MedChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

Synthesis, Antiribosomal and Antibacterial Activity of 4□-*O*-Glycopyranosyl Paromomycin Aminoglycoside Antibiotics

Weiwei Chen,[a] *Takahiko Matsushita,*[a] *Dimitri Shcherbakov,*[b] *Heithem Boukari,*[b] *Andrea Vasella,*[c,*] *Erik C. Böttger,*[b,*] *and David Crich*[a,*]

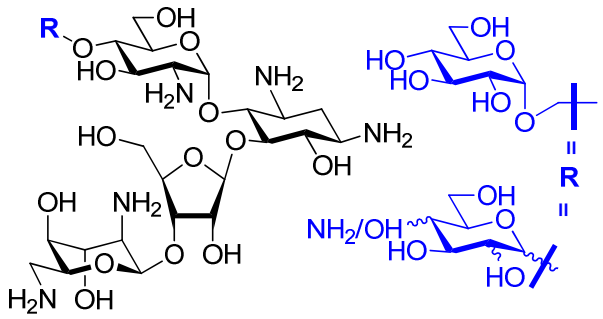
a) Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

b) Institut für Medizinische Mikrobiologie, Universität Zürich, 8006 Zürich, Switzerland

c) Laboratorium für Organische Chemie, ETH Zürich, 8093 Zürich, Switzerland

vasella@org.chem.ethz.ch; dcrich@chem.wayne.edu; boettger@imm.uzh.ch

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)



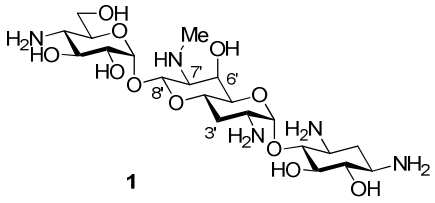
4□-*O*-Glycopyranosylation of the aminoglycoside paromomycin affects differentially the inhibition of prokaryotic and eukaryotic ribosomes and influences antibacterial activity against MRSA.

Abstract. A series of 4□-*O*-glycopyranosyl paromomycin analogs and a 4□-*O*-(glucosyloxymethyl) analog were synthesized and evaluated for their ribosomal activity to determine the influence of the glycosyl moiety on drug activity and selectivity. Antibacterial activity against clinical strains of *Escherichia coli* and *Staphylococcus aureus* was also investigated. While all compounds were less active than paromomycin itself, differences in activity were seen between the gluco-, manno-, and galactopyranosyl series and between individual anomers. These differences in activity, which are discussed in terms of variations in affinity for the ribosomal decoding A site, may prove useful in the design of subsequent generations of improved aminoglycoside antibiotics with reduced toxicity.

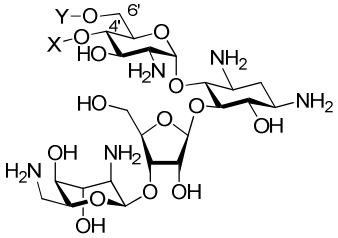
Introduction.

The aminoglycosides (AGAs) are an established class of broad spectrum antibiotics with proven efficacy against a number of clinical pathogens.[1-3] The evolution of widespread resistance mechanisms coupled with concerns arising from nephrotoxicity and ototoxicity together with the advent of combinatorial chemistry and high throughput screening methods, however, combined to end the classical phase of semi-synthetic AGA discovery and development in the pharmaceutical industry in the 1980 and 1990's.[1-3] The continued emergence of drug-resistant pathogens coupled with recent advances in the understanding of the mode of action of the AGAs, and the paucity of new antibiotic drug classes to emerge from the screening efforts of the major pharmaceutical companies suggests that the time is now ripe for the further exploration of the AGAs.[3, 4] Indeed, the semi-synthetic 4,6-AGA plazomicin, currently under investigation for the treatment of drug resistant bacteria, is the first demonstration of the renewed potential of the AGAs.[5] The molecular basis of resistance mechanisms to AGAs is for the most part well-understood such that resistance can be overcome by design, and AGA nephrotoxicity may be circumvented in the clinic by careful monitoring and especially administration in the form of a single large dose daily, which leaves AGA ototoxicity as the major area of concern in the future development of novel AGAs.[1-3, 6] AGAs bind to the ribosomal decoding A-site and recent advances in molecular biology, most notably in the engineering of eukaryotic ribosomal A-site cassettes into bacterial ribosomes,[7] have led to the hypothesis that ototoxicity relates to limitations in drug target selectivity.

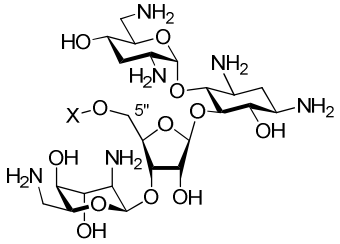
Thus, AGA binding to the decoding A-site of eukaryotic ribosomes,[8-11] in particular the mitochondrial decoding A site is the root cause of ototoxicity.[8-10] On this basis a series of functional ribosomal assays using mutant hybrid ribosomes enables the prediction of ototoxic activity alongside antibacterial activity in collections of semisynthetic AGAs and other A site binders.[7-10] Using this method we recently predicted that the natural atypical AGA apramycin **1** would have reduced ototoxicity and demonstrated that this is indeed the case in the ex-vivo murine cochlear explant model and in the in-vivo guinea pig auditory brainstem response model.[12] Working in the same manner we next discovered that the target selectivity of the potent natural pseudotetrasaccharide paromomycin **2**, a member of the 4,5-AGA class of molecules can be increased by simple substitution at the 4□- and 4□,6□-positions as exemplified by **3** and **4**, respectively.[13] Recognizing the structural similarity of apramycin **1** with **3** and **4**, both in solution and in complex with fully constituted bacterial ribosomes,[13] we were spurred to prepare and investigate the series of 4□-*O*-glycopyranosyl paromomycin derivatives on which we report here, whose substituent is positioned so as to mimic the terminal apramycin 4-amino-4-deoxyglucopyranosyl moiety. In previous work Baasov and coworkers have synthesized a series of 5□□-*O*-glycosylated derivatives **5**-**8** of neomycin B **9**, which were active against various bacterial strains; their ototoxic potential however was not assessed. [14] All of these compounds may be considered regiosiomeric analogs of the pseudopentasaccharide lividomycin A **10**, a 3□-deoxyparomomycin derivative characterized in the early AGA literature as being less ototoxic than paromomycin itself.[15]



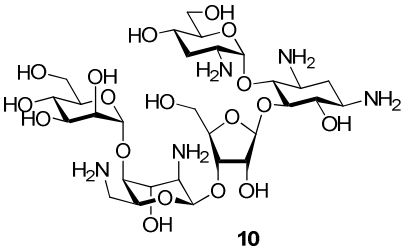
1



2: X = Y =H
3: X = $(CH_2)_3Ph$, Y = H
4: X-Y = $CH(CH_2)_2Ph$



5: X = 3,4-diamino-3,4-dideoxy-β-D-allopyranosyl
6: X = 4,6-diamino-4,6-dideoxy-β-D-glucopyranosyl
7: X = 2-amino-2-deoxy-β-D-glucopyranosyl
8: X = β-D-ribofuranosyl
9: X = H, neomycin B



10

Results and Discussion.

Chemical Synthesis. Coupling of the known selectively protected paromomycin derivative[16] **11** with a series of four known thioglycosides **12-15**[17-20] with low temperature activation by the diphenyl sulfoxide/trifluoromethanesulfonic anhydride (Tf_2O) couple[21] in dichloromethane in the presence of the hindered non-nucleophilic base tri-*tert*-butylpyrimidine (TTBP)[22] at temperatures between -72 °C and room temperature gave the α- and β-glycosides **16-19** (Scheme 1) with the yields and anomeric ratios indicated in Table 1. No attempt was made to improve stereoselectivity in these coupling reactions, but the anomeric mixtures were separated by standard chromatographic techniques and subject to deprotection by treatment with trimethylphosphine and subsequent hydrogenolysis leading to the isolation of the 4□-*O*-paromomycin derivatives **24α,β - 27α,β** (Scheme 1) with the yields indicated in Table 1.

Scheme 1. Synthesis of 4□-*O*-glycosyl paromomycin derivatives.

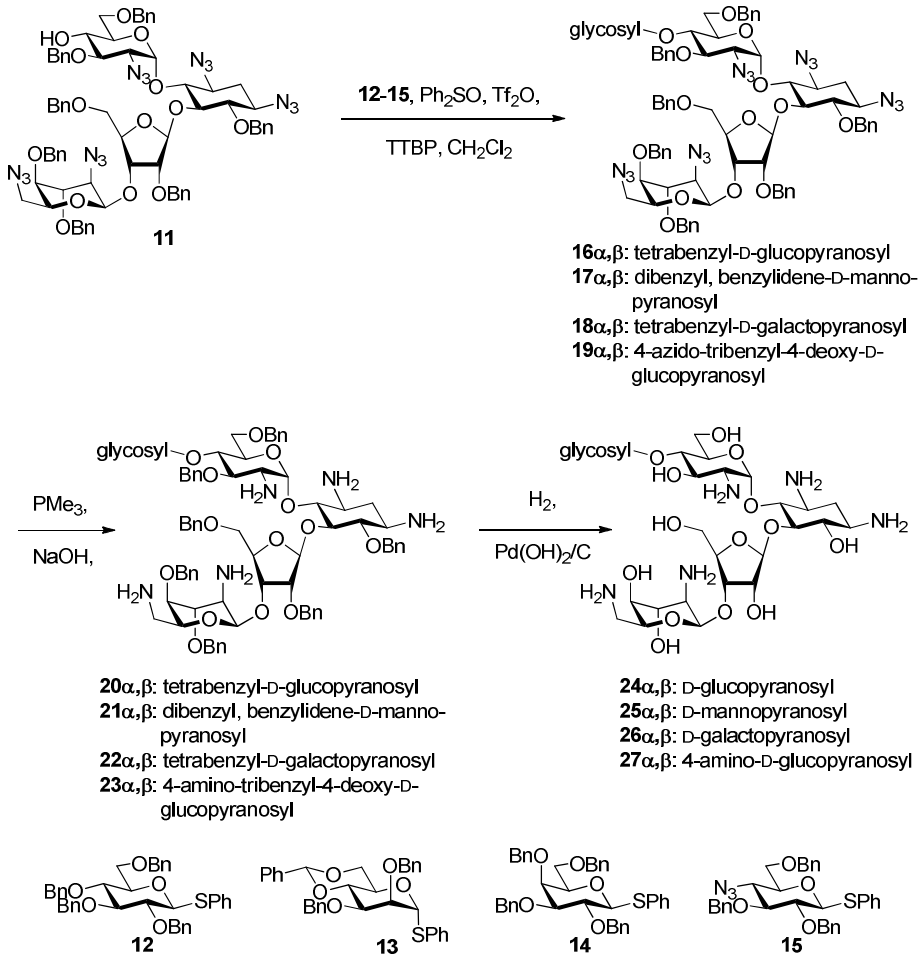
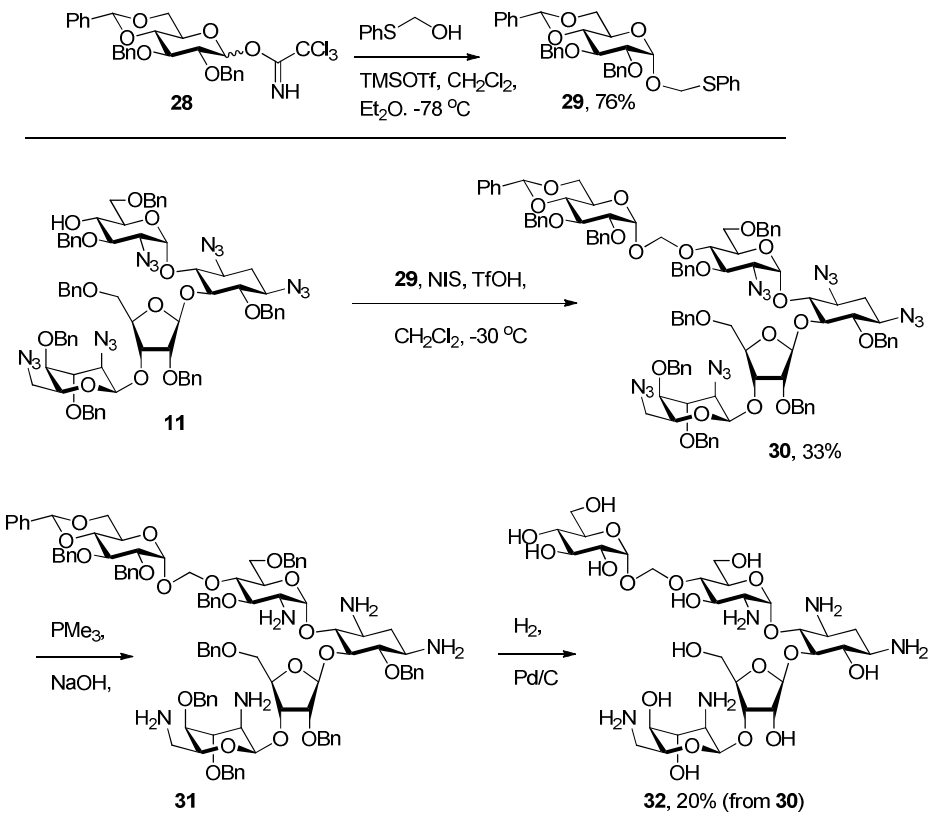


Table 1. Synthesis of 4□-*O*-glycosyl paromomycin derivatives.

Entry	Donor	Glycosylation Yield, ratio	Staudinger Yield	Hydrogenolysis Yield
1	**12**	**16**, 42%, α:β = 3.2:1	**20α**, 64%	**24α**, 39%
2			**20β**, 55%	**24β**, 87%
3	**13**	**17**, 51%, α:β = 1:3.6	**21α**, 91%	**25α**, 33%
4			**21β**, 66%	**25β**, 25%
5	**14**	**18**, 60%, α:β = 6.4:1	**22α**, 81%	**26α**, 62%
6			**22β**, 78%	**26β**, 39%
7	**15**	**19**, 50%, α:β = 4.5:1	**23α**, 64%	**27α**, 64%
8			**23β**, 48%	**27β**, 29%

In order to mimic more closely the trehalose-type linkage in apramycin, the known trichloroacetimidate **28**[23] was coupled[24] with phenylthiomethanol[25] to give the phenylthiomethyl α-D-glucoside **29** (Scheme 2). Subsequent *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) mediated coupling[24] of **29** with the acceptor **11** gave the derivative **30**, which, on subjection to the Staudinger protocol followed by hydrogenolysis in the standard manner afforded the 4□-*O*-α-D-glucopyranosyloxymethyl derivative **31** of paromomycin (Scheme 2).

Scheme 2. Synthesis of a 4□-*O*-glucosyloxymethyl paromomycin derivative



Antiribosomal Activity. The ability of the synthetic AGAs to prevent translation in cell-free ribosome assays was assessed using the parental drug paromomycin (**2**), apramycin (**1**), and the 4□-paromomycin derivatives **3** and **4** as comparators. The data presented in Table 2 indicate that, with the single exception of the 4-amino-α-D-glucopyranosyl derivative **27α**, all compounds display an IC_{50} value in μg/ml ~ten or more times greater than the parental compound **2** for bacterial wild-type ribosomes. Even taking into account the approximately 20% increase in molecular weight due to the incorporation of the additional sugar units, these IC_{50} values are predictive of a substantial decrease of antibacterial activity. Closer inspection of the data suggests that the α-anomers, with their axial glycosidic linkage to the newly introduced hexopyranosyl residue, have lower IC_{50} values than the corresponding equatorial (β-) glycosides, although the difference is small and perhaps only significant for compounds **26α,β** and **27α,β** (2.7 and 2.4-fold, respectively). The ability of **27α** to inhibit translation in bacterial ribosomes,

which approaches that of paromomycin and apramycin, may be in part attributed to the incorporation of the additional amino group and thus to increased electrostatic attraction between the positively charged AGA and the negatively charged ribosome.[26-28] However, the difference in IC_{50} with its anomer **27β** suggests that other, more specific, interactions also play a role.

Table 2. Compound interaction with polymorphic residues in the drug binding pocket (IC_{50}, μg/ml)[a]

Cmpd	4□-*O*-Substitution Type	Bacterial A Site			
		Wild Type	G1491C	G1491A	A1408G
2	none (paromomycin)	0.03±0.02	10.42±2.86	0.57±0.09	0.26±0.04
1	na (apramycin)	0.06±0.04	31.21±7.27	5.00±1.36	128.89±41.49
3	phenylpropyl ether	0.20±0.07	210.73±39.98	51.26±15.72	14.52±8.60
4	4□,6□-*O*-phenylpropylidene acetal	0.10±0.03	124.07±0.03	43.01±11.31	3.81±2.44
24α	α-D-glucopyranosyl	0.32±0.05	109.01±33.61	27.08±10.41	21.84±6.87
24β	β-D-glucopyranosyl	0.37±0.06	294.83±21.70	15.50±2.72	9.32±1.36
25α	α-D-mannopyranosyl	0.29±0.03	81.32±50.80	8.79±0.12	5.10±0.78
25β	β-D-mannopyranosyl	0.46±0.02	297.39±21.16	51.48±17.39	27.91±2.04
26α	α-D-galactopyranosyl	0.55±0.08	118.67±8.58	18.68±2.37	12.01±1.17
26β	β-D-galactopyranosyl	1.52±0.30	623.16±46.00	78.05±2.35	36.46±2.23
27α	4-amino-α-D-glucopyranosyl	0.12±0.04	32.79±2.05	6.07±0.06	7.06±0.52
27β	4-amino-β-D-glucopyranosyl	0.29±0.07	32.03±2.21	4.87±0.46	2.39±0.21
32	α-D-glucopyranosyloxymethyl	0.31±0.01	200.30±0.42	33.45±2.21	17.16±1.89

a) All measurements were made in triplicate

X-ray crystallographic studies of paromomycin in complex with the bacterial ribosomal 30S subunit[29] and with truncated oligonucleotide models of it's decoding A-site[30] indicate a hydrogen bond between O4□ of the drug and the phosphate backbone of the flipped out base A1493. However, 4□-deoxyparomomycin experiences only a minimal loss of antibacterial activity (MIC values)[16, 31] with respect to paromomycin suggesting that the 4□-OH---A1493 H bond is only a minor component of the overall binding energy. Suppression of the 4□-OH---A1493 H bond by derivatization of the 4□-OH group is thus not the major cause of the loss of activity seen in the 4□-*O*-substituted paromomycin derivatives. Presumably, the substitution pattern at the 4□-position affects the crystallographically revealed[29, 30] CH-π[32, 33] interactions of the β-face of paromomycin ring with G1491 at the bottom of the binding pocket, and the interplay between the AGA ring 1 and its hydration shell. Alteration of the

electron density at C4□ either by removal of O4□ or by substitution at O4□ may modulate the CH-π interaction between H4□ and G1491 by a combination of electronic, hydrophobic, and steric effects. For example, alkylation of O4', as in derivative **3**, will have the effect of lowering the electron density at O4□. Inclusion of O4□ in either an acetal linkage (compounds **4**, **32**) or a glycosidic bond (compounds **24**-**27**) will further reduce the electron density at that locus beyond simply alkylation. In addition, the various substitutions of O4□ may result in an unfavourable steric interaction with G1491 and so further reduce the CH-π interaction with H4□, indeed, this may be the origin of the observed differences in antiribosomal activity between the α- and β-anomers of the various glycosides studied.

X-ray crystallographic studies of apramycin (**1**),[12] of the 4□-phenylpropyl paromomycin ether (**3**)[13] and of the paromomycin 4□,6□-*O*-phenylpropylidene acetal (**4**)[13] all reveal a common mode of binding with paromomycin to the ribosomal RNA decoding A site. The aminoglucosyl residue of apramycin and the 4□(6□)-substituents in **3** and **4** also share a common location extending beyond the main binding pocket into the bulk solvent between A1492 and G1491. The glucosyl (**24**) and mannosyl (**25**) derivatives exhibit very similar antiribosomal activity for a given anomer, while the galactosyl isomers (**26**) are marginally less active. These results are consistent with the notion that gluco- and mannopyranosides may better fit into and disrupt the structure of organized water less than galactopyranosides;[34-37] thus, the galactosyl derivatives **26α,β** are the most disruptive of the solvent shell in the exposed region between A1492 and G1491.

In order to determine the influence of individual bases on the ribosome-drug interaction, the 4□-substituted AGA derivatives were assayed for their ability to inhibit mutant ribosomes in each of which a single base in the bacterial decoding A site was replaced by that found in human mitochondrial rRNA or in human cytosolic rRNA. In Figure 1 the decoding A site of the bacterial ribosome is aligned with those of human cytosolic and human mitochondrial rRNA, and with that of a human population that suffers from an enhanced incidence of drug-induced deafness.[38] Thus, the G1491C mutant (Table 2) is representative of the human mitochondrial A site, while the G1491A mutant (Table 2) mimics one of the

two changes in the human cytosolic decoding A site, with the importance of the second change independently being examined with the aid of the A1408G mutant (Table 2).

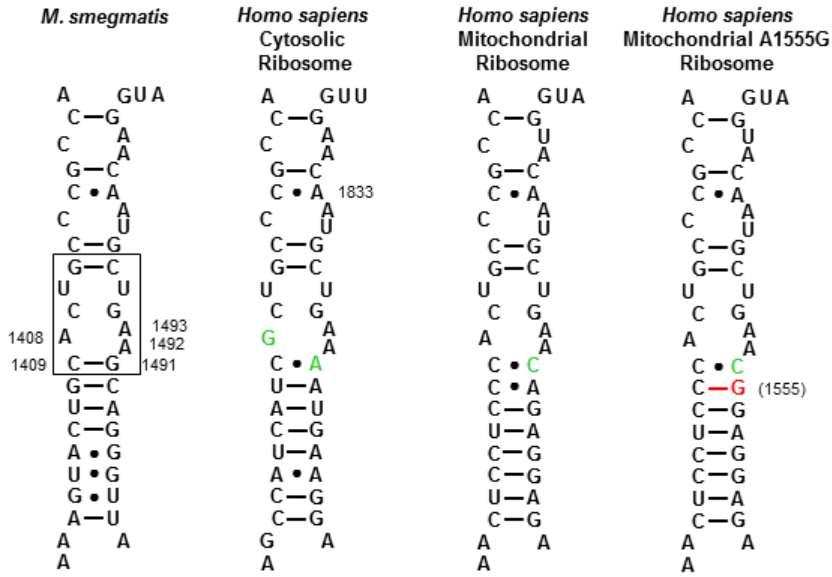


Figure 1. Decoding A sites of bacterial and eukaryotic ribosomes. (The AGA binding pocket is boxed. The prokaryotic numbering scheme is illustrated for the AGA binding pocket. Changes in the binding pocket from the bacterial ribosome are coloured green. The A1555G mutant conferring hypersusceptibility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

The data (Table 2) reveals that all compounds exhibit a significant loss of antiribosomal activity on going from the wild type to the G1491C mutant, as is the case for the parental compound paromomycin **2**, for apramycin **1**, and for the paromomycin derivatives **3** and **4**. With the exception of the 4□-*O*-(4-amino-4-deoxyglucosyl) derivatives **27α**- and **β**- which show comparable affinity, the α-anomers inhibit the G1491C mutant to a greater extent than the corresponding β-anomers. A very similar pattern is seen for inhibition of the G1491A mutant ribosomal activity, namely an overall decrease in activity and for the mannose and galactose series (**25** and **26**) preferential binding of the α-anomer (the two anomers of the glucose and 4-amino-4-deoxyglucose series exhibiting little or no difference). Overall this pattern suggests a related binding mode for all compounds studied in the bacterial wild type drug binding pocket, with the cognate C1409=G1491 base pair at the base of the ribosomal decoding A site, in the

G1491C and G1491A mutants with the noncanonical C1409●C1491 and C1409●A1491 bases pair interactions, respectively. The greater loss of activity seen for all compounds on going from the wild type to the G1491C mutant than to the G1491A mutant is indicative of greater disruption to binding on replacement of the purine G by the pyrimidine C than by the purine A. This may be the result of greater distortion of the binding pocket geometry, of a larger perturbation of the CH-π interaction with the β-face of the AGA ring 1, or of changing interactions with the varying hydration shells around the different bases.

The A1408G mutation causes a reduction in ribosomal activity in all compounds (Table 2). In the wild type A1408 forms a pseudobase interaction with the ring oxygen and the 6□-OH of paromomycin ring 1 with its hydroxymethyl group in the *gt* conformation.[29] A comparable pseudobase pair is formed with apramycin,[12] albeit the *gg* conformation about the C5□-C6□ bond is enforced by the rigid bicyclic nature of its ring1. Modelling studies[39] suggest that an alternative approximately isosteric pseudobase interaction is possible following the A1408G exchange at least in the case of paromomycin. The lower antiribosomal activity of paromomycin **2** and of its 4□-phenylpropyl ether **3** against the A1408G mutant than the wild type indicates that the alternative pseudobase interaction of ring 1 with G1408-ring 1 is weaker than that with A1408. The greatly reduced activity of apramycin toward the A1408G mutant ribosome suggests that the G-ring 1 pseudobase interaction is less adaptable than the A-ring interaction and does not accommodate the *gg* conformation of the C5□-C6□ bond. In the case of the 4□,6□-*O*-alkylidene paromomycin derivative **4** a single hydrogen bond remains possible between either A1408 N6 or G1408 N1 and O5□, despite the enforced *tg* conformation of the C5□-C6□ bond, without distortion of the overall geometry of the pseudobase pair interaction.[13] Glycosylation at O4□ presumably disrupts the A1408-ring 1 pseudobase interaction, thereby contributing to the loss of activity in the wild type through the involvement of the 6□-OH group in inter-residue hydrogen bonds with the 4□-*O*-glycosyl ring, altering the conformational preference of the C5□-C6□ bond, and reducing the availability of the 6□-OH group for hydrogen bonding to the ribosomal base. Any such modulation of

this pseudobase interaction will impact the weaker G1408-ring 1 system to a greater extent leading to the loss of activity seen with the A1408G mutant.

Having examined the influence of single point mutations in the bacterial A site on the drug ribosome interaction we turned to the use of hybrid mutant ribosomes in which complete eukaryotic A site cassettes are inserted into the bacterial ribosome.[7] The three constructs employed in this manner representative of the mitochondrial wild type, the mitochondrial mutant A1555G (deafness allele),[38] and the cytosolic wild type (Figure 2, Table 3), were complemented by the use of native eukaryotic cytosolic ribosomes from rabbit reticulocytes.

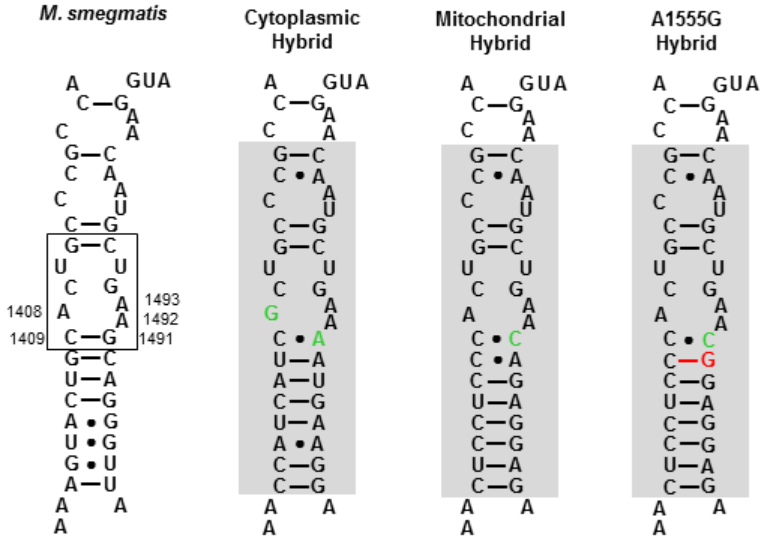


Figure 2. Decoding A sites of the bacterial and mutant hybrid ribosomes. (The AGA binding pocket is boxed. Eukaryotic A sites replacing bacterial h44 are shaded. The prokaryotic numbering scheme is illustrated for the AGA binding pocket. Changes in the binding pocket from the bacterial ribosome are coloured green. The A1555G mutant conferring hypersusceptibility to aminoglycoside ototoxicity is coloured red).

Table 3. Compound interaction with hybrid ribosomes (IC_{50}, μg/ml)[a]

Cmpd	Substitution Type	Bacterial A Site Wild Type	Eukaryotic A Site Mitochondrial Wild Type	Mitochondrial Mutant A1555G	Cyt 14	RR[illegible]
2	none (paromomycin)	0.03±0.02	50.54±13.04	5.83±2.27	10.39±2.57	9.78±4.47
1	na (apramycin)	0.06±0.04	58.03±18.80	26.06±7.08	49.17±26.01	23.77±7.48
3	phenylpropyl	0.20±0.07	49.85±15.39	105.68±39.23	268.57±54.97	nd
4	4□,6□-*O*-phenyl-propylidene	0.10±0.03	330.74±115.30	126.88±54.36	183.86±84.90	nd
24α	α-D-glucosyl	0.32±0.05	1.86±0.36	13.98±2.56	46.26±4.65	30.64±1.40
24β	β-D-glucosyl	0.37±0.06	59.12±6.57	83.64±24.45	117.53±29.68	60.75±1.21
25α	α-D-mannosyl	0.29±0.03	2.19±0.07	18.05±4.68	51.72±16.80	36.82±0.9
25β	β-D-mannosyl	0.46±0.02	54.60±8.3	85.70±27.23	90.35±26.69	47.64±2.7
26α	α-D-galactosyl	0.55±0.08	2.23±0.33	14.95±0.11	35.63±2.23	23.75±1.20
26β	β-D-galactosyl	1.52±0.30	141.35±3.08	209.22±52.33	286.76±32.27	104.57±0.54
27α	4-amino-α-D-glucosyl	0.12±0.04	0.34±0.01	3.62±1.22	18.95±5.37	19.59±4.54
27β	4-amino-β-D-glucosyl	0.29±0.07	7.62±3.30	17.38±5.12	21.43±8.53	32.41±5.11
32	α-D-glucosyl-oxymethyl	0.31±0.01	24.11±2.31	96.03±14.50	130.61±13.31	43.14±5.40

a) All measurements were made in triplicate

Compared to bacterial ribosomes, all compounds prepared in this study showed reduced antiribosomal activity toward the hybrid ribosome containing the mitochondrial A site (Table 3). A somewhat greater loss of activity is seen in the case of the mitochondrial mutant A1555G in which the non-Watson-Crick C1494●A1555 base pair immediately below the C1493●C1491 noncanonical pair at the base of the decoding A site is replaced by the Watson-Crick C1494=G1555 base pair (Figure 2). Thus, in the deafness allele the two sequential noncanonical pairs at the base of the mitochondrial A site are replaced by a single non-Watson-Crick pair. Presumably, the more flexible mitochondrial A site is better able to accommodate substitution at the 4□-position of the AGA than is its A1555G deafness allele. As with the bacterial wild type and the G1491C single point mutation both the mitochondrial hybrid ribosome

and A1555G its deafness allele were inhibited to a greater extent by the α- than by the β-anomers of the 4'-*O*-glycosides.

The Cyt 14 hybrid ribosome carrying the A site of human cytosolic rRNA incorporates both of the A1491A and A1408G single point mutations. Not surprisingly therefore all compounds studied have lower antiribosomal activity toward the Cyt 14 hybrid than toward the bacterial wild type. SAR data obtained with eukaryotic ribosomes from rabbit reticulocytes were comparable to that from the Cyt 14 hybrid ribosome. The pattern of greater inhibition by the α-anomers of 4□-*O*-glycosides than by the β-isomers, which was partially obscured for the G1491A and A1408G single point mutants, is restored for the Cyt 14 hybrid and rabbit reticulocyte ribosomes.

The difference in antiribosomal activity between the individual anomers, while typically favouring the α-anomer, is small for bacterial, cytosolic, and rabbit reticulocyte ribosomes but noticeably larger for the mitochondrial wild type and deafness alleles (Table 3). The large difference in activity of the anomers seen with the mitochondrial ribosomes results in the β-anomers displaying greater bacterial/mitochondrial selectivity. While still significant the activity difference between pairs of anomers for the deafness allele is smaller, which can be attributed to the less accommodating nature of the less flexible binding site of the deafness allele.

The small differences in antiribosomal activity (Tables 2 and 3) observed between the 4□-*O*-gluco-, manno-, and galacto- pyranosides and between the individual anomers in each series recalls the modest influence of substituents in SAR studies of 4□,6□-*O*-alkylidene and 4□-*O*-arylalkyl derivatives of paromomycin related to compounds **3** and **4**.[13] Together this suggests the absence of a specific interaction between the 4□-(6□-)-*O*-substituent and the ribosomal decoding A site, but rather the existence of one or more less specific interactions none of which is dominant.

Antibacterial Activity. All glycosides prepared were assessed for their antibacterial activity by the determination of MIC values against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* (Table 4). The parental drug paromomycin **2**, apramycin **1**, and the 4'-*O*-(phenylpropyl) and 4□,6□-*O*-

alkylidene derivatives **3** and **4** of paromomycin were used as comparators (Table 4). With the exception of two strains of *S aureus* which were resistant to paromomycin, all compounds other than the 4-amino-4-deoxy glucoside **27α** were less active than the comparators reflecting the IC_{50} data for inhibition of the wild type bacterial ribosome (Table 2). Consistent with the IC_{50} data for the wild type bacterial ribosome (Table 2) the α-anomers of all 4□-*O*-glycosides are more active than their β-counterparts. No advantage is gained by the inclusion of a spacer group between the AGA 4□-position and the glycoside. 4□-*O*-(4-Amino-4-deoxy)-α-D-glucopyranosyl paromomycin **27α** has comparable activity to the 4□,6□-*O*-(2-phenylpropylidene) derivative **4** of paromomycin, which we attribute to the presence of the additional amino group in the sugar moiety and which is consistent with the wild type antiribosomal activity. Comparators **1**, **3**, and **4**, the 4□-*O*-α-glucoside **24α**, and both anomers of the 4-amino-4-deoxy glucoside **27α** and **27β**, unlike paromomycin itself **2**, are active against the *S aureus* strains AG 039 and AG 042. Presumably, AG 039 and AG 042 achieve resistance through the operation of a 4□- or a 3'-aminoglycoside modifying enzyme.[5, 40, 41]

Table 4. Minimal inhibitory concentrations (MIC, μg/ml) of clinical isolates

Compound	Strain							
	Staphylococcus aureus (MRSA)				*Escherichia coli*			
	AG 038	**AG 039**	**AG 042**	**AG 044**	**AG 006**	**AG 001**	**AG 055**	**AG 003**
2	4	>256	>256	4-8	2-4	16-32	8	8-16
1	8	8	8	16	4	16	8	8-16
3	2-4	8	4	4-8	8	16	16	8-16
4	4-8	8	4-8	16	16-32	32-64	32	32
24α	32	32	32	16-32	16-32	128	128	128
24β	128	≥128	>128	128	32	>128	>128	>128
25α	64	≥128	>128	32-64	16	>128	>128	≥128
25β	≥128	≥128	≥128	≥128	64	>128	>128	>128
26α	64	128	64	64	32	>128	>128	>128
26β	≥128	≥128	>128	>128	128	>128	>128	>128
27α	4	8-16	8-16	4	2-4	32	32	16-32
27β	32	>64	>64	32	16	>64	>64	>64
32	64-128	≥128	128	64-128	64	≥128	≥128	≥128

Conclusion

A series of 4□-*O*-D-hexopyranosyl analogues of the AGA paromomycin have been prepared by chemical synthesis and their individual α- and β-anomers assayed for their ability to inhibit ribosomal protein synthesis by bacterial ribosomes and their mutants designed to mimic the various eukaryotic drug binding pockets. All compounds were also assayed for antibacterial activity against clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. With the exception of the 4-amino-4-deoxy glucosides, all glycosides showed less antibacterial activity than paromomycin, apramycin, and two simple 4□-*O*-derivatives of paromomycin. Among the glycosides prepared the α-anomers were generally more active than their β-isomers and the glucopyranosides more active than the manno- and galactopyranosides.

Experimental Part.

General procedure for glycosylation. A dichloromethane solution of glycosyl donor (0.47 mmol, 1.3 eq), diphenyl sulfoxide (0.55 mmol, 1.5 eq), 2,4,6-tri-*tert*-butylpyrimidine (1.05 mmol, 2.9 eq) and activated 3 Å molecular sieves in dichloromethane (3 mL) was stirred at room temperature for 3 h before cooling to the indicated temperature (see Supplementary Information). Trifluoromethanesulfonic anhydride (0.55 mmol, 1.5 eq) was added slowly to the solution. After 1 h at the same temperature a solution of acceptor **11**[16] (0.36 mmol, 1 eq) in dichloromethane (1.5 mL) was added dropwise to the reaction mixture. The resulting solution was stirred at the same temperature for 2 h before the reaction was quenched by the addition of saturated aqueous $NaHCO_3$. The reaction mixture was diluted with EtOAc and the organic phase was washed with saturated aqueous $NaHCO_3$ three times. The organic layer was dried (Na_2SO_4), concentrated and purified by column chromatography over silica gel.

General procedure for the Staudinger reduction of azides to amines.[16] The substrate (0.06 mmol, 1 eq) in THF (5 mL) was treated at room temperature with 0.1 M aqueous NaOH (0.3 mL, 0.03 mmol, 5 eq) and 1 M trimethylphosphine in THF (0.6 mmol, 10 eq). The reaction mixture was stirred for 2 h at 50 °C, and then cooled to room temperature and neutralized with 1 M aqueous AcOH to pH 7 before

concentration. The resulting slurry was subjected to silica gel chromatography, eluting first with MeOH (100 mL), followed by 0.5% aqueous NH_4OH in MeOH (150 mL) to give the product.

General procedure for hydrogenolysis. The substrate (25 mg) was dissolved in a mixture of methanol (1 mL), deionized water H_2O (2 mL), and glacial acetic acid (0.1 mL). A catalytic amount of $Pd(OH)_2$ on carbon (20 wt. %) was added, and the reaction mixture was stirred at room temperature under 1 atm of hydrogen (balloon) overnight. After completion, the reaction mixture was filtered over Celite® and filtrate was neutralized by the addition of Amberlite-IRA400 to pH 7 and filtered. The filtrate was concentrated to dryness and dissolved in 0.002 M aqueous AcOH (pH 4, 2 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The Sephadex column was flushed sequentially with deionized water H_2O (50 mL), 0.5% aqueous NH_4OH (20 mL), and 1.5% NH_4OH (40 mL). The fractions containing the hydrogenolysis product were combined and evaporated to give a sticky white solid, which was dissolved in 0.00002 M acetic acid (pH 5, 1 mL). The resulting solution was frozen by immersion in a dry ice acetone bath, and then the water was removed by lyophilization to give the product in the form of its acetate salt.

Bacterial strains. Clinical isolates of *E. coli* and *S. aureus* were obtained from the Diagnostic Department, Institute of Medical Microbiology, University of Zurich. MIC values were determined by broth microdilution assays.

Recombinant microorganisms. The construction of these strains derived from single rRNA allelic *M. smegmatis* ΔrrnB, has been described previously.[7, 38]

Cell-free translation assays. Rabbit reticulocyte lysate (Promega), S-30 extracts and purified ribosomes were used for cell-free translation assays as described previously.[12] Firefly luciferase mRNA was used as reporter to monitor translation activity. Luminescence was measured using a luminometer Flx800 (Bio-Tek Instruments).

Supplementary Information

Complete characterization data for all new compounds. Copies of the ^{1}H and ^{13}C NMR spectra of **20α,β-23α,β** and **32**.

Acknowledgments. We gratefully acknowledge support from the University of Zurich and Wayne State University.

References

1. E. S. Armstrong, C. F. Kostrub, R. T. Cass, H. E. Moser, A. W. Serio and G. H. Miller, in *Antibiotic Discovery and Development*, eds. T. J. Dougherty and M. J. Pucci, Springer Science+Business Media, 2012, pp. 229-269.
2. B. Becker and M. A. Cooper, *ACS Chem. Biol.*, 2013, **8**, 105-115.
3. J. L. Houghton, K. D. Green, W. Chen and S. Garneau-Tsodikova, *ChemBioChem*, 2010, **11**, 880-902.
4. S. B. Vakulenko and S. Mobashery, *Clin. Microbiol. Rev.*, 2003, **16**, 430-450.
5. J. B. Aggen, E. S. Armstrong, A. A. Goldblum, P. Dozzo, M. S. Linsell, M. J. Gliedt, D. J. Hildebrandt, L. A. Feeney, A. Kubo, R. D. Matias, S. Lopez, M. Gomez, K. B. Wlasichuk, R. Diokno, G. H. Miller and H. E. Moser, *Antimicrob. Agent. Chemother.*, 2010, **54**, 4636-4642.
6. S. Magnet and J. S. Blanchard, *Chem. Rev.*, 2005, **205**, 477-497.
7. S. N. Hobbie, S. K. Kalapala, S. Akshay, C. Bruell, S. Schmidt, S. Dabow, A. Vasella, P. Sander and E. C. Böttger, *Nucl. Acids Res.*, 2007, **35**, 6086-6093.
8. S. N. Hobbie, S. Akshay, S. K. Kalapala, C. Bruell, D. Shcherbakov and E. C. Böttger, *Proc. Natl. Acad. Sci., USA*, 2008, **105**, 20888-20893.
9. R. Akbergenov, D. Shcherbakov, T. Matt, S. Duscha, M. Meyer, D. Perez-Fernandez, R. Pathak, S. Harish, I. Kudyba, S. R. Dubbaka, S. Silva, M. Ruiz Ruiz, S. Salian, A. Vasella and E. C. Böttger, in *Ribosomes: Structure, Function, and Dynamics*, eds. M. V. Rodnina, W. Wintermeyer and R. Green, Springer-Verlag, Vienna, 2011, pp. 249-261.
10. E. C. Böttger and J. Schacht, *Hearing Research*, 2013, **303**, 12-19.
11. S. P. Francis, J. Katz, K. D. Fanning, K. A. Harris, B. D. Nicholas, M. Lacy, J. Pagana, P. F. Agris and J.-B. Shin, *J. Neuroscience*, 2013, **33**, 3079-3093.
12. T. Matt, C. L. Ng, K. Lang, S.-H. Sha, R. Akbergenov, D. Shcherbakov, M. Meyer, S. Duscha, J. Xie, S. R. Dubbaka, D. Perez-Fernandez, A. Vasella, V. Ramakrishnan, J. Schacht and E. C. Böttger, *Proc. Natl. Acad. Sci., USA*, 2012, **109**, 10984-10989.
13. D. Perez-Fernandez, D. Shcherbakov, T. Matt, N. C. Leong, I. Kudyba, S. Duscha, H. Boukari, R. Patak, S. R. Dubbaka, K. Lang, M. Meyer, R. Akbergenov, P. Freihofer, S. Vaddi, P. Thommes, V. Ramakrishnan, A. Vasella and E. C. Böttger, *Nature Commun.*, 2014, **5**, 3112.
14. M. Fridman, V. Belakhov, S. Yaron and T. Baasov, *Org. Lett.*, 2003, **5**, 3575-3578.
15. H. Tamura, Y. Fukakusa, Y. Ogawa and J. Ouchi, *Oyo Yakuri* 1972, **6**, 845-855.
16. R. Pathak, D. Perez-Fernandez, R. Nandurdikar, S. k. Kalapala, E. C. Böttger and A. Vasella, *Helv. Chim. Acta*, 2008, **91**, 1533-1552.
17. J. Dinkelaar, A. R. de Jong, R. van Meer, M. Somers, G. Lodder, H. S. Overkleeft, J. D. C. Codée and G. A. van der Marel, *J. Org. Chem.*, 2009, **74**, 4982-4991.
18. D. Crich and S. Sun, *Tetrahedron*, 1998, **54**, 8321-8348.
19. C. M. Taylor, C. A. Weir and C. G. Jørgensen, *Aust. J. Chem.*, 2002, **55**, 135-140.
20. W. A. Greenberg , E. S. Priestley , P. S. Sears , P. B. Alper , C. Rosenbohm , M. Hendrix , S.-C. Hung and C.-H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 6527-6541.
21. J. D. C. Codée, L. J. van den Bos, R. E. J. N. Litjens, H. S. Overkleeft, C. A. A. van Boeckel, J. H. van Boom and G. A. van der Marel, *Tetrahedron*, 2004, **60**, 1057-1064.
22. D. Crich, M. Smith, Q. Yao and J. Picione, *Synthesis*, 2001, 323-326.
23. H. Waldmann, G. Böhm, U. Schmid and H. Röttele, *Angew. Chem. Int. Ed.*, 1994, **33**, 1944-1946.
24. D. Crich and F. Yang, *Angew. Chem. Int. Ed.*, 2009, **48**, 8896-8899.
25. L. L. Gundersen and T. Benneche, *Acta Chem. Scand.*, 1991, **45**, 975-977.
26. R. Benveniste and J. Davies, *Antimicrob. Agent. Chemother.*, 1973, **4**, 402-409.
27. M. Kaul, C. M. Barbieri, J. E. Kerrigan and D. S. Pilch, *J. Mol. Biol.*, 2003, **326**, 1373-1387.

28. S. Salian, T. Matt, R. Akbergenov, S. Harish, M. Meyer, S. Duscha, D. Shcherbakov, B. B. Bernet, A. Vasella, E. Westhof and E. C. Böttger, *Antimicrob. Agents Chemother.*, 2012, **56**, 6104-6108.
29. A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly and V. Ramakrishnan, *Nature*, 2000, **407**, 340-348.
30. Q. Vicens and E. Westhof, *Structure*, 2001, **9**, 647-658.
31. C. Battistini, G. Cassinelli, G. Franceschi, F. Arcamone and R. Mazzoleni, *J. Antibiotics*, 1981, **34**, 240-242.
32. J. L. Asensio, A. Ardá, F. J. Cañada and J. Jiménez-Barbero, *Acc. Chem. Res.*, 2013, **46**, 946-954.
33. T. Vacas, F. Corzana, G. Jiménez-Osés, C. González, A. M. Gómez, A. Bastida, J. Revuelta and J. L. Asensio, *J. Am. Chem. Soc.*, 2010, **132**, 12074-12090.
34. S. A. Galema, J. B. F. N. Engberts, H. Hoiland and G. M. Ferrland, *J. Phys. Chem.*, 1993, **97**, 6885-6889.
35. S. A. Galema, M. J. Blandamer and J. B. F. N. Engberts, *J. Org. Chem.*, 1992, **57**, 1995-2001.
36. S. A. Galema and H. Hoiland, *J. Phys. Chem.*, 1991, **95**, 5321-5326.
37. C. J. Capicciotti, M. Leclere, F. A. Perras, D. L. Bryce, H. Paulin, J. Harden, Y. Liu and R. N. Ben, *Chem. Sci.*, 2012, **3**, 1408-1416.
38. S. N. Hobbie, C. M. Bruell, S. Akshay, S. K. Kalapala, D. Shcherbakov and E. C. Böttger, *Proc. Natl. Acad. Sci., USA*, 2008, **105**, 3244-3249.
39. P. Pfister, S. Hobbie, Q. Vicens, E. C. Böttger and E. Westhof, *ChemBioChem*, 2003, **4**, 1078-1088.
40. T. Ida, R. Okamoto, C. Shimauchi, T. Okubo, A. Kuga and M. Inoue, *J. Clin. Microbiol.*, 2001, **39**, 3115-3121.
41. R.-B. Yan, M. Yuan, Y. Wu, X. You and X.-S. Ye, *Bioorg. Med. Chem.*, 2011, **19**, 30-40.