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Modification at the 2′-Position of the 4,5-Series of 2-Deoxystreptamine Aminoglycoside Antibiotics To Resist Aminoglycoside Modifying Enzymes and Increase Ribosomal Target Selectivity

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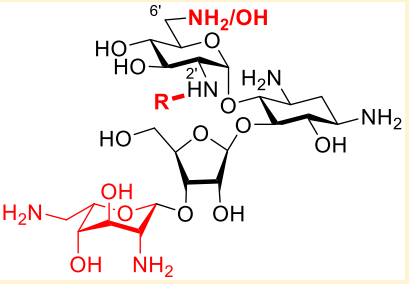
Supporting Information

ABSTRACT: A series of derivatives of the 4,5-disubstituted class of 2-deoxystreptamine aminoglycoside antibiotics neomycin, paromomycin, and ribostamycin was prepared and assayed for (i) their ability to inhibit protein synthesis by bacterial ribosomes and by engineered bacterial ribosomes carrying eukaryotic decoding A sites, (ii) antibacterial activity against wild type Gram negative and positive pathogens, and (iii) overcoming resistance due to the presence of aminoacyl transferases acting at the 2′-position. The presence of five suitably positioned residual basic amino groups was found to be necessary for activity to be retained upon removal or alkylation of the 2′-position amine. As alkylation of the 2′-amino group overcomes the action of resistance determinants acting at that position and in addition results in increased selectivity for the prokaryotic over eukaryotic ribosomes, it constitutes an attractive modification for introduction into next generation aminoglycosides. In the neomycin series, the installation of small (formamide) or basic (glycinamide) amido groups on the 2′-amino group is tolerated.



KEYWORDS: *aminoglycosides, multidrug-resistant infectious diseases, decoding A site, selectivity, synthesis*

The increasing threat of multidrug-resistant infectious diseases demands continued development of new and improved anti-infective agents.[1−6] In this regard, aminoglycosides (AGAs)[7−11] are strong candidates for further development because of their widespread availability, innate potency, and the extensive knowledge base covering their mechanism of action[10,12,13] and chemistry.[7,14−16] Together with the deep understanding of the mechanism of resistance, this knowledge base has long informed the structure-based design of new generations of AGAs,[17−24] as exemplified by the recent introduction of the semisynthetic AGA plazomicin **1** into clinical practice.[25,26] In addition to many aminoglycoside modifying enzymes (AMEs),[27−30] a second important and growing mechanism of AGA resistance is the modification of G1405 in the drug binding pocket in the decoding A site of the bacterial ribosome by the ribosomal methyl transferases (RMTs).[31] The action of the G1405 RMTs greatly diminishes the activity of all members of the 4,6-disubstituted 2-deoxystreptamine (DOS) class of AGAs, that is, all AGAs in current clinical use including plazomicin,[25,26,32] and is especially problematic when the responsible genes are encoded on the same plasmid as those for a metallocarbapenemase.[31,33−35] Fortunately, DOS-type AGAs lacking a ring substitution at the 6-position do not make direct contact with G1405 and are not susceptible to the action of RMTs.[36] Such AGAs include the 4,5-disubstituted DOS class such as paromomycin **2** and neomycin **3**, and the unusual monosubstituted DOS AGA apramycin **4**,[37−41] currently a clinical candidate for the treatment of complicated urinary tract infections.[42]

Accordingly, we have focused our efforts on the design and development of semisynthetic AGAs in the 4,5-DOS class with emphasis on modification at the 4′- and 6′-positions of paromomycin,[43−46] as exemplified by propylamycin **5** (4′-deoxy-4′-propylparomomycin).[47] In addition to thwarting the action of several AMEs, we discovered that the introduction of appropriate substituents to the 4′-position of paromomycin disproportionately reduces affinity for the drug binding site in the eukaryotic ribosomes, cytoplasmic and mitochondrial, thereby increasing target selectivity and reducing ototoxicity, an important side effect of AGA therapy,[48−50] as borne out by in vivo studies with guinea pigs.[44,47] Stimulated by early observations in the literature on the derivatization of the 2′-position of paromomycin and neomycin directed at circumventing the action of the AAC(2′) class of aminoglycoside acetyl transferase AMEs,[51−53] we now turn our attention to and report

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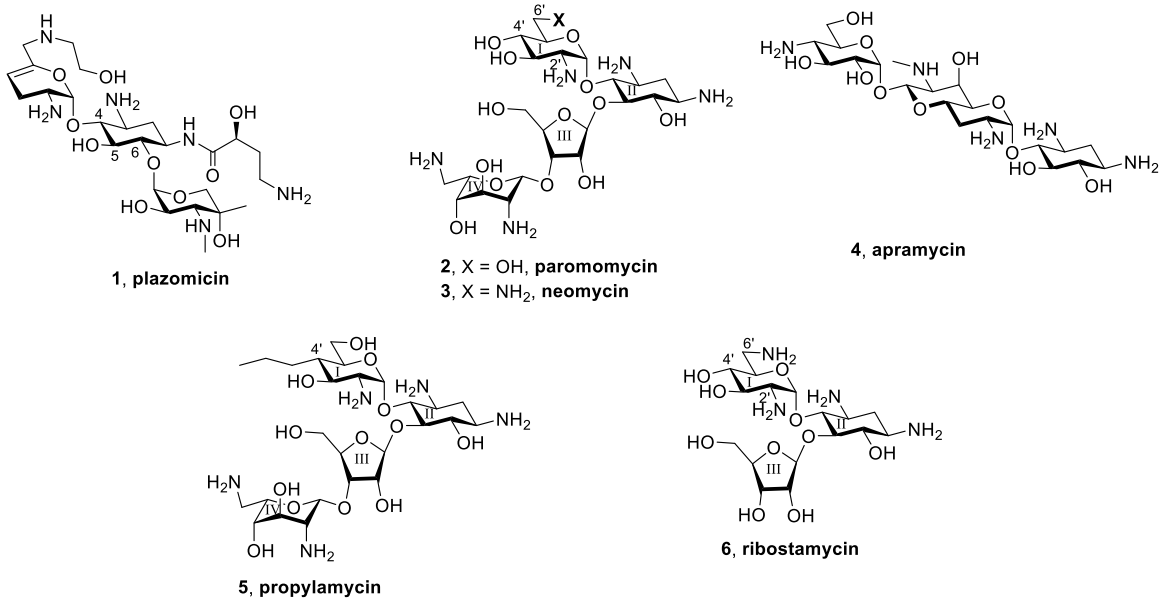
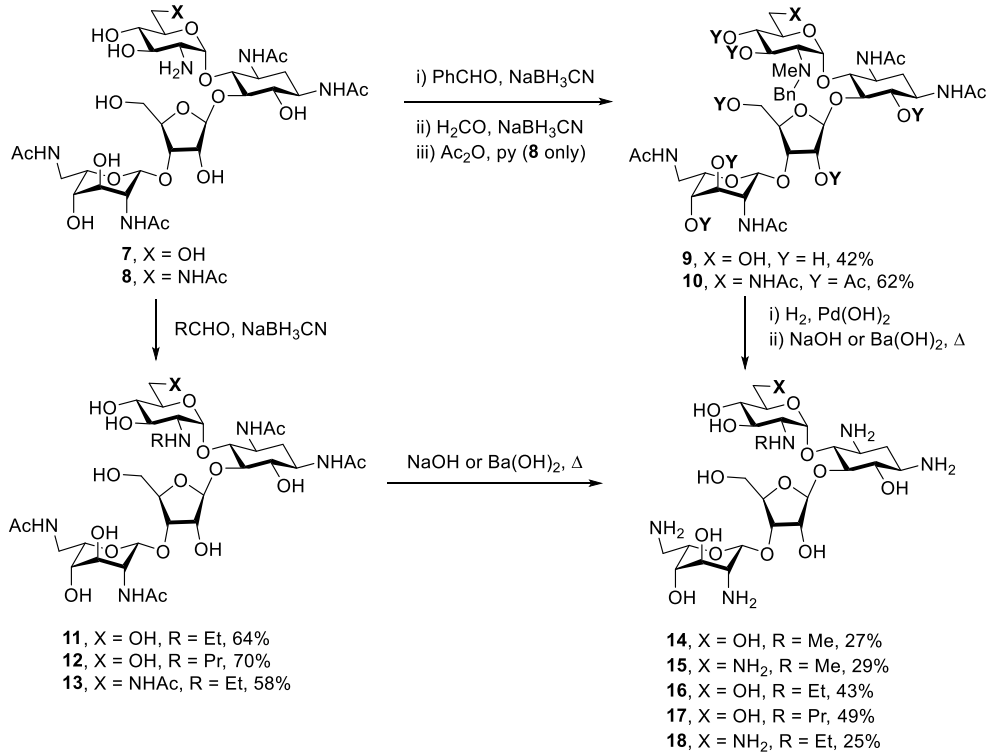


Figure 1. Some natural and semisynthetic aminoglycoside antibiotics.

Scheme 1. Synthesis of 2′-*N*-Alkyl Derivatives of Paromomycin and Neomycin



here on the possibility of circumventing AME action in conjunction with improvements in target selectivity by modification at the 2′-position of paromomycin **2**, neomycin **3**, and ribostamycin **6** (Figure 1).

RESULTS

Chemical Synthesis. By adapting a literature procedure for the regioselective tetra-*N*-acetylation of paromomycin,[51] treatment of a solution of paromomycin **2** and neomycin B **3** free bases in methanol containing 1N aqueous HCl with acetic anhydride gave 1,3,2‴,6‴-tetra-*N*-acetyl paromomycin **7** and 1,3,6′,2‴,6‴-penta-*N*-acetyl neomycin **8** as the major products in 41 and 39% isolated yields, respectively. Reductive amination of both **7** and **8** with benzaldehyde, followed by a second reductive amination with formaldehyde, and, in the case of the neomycin derivative by peracetylation, afforded the 2′-*N*-benzyl-2′-*N*-methyl paromomycin and neomycin derivatives **9** and **10** in 48 and 62% yield, respectively (Scheme 1). Reductive amination of **7** with acetaldehyde and propionaldehyde gave **11** and **12**, whereas treatment of **8** with acetaldehyde and sodium cyanoborohydride gave **13**, all in moderate to good yield. Hydrogenolysis of **9** and **10** over palladium hydroxide in

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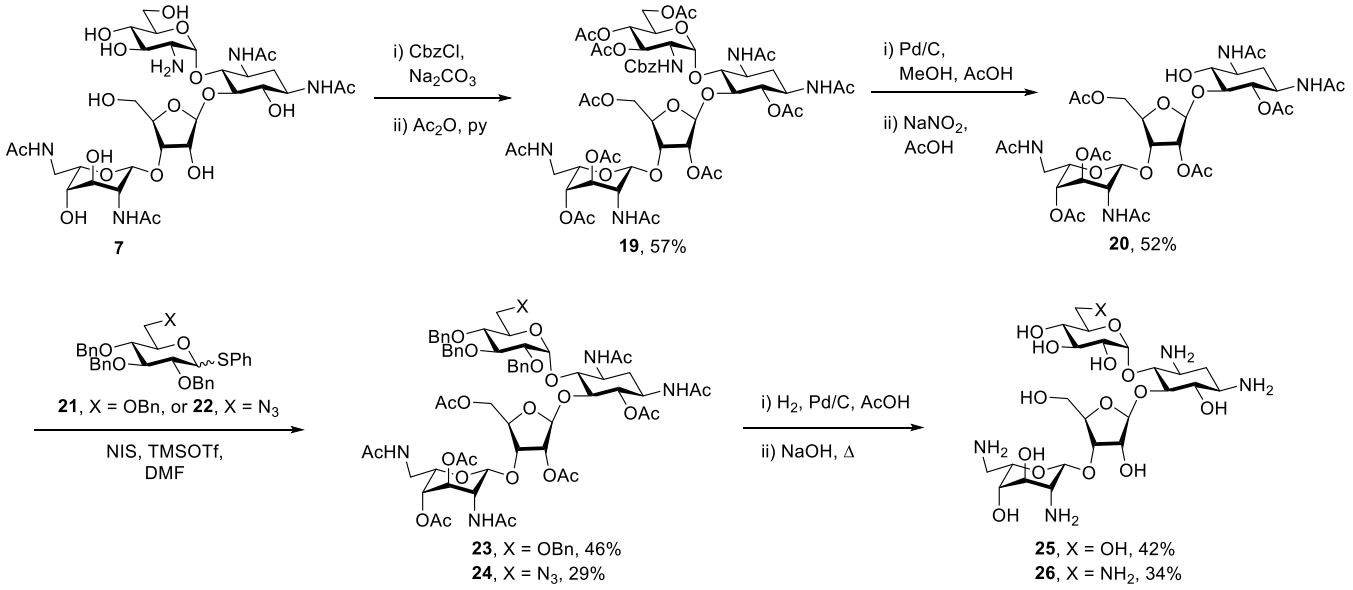
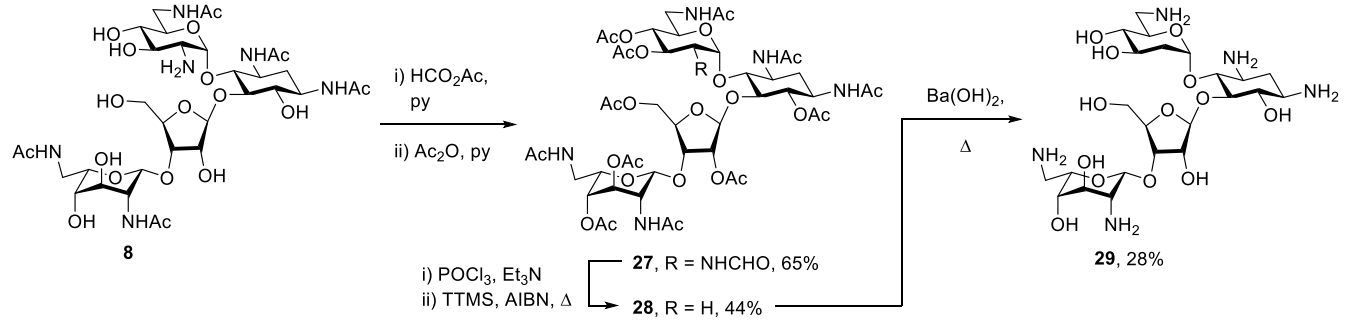
Scheme 2. Synthesis of 2′-Desamino-2′-hydroxy Paromomycin and Neomycin Derivatives



Scheme 3. Synthesis of 2′-Desamino Neomycin



methanol followed by heating to reflux with either aqueous sodium hydroxide or barium hydroxide and then Sephadex chromatography gave the 2′-*N*-methyl derivatives **14** and **15** of paromomycin and neomycin, respectively. Simple heating of **11**−**13** with aqueous sodium or barium hydroxide followed by Sephadex chromatography afforded the known[51] 2′-*N*-ethyl paromomycin derivative **16**, the corresponding neomycin derivative **18**, and the 2′-*N*-propyl paromomycin derivative **17**. All compounds in this and subsequent schemes were isolated as their peracetate salts following lyophilization from aqueous acetic acid.

Following the Farmitalia protocol,[52] installation of a benzyl carbamate on the amino group of **7** followed by peracetylation gave **19** in 57% yield. Hydrogenolysis of **19** followed by diazotization in aqueous acetic acid gave the known[52] pseudotrisaccharide **20** in 52% yield. Glycosylation of acceptor **20** with donors **21** and **22**, prepared as described in the literature,[54,55] with activation by *N*-iodosuccinimide and trimethylsilyl triflate in a mixture of dichloromethane and dimethylformamide, so as to obtain the axial glycosides selectively,[56] gave **23** and **24** in 46 and 29% yield, respectively. Hydrogenolysis, heating to reflux in aqueous sodium hydroxide, and Sephadex chromatography then gave the 2′-desamino-2′-hydroxy paromomycin and neomycin derivatives **25** and **26**, respectively (Scheme 2). Glycosylation with donor **21** leading directly to the glycoside **23** was considered preferable to the more elaborate sequence employed previously in the preparation of **25** from **20** by the Farmitalia group who nevertheless favored the use of DMF as solvent in their glycosylation reaction.[52] The cleavage of ring I from **19** by the nitrosylation protocol confirms the regioselectivity of the initial partial acetylation of **2** giving **7** (Scheme 1) and of all subsequent derivatives of it.

Formylation of **8** with formic acetic anhydride followed by acetylation gave the 2′-*N*-formyl neomycin derivative **27**. This was converted by reaction with phosphorus oxychloride and triethylamine to the corresponding isocyanate, which without characterization was subject to the Barton deamination reaction[57−59] using tris(trimethylsilyl)silane[60] in place of the original tributyltin hydride, to afford the 2′-desamino derivative **28** in 44% yield for the two steps. Hydrolysis with hot barium hydroxide then afforded **29** in the standard manner (Scheme 3). No attempt was made to prepare the corresponding 2′-desamino paromomycin derivative in view of the modest antibacterial activity of the 2′-desamino-2′-hydroxy paromomycin derivative **25**.

Turning to the preparation of 2′-*N*-acyl derivatives, neomycin B **3** was treated with acetic anhydride in the presence of HCl to give crude **8**, which was subjected to reaction with imidazole sulfonyl azide[61−64] and potassium carbonate in the presence of copper sulfate to give the corresponding 2′-azido derivative. Without isolation, and adopting the Grieco protocol for acetamide cleavage,[65] this compound was heated to reflux in tetrahydrofuran with Boc_2O and DMAP, followed by acetylation

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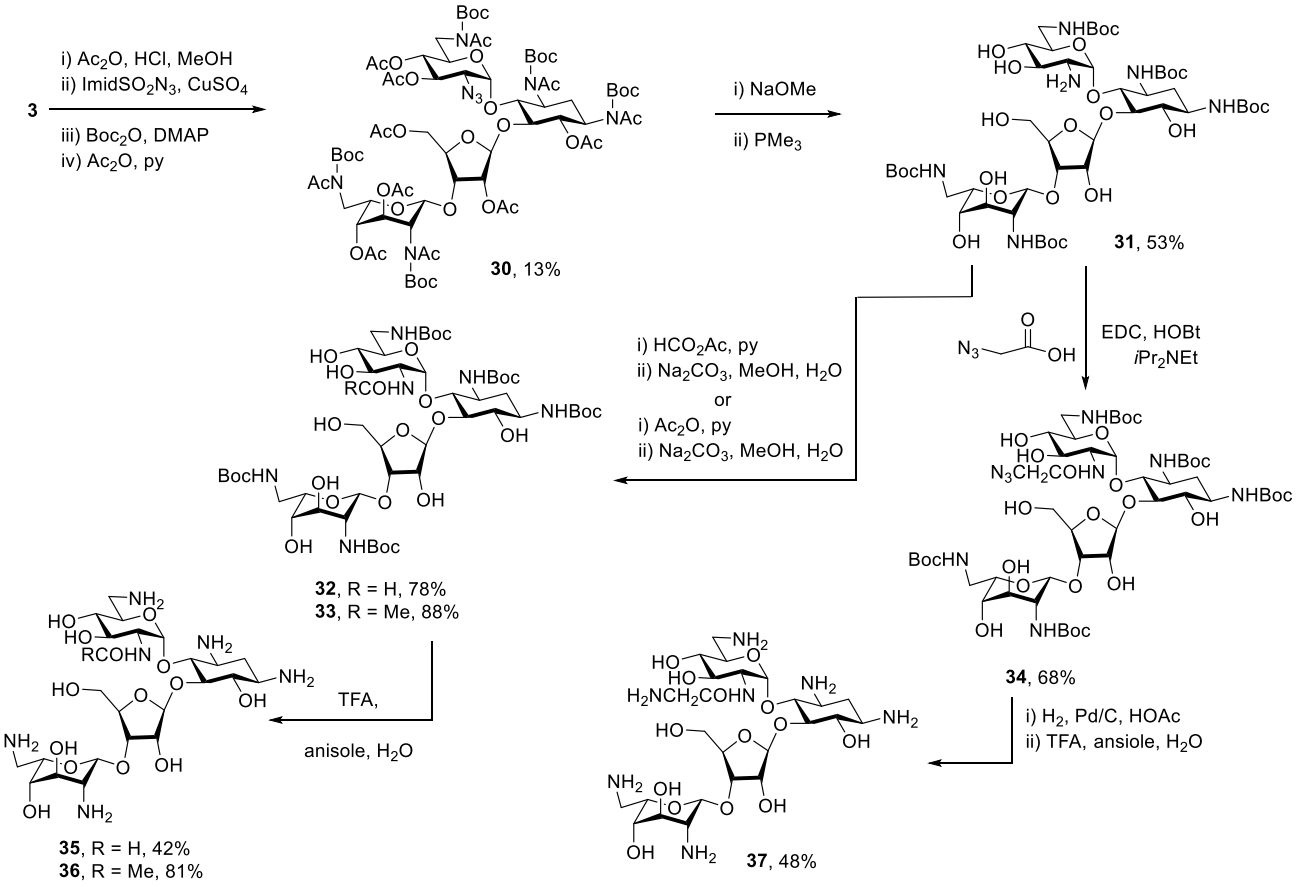
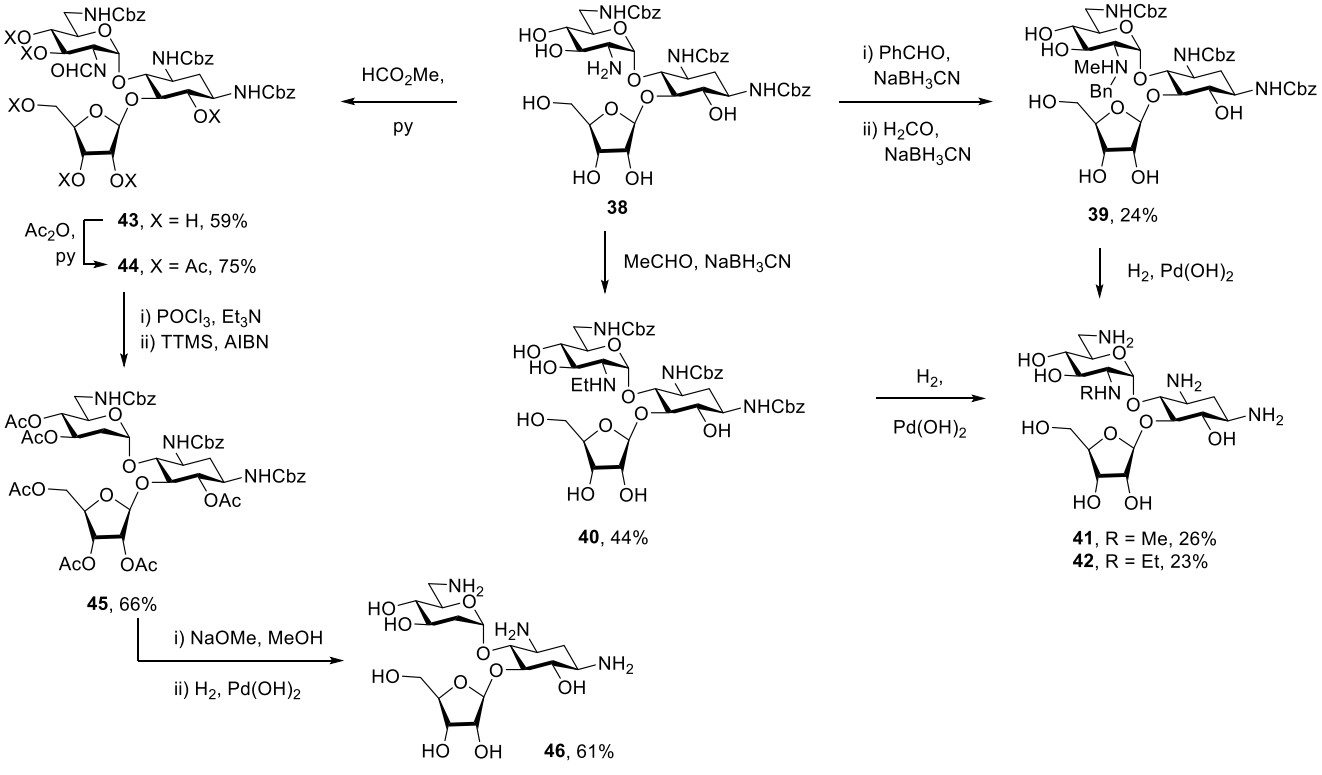
Scheme 4. Synthesis of Neomycin 2′-Amides



Scheme 5. Synthesis of Ribostamycin Derivatives 41, 42, and 46



with acetic anhydride in pyridine to give **30** in 13% overall yield for the four steps from **3** (Scheme 4). Treatment with sodium methoxide in methanol followed by Staudinger reduction of the azide with trimethylphosphine[66] then afforded a 2′-amine **31**

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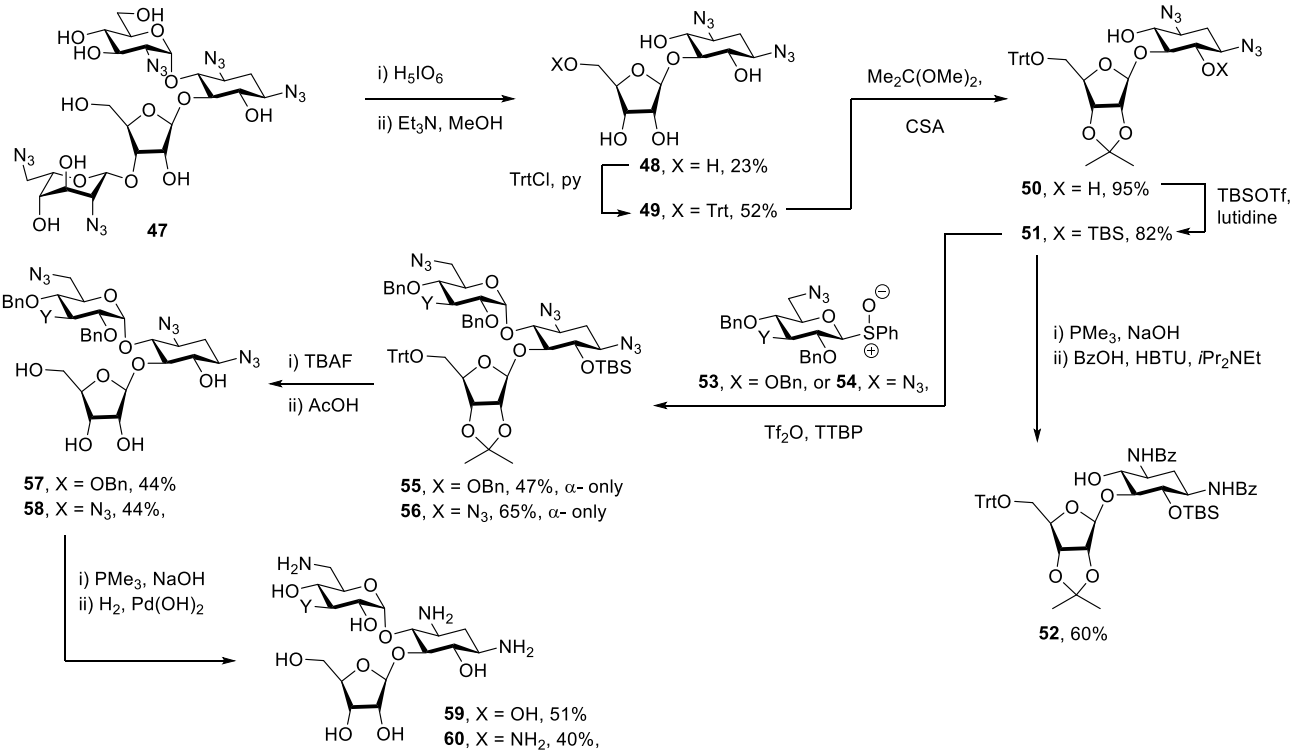
Scheme 6. Synthesis of Ribostamycin Derivatives 59 and 60



suitable for installation of various amides. Treatment of **31** with formic acetic anhydride or acetic anhydride in pyridine followed by aqueous methanolic sodium carbonate then gave amides **32** and **33** in 78 and 88% yield, respectively. Coupling of **31** with azidoacetic acid[67] by means of EDC and HOBt gave the azido acetamide **34** in 68% yield. Finally, stirring of **32** and **33** with wet trifluoroacetic acid in the presence of anisole, followed by sephadex chromatography and lyophilization from acetic acid gave the 2′-*N*-formamide **35**, and an authentic sample of the acetamide **36** in 42, and 81% yields, respectively. In the case of the glycinamide **37**, isolated in 48% yield, the removal of the carbamate groups was preceded by reduction of the azide (Scheme 4). It is of interest, although not inconsistent with the literature,[68−70] that the formamide **35** exists in D_2O solution as an unassigned 7:3 mixture of rotamers.

In the ribostamycin series, the parent **6** was regioselectively protected as the 1,3,6′-tris(benzyloxycarbamate) **38** by treatment with *N*-(benzyloxycarbonyloxy)-5-norborene-endo-2,3-dicarboximide (NBD)[71] in the presence of sodium carbonate in 36% yield. Sequential reductive amination with benzaldehyde and then with formaldehyde gave the 2′-*N*-benzyl-2′-*N*-methyl derivative **39** in 24% yield, while parallel treatment with acetaldehyde and sodium cyanoborohydride gave the 2′-*N*-ethyl derivative **40** in 44% yield. Hydrogenolysis of **39** and **40** over palladium hydroxide then afforded 2′-*N*-methyl and 2′-*N*-ethyl ribostamycin, **41** and **42**, in 26 and 23% yield, respectively (Scheme 5). Reaction of **38** with formic acetic anhydride and pyridine provided the 2′-formamide **43** in 59%, which was converted to the peracetate **44** in 75% yield in the standard manner. Dehydration of **44** with phosphorusoxy chloride and triethylamine afforded the corresponding 2′-isonitrile, which was subjected to tris(trimethylsilyl)silane and AIBN in hot toluene to give the 2′-desamino derivative **45** in 66% yield for the two steps. Deprotection with sodium methoxide in methanol followed by hydrogenloysis then afforded 2′-desamino ribostamycin **46** in 61% yield.

To prepare 2′-desamino-2′-hydroxy ribostamycin derivatives, we again took an approach based on glycosylation of a 2-deoxystreptamine derivative. Thus, adapting Hanessian's protocol for the degradation of rings I and IV,[72] the perazido paromomycin derivative **47**[73] was treated with periodic acid followed by triethylamine to afford the 5-*O*-ribosyl-2-deoxystreptamine derivative **48** in 23% yield (Scheme 6). Installation of a trityl group on the primary alcohol to give **49** in 52% was followed by conversion of the *cis*-diol to the corresponding acetonide **50** in 95% yield with 2,2-dimethoxypropane under catalysis by camphor-10-sufonic acid. Treatment of **50** with *tert*-butyldimethylsilyl triflate in the presence of 2,6-lutidine gave a single monosilyl ether **51** in 82% yield. Notwithstanding the literature precedent for the regioselective monofunctionalization of **50**-like diols at the 6-position,[74] **51** was converted to the crystalline derivative **52** in 60% yield by Staudinger reduction of the azides followed by amide formation, whose structures were confirmed by X-ray crystallography (Supporting Information, Figure S1, CCDC 1908319). The glycosyl acceptor **51** was converted to the α-glycoside **55** in 47% yield by means of reaction with sulfoxide **53** (Supporting Information) on activation with triflic anhydride. Similarly, glycosylation of **51** with sulfoxide **54** (Supporting Information) gave the α-glycoside **56** in 65% yield. Treatment of **55** and **56** with tetrabutylammonium fluoride and then acetic acid affording the triols **57** and **58**, both in 44% yield, was followed by Staudinger reaction and hydrogenolysis to give 2′-desamino-2′-hydroxy ribostamycin **59** and the ribostamycin regioisomer **60** in 51 and 40% yields, respectively (Scheme 6).

Activity and Selectivity at the Target Level. All compounds were studied for their ability to inhibit protein synthesis in a series of cell-free translation assays as described previously.[43] These assays employed wild-type bacterial

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Mycobacterium smegmatis ribosomes and humanized hybrid *M. smegmatis* ribosomes carrying complete eukaryotic decoding A sites, that is, the crystallographically characterized drug binding pocket (Figure 3),[36,75] from human mitochondrial ribosomes (Mit 13), A1555G mutant mitochondrial ribosomes (A1555), and human cytoplasmic ribosomes (Cyt 14) (Figure 2 and Table 1).[76] This model system, developed in our laboratories, was previously employed to identify the mitoribosome as target in aminoglycoside ototoxicity,[77] to study the mechanisms of mutant rRNA-associated deafness,[78] and to rationalize aminoglycoside activity against protozoa.[79] In the series of experiments reported in Table 1, inhibition of the mitochondrial ribosome, together with slow clearance from the inner ear, is thought to be the root cause of AGA-induced ototoxicity; the hypersusceptibility to AGA-induced ototoxicity found in a subset of the population arises from the presence of the A1555G mutation in the mitochondrial decoding A site.[77,78,80,81] Inhibition of the human cytoplasmic ribosome on the other hand is expected to result in more systemic toxicity.

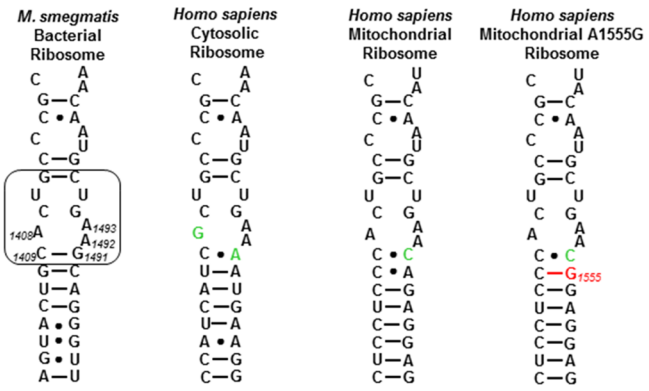

Figure 2. Decoding A sites of prokaryotic and eukaryotic ribosomes. The bacterial AGA binding pocket is boxed. The bacterial numbering scheme is illustrated for the AGA binding pocket. Changes from the bacterial ribosome binding pocket are colored green. The A1555G mutant conferring hypersusceptibility to AGA ototoxicity is colored red.

As shown in Table 1, 2′-*N*-methylation and ethylation have little to no effect on the ability of paromomycin or neomycin to inhibit translation by the bacterial ribosome. Even 2′-*N*-propylation has a minimal effect as demonstrated with the paromomycin derivative **17**. The effects of these 2′-*N*-alkylations on translation by the mitochondrial and cytoplasmic hybrid ribosomes are minimal for paromomycin but result in significant increases in selectivity for neomycin. In addition, these modifications have a marked effect on A1555G mutant mitochondrial ribosomes resulting in increases in selectivity of between 3- and 10-fold. The minimal loss of inhibitory activity of 2′-*N*-ethyl paromomycin for the bacterial ribosome is consistent with the limited reduction in antibacterial activity observed previously for this modification.[51] In contrast to the minimal influence of 2′-*N*-alkylation in the paromomycin and neomycin series, the comparable 2′-*N*-alkylated ribostamycin derivatives **41** and **42** were found to be 10-fold less active than the parent against the bacterial ribosome. In view of the low antibacterioribosomal activity of **41** and **42** and all subsequent ribostamycin derivatives, the activity of ribostamycin compounds against the eukaryotic ribosomes was not investigated.

Substitution of a hydroxyl group for an amino group at the 2′-position has minimal influence on antiribosomal activity in the neomycin series (analogue **26**), consistent with the antibacterial activity reported for this compound previously.[52] Notably, a significant increase in selectivity for all eukaryotic decoding sites

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

	substituent			IC_{50}, μM				selectivity		
compound	6′	2′	basic amino groups	bacterial	Mit13	A1555G	Cyt14	Mit13	A1555G	Cyt14
Neomycin Series										
3	NH_2	NH_2	6	0.04	4.3	0.4	36	108	10	900
15	NH_2	NHMe	6	0.01	4.7	1.1	37	470	110	3700
18	NH_2	NHEt	6	0.02	11	1.6	43	550	80	2150
26	NH_2	OH	5	0.03	36	2.9	108	1200	97	3600
29	NH_2	H	5	0.03	22	0.9	85	773	30	2833
35	NH_2	NHCHO	5	0.12	54	13	127	450	108	1058
36	NH_2	NHAc	5	5.3	93	28	147	18	5.3	28
37	NH_2	NHglycyl	5	0.16	11	1.2	25	69	8	156
Paromomycin Series										
2	OH	NH_2	5	0.04	142	12	31	3550	300	775
14	OH	NHMe	4	0.05	150	54	60	3000	1080	1200
16	OH	NHEt	5	0.05	220	84	43	4400	1680	860
17	OH	NHPr	5	0.07	223	58	32	3186	829	457
25	OH	OH	4	2.2	662	313	446	301	142	203
Ribostamycin Series										
6	NH_2	NH_2	4	0.10						
41	NH_2	NHMe	4	1.13						
42	NH_2	NHEt		1.93						
59	NH_2	OH	3	2.66						
46	NH_2	H	3	9.93						
60[b]	NH_2	OH	4	>20						

[a]Selectivities are obtained by dividing the eukaryotic by the bacterial values. [b]Compound **60** is additionally modified at the 3′-position by replacement of the hydroxyl group by an amino group.

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

	substituent		MRSA	*E. coli*		*K. pneu*	*E. cloa*	*A. baum*
compound	6′	2′	AG038	AG001	AG003	AG215	AG290	AG225
Neomycin Series								
3	NH_2	NH_2	0.5	1	1	0.25−0.5	1	1−2
15	NH_2	NHMe	0.5	2	1	0.5	0.5−1	1
18	NH_2	NHEt	0.5	1−2	1	0.5	0.5−1	1
26	NH_2	OH	2	2−4	2−4	0.5	1	1
29	NH_2	H	1	2	1−2	0.5	1	1
35	NH_2	NHCHO	2−4	4−8	4−8	1−2	2	2
36	NH_2	NHAc	>128	>128	>128			
37	NH_2	NHglycyl	4	16	32			
Paromomycin Series								
2	OH	NH_2	4	2−4	4−8	1	2	2
16	OH	NHEt	8	16	16			
14	OH	NHMe	8	8	16			
17	OH	NHPr	8−16	16	16			
25	OH	OH	64−128	>128	>128			
Ribostamycin Series								
6	NH_2	NH_2	4	4−8	4−8	2	4	4
41	NH_2	NHMe	64−128	64−128	64			
42	NH_2	NHEt	>128	128	128			
59	NH_2	OH	>128	>128	>128			
46	NH_2	H	≥256					
60[b]	NH_2	OH	>128	>128	>128			

[a]All values were determined in duplicate using 2-fold dilution series. [b]Compound **60** is additionally modified at the 3′-position by replacement of the hydroxyl group by an amino group.

Table 3. Antibacterial Activities Against *E. coli* Strains with Acquired AAC(2′) Resistance and Mycobacteria with Intrinsic AAC(2′) Resistance (MIC, μg/mL)[a]

	substituent		*E. coli* AG001	*E. coli* AG106	*E. coli* pH434	*M. abscessus* ATCC 19977 (AAC2′)	ratio *M. abscessus* wt/*E. coli* wt
compound	6′	2′	wt	AAC(2′)-1a	AAC(2′)-1b	wt	
Neomycin Series							
3	NH_2	NH_2	1	16	>64	16	16
15	NH_2	NHMe	2	2.0	2−4	0.25	0.125
18	NH_2	NHEt	1−2	1	2	0.25	0.125−0.25
26	NH_2	OH	2−4	2	4	2	0.5−1.0
29	NH_2	H	2	2	4	2	1
35	NH_2	NHCHO	4−8	8	8−16		
37	NH_2	NHglycyl	16	16−32			
Paromomycin Series							
2	OH	NH_2	2−4	>64	>64	8	2−4
14	OH	NHMe	8	8	8	16	2
16	OH	NHEt	16		8	32	2
17	OH	NHPr	16		8	32	2
Ribostamycin Series							
6	NH_2	NH_2	4−8	128	>128		
plazomicin			0.5−1	8−16	8		
amikacin (2′OH)			2	2	2		

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

(mitochondrial, A1555G, and cytosolic) is achieved by the 2′-hydroxyl modification. However, the identical substitution is detrimental for both paromomycin and ribostamycin as evidenced by the corresponding paromomycin and ribostamycin analogues **25** and **59**. The high activity of **26** suggested that further such modifications would be tolerated in the neomycin series, as was borne out by the 2′-desamino neomycin derivative **29**, which again displays increased across the board selectivity compared to the parent. The substantially reduced activity of 2′-desamino-2′-hydroxy paromomycin **25** and of 2′-desaminoribostamycin **46** discouraged us from making any further modifications to the 2′-position of paromomycin and ribostamycin that result in loss of the basic amine. In an attempt to remove the 2′-amino group without a change in the number of basic amines present, ribostamycin was converted to the regioisomer **60** in which the 2′-amino group has been substituted by an hydroxyl group, while the inverse modification was affected at the 3′-position. Unfortunately, this modification was associated with a substantial loss of activity.

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Turning to the installation of amides at the 2′-position, and restricting ourselves to the neomycin series, formylation giving the neomycin derivative **35** resulted in little loss of activity against the bacterial ribosome coupled with a substantial increase in selectivity for the mitochondrial and mutant mitochondrial ribosomes. This is in contrast to 2′-*N*-acetylation, the modification introduced by the AAC(2′) family of AMEs, which led to a greater than 100-fold loss in activity in derivative **36**. The negative influence of the 2′-*N*-acetyl modification on activity can be largely overcome by its coupling with the reinstallation of a basic amine as in the 2′-*N*-glycyl neomycin derivative **37**, which shows only a 4-fold loss of activity against the bacterial ribosome compared to the parent. However, this modification comes with a substantial loss in selectivity with regard to the cytoplasmic ribosome. These observations on the influence of amide formation at the 2′-position are consistent with the prior literature describing other 2′-amides. Thus, it has been reported that the 2′-*N*-glycinamide derivatives of simple aminoalkyl 2,6-diamino-α-D-glucopyranosides, and of fortimicin B, retain antibacterial activity as does 2′-formamido sisomicin.[82−84]

Antibacterial Activity in the Absence and Presence of Aminoglycoside Modifying Enzymes. The antibacterial activities displayed by the various paromomycin, neomycin, and ribostamycin derivatives against clinical strains of the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and the Gram-negative *Escherichia coli* (Table 2) were consistent with their ability to inhibit bacterial protein synthesis in the translation assays (Table 1). The more potent compounds were also screened for activity against a single wild-type clinical strain each of the ESKAPE pathogens, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii*, with MICs determined to be ≤4 μg/mL (Table 2).

As the 2′-amino group is the target for acetylation by the AAC(2′) class of AMEs, the more active compounds were screened for activity against two clinical strains of *E. coli* carrying different isoforms of AAC(2′) (Table 3). The presence of either of these AAC(2′) isoforms greatly abrogates the activity of both paromomycin and neomycin, consistent with the minimal activity of authentic 2′-*N*-acetyl neomycin **36**. In contrast, 2′-*N*-methylation, ethylation, and propylation, compounds **14**−**18**, maintains activity in the presence of AAC(2′). Similarly, the 2′-desamino-2′-hydroxy and 2′-desamino modifications in the neomycin series, compounds **26** and **29**, resist the AAC(2′) class of AMEs in *E. coli*, as does the 2′-*N*-formyl modification displayed in compound **35**. Plazomicin, obtained by an improved synthesis,[32] and amikacin, one carrying a 2′-amino group and the other a 2′-hydroxy group, served as controls for the AAC(2′) AMEs in *E. coli*.

Mycobacterium abscessus is a growing threat in hospitalized patients with chronic pulmonary disease or cystic fibrosis.[85] The innate AGA susceptibility of *M. abscessus* is determined by the presence of a functional AAC(2′) AME,[86] prompting us to test the more active compounds against the *M. abscessus* reference strain ATCC 19977. To illustrate the effect of the mycobacterial AAC2′ on compound activity, we also calculated the ratio of *M. abscessus* wt (AAC2′ present) versus *E. coli* wt (no AAC2′ present). Inherently, the mycobacterial AAC2′ shows little activity toward paromomycin but significantly reduces the antimycobacterial activity of neomycin. In line with expectation, the 2′-*N*-alkyl derivatives of paromomycin **14**, **16**, and **17** did not show any difference compared to the parent; however, the same modifications in the neomycin series, as in compounds **15** and **18**, were highly efficacious resulting in a 64-fold reduction in MIC values (Table 3). The 2′-desamino-2′-hydroxy and 2′-desamino derivatives **26** and **29** of neomycin also showed much greater activity against *M. abscessus* than the parent.

Finally, selected compounds were screened for activity in the presence of AAC(3), AAC(6′), ANT(4′,4′′), and APH(3′). As expected, none of the modifications investigated was effective at suppressing the activity of these AMEs.

DISCUSSION

Classically, the activity of AGAs is considered to be correlated to the number of basic amino groups[87] and so, following protonation at physiological pH, to the degree of electrostatic attraction with the negatively charged drug binding pocket.[88,89] The electrostatic component of the binding energy is supplemented by a number of direct and water-mediated hydrogen bonding contacts and other directional intermolecular noncovalent interactions between the drug and the binding pocket (Figure 3).[27,75,87,90−92] The partitioning of the contribution of an individual protonated amine to the binding energy between electrostatic and directed components necessarily varies with the location of the amine in the AGA. Thus, while the amines of rings I and II of the 4,5-AGAs are involved in multiple H-bonding interactions with the drug-binding pocket, those of ring IV do so to a lesser extent (Figure 3). Indeed, it has been suggested that ring IV serves simply as a concentration of positive charge that makes a strong electrostatic contribution to the binding energy.[93] Finally, intramolecular conformation-restricting noncovalent interactions within the body of the drug itself have also been suggested to be an important component of the binding energy.[94] Among the hydrogen bonding interactions between rings I and III of the 4,5-AGAs, specifically those between the protonated 2′-amino group and O4′′ or O5′′ of the ribofuranosyl moiety (Figure 3)[75,95] are featured prominently. On the basis of these interactions, Hermann and co-workers prepared the conformationally restricted paromomycin and neomycin analogues **61** and **62** and studied their binding to the bacterial decoding A site.[96,97] Contemporaneously, and with a

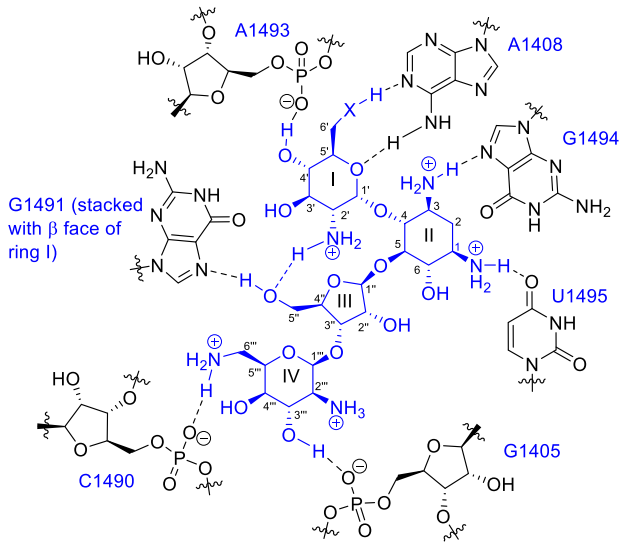

Figure 3. Schematic of the crystallographically determined interactions of neomycin **3** (X = NH_2^+) and paromomycin **2** (X = O) with the AGA binding pocket. Ribostamycin **6** (X = NH_2^+) binds identically but lacks ring IV.

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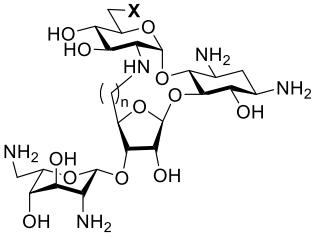
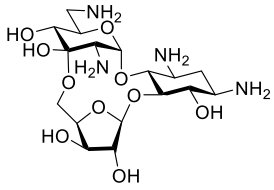
view to exploiting the differing conformations of AGAs bound to the decoding A site and that of the ANT(4′,4′′) AMEs, Asensio and co-workers prepared **62** and the higher homologue **63**.[98,99] Other workers have prepared complex aminoglycosides by ribofuranosylation of the 5-OH in the 4,6-AGAs to take advantage of this intramolecular interaction and other contacts.[100,101] Unfortunately, each of **61**−**63** showed reduced affinity for decoding A site models and reduced antibacterial activity, the latter in common with an earlier conformationally restricted analogue **64** of the ribostamycin isomer xylostacin.[102]



61: X = O, n = 1
62: X = NH_2, n = 1
63: X = NH_2, n = 3



64

The pattern of antibacterioribosomal activity (Table 1) and antibacterial activity (Table 2) of the present series of compounds, together with the reduced activity of **61**−**63**, provides the opportunity to examine the interplay between electrostatic and hydrogen bonding interactions in greater detail. The parity in the ability of neomycin and its analogues **26** and **29** lacking the 2′-amino group to inhibit the function of the bacterial ribosome (Table 1) and the growth of wild-type Gram-negative pathogens (Table 2) indicates that the presence of five appropriately located protonated amino groups provides sufficient electrostatic attraction with the negatively charged ribosome to overcome the absence of the 2′-amino group for this scaffold. For paramomycin and ribostamycin on the other hand, with only five and four such basic amino groups, respectively, the reduction in the electrostatic component conferred by removal of the 2′-amino group is critical, as borne out by the reduced activity of 2′-desamino-2′-hydroxy paromomycin **25** with its four protonated amino groups and by the ribostamycin analogues **46** and **59**, both with only three protonated amino groups (Tables 1 and 2). Compensating for the loss of C(2′)NH_2 in **59** by reintroducing an amino group at the 3′ position, resulting in compound **60**, did not restore activity. This indicates that in the ribostamycin series, with only four basic amino groups, electrostatic attraction alone is not sufficient for activity but must be complemented by location of one of these groups at the 2′-position.

Alkylation of N2′ is tolerated when the molecule possesses five or more suitably placed basic amines as is clear from the activity of the neomycin and paromomycin analogs **14**, **15**, **16**, **17**, and **18** (Tables 1 and 3). The loss of activity observed for the 2′-*N*-alkylated ribostamycin analogues **41** and **42** on the other hand indicates that even the correct placement of four basic amino groups is insufficient to compensate for the destabilizing effect of the alkyl groups. The retention of activity of **14**, **15**, **16**, **17**, and **18** contrasts with the reduced activity of the cyclic (2′-*N*-alkylated) neomycin and paromomycin derivatives **61**−**63**. Cyclization of the 2′-amine onto the 5′′-position was reported to result in a reduction in basicity of the 2′-amine in **61** and **62** by ∼1.5 p*K*a units. This reduction in p*K*a is most likely due to the inductively electron-withdrawing O4′′ ribosyl ring oxygen, which is *β*- to N2′ in **61** and **62**,[103] albeit the authors suggested steric inhibition of solvation of the protonated amino group as the cause.[96,97,104] Whatever the underlying reason for the reduction in basicity, affinity for the ribosome was restored under more acidic conditions, illustrating the importance of protonation of N2′ in overcoming the negative effect of the cyclic modification. Simple alkylation, as in **14**, **15**, **16**, **17**, and **18**, on the other hand is expected to increase the basicity of N2′ consistent with their high levels of activity.[103] The homologue **63** of **62** lacks the basicity-reducing vicinal relationship between N2′ and O4′′ present in **61** and **62** but also shows a significant reduction in antibacterial activity and affinity for a decoding A site model.[99] This suggests that the diminished activity of **61**−**63** is at least in part due to other factors such as the loss of a crystallographically observed (Figure 3)[75] hydrogen bond from 5′′−OH in the parent to N7 of G1491 at the bottom of the binding site.[96−99] Overall, in the absence of cyclic constraints, the presence of a basic amino group at the 2′-position of the 4,5-AGAs is not necessary provided five other appropriately located basic amino groups are present in the molecule. When a 4,5-series AGA contains only four basic amino groups, one of them must be at the 2′-position. Alkylation of the 2′-amino group is tolerated in the absence of cyclic constraints provided there are a minimum of five basic amino groups in the molecule.

With regard to the 2′-*N*-acyl derivatives of neomycin, the loss of activity observed with the acetamide **36** was anticipated in view of the well-known ability of the AAC(2′) AMEs to significantly reduce the activity of AGAs (Table 2). In view of the high levels of activity retained by neomycin derivatives **26** and **29**, both of which lack a basic nitrogen at the 2′-position, the loss of activity due to 2′-acetamide formation cannot result from a reduction in electrostatic binding energy in neomycin. The loss of activity on acetamide formation can also not be due to the inclusion of a hydrophobic methyl group, as simple 2′-*N*-alkylation is well tolerated in both paromomycin and neomycin. By a process of elimination, the detrimental effect of the 2′-acetamido group in the neomycin series must arise either from a steric interaction or an unfavorable electrostatic interaction involving the amide carbonyl group. The steric component of this interaction is more important for the 2′-acetamide than for the 2′-*N*-alkyl derivatives because of the rigid nature of the amide function and its well-known orientation with respect to the pyranose ring in all 2-deoxy-2-acetamido glucopyranose derivatives.[69] Further, it results mainly from the presence of the acetamide methyl group as the corresponding formamide **36** is considerably more active. In addition to its smaller size, the formamide **36** is also capable of avoiding any unfavorable electrostatic interaction by population of the *cis*- rather than the *trans*-rotamer.[70] Finally, the unfavorable interaction in the acetamide is not so large as to prevent docking of the drug into the binding site, as it can be partially overcome by the introduction of a further basic amino group as in the glycinamide **37**.

■ CONCLUSION

Extensive structure activity relationships (SAR) have been provided, resulting from modification of the 2′-position by deletion, replacement by a hydroxy group, alkylation, and in part acylation of the AGAs neomycin, paromomycin, and ribostamycin. This SAR leads to a series of conclusions on the influence of modification at this position on ribosomal affinity and antibacterial activity. Thus, the presence of a basic amino group at the 2′-position of the 4,5-AGAs is not essential for activity provided five other suitably placed basic amines are present in the molecule. In contrast, a basic amino group is required at the

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2′-position when a 4,5-series AGA contains only four such basic amines. With five correctly placed basic amino groups present, alkylation of the 2′-amine is tolerated. Most notably, in paromomycin and in particular neomycin modification of the 2′-amino group also results in a gain in selectivity for the bacterial over the eukaryotic ribosomes, thereby providing an attractive means of both overcoming resistance due to the presence of AAC(2′)-type resistance determinants and increasing ribosomal target selectivity by a single modification.

Finally, the changes in selectivity between the prokaryotic and the different eukaryotic ribosomes occasioned by the various modifications introduced at the 2′-position (Table 1) presumably arise by indirect effects as the 2′-amine makes no direct contact with the drug binding pocket (Figure 3). These differential effects likely arise from interactions of the AGAs with the ribosomal base 1491, which is G in the bacterial ribosomes and A or C in the humanized ribosomes (Figure 2). Thus, modification of the 2′-substituent necessarily affects the CH−π interaction of the 4′−C−H bond with the base 1491 and, because of the H-bond network, the H-bond of the 5′′-hydroxyl group with the same base (Figure 3), both in a base-dependent manner.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.9b00128.

Full experimental details and copies of ^{1}H and ^{13}C NMR spectra for all new AGAs (PDF)

Crystallographic data (CIF)

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Notes

The authors declare the following competing financial interest(s): D.C., A.V., S.N.H., and E.C.B. are cofounders of and have an equity interest in Juvabis AG, a biotech startup operating in the field of aminoglycoside antibiotics.

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