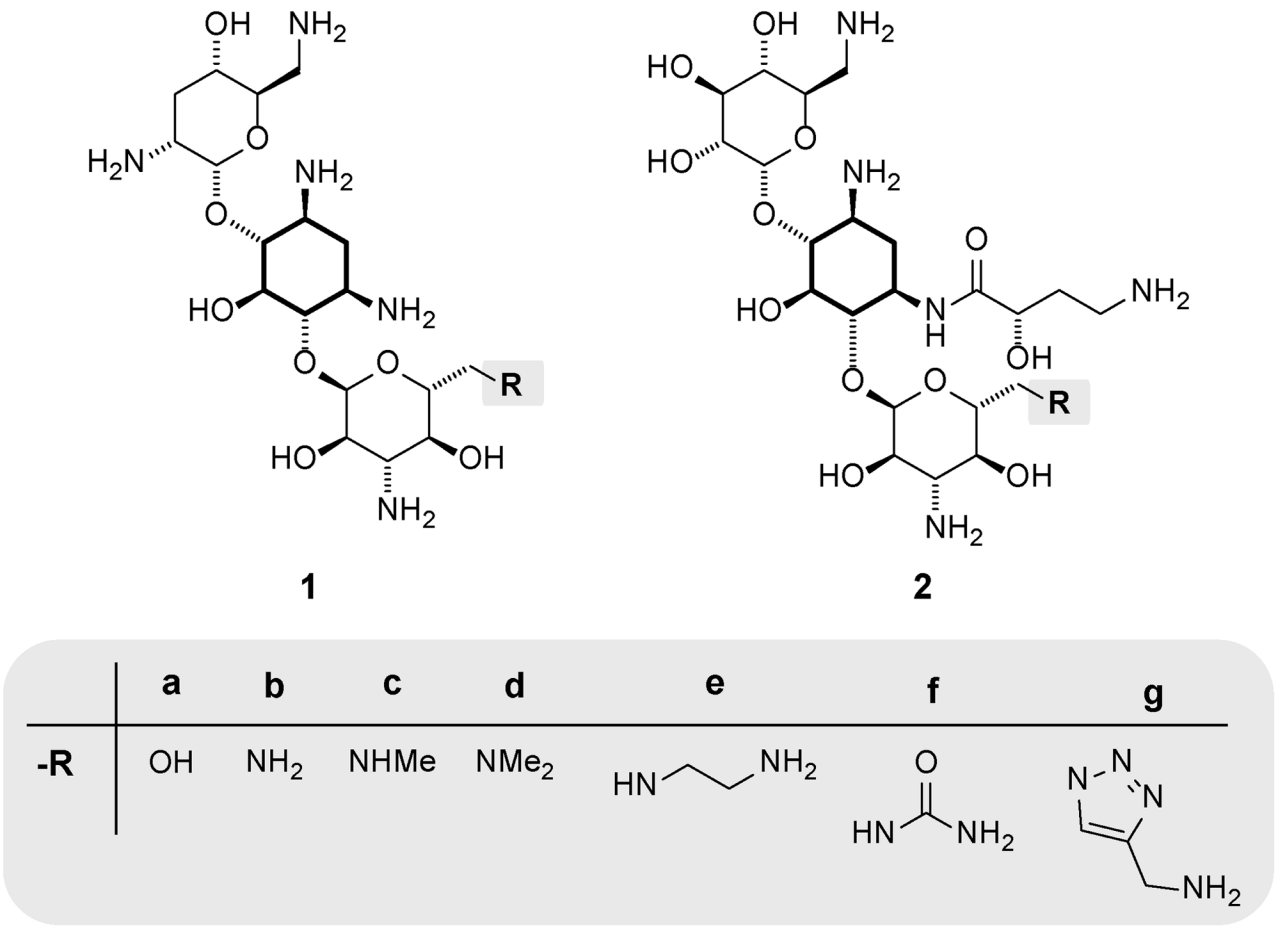


Figure 1.

Tobramycin (**1a**), amikacin (**2a**) and derivatives prepared and studied. The 2-deoxystreptamine (2-DOS) ring is bold. The 6″ modification position is highlighted in grey.

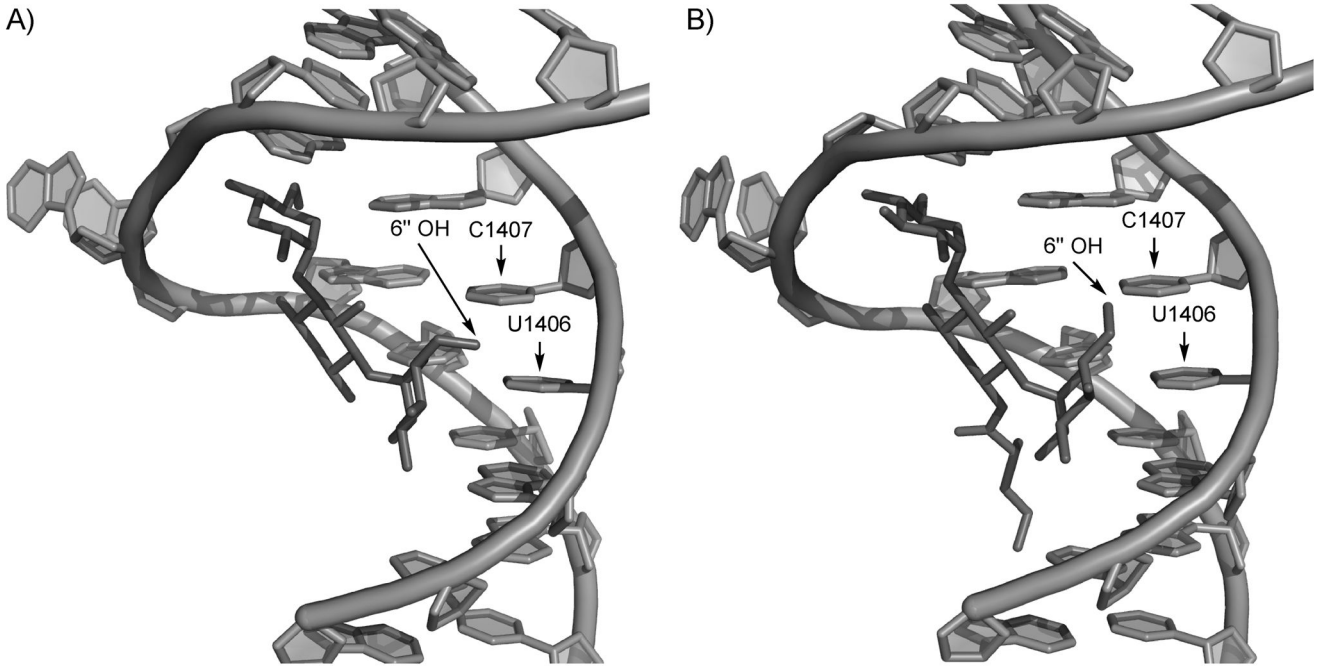


Figure 2.

A) Crystal structure of tobramycin (**1a**) with A-site rRNA. B) Crystal structure of amikacin (**2a**) with A-site rRNA. Aminoglycoside 6″ alcohols and A-site bases U1406 and C1407 are labeled. Figures were adapted from PDB files: tobramycin (1LC4), amikacin (2GSQ)[12] and made using PyMOL Molecular Graphics Systems, Version 1.4.1, Schrödinger, LLC.

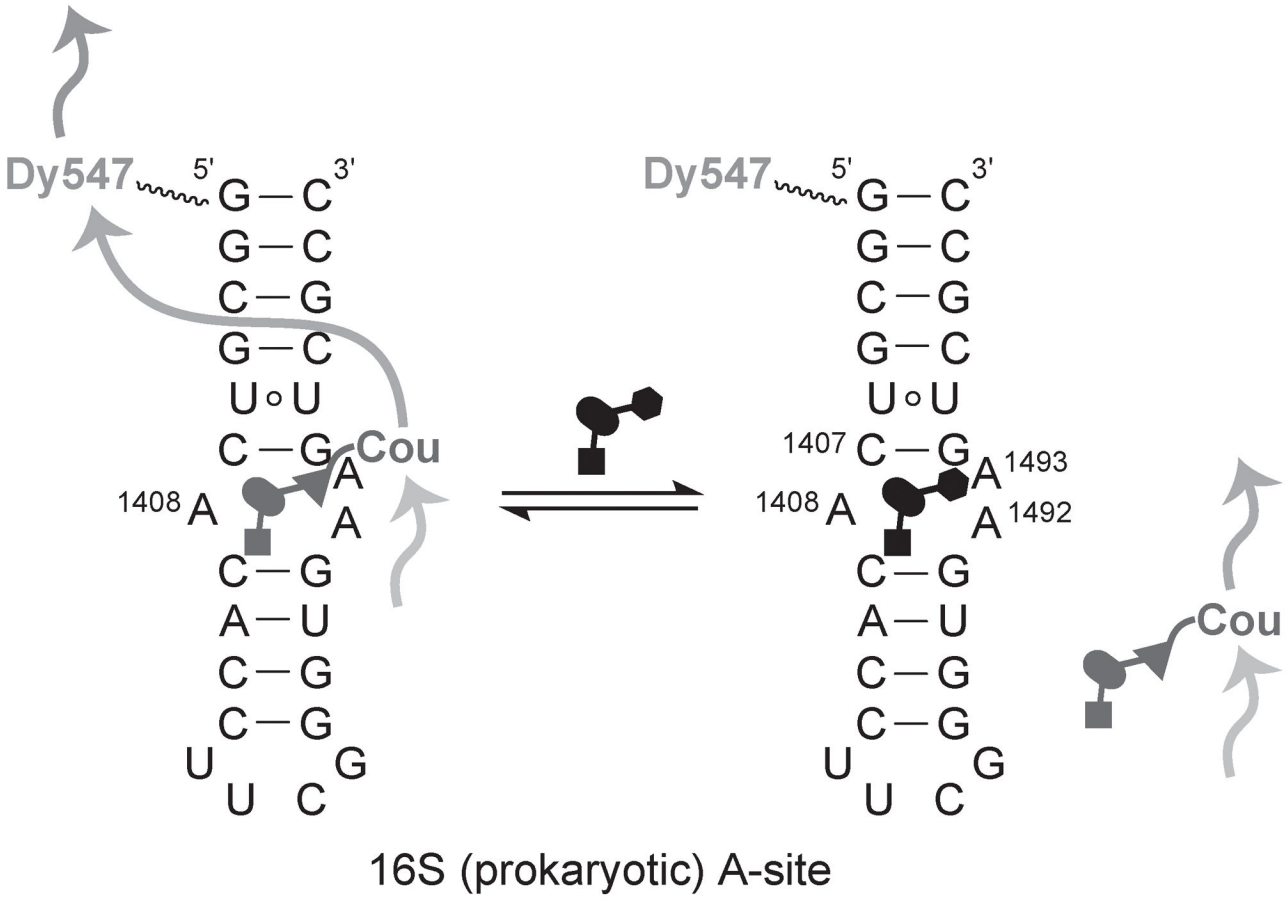


Figure 3.

Secondary structure of the 16S (prokaryotic) A-sites RNA labeled with the FRET acceptor Dy547 (grey). The place-holding coumarin labeled aminoglycoside (Neomycin-Coumarin or Kanamycin-Coumarin, dark grey). As the aminoglycoside-coumarin conjugate is displaced by an unlabeled aminoglycoside, the affinity and selectivity of unlabeled aminoglycosides for the 16S A-site can be accurately monitored using FRET by following a decrease in emission of the acceptor (Dy547).

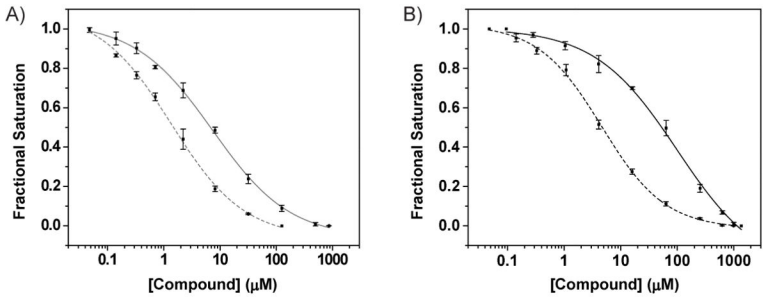
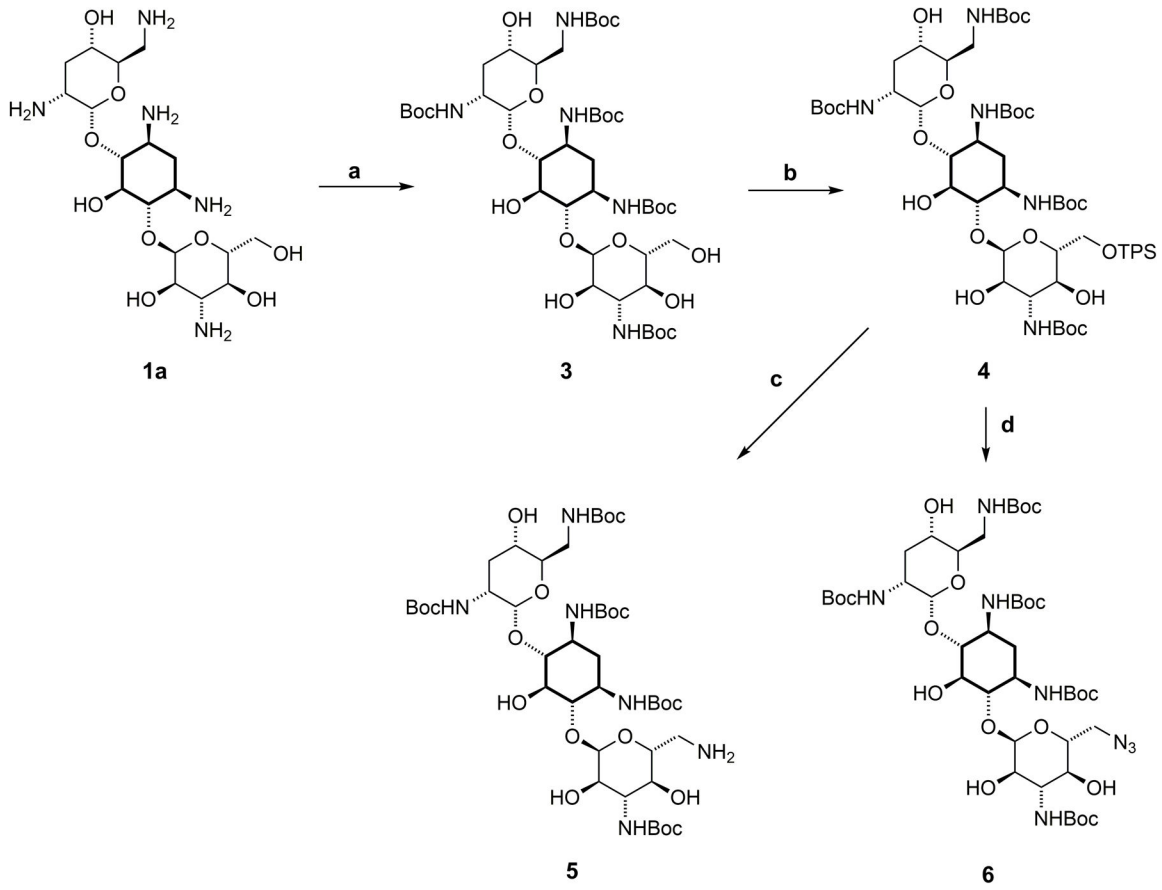


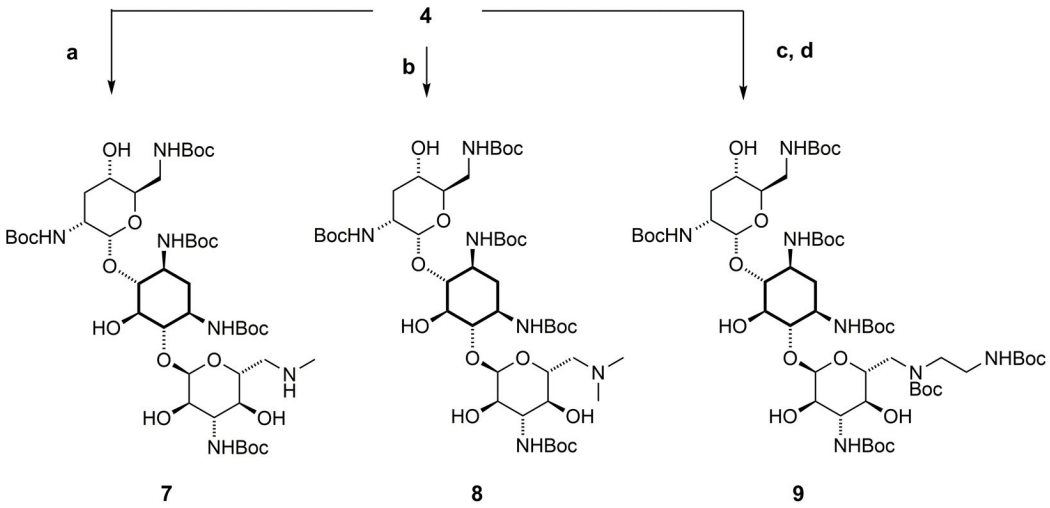
Figure 3.

Representative displacement curves of A) Kanamycin-Coumarin by **2a** (grey solid) and **2c** (grey dashed), with IC_{50} values of 6.7 ± 0.7 and 1.5 ± 0.2, respectively. B) Neomycin-Coumarin by **1a** (black solid) and **1b** (black dashed) with IC_{50} values of 53.0 ± 6.0 and 4.7 ± 0.4, respectively.



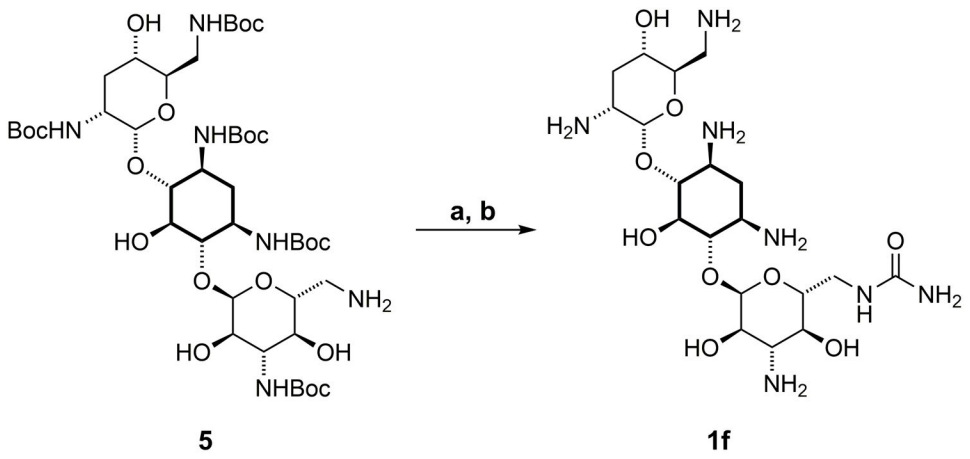
Scheme 1.

Synthesis of key intermediates **4**, **5**, and **6**. *Reagents and conditions:* a) Boc_2O, Et_3N, H_2O, DMF, 55 °C, 96%; b) TPSCl, pyridine, RT; 72% c) NH_3, MeOH, 80 °C, 94%; d) NaN_3, DMF, 55 °C, 71%.



Scheme 2.

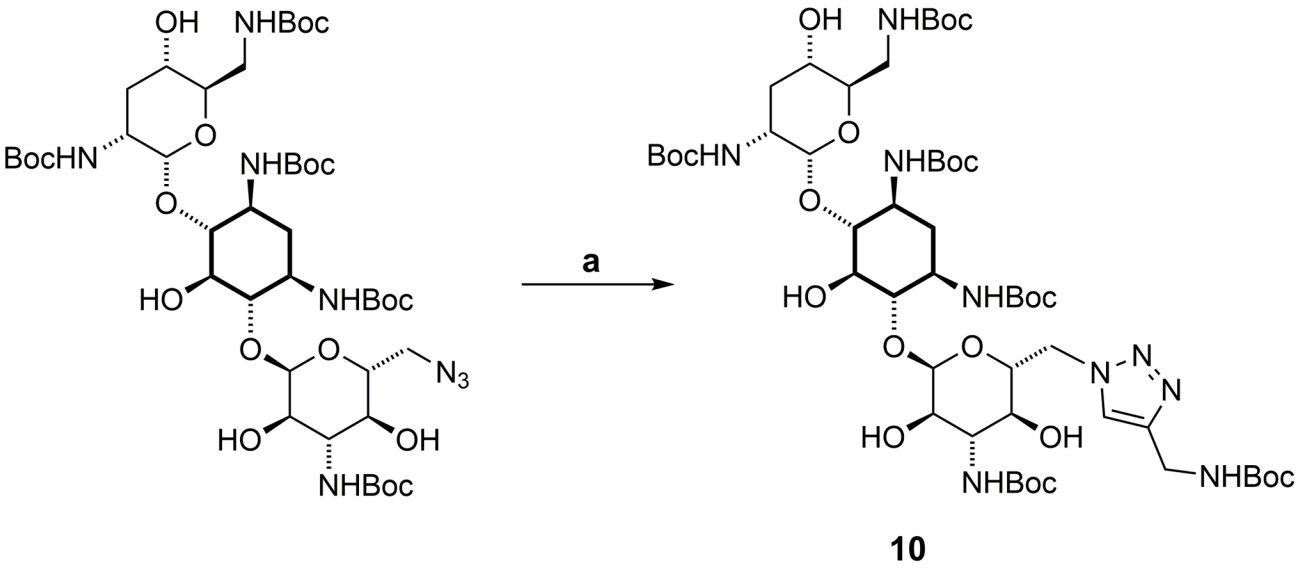
Substitution reactions of 6″-deoxy-6″-triisopropylbenzylsulfonyl(Boc)$_5$tobramycin (**4**). *Reagents and conditions:* a) Methylamine, EtOH, 80 °C, 88%; b) Dimethylamine, THF, DMF, 80 °C, 91%; c) Ethylene diamine, MeOH, 80 °C; d) Boc_2O, Et_3N, H_2O, DMF, 55 °C, 58% (two steps).



5 1f

Scheme 3.

Synthesis 6″-deoxy-6″-ureidotobramycin (**1f**). *Reagents and conditions:* a) 2,4-Dimethoxybenzyl isocyanate, pyridine, RT; b) TFA, TIPS, CH_2Cl_2, RT, 58% (two steps).



Scheme 4.

Synthesis 6″-deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)-(Boc)$_6$tobramycin (**10**). *Reagents and conditions:* a) Propargyl (Boc)amine, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF, *t*-BuOH, H_2O, RT, 81%.

Table 1

IC_{50} Values for Competing Off Kanamycin-Coumarin[a]

Compound	IC_{50} (μM)
Tobramycin (**1a**)	1.5 ± 0.2
Amikacin (**2a**)	6.7 ± 0.7
6″-Deoxy-6″-aminoamikacin (**2b**)	2.1 ± 0.2
6″-Deoxy-6″-methylaminoamikacin (**2c**)	1.5 ± 0.2
6″-Deoxy-6″-dimethylaminoamikacin (**2d**)	2.2 ± 0.2
6″-Deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**)	1.7 ± 0.03
6″-Deoxy-6″-ureidoamikacin (**2f**)	50.7 ± 5.5
6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**)	2.2 ± 0.1

[a]Conditions: A-site RNA (1 μM), kanamycin-coumarin (0.53 μM), cacodylate buffer pH 7.0 (20 mM), NaCl (100 mM), EDTA (0.5 mM)

Table 2

IC_{50} Values for Competing Off Neomycin-Coumarin[a]

Compound	IC_{50} (μM)
Tobramycin (**1a**)	53.0 ± 6.0
6″-Deoxy-6″-aminotobramycin (**1b**)	4.7 ± 0.4
6″-Deoxy-6″-methylaminotobramycin (**1c**)	7.4 ± 0.6
6″-Deoxy-6″-dimethylaminotobramycin (**1d**)	6.8 ± 0.8
6″-Deoxy-6″-(2-(aminoethyl)amino)tobramycin (**1e**)	5.3 ± 0.5
6″-Deoxy-6″-ureidotobramycin (**1f**)	30.0 ± 4.0
6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)tobramycin (**1g**)	9.8 ± 1.0
Amikacin (**2a**)	>100
6″-Deoxy-6″-aminoamikacin (**2b**)	46.7 ± 1.5
6″-Deoxy-6″-methylaminoamikacin (**2c**)	45.7 ± 5.8
6″-Deoxy-6″-dimethylaminoamikacin (**2d**)	46.4 ± 5.4
6″-Deoxy-6″-(2-(aminoethyl)amino)amikacin (**2e**)	20.2 ± 2.6
6″-Deoxy-6″-ureidoamikacin (**2f**)	>100
6″-Deoxy-6″-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)amikacin (**2g**)	47.6 ± 2.6

[a]Conditions: A-site RNA (1 μM), neomycin-coumarin (0.53 μM), cacodylate buffer pH 7.0 (20 mM), NaCl (100 mM), EDTA (0.5 mM)

Table 3

Inhibitory activities of tobramycin (**1a**) and derivatives against a panel of bacterial strains (μg mL^{-1})[a]

Bacterial Strain	1a	1b	1c	1d	1e	1f	1g
E. coli (ATCC25922)	3.13	25–50	≥50	≥50	6.25–12.5	6.25	6.25
P. aeruginosa (P4)	0.78	25	>50	>50	3.13–6.25	3.13–6.25	1.56
P. aeruginosa (PA01)	0.39	12.5	50	50	1.56	0.78	*0.39*
P. aeruginosa (ATCC27853)	0.39	12.5–50	50	>50	3.13	0.78–1.56	0.78
K. pneumoniae (ATCC700603)	6.25	12.5	25	25–50	*6.25*	12.5	12.5
K. pneumoniae (GNR1100)	>50	>50	>50	>50	>50	>50	>50
MRSA (TCH1516)	0.78–1.56	6.25–12.5	12.5–25	25	6.25	3.13–6.25	6.25
MRSA (ATCC33591)	3.13	6.25	12.5–25	25	3.13–6.25	*3.13*	**0.78–1.56**

[a]Minimum inhibitory concentration (MIC) values [μg mL^{-1}]. MIC value equal to tobramycin (*italics*); MIC value lower than tobramycin (**bold**).

Table 4

Inhibitory activities of amikacin (**2a**) and derivatives against a panel of bacterial strains (μg mL^{-1})[a]

Bacterial Strain	2a	2b	2c	2d	2e	2f	2g
E. coli (ATCC25922)	6.25–12.5	*6.25–12.5*	12.5–25	25–50	12.5	50	12.5
P. aeruginosa (P4)	25	**6.25**	**12.5**	>50	**6.25**	>50	25 – 50
P. aeruginosa (PA01)	1.56–3.13	*1.56–3.13*	3.13	12.5–25	**1.56**	25–50	*1.56–3.13*
P. aeruginosa (ATCC27853)	3.13	**1.56–3.13**	3.13–6.25	25	**1.56**	25–50	*3.13*
K. pneumoniae (ATCC700603)	0.78	0.78–1.56	1.56	3.13	*0.78*	6.25–12.5	*0.78*
K. pneumoniae (GNR1100)	50	*50*	>50	>50	**12.5 – 25**	>50	**12.5 – 25**
MRSA (TCH1516)	6.25–12.5	12.5–25	50	>50	12.5–25	>50	12.5–25
MRSA (ATCC33591)	25	**12.5 – 25**	*25*	>50	**12.5**	>50	**12.5**

[a]Minimum inhibitory concentration (MIC) values [μg mL^{-1}]. MIC value equal to amikacin (*italics*); MIC value lower than amikacin (**bold**).