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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

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DISSERTATION

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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.

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helpful staff in the chemistry department, as well as all my supportive friends and

family.

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A	Adenine
AAC	Aminoglycoside acetyltransferases
Ac	Acetyl
ACN	Acetonitrile
AcOH	Acetic acid
ADME	Absorption, distribution, metabolism, and excretion
ADP	Adenosine diphosphate
AGA	Aminoglycoside antibiotics
AIBN	Azobisisobutyronitrile
AME	Aminoglycoside modifying enzymes
ANT	Aminoglycoside nucleotidytransferases
APH	Aminoglycoside phosphotransferases
ATP	Adenosine triphosphate
Bn	Benzyl
Bz	Benzoyl
c	Concentration
C	Cytosine
^{o}C	Celsius
Calcd.	Calculated
Cbe	Ethoxycarbonyl

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 ribonucl
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Molecular sieves
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
Ph	Phenyl
PMe$_3$	Trimethylphosphine
PTSA	*p*-Toluenesulfonic acid
Py	Pyridine
ROS	Reactive oxygen species
RNA	Ribonucleic acid
RT	Room temperature

Stick's reagent	Imidazolesulfonyl azide hydrochloride salt
Su	Succinimide
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanefulfonic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TMSSPh	Trimethyl(phenylthio)silane
tRNA	Transfer ribonucleic acid
Ts	*p*-Toluenesulfonyl
TTBP	2,4,6-Tri-*tert*-butylpyrimidine
TTMSS	Tris(trimethylsilyl)silane
U	Uracil
UV	Ultraviolet
WHO	World Health Organization

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 (Telavancin[7] and Ceftaroline fosamil[8]) 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,[9] we hope to be able to

bypass those resistance mechanisms and achieve novel antibiotics.

Number of Antibiotics Approvals per Year
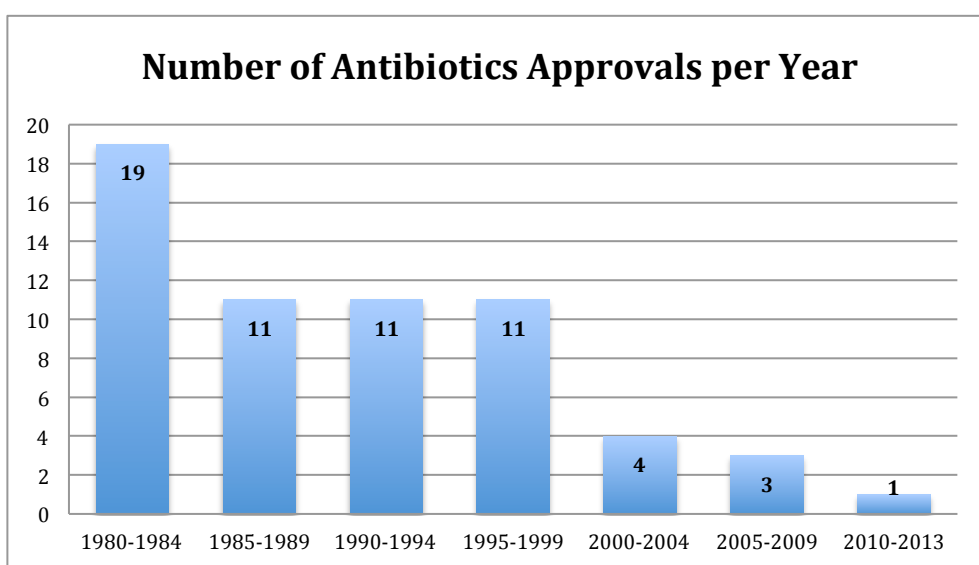
Figure 1. **Numbers of antibacterial approvals from 1980 till 2013**

Antibacterials are a type of antimicrobial agent used in the treatment of bacterial

infections. They either inhibit the rapid grow of or kill bacteria. Based on their

mechanisms of action, antibiotics can be divided into four different categories (Figure

2):[10]

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)
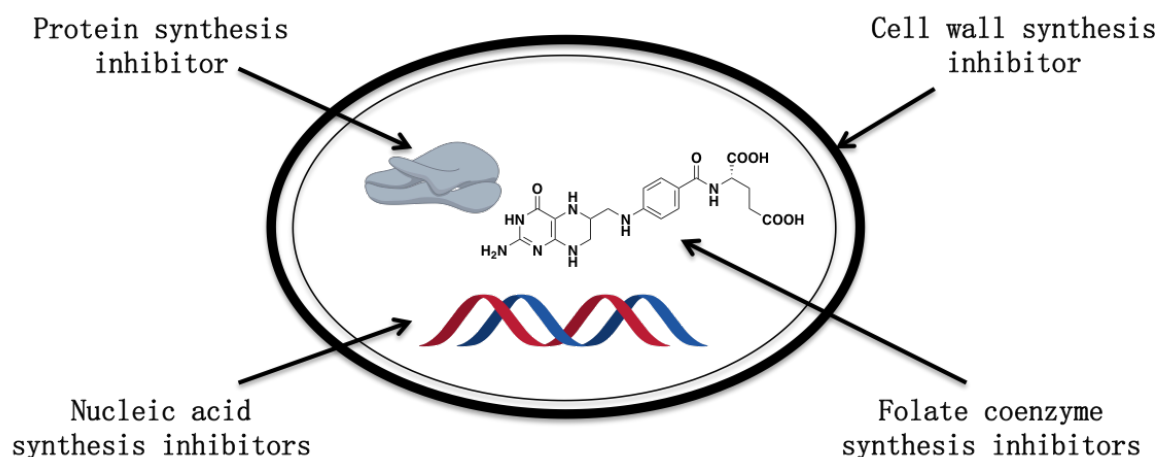
Figure 2. **Four major categories of antibiotics**

Aminoglycosides (AGAs) are a family of water-soluble, poly-cationic amino sugar molecules that can inhibit the protein synthesis of bacteria.[11] The first AGA was called streptomycin **1** (Figure 3), and was discovered by Nobel Prize winner Selman Waksman in 1944.[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.[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 *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.[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,[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]
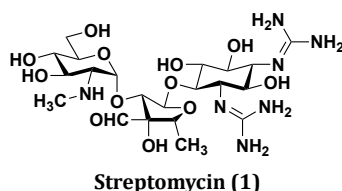
Streptomycin (1)

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).
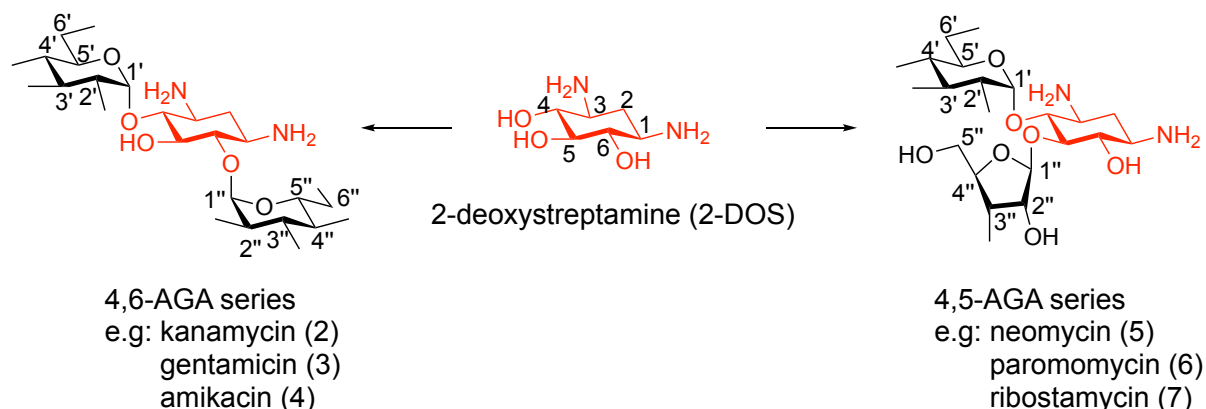
Figure 4. Structure and classification of major AGAs

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*,[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[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)[25] and Spectinomycin **10** (three fused ring system and an aminocyclotol ring called spectinamine)[26-27] are another two examples of AGAs that have unusual structures.
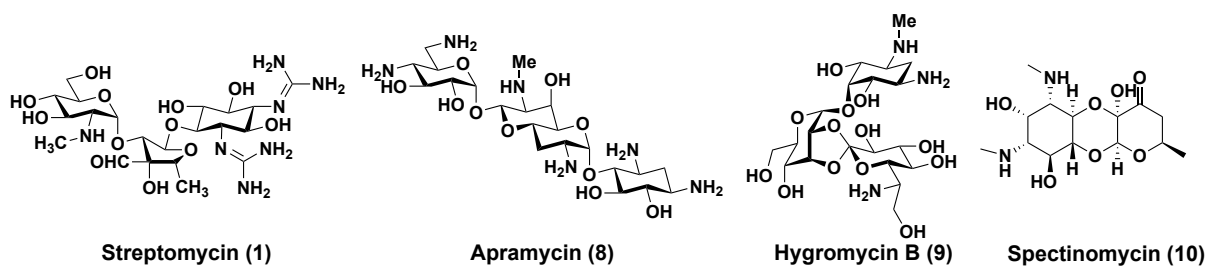
Figure 5. Unusual aminoglycoside structures

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.[28] After decades of intense study, scientists confirmed that the activities of the aminoglycosides can be mainly attributed to their action as protein synthesis inhibitors,[21, 29-30] 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).[31-32] 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).
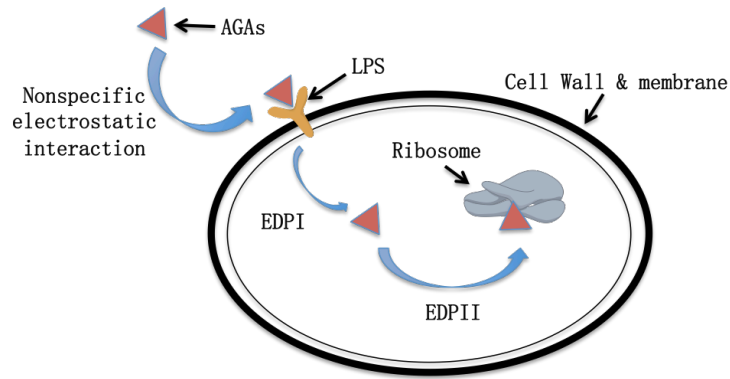
Figure 6. **The uptake of AGAs**

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.
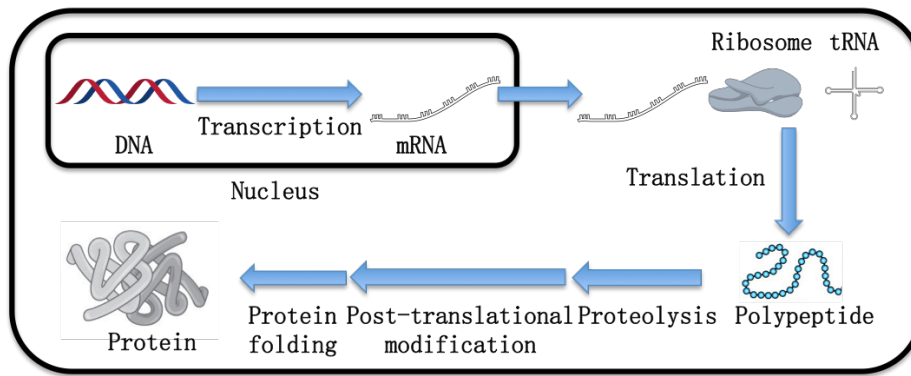
Figure 7. The overall process of protein synthesis

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 the 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.
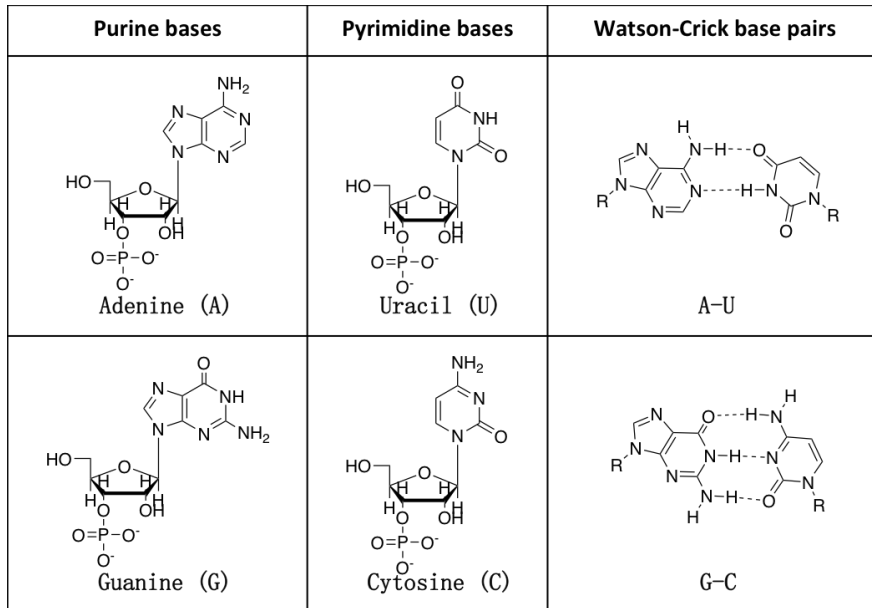
Figure 8. **Structure of four nucleotides found in RNA and their Watson-Crick base pairs**

The high accuracy of the translation process, with errors estimated at 4×10^{-4} per codon,[33] 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).[34] The A-site recognizes and binds the tRNA that carries the corresponding amino acid based on the codon on mRNA[35]; 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).
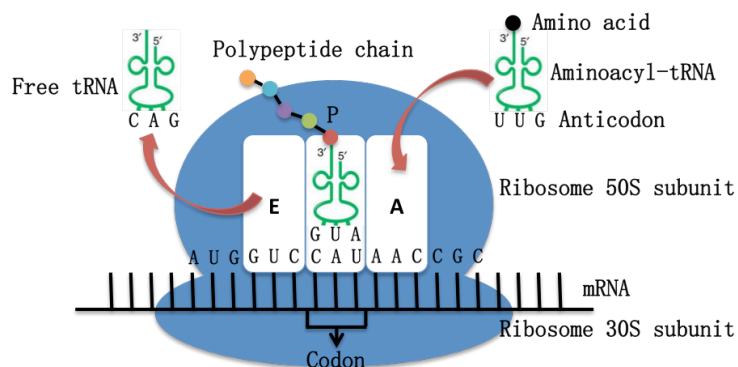
Figure 9. **Ribosomes in protein synthesis**

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).[36] This is a conserved loop that belongs to the small ribosomal subunit's A-site.[37] AGAs can selectivity 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 NH_2 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).[37-40] 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).[37] 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).[41-42]
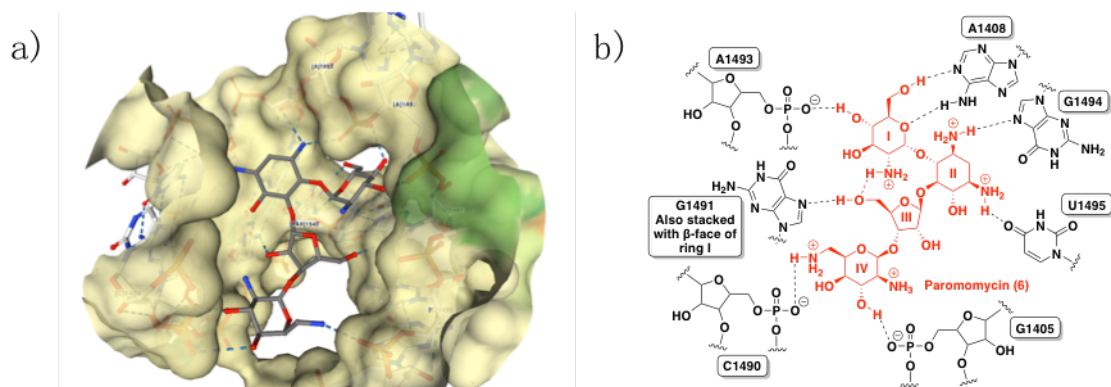
Figure 10. **a) Crystal structure of paromomycin (6) binding to bacterial ribosome A-site (PDB: 1FJG). b) Key hydrogen bonding interactions between paromomycin (6) and A-site.**

- Tobramycin (11), a 4,6-AGA
- Paromomycin (6), a 4,5-AGA
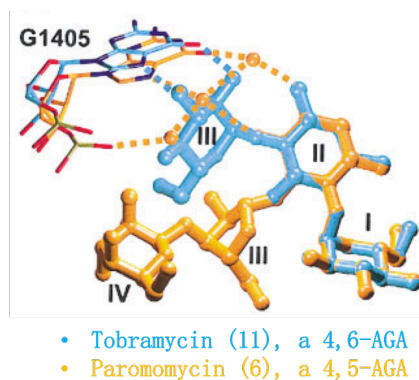
Figure 11. **The binding similarity and difference between 4,5- and 4,6-AGAs**

The A-site contains three unpaired adenine residues (A1408, A1492 and A1493) and adapts a 'flipped-out' conformation when tRNA binds (Figure 12).[43-44] 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.[45] The interactions between AGAs and ribosomal A-site lock the decoding loop into a similar conformation to that observed during mRNA decoding (Figure 12).[46-47] 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.[43-44, 48] 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).[49-50]
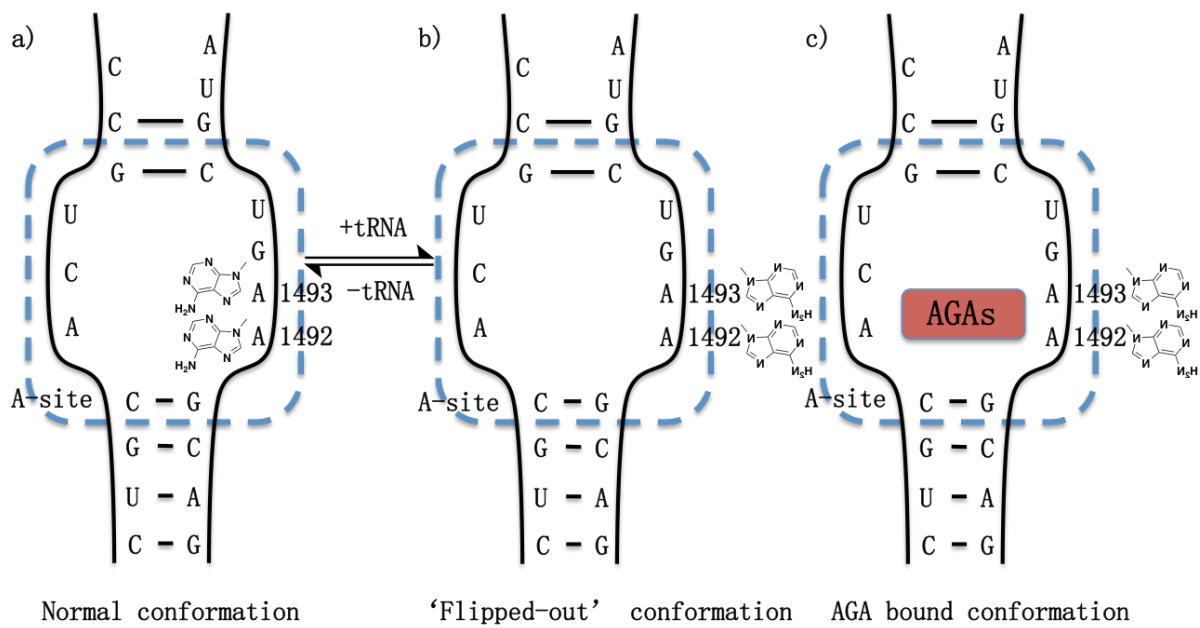
Figure 12. **Conformational changes of A-site: a) normal conformation; b) 'flipped-out' conformation; c) AGAs bound conformation**
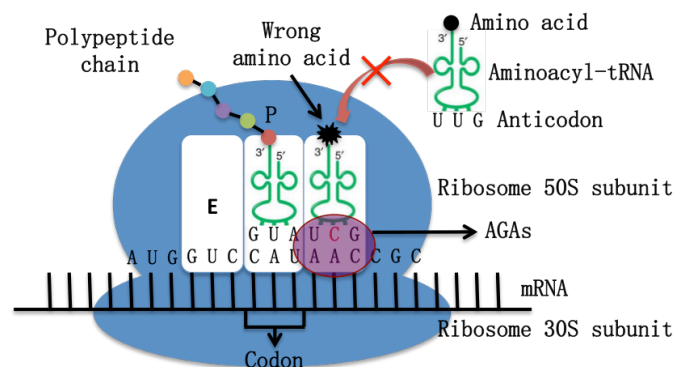
Figure 13. **The binding of AGAs interferes with protein synthesis**

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.[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.[53]

1.4. Selectivity and toxicity problems

AGAs are useful in treating infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas, Acinetobacter*, and *Enterobacter*. But severe toxicity problems, including ototoxicity (irreversible hearing loss) and nephrotoxicity (reversible kidney damage),[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.[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[58] after glomerular filtration.[59] AGAs bind to the acidic phospholipids on the brush-border cell membrane due to their cationic form under physiological condition,[60] and then are transferred to the transmembrane protein megalin, with which they become internalized in endosomes.[61] Once passing through cell membranes, the accumulated AGAs are mainly localized with in endosomal and lysosomal vacuoles,[62-63] where they inhibit the activity of lysosomal

phospholipase[64] and cause an abnormal increase in the size and number of lysosomals

that eventually leads to death of proximal tubular cells and kidney damage.[65]

Nephrotoxicity is reversible, but can be fatal owing to permanent kidney damage

and kidney failure if untreated.[17] 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.[66] 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.[57, 67] 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 **4**)[68] or by co-administration of other drugs that can

competitively bind to the phospholipids (i.e. polyaspartic acid).[69] Some reports also

show that hydration therapy can alleviate the symptoms of AGA-induced

nephrotoxicity.[17]

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 ototoxicty.[70] 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.[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.[83]

1.4.3.   Cell-free functional ribosomal assays (IC$_{50}$)

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).[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.[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 Univeristy 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).[84] Those recombinant hybrids are used in cell free translation assays and the IC_{50} value of each synthesized AGAs can be easily tested.
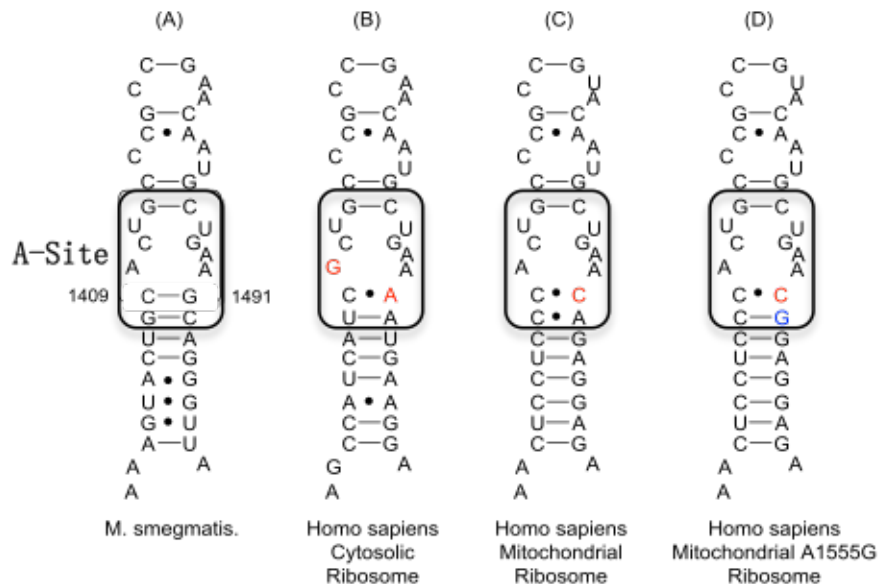
Figure 14. **Different rRNA sequences of decoding-sites in the small ribosomal subunits: (A) Wild-type ribosomes of *M. smegmatis*. (B) Human cytoplasmic ribosomes. (C) Human mitochondrial ribosomes. (D) Mitochondrial ribosomes**

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).
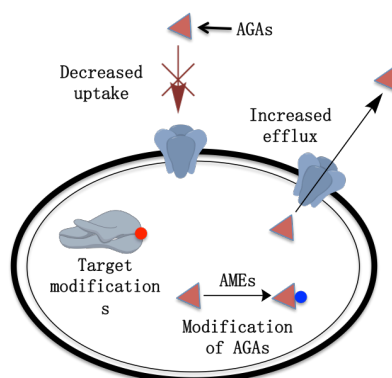
Figure 15. **Resistance mechanisms of AGAs**

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.[85-87] Other modifications include adding phosphoethanolamine[88] or an unusual lipid called diacylphosphatidyllinositol dimannoside.[89] 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 chacpter.[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.[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.
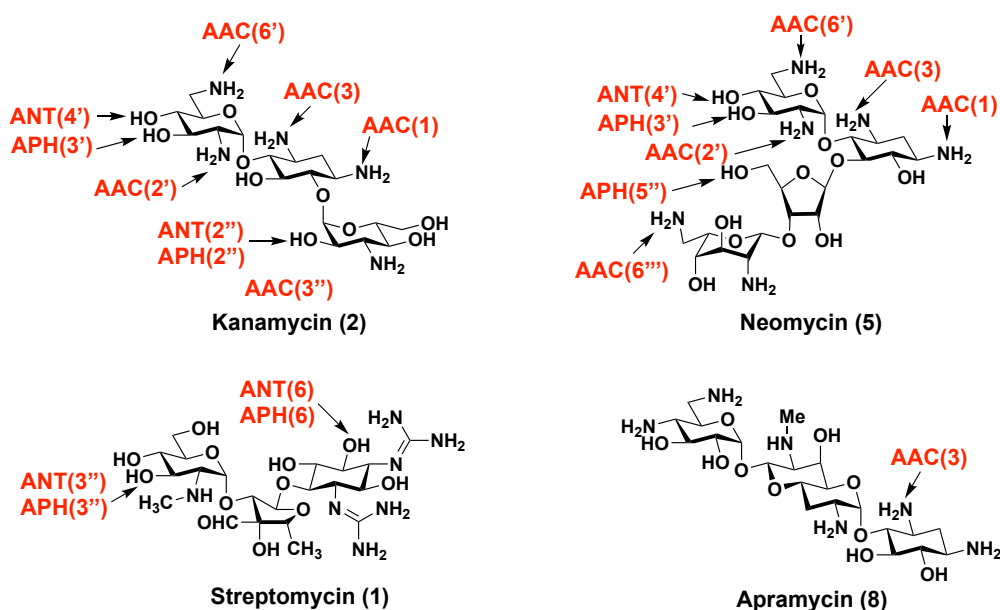
Figure 16. Target sites of AMEs on kanamycin (2), neomycin (5), streptomycin (1), and apramycin (8)

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.[107] 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.[108-109] There are nine subclasses of AAC(3) described to date, which are all in Gram-negative bacteria.[109] They confer resistance to most 4,6-AGAs including gentamicin **3**, tobramycin **11**, sisomicin **14**, fortimicin **15**, dibekacin **16**, and netilmicin **17**.[109-110] AAC(2') have been found in Gram-negative and *Mycobacteria*, they also mediate modifications of 4,6-AGAs.[109, 111] 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.[112]

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.[113-114] 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.[109]

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,[17] and only five classes have been identified so far [ANT(6), ANT(9), ANT(4'), ANT(2''), and ANT(3'')].[109] The most commonly found ANTs are ANT(3''), but they are not considered as serious problems because they specify resistant to streptomycin **1** and spectinomycin **10**. ANT(2''), on the other hand, are of significant clinical importance for their abilities to inactivate gentamicin **3**, amikacin **4** and tobramycin **11**.[115]

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,[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.[116-121] In another study, cationic antimicrobial peptides, such as bovine peptide indolicidin, showed inhibition activities against APHs and AACs.[122] Two non-carbohydrate di-amine derivatives[123] and some known eukaryotic protein kinase inhibitors[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.[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.
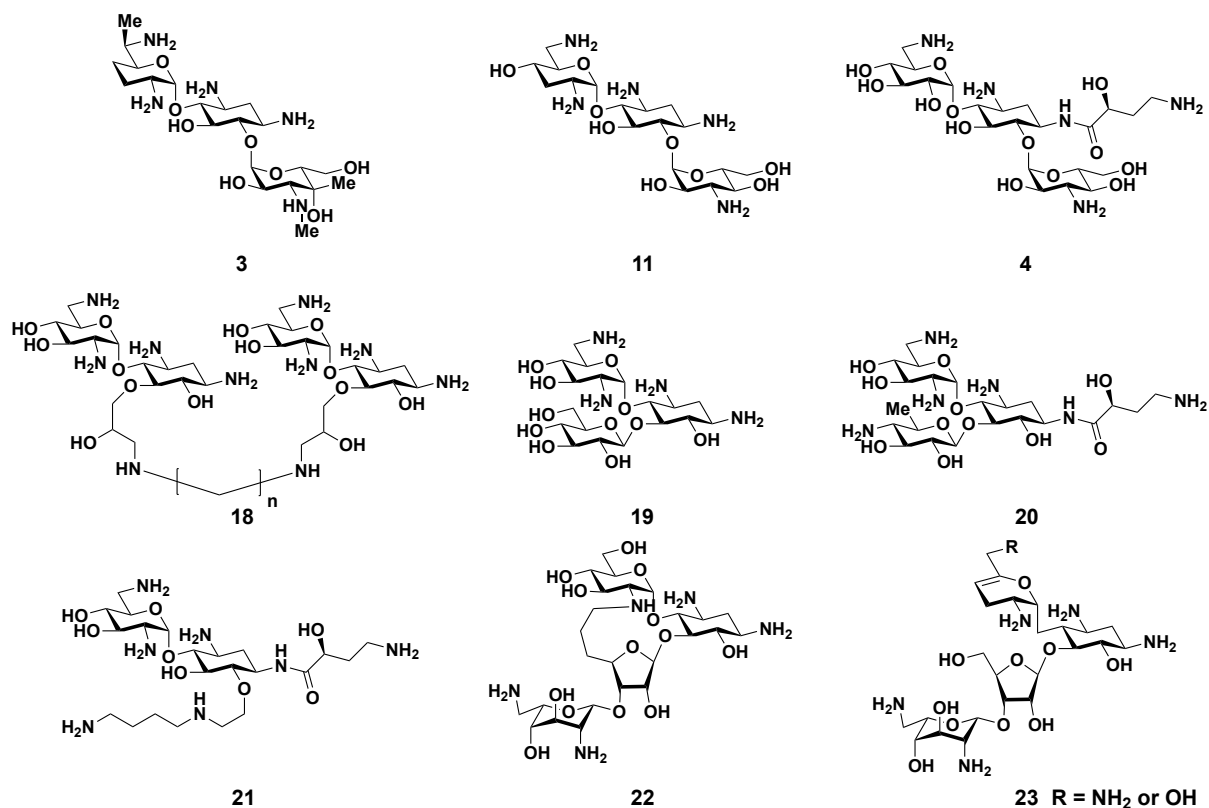
Figure 17. Modified AGAs that overcome AME resistance mechanisms

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.[140-141] 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.[142] 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.[143-144] The intrinsic level of expression of efflux pumps is so small that it contributes little to resistance in normal situations.[145] 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.[145] 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.[145]

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 **6** 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).[146-149]
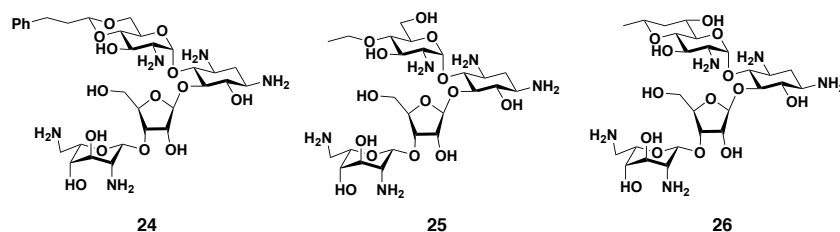
Figure 18. **Structure of 4',6'-O-alkyldiene (24), 4'-O-alkyl (25) and bicyclic ring I paromomycin derivatives (26) that exhibit increased selectivity toward bacterial A-site**

Apramycin **8** 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.[150] 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.[151] Now, apramycin is expected to go into a phase I clinical trial in Europe.

Plazomicin **27** is a semisynthetic aminoglycoside that has been developed by the Achaogen Company[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.[153] It is 6'-(2-hydroxyethyl)-1-(4-amino-2(S)-hydroxybutyryl) sisomicin,[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'').
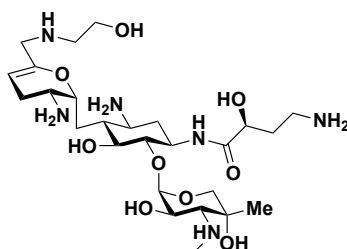
Plazomicin (27)

Figure 19. **Structure of plazomicin (27)**

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'-MODIFICATON 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 NH_2) 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.[37-40] 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,[109] 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.[109] 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**.[149] These existing

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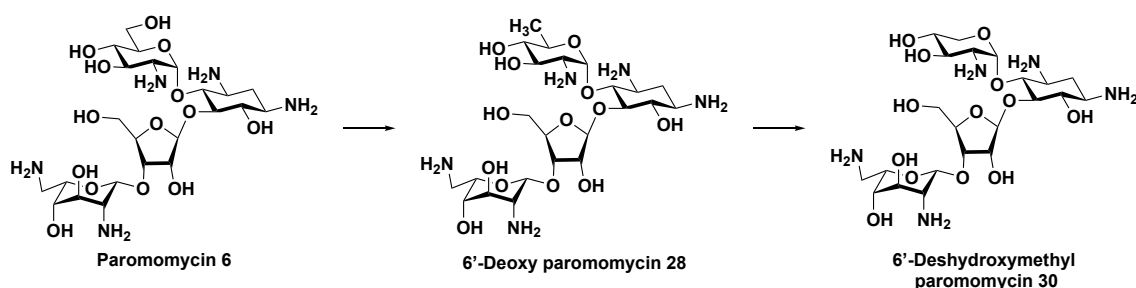
Figure 20. Rational design of 6'-deshydroxymethyl paromomycin (30)

2.2   Chemistry

The synthesis of 6'-deshydroxymethyl paromomycin **30** started from the fully

protected paromomycin intermediate **31**,[154] 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;[155] and borane dimethyl sulfide with copper(II) triflate,[155] 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),[147] which gave compound **32** in

60% yield. Alcohol **32** was oxidized with catalytic TEMPO, potassium bromide, and

bleach[156] to the uronic acid **33** in 79% yield. Barton's decarboxylation reaction was utilized to remove the 5'-carboxylic acid.[157] The reaction was carried out under white light photolysis conditions with tert-dodecanethiol as hydrogen atom source,[158] and gave the fully protected 6'-deshydroxymethyl derivative **34** in 55% yield. A photocatalytic decarboxylation method developed by Macmillan[159-160] 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 **30** (Scheme 1).
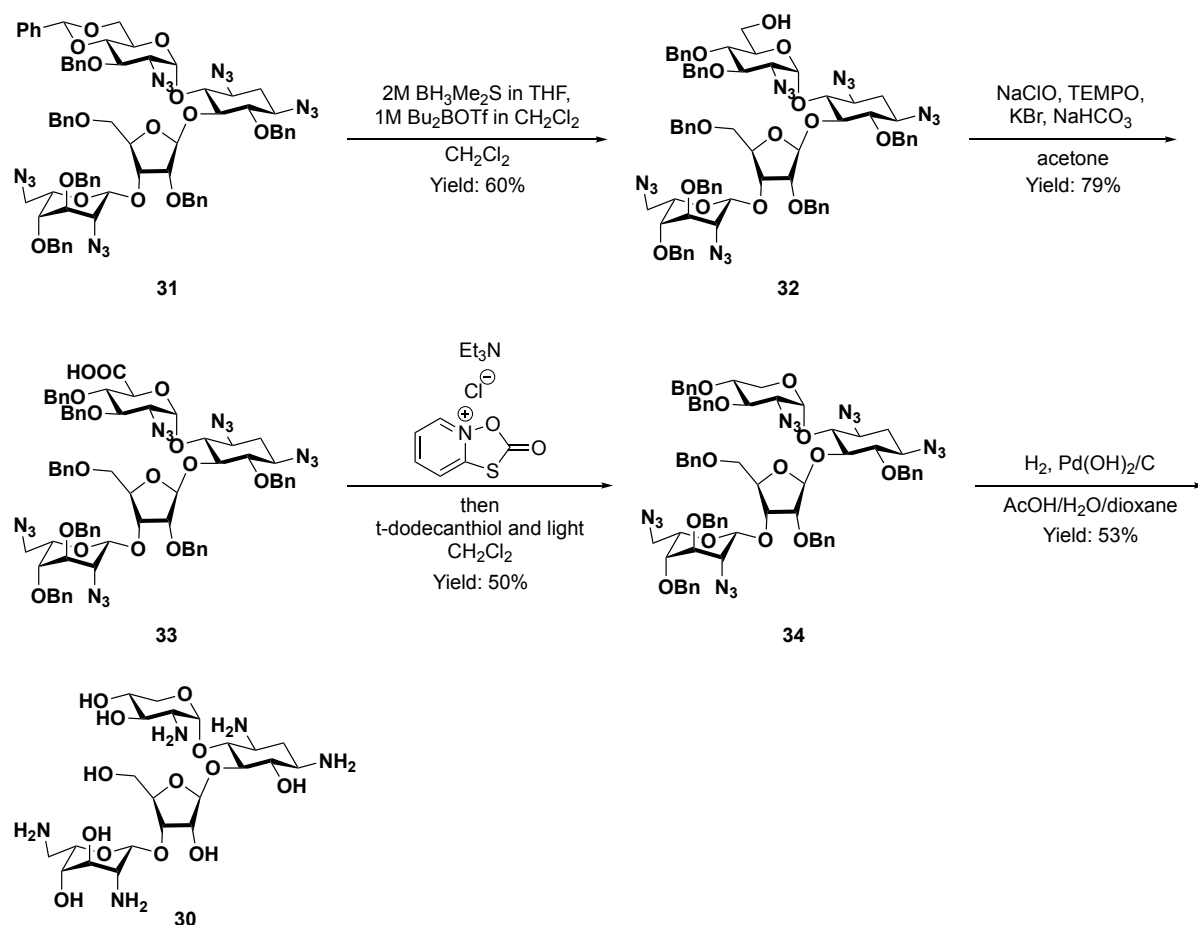
Scheme 1. **Synthesis of 6'-deshydroxymethyl paromomycin 30**

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 *Staphylococccus 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)**

Compound	IC$_{50}$ / (μg/mL)						
	Bacterial activity	Mit13	Sel.	A1555G	Sel.	Cyt14	Sel.
Paromomycin 6	0.03	50.6	1688	5.5	184	10.6	353
6'-Deoxy paromomycin 28	0.09	166.2	1846	93.2	1036	152.1	1690
6'-Deshydroxymethyl paromomycin 30	0.23	89.4	389	38.0	165	42.9	186

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)

	MRSA				E coli				P. aeruginosa			
Compound	AG 038	AG 039	AG 042	AG 044	AG 006	AG 001	AG 055	AG 003	AG 031	AG 032	AG 033	AG 086
Paromomyci n 6	2	≥256	256	8-16	2-4	16-32	8	8-16	>128	>128	>128	>128
6'-Deoxy paromomyci n 28	16	>128	>128	8	4	32	16-32	16-32	-	-	-	-
6'-Deshydro xymethyl paromomyci n 30	16	>128	>128	8	8	64	64	64	>128	>128	>128	>128

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.[161] 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**).[109] 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**.[109] APH(3')-IIa is well studied and its crystal structure in complex with kanamycin **2** has been resolved.[162] The epidemiological data of APH(3')-IIIa has been extensively reviewed.[162] 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).[109] 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.

Isepamicin 13   Lividomycin 35   Butirosin 36
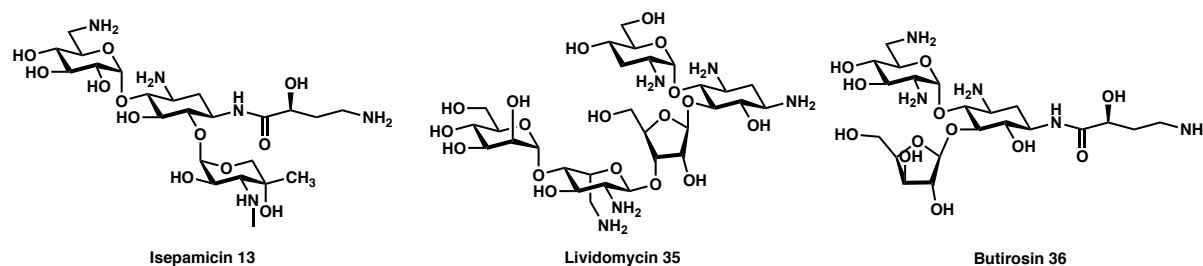
Figure 21. **Structure of isepamicin 13, lividomycin 35 and buirosin 36**

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).[163] Tobramycin **11** has also been prepared directly from kanamycin **2** with selective functionalization at the 3'-position (Scheme 2B).[164] However, the reported methods have disadvantages such as low yields for the key reactions,[163] and limited substrate scope.[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.
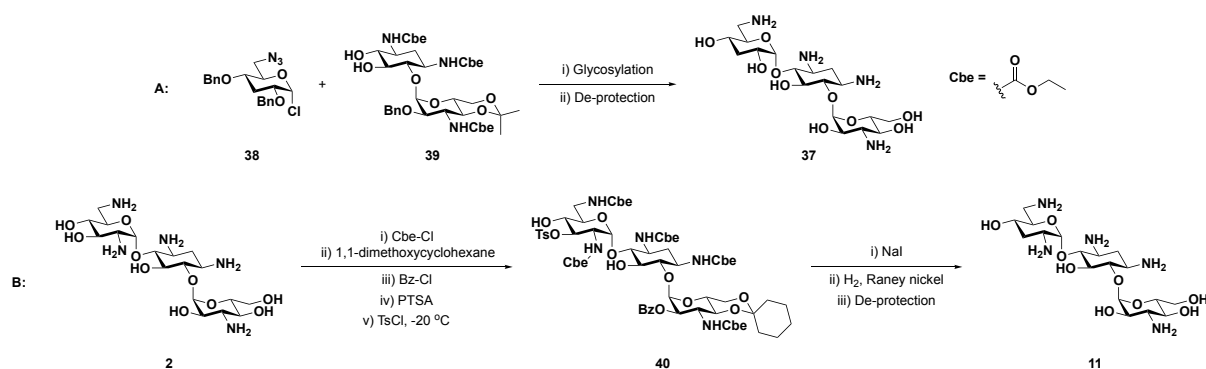
Scheme 2. **Some reported chemical approaches to 3'-deoxy modified AGAs**

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.[165] The procedure comprised the protection of lividomycin A **35**, followed by degradation of the mannose moiety with periodate and phenylhydrazine and subsequent de-protection.[165] 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).
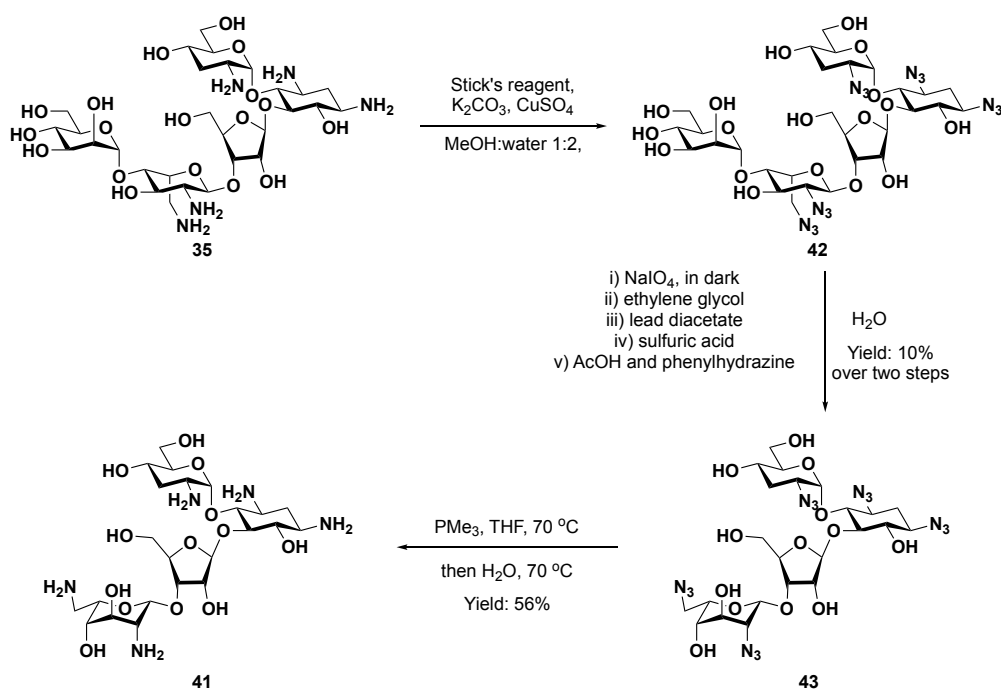
Scheme 3. Synthesis of 3'-deoxy paromomycin 41 from lividomycin A 35

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.
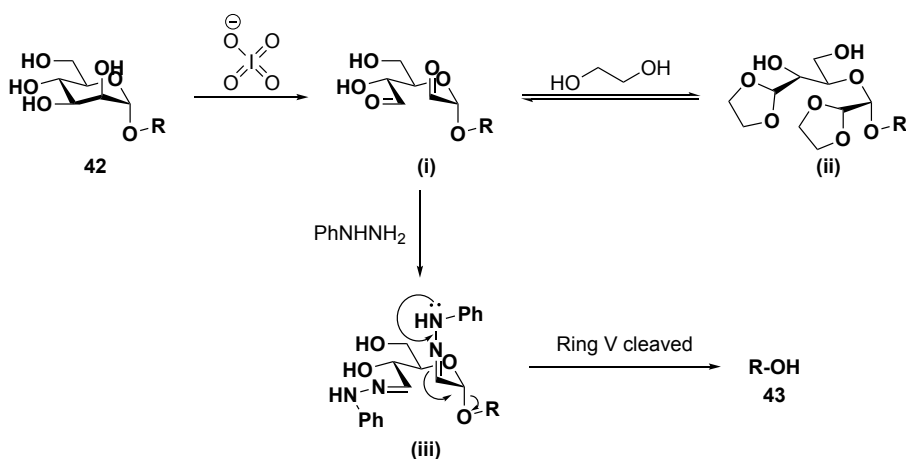
Figure 22. **Mechanism of the degradation of ring V**

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.[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).[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.
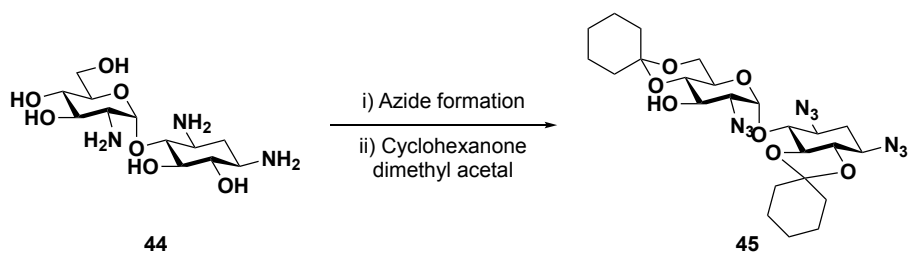
Scheme 4. Methodology to expose the 3'-position of paromamine **44**

Samarium iodide is a one-electron reducing reagent well known for its many chemoselective reductions.[167] Its applications include reductive cleavage of α-alkoxy groups from α-alkoxy ketones and the reduction of ketones to corresponding alcohols[168] (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.

X = heterosubstitution

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.[148, 154] It has been reported that azide groups can be reduced to the corresponding primary amines upon treatment with excess samarium iodide,[169-170] 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*-(benzyloxycarbonyloxy)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).
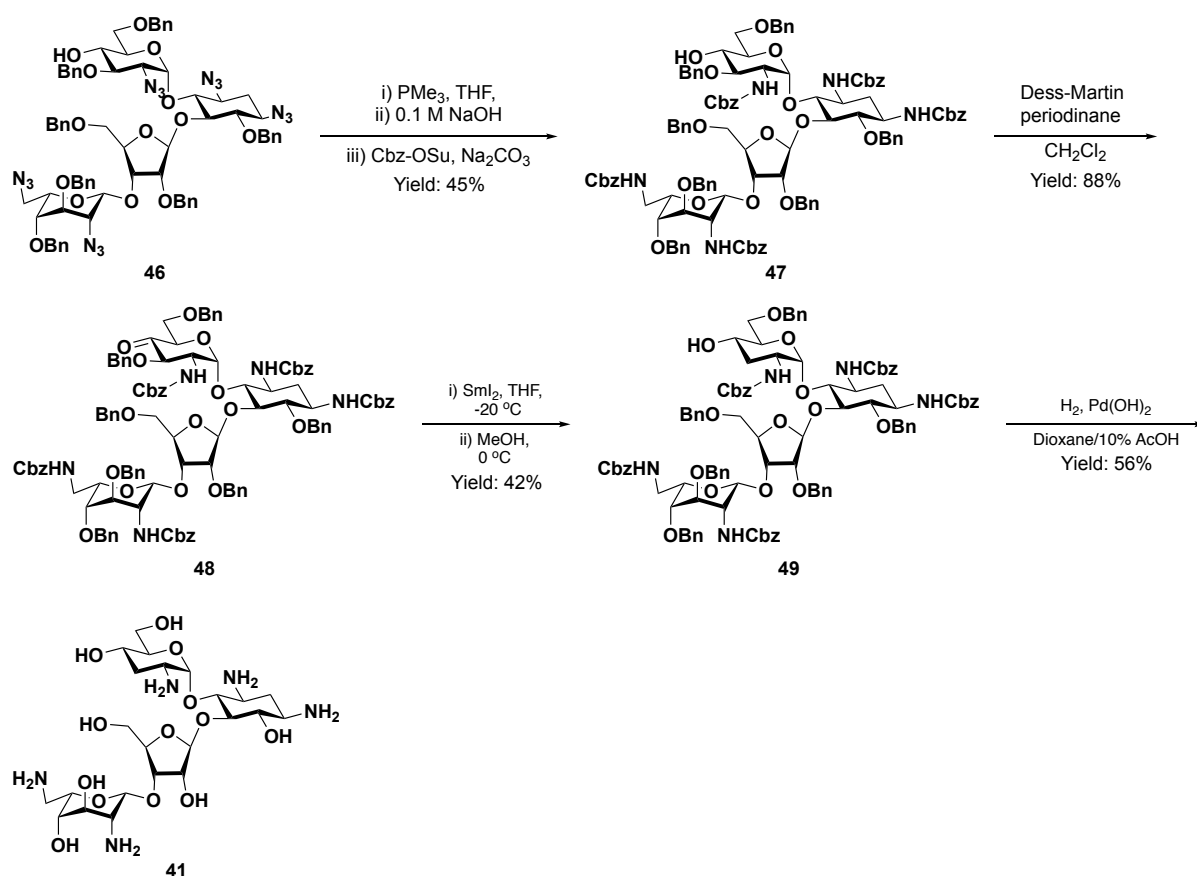
***Scheme 5.* Synthesis of 3'-deoxy paromomycin 41 using samarium iodide reduction**

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**.
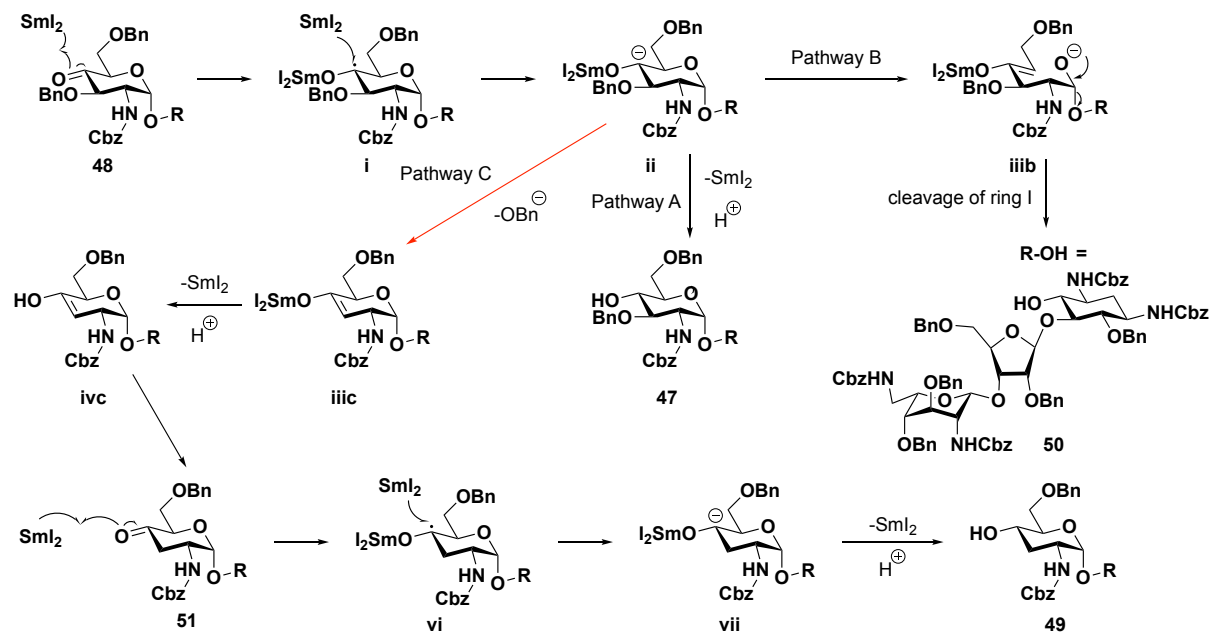
Figure 24. **The proposed mechanism of reductive cleavage of α-alkoxy ketones**

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

Entry	Reaction temperature	Reaction time	Product observed	Yield
1	-78 °C	1 h	Starting material (48)	-
2	-78 °C	Overnight	3'-deoxy-4'-keto product (51)	-
3	-20 °C	1h	3'-deoxy-4'-keto product (51)	70%
4	Room temperature	2 h	Ring I degraded product (50)[a]	-

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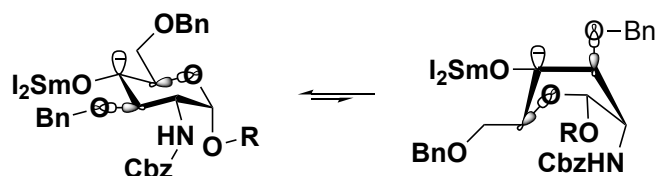
Figure 25. **The orbital overlapping of chair and boat conformation**

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**

Entry	Amount of SmI$_2$ (eq.)	Amount of MeOH (eq.)	Time -20 ^{o}C + 0 ^{o}C	Product observed
1	4	10	1 h + 2 h	Compound 50[a] & 51
2	6	10	1 h + 2 h	Compound 49 in 42%
3	6	10	1 h+ 1 h	Compound 50[a] & 51
4	Large excess	10	1 h + 2 h	Compound 49 in 35%
5	Large excess	5	1 h + 2 h	Compound 50[a] & 51
6	Large excess	20	1 h + 2 h	Compound 50[a]

[a] 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 in-complete 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).
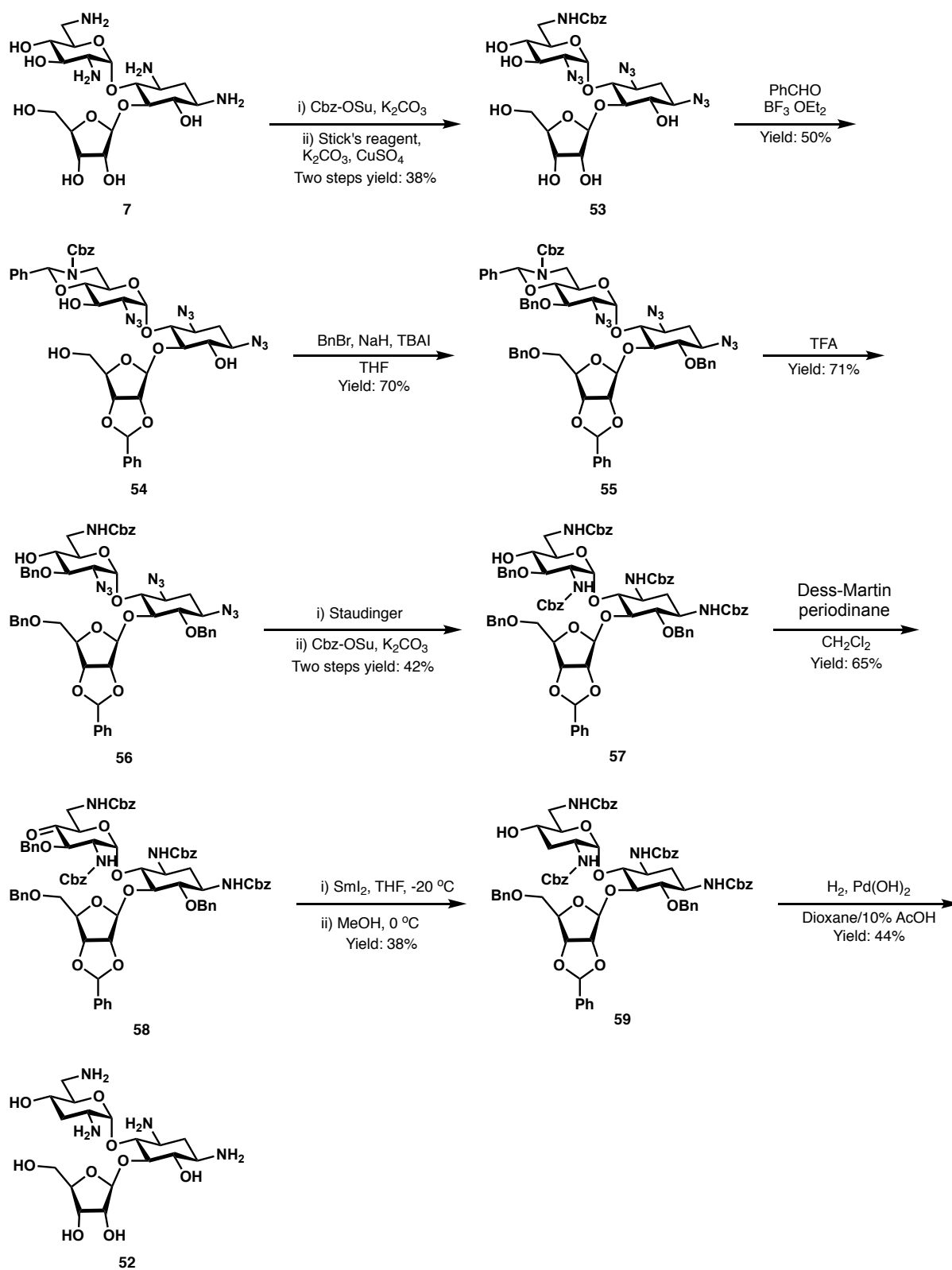
Scheme 6. *Scheme 6.* **Synthesis of 3'-deoxy ribostamycin 52**

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 *N*6'-benzyl carbamate, *N*6'''-benzyl carbamate and *N*6',*N*6'''-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*-(benzyloxycarbonyloxy)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).
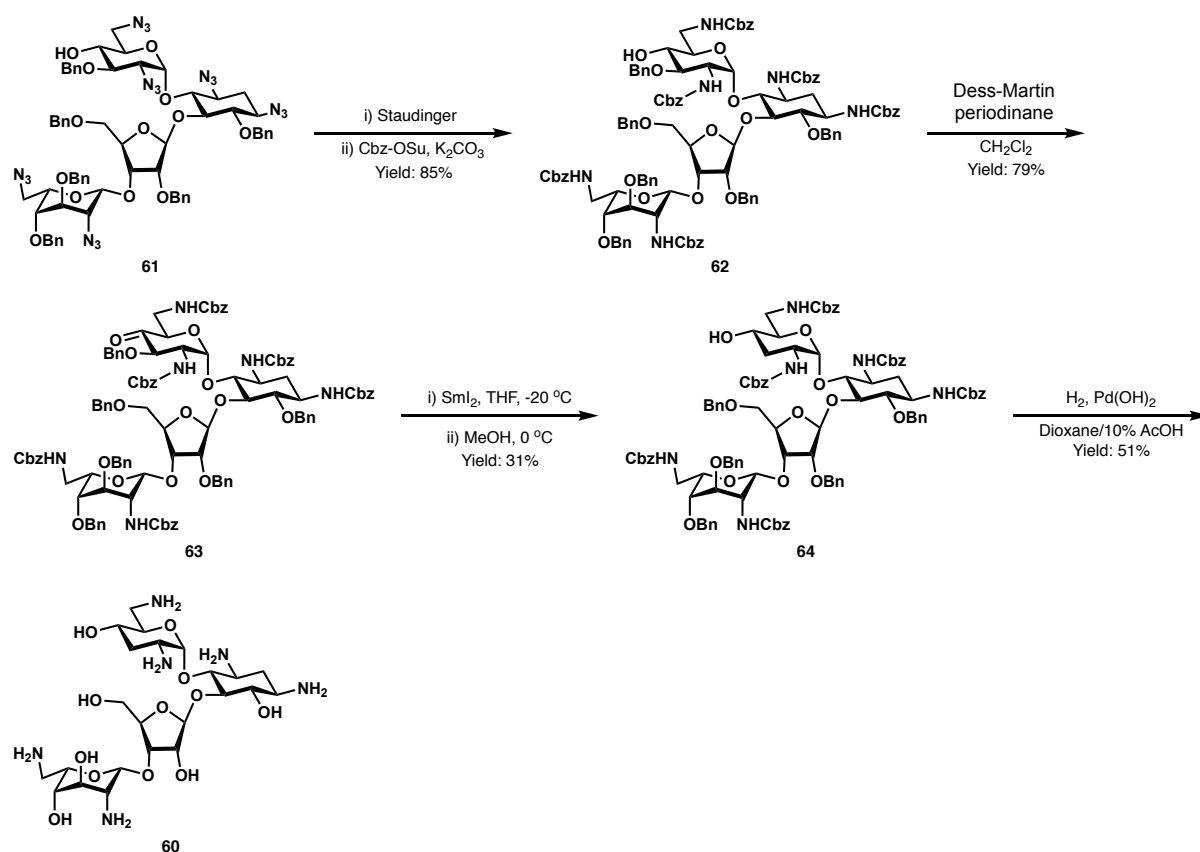
Scheme 7. **Synthesis of 3'-deoxy neomycin 60**

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)**

Compound	IC$_{50}$ / (μg/mL)						
	Bacterial activity	**Mit13**	**Sel.**	**A1555G**	**Sel.**	**Cyt14**	**Sel.**
Paromomycin 6	0.03	50.6	1688	5.5	184	10.6	353
3'-Deoxy paromomycin 41	0.04	109.0	2725	15.2	380	52.9	1322
Ribostamycin 7	0.04	203.9	5097	37.6	940	199.2	4979
3'-Deoxy ribostamycin 52	0.05	163.2	3264	59.0	1179	167.6	3352
Neomycin 5	0.01	2.0	197	0.26	26	21.8	2175
3'-Deoxy neomycin 60	0.02	2.6	130	0.28	28	34.4	1718

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 6. Antibacterial activities of compound 41, 52 and 60 (MIC, μg/mL)

Compound	MRSA				E coli				P. aeruginosa			
	AG038	AG039	AG042	AG044	AG006	AG001	AG055	AG003	AG031	AG032	AG033	AG086
Paromomycin 6	4	≥256	256	4-8	2-4	16-32	8	8-16	>128	>128	>128	>128
3'-Deoxy paromomycin 41	4	>128	>128	4	1-2	16	16	8-16	16	16	>128	>128
Ribostamcin 7	4-8	>128	>128	8	2	8	8	4-8	>128	>128	>128	>128
3'-Deoxy ribostamycin 52	8-16	>128	>128	8	1-2	8	8	8	4	4	>128	16-32
Neomycin 5	0.5-1	128	128	0.5-1	1	8-16	2-4	4	32	32-64	>128	>128
3'-Deoxy neomycin 60	1-2	64-128	64	0.5-1	0.5-1	4	4	4	2	2	32-64	16

The antibacterial activity results revealed that for the _MRSA_ and _E. coli_ strains that were tested, the 3'-deoxy modification did not changed 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 paromomyicn **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)

Strains	AG006	AG007	AG008	AG009	AG036	AG037	AG103	AG105
Resistance mechanism	-	AAC(3)	ANT(2'')	AAC(6')	ANT(4',4'')	APH(3',5'')	armA	AAC(2')
Paromomycin 6	2	8	4-8	4-8	>128	>128	4	2-4
3'-Deoxy paromomycin 41	1-2	4-8	2	8	128	>128	8	2-4
3'-Deoxy ribostamycin 52	2-4	4	1	128	64	>128	4	2-4

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'-postion 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.
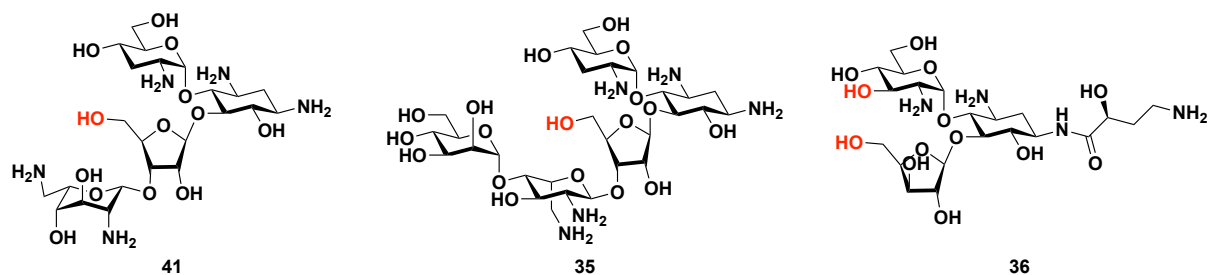
Figure 26. **Some known AGAs that are deactivated by APH(3')-IIIa**

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).[175] Similar results also appear in the cases of the 5''-carboxylic acid paromomycin derivative **68**[138] and 5''-carboxamide paromomycin derivative **69**[176] (Figure 27).

R = H, 5''-Deoxy paromomycin (65)
R = alkoxy group, 5''-*O*-Alkyl paromomycin (66)
R = F, 5''-Deoxy-5''-fluoro paromomycin (67)

R = OH, paromomycin 5''-carboxylic acid (68)
R = NH₂, paromomycin 5''-carboxamide (69)
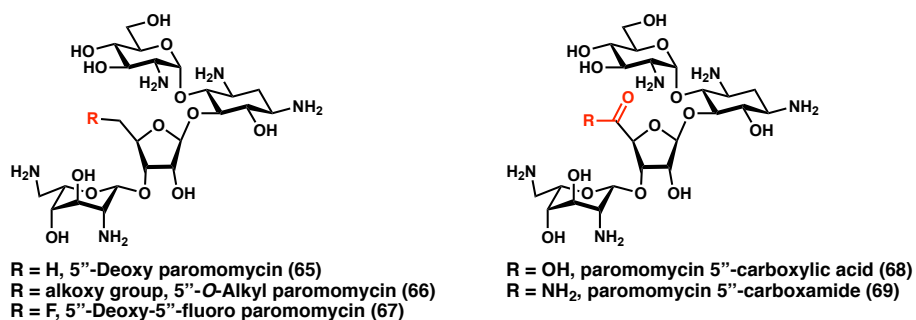
Figure 27. **Inactive modifications at 5''-position of 4,5-aminoglycosides**

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).[177-178] 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**),[179] neomycin–anthroquinone conjugate **74**,[180] and neomycin dimer **75**[181] that are linked at the 5''-position through urea or thiourea linkages also exhibited good antibacterial activity (Figure 28).

R = OH, 5''-Deoxy-5''-amino paromomycin (70)
R = NH₂, 5''-Deoxy-5''-amino neomycin (71)

5''-Deoxy-5''-amino butirosin (72)

R = CH₂NH₂ or CH₂NH(CO)CH₂NH₂
Neomycin peptide analogs (73)

Neomycin-anthroquinone conjugate (74)

Neomycin dimer (75)
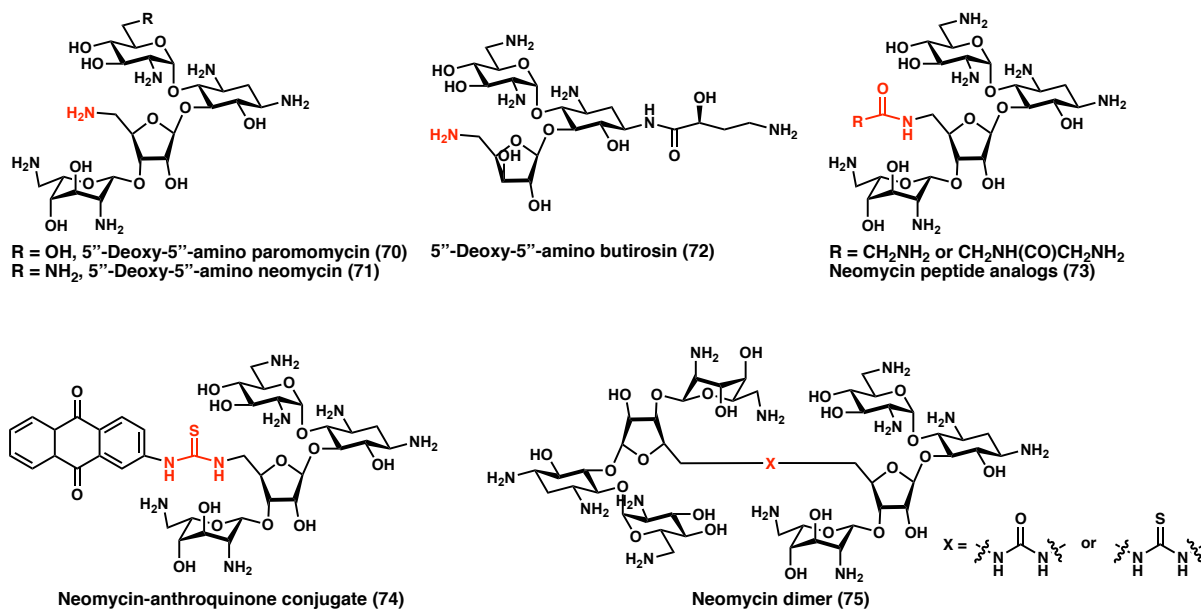
***Figure 28.* Active modifications at 5''-position of 4,5-aminoglycosides**

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.
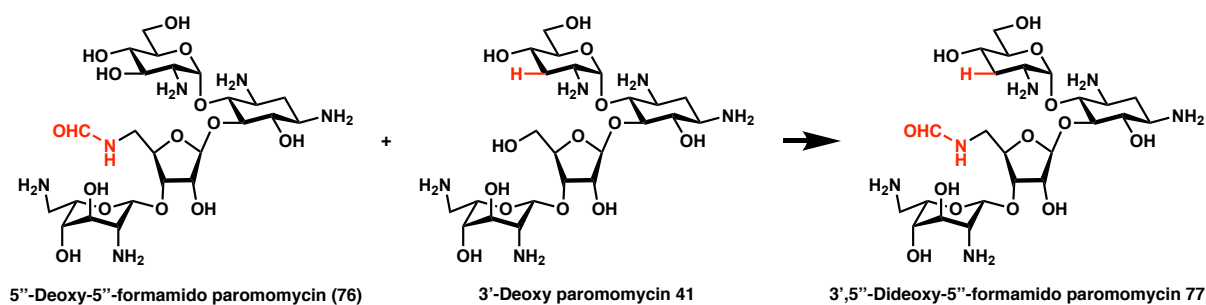
Figure 29. **Design of the doubly modified paromomycin analog 77**

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.[154] 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*-(benzyloxycarbonyloxy)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).
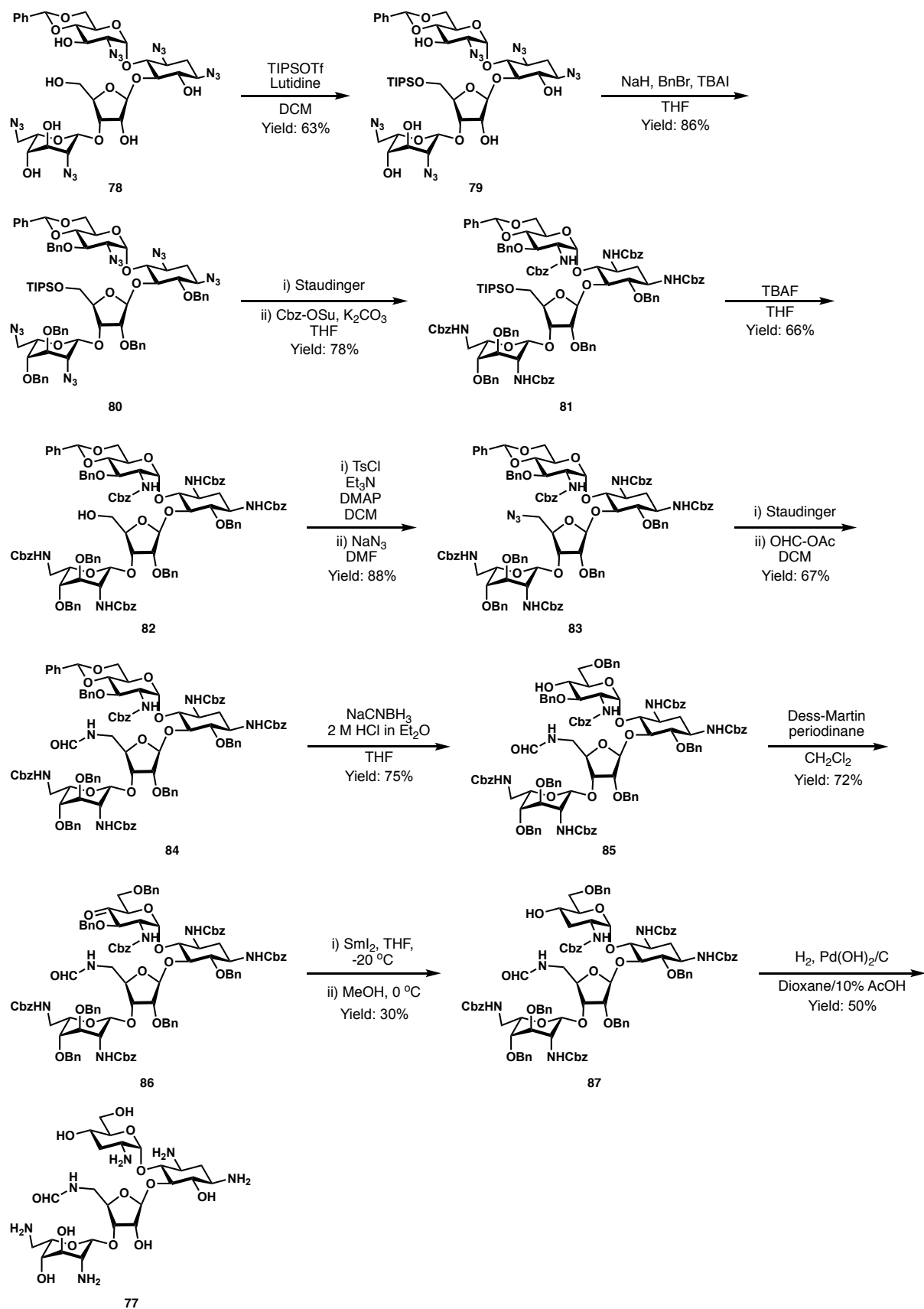
Scheme 8. Synthesis of 3',5''-dideoxy-5''-formamido paromomycin 77

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'-postion 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 tosylated 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).
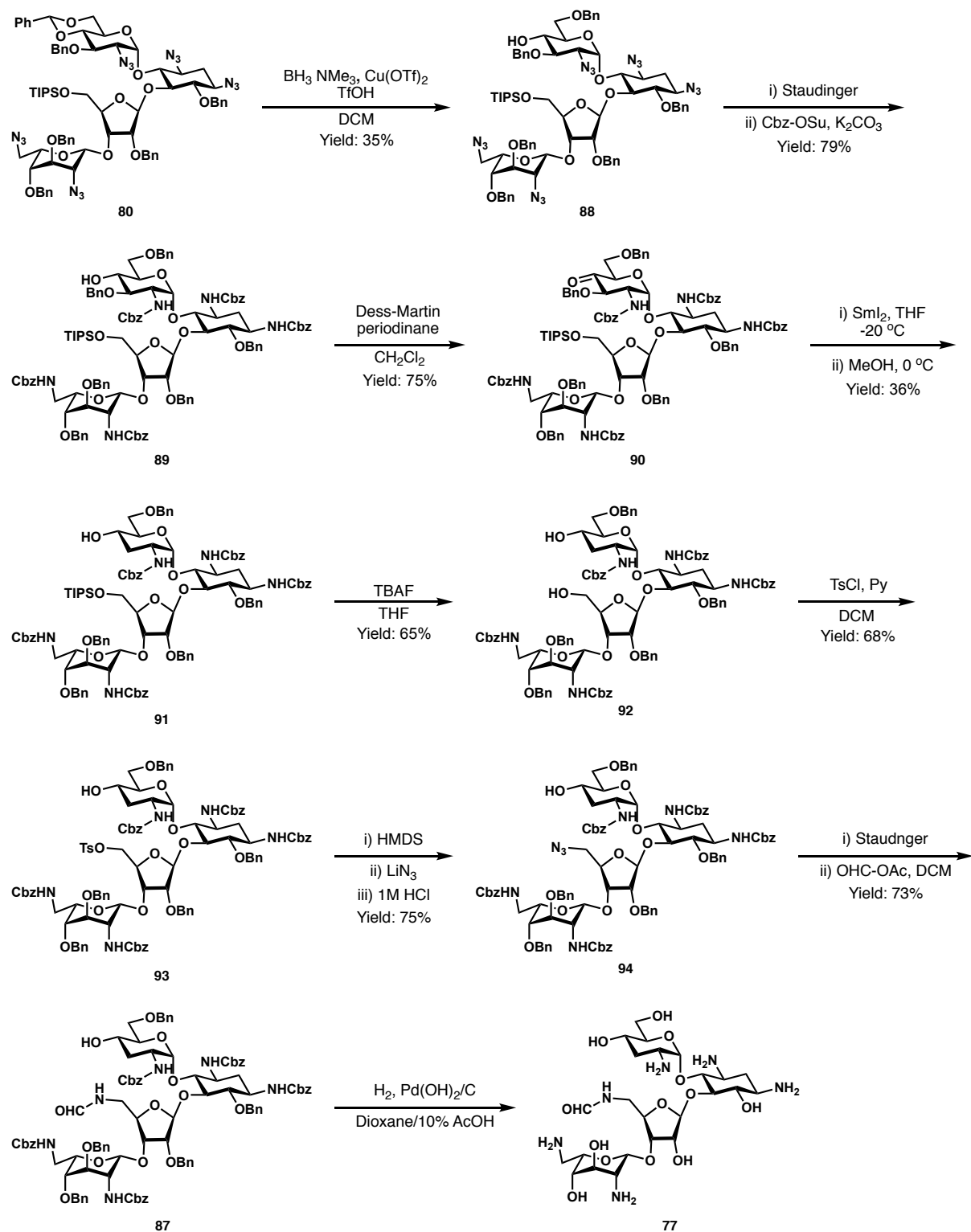
Scheme 9. Alternative synthesis of 3',5''-dideoxy-5''-formamido paromomycin **77**

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. **Antiribomomal activity of compound 77 (IC$_{50}$, μg/mL)**

Compound	Bacterial activity	Mit13	Sel.	A1555G	Sel.	Cyt14	Sel.
Paromomycin 6	0.03	50.6	1688	5.5	184	10.6	353
3'-Deoxy paromomycin 41	0.04	109.0	2725	15.2	380	52.9	1322
5''-Deoxy-5''-formamido paromomycin 76	0.03	80.6	2686	19.0	635	50.3	1676
3',5''-Dideoxy-5''-formamido paromomycin 77	0.04	70.5	1763	37.1	928	78.5	1963

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 IC$_{50}$ 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)**

Compound	MRSA				E. coli			P. aeruginosa		
	AG0 38	AG0 39	AG0 42	AG0 44	AG0 01	AG0 55	AG0 03	AG0 31	AG0 32	AG0 33
Paromomycin 6	4	>12 8	>12 8	4-8	16	8	8-16	>12 8	>12 8	>12 8
3'-Deoxy paromomycin 41	4	>12 8	>12 8	4	16	16	8-16	16	16	128
5''-Deoxy-5''-for mamido paromomycin 76	4	>12 8	>12 8	2-4	4	4	4	128	128	>12 8
3',5''-Dideoxy-5''-formamido paromomycin 77	4	>12 8	>12 8	4	8-16	8-16	8	32-6 4	32	>32

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)**

Strains	AG006	AG009	AG036	AG037	AG103
Resistance mechanism	-	AAC(6')	ANT(4', 4'')	APH(3', 5'')	armA
Paromomycin 6	2	4-8	>128	>128	4
3'-Deoxy paromomycin 41	1-2	8	128	>128	8
5''-Deoxy-5''-formamido paromomycin 76	1-2	-	-	128	-
3',5''-Dideoxy-5''-formami do paromomycin 77	2	8	64-128	4	32

Paromoycin **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'-deoxygenation, 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'-positon 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''-postion. 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.[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.[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.[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]

R =H, 4'-deoxy neomycin 95
R = F, 4'-deoxy-4'-fluoro neomycin 96

R = aralkyl, 4'-*O*-aralkyl paromomycin 97
R = alkyl, 4'-*O*-alkyl paromomycin 98

4′-*O*-β-D-xylopyranosyl paromomycin 99
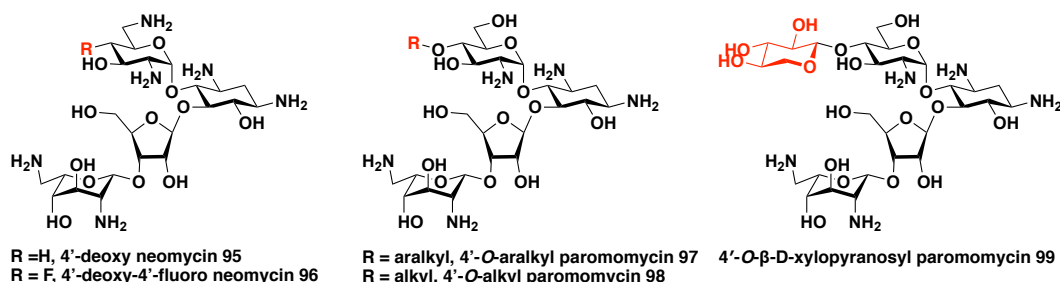
Figure 30. **Some 4'-modified 4,5-aminoglycosides derivatives**

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 paromocyin **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.
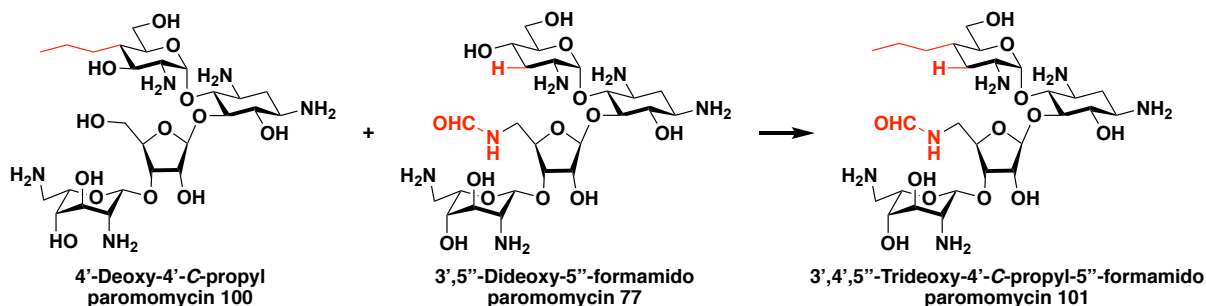
Figure 31. Design of the triply modified paromomycin derivative 101

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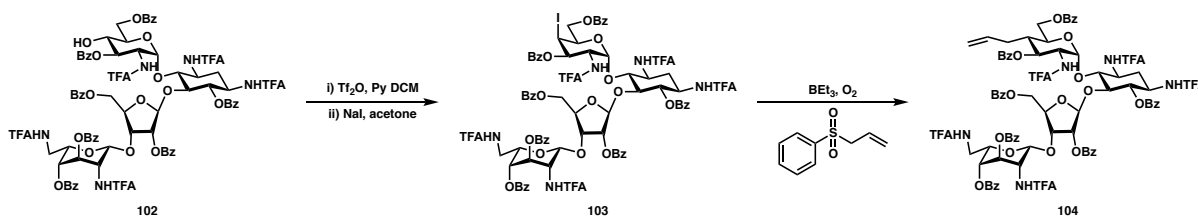
Scheme 10. The key reactions to synthesize 4'-deoxy-4'-C-propyl paromomycin

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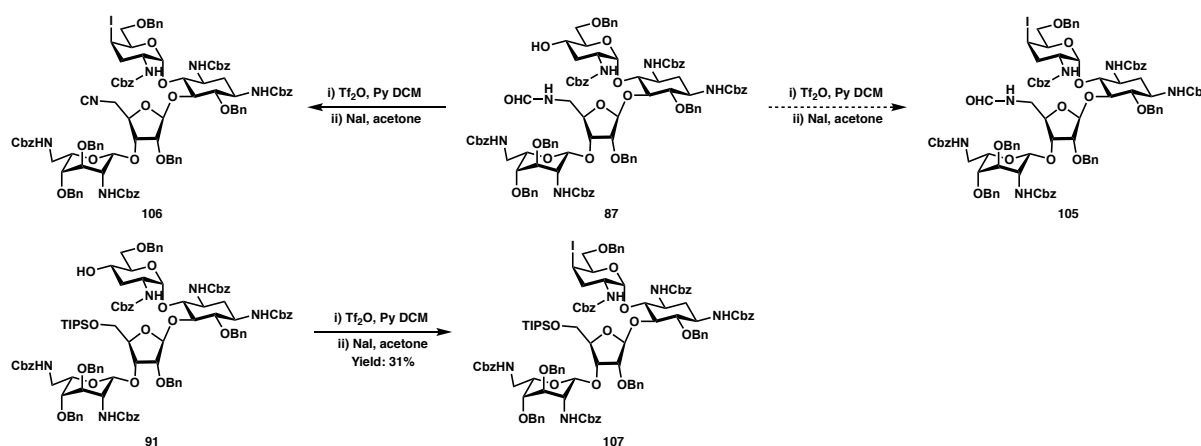
Scheme 11. **Attempted iodination at the 4'-position of compound 87 and 91**

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**.
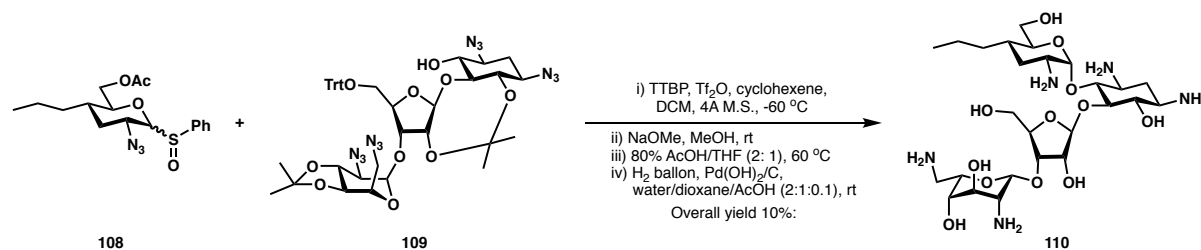
Scheme 12. **Synthesis of compound 110 through glycosylation**

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 hydrogenloysis, 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,[185] 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).
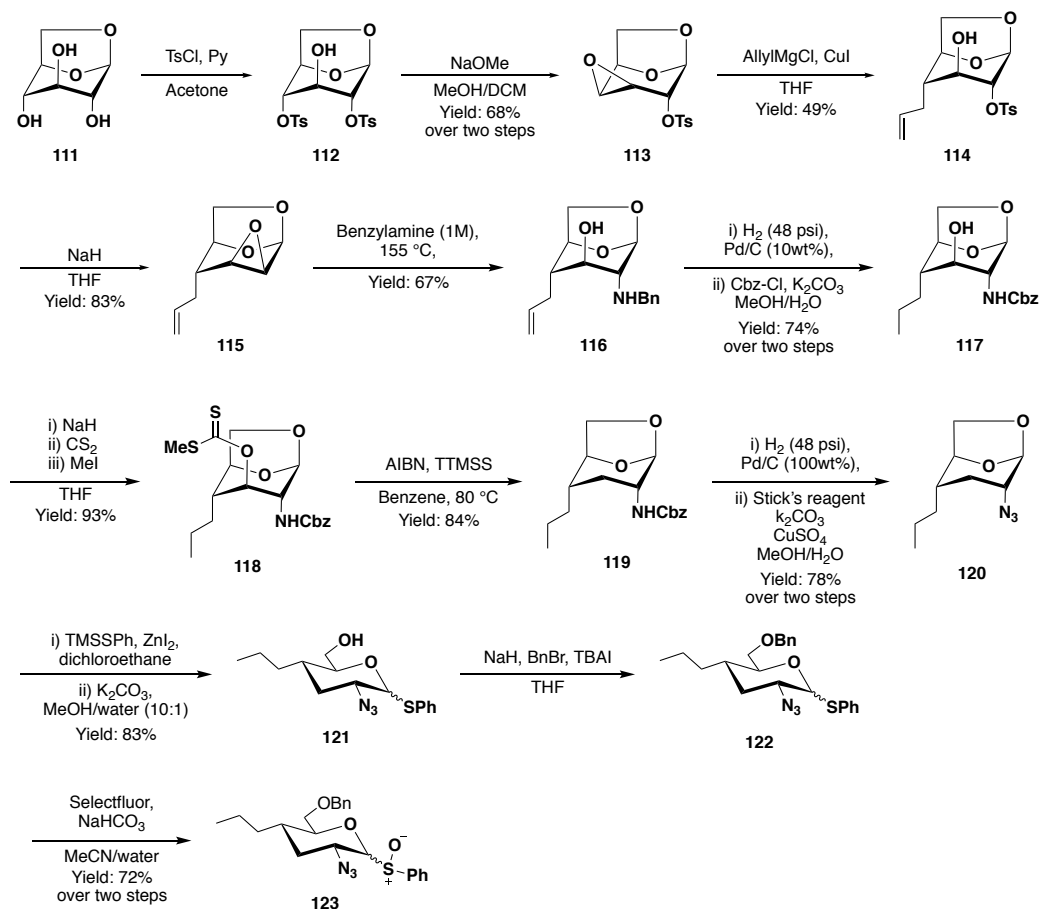
Scheme 13. **Synthesis of the glycosyl donor 123**

5.4   Acceptor synthesis

The straightforward synthesis of a suitable glycosyl acceptor has been described in the literature.[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.
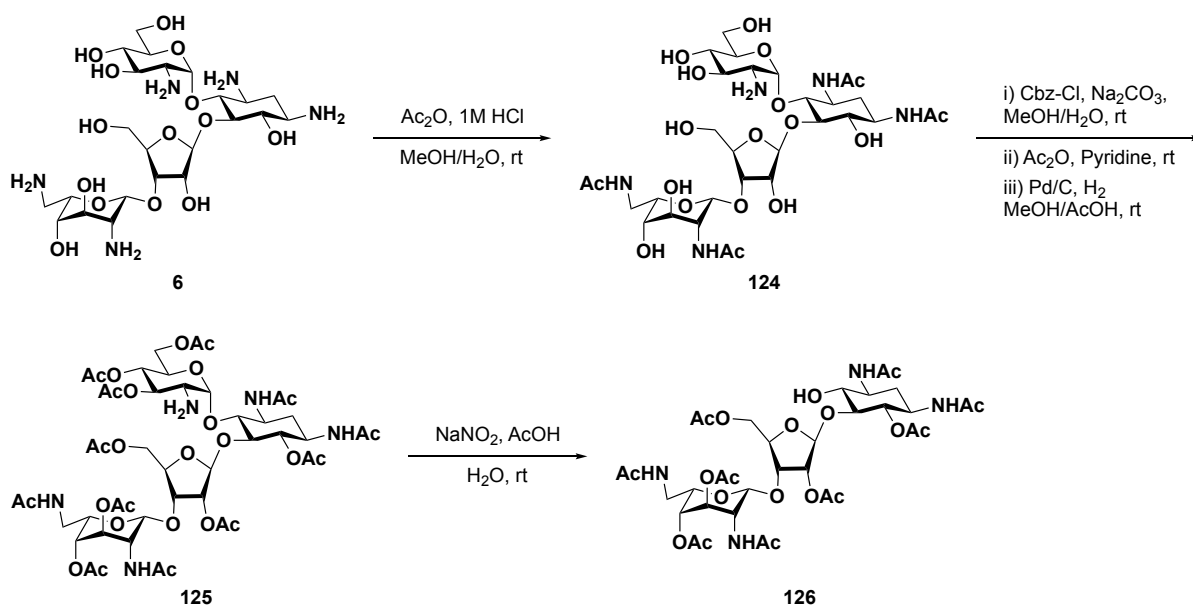
Scheme 14. **Literature protocol to degrade ring I of paromomycin 6**

Compound **80**, discussed in chapter four, was treated with *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.[188] 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).
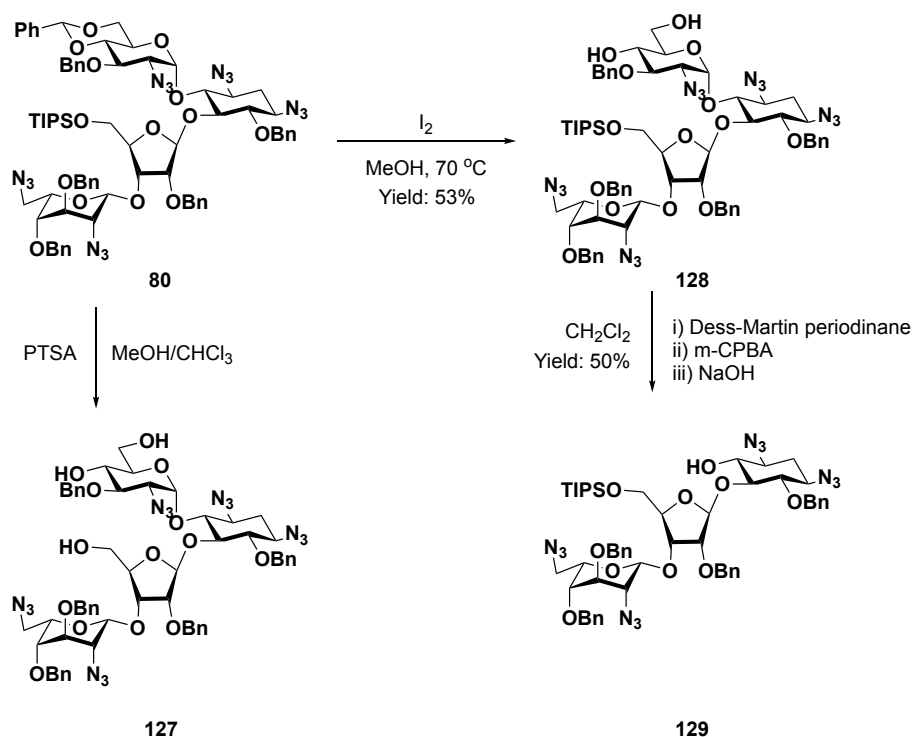
Scheme 15. **Synthesis of the glycosyl acceptor 129**

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**).
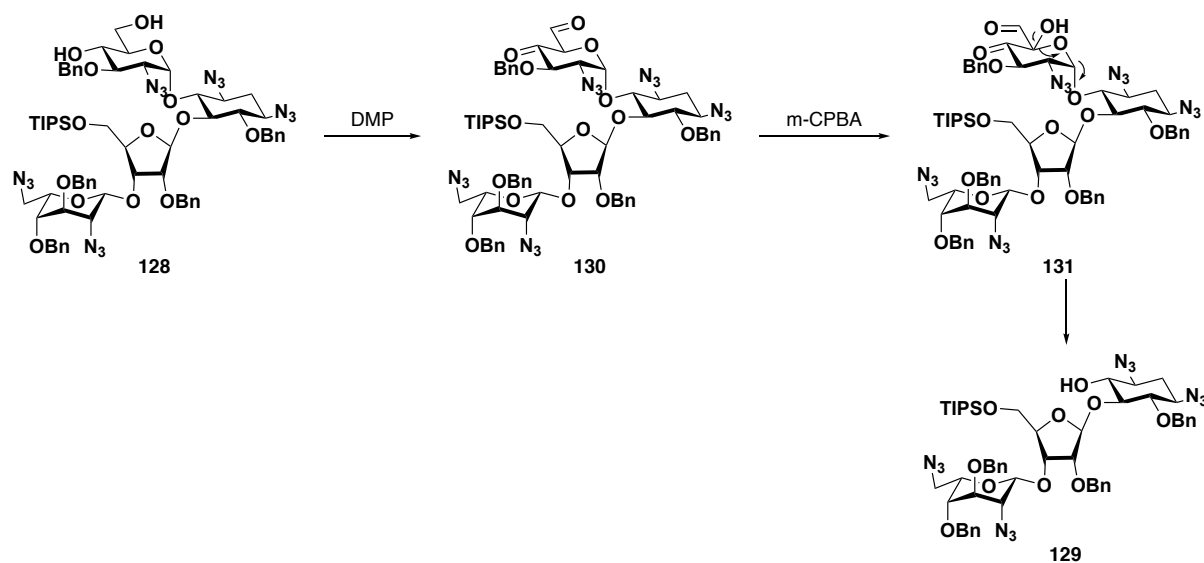
Figure 32. **Proposed mechanism of the degradation of ring I**

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.[189] 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 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.
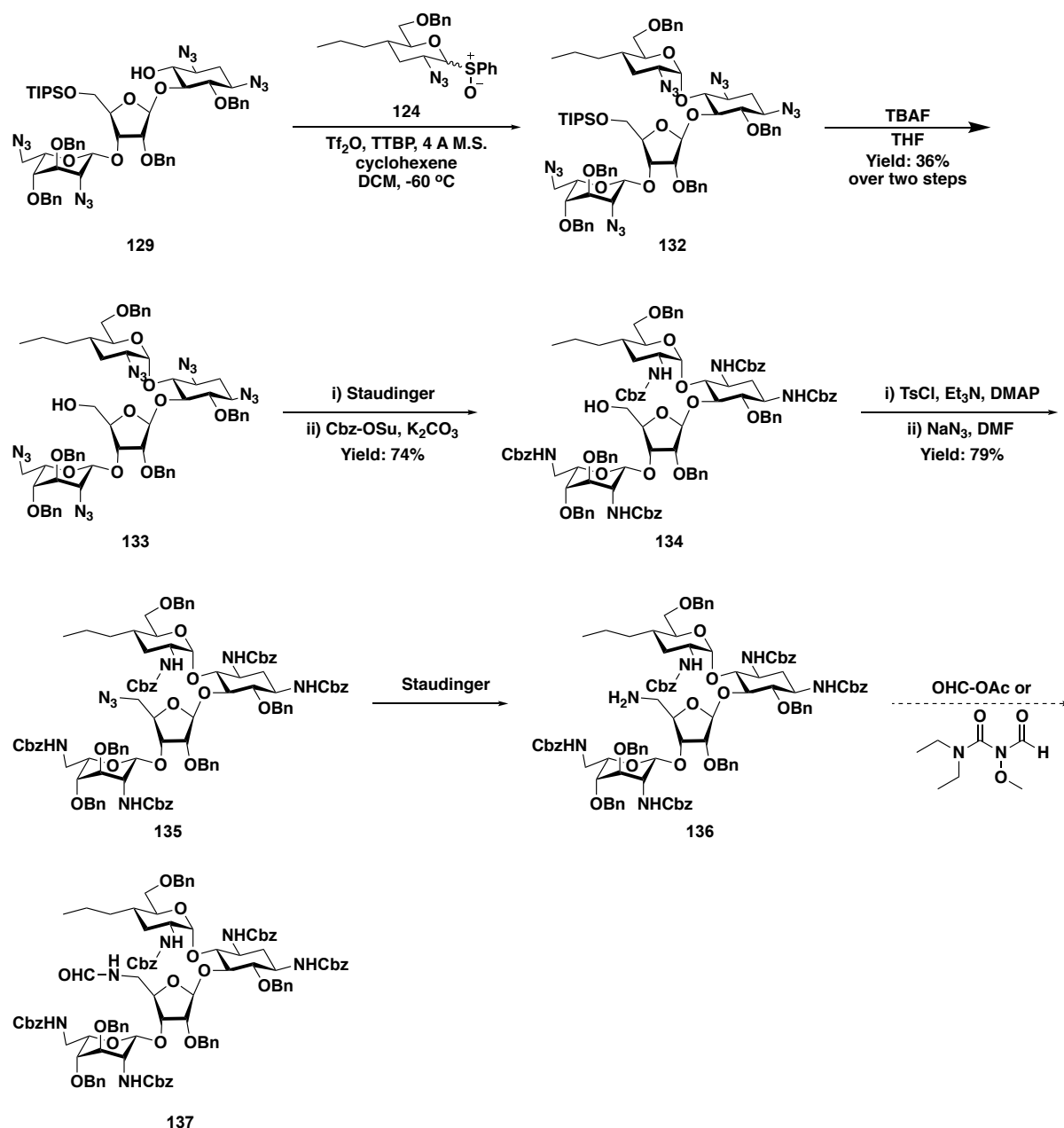
Scheme 16. **Glycosylation and the first approach to modify the 5''-position**

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).
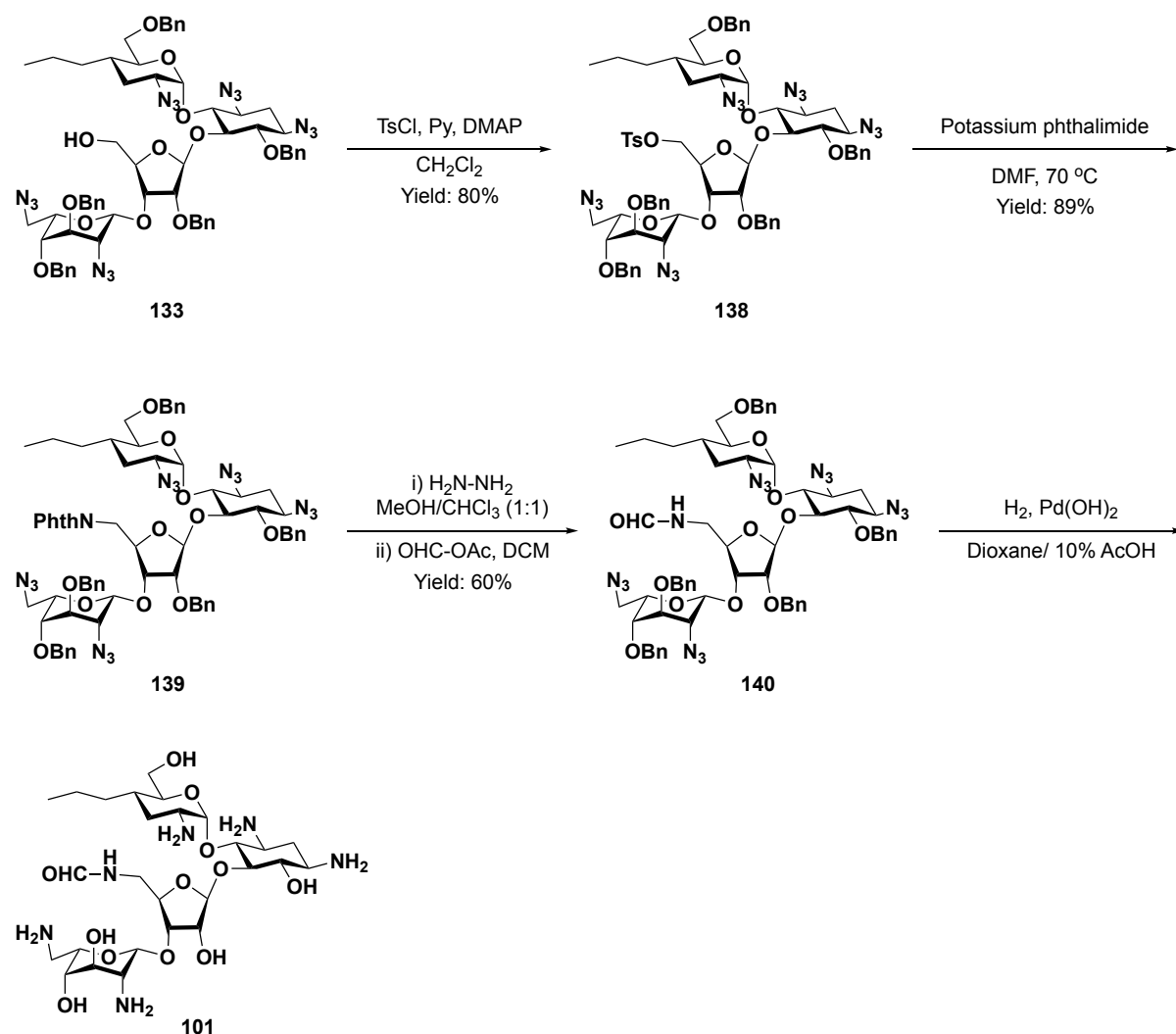
***Scheme 17.* Synthesis of the triply modified paromomycin derivative 101**

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 (IC$_{50}$, μg/mL)**

Compound	IC$_{50}$ / (µg/mL)						
	Bacterial activity	Mit13	Sel.	A1555G	Sel.	Cyt14	Sel.
Paromomycin 6	0.03	50.6	1688	5.5	184	10.6	353
3',5''-Dideoxy-5''-formamido paromomycin 77	0.04	70.5	1763	37.1	928	78.5	1963
4'-Deoxy-4'-*C*-propyl paromomycin 100	0.02	107	5350	29	1450	41	2050
3',4',5''-trideoxy-4'-*C*-propyl-5''- formamido paromomycin 101	0.51	686	1342	293	573	411	804

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)**

Compound	MRSA			E. coli			P. aeruginosa		
	AG0 38	AG0 39	AG0 42	AG0 01	AG0 55	AG0 03	AG0 31	AG0 32	AG0 33
Paromomycin 6	4	>12 8	>12 8	16	8	8-1 6	>12 8	>12 8	>12 8
3',5''-Dideoxy-5''-formamido paromomycin 77	4	>12 8	>12 8	8-1 6	8-1 6	8	32- 64	32	>32
4'-Deoxy-4'-*C*-propyl paromomycin 100	1-2	1-2	2	2	2-4	2-4	8	8	64
3',4',5''-Trideoxy-4'-C-propyl-5''-formamido paromomycin 101	16- 32	32	16	32	32	32	>64	>64	>64

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. col*i 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. Further more, 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'-deoxygenation 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 [$Ce(SO_4)_2$: 4 g, $(NH_4)_6Mo_7O_{24}$: 10 g, H_2SO_4: 40 mL, H_2O: 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, ^{13}C 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‴-pentadeamino-1,3,2′,2‴,6‴-pentaazido 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. $[\alpha]_D^{23}$ = + 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, -OC*H*$_2$Ph), 4.92 (d, $J_{1'''-2'''}$ = 1.6 Hz, 1H, H1′′′), 4.90 – 4.81 (m, 3H, - OC*H*$_2$Ph), 4.74 (d, *J* = 10.6 Hz, 1H, - OC*H*$_2$Ph), 4.68 – 4.58 (m, 3H, - OC*H*$_2$Ph), 4.54 – 4.46 (m, 3H, - OC*H*$_2$Ph), 4.43 (d, *J* = 12.0 Hz, 1H, - OC*H*$_2$Ph), 4.37-4.31 (m, 3H, - OC*H*$_2$Ph, H3′′, H4′′), 4.27 (d, *J* = 12.1 Hz, 1H, - OC*H*$_2$Ph), 4.08 (t, $J_{3'-2'}$ = 10.0 Hz, $J_{3'-4'}$ = 10.0 Hz, 1H, H3′), 4.03 (t, $J_{2''-1''}$ = 5.2 Hz, $J_{2''-3''}$ = 5.2 Hz, 1H, H2′′), 4.00 - 3.93 (m, 2H, 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, H2$_{eq}$), 1.42 (q, $J_{2ax-2eq}$ = 12.7 Hz, J_{2ax-1} = 12.7 Hz, J_{2ax-3} = 12.7 Hz, 1H, H2$_{ax}$). ^{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 (Ph*C*H$_2$), 75.0 (Ph*C*H$_2$), 74.9 (C4), 74.7 (Ph*C*H$_2$), 74.4 (C5′′′), 73.2 (Ph*C*H$_2$), 73.2 (Ph*C*H$_2$), 72.8 (C3′′′), 72.4 (Ph*C*H$_2$), 71.7 (Ph*C*H$_2$), 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]$^+$

1398.5672, found 1398.5676.

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. $[\alpha]_D^{23}$ = + 5.9° (*c* = 1.0, chloroform). ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.43 – 7.12 (m, 35H, aromatic), 6.15 (d, $J_{1'-2'}$ = 3.6 Hz, 1H, H1′), 5.65 (d, $J_{1''-2''}$ = 5.3 Hz, 1H, H1″), 4.95 (d, *J* = 10.7 Hz, 1H, -OC*H*$_2$Ph), 4.88 (d, $J_{1'''-2'''}$ = 1.5 Hz, 1H, H1‴), 4.86 - 4.78 (m, 3H, -OC*H*$_2$Ph), 4.77 – 4.68 (m, 3H, -OC*H*$_2$Ph, H5′), 4.65 (d, *J* = 12.0 Hz, 1H, - OC*H*$_2$Ph), 4.60 - 4.55 (m, 2H, -OC*H*$_2$Ph), 4.49 (d, *J* = 11.9 Hz, 1H, -OC*H*$_2$Ph), 4.46 – 4.42 (m, 2H, -OC*H*$_2$Ph), 4.37 – 4.26 (m, 4H, - OC*H*$_2$Ph, H3″, H4″), 4.06 (t, $J_{3'-2'}$ = 9.5 Hz, $J_{3'-4'}$ = 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, H2$_{eq}$), 1.46 (q, $J_{2ax-2eq}$ = 12.7 Hz, J_{2ax-1} = 12.7 Hz, J_{2ax-3} = 12.7 Hz, 1H, H2$_{ax}$). ^{13}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 (PhCH_2), 75.0 (PhCH_2), 74.7 (PhCH_2), 74.2 (C5′′′), 73.3 (PhCH_2), 73.2 (C3′′′), 72.9 (PhCH_2), 72.4 (PhCH_2), 71.8 (PhCH_2), 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 calcd for $C_{72}H_{75}N_{15}NaO_{15}$ [M+Na]$^+$ 1412.5465, found 1412.5470.

6,3′,4′,2′′,5′′,3′′′,4′′′-Hepta-O-benzyl-1,3,2′,2′′′,6′′′-pentadeamino-1,3,2′,2′′′,6′′′-pentaazido-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 atomesphere, 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%). $[\alpha]_D^{23}$ = + 6.0° (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, -OC*H*$_2$Ph), 4.90 (d, *J* = 10.8 Hz, 1H, -OC*H*$_2$Ph), 4.86 (d, $J_{1'''-2'''}$ = 1.5 Hz, 1H, H1′′′), 4.82 (d, *J* = 10.9 Hz, 1H, -OC*H*$_2$Ph), 4.74 – 4.67 (m, 2H, -OC*H*$_2$Ph), 4.66 – 4.61 (m, 2H, -OC*H*$_2$Ph), 4.59 (d, *J* = 11.7 Hz, 1H, -OC*H*$_2$Ph), 4.54 (d, *J* = 11.9 Hz, 1H, -OC*H*$_2$Ph), 4.48 – 4.39 (m, 3H, -OC*H*$_2$Ph), 4.36 – 4.22 (m, 4H, -OC*H*$_2$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, $J_{2eq-2ax}$ = 12.7 Hz, J_{2eq-1} = 4.0 Hz, J_{2eq-3} = 4.0 Hz,1H, H2$_{eq}$), 1.39 (q, *J* = $J_{2ax-2eq}$ = 12.7 Hz, J_{2ax-1} = 12.7 Hz, J_{2ax-3} = 12.7 Hz, H2$_{ax}$). ^{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 (Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 74.3 (C5′′′), 73.3 (Ph*C*H$_2$), 73.2 (Ph*C*H$_2$), 73.0 (C3′′′), 72.9 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 71.7 (Ph*C*H$_2$), 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). $[\alpha]_D^{23}$ = + 28.8° (*c* = 0.3, H_2O). ^1H NMR (500 MHz, D_2O) δ 5.26 (d, $J_{1'-2'}$ = 2.1 Hz, 1H, H1′), 5.17 (d, $J_{1'''-2'''}$ = 1.5 Hz, 1H, H1‴), 5.12 (d, $J_{1''-2''}$ = 2.1 Hz, 1H, H1″), 4.41 (dd, $J_{3''-4''}$ = 5.0 Hz, $J_{3''-2''}$ = 2.0 Hz, 1H, H3″), 4.24 (dd, $J_{2''-1''}$ = 4.5 Hz, $J_{2''-3''}$ = 2.0 Hz, 1H, H2″), 4.21 – 4.17 (m, 1H, H5‴), 4.12 (t, $J_{3'''-4'''}$ = 3.0 Hz, $J_{3'''-2'''}$ = 3.0 Hz, 1H, H3‴), 4.07 – 4.02 (m, 1H, H4″), 4.01 (t, $J_{3'-2'}$ = 4.7 Hz, $J_{3'-4'}$ = 4.7 Hz, 1H, H3′), 3.92 – 3.86 (m, 1H, H5′), 3.83 (t, J_{4-3} = 9.5 Hz, J_{4-5} = 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, H4′), 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, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-1} = 4.0 Hz, J_{2eq-3} = 4.0 Hz, 1H, H2eq), 1.81 (s, 15H, CH_3CO_2H), 1.74 – 1.62 (m, 1H, H2ax). ^{13}C NMR (125 MHz, D_2O) δ: 180.9 (CH₃*C*OOH), 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 (CH₃*C*OOH). ESI-HRMS: *m/z* calcd for $C_{22}H_{44}N_5O_{13}$ $[M+H]^+$ 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° (c = 0.6, methanol). *Rf* (ethyl acetate: isopropyl alcohol 50:1) 0.50. ESI-HRMS: *m/z* calcd for $C_{23}H_{35}N_5NaO_{13}$ [M+Na]$^+$ 752.2431, found 752.2433. ^{1}H NMR (600 MHz, CD$_3$OD) δ 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, $J_{2'\text{-}3'a}$ = 12.0 Hz, $J_{2'\text{-}3'b}$ = $J_{2'\text{-}1'}$ = 3.5 Hz, 1H, H2'), 2.14 – 2.04 (m, 2H, H3'a, H3'b), 2.18 (dt, $J_{2eq\text{-}2ax}$ = 12.6 Hz, $J_{2eq\text{-}1}$ = $J_{2eq\text{-}3}$ = 4.2 Hz, 1H, H2eq), 1.38 (q, $J_{2ax\text{-}1}$ = $J_{2ax\text{-}3}$ = $J_{2ax\text{-}2eq}$ = 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)[165] 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). $[\alpha]_D^{23}$ = + 62.5° (*c* = 1.0, H$_2$O). Lit $[\alpha]_D^{23}$ = + 62.0° (*c* = 1.0, H$_2$O).[165] ^{1}H NMR (600 MHz, D$_2$O) δ 5.37 (d, $J_{1'\text{-}2'}$ = 3.5 Hz, 1H, H1'), 5.13 (d, $J_{1''\text{-}2''}$ = 2.0 Hz, 1H, H1''), 5.09 (d, $J_{1'''\text{-}2'''}$ = 1.5 Hz, 1H, H1'''), 4.31 (dd, $J_{3''\text{-}2''}$ = 5.0 Hz, $J_{3''\text{-}4''}$ = 6.0 Hz, 1H, H3''), 4.17 (dd, $J_{2''\text{-}3''}$ = 5.0 Hz, $J_{2''\text{-}1''}$ = 2.0 Hz, 1H, H2''), 4.14 – 4.09 (m, 1H, H5'''), 4.04 (t, $J_{3'''\text{-}2'''}$ = $J_{3'''\text{-}4'''}$ = 3.0 Hz, 1H, H3'''), 4.02 – 3.97 (m, 1H, H4''), 3.80 (t, $J_{4\text{-}3}$ = $J_{4\text{-}5}$ = 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, $J_{3'eq-3'ax}$ = 11.2 Hz, $J_{3'eq-2'}$ = $J_{3'eq-4'}$ = 4.2 Hz, 1H, H3'eq), 1.86 – 1.69 (m, 16H, H3'ax, CH_3CO$_2$H), 1.65 (q, $J_{2ax-2eq}$ = J_{2ax-3} = J_{2ax-1} = 11.2 Hz, 1H, H2ax). ^{13}C NMR (151 MHz, D$_2$O) δ 180.49 (CH$_3$$CO_2$H), 110.0 (C1''), 95.3 (C1'''), 94.2 (C1'), 84.0 (C5), 81.2 (C4''), 77.8 (C5'), 75.8 (C4), 75.3 (C3''), 73.2 (C2''), 72.0 (C6), 70.1 (C5'''), 67.5 (C4'''), 67.2 (C3'''), 62.9 (C4'), 60.4 (C5''), 59.8 (C6'), 50.7 (C1), 49.6 (C2'''), 48.7 (C3), 47.7 (C2'), 40.3 (C6'''), 29.4 (C3'), 28.1 (C2), 22.7 (CH$_3$CO$_2$H). ESI-HRMS: m/z calcd for C$_{23}$H$_{46}$N$_5$O$_{13}$ [M+H]$^+$ 600.3087, found 600.3083.

6,3',6',2'',5'',3''',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%). $[\alpha]_D^{23}$ = + 17.4° (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%). $[\alpha]_D^{23}$ = + 28.6° (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′-de oxy 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%). $[\alpha]_D^{23}$ = + 17.5° (*c* = 0.9, dichloromethene). *Rf* (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*-Benzyloxycarbonyl-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%). $[\alpha]_D^{23}$ = + 73.0° (*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$_2$C*H*$_2$Ph), 4.16 (dd, *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, $J_{3'-4'}$ = 8.9 Hz, 1H, H3'), 3.79 – 3.74 (m, 1H, H5''a), 3.67 – 3.60 (m, 4H, H6'a, H5''b, 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 (-N*C*O), 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$_2$*C*H$_2$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*-Benzyloxycarbonyl-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%). $[\alpha]_D^{23}$ = + 41.6° (c = 1.0, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.35. ^{1}H NMR (600 MHz, chloroform-*d*) δ 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$_2$C*H$_2$*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, $J_{5'-4'}$ = $J_{5'-6'ax}$ = 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). ^{13}C NMR (151 MHz, chloroform-*d*) δ 170.5, (-N*C*O), 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 (OO-benzylidene), 104.1 (C1'), 86.5 (C4''), 85.4 (C2''), 82.6 (NO-benzylidene), 82.0

(C5), 80.5 (C3''), 76.8 (C4), 75.0 (C4'), 74.7 (C6), 69.0 (C5'), 68.2 (-CO$_2$CH$_2$Ph), 64.3 (C3'),

63.7 (C2'), 63.0 (C5''), 60.0 (C1), 59.2 (C3), 41.7 (C6'), 31.5 (C2). ESI-HRMS: m/z calcd

for C$_{39}$H$_{42}$N$_{10}$NaO$_{12}$ [M+Na]$^+$ 865.2881, found 865.2878.

6,3',5''-Tri-*O*-benzyl-6'-*N*-benzyloxycarbonyl-4'-*O*-6'-*N*-benzylidene-2'',3''-*O*-

benzylidene-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 ^{o}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 ^{o}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%). $[\alpha]_D^{23}$ = + 51.4° (c = 0.5,

dichloromethane). Rf (hexane: ethyl acetate 4: 1) 0.50. ^{1}H NMR (600 MHz, chloroform-*d*)

δ 7.57 – 7.04 (m, 35H), 6.86 (s, 1H, NO-benzylidene), 5.74 (s, 1H, OO-benzylidene), 5.69

(d, $J_{1'-2'}$ = 3.8 Hz, 1H, H1'), 5.60 (br s, 1H, H1''), 5.36 – 5.18 (m, 2H, -CO$_2$CH$_2$Ph), 5.12 –

5.05 (d, J = 11.8 Hz, 1H, -CH$_2$Ph), 4.88 – 4.80 (m, 2H, -CH$_2$Ph), 4.77 – 4.72 (m, 1H, H2''),

4.64 (d, J = 10.2 Hz, 1H, -CH$_2$Ph), 4.57 – 4.53 (m, 1H, H3''), 4.53 – 4.42 (m, 3H, -CH$_2$Ph,

H6'eq), 4.32 (br s, 1H, H4''), 4.16 – 4.03 (m, 1H, H5'), 3.90 (t, $J_{3'-2'}$ = $J_{3'-4'}$ = 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) δ 155.2 (-NCO), 138.2 - 135.5 (aromatic), 129.5 - 126.6 (aromatic), 110.2 (C1'), 104.0 (OO-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 (-CH$_2$Ph), 75.1 (-CH$_2$Ph), 73.3 (-CH$_2$Ph), 70.4 (C5''), 68.1 (-CO$_2$$CH_2$Ph), 64.3 (C5'), 63.2 (C2'), 60.5 (C1), 59.3 (C3), 41.9 (C6'), 32.2 (C2). ESI-HRMS: m/z calcd for C$_{60}$H$_{60}$N$_{10}$NaO$_{12}$ [M+Na]$^+$ 1135.4290, found 1135.4288.

6,3',5''-Tri-O-benzyl-6'-N-benzyloxycarbonyl-2'',3''-O-benzylidene-1,3,2'-trideami no-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 ^{o}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) δ 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_2Ph), 4.90 – 4.83 (m, 3H, H2'', -CH_2Ph), 4.71 (d, J = 10.9 Hz, 1H, -CH_2Ph), 4.64 – 4.57 (m, 3H, H3'', -CH_2Ph), 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_{5-4} = 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

(OO-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 (-CH$_2$Ph), 75.4 (-CH$_2$Ph), 73.3 (-CH$_2$Ph), 71.6 (C5'), 71.2 (C4'),

70.5 (C5''), 67.4 (-CO$_2$$CH_2$Ph), 63.3 (C2'), 60.5 (C1), 59.5 (C3), 41.2 (C6'), 32.3 (C2).

ESI-HRMS: m/z calcd for C$_{53}$H$_{56}$N$_{10}$NaO$_{12}$ [M+Na]$^+$ 1047.3977, found 1047.3980.

6,3',5''-Tri-O-benzyl-1,3,2',6'-tetra-N-benzyloxycarbonyl-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 ^{o}C

for 1 h, and then 0.1 M sodium hydroxide soln. (9 mL) was added. The reaction mixture

was kept stirring at 70 ^{o}C for 6 h. All solvent was removed under reduced pressure and

the residue was dissolved in methanol (20 mL). N-(Benzyloxycarbonyloxy)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%). $[\alpha]_D^{23}$ = + 12.7 ° (c = 1.0,

dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.45. ESI-HRMS: *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.

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%). $[\alpha]_D^{23}$ = + 17.8° (*c* =

1.4, dichloromethane). *Rf* (hexane: ethyl acetate 3: 2) 0.30. ESI-HRMS: *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.

6,5''-Di-*O*-benzyl-1,3,2',6'-tetra-*N*-benzyloxycarbonyl-2'',3''-*O*-benzylidene-3'-deox

y 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 ºC. Samarium

iodide solution (0.1 M in tetrahydrofuran, 7 mL, 0.7 mmol) was added and the mixture

was kept stirring at -20 ºC for 1 h. Dry methanol (48 µL, 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%). $[a]_D^{23}$ = + 8.7° (*c* = 1.7, dichloromethane). *Rf* (chloroform: ethyl

acetate 3: 1) 0.22. ESI-HRMS: *m/z* calcd for $C_{70}H_{74}N_4NaO_{17}$ [M+Na]+ 1265.4947, 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° (*c* = 0.2, H_2O). Lit

$[\alpha]_D^{23}$ = + 41° (*c* = 1.0, H_2O).[190] 1H NMR (600 MHz, D_2O) δ 5.66 (d, $J_{1'-2'}$ = 3.4 Hz, 1H, H1'),

5.13 (d, $J_{1''-2''}$ = 0.3 Hz, 1H, H1''), 4.00 (dd, $J_{2''-3''}$ = 3.7 Hz, $J_{2''-1''}$ = 0.3 Hz, 1H, H2''), 3.97 –

3.94 (m, 1H, H3''), 3.85 – 3.78 (m, 2H, H4'', H4), 3.72 – 3.63 (m, 3H, 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, $J_{3'eq-3'ax}$ = 11.7 Hz, $J_{3'eq-2'}$ = $J_{3'eq-4'}$ = 3.6 Hz, 1H, H3'eq), 1.80 (q, $J_{3'ax-3'eq}$

= $J_{3'ax-2'}$ = $J_{3'ax-4'}$ = 11.7 Hz, 1H, H3'ax), 1.72 (s, 12H, CH_3CO$_2$H), 1.62 (q, $J_{2ax-2eq}$ = J_{2ax-1} =

J_{2ax-3} = 12.6 Hz, 1H, H2ax). ^{13}C NMR (151 MHz, D$_2$O) δ 180.8 (CH$_3$$CO_2$H), 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$_3$CO$_2$H). 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-*O*-benzyl-1,3,2′,6′,2‴,6‴-hexa-*N*-benzyloxycarbonyl

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 ºC for 1 h. Then 0.1 M sodium hydroxide solution (6.5 mL) was added and the

mixture was heated at 70 ºC for 8 h. *N*-(Benzyloxycarbonyloxy)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%). $[\alpha]_D^{23}$ = +

10.0° (*c* = 1.0, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.25. ESI-HRMS: m/z

calcd for $C_{113}H_{118}N_6NaO_{25}$ [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*-benzyloxycarbonyl-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%) $[\alpha]_D^{23}$ = + 20.2° (*c* = 1.1, dichloromethane). *Rf* (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*-benzyloxycarbonyl-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 °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 °C and stirred for another 2 h. Saturated aqueous sodium bicarbonate was added at 0 °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° (*c* = 1.3, dichloromethane). *Rf* (chloroform: ethyl acetate 1: 1) 0.70. ESI-HRMS: *m/z* calcd for $C_{106}H_{112}N_6NaO_{24}$ [M+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)[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° (*c* = 0.4, H$_2$O). Lit $[\alpha]_D^{15}$ = + 52° (*c* = 1.0, H$_2$O).[191] ^{1}H NMR (600 MHz, D$_2$O) δ 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, $J_{3''-2''}$ = $J_{3''-4''}$ =

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, $J2eq\text{-}2ax$ = 11.7 Hz, J_{2eq-1} = J_{2eq-3} = 3.9 Hz, 1H, H2eq), 2.07 (dt, $J_{3'eq-3'ax}$ = 12.0 Hz, $J_{3'eq-2'}$ = $J_{3'eq-4'}$ = 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 CH_3CO$_2$H), 1.71 – 1.62 (q, $J_{2ax-2eq}$ = J_{2ax-1} = J_{2ax-3} = 11.7 Hz, 1H, H2ax). ^{13}C NMR (151 MHz, cdcl$_3$) δ 180.6 (CH$_3$$CO_2$H), 109.8 (C1''), 96.0 (C1'''), 93.0 (C1'), 84.5 (C5), 81.1 (C4''), 75.1 (C4), 75.0 (C3''), 73.3 (C2''), 71.9 (C6), 69.8 (C5'), 69.7 (C5'''), 67.1 (C3'''), 66.9 (C4'''), 64.4 (C4'), 60.1 (C5''), 50.2 (C2'''), 49.4 (C1), 48.2 (C3), 47.5 (C2'), 39.9 (C6''), 39.7 (C6'), 28.7 (C3'), 27.8 (C2), 22.1 (CH$_3$CO$_2$H). ESI-HRMS: m/z calcd for C$_{23}$H$_{47}$N$_6$O$_{12}$ [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%). $[\alpha]_D^{23}$ = + 90.5° (*c* = 0.5, chloroform). *Rf* (hexane:

ethyl acetate 1: 4) 0.45. ¹H NMR (600 MHz, chloroform-*d*) δ 7.51 – 7.47 (m, 2H,

aromatic), 7.40 – 7.37 (m, 3H, aromatic), 5.68 (d, $J_{1'\text{-}2'}$ = 3.9 Hz, 1H, H1'), 5.53 (s, 1H,

benzyllidene-H), 5.24 (d, $J_{1''\text{-}2''}$ = 4.6 Hz, 1H, H1''), 5.11 (d, $J_{1'''\text{-}2'''}$ = 1.3 Hz, 1H, H1'''), 4.42

(dd, $J_{3''\text{-}2''}$ = 5.0 Hz, $J_{3''\text{-}4''}$ = 3.2 Hz, 1H, H3''), 4.31 – 4.24 (m, 3H, H6', H2'', H4''), 4.19 – 4.10

(m, 2H, H3', H5'), 4.02 (t, $J_{3'''\text{-}2'''}$ = $J_{3'''\text{-}4'''}$ = 3.5 Hz, 1H, H3'''), 4.00 – 3.97 (m, 1H. H5'''), 3.88

(d, $J_{5''\text{-}4''}$ = 3.2 Hz, 2H. H5''), 3.78 (dd, $J_{2'''\text{-}1'''}$ = 1.3 Hz, $J_{2'''\text{-}3''}{}'$ = 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, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$ = 9.4 Hz, 1H, H4'),

3.43 – 3.31 (m, 4H, H6''', H1, H3, H6), 3.24 (dd, $J_{2'\text{-}3'}$ = 10.1 Hz, $J_{2'\text{-}1'}$ = 3.9 Hz, 1H, H2'), 2.17

(dt, $J_{2eq\text{-}2ax}$ = 12.8 Hz, $J_{2eq\text{-}1}$ = $J_{2eq\text{-}3}$ = 4.5 Hz, 1H, H2eq), 1.40 (q, $J_{2ax\text{-}2eq}$ = $J_{2ax\text{-}1}$ = $J_{2ax\text{-}3}$ = 12.8

Hz, 1H, H2ax), 1.18 – 1.01 (m, 21H, -Si(CH(CH₃)₂)₃). ¹³C NMR (151 MHz, chloroform-*d*) δ

136.9 (aromatic), 129.5 (aromatic), 128.5 (aromatic), 126.4 (aromatic), 106.3 (C1''),

102.1 (benzylidene C), 99.0 (C1'''), 97.9 (C1'), 84.6 (C4), 83.6 (C2''), 81.5 (C4'), 77.0

(C3''), 76.4 (C4'''), 75.1 (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(CH₃)₂), 12.0 (-CH(CH₃)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*-benzyl-4',6'-*O*-benzylidene-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° (*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, -OC*H*$_2$Ph), 4.89 – 4.84 (m, 2H, H1''', -OC*H*$_2$Ph), 4.80 (d, *J* = 10.8 Hz, 1H, -OC*H*$_2$Ph), 4.76 (d, *J* = 10.8 Hz, 1H, -OC*H*$_2$Ph), 4.62 (d, *J* = 11.9 Hz, 1H, -OC*H*$_2$Ph), 4.51 (d, *J* = 11.8 Hz, 1H, -OC*H*$_2$Ph), 4.43 – 4.36 (m, 3H, H3'', -OC*H*$_2$Ph), 4.35 – 4.28 (m, 3H, H6', -OC*H*$_2$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, H2$_{eq}$), 1.45 (q, $J_{2ax-2eq}$

= J_{2ax-1} = J_{2ax-3} = 13.2 Hz, 1H, H2$_{ax}$), 1.11 – 1.03 (m, 21H, -Si(CH(CH$_3$)$_2$)$_3$). ^{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 (-CH$_2$Ph), 74.7 (-CH$_2$Ph), 73.8 (C5'''), 73.0 (C3'''), 72.6 (-CH$_2$Ph), 72.5 (-CH$_2$Ph), 71.8 (-CH$_2$Ph), 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(CH$_3$)$_2$), 12.0 (-CH(CH_3)$_2$). ESI-HRMS: m/z calcd for C$_{74}$H$_{89}$N$_{15}$NaO$_{14}$Si [M+Na]$^+$ 1462.6380, found 1462.6384.

6,3',2'',3''',4'''-Penta-O-benzyl-1,3,2',2''',6'''-penta-N-benzyloxycarbonyl-4',6'-O-benzylidene-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-(benzyloxycarbonyloxy)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%). [α]$_D^{23}$ = + 12.5° (c = 1.0, dichloromethane). Rf (hexane: ethyl

acetate 2: 1) 0.25. ESI-HRMS: m/z calcd for $C_{114}H_{129}N_5NaO_{24}Si$ [M+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*-be nzylidene 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° (*c* = 0.7, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.32. ESI-HRMS: m/z calcd for $C_{105}H_{109}N_5NaO_{24}$ [M+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° (*c* = 0.3, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1)

0.60. ESI-HRMS: *m/z* calcd for $C_{105}H_{109}N_8NaO_{23}$ [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-*O*-benzyl-1,3,2',2''',6'''-penta-*N*-benzyloxycarbonyl-4',6'-*O*-be

nzylidene-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° (*c* = 0.3, dichloromethane). *Rf* (hexane: ethyl acetate 1: 2) 0.70. ESI-HRMS: *m/z* calcd for $C_{106}H_{110}N_6NaO_{24}$ [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.

6,3',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° (*c* = 0.7, dichloromethane). *Rf* (hexane: ethyl acetate 1: 2) 0.25. ESI-HRMS: *m/z* calcd for $C_{106}H_{112}N_6NaO_{24}$ [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*-benzyloxycarbonyl-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%). $[\alpha]_D^{23} = + 19.3°$ ($c = 0.9$, dichloromethane). *Rf* (hexane: ethyl acetate 1: 2) 0.45. ESI-HRMS: *m/z* calcd for $C_{106}H_{110}N_6NaO_{24}$ $[M+Na]^+$ 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*-benzyloxycarbonyl-3',5''-dide oxy-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 $C_{99}H_{106}N_6NaO_{23}$ [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, H$_2$O). ^{1}H NMR (600 MHz, D$_2$O) δ 7.99 (s, 1H, C*H*O), 5.31 (d, $J_{1'-2'}$ = 2.4 Hz, 1H, H1'), 5.10 (br s, 2H, H1'', H1'''), 4.29 (t, $J_{3''-2''}$ = $J_{3''-4''}$ = 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, H4''', 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, H2$_{eq}$), 2.09 – 2.05 (m, 1H, H3'$_{eq}$), 1.88 – 1,84 (m, 1H, H3'$_{ax}$), 1.79 (s, 15H, CH_3CO$_2$H), 1.66 – 1.62 (m, 1H, H2$_{ax}$). ^{13}C NMR (150 MHz, D$_2$O) δ 180.3 (CH$_3$$C$OOH), 164.8 ($C$HO), 109.9 (C1''), 95.5 (C1'), 95.4 (C1'''), 83.2 (C5), 79.4 (C4''), 77.2 (C4), 74.1 (C5'), 72.9 (C2''), 72.1 (C6), 70.1 (C5'''), 69.0 (C4'), 67.6 (C3'''), 67.3 (C4'''), 62.7 (C6'), 53.6 (C3''), 50.7 (C2'''), 49.6 (C1), 48.9 (C3), 48.7 (C2'), 40.4 (C6'''), 38.8 (C5''), 29.2 (C2), 28.5 (C3'), 22.6 (CH$_3$COOH), ESI-HRMS: m/z calcd for C$_{24}$H$_{47}$N$_6$NaO$_{13}$ [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-*O*-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]$_D^{23}$ = + 43.2° (c = 1.8, dichloromethane). Rf (toluene: ethyl acetate 20: 1) 0.60. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.48 – 7.20 (m, 35H), 6.06 (d, $J_{1'-2'}$ = 3.5 Hz, 1H, H1'), 5.63 (d, $J_{1''-2''}$ = 4.4 Hz, 1H, H1''), 5.00 – 4.91 (m, 3H, H1''', -CH_2Ph), 4.84 (d, J = 11.1 Hz, 1H, -CH_2Ph), 4.75 (d, J = 11.0 Hz, 1H, -CH_2Ph), 4.70 – 4.56 (m, 4H, -CH_2Ph), 4.49

(d, *J* = 12.4 Hz, 1H, -C*H*₂Ph), 4.45 – 4.37 (m, 3H, H3", -C*H*₂Ph), 4.34 (d, *J* = 12.3 Hz, 1H, -C*H*₂Ph), 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, 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, *J*₂ₑq₋₂ₐₓ = 12.7 Hz, *J*₂ₑq₋₁ = *J*₂ₑq₋₃ = 4.0 Hz, 1H, H2eq), 1.41 (q, *J*₂ₐₓ₋₂ₑq = *J*₂ₐₓ₋₁ = *J*₂ₐₓ₋₃ = 12.7 Hz, 1H, H2ax), 1.21 – 0.97 (m, 21H, -Si(C*H*(C*H*₃)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 (-*C*H₂Ph), 73.72 (-*C*H₂Ph), 73.51 (-*C*H₂Ph), 73.20 (C5‴), 72.83 (C3‴), 72.57 (-*C*H₂Ph), 72.24 (-*C*H₂Ph), 71.91 (-*C*H₂Ph), 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 (-C*H*(CH₃)₂), 12.0 (-CH(C*H*₃)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*-benzyloxycarbonyl-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*-(Benzyloxycarbonyloxy)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%). $[\alpha]_D^{23}$ = + 17.6° (*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%). $[\alpha]_D^{23}$ = + 38.6° (*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*-triiso

propylsilyl-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%). $[\alpha]_D^{23}$ = + 27.6° (*c* = 1.0, dichloromethane). *Rf* (hexane: ethyl acetate 1:

1) 0.25. ESI-HRMS: *m/z* calcd for $C_{107}H_{125}N_5NaO_{23}Si$ [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° (c = 0.6, dichloromethane). *Rf* (hexane: ethyl acetate 1: 2) 0.45. ESI-HRMS: *m/z* calcd for $C_{98}H_{105}N_5NaO_{23}$ [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*-benzyloxycarbonyl-3'-deoxy-5''-*O*-tosyl paromomycin (93) Compound **92** (240 mg, 0.14 mmol) was dissolved 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° (c = 0.6, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.27. ESI-HRMS: *m/z* calcd for $C_{105}H_{111}N_5NaO_{25}S$ [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*-benzyloxycarbonyl-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%). $[\alpha]_D^{23}$ = + 16.0° (*c* = 0.4, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.16. ESI-HRMS: *m/z* calcd for $C_{98}H_{104}N_8NaO_{22}$ [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*-benzyloxycarbonyl-3',5''-dideoxy-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)[192] 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.[192]

1,6-Anhydro-3,4-epoxy-2-tosyl-β-D-glucopyranose (113)[192] 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.[192]

1,6-Anhydro-4-deoxy-4-*C*-allyl-2-tosyl-β-D-glucopyranose (114)[193] 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°

(c = 1.00, chloroform). *Rf* (hexane: ethyl acetate 1: 1) 0.41. ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.38 – 7.23 (m, 5H: aromatic), 5.83 (ddq, *J* = 16.4, 14.4, 6.2, 5.3 Hz, 1H: CH$_2$C*H*CH$_2$-C4), 5.47 (s, 1H: H1), 5.21 – 5.10 (m, 2H: C*H$_2$*CHCH$_2$-C4), 4.40 (d, *J* = 5.0 Hz, 1H: H5), 4.06 (d, *J* = 7.0 Hz, 1H: H6a), 3.93 – 3.85 (m, 2H: PhC*H$_2$*), 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: CH$_2$CHC*H$_2$*-C4), 1.77 (t, *J* = 7.2 Hz, 1H: H4). ^{13}C NMR (125 MHz, chloroform-*d*) δ 139.9, 136.1 (CH$_2$*C*HCH$_2$-C4), 128.5, 128.1, 127.2, 117.5 (*C*H$_2$CHCH$_2$-C4), 102.6 (C1), 74.6 (C5), 70.3 (C3), 68.5 (C6), 62.2 (C2), 51.7 (Ph*C*H$_2$), 44.6 (C4), 36.5 (CH$_2$CH*C*H$_2$-C4). 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*-benzyloxycarbonyl-2,4-dideoxy-4-*C*-propyl-β-D-glucopyranose (117) 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%). $[\alpha]_D^{23}$ = -34.3°

(c = 1.00, chloroform). *Rf* (hexane: ethyl acetate 1: 1) 0.36. ^{1}H NMR (600 MHz, chloroform-*d*) δ 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: PhC*H*$_2$), 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$_2$C*H*$_2$), 1.49 – 1.38 (m, 2H: CH$_3$C*H*$_2$CH$_2$, CH$_3$CH$_2$C*H*$_2$), 1.38 – 1.26 (m, 1H: CH$_3$C*H*$_2$CH$_2$), 0.91 (t, *J* = 7.0 Hz, 3H: C*H*$_3$CH$_2$CH$_2$). ^{13}C NMR (150 MHz, chloroform-*d*) δ 155.7 (*C*=O), 136.1, 128.5, 128.2, 128.2, 101.2 (C1), 74.9 (C5), 71.8 (C3), 68.3 (C6), 67.1 (Ph*C*H$_2$), 54.8 (C2), 44.1 (C4), 34.0 (CH$_3$CH$_2$*C*H$_2$), 20.8 (CH$_3$*C*H$_2$CH$_2$), 14.0 (*C*H$_3$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*-benzyloxycarbonyl-2,4-dideoxy-4-*C*-propyl-3-*O*-[(methylthio)thiocarbonyl]-β-D-glucopyranose (118) A soln. of **117** (1.54 g, 4.8 mmol) in dry tetrahydrofuran (40 mL) was cooled to 0 ^{o}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%). [α]$_D^{23}$ = -60.0° (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: PhC*H*₂), 5.10 – 5.03 (m, 2H: PhC*H*₂, 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: C*H*₃S), 1.85 (t, *J* = 7.5 Hz, 1H: H4), 1.59 (q, *J* = 7.5 Hz, 2H: CH₃CH₂C*H*₂), 1.51 (dt, *J* = 14.6, 7.1 Hz, 1H: CH₃C*H*₂CH₂), 1.36 (dq, *J* = 14.9, 7.3 Hz, 1H: CH₃C*H*₂CH₂), 0.92 (t, *J* = 7.2 Hz, 3H: C*H*₃CH₂CH₂). ^{13}C 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 (Ph*C*H₂), 51.8 (C2), 41.4 (C4), 33.0 (CH₃*C*H₂CH₂), 20.7 (CH₃*C*H₂CH₂), 18.9 (*C*H₃), 13.9 (*C*H₃CH₂CH₂). ESI-HRMS: *m/z* calcd for $C_{19}H_{25}NO_5S_2Na$ [M+Na]$^+$ 434.1072, found 434.1072.

1,6-Anhydro-2-*N*-benzyloxycarbonyl-2,3,4-trideoxy-4-*C*-propyl-β-D-glucopyranos e (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]$_D^{23}$ = -17.6° (c = 1.00, chloroform). *Rf* (hexane: ethyl acetate 4: 1) 0.22. ^{1}H 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, PhC*H*₂), 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: H3$_{eq}$), 1.77 – 1.58 (m, 1H: CH$_3$CH$_2$CH_2), 1.57 – 1.26 (m, 5H: H3$_{ax}$, H4, CH$_3$CH_2CH$_2$, CH$_3$CH$_2$CH_2, CH$_3$CH_2CH$_2$), 0.91 (t, J = 7.2 Hz, 3H: CH_3CH$_2$CH$_2$). ^{13}C 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 (PhCH$_2$), 48.8 (C2), 36.6 (C4), 35.5 (CH$_3$CH$_2$CH$_2$), 25.6 (C3), 20.9 (CH$_3$$CH_2CH_2$), 14.0 ($CH_3CH_2CH_2$). ESI-HRMS: m/z calcd for C$_{17}$H$_{23}$NO$_4$Na [M+Na]$^+$ 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° (c = 1.00, chloroform). *Rf* (hexane: ethyl acetate 4: 1) 0.58. ^{1}H NMR (500

MHz, chloroform-*d*) δ 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$_2$C*H*$_2$), 1.68 – 1.57 (m, 2H: H3$_{ax}$,

CH$_3$CH$_2$C*H*$_2$), 1.53 – 1.45 (m, 1H: H4), 1.53 – 1.45 (m, 1H: H4), 1.47 – 1.31 (m, 2H:

CH$_3$C*H*$_2$CH$_2$), 0.95 (t, *J* = 7.3 Hz, 3H: C*H*$_3$CH$_2$CH$_2$). ^{13}C NMR (125 MHz, chloroform-*d*) δ

100.6 (C1), 76.0 (C5), 68.2 (C6), 57.6 (C2), 36.0 (C4), 34.5 (CH$_3$CH$_2$*C*H$_2$), 24.2 (C3), 20.8

(CH$_3$*C*H$_2$CH$_2$), 14.0 (*C*H$_3$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: ^{1}H 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: CH$_3$C*H$_2$*CH$_2$, CH$_3$CH$_2$C*H$_2$*, CH$_3$CH$_2$C*H$_2$*, CH$_3$C*H$_2$*CH$_2$), 0.94 (t, *J* = 7.1 Hz, 3H:

C*H$_3$*CH$_2$CH$_2$). ^{13}C 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 (CH$_3$CH$_2$*C*H$_2$), 29.8 (C3), 19.1

(CH$_3$*C*H$_2$CH$_2$), 14.2 (*C*H$_3$CH$_2$CH$_2$). β anomer: ^{1}H 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: HO-C6), 1.68 – 1.58 (m, 1H: H4), 1.50 – 1.15 (m, 5H: CH$_3$C*H$_2$*CH$_2$, CH$_3$CH$_2$C*H$_2$*,

CH$_3$C*H$_2$*CH$_2$), 1.12 – 1.00 (m, 1H: CH$_3$CH$_2$C*H$_2$*), 0.90 (t, *J* = 7.1 Hz, 1H: C*H$_3$*CH$_2$). ^{13}C 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 (CH$_3$CH$_2$*C*H$_2$), 19.2 (CH$_3$*C*H$_2$CH$_2$), 14.2 (*C*H$_3$CH$_2$CH$_2$).

ESI-HRMS: *m/z* calcd for C$_{15}$H$_{21}$N$_3$O$_2$SNa [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 anomeric mixtures, which are also a

mixture of diastereomers at sulfur. *Rf* (hexane: ethyl acetate 3: 1) 0.17. ESI-HRMS: *m/z*

calcd for $C_{22}H_{27}N_3NaO_3S$ $[M+Na]^+$ 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*-benzyl-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%). $[\alpha]_D^{23}$ = +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, $J_{1''-2''}$ = 4.6 Hz, 1H, H1''), 4.96 (d, J = 11.3 Hz, 1H, -CH_2Ph), 4.91 (d,

J = 10.9 Hz, 1H, -CH_2Ph), 4.88 (d, $J_{1'''-2'''}$ = 1.3 Hz, 1H, H1'''), 4.75 – 4.68 (m, 2H, -CH_2Ph),

4.61 (d, J = 11.9 Hz, 1H, -CH_2Ph), 4.53 (d, J = 11.8 Hz, 1H, -CH_2Ph), 4.44 – 4.39 (m, 2H,

-CH_2Ph), 4.36 (t, $J_{3''-2''}$ = $J_{3''-4''}$ = 4.6 Hz, 1H, H3''), 4.33 (d, J = 11.9 Hz, 1H, -CH_2Ph), 4.29 (d,

J = 12.0 Hz, 1H, -CH_2Ph), 4.25 (dd, $J_{4''-5''}$ = 7.6 Hz, $J_{4''-3''}$ = 4.6 Hz, 1H, H4''), 3.96 (td, $J_{5'-4'}$ =

$J_{5'-6'a}$ = 9.6 Hz, $J_{5'-6'b}$ = 2.4 Hz, 1H, H5'), 3.92 (t, $J_{2''-1''}$ = $J_{2''-3''}$ = 4.6 Hz, 1H, H2''), 3.89 – 3.80

(m, 5H, H3', H6'a, H5'', 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 (-CH_2Ph), 73.7 (-CH_2Ph), 73.1 (C5'''), 72.7 (C3'''), 72.6 (-CH_2Ph), 72.5 (-CH_2Ph), 71.8 (C4'''), 71.6 (-CH_2Ph), 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(CH_3)$_2$), 12.0 (-CH(CH_3)$_2$). ESI-HRMS: m/z calcd for $C_{67}H_{85}N_{15}NaO_{14}Si$ [M+Na]$^+$ 1374.6067, found 1374.6071.

5-O-[3-O-(2,6-Diazido-2,6-dideoxy-3,4-di-O-benzyl-α-L-idopyranosyl)-2-O-benzyl-5-O-triisopropylsilyl-β-D-ribofuranosyl]-1,3-dideamino-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%). $[\alpha]_D^{23}$ = +83.2° (c = 0.8, dichloromethane). Rf (hexane: ethyl acetate 2: 1) 0.66. ^{1}H NMR (600 MHz, chloroform-d) δ 7.40 – 7.11 (m, 20H), 5.31 (d, $J_{1''-2''}$ = 3.3 Hz, 1H, H1''), 4.95 (d, $J_{1'''-2'''}$ = 1.9 Hz, 1H. H1'''), 4.89 (d, J = 10.9 Hz, 1H, -CH_2Ph), 4.83(d, J = 11.2 Hz, 1H, -CH_2Ph), 4.64 (d, J = 12.0 Hz, 1H, -CH_2Ph), 4.51 (t, $J_{3''-2''}$ = $J_{3''-4''}$ = 4.7 Hz, 1H, H3''), 4.47 (d, J = 11.8 Hz, 1H, -CH_2Ph), 4.43 (d, J = 11.8 Hz, 1H, -CH_2Ph),

4.37 (d, J = 9.6 Hz, 1H, -CH_2Ph), 4.34 – 4.32 (m, 1H, H4''), 4.31 – 4.27 (m, 2H, -CH_2Ph), 4.04 (dd, $J_{2''\text{-}3''}$ = 4.7 Hz, $J_{2''\text{-}1''}$ = 3.3 Hz, 1H, H2''), 3.94 (dd, $J_{5''a\text{-}4''}$ = 2.7 Hz, $J_{5''a\text{-}5''b}$ = 11.7 Hz, 1H, H5''a), 3.90 – 3.86 (m, 1H, H5'''), 3.83 (dd, $J_{5''a\text{-}5''b}$ = 11.7 Hz, $J_{5''b\text{-}4''}$ = 3.6 Hz, 1H, H5''b), 3.81 – 3.78 (m, 1H, H3'''), 3.69 (dd, $J_{6'''a\text{-}6'''b}$ = 12.9 Hz, $J_{6'''a\text{-}5'''}$ = 8.4 Hz, 1H, H6'''a), 3.53 (t, $J_{2'''\text{-}3'''}$ = $J_{2'''\text{-}1'''}$ = 1.9 Hz, 1H, H2'''), 3.48 (t, $J_{5\text{-}6}$ = $J_{5\text{-}4}$ = 9.3 Hz, 1H, H5), 3.46 – 3.40 (m, 2H, H1, H4), 3.40 – 3.34 (m, 1H, H3), 3.29 (t, $J_{6\text{-}5}$ = $J_{6\text{-}1}$ = 9.3 Hz, 1H, H6), 3.19 (t, $J_{4'''\text{-}5'''}$ = $J_{4'''\text{-}3'''}$ = 2.4 Hz 1H, H4'''), 3.07 (dd, $J_{6'''b\text{-}6'''a}$ = 12.9 Hz, $J_{6'''b\text{-}5'''}$ = 4.3 Hz, 1H, H6'''b), 2.09 (dt, $J_{2eq\text{-}2ax}$ = 13.2 Hz, $J_{2eq\text{-}1}$ = $J_{2eq\text{-}3}$ = 4.4 Hz, 1H, H2eq), 1.28 (q, $J_{2ax\text{-}2eq}$ = $J_{2ax\text{-}1}$ = $J_{2ax\text{-}3}$ = 13.2 Hz, 1H, H2ax). ^{13}C NMR (151 MHz, cdcl$_3$) 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 (-CH$_2$Ph), 75.1 (C3''), 74.1 (C5'''), 72.8 (C3'''), 72.5 (-CH$_2$Ph), 72.4 (-CH$_2$Ph), 71.7 (-CH$_2$Ph), 71.5 (C4'''), 63.2 (C5''), 60.5 (C1), 59.5 (C3), 57.5 (C2''), 51.1 (C6'''), 18.0 (-CH(CH$_3$)$_2$), 11.9 (-CH(CH$_3$)$_2$). ESI-HRMS: m/z calcd for $C_{54}H_{70}N_{12}NaO_{10}Si$ [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-benzyl-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 flitered 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%).

$[\alpha]_D^{23}$ = + 39.0° (c = 0.2, dichloromethane). *Rf* (hexane: ethyl acetate 3: 1) 0.24. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.41 – 7.11 (m, 25H), 5.90 (d, $J_{1'-2'}$ = 3.4 Hz, 1H, H1'), 5.71 (d, $J_{1''-2''}$ = 6.2 Hz, 1H, H1''), 4.97 – 4.92 (m, 2H, -CH_2Ph, H1'''), 4.74 – 4.64 (m, 3H, -CH_2Ph), 4.61 (d, *J* = 12.1 Hz, 1H, -CH_2Ph), 4.55 (d, *J* = 11.6 Hz, 1H, -CH_2Ph), 4.48 (d, *J* = 12.2 Hz, 1H, -CH_2Ph), 4.38 (d, *J* = 12.1 Hz, 1H, -CH_2Ph), 4.35 – 4.31 (m, 1H, H4''), 4.31 – 4.22 (m, 2H, -CH_2Ph), 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 Hz, J_{2eq-1} = J_{2eq-3} = 4.3 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, CH_2CH$_3$), 1.30 – 1.16 (m, 2H, CH_2CH$_3$, CH_2CH$_2$CH$_3$), 1.15 – 1.05 (m, 1H, CH_2CH$_2$CH$_3$), 0.86 (t, J = 7.0 Hz, 3H, CH$_3$). ESI-HRMS: m/z calcd for $C_{61}H_{71}N_{15}NaO_{12}$ [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'-dideoxy-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 oC for 1 h under an argon atmosphere, deionized water (0.8 mL) was added and the mixture was kept stirring at 70 ^{o}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%). $[\alpha]_D^{23}$ = + 12.3° (c = 1.0, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1) 0.62. ESI-HRMS: m/z calcd for $C_{101}H_{111}N_5NaO_{22}$ [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*-(benzyloxycarbonyl)-3',4',5''-t

rideoxy-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,

0.007mmol). 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%). $[\alpha]_D^{23}$ = + 17.9 (c = 1.1, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1)

0.50. ESI-HRMS: *m/z* calcd for $C_{101}H_{110}N_8NaO_{21}$ [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, 0.008mmol). 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° (c = 0.2, dichloromethane). *Rf* (hexane: ethyl acetate 3: 1) 0.45. ^{1}H NMR (600 MHz, chloroform-*d*) δ 7.77 (dd, *J* = 12.0, 5.5 Hz, 2H, aromatic), 7.40 – 7.17 (m, 27H), 5.82 (d, $J_{1'-2'}$ = 3.5 Hz, 1H, H1'), 5.54 (d, $J_{1''-2''}$ = 5.0 Hz, 1H, H1''), 4.89 (d, *J* = 10.9 Hz, 1H, -C*H*$_2$Ph), 4.69 (d, *J* = 12.1 Hz, 1H, -C*H*$_2$Ph), 4.68 – 4.64 (m, 2H, H1''', -C*H*$_2$Ph), 4.59 (d, *J* = 12.0 Hz, 1H, -C*H*$_2$Ph), 4.55 – 4.51 (m, 2H, -C*H*$_2$Ph), 4.47 (d, *J* = 11.7 Hz, 1H, -C*H*$_2$Ph), 4.42 (d, *J* = 11.9 Hz, 1H, -C*H*$_2$Ph), 4.34 (d, *J* = 11.9 Hz, 1H, -C*H*$_2$Ph), 4.31 – 4.24 (m, 3H, H4'', H5''a, -C*H*$_2$Ph), 4.22 (dd, $J_{5''b-5''a}$ = 10.3 Hz, $J_{5''b-4''}$ = 2.5 Hz, 1H, H5''b), 4.09 (dd, $J_{3''-4''}$ = 5.1 Hz, $J_{3''-2''}$ = 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, $J_{6'''a-5'''}$ = 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, $J_{6'''b-6'''a}$ = 12.6 Hz, $J_{6'''b-5'''}$ = 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, -PhC*H*$_3$), 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, C*H*$_2$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, C*H*$_2$CH$_3$, C*H*$_2$CH$_2$CH$_3$), 1.13 – 1.06 (m, 1H, C*H*$_2$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''), 75.7 (C3''), 75.0 (-CH_2Ph), 74.6 (C4), 73.7 (-CH_2Ph), 73.3 (-CH_2Ph), 73.2 (C3'''), 73.1 (C5''), 72.5 (-CH_2Ph), 72.2 (C5'), 72.0 (-CH_2Ph), 71.9 (-CH_2Ph), 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 ($CH_2CH_2CH_3$), 32.7 (C2), 27.2 (C3'), 21.6 (CH_3Ph), 19.1 (CH_2CH_3), 14.3 (CH_2CH_3). ESI-HRMS: *m/z* calcd for $C_{68}H_{77}N_{15}NaO_{14}S$ [M+Na]$^+$ 1382.5393, found 1382.5390.

1,3,2',2''',6'''-Pentadeamino-1,3,2',2''',6'''-pentaazido-6,6',2'',3''',4'''-penta-*O*-benzyl-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, -CH_2Ph), 4.56 – 4.52 (m, 3H, -CH_2Ph), 4.49 (d, *J* = 11.9 Hz, 1H, -CH_2Ph), 4.44 – 4.41

(m, 1H, H4''), 4.40 (d, J = 12.5 Hz, 1H, -CH_2Ph), 4.37 (t, $J_{3''\text{-}2''}$ = $J_{3''\text{-}4''}$ = 4.8 Hz, 1H, H3''),

4.33 (d, J = 12.0 Hz, 1H, -CH_2Ph), 4.26 (d, J = 12.0 Hz, 1H, -CH_2Ph), 4.16 (dd, $J_{5''a\text{-}5''b}$ = 14.1

Hz, $J_{5''a\text{-}4''}$ = 4.7 Hz, 1H, H5''a), 3.96 (t, $J_{2''\text{-}1''}$ = $J_{2''\text{-}3''}$ = 3.6 Hz, 1H, H2''), 3.91 (dd, $J_{5''b\text{-}5''a}$ =

14.1 Hz, $J_{5''b\text{-}4''}$ = 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, $J_{5\text{-}4}$ = $J_{5\text{-}6}$ = 9.1 Hz, 1H, H5), 3.42 (t, $J_{4\text{-}5}$ = $J_{4\text{-}3}$ =

9.1 Hz, 1H, H4), 3.38 – 3.27 (m, 3H, H6''', H1, H3), 3.26 (t, $J_{2'''\text{-}1'''}$ = $J_{2'''\text{-}3'''}$ = 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, $J_{2eq\text{-}2ax}$ =

13.2 Hz, $J_{2eq\text{-}3}$ = $J_{2eq\text{-}1}$ = 4.6 Hz, 1H, H2eq), 1.97 (dt, $J_{3'eq\text{-}3'ax}$ = 12.3 Hz, $J_{3'eq\text{-}4'}$ = $J_{3'eq\text{-}2'}$ = 4.0

Hz, 1H, H3'eq), 1.86 – 1.79 (m, 1H, H4'), 1.74 (q, $J_{3'ax\text{-}3'eq}$ = $J_{3'ax\text{-}2'}$ = $J_{3'ax\text{-}4'}$ = 12.3 Hz, 1H,

H3'ax), 1.47 – 1.35 (m, 1H, CH_2CH$_3$), 1.33 – 1.16 (m, 3H, H2ax, CH_2CH$_3$, CH_2CH$_2$CH$_3$), 1.14

– 1.05 (m, 1H, CH_2CH$_2$CH$_3$), 0.86 (t, J = 7.1 Hz, 3H, CH$_3$). ^{13}C 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 (-CH$_2$Ph), 73.2 (-CH$_2$Ph), 73.1 (C3'''), 73.0 (C5'''), 72.4 (-CH$_2$Ph),

72.3 (C5'), 71.9 (-CH$_2$Ph), 71.7 (-CH$_2$Ph), 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 (CH_2CH$_2$CH$_3$), 32.4 (C2),

27.2 (C3'), 19.1 (CH_2CH$_3$), 14.2 (CH$_3$). ESI-HRMS: m/z calcd for $C_{69}H_{74}N_{16}NaO_{13}$ [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-benzyl-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%). $[\alpha]_D^{23}$ = +77.2° (c = 0.5, dichloromethane). *Rf* (hexane: ethyl acetate 1: 1)

0.44. ^{1}H NMR (499 MHz, chloroform-*d*) δ 8.17 (s, 1H), 7.40 – 7.19 (m, 25H), 5.90 (d, $J_{1'-2'}$

= 3.5 Hz, 1H, H1'), 5.61 (d, $J_{1''-2''}$ = 4.9 Hz, 1H, H1''), 4.91 – 4.82 (m, 2H, H1''', -CH_2Ph),

4.80 – 4.68 (m, 2H, -CH_2Ph), 4.64 (d, J = 11.9 Hz, 1H, -CH_2Ph), 4.58 – 4.47 (m, 3H,

-CH_2Ph), 4.44 (d, J = 12.0 Hz, 1H, -CH_2Ph), 4.41 – 4.32 (m, 2H, -CH_2Ph), 4.18 (dd, $J_{4''-5''}$ =

9.3 Hz, $J_{4''-3''}$ = 4.5 Hz, 1H, H4''), 4.10 (t, $J_{3''-2''}$ = $J_{3''-4''}$ = 4.5 Hz, 1H, H3''), 3.94 – 3.88 (m, 1H,

H5'), 3.85 (t, J_{5-4} = J_{5-6} = 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, $J_{5''a-5''-b}$ = 5.0 Hz, $J_{5''b-4''}$ = 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, $J_{2'-3'ax}$ = 13.0 Hz,

$J_{2'-1'}$ = $J_{2'-3eq}$ = 3.5 Hz, 1H, H2'), 2.25 (dt, $J_{2eq-2ax}$ = 13.0 Hz, J_{2eq-1} = J_{2eq-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,

CH_2CH$_3$), 1.33 – 1.21 (m, 2H, CH_2CH$_3$, CH_2CH$_2$CH$_3$), 1.16 – 1.06 (m, 1H, CH_2CH$_2$CH$_3$), 0.89

(t, J = 7.0 Hz, 3H, CH$_3$). ^{13}C NMR (126 MHz, chloroform-*d*) δ 161.3 (-*C*HO), 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 (-*C*H$_2$Ph), 74.9 (C4), 73.9 (C5'''), 73.3 (C5'),

73.2 (-CH_2Ph), 73.0 (C3'''), 72.5 (-CH_2Ph), 72.4 (-CH_2Ph), 72.0 (-CH_2Ph), 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 ($CH_2CH_2CH_3$), 32.6 (C2), 26.8 (C3'), 19.1 (CH_2CH_3), 14.2 (CH$_3$). ESI-HRMS: m/z calcd for $C_{62}H_{72}N_{16}NaO_{12}$ [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%). $[a]_D^{23}$ = + 66.4° (c = 0.2, H$_2$O). ^{1}H NMR (600 MHz, D$_2$O) δ 8.00 (s, 1H, -CHO), 5.39 (d, $J_{1'-2'}$ = 3.7 Hz, 1H, H1'), 5.15 (d, $J_{1''-2''}$ = 3.0 Hz, 1H, H1''), 5.11 (br s, 1H, H1'''), 4.27 (t, $J_{3''-2''}$ = $J_{3''-4''}$ = 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, J_{4-3} = J_{4-5} = 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, J_{1-2ax} = J_{1-6} = 12.4 Hz, J_{1-2eq} = 3.9 Hz, 1H, H1), 2.24 (dt, $J_{2eq-2ax}$ = 12.0 Hz, J_{2eq-1} = J_{2eq-3} = 3.9 Hz, 1H, H2eq), 1.93 (dt, $J_{3'eq-3'ax}$ = 12.1 Hz, $J_{3'eq-2'}$ = $J_{3'eq-4'}$ = 4.1 Hz, 1H, H3'eq), 1.76 (s, 15H,

CH_3CO$_2$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, CH_2CH$_2$CH$_3$, CH_2CH$_3$), 1.11 – 1.05 (m, 1H, CH_2CH$_3$), 1.01 (dd, J = 18.8, 9.4 Hz, 1H, CH_2CH$_2$CH$_3$), 0.70 (t, J = 6.9 Hz, 1H, CH$_3$). ^{13}C NMR (151 MHz, D$_2$O) δ 179.8 (CH$_3$$CO_2$H), 164.9 (-$C$HO), 109.8 (C1''), 95.3 (C1'''), 94.7 (C1'), 83.8 (C5), 79.5 (C4''), 78.3 (C4), 76.7 (C3''), 75.5 (C6), 72.8 (C2''), 71.6 (C5'), 70.1 (C5'''), 67.6 (C4'''), 67.3 (C3'''), 61.2 (C6'), 50.7 (C2'''), 49.5 (C1), 49.1 (C3), 48.9 (C2'), 40.4 (C6''), 39.5 (C5''), 33.7 (C4'), 32.1 (CH$_2$CH$_2$CH$_3$), 28.1 (C2), 26.4 (C3'), 22.3 (CH$_3$CO$_2$H), 18.3 (CH$_2$CH$_3$), 13.2 (CH$_3$). ESI-HRMS: m/z calcd for C$_{27}$H$_{54}$N$_6$O$_{12}$ [M+H]$^+$ 653.3721, found 653.3724.

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ABSTRACT

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

by

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Major: Chemistry

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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'-deoxygenation 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.

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EDUCATION

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RESEARCH PUBLICATIONS

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