Modification Of 4,5- Aminoglycosides To Overcome Drug Resistance Bacteria And Toxic Side Effect

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MODIFICATION OF 4,5-AMINOGLYCOSIDES TO OVERCOME DRUG RESISTANCE BACTERIA AND TOXIC SIDE EFFECT

by

GUANYU YANG

DISSERTATION

Submitted to the Graduate School of Wayne State University, Detroit, Michigan

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

2018

MAJOR: CHEMISTRY (Organic)

Approved By:

____________________________________
Advisor Date
DEDICATION

I dedicate my PhD. work to my parents Mr. Guoqiu Yang and Mrs. Liping Song, my wife Qian Lin and my friends for their endless love, support and guidance.
ACKNOWLEDGEMENTS

First of all, I would like to express my deep admiration and gratitude to my advisor, Professor David Crich, for his constant guidance, support, motivation and patience during the course of my researches in his laboratory. His passion for new scientific discoveries, diligent and rigorous working attitude always encourage me to do better. Without his timely support and guidance, it would have been extremely difficult to finish this thesis.

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<tbody>
<tr>
<td>A</td>
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</tr>
<tr>
<td>AAC</td>
<td>Aminoglycoside acetyltransferases</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption, distribution, metabolism, and excretion</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AGA</td>
<td>Aminoglycoside antibiotics</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>AME</td>
<td>Aminoglycoside modifying enzymes</td>
</tr>
<tr>
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<tr>
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<tr>
<td>ATP</td>
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<td>Benzyl</td>
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<td>Bz</td>
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</tr>
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</tr>
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</tr>
<tr>
<td>Cbe</td>
<td>Ethoxycarbonyl</td>
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Cbz  benzyloxycarbonyl
CDC  Centers of Disease Control and Prevention
cm  Centimeter
COSY  Homonuclear correlation spectroscopy
m-CPBA  m-Chloroperbenzoic acid
DCM  Dichloromethane
DMAP  4-Dimethylaminopyridine
DMF  N,N-Dimethylformamide
DNA  Deoxyribonucleic acid
DOS  Deoxystreptamine
EDP  Energy-dependent phase
ESI  Electrospray ionization
ESIHRMS  Electrospray ionization high resolution mass spectrometry
Et  Ethyl
Et$_3$N  Triethylamine
EtOH  Ethanol
EtOAc  Ethyl acetate
FT/IR  Fourier transform infrared
G  Guanine
h  Hour
HMBC  Heteronuclear multiple bond correlation
HMDS  Hexamethyldisilazane
HSQC  Heteronuclear single quantum coherence
Hz  Hertz
LPS  Lipopolysaccharides
m-CPBA  3-Chloroperbenzoic acid
MDR  Multi-drug-resistant
Me  Methyl
MeOH  Methanol
mmol  Millimole
mRNA  Messenger ribonuc
MRSA  Methicillin-resistant *Staphylococcus aureus*
MS  Molecular sieves
NADPH  Nicotinamide adenine dinucleotide phosphate
NMR  Nuclear magnetic resonance
Ph  Phenyl
PMe₃  Trimethylphosphine
PTSA  *p*-Toluenesulfonic acid
Py  Pyridine
ROS  Reactive oxygen species
RNA  Ribonucleic acid
RT  Room temperature
<table>
<thead>
<tr>
<th>Term</th>
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<tr>
<td>Stick’s reagent</td>
<td>Imidazolesulfonyl azide hydrochloride salt</td>
</tr>
<tr>
<td>Su</td>
<td>Succinimide</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBAI</td>
<td>Tetrabutylammonium iodide</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-Tetramethyl-1-piperidinyloxy</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TfOH</td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TMSSPh</td>
<td>Trimethyl(phenylthio)silane</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>Ts</td>
<td>p-Toluenesulfonyl</td>
</tr>
<tr>
<td>TTBP</td>
<td>2,4,6-Tri-tert-butylpyrimidine</td>
</tr>
<tr>
<td>TTMSS</td>
<td>Tris(trimethylsilyl)silane</td>
</tr>
<tr>
<td>U</td>
<td>Uracil</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>WHO</td>
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CHAPTER 1. INTRODUCTION

1.1. Background and significance

Infectious diseases are one of the major threats to human health and society. Over nine millions deaths were caused by infection in 2013, which were about 17% of all deaths in the world.¹ The major cause of these diseases is pathogenic bacteria. Since 1928, when Sir Alexander Fleming first discovered penicillin-G, antibiotics became the most powerful and effective weapon against bacterial infections. However, widespread antibiotic usage, especially uncontrolled, improper and incomplete use in many low or middle-income countries, has made bacteria resilient to most of the existing antibiotic drugs.²⁻³ According to a CDC report in 2013, antibiotic-resistant bacteria infected 2 million people and at least 23,000 people die each year in the USA as a direct result of them.⁴ Infectious diseases also lead to $20 billion in excess direct healthcare costs and $35 billion additional costs to society for lost of productivity.⁴ These facts demand the development of new generations of antibacterial drugs.

Unfortunately, in recent years the pace of developing new antibiotics is not increasing, but decreasing (Figure 1). Bacterial metabolism pathways provide only a few suitable targets for drugs; so that only two new classes of antibiotics have been developed since the 1970s.⁵⁻⁶ Because of marketing risks, big pharmaceutical companies have mostly cut off their investment in developing new antibacterial agents. A report written by the Infectious Diseases Society of America (IDSA) in 2013 pointed out that only two new antibiotics (Telavancin⁷ and Ceftaroline fosamil⁸) have been
approved in the United State since 2009, which is far from sufficient to match bacteria's increasing ability to develop resistance. These are the reasons why we need to devote our efforts to discovering new antibiotics. We choose to reinvestigate existing classes of antibiotics because their mechanisms of action and of resistance are known. By rational design and utilizing modern synthetic organic chemistry tools, we hope to be able to bypass those resistance mechanisms and achieve novel antibiotics.

Figure 1. Numbers of antibacterial approvals from 1980 till 2013

Antibacterials are a type of antimicrobial agent used in the treatment of bacterial infections. They either inhibit the rapid grow or kill bacteria. Based on their mechanisms of action, antibiotics can be divided into four different categories (Figure 2):

1. Cell wall synthesis inhibitors (cephalosporins, penicillins, etc.)
2. Protein synthesis inhibitors (aminoglycosides, macrolides, oxazolidinones, tetracyclines)
3. Folate coenzyme synthesis inhibitors (sulfonamides)
4. Nucleic acid synthesis inhibitors (quinolones, fluoroquinolones)

![Figure 2. Four major categories of antibiotics](image)

Aminoglycosides (AGAs) are a family of water-soluble, poly-cationic amino sugar molecules that can inhibit the protein synthesis of bacteria.\textsuperscript{11} The first AGA was called streptomycin 1 (Figure 3), and was discovered by Nobel Prize winner Selman Waksman in 1944.\textsuperscript{12-13} It was a landmark in antibiotic history because streptomycin was the first effective drug against tuberculosis; and it is still in use today.\textsuperscript{14} Compared with other types of antibiotics, AGAs are attractive to our lab for several advantages. They are considered as highly potent broad-spectrum antibacterial agents and have been used against Gram-negative bacteria, methicillin-resistant \textit{Staphylococcus aureus} (MRSA), multidrug-resistant tuberculosis, and many other complex infectious diseases. Unlike penicillin type or sulfonamide type antibiotics, which usually cause severe allergic problems, aminoglycosides lack drug-related allergic effect and have little impact on host’s intestinal microbiome.\textsuperscript{15} Moreover, the properties of AGAs, including how they kill bacteria, how they are deactivated, their pharmacokinetics and pharmacodynamics, and ADME properties are well-understood and predictable,\textsuperscript{15-18} which provides a strong
basis for rational design of next generation of aminoglycosides (less toxic and resistance proof). Last but not least, the availability of many AGAs on large scale and their well-established chemistry make them good candidates for modification.19-20

Figure 3. Structure of streptomycin (1)

1.2. Structure and classification

Aminoglycosides are hydrophilic pseudo-oligosaccharides that have a core structure called the 2-deoxystreptamine (2-DOS) ring (Figure 4).21 Amino sugars are linked to this 2-DOS ring by glycosidic bonds to generate different types of aminoglycosides.21 Most of the clinically used AGAs have a di-substituted 2-DOS ring and can be classified into two series based on the positions of the glycosidic linkages. One class is named as 4,6-aminoglycosides, and includes kanamycin 2, gentamicin 3 and amikacin 4, in which the 2-DOS ring (ring II) is di-substituted in positions 4 and 6. Neomycin 5, paromomycin 6 and ribostamycin 7 belong to another series of AGAs called 4,5-aminoglycosides because the 2-DOS ring (ring II) is di-substituted in positions 4 and 5 (Figure 4).
Some other AGAs have unique structures and do no fit in the normal classification. Streptomycin 1, the first discovered AGA, has a streptamine core that is mono-substituted, where the amino groups have been modified to guanidino groups (Figure 5). Apramycin 8, which is produced by *Streptomyces tenebrarius*,\(^\text{22}\) is an aminoglycoside that consists of a mono-substituted 2-DOS ring core and a bicyclic rings system (Figure 5). This compound shows little or no ototoxicity in animal models\(^\text{23-24}\) and avoids modification by most known aminoglycoside modifying enzymes, making it good candidate for the next generation of AGAs that are active against multidrug resistant bacteria. Hygromycin B 9 (5-substituted-2-deoxystrepaamine ring)\(^\text{25}\) and Spectinomycin 10 (three fused ring system and an aminocyclotol ring called spectinamin)\(^\text{26-27}\) are another two examples of AGAs that have unusual structures.

\[ \text{Figure 5. Unusual aminoglycoside structures} \]
1.3. Mechanism of action of AGAs

Early studies on the mechanism of action of streptomycin against *Mycobacterium tuberculosis* showed that the production of labeled protein by cell-free extracts was blocked, which suggested that AGAs inhibit protein synthesis. After decades of intense study, scientists confirmed that the activities of the aminoglycosides can be mainly attributed to their action as protein synthesis inhibitors and acquired significant knowledge of all different stages of AGAs action from uptake to killing bacteria.

1.3.1. AGA uptake

The typical molecular weight of an aminoglycosides molecule is around 300-800, which excludes the possibility of AGAs spontaneously penetrating the cell membrane of bacteria. In fact, AGAs are taken into cells by a specific mechanism. Under physiological conditions, aminoglycosides have a cationic nature, as the free amines of the molecules are protonated. This phenomenon provides a nonspecific electrostatic interaction between the positively charged AGAs and negatively charged biomolecules, including lipopolysaccharides in the outer bacterial membrane (Figure 6). The energy-dependent phase I is the uptake step after AGAs interact with LPS, which helps transport AGAs from the outer membrane to the cytosol (Figure 6). The rate of uptake is AGA concentration dependent, and can be interfered by inhibition of oxidative phosphorylation or of electron transport. In the next step, which is called energy-dependent phase II, AGAs bind to the 30S ribosomal subunit rapidly and disturb the protein synthesis process (Figure 6).
1.3.2. **Protein synthesis and the action of AGAs**

In order to understand the action of AGAs, we need to know how proteins are synthesized in cells. Normally, protein synthesis goes through several steps, which are called sequentially transcription, translation, proteolysis, post-translation modification and protein folding (Figure 7). In the transcription stage, messenger RNA is synthesized from the template strand of a DNA double helix in the genome. This process is followed by a step called translation in which amino acids are assembled by ribosomal RNA and generate the peptide sequence based on the genetic information encoded in the mRNA. Proteolysis may remove the N-terminal, C-terminal or internal amino acid residues or peptides from the polypeptide chains that synthesized in translation stage. These polypeptides fold to secondary and tertiary structure to generate fully functionalize proteins after their terminal and side chains are modified in post-translational modification stage.
Figure 7. The overall process of protein synthesis

During translation, mRNA binds to the smaller ribosomal subunit and then recognizes the aminoacyl transfer RNA (tRNA) that carries the specific amino acid. The large ribosomal subunit binds to form an initiation complex and starts elongate the polypeptide chain by covalent attaching amino acids. The structure of mRNA and tRNA ensures the accuracy of translation. Ribonucleic acid consists of three parts: the phosphate backbone, the ribose sugar and nitrogenous bases. There are four bases found in RNA: adenine (A), guanine (G) cytosine (C) and uracil (U). A and G belong to purine bases while C and U are pyrimidine bases (Figure 8). Watson and Crick discovered that those bases form specific pairs through unique hydrogen bonds based on their structure in 1953. This is called the Watson-Crick rule, according to which adenine pairs with uracil by two hydrogen bonds, while guanine pairs with cytosine by three hydrogen bonds (Figure 8). A specific mRNA bears sequences of nucleobases called codons, which determine the polypeptide that needs to be synthesized. tRNA bears complimentary nucleobases called anticodons. By the specific codon/anticodon recognition through Watson-Crick rule, tRNA brings in the required amino acid to synthesize the correct polypeptide that encoded by the mRNA.
The high accuracy of the translation process, with errors estimated at $4 \times 10^{-4}$ per codon, implies that beside codon/anticodon recognition, ribosomes also play an important role in securing translation accuracy. The ribosome contains three RNA binding sites: (i) aminoacyl-tRNA binding site (A-site), (ii) peptidyl tRNA binding site (P-site) and (iii) empty tRNA binding site (E-site) (Figure 9). The A-site recognizes and binds the tRNA that carries the corresponding amino acid based on the codon on mRNA; the P-site binds the tRNA bound to the peptide chain being synthesized; and the E-site binds a free tRNA before it exits the ribosome (Figure 9).
AGAs carry positive charges under physiological conditions, so they have strong electrostatic interactions with the negatively charged nucleotides. In the late 1980s, it was discovered that aminoglycosides bind to the 16S rRNA subunit of the 30S bacterial ribosome (Figure 10a). This is a conserved loop that belongs to the small ribosomal subunit’s A-site. AGAs can selectively bind to this ribosomal A-site by specific hydrogen bonding interactions. For example, the ring I oxygen (O5') and the 6'-substituents (OH in paromomycin 6 and NH₂ in neomycin 5) of 4,5-AGAs form hydrogen bonds with N-1 and N-6 of A1408 and make a pseudo base-pair type interaction (Figure 10b). There is also a CH-π interaction between the β-face of ring I and G1491. The 2-DOS ring (ring II) also contributes to binding and interacts with A1406, G1494 and U1495 through hydrogen bonds (Figure 10b). Rings III and IV of the 4,5-AGAs provide additional hydrogen bonds with G1491 and C1490 as they reach to the base pair of 1409-1491 and 1410-1490 (Figure 10b). For 4,6-AGAs, the binding pattern of ring I and ring II to A-site is similar to 4,5-AGAs, but the position of ring III is different (Figure 11). Ring III of the 4,6-AGAs tobramycin 11 points to another direction in the binding pocket and forms a hydrogen bond with G1405 (Figure 11).
Figure 10. a) Crystal structure of paromomycin (6) binding to bacterial ribosome A-site (PDB: 1FJG). b) Key hydrogen bonding interactions between paromomycin (6) and A-site.

Figure 11. The binding similarity and difference between 4,5- and 4,6-AGAs

The A-site contains three unpaired adenine residues (A1408, A1492 and A1493) and adapts a ‘flipped-out’ conformation when tRNA binds (Figure 12). A faster step includes other conformational changes occurs after the ‘flipped-out’ conformation forms. It leads to tight binding of tRNA to the A-site. The interactions between AGAs and ribosomal A-site lock the decoding loop into a similar conformation to that observed during mRNA decoding (Figure 12). This conformational lock up reduces the energy barrier for the binding of tRNA, which allows both cognate and non-cognate tRNA to bind to mRNA (Figure 13). There are two consequences: (i) prevention of the
correct initiation of protein synthesis; and (ii) synthesis of non-functional or abnormal proteins as mRNA is misread and incorrect amino acid is incorporated. In addition, the binding affinity of tRNA for the A-site is increased due to the ‘flipped-out’ conformation being locked up. This results in stabilization of the pre-translocation state of the ribosome, increase of the energy barrier of translocation and stopping of movement of peptidyl tRNA from the A-site to the P-site (Figure 13).

**Figure 12.** Conformational changes of A-site: a) normal conformation; b) ‘flipped-out’ conformation; c) AGA bound conformation

**Figure 13.** The binding of AGAs interferes with protein synthesis

There are several hypothesizes about how incorrect protein synthesis leads to
cell death. One assumption is that the abnormal protein causes cell death by inserting in the inner membrane of bacteria and destabilizing it.\textsuperscript{51-52} Another theory suggests that reactive oxygen species (ROS), the key factor associated with cell apoptosis, are generated by defective metabolic and respiratory enzymes.\textsuperscript{53}

### 1.4. Selectivity and toxicity problems

AGAs are useful in treating infections involving aerobic, Gram-negative bacteria, such as \textit{Pseudomonas}, \textit{Acinetobacter}, and \textit{Enterobacter}. But severe toxicity problems, including ototoxicity (irreversible hearing loss) and nephrotoxicity (reversible kidney damage),\textsuperscript{20,54-56} limit the therapeutic use of AGAs and have fueled the decline of interest in developing new aminoglycosides.

#### 1.4.1. Nephrotoxicity

Nephrotoxicity is one of the major side effects caused by treatment with AGAs. It is classified as nonoliguric renal failure, with a slow rise in serum creatinine and a hypoosmolar urinary output.\textsuperscript{57} During the process of AGA excretion, approximately 5\% (small but not negligible) of the administered dose is retained in the epithelial cells lining the S1 and S2 segments of the proximal tubules\textsuperscript{58} after glomerular filtration.\textsuperscript{59} AGAs bind to the acidic phospholipids on the brush-border cell membrane due to their cationic form under physiological condition,\textsuperscript{50} and then are transferred to the transmembrane protein megalin, with which they become internalized in endosomes.\textsuperscript{61} Once passing through cell membranes, the accumulated AGAs are mainly localized with in endosomal and lysosomal vacuoles,\textsuperscript{62-63} where they inhibit the activity of lysosomal
phospholipase and cause an abnormal increase in the size and number of lysosomes that eventually leads to death of proximal tubular cells and kidney damage.

Nephrotoxicity is reversible, but can be fatal owing to permanent kidney damage and kidney failure if untreated. Several strategies have been developed to reduce nephrotoxicity, of which the most common method used in the clinic is the administration of a single large daily dose instead of separate smaller doses or continuous infusion. The logic behind this strategy is that the large excess of drug can be excreted without causing toxicity as the uptake of AGAs by renal cells is saturated at relatively low concentrations. At the same time the large dose is lethal to the bacterial infection. Another useful strategy is to decrease the binding of AGAs with lysosomal phospholipids, either by modification of AGAs (i.e. by acylation of N-1-position, as in amikacin) or by co-administration of other drugs that can competitively bind to the phospholipids (i.e. polyaspartic acid). Some reports also show that hydration therapy can alleviate the symptoms of AGA-induced nephrotoxicity.

1.4.2. Ototoxicity

Ototoxicity is another important and severe side effect of aminoglycoside therapy. According to reports, nearly 20% of patient populations, who undergo AGAs treatment, suffer from irreversible ototoxicity. There are two symptoms of ototoxicity, including imbalance disorder that is caused by damage of the vestibular system, and permanent hearing loss due to the disruption of the cochlea. The magnitude of
ototoxicity is related to a couple of factors. For instance, different AGAs have different ototoxic potentials. Thus, neomycin 5, a representative of the 4,5-AGAs, is known as more ototoxic than gentamicin 3 or tobramycin 11 (both belong to 4,6-AGAs). Additionally, in longer therapeutic AGAs regimes, as in the treatment of tuberculosis, kidney malfunction, and nutritional and physiological states of the patients also contribute to the severity of ototoxicity.71

AGAs disrupt sensory hair cells of the inner ear, leading to irreversible effects because once damaged, such cells cannot be regenerated.17,65,72 The uptake of AGAs into the inner ear is very fast (a few minutes), meanwhile the half-life of AGAs in the inner ear can reach a month (as compared to 3-5 h in the plasma), which makes the inner ear a vulnerable target of AGAs. The sensory hair cells of the inner ear are essential for the transduction of auditory stimuli (they convert sound waves to electric impulses that can be transferred to the brain to give the hearing sensation) and balance sensation (type-I and type-II vestibular hair cells).73

The molecular mechanism of AGA damage to the cochlea is still unclear,74-76 but recent studies suggest that the AGAs bind to the human mitochondrial ribosome A-site of those cells, cause inhibition of mitochondrial protein synthesis, or promotion of abnormal mitochondrial protein synthesis, which result in ROS formation and cell apoptosis.77-80 There are two ways that ototoxicity occurs: (i) a sporadic does-dependent manner in general patients; and (ii) an aggravated manner in genetically susceptible people, whose mitochondrial A-site is slightly mutated (deafness
mutation).

Unlike nephrotoxicity, there is still no practical clinical regime to prevent AGA-induced ototoxicity. However, antioxidant therapy shows potential to attenuate ototoxicity by blocking the formation of ROS.\textsuperscript{81-82} Salicylic acid, which acts as both an antioxidant and iron-chelator, has been shown to reduce the auditory threshold change caused by the treatment of gentamicin from more than 60 dB to less than 20 dB.\textsuperscript{93}

1.4.3. Cell-free functional ribosomal assays (IC\textsubscript{50})

The selectivity of aminoglycosides relies on the small sequence differences between the ribosome A-site of eukaryotic cells and prokaryotic cells. At the 1408 position, an adenine residue (A) is found in bacteria, but a guanine residue (G) is found in human cytoplasmic RNA (Figure 14). As discussed in the previous section, the A residue provides key hydrogen bond interactions with the 6'-substituent of aminoglycosides, which helps AGAs bind tighter with bacteria ribosome than human cytosolic ribosome. Another structural difference appears at the 1409-1491 (C-G pair in bacteria and C-A pair in human cytosolic) and 1410-1490 (G-C pair in bacteria and U-A pair in human cytosolic) base pairs (Figure 14).\textsuperscript{42,80} Human mitochondrial ribosome A site, on the other hand, is more similar to bacteria A-site. They share the same key A1408 residue (Figure 14), which leads to lower selectivity for the bacteria A-site over the mitochondrial A-site and results in ototoxicity.\textsuperscript{80} In the case of deafness mutant, compare with the normal mitochondrial A-site, apart from the same A1408 residue, residue 1555 is mutated from A to G, which forms a Watson-Crick pair, tightens up the
binding pocket and increases the binding affinity of AGAs to those mutated ribosome A-site (Figure 14).

Prof. Böttger, our collaborator in the University of Zurich, has developed an efficient genetic tool to study the interaction between eukaryotic rRNA with aminoglycosides and to screen AGAs derivatives for their antibacterial activities and selectivity. His group replaced the A-site of 16S rRNA of *M. smegmatis* with its eukaryotic counterpart, and obtained the purified 70S hybrid bacterial ribosomes with fully functional human cytosolic A-site (Cyt 14), human mitochondrial A-site (Mit 13) or mitochondrial A1555G A-site (deafness mutation, A1555G).

Those recombinant hybrids are used in cell free translation assays and the IC₅₀ value of each synthesized AGAs can be easily tested.

*Figure 14. Different rRNA sequences of decoding-sites in the small ribosomial subunits: (A) Wild-type ribosomes of *M. smegmatis*. (B) Human cytoplasmic ribosomes. (C) Human mitochondrial ribosomes. (D) Mitochondrial ribosomes.*
with mutation A1555G conferring hypersusceptibility to AGA ototoxicity. The AGA binding site is boxed.

1.5. Resistance problems

One of the main properties of next generation of aminoglycosides must be circumvention of antibiotic resistance. The most common resistance mechanisms include: decreased AGAs uptake; target modification; aminoglycoside modifying enzymes and increased efflux (Figure 15).

![Figure 15. Resistance mechanisms of AGAs](image)

1.5.1. Decreased uptake

The uptake of AGAs is highly relying on the interaction between positively charged AGA molecules and the negatively charged LPS, so modifications of LPS diminish the AGAs uptake. Incorporation with 4-amino-4-deoxy-L-arabinose is the most common modification of LPS, which reduces the net negative charge of LPS and leads to the decrease of AGAs uptake. Other modifications include adding phosphoethanolamine or an unusual lipid called diacylphosphatidylinositol dimannoside. Because the transportation of AGAs is energy-dependent, mutations to the ATP-synthase in *E. coli*, *S. aureus* and *P. aeruginosa* have been shown to decrease
their susceptibility of AGAs.90

1.5.2. Target modification

Alteration of the A-site of bacterial ribosome is common mechanism of resistance, which interferes with the binding between aminoglycosides and their target. There are two types of modifications: nucleotide mutation and nucleotide methylation. One of the nucleotide mutation examples is the A1408G mutation, which leads to high level of resistance to kanamycin 2, gentamicin 3, amikacin 4, tobramycin 11, arbekacin 12, isepamicin 13 and neomycin 5 by interrupting the key hydrogen bond interactions with AGAs as mention in previous chapter.91-92

Aminoglycosides are produced by certain bacteria strains (Streptomyces and Micromonospora). In order to protect themselves, those bacteria develop a defensive mechanism by methylation of their 16S RNA A-site with rRNA methylases.93-94 For example, *M. purpurea* (producer of gentamicin 3) and *S. tenebrarius* (producer of tobramycin 11) modify the G1405 and A1408 residues in their ribosome A-site to the 7-methyl derivatives.95 This mechanism is not considered as a threat until it spreads to various pathogenic bacteria recently and affects all the clinical used 4,6-AGAs as methylated G1405 disrupt the key interaction between the binding site and ring III of that series.16, 96-98 Common examples of these rRNA methylases include: RmtA in *P. aeruginosa*;99 RmtB in *S. marcescens*, *A. baumannii*, *P. aeruginosa*, *E. coli*, and *K. pneumonia*;100 RmtC in *K. pneumonia*;101 and ArmA in *S. marcescens*, *E. coli*, and *K. pneumonia*.93,102-103
1.5.3. Aminoglycoside modifying enzymes (AMEs)

AMEs are enzymes that are expressed by bacteria to catalyze the modification of aminoglycosides and inactivate the drug molecule.\textsuperscript{27, 36, 104-106} They are the most widespread and clinically relevant mechanism of AGA-resistance. There are three major categories of AMEs: (i) aminoglycoside acetyltransferases (AACs), (ii) aminoglycoside phosphotransferases (APHs), and (iii) aminoglycoside nucleotidyltransferases (ANTs), which can modify AGA molecules by acetylating the free amino groups (AACs) or phosphorylating the free hydroxy groups (APHs and ANTs). The modified AGAs are blocked from binding to the ribosome A-site and lose their antibacterial activity. Figure 16 shows the potential modification sites of some commonly used AGAs.

Figure 16. Target sites of AMEs on kanamycin (2), neomycin (5), streptomycin (1), and apramycin (8)

1.5.3.1. Aminoglycoside acetyltransferases (AACs)

This is the largest family of AMEs, and more than fifty members of AACs have
been identified in both Gram-positive and Gram-negative bacteria. They belong to ubiquitous GCN5-related N-acetyltransferase superfamily of protein. The AACs catalyze acetylation at the 1 [AAC(1)], 3 [AAC(3)], 2’ [AAC(2’)] or 6’ [AAC(6’)] position of AGAs, reduce the positive charge of the molecules and generate steric hindrance to block the binding. AAC(1) enzymes are not the major threat because they do not cause significant reduction of the antibacterial activity and are rarely found in clinical isolates. There are nine subclasses of AAC(3) described to date, which are all in Gram-negative bacteria. They confer resistance to most 4,6-AGAs including gentamicin, tobramycin, sisomicin, fortimicin, dibekacin, and netilmicin. AAC(2’) have been found in Gram-negative and *Mycobacteria*, they also mediate modifications of 4,6-AGAs. By far, the most common and important AACs are AAC(6’), which have been found in Gram-negative as well as Gram-positive bacteria. Acetylation at the 6’-amino group disturbs the key hydrogen bond interactions between A1408 and AGA molecules and renders the AGA inactive.

1.5.3.2. Aminoglycoside phosphotransferases (APHs)

APHs catalyze the regiospecific transfer of the γ-phosphoryl group of the ATP to the hydroxy group of aminoglycosides. Phosphorylation of specific hydroxy groups leads to: (i) reduction of the binding affinity of AGAs by introducing negative charge, and (ii) disruption of binding by generating steric hindrances and blocking the hydrogen bond donors. There are seven classes of APHs: APH(4), APH(6), APH(9), APH(2’), APH(3’), APH(3’’), and APH(7’’), among which APH(3’) is the most widespread.
and well-studied APH.\textsuperscript{109}

\subsection*{1.5.3.3. Aminoglycoside nucleotidyltransferases (ANTs)}

ANTs deactivate aminoglycosides by modifying hydroxy groups of AGAs with AMP group from the donor substrate ATP. This is the smallest AMEs family,\textsuperscript{17} and only five classes have been identified so far [ANT(6), ANT(9), ANT(4'), ANT(2''), and ANT(3'')].\textsuperscript{109} The most commonly found ANTs are ANT(3''), but they are not considered as serious problems because they specify resistant to streptomycin \textsuperscript{1} and spectinomycin \textsuperscript{10}. ANT(2''), on the other hand, are of significant clinical importance for their abilities to inactivate gentamicin \textsuperscript{3}, amikacin \textsuperscript{4} and tobramycin \textsuperscript{11}.\textsuperscript{115}

\subsection*{1.5.3.4. Avoiding AME resistance}

There are three major strategies to overcome AMEs resistance: inhibit the action or biosynthesis of aminoglycoside modifying enzymes,\textsuperscript{109} and modification of aminoglycoside molecules to make them resistance-proof. In order to inhibit the activities of AMEs, several methods have been studied. For example, aminoglycoside-CoA bi-substrates can inhibit AAC(3) and AAC(6') by mimicking the intermediate complex or random binding to the enzymes.\textsuperscript{116-121} In another study, cationic antimicrobial peptides, such as bovine peptide indolicidin, showed inhibition activities against APHs and AACs.\textsuperscript{122} Two non-carbohydrate di-amine derivatives\textsuperscript{123} and some known eukaryotic protein kinase inhibitors\textsuperscript{124} are also active against ANTs and APHs. A number of antisense oligonucleotides or oligonucleotides analogs have been explored in bacteria to inhibit the gene expression of AMEs.\textsuperscript{125-128} Phosphorothioate
deoxyribozymes are another example that can target genes coding AMEs and restore susceptibility of AGAs.129-130

Modified AGAs show promising results in defeating AGA resistant bacterial strains (Figure 17). Gentamicin 3 and tobramycin 11 are not susceptible to APH(3’) because they do not have the 3’-hydroxy group. Amikacin 4, a semisynthetic derivative of kanamycin 2, has a L-hydroxyaminobutyroyl amide (L-HABA) side chain attached to the N-1 position, which prevents the molecule from binding to AMEs.131-132 There is only one site of amikacin 4 can be attacked by AMEs, while gentamicin 3 and tobramycin 11 have six.133 Neamine analogs, such as dimer 18,134 semisynthetic derivatives pyranmycin 19,135 pyrankacin 20,136 and amikacin mimetic 21,137 are not affected by certain AMEs [AAC(6’), APH(2’), and APH(3’)] and are active against AGA resistant strains. Neomycin binds to bacterial A-site and ANT(4’) active site with different conformation. So a conformationally locked up derivative of neomycin 22 was designed, synthesized and tested, and showed no binding affinity to ANT(4’) and AAC(2’).138 AGA hybrid 23 that contains ring I of sisomicin 14 and ring II, III, and IV of neomycin 5 shows good activity against bacterial strain with APH(3’) and ANT(4’) resistance mechanism.139 Those results prove that rational modifications of known AGAs are very good strategies to develop next generation of aminoglycosides.
1.5.4. Increased efflux

Increasing the efflux of the drug molecules is an additional mechanism of resistance of AGAs. Due to the polycationic structure of aminoglycosides, efflux pumps are needed to transport AGAs out of the cell, and only a few of them have been identified. AcrAD is the main AGAs efflux pump in Gram-negative bacteria that belongs to the resistance-nodulation-division (RND)-type transporter superfamily and corresponds to AGAs resistance. Besides that, the multidrug and toxic compound extrusion (MATE) efflux pumps family in *Vibrio cholerea* and the major facilitator superfamily (MFS) of transporters in *M. tuberculosis* have also been demonstrated to transport AGAs. The intrinsic level of expression of efflux pumps is so small that it contributes little to resistance in normal situations. It is the overexpression of efflux
pumps, the mutations of efflux pumps that increase the substrate affinity, and the synergy with other resistance mechanisms that causes high level of AGAs resistance. For example, in *P. aeruginosa* or *M. tuberculosis*, which have slow rates of AGAs uptake, the increased level of efflux becomes a large contributor to AGAs resistance.

1.6. Recent advances

In the last few years, several research groups have begun to develop the next generation of aminoglycosides antibiotics. The Crich and Vasella groups collaborated and focus on modification of existing AGAs. They synthesized a series of 4',6'-position, 4'-position and bicyclic ring I modified paromomycin analogs, which retained the full antibacterial activity of the parent and were more selective toward bacterial over the eukaryotic mitochondrial and cytosolic ribosomes (Figure 18).

![Figure 18. Structure of 4',6'-O-alkyldiene (24), 4'-O-alkyl (25) and bicyclic ring I paromomycin derivatives (26) that exhibit increased selectivity toward bacterial A-site](image)

Apramycin is a good candidate for the next generation AGAs. It has been used in animals for decades, and recent study shows that it is one of the least ototoxic aminoglycosides, probably due to the unique bicyclic core structure. The Crich and Vasella groups also put much of effort into increasing the activity and selectivity of
apramycin by modifying the 6' and 7'-positions.\textsuperscript{151} Now, apramycin is expected to go into a phase I clinical trial in Europe.

Plazomicin \textsuperscript{27} is a semisynthetic aminoglycoside that has been developed by the Achaogen Company\textsuperscript{152} and has been approved by FDA for adults with complicated urinary tract infections (cUTI) in patients who have limited or no alternative treatment options in June 2018. This is an AGA that designed to respond to the uprising threat of multidrug-resistant infectious diseases and fight against bacteria with AMEs resistance mechanism.\textsuperscript{153} It is 6'-(2-hydroxyethyl)-1-(4-amino-2(S)-hydroxybutyryl) sisomicin,\textsuperscript{152} in which the 6'-modification can protect the molecule from AAC(6'), and the 1-modification makes the drug not susceptible to AAC(1), AAC(3), ANT(2''), and APH(2'').

![Figure 19. Structure of plazomicin (27)](image)

1.7. Overall goals

The overall goal of this thesis was to develop the next generation of aminoglycosides that are nontoxic and resistant-proof by rational design and novel organic synthesis. We aimed to exploit the known structural differences between bacterial ribosome A-site and mitochondrial ribosome A-site to design more selective and less toxic AGAs. Meanwhile, the well-studied resistance mechanism of AGAs,
especially the AMEs mechanism, can help us develop new aminoglycosides that remain active against AGA resistant bacterial strains. We choose to modify 4,5-AGAs as the A-site G1405 methylation resistance mechanism mentioned in previous chapter affects all 4,6-AGAs. Paromomycin 6, compared with neomycin 5, is less toxic because it has less free amine groups, which makes it good substrate for modifications. Several modifications, including single modification at the 6’ and 3’ positions, double modification at the 3’,5” positions, and triple modification at the 3’, 4’, 5” positions were made and tested for their selectivity to bacterial ribosome A-site and activity against AGAs resistant bacterial strains.
CHAPTER 2. 6’-MODIFICATION OF PAROMOMYCIN

2.1  Rationale

The 6’-position of aminoglycosides is crucial for AGA binding to the ribosome A-site. As discussed in the previous chapter, the 6’-substituents (OH or NH₂) and ring I oxygen (O5’) form hydrogen bonds with N-1 and N-6 of the A1408 residue in the bacterial ribosome A-site, making a pseudo base-pair type interaction between 16S RNA and the drug molecule. The importance of the 6’-position makes it a good target for resistance mechanisms. AAC(6’), one of the most common aminoglycoside modifying enzymes, can catalyze acetylation at the 6’-position using acetyl coenzyme A as donor substrate, and deactivate AGAs that have a 6’-amino group. The 16S Ribosomal RNA methyltransferases (RMTases), which some bacteria use to protect themselves from aminoglycoside action, are another resistance mechanism that can involve the 6’-position. NpmA, a RMTase that methylates the N-1 position of A1408, has low clinical prevalence, but is a considerable potential threat because it affects not only 4,6-AGAs, but also 4,5-AGAs. The methylated A1408 residue loses the ability to form the important pseudo base-pair hydrogen bond interaction and also sterically blocks the binding pocket of ring I.

Some modifications have been made at the 6’-position of paromomycin 6 in the Crich laboratory. 6’-Deoxy paromomycin 28 and 6’-deoxy-6’-fluoro paromomycin 29 have good selectivity for the bacterial ribosome A-site over the eukaryotic ribosome without losing too much antibacterial activity, which suggested that there was room to
do more modifications at this position of paromomycin 6. These existing paromomycin analogs retain a bulky methyl or fluoromethyl group that could be affected by the NpmA resistance mechanism, so 6'-deshydroxymethyl paromomycin 30, which lacks the 6'-carbon of paromomycin 6 (Figure 20) were synthesized and screened for ribosomal activity and antibacterial activity. Additionally, the new compound would lack possible unfavorable hydrophobic interactions and was expected to be active against bacterial strains containing the NpmA resistance mechanism.

![Chemical structures](image)

**Figure 20. Rational design of 6'-deshydroxymethyl paromomycin (30)**

### 2.2 Chemistry

The synthesis of 6'-deshydroxymethyl paromomycin 30 started from the fully protected paromomycin intermediate 31, whose selective reduction was needed to afford the free 6'-monohydroxy intermediate 32. Several different conditions, including borane trimethylamine with dibutylboron triflate; borane tetrahydrofuran complex with copper(II) triflate; and borane dimethyl sulfide with copper(II) triflate, were tried in an attempt to optimize selectivity and obtain a good yield. The best conditions we found were borane dimethyl sulfide (2 M solution in tetrahydrofuran) and dibutylboron triflate (1 M solution in dichloromethane), which gave compound 32 in 60% yield. Alcohol 32 was oxidized with catalytic TEMPO, potassium bromide, and
bleach\textsuperscript{156} to the uronic acid \textbf{33} in 79\% yield. Barton’s decarboxylation reaction was utilized to remove the 5’-carboxylic acid.\textsuperscript{157} The reaction was carried out under white light photolysis conditions with tert-dodecanethiol as hydrogen atom source,\textsuperscript{158} and gave the fully protected 6’-deshydroxymethyl derivative \textbf{34} in 55\% yield. A photocatalytic decarboxylation method developed by Macmillan\textsuperscript{159}-\textsuperscript{160} was also examined for comparison purposes. The results showed that Barton’s decarboxylation was significantly more effective on this substrate than the more recent photocatalytic decarboxylation reactions. This may be because Macmillan’s method relies on electron transfer chain reactions, which were interrupted by the multiple electron-withdrawing azide groups, while the Barton decarboxylation was not affected due to its radical chain nature. Hydrogenolysis over palladium hydroxide in a mixture of dioxane, water, and acetic acid, followed by lyophilization then gave 6’-deshydroxymethyl paromomycin \textbf{30} (Scheme 1).
Scheme 1. Synthesis of 6'-deshydroxymethyl paromomycin 30

2.3 Biological evaluations

The above synthesized paromomycin derivative 30 was tested for ribosomal activity and antibacterial activity by the Böttger group in Zurich, together with the parent molecule paromomycin 6 and 6'-deoxy paromomycin 28. The ribosomal activity is obtained from the IC$_{50}$ values of functional cell-free ribosomal assays with the different ribosomes as discussed in the previous chapter. Four different 70S purified ribosomes that carry the rRNA binding A-site, including the wild-type bacterial A-site (Bacterial), the human cytosolic A-site (Cyt14), the human mitochondrial A-site (Mit13), and the deafness mutation A-site (A1555G), were screened,$^{79,84}$ and the data are shown
in Table 1. The in vivo minimal inhibitory concentration (MIC) values for Methicillin-resistant Staphylococcus aureus (MRSA), E. coli and P. aeruginosa were also determined.77

From the ribosomal activities and selectivity results (Table 1), we can see that by comparison with the parent paromomycin 6 and 6'-deoxy paromomycin 28, compound 30 showed a moderate lost of activity against the bacterial ribosome. It also suffered a noticeable decrease in selectivity as the reduction of activity toward all three hybrid ribosomes (Cyt14, Mit13 and A1555G) is smaller than that toward the bacterial ribosome.

**Table 1. Antiribosomal activity of compound 30 (IC$_{50}$, µg/mL)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$/ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paromomycin 6</td>
<td>0.03</td>
</tr>
<tr>
<td>6'-Deoxy paromomycin 28</td>
<td>0.09</td>
</tr>
<tr>
<td>6'-Deshydroxymethyl paromomycin 30</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Similar trends also appeared in the antibacterial activity (Table 2). The paromoycin analogue 30 exhibited activity against two paromomycin-susceptible strains of MRSA and against E. coli, but its activity was almost ten times less than that of the parent. The lost of antibacterial activity of compound 30 made it unnecessary to test its activity against bacterial strains with the NpmA resistance mechanism.
Table 2. Antibacterial activity of compound 30 (MIC, μg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRSA</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG 038</td>
<td>AG 039</td>
<td>AG 042</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>2</td>
<td>≥25</td>
<td>6</td>
</tr>
<tr>
<td>6'-Deoxyparomomycin 28</td>
<td>16</td>
<td>&gt;12</td>
<td>8</td>
</tr>
<tr>
<td>6'-Deshydroxymethylparomomycin 30</td>
<td>16</td>
<td>&gt;12</td>
<td>8</td>
</tr>
</tbody>
</table>

2.4 Discussion

The removal of hydrophobic 6'-methyl group of 6'-deoxy paromomycin 28 cannot be considered as an effective modification. The lost of antiribosomal activity and selectivity suggested that even without the critical hydroxy group (hydrogen bond donor), the 6'-methyl group in 28 still plays an important role in the binding to the bacterial ribosome A-site. One of the hypotheses is that the 6'-methyl group can serve as an electron-donating group and increase the electron density of the ring oxygen, which makes the oxygen a better hydrogen bond acceptor. The absence of the 6'-methyl group weakens the hydrogen bond between O5' and N-6 of the A1408 residue and leads to the decrease of activity. Another possible explanation would be that the 6'-methyl group therefore provides a certain hydrophobic interaction to stabilize the binding. The disappearance of this hydrophobic group of compound 30 results in loss of this stabilization and decreased binding affinity. However, compound 30 was not completely inactive and has the potential to avoid the NpmA resistance mechanism,
which could affect all 4,6 and 4,5-AGAs. In combination with proper modifications at other positions that can increase antibacterial activity, there remains the possibility to develop drug candidates that can fight against the NpmA resistance mechanism.
CHAPTER 3. 3’-MODIFICATIONS OF 4,5-AMINOGLYCOSIDES

3.1 Rationale

The APH(3’)s are the most widespread aminoglycoside phosphotransferases. They form a large family and can be divided into seven different subclasses (I through VII). The APH(3’)-I subclass is composed of three enzymes that are widely distributed in Gram-negative bacteria within a wide host range of plasmids and transposons. This class of APH(3’) shows a resistance profile that includes most 4,5-AGAs (neomycin 5, paromomycin 6, ribostamycin 7 and lividomycin A 35) and some 4,6-AGAs (kanamycin 2). The APH(3’)-II subclass also has three isozymes (APH(3’)-IIa, APH(3’)-IIb and APH(3’)-IIc) and targets kanamycin 2, neomycin 5, paromomycin 6, ribostamycin 7 and butirosin 36. APH(3’)-IIa is well studied and its crystal structure in complex with kanamycin 2 has been resolved. The epidemiological data of APH(3’)-IIa has been extensively reviewed. It is highly disseminated within Gram-positive bacteria and deactivates kanamycin 2, amikacin 4, neomycin 5, paromomycin 6, isepamicin 13, lividomycin A 35 and butirosin 36 (Figure 21). This enzyme also shows APH(5’’) activity, which will be discussed in the next chapter. The remaining subclasses share similar resistance profiles, but are less important than the first three.

Figure 21. Structure of isepamicin 13, lividomycin 35 and butirosin 36
Since APH(3')s are widely distributed and their resistance spectra are large, strategies that can fight against this resistance mechanism are desperately needed. Modifying the 3’-position to remove the hydroxy group that is susceptible to the enzymes has been proved to be effective. Gentamicin 3 and tobramycin 11 are two examples of clinically used 3’-deoxy aminoglycosides that are active against bacterial strains with the APH(3’) resistance mechanism. Although some 3’-deoxy AGAs are cheaply available in large scale by fermentation, chemical approaches to achieve 3’-deoxygenation modification on aminoglycosides have been studied in order to gain more knowledge about how this modification can affect the activity and selectivity of different AGAs. For example, 3’-deoxy kanamycin A 37 can be synthesized through a glycosylation reaction between the 3’-deoxy ring I glycosyl donor 38 and acceptor 39 (Scheme 2A).\textsuperscript{163} Tobramycin 11 has also been prepared directly from kanamycin 2 with selective functionalization at the 3’-position (Scheme 2B).\textsuperscript{164} However, the reported methods have disadvantages such as low yields for the key reactions,\textsuperscript{163} and limited substrate scope.\textsuperscript{164} In this chapter, two different methods to synthesize 3’-deoxy paromomycin 41 are discussed, and three different 3’-deoxy 4,5-AGAs are synthesized. All these compounds were screened for their antiribosomal activity and antibacterial activity.
Scheme 2. Some reported chemical approaches to 3’-deoxy modified AGAs

3.2 Synthesis of 3’-deoxy paromomycin 41 (starting from lividomycin A 35)

3’-Deoxy paromomycin 41, also known as lividomycin B, was prepared from lividomycin A 35, a paromomycin analog that contains the 3’-deoxy ring I and a mannose ring (ring V) at the 4‴ position, by a slightly modified procedure of the chemical conversion reported by Mori et al. in 1972. The procedure comprised the protection of lividomycin A 35, followed by degradation of the mannose moiety with periodate and phenylhydrazine and subsequent de-protection. Lividomycin A 35 was reacted with imidazolesulfonyl azide hydrochloride salt in the presence of potassium carbonate and catalytic copper(II) sulfate pentahydrate to give perazide intermediate 42. The degradation of the mannose ring V was accomplished by sequential treatment with sodium periodate, ethylene glycol, lead diacetate, sulfuric acid and phenylhydrazine in acetic acid condition. Intermediate 43 was obtained in 10% yield over two steps, and which Staudinger reduction was applied to give 3’-deoxy paromomycin 41 after filtration through Sephadex and lyophilization (Scheme 3).
Scheme 3. Synthesis of 3’-deoxy paromomycin 41 from lividomycin A 35

Figure 22 shows the mechanism of the degradation of the ring V. The treatment with sodium periodate oxidized the 2‴‴, 3‴‴-cis-diol of the mannose ring to a di-aldehyde (i), in which the two carbonyl groups were temporarily protected by forming cyclic acetals (ii) with ethylene glycol. After removal of iodate by precipitation with lead acetate, the excess lead was removed by treatment with sulfuric acid and filtration. The di-aldehyde (i) was regenerated by hydrolyzing the acetals and reacted with phenylhydrazine. The phenylhydrazone (iii) that formed in this reaction decomposed rapidly leading to the cleavage of ring V.
3.3 Synthesis of 3′-deoxy paromomycin 41 (samarium iodide reduction)

The synthetic scheme discussed in previous section had a drawback as the starting material lividomycin 35 was not commercial available. A methodology to expose the 3′-position of paromamine 44 for individual functional group transformation was reported in 2006.\(^\text{166}\) Cyclohexanone dimethyl acetal was used to form di-cyclohexylidene intermediate 45, in which all functional groups were protected except for the 3′-hydroxy group (Scheme 4).\(^\text{166}\) However, utilizing this strategy on synthesize more complex 3′-deoxy aminoglycosides requires controlled degradation of AGA to paromoamine 44 and re-glycosylation after the 3′-modification is completed, which makes a tedious synthetic scheme. A chemical approach to achieve de-oxygenation at the 3′-position, which is compatible with the complex AGA substrates, is still required.

*Figure 22. Mechanism of the degradation of ring V*
Scheme 4. Methodology to expose the 3'-position of paromamine 44

Samarium iodide is a one-electron reducing reagent well known for its many chemoselective reductions. Its applications include reductive cleavage of α-alkoxy groups from α-alkoxy ketones and the reduction of ketones to corresponding alcohols (Figure 23). To exploit this reagent in the synthesis of the targeted 3'-deoxy 4,5-AGAs, an O-selective oxidation of the 4'-hydroxy group of ring I of AGAs is necessary. The 4'-ketone intermediate, for which the 3'-substituent could be considered as the α-alkoxy group, can react with samarium iodide and give the 3'-deoxy product thereby providing an easy route to the 3'-deoxy series.

Figure 23. Two applications of samarium iodide reduction

The synthetic scheme started from protected paromomycin derivative 46 that has been described in the literature, in which only the 4'-hydroxy group is exposed. It has been reported that azide groups can be reduced to the corresponding primary amines upon treatment with excess samarium iodide, so changing of the amino protecting groups before the treatment with samarium iodide became necessary. Alcohol 46 was first treated with trimethylphosphine and then 0.1M sodium hydroxide
solution to give the free amines, which were protected by carboxybenzyl groups under direct treatment with $N$-(benzyloxy carbonyloxy)succinimide and sodium carbonate. Alcohol 47 was obtained in 45% yield over two steps and was oxidized with Dess-Martin periodinane to afford the corresponding ketone 48 in 88% yield. Treatment with samarium iodide at -20 °C, followed by addition of methanol at 0 °C gave the fully protected 3'-deoxy paromomycin derivative 49 in 42% yield. Compound 41 was acquired by hydrogenolysis of 49 over palladium hydroxide in aqueous dioxane and acetic acid, followed by filtration through Sephadex and lyophilization (Scheme 5).

Scheme 5. Synthesis of 3'-deoxy paromomycin 41 using samarium iodide reduction
Some interesting discoveries were made in this samarium iodide reductive cleavage reaction. The proposed mechanism of the reductive cleavage of α-alkoxy group of ketone is shown in Figure 24. This process may be initiated by electron transfer from samarium iodide to the carbonyl moiety of compound 48 and generates samarium alkoxide radical i. A second reduction immediately follows, after which samarium alkoxide anion ii is formed. This anion can be trapped by protonation, which leads to the 4'-alcohol 47 outlined in Scheme 5 (Figure 24, pathway A). Two possible elimination reactions can also occur, as the 3'- and 5'-positions are both at the α-position of the 4'-ketone. The cleavages of 5'-carbon-oxygen bond forms alcohol 50 by generating a ring I opened samarium alkoxide enolate type intermediate iiib, which rearranges rapidly and causes the degradation of ring I (Figure 24, pathway B). On the other hand, the cleavage of the 3’-carbon-oxygen bond results in de-oxygenation at the 3’-position and affords another samarium alkoxide enolate type intermediate iiic (Figure 24, pathway C). After the hydrolysis of the samarium-oxygen single bond, enolate iv is formed and easily tautomerizes to give 3’-deoxy-4’-keto paromomycin derivative 51. Excess samarium iodide initiates second reaction at the 4'-ketone, generates samarium alkoxide radical vi and samarium alkoxide anion vii sequentially. Quenching then affords the protected 3’-deoxy paromomycin analog 49.
Figure 24. The proposed mechanism of reductive cleavage of α-alkoxy ketones using samarium iodide

The reaction was conducted at different temperatures and different products were observed. At -78 °C, reaction proceeded slowly and 3'-deoxy-4'-keto paromomycin derivative 51 was formed through pathway C without any sign of reduction of the 4'-ketone or of decomposition of the ring I. It was found that increasing the temperature accelerates the reaction. Compared with stirring at -78 °C overnight, the same 3'-deoxy-4'-keto product 51 was obtained in good yield in 1 h on treatment of 48 with samarium iodide at -20 °C. However, further increasing the temperature affects not only the reaction rate, but also the reaction mechanism. Alcohol 50, which was generated from degradation of ring I through pathway B, was the major product when the samarium iodide reduction was conducted at room temperature.
Table 3. The result of samarium iodide reduction proceeded at different temperature

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
<th>Product observed</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-78 °C</td>
<td>1 h</td>
<td>Starting material (48)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-78 °C</td>
<td>Overnight</td>
<td>3′-deoxy-4′-keto product (51)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-20 °C</td>
<td>1h</td>
<td>3′-deoxy-4′-keto product (51)</td>
<td>70%</td>
</tr>
<tr>
<td>4</td>
<td>Room temperature</td>
<td>2 h</td>
<td>Ring I degraded product (50)(^a)</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) No isolated, but identified by high-resolution mass spectrometry.

The cleavage of the α-alkoxy groups requires an orbital overlapping between the p orbital that contains a pair of electrons at the 4′-position and the anti-bonding orbital of the 3′- or 5′-carbon-oxygen bond. When the reaction proceeds at very low temperature (-78 °C), the dominant conformation of ring I is the stable chair conformation, which provides no orbital overlapping (Figure 25, left). So the reaction progress is very slow at this temperature. As the reaction temperature increases, the chair conformation is able to convert to boat conformations (Figure 25, right), which affords an orbital overlapping between the 4′-position and 3′-position, making the cleavage of the 3′-O-benzyl group become easier and accelerating the reaction. Meanwhile, there is no orbital overlapping between the 4′ and 5′-position in chair conformation or boat conformation. In order to break the 5′-carbon-oxygen bond, more energy is required to achieve an unstable conformation that allows the overlap between 4′- and 5′-position. In this case, when the reaction is proceeded at -20 °C, there is enough energy to achieve the boat conformation and the 3′-O-benzyl group is cleave
without affecting the 5’-position, but a higher reaction temperature (room temperature) makes the orbital overlap between 4’-and 5’-position become possible so that the ring I cleavage product is observed.

**Figure 25. The orbital overlapping of chair and boat conformation**

Having identified the best temperature to operate the samarium iodide reduction, other parameters of this reaction were optimized to generate the 3’-deoxy-4’-hydroxy paromomycin derivative 49 in one pot. Based on the proposed mechanism, at least four equivalents of samarium iodide are needed to achieve alcohol 49 in good yield. An extra proton source is also required to hydrolyze the samarium alkoxide intermediate vii to generate alcohol 49. Combinations of different amounts of samarium iodide, methanol (as proton source), and different reaction times were examined and the results are shown in Table 4.
### Table 4. Optimized conditions of samarium iodide reduction and their results

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amount of SmI₂ (eq.)</th>
<th>Amount of MeOH (eq.)</th>
<th>Time -20 °C + 0 °C</th>
<th>Product observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>10</td>
<td>1 h + 2 h</td>
<td>Compound 50&lt;sup&gt;a&lt;/sup&gt; &amp; 51</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>10</td>
<td>1 h + 2 h</td>
<td>Compound 49 in 42%</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>10</td>
<td>1 h+ 1 h</td>
<td>Compound 50&lt;sup&gt;a&lt;/sup&gt; &amp; 51</td>
</tr>
<tr>
<td>4</td>
<td>Large excess</td>
<td>10</td>
<td>1 h+ 2 h</td>
<td>Compound 49 in 35%</td>
</tr>
<tr>
<td>5</td>
<td>Large excess</td>
<td>5</td>
<td>1 h + 2 h</td>
<td>Compound 50&lt;sup&gt;a&lt;/sup&gt; &amp; 51</td>
</tr>
<tr>
<td>6</td>
<td>Large excess</td>
<td>20</td>
<td>1 h + 2 h</td>
<td>Compound 50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>No isolated, but identified by high-resolution mass spectrometry.

The reaction was first conducted at -20 °C for 1 h to complete the de-oxygenation reaction at the 3'-position, then the temperature was slightly increased and methanol was added so that the carbonyl group at the 4'-position can be reduced to the alcohol. The results indicated that four equivalents of samarium iodide are insufficient to reduce the ketone. Adding more reagents helped complete the conversion, but the yield was not optimal because compound 50, generated from decomposition of ring I, was found after addition of methanol. The reaction time of the reduction was also important. It required at least two hours for the reduction to complete, otherwise ketone 51 and alcohol 50 were the dominant products. The amount of methanol added was critical to achieve the correct product. Lower amounts of methanol (5 equivalents) lead to low concentrations of protons and incomplete reduction of the 4'-ketone; meanwhile more
methanol (20 equivalents) increased the reactivity of samarium iodide and resulted in the ring I degradation product 50 becoming major. In conclusion, the best conditions were using six equivalents of samarium iodide, first reacting at -20 °C for one hour, then addition of 10 equivalents of methanol with stirring for another two hours at 0 °C, which afforded the desired product in a modest 35% yield.

3.4 Synthesis of 3’-deoxy ribostamycin 52

To test whether the same de-oxygenation method could be applied to other aminoglycosides, 3’-deoxy ribostamycin 52 was chosen as a second target. Starting from commercially available ribostamycin 7, one equivalent of N-(benzyloxycarbonyloxy)succinimide and potassium carbonate were used to selectively protect the 6’-amino group, as this is the only primary amine group in the molecule and is the most reactive one. Without further work up and purification, imidazolesulfonyl azide hydrochloride salt, potassium carbonate and a catalytic amount of copper(II) sulfate were added into the reaction mixture to give the protected ribostamycin derivative 53 in 38% overall yield. Treatment of 53 with freshly distilled benzaldehyde in the presence of boron trifluoride diethyl etherate generated intermediate 54 with the 4’,6’-benzylidene aminal group and a 2”,3”-benzylidene acetal in 45% yield. All remaining free hydroxy groups were protected as benzyl ethers using benzyl bromide, sodium hydride and a catalytic amount of tetrabutylammonium iodide in 62% yield. The fully protected ribostamycin analog 55 was treated with trifluoroacetic acid at low temperature to hydrolyze the 4’,6’-benzylidene aminal and
provide alcohol 56 in 71% yield. A similar protocol to that developed for 3’-deoxy paromomycin 41 outlined in Scheme 5 was applied to convert the amine protecting groups from azides to benzylcarbamates. After the conversion, alcohol 57 was oxidized to ketone 58 with Dess-Martin periodinane in 62% yield, which was treated with samarium iodide under the optimized conditions to form the protected 3’-deoxy ribostamycin analog 59 in 38% yield. De-protection of 59 by hydrogenolysis using palladium hydroxide as catalyst gave the final product 52 (Scheme 6).
Scheme 6. Synthesis of 3'-deoxy ribostamycin 52
3.5  **Synthesis of 3′-deoxy neomycin 60**

3′-Deoxy neomycin 60 was the third target. Based on the previous experience, using neomycin 5 as starting material was the first option. However, compared with ribostamycin 7, neomycin 5 has two primary amino groups at the 6′- and 6″′-positions. The selective protection strategy that worked on ribostamycin 7 gave a mixture of N6′-benzyl carbamate, N6″′-benzyl carbamate and N6′,N6″′-bis(benzyl carbamate) derivatives when applied to neomycin 5, which was difficult to purify and led to a low yield of the desired product. Thus, paromomycin 6 was chosen as alternative starting material to synthesize the target compound. Neomycin derivative 61, in which the secondary hydroxy group at the 4′-position was exposed for further modification, was prepared from paromomycin 6 following a literature protocol.\(^{40,154}\) Staudinger reaction was applied to alcohol 61 to reduce all the azide groups, then the amines were all protected by benzylcarbamates by treatment with excess of N-(benzyloxy carbonyloxy)succinimide and potassium carbonate. The two step reaction gave alcohol 62 in 85% yield. Oxidation of 62 using Dess-Martin periodinane generated ketone 63 in 79% yield, which was subjected to the optimized samarium iodide reaction and provided the protected 3′-deoxy neomycin 64 in 31% yield. The target molecule 60 was obtained by hydrogenolysis of 64 over palladium hydroxide on carbon in the final de-protection step (Scheme 7).
Scheme 7. Synthesis of 3'-deoxy neomycin 60

3.6 Biological evaluations

All the 3'-deoxy 4,5-AGA derivatives were submitted to the Böttger group in Zurich, where they were tested for antiribosomal selectivity (Table 5) and antibacterial activity (Table 6), together with the parents paromomycin 6, neomycin 5, and ribostamycin 7. The methods were the same as outlined in chapter two.
Table 5. Antiribosomal activities of compound 41, 52 and 60 (IC$_{50}$, μg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$/ (μg/mL)</th>
<th>Bacterial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paromomycin 6</td>
<td>0.03</td>
<td>50.6</td>
</tr>
<tr>
<td>3'-Deoxy paromomycin 41</td>
<td>0.04</td>
<td>109.0</td>
</tr>
<tr>
<td>Ribostamycin 7</td>
<td>0.04</td>
<td>203.9</td>
</tr>
<tr>
<td>3'-Deoxy ribostamycin 52</td>
<td>0.05</td>
<td>163.2</td>
</tr>
<tr>
<td>Neomycin 5</td>
<td>0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>3'-Deoxy neomycin 60</td>
<td>0.02</td>
<td>2.6</td>
</tr>
</tbody>
</table>

All three 3’-deoxy modified 4,5-AGA derivatives showed similar activity against the bacterial ribosome as their parents. For 3’-deoxy paromomycin 41, the ribosomal selectivity was increased because its activity against mitochondrial ribosome (Mit13), the deafness mutation ribosome (A1555G) and the cytosolic ribosome (Cyt14) were all decreased. On the other hand, compared with their parents, 3’-deoxy ribostamycin 52 and 3’-deoxy neomycin 60 both exhibited a loss of selectivity in the case of mitochondrial ribosome (Mit13) and cytosolic ribosome (Cyt14), while their selectivity to the deafness mutation ribosome were almost the same. Unlike compound 41 and compound 52, 3’-deoxy neomycin 60 was strongly active against the Mit13 hybrid ribosome and the A1555G hybrid ribosome, which indicated that this compound might cause a severe ototoxic problem.
<table>
<thead>
<tr>
<th>Compound</th>
<th>MRSA</th>
<th>E coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG0 38</td>
<td>AG0 39</td>
<td>AG0 42</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>4</td>
<td>≥25</td>
<td>256</td>
</tr>
<tr>
<td>3’-Deoxy paromomycin 41</td>
<td>4</td>
<td>&gt;12</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Ribostamycin 7</td>
<td>4-8</td>
<td>&gt;12</td>
<td>&gt;12</td>
</tr>
<tr>
<td>3’-Deoxy ribostamycin 52</td>
<td>8-1</td>
<td>&gt;12</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Neomycin 5</td>
<td>0.5-1</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>3’-Deoxy neomycin 60</td>
<td>1-2</td>
<td>64-128</td>
<td>64</td>
</tr>
</tbody>
</table>

The antibacterial activity results revealed that for the MRSA and E. coli strains that were tested, the 3’-deoxy modification did not change the antibacterial activity compared with their parents. For P. aeruginosa, compound 41, 52 and 60 all showed activity against some strains that were not susceptible to their mother compounds, which suggested that they were able to circumvent AGA resistance mechanisms. 3’-Deoxy paromomycin 41 and 3’-deoxy ribostamycin 52 were screened for their antibacterial activities against engineered E. coli that expressed different AMEs (Table 7), while 3’-deoxy neomycin 60 was not submitted into this test as it was considered ototoxic.
Table 7. Antibacterial activities of compound 41 and 52 against engineered strains of E. coli carrying specific resistant determinants (MIC, μg/mL)

<table>
<thead>
<tr>
<th>Strains</th>
<th>AG006</th>
<th>AG007</th>
<th>AG008</th>
<th>AG009</th>
<th>AG036</th>
<th>AG037</th>
<th>AG103</th>
<th>AG105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance mechanism</td>
<td>-</td>
<td>AAC(3)</td>
<td>ANT(2'')</td>
<td>AAC(6')</td>
<td>ANT(4',4'')</td>
<td>APH(3',5'')</td>
<td>armA</td>
<td>AAC(2')</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>2</td>
<td>8</td>
<td>4-8</td>
<td>4-8</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>4</td>
<td>2-4</td>
</tr>
<tr>
<td>3'-Deoxy paromomycin 41</td>
<td>1-2</td>
<td>4-8</td>
<td>2</td>
<td>8</td>
<td>128</td>
<td>&gt;128</td>
<td>8</td>
<td>2-4</td>
</tr>
<tr>
<td>3'-Deoxy ribostamycin 52</td>
<td>2-4</td>
<td>4</td>
<td>1</td>
<td>128</td>
<td>64</td>
<td>&gt;128</td>
<td>4</td>
<td>2-4</td>
</tr>
</tbody>
</table>

Compound 41 and 52 were both active against the wild type AG006, and the strains that expressed AAC(3) (AG007), ANT(2'') (AG008), armA (AG103), and AAC(2') (AG105) resistance mechanisms, which were similar to the case of paromomycin 6. However, all three compounds were deactivated by ANT(4',4'') (AG036) as well as APH(3',5'') (AG037). 3'-Deoxy ribostamycin 52 was also suffered from a loss of activity to AG009 strains, which contained the AAC(6') resistance mechanism and was susceptible to paromomycin 6 and 3'-deoxy paromomycin 41.

3.7 Discussion

In this chapter, two different methods to synthesize 3'-deoxy paromomycin 41 were established. The second method, which utilized samarium iodide reduction as a key reaction, was proven to be useful in different aminoglycosides. Even though the overall yield was only modest, it provided a possible way to effectively achieve 3'-deoxy modification on complex aminoglycoside molecules. 3'-Deoxy ribostamycin 52 and
3'-deoxy neomycin 60 were also synthesized through this samarium iodide reduction method. All the 3'-deoxy modified 4,5-AGAs were tested for their antiribosomal activities and antibacterial activities, and some of the results were promising. In general, the 3'-deoxy modification did not cause a reduction of activity against the bacterial ribosome, which suggested that the 3'-hydroxy group is not so critical for binding to the decoding A-site of bacteria. For the selectivity results, 3'-deoxy paromomycin 41 was more selective toward the bacterial ribosomal A-site, while 3'-deoxy ribostamycin 52 and 3'-deoxy neomycin 60 suffered a slight loss in their selectivity. The selectivity difference may be due to the different substitution groups at the 6'-position (hydroxyl group on paromomycin versus amino group on neomycin and ribostamycin). 3'-Deoxy neomycin 60 was considered ototoxic because it exhibited good activity toward the mitochondrial ribosome and the deafness mutation ribosome. The reason for this is that compound 60 has an extra amine group, as its parent neomycin 5, which helps the molecule bind more tightly not only to the bacteria decoding A-site, but also to the A-sites of mitochondrial and deafness mutation, by providing stronger electrostatic interaction under physiological conditions through protonation. The 3'-deoxy modification does not affect antibacterial activity, as compounds 41, 52 and 60 showed similar activity against MRSA and E. coli compared with their parents. What is more, 41, 52 and 60 were all active against P. Aeruginosa strains that were not susceptible to paromomycin 6, ribostamycin 7 and neomycin 5, supporting the hypothesis of restoring 4,5-aminoglycoside activity against bacteria strains that carry APH(3') resistance.
mechanism by 3’-deoxy modification. A further antibacterial experiment was undertaken to test the effects of different AMEs on compound 41 and 52. 3’-Deoxy ribostamycin 52 was affected by AAC(6’) due to the 6’-amino group. Beside that, two 3’-deoxy 4,5-AGA derivatives did not show significant changes in antibacterial activities against the engineered E. coli strains. It is predictable that all the compounds were deactivated by ANT(4’,4’’) as the 4’-position of these compounds are exposed to be modified. But the effect of APH(3’,5’’) on the 3’-deoxy 4,5-AGA analogs suggests that 5’’-position is also an important target of this enzyme and only modifying 3’-position is insufficient to completely circumvent this resistance mechanism. In order to develop a molecule that can active against all different APH(3’), additional modification at the 5’’-position of the 3’-deoxy 4,5-AGA series is required.
CHAPTER 4. 3’,5’'-DOUBLE MODIFICATION OF PAROMOMYCIN

4.1 Rationale

The 3’-deoxy paromomycin 41 (Figure 26) showed enhanced selectivity toward the bacterial decoding A-site without losing antiribosomal and antibacterial activity, which made it a good substrate for further modification. A modification at the 5’’-position was decided for two main reasons. First, the 5’’-hydroxy group serves as hydrogen bond donor and makes a hydrogen bond with N-7 of the A1491G residue (Figure 10b), which is important for the binding of 4,5-AGAs. Some studies also hypothesize that the 5’’-hydroxy group forms an intermolecular hydrogen bond with the protonated 2’-amine group to enforce the correct conformation for binding with bacterial ribosome (Figure 10b).138,171 Second, the 5’’-hydroxy group can be modified by AMEs. The highly disseminated APH(3’)-IIIa has the capability to phosphorylate the 5’’-position, in addition to the normal 3’-position. Thus APH(3’)-IIIa deactivated 3’-deoxy 4,5-AGAs that have a 5’’-hydroxy group as for example lividomycin A 35 (Figure 26).172 The APH(3’)-IIIa is also able to di-phosphorylate neomycin 5 and butirosin 36 (Figure 26), which have free 3’- and 5’’-hydroxy groups.173-174 In summary, in order to circumvent the action of the widespread APH(3’)-IIIa, 3’,5’’-double modified 4,5-aminoglycosides are needed.
Figure 26. **Some known AGAs that are deactivated by APH(3')-IIIa**

Several attempts have been made to modify the 5″-position of 4,5-AGAs. The 5″-deoxy paromomycin 65, 5″-O-alkyl paromomycin 66, and 5″-deoxy-5″-fluoro paromomycin 67 derivatives suffer a significant loss of antibacterial activity (Figure 27). Similar results also appear in the cases of the 5″-carboxylic acid paromomycin derivative 68 and 5″-carboxamide paromomycin derivative 69 (Figure 27).

**Figure 27. Inactive modifications at 5″-position of 4,5-aminoglycosides**

In contrast, 5″-deoxy-5″-amino modified 4,5-aminoglycosides 70, 71 and 72 retain or even have increased antibacterial activity compared with their parents (Figure 28). These results indicate that the presence of a hydrogen bond donor substituent at the 5″-position is critical for binding to the bacterial ribosomal A-site. However, the introduction of the amine group results in a loss of selectivity toward the bacterial ribosome and an increase toxicity, because the extra primary amine group is protonated under physiological conditions and provides stronger electrostatic
attraction. Simple peptide analogs of 3’-deoxy-5”-amino neomycin 71 (compound 73), neomycin–anthroquinone conjugate 74, and neomycin dimer 75 that are linked at the 5”-position through urea or thiourea linkages also exhibited good antibacterial activity (Figure 28).

![Chemical structures](image)

**Figure 28. Active modifications at 5”-position of 4,5-aminoglycosides**

Based on these observations, the Crich laboratory recently discovered that 5”-deoxy-5”-formamido paromomycin 76 (Figure 29) retains the antibacterial activity of the parent but also shows increased selectivity. Accordingly, in combination with the samarium iodide reduction method developed in chapter three, 3’,5’-dideoxy-5”-formamido paromomycin 77 (Figure 29) was targeted for synthesis and screening for antiribosomal activity and antibacterial activity.
4.2 Chemistry

The 3',5''-dideoxy-5''-formamido paromomycin derivative 77 was prepared from intermediate 78, which was readily obtained from paromomycin 6 by following literature protocol. Selective silylation at the 5''-position with one equivalent of triisopropylsilyl triflate in the presence of 2,6-lutidine gave silyl ether 79 in 63% yield, in which all hydroxy groups were protected as benzyl ethers using excess of benzyl bromide, sodium hydride and a catalytic amount of tetrabutylammonium iodide in 86% yield. When the fully protected intermediate 80 was obtained, the 5''-position was modified first, followed by modification of the 3'-position. Trimethylphosphine and 0.1M sodium hydroxide were used to reduce the azide groups of compound 80 to amines, which were reacted with N-(benzylxoycarbonyloxy)succinimide and potassium carbonate to afford the protected paromomycin derivative 81 in 86% yield over two steps. The silyl ether was hydrolyzed by treatment with tetrabutylammonium fluoride, and alcohol 82 was generated in 64% yield. The 5''-hydroxy group was first tosylated with p-toluenesulfonyl chloride in the presence of triethylamine and a catalytic amount of 4-dimethylaminopyridine, and then substituted by an azido group using sodium azide. The azido intermediate 83 was achieved in 88% yield over two steps, after which
Staudinger reaction, and freshly prepared formic acetic anhydride were applied sequentially to give the corresponding formamide 84 in 67% yield over two steps. Alcohol 85 was acquired in 75% yield by treating compound 84 with sodium cyanoborohydride and 2 M hydrogen chloride solution in diethyl ether. Oxidation of 85 with Dess-Martin periodinane gave the corresponding ketone 86 in 67% yield, to which the optimized samarium iodide reduction conditions discussed in chapter three were applied and gave the protected 3’,5’-dideoxy-5’-formamido paromomycin derivative 87 in 30% yield. Global de-protection by hydrogenolysis, followed by purification on Sephadex and lyophilization then afforded the final product 77 (Scheme 8).
Scheme 8. Synthesis of 3',5''-dideoxy-5''-formamido paromomycin 77
Another approach, in which the modification of 5''-position was undertaken after the 3'-deoxy modification, was also examined for comparison purposes. Since the common regioselective 4',6'-O-benzylidene acetal hydrolysis reaction that provides the secondary alcohol at 4'-position takes place in strong acidic conditions, which silyl ether at 5''-position could not survive, the alternative reagent combination of borane trimethylamine, copper(II) triflate and a catalytic amount of triflic acid was utilized and alcohol 88 was formed in 35% yield. The two step protocol outlined in Scheme 8, which transformed the azides into N-benzylcarbamates, was applied to alcohol 88 and provided intermediate 89 in 79% yield. Ketone 90 was obtained by treatment of 89 with Dess-Martin periodinane in 75% yield, and was subjected to the samarium iodide reduction to cleave the 3'-O-benzyl group. The samarium iodide reduction gave alcohol 91 in 35% yield, from which the silyl ether at the 5''-position was hydrolyzed using tetrabutylammonium fluoride giving the 4',5''-diol intermediate 92 in 75% yield. The primary hydroxy group at the 5''-position was selectively tosyalted in 68% yield by treatment with p-toluenesulfonyl chloride and pyridine. Heating with sodium azide to displace the 5''-tosyl group gave only a low yield of the desired azide. This could be improved by first protecting the 4'-hydroxy group of compound 93 as a trimethylsilyl ether using hexamethyldisilazane, and then reacting with lithium azide and finally washing with 1 M hydrochloric acid to hydrolyze the trimethylsilyl ether protecting group after the reaction was completed. Intermediate 94 was treated with trimethylphosphine, 0.1 M sodium hydroxide and freshly prepared formic acetic
anhydride to give compound 87 in 75% yield, to which same de-protection protocol outlined in Scheme 8 was applied and the final product 77 was achieved (Scheme 9).

Scheme 9. Alternative synthesis of 3',5"'-dideoxy-5"'-formamido paromomycin 77
4.3 Biological evaluations

Compound 77 was evaluated for antiribosomal activity and antibacterial activity by the Böttger group in Zurich using the same methods outlined in chapter two. The results were compared with those of the parent paromomycin 6, and two singly modified compounds 3’-deoxy paromomycin 41 and 5″-deoxy-5″-formamido paromomycin 76 and the data are presented in Table 8 and Table 9.

*Table 8. Antiribosomal activity of compound 77 (IC₅₀, µg/mL)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ / (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial</td>
</tr>
<tr>
<td></td>
<td>activity</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>0.03</td>
</tr>
<tr>
<td>3′-Deoxy paromomycin 41</td>
<td>0.04</td>
</tr>
<tr>
<td>5″-Deoxy-5″-formamido paromomycin 76</td>
<td>0.03</td>
</tr>
<tr>
<td>3′,5″-Dideoxy-5″-formamido paromomycin 77</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The cell-free translation assays indicated that the combination of 3′-deoxy and 5″-deoxy-5″-formamido modifications did not cause reduction in the inhibition of the bacterial ribosome. For the mitochondrial ribosome, the IC₅₀ value of compound 77 was bigger than that of paromomycin 6, but smaller than the singly modified compounds 41 and 76. These results indicated that compared with paromomycin 6, the doubly modified compound 77 showed a slight increase of selectivity in the case of mitochondrial binding A-site, but the increase is not as large as for the two singly modified compounds. On the other hand, for the deafness mutation ribosome and
cytosolic ribosomes, compound 77 exhibited highest IC_{50} values, which suggested that it was the most selective compound toward the deafness mutation A-site and cytosolic A-site.

Table 9. Antibacterial activity of compound 77 (MIC, μg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRSA</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG0 38</td>
<td>AG0 39</td>
<td>AG0 42</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
<tr>
<td>3’-Deoxy paromomycin 41</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
<tr>
<td>5’-Deoxy-5’-formamido paromomycin 76</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
<tr>
<td>3’,5’-Dideoxy-5’-formamido paromomycin 77</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
</tbody>
</table>

The antibacterial assays showed that 3’5’-dideoxy-5’-formamido paromomycin 77 was active against two strains of MRSA (AG038 and AG044) and three strains of E. coli that were tested, which was the same as the parent paromomycin 6, and the singly modified compounds 41 and 76. For the P. aeruginosa strains, compound 77 exhibited modest activities against all three strains, while paromomycin 6 and compound 76 were completely inactive and compound 41 was only active against two of them (AG031 and AG032). Since compound 77 retained antibacterial activity against the clinical isolates of E. coli, engineered E. coli strains that contain different resistance mechanisms were used to test the susceptibility of compound 77 to different AMEs, especially to APH(3’)-IIIa.
Table 10. Antibacterial activity of compound 77 against engineered strains of *E. coli* carrying specific resistant determinants (MIC, μg/mL)

<table>
<thead>
<tr>
<th>Strains</th>
<th>AG006</th>
<th>AG009</th>
<th>AG036</th>
<th>AG037</th>
<th>AG103</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance mechanism</td>
<td>-</td>
<td>AAC(6’)</td>
<td>ANT(4’, 4’”)</td>
<td>APH(3’, 5’”)</td>
<td>armA</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>2</td>
<td>4-8</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>4</td>
</tr>
<tr>
<td>3’-Deoxy paromomycin 41</td>
<td>1-2</td>
<td>8</td>
<td>128</td>
<td>&gt;128</td>
<td>8</td>
</tr>
<tr>
<td>5’’-Deoxy-5’’-formamido paromomycin 76</td>
<td>1-2</td>
<td>-</td>
<td>-</td>
<td>128</td>
<td>-</td>
</tr>
<tr>
<td>3’,5’’-Dideoxy-5’’-formamido paromomycin 77</td>
<td>2</td>
<td>8</td>
<td>64-128</td>
<td>4</td>
<td>32</td>
</tr>
</tbody>
</table>

Paromomycin 6, two singly modified derivatives 41 and 76, and the double modified compound 77 were screened for their antibacterial activities against engineered *E. coli* and the results are presented in Table 10. All four compounds shared similar activity against the parent wild type AG006 strain and the AG009 strain that expressed AAC(6’). In the meantime, all the compounds were deactivated by the ANT(4’, 4”') resistance mechanism. Excitingly, 3’,5’’-dideoxy-5’’-formamido paromomycin 77 exhibited good activity against the AG037 strain that contains the APH(3’, 5’”) resistance mechanism, which affects not only the parent paromomycin 6, but also the singly modified compounds 41 and 76. Another interesting discovery was that compound 77 exhibited reduced activity against the AG103 strain that expressed the armA resistance mechanism whereas neither paromomycin 6 nor 3’-deoxy paromomycin 41 were affected by this mechanism.

4.4 Discussion

In this chapter, the synthesis of 3’,5’’-dideoxy-5’’-formamido paromomycin 77 was discussed. The samarium iodide reduction outlined in chapter three was utilized to
achieve 3'-deoxyxygenation, while 5''-modification was obtained by tosylation followed by azide displacement, reduction of the azide, and treatment with formic acetic anhydride. Two different modification schemes were carried out for comparison purpose. In the first one the 5''-position was modified before the 3'-position, while the second one reversed the sequence. The first route required ten steps starting from the common intermediate 80 and gave the final product in an overall yield of 2.4%. In comparison, the second route needed an extra step to protect the hydroxy group at the 4'-position while modifying the 5''-position, and the overall yield was only 0.9%. The major difference came from the regioselective cleavage of the benzylidene acetal at the 4',6'-position. The commonly used sodium cyanoborohydride conditions that expose the secondary hydroxy group at the 4'-position selectively require the use of strong acid, which was not compatible with the second route because of the silyl ether group at 5''-position. The yield of the alternative reaction was unsatisfactory and decreased the overall efficiency of the second route.

Most bioactivity data indicated that the doubly modified paromoycin analog 77 was a promising compound. The combination of 3'-deoxy and 5''-deoxy-5''-formamido modifications into a single compound did not affect the affinity of compound 77 toward the bacterial ribosomal A-site, which was the same as that of the two singly modified compounds 41 and 76. What is more, compound 77 showed increased selectivity toward the cytosolic ribosomal A-site and the deafness mutation ribosomal A-site. For mitochondrial ribosomal A-site, a reduction of selectivity of compound 77 was observed
compared with compound 41 and 76, but compound 77 was still more selective than paromomycin 6. The antibacterial activity of compound 77 against the clinical isolates MRSA and E. coli was the same as that of the parent and the two singly modified compounds. Compound 77 also exhibited modest activities against all three P. aeruginosa strains tested, which was important because these strains are not susceptible to paromomycin 6 and 5''-deoxy-5''-formamido paromomycin 76, while 3'-deoxy paromomycin 41 was only active against two of them (AG031 and AG032). These results indicated that the doubly modified paromomycin derivative 77 is a better antibiotic compared with two singly modified compounds 41 and 76. Further experiments on the engineered E. coli strains demonstrated the efficacy of the modifications made on paromomycin 6 in overcoming different resistance mechanisms. The doubly modified compound 77, as well as paromoycin 6 and two singly modified compound 41 and 76, inhibited the growth of bacteria that expressed AAC(6'), which would be expected because all these compounds are paromomycin or its analogs and do not have a 6'-amino group. Deactivation of all the tested compounds by ANT(4', 4'') was also predictable as the 4'-hydroxy group of these compounds is available for modification by this enzyme. The most valuable result was the demonstration that the 3',5''-double modified paromomycin derivative 77 showed good activity against bacteria strains that carried the APH(3', 5'') resistance mechanism, which affected not only paromomycin 6, but also 3'-deoxy paromomycin 41, and 5''-deoxy-5''-formamido paromomycin 76. An unexpected result was the loss of activity in the presence of the
armA resistance mechanism (AG103). This mechanism is not supposed to deactivate 4,5-AGAs, as is demonstrated for paromomycin 6 and 3’-deoxy paromomycin 41. However, the activity of compound 77 against the AG103 strain was eight times less than that of paromomycin 6. Perhaps, modifying the 3’-position and 5”-positions at the same time may change the binding mode of compound 77, making rings III and IV closer to the G1405 residue. Despite this setback, 3’,5”-dideoxy-5”-formamido paromomycin 77 was proved to circumvent the APH(3’)-IIIa mechanism, which makes it a good drug candidate and a good substrate for further modification.
CHAPTER 5. 3’, 4’, 5’’-TRIPLE MODIFICATION OF PAROMOMYCIN

5.1 Rationale

The 3’, 5’’-dideoxy-5’’-formamido paromomycin 77 exhibited good activity toward the bacterial ribosome, as well as good ribosomal selectivity and antibacterial activity. The antibacterial experiments against engineered E. coli strains proved that this compound was active against bacteria that contain the APH(3’)-IIIa resistance mechanism as expected. However, compound 77 still suffered from susceptibility to ANT(4’, 4’’), making further modification necessary.

The 4’-hydroxy group forms a hydrogen bond with the phosphate oxygen of the A1493 residue of the ribosomal A-site of bacteria (Figure 10b), which makes it critical for AGA binding and a target for AMEs. Aminoglycoside nucleotidyltransferases (ANTs) are AMEs that can catalyze an O-adenylation reaction between ATP and aminoglycoside in the presence of Mg^{2+} ions.\textsuperscript{17} ANT(4’) has been found in a variety of bacteria and produces resistance to a broad array of aminoglycosides such as amikacin 4, tobramycin 11 and kanamycin 2 by O-adenylation of the 4’-hydroxy group.\textsuperscript{109}

Several attempts have been made to modify the 4’-position of 4,5-AGAs in order to block the ANT(4’) resistance mechanism, and to increase the selectivity for the bacterial ribosome thereby alleviating the toxicity problems. 4’-Deoxy neomycin 95 (Figure 30) maintains the intrinsic activity of neomycin 5, while its activity against various strains of E. coli and P. Aeruginosa, which are known to produce inactivating enzymes, is improved.\textsuperscript{182} The replacement of the 4’-hydroxy group of neomycin 5 with
fluorine (Figure 30, 96) increases the potency against aminoglycoside susceptible bacterial strains and leads to the evasion of ANT(4').40 4’-O-Aralkyl (Figure 30, 97), as well as 4’-O-alkyl (Figure 30, 98) modifications of paromomycin 6 confer substantial antibacterial activity in vitro and in vivo, accompanied by little hearing loss or morphological cochlear damage.146-147 The 4’-O-β-D-xylopyranosyl paromomycin 99 (Figure 30) inhibits the bacterial ribosome comparably to paromomycin 6, but is significantly more selective. Meanwhile, it retains activity against MRSA strains that are resistant to paromomycin 6, which is a consequence of 4’-O-glycosylation blocking the action of ANT(4’).183

Figure 30. Some 4’-modified 4,5-aminoglycosides derivatives

4’-Deoxy-4’-C-propyl paromomycin 100 (Figure 31) is a compound developed by the Crich laboratory that increases both activity and selectivity against the bacterial ribosome compared with the parent paromocycin 6. The absence of the 4’-hydroxy group also makes it not susceptible for the ANT(4’) resistance mechanism. In this chapter, the synthesis, as well as the bioactivity data, of triply modified paromomycin derivative 101 (Figure 31), combining with 3’-deoxy, 4’-deoxy-4’-C-propyl and 5’’-deoxy-5’’-formamido modifications are presented.
5.2 Direct synthesis from paromomycin 6 using samarium iodide reduction

An effective procedure was developed by the Crich laboratory to synthesize 4'-deoxy-4'-C-propyl paromomycin 100 starting from paromomycin 6. One of the key steps in this procedure installed an equatorial C-allyl group at the 4'-position (104) through a radical reaction between the axial iodide 103, which was generated from alcohol 102, and (allylsulfonyl)benzene in the presence of triethylborane and air (Scheme 10).

Scheme 10. The key reactions to synthesize 4'-deoxy-4'-C-propyl paromomycin 100

The same strategy was applied to intermediate 87, which was obtained as outlined in chapter four. Unfortunately, treatment of 87 with triflic anhydride and pyridine followed by S_N2 substitution with sodium iodide did not afford the desired axial iodide intermediate 105. The major product observed in this reaction had a molecular weight that was eighteen amu less than the expected product, possibly as a
result of dehydration of the formamide group at the 5''-position (106, Scheme 11).

Instead of triflation, tosylation and mesylation at the 4'-position were also tested, but the displacement with iodide did not proceed even under heating conditions. Intermediate 91 was also subjected to the iodide installation protocol. Even though the axial iodide intermediate 107 was observed, the overall yield was too low to enable the necessary subsequent modifications at the 4'-position and 5''-position (Scheme 11).

Scheme 11. Attempted iodination at the 4'-position of compound 87 and 91

Eventually, a glycosylation strategy, which was also developed by the Crich laboratory in order to synthesize 3',4'-dideoxy-4'-C-propyl paromomycin 110 using donor 108 and acceptor 109 (Scheme 12), was utilized as alternative approach to complete the synthesis of the required paromomycin derivative 101.

Scheme 12. Synthesis of compound 110 through glycosylation
5.3 Donor synthesis

The Crich laboratory developed the synthetic method for donor 108 when compound 110 was synthesized. A literature protocol was followed to prepare intermediate 115 from commercially available levoglucosan 111.184 Two equivalents of p-toluenesulfonyl chloride were used to selectively tosylate positions 2 and 4 of levoglucosan 111 in the presence of pyridine at low temperature to achieve intermediate 112, which was treated with sodium methoxide to give epoxide 113 in 68% yield over two steps. The allyl Grignard reagent was applied and an allyl group was installed at the 4-position with epoxide ring opening in 49% yield. Alcohol 114 then was reacted with sodium hydride and epoxide 115 was formed in 83% yield. Heating with benzylamine opened the epoxide ring of intermediate 115 and the 2-benzylamino substituent was introduced in 67% yield. Hydrogenolysis over palladium on carbon, followed by treatment with benzyl chloroformate and sodium carbonate afforded intermediate 117 from compound 116 in 74% yield over two steps. Alcohol 117 was first treated with sodium hydride, and then reacted with carbon disulfide and methyl iodide to form the xanthate 118 in 93% yield, which was subjected to radical de-oxygenation with azobisisobutyronitrile as radical initiator and tris(trimethylsilyl)silane as hydrogen radical donor in benzene at reflux. The de-oxygenation product 119 was obtained in 84% yield from this reaction. The carboxybenzyl group was removed by hydrogenolysis, and then was followed by treatment with imidazolesulfonyl azide hydrochloride salt, potassium carbonate and a
catalytic amount of copper(II) sulfate, when azide 120 was formed in 78% yield over two steps. Preparation of intermediate 121 was completed by anomeric sulfide formation reaction using trimethyl(phenylthio)silane and zinc(II) iodide, and basic work up with potassium carbonate in 83% yield. A small adjustment was made in order to take advantage of the synthetic intermediate outlined in previous chapter and simplify the de-protection step. Instead of installing an ester, the 6-hydroxy group of compound 121 was protected as a benzyl ether in the presence of sodium hydride, benzyl bromide and tetrabutylammonium iodide. Finally, oxidation of intermediate 122 using L-selectfluor gave the glycosyl donor 123 in 72% yield over two steps (Scheme 13).

**Scheme 13. Synthesis of the glycosyl donor 123**
5.4 Acceptor synthesis

The straightforward synthesis of a suitable glycosyl acceptor has been described in the literature.\textsuperscript{186-187} The strategy is constituted of regioselective protection of four amine groups in paromomycin 6 followed by diazotization at the 2''-position with sodium nitrite in aqueous acetic acid to degrade the ring I (Scheme 14). However, this synthesis does not permit selective functionalization of the 5''-position as needed for the target compound 101. Therefore an alternative method developed in the Crich laboratory for the cleavage of the paromomycin ring I under mild conditions was employed.

\textbf{Scheme 14. Literature protocol to degrade ring I of paromomycin 6}

Compound 80, discussed in chapter four, was treated with \textit{p}-toluenesulfonic acid in order to hydrolyze the benzylidene acetal. But this was accompanied by the hydrolysis of the 5''-silyl ether group giving the undesired triol product 127. Fortunately, heating with iodine in methanol converted intermediate 80 into diol 128 in
53% yield. Ring I then was degraded to glycosyl acceptor 129 by sequential treatment with Dess-Martin periodinane, 3-chloroperbenzoic acid and 3M sodium hydroxide in 50% yield (Scheme 15).

Scheme 15. Synthesis of the glycosyl acceptor 129

The proposed mechanism for the degradation of ring I by this protocol is shown in Figure 32. The 4’- and 6’-hydroxy groups of compound 128 were oxidized to the corresponding ketone and aldehyde (130) using the Dess-Martin periodinane, after which a hydroxy group was installed at the 5’-position on treatment with 3-chloroperoxybenzoic acid through a Rubottom oxidation-type reaction. An unstable hemiacetal 131 was formed and decomposed easily under basic conditions resulting in degradation of ring I (129).
5.5 Glycosylation and 5''-modification

Triflic anhydride was used to activate glycosyl donor 123 in the presence of 2,4,6-tri-tert-butylpyrimidine, 4Å molecular sieves and cyclohexene at low temperature in dry dichloromethane\textsuperscript{199}. The addition of glycosyl acceptor 129 gave the glycosylation product 132, with only the α-isomer observed. Hydrolysis of the triisopropylsilyl ether protecting group at the 5''-position using tetrabutylammonium fluoride afforded alcohol 133 in 36% yield over two steps (Scheme 16). Two different synthetic routes were tried to access the 5''-deoxy-5''-formamido modification. The first route included azide displacement at the 5''-position followed by reduction and formylation, as discussed in chapter four. In order to avoid side reactions caused by the other azide groups, intermediate 133 was treated with trimethylphosphine and 0.1 M sodium hydroxide, and then N-(benzyloxycarbonyloxy)succinimide and potassium carbonate were added to form alcohol 134 in 74% yield. A tosyl group was installed at the 5''-position using p-toluenesulfonyl chloride, triethylamine and a catalytic amount of
4-dimethylaminopyridine, and then displaced by an azido group using sodium azide. The azide intermediate **135**, obtained in 79% yield, was subjected to the Staudinger reaction to afford amine **136**. Unfortunately, formylation of amine **136** with formic acid, acetic anhydride or \(N\)-(diethylcarbamoyl)-\(N\)-methoxyformamide generated two inseparable isomers, both of which had the correct molecular weight according to mass spectrometer (Scheme 16). The \(^1\)H-NMR spectra of these isomers were unresolvable due to the presence of multiple carboxybenzyl groups, such that these two isomers remain unidentified.
Scheme 16. Glycosylation and the first approach to modify the 5''-position

In the light of this failure, potassium phthalimide, instead of sodium azide, was used to introduce the amino group to the 5''-position. Alcohol 133 was tosylated following the procedure outlined in Scheme 16 in 80% yield. On heating with potassium phthalimide in DMF, the tosyl group was displaced by the phthalimido group and intermediate 139 was formed in 89% yield. Hydrazine was used to hydrolyze the
phthalimido group generating the free amine, which, on treatment with formic acetic anhydride, gave formamide 140 in 60% yield. The final product 101 was obtained by single-step hydrogenolysis de-protection, filtration through Sephadex, and lyophilization (Scheme 17).

Scheme 17. Synthesis of the triply modified paromomycin derivative 101

5.6 Biological evaluations

The triply modified paromomycin derivative 101 was submitted to the Böttger group in Zurich to test for antiribosomal activity and antibacterial activity using the same methods outlined in chapter two. The data are presented in Tables 11 and 12.
together with the parent paromomycin 6, 4'-deoxy-4'-C-propyl paromomycin 100 and 3', 5''-dideoxy-5''-formamido paromomycin 77.

Table 11. Antiribosomal activity of compound 101 (IC50, μg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 / (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial activity</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>0.03</td>
</tr>
<tr>
<td>3',5''-Dideoxy-5''-formamido paromycin 77</td>
<td>0.04</td>
</tr>
<tr>
<td>4'-Deoxy-4'-C-propyl paromycin 100</td>
<td>0.02</td>
</tr>
<tr>
<td>3',4',5''-trideoxy-4'-C-propyl-5''-formamido paromycin 101</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Compared with paromomycin 6, singly modified derivative 100 and doubly modified derivative 77, the triply modified compound 101 exhibited a significant lost of antiribosomal activity. The activity of compound 101 against bacterial ribosomes was seventeen times less than that of the paromomycin 6. The activity of compound 101 toward the mitochondrial ribosome A-site was comparably reduced, which leads similar selectivity compared with paromoycin 6. A slightly increase in selectivity toward deafness mutation ribosomal A-site and cytosolic ribosomal A-site was observed, as the reduction of activity toward bacterial ribosome was smaller than it was toward the A1555G and Cyt14 hybrid ribosomes.
Table 12. Antibacterial activity of compound 101 (MIC, μg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRSA</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG0 38</td>
<td>AG0 39</td>
<td>AG0 42</td>
</tr>
<tr>
<td>Paromomycin 6</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
<tr>
<td>3',5''-Dideoxy-5''-formamido paromomycin 77</td>
<td>4</td>
<td>&gt;12 8</td>
<td>&gt;12 8</td>
</tr>
<tr>
<td>4'-Deoxy-4'-C-propyl paromomycin 100</td>
<td>1-2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3',4',5''-Trideoxy-4'-C-propyl-5''-formamido paromomycin 101</td>
<td>16-32</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

In general, the combination of three different modifications resulted in reduced antibacterial activity; especially compared with the singly modified compound 100. Compound 101 was active against two MRSA strains (AG039 and AG042) that were not susceptible to paromomycin 6 and double modified derivative 77, but still eight times to sixteen times less than that of the singly modified derivative 100. A similar activity loss was also observed in the E. coli and P. aeruginosa strains tested. The lost of antibacterial activity of compound 101 made it unnecessary to test its activity against engineered bacteria strains that express different resistance mechanisms.

5.7 Discussion

In this chapter, two synthetic approaches were tried to synthesize triply modified paromomycin analog 101. The first scheme involved samarium iodide reduction as discussed in chapter three; azide substitution, reduction and formylation at the 5''-position as outlined in chapter four; and a radical alkylation developed by the
Crich laboratory using (allylsulfonyl)benzene. Introduction of an axial iodide to the 4’-position is a prerequisite reaction for the radical alkylation, which did not proceed well in this scheme. The second scheme utilized a glycosylation reaction to construct the modified ring I with glycosyl donor 123 and acceptor 129 synthesized in acceptable yields. Potassium phthalimide, rather than sodium azide, was used to introduce amino group at the 5”-position as it eliminated steps and provided better yields with less side products.

Unfortunately, the combination of 3’-deoxy, 4’-deoxy-4’-C-propyl and 5”-deoxy-5”-formamido modifications into a single molecule caused a significant reduction of activity against the prokaryotic and eukaryotic ribosomes, such that the triply modified compound 101 was not considered as active enough to be useful. The antibacterial activity results provided the same conclusion. Compound 101 showed noticeably decreased activity against clinical isolates of MRSA and E. coli that were tested compared with the parent paromomycin 6, the singly modified compound 100 and the doubly modified compound 77. Although this compound inhibited the growth of AG039 and AG042 strains, which were not susceptible to paromomycin 6 and the doubly modified derivative 77, the activity was much worse than that of the singly modified compound 100. Perhaps the combination of 3’-deoxy, 4’-deoxy-4’-C-propyl and 5”-deoxy-5”-formamido modifications caused a conformational change of the molecule, which destroyed the interaction between the molecule and the binding A-site.

In conclusion, a chemical approach to modify three different positions of
paromomycin 6 was successfully executed, but the unexpected loss of activity in the triply modified paromomycin derivative 101 indicated the 4’-deoxy-4’-C-propyl modification was not compatible with 3’-deoxy-5′-deoxy-5′-formamido double modifications.
CHAPTER 6. OVERALL CONCLUSION

With a goal of developing next generation aminoglycosides that are nontoxic and resistant-proof, the 4,5-series AGAs were selected for modifications by rational design and novel organic synthesis. Several modifications, including single modification at the 6’- and 3’-positions, double modification at the 3’,5”-positions, and triple modification at the 3’, 4’, 5”-positions were made to these AGAs. Cell-free functional ribosomal assays were applied to determine the influences of these modifications on antiribosomal activity and selectivity. Antibacterial experiment against clinical isolates of Methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli and Pseudomonas aeruginosa were also used to test the antibacterial activity of the modified compounds. Furthermore, some of the active compounds were screened against engineered E. coli strains that expressed different AMEs in order to investigate their ability to circumvent common AMEs. Such studies revealed the efficacy of different modifications and ultimately lead to new AGA derivatives, which are resistance-proof and less toxic.

For the 6’-position of paromomycin, the 6’-deshydroxymethyl modification was accomplished through Barton decarboxylation. Unfortunately, this novel paromomycin derivative exhibited a significant loss of antiribosomal activity and antibacterial activity, which means it was not an effective modification. But this newly synthesized compound has the potential to evade the critical NpmA resistance mechanism that could affect all 4,6 and 4,5-AGAs, so further investigations on this compound are still necessary.

For the 3’-position of common 4,5-AGAs, 3’-deoxy paromomycin, 3’-deoxy
ribostamycin and 3’-deoxy neomycin were synthesized through a novel method, which the 3’-deoxy oxygenation modification was achieved by samarium iodide reduction. Even though the overall yield was modest, this novel synthetic method showed good compatibility to the complex AGA molecules. All the 3’-deoxy modified compounds retained their antiribosomal and antibacterial activity and were active *P. Aeruginosa* strains that were not susceptible to their parents. Further biological experiment on 3’-deoxy paromomycin and 3’-deoxy ribostamycin showed that these singly modified compounds were still affected by APH(3’,5”) resistance mechanism, so double modification is required.

For the double modification of paromomycin, a novel 3’,5”-dideoxy-5”-formamido paromomycin derivatives was synthesized. The samarium iodide reduction method showed its wide application potency and helped to achieve the 3’-deoxy modification in the synthetic scheme. The doubly modified compound retained the activity against bacterial ribosomal A-site and increased selectivity toward the cytosolic ribosomal A-site and the deafness mutation ribosomal A-site. It was also active against all three *P. aeruginosa* strains tested in the antibacterial experiment. Further experiments demonstrated that this 3’,5”-double modified paromomycin derivative circumvent the APH(3’,5”) resistance mechanism. All these results showed that 3’,5”-dideoxy-5”-formamido paromomycin can be a good drug candidate and a good substrate for further modification.

3’,4’,5”-Trideoxy-4’-C-propyl-5”-formamido paromomycin were synthesized
through a glycosylation strategy. But this triply modified compound was not considered as active enough to be useful because its activity against the prokaryotic and eukaryotic ribosomes were significantly reduced. The antibacterial experiment against clinical isolates provided same conclusion. These result suggested that the 4'-deoxy-4'-C-propyl modification was not compatible with 3',5''-dIDEOXY-5''-FORMAMIDO double modification.

Overall, several modifications have been made on 4,5-AGAs, including paromomycin, ribosatmycin and neomycin. Some modified compounds show good antibacterial activity and circumvent known AMEs. These results prove that the next generation of AGAs, which are less toxic and resistance-proof, can be developed by rational design and novel organic synthesis.
CHAPTER 7. EXPERIMENTAL SECTION

General experimental:

All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Chromatographic purifications were carried out over silica gel (230 – 400 mesh) and Sephadex C-25. Analytical thin-layer chromatography (TLC) was preformed with pre-coated glass backed plates (w/UV 254). TLC was visualized by UV irradiation (254 nm) or by staining with sulfuric acid in ethanol (20:80, v/v), ceric ammonium molybdate solution [Ce(SO₄)₂: 4 g, (NH₄)₆Mo₇O₂₄: 10 g, H₂SO₄: 40 mL, H₂O: 360 mL] or ninhydrin solution (ninhydrin: 1.5 g, n-butanol: 100 mL, glacial acetic acid: 3 mL). Specific rotations were obtained at 589 nm and 23 °C on an Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ) with a path length of 10 cm. High resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer (Waters LCT Premier Xe TOF mass spectrometer). 1 and 2D ¹H, ¹³C NMR spectra of all compounds were recorded at 600, 500 or 400 MHz instrument as stated.

6,3′,4′,2″,5″,3‴,4‴-Hepta-O-benzyl-1,3,2′,2″″,6″″-pentadecamino-1,3,2′,2″″,6″″-pentaaazido paromomycin (32): Under an argon atmosphere, a soln. of 31 (2.7 g, 2.0 mmol) in dry dichloromethene (55 mL) was cooled to 0 °C, treated with 2 M borane dimethyl sulfide in tetrahydrofuran (9.5 mL, 19 mmol) and 1 M dibutylboron triflate in dichloromethane (1.9 mL, 1.9 mmol), and stirred at room temperature for 4 h. Saturated aqueous sodium bicarbonate was added at 0 °C, and the layers were separated. The
organic layer was washed with brine, dried with sodium sulfate, filtered, and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 4:1) gave 32 as a white solid (2.35 g, 87%). Rf (hexane: ethyl acetate 2:1) 0.50. [α]$_D^{23}$ = +6.4° (c = 1.0, chloroform). $^1$H NMR (500 MHz, chloroform-d) δ 7.52 – 7.03 (m, 35H, aromatic), 6.17 (d, $J_{1′′′-2′′′}$ = 3.6 Hz, 1H, H1′), 5.71 (d, $J_{1′′-2′}$ = 5.6 Hz, 1H, H1′), 5.00 (d, $J$ = 10.6 Hz, 1H, -OCH$_2$Ph), 4.92 (d, $J_{1′′′-2′′′}$ = 1.6 Hz, 1H, H1′′′), 4.90 – 4.81 (m, 3H, -OCH$_2$Ph), 4.74 (d, $J$ = 10.6 Hz, 1H, -OCH$_2$Ph), 4.68 – 4.58 (m, 3H, -OCH$_2$Ph), 4.54 – 4.46 (m, 3H, -OCH$_2$Ph), 4.43 (d, $J$ = 12.0 Hz, 1H, -OCH$_2$Ph), 4.37–4.31 (m, 3H, -OCH$_2$Ph, H3′′, H4′′), 4.27 (d, $J$ = 12.1 Hz, 1H, -OCH$_2$Ph), 4.08 (t, $J_{3′-4′}$ = 10.0 Hz, $J_{3′-4′}$' = 10.0 Hz, 1H, H5′, H5), 3.88 – 3.83 (m, 1H, H5′′), 3.83 – 3.74 (m, 3H, H6′, H3′′′, H5′′′), 3.73 – 3.59 (m, 4H, H6′, H4, H5′′, H6′′′), 3.49 - 3.42 (m, 2H, H1, H3), 3.41 - 3.37 (m, 2H, H4′, H2′′′), 3.32 (t, $J_{6-5}$ = 9.3 Hz, $J_{6-1}$ = 9.3 Hz, 1H, H6), 3.14 (br s, 1H, H4′′′), 2.96 (dd, $J_{2′-3′}$ = 10.0 Hz, $J_{2′-1′}$ = 3.6 Hz, 1H, H2′), 2.89 (dd, $J$ = 13.0, 3.7 Hz, 1H, H6′′′), 2.25 (dt, $J_{2eq-2ax}$ = 12.7 Hz, $J_{2eq-1}$ = 4.6 Hz, $J_{2eq-3}$ = 4.6 Hz, 1H, H2eq), 1.42 (q, $J_{2ax-2eq}$ = 12.7 Hz, $J_{2ax-1}$ = 12.7 Hz, $J_{2ax-3}$ = 12.7 Hz, 1H, H2ax). $^{13}$C NMR (125 MHz, chloroform-d) δ: 138.4 – 136.9 (tertiary aromatic), 128.70 - 127.1 (aromatic), 106.2 (C1′′′), 98.6 (C1′′′′), 95.8 (C1′), 84.2 (C6), 82.5 (C2′′′), 82.1 (C5), 82.0 (C4′′); 79.8 (C3′), 77.6 (C4′), 75.5 (C3′′), 75.4 (PhCH$_2$), 75.0 (PhCH$_2$), 74.9 (C4), 74.7 (PhCH$_2$), 74.4 (C5′′′), 73.2 (PhCH$_2$), 73.2 (PhCH$_2$), 72.8 (C3′′), 72.4 (PhCH$_2$), 71.7 (PhCH$_2$), 71.6 (C5′), 71.5 (C4′′′′), 70.3 (C5′′), 63.2 (C2′), 61.5 (C6′), 60.4 (C3), 60.1 (C1), 57.3 (C2′′′), 51.2 (C6′′′′), 32.5 (C2). ESI-HRMS: m/z calcd for C$_{72}$H$_{77}$N$_{15}$NaO$_{14}$ [M+Na]$^+$
6,3',4',2'',5'',3'',4''-Hepta-O-benzyl-1,3,2',2'',6''-pentadeamino-1,3,2',2'',6''-pentaazido-paromomycin-6'-carboxylic Acid (33): Potassium bromide (18 mg, 0.15 mmol), sodium bicarbonate soln. (5%, 1 mL), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 24 mg, 0.15 mmol) and sodium hypochlorite soln. (5%, 0.3 mL) were added at 0 °C to a stirred soln. of 33 (200 mg, 0.14 mmol) in acetone (4 mL). After stirring for 1 h at 0 °C, additional sodium hypochlorite soln. (5%, 0.6 mL) was added, and the mixture was stirred at room temperature for another 18 h. The reaction mixture was diluted with ethyl acetate, washed with 1 M hydrochloric acid and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 2:1) gave 33 as a light yellow solid (133 mg, 68%). Rf (hexane: ethyl acetate 1:2) 0.60. [α]$_{D}^{23}$ = + 5.9° (c = 1.0, chloroform). $^1$H NMR (500 MHz, chloroform-d$_6$) δ 7.43 – 7.12 (m, 35 H, aromatic), 6.15 (d, $J_{1'\cdot 2'}$ = 3.6 Hz, 1H, H1'), 5.65 (d, $J_{1''\cdot 2''}$ = 5.3 Hz, 1H, H1''), 4.95 (d, $J = 10.7$ Hz, 1H, -OCH$_2$Ph), 4.88 (d, $J_{1''\cdot 2''}$ = 1.5 Hz, 1H, H1'''), 4.86 - 4.78 (m, 3H, -OCH$_2$Ph), 4.77 - 4.68 (m, 3H, -OCH$_2$Ph, H5'), 4.65 (d, $J = 12.0$ Hz, 1H, -OCH$_2$Ph), 4.60 - 4.55 (m, 2H, -OCH$_2$Ph), 4.49 (d, $J = 11.9$ Hz, 1H, -OCH$_2$Ph), 4.46 - 4.42 (m, 2H, -OCH$_2$Ph), 4.37 - 4.26 (m, 4H, -OCH$_2$Ph, H3'', H4''), 4.06 (t, $J_{3''\cdot 2''}$ = 9.5 Hz, $J_{3''\cdot 4''}$ = 9.5 Hz, 1H, H3''), 3.98 - 3.90 (m, 2H, H2'', H5), 3.84 - 3.75 (m, 3H, H5'', H3''', H5'''), 3.74 - 3.68 (m, 2H, H4, H4''), 3.67 - 3.57 (m, 2H, H5'', H6'''), 3.51 - 3.39 (m, 2H, H1, H3), 3.38 - 3.36 (m, 1H, H2''), 3.29 (t, $J_{6.5}$ = 9.5 Hz, $J_{6.1}$ = 9.5 Hz, 1H, H6), 3.19 - 3.13 (m, 2H, H4''', H2'), 2.95 (dd, $J = 12.9, 4.2$ Hz, 1H, H6'''), 2.27
(dt, $J_{2eq-2ax} = 12.7$ Hz, $J_{2eq-1} = 4.3$ Hz, $J_{2eq-3} = 4.3$ Hz, 1H, H2eq), 1.46 (q, $J_{2ax-2eq} = 12.7$ Hz, $J_{2ax-1} = 12.7$ Hz, $J_{2ax-3} = 12.7$ Hz, 1H, H2ax). $^1$C NMR (125 MHz, chloroform-d) δ: 173.12 (COOH), 138.2 - 137.0 (tertiary aromatic), 128.7 - 127.5 (aromatic), 106.2 (C1”), 98.6 (C1””), 96.4 (C1’), 84.0 (C6), 82.2 (C2””), 81.9 (C5), 81.8 (C4”), 79.5 (C3”), 79.4 (C4”), 75.8 (C4), 75.4 (C3”), 75.4 (PhCH2), 75.0 (PhCH2), 74.7 (PhCH2), 74.2 (C5””), 73.3 (PhCH2), 73.2 (C3””), 72.9 (PhCH2), 72.4 (PhCH2), 71.8 (PhCH2), 71.5 (C4””), 70.9 (C5’), 70.0 (C5’”), 62.6 (C2’), 60.4 (C3), 59.7 (C1), 57.3 (C2’”), 51.0 (C6””), 32.2 (C2). ESI-HRMS: m/z calcld for C72H75N15NaO15 [M+Na]+ 1412.5465, found 1412.5470.

6,3’,4’,2”,5”,3’”,4’”-Hepta-O-benzyl-1,3,2’,2’”,6”'-pentadecamino-1,3,2’’,2’”’,6’’'-pentaaazido-6’-deshydroxymethyl paromomycin (34): Under an argon atmosphere and in the dark, a stirred soln. of 33 (140 mg, 0.1 mmol) in dry dichloromethane (3 mL) was cooled to -5 °C, treated with 1-oxa-2-oxo-3-thiaindolizinium chloride (95 mg, 0.5 mmol) and triethylamine (0.11 mL, 0.8 mmol). After stirring for 3 h at -5 °C, tert-dodecylmercaptan (0.11 mL, 0.5 mmol) was added under an argon atomosphere, and the reaction mixture was irradiated with a Utilitech Bright White Spiral CFL Light Bulb (Color temperature: 3500 K; Lumens: 1600; 120 V; 60 Hz; 23 W, 0.308 A) for 1.5 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 4:1) gave 34 as a light yellow solid (83 mg, 62%). $[\alpha]_{23}^{D} = + 6.0^\circ$ (c = 1.3, chloroform). Rf (hexane: ethyl acetate 2:1) 0.24. $^1$H NMR (500 MHz,
chloroform-d) δ 7.45 – 7.06 (m, 35H), 6.09 (d, J$_{1''-2'''}$ = 3.5 Hz, 1H, H1’), 5.66 (d, J$_{1''-2'''}$ = 5.7 Hz, 1H, H1’’), 4.96 (d, J = 10.6 Hz, 1H, -OCH$_2$Ph), 4.90 (d, J = 10.8 Hz, 1H, -OCH$_2$Ph), 4.86 (d, J$_{1''-2'''}$ = 1.5 Hz, 1H, H1’’’), 4.82 (d, J = 10.9 Hz, 1H, -OCH$_2$Ph), 4.74 – 4.67 (m, 2H, -OCH$_2$Ph), 4.66 – 4.61 (m, 2H, -OCH$_2$Ph), 4.59 (d, J = 11.7 Hz, 1H, -OCH$_2$Ph), 4.54 (d, J = 11.9 Hz, 1H, -OCH$_2$Ph), 4.48 – 4.39 (m, 3H, -OCH$_2$Ph), 4.36 – 4.22 (m, 4H, -OCH$_2$Ph, H3’’’, H4’’’), 4.00 (t, J$_{3'-2'}$ = 9.0 Hz, J$_{3'-4'}$ = 9.0 Hz, 1H, H3’), 3.97 – 3.92 (m, 2H, H2’’, H5), 3.86 – 3.73 (m, 4H, H5’, H5’’, H3’’’, H5’’’), 3.70 – 3.59 (m, 3H, H5’, H6’’’, H4), 3.59 – 3.54 (m, 1H, H5’’’), 3.51 – 3.40 (m, 3H, H4’, H1, H3), 3.34 (br s, 1H, 2’’’), 3.29 (t, J$_{6-5}$ = 9.3 Hz, J$_{6-1}$ = 9.3 Hz, 1H, H6), 3.12 (br s, 1H, H4’’’), 3.01 (dd, J$_{2'-3'}$ = 9.0 Hz, J$_{2'-1}$ = 3.5 Hz, H2’), 2.90 (dd, J = 12.9, 3.8 Hz, 1H, H6’’’), 2.26 (dt, J$_{2ax-2eq}$ = 12.7 Hz, J$_{2ax-1}$ = 12.7 Hz, J$_{2ax-3}$ = 12.7 Hz, H2ax). $^{13}$C NMR (125 MHz, chloroform-d) δ: 138.3 – 136.9 (tertiary aromatic), 128.7 – 127.4 (aromatic), 106.0 (C1’’’), 98.6 (C1’’), 95.9 (C1’), 84.2 (C6), 82.5 (C2’’), 82.0 (C5), 81.8 (C4’’’), 79.0 (C3’), 78.0 (C4’), 75.5 (C4), 75.3 (C3’’’), 75.0 (PhCH$_2$), 74.9 (PhCH$_2$), 74.3 (C5’’’), 73.3 (PhCH$_2$), 73.2 (PhCH$_2$), 73.0 (C3’’’), 72.9 (PhCH$_2$), 72.4 (PhCH$_2$), 71.7 (PhCH$_2$), 71.4 (C4’’’), 70.1 (C5’’), 62.9 (C2’), 60.8 (C5’), 60.3 (C3), 60.0 (C1), 57.3 (C2’’’), 51.0 (C6’’’), 32.6 (C2).

ESI-HRMS: m/z calcd for C$_{71}$H$_{75}$N$_{15}$NaO$_{13}$ [M+Na]$^+$ 1368.5566, found 1368.5564.

6’-Deshydromethyl paromomycin pentaacetate salt (30): A stirred soln. of 34 (33 mg 0.024 mmol) in a mixture of (1,4-dioxane/water/acetic acid 1/2/0.2, 0.35 mL) was treated with palladium hydroxide on carbon (33 mg), and stirred under a hydrogen atmosphere (40 psi) at room temperature for 24 h. The mixture was filtered,
concentrated under reduced pressure and purified by Sephadex C-25 column chromatography (0.17% ammonium hydroxide). The product-containing fractions were combined, glacial acetic acid (41 μL, 0.69 mmol) was added, and the mixture was freeze dried to give 11 as a white solid (13.5 mg, 62% as the pentaacetate salt). [α]D23° = +28.8° (c = 0.3, H2O). 1H NMR (500 MHz, D2O) δ 5.26 (d, J1′-2′ = 2.1 Hz, 1H, H1′), 5.17 (d, J1′′-2′′ = 1.5 Hz, 1H, H1′′′), 5.12 (d, J1′''-2′'' = 2.1 Hz, 1H, H1′′′), 4.41 (dd, J3′′-4′′ = 5.0 Hz, J3′-2′ = 2.0 Hz, 1H, H3′′), 4.24 (dd, J2′′-1′′ = 4.5 Hz, J2′-3′ = 2.0 Hz, 1H, H2′′), 4.21 – 4.17 (m, 1H, H5′′′), 4.07 – 4.02 (m, 1H, H4′′′), 4.01 (t, J3′-2′ = 4.7 Hz, J3′-4′ = 4.7 Hz, 1H, H3′), 3.92 – 3.86 (m, 1H, H5′), 3.83 (t, J4-3 = 9.5 Hz, J4-5 = 9.5 Hz, 1H, H4), 3.80 – 3.74 (m, 2H, H5′′, H5′′′), 3.72 – 3.69 (m, 1H, H4′′′), 3.67 – 3.60 (m, 3H, H5′, H5′′, H5′′′), 3.55 – 3.50 (m, 2H, H6, H2′), 3.48 – 3.46 (m, 1H, H2′′′), 3.38 – 3.28 (m, 2H, H3, H6′′′), 3.28 – 3.16 (m, 2H, H1, H6′′′), 2.35 (dt, J2eq-2eq′ = 13.0 Hz, J2eq-1 = 4.0 Hz, J2eq-3 = 4.0 Hz, 1H, H2eq), 1.81 (s, 15H, CH3CO2H), 1.74 – 1.62 (m, 1H, H2ax). 13C NMR (125 MHz, D2O) δ: 180.9 (CH3COOH), 110.2 (C1′), 98.1 (C1′), 95.4 (C1′′′), 83.5 (C5), 81.2 (C4′′), 78.4 (C4), 75.7 (C3′′′), 73.2 (C2′′), 71.8 (C6), 70.2 (C5′′′), 67.8 (C3′), 67.6 (C3′′), 67.2 (C4′), 66.8 (C4′′), 65.3 (C5′), 60.7 (C5′′), 51.4 (C2′′′), 50.8 (C2′), 49.7 (C1′), 48.4 (C3′), 40.4 (C6′′), 28.2 (C2′), 22.9 (CH3CO2H). ESI-HRMS: m/z calcd for C22H44N5O13 [M+H]+ 586.2936, found 586.2933.

1,3,2′,2″′,6″′-Pentadeamino-1,3,2′,2″′,6″′-pentaazido lividomycin B (43) A soln. of lividomycin A 35 (760 mg, 1 mmol) in a mixture of methanol and water (1:2, 60 mL) was cooled to 0 °C, treated with imidazolesulfonyl azide hydrochloride salt (1.3 g, 6.1
mmol), potassium carbonate (842 mg, 6.1 mmol) and copper(II) sulfate pentahydrate (15 mg, 0.061 mmol), and stirred at room temperature for 24 h. All solvent was removed under reduced pressure, the remaining residue was dissolved in methanol and filtered. The filtrate was concentrated under reduced pressure. After purification by flash column chromatography over silica gel (ethyl acetate: methanol 5:1), the resulting compound (401 mg) was dissolved in water (22 mL) and sodium periodate (225 mg, 1.1 mmol) was added. The reaction mixture was stirred in the dark at room temperature for 18 h. Ethylene glycol (0.11 mL) was added and stirring was continued at room temperature for another 2 h. Lead carbonate (225 mg) was added and the mixture was stirred for 0.5 h. After filtration, the filtrate was treated with 5% sulfuric acid (0.11 mL) for 0.5 h and filtered again. The lead free filtrate was heated at 100 °C with 10% acetic acid (0.9 mL) and phenylhydrazine (0.9 mL) for 3 h. The mixture was extracted with chloroform, and the aqueous layer was saturated with sodium chloride and further extracted with ethyl acetate. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (ethyl acetate) gave 43 as a light yellow solid (78 mg, 10%). $[\alpha]_{D}^{23} = + 158.2^\circ$ (c = 0.6, methanol). $R_f$ (ethyl acetate: isopropyl alcohol 50:1) 0.50. ESI-HRMS: $m/z$ calcd for C$_{23}$H$_{35}$N$_5$NaO$_{13}$ [M+Na]$^+$ 752.2431, found 752.2433. $^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 5.68 (d, $J_{1'-2'} = 3.5$ Hz, 1H, H1'), 5.40 (d, $J_{1''-2''} = 1.2$ Hz, 1H, H1''), 5.11 (d, $J_{1'''-2'''} = 1.6$ Hz, 1H, H1'''), 4.45 (dd, $J_{3''-4''} = 6.9$ Hz, $J_{3''-2''} = 4.5$ Hz, 1H, H3''), 4.28 (dd, $J_{2''-3''} = 4.5$ Hz, $J_{2''-1''} = 1.2$ Hz, 1H, H2''), 4.15 – 4.09 (m, 1H, H4''), 4.03 – 3.97 (m, 1H, H5''), 3.94 – 3.89 (m, 1H, H3'''), 3.84 – 
3.75 (m, 4H, H5', H5'', H6', H5), 3.73 – 3.57 (m, 6H, H4', H2'', H6'', H6', H4, H5''), 3.54 – 3.48 (m, 2H, H3, H6), 3.45 – 3.35 (m, 3H, H4'', H6'', H1), 3.14 (dt, $J_{2'-3'a} = 12.0$ Hz, $J_{2'-3'b} = J_{2'-1'} = 3.5$ Hz, 1H, H2'), 2.14 – 2.04 (m, 2H, H3'a, H3'b), 2.18 (dt, $J_{2eq-2ax} = 12.6$ Hz, 1H, H2ax).

$^{13}$C NMR (151 MHz, CD$_3$OD) δ 107.1 (C1''), 98.2 (C1'''), 95.3 (C1'), 83.8 (C4), 81.9 (C4''), 75.5 (C6), 75.4 (C3''), 74.7 (C5), 74.1 (C5'''), 73.7 (C2''), 73.4 (C5'), 69.7 (C3'''), 68.1 (C4'''), 64.4 (C4'), 61.8 (C6'), 61.0 (C5'), 60.4 (C3), 60.3 (C1), 60.2 (C2'''), 56.3 (C2'), 51.0 (C6''), 31.6 (C2), 30.8 (C3').

**Lividomycin B (41)**

A stirred soln. of 43 (20 mg, 0.03 mmol) in tetrahydrofuran (0.5 mL) was treated with 1.0 M trimethylphosphine in tetrahydrofuran (0.2 mL, 0.2 mmol) and heated at 60 °C for 1 h. Then deionized water (1 mL) was added and reaction mixture was heated at 60 °C for 16 h. The reaction mixture was concentrated under reduced pressure and purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water). The product-containing fractions were combined, glacial acetic acid (50 μL, 0.81 mmol) added, and freeze dried to give 41 as a white solid (15.1 mg, 56% as pentaacetate salt).

$[\alpha]^{23}_D = +62.5^\circ$ (c = 1.0, H$_2$O). Lit $[\alpha]^{23}_D = +62.0^\circ$ (c = 1.0, H$_2$O).$^{165}$ $^1$H NMR (600 MHz, D$_2$O) δ 5.37 (d, $J_{1'-2'} = 3.5$ Hz, 1H, H1'), 5.13 (d, $J_{1''-2''} = 2.0$ Hz, 1H, H1''), 5.09 (d, $J_{1'''-2'''} = 1.5$ Hz, 1H, H1''''), 4.31 (dd, $J_{3''-2''} = 5.0$ Hz, $J_{3''-4''} = 6.0$ Hz, 1H, H3'''), 4.17 (dd, $J_{2''-3''} = 5.0$ Hz, $J_{2''-1''} = 2.0$ Hz, 1H, H2'''), 4.14 – 4.09 (m, 1H, H5''''), 4.04 (t, $J_{3''-2''} = 3.0$ Hz, 1H, H3''''), 4.02 – 3.97 (m, 1H, H4'''), 3.60 (t, $J_{4-3} = J_{4-5} = 9.4$ Hz, 1H, H4), 3.71 – 3.59 (m, 5H, H6'a,
H5″a, H5, H4′′′′, H5′), 3.58 – 3.52 (m, 3H, H4′, H6′b, H5″″b), 3.52 – 3.46 (m, 2H, H2′, H6), 3.41 – 3.37 (m, 1H, H2″″″″), 3.36 – 3.30 (m, 1H, H3), 3.27 – 3.19 (m, 1H, H6″″″″a), 3.19 – 3.12 (m, 2H, H1, H6″″″″b), 2.28 (dt, \( J_{2eq-2ax} = 11.2 \) Hz, \( J_{2eq-3} = J_{2eq-1} = 3.9 \) Hz, 1H, H2eq), 2.06 (dt, \( J_{3eq-3′ax} = 11.2 \) Hz, \( J_{3eq-2′} = J_{3eq-4′} = 4.2 \) Hz, 1H, H3′eq), 1.86 – 1.69 (m, 16H, H3′ax, CH₃CO₂H), 1.65 (q, \( J_{2ax-2eq} = J_{2ax-3} = J_{2ax-1} = 11.2 \) Hz, 1H, H2ax). 

\(^{13}\)C NMR (151 MHz, D₂O) \( \delta \)
- 180.49 (CH₃CO₂H), 110.0 (C1″″), 95.3 (C1″′′), 94.2 (C1′), 84.0 (C5), 81.2 (C4″), 77.8 (C5″), 75.8 (C4), 75.3 (C3″′′), 73.2 (C2″″), 72.0 (C6), 70.1 (C5″″), 67.5 (C4″″″), 67.2 (C3″″″), 62.9 (C4″), 60.4 (C5″), 59.8 (C6″), 50.7 (C1), 49.6 (C2″″″), 48.7 (C3), 47.7 (C2″), 40.3 (C6″″″), 29.4 (C3″), 28.1 (C2), 22.7 (CH₃CO₂H).

ESI-HRMS: \( m/z \) calcd for C₁₂₃H₄₆N₅O₁₃ [M+H]⁺ 600.3087, found 600.3083.

6,3′,6′,2″″,5″″,4′′′′-Hepta-O-benzyl-1,3,2′,2′′″,6′′″-penta-N-benzyloxycarbonyl paromomycin (47) 1.0 M trimethylphosphine in tetrahydrofuran (6 mL, 6 mmol) was added into a stirred soln. of compound 46 (800 mg, 0.6 mmol) in tetrahydrofuran (16 mL), and the mixture was heated at 70 °C for 0.5 h. Then 0.1 M sodium hydroxide solution (6 mL) was added and the temperature was maintained at 70 °C for 6 h. All solvent was evaporated under reduced pressure and the residue was dissolved in methanol (20 mL). Sodium carbonate (900 mg, 8.4 mmol) and benzyl chloroformate (0.6 mL, 4.2 mmol) were added at 0 °C and the mixture was warmed up to room temperature and stirred for 24 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel
(hexane: ethyl acetate 1:1) gave 47 as colorless oil (518 mg, 45%). \([\alpha]^{23}_D = + 17.4^\circ\) (c = 0.8, dichloromethane). \(Rf\) (hexane: ethyl acetate 1:1) 0.55. ESI-HRMS: \(m/z\) calcd for \(C_{112}H_{117}N_5NaO_{24} [M+Na]^+\) 1939.8020, found 1939.8017. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,3',6',2'',5'',3'''4''''-Hepta-O-benzyl-1,3,2',2'',6''''-penta-N-benzyloxycarbonyl-4'-keto paromomycin (48)** A soln. of 47 (470 mg, 0.25 mmol) in dichloromethane (10 mL) was treated with Dess-Martin periodinane (125 mg, 0.3 mmol) and stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane: ethyl acetate 2:1) to give 48 as a white solid (420 mg, 88%). \([\alpha]^{23}_D = + 28.6^\circ\) (c = 0.6, dichloromethane). \(Rf\) (hexane: ethyl acetate 1:1) 0.71. ESI-HRMS: \(m/z\) calcd for \(C_{112}H_{115}N_5NaO_{24} [M+Na]^+\) 1937.7863, found 1937.7868. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,6',2'',5'',3'''4''''-Hexa-O-benzyl-3'1,3,2',2'',6''''-penta-N-benzyloxycarbonyl-3'-deoxy paromomycin (49)** Under an argon atmosphere, a soln. of compound 48 (90 mg, 0.05 mmol) in tetrahydrofuran (0.5 mL) was cooled to -20 °C, and treated with samarium iodide solution (0.1 M in tetrahydrofuran, 3 mL, 0.3 mmol). The reaction
mixture was stirred at -20 °C for 1 h before dry methanol (20 μL, 0.5 mmol) was added drop-wise. After stirring for another 2 h at 0 °C, the reaction was quenched by addition of saturated aqueous sodium bicarbonate. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (chloroform: ethyl acetate 5:1) gave 49 as colorless oil (38 mg, 42%). \([\alpha]^{23}_D = +17.5^\circ (c = 0.9, \text{dichloromethene})\). \(R_f\) (chloroform: ethyl acetate 5:1) 0.18. ESI-HRMS: \(m/z\) calcd for \(C_{105}H_{111}N_5NaO_{23}\) [M+Na]+ 1833.7601, found 1833.7599. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**Lividomycin B (41)** Compound 49 (20 mg, 0.011 mmol) was dissolved in a mixture of 1,4-dioxane/water/acetic acid 1/2/0.2 (0.5 mL) and stirred with palladium hydroxide on carbon (20 mg) under hydrogen atmosphere (40 psi) at room temperature for 24 h. The mixture was filtered, concentrated under reduced pressure and purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water). The product-containing fractions were combined, glacial acetic acid (40 μL, 0.69 mmol) was added, and the mixture was freeze dried to give 41 as a white solid (5 mg, 50% as pentaacetate salt) with spectral data identical to the above sample.

**6’-N-Benzylloxycarbonyl-1,3,2’-trideamino-1,3,2’-triazido ribostamycin (53)** A stirred soln. of ribostamycin 7 (5.5 g, 10 mmol) in water (50 mL) was cold to 0 °C, then
potassium carbonate (1.6 g, 11 mmol), N-(benzyloxycarbonyloxy)succinimide (2.5 g, 10 mmol) and methanol (50 mL) was added. The reaction mixture was stirred at room temperature for 18 h. Then the mixture was cooled to 0 °C, followed by addition of imidazolesulfonyl azide hydrochloride salt (8.4 g, 40 mmol), potassium carbonate (5.6 g, 40 mmol) and copper(II) sulfate pentahydrate (125 mg, 0.5 mmol). After stirring at room temperature for another 24 h, 0.5 M hydrochloric acid was added slowly into the reaction mixture at 0 °C until pH ≈ 3. Then the solution was saturated with sodium chloride and extracted by ethyl acetate twice. The organic layer was washed with brine, dried with sodium sulfate, filtrated and concentrated under reduced pressure. Flash column chromatography over silica gel (ethyl acetate: isopropanol 20: 1) gave 53 as white solid (2.55 g, 38%). $\left[\alpha\right]_{D}^{23} = +73.0^\circ$ ($c = 0.4$, methanol). $Rf$ (ethyl acetate) 0.15. $^1$H NMR (600 MHz, CD$_3$OD) δ 7.41 – 7.23 (m, 5H, aromatic), 5.70 （d, $J_{1'-2'} = 3.7$ Hz, 1H, H1'), 5.34 （br s, 1H, H1''), 5.12 – 5.02 （m, 2H, -CO$_2$CH$_2$Ph), 4.16 （d, $J = 7.0$, 4.7 Hz, 1H, H3''), 4.10 – 4.05 （m, 1H, H2''), 4.00 – 3.95 （m, 1H, H5''), 3.95 – 3.92 （m, 1H, H4''), 3.89 （dd, $J_{3'-2'} = 10.3$ Hz, 1H, H3''), 3.79 – 3.74 （m, 1H, H5''a), 3.67 – 3.60 （m, 4H, H6'a, H5''h, H4, H5), 3.49 – 3.44 （m, 1H, H3), 3.42 （t, $J_{6-5} = J_{6-1} = 9.6$ Hz, 1H, H6), 3.38 – 3.33 （m, 1H, H1), 3.27 – 3.23 （m, 1H, H6'b), 3.21 （dd, $J_{4'-5'} = 9.9$ Hz, $J_{4'-3'} = 8.9$ Hz, 1H, H4''), 3.06 （dd, $J_{2'-1'} = 10.3$ Hz, $J_{2'-3'} = 3.7$ Hz, H2''), 2.09 （dt, $J_{2eq-2ax} = 12.6$ Hz, $J_{2eq-1} = J_{2eq-3} = 4.2$ Hz, 1H, H2eq), 1.25 – 1.17 （m, 1H, H2ax). $^{13}$C NMR (151 MHz, CD$_3$OD) δ 157.7 (-NCO), 128.2, 127.7, 127.2, 106.9 （C1'''), 96.6 （C1''), 83.4 （C5), 83.0 （C4''), 75.6 （C2''), 75.4 （C6), 75.0 （C4), 71.9 （C4'), 71.3 （C5'), 70.6 （C3'), 70.0 （C3''), 66.2 （-CO$_2$CH$_2$Ph), 63.1 （C2''), 61.7
(C5’), 60.3 (C1), 59.9 (C3), 41.6 (C6’), 31.7 (C2). ESI-HRMS: m/z calcd for C_{25}H_{34}N_{10}NaO_{12} [M+Na]^+ 689.2255, found 689.2255.

6′-N-Benzylloxycarbonyl-4′-O-6′-N-benzylidene-2′,3′-O-benzylidene-1,3,2′-tridea mino-1,3,2′-triazido ribostamycin (54) Compound 53 (500 mg, 0.75 mmol) was dissolved in freshly distilled benzaldehyde (10 mL). The mixture was cooled to 0 °C and boron trifluoride diethyl etherate (0.2 mL, 1.6 mmol) was added drop-wise under an argon atmosphere. Reaction mixture was stirred at 0 °C for 1.5 h before work up. The mixture was added into ice-cold saturated aqueous sodium bicarbonate slowly with stirring, and then extracted with ethyl acetate twice. The combined organic layer was washed with brine and dried with sodium sulfate. Filtration and concentration under reduced pressure, followed by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) afforded 54 as a white solid (320 mg, 50%). [α]_{23}^{23}D = + 41.6° (c = 1.0, dichloromethane). Rf (hexane: ethyl acetate 1: 1) 0.35. \(^1\)H NMR (600 MHz, chloroform-d) \(\delta\) 7.50 – 7.26 (m, 15H), 6.90 (s, 1H, NO-benzylidene), 5.90 (br s, 1H, H1’), 5.75 (s, 1H, OO-benzylidene), 5.64 (br s. 1H, H1’’), 5.24 – 5.13 (m, 2H, -CO_{2}CH_{2}Ph), 4.98 – 4.93(m, 1H, H2’’), 4.83 – 4.74 (m, 1H, H3’’), 4.57 – 4.38 (m, 2H, H6’eq, H4’’), 4.15 (td, \(J_{5′-4′} = J_{5′-6′eq} = 9.7\) Hz, \(J_{5′-6′eq} = 3.9\) Hz, 1H, H5’), 4.13 – 4.02 (m, 1H, H3’), 3.78 – 3.68 (m, 2H, H5’’a, H5), 3.66 (dd, \(J = 12.2, 5.7\) Hz, 1H, H5’’b), 3.62 – 3.45 (m, 2H, H4’’, H4), 3.45 – 3.26 (m, 3H, H1, H3, H6), 3.17 – 3.08 (m, 1H, H2’), 2.97 – 2.80 (m, 1H, H6’ax), 2.33 – 2.18 (m, 1H, H2eq), 1.56 – 1.31 (m, 1H, H2ax). \(^1\)C NMR (151 MHz, chloroform-d) \(\delta\) 170.5, (-NCO), 133.6, 130.1, 130.0, 129.2 128.5, 128.45, 128.4, 128.3, 128.0, 126.9, 126.7, 126.6, 108.6 (C1’’),
106.3 (O0-benzyldiene), 104.1 (C1’), 86.5 (C4”), 85.4 (C2”), 82.6 (NO-benzyldiene), 82.0 (C5), 80.5 (C3”), 76.8 (C4), 75.0 (C6), 69.0 (C5”), 68.2 (-CO2CH2Ph), 64.3 (C3”), 63.7 (C2”), 63.0 (C5”), 60.0 (C1), 59.2 (C3), 41.7 (C6”), 31.5 (C2).


6,3’,5”-Tri-O-benzyl-6’-N-benzyloxycarbonyl-4’-O-6’-N-benzyldiene-2”,3”-O-benzyldene-1,3,2’-trideamino-1,3,2’-triazido ribostamycin (55) Under an argon atmosphere, a soln. of 54 (320 mg, 0.38 mmol) in dry tetrahydrofuran (6 mL) was cooled to 0 °C. Sodium hydride (60% in mineral oil, 64 mg, 1.6 mmol), benzyl bromide (0.2 mL, 1.6 mmol) and tetrabutylammonium iodide (15 mg, 0.04 mmol) were added and the reaction mixture was warmed up to room temperature and stirred for 24 h. The reaction was quenched by addition of methanol at 0 °C until all solid was dissolved, then was diluted with ethyl acetate, washed with brine, and dried with sodium sulfate. After removing all sodium sulfate by filtration, the residue was concentrated under reduced pressure and purified by flash column chromatography over silica gel (hexane: ethyl acetate 8: 1) to give 55 as colorless oil (300 mg, 70%). [α]D23 = + 51.4° (c = 0.5, dichloromethane). Rf (hexane: ethyl acetate 4: 1) 0.50. 1H NMR (600 MHz, chloroform-d) δ 7.57 – 7.04 (m, 35H), 6.86 (s, 1H, NO-benzyldiene), 5.74 (s, 1H, O0-benzyldiene), 5.69 (d, J1-2’ = 3.8 Hz, 1H, H1’), 5.60 (br s, 1H, H1”), 5.36 – 5.18 (m, 2H, -CO2CH2Ph), 5.12 – 5.05 (d, J = 11.8 Hz, 1H, -CH2Ph), 4.88 – 4.80 (m, 2H, -CH2Ph), 4.77 – 4.72 (m, 1H, H2”), 4.64 (d, J = 10.2 Hz, 1H, -CH2Ph), 4.57 – 4.53 (m, 1H, H3”), 4.53 – 4.42 (m, 3H, -CH2Ph, H6’eq), 4.32 (br s, 1H, H4”), 4.16 – 4.03 (m, 1H, H5”), 3.90 (t, J3’-2’ = J3’-4’ = 9.6 Hz, 1H, H3’),
3.67 (t, \(J_{5,4} = J_{5,6} = 9.0\) Hz, 1H, H5), 3.58 – 3.49 (m, 3H, H5", H4), 3.49 – 3.48 (m, 3H, H4’, H1, H3), 3.18 – 3.10 (m, 2H, H2’, H6), 2.93 – 2.78 (m, 1H, H6’ax), 2.34 – 2.22 (m, 1H, H2eq), 1.55 – 1.31 (m, 1H, H2ax). \(^{13}\)C NMR (151 MHz, chloroform-d) \(\delta 155.2 (-\text{NCO}), 138.2 - 135.5\) (aromatic), 129.5 - 126.6 (aromatic), 110.2 (C1’), 104.0 (00-benzylidene), 96.9 (C1’), 84.2 (C2”), 83.9 (C4”), 83.2 (C6), 82.4 (NO-benzylidene), 81.7 (C5), 80.9 (C3”), 77.0 (C3’), 76.8 (C4), 75.4 (C4’), 75.2 (-CH2Ph), 75.1 (-CH2Ph), 73.3 (-CH2Ph), 70.4 (C5”), 68.1 (-CO2CH2Ph), 64.3 (C5’), 63.2 (C2’), 60.5 (C1), 59.3 (C3), 41.9 (C6’), 32.2 (C2).

ESI-HRMS: \(m/z\) calcd for C\(_{60}\)H\(_{60}\)N\(_{10}\)NaO\(_{12}\) [M+Na]+ 1135.4290, found 1135.4288.

6,3’,5”-Tri-0-benzyl-6’-N-benzyloxycarbonyl-2”,3”-O-benzylidene-1,3,2’-trideamino-1,3,2’-triazido ribostamycin (56) Compound 55 (600 mg, 0.54 mmol) was dissolved in ice-cold trifluoroacetic acid (6 mL) and stirred at 0 °C for 0.5 h under an argon atmosphere. After the reaction was completed, the mixture was added drop-wise into ice-cold saturated potassium carbonate soln. and extracted with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 4: 1) provided 56 as colorless oil (390 mg, 71%). \([\alpha]\)\(_D^{23}\) = + 34.1° (c = 0.3, dichloromethane). \(Rf\) (hexane: ethyl acetate 4: 1) 0.36. \(^1\)H NMR (600 MHz, chloroform-d) \(\delta 7.50 – 7.24\) (m, 25H), 5.84 (d, \(J_{1'-2'} = 3.9\) Hz, 1H, H1’), 5.81 (s, 1H, benzylidene) 5.69 (br s, 1H, H1”), 5.20 – 5.15 (m, 1H, -CO\(_2\)CH\(_2\)Ph), 5.14 – 5.08 (m, 1H, -CO\(_2\)CH\(_2\)Ph), 5.05 – 4.99 (m, 1H, -CH\(_2\)Ph), 4.90 – 4.83 (m, 3H, H2”, -CH\(_2\)Ph), 4.71 (d, \(J = 10.9\) Hz, 1H, -CH\(_2\)Ph), 4.64 – 4.57 (m, 3H, H3’”, -CH\(_2\)Ph), 4.40 (td, \(J = 5.5, 2.2\) Hz, 1H, H4’”).
4.03 (dt, $J_{5'4'} = J_{5'6'a} = 9.6$ Hz, $J_{5'6'b} = 3.0$ Hz, 1H, H5’), 3.93 – 3.85 (m, 1H, H3’), 3.85 – 3.77 (m, 1H, H6’a), 3.75 (t, $J_{5'6} = J_{54} = 9.0$ Hz, 1H, H5), 3.68 – 3.53 (m, 3H, H5”, H4), 3.49 – 3.34 (m, 3H, H4’, H1, H3), 3.30 – 3.15 (m, 3H, H2’, H6’b, H6), 2.29 – 2.24 (m, 1H, H2eq), 1.43 (q, $J_{2ax-2eq} = J_{2ax-1} = J_{2ax-3} = 12.7$ Hz, 1H, H2ax). $^{13}$C NMR (151 MHz, chloroform-d) δ 157.9 (-NCO), 138.3 - 135.9 (aromatic), 128.6 - 127.6 (aromatic), 109.9 (C1”), 104.1 (00-benzylidene), 96.6 (C1’), 84.3 (C2”), 83.8 (C4”), 83.3 (C6), 81.4 (C5), 81.0 (C3”), 78.9 (C3’), 76.6 (C4), 75.6 (-CH2Ph), 75.4 (-CH2Ph), 73.3 (-CH2Ph), 71.6 (C5’), 71.2 (C4’), 70.5 (C5”), 67.4 (-CO2CH2Ph), 63.3 (C2’), 60.5 (C1), 59.5 (C3), 41.2 (C6’), 32.3 (C2).


6,3’,5”-Tri-O-benzyl-1,3,2’,6’-teta-N-benzyloxy carbonyl-2”,3”-O-benzylidene ribostamycin (57) A stirred soln. of 56 (920 mg, 0.9 mmol) in tetrahydrofuran (20 mL) was treated with 1.0 M trimethylphosphine in tetrahydrofuran (9 mL, 9 mmol) at 70 °C for 1 h, and then 0.1 M sodium hydroxide soln. (9 mL) was added. The reaction mixture was kept stirring at 70 °C for 6 h. All solvent was removed under reduced pressure and the residue was dissolved in methanol (20 mL). N-(Benzyloxy carbonyloxy)succinimide (1.5 g, 6 mmol) and potassium carbonate (840 mg, 6 mmol) were added and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate, washed with water and brine. The combined organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) and 57 was obtained as a white solid (516 mg, 42%). $[\alpha]^{23}_D = + 12.7$ ° ($c = 1.0,$
dichloromethane). \textit{Rf} (hexane: ethyl acetate 1: 1) 0.45. ESI-HRMS: \textit{m/z} calcd for \( C_{77}H_{80}N_4NaO_{18} [M+Na]^+ \) 1371.5365, found 1371.5368. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

\textit{6,3',5''-Tri-O-benzyl-1,3,2',6'-tetra-N-benzyloxycarbonyl-2'',3''-O-benzylidene-4'-keto ribostamycin (58)} Dess-Martin periodinane (212 mg, 0.5 mmol) was added into a soln. of 57 (234 mg, 0.17 mmol) in dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 20 h, and then quenched by adding saturated aqueous sodium bicarbonate. The mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 3: 2) gave 58 as a white solid (150 mg, 66\%). \([\alpha]_{23}^D = +17.8^\circ (c = 1.4, \text{dichloromethane})\). \textit{Rf} (hexane: ethyl acetate 3: 2) 0.30. ESI-HRMS: \textit{m/z} calcd for \( C_{77}H_{78}N_4NaO_{18} [M+Na]^+ \) 1369.5209, found 1369.5206. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

\textit{6,5''-Di-O-benzyl-1,3,2',6'-tetra-N-benzyloxycarbonyl-2'',3''-O-benzylidene-3'-deoxy ribostamycin (59)} Compound 58 (150 mg, 0.12 mmol) was dissolved in dry tetrahydrofuran (0.7 mL) and stirred under an argon atmosphere at -20 °C. Samarium iodide solution (0.1 M in tetrahydrofuran, 7 mL, 0.7 mmol) was added and the mixture was kept stirring at -20 °C for 1 h. Dry methanol (48 \( \mu \)L, 1.2 mmol) was added drop-wise
and the mixture was brought to 0 °C and stirred for another 2 h. Saturated aqueous sodium bicarbonate was added at 0 °C and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (chloroform: ethyl acetate 3: 1) to give 59 as a white solid (72.4 mg, 32%). \([\alpha]_D^{23} = + 8.7^\circ \) \((c = 1.7, \text{dichloromethane}). Rf (chloroform: ethyl acetate 3: 1) 0.22. ESI-HRMS: \(m/z\) calcd for \(C_{70}H_{74}N_4O_{17}\) \([M+Na]^+ 1265.4947, \text{found 1265.4950.}\) This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

3'-Deoxy ribostamycin (52)\(^{190}\) A soln. of 59 (35 mg, 0.03 mmol) in a mixture of 1,4-dioxane/water/acetic acid 1/2/0.2 (0.35 mL) was treated with palladium hydroxide on carbon (35 mg) under hydrogen atmosphere (48 psi) at room temperature for 22 h. The mixture was filtered, concentrated under reduced pressure and purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water). The product-containing fractions were combined and glacial acetic acid (40 μL, 0.7 mmol) was added. Lyophilization of the mixture gave 52 as a white solid (8.9 mg, 44% as the pentaacetate salt). \([\alpha]_D^{23} = + 21.7^\circ \) \((c = 0.2, \text{H}_2\text{O}).\) Lit \([\alpha]_D^{23} = + 41^\circ \) \((c = 1.0, \text{H}_2\text{O}).\)^{190} 1H NMR (600 MHz, D\(_2\)O) \(\delta \) 5.66 \((d, J_{1'-2'} = 3.4 \text{ Hz}, 1\text{H}, H1'\)), 5.13 \((d, J_{1''-2''} = 0.3 \text{ Hz}, 1\text{H}, H1''\)), 4.00 \((dd, J_{2''-3''} = 3.7 \text{ Hz}, J_{2''-1''} = 0.3 \text{ Hz}, 1\text{H}, H2'')\), 3.97 – 3.94 \((m, 1\text{H}, H3'')\), 3.85 – 3.78 \((m, 2\text{H}, H4'', H4)\), 3.72 – 3.63 \((m, 3\text{H}, H5', H5''a, H5)\), 3.52 –
3.42 (m, 4H, H2', H4', H5''b, H6), 3.28 – 3.18 (m, 2H, H6'a, H3), 3.15 – 3.09 (m, 1H, H1), 3.01 (dd, J = 13.5, 7.3 Hz, 1H, H6'b), 2.27 – 2.21 (dt, J_{2eq-2ax} = 12.6 Hz, J_{2eq-1} = J_{2eq-3} = 3.9 Hz, 1H, H2eq), 2.06 (dt, J_{3eq-3'ax} = 11.7 Hz, J_{3'ax-3'eq} = J_{3'ax-4'} = 11.7 Hz, 1H, H3'ax), 1.72 (s, 12H, CH_3CO_2H), 1.62 (q, J_{2ax-2eq} = J_{2ax-1} = J_{2ax-3} = 12.6 Hz, 1H, H2ax). 13C NMR (151 MHz, D_2O) δ 180.8 (CH_3CO_2H), 110.3 (C1’”), 93.4 (C1’), 84.9 (C5), 82.4 (C4”), 75.8 (C6), 75.1 (C5”), 72.4 (C4), 70.0 (C2’”), 69.0 (C3’”), 64.5 (C4”), 60.9 (C5”), 49.8 (C1), 48.4 (C3), 47.7 (C2”), 39.8 (C6’), 29.1 (C3’), 28.6 (C2), 22.9 (CH_3CO_2H). ESI-HRMS: m/z calcd for C_{17}H_{35}N_{4}O_9 [M+H]^+ 439.2399, found 439.2402.

6,3′,2′″,5′″,3′′′,4′′′-Hexa-0-benzyl-1,3,2′,6′,2′′′,6′′′-hexa-N-benzylxoycarbonyl neomycin (62) Compound 61 (850 mg, 0.65 mmol) was dissolved in tetrahydrofuran (17 mL) and heated with 1 M trimethylphosphine in tetrahydrofuran (6.5 mL, 6.5 mmol) at 70 °C for 1 h. Then 0.1 M sodium hydroxide solution (6.5 mL) was added and the mixture was heated at 70 °C for 8 h. N-(Benzylxoycarbonyloxy)succinimide (2.0 g, 8.0 mmol) and potassium carbonate (1.2 g, 8.7 mmol) were added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was extracted with ethyl acetate and the separated organic layer was washed with 1 M hydrochloric acid and brine. After drying with sodium sulfate, filtration and concentration under reduced pressure, the residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) and provided 62 as a white solid (1.08 g, 85%). [α]_D^{23} = + 10.0° (c = 1.0, dichloromethane). Rf (hexane: ethyl acetate 1: 1) 0.25. ESI-HRMS: m/z
calcd for $C_{113}H_{116}N_6NaO_{25} [M+Na]^+$ 1982.8078, found 1982.8073. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,3',2'',5'',3'''4''''-Hexa-O-benzyl-1,3,2',6',2'',6''''-hexa-N-benzyloxy carbonyl-4'-keto neomycin (63) A soln. of 62 (1.05 g, 0.54 mmol) in dichloromethane (20 mL) was treated with Dess-Martin periodinane (460 mg, 1.08 mmol) and stirred at room temperature for 24 h. The reaction was quenched by addition of saturated aqueous sodium bicarbonate and then extracted with ethyl acetate. The separated organic layer was washed with water and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) gave 63 as a white solid (830 mg, 79%) $[\alpha]_{23}^D = +20.2^\circ$ ($c = 1.1$, dichloromethane). $R_f$ (hexane: ethyl acetate 1: 1) 0.45. ESI-HRMS: $m/z$ calcd for $C_{113}H_{116}N_6NaO_{25} [M+Na]^+$ 1980.7921, found 1980.7920. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,2'',5'',3'''4''''-Penta-O-benzyl-1,3,2',6',2'',6''''-hexa-N-benzyloxy carbonyl-3'-deoxy neomycin (64) Samarium iodide soln. (0.1 M in tetrahydrofuran, 10 mL, 1 mmol) was added into a stirred soln. of 63 (243 mg, 0.12 mmol) in tetrahydrofuran (1.2 mL) at -20 $^\circ$C under an argon atmosphere. The mixture was stirred for 1 h and then dry methanol (48 µL, 1.2 mmol) was added. The reaction mixture was allowed to warm up to 0 $^\circ$C and stirred for another 2 h. Saturated aqueous sodium bicarbonate was added at 0 $^\circ$C to
quench the reaction. After that the mixture was extracted with ethyl acetate. The organic layer was washed with brine and then dried with sodium sulfate. Filtration and concentration under reduced pressure, followed by flash column chromatography over silica gel (chloroform: ethyl acetate 3: 1) gave 64 as a white solid (80 mg, 31%). \([\alpha]_{D}^{23} = +12.8^\circ (c = 1.3, \text{dichloromethane}).\) \(R_f\) (chloroform: ethyl acetate 1: 1) 0.70. ESI-HRMS: \(m/z\) calcd for \(\text{C}_{106}\text{H}_{112}\text{N}_{6}\text{NaO}_{24} [\text{M}+\text{Na}]^+ 1876.7659,\) found 1876.7657. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**3'-Deoxy neomycin (60)**\(^\text{191}\) A soln. of 64 (80 mg, 0.04 mmol) in dioxane (1 mL) was mixed with palladium hydroxide on carbon (40 mg) and stirred at room temperature under a hydrogen atmosphere (40 psi) for 12 h. Deionized water (2 mL) and glacial acetic acid (0.2 mL) was added and the mixture was kept stirring for another 12 h at room temperature under a hydrogen atmosphere (40 psi). After filtration through a Celite pad, the cake was washed with deionized water and the filtrate was combined and concentrated under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water), and the product-containing fractions were combined and glacial acetic acid (40 μL, 0.7 mmol) was added. Lyophilization of the mixture gave 60 as a white solid (21 mg, 51 % as the pentaacetate salt). \([\alpha]_{D}^{23} = +23.6^\circ (c = 0.4, \text{H}_{2}\text{O}).\) Lit \([\alpha]_{D}^{15} = +52^\circ (c = 1.0, \text{H}_{2}\text{O}).\) \(^\text{191}\)\(^1\)H NMR (600 MHz, D₂O) \(\delta\) 5.67 (d, \(J_{1'-2'} = 3.5\) Hz, 1H, H1'), 5.20 (d, \(J_{1''-2''} = 2.4\) Hz, 1H, H1''), 5.10 (d, \(J_{1'''-2'''} = 1.2\) Hz, 1H, H1'''), 4.29 (t, \(J_{3''-4''} = 5.29\) Hz, 2H, H3''-4'').
5.4 Hz, 1H, H3''), 4.19 (dd, \( J_{2'-3'} = 5.4 \) Hz, \( J_{2''-1''} = 2.4 \) Hz, 1H, H2''), 4.12 (t, \( J = 4.5 \) Hz, 1H, H5''), 4.06 – 4.01 (m, 2H, H4'', H3''), 3.87 (t, \( J_{4+5} = J_{4-3} = 9.6 \) Hz, 1H, H4), 3.72 – 3.67 (m, 3H, H5', H5'', H5), 3.63 – 3.62 (m, 1H, H4'''), 3.57 – 3.43 (m, 4H, H2', H4', H5'', H6), 3.39 (t, \( J_{2'-1''} = J_{2''-3''} = 1.2 \) Hz, 1H, H2''), 3.34 – 3.27 (m, 1H, H3), 3.26 – 3.12 (m, 4H, H6'a, H1, H6''), 3.04 (dd, \( J = 13.6, 7.3 \) Hz, 1H, H6'b), 2.28 (dt, \( J_{2eq-2ax} = 11.7 \) Hz, \( J_{2eq-1} = J_{2eq-3} = 3.9 \) Hz, 1H, H2eq), 2.07 (dt, \( J_{3eq-3'ax} = 12.0 \) Hz, \( J_{3eq-2'} = J_{3eq-4'} = 4.2 \) Hz, 1H, H3'eq), 1.84 (q, \( J_{3'ax-3'eq} = J_{3'ax-2'} = J_{3'ax-4'} = 12.0 \) Hz, 1H, H3'ax), 1.75 (s, 18H CH3CO2H), 1.71 – 1.62 (q, \( J_{2ax-2eq} = J_{2ax-1} = J_{2ax-3} = 11.7 \) Hz, 1H, H2ax). ^13C NMR (151 MHz, cdcl3) δ 180.6 (CH3CO2H), 109.8 (C1'), 96.0 (C1''), 93.0 (C1''), 84.5 (C5), 81.1 (C4''), 75.1 (C4), 75.0 (C3''), 73.3 (C2''), 71.9 (C6), 69.8 (C5'), 69.7 (C5'''), 67.1 (C3'''), 66.9 (C4'''), 64.4 (C4'), 60.1 (C5''), 50.2 (C2''), 49.4 (C1), 48.2 (C3), 47.5 (C2'), 39.9 (C6''), 39.7 (C6'), 28.7 (C3'), 27.8 (C2), 22.1 (CH3CO2H). ESI-HRMS: \( m/z \) calcd for C23H47N6O12 [M+H]^+ 599.3246, found 599.3241.

61,3,2',2'',6''-Pentadeamino-1,3,2',2'',6''-pentaazido-4',6'-O-benzylidene-5''-O-triisopropylsilyl paromomycin (79) A soln. of 78 (4.16 g, 5 mmol) in dry dichloromethane (80 mL) was treated with 2,6-lutidine (3.07 mL, 25 mmol) and triisopropylsilyl trifluoromethanesulfonate (1.51 mL, 6 mmol) and stirred at room temperature for 1.5 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 4) to
give 79 as a white solid (4.42 g, 89%). \([\alpha]^{23}_{D} = +90.5^\circ \) (c = 0.5, chloroform). \(Rf\) (hexane: ethyl acetate 1: 4) 0.45. \(^1\)H NMR (600 MHz, chloroform-\(d\)) \(\delta 7.51 - 7.47\) (m, 2H, aromatic), \(7.40 - 7.37\) (m, 3H, aromatic), \(5.68\) (d, \(J_{1'\cdots2'} = 3.9\) Hz, 1H, H1'), \(5.53\) (s, 1H, benzyllidene-H), \(5.24\) (d, \(J_{1'\cdots2'} = 4.6\) Hz, 1H, H1''), \(5.11\) (d, \(J_{1'\cdots2'} = 1.3\) Hz, 1H, H1'''). \(4.42\) (dd, \(J_{3''\cdots4'} = 5.0\) Hz, \(J_{3''\cdots4''} = 3.2\) Hz, 1H, H3''), \(4.31 - 4.24\) (m, 3H, H6', H2'', H4''), \(4.19 - 4.10\) (m, 2H, H3', H5'), \(4.02\) (dd, \(J_{3''\cdots4'} = 5.0\) Hz, \(J_{3''\cdots4''} = 3.2\) Hz, 1H, H3''), \(4.00 - 3.97\) (m, 1H, H5'''), \(3.88\) (d, \(J_{5''\cdots4'} = 3.2\) Hz, 2H, H5'''), \(3.78\) (dd, \(J_{2\cdots1''} = 1.3\) Hz, \(J_{2\cdots3'} = 3.5\) Hz, 1H, H2'''), \(3.73 - 3.67\) (m, 2H, H6', H4), \(3.61 - 3.53\) (m, 3H, H4'', H6'', H5), \(3.51\) (t, \(J_{4\cdots3} = J_{4\cdots5'} = 9.4\) Hz, 1H, H4''), \(3.43 - 3.31\) (m, 4H, H6'', H1, H3, H6), \(3.24\) (dd, \(J_{2\cdots3'} = 10.1\) Hz, \(J_{2\cdots1'} = 3.9\) Hz, 1H, H2'''), \(2.17\) (dt, \(J_{2eq\cdots2ax} = 12.8\) Hz, \(J_{2eq\cdots1} = J_{2eq\cdots3} = 4.5\) Hz, 1H, H2eq), \(1.40\) (q, \(J_{2ax\cdots2eq} = J_{2ax\cdots1} = J_{2ax\cdots3} = 12.8\) Hz, 1H, H2ax), \(1.18 - 1.01\) (m, 21H, -Si(CH(CH\(_3\))\(_2\))\(_3\)). \(^{13}\)C NMR (151 MHz, chloroform-\(d\)) \(\delta 136.9\) (aromatic), \(129.5\) (aromatic), \(128.5\) (aromatic), \(126.4\) (aromatic), \(106.3\) (C1''), \(102.1\) (benzyllidene C), \(99.0\) (C1''), \(97.9\) (C1'), \(84.6\) (C4), \(83.6\) (C2''), \(81.5\) (C4'), \(77.0\) (C3''), \(74.0\) (C5''), \(75.0\) (C4'''), \(74.0\) (C5'''), \(69.2\) (C3''''), \(69.0\) (C6), \(68.8\) (C6'), \(68.5\) (C3'), \(63.7\) (C2'), \(63.2\) (C5'''), \(62.8\) (C5''), \(60.8\) (C2'''), \(59.4\) (C3), \(59.2\) (C1), \(51.2\) (C6'), \(31.8\) (C2), \(18.0\) (-CH(CH\(_3\))\(_2\)), \(12.0\) (-CH(CH\(_3\))\(_2\)). ESI-HRMS: \(m/z\) calcd for C\(_{39}\)H\(_{59}\)N\(_{15}\)NaO\(_{14}\)Si [M+Na]\(^+\) 1012.4033, found 1012.4030.

1,3,2',2'',6'''-Pentadeamino-1,3,2',2'',6'''-pentaazido-6,3',2'',3''',4''''-penta-O-benzy1-4',6'-O-benzyllidene-5''-O-triisopropylsilyl paromomycin (80) Compound 79 (3.94 g 3.97 mmol) was dissolved in dry tetrahydrofuran (40 mL) and cooled to 0 °C in an ice bath. Sodium hydride (60% in mineral oil, 1.27 g, 31.8 mmol) was added slowly with
stirring followed by addition of benzyl bromide (4 mL, 31.8 mmol) and tetrabutylammonium iodide (148 mg, 0.4 mmol). The reaction mixture was warmed up to room temperature and stirred for 15 h under argon atmosphere. Methanol was added to quench the reaction at 0 °C, and then saturated aqueous sodium bicarbonate was added. The mixture was extracted with ethyl acetate and the combined organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 4: 1) afforded 80 as a white solid (4.2 g, 73%). $[\alpha]_{D}^{23} = + 45.3^\circ$ (c = 1.4, dichloromethene). Rf (hexane: ethyl acetate 4: 1) 0.50. $^1$H NMR (600 MHz, chloroform-d) δ 7.50 – 7.47 (m, 2H, aromatic), 7.41 – 7.16 (m, 28H, aromatic), 6.01 (d, $J_{1',2'} = 4.1$ Hz, 1H, H1'), 5.56 (s, 1H, benzylidene-H), 5.53 (d, $J_{1',2'} = 4.8$ Hz, 1H, H1''), 4.93 (d, $J = 11.3$ Hz, 1H, -OCH$_2$Ph), 4.89 – 4.84 (m, 2H, H1'''', -OCH$_2$Ph), 4.80 (d, $J = 10.8$ Hz, 1H, -OCH$_2$Ph), 4.76 (d, $J = 10.8$ Hz, 1H, -OCH$_2$Ph), 4.62 (d, $J = 11.9$ Hz, 1H, -OCH$_2$Ph), 4.51 (d, $J = 11.8$ Hz, 1H, -OCH$_2$Ph), 4.43 – 4.36 (m, 3H, H3''', -OCH$_2$Ph), 4.35 – 4.28 (m, 3H, H6', -OCH$_2$Ph), 4.26 – 4.23 (m, H4''), 4.13 (td, $J_{5'-4'} = 10.0$ Hz, $J_{5'-6'} = 4.9$ Hz, 1H, H5'), 4.04 (t, $J_{3'-2'} = J_{3'-4'} = 9.6$ Hz, 1H, H3'), 3.95 (t, $J_{2'''.1'''} = J_{2'''-3'''} = 4.8$ Hz, 1H, H2''), 3.91 – 3.87 (m, 1H, H5''), 3.85 - 3.81 (m, 1H, H5''), 3.78 – 3.74 (m, 3H, H3''', H5''', H5), 3.72 – 3.67 (m, 1H, H6'), 3.66 – 3.60 (m, 2H, H4', H4), 3.60 – 3.57 (m, 1H, H6''a), 3.43 – 3.39 (m, 2H, H1, H3), 3.37 (dd, $J_{2'-3'} = 10.0$ Hz, $J_{2'-1'} = 4.1$ Hz, 1H, H2'), 3.33 (t, $J_{2''-1''} = J_{2''-3''} = 2.3$ Hz, 1H, H2''), 3.26 (t, $J_{6-5} = J_{6-1} = 9.1$ Hz, 1H, H6), 3.18 (t, $J_{4'-3''} = J_{4'-5''} = 2.1$ Hz, 1H, H4''), 3.14 (dd, $J_{6-5''} = 12.7$ Hz, $J_{6'b-6''a} = 5.1$ Hz, 1H, H6''b), 2.26 (dt, $J_{2eq-2ax} = 13.2$ Hz, $J_{2eq-1} = J_{2eq-3} = 4.7$ Hz, 1H, H2eq), 1.45 (q, $J_{2ax-2eq} = 7.2$ Hz, 6H, Me).
$J_{2ax-1} = J_{2ax-3} = 13.2 \text{ Hz, } 1H, \text{ H}_{2ax}$, 1.11 – 1.03 (m, 21H, -Si(CH(CH₃)₂)₃). $^{13}$C NMR (151 MHz, chloroform-d) δ 138.1 - 137.0 (aromatic), 129.0 - 126.1 (aromatic), 107.2 (C1’’), 101.4 (benzylidene-H), 98.3 (C1’’’), 96.7 (C1’), 83.2 (C4’’’), 83.2 (C6), 82.4 (C4’), 82.3 (C5), 81.3 (C2’’), 76.6 (C4), 76.5 (C3’), 74.9 (C3’’’), 74.8 (–CH₂Ph), 74.7 (–CH₂Ph), 73.8 (C5’’’), 73.0 (C3’’’), 72.6 (–CH₂Ph), 72.5 (–CH₂Ph), 71.8 (–CH₂Ph), 71.6 (C4’’’), 69.0 (C6’), 64.0 (C5’), 63.2 (C2’), 62.9 (C5’), 60.4 (C3), 59.4 (C1), 57.6 (C2’’’), 51.0 (C6’’’), 32.0 (C2), 18.1 (–CH(CH₃)₂), 12.0 (–CH(CH₃)₂). ESI-HRMS: m/z calcd for C₇₄H₈₉N₁₅NaO₁₄Si [M+Na]⁺ 1462.6380, found 1462.6384.

6,3’’,2’’’-Penta-O-benzyl-1,3,2’,2’’’,6’’-penta-benzoylcarbonyl-4’,6’-O-be nzylidene-5’’-O-triisopropylsilyl paromomycin (81) A soln. of 80 (4.2 g 2.9 mmol) in tetrahydrofuran (42 mL) was treated with trimethylphosphine solution (1 M in tetrahydrofuran, 23.3 mL, 23.3 mmol) at 65 °C for 1 h. Sodium hydroxide solution (0.1 M in water, 42 mL) was added and the mixture was kept stirring at 65 °C for another 3 h. The reaction mixture was cooled to room temperature, and then N-(benzoylcarbonyloxy)succinimide (7.27 g, 29.2 mmol) and potassium carbonate (4.03 g, 29.2 mmol) were added. After stirring at room temperature for 15 h, saturated aqueous sodium bicarbonate was added, and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) and gave 81 as a white solid (4.52 g, 78%). $[\alpha]^{23}_D = +12.5°$ (c = 1.0, dichloromethane). Rf (hexane: ethyl
acetate 2: 1) 0.25. ESI-HRMS: \( m/z \) calcd for \( \text{C}_{114}\text{H}_{129}\text{N}_{5}\text{NaO}_{24}\text{Si} \) \([\text{M}+\text{Na}]^+ \) 2003.8728, found 2003.8725. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,3',2'',3''',4'''-Penta-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxycarbonyl-4',6'-O-benzylidene paromomycin (82) Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 2 mL) was added into a soln. of 81 (1.98 g, 1 mmol) in tetrahydrofuran (20 mL). The reaction mixture was stirred at room temperature for 1 h, and then diluted with ethyl acetate. The mixture was washed with saturated sodium aqueous bicarbonate and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) afforded 82 as a white solid (1.21 g, 66%). \([\alpha]_{D}^{23} = +9.2^\circ \ (c = 0.7, \text{dichloromethane})\). \( Rf \) (hexane: ethyl acetate 1: 1) 0.32. ESI-HRMS: \( m/z \) calcd for \( \text{C}_{105}\text{H}_{109}\text{N}_{5}\text{NaO}_{24} \) \([\text{M}+\text{Na}]^+ \) 1847.7394, found 1847.7396. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

5''-Azido-6,3',2'',3''',4'''-Penta-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxycarbonyl-4',6'-O-benzylidene-5''-deoxy paromomycin (83) A soln. of 82 (1.21 g, 0.66 mmol) in dichloromethane (12 mL) was treated with \( p \)-toluenesulfonyl chloride (1.28 g, 6.6 mmol), triethylamine (1.8 mL, 13.2 mmol) and 4-dimethylaminopyridine (80 mg, 0.66 mmol). The mixture was stirred at room temperature for 16 h, and then diluted with
ethyl acetate, washed sequentially with saturated aqueous sodium bicarbonate, 1 M hydrochloric acid and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dry dimethylformamide (12 mL) and sodium azide (430 mg, 6.6 mmol) was added. The reaction mixture was stirred at 65 °C for 15 h, and then cooled to room temperature and diluted with ethyl acetate. The mixture was washed with saturated sodium aqueous bicarbonate and brine, and the organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) to give 83 as a white solid (1.08 g, 88%). $[\alpha]_D^{23} = +23.1^\circ$ (c = 0.3, dichloromethane). $Rf$ (hexane: ethyl acetate 1: 1) 0.60. ESI-HRMS: $m/z$ calcd for C$_{105}$H$_{109}$N$_8$NaO$_{23}$ [M+Na]$^+$ 1872.7459, found 1872.7458.

This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,3’,2”,3”’,4”’-Penta-0-benzyl-1,3,2’,2”’,6”’-penta-N-benzyloxy carbonyl-4’,6’-O-benzylidene-5”-deoxy-5”-formamido paromomycin (84)** Compound 83 (1.57 g, 0.85 mmol) was dissolved in tetrahydrofuran (16 mL) and treated with trimethylphosphine (1 M in tetrahydrofuran, 1.7 mL, 1.7 mmol) at 65 °C for 1 h. Deionized water (16 mL) was added and the mixture was heated at 65 °C for another 3 h. The solvent was removed under reduced pressure, and then the residue was dissolved in dichloromethane (16 mL). Freshly prepared formic acetic anhydride (1.6 mL) was
added and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness and the residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) to afford 84 as a white solid (1.06 g, 67%). $[\alpha]_{D}^{23} = +10.0^\circ \ (c = 0.3, \text{dichloromethane}).$ $Rf$ (hexane: ethyl acetate 1: 2) 0.70. ESI-HRMS: $m/z$ calcd for $\text{C}_{106}\text{H}_{110}\text{N}_6\text{NaO}_{24} [\text{M+Na}]^+$ 1874.7503, found 1874.7505. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

$6,3',6',2'',3''',4'''$-Hexa-$O$-benzyl-$1,3,2',2''',6'''$-penta-$N$-benzyloxycarbonyl-$5''$-deoxy-$5''$-formamido paromomycin (85) A soln. of 84 (1.06 g, 0.57 mmol) in dry tetrahydrofuran (10 mL) was stirred with activated 4 Å molecular sieves and sodium cyanoborohydride (730 mg, 11.5 mmol) at room temperature under an argon atmosphere for 1 h. The mixture was cooled to 0 °C and 2 M hydrogen chloride in diethyl ether soln. (11.5 mL, 23 mmol) was added drop-wise. After stirring under an argon atmosphere at 0 °C for 2 h, the reaction mixture was added into ice-cold saturated aqueous sodium bicarbonate slowly and then extracted with ethyl acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered, and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 1: 2) gave 85 as a white solid (804 mg, 75%). $[\alpha]_{D}^{23} = +8.1^\circ \ (c = 0.7, \text{dichloromethane}).$ $Rf$ (hexane: ethyl acetate 1: 2) 0.25. ESI-HRMS: $m/z$ calcd for $\text{C}_{106}\text{H}_{112}\text{N}_6\text{NaO}_{24} [\text{M+Na}]^+$ 1876.7659, found 1876.7663. This compound was employed
in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,3',6',2'',3''',4'''-Hexa-O-benzyl-1,3,2',2'',6''-penta-N-benzylxycarbonyl-5''-deoxy-5''-formamido-4'-keto paromomycin (86)**

Dess-Martin periodinane (276 mg, 0.65 mmol) was added into a soln. of 85 (804 mg, 0.43 mmol) in dichloromethane (8 mL). The mixture was stirred at room temperature for 7 h, and then diluted with ethyl acetate, washed with saturated sodium aqueous bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) to give 86 as a white solid (574 mg, 72%). $[\alpha]_{D}^{23} = +19.3^\circ$ (c = 0.9, dichloromethane). $R_f$ (hexane: ethyl acetate 1: 2) 0.45. ESI-HRMS: $m/z$ calcd for $C_{106}H_{110}N_{6}NaO_{24}$ [M+Na]$^+$ 1874.7503, found 1874.7505. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,6',2'',3''',4'''-Penta-O-benzyl-1,3,2',2'',6''-penta-N-benzylxycarbonyl-3',5''-dideoxy-5''-formamido paromomycin (87)**

A solution of 86 (300 mg, 0.16 mmol) in dry tetrahydrofuran (1 mL) was cooled to -20 °C and treated with samarium iodide (0.1 M in tetrahydrofuran, 10 mL, 1 mmol) under an argon atmosphere for 1 h. Dry methanol (61 μL, 1.6 mmol) was added and the reaction mixture was allowed to warm up to 0 °C and stirred for 2 h under an argon atmosphere. Saturated aqueous sodium bicarbonate was added at 0 °C to quench the reaction and the mixture was extracted with ethyl
acetate. The combined organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 1: 3) gave 87 as a white solid (84 mg, 30%). \([\alpha]\)D 23 = + 11.6° (c = 1.3, dichloromethane). Rf (hexane: ethyl acetate 1: 4) 0.55. ESI-HRMS: m/z calcd for C99H106N6NaO23 [M+Na]+ 1770.7241, found 1770.7245. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

3’,5’’-Dideoxy-5’’-formamido-paromomycin (77) A stirred soln. of compound 87 (19 mg, 0.01 mmol) in a mixture of dioxane/water/acetic acid 2/2/0.1 (0.5 mL) was treated with palladium hydroxide on carbon (20 mg). The reaction mixture was stirred under a hydrogen atmosphere (48 psi) for 24 h. After filtration through a Celite pad, the cake was washed with deionized water and the filtrate was combined and concentrated under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water). Glacial acetic acid (41 μL, 0.69 mmol) was added into the product-containing fractions, and lyophilization of the mixture gave 77 as a white solid in the form of the pentaacetate salt (4.6 mg, 50%). \([\alpha]\)D 23 = + 56.0° (c = 0.1, H2O). 1H NMR (600 MHz, D2O) δ 7.99 (s, 1H, CHO), 5.31 (d, J1’-2’ = 2.4 Hz, 1H, H1’), 5.10 (br s, 2H, H1”, H1’’’), 4.29 (t, J3’-2’’ = J3’-4’’ = 5.1 Hz, 1H, H3’’), 4.20 (m, 1H, H2’’), 4.15 (t, J = 5.2 Hz, 1H, H5’’’), 4.05 (m, 2H, H4”, H3”), 3.85 - 3.74 (m, 2H, H4, H5’), 3.73 – 3.57 (m, 4H, H5”, H5’’a, H6’a), 3.56 – 3.34 (m, 6H, H2”’, H2’, H6, H4’, H5”b, H6’b), 3.34-3.11 (m, 4H, H1, H3, H6”’), 2.30 – 2.26
(m, 1H, H2eq), 2.09 – 2.05 (m, 1H, H3′eq), 1.88 – 1.84 (m, 1H, H3′ax), 1.79 (s, 15H, CH3CO2H), 1.66 – 1.62 (m, 1H, H2ax). 13C NMR (150 MHz, D2O) δ 180.3 (CH3COOH), 164.8 (CHO), 109.9 (C1′′), 95.5 (C1′), 95.4 (C1′′′), 83.2 (C5), 79.4 (C4′′), 77.2 (C4), 74.1 (C5′), 72.9 (C2′′), 72.1 (C6), 70.1 (C5′′′), 69.0 (C4′), 67.6 (C3′′′), 67.3 (C4′′′), 62.7 (C6′), 53.6 (C3′), 50.7 (C2′′′), 49.6 (C1), 48.9 (C3), 48.7 (C2′), 40.4 (C6′′′), 38.8 (C5′′), 29.2 (C2), 28.5 (C3′), 22.6 (CH3COOH), ESI-HRMS: m/z calcd for C25H47N6NaO13 [M+H]+ 627.3201, found 627.3197.

1,3,2′,2″′,6″′-Pentadeamino-1,3,2′,2″′,6″′-pentaazido-6,3′,6′,2″′,3″′,4″′-hexa-0-benzyl-5″-O-triisopropylsilyl paromomycin (88) Compound 80 (700 mg, 0.49 mmol) was dissolved in dry dichloromethane (15 mL) and treated with borane trimethylamine (150 mg, 2 mmol), freshly dried copper(II) triflate (18 mg, 0.05 mmol) and a catalytic amount of triflic acid (0.1 mL) under argon atmosphere. After stirring at room temperature for 2 h, the reaction was quenched by adding into ice-cold saturated aqueous sodium bicarbonate slowly. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification with flash column chromatography over silica gel (toluene: ethyl acetate 40:1) gave 88 as a white solid (250 mg, 35%) [α]D23 = +43.2° (c = 1.8, dichloromethane). Rf (toluene: ethyl acetate 20:1) 0.60. 1H NMR (400 MHz, chloroform-d) δ 7.48 – 7.20 (m, 35H), 6.06 (d, J1′-2′ = 3.5 Hz, 1H, H1′), 5.63 (d, J1″′-2″′ = 4.4 Hz, 1H, H1″′), 5.00 – 4.91 (m, 3H, H1″′, -CH2Ph), 4.84 (d, J = 11.1 Hz, 1H, -CH2Ph), 4.75 (d, J = 11.0 Hz, 1H, -CH2Ph), 4.70 – 4.56 (m, 4H, -CH2Ph), 4.49
(d, J = 12.4 Hz, 1H, -CH2Ph), 4.45 – 4.37 (m, 3H, H3'', -CH2Ph), 4.34 (d, J = 12.3 Hz, 1H, -CH2Ph), 4.32 – 4.27 (m, 1H, H4''), 4.20 – 4.13 (m, 1H, H5''), 4.00 – 3.66 (m, 11H, H3', H2'', H5'', H3''', H5''', H5, H4', H6'), 3.66 – 3.56 (m, 1H, H6''a), 3.49 – 3.38 (m, 2H, H1, H3), 3.36 (br s, 1H, H2'''), 3.32 – 3.17 (m, 4H, H6, H2', H4''', H6'''b), 2.24 (dt, J2eq-2ax = 12.7 Hz, J2eq-1 = J2eq-3 = 4.0 Hz, 1H, H2eq), 1.41 (q, J2ax-2eq = J2ax-1 = J2ax-3 = 12.7 Hz, 1H, H2ax), 1.21 – 0.97 (m, 21H, -Si(CH(CH3)2)3). 13C NMR (101 MHz, chloroform-d) δ 138.26 - 137.06 (aromatic), 128.69 - 127.48 (aromatic), 106.72 (C1''), 98.51 (C1'''), 96.33 (C1'), 83.50 (C4''), 83.23 (C6), 82.15 (C5), 81.59 (C2''), 79.77 (C3''), 75.86 (C4), 75.03 (C3'''), 74.87 (-CH2Ph), 73.72 (-CH2Ph), 73.51 (-CH2Ph), 73.20 (C5'''), 72.83 (C3'''), 72.57 (-CH2Ph), 72.24 (-CH2Ph), 71.91 (-CH2Ph), 71.76 (C4'''), 70.26 (C4'), 63.94 (C5'''), 62.84 (C2'), 60.51 (C3), 59.93 (C1), 57.76 (C2''), 50.97 (C6'''), 47.91 (C6'), 32.26 (C2), 18.15 (-CH(CH3)2), 12.0 (-CH(CH3)2). ESI-HRMS: m/z calcd for C74H91N15NaO14Si [M+Na]+ 1464.6537, found 1464.6532.

6,3',6',2'',3''',4'''-Hexa-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxy carbonyl-5''-O-triisopropylsilyl paromomycin (89) A soln. of 88 (250 mg, 0.17 mmol) in tetrahydrofuran (5 mL) was heated at 70 °C with trimethylphosphine (1 M in tetrahydrofuran, 1.4 mL, 1.4 mmol) for 1 h, and then 0.1 M sodium hydroxide (5 mL) was added. After stirring at 70 °C for another 3 h, the mixture was cooled to room temperature. N-(Benzyloxy carbonyloxy)succinimide (350 mg, 1.4 mmol) and potassium carbonate (200 mg, 1.4 mmol) were added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched by addition of 1 M hydrochloric acid
and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) afforded 89 as a white solid (265 mg, 79%). \([\alpha]^{23}_D = +17.6^\circ\) (c = 1.4, dichloromethane). \(Rf\) (hexane: ethyl acetate 1: 1) 0.60. ESI-HRMS: \(m/z\) calcd for C_{114}H_{131}N_5NaO_{24}Si [M+Na]^+ 2005.8885, found 2005.8888. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

**6,3',6',2'',3''',4'''-Hexa-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxycarbonyl-5''-O-triisopropylsilyl-4'-keto paromomycin (90)** Compound 89 (265 mg, 0.13 mmol) was dissolved in dichloromethane (5 mL) and treated with Dess-Martin periodinane (114 mg, 0.26 mmol). The mixture was stirred at room temperature for 20 h. Saturated aqueous sodium bicarbonate was added to quench the reaction, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification with flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) gave 90 as a white solid (192 mg, 75%). \([\alpha]^{23}_D = +38.6^\circ\) (c = 0.8, dichloromethane). \(Rf\) (hexane: ethyl acetate 1: 1) 0.55. ESI-HRMS: \(m/z\) calcd for C_{114}H_{129}N_5NaO_{24}Si [M+Na]^+ 2003.8728, found 2003.8723. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.
6,6′,2″,3‴,4″″-Penta-O-benzyl-1,3,2′,2″,6″″-penta-N-benzyloxycarbonyl-5″-O-triisopropylsilyl-3′-deoxy paromomycin (91) Samarium iodide (0.1 M in tetrahydrofuran, 15 mL, 1.5 mmol) was added into a soln. of 90 (490 mg, 0.25 mmol) in tetrahydrofuran (1.5 mL) under an argon atmosphere at -20 °C. After stirring at -20 °C for 1 h, methanol (100 μL, 2.5 mmol) was added drop-wise and the mixture was warmed up to 0 °C and stirred under an argon atmosphere for 1.5 h. Saturated aqueous sodium bicarbonate was added at 0 °C to quench the reaction and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 3: 1) to give 91 as a colorless oil (171 mg, 36%). \([\alpha]^{23}_D = + 27.6°\) (c = 1.0, dichloromethane). \(Rf\) (hexane: ethyl acetate 1: 1) 0.25. ESI-HRMS: \(m/z\) calcd for \(\text{C}_{107}\text{H}_{125}\text{N}_5\text{NaO}_{23}\text{Si}\) [M+Na]⁺ 1899.8466, found 1899.8469. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,6′,2″,3‴,4″″-Penta-O-benzyl-1,3,2′,2″,6″″-penta-N-benzyloxycarbonyl-3′-deoxy paromomycin (92) A soln. of 91 (171 mg, 0.1 mmol) in tetrahydrofuran (3 mL) was treated with tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.2 mL, 0.2 mmol) at room temperature for 3.5 h. The reaction mixture was diluted with ethyl acetate, and then washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash
column chromatography over silica gel (hexane: ethyl acetate 1: 1) afforded 92 as a white solid (100 mg, 65%). $[\alpha]_{D}^{23} = + 8.8^\circ$ ($c = 0.6$, dichloromethane). $Rf$ (hexane: ethyl acetate 1: 2) 0.45. ESI-HRMS: $m/z$ calcd for $C_{98}H_{105}N_{5}NaO_{23}$ [M+Na]$^+$ 1743.7132, found 1743.7133. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,6',2'',3''',4'''-Penta-O-benzyl-1,3,2',2'''',6'''-penta-N-benzyloxy carbonyl-3'-deoxy-5''''-O-tosyl paromomycin (93) Compound 92 (240 mg, 0.14 mmol) was dissolved in dichloromethane (5 mL). Pyridine (112 μL, 1.4 mmol) and p-toluenesulfonyl chloride (267 mg, 1.4 mmol) were added and the mixture was stirred at room temperature for 21 h. The reaction mixture was diluted with ethyl acetate, and then washed by 1 M hydrochloric acid and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) gave 93 as a white solid (180 mg, 68%). $[\alpha]_{D}^{23} = + 11.8^\circ$ ($c = 0.6$, dichloromethane). $Rf$ (hexane: ethyl acetate 1: 1) 0.27. ESI-HRMS: $m/z$ calcd for $C_{105}H_{111}N_{5}NaO_{25}S$ [M+Na]$^+$ 1897.7220, found 1897.7723. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,6',2'',3''',4''''-Penta-O-benzyl-1,3,2',2''''',6''''-penta-N-benzyloxy carbonyl-3',5'''-dideoxy-5''''-azido paromomycin (94) A soln. of 93 (200 mg, 0.11 mmol) in dry acetonitrile (4 mL) was stirred at room temperature with hexamethyldisilazane (45 μL, 0.22 mmol)
for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in dry dimethylformamide (2 mL). Lithium azide (160 mg, 3.3 mmol) was added and the mixture was heated at 65 °C for 2 h. After cooling down to room temperature, the reaction mixture was diluted with ethyl acetate, and then washed with 1 M hydrochloric acid twice. The organic layer was washed with brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification by flash column chromatography over silica gel (hexane: ethyl acetate 1: 1) afforded 94 as a colorless oil (145 mg, 75%). $[\alpha]^{23}_D = +16.0^\circ$ (c = 0.4, dichloromethane). $R_f$ (hexane: ethyl acetate 1: 1) 0.16. ESI-HRMS: $m/z$ calcd for C$_{98}$H$_{104}$N$_8$NaO$_{22}$ [M+Na]$^+$ 1768.7196, found 1768.7195. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

6,6',2'',3''',4'''-Penta-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxy carbonyl-3',5''-dide oxy-5''-formamido paromomycin (87) Trimethylphosphine (1 M in tetrahydrofuran, 60 μL, 0.06 mmol) was added into a soln. of 94 (50 mg, 0.03 mmol) in tetrahydrofuran (1 mL). The mixture was stirred at 65 °C for 1 h, and then 0.1 M sodium hydroxide (1 mL) was added. After stirring at 65 °C for 7 h, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane (1 mL) and freshly prepared formic acetic anhydride (1 mL) was added. The mixture was stirred at room temperature for 1 h and then evaporated to
dryness under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 1: 3) to afford 87 as a white solid (36 mg, 73%), with physical data identical to the previously described sample.

**1,6-Anhydro-2,4-O-ditosyl-β-D-glucopyranose (112)**

A soln. of 111 (4.9 g, 30 mmol) in a mixture of acetone/pyridine (1: 1, 96 mL) was treated with p-toluenesulfonyl chloride (13.3 g, 70 mmol) at 0 °C. The mixture was slowly warmed up to room temperature and kept stirring for 16 h. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The mixture was washed with 1 M hydrochloric acid, water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure and compound 112 was obtained as colorless oil (12 g, 85%) with data identical to the literature.

**1,6-Anhydro-3,4-epoxy-2-tosyl-β-D-glucopyranose (113)**

Compound 112 (12 g, 25.5 mmol) was dissolved in a mixture of methanol/dichloromethane (1: 2, 60 mL) and sodium methoxide (4.25 g, 78.7 mmol) was added at 0 °C. The reaction mixture was slowly warmed up to room temperature and stirred for 2 h. The mixture was diluted with dichloromethane and then washed with water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure to give 113 as white solid (6.08 g, 80%) with data identical to the literature.

**1,6-Anhydro-4-deoxy-4-C-allyl-2-tosyl-β-D-glucopyranose (114)**

Freshly prepared allyl Grignard reagent (2 M in tetrahydrofuran, 50 mL) and copper (I) iodide (360 mg, 1.89 mmol) were added into a soln. 113 (6.08 g, 20.4 mmol) in dry
tetrahydrofuran (50 mL) slowly at 0 °C under an argon atmosphere. After stirring at room temperature for 19 h, the mixture was diluted with ethyl acetate, washed with 1 M hydrochloric acid, saturated aqueous ammonium chloride, saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) to afford 114 as light yellow oil (3.37 g, 49%) with data identical to the literature.193

1,6-Anhydro-4-deoxy-4-C-allyl-2,3-epoxy-β-D-glucopyranose (115)194 A soln. of 114 (3.37 g, 9.9 mmol) in dry tetrahydrofuran (60 mL) was cooled to 0 °C and treated with sodium hydride (60% in mineral oil, 793 mg, 19.8 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature for 20 h, and then cooled to 0 °C. Saturated aqueous ammonium chloride was added to quench the reaction, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 3: 1) gave 115 as light yellow oil (1.38 g, 83%) with data identical to the literature.194

1,6-Anhydro-2-N-benzyl-2,4-dideoxy-4-C-allyl-β-D-glucopyranose (116)

Compound 115 (1.38 g, 8.2 mmol) was dissolved in benzylamine (7.9 mL) and stirred at 155 °C under an argon atmosphere for 22 h. All solvent was removed under reduced pressure and the residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) to afford 116 as a white solid (1.52 g, 67%). $[\alpha]_{D}^{23} = -49.4^\circ$
(c = 1.00, chloroform). \(Rf\) (hexane: ethyl acetate 1: 1) 0.41. \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) 7.38 – 7.23 (m, 5H: aromatic), 5.83 (ddq, \(J = 16.4, 14.4, 6.2, 5.3\) Hz, 1H: \(\text{CH}_2\text{CH}_2\text{C}-\text{C}4\)), 5.47 (s, 1H: H1), 5.21 – 5.10 (m, 2H: \(\text{CH}_2\text{CHCH}_2\text{C}-\text{C}4\)), 4.40 (d, \(J = 5.0\) Hz, 1H: H5), 4.06 (d, \(J = 7.0\) Hz, 1H: H6a), 3.93 – 3.85 (m, 2H: PhCH2), 3.80 – 3.72 (m, 1H, H6b), 3.65 (td, \(J = 2.9, 1.6\) Hz, 1H: H3), 2.68 – 2.63 (m, 1H: H2), 2.49 – 2.33 (m, 2H: \(\text{CH}_2\text{CH}_2\text{C}-\text{C}4\)), 1.77 (t, \(J = 7.2\) Hz, 1H: H4). \(^{13}\)C NMR (125 MHz, chloroform-\(d\)) \(\delta\) 139.9, 136.1 (\(\text{CH}_2\text{CHCH}_2\text{C}-\text{C}4\)), 128.5, 128.1, 127.2, 117.5 (\(\text{CH}_2\text{CHCH}_2\text{C}-\text{C}4\)), 102.6 (C1), 74.6 (C5), 70.3 (C3), 68.5 (C6), 62.2 (C2), 51.7 (PhCH2), 44.6 (C4), 36.5 (\(\text{CH}_2\text{CHCH}_2\text{C}-\text{C}4\)). ESI-HRMS: \(m/z\) calcd for C\(_{16}\)H\(_{22}\)NO\(_3\)Na [M+Na]\(^{+}\) 276.1600, found 276.1600.

1,6-Anhydro-2-N-benzylxoycarbonyl-2,4-dideoxy-4-C-propyl-\(\beta\)-D-glucopyranose

To a soln. of 116 (2.83 g, 10.3 mmol) in a mixture of methanol/acetic acid (9: 1, 30 mL) was added palladium on carbon (300 mg) and the mixture was stirred under a hydrogen atmosphere (48 psi) at room temperature for 16 h. The mixture was filtered through a Celite pad and the solid was washed with methanol. The combined filtrate was concentrated under reduced pressure and the residue was dissolved in a mixture of methanol/water (3: 1, 28 mL). Potassium carbonate (7.1 g, 51.5 mmol) and benzyl chloroformate (2.2 mL, 15 mmol) were added and the mixture was stirred at room temperature for 16 h. The mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel gave 117 as light yellow oil (2.46 g, 74%). \([\alpha]^{23}_{D} = -34.3^\circ\)
(c = 1.00, chloroform). \( Rf \) (hexane: ethyl acetate 1: 1) 0.36. \(^1\)H NMR (600 MHz, chloroform-\(d\)) \( \delta \) 7.33 – 7.22 (m, 5H: aromatic), 5.37 (s, 1H: H1), 5.18 (d, \( J = 9.0 \) Hz, 1H: NH), 5.08 (t, \( J = 6.9 \) Hz, 2H: PhCH\(_2\)), 4.36 (d, \( J = 5.3 \) Hz, 1H: H5), 4.21 (d, \( J = 6.9 \) Hz, 1H: H6a), 3.74 (t, \( J = 6.1 \) Hz, 1H: H6b), 3.69 (d, \( J = 9.1 \) Hz, 1H: H2), 3.61 (d, \( J = 3.9 \) Hz, 1H: H3), 3.24 (d, \( J = 4.8 \) Hz, 1H: HO-C3), 1.69 – 1.61 (m, 1H: H4), 1.52 (q, \( J = 12.9, 10.8 \) Hz, 1H: CH\(_3\)CH\(_2\)CH\(_2\)), 1.49 – 1.38 (m, 2H: CH\(_3\)CH\(_2\)CH\(_2\), CH\(_3\)CH\(_2\)CH\(_2\)), 1.38 – 1.26 (m, 1H: CH\(_3\)CH\(_2\)CH\(_2\)), 0.91 (t, \( J = 7.0 \) Hz, 3H: CH\(_3\)CH\(_2\)CH\(_2\)). \(^{13}\)C NMR (150 MHz, chloroform-\(d\)) \( \delta \) 155.7 (C=O), 136.1, 128.5, 128.2, 128.1, 101.2 (C1), 74.9 (C5), 71.8 (C3), 68.3 (C6), 67.1 (PhCH\(_2\)), 54.8 (C2), 44.1 (C4), 34.0 (CH\(_3\)CH\(_2\)CH\(_2\)), 20.8 (CH\(_3\)CH\(_2\)CH\(_2\)), 14.0 (CH\(_3\)CH\(_2\)CH\(_2\)).

ESI-HRMS: \( m/z \) calcd for C\(_{17}\)H\(_{23}\)NO\(_5\)Na [M+Na]\(^+\) 344.1474, found 344.1480.

1,6-Anhydro-2-N-benzylxycarbonyl-2,4-dideoxy-4-C-propyl-3-O-[(methylthio)thiocarbonyl]-\(\beta\)-D-glucopyranose (118) A soln. of 117 (1.54 g, 4.8 mmol) in dry tetrahydrofuran (40 mL) was cooled to 0 °C and sequentially treated with sodium hydride (60% in mineral oil, 230 mg, 5.8 mmol) and carbon disulfide (5.3 mL, 4.8 mmol) under argon atmosphere. After stirring for 0.5 h at room temperature, methyl iodide (1.3 mL, 24 mmol) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with diethyl ether, and then washed with water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 5: 1) to give 118 as light yellow oil (1.83 g, 93%). \([\alpha]^{23}_D = -60.0^\circ \) (c = 1.00, chloroform). \( Rf \) (hexane: ethyl acetate 6: 1) 0.17.\(^1\)H NMR (600 MHz,
chloroform-d) δ 7.41 – 7.26 (m, 5H: aromatic), 5.39 (s, 1H: H1), 5.34 (s, 1H: H3), 5.14 (d, J = 11.7 Hz, 1H: PhCH₂), 5.10 – 5.03 (m, 2H: PhCH₂, NH), 4.41 (d, J = 5.4 Hz, 1H: H5), 4.02 (d, J = 7.1 Hz, 1H: H6a), 3.90 (d, J = 9.6 Hz, 1H: H2), 3.83 (dd, J = 7.1, 5.5 Hz, 1H: H6b), 2.56 (s, 3H: CH₃S), 1.85 (t, J = 7.5 Hz, 1H: H4), 1.59 (q, J = 7.5 Hz, 2H: CH₃CH₂CH₂), 1.51 (dt, J = 14.6, 7.1 Hz, 1H: CH₃CH₂CH₂), 1.36 (dq, J = 14.9, 7.3 Hz, 1H: CH₃CH₂CH₂), 0.92 (t, J = 7.2 Hz, 3H: CH₃CH₂CH₂). ¹³C NMR (150 MHz, chloroform-d) δ 214.1 (C=S), 155.1 (C=O), 136.0, 128.6, 128.3, 100.8 (C1), 81.5 (C3), 73.6 (C5), 68.2 (C6), 67.3 (PhCH₂), 51.8 (C2), 41.4 (C4), 33.0 (CH₃CH₂CH₂), 20.7 (CH₃CH₂CH₂), 18.9 (CH₃), 13.9 (CH₃CH₂CH₂).

ESI-HRMS: m/z calcd for C₁₉H₂₅NO₅S₂Na [M+Na]⁺ 434.1072, found 434.1072.

1,6-Anhydro-2-N-benzylxycarbonyl-2,3,4-trideoxy-4-C-propyl-β-D-glucopyranose (119) Compound 118 (1.83 g, 4.4 mmol) was dissolved in dry benzene (40 mL) and heated with tris(trimethylsilyl)silane (1.64 mL, 5.3 mmol) and azobisisobutyronitrile (215 mg, 1.3 mmol) at 80 °C under an argon atmosphere for 18 h. The reaction mixture was cooled to room temperature and tetrabutylammonium fluoride (1 M in tetrahydrofuran, 13.5 mL, 13.5 mmol) was added. After stirring at room temperature for 0.5 h, the mixture was diluted with diethyl ether and washed with water and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 4: 1) afforded 119 as light yellow oil (1.14 g, 84%). [α]D²³ = -17.6° (c = 1.00, chloroform). 
Rf (hexane: ethyl acetate 4: 1) 0.22. ¹H NMR (400 MHz, chloroform-d) δ 7.42 – 7.28 (m, 5H: aromatic), 5.31 (s, 1H: H1), 5.17 – 5.07 (d, J = 5.5 Hz, 2H: NH, PhCH₂), 4.37 (d, J = 5.2
Hz, 1H: H5), 3.94 – 3.79 (m, 2H: H6a, H6b), 3.69 (t, J = 7.6 Hz, 1H: H2), 2.14 (dt, J = 15.0, 6.1 Hz, 1H: H3eq), 1.77 – 1.58 (m, 1H: CH₃CH₂CH₂), 1.57 – 1.26 (m, 5H: H3ax, H4, CH₃CH₂CH₂, CH₃CH₂CH₂CH₃CH₂CH₂), 0.91 (t, J = 7.2 Hz, 3H: CH₃CH₂CH₂). ¹³C NMR (100 MHz, chloroform-d) δ 155.6 (C=O), 128.5, 128.2, 128.1, 101.8 (C1), 75.9 (C5), 68.4 (C6), 66.8 (PhCH₂), 48.8 (C2), 36.6 (C4), 35.5 (CH₃CH₂CH₂), 25.6 (C3), 20.9 (CH₃CH₂CH₂), 14.0 (CH₃CH₂CH₂). ESI-HRMS: m/z calcd for C₁₇H₂₃NO₄Na [M+Na]⁺ 328.1525, found 328.1531.

1,6-Anhydro-2-azido-2,3,4-trideoxy-4-C-propyl-β-D-glucopyranose (120)

Palladium on carbon (1.44 g) was added into a soln. of 119 (1.44 g, 4.4 mmol) in a mixture of ethanol/acetic acid (3: 0.1, 15.5 mL). The mixture was stirred at room temperature under a hydrogen atmosphere (48 psi) for 24 h and then filtered through a Celite pad. The cake was washed with ethanol and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture of methanol/water (1: 1, 28 mL). Imidazolesulfonyl azide hydrochloride salt (1.38 g, 6.6 mmol), potassium carbonate (3.04 g, 22 mmol) and copper(II) sulfate pentahydrate (110 mg, 0.44 mmol) were added at 0 °C and the mixture was warmed up to room temperature and stirred for 17 h. The reaction mixture was acidified by adding 1 M hydrochloric slowly until pH = 2-3 at 0 °C, and then extracted with ethyl acetate. The organic layer was washed with brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification by flash column chromatography (hexane: ethyl acetate 6: 1) gave 120 as a colorless oil (674 mg, 78%).
$[\alpha]_{D}^{23} = -108.2^\circ$ (c = 1.00, chloroform). $R_f$ (hexane: ethyl acetate 4: 1) 0.58. $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ 5.32 (dt, $J$ = 2.3, 1.3 Hz, 1H: H1), 4.42 (d, $J$ = 5.2 Hz, 1H: H5), 3.92 – 3.82 (m, 2H: H6), 3.50 (dt, $J$ = 5.5, 1.8 Hz, 1H: H2), 2.15 (ddd, $J$ = 15.4, 6.8, 5.4 Hz, 1H: H3$_{eq}$), 1.81 (dddd, $J$ = 13.7, 10.0, 7.9, 5.9 Hz, 1H: CH$_3$CH$_2$CH$_2$), 1.68 – 1.57 (m, 2H: H3$_{ax}$, CH$_3$CH$_2$CH$_2$), 1.53 – 1.45 (m, 1H: H4), 1.53 – 1.45 (m, 1H: H4), 1.47 – 1.31 (m, 2H: CH$_3$CH$_2$CH$_2$), 0.95 (t, $J$ = 7.3 Hz, 3H: CH$_3$CH$_2$CH$_2$). $^{13}$C NMR (125 MHz, chloroform-$d$) $\delta$ 100.6 (C1), 76.0 (C5), 68.2 (C6), 57.6 (C2), 36.0 (C4), 34.5 (CH$_3$CH$_2$CH$_2$), 24.2 (C3), 20.8 (CH$_3$CH$_2$CH$_2$), 14.0 (CH$_3$CH$_2$CH$_2$). ESI-HRMS: $m/z$ calcd for C$_9$H$_{15}$N$_3$O$_2$Na [M+Na]$^+$ 220.1062, found 220.1056.

**Phenyl 2-azido-2,3,4-trideoxy-4-C-propyl-1-thio-β-D-glucopyranoside (121)** To a soln. of 120 (264 mg, 1.34 mmol) in 1,2-dichloroethane (8 mL) were added zinc(II) iodide (1.27 g, 4 mmol) and trimethyl(phenylthio)silane (0.76 mL, 4 mmol). The mixture was stirred at room temperature for 20 h. After diluting with 1,2-dichloroethane, the mixture was filtered through a Celite pad. The solid was washed with 1,2-dichloroethane and the combined filtrate was washed with saturated aqueous sodium bicarbonate and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in a mixture of methanol/water (10: 1, 11 mL) and stirred with potassium carbonate (372 mg, 2.7 mmol) at room temperature for 1 h. The mixture was diluted with ethyl acetate, washed with brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification by flash column chromatography over silica gel (hexane: ethyl acetate 6: 1)
afforded **121** as colorless oil and an anomeric mixture (343 mg, 83%, α: β = 2.4: 1). *Rf* (hexane: ethyl acetate 3: 1) 0.38. α anomer: ¹H NMR (500 MHz, chloroform-*d*) δ 7.58 – 7.48 (m, 2H: aromatic), 7.37 – 7.24 (m, 3H: aromatic), 5.55 (dd, *J* = 4.9, 1.3 Hz, 1H: H1), 4.04 (ddd, *J* = 10.5, 5.8, 2.5 Hz, 1H: H5), 3.84 (dt, *J* = 12.5, 4.6 Hz, 1H: H2), 3.81 – 3.73 (m, 1H: H6a), 3.67 – 3.61 (m, 1H: H6b), 2.07 (dtd, *J* = 13.0, 4.3, 1.5 Hz, 1H: H3(eq), 1.82 (t, *J* = 6.2 Hz, 1H: HO-C6), 1.78 – 1.68 (m, 1H: H4), 1.54 (q, *J* = 12.5 Hz, 1H: H3(ax), 1.50 – 1.15 (m, 4H: CH₃CH₂CH₂, CH₃CH₂CH₂, CH₃CH₂CH₂, CH₃CH₂CH₂), 0.94 (t, *J* = 7.1 Hz, 3H: CH₃CH₂CH₂). ¹³C NMR (125 MHz, chloroform-*d*) δ 133.6, 132.6, 129.1, 127.7, 88.6 (C1), 73.8 (C5), 62.8 (C6), 59.3 (C2), 35.2 (C4), 33.1 (CH₃CH₂CH₂), 29.8 (C3), 19.1 (CH₃CH₂CH₂), 14.2 (CH₃CH₂CH₂). β anomer: ¹H NMR (500 MHz, chloroform-*d*) δ 7.58 – 7.48 (m, 2H: aromatic), 7.37 – 7.24 (m, 3H: aromatic), 4.50 (d, *J* = 9.9 Hz, 1H: H1), 3.81 – 3.73 (m, 1H: H6a), 3.61 – 3.55 (m, 1H: H6b), 3.31 (ddd, *J* = 11.5, 9.9, 4.8 Hz, 1H: H2), 3.24 (ddd, *J* = 9.6, 6.6, 2.6 Hz, 1H: H5), 2.33 (dt, *J* = 13.0, 4.4 Hz, 1H: H3(eq), 2.16 (t, *J* = 6.6 Hz, 1H: H3(ax), 1.68 – 1.58 (m, 1H: H4), 1.50 – 1.15 (m, 5H: CH₃CH₂CH₂, CH₃CH₂CH₂, CH₃CH₂CH₂, CH₃CH₂CH₂), 1.12 – 1.00 (m, 1H: CH₃CH₂CH₂), 0.90 (t, *J* = 7.1 Hz, 1H: CH₃CH₂). ¹³C NMR (125 MHz, chloroform-*d*) δ 132.7, 132.2, 129.0, 128.0, 88.2 (C1), 83.3 (C5), 63.1 (C6), 59.7 (C2), 35.6 (C3), 35.3 (C4), 32.7 (CH₃CH₂CH₂), 19.2 (CH₃CH₂CH₂), 14.2 (CH₃CH₂CH₂). ESI-HRMS: *m/z* calcd for C₁₅H₂₁N₃O₂SNa [M+Na]⁺ 330.1252, found 330.1252.

**Phenyl 6-O-benzyl-2-azido-2,3,4-trideoxy-4-C-propyl-1-thio-β-D-glucopyranosyl sulfoxide (123)** A soln. of 121 (343 mg, 1.12 mmol) was treated with sodium hydride (60% in mineral oil, 67 mg, 1.7 mmol), benzyl bromide (0.2 mL, 1.7 mmol) and
tetrabutylammonium iodide (41 mg, 0.11 mmol) at 0 °C under an argon atmosphere. The mixture was allowed to warm up to room temperature and stirred for 16 h. After quenching by addition of methanol at 0 °C, the reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. After purification by flash column chromatography over silica gel (hexane: ethyl acetate 12: 1), the result compound (400 mg) was dissolved in a mixture of acetonitrile/water (10: 1, 5 mL). A soln. of L-selectfluor (242 mg, 0.68 mmol) in a mixture of acetonitrile/water (10: 1, 5 mL) and sodium bicarbonate (240 mg, 2.85 mmol) were added. After stirring at room temperature for 1.5 h, the mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane and washed with saturated aqueous sodium bicarbonate/water (1: 9) and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) afforded 123 as colorless oil (192 mg, 73%) and an inseparable anomic mixtures, which are also a mixture of diastereomers at sulfur. Rf (hexane: ethyl acetate 3: 1) 0.17. ESI-HRMS: m/z calcd for C_{22}H_{27}N_{3}NaO_{3}S [M+Na]^{+} 436.1671, found 436.1676. This compound was employed in the next step without further characterization because the NMR spectrum showed a complex mixture of isomers.

1,3,2',2''',6'''-Pentadeamino-1,3,2',2''',6'''-pentaazido-6,3',2'',3''',4''''-penta-O-benzy 1-5'''-O-triisopropylsilyl paromomycin (128) Compound 80 (3 g, 2.1 mmol) was
dissolved in methanol (60 mL) and iodine (600 mg, 2.4 mmol) was added. The reaction mixture was stirred at 70 °C for 3 h under an argon atmosphere. After cooling down to room temperature, 20% sodium thiosulfate soln. was added to quench the reaction. The mixture was extracted with ethyl acetate and the organic layer was washed with saturated aqueous sodium bicarbonate and brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) gave 128 as a white solid (1.5 g, 53%). [α]D^23 = +58.3° (c = 1.2, dichloromethane). Rf (hexane: ethyl acetate 2: 1) 0.28. \(^1\)H NMR (600 MHz, chloroform-\(d\)) δ 7.42 – 7.15 (m, 25H, aromatic), 6.10 (d, \(J_{1'-2'} = 3.7\) Hz, 1H, H1'), 5.57 (d, \(J_{1''-2''} = 1.3\) Hz, 1H, H1''), 4.96 (d, \(J = 11.3\) Hz, 1H, -CH\(_2\)Ph), 4.91 (d, \(J = 10.9\) Hz, 1H, -CH\(_2\)Ph), 4.88 (d, \(J_{1''-2''} = 1.3\) Hz, 1H, H1''), 4.75 – 4.68 (m, 2H, -CH\(_2\)Ph), 4.61 (d, \(J = 11.9\) Hz, 1H, -CH\(_2\)Ph), 4.53 (d, \(J = 11.8\) Hz, 1H, -CH\(_2\)Ph), 4.44 – 4.39 (m, 2H, -CH\(_2\)Ph), 4.36 (t, \(J_{3''-4''} = J_{3''-5''} = 4.6\) Hz, 1H, H3''), 4.33 (d, \(J = 11.9\) Hz, 1H, -CH\(_2\)Ph), 4.29 (d, \(J = 12.0\) Hz, 1H, -CH\(_2\)Ph), 4.25 (dd, \(J_{4''-5''} = 7.6\) Hz, \(J_{4''-3''} = 4.6\) Hz, 1H, H4''), 3.96 (td, \(J_{5''-4''} = J_{5''-6''} = 9.6\) Hz, \(J_{5''-6''} = 2.4\) Hz, 1H, H5''), 3.92 (t, \(J_{2''-1''} = J_{2''-3''} = 4.6\) Hz, 1H, H2''), 3.89 – 3.80 (m, 3H, H6'b, H3'', H5''), 3.80 – 3.74 (m, 3H, H6'b, H3'', H5'''), 3.65 (t, \(J_{4,5} = J_{4,3} = 9.0\) Hz, 1H, H4), 3.61 – 3.51 (m, 2H, H4', H6''), 3.46 – 3.39 (m, 2H, H1, H3), 3.32 (t, \(J_{2''-1''} = J_{2''-3''} = 1.3\) Hz, 1H, H2'''), 3.28 (t, \(J_{6-5} = J_{6-1} = 9.0\) Hz, 1H, H6), 3.22 – 3.14 (m, 3H, H2', H4''', H6'''), 2.24 (dt, \(J_{2eq,2ax} = 13.2\) Hz, \(J_{2eq-1} = J_{2eq-3} = 4.5\) Hz, 1H, H2eq), 1.42 (q, \(J_{2ax,2eq} = J_{2ax-3} = J_{2ax,1} = 13.2\) Hz, 1H, H2ax), 1.11 – 1.00 (m, 21H, -Si(CH(CH\(_3\))\(_2\))\(_3\)). \(^{13}\)C NMR (151 MHz, cdcl\(_3\)) δ 138.0 - 137.0 (aromatic), 128.6 - 127.4 (aromatic), 106.8 (C1''), 98.5 (C1'''), 96.2 (C1').
83.5 (C6), 83.3 (C4''), 82.4 (C5), 81.5 (C2''), 79.9 (C3'), 75.9 (C4), 74.9 (C3''), 74.8 (-CH₂Ph), 73.7 (-CH₂Ph), 73.1 (C5''), 72.7 (C3''), 72.6 (-CH₂Ph), 72.5 (-CH₂Ph), 71.8 (C4''), 71.6 (-CH₂Ph), 71.5 (C5'), 70.6 (C4'), 64.0 (C5''), 63.0 (C2'), 62.3 (C6'), 60.5 (C3''), 60.0 (C1), 57.6 (C2''), 51.0 (C6''), 32.2 (C2), 18.1 (-CH(CH₃)₂), 12.0 (-CH(CH₃)₂).

ESI-HRMS: m/z calcd for C₆₇H₈₅N₁₅O₁₄Si [M+Na]+ 1374.6067, found 1374.6071.

5-O-[3-O-(2,6-Diazo-2,6-dideoxy-3,4-di-O-benzyl-α-L-idopyranosyl)-2-O-benzyl-5-O-triisopropylsilyl-β-D-ribofuranosyl]-1,3-dideoxy-1,3-diazido-6-O-benzyl-2-deoxystreptamine (129) A soln. of 128 (1.5 g, 1.1 mmol) in dry dichloromethane (30 mL) was treated with Dess-Martin periodinane (1.0 g, 2.4 mmol) at room temperature and stirred for 1 h. 3-Chloroperoxybenzoic acid (296 mg, 1.3 mmol) was added and the mixture was kept stirring at room temperature for 16 h. After adding 3 M sodium hydroxide soln. (4.9 mL) and 20% sodium thiosulfate soln. (4.9 mL), the mixture was stirred at room temperature for 1 h. The reaction mixture was then extracted with ethyl acetate and the organic layer was washed with water and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (hexane: ethyl acetate 4: 1) to give 129 as colorless oil (586 mg, 50%). [α]D²³ = +83.2° (c = 0.8, dichloromethane). Rf (hexane: ethyl acetate 2: 1) 0.66. ¹H NMR (600 MHz, chloroform-d) δ 7.40 – 7.11 (m, 20H), 5.31 (d, J₁₋₂'' = 3.3 Hz, 1H, H1''), 4.95 (d, J₁''₋₂'' = 1.9 Hz, 1H, H1'''), 4.89 (d, J = 10.9 Hz, 1H, -CH₂Ph), 4.83(d, J = 11.2 Hz, 1H, -CH₂Ph), 4.64 (d, J = 12.0 Hz, 1H, -CH₂Ph), 4.51 (t, J₃₋₄'' = J₃₋₄ = 4.7 Hz, 1H, H3''), 4.47 (d, J = 11.8 Hz, 1H, -CH₂Ph), 4.43 (d, J = 11.8 Hz, 1H, -CH₂Ph),
4.37 (d, J = 9.6 Hz, 1H, -CH2Ph), 4.34 – 4.32 (m, 1H, H4”), 4.31 – 4.27 (m, 2H, -CH2Ph), 4.04 (dd, J2”-3” = 4.7 Hz, J2’-1’ = 3.3 Hz, 1H, H2”), 3.94 (dd, J5”a-4” = 2.7 Hz, J5”a-5”b = 11.7 Hz, 1H, H5”a), 3.90 – 3.86 (m, 1H, H5’”), 3.83 (dd, J6”a-6”b = 11.7 Hz, J6”b-4” = 3.6 Hz, 1H, H5”b), 3.81 – 3.78 (m, 1H, H3’”), 3.69 (dd, J6”a-6”b = 12.9 Hz, J6”a-5” = 8.4 Hz, 1H, H6”a), 3.53 (t, J2”-3” = J2’-1’ = 1.9 Hz, 1H, H2”), 3.48 (t, J5”a-5”b = 9.3 Hz, 1H, H5), 3.46 – 3.40 (m, 2H, H1, H4), 3.40 – 3.34 (m, 1H, H3), 3.29 (t, J6-5 = J6-1 = 9.3 Hz, 1H, H6), 3.19 (t, J4”-5” = J4”-3” = 2.4 Hz 1H, H4”), 3.07 (dd, J6”-b-6”a = 12.9 Hz, J6”-b-5” = 4.3 Hz, 1H, H6”b), 2.09 (dt, J2eq-2ax = 13.2 Hz, J2eq-1eq = 4.4 Hz, 1H, H2eq), 1.28 (q, J2ax-2eq = J2ax-1eq = J2ax-3 = 13.2 Hz, 1H, H2ax). 13C NMR (151 MHz, cdcl3) δ 137.9 – 136.8 (aromatic), 128.7 – 127.3 (aromatic), 107.0 (C1”), 98.5 (C1’”), 86.4 (C5), 83.8 (C4”), 83.0 (C6), 81.7 (C2”), 75.4 (C4), 75.3 (-CH2Ph), 75.1 (C3”), 74.1 (C5’”), 72.8 (C3”), 72.5 (-CH2Ph), 72.4 (-CH2Ph), 71.7 (-CH2Ph), 71.5 (C4’”), 63.2 (C5”), 60.5 (C1), 59.5 (C3), 57.5 (C2””), 51.1 (C6”), 18.0 (-CH(CH3)2), 11.9 (-CH(CH3)2). ESI-HRMS: m/z calcd for C54H70N12NaO10Si [M+Na]+ 1097.5005, found 1097.5008.

1,3,2’,2”’,6”’-Pentadeamino-1,3,2’,2”’,6”’-pentaazido-6,6’,2”’,3”’,4”’-penta-O-benzy

l-3’-4’-dideoxy-4’-C-propyl paromomycin (133) Glycosyl donor 123 (192 mg, 0.46 mmol), glycosyl acceptor 129 (444 mg, 0.41 mmol), 2,4,6-tri-tert-butylpyrimidine (571 mg, 2.3 mmol), and activated 4Å molecular sieves (700 mg) were put in a flame dried flask and dry dichloromethane (7.2 mL) was added under an argon atmosphere. The mixture was stirred at room temperature for 1 h, and cyclohexene (48 μL, 0.47 mmol) was added at -60 °C. After stirring at -60 °C for 0.5 h, freshly distilled
trifluoromethanesulfonic anhydride (81 μL, 0.48 mmol) was added and the mixture was stirred at -60 °C under an argon atmosphere for 17 h. Triethylamine (0.6 mL) was added to quench the reaction and the mixture was allowed to warm up to room temperature. The mixture was filtered through a Celite pad and washed with dichloromethane. The combined filtrate was washed with saturated aqueous sodium bicarbonate and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. After purification by flash column chromatography over silica gel (hexane: ethyl acetate 9: 1), the resulting compound (223 mg,) was dissolved in tetrahydrofuran (5 mL) and tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.2 mL) was added. After stirring at room temperature for 1 h, the mixture was concentrated under reduced pressure and purified by flash column chromatography over silica gel (hexane: ethyl acetate 3: 1) to afford 133 as colorless oil (107 mg, 36%).

\[ [\alpha]^{23}_D = +39.0^\circ \ (c = 0.2, \text{dichloromethane}) \]

\[ Rf \; (\text{hexane: ethyl acetate 3: 1}) \; 0.24 \]

\[ ^1H \text{NMR} \; (400 \text{ MHz, chloroform-}d) \delta 7.41 - 7.11 \; (m, 25H), \; 5.90 \; (d, J_{1'-2'} = 3.4 \text{ Hz, } 1H, \; H1'), \; 5.71 \; (d, J_{1'-2'} = 6.2 \text{ Hz, } 1H, \; H1''), \; 4.97 - 4.92 \; (m, 2H, \; -CH}_2\text{Ph, H1''}), \; 4.74 - 4.64 \; (m, \; 3H, \; -CH}_2\text{Ph),} \; 4.61 \; (d, \; J = 12.1 \text{ Hz, } 1H, \; -CH}_2\text{Ph),} \; 4.55 \; (d, \; J = 11.6 \text{ Hz, } 1H, \; -CH}_2\text{Ph),} \; 4.48 \; (d, \; J = 12.2 \text{ Hz, } 1H, \; -CH}_2\text{Ph),} \; 4.38 \; (d, \; J = 12.1 \text{ Hz, } 1H, \; -CH}_2\text{Ph),} \; 4.35 - 4.31 \; (m, \; 1H, \; H4''), \; 4.31 - 4.22 \; (m, \; 2H, \; -CH}_2\text{Ph),} \; 4.12 - 4.08 \; (m, \; 1H, \; H3''), \; 3.99 - 3.93 \; (m, \; 1H, \; H2''), \; 3.93 - 3.86 \; (m, \; H5'), \; 3.85 - 3.78 \; (m, \; 1H, \; H5''a), \; 3.78 - 3.65 \; (m, \; 5H, \; H3'''', \; H5''', \; H4, \; H5, \; H5''b), \; 3.65 - 3.55 \; (m, \; 3H, \; H6', \; H6''''a), \; 3.50 - 3.35 \; (m, \; 2H, \; H1, \; H3), \; 3.33 - 3.24 \; (m, \; 2H, \; H6, \; H2'''), \; 3.13 \; (br s, \; 1H, \; H4''''), \; 3.07 - 2.99 \; (m, \; 2H, \; H6''''b, \; H2'), \; 2.24 \; (dt, \; J_{2eq-2ax} = 13.0 \text{ Hz,} \; J_{2eq-1} = J_{2eq-3} = 4.3 \text{ Hz, } 1H,
H2eq), 2.02 – 1.94 (m, 1H, H3’eq), 1.93 – 1.75 (m, 2H, H3’ax, H4’), 1.48 – 1.30 (m, 2H, H2ax, CH2CH3), 1.30 – 1.16 (m, 2H, CH2CH3, CH2CH2CH3), 1.15 – 1.05 (m, 1H, CH2CH2CH3), 0.86 (t, J = 7.0 Hz, 3H, CH3). ESI-HRMS: m/z calcd for C61H71N15NaO12 [M+Na]+ 1228.5304, found 1228.5309.

6,6’,2”,3””,4””-Penta-O-benzyl-1,3,2’,2”’,6”’-penta-N-(benzyloxycarbonyl)-3’,4’-did epoxy-4’-C-propyl paromomycin (134) A soln. of 133 (107 mg, 0.09 mmol) in tetrahydrofuran (2 mL) was treated with trimethylphosphine (1 M in tetrahydrofuran, 0.72 mL, 0.72 mmol). After heating at 70 °C for 1 h under an argon atmosphere, deionized water (0.8 mL) was added and the mixture was kept stirring at 70 °C for 3 h. All solvent was evaporated under reduced pressure and the residue was dissolved in a mixture of methanol/water (1: 1, 2 mL). The mixture was stirred with potassium carbonate (99 mg, 0.72 mmol) and N-(benzyloxycarbonyloxy)succinimide (180 mg, 0.72 mmol) at room temperature for 16 h, and then extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) afforded 134 as colorless oil (117 mg, 74%). [α]23° = + 12.3° (c = 1.0, dichloromethane). Rf (hexane: ethyl acetate 1: 1) 0.62. ESI-HRMS: m/z calcd for C101H111N5NaO22 [M+Na]+ 1769.7652, found 1769.7651. This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.
6,6',2'',3''',4'''-Penta-O-benzyl-1,3,2',2'',6'''-penta-N-(benzyloxy carbonyl)-3',4',5''-trIDEOXY-5''-azido-4'-C-propyl paromomycin (135) To a soln. of 134 (117 mg, 0.07 mmol) in dry dichloromethane (2 mL) were added p-toluenesulfonyl chloride (127 mg, 0.7 mmol), triethylamine (0.16 mL, 1.3 mmol) and 4-dimethylaminopyridine (0.8 mg, 0.007 mmol). After stirring at room temperature for 22 h, the mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dimethylformamide (2 mL) and stirred with sodium azide (78 mg, 1.2 mmol) at 70 °C for 19 h. After cooling down to room temperature, the mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane: ethyl acetate 2: 1) to give 135 as a colorless oil (94 mg, 79%). [α]$_D^{23}$ = +17.9 (c = 1.1, dichloromethane). $R_f$(hexane: ethyl acetate 1: 1) 0.50. ESI-HRMS: $m/z$ calcd for C$_{101}$H$_{110}$N$_8$NaO$_{21}$ [M+Na]$^+$ 1794.7717, found 1794.7714.

This compound was employed in the next step without further characterization. The presence of rotamers in the multiple Cbz groups rendered the NMR spectrum uninterpretable.

1,3,2',2'',6'''-Pentadeamino-1,3,2',2'',6'''-pentaaZido-6,6',2'',3''',4'''-penta-O-benzy l-3',4'-dideoxy-4'-C-propyl-5''-O-tosyl paromomycin (138) Compound 133 (92 mg, 0.08 mmol) was dissolved in dry dichloromethane (2 mL) and treated with
p-toluenesulfonyl chloride (146 mg, 0.8 mmol), triethylamine (0.18 mL, 1.5 mmol) and 4-dimethylaminopyridine (0.9 mg, 0.008 mmol). The reaction mixture was stirred at room temperature for 24 h, and then extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate and brine and dried with sodium sulfate. Filtration, concentration under reduced pressure and purification by flash column chromatography over silica gel (hexane: ethyl acetate 4: 1) gave 138 as a colorless oil (83 mg, 80%). $[\alpha]_{D}^{23} = +42.8^\circ$ (c = 0.2, dichloromethane). $R_f$ (hexane: ethyl acetate 3: 1) 0.45. $^1$H NMR (600 MHz, chloroform-d) $\delta$ 7.77 (dd, $J = 12.0$, 5.5 Hz, 2H, aromatic), 7.40 – 7.17 (m, 27H), 5.82 (d, $J = 3.5$ Hz, 1H, H1’), 5.54 (d, $J = 5.0$ Hz, 1H, H1”), 4.89 (d, $J = 10.9$ Hz, 1H, -CH$_2$Ph), 4.69 (d, $J = 12.1$ Hz, 1H, -CH$_2$Ph), 4.68 – 4.64 (m, 2H, H1”’), 4.59 (d, $J = 12.0$ Hz, 1H, -CH$_2$Ph), 4.55 – 4.51 (m, 2H, -CH$_2$Ph), 4.47 (d, $J = 11.7$ Hz, 1H, -CH$_2$Ph), 4.42 (d, $J = 11.9$ Hz, 1H, -CH$_2$Ph), 4.34 (d, $J = 11.9$ Hz, 1H, -CH$_2$Ph), 4.31 – 4.24 (m, 3H, H4”, H5”a, -CH$_2$Ph). 4.22 (dd, $J_{5''b-5''a} = 10.3$ Hz, $J_{5''b-6''} = 2.5$ Hz, 1H, H5”b), 4.09 (dd, $J_{3''-4''} = 5.1$ Hz, $J_{3''-5''} = 3.4$ Hz, 1H, H3”), 3.90 – 3.84 (m, 2H, H2”, H5”), 3.76 – 3.69 (m, 4H, H3””, H5””, H4, H5), 3.67 – 3.62 (m, 2H, H6”), 3.58 (dd, $J_{6''-a-6''b} = 12.6$ Hz, $J_{6''-b-6''} = 7.8$ Hz, 1H, H6”’a), 3.39 – 3.33 (m, 2H, H1, H3), 3.26 (d, $J_{2''-1''} = 2.1$ Hz, 1H, H2”’), 3.21 – 3.14 (m, 2H, H4””, H6), 3.12 (dd, $J_{6''-a-6''b} = 12.6$ Hz, $J_{6''-b-6''} = 5.4$ Hz, 1H, H6””b), 2.92 – 2.86 (dt, $J_{2''-3''ax} = 12.0$ Hz, $J_{2''-1''} = J_{2''-3''eq} = 3.5$ Hz, H2’), 2.35 (s, 3H, -PhCH$_3$), 2.22 – 2.14 (m, 1H, H2eq), 1.90 – 1.86 (m, 1H, H3’eq), 1.79 – 1.72 (m, 2H, H3’ax, H4’), 1.46 – 1.37 (m, 1H, CH$_2$CH$_3$), 1.29 (q, $J_{2ax-1} = J_{2ax-3} = J_{2ax-2eq} = 12.6$ Hz, 1H, H2ax) 1.26 – 1.18 (m, 2H, CH$_2$CH$_3$, CH$_2$CH$_2$CH$_3$), 1.13 – 1.06 (m, 1H, CH$_2$CH$_2$CH$_3$), 0.87 (t, $J = 7.2$ Hz, 3H, CH$_3$). $^{13}$C NMR (151
MHz, chloroform-d) δ 144.7 (aromatic), 138.5 - 136.9 (aromatic), 132.9 (aromatic), 129.9 - 127.5 (aromatic), 106.1 (C1''), 99.1 (C1''''), 95.7 (C1'), 83.8 (C6), 81.6 (C5), 81.3 (C2''), 80.3 (C4'''), 77.5 (C3''), 75.0 (-CH2Ph), 74.6 (C4), 73.7 (-CH2Ph), 73.3 (-CH2Ph), 73.2 (C3''''), 73.1 (C5'''), 72.5 (-CH2Ph), 72.2 (C5'), 72.0 (-CH2Ph), 71.9 (-CH2Ph), 71.2 (C4'''), 70.0 (C6'), 69.2 (C5''), 60.5 (C3), 60.5 (C1), 57.4 (C2''''), 57.1 (C2'), 50.7 (C6'''), 35.1 (C4'), 33.3 (CH2CH2CH3), 32.7 (C2), 27.2 (C3'), 21.6 (CH3Ph), 19.1 (CH2CH3), 14.3 (CH2CH3). ESI-HRMS: m/z calcd for C68H77N15NaO14S [M+Na]+ 1382.5393, found 1382.5390.

**1,3,2''',6'''-Pentadeamino-1,3,2''',6'''-pentaazido-6,6',2'',3''',4'''-penta-O-benzyll-3',4',5''-trideoxy-4'-C-propyl-5''-phthalimido paromomycin (139)** A soln. of 138 (83 mg, 0.06 mmol) in dry dimethylformamide was stirred with potassium phthalimide (222 mg, 1.2 mmol) at 70 °C for 24 h under an argon atmosphere. After cooling down to room temperature, the mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried with sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography over silica gel (hexane: ethyl acetate 3: 1) gave 139 as a white solid (72.3 mg, 89%). [α]$_D^{23}$ = +71.6° (c = 0.5, dichloromethane). Rf (hexane: ethyl acetate 3: 1) 0.18. $^{1}$H NMR (600 MHz, chloroform-d) δ 7.84 (dd, J = 5.3, 3.1 Hz, 2H, aromatic), 7.58 (dd, J = 5.4, 3.0 Hz, 2H, aromatic), 7.40 – 7.14 (m, 25H, aromatic), 5.65 (d, J$_{1''-2'''}$ = 3.5 Hz, 1H, H1'), 5.43 (d, J$_{1'-2'''}$ = 3.6 Hz, 1H, H1''), 4.80 (d, J$_{1''-2'''}$ = 1.9 Hz, 1H, H1'''), 4.74 – 4.63 (m, 3H, -CH2Ph), 4.56 – 4.52 (m, 3H, -CH2Ph), 4.49 (d, J = 11.9 Hz, 1H, -CH2Ph), 4.44 – 4.41
(m, 1H, H4’’), 4.40 (d, J = 12.5 Hz, 1H, -CH₂Ph), 4.37 (t, J₃''-₂'' = J₃''-₄'' = 4.8 Hz, 1H, H3’’), 4.33 (d, J = 12.0 Hz, 1H, -CH₂Ph), 4.26 (d, J = 12.0 Hz, 1H, -CH₂Ph), 4.16 (dd, J₅''a-₅''b = 14.1 Hz, J₅''-₄'' = 4.7 Hz, 1H, H5’’a), 3.96 (t, J₂''-₁'' = J₂''-₃'' = 3.6 Hz, 1H, H2’’), 3.91 (dd, J₅''b-₅''a = 14.1 Hz, J₅''-₄'' = 7.2 Hz, 1H, H5’’b), 3.87 (dt, J = 6.8, 3.2 Hz, 1H, H5’’), 3.76 – 3.69 (m, 2H, H₃’’’,H₅’’’), 3.67 – 3.64 (m, 2H, H₆’’), 3.60 (t, J₅-₄ = J₅-₆ = 9.1 Hz, 1H, H5), 3.42 (t, J₄-₅ = J₄-₃ = 9.1 Hz, 1H, H4), 3.38 – 3.27 (m, 3H, H6’’’, H1, H3), 3.26 (t, J₄-₃ = J₄-₂ = 1.9 Hz, 1H, H2’’), 3.21 – 3.14 (m, 3H, H6’’’, H2’, H4’’’), 2.81 (t, J = 9.1 Hz, 1H, H6), 2.15 (dt, Jeq-2ax = 13.2 Hz, Jeq-3 = Jeq-1 = 4.6 Hz, 1H, H2eq), 1.97 (dt, J₃eq-₃ax = 12.3 Hz, J₃eq-4’ = J₃eq-2’ = 4.0 Hz, 1H, H3’eq), 1.86 – 1.79 (m, 1H, H4’), 1.74 (q, J₃ax-₃eq = J₃ax-2' = J₃ax-4' = 12.3 Hz, 1H, H3'ax), 1.47 – 1.35 (m, 1H, CH₂CH₃), 1.33 – 1.16 (m, 3H, H2ax, CH₂CH₃, CH₂CH₂CH₃), 1.14 – 1.05 (m, 1H, CH₂CH₂CH₂CH₃), 0.86 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (151 MHz, chloroform-d) δ 168.3 (-CON-), 138.4 – 132.3 (aromatic), 128.6 – 127.1 (aromatic), 106.2 (C1’), 98.7 (C1’’’), 96.0 (C1’), 83.2 (C6), 80.9 (C4’’’), 80.4 (C2’’’), 79.8 (C5), 77.4 (C3’’’), 75.5 (C4), 74.5 (-CH₂Ph), 73.2 (-CH₂Ph), 73.1 (C3’’’), 73.0 (C5’’’), 72.4 (-CH₂Ph), 72.3 (C5’), 71.9 (-CH₂Ph), 71.7 (-CH₂Ph), 71.2 (C4’’’), 70.0 (C6’), 60.5 (C3), 60.0 (C1), 57.8 (C2’’), 57.5 (C2’’’), 50.4 (C6’’’), 39.5 (C5’’’), 35.1 (C4’’’), 33.3 (CH₂CH₂CH₂CH₃), 32.4 (C2), 27.2 (C3’’), 19.1 (CH₂CH₃), 14.2 (CH₃). ESI-HRMS: m/z calcd for C₆₉H₇₄N₁₆O₁₃ [M+Na]^+ 1357.5519, found 1357.5520.

1,3,2’,2’’’,6’’’-Pentadeamino-1,3,2’,2’’’,6’’’-pentaazido-6,6’’,2’’,3’’’,4’’’-penta-O-benzy
l-3’,4’’,5’’-trideoxy-4’-C-propyl-5’’-formamido paromomycin (140) Compound 139 (36 mg, 0.03 mmol) was dissolved in a mixture of methanol/chloroform (1: 1, 1mL) and
treated with hydrazine hydrate (10 μL, 0.18 mmol). The reaction mixture was stirred at room temperature for 4 h, and then concentrated under reduced pressure. The residue was dissolved in dry dichloromethane (2 mL) and freshly prepared formic acetic anhydride (0.1 mL) was added. After stirring at room temperature for 0.5 h, the mixture was concentrated under reduced pressure and purified by flash column chromatography over silica gel (hexane: ethyl acetate 2: 1) to afford 140 as colorless oil (22 mg, 60%). [α]23° = +77.2° (c = 0.5, dichloromethane). Rf (hexane: ethyl acetate 1: 1) 0.44. 1H NMR (499 MHz, chloroform-d) δ 8.17 (s, 1H), 7.40 – 7.19 (m, 25H), 5.90 (d, J1′-2′ = 3.5 Hz, 1H, H1′), 5.61 (d, J1′-2′ = 4.9 Hz, 1H, H1′′), 4.91 – 4.82 (m, 2H, H1′′′, -CH2Ph), 4.80 – 4.68 (m, 2H, -CH2Ph), 4.64 (d, J = 11.9 Hz, 1H, -CH2Ph), 4.58 – 4.47 (m, 3H, -CH2Ph), 4.44 (d, J = 12.0 Hz, 1H, -CH2Ph), 4.41 – 4.32 (m, 2H, -CH2Ph), 4.18 (t, J = 4''-5'' = 9.3 Hz, J4''-3'' = 4.5 Hz, 1H, H4′′′), 4.10 (t, J3''-2'' = J3''-4'' = 4.5 Hz, 1H, H3′′′), 3.94 – 3.88 (m, 1H, H5′), 3.85 (t, J5'-4' = J5-6 = 8.9 Hz, 1H, H5), 3.81 – 3.71 (m, 5H, H2''′, H3''′, H5''′, H6''′, H4), 3.68 – 3.60 (m, 3H, H6′, H5''′a), 3.55 (dt, 1H, Js-a.5-b = 5.0 Hz, J5-b-4 = 9.3 Hz, H5''′b), 3.48 – 3.37 (m, 2H, H1, H3), 3.36 – 3.21 (m, 4H, H2''′, H4''′, H6''′, H6), 3.07 (dt, J2-3'' = 13.0 Hz, J2-1'' = J2-3eq = 3.5 Hz, 1H, H2′), 2.25 (dt, J2eq-2ax = 13.0 Hz, J2eq-1 = J2eq-3 = 4.4 Hz, 1H, H2eq), 2.03 (2.09 – 1.99, m, 1H, H3′eq), 1.94 – 1.74 (m, 2H, H3′ax, H4′), 1.49 – 1.33 (m, 2H, H2ax, CH2CH3), 1.33 – 1.21 (m, 2H, CH2CH3, CH2CH2CH3), 1.16 – 1.06 (m, 1H, CH2CH2CH3), 0.89 (t, J = 7.0 Hz, 3H, CH3). 13C NMR (126 MHz, chloroform-d) δ 161.3 (-CHO), 138.2 - 137.0 (aromatic), 128.7 - 127.3 (aromatic), 105.7 (C1′′′), 99.2 (C1′′′′), 96.0 (C1′), 83.9 (C6), 81.7 (C5), 81.5 (C2′′), 80.9 (C4′′), 76.2 (C3′′), 75.0 (-CH2Ph), 74.9 (C4), 73.9 (C5′′′), 73.3 (C5′),
73.2 (-CH₂Ph), 73.0 (C3''), 72.5 (-CH₂Ph), 72.4 (-CH₂Ph), 72.0 (-CH₂Ph), 71.4 (C4''), 69.4 (C6'), 60.5 (C3), 60.4 (C1), 57.5 (C2'), 57.2 (C2''), 50.8 (C6'''), 39.5 (C5''), 35.0 (C4'), 33.2 (C₆H₂CH₂CH₃), 32.6 (C2), 26.8 (C3'), 19.1 (CH₂CH₃), 14.2 (CH₃). ESI-HRMS: m/z calcld for C₆₂H₇₂N₁₆NaO₁₂ [M+Na]⁺ 1255.5413, found 1255.5415.

3',4',5''-Trideoxy-4'-C-propyl-5''-formamido paromomycin 101 Palladium hydroxide on carbon (24 mg) was added into a soln. of 140 (12 mg, 0.01 mmol) in a mixture of dioxane/10% AcOH (2: 1, 0.6 mL). The mixture was stirred under a hydrogen atmosphere (48 psi) for 7 d, and then filtered through a Celite pad. The solid was washed with deionized water and the combined filtrate was concentrated under reduced pressure and purified by Sephadex C-25 column chromatography (gradient elution of 0.1% - 1.0% ammonium hydroxide in deionized water). The product-containing fraction was treated with acetic acid (30 μL, 0.50 mmol) and then lyophilized to give 101 as a white powder in the form of pentaacetate salt (4.0 mg, 42%).

[a]D²³ = +66.4° (c = 0.2, H₂O). ¹H NMR (600 MHz, D₂O) δ 8.00 (s, 1H, -CHO), 5.39 (d, J₁''-₂'' = 3.7 Hz, 1H, H1'), 5.15 (d, J₁''-₂'' = 3.0 Hz, 1H, H1''), 5.11 (br s, 1H, H1'''), 4.27 (t, J₃''-₂'' = J₃''-₄'' = 5.4 Hz, 1H, H3''), 4.18 (dd, J = 5.0, 3.0 Hz, 1H, H2''), 4.14 (t, J = 4.7 Hz, 1H, H5'''), 4.09 – 4.04 (m, 2H, H4'', H3''''), 3.74 (t, J₄-₃ = J₄-₅ = 9.3 Hz, 1H, H4), 3.71 – 3.65 (m, 3H, H6'a, H5, H4''''), 3.56 – 3.52 (m, 2H, H5', H6'b), 3.52 – 3.47 (m, 3H, H5'', H6), 3.45 – 3.41 (m, 2H, H2', H2''''), 3.40 – 3.36 (m, 1H, H, H3), 3.29 – 3.19 (m, 2H, H6''''), 3.15 (td, J₁-₂ax = J₁-₆ = 12.4 Hz, J₁-2eq = 3.9 Hz, 1H, H1), 2.24 (dt, J₂eq-₂ax = 12.0 Hz, J₂eq-₁ = J₂eq-₃ = 3.9 Hz, 1H, H2eq), 1.93 (dt, J₃eq-₃ax = 12.1 Hz, J₃eq-₂'' = J₃eq-₄' = 4.1 Hz, 1H, H3'eq), 1.76 (s, 15H,
\(CH_3CO_2\text{H})\), 1.66 – 1.53 (m, 2H, H2ax, H4’), 1.43 (q, \(J_{3'ax-3'eq} = J_{3'ax-2'} = J_{3'ax-4'} = 12.1\) Hz, 1H, H3’ax), 1.28 – 1.20 (m, 2H, CH2CH2CH3, CH2CH3), 1.11 – 1.05 (m, 1H, CH2CH3), 1.01 (dd, \(J = 18.8, 9.4\) Hz, 1H, CH2CH2CH3), 0.70 (t, \(J = 6.9\) Hz, 1H, CH3). \(^{13}\)C NMR (151 MHz, D2O) \(\delta\)

179.8 (CH3CO2H), 164.9 (-CHO), 109.8 (C1’’’), 95.3 (C1’’’’), 94.7 (C1’), 83.8 (C5), 79.5 (C4’’’), 78.3 (C4), 76.7 (C3’’’), 75.5 (C6), 72.8 (C2’’’), 71.6 (C5’), 70.1 (C5’’’), 67.6 (C4’’’’), 67.3 (C3’’’’), 61.2 (C6’), 50.7 (C2’’’’), 49.5 (C1), 49.1 (C3), 48.9 (C2’), 40.4 (C6’’’’), 39.5 (C5’’’), 33.7 (C4’), 32.1 (CH2CH2CH3), 28.1 (C2), 26.4 (C3’), 22.3 (CH3CO2H), 18.3 (CH2CH3), 13.2 (CH3). ESI-HRMS: \(m/z\) calcd for C27H54N6O12 [M+H]^+ 653.3721, found 653.3724.
REFERENCES


(3), 317-326.


73. Fausti, S. A.; Rappaport, B. Z.; Schechter, M. A.; Frey, R. H.; Ward, T. T.; Brummett, R.


86. Fernandez, L.; Gooderham, W. J.; Bains, M.; McPhee, J. B.; Wiegand, I.; Hancock, R. E.,


4603-4606.


159


180. Degtyareva, N. N.; Gong, C.; Story, S.; Levinson, N. S.; Oyelere, A. K.; Green, K. D.;


ABSTRACT

MODIFICATION OF 4,5-AMINOGLYCOSIDES TO OVERCOME DRUG RESISTANCE BACTERIA AND TOXIC SIDE EFFECT

by

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Infectious diseases causing by antibiotic resistant pathogen are one of the major threat to human health and society today. Many researchers tried to develop next generation of antibiotics by reinvesting the existing antibacterial drugs. Aminoglycosides have long been used as highly potent and broad-spectrum antibiotics for treating bacterial infections. But their side effect, especially the irreversible ototoxicity, and the fast-growing resistant problem limit their application. The goal of this research was to develop next generation of AGAs that are less toxic and resistance-proof by modifying known aminoglycosides.

Chapter one briefly explains the MDR bacterial infection problem and its influence. Aminoglycosides are also well discussed in this chapter, including their history, classifications, mechanism of action, toxicity and resistance problems, as well as the recent research advances.

Chapter two discusses the synthesis and biological evaluation of
6′-deshydroxymethyl paromomycin The loss of activity shows in the biological test suggested that the 6′-deshydroxymethyl modification was not an effective modification.

Chapter three discusses the 3′-deoxy modification on different 4,5-AGAs. A novel synthetic method utilizing samarium iodide reduction to achieve 3′-deoxyxygenation modification is introduced. This new method shows good substrate compatibility and avoids the tedious scheme in the traditional method. The 3′-deoxy 4,5-AGAs retain their antibacterial activity and exhibit activity against some AGA resistance strains. But they still suffers from APH(3′,5″) resistance mechanism.

Chapter four describes the synthesis and biological test results of the 3′,5″-dideoxy-5″-formamido paromomycin. The synthesis of this doubly modified compound demonstrates the wide application potency of the samarium iodide reduction for 3′-deoxy modification. The biological experiment results show that the doubly modified compound has good antibacterial activity even in the presence of some common AMEs.

Chapter five discussed the synthesis and biological evaluation of a triply modified paromomycin derivative. The combination of 3′-deoxy, 4′-deoxy-4′-C-propyl and 5″-deoxy-5″-formamido modification into paromomycin leads to unexpected loss of antiribosomal and antibacterial activity.

Finally, chapter six documents the experiment procedure and characterization data for the synthesized compounds and chapter seven presents the overall conclusion.
AUTOBIOGRAPHICAL STATEMENT

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EDUCATION

2013-Present
PhD. in Organic Chemistry
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