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Structure-Based Design and Synthesis of Apramycin-Paromomycin Analogues. Importance of the Configuration at the 6′-Position and Differences Between the 6′-Amino and Hydroxy Series.

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ABSTRACT: The preparation of a series of four analogues of the aminoglycoside antibiotics neomycin and paromomycin is described in which ring I, involved in critical binding interactions with the ribosomal target, is replaced by an apramycin-like dioxabicyclo[4.4.0]octane system. The effect of this modification is to lock the hydroxymethyl side chain of the neomycin or paromomycin ring I, as part of dioxabicyclooctane ring, into either the *gauche-gauche* or the *gauche-trans* conformation (respectively axial or equatorial to the bicyclic system). The antiribosomal activity of these compounds is investigated with cell-free translation assays using both bacterial ribosomes and recombinant hybrid ribosomes carrying eukaryotic decoding A site cassettes. Compounds substituted with an equatorial hydroxyl or amino group in the newly formed ring are considerably more active than their axial diastereomers lending strong support to crystallographically-derived models of aminoglycoside-ribosome interactions. One such bicyclic compound carrying an equatorial hydroxyl group has activity equal to that of the parent, yet displays better ribosomal selectivity, predictive of an enhanced therapeutic index. A paromomycin analog lacking the hydroxymethyl ring I side chain is considerably less active than the parent. Antibacterial activity against model Gram negative and Gram positive bacteria is reported, for selected compounds, as is activity against ESKAPE pathogens and recombinant bacteria carrying specific resistance determinants. Analogues with a bicyclic ring I carrying equatorial amino or hydroxyl groups mimicking the bound side

chains of neomycin and paromomcyin, respectively, show excellent activity and by virtue of their novel structure retain this activity in strains that are insensitive to the parent compounds.

Introduction.

Multidrug-resistant infectious diseases present a significant and ever increasing threat to society and demand the development of new generations of antibiotic agents.[1-4] One approach to the development of new and improved antibiotics is the reinvestigation of existing drug classes, taking advantage of the power of modern synthetic organic chemistry[5] and of the accrued knowledge of the mechanisms of action of the antibiotics and of their inactivation by resistance determinants. The aminoglycoside antibiotics (AGAs) are particularly attractive in this light as both their mechanism of antibacterial action and the common causes of resistance to them are well-understood.[6-9] Advantages of the AGAs as parents for optimization include their broad spectrum of activity, their non-allergenic character, and their lack of effect on the host intestinal microbiome.[9] In addition, AGAs have physicochemical properties consistent with suggested guidelines for the development of new antibacterial compounds,[10] and following decades of use in the clinic, their PK, PD and ADME properties are well-understood and largely predictable.[9] Finally, existing AGAs are available on a large scale by fermentation, and their chemistry is well-documented,[11-12] making them good candidates for optimization by chemical modification. Indeed, these factors underlie the development of plazomicin,[9,13-14] a new generation semi-synthetic AGA currently in phase three clinical trials, and of related compounds[15] designed to circumvent most current mechanisms of AGA resistance.

In addition to widespread resistance, clinical use of existing AGAs is limited by nephrotoxicity and ototoxicity (drug-induced hearing loss).[9,16-17] Previous studies suggested that increased antibacterial activity frequently comes at the cost of increased toxicity, particularly when

achieved by the introduction of additional basic amines, thereby highlighting the difficulty of the problem.[18-20] Fortunately, nephrotoxicity can be adequately managed and rendered effectively reversible by regimes based on the use of a single daily dose.[21-22] Ototoxicity on the other hand is irreversible and affects up to 20% of the patient population,[16,23] with estimates as high as 37% in patients taking AGAs for prolonged periods.[24] Ototoxicity arises through damage to cochlea hair cells, which take up AGAs through mechanotransducer channels,[25-26] and in which AGAs persist for days after administration in contrast to the rapid clearance of AGAs from the body as a whole.[9,16-17] In the general patient population ototoxicity occurs in a sporadic dose-dependent manner; an aggravated form occurs in genetically susceptible individuals and is linked to mutations in mitochondrial rRNA, especially the transition mutation A1555G in the A-site of the mitoribosomal small subunit.[27] Genetically susceptible individuals constitute approximately 1 in 500 of the native European population, and it has been suggested that children be screened for genetic susceptibility before treatment with AGAs.[28-29]

Clearly, future generations of AGAs must be engineered address the issue of toxicity, in particular ototoxicity, and to overcome resistance mechanisms, without compromising antibacterial activity. Optimization of AGAs for enhanced activity and reduced ototoxicity is aided by detailed knowledge of the drug binding site: the bacterial decoding A site in helix 44 of the small ribosomal subunit,[30-33] and the analogous human decoding A site[17,25,34-36] whose complexation with AGAs is a significant cause of ototoxicity (Figure 1).

In our laboratories, optimization of ribosomal selectivity is guided by cell-free translation assays based on a set of wild type bacterial and mutant recombinant ribosomes carrying single point mutations either in the decoding A site characteristic of the eukaryotic ribosomes, or on hybrid ribosomes incorporating complete eukaryotic decoding A site cassettes.[37] Indeed, the use of such

mutant and hybrid ribosomes led us to the unusual monosubstituted 2-deoxystreptamine AGA apramycin **1**, with its bicyclic ring I, as a first generation AGA with excellent selectivity for the bacterial ribosome over the human mitochondrial and cytosolic ribosomes, that is essentially devoid of ototoxicity in the guinea pig model.[38-3940] Synthetic derivatives of the 4,5-AGA paromomycin **2** substituted in ring I at the 4′-position or at both the 4′- and 6′-positions, designed to mimic apramycin, also showed reduced affinity for the mitochondrial and cytosolic ribosomes. Animal models confirm the expected significantly reduced ototoxicity of selected paromomycin derivatives.[41-42] However, except for certain 4′-*O*-glycosyl derivatives,[43] this was accompanied by reduced antibacterial activity.

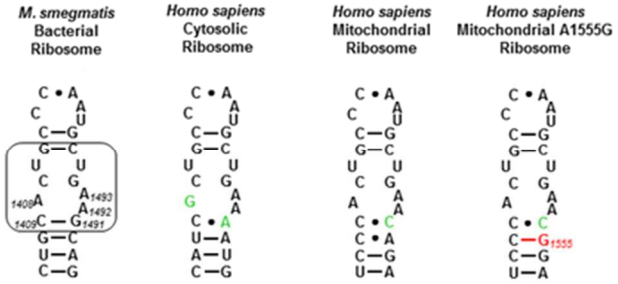
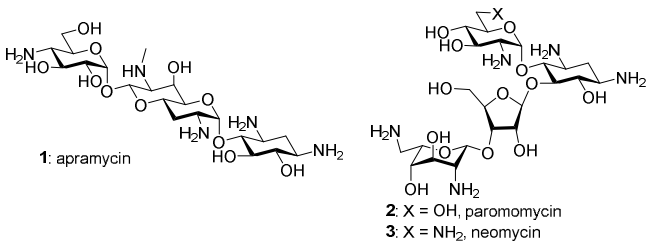


Figure 1.[43] Decoding A sites of prokaryotic and eukaryotic ribosomes. The AGA binding pocket is boxed. The bacterial numbering scheme is illustrated for the AGA binding pocket. Changes from the bacterial ribosome binding pocket are coloured green. The A1555G mutant conferring hypersusceptibility to AGA ototoxicity is coloured red.

We report on the structure-based design of an improved paromomycin derivative, incorporating both an apramycin-like bicyclic ring I and a conformationally-restricted hydroxylmethyl group ideally poised to mimic the paromomcyin A1408 interaction. In contrast to previous conformationally-restricted AGA analogues,[44-46] this derivative retains the full antibacterial

activity of the parent and displays exquisite selectivity for the bacterial over the eukaryotic mitochondrial and cytosolic ribosomes.



Design.

X-Ray crystallographic studies of both the 4,5- and 4,6-disubstituted 2-deoxystreptamine classes of aminoglycosides bound to the decoding A site of the bacterial 30S ribosomal subunit and its truncated models reveal a pseudo-base interaction, pairing N1 and N6 of A1408 with the 6′-substituent (OH in paromomycin **2** or NH_2 in neomycin **3**) and the ring I oxygen (O5′) of the AGA.[30,41,47-48] In these structures the ring I side chain invariably adopts the *gauche*,*trans* (*gt*) conformation (Figure 2)[49] in the pseudo-base pair. We classify this ring I *gt* conformation-A1408 interaction as type I pseudo-base pair (Figure 3a) to distinguish it from the apramycin ring I-A1408 interaction,[38,50] here called a type II pseudo-base pair (Figure 3b and 3c), in which the 6′-hydroxy group takes up the *gauche*,*gauche* (*gg*) conformation.

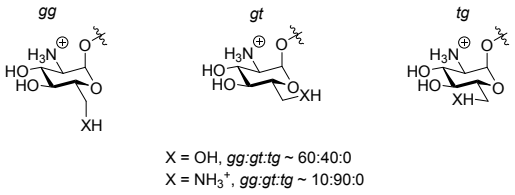


Figure 2. Side Chain conformations of Ring I and estimated populations based on methyl 2-amino-2-deoxy-α-D-glucopyranoside and methyl 2,6-diamino-2,6-dideoxy-α-D-glucopyranoside.

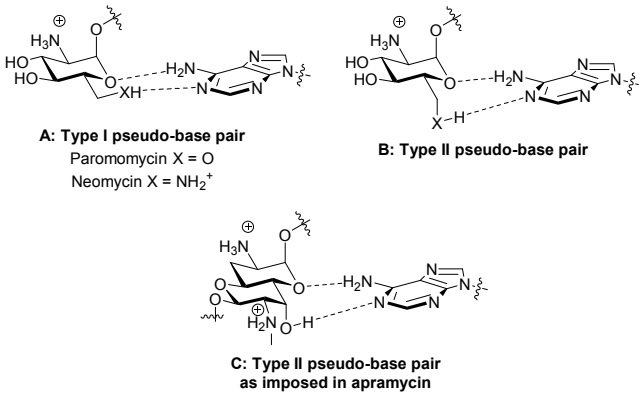
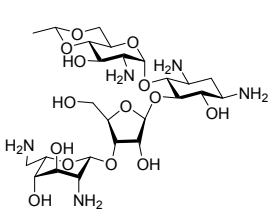


Figure 3. Type I and II Pseudo-base Pairs

Previous work with a series of 4′-*O*-alkyl paromomcyin derivatives revealed a chain length of 2 or 3 carbons in the appended alkyl group to afford the optimum reduction in mitoribosomal activity with the least loss of antibacterioribosomal activity.[42] In the 4′,6′-*O*-alkylidene paromomycin derivatives studied the ethylidene derivative **4** had a better profile than the comparable methylidene derivative lacking the terminal methyl group, again suggesting a 2 carbon chain on O4′ to be optimal.[42] Therefore, in designing further apramycin-paromomycin hydrids we opted to construct a *trans*-dioxadecalin-like structure carrying a methyl substituent designed to overlay the ethylidene acetal **4**. We further reasoned that the antibacterial activity of these new derivatives would be enhanced by the incorporation of a hydroxyl group at the 6′-position so as to permit complete pseudo-base pair formation with A1408. Finally, the introduction of additional basic amines to increase antibacterial activity owing to anticipated problems with correspondingly increased toxicity.[18-20] These considerations led us to **5** and **6** as targets, with the axial isomer **5** mimicking the *gg* conformation of the paromomycin side chain (type II pseudo-base – apramycin interaction) and the equatorial isomer **6** mimicking the *gt* conformation (type I pseudo-base interaction). We also targeted the 6′-amino analogs **7** and **8** in

the hope of harnessing the neomycin-like increased activity resulting from the extra amino group.

Consideration of the differences in geometry of the Type I and Type II pseudo-base pairs (Figure 3) lead us to re-evaluate the effect of deoxygenation at the 6′-position in paromomycin. Thus, 6′-deoxyparomomycin **9** and 6′-deoxy-6′-fluoroparomomycin **10**, previously only investigated for their antibacterial activity,[51] were also screened for activity and selectivity at the target level with the cell-free translation assays. Seeking to remove any possibly unfavorable hydrophobic interactions in the **9**-ribosome complexes, we also prepared and investigated the novel 6′-deshydroxymethyl paromomycin derivative **11**.



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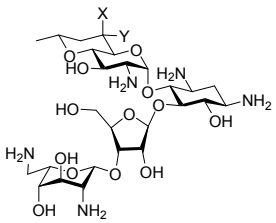
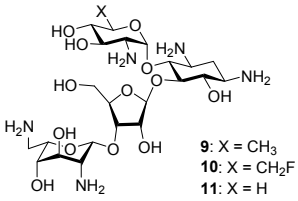


5: X = OH, Y = H
6: X = H, Y = OH
7: X = H, Y = NH_2
8: X = NH_2, Y = H

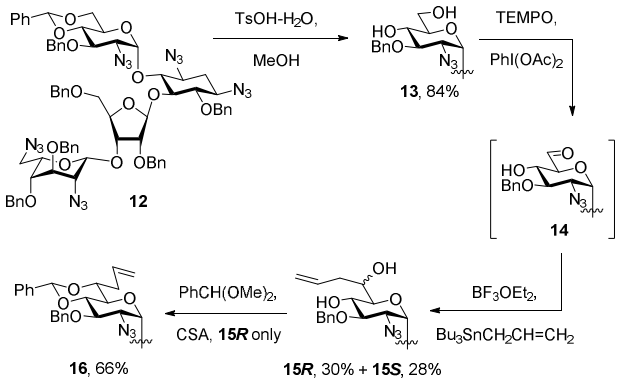


9: X = CH_3
10: X = CH_2F
11: X = H

Synthesis.

Synthetic work began with the fully protected paromomycin derivative **12**,[52] which on treatment with toluenesulfonic acid in methanol afforded the known diol **13**.[52] Controlled selective oxidation of the primary alcohol in **13** with catalytic tetramethylpiperidinoxyl (TEMPO) and bis(acetyoxy)iodobenzene[53] then gave an unstable aldehyde **14**, which on exposure to allyltributylstannane and boron trifluoride etherate[54] in dichloromethane at 0 ^{o}C gave the *R* and

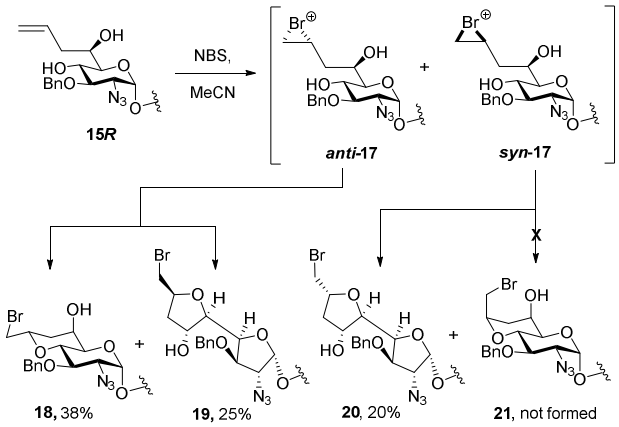
S-isomers of **15** in 30% and 28%, respectively, for the two steps. Transacetalization of one isomer with benzaldehyde dimethylacetal gave the benzylidene acetal **16**, whose all equatorial configuration was established by nuclear Overhauser correlations between the three axial protons on the upper rim thereby identifying its immediate precursor as the *R*-alcohol **15*R*** (Scheme 1). The diastereoisomer **15*S*** was not converted to the corresponding benzylidene acetal under the same mild conditions.



Scheme 1. Synthesis of Alcohols **15*R*** and **15*S***

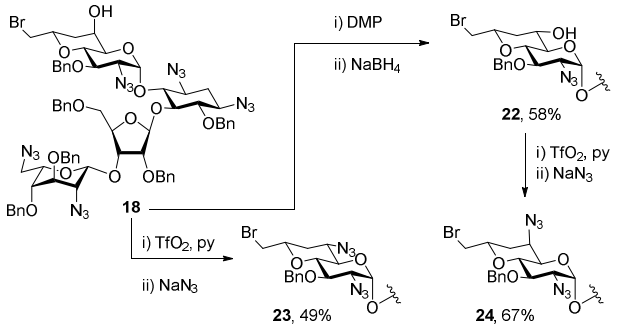
Treatment of alcohol **15*R*** with *N*-bromosuccinimide in acetonitrile at 0 °C gave the desired cycloetherification product **18** in 38% yield along with two byproducts **19** and **20** in 25 and 20% yield, respectively (Scheme 2). The bicyclic derivative **18**, whose relative configuration rests on coupling constant analysis and the observation of nuclear Overhauser effects around the newly formed ring, arises from NBS attack on the *Re*-face of the alkene **15*R*** to give the cyclic bromonium ion *anti*-**17** followed by nucleophilic ring closure with the 4′-hydroxy group. The diastereoisomeric product **21** arising from NBS attack on the *Si*-face of **15*R*** with subsequent six-membered ring formation was not observed. The two rearranged products **19** and **20** are formed by initial attack of the pyranosyl ring oxygen on the cyclic bromonium ions *anti*- and *syn*-**17**, respectively, with subsequent pyranoside to furanoside rearrangement. Related rearrangements

arising from intramolecular nucleophilic attack of pyranoside ring oxygens on cyclic bromonium ions have been described.[55-56] The relative configurations of the two rearranged products **19** and **20** were determined by extensive NMR spectroscopic studies following deprotection.



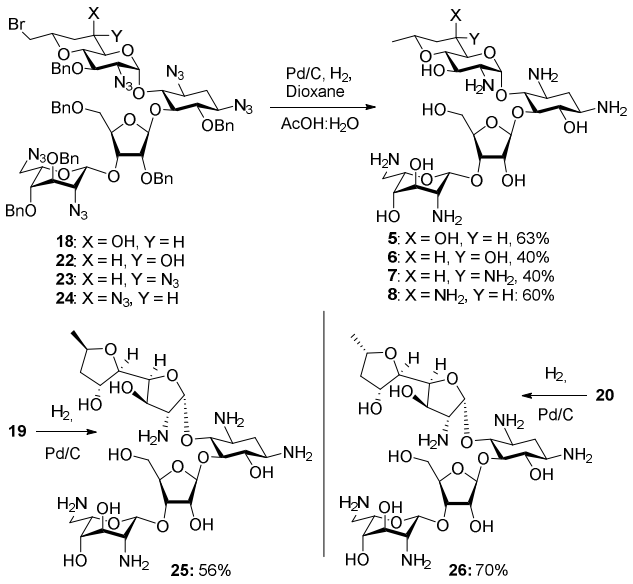
Scheme 2. Cycloetherification of Alcohol **15*R***

Bromoetherification of the diastereoisomeric homoallylic alcohol **15*S*** did not give the desired fused bicyclic system **22** in any measureable yield, but afforded several rearranged products similar to **19** and **20**, as judged by the inspection of their NMR spectra, which were not pursued further. Accordingly, **22** was obtained by oxidation of **18** with the Dess Martin periodinane[57] followed by sodium borohydride reduction (Scheme 3). Alcohols **18** and **22** were then converted to the corresponding inverted azides **23** and **24** by reaction with trifluoromethanesulfonic anhydride followed by displacement with sodium azide (Scheme 3).



Scheme 3. Preparation of Alcohol **22** and Azides **23** and **24**

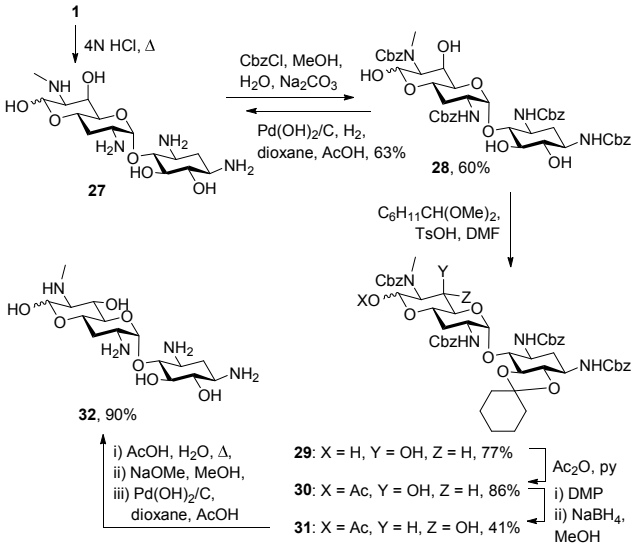
Hydrogenolysis of alcohols **18** and **22**, and of azides **23** and **24** over Pd/C in aqueous dioxane and acetic acid, followed by filtration through Sephadex and lyophilization from aqueous acetic acid gave the target compounds **5**-**8**, respectively, in the form of their acetate salts (Scheme 4). Analogous treatment of the furanosides **19** and **20** gave compounds **25** and **26**, whose relative configurations were established by extensive NMR studies as described in the supporting information. 6′-Deoxyparomomycin **9** and 6′-deoxy-6′-fluoroparomomycin **10** were obtained in the form of their trifluoroacetate salts as described previously, then converted to their acetate salts by Sephadex chromatography and lyophilization from aqueous acetic acid.



Scheme 4. Deprotection of Bicyclic Paromomycin Analogs **5**-**8** and of Rearranged Products **25** and **26**.

Aprosamine **27** was obtained in admixture with 4-aminoglucose by heating apramycin **1** in 4N HCl as described previously.[58] Treatment of this mixture with benzyloxycarbonyl chloride and sodium carbonate enabled separation of the tetra carbamate **28** in 60% overall yield, from which

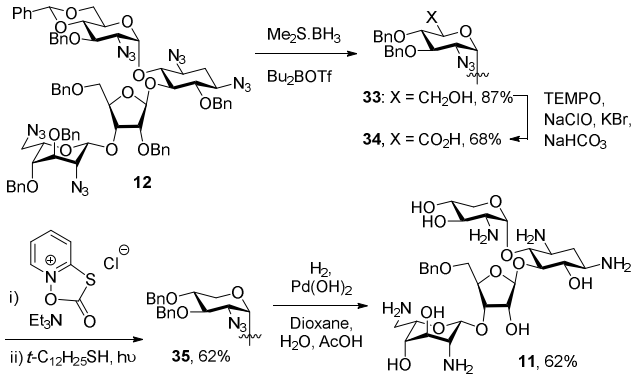
a pure sample of aprosamine **27** was obtained in the form of an anomeric mixture by hydrogenolysis (Scheme 5). Reaction of **28** with the dimethylacetal of cyclohexanecarbaldehyde in DMF with catalytic toluenesulfonic acid gave 77% of the acetal **29**, which on exposure to acetic anhydride in pyridine was converted to the equatorial anomeric acetate **30** in 86% yield.[59] Dess Martin oxidation[57] followed by reduction with sodium borohydride then afforded the 6′-epiaprosamine derivative **31** in 41% yield, from which 6′-epiaprosamine **32** was obtained by a three step deprotection sequence in 90% overall yield (Scheme 5).



Scheme 5. Synthesis of Aprosamine **27** and its 6′-Epimer **32**.

Reduction of the benzylidene acetal **12**[52] with borane dimethyl sulfide and dibutylboron triflate[60] gave the paromomycin derivative **33**, which was oxidized with catalytic TEMPO, potassium bromide, and bleach[61] to the uronic acid **34** in 79% yield. Application of Barton′s decarboxylation reaction,[62] using *tert*-dodecanethiol[63] as hydrogen atom source under white light photolysis conditions, gave the fully protected 6′-deshydroxymethyl derivative **35** in 55% yield. This classical radical chain decarboxylation was significantly more effective on this substrate than more recent photocatalytic decarboxylation reactions[64-65] relying on electron transfer chain

reactions, possibly because of the presence of multiple azide groups. Hydrogenolysis over palladium hydroxide in a mixture of dioxane, water, and acetic acid followed by lyophilization then gave **11** (Scheme 6).



Scheme 6. Synthesis of the 6′-deshydromethyl paromomycin derivative **11**.

Antiribosomal and Antibacterial Activity.

All compounds were screened in cell-free assays for their ability to inhibit translation by bacterial wild-type and recombinant hybrid ribosomes carrying complete A site cassettes of the human mitochondrial (Mit13), mitochondrial A1555G, and cytoplasmic ribosomes (Cyt14). For all compounds except the rearranged products **25** and **26**, which showed no significant activity, the experimental data together with those of the comparators **1**-**4**, 6′-epiapramycin **36**[50] and 6′-deoxyapramycin **37**[50] are reported in Table 1.[37] The antibacterial activity of all compounds was determined against clinical isolates of methicillin-resistant strains of the Gram-positive bacterium *Staphylococcus aureus* and of the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* as reported in Table 2, with the exception of the inactive compounds **25** and **26**. The more active compounds **6** and **7** were also screened for activity against the ESKAPE pathogens *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter cloacae* (*E. cloacae*), and *Acinetobacter baumannii* (*A. baumannii*) (Table 3). In order to determine

susceptibility to common resistance determinants compounds **6** and **7** additionally were screened for activity against wild type and recombinant *E coli* strains carrying defined resistance determinants (Table 4).[38]

Table 1. Antiribosomal Activities (IC_{50}, μg/mL) and Selectivities.[a]

	IC_{50}, μg/mL				**Selectivity**		
Compound	**bacterial**	**Mit13**	**A1555G**	**Cyt14**	**Mit 13**	**A1555G**	**Cyt 14**
Paromomycin **2**	0.03	50.64	5.52	10.58	1688	184	353
Neomycin **3**	0.02	1.62	0.22	17.12	81	11	856
Apramycin **1**	0.07	60.97	26.73	55.89	871	382	798
6′-Epiapram. **36**	0.74	124.21	45.08	90.01	168	61	122
6′-Deoxyapram. **37**	>20	101.15	85.26	103.05	<5.1	<4.3	<5.1
4	0.12	226.38	76.97	-	1886	641	-
5	0.47	193.33	213.00	169.16	411	453	360
6	0.02	231.85	11.82	15.07	11593	591	753
7	0.08	1.15	0.18	12.03	14	2.3	150
8	0.37	2.99	1.66	10.31	8.1	4.5	28
Aprosamine **27**	1.99	-	-	-	-	-	-
32	>10	-	-	-	-	-	-

9	0.09	166.16	93.23	152.14	1846	1036	1690
10	0.06	107.56	92.51	80.06	1792	1542	1334
11	0.23	89.43	37.95	42.88	389	165	186

Table 2. Antibacterial Activities (MIC, μg/mL)

	MRSA				*E coli*			*P aeruginosa*			
Compound	AG038	AG039	AG042	AG044	AG001	AG055	AG003	AG031	AG032	AG033	AG086
Paromomycin **2**	4	>256	>256	4-8	16-32	8	8-16	>128	>128	>128	>128
Neomycin **3**	0.5-1	128	128	0.5-1	8-16	4	4	32	32-64	>128	>128
Apramycin **1**	8	8	8	16	16	8	8-16	8	8	8	4
6′-Epiapramycin **36**	32-64	64	64	32-64	32	32	32	-	-	-	-
6′-Deoxyapramycin **37**	>128	>128	>128	>128	>128	>128	>128	-	-	-	-
4	32	64	32	32	>128	-	64-128				
5	32	32-64	16-32	32	≥128	64-128	64-128	>128	>128	>128	>128
6	8-16	8	8	4	8	8	8	32	16-32	>128	>128
7	4	4	4	2	2	2	2	32	32	≥128	≥128
8	>128	>128	128	128	>128	>128	>128	>128	128	>128	>128

Aprosamine **27**	32-64	32-64	32	32	64-128	64-128	32-64	32	32	32	32-64
32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
9	16	>128	>128	8	32	16-32	16-32	-	-	-	-
10	16	>128	>128	8	32	16-32	16-32	-	-	-	-
11	16	>128	>128	8	64	64	64	-	-	-	-

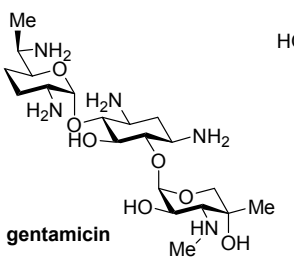
Table 3. Antibacterial Activities (MIC, μg/mL) **Against ESKAPE Pathogens**

	K. pneumoniae **(AG215)**	***A. baumannii*** **(AG225)**	***E. cloacae*** **(AG290)**
Paromomycin **2**	1	2	2
Neomycin **3**	0.5	1-2	1
Apramycin **1**	2	4	4
6	2	4	2
7	0.5-1	4	1

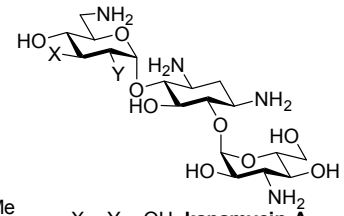
Table 4. Antimicrobial Data Against Wild Type and Engineered Strains of *E coli* Carrying Specific Resistance Determinants for Neomycin and Selected Derivatives (MIC, μg/mL)[a].

Strain	**BM13 (AG006)**	**AG007**	**AG105**	**AG009**	**AG036**	**AG037**	**AG103**
Resistance Mechanism	**-**	**AAC(3)**	**AAC(2′)**	**AAC(6′)**	**ANT(4′, 4″)**	**APH(3′, 5″)**	**ArmA**
Gentamicin[b]	0.5	32	32	nd	0.5	0.5	>128
Kanamycin A[b]	1-2	nd	1-2	128	16	>256	>128
Tobramycin[b]	0.5	nd	nd	64	16	1	>128
Apramycin **1**	4	8	4	8	4	4	2
Neomycin **3**	1	4	2	8	32	>256	0.5
Paromomycin **2**	2-4	4-8	2-4	8-16	256	>256	4
6	2	8	2-4	4	2-4	64	4
7	1-2	4	2-4	2	2	32-64	2

a) All values were determined in duplicate using two dilution series. b) These AGAs were used to verify the resistance phenotype type of the recombinant strains.



gentamicin



X = Y = OH. **kanamycin A**
X = H, Y = NH_2, **tobramycin**

Discussion

Comparison of the antibacterioribosomal activities of paromomycin and of its bicyclic derivatives, the ethylidene acetal **4** and the 6′-hydroxylated analogues **5** and **6**, reveals the essential role of a 6′-hydroxyl group in the correct orientation for tight binding to the bacterial decoding A site (Table 1). Thus, the equatorial 6′-OH group in the conformationally locked bicyclic ring I of **6** occupies the identical space to the 6′-hydroxyl group of paromomycin in the *gt* conformation as found in its complex with the decoding A site (Figure 3A, type I pseudo-base pair).[30,41] Furthermore, the advantage gained from imposing the *gt* conformation is sufficient to overcome any detrimental effect on binding of the additional ring and the concomitant loss of the hydrogen bond between the paromomycin 4′-OH and the backbone phosphate linking G1491 to A1492.

In the analogous 6′-amino series, **7** with the enforced equatorial amino group is more active than the axial analog **8**, consistent with the 6′-amino AGA neomycin binding to the decoding A site with its side-chain in the *gt* conformation (Table 1). Nevertheless, the difference in IC_{50} values of the two 6′-amino epimers **7** and **8** (4-5 fold) is strikingly less than that of the two 6′-hydroxy derivatives **5** and **6** (23 fold). Furthermore, unlike the 6′-equatorial hydroxy derivative **6**, which has comparable activity to the parent paromomycin, the 6′-equatorial amino derivative **7** is some four times less active toward the bacterial ribosome than the parent neomycin.

The differences between the 6′-hydroxy and 6′-amino series can be largely attributed to the differing ground state conformations of the ring one side chains in 6′-hydroxy and 6′-aminopyranosides (Figure 2) and the corresponding energetic penalties paid on binding to the decoding A site in the *gt* conformation. Thus, adopting literature data on the side chain conformations of methyl 2-amino-2-deoxy-α-D-glucopyranoside and of methyl 2,6-diamino-2,6-

dideoxy-α-D-glucopyranoside,[49,66] the hydroxymethyl and protonated aminomethyl side chains of paromomycin **2** and neomycin **3** are approximately 60:40:0 and 10:90:0 mixtures, respectively, of the *gg*, *gt* and *tg* conformers. Thus, on binding to the decoding A site in the *gt* conformation, paromomycin pays a penalty for restricting the side chain to a higher energy conformer. Artificially locking the paromomycin side chain into the required *gt* conformer eliminates this penalty and results in the highly active compound **6**. Conversely, no such gain is obtained on locking the neomycin side chain in the already largely predominant *gt* conformation and the 6′-equatorial amino derivative **7** consequently does not achieve the same level of affinity for the bacterial ribosome as the parent. Clearly, other factors such as changes in relative solvation levels may also contribute to the observed pattern of activity changes.

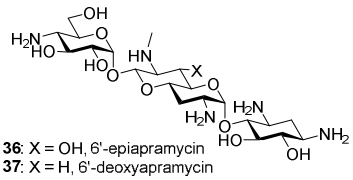
Compound **6** with the equatorial 6′-hydroxy group displays outstanding selectivity for the bacterial ribosome over the eukaryotic mitochondrial ribosome and good selectivity over the A1555G mitochondrial mutant ribosome found in individuals genetically predisposed to ototoxicity.[67-68] These differences in affinity for the prokaryotic and mitochondrial ribosomes result from differing interactions of the β-face of ring I with the bases at the bottom of the decoding A site. Thus, on the one hand, the G1491=C1409 base pair at the foot of the bacterial site, in which G1491 interacts with the β-face of the ligand ring I by a CH-π interaction, compensates for any loss of affinity due to the introduction of the hydrophobic bicyclic moiety. On the other hand, the mitochondrial ribosome, with its two consecutive non Watson-Crick pairs (C1491•C1409 and A1490•C1410), and consequently looser binding site, compensates much less effectively for the hydrophobic nature of the bicyclic ring I. With only one non-cognate base pair (C1491•C1409) in the floor of the decoding A site, the mitochondrial A1555G hybrid ribosome binds **6** with intermediate affinity between those of the bacterial and wild-type

mitochondrial ribosomes. In the 6′-amino series both the equatorial isomer **7** and the axial isomer **8**, like the parent neomycin, strongly inhibit the mitochondrial ribosome and its A1555G mutant, reflecting the presence of the additional basic amino group.[69]

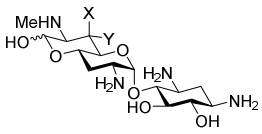
The A1408G mutation in the eukaryotic cytoplasmic decoding A site permits the formation of a complementary pseudo-base pair of the AGA ring I oxygen and the 6′-hydroxy group with N1 and N2, respectively, of G1408 that is analogous to the A1408=ring I pseudo-base pair in the bacterial ribosome-AGA complex. Not surprisingly, therefore, **6** with the equatorial hydroxy group binds more tightly to the cytoplasmic ribosome than does **5** with the axial hydroxy group. Nevertheless, because of its high affinity for the bacterial ribosome, **6** still shows excellent selectivity over the cytosolic ribosome. In contrast, the A1408G mutation does not accommodate a pseudo-base pair with ring I of the 6′-amino AGAs owing to the repulsion between the base and the AGA 6′-amine.[70] This discordance accounts for the much lower affinity of neomycin and its bicyclic analogs **7** and **8** for cytoplasmic ribosome compared to the either mitochondrial or bacterial ribosomes. The comparable affinity of both the axial amino derivative **8** and its equatorial isomer **7** for the cytoplasmic ribosome presumably reflects the inability of either isomer to take part in an effective pseudo base interaction with G1408.

Overall, with the exception of the interaction of the 6′-amino-derivatives **7** and **8** with the cytoplasmic ribosome, it is clear that the rigid bicyclic analogues with equatorial 6′-hydroxyl and 6′-amino groups, **6** and **7** respectively, bind more tightly to the ribosomal decoding A site than their axial analogues **5** and **8**. This observation is consistent with *gt* conformation of the AGA ring I side in crystallographically observed AGA ribosome complexes and the preferential formation of a type I pseudo-base pair (Figure 3A). This situation is to be contrasted with that of the natural bicyclic AGA apramycin **1** and its synthetic 6′-epi-isomer **36**. In this series, as

previously reported,[50] apramycin, with the axial 6′-hydroxy group, binds more effectively to the ribosome than the 6′-epimer with its equatorial hydroxy group. Crystallographic studies reveal that apramycin binds to the complete bacterial decoding A site in the typical manner,[38] except for the type II pseudo base pair between its ring I oxygen and axial 6′-hydroxy group, and A1408 N1 and N6. The same situation pertains with aprosamine **27**, lacking the terminal 4-aminoglucosyl of apramycin, and 6′-epiaprosamine **32** (Table 2). The exact reasons for the preference of an axial 6′-hydroxy group in the pseudo-base pair between apramycin and the decoding site A1408 in the apramycin series as opposed to the equatorial 6′-hydroxy group in the bicyclic paromomycin analogs have yet to be elucidated. It is clear from the relative activities of aprosamine **27** and its isomer **32**, however, that they involve the 7′-methylamino group of apramycin and its interaction with the 6′-hydroxyl group and the phosphate bridge between A1492 and A1493, and are not related to the presence of the extra 4-aminoglucosyl ring.



36: X = OH, 6'-epiapramycin
37: X = H, 6'-deoxyapramycin



27:X = OH, Y = H, aprosamine
32: X = H, Y = OH, 6'-epiaprosamine

Turning to the 6′-deoxy compounds **9** and **10**, the antibacterioribosomal activity of paromomycin, with its Type I pseudo-base pair interaction is much less affected by deoxygenation than that of apramycin, whose 6′-deoxy derivative **36**[50] is essentially devoid of activity (Table 1). Removal of the hydrophobic methyl group from **9** to give **11** results in a significant loss of activity against the bacterial ribosome (Table 1), but less than the simple removal of the 6′-hydroxy group from apramycin. Evidently, the 6′-hydroxy group is a more critical part of the interaction of apramycin with the bacterial decoding A than it is in paromomycin. It is striking that **11**, like 6′-deoxyapramycin **37**, suffers a significantly smaller

reduction in activity toward the hybrid ribosomes carrying the eukaryotic binding sites, than it does toward the bacterial ribosome resulting in a marked loss of selectivity (Table 1).

The antibacterial activities of the bicyclic derivatives **5**, **6**, **7**, and **8** against clinical isolates of methicillin-resistant strains of the Gram-positive bacterium *Staphylococcus aureus* and of the Gram-negative bacterium *Escherichia coli* reflect the trends observed at the target level, with the exception that **6** and **7** with the equatorial 6′-hydroxy and amino groups, respectively show moderately increased activity against *E. coli* in comparison to the parent compounds. The corresponding axial isomers **5** and **8** are significantly less active, again stressing the importance of configuration at the 6′-position. As with all previous 4′-derivatives of paromomycin prepared in our laboratories,[41,43,71] and comparable to apramycin, compounds **5**, **6** and **7** show good activity against two clinical strains of MRSA that are resistant to the parent paromomycin. This increase in activity arises from the ability to block the resistance mechanism of these two MRSA strains, which most likely arises from drug modification by either ANT(4′) or APH(3′) aminoglycoside modifying enzymes. With respect to the four clinical strains of the Gram-negative bacterium *Pseudomonas aeruginosa* tested, paromomycin itself is inactive and neomycin only moderately active against two of the four. Bicyclic compounds **6** and **7**, with their equatorial hydroxyl and amino groups respectively, show modest neomycin-like activity against two of the four strains but this activity is still inferior to that exhibited by apramycin. The bicyclic compounds **6** and **7** were also screened for activity against clinical isolates of the ESKAPE pathogens *K pneumoniae*, *E cloacae*, and *A baumannii* and were found to have activities comparable to the comparators paromomycin, apramycin and neomycin (Table 3).

Consistent with their antibacterioribosomal activities the 6′-deoxy, 6′-deoxy-6′-fluoro and 6′-(deshydroxymethyl) paramomycin derivatives **9**, **10**, and **11** show moderately reduced activity against the two paromomycin-susceptible strains of *MRSA* and against *E coli*.

When screened against a series of recombinant *E coli* strains carrying specific resistance determinants the structural modifications inserted into compounds **6** and **7** were found to be more active than the parents paromomycin and neomycin in the presence of ANT(4′,4″) and, to a lesser extent, APH(3′,5″) and AAC(6′) AMEs (Table 4). The ability of **6** and **7** to thwart the action of the ANT(4,4″) class of AMEs clearly arises from the absence of a derivatizable 4′-hydroxy group, while the enhanced activity in the presence of APH(3′,5″) is presumably a function of the reduced accessibility of the 3′-hydroxy group. A combination of conformational constraint and steric shielding of the 6′-hydroxy and 6′-amino groups in **6** and **7**, respectively, accounts for the increased activity in the presence of AAC(6′). As expected,[72] compounds **6**, **7** and the parents paromomycin and neomycin are little affected by AAC3 and AAC2′ AMEs (Table 4). In addition, as is characteristic for members of the 4,5-series of deoxystreptamine aminoglycosides neither **6** nor **7** show any loss of activity in the presence of the ribosomal methyl transferase ArmA[73-76] that, in contrast, completely inhibits the activity of all 4,6-AGAs including the current late-stage clinical candidate plazomicin.[77]

Conclusion. Designed on the basis of analogy with apramycin and previous experience with 4′-*O*-substituted analogs of parmomycin, the structurally novel compound **6** was synthesized and shown to exhibit enhanced inhibitory activity of the bacterial ribosome with respect to the parent paromomycin. Semisynthetic AGA **6** displays comparable antibacterial activity to the parent against clinical strains of *E coli* and *MRSA* and enhanced activity against recombinant *E coli* carrying the AAC(4′,4″), APH(3′,5″), and AAC(6′) resistance determinants. Additionally, **6**

shows reduced binding to eukaryotic mitochondrial and cytosolic ribosomes, which is predictive of an enhanced therapeutic index. These results clearly demonstrate that existing readily available AGAs can be modified to overcome common resistance mechansims and reduce common clinical side effects, without significant loss of antibacterial activity. The enhanced bacterioribosomal activity of **6** is demonstrated to be a function of the equatorial hydroxyl group at the 6′-position, thereby providing support for the crystallographically-derived models of AGA-ribosome interactions. Importantly, these results demonstrate that aminoglycosides with increased ribosomal activity can be designed without the need for the incorporation of additional basic amines and the concomitant increases in toxicity.

Supporting Information. Full experimental details and copies of the ^{1}H and ^{13}C NMR spectra of all new compounds. The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

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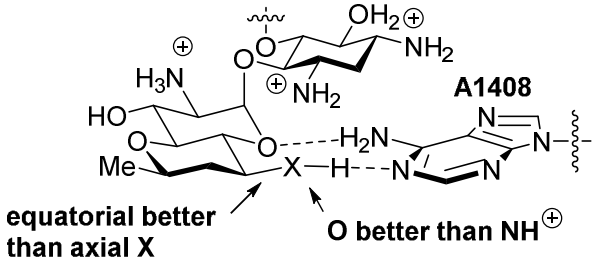
TOC Graphic



bicyclic system: • locks conformation, • blocks resistance, • enhances rselectivity