# Aminoglycoside Antibiotics in the 21st Century

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ABSTRACT: Aminoglycoside antibiotics were among the first antibiotics discovered and used clinically. Although they have never completely fallen out of favor, their importance has waned due to the emergence of other broad-spectrum antibiotics with fewer side effects. Today, with the dramatically increasing rate of infections caused by multidrug-resistant bacteria, focus has returned to aminoglycoside antibiotics as one of the few remaining treatment options, particularly for Gram-negative pathogens. Although the mechanisms of resistance are reasonably well understood, our knowledge about the mode of action of aminoglycosides is still far from comprehensive. In the face of emerging bacterial infections that are virtually untreatable, it is time to have a fresh look at this old class to reinvigorate the struggle against multidrug-resistant pathogens.
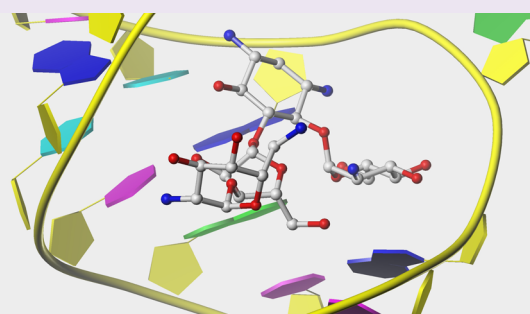
1. INTRODUCTION

Aminoglycoside antibiotics (AGAs) are first and foremost agents of bacterial warfare. Similar to other antibiotics and mycotoxins they are secondary metabolites that possess the ability to kill other bacteria or fungi in the evolutionary struggle to gain an advantage over other species competing for the same ecological niche. Their activity against human pathogens is a fortunate coincidence that has allowed broad clinical application against microbial infections in humans.

However, their origin is also the source for almost all of the resistance problems encountered today, as most of the AGA-producing species have developed strategies to avoid the deleterious effects of the antimicrobial metabolites they produce themselves or that are produced by others.

The first AGAs were isolated from the soil-dwelling bacteria species *Streptomyces* and *Micromonospora*. Streptomycin (**1**) was the first AGA discovered and was isolated in 1943 from *Streptomyces griseus*;[1] it was the first antibiotic used to successfully treat tuberculosis and was introduced into the clinic in the mid 1940s.

After the initial discovery of streptomycin and its successful introduction into medical practice, several others followed, and the development of resistance was largely overcome by the then rapid discovery and introduction of new AGAs. Notable active early AGAs include neomycin (1949), gentamicin (1963), tobramycin (1967), and sisomycin (1970). At the time, they all showed good intrinsic efficacy against Gram-negative and selected Gram-positive bacterial infections as well as *Pseudomonas* spp.

As the use of these antibiotics in clinical practice became widespread, resistance was observed more frequently, and toxicological liabilities, in particular ototoxicity and nephrotoxicity, became more obvious. This led to efforts to improve the pharmacological profile of AGAs and culminated in the discovery and subsequent introduction of a second generation

of AGAs, namely, the semisynthetic derivatives dibekacin (1971), amikacin (1972), arbekacin (1973), isepamicin (1975), and netilmicin (1976). Some of these newer aminoglycosides, notably amikacin, appeared to be less susceptible to the more common AMEs.[2]

In the late 1970s the commercial launch of other broad-spectrum antibiotics with fewer side effects, such as fluoroquinolones, carbapenems, cephalosporins, and β-lactam/β-lactamase inhibitor combinations led to a decline in interest in the search for new AGAs. Since the approval of isepamycin in 1988 and arbekacin in 1990 no new AGAs have made it to the market, and the total share of AGAs in the antibiotics market was only 2.7% in 2010.[3]

Although AGAs have never completely vanished from the clinic, the ever-increasing resistance to all other common antibiotics, especially in nosocomial infections, has once again focused clinical interest in AGAs and in particular their use in serious Gram-negative infections.

2. AMINOGLYCOSIDE STRUCTURE

The AGAs can be divided into a number of different classes based on their chemical structure and their biosynthesis. The structure of an AGA determines its susceptibility to various aminoglycoside-modifying enzymes and hence the development of resistance (see Section 4).

The general structural motif consists of an inositol derivative linked to at least one aminosugar, the whole structure containing a number of free hydroxyl and at least two amino groups (Figure 1). The hydroxyl and amino groups, which can also contain further substituents, are the key binding elements that interact with the RNA of the 30S subunit of the ribosome
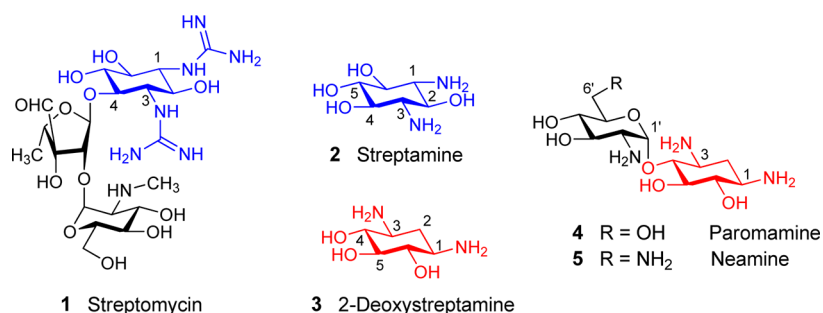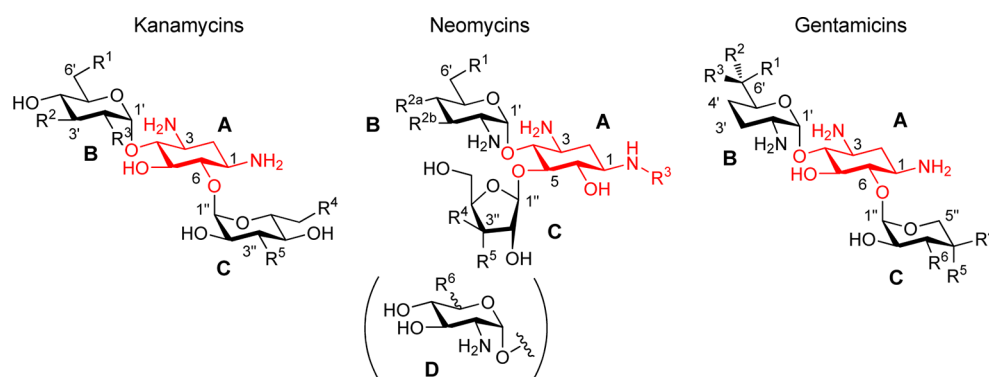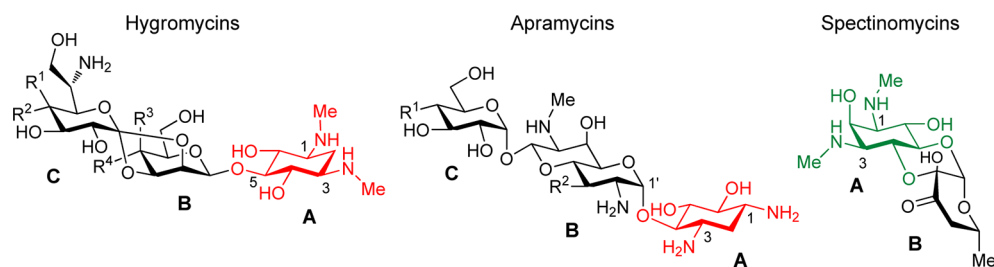
Figure 1. Streptomycins and core AGA structural elements.

Figure 2. Structures of the three main AGA classes based on paromanine: kanamycins, neomycins, and gentamicins.

Figure 3. Unusual AGA structures include the spiro ring system in the hygromycins and the fused bi- and tricyclic systems in the apramycins and in spectinomycin.

where they interfere with protein translation (see Section 3). For an excellent review of the biochemistry and genetics of aminoglycoside producers illustrating the relationships of the different biosynthetic pathways see Piepersberg et al.[4]

The first discovered AGA was streptomycin (**1**, Figure 1), which is made up of a disaccharide unit linked to the 4-position of a guanidinylated streptamine (**2**); however, there are only a few members in this class of AGAs.

A large number of AGAs contain 2-deoxystreptamine (**3**; 2-DOS) as a core scaffold and are biosynthetically derived from paromamine (**4**). AGA classes derived from paromamine include the kanamycins, neomycins, and gentamicins (Figure 2).

The kanamycin class consists of 4,6-substituted 2-DOS derivatives generally with a 3-aminoglucose as ring C and 2-amino- or 2,6-diamino-glucose as ring B.

The neomycin class has one or two hexoses and one furanose attached to the 2-DOS core in positions 4 and 5, and the amine groups are solely located on the hexoses. The third class of AGAs derived from paromamin are the gentamicins. The gentamicins consist of a 4,6-substituted 2-DOS and two

hexoses that may also contain some additional carbon side chains or an unsaturated B ring.

The majority of clinically relevant AGAs fall into the broad class of paromamine-derived AGAs, and these also form the basis of a number of semisynthetic derivatives in clinical use today.

Other classes of AGAs not derived from paromamine are the hygromycins and apramycins as well as a number of different pseudodisaccharides (Figure 3). Both the hygromycins and the apramycins still contain the 2-DOS unit as central core with substitutions in the 5- and 4-positions, respectively. The pseudodisaccharides, such as the spectinomycins, kasugamycins, fortimicins, istamycins, and sporamycins, have a number of different substituted inositols at the core with another hexose attached via the 4- or 5-position and are generally of little or no clinical importance with the exception of spectinomycin, which has actinamine as the diamino inositol unit.

3. UPTAKE AND MODE OF ACTION

3.1. Uptake. For aminoglycosides to reach their molecular target, they must first penetrate into the cytoplasm of bacteria. The mechanisms by which aminoglycoside antibiotics penetrate
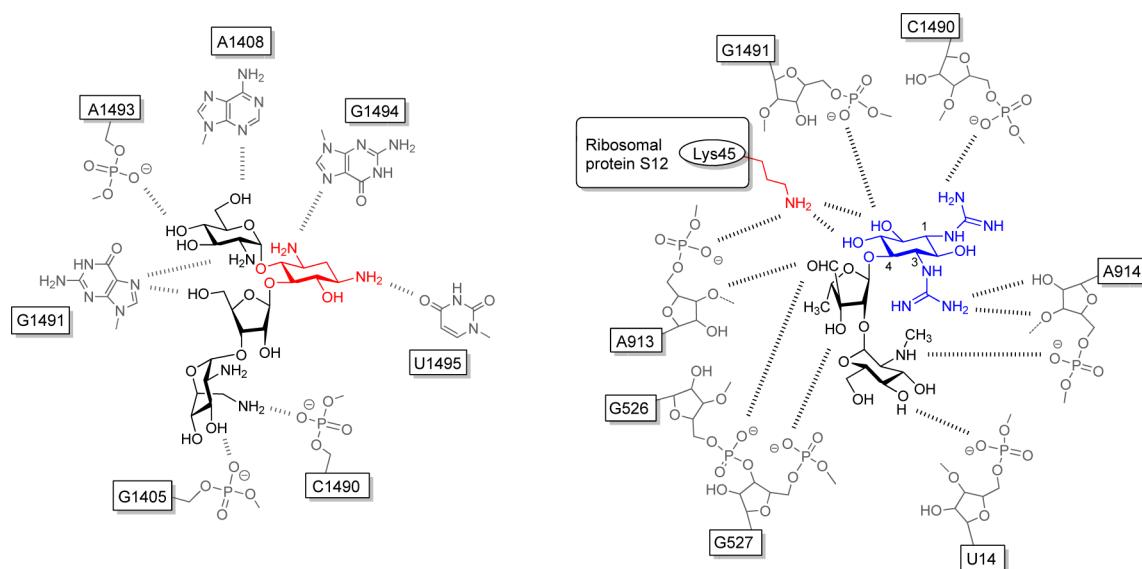
Figure 4. Interactions of paromomycin with the 16S rRNA in the A-site (left) and in the binding site for streptomycin including interactions with ribosomal protein S12 (right) (adapted from ref 31).

Gram-negative bacteria remain elusive, but a model of cellular uptake has been proposed that consists of three different stages.[5−8] The vast majority of the studies have been carried out using only two AGAs (streptomycin and gentamicin). The uptake of other AGAs is not well characterized, and as such the mechanisms that drive uptake of other AGAs may well have considerable differences.

According to the current model the first stage of AGA uptake is simply an electrostatic interaction between the positively charged AGAs and the negatively charged lipopolysaccharides (LPS) of the outer bacterial membrane.[8,9] This is largely nonspecific and solely due to the cationic nature of the AGAs resulting from a predominance of basic, ionizable amino groups within the class. The two subsequent stages are the energy-dependent phase I (EDPI) and energy-dependent phase II (EDPII).

EDPI is characterized by a slow rate of energy-dependent uptake and is correlated with AGA concentration.[10] It can also be blocked by inhibitors of oxidative phosphorylation or electron transport inhibitors.[11]

EDPII involves a rapid energy-dependent accumulation of AGAs following EDPI that uses energy from electron transport and ATP hydrolysis. However, the exact mechanism still remains unclear, as EDPII can also be reduced or completely inhibited by some inhibitors of protein synthesis, suggesting that protein synthesis is a requirement for EDPII.[12]

Due to their lack of a membrane potential and the electron transport mechanisms required for its upkeep, anaerobes are generally immune to AGAs as EDPI and EDPII cannot take place.

In the most widely accepted model the increased uptake of AGAs following the entry of the first few molecules is attributed to misreading in protein translation, which compromises cytoplasmic membrane integrity and function due to faulty proteins, leading to an autocatalytic cycle of AGA uptake, followed by cell death[13] (see Mode of Action, below).

3.2. Mode of Action. The elucidation of the mode of action of AGAs went hand-in-hand with the biochemical understanding of protein synthesis and especially the molecular basis of translation fidelity. The high level of accuracy with which translations occurs (the error rate of transcription *in vivo* in *E. coli* has been estimated to be 1.4×10^{-4} per nucleotide and thus around 4×10^{-4} per codon)[14,15] gave an early indication that more than just codon-anticodon recognition between the mRNA and the stem loop of tRNA was at the heart of protein translation (for recent reviews see, for example, refs 16−20).

The essential part of the A-site in the 30S ribosomal subunit consists of an asymmetric internal loop made up from three adenines: A1408 on one strand and A1492 and A1493 on the other strand, framed by two G-C pairs (*E. coli* nomenclature).[21] Binding of a cognate tRNA to the A-site of the 30S ribosomal unit is composed of two distinct events.[22] Before binding the A-site is conformationally dynamic (resting state or "off") and a first decoding step leads to identification of the cognate tRNA in a fast equilibrium reaction. Binding to the cognate tRNA results in a major rearrangement within the A-site in which A1492 and A1493 flip out of the internal loop. This in turn induces a much slower second step leading to a tight binding involving a number of conformational changes within the ribosome that enable a precise fit of the tRNA within the A-site (decoding state or "on").[23]

AGAs that bind into the A-site stabilize a conformation of the internal loop very similar to the "on" state with the A1492 and A1493 flipped out of the internal loop.[24,25] This allows other noncognate tRNA to bind and leads to a misreading of the mRNA and synthesis of faulty proteins. Although the interactions of the 2-deoxy streptamine AGA cores within the A-site are highly conserved across almost all AGAs, each individual AGA affects the dynamic structural changes within the ribosome occurring during translocation in a distinctly different way.[26] Interestingly, the magnitude of the binding affinity of the AGA for the A-site itself seems to be less crucial for antibacterial potency, and the actual reduction of the mobility of A1492 is the determining factor with higher reduction of mobility leading to more potent compounds.[27]

Recent evidence also shows allosteric binding sites within the ribosome that affect the mobility of ribosomal subunits, which leads to reduced translation factor binding and translational activity as well as ribosome recycling.[28]

Spectinomycin (**7**) and hygromycin B (**10**) differ slightly from the other AGAs as they bind in a different location but still very close to the A-site and interfere with translocation in this way.[29,30]

Streptomycin (**1**), the only AGA to contain a guanidinylated streptamin at its core, has a different binding site and interferes with initial tRNA selection.[31]

The crystal structures for a number of AGAs bound to the ribosome have been resolved, and Figure 4 shows the interactions of paromomycin and streptomycin within the A-site as examples. Although the binding interactions for most AGAs are different, they lead to the same loss of translational fidelity.

The details of how the loss of translational fidelity leads to cell death are less clear, as for example ribosomal mutants with reduced translational accuracy are still viable.[32,33] The main reasons for bacterial cell death following aminoglycoside uptake are thought to be either due to insertion of misread proteins into the inner membrane leading to destabilization[13,34] or uptake of AGAs to a level that leads to complete inhibition of ribosomal activity.[5] Another model suggests that the perturbed metabolism and respiration results in oxidative stress due to increased superoxide production and formation of highly toxic hydroxyl radicals.[35] Recent evidence also points to the involvement of the protein translocation machinery, the Cpx envelope stress-response, and the redox-responsive Arc two-component systems that play an important role in the gene regulation of processes important for membrane composition and membrane integrity.[36] Gene expression analysis and an *E. coli* single-gene knockout library were used to reveal some of the genes and proteins involved in AGA lethality; up-regulation of the Arc-regulated elements of the electron transport chain, the tricarboxylic acid cycle, and respiration were observed.[36]

4. MECHANISMS OF RESISTANCE

4.1. Aminoglycoside Modifying Enzymes (AME). Although 16S rRNA is the main target of all AGAs, the most prevalent mechanism of resistance is not the mutation or modification of rRNA, the function of which is highly conserved across all genera, but instead the enzymatic modification of the AGAs themselves.

There are three different families of AMEs that modify AGAs, these are ATP (and/or GDP)-dependent aminoglycoside phosphotransferases (APHs), the acetyl-CoA-dependent aminoglycoside acetyltransferases, and the ATP-dependent aminoglycoside nucleotidyltransferases. Many of these AMEs are encoded on plasmids, transposons, and integrons, which makes them highly mobile and facilitates the spreading of resistance.

The AMEs most likely evolved from enzymes of normal cellular metabolism due to selective pressure from AGAs. This is for instance supported by the observation that a mutation in the *aac(2′)*-Ia gene that encodes for an aminoglycoside acetyltransferase in *Providencia stuartii* can cause increased levels of peptidoglycan *O*-acetylation, suggesting that this could have been the original function of this enzyme.[37]

There are two different systems of nomenclature in use for the AMEs. One is from an enzymatic perspective and consists of a three-letter code to identify the activity (APH, ANT, AAC), followed by a number in parentheses that identifies the site of modification, then a roman numeral that describes a particular resistance profile that is evoked in the host (subclass), and last a lower case letter as individual identifier; the

parentheses and the subclass are separated by a hyphen, *e.g.*, AAC(3)-IIa. The other nomenclature system has a genetic perspective, with a three lower case letter code in italics for the type of activity (*aph*, *aac*, *aad*), a capital letter for the site of modification and a number as unique identifier of the individual genes.

The most important classes and subclasses of AMEs, the AGAs they confer resistance against, and their prevalence in either Gram-negative or Gram-positive bacteria are summarized in Table 1. For an excellent in-depth review on function and prevalence of AMEs, see Ramirez *et al.*[38]

Table 1. Main AMEs, Their Substrates, and Dissemination[a]

| classification	type	AGAs affected[b]	bacteria
O-Phosphotransferases			
APH(3′)	I	K, Ne, L, P, R	G+, G−
	II	K, Ne, B, P, R	
	III	K, Ne, L, P, R, B, A, I	
	IV	K, Ne, B, P, R	
	V	Ne, P, R	
	VI	K, Ne, B, P, R, A, I	
	VII	K, Ne	
APH(2″)	Ia[c]	K, G, T, S, D	G+
	I	K, G, T, N[d], D[d]	
APH(3″)	I	St	G+, G−
APH(7″)	I	H	G+
APH(4)	I	H	G−, G+
APH(6)	I	St	G+, G−
APH(9)	I	Sp	G−, G+
N-Acetyltransferases			
AAC(6′)	I	T, A, N, D, S, K, I	G−, G+
	II	T, G, N, D, S, K	
AAC(3)	I	G, S, F	G−
	II	T, G, N, D, S	
	III	T, G, D, S, K, N, P, L	
	IV	T, S, N, D, S, A	
	VII	G	
AAC(1)		P, L, R, Ap	G−, G+
AAC(2′)	I	T, S, N, D, Ne	G−, G+
O-Nucleotidyltransferases			
ANT(2″)	I	T, G, D, S, K	G−
ANT(3″)	I	St, Sp	G−
ANT(4′)	I	T, A, D, K, I	G+, G−
	II	T, A, K, I	
ANT(6)	I	St	G−, G+
ANT(9)	I	Sp	G+

[a]Adapted from refs 38−40. [b]A amikacin, Ap apramycin, B butirosin, D dibekacin, G gentamicin, H hygromycin, I isepamicin, K kanamycin, L lividomycin, N netilmicin, Ne neomycin, P paromomycin, R ribostamycin, S sisomicin, Sp spectinomycin, St streptomycin, T tobramycin. [c]from the bifunctional enzyme AAC(6′)-APH(2″). [d]not APH(2″)-Ic.

4.1.1. Aminoglycoside Acetyltransferases (AACs). AACs catalyze the acetylation of amino groups in aminoglycosides and belong to the GCN5-related *N*-acetyltransferase (GNAT) superfamily of proteins. A large number of AACs has been identified to date, the structures of several AACs have been resolved, and structural aspects and mechanism of these and other members of the family have been studied (see refs 38 and 41 and references therein). There are four classes and a number

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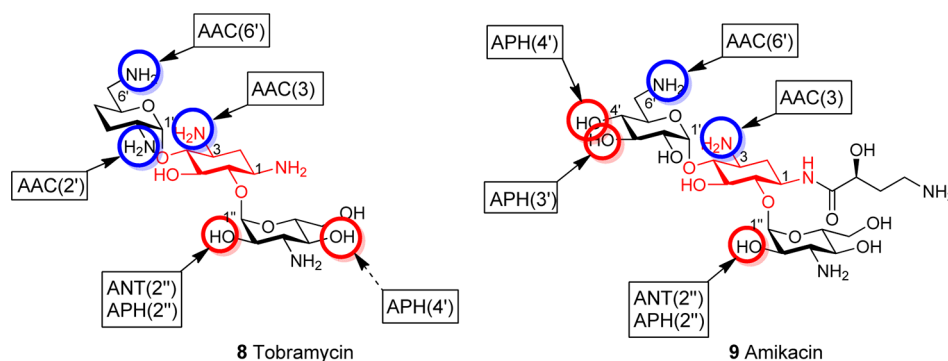
Figure 5. Sites of enzymatic modifications on tobramycin and amikacin by various AACs, APHs, and ANTs.
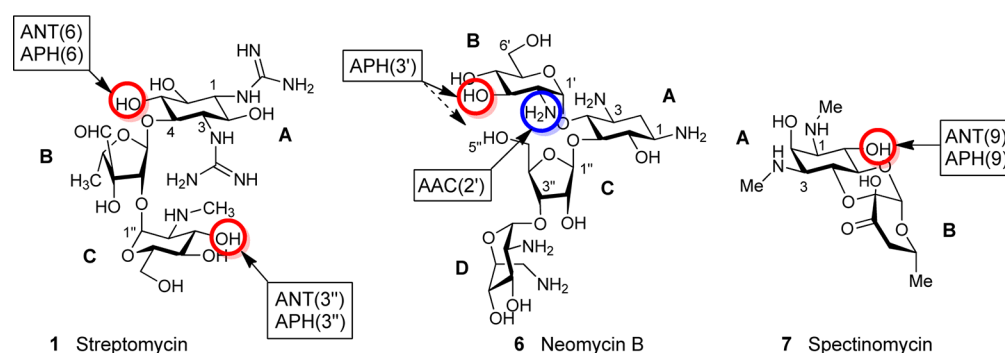
Figure 6. Sites of enzymatic modifications on streptomycin, neomycin B, and spectinomycin.

of subclasses of AACs, namely, AAC(1), which has no subclasses, AAC(3)-I to X, AAC(2′)-I, and AAC(6′)-I and -II.

Acetylation of AGAs by AAC(1) enzymes does generally not lead to a significant reduction in antibiotic activity,[42] and AAC(1) enzymes are extremely rare in clinical isolates.

AAC(3) enzymes of all nine subclasses (the only AAC(3)-V enzymes isolated were found to be identical to AAC(3)-II and the subclass was eliminated) are found only in Gram-negatives. While the AAC(3)-II enzymes and especially AAC(3)-IIa are found in a large variety of genera, the other subclasses are less common, and the subclasses VII−X have only been found in actinomycetes.[38,43,44]

AAC(2′)-I is the only subclass within the AAC(2′) enzymes and is found in Gram-negatives and *Mycobacterium*, and members of this class confer resistance to a large number of different members in the neomycin, kanamycin, and gentamicin classes of AGAs.

AAC(6′) enzymes are present in Gram-negatives as well as Gram-positives and are by far the most common of all AMEs. The 6′-amino group in AGAs plays an important role in rRNA binding, and acetylation of this amino group causes resistance to the majority of useful AGAs.[45] The AAC(6′) genes are often part of mobile genetic elements and have been found in plasmids and chromosomes. These enzymes are also a well-studied class of AME, and the AAC(6′)-I class is so highly populated that double lower case letters are required for the identification of individual enzymes.[38]

The most clinically relevant enzyme in this class is probably AAC(6′)-Ib. It is present in over 70% of AAC(6′)-I-producing Gram-negative clinical isolates[40] causing resistance to the majority of useful AGAs, with the exception of gentamicin, and some of its variants show an extended spectrum of resistance. Some significant progress in the understanding of AAC(6′)-Ib and its variants has been made by elucidation of the crystal

structure and the construction of a molecular model.[46] AAC(6′) enzymes can also exist as fusion proteins,[47] for example, AAC(6′)-Ie/APH(2″)-Ia[48,49] or ANT(3′)-Ii/AAC(6′)-IId,[50] which further expands the resistance profile of organisms carrying these genes and make them a serious obstacle to treatment.

4.1.2. Aminoglycoside Phosphotransferases (APHs). Phosphorylation of hydroxyl groups in AGAs introduces a negative charge into the molecule, which results in a dramatic change in their ability to bind to the A-site in the ribosome. The genes for APHs are often found on multidrug-resistant R plasmids, transposons, and integrons, leading to problems in the treatment of some enterococcal and staphylococcal species.[40] The different classes and subclasses of APHs are APH(4)-I, APH(6)-I, APH(9)-I, APH(3′)-I to -VII, APH(2″)-I to -IV, APH(3″)-I, and APH(7″)-I.

APH(4) enzymes only mediate resistance to hygromycin (see Figure 7) and are not clinically important, the same as APH(9), which mediates resistance to spectinomycin (see Figure 5).

APH(6) confers resistance to streptomycin (see Figure 6) and is important as part of the Tn5 composite transposon used for molecular genetics.

The largest class of the APH family are the APH(3′) enzymes, which phosphorylate the 3-hydroxyl in the B-ring in many AGAs. APH(3′)-IIIa is widely disseminated within Gram-positives, and in some 4,5-substituted AGAs, such as neomycin B (see Figure 6) and butirosin, both the 3′- and the 5″-positions can be phosphorylated by APH(3′)-IIIa.[51] Even in the case of lividomycin A, where there is no 3-hydroxyl, the phosphorylation by this enzyme may still occur only at the 5″-position of the ribose ring.[51] The other subclasses are of lesser clinical importance, but the APH(3′)-I enzymes are widely distributed largely among Gram-negatives, and some of the

aph(3′)-I genes as well as *aph(3′)-II* genes are used in cloning vehicles and vectors.[38] The remaining APH(3′) subclasses are less common.

APH(2″) enzymes play an important role in the gentamicin resistance in Gram-positive bacteria (see Figure 7), and APH(2″)-Ia also exists as part of the bifunctional fusion protein AAC(6′)-Ie/APH(2″)-Ia, which confers broad spectrum AGA resistance.[52]
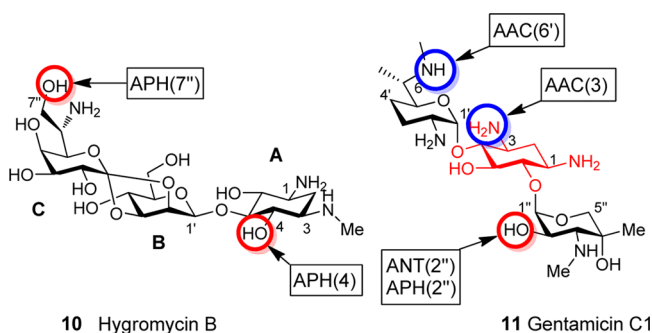
Figure 7. Sites of enzymatic modifications on hygromycin B and gentamicin C1.

APH(3″) and APH(7″) mediate resistance to streptomycin (Figure 6) and hygromycin B (Figure 7), respectively, and are of little practical importance.

4.1.3. Aminoglycoside Nucleotidyltransferases (ANTs). ANTs catalyze the transfer of an AMP group from ATP to a hydroxyl group in the AGA. The different classes of ANTs are ANT(6), ANT(9), ANT(4′), ANT(2″), and ANT(3″); there are no subclasses with the exception of ANT(4), which includes the subclasses I and II. Although they are the smallest AME family by number, ANTs are of significant clinical importance because both tobramycin and amikacin (Figure 5) as well as gentamicin (Figure 7) are susceptible to ANT(2″).

The genes for ANT(6) are widely spread among Grampositives and confer resistance to streptomycin (Figure 6).[40,53]

ANT(9) enzymes are able to modify spectinomycin (Figure 6) and are found in some Enterococci.[38]

ANT(4′) can also in some cases modify the 4″-position[54] and the subclass ANT(4′)-I is found in plasmids of Staphylococci, Enterococci, and *Bacillus* spp., whereas ANT(4′)-II is found in some Gram-negatives.

The gene for ANT(2″)-Ia is widely distributed and present in enterobacteria and nonfermentative Gram-negative bacilli, is commonly encoded by plasmids and transposons,[40,55] and confers resistance to a number of AGAs in the kanamycin class.

The most commonly found ANT enzymes are from the ANT(3″) class, which confers resistance to streptomycin and spectinomycin (see Figure 6). The gene for ANT(3″) exist as gene cassettes and are part of a large number of integrons, plasmids, and transposons and can also be part of gene fusions.[38]

4.2. Target Modification. Alteration of the target binding site can occur by either mutation or enzymatic modification. For the 2-DOS containing classes of AGAs these changes usually involve the 16S rRNA in the codon-decoding A-site of the ribosomal 30S subunit,[56] but mutations directly in the highly conserved A-site are not very common, as most mutations in this area are lethal.

4.2.1. Target Modification by Mutation. The prototypical example for a successful mutation in the ribosomal 30S subunit

binding site is the mutation of A1408 in the single-stranded region of the 16S rRNA loop that confers high-level resistance to neomycin and the gentamicins, as well as other members of the 2-DOS class, by interrupting key interactions with the AGAs (see Figure 4).[57]

In the streptomycin class of AGAs there also is an interaction with the ribosomal protein S12 (see Figure 4), and mutations in this protein can affect binding and lead to resistance. This is for example the case for *Mycobacterium tuberculosis* where mutations in the 16S rRNA and the S12 protein give rise to high-level resistance.[58,59]

Mutations of RNA or protein sites not directly involved in binding can in some cases also confer resistance, possibly due to a conformational change in the binding site caused by these changes. Examples of this type of influence on binding by remote-site mutations is the streptomycin resistance in *Thermus thermophilus*, which is caused by a A1408G mutation not proximal to the binding site,[60] and a mutation in the S4 protein in *Salmonella typhimurium* that also leads to streptomycin resistance.[61]

4.2.2. Ribosomal Methyltransferases. Enzymatic modification of the target binding site can be mediated by methyltransferases (MTases) that transform nucleotides in the binding site into the corresponding 7-methyl derivatives.[62,63] DNA methylation in bacteria controls numerous processes including replication, regulation of transcription and transposition, and mismatch repair. MTases that target the 16S rRNA can be found in actinomycetes, natural producers of AGAs, and are one of the mechanisms to protect them from the toxicity of their own metabolites.

The 16S rRNA MTases exist in two distinct groups based on their target nucleotides, G1405 and A1408, and are further subdivided by origin. Together they form the Rma (resistance methyltransferases for aminoglycosides) superfamily. The presence of Rmas was thought to be restricted to AGA-producing bacteria, but recently plasmid-mediated MTases that lead to very high-level resistance to AGAs have been reported in a number of pathogens[64] including *Pseudomonas aeruginosa*,[65,66] *Klebsiella pneumoniae*,[67] *Escherichia coli*,[68,69] *Serratia marcescens*,[70] *Proteus mirabilis*,[71] and also *Acinetobacter baumannii*,[72] making further spread to other strains likely.

Although the Rmas are not of clinical significance at the moment, they do pose a considerable potential threat because of the almost complete resistance against AGAs that they can confer. One notable exception here is apramycin (**12**, Figure 8), an AGA used in veterinary medicine, that still is active against Enterobacteriaceae carrying the genes for 16s rRNA MTases.[73] The unusual structure of apramycin with the unsubstituted 6-position in the 2-DOS ring and the unusual fused ring system
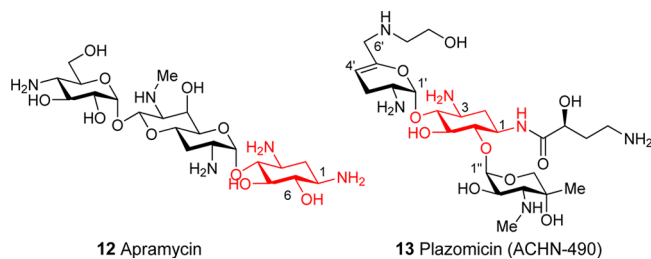
Figure 8. Structure of apramycin, a veterinary AGA active against 16S rRNA methyltransferase carrying bacteria, and plazomicin, a candidate that has successfully completed phase II clinical trials.

may hold the key to overcoming resistance mediated by MTases.

4.3. Change of Uptake and Efflux. Increased resistance to AGAs was also observed in *P. aeruginosa* strains resistant against antimicrobial lipopeptides (such as colistin) as a result of changes to the outer membrane lipopolysaccharides (LPS). The PhoP-PhoQ two-component regulatory system responsible for these changes can be up-regulated by Mg^{2+} starvation[74] or the presence of polyamines.[75] As the changes to the LPS for the resistant strains reduce its net negative charge,[76] one possible reason for the increased resistance against AGs could be due simply to a reduced electrostatic interaction in the first stage of uptake.

As transport across the membrane into the cell requires energy and involves the proton motive force (see Section 3.1), any mutations that lead to defect electron transport chain components will confer resistance.[77−79] Recently it has been shown that NO-mediated repression of respiratory activity was able to block EPDI and EPDII in *Salmonella*, *P. aeruginosa*, and *S. aureus*[80] as well as *Bacillus*.[81] As NO is associated with host inflammatory responses,[82,83] this may diminish the effectiveness of aminoglycoside therapy.

Although the polar nature of AGAs originally led to the assumption that they would not be likely to be subject to multidrug efflux pumps, it is today recognized that efflux is a general mechanism of resistance that affects many different types of compounds, particularly in Gram-negative bacteria.[84−87] The most prominent members of the five classes of efflux pumps able to transport AGAs are in the resistance nodulation division (RND) family, which is mainly present in Gram-negative bacteria.[88] Their action contributes to resistance in *Pseudomonas*, *Acinetobacter*, *Brucella*, *Burkholderia*, *Enterobacter*, *Escherichia*, *Helicobacter*, and *Stenotrophomonas* spp.[84,85]

Efflux proteins from the major facilitator superfamily (MFS) have been found to be involved in AGA resistance in *A. baumannii*[89] and *V. cholera*.[90] There is also some evidence that MFS activity may explain the streptomycin resistance of some *M. tuberculosis* strains that cannot be assigned to other resistance mechanisms.[91]

However, the overall contribution of efflux to resistance against AGAs is modest at best and seems to play a more important role only in the adaptive aminoglycoside resistance in *P. aeruginosa*.[92] This is likely due to the fact that nonefflux-based resistant strains are available in the local pathogen population and are likely to become prevalent as they impose less "cost" on the bacteria than a broad specificity efflux does.[84]

4.4. Membrane Proteases. After translocation of proteins across the membrane, several overlapping cell envelope maintenance and stress response systems are responsible for protein biosynthesis quality control. This includes signal sequence cleavage, regulation of protein abundance and degradation of misfolded and mistargeted proteins.[93] Although usually not considered a mechanism of resistance *per se*, the bacterial membrane proteases that are part of this system are responsible for the proteolysis of mistranslated proteins due to the presence of AGAs. Only if expression levels of misread proteins are higher than the ability of the membrane proteases to identify and degrade these proteins will faulty proteins be able to accumulate enough to perturb membrane integrity.

In *E. coli* deletion of the genes *hflK* or *hflC*, regulators for the membrane protease FtsH, led to increased susceptibility toward gentamicin compared to the wild-type, whereas treatment with the DNA gyrase inhibitor norfloxacin showed no significant difference, demonstrating the importance of FtsH for survival of the bacteria when challenged with an AGA.[36]

It could also be demonstrated that proteolysis does form part of the intrinsic aminoglycoside resistance in *P. aeruginosa*.[94] The membrane protease FtsH was again identified as a major determinant of resistance and inactivating genes relating to FtsH, and its regulation led to markedly increased sensitivity to tobramycin. Multiple mutations led to synergistic effects, increasing the sensitivity against several classes of antibiotics and tobramycin sensitivity up to 500-fold.[94]

5. NEW DEVELOPMENTS

With the increased occurrence of nosocomial infections caused by multidrug resistant strains, especially Gram-negative bacteria, the interest in AGAs for clinical use has been rekindled and led to increased research efforts both in academia and industry (for recent reviews see refs 41 and 95−100). Current research is aimed mainly at overcoming resistance by blocking sites of AME action or designing AME inhibitors,[99,100] modulating AGA pharmacology,[101] accessing new binding modes,[102] and understanding and overcoming toxicity issues such as ototoxicity[103,104] and nephrotoxicity.[105] Related research aims to exploit the ability of AGAs to interact with RNA for the generation of antivirals[41] and the potential to use them in the treatment of certain genetic diseases.[97]

The most advanced of these novel AGAs is Achaogen's plazomicin[106] (**13**, Figure 8). Plazomicin (formerly ACHN-490) is obtained through chemical synthesis starting from sisomicin. It combines structural features of several AGAs, and the presence of deoxy sugars and the substitution of some of the amines reduces the number of structural elements susceptible to AMEs. A hydroxy aminobutyric acid (HABA) side chain in the 1-position of the 2-DOS ring generates the same structural feature that gives amikacin, arbekacin, and isepamicin improved activity against many resistant strains, and a hydroxyethyl chain in the 6′-postion leads to further improvement of activity and desirable pharmacological properties.

Plazomicin was tested against panels of Gram-negative and Gram-positive pathogens, including bacteria containing various resistance mechanisms. It was active against strains expressing known AMEs affecting amikacin and gentamicin, including the three most common such enzymes found in Enterobacteriaceae. However, resistance due to changed membrane permeability and MTases still affected plazomicin in the same way as amikacin and gentamicin and led to high MICs.[106,107]

Plazomicin has successfully completed its phase II clinical trial for complicated urinary tract infections in early 2012. Neither nephrotoxicity nor ototoxicity was observed in the clinical trials so far.

Other AGA derivatives in early development in industry include arbekacin derivatives that showed promising MICs against some resistant strains as well as derivatives of kanamycin, some derivatives of neomycin and paromomycin, and some gentamicin derivatives.[96] However, none of these compounds are as far advanced as plazomicin.

6. CONCLUSIONS

Increased clinical use of AGAs in recent years has been sparked by the limitations imposed on treatment of severe bacterial infections by multidrug-resistant pathogens. Improved dosage regimens and therapeutic drug monitoring have made the use

of AGAs much safer for empirical and directed therapy.[108] On the other hand the clinical toxicity of the classic AGAs with its potentially severe side effects, namely, nephrotoxicity, ototoxicity, and to a lesser extent neuromuscular toxicity, still remains a concern.

We have highlighted renewed research interest in the AGAs. Today our knowledge of the mechanisms of resistance and AGA mode of action is greater than ever before. Efforts from academia and industry in the past decade have already produced new clinical candidates active against many resistant strains and with possibly fewer side effects. With advances in the chemistry of AGAs the synthesis of specific derivatives to probe mode of action and to allow systematic fine-tuning of the activity and pharmacological profiles has become easier, and the realization of a fully synthetic AGA in the clinical pipeline in the near future is a distinct possibility.

Armed with a better understanding of their biology, chemistry, and pharmacology, we are now in a good position to take advantage of the excellent antibacterial scaffold that nature has provided to generate "third generation" AGAs in order to address issues associated with this class of antibiotics. Faced with a severe paucity of novel antibiotics in the clinical pipeline, particularly those possessing activity against resistant Gram-negative bacteria, it is time to have a fresh look at this old class of antibiotics to reinvigorate the struggle against multidrug-resistant bacterial pathogens.

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Notes
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