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Importance of Co-operative Hydrogen Bonding in the Apramycin-Ribosomal Decoding A-Site Interaction

Michael G. Pirrone,[b] Chennaiah Ande,[b] Klara Haldimann,[c] Sven N. Hobbie,[c] Andrea Vasella,[d] Erik C. Böttger,[c] and David Crich*[a]

An intramolecular hydrogen bond between the protonated equatorial 7′-methylamino group of apramycin and the vicinal axial 6′-hydroxy group acidifies the 6′-hydroxy group leading to a strong hydrogen bond to A1408 in the ribosomal drug binding pocket in the decoding A site of the small ribosomal subunit. In 6′-epiapramycin, the trans-nature of the 6′-hydroxy group and the 7′-methylamino group results in a much weaker intramolecular hydrogen bond, and a consequently weaker cooperative hydrogen bonding network with A1408, resulting overall in reduced inhibition of protein synthesis and antibacterial activity.

Introduction

Apramycin **1**, an atypical 2-deoxystreptamine (DOS) type aminoglycoside antibiotic (AGA),[1] differs structurally from the more common 4,5-disubstituted DOS type AGAs paromomycin **2** and neomycin **3**, and the clinically important 4,6-disubstituted DOS type AGA gentamicin **4**, by the singly substituted nature of its DOS ring and by its bicyclic octodialdose ring I that additionally carries a 4-aminoglucosyl residue. With the single exception of the uncommon aminoglycoside acetyltransferase 3 isoform IV (AAC(3)IV) aminoglycoside modifying enzyme (AME) that acts on N3, the unique structure of apramycin ensures that it is not a substrate for any of the common AMEs and so does not lose antibacterial activity in their presence. Furthermore, because of its unusual structure, its antibacterial activity is not blocked by the action of the common ribosomal methyltransferases (RMTs) acting on G1405 in the bacterial AGA binding site. Therefore, apramycin retains activity in the face of most of the resistance determinants that are responsible for the widespread clinical resistance against both the 4,5- and 4,6-DOS classes of AGA.[2]

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Like the 4,5- and 4,6-AGAs paromomycin **2**, neomycin **3**, and gentamicin **4**, apramycin derives its antibacterial activity from its ability to bind to the decoding A site in helix 44 of the small ribosomal subunit of the bacterial ribosome and its consequent interference with bacterial protein synthesis.[2a,3] In particular, the pyranosyl ring oxygen (O5′) and the 6′-hydroxy group of the apramycin ring I form a pseudo-base pair interaction with the conserved A1408 residue in the decoding A site that resembles that between A1408 and the equivalent atoms in rings I of paromomycin **2** and neomycin **3**.[3] Closer inspection, however, reveals a difference in geometry of these pseudo-base interactions: in **2** and **3** the side chain of ring I is held in the *gt* conformation[4] in the confines of the pseudo-base pair, which we term a type I interaction,[5] whereas in apramycin the axial C–O6′ bond is locked in the *gg* conformation[4] enforcing what we have called a type II pseudo-base pair[5] (Figure 1).

Seeking to probe the importance of the pseudo-base pair interaction and its geometry (Type I or Type II), we first synthesized 6′-epipramycin **5** (Figure 2), with the enforced *gt* conformation of its side chain and found it to be less active than apramycin itself in inhibiting protein synthesis by bacterial ribosomes in cell-free translation assays, and in antibacterial assays.[6] Subsequently, we synthesized paromomycin analog **6** with the enforced *gg* conformation of its side chain, and its 6′-epimer **7** with the enforced *gt* conformation. We found the *gt* isomer **7**, which necessarily adopts the type I pseudo-base pair interactions with A1408 in the decoding A site to be more active than **6**.[5,7] We also found that aprosamine **8**, while less active than the parent apramycin, was more active than its 6′-epi isomer **9**, suggesting that the greater activity of apramycin **1** over it 6′-epimer **5** is not related to the presence of the pendant 4-aminoglucosyl ring (Figure 2).[5]

Clearly, further insight into the approximately 10-fold difference in activity between apramycin **1** and its respective 6′-epimer **5**, and the inverted but also approximately 10-fold difference in activity between the bicyclic 4,5-AGA analog **6**, and its 6′-epimer **7** is central to a complete understanding of the interaction of these AGAs with the decoding A site, and

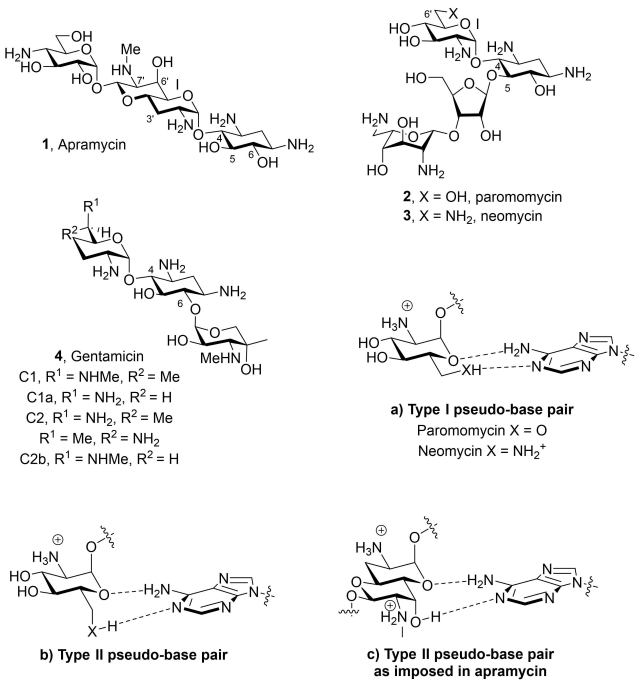


Figure 1. Structures of apramycin, paromomycin, neomycin, and gentamicin and the Types I (a) and II (b and c) pseudo-base pair interactions.

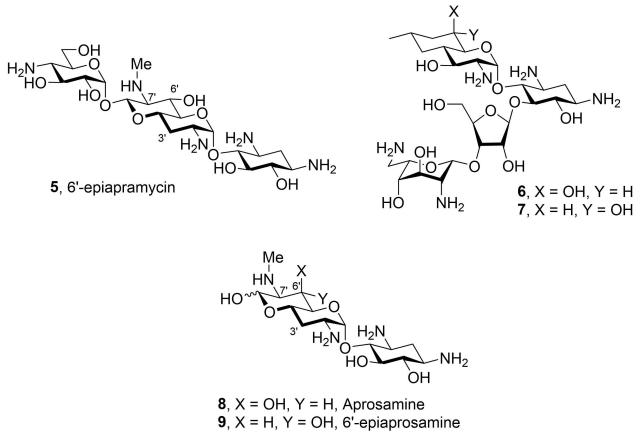


Figure 2. Structures of 6′-epiapramycin **5**, bicyclic paromomycin analogs **6** and **7**, and of aprosamine **8**, and 6′-epiaprosamine **9**.

should inform the design of improved AGAs. Consequently, we turn our attention here to the C and D ring moiety (paromobiosyl or 2,6-diamino-2,6-dideoxy-β-L-idopyranosyl-(1→3)-β-D-ribofuranosyl) of the 4,5-AGAs and to the protonated 7′-methylamino group of the apramycin series as likely reasons for the change in preference from the type-I base pair in bicyclic paromomycin derivatives to type-II in apramycin derivatives Ultimately, we find that the cis-vicinal 6′-hydroxy group and the protonated 7′-methylamino group in apramycin participate in a co-operative hydrogen bond spanning N7′–O6′ and A1408, which enhances the strength of the type-II base pair interaction.

Results and Discussion

Recalling our earlier syntheses of the apralogs, apramycin-paromomycin hybrids,[8] we targeted the 7′-methylaminoapralogs **10** and **11**, differing only in configuration at the 6′-position (Figure 3), for synthesis. In designing **10** and **11** we elected not only to remove the 4-aminoglucosyl ring from the starting material apramycin as in the aprosamines **8** and **9**, but to remove it by reduction at the 8′-position to give what are effectively 4′,8′-anhydroalditol derivatives of aprosamine.

Synthesis

Treatment of apramycin **1** with Stick's reagent[9] in the presence of catalytic copper sulfate gave apramycin tetraazide,[6] which was acetylated to facilitate isolation, followed by heating to 85 °C in methanolic 4 N HCl resulting in the cleavage of the aminoglucosyl ring[1,5] and formation of the aprosamine derivative **12**. Without purification, **12** was converted with benzyl chloroformate and aqueous potassium carbonate to the corresponding benzyl carbamate, which was stirred with *p*-thiocresol, 1,3-dimethyl-2-chloroimidzolinium chloride and triethylamine according to the Shoda protocol for thioglycoside formation,[10] followed by stirring with sodium hydride in THF to give the aprosamine thioglycoside **13** in 18% overall yield for the six step sequence (Scheme 1). Treatment with cyclohexanone dimethyl acetal in the presence of camphor-10-sulfonic acid then gave the fully protected derivative **14** in 75% yield. Conversion to the 4′,8′-anhydroalditol derivative **15** was achieved in 68% overall yield by controlled oxidation of **14** to the corresponding sulfoxides with *m*CPBA, followed by activation with trifluoromethanesulfonic anhydride and then reduction with triethylsilane. Exposure of **15** to methanol in the presence of toluenesulfonic acid then afforded diol **16** in 57% yield, which on controlled stirring with benzoyl chloride in pyridine gave the regioselectively mono-esterified derivative **17** in 45% yield consistent with the established reactivity pattern of comparable diols.[8a,11] To obtain the 6′-epimer, **14** was heated to 65 °C with sodium hydroxide in ethanol followed by treatment with phenyl chloroformate, oxidation with the Dess-Martin periodinane,[12] reduction with sodium borohydride, and cyclization with sodium hydride giving **18** in 40% overall yield

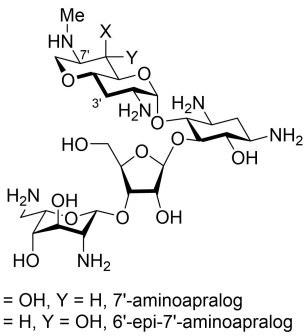
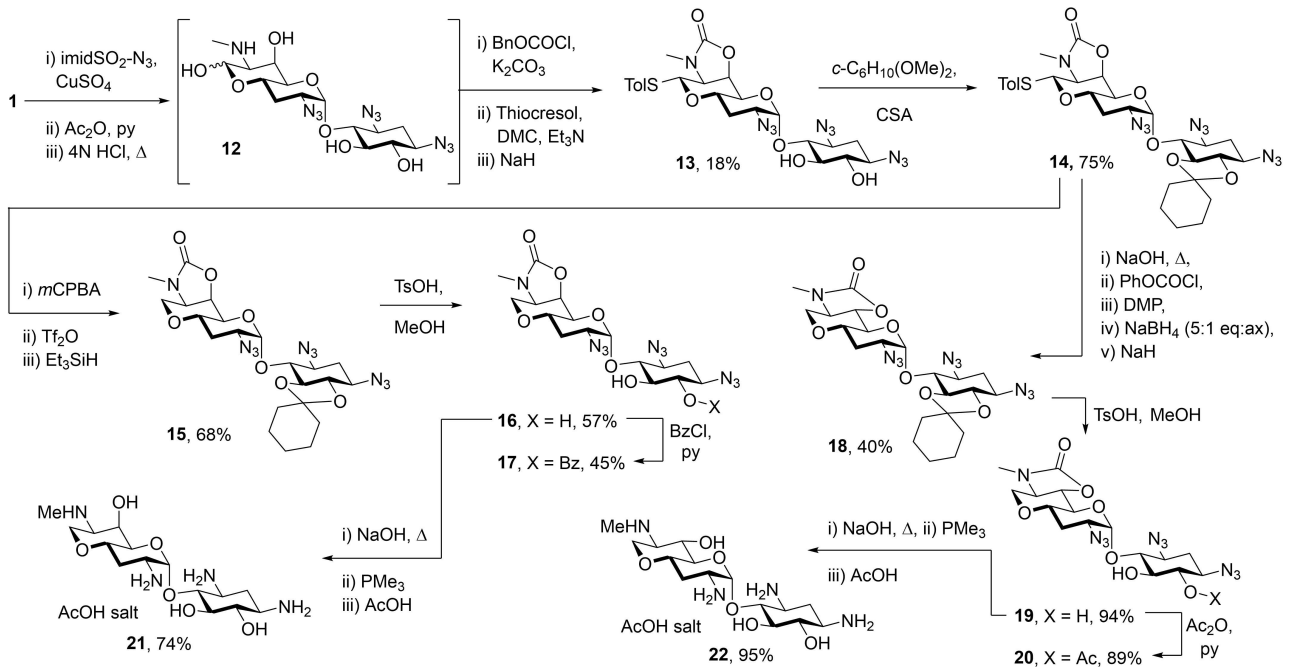


Figure 3. Target molecules **10** and **11**.

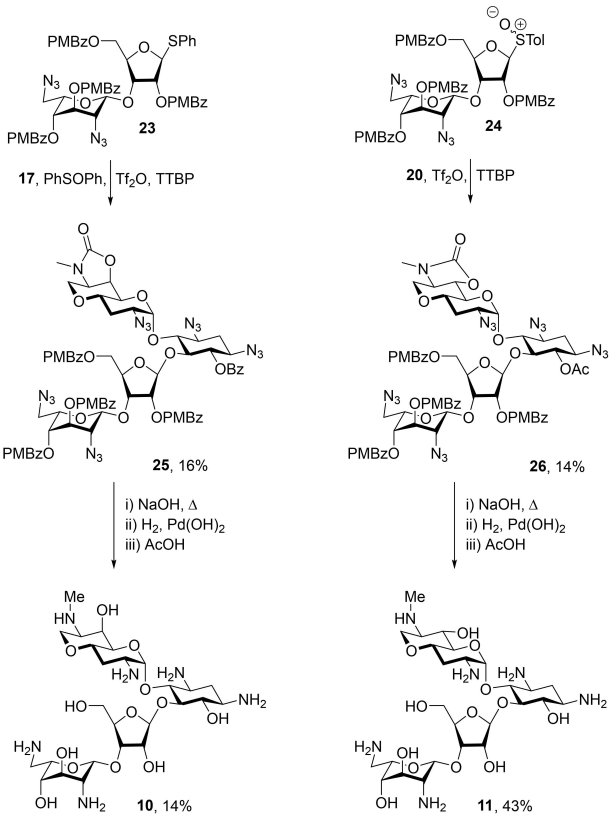


Scheme 1. Synthesis of the glycosyl acceptors **17** and **20** and the aprosamine alditols **21** and **22**.

for the six step sequence. Methanolysis of the acetal then gave diol **19** in 94% yield and was followed by controlled acetylation to afford the mono-acetate **20** in 89% yield (Scheme 1). Heating of diols **16** and **19** with sodium hydroxide in aqueous dioxane followed by addition of trimethylphosphine and continued heating gave the aprosamine alditols **21** and **22** in 74 and 95% yield, respectively, in the form of their peracetate salts after lyophilization from acetic acid solution (Scheme 1).

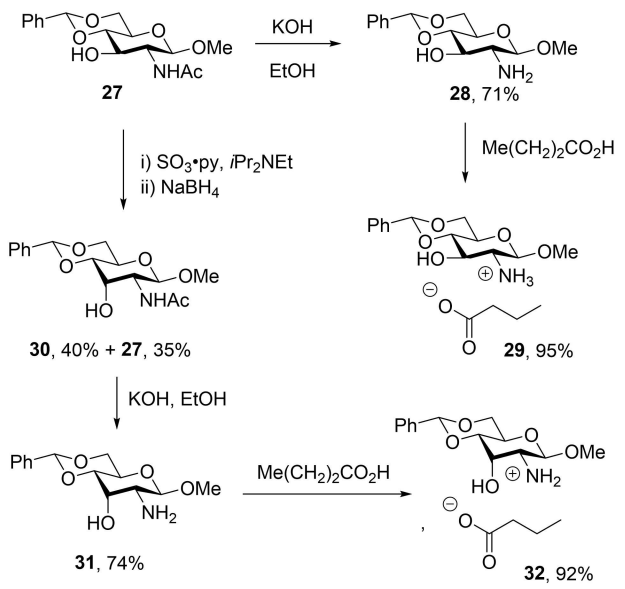
Paromobiosyl thioglycoside **23** was accessed from paromomycin, based on precedent by the Hanessian, Swayze and Wong laboratories,[13] and sulfoxide **24** was prepared as described previously.[8a] A mixture of acceptor **17**, donor **23**, the hindered non-nucleophilic base 2,4,6-tri-*tert*-butylpyrimidine (TTBP), and diphenyl sulfoxide[14] was stirred in dichloromethane at −60 °C and treated with triflic anhydride followed by warming to room temperature and quenching with triethylamine, enabling isolation of the saccharide **25**, albeit only in 16% yield (Scheme 2). A mixture of acceptor **20**, sulfoxide **24**, and TTBP was similarly stirred at −60 °C in dichloromethane and activated with triflic anhydride before warming to room temperature and eventual quenching, leading to the isolation of **26** in 14% yield. Finally, heating of **25** and **26** with sodium hydroxide, followed by Staudinger cleavage of the azides and lyophilization in the presence of acetic acid gave the target molecules **10** and **11** in 14 and 43% yield, respectively (Scheme 2).

A pair of simple diastereomeric vicinal amino alcohols modelling the bicyclic core of apramycin **1** and of 6′-epiapramycin **5** was obtained by a variation on a literature method[15] from methyl 4,6-*O*-benzylidene-2-acetamido-β-D-glucoside **27**.[16] Treatment of **27** with ethanolic potassium hydroxide gave the amino alcohol **28**,[17] which on acidification



Scheme 2. Synthesis of the epimeric 8′-deoxyapralogs **10** and **11**.

with butyric acid gave the butyrate salt **29**. Parikh-Doering[18] oxidation of **27** followed by reduction with sodium borohydride



Scheme 3. Preparation of the *trans*- and *cis*-vicinal amino alcohols **28** and **31** and their butyrate salts **29** and **32**.

gave 35% of the gluco isomer **27** and 40% of the desired allo isomer **30**, which was then converted to the amine **31** and its butyrate salt **32** analogously to the gluco isomer (Scheme 3). The butyrate salts **29** and **32** were preferred as the corresponding acetates, especially the allo isomer, were insufficiently soluble in chloroform for the subsequent studies.

Antiribosomal activity and selectivity

All compounds depicted in Figure 4 were screened for their ability to disrupt protein synthesis in cell-free translation assays using wild-type *Mycobacterium smegmatis* ribosomes (Table 1).

Two extremes are apparent with regard to the influence of configuration at the 6′-position. The one extreme is represented by apramycin **1** and its 6′-epimer **5**, by aprosamine **8** and its 6′-epimer **9**, and by 8′-deoxy aprosamine **21** and its 6′-epimer **22**: each of these compounds carries an equatorial methylamino group at the 7′-position, but lacks substitution at the 5-position on the DOS ring. For each of these three pairs the isomer with the axial 6′-hydroxy group is the more active than that with an equatorial 6′-hydroxy group, indicative of a preference for the

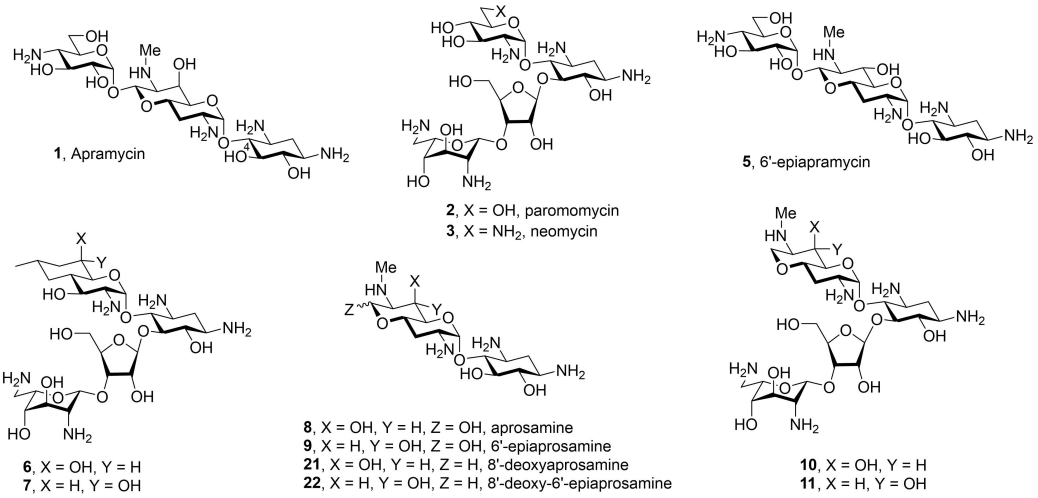


Figure 4. Compounds screened for antiribosomal and antimicrobial activity.

Table 1. Antiribosomal activity (IC_{50}, μM) and selectivity against wild-type and A1408G mutant (*M. smegmatis*) ribosomes.

Entry	Cmpd	5-Substituent	6′-Substituent	7′-Substituent	IC_{50} [μM][a] wt	A1408G	Selectivity ($IC_{50\ A1408G}/IC_{50\ wild\ type}$)
1	paromomycin, **2**	paromobiosyl-O	OH	–	0.033	0.37	11
2	neomycin, **3**	paromobiosyl-O	NH_2	–	0.035	28	800
3	apramycin, 1	OH	ax OH	eq NHMe	0.15	790	5339
4	**5**	OH	eq OH	eq NHMe	1.6	315	198
5	**6**	paromobiosyl-O	ax OH	–	0.87	43	49
6	**7**	paromobiosyl-O	eq OH	–	0.060	0.82	14
7	**10**	paromobiosyl-O	ax OH	eq NHMe	0.034	46	1358
8	**11**	paromobiosyl-O	eq OH	eq NHMe	0.043	1.5	35
9	**8**	OH	ax OH	eq NHMe	5.9	425	72
10	**9**	OH	eq OH	eq NHMe	50	556	11
11	**21**	OH	ax OH	eq NHMe	7.0	>1000	>143
12	**22**	OH	eq OH	eq NHMe	132	>1000	>8

[a] All values were determined in at least duplicate using 2-fold dilution series.

type II pseudo-base pair interaction. The other extreme is represented by the bicyclic paromomycin analogs **6** and **7**, lacking substitution at the 7′-position but carrying the paromobiosyl disaccharide at the DOS 5-position. In this pair, and other similar compounds reported previously,[5,7] it is the equatorial isomer that is the more active pointing to the importance of the type I pseudo-base pair interaction. Between the two extremes lie compounds, **10** and **11**, which both carry the paromobiosyl disaccharide on the DOS ring and the 7′-methylamino group, and for which both isomers have comparable high levels of activity. Overall, it is apparent that the presence of the paromobiosyl group at the DOS 5-position results in a preference for the equatorial isomers at the 6′-position and for the associated type-I pseudo-base pair interaction, while the presence of a 7′-methylamino group favors the axial 6′-alcohol and the type II pseudo-base pair interaction. When both groups are present their contrasting influences are balanced resulting in little or no difference in activity between the axial and equatorial isomers.

In X-ray crystal structures of their complexes with the *Thermus thermophilus* 30S ribosomal subunit, within the limits imposed by the ~3.5 Å resolution, the paromamine core (ring I and the DOS ring) of paromomycin and the aprosamine core of apramycin take up the same position in the decoding A site.[2a,3a] With simple 4′,6′-*O*-benzylidene derivatives of paromomycin the resemblance of the binding mode to that of apramycin is even more obvious.[19] Yet, as discussed above (Figure 1), subtle differences in binding modes exist, with paromomycin forming a type I pseudo-base pair with A1408 and apramycin a type II interaction. 6′-Epiapramycin **5** necessarily forms a type-I pseudo-base pair interaction with A1408 and is less active than apramycin itself, whereas in bicyclic derivatives of paromomycin such as **6** and **7** it is the equatorial isomer capable of forming the type-I pseudo-base pair interaction that is the more effective inhibitor. Evidently, the presence of the paromobiosyl disaccharide at O5 in the DOS ring favors the formation of type-I pseudo-base pair interactions, whereas that of an equatorial 7′-methylamino group in systems carrying a bicyclic ring I leads to a preference for a type-II pseudo-base pair interaction. As we have now shown, when both the 5-*O*-paromobiosyl group and the 7′-methylamino group are incorporated into the same compound, their opposing influences essentially cancel each other such that the axial and equatorial isomers **10** and **11** have comparable activity against the bacterial ribosome.

The 2,6-diamino-2,6-dideoxy-L-idopyranosyl ring of the paromobiosyl disaccharide has been considered to function in its protonated state as essentially a sphere of positive charge that fits in a convenient cavity in the decoding A site providing mainly electrostatic stabilization to the drug-ribosome complex.[20] Indeed, replacement of the 2,6-diamino-2,6-dideoxy-L-idopyranosyl moiety by simple alkylamino groups results in compounds with comparable activity.[8a,21] In contrast, the ribofuranosyl moiety that bridges between the paromamine core and the protonated 2,6-diamino-2,6-dideoxy-L-idopyranosyl ring is involved in two critical hydrogen bonds (Figure 6). In the first of these its primary hydroxy group donates a hydrogen bond to G1491, while in the second it accepts an intramolecular hydrogen bond from the protonated 2′-amino group of ring I: this latter hydrogen bond, which is found in free solution as well as in X-ray structures of drug-ribosome complexes,[22] is essential as its elimination in the paromomycin series by suitable modification of either N2′ or O5″ results in a significant reduction in activity.[23] This N2′–H–O5″ hydrogen bond is less critical, however, in neomycin derivatives and in the apralog series,[8a,23b] with their 7′-methylamino groups. For neomycin derivatives this is because the stronger pseudo-base pair interaction with A1408 arising from the involvement of the protonated neomycin 6′-amino group compensates for loss of any N2′–H–O5″ hydrogen bonds, and the apralogs.[23] The 7′-methylamino group of apramycin and the apralog series, however, makes no direct contact with the drug binding pocket,[2a] leading us to suggest that in its protonated state it is involved in an intramolecular hydrogen bond with O6′ that in turn acidifies the 6′-hydroxy group of apramycin and strengthens the pseudo-base pair interaction with A1408 (Figure 6). In other words, the protonated 7′-methylamino-6′-hydroxy moiety of apramycin is effectively a surrogate for the protonated 6′-amino group of neomycin.

To challenge our interpretation of the data obtained with the wild-type bacterial ribosomes, we tested the compounds for their ability to disrupt protein synthesis by *M. smegmatis* ribosomes in which the adenosine residue at position 1408 is replaced by a guanosine moiety (A1408G) (Table 1 and Figure 5).[24] A guanine exchange for adenine in the 16S RNA position 1408 is the single critical polymorphism that distinguishes the AGA drug binding pocket in the eukaryotic cytoplasmic from that in bacterial ribosomes.[25] It is known that neomycin exhibits better selectivity than paromomycin for inhibition of the wild type bacterial ribosome over the A1408G mutant, where selectivity is defined as the ratio of the IC_{50} for inhibition of the A1408G mutant ribosome to that for wild-type ribosome. This difference in activity for A1408G ribosomes is rationalized in terms of the 6′-hydroxy AGA paromomycin retaining a favorable pseudo-base air interaction with 1408G, whereas for the protonated 6′-amino AGA a repulsive interaction occurs with 1408G (Figure 6).[26] Like neomycin, apramycin exhibits excellent selectivity (Table 1) for the wild-type over

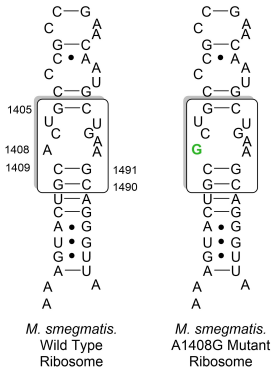


Figure 5. Decoding A sites of prokaryotic and eukaryotic ribosomes. The bacterial AGA binding pocket is boxed and the A1408G substitution is colored green.

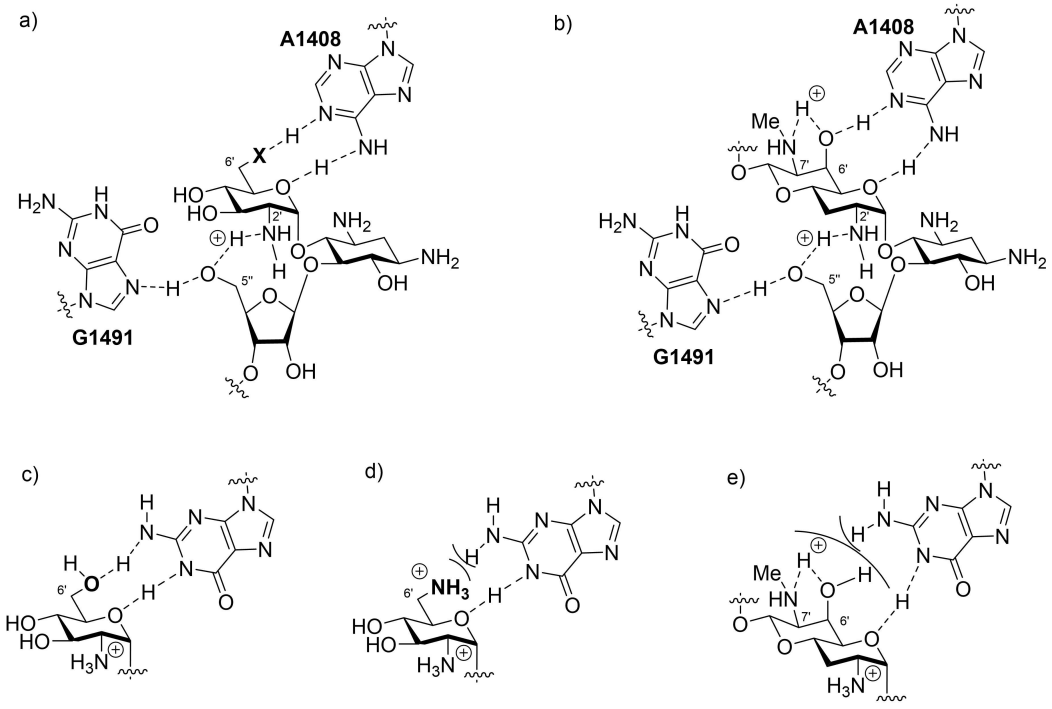


Figure 6. Pseudo-base pair interactions; a) paromomycin (X = O) and neomycin (X = N^+H_2) with A1408; b) apramycin with A1408; c) paromomycin with 1408G; d) neomycin with 1408G with indicated steric clash; and e) apramycin with 1408G with indicated steric clash.

the A1408G mutant of the bacterial ribosome,[2a] which can now be understood in terms of the repulsive interaction between the base and the protonated 7′-methylamino-6′-hydroxy moiety of apramycin (Figure 6). Moreover, the apramycin-1408G repulsive interaction can be expected to be greater than the corresponding neomycin-1408G interaction because of the reduced degrees of freedom arising from the constraints imposed by the rigid bicyclic ring and the N7′–O6′ hydrogen bond.

The selectivities for inhibition of the wild-type and A1408G mutant ribosomes by 6′-epiapramycin **5**, and by the potent apralogs **10** and **11** are 198, 1358, and 35, respectively. The strong repulsive interaction between apramycin and 1408G is therefore reproduced in **10**, with its identical *cis*-7′-methylamino-6′-hydroxy moiety, but is much reduced in **5** and **11**, which are characterized by the stereoisomeric *trans*-7′-methylamino-6′-hydroxy functionality. In contrast, for the bicyclic paromomycin analogs **6** and **7**, lacking the 7′-methylamino group, both the axial and equatorial isomers have low, paromomycin-like selectivity for inhibition of the wild-type over the A1408G mutant ribosome (Table 1). We suggest therefore that the influence of relative configuration (*cis* vs *trans*) in the interaction of the 7′-methylamino-6′-hydroxy moiety with both A1408 in the wild-type ribosome and 1408G in the A1408G mutant ribosomes is related to the strength of the intramolecular N7′–H–O6′ hydrogen bond and its acidifying effect on the 6′-hydroxy group. It is well-established that intramolecular hydrogen bonding between a pair of *cis*-vicinal hydroxy groups, or between an amide and a *cis*-vicinal alcohol, on a cyclohexane or related framework is stronger than that between the corresponding *trans*-diequatorial pair,[27] that the more strongly intramolecular hydrogen bonded *cis*-diol is a better donor of an intermolecular co-operative hydrogen bond to a suitable acceptor,[28] and that cooperativity enhances hydrogen bonds in general.[27b,28–29] We considered that intramolecular hydrogen bonding between a protonated *cis*-vicinal amino alcohol such as apramycin **1** or apralog **10** would similarly be stronger than in the *trans*-isomers **5** and **11** and turned to the ^{1}H NMR spectra of the model compounds **29** and **32** for evidence.[30] We plotted the chemical shift of the ammonium resonance in the ammonium salts **29** and **32** in deuterodichloromethane solution as a function of concentration (Figure 7), and found a strong concentration dependence for the gluco isomer **29** with its *trans*-vicinal amino alcohol moiety, but only a minor concentration dependence for the *cis*-vicinal allo isomer **32**. Consistent with earlier studies on hydrogen bonding in *cis*- and *trans*-vicinal hydroxy amides, probed inter alia with the aid of studies on the concentration dependence of the H-bond,[31] the ammonium ion in the *cis*-system **32** is therefore mainly involved in a strong intramolecular hydrogen bond, whereas that in the *trans*-isomer **29** is mainly intermolecularly hydrogen bonded. The established pattern of hydrogen bonding for vicinal diols and amido alcohols is therefore reproduced in vicinal ammonio alcohols. Gas phase computational work on the relative strengths of the intramolecular hydrogen in protonated *cis*- and *trans*-2-aminocyclohexanol concur with this conclusion.[32]

Overall, we conclude that the greater effectiveness of apramycin **1** as an inhibitor of the wild-type bacterial ribosome as compared to its trans-isomer **5** is due to its stronger

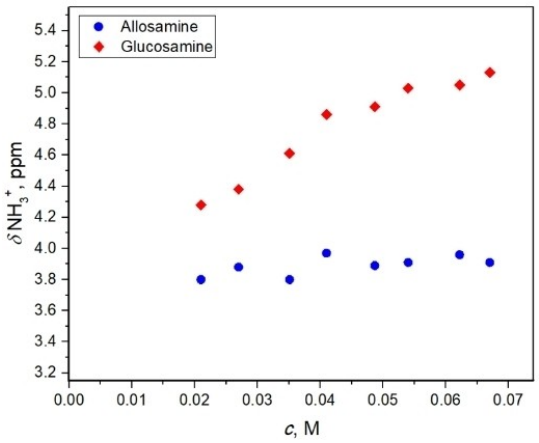


Figure 7. Concentration dependence of the ammonium resonance in protonated the *trans*- (gluco-) and *cis*-vicinal (allo-) amino alcohols **29** and **32**.

intramolecular N7′–O6′ hydrogen bond, which in turn results in greater acidification of its 6′-hydroxy group and a stronger cooperative hydrogen bond to A1408 in the context of the type-II pseudo-base pair interaction. The greater repulsive interaction of the cis-amino alcohols **1** and **10** with 1408G is similarly explained (Figure 6).

Antibacterial activity against wild-type and resistant bacterial strains

While the focus of this study has been on activity at the target level and the influence of the 7′-methylamino group, compounds were nevertheless screened for antibacterial activity against a limited set of representative Gram-negative bacteria. Consistent with the pattern observed for the inhibition of the wild-type bacterial ribosome (Table 1), in the absence of an amino group at the 7′-position, all compounds with a bicyclic ring 1 and an equatorial 6′-hydroxy group are more active than their axial counterparts. In the presence of a 7′-methylamino group on the other hand it is the compounds with a 6′-axial alcohol that are more active (Table 2). Finally, consistent with previous observations,[8] the presence of the paromobiosyl disaccharide at the DOS 5-position removes the susceptibility to inhibition of antibacterial activity by the presence of the AAC(3)-IV AME,[33] which is the only significant mechanism of resistance to apramycin.[2]

Conclusion

The increased antiribosomal and antibacterial activity of apramycin **1** over its 6′-epi isomer **5** is demonstrated to be the result of a cooperative hydrogen bonding network in which an intramolecular hydrogen bond between the protonated equatorial 7′-methylamino group and the axial 6′-hydroxy group acidifies the latter and so reinforces its ability to donate a hydrogen bond to A1408 in the drug binding pocket in the decoding A site of the small ribosomal subunit. In the 6′-epi-isomer **5**, the intramolecular hydrogen bond is much weaker leading to a weaker hydrogen bond with A1408. Acidification of the axial 6′-hydroxy group by an equatorial 7′-methylamino group fosters selectivity of apramycin derivatives by two mechanisms that act in concert: it increases interaction with bacterial A1408 ribosomes and it results in a repulsive interaction with a 1408G residue, the single critical rRNA exchange that distinguishes the bacterial AGA drug binding pocket from that in eukaryotic cytosolic ribosomes. These observations are significant for the design of improved next generation aminoglycoside antibiotics.

Conflict of Interest

S. N. H., A. V., E. C. B. and D. C. are founders of an equity holders in Juvabis AG, a biotech company developing aminoglycoside antibiotics

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Table 2. Antibacterial activity [μg/mL].

Compound	5-substituent	6′-substituent	7′-substituent	Strain AG001 *E. coli*	AG173 *E. coli* AAC(3)-IV	AG215 *K. pneu.*	AG220 *P. aerug.*	AG309 *A. baum.*
Paromomycin, **2**	parombiosyl-O	OH	–	2-4	4–8	1	>128	2
Apramycin, **1**	OH	ax OH	eq NHMe	4	>128	1-2	4	4
5	OH	eq OH	eq NHMe	16–32	≥128	8	32	16–32
6	paromobiosyl-O	ax OH	–	32–64	>64	16	>128	32
7	paromobiosyl-O	eq OH	–	4	4	1-2	16	4-8
10	paromobiosyl-O	ax OH	eq NHMe	1	2–4	1	1–2	1–2
11	paromobiosyl-O	eq OH	eq NHMe	2-4	8	2	4	4
8	OH	ax OH	eq NHMe	16-32	>128	8-16	16	16
9	OH	eq OH	eq NHMe	≥128	>128	64-128	128	128
21	OH	ax OH	eq NHMe	16-32	>64	8	32	32–64
22	OH	eq OH	eq NHMe	>128	>128	128	>128	>128

Keywords: hydrogen bonding · aminoglycosides · antibacterial · antiribosomal activity · aminoglycoside modifying enzymes

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