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Synthesis Of Netilmicin And Apramycin Derivatives For The Treatment Of Multidrug-Resistant Infectious Diseases

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SYNTHESIS OF NETILMICIN AND APRAMYCIN DERIVATIVES FOR THE TREATMENT OF MULTIDRUG-RESISTANT INFECTIOUS DISEASES

by

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Advisor Date

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DEDICATION

I dedicate my PhD work to my parents Sayed Sonousi and Hoda Fayed for nursing me with affection and love and for their dedicated partnership for success in my life. I also dedicate my work to my wife Tasnim Kandeel for her endless love and support for me throughout the process.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

A	Adenine
AAC	Aminoglycoside acetyltransferases
Ac	Acetyl
ACN	Acetonitrile
ADP	Adenosine diphosphate
AGA	Aminoglycoside antibiotics
AIBN	Azobisisobutyronitrile
AME	Aminoglycoside modifying enzyme
ANT	Aminoglycoside nucleotidyltransferases
APH	Aminoglycoside acetyltransferases
Ar	Aryl
ATP	Adenosine triphosphate
BAIB	Bis(acetoxy)iodobenzene
Boc	tert-Butyloxycarbonyl
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
c	Concentration
C	Cytosine
^{o}C	Celsius
Calcd.	Calculated
Cbz	Benzyloxycarbonyl

COSY	Homonuclear correlation spectroscopy
m-CPBA	*m*-Chloroperbenzoic acid
DAST	Diethylaminosulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin Periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOS	Deoxystreptamine
dppf	1,1'-Bis(diphenylphosphino)ferrocene
ESI	Electrospray ionization
EDP	Energy-dependent phase
ESIHRMS	Electrospray ionization high resolution mass spectrometry
Et	Ethyl
Fmoc	9-Fluorenylmethoxycarbonyl
FT/IR	Fourier transform infrared
G	Guanine
Gal	Galactose
h	Hour

HMBC	Heteronuclear multiple bond correlation
HMPA	Hexamethylphosphoramide
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
KHMDS	Potassium bis(trimethylsilyl)amide
L-HABA	L-γ-amino-α-hydroxybutyryl
LPS	lipopolysaccharides
MDR	Multi-drug-resistant
Me	Methyl
mmol	Millimole
mp	Melting point
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Molecular sieves
Ms	Methanesulfonyl
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	*N*-Bromosuccinamide
NIS	*N*-Iodosuccinamide
NMMO	*N*-Methylmorpholine-*N*-oxide
nOe	Nuclear Overhauser effect
NOS	*N*-*O*-succinimde
OHC	Outer hair cells
PCC	Pyridinium chlorochromate

Ph	Phenyl
Phth	Phthaloyl
PMB	*p*-Methoxybenzyl
ppm	Parts per million
pTSA	4-Toluene sulfonic acid
Py	Pyridine
ROS	Reactive oxygen species
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Stick's reagent	Imidazole-1-sulfonyl azide hydrochloride
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TEA	Triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
tRNA	Transfer ribonucleic acid
Troc	2,2,2-Trichloroethoxycarbonyl
TTMS	Tris(trimethylsilyl)silane
U	Uracil

CHAPTER 1. INTRODUCTION

1.1. Background and Significance

Infectious bacteria are becoming progressively more resilient to existing antibiotic drugs. It has been estimated that multi-drug resistant bacterial diseases are directly responsible for 23000 deaths annually in the United States and more than 25000 in the European Union.[1-2] These multi-drug resistant diseases are also estimated to cause economic loses of $55 billion dollars annually in the United States.[1] Despite the need for new antibiotics to overcome the huge loses in lives and money, the development pipeline is constrained (Figure 1).[1, 3] Pharmaceutical companies are unwilling to develop novel antibiotics because of risky market failures. Therefore many incentive strategies have been proposed to encourage research facilities and pharmaceutical companies to develop new antibiotics.[4]

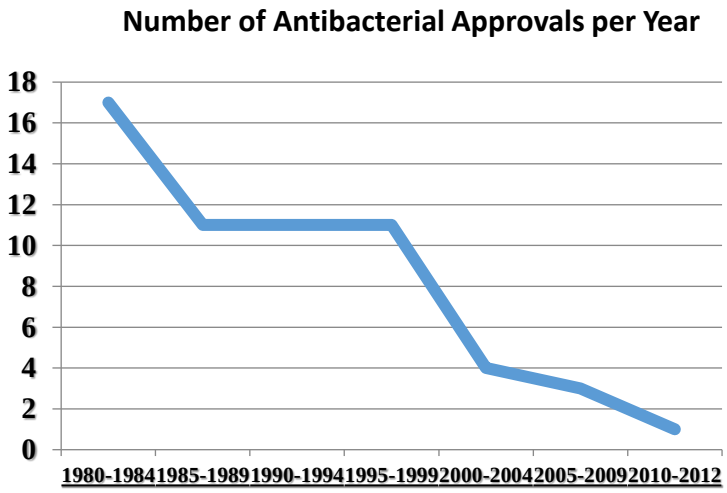


Figure 1. **A graphical representation showing the number of antibacterial approvals from 1980 till 2012**[1]

Antibiotics "opposing life" are compounds that are produced by microorganisms that selectively inhibit the growth of or kill other microorganisms. Antibiotics can be classified according to their chemical structure, mechanism of action, spectrum of activity or their source.

Based on their mechanism of action, antibiotics are classified into four main categories: cell wall synthesis inhibitors; protein synthesis inhibitors; DNA/RNA replication and repair inhibitors; and folate coenzyme biosynthesis inhibitors (Figure 2).

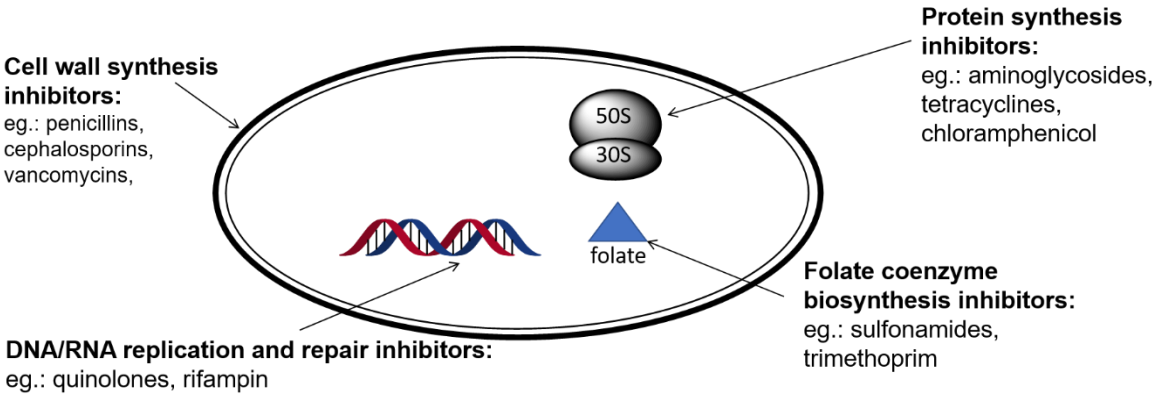


Figure 2. **A graphical representation showing the classification of antibiotics according to their mode of action**

Aminoglycoside antibiotics, which are classified as protein synthesis inhibitors, were among the first "weapons" against bacterial warfare revealed to mankind. Their history began when Waksman discovered streptomycin **1** (Figure 3),[5] the first useful antibiotic isolated from a bacterial source, in 1944. Streptomycin was the first effective therapeutic for tuberculosis, a disease that for centuries caused human morbidity and mortality unsurpassed by wars or any other pestilence.[6-7] Streptomycin **1** opened the door for the successive introduction of a series of milestone aminoglycosides which definitively established the usefulness of this class of antibiotics in the treatment of serious life-threatening bacterial infections.[8] Unfortunately, as the use of AGAs in clinical practice became widespread, resistance came to be observed more frequently. This expanding bacterial resistance together with adverse effects, in particular ototoxicity and nephrotoxicity, decreased the use of AGAs in clinics and led to them being progressively replaced broad-spectrum antibiotics with fewer side effects, such as fluoroquinolones, carbapenems, and cephalosporins. Consequently, AGAs share in the

antibiotics market declined to only 2.7% in 2010.[9] Recently, with the ever-growing bacterial resistance to the newer classes of antibiotics, many researchers decided to revisit AGAs with renewed emphasis on chemical modification, which includes structural modifications,[10-11] dimerization,[12] and conjugation to other antibiotics[13] or biomolecules.[14]

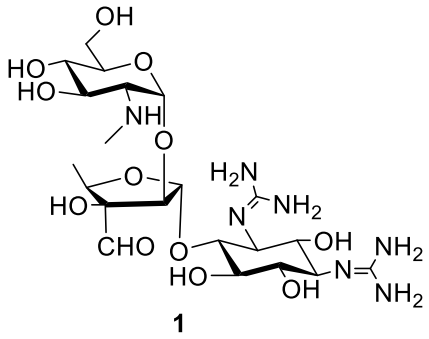


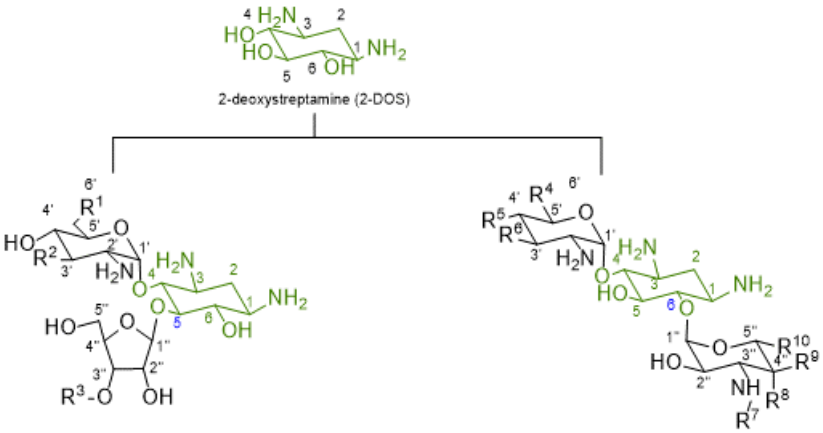
Figure 3. **Streptomycin (1)**

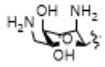
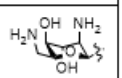
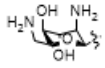
1.2. Structural features and classifications

Structurally aminoglycosides are low molecular weight (300-800 Dalton) pseudosaccharide molecules consisting of a central aminocyclitol ring, mostly a 2-deoxystreptamine (2-DOS) ring, linked to one or more amino sugars by glycosidic bonds. The most prominent features of AGAs are the presence of multiple amines attached to their rings. These amines together with the various hydroxyl groups gave them high water solubility and basic characteristics. As a result of their high polarity, AGAs are poorly absorbed orally with less than 1% reaching the blood stream through the gastrointestinal tract, which makes parenteral injections the common mode of administration for systemic diseases. Moreover, their high polarity prevents them from crossing the blood-brain barrier and reaching the central nervous system.[15]

Aminoglycosides are classified according to the linkage type with the 2-deoxystreptamine ring into two major classes: 4,5-aminoglycosides and 4,6-aminoglycosides in

which the 2-deoxystreptamine ring is disubstituted at the positions 4 and 5, or 4 and 6 (Figure 4). Although most of the aminoglycosides fit to this classification, some others have unusual structures (Figure 5) such as bicyclic rings (e.g. apramycin **9**), mono-substitution of the 2-DOS ring (e.g. apramycin **9** and garamine **10**) or incorporation of a streptamine ring instead of 2-DOS (e.g. streptomycin **1**). In addition, aminoglycosides that are derived from bacteria of the *Streptomyces* genus are named with the suffix *mycin*, whereas those that are derived from *Micromonospora* are named with the suffix *micin.*



Year discovered	Name	R^1	R^2	R^3
1949	Neomycin (**2**)	NH_2	OH	
1956	Paromomycin (**3**)	OH	OH	
1970	Ribostamycin (**4**)	NH_2	OH	H
1971	Lividomycin B (**5**)	OH	H	

Year discovered	Name	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}
1957	Kanamycin (**6**)	CH_2NH_2	OH	OH	H	H	OH	CH_2OH
1963	Gentamicin (**7**)	$CH(CH_3)NH_2$	H	H	CH_3	OH	CH_3	H
1970	Sisomicin (**8**)	CH_2NH_2	H $C^{4'}=C^{5'}$	H	CH_3	OH	CH_3	H

Figure 4. **Classification of aminoglycosides**

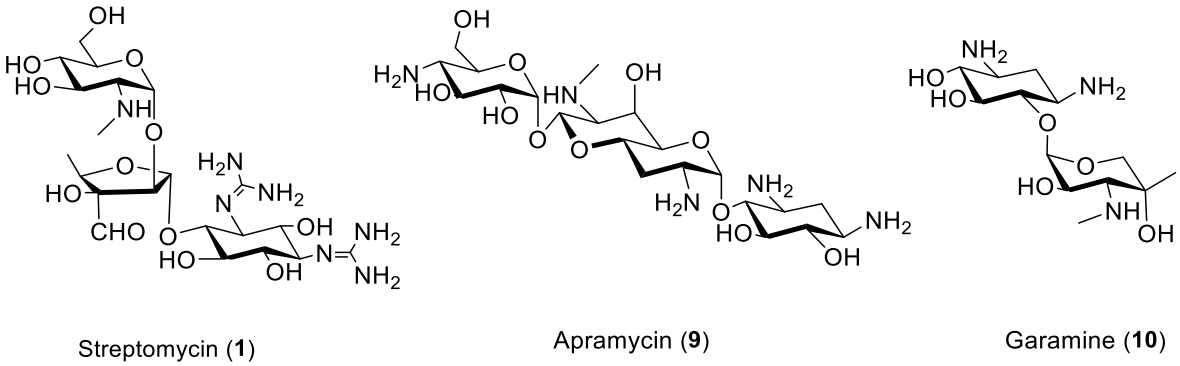


Figure 5. **Unusual aminoglycoside structures**

1.3. Mechanism of action of aminoglycosides

Aminoglycosides are of outstanding value as they are highly active broad-spectrum antibiotics that act against a wide range of bacteria including Gram-positive, and especially Gram-negative bacteria, as well as various parasites such as plasmodiums. They are also bactericidal in that they kill the bacteria, rather than simply stopping their growth. Early studies showed that protein synthesis is the primary target of AGAs, as production of labeled protein by cell-free extracts of *Mycobacterium tuberculosis* was blocked by streptomycin **1**.[16] Today there is significant knowledge of all the different stages of AGAs action from uptake until bacterial death.

1.3.1. AGA uptake

The large size of aminoglycosides excludes their penetration through bacterial porin channels. However, AGAs with their several basic amine groups are protonated, and have cationic nature, which results in nonspecific electrostatic interactions between the positively charged AGAs and the negatively charged lipopolysaccharides (LPS) in the outer bacterial membrane.[17-18] This electrostatic interaction is followed by two energy-dependent uptake phases: energy-dependent phase I (EDPI) and energy-dependent phase II (EDPII).

Energy-dependent phase I (EDPI) is a slow rate uptake to the cytosol and depends on AGA concentration. Inhibitors of oxidative phosphorylation or electron transport can halt this phase (Figure 6). In the energy-dependent phase II (EDPII), aminoglycosides bind to the 30S ribosomal subunit through a rapid process, and this binding perturbs the translational accuracy (misreading) and leads to defective proteins. The so-formed defective cell membrane proteins eventually alter cell membrane permeability, stimulating further aminoglycoside influx and leading to an autocatalytic cycle of AGA uptake and protein synthesis disruption, followed by cell death.[19]

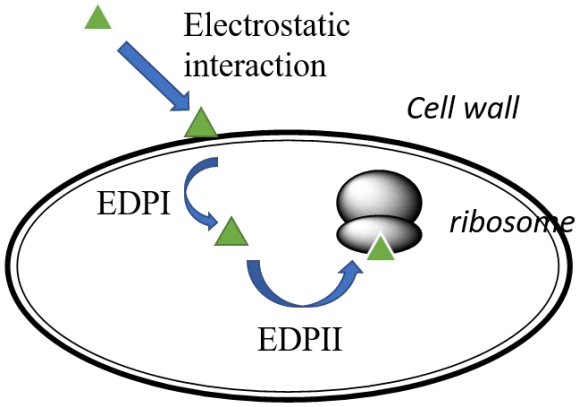


Figure 6. **Schematic diagram showing the stages of AGAs uptake**

1.3.2. Protein synthesis and AGA binding to the ribosomes

Normal protein synthesis starts by producing a mRNA copy of the genetic information in the DNA in a process called transcription. This process is followed by the translation process in which the ribosomes translate mRNA-encoded genetic information to proteins. RNA is assembled as a single-stranded chain of nucleotides that fold upon themselves, rather than as paired double-strands as in the DNA. Ribonucleotides consist of three parts: the phosphate backbone, ribofuranose sugars, and nucleobases. There are four nucleobases found in RNA: adenine, guanine, cytosine and uracil. Adenine and guanine are purine bases while cytosine and

uracil are pyrimidine bases. The bases usually pair together according to the Watson-Crick rule in which adenine associates with uracil by two hydrogen bonds, and guanine pairs with cytosine by three hydrogen bonds (Figure 7).

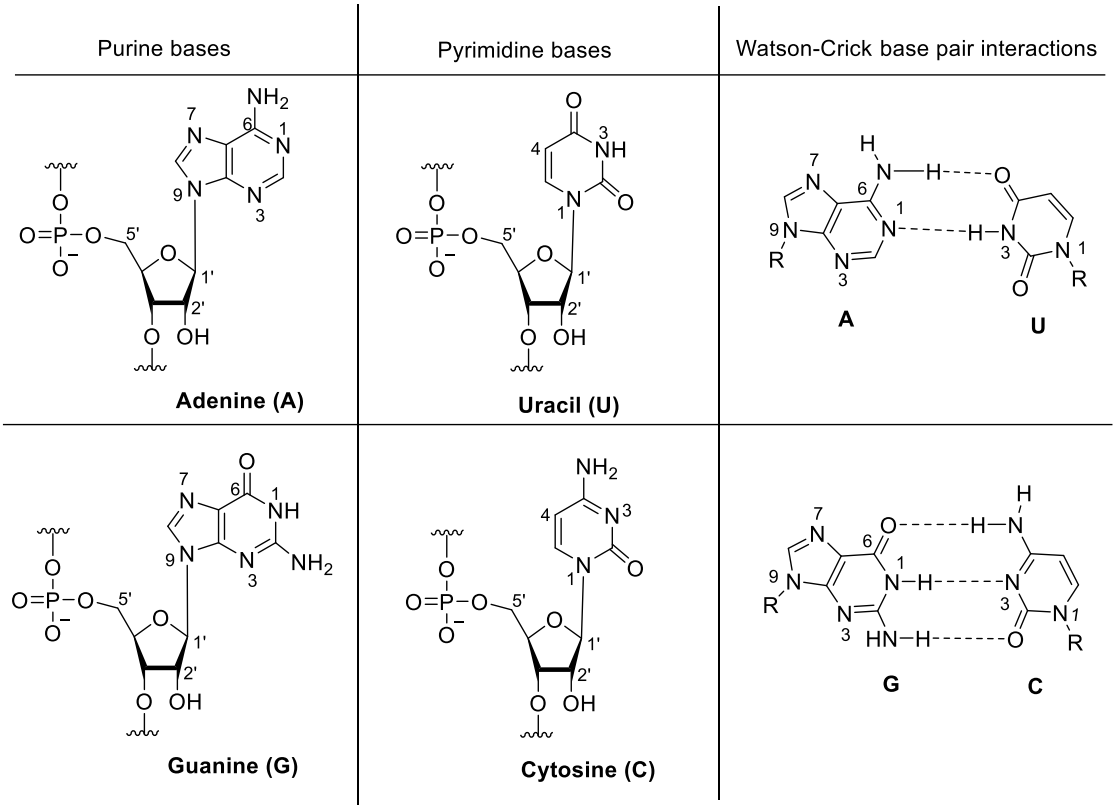


Figure 7. **The four fundamental nucleotides found in RNA and their Watson-Crick base pairs.**

The high accuracy of protein translation, with errors estimated at only 4×10^{-4} per codon,[20] suggested that fidelity does not simply come from mRNA-codon/tRNA-anticodon recognition, but that ribosomes also play a crucial role in translation accuracy and do not just act as an inert platform. The fidelity of protein synthesis requires the binding of a correct tRNA to the A-site, which is a small loop in the small ribosomal subunit that serves as aminoacyl-tRNA acceptor site. tRNA interacts with the ribosomal A-site containing three unpaired adenines (A1408, A1492 and A1493) and makes it adopt a "flipped-out" conformation. This leads to a

faster step in which other conformational changes occur, and results in tight binding of the cognate tRNA to the A-site.[21] The amino acid that the tRNA was carrying is transferred and bonds to the growing polypeptide chain (Figure 8). The ribosome then moves one codon step along the mRNA to accept the new tRNA, which codes to the next codon.

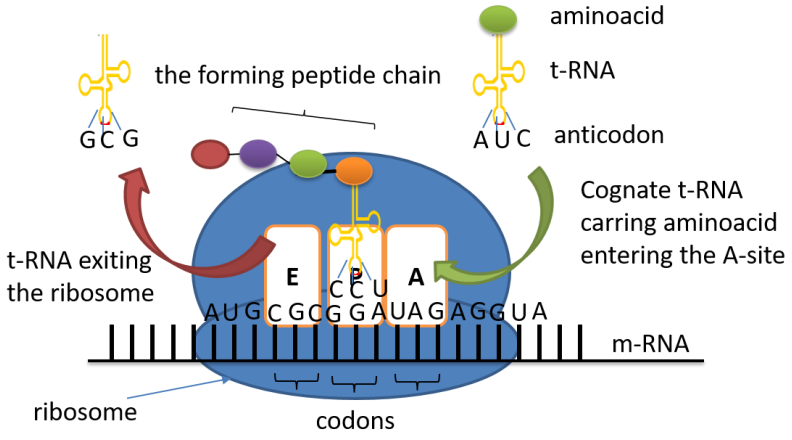


Figure 8. **Schematic diagram showing ribosomes in protein synthesis**

Aminoglycoside bactericidal activity is attributed to their binding of aminoacyl-tRNA acceptor site A-site in the bacterial 16S rRNA.[22-27] Recognition and binding of aminoglycosides to their target is due to two primary types of interactions. The most prominent interaction comes from the electrostatic interaction between the cationic aminoglycosides and the negatively charged backbone of rRNA. Other interactions mainly arise from hydrogen bonding of aminoglycosides with rRNA bases. The location of these hydrogen bonds differs between aminoglycosides, but some are common. For example, the 2-DOS ring (ring II) of paromomycin interacts with bases A1406, U1495 and G1494 by hydrogen bonds.[23] Moreover, hydrogen bonds are formed between the paromomycin ring 1 and A1408, A1492, A1493 and G1491 in the bacterial rRNA (Figure 9). On the other hand, neither rings III nor IV have any direct interaction with rRNA.[23]

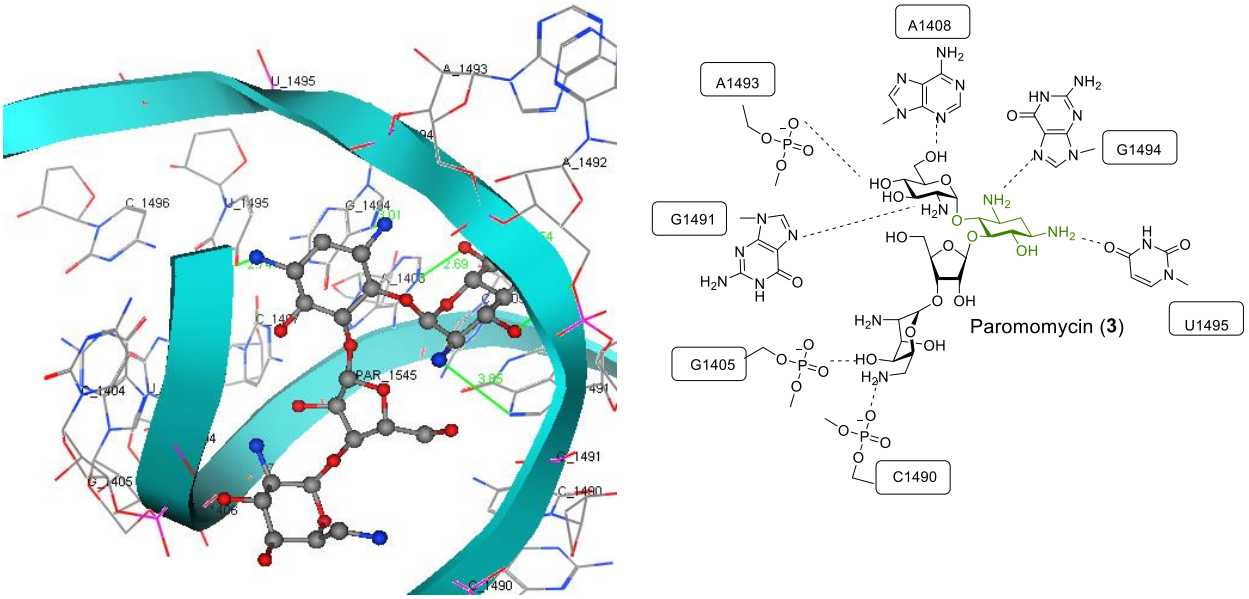


Figure 9. **a) Crystal structure of paromomycin binding to the bacterial A site (PDB code: 1FJG). b) Schematic diagram showing the binding of paromomycin (3) with rRNA nucleobases.**

When bound to the A-site, AGAs stabilize the conformation of the internal loop with A1492 and A1493 "flipped out" (Figure 10). This reduces the energetic cost for both cognate and noncognate tRNA to bind, thereby reducing the ability of the ribosome to recognize the correct tRNA, and leads to misreading of the mRNA and synthesis of defective proteins (Figure 11).[28-30] In addition, this "flipped out" conformation increases the affinity of the tRNA for the A site, thus stabilizing the pre-translocation state and increasing the energy barrier for translocation.[31-32]

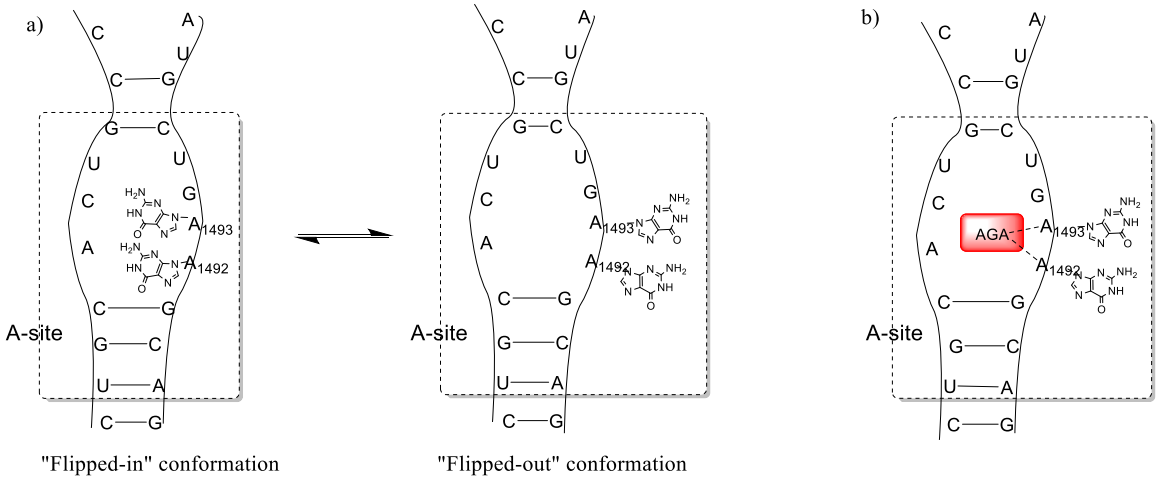


Figure 10. **Schematic diagram showing a) the equilibrium of A-site between "flipped in" and "flipped out" conformations in protein translation and b) aminoglycoside bound to ribosome A site stabilizing the "flipped out" conformation**

The connection between the resulting faulty proteins and bacterial cell death is a subject of debate. One hypothesis is that the damaged protein can insert in the bacterial inner membrane and cause its destabilization and so cell death.[33-34] Another hypothesis suggests that defective metabolic and respiratory enzymes lead to oxidative stress and production of toxic free radicals.[35]

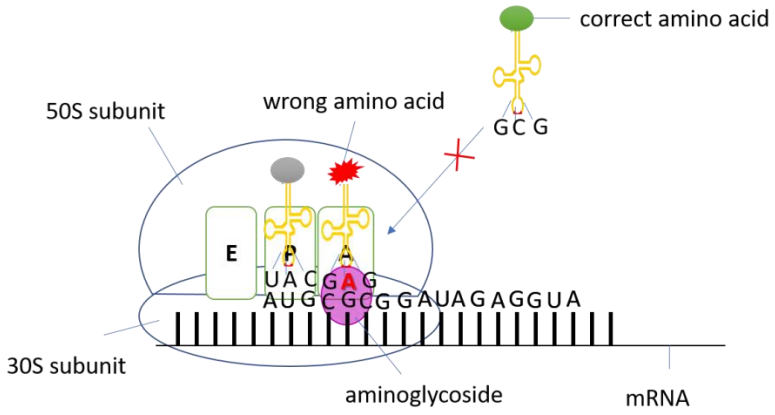


Figure 11. **Schematic diagram showing aminoglycoside bound to ribosome and causing codon misreading.**

1.4. Aminoglycoside resistance and toxicity

The excellent characteristics of AGAs as broad-spectrum antibacterial agents, with desirable bactericidal activity against difficult-to-treat Gram-negative bacteria and mycobacteria, are counterbalanced by two major problems, namely resistance and toxicity (ototoxicity, i.e., damage to the inner ear, and nephrotoxicity, i.e., damage to the kidney).[36] Due to these problems the importance of AGAs has waned, but a deep understanding of these problems may help to overcome them.

1.4.1. Aminoglycoside resistance

AGAs are isolated from soil-dwelling bacteria in particular *Streptomyces* and *Micromonospora* species. However, their bacterial origins also are the source of most of the resistance problems encountered today, as most of the AGA producing species have established strategies to prevent the deleterious effects of the antimicrobial metabolites they produce themselves.[9] Resistance problems also arise because of the frequent use of AGAs against human and animal pathogens. Improper and incomplete treatment with AGAs will allow mutant resistant bacteria to flourish. Thus, establishment of regulations to address proper use of antibiotics, promotion of public awareness of rational administration of antibiotics, and encouragement of the development of new antibiotics are three strategies that Food and Drug Administration (FDA) is pursuing to solve bacterial resistance problems.[37]

Resistance mechanisms can be categorized into three types (Figure 12). First, bacteria can reduce the internal concentration of AGAs by decreasing the drug uptake (influx) or increasing the drug expulsion (efflux). Second, some bacteria are able to modify their ribosomal A-site so that AGAs can no longer bind to it. Finally, the most common mechanism for bacterial resistance arises from the structural modification of the aminoglycosides themselves by specific enzymes

expressed by resistant strains. There are three classes of these aminoglycoside modifying enzymes (AME): aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs) and aminoglycoside nucleotidyltransferases (ANTs).[38-39]

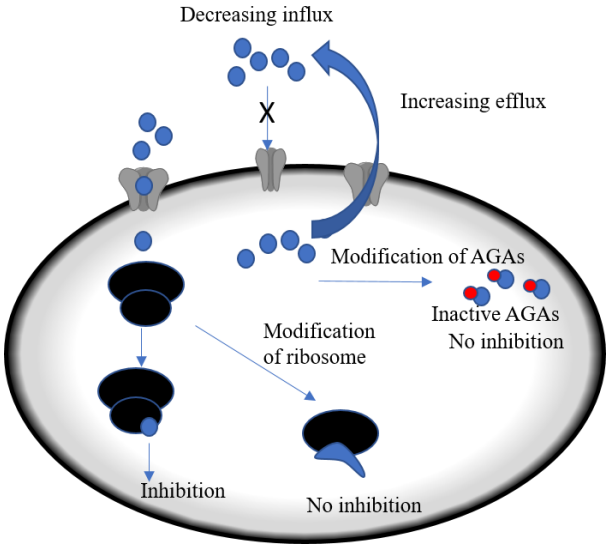


Figure 12. **Schematic diagram showing different resistance mechanisms**

1.4.1.1. Reduction of aminoglycoside internal concentration

Bacteria can reduce aminoglycoside concentration by decreasing the drug uptake (influx) or by increasing the drug expulsion (efflux). As discussed in the previous section AGA uptake goes through three stages. While the first step is electrostatic attraction between AGAs and the bacterial cell surface, the other two steps are energy and oxygen dependent which give anaerobic bacteria an inherent resistance to AGAs.[40] Also mutations to the ATP synthases of *E. coli*, *S. aureus*, and *P. aeruginosa* have been shown to decrease their susceptibility to AGAs.[41]

The other strategy of decreasing AGA concentration in the bacterial cells is by increasing the drug expulsion (efflux). This is done by efflux energy-dependent active pumps. There are many types of transporters, such as the resistance nodulation cell division (RND)-type transporter superfamily,[42-43] which plays an important role in Gram-negative bacteria like *P.*

aeruginosa. Another example is the major facilitator superfamily (MFS) of transporters which contributes to aminoglycoside resistance of *E. coli*.[44]

1.4.1.2. Ribosomal binding site modifications

Alteration of the aminoglycosides target, the 16S RNA by bacteria, is another mode of resistance. There are two types of target modifications: nucleotide mutation and nucleotide methylation. The most common example of nucleotide mutations is the A1408G mutation in the ribosomal A-site. This mutation gives bacteria resistance to the 6'-NH_2 aminoglycosides by interrupting key interactions with the AGAs.[45]

Many aminoglycoside-producing bacteria (*Streptomyces* and *Micromonospora*) protect themselves from their own AGAs by producing rRNA methylases, which can methylate the 16S rRNA.[46-47] Examples of these methylase enzymes that are now well known include RmtA, in *P. aeruginosa*,[48] the RmtB that was found to be responsible for aminoglycoside resistance in *Serratia marcescens*,[46] and ArmA, that was first found in a *Klebsiella pneumoniae* clinical isolate.[49] These mutations are of low clinical importance at present, but they pose a potential threat because of the almost complete resistance they bring against AGAs, especially 4,6-disubstituted AGAs. The mono-substituted 2-DOS AGA apramycin (**9**), on the other hand, is not susceptible to the ArmA methylation mechanism due to its unusual bicyclic structure.[50]

1.4.1.3. Aminoglycoside modifying enzymes (AMEs)

Aminoglycoside modifying enzymes catalyze covalent modification at hydroxyl or amino groups of both the 2-deoxystreptamine nucleus and the sugar moieties. The modified drugs fail to properly bind to the ribosomes. As AMEs are encoded on plasmids they are highly mobile and are easily spread between bacterial species. There are three classes of aminoglycoside modifying enzymes (AME): aminoglycoside acetyltransferases (AACs), aminoglycoside

phosphotransferases (APHs) and aminoglycoside nucleotidyltransferases (ANTs). Typical positions for structural modification by AMEs in kanamycin **6**, neomycin **2**, netilmicin **11** and apramycin (**9**) are shown in (Figure 13).[51] The common nomenclature of these enzymes identifies their class by three capital letters followed by a number in parentheses that indicates the site of modification. Sometimes there is a roman numeral that describes a particular resistance profile and then a lower-case letter that acts as an individual identifier (e.g. AAC(3)-IIa).

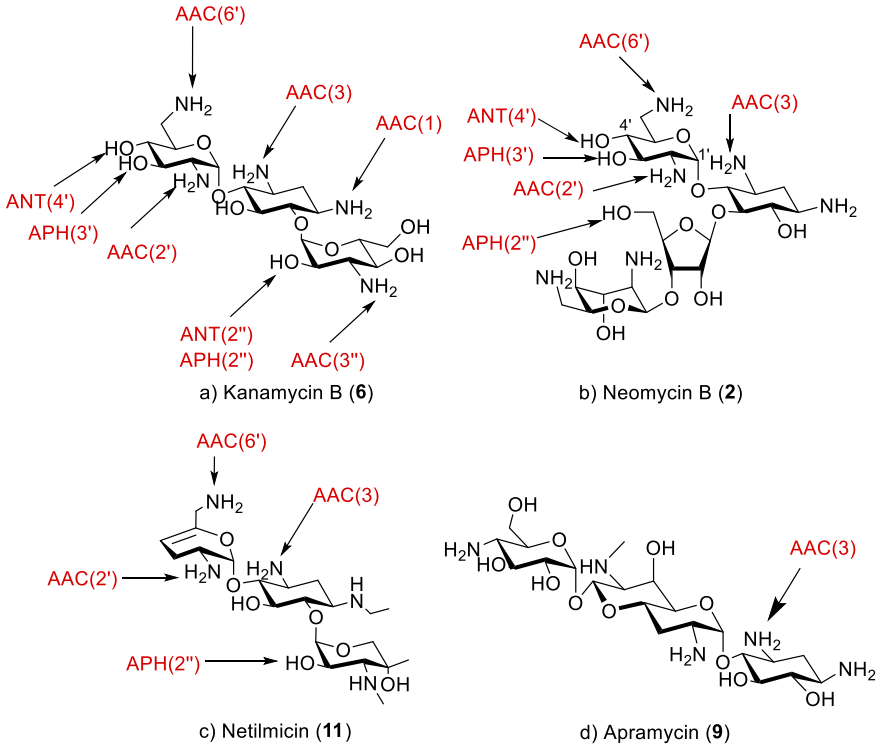


Figure 13. **Target sites of aminoglycosides modifying enzymes on a) kanamycin B b) neomycin B c) netilmicin and d) apramycin**

1.4.1.3.1. Aminoglycoside acetyltransferases (AACs)

AACs belong to the GCN5-related *N*-acetyltransferase (GNAT) superfamily of proteins. They are acetyl-CoA-dependent and catalyze the acetylation of amino groups in aminoglycosides. Over 50 members of the AAC family have been identified that are sub-

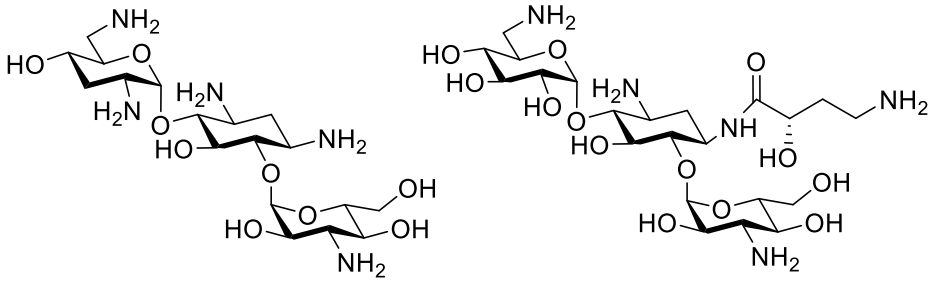
classified to AAC(1), which has no further subclasses, AAC(3)-I to X, AAC(2′)-I, and AAC(6′)-I and -II. AAC(1) enzymes do not cause a substantial drop in antibiotic activity and are of little importance as they are rarely found in clinical isolates.[52] AAC(3) enzymes are found only in Gram-negative bacteria where AAC(3)-IIa are found in a large variety of genera, AAC(2′)-I confers resistance to neomycin, kanamycin, and gentamicin and is found in Gram-negative bacteria and mycobacteria.[53] AAC(6′) enzymes are present in Gram-negative as well as Gram-positive bacteria and are by far the most common of all AMEs as acetylation of the 6'-amino group blocks a crucial interaction with A1408 and renders the AGA inactive.[54]

1.4.1.3.2. Aminoglycoside phosphotransferases (APHs)

APHs are ATP-dependent enzymes that catalyze the regiospecific transfer of the γ-phosphoryl group of the ATP to hydroxyl groups of AGAs. This phosphorylation introduces a negative charge into the molecule, which decrease the ability to bind to the A-site in the ribosome. APHs are often found on multidrug-resistant R plasmids leading to problems of gene transfer between Gram-positive and Gram-negative bacteria. Seven classes of such enzymes, APH(3'), APH(2''), APH(3''), APH(4), APH(7''), APH(6), and APH(9) have been identified in clinical isolates of which the APH(3′) class is the most common.[55]

1.4.1.3.3. Aminoglycoside nucleotidyltransferases (ANTs)

ANTs are another class of ATP-dependent enzyme that catalyze the transfer of an AMP group to a hydroxyl group in the AGA. Different classes of ANTs, the ANT(6), ANT(9), ANT(4′), ANT(2″), and ANT(3″), are now known. Although they are the smallest AME family by number, ANTs are of significant clinical importance because of the ability of ANT(2″) to neutralize tobramycin **12** and amikacin **13** as well as gentamicin **7** (Figure 14).[56]



Tobramycin (**12**) Amikacin (**13**)

Figure 14. **Structures of tobramycin (12) and amikacin (13)**

1.4.1.3.4. Avoiding AME resistance

There are two viable ways to avoid the resistance from AMEs. One way is to develop inhibitors of the modifying enzymes that can be co-administered with the AGA. Alternatively, a better way is to synthesize analogs of natural aminoglycosides resistant to the modifying enzymes. In comparison to kanamycin B (**6**), netilmicin (**11**) suffers from fewer resistance enzymes as the installation of the N1-ethyl group sterically protects it from aminoglycoside modifying enzymes at this position (Figure 13). Netilmicin (**11**) is also protected from several AMEs, such as APH(3′) and ANT(4′), by the absence of the 3′- and 4′-OH groups in ring I. Aminoglycoside modifying enzymes for apramycin also are rare, with only AAC(3) known thus far. This absence of resistance makes apramycin (**9**) a candidate for human use and a good candidate for modification and development.[57]

1.4.2. Adverse effects of aminoglycosides

The adverse effects of AGAs are one of the main problems that prevent their wide use in clinics. The main side effects a patient can encounter when given AGAs are nephrotoxicity, or kidney damage, and ototoxicity, or hearing loss. Along with these main side effects, there are minor acute side effects like neuromuscular blocking action that can be referred to as curare-like activity. The mechanism of this latter effect was confirmed to be action as a calcium channel

blocker; subsequently AGAs have been used as chemical tools to explore the functions of calcium channels.[58]

1.4.2.1. Nephrotoxicity

Nephrotoxicity is kidney damage cause by AGAs and is clinically presented as non-oligouric kidney injury. Kidney damage can lead to the inability of the body to clear urine and other wastes. Its manifestations include aminoaciduria, glycosuria, enzymuria, hypomagnesemia, hypocalcemia, and hypokalemia. Although nephrotoxicity is reversible, if it is untreated it causes increased electrolyte levels in the body and may lead to permanent kidney damage and eventually kidney failure.[27] This toxicity can be explained by the accumulation of AGAs in the renal cortical tissue especially the proximal tubules. AGAs are absorbed by endocytosis and once transferred to the lysosome, the positively-charged AGAs strongly bind to the negatively-charged phospholipids resulting in a decrease of lysosomal phospholipase activity.[59] An abnormal increase in size and number of lysosomes was found with decreased lysosome stability can lead eventually to cell death.[60]

Several strategies have been used to prevent nephrotoxicity including: 1) hydration therapy, which can often decrease the symptoms of aminoglycoside-induced nephrotoxicity. 2) The use of a once daily large dose as opposed to the same daily dose taken as separate three doses or by continuous infusion.[61] This latter is explained by the finding that uptake by the renal cells will be saturable at relatively low concentrations such that the excess drug passes the lumen, is not reabsorbed, and is excreted without causing toxicity. 3) Aminoglycoside modifications in which the *N*-1 atom has been made non-ionizable (i.e., by acylation) decrease AGA basicity and thus reduce binding to acidic phospholipids, and decrease inhibition of the lysosomal phospholipases.[39] 4) Co-administration of polyaspartic acid prevents aminoglycoside

binding to negatively charged phospholipids bilayers and thereby prevents the drug from inhibiting the activities of lysosomal phospholipases.[62] Overall, nephrotoxicity is reversible, can be easily monitored and can largely be prevented.

1.4.2.2. Ototoxicity

Unlike nephrotoxicity, ototoxicity is irreversible and difficult to monitor. It is reported to affect as much as 20% of the patient population, which makes it the main concern.[63] Ototoxicity includes damage to the vestibular system, resulting in imbalance disorders, and damage to the cochlea, resulting in tinnitus and hearing loss. There is no apparent correlation between nephrotoxicity potential or with the concentrations reached in the inner ear by different aminoglycosides with the magnitude of their ototoxic potential.[63] However, longer AGA treatments, kidney malfunction and the nutritional state of the patient may also contribute to the magnitude of ototoxicity.[64] While some AGAs (e.g. gentamicin) are more vestibulotoxic than cochleotoxic, which can be used for vestibular chemical ablation, others (e.g. amikacin and neomycin) can be more cochleotoxic than vestibulotoxic.

AGAs quickly penetrate to the inner ear within minutes of parenteral administration, and although the half-life of the AGAs in the plasma ranges from 3-5 h, their half-lives in the inner ear can reach 30 days. This long resident time was earlier misinterpreted as accumulation of AGAs in the inner ear, but this was ruled out by the fact the concentration of the drug in the inner ear was the same as in other organs and never reached the plasma concentration.[65]

AGAs cause damage to hair cells in the cochlea located in the inner ear. The cochlear hair cells function is to convert sound waves to electric impulses, which are transferred to the brain to give the hearing sensation. AGAs affect first the cochlear hair cells in the basal part that are responsible for the higher frequency (high pitched) sound, and so causing higher frequency

deafness, before they affect the cochlear hair cells in the apical part that are responsible of the lower frequency (low pitched) sound.[66] The fact that the damaged hair cells do not regenerate makes ototoxicity irreversible.

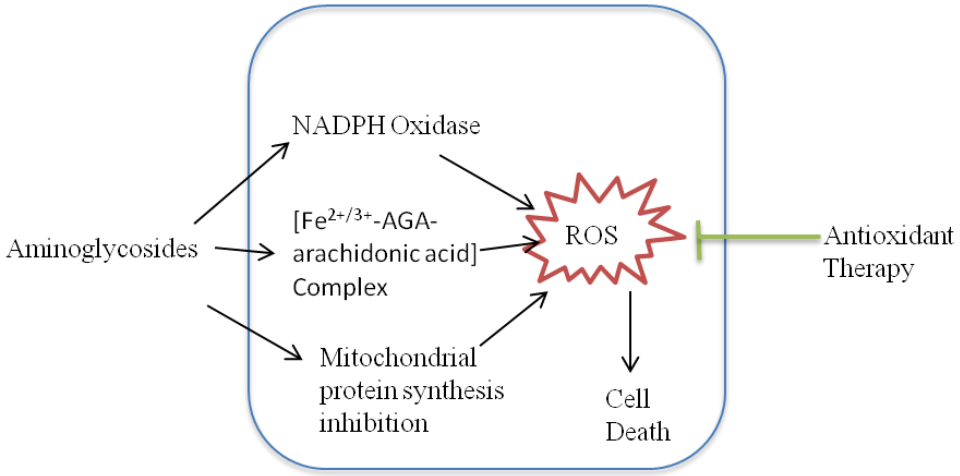


Figure 15. **A schematic representation of the mechanisms of aminoglycoside ototoxicity.**

Ototoxicity caused by AGAs involves the formation of reactive oxygen species (ROS) in the inner ear tissues that lead to ultimate cell death (Figure 15). The mode of formation of ROS has not been clear,[67-69] but some hypothesis will be outlined: 1) a non-catalytical pathway, in which AGAs binds to iron and mediate the formation a reactive oxygen species from arachidonic acid. Electrospray Ionization Mass Spectrometry (ESIMS) confirmed the existence of ternary complexes between $Fe^{2+/3+}$, gentamicin **7**, and arachidonic acid.[70] 2) A catalytical pathway, in which AGAs activate Rho-GTPase (Rac-1), which in turn activates the NADPH oxidase complex to form superoxide radicals.[71] However, recent evidence suggests that inhibition of mitochondrial protein synthesis is the main reason.[24, 72-74] The potency of a series of AGAs in inhibiting mitochondrial hybrid ribosome function correlated with the relative cochleotoxicity of the respective compounds in humans.[75] The molecular rationale for the increased affinity to mitochondrial rRNA is that its A site retains the critical A1408 residue, similar to the bacterial A

site (Figure 16). This rationale is bolstered by the hyper-susceptibility of individuals with mutations in mitochondrial rRNA to ototoxicity, in particular the transition mutation A1555G in the A-site of the mitoribosomal small subunit which renders such A sites even more similar to the bacterial A site.[73, 75] The problem of efficient genetic tools to study the interaction between eukaryotic rRNA with aminoglycosides has been overcome by the pioneering work of the Böttger group. They replaced the bacterial drug binding site in 16S rRNA with its eukaryotic counterpart, resulting in bacterial hybrid ribosomes with a fully functional eukaryotic rRNA decoding site (Figure 17).[76] This technique allows the fast screening of aminoglycosides derivatives *in vitro* to evaluate their potential ototoxicity in addition to evaluation of their bactericidal activity against wild type and clinically resistant strains. It is one of the key techniques in this collaborative project with the Böttger group.

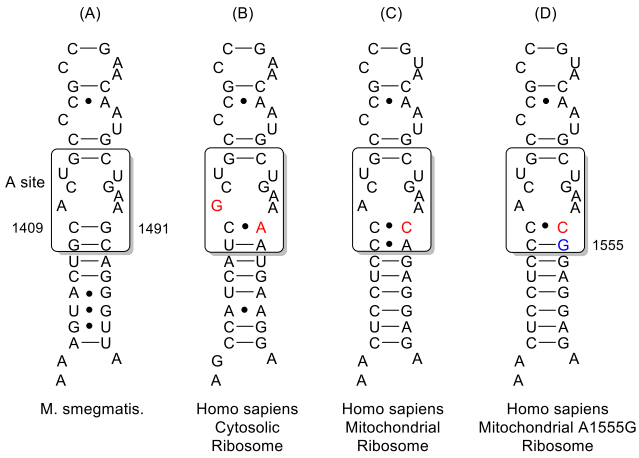


Figure 16. **Secondary-structure comparison of decoding-site rRNA sequences in the small ribosomal subunit. (A) Decoding region of 16S rRNA helix 44 in wild-type ribosomes of *M. smegmatis*. (B) Homologous 18S rRNA sequence in human ribosomes. (C) Homologous 12S rRNA sequence in human mitochondrial ribosomes. (D) Mitochondrial 12S rRNA sequence with mutation A1555G conferring hypersusceptibility to AGA ototoxicity.**

Ototoxicity side effects can be reduced by concurrent administration of antioxidants which act as radical scavengers to neutralize any ROS formed and prevent their delirious actions. Antioxidants do not affect the serum concentration or the antibacterial efficacy of

aminoglycosides.[77] Indeed, salicylic acid, which acts as both an antioxidant and an iron-chelator has been shown to reduce the gentamicin-induced auditory threshold from more than 60 dB to less than 20 dB.[78]

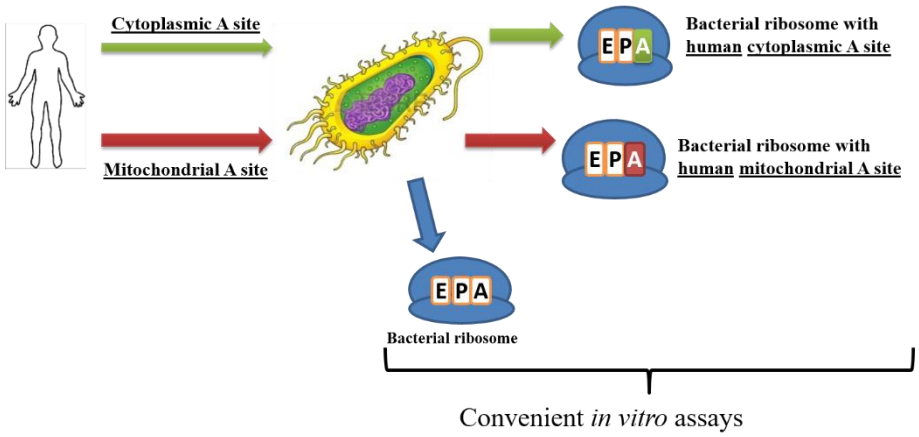


Figure 17. **Bacterial hybrid ribosomes with fully functionally eukaryotic rRNA A site**

1.5. Recent advances

In the last few years, several research groups have begun to develop aminoglycoside derivatives that can be more active, less toxic, and suffer less resistance from bacteria. The Crich and Vasella groups collaborated to synthesize modifications on the 4',6'- and 4'-positions of paromomycin **3**. These derivatives were more selective toward bacterial ribosomes over human mitoribosomes, with a concomitant decrease in ototoxicity but not in antibiotic activity (Figure 18). [10, 79-80]

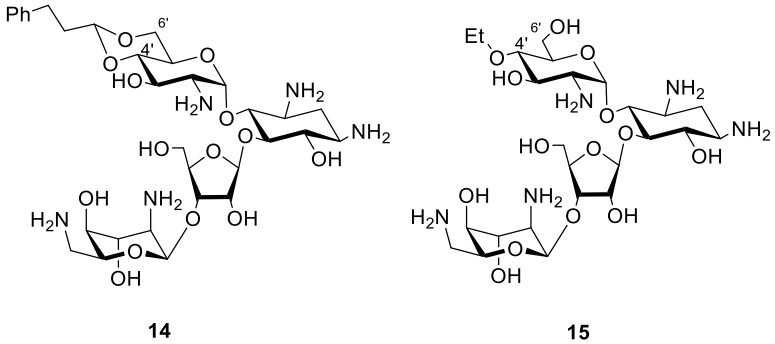


Figure 18. **Examples of 4',6'-*O*-alkylidene (14) and 4'-*O*-alkyl paromomycin (15) derivatives that showed increased selectivity for bacterial ribosomes over human mitoribosomes.**

Apramycin (**9**) is considered to be one of the least ototoxic AGAs, which this can possibly be attributed to its unique bicyclic aminosugar moiety.[81] Considerable work has been done by the Crich group in an effort to increase apramycin activity as well as it's selectivity by modification of 6' and 7'-positions.[82] These studies, however, showed these positions to be essential for activity as most manipulations of them decreased the activity.

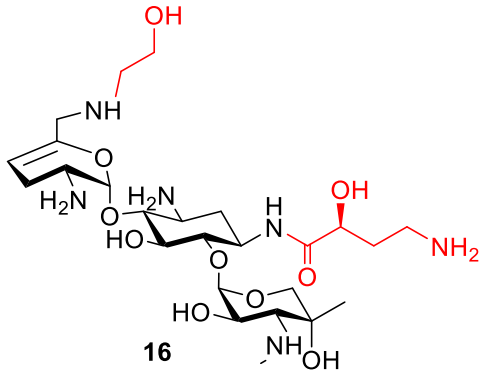


Figure 19. **The structure of plazomicin [6'-*N*-(2-hydroxyethyl)-1-*N*-(4-amino-2(*S*)-hydroxybutyryl) sisomicin] (16)**

Plazomicin, **16** (Figure 19) developed by the Achaogen company, is a semisynthetic aminoglycoside antibiotic currently in phase 3 clinical trials for complicated urinary tract infections.[83-84] Like netilmicin, plazomicin is prepared from the readily available sisomicin.[83-84] Plazomicin **16** was designed to thwart the most common aminoglycoside modifying enzymes (AMEs) by the judicious positioning of substituents and so to respond to the ever-growing problem of drug-resistant infectious diseases.[85] The placement of the 4-amino-2(*S*)-hydroxybutyryl (L-HABA) unit on the 1-position not only protect the drug against enzymes acting on position 1 [AAC(1) enzymes], but also on both position 3 [AAC(3) enzymes] and position 2" [ANT(2'') and APH(2'')]. L-HABA substitution also serves to decrease the drug nephrotoxicity as discussed before. The inclusion of a 2-hydroxyethyl group on the 6'-position protects the drug against enzymes acting on position 6'.

1.6. Overall goals

Toward the goal of preparing next generation AGAs two sub-projects were defined: 1) examining the effects on 4'-modification in the 4,6-series on the netilmicin skeleton **11**. Netilmicin **11** was chosen as substrate for this investigation because its ototoxicity is low, which makes it ideal for further investigation.[86-88] Likewise, the knowledge that modification of the 4'-position of paromomycin[10, 79-80] reduces its ototoxicity but not it's antibiotic activity, suggested derivatization of netilmicin at that position.

2) Improvement of apramycin **9** by hybridization: A number of reports have described the synthesis and evaluation of hybrid AGAs combining fragments from both the 4,5- and 4,6-series of 2-deoxystrptamine AGAs.[89-92] Our attention was captured by an early report on the moderately increased activity of the 5-*O*-(β-D-ribofuranosyl) derivative of apramycin against selected Gram-negative organisms,[93] suggesting derivatization at the 5-position of apramycin **9** and apramycin-paromomycin hybrids; the goal being to combine paromomycin's high activity with apramycin's low ototoxicity as a possible avenue for further improving the profile of this promising antibiotic.

CHAPTER 2. INVESTIGATIONS IN THE 4,6-CLASS OF AGAs

2.1. Mode of binding of netilmicin to the bacterial ribosomal A-site

Netilmicin **11** is a N1-ethyl semisynthetic analog of sisomicin, an antibiotic extracted from *Micromonospora inyoensis.*[94] Netilmicin was developed and marketed in the United States as Netromycin® by the Schering Corporation.[95] Netilmicin belongs to the 4,6-disubstituted class of aminoglycosides and is active against most Gram-negative and some Gram-positive bacteria, including many gentamicin-resistant strains.[96] The N1-ethyl modification protects netilmicin from both adenylating and phosphorylating enzymes and consequently maintains its activity against most strains of bacteria that harbor these enzymes.[97] It is also reported to be less ototoxic than amikacin and gentamicin in *in vivo* rabbit studies.[87]

Although a crystal structure of the rRNA A-site bound to netilmicin has yet to be determined, it is assumed that netilmicin has the same binding mode as its congener sisomicin, which specifically binds to the deep/major groove of the bacterial A site and makes 11 hydrogen bonds to base and phosphate oxygen atoms (Figure 20).[98] Sisomicin **8** has a double bond between the C4′ and the C5′ atoms, which force ring I to adopt a flattened conformation with the possibility of classical π-stacking with the G1491 base. This is in contrast to AGAs with a saturated carbohydrate ring I, which adopt a chair conformation that interacts with the aromatic of G1491 through CH/π interactions.[98] Ring I also forms a pseudo base-pair interaction with A1408 in which the ring oxygen of ring 1 accepts a hydrogen bond from N6-adenine and the protonated 6'-amino group donates a hydrogen bond to adenine N1. The C3-amine in ring II makes hydrogen bonds with the phosphate oxygen atoms of A1493 and A1494 so that A1492 and A1493 can take flipped-out conformations. Ring III binds to the upper side of the A-site helix through four hydrogen bonds.[98]

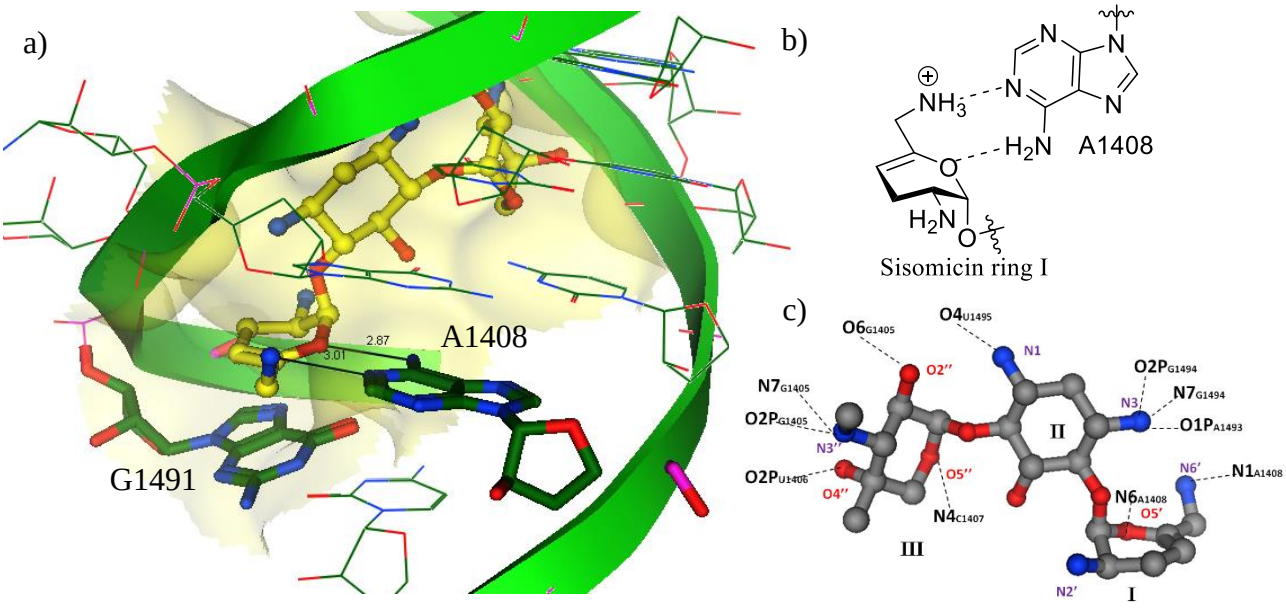
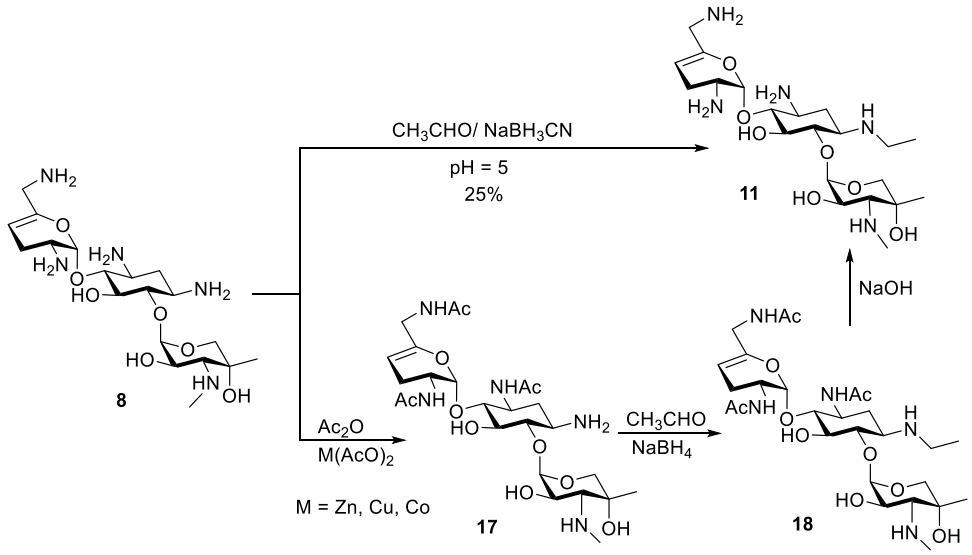


Figure 20. **a) Crystal structure of sisomicin bound to the deep/major groove of the bacterial A site showing π-stacking with G1491 base and pseudo base-pair interaction with A1408 (PDB code: 4F8U), b) a schematic diagram showing the pseudo base-pair interaction of ring I with A1408 and c) detailed interaction of sisomicin with the bacterial A site.**

2.2. Synthesis of netilmicin

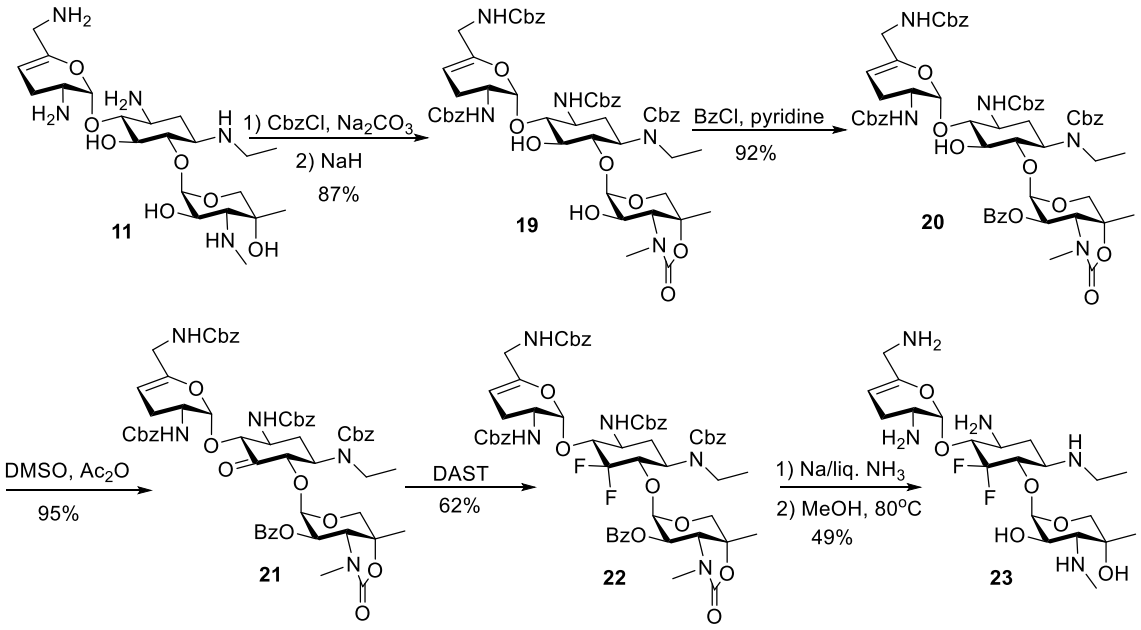
Netilmicin **11** was first synthesized in one step by reductive alkylation of sisomicin[95] without the conventional protection of the other amine groups (Scheme 1). The synthesis took advantage of the fact that the 1-amino group is the least basic and under acidic conditions (pH = 5), all other amines are protonated and so unreactive. The only problem with this direct synthesis was its low yield (25%). Subsequently, several papers and patents were published that describe improved overall yields albeit employing three main steps.[99-101] In these sequences the first step was selective protection of the 3,2',6'-amino groups mediated by transition metal acetates such as zinc, copper or cobalt acetates to give **17**. This was followed by reductive alkylation of the *N*1-amine to afford compound **18** and finally deprotection; the best overall yield (73%) was obtained using zinc acetate in the first step (Scheme 1).[101]



Scheme 1. **Synthesis of netilmicin**

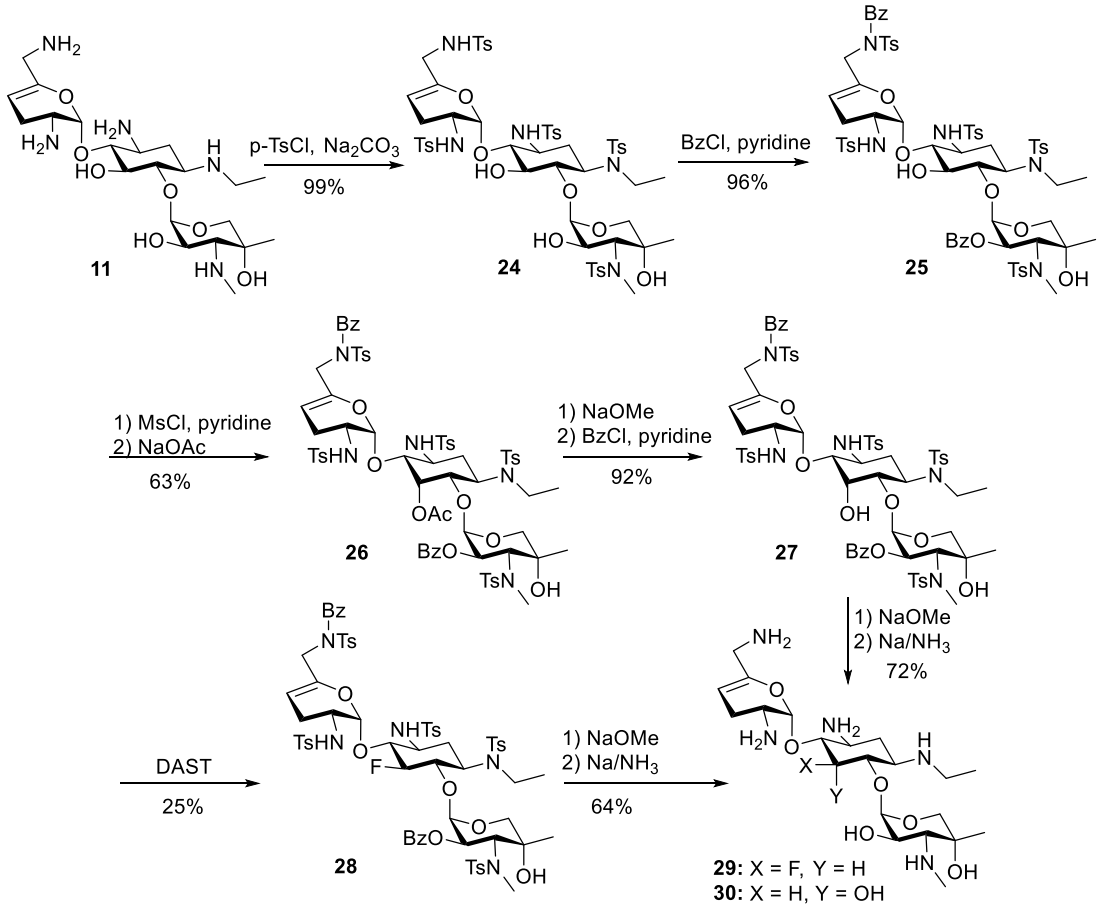
2.3. Modifications of netilmicin

Modification of netilmicin was done primarily by deoxyfluorination[102] of the 5-position and acylation of *N*6'-position.[103] The synthesis of 5-deoxy-5,5-difluoro-netilmicin **23** started by Cbz-protection of the sisomicin amines and was followed by cyclization using sodium hydride to provide the 3'',4''-oxazolidinone **19** in 87% yield (Scheme 2). Selective benzoylation was done at the 2''-position to give **20,** which was oxidized to give the ketone **21**, followed by difluorodeoxygenation using diethylaminosulfur trifluoride (DAST) to give **22.** Birch reduction removed the Cbz-groups and gave the product **23**.[102]



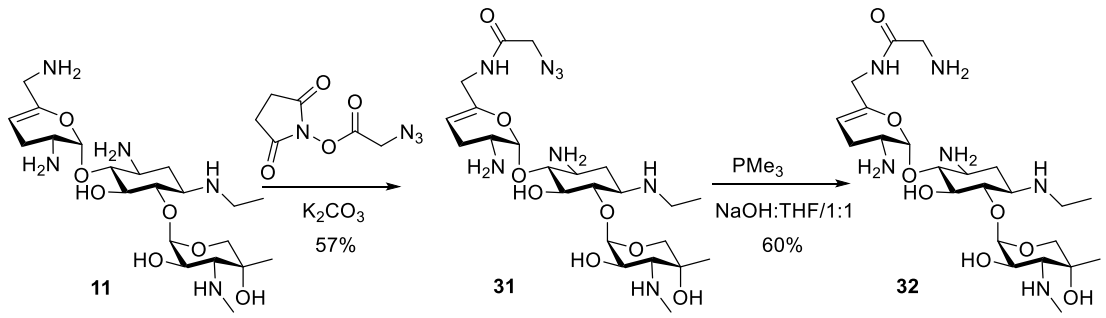
Scheme 2. **Synthesis of 5-deoxy-5,5-difluoro-netilmicin (23)**

The synthesis of 5-deoxy-5-fluoronetilmicin **29** started by tosyl protection of the sisomicin amines to give the pentatosyl derivative **24**, which was benzoylated at the N1' and 2''-positions to afford **25** (Scheme 3). Inversion of configuration of the 5-hydroxyl group and adjustment of the protecting groups was done by a sequence of the following steps: mesylation of the 5-hydroxyl group, substitution of the mesylate with sodium acetate, cleavage of acetate and benzoate esters, and finally benzoylation at the N1' and 2''-positions to give the 5-epi-netilmicin derivative **27**. Deoxyfluorination then was done using DAST to afford the 5-fluoro-netilmicin derivative **28** in 25%, which upon deprotection with sodium in liquid ammonia yielded the desired product **29**. In the same fashion, **27** was deprotected to afford 5-epi-netimicin **30** in 72% yield. 5-Deoxy-5-fluoronetilmicin **29** and 5-epi-netimicin **30** showed higher antibacterial activity than the parent, while 5-deoxy-5,5-difluoro-netilmicin **23** was slightly less active. Moreover, in acute toxicity studies, compound **30** showed less toxicity that netilmicin.[102]



Scheme 3. **Synthesis of 5-deoxy-5-fluoronetilmicin 29 and 5-epi-netilmicin 30**

6'-*N*-Glycyl-netilmicin **32** was synthesized in two steps (Scheme 4): first, netilmicin was selectively acylated at the 6'-position with 2-azidoacetyl-*N*-hydroxysuccinimide to form derivative **31**, and this was followed by Staudinger reaction, which reduced the azide into the corresponding amine **32**. 6'-*N*-Glycyl-netilmicin showed less antibacterial activity than netilmicin.



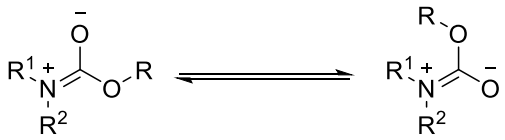
Scheme 4. **Synthesis of 6'-N-glycyl-netilmicin 32**

2.4. Rational

As discussed in the previous chapter, because modification of the 4'-position of paromomycin[10, 79-80] reduces its ototoxicity but not its antibiotic activity, it was of interest to examine if these effects on 4'-modification in the 4,5-series (i.e. paromomycin) extends to 4,6-series (i.e. netilmicin). Netilmicin was chosen as substrate for modification in this manner because it is considered to be one of the least ototoxic aminoglycosides, which makes it ideal for further investigation.[86-88]

2.5. Chemistry

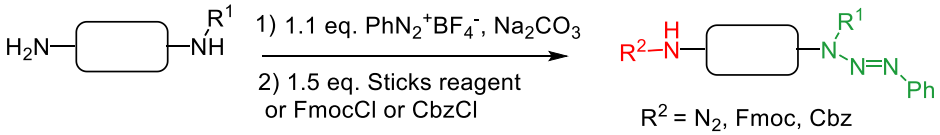
2.5.1. Triazenes as a selective protecting group for secondary amines



Scheme 5. **Rotamers of secondary amine carbamates**

Modification of netilmicin at the 4'-position requires prior protection of the amino and hydroxyl groups present. Work initially proceeded with a benzyl carbamate protection route, however, severe rotamer problems prevented sharp NMR spectra from being obtained (Scheme 5). High temperature NMR was done to facilitate rotamer exchange to give average peaks and hence sharper spectra, but unfortunately to no avail – presumably because of the number of carbamates present.

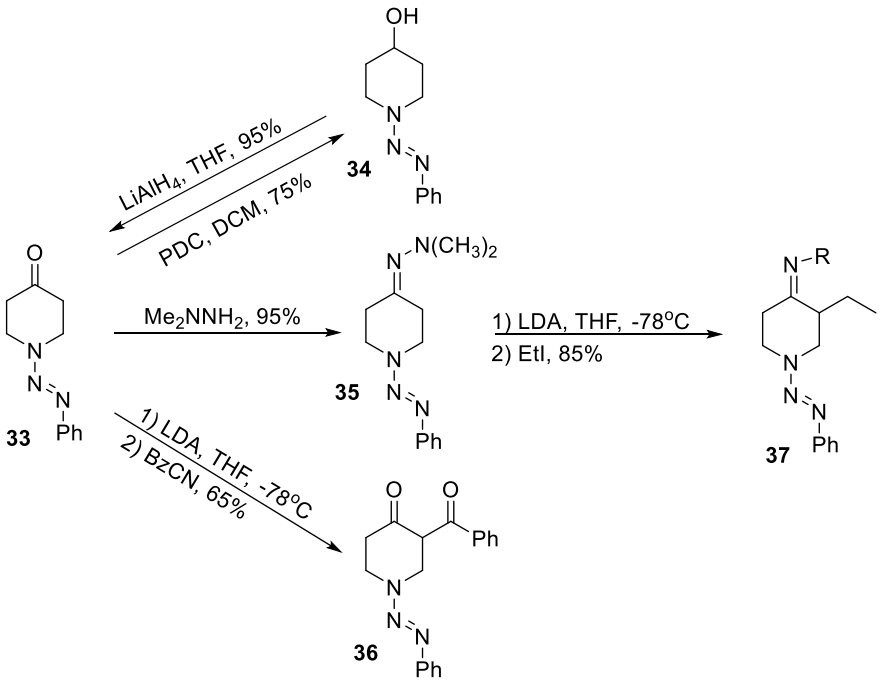
The use of triazenes as protecting groups[104] was considered to be a possible solution to the problem. A search of the literature[105-106] showed that this protecting group is only used for secondary amines. Triazenes derived from primary amines are unstable and are more commonly exploited as nucleophiles in the capture of a range of electrophiles, either inter- or intramolecularly.[107-108] Taking an advantage of this feature it was possible to protect secondary amines selectively in the presence of primary amines (Scheme 6).[109]



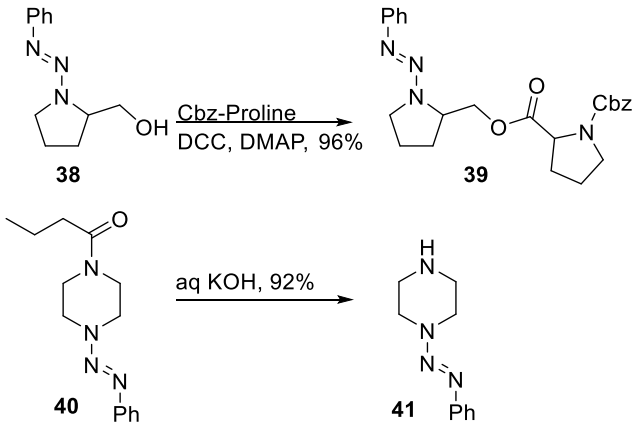
Scheme 6. **General protocol for selective protection of secondary amines as the *N*-phenyl triazenes**

2.5.2. Aryl triazenes

Trisubstituted triazenes have been widely employed in organic synthesis for the protection and/or derivatization of aryl amines.[104-105, 109-110] As a protecting group, aryl triazenes are compatible with oxidizing agents (e.g. pyridinium dichromate (PDC)) and reducing agents (e.g. $LiAlH_4$ and $NaBH_4$) as well as with basic conditions, acylating and alkylating reagents (Schemes 7 and 8). The free amine can be easily regenerated by treatment with trifluoroacetic acid.[105, 111]

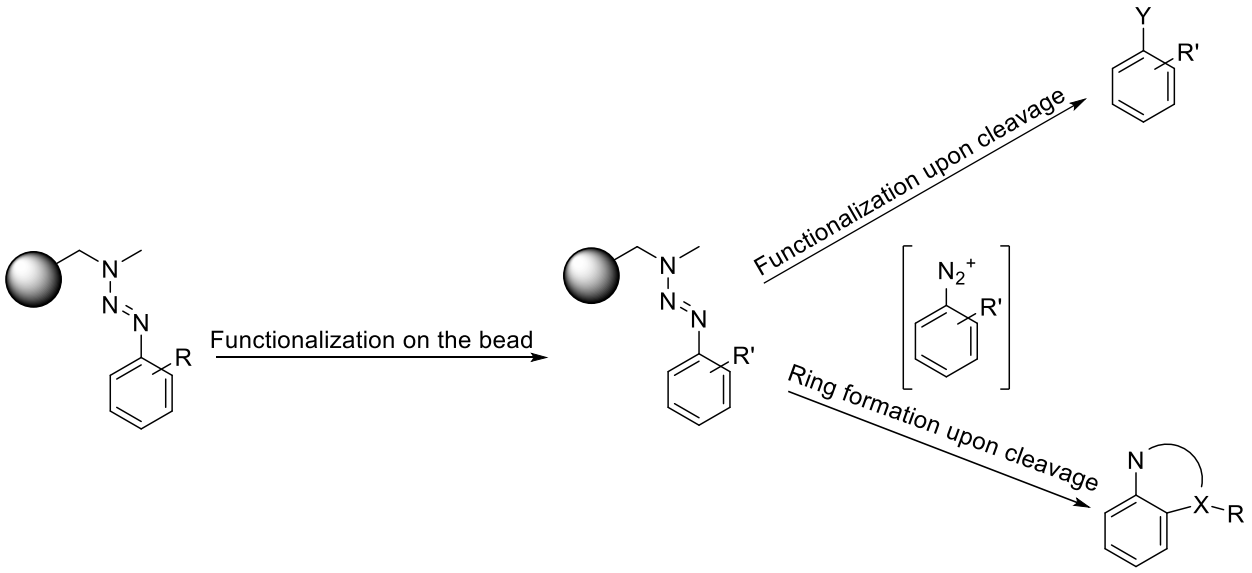


Scheme 7. **Literature reactions showing the compatibility of phenyltriazenes with oxidizing, reducing and alkylating agents**

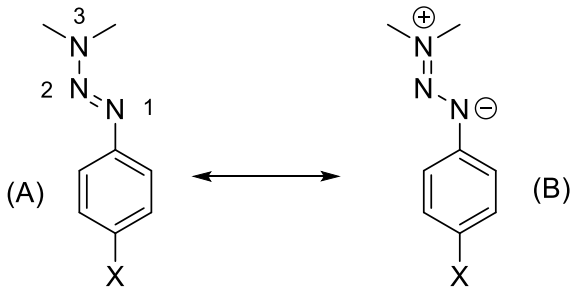


Scheme 8. **Literature reactions showing the compatibility of phenyltriazenes with acylating reagents and basic conditions**

Triazenes are also used as linkers to solid supports in solid-phase organic synthesis (SPOS) (Scheme 9). After functionalization on the bead, the triazene linker can be easily cleaved from the solid support using acidic conditions. Triazenes can also act as concealed diazonium salts upon cleavage from the resin and as such are used for conversion of aryl amines to many functionalized arenes as well as in cyclizations to generate various heterocyclic structures, namely, benzoannelated nitrogen heterocycles.[104, 110]



Scheme 9. **Uses of triazenes in solid-phase organic chemistry (SPOS)**



X	Tc_{CDCl_3}	Rotation barrier (kcal/mol)
OCH_3	-44	12.7
CH_3	-31.5	13.0
H	-23.5	13.7
Cl	-13	13.9
NO_2	+37	15.7

Figure 21. **Resonance structures of aryl triazenes and the coalescence temperatures of triazene *N*-methyl protons**

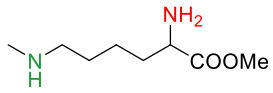
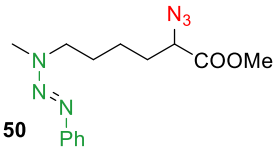
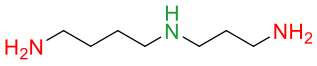
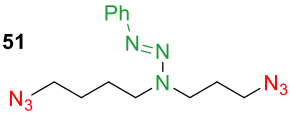
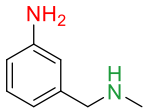
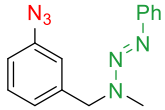
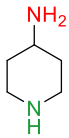
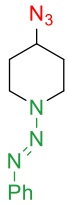
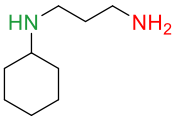
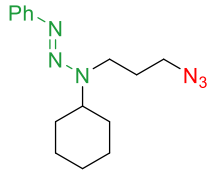
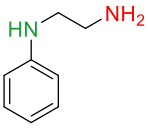
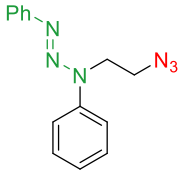
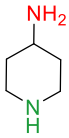
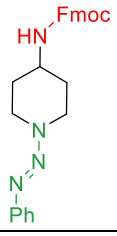
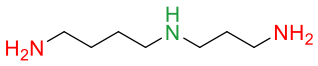
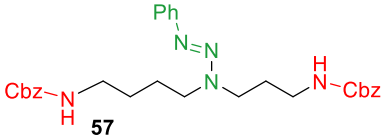
Aryl triazenes contain an extended π system in which there is considerable delocalization of charge density and so can be presented by two resonance structures (A) and (B) (Figure 21). An observable effect of this resonance is an increase in the effective barrier to rotation about the N(2)-N(3) bond due to the double bond character contribution of the 1,3-dipolar resonance form (B). The barriers to rotation about the N(2)-N(3) bond, which are determined by variable temperature NMR, show that the more electron-withdrawing the substituent attached at the para-position, the more important the 1,3 dipolar form (B) of these compounds and hence the higher the rotation barrier about the N(2)-N(3) bond (Figure 21).[112-113] In the *p*-NO_2 derivative, the coalescence temperature (*Tc*) is higher than room temperature and the two methyl groups appear as distinct resonances at room temperature in the NMR spectra. The barrier to rotation for trialkyltriazenes was found to be about 10.5-11 kcal/mol, approximately 3 kcal/mol lower than that of most aryldialkyltriazenes, due to absence of stabilization of the 1,3-dipolar form.[114] Generally, steric bulk is reported to have little effect on the barrier to rotation about the N(2)-

N(3) bond, but in the extreme case of 2,2-dimethyl- and 2,2,6,6-tetramethylpiperidine-based triazenes the barrier is reduced to ~11 kcal mol^{-1} in CS_2.[115]

2.5.3. Examples of Selective Protection

A general reaction protocol was established for this synthetic method. A series of primary and secondary diamines were treated with 1.1 equivalents of benzenediazonium tetrafluoroborate in methanol/water in the presence of powdered potassium or sodium carbonate followed by protection of the primary amines as the azides, benzyloxy carbamates or fluorenyl methyl carbamates. Workup and silica gel chromatography then gave the product in moderate to good yield. Yields were not improved by the use of excess benzenediazonium tetrafluoroborate as this leads to complications in isolation arising from the decomposition of the reagent. All these results are summarized in Table 1.

Table 1. **Examples of selective protection of secondary amines as the *N*-phenyl triazenes**

Substrate	Product	% Yield
 **42**	 **50**	58%
 **43**	**51** 	61%
44 	**52** 	69%
45 	**53** 	70%
46 	**54** 	60%
47 	**55** 	41% decomp
48 	**56** 	77%
 **49**	 **57**	54%

Consistent with expectations, the ^{1}H NMR spectra of the azido triazenes are mostly sharp in $CDCl_3$ and CD_3OD at room temperature with the exception of those compounds containing multiple Cbz groups. The contrast between the ^{1}H NMR spectra of phenyl triazene protected dissymmetric secondary amines and those of the corresponding carbamates is illustrated in (Figure 22). The room-temperature ^{1}H NMR spectrum of **58**, obtained by sequential treatment of spermidine with imidazole sulfonyl azide and benzyl chloroformate, displays significant broadening of all resonances in this pseudosymmetric secondary carbamate (Figure 22a). In contrast, the ^{1}H NMR spectrum of the corresponding diazido triazene **51** is sharp (Figure 22b).

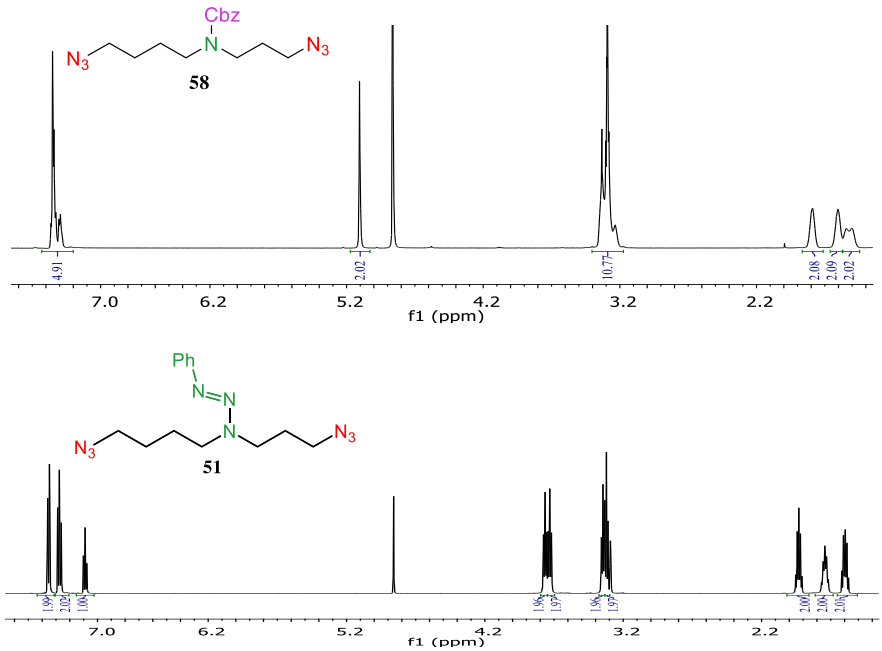
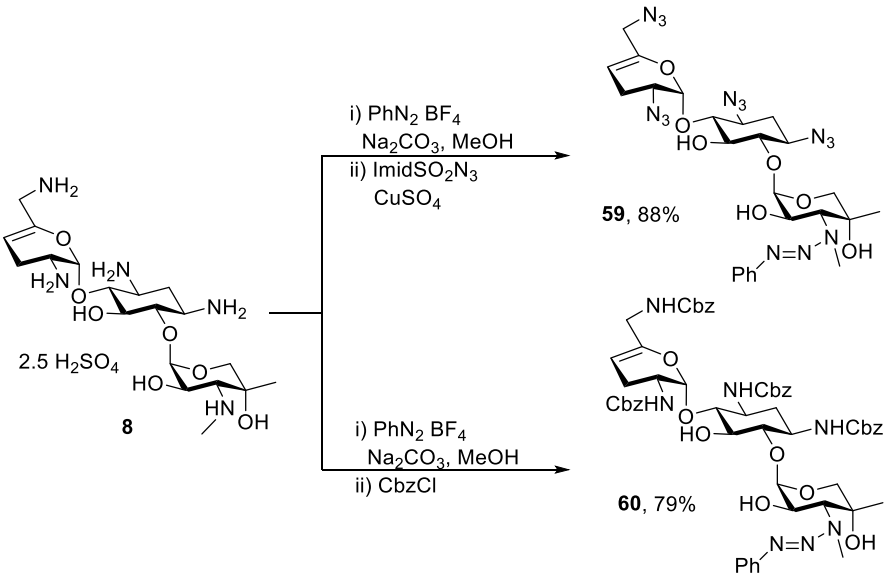


Figure 22. **Room-temperature 600 MHz ^{1}H NMR spectra of (a) 58 and (b) 51 in CD_3OD.**

2.5.4. Application to Aminoglycosides

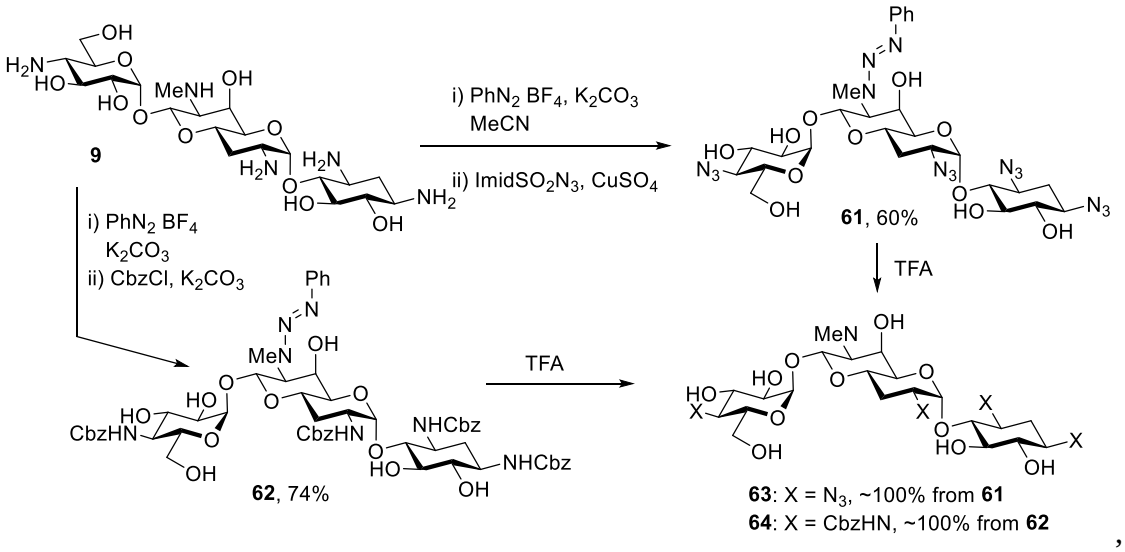
Having established the viability of the method, its application to the aminoglycosides was explored. First, sisomicin **8** with its single secondary and four primary amino groups was

investigated. Reaction with one equivalent of benzenediazonium tetrafluoroborate under the standard conditions was followed by treatment with either an excess of imidazole sulfonyl azide or benzyloxy carbonyl chloride resulting in the isolation of **59** and **60** respectively, in excellent yields (Scheme 10).



Scheme 10. **Application to sisomicin**

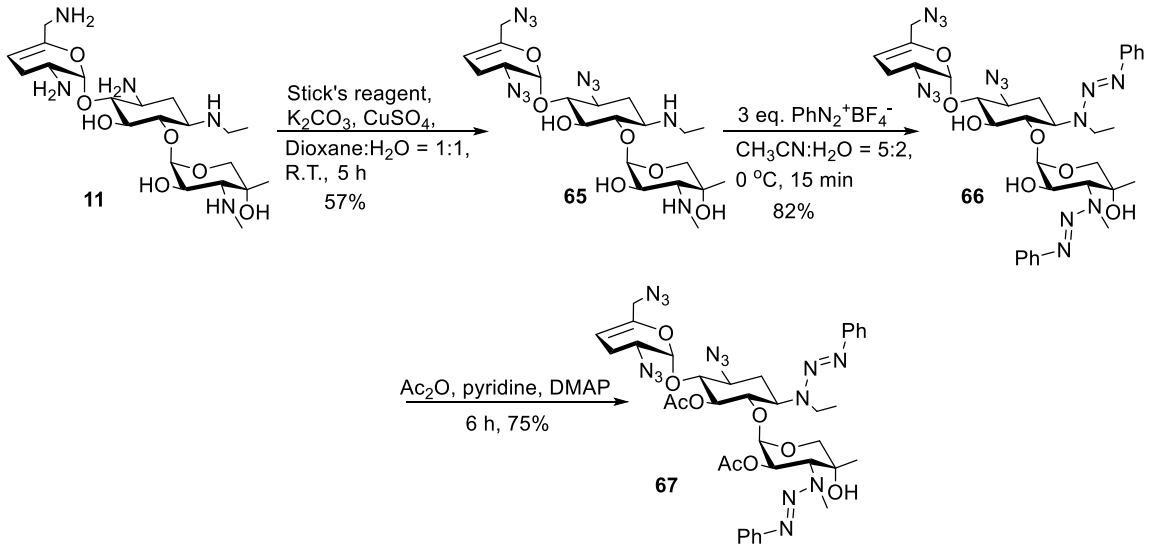
The method was also applied to the monosubstituted deoxystreptamine aminoglycoside apramycin **9**, when the azido and carbamate-protected triazenes **61** and **62** were both obtained in moderate yield (Scheme 11). Triazenes can be easily deprotected using trifluoroacetic acid in essentially quantitative yield.



Scheme 11. **Application to Apramycin**

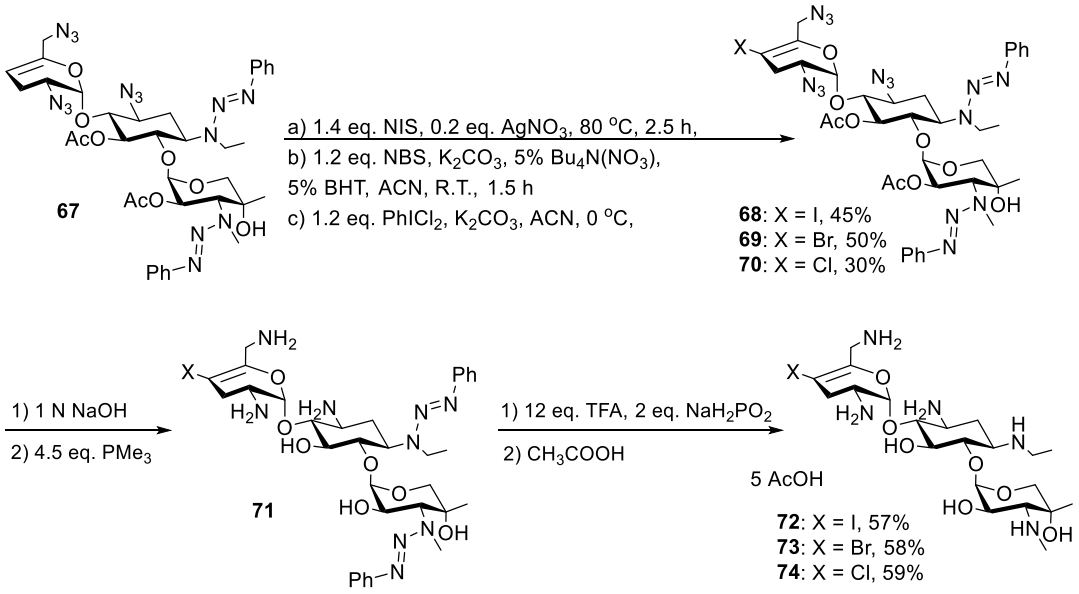
2.5.5. Synthesis of Netilmicin derivatives

The protected netilmicin **67** was prepared in three steps (Scheme 12). First, netilmicin was reacted with imidazole-1-sulfonyl azide hydrochloride (Stick's reagent)[116] in the presence of excess K_2CO_3 and catalytic $CuSO_4$ to form the triazido derivative **65** through diazo transfer.[117-118] The secondary amines were then protected as triazenes using benzenediazonium tetrafluoroborate to give derivative **66**. Intermediate **66** was finally acetylated to give the diacetylated derivative **67**.

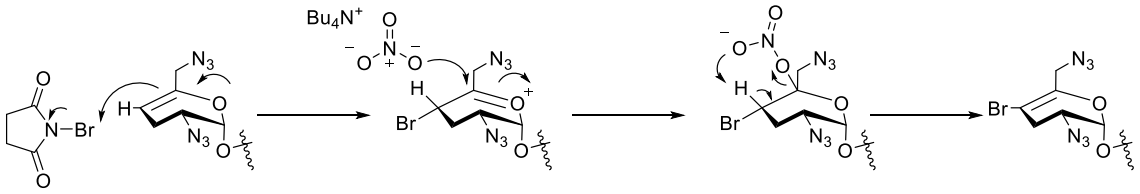


Scheme 12. **Synthesis of a protected netilmicin intermediate**

After the common intermediate **67** was obtained, iodination using *N*-iodosuccinimide[119] in the presence of silver nitrate installed iodine in the 4'-position. Attempted bromination of the 4'-position using bromine[120] was unsuccessful. However, the reaction proceeded using *N*-bromosuccinimide and K_2CO_3 as a base in acetonitrile as a solvent in 29% yield. The yield increased to 50% when a catalytic amount of tetrabutylammonium nitrate was added (Scheme 13). This can be explained either by the lipophilic tetrabutylammonium ion acting as phase transfer catalyst facilitating the deprotonation or by the effect of nitrate ion which traps the oxocarbenium ion and facilitates subsequent deprotonation (Scheme 14) as has been proposed in related processes.[121] Chlorination was best achieved with iodobenzene dichloride[122] (Scheme 13). Intermediates **68**, **69** and **70** were subjected to a stepwise sequential deprotection: deacetylation, reduction of the azide groups under Staudinger conditions,[123] and removal of the triazene group with trifluoroacetic acid to give the final products **72**, **73** and **74**.



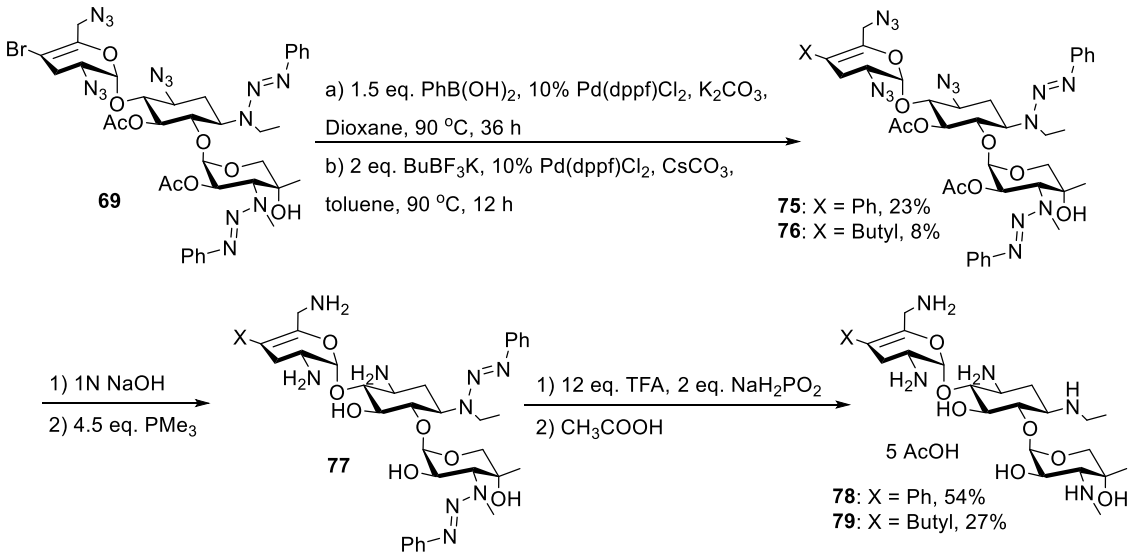
Scheme 13. **Synthesis of 4'-iodo netilmicin, 4'-bromo netilmicin and 4'-chloro netilmicin**



Scheme 14. **A proposed mechanism for nitrate catalysis of bromination of glycals**

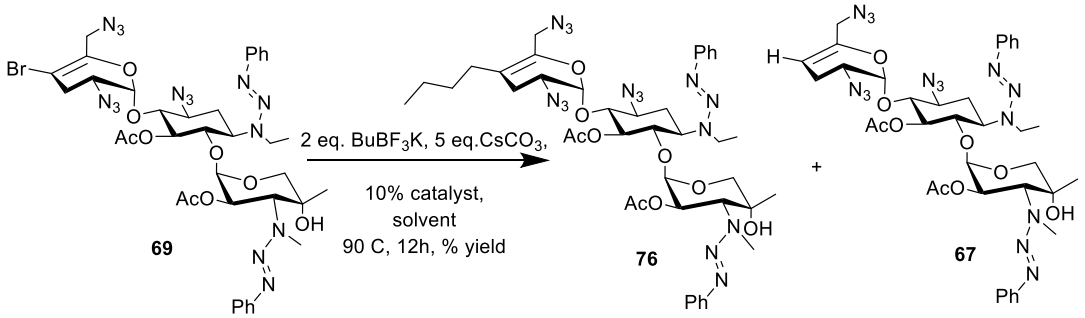
The 4'-phenyl derivative **75** was prepared from intermediate **69** via Suzuki coupling using Pd(dppf).CH_2Cl_2[124] as catalyst in 23% yield (Scheme 15). The 4'-butyl derivative **70** was achieved by *B*-alkyl Suzuki coupling of intermediate **69**. This reaction was challenging as the major product was the debrominated derivative **67**. Different large bite angle catalysts were tried in order to enforce the necessary reductive elimination in the catalytic cycle instead of the β-hydride elimination that results in debromination.[125] However, the best yield, 8%, was obtained with Pd(dppf).CH_2Cl_2 (Table 2). Nevertheless, this was deemed sufficient to obtain a sample for screening, with further reaction optimization deferred until required by good biological results.

Deprotection of intermediates **75** and **76** was done as described in (Scheme 13) to give the desired products **78** and **79**.



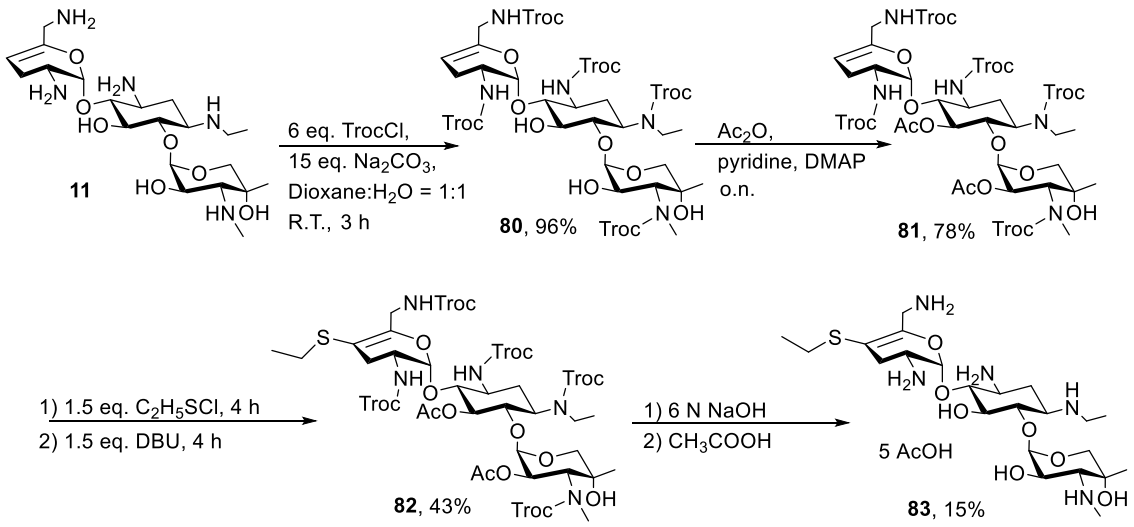
Scheme 15. **Synthesis of 4'-phenyl netilmicin and 4'-butyl netilmicin**

Table 2. **Catalysts used in the *B*-alkyl Suzuki reaction and their yields**



Entry	Catalyst	Solvent	% Yield of **76**	% Yield of **69**
1	$Pd(dppf).CH_2Cl_2$	dioxane:water (3:1)	0	-
2	$Pd(dppf).CH_2Cl_2$	toluene:water (3:1)	8	32
3	$Pd(PPh_3)_4$	toluene:water (4:1)	traces	-
4	$Pd(PCy_3)\ Cl_2$	toluene:water (4:1)	5	14

Triazene protecting groups were found to be incompatible with the ethylsulfenyl chloride needed to prepare 4'-(ethylsulfanyl) netilmicin **83**; therefore, all amines were protected as the 2,2,2-trichloroethyl carbamate (Troc) group,[126] followed by acetylation of the hydroxyl groups (Scheme 16). The resulting compound **81** was reacted with ethylsulfenyl chloride,[127] formed *in situ* from diethyl disulfide and sulfuryl chloride, to give the desired intermediate **82**. One step deprotection using 6 N NaOH afforded the desired product **83**.



Scheme 16. **Synthesis of 4'-(ethylsulfanyl) netilmicin**

2.5.6. Synthesis of plazomicin

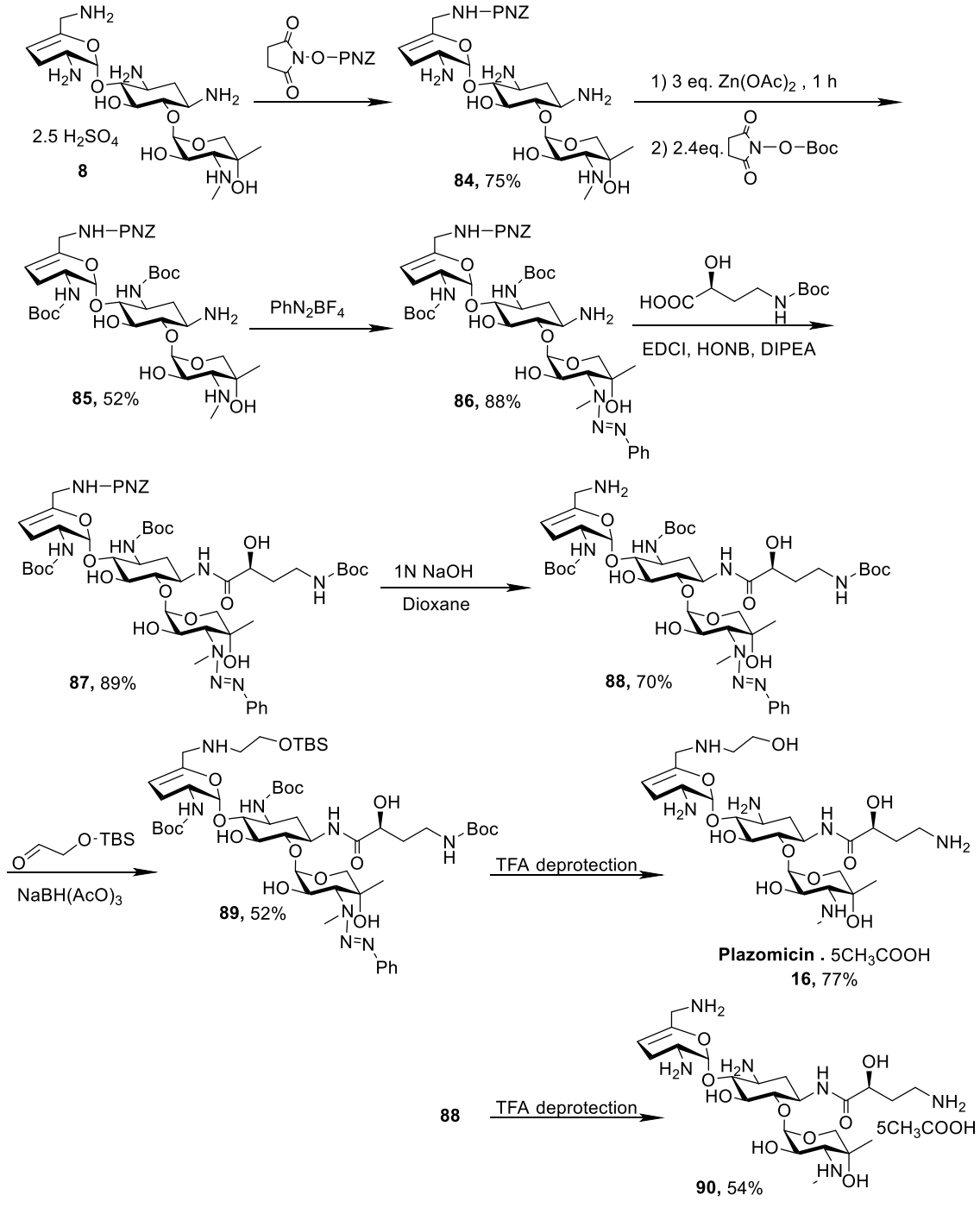
As discussed in the introduction, plazomicin **16** is one of the recently developed AGAs and is currently in phase 3 clinical trials for complicated urinary tract infections.[83-84] An authentic sample was needed to serve as a standard for the comparison of biological activity of the synthesized compounds. However, although the synthesis of plazomicin from sisomicin has been outlined in the patent[84] and in the open literature,[83] few details and characterization data were available. Accordingly, the semisynthesis of plazomicin from sisomicin was undertaken by a novel synthetic route featuring the use of the phenyltriazenyl protecting group for secondary

amines in only 7 steps and 8.5% overall yield, which compares favorably to the published 0.16% yield.[83]

The synthesis of plazomicin started by selective protection of the 6'-NH_2 group of sisomicin **8** using 4-nitrobenzyl *N*-hydroxysuccinimidyl carbonate[128] to give 6'-*N*-(4-nitrobenzyloxycarbonyl) sisomicin **84** in 75% yield. The amino substituents at positions 2' and 3 were then selectively protected as the Boc derivatives by the temporary protection of the other amines in the form of zinc chelates, resulting in the formation of **85** in 52% yield.[129] The reaction of **85** with a 10 mol% excess of phenyl diazonium tetrafluoroborate in acetonitrile in the presence of potassium carbonate gave an 88% yield of the desired triazene **86** (Scheme 17), whose sharp, well-resolved NMR spectra facilitated spectral elucidation in this and the following steps. Thus, the triazene moiety fulfills the expectation of a disymmetric secondary amine protecting group, free of the rotamer problems associated with the more common carbamates. Furthermore, as suggested by the model studies, it was selectively introduced onto a secondary amine in the presence of a primary amine without the need for an excess of the diazonium salt.

The remaining amine at position N1 was then coupled to *N*-Boc-4-amino-2(*S*)-hydroxy-butyric acid[130] under standard carbodiimide conditions in the presence of hydroxy-5-norbornene-2,3-dicarboximide[131] and afforded the amide **87** in high yield. The 4-nitrobenzyl carbamate group was then cleaved with aqueous sodium hydroxide in dioxane to yield 70% of the amine **88**, which was subjected to reductive amination with *tert*-butyldimethylsilyloxy acetaldehyde and sodium triacetoxyborohydride[132] to give **89** in 52% yield. The reductive amination was conducted in the presence of Hünig's base[133] so as to avoid premature cleavage of the triazene group. Finally, removal of the Boc and triazene groups was achieved with 50% trifluoroacetic acid in dichloromethane to give plazomicin **16** in 77% yield after purification by

chromatography over Sephadex C-25. A sample of the analog *N*1-(4-amino-2(*S*)-hydroxybutyryl sisomycin **88** was obtained in 54% by simple acid-mediated deprotection of **90**.



Scheme 17. **Synthesis of plazomicin.**

2.6. Biological Evaluation

The above synthesized samples were screened for ribosomal selectivity and antibacterial activity by the Böttger lab in Zurich. To assess the effect of the antibiotic on translation, IC_{50} values of cell-free translation assays with purified 70S ribosomes of both wild-type and mutant *M. smegmatis* strains that carry the rRNA A sites of either eukaryotic cytoplasmic or mitochondrial ribosomes were determined. The IC_{50} value is the concentration required to inhibit protein synthesis by 50% (Table 3).[134] In addition, the MIC values for *Mycobacterium smegmatis* (Sms), Methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli* and *P. aeruginosa* were recorded.[72] The minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent (AGA) that inhibits 50% of the visible growth of a microorganism (Table 4).

Table 3. **Inhibition of wild-type bacterial and hybrid ribosomes (IC_{50}, μM).**

IN VITRO (IC_{50}, **μM**)	**Sms (μM)**	**Mit (μM) (selectivity)**	**A1555G (μM) (selectivity)**	**Cyt (μM) (selectivity)**
Sisomicin **(8)**	0.02	25.4 (1137)	1.4 (64)	93.8 (4196)
Netilmicin **(11)**	0.02	31.3 (1490)	1.4 (66)	98.8 (4698)
Apramycin **(8)**	0.17	124.8 (747)	51.5 (308)	108.7 (651)
Gentamicin **(7)**	0.03	19 (594)	1.1 (34)	58 (1812)
4'-Iodo netilmicin **(72)**	0.15	81.7 (545)	2.6 (17)	366.8 (2451)
4'-Bromo netilmicin **(73)**	0.09	71.3 (791)	1.8 (20)	261.5 (2900)
4'-Chloro netilmicin **(74)**	0.18	305.9 (1733)	84.8 (481)	980.3 (5555)
4'-Phenyl netilmicin **(78)**	0.27	28.9 (106)	3.5 (13)	81.4 (299)
4'-Butyl netilmicin **(79)**	0.58	407.6 (699)	21.9 (37)	940.4 (1612)
4-(Ethylsulfanyl) netilmicin **(83)**	0.67	256.3 (381)	46.3 (69)	783.3 (1165)
Plazomicin **(16)**	0.08	68.2 (808)	4.7 (56)	388.5 (4605)
1-[4-Amino-2-hydroxy-butyryl] sisomicin **(90)**	0.02	8.5 (467)	0.4 (24)	169.1 (9278)

Sms: *M. Smegmatis* ribosome, Cyt: *M. Smegmatis* ribosome with human cytosolic A-site, Mit: *M. Smegmatis* ribosome with human mitochondrial A-site, A1490G: *M. Smegmatis* ribosome with human mitochondrial A-site with A1555G mutation. Selectivities are obtained by dividing the hybrid ribosomal activity by *M. Smegmatis* ribosomal activity

The addition of an iodo group at the 4’-position of apramycin results in a 7-fold reduction in ribosomal activity and reduction of overall selectivities. When compared to the parent netilmicin, 4'-bromo netilmicin **73** exhibits a 4 to 5-fold loss in activity and a smaller (2 to 3-fold) reduction in activities against the mitochondrial wild-type, the A1555G mitochondrial mutant, and the cytosolic hybrid. On the other hand, 4'-chloro netilmicin **74** showed a 9-fold loss in activity against the bacterial ribosome, but an increase in selectivity overall, especially over the A1555G mitochondrial mutant hybrid that is considered to be the allele responsible for hyper-susceptibility to AGAs ototoxicity. A clear trend is visible in which the selectivities increase as the electronegativity of the halogen, introduced at the 4’-position, increases. The inclusion of phenyl, butyl and ethylsulfide groups at 4’-position of netilmicin is detrimental for activities against the wild-type bacterial ribosome, and also causes an overall reduction in ribosomal selectivities.

1-L-HABA sisomicin **90** shows a similar bacterial ribomosal activity when compared to sisomicin and netilmicin. The L-HABA group decreases the selectivities in the mitochondrial and A1555G mutant hybrid ribosomes but increases the selectivity over the cytosolic hybrid. Plazomicin **16**, on the other hand, showed a 4-fold reduction in the bacterial ribomosal activity and decreased selectivities over the mitochondrial and A1555G hybrid ribosomes but to a lesser extent than 1-L-HABA sisomicin **90**. Therefore, the 6’-(2-hydroxyethyl) group results in decreased antibacterioribosomal activity probably due to moderation of the hydrogen bond between the 6’-amine and A1408, resulting in a weakened pseudobase pair interaction. Inclusion of the 6’-*N*-(2-hydroxyethyl) does however increase selectivity over the mitoribosomes when compared to L-HABA sisomicin **90**. A similar effect of 6’-*N*-(2-hydroxyethyl) substituent on the selectivities of neomycin was recently reported by the Crich group.[135]

***Table 4. In vivo* minimal inhibitory concentrations (MIC, µg/ml) of clinical isolates.**

IN VIVO (MIC, **µg/ml**)	Sms	*MRSA*				*E coli*			*P aeruginosa*			
		AG038	**AG039**	**AG042**	**AG044**	**AG001**	**AG055**	**AG003**	**AG031**	**AG032**	**AG033**	**AG086**
Sisomicin	1	0.25-0.5	0.25-0.5	16-32	4	1-2	1-2	32	16	0.5	>128	4
Netilmicin	2	0.5	0.5	2	1	2	2	8	2	2	>128	>128
4'-Iodo netilmicin **(72)**	2	4-8	16	64	16	2	1-2	16	4-8	4-8	>128	>128
4'-Bromo netilmicin **(73)**	2	4	8-16	64	8-16	2	1-2	16	8	8	64	2
4'-Chloro netilmicin **(74)**	2	2-4	8	32	8	4	2-4	16-32	4	64	>128	n.d.
4'-Phenyl netilmicin **(78)**	8	8	16	64	16	4	2	64	16-32	16-32	>64	2
4'-Butyl Netilmicin **(79)**	32	16	16	64	32	8-16	5	8-16	64	64	>128	8-16
4-(Ethylsulfanyl) netilmicin **(83)**	64	32	32	128	32-64	16	8-16	8-16	>128	128	>128	16
Plazomicin **(16)**	≤0.25	2	2	2	2	2	2	2	4	4	16	32
1-[4-amino-2-hydroxy-1-oxobutyl] sisomicin **(90)**	≤0.25	1	1-2	1	1	2	2	1	2	2	>128	16

MIC (µg/ml) *in vivo*: minimal inhibitory concentrations of netilmicin derivatives to inhibit Sms (*M smegmatis*), MRSA (Methicillin-resistant *S aureus*), *E coli* and *P aeruginosa.*

Consideration of the MIC values reveals that the 4'-iodo, bromo and chloro derivatives of netilmicin have almost the same activity against *Sms* and *E. coli* as netilmicin, but that they are less active against MRSA. On the other hand, the presence of phenyl, butyl and ethylsulfanyl groups in the 4'-position of netilmicin results in considerable loss of activity in most strains. All

4’-derivatives synthesized (except the 4’-iodo derivative) have good to moderate activity against the AG086 clinical strain of *P. aeruginosa* (Table 4). This is relevant as this strain is resistant to both netilmicin and sisomicin, and indicates that modification at the 4’-position gives some protection against the aminoglycoside modifying enzyme responsible for resistance to netilmicin and sisomicin in this strain. Overall it is apparent that 4’-modifications reduce activity in the 4,6-series to a greater extent than in the 4,5-series. The same effect was encountered with 4’-modifications of the 4,6-aminoglycoside kanamycin B.[11]

As expected, plazomicin **16** has good activity with all strains tested including the strains that are resistant to netilmicin, suggesting that L-HABA and hydroxyethyl groups were successfully able to shield the drug from most AMEs. Compound **90**, lacking the 6’-*N*-hydroxyethyl group displayed slightly better activity in all strains than plazomicin, except for the *P aeruginosa* strain AG033, which was completely resistant to it.

A mouse cochlear explant study, conducted by Professor Jochen Schacht at the Kresge hearing institute of the University of Michigan, was done to compare the ototoxicities of plazomicin **16**, apramycin **9** and gentamicin **7**. This study depends on extracting the cochleae of mice and soaking them in various concentrations of the drug. The extent of the loss of outer hair cells (OHC) is examined and quantified (Figure 23). The results showed that plazomicin is more cochleotoxic than apramycin and comparable to gentamicin (Figure 24). The cochlear damage caused by plazomicin in comparison to apramycin and gentamicin is consistent with predictions from the A1555G hypersusceptibility mutant of the mitochondrial ribosome, albeit the differences are less apparent on the mitochondrial ribosomes themselves.

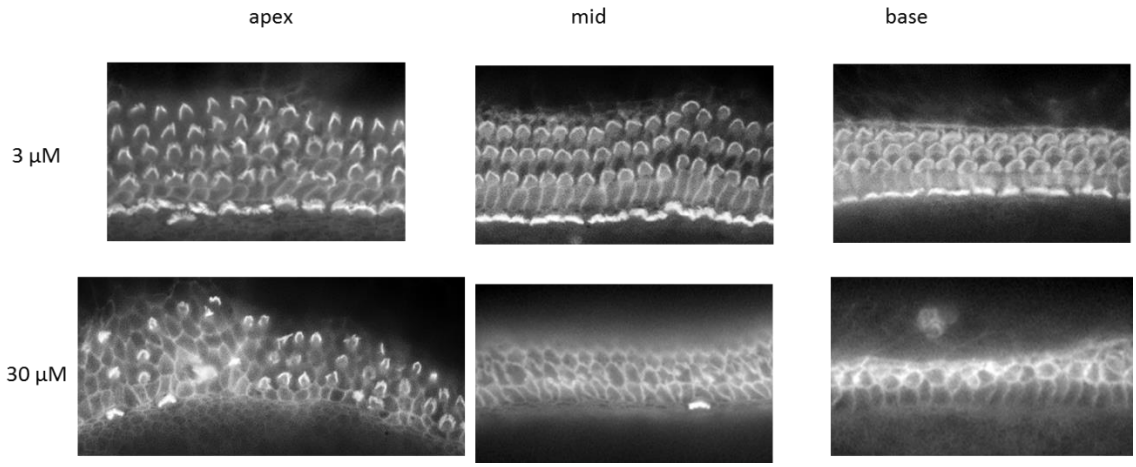


Figure 23. **The outer hair cells (OHC) in different parts of the cochlea (apex, mid and base) at 3 µM (OHC are still intact) and 30 µM (considerable loss in OHC) of plazomicin.**

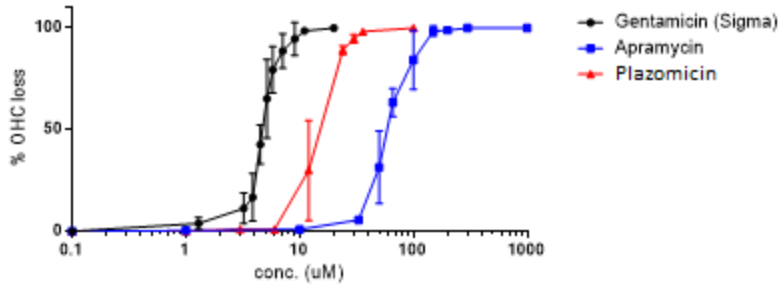


Figure 24. **The effect of concentration of plazomicin (16) in comparison to gentamicin (7) and apramycin (9) in the percentage of outer hair cell (OHC) loss.**

2.7. Conclusion

This chapter elaborated the use of phenyl triazenes as selective protecting groups for secondary amines in the presence of primary amines providing sharp ^{1}H NMR spectra at room temperature. The protecting group enabled the synthesis of several 4'-netilmicin derivatives, which were examined for antibacterial activities as well as ribosomal selectivities. Unfortunately, the 4'-netilmicin derivatives showed reduced antiribosomal and antibacterial activities compared to the parent and to a greater extent than in the 4,5-series. The triazene protecting group also facilitated the synthesis of plazomicin, an aminoglycoside in the phase III clinical trials, in less steps and

higher yield than the published synthesis, and enabled the study of the ribosomal selectivity and ototoxicity of this compound.

CHAPTER 3. DEVELOPMENT OF APRAMYCIN DERIVATIVES WITH MODIFICATION AT THE 5-POSITION AND EXAMINATION OF THEIR ANTIRIBOSOMAL AND ANTIBACTERIAL ACTIVITY

3.1. Mode of binding of apramycin to the bacterial ribosomal A-site

Apramycin **9**, which was previously called nebramycin factor 2, is produced by *Streptomyces tenebrarius* bacteria. Apramycin **9**, with a unique bicyclic structural feature, is active against many Gram-positive and Gram-negative MDR infectious bacteria[136-139] and overcomes all of the known AME except AAC(3).[57] Although apramycin has not been used in humans yet, it has found wide application in veterinary medicine,[140] as a consequence of which much pharmacological data is available.[141] Recent reports showed that in animal models apramycin displays minimal ototoxicity, the main adverse effect of AGAs, and suggesting its re-evaluation for human use.[134, 142]

The X-ray crystal structure of apramycin in complex with the 30S ribosomal subunit of *Thermus thermophilus* bacteria showed that the bicyclic ring I of apramycin is bound in a similar way to ring I of the 4,5- and 4,6-AGAs. Ring I (bicyclic ring) interacts through CH-π interactions with G1491 in the ribosomal A-site. Thus, ring I forms a pseudo base-pair interaction with A1408, as presented for netilmicin in chapter 2. The importance of the 6'- and N7'-positions in the pseudo base-pair interaction was emphasized by the Crich group whose modifications at these positions negated activity of apramycin.[143] This casts doubt on an alternative binding mode, from an NMR structure of a short RNA oligonucleotide complex with apramycin, in which the 6'- and N7'-positions have minimal role in binding.[144]

In the X-ray of apramycin with the actual 30S ribosomal subunit, ring III is oriented so as to allow the formation of direct hydrogen bonds with the highly flexible A1492 (Figure 25).[134] This interaction may stabilize a partially flipped-out A1492 conformation, that makes it difficult

for A1492 to contact with the minor groove of the codon-anticodon helix and cause misreading. This explains the failure of apramycin to induce misreading leaving the inhibition of translocation as the main cause of bacterial protein synthesis inhibition.[79] This rationale for the absence of misreading by apramycin is supported by work on the cleavage of ring III to give aprosamine **91** (Figure 26), which, albeit with lower levels of activity, induces misreading.[23]

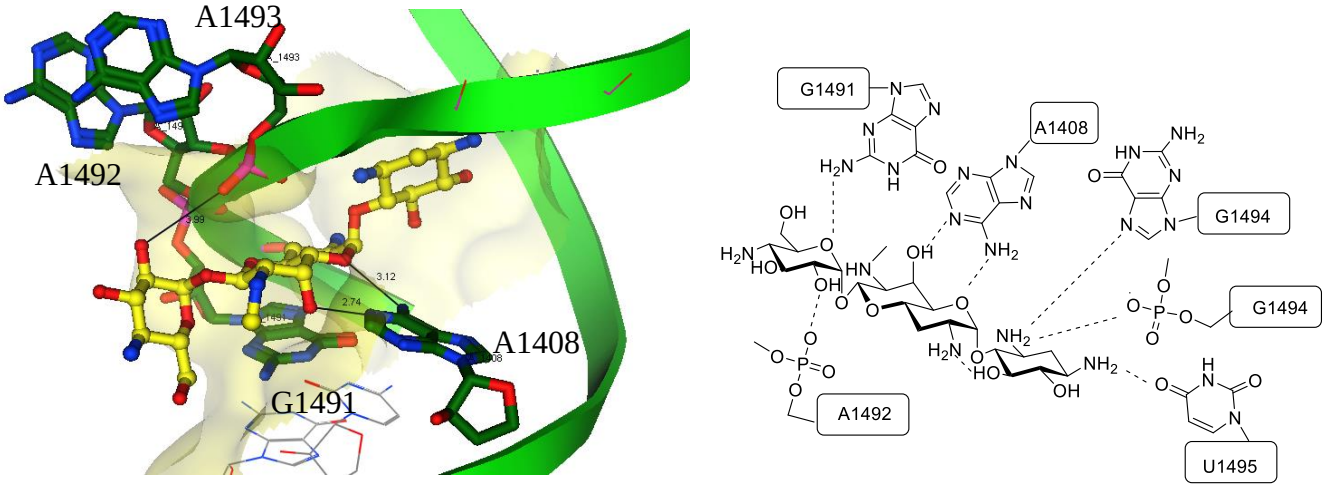


Figure 25. **a) X-Ray crystal structure of apramycin bound to the bacterial A site showing CH/π-stacking of ring I with G1491 base, pseudo base-pair interaction of ring I with A1408 and interaction of ring III with A1492 (PDB code: 4AQY) and b) detailed interaction of apramycin with the bacterial A site.**

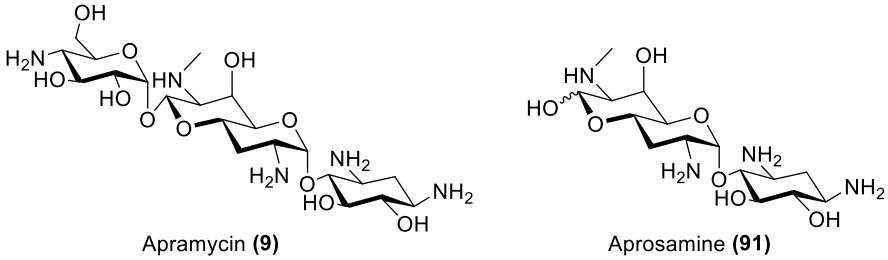


Figure 26. **Structures of apramycin (9) and aprosamine (91)**

3.2. Modifications of apramycin

Many modifications of apramycin have been done in order to increase its potency. Most are reported in patents and include modifications at the 5- and 6-positions,[93, 145-146] modifications

at the *N*1, *N*2', *N*7', and *N*4"-positions,[142-143, 147-150] modifications at the 6"-position,[151] and modifications at the *N*7'-Me and 6'-position.[143] These modifications are summarized in (Figure 27) and show how apramycin has started to gain attention recently after having been neglected since the 1980s. This ensemble of studies reveals that enhanced bacterial activity might best be achieved through modifications to ring II (2-DOS), and most modifications to ring I (bicyclic ring) decreased the bacterial activity. The following sub-sections will discuss the art of selective modification at these positions.

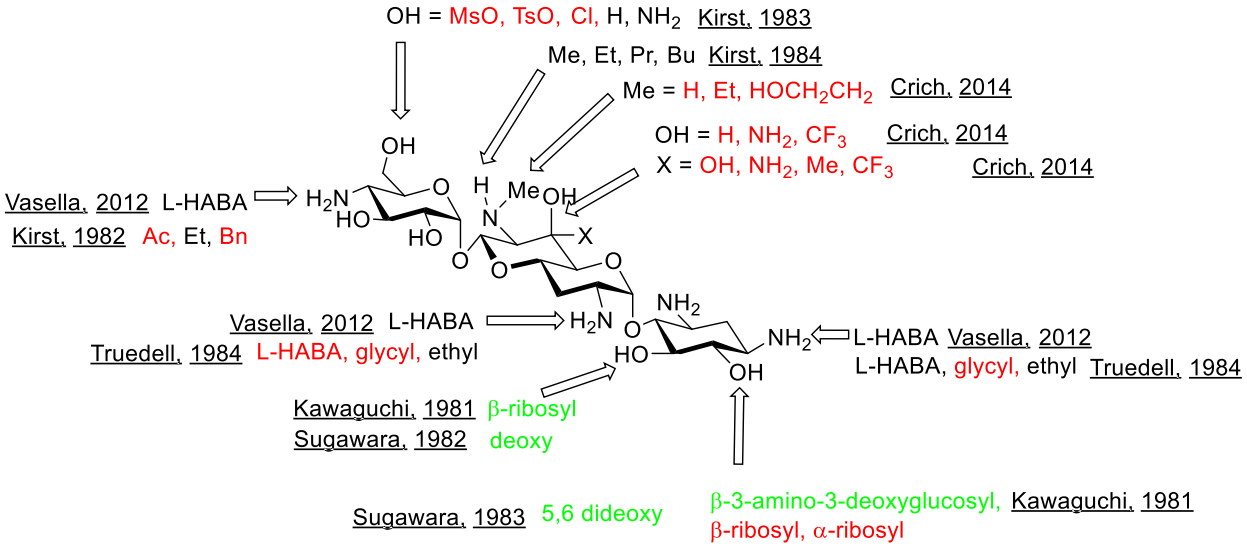
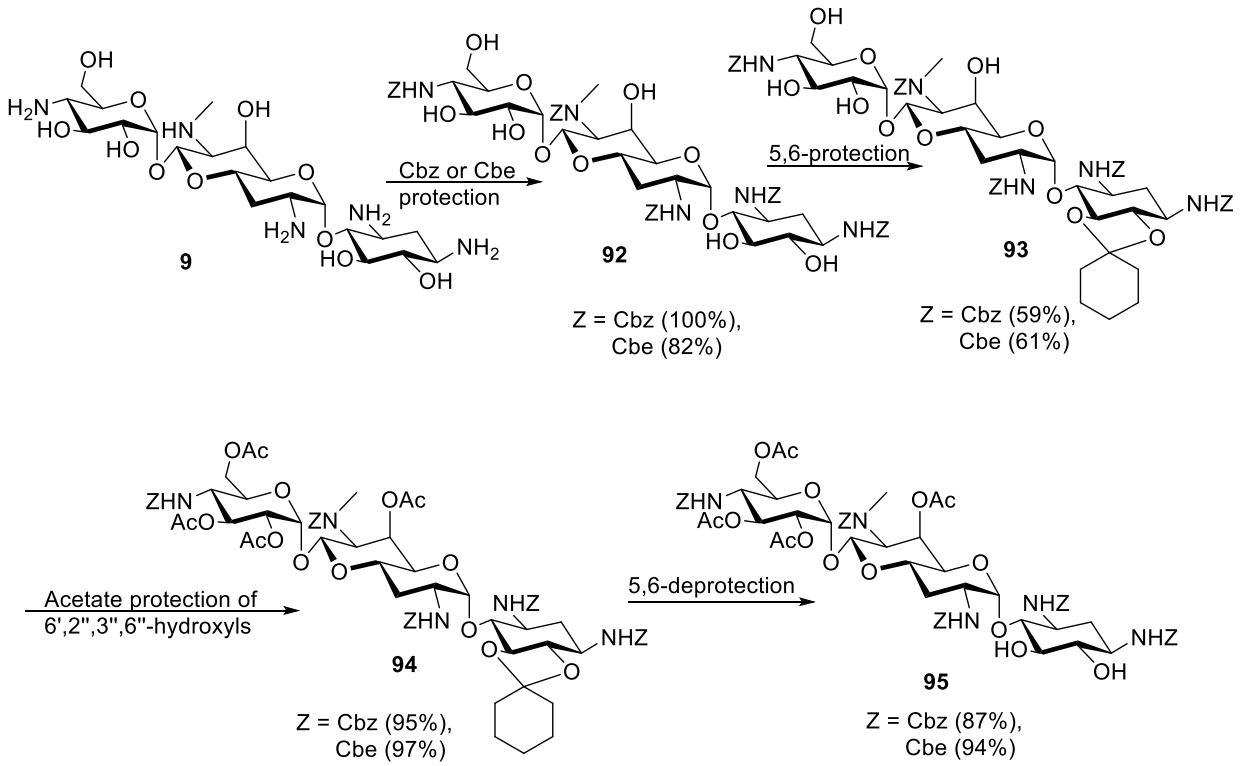


Figure 27. **A schematic diagram showing known modifications of apramycin and the year modified; modifications in green showed increased bacterial activity, modifications in red showed a decreased bacterial activity, and modifications in black showed an unreported or comparable bacterial activity.**

3.2.1. Modifications at the 5- and 6-positions of apramycin

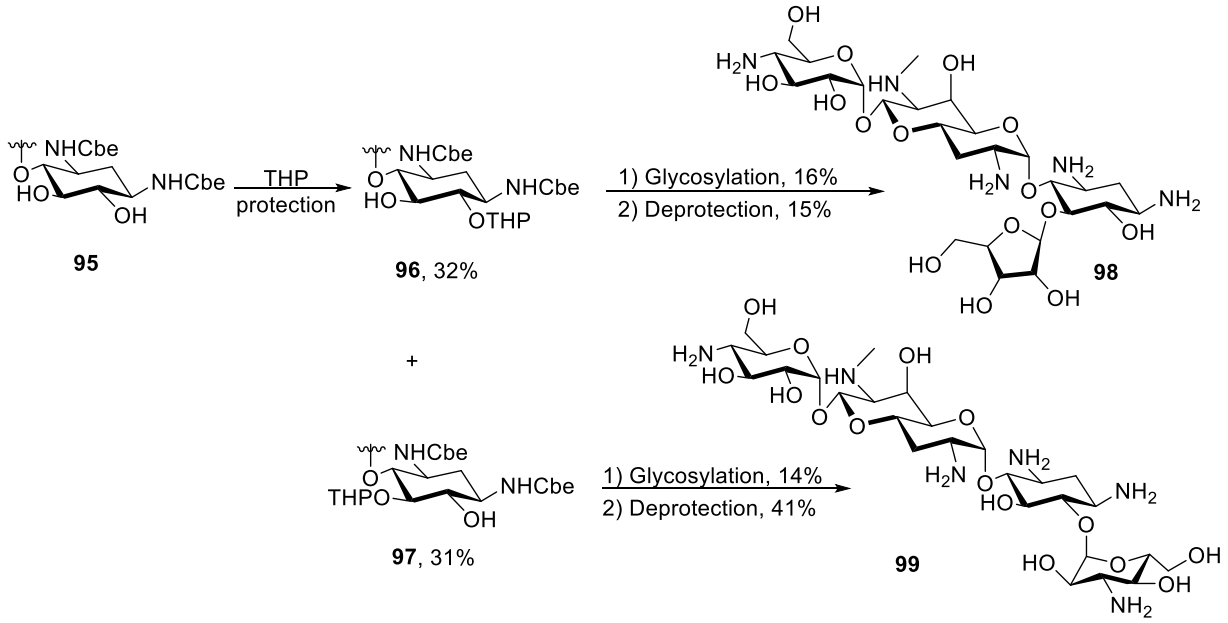
A method of derivatizing apramycin at the 5- and/or 6-positions was reported that passes through a common intermediate **95**, which requires only four steps to synthesize**.** In this sequence, all amines were first protected as carbamates, which was followed by protection of the 5- and 6-hydroxyl groups as a cyclohexylidene ketal. Subsequent protection of the remaining

hydroxyl groups as esters was followed by acid hydrolysis of the ketal to give the 5,6-diol compound **95**, that is the key intermediate for modifications at these positions (Scheme 18).[93, 145-146]



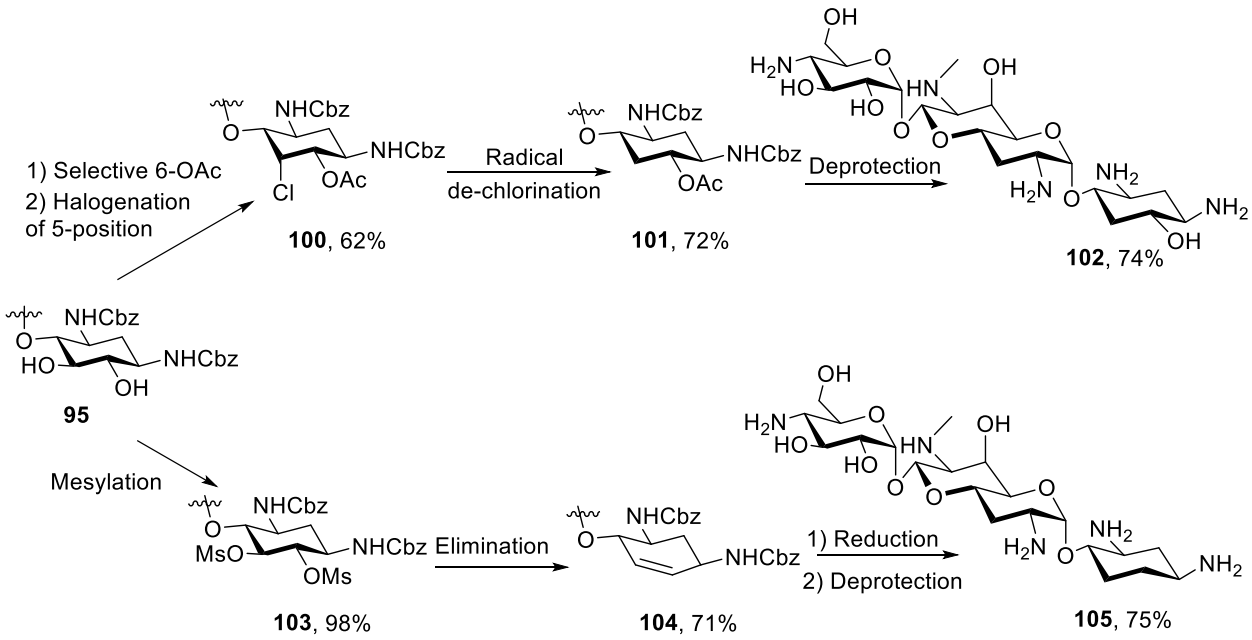
Scheme 18. **Preparation of the key intermediate 95 for modification at the 5,6-positions**

Reaction of **95** with dihydropyran was unselective and gave both the 5- and 6-protected alcohols **96** and **97**. The alcohols **96** and **97** were separated, glycosylated and deprotected to achieve the 5-*O*-glycoside **98** and the 6-*O*-glycosides **99** (Scheme 19).[93]



Scheme 19. **Synthesis of 5-*O*-glycosides 98 and 6-*O*-glycosides 99**

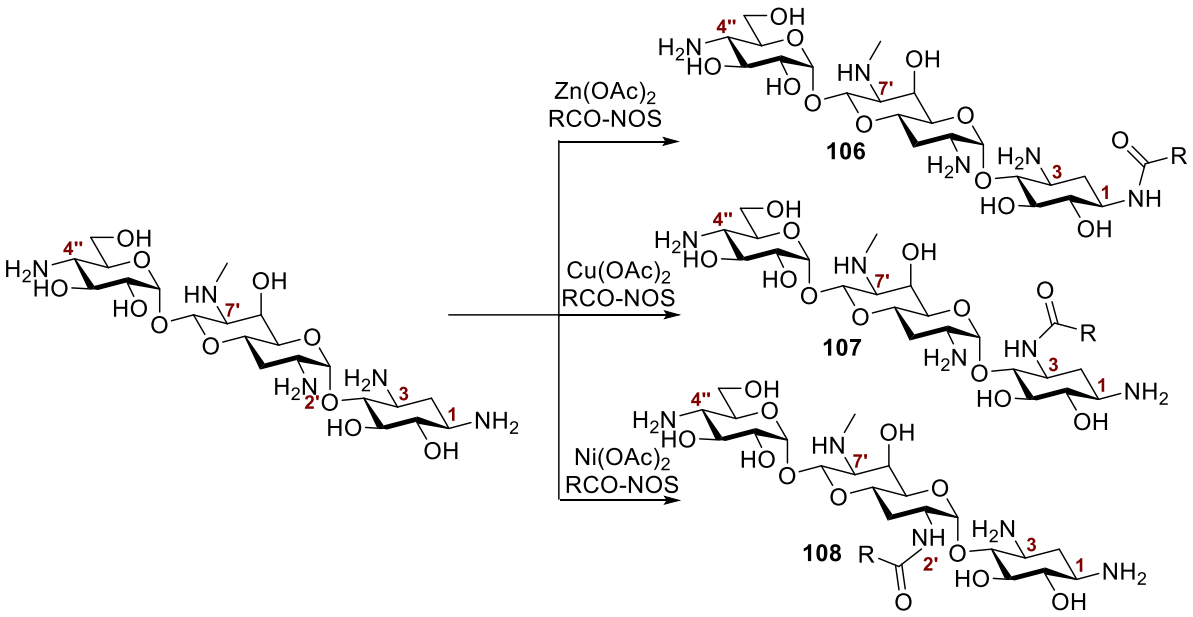
Additionally, the 5,6-diol intermediate **95** was selectively acetylated at the 6-position followed by chlorination at 5-position to afford the compound **100**. This was followed by radical de-chlorination to give the compound **101**. Global deprotection by catalytic hydrogenolysis and basic hydrolysis afforded the 5-deoxyapramycin **102**.[145] Further, the key intermediate **95** was subjected to mesylation to give **103**, which was reacted with sodium iodide and then zinc dust to give the 5,6-alkene **104**. Finally, hydrolysis and catalytic hydrogenation gave 5,6-dideoxyapramycin derivative **105** (Scheme 20).[146]



Scheme 20. **Synthesis of 5-deoxyapramycin 102 and 5,6-dideoxyapramycin 105**

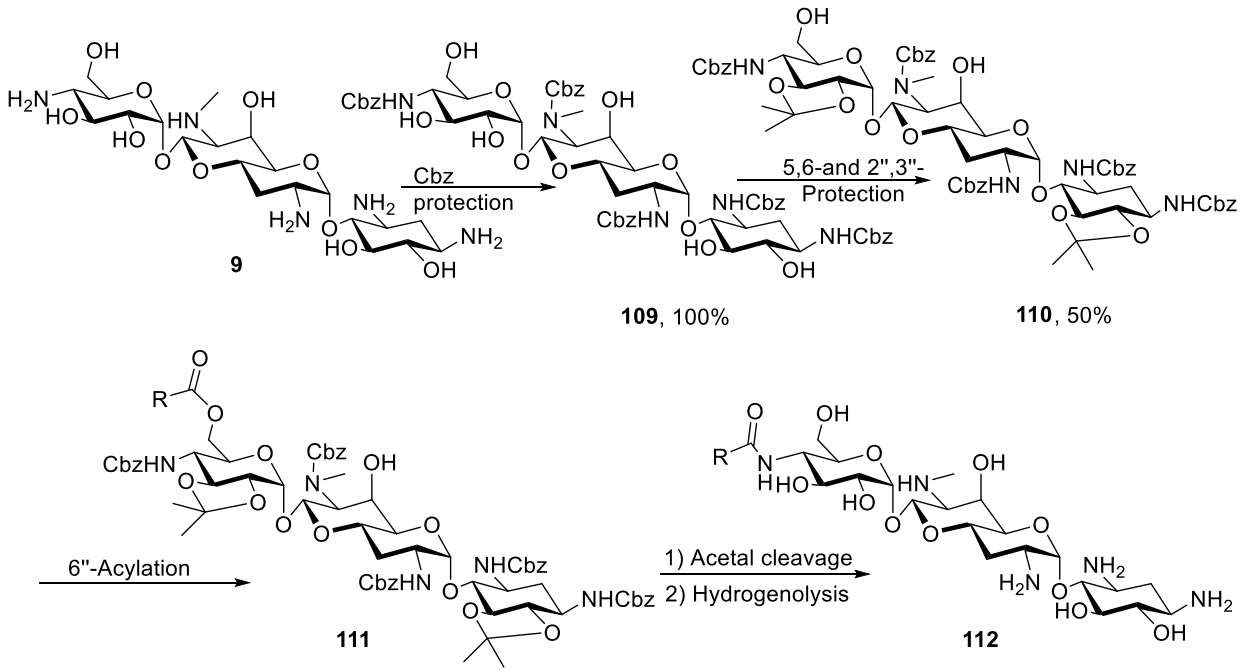
3.2.2. Modifications at the *N*1,*N*2',*N*7',*N*4''-positions of apramycin

A novel method for regioselective derivatization at the *N*1, *N*3 or *N*2'-positions of apramycin was introduced by Kirst and co-workers.[152] The method reported facilitates the synthesis of *N*1, *N*3 or *N*2'-derivatives of apramycin by transition metal-directed acylations of apramycin. The transition metals co-ordinate with all except the amine to be acylated and so act as transient protecting groups that save the conventional steps of protection and deprotection. Selectively among the different amines is achieved by changing the transition metal cations. Zinc salts are used to achieve regioselective acylation and alkylation of *N*1 of apramycin in a single reaction. Copper salts are used for the synthesis of *N*3-acyl derivatives of apramycin, while nickel salts are used to accomplished the regioselective acylation of *N*2' of apramycin (Scheme 21). The so-formed acyl derivatives can be reduced subsequently by diborane or lithium aluminium hydride to give the corresponding *N*-alkyl derivatives.[149-150, 152]



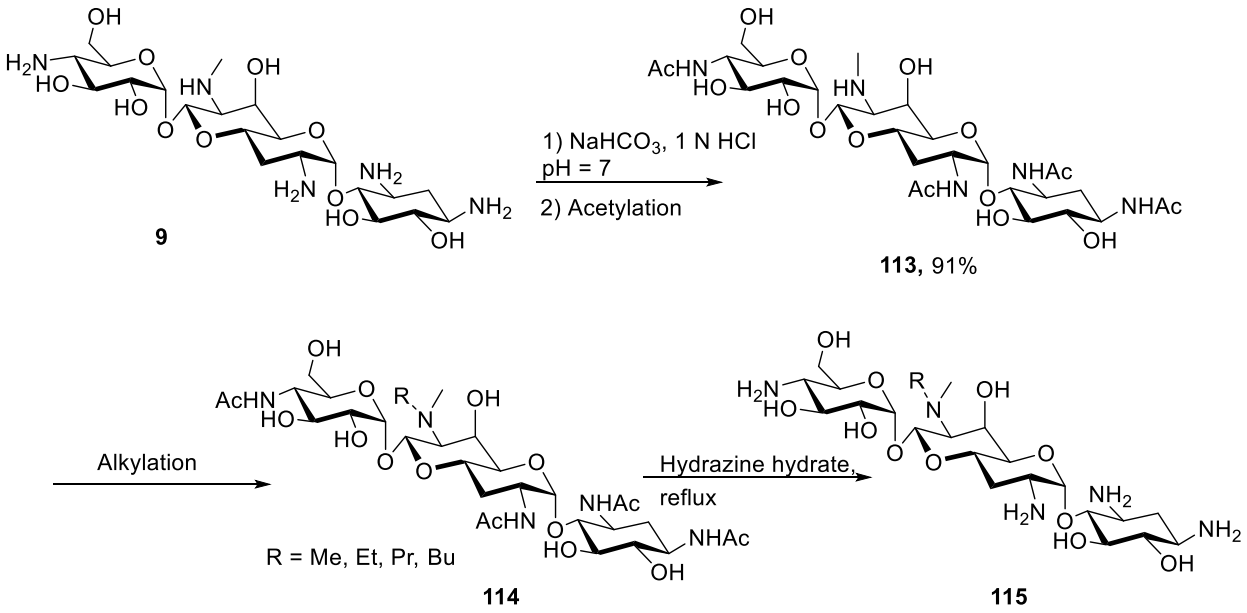
Scheme 21. **Transition metal directed derivatization**

The regioselective derivatization at the 4" position of apramycin is reported in a US patent.[147] The approach for the selective modification at 4" position involves protection of all amines as their Cbz derivatives, then the 5,6- and 2",3"- vicinal diols are both protected with an isopropylidine group. The 6"-hydroxyl group is next acylated prior to deprotection of the isopropylidine groups. The key step in this method involves releasing the free amines by hydrogenolysis so that migration of 6"-*O*-acyl group to the proximal 4''-amine takes place and gives the 4"-derivatives of apramycin. The 4"-*N*-alkyl analogues are prepared by reduction of corresponding acyl derivatives with diborane or lithium aluminum hydride (Scheme 22).



Scheme 22. **Synthetic scheme for the modification at the 4'-position**

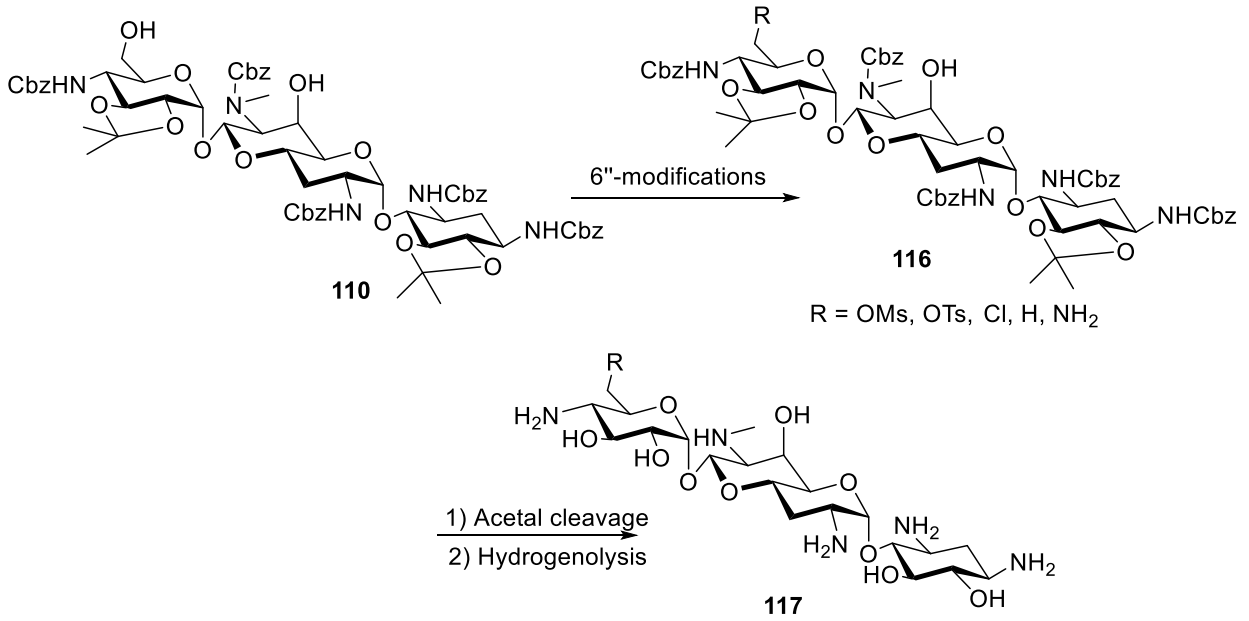
The synthesis of 7'-*N*-alkylapramycin derivatives proceeded by the initial preparation of an apramycin-carbon dioxide complex that then was reacted with acetic anhydride to form 1,3,2',4"-tetra-*N*-acetyl apramycin **113** (Scheme 23). This was followed by alkylation of the acetylated intermediate to provide the 7'-*N*-alkyl-1,3,2',4"-tetra-*N*-acetyl apramycin derivatives **114**. General deprotection strategies finally yielded the 7'-*N*-alkyl apramycins **115**.[148]



Scheme 23. **Synthetic scheme for the modification at the 7'-position**

3.2.3. Modifications at the 6''-position of apramycin

Modification at the 6"-position of apramycin is achieved by a comparable approach to that used to modify the N4''-position. In this chemistry, instead of 6''-acylation of the derivative **110**, the 6''-hydroxyl is converted to the corresponding mesylates, tosylates, chlorides, and amines or is deoxygenated. Unlike acyl groups, these groups were not to migrate after unmasking of the apramycin amines (Scheme 24).[151]

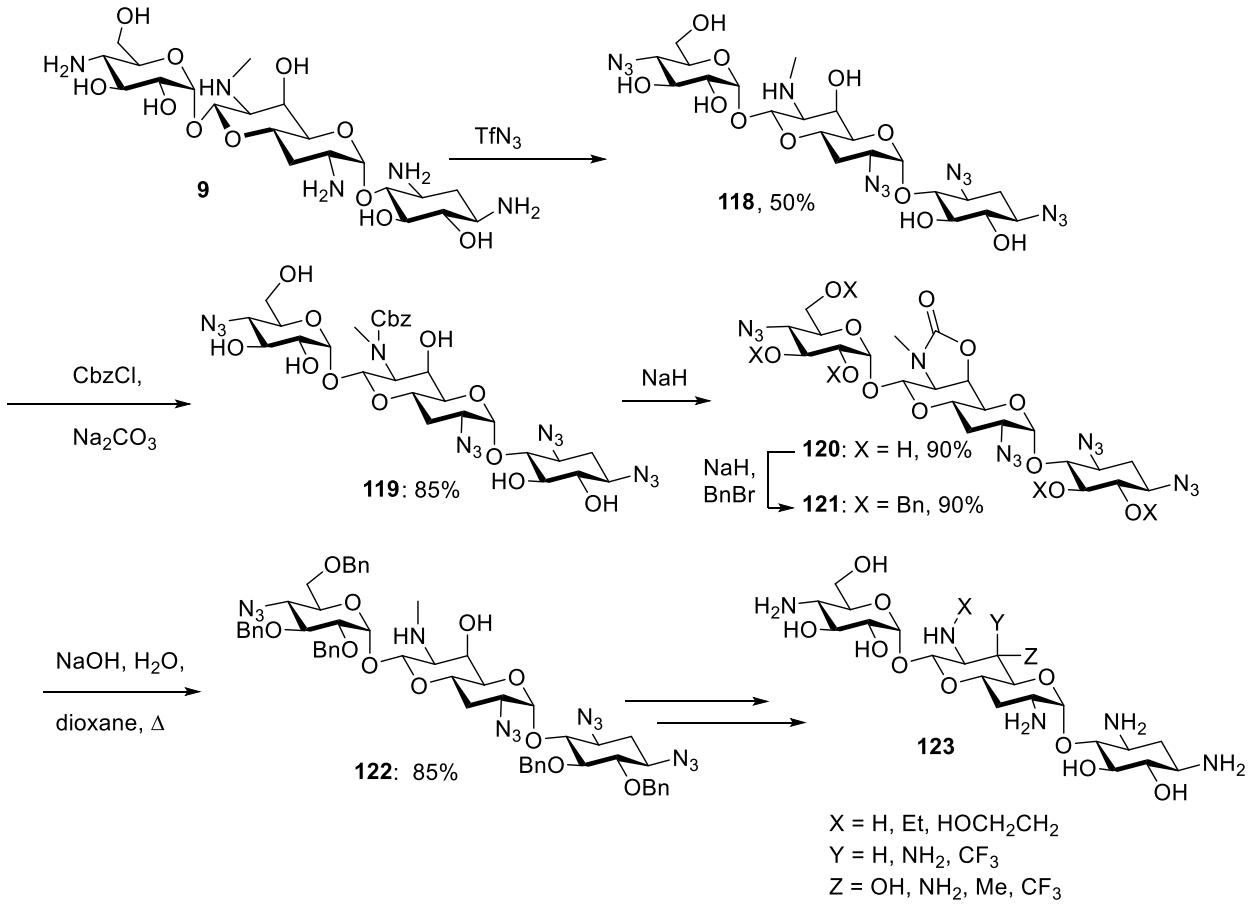


Scheme 24. **Synthetic scheme for the modification at the 4''-position**

3.2.4. Modifications at the N7'-Me and 6'-positions of apramycin

Crich and co-workers reported modifications at N7' and 6'-postions (Scheme 25).[143] All amines and hydroxyl groups except at the N7' and 6'-*O*-postions of apramycin were selectively protected in five steps. First, all the primary amines were protected as azides using triflyl azide. Subsequently, the secondary amine was protected as the benzyl carbamate, which upon cyclization using sodium hydride provided the 6',7'-oxazolidinone ring. Benzylation of all the remaining hydroxyl groups and finally the cleavage of oxazolidinone ring afforded the key intermediate **122**. This key intermediate allowed the subsequent modifications at the 6'-position, including the inversion of the hydroxyl group configuration or replacement of hydroxyl group with both inversion and retention of configuration by an amine group. The introduction of CH_3 and CF_3 groups geminal to the 6'-alcohol was also achieved in both configurations. Modifications can also be made at the 7'-position from intermediate **122**, including the

preparation of analogues in which the methyl group is removed or replaced by longer alkyl chains.



Scheme 25. Synthetic scheme for the modification at the *N*7'-Me and 6'-positions

3.3. Rationale

Comparison of the antiribosomal and antibacterial activities of 4,5- or 4,6-disubstituted 2-deoxystreptamine derivatives with the corresponding 4-monosubstituted 2-deoxystreptamine derivatives generally shows the higher activity of the disubstituted compounds. For example, AGAs like kanamycin B, neomycin and ribostamycin are more active than neamine (Figure 28 and Table 5).[153] Correspondingly, in apramycin, there was an enhancement of activity when it was glycosylated at the 6-position to give 6-*O*-(3-amino-deoxy-α-D-glucopyranosyl) apramycin **99**, and when it was glycosylated at the 5-position to give 5-*O*-(β-D-ribofuranosyl) apramycin **98**

(Table 6).[93] Thus, modification of apramycin at the 5- and/or 6-positions was considered to offer the most promise for the development of improved derivatives. The knowledge that bacterial resistance arising from the presence of ribosomal methyltransferases (e.g., ArmA) renders most of the 4,6-disubstituted AGAs obsolete focused the project on substitution of apramycin at the 5-position. Consequently, it was decided to re-evaluate the 5-*O*-β-D-ribofuranosyl apramycin (**98**, Scheme 19) previously prepared by a Japanese group,[93] preferably by a shortened and improved route. Extrapolating further, the attachment of aminosugars such as the paromomycin CD ring to the 5-position was projected to give an apramycin-paromomycin hybrid; the goal being to combine paromomycin's high activity with apramycin's low ototoxicity (Figure 28). A simplified form of this hybrid, in which a 2-aminoethyl group replaces the D ring paromomycin, was also designed.

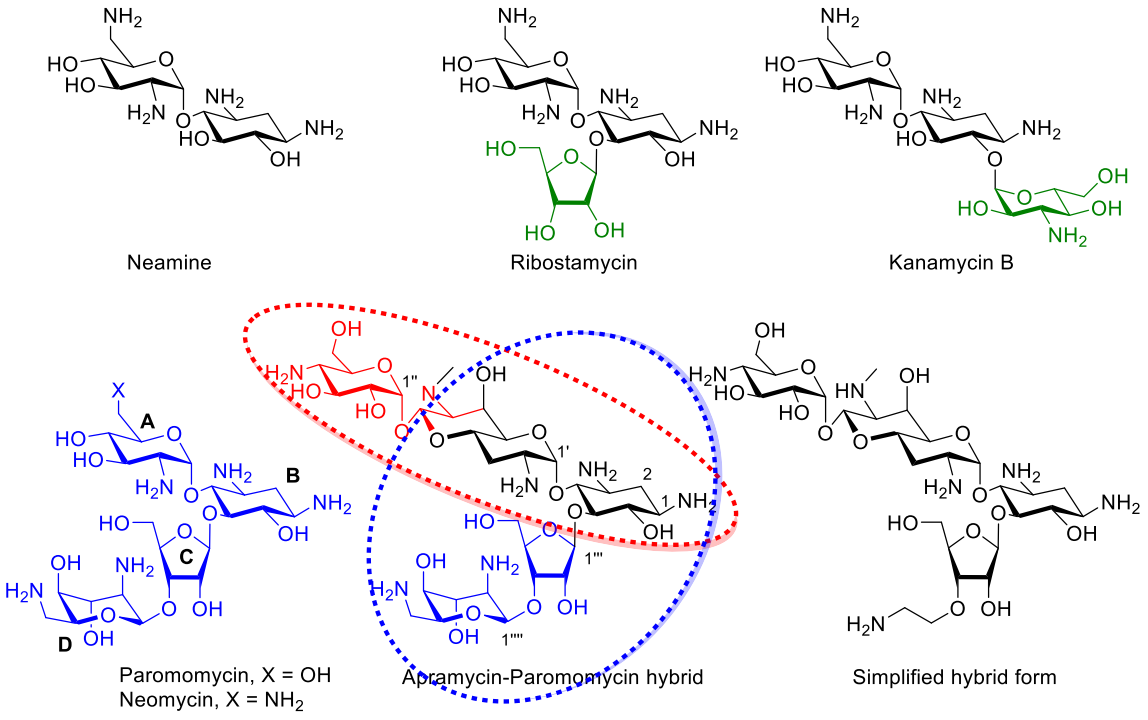


Figure 28. **Paramomycin structure showing rings A-D, its hybrid with apramycin and a simplified form of the hybrid.**

***Table 5.* %Inhibition of *in vitro* R17 phage RNA-directed polypeptide synthesis by various aminoglycoside antibiotics at four concentrations.**

	Drug concentration (μg/ml)			
Antibiotic	0.1	1	10	50
Neamine	2	6	37	75
Ribostamycin	5	45	65	92
Kanamycin B	22	48	58	82
Neomycin	28	55	80	96

***Table 6. In vivo* minimal inhibitory concentrations (MIC, μg/ml) of clinical isolates.**

IN VIVO (MIC, μg/ml)	*E coli* **NIHJ**	*P. aeruginosa* **A9930**	*K. pneumoniae* **D11**
Apramycin	3.1	3.1	0.8
5-*O*-(β-D-ribofuranosyl) apramycin **(98)**	1.6	0.8	0.8
6-*O*-(3-amino-deoxy-α-D-glucopyranosyl) apramycin **(99)**	1.6	1.6	0.4

While this work was underway, Fridman and coworkers described the ribosylation of the 4,6-AGAs at the 5-position to give 4,5,6-trisubstitued AGAs (Figure 29).[92] The resulting compounds were slightly less active than the parents, but apparently gave improved ribosomal selectivity in the 5-*O*-ribosylated kanamycin B (X = OH, Y = NH_2, Z = H) and tobramycin (X = H, Y = NH_2, Z = H) series, both of which contain 2′-amines. The 2′-amine in these AG structures forms a hydrogen bond with the ribofuranose sugar ring oxygen,[154] which may assist in orienting the ribofuranose ring in the A-site. However, these compounds are still expected to have the ArmA resistance problem of 4,6 AGAs.

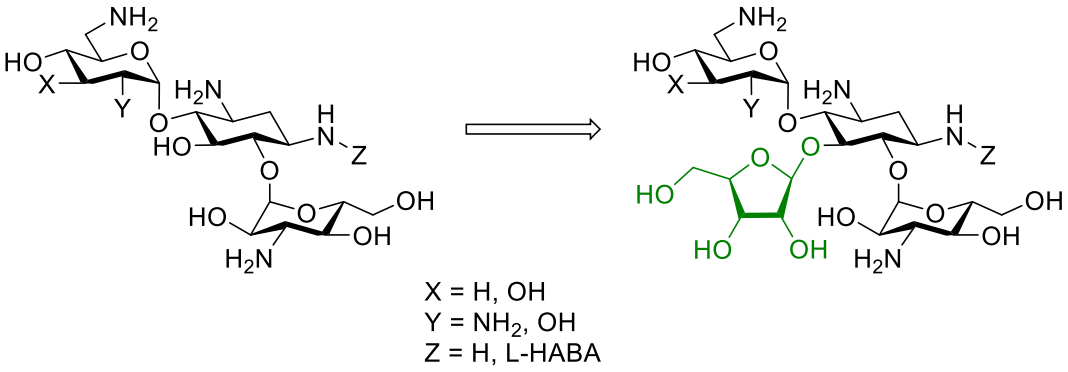
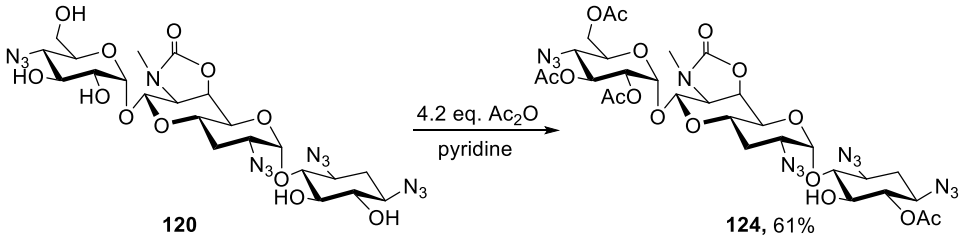


Figure 29. **A Schematic diagram showing the 5-ribosylation of the 4,6 AGAs**

3.4. Synthesis of apramycin derivatives

3.4.1. Synthesis of a key apramycin intermediate

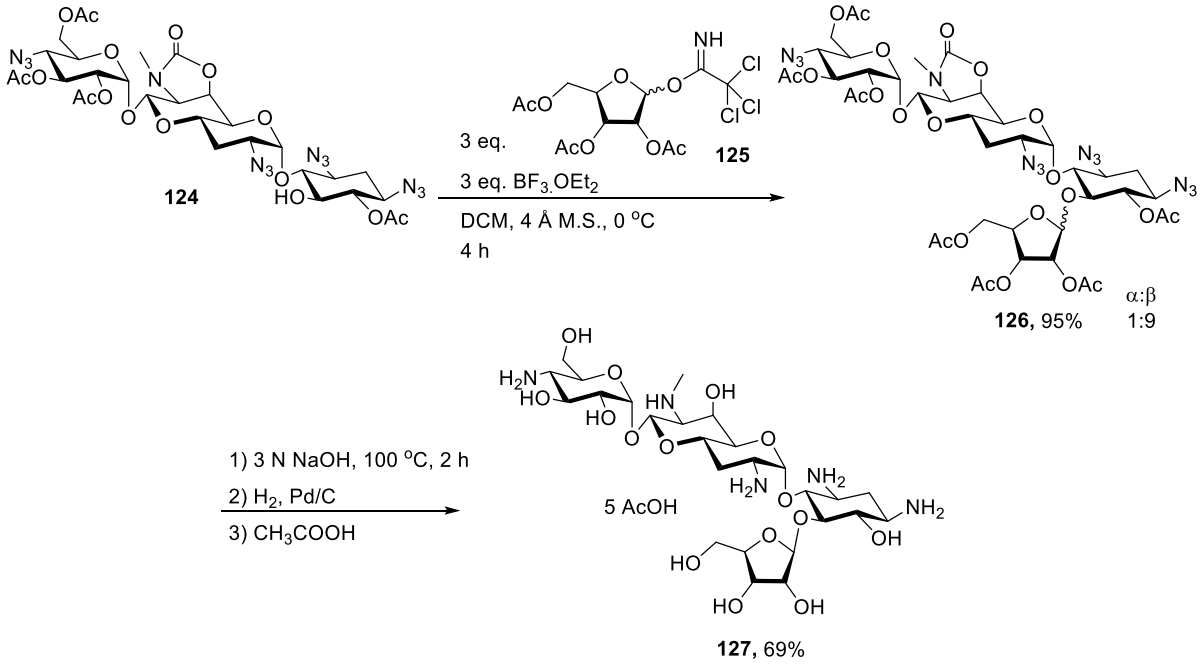
The synthesis of the apramycin derivatives started by preparing the key intermediate **124**, which has all functionality blocked except for the crucial 5-hydroxyl group. The apramycin primary amines were protected as azides and both the N7' amino and 6-hydroxyl groups were tied up in an oxazolidinone ring as described previously.[82] Consistent with previous reports on the regioselective acetylation of neamine,[155-156] treatment of **120** with a controlled amount of acetic anhydride in pyridine then gave the 6, 2'',3''-6''-tetra-*O*-acetate **124** in 61% yield (Scheme 26). This method, which gives access to a simple 5-hydroxy apramycin derivative in 4 steps and 26% overall yield, is a considerable improvement over the earlier chemistry outlined in (Schemes 18 and 19).



Scheme 26. **Synthesis of key apramycin intermediate 124.**

3.4.2. Synthesis of 5-*O*-β-ribofuranosyl apramycin, 5-*O*-β-paromobiosyl apramycin and 5-*O*-β-[3-*O*-(2-aminoethyl) ribofuranosyl] apramycin

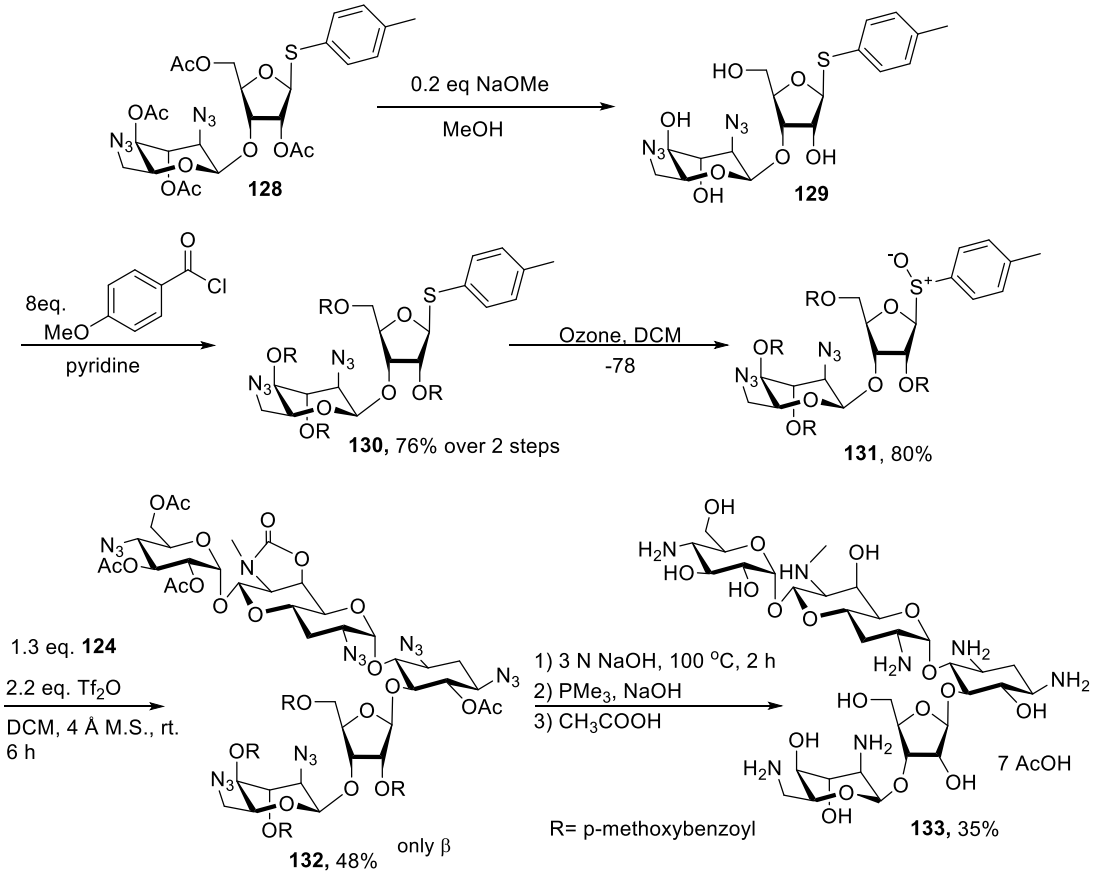
Glycosylation of **124** with 2,3,5-tri-*O*-acetyl-α,β-D-ribofuranosyl trichloroacetimidate[157] **125** with promotion by $BF_3.OEt_2$ afforded the glycoside **126** in 95% yield as a separable anomeric mixture (α:β = 1:9). Deprotection of the β-anomer by saponification then hydrogenolysis allowed the formation of **127** (Scheme 27).



Scheme 27. **Synthesis of 5-*O*-β-ribofuranosyl apramycin 127.**

Treatment of pentaazido per-*O*-acetylparomomycin[158] with *p*-thiocresol and $BF_3.OEt_2$, as described for neomycin B,[159-160] gave a 43% yield of the parombiosyl thioglycoside **128**. Complications of anomer separation following glycosylations of **124** with **128** prompted its conversion to the 4-methoxybenzoate **130**, after which oxidation with ozone gave the disaccharyl sulfoxide **131** (Scheme 28). Triflic anhydride activation[161-162] of **131** and exposure to **124** then afforded the anticipated saccharide **132** in 48% yield as a single β-anomer. Finally, saponification of the carbamate and ester groups followed by Staudinger reduction of the azides

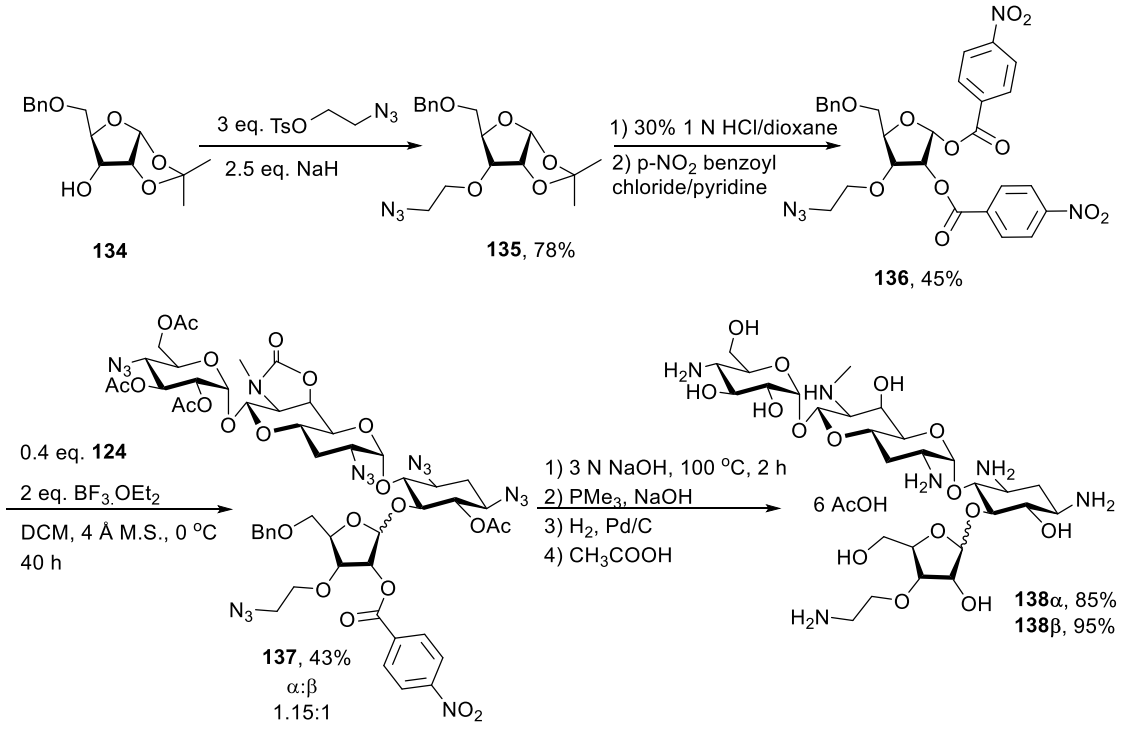
gave the apramycin-paromomycin chimera **133** in 35% yield, which was isolated in the form of the peracetate salt (Scheme 28).



Scheme 28. **Synthesis of 5-*O*-β-paromobiosyl apramycin 133.**

Preparation of the 3-*O*-(2-azidoethyl) ribofuranosyl donor **136** from 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose **134** was achieved by alkylation with 2-azidoethyl tosylate[163] to give **135**. Acidic hydrolysis of the acetonide, *p*-nitrobenzoylation gave the donor **136** in 45% yield. This is a considerable improvement over an earlier synthesis of the related 3-*O*-(2-azidoethyl) ribofuranosyl groups reported by the Wong group.[164] Activation of **136** with $BF_3.OEt_2$ in the presence of **124** gave a separable anomeric mixture (α:β = 1.15:1) of the glycosides **137** in 43% yield. Deprotection of individual isomers resulted in the formation of

138α and **138β**, both of which were isolated as the peracetate salts in excellent yields (Scheme 29).



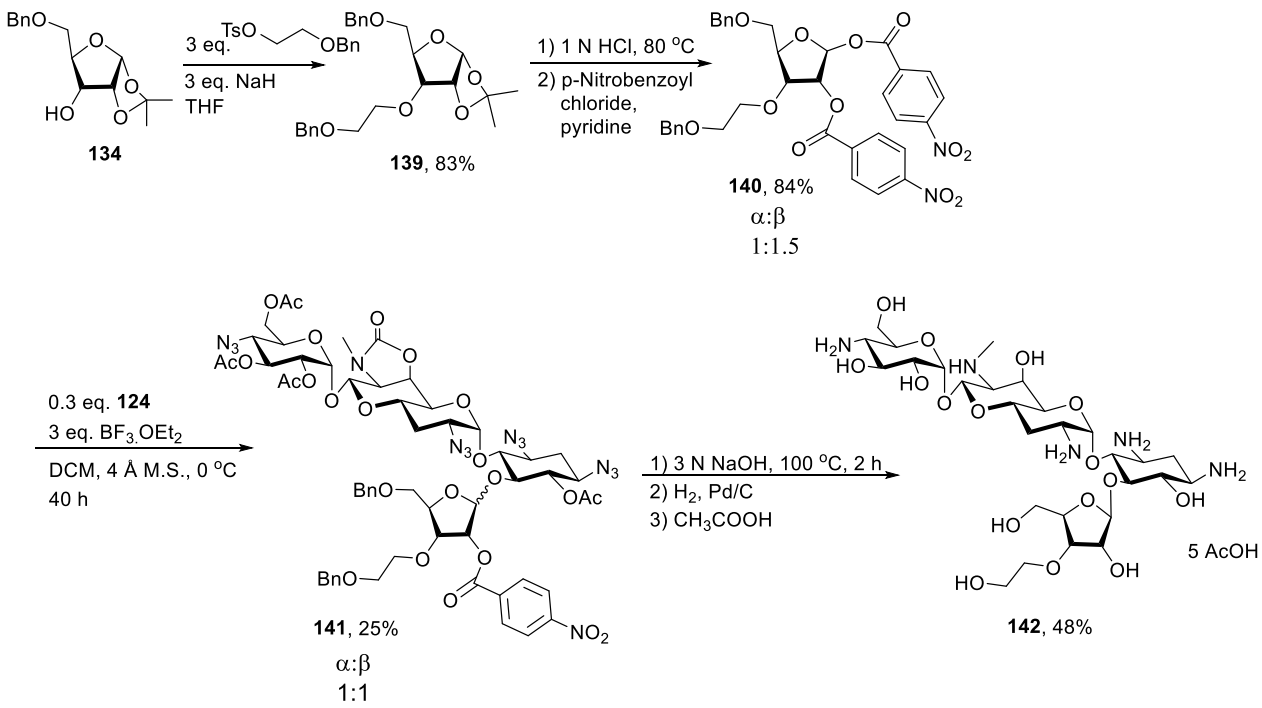
Scheme 29. **Synthesis of compounds 138α and 138β.**

The synthesized compounds **127**, **133**, **138α** and **138β** were screened for the antiribosomal and antibacterial activities. Compared to apramycin, the 5-*O*-β-D-ribofuranosyl apramycin **127** showed moderately increased antiribosomal activity and especially selectivity over the mitoribosome (Table 7), but reduced antibacterial activity against all strains tested (Table 8). On the other hand, the 5-*O*-paromobiosyl apramycin **133** shows better antiribosomal activity and better antibacterial activity towards *MRSA* and *E coli*. Unfortunately, **133** has less mitoribosomal selectivity and less activity towards *P aeruginosa*. 5-*O*-[3-(2-Aminoethyl)-β-D-ribofuranosyl] apramycin **(138β)** shows an enhanced antiribosomal activity and mitoribosomal selectivity with comparable antibacterial activity towards *MRSA* and *E coli* but is less active towards *P aeruginosa*. In addition, both compounds **133** and **138β** were active towards AAC(3)

containing bacteria, that inactivate apramycin by acetylation at the 3-position. Biological results will be discussed in detail in Section 3.5. In view of the increased activity of paromobiosyl and aminoethylribosyl apramycin compared to the parent compound, and their ability to overcome AAC(3) AME resistance, more derivatives were explored and evaluated.

3.4.3 Synthesis of 5-*O*-β-[3-*O*-(2-hydroxyethyl) ribofuranosyl] apramycin

Two hypotheses were prompted by the observation that 5-[3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **138β** shows activity towards AAC(3) containing bacteria: i) the aminoethyl group was bulky enough to block the AAC(3) enzyme's active site or ii) the increased affinity for the ribosome resulting from the addition of the extra amine outweighs the reduction in activity due to N3 acetylation by the AME. To differentiate between these hypotheses, a similar compound was synthesized in which a hydroxyethyl side chain replaced the aminoethyl group of **138β**. The preparation of compound **142** started by alkylation of the reported intermediate **134**[165] with 2-(benzyloxy)ethyl tosylate[166] to afford the compound **139** in 83% yield (Scheme 30). Acetonide cleavage then *p*-nitrobenzoylation of intermediate **139** enabled the preparation of the donor **140**, which was glycosylated with **124** to allow the formation of the ribofuranoside product **141** in 25% yield as an anomeric mixture (α:β = 1:1). Finally, deprotection of the β-isomer allowed the formation of the target compound **142**. When this compound was screened for activity against AAC(3) containing bacteria (Table 9) it showed a loss in activity, which supports the hypothesis that the amino group of **138β** is essential and plays role in increasing the overall activity of the aminoglycoside. Biological results will be discussed in detail in Section 3.5.



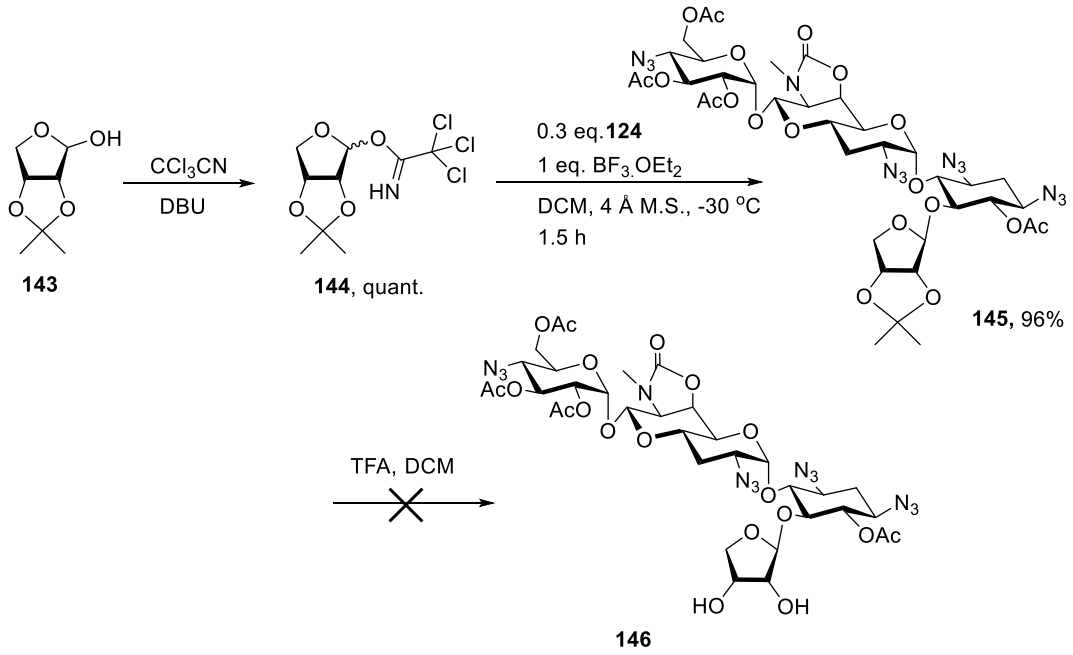
Scheme 30. Synthesis of compound 142.

3.4.4. Modification of the 5''-hydroxyl group of 5-*O*-β-ribofuranosyl apramycin and 5-*O*-β-[3-*O*-(2-aminoethyl) ribofuranosyl] apramycin

The presence of the ribosyl 5-hydroxyl group in compounds **127** and **138β** renders them prone to antibacterial resistance due to the action of the APH(3',5'') AMEs. Previous modifications to the ribosyl 5-hydroxyl group in the 4,5-aminoglycoside series carried out with a view to circumventing this AME include deoxygenation accompanied with cyclization with the 2'-amine[167] or substitution by a halogen atom.[168] However, these modifications were reported to reduce antibacterial activity substantially and so were not considered useful. Therefore, two alternative approaches to the solution of this problem were developed. Thus, it was decided i) to explore the effect of removing the offending CH_2OH group from the ribosyl ring altogether, and ii) to substitute a formamido group for the 5-hydroxyl group in the ribose ring. Consequently, a compound analogous to the 5-*O*-β-ribofuranosyl apramycin **127** lacking the 5''-hydroxymethyl group (5-*O*-β-D-erythrofuranosyl apramycin) **152** was synthesized and screened. The 5-*O*- (5"-

formamido-5"-deoxy-β-D-ribofuranosyl) and the 5-*O*-[3-*O*-(2-aminoethyl)-5-deoxy-5-formamido-β−D-ribofuranosyl] apramycin derivatives **157** and **166**, in both of which the primary hydroxyl group of the ribose ring was substituted with a formamido group, also were prepared and screened.

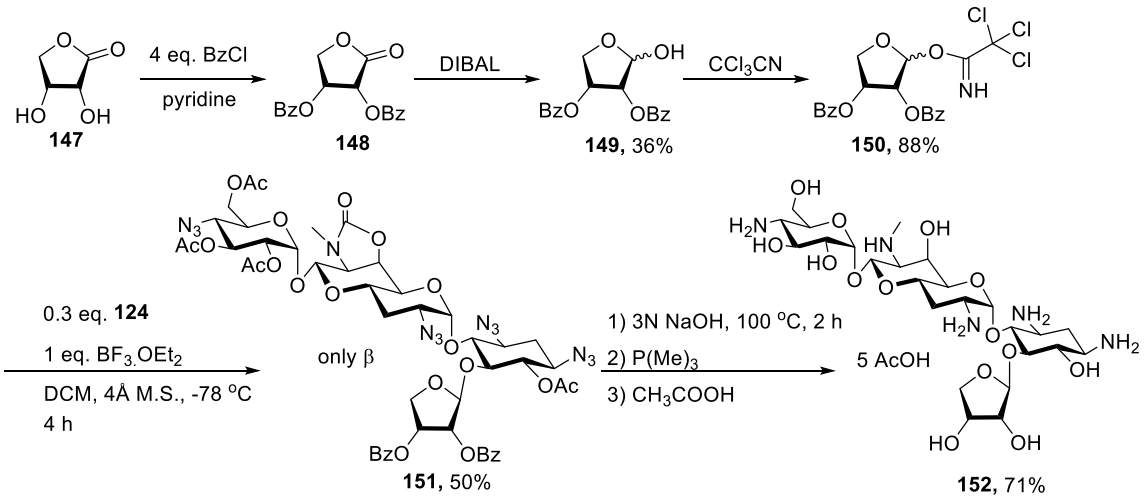
To synthesize the erythrofuranose derivative, 2,3-*O*-isopropylidene-β-D-erythrofuranose[169] **143** was treated with trichloroacetonitrile in basic conditions to give the trichloroacetimidate derivative **144,** which was glycosylated with **124** to allow the formation of the ribofuranoside product **145** in 96% yield as a single β anomer. Unfortunately, all attempts to cleave the acetonide from the furanose sugar, ended up cleaving the furanose ring altogether (Scheme 31)



Scheme 31. **Synthesis of compound 145.**

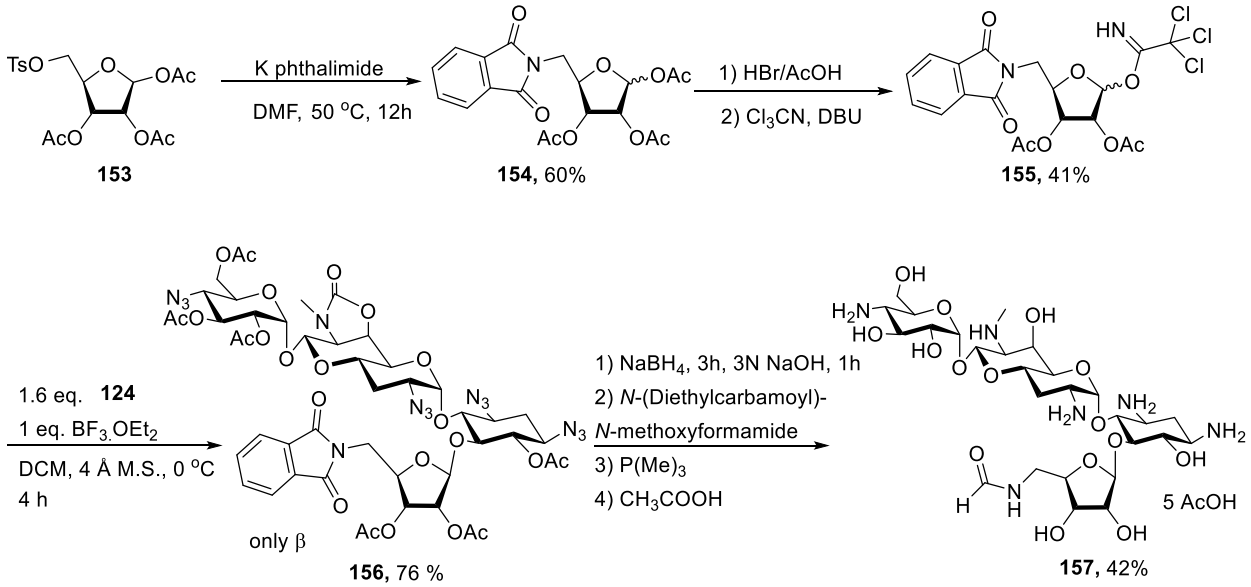
Alternatively, erythrolactone[170] **147** was benzoylated followed by selective DIBAL reduction of the lactone to give **149**. The product was treated with trichloroacetonitrile in basic conditions to give the trichloroacetimidate derivative **150** (Scheme 32). This donor was then

activated with $BF_3.OEt_2$ in the presence of the apramycin acceptor **124** to afford the 5-*O*-β-D-erythrofuranoside **151** in 50% yield as a single β anomer. Deprotection of **151** gave 5-eythrofuranosyl apramycin **152** in 71% yield as its peracetate salt.



Scheme 32. **Synthesis of compound 152.**

The preparation of 5-*O*-(5-deoxy-5-formamido-β-D-ribofuranosyl) apramycin **157** started with the synthesis of 1,2,3-tri-*O*-acetyl-5-*O*-p-tolylsulfonyl-D-ribofuranose **153** following the reported procedure.[171] Subsequent reaction with potassium phthalimide gave intermediate **154**. Acidic treatment cleaved the anomeric acetate for conversion to trichloroacetimidate **155** using trichloroacetonitrile (Scheme 33). Glycosylation of **155** with **124** allowed the formation of the β-anomer of the 5-phthalimido ribofuranoside **156** in 76% yield. Partial deprotection using $NaBH_4$ freed the amino group from the phthalimide and also liberated the 7'-amino group from the oxazolidinone. This was followed by selective formylation at the 5'''-amine with *N*-(diethylcarbamoyl)-*N*-methoxyformamide.[172] Reduction of the remaining azides to amines enabled the preparation of the desired compound **157** as a pentaacetate salt in 42% yield.

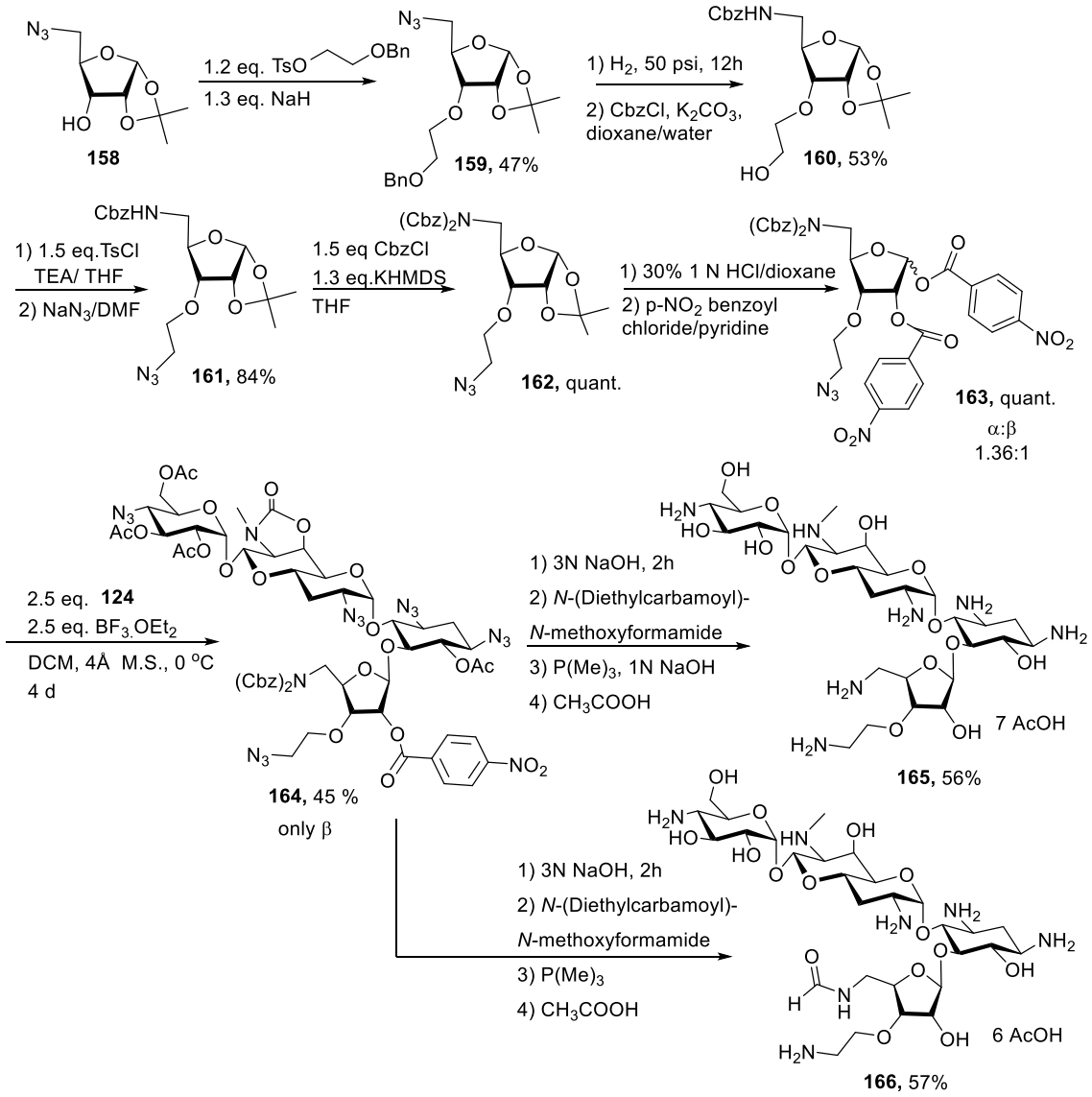


Scheme 33. Synthesis of compound 157.

The synthesis of compound **166** began with the preparation of the reported compound **158**,[173] which was alkylated with 2-(benzyloxy)ethyl tosylate to give **159** (Scheme 34). Subsequent hydrogenolysis converted the azide to an amine and the benzyl ether to a hydroxyl group, and was followed by Cbz protection of the amine to give compound **160**. Conversion of the free hydroxyl group to an azide was done by tosylation then substitution with sodium azide to give the intermediate **161** in 84% yield.

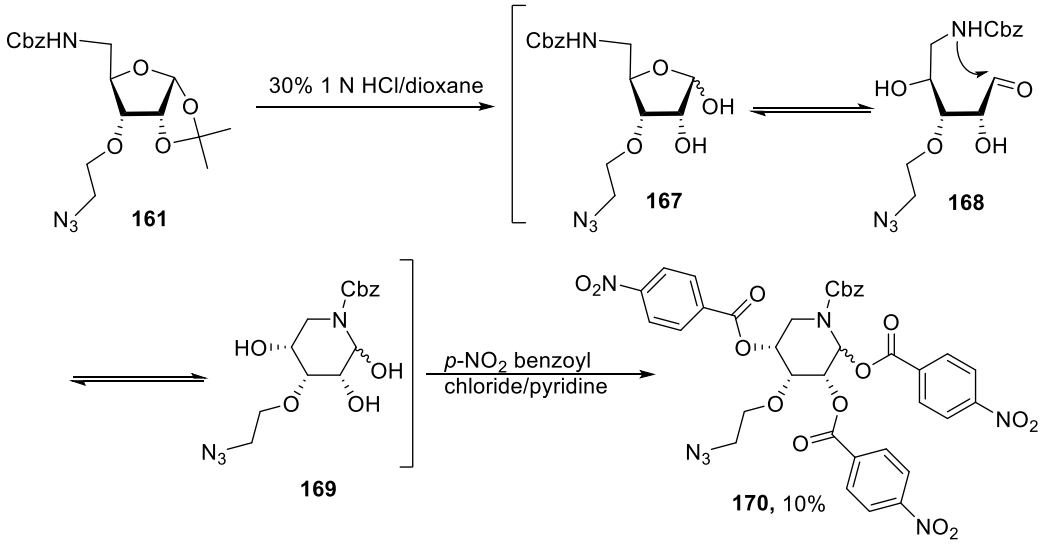
The NHCbz group was then converted to an imide **162** by introduction of a second Cbz group using CbzCl and KHMDS. This *N*,*N*-di-Cbz moiety is functionally analogous to the phthalimido group but affords greater versatility in deprotection. Acidic hydrolysis of the acetonide followed by *p*-nitrobenzoylation then gave the donor **163** in quantitative yield, which was employed to glycosylate **124** giving **164** in 45% yield as a single β-anomer. Partial deprotection of **164** under basic conditions freed the ribosyl amine as well as the 7'-apramycin secondary amine and was followed by selective formylation of the ribosyl amine. Reduction of the azide using the Staudinger reaction in 1 N NaOH unfortunately cleaved the formamide and

provided 5-*O*-[5-amino-3-*O*-(2-aminoethyl)-5-deoxy-β-D-ribofuranosyl] apramycin **165**. The cleavage of the formamide was avoided by conducting the Staudinger reaction under neutral conditions when the desired product **166** was obtained in 57% yield as the peracetate salt (Scheme 34). The ^{1}H-NMR spectrum of compound **166** showed two rotamers about the formamide N-CHO bond in a ratio of 9:1. NMR analysis of the major rotamer showed a $^{3}J_{H\text{-}5'',C=O}$ = 3.1 and $^{2}J_{CHO,C=O}$ = 197.1, which are in agreement with it being the expected trans-rotamer.[174-175]



Scheme 34. **Synthesis of the compounds 165 and 166**

In an attempt to cleave the acetonide of **161** followed by *p*-nitrobenzoylation of the resulting diol**,** complications arose from the formation of a pyranose side product **170** due to ring closure of the 6-amino group with the aldose moiety of **168** (Scheme 35).[176] This problem was solved by introduction of a second Cbz onto the amine to give the imide **162** as presented above in (Scheme 34).



Scheme 35. **The formation of the pyranose side product 170**

Compared to 5-*O*-β-ribofuranosyl apramycin **127**, both the dehydroxmethyl analog, 5-*O*-(β-D-erythrofuranosyl) apramycin **152**, and its 5’’’-formamido analog, 5-*O*-(5-deoxy-5-formamido-β-D-ribofuranosyl) apramycin **157**, were more active towards *MRSA* and *P aeruginosa*, suggesting that the presence of an APH (3’,5’’) resistance determinant may at least in part be responsible for the poor activity of the simple ribofuranosyl derivative **127** (Tables 8 and 9). Similarly, when compared to 5-*O*-[3-*O*-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **138β**, the 5-amino-5-deoxy-ribosyl analog **165** and 5-deoxy-5-formamido ribosyl analog **166** showed a higher activity in the presence of the APH(3’,5’’) AME (Table 9). In addition, both of these compounds, but especially **165** showed i) potent antibacterial activity exceeding that of

apramycin in all strains and ii) better ribosomal selectivity. Biological results will be discussed in detail in Section 3.5.

3.4.5. Synthesis of 6-*O*-propyl apramycin, 6-*O*-(2,3-dihydroxypropyl) apramycin and 6-*O*-(2-hydroxyethyl) apramycin

Introduction of some simpler modifications at the 5-position of apramycin was attempted. Thus, compound **124** was allylated using allyl bromide and a base, however the 6-*O*-allyl derivative **172** was obtained instead of the desired 5-*O*-allyl isomer. Clearly, under the basic conditions the 6-*O*-acetyl group migrated to the adjacent 5-hydroxyl group drive the allylation reaction toward the more reactive 6-hydroxy group following the Curtin-Hammett principle (Figure 30).[177-178] Different bases were tried and but even under the mild silver oxide conditions the 6-*O*-allyl derivative **172** was separated in 59% yield.

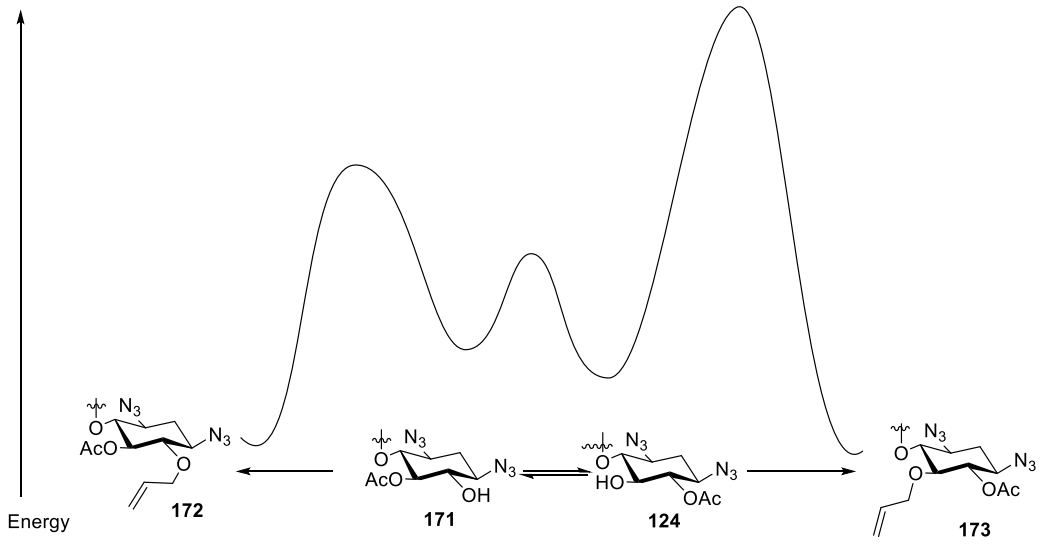
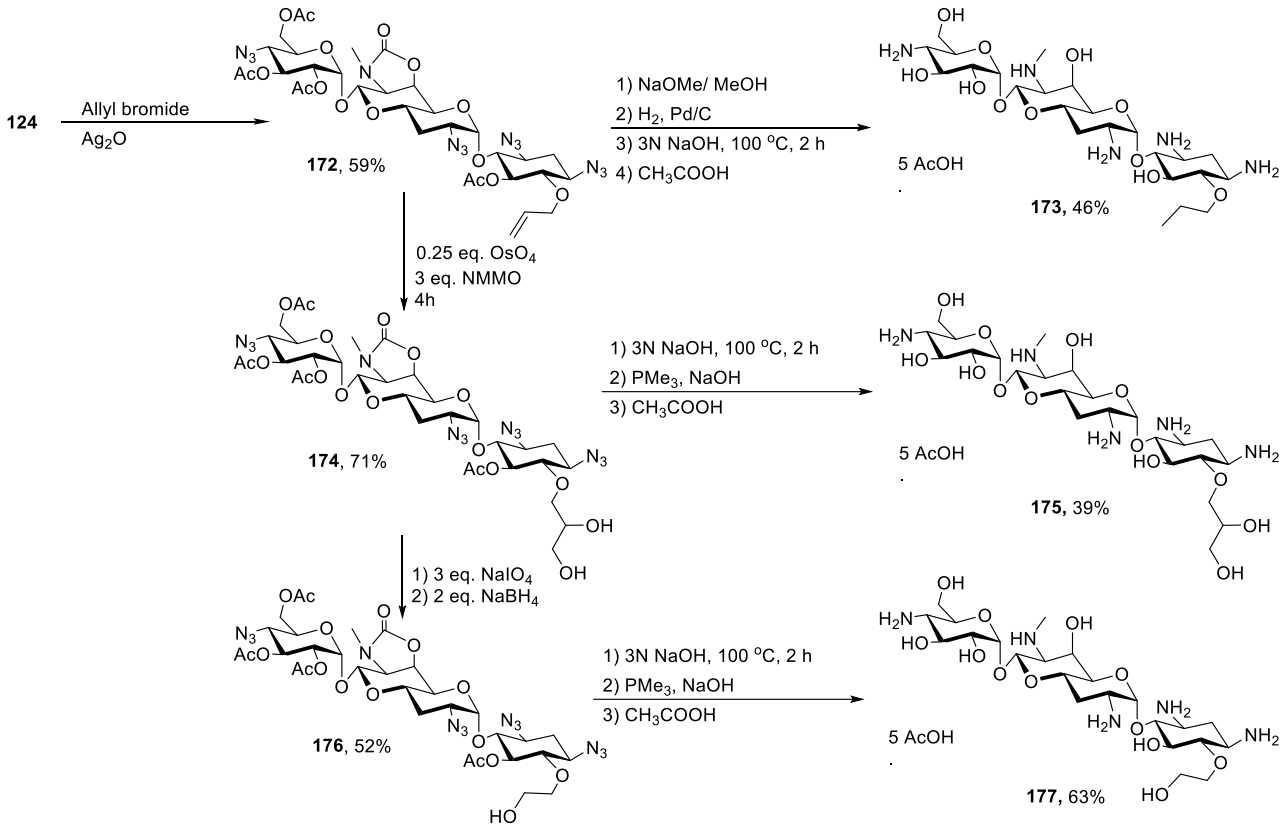


Figure 30. **Reaction co-ordinate diagram showing the formation of 6-*O*-allyl apramycin derivative 172 under Curtin-Hammett control**

Nevertheless, compound **172** was deprotected to give the 6-*O*-propyl apramycin derivative **173** (Scheme 36). In addition, dihydroxylation of intermediate **172** by OsO_4 gave the dihydroxylated intermediate **174** in 71% yield, which was either deprotected to give 6-*O*-(2,3-

dihydroxypropyl) apramycin **175** or exposed to oxidative cleavage then reduction before deprotection to allow the formation of 6-*O*-(2-hydroxyethyl) apramycin **177**. Biological screening of **173**, **175** and **177** didn't show any advantage over the parent apramycin (Tables 7 and 8).

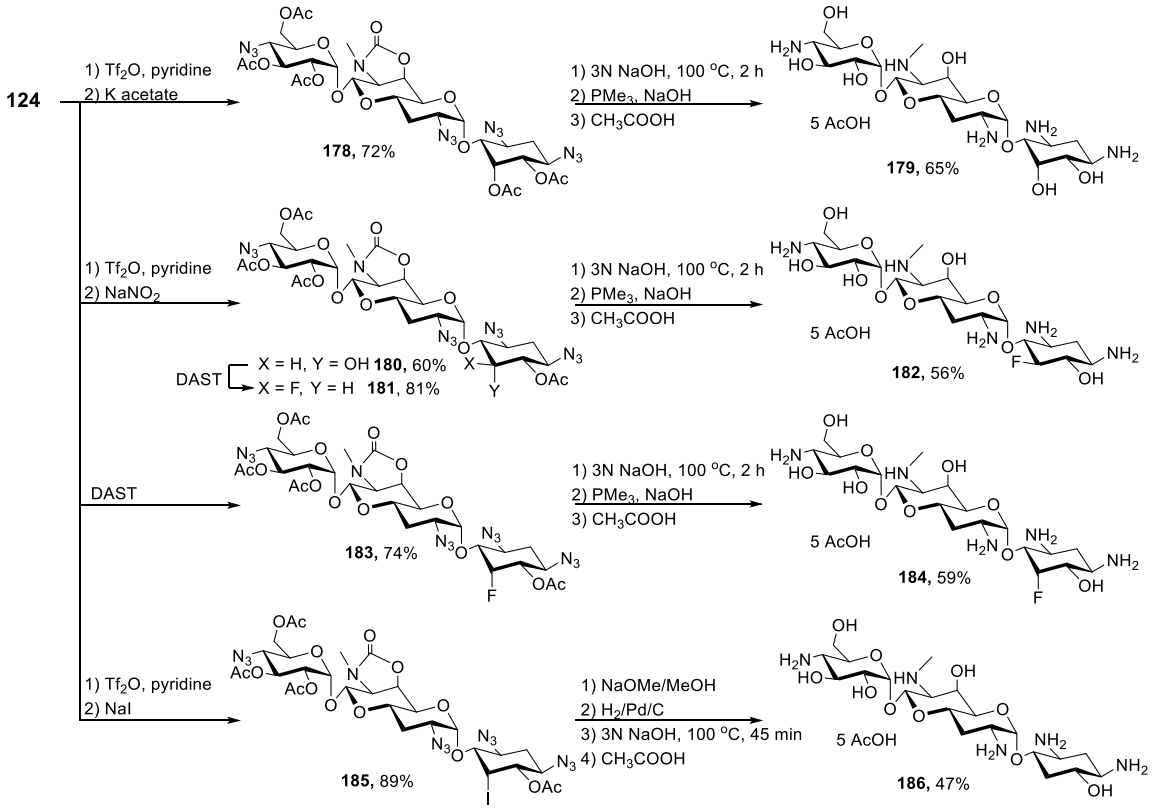


Scheme 36. **Synthesis of compounds 173, 175 and 177.**

3.4.6. Synthesis of 5-epi-apramycin, 5-deoxy-5-fluoro apramycin, 5-deoxy-5-epi-fluoro-apramycin and 5-deoxy apramycin

A previous report showed that the 5-hydroxyl group is not essential for activity, and that removing it even increases antibacterial activity,[145] suggesting further exploration of this position. Thus, inversion of configuration at the 5-position and replacement of the 5-hydroxy group with fluoride in both configurations were explored. Moreover, 5-deoxyapramycin was also

synthesized as a comparator. To synthesize 5-epi-apramycin **179**, compound **124** was triflated at the 5-position and the triflate displaced by potassium acetate to afford the inverted acetate intermediate **178** (Scheme 37). Basic hydrolysis followed by Staudinger reaction of the intermediate **178** enabled the preparation of compound **179** in 65% yield as the pentaacetate salt. Preparation of 5-deoxy-5-fluoro apramycin **182** started by triflation and was followed by substitution with sodium nitrite, which upon workup gave the inverted hydroxyl group intermediate **180**.[179] Subsequent treatment with diethylaminosulfur trifluoride (DAST) substituted the hydroxyl group with fluoride with inversion of configuration giving **181** in 81% yield. Deprotection of **181** by basic hydrolysis of the esters and subsequent Staudinger reaction enabled the preparation of **182** in 58% yield. The 5-deoxy-5-epi-fluoro apramycin derivative **183** was accessed from **124** in 74% yield by displacement of the 5-hydroxyl group with fluoride using DAST. Global deprotection of **183** gave the 5-deoxy-5-epi-fluoro apramycin pentaacetate salt **184** in 59% yield. Finally, 5-deoxyapramycin was synthesized by the initial displacement of the 5-hydroxyl group of **124** with iodide via the triflate intermediate giving **185**. This was followed by deacetylation and parallel hydrogenolysis of the azides and of the C-I bond. Basic hydrolysis of the oxazolidinone ring gave **186**, which was isolated as the peracetate salt in 47% yield. Biological screening (Table 7 and Table 8) of 5-deoxyapramycin was consistent with the previous report.[145] All compounds tested in this series (5-epi-OH, 5-fluoro, 5-epi-fluoro and 5-deoxy) showed better antiribosomal activity than apramycin. Derivatives 5-epi apramycin **179**, 5-fluoro-5-deoxy apramycin **182**, 5-epifluoro apramycin **184** and 5-deoxy apramycin **186** had better antibacterial effect against *MRSA* and *E. coli* strains and comparable antibacterial effects toward *P aeruginosa* compared to the parent compound (Table 7 and Table 8). Biological results will be discussed in detail in Section 3.5.

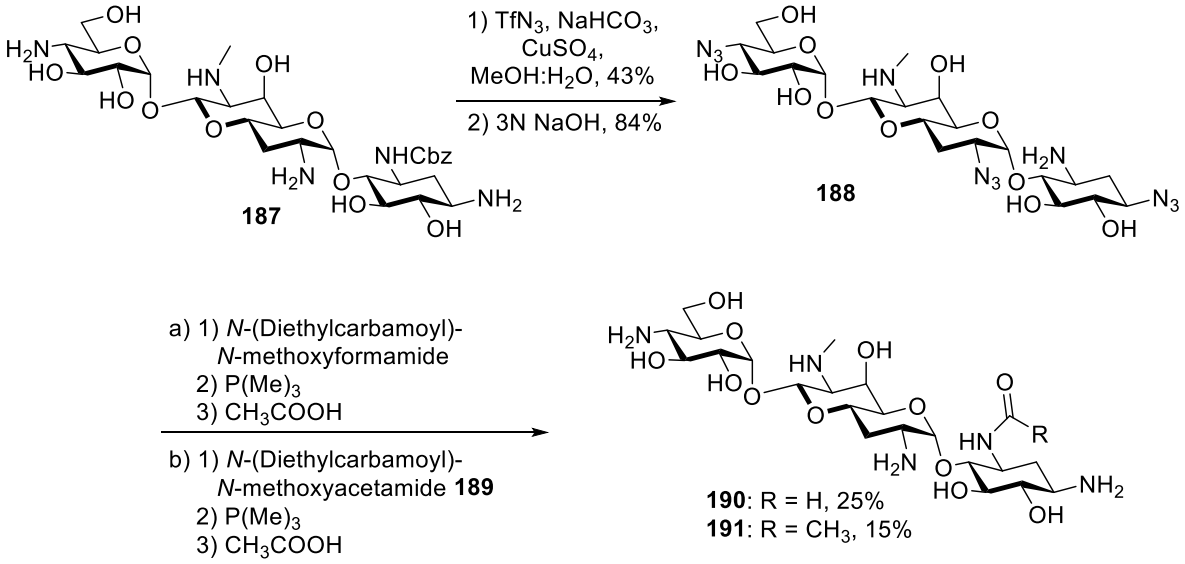


Scheme 37. **Synthesis of compounds 179, 182, 184 and 186.**

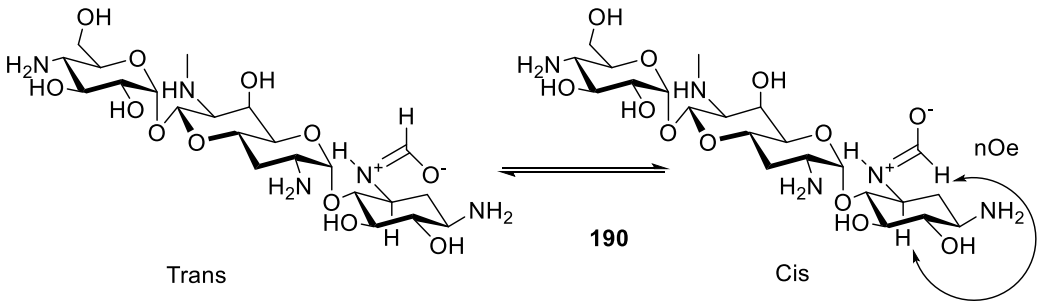
3.4.7. Synthesis of 3-*N*-formyl apramycin and 3-*N*-acetyl apramycin

The only known AME that affects apramycin is AAC(3)-IV that acetylates the 3-amino group of apramycin.[57] As formamido groups are tolerated at other positions on aminoglycoside skeletons when acetamido groups are not,[180] it was possible that installation of a formamide group at the 3-amino group of apramycin would block the action of AAC(3)-IV but not compromise the antibacterial activity. Consequently, 3-*N*-formyl apramycin was synthesized together with 3-*N*-acetyl apramycin that was needed as a comparison standard. The synthesis of these derivatives began with the preparation of the compound **187** using copper-based selective acylation as reported.[152] Subsequently, the remaining primary amines were protected as azides and the 3-amino group was freed in basic conditions to afford the compound **188**. Selective formylation at the 3-position was achieved with using *N*-(diethylcarbamoyl)-*N*-

methoxyformamide.[172] Reduction of the remaining azides to amines enabled the preparation of the desired compound **190**. In a similar way, 3-amino group of **188** was acetylated using *N*-(diethylcarbamoyl)-*N*-methoxyacetamide **189** and the azides were reduced to afford compound **191** (Scheme 38). Interestingly, the ^{1}H-NMR spectra of 3-*N*-formyl apramycin shows two rotamers in 4:3 ratio favoring the trans-rotamer, with the assignment-based on an nOe spectrum of **190**, which showed clear enhancement of the resonances for H-3 and CHO in the cis-form but not of H-3 on irradiation of the formyl proton in the trans-rotomer (Scheme 39). The biological data for compounds **190** and **191** showed complete loss of antibacterial activity (Table 7 and Table 8) thereby negating the hypothesis that a formamido group might be tolerated at the 3-position of apramycin.



Scheme 38. **Synthesis of compounds 190 and 191.**



Scheme 39. **Diagnostic nOe interactions in the cis and trans rotamers of 3-*N*-formyl apramycin**

3.5. Biological Evaluation

All the synthesized compounds were submitted to the Böttger lab in Zurich, where they were screened for antiribosomal activity (Table 7) and antibacterial activity against strains of *E. coli*, *MRSA* and *P aeruginosa* (Table 8). The methods were identical to the ones applied in the netilmicin series (Chapter 2). In addition, the MIC of the compounds were obtained against engineered *E. coli* strains with known AME resistance mechanisms.

Table 7. **Inhibition of wild-type bacterial and hybrid ribosomes (IC_{50}, μM).**

IN VITRO (IC_{50}, **μM**)	**Sms (μM)**	**Mit (μM)**	**A1555G (μM)**	**Cyt (μM)**
Apramycin **(9)**	0.17	124.8 (747)	51.5 (308)	108.7 (651)
Neomycin **(2)**	0.03	3.8 (127)	0.4 (14)	32.8 (1094)
5-*O*-(β-D-Ribofuranosyl) apramycin **(127)**	0.09	368.9 (4130)	272.8 (3054)	436.2 (4883)
5-*O*-(β-Paramobiosyl) apramycin **(133)**	0.13	2.1 (16)	0.5 (4)	29.7 (225)
5-*O*-[3-(2-Aminoethyl)-β-ribofuranosyl] apramycin **(138β)**	0.07	77.4 (1106)	16.9 (241)	202.6 (2896)
5-*O*-[3-(2-Aminoethyl)-α-ribofuranosyl] apramycin **(138α)**	0.35	192.4 (550)	284.8 (814)	257.2 (735)

5-*O*-[3-(2-Hydroxyethyl)-β-D-ribofuranosyl] apramycin **(142)**	0.31	904.0 (2945)	456.0 (1483)	1227.0 (3991)
5-*O*-(β-D-Erythrofuranosyl) apramycin **(152)**	0.12	312.2 (2504)	235.0 (1885)	448.6 (3598)
5-*O*-β-(5'''-Formamido-5'''-deoxy-D-ribofuranosyl) apramycin **(157)**	0.33	571.5 (1744)	263.2 (803)	>
5-*O*-[5-Deoxy-5-amino-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(165)**	0.04	48.0 (1143)	22 (524)	37.0 (881)
5-*O*-[5-Deoxy-5-formamido-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(166)**	0.09	93.0 (1094)	11.0 (129)	142.0 (1671)
6-*O*-Propyl apramycin **(173)**	2.49	448.7 (180)	828.5 (332)	646.0 (259)
6-*O*-(2,3-Dihydroxypropyl) apramycin **(175)**	0.57	403.1 (707)	565.3 (991)	462.4 (811)
6-*O*-(2-Hydroxyethyl) apramycin **(177)**	0.39	638.1 (1619)	388.6 (986)	425.34 (1079)
5-Epi apramycin **(179)**	0.11	39.9 (359)	17.8 (160)	25.5 (229)
5-Deoxy-5-fluoro-apramycin **(182)**	0.12	113.7 (978)	125.2 (1076)	136.6 (1175)
5-Deoxy-5-epifluoro-apramycin **(184)**	0.10	55.8 (559)	50.4 (506)	51.7 (519)
5-Deoxy apramycin **(186)**	0.11	86.5 (824)	67.4 (642)	80.2 (764)
3-*N*-Formyl apramycin **(190)**	69	> 2000	> 2000	> 2000
3-*N*-Acetyl apramycin **(191)**	n.d.	n.d.	n.d.	n.d.

Sms: *M. Smegmatis* ribosome, Cyt: *M. Smegmatis* ribosome with human cytosolic A-site, Mit: *M. Smegmatis* ribosome with human mitochondrial A-site, A1490G: *M. Smegmatis* ribosome with human mitochondrial A-site with A1555G mutation. Selectivities are obtained by dividing the hybrid ribosomal activity by *M. Smegmatis* ribosomal activity.

The inhibitory activity of apramycin against the bacterial ribosome is less than that of neomycin but much greater selectivities is displayed toward the wild-type mitochondrial and the A1555G mutant mitochondrial ribosomes, which is reflected in lower cochleotoxicity of apramycin compared to neomycin[75, 134] Compared to apramycin, the installation of a ribosyl

group at the 5-position, as in compound **127**, resulted in moderately increased antiribosomal activity (2-fold) and greater selectivity over the wild-type mitoribosome hybrid (5- to 6-fold) and the A1555G mutant mitoribosome hybrid (10-fold). On the other hand, the 5-*O*-paromobiosyl group shifted the activities of apramycin to resemble more those of neomycin: like neomycin, 5-*O*-paromobiosyl apramycin **133** exhibits strong antibacterioribosomal activity but very low mitoribosomal selectivity. 5-*O*-[3-(2-Aminoethyl)-β-D-ribofuranosyl] apramycin **138β** shows an enhanced antiribosomal activity (2 to 3-fold) and mitoribosomal selectivity in wild-type but not in the A1555G mutant hybrid. Both changing the anomeric configuration of the ribose ring as in compound **138α**, or subtituting the 2-aminoethyl group with hydroxyethyl group **142** had a detrimental effect on the ribosomal activity. On the other hand, the 5’’’-amine analog **165** of compound **138β** showed enhanced antibacterioribosomal activity (2-fold) and a comparable mitoribosomal selectivity when compared to the parent. Replacing the 5’’’-hydroxyl group of **138β** with a 5’’’-fomamido group as in compound **166** did not change much the ribosomal activity and selectivities. Compared to 5-*O*-β-ribofuranosyl apramycin **127**, both the dehydroxmethyl analog **152**, and its 5’’’-formamido analog **157**, exhibited reduced antibacterioribosomal activities and mitoribosomal selectivities.

Derivatives 5-epi apramycin **179,** 5-fluoro-5-deoxy apramycin **182**, 5-epifluoro apramycin **184** and 5-deoxy apramycin **186** showed better antiribosomal activity than apramycin. Enhancement of selectivity over the wild-type and mutant mitoribosomal were also obtained in case of 5-fluoro-5-deoxy apramycin **182** and 5-deoxy apramycin **186**.

All the compounds synthesized with substitutions at the 6-position and the 3-position abolish the ribosomal activity.

Table 8. **Minimal inhibitory concentrations (MIC, µg/mL) of clinical isolates.**

(MIC, **µg/ml**)	*MRSA*				*E coli*			*P aeruginosa*			
	AG038	**AG039**	**AG042**	**AG044**	**AG001**	**AG055**	**AG003**	**AG031**	**AG032**	**AG033**	**AG086**
Apramycin **(9)**	8	8	8	16	16	8	8-16	8	8	8	4
Neomycin **(2)**	0.5-1	128	128	0.5-1	----	4	4	32	32-64	≥128	≥128
5-*O*-(β-D-Ribofuranosyl) apramycin **(127)**	64	64	64	32-64	16	16-32	16	64	32-64	128	>128
5-*O*-(β-Paramobiosyl) apramycin **(133)**	2-4	2-4	2	2	4	8	4	64	64	64	≥128
5-*O*-[3-(2-Aminoethyl)-β-ribofuranosyl] apramycin **(138β)**	8-16	8	8	8-16	8	8	4-8	32	32	128	>128
5-*O*-[3-(2-Aminoethyl)-α-ribofuranosyl] apramycin **(138α)**	16	16	16	16	32	16-32	16-32	32-64	64	128	>128
5-*O*-[3-(2-Hydroxyethyl)-β-D-ribofuranosyl] apramycin **(142)**	32	32	32	n.d.	32	16	16	>32	>32	>32	n.d.
5-*O*-(β-D-Erythrofuranosyl) apramycin **(152)**	16-32	16-32	16	16	16	16	16	32	32	64-128	>128
5-*O*-β-(5'''-Formamido-5'''-deoxy-D-ribofuranosyl) apramycin **(157)**	32	16-32	32	n.d.	8	16	8	>32	>32	>32	n.d.
5-*O*-[5-Deoxy-5-amino-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(165)**	1	1	1	n.d.	2	2	1-2	2	2	2	n.d.
5-*O*-[5-Deoxy-5-formamido-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(166)**	4	4-8	4-8	n.d.	2	4	2	16	32	64	n.d.
6-*O*-Propyl apramycin **(173)**	≥128	≥128	64-128	128	64-128	64-128	64-128	>128	>128	>128	>128
6-*O*-(2,3-Dihydroxypropyl) apramycin **(175)**	64	64	64	64-128	32	32	32	64	64	≥128	≥128

6-*O*-(2-Hydroxyethyl) apramycin **(177)**	16-32	16-32	64	64	16	16	16	32	32	64-128	4-8
5-Epi apramycin **(179)**	4	4	2-4	4	4	4	4	4	4	8	32
5-Deoxy-5-fluoro-apramycin **(182)**	4-8	4-8	8	n.d.	8	4	8	8-16	16	32	n.d.
5-Deoxy-5-epifluoro-apramycin **(184)**	2-4	2-4	4	n.d.	8	4	4	8-16	8-16	32	n.d.
5-Deoxy apramycin **(186)**	2-4	2	4	n.d.	4	4	4	8	8	16	n.d.
3-*N*-Formyl apramycin **(190)**	>64	n.d.	n.d.	n.d.	>64	n.d.	n.d.	>64	n.d.	n.d.	n.d.
3-*N*-Acetyl apramycin **(191)**	>64	>32	>32	n.d.	>32	>32	>32	>32	>32	>32	n.d.

MIC (μg/mL) of apramycin derivatives to inhibit *Sms* (*Mycobacterium smegmatis*), *MRSA* (Methicillin-resistant *Staphylococcus aureus*), *E coli* and *P aeruginosa*.

Compared to apramycin, the 5-*O*-β-D-ribofuranosyl apramycin **127** showed a reduced antibacterial activity against all strains tested (Table 8). On the other hand, the 5-*O*-paromobiosyl apramycin **133** shows an increase in antibacterial activity towards *MRSA* and *E coli* but a reduced activity towards *P aeruginosa*. 5-*O*-[3-(2-Aminoethyl)-β-D-ribofuranosyl] apramycin **138β** exhibits comparable antibacterial activity towards *MRSA* and *E coli* but is less active towards *P aeruginosa*. Both the dehydroxmethyl analog, 5-*O*-(β-D-erythrofuranosyl) apramycin **152**, and the 5''-formamido analog, 5-*O*-(5''-deoxy-5'''-formamido- β-D-ribofuranosyl) apramycin **157**, were more active than their ribosyl congener towards *MRSA* and *P aeruginosa*, suggesting that the presence of an APH (3',5'') resistance determinant may at least in part be responsible for the poor activity of the simple ribofuranosyl derivative **127**. Similarly, when compared to 5-*O*-[3-*O*-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **138β**, the 5-amino-5-deoxy-ribosyl analog **165** and 5-deoxy-5-formamido ribosyl analog **166** showed a higher activity towards *MRSA* and *E coli*.

Compared to apramycin, 5-epi apramycin **179,** 5-fluoro-5-deoxy apramycin **182**, 5-

epifluoro apramycin **184** and 5-deoxy apramycin **186** had better antibacterial effects against *MRSA* and *E. coli* strains and comparable antibacterial effects toward *P aeruginosa*. This highlights the fact that a 5-hydroxy group is not necessary for activity and that its removal or substitution may be beneficial.

All the compounds synthesized with the substitution at the 6-position (6-*O*-propyl **173**, 6-*O*-(2,3-dihydroxypropyl) **175** and 6-*O*-(2-hydroxyethyl) **177**) were less active in all tested bacterial strains. Similarly, 3-*N*-formyl apramycin **190** and 3-*N*-acetyl apramycin **191** showed greatly reduced activity.

Table 9. **Minimal inhibitory concentrations (MIC, μg/mL) of engineered *E coli* strains carrying known AMEs**

(MIC, **μg/ml**)	*E coli*				
	AG173	**AG163**	**AG166**	**AG182**	**AG037**
	AAC(3)-IV	**APH(3')-Ia**	**APH(3')-IIa**	**AAC(3)-IV APH(3')-Ia**	**APH(3')-IIIa**
Apramycin **(9)**	256	4	4-8	>128	1
Neomycin **(2)**	n.d.	>64	>64	n.d.	>64
5-*O*-(β-D-Ribofuranosyl) apramycin **(127)**	16-32	8-16	8	64	4
5-*O*-(β-Paramobiosyl) apramycin **(133)**	1-2	8	2	64	1
5-*O*-[3-(2-Aminoethyl)-β-ribofuranosyl] apramycin **(138β)**	4	4-8	2	16	0.5
5-*O*-[3-(2-Aminoethyl)-α-ribofuranosyl] apramycin **(138α)**	n.d.	n.d.	n.d.	n.d.	n.d.
5-*O*-[3-(2-Hydroxyethyl)-β-D-ribofuranosyl] apramycin **(142)**	32-64	8	8	n.d.	n.d.
5-*O*-(β-D-Erythrofuranosyl) apramycin **(152)**	128	8	8	>128	1-2
5-*O*-β-(5'''-Formamido-5'''-deoxy-D-ribofuranosyl) apramycin **(157)**	>64	8	8-16	n.d.	n.d.
5-*O*-[5-Deoxy-5-amino-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(165)**	4	2	2	8	0.25
5-*O*-[5-Deoxy-5-formamido-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **(166)**	32	2	4	32-64	0.25
6-*O*-Propyl apramycin **(173)**	n.d.	n.d.	n.d.	n.d.	n.d.
6-*O*-(2,3-Dihydroxypropyl) apramycin **(175)**	n.d.	n.d.	n.d.	n.d.	n.d.
6-*O*-(2-Hydroxyethyl) apramycin **(177)**	>128	8-16	8-16	>128	2

5-Epi apramycin **(179)**	64	n.d.	n.d.	>128	n.d.
5-Deoxy-5-fluoro-apramycin **(182)**	64	n.d.	n.d.	>128	n.d.
5-Deoxy-5-epifluoro-apramycin **(184)**	128	n.d.	n.d.	>128	n.d.
5-Deoxy apramycin **(186)**	32	n.d.	n.d.	128	n.d.
3-*N*-Formyl apramycin **(190)**	n.d.	n.d.	n.d.	n.d.	n.d.
3-*N*-Acetyl apramycin **(191)**	n.d.	n.d.	n.d.	n.d.	n.d.

AAC(3)-IV AME is the only resistance determinant currently effective in the abrogation of apramycin activity,[57] consequently it was important to examine the synthesized compounds against bacteria known to carry this and other enzymes. Interestingly, adding the β-D-ribofuranosyl group **127** at the 5-position protects the compound from the action of AAC(3)-IV. This protection is increased at least 4-fold by further incorporation of a 2-aminoethyl group at the 3’’’-position of compound **138β** and 8-fold if a diaminoidose sugar is incorporated instead as in 5-*O*-(β-paramobiosyl) apramycin **133**. Compared to 5-*O*-(β-D-ribofuranosyl) apramycin **127**, removal of the 5’’’-CH_2OH **152** or substituting the 5’’’-OH with a formamido group **157** made the compounds more prone to AAC(3)-IV. Similarly, when compared to 5-*O*-(2-aminoethyl)-β-ribofuranosyl] apramycin **138β**, its 5’’’ formamide analog **166** or substituting the 3’’’-aminoethyl group with a hydroxyethyl group **142** reduced the activity at least 8-fold against AAC(3)-IV containing bacteria. On the other hand, activity was not reduced in the 5’’’ amine analog **165** of 5-*O*-[3-(2-aminoethyl)-β-ribofuranosyl] apramycin **138β**.

Compared to apramycin, the 5-epi apramycin **179,** 5-fluoro-5-deoxy apramycin **182**, and 5-epifluoro apramycin **184** showed moderately increased activity (2- to 4-fold) against AAC(3)-IV containing *E coli* , whereas 5-deoxy apramycin **186** showed a more substantial increase in activity (8-fold).

Apramycin is inheritably not susceptible to APH(3') due to the absence of a hydroxy group at the 3'-position, thus all synthesized compounds tested showed results with APH(3')-Ia and APH(3')-IIa containing bacteria comparable to the wild type. However, APH(3')-IIIa enzymes are also known to phosphorylate the 5-hydroxyl of the ribose ring in 4,5-AGAs.[38] Thus, the presence of APH (3')-IIIa results in a 4-fold reduction activity in antibacterial activity when a ribofuranosyl group is attached at the 5-position as in compound **127**. Removal of the ribosyl 5-OH as in the erythrofuranose restores activity in the presence of the APH(3')-IIIa. Similarly, the replacement of the ribosyl 5-OH by a formamido or amino group overcomes the presence of APH(3')-IIIa as is clear from the comparison of 5-*O*-[3-(2-aminoethyl)-β-ribofuranosyl] apramycin **138β**, with its formamido **166** and amino **165** analogs.

3.6. Conclusion

An easy and efficient method has been developed for derivatization of apramycin at the 5-position. Compounds 5-*O*-[3-(2-aminoethyl)-β-ribofuranosyl] apramycin **138β**, 5-*O*-(β-paramobiosyl) apramycin **133**, 5-*O*-[5-deoxy-5-formamido-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **166** and 5-Deoxy apramycin **186** showed improved antibacterial activity against *MRSA* and *E. coli* strains and reduced antibacterial activity toward *P aeruginosa* in comparison to the parent apramycin. 5-Epi apramycin **179** exhibits higher activity against all bacterial strains, albeit with less mitoribosomal selectivity. The apramycin-paromomycin hybrid **133** and its simplified form **138β** were active in the presence of the AAC(3)-IV class of AME that is the only resistance determinant currently effective in the abrogation of apramycin activity. The 5-*O*-[5-deoxy-5-amino-3-(2-aminoethyl)-β-D-ribofuranosyl] apramycin **165** is considered a breakthrough in so far as it satisfies all the project needs with improved antibacterial activity compared to apramycin against all bacterial strains screened including *P aeruginosa,* and strains

carrying the AAC(3) and APH(3’,5’’) AMEs. In addition, compound **165** showed improved ribosomal selectivity. Moreover, this study showed that a hydroxy group at the 5-position of apramycin is not essential, in fact its removal, inversion of configuration, or replacement by a fluoro group as in compounds **179**, **182**, **184** and **186** increases activity. Overall, new apramycin derivatives were developed which are more active than the parent drug. Moreover, aminoglycosides with better mitoribosomal selectivity and the ability to overcome the AAC(3)resistance determinant were developed for potential treatment of multidrug resistant bacterial infections.

CHAPTER 4. CONCLUSIONS

In order to investigate the effect of 4’-modifications of the 4,6-AGA netilmicin on the reduction of ototoxicity but not antibiotic activity several netilmicin derivatives have been prepared. These derivatives were screened for antiribosomal activity in cell-free translation assays as well as antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* (*E coli*) and *Pseudomonas aeruginosa*. Unfortunately, all modifications of netilmicin at the 4’-position showed similar or reduced antibacterial activities to the parent. All derivatives except 4'-chloro netilmicin showed less selectivity than netilmicin for inhibition of the bacterial ribosome as compared to the eurkaryotic ribosomes. Overall, the 4’-modifications reduce activity in 4,6-series to a greater extent than in the 4,5-series. This conclusion is consistent with the results observed in a series of 4’-modifications of the 4,6-aminoglycoside kanamycin B.[11]

This work made use of phenyl triazenes as selective protecting groups for secondary amines in the presence of primary amines. A number of polyamine substrates were used in the study including aliphatic and heterocyclic polyamines, amino acids and aminoglycosides. The secondary amines in these substrates were selectively protected as phenyl triazenes, and the primary amines were subsequently protected as the azides, benzyloxy carbamates or fluorenylmethyl carbamates. Phenyl triazenes also gave the advantage over carbamates of showing sharp ^{1}H NMR spectra at room temperature. This protecting group enabled the synthesis of plazomicin, an aminoglycoside in the 3rd phase of clinical trials, in fewer steps and higher yield than previously reported.

A series of novel apramycin derivatives were designed by focusing on derivatization and modifications at the 5-position. An apramycin-paromomycin hybrid, in which the paromobiosyl

group (rings III and IV) of paromomycin was attached to the 5-postion of apramycin, was synthesized with the aim of combining paromomycin's high activity with apramycin's low ototoxicity. The hybrid **133** showed better antibacterial activity against *MRSA* and *E coli* strains compared to the parent apramycin, but unfortunately with reduced antibacterial activity toward *P aeruginosa* and reduced ribosomal selectivity. Further truncation and modifications of this hybrid led to the development of 5-*O*-[5-amino-3-*O*-(2-aminoethyl)-5-deoxy-β-D-ribofuranosyl] apramycin **165**, 5-*O*-[3-*O*-(2-aminoethyl)-5-deoxy-5-formamido-β-D-ribofuranosyl] apramycin **166**, 5-epi apramycin **179** and 5-deoxy apramycin **186** that showed an overall better antibacterial effect with better ribosomal selectivity than the parent. Compound **165** is considered the best of all derivatives synthesized as it was more active against all bacterial strains than apramycin including *P aeruginosa* and AAC(3) and APH(3')-IIIa- containing bacteria, and also showed a better ribosomal selectivity. Overall, apramycin-class aminoglycoside derivatives with better antibacterial activities and ribosomal selectivities, and the ability to overcome the AAC(3) and APH(3')-IIIa resistance determinants were developed with for the treatment of multidrug resistant bacterial infections.

CHAPTER 5. EXPERIMENTAL SECTION

General Information

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. Analytical thin-layer chromatography was performed with pre-coated glass backed plates (w/UV 254) and visualized by UV irradiation (254 nm) or by staining with 25% H_2SO_4 in EtOH or ceric ammonium molybdate (ceric sulfate: (4.0 g); ammonium molybdate (10 g); H_2SO_4: 40 mL, H_2O: 360 mL) solution. **S**pecific rotations were obtained using a digital polarimeter in the solvent specified at 589 nm and 23 ºC on an Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ) with a path length of 10 cm. Infrared spectra were recorded on a FT/IR instrument. High resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer (Waters). ^{1}H, ^{13}C and 2D NMR spectra were recorded on 400 MHz, 500 MHz or 600 MHz instruments as specified. Assignments in ^{1}H and ^{13}C NMR were done by the assistance of H−H COSY, HSQC and/or HMBC experiments

General procedure A. Selective protection of secondary amines as phenyl triazenes with subsequent protection of primary amines as azides: An ice cooled solution of the amine (1 equiv) in a water/methanol mixture (3:7, 0.1 M) was treated with Na_2CO_3 (8 equiv), and then dropwise over 0.5 h with a solution of phenyldiazonium tetrafluoroborate (1.1 equiv) in water (0.1 M). After completion of the addition, Stick's reagent (imidazole-1-sulfonyl azide hydrochloride, 0.3 mmol per primary amine) and a catalytic amount of $CuSO_4$ were added. The reaction mixture was allowed to warm to rt and stirred overnight before it was diluted with ethyl acetate and washed with water and brine. The organic layer was dried and concentrated. The residue was purified by chromatography over silica gel.

General procedure B: Selective protection of secondary amines as phenyl triazenes with subsequent protection of primary amines as azides: The substrate (1 equiv) was dissolved in acetonitrile:H_2O (1:1, 0.1 M). K_2CO_3 (8 equiv) was added and the reaction mixture was cooled to 0 ^oC using an ice bath. A solution of phenyldiazonium tetrafluoroborate (1.1 equiv) in acetonitrile (0.1 M) was added using a syringe pump over 0.5 h. After completion of the addition, 0.22 mmol per amine of imidazole-1-sulfonyl azide hydrochloride and a catalytic amount of $CuSO_4$ were added. The reaction mixture was allowed to warm to rt and stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried and concentrated. The residue was purified by chromatography over silica gel.

General procedure C: Selective protection of secondary amines as phenyl triazenes with subsequent protection of primary amines as benzyl carbamates: An ice cooled solution of the amine (1 equiv) in a water/methanol mixture (3:7, 0.1 M) was treated with Na_2CO_3 (8 equiv), and then dropwise over 0.5 h with a solution of phenyldiazonium tetrafluoroborate (1.1

equiv) in water (0.1 M). After completion of the addition, 0.24 mmol per amine of benzyl chloroformate was added. The reaction mixture was allowed to warm to rt and stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried and concentrated. The residue was purified by chromatography over silica gel.

General procedure D: Deprotection by deacetylation, Staudinger and acid cleavage of phenyl triazenes: A stirred solution of substrate (0.04 mmol, 1 equiv) in dioxane (1.5 mL) was treated with 1N NaOH (0.5 mL) and heated with stirring at 60 °C for 1.5 h. The reaction mixture was cooled to rt before 1M $P(CH_3)_3$ in THF (4.5 equiv) was added and the reaction mixture stirred at rt for 6h. The reaction mixture was then concentrated and purified by column chromatography (eluent: 5% to 12% ammonical MeOH in DCM). The product-containing fractions were concentrated, dissolved in ethanol (0.9 mL) and treated with sodium hypophosphite (2 equiv) and trifluoroacetic acid (12 equiv), and stirred at rt for 8 h. The reaction mixture was neutralized using Amberlite® IRA400 hydroxide form, filtered and dried. The crude product was dissolved in D.I. water (1 mL), acidified by glacial acetic acid till pH = 3-4 and loaded to a Sephadex column (CM Sephadex C-25) from which it was flushed with D.I. water (20 mL), then by gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to give a white solid.

Methyl 2-azido-6-(1-methyl-3-phenyltriaz-2-en-1-yl) hexanoate (50). This compound was prepared according to the general procedure A using compound **42** (38 mg, 0.22 mmol) to afford the desired product **7** (38 mg, 58 %) as a yellow oil; *Rf* = 0.8 (40% EtOAc in hexanes); $[\alpha]_D^{25}$= -26.1 (*c* 0.025, MeOH); IR (film) (cm^{-1}): 2105, 1745, 1594 (w); ^1H NMR (400 MHz,

CD_3OD) δ 7.36 (dd, *J* = 8.5, 1.2 Hz, 2H), 7.33 – 7.24 (m, 2H), 7.09 (t, *J* = 7.3 Hz, 1H), 4.00 (dd, *J* = 8.2, 5.3 Hz, 1H), 3.77 (t, *J* = 7.0 Hz, 2H), 3.71 (s, 3H), 3.19 (s, 3H), 1.91 – 1.81 (m, 1H), 1.79-1.66 (m, 3H), 1.47 – 1.36 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 171.2, 151.0, 128.3, 124.9, 120.0, 61.6, 51.6, 30.5, 27.2, 22.4; ESI-HRMS: *m/z* calcd. for $C_{14}H_{20}N_6NaO_2$ $[M+Na]^+$ 327.1545; found, 327.1561.

3-(4-Azidobutyl)-3-(3-azidopropyl)-1-phenyltriaz-1-ene (51). This compound was prepared according to the general procedure A using compound **43** (50 mg, 0.20 mmol) to afford the desired product **51** (36 mg, 61 %) as a yellow oil; *Rf* = 0.5 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 2093, 1593 (w); ^1H NMR (600 MHz, CD_3OD) δ 7.35 (d, *J* = 7.4 Hz, 2H), 7.28 (t, *J* = 7.9 Hz, 2H), 7.09 (t, *J* = 7.3 Hz, 1H), 3.77 (t, *J* = 7.0 Hz, 2H), 3.73 (t, *J* = 7.2 Hz, 2H), 3.35 (t, *J* = 6.6 Hz, 2H), 3.32 (t, *J* = 6.8 Hz, 2H), 1.93 (p, *J* = 6.7 Hz, 2H), 1.74 (p, *J* = 7.3 Hz, 2H), 1.60 (p, *J* = 7.1 Hz, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ 150.8, 128.3, 125.0, 120.1, 50.7, 48.7, 27.7, 25.9, 23.8; ESI-HRMS: *m/z* calcd. for $C_{13}H_{20}N_9$ $[M+H]^+$, 302.1842; found, 302.1845.

3-(3-Azidobenzyl)-3-methyl-1-phenyltriaz-1-ene (52). This compound was prepared according to the general procedure A using compound **44** (100 mg, 0.74 mmol) to afford the desired product **52** (135 mg, 69 %) as a yellow oil; *Rf*= 0.7 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 2109, 1590 (w); ^1H NMR (400 MHz, CD_2Cl_2) δ 7.53 (d, *J* = 8.2 Hz, 2H), 7.42-7.31 (m, 3H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 7.05 – 6.92 (m, 2H), 4.97 (s, 2H), 3.21 (br s, 3H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 150.6, 140.5, 139.0, 130.1, 128.9, 125.8, 124.4, 120.8, 118.5, 118.3, 59.0, 34.9; ESI-HRMS: *m/z* calcd. for $C_{14}H_{15}N_6$ $[M+H]^+$, 267.1358; found, 267.1363.

4-Azido-1-(phenyldiazenyl)piperidine (53). This compound was prepared according to the general procedure A using compound **45** (100 mg, 1 mmol) to afford the desired product **53**

(160 mg, 70 %) as a yellow oil; *Rf*= 0.65 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 2092, 1593 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 8.4, 1.4 Hz, 2H), 7.40 – 7.33 (m, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 4.16 (ddd, *J* = 13.6, 6.4, 4.3 Hz, 2H), 3.74 (tt, *J* = 8.3, 3.7 Hz, 1H), 3.56 (ddd, *J* = 13.6, 8.8, 3.9 Hz, 2H), 2.06 – 1.96 (m, 2H), 1.77 (dtd, *J* = 13.1, 8.7, 4.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.3, 128.9, 126.3, 120.7, 57.3, 44.2, 29.9; ESI-HRMS: *m/z* calcd. for $C_{11}H_{15}N_6$ $[M+H]^+$, 231.1358; found, 231.1367.

3-(3-Azidopropyl)-3-cyclohexyl-1-phenyltriaz-1-ene (54). This compound was prepared according to the general procedure A using compound **46** (100 mg, 0.64 mmol) to afford the desired product **54** (110 mg, 60 %) as a yellow oil; *Rf*= 0.75 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 2094, 1594 (w); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.43 (dd, *J* = 8.5, 1.2 Hz, 2H), 7.37 – 7.32 (m, 2H), 7.14 (t, *J* = 7.3 Hz, 1H), 3.77 – 3.72 (m, 2H), 3.66 (br s, 1H), 3.37 (t, *J* = 6.7 Hz, 2H), 2.05-1.94 (m, 4H), 1.90 (d, *J* = 13.6 Hz, 2H), 1.76 – 1.61 (m, 3H), 1.42 (qt, *J* = 13.1, 3.3 Hz, 2H), 1.24 (qt, *J* = 13.0, 3.7 Hz, 1H); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 151.2, 128.7, 125.0, 120.4, 64.4, 49.6, 43.6, 32.4, 26.6, 25.8, 25.5; ESI-HRMS: *m/z* calcd. for $C_{15}H_{23}N_6$ $[M+H]^+$, 287.1984; found, 287.1979.

3-(2-Azidoethyl)-1,3-diphenyltriaz-1-ene (55). This compound was prepared according to the general procedure A using compound **47** (100 mg, 0.74 mmol) to afford the desired product **55** (81 mg, 41 %) as a red oil; *Rf*= 0.7 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 2098, 1601; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.2 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.47-7.37 (m, 4H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.17 (t, *J* = 7.3 Hz, 1H), 4.46 (t, *J* = 6.4 Hz, 2H), 3.69 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.8, 144.6, 129.3, 129.0, 127.2, 123.9, 121.5, 117.4, 47.4, 44.7; ESI-HRMS: *m/z* calcd. for $C_{14}H_{15}N_6$ $[M+H]^+$, 267.1358; found, 267.1350.

4-Fluorenylmethyloxycarbonylamino-1-(phenyldiazenyl)piperidine (56). An ice cooled solution of **45** (100 mg, 1 mmol) in a water/methanol mixture (3:7, 4 mL) was treated with Na_2CO_3 (5 mmol), and then dropwise over 0.5 h with a solution of phenyldiazonium tetrafluoroborate (1.1 mmol) in water (4 mL). After completion of the addition, Fmoc-Cl (1.1 mmol) was added. The reaction mixture was allowed to warm to rt and stirred 7 h before it was diluted with brine and extracted with DCM thrice. The organic layer was dried and concentrated. The residue was purified by column chromatography (15% EtOAc in hexanes) over silica gel to give the desired product **56** (329 mg, 77 %) as a yellow solid. *Rf*= 0.25 (20% EtOAc in hexanes); IR (film) (cm^{-1}): 1687, 1538; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.47 – 7.30 (m, 8H), 7.19 (t, *J* = 7.3 Hz, 1H), 4.71 (br d, *J* = 6.1 Hz, 1H), 4.45 (d, *J* = 6.0 Hz, 4H), 4.22 (t, *J* = 6.3 Hz, 1H), 3.90 – 3.75 (br s, 1H), 3.32 – 3.14 (m, 2H), 2.16 – 2.04 (m, 2H), 1.59 – 1.34 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.5, 150.3, 143.9, 141.4, 128.9, 127.7, 127.1, 126.2, 124.9, 120.7, 120.0, 66.5, 48.3, 47.3, 45.7, 31.5; ESI-HRMS: *m/z* calcd. for $C_{26}H_{26}N_4NaO_2$ $[M+H]^+$, 449.1953; found, 449.1939.

Benzyl *N*-(4-(1-(3-(benzyloxycarbonylamino)propyl)-3-phenyltriaz-2-en-1-yl)butyl) carbamate (57). This compound was prepared according to the general procedure C using compound **43** (50 mg, 0.2 mmol) to afford the desired product **57** (55 mg, 54 %) as a yellow oil; *Rf*= 0.5 (60% EtOAc in hexanes); IR (film) (cm^{-1}): 1700, 1591 (w); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.42 (d, *J* = 7.6 Hz, 2H), 7.33 (m, 12H), 7.14 (t, *J* = 7.3 Hz, 1H), 5.09 (m, 4H), 3.76 (t, *J* = 6.5 Hz, 2H), 3.74 – 3.68 (m, 2H), 3.25 – 3.20 (m, 2H), 3.19 (br s, 2H), 1.84 (br s, 2H), 1.72 (br s, 2H), 1.59 – 1.47 (m, 2H); ^{13}C NMR (101 MHz, CD_2Cl_2) δ 156.3, 150.7, 137.1, 137.0, 128.8, 127.92, 127.88, 127.85, 127.8, 125.3, 120.3, 66.3, 40.5, 38.4, 27.9, 27.2, 24.7; ESI-HRMS: *m/z* calcd. for $C_{29}H_{36}N_5O_4$ $[M+H]^+$, 518.2767; found, 518.2770.

Benzyl (4-azidobutyl)(3-azidopropyl)carbamate (58). A solution of **43** (100 mg, 0.39 mmol) in a water/methanol mixture (1:1, 4 mL) was treated with Na_2CO_3 (3.9 mmol), imidazole-1-sulfonyl azide hydrochloride (3 equiv) and a catalytic amount of $CuSO_4$. The mixture was stirred for 3 h, after which 5 equiv of benzyl chloroformate was added. The reaction mixture was stirred for another hour before it was diluted with brine and extracted with DCM thrice. The organic layer was dried using Na_2SO_4 and concentrated. The residue was purified by column chromatography to give the desired product **58** (77 mg, 59 %) as a colorless oil; *Rf*= 0.4 (25% EtOAc in hexanes); ^{1}H NMR (600 MHz, CD_3OD) δ 7.43 – 7.20 (m, 5H), 5.11 (s, 2H), 3.40 – 3.17 (m, 11H), 1.87 – 1.71 (m, 2H), 1.66 – 1.57 (m, 2H), 1.57 – 1.45 (m, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ 156.5, 136.3, 128.1, 127.7, 127.6, 66.9, 50.7, 48.63, 48.59, 46.7, 46.4, 44.7, 44.2, 27.6, 27.0, 25.7, 25.4, 24.8. (151 MHz, CD_3OD); ESI-HRMS: *m/z* calcd. for $C_{15}H_{21}N_7NaO_2$ $[M+Na]^+$, 354.1654; found, 154.1666.

1,3,2',6'-Tetra-deamino-1,3,2',6'-tetraazido-3''-*N*-(phenylazo)sisomicin (59). This compound was prepared according to the general procedure A using compound **8** (100 mg, 0.14 mmol) to afford the desired product **59** (83 mg, 88 %) as a yellow solid; *Rf*= 0.4 (5% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$= +143.4 (*c* 0.037, MeOH); IR (film) (cm^{-1}):3442, 2102, 1594 (w); ^{1}H NMR (400 MHz, CD_3OD) δ 7.40 (d, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.11 (t, *J* = 7.3 Hz, 1H), 5.89 (d, *J* = 2.3 Hz, 1H), 5.48 (d, *J* = 4.2 Hz, 1H), 4.98 (dd, *J* = 5.2, 2.2 Hz, 1H), 4.40 (dd, *J* = 11.4, 3.8 Hz, 1H), 4.30 (d, *J* = 12.1, 1H), 4.29 (d, *J* = 11.2, 1H) 3.83 – 3.60 (m, 6H), 3.43 (m, 3H), 3.34 (s, 3H), 2.45 (dd, *J* = 15.5, 11.5 Hz, 1H), 2.28 (m, 2H), 1.42 (q, *J* = 12.6 Hz, 1H), 1.11 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 151.1, 145.7, 128.4, 125.0, 120.3, 98.9, 98.0, 97.0, 80.1, 79.7, 74.6, 73.2, 68.8, 68.5, 65.3, 60.7, 60.2, 55.1, 54.6, 51.9, 32.6, 21.1, 20.8; ESI-HRMS: *m/z* calcd. for $C_{25}H_{34}N_{15}O_7$ $[M+H]^+$, 656.2766; found, 656.2747.

1,3,2',6'-Tetra-*N*-(benzyloxycarbonyl)-3''-*N*-(phenylazo)sisomicin (60). This compound was prepared according to the general procedure C using compound **8** (100 mg, 0.14 mmol) to afford the desired product **60** (125 mg, 79 %) as a yellow solid; *Rf*= 0.33 (5% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$= +9.8 (*c* 5.7, CH_2Cl_2); IR (film) (cm^{-1}): 3416, 1702, 1592 (w); ^{1}H NMR (600 MHz, CD_2Cl_2 + CD_3OD) δ 7.42 – 7.22 (m, 24H), 7.16 (t, *J* = 7.0 Hz, 1H), 5.41 (br s, 1H), 5.18 – 4.94 (m, 9H), 4.56 (br s, 1H), 4.37 – 4.30 (m, 1H), 3.99 – 3.88 (m, 2H), 3.86 – 3.79 (m, 1H), 3.72 – 3.59 (m, 3H), 3.59 – 3.45 (m, 3H), 3.43 – 3.28 (m, 5H), 2.31 (br s, 1H), 2.11 (s, 1H), 2.00 (br s, 1H), 1.30 (br s, 1H), 1.08 (br s, 3H); ^{13}C NMR (151 MHz, CD_2Cl_2 + CD_3OD) δ 156.8, 156.2, 150.7, 146.2, 136.7, 136.5, 128.9, 128.5, 128.4, 128.3, 128.03, 127.95, 127.9, 127.7, 125.7, 120.5, 100.8, 97.3, 96.3, 85.6, 79.7, 75.8, 73.1, 69.1, 68.9, 67.3, 66.7, 66.5, 50.3, 49.6, 47.3, 42.5, 36.4, 33.8, 22.7, 22.2; ESI-HRMS: *m/z* calcd. for $C_{57}H_{65}N_7NaO_{15}$ $[M+Na]^+$ 1110.4436; found, 1110.4463.

1,3,2',4''-Tetra-deamino-1,3,2',4''-tetraazido-7'-*N*-(phenylazo) apramycin (61). This compound was prepared according to the general procedure B using compound **9** (free base, 100 mg, 0.19 mmol) to afford the desired product **61** (83 mg, 60 %) as a buff solid; *Rf*= 0.5 (40% EtOAc in hexanes); $[\alpha]_D^{25}$= +52.3 (*c* 0.7, MeOH); IR (film) (cm^{-1}): 3410, 2105, 1595 (w); ^{1}H NMR (600 MHz, CD_3OD) δ 7.35 (d, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.13 (d, *J* = 7.2 Hz, 1H), 5.60 (d, *J* = 3.2 Hz, 1H), 5.53 (d, *J* = 8.4 Hz, 1H), 5.26 (d, *J* = 3.7 Hz, 1H), 4.35 (s, 1H), 4.03 – 3.98 (m, 1H), 3.95 (dd, *J* = 10.8, 4.3 Hz, 1H), 3.92 (t, *J* = 6.3 Hz, 1H), 3.56 (t, *J* = 9.6 Hz, 1H), 3.53 – 3.46 (m, 3H), 3.45 – 3.36 (m, 3H), 3.33 (s, 1H), 3.32 – 3.30 (m, 4H), 3.29 – 3.19 (m, 4H), 3.19 – 3.10 (m, 2H), 2.28 – 2.19 (m, 2H), 2.08 (q, *J* = 11.3 Hz, 1H), 1.47 – 1.36 (m, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 150.8, 128.4, 125.3, 120.6, 97.7, 95.3, 93.9, 79.4, 76.6, 76.5,

72.0, 71.4, 71.0, 70.1, 68.8, 67.8, 66.5, 61.2, 60.3, 60.1, 59.7, 56.4, 35.2, 31.8, 28.0; ESI-HRMS: *m/z* calcd. for $C_{27}H_{37}N_{15}NaO_{11}$ $[M+Na]^+$, 770.2695; found, 770.2660.

1,3,2',4''-Tetra-*N*-(benzyloxycarbonyl)-7'-*N*-(phenylazo)apramycin (62).This compound was prepared according to the general procedure C using compound **9** (free base, 100 mg, 0.19 mmol) to afford the desired product **62** (162 mg, 74 %) as a buff solid; *Rf*= 0.6 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$= +19.8 (*c* 2.6, MeOH); IR (film) (cm^{-1}): 3396, 1691, 1532 (w); ^1H NMR (600 MHz, CD_3OD) δ 7.35 – 7.16 (m, 24H), 7.04 (t, *J* = 7.3 Hz, 1H), 5.53 (d, *J* = 8.4 Hz, 1H), 5.33 – 5.25 (m, 3H), 5.09 – 5.02 (m, 5H), 4.97 (d, *J* = 12.5 Hz, 1H), 4.87 (d, *J* = 12.9 Hz, 1H), 4.39 (s, 1H), 3.97 (d, *J* = 7.7 Hz, 1H), 3.95 – 3.88 (m, 1H), 3.80 – 3.70 (m, 2H), 3.66 – 3.37 (m, 9H), 3.35 – 3.30 (m, 1H), 3.27 – 3.13 (m, 4H), 2.09 – 1.96 (m, 2H), 1.82 - 1.71 (m, 1H), 1.50 – 1.39 (m, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 157.7, 157.2, 156.9, 156.7, 150.6, 136.8, 128.5, 128.1, 128.0, 127.6, 127.5, 127.4, 125.3, 120.5, 98.2, 95.5, 93.9, 81.4, 77.1, 75.1, 72.2, 72.0, 70.4, 70.2, 69.3, 67.5, 66.5, 66.3, 66.2, 66.1, 60.8, 52.8, 51.4, 50.3, 49.9, 35.0, 34.0, 30.0; ESI-HRMS: *m/z* calcd. for $C_{59}H_{69}N_7NaO_{19}$ $[M+Na]^+$, 1202.4546; found, 1202.4500.

1,3,2',4''-Tetra-deamino-1,3,2',4''-tetraazidoapramycin (63). Compound **61** (10 mg, 0.015 mmol)) was dissolved in a CH_2Cl_2/ethanol mixture (1:1, 0.3 mL) and cooled in an ice bath before trifluoroacteic acid (0.05 mL) was added. The reaction mixture was allowed to warm to rt and was stirred for 0.5 h. After completion the solution was concentrated, toluene (1 mL) was added and the solution concentrated again. The crude product was purified using column chromatography (10% methanol/CH_2Cl_2) to afford **63** (8.5 mg, 98 %) as a buff solid with spectral data identical to the literature.[181]

1,3,2',4''-Tetra-*N*-(benzyloxycarbonyl)apramycin (64). Compound **62** (40 mg, 0.034 mmol)) was dissolved in a CH_2Cl_2/ethanol mixture (1:1, 0.5 mL) and cooled in an ice bath before

trifluoroacteic acid (0.1 mL) was added. The reaction mixture was allowed to warm to rt and was stirred for 0.5 h. After completion the solution was concentrated, toluene (1 mL) was added and the solution concentrated again. The crude product was purified using column chromatography (10% methanol/CH_2Cl_2) to afford **64** (35 mg, 96 %) as a buff solid; *Rf*= 0.2 (10% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$= +12.5 (*c* 0.002, MeOH); ^{13}C NMR (101 MHz, CD_3OD) δ 157.3, 156.9, 156.7, 136.8, 128.3, 128.1, 128.1, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 97.6, 94.3, 92.1, 80.4, 77.2, 75.1, 71.7, 69.6, 69.1, 66.8, 66.4, 66.3, 66.1, 63.0, 61.5, 60.7, 53.7, 51.5, 50.2, 49.7, 33.9, 29.7, 29.5, 29.3; ESI-HRMS: *m/z* calcd. for $C_{53}H_{66}N_5O_{19}$ $[M+H]^+$, 1076.4352; found, 1076.4324. ^{1}H-NMR spectral data are not listed for this compound owing to complications owing to the presence of multiple rotamers arising from the presence of the four Cbz groups.

3,2',6'-Triazido netilmicin (65). K_2CO_3 (8.06 g, 58.3 mmol) was added slowly to a stirred solution of netilmicin sulfate (5.00 g, 6.94 mmol) in 1:1 dioxane:water (150 mL). A catalytic amount of copper sulfate (110.7 mg, 0.69 mmol) was added before the solution was ice cooled. Stick's reagent (5.27 g, 25.0 mmol) was added and the reaction mixture was stirred for 4 h at rt. After completion, the reaction mixture was concentrated *in vacuo* and purified by gradient chromatography over silica gel (eluent: 5% to 12% ammonical MeOH in DCM) to give **65** (2.2 g, 57%) as a white solid; $[\alpha]_D^{25}$= +94.1 (*c* 2.8, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.89 (s, 1H, H-1'), 4.93 (d, *J* = 5.1 Hz, 1H, H-4'), 4.85 (d, *J* = 3.3 Hz, 1H, H-1''), 3.78 – 3.68 (m, 3H, H-4, H-6', H-5''), 3.63 (m, 2H, H-5, H-6'), 3.58 – 3.52 (m, 2H, H-2'', H-5''), 3.37 (ddd, *J* = 11.0, 6.1, 2.0 Hz, 1H, H-2'), 3.33 – 3.26 (m, 1H, H-3), 3.05 (t, *J* = 9.5 Hz, 1H, H-6), 2.86 – 2.78 (m, 1H, NCH_2), 2.62 – 2.49 (m, 5H, H-1, NCH_2, NCH_3), 2.48 – 2.42 (m, 1H, H-3'), 2.41 (d, *J* = 10.2 Hz, 1H, H-3''), 2.33 (m, 1H, H-2), 2.27 (dt, *J* = 16.2, 5.8 Hz, 1H, H-3'), 1.13 (s, 3H, 4''CH_3), 1.12 – 1.06 (m, 3H, NCH_2CH_3, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 145.4 (C-5'), 102.7 (C-

1’’), 98.3 (C-4’), 96.4 (C-1’), 88.3 (C-6), 79.4 (C-4), 76.6 (C-5), 71.1 (C-4’’), 69.7 (C-2’’), 67.4 (C-5’’), 65.7 (C-3’’), 60.6 (C-3), 56.0 (C-1), 54.6 (C-2’), 52.3 (C-6’), 41.5 (N*C*H_2), 39.0 (N*C*H_3), 33.3 (2), 24.3 (4’’*C*H_3), 21.1 (C-3’), 15.0 (NCH_2*C*H_3); ESI-HRMS: *m/z* calcd. for $C_{21}H_{36}N_{11}O_7$ $[M+H]^+$ 554.2799; found, 554.2789.

Triazido-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (66). A stirred solution of **65** (2.26 g, 4.07 mmol) in 2:1 acetonitrile:water (50 mL) was treated with K_2CO_3 (5.61 g, 40.7 mmol) and cooled to 0 °C. Benzenediazonium tetrafluoroborate (2.34 g, 12.2 mmol) was added slowly and the reaction mixture was stirred for 2 h. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine, then dried with sodium sulfate, filtered and concentrated. The crude product was purified by gradient chromatography over silica gel (eluent: 20% to 40% EtOAc/hexanes) to yield **66** (2.5 g, 82%) as a buff solid; $[\alpha]_D^{25}$= +33.0 (*c* 0.3, DCM); ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.42 – 7.37 (m, 2H, Ar*H*), 7.35 – 7.28 (m, 6H, Ar*H*), 7.19 – 7.12 (m, 2H, Ar*H*), 5.93 (d, *J* = 2.4 Hz, 1H, H-1’), 5.32 (t, *J* = 1.1 Hz, 1H, OH), 5.08 (d, *J* = 3.9 Hz, 1H, H-1’’), 4.99 (dd, *J* = 5.5, 2.3 Hz, 1H, H-4’), 4.66 (s, 1H, OH), 4.26 (td, *J* = 10.9, 3.8 Hz, 1H, H-2’’), 4.00 – 3.93 (m, 2H, H-6, H-5’’), 3.93 – 3.86 (m, 2H, H-5, NC*H*$_2$), 3.82 (t, *J* = 9.4 Hz, 1H, H-4), 3.78 (d, *J* = 14.0 Hz, 1H, H-6), 3.71 (d, *J* = 14.0 Hz, 1H, H-6), 3.66 – 3.55 (m, 3H, H-3’’, H-5’’, NC*H*$_2$), 3.52 (ddd, *J* = 12.5, 9.7, 4.5 Hz, 1H, H-3), 3.46 (ddd, *J* = 11.1, 6.2, 2.4 Hz, 1H, H-2’), 3.09 (s, 3H, NC*H*$_3$), 2.58 – 2.45 (m, 1H, H-3’), 2.37 – 2.26 (m, 2H, H-2, H-3’), 1.82 (q, *J* = 13.0 Hz, 1H, H-2), 1.68 (d, *J* = 11.0 Hz, 1H, OH), 1.26 (t, *J* = 7.2 Hz, 3H, NCH$_2$C*H*$_3$), 1.14 (s, 3H, 4’’CH$_3$); ^{13}C NMR (151 MHz, CD_2Cl_2): δ 150.6 (Ar*C*), 150.3 (Ar*C*), 145.5 (Ar*C*), 128.8 (Ar*C*), 126.1(Ar*C*), 125.8 (Ar*C*), 120.6 (Ar*C*), 120.5 (Ar*C*), 101.0 (C-1’’), 98.3 (C-4’), 96.9 (C-1’), 84.5 (C-6), 79.9 (C-4), 76.5 (C-5), 73.3 (C-4’’), 69.2 (C-5’’, C-3’’), 67.7 (C-2’’), 60.5 (C-3), 54.9 (C-2’), 52.4 (C-6’), 42.4 (N*C*H$_2$), 37.0 (N*C*H$_3$), 32.8 (C-2), 22.5

(4’’*C*H_3), 21.3 (C-3’), 12.5 (NCH_2*C*H_3); ESI-HRMS: *m/z* calcd. for $C_{33}H_{43}N_{15}NaO_7$ $[M+Na]^+$ 784.3368; found:784.3348.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (67). 4-Dimethylaminopyridine (7.95 g, 65.1 mmol) and **66** (2.48 g, 3.2 mmol) were dissolved in dry DCM and the resulting solution was stirred and ice cooled before acetic anhydride (1.2 mL, 13.0 mmol) was added dropwise. The reaction mixture was stirred overnight at rt under argon, then it was diluted with DCM and the organic layer was washed with aqueous $NaHCO_3$ followed by brine, dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified via silica gel chromatography eluting with 15% to 40 % EtOAc in hexanes to give **67** (2.10 g, 75%) as a white foam; $[\alpha]_D^{25}$= -19.7 (*c* 1.0, DCM); ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.39 – 7.28 (m, 8H, Ar*H*), 7.19 – 7.13 (m, 2H, Ar*H*), 5.43 (d, *J* = 10.4 Hz, 1H, H-2’’), 5.34 (t, *J* = 9.7 Hz, 1H, H-5), 5.32 (s, 1H, H-1’), 5.08 (d, *J* = 2.8 Hz, 1H, H-1’’), 5.03 (dd, *J* = 5.5, 1.8 Hz, 1H, H-4’), 4.10 (s, 3H, H-6, H-3’’, NCH_2), 3.88 – 3.80 (m, 2H, H-1, H-5’’), 3.79 (d, *J* = 14.0 Hz, 2H, H-6’, H-1), 3.73 (d, *J* = 14.0 Hz, 1H, H-6’), 3.68 – 3.60 (m, 1H, NCH_2), 3.60 – 3.53 (m, 1H, H-3), 3.42 (ddd, *J* = 11.3, 6.1, 2.4 Hz, 1H, H-2’), 3.35 (d, *J* = 12.3 Hz, 1H, H-5’’), 3.15 (s, 3H, NCH_3), 2.54 – 2.46 (m, 1H, H-3’), 2.33 (dt, *J* = 15.4, 5.5 Hz, 1H, H-3’), 2.31 – 2.25 (m, 1H, H-2), 2.20 (s, 3H, COCH_3), 2.08 – 1.95 (m, 1H, H-2), 1.69 (s, 3H, COCH_3), 1.27 (s, 3H, NCH_2CH_3), 1.14 (s, 3H, 4’’CH_3); ^{13}C NMR (151 MHz, CD_2Cl_2): δ 170.1 (C=O), 169.8 (C=O), 150.5 (Ar*C*), 150.4 (Ar*C*), 145.3 (C-5’), 128.8 (Ar*C*), 128.8 (Ar*C*), 125.9 (Ar*C*), 125.8 (Ar*C*), 120.6 (Ar*C*), 120.5 (Ar*C*), 98.6 (C-1’’), 98.3 (C-4’), 97.1 (C-1’), 81.4 (C-6), 77.8 (C-4), 74.9 (C-5), 73.6 (C-4’’), 68.9 (C-2’’, C-5’’), 65.9 (C-3’’), 61.6 (C-1), 60.7 (C-3), 54.4 (C-2’), 52.2 (C-6’), 43.9 (N*C*H_2), 38.2 (N*C*H_3), 34.2 (C-2), 22.4 (4’’*C*H_3), 21.5 (CO*C*H_3), 20.6 (CO*C*H_3), 20.1 (C-3’); ESI-HRMS: *m/z* calcd. for $C_{37}H_{48}N_{15}O_9$ $[M+H]^+$ 846.3759; found, 846.3747.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-4'-iodo-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (68). To a stirred solution of compound **67** (100 mg, 0.11 mmol) in dry acetonitrile (1 mL), *N*-iodosuccinimide (37 mg, 0.16 mmol) and silver nitrate (500 mg, 0.59 mmol) were added. The reaction mixture was stirred at 80 °C for 2.5 h then cooled to rt and filtered through Celite®. The filtrate was diluted with EtOAc and washed with saturated aqueous $Na_2S_2O_3$ and brine, dried and concentrated. The residue was purified by silica gel column chromatography eluting with 12% acetone/hexanes to give **68** (52 mg, 45%) as white solid; $[\alpha]_D^{25}$= +33.5 (*c* 0.4, DCM); ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.38 – 7.28 (m, 8H, Ar*H*), 7.20 – 7.12 (m, 2H, Ar*H*), 5.48 – 5.39 (m, 2H, H-1', H-2''), 5.35 (t, *J* = 9.6 Hz, 1H, H-5), 5.12 – 5.05 (s, 1H, H-1''), 4.24 (d, *J* = 13.8 Hz, 1H, H-6'), 4.17 – 4.06 (m, 2H, H-6, NC*H*$_2$), 4.04 (d, *J* = 13.8 Hz, 1H, H-6'), 3.90 – 3.74 (m, 3H, H-4, H-3'', H-5''), 3.69 – 3.58 (m, 2H, H-3, NC*H*$_2$), 3.55 (ddd, *J* = 11.1, 6.2, 2.5 Hz, 1H, H-2'), 3.35 (d, *J* = 12.3 Hz, 1H, H-5''), 3.15 (s, 3H, NC*H*$_3$), 2.95 (dd, *J* = 16.1, 11.5 Hz, 1H, H-3'), 2.80 (dd, *J* = 16.3, 6.2 Hz, 1H, H-3'), 2.33 – 2.25 (m, 1H, H-2), 2.19 (s, 3H, COC*H*$_3$), 2.09 – 2.00 (m, 1H, H-2), 1.69 (s, 3H, COC*H*$_3$), 1.27 (s, 3H, NCH$_2$C*H*$_3$), 1.14 (s, 3H, 4''CH$_3$); ^{13}C NMR (151 MHz, CD_2Cl_2): δ 170.0 (C=O), 169.8 (C=O), 150.5 (Ar*C*), 150.4 (Ar*C*), 145.3 (C-5'), 128.8 (Ar*C*), 128.8 (Ar*C*), 125.9 (Ar*C*), 125.8 (Ar*C*), 120.6 (Ar*C*), 120.5 (Ar*C*), 98.6 (C-1''), 97.1 (C-1'), 81.4 (C-6), 78.3 (C-4), 74.8 (C-5), 73.6 (C-4''), 68.9 (C-2'', 5''), 66.1 (C-4'), 65.9 (C-3''), 61.5 (C-1), 60.7 (C-3), 55.0 (C-2'), 54.5 (C-6'), 43.9 (N*C*H$_2$), 34.0 (C-3'), 22.4 (4''*C*H$_3$), 21.4 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 11.9 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{37}H_{47}IN_{15}O_9$ $[M+H]^+$ 972.2726; found, 972.2701.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-4'-bromo-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (69). In a round bottom flask, protected netilmicin **67** (500 mg, 0.59 mmol), K_2CO_3 (408 mg, 2.95 mmol), tetrabutylammonium nitrate (5 mol%) and BHT (5 mol%) were dissolved

with stirring in dry acetonitrile (5 mL) and the flask was wrapped with aluminum foil before *N*-bromosuccinimide (126 mg, 0.71 mmol) was added. The reaction mixture was stirred for 1.5 h for completion, then was diluted with EtOAc and washed with saturated aqueous $Na_2S_2O_3$ and brine, dried and concentrated. The resulting crude product was purified using silica gel chromatography (eluent: 25% to 50% EtOAc/hexanes) to yield **69** (270 mg, 50%) as an orange solid; $[\alpha]_D^{25}$= +14.03 (*c* 0.5, DCM); ^{1}H NMR (499 MHz, CD_2Cl_2): δ 7.36 (p, *J* = 10.7, 9.4 Hz, 8H, Ar*H*), 7.21 – 7.15 (m, 2H, Ar*H*), 5.46 (dd, *J* = 11.0, 3.0 Hz, 1H, H-2’’), 5.41 (d, *J* = 2.5 Hz, 1H, H-1’), 5.38 (t, *J* = 11.9 Hz, 1H, H-5), 5.10 (d, *J* = 3.1 Hz, 1H, H-1’’), 4.24 (d, *J* = 13.8 Hz, 1H, H-6’), 4.19 – 4.03 (m, 2H, H-6, NC*H*$_2$), 4.01 – 3.93 (m, 2H, H-6’, H-3’’), 3.85 (t, *J* = 9.9 Hz, 1H, H-4), 3.89 – 3.83 (m, 1H, H-1), 3.81 (d, *J* = 13.6 Hz, 1H, H-5’’), 3.71 – 3.62 (m, 2H, H-3, NC*H*$_2$), 3.59 (ddd, *J* = 11.2, 6.3, 2.6 Hz, 1H, H-2’), 3.37 (d, *J* = 12.4 Hz, 1H, H-5’’), 3.17 (s, 3H, NC*H*$_3$), 2.95 (dd, *J* = 16.6, 11.1 Hz, 1H, H-3’), 2.75 (dd, *J* = 16.2, 6.4 Hz, 1H, H-3’), 2.36 – 2.26 (m, 1H, H-2), 2.22 (s, 3H, COC*H*$_3$), 2.11 – 2.01 (m, 1H, H-2), 1.72 (s, 3H, COC*H*$_3$), 1.29 (t, *J* = 5.7 Hz, 3H, NCH$_2$C*H*$_3$), 1.16 (s, 3H, 4’’CH$_3$); ^{13}C NMR (126 MHz, CD_2Cl_2): δ 170.1 (C=O), 169.8 (C=O), 150.5 (Ar*C*), 143.3 (C-5’), 128.9 (Ar*C*), 128.8 (Ar*C*), 126.0 (Ar*C*), 125.8 (Ar*C*), 120.6 (Ar*C*), 120.5 (Ar*C*), 98.7 (C-1’’), 97.0 (C-4’), 96.2 (C-1’), 81.5 (C-6), 78.5 (C-4), 74.8 (C-5), 73.6 (C-4’’), 68.9 (C-2’’), 66.0 (C-3’’), 61.6 (C-1), 60.6 (C-3), 54.7 (C-2’), 50.9 (C-6’), 41.9 (N*C*H$_2$), 33.9 (C-2), 30.4 (C-3’), 22.4 (4’’*C*H$_3$), 21.5 (CO*C*H$_3$), 20.6 (CO*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{37}H_{47}BrN_{15}O_9[M+H]^+$ 924.2865; found, 924.2836.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-4'-chloro-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (70). A mixture of K_2CO_3 (123 mg, 0.89 mmol) and compound **67** (150 mg, 0.18 mmol) in dry acetonitrile (2 mL) was dried over 3Å molecular sieves for 1 h, then cooled to 0 °C and the flask wrapped with aluminium foil before iodobenzene dichloride[122] (58.5 mg, 0.21 mmol) was added.

The reaction mixture was stirred for 45 min for complete consumption of the starting material, then was diluted with EtOAc and washed with saturated aqueous $Na_2S_2O_3$ and brine, dried and concentrated. The crude product was purified by gradient chromatography over silica gel (eluent: 15% to 30% EtOAc/hexanes) to yield **70** (45 mg, 30%) as a foam; $[\alpha]_D^{25}$= +17.25 (*c* 0.1, DCM); ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.41 – 7.27 (m, 8H, Ar*H*), 7.19 – 7.13 (m, 2H, Ar*H*), 5.43 (d, *J* = 11.7 Hz, 1H, H-2'') 5.39 – 5.31 (m, 2H, H-1', H-5), 5.08 (d, *J* = 2.9 Hz, 1H, H-1''), 4.19 (d, *J* = 13.9 Hz, 1H, H-6'), 4.16 – 3.97 (m, 2H, H-6, NC*H*$_2$), 3.92 (d, *J* = 13.9 Hz, 1H, H-6'), 3.89 – 3.75 (m, 3H, H-4, H-3'', H-5''), 3.64 – 3.59 (m, 2H, H-3, NC*H*$_2$), 3.56 (ddd, *J* = 11.3, 6.3, 2.5 Hz, 1H, H-2'), 3.34 (d, *J* = 12.4 Hz, 1H, H-5''), 3.15 (s, 3H, NC*H*$_3$), 2.84 (dd, *J* = 16.0, 11.4 Hz, 1H, H-3'), 2.61 (dd, *J* = 16.1, 6.3 Hz, 1H, H-3'), 2.33 – 2.24 (m, 1H, H-2), 2.19 (s, 3H, COC*H*$_3$), 2.08 – 1.99 (m, 1H, H-2), 1.69 (s, 3H, COC*H*$_3$), 1.31 – 1.23 (m, 3H, NCH$_2$C*H*$_3$), 1.14 (s, 3H, 4''C*H*$_3$); ^{13}C NMR (151 MHz, CD_2Cl_2): δ 170.1 (C=O), 169.8 (C=O), 150.5 (Ar*C*), 150.4 (Ar*C*), 142.3 (C-5'), 128.8 (Ar*C*), 128.8 (Ar*C*), 126.0 (Ar*C*), 125.8 (Ar*C*), 120.5 (Ar*C*), 120.5 (Ar*C*), 107.9 (C-4'), 98.7 (C-1''), 96.9 (C-1'), 81.6 (C-6), 78.5 (C-4), 74.8 (C-5), 73.6 (C-4''), 69.3 (C-2''), 68.9 (C-5''), 65.9 (C-3''), 61.5 (C-1), 60.6 (C-3), 54.3 (C-2'), 48.8 (C-6'), 44.0 (N*C*H$_2$), 34.0 (C-2), 28.5 (C-3'), 22.4 (4''*C*H$_3$), 21.5 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 11.8 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{37}H_{46}ClN_{15}NaO_9$ $[M+Na]^+$ 902.3189; found, 902.3177.

4'-Iodonetilmicin pentaacetate salt (72). Substrate **68** (30 mg, 0.03 mmol) was deprotected using the general procedure D to yield **72** (13 mg, 57%) as a white solid; $[\alpha]_D^{25}$= +87.3 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.63 (s, 1H, H-1'), 4.91 (s, 1H, H-1''), 4.09 (d, *J* = 10.8 Hz, 1H, H-2''), 3.95 (d, *J* = 14.6 Hz, 1H, H-6'), 3.81 (d, *J* = 13.4 Hz, 1H, H-5''), 3.79 – 3.68 (m, 3H, H-4, H-2', H-6'), 3.64 – 3.55 (m, 2H, H-5, H-6), 3.37 – 3.28 (m, 3H, H-1, H-3'', H-5''), 3.23 (t, *J* = 10.0 Hz, 1H, H-3), 3.12 (dq, *J* = 13.1, 7.2 Hz, 1H, NC*H*$_2$), 2.97 (dd, *J* = 18.3, 5.3

Hz, 1H, H-3'), 2.92 (dt, *J* = 13.4, 7.3 Hz, 1H, NC*H*$_2$), 2.76 (s, 3H, NC*H*$_3$), 2.67 (dd, *J* = 18.2, 4.1 Hz, 1H, H-3'), 2.44 – 2.35 (m, 1H, H-2), 1.66 (q, *J* = 13.0 Hz, 1H, H-2), 1.18 (s, 3H, 4''C*H*$_3$), 1.14 (t, *J* = 6.6 Hz, 3H, NCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O): δ 180.9 (C=O), 142.6 (C-5'), 101.6 (C-1''), 96.7 (C-1'), 83.2 (C-6), 79.6 (C-4), 73.6 (C-5), 69.8 (C-4''), 68.3 (C-4'), 67.6 (C-5''), 66.4 (C-2''), 63.6 (C-3''), 56.1 (C-1), 48.0 (C-3), 47.9 (C-2'), 43.1 (N*C*H$_2$), 40.8 (C-6'), 36.2 (C-3'), 34.6 (N*C*H$_3$), 26.2 (C-2), 23.0 (CO*C*H$_3$), 20.7 (4''CH3), 10.9 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{21}H_{41}IN_5O_7$ $[M+H]^+$ 602.2051; found, 602.2043.

4'-Bromonetilmicin pentaacetate salt (73). Substrate **69** (40 mg, 0.04 mmol) was deprotected using the general procedure D to yield **73** (16.2 mg, 58%) as a white solid; $[\alpha]_D^{25}$= +50.0 (*c* 1.1, H$_2$O); ^{1}H NMR (600 MHz, D$_2$O): δ 5.56 (s, 1H, H-1'), 4.89 (s, 1H, H-1''), 4.07 (dt, *J* = 10.8, 3.0 Hz, 1H, H-2''), 3.91 (d, *J* = 14.5 Hz, 1H, H-6'), 3.82 (d, *J* = 12.9 Hz, 1H, H-5''), 3.70 – 3.64 (m, 2H, H-6', H-2'), 3.64 – 3.59 (m, 1H, H-4), 3.59 – 3.51 (m, 2H, H-5, H-6), 3.36 – 3.23 (m, 3H, H-1, H-3'', H-5''), 3.12 – 3.01 (m, 2H, H-3, NC*H*$_2$), 2.92 – 2.81 (m, 2H, H-3', NC*H*$_2$), 2.75 (s, 3H, NC*H*$_3$), 2.63 – 2.54 (m, 1H, H-3'), 2.33 – 2.25 (m, 1H, H-2), 1.49 (q, *J* = 12.7, 12.3 Hz, 1H, H-2), 1.17 (s, 3H, 4''C*H*$_3$), 1.11 (t, *J* = 7.2 Hz, 3H, NCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O): δ 181.2 (C=O), 140.3 (C-5'), 101.5 (C-1''), 98.1 (C-4'), 96.8 (C-1'), 83.8 (C-6), 80.8 (C-4), 73.9 (C-5), 69.8 (C-4''), 67.6 (C-5''), 66.5 (C-2''), 63.6 (C-3''), 56.4 (C-1), 48.1 (C-3), 47.3 (C-2'), 40.7 (N*C*H$_2$), 39.5 (C-6'), 34.6 (N*C*H$_3$), 32.6 (C-3'), 27.4 (C-2), 23.1 (CO*C*H$_3$), 20.8 (4''*C*H$_3$), 11.1 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{21}H_{41}BrN_5O_7$ $[M+H]^+$ 554.2189; found, 554.2200.

4'-Chloronetilmicin pentaacetate salt (74). Substrate **70** (37 mg, 0.04 mmol) was deprotected using the general procedure D to yield **74** (19.6 mg, 59%) as a white solid; $[\alpha]_D^{25}$= +41.8 (*c* 1.2, H$_2$O); ^{1}H NMR (600 MHz, D$_2$O): δ 5.56 (s, 1H, H-1'), 4.91 (d, *J* = 3.7 Hz, 1H, H-

1’’), 4.09 (dd, *J* = 10.8, 3.7 Hz, 1H, H-2’’), 3.93 (d, *J* = 14.5 Hz, 1H, H-6’), 3.82 (d, *J* = 12.9 Hz, 1H, H-5’’), 3.79 (t, *J* = 5.9 Hz, 1H, H-2’), 3.77 – 3.73 (m, 1H, H-4), 3.65 (d, *J* = 14.5 Hz, 1H, H-6’), 3.63 – 3.55 (m, 2H, H-5, H-6), 3.36 –3.28 (m, 3H, H-1, H-3’’, H-5’’), 3.20 (td, *J* = 11.4, 10.7, 3.9 Hz, 1H, H-3), 3.11 (dq, *J* = 14.2, 7.1 Hz, 1H, NC*H*$_2$), 2.91 (dq, *J* = 14.4, 7.2 Hz, 1H, NC*H*$_2$), 2.84 (dd, *J* = 18.1, 6.1 Hz, 1H, H-3’), 2.76 (s, 3H, NC*H*$_3$), 2.52 (dd, *J* = 18.0, 4.8 Hz, 1H, H-3’), 2.38 (dt, *J* = 12.4, 4.1 Hz, 1H, H-2), 1.63 (q, *J* = 12.5 Hz, 1H, H-2), 1.18 (s, 3H, 4’’C*H*$_3$), 1.13 (t, *J* = 7.2 Hz, 3H, NCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O): δ 181.1 (C=O), 139.7 (C-5’), 110.0 (C-4’), 101.5 (C-1’’), 96.8 (C-1’), 83.3 (C-6), 80.0 (C-4), 73.6 (C-5), 69.8 (C-4’’), 67.6 (C-5’’), 66.4 (C-2’’), 63.6 (C-3’’), 56.2 (C-1), 48.0 (C-3), 46.9 (C-2), 40.8 (N*C*H$_2$), 37.4 (C-6’), 34.6 (N*C*H$_3$), 30.7 (C-3’), 26.4 (C-2), 23.0 (CO*C*H$_3$), 20.8 (4’’*C*H$_3$), 10.9 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{21}H_{41}ClN_5O_7$ $[M+H]^+$ 510.2695; found, 510.2687.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-4'-phenyl-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (75). A round bottom flask was charged with compound **69** (146 mg, 0.16 mmol), K_2CO_3 (66 mg, 0.47 mmol), phenylboronic acid (29 mg, 0.24 mmol) and Pd(dppf)Cl_2 (10 mol%) then evacuated and and flushed with argon three times. Dry dioxane (3 mL), dried over 5 Å MS, was added and the reaction mixture was heated at 80 °C with stirring for 36 h. The reaction mixture was concentrated and purified by gradient chromatography over silica gel (eluent: 3% to 5% IPA in hexanes) to give **75** in 23% yield; $[\alpha]_D^{25}$= +54.6 (*c* 0.3, DCM); ^{1}H NMR (500 MHz, CD_2Cl_2): δ 7.45 – 7.23 (m, 13H, Ar*H*), 7.22 – 7.13 (m, 2H, Ar*H*), 5.47 (dd, *J* = 11.7, 2.3 Hz, 1H, H-2’’), 5.44 (d, *J* = 2.5 Hz, 1H, H-1’), 5.40 (t, *J* = 9.3 Hz, 1H, H-5), 5.12 (d, *J* = 3.0 Hz, 1H, H-1’’), 4.21 – 3.99 (m, 3H, H-6, H-3’’, NC*H*$_2$), 3.96 (t, *J* = 9.9 Hz, 1H, H-4), 3.88 (d, *J* = 13.5 Hz, 1H, H-6’), 3.84 (d, *J* = 12.1 Hz, 1H, H-5’’), 3.78 (d, *J* = 13.4 Hz, 1H, H-6’), 3.72 – 3.64 (m, 2H, H-3, NC*H*$_2$), 3.63 (ddd, *J* = 11.4, 6.0, 2.6 Hz, 1H, H-2’), 3.38 (d, *J* = 12.4 Hz, 1H, H-5’’), 3.18

(s, 3H, NC*H*$_3$), 2.90 (dd, *J* = 15.9, 11.7 Hz, 1H, H-3'), 2.60 (dd, *J* = 16.1, 6.0 Hz, 1H, H-3'), 2.40 – 2.29 (m, 1H, H-2), 2.25 (s, 3H, COC*H*$_3$), 2.18 – 2.08 (m, 1H, H-2), 1.73 (s, 3H, COC*H*$_3$), 1.37 – 1.25 (m, 3H, NCH$_2$C*H*$_3$), 1.17 (s, 3H, 4''CH$_3$); ^{13}C NMR (126 MHz, CD_2Cl_2): δ 170.1 (C=O), 169.9 (C=O), 150.5 (Ar*C*), 142.4 (C-5'), 138.4 (Ar*C*), 128.9 (Ar*C*), 128.8 (Ar*C*), 128.6 (Ar*C*), 128.4 (Ar*C*), 127.4 (Ar*C*), 126.0 (Ar*C*), 125.8 (Ar*C*), 120.6 (Ar*C*), 113.0 (C-4'), 98.6 (C-1''), 96.9 (C-1'), 81.6 (C-6), 78.2 (C-4), 75.0 (C-5), 73.6 (C-4''), 68.9 (C-5''), 66.0 (C-3''), 61.7 (C-1), 60.6 (C-3), 54.6 (C-2'), 49.9 (C-6'), 33.9 (N*C*H$_2$), 26.3 (C-3'), 22.4 (4''*C*H$_3$), 21.5 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 11.9 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{43}H_{52}N_{15}O_9$ $[M+H]^+$ 922.4072; found, 922.4070.

5,2'''-Di-*O*-acetyl-3,2',6'-triazido-4'-butyl-1,3''-bis(phenyltriaz-2-en-1-yl) netilmicin (76). A round bottom flask was charged with compound **69** (300 mg, 0.32 mmol), cesium carbonate (527 mg, 1.62 mmol), potassium butyltrifluoroborate (106 mg, 0.64 mmol) and Pd(dppf)Cl_2 (10 mol%) then evacuated and flushed with argon three times. Degassed toluene (2 mL) and degassed water (1 mL) were added and the reaction mixture was heated at 90 °C for 12 h under argon. On completion, the reaction mixture was cooled and directly loaded in a silica gel column and eluted with 15% acetone in hexanes to give **76** in 8% yield as white solid; $[\alpha]_D^{25}$= +23.14 (*c* 0.5, DCM); ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.41 – 7.24 (m, 8H, Ar*H*), 7.21 – 7.11 (m, 2H, Ar*H*), 5.43 (d, *J* = 11.6 Hz, 1H, H-2''), 5.32 – 5.29 (m, 1H, H-5), 5.27 (d, *J* = 2.4 Hz, 1H, H-1'), 5.07 (d, *J* = 2.8 Hz, 1H, H-1''), 4.23 – 4.00 (m, 3H, H-6, H-3'', NC*H*$_2$), 3.96 (d, *J* = 13.7 Hz, 1H, H-6'), 3.87 – 3.79 (m, 2H, H-4, H-5''), 3.77 (d, *J* = 13.6 Hz, 1H, H-6'), 3.68 – 3.60 (m, 1H, NC*H*$_2$), 3.60 – 3.54 (m, 1H, H-3), 3.40 (dq, *J* = 8.7, 2.8, 2.3 Hz, 1H, H-2'), 3.34 (d, *J* = 12.3 Hz, 1H, H-5''), 3.14 (s, 3H, NC*H*$_3$), 2.45 (dd, *J* = 16.0, 11.8 Hz, 1H, H-3'), 2.27 (m, 2H, H-2, H-3'), 2.19 (s, 3H, COC*H*$_3$), 2.11 (m, 1H, C*H*$_2$CH$_2$CH$_2$CH$_3$), 2.07 – 1.99 (m, 1H, H-2), 1.95 (M, 1H,

C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.69 (s, 3H, COC*H*$_3$), 1.46 – 1.23 (m, 7H, CH$_2$C*H*$_2$CH$_2$CH$_3$, CH$_2$CH$_2$C*H*$_2$CH$_3$, NCH$_2$C*H*$_3$), 1.13 (s, 3H, 4’’C*H*$_3$), 0.91 (t, *J* = 5.7 Hz, 3H, CH$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, CD$_2$Cl$_2$): δ 169.9 (C=O), 150.4 (Ar*C*), 139.5 (C-5), 128.8 (Ar*C*), 128.8 (Ar*C*), 125.9 (Ar*C*), 125.8 (Ar*C*), 120.5 (Ar*C*), 111.3 (C-4’), 98.5 (C-1’’), 96.6 (C-1’), 81.53 (C-6), 77.9 (C-4), 74.9 (C-5), 73.6 (C-4’’), 68.9 (C-5’’), 65.9 (C-3’’), 61.6 (C-1), 60.6 (C-3), 54.7 (C-2’), 48.6 (C-6’), 43.9 (N*C*H$_2$), 34.1 (C-2), 31.3 (*C*H$_2$CH$_2$CH$_2$CH$_3$), 30.9 (CH$_2$*C*H$_2$CH$_2$CH$_3$), 23.9 (C-3’), 22.5 (CH$_2$CH$_2$*C*H$_2$CH$_3$), 22.4 (4’’*C*H$_3$), 21.5 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 13.7 (CH$_2$CH$_2$CH$_2$*C*H$_3$), 11.8 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for C$_{41}$H$_{56}$N$_{15}$O$_9$ [M+H]$^+$ 902.4385; found, 902.4344.

4'-Phenylnetilmicin pentaacetate salt (78). Substrate **75** (29 mg, 0.03 mmol) was deprotected using the general procedure D to yield **78** (14 mg, 54%) as a white solid; [α]$_D^{25}$= +64.09 (*c* 0.5, H$_2$O); 1H NMR (600 MHz, D$_2$O): δ 7.36 – 7.26 (m, *J* = 26.9, 7.3 Hz, 3H, Ar*H*), 7.14 (d, *J* = 7.5 Hz, 2H, Ar*H*), 5.66 (s, 1H, H-1’), 4.95 (d, *J* = 3.7 Hz, 1H, H-1’’), 4.12 (dd, *J* = 10.7, 3.7 Hz, 1H, H-2’’), 3.92 – 3.83 (m, 2H, H-2’, H-5’’), 3.83 – 3.77 (m, 1H, H-4), 3.69 – 3.63 (m, 2H, H-5, H-6), 3.61 (d, *J* = 14.4 Hz, 1H, H-6’), 3.53 (d, *J* = 14.3 Hz, 1H, H-6’), 3.41 – 3.32 (m, 3H, H-1, H-3’’, H-5’’), 3.22 (td, *J* = 11.9, 4.2 Hz, 1H, H-3), 3.15 (dq, *J* = 14.5, 7.2 Hz, 1H, NC*H*$_2$), 2.95 (dq, *J* = 14.7, 7.3 Hz, 1H, NC*H*$_2$), 2.86 (dd, *J* = 18.2, 5.8 Hz, 1H, H-3’), 2.79 (s, 3H, NC*H*$_3$), 2.55 (dd, *J* = 18.3, 5.2 Hz, 1H, H-3’), 2.41 (dt, *J* = 12.3, 4.0 Hz, 1H, H-2), 1.70 – 1.61 (m, 1H, H-2), 1.21 (s, 3H, 4’’C*H*$_3$), 1.17 (t, *J* = 7.2 Hz, 3H, NCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O): δ 180.9 (C=O), 139.1 (C-5’), 136.5 (Ar*C*), 129.0 (Ar*C*), 128.5 (Ar*C*), 128.3 (Ar*C*), 114.7 (C-4’), 101.6 (C-1’’), 96.3 (C-1’), 83.5 (C-6), 80.1 (C-4), 73.9 (C-5), 69.9 (C-4’’), 67.7 (C-5’’), 66.5 (C-2’’), 63.6 (C-3’’), 56.4 (C-1), 48.2 (C-3), 46.7 (C-2’), 40.8 (N*C*H$_2$), 37.9 (C-6’), 34.6

(N*C*H$_3$), 29.2 (C-3'), 26.8 (C-2), 23.0 (CO*C*H$_3$), 20.8 (4''CH3), 11.0 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{27}H_{46}N_5O_7$ $[M+H]^+$ 552.3397; found, 552.3391.

4'-Butylnetilmicin pentaacetate salt (79). Substrate **76** (27 mg, 0.03 mmol) was deprotected using the general procedure D to yield **79** (7 mg, 27%) as a white solid;; $[\alpha]_D^{25}$= +38.02 (*c* 0.3, H_2O); 1H NMR (600 MHz, D_2O): δ 5.32 (s, 1H, H-1'), 4.86 (d, *J* = 3.7 Hz, 1H, H-1''), 3.93 (dd, *J* = 10.7, 3.6 Hz, 1H, H-2''), 3.86 (d, *J* = 12.8 Hz, 1H, H-5''), 3.69 (d, *J* = 14.2 Hz, 1H, H-6''), 3.49 (t, *J* = 9.1 Hz, 1H, H-6), 3.44 (d, *J* = 14.4 Hz, 1H, H-6''), 3.41 – 3.37 (m, 1H, H-4), 3.35 (t, *J* = 9.6 Hz, 1H, H-5), 3.30 – 3.22 (m, 2H, H-5'', H-2'), 3.01 (d, *J* = 10.9 Hz, 1H, H-3''), 2.98 – 2.90 (m, 1H, H-1), 2.89 – 2.80 (m, 1H, NC*H*$_2$), 2.80 – 2.71 (m, 1H, H-3), 2.69 – 2.57 (m, 4H, NC*H*$_3$, NC*H*$_2$), 2.19 (dd, *J* = 17.1, 5.9 Hz, 1H, H-3'), 2.11 (dt, *J* = 12.9, 3.9 Hz, 1H, H-2), 2.06 (dd, *J* = 17.6, 9.3 Hz, 1H, H-3'), 1.96 (dt, *J* = 14.4, 7.4 Hz, 1H, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.91 – 1.85 (m, 1H, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.28 – 1.15 (m, 3H, CH$_2$C*H*$_2$CH$_2$CH$_3$, H-2), 1.14 (s, 3H, 4''C*H*$_3$), 1.10 (dq, *J* = 14.8, 7.6 Hz, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 1.03 (t, *J* = 7.1 Hz, 3H, NCH$_2$C*H*$_3$), 0.71 (t, *J* = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D_2O): δ 181.4 (C=O), 135.9 (C-5'), 113.4 (C-4'), 101.3 (C-1''), 96.8 (C-1'), 84.9 (C-5), 81.4 (C-4), 74.4 (C-6), 70.5 (C-4''), 67.5 (C-5''), 67.4 (C-2''), 63.7 (C-3''), 56.9 (C-1), 48.7 (C-3), 46.5 (C-2'), 40.5 (N*C*H$_2$), 37.0 (C-6'), 35.2 (N*C*H$_3$), 30.0 (*C*H$_2$CH$_2$CH$_2$CH$_3$), 29.8 (CH$_2$*C*H$_2$CH$_2$CH$_3$), 29.7 (C-2), 27.5 (C-3'), 23.1 (CO*C*H$_3$), 21.6 (CH$_2$CH$_2$*C*H$_2$CH$_3$), 20.9 (4''CH3), 13.0 (CH$_2$CH$_2$CH$_2$*C*H$_3$), 12.1 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{25}H_{50}N_5O_7$ $[M+H]^+$ 532.3710; found, 532.3694.

1,3,2',6',3''-Penta(trichloroethyloxycarbonyl) netilmicin (80). A stirred solution of netilmicin sulfate (400 mg, 0.55 mmol) in 1:1 dioxane:water (10 mL) was ice-cooled and treated with Na_2CO_3 (883 mg, 8.33 mmol) and 2,2,2-trichloroethyl chloroformate (0.46 mL, 3.33 mmol). The reaction mixture was stirred at rt for 4 h. After completion, the reaction mixture was

concentrated *in vacuo* then diluted with EtOAc and washed with brine, dried and concentrated. The crude product was purified by silica gel column chromatography eluting with 4% methanol/DCM to give **80** (722 mg, 96%) as a white solid; ESI-HRMS: *m/z* calcd. for $C_{36}H_{46}Cl_{15}N_5NaO_{17}$ $[M+Na]^+$ 1367.8114; found, 1367.8109.

5,2'''-Di-*O*-acetyl-1,3,2',6',3''-penta(trichloroethyloxycarbonyl) netilmicin (81). A stirred solution of compound **80** (1.06 g, 0.78 mmol) in pyridine (10 mL) was cooled to 0 °C and treated with acetic anhydride (1 mL). The resulting solution was stirred at rt for 12 h after which it was diluted with EtOAc and the organic layer was washed with aqueous $NaHCO_3$ followed by brine, dried with Na_2SO_4, and concentrated. The crude product was purified via silica gel chromatography eluting with 20% to 35 % EtOAc in hexanes to give **81** (880 mg, 78%) as a white solid; ESI-HRMS: *m/z* calcd. for $C_{40}H_{50}Cl_{15}N_5NaO_{19}$ $[M+Na]^+$ 1367.8114; found, 1367.8109.

5,2'''-Di-*O*-acetyl-1,3,2',6',3''-penta(trichloroethyloxycarbonyl)-4'-(ethylsulfanyl)-netilmicin (82). Ethanesulfanyl chloride was freshly prepared for the reaction as described. Diethyl disulfide (1.22 mL, 10 mmol) was dissolved in dry DCM (20 mL) and cooled to -30 °C before sulfuryl chloride (0.80 mL, 10 mmol) was added dropwise. The resulting ethanesulfanyl chloride solution (0.52 mL, 0.52 mmol) was added to a stirred solution of **81** (500 mg, 0.35 mmol) previously cooled to -50 °C. The reaction mixture was stirred for 1 h before DBU (78 μL, 0.52 mmol) was added, then it was stirred for an additional 4 h. After completion, the reaction mixture was diluted with DCM and washed with aqueous $NaHCO_3$ followed by brine, dried, and concentrated. The crude product was purified via silica gel chromatography eluting with 20% to 30 % EtOAc in hexanes to give **82** (225 mg, 43%) as a white foam; ESI-HRMS: *m/z* calcd. for $C_{42}H_{54}Cl_{15}N_5NaO_{19}S$ $[M+Na]^+$ 1511.8359; found, 1511.8390.

4'-(Ethylsulfanyl) netilmicin pentaacetate salt (83). Compound **82** (100 mg, 0.066 mmol) was suspended in 6N NaOH (4 mL) in a closed vial and heated to 120 °C for 4 h during which the reaction mixture become clear. Upon completion, the reaction mixture was neutralized by 12 N H_2SO_4 till pH = 9 before it was lyophilized. The resulting solid residue was extracted with isopropyl alcohol (10 mL) and concentrated. The crude product was desalted and purified using Sephadex column (elution: D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water). The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **83** (8 mg, 15%) as the pentaacetate salt in the form of white solid; $[\alpha]_D^{25}$= +87.4 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.54 (s, 1H, H-1'), 4.89 (d, *J* = 3.5 Hz, 1H, H-1''), 4.15 (d, *J* = 14.3 Hz, 1H, H-6'), 4.07 (dd, *J* = 10.8, 3.6 Hz, 1H, H-2''), 3.81 (d, *J* = 12.9 Hz, 1H, H-5''), 3.75 (t, *J* = 5.7 Hz, 1H, H-2'), 3.73 – 3.65 (m, 2H, H-6', H-4), 3.60 – 3.54 (m, 2H, H-5, H-6), 3.37 – 3.25 (m, 3H, H-1, H-3'', H-5''), 3.16 – 3.05 (m, 2H, H-3, NC*H*$_2$), 2.94 – 2.84 (m, 1H, NC*H*$_2$), 2.74 (s, 4H, H-3', NC*H*$_3$), 2.51 (hept, *J* = 6.5, 5.9 Hz, 2H, SC*H*$_2$CH$_3$), 2.42 (dd, *J* = 18.0, 5.5 Hz, 1H, H-3'), 2.34 (dt, *J* = 14.1, 4.3 Hz, 1H, H-2), 1.62 – 1.51 (m, 1H, H-2), 1.16 (s, 3H, 4''C*H*$_3$), 1.11 (t, *J* = 7.2 Hz, 3H, NCH$_2$C*H*$_3$), 1.00 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, D_2O): δ 181.1(C=O), 145.2 (C-5''), 106.1 (C-4''), 101.5 (C-1''), 96.5 (C-1'), 83.4 (C-6), 80.2 (C-4), 73.7 (C-5), 69.8 (C-4''), 67.6 (C-5''), 66.4 (C-2''), 63.5 (C-3''), 56.3 (C-1), 48.0 (C-3), 46.8 (C-2'), 40.7 (N*C*H$_2$), 38.2 (C-6'), 34.5 (N*C*H$_3$), 28.4 (C-3'), 26.7 (C-2), 26.0 (S*C*H$_2$CH$_3$), 23.0 (CO*C*H$_3$), 20.7 (4''CH3), 14.0 (SCH$_2$*C*H$_3$), 10.9 (NCH$_2$*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{23}H_{46}N_5O_7S$ $[M+H]^+$ 536.3118; found, 536.3099.

6'-*N*-(*p*-Nitrobenzyloxycarbonyl) sisomicin (84). Sisomicin sulfate **8** (2.00 g, 2.88 mmol) was added to a stirred suspension of Amberlite IRA-400 (OH form) (20 g) in methanol (15 mL) and stirred overnight. The suspension was filtered from the resin and the filtrate was

concentrated to give sisomicin free base (1.54 g). A solution of sisomicin free base (1.29 g) in methanol (30 mL) was treated with $Zn(AcO)_2.2H_2O$ (1.90 g, 8.67 mmol) and stirred for 1 h. A solution of N-(4-nitrobenzyloxycarbonyloxy) succinimide[128, 182] (0.85 g, 2.88 mmol) in DCM (10 mL) was added using syringe pump over 3h and the reaction mixture was left to stir overnight. The reaction mixture was concentrated to dryness then dissolved in 10% aqueous NH_4OH solution (8 mL). The aqueous layer was washed with DCM (3 mL) four times and extracted with 30% IPA in DCM which was washed with a 10% NH_4OH:brine (7:3) mixture. The organic layer was dried over Na_2SO_4, filtered and dried to give **84** (1.35 g, 75%) as a foam; *Rf* = 0.55 (DCM:MeOH:NH_4OH = 3:2:1); $[\alpha]_D^{25}$ = +101.1 (*c* = 0.90, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.49 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 5.44 (s, 1H, H-1'), 5.21-5.15 (m, 2H, C*H*$_2$-4-$NO_2C_6H_4$), 4.90 (d, *J* = 2.6 Hz, 1H, H-1''), 4.80 (d, *J* = 2.9 Hz, 1H, H-4'), 3.89 (dd, *J* = 13.9 Hz, 5.9 Hz, 1H, H-6'), 3.79 (d, *J* = 12.1 Hz, 1H, H-5''), 3.57-3.44 (m, 5H, H-2'', H-4, H-5, H-5'', H-6'), 3.07-3.04 (m, 1H, H-2'), 3.01 (t, *J* = 9.4 Hz, 1H, H-6), 2.82-2.77 (m, 2H, H-1, H-3), 2.57 (s, 3H, NC*H*$_3$), 2.45 (d, *J* = 9.9 Hz, 1H, H-3''), 2.16-2.12 (m, 1H, H-3'), 2.00-1.91 (m, 2H, H-2, H-3'), 1.23-1.15 (m, 1H, H-2), 1.13 (s, 3H, 4''-C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.2 (s, C=O), 147.4 (s, C-5'), 145.8, 144.5, 128.0, 123.7 (s, arom.), 101.2 (s, C-1''), 99.1 (s, C-1'), 98.3 (s, C-4'), 89.5 (s, C-6), 82.6 (s, C-4), 75.6 (s, C-5), 71.1 (s, C-4''), 69.4 (s, C-2''), 67.3 (s, C-5''), 65.2 (s, C-3''), 64.8 (s, *C*H_2-4-$NO_2C_6H_4$), 50.6 (s, C-1), 49.7 (s, C-3), 47.0 (s, C-2'), 43.5 (s, C-6'), 39.3 (s, C-2), 38.7 (s, C- N*C*H_3), 25.9 (s, C-3'), 24.2 (s, C-4''-*C*H_3); ESI-HRMS: *m/z* calcd. for $C_{27}H_{43}N_6O_{11}$ $[M+H]^+$ 627.2990, found: 627.2971.

6'-*N*-(*p*-Nitrobenzyloxycarbonyl)-2',3-di-*N*-(*tert*-butyloxycarbonyl) sisomicin (85). A solution of **84** (1.00 g, 1.60 mmol) and $Zn(AcO)_2.2H_2O$ (1.05 g, 8.67 mmol) in methanol (20 mL) was stirred for 1 h before a solution of *N*-(*tert*-butoxycarbonyloxy)succinimide (0.65 g, 3.02

mmol) in THF (10 mL) was added using a syringe pump over 4 h. After stirring overnight (TEA 0.17 mL) was added, followed by *N*-(*tert*-butoxycarbonyloxy)succinimide (0.18 g, 0.81 mmol) in THF (2 mL) and the resulting solution was stirred for 24 h. The reaction mixture was quenched by addition of glycine (0.48 g, 6.4 mmol) and evaporated to dryness. The crude product was dissolved in DCM (50 mL) and washed with 30% aqueous NH_4OH and dried over Na_2SO_4 and concentrated. The residue was purified by chromatography over silica gel (eluent: gradient of 6% to 8% of ammonical methanol in dichloromethane) to give **85** (0.69 g, 52%) as a white solid *Rf*= 0.5 (25% ammonical MeOH in DCM); $[\alpha]_D^{25}$= +131.7 (*c* = 0.25, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.21 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.58 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 5.40 (s, 1H, H-1'), 5.26-5.17 (m, 2H, C*H*$_2$-4-$NO_2C_6H_4$), 4.95 (d, *J* = 3.7 Hz, 1H, H-1"), 4.73 (br s, 1H, H-4'), 3.97 (d, *J* = 12.1 Hz, 1H, H-5"), 3.87 (d, *J* =15.4, 1H, H-6'), 3.75 (dd, *J* = 10.5 Hz, 3.5 Hz, 1H, H-2"), 3.69 (t, J = 7.9, 1H, H-5), 3.65 (d, *J* = 15.4, 1H, H-6'), 3.50 (t, *J* = 8.1, 1H, H-5), 3.45 (m, 2H, H-3, H-4), 3.32 (d, *J* = 12.1 Hz, 1H, H-5"), 3.08 (t, *J* = 9.4, 1H, H-6), 2.82 (t, *J* = 8.4, 1H, H-1), 2.57 (s, 3H, NC*H*$_3$), 2.53 (d, *J* = 10.3, 1H, H-3"), 2.08-2.03 (m, 2H, H-3'), 1.99 (br d, *J* =11.4, 1H, H-2), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.26(m, 1H, H-2), (, *J* =, H, H-'), 1.16 (s, 3H, 4"-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 157.0, 156.5, 156.3(s, C=O), 147.4 (s, arom.), 146.4 (s, C-5'), 144.7 (s, arom.) 127.6, 123.2 (s, arom.), 101.0 (s, C-1"), 97.0 (s, C-1'), 95.9 (s, C-4'), 88.6 (s, C-6), 80.2 (s, C-4), 78.8 (s, *C*(CH$_3$)$_3$), 75.6 (s, C-5), 71.4 (s, C-4"), 69.2 (s, C-2"), 68.0 (s, C-5"), 64.7 (s, *C*H$_2$-4-$NO_2C_6H_4$), 64.0 (s, C-3"), 50.3 (s, C-1), 49.3 (s, C-3), 46.9 (s, C-2'), 42.6 (s, C-6'), 36.9 (s, N*C*H$_3$), 35.4 (s, C-2), 27.4 (s, C(*C*H$_3$)$_3$), 27.3 (s, C(*C*H$_3$)$_3$), 22.1 (s, C-3'), 22.0 (s, C-4"-*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{37}H_{59}N_6O_{15}$ $[M+H]^+$ 827.4038, found: 827.4023.

6'-*N*-(*p*-Nitrobenzyloxycarbonyl)-3''-*N*-phenylazo-2',3-di-*N*-(*tert*-butyloxycarbonyl) sisomicin (86). A stirred solution of **85** (675 mg, 0.82 mmol) in acetonitrile:H_2O (1:1, 14 mL) was treated with K_2CO_3 (1.13 g, 8.17 mmol) and cooled to 0 ^oC in an ice bath. A solution of phenyldiazonium tetrafluoroborate (173 mg, 0.90 mmol) in acetonitrile (7 mL) was added using a syringe pump over 1 h at 0 ^oC, after which the reaction was complete. The reaction mixture was diluted with ethyl acetate and washed with water, and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography over silica gel (eluent: gradient of 6% to 10% of methanol in dichloromethane) to give **86** (0.67 g, 88%) as a buff gum; *Rf* = 0.3 (10% MeOH in DCM); $[\alpha]_D^{25}$ = +127.6 (*c* = 2.7, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.22 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.58 (d, *J* = 8.1 Hz, 2H, PNZ-*o*Hs), 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.7 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.41 (s, 1H, H-1'), 5.26-5.17 (m, 2H, C*H*$_2$-4-$NO_2C_6H_4$), 5.16 (d, *J* = 3.7 Hz, 1H, H-1''), 4.73 (br s, 1H, H-4'), 4.44 (dd, *J* = 11 Hz, 3.7 Hz, 1H, H-2''), 4.21 (d, *J* = 11 Hz, 1H, H-3''), 4.13 (d, *J* =11.7, 1H, H-5''), 3.88 (d, *J* = 15.4, 1H, H-6'), 3.71 (t, *J* = 8.1, 1H, H-2'), 3.65 (d, *J* = 15.4, 1H, H-6'), 3.56 (m, 1H, H-5), 3.46 (m, 2H, H-3, H-4), 3.38 (d, *J* =11.7, 1H, H-5''), 3.31(s, 3H, NC*H*$_3$), 3.17 (t, *J* = 9.4, 1H, H-6), 2.88 (t, *J* = 8.4, 1H, H-1), 2.10-2.06 (m, 2H, H-3'), 1.99 (br d, *J* =13.2, 1H, H-2), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.41 (s, 9H, C(C*H*$_3$)$_3$), 1.31-1.25(m, 1H, H-2), 1.09 (s, 3H, 4''-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 157.1, 156.5, 156.4(s, C=O), 151.0, 147.4 (s, arom.), 146.5 (s, C-5'), 144.7, 128.5, 128.3, 127.7, 127.6, 124.9, 123.2, 120.2 (s, arom.), 101.5 (s, C-1''), 97.1 (s, C-1'), 95.9 (s, C-4'), 88.4 (s, C-6), 80.2 (s, C-4), 78.9 (s, *C*$(CH_3)_3$), 75.6 (s, C-5), 72.9 (s, C-4''), 69.1 (s, C-5''), 68.0 (s, C-3''), 65.6 (s, C-2''), 64.7 (s, *C*H_2-4-$NO_2C_6H_4$), 50.4 (s, C-1), 49.3 (s, C-3), 46.9 (s, C-2'), 42.6 (s, C-6'), 35.4 (s, C-2), 32.6 (s, N*C*H_3), 27.4 (s, C(*C*H_3)$_3$),

27.2 (s, C(CH$_3$)$_3$), 22.1 (s, C-3'), 21.2 (s, C-4"-CH$_3$); ESI-HRMS: *m/z* calcd. for $C_{43}H_{63}N_8O_{15}$ $[M+H]^+$ 931.4413, found: 931.4388.

1-*N*-(*N*-*tert*-butyloxycarbonyl-4-amino-2(S)-hydroxybutyryl)-6'-*N*-(*p*-nitrobenzyloxycarbonyl)-3''-*N*-phenylazo-2',3-di-*N*-(tert-butyloxycarbonyl) sisomicin (87). A solution of *N*-Boc-4-amino-2(S)-hydroxy-butyric acid (15.3 mg, 0.069 mmol), HONB[183] (12.5 mg, 0.069 mmol), DIPEA (18.0 mg, 0.14 mmol) and EDCI (13.4 mg, 0.069 mmol) in DMF (0.2 mL) was stirred for 0.5 h before **86** (50 mg, 0.053 mmol) in DMF (2 mL) was added. Stirring was continued for 24 h at room temperature before the reaction mixture was cooled to 0°C, quenched with saturated aqueous $NaHCO_3$ (0.5 mL), and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified via silica gel chromatography eluting with 25% to 40% acetone in hexanes to give **87** (54 mg, 89%) as a white foam; *Rf* = 0.3 (50% Acetone in hexanes); $[\alpha]_D^{25}$ = +83.0 (*c* = 1.4, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 8.23 (d, *J* = 8.4 Hz, 2H, PNZ-*m*Hs), 7.59 (d, *J* = 8.4 Hz, 2H, PNZ-*o*Hs), 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.9 Hz, 2H, Ph-*m*Hs), 7.09 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.40 (s, 1H, H-1'), 5.27-5.18 (m, 3H, CH_2-4-$NO_2C_6H_4$, H-1"), 4.74 (br s, 1H, H-4'), 4.34 (dd, *J* = 11.2 Hz, 3.5 Hz, 1H, H-2"), 4.19 (m, 2H, H-3", H-5"), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.88 (m, 2H, H-1, H-6'), 3.71 (t, *J* = 8.1, 1H, H-2'), 3.68-3.60 (m, 3H, H-5, H-6, H-6'), 3.51-3.47 (m, 2H, H-3, H-4), 3.33 (d, *J* = 12.1, 1H, H-5"), 3.29(s, 3H, NCH_3), 3.13-3.00 (m, 2H, CH(OH)CH_2CH_2), 2.11-2.00 (m, 3H, H-3', H-2), 1.85-1.82 (m, 1H, CH(OH)CH_2CH_2), 1.73-1.67 (m, 1H, CH(OH)CH_2CH_2), 1.54-1.51 (m, 1H, H-2), 1.42- 1.40 (m, 27H, C(CH_3)$_3$), 1.08 (s, 3H, 4"-CH_3); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.2, 157.1, 156.4 (s, C=O), 151.0, 147.5 (s, arom.), 146.5 (s, C-5'), 144.7, 128.3, 127.6, 124.9, 123.2, 120.2 (s, arom.), 95.6 (s, C-1"), 97.2 (s, C-1'),

96.0 (s, C-4'), 81.5 (s, C-6), 79.8 (s, C-4), 79.0, 78.6 (s, *C*$(CH_3)_3$), 75.9 (s, C-5), 73.0 (s, C-4"), 69.4 (s, *C*H(OH)CH_2CH_2), 69.1 (s, C-5"), 68.6 (s, C-3"), 65.4 (s, C-2"), 64.7 (s, *C*H_2-4-$NO_2C_6H_4$), 49.2 (s, C-3), 49.0 (s, C-1), 47.0 (s, C-2'), 42.6 (s, C-6'), 36.3 (s, CH(OH)CH_2*C*H_2), 33.9 (s, CH(OH)*C*H_2CH_2), 33.7 (s, C-2), 32.8 (s, N*C*H_3), 27.4 (s, C(*C*$H_3)_3$), 27.2 (s, C(*C*$H_3)_3$), 22.1 (s, C-3'), 21.2 (s, C-4"-*C*H_3); ESI-HRMS: *m/z* calcd. for $C_{52}H_{77}N_9NaO_{19}$ $[M+Na]^+$ 1154.5233, found: 1154.5212.

1-*N*-(*N*-tert-Butyloxycarbonyl-4-amino-2(S)-hydroxybutyryl)-3''-*N*-phenylazo-2',3-di-*N*-(*tert*-butyloxycarbonyl) sisomicin (88). A stirred solution of **87** (50 mg, 0.08 mmol) in 1,4-dioxane (1 mL) was treated with 1 N aqueous NaOH (1 mL) and heated to 40 ^oC for 14 h. The reaction mixture was diluted with H_2O (2 mL) and extracted with ethyl acetate twice. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography over silica gel (eluent: gradient of 6% to 15% of ammonical methanol in dichloromethane) to give **88** (38 mg, 70%) as a white solid; *Rf* = 0.5 (20% ammonical MeOH in DCM); $[\alpha]_D^{25}$ = +116.5 (*c* = 0.086, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.9 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.2 Hz, 1H, Ph-*p*H), 5.44 (s, 1H, H-1'), 5.20 (d, *J* = 3.3, H-1"), 4.72 (br s, 1H, H-4'), 4.35 (dd, *J* = 11.4 Hz, 3.7 Hz, 1H, H-2"), 4.23-4.19 (m, 2H, H-3", H-5"), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.90 (m, 1H, H-1), 3.75-3.69 (m, 2H, H-6, H-2'), 3.65-3.60 (m, 1H, H-5), 3.55 (t, 1H, *J* = 9.4, H-4), 3.51-3.45 (m, 1H, H-3), 3.33 (d, *J* =12.1, 1H, H-5"), 3.29(s, 3H, NC*H*$_3$), 3.23-3.15 (m, 2H, H-6'), 3.13-3.02 (m, 2H, CH(OH)CH_2C*H*$_2$), 2.14-2.00 (m, 3H, H-3', H-2), 1.88-1.82 (m, 1H, CH(OH)C*H*$_2CH_2$), 1.73-1.67 (m, 1H, CH(OH)C*H*$_2CH_2$), 1.57-1.51 (m, 1H, H-2), 1.42- 1.40 (m, 27H, C(C*H*$_3)_3$), 1.08 (s, 3H, 4"-C*H*$_3$); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.1, 156.4, 156.3 (s, C=O), 151.0 (s, arom.), 149.4 (s, C-5'), 128.3, 124.9, 120.2 (s,

arom.), 95.6 (s, C-1''), 97.3 (s, C-1'), 95.2 (s, C-4'), 81.5 (s, C-6), 80.1 (s, C-4), 78.9, 78.6 (s, *C*(CH_3)$_3$), 75.9 (s, C-5), 73.0 (s, C-4''), 69.4 (s, *C*H(OH)CH_2CH_2), 69.1 (s, C-5''), 68.6 (s, C-3''), 65.4 (s, C-2''), 49.2 (s, C-3), 49.0 (s, C-1), 47.0 (s, C-2'), 43.4 (s, C-6'), 36.4 (s, CH(OH)CH_2*C*H_2), 33.9 (s, CH(OH)*C*H_2CH_2), 33.7 (s, C-2), 32.7 (s, N*C*H_3), 27.43 (s, C(*C*H_3)$_3$), 27.42 (s, C(*C*H_3)$_3$), 22.1 (s, C-3'), 21.2 (s, C-4''-*C*H_3); ESI-HRMS: *m/z* calcd. for $C_{44}H_{72}N_8NaO_{15}$ $[M+Na]^+$ 975.5015, found: 975.4996.

6'-*N*-(2-*tert*-Butyldimethylsiloxyethyl)-1-*N*-(*N*-*tert*-butyloxycarbonyl-4-amino-2(S)-hydroxybutyryl)-3''-*N*-phenylazo-2',3-di-*N*-(*tert*-butyloxycarbonyl) sisomicin (89). *tert*-Butyldimethylsilyloxy acetaldehyde (Sigma-Aldrich, 20 μL, 0.11 mmol) and DIPEA (27.1 mg, 0.21 mmol) were added to a stirred solution of **88** (100 mg, 0.11 mmol) in dry THF (2.5 mL). The reaction mixture was stirred for 10 min before sodium triacetoxyborohydride (26.7 mg, 0.13 mmol) was added, after which stirring was continued for 24 h at room temperature. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel eluting with gradient of 4% to 15% ammonical methanol in dichloromethane to give **89** (59mg, 51%) as a white foam; *Rf* = 0.5 (10% ammonical MeOH in DCM); $[\alpha]_D^{25}$ = +95.3 (*c* = 0.01, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 7.38 (d, *J* = 7.7 Hz, 2H, Ph-*o*Hs), 7.28 (t, *J* = 7.7 Hz, 2H, Ph-*m*Hs), 7.10 (t, *J* = 7.3 Hz, 1H, Ph-*p*H), 5.43 (s, 1H, H-1'), 5.20 (d, *J* = 3.3, H-1''), 4.76 (br s, 1H, H-4'), 4.34 (dd, *J* = 11.4 Hz, 3.3 Hz, 1H, H-2''), 4.22-4.19 (m, 2H, H-3'', H-5''), 3.96 (dd, *J* = 8.4 Hz, 3.7 Hz, 1H, C*H*(OH)CH_2CH_2), 3.88 (m, 1H, H-1), 3.77-3.67 (m, 4H, NHCH_2C*H*$_2$O, H-6, H-2'), 3.65-3.60 (m, 1H, H-5), 3.57 (t, 1H, *J* = 8.4, H-4), 3.51-3.46 (m, 1H, H-3), 3.33 (d, *J* =12.1, 1H, H-5''), 3.30(m, 1H, H-6'), 3.29(s, 3H, NC*H*$_3$), 3.16 (d, *J* =13.6, 1H, H-6'), 3.12-3.02 (m, 2H, CH(OH)CH_2C*H*$_2$), 2.80-2.64 (m, 2H, NHC*H*$_2$$CH_2$O), 2.14-2.00 (m, 3H, H-3', H-2), 1.88-1.82 (m, 1H, CH(OH)C*H*$_2$$CH_2$), 1.73-1.67 (m, 1H, CH(OH)C*H*$_2$$CH_2$), 1.59-

1.51 (m, 1H, H-2), 1.42-1.40 (m, 27H, C(C*H*$_3$)$_3$), 1.08 (s, 3H, 4"-C*H*$_3$), 0.91 (s, 9H, SiC(C*H*$_3$)$_3$), 0.09 (s, 6H, Si(C*H*$_3$)$_2$); ^{13}C NMR (151 MHz, CD_3OD): δ 175.7, 157.1, 156.5, 156.4 (s, C=O), 151.0 (s, arom.), 146.5 (s, C-5'), 128.3, 124.9, 120.2 (s, arom.), 99.6 (s, C-1"), 97.6 (s, C-4'), 97.2 (s, C-1'), 81.5 (s, C-6), 79.8 (s, C-4), 78.9, 78.8, 78.6 (s, *C*(CH$_3$)$_3$), 75.9 (s, C-5), 73.0 (s, C-4"), 69.4 (s, *C*H(OH)CH$_2$CH$_2$), 69.2 (s, C-5"), 68.6 (s, C-3"), 65.4 (s, C-2"), 61.6 (s, NHCH$_2$*C*H$_2$O), 50.7 (s, C-6'), 49.9 (s, NH*C*H$_2$CH$_2$O), 49.3 (s, C-3), 49.0 (s, C-1), 47.1 (s, C-2'), 36.4 (s, CH(OH)CH$_2$*C*H$_2$), 33.93 (s, CH(OH)*C*H$_2$CH$_2$), 33.87 (s, C-2), 32.7 (s, N*C*H$_3$), 27.5 (s, C(*C*H$_3$)$_3$), 27.4 (s, C(*C*H$_3$)$_3$), 25.0(s, SiC(*C*H$_3$)$_3$), 22.2 (s, C-3'), 21.2 (s, C-4"-*C*H$_3$), 17.8 (s, Si*C*(CH$_3$)$_3$), -6.6(s, Si(*C*H$_3$)$_2$); ESI-HRMS: *m/z* calcd. for $C_{52}H_{91}N_8O_{16}Si$ $[M+H]^+$ 1111.6322, found: 1111.6307.

6'-*N*-(2-Hydroxyethyl)-1-*N*-(4-amino-2(S)-hydroxybutyryl)sisomicin pentaacetate salt [Plazomicin.5AcOH] (16). Compound **89** (59 mg, 0.054 mmol) was dissolved in DCM (1 mL) and cooled to 0 °C before trifluoroacetic acid (1 mL) was added. The reaction mixture was stirred for 6 h. Upon completion of the reaction, the reaction mixture was diluted with dry DCM (10 mL) and toluene (10 mL) and concentrated. The crude product was diluted with dry DCM (10 mL) and concentrated twice and the residue was purified by Sephadex C-25 chromatography eluting with a gradient of 0.1% to 3% ammonia in deionized H_2O. Fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to give **16** (19 mg, 40%) as a white solid; $[\alpha]_D^{25}$ = +46.5 (*c* = 0.67, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.51 (s, 1H, H-1'), 5.16 (t, *J* = 3.5 Hz, H, H-4'), 4.99 (d, *J* = 4.0 Hz, 1H, H-1"), 4.11(dd, *J* =9.4 Hz, 3.9 Hz, 1H, C*H*(OH)CH$_2$CH$_2$), 4.00 (d, *J* = 12.8 Hz, 1H, H-5"), 3.95 (m, 1H, H-1), 3.84 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H, H-2"), 3.81 (t, *J* = 9.9 Hz, 1H, H-4), 3.77 (t, *J* = 5.3 Hz, 1H, H-2'), 3.71(t, *J* = 5.1 Hz, 2H, NHCH$_2$C*H*$_2$O), 3.69-3.65 (m, 2H, H-6, H-6'), 3.64-3.94 (m, 2H, H-5, H-6'), 3.31 (m, 1H,

H-3), 3.24 (d, *J* = 12.8 Hz, 1H, H-5''), 3.15 (d, *J* = 11.0 Hz, 1H, H-3''), 3.07 (q, *J* = 4.9, 2H, NHC*H*$_2$CH$_2$O) 3.01 (t, *J* =7.2 Hz, 2H, CH(OH)CH$_2$C*H*$_2$), 2.74 (s, 3H, NC*H*$_3$), 2.58-2.52 (m, 1H, H-3'), 2.29-2.24 (m, 1H, H-3'), 2.07(dt, *J* = 13.2 Hz, 4.4 Hz, 1H, H-2), 2.04-1.98 (m, 1H, CH(OH)C*H*$_2$CH$_2$), 1.84-1.79 (m, 1H, CH(OH)C*H*$_2$CH$_2$), 1.63-1.57 (q, 1H, *J* = 12.5 Hz, H-2), 1.17 (s, 3H, 4''-C*H*$_3$); ^{13}C NMR (151 MHz, D$_2$O): δ 181.2 (s, CH$_3$*C*OOH), 175.4 (s, NHCO), 141.7 (s, C-5'), 102.5 (s, C-4'), 98.0 (s, C-1''), 96.9 (s, C-1'), 79.8 (s, C-4), 78.8 (s, C-6), 73.8 (s, C-5), 69.8 (s, C-4''), 69.4 (s, *C*H(OH)CH$_2$CH$_2$), 66.8 (s, C-5''), 65.9 (s, C-2''), 64.2 (s, C-3''), 56.4 (s, NHCH$_2$*C*H$_2$O), 48.8 (s, C-1), 48.31 (s, NH*C*H$_2$CH$_2$O), 48.26 (s, C-3), 47.9 (s, C-6'), 45.9 (s, C-2'), 36.8 (s, CH(OH)CH$_2$*C*H$_2$), 34.9 (s, N*C*H$_3$), 30.7 (s, CH(OH)*C*H$_2$CH$_2$), 30.4 (s, C-2), 23.1 (s, *C*H$_3$COOH), 23.0 (s, C-3'), 20.8 (s, 4''-*C*H$_3$); ESI-HRMS: *m/z* calcd. for $C_{25}H_{49}N_6O_{10}$ $[M+H]^+$ 593.3510, found: 593.3481.

1-*N*-(4-Amino-2(S)-hydroxy-butyryl) sisomicin pentaacetate salt (90). Compound **88** (50 mg, 0.052 mmol) was dissolved in DCM (1 mL) and cooled to 0 °C before trifluoroacetic acid (1 mL) was added. The reaction mixture was stirred for 45 min. Upon completion of the reaction, the reaction was diluted with dry DCM (10 mL) and toluene (10 ml) and concentrated. The crude product was diluted with dry DCM (10 mL) and concentrated twice and the residue was purified by a Sephadex column eluting with gradient of 0.1% to 3% ammonia in deionized H$_2$O. Fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to give **90** (24 mg, 54%) as a white solid; $[\alpha]_D^{25}$= +34.5 (*c*=0.07, H$_2$O); ^{1}H NMR (600 MHz, D$_2$O): δ 5.43 (s, 1H, H-1'), 5.02 (t, *J* = 3.5 Hz, H, H-4'), 4.96(d, *J* = 4.0 Hz, 1H, H-1''), 4.08(dd, *J* =9.2 Hz, 3.7 Hz, 1H, C*H*(OH)CH$_2$CH$_2$), 3.98 (d, *J* = 12.8 Hz, 1H, H-5''), 3.92 (m, 1H, H-1), 3.84(dd, *J* = 11.0 Hz, 3.9 Hz, 1H, H-2''), 3.74 (t, *J* = 9.7 Hz, 1H, H-4), 3.70 (t, *J* = 5.1 Hz, 1H, H-2'), 3.64 (m, 1H, H-6), 3.57 (m, 1H, H-5), 3.49 (s, 2H, H-6'), 3.25 (m, 1H, H-3), 3.21

(d, *J* = 12.8 Hz, 1H, H-5"), 3.11(d, *J* = 11.0 Hz, 1H, H-3"), 2.98 (t, *J* =7.3 Hz, 2H, CH(OH)$CH_2C\mathit{H}_2$), 2.71 (s, 3H, N$C\mathit{H}_3$), 2.52-2.46 (m, 1H, H-3'), 2.23-2.17 (m, 1H, H-3'), 2.03-1.95 (m, 2H, H-2, CH(OH)$C\mathit{H}_2CH_2$), 1.82-1.75 (m, 1H, CH(OH)$C\mathit{H}_2CH_2$), 1.63-1.57 (m, 1H, H-2), 1.14 (s, 3H, 4"-$C\mathit{H}_3$); ^{13}C NMR (151 MHz, D_2O): δ 181.2 (s, $CH_3\mathit{C}OOH$), 175.4 (s, NHCO), 143.3 (s, C-5'), 100.3 (s, C-4'), 98.1 (s, C-1"), 97.0 (s, C-1'), 80.0 (s, C-4), 78.8 (s, C-6), 73.8 (s, C-5), 69.8 (s, C-4"), 69.5 (s, *C*H(OH)CH_2CH_2), 66.8 (s, C-5"), 66.0 (s, C-2"), 64.2 (s, C-3"), 48.8 (s, C-1), 48.4 (s, C-3), 46.0 (s, C-2'), 40.5 (s, C-6'), 36.8 (s, CH(OH)$CH_2\mathit{C}H_2$), 34.9 (s, N$\mathit{C}H_3$), 30.7 (s, CH(OH)$\mathit{C}H_2CH_2$), 30.4 (s, C-2), 23.2 (s, $\mathit{C}H_3COOH$), 23.0 (s, C-3'), 20.8 (s, 4"-$\mathit{C}H_3$); ESI-HRMS: *m/z* calcd. for $C_{23}H_{45}N_6O_9$ $[M+H]^+$ 549.3248, found: 549.3234.

6,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-6′,7′-oxazolidino-apramycin (124). A stirred solution of 1,3,2′,4′′-tetraazido-6′,7′-oxazolidino-apramycin[82] **120** (100 mg, 0.15 mmol) in dry pyridine (0.3 mL) was cooled to 0 °C and treated with acetic anhydride (60 μL, 0.62 mmol). The reaction mixture was allowed to warm up to rt and stirred overnight. The reaction progress was monitored by TLC and additional acetic anhydride (0.5-1 equiv) was added as needed. After completion, the reaction mixture was diluted with EtOAc and the organic layer was washed with aqueous $NaHCO_3$ followed by brine, dried with Na_2SO_4, and concentrated. The crude product was purified via silica gel chromatography eluting with 0.6% to 0.8 % methanol in DCM to give **124** (76 mg, 61%) as a white solid; $[\alpha]_D^{25}$= +101.6 (*c* 3.1, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.35 (t, *J* = 9.9 Hz, 1H, H-3"), 5.30 (d, *J* = 3.7 Hz, 1H, H-1"), 5.15 (d, *J* = 3.5 Hz, 1H, H-1'), 4.93 (d, *J* = 2.9 Hz, 1H, H-8'), 4.93 – 4.87 (m, 2H, H-6, H-2"), 4.81 (dd, *J* = 8.6, 3.2 Hz, 1H, H-6'), 4.61 (dd, *J* = 10.5, 3.2 Hz, 1H, H-5'), 4.31 (dd, *J* = 12.3, 2.2 Hz, 1H, H-6"), 4.20 (dd, *J* = 12.3, 5.1 Hz, 1H, H-6"), 3.84 (dd, *J* = 8.7, 3.0 Hz, 1H, H-7'), 3.80 (dt, *J* = 10.9, 5.5 Hz, 1H, H-4'), 3.72 (ddd, *J* = 10.7, 5.1, 2.3 Hz, 1H, H-5"), 3.66 (ddd, *J* = 12.3, 9.9, 4.7 Hz, 1H, H-3), 3.62 – 3.53 (m,

3H, H-2', H-4", H-5), 3.49 (ddd, *J* = 12.5, 10.0, 4.5 Hz, 1H, H-1), 3.45 (t, *J* = 9.5 Hz, 1H, H-4), 2.89 (s, 3H, NCH_3), 2.41 (dt, *J* = 13.3, 4.6 Hz, 1H, H-2), 2.29 (dt, *J* = 10.9, 4.5 Hz, 1H, H-3'), 2.13 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.89 (q, *J* = 11.7 Hz, 1H, H-3'), 1.60 (q, *J* = 12.7 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.37 (C=O), 170.34 (C=O), 169.91 (C=O), 169.88 (C=O), 157.0 (NC=O), 98.8 (C-1'), 94.9 (C-8'), 94.5 (C-1"), 83.8 (C-4), 74.9 (C-6), 74.3 (C-5), 70.7 (C-3"), 70.1 (C-2"), 69.8 (C-6'), 68.8 (C-5"), 65.5 (C-5'), 65.1 (C-4'), 62.8 (C-6"), 60.1 (C-2'), 59.9 (C-7'), 58.4 (C-3), 58.0 (C-1), 57.6 (C-4"), 32.0 (C-2), 30.6 (C-3'), 29.8 (C- NCH_3), 20.9 ($COCH_3$), 20.7 ($COCH_3$); ESI-HRMS: *m/z* calcd. for $C_{30}H_{39}N_{13}NaO_{16}$ $[M+Na]^+$ 860.2535; found, 860.2522.

5-*O*-β-[2’’’,3’’’,5’’’-Tri-*O*-acetyl-D-ribofuranosyl]-6,2’’,3’’,6’’-tetra-*O*-acetyl-1,3,2’,4’’-tetraazido-6’,7’-oxazolidino-apramycin (126). A suspension of 2’’’,3’’’,5’’’-tri-*O*-acetyl-D-ribofuranosyl trichloroacetimidate[157] **125** (150 mg, 0.36 mmol), acceptor **124** (100 mg, 0.12 mmol) and activated 4 Å MS in dry DCM was stirred at rt for 1 h before cooling to 0 °C and addition of $BF_3.OEt_2$ (132 μL, 0.54 mmol). After 4 h of stirring at 0 °C, the reaction was quenched with triethylamine (0.5 mL) and filtered through Celite®. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 20% - 40% EtOAc/hexanes) to give **126** α:β = 1:9 (126 mg, 95%), further purification was done to give **126** (47 mg) as the β-anomer in the form of a white solid while the rest remained as a mixture. $[\alpha]_D^{25}$= +47.2 (*c* 2.7, DCM); ^1H NMR (600 MHz, $CDCl_3$): δ 5.67 (d, *J* = 3.5 Hz, 1H, H-1'), 5.43 (d, *J* = 10.1 Hz, 1H, H-3"), 5.40 (d, *J* = 2.3 Hz, 1H, H-1'"), 5.37 (d, *J* = 3.8 Hz, 1H, H-1"), 5.11 (t, *J* = 5.0 Hz, 1H, H-3'"), 5.07 (dd, *J* = 4.9, 3.3 Hz, 1H, H-2'"), 4.92 (t, *J* = 9.9 Hz, 1H, H-6), 4.85 (dd, *J* = 10.4, 3.9 Hz, 1H, H-2"), 4.82 (d, *J* = 4.6 Hz, 1H, H-8'), 4.76 (dd, *J* = 7.4, 3.3 Hz, 1H, H-

6'), 4.41 (dd, *J* = 10.3, 3.3 Hz, 1H, H-5'), 4.32 (m, 2H, H-6", H-5'"), 4.24 (q, *J* = 4.0 Hz, 1H, H-4'"), 4.21 (dd, *J* = 12.2, 5.4 Hz, 1H, H-6"), 4.09 (dd, *J* = 12.1, 4.1 Hz, 1H, H-5'"), 3.79 (d, *J* = 9.2 Hz, 1H, H-5), 3.77 – 3.64 (m, 4H, H-4, H-7', H-4', H-5"), 3.60 – 3.53 (m, 2H, H-4", H-3), 3.46 – 3.38 (m, 1H, H-1), 3.22 (dt, *J* = 12.9, 4.1 Hz, 1H, H-2'), 2.93 (s, 3H, NCH_3), 2.41 (dt, *J* = 13.0, 4.5 Hz, 1H, H-2), 2.26 – 2.21 (m, 1H, H-3'), 2.20 (s, 3H, $COCH_3$), 2.14 – 2.02 (m, 18H, 6*$COCH_3$), 1.93 (q, *J* = 11.7 Hz, 1H, H-3'), 1.58 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.8 (C=O), 170.4 (C=O), 170.2 (C=O), 169.8 (C=O), 169.6 (C=O), 169.5 (C=O), 169.5 (C=O), 157.0 (C=O), 106.1 (C-1'"), 97.4 (C-8'), 96.4 (C-1'), 94.0 (C-1"), 80.4 (C-5), 79.5 (C-4'"), 77.5 (C-4), 74.5 (C-6), 74.1 (C-2'"), 71.0 (C-6'), 70.9 (C-3'"), 70.3 (C-3"), 69.9 (C-2"), 69.1 (C-5"), 66.0 (C-5'), 65.7 (C-4'), 63.4 (C-5'"), 62.9 (C-6"), 60.2 (C-7'), 60.1 (C-4"), 59.1 (C-3), 58.1 (C-1), 56.6 (C-2'), 31.5 (C-2), 30.1 (NCH_3), 29.9 (C-3'), 20.8 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$), 20.7 ($COCH_3$), 20.7 ($COCH_3$), 20.4 ($COCH_3$), 20.4 ($COCH_3$); ESI-HRMS: m/z calcd. for $C_{41}H_{54}N_{13}O_{23}$ $[M+H]^+$ 1118.3275; found, 1118.3234.

***5-O*-(β-D-Ribofuranosyl) apramycin pentaacetate salt (127).** A stirred solution of substrate **129** (47 mg, 0.04 mmol) in dioxane (0.5 mL) was treated with 3 N NaOH (0.25 mL) and heated at 100 °C for 12 h. The reaction mixture was cooled to rt and neutralized with glacial acetic acid before it was concentrated *in vacuo*. The crude product was purified through a silica gel column (eluent: 10-20% methanol/DCM). The product-containing fractions were concentrated, dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL) and Pd/C (0.5 equiv) was added. The reaction mixture was stirred at room temperature under 1 atm of hydrogen (balloon) for 1 h. After completion, the reaction mixture was filtered over Celite® and the filtrate concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water

(20 mL), then eluted with a gradient of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **127** (29 mg, 69%) as pentaacetate salt in the form of a white solid $[\alpha]_D^{25}$= +66.25 (*c* 0.8, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.66 (d, *J* = 4.0 Hz, 1H, H-1'), 5.29 (d, *J* = 3.8 Hz, 1H, H-1''), 5.14 (s, 1H, H-1'''), 5.01 (d, *J* = 8.5 Hz, 1H, H-8'), 4.38 (s, 1H, H-6'), 4.00 (d, *J* = 4.7 Hz, 1H, H-2'''), 3.98 – 3.93 (m, 1H, H-3'''), 3.86 – 3.74 (m, 3H, H-4''', H-4, H-3''), 3.74 – 3.63 (m, 5H, H-4', H-5, H-5'', H-5''', H-6''), 3.58 (dd, *J* = 12.4, 4.4 Hz, 1H, H-6''), 3.53 – 3.41 (m, 5H, H-5', H-2', H-2'', H-6, H-5'''), 3.29 – 3.21 (m, 1H, H-3), 3.19 (dd, *J* = 8.5, 2.4 Hz, 1H, H-7'), 3.12 (td, *J* = 11.6, 4.3 Hz, 1H, H-1), 3.06 (t, *J* = 10.4 Hz, 1H, H-4''), 2.58 (s, 3H, NCH_3), 2.24 (dt, *J* = 11.3, 3.1 Hz, 1H, H-2), 2.21 – 2.10 (m, 1H, H-3'), 1.92 – 1.81 (m, 1H, H-3'), 1.66 – 1.57 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 110.3 (C-1'''), 94.5 (C-1'), 94.3 (C-1''), 92.8 (C-8'), 84.9 (C-5), 82.3 (C-4'''), 75.9 (C-4), 75.1 (C-2''') 72.5 (C-6), 70.2 (C-5''), 69.6 (C-2''), 69.6 (C-4'), 68.9 (C-3''), 68.4 (C-3'''), 66.0 (C-5'), 62.6 (C-6'), 60.8 (C-5'''), 60.2 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 49.8 (C-3), 48.3 (C-1), 47.8 (C-2'), 30.0 (NCH_3), 28.6 (C-2), 26.7 (C-3'); ESI-HRMS: m/z calcd. for $C_{26}H_{49}N_5NaO_{15}$ $[M+Na]^+$ 694.3123; found, 694.3122.

***p*-Cresyl-2',6'-diazido-2,5,3'4'-tetra(4-methoxybenzoyl)-1-thio-β-paromobioside (130).** *p*-Cresyl-2',6'-diazido-2,5,3'4'-tetra-*O*-acetyl-α-thioparomobioside[159-160] **128** (375 mg, 0.59 mmol) was dissolved in dry methanol, then NaOMe (4.7 mg, 0.12 mmol) was added and the reaction mixture was stirred for 1.5 h. The reaction was quenched with glacial acetic acid and concentrated till dryness. The crude product was dissolved in pyridine (5 mL) and *p*-methoxybenzoyl chloride (802 mg, 4.72 mmol) was added followed by stirring for 48 h then diluting with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ followed by brine, dried with Na_2SO_4, and concentrated. The crude product was purified via silica gel

chromatography eluting with 10% to 40 % EtOAc in hexanes to give **130** (398 mg, 76%) as a white solid; $[\alpha]_D^{25}$= -14.2 (c 13.2, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.08 (d, *J* = 8.8 Hz, 2H, ArH), 8.04 (d, *J* = 8.8 Hz, 2H, ArH), 7.95 (d, *J* = 8.8 Hz, 2H, ArH), 7.85 (d, *J* = 8.9 Hz, 2H, ArH), 7.40 (d, *J* = 8.0 Hz, 2H, ArH), 7.02 (d, *J* = 7.8 Hz, 2H, ArH), 6.97 – 6.88 (m, 6H, ArH), 6.78 (d, *J* = 8.9 Hz, 2H, ArH), 5.51 (d, *J* = 4.5 Hz, 1H, H-1), 5.43 (t, *J* = 4.9 Hz, 1H, H-2), 5.23 (t, *J* = 2.8 Hz, 1H, H-3'), 5.16 (d, *J* = 1.8 Hz, 1H, H-1'), 5.04 (d, *J* = 1.8 Hz, 1H, H-4'), 4.78 (t, *J* = 5.6 Hz, 1H, H-3), 4.68 (dd, *J* = 12.0, 2.7 Hz, 1H, H-5), 4.56 (td, *J* = 5.8, 5.2, 2.7 Hz, 1H, H-4), 4.50 (dd, *J* = 12.1, 4.6 Hz, 1H, H-5), 4.24 (ddd, *J* = 8.5, 4.1, 1.9 Hz, 1H, H-5'), 3.87 (s, 3H, OCH_3), 3.85 – 3.73 (m, 9H, 3 OCH_3), 3.57 (dd, *J* = 13.1, 8.4 Hz, 1H, H-6'), 3.41 (t, *J* = 2.2 Hz, 1H, H-2'), 3.27 (dd, *J* = 13.1, 4.0 Hz, 1H, H-6'), 2.22 (s, 3H, CH_3); 13C NMR (151 MHz, $CDCl_3$): δ 166.0 (C=O), 165.4 (C=O), 165.0 (C=O), 164.03 (C=O), 163.97 (Ar-C), 163.9 (Ar-C), 163.7 (Ar-C), 163.4 (Ar-C), 138.6 (Ar-C), 133.9 (Ar-C), 132.3 (Ar-C), 132.0 (Ar-C), 131.9 (Ar-C), 131.8 (Ar-C), 129.8 (Ar-C), 127.7 (Ar-C), 122.3 (Ar-C), 121.1 (Ar-C), 120.9 (Ar-C), 120.8 (Ar-C), 99.3 (C-1'), 88.2 (C-1), 81.2 (C-4), 76.7 (C-3), 74.5 (C-2), 74.2 (C-5'), 69.1 (C-3'), 65.7 (C-4'), 63.8 (C-5), 56.9 (C-2'), 55.52 (OCH_3), 55.46 (OCH_3), 55.4 (2 OCH_3), 50.8 (C-6'), 21.1 (CH_3); ESI-HRMS: m/z calcd. for $C_{50}H_{48}N_6NaO_{15}S$ $[M+Na]^+$ 1027.2796; found, 1027.2749.

***p*-Cresyl-2',6'-diazido-2,5,3'4'-tetra(4-methoxybenzoyl)-β-thio-paromobiosyl *S*-oxide (131).** A solution of compound **130** (198 mg, 0.2 mmol) in dry DCM (15 mL) was cooled to -78 °C before ozone gas was bubbled in for 5 min till the solution turned blue. The solution was then warmed to rt, concentrated and the crude product purified by gradient chromatography over silica gel (eluent: 40% EtOAc/hexanes) to give **131** (162 mg, 80%) as a white solid; $[\alpha]_D^{25}$= +60.0 (c 0.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 9.0 Hz, 2H, ArH), 8.12 – 8.03 (m, 4H, ArH), 7.68 (d, *J* = 9.0 Hz, 2H, ArH), 7.51 (d, *J* = 8.2 Hz, 2H, ArH), 7.24 (d, *J* = 7.9 Hz,

2H, ArH), 7.00 (d, *J* = 9.0 Hz, 2H, ArH), 6.96 (d, *J* = 4.3 Hz, 2H, ArH), 6.93 (d, *J* = 4.4 Hz, 2H, ArH), 6.78 (d, *J* = 9.0 Hz, 2H, ArH), 6.14 (dd, *J* = 5.0, 1.9 Hz, 1H, H-2), 5.28 (d, *J* = 1.6 Hz, 1H, H-1'), 5.23 (t, *J* = 2.7 Hz, 1H, H-3'), 5.09 – 5.03 (m, 1H, H-4'), 5.00 (dd, *J* = 7.1, 5.1 Hz, 1H, H-3), 4.90 (d, *J* = 1.9 Hz, 1H, H-1), 4.76 (dd, *J* = 11.3, 1.5 Hz, 1H, H-5), 4.64 – 4.52 (m, 2H, H-5, H-4), 4.28 (ddd, *J* = 7.7, 4.4, 1.7 Hz, 1H, H-5'), 3.92 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.54 (dd, *J* = 13.0, 8.1 Hz, 1H, H-6'), 3.42 – 3.36 (m, 1H, H-2'), 3.32 (dd, *J* = 13.0, 4.5 Hz, 1H, H-6'), 2.30 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.0 (C=O), 165.0 (C=O), 164.8 (C=O), 164.1 (C=O), 163.94 (Ar-C), 163.86 (Ar-C), 163.6 (Ar-C), 142.2 (Ar-C), 136.6 (Ar-C), 132.3 (Ar-C), 132.1 (Ar-C), 131.9 (Ar-C), 131.7 (Ar-C), 130.0 (Ar-C), 124.6 (Ar-C), 122.1 (Ar-C), 121.1 (Ar-C), 121.0 (Ar-C), 120.6 (Ar-C), 113.94 (Ar-C), 113.86 (Ar-C), 113.8 (Ar-C), 113.7 (Ar-C), 100.1 (C-1), 99.1 (C-1'), 81.6 (C-4), 75.9 (C-3), 74.1 (C-5'), 70.1 (C-2), 69.1 (C-3'), 65.7 (C-4'), 62.7 (C-5), 56.7 (C-2'), 55.54 (OCH_3), 55.46 (OCH_3), 55.4 (OCH_3), 50.7 (C-6'); ESI-HRMS: m/z calcd. for $C_{50}H_{48}N_6NaO_{16}S$ $[M+Na]^+$ 1043.2745; found, 1043.2717.

5-*O*-β-[2’’’’,6’’’’-Diazido-2’’’,5,3’’’’4’’’’-tetra(4-methoxybenzoyl) paromobiosyl]-6,2’’,3’’,6’’-tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-6′,7′-oxazolidino-apramycin (132). A suspension of donor **131** (140 mg, 0.14 mmol), acceptor **124** (150 mg, 0.18 mmol) and activated 4 Å MS in dry DCM (2 mL) was stirred at rt for 1 h before addition of freshly distilled triflic anhydride (33 μL, 0.2 mmol). After 5 h of stirring at rt, the reaction was quenched with triethylamine (0.1 mL) and filtered through Celite® then concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (20% EtOAc/toluene) to afford **132** (113 mg, 48%) as a white foam; $[\alpha]_D^{25}$= +84.9 (c 0.3, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10 – 8.04 (m, 4H, ArH), 8.02 (d, *J* = 8.9 Hz, 2H, ArH), 7.83 (d, *J* = 8.9 Hz, 2H,

ArH), 7.01 – 6.94 (m, 4H, ArH), 6.92 (d, *J* = 8.9 Hz, 2H, ArH), 6.82 (d, *J* = 9.0 Hz, 2H, ArH), 5.58 (d, *J* = 3.5 Hz, 1H, H-1'), 5.44 (d, *J* = 1.7 Hz, 1H, H-1'''), 5.39 (t, *J* = 10.0 Hz, 1H, H-3''), 5.33 (d, *J* = 3.8 Hz, 1H, H-1''), 5.24 – 5.18 (m, 2H, H-2''', H-3''''), 5.14 (d, *J* = 1.5 Hz, 1H, H-1''''), 5.07 – 5.04 (m, 1H, H-4''''), 4.89 – 4.80 (m, 4H, H-2'', H-5''', H-6, H-8'), 4.77 – 4.72 (m, 2H, H-3''', H-6'), 4.50 – 4.44 (m, 2H, H-4''', H-5'''), 4.43 (dd, *J* = 10.4, 3.3 Hz, 1H, H-5'), 4.32 – 4.26 (m, 2H, H-5'''', H-6''), 4.20 (dd, *J* = 12.2, 5.4 Hz, 1H, H-6''), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.75 – 3.68 (m, 3H, H-5, H-5'', H-7'), 3.64 – 3.49 (m, 4H, H-3, H-4', H-4'', H-6''''), 3.41 – 3.30 (m, 4H, H-1, H-2'''', H-4, H-6''''), 3.12 (dt, *J* = 12.9, 4.1 Hz, 1H, H-2'), 2.92 (s, 3H, NCH_3), 2.38 (dt, *J* = 12.9, 4.4 Hz, 1H, H-2), 2.17 – 2.12 (m, 1H, H-3'), 2.11 – 2.07 (m, 9H, $3COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.88 – 1.81 (m, 1H, H-3'), 1.44 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 165.9 (C=O), 165.5 (C=O), 165.0(C=O), 164.1(C=O), 164.0 (Ar-C), 163.9 (Ar-C), 163.8 (Ar-C), 163.6 (Ar-C), 157.1(C=O), 132.3 (Ar-C), 132.1 (Ar-C), 131.8 (Ar-C), 122.2 (Ar-C), 121.1 (Ar-C), 121.0 (Ar-C), 120.7 (Ar-C), 113.9 (Ar-C), 113.9 (Ar-C), 113.9 (Ar-C), 113.8 (Ar-C), 106.8 (C-1'''), 99.3 (C-1''''), 96.9 (C-1'), 96.6 (C-8'), 94.1 (C-1''), 81.4 (C-5), 79.9 (C-4'''), 78.0 (C-4), 75.7 (C-3'''), 74.9 (C-6), 74.5 (C-5''''), 74.2 (C-3''), 70.8 (C-3''''), 70.5 (C-6'), 69.9 (C-2''), 69.2 (C-2'''), 69.0 (C-5''), 65.7 (C-4''''), 65.6 (C-5'), 63.0 (C-5'''), 62.9 (C-6''), 60.1 (C-7', C-3), 58.8 (C-4''), 58.1 (C-1), 56.8 (C-2''''), 56.6 (C-2'), 55.5 (OCH_3), 55.5 (OCH_3), 50.7 (C-6''''), 31.2 (C-2'), 30.4 (C-3'), 30.0 (NCH_3), 21.0 ($COCH_3$), 20.8 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$); ESI-HRMS: m/z calcd. for $C_{73}H_{79}N_{19}NaO_{31}$ $[M+Na]^+$ 1740.5087; found, 1740.5109.

5-*O*-β-(Paromobiosyl) apramycin heptaacetate salt (133). A stirred solution of compound **132** (40 mg, 0.02 mmol) in dioxane (0.5 mL) was treated with 3N NaOH (0.5 mL) and heated at 120 °C for 2 h. The reaction mixture was cooled to rt and neutralized with glacial

acetic acid before it was concentrated *in vacuo*. The crude product was purified with silica gel column chromatography (eluent: 5%-15% methanol/DCM) to give a residue that was directly subjected to Staudinger reaction by dissolving in THF (0.6 mL) followed by the addition of 0.3N NaOH (0.3 mL) and 1M $P(CH_3)_3$ in THF (0.3 mL). The reaction mixture was stirred at 55 °C for 2h, then concentrated and purified by column chromatography (eluent: 5% to 50% ammonia/MeOH). The product-containing fractions were concentrated and dissolved in D.I. water (1 mL), acidified by glacial acetic acid till pH = 3-4 and loaded to Sephadex column (CM Sephadex C-25) from which the product was flushed with D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **133** (8 mg, 35%) as a peracetate salt in the form of a white foam; $[\alpha]_D^{25}$ = +54.4 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.68 (d, *J* = 3.9 Hz, 1H, H-1'), 5.32 (d, *J* = 3.9 Hz, 1H, H-1''), 5.22 (d, *J* = 2.4 Hz, 1H, H-1'''), 5.10 (d, *J* = 1.4 Hz, 1H, H-1''''), 5.03 (d, *J* = 8.5 Hz, 1H, H-8'), 4.41 (s, 1H, H-6'), 4.31 (t, *J* = 5.7 Hz, 1H, H-3'''), 4.21 (dd, *J* = 3.8, 2.8 Hz, 1H, H-2'''), 4.15 – 4.09 (m, 1H, H-5''''), 4.08 – 4.00 (m, 2H, H-3'''', H-4'''), 3.83 (t, *J* = 9.7 Hz, 1H, H-4), 3.79 (dt, *J* = 10.2, 3.9 Hz, 1H, H-5''), 3.77 – 3.65 (m, 5H, H-5''', H-3'', H-4', H-5, H-6''), 3.65 – 3.58 (m, 2H, H-4'''', H-6''), 3.58 – 3.44 (m, 5H, H-6, H-2', H-5', H-2'', H-5'''), 3.39 (s, 1H, H-2''''), 3.32 – 3.25 (m, 1H, H-3), 3.25 – 3.19 (m, 2H, H-7', H-6''''), 3.19 – 3.12 (m, 2H, H-1, H-6''''), 3.09 (t, *J* = 10.3 Hz, 1H, H-4''), 2.60 (s, 3H, NCH_3), 2.27 (dt, *J* = 11.8, 3.1 Hz, 1H, H-2), 2.22 – 2.13 (m, 1H, H-3'), 1.87 (dd, *J* = 24.1, 11.8 Hz, 1H, H-3'), 1.64 (q, *J* = 13.0 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 110.1 (C-1'''), 95.3 (C-1''''), 94.5 (C-1'), 94.4 (C-1''), 92.8 (C-8'), 84.9 (C-5), 81.3 (C-4'''), 76.0 (C-4), 75.2 (C-3'''), 73.4 (C-2'''), 72.5 (C-2''), 70.2 (C-5''''), 70.1 (C-5''), 69.7 (C-6), 69.5 (C-5'), 68.4 (C-3''), 67.5 (C-3''''), 67.2 (C-4''''), 65.9 (C-4'), 62.6 (C-6'), 60.2 (C-5'''), 60.0 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 50.7

(C-2""), 49.8 (C-1), 48.4 (C-3), 47.8 (C-2'), 40.3 (C-6""), 30.0 (NCH_3), 28.6 (C-2), 26.7 (C-3'); ESI-HRMS: m/z calcd. for $C_{32}H_{62}N_7O_{18}$ $[M+H]^+$ 832.4151; found, 832.4131.

3-*O*-(2-Azidoethyl)-5-*O*-benzyl-1,2-*O*-isopropylidene–α-D-ribofuranose (135). 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose[164] **134** (1000 mg, 3.57 mmol) was dissolved in dry THF (3 mL) and NaH (214 mg, 5.36 mmol) was added under argon. After stirring for 15 min, a solution of 2-azidoethyl tosylate[163] (1.72 g, 7.14 mmol) in dry THF (3 mL) was added dropwise followed by stirring for 12 h. More NaH (150 mg, 3.75 mmol) and 2-azidoethyl tosylate (860 mg, 3.57 mmol) were added and stirring continued for 24 h. After completion, the reaction was quenched with methanol and concentrated *in vacuo* and the crude product was purified by column chromatography (eluent: 5% to 30% EtOAc/hexanes) to give **135** (974 mg, 78%) as a gum; $[\alpha]_D^{25}$ = = +71.2 (*c* = 1.0); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 – 7.26 (m, 5H, Ar*H*), 5.81 (d, *J* = 3.8 Hz, 1H, H-1), 4.67 – 4.61 (m, 2H, H-2, $PhCH_2$), 4.55 (d, *J* = 12.2 Hz, 1H, $PhCH_2$), 4.12 (ddd, *J* = 9.0, 3.7, 2.1 Hz, 1H, H-4), 3.89 – 3.84 (m, 2H, H-3, H-5), 3.81 (dd, *J* = 11.5, 2.2 Hz, 1H, OCH_2), 3.67 – 3.58 (m, 2H, OCH_2, H-5), 3.45 (ddd, *J* = 13.3, 7.4, 3.5 Hz, 1H, CH_2N_3), 3.28 (ddd, *J* = 13.3, 5.6, 3.5 Hz, 1H, CH_2N_3), 1.57 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.99 (Ar-C), 128.37 (Ar-C), 127.78 (Ar-C), 127.68 (Ar-C), 112.98 (CMe_2), 104.13 (C-1), 78.61 (C-2), 77.89 (C-3), 77.17 (C-4), 73.52 ($PhCH_2$), 69.38 (C-5), 67.65 (OCH_2), 50.59 (CH_2N_3), 26.69 (CH_3), 26.58 (CH_3); ESI-HRMS: *m*/*z* calcd for $C_{17}H_{23}N_3O_5Na$ $[M+Na]^+$ 372.1535, found 372.1533.

3-*O*-(2-Azidoethyl)-5-*O*-benzyl-1,2-di-*O*-(4-nitrobenzoyl)-α-D-ribofuranose (136). A solution of **135** (822 mg, 2.36 mmol) in a 1:3 mixture of 1 N aqueous hydrochloric acid and *p*-dioxane (16 mL) was stirred for 1.5 h at ambient temperature then was concentrated and dried under reduce pressure. The residue was diluted with pyridine (40 mL) and treated with 4-

dimethylaminopyridine (30 mg, 0.236 mmol) and *p*-nitrobenzoyl chloride (1.53 g, 8.26 mmol) at ambient temperature and stirred for 10 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (hexane: ethyl acetate 8:1 to 4:1). to give **136** as a white foam (643 mg, 1.06 mmol, 45%); $[\alpha]_D^{25}$ = +66.9 (*c* = 1.0); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.26 (s, 4H, Ar*H*); 8.20 (d, *J* = 8.8 Hz, Ar*H*); 8.12 (d, *J* = 8..8 Hz, Ar*H*); 7.39-7.26 (m, 5H, Ar*H*); 6.80 (d, *J* = 4.4 Hz, 1H, H-1); 5.45 (dd, *J* = 4.4 Hz, 6.6 Hz, 1H, H-2); 4.65 (d, *J* = 11.7 Hz, 1H, PhCH); 4.58 (d, *J* = 11.7 Hz, 1H, $PhCH_2$); 4.58 (m, 1H, H-4); 3.86 (dd, *J* = 6.6 Hz, 2.9 Hz, 1H, H-3); 3.74-3.65 (m, 4H, H-5, C*H*$_2$$CH_2N_3$); 3.33 (m, 1H, CH_2C*H*$_2$$N_3$); 3.28 (m, 1H, CH_2C*H*$_2$$N_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 163.72, 163.56, 150.84, 150.79, 137.47, 131.00, 130.80, 128.55, 127.96, 127.70, 123.65 (18C, Ar*C*.); 95.82 (C-1); 84.70 (C-4); 76.91 (C-3); 73.73 ($PhCH_2$); 73.14 (C-2); 70.33 (-*C*$H_2CH_2N_3$); 69.38 (C-5); 51.12 (CH_2*C*H_2N_3); ESI-HRMS: *m*/*z* calcd for $C_{28}H_{25}N_5O_{11}Na$ $[M+Na]^+$ 630.1448, found 630.1453.

5-*O*-α-[3'''-*O*-(2-Azidoethyl)-5'''-*O*-benzyl-2'''-*O*-(4-nitrobenzoyl)-D-ribofuranosyl]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (137α) and **5-*O*-β-[3'''-*O*-(2-Azidoethyl)-5'''-*O*-benzyl-2'''-*O*-(4-nitrobenzoyl)-D-ribofuranosyl]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (137β)** 3-*O*-(2-Azidoethyl)-5-*O*-benzyl-1,2-di-*O*-(4-nitrobenzoyl)-α-D-ribofuranose **136** (181 mg, 0.30 mmol) and apramycin derivative **124** (100 mg, 0.12 mmol) was were charged to a round bottom flask, co-evaporated with toluene three times and dried *in vacuo* overnight. The flask was purged with argon and the mixture dissolved in dry DCM (1.5 mL) before cooling to 0 °C, treatment with $BF_3.OEt_2$ (0.23 mL, 0.63 mmol) and stirring for 12 h. The reaction was quenched with triethylamine (0.2 mL), diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column

chromatography (eluent: 20% - 40% EtOAc/hexanes) to give **137α** (35 mg, 23%) and **137β** (31 mg, 20%); **α anomer:** $[\alpha]_D^{25}$= +79.1 (*c* 1.1, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.32 (d, *J* = 8.6 Hz, 2H, Ar*H*), 8.23 (d, *J* = 8.9 Hz, 2H, Ar*H*), 7.36 – 7.29 (m, 5H, Ar*H*), 5.68 – 5.65 (m, 1H, H-2'''), 5.56 (d, *J* = 3.8 Hz, 1H, H-1'''), 5.38 (t, *J* = 10.0 Hz, 1H, H-3''), 5.32 (d, *J* = 3.8 Hz, 1H, H-1''), 5.15 (d, *J* = 3.5 Hz, 1H, H-1'), 4.94 – 4.87 (m, 3H, H-2'', H-6, H-8'), 4.75 (dd, *J* = 8.4, 3.1 Hz, 1H, H-6'), 4.66 (dd, *J* = 10.5, 3.1 Hz, 1H, H-5'), 4.60 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.54 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.31 (dd, *J* = 12.1, 1.8 Hz, 1H, H-6''), 4.25 (dt, *J* = 7.0, 3.3 Hz, 1H, H-4'''), 4.21 (dd, *J* = 12.2, 5.2 Hz, 1H, H-6''), 4.04 (dd, *J* = 7.6, 5.1 Hz, 1H, H-3'''), 3.79 (dd, *J* = 8.4, 3.0 Hz, 1H, H-7'), 3.75 – 3.69 (m, 4H, H-4', H-5, H-5', H-5''), 3.68 – 3.62 (m, 1H, H-3), 3.62 – 3.57 (m, 3H, H-5''', H-4'', OC$H_2$$CH_2$), 3.56 – 3.48 (m, 3H, H-1, H-4, OC$H_2$$CH_2$), 3.29 (dt, *J* = 12.8, 4.1 Hz, 1H, H-2'), 3.18 (t, *J* = 4.9 Hz, 2H, OCH_2CH_2), 2.91 (s, 3H, NCH_3), 2.42 (dt, *J* = 12.9, 4.4 Hz, 1H, H-2), 2.25 (dt, *J* = 10.6, 4.3 Hz, 1H, H-3'), 2.13 (s, 3H, COCH_3), 2.11 (s, 3H, COCH_3), 2.09 (m, 6H, COCH_3), 1.86 (q, *J* = 11.5 Hz, 1H, H-3'), 1.55 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.3 (C=O), 169.9 (C=O), 169.8 (C=O), 164.3 (C=O), 156.9 (C=O), 150.9 (Ar-C), 137.5 (Ar-C), 134.6 (Ar-C), 131.1 (Ar-C), 128.5 (Ar-C), 127.9 (Ar-C), 127.8 (Ar-C), 123.8 (Ar-C), 102.8 (C-1'''), 97.8 (C-1'), 95.0 (C-8'), 94.2 (C-1''), 82.5 (C-5), 80.4 (C-4'''), 80.1 (C-4), 77.2 (C-3'''), 73.6 (C-6), 73.6 (CH_2Ph), 71.3 (C-2'''), 70.7 (C-3''), 70.0 (C-2''), 69.9 (C-6'), 69.9 (OC$H_2$$CH_2$), 68.9 (C-5'''), 68.7 (C-5''), 65.5 (C-5'), 65.2 (C-4'), 62.8 (C-6''), 60.2 (C-7'), 60.1 (C-4''), 58.3 (C-3), 58.1 (C-1), 56.7 (C-2'), 50.6 (OCH_2CH_2), 31.4 (C-2), 30.3 (C-3'), 29.8 (NCH_3), 21.2 (COCH_3), 20.8 (COCH_3), 20.8 (COCH_3), 20.7 (COCH_3); ESI-HRMS: m/z calcd. for $C_{51}H_{59}N_{17}O_{23}$ $[M+Na]^+$ 1300.3867; found, 1300.3887; **β anomer:** $[\alpha]_D^{25}$= +70.8 (*c* 1.8, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.28 (d, *J* = 8.6 Hz, 2H, Ar*H*), 8.20 (d, *J* = 8.7 Hz, 2H, Ar*H*), 7.46 – 7.26 (m, 5H, Ar*H*), 5.79 (d, *J* = 3.4 Hz, 1H, H-1'), 5.42 (t, *J* = 10.0 Hz, 1H, H-3''),

5.38 (s, 1H, H-1'''), 5.37 (d, *J* = 3.8 Hz, 1H, H-1''), 5.23 (d, *J* = 4.2 Hz, 1H, H-2'''), 4.92 (t, *J* = 9.7 Hz, 1H, H-6), 4.86 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2''), 4.81 (d, *J* = 4.4 Hz, 1H, H-8'), 4.76 (dd, *J* = 7.5, 3.2 Hz, 1H, H-6'), 4.60 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.51 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.39 (dd, *J* = 10.3, 3.2 Hz, 1H, H-5'), 4.33 (d, *J* = 12.0 Hz, 1H, H-6''), 4.26 – 4.16 (m, 2H, H-6'', H-4'''), 4.14 (dd, *J* = 7.1, 4.5 Hz, 1H, H-3'''), 3.84 – 3.71 (m, 3H, H-5, H-7', H-5''), 3.71 – 3.52 (m, 8H, H-4, H-4', H-4'', H-3, H-5''', OC*H*$_2$CH$_2$), 3.47 – 3.37 (m, 1H, H-1), 3.17 (m, 2H, OCH$_2$C*H*$_2$), 3.09 (dt, *J* = 12.8, 3.9 Hz, 1H, H-2'), 2.94 (s, 3H, NHC*H*$_3$), 2.41 (dt, *J* = 12.9, 4.3 Hz, 1H, H-2), 2.19 – 1.99 (m, 13H, 4COC*H*$_3$, H-3'), 1.88 (q, *J* = 11.8 Hz, 1H, H-3'), 1.57 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4(C=O), 170.1(C=O), 169.8 (C=O), 169.7 (C=O), 163.8 (C=O), 157.1 (C=O), 150.8 (Ar-C), 137.7 (Ar-C), 134.5 (Ar-C), 131.1 (Ar-C), 130.9 (Ar-C), 128.5 (Ar-C), 127.8 (Ar-C), 127.7 (Ar-C), 123.7 (Ar-C), 123.6 (Ar-C), 106.9 (C-1'''), 97.3 (C-1'), 96.4 (C-8'), 94.2 (C-1''), 82.0 (C-5), 80.5 (C-4'''), 77.8 (C-4), 76.8 (C-3'''), 75.7 (C-6), 75.0 (*C*H$_2$Ph), 73.4 (C-2'''), 71.0 (C-3''), 70.4 (C-2''), 70.2 (C-6'), 70.0 (O*C*H$_2$CH$_2$), 69.7 (C-5'''), 69.1 (C-5''), 65.9 (C-5'), 65.7 (C-4'), 62.9 (C-6''), 60.2 (C-7'), 60.1 (C-4''), 59.1 (C-3), 58.1 (C-1), 56.5 (C-2'), 50.6 (OCH$_2$*C*H$_2$), 31.3 (C-2), 30.1 (C-3'), 29.7(N*C*H$_3$), 20.9 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{51}H_{59}N_{17}O_{23}$ $[M+Na]^+$ 1300.3867; found, 1300.3835.

***5-O*-α-[3-*O*-(2-Aminoethyl)-D-ribofuranosyl] apramycin hexaacetate salt (138α).** A stirred solution of compound **137α** (30 mg, 0.02 mmol) in dioxane (0.5 mL) was treated with 3N NaOH (0.25 mL) and heated at 100 °C for 18 h. The reaction mixture was cooled to rt and neutralized with glacial acetic acid and concentrated *in vacuo*. The crude product was passed through a silica gel column (eluent: 50% methanol/DCM). The product-containing fractions was concentrated and dissolved in THF (0.6 mL) followed by the addition of 0.3N NaOH (0.3 mL)

and 1M $P(CH_3)_3$ in THF (0.3 mL). The reaction mixture was stirred at 55 °C for 2 h then concentrated and purified by column chromatography (eluent: 5% to 50% ammonia/MeOH). The product-containing fractions were concentrated, dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL). $Pd(OH)_2/C$ (0.5 equiv) was added and the reaction mixture was stirred at room temperature under 1 atm of hydrogen (balloon) for 4 h. After completion, the reaction mixture was filtered over Celite®, concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then eluted with a gradient of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **138α** (21 mg, 85%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +52.3 (*c* 1.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.65 (d, *J* = 3.4 Hz, 1H, H-1'), 5.33 (s, 1H, H-1''), 5.24 (d, *J* = 4.4 Hz, 1H, H-1'''), 5.03 (d, *J* = 8.4 Hz, 1H, H-8'), 4.40 (s, 1H, H-6'), 4.23 – 4.18 (m, 1H, H-2'''), 4.16 (q, *J* = 3.6 Hz, 1H, H-4'''), 3.87 – 3.80 (m, 1H, H-4), 3.80 – 3.64 (m, 8H, H-3'', H-4', H-5, H-5'', H-3''', H-6'', OC*H*$_2$CH$_2$), 3.64 – 3.41 (m, 7H, H-6'', H-5', H-6, H-5''', H-2', H-5', H-2''), 3.29 – 3.11 (m, 3H, H-1, H-3, H-7'), 3.05 (m, 3H, OCH_2CH_2, H-4''), 2.60 (s, 3H, NCH_3), 2.29 – 2.16 (m, 2H, H-2, H-3'), 1.91 – 1.80 (m, 1H, H-3'), 1.66 – 1.56 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 102.8 (C-1'''), 95.1 (C-1'), 94.4 (C-1''), 92.9 (C-8''), 84.3 (C-5), 83.2 (C-4'''), 78.4 (C-4), 78.0 (C-3'''), 71.6 (C-6), 70.9 (C-2'''), 70.3 (C-5''), 69.8 (C-2''), 68.7 (C-4'), 66.8 (OCH_2CH_2), 66.0 (C-5'), 62.8 (C-6'), 61.5 (C-5'''), 60.3 (C-6''), 59.5 (C-7'), 52.0 (C-4''), 50.0 (C-3), 48.6 (C-1), 47.8 (C-2'), 39.1 (OCH_2CH_2), 30.1 (NCH_3), 29.2 (C-2), 27.0 (C-3'); ESI-HRMS: m/z calcd. for $C_{28}H_{55}N_6O_{15}$ $[M+H]^+$ 715.3725; found, 715.3742.

5-*O*-β-(3'''-*O*-(2-Aminoethyl)-D-ribofuranosyl) apramycin hexaacetate salt (138β). Substrate **137β** (35 mg, 0.03 mmol) was deprotected in the same manner as compound **137α** to

yield **138β** (30 mg, 95%) as a pentaacetate salt in the form of a white solid; $[\alpha]_D^{25}$= 68.92 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.64 (d, *J* = 3.6 Hz, 1H, H-1'), 5.28 (d, *J* = 3.7 Hz, 1H, H-1''), 5.16 (s, 1H, H-1'''), 5.00 (d, *J* = 8.5 Hz, 1H, H-8'), 4.37 (s, 1H, H-6'), 4.19 (d, *J* = 2.9 Hz, 1H, H-2'''), 3.97 – 3.88 (m, 1H, H-4'''), 3.86 – 3.54 (m, 12H, H-4, H-3''', H-3'', H-5''', H-6'', H-5'', H-4', H-5, H-2', OC*H*$_2$CH$_2$), 3.53 – 3.39 (m, 5H, H-2', H-5', H-5''', H-2'', H-6), 3.31 – 3.21 (m, 1H, H-3), 3.18 (d, *J* = 8.4 Hz, 1H, H-7'), 3.16 – 3.08 (m, 1H, H-1), 3.05 (t, *J* = 10.3 Hz, 1H, H-4''), 3.01 (t, *J* = 4.8 Hz, 2H, OCH$_2$C*H*$_2$), 2.57 (s, 3H, NHC*H*$_3$), 2.24 (dd, *J* = 8.6, 4.0 Hz, 1H, H-2), 2.20 – 2.09 (m, 1H, H-3'), 1.84 (q, *J* = 11.9 Hz, 1H, H-3'), 1.66 – 1.59 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 110.5 (C-1'''), 94.5 (C-1'), 94.3 (C-1''), 92.8 (C-8'), 84.9 (C-5), 81.0 (C-4'''), 77.0 (C-3'''), 75.8 (C-4), 73.2 (C-2'''), 72.5 (C-6), 70.2 (C-5''), 69.6 (C-2''), 69.5 (C-3''), 68.4 (C-4'), 66.0 (C-5'), 65.9 (O*C*H$_2$CH$_2$), 62.6 (C-6'), 61.0 (C-5'''), 60.2 (C-6''), 59.3 (C-7'), 51.9 (C-4''), 49.8 (C-3), 48.3 (C-1), 47.8 (C-2'), 39.2 (OCH$_2$*C*H$_2$), 30.0 (NCH$_3$), 28.5 (C-2), 26.7 (C-3); ESI-HRMS: m/z calcd. for $C_{28}H_{55}N_6O_{15}$ $[M+H]^+$ 715.3725; found, 715.3690.

5-*O*-Benzyl-3-*O*-(2-benzyloxyethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (139). 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose[164] **134** (1000 mg, 3.57 mmol) was dissolved in dry THF (20 mL) and NaH (185 mg, 4.64 mmol) was added under argon. After stirring for 15 min, 2-benzyloxyethyl tosylate[166] (1.31 g, 4.29 mmol) was added and stirring continued for 12 h. More NaH (185 mg, 4.64 mmol) and 2-benzyloxyethyl tosylate (1.31 g, 4.29 mmol) were added and the mixture stirred for 24 h. After completion, the reaction was quenched with methanol, diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5% to 12% EtOAc/hexanes) to give **139** (1.24 g, 83%) in the form of a colorless oil; $[\alpha]_D^{25}$= +42.22 (*c* 2.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 – 7.26 (m, 10H,

Ar*H*), 5.77 (d, *J* = 3.7 Hz, 1H, H-1), 4.63 (d, *J* = 4.1 Hz, 1H, H-2), 4.61 (d, *J* = 4.1 Hz, 1H, C*H*$_2$Ph), 4.58 – 4.52 (m, 3H, C*H*$_2$Ph), 4.14 (ddd, *J* = 9.1, 4.1, 2.0 Hz, 1H, H-4), 3.92 – 3.76 (m, 3H, H-3, H-5, CH_2CH_2), 3.76 – 3.56 (m, 4H, H-5, 3H-CH_2CH_2), 1.57 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.2 (Ar*C*), 138.1 (Ar*C*), 128.4 (Ar*C*), 128.3 (Ar*C*), 127.73 (Ar*C*), 127.69 (Ar*C*), 127.67 (Ar*C*), 127.63 (Ar*C*), 127.59 (Ar*C*), 112.8 (*C*$(CH_3)_2$), 104.0 (C-1), 79.0 (C-3), 77.9 (C-4), 77.5 (C-2), 73.5 (*C*H_2Ph), 73.2 (*C*H_2Ph), 70.7 (CH_2CH_2), 70.1 (CH_2CH_2), 68.2 (C-5), 26.8 (CH_3), 26.5 (CH_3); ESI-HRMS: m/z calcd. for $C_{24}H_{30}NaO_6$ $[M+Na]^+$ 437.1940; found, 437.1939.

5-*O*-Benzyl-3-*O*-(2-benzyloxyethyl)-1,2-di-*O*-(4-nitrobenzoyl)-α-D-ribofuranose (140α) and **5-*O*-Benzyl-3-*O*-(2-benzyloxyethyl)-1,2-di-*O*-(4-nitrobenzoyl)-β-D-ribofuranose (140β).** To a stirred solution of compound **139** (600 mg, 1.45 mmol) in dioxane (10 mL), 1 N HCl (5 mL) was added and the reaction mixture was heated at 80 °C for 45 min. The reaction mixture was cooled, neutralized with solid $NaHCO_3$ then the solvent was evaporated. The residue was dissolved in EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated. To a solution of the residue in dry pyridine (10 mL), *p*-nitrobenzoyl chloride (672 mg, 3.6 mmol) and a catalytic amount of DMAP were added followed by stirring overnight. The reaction mixture was concentrated then diluted with EtOAc and washed with aqueous $NaHCO_3$, brine, dried with Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5% - 25% EtOAc/hexanes) to give **140** α:β = 1:1.5 (820 g, 84%, yellow oil). Further purification was done to separate analytical sample of anomers: **140α** (86 mg, 9%, yellow oil), **140β** (63 mg, 6%, yellow oil); **α anomer:** $[\alpha]_D^{25}$= +74.69 (*c* 5.7, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 – 8.21 (m, 2H, Ar*H*), 8.19 – 8.14 (m, 2H, Ar*H*), 8.11 – 8.07 (m, 4H, Ar*H*), 7.42 – 7.27 (m, 5H, Ar*H*), 7.27 – 7.22 (m, 3H,

Ar*H*), 7.20 – 7.06 (m, 2H, Ar*H*), 6.81 (d, *J* = 4.4 Hz, 1H, H-1), 5.47 (dd, *J* = 6.3, 4.4 Hz, 1H, H-2), 4.66 – 4.53 (m, 3H, H-4, C*H*$_2$Ph), 4.49 – 4.41 (m, 2H, C*H*$_2$Ph), 4.38 (dd, *J* = 6.3, 2.8 Hz, 1H, H-3), 3.81 – 3.73 (m, 2H, CH_2CH_2), 3.71 – 3.65 (m, 2H, H-5), 3.66 – 3.56 (m, 2H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.7 (C=O), 163.6 (C=O), 150.7 (Ar*C*), 150.7 (Ar*C*), 137.8 (Ar*C*), 137.6 (Ar*C*), 135.1 (Ar*C*), 134.5 (Ar*C*), 131.0 (Ar*C*), 130.7 (Ar*C*), 128.5 (Ar*C*), 128.4 (Ar*C*), 127.9 (Ar*C*), 127.7 (Ar*C*), 127.3 (Ar*C*), 123.6 (Ar*C*), 96.0 (C-1), 85.0 (C-4), 76.9 (C-3), 73.7 (*C*H_2Ph), 73.4 (*C*H_2Ph), 73.2 (C-2), 71.2 (CH_2CH_2), 69.9 (CH_2CH_2), 69.5 (C-5); ESI-HRMS: m/z calcd. for $C_{35}H_{32}N_2NaO_{12}$ $[M+Na]^+$ 695.1853; found, 695.1859; **β anomer:** $[\alpha]_D^{25}$= -11.33 (*c* 0.042, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (m, 4H, Ar*H*), 8.12 – 8.05 (m, 2H, Ar*H*), 8.05 – 7.99 (m, 2H, Ar*H*), 7.29 – 7.16 (m, 10H, Ar*H*), 6.55 (s, 1H, H-1), 5.74 (d, *J* = 4.4 Hz, 1H, H-2), 4.66 (dd, *J* = 7.7, 4.4 Hz, 1H, H-3), 4.51 (s, 2H, C*H*$_2$Ph), 4.46 – 4.39 (m, 3H, H-4, C*H*$_2$Ph), 3.85 (dd, *J* = 11.0, 2.7 Hz, 1H, H-5), 3.83 – 3.75 (m, 2H, CH_2CH_2), 3.72 (dd, *J* = 11.1, 3.5 Hz, 1H, H-5), 3.64 – 3.53 (m, 2H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.6 (C=O), 163.0 (C=O), 150.7 (Ar*C*), 150.6 (Ar*C*), 137.9 (Ar*C*), 137.8 (Ar*C*), 134.6 (Ar*C*), 131.0 (Ar*C*), 130.9 (Ar*C*), 128.4 (Ar*C*), 128.3 (Ar*C*), 127.7 (Ar*C*), 127.6 (Ar*C*), 127.5 (Ar*C*), 123.6 (Ar*C*), 123.5 (Ar*C*), 99.5 (C-1), 82.2 (C-4), 77.3 (C-3), 75.2 (C-2), 73.5 (*C*H_2Ph), 73.2 (*C*H_2Ph), 71.1 (CH_2CH_2), 69.7 (CH_2CH_2), 68.8 (C-5); ESI-HRMS: m/z calcd. for $C_{35}H_{32}N_2NaO_{12}$ $[M+Na]^+$ 695.1853; found, 695.1846.

5-*O*-β-[5'''-*O*-Benzyl-3'''-*O*-(2-benzyloxyethyl)-1''',2'''-di-*O*-(4-nitrobenzoyl)-D-ribofuranosyl]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (141). Donor **140** (161 mg, 0.24 mmol), acceptor **124** (67 mg, 0.08 mmol) and activated 4 Å MS were stirred in dry DCM at rt for 1 h before cooling to 0 °C. $BF_3.OEt_2$ (100 μL, 0.27 mmol) was added and reaction mixture was stirred for 48 h at 0 °C. The reaction was quenched with

triethylamine (0.5 mL) and filtered through Celite® before dilution with EtOAc and washing with aqueous $NaHCO_3$ and brine and concentration. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.5% methanol/DCM) to give the β anomer **141** (14 mg, 13%) as a white solid; $[\alpha]_D^{25}$= +56.85 (*c* 0.7, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.20 – 8.11 (m, 4H, Ar*H*), 7.40 –7.30 (m, 5H, Ar*H*), 7.24 – 7.10 (m, 5H, Ar*H*), 5.80 (d, *J* = 3.6 Hz, 1H, H-1'), 5.43 (t, *J* = 10.0 Hz, 1H, H-3''), 5.39 – 5.34 (m, 2H, H-1'', H-1'''), 5.26 (d, *J* = 4.3 Hz, 1H, H-2'''), 4.91 (t, *J* = 9.7 Hz, 1H, H-6), 4.87 (dd, *J* = 10.3, 3.9 Hz, 1H, H-2''), 4.81 (d, *J* = 4.5 Hz, 1H, H-8'), 4.76 (dd, *J* = 7.4, 3.3 Hz, 1H, H-6'), 4.57 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.51 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.39 (dd, *J* = 10.3, 3.4 Hz, 1H, H-5'), 4.37 – 4.31 (m, 3H, C*H*$_2$Ph, H-6''), 4.27 – 4.19 (m, 2H, H-6'', H-4'''), 4.14 (dd, *J* = 7.5, 4.4 Hz, 1H, H-3'''), 3.87 – 3.71 (m, 4H, H-5, H-7', H-5'', H-5'''), 3.71 – 3.63 (m, 3H, H-4, CH_2CH_2), 3.63 – 3.52 (m, 4H, H-3, H-5'' H-4', H-4''), 3.51 – 3.37 (m, 3H, H-1, CH_2CH_2), 3.08 (dt, *J* = 12.9, 4.2 Hz, 1H, H-2'), 2.95 (s, 3H, NHC*H*$_3$), 2.41 (dt, *J* = 13.1, 4.5 Hz, 1H, H-2), 2.23 – 1.97 (m, 13H, 4COCH_3, H-3'), 1.87 (q, *J* = 11.7 Hz, 1H, H-3'), 1.57 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.3 (C=O), 170.1 (C=O), 169.8 (C=O), 169.7 (C=O), 163.8 (C=O), 157.1 (Ar*C*), 150.7 (Ar*C*), 137.9 (Ar*C*), 137.9 (Ar*C*), 134.7 (Ar*C*), 130.8 (Ar*C*), 128.5 (Ar*C*), 128.2 (Ar*C*), 127.7 (Ar*C*), 127.6 (Ar*C*), 127.5 (Ar*C*), 127.4 (Ar*C*), 123.6 (Ar*C*), 107.2 (C-1'''), 97.3 (C-8'), 96.4 (C-1'), 94.2 (C-1''), 82.0 (C-5), 80.7 (C-4'''), 77.8 (C-4), 77.7 (C-3'''), 75.7 (C-2''), 75.0 (C-6), 73.4 (*C*H$_2$Ph), 73.0 (*C*H$_2$Ph), 71.0 (C-6'), 70.8 (C-3''), 70.4 (CH_2CH_2), 70.3 (C-5'''), 69.9 (C-2''), 69.2 (C-5''), 69.1 (CH_2CH_2), 65.9 (C-5'), 65.7 (C-4'), 62.9 (C-6''), 60.20 (C-4''), 60.17 (C-7'), 59.1 (C-3), 58.1 (C-1), 56.5 (C-2'), 31.3 (C-2), 30.1 (C-3'), 29.7 (N*C*H$_3$), 20.9 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{58}H_{66}N_{14}NaO_{24}$ $[M+Na]^+$ 1365.4272; found, 1365.4260.

5-*O*-β-[3'''-*O*-(2-Hydroxyethyl)-D-ribofuranosyl] apramycin pentaacetate salt (142). A stirred solution of compound **141** (10 mg, 0.01 mmol) in dioxane (0.5 mL) was treated with 3N NaOH (0.25 mL) and heated at 100 °C for 3 h. The reaction mixture was cooled to rt and neutralized with Amberlyst® before concentration *in vacuo*. The crude product was dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL) and $Pd(OH)_2/C$ (0.5 equiv) was added. The reaction mixture was stirred at room temperature under 50 psi of hydrogen for 12 h. After completion, the reaction mixture was filtered through Celite® and concentrated to dryness. The residue was then dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **142** (3.5 mg, 48%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +72.0 (*c* 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.72 (d, *J* = 3.6 Hz, 1H, H-1'), 5.34 (d, *J* = 3.8 Hz, 1H, H-1''), 5.20 (s, 1H, H-1'''), 5.06 (d, *J* = 8.5 Hz, 1H, H-8'), 4.43 (s, 1H, H-6'), 4.21 (d, *J* = 4.3 Hz, 1H, H-2'''), 3.97 – 3.92 (m, 1H, H-4'''), 3.91 – 3.86 (m, 1H, H-4), 3.86 – 3.79 (m, 2H, H-5'', H-3'''), 3.78 – 3.66 (m, 7H, H-5, H-4', H-5''', H-6'', H-3'', CH_2CH_2), 3.63 (dd, *J* = 12.4, 4.6 Hz, 1H, H-6''), 3.60 – 3.55 (m, 3H, H-2'',CH_2CH_2), 3.55 – 3.45 (m, 4H, H-6, H-2', H-6', H-5'''), 3.42 – 3.33 (m, 1H, H-3), 3.24 (d, *J* = 8.9 Hz, 1H, H-7'), 3.21 – 3.09 (m, 2H, H-, H-1, H-4''), 2.63 (s, 3H, NCH_3), 2.32 (dt, *J* = 12.3, 3.8 Hz, 1H, H-2), 2.23 – 2.15 (m, 1H, H-3'), 1.94 – 1.90 (m, 1H, H-3'), 1.70 (q, *J* = 12.8 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 110.4 (C-1'''), 94.6 (C-1'), 94.4 (C-1''), 92.8 (C-8'), 84.8 (C-5), 81.1 (C-4'''), 77.0 (C-3'''), 75.2 (C-4), 73.4 (C-2'''), 72.4 (C-6), 71.4 (CH_2CH_2), 70.2 (C-5''), 69.7 (C-2''), 69.3 (C-3''), 68.2 (C-4'), 66.0 (C-5'), 62.6 (C-6'), 60.9 (C-5'''), 60.5 (CH_2CH_2), 60.3 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 49.7 (C-3), 48.3 (C-1),

47.8 (C-2'), 30.0 (N*C*H$_3$), 28.0 (C-2), 26.6 (C-3'); ESI-HRMS: m/z calcd. for $C_{28}H_{54}N_5O_{16}$ $[M+H]^+$ 716.3566; found, 716.3541.

2,3-*O*-Isopropylidene-β-D-erythrofuranosyl trichloroacetimidate (144). 2,3-*O*-Isopropylidene-β-D-erythrofuranose[169] **143** (300 mg, 1.88 mmol) and trichloroacetonitrile (2 mL) were dissolved with stirring in dry DCM (2 mL) and ice-cooled before addition of DBU (2 drops). The reaction mixture was stirred at rt for 5 min and concentrated. The crude mixture was passed through a silica gel column that had been basified with 0.5% triethylamine/hexanes, eluting with 0.5% triethylamine in EtOAc/hexanes to give **144** (564 mg, quant.) in the form of a yellow oil; $[\alpha]_D^{25}$= -84.04 (*c* 0.5, DCM);^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (s, 1H, NH), 6.29 (s, 1H, H-1), 4.93 (dd, *J* = 5.9, 3.5 Hz, 1H, H-3), 4.83 (d, *J* = 5.9 Hz, 1H, H-2), 4.20 (d, *J* = 10.5 Hz, 1H, H-4), 4.09 (dd, *J* = 10.6, 3.6 Hz, 1H, H-4), 1.50 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.7(C=N), 113.1 (*C*$(CH_3)_2$), 105.4 (C-1), 84.4 (C-2), 79.4 (C-3), 74.0 (C-4), 26.2 (CH_3), 24.9 (CH_3); ESI-HRMS: m/z calcd. for $C_9H_{12}Cl_3NNaO_4$ $[M+Na]^+$ 325.9730; found, 325.9722.

5-*O*-β-[2''',3'''-*O*-Isopropylidene-D-erythrofuranosyl]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (145). Donor **144** (109 mg, 0.36 mmol), acceptor **124** (100 mg, 0.12 mmol) and activated 4 Å MS were added to a flame-dried round-bottom flask and stirred in dry DCM (2.5 mL) at rt for 1 h before cooling to -78 °C, addition of $BF_3.OEt_2$ (200 μL, 0.54 mmol) and stirring for 1.5 h at -30 °C. The reaction was allowed to warm up to 0 °C before quenching with triethylamine (0.5 mL) and filtered through Celite®. The reaction mixture was diluted with EtOAc, and washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.4% - 0.8% Methanol/DCM) to give **145** (112 mg, 96%) as white solid; $[\alpha]_D^{25}$= +77.97 (*c* 0.7,

DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45 – 5.35 (m, 3H, H-1', H-1'', H-3''), 5.23 (s, 1H, H-1'''), 4.93 (t, *J* = 9.8 Hz, 1H, H-6), 4.87 – 4.80 (m, 2H, H-8', H-2''), 4.76 (dd, *J* = 7.5, 3.5 Hz, 1H, H-6'), 4.72 (dd, *J* = 5.9, 3.5 Hz, 1H, H-3'''), 4.45 – 4.36 (m, 2H, H-5', H-2'''), 4.31 (dd, *J* = 12.2, 2.3 Hz, 1H, H-6''), 4.20 (dd, *J* = 12.2, 5.2 Hz, 1H, H-6''), 4.04 (d, *J* = 10.7 Hz, 1H, H-4'''), 3.90 (dd, *J* = 10.8, 3.6 Hz, 1H, H-4'''), 3.79 – 3.68 (m, 3H, H-5, H-7', H-5''), 3.68 – 3.62 (m, 2H, H-4, H-4'), 3.61 – 3.51 (m, 2H, H-3, H-4''), 3.45 (ddd, *J* = 12.3, 10.1, 4.3 Hz, 1H, H-1), 3.11 (dt, *J* = 12.8, 4.0 Hz, 1H, H-2'), 2.92 (s, 3H, N*C*H_3), 2.42 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2), 2.24 (dt, *J* = 11.1, 4.4 Hz, 1H, H-3'), 2.19 (s, 3H, COC*H*$_3$), 2.14 – 2.04 (m, 6H, 2*COC*H*$_3$), 2.05 (s, 3H, COC*H*$_3$), 2.03 – 1.93 (m, 1H, H-3'), 1.59 (q, *J* = 12.6 Hz, 1H, H-2), 1.40 (s, 3H, C*H*$_3$), 1.25 (s, 3H, C*H*$_3$); ^{13}C NMR (101 MHz, CDCL3): δ 170.4 (C=O), 170.1 (C=O), 169.8 (C=O), 156.9 (C=O), 112.5 (*C*$(CH_3)_2$), 109.9 (C-1'''), 97.7 (C-8'), 97.4 (C-1'), 94.3 (C-1''), 85.6 (C-2'''), 80.9 (C-5), 79.7 (C-3'''), 77.2 (C-4), 75.6 (C-6), 73.4 (C-4'''), 71.0 (C-6'), 70.3 (C-3''), 69.9 (C-2''), 69.1 (C-5''), 66.2 (C-5'), 65.8 (C-4'), 62.8 (C-6''), 60.2 (C-7'), 60.1 (C-4''), 59.1 (C-3), 57.9 (C-1), 55.9 (C-2'), 31.5 (C-2), 30.2 (N*C*H_3), 29.4 (C-3'), 26.2 (CH_3), 24.8 (CH_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.7 (CO*C*H_3); ESI-HRMS: m/z calcd. for $C_{37}H_{49}N_{13}NaO_{19}$ $[M+Na]^+$ 1002.3165; found, 1002.3137.

Erythrolactone-2,3-dibenzoate (148). An aqueous solution of isoascorbic acid (7.04 g, 40 mmol) in water (100 mL) was ice-cooled and Na_2CO_3 (8.48 g, 80 mmol) was added slowly followed by aqueous solution of H_2O_2 (30%, 9.2 mL). The reaction mixture was stirred at 42 °C for 30 min after which charcoal (2 g) was added and the mixture stirred at 75 °C for 30 min to destroy excess H_2O_2. The reaction mixture was filtered while hot and neutralized with 6 N HCl then concentrated till dryness. The resulting residue was dissolved in dry pyridine (50 mL) and cooled to 0 °C before addition of benzoyl chloride dropwise (11.6 mL, 100 mmol). The reaction

mixture was stirred at rt for 12 h before it was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by gradient chromatography over silica gel (eluent: 10% to 35% EtOAc in hexanes) to give **148** (9.0 g, 69%) as a white solid; $[\alpha]_D^{25}$= -145.78 (c 1.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (m, 4H), 7.65 – 7.50 (m, 2H, Ar*H*), 7.41 (m, 2H, Ar*H*), 7.35 (m, 2H, Ar*H*), 6.07 – 5.95 (m, 2H, H-2, H-3), 4.73 (dd, *J* = 11.4, 3.1 Hz, 1H, H-4), 4.65 (d, *J* = 11.4 Hz, 1H, H-4); ^{13}C NMR (101 MHz, CDCL3): δ 170.3 (C=O), 165.3 (Ar*C*), 164.9 (Ar*C*), 133.9 (Ar*C*), 130.1 (Ar*C*), 129.8 (Ar*C*), 128.6 (Ar*C*), 128.5 (Ar*C*), 128.5 (Ar*C*), 128.0 (Ar*C*), 69.9 (C-4), 69.7 (C-2), 67.7(C-3); ESI-HRMS: m/z calcd. for $C_{18}H_{14}NaO_6$ $[M+Na]^+$ 349.0688; found, 349.0691.

2,3-Di-*O*-benzoyl-α/β-D-erythrofuranose (149). A stirred solution of erythrolactone-2,3-dibenzoate **148** (1000 mg, 3.06 mmol) in dry THF was cooled to -78 °C and DIBAL (1 M in hexanes, 6 mL) was added. The mixture was stirred for 4 h before it was quenched with methanol (20 mL). The so-formed residue was filtered through Celite® and the filtrate was concentrated and dissolved in EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by gradient chromatography over silica gel (eluent: 10% to 25% EtOAc in hexanes) to give **149** (360 mg, 36%) as an α:β mixture (0.5:1) in the form of a gum; $[\alpha]_D^{25}$= -29.1 (*c* 0.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 – 8.01 (m, 2H, Ar*H*), 8.00 – 7.93 (m, 2H, Ar*H*), 7.92 – 7.84 (m, 2H, Ar*H*), 7.57 – 7.42 (m, 2H, Ar*H*), 7.42 – 7.24 (m, 7H, Ar*H*), 5.90 – 5.78 (m, 1.5H, H-3b, H-3α), 5.74 (dd, *J* = 7.2, 4.7 Hz, 0.5H, H-1α), 5.72 – 5.67 (m, 1H, H-1β), 5.59 (dd, *J* = 5.3, 1.6 Hz, 1H, H-2β), 5.33 (dd, *J* = 5.9, 4.7 Hz, 0.5H, H-2β), 4.72 – 4.63 (m, 1H, OH), 4.58 (dd, *J* = 10.0, 6.0 Hz, 1H, H-4β), 4.37 – 4.26 (m, 1H, H-4α), 4.13 (dd, *J* = 10.0, 4.2 Hz, 1H, H-4β); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.8 (C=O), 165.7 (C=O), 133.5 (Ar*C*), 133.5 (Ar*C*), 133.4 (Ar*C*), 133.4

(Ar*C*), 133.3 (Ar*C*), 130.00 (Ar*C*), 139.95 (Ar*C*), 129.9 (Ar*C*), 129.83 (Ar*C*), 129.79 (Ar*C*), 129.7 (Ar*C*), 129.18 (Ar*C*), 129.15 (Ar*C*), 128.6 (Ar*C*), 128.51 (Ar*C*), 128.45 (Ar*C*), 128.41 (Ar*C*), 128.36 (Ar*C*), 100.4 (C-1β), 94.9 (C-1α), 77.0 (C-2β), 76.9 (C-2α), 72.3 (C-3β), 72.2 (C-3α), 69.9 (C-4β), 69.8 (C-4α); ESI-HRMS: m/z calcd. for $C_{18}H_{16}NaO_6$ $[M+Na]^+$ 349.0688; found, 349.0691.

2,3-Di-*O*-benzoyl-β-D-erythrofuranosyl trichloroacetimidate (150). 2,3-*O*-Dibenzoyl-α/β-D-erythrofuranose **(149)** (360 mg, 1.10 mmol) and trichloroacetonitrile (2 mL) were dissolved in dry DCM (2 mL) and ice-cooled before addition of DBU (2 drops). The reaction mixture was stirred at rt for 5 min and concentrated. The crude mixture was passed through a silica gel column that had been basified with 0.5% triethylamine/hexanes, eluting with 0.5% triethylamine in EtOAc/hexanes to give compound **150** (458 mg, 88%) as a gum; $[\alpha]_D^{25}$= -78.9 (*c* 1.6, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1H, NH), 8.05 – 7.97 (m, 2H, Ar*H*), 7.93 – 7.86 (m, 2H, Ar*H*), 7.54 (m, 2H, Ar*H*), 7.40 (m, 2H, Ar*H*), 7.32 (m, 2H, Ar*H*), 6.58 (s, 1H, H-1), 5.94 – 5.84 (m, 2H, H-2, H-3), 4.66 – 4.58 (m, 1H, H-4), 4.30 (dd, *J* = 9.8, 3.4 Hz, 1H, H-4); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.6 (Ar*C*), 165.1 (Ar*C*), 160.8 (C=N), 133.6 (Ar*C*), 133.4 (Ar*C*), 129.9 (Ar*C*), 129.7 (Ar*C*), 128.9 (Ar*C*), 128.8 (Ar*C*), 128.5 (Ar*C*), 128.4 (Ar*C*), 103.2 (C-1), 77.2 (CCl_3), 75.5 (C-2), 71.8 (C-4), 71.5 (C-3); ESI-HRMS: m/z calcd. for $C_{20}H_{16}Cl_3NaO_6$ $[M+Na]^+$ 493.9941; found, 493.9945.

5-*O*-β-[2''',3'''-Di-*O*-benzoyl-D-erythrofuranosyl]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (151). Donor **150** (109 mg, 0.36 mmol), acceptor **124** (100 mg, 0.12 mmol) and activated 4 Å MS were stirred in dry DCM (2.5 mL) at rt for 1 h before cooling to -78 °C. $BF_3.OEt_2$ (200 μL, 0.54 mmol) was added and reaction mixture was stirred for 3 h at -78 °C. The reaction was quenched at -78 °C with triethylamine (0.5 mL)

and filtered through Celite® before it was diluted with EtOAc. The organic layer was washed with $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.4% - 0.8% Methanol/DCM) to give the β anomer **151** (68 mg, 50%) in the form of white solid; $[\alpha]_D^{25}$= +64.1 (*c* 4.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (m, 4H, Ar*H*), 7.53 (m, 2H, Ar*H*), 7.35 (m, 4H, Ar*H*), 5.63 (m, 2H, H-3''', H-1'''), 5.54 (d, *J* = 3.5 Hz, 1H, H-1'), 5.51 (dd, *J* = 5.0, 1.4 Hz, 1H, H-2'''), 5.44 (t, *J* = 10.0 Hz, 1H, H-3''), 5.39 (d, *J* = 3.8 Hz, 1H, H-1''), 5.02 (t, *J* = 9.9 Hz, 1H, H-6), 4.93 – 4.86 (m, 2H, H-2'', H-8'), 4.82 (dd, *J* = 7.7, 3.3 Hz, 1H, H-6'), 4.62 – 4.50 (m, 2H, H-5', H-4'''), 4.33 (dd, *J* = 12.1, 1.9 Hz, 1H, H-6''), 4.23 (dd, *J* = 12.2, 5.3 Hz, 1H, H-6''), 4.16 (dd, *J* = 9.9, 4.9 Hz, 1H, H-4'''), 3.93 (t, *J* = 9.2 Hz, 1H, H-5), 3.84 – 3.70 (m, 4H, H-4, H-4', H-5'', H-7'), 3.70 – 3.54 (m, 2H, H-3, H-4''), 3.49 (ddd, *J* = 12.5, 10.5, 4.3 Hz, 1H, H-1), 3.34 (dt, *J* = 12.9, 4.1 Hz, 1H, H-2'), 2.94 (s, 3H, NCH_3), 2.47 (dt, *J* = 12.9, 4.5 Hz, 1H, H-2), 2.33 – 2.23 (m, 1H, H-3'), 2.11 (m, 12H, 4COC*H*$_3$), 1.97 (q, *J* = 11.8 Hz, 1H, H-3'), 1.65 (q, *J* = 12.5 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4 (C=O), 170.2 (C=O), 169.8 (C=O), 169.6 (C=O), 165.5 (Ar-C), 165.3 (Ar-C), 157.0 (Ar-C), 133.6 (Ar-C), 133.4 (Ar-C), 129.6 (Ar-C), 129.0 (Ar-C), 128.9 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 106.4 (C-1'''), 97.4 (C-1'), 96.8 (C-8'), 94.1 (C-1''), 79.2 (C-5), 78.1 (C-4), 75.8 (C-2'''), 74.8 (C-6), 71.6 (C-3'''), 70.8 (C-6'), 70.6 (C-2''), 70.4 (C-4'''), 69.9 (C-3''), 69.1 (C-5''), 66.0 (C-5'), 65.6 (C-4'), 62.9 (C-6''), 60.2 (C-7'), 60.1 (C-3), 59.0 (C-4''), 58.2 (C-1), 56.5 (C-2'), 31.5 (C-2), 30.1 (N*C*H$_3$), 29.7(C-3') 20.9 (2CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{48}H_{53}N_{13}NaO_{21}$ $[M+Na]^+$ 1170.3377; found, 1170.3353.

5-*O*-β-D-(Erythrofuranosyl) apramycin pentaacetate salt (152). A stirred solution of compound **151** (60 mg, 0.02 mmol) in dioxane (1mL) was treated with 3N NaOH (1 mL) and heated at 100 °C for 2 h. The reaction mixture was cooled to 55 °C and 1M $P(CH_3)_3$ in THF (0.3

mL) was added and stirring continued for 2 h. The reaction mixture was neutralized with glacial acetic acid, concentrated *in vacuo*, the residue dissolved in aqueous acetic acid solution (pH 4, 1 mL) and was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and lyophilized to afford compound **152** (35 mg, 71%) as peracetate salt in the form of white solid; $[\alpha]_D^{25}$= +58.71 (*c* 2.3, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.67 (d, *J* = 4.1 Hz, 1H, H-1'), 5.28 (d, *J* = 3.9 Hz, 1H, H-1''), 5.18 (d, *J* = 3.8 Hz, 1H, H-1'''), 5.00 (d, *J* = 8.5 Hz, 1H, H-8'), 4.38 (s, 1H, H-6'), 4.16 (q, *J* = 4.4 Hz, 1H, H-3'''), 4.04 (dd, *J* = 9.6, 4.9 Hz, 1H, H-4'''), 3.98 (t, *J* = 4.2 Hz, 1H, H-2'''), 3.85 (t, *J* = 9.7 Hz, 1H, H-4), 3.77 (dt, *J* = 10.2, 3.9 Hz, 1H, H-5''), 3.74 – 3.65 (m, 3H, H-4', H-5, H-3''), 3.74 – 3.65 (m, 2H, H-4''', H-6''), 3.57 (dd, *J* = 12.5, 4.5 Hz, 1H, H-6''), 3.54 – 3.43 (m, 4H, H-2', H-2'', H-5', H-6), 3.34 – 3.27 (m, 1H, H-3), 3.18 (dd, *J* = 8.5, 2.7 Hz, 1H, H-7'), 3.13 (td, *J* = 12.0, 4.2 Hz, 1H, H-1), 3.08 (t, *J* = 10.4 Hz, 1H, H-4''), 2.57 (s, 3H, NCH_3), 2.26 (dt, *J* = 12.5, 4.1 Hz, 1H, H-2), 2.16 (dt, *J* = 9.7, 4.4 Hz, 1H, H-3'), 1.86 – 1.80 (m, 1H, H-3'), 1.65 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 110.0 (C-1'''), 94.3 (C-1'), 93.9 (C-1''), 92.7 (C-8'), 84.4 (C-5), 75.6 (C-2'''), 75.1 (C-4), 72.4 (C-6), 71.4 (C-4'''), 70.1 (C-5''), 69.6 (C-2''), 69.6 (C-4'), 69.3 (C-3''), 68.1 (C-3'''), 65.7 (C-5'), 62.5 (C-6'), 60.2 (C-6''), 59.2 (C-7'), 51.9 (C-4''), 49.4 (C-3), 48.4 (C-1), 47.4 (C-2'), 29.9 (NCH_3), 28.0 (C-2), 26.8 (C-3'); ESI-HRMS: m/z calcd. for $C_{25}H_{48}N_5O_{14}$ $[M+H]^+$ 642.3198; found, 642.3182.

1,2,3-Tri-*O*-acetyl-5-deoxy-5-phthalimido-α-D-ribofuranose (154). 1,2,3-Tri-*O*-acetyl-5-*O*-p-tolylsulfonyl-D-ribofuranose[171] **153** (1000 mg, 2.3 mmol) was dissolved in dry DMF (20 mL) and treated with potassium phthalimide (1000 mg, 5.4 mmol). The reaction mixture was stirred at 50 °C for 12 h before it was diluted with water and extracted with DCM

three times. The organic layer wash then washed with 5% aqueous NaOH and brine, dried over Na_2SO_4, and concentrated. The residue was purified using silica gel column chromatography (eluent: 15% - 35% EtOAc/hexanes) to give **154** (566 mg, 60%) as a white solid; $[\alpha]_D^{25}$= +49.66 (*c* 1.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 – 7.73 (m, 2H, Ar*H*), 7.70 – 7.62 (dd, *J* = 5.4, 3.1 Hz, 2H, Ar*H*), 6.34 (d, *J* = 4.5 Hz, 1H, H-1), 5.23 (dd, *J* = 6.8, 4.5 Hz, 1H, H-2), 5.15 (dd, *J* = 6.7, 3.4 Hz, 1H, H-3), 4.48 (td, *J* = 6.8, 3.4 Hz, 1H, H-4), 3.86 (dd, *J* = 6.8, 5.2 Hz, 2H, H-5), 2.03 – 1.95 (m, 9H, CO*C*H$_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.9 (C=O), 169.4 (C=O), 169.2 (C=O), 168.0 (C=O), 134.1 (Ar*C*), 131.8 (Ar*C*), 123.4 (Ar*C*), 93.7 (C-1), 80.4 (C-4), 70.7 (C-3), 69.6 (C-2), 39.3 (C-5), 20.9 (CO*C*H$_3$), 20.5 (CO*C*H$_3$), 20.2 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{19}H_{19}NNaO_9$ $[M+Na]^+$ 428.0958; found, 428.0964.

2,3-Di-*O*-acetyl-5-deoxy-5-phthalimido-D-ribofuranose trichloroacetimidate (155). To an ice-cooled solution of **154** (550 mg, 1.36 mmol) in DCM (5 mL), 33% HBr/acetic acid (0.7 mL, 4.07 mmol) was added followed by stirring for 45 min. After completion, solid $NaHCO_3$ was added to neutralize the reaction, then water was added and the aqueous layer was extracted with DCM three times. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified using silica gel column chromatography (eluent: 20% - 60% EtOAc/hexanes) to give 2,3-di-*O*-acetyl-5-deoxy-5-phthalimido-α/β-D-ribofuranose as a mixture of anomers α:β = 1:3 (200 mg, 41%) that was used directly in the next step. 2,3-Di-*O*-acetyl-5-deoxy-5-phthalimido-α/β-D-ribofuranose (190 mg, 0.53 mmol) and trichloroacetonitrile (2 mL) were dissolved in dry DCM (2 mL) and ice-cooled before addition of DBU (2 drops). The reaction mixture was stirred at rt for 5 min and concentrated. The crude product was passed through a silica gel column, basified with 0.5% triethylamine/hexanes, eluting with 0.5%

triethylamine in EtOAc/hexanes to give compound **155** (270 mg, quant) which was used in the next step without further purification.

5-*O*-β-(2''',3'''-Di-*O*-acetyl-5'''-deoxy-5'''-phthalimido-D-ribofuranosyl)-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (156). Donor **155** (190 mg, 0.52 mmol), acceptor **124** (701 mg, 0.84 mmol) and activated 4 Å MS were stirred in dry DCM (3 mL) at rt for 1 h before cooling to 0 °C. $BF_3.OEt_2$ (400 μL, 1.08 mmol) was added and reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched with triethylamine (0.5 mL) and filtered through Celite® before it was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.5% methanol/DCM) to give the glycoside **156** (470 mg, 76%) as the β anomer in the form of white solid; $[\alpha]_D^{25}$= +131.96 (*c* 5.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 – 7.88 (m, 2H, Ar*H*), 7.77 – 7.70 (m, 2H, Ar*H*), 5.39 (t, *J* = 9.9 Hz, 1H, H-3''), 5.34 (d, *J* = 3.8 Hz, 2H, H-1', H-1''), 5.30 (s, 1H, H-1'''), 5.12 (d, *J* = 4.9 Hz, 1H, H-2'''), 5.06 (dd, *J* = 7.2, 4.8 Hz, 1H, H-3'''), 4.95 – 4.88 (m, 2H, H-8', H-2''), 4.83 (dd, *J* = 8.2, 2.9 Hz, 1H, H-6'), 4.66 (dd, *J* = 10.5, 2.9 Hz, 1H, H-5'), 4.46 (t, *J* = 9.9 Hz, 1H, H-6), 4.42 – 4.29 (m, 2H, H-6'', H-4'''), 4.22 (dd, *J* = 12.2, 5.2 Hz, 1H, H-6''), 3.96 (d, *J* = 5.0 Hz, 2H, H-5'''), 3.84 – 3.68 (m, 3H, H-4', H-6', H-5''), 3.65 – 3.53 (m, 3H, H-3, H-4, H-4''), 3.46 – 3.28 (m, 3H, H-1, H-5, H-2'), 2.94 (s, 3H, NCH_3), 2.41 (dt, *J* = 12.6, 4.3 Hz, 1H, H-2), 2.23 (s, 4H, H-3', $COCH_3$), 2.13 – 1.98 (m, 15H, 5*$COCH_3$), 1.78 (q, *J* = 11.7 Hz, 1H, H-3'), 1.43 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3 (C=O), 170.2 (C=O), 169.9 (C=O), 169.8 (C=O), 169.5 (C=O), 168.2 (C=O), 157.2 (C=O), 134.1 (Ar*C*), 132.0 (Ar*C*), 123.7 (Ar*C*), 106.9 (C-1'''), 97.0 (C-1'), 94.8 (C-8'), 93.8 (C-1''), 79.8 (C-5), 79.1 (C-4'''), 78.9 (C-4), 74.0 (2'''), 73.4 (C-6), 72.6 (C-3'''), 70.7 (C-6'), 70.3 (C-

3’’), 69.9 (C-2’’), 68.9 (C-5’’), 65.4 (C-5’), 65.3 (C-4’), 62.9 (C-6’’), 60.21 (C-7’), 60.16 (C-4’’), 58.4 (C-3), 58.2 (C-1), 57.7 (C-2), 39.5 (C-5’’’), 31.4 (C-2), 31.3 (C-3’), 29.9 (N*C*H_3), 20.9 (CO*C*H_3), 20.8 (CO*C*H_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.6 (CO*C*H_3), 20.4 (CO*C*H_3); ESI-HRMS: m/z calcd. for $C_{47}H_{54}N_{14}NaO_{23}$ $[M+Na]^+$ 1205.3384; found, 1205.3359.

5-*O*-β-(5'''-Formamido-5'''-deoxy-D-ribofuranosyl) apramycin pentaacetate salt (157). To a stirred solution of compound **156** (50 mg, 0.04 mmol) in an IPA:water mixture (7:3, 1.5 mL), $NaBH_4$ (90 mg, 2.4 mmol) was added followed by stirring for 2 h. The reaction mixture was diluted with methanol and glacial acetic acid was added dropwise until effervescence stopped. The reaction mixture was concentrated *in vacuo* followed by the addition of 3 N NaOH (0.5 mL) and water (0.5 mL). The reaction mixture was heated at 100 °C for 1 h before it was cooled, neutralized with glacial acetic acid and concentrated. The crude mixture was desalted using a Sephadex column and the product-containing fractions were concentrated. A part of the solid residue (8.2 mg, 0.009 mmol) was dissolved in water (0.2 mL) and treated with *N*-(diethylcarbamoyl)-*N*-methoxyformamide[172] (2.4 μL, 0.014 mmol) and triethylamine (1 μL). The reaction mixture was stirred for 2 h and quenched with ammonium hydroxide (0.25 mL) followed by addition of 1M $P(CH_3)_3$ in THF (0.3 mL) and stirring at 60 °C for 3h. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with acetic acid and lyophilized to afford **157** in (4.5 mg, 42%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +82.2 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 7.98 (s, 1H, CHO), 5.67 (d, *J* = 3.9 Hz, 1H, H-1’), 5.34 (d, *J* = 3.9 Hz, 1H, H-1’’), 5.14 (d, *J* = 2.9 Hz, 1H, H-1’’’), 5.06 (d, *J* = 8.5 Hz, 1H, H-8’), 4.46 – 4.39

(m, 1H, H-6'), 4.02 (dd, *J* = 4.4, 3.2 Hz, 1H, H-2'''), 3.94 (t, *J* = 5.3 Hz, 1H, H-3'''), 3.90 (q, *J* = 5.7 Hz, 1H, H-3'''), 3.85 – 3.67 (m, 6H, H-4, H-5, H-4', H-3'', H-5'', H-6), 3.63 (dd, *J* = 12.5, 4.6 Hz, 1H, H-6''), 3.56 (dd, *J* = 9.8, 3.8 Hz, 1H, H-2''), 3.54 – 3.50 (m, 3H, H-6, H-2', H-5'), 3.42 (dd, *J* = 14.6, 4.2 Hz, 1H, H-5'''), 3.32 (dd, *J* = 14.6, 6.2 Hz, 1H, H-5'''), 3.29 – 3.23 (m, 1H, H-3), 3.22 (dd, *J* = 8.5, 2.7 Hz, 1H, H-7'), 3.16 (td, *J* = 11.6, 10.9, 4.3 Hz, 1H, H-1), 3.10 (t, *J* = 10.3 Hz, 1H, H-4''), 2.63 (s, 3H, NCH_3), 2.30 – 2.16 (m, 2H, H-2, H-3'), 1.94 – 1.83 (m, 1H, H-3'), 1.68 – 1.55 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 164.7(CHO), 110.0 (C-1'''), 94.4 (C-1''), 94.0 (C-1'), 92.9 (C-8'), 85.1 (C-5), 80.9 (C-4'''), 76.4 (C-4), 74.7 (C-2'''), 72.5 (C-6), 70.8 (C-3'''), 70.2 (C-2''), 69.7 (C-5''), 69.7 (C-4'), 68.6 (C-3''), 65.9 (C-5'), 62.7 (C-6'), 60.3 (C-6''), 59.4 (C-7'), 52.0 (C-4''), 49.8 (C-3), 48.5 (C-1), 47.7 (C-2'), 40.0 (C-5'''), 30.0 (N*C*H_3), 28.9 (C-2), 26.8 (C-3'); ESI-HRMS: m/z calcd. for $C_{27}H_{51}N_6O_{15}$ $[M+H]^+$ 699.3412; found, 699.3410.

5-Azido-3-*O*-(2-benzyloxyethyl)-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (159). 5-Azido-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose[173] **158** (4.0 g, 18.6 mmol) was dissolved in dry THF (100 mL) and NaH (100 mg, 24.5 mmol) was added. After stirring for 15 min, 2-benzyloxyethyl tosylate (6.83 g, 22.3 mmol) was added and stirring continued for 36 h. After completion, the reaction was quenched with methanol, diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 10% to 20% EtOAc/hexanes) to give **159** (3.08 g, 47%) in the form of a colorless oil; $[\alpha]_D^{25}$= +119.83 (*c* 1.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 – 7.21 (m, 5H, Ar*H*), 5.76 (d, *J* = 3.5 Hz, 1H, H-1), 4.64 (t, *J* = 3.9 Hz, 1H, H-2), 4.56 (s, 2H, C*H*$_2$Ph), 4.14 (dt, *J* = 8.5, 3.2 Hz, 1H, H-4), 3.91– 3.71 (m, 2H, H-3, CH_2CH_2), 3.76 – 3.64 (m, 4H, H-, CH_2CH_2, CH_2CH_2, H-5), 3.32 (dd, *J* = 13.5, 4.0 Hz, 1H, H-5), 1.57 (s, 3H, CH_3), 1.35 (s,

3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.0 (Ar*C*), 128.4 (Ar*C*), 127.7 (Ar*C*), 113.2(*C*$(CH_3)_2$), 103.9 (C-1), 79.5 (C-3), 77.4 (C-4), 77.3 (C-2), 73.3 (*C*H_2Ph), 70.1 (CH_2CH_2), 69.7 (CH_2CH_2), 50.6 (C-5), 26.8(CH_3), 26.5(CH_3). ; ESI-HRMS: m/z calcd. for $C_{17}H_{23}N_3NaO_5$ $[M+Na]^+$ 372.1535; found, 372.1538.

5-Benzyloxycarbonylamino-5-deoxy-3-*O*-(2-hydroxyethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (160). To a solution of compound **(159)** (3.0 g, 8.6 mmol) in dioxane:water = 5:1 (30 mL), 20% $Pd(OH)_2/C$ (3.0 g, 0.5 equiv) was added and the reaction mixture stirred at room temperature under 50 psi of hydrogen for 18 h. After completion, the reaction mixture was filtered over Celite®, concentrated to dryness and dissolved in dioxane:water = 3:1 (50 mL). K_2CO_3 (6.0 g, 43.5 mmol) and benzyloxychloroformate (2.5 mL, 17.2 mmol) were added and the reaction mixture was stirred for 4 h. After completion, the reaction mixture was concentrated and purified using silica gel column chromatography (eluent: 0.8% to 1% methanol/DCM) to give **160** (1.67 g, 53%) as a colorless oil; $[\alpha]_D^{25}$= +35.47 (*c* 1.5, DCM); ^1H NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.28 (m, 5H, Ar*H*), 5.73 (d, *J* = 3.8 Hz, 1H, H-1), 5.10 (d, *J* = 1.4 Hz, 2H (*C*H_2Ph)), 4.60 (t, *J* = 4.1 Hz, 1H, H-2), 4.03 (dt, *J* = 9.0, 3.6 Hz, 1H, H-4), 3.77 – 3.61 (m, 5H, CH_2CH_2, CH_2CH_2, H-5), 3.55 (dd, *J* = 9.0, 4.4 Hz, 1H, H-3), 3.45 (dt, *J* = 14.6, 4.2 Hz, 1H, H-5), 3.04 (t, *J* = 5.8 Hz, 1H, OH), 1.56 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.9 (C=O), 136.3 (Ar*C*), 128.5 (Ar*C*), 128.2 (Ar*C*), 113.1(*C*$(CH_3)_2$), 104.1 (C-1), 79.3 (C-3), 77.1 (C-2), 77.0 (C-4), 72.0 (CH_2CH_2), 67.0 (*C*H_2Ph), 61.6 (CH_2CH_2), 40.6 (C-5), 26.6 (CH_3), 26.5 (CH_3); ESI-HRMS: m/z calcd. for $C_{18}H_{25}NNaO_7$ $[M+Na]^+$ 390.1529; found, 390.1537.

3-*O*-(2-Azidoethyl)-5-benzyloxycarbonylamino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (161). To a stirred solution of the alcohol **160** (1.0 g, 2.7 mmol) in dry THF (5 mL), triethylamine (2.8 mL, 20.4 mmol). The reaction mixture was ice-cooled before addition of

p-tolylsulfonyl chloride (975 mg, 5.13 mmol) in dry THF (5 mL). The reaction mixture was stirred at 30 °C for 48 h before it was concentrated *in vacuo*. The crude product was dissolved in EtOAc and washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The resulted solid was dissolved in dry DMF (10 mL) and treated with NaN_3 (1.05 g, 16.3 mmol) and stirred at 40 °C for 48 h. After completion, the reaction mixture was diluted with acetone and excess NaN_3 was filtered off. The solvent was partially removed under vacuum and the residue was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed under vacuum and the resulting product was purified using silica gel column chromatography (eluent: 10% to 25% EtOAc/hexanes) to give **161** (900 mg, 84% over two steps) as a viscous oil; $[\alpha]_D^{25}$= +35.38 (*c* 1.9, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 – 7.27 (m, 5H, Ar*H*), 5.73 (d, *J* = 3.7 Hz, 1H, H-1), 5.10 (s, 2H, CH_2Ph), 4.60 (t, *J* = 4.1 Hz, 1H, H-2), 4.05 (dt, *J* = 8.6, 4.1 Hz, 1H, H-4), 3.82 (ddd, *J* = 10.1, 6.0, 3.8 Hz, 1H, CH_2O), 3.67 (ddd, *J* = 10.3, 6.5, 3.9 Hz, 1H, CH_2O), 3.63 – 3.47 (m, 3H, H-3, H-5), 3.47 – 3.32 (m, 2H, CH_2N_3), 1.56 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.5 (C=O), 136.4 (Ar*C*), 128.5 (Ar*C*), 128.1 (Ar*C*), 113.3(*C*$(CH_3)_2$), 104.1 (C-1), 80.0 (C-3), 77.1 (C-2), 77.0 (C-4), 69.4 (*C*H_2O), 66.9 (*C*H_2Ph), 50.7 (*C*H_2N_3), 41.1 (C-5), 26.7 (CH_3), 26.6 (CH_3); ESI-HRMS: m/z calcd. for $C_{18}H_{24}N_4NaO_6$ $[M+Na]^+$ 415.1594; found, 415.1589.

3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (162). A stirred solution of the compound **161** (200 mg, 0.51 mmol) in dry THF (8 mL) and HMPA (2 mL), was cooled to -78 °C under argon before KHMDS (0.5 M in toluene, 1.5 mL, 0.66 mmol) and benzyloxychloroformate (0.3 mL, 2.1 mmol) were added. The reaction mixture was stirred at -78 °C for 2 h before additional KHMDS (0.5 M in toluene, 3 mL, 1.5 mmol) was added. The reaction was stirred for 30 min and quenched with NH_4Cl, diluted with

EtOAc, and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 10% to 25% EtOAc/hexanes) to give **162** (272 mg, quant) $[\alpha]_D^{25}$= +14.73 (*c* 1.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 – 7.28 (m, 10H, Ar*H*), 5.70 (d, *J* = 3.8 Hz, 1H, H-1), 5.42 – 5.12 (m, 4H, CH_2Ph), 4.56 (t, *J* = 4.1 Hz, 1H, H-2), 4.20 (dt, *J* = 8.9, 5.4 Hz, 1H, H-4), 4.07 (dd, *J* = 5.4, 1.2 Hz, 2H, H-5), 3.72 (ddd, *J* = 9.9, 6.0, 4.0 Hz, 1H, CH_2O), 3.57 (dd, *J* = 8.8, 4.4 Hz, 1H, H-3), 3.45 (ddd, *J* = 10.1, 6.3, 4.2 Hz, 1H, CH_2O), 3.34 – 3.16 (m, 2H, CH_2N_3), 1.51 (s, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.6 (C=O), 135.2 (Ar*C*), 128.5 (Ar*C*), 128.3 (Ar*C*), 128.2 (Ar*C*), 128.1 (Ar*C*), 113.1(*C*$(CH_3)_2$), 104.2 (C-1), 81.3 (C-3), 77.4 (C-2), 77.1 (C-4), 68.9 (*C*H_2O), 68.8 (*C*H_2Ph), 66.9 (*C*H_2Ph), 50.5 (*C*H_2N_3), 47.1 (C-5), 26.7 ($2CH_3$); ESI-HRMS: m/z calcd. for $C_{26}H_{30}N_4NaO_8$ $[M+Na]^+$ 549.1961; found, 549.1962.

3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-1,2-di-*O*-(*p*-nitrobenzoyl)-α/β-D-ribofuranose (163). To a stirred solution of compound **162** (268 mg, 0.51 mmol) in dioxane (10 mL), 1 N HCl (4 mL) was added and the reaction mixture was heated at 80 °C for 2 h. The reaction mixture was cooled, neutralized with solid $NaHCO_3$ and the solvent was evaporated. The residue was dissolved in EtOAc and washed with water and brine, dried with Na_2SO_4 and evaporated. To a solution of the crude mixture in dry pyridine (10 mL), p-nitrobenzoyl chloride (672 mg, 3.6 mmol) and a catalytic amount of DMAP were added followed by stirring overnight. The reaction mixture was diluted with EtOAc and washed with $NaHCO_3$, brine, dried with Na_2SO_4 then concentrated. The crude product was purified using silica gel column chromatography (eluent: 15% - 40% EtOAc/hexanes) to give the α isomer (235 mg, 59%) as a white solid and the β isomer (165 mg, 41%) as a white solid; **α isomer:** $[\alpha]_D^{25}$= +13.85 (*c* 0.4, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 – 8.17 (m, 6H, Ar*H*), 8.12 (d, *J* = 8.8

Hz, 2H, Ar*H*), 7.48 – 7.17 (m, 10H, Ar*H*), 6.64 (d, *J* = 4.4 Hz, 1H, H-1), 5.36 (dd, *J* = 6.6, 4.4 Hz, 1H, H-2), 5.34 – 5.21 (m, 4H, 2*CH_2Ph), 4.61 (td, *J* = 6.2, 3.8 Hz, 1H, H-4), 4.18 (dd, *J* = 6.6, 3.8 Hz, 1H, H-3), 4.15 – 3.99 (m, 2H, H-5), 3.47 (t, *J* = 4.8 Hz, 2H, $CH_2C\mathit{H}_2O$), 3.20 – 3.12 (m, 2H, CH_2N_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.6 (C=O), 163.3 (C=O), 153.5 (C=O), 150.9 (Ar*C*), 150.8 (Ar*C*), 148.4 (Ar*C*), 137.3 (Ar*C*), 134.9 (Ar*C*), 134.8 (Ar*C*), 134.1 (Ar*C*), 131.0 (Ar*C*), 130.9 (Ar*C*), 130.8 (Ar*C*), 128.6 (Ar*C*), 128.4 (Ar*C*), 123.7 (Ar*C*), 123.4 (Ar*C*), 95.4 (C-1), 82.6 (C-4), 77.3 (C-3), 72.4 (C-2), 70.1 ($CH_2\mathit{C}H_2O$), 69.3 ($\mathit{C}H_2$Ph), 50.9 ($\mathit{C}H_2N_3$), 47.7 (C-5); ESI-HRMS: m/z calcd. for $C_{37}H_{32}N_6NaO_{14}$ $[M+Na]^+$ 807.1874; found, 807.1852; **β isomer:** $[\alpha]_D^{25}$= -32.63 (*c* 0.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 – 7.91 (m, 8H, Ar*H*), 7.29 (s, 10H, Ar*H*), 6.52 (s, 1H, H-1), 5.71 (d, *J* = 4.1 Hz, 1H, H-2), 5.19 (q, *J* = 12.3 Hz, 4H, 2*CH_2Ph), 4.46 (dt, *J* = 8.1, 4.9 Hz, 1H, H-4), 4.39 (dd, *J* = 8.0, 4.2 Hz, 1H, H-3), 4.30 – 4.14 (m, 2H, H-5), 3.73 (ddd, *J* = 9.9, 6.8, 3.3 Hz, 1H, $CH_2C\mathit{H}_2O$), 3.57 (ddd, *J* = 9.5, 6.0, 3.3 Hz, 1H, CH_2O), 3.30 – 3.04 (m, 2H, CH_2N_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.5 (C=O), 162.9 (C=O), 153.9 (C=O), 150.9 (Ar*C*), 150.7 (Ar*C*), 134.9 (Ar*C*), 134.4 (Ar*C*), 134.3 (Ar*C*), 131.1 (Ar*C*), 131.0 (Ar*C*), 128.6 (Ar*C*), 128.5 (Ar*C*), 128.2 (Ar*C*), 128.1 (Ar*C*), 123.7 (Ar*C*), 123.6 (Ar*C*), 99.6 (C-1), 80.2 (C-4), 79.2 (C-3), 74.5 (C-2), 70.4 ($CH_2\mathit{C}H_2O$), 69.2 ($\mathit{C}H_2$Ph), 50.6 ($\mathit{C}H_2N_3$), 47.2 (C-5); ESI-HRMS: m/z calcd. for $C_{37}H_{32}N_6NaO_{14}$ $[M+Na]^+$ 807.1874; found, 807.1877.

5-*O*-β-[3-*O*-(2-Azidoethyl)-5-di(benzyloxycarbonyl)amino-5-deoxy-2-*O*-*p*-nitrobenzoyl-D-ribofuranose]-6,2'',3'',6''-tetra-*O*-acetyl-1,3,2',4''-tetraazido-6',7'-oxazolidino-apramycin (164). Donor **163** (β isomer, 165 mg, 0.21 mmol), acceptor **124** (440 mg, 0.52 mmol) and activated 4 Å MS were stirred in dry DCM (3 mL) at rt for 1 h before cooling to 0 °C. $BF_3.OEt_2$ (300 μL, 0.78 mmol) was added and reaction mixture was stirred for 48 h at 0 °C. The reaction was quenched with triethylamine (0.5 mL) and filtered through Celite®

before it was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.5% Methanol/DCM) to give the glycoside **164** (136 mg, 45%) as the β anomer in the form of a white solid; $[\alpha]_D^{25}$= +46.26 (*c* 0.9, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.29 – 8.21 (m, 2H, Ar*H*), 8.19 – 8.09 (m, 2H, Ar*H*), 7.39 – 7.32 (m, 4H, Ar*H*), 7.32 – 7.25 (m, 6H, Ar*H*), 5.43 – 5.35 (m, 3H, H-1', H-3'', H-1''), 5.32 (d, *J* = 3.9 Hz, 1H, H-1'''), 5.31 – 5.26 (m, 4H, 2*CH_2Ph), 5.25 (d, *J* = 4.1 Hz, 1H, H-2'''), 4.89 (dd, *J* = 10.3, 3.9 Hz, 1H, H-2''), 4.87 (d, *J* = 3.4 Hz, 1H, H-8'), 4.84 (t, *J* = 9.8 Hz, 1H, H-6), 4.78 (dd, *J* = 8.2, 3.2 Hz, 1H, H-6'), 4.60 (dd, *J* = 10.5, 3.2 Hz, 1H, H-5'), 4.31 (dd, *J* = 12.3, 2.3 Hz, 1H, H-6''), 4.28 – 4.18 (m, 2H, H-6'', H-4'''), 4.18 – 4.10 (m, 2H, H-5'''), 4.08 (dd, *J* = 7.8, 4.5 Hz, 1H, H-3'''), 3.79 (dd, *J* = 8.2, 3.5 Hz, 1H, H-7'), 3.71 (ddd, *J* = 10.7, 5.3, 2.3 Hz, 1H, H-5''), 3.66 (td, *J* = 10.9, 4.3 Hz, 1H, H-4'), 3.63 – 3.52 (m, 4H, H-3, H-5, H-4'', $CH_2C\mathit{H}_2O$), 3.49 – 3.43 (m, 2H, H-4, $CH_2C\mathit{H}_2O$), 3.39 (ddd, *J* = 12.5, 10.2, 4.2 Hz, 1H, H-1), 3.27 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2'), 3.12 (ddd, *J* = 13.3, 7.3, 3.2 Hz, 1H, CH_2N_3), 3.02 (ddd, *J* = 13.3, 5.7, 3.2 Hz, 1H, CH_2N_3), 2.92 (s, 3H, NC*H*$_3$), 2.38 (dt, *J* = 12.9, 4.5 Hz, 1H, H-2), 2.20 (s, 3H, $COCH_3$), 2.17 – 2.11 (m, 1H, H-3'), 2.08 (d, *J* = 4.4 Hz, 6H, 2*$COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.77 (q, *J* = 11.8 Hz, 1H, H-3'), 1.41 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.3 (C=O), 170.0 (C=O), 169.9 (C=O), 169.4 (C=O), 163.9 (C=O), 157.0 (Ar*C*), 153.7 (Ar*C*), 150.8 (Ar*C*), 135.3 (Ar*C*), 134.4 (Ar*C*), 130.9 (Ar*C*), 128.5 (Ar*C*), 128.2 (Ar*C*), 127.9 (Ar*C*), 123.7 (Ar*C*), 106.7 (C-1'''), 96.8 (C-1'), 95.4 (C-8'), 94.0 (C-1''), 80.4 (C-5), 79.5 (C-3'''), 79.3 (C-4), 79.1 (C-4'''), 74.8 (C-2'''), 74.3 (C-6), 70.6 (C-3''), 70.3 (C-6'), 70.0 ($CH_2\mathit{C}H_2O$), 69.9 (C-2''), 68.9 (2*$\mathit{C}H_2$Ph), 65.6 (C-5'), 65.3 (C-4'), 62.9 (C-6''), 60.14 (C-7'), 60.11 (C-4''), 58.3 (C-3), 58.1 (C-1), 57.4 (C-2'), 50.6 ($\mathit{C}H_2N_3$), 48.2 (C-5'''), 31.3 (C-3'), 31.0 (C-2), 29.9 (N$\mathit{C}H_3$), 21.0 (CO$\mathit{C}H_3$), 20.9 (CO$\mathit{C}H_3$), 20.8

($CO\mathit{C}H_3$), 20.7 ($CO\mathit{C}H_3$); ESI-HRMS: m/z calcd. for $C_{60}H_{66}N_{18}NaO_{26}$ $[M+Na]^+$ 1477.4293; found, 1477.4232.

5-*O*-β-[5-Amino-3-*O*-(2-aminoethyl)-5-deoxy-D-ribofuranosyl] apramycin heptaacetate salt (165). A stirred solution of substrate **164** (67 mg, 0.046 mmol) in dioxane (1.5 mL) was treated with 3 N NaOH (1.5 mL) and heated at 100 °C for 18 h. The reaction mixture was cooled to 0 °C and neutralized with glacial acetic acid before it was concentrated *in vacuo*. The crude mixture was passed through a silica gel column (eluent: 25% methanol/DCM). The resulting solid (20 mg, 0.023 mmol) was dissolved in a water methanol:water mixture (1:1, 0.5 mL) and treated with *N*-(diethylcarbamoyl)-*N*-methoxyformamide[172] (30 μL, 0.17 mmol) and triethylamine (2 μL). The reaction mixture was stirred for 2 h and quenched with aqueous ammonium hydroxide (0.25 mL) and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5% to 15% ammonical MeOH in DCM). A part of the solid residue (12 mg, 0.014 mmol) dissolved in dioxane (3 mL) followed by the addition of 1 N NaOH (0.5 mL) and 1 M $P(CH_3)_3$ in THF (0.2 mL), and stirred at 50 °C for 45 min. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the products were combined, acidified with glacial acetic acid and lyophilized to afford **165** in (14.5 mg, 56%) as a white solid; $[\alpha]_D^{25}$= +72.53 (*c* 0.7, H_2O); ^1H NMR (600 MHz, D_2O): δ 5.73 (d, *J* = 4.0 Hz, 1H, H-1’), 5.31 (d, *J* = 3.7 Hz, 1H, H-1’’), 5.26 (s, 1H, H-1’’’), 5.03 (d, *J* = 8.6, 1H, H-8’), 4.40 (s, 1H, H-6’), 4.26 (d, *J* = 4.4 Hz, 1H, H-2’’’), 4.03 (td, *J* = 7.5, 3.8 Hz, 1H, H-4’’’), 3.93 – 3.86 (m, 2H, H-4, H-3’’’), 3.83 – 3.71 (m, 4H, H-5, H-4’, H-3’’, H-5’’), 3.69 – 3.57 (m, 4H, $CH_2C\mathit{H}_2O$, H-6’’), 3.56 – 3.44 (m, 4H, H-6, H-2’, H-5’, H-2’’), 3.35 – 3.26

(m, 1H, H-3), 3.23 – 3.11 (m, 3H, H-1, H-7', H-5'''), 3.11 – 2.99 (m, 4H, H-4'', H-5''', C*H*$_2$CH$_2$O), 2.59 (s, 3H, NC*H*$_3$), 2.24 (dt, *J* = 13.1, 4.4 Hz, 1H, H-2), 2.20 (dt, *J* = 10.1, 4.7 Hz, 1H, H-3'), 1.93 – 1.83 (m, 1H, H-3'), 1.68 – 1.59 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 108.9 (C-1'''), 94.4 (C-1'), 92.7 (C-1''), 92.5 (C-8'), 83.2 (C-5), 79.2 (C-3'''), 77.0 (C-4'''), 73.6 (C-4), 72.7 (C-2'''), 72.0 (C-6), 70.2 (C-2''), 69.5 (C-5'', C-5'), 68.3 (C-3''), 65.9 (C-4'), 65.8 (CH$_2$*C*H$_2$O), 62.6 (C-6'), 60.2 (C-6''), 59.5 (C-7'), 52.0 (C-4''), 50.0 (C-1), 48.7 (C-3), 47.6 (C-2'), 42.1 (C-5'''), 39.2 (*C*H$_2$CH$_2$O), 30.0 (NCH$_3$), 28.2 (C-2), 27.0 (C-3'); ESI-HRMS: m/z calcd. for $C_{28}H_{56}N_7O_{14}$ $[M+H]^+$ 714.3885; found, 714.3868.

5-*O*-β-[3-*O*-(2-Aminoethyl)-5-deoxy-5-formamido-D-ribofuranosyl] apramycin hexaacetate salt (166). A stirred solution of substrate **164** (67 mg, 0.046 mmol) in dioxane (1.5 mL) was treated with 3 N NaOH (1.5 mL) and heated at 100 °C for 18 h. The reaction mixture was cooled to 0 °C and neutralized with glacial acetic acid before it was concentrated *in vacuo*. The crude mixture was passed through a silica gel column (eluent: 25% methanol/DCM). The resulting solid (20 mg, 0.023 mmol) was dissolved in a water methanol:water mixture (1:1, 0.5 mL) and treated with *N*-(diethylcarbamoyl)-*N*-methoxyformamide[172] (30 μL, 0.17 mmol) and triethylamine (2 μL). The reaction mixture was stirred for 2 h and quenched with aqueous ammonium hydroxide (0.25 mL) and concentrated. The crude product was purified using silica gel column chromatography (eluent: 5% to 15% ammonical MeOH in DCM). A part of the solid residue (20 mg, 0.022 mmol) was dissolved in dioxane:water (1:1, 0.6 mL) followed by the addition 1 M P(CH$_3$)$_3$ in THF (0.3 mL), and stirred at 50 °C for 45 min. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions

containing the product were combined, acidified with acetic acid, and lyophilized to afford **166** in (13.9 mg, 57%) as a white solid; $[\alpha]_D^{25}$= +55.35 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 7.94 (s, 1H, CHO), 5.69 (d, *J* = 3.9 Hz, 1H, H-1'), 5.30 (d, *J* = 4.0 Hz, 1H, H-1''), 5.15 (d, *J* = 3.0 Hz, 1H, H-1'''), 5.03 (d, *J* = 8.6 Hz, 1H, H-8'), 4.40 (t, *J* = 2.7 Hz, 1H, H-6'), 4.16 (dd, *J* = 4.9, 3.0 Hz, 1H, H-2'''), 3.97 (q, *J* = 5.6 Hz, 1H, H-4'''), 3.88 (t, *J* = 9.6 Hz, 1H, H-4), 3.83 – 3.68 (m, 5H, H-5, H-4', H-3'', H-5'', H-3'''), 3.68 – 3.55 (m, 4H, H-6'', CH_2CH_2O), 3.54 – 3.44 (m, 4H, H-6, H-2', H-5', H-2''), 3.40 (dd, *J* = 14.5, 4.6 Hz, 1H, H-5'''), 3.33 (ddd, *J* = 14.3, 10.4, 4.3 Hz, 1H, H-3), 3.29 (dd, *J* = 14.5, 6.1 Hz, 1H, H-5'''), 3.20 (dd, *J* = 8.6, 2.8 Hz, 1H, H-7'), 3.18 – 3.12 (m, 1H, H-1), 3.10 (t, *J* = 10.3 Hz, 1H, H-4''), 3.05 – 2.98 (m, 2H, CH_2CH_2O), 2.59 (s, 3H, NCH_3), 2.28 (dt, *J* = 12.6, 4.3 Hz, 1H, H-2), 2.18 (dt, *J* = 11.2, 4.6 Hz, 1H, H-3'), 1.88 (d, *J* = 11.8 Hz, 1H, H-3'), 1.72 – 1.63 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 164.8 (CHO), 110.3 (C-1'''), 94.4 (C-1'), 93.6 (C-1''), 92.8 (C-8'), 84.8 (C-5), 79.3 (C-4'''), 78.8 (C-3'''), 74.8 (C-4), 73.1 (C-2'''), 72.3 (C-6), 70.2 (C-2''), 69.7 (C-5''), 69.3 (C-5'), 68.2 (C-3''), 65.9 (C-4'), 65.8 (CH_2CH_2O), 62.6 (C-6'), 60.2 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 49.6 (C-1), 48.5 (C-3), 47.6 (C-2'), 40.1 (C-5'''), 39.2 (CH_2CH_2O), 30.0 (NCH_3), 27.9 (C-2), 26.7 (C-3'); ESI-HRMS: m/z calcd. for $C_{29}H_{56}N_7O_{15}$ $[M+H]^+$ 742.3834; found, 742.3861.

3-*O*-(2-Azidoethyl)-5-(benzyloxy)-5-deoxy-1,2,4-tri-*O*-(*p*-nitrobenzoyl)-1,5-imino-D ribopyranose (170). To a stirred solution of compound **161** (350 mg, 0.89 mmol) in dioxane (10 mL), 1 N HCl (4 mL) was added and the reaction mixture was heated at 80 °C for 45 min. The reaction mixture was cooled, neutralized with solid $NaHCO_3$ and the solvent was evaporated. The residue was dissolved in EtOAc and washed with water and brine, dried with Na_2SO_4 and concentrated *in vacuo*. A solution of the crude product in dry pyridine (10 mL), was treated with p-nitrobenzoyl chloride (560 mg, 3.01 mmol) and a catalytic amount of DMAP and stirred

overnight. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine, dried with Na_2SO_4 then concentrated. The crude product was purified using silica gel column chromatography (eluent: 10% - 40% EtOAc/hexanes) to give **170** (69 mg, 10 %) as a yellow oil; $[\alpha]_D^{25}$= +15.99 (c 3.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 – 8.25 (m, 8H, Ar*H*), 8.25 – 8.19 (m, 2H, Ar*H*), 8.12 – 8.02 (m, 2H, Ar*H*), 7.37 (dd, *J* = 37.0, 19.9 Hz, 5H, Ar*H*), 5.42 (dd, *J* = 4.2, 3.0 Hz, 1H, H-2), 5.32 (ddd, *J* = 11.4, 5.2, 2.5 Hz, 1H, H-4), 5.26 (d, *J* = 12.4 Hz, 1H, OCH_2Ph), 5.18 (d, *J* = 12.3 Hz, 1H, OCH_2Ph), 4.70 (br s, 1H, H-1), 4.49 (t, *J* = 2.9 Hz, 1H, H-3), 4.39 (br s, 1H, H-5), 3.93 – 3.74 (m, 3H, H-5, CH_2O), 3.42 – 3.23 (m, 2H, CH_2N_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.0 (Ar*C*), 131.0 (Ar*C*), 130.8 (Ar*C*), 128.6 (Ar*C*), 123.9 (Ar*C*), 123.8 (Ar*C*), 76.0 (C-3), 75.4 (C-1), 72.6 (CH_2CH_2O), 70.2 (C-2), 69.6 (C-4), 68.8 (OCH_2Ph), 51.4 (CH_2CH_2O), 37.5 (C-5); ESI-HRMS: m/z calcd. for $C_{36}H_{29}N_7NaO_{15}$ $[M+Na]^+$ 822.1619; found, 822.1601.

5,2'',3'',6''-Tetra-*O*-acetyl-6-*O*-allyl-1,3,2′,4′′-tetraazido-6′,7′-oxazolidino-apramycin (172). 6,2'',3'',6''-Tetra-O-acetyl-1,3,2′,4′′-tetraazido-6′,7′-oxazolidino-apramycin **124** (100 mg, 0.12 mmol) was dissolved in dry DCM (0.5 mL) and treated with allyl bromide (0.5 mL, 5.9 mmol) and silver oxide (400 mg, 1.7 mmol). The reaction mixture was cover with aluminium foil and stirred at rt for 12 h. After completion, the reaction was filtered through Celite® and concentrated to dryness. The crude product was purified by column chromatography (eluent: 5% to 30% EtOAc/hexanes) to give **172** (60 mg, 59%) as a white solid; $[\alpha]_D^{25}$= +60.0 (*c* 0.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (ddt, *J* = 16.3, 10.3, 5.9 Hz, 1H, CH_2CHCH_2O), 5.45 – 5.34 (m, 1H, H-3''), 5.32 (d, *J* = 3.8 Hz, 1H, H-1''), 5.25 (dd, *J* = 17.2, 1.5 Hz, 1H, CH_2CHCH_2O), 5.22 – 5.16 (m, 1H, CH_2CHCH_2O), 5.05 (t, *J* = 9.9 Hz, 1H, H-5), 4.99 – 4.90 (m, 2H, H-8', H-2''), 4.87 (d, *J* = 3.6 Hz, 1H, H-1'), 4.81 (dd, *J* = 8.6, 3.2 Hz, 1H, H-6'), 4.74 (dd, *J* = 10.5, 3.2

Hz, 1H, H-5'), 4.36 – 4.17 (m, 3H, H-6'', CH_2CHCH_2O), 4.09 (dd, *J* = 12.2, 6.1 Hz, 1H, CH_2CHCH_2O), 3.92 – 3.82 (m, 2H, H-4', H-7'), 3.82 – 3.76 (m, 1H, H-3), 3.76 – 3.67 (m, 1H, H-5''), 3.66 – 3.52 (m, 2H, H-1, H-4''), 3.48 (t, *J* = 9.9 Hz, 1H, H-4), 3.39 (dt, *J* = 12.7, 4.1 Hz, 1H, H-2'), 3.19 (t, *J* = 9.8 Hz, 1H, H-6), 2.92 (s, 3H, NCH_3), 2.46 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2), 2.26 (dt, *J* = 10.7, 4.1 Hz, 1H, H-3'), 2.21 – 2.02 (m, 12H, 4*$COCH_3$), 1.85 (q, *J* = 11.4 Hz, 1H, H-3'), 1.49 (q, *J* = 12.5 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3 (C=O), 170.3 (C=O), 170.0 (C=O), 169.4 (C=O), 156.9 (C=O), 133.6 (CH_2CHCH_2O), 118.1 (CH_2CHCH_2O), 99.1 (C-1'), 94.2 (C-8', C-1''), 81.9 (C-6), 80.3 (C-4), 74.3 (CH_2CHCH_2O), 74.0 (C-5), 70.7 (C-3''), 69.9 (C-2''), 69.7 (C-6'), 68.8 (C-5''), 65.4 (C-5'), 65.2 (C-4'), 62.9 (C-6''), 60.2 (C-4''), 60.0 (C-7'), 59.7 (C-1), 58.4 (C-3), 56.6 (C-2'), 32.1 (C-2), 30.0 (NCH_3), 29.8 (C-3'), 21.1 ($COCH_3$), 21.0 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$); ESI-HRMS: m/z calcd. for $C_{33}H_{43}N_{13}NaO_{16}$ $[M+Na]^+$ 900.2848; found, 900.2841.

6-*O*-Propyl apramycin pentaacetate salt (173). To a solution of compound **172** (20 mg, 0.02 mmol) in dry methanol (0.4 mL), sodium methoxide (5 mg, 0.03 mmol) was added and reaction mixture was stirred for 3 h. The reaction was quenched with glacial acetic acid, filtered and the solvent was evaporated *in vacuo*. The crude mixture was purified using silica gel column chromatography (eluent: 0.5% - 3.5% methanol/DCM) and the product-containing fractions were concentrated *in vacuo.* The resulting solid was dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL) and 10% Pd/C (15 mg) was added. The reaction was stirred at room temperature under 1 atm of hydrogen (balloon) for 12 h. After completion, the reaction mixture was filtered over Celite® and filtrate concentrated to dryness. The residue was dissolved in dioxane (0.5 mL) and treated with 3N NaOH (0.25 mL) and heated at 100 °C for 2 h. The reaction mixture was cooled to rt and neutralized with glacial acetic acid before concentration *in*

vacuo. The crude product was dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **173** (9.2 mg, 46%) as a white solid; $[\alpha]_D^{25}$= +93.33 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.57 (d, *J* = 3.8 Hz, 1H, H-1'), 5.33 (d, *J* = 4.0 Hz, 1H, H-1''), 5.05 (d, *J* = 8.5 Hz, 1H, H-8'), 4.40 (s, 1H, H-6'), 3.84 – 3.72 (m, 4H, H-4, H-4', H-3'', H-5''), 3.72 – 3.52 (m, 5H, H-5, H-5', H-2'', H-6'', $CH_3CH_2CH_2O$), 3.52 – 3.42 (m, 2H, H-2', $CH_3CH_2CH_2O$), 3.34 – 3.24 (m, 2H, H-3, H-6), 3.24 – 3.10 (m, 3H, H-1, H-7', H-4''), 2.62 (s, 3H, NCH_3), 2.32 (dt, *J* = 12.5, 4.3 Hz, 1H, H-2), 2.20 (dt, *J* = 10.6, 4.7 Hz, 1H, H-3'), 1.69 (q, *J* = 12.5 Hz, 1H, H-2), 1.44 (h, *J* = 7.2 Hz, 2H, $CH_3CH_2CH_2O$), 0.69 (t, *J* = 7.4 Hz, 3H, $CH_3CH_2CH_2O$); ^{13}C NMR (151 MHz, D_2O): δ 95.3 (C-1'), 94.4 (C-1''), 92.8 (C-8'), 80.5 (C-6), 77.7 (C-4), 75.6 ($CH_3CH_2CH_2O$), 75.2 (C-5), 70.1 (C-2''), 69.6 (C-5'), 69.3 (C-5''), 68.2 (C-3''), 66.0 (C-4'), 62.7 (C-6'), 60.2 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 48.8 (C-1), 48.3 (C-3), 47.8 (C-2'), 28.2 (C-2), 26.7 (C-3'), 30.0 (NCH_3), 22.5 ($CH_3CH_2CH_2O$), 9.3 ($CH_3CH_2CH_2O$); ESI-HRMS: m/z calcd. for $C_{24}H_{48}N_5O_{11}$ $[M+H]^+$ 582.3350; found, 582.3342.

5,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-6-*O*-(2,3-dihydroxypropyl)-6′,7′-oxazolidino-apramycin (174). A stirred solution of compound **172** (20 mg, 0.02 mmol) in THF (0.4 mL) and water (0.1 mL) was treated with *N*-Methylmorpholine-*N*-oxide (8 mg, 0.07 mmol) and 2.5% OsO_4 in *tert*-butanol (60 μL mg, 0.005 mmol). The reaction mixture was stirred at rt for 4 h. After completion, the reaction mixture was diluted with EtOAc and the organic layer was washed with aqueous $NaHCO_3$ followed by brine, dried with Na_2SO_4, and concentrated. The crude product was purified via silica gel chromatography eluting with 0.7% to 3% methanol in

DCM to give **174** (15 mg, 71%) as a white solid; $[\alpha]_D^{25}$= +63.75 (*c* 1.3, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (t, *J* = 9.9 Hz, 1H, H-3'), 5.32 (d, *J* = 3.8 Hz, 1H, H-1''), 5.08 – 5.01 (m, 1H, H-5), 4.98 – 4.91 (m, 2H, H-8', H-2''), 4.88 (d, *J* = 3.0 Hz, 1H, H-1'), 4.80 (dd, *J* = 8.6, 3.1 Hz, 1H H-6'), 4.71 (dd, *J* = 10.5, 3.1 Hz, 1H, H-5'), 4.36 – 4.27 (m, 1H, H-6''), 4.22 (dd, *J* = 12.2, 5.0 Hz, 1H, H-6''), 3.90 – 3.68 (m, 6H, H-3, H-4', H-7', H-5'', $CH_2OHCHOHCH_2O$, $CH_2OHCHOHCH_2O$), 3.66 – 3.43 (m, 6H, H-1, H-4, H-4'', $CH_2OHCHOHCH_2O$, $CH_2OHCHOHCH_2O$), 3.37 (dt, *J* = 12.6, 3.6 Hz, 1H, H-2'), 3.19 (t, *J* = 9.8 Hz, 1H, H-6), 2.91 (s, 3H, NCH_3), 2.48 (dt, *J* = 12.7, 4.2 Hz, 1H, H-2), 2.29 – 2.23 (m, 1H, H-3'), 2.17 – 2.07 (m, 12H, 4*$COCH_3$), 1.90 – 1.80 (m, 1H, H-3'), 1.59 – 1.46 (m, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4 (C=O), 170.2 (C=O), 170.0 (C=O), 157.0 (C=O), 99.1 (C-1'), 94.5 (C-8'), 94.3 (C-1''), 82.9 (C-6), 80.0 (C-4), 74.8 (C-5), 74.4 ($CH_2OHCHOHCH_2O$), 70.7 (C-3''), 70.6 (C-2''), 69.9 ($CH_2OHCHOHCH_2O$), 69.7 (C-6'), 68.8 (C-5''), 65.4 (C-5'), 65.2 (C-4'), 63.1 ($CH_2OHCHOHCH_2O$), 62.9 (C-6''), 60.1 (C-4''), 60.0 (C-7'), 59.6 (C-1), 58.2 (C-3), 56.4 (C-2'), 31.8 (C-2), 29.9 (NCH_3), 29.8 (C-3'), 21.1 ($COCH_3$), 20.9 ($COCH_3$), 20.9 ($COCH_3$), 20.7 ($COCH_3$); ESI-HRMS: m/z calcd. for $C_{33}H_{45}N_{13}NaO_{18}$ $[M+Na]^+$ 934.2903; found, 934.2905.

6-*O*-(2,3-Dihydroxypropyl)-apramycin pentaacetate salt (175). A stirred solution of compound **174** (14 mg, 0.015 mmol) in dioxane (0.2 mL) was treated with 3N NaOH (0.2 mL) and heated at 100 °C for 2 h. 1M $P(CH_3)_3$ in THF (0.15 mL) was added and the reaction mixture was stirred at 55 °C for 2 h. The reaction mixture was cooled to 0 °C, neutralized with glacial acetic acid and concentrated. The crude product was dissolved in aqueous acetic acid solution (pH 4, 1 mL) then charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and

lyophilized to afford **175** (5.5 mg, 39%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +100.96 (*c* 0.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.51 (s, 1H, H-1'), 5.28 (d, *J* = 4.0 Hz, 1H, H-1''), 5.00 (dd, *J* = 8.4, 1.5 Hz, 1H, H-8'), 4.35 (s, 1H, H-6'), 3.86 – 3.66 (m, 6H, H-4, H-4', H-3'', H-5'', $CH_2OHC\mathit{H}OHCH_2O$, $CH_2OHCHOHC\mathit{H}_2O$), 3.66 – 3.52 (m, 5H, H-5, H-5', H-6'', $CH_2OHC\mathit{H}OHCH_2O$), 3.50 (dd, *J* = 9.3, 3.9 Hz, 1H, H-2''), 3.48 – 3.34 (m, 4H, H-2', $C\mathit{H}_2OHCHOHCH_2O$), 3.32 – 3.22 (m, 2H, H-3, H-6), 3.23 – 3.14 (m, 2H, H-1, H-7'), 3.08 (t, *J* = 10.2 Hz, 1H, H-4''), 2.57 (s, 3H, $NC\mathit{H}_3$), 2.31 – 2.23 (m, 1H, H-2), 2.19 – 2.10 (m, 1H, H-3'), 1.66 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O) δ 95.4 (C-1'), 94.3 (C-1''), 92.7 (C-8'), 81.3 (C-6), 77.7 (C-4), 75.2 (C-5), 73.7 ($CH_2OHCHOHC\mathit{H}_2O$), 70.3 ($CH_2OHC\mathit{C}HOHCH_2O$), 70.1 (C-2''), 69.6 (C-5'), 69.2 (C-5''), 68.2 (C-3''), 65.9 (C-4'), 62.6 (C-6'), 62.1 ($C\mathit{C}H_2OHCHOHCH_2O$), 60.2 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 48.8 (C-1), 48.2 (C-3), 47.8 (C-2'), 29.9 ($NC\mathit{C}H_3$), 28.1 (C-2), 26.6 (C-3'); ESI-HRMS: m/z calcd. for $C_{24}H_{48}N_5O_{13}$ $[M+H]^+$ 614.3249; found, 614.3242.

5,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-6-*O*-(2-hydroxyethyl)-6′,7′-oxazolidino-apramycin (176). To a stirred solution of compound **174** (22 mg, 0.024 mmol) in THF (0.4 mL) and water (0.1 mL), $NaIO_4$ (15.5 mg, 0.07 mmol) was added and the reaction mixture was stirred at rt for 12 h. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. To a solution of the residue in in THF (0.4 mL) and water (0.1 mL), $NaBH_4$ (1.8 mg, 0.048 mmol) and the reaction mixture was stirred at rt for 45 min. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$, brine, dried with Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.75% - 3% methanol/DCM) to give **176** (11 g, 52%) as a white solid; $[\alpha]_D^{25}$= +60.6 (c 0.3, DCM); ^{1}H NMR

(600 MHz, CD_3OD): δ 5.47 – 5.40 (m, 1H, H-3’’), 5.38 (d, *J* = 3.8 Hz, 1H, H-1’’), 5.13 (d, *J* = 2.1 Hz, 1H, H-8’), 5.02 (t, *J* = 9.7 Hz, 1H, H-5), 4.97 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2’’), 4.92 – 4.86 (m, 3H, H-1’, H-5’, H-6’), 4.35 (d, *J* = 12.1 Hz, 1H, H-6’’), 4.25 (dd, *J* = 12.3, 4.1 Hz, 1H, H-6’’), 4.10 (dd, *J* = 8.9, 2.1 Hz, 1H, H-7’), 3.89 – 3.79 (m, 4H, H-4’, H-4’’, H-5’’, $CH_2OHC\mathit{H}_2O$), 3.79 – 3.73 (m, 1H, H-3), 3.72 – 3.63 (m, 2H, H-4, $CH_2OHC\mathit{H}_2O$), 3.63 – 3.54 (m, 3H, H-1, $C\mathit{H}_2OHCH_2O$), 3.57 – 3.49 (m, 1H, H-2’), 3.37 (t, *J* = 9.8 Hz, 1H, H-6), 2.91 (s, 3H, $NC\mathit{H}_3$), 2.44 (dt, *J* = 12.7, 4.6 Hz, 1H, H-2), 2.28 (dt, *J* = 8.8, 4.2 Hz, 1H, H-3’), 2.18 – 2.04 (m, 12H, 4*$COCH_3$), 1.85 – 1.79 (m, 1H, H-3’), 1.61 (q, *J* = 12.5 Hz, 1H, H-2); ^{13}C NMR (151 MHz, CD_3OD): δ 170.9 (C=O), 170.5 (C=O), 170.4 (C=O), 158.2 (C=O), 99.1 (C-1’), 93.9 (C-1’’), 93.0 (C-8’), 82.5 (C-6), 80.2 (C-4), 74.5 (C-5), 74.1 ($CH_2OH\mathit{C}H_2O$), 70.9 (C-3’’), 70.2 (C-2’’), 70.0 (C-6’), 68.7 (C-5’’), 65.1 (C-5’), 65.1 (C-4’), 62.9 (C-6’’), 60.8 ($\mathit{C}H_2OHCH_2O$), 60.1 (C-4’’), 59.9 (C-7’), 59.8 (C-1), 58.2 (C-3), 56.6 (C-2’), 31.1 (C-2), 30.1 (C-3’), 28.6 ($NC\mathit{H}_3$), 20.1 ($CO\mathit{C}H_3$), 20.0 ($CO\mathit{C}H_3$), 19.4 ($CO\mathit{C}H_3$), 19.2 ($CO\mathit{C}H_3$); ESI-HRMS: m/z calcd. for $C_{32}H_{43}N_{13}NaO_{17}$ $[M+Na]^+$ 904.2798; found, 904.2801.

6-*O*-(2-hydroxyethyl)-apramycin pentaacetate salt (177). A stirred solution of compound **176** (10 mg, 0.011 mmol) in dioxane (0.2 mL) was treated with 3N NaOH (0.2 mL) and heated at 100 °C for 1 h. 1M $P(CH_3)_3$ in THF (0.15 mL) was added and the reaction mixture was stirred at 55 °C for 2 h. The reaction mixture was cooled to 0 °C, neutralized with glacial acetic acid and concentrated. The crude product was dissolved in aqueous acetic acid solution (pH 4, 1 mL) then charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and lyophilized to afford **177** (6.3 mg, 63%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$=

+107.38 (c 0.4, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.52 (d, *J* = 3.8 Hz, 1H, H-1’), 5.32 (d, *J* = 4.0 Hz, 1H, H-1’’), 5.04 (d, *J* = 8.5 Hz, 1H, H-8’), 4.38 (s, 1H, H-6’), 3.90 – 3.84 (m, 1H, $CH_2OHC\mathit{H}_2O$), 3.80 – 3.66 (m, 5H, H-4, H-4’, H-3’’, H-5’’, H-6’’), 3.66 – 3.55 (m, 6H, H-5, H-5’, H-6’’, $CH_2OHC\mathit{H}_2O$, $C\mathit{H}_2OHCH_2O$), 3.54 (dd, *J* = 9.7, 4.0 Hz, 1H, H-2’’), 3.47 (dt, *J* = 13.0, 4.1 Hz, 1H, H-2’), 3.33 (t, *J* = 9.7 Hz, 1H, H-6), 3.27 – 3.15 (m, 3H, H-1, H-3, H-7’), 3.08 (t, *J* = 10.3 Hz, 1H, H-4’’), 2.61 (s, 3H, $NC\mathit{H}_3$), 2.31 – 2.24 (m, 1H, H-2), 2.20 (dt, *J* = 8.7, 3.9 Hz, 1H, H-3’), 1.86 (q, *J* = 11.6 Hz, 1H, H-3’), 1.64 (q, *J* = 11.7, 11.0 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 95.6 (C-1’), 94.4 (C-1’’), 92.9 (C-8’), 81.5 (C-6), 79.0 (C-4), 75.3 (C-5), 74.0 ($CH_2OH\mathit{C}H_2O$), 70.2 (C-2’’), 69.7 (C-5’), 69.6 (C-5’’), 68.6 (C-3’’), 66.0 (C-4’), 62.8 (C-6’), 60.8 ($\mathit{C}H_2OHCH_2O$), 60.3 (C-6’’), 59.4 (C-7’), 52.0 (C-4’’), 49.1 (C-1), 48.3 (C-3), 47.9 (C-2’), 30.0 (NCH_3), 28.9 (C-2), 26.8 (C-3’); ESI-HRMS: m/z calcd. for $C_{24}H_{48}N_5O_{13}$ $[M+H]^+$ 584.3143; found, 584.3129.

5,6,2’’,3’’,6’’-Penta-*O*-acetyl-1,3,2′,4′′-tetraazido-5-epi-6′,7′-oxazolidino-apramycin (178). To a stirred solution of compound **124** (100 mg, 0.12 mmol) in dry DCM (1.5 mL), pyridine (0.1 mL) was added and reaction mixture was cooled to 0 °C before triflic anhydride (40 μL, 0.24 mmol) was added. The reaction mixture was stirred for 1 h and additional triflic anhydride (40 μL, 0.24 mmol) was added. After 2 h, the reaction mixture was poured into an iced aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4 and concentrated *in vacuo*. The crude was dissolved in dry DMF (1 mL), treated with potassium acetate (174 mg, 1.78 mmol) and stirred at 50 °C for 1 h. After completion, the reaction was diluted with EtOAc and washed with $NaHCO_3$ and brine then concentrated. The crude was purified using silica gel column chromatography (eluent: 0.6% - 1.0% methanol/DCM) to give compound **178** (75 mg, 72%) as a white solid; $[\alpha]_D^{25}$= +154.25 (*c*

1.2, DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (t, *J* = 2.7 Hz, 1H, H-5), 5.51 – 5.38 (m, 2H, H-1'', H-3''), 5.06 (d, *J* = 3.5 Hz, 1H, H-1'), 4.87 – 4.68 (m, 4H, H-6, H-6', H-8', H-2'), 4.34 (dd, *J* = 12.3, 2.2 Hz, 1H, H-6''), 4.22 (dd, *J* = 12.2, 5.4 Hz, 1H, H-6''), 3.97 (dd, *J* = 10.2, 3.7 Hz, 1H, H-5'), 3.91 – 3.83 (m, 2H, H-1, H-3), 3.80 (dd, *J* = 10.2, 2.6 Hz, 1H, H-4), 3.78 – 3.71 (m, 2H, H-7', H-5''), 3.66 (td, *J* = 10.8, 4.4 Hz, 1H, H-4'), 3.56 (t, *J* = 10.1 Hz, 1H, H-4''), 3.20 (dt, *J* = 12.7, 4.0 Hz, 1H, H-2'), 2.97 (s, 3H, NCH_3), 2.38 (dt, *J* = 13.5, 4.7 Hz, 1H, H-2), 2.27 (dt, *J* = 11.4, 4.4 Hz, 1H, H-3'), 2.17 (s, 3H, $COCH_3$), 2.12 (d, *J* = 6.5 Hz, 6H, 2*$COCH_3$), 2.08 – 1.94 (m, 7H, H-3', 2*$COCH_3$), 1.41 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4 (C=O), 170.2 (C=O), 169.7 (C=O), 169.6 (C=O), 169.5 (C=O), 156.9 (C=O), 99.9 (C-8'), 94.1 (C-1''), 93.8 (C-1'), 74.6 (C-4), 72.9 (C-6), 71.8, (C-6') 69.9 (C-3'', 2''), 69.4 (C-5''), 66.9 (C-5'), 66.1 (C-5), 65.9 (C-4'), 62.8 (C-6''), 60.2 (C-4''), 60.1 (C-7'), 58.0 (C-1), 56.2 (C-3), 55.5 (C-2'), 32.2 (C-2), 30.5 (NCH_3), 28.1 (C-3'), 20.7 (COCH_3), 20.6 (COCH_3), 20.5 (COCH_3); ESI-HRMS: m/z calcd. for $C_{32}H_{41}N_{13}NaO_{17}$ $[M+Na]^+$ 902.2641; found, 902.2639.

5-Epi-apramycin pentaacetate salt (179). A stirred solution of compound **(178)** (60 mg, 0.057 mmol) in dioxane (0.2 mL) was treated with 3N NaOH (0.2 mL) and heated at 100 °C for 2 h. The reaction mixture was treated with 1M $P(CH_3)_3$ in THF (0.15 mL) and stirred at 55 °C for 2h. The reaction mixture was then concentrated and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and lyophilized to afford **179** (39 mg, 65%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +90.0 (c 0.7, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.29 (d, *J* = 4.0 Hz, 1H, H-1''), 5.21 (d, *J* = 3.8 Hz, 1H, H-1'), 5.02 (d, *J* = 8.5 Hz, 1H, H-8'), 4.34 (s, 1H, H-6'), 4.29 (s, 1H, H-5), 3.82

– 3.69 (m, 4H, H-4, H-4’, H-3’’, H-5’’), 3.65 (dd, *J* = 12.5, 3.5 Hz, 1H, H-6’’), 3.58 (dd, *J* = 13.0, 4.5 Hz, 1H, H-6’’), 3.56 – 3.47 (m, 4H, H-3, H-6, H-2’, H-2’’), 3.44 (dd, *J* = 10.0, 2.6 Hz, 1H, H-5’), 3.34 (ddd, *J* = 12.3, 10.6, 4.5 Hz, 1H, H-1), 3.15 (dd, *J* = 8.5, 2.8 Hz, 1H, H-7’), 3.09 (t, *J* = 10.4 Hz, 1H, H-4’’), 2.58 (s, 3H, NCH_3), 2.27 (dt, *J* = 12.5, 4.4 Hz, 1H, H-2), 2.20 (dt, *J* = 11.4, 4.6 Hz, 1H, H-3’), 1.92 – 1.86 (m, 1H, H-3’), 1.55 (q, *J* = 12.5 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 94.3 (C-1’’), 92.7 (C-8’), 89.9 (C-1’), 72.9 (C-4), 70.1 (C-6), 69.8 (C-2’’), 69.4 (C-5’), 69.3 (C-5’’), 68.2 (C-3’’), 66.0 (C-5), 65.8 (C-4’), 62.5 (C-6’), 60.2 (C-6’’), 59.4 (C-7’), 52.0 (C-4’’), 48.1 (C-1), 47.4 (C-2’), 46.7 (C-3), 29.9 (N*C*H_3), 28.1 (C-2), 26.9 (C-3’); ESI-HRMS: m/z calcd. for $C_{21}H_{42}N_5O_{11}$ $[M+H]^+$ 540.2881; found, 540.2855.

6,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-5-epi-6′,7′-oxazolidino-apramycin (180). To a stirred solution of compound **124** (100 mg, 0.12 mmol) in dry DCM (1.5 mL), pyridine (0.1 mL) was added and reaction mixture was cooled to 0 °C before triflic anhydride (40 μL, 0.24 mmol) was added. The reaction mixture was stirred for 1 h and additional triflic anhydride (40 μL, 0.24 mmol) was added. After 2 h, the reaction mixture was poured into an iced aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was dissolved in dry DMF (1 mL), treated with sodium nitrite (40 mg, 0.60 mmol) and stirred at 50 °C for 1 h. After completion, the reaction was diluted with EtOAc and washed with brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.0% Methanol/DCM) to give compound **180** (60 mg, 60%) as a white solid; $[\alpha]_D^{25}$= +100.5 (*c* 0.6, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.43 (t, *J* = 10.1 Hz, 1H, H-3’’), 5.39 (d, *J* = 3.8 Hz, 1H, H-1’’), 4.99 (d, *J* = 3.5 Hz, 1H, H-1’), 4.83 (dd, *J* = 10.4, 3.8 Hz, 1H, H-2’’), 4.81 – 4.76 (m, 2H, H-6’, H-8’), 4.64 (dd, *J* = 10.4, 2.6 Hz, 1H, H-6), 4.39 (q, *J* = 2.7 Hz, 1H, H-5), 4.32 (dd, *J* =

12.2, 2.2 Hz, 1H, H-6’’), 4.25 – 4.19 (m, 2H, H-6’’, H-5’), 4.01 (ddd, *J* = 12.3, 10.4, 4.7 Hz, 1H, H-1), 3.92 (ddd, *J* = 12.4, 9.9, 4.9 Hz, 1H, H-3), 3.77 (dd, *J* = 7.3, 5.0 Hz, 1H, H-7’), 3.72 (ddd, *J* = 10.6, 5.3, 2.2 Hz, 1H, H-5’’), 3.67 (td, *J* = 10.9, 4.4 Hz, 1H, H-4’), 3.61 (dd, *J* = 9.9, 2.7 Hz, 1H, H-4), 3.57 (t, *J* = 10.2 Hz, 1H, H-4’’), 3.45 (dt, *J* = 12.6, 4.1 Hz, 1H, H-2’), 3.13 (d, *J* = 2.7 Hz, 1H, OH), 2.94 (s, 3H, NC*H*$_3$), 2.36 – 2.26 (m, 2H, H-2, H-3’), 2.16 (s, 3H, COCH$_3$), 2.15 – 2.05 (m, 6H, 2*COCH$_3$), 1.99 (dd, *J* = 23.9, 12.0 Hz, 1H, H-3’), 1.33 (q, *J* = 12.7 Hz, 1H, H-2); ^{13}C NMR (151 MHz, CDCl$_3$): δ 170.4 (C=O), 170.2 (C=O), 169.7 (C=O), 157.1 (C=O), 98.0 (C-8’), 94.5 (C-1’), 94.2 (C-1’’), 78.6 (C-4), 74.8 (C-6), 71.3 (C-6’), 70.1 (C-2’’), 69.9 (C-3’’), 69.3 (C-5’’), 66.7 (C-5), 66.6 (C-5’), 65.6 (C-4’), 62.8 (C-6’’), 60.2 (C-7’), 60.1 (C-4’’), 57.15 (C-3), 57.07 (C-2’), 55.7 (C-1), 32.0 (C-2), 30.2 (N*C*H$_3$), 29.4 (C-3’), 20.9 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{30}H_{39}N_{13}NaO_{16}$ $[M+Na]^+$ 860.2535; found, 860.2530.

6,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-5-deoxy-5-fluoro-6′,7′-oxazolidino-apramycin (181). A stirred ice-cooled solution of compound **180** (36 mg, 0.04 mmol) in dry DCM (0.2 mL), was treated with diethylaminosulfur trifluoride (45 μL, 0.34 mmol) and stirred at 0 °C for 1 h and at rt for 30 min. After completion, the reaction mixture was purified by gradient chromatography over silica gel (eluent: 0.7% to 0.8% MeOH in DCM) to give **181** (29 mg, 81%) as a white solid; $[\alpha]_D^{25}$= +127.76 (*c* 1.9, DCM); ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.40 (t, *J* = 10.0 Hz, 1H, H-3’’), 5.35 (d, *J* = 3.8 Hz, 1H, H-1’’), 5.21 – 5.07 (m, 2H, H-6, H-1’), 4.96 – 4.86 (m, 2H, H-8’, H-2’’), 4.82 (dd, *J* = 8.2, 3.4 Hz, 1H, H-6’), 4.57 (dd, *J* = 10.4, 3.3 Hz, 1H, H-5’), 4.45 (dt, *J* = 50.3, 9.1 Hz, 1H, H-5), 4.34 (dd, *J* = 12.2, 2.4 Hz, 1H, H-6’’), 4.23 (dd, *J* = 12.2, 5.1 Hz, 1H, H-6’’), 3.86 – 3.66 (m, 5H, H-3, H-4, H-4’, H-7’, H-5’’), 3.65 – 3.58 (m, 1H, H-4’’), 3.53 (ddd, *J* = 12.3, 10.2, 4.4 Hz, 1H, H-1), 3.30 (dt, *J* = 12.9, 4.1 Hz, 1H, H-2’), 2.94 (s, 3H, NC*H*$_3$),

2.47 (dt, *J* = 13.2, 4.4 Hz, 1H, H-2), 2.27 (dt, *J* = 11.4, 4.4 Hz, 1H, H-3'), 2.20 – 2.06 (m, 12H, 4*$COCH_3$), 1.94 (q, *J* = 11.5 Hz, 1H, H-3'), 1.67 (q, *J* = 12.5 Hz, 1H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3 (C=O), 170.0 (C=O), 169.9 (C=O), 169.5 (C=O), 156.9 (C=O), 98.6 (C-1'), 96.1 (C-8'), 94.6 (C-1''), 93.34 (d, *J* = 187.8 Hz, C-5), 79.78 (d, *J* = 17.1 Hz, C-4), 73.05 (d, *J* = 18.5 Hz, C-6), 70.6 (C-3''), 70.2 (C-2''), 70.0 (C-6'), 69.0 (C-5''), 65.8 (C-5'), 65.4 (C-4'), 62.8 (C-6''), 60.2 (C-7'), 60.1 (C-4''), 57.58 (d, *J* = 10.6 Hz, C-3), 57.00 (d, *J* = 9.3 Hz, C-1), 56.4 (C-2'), 31.7 (C-2), 30.0 (N*C*H_3), 29.7 (C-3'), 20.9 (CO*C*H_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.7 (CO*C*H_3); ^{19}F NMR (376 MHz, CDCl3): δ -196.30 (dt, *J* = 50.4, 11.5 Hz); ESI-HRMS: m/z calcd. for $C_{30}H_{38}FN_{13}NaO_{15}$ $[M+Na]^+$ 862.2492; found, 862.2511.

5-Deoxy-5-fluoro apramycin pentaacetate salt (182). A stirred solution of compound **181** (29 mg, 0.035 mmol) in dioxane (0.2 mL) was treated with 3N NaOH (0.2 mL) and heated at 100 °C for 2 h. 1M $P(CH_3)_3$ in THF (0.3 mL) was added and the reaction mixture was stirred at 55 °C for 2h. The reaction mixture was then concentrated and dissolved in aqueous acetic acid solution (pH 4, 1 mL) then charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and lyophilized to afford **182** (16 mg, 56%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +89.91 (*c* 1.1, H_2O); ^1H NMR (600 MHz, D_2O): δ 5.35 (d, *J* = 3.4 Hz, 1H, H-1'), 5.28 (d, *J* = 3.8 Hz, 1H, H-1''), 5.00 (d, *J* = 8.5 Hz, 1H, H-8'), 4.43 (dt, *J* = 50.8, 9.0 Hz, 1H, H-5), 4.35 (s, 1H, H-6'), 4.04 (q, *J* = 9.6 Hz, 1H, H-4), 3.79 – 3.69 (m, 4H, H-6, H-4', H-3'', H-5''), 3.63 (dd, *J* = 12.3, 2.9 Hz, 1H, H-6''), 3.56 (dd, *J* = 12.5, 4.5 Hz, 1H, H-6''), 3.52 (d, *J* = 10.3 Hz, 1H, H-5'), 3.49 (dd, *J* = 9.8, 3.9 Hz, 1H, H-2''), 3.44 (dt, *J* = 12.3, 3.9 Hz, 1H, H-2'), 3.34 (td, *J* = 11.7, 10.9, 4.0 Hz, 1H, H-3), 3.21 – 3.14 (m, 2H, H-1, H-7'), 3.07 (t, *J* = 10.4 Hz, 1H, H-4''),

2.57 (s, 3H, NC*H*$_3$), 2.29 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2), 2.15 (dt, *J* = 10.1, 4.5 Hz, 1H, H-3'), 1.87 – 1.83 (m, 1H, H-3'), 1.73 – 1.66 (m, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 95.33 (d, *J* = 190.2 Hz, C-5), 94.8 (C-1'), 94.3 (C-1''), 92.7 (C-8'), 75.72 (d, *J* = 16.8 Hz, C-4), 70.27 (d, *J* = 20.5 Hz, C-6), 70.2 (C-2''), 69.5 (C-5'), 69.3 (C-5''), 68.2 (C-3''), 65.8 (C-4'), 62.6 (C-6'), 60.2 (C-6''), 59.3 (C-7'), 52.0 (C-4''), 48.67 (d, *J* = 11.5 Hz, C-1), 47.6 (C-2'), 47.34 (d, *J* = 11.3 Hz, C-3), 29.9 (N*C*H$_3$), 28.0 (C-2), 26.6 (C-3'). ^{19}F NMR (376 MHz, D_2O): δ -195.08 (dt, *J* = 50.9, 11.7 Hz); ESI-HRMS: m/z calcd. for $C_{21}H_{41}FN_5O_{10}$ $[M+H]^+$ 542.2837; found, 542.2838.

6,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-5-deoxy-5-epifluoro-6′,7′-oxazolidino-apramycin (183). To a stirred ice-cooled solution of compound **124** (50 mg, 0.06 mmol) in dry DCM (0.2 mL), diethylaminosulfur trifluoride (65 μl, 0.48 mmol) was added, and the reaction mixture was stirred at rt for 3 h. After completion, the reaction mixture was purified by gradient chromatography over silica gel (eluent: 0.7% to 0.8% MeOH in DCM) to give **183** (37 mg, 74%) as a white solid; $[\alpha]_D^{25}$= +104.39 (*c* 2.5, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.42 (t, *J* = 10.0 Hz, 1H, H-3''), 5.38 (d, *J* = 3.8 Hz, 1H, H-1''), 5.09 – 4.96 (m, 2H, H-5, H-1'), 4.90 – 4.84 (m, 2H, H-8', H-2''), 4.80 (dd, *J* = 7.7, 3.5 Hz, 1H, H-6'), 4.70 (ddd, *J* = 27.3, 10.5, 1.8 Hz, 1H, H-6), 4.35 (dd, *J* = 10.4, 3.4 Hz, 1H, H-5'), 4.32 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6''), 4.21 (dd, *J* = 12.2, 5.3 Hz, 1H, H-6''), 4.02 – 3.92 (m, 2H, H-1, H-3), 3.80 (dd, *J* = 7.6, 4.3 Hz, 1H, H-7'), 3.78 – 3.69 (m, 2H, H-4', H-5''), 3.67 – 3.55 (m, 2H, H-4, H-4''), 3.34 (dt, *J* = 12.8, 4.0 Hz, 1H, H-2'), 2.94 (s, 3H, NC*H*$_3$), 2.45 (dt, *J* = 13.5, 4.9 Hz, 1H, H-2), 2.29 (dt, *J* = 11.3, 4.4 Hz, 1H, H-3'), 2.17 (s, 3H, $COCH_3$), 2.10 (d, *J* = 10.8 Hz, 6H, 2*$COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.01 – 1.94 (m, 1H, H-3'), 1.42 (q, *J* = 12.7 Hz, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4 (C=O), 169.9 (C=O), 169.7 (C=O), 156.9 (C=O), 96.7 (C-8'), 96.2 (C-1'), 94.1 (C-1''), 87.65 (d, *J* = 184.1 Hz, C-5), 77.55 (d, *J* = 17.9 Hz, C-4), 73.45 (d, *J* = 17.1 Hz, C-6),

70.6 (C-6’), 70.3 (C-3’’), 69.8 (C-2’’), 69.1 (C-5’’), 66.4 (C-5’), 65.5 (C-4’), 62.9 (C-6’’), 60.2 (C-4’’), 60.0 (C-7’), 57.03 (d, *J* = 3.8 Hz, C-3), 56.0 (C-2’), 55.76 (d, *J* = 4.1 Hz, C-1), 32.0 (C-2), 30.1 (N*C*H$_3$), 29.2 (C-3’), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); ^{19}F NMR (376 MHz, CDCl$_3$): δ -213.48 (dt, *J* = 52.6, 26.8 Hz); ESI-HRMS: m/z calcd. for $C_{30}H_{38}FN_{13}NaO_{15}$ $[M+Na]^+$ 862.2492; found, 862.2502.

5-Deoxy-5-epi-fluoro apramycin pentaacetate salt (184). A stirred solution of compound **183** (37 mg, 0.044 mmol) in dioxane (0.2 mL) was treated with 3 N NaOH (0.2 mL) and heated at 100 °C for 2 h. 1 M P(CH$_3$)$_3$ in THF (0.3 mL) was added and the reaction mixture stirred at 55 °C for 2h. The reaction mixture was then concentrated and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH$_4$OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid, and lyophilized to afford **184** (22 mg, 59%) as peracetate salt in the form of a white solid; $[\alpha]_D^{25}$= +152.0 (*c* 0.1, H$_2$O); ^{1}H NMR (600 MHz, D$_2$O): δ 5.25 (d, *J* = 3.9 Hz, 1H, H-1’’), 5.24 (d, *J* = 3.8 Hz, 1H, H-1’), 5.13 (d, *J* = 51.7 Hz, 1H, H-5), 4.98 (d, *J* = 8.5 Hz, 1H, H-8’), 4.31 (s, 1H, H-6’), 3.94 (dd, *J* = 26.1, 10.7 Hz, 1H, H-4), 3.78 – 3.63 (m, 4H, H-6, H-4’, H-3’’, H-5’’), 3.61 (dd, *J* = 12.5, 3.5 Hz, 1H, H-6’’), 3.57 – 3.50 (m, 2H, H-3, H-6’’), 3.49 – 3.44 (m, 2H, H-2’, H-2’’), 3.42 (dd, *J* = 10.1, 2.6 Hz, 1H, H-5’), 3.33 (td, *J* = 11.7, 4.3 Hz, 1H, H-1), 3.12 (dd, *J* = 8.5, 2.8 Hz, 1H, H-7’), 3.05 (t, *J* = 10.4 Hz, 1H, H-4’’), 2.54 (s, 3H, NC*H*$_3$), 2.29 (dt, *J* = 12.6, 4.4 Hz, 1H, H-2), 2.19 – 2.10 (m, 1H, H-3’), 1.90 – 1.82 (m, 1H, H-3’), 1.59 (q, *J* = 12.6 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D$_2$O): δ 94.3 (C-1’’), 92.7 (C-8’), 90.3 (C-1’), 87.37 (d, *J* = 181.7 Hz, C-5), 72.01 (d, *J* = 17.8 Hz, C-4), 70.1 (C-2’’), 69.5 (C-5’), 69.2 (C-5’’), 68.55 (d, *J* = 17.2 Hz, C-6), 68.1 (C-3’’), 65.7 (C-4’), 62.5 (C-6’), 60.2 (C-6’’), 59.3 (C-7’), 52.0 (C-4’’),

48.09 (d, *J* = 4.4 Hz, C-1), 47.3 (C-2'), 46.61 (d, *J* = 4.2 Hz, C-3), 29.9 (N*C*H$_3$), 27.9 (C-2), 26.7 (C-3'); ^{19}F NMR (376 MHz, D$_2$O): δ -217.88 (dt, *J* = 51.8, 27.2 Hz); ESI-HRMS: m/z calcd. for $C_{21}H_{41}FN_5O_{10}$ $[M+H]^+$ 542.2837; found, 542.2825.

6,2'',3'',6''-Tetra-*O*-acetyl-1,3,2′,4′′-tetraazido-5-deoxy-5-epiiodo-6′,7′-oxazolidino-apramycin (185). To a stirred solution of compound **124** (100 mg, 0.12 mmol) in dry DCM (1.5 mL), pyridine (0.1 mL) was added and reaction mixture was cooled to 0 °C before triflic anhydride (40 μL, 0.24 mmol) was added. The reaction mixture was stirred for 1 h and additional triflic anhydride (40 μL, 0.24 mmol) was added. After 2 h, the reaction mixture was poured into an iced aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was dissolved in dry acetone (25 mL), heated to reflux under stirring with sodium iodide (266 mg, 1.78 mmol) for 12 h. After completion, the reaction mixture was concentrated, diluted with EtOAc and washed with brine then concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.6% - 1.0% Methanol/DCM) to give compound **185** (93 mg, 89%) as a white solid; $[\alpha]_D^{25}$= +172.23 (*c* 6.2, DCM); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.46 – 5.38 (m, 2H, H-1'', H-3''), 4.92 (d, *J* = 3.4 Hz, 1H, H-1'), 4.84 (t, *J* = 3.2 Hz, 1H, H-5), 4.80 – 4.72 (m, 3H, H-6', H-8', H-2''), 4.31 (dd, *J* = 12.3, 2.2 Hz, 1H, H-6''), 4.20 (dd, *J* = 12.2, 5.4 Hz, 1H, H-6''), 4.04 – 3.98 (m, 2H, H-3, H-4), 3.98 – 3.90 (m, 2H, H-1, H-5'), 3.80 – 3.70 (m, 2H, H-7', H-5''), 3.66 (td, *J* = 10.8, 4.5 Hz, 1H, H-4'), 3.55 (t, *J* = 10.2 Hz, 1H, H-4''), 3.25 (dt, *J* = 12.8, 3.9 Hz, 1H, H-2'), 3.06 (dd, *J* = 9.7, 3.6 Hz, 1H, H-6), 2.94 (s, 3H, NC*H*$_3$), 2.28 (ddt, *J* = 12.6, 8.7, 4.6 Hz, 2H, H-2, H-3'), 2.15 (s, 3H, $COCH_3$), 2.13 – 2.03 (m, 7H, H-3', 2*$COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.41 – 1.29 (m, 1H, H-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.4 (C=O), 170.2 (C=O), 169.6 (C=O), 169.6 (C=O), 156.8 (C=O), 99.8 (C-8'), 94.3 (C-1''), 93.9 (C-1'), 74.1 (C-6), 72.6

(C-4), 71.7 (C-6'), 69.9 (C-2''), 69.8 (C-3''), 69.4 (C-5''), 67.2 (C-5'), 65.8 (C-4'), 62.8 (C-6''), 60.6 (C-1), 60.2 (C-4''), 60.1 (C-7'), 59.2 (C-3), 55.4 (C-2'), 32.8 (C-5), 32.6 (C-2), 30.4 (N*C*H_3), 28.0 (C-3'), 20.9 (CO*C*H_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.7 (CO*C*H_3); ESI-HRMS: m/z calcd. for $C_{30}H_{38}IN_{13}NaO_{15}$ $[M+Na]^+$ 970.1553; found, 970.1571.

5-Deoxy-apramycin pentaacetate salt (186). To a solution of compound **185** (45 mg, 0.05 mmol) in dry methanol (5 mL), sodium methoxide (10 mg, 0.06 mmol) was added and reaction mixture was stirred for 30 min. The reaction was quenched with Amberlyst®, filtered and the solvent was evaporated *in vacuo*. The crude product was dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL) and 10% Pd/C (60 mg, 1.1 equiv.) was added. The reaction was stirred at room temperature under 1 atm of hydrogen (balloon) for 12 h. After completion, the reaction mixture was filtered over Celite® and filtrate concentrated to dryness. The residue was dissolved in dioxane (0.5 mL) and treated with 3N NaOH (0.25 mL) and heated at 100 °C for 30 min. The reaction mixture was cooled to rt and neutralized with glacial acetic acid before concentration *in vacuo*. The crude product was dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient eluted with 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with glacial acetic acid and lyophilized to afford **186** (18.5 mg, 47%) as a white solid; $[\alpha]_D^{25}$= +105.0 (*c* 0.2, H_2O); 1H NMR (600 MHz, D_2O): δ 5.35 (d, *J* = 4.0 Hz, 1H, H-1''), 5.23 (d, *J* = 3.8 Hz, 1H, H-1'), 5.07 (d, *J* = 8.5 Hz, 1H, H-8'), 4.40 (s, 1H, H-6'), 3.87 – 3.73 (m, 4H, H-4, H-4', H-3'', H-5''), 3.70 (dd, *J* = 12.5, 3.3 Hz, 1H, H-6''), 3.63 (dd, *J* = 12.5, 4.7 Hz, 1H, H-6''), 3.62 – 3.52 (m, 2H, H-6, H-2''), 3.52 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2'), 3.48 (dd, *J* = 10.0, 3.0 Hz, 1H, H-5'), 3.34 (ddd, *J* = 12.4, 10.1, 4.1 Hz, 1H, H-3), 3.21 (dd, *J* = 8.5, 2.9 Hz, 1H, H-7'), 3.17 – 3.10 (m,

2H, H-1, H-4''), 2.63 (s, 3H, NC*H*$_3$), 2.55 (dt, *J* = 12.2, 4.3 Hz, 1H, H-5), 2.33 (dt, *J* = 12.6, 4.3 Hz, 1H, H-2), 2.23 (dt, *J* = 10.8, 4.5 Hz, 1H, H-3'), 1.90 (q, *J* = 11.8, 11.0 Hz, 1H, H-3'), 1.61 (q, *J* = 12.5 Hz, 1H, H-2), 1.34 (q, *J* = 11.7 Hz, 1H, H-5); ^{13}C NMR (151 MHz, D_2O): δ 94.3 (C-1''), 92.7 (C-8'), 90.1 (C-1'), 70.8 (C-4), 70.2 (C-6), 69.4 (C-2''), 69.3 (C-5'), 68.2 (C-5''), 67.2 (C-3''), 65.8 (C-4'), 62.6 (C-6'), 60.2 (C-6''), 59.4 (C-7'), 52.5 (C-1), 52.0 (C-4''), 50.8 (C-3), 47.3 (C-2'), 33.8 (C-5), 30.0 (N*C*H_3), 28.5 (C-2), 26.9 (C-3'); ESI-HRMS: m/z calcd. for $C_{21}H_{42}N_5O_{10}$ $[M+H]^+$ 524.2932; found, 524.2924.

1,2′,4″-Triazido-apramycin (188). Trifluoromethanesulfonyl azide was prepared fresh for each reaction as described here. Sodium azide (995.0 mg, 14.5 mmol) was dissolved in water (5.0 mL) and an equal volume of dichloromethane (5.0 mL) was added while stirring at room temperature. The resulting suspension was cooled to 0 ºC and Tf_2O (2.0 g, 7.4 mol) was added drop wise over 45 min with vigorous stirring. The mixture was stirred at 0 ºC for 3 h before sat. $NaHCO_3$ (5.0 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with dichloromethane (5.0 mL). The organic layers were combined (triflyl azide solution) and kept at 0 ºC until needed. In a 100 mL round bottom flask, 3-*N*-(benzyloxycarbonyloxy) apramycin[152] **187** (500.0 mg, 0.74 mmol), $NaHCO_3$ (524.0 mg, 6.20 mmol) and $CuSO_4.5H_2O$ (18.0 mg, 0.17 mmol) were dissolved in H_2O (3.0 mL) and cooled to 0 $^{\circ}$C. Triflyl azide solution (freshly prepared dichloromethane solution) was added slowly to the reaction mixture at 0 ºC over 0.5 h, followed by drop wise addition of MeOH (5.0 mL) over 0.5 h. The reaction mixture was allowed to come to room temperature and was stirred for 8 h before *n*-butylamine (500.0 mg) was added to quench the excess TfN_3. The solvent was evaporated under vacuum and the residue was purified by column chromatography over silica gel to give 2 (135.0 mg, 43%) as a white solid and which used for further without any characterization. A

stirred solution of 2 (134.0 mg, 0.18 mmol) in *p*-dioxane (2 mL) was treated with 3N NaOH (2.0 mL) at rt. The resulting reaction mixture was stirred at 100 ºC for 2 h and was quenched with glacial acetic acid. The reaction mixture was concentrated to afford a yellow oil that was purified by chromatography over silica gel eluting with gradient of 10% to 40% ammonical methanol in dichloromethane to give 3 (92.0 mg, 84%) as a white foam. $[\alpha]_D^{25}$ = +114.28 (*c* 0.14, MeOH); ^{1}H NMR (600 MHz, CD_4OD): δ 5.68 (d, *J* = 3.5 Hz, 1H), 5.32 (d, *J* = 3.9 Hz, 1H), 4.99 (d, *J* = 8.3 Hz, 1H), 4.31 (t, *J* = 2.8 Hz, 1H), 3.87-3.78 (m, 2H), 3.80-3.74 (m, 1H), 3.70-3.62 (m, 2H), 3.59-3.49 (m, 3H), 3.45 (td, *J* = 9.5, 2.8 Hz, 1H), 3.39 (td, *J* = 10.2, 3.7 Hz, 2H), 3.33 (d, *J* = 4.6 Hz, 1H), 3.31-3.24 (m, 2H), 3.01-2.90 (m, 2H), 2.60 (s, 3H), 2.20 (dt, *J* = 9.6, 4.5 Hz, 1H), 2.14-2.02 (m, 2H), 1.91 (s, 2H), 1.33 (q, *J* = 12.5 Hz, 1H); ^{13}C NMR (151 MHz, CD_4OD) δ 97.02, 94.51, 94.37, 81.82, 77.02, 76.34, 72.24, 71.46, 71.28, 70.21, 66.58, 64.32, 62.04, 60.98, 60.93, 60.84, 56.72, 49.31, 33.19, 31.07, 27.70; ESI-HRMS: *m/z* calcd. for $C_{21}H_{35}N_{11}O_{11}$ $[M+H]^+$ 640.2415, found: 640.2399.

***N*-(Diethylcarbamoyl)-*N*-methoxyacetamide (189).** To a mixture of 1,1-diethyl-3-methoxyurea[172] (500 mg, 3.4 mmol), 4-dimethylaminopyridine (1250 mg, 10.2 mmol) and DCM (5 mL) cooled in an ice-bath was added acetic anhydride (970 μL, 10.2 mmol) over 20 min. The mixture was stirred for 10 h before diluting with EtOAc. The organic layer washed with 5% aqueous HCl, aqueous Na_2HCO_3 and brine, dried with Na_2SO_4 and concentrated. The crude product was purified using silica gel column chromatography (eluent: 50% EtOAc/hexanes) to give **189** (385 mg, 65%) as colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H, OCH_3), 3.25 (br s, 4H, 2*NCH_2CH_3), 2.08 (s, 3H, $COCH_3$), 1.08 (t, *J* = 7.2 Hz, 6H, 2*NCH_2CH_3); ^{13}C NMR (101 MHz, $CDCL_3$) δ 170.0 ($COCH_3$), 153.3 (NCON), 63.2 (OCH_3), 41.6 (NCH_2CH_3), 21.9

($CO\mathit{C}H_3$), 13.0 ($NCH_2\mathit{C}H_3$); ESI-HRMS: m/z calcd. for $C_8H_{16}N_2NaO_3$ $[M+Na]^+$ 211.1059; found, 211.1050.

3-*O*-Formyl-apramycin tetraacetate salt (190). To a stirred solution of 1,2',4"-triazido-apramycin **188** (15 mg, 0.02 mmol) in water (0.5 mL), *N*-(diethylcarbamoyl)-*N*-methoxyformamide[172] (16 μL, 0.09 mmol) and triethylamine (2 μL) were added. The reaction mixture was stirred for 48 h and quenched with aqueous ammonium hydroxide (0.5 mL) and concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.5% to 3% ammonical MeOH in DCM). The product-containing fractions was concentrated and dissolved in dioxane:water (1:1, 0.4 mL) followed by the addition 1 M $P(CH_3)_3$ in THF (0.2 mL), and stirred at 50 °C for 2 h. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with acetic acid, and lyophilized to afford **190** (5 mg, 25%) as a white solid; $[\alpha]_D^{25}$= +103.2 (*c* 0.3, H_2O); Major (trans): Minor (Cis) = 4:3; ***Major (trans)***: ^{1}H NMR (600 MHz, D_2O) δ 5.28 (d, *J* = 3.5 Hz, 1H, H-1't), 3.99 – 3.91 (m, 1H, H-3t), 3.04 (dd, *J* = 8.6, 2.4 Hz, 1H, 7't) 2.64 (s, 3H, $NC\mathit{H}_3$t), 2.08 (dt, *J* = 13.3, 3.9 Hz, 1H, H-2); ^{13}C NMR (151 MHz, d_2o) δ 163.8 (CHOt), 96.1 (C-1't), 81.1 (C-4t), 46.4 (C-3t), 30.2 (C-2t); ***Minor (Cis)***: ^{1}H NMR (600 MHz, Deuterium Oxide): δ 5.20 (d, *J* = 3.6 Hz, 0.75H, H-1'c), 2.63 (s, 1.5H, $NC\mathit{H}_3$c); ^{13}C NMR (151 MHz, d_2o): δ 167.2 (CHOc), 96.6 (C-1'c), 81.2 (C-4c), 51.0 (C-3c), 31.5 (C-2c); ***Not resolved rotamers***; ^{1}H NMR (600 MHz, D_2O): δ 8.00 (s, 1H, CHO), 7.93 (s, 1H, CHO), 5.32 (t, *J* = 4.3 Hz, 1.75H, H-1''), 5.04 (t, *J* = 7.5 Hz, 1.75H, H-8'), 4.27 (s, 1.75H, H-6'), 3.84 – 3.78 (m, 1.75H, H-5''), 3.78 – 3.71 (m, 3.5H, H-4', H-3''), 3.69 (dd, *J* = 12.5, 3.3 Hz, 1.75H, H-6''), 3.62 (dd, *J* = 12.5, 4.5 Hz,

1.75H, H-6’’), 3.59 – 3.51 (m, 3.5H, H-3c, H-4t, H-2’’), 3.50 – 3.39 (m, 6H, H-4c, H-5, H-2’, H-5’), 3.38 – 3.31 (m, 1.75H, 6), 3.19 – 3.06 (m, 4.25H, H-1, H-4’’, H-7c), 2.19 (ddt, *J* = 16.3, 8.8, 4.4 Hz, 2.25H, H-3’, H-2c), 1.59 (q, *J* = 12.5 Hz, 1.75H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 94.4 (C-1’’), 92.9 and 92.7 (C-8’), 75.5 and 75.2 (C-5), 72.5 (C-6), 70.2 (C-2’’), 69.4 (C-5’’), 69.5 and 69.0 (C-5’), 68.4 (C-3’’), 65.9 (C-4’), 62.8 and 62.5 (C-6’), 60.3 (C-6’’), 59.6 (C-7’), 52.0 (C-4’’), 50.1 and 50.0 (C-1), 48.2 and 48.0 (C-2’), 30.1 and 30.0, (N*C*H_3), 27.1 and 26.9 (C-3’); ESI-HRMS: m/z calcd. for $C_{22}H_{42}N_5O_{10}$ $[M+H]^+$ 568.2830; found, 568.2833.

3-*O*-Acetyl-apramycin tetraacetate salt (191). To a stirred solution of 1,2',4"-triazido-apramycin **188** (15 mg, 0.02 mmol) in water (0.5 mL), *N*-(diethylcarbamoyl)-*N*-methoxyacetamide **189** (50 μL, 0.26 mmol) was added. The reaction mixture was stirred for 3 h and quenched with aqueous ammonium hydroxide (0.5 mL) and concentrated. The crude product was purified using silica gel column chromatography (eluent: 0.5% to 3% ammonical MeOH in DCM). The crude product was dissolved in dioxane:water:glacial acetic acid = 1:2:0.2 (0.3 mL) and 10% Pd/C (20 mg, 1 equiv.) was added. The reaction was stirred at room temperature under 1 atm of hydrogen (balloon) for 1 h. After completion, the reaction mixture was filtered over Celite® and filtrate concentrated to dryness. The reaction mixture was then concentrated to dryness and dissolved in aqueous acetic acid solution (pH 4, 1 mL) before it was charged to a Sephadex column (CM Sephadex C-25). The column was flushed with D.I. water (20 mL), then gradient elution of 0.1% - 1.0% NH_4OH in D.I. water. The fractions containing the product were combined, acidified with acetic acid, and lyophilized to afford **191** (3 mg, 15%) as a white solid; $[\alpha]_D^{25}$= +113.0 (*c* 0.1, H_2O);^{1}H NMR (600 MHz, D_2O): δ 5.41 (d, *J* = 3.3 Hz, 1H, H-1’), 5.36 (d, *J* = 4.0 Hz, 1H, H-1’’), 5.07 (d, *J* = 8.4 Hz, 1H, H-8’), 4.33 (s, 1H, H-6’), 3.89 (ddd, *J* = 12.5, 9.6, 4.4 Hz, 1H, H-3), 3.84 – 3.74 (m, 3H, H-4’, H-3’’, H-5’’), 3.71 (dd, *J* = 12.5, 3.3 Hz, 1H, H-

6’’), 3.64 (dd, *J* = 12.4, 4.7 Hz, 1H, H-6’’), 3.57 (dd, *J* = 9.8, 4.0 Hz, 1H, H-2’’), 3.54 – 3.44 (m, 2H, H-4, H-5), 3.44 – 3.34 (m, 2H, H-2’, H-5’), 3.35 (t, *J* = 9.7 Hz, 1H, H-6), 3.17 – 3.07 (m, 3H, H-1, H-7’, H-4’’), 2.65 (s, 3H, NC*H*$_3$), 2.19 (dt, *J* = 11.1, 4.7 Hz, 1H, H-3’), 2.08 (dt, *J* = 12.9, 4.4 Hz, 1H, H-2), 1.89 (s, 3H, COC*H*$_3$), 1.74 (q, *J* = 11.9 Hz, 1H, H-3’), 1.53 (q, *J* = 12.7 Hz, 1H, H-2); ^{13}C NMR (151 MHz, D_2O): δ 173.4 (C=O), 95.3 (C-1’), 94.3 (C-1’’), 92.9 (C-8’), 79.6 (C-4), 75.8 (C-5), 72.7 (C-6), 70.2 (C-2’’), 69.6 (C-5’’), 69.5 (C-5’), 68.5 (C-3’’), 65.9 (C-4’), 62.9 (C-6’), 60.3 (C-6’’), 59.8 (C-7’), 52.1 (C-4’’), 50.1 (C-1), 48.3 (C-2’), 47.5 (C-3), 30.3 (C-2), 30.1 (N*C*H$_3$), 26.8 (C-3’), 22.4 (CO*C*H$_3$); ESI-HRMS: m/z calcd. for $C_{23}H_{44}N_5O_{12}$ $[M+H]^+$ 582.2986; found, 582.2983.

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ABSTRACT

SYNTHESIS OF NETILMICIN AND APRAMYCIN DERIVATIVES FOR THE TREATMENT OF MULTIDRUG-RESISTANT INFECTIOUS DISEASES

by

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The ever-growing bacterial resistance to existing antibiotics is alarming to humanity. Many researchers decided to revisit aminoglycosides with renewed emphasis on chemical modification as they have long been used as highly potent antibiotics for treating severe bacterial infections. The bactericidal effect of aminoglycosides is mainly due to protein synthesis inhibition by binding to the A-site of the bacterial ribosomes. However, the high potency and the broad spectrum of aminoglycosides has been outweighed by their side effects, especially ototoxicity, and by the resistance of pathogens. The goal of this research was the modification of existing aminoglycosides to develop derivatives which are less toxic and that evade resistance. The chapters in the thesis discuss the chemical synthesis as well as the biological evaluation of the newly synthesized analogs. This study has focused on the modification of aminoglycosides netilmicin and apramycin.

Chapter one introduces the MDR bacterial infection problem and its influence. Chapter one also introduces the aminoglycosides elaborating their history, classifications, and their mechanism of action. The resistance mechanisms against aminoglycosides and their adverse effects, as well as the ways to prevent them are briefly explained.

Chapter two discusses modifications of netilmicin at the 4’-position conducted with a view to reducing the ototoxicity but not the antibiotic activity, as was previously done in the 4,5-series with paromomycin. The antibacterial activity and antiribosomal activity of the six netilmicin derivatives synthesized were determined. The 4’-position is more sensitive to modification in 4,6-series than in the 4,5-series to the extent that such modifications are ineffective. Chapter two also highlights the use of phenyl triazenes as selective protecting groups for secondary amines in the presence of primary amines. Several polyamine substrates were selectively protected as phenyl triazenes, and primary amines were subsequently protected as azides, benzyloxy carbamates, or fluorenylmethyl carbamates. Phenyl triazenes enabled the synthesis of plazomicin, an aminoglycoside in phase III clinical trials, in fewer steps and higher yield than previously reported.

Chapter three describes derivatization and modification of apramycin at the 5-position. The influence of these modifications was investigated using cell-free translation assays and antibacterial assays. An apramycin-paromomycin hybrid was synthesized with the aim of combining paromomycin’s high activity with apramycin’s low ototoxicity. Eighteen compounds were synthesized with modifications mainly at the 5-position leading to the development of a potent derivative that was more active than apramycin against all bacterial strains tested and which also showed better ribosomal selectivity. This investigation affords proof of concept for the development of more potent and selective aminoglycosides in the apramycin class.

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PUBLICATIONS

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POSTER PRESENTATION

- Presented a poster at 252nd ACS National Meeting in Philadelphia, Pennsylvania held in August 21-25, 2016. Title: Design, synthesis, and evaluation of improved apramycin derivatives for the treatment of MDR infectious diseases.