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# Selectively Guanidinylated Aminoglycosides as Antibiotics

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The emergence of virulent, drug-resistant bacterial strains coupled with a minimal output of new pharmaceutical agents to combat them makes this a critical time for antibacterial research. Aminoglycosides are a well-studied, highly potent class of naturally occurring antibiotics with scaffolds amenable to modification, and therefore, they provide an excellent starting point for the development of semisynthetic, next-generation compounds. To explore the potential of this approach, we synthesized a small library of aminoglycoside derivatives selectively and minimally modified at one or two positions with a guanidine group replacing the corresponding amine or hydroxy functionality. Most guanidino-aminoglycosides showed increased affinity for the ribosomal decoding rRNA site, the cognate biological target of the natural products, when compared with their parent antibiotics, as measured by an in vitro fluorescence resonance energy transfer (FRET) A-site binding assay. Additionally, certain analogues showed improved minimum inhibitory concentration (MIC) values against resistant bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA). An amikacin derivative holds particular promise with activity greater than or equal to the parent antibiotic in the majority of bacterial strains tested.

## Introduction

The discovery of antibiotics—small molecules of natural or synthetic origin that specifically interfere with vital processes in bacteria—can be viewed as one of the major medical breakthroughs of the 20th century. Indeed, antibiotics facilitated the cure of previously untreatable life-threatening infectious diseases. However, many of the originally identified antibiotics are no longer clinically useful as they are compromised by bacterial resistance mechanisms, which include modification of the drug molecules, mutation of the molecular drug targets, or increased cellular drug efflux by small-molecule transporters and biofilm formation.[1] The emergence of pathogens resistant to nearly all antibiotics in current use is of particular concern to clinicians. While infections caused by Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), remain a major problem worldwide, the emergence within the last decade of multiple-drug-resistant Gram-negative organisms, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, is equally worrisome.[2] In fact, Gram-negative resistance to drugs of last resort, such as colistin, has become alarmingly more commonplace in the clinical setting.[3] The widespread and frequently indiscriminate use of antibiotics in human and veterinary medicine has further accelerated the emergence of these resistant, highly pathogenic bacteria that can cause life-threatening infections.[4] Unfortunately, the surge seen in the appearance of resistant bacteria has not been met by a parallel development of new effective, broad-spectrum antibiotics, and only two novel classes of antibacterial agents, fluoroquinolones and oxazolidinones, have been identified over the past few decades.[5] It is apparent, therefore, that new potent antibiotics are required to complement or even replace currently used drugs, whose utility is ever increasingly compromised by bacterial resistance.

Diverse approaches to the discovery of new antibiotics exist. High-throughput screening campaigns of novel natural products and synthetic libraries can, in principle, lead to the identification of potent novel agents.[6] Such screening strategies can involve empirical, whole-cell testing or target-based in vitro assays for known or underutilized, genomically identified targets with the former approach having the potential to identify antibacterial agents with distinct modes of action.[7] Unfortunately, such efforts have resulted in minimal output and lead compound discovery. Alternatively, modifications of existing and perhaps retired antibiotics can revive some of their utility through systematic structural modifications.[8] While incremental, such efforts rely on established bioactive scaffolds targeting known bacterial pathways, with the prospective of generating potent agents, which can potentially evade deactivation by prevailing resistance mechanisms. Here, we address the selective modification of aminoglycoside antibiotics, a large family of natural products active primarily against Gram-negative bacteria.[9]

Aminoglycosides are polycationic antibiotics, and most bind to the 16S ribosomal A-site RNA, leading to diminished translational fidelity.[10] Being a particularly well-studied antibacterial class, a wealth of information exists regarding their interactions with their intracellular target and bacterial resistance mechanisms.[1a,b,d,11] This vast knowledge makes the aminoglycoside

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scaffold an ideal starting point for the development of new, potentially more effective antibiotics.[12] Indeed, semisynthetic, second-generation aminoglycosides such as amikacin have proven very successful in the clinic.[9] With this in mind, we set out to make minor structural modifications to selected members of these known antibiotics in an effort to retain or even improve upon their affinity for the 16S A-site, while potentially decreasing their susceptibility to the most prevalent modes of bacterial deactivation.

Here we disclose the synthesis of a small focused library of aminoglycoside derivatives selectively modified at one or two positions. We strategically replace amine or hydroxy functionalities with a guanidine group in tobramycin, amikacin, kanamycin A, neomycin B neamine, paromomycin, and apramycin (Figure 1).[12a,c] Most of the newly synthesized guanidino-aminoglycosides displayed enhanced affinity for the ribosomal A-site, the biological target of the parent derivatives, as determined by an in vitro fluorescence resonance energy transfer (FRET)-based binding assay. The results of antibacterial tests on a diverse collection of regular and resistant strains illustrate that certain analogues exhibit improved potency against resistant strains, including MRSA. An amikacin analogue shows particular promise with activities greater or equal to those of the parent antibiotic in the majority of strains tested.

## Results

### Design strategy

The bacterial A-site is a highly discriminating RNA target that is not tolerant of major structural changes to its cognate ligands.[12n,13] Therefore, we decided to selectively and strategically functionalize aminoglycosides at positions that are less likely to perturb binding and, at the same time, are synthetically accessible.[12a,c] A relatively small modification, which would retain or enhance the overall charge of the RNA-targeting antibiotic, could be achieved by replacing a hydroxy or amine group with a guanidine functionality. In contrast to amines, the planar guanidine functional group is highly basic and can participate in well-defined directional hydrogen bonds. To probe this strategy, we derivatized several aminoglycoside antibiotics, including neamine, kanamycin A, tobramycin, paromomycin, neomycin B, amikacin, and apramycin, by converting selected primary alcohols into guanidine groups, or turning an existing aminomethyl group into the corresponding guanidine derivative. We hypothesized that beyond yielding a greater affinity for the A-site, functional group changes at some sites could potentially lead to decreased recognition by aminoglycoside-modifying enzymes, one of the major bacterial resistance mechanisms.

### Synthesis

A general synthetic approach for the conversion of aminoglycoside primary alcohols to guanidinium groups is illustrated using tobramycin (**1**) as an example (Scheme 1). First, all amines were globally *tert*-butyloxycarbonyl (Boc)-protected using di-*tert*-butyl dicarbonate. The single primary alcohol of $(Boc)_5$tobramycin (**17**) was then selectively converted to a sterically demanding sulfonate by treatment with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in pyridine.[12f] Reflux in methanolic ammonia then afforded 6″-deoxy-6″-amino$(Boc)_5$tobramycin (**19**). This three-step process, converting primary alcohols into amines, has been previously used in our laboratory for the synthesis of other modified aminoglycosides.[14] Treatment of the single free amine with 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine in the presence of triethylamine gave fully Boc-protected guanidino-aminoglycoside, 6″-deoxy-6″-guanidino$(Boc)_7$tobramycin (**20**). Acidic deprotection of all Boc groups using a one to one mixture of trifluoroacetic acid (TFA) and tri-*iso*-propyl silane (TIPS) in dichloromethane, followed by HPLC purification, afforded the analytically pure 6″-deoxy-6″-guanidinotobramycin (**2**).

A slightly different approach was employed for the synthesis of 6″-deoxy-6″-guanidinoapramycin (**16**). Care had to be taken in preparing the sulfonate to prevent activation of multiple hydroxy groups. This was achieved by using fewer equivalents of the sulfonyl chloride (Step b, Scheme 2), in comparison with the other aminoglycosides. In addition, unlike other aminoglycoside derivatives, 6″-deoxy-6″-triisopropylbenzylsulfonyl$(Boc)_5$-apramycin (**22**) was found to degrade in refluxing methanolic ammonia. Instead, it was converted to the amino intermediate via a two-step process wherein the sulfonyl functionality was first substituted for an azide using sodium azide and then subsequently reduced in a palladium-catalyzed hydrogenation to give 6″-deoxy-6″-guanidino$(Boc)_7$apramycin (**23**).

A third protocol was used for selectively converting aminomethyl groups in aminoglycosides to the corresponding guanidine derivatives, relying on their higher nucleophilicity compared with the other more sterically hindered amines. Treatment of unprotected tobramycin with sub-stoichiometric quantities of 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine followed by deprotection in a one to one mixture of TFA in dichloromethane provided desired derivative **3** (Scheme 3). Similar protocols were applied to amikacin and neamine. Note, all guanidino-aminoglycoside derivatives were first converted to their free-base form by exposure to a strong basic anion ($OH^-$) exchange resin (Monosphere 550A, Dowex) prior to their evaluation in any A-site binding assays or antibacterial experiments.

### 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.[15] In this assay, a coumarin–aminoglycoside conjugate placeholder binds to a Dy-547-labeled 16S A-site construct. Coumarin acts as a FRET donor to its matched Dy-547 acceptor. The 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 placeholder, resulting in a decreased sensitized acceptor emission. Different coumarin–ami-

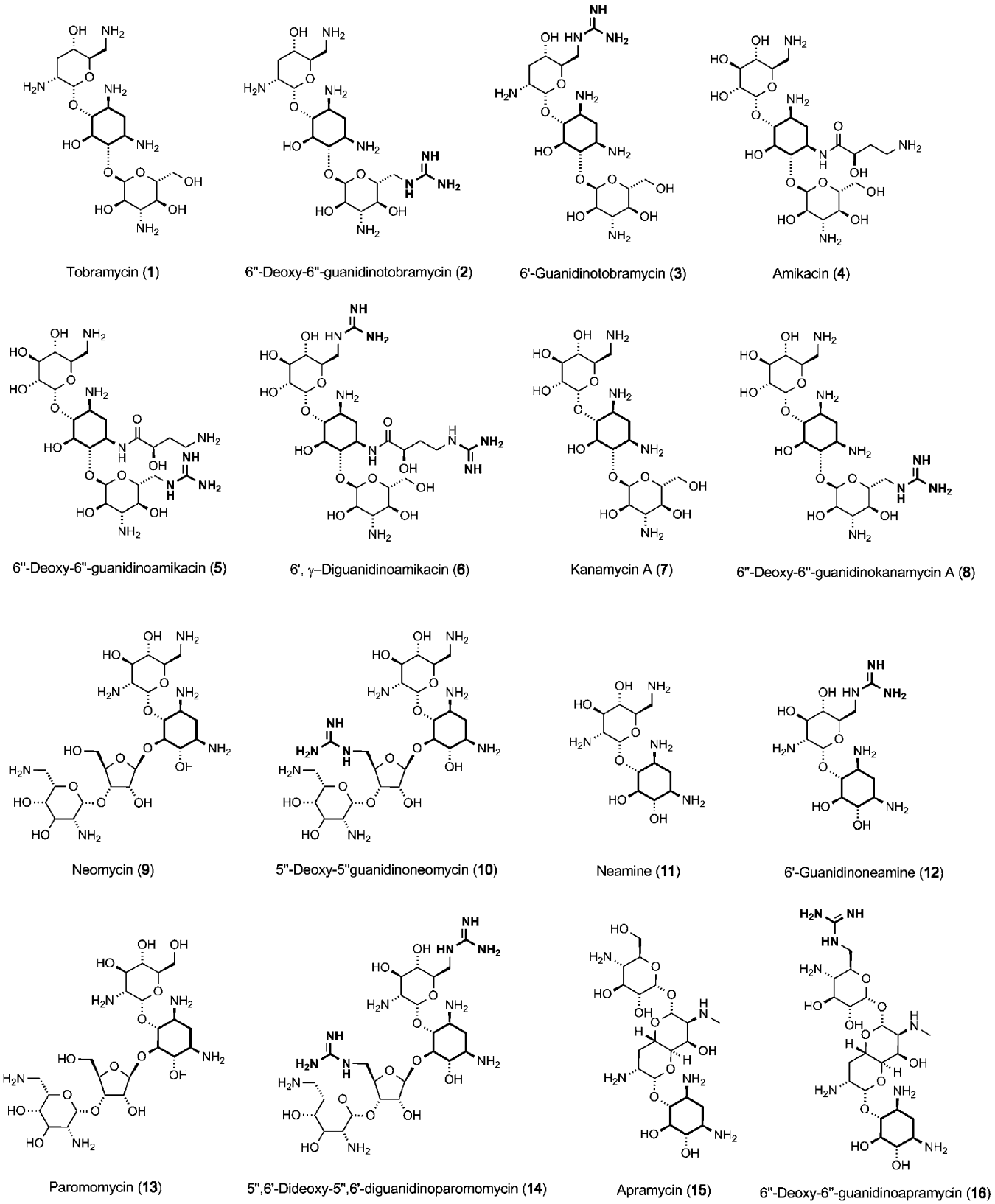
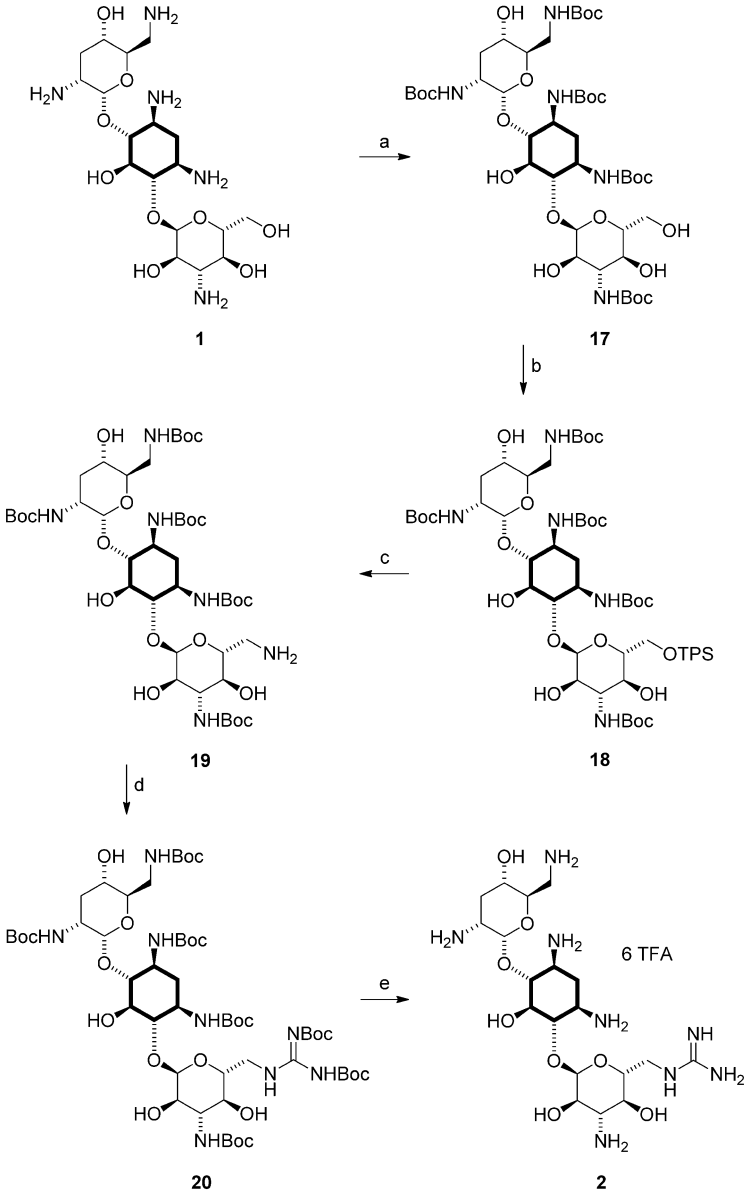



**Figure 1.** Parent aminoglycosides and guanidino-aminoglycosides. The guanidine and 2-deoxystreptamin (2-DOS) moieties are highlighted in bold.

noglycoside conjugates can be used to cover distinct affinity ranges of putative competitor antibiotics.

Initial titrations were performed with a coumarin–kanamycin derivative, the lowest affinity placeholder aminoglycoside con-




**Scheme 1.** Primary alcohol to guanidinium conversions. *Reagents and conditions*: a) $Boc_2O$, $Et_3N$, $H_2O$, DMF, 55 °C, 4–6 h, 91–97%; b) TPSCl, pyridine, RT, 2 days, 51–66%; c) $NH_3$, MeOH, 80 °C, 2–2.5 days, 57–93%; d) 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine, $Et_3N$, $CH_2Cl_2$, MeOH, RT, 3 days, 71–85%; e) TFA, TIPS, $CH_2Cl_2$, RT, 2.5–3 h, 79–96%.

jugate (Table 1). Potent A-site binders, such as neomycin and paromomycin derivatives **10** and **14**, respectively, were titrated against a coumarin–neomycin derivative (Table 2). In all cases, titration curves were generated by plotting the fractional fluorescence saturation of the acceptor against the concentration of the molecule of interest (for a representative example, see Figure 2 and the Supporting Information).

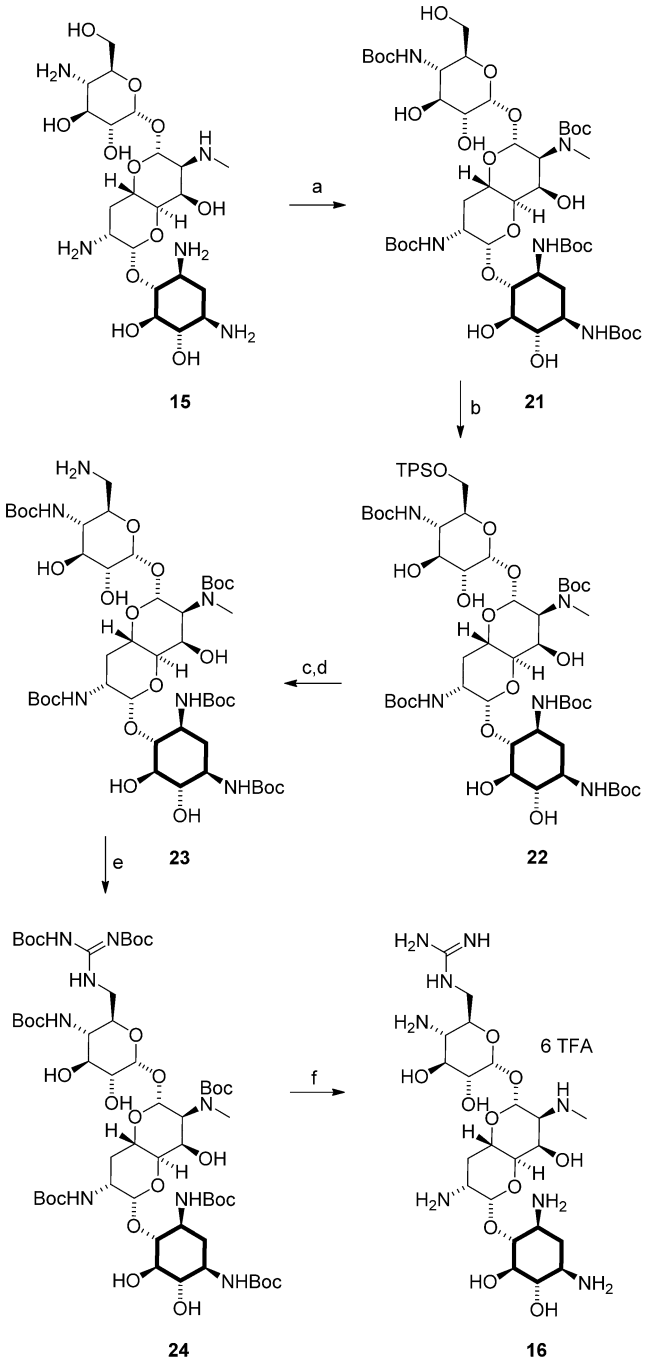
In general, derivatives with primary alcohols converted to guanidinium groups were found to have significantly higher affinities for the A-site in comparison with their parent aminoglycosides. In particular, 6″-deoxy-6″-guanidinoamikacin (**5**) and 6″-deoxy-6″-guanidinokanamycin A (**8**) showed marked improvements compared with their parent compounds, which were the weakest binders tested. 5″-Deoxy-5″-guanidinoneomycin (**10**) and 5″,6′-dideoxy-5″,6′-diguanidinoparomomycin (**14**) both showed higher affinities for the A-site than neomycin (**9**), which was the highest affinity binder among the natural aminoglycosides. The only exception to this trend was 6″-deoxy-6″-guanidinoapramycin (**16**), which has a similar affinity to the unmodified antibiotic. Most of the aminoglycoside derivatives, where amines at primary carbon centers are replaced with guanidinium groups, show comparable affinity to their parent antibiotics. This is not surprising since the overall positive charge is unlikely to drastically change; this is in contrast to derivatives where primary alcohols were converted to a guanidine group, which would have increased positive character.

### Antibacterial activities

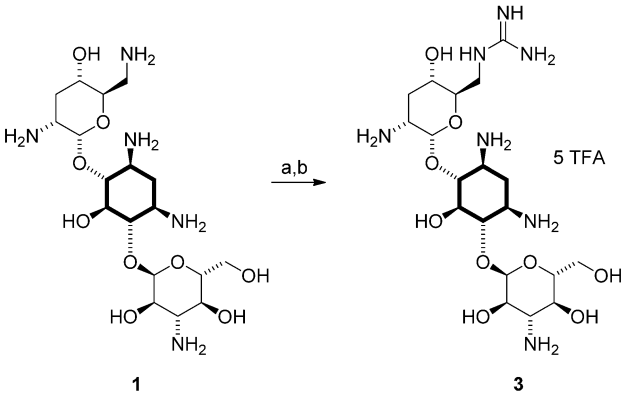
While the structure–activity relationship (SAR) data generated is intriguing, the ultimate test is the actual efficacy against bacteria. To assess the relative antibacterial activities of the synthetic derivatives, minimum inhibitory concentration (MIC) values of both the modified and parent antibiotics were determined against a wide variety of bacterial strains (Table 3). The compounds were first tested against the antibacterial-agent-susceptible control *E. coli* strain ATCC25922. We also used the clinically relevant Gram-positive MRSA strain ATCC33591. Most of the guanidino-aminoglycosides showed improved efficacy over their respective parent aminoglycosides against MRSA. The two compounds showing the greatest improvements were 6″-deoxy-6″-guanidinokanamycin A (**8**) with an MIC value dropping to 3.125–6.25 $\mu g\,mL^{-1}$ from 25 $\mu g\,mL^{-1}$ for the parent compound, and 6″-deoxy-6″-guanidinoamikacin (**5**), which improved to 3.125 $\mu g\,mL^{-1}$ from a parent MIC value of 12.5–25 $\mu g\,mL^{-1}$.

All synthetic derivatives were also tested against a variety of antibacterial-drug-resistant, Gram-negative, clinical isolates including strains of *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii*. Compound **5** showed the broadest spectrum activity, with potency greater or equal to its parent compound in six out of the eight strains tested. For example, the MIC values of compound **5** against the two *P. aeruginosa* strains were 1.56 $\mu g\,mL^{-1}$ and 3.125 $\mu g\,mL^{-1}$, respectively, compared with 3.125 $\mu g\,mL^{-1}$ and 6.25 $\mu g\,mL^{-1}$ for the parent compound—a twofold increase in potency in both cases. With the exception of the aforementioned derivative, the guanidino-aminoglycosides did not perform as well in general against these strains. Surprisingly, 5″,6′-dideoxy-5″,6′-diguanidinoparomomycin (**14**) showed a vast improvement against the *A. baumannii* strain GNR1753 compared with paromomycin (**13**), dropping from an MIC value of greater than 50 $\mu g\,mL^{-1}$ to 6.25–12.5 $\mu g\,mL^{-1}$.




**Scheme 2.** 6′′-Deoxy-6′′guanidinoapramycin synthesis. *Reagents and conditions*: a) $Boc_2O$, $Et_3N$, $H_2O$, DMF, 55 °C, 8 h, 92%; b) TPSCl, pyridine, RT, 1.5 days, 31%; c) $NaN_3$, DMF, 55 °C, 2 days; d) Pd/C, $H_2$, MeOH, RT, overnight, 80% (two steps); e) 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine, $Et_3N$, $CH_2Cl_2$, MeOH, RT, 3 days, 74%; f) TFA, TIPS, $CH_2Cl_2$, RT, 2 h, 78%.




**Scheme 3.** Amine to guanidinium conversions. *Reagents and conditions*: a) 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine, $Et_3N$, $CH_2Cl_2$, MeOH, RT, 5 days; b) TFA, TIPS, $CH_2Cl_2$, RT, 2 h, 12–22% (two steps).

**Table 1.** Initial evaluation of inhibitory concentrations determined by titration against the coumarin–kanamycin derivative.[a]

| Compd | $IC_{50}$ [μM] |
|---|---|
| Tobramycin (**1**) | 1.6 ± 0.2 |
| 6′′-Deoxy-6′′-guanidinotobramycin (**2**) | 1.0 ± 0.1 |
| 6′-Guanidinotobramycin (**3**) | 1.6 ± 0.1 |
| Amikacin (**4**) | 6.7 ± 0.7 |
| 6′′-Deoxy-6′′-guanidinoamikacin (**5**) | 1.4 ± 0.04 |
| 6′,γ-Diguanidinoamikacin (**6**) | 3.2 ± 0.2 |
| Kanamycin A (**7**) | 7.0 ± 0.7 |
| 6′′-Deoxy-6′′-guanidinokanamycin A (**8**) | 1.8 ± 0.1 |
| Neamine (**11**) | 4.5 ± 0.4 |
| 6′-Guanidinoneamine (**12**) | 4.4 ± 0.5 |
| Apramycin (**15**) | 1.7 ± 0.1 |
| 6′′-Deoxy-6′′-guanidinoapramycin (**16**) | 1.9 ± 0.2 |

[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). Values represent the mean ± SD of $n = 3$–5 experiments.

**Table 2.** Inhibitory concentrations of potent A-site binders determined by titration against the coumarin–neomycin derivative.[a]

| Compd | $IC_{50}$ [μM] |
|---|---|
| Neomycin (**9**) | 4.4 ± 0.3 |
| 5′′-Deoxy-5′′-guanidinoneomycin (**10**) | 1.5 ± 0.1 |
| Paromomycin (**13**) | 19.6 ± 2.0 |
| 5′′,6′-Dideoxy-5′′,6′-diguanidinoparomomycin (**14**) | 1.8 ± 0.1 |

[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). Values represent the mean ± SD? of $n = 3$ experiments.

## Discussion

Aminoglycoside derivatives with guanidinylated aminomethyl groups or primary alcohols selectively converted to guanidinium groups were synthesized. Analogues with guanidinylated amines showed modest, if any, improvement in affinity for the bacterial A-site RNA. These analogues did not show any improvement over their parent compounds when tested for antibacterial activity. In contrast, analogues with primary alcohols converted to guanidinium groups, particularly those of the kanamycin class, consistently showed marked increases in A-site affinity, which was coupled, in most cases, with improved antibacterial activity.

**Table 3.** Inhibitory activities of both the modified and parent compounds against a panel of bacterial strains.[a]

| Compd | *E. coli* (ATCC25922) | *S. aureus* (ATCC33591) | *P. aeruginosa* (PA01) | *P. aeruginosa* (GNR0697) | *K. pneumoniae* (GNR0713) | *K. pneumoniae* (GNR1100) | *A. baumannii* (GNR0717) | *A. baumannii* (GNR1753) |
|---|---|---|---|---|---|---|---|---|
| **1** | 0.78–1.56 | 3.125 | 0.39 | 0.78 | >50 | >50 | 6.25–12.5 | 0.78 |
| **2** | 3.125–6.25 | **1.56** | 6.25 | 25 | >50 | >50 | >50 | 1.56 |
| **3** | 25–50 | 25 | 50 | >50 | >50 | >50 | 12.5 | 25 |
| **4** | 1.56–3.125 | 12.5–25 | 3.125 | 6.25 | 25 | 25 | 1.56 | 0.39 |
| **5** | *1.56–3.125* | **3.125** | **1.56** | **3.125** | 25–50 | *25* | 3.125 | *0.39* |
| **6** | >50 | >50 | >50 | >50 | >50 | >50 | 6.25 | 6.25 |
| **7** | 6.25 | 25 | >50 | >50 | >50 | >50 | 12.5 | >50 |
| **8** | *6.25* | **3.125–6.25** | >50 | >50 | >50 | >50 | *12.5* | >50 |
| **9** | 0.78 | 1.56–3.125 | 3.125–6.25 | 12.5–25 | >50 | 6.25 | 0.39–0.78 | 6.25 |
| **10** | 1.56 | **0.78–1.56** | 6.25–12.5 | >50 | >50 | *6.25* | 3.125–6.25 | *6.25* |
| **13** | 6.25 | 12.5–25 | >50 | >50 | >50 | 3.125 | 0.39 | >50 |
| **14** | 12.5 | **6.25–12.5** | **25–50** | >50 | >50 | >50 | 6.25 | **6.25–12.5** |
| **15** | 6.25 | 25–50 | 6.25 | 12.5 | >50 | >50 | 6.25 | 0.39 |
| **16** | 12.5–25 | >50 | 50 | >50 | >50 | >50 | 25 | *0.39* |

[a] Minimum inhibitory concentration (MIC) values [μg mL$^{-1}$]. MIC value equal to parent compound (*italics*); MIC value lower than parent compound (**bold**). Compounds **11** and **12** showed no activity.

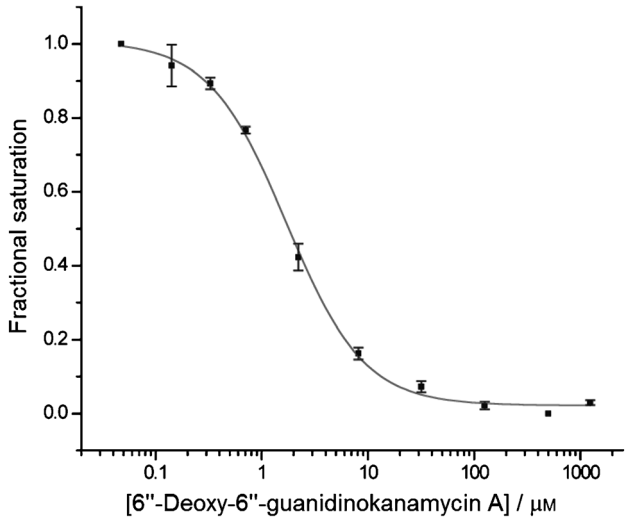



**Figure 2.** Representative A-site competitive displacement curve.

The marked increase in the affinity of the kanamycin class of derivatives upon replacing the 6″ hydroxy with a guanidinium group is interesting since the 6″ hydroxy group in the parent compounds in not involved in hydrogen bonding with the A-site, at least not in the published co-crystal structures, but the 6″ hydroxy group is in close proximity to U1406 and C1407. This could suggest that the new guanidine group in these derivatives, in addition to its overall electrostatic contribution, might be extended far enough to make new contacts with these RNA nucleobases (Figure 3).[11b,c,e] The increased binding affinity of 5″,6′-dideoxy-5″,6′-diguanidinoparomomycin (**14**) is most likely due to the increased overall charge and perhaps also replacement of the hydroxy-based hydrogen bonds observed for the parent molecule paromomycin (**13**), which is known to make A-site contacts at both the 5″ and 6′ positions,[9d] with stronger (charged) hydrogen bonds. The affinity of 6″-deoxy-6″-guanidinoapramycin (**16**) can also be potentially rationalized by examining the crystal structure of the parent aminoglycoside. A crystal structure of apramycin (**15**) with the 16S A-site shows that the 6″-hydroxy group forms a unique hydrogen-bonding interaction in which it functions as both a donor and an acceptor along the edge of the G1491–C1409 base pair.[11a] Disrupting these interactions could explain why an alteration at that site was not as well tolerated (Figure 3).

The lack of improvement in binding seen for derivatives with modifications to the 6′ amine is not entirely surprising. We recognize that the guanidine groups, being highly basic, can also somewhat attenuate the p$K_a$ of neighboring ammonium groups, leading to derivatives with similar overall charge.[12a] Additionally, all of these derivatives are modified at the 6′ amine, which is known to make critical contacts with A1408 within the A-site binding pocket.[11b,e] 6′,γ-Diguanidinoamikacin (**6**) was the only derivative of this class to show statistically significant improvements in affinity compared with the parent compound. However, 6′,γ-diguanidinoamikacin (**6**) still showed weaker affinity than 6″-deoxy-6″-guanidinoamikacin (**5**), the corresponding derivative with a guanidinium group replacing an alcohol.

When analyzing the potency and MIC values, it is important to remember that affinities to the A-site do not necessarily correlate with antibacterial potency.[16] It is interesting to note that all but two of the synthesized compounds, 6″-deoxy-6″-guanidinoamikacin (**5**) and 6″-deoxy-6″-guanidinokanamycin A (**8**), showed inferior antibacterial activity against the control *E. coli* strain ATCC25922, suggesting that improvement in activity against resistant strains is at least partially due to overcoming bacterial resistance mechanisms. This makes the broad-spectrum improvement of 6″-deoxy-6″-guanidinoamikacin (**5**) a particularly intriguing observation given that amikacin is a semi-synthetic aminoglycoside structurally derived from kanamycin A with an amino 2-hydroxybutyryl (AHB) side chain, which lowers its susceptibility to aminoglycoside-modifying enzymes. It is possible that the AHB and guanidinium modifications operate synergistically to further decrease its affinity for

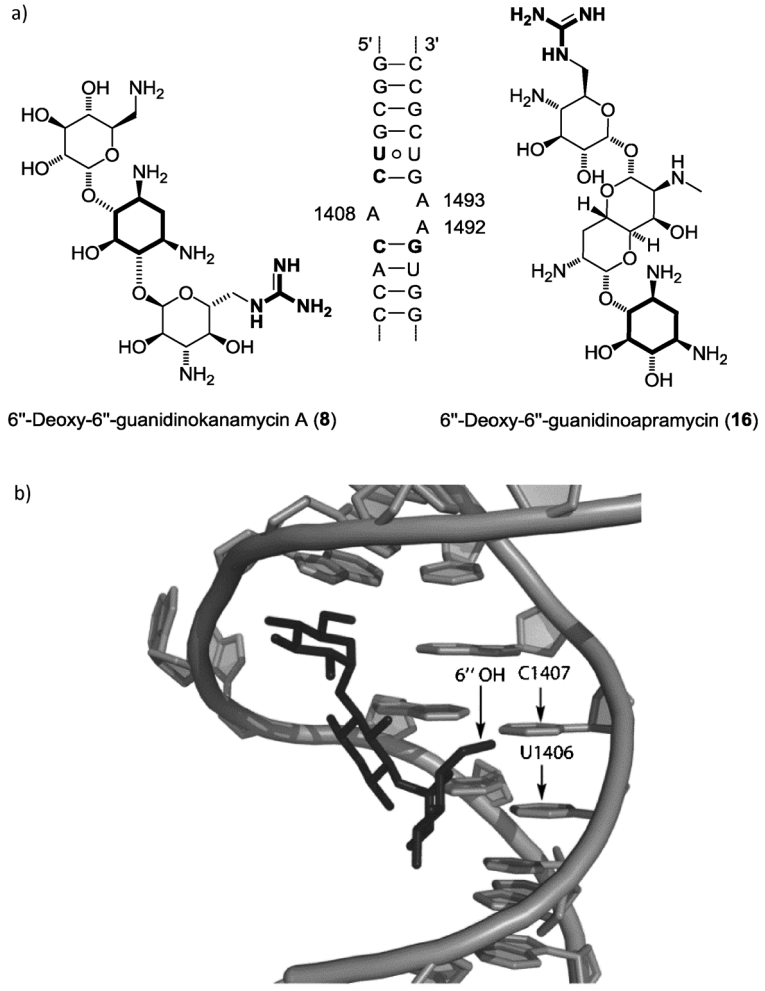



**Figure 3.** Proposed interactions between guanidinium groups of guanidino-aminoglycosides and A-site RNA bases. a) 6′′-Deoxy-6′′-guanidinokanamycin A (**8**) interactions with U1406 and C1407 and 6′′-deoxy-6′′-guanidinoapramycin (**16**) interactions with C1409 and G1491 (in bold). b) Kanamycin A (**7**) crystal structure with 6′′ OH modification site and potential new contacts of 6′′-deoxy-6′′-guanidinokanamycin A (**8**) highlighted.

modifying enzymes. Derivatives where amines were guanidinylated generally show poor antibacterial activity, in all cases inferior to the parent aminoglycosides, which could suggest that the amine at position 6′ plays an important role in antibacterial activity. This is somewhat surprising, since the majority of aminoglycoside-modifying enzymes make alterations to ring I including the AAC(6′) enzyme which directly modifies 6′ amines.[1a] In contrast, 5′′,6′-dideoxy-5′′,6′-diguanidino-paromomycin (**14**), which contains a 6′ hydroxy functionality, exhibits several improved antibacterial activities.

## Conclusions

A series of guanidino-aminoglycosides, selectively modified aminoglycosides, was synthesized. In almost all cases, these derivatives have proven to be superior binders of the bacterial A-site compared with their parent antibiotics when tested in an in vitro FRET-based assay. Some of the compounds showed potent antibacterial activity, frequently performing as well or even better than the parent aminoglycosides. In particular, 6′′-deoxy-6′′-guanidinoamikacin (**5**) proved to be particularly promising; showing equal or better activity than amikacin (**4**) against almost all of the bacterial strains tested, including several clinical isolates.

## Experimental Section

**Materials**: Unless otherwise specified, materials purchased from commercial suppliers were used without further purification. All aminoglycosides were obtained from Sigma–Aldrich as their sulfate salts. Tobramycin sulfate was converted to the trifluoroacetic acid (TFA) salt by first passing it over an anion ($OH^-$) exchange resin (Monosphere 550A, Dowex) to get the free base, then stirring in 0.1% TFA/$H_2O$. Neamine hydrochloride was made by methanolysis of commercially available neomycin sulfate.[17] 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine 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. 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 two reference strains from the American Type Culture Collection (Manassas, VA, USA): hospital-associated MRSA strain 33591 rendered resistant to rifamicin by serial passage 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: *P. aeruginosa* strain GNR0697 (blood isolate), *K. pneumoniae* strain GNR0713 (blood isolate), *K. pneumoniae* strain GNR1100 (respiratory isolate), *A. baumannii* strain GNR0717 (urine isolate), and *A. baumannii* strain GNR1753 (wound isolate).

**Instrumentation**: NMR spectra were recorded on Varian Mercury 300 and 400 MHz, Varian VX 500 MHz, and Jeol ECA 500 MHz spectrometers. All two-dimensional NMR spectra were recorded on a Jeol ECA 500 MHz spectrometer and processed using the Delta NMR Processing and Control Software (version 4.3.6). 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, Moun-

tain View, CA, USA) set at 600 nm wavelength was used for MIC assays.

**Synthesis**: Full synthetic procedures and characterization of all compounds can be found in the Supporting Information.

**Desalting**: Guanidino-aminoglycoside·TFA (up to 40 mg) was dissolved in autoclaved $H_2O$ (0.6 mL) in a sterile eppendorf tube. Monosphere 550 A (75 mg) was added, and the suspension was shaken lightly on a Fisher Vortex Genie 2 overnight. The resin was removed by centrifugal filtration and washed twice with autoclaved $H_2O$. The desalted solutions were lyophilized, and the 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]

**Parent aminoglycoside crystal structures**: PyMOL representations of all aminoglycoside/A-site crystal structures with modification sites and relevant bases highlighted can be found in the Supporting Information.

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