Drug Design

Antibacterial Aminoglycosides with a Modified Mode of Binding to the Ribosomal-RNA Decoding Site**

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The past decade has witnessed a phenomenal advance in our understanding of the structure and function of ribosomal RNAs as they relate to the mode of action of clinically relevant antibiotics.[1–6] The aminoglycosides are a group of well-known bactericidal antibiotics.[7] Their widespread use in clinical practice has been curtailed as a result of their oxotoxicity, nephrotoxicity, and susceptibility to enzymatic inactivation;[8] hence the need for careful patient monitoring in a hospital environment.[9] By binding specifically to the bacterial decoding A site, which is responsible for fidelity during protein synthesis by monitoring correct Watson–Crick base pairing between the mRNA codon and the tRNA anticodon, aminoglycoside antibiotics increase the error rate of translation.[10–12] Paromomycin (**1**) has been a benchmark for structural studies owing to its excellent binding affinity for the highly conserved set of nucleotides in the decoding 16S rRNA (A site) region of the 30S subunit of bacterial ribosomes (Figure 1).[13] X-ray crystallographic studies of paromomycin complexed with the 30S subunit of rRNA from *Thermus thermophilus*[2] as well as with a sequence of oligonucleotides corresponding to the ribosomal A site of *E. coli*[14] have delineated the basis for the molecular recognition.[15]

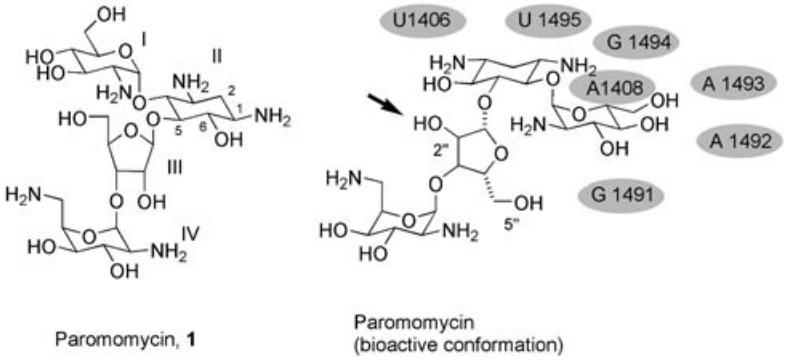

Figure 1. Structure of paromomycin, its bioactive conformation with relevant nucleotides in the A-site binding domain, and proposed site for diversification (arrow).

Crystal structures[2,14,16,17] have shown that upon binding to the A site the four rings in paromomycin adopt an L shape, which represents its bioactive conformation. Ring I intercalates into the A site and forms two H bonds with A1408, whereas the invariant ammonium nitrogen atoms of ring II form constant interactions with A1493, G1494, and U1495. The binding of rings I and II force A1492 and A1493 to bulge out of the deep/major groove. Rings III and IV interact with the lower stem of the A site mainly through more variable charge and H-bonding interactions with neighboring nucleotides. These hallmark structural events, characteristic of all bioactive aminoglycosides that bind at the A site,[14,16,17] are supported by the observed in vivo drug susceptibilities of several ribosomal-RNA mutants.[18]

We reasoned that a structure-based design approach with a chemically modified paromomycin derivative could exploit uncharted areas of the A site, with the possible location of new modes of binding.[19–23] Examination of the crystal structure of the paromomycin complex[14] reveals that the hydroxy group at C2″ of ring III is favorably disposed for appropriate functional diversification (Figure 1). With this objective in mind, the main challenge was to develop methods of regioselective functionalization of the complex polyol system in paromomycin, so as to access the C2″ hydroxy group selectively.

The readily available paromomycin derivative **2**[24] was silylated at C5″ and the product was subjected to O allylation under standard conditions (Scheme 1). Remarkably, after TBS protection of the C5″ hydroxy group, a highly regioselective allylation of the C2″ hydroxy group led to **3** in good yield. Protection of the hydroxy groups on rings II and IV by benzoylation and oxidative cleavage of the allyl group afforded aldehyde **4**. Reductive amination with three representative alkyl amines gave the protected ω-(aminoalkyl) ether analogues **5**–**7**. Treatment with a catalytic amount of sodium methoxide in methanol afforded the corresponding

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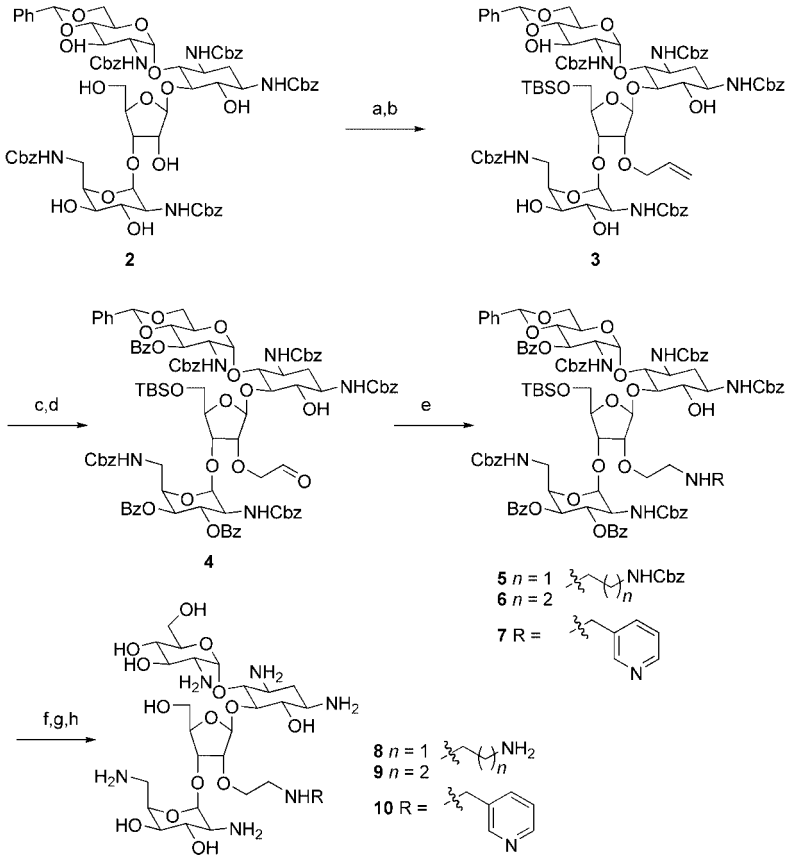

Scheme 1. Reagents and conditions: a) TBSOTf, 2,4,6-collidine, CH_2Cl_2, 75%; b) CH_2=$CHCH_2I$, KHMDS, THF, 70%; c) BzCl, pyridine, DMAP, 95%; d) 1. O_3, CH_2Cl_2, −78°C; 2. Ph_3P, −78°C→RT, 80%; e) amine, $NaBH_3CN$, MeOH/AcOH (30:1), 90%; f) NaOMe, MeOH, 80%; g) AcOH, 80%, 60°C, 2 h; h) $Pd(OH)_2$/C, H_2, AcOH 80%, 70% (2 steps). Bz = benzoyl, Cbz = benzyloxycarbonyl, HMDS = hexamethyldisilazide, TBS = *tert*-butyldimethylsilyl.

polyols. Hydrolysis of the *O*-benzylidene acetal and the TBS ether with aqueous acetic acid, followed by catalytic hydrogenation gave the paromomycin derivatives **8**–**10**.

The amino ether analogues **8**–**10** bonded to the 16S rRNA subunit and inhibited bacterial translation/transcription (T/T)[25] with similar potencies. This result was gratifying given our prediction from modeling studies that the C2″ position is suitable for substitution. Most rewarding, however, was that all of the substituted compounds maintained activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Table 1). Whereas in the case of **8** the addition of a cationic side chain to the paromomycin core afforded a compound with near-identical activity to that of paromomycin, the extension of this chain by one methylene group led to a compound **9** with similar activity against *S. aureus* but diminished activity against *E. coli*. As the binding and T/T activity were largely unaffected, the diminished activity is probably a result of decreased uptake and/or increased efflux by the *E. coli* strain. Most interesting was the 3-(aminomethyl)pyridyl analogue **10**, which was slightly more potent than the parent antibiotic paromomycin against the Gram-positive *S. aureus* strain. Compound **10** was further evaluated against *Streptococcus pyogenes*, *Proteus vulgaris*, and *Klebsiella pneumoniae* strains, and was found to maintain inhibitory activity similar to that of the parent paromomycin.

Extensive studies on the nature of the C2″ tether and of the distal group have led to analogues that exhibit excellent antibacterial activities and interesting structure–activity relationships.[26] Importantly, it might be expected that the incorporation of a large side chain could prevent resistance enzymes from modifying the compounds and rendering them inactive, which is a key element in resistance to aminoglycosides. This hypothesis was partially supported by the activity (MIC 25–50 μM) of **10** against a multidrug- and methicillin-resistant *S. aureus* (MRSA) strain (ATCC BAA-44). This activity is comparable to that observed for the semisynthetic aminoglycoside amikacin, which is modified with an N1 side chain known to combat resistance. In contrast, paromomycin, neomycin, kanamycin, tobramycin, gentamicin, sisomicin, and streptomycin were completely ineffective against MRSA. These results suggest that a C2″ substituent with an aromatic terminal group in 4,5-disubstituted aminoglycosides offers distinct advantages in the fight against the emergence of drug-resistant bacteria. However, it remains to be seen if this type of modification does in fact hinder interaction with one or more resistance enzymes, while allowing strong binding to the target RNA.

Further insight into structure-based design was gathered from X-ray cocrystal data, which revealed a new mode of binding to the decoding A site of rRNA. Crystal structures of **9** and **10** complexed with the same A-site oligonucleotide sequence of *E. coli* RNA as that used for the paromomycin structure were solved at a resolution of 2.6 Å. The two structures are isomorphous and both reveal a normal mode of binding for rings I and II, with the characteristic bulging of A1492 and A1493, and the invariant contacts between the paromamine part (rings I and II) and A1408, or the sugar–phosphate backbone, as shown in Figure 2 for analogue **10**. An important difference is the *direct* contact between the O6 hydroxy group of the deoxystreptamine (DOS) unit and the O4 atom of U1406; the equivalent interaction is mediated by a water molecule in the case of the parent paromomycin.[14] Furthermore, the non-Watson–Crick U1406·U1495 base pair is

Table 1: Activities of C2″-*O*-(aminoalkyl) ether analogues of paromomycin.[a]

Compound	16S	T/T	MIC [μM][b]				
	K_D [μM]	IC_{50} [μM]	*E. coli*	*S. aureus*	*S. pyog.*	*P. vulg.*	*K. pneum.*
1	0.13	0.15	5	3	3	1	1
8	0.23	0.29	6	2	nd[c]	nd	nd
9	0.10	0.29	25	3	nd	nd	nd
10	0.13	0.23	5	1	6	3	3

[a] For details of K_D, T/T, and MIC testing, see reference [25]. [b] Bacterial strains used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 13709), *Streptococcus pyogenes* (ATCC 49399), *Proteus vulgaris* (ATCC 8427), *Klebsiella pneumoniae* (ATCC 13883). [c] nd = not determined. MIC = minimum inhibitory concentration.

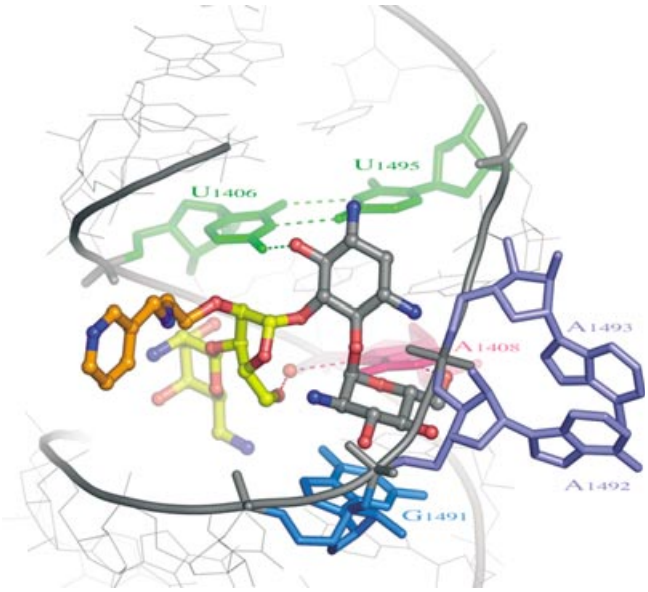

Figure 2. Top view of the complex between **10** and the A-site RNA oligonucleotide. Adenine residues A1492 and A1493 are dark blue, G1491 is paler blue, A1408 is pink, and the U1406·U1495 base pair is green. The bridging water molecule between the hydroxy group at C5″ of ring III and N7 of A1408 is also shown in pink. Note the U·U pair with standard H bonds and the pseudopair of ring I and A1408.

not bifurcated as in all other aminoglycoside/A-site complexes,[14,16,17] but instead adopts a common geometry with two direct H bonds O4···H–N3 and N3–H···O2[27] (Figure 2).

Rings III and IV of **10** are oriented very differently in the A-site complex with respect to the parent compound paromomycin (and all known 4,5-disubstituted aminoglycosides)[14–16] (Figure 3). In the structure of both analogues **9** and **10**, there is a 40° flip about the β-D-ribofuranosyl linkage to the paromamine unit, which, together with a change in sugar pucker (from C2″-*endo* in paromomycin to C3″-*endo* in **9** and **10**) results in a 90° change in the orientation of ring IV.

The oxygen atom in ring III in **9** and **10** still forms an intramolecular H bond with the amino group at C2′ of ring I, as in the paromomycin complex[14] (Figure 4). However, the hydroxymethyl group at C5″ in **9** and **10** forms a hydrogen

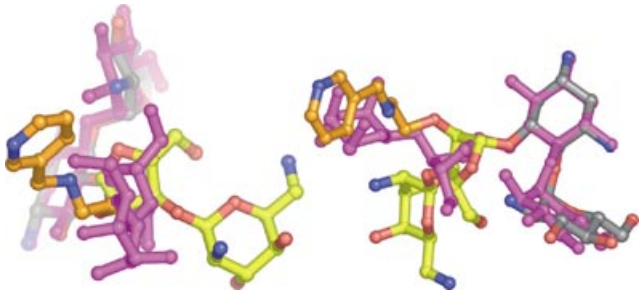

Figure 3. Superposition of paromomycin and **10** complexed to the A site with the DOS rings II superimposed. The left-hand drawing illustrates the 90° rotation of ring IV in **10** relative to that in paromomycin (magenta). The superposition on the right highlights the difference in sugar pucker of ring III from C2″-*endo* in paromomycin (magenta) to C3″-*endo* in **10**.

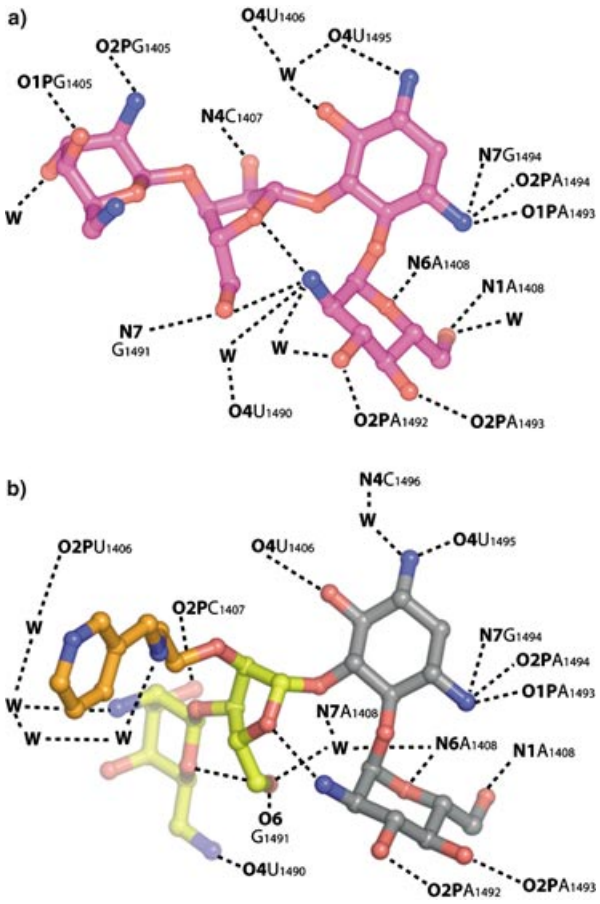

Figure 4. a) Schematic representation of the contacts between paromomycin and the RNA in the cocrystal with the A-site RNA fragment.[14] b) Schematic representation of the direct and water-mediated contacts observed in the cocrystal of the A-site RNA fragment with **10**.

bond with O6 of G1491 instead of N7 of G1491 as in the case of paromomycin. Because of the rotation of ring III, the C5″ hydroxymethyl group forms an additional water-mediated H bond with N7 of A1408. In fact, the central A1408 residue forms three (instead of the usual two) H-bonded contacts with the aminoglycoside unit of the two analogues **9** and **10**. The new orientation of ring IV results in a new set of H-bonding interactions. Thus, a network of H bonds links rings I and IV via the C5″ hydroxymethyl group, a water molecule, and A1408.

The ether chain at C2″ with the distal amino or pyridine group in **9** and **10**, respectively, extends across the deep/major groove of the A site, points into the solvent, and forms no direct contact with the RNA (Figure 4b). Additional contacts are mediated by bridging water molecules that extend in one binding site from the aminoethyl group at C2″ to an oxygen atom of an anionic phosphate in U1406. These unprecedented conformational changes and new interactions brought about by the presence of the ether chains at C2″ of **9** and **10** result in a compactly folded aminoglycoside structure with unique binding sites (Figure 4b).

We have uncovered a new mode of binding for paromomycin analogues that contain diversely substituted amino-

alkyl ethers at C2″. Cocrystal structures of compounds **9** and **10** with the A site of *E. coli* RNA revealed a new position for the C2″ substituent in the deep/major groove. The orientation of rings III and IV is dramatically changed in the bound conformation, thus resulting in a new set of intramolecular networks of H bonds. The pyridine-substituted aminoethyl ether analogue **10** of paromomycin exhibited potent inhibition of *S. aureus* and promising activity against a panel of resistant bacterial strains, including MRSA. Thus, the introduction of a novel aryl aminoalkyl functionality at C2″ of paromomycin has resulted in the discovery of an excellent first-generation lead compound for the development of new and potent bactericidal aminoglycoside analogues in this series.

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