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Redesign of aminoglycosides for treatment of human genetic diseases caused by premature stop mutations

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Abstract—A series of new derivatives of the clinically used aminoglycoside antibiotic paromomycin were designed, synthesized, and their ability to read-through premature stop codon mutations was examined in both in vitro translation system and ex vivo mammalian cultured cells. One of these structures, a pseudo-trisaccharide derivative, showed notably higher stop codon read-through activity in cultured cells compared to those of paromomycin and gentamicin.

A large number of human genetic disorders result from nonsense mutations, single point alterations in the DNA, where one of the three stop codons (TAA, TAG or TGA) replaces an amino acid-coding codon, leading to premature termination of the translation and eventually to truncated, nonfunctional proteins. Currently, hundreds of such mutations are known,[1] and for many of those diseases there is presently no effective treatment.

Aminoglycosides are highly potent, broad-spectrum antibiotics that exert their antibacterial therapeutic effect by selectively binding to the decoding aminoacyl site (A-site) of the bacterial 16S rRNA, and interfering with translational fidelity during protein synthesis.[2] Interestingly, in the last several years, numerous experiments performed either in vitro in mammalian translation systems, cultured cell lines, or animal models confirmed the ability of certain types of aminoglycoside antibiotics (Fig. 1) to induce mammalian ribosomes to read-through stop codon mutations via insertion of a random amino acid by a nearcognate tRNA. This unique activity was shown to generate full-length functional proteins in several genetic disorders.[3] Furthermore, clinical trials with cystic fibrosis patients clearly showed that aminoglycosides can suppress premature stop mutations in affected patients.[4] However, unfortunately, this great excitement which continues over the last two decades was largely hampered because of the following reasons. First and foremost, aminoglycosides are highly toxic to mammals (nephrotoxicity and ototoxicity), and the use of subtoxic doses in clinical trials resulted with the reduced read-through efficiency probably insufficient for

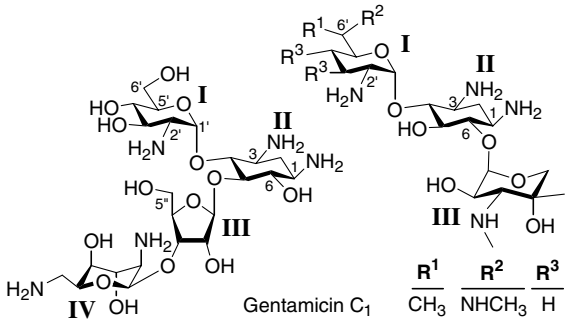

Figure 1. Aminoglycosides with stop codon read-through activity.

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successful therapy. Second, unlike recent insights into our understanding of how aminoglycosides might induce deleterious misreading of the genetic code in prokaryote cells,[5] the molecular mechanism of aminoglycoside-induced nonsense mutation suppression in mammalian cells remains to be established. Third, to date, nearly all suppression experiments have been performed with clinical, commercially available aminoglycosides,[6] and no efforts have been made to optimize their activity as stop codon read-through inducers. Clearly, a systematic search for new structures with improved termination suppression activity and lower toxicity is required to extrapolate the approach to the point where it can actually help patients.

For this purpose, as an initial trial, we have modified paromomycin and prepared a series of derivatives, **1**–**9** (Fig. 2). In selecting paromomycin as the modification target we have taken into consideration the following points. First, although to date there are not enough data to answer the question why some aminoglycosides induce termination suppression, while others do not, from the available data, it turns out that aminoglycosides with a C6′ hydroxyl group on ring I (such as G-418 and paromomycin, Fig. 1) are generally more effective than those with the amine functionality at the same position.[6] Second, paromomycin is the least toxic among the aminoglycosides that show considerably high suppression activity.[7] Based on these data we reasoned that by dissecting paromomycin structure (via selectively removing one or two sugar rings) we could identify a minimal structural motif with significant suppression activity, which then can be used as a scaffold for the construction of diverse structures with improved termination suppression and probably with lower toxicity.

Structures **2**–**7** preserve rings I and II of paromomycin (paromamine **1** moiety) as the minimal structural motif of paromomycin that binds to the mammalian ribosome and has significant suppression activity (vide infra). The extended sugar ring (ring III) in each structure is either the plain ribose (structures **2**, **4**, and **6**) or 5-amino ribose (structures **3**, **5**, and **7**) attached at C5, C6, and C3′ of **1**, respectively. The rational in selecting the ribose as a third sugar ring in **4** and **6** was to retain the identity of this sugar as in the parent paromomycin, and in parallel to explore another hitherto unknown areas of the mammalian A-site rRNA with possible location of new modes of binding. Since at physiological pH aminoglycosides are highly charged and their interaction with rRNA is mainly determined by electrostatic interaction,[8] we reasoned that by adding additional aminosugar to the paromamine **1** moiety, superior binding to mammalian rRNA and probably better suppression activity will result. Therefore, we selected 5-amino ribose as a third sugar ring and prepared the new generation of pseudotrisaccharides **3**, **5**, and **7** with the expectation that they will possibly function better as read-through inducers than the parallel structures containing the plain ribose ring (structures **2**, **4**, and **6**). Similar arguments, the direct addition of an extra amino group to the paromamine **1** moiety and its dimerization, served as a basis for the preparation of compounds **8** and **9**, respectively. Enhanced RNA binding by using dimerized aminoglycosides[9] and amino-aminoglycosides[10] supports these designs.

All the designed structures **1**–**9** were synthesized according to the general strategy (Fig. 2) that involves direct Lewis acid promoted cleavage of paromomycin into the pseudo-disaccharide **1**, which is then used as a common starting material for the preparation of all the designed structures. For the construction of pseudotrisaccharides **2**–**7**, we employed the appropriately protected three different paromamine acceptors, **11**–**13**, which selectively expose C5, C6, and C3′ hydroxyl groups of the paromamine moiety, respectively, for glycosidation reactions. These acceptor molecules were readily accessible from paromamine **1** as illustrated in Scheme 1. Simultaneous conversion of all the amino groups of **1** into the corresponding azides was done by treatment with TfN_3 to afford **10**. Regioselective

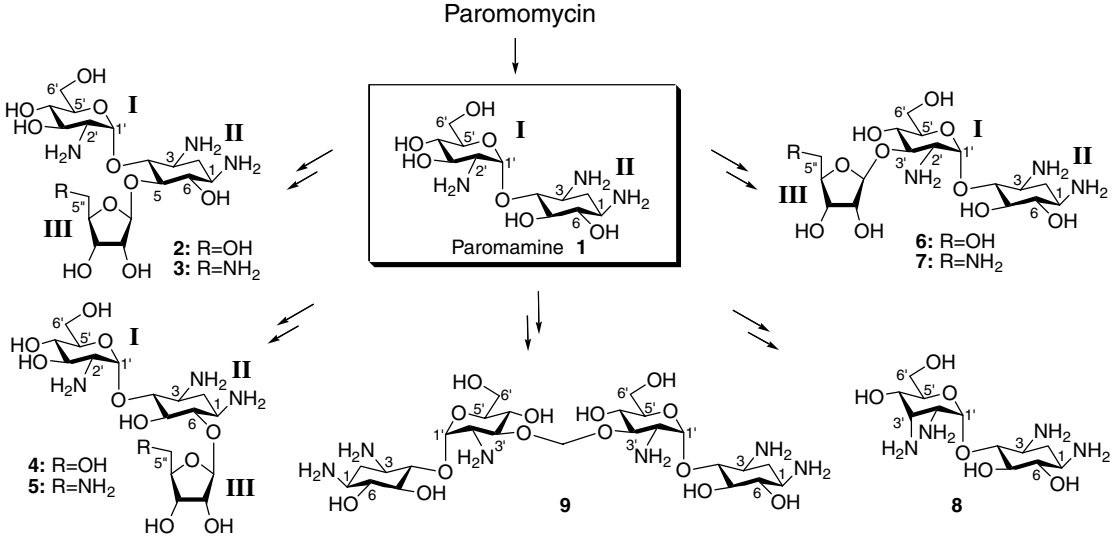

Figure 2. Structures of paromamine **1** and its synthetic derivatives **2**–**9**.